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FACULTY OF ENGINEERING, SCIENCES AND MATHEMATICS

School of Chemistry

Synthesis of cyclic peptide natural products and inhibitors of histone modifying enzymes

by

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Thesis for the degree of Doctor of Philosophy

ABSTRACT

Natural products have been the source of numerous leads for several drugs. As these natural products are often isolated in small quantities, it is necessary to produce them synthetically to allow testing for biological activity. Furthermore, synthesis allows the preparation of unnatural analogues for SAR studies. Cyclic peptides represent an important family of biologically active natural products.

The hepta- and octacyclopeptides sanguinamide A and sanguinamide B were recently isolated in submicromolar amounts by the Molinski group. The lack of material prevented biological evaluation of the natural products. For this reason and to confirm the structural elucidation we have targeted the total synthesis of sanguinamides. In addition to two proline residues, sanguinamides A and B include heterocycles and natural L-amino acid residues. We have completed the total syntheses of sanguinamides A and B; however the synthetic rotamers differed in both cases from the natural rotamers. We have investigated the influence of macrocyclisation on *cis/trans* conformational preference of the proline residues for the synthesis of sanguinamide A. We attempted several isomerisations and calculated the relative energies of the different sanguinamide conformers. [D-Ile]-Sanguinamide A, Cys(*t*Bu) analogue of sanguinamide A and the synthetic sanguinamide B displayed antibacterial activity while the synthetic *trans, trans*-sanguinamide A displayed mild tyrosine kinase inhibitory activity. While extracted stylissamide A showed inhibition of translation during the elongation step, even though being structurally identical to the natural product, the synthetic compound prepared by macrocyclisation from a linear precursor was found to be totally inactive.

Histones undergo different types of covalent modifications on the *N*-terminal tails such as acetylation, phosphorylation and methylation. Histone modification is a major mechanism of regulation in gene expression, replication and repair. Deregulation of histone modifications leads to cancer progression and therefore, inhibitors of enzymes which are able to catalyse the addition and removal of these epigenetic marks have therapeutic potential for treating cancer. An enzyme of particular interest is the family of zinc-dependent histone deacetylases (HDACs) that remove acetyl groups from acetylated lysine residues. Depsipeptides were prepared as HDAC inhibitors. We will

present our total synthesis of largazole along with a range of analogues and discuss the SAR obtained from HDAC and cell proliferation assays. We elucidated the stereochemistry of burkholdac B by total synthesis of three diastereomers. The diastereomers made along with the natural product were tested as HDAC inhibitors.

We are interested in inhibitors of lysine-specific demethylase 1 (LSD1) which is a different kind of epigenetic enzyme involved in demethylation of histone proteins in chromatin. Tranylcypromine is known to be an LSD1 inhibitor. Analogues of PCPA have been synthesised in order to explore the structure-activity relationships of this inhibitor. Analogues were also prepared and tested as LSD1 inhibitors.

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I, Hanae Benelkebir declare that the thesis entitled *"Synthesis of cyclic peptide natural products and inhibitors of histone modifying enzymes"* and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

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Total synthesis and stereochemical assignment of burkholdac B, a depsipeptide HDAC inhibitor
Benelkebir, H.; Donlevy, A. M.; Packham, G.; Ganesan, A. *Org. Lett.* **2011**, *13*, 6334-6337.

Total synthesis of largazole and analogues: HDAC inhibition, antiproliferative activity and metabolic stability Benelkebir H.; Marie S.; Hayden A.; Lyle J.; Loadman P.; Packham G.; Ganesan A. *Bioorg. Med. Chem.* **2011**, *19*, 3650-3658.

Enantioselective synthesis of tranylcypromine analogues as lysine demethylase (LSD1) inhibitors Benelkebir H.; Hodgkinson C.; Duriez P. J.; Hayden A.; Bullied R.; Crabb S. J.; Packham G.; Ganesan A. *Bioorg. Med. Chem.* **2011**, *19*, 3709-3716.

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ABBREVIATIONS

[α]	specific rotation
Ac	acetyl
Aib	aminoisobutyric acid
All	allyl
Alloc	allyloxycarbonyl
Aoe	(*S*)-2-amino-9,10-epoxy-8-oxodecanoic acid
aq	aqueous
AR	androgen receptor
Boc	*tert*-butoxycarbonyl
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
bp	boiling point
br	broad
calcd	calculated
COSMO	conductor-like screening model
COSY	correlation spectroscopy
δ	chemical shift in parts per million
d	day(s), doublet (NMR)
DABCO	1,4-diazabicyclo[2.2.2]octane
DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethylazocarboxylate
DEPT	distortionless enhancement through polarization transfer
DFT	density functional theory
Dhb	dehydrobutyrine
DIBAL	diisobutylaluminium hydride
DIC	*N,N'*-diisopropylcarbodiimide
DIAD	diisopropylazodicarboxylate
DIPA	diisopropylamine
DIPEA	*N,N'*-diisopropylethylamine
DMAP	4-(*N,N*-dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid

DPPA	diphenylphosphoryl azide
dr	diastereomeric ratio
DTT	dithiothreitol
EAM	energy absorbing molecule
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDT	ethane dithiol
ee	enantiomeric exces
EI	electron impact
equiv	equivalent
ER	estrogen receptor
ES^-	negative electrospray
ES^+	positive electrospray
ESI	electrospray ionisation
Et	ethyl
FAD	flavin adenine dinucleotide
Fmoc	9-fluorenylmethoxycarbonyl
h	hour
HAT	histone acetyltransferases
H3K4	lysine residue 4 of histone protein 3
HATU	2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HCV	hepatitis C virus
HDAC	histone deacetylase inhibitor
HIV	Human immunodeficiency virus
HMT	histone methyltransferase
HKMT	histone lysine methyltransferase
HRMT	histone arginine methyltransferase
HOBt	hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HRP	horseradish peroxidase
Hz	hertz
IC_{50}	half maximal inhibitory concentration
IPTG	isopropyl-β-D-thiogalactopyranoside
IR	infrared
IRES	internal ribosomal entry sites
J	coupling constant
Ki	inhibition constant
LDA	lithium diisopropylamide
LSD1	lysine-specific demethylase 1
m	multiplet

MAO	monoamine oxidase
MCF7	Michigan Cancer Foundation 7
Me	methyl
MIC	minimum inhibitory concentration
min	minute
MNBA	2-methyl-6-nitrobenzoic anhydride
mp	melting point
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSNT	1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole
Mult	multiplicity
MW	Molecular weight or microwave
NAD^+	nicotinamide adenine dinucleotide
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
OPA	*o*-phthalaldehyde
Oxz	oxazole
PAL	5-[4-(9-fluorenylmethyloxycarbonyl)aminomethyl-3,5-dimethoxy-phenoxy] valeric acid
PCPA	*trans*-2-phenylcyclopropylamine = tranylcypromine
PFP	pentafluorophenol
PG	protecting group
Ph	phenyl
Pi	isoelectric point
PMB	*p*-methoxybenzyl
PMP	*p*-methoxyphenyl
ppm	parts per millions
PPTS	pyridinium *para*-toluenesulfonate
PSA	prostate-specific antigen
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
q	quartet
quin	quintet
Red-Al	sodium bis(2-methoxyethoxy)aluminumhydride
R_f	retention factor
RFU	relative fluorescence unit
RNA	ribonucleic acid
rt	room temperature
s	singlet
SAHA	suberoylanilide hydroxamic acid
SAM	*S*-adenosylmethionine
SAR	structure-activity relationships
SCX	strong cation-exchanger

SELDI	surface-enhanced laser desorption/ionization
SEM	standard error of the mean
siRNA	small interfering ribonucleic acid
SPPS	solid phase peptide synthesis
spt	septet
STDEV	standard deviation
sxt	sextet
t	triplet
TBAF	tetra-*n*-butylammonium fluoride
TBDA	*tert*-butyldiazoacetate
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
Tce	trichloroethyl
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic acid anhydride
THF	tetrahydrofuran
Thz	thiazole
TIS or TIPS	triisopropylsilane
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSE	trimethylsilyl ethyl
TOF	time-of-flight
Tr	triphenylmethyl (trityl)
Ts	*para*-toluenesulfonyl (tosyl)
TSA	trichostatin A

1. PROLINE-CONTAINING CYCLIC PEPTIDE NATURAL PRODUCTS

1.1. INTRODUCTION

1.1.1. Natural products

Natural products are produced by living organisms and come from various origins such as plants, animals or microorganisms.[1] They have been the source of numerous leads for medicinally important drugs for many years.[2]

For a time, combinatorial chemistry became the method of choice for drug discovery in pharmaceutical companies. Some companies stopped developing their natural product database and concentrated their efforts on synthetic compounds. With time, they realised that natural products are very important in drug discovery. In fact, statistics show that the numbers of drugs discovered decreased and that natural products remained the major source of new drugs. A total of 24 natural products isolated between 1970 and 2006 led to an approved drug.[3] A study was carried out in order to compare the chemical space occupied by combinatorial compounds to natural product and drug databases (Figure 1.1).[2] It showed that natural products present a greater chemical diversity and cover a larger chemical space than synthetic compounds which only occupies a limited chemical space. The pattern of the chemical space of natural product database and drug database displays more similarities.
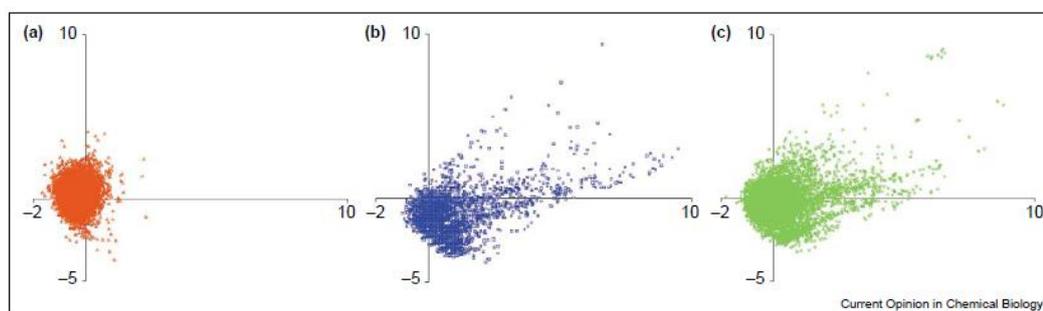
Figure 1.1: Representation of the chemical space occupied by combinatorial compounds (a), natural product database (b) and drugs database (c)[2] "Reprinted from Current Opinion in Chemical Biology, *8*, Ortholand J.-Y.; Ganesan A., Natural products and combinatorial chemistry: back to the future, 271-280, Copyright (2004), with permission from Elsevier."

Natural products contain more oxygen atoms but fewer halogen or sulfur atoms compared to synthetic compounds. They also display more complex features with numerous rings and chiral centres. For example, the well-known taxol (**1.1**), isolated from the bark of the Pacific yew tree, *Taxus brevifolia*, has a highly complex structure with eleven stereocentres and seven rings.[4] It is a mitotic inhibitor involving the stabilization of microtubules and clinically used for treating several types of cancer including lung, breast and ovarian cancer.[4] Trabectedin (**1.2**) was isolated from a marine source: the sea squirt *Ecteinascidia turbinata*.[5] It is an anti-tumour agent used for the treatment of advanced soft tissue sarcoma. Its complex structure contains nine rings and seven chiral centres. Cyclosporin A (**1.3**), isolated from the fungus *Tolypocladium inflatum*, is a cyclopeptide containing eleven amino acids.[6] It is an immunosuppressant drug used during organ transplants to reduce the risk of organ rejection. Cyclosporin A is a drug even though its characteristics do not follow the Lipinski's rule of five.[7] These three natural products became drugs without modification and illustrate the diverse structures of natural products along with their various origins and biological activities.
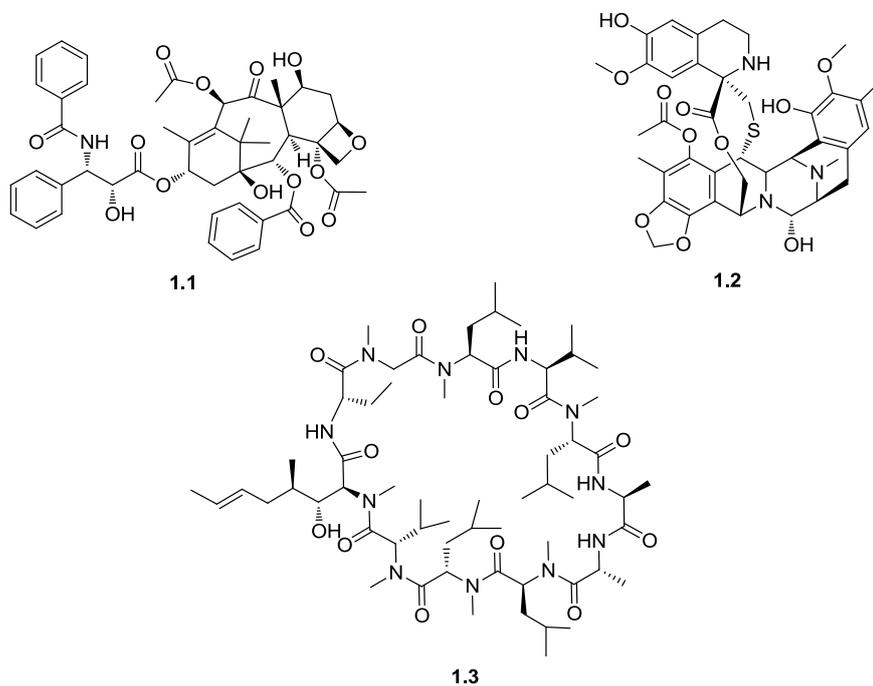
Figure 1.2 : Structures of taxol (1.1), trabectedin (1.2) and cyclosporin A (1.3)

As these natural products are often isolated in small quantities, it is necessary to synthesise them to allow them to be tested for their biological activity. Furthermore, synthesis allows the preparation of unnatural analogues for SAR studies.

1.1.2. Proline-containing cyclic peptides

The amide group, abundant in all living organisms, is a very important group as it is the link between the amino acids in peptides and proteins. Several coupling reactions are available to synthetically prepare amides.[8] Delocalization of the lone pair electrons from nitrogen to the carbonyl group is responsible for the stability of the proteins.[9] This gives the C-N bond some double-bond character due to conjugation and thus, no rotation is possible around this bond.[9] Due to this restricted rotation, two isomers are possible: the *cis* and *trans* isomer (Figure 1.3). The latter is more common in nature as the hydrogen of the amide has less interaction with the substituent of the carbonyl group.
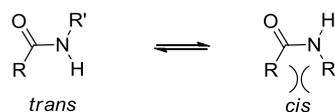
Figure 1.3 : The two possible conformations for the amide peptide bond

Cyclic peptides represent an important family of biologically active natural products. They have a wide range of pharmaceutical properties such as cytotoxicity, anticancer, antiviral, antifungal and anti-inflammatory activity.[10] The fact that cyclopeptides do not possess the ionised *C*- and *N*-termini present in linear peptides makes them more cell permeable.[10] They display a greater bioavailability and resistance to enzymatic degradation than their non cyclic analogues and possess a more rigid structure.[10]

Many biologically important cyclic peptide sequences and natural products contain multiple proline residues. Proline is the only amino acid with a secondary amino group based on a pyrrolidine. As seen previously for peptide bonds, the proline amide bond can also exist in *trans* or *cis* conformations (Figure 1.4). Although the *trans* amide bond is more common, the occurrence of *cis* geometry is more frequent for the proline peptide bond than for other amino acids.[11] The frequency of *cis* proline peptide bond is higher in cyclic peptides than in linear peptides.[12] A statistical study performed on the

Cambridge Structural Database showed that 57.4% of proline residues present in cyclic peptides were in the *cis* conformation compared to only 5.6% in acyclic peptides.[12] The reason for this high proportion of *cis* proline in cyclopeptides is due to the conformational restrictions during the cyclisation step.[12]
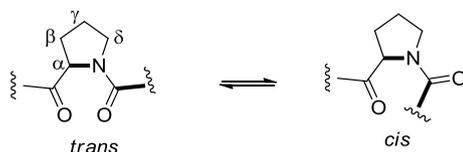
Figure 1.4: The two possible conformations for the proline peptide bond

The geometry of the proline amide can be determined on the basis of the difference in ^{13}C chemical shifts between $C\beta$ and $C\gamma$ signals ($\Delta\delta\beta\gamma = \delta\beta - \delta\gamma$).[13] A small ^{13}C chemical shift difference indicates that the proline peptide bond is *trans* while a large ^{13}C chemical shift difference indicates a *cis* proline residue.

Marine sponges are the main source of proline-rich cyclopeptides. Several examples of isolation, synthesis and structural aspects of proline-containing cyclic peptides are described in the next section.

1.1.2.1. Yunnanins

Yunnanins A and C are cyclic heptapeptides isolated from the roots of the plant *Stellaria yunnanensis* containing two and one proline residues respectively (Figure 1.5). Paloma *et al.* prepared these cyclic peptides by solid phase synthesis.[14] Synthetic yunnanin A displayed the identical proline peptide bond conformation when compared to the natural product (both *trans*). Synthesis of yunnanin C gave a complex mixture of a major isomer (*trans*-Pro) identical to the natural yunnanin C and two different rotamers with both *cis* conformation for the unique proline residue. The synthetic products displayed weaker biological activity against cancer cell lines even though they are identical to natural products. The two conformers of yunnanin C presented a similar biological result. Paloma and co-workers gave two different hypotheses to explain this surprising result. The first is that traces of a cytotoxic compound responsible for the biological activity are retained by the natural cyclopeptides. The second explanation is

that subtle conformational changes of the prolyl residues are responsible for the activity of the natural product.
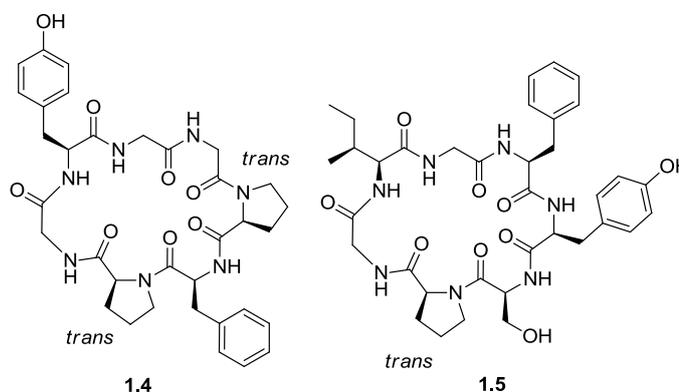
Figure 1.5 : Structures of yunnanins A (1.4) and C (1.5)

1.1.2.2. Phakellistatins

Phakellistatins were isolated from a marine sponge *Phakellia*.[15,16]

Paloma and co-workers also prepared phakellistatins 1 and 10, a hepta- and octapeptide both containing three proline residues (Figure 1.6).[14] They used solid phase peptide synthesis with 2-chlorotrityl chloride resin and Fmoc/*t*Bu strategy. Proline structural conformation in the synthetic phakellistatin 1 is identical to the natural product (all *cis*-Pro). The synthetic octacyclopeptide phakellistatin 10 also possesses identical proline geometries as the natural product (all *trans*-Pro). Even though the synthetic compounds have identical connectivity and rotamers to the natural product, the synthetic and natural products did not exhibit the same biological activities against cancer cell lines. The conclusion they gave is identical to that of yunnanins.
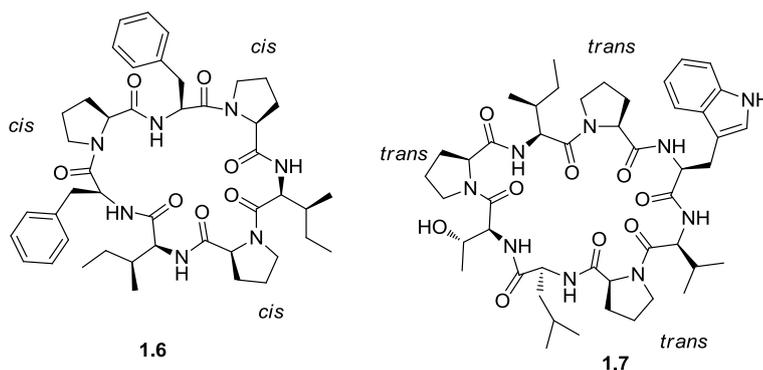
Figure 1.6 : Structures of phakellistatins 1 (1.6) and 10 (1.7)

Phakellistatin 2 (**1.8**) was isolated by Pettit (Figure 1.7).[17] The natural product was found to possess all *cis* proline geometry for the peptide bonds.[18] Kessler prepared phakellistatin 2 by solid phase synthesis with the macrolactamisation between the two isoleucine residues (Ile[3]-Ile[4]).[19] The NMR they obtained did not match the NMR of the natural product. They concluded that a mistake in the structure assignment was previously made. They actually made a different conformer (*trans*-Pro[2], *trans*-Pro[5], *cis*-Pro[7]).[18] Later on, Pettit prepared phakellistatin 2 in solution phase with cyclisation at Pro[2]-Ile[3] peptide linkage.[20] They believed that the cyclisation at the proline linkage is turn inducing. The NMR they obtained matched the NMR of the natural compound exactly however the biological activities of the synthetic and natural product differed. They believed that the cyclic peptide is able to complex a potent antineoplastic agent which is not detectable by spectroscopic analysis and is responsible for the biological potency of the natural product.
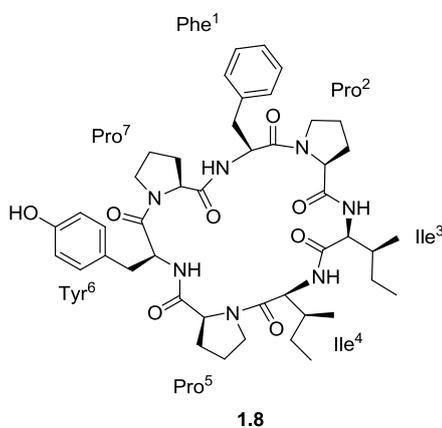
Figure 1.7 : Structure of phakellistatin 2 (1.8)

Phakellistatin 2 was isolated as two separable conformers.[18] They studied their conformations and realised that one conformation had all *cis* proline like the natural product while the other had *trans*-Pro^2, *cis*-Pro^5, *cis*-Pro^7. The conformation was changing depending on the polarity of the solvent used: in polar solvent, (methanol) the first form was obtained and in less polar solvent (chloroform) the second form is detected. They tested the compounds in solvents of different polarity to find which gave the best biological result; this was obtained in the polar solvent methanol.

Pettit and co-workers prepared phakellistatin 5 (**1.9**) which structure is a perfect match with the natural product. They obtained the same conformer in two different ways, by solution phase (macrolactamisation between proline and phenylalanine residues) and by solid phase synthesis with PAL resin (macrolactamisation between asparagine and alanine residues). Nonetheless, the synthetic compound is not biologically identical to the natural product in cancer cell growth inhibition.[21] They gave the same hypothesis as for phakellistatin 2 that the cyclopeptide is capturing an active and undetectable antineoplastic agent.

1.9
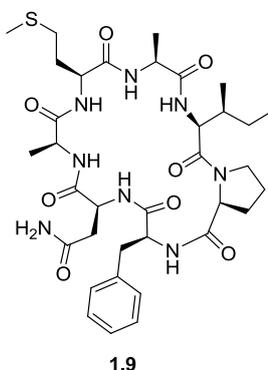
Figure 1.8 : Structure of phakellistatin 5 (1.9)

Paloma *et al.* also prepared the decapeptides phakellistatins 7-9 by Fmoc/*t*Bu solid phase method with 2-chlorotrityl chloride resin.[22] All three compounds contain four proline residues present as two prolylproline fragments. The synthesis of phakellistatin 7 gave a unique conformer identical to the natural product (*trans*-Pro^1, *cis*-Pro^2, *trans*-Pro^7, *cis*-Pro^8), whereas the proline geometry in the synthetic phakellistatins 8 and 9 differed from the natural product. Both syntheses of phakellistatins 8 and 9 gave a mixture of a major isomer (all *trans*-Pro) and a rotamer with *trans*-Pro^1, *cis*-Pro^2, *cis*-

Pro^7, *trans*-Pro^8 bonds. The natural product phakellistatins 8 and 9 displayed *trans*-Pro^1, *cis*-Pro^2, *trans*-Pro^7, *cis*-Pro^8 peptide bonds. They also remarked that the biological activity of the natural and synthetic compounds differed, the synthetic phakellistatins being less potent in cancer cell line growth inhibition. This was expected for phakellistatins 8 and 9 as their conformations differed from that of the natural products but it is more difficult to understand for phakellistatin 7. They believe that the conformational changes occurring in the natural product are responsible for the biological activity.
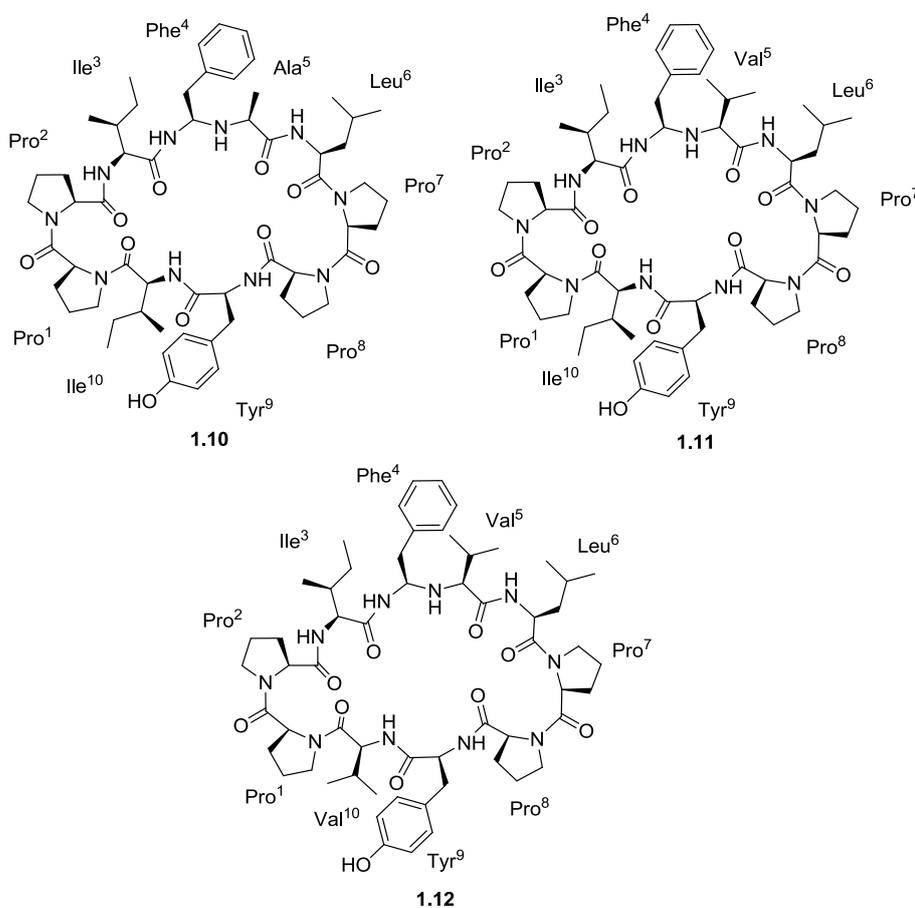
Figure 1.9 : Structures of natural product phakellistatins 7 (1.10), 8 (1.11) and 9 (1.12)

Synthesis of phakellistatin 11 (**1.13**) was first attempted by solution phase but issues with the protecting groups led them to use solid phase synthesis which gave the cyclopeptide. Both synthetic and natural phakellistatin 11 displayed identical chemical characteristics.[23] The compounds gave however different biological results against

cancer cell lines: the natural product being more active. Pettit *et al.* once more gave the same hypothesis of a potent but undetectable antineoplastic agent being present.
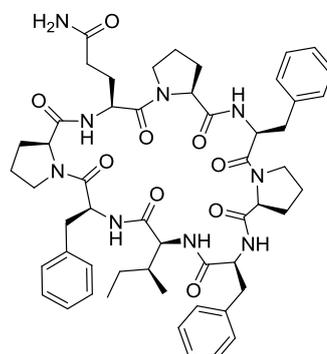
1.13

Figure 1.10 : Structure of phakellistatin 11 (1.13)

1.1.2.3. Hymenamide C

Hymenamides were isolated from a marine sponge *Hymeniacidon*. Synthetic hymenamide C (**1.14**) was obtained as a mixture of a major product (*cis*-Pro[3], *trans*-Pro[6]) identical to natural product and a minor isomer with *cis*-Pro[3], *cis*-Pro[6].[24] Because of the presence of glutamine and tryptophan, they used a three-dimensional orthogonal solid phase method (Fmoc/*t*Bu/Allyl) with PAL as resin. Hymenamide C is a micromolar inhibitor of the elastase degranulation release which is important in immunomodulation. It could be used to complement the immunosuppressant drug cyclosporine which has a weaker effect on elastase degranulation release.
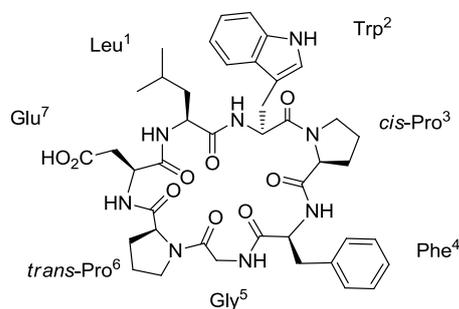
1.14

Figure 1.11 : Structure of the major isomer hymenamide C (1.14)

1.1.2.4. Stylopeptides, axinellins A and axinastatins

Several other cyclopeptides have been discovered over recent years such as stylopeptides, axinellins and axinastatins, a selection of these is shown in figure 1.12.[25,26] Stylopeptides were isolated from the marine sponge *Stylotella sp.*[27] For example, stylopeptide 1 (**1.15**), a decapeptide with four proline residues, displayed micromolar growth inhibition activity towards breast cancer cell lines.[27] The heptapeptides axinellin A (**1.16**) and the octapeptide axinellin B (**1.17**), possessing two and three proline residues respectively, were isolated from the marine sponge *Axinella carteri*. Both compounds showed moderate antitumor activity against a lung cancer cell line.[26] Axinastatins contain two proline residues and were able to inhibit six different cancer cell lines. The synthetic compounds were found to have a significantly lower cancer cell growth inhibition activity than the natural products.
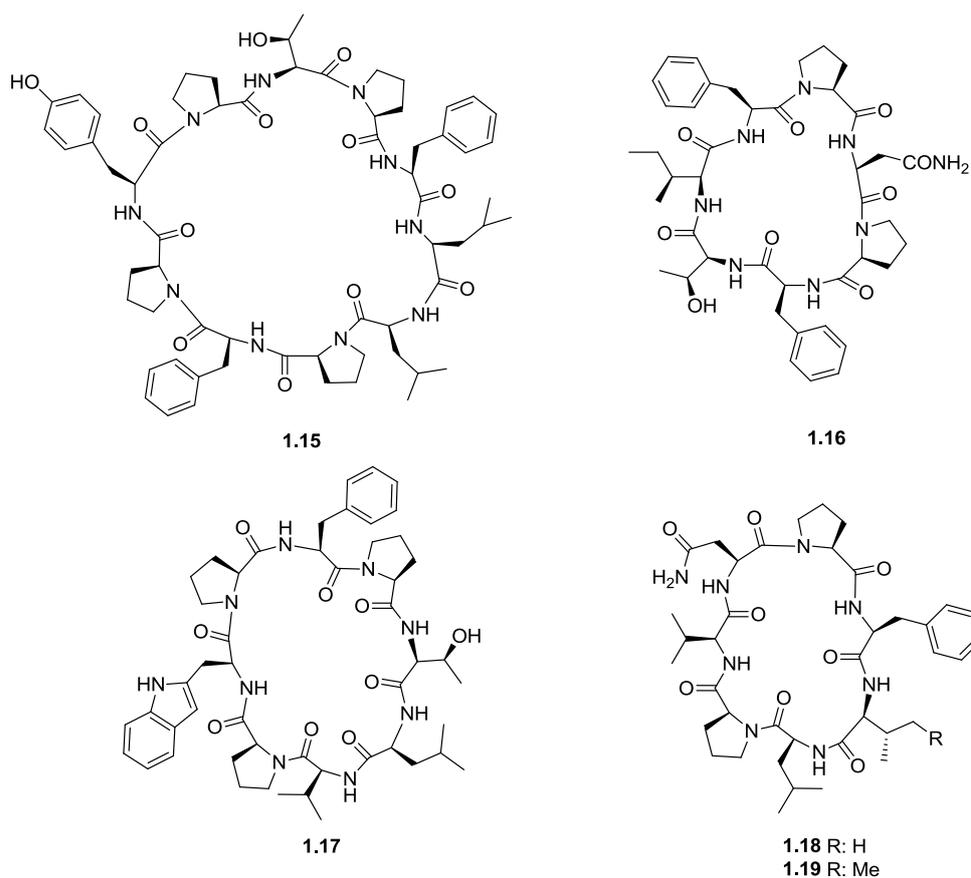
Figure 1.12 : Structures of stylopeptide 2 (1.15), axinellins A (1.16) and B (1.17) and axinastatins 2 (1.18) and 3 (1.19)

1.1.2.5. Stylissamides and stylisins

The cyclic heptapeptides stylissamides A-D were isolated from the Caribbean sponge *Stylissa caribica* (Figure 1.13).[28] Stylissamides A, C and D contain three proline residues including one prolylproline segment. Stylissamide B possesses four proline residues with three of them linked together (Pro-Pro-Pro linkage). Their structures were elucidated by spectroscopic analysis (NMR and mass spectrometry). Marfey's method and the *o*-phthalaldehyde method (OPA method) after hydrolysis were employed to assign the L-configuration of all the amino acid residues. The conformation of the proline in the prolylproline segment is *trans, cis* as often observed in other natural products such as phakellistatins 7-9. Stylissamide A was evaluated for antimicrobial or cytotoxic activity but it was found to be inactive.
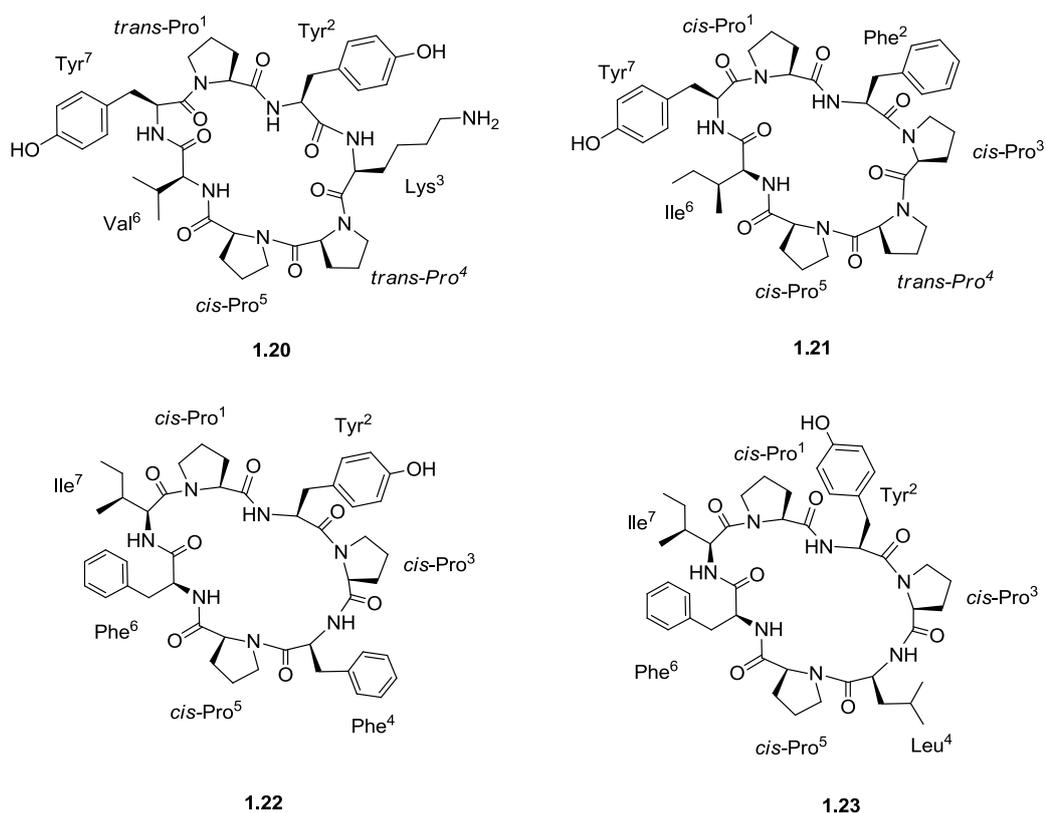
Figure 1.13 : Structures of stylissamides A - D

It was unexpected to see that stylisins 1 (**1.24**) and 2 (**1.25**), which were isolated from the same sponge *Stylissa caribica* as stylissamides, were found to be inactive even though they are very similar to phakellistatin 2.[28] They were evaluated for antimicrobial,

anti-malarial, anti-cancer, anti-HIV-1, anti-*Mycobacterium tuberculosis (*anti *Mtb*) and anti-inflammatory activity yet showed no activity.
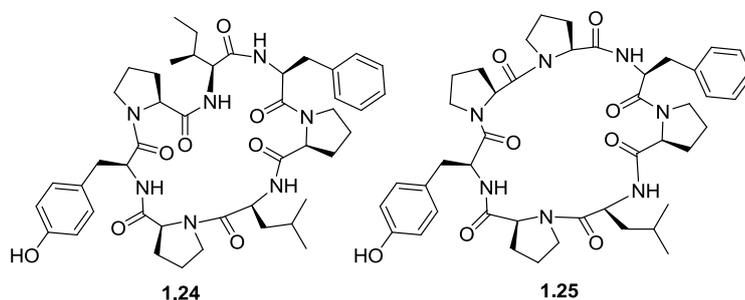
Figure 1.14 : Structures of stylisins 1 (1.24) and 2 (1.25)

Several cyclic peptides isolated from marine organisms contain in addition to natural or unnatural amino acids, five-membered heterocycles such as oxazole, oxazoline, thiazole and thiazoline. These five-membered rings are obtained from the biosynthetic cyclisation of amino acids such as serine, threonine and cysteine.

1.1.2.6. Ceratospongamide

Ceratospongamide was isolated as two different isomers: the major *cis, cis* rotamer **1.26**, and the minor *trans, trans* rotamer **1.27**, from the Indonesian red alga *Ceratodictyon* and its symbiotic sponge *Sigmadocia symbiotica*, by Gerwick *et al.*[29] They managed to interconvert the *cis, cis* isomer to the *trans, trans* isomer by heating at 175 ºC in DMSO for 30 min. *Trans, trans*-Ceratospongamide has anti-inflammatory activity whereas *cis, cis*-ceratospongamide is inactive. The *trans, trans*-isomer inhibits secreted phospholipase A_2 (sPLA$_2$) expression in cell-assay which is known to promote inflammation (ED$_{50}$ 32 nM). [29]
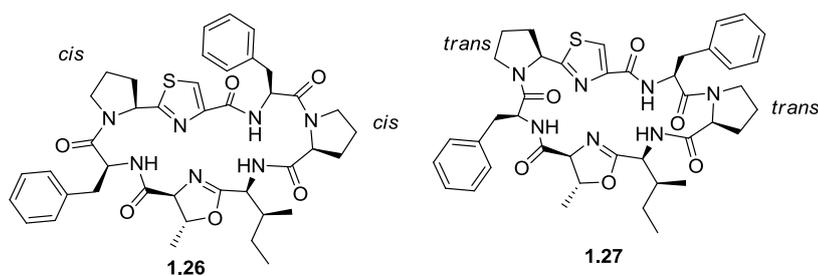
Figure 1.15 : Structures of *cis, cis*-ceratospongamide (1.26) and *trans, trans*-ceratospongamide (1.27)

Ceratospongamide was then prepared in solution phase synthesis by Yokokawa and co-workers.[30] They obtained the *cis, cis* isomer with two different macrolactamisation strategies: cyclisation between thiazole and phenylalanine residue and between isoleucine and proline peptide linkage. They attempted the thermal interconversion of *cis, cis* to the *trans, trans* isomer using the conditions described by Gerwick (175 °C in DMSO for 30 min) but only obtained a low yield of 10-30%.[30] They obtained an excellent yield (100%) with heating in DMSO at 100 °C for a longer time (8 days). PPTS as additive reduced the time required for the interconversion to 40 min with a 75% yield. They have demonstrated that the natural product and the thermodynamic compound is not the *trans, trans* isomer as postulated by Gerwick *et al.* but the *trans, trans*-[D-*allo*-Ile] isomer.[30] An epimerization occurred at the α-stereocentre of the isoleucine residue next to the oxazoline.

1.1.2.7. Mollamide

Mollamide (**1.28**) was isolated from the sea squirts *Didemnum molle*.[31] It contains a thiazoline ring, several natural amino acids and a modified serine residue with an unusual reverse prenyl unit. This compound is a micromolar inhibitor of several cancer cell lines (i.e. murine leukaemia, human lung carcinoma).[31] They obtained the same conformer as the natural product using solution phase synthesis.[31]

1.28
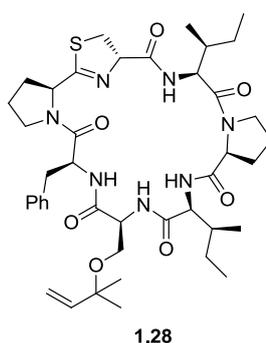
Figure 1.16 : Structure of mollamide (1.28)

1.1.2.8. Sanguinamides A and B

Sanguinamides A and B have been isolated in microgram quantities from a single specimen of *Hexabranchus sanguineus*, a nudibranch collected from the Indo-Pacific in 1987 (Figure 1.17).[32]
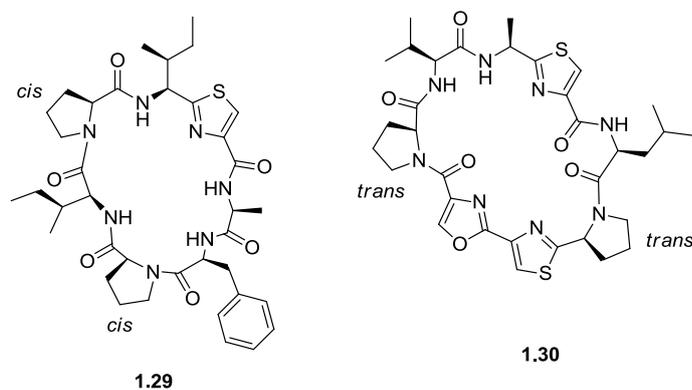
Figure 1.17 : Structures of sanguinamides A and B

The structure elucidation of the cyclic peptides was only reported recently by Molinski *et al* in 2009.[32] The quantities isolated for sanguinamides A (390 µg) and B (190 µg) were insufficient for biological testing. The structures were elucidated using spectrometric analysis such as NMR and mass spectrometry. They used a 600 MHz NMR spectrometer with high-temperature superconducting (HTS) NMR cryoprobe. The L-configuration was determined by Marfey's method. Sanguinamide A is a thiazole-containing cyclic peptide with seven amino acids of the L-series comprising of phenylalanine, proline, isoleucine and alanine. This octapeptide contains three five-membered ring heterocycles (two thiazole rings and one oxazole ring) and five amino acid residues comprising of valine, proline, leucine and alanine. Both sanguinamides contain two proline residues.

The cyclopeptides presented above possess several similarities pointed out by Paloma.[26] They all come from marine sources and mostly all come from the south Pacific. These proline-rich cyclopeptides in general contain seven to eight amino acids even though some are larger with ten amino acid residues. They usually include two to four proline residues. Most of the amino acids have an L-configuration. Several apolar amino acids

are present in each of these cyclic peptides such as alanine, valine, leucine and isoleucine. Most of these cyclopeptides also contain aromatic residues such as phenylalanine or tyrosine which are next to a proline residue in most cases. They sometimes also contain aspartate or asparagine and glutamate or glutamine. A high frequency of five-membered heterocyclic rings is observed within these cyclopeptides. The biological activity of these cyclic peptide natural products is in general interesting even though some were reported to be inactive or less active. It is surprising to see that in many cases the biological activity is not identical for the natural product and the synthetic product. [14,21-23] Numerous groups mentioned that cyclic peptide natural products may contain a chemically undetectable amount of a strongly active cytotoxic agent.[14,21,23] It has also been hypothesised that proline interconversion occurs in the natural products to give the bioactive molecule with a specific conformation for the proline residues.[14,22] Synthetic methods may not form the potential active conformer and therefore the synthetic compound shows no activity.

1.2. AIMS

We were planning to synthesise sanguinamides A and B in order to carry out biological testing as none has ever been reported on these two compounds. We also wanted to check the accuracy of the characterisation of the sanguinamide structures by Molinski *et al.* as they used very little material to do so. We planned to study the conformations adopted by the proline in the synthesised product and to compare it to that reported for the natural products in the literature.

We were also planning to prepare synthetically stylissamide A in order to study the *cis*/*trans* geometry of the proline in the synthetic product. We were working in collaboration with a group who was planning to further investigate the biological activity of this compound even though preliminary studies on this compound were not promising.

1.3. TOWARDS THE SYNTHESIS OF THE HEPTAPEPTIDE SANGUINAMIDE A

Initial work was performed by an undergraduate in the group, Wan-Jun Chung. The strategy she used was to prepare the thiazole-containing fragment **1.31** and the pentapeptide **1.32** (Scheme 1.1). She successfully prepared the pentapeptide **1.32** but encountered several difficulties in the synthesis of the Fmoc protected thiazole-containing fragment **1.31**.
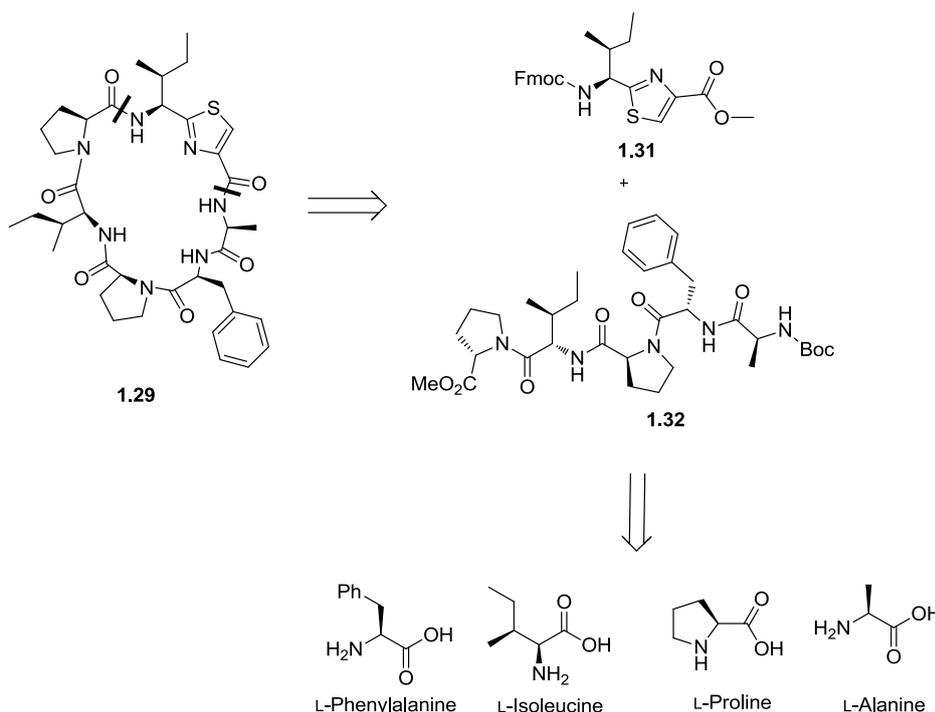
Scheme 1.1 : Previous retrosynthetic analysis of sanguinamide A

1.3.1. Strategy 1 : macrolactamisation between thiazole and alanine residue

Several disconnections are possible at the six different amide bonds. The natural product will be assembled from the two following fragments: the thiazole-containing fragment **1.33** and the tetrapeptide **1.34** prepared from L-phenylalanine, L-isoleucine, L-proline and L-alanine (Scheme 1.2). In order to increase efficiency and yield, a convergent synthesis is proposed from two fragments **1.33** and **1.34** of approximately equal size. The macrolactamisation would be performed between the thiazole and alanine which we considered to be the least hindered position.

17
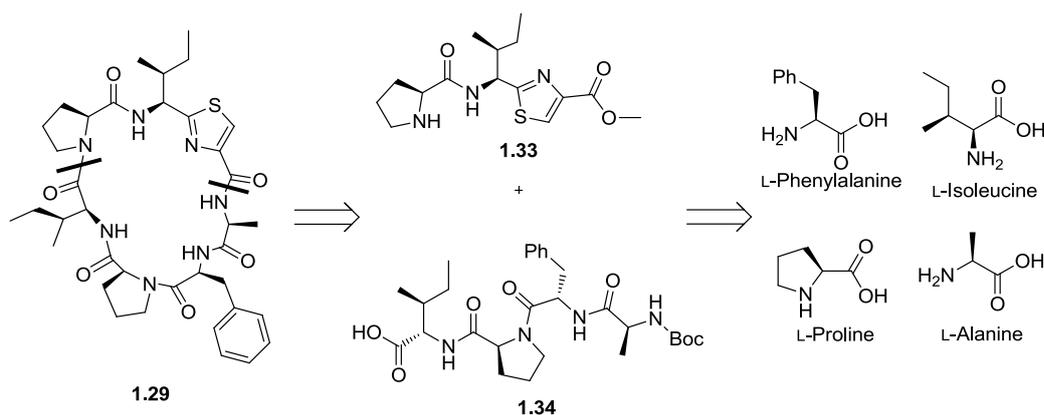
Scheme 1.2 : Retrosynthetic analysis of sanguinamide A

The coupling reagents we decided to use were a substoichiometric amount of HOBt with the carbodiimide EDCI (**1.35**) because of its water-soluble by-product urea which can be easily removed by aqueous washes.[8] For couplings that we considered more difficult, we decided to use PyBOP (**1.36**) a more efficient coupling reagent.[8]
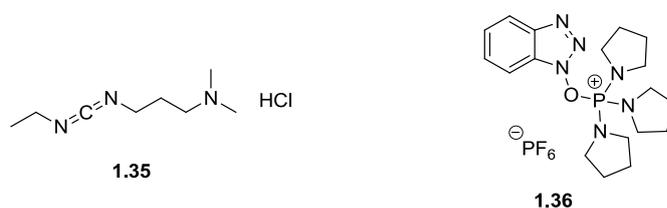
Figure 1.18 : Structures of EDCI.HCl (1.35) and PyBOP (1.36)

We planned to synthesise the thiazole containing fragment **1.33** starting from L-isoleucinamide **1.37**, the first step being the protection of the amine with a Boc group followed by the conversion of the amide to thioamide **1.39** using Lawesson's reagent (Scheme 1.3). A Hantzsch thiazole synthesis is then performed with methyl bromopyruvate to form the thiazole **1.40**. The Boc is then removed and the resulting amine is coupled to Boc-L-Pro-OH using PyBOP to give **1.42**. Finally, the Boc group is removed from the proline to afford the thiazole-containing fragment **1.33**. We chose the Boc group because of its compatibility with the subsequent reaction conditions and also because of its easy incorporation and removal.
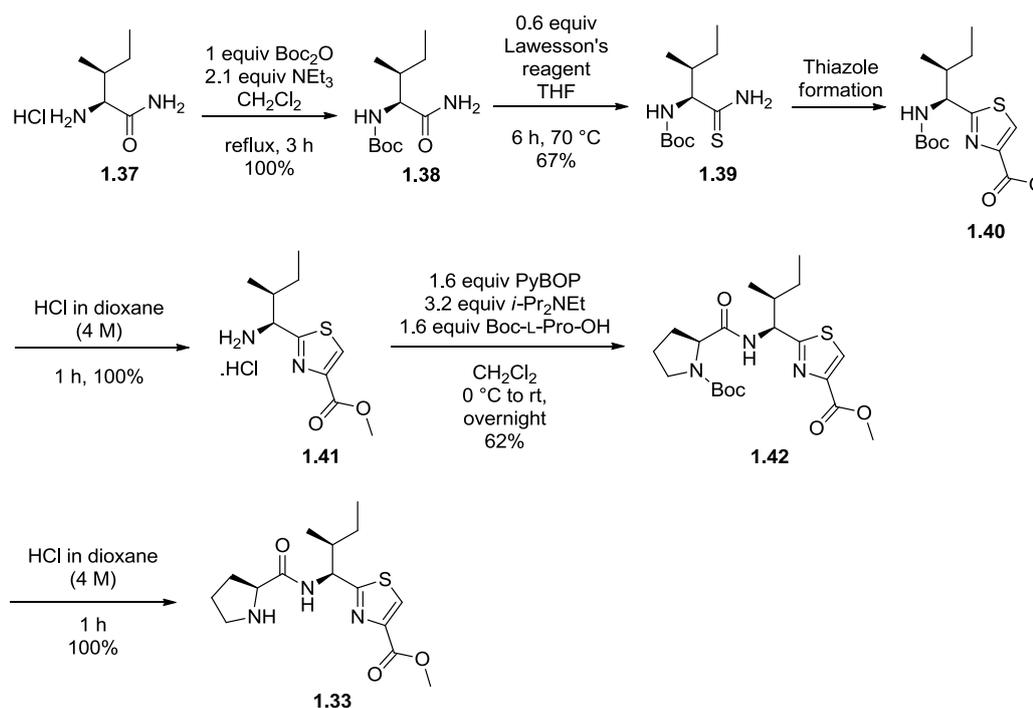
Scheme 1.3 : Synthesis of the thiazole-containing fragment 1.33

We encountered some difficulties in the formation of the thiazole ring as epimerization occurred at the α-centre of the thiazole ring. Epimerisation was shown to take place due to acid catalysed imine-enamine tautomerisation of thiazoline **1.43** by treatment of the *N*-protected thioamide with methyl bromopyruvate in the presence of 0.5 equivalent of calcium carbonate (Scheme 1.4).[33,34] Racemisation depends on the rate of the dehydration step leading to the aromatised compound, and this depends on the stability of the carbocation intermediate **1.46**.[34]
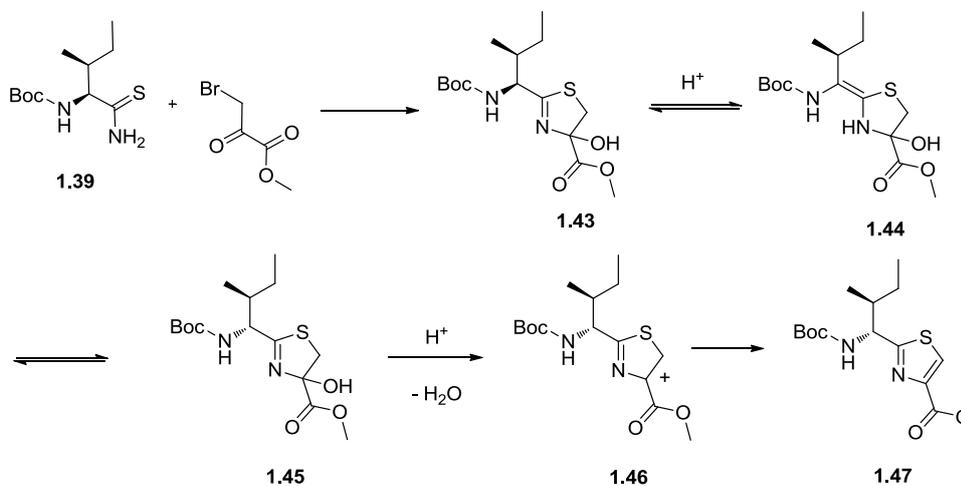
Scheme 1.4 : Epimerisation at the α-chiral centre of the thiazole ring

A mixture of the two diastereomers, **1.40** and **1.47**, was obtained and they were not separable by flash chromatography as both diastereomers have the same retention factor (R_f) on TLC. ^{1}H NMR showed the presence of diastereomers as some of the peaks are doubled. We nevertheless managed to separate the two different diastereomers after coupling with proline and continued the synthesis on the major diastereomer **1.42** (Scheme 1.5).
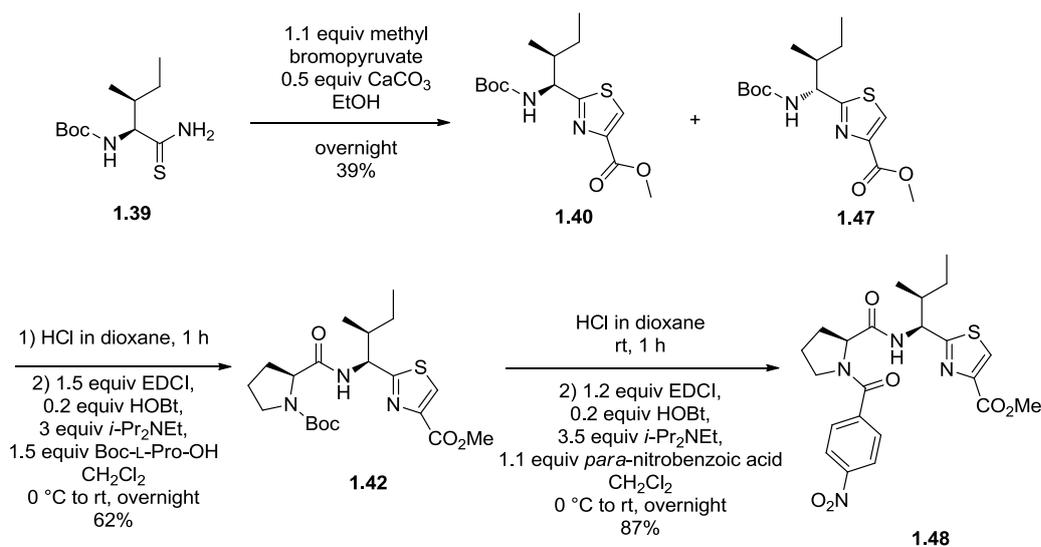
Scheme 1.5 : Synthesis of the *para*-nitro compound 1.48

The major diastereomer was presumed to possess the correct stereochemistry. We nevertheless decided to check the stereochemistry of **1.42** by coupling it to *para*-nitrobenzoic acid after Boc removal in order to obtain the X-ray crystal structure of the compound. Lath-shaped crystals of the *para*-nitrobenzoic acid derivative **1.48** were grown from a mixture of acetonitrile and water. The X-ray structure of the *para*-nitrobenzamide derivative of the amine was obtained and confirmed the stereochemistry of the major diastereomer with the desired stereochemistry (Figure 1.19). The stereochemistry of the isoleucine residue was indeed shown to be the (*S*, *S*) corresponding to the natural L-isoleucine.

20
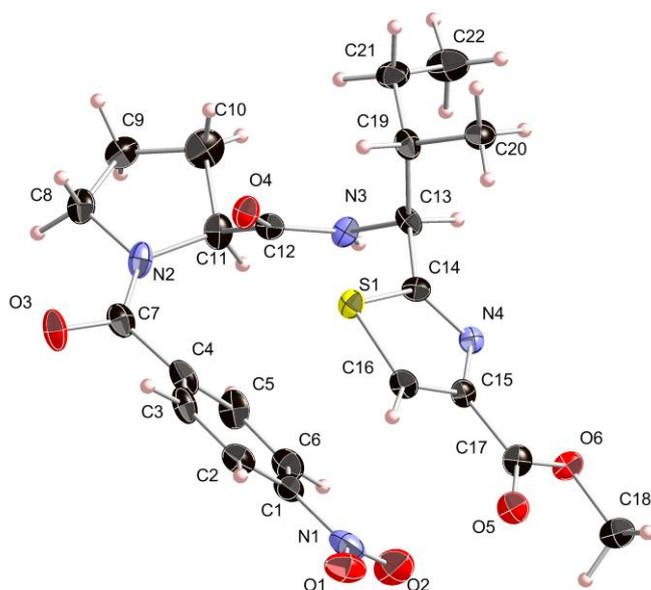
Figure 1.19 : X-Ray structure of the *para*-nitro compound 1.48

A modified Hantzsch reaction described in the literature for epimerisation-free thiazole formation was then attempted.[34,35] The reaction of the thioamide with methyl bromopyruvate in dimethoxyethane in the presence of finely powdered KHCO$_3$ leads to the formation of hydroxythiazoline derivative **1.43**. The HBr formed is neutralised by the excess KHCO$_3$. Without isolation of this product, the hydroxyl group is activated with trifluoroacetic acid anhydride (TFAA) in pyridine followed by formation of the thiazole by immediate aromatisation with complete retention of configuration.
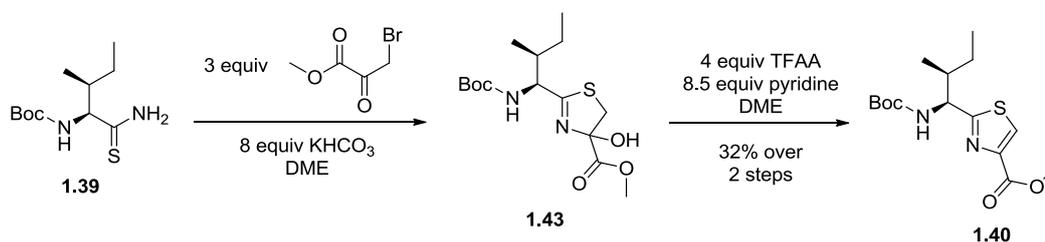
Scheme 1.6 : Thiazole formation using epimerization-free conditions

TFAA reacts with pyridine to form trifluoroacetylpyridinium trifluoroacetate **1.49** which is in equilibrium with tetrahedral intermediate **1.50** (Figure 1.20).[36] The hydroxyl

group of the intermediate thiazoline compound **1.43** is trifluoroacetylated to form a good leaving group.
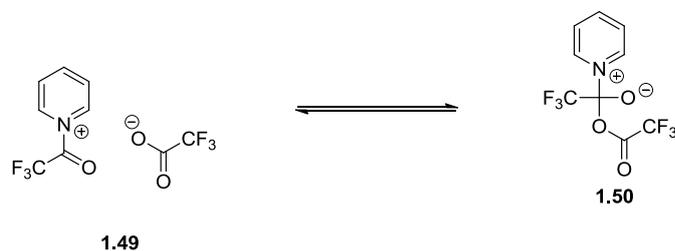
Figure 1.20 : Equilibrium between the two forms of the TFAA.pyridine complex

The tetrapeptide Ile-Pro-Phe-Ala **1.34** was prepared using a sequence of coupling and deprotection steps (Scheme 1.7). The coupling reagent used is EDCI with a catalytic amount of HOBt and the Boc deprotection is performed by treatment with HCl in dioxane. We believed that **1.54**, **1.55**, **1.56** and **1.34** were obtained as a mixture of conformers due to the presence of the proline residue.
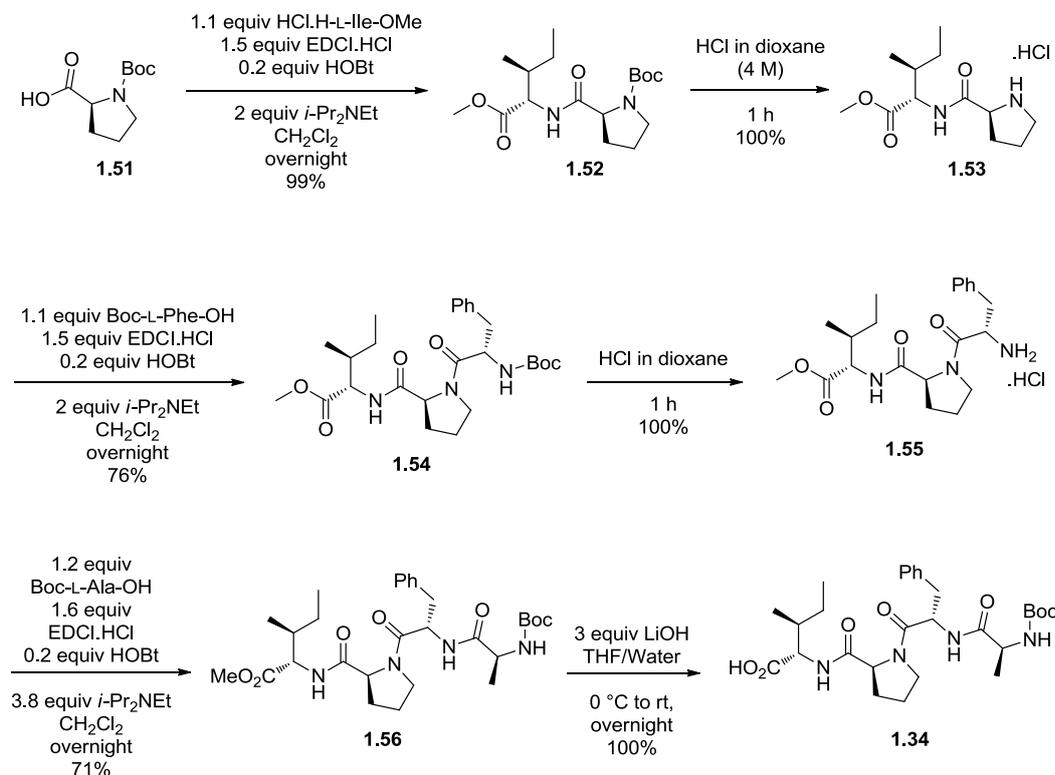
Scheme 1.7 : Synthesis of the tetrapeptide 1.34

HATU (**1.57**), which can exist as uronium (*O*-form) salt, is generally the coupling reagent of choice for macrolactamisation and will be employed for all our macrolactamisations.[8]

1.57
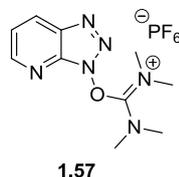
Figure 1.21 : Structure of HATU (1.57)

Coupling of the thiazole containing fragment **1.33** with the tetrapeptide **1.34** using PyBOP gave compound **1.58** in which the acid and amine were successively deprotected (Scheme 1.8). Difficulties to obtain a good NMR for this compound were encountered. The PyBOP by-product was difficult to remove, so we decided to carry on and to purify the final product. The macrolactamisation was performed using HATU as coupling reagent and HATU by-product was difficult to remove by flash chromatography. Several attempts to purify the compound led to a low yield for the macrolactamisation. HPLC was required and was run by our collaborators Marcel Jaspars and Mostafa Rateb at the University of Aberdeen.
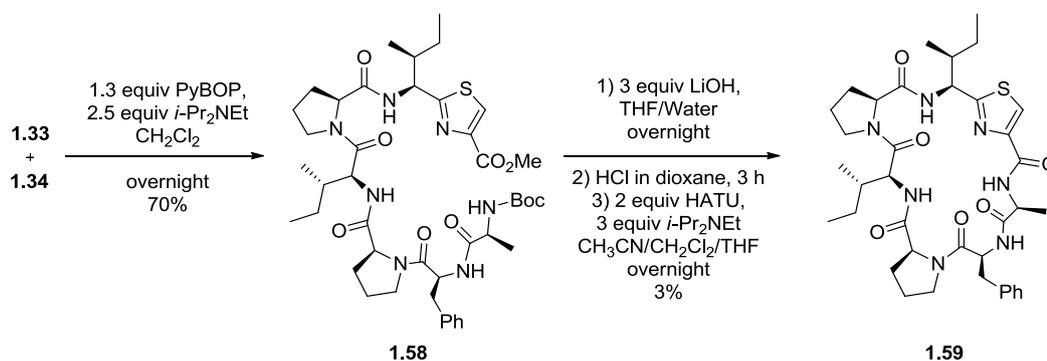
Scheme 1.8 : Synthesis of *trans, trans*-sanguinamide A

The NMR of synthetic sanguinamide A reported in table 1.1 shows significant differences with that reported for the natural product.[32]
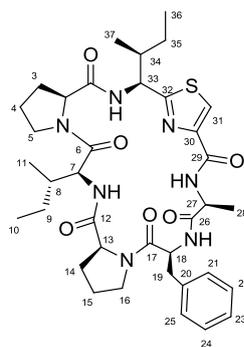
1.59

Figure 1.22 : Atom numbering used for sanguinamide A

The two proline residues have *trans* geometry in the synthetic compound **1.59**. The conformation was assigned by NOESY experiments performed with the help of Professor Marcel Jaspars.

Broad signals for the ^{1}H and ^{13}C NMR spectra were obtained at rt in $CDCl_3$. Marcel Jaspars attempted to run the NMR at higher temperature but the signals were still broad. Heating usually averages out the rotamers. However in our case, the average signals were broad and represented a number of conformers. Running the NMR at lower temperature (−10 ℃) gave sharper signals in the ^{1}H NMR; the low temperature enabled to freeze out a conformer. We were able to see one conformer using low temperature NMR rather than the average. The NMR spectra at 25 ℃ and −10 ℃ are considerably different (Figures 1.23 and 1.24).

HB6-15.ESP
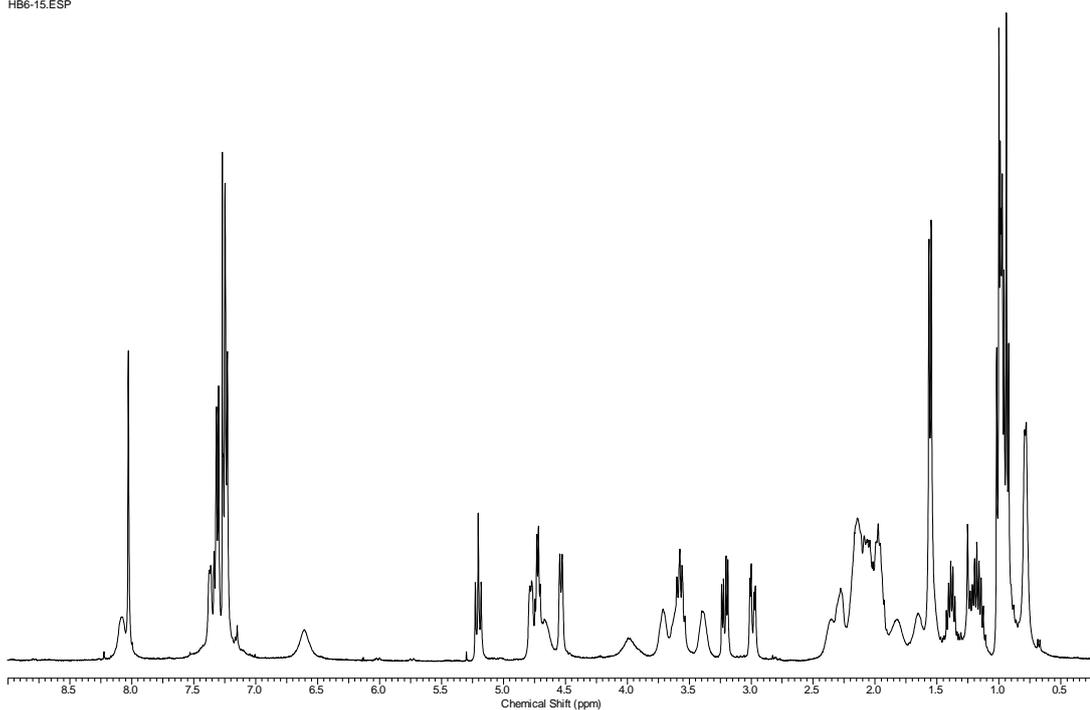
Figure 1.23 : ^{1}H NMR of *trans, trans*-sanguinamide A (1.59) in CDCl$_3$ run at 25 ºC
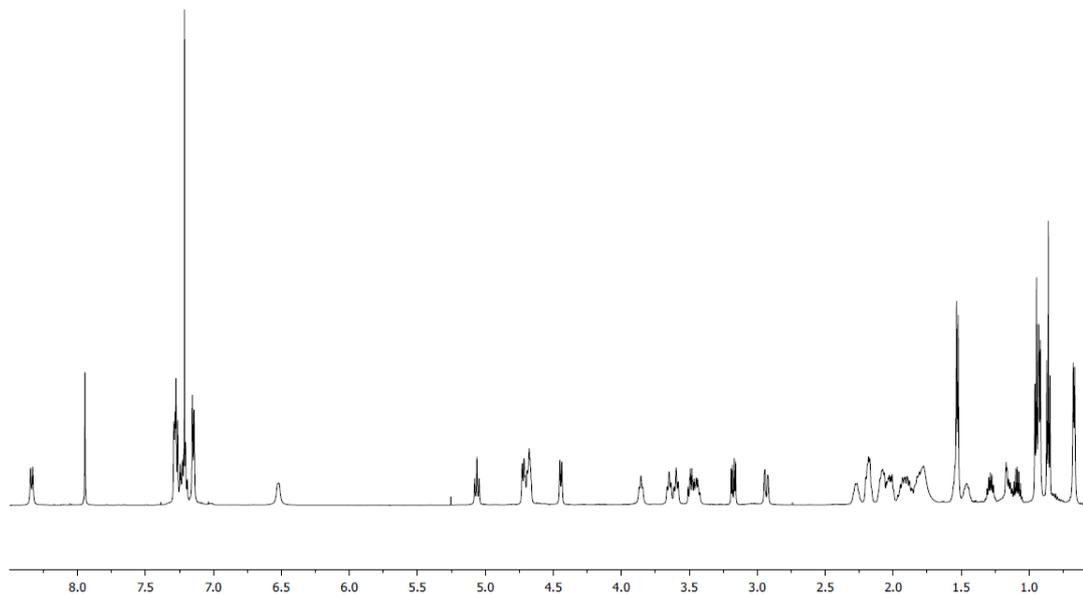
Figure 1.24 : ^{1}H NMR of *trans, trans*-sanguinamide A (1.59) in CDCl$_3$ run at –10 ºC

Residue	#	$\delta^{13}C$	^{13}C mult.	Reported[32] $\delta^{13}C$	δ^1H and mult.	Reported[32] δ^1H and mult.
Pro	1	171.4	C	170.6		
	2	61.4	CH	60.8	4.44 (d)	4.67 (d)
	3	30.6	CH$_2$	30.5	2.19, 2.05 (m, m)	1.81, 2.68 (m, dd)
	4	24.1	CH$_2$	25.0	2.01, 1.89 (m, m)	1.90, 2.02 (m, m)
	5	46.4	CH$_2$	48.1	3.59, 3.48 (t, m)	3.67, 4.00 (m, m)
Ile	6	170.63	C	174.5		
	7	53.0	CH	56.3	4.72 (d)	4.26 (dd)
	8	37.7	CH	35.5	1.56 (m)	2.30 (m)
	9	27.5	CH$_2$	25.3	1.29, 1.08 (m, m)	1.24, 1.70 (m, m)
	10	12.2	CH$_3$	10.3	0.95 (t)	0.91 (t)
	11	13.8	CH$_3$	15.1	0.67 (d)	1.02 (d)
Pro	12	171.3	C	171.5		
	13	62.2	CH	60.9	3.85 (t)	3.38 (d)
	14	29.8	CH$_2$	30.4	2.17, 1.93 (m, m)	0.93, 2.10 (m, dd)
	15	25.9	CH$_2$	22.0	2.08, 1.81 (m, m)	1.70, 1.50 (m, m)
	16	47.6	CH$_2$	46.3	3.64, 3.44 (t, m)	3.50, 3.53 (m, m)
Phe	17	168.9	C	169.2		
	18	51.3	CH	54.5	4.67 (m)	4.22 (ddd)
	19	35.8	CH$_2$	37.9	3.17, 2.93 (dd, dd)	2.98, 3.08 (dd, dd)
	20	135.3	C	134.3		
	21/25	131.1	2CH	129.3	7.15 (d)	7.16 (d)
	22/24	128.0	2CH	127.8	7.27 (m)	7.24 (m)
	23	126.7	CH	129.1	7.21 (m)	7.28 (m)
Ala	26	170.9	C	171.0		
	27	48.9	CH	49.1	4.68 (m)	4.69 (qd)
	28	18.3	CH$_3$	17.9	1.53 (d)	1.48 (d)
Thz	29	160.8	C	160.3		
	30	146.6	C	148.5		
	31	125.6	CH	123.1	7.94 (s)	8.01 (s)
	32	170.58	C	168.1		
Ile	33	53.1	CH	56.3	5.06 (t)	5.09 (dd)
	34	34.1	CH	40.9	2.27 (br m)	1.80 (m)
	35	25.3	CH$_2$	25.9	1.46, 1.14 (m, m)	1.17, 1.50 (m, m)
	36	10.0	CH$_3$	10.3	0.86 (t)	0.91 (t)
	37	17.0	CH$_3$	15.0	0.92 (d)	0.77 (d)

Table 1.1 : ^1H and ^{13}C NMR data in ppm for synthetic *trans, trans*-sanguinamide A and natural[32] *cis, cis*-sanguinamide A in CDCl$_3$ (see figure 1.22 for numbering)

We obtained an X-ray crystal structure to confirm our structure (Figure 1.25). Crystalline rods of sanguinamide A were grown from a mixture of methanol/water. The X-ray structure confirmed the structure of the synthetic sanguinamide A, the stereochemistry of its stereogenic centers along with the *trans* proline peptide bonds.
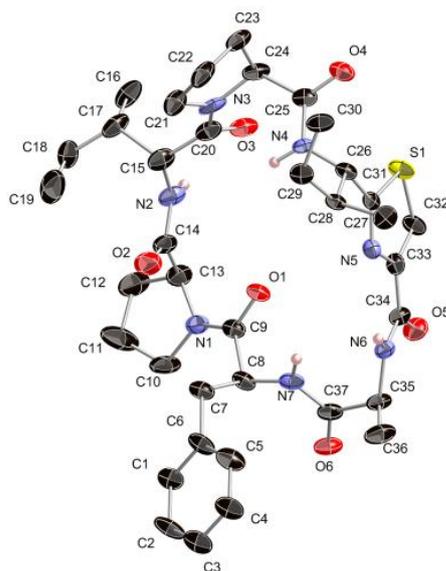
Figure 1.25 : X-Ray structure of *trans, trans*-sanguinamide A (1.59)

In the ^{13}C NMR, the synthetic compound with both prolines in the *trans* rotamer, determined by NOESY experiment, has Pro^1 ($\Delta\delta\beta\gamma = \delta\beta - \delta\gamma = 6.5$ ppm) and Pro^2 ($\Delta\delta\beta\gamma$ = 3.9 ppm). The reported values for the natural product with both prolines in the *cis* rotamer are Pro^1 ($\Delta\delta\beta\gamma = 5.5$ ppm) and Pro^2 ($\Delta\delta\beta\gamma = 8.4$ ppm).[32] Although the $\Delta\delta\beta\gamma$ is widely used to assign proline rotamers in the literature, this example shows the limitations of the calculation method (Figure 1.26). *Cis* proline residues should have a larger ^{13}C chemical shifts difference than *trans* proline residues. According to the NOESY experiment, the Pro^1 is in *trans* conformation but the value obtained by the calculation method is larger than the literature value for the *cis* rotamer.
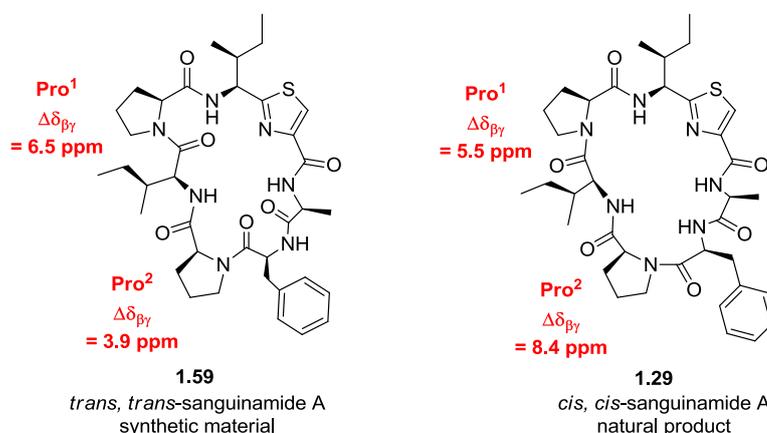
Figure 1.26 : Structures of the synthetic material 1.59 and the natural product *cis, cis*-sanguinamide A (1.29)

DFT calculations performed by our collaborator Bruce Milne at the University of Coimbra suggest the *cis, cis* rotamer is higher in energy than *trans, trans* rotamer. We decided to attempt a different synthetic approach in order to obtain the identical rotamer to the natural product.

1.3.2. Strategy 2: macrolactamisation between Pro[1] and Ile[2]

We decided to try to perform the macrolactamisation at a different position as it is known that coupling next to a proline is a turn inducer allowing *cis* bond formation.[37,38] We decided to try to perform the macrolactamisation next to the proline residues and began with the disconnection between proline and isoleucine residues. As fragments **1.61** and **1.62** would easily be prepared from available fragments **1.42** and **1.56**, the macrolactamisation between Pro[1] and Ile[2] residues would easily be performed after amide bond formation between our thiazole and alanine (Scheme 1.9).
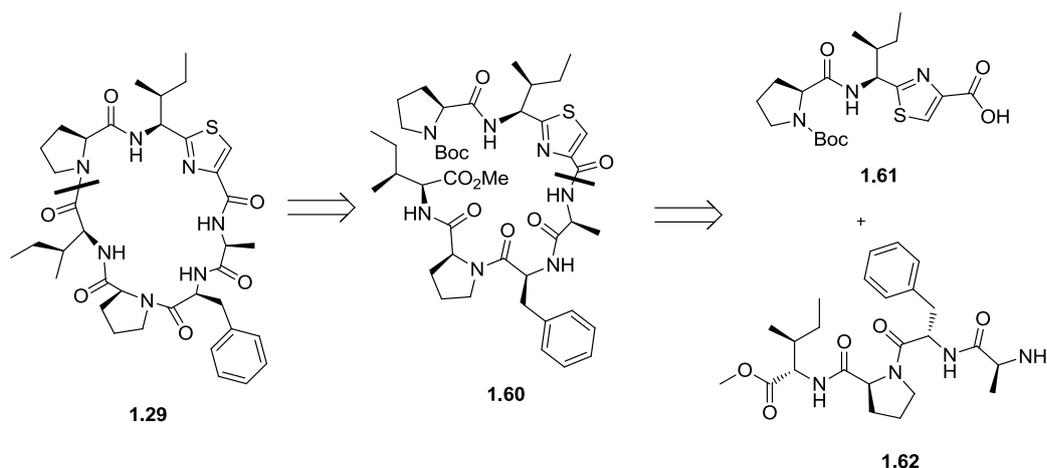
Scheme 1.9 : New retrosynthesis of sanguinamide A

Saponification of fragment **1.42** was performed by treatment with LiOH and Boc removal on **1.56** was carried out. Fragments **1.61** and **1.62** were coupled to give the compound **1.60** which was only characterised by mass spectrometric analysis as we were not able to remove the PyBOP by-product (Scheme 1.10). Sequential deprotection of *C*- and *N*- termini and macrolactamisation with HATU afforded **1.63** in low yield.
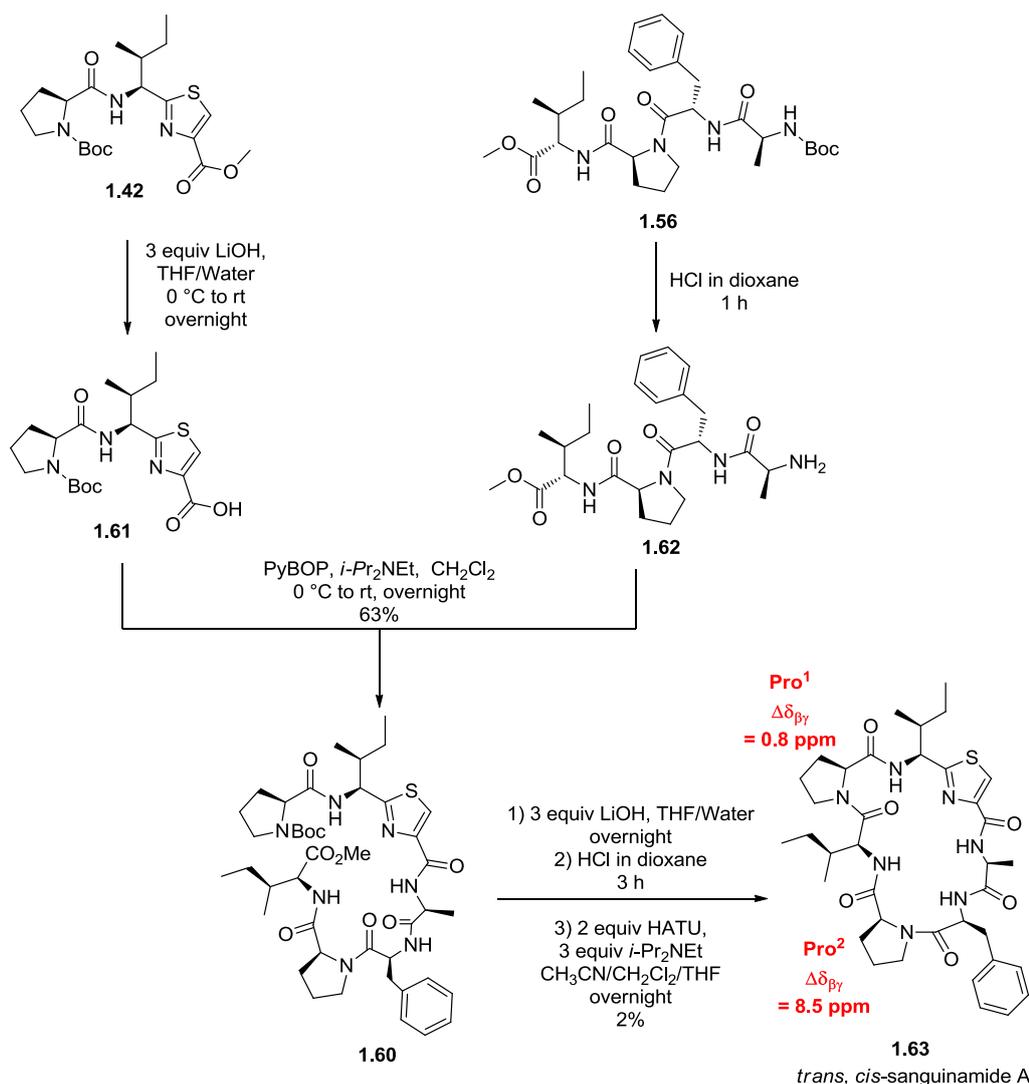
Scheme 1.10 : Synthesis of *trans, cis*-sanguinamide A (1.63) by macrolactamisation between Pro[1] and Ile

In our second synthesis, the ^{1}H and ^{13}C NMR data that we obtained once again gave significant variations to that reported by Molinski for the natural product *cis, cis*-sanguinamide A (Figure 1.22 and table 1.2).[32] This time, we had synthesised the *trans* conformation for Pro[1] ($\Delta\delta\beta\gamma$ = 0.8 ppm) and *cis* for Pro[2] ($\Delta\delta\beta\gamma$ = 8.5 ppm). The determination of the proline residue conformations was done using NOESY experiment.

Residue	#	$\delta^{13}C$	^{13}C mult.	Reported[32] $\delta^{13}C$	δ^{1}H and mult.	Reported[32] δ^{1}H and mult.
Pro	1	170.7	C	170.6		
	2	60.9	CH	60.8	4.69 (d)	4.67 (d)
	3	25.8	CH$_2$	30.5	2.69, 1.91 (dd, m)	1.81, 2.68 (m, dd)
	4	25.0	CH$_2$	25.0	2.04, 1.91 (m, m)	1.90, 2.02 (m, m)
	5	48.3	CH$_2$	48.1	4.00, 3.63 (td, t)	3.67, 4.00 (m, m)
Ile	6	174.4	C	174.5		
	7	56.4	CH	56.3	4.28 (dd)	4.26 (dd)
	8	35.5	CH	35.5	2.29 (m)	2.30 (m)
	9	25.5	CH$_2$	25.3	1.82, 1.28 (m, m)	1.24, 1.70 (m, m)
	10	10.4	CH$_3$	10.3	0.92 (t)	0.91 (t)
	11	15.2	CH$_3$	15.1	1.03 (d)	1.02 (d)
Pro	12	171.4	C	171.5		
	13	61.1	CH	60.9	3.41 (d)	3.38 (d)
	14	30.4	CH$_2$	30.4	2.11, 0.96 (dd, m)	0.93, 2.10 (m, dd)
	15	21.9	CH$_2$	22.0	1.82, 1.71 (m, m)	1.70, 1.50 (m, m)
	16	46.4	CH$_2$	46.3	3.54, 3.47 (td, m)	3.50, 3.53 (m, m)
Phe	17	169.9	C	169.2		
	18	54.6	CH	54.5	4.25 (ddd)	4.22 (ddd)
	19	37.8	CH$_2$	37.9	3.11, 3.02 (t, dd)	2.98, 3.08 (dd, dd)
	20	134.7	C	134.3		
	21/25	129.4	2CH	129.3	7.17 (d)	7.16 (d)
	22/24	129.1	2CH	129.1	7.29 (d)	7.28 (m)
	23	127.7	CH	127.8	7.26 (m)	7.24 (m)
Ala	26	171.3	C	171.0		
	27	49.2	CH	49.1	4.77 (ddd)	4.69 (qd)
	28	18.0	CH$_3$	17.9	1.48 (d)	1.48 (d)
Thz	29	160.5	C	160.3		
	30	148.5	C	148.5		
	31	123.1	CH	123.1	8.01 (s)	8.01 (s)
	32	168.2	C	168.1		
Ile	33	56.3	CH	56.3	5.10 (dd)	5.09 (dd)
	34	41.0	CH	40.9	1.71 (m)	1.80 (m)
	35	25.8	CH$_2$	25.9	1.53, 1.18 (m, m)	1.17, 1.50 (m, m)
	36	11.5	CH$_3$	10.3	0.93 (t)	0.91 (t)
	37	15.1	CH$_3$	15.0	0.78 (d)	0.77 (d)

Table 1.2 : ^{1}H and ^{13}C NMR data in ppm for synthetic *trans, cis*-sanguinamide A and natural[32] *cis, cis*-sanguinamide A in CDCl$_3$ (see figure 1.22 for numbering)

Choosing a different macrolactamisation position, the conformation obtained for the proline peptide bonds differed.

Cis amide bonds in proteins are apparently more frequent in aromatic amino acid-proline residue pairs because of an interaction occurring between the aromatic ring and the proline.[39] In accordance with this observation, we obtained the *cis* peptide linkage for the proline-phenylalanine bond.

1.3.3. Strategy 3: macrolactamisation between Phe and Pro^2

We thought of performing the macrolactamisation next to the other proline (Pro^2-Phe) to check the geometry of the proline residues in the final cyclic compound. We decided to prepare intermediate **1.65** that we could obtain from the coupling of thiazole-containing fragment **1.61** and the dipeptide **1.67** (Scheme 1.11). We had already prepared **1.61** and just had to prepare dipeptide **1.67** in order to prepare **1.65**. The already made dipeptide **1.52** will be saponified to give **1.66** and will be used for synthesis of fragment **1.64**.
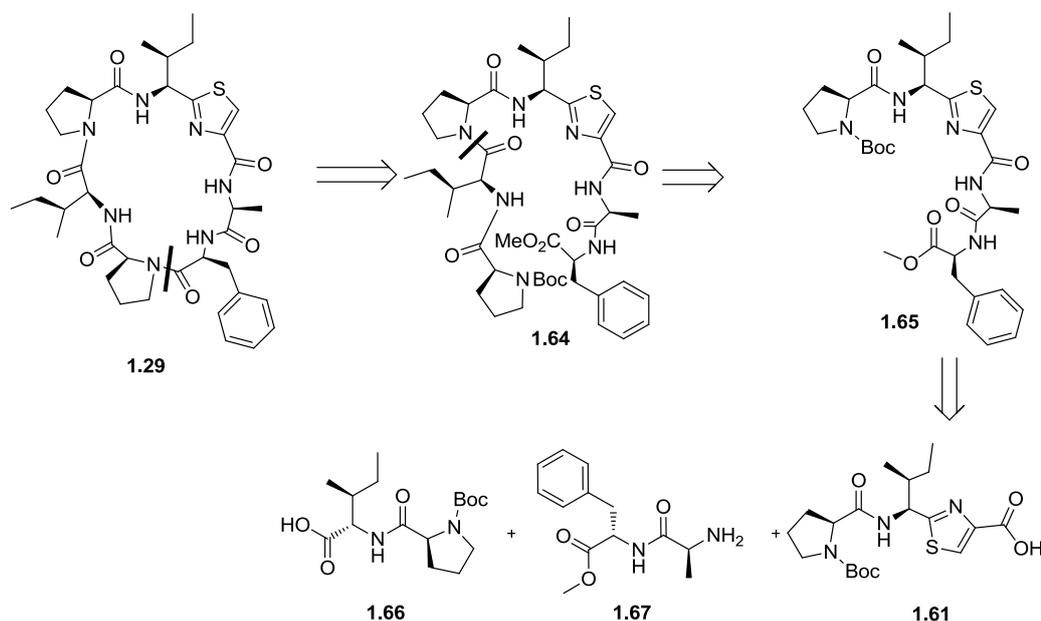
Scheme 1.11 : Alternative retrosynthesis of sanguinamide A

Dipeptide **1.69** was prepared in excellent yield by coupling of Boc-Ala-OH to H-Phe-OMe using PyBOP (Scheme 1.12). The dipeptides **1.70** and **1.66** were prepared by respective deprotection of **1.69** and **1.52**.
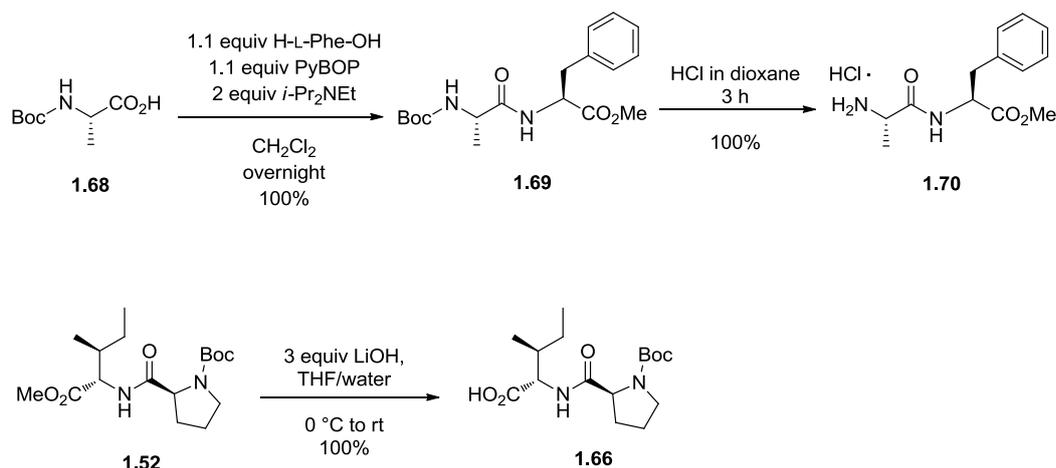
Scheme 1.12 : Synthesis of the dipeptides 1.70 and 1.66

We coupled the thiazole-containing fragment **1.61** with the dipeptide alanine-phenylalanine **1.70** (Scheme 1.13). After Boc removal, fragment **1.65** is then coupled to the dipeptide proline-isoleucine **1.66**. Deprotections followed by macrolactamisation furnished a mixture of conformers: *trans, trans-* and *trans, cis-*sanguinamide A.
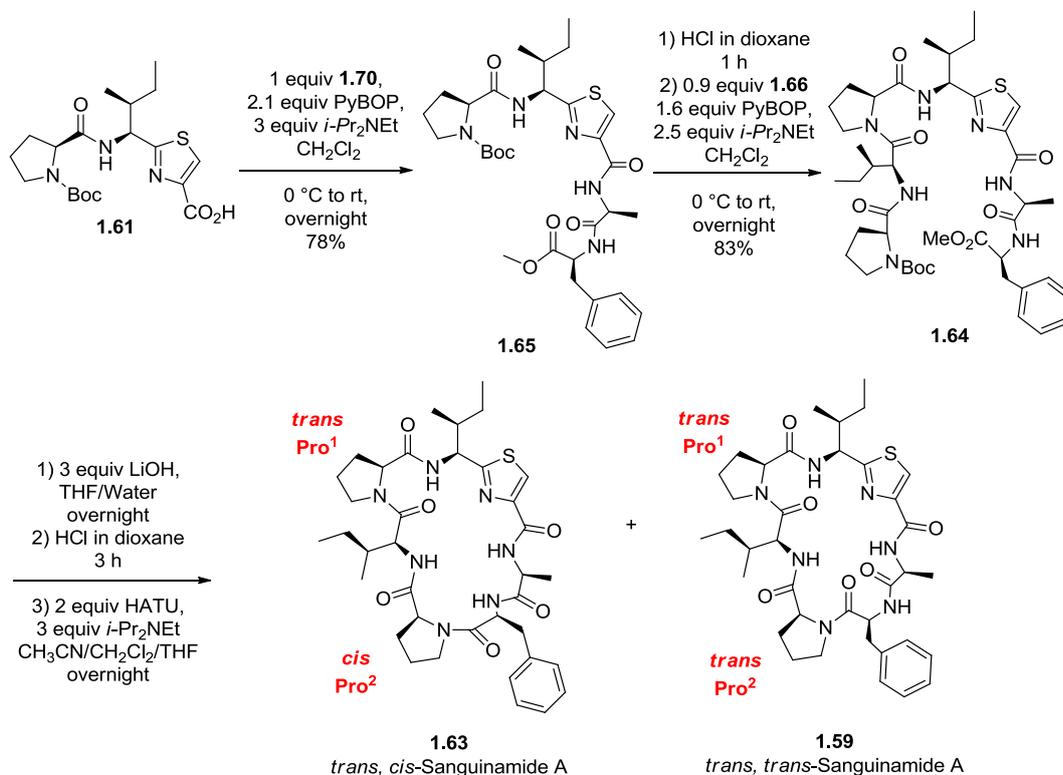
Scheme 1.13 : Synthesis of a mixture of conformers of sanguinamide A by macrolactamisation between Phe and Pro[2]

Thus, our three syntheses gave different conformers depending on the macrolactamisation position. However, the *cis, cis* conformer reported for the natural product was not observed.

1.3.4. Modification of the stereochemistry of the isoleucine residue next to the thiazole

A chiral α-stereocentre next to thiazole tends to epimerise. Epimerisation might have occurred during Molinski's stereochemical assignment of the natural product by degradation and derivatisation by Marfey's method. There are precedents for such wrong assignments as in the case of ceratospongamide. The stereochemistry of the isoleucine residue was assigned as D-isoleucine and was instead D-*allo*-isoleucine.[30]

We started with the D-isoleucine to see if there would be a difference in the proline conformations. The synthesis is identical to the prior synthesis of *trans, trans*-sanguinamide A. The first step however differs as the starting material is Boc-D-Ile-OH instead of the isoleucinamide. The first step is an amidation performed using ammonia in dioxane, EDCI, HOBt and Hünig's base (Scheme 1.14). We also in that case used the epimerisation-free thiazole formation method and obtained **1.73** as a unique diastereomer. For the preparation of the amide **1.74**, PyBOP was replaced by EDCI in order to obtain a cleaner intermediate. The NMR is cleaner but the peaks are very broad due to a mixture of rotamers.
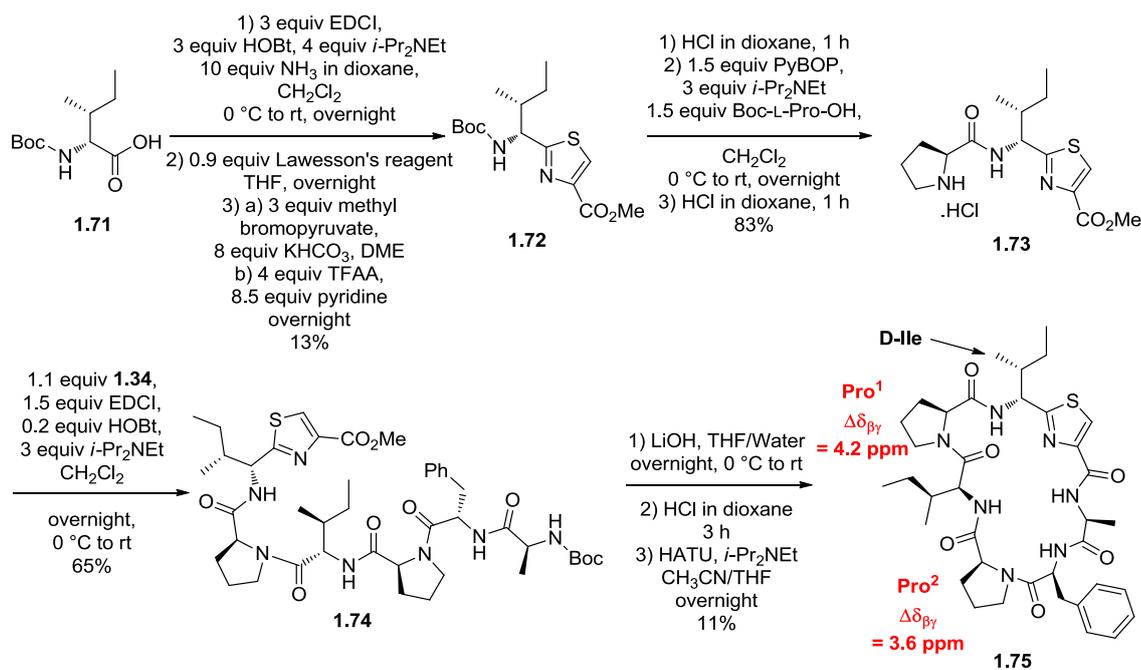
Scheme 1.14 : Synthesis of [D-Ile]-sanguinamide A

Pro^1 has a *trans* conformation ($\Delta\delta\beta\gamma$ = 4.2 ppm) and Pro^2 has a *cis* geometry ($\Delta\delta\beta\gamma$ = 3.6 ppm). The geometries of the proline residues were attributed by NOESY experiment and the values ($\Delta\delta\beta\gamma$) obtained by calculation showed once again the limitation of the method based on the difference in ^{13}C chemical shifts between Cβ and Cγ signals. The *cis* proline peptide bond is supposed to give large ^{13}C chemical shifts difference but in this case, it is lower than that obtained for the *trans*. We obtained the *trans, cis*-[D-Ile]-sanguinamide A using the same macrolactamisation that previously gave us the *trans, trans*-sanguinamide A.

We decided to make sanguinamide A with D-*allo*-isoleucine as we obtained different rotamers when using either D-Ile and L-Ile but the same macrolactamisation position. Synthesis was carried out as described previously from D-*allo*-isoleucine. Boc protection with NaOH as a base in dioxane gave excellent yield (100%) compared to the conditions with Et$_3$N in dichloromethane that gave moderate yields (Scheme 1.15).[40] This difference might be explained by the higher solubility of D-*allo*-isoleucine in dioxane or by the strength of the base (NaOH vs. Et$_3$N). We used ethyl bromopyruvate in this case instead of methyl bromopyruvate only for availability reasons.
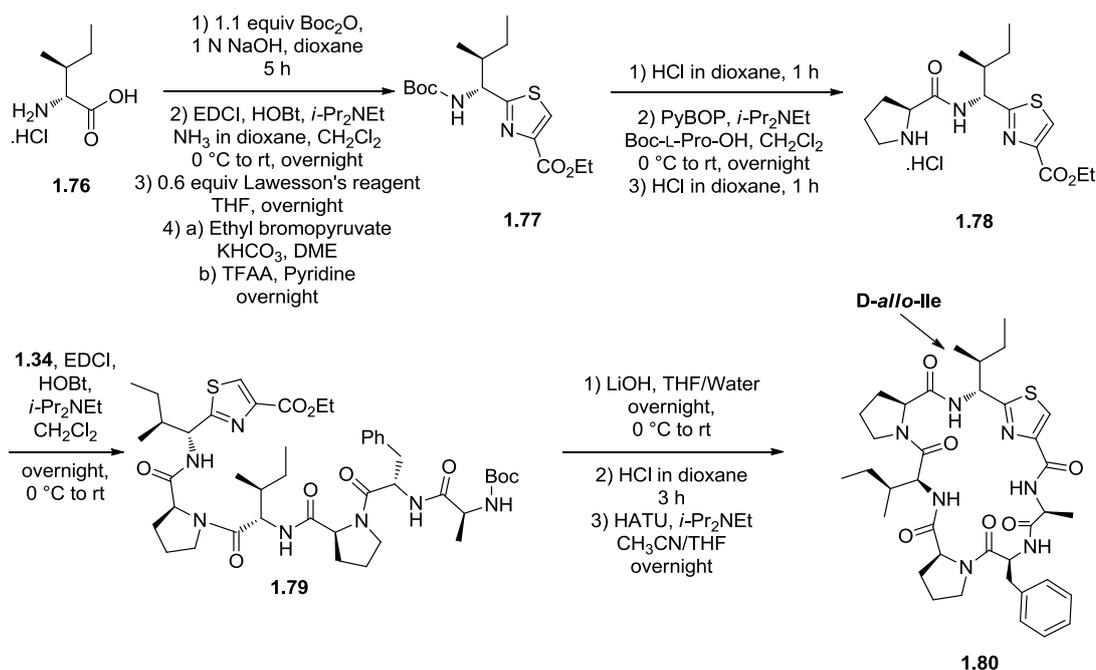
Scheme 1.15 : Synthesis of [D-*allo*-Ile]-sanguinamide A

The compound **1.80** was sent to Marcel Jaspars for HPLC purification but not enough material was recovered to elucidate the proline conformations.

1.3.5. Following the biomimetic pathway

We thought of following the likely biosynthesis of sanguinamide A. It is very likely that the last step of the biomimetic pathway is the thiazole formation. To do so, we envisaged a new strategy, in which the thiazole ring formation will be performed after the macrolactamisation. We cannot use a Boc group in this case as it will not be compatible with the trityl group. We planned to prepare the linear heptapeptide **1.82** obtained by coupling the tetrapeptide **1.62** to the tripeptide **1.83** (Scheme 1.16). We also decided to perform the macrolactamisation between Pro[1] and Ile which has given the closest conformation to the natural product so far, as only the *trans, cis* isomer is obtained.
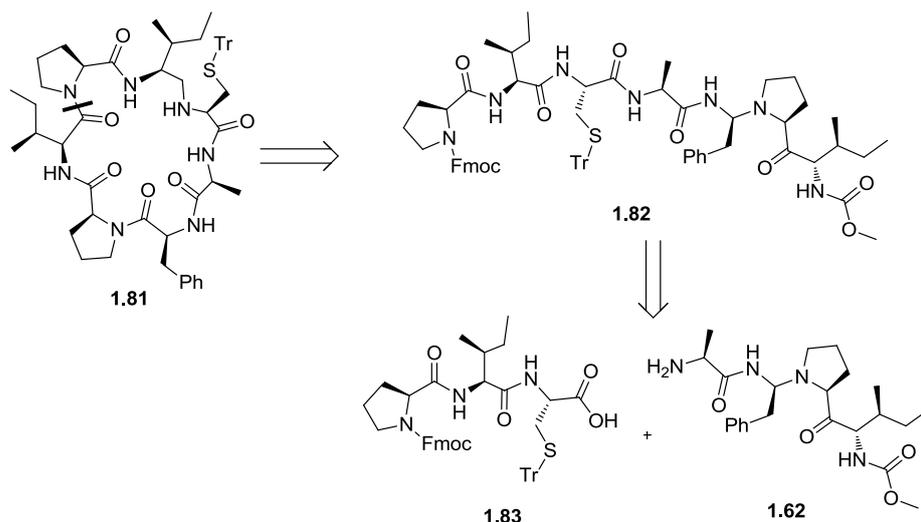
Scheme 1.16 : Retrosynthesis of compound 1.81

Tetrapeptide **1.62** had been previously made and some was still available. The dipeptide Fmoc-Pro-Ile-OMe **1.85** was prepared. Ester hydrolysis with LiOH in THF/water also deprotected the Fmoc group. The hydrolysis of the ester, following a milder method using Me$_3$SnOH, was attempted but the Fmoc group was also removed.[41] As we got H-Pro-Ile-OH, the amine was reprotected with Fmoc chloride to give **1.86**.[42] The acid is then coupled to H-Cys(Tr)-OMe which had been obtained after trityl protection of H-Cys-OMe. The same problem was encountered during the deprotection of the ester **1.87** as Fmoc group was also removed however the same procedure as earlier was employed to reprotect the amine in order to obtain **1.88**. The coupling of **1.88** with **1.62** was tried but this coupling failed because of solubility issues.
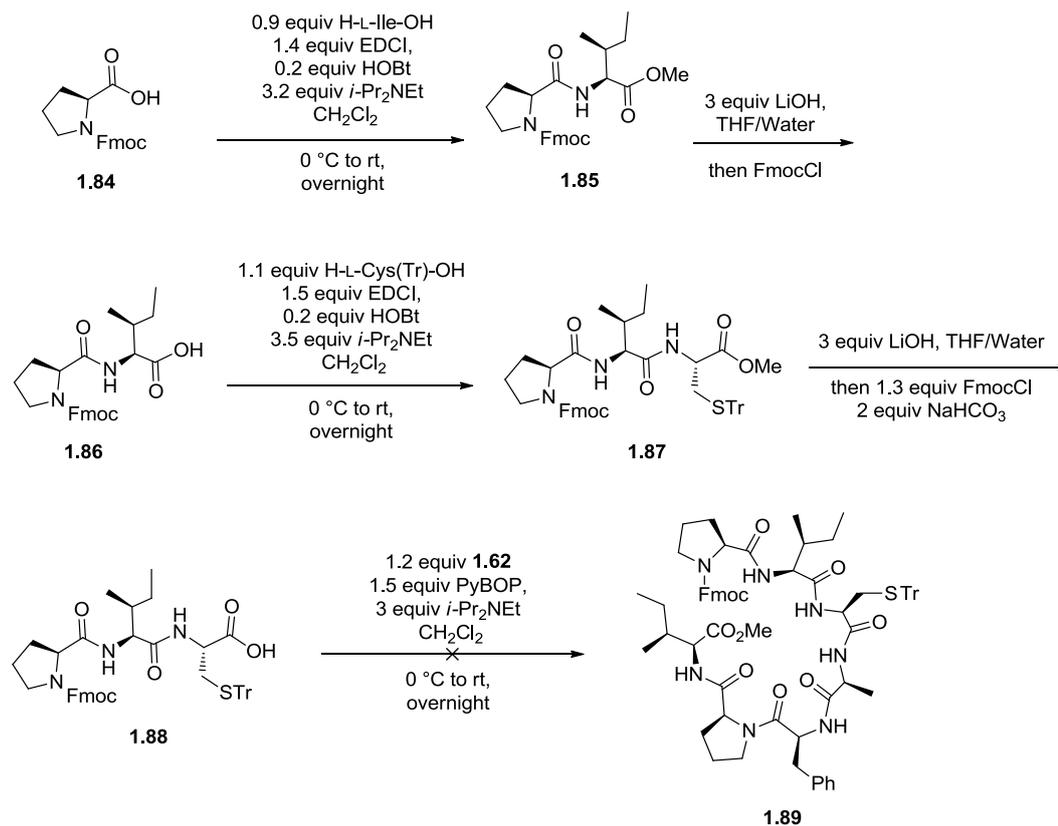
Scheme 1.17 : Attempt to prepare 1.89 in solution phase

We decide to change the strategy and to prepare the linear peptide **1.90** using solid phase peptide synthesis (SPPS).
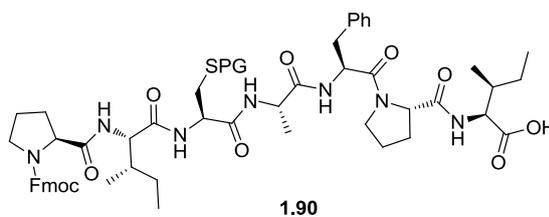
Figure 1.27 : Structure of the peptide 1.90

We employed Fmoc/*t*Bu SPPS method to prepare peptide **1.90** with the Wang resin (Figure 1.28). We used the procedure described by White and Wang.[43]
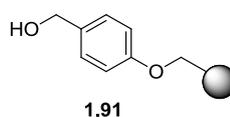
Figure 1.28 : Structure of the Wang resin

The first step consists of swelling the resin. We loaded the resin with isoleucine using diisopropylcarbodiimide (DIC, figure 1.29) to prepare the symmetrical anhydride of Fmoc-Ile-OH.

1.92
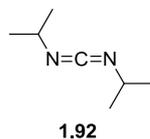
Figure 1.29 : Structure of DIC (1.92)

Deprotection of the Fmoc group was then performed using a mixture of piperidine in DMF (20%) followed by coupling of Fmoc-Pro-OH to the isoleucine using PyBOP. This is followed by deprotection and successive couplings with the corresponding amino acids (Fmoc-Phe-OH, Fmoc-Ala-OH, Fmoc-Cys(*t*Bu)-OH, Fmoc-Ile-OH and Fmoc-Pro-OH) and deprotection steps (Scheme 1.18). The deprotection and coupling steps were monitored using a qualitative ninhydrin assay (Kaiser test) which is a colorimetric test based on the reaction of ninhydrin with amino groups. We also monitored the reactions by ESI-MS if necessary. In the case of incomplete couplings, the coupling step was repeated or if necessary, the peptide was capped by *N*-acetylation. The cleavage of the resin was done using a mixture of TFA, EDT (ethane dithiol), anisole, TIPS and water in CH_2Cl_2 and allowed us to obtain the heptapeptide **1.100**. We used Fmoc-Cys(*t*Bu)-OH because of its availability in the laboratory and its stability during the TFA cleavage of the resin. Solid phase synthesis on Wang resin allowed us to obtain the linear peptide **1.100**. The carboxylic acid **1.100** was treated with piperidine in DMF to form the unprotected linear peptide. HATU-mediated macrolactamisation afforded Cys(*t*Bu) analogue **1.101**.
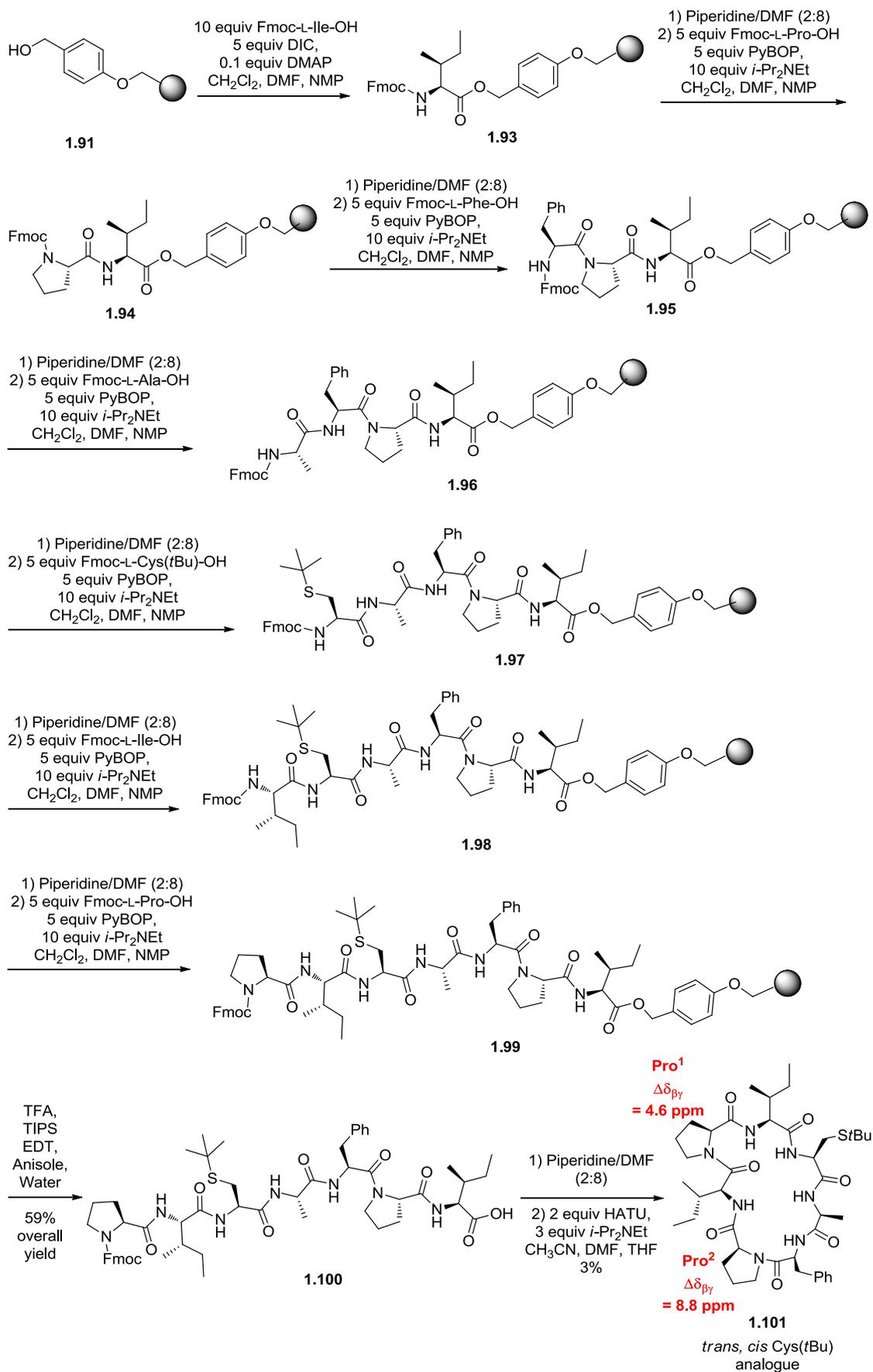
Scheme 1.18 : Solid phase synthesis of the Cys(*t*Bu) compound 1.101

We checked the conformation of the proline residues of the Cys(*t*Bu) compound **1.101** by ^{13}C NMR and NOESY experiment. The proline peptide bonds have *trans* and *cis* conformations (Pro^1: $\Delta\delta\beta\gamma$ = 4.6 ppm; Pro^2: $\Delta\delta\beta\gamma$ = 8.8 ppm). We attempted to convert **1.101** to the thiazoline using Kelly's method (OPPh$_3$/Tf$_2$O).[44,45] In the synthesis of telomestatin, Takahashi performed the cyclodehydration of a Cys(*t*Bu) moiety using Kelly's method with anisole.[45] In our case the reaction was unsuccessful and no product was formed. We decided to try another well-known method using TiCl$_4$ which required the free thiol as starting material.[46] To do so, we have to deprotect the *t*Bu thioether. Deprotection of the *t*Bu protected thiol is not possible using TFA. Harsher conditions are required, such as Hg(OAc)$_2$/TFA then H$_2$S or HF/anisole or BBr$_3$ with AcCl or Br$_2$, AcCl and AcOH.[47] We attempted the deprotection with catalytic bromine (Br$_2$, AcCl and AcOH) but it gave a messy reaction mixture and no thiol **1.104** was isolated after hydrolysis of the thioacetate **1.103**.[48] The thioether deprotection was problematic.
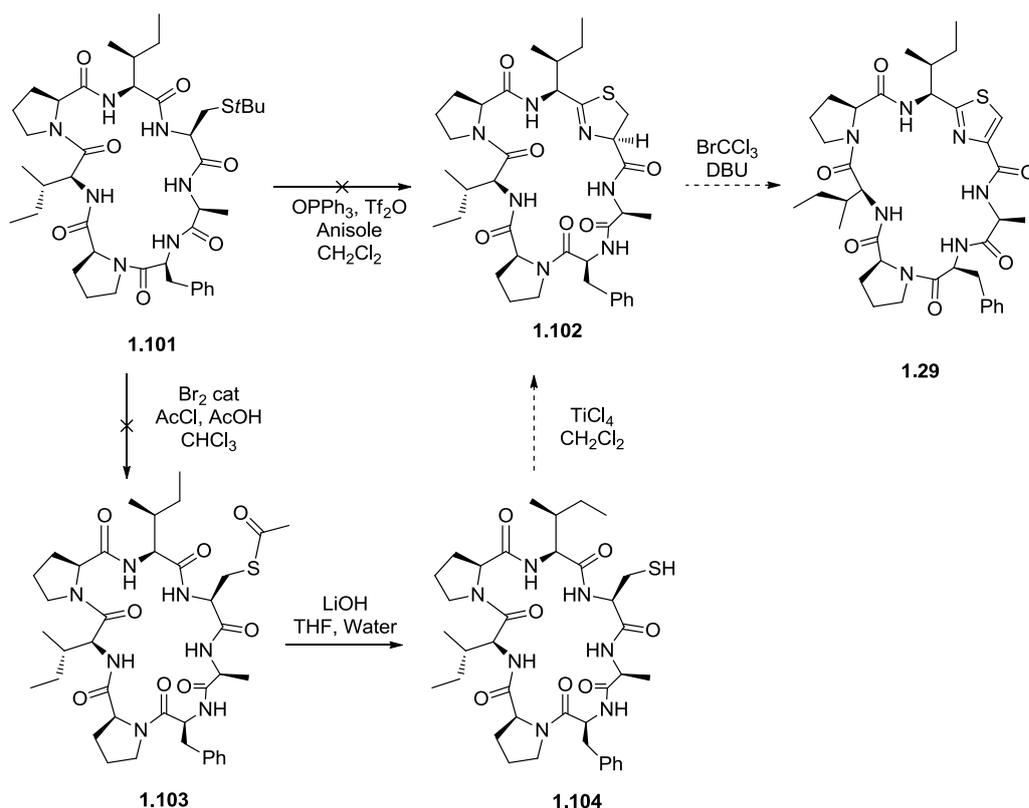
Scheme 1.19 : Attempts to prepare sanguinamide A by late thiazole formation from Cys(*t*Bu) compound 1.101

Fmoc-Cys(*t*Bu)-OH was only used for availability reasons. Several thiazoline syntheses from Cys(Tr) are described in the literature. We planned to make the cyclic peptide with Cys(Tr) and then try to form the thiazoline using first the Kelly method and if necessary other methods.

We decided to move on to another protecting group on the cysteine residue: the trityl group instead of the *t*Bu. I have repeated the SPPS using Fmoc-Cys(Tr) instead of Fmoc-Cys(*t*Bu). Cleavage of the resin with TFA/TIPS/water also deprotected the trityl group which had to be reprotected by treatment with TFA and TrOH.[49] Fmoc deprotection followed by macrolactamisation affords the compound **1.107** but a low yield is obtained (Scheme 1.20).
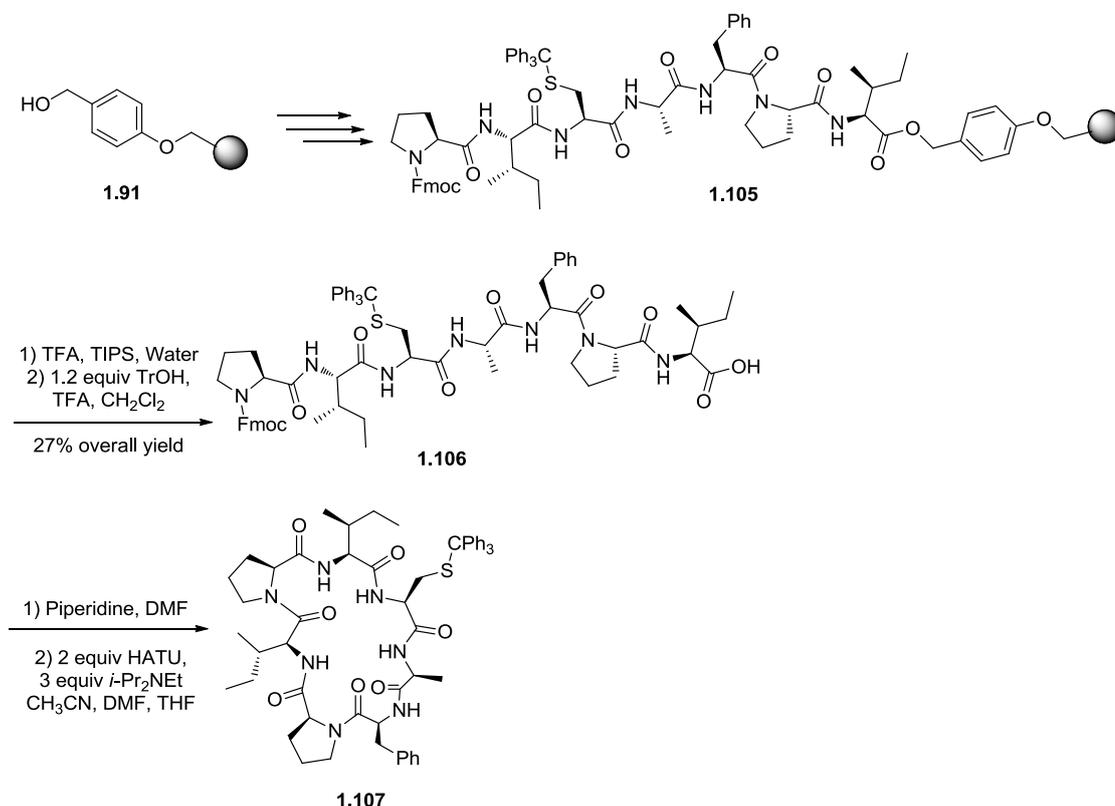
Scheme 1.20 : Solid phase synthesis of the Cys(Tr) compound 1.107

The Kelly method (OPPh$_3$/Tf$_2$O) failed on compound **1.107** (Scheme 1.21). We deprotected the thiol by TFA treatment and use of triisopropylsilane as a scavenger. Compound **1.104** was treated with TiCl$_4$ but no product was formed. We tried another reaction condition found in the literature using (NH$_4$)MoO$_{24}$.H$_2$O which gave the

thiazoline **1.102**.[50] Activated manganese dioxide failed to oxidize the thiazoline to thiazole ring. Oxidation with bromotrichloromethane and DBU furnished the thiazoline but the reaction did not go to completion. Separation of the product from the starting material was not possible. We decided to abandon this route as we were not encouraged by the conformation of the intermediate **1.101**.
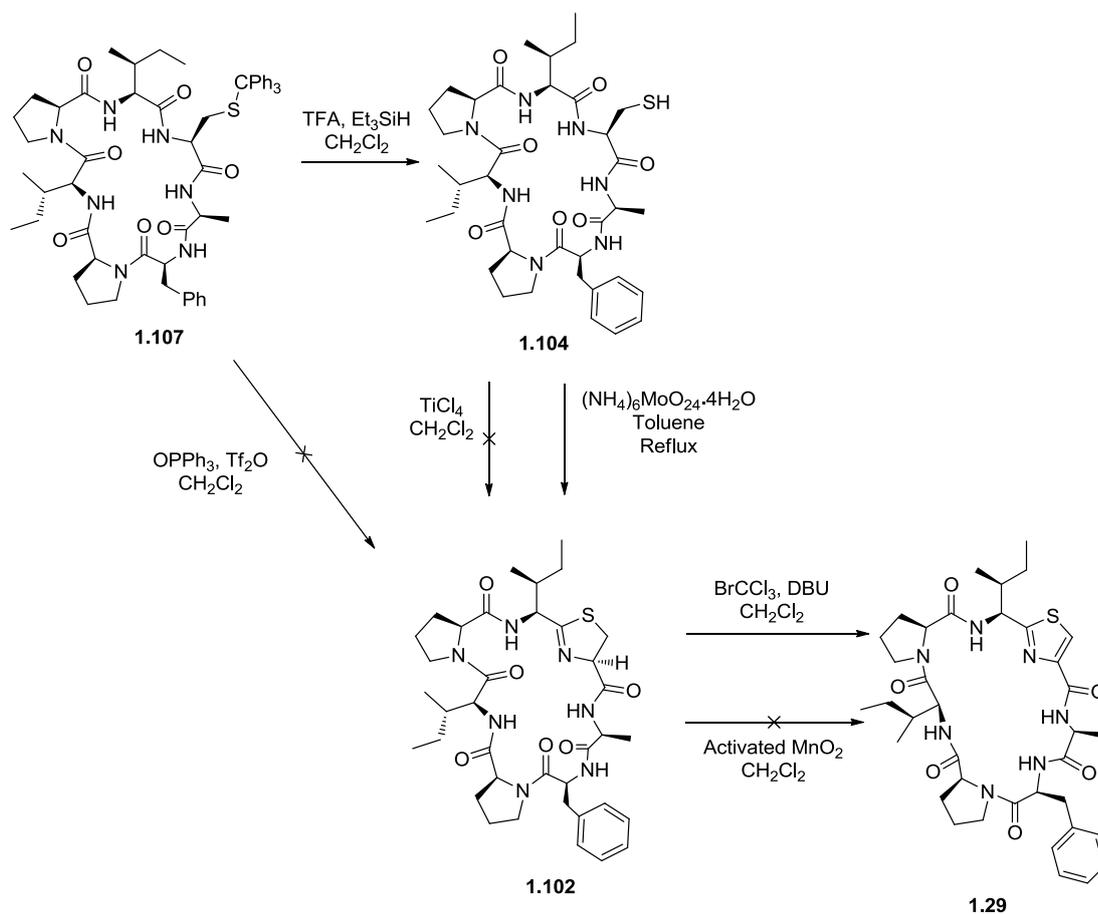
Scheme 1.21 : Attempts to prepare sanguinamide A by late thiazole formation from Cys(Tr) compound 1.107

1.4. TOWARDS THE SYNTHESIS OF THE OCTAPEPTIDE SANGUINAMIDE B

In addition to five amino acids in the L series, sanguinamide B contains one oxazole and two thiazole rings. Five disconnections at the amide linkage are conceivable. We decided to prepare a fragment with the thiazole moiety and another with the thiazole-oxazole unit. The bond-disconnection strategy provided three key synthetic precursors: the dipeptide **1.108**, the thiazole-containing fragment **1.109** and the thiazole-oxazole moiety **1.110** (Scheme 1.22). We decide to couple the thiazole-containing fragment **1.109** to the thiazole-oxazole moiety **1.110** and then to dipeptide **1.108**. The macrolactamisation would be performed between alanine and valine as this is a less hindered position compared to the alternative.
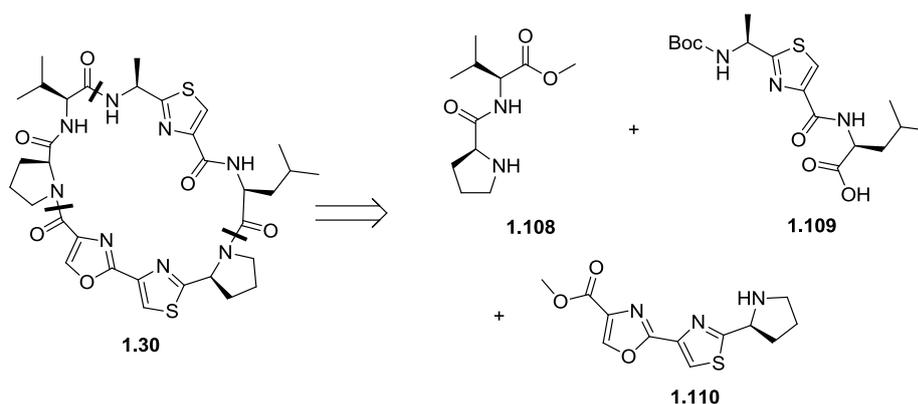
Scheme 1.22 : Retrosynthetic analysis of sanguinamide B

Synthesis of the proline-valine dipeptide **1.112** was performed by coupling Boc-Pro-OH and H-Val-OMe using EDCI as coupling reagent in excellent yield (Scheme 1.23). Boc deprotection afforded the hydrochloride salt of the desired fragment **1.113**.
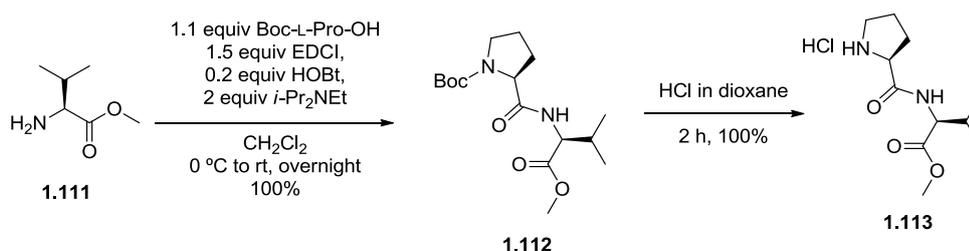
Scheme 1.23 : Synthesis of dipeptide 1.113

We synthesised the thiazole-containing fragment **1.119** starting from L-alaninamide (**1.114**) in which the amine is first protected with a Boc group and the amide converted to the thioamide **1.116** (Scheme 1.24). A Hantzsch thiazole synthesis is then performed with methyl bromopyruvate to form the desired product **1.117**. We used the modified version in order to avoid the epimerization (TFAA/pyridine). The ester is deprotected and the resulting acid **1.118** is coupled to H-L-Leu-OMe using PyBOP to afford the thiazole-containing fragment **1.119**.
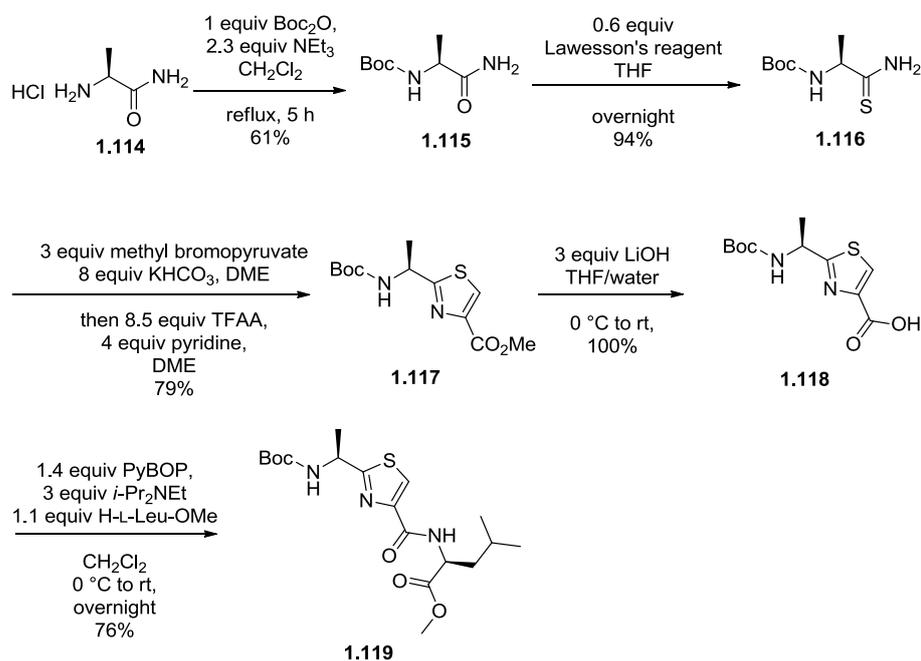
Scheme 1.24 : Synthesis of thiazole-containing fragment 1.119

We started the synthesis of **1.127** from Boc-Pro-OH (**1.51**) which was converted to thioamide **1.121** in two steps (Scheme 1.25). Synthesis of the thiazole ring was carried out as described previously to form compound **1.122**; this was saponified to give the carboxylic acid **1.123** which was then coupled with H-L-Ser-OMe without needing to protect the hydroxyl group of serine. Activation of the hydroxyl group using diethyl(amino)sulfur trifluoride (DAST) at −78 ℃ followed by cyclisation under basic conditions with K_2CO_3 furnished the oxazoline **1.125**.[51] This compound was converted to the desired oxazole **1.126** by oxidation in the presence of bromotrichloromethane and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The expected thiazole-oxazole fragment

1.126 was obtained in 64% after cyclisation-oxidation. Boc deprotection affords the thiazole-oxazole fragment **1.127**.
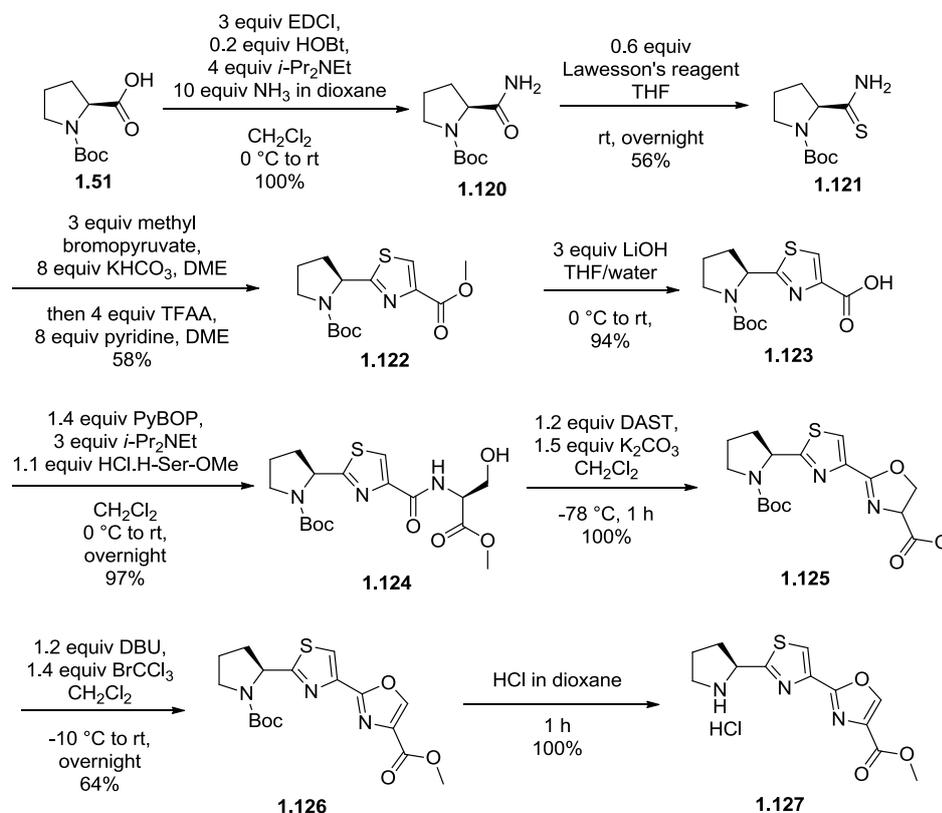
Scheme 1.25 : Synthesis of thiazole-oxazole fragment 1.127

After ester hydrolysis, the thiazole-containing fragment **1.119** is coupled to the thiazole-oxazole fragment **1.127** (Scheme 1.26). The ester of the compound **1.128** is saponified and coupled to the dipeptide **1.113**. Compounds **1.128** and **1.129** gave mixture of conformers. After deprotections, the macrolactamisation with HATU is performed to form the desired octapeptide **1.130**. After several attempts to purify the mixture of two compounds by flash chromatography, we decided to send it to our collaborators for HPLC purification.
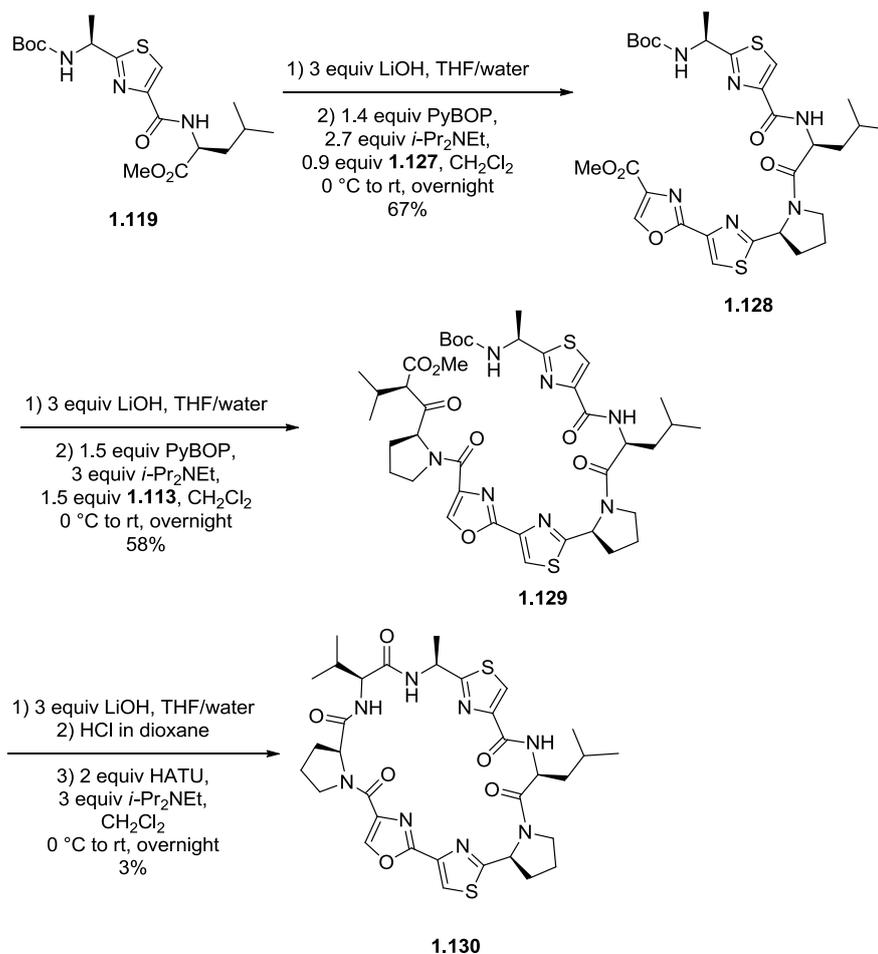
Scheme 1.26 : Synthesis of sanguinamide B

Comparison of the ^{1}H and ^{13}C NMR data of the synthetic compound with that reported in the literature for the natural product showed significant differences and suggest that the geometry of the proline residues in the natural and synthetic compounds is different (Figure 1.30, table 1.3). NMR analysis and NOESY experiment showed that the compound is *cis*, *cis*-sanguinamide B (Pro[1]: $\Delta\delta\beta\gamma$ = 4.2 ppm; Pro[2]: $\Delta\delta\beta\gamma$ = 3.6 ppm). The natural product has the opposite proline conformations as Pro[1] is *trans* ($\Delta\delta\beta\gamma$ = 4.2 ppm) and Pro[2] is *trans* ($\Delta\delta\beta\gamma$ = 2.4 ppm). The limitation of the method of ^{13}C NMR chemical shifts differences is once again observed as even though some of the proline residues are *cis* and some are *trans*, all the values obtained by this calculation method are small. Synthesis of sanguinamide B also gave a different conformer than the natural product as was the case for the synthesis of sanguinamide A.
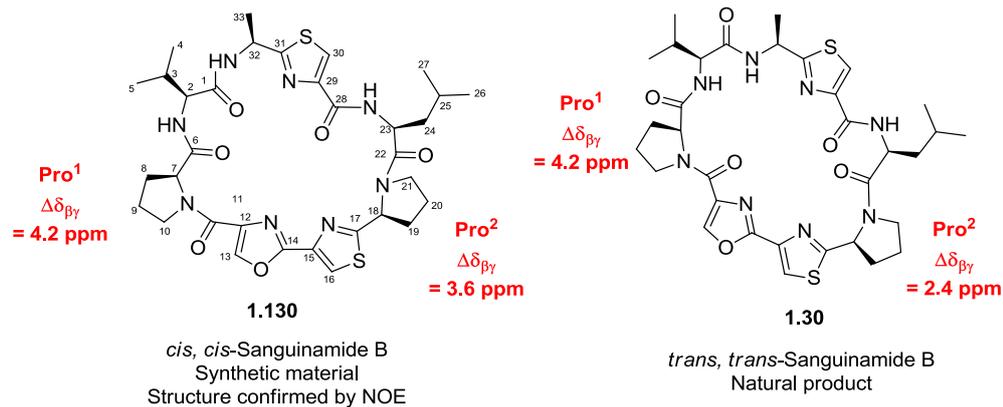
Figure 1.30 : Structures of the synthetic material 1.130 with the atom numbering used and the natural product sanguinamide B (1.30)

Residue	#	$\delta^{13}C$	^{13}C mult.	Reported[32] $\delta^{13}C$	δ^1H and mult.	Reported[32] δ^1H and mult.
Val	1	170.96	C	159.1		
	2	56.9	CH	58.7	4.78 (dd)	4.61 (d)
	3	28.4	CH	30.6	2.88 (dq)	2.93 (m)
	4	16.5	CH$_3$	20.4	0.86 (d)	0.87 (d)
	5	19.9	CH$_3$	20.2	0.98 (m)	1.15 (d)
	NH				6.27(d)	9.26 (d)
Pro	6	173.6	C	170.4		
	7	64.3	CH	60.9	4.72 (dd)	4.39 (d)
	8	32.0	CH$_2$	29.4	2.29 (m)	1.74, 2.80 (m, m)
	9	22.7	CH$_2$	25.2	2.13, 2.00 (m, m)	2.22, 1.94 (m, m)
	10	48.1	CH$_2$	45.4	3.88, 3.83 (m, m)	3.75, 3.34 (m, m)
Oxz	11	162.1	C	169.2		
	12	136.7	C	136.7		
	13	143.7	CH	141.5	8.37 (s)	8.27 (s)
	14	157.7	C	157.7		
Thz	15	140.7	C	142.3		
	16	122.6	CH	121.8	7.43 (s)	7.95 (s)
	17	172.8	C	172.9		
Pro	18	59.0	CH	58.7	5.43 (d)	4.57 (d)
	19	35.8	CH$_2$	31.1	2.32, 1.91 (m, m)	2.94, 2.07 (m, m)
	20	20.3	CH$_2$	28.7	1.95, 1.77 (m, m)	2.10, 1.81 (m, m)
	21	46.7	CH$_2$	48.0	3.87, 3.80 (m, m)	3.81, 3.97 (m, m)
Leu	22	168.9	C	170.8		
	23	49.1	CH	48.4	5.08 (m)	5.29 (dd)
	24	41.6	CH$_2$	41.4	1.95, 1.57 (m, m)	2.02 (m)
	25	24.4	CH	25.6	1.57 (m)	1.63 (m)
	26	22.3	CH$_3$	23.2	0.98 (m)	0.99 (d)
	27	23.5	CH$_3$	22.0	0.92 (d)	1.03 (d)
	NH				7.02 (d)	9.60 (d)
Thz	28	160.0	C	160.1		
	29	146.7	C	149.2		
	30	125.7	CH	124.1	7.92 (s)	8.12 (s)
	31	171.02	C	170.6		
	32	45.9	CH	47.8	5.10 (m)	5.56 (qd)
	33	16.3	CH$_3$	24.8	1.00 (m)	1.68 (d)
	NH				7.24 (d)	8.42 (d)

Table 1.3 : ^{1}H and ^{13}C NMR data in ppm for synthetic *cis, cis*-sanguinamide B and natural[32] *trans, trans*-sanguinamide B in CDCl$_3$

1.5. *CIS-TRANS* ISOMERISATION AND ATTEMPTED REISOLATION

Sanguinamide A

The energies calculated by Dr Bruce Milne at the University of Coimbra and shown in table 1.4 suggest that *cis, trans* is the only significantly populated conformer at room temperature in chloroform solution. Three different levels of theory, hybrid DFT (B3LYP), double-hybrid DFT (B2PLYP) and second order perturbation theory (MP2), were used and support this fact. The COSMO solvent model used during the DFT geometry optimisations is chloroform. The *trans, cis* rotamer, that we obtained synthetically, is also lower in energy followed by the *trans, trans* rotamer followed by the natural product (*cis, cis*) with the highest energy.

Conformer sanguinamide A	Relative energy (kJ/mol)		
	B3LYP-D	**B2PLYP-D**	**MP2**
Cis, trans	0.00	0.00	0.00
Trans, cis (Synthetic)	7.80	13.30	18.21
Trans, trans (Synthetic)	19.72	21.49	27.04
Cis, cis (Natural product)	22.09	24.67	22.73

Table 1.4 : Single point energies of sanguinamide A calculated at BLYP-D/SV(P) geometries with solvation in chloroform

We tried to interconvert *trans, cis*-sanguinamide A to *cis, cis*-sanguinamide A by heating the cyclic peptide in the microwave. *Trans, cis*-Sanguinamide A was dissolved in isopropanol, a polar solvent, and heated at 80 ºC under microwave radiation for 4 h but no interconversion occurred. ^{1}H and ^{13}C NMR data were identical to the starting material *trans, cis*-sanguinamide A. We are trying to interconvert the thermodynamic product to the kinetic isomer and this may explain the lack of success. We are using the intermediate compound *trans, cis*-sanguinamide A and by heating it up we might interconvert it to *trans, trans*-sanguinamide A or *cis, trans*-sanguinamide A. Alternatively, it is possible that interconversion occurs during cooling since Molinski's sample was kept in the freezer for 20 years. We decided to take *trans, cis*-sanguinamide

A and leave it in the freezer in isopropanol for two weeks in order to check if one rotamer would froze out. NMR showed that no isomerisation occurred.

Sanguinamide B

Calculations by Dr Bruce Milne for the sanguinamide B rotamers showed that the *cis, cis* conformer that we obtained synthetically has the lowest energy by all three methods (Table 1.5). Variations are obtained depending on the calculation method for the *trans, trans*-natural product however, the relative energies are higher than that of the *cis, cis*-rotamer.

Conformer sanguinamide B	Relative energy (kJ/mol)		
	B3LYP-D	**B2PLYP-D**	**MP2**
Cis, cis **(Synthetic)**	0.00	0.00	0.00
Trans, trans **(Natural product)**	9.95	17.50	27.42
Trans, cis	16.04	12.81	7.27
Cis, trans	22.21	23.24	24.93

Table 1.5 : Single point energies of sanguinamide A calculated at BLYP-D/SV(P) geometries with solvation in chloroform

Variable-temperature NMR was carried out on sanguinamide B to see if there was any interconversion between rotamers. The NMR was run from −10 °C to 55 °C in CDCl$_3$ but did not show any significant variation in the conformation. *Cis-trans* Isomerisation by adding small amounts of water was attempted since this has been reported to have an effect in rotamer population with a synthetic peptide.[52] However the NMR run in CDCl$_3$ with water did not show any variation in the proline conformations.

The various isomerisation attempts were unsuccessful. Synthesis is leading to the formation of different rotamers than that of the natural products.

Sanguinamides were not detected by our collaborators: Marianna Carbone and Ernesto Mollo in attempted reisolation from a different sample of the same nudibranch isolated. This is raising a new hypothesis that the natural products might be produced by a symbiotic component which was in the sample of the sponge *Hexabranchus sanguineus* at the time used in Molinski's isolation.

1.6. BIOLOGICAL ACTIVITY OF SANGUINAMIDES

Biological assays of the synthetic rotamers of sanguinamide A, the [D-Ile]-sanguinamide A, the Cys(*t*Bu) analogue of sanguinamide A and the synthetic rotamer of sanguinamide B were performed by our collaborator Dr Wael Houssen and Prof Jaspars from the University of Aberdeen (Figure 1.31). They evaluated the antibacterial activity against several bacterial strains and the tyrosine kinase inhibitory activity of the different compounds.

1.59
trans, trans-Sanguinamide A

1.63
trans, cis-Sanguinamide A

1.75
trans, cis-[D-Ile]-Sanguinamide A

1.101
trans, cis Cys(*t*Bu) analogue

1.130
cis, cis-Sanguinamide B
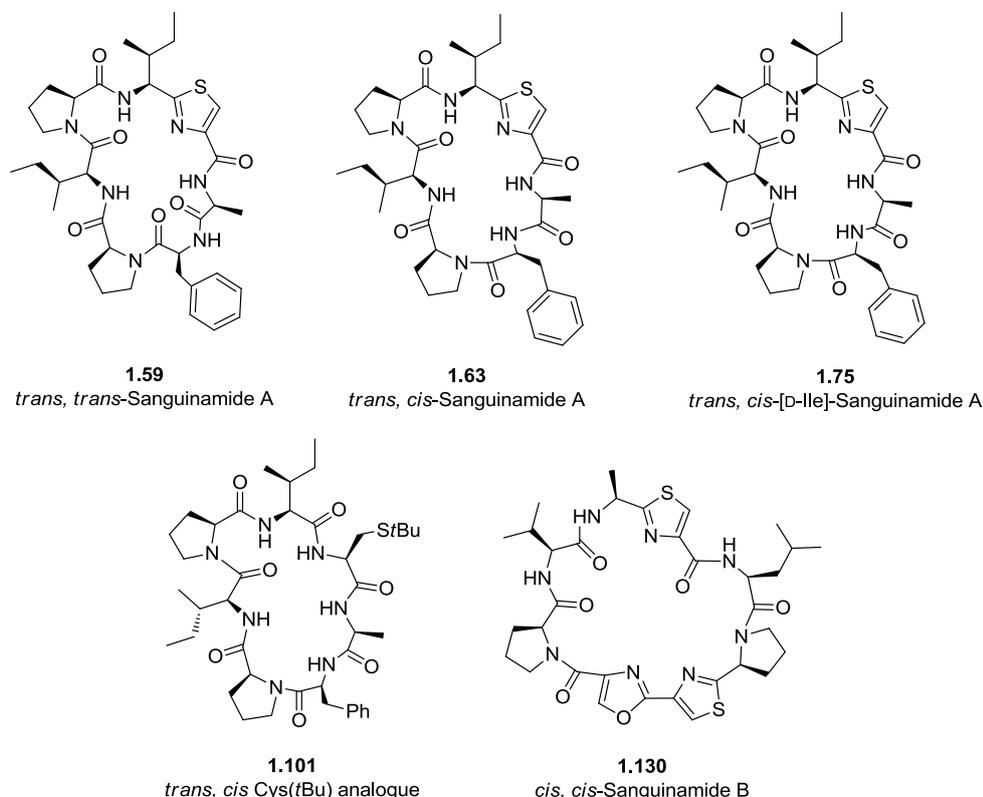
Figure 1.31 : Structures of the different compounds tested

1.6.1. Antibacterial activity

Antibacterial testing was evaluated against bacterial strains: the Gram positive *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* NCIMB 13726 and *Bacillus subtilis* NCIMB 9118 and the Gram negative *Escherichia coli* ATCC 25922 and *Vibrio parahaemolyticus* NCTC 10441. Minimum inhibitory concentrations (MIC, μM) are reported in table 1.6. The Cys(*t*Bu) analogue, synthetic sanguinamide B and the

D-isoleucine modified sanguinamide A were only found to display a micromolar antibacterial activity against one bacterial strain: *Escherichia coli* ATCC 25922. The synthetic rotamers of sanguinamide A were inactive against all the tested bacterial strains.

Compound	MIC (μM)				
	S. aureus ATCC 25923	*L. monocytogenes* NCIMB 13726	*B. subtilis* NCIMB 9118	*E. coli* ATCC 25922	*V. parahaemolyticus* NCTC 10441
***Trans, trans*-sanguinamide A**	Not active	Not active	Not active	Not active	Not active
***Trans, cis*-sanguinamide A**	Not active	Not active	Not active	Not active	Not active
D-Ile derivative 1.75	Not active	Not active	Not active	**25**	Not active
Cys(*t*Bu) compound 1.101	Not active	Not active	Not active	**11.3**	Not active
Sanguinamide B (1.130)	Not active	Not active	Not active	**12.7**	Not active

Table 1.6 : Minimum inhibitory concentrations in μM for different rotamers and derivatives of sanguinamides A and B

1.6.2. Tyrosine kinase inhibitory activity

The compounds were tested for their ability to inhibit the activity of tyrosine kinase. The inhibition of the epidermal growth factor receptor (EGFR) was evaluated with the activities of the compounds compared at the same concentration (100 μM) to the known tyrosine kinase inhibitor genistein. Mild to low inhibitory activity was obtained for all the tested compounds (Table 1.7). The rotamer *trans, trans*-sanguinamide A is the most active compound with 52.5% of inhibition.

Compound	% Inhibition
Trans, trans-sanguinamide A	52.5
Trans, cis-sanguinamide A	27.5
D-Ile derivative 1.75	32.5
Cys(*t*Bu) compound 1.101	25
Sanguinamide B (1.130)	30

Table 1.7 : Inhibition in percentage of tyrosine kinase by the different rotamers and derivatives of sanguinamides A and B at a concentration of 100 μM

Unfortunately no biological results were published for the natural products and comparison between the biological activity of natural and synthetic compounds is not possible.

1.7. SYNTHESIS OF STYLISSAMIDE A

In collaboration with Professor Mark Hamann from the University of Mississippi we worked on the synthesis of stylissamide A. They isolated the natural product from the sponge *Stylissa caribica* and wanted to compare the isolated product to the synthetic compound. They were interested to verify that the biological activity of both natural and synthetic compound is the same.

Stylissamide A was prepared by macrocyclisation from a commercial linear precursor **1.131** followed by deprotection with TFA treatment of the Boc and *t*Bu protecting groups respectively present on lysine and tyrosine residues (Scheme 1.27). I purified the compound by flash chromatography and sent it to Hamann's group for HPLC purification.
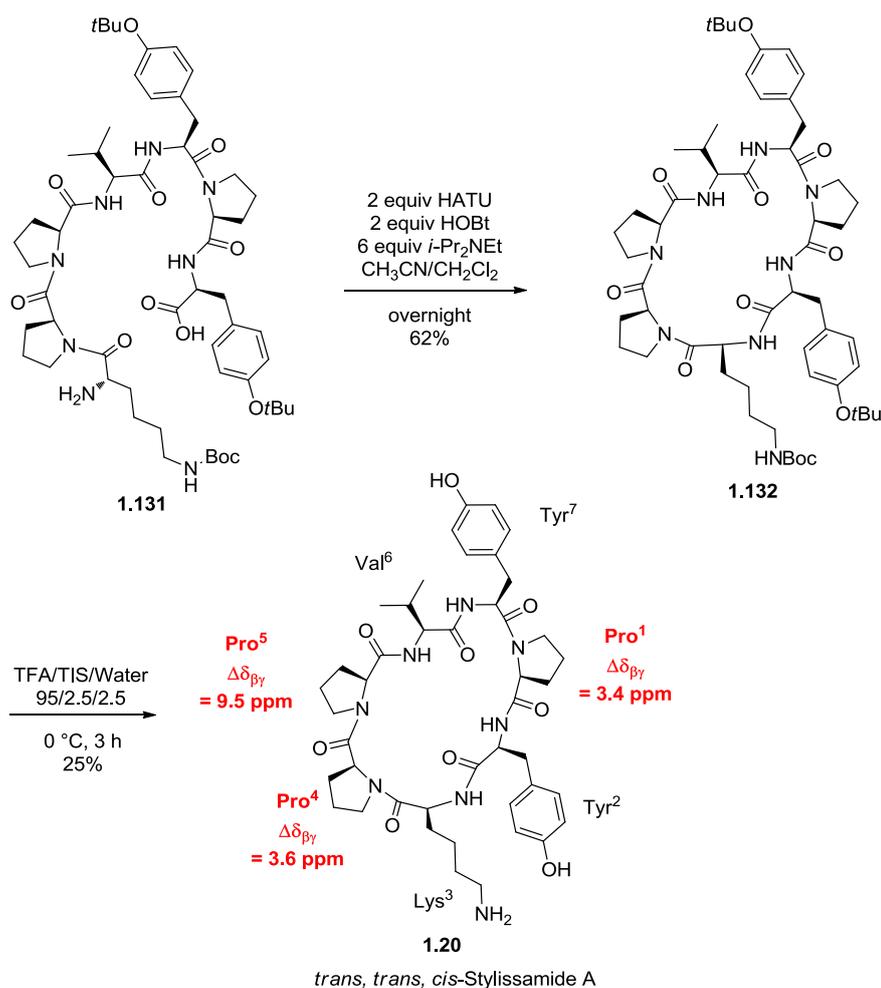
Scheme 1.27 : Synthesis of stylissamide A (1.20)

The synthetic compound was a perfect match with the natural product displaying the same geometry for the proline residues (Figures 1.32 and 1.33). The structure of the heptapeptide was confirmed as *cyclo*(Pro^1-Tyr^2-Lys^3-Pro^4-Pro^5-Val^6-Tyr^7). ^{13}C NMR data of stylissamide A indicated two proline peptide bonds are *trans* (Pro^1 $\Delta\delta\beta\gamma = 3.4$ ppm, Pro^4 $\Delta\delta\beta\gamma = 3.6$ ppm) and one *cis* for Pro^5 ($\Delta\delta\beta\gamma = 9.5$ ppm). Köck and co-workers reported the $\Delta\delta\beta\gamma$ of Pro^1 and Pro^4 to be *trans* ($\Delta\delta\beta\gamma = 3.5$ ppm) and Pro^5 to be *cis* with $\Delta\delta\beta\gamma = 9.5$ ppm.[28] Adjacent *cis* and *trans* Pro residues were also found in a few other natural products such as phakellistatin 8 and wainunuamide and were found to be powerful β turn inducers.[53,54]
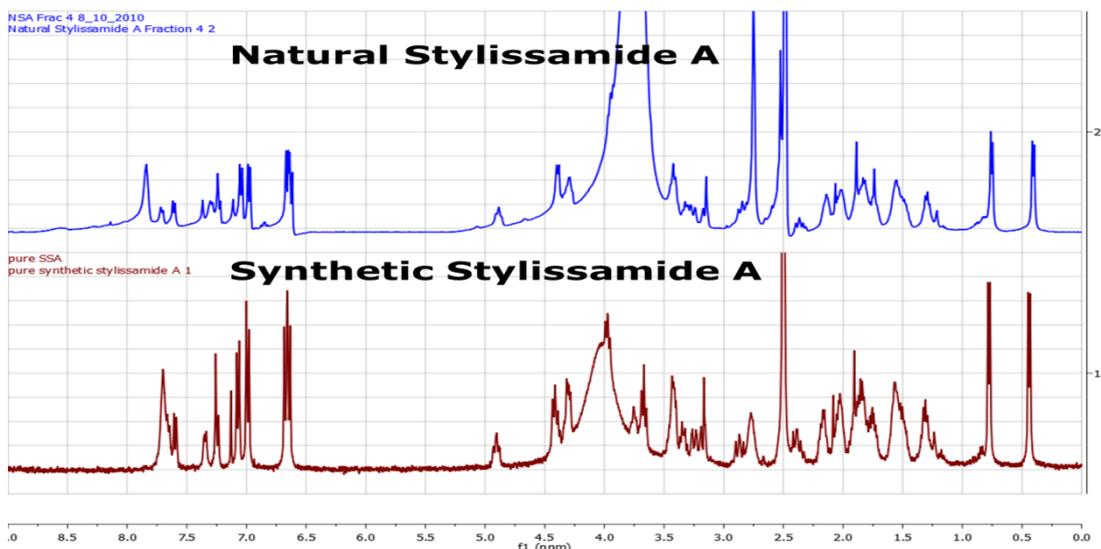
Figure 1.32 : Comparison of the ^{1}H NMR of natural (blue) and synthetic (red) stylissamide A in DMSO-*d*6[55]
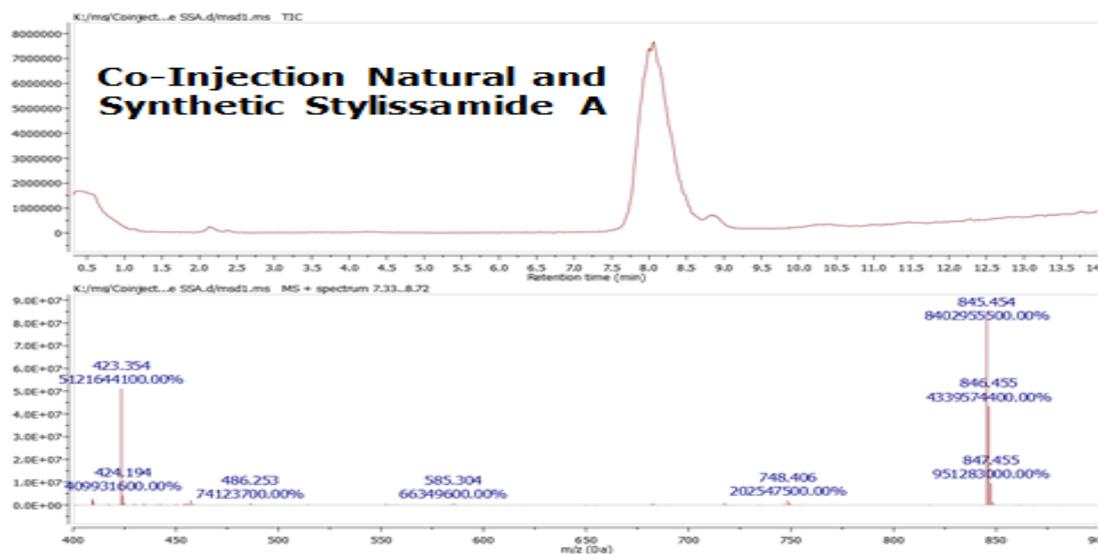
Figure 1.33 : Co-injection on LC/MS of natural and synthetic stylissamide A in equal concentration ratio[55]

1.8. BIOLOGICAL ACTIVITY OF STYLISSAMIDE A

Professor Mark Hamann and his group are interested in inhibitors of protein translation. Translation is extremely important in protein synthesis and is constituted of three main phases: initiation, elongation and termination. Two different ways of initiating translation are possible; the most common process is the cap-dependent initiation and the alternative mechanism known as cap-independent initiation, requires the involvement of the internal ribosomal entry sites (IRES). During the cap-dependent initiation step, messenger RNA is recognised at the 5'-end (5'-cap) by specific initiation factors (IF) such as eIF4E. The cap-independent initiation (IRES method) does not require the interaction of eIF4E with the cap but occur through the internal ribosomal entry sites (IRES) present on mRNA. Several protein factors regulating the cap-dependent translation initiation are overexpressed and associated with cancers.

The following work has been done by Anna J. Kochanowska, Amanda L. Waters and Professor Hamann. They discovered that the sponge *Stylissa caribica* is able to inhibit the protein translation.[55] They identified stylissamide A to be the active component responsible for this inhibitory activity towards protein translation, even though it has been reported by Schmidt *et al.* that the compound had no antimicrobial or cytotoxic activity. They purified the active fractions of the sponge and isolated stylissamide A. They showed that extracted stylissamide A inhibits HCV IRES-dependent and cap-dependent translation in Krebs extracts (Figure 1.34) and in rabbit reticulocytes lysate (Figure 1.35). It also inhibited the cap-dependent translation in the wheat germ extracts (Figure 1.36). The translation was also inhibited in yeast extract by stylissamide A (Figure 1.37). The inhibition is dependent on the concentration.
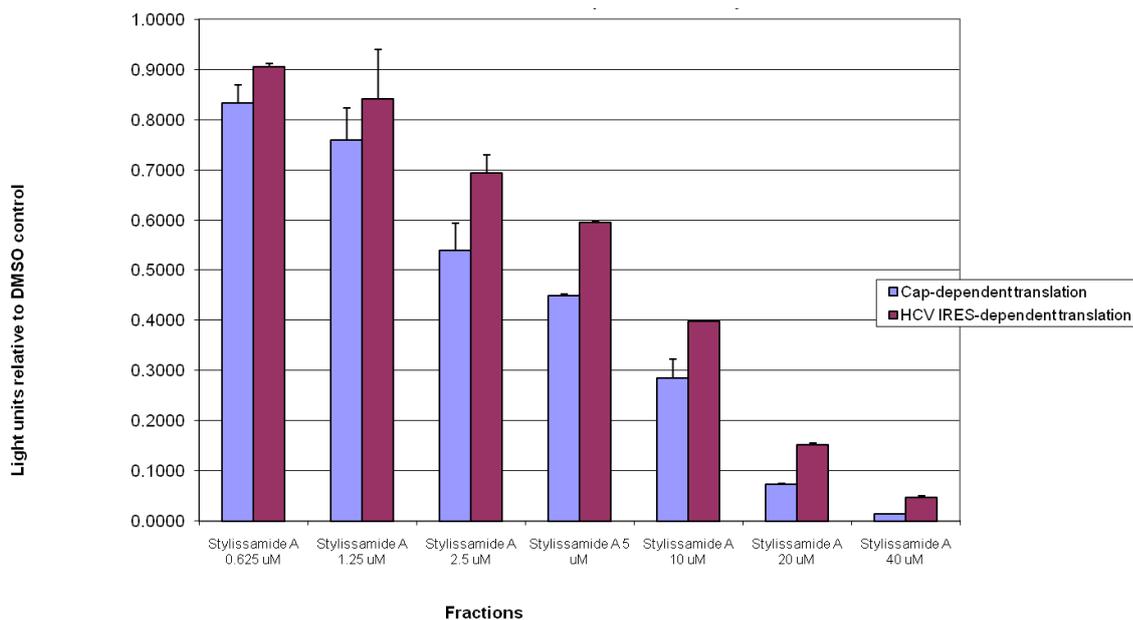
Figure 1.34 : Effect of stylissamide A on cap-dependent and HCV IRES-dependent translation in Krebs-2 cell extracts[55]
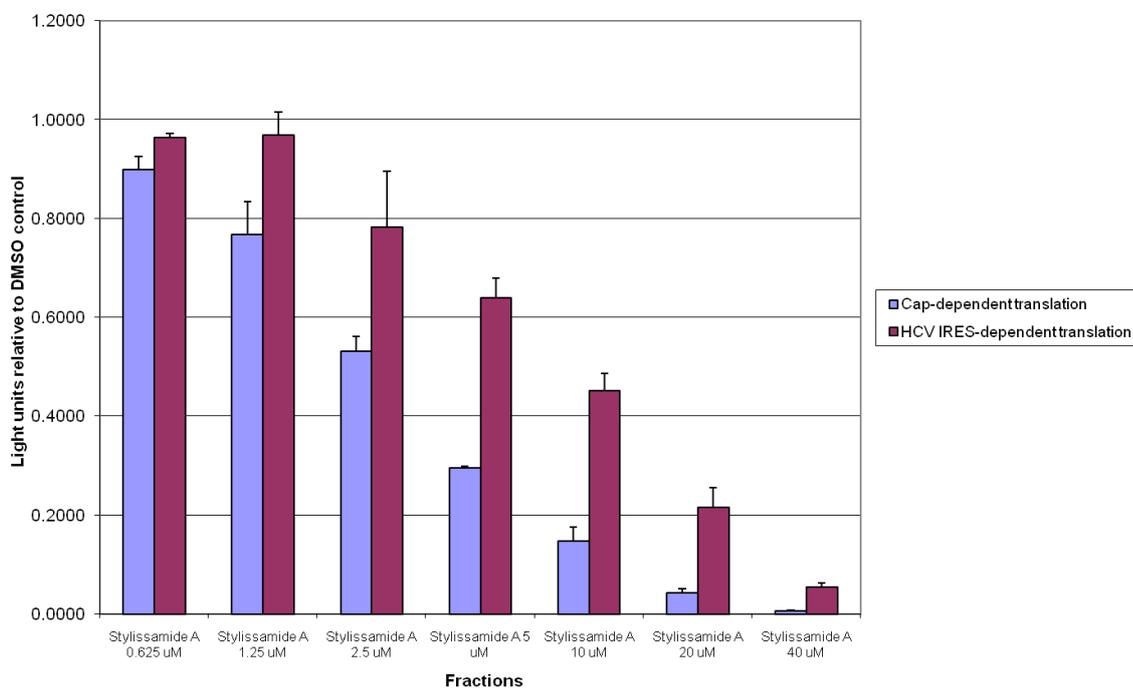
Figure 1.35 : Effect of stylissamide A on cap-dependent and HCV IRES-dependent translation in rabbit reticulocytes lysate[55]
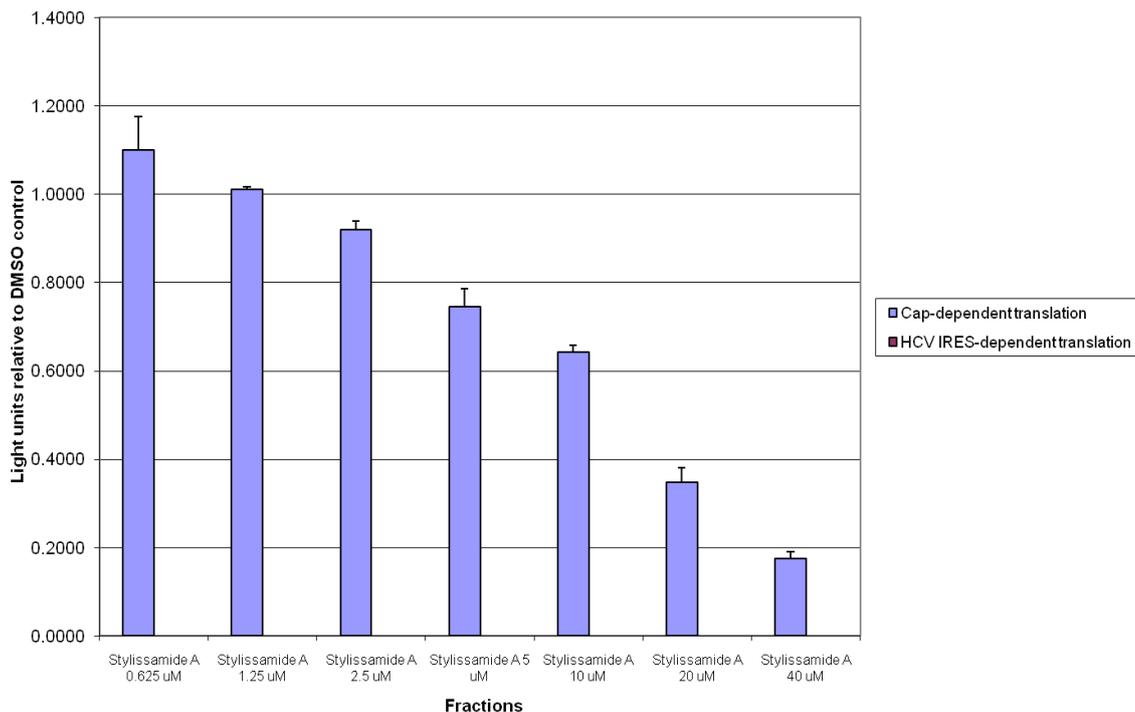
Figure 1.36 : Effect of stylissamide A on cap-dependent translation in wheat germ extract[55]
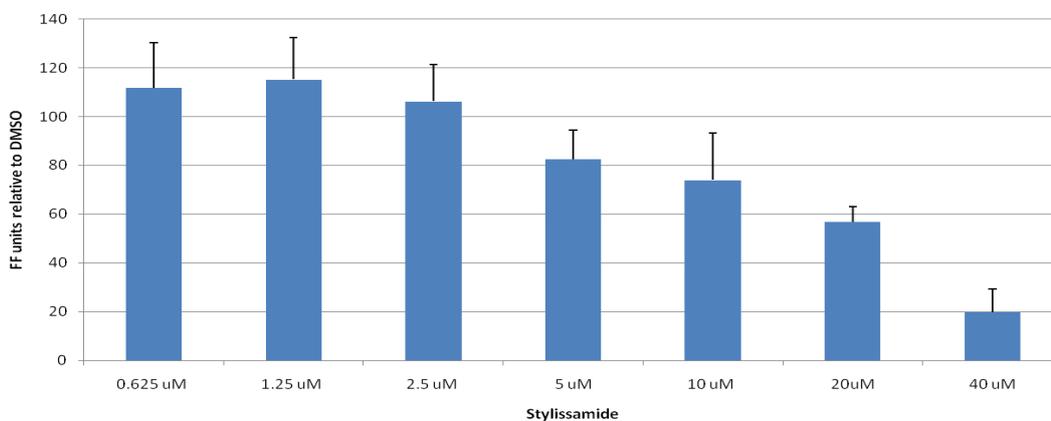
Figure 1.37 : Effect of stylissamide A on translation in yeast extract[55]

The Hamann group studied the mechanism of the translation inhibitory activity by the natural stylissamide A in order to determine which step is inhibited. They started with the initiation step and checked the influence of the inhibitor on the formation of the initiation complex (80S complex). Stylissamide A did not inhibit the translation during the initiation step as the 80S complex is formed (Figure 1.38).
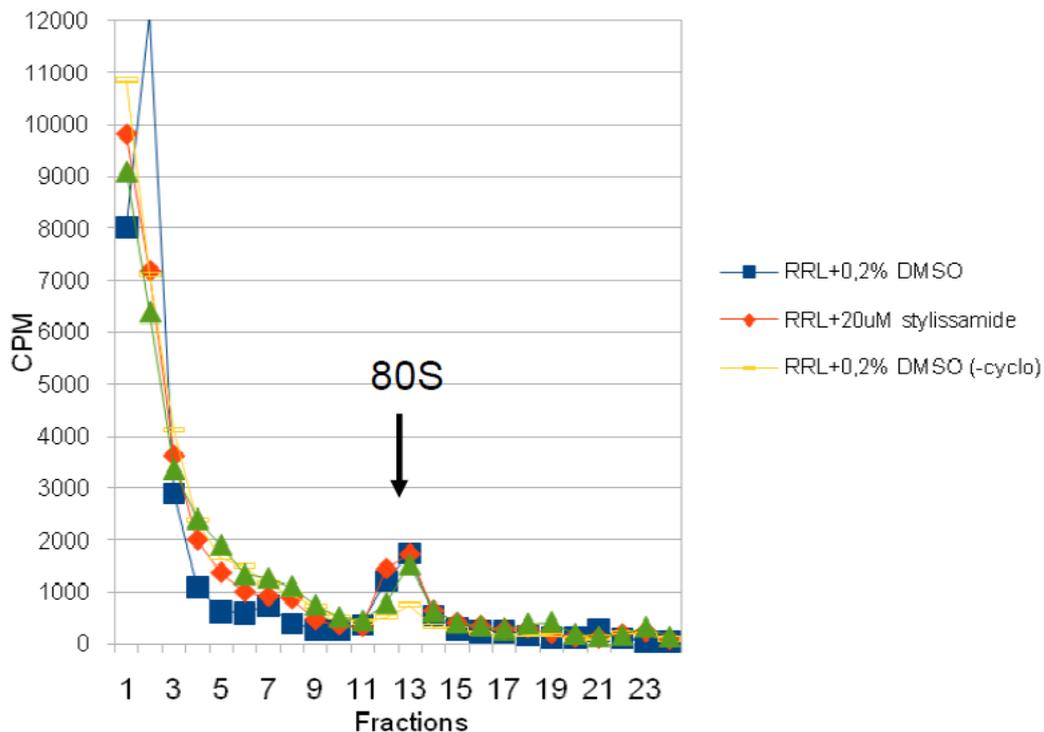
Figure 1.38 : Effect of stylissamide A on the assembly of initiation complex[55]

The natural stylissamide A was shown to block the translation at the elongation step. They added stylissamide A to actively translating ribosomes five minutes after the initiation of translation and also ran the experiments with cycloheximide as positive and with DMSO as negative control. These experiments showed that the translation is immediately stopped by the two compounds: stylissamide A and cycloheximide (Figure 1.39). The latter is known to be a translation inhibitor during the elongation step.
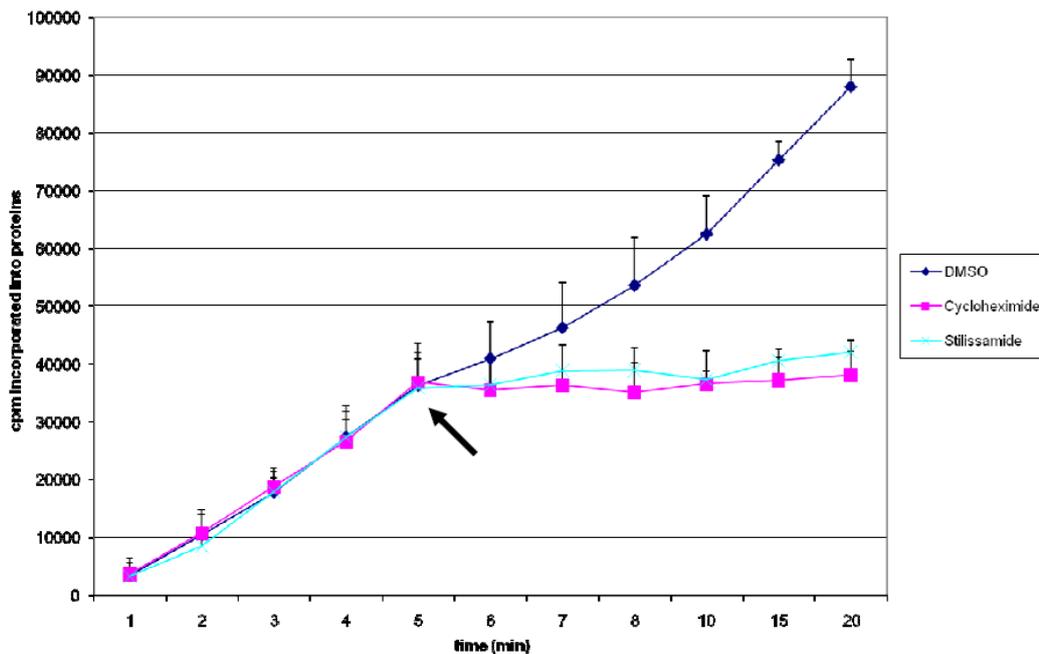
Figure 1.39 : Effect of stylissamide A on actively translating ribosomes[55]

Natural stylissamide A showed very interesting results in *in vitro* studies but very poor results were obtained *in vivo*. They believe that the membrane crossing of this cyclopeptide is responsible for the absence of inhibitory activity towards protein translation *in vivo* as once injected into oocytes stylissamide A was active (Figure 1.40).
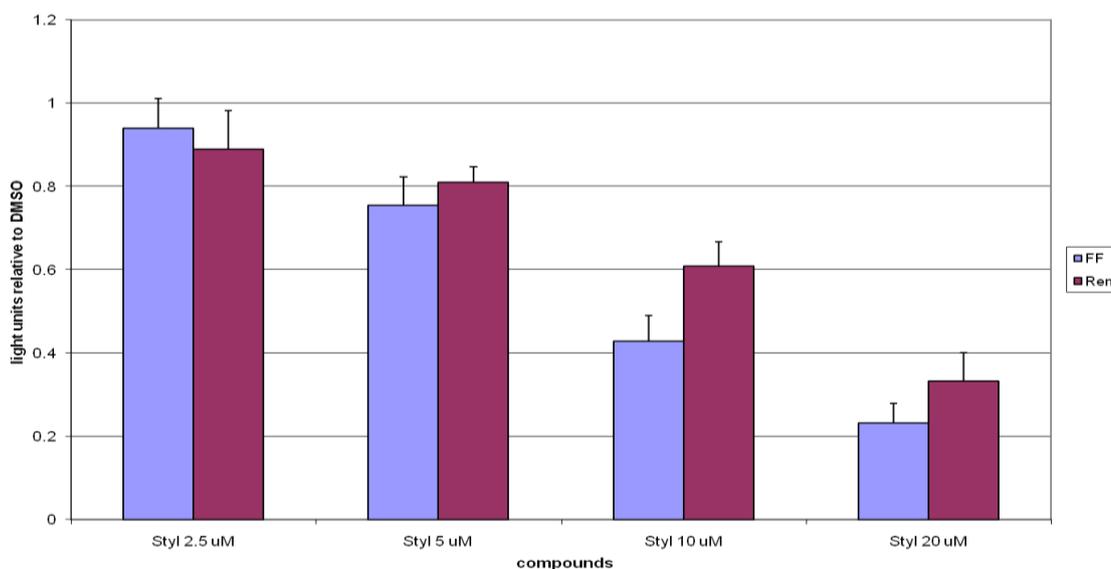
Figure 1.40 : Effect of stylissamide A on the translation after injection into oocytes[55]

61

The synthetic stylissamide A which is identical to the natural product was also tested but no activity in oocytes was observed. They believed that another metabolite is responsible for the cell permeability of stylissamide A and their attempt to identify this compound was unsuccessful. Crude fractions containing the heptapeptide were tested for antimicrobial activity against *Cryptococcus neoformans* (IC_{50} 0.79 to 10.51 µg/mL depending on fractions). The synthetic purified stylissamide was inactive and this supports the hypothesis that a potent metabolite is acting with stylissamide and helping its cell entrance. It is nevertheless possible that tertiary structural differences between the synthetic and natural product are responsible of this biological activity. Discovering the minor metabolite or other factor responsible for this biological activity is the focus of ongoing research in the Hamann group.

1.9. CONCLUSION

Syntheses of sanguinamides gave different conformers to that of the natural products. Synthesis of sanguinamide A led to the formation of two conformers: the *trans, trans* and *trans, cis* that differed from the natural product which displayed a *cis, cis* geometry. We noticed that the position of the macrolactamisation led to the formation of different conformers. We checked that no epimerisation occurred at the α-stereocentre next to the thiazoline ring. We also attempted to follow the likely biosynthesis of sanguinamide A in order to obtain the same geometry as in the natural product but with no success. Synthetic and natural sanguinamide B presented also opposite geometry in their proline peptide bonds. The synthetic *trans, trans*-sanguinamide B was obtained whereas the natural product has both *cis* proline peptide linkage. The yields for the macrolactamisation are low due to the PyBOP impurity which is difficult to separate from the product. The synthetic conformers of sanguinamides along with the [D-Ile]-sanguinamide A and the Cys(*t*Bu) analogue of sanguinamide A were tested in different biological assays. [D-Ile]-Sanguinamide A, Cys(*t*Bu) analogue of sanguinamide A and the sanguinamide B showed antibacterial activity. The rotamer *trans, trans*-sanguinamide A showed mild tyrosine kinase inhibitory activity.

Our results show that NOE experiments are the most reliable in assigning the proline rotamer conformation. Rotamer assignment based on ^{13}C NMR chemical shift of proline β,γ CH$_2$ carbons is not enough as several examples show smaller values for the *cis* peptide bond instead of *trans*.

Attempts to interconvert the synthetic conformers to the natural conformers were performed with no success. Biosynthesis or prolonged storage may well produce a different rotamer which is then apparently locked.

The natural and synthetic stylissamide A displayed the same structural characteristics but different translation inhibitory activity.

Cyclic peptides are important biologically active natural products. In this first part, we have seen some proline-rich cyclopeptides with various biological activities. In the next part, we will see the synthesis of a different type of cyclic peptide: cyclic depsipeptide natural products. They contain one ester bond and a specific biological activity.

2. HISTONE DEACETYLASE INHIBITORS

2.1. INTRODUCTION

2.1.1. Post translational modifications

Within eukaryotic cells, DNA is condensed into a highly compact structure known as chromatin, which is a stainable material of interphase nuclei consisting of nucleic acids and associated histone proteins packed into nucleosomes. The fundamental repeating unit of chromatin is the nucleosome which is composed of 145-147 DNA base pairs. The DNA double helix is wrapped twice around a central core of eight histone protein molecules. The core consists of two of each of the four histone protein subunits: H2A, H2B, H3 and H4 as represented in figure 2.1. The linker histone H1 allows the condensation of this fundamental unit into structures of higher order.[56]
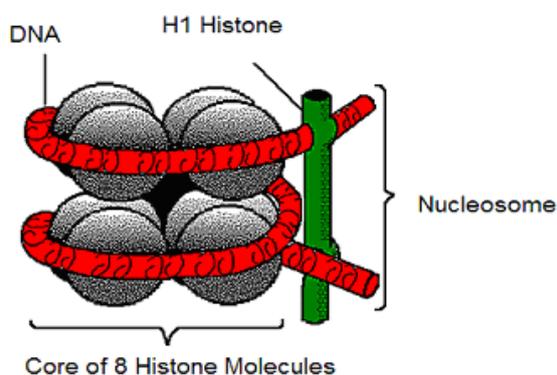
Figure 2.1 : Simplified representation of the nucleosome "Reprinted with permission from Access excellence the national health museum."

The basic *N*-terminal tails of these histones are exposed to different types of covalent modifications such as: acetylation occurring on lysine residues; ubiquitination and sumoylation on lysine residues; methylation on lysine or arginine residues and phosphorylation on serine residues.[57] These post translational modifications are a major mechanism of regulation in gene expression, DNA replication and DNA damage repair. Such modifications also affect histone interactions with DNA and other proteins and are categorised as epigenetic marks which also include DNA methylation as well as histone

modifications. The term epigenetic describes the heritable changes that affect gene expression without modifying the DNA sequence.[58] The accessibility of the DNA to the transcription machinery is determined by the state of modification of the *N*-terminal tails. Alterations of histone modifications lead to errors in gene transcription or repression. Since most human diseases, including cancer, involve mis-timed transcription this suggests that the modulation of epigenetic events will be an attractive therapeutic strategy.

2.1.2. Histone deacetylases

Histone acetylation is one of the best understood histone modifications. It is a reversible modification: histone acetyltransferases (HATs) add acetyl groups to lysine residues whereas histone deacetylases (HDACs) catalyse the removal of acetyl groups from acetylated lysine residues. The positively charged lysine residues interact with the negatively charged DNA; this strong interaction results in a closed chromatin structure which prevents transcription from occurring.[58] The acetylation of lysine by HATs converts the charged lysine to a neutral acetamide, promoting a more relaxed chromatin structure which permits transcriptional activation.[58] The balance between acetylation and deacetylation of histone residues, mediated by HATs and HDACs, is generally deregulated in cancer diseases.
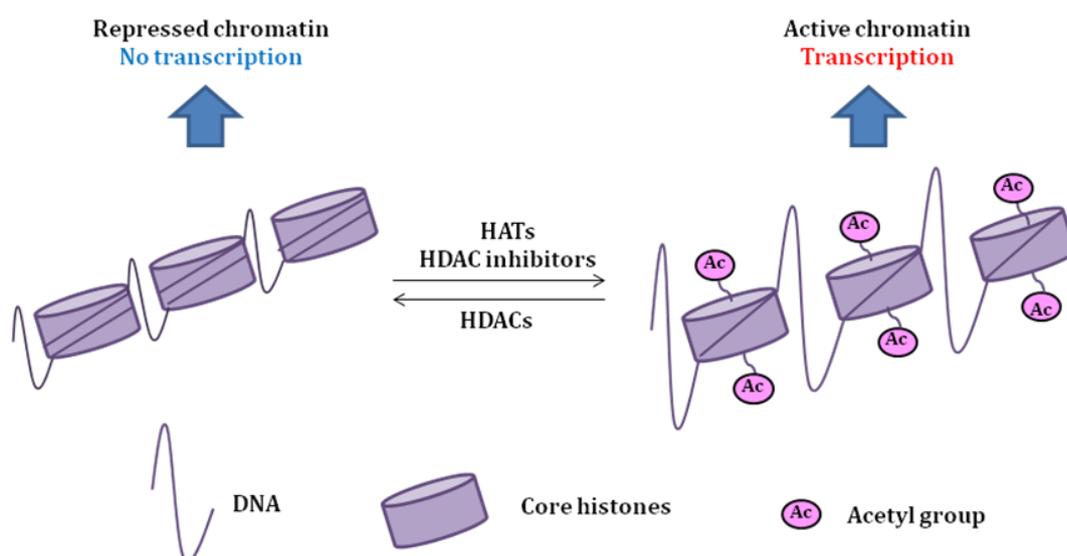
Figure 2.2 : Regulation of the transcription by acetylation/deacetylation

To date, 18 HDACs have been identified in the human genome and are grouped into four classes based on their homology to yeast histone deacetylases. Class I HDACs, located in the nucleus, are homologous to yeast Rpd3 and comprises of HDAC1, -2, -3 and -8.[59] The catalytic cycle of class II members share high similarity with that of yeast Hda1 and comprises of HDAC4, -5, -6, -7, -9 and -10; these HDACs shuttle between the nucleus and cytoplasm.[59] Class III is formed of seven sirtuins (SIRT1-7).[60,61] Class IV only contains HDAC11 which possesses a catalytic cycle which bears similarities to both Rpd3 and Hda1. Classes I, II and IV are zinc-dependent enzymes whereas class III enzymes are metal-free and require nicotinamide adenine dinucleotide (NAD^+) as a cofactor. As class III HDACs are not relevant to this work they will not be discussed further.

As deregulation of histone modifications is implicated in many diseases, including cancer, the inhibition of enzymes which are able to catalyse the addition and removal of these epigenetic marks have therapeutic potential. Class I and II HDACs are mainly associated with haematological and solid cancers.[62] In addition HDACs are usually over-expressed in multiple cancers: for example, HDAC1 is over-expressed in colon, prostate, gastric and breast cancers; high levels of HDAC2 are observed in colorectal, cervical and gastric cancers and over-expressed HDAC3 and -6 are detected in colon and breast cancers.[63]

Over-expression of HDACs can also be indicative of the outcome of the disease.[62] High levels of class I HDACs in colorectal cancer (HDAC1, -2 and -3) are associated with reduced survival rates whilst over-expression of HDAC6 in breast cancer is associated with improved survival rates.

HDAC inhibitors are also able to regulate p21, which is a cyclin-dependent kinase (CDK) inhibitor responsible for the inhibition of cell-cycle progression.[62,64] Its expression is generally inactivated in tumour cells because of the deacetylation of the p21 promoter.[62] The up-regulation of p21 by HDAC led to tumour growth inhibition by apoptosis and cell cycle arrest.[63,64]

In cancer, HDACs have an abnormal activity that leads to hyperacetylation and aberrant silencing of genes.[65] The inhibition mechanism of HDAC inhibitors has different effects, including differentiation and/or apoptosis of transformed cells. Due to their pharmacological activity HDAC inhibitors have the potential to treat cancer, as they are able to reactivate gene expression and induce cancer cell death.

2.1.3. Histone deacetylase inhibitors

A variety of inhibitors of zinc-dependent HDACs have been discovered so far and they can be divided into different classes including hydroxamates, aliphatic acids, benzamides and cyclic peptides.[66]

2.1.3.1. Acyclic inhibitors

A) Hydroxamates

Hydroxamate HDAC inhibitors include suberoylanilide hydroxamic acid (SAHA, Zolinza or vorinostat, **2.1**) and trichostatin A (TSA, **2.2**). TSA was the first natural hydroxamate discovered to be an HDAC inhibitor.[67] SAHA, a synthetic compound, was the first FDA approved HDAC inhibitor for the treatment of cutaneous T-cell lymphoma.[68] Both TSA and SAHA are nanomolar inhibitors and non selective as they target all zinc-dependent HDACs.

Figure 2.3 : Structures of SAHA (2.1) and TSA (2.2)

The first X-ray structure of a bacterial histone deacetylase-like protein (HDLP), which shares 35% sequence homology with human HDAC1, was solved by Finnin and co-workers.[69] A solved X-ray structure of co-crystals of this HDAC-like protein and TSA has shown that the *A. aeolicus* HDLP active site contains a deep narrow pocket where TSA is bound (Figure 2.4).

Figure 2.4 : X-ray structure of TSA in the active site of HDLP[69] "Reprinted by permission from Macmillan Publishers Ltd: [Nature] (Finnin, M. S. *et al., 401*, 1999, 188-193), copyright (1999)."

A mechanism for the deacetylation of acetylated lysine by HDAC1 has been proposed based on the X-ray structure (Scheme 2.1).[66,69,70] The zinc atom coordinates to two asparagine residues, one histidine residue and a molecule of water. The latter, chelated by a histidine residue, attacks the carbonyl group of the acetylated lysine residue to form an intermediate where the zinc coordinates to two asparagine residues, one histidine residue and two oxygens. The tetrahedral intermediate degrades to a molecule of acetic acid and the deacetylated lysine residue which had abstracted a proton from His132.
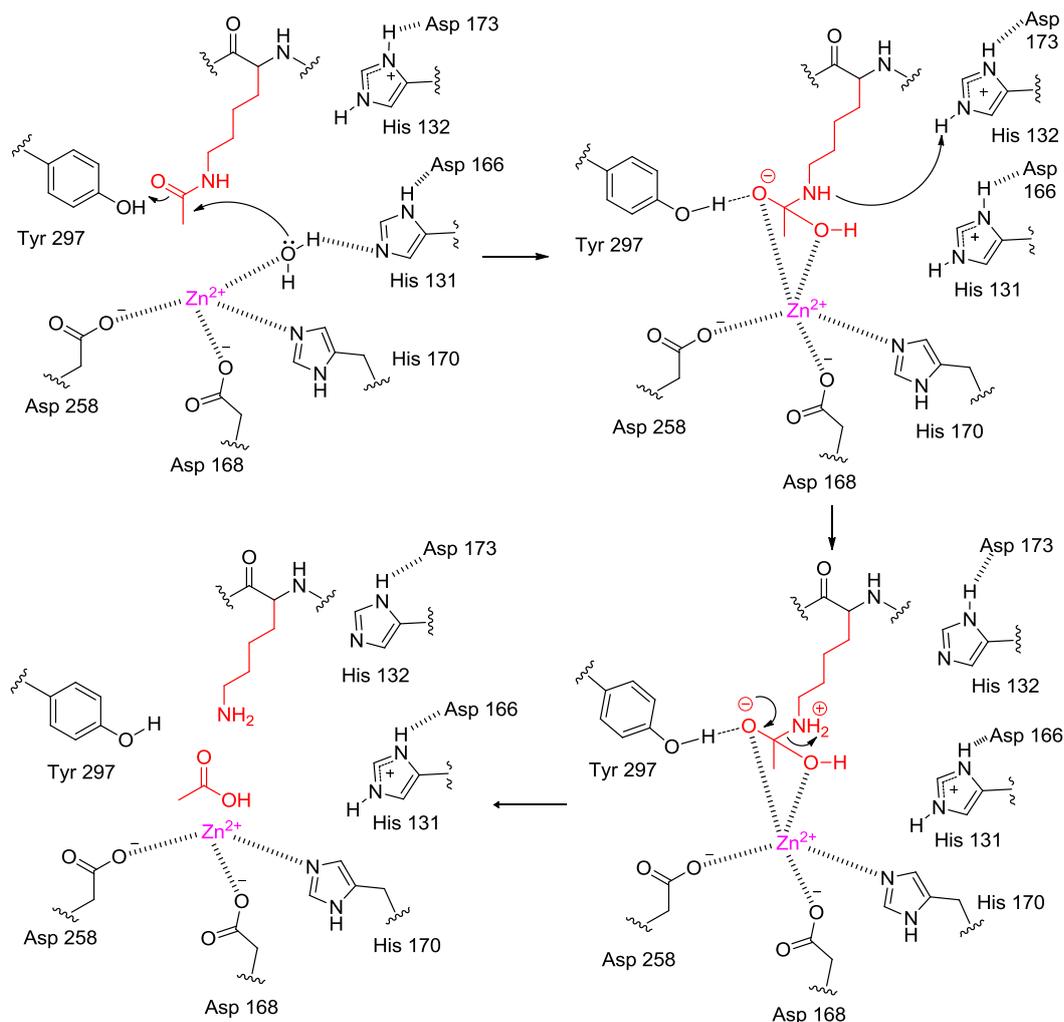
Scheme 2.1 : Proposed catalytic mechanism of lysine deacetylation[69]

The classic pharmacophore for HDAC inhibitors is deduced from the transition state existing between the acetylated lysine residue and an HDAC. An HDAC inhibitor consists of three distinct domains: a metal binding domain which chelates the zinc; a linker which occupies the channel and a hydrophobic cap which interacts with residues present on the rim of the active site. The HDAC inhibitor binds directly and competitively to the HDAC active site blocking the substrate access.

In hydroxamates, the hydroxamic acid moiety chelates the zinc in a bidentate manner through its carbonyl and hydroxyl functions.

B) Aliphatics

Some short chain aliphatic carboxylic acids are able to inhibit class I and II HDACs. For example, valproic acid (**2.3**), previously used as an anticonvulsant drug, is in clinical trials for cancer as a HDAC inhibitor and additionally phenylbutyrate and its derivatives also exhibit activity (Figure 2.5). Nevertheless, these compounds are relatively weak inhibitors and not very selective.
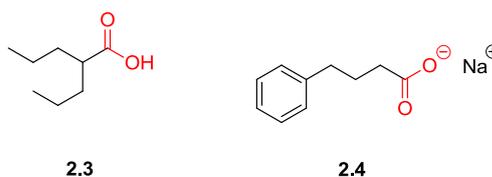
Figure 2.5 : Structures of valproic acid (2.3) and sodium phenylbutyrate (2.4)

C) Benzamides

Benzamides are sub-micromolar HDAC inhibitors in which the benzamide function is the zinc-binding group. Entinostat (MS-275, SNDX-275, figure 2.6) is a synthetic benzamide with micromolar inhibition activity for class I HDAC1, 2 and 3 and is undergoing clinical trials for various cancers.[63]
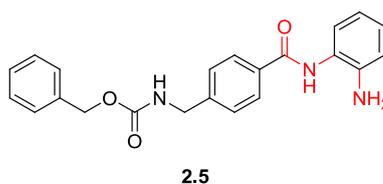
Figure 2.6 : Structure of Entinostat (2.5)

2.1.3.2. Cyclic peptides

A number of cyclic peptides are known to be HDAC inhibitors; they all contain a macrocycle and a side chain arm containing a zinc-binding warhead.

A) Cyclic peptides containing an epoxyketone

Cyclic peptides containing the (*S*)-2-amino-9,10-epoxy-8-oxodecanoic acid moiety (L-Aoe moiety) are known to be HDAC inhibitors.[71] The zinc binding site in these cyclic peptides is the α-epoxy ketone which coordinates irreversibly to the zinc ion. The following figure represents a selection of HDAC inhibitors with an L-Aoe side chain arm: HC-toxin, trapoxin B and chlamydocin.

2.6

2.7

2.8
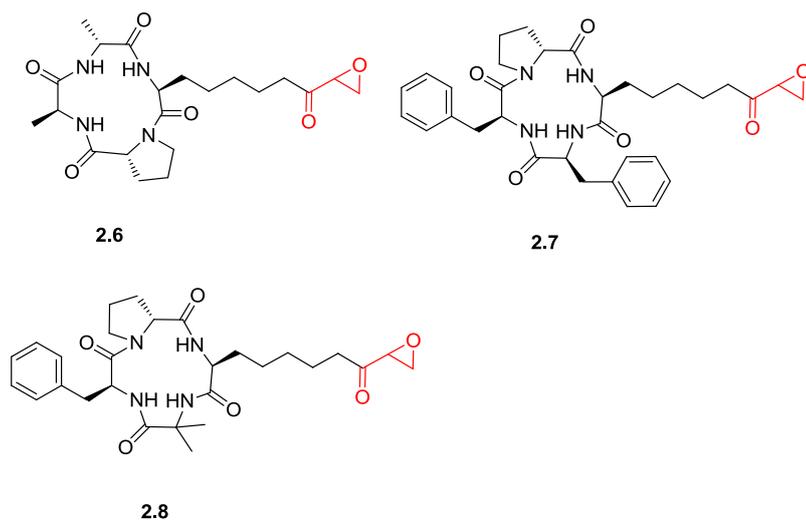
Figure 2.7: Structures of HC-toxin (2.6), trapoxin B (2.7) and chlamydocin (2.8)

B) Azumamides

Azumamides A-E, isolated from the marine sponge *Mycale izuensis,* are cyclic tetrapeptides with three D-amino acid residues and one β-amino acid (Figure 2.8).[72]
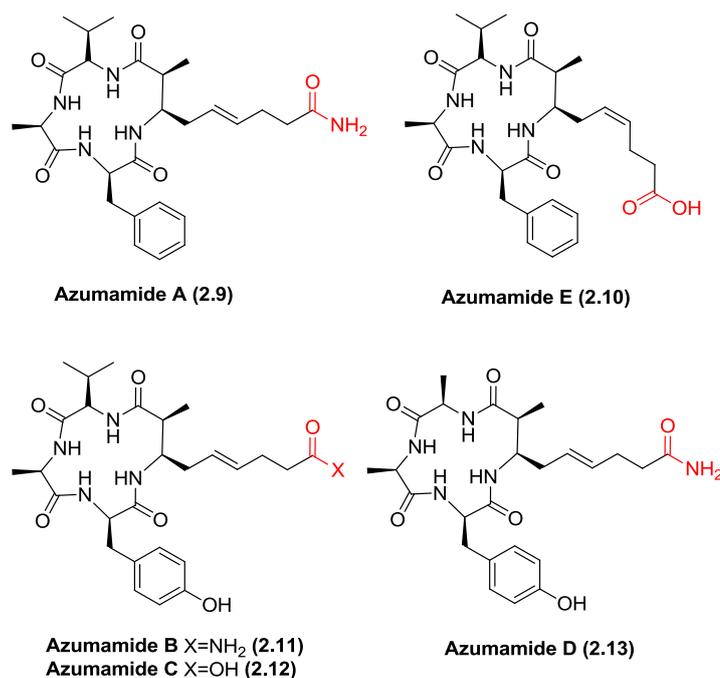
Figure 2.8 : Structures of azumamides A-E

Azumamides A, B and D possess a carboxamide warhead whereas azumamides C and E contain a carboxylic acid warhead. As expected azumamide E, bearing the carboxylic acid function as the zinc-binding group, displays a better inhibition activity (IC_{50} 1.22 µM against HDAC1) than azumamide A with the carboxamide ($IC_{50} > 50$ µM against HDAC1).[73] Azumamide E is much more potent than the simple aliphatic acids, such as valproic acid, indicating that interactions of the macrocycle with the rim of the enzyme contribute to HDAC binding. Nevertheless, the carboxylic acid or carboxamide present in the natural products are weaker zinc-binding groups when compared to hydroxamic acids. The Ganesan group prepared the hydroxamic acid derivative of azumamide E and showed that it is significantly more potent than both natural products azumamides A or E (IC_{50} 7 nM total HDACs versus 5800 nM for azumamide A and 110 nM for azumamide E).[73]

C) Cyclic depsipetides

a) FK228, FR901375, spiruchostatins and largazole

FK228, FR901375, spiruchostatins and largazole are natural product cyclic depsipetides known to be HDAC inhibitors (Figure 2.9). A depsipeptide is a peptide where at least

one internal, secondary amide has been replaced by an ester. These cyclic depsipetides all contain only one ester bond in their macrocycle and the β-hydroxy acid, (3*S*, 4*E*)-3-hydroxy-7-mercaptohept-4-enoic acid. FK228, FR901375 and spiruchostatins are bicyclic depsipeptide molecules which contain a disulfide bridge which is not present in largazole. All the members of this depsipeptide family have been isolated from extracts of bacterial fermentation. There are however several differences between FK228, FR901375 and spiruchostatins within their caps.

FK228, also known as romidepsin or FR901228, was isolated in the culture broth of *Chromobacterium violaceum* by Fujisawa Pharmaceutical Company.[74,75] Its structure was elucidated by spectroscopic analysis, chiral chromatography and X-ray crystallographic analysis. FK228 contains the β-hydroxy acid, a D-cysteine, a D-valine and L-valine residues and a (*Z*)-dehydrobutyrine residue which is a potential site for covalent modifications by Michael addition.

FR901375 was discovered in the fermentation broth of *Pseudomonas chloroaphis* and also in *Chromobacterium violaceum* by Fujisawa Pharmaceutical Company.[76] In addition to the β-hydroxy acid, FR901375 contains a L-threonine, a D-cysteine and two D-valine residues. Despite the similarity to FK228, the HDAC inhibitory activity of FR901375 has not been reported.

Spiruchostatins were isolated from a *Pseudomonas* extract in 2001 by Shin-ya *et al.*[77] They determined the structure using spectroscopic analysis which showed that the spiruchostatins include the β-hydroxy acid, a statine unit and three amino acids (D-alanine, D-cysteine, D-valine/D-*allo*-isoleucine). Spiruchostatins differ by the amino acid next to the statine subunit: spiruchostatin A contains a valine residue whereas spiruchostatin B contains an isoleucine residue. The stereochemistry of the alanine and cysteine residues was determined by chemical degradation. The stereochemistry of the statine and the 3-hydroxy-7-mercaptohept-4-enoic acid has not been assigned in the isolation paper but was elucidated only later by total synthesis of spiruchostatins A and B.[78,79]

Largazole was isolated from the marine cyanobacterium *Symploca* species collected in the Florida Keys.[80] H. Luesch and co-workers elucidated the structure by a combination of chemical degradation, chiral chromatography and spectroscopic analysis. Largazole is a flat 16-membered macrocycle with a side chain arm. It possesses a 4-

methylthiazoline linearly fused to a thiazole in its cyclic core, a L-valine residue and a thioester of the β-hydroxy acid unit.
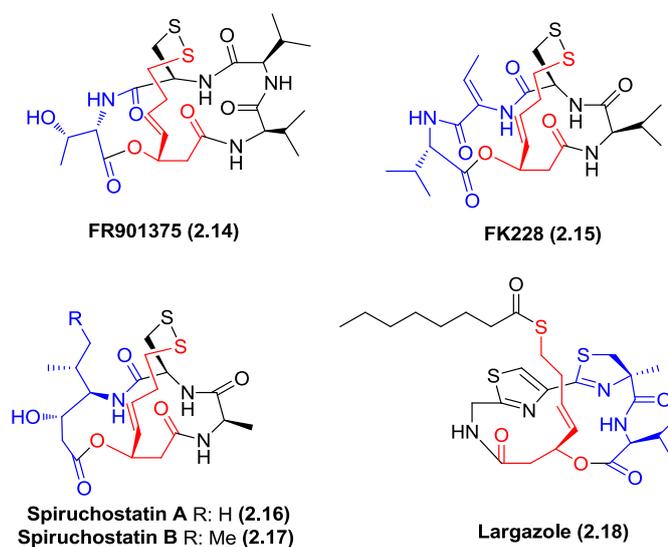
Figure 2.9 : Structures of FR901375 (2.14), FK228 (2.15), spiruchostatins (2.16-2.17) and largazole (2.18)

The complexity of their cap is responsible for their high potency (nanomolar order) and isoform selectivity towards class I HDACs. FK228 was FDA approved in 2009 for the treatment of peripheral and cutaneous T-cell lymphoma (CTCL) and is the most thoroughly studied among the depsipeptides. Cheng and co-workers have managed to identify the gene cluster responsible for the biosynthesis of FK228 (Figure 2.10).[81]
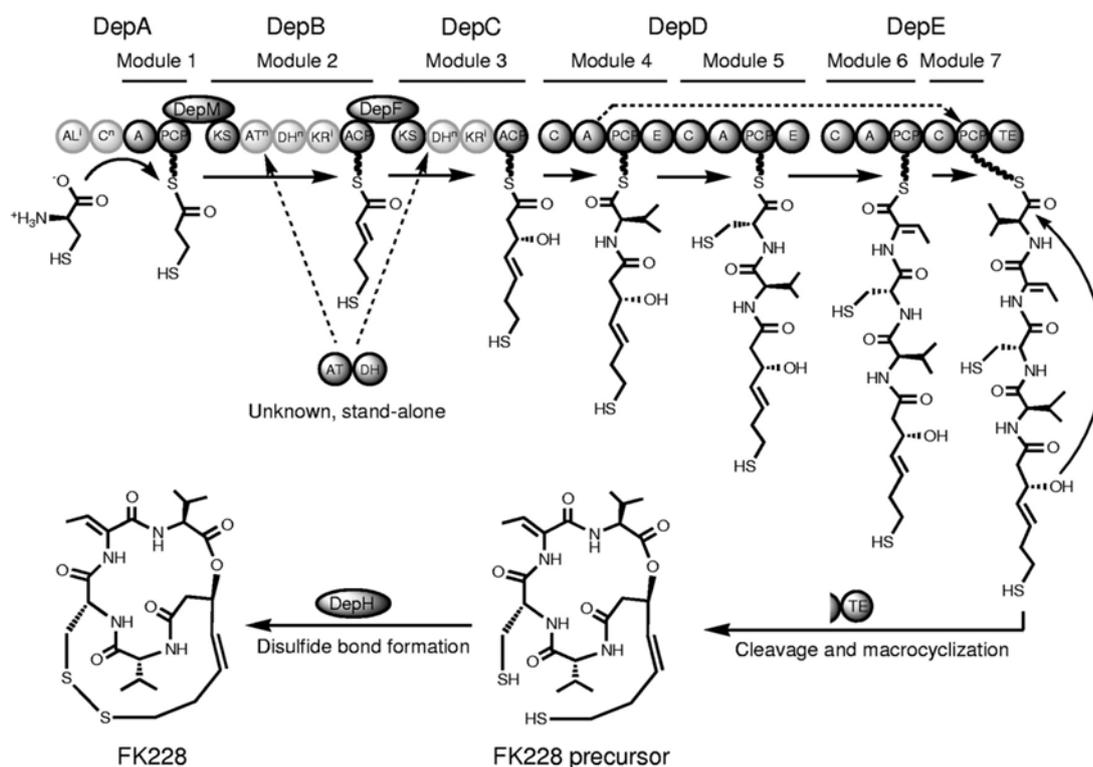
Figure 2.10 : Proposed biosynthesis of FK228[81] "Reprinted from Applied and Environmental Microbiology, *73*, Cheng, Y.-Q.; Matter A. M., Characterization of a Gene Cluster Responsible for the Biosynthesis of Anticancer Agent FK228 in Chromobacterium violaceum No. 968, 3460-3469, Copyright (2007), with permission from American Society for Microbiology."

Spiruchostatins are also very potent HDAC inhibitors (nanomolar).[78,82] Largazole presents potent antiproliferative activity (nanomolar) and an excellent selectivity at targeting cancer cells over non-transformed cells. As reported by Luesch and co-workers, the growth inhibitory activity against the transformed mammary epithelial cells (MDA-MB-231, GI_{50} 7.7 nM) is considerably higher than against non-transformed mammary epithelial cells (NMuMG, 122 nM).[80] This natural product shows strong class I HDAC selectivity.

The warhead in these natural products is the sulfur containing β-hydroxy acid (3*S*, 4*E*)-3-hydroxy-7-mercaptohept-4-enoic acid. FR901375, FK228 and spiruchostatins contain an identical disulfide bridge which is reduced intracellularly to provide the free thiol as shown for FK228 in scheme 2.2.[81] FR901375, FK228 and spiruchostatins are prodrugs and their reduced forms are the active metabolites in which the thiol is able to chelate the active-site Zn^{2+} residue of the HDACs and by doing so inhibit the enzymes. Although the thiols are weaker zinc-binding groups than the hydroxamic acids, the caps of these cyclic depsipeptides are able to interact strongly with the enzyme rim and are

responsible for the high potency of these natural products. Largazole was also believed to be a prodrug as the octanoic thioester would be metabolised *in vivo* into an active metabolite. As predicted, the thioester function is rapidly hydrolysed under physiological conditions and the thiol residue coordinates to Zn^{2+} as shown in the following scheme.
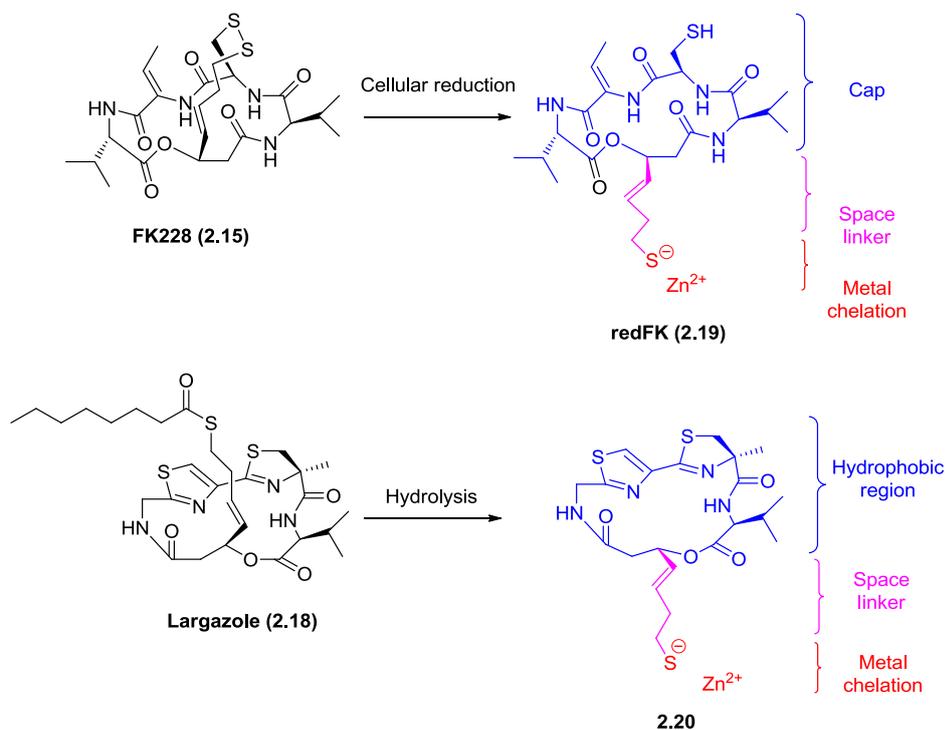
Scheme 2.2 : Cellular metabolism and mechanisms of HDAC inhibition of FK228 (2.15) and largazole (2.18)

b) Thailandepsins

In 2008, Cheng *et al.* initially reported in a patent the following structures of new depsipeptide HDAC inhibitors: the natural products thailandepsins A, B and C (Figure 2.11).[83] They also contain the (3*S*, 4*E*)-3-hydroxy-7-mercaptohept-4-enoic acid moiety and the disulfide bridge present in FK228 and spiruchostatins. Thailandepsins A and B contain a hemiaminal function, whilst thailandepsin C includes an imine. They differ from spiruchostatins by the phenylalanine and imine or hemiaminal function. The structures were deduced by analysis of the biosynthesis gene cluster of thailandepsins.[83]
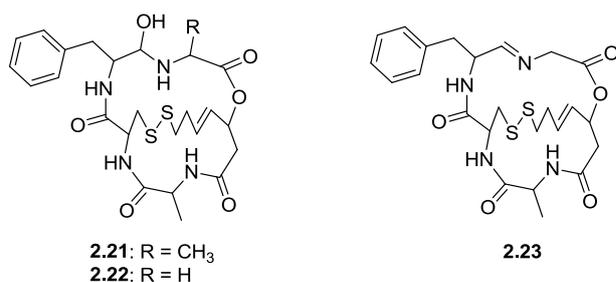
Figure 2.11 : Structures of thailandepsins A (2.21), B (2.22) and C (2.23)

In 2010, Cheng presented revised structures for thailandepsins A and B, which are very similar to spiruchostatins; however their stereochemistry was only predicted and is shown in figure 2.12. The hemiaminal function and imine are replaced by the statine moiety in the revised structures of thailandepsins. Thailandepsin A contains a methionine residue, whereas thailandepsin B has a norleucine residue.
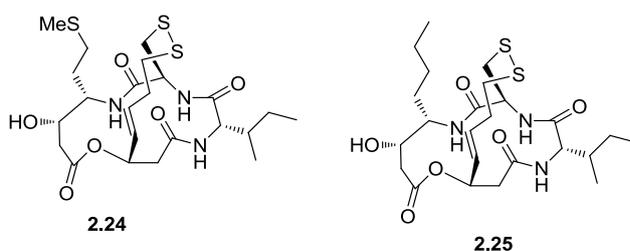
Figure 2.12 : Structures of thailandepsins A (2.24) and B (2.25)

c) Burkholdacs

Burkholdacs A and B were isolated by Brady and co-workers from *Burkholderia thailandensis* E264 in 2011.[84] Their structures were elucidated by spectroscopic analysis (HRMS and NMR) and confirmed by the biosynthetic gene cluster. Burkholdacs A and B are also bicyclic depsipetides containing a statine unit like the spiruchostatins, from which they only differ by the replacement of the valine residue with a methionine residue.
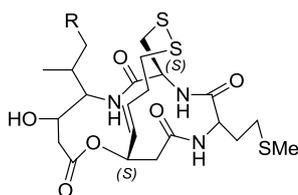
2.26 R:H Burkholdac A
2.27 R:Me Burkholdac B

Figure 2.13 : Structures of Burkholdacs

We believed that burkholdacs and spiruchostatins are the same molecules as they were isolated from the same bacterial strain.

The stereochemistry of these natural products has not been definitively assigned, only predicted from the gene cluster and biosynthesis of burkholdacs (Figure 2.14). Only one epimerase domain is present in the gene cluster in the cysteine incorporating module. According to this result, the stereochemistry was predicted by Brady to be the following L-Val or L-Ile, D-Cys and L-Met (Figure 2.15).
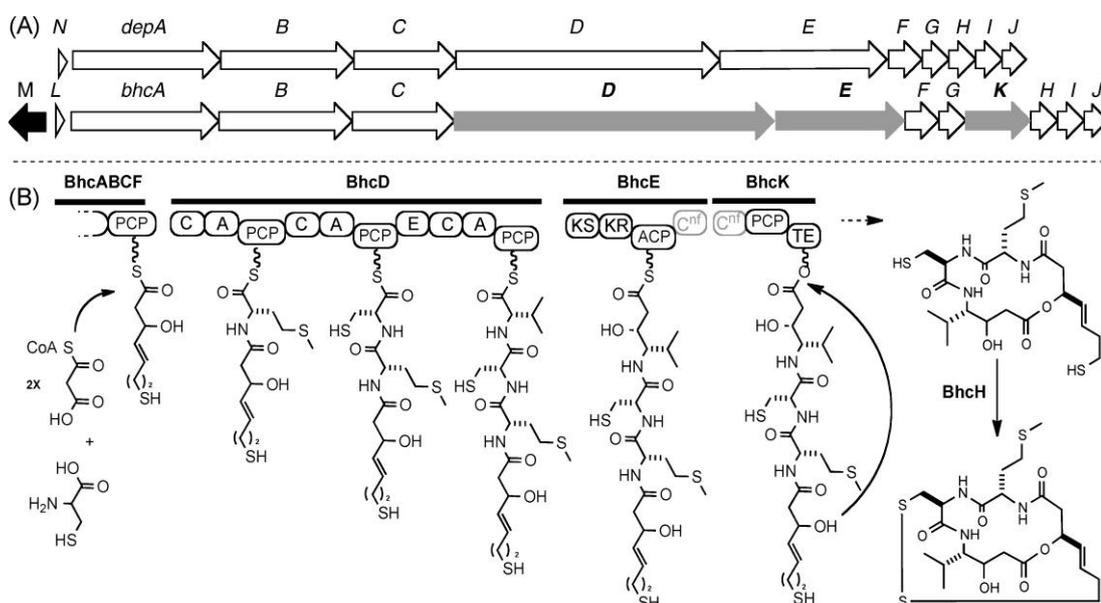
Figure 2.14 : Gene cluster and proposed biosynthesis of burkholdacs[84] "Reprinted with permission from Biggins, J. B.; Gleber, C. D.; Brady, S. F. *Org. Lett.* **2011**, *13*, 1536-1539, copyright (2011) American Chemical Society."
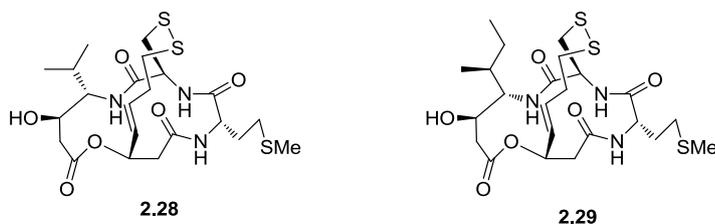
Figure 2.15 : Predicted stereochemistry of burkholdacs A (2.28) and B (2.29)

d) New thailandepsins structures

Finally, very recently during the writing of this thesis, Cheng published new structures for the thailandepsins isolated from the same bacterial strain: *Burkholderia thailandensis* E264 (Figure 2.16).[85] Thailandepsin A is identical to burkholdac B but thailandepsin B is different to burkholdac A. Instead of the methionine residue, thailandepsin B possesses a norleucine residue, NMR and MS have confirmed their structures. The stereochemistry is the same as in spiruchostatins.
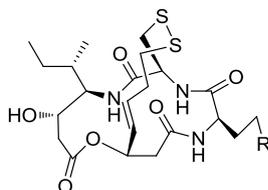
2.30 R:SMe Thailandepsin A = Burkholdac A
2.31 R:Et Thailandepsin B

Figure 2.16 : Final structures of thailandepsins A (2.30) and B (2.31)

2.1.4. The syntheses of depsipeptides

Whilst no syntheses have been reported for thailandepsins or burkholdacs, several syntheses are described in the literature for the other depsipeptides.[42,78,79,82,86-92]

2.1.4.1. Preparation of the β-hydroxy acid moiety

Different strategies were employed to prepare the (3*S*, 4*E*)-3-hydroxy-7-mercaptohept-4-enoic acid moiety **2.32** (Figure 2.17).
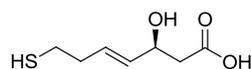
2.32

Figure 2.17 : Structure of the β-hydroxy acid 2.32

In the first synthesis of FK228, Simon prepared the β-hydroxy acid in five steps and employed the Carreira catalytic asymmetric aldol reaction (Scheme 2.3).[86] They required the enantiomer of the β-hydroxy acid fragment as a Mitsunobu reaction is later employed for their macrolactonisation. Cesium triphenylmethylthiolate anion was added to methyl 2,4-pentadienoate **2.33** by conjugate addition to afford the α,β-unsaturated methyl ester. Reduction with DIBAL followed by Swern oxidation afforded the α,β-unsaturated aldehyde **2.34** which then underwent an aldol reaction using a chiral Ti-catalyst prepared in situ from Ti(O-iPr)$_4$ and ligands di-*tert*-butyl salicylaldehyde and the Schiff base derived from (*R*)-(+)-binaphthyl amino alcohol. The aldol adduct was obtained in 98% yield and 99% enantiomeric excess. The benzyl ester of **2.35** was hydrolysed by LiOH in a mixture of methanol/water to afford the β-hydroxy acid. However several groups failed to reproduce this Carreira catalytic asymmetric aldol reaction.[78,88]
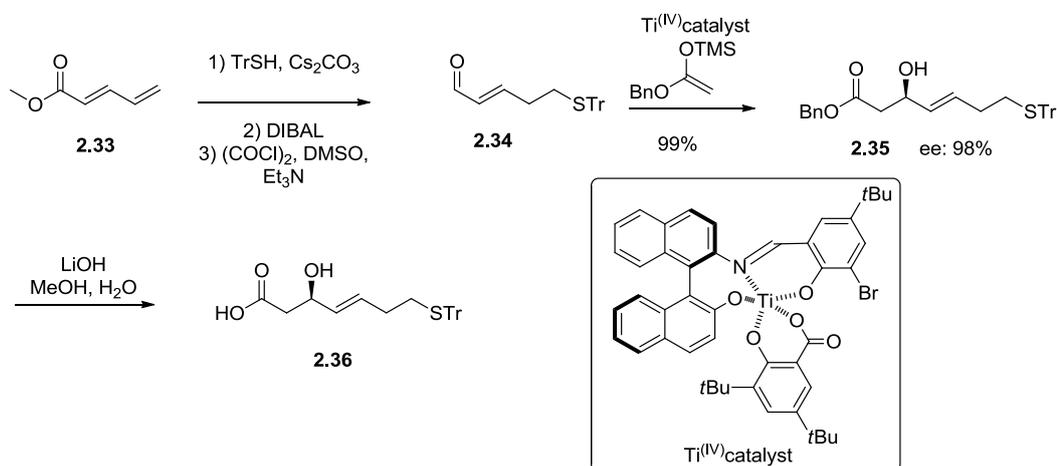
Scheme 2.3 : Simon's synthesis of the β-hydroxy acid 2.36 with the (*R*) stereochemistry via Carreira catalytic asymmetric aldol reaction

For the preparation of FR901375, Wentworth and Janda also required the enantiomer of the β-hydroxy acid as the Mitsunobu strategy was chosen for their macrolactonisation.[88] They prepared the α,β-unsaturated aldehyde **2.38** in 2 steps by 1,4-addition of trityl

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mercaptan to acrolein, followed by Wittig reaction using the commercially available (triphenylphosphoranylidene)-acetaldehyde in benzene (Scheme 2.4).
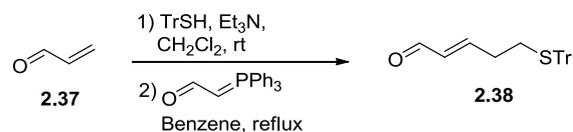
Scheme 2.4 : Wentworth and Janda's synthesis of *α,β*-unsaturated aldehyde 2.38

They attempted the aldol reaction using oxazolidinethione auxiliary reported by Phillips but the obtained yield was low (35%).[93]
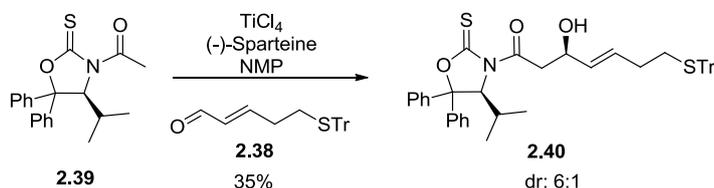
Scheme 2.5 : Attempt of aldol reaction using oxazolidinethione auxiliary

It is known that acetate aldol reactions have poor diastereoselectivity when using the classical Evans' oxazolidin-2-one auxiliary; an alternative approach is to use haloacetyloxazolidinones.[94] Wentworth and Janda decided to use a chloroacetyloxazolidinone auxiliary to perform the Evans' aldol reaction which would be followed by dechlorination (Scheme 2.6).[88] The aldol reaction of the dibutylboron enolate derivative of chloroacetyloxazolidinone **2.41** with the aldehyde **2.38** produced compound **2.42** in good yield (69%) and high diastereoselectivity (>90%). Dechlorination, performed by treatment with aluminium amalgam, followed by hydrolysis, using LiOH and H_2O_2 in THF, afforded the β-hydroxy acid. The latter was obtained in a total of five steps.
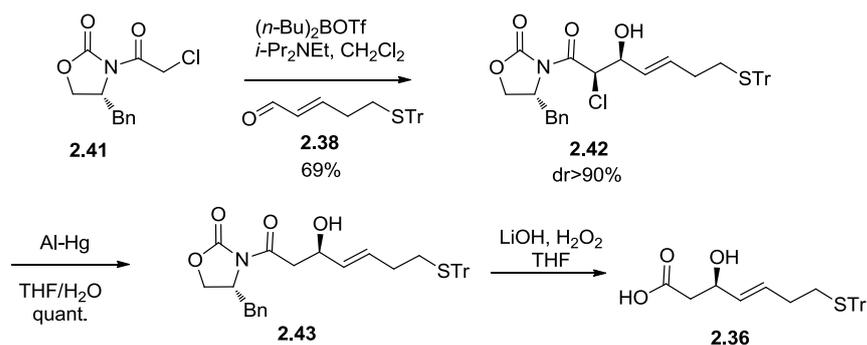
Scheme 2.6 : Wentworth and Janda's synthesis of the β-hydroxy acid 2.36 with the (*R*) stereochemistry

Doi and Takahashi studied the stereoselectivity of different enolates with Seebach's *N*-acetyl-oxazolidin-2-one auxiliary (Scheme 2.7).[90] They looked at the lithium enolate, the titanium enolate obtained by addition of TiCl(O*i*-Pr)$_3$ and the zirconium enolate obtained with Cp$_2$ZrCl$_2$.[90] The zirconium enolate obtained by transmetallation gave the best results whereas the lithium and titanium enolates only gave moderate diastereoselectivity. They also used Seebach's bromoacetyloxazolidinone auxiliary, followed by a samarium-Reformatsky reaction to compare the influence of the bromo group on the diastereoselectivity. Better results were obtained using Seebach's *N*-acetyl-oxazolidin-2-one auxiliary with Cp$_2$ZrCl$_2$ as additive.[90]

no additive	yield :89% and dr: 77%
with TiCl(O*i*-Pr)$_3$	yield: 94% and dr: 76%
with Cp$_2$ZrCl$_2$	yield :55% and dr: 93%
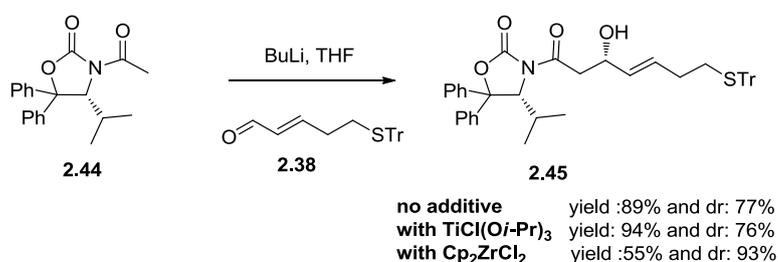
Scheme 2.7 : Stereoselectivity of different enolates with 2.38

To prepare the β-hydroxy acid for their synthesis of spiruchostatin A, Ganesan and co-workers used an aldol reaction between aldehyde **2.38** and Nagao's chiral *N*-acetylthiazolidine-2 thione **2.46** under Vilarrasa's conditions (Scheme 2.8).[78] The aldol reaction gave a good yield (84%) and a high diastereoselectivity (95%). Aldehyde **2.38** was prepared in two steps from acrolein as described previously by Wentworth and Janda.[88] The reaction sequence is scalable and the β-hydroxy acid is obtained in only three steps as they directly used compound **2.47** in their synthesis of spiruchostatin A.

This method was employed by several other groups for the synthesis of the β-hydroxy acid fragment.[95-98]
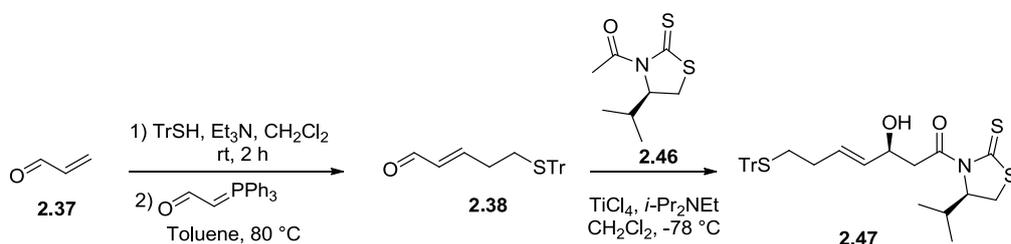
Scheme 2.8 : Ganesan's synthesis of the β-hydroxy acid derivative 2.47

Williams' preparation of the β-hydroxy acid involved a different approach using an enantioselective synthesis.[87] The key step is a Noyori asymmetric hydrogen transfer reaction performed on compound **2.50**, which was obtained in two steps from methyl 3,3-dimethoxypropionate **2.48** via formation of Weinreb amide **2.49** (Scheme 2.9).[99] Noyori's asymmetric transfer hydrogenation using **2.51** as a catalyst gave a good yield (71%) and excellent enantioselectivity (99%). The alkyne was reduced by Red-Al to the (*E*)-alkene **2.52** prior to conversion of the silyl ether to the tosylate. Hydrolysis of the acetal to the aldehyde followed by oxidation using the Pinnick conditions afforded the carboxylic acid **2.36**. The tosylate was displaced by trityl mercaptan to afford the β-hydroxy acid in an overall yield of 13%. This method is slightly longer than the others (nine steps) but it is scalable.
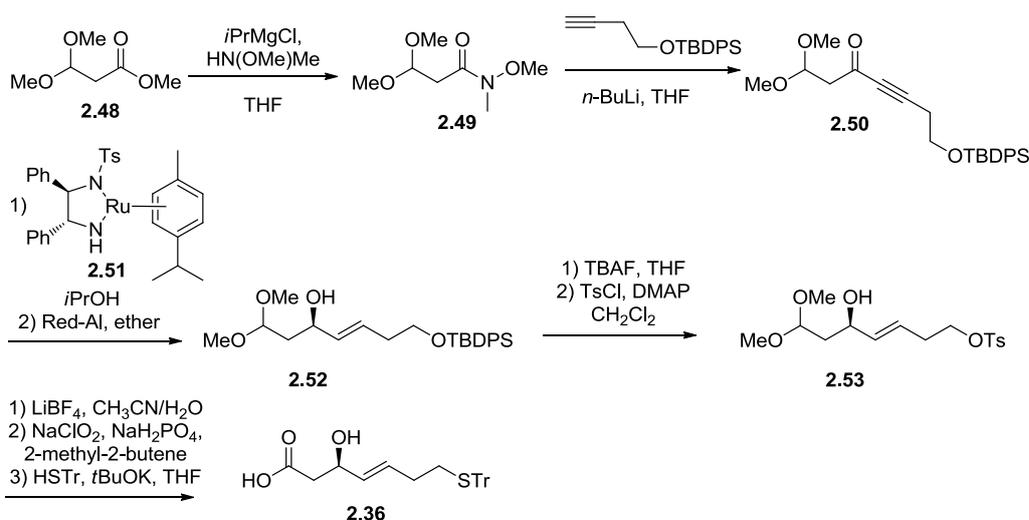
Scheme 2.9 : Williams' synthesis of the β-hydroxy acid 2.36 with the (*R*) stereochemistry via Noyori asymmetric hydrogen transfer reaction

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Katoh and co-workers used a Julia-Kocienski olefination of sulfone **2.56** with aldehyde **2.57** prepared from maleic acid (Scheme 2.10).[79,82] The sulfone **2.56** was obtained from mono-protected 1,3-propanediol: a Mitsunobu reaction to introduce the *S*-tetrazole was followed by molybdenum-mediated oxidation, deprotection of the PMB group and finally another Mitsunobu reaction to insert the *S*-trityl group. The Julia-Kocienski olefination of sulfone **2.56** with aldehyde **2.57** led to the inseparable mixture of *E/Z*-stereoisomers **2.59** and **2.60** (5:1) in good yield (66%). The mixture was treated by DIBAL to regioselectively open the acetal to form the separable *E* and *Z* isomers. The primary alcohol group was converted into the carboxylic acid in two steps. The PMB protected *seco*-acid was obtained in eight steps and used as such in their synthesis of spiruchostatin B.
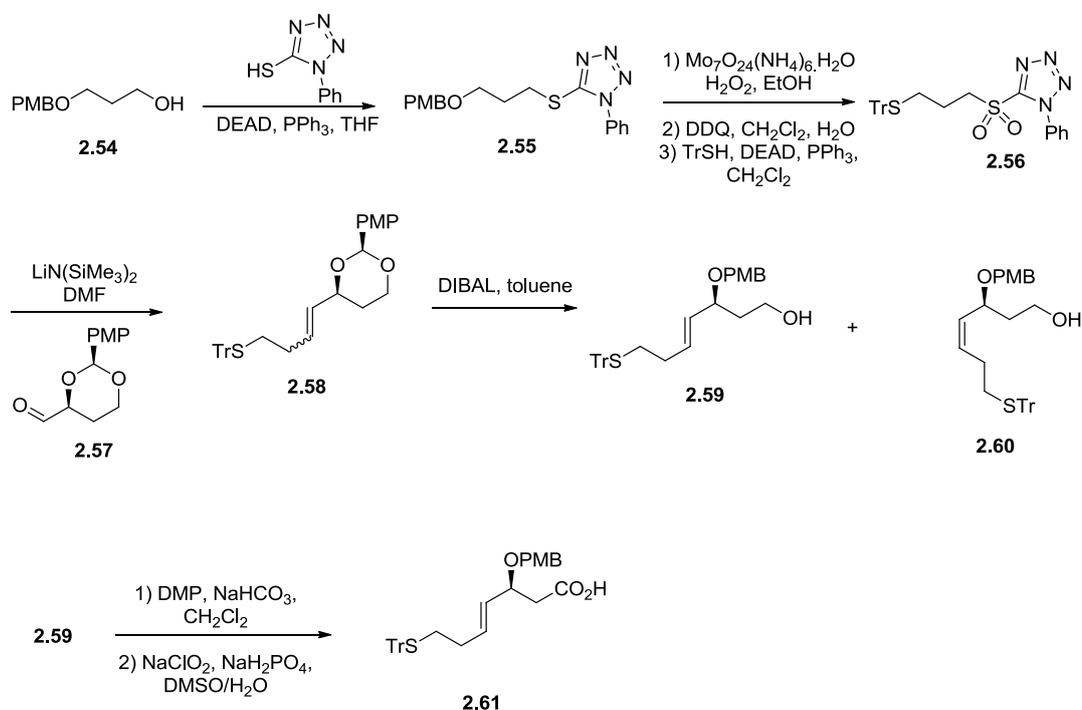
Scheme 2.10 : Katoh's synthesis of the β-hydroxy acid 2.61 via Julia-Kocienski olefination

Several groups used kinetic resolution to prepare the β-hydroxy acid for their synthesis of largazole (Scheme 2.11).[100-103] The racemic β-hydroxy ester **2.63** is obtained by aldol reaction between the lithium enolate of *tert*-butyl-acetate and acrolein. The enzymatic resolution of the racemic mixture **2.63**, using PS-Amano Lipase, led to the formation of the (*S*)-acetate only. The yield of the enzymatic resolution is almost 50% and the enantiomeric excess is excellent. The (*S*)-acetate is then hydrolysed to give the β-

hydroxy ester. Metathesis is then used to introduce the thiol containing side chain at a later stage, such as in the total synthesis of largazole.
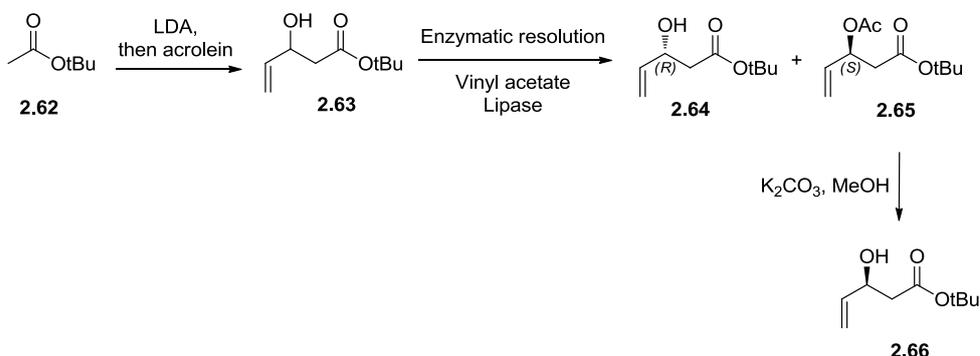
Scheme 2.11 : Synthesis of the β-hydroxy ester 2.66 via enzymatic resolution

2.1.4.2. Macrolactonisation in the cyclic depsipeptides

Simon's synthesis of FK228 is the first synthesis of a member of this class of depsipeptides to be described in the literature (Scheme 2.12).[86] Synthesis of the tetrapeptide **2.69**, constituting of valine, cysteine, threonine and valine, involved a standard sequence of couplings. Tosylation of the alcohol followed by elimination after treatment with DABCO formed the dehydrobutyrine residue; diethylamine was also used in the same step in order to remove the Fmoc protecting group. The tetrapeptide **2.70** was then coupled to the β-hydroxy acid **2.36**. Ester hydrolysis followed by macrolactonisation was performed by the Mitsunobu reaction. Finally the disulfide bridge was formed by treatment with iodine and methanol.
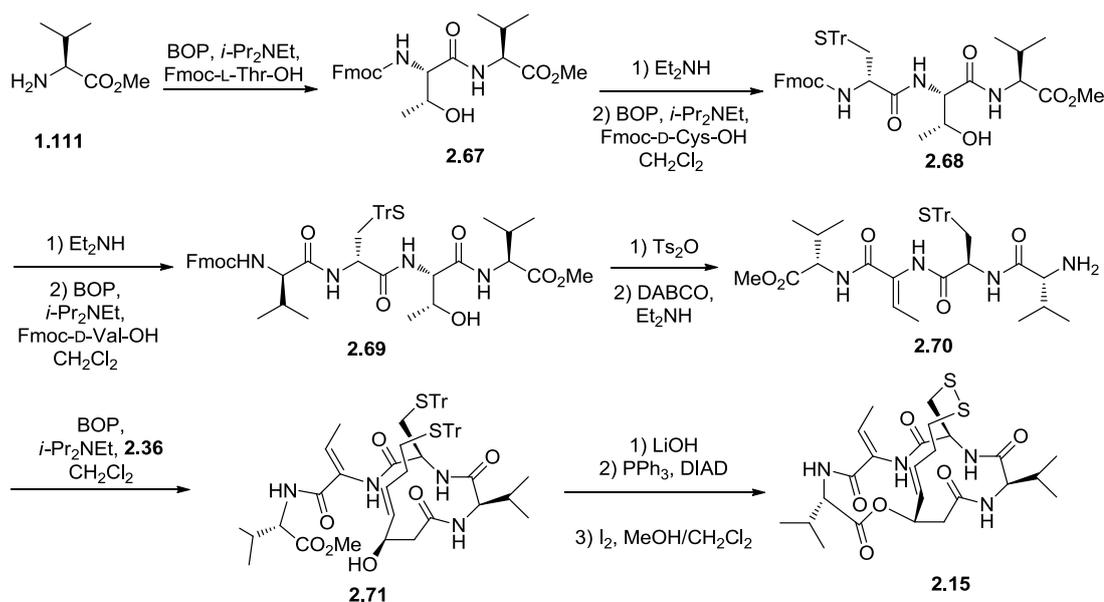
Scheme 2.12 : First synthesis of FK228 (2.15)

Macrolactonisation appeared to be very difficult in the synthesis of FK228. Simon and co-workers attempted the esterification using acid activation with various coupling reagents were unsuccessful.[86] The Keck modification of the Steglich esterification gave the product with a very low yield (<5%). They then resorted to alcohol activation by Mitsunobu reaction to form the product in 62% yield following reaction optimisation. They noticed that the use of TsOH was essential to reduce the β-elimination occurring on the β-hydroxy acid. Since the Mitsunobu reaction proceeds with stereochemical inversion, the total synthesis required the enantiomer of the β-hydroxy acid prior to macrolactonisation. Katoh *et al.* managed to reproduce the 62% yield for their macrolactonisation in their synthesis of spiruchostatins.[82] The Mitsunobu reaction was also tried by Williams *et al.* who reported 24% yield, and by Ganesan who also reported a low yield (10-20%).[42,87] This shows that this Mitsunobu macrolactonisation is complex and not easily reproducible.

Wentworth and Janda were the only group who prepared FR901375 (Scheme 2.13).[88] They used the same Mitsunobu conditions described by Simon and obtained a yield of 59%.[86] Firstly they prepared the tetrapeptide **2.75**, constituting of threonine, cysteine and two valine residues, which was then coupled to the β-hydroxy acid **2.36** with the (*R*)-stereochemistry. After ester deprotection, they performed the macrolactonisation under Mitsunobu conditions and then formed the disulfide bond. The final step was the

deprotection of the hydroxyl group of the threonine residue; the protecting group was necessary earlier in the synthesis in order to avoid side reactions occurring during the Mitsunobu macrolactamisation.
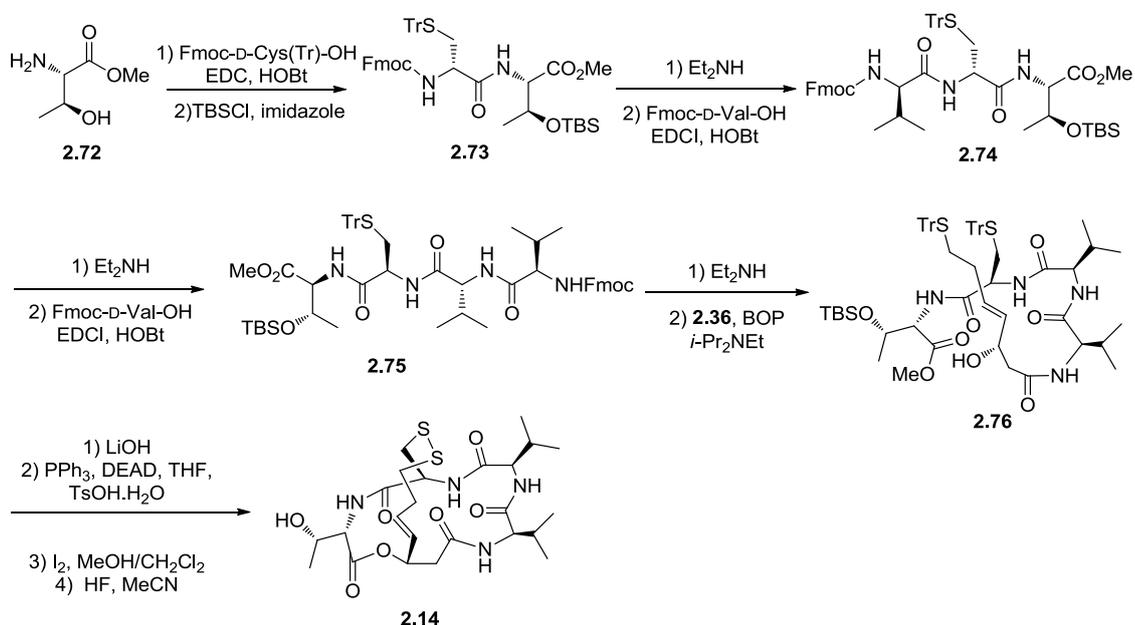
Scheme 2.13 : Wentworth and Janda's synthesis of FR901375 (2.14)

In the synthesis of spiruchostatin, the macrolactonisation by carbonyl activation rather than Mitsunobu reaction is possible as the carboxylic acid is unhindered at the α-position. This was first realised by Yamaguchi esterification (53%) in the total synthesis of spiruchostatin A performed in 2004 by Ganesan *et al.* (Scheme 2.14).[78] They performed a condensation of Boc-D-valine pentafluorophenyl ester with methyl acetate followed by diastereoselective reduction to obtain the valine statine fragment. The methyl ester has to be deprotected and reprotected with a Tce protecting group which is compatible with acidic and basic conditions. The valine statine derivative was coupled with Fmoc-D-Cys-OH, then with Fmoc-D-Ala-OH and finally with the β-hydroxy acid. The Yamaguchi esterification was used to form the macrocycle. Formation of the disulfide bridge by treatment with iodine in a mixture of MeOH and CH_2Cl_2, followed by deprotection of the hydroxyl group afforded spiruchostatin A.
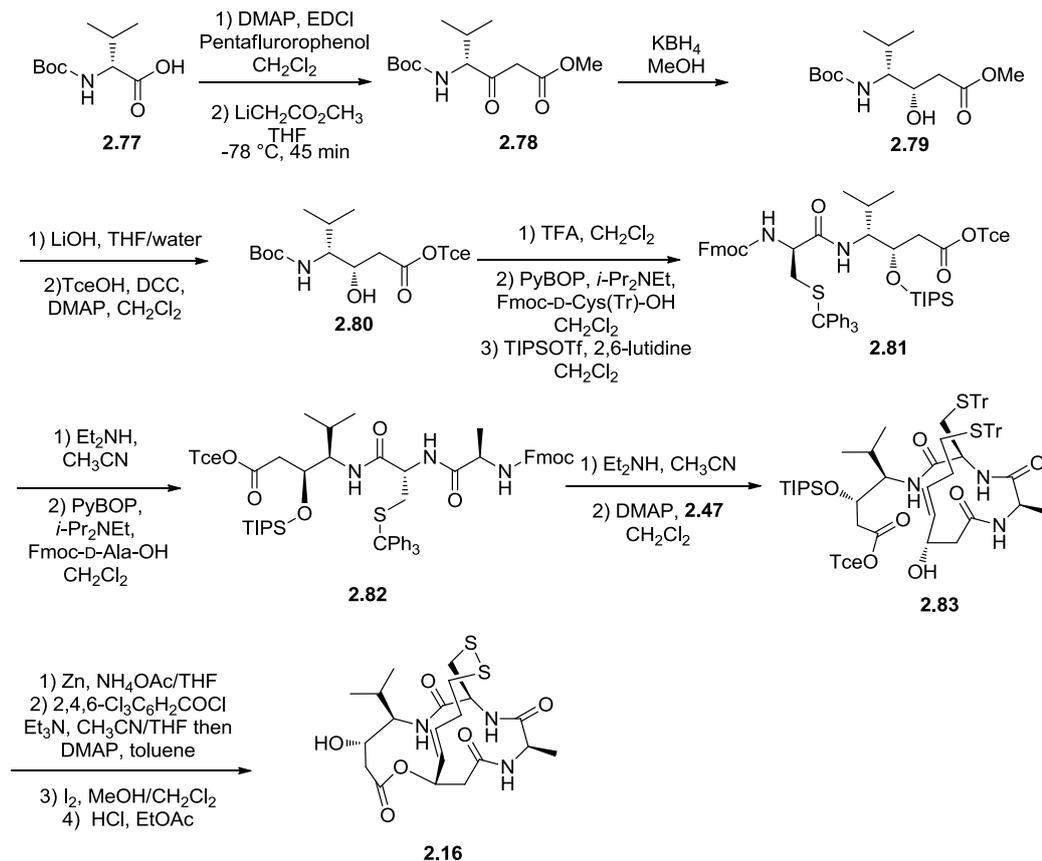
Scheme 2.14 : First synthesis of spiruchostatin A (2.16)

However, improved yields (67 and 90% respectively) for the macrolactonisation were later obtained by Doi and Takahashi followed by Katoh with the use of 2-methyl-6-nitrobenzoic anhydride (MNBA, figure 2.18).[82,90] This reagent, also known as the Shiina reagent, is an effective condensation reagent for the DMAP-promoted lactonisation of *seco*-hydroxycarboxylic acids, as reported by Shiina *et al.*[41] These mild conditions do not require a protecting group on the hydroxyl group of the statine and also reduce the formation of side products.
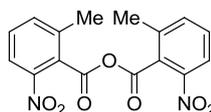
Figure 2.18 : Structure of MNBA (2.84)

Doi and Takahashi also showed that the order of reaction between macrolactonisation and disulfide formation is important as attempted macrolactonisation after the disulfide bond formation failed.[90]

Ganesan *et al.* tried to perform the macrolactonisation of FK228 using the Shiina reagent but no product was formed.[42] They also tried several coupling reagents and the best results were obtained with MSNT in a low 34% yield. These low yields for the macrolactonisation are due not only to the presence of valine in the α-position, but also due to the rest of the peptide. They decided to change their strategy and to perform the macrocyclisation by forming an amide bond instead of the ester bond. They started with the high yielding coupling of Fmoc-Val-OH with the β-hydroxy acid derivative **2.85** that they obtained in two steps from compound **2.47** (Scheme 2.15).
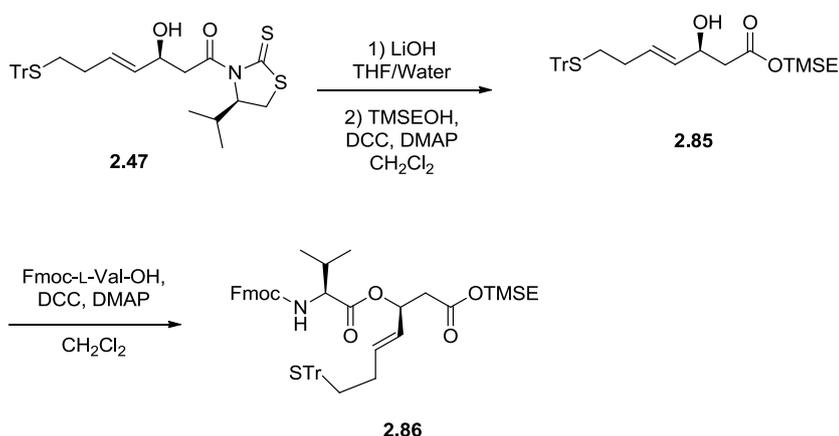
Scheme 2.15 : Synthesis of fragment 2.86

This confirmed that not only is the hindrance of the valine responsible for the low yield of the macrolactonisation, but also the rest of the peptide. The tripeptide **2.89** formed of valine, cysteine and threonine is prepared by standard couplings (Scheme 2.16). The dehydrobutyrine **2.90** is formed by dehydration using DABCO and is then coupled to **2.86** after ester hydrolysis. The macrolactamisation is performed after deprotection of both sides of the linear peptide **2.92** and is followed by the disulfide bridge formation.
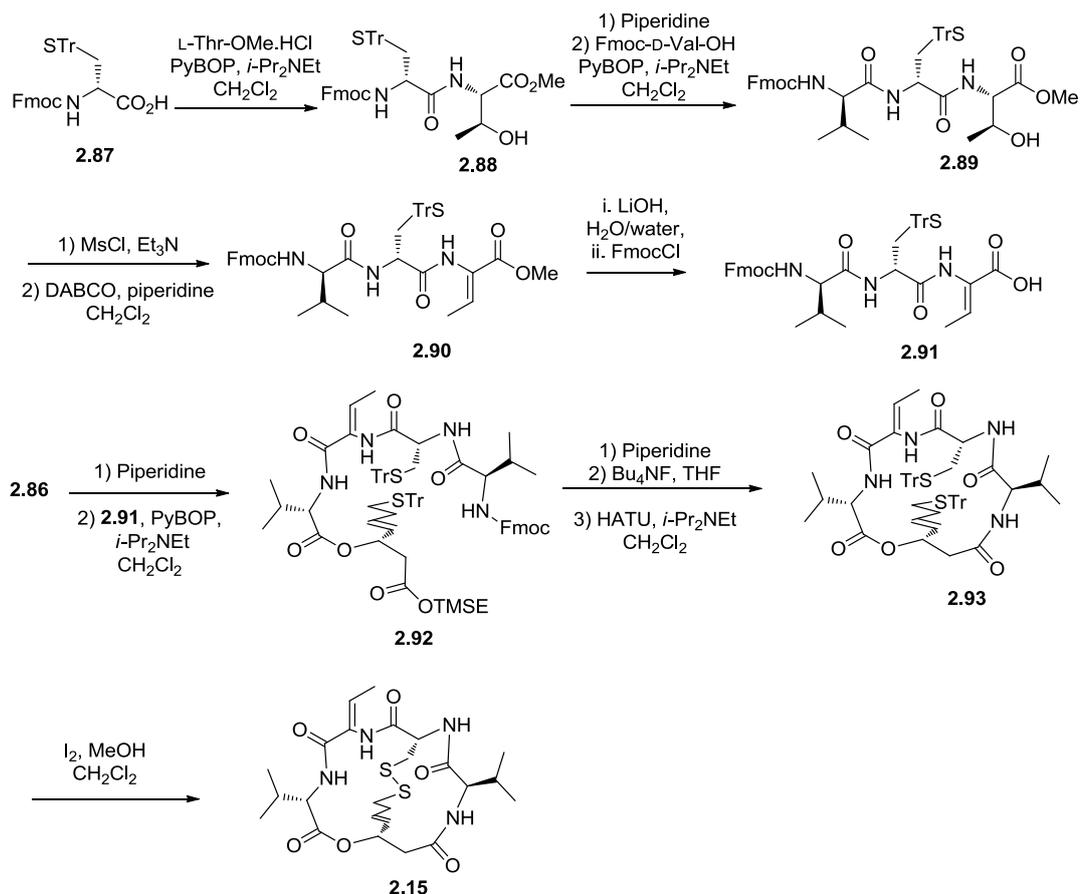
Scheme 2.16 : Macrolactamisation strategy for FK228 preparation

During the course of my PhD, several total syntheses of largazole were published. To form the macrocycle of largazole, the possible disconnections are the two amide bonds and the ester bond. Several groups performed the macrolactamisation between the thiazole ring and the β-hydroxy acid (Figure 2.19).[96,97,100-102,104] It is interesting to notice that Luesch *et al.* successfully performed the macrolactamisation between the 4-methylthiazoline ring and the valine residue with good yield despite the position being relatively hindered.[105] Several other groups also prepared the macrocycle by forming this bond.[95,103]

2.18
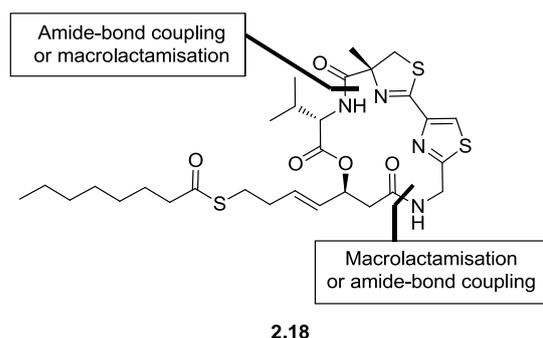
Figure 2.19 : Different strategies employed for the preparation of largazole

Many groups mentioned their attempts to perform the macrocyclisation by forming the ester bond. Forsyth mentioned that they tried the Yamaguchi, Keck, Mukaiyama and Mitsunobu procedures.[104] The macrolactonisation is not possible for the synthesis of largazole due to the hindrance of the valine and the rest of the peptide as observed in the synthesis of FK228.

Xu and Tao used OTBS as precursor for the thiol but their macrolactamisation was unsuccessful (Figure 2.20).[97] They proposed that the homoallylic alcohol is involved in protecting group migration or intramolecular cyclisation and it is necessary to insert the *S*-trityl group before the macrolactamisation.

2.94   **2.95**
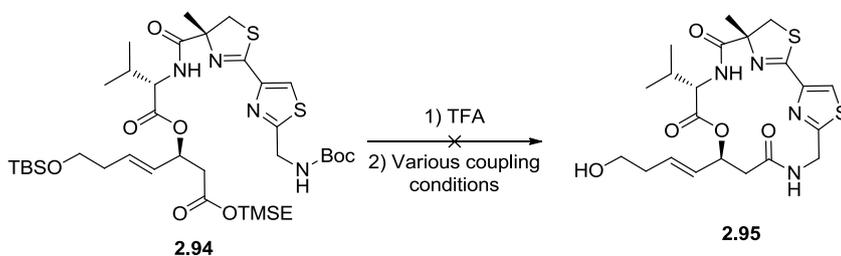
Figure 2.20 : Macrolactamisation failed because of the presence of –OTBS group

2.1.4.3. Statine unit of spiruchostatins

Spiruchostatins contain an isoleucine statine derivative (Figure 2.21).

2.96

R = H, Me
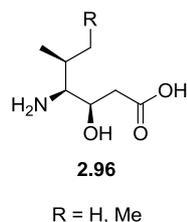
Figure 2.21 : Structure of the statine subunit

Several natural products contain this moiety including tamandarins, melleumin A and miraziridine A (Figure 2.22).[106-108] Tamandarins and melleumin A are also cyclic depsipeptides.

Tamandarin A R = Me
Tamandarin B R = H

Melleumin A
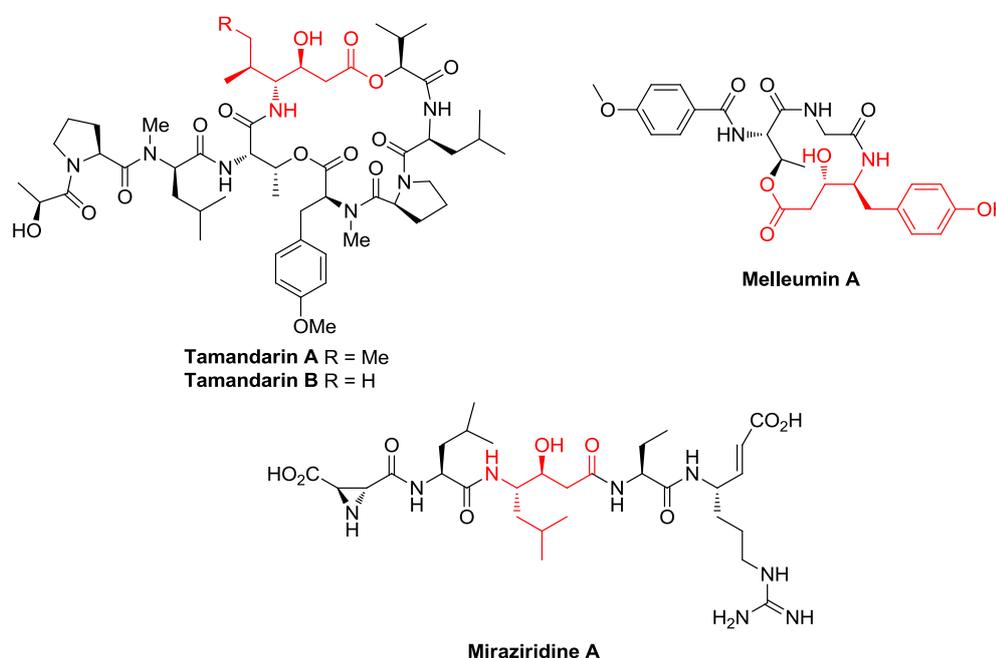
Miraziridine A

Figure 2.22 : Structures of tamandarins A and B, melleumin A and miraziridine A

The *syn*-stereochemistry of the statine present in spiruchostatin was not originally assigned and was elucidated by synthesis.[77,78] The statine unit can undergo *β*-elimination and form a similar function to the dehydrobutyrine moiety present in FK228.

Ganesan *et al.* performed a Claisen condensation of Boc-D-valine pentafluorophenyl ester with methyl acetate, followed by diastereoselective reduction to obtain the *syn* diastereomer (Scheme 2.17).[78,106] The methyl ester has to be deprotected and

93

reprotected with a Tce protecting group which is compatible with acidic and basic conditions.
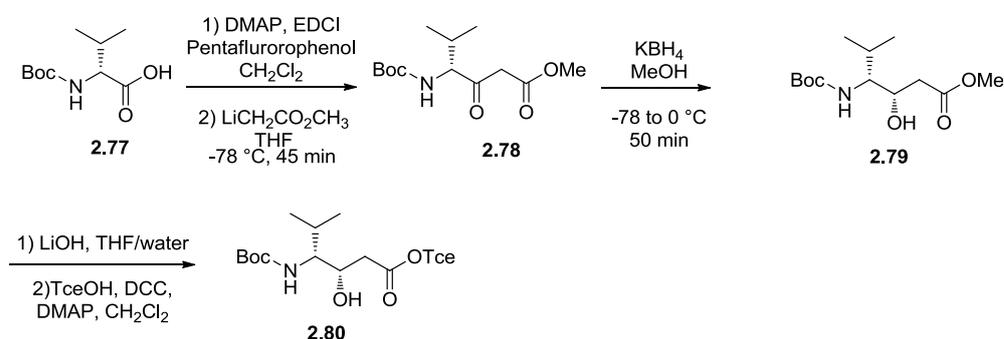
Scheme 2.17 : Formation of the statine unit 2.80

Katoh investigated the best conditions for the reduction by comparing different reducing agents (LiBH$_4$, NaBH$_4$ and KBH$_4$); it was determined that KBH$_4$ at low temperature (–40 °C) gave the best yield and diastereomeric ratio.[82]

2.1.4.4. Metathesis in largazole synthesis

Several groups used the cross-metathesis reaction to insert the side chain (Scheme 2.18).[101-103,105] This method is interesting as it allowed the rapid preparation of analogues. Most of the groups used Grubbs' second generation catalyst. Optimisation of this step was undertaken by Cramer *et al.* and Grela's catalyst **2.98** appeared to give the best results.[102] Largazole is obtained with a good yield (75%) with a *trans/cis* ratio 4:1.
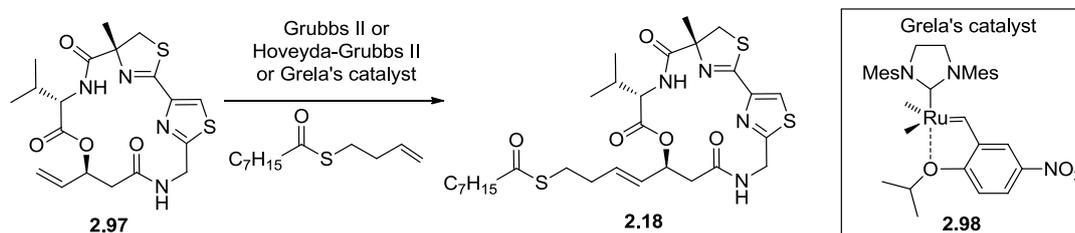
Scheme 2.18 : Incorporation of the side chain on largazole

2.1.4.5. Thiazoline formation in largazole synthesis

Most of the groups used basic conditions to form the thiazoline ring by condensation of the nitrile derivative **2.100** and (*R*)-methylcysteine hydrochloride **2.99**, using either triethylamine as a base or sodium hydrogenocarbonate and a phosphate buffer solution (Scheme 2.19).[96,97,101,102,105]
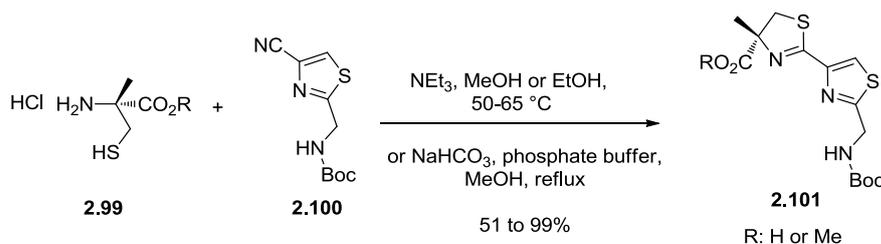
Scheme 2.19 : Thiazoline formation via basic condensation

Xie used the Charette conditions to form the thiazoline moiety by condensation between the thiazolyl amide **2.103** and the (*R*)-methylcysteine methyl ester hydrochloride **2.102** by treatment with triflic anhydride and pyridine in good yield (Scheme 2.20).[95,109]
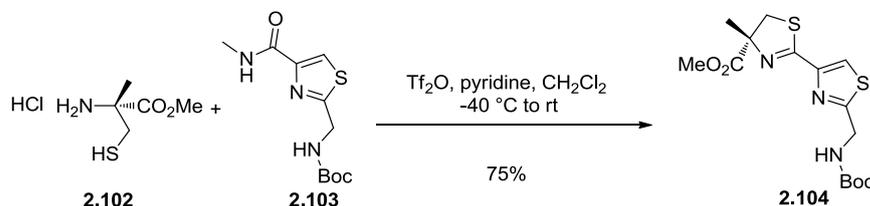
Scheme 2.20 : Thiazoline formation using Charette conditions

Forsyth employed a Staudinger reduction to obtain the thiazoline ring (Scheme 2.21).[104] The azido thioester **2.105** was treated with triphenylphosphine in acetonitrile under microwave irradiation to form **2.106**.
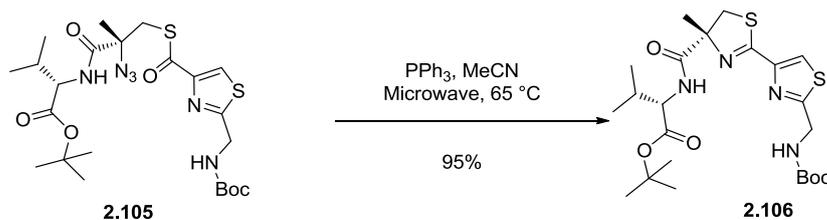
Scheme 2.21 : Thiazoline formation using Staudinger reduction

Kelly's method was employed on **2.107** by both Ghosh and Doi to form the thiazoline in excellent yield (Scheme 2.22).[44,100,110]
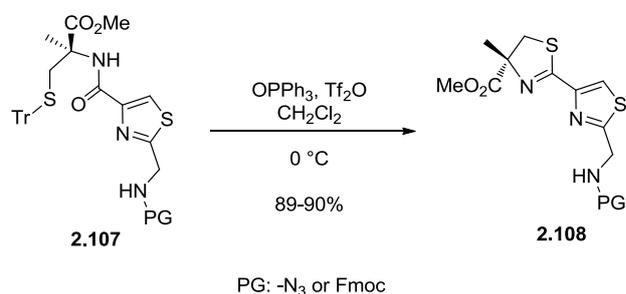
Scheme 2.22 : Thiazoline formation by Kelly's method

Zhou and Jiang performed the simultaneous formation of the thiazole and thiazoline by treatment of the tripeptide, which was prepared by solid phase synthesis using 2-chloro-trityl chloride resin, with $TiCl_4$ (Scheme 2.23).[111] The thiazoline ring was then oxidised to the thiazole using activated manganese dioxide.
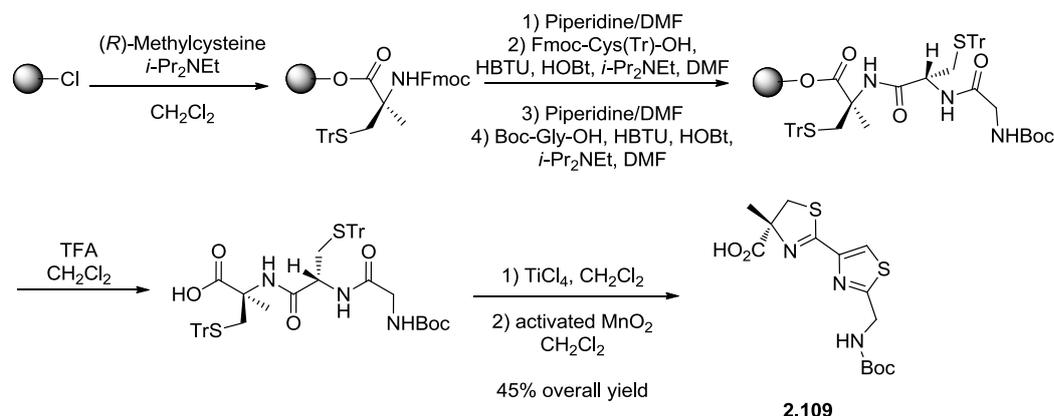
Scheme 2.23 : Simultaneous formation of the thiazole and thiazoline rings

2.1.5. Analogues of the cyclic depsipetides

2.1.5.1. FK228 analogues

Ganesan and co-workers prepared several analogues of FK228 (Figure 2.23).[112] They made hybrids of FK228 and spiruchostatin A that contain in position 1: a glycine residue, in position 2: a valine residue and in position 3: an alanine or a valine residues. FK228 possesses instead in position 1: a valine residue, in position 2: a dehydrobutyrine

residue (Dhb) and in position 3: a valine residue and spiruchostatin A comprises in position 1: the statine moiety, in position 2: a valine and an alanine residue in position 3. Both the analogues **2.110** and **2.111** are potent HDAC inhibitors with similar activity to FK228. It is important to highlight the fact that the Dhb residue is not an essential element for the activity of FK228 and can be replaced, for example by a valine residue. Similarly, in spiruchostatin the presence of the statine functional unit is not necessary for biological activity. Biological activities of analogues **2.110** and **2.111** have shown that a bulky group in position 3 is favourable. The linear compound **2.112** was found to be totally inactive as an HDAC inhibitor, demonstrating the importance of the macrocycle in the activity of the compounds. They decided to make analogues **2.113** and **2.114**, where the cysteine is replaced by an alanine residue, to check the importance of the disulfide bridge. They tested compound **2.114** and found it to give a good result in growth inhibition but poor result in HDAC inhibition. Compound **2.113** gave the opposite results: poor growth inhibition but potent as an HDAC inhibitor. This showed the importance of both thiols, one binds the zinc and the other is involved in the cellular uptake.

FK228 (2.15)
MCF7 growth inhibition IC50 1 nM
Total HDACs inhibition IC50 24 nM

Spiruchostatin A R: H **(2.16)**
Spiruchostatin B R: Me **(2.17)**

2.110 R: Me
MCF7 growth inhibition IC50 37 nM
Total HDACs inhibition IC50 15 nM

2.111 R: *i*Pr
MCF7 growth inhibition IC50 5 nM
Total HDACs inhibition IC50 7 nM

2.112
MCF7 growth inhibition IC50 >1000 nM
Total HDACs inhibition IC50 >3200 nM

2.113 R: H
MCF7 growth inhibition IC50 >1000 nM
Total HDACs inhibition IC50 7 nM

2.114 R: Ac
MCF7 growth inhibition IC50 55 nM
Total HDACs inhibition IC50 242 nM

Figure 2.23 : Reported analogues of FK228

2.1.5.2. Spiruchostatin analogues

Epi-Spiruchostatin A (**2.115**) was prepared by Ganesan with the (*R*)-stereochemistry for the β-hydroxy acid.[78] Biological activity showed that the stereochemistry (*S*) at this position is essential as the *epi*-spiruchostatin lost considerable activity. The inhibition activity against growth of breast cancer cell lines dropped from 10 nM for spiruchostatin A to 10 μM for *epi*-spiruchostatin A.[78]

5''-*epi*-Spiruchostatin B (**2.116**) was prepared by Katoh *et al.* with the biological activity showing that the stereochemistry at this position can be either (*R*) or (*S*).[82] The 5''-*epi*-spiruchostatin B appeared to show even greater selectivity towards HDAC1 than HDAC6, than spiruchostatin B (1625-fold to 636-fold).

Spiruchostatin A (2.16) R:H
Spiruchostatin B (2.17) R:Me

2.115
epi-**Spiruchostatin A**

2.116
5"-*epi*-Spiruchostatin B
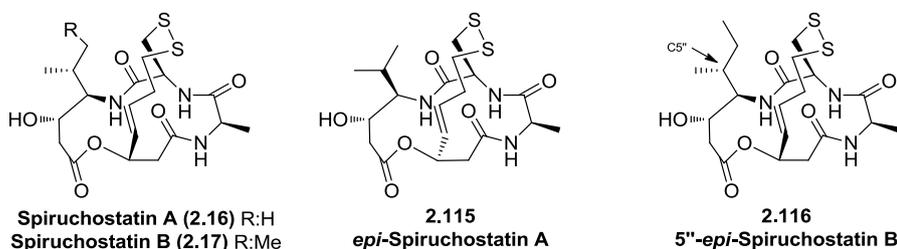
Figure 2.24 : Spiruchostatins and epimers

2.1.5.3. Largazole analogues prepared by other groups

According to Luesch and Cramer, a four-atom linker, between the macrocycle and the octanoyl group in the side chain, is optimal for HDAC inhibitory activity to be a good mimic of the lysine side chain of substrate.[98,102] Studies showed the necessity of the thiobutenyl group for the antiproliferative activity of the molecule, as a shorter or longer spacer resulted in a loss of inhibitory activity. Zhou and Jiang confirmed the essential (*E*)-geometry for the alkene; (*Z*)-alkenes are inactive as HDAC inhibitors.[111]

2.117
n = 1-4

Figure 2.25 : Variation of largazole side chain

Analogues bearing *S*-acetyl **2.118** or disulfide derivative **2.119** present a similar inhibition activity against HDAC when compared to largazole with its *S*-octanoyl chain.[110] Phillips was able to confirm that the thiol is a zinc chelating group by preparing inactive derivatives with an ester or ketone function instead of the thioester (**2.120** and **2.121**).[101] Williams replaced the zinc-binding group of largazole by a carboxylic acid function to form analogue **2.122**, such as in azumamide.[113] As expected the thiol is a better zinc-binding group than the carboxylic acid function. Williams also prepared analogues with benzamide and thioamide as the zinc binding group (**2.123**, **2.124**, **2.125** and **2.126**).[113] All these analogues are potent inhibitors, but a decrease of activity is nonetheless observed.

2.118 n=2, R: SAc

2.119 n=2, R: (disulfide pyridyl amine structure)

2.120 n=2, R: (ester) O C_7H_{15}

2.121 n=2, R: (ketone) O C_7H_{15}

2.122 n=2, R: CO_2H

2.123 n=2, R: (benzamide, NH_2)

2.124 n=3, R: (benzamide, NH_2)

2.125 n=1, R: (thioamide, SH)

2.126 n=2, R: (thioamide, SH)

Figure 2.26 : Reported analogues of largazole with variation in the side chain

Luesch *et al.* demonstrated that the (*S*)-configuration at the C17-position is crucial as the 17-*epi*-largazole exhibited a more than 500-fold loss in activity.[98] Williams and co-workers modified the thiazoline ring.[113] They prepared the C-7 epimer, the cysteine analogue **2.128** and the thiazole-thiazole derivative **2.131**. These analogues are potent HDAC inhibitors but their activities are lower than largazole. The oxazoline-oxazole **2.132** and pyridine-thiazole **2.133** analogues were also synthesised. They both displayed greater inhibitory activity than largazole, especially the pyridine-thiazole derivative **2.133**. De Lera prepared analogues with a substituent in C-7 position (H, Et, Bn) and the same thiazole-thiazole derivative **2.131**.[103] The C7-benzyl and the thiazole-thiazole

derivative were more potent inhibitors than largazole, however the biological result for the thiazole-thiazole analogue differed from that previously reported by Williams.[113] Williams and co-workers prepared the enantiomer of largazole which was found to be less potent as an HDAC inhibitor than largazole (100-fold decrease).[113]
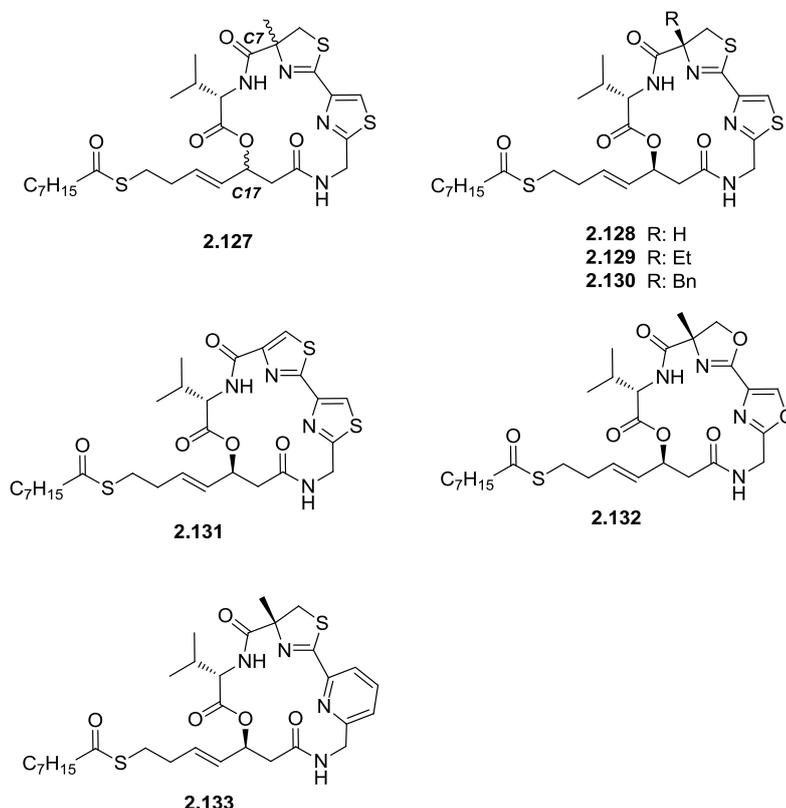
Figure 2.27 : Reported analogues of largazole with variation in the macrocycle

Luesch *et al.* showed that it is possible to replace the valine residue with an alanine residue with little alteration to the inhibition activity.[98] Williams and co-workers replaced the valine with a proline to add more rigidity in the macrocycle and observed a significant decrease in activity.[113] Zhou and Jiang showed that replacing valine by a tyrosine resulted in a slightly lower activity but an increase of selectivity towards cancer cell lines over normal cell lines of more than 100-fold.[111]

The linear compound **2.134** prepared by Phillips and co-workers presented no biological activity as an HDAC inhibitor and emphasised the importance of the macrolide ring (Figure 2.28).[101]
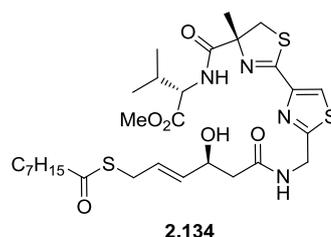
2.134

Figure 2.28 : Structure of linear compound 2.134

Bradner decided to make the amide isosteres of the depsipeptides FK228 and largazole (Figure 2.29).[114] These compounds should be more stable with more rigid conformations.

2.135

2.136
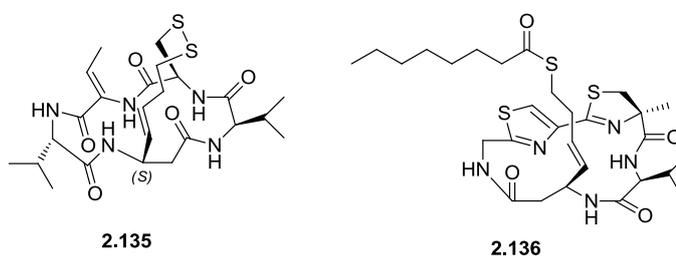
Figure 2.29 : Amide isosteres of FK228 and largazole

Having prepared the compound **2.138** from *N*-Boc(Asp)-O*t*Bu-OH (**2.137**) in seven steps they coupled it to the tetrapeptide **2.139**. However, in the case of the FK228 analogue, the macrolactamisation was unsuccessful. They reversed the order of the steps, forming the disulfide bridge followed by the macrolactamisation in 53% yield.
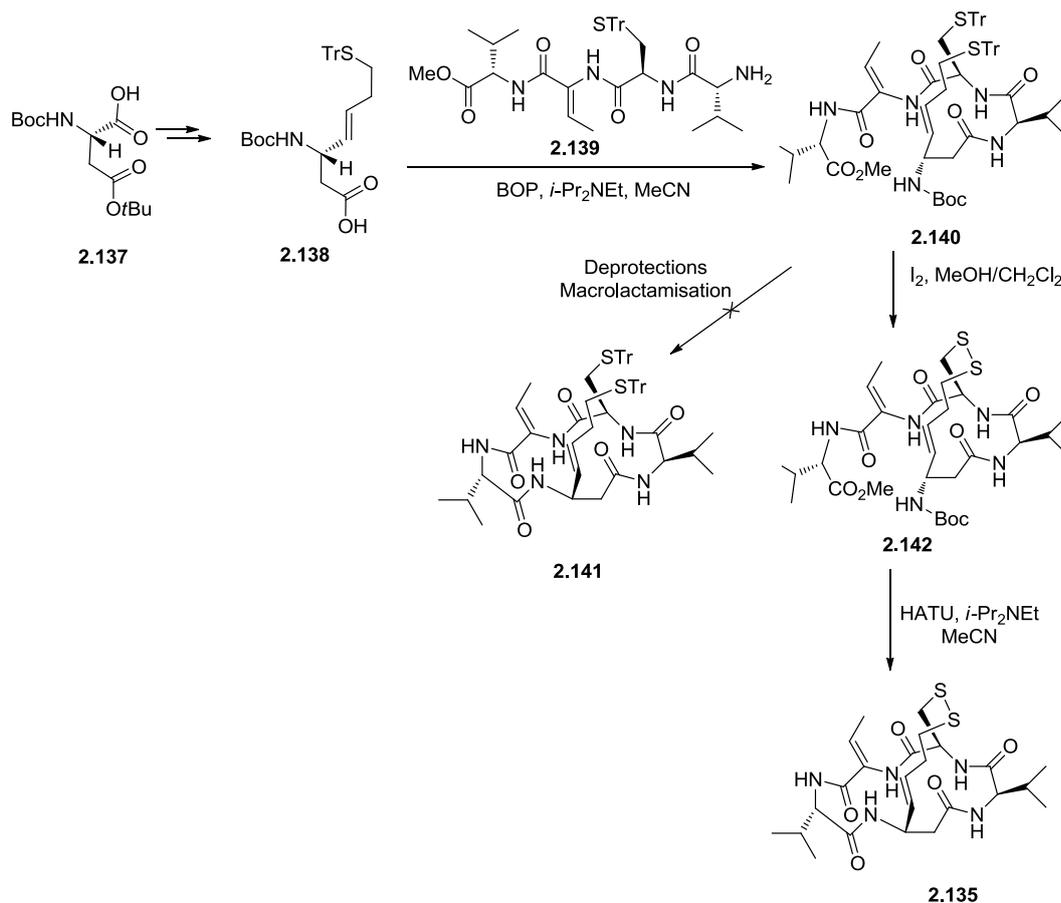
Scheme 2.24 : Synthesis of the amide isostere of FK228

Concerning the preparation of largazole analogue **2.136**, the macrolactamisation between the thiazole ring and the β-hydroxy acid worked well following neutralization of the TFA salt obtained after Boc deprotection of the linear precursor. The amide isostere of FK228 lost considerable inhibition activity when compared to FK228 with a 50-fold less in potency. The amide isostere of largazole also showed a loss in inhibition activity, but only a decrease of 9-fold when compared to largazole. The replacement of the ester function by an amide modifies the accessible conformational space.

2.2. AIMS

HDAC depsipeptide inhibitors are molecules of interest in our group and we had previously prepared FK228, spiruchostatin A and analogues of these natural products.

We decided to prepare largazole as it was an attractive target as an HDAC inhibitor. We also planned to prepare some related analogues in order to study the structure-activity relationships (SAR) of largazole.

During the course of my PhD, the thailandepsins/burkholdacs were disclosed by Cheng and Brady. These natural products were also targeted for total synthesis. We started with Cheng's originally reported hemiaminal structure for thailandepsins as target molecules, followed later by his revised structures. Later, Brady's burkholdac B became our target as we believed it was identical to Cheng's revised structure for thailandepsin A. Since neither Cheng nor Brady determined the stereochemistry of the natural products, this would need to be deduced and confirmed by comparison of synthetic and naturally isolated material.

2.3. SYNTHESIS AND BIOLOGICAL ACTIVITY OF LARGAZOLE AND ANALOGUES

2.3.1. Total synthesis of largazole

2.3.1.1. Retrosynthesis of largazole

The strategy proposed to prepare largazole is to synthesise the following two fragments: the thiazole-thiazoline fragment **2.109** and the β-hydroxy acid derivative **2.143** (Scheme 2.25). A convergent synthesis is to be employed that will make the synthesis of analogues easier. The macrolactamisation can be performed between the thiazole and the carboxylic acid of the β-hydroxy acid (position 1) and between the thiazoline and valine (position 2). We decided to perform the macrolactamisation between the thiazole and the carboxylic acid of the β-hydroxy acid because this position is less hindered than the position between the thiazoline and valine. Amide bond formation will be performed in position 2 prior to the macrolactamisation. The fragment containing the 4-methylthiazoline linearly fused to the thiazole **2.109**, can be prepared from (*R*)-methylcysteine and glycine. The β-hydroxy acid derivative **2.143** will be synthesised using the method previously employed in our group to prepare spiruchostatin A and FK228. The thioester will be formed in the last step as thioesters are known to be easily hydrolysed.
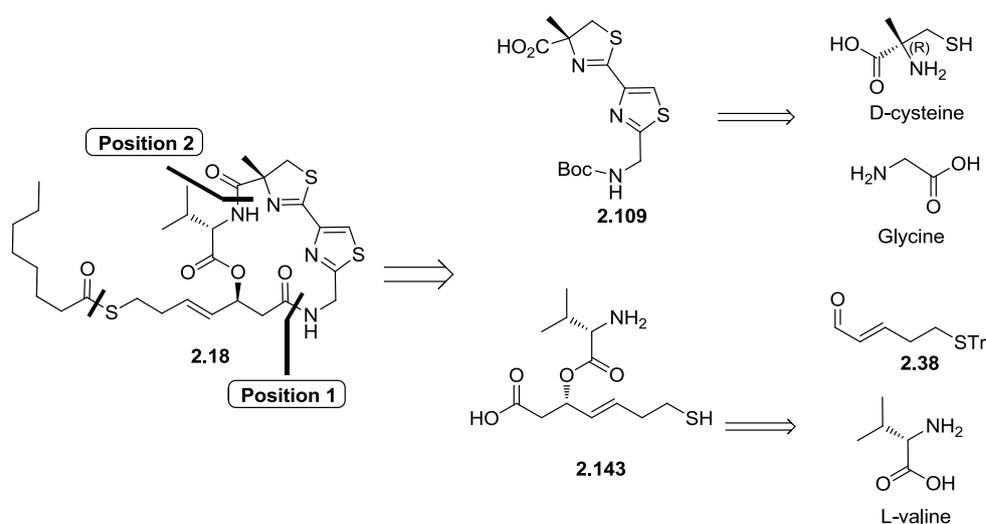
Scheme 2.25 : Retrosynthetic analysis of largazole (2.18)

2.3.1.2. Synthesis of the β-hydroxy acid derivative

The Fujita-Nagao auxiliary was obtained in two steps from D-valine (Scheme 2.26). The reduction of valine, using a mixture of sodium borohydride and iodine in THF, yielded (R)-valinol in good yield.[115] The alcohol is then condensed with carbon disulfide in excellent yield.[116] Treatment of **2.145** with sodium hydride and acetyl chloride gave the desired acetylated Fujita-Nagao auxiliary **2.46** (89%).[117]
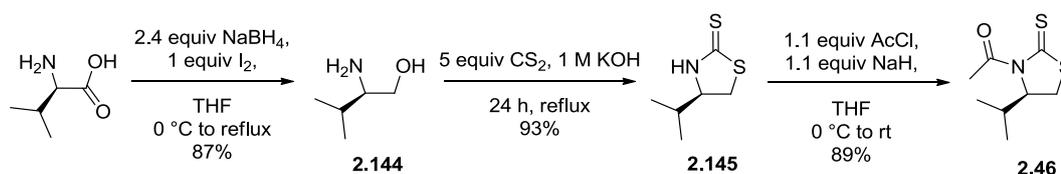
Scheme 2.26 : Synthesis of acetylated Fujita-Nagao auxiliary 2.46

The aldehyde **2.38** was prepared in two steps, 1,4-addition and Wittig reaction, from acrolein **2.37** as described in the Wentworth-Janda synthesis.[88] Reaction of the aldehyde **2.38** with the *N*-acetylthiazolidinethione Nagao auxiliary **2.46**, under Vilarrasa's conditions, gives the desired diastereomer **2.47** in moderate yield. The chiral auxiliary is then removed and replaced by a trimethylsilyl ethyl protecting group (TMSE) in one step. The fragment **2.86** is finally prepared by esterification of *N*-Fmoc-L-valine with the *seco*-acid **2.85** (Scheme 2.27).
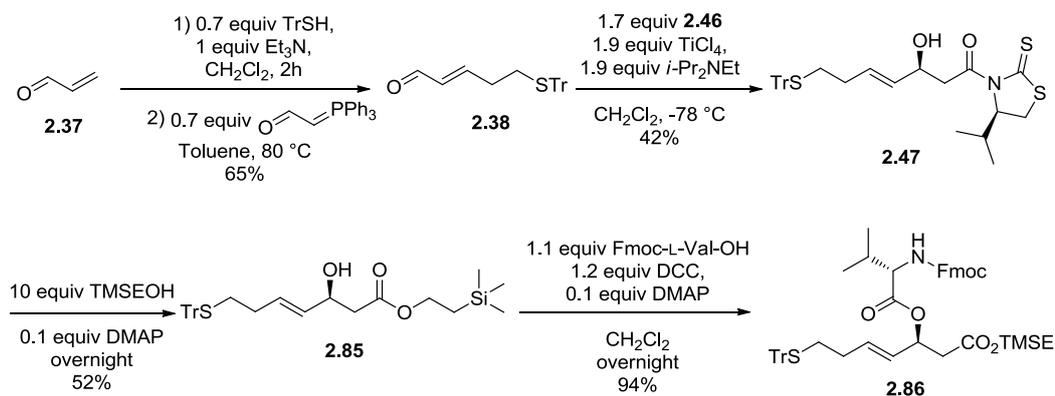
Scheme 2.27 : Synthesis of β-hydroxy acid derivative 2.86

2.3.1.3. Synthesis of the thiazole-thiazoline fragment

In order to prepare the thiazole-thiazoline fragment **2.104**, we planned to prepare the thiazole fragment **2.147** from the thioamide **2.149**, methyl bromopyruvate and (*R*)-2-methylcysteine. The thiazoline ring will be prepared via formation of the amide **2.146**.
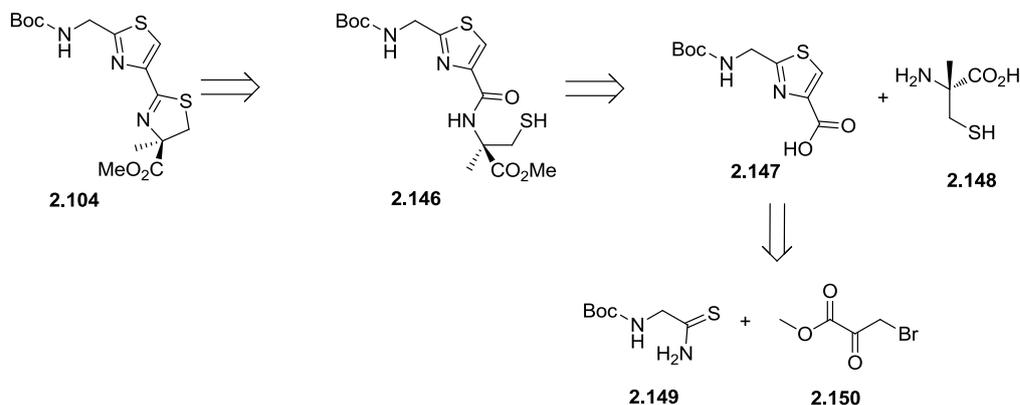
Scheme 2.28 : Retrosynthetic analysis of fragment 2.104

The commercially available thioamide **2.149** was treated with methyl bromopyruvate to give the thiazole derivative **2.151** in moderate yield (50%).[33] Saponification of **2.151** with lithium hydroxide in a mixture of water/THF at 0 °C generated the carboxylic acid **2.147** in excellent yield (Scheme 2.29). In this case, we do not need to use epimerisation free conditions for the thiazole formation as a glycine derivative is used.
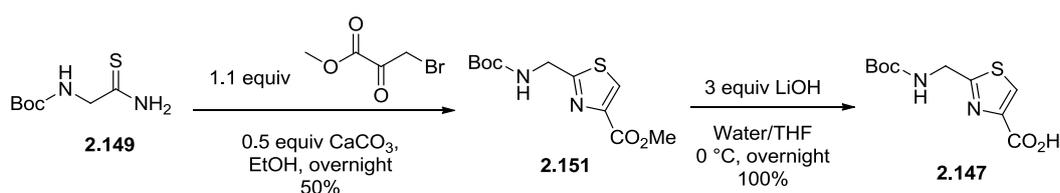
Scheme 2.29 : Synthesis of fragment 2.147

Amide **2.146** was prepared by coupling of **2.147** with (*R*)-2-methylcysteine methyl ester, although protection of the thiol would have avoided side reactions such as dimerisation. Formation of the thiazoline was attempted using TiCl$_4$ but with no success (Scheme 2.30).[44]

106
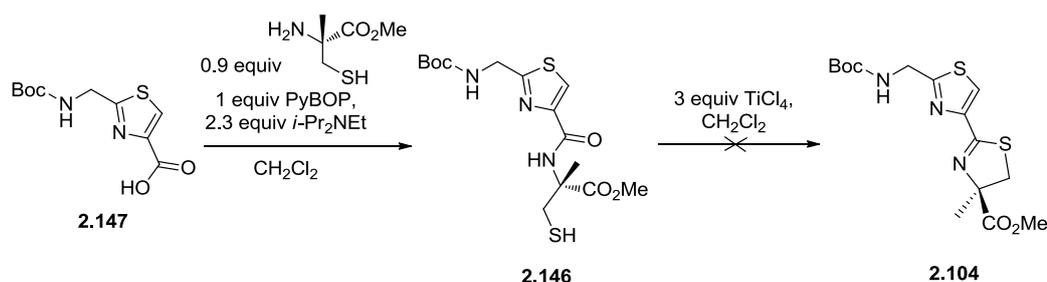
Scheme 2.30 : Attempt to synthesise fragment 2.104

We proposed that the lack of success was due to loss of the Boc group during this step. We decided to use a different protecting group: the allyloxycarbonyl (Alloc) protecting group.

The synthesis of the Alloc fragment **2.156** is slightly different as the thioamide is not commercially available (Scheme 2.31). The first step is to convert the amine function of glycinamide **2.152** to its *N*-Alloc derivative **2.153** using allyl chloroformate and Hünig's base in the presence of DMAP as a catalyst (Scheme 2.31).[118] The amide **2.153** is then transformed into the thioamide **2.154** using Lawesson's reagent.[119] The thiazole ring is formed using the conditions described previously and finally the ester **2.155** hydrolysed to afford **2.156**.[33]
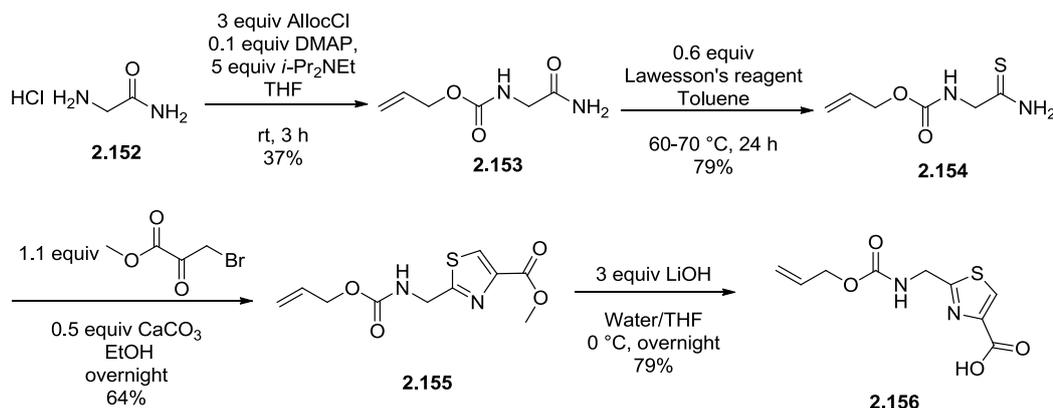
Scheme 2.31 : Synthesis of fragment 2.156

The thiol function of commercial (*R*)-2-methylcysteine hydrochloride was first protected with a trityl group by using triphenylmethanol and boron trifluoride diethyl etherate in acetic acid (Scheme 2.32).[120] Esterification of the *S*-triphenylmethyl thioether **2.158** with thionyl chloride in methanol provided the methyl ester **2.159**.[121]
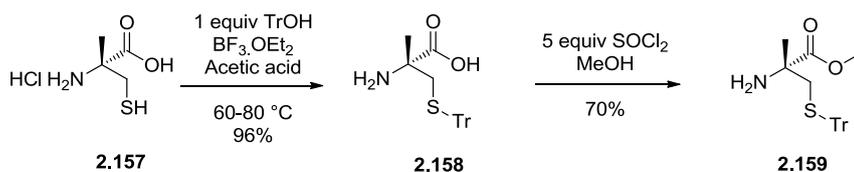
Scheme 2.32 : Protection of (*R*)-2-methylcysteine

We prepared amide **2.160** and attempted to form the thiazoline ring using the Kelly method (triphenylphosphine oxide and triflic anhydride), however no product was obtained (Scheme 2.33). We thought that this method was not suitable for 4-methyl thiazolines as no examples were described in the literature. However Doi and Gosh later published their syntheses of largazole where they employed the Kelly method to form the 4-methylthiazoline ring, respectively using the azide and Fmoc protecting group.[100,110] We hoped that the Alloc protecting group should be compatible with the Kelly conditions, however the reaction was unsuccessful. It is possible that the failed reaction was due to the use of an open bottle of triflic anhydride. After trityl deprotection of **2.160**, we tried to cyclise **2.161** with TiCl$_4$ but obtained a mixture of starting material and product that was difficult to separate.
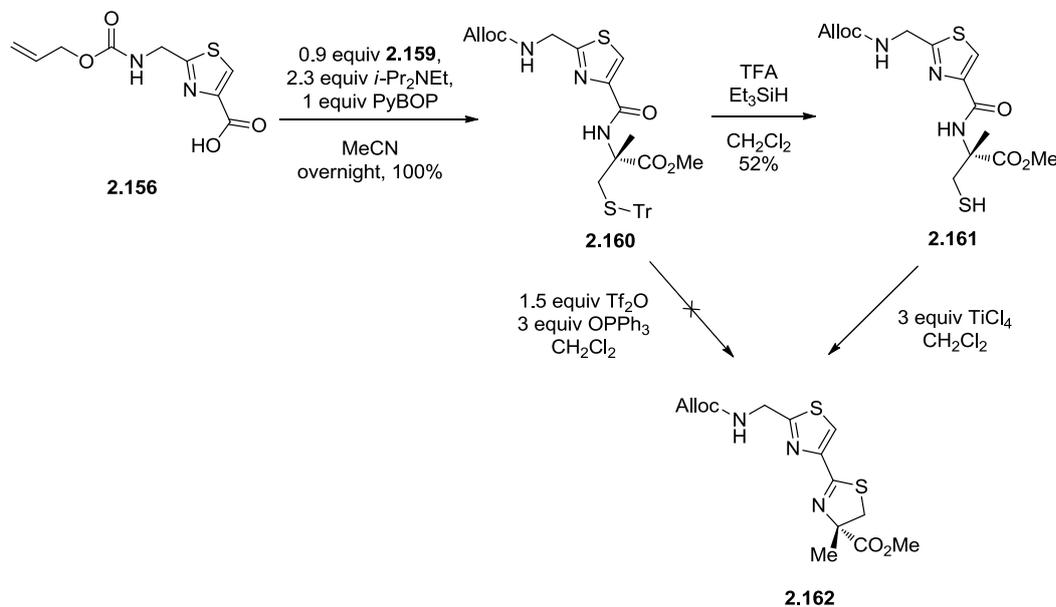
Scheme 2.33 : Attempt to synthesise fragment 2.162

As difficulties were encountered in the formation of the thiazoline ring, some modifications were necessary to the previous pathway. Conversion of the carboxylic acid **2.156** into the amide **2.163**, followed by dehydration with trifluoroacetic anhydride and triethylamine, resulted in the cyano- compound **2.164** in two steps (Scheme 2.34).[73,101] The latter was directly coupled to (*R*)-2-methylcysteine under mild conditions (phosphate buffer solution pH 6, methanol, 70 ºC, 2 h).[122] This procedure allowed the direct use of (*R*)-methylcysteine hydrochloride without addition of any protecting groups.
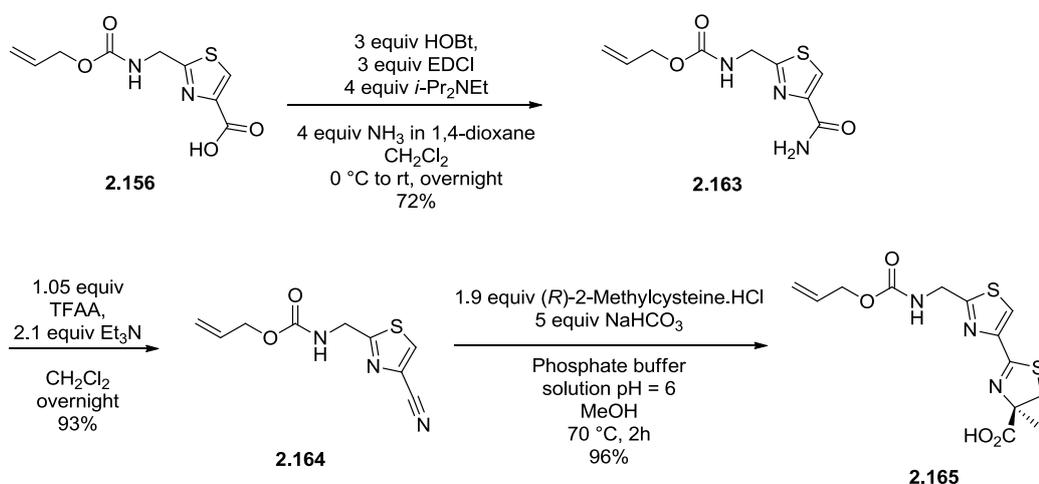
Scheme 2.34 : Synthesis of thiazole-thiazoline fragment 2.165

Removal of the Fmoc protecting group on the fragment **2.86**, using diethylamine, and PyBOP-mediated coupling to the thiazoline-thiazole carboxylic acid **2.165** provided the acyclic precursor **2.166** (Scheme 2.35). The Alloc protecting group was then removed using tetrakis(triphenylphosphine)palladium, with morpholine as a scavenger, in poor yield (30%). The TMSE group was deprotected to form the carboxylic acid using TFA, after which the macrolactonisation, using HATU under highly diluted conditions in order to avoid intermolecular reactions, was performed to give **2.168**. The quantity of final product obtained was very low.
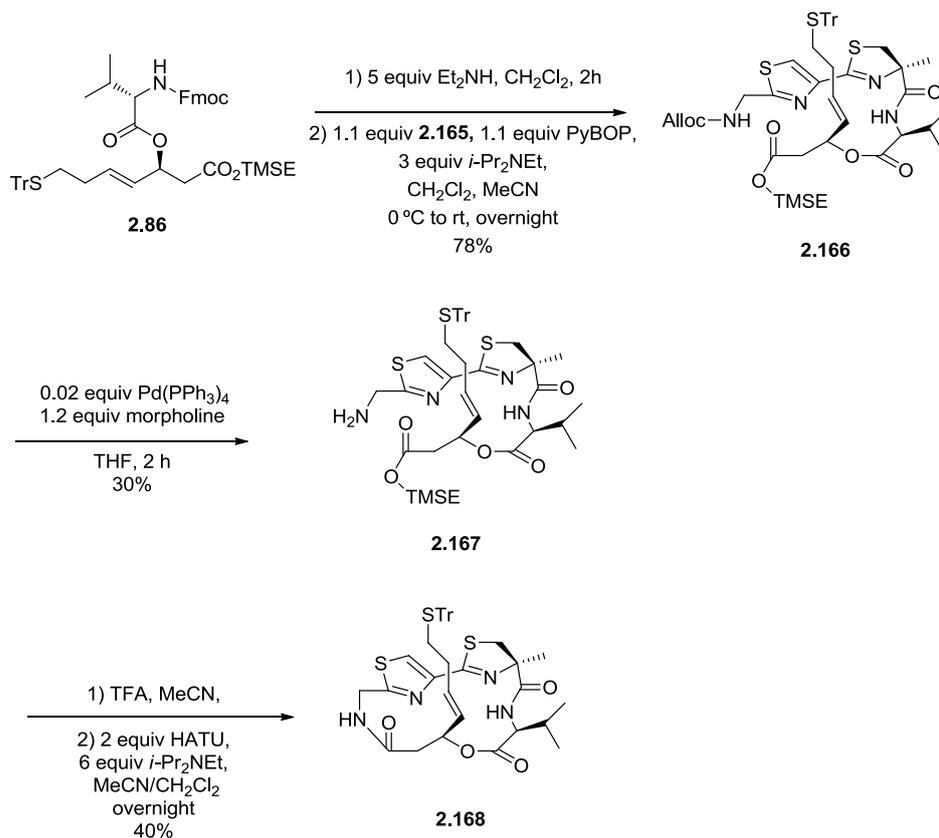
Scheme 2.35 : Attempts to synthesise largazole

Using compound **2.169** as a model, an undergraduate student, Emma Packard, attempted to deprotect the Alloc group. However, the reaction yielded a multi-component crude product from which low yields of desired product were obtained (Scheme 2.36). We believe that this is due to interference in the reaction by the β-hydroxy acid moiety and a different amine protecting group would be more effective.
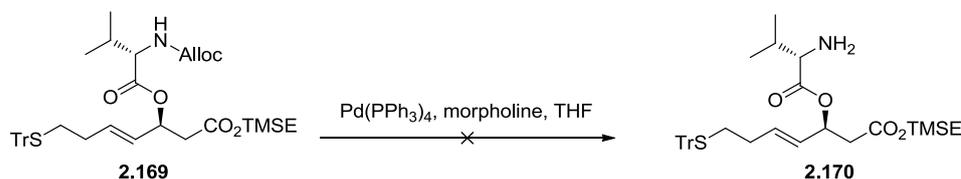
Scheme 2.36 : Removal of Alloc protecting group

As a result of changing our strategy to form the thiazoline ring, by using basic conditions, the Boc group became a suitable protecting group. We started the synthesis from the cheap starting material glycinamide hydrochloride instead of the more expensive, commercially available thioamide **2.149**. Glycinamide hydrochloride **2.152** was protected with a Boc group and the resulting amide **2.171** converted into a thioamide **2.149** at rt using Lawesson's reagent (Scheme 2.37).[123] Initial attempts to convert to the thioamide in refluxing toluene and then in refluxing THF were unsuccessful with decomposition observed in both cases. Classic thiazole formation followed by hydrolysis of the ester yielded the desired product **2.147**.
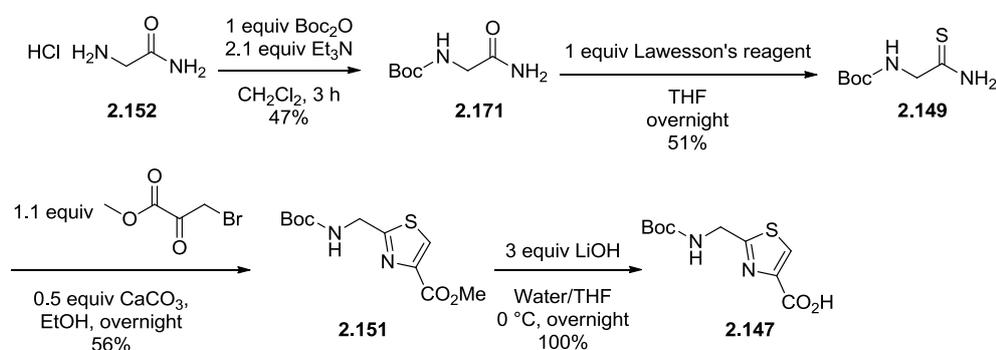
Scheme 2.37 : Synthesis of thiazole fragment 2.147

Due to the high costs of (*R*)-2-methylcysteine hydrochloride (£200 for 1 g) we decided to prepare it ourselves from commercially available starting materials. (*R*)-2-Methylcysteine hydrochloride **2.157** was prepared in four steps from cysteine using the Pattenden method (Scheme 2.38).[124,125] Condensation of (*R*)-cysteine methyl ester **2.172** with pivaldehyde yielded the thiazolidine **2.173** with a mixture of (2*R*, 4*R*) and (2*S*, 4*R*) diastereomers in a 1.9:1 ratio. *N*-Formylation of the thiazolidine **2.173** using sodium formate in the presence of formic acid led to a single (2*R*, 4*R*) *syn*-diastereomer **2.174** in 79% yield. Treatment of a solution of **2.174** in tetrahydrofuran at –90 ºC with lithium diisopropylamide in the presence of the additive 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU), followed by quenching of the resulting enolate with iodomethane at –90 ºC produced the corresponding 4-methylthiazolidine **2.175** in 51% yield. Methylation occurred anti- to the bulky *t*-butyl group with excellent diastereoselectivity observed. Hydrolysis of **2.175** in the presence of 5 M HCl solution

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produced the desired product (*R*)-2-methylcysteine hydrochloride **2.157** in excellent yield.
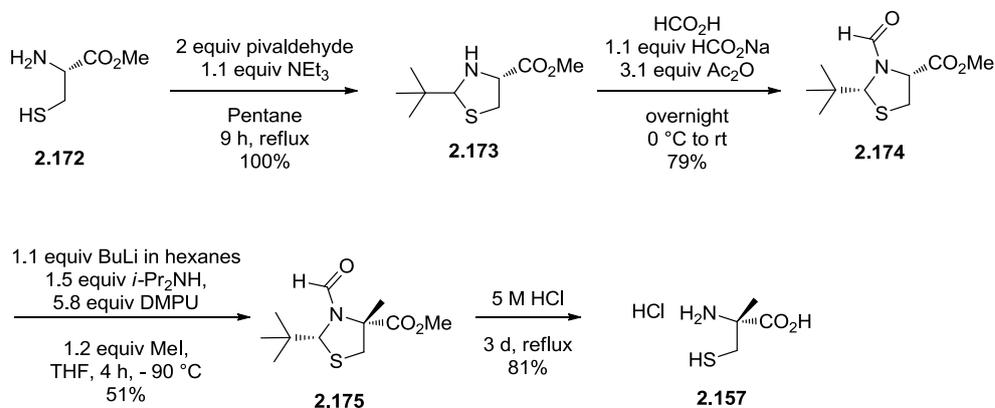
Scheme 2.38 : Preparation of (*R*)-2-methylcysteine hydrochloride (2.157)

The nitrile derivative **2.100** was prepared as previously described from the carboxylic acid **2.147** via the amide **2.176**, and then directly coupled to the (*R*)-methylcysteine under basic conditions to form the thiazole-thiazoline fragment **2.109** in excellent yield (Scheme 2.39).[122]
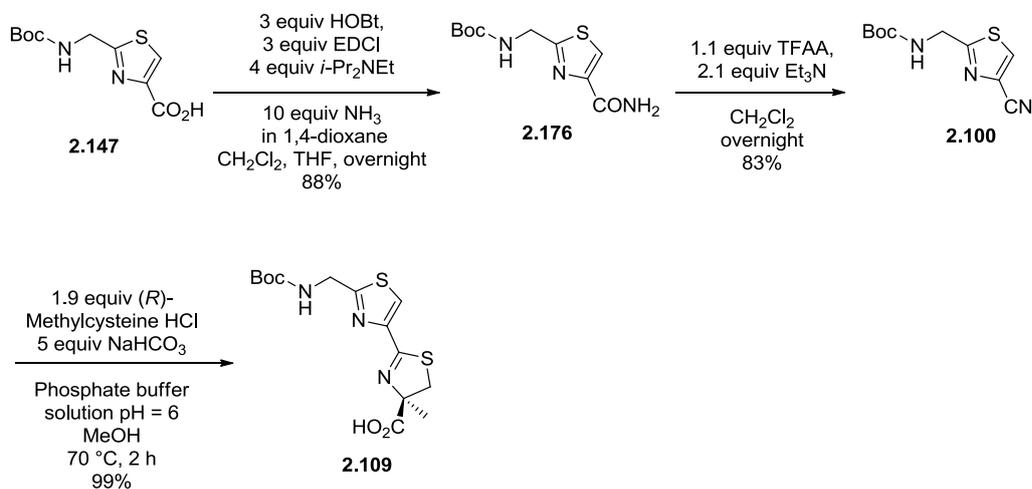
Scheme 2.39 : Synthesis of thiazole-thiazoline fragment 2.109

2.3.1.4. Coupling of the fragments

Fragment **2.86** was Fmoc deprotected and coupled to the carboxylic acid **2.109** using PyBOP as the coupling reagent (Scheme 2.40). TFA treatment removed the Boc and

TMSE protecting groups on the linear fragment of largazole.[96] It was necessary to extend the reaction times as the TMSE protecting group proved to be more difficult to remove than the Boc protecting group. The macrolactamisation was then performed under high dilution using HATU and HOBt (65% over 2 steps).[96] The *S*-trityl protecting group was removed using TFA and triethylsilane as scavenger to afford the active metabolite: largazole thiol in good yield (79%). Formation of the thioester using octanoyl chloride and triethylamine completed the total synthesis of largazole.[96]
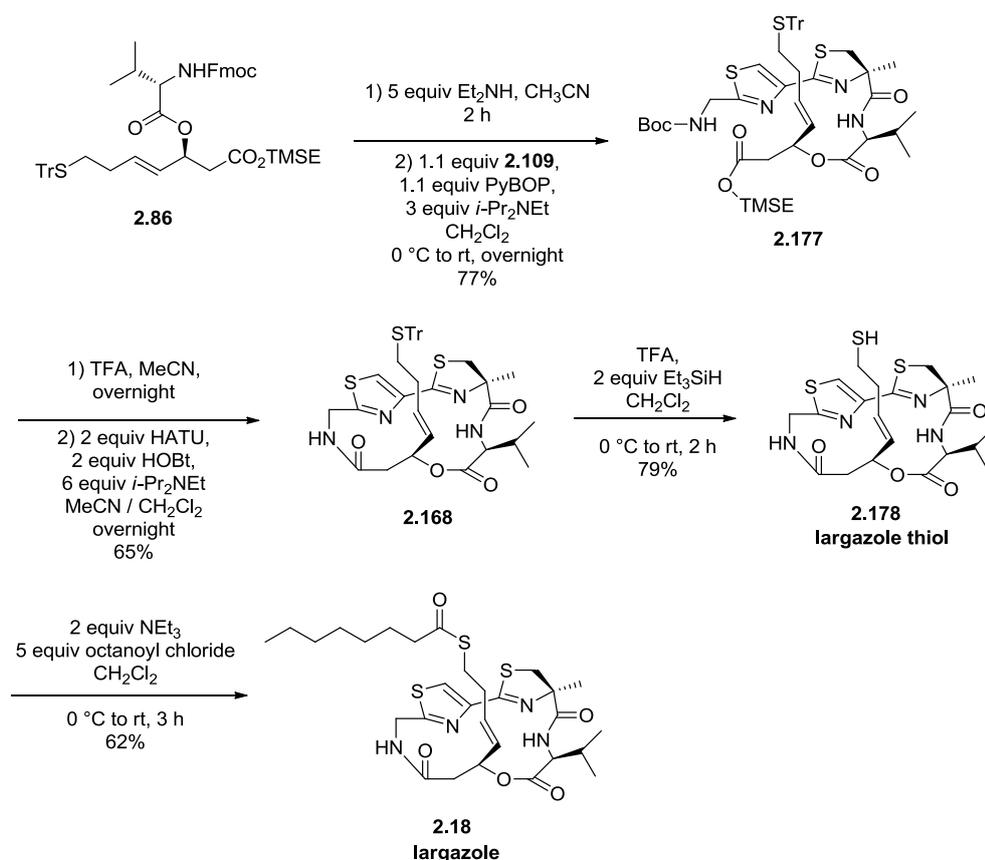
Scheme 2.40 : Synthesis of largazole (2.18)

Largazole was obtained in 2.4% overall yield in the longest linear sequence (11 steps).[126] The structure was confirmed by comparison of the ^{1}H and ^{13}C NMR data of the synthetic compound with that reported in the literature for the natural product (Figure 2.30, table 2.1).[80]
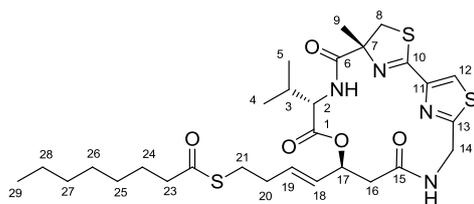
2.18

Figure 2.30 : Atom numbering used for largazole

#	δ^{1}H and mult.	Reported[80] ^{1}H and mult.	δ^{13}C and mult.	Reported[80] δ^{13}C and mult.
1			168.8, C	168.9, C
2	4.61, dd (9.3, 3.3)	4.61, dd (9.2, 3.3)	57.8, CH	57.7, CH
3	2.11, td (6.8, 3.5)	2.10, m	34.2, CH	34.2, CH
4	0.69, d (7.0)	0.68, d (7.2)	18.8, CH$_3$	18.9, CH$_3$
5	0.53, d (7.0)	0.50, d (7.2)	16.7, CH$_3$	16.6, CH$_3$
NH	7.17, d (9.0)	7.15, d (9.2)		
6			173.5, C	173.5, C
7			84.4, C	84.4, C
8a	4.05, d (11.5)	4.04, d (11.4)	43.3, CH$_2$	43.3, CH$_2$
8b	3.28, d (11.5)	3.27, d (11.4)		
9	1.88, s	1.87, br s	24.1, CH$_2$	24.2, CH$_3$
10			164.3, C	164.6, C
11			147.4, C	147.4, C
12	7.77, s	7.76, s	124.2, CH	124.2, CH
13			167.9, C	167.9, C
14a	5.29, dd (17.8, 9.3)	5.29, dd (17.4, 9.6)	41.1, CH$_2$	41.1, CH$_2$
14b	4.28, dd (17.6, 3.0)	4.27, dd (17.4, 2.5)		
NH	6.44, dd (9.3, 2.8)	6.45, dd (9.6, 2.5)		
15			169.4, C	169.4, C
16a	2.84, dd (16.6, 10.5)	2.86, dd (16.5, 10.5)	40.5, CH$_2$	40.5, CH$_2$
16b	2.69, dd (16.1, 2.5)	2.68, dd (16.5, 1.8)		
17	5.68, m	5.66, ddd (10.5, 7.2, 1.8)	72.0, CH	72.0, CH
18	5.52, dd (15.6, 7.0)	5.51, dd (15.6, 7.2)	128.4, CH	128.4, CH
19	5.84, m	5.82, dt (15.6, 7.2)	132.7, CH	132.7, CH
20	2.32, q (6.5)	2.31, br q (7.2)	32.3, CH$_2$	32.3, CH$_2$
21	2.91, t (7.5)	2.90, t (7.2)	27.9, CH$_2$	27.9, CH$_2$
22			199.4, C	199.4, C
23	2.54, t (7.5)	2.52, t (7.5)	44.1, CH$_2$	44.1, CH$_2$
24	1.63, m	1.64, m	25.6, CH$_2$	25.6, CH$_2$
25	1.28, m	1.29, m	28.9 or 29.0, CH$_2$	28.9, CH$_2$
26	1.28, m	1.25, m	28.9 or 29.0, CH$_2$	28.9, CH$_2$
27	1.28, m	1.26, m	31.6, CH$_2$	31.6, CH$_2$
28	1.28, m	1.28, m	22.6, CH$_2$	22.6, CH$_2$
29	0.88, t (6.8)	0.87, br t (6.9)	14.0, CH$_3$	14.0, CH$_3$

Table 2.1 : Collected and reported[80] ^{1}H and ^{13}C NMR data in ppm for largazole in CDCl$_3$

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2.3.2. Synthesis of largazole analogues

To study the structure-activity relationships of largazole, several modifications were envisaged, such as simplification of largazole or increasing its lipophilicity.

We decided to simplify the structure by replacing the valine with a glycine and also a β-alanine residue, which would increase the size of the macrocycle by one carbon. Replacing the 4-methyl thiazoline by the achiral Aib (aminoisobutyric acid) moiety was also envisaged to determine the influence of the thiazoline ring on the inhibition activity. Comparison of the glycine position in largazole with the other depsipeptides shows that they possess bigger substituents (alanine or valine). In addition, the phenylalanine derivative has been prepared by Sabrina Marie in order to incorporate a large substituent at this position and to increase the lipophilicity of the compound. It was proposed that it would be possible to improve the inhibition activity in a cell assay by modifying the thioester function of largazole through insertion of a valine subunit that can be recognised by a transporter.

FR901375 (2.14)

FK228 (2.15)

Spiruchostatin A R: H (2.16)
Spiruchostatin B R: Me (2.17)
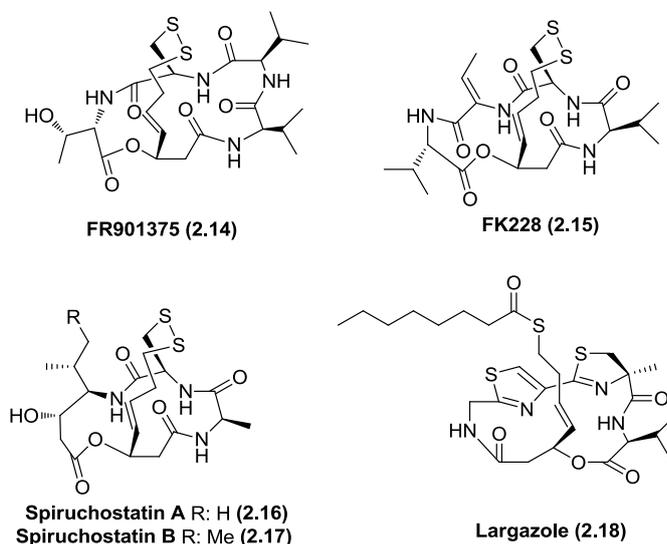
Largazole (2.18)

Figure 2.31 : Structures of FR901375, FK228, spiruchostatins and largazole

We were planning to prepare a total of five analogues (Figure 2.32).
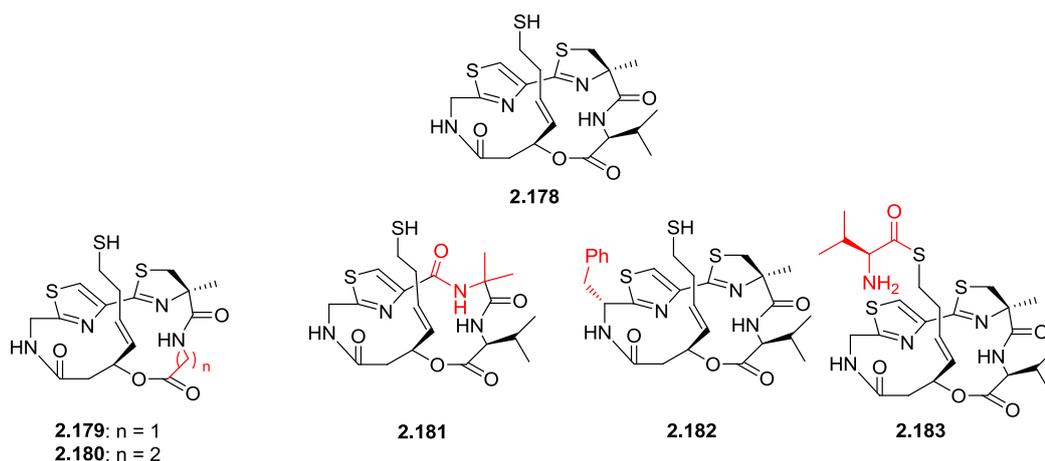
Figure 2.32 : Structures of largazole thiol and the proposed analogues

2.3.2.1. Synthesis of glycine and the β-alanine analogues

In order to make the glycine and the β-alanine derivatives **2.179** and **2.180**, it was necessary to prepare the fragments **2.184** and **2.185** by esterification of *N*-Fmoc-glycine or *N*-Fmoc-β-alanine with the previously prepared β-hydroxy acid derivative **2.85** (Scheme 2.41). The following steps were undertaken in the same way as those undertaken in the synthesis of largazole. The yield obtained for the 18-membered ring formation with the β-alanine residue is lower than that obtained for either of the 17-membered ring formations of largazole or the glycine analogue (18% yield versus 65% or 53% respectively).
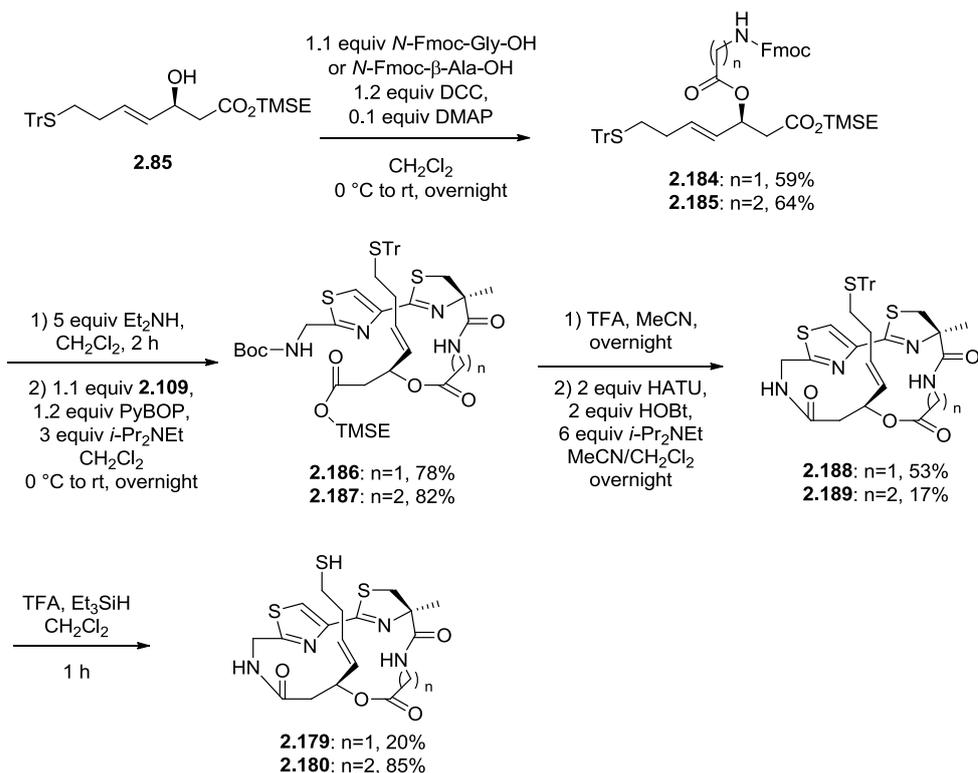
Scheme 2.41 : Synthesis of largazole analogues 2.179 and 2.180

2.3.2.2. Synthesis of the Aib analogue

The previously prepared carboxylic acid **2.147** was coupled to 2-aminoisobutyric acid to form the methyl ester **2.190**, which was then hydrolysed to afford the carboxylic acid **2.191** (Scheme 2.42). The Fmoc deprotected β-hydroxy acid derivative was coupled to the carboxylic acid **2.191** using PyBOP as a coupling reagent (62% over 2 steps). The following steps to obtain the Aib analogue **2.181** are repeated as above.
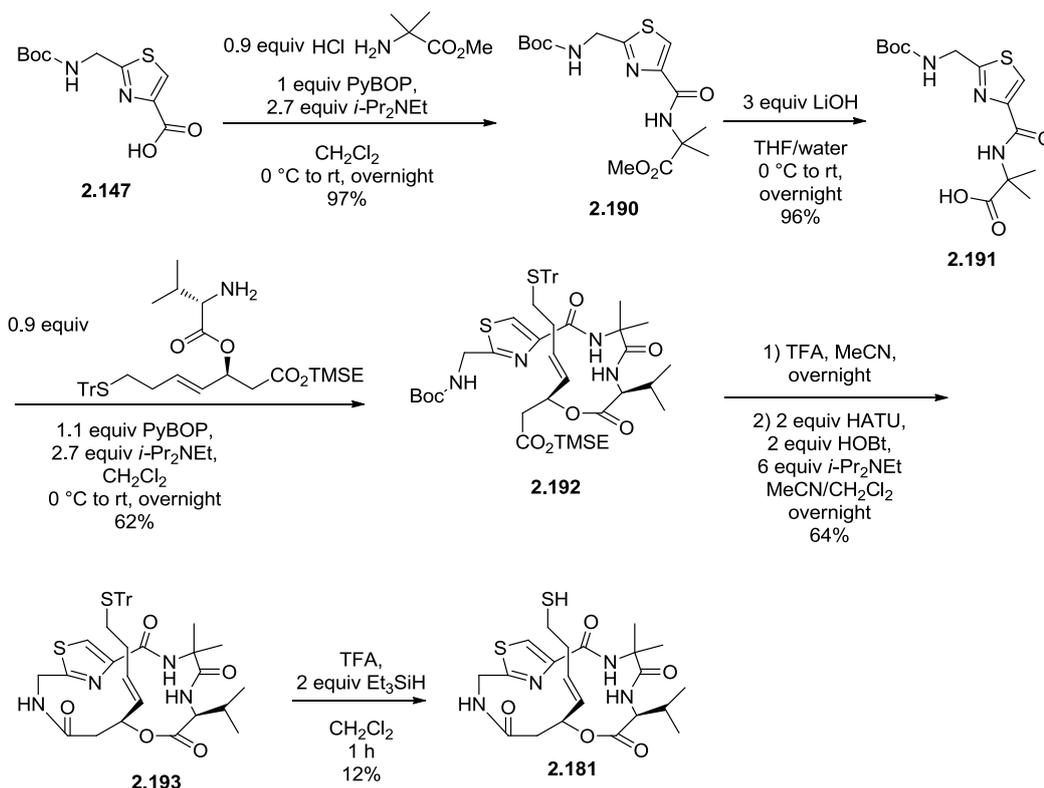
Scheme 2.42 : Synthesis of largazole analogue 2.181

2.3.2.3. Attempt to synthesise the valine thioester analogue

We coupled largazole thiol **2.178** with Boc-L-valine to form **2.194** and then removed the Boc group to afford the desired largazole analogue **2.183** (Scheme 2.43).
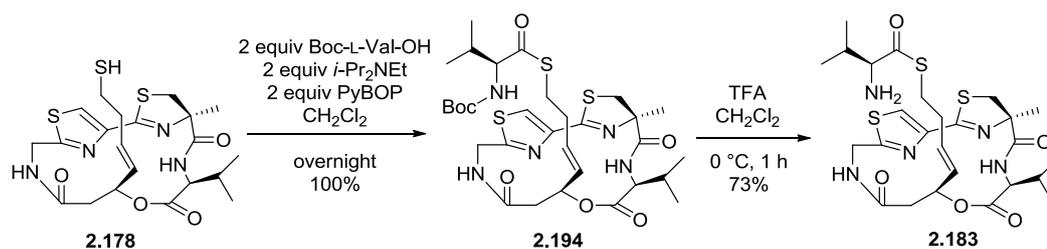
Scheme 2.43 : Attempt to synthesise the valine thioester analogue

The thioester **2.183** was found to be unstable over time as partial hydrolysis occurred despite the compound being stored in the freezer. Further quantities of this analogue were not prepared due to the cell assay showing similar inhibition activity to the largazole thiol.

2.3.3. Biological results and metabolic studies of largazole and its analogues

A Western blot experiment was performed by Annette Hayden at the Cancer Research UK centre in Southampton using an antibody which binds only to the acetylated form of histone 4 lysine 8 (acH4K8) (Figure 2.33). This experiment measures acetylated histone proteins levels in protein extracts from three different breast cancer cell lines: MCF7, MDA-MB-231 and SKBr3. These cancer cell lines are representative of three major subtypes of breast cancer cell lines. MCF7 are estrogen receptor positive cancer cell lines; MDA-MB-231 are triple negative and SKBr3 are HER2 positive cancer cell lines. Largazole and largazole thiol were tested in comparison with untreated cells. The strong bands correspond to the presence of acetylated H4K8. This experiment shows that in the presence of inhibitor, the deacetylation activity of HDACs decreases for the three cancer cell lines as shown by enhancement of the level of the acetylated form of H4K8. As expected largazole, which is more cell permeable, has a more intense band than largazole thiol.
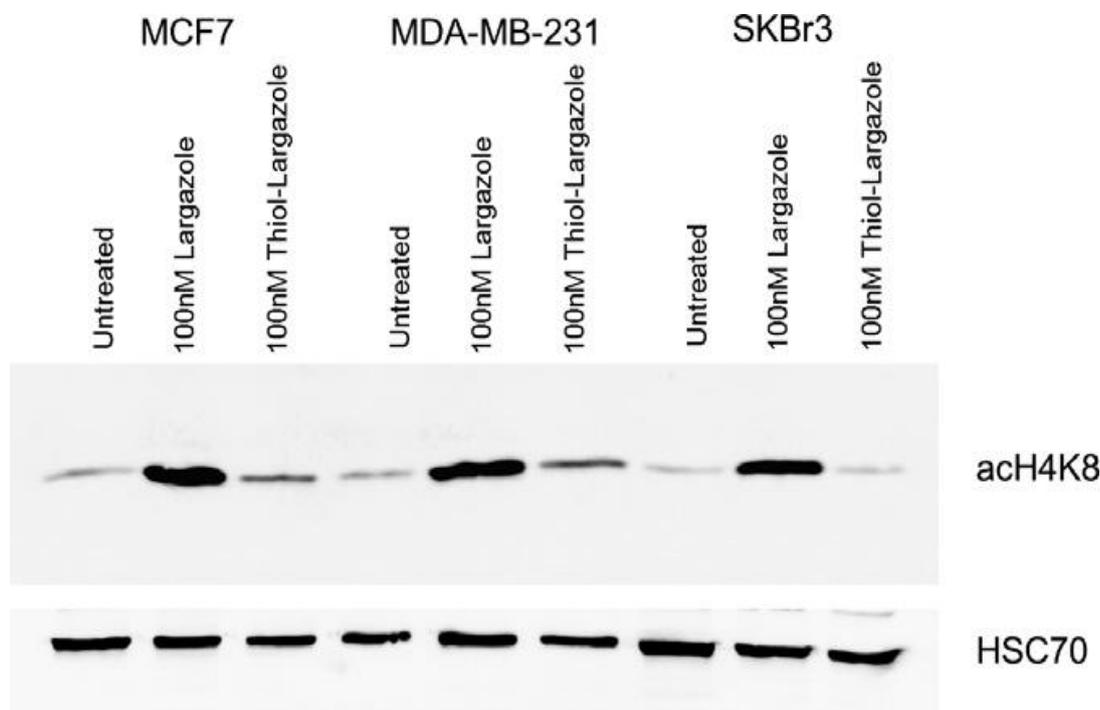
Figure 2.33 : Western blotting of acH4K8 in MCF7, MDA-MB-231 and SKBr3 cell lines with DMSO control, largazole and largazole thiol

The compounds were tested in both enzyme and cell assays by Annette Hayden. The cell assay involves the measurement of growth inhibition activity of the compounds against the breast cancer cell line MCF7.

The Fluor de Lys® (Fluorescent deacetylation of lysine) assay system is used to measure the inhibition of histone deacetylase activity in HeLa cell extracts. Deacetylation of a Fluor de Lys® substrate by a deacetylase sensitizes the substrate which then produces a fluorophore after treatment with the Fluor de Lys® developer. The fluorophore is excited with light (wavelength 360 nm) and the emitted light (460 nm) is detected on a fluorometric plate reader.
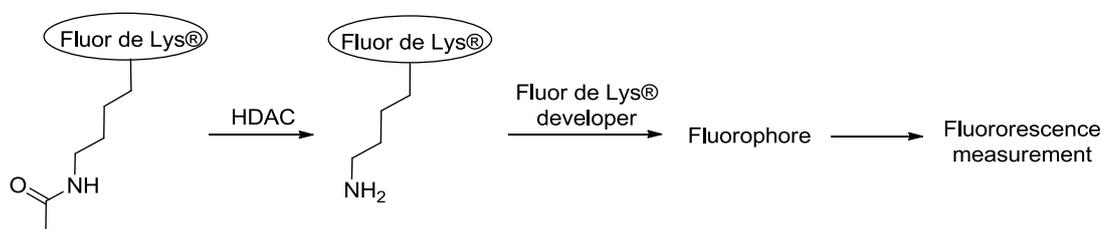
Scheme 2.44 : Simplified reaction scheme of the Fluor de Lys® fluorometric assay

Largazole and largazole thiol were first tested in the Fluor-de-Lys® HDAC enzyme assay and the cell line growth inhibition assay in the breast cancer cell line MCF7 (Figure 2.34). Largazole thiol gives excellent inhibition activity in the enzyme assay (43 pM) and largazole had potent antiproliferative activity. Largazole is hydrolysed to its active metabolite, largazole thiol, under physiological conditions. Nevertheless, we are surprised to see that largazole thiol is such a potent HDAC inhibitor in the enzyme assay but it is not so good in the cell assay, as we expected a picomolar inhibitor to give better results in the cell assay (5 nM for largazole versus 0.8 nM for FK228).

		2.178		2.18	
largazole thiol				largazole	
HDAC IC$_{50}$	43 pM		HDAC IC$_{50}$	572 nM	
MCF7 IC$_{50}$	277 nM		MCF7 IC$_{50}$	5 nM	
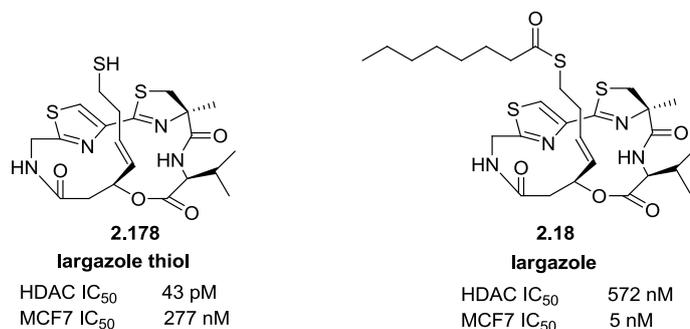
Figure 2.34 : HDAC inhibition and growth inhibition of largazole thiol (2.178) and largazole (2.18)

The largazole analogues were tested in a similar manner in comparison with largazole thiol (Table 2.2). The glycine analogue **2.179** is a subnanomolar HDAC inhibitor which suggests that the valine is not indispensable to the activity and can be replaced by a glycine residue. The β-alanine analogue **2.180** is also a nanomolar HDAC inhibitor and shows that an increase in the size of the macrocycle is tolerated. The Aib derivative **2.181** exhibits a nanomolar inhibition activity. Nevertheless, the cell assay results are very poor for these three compounds which was as expected since they are free thiols and not prodrugs. On the contrary, the phenylalanine analogue **2.182** is a weaker HDAC inhibitor in the enzyme assay (a 400 fold reduction) even though it has a similar antiproliferative activity to largazole thiol.

Compound	HDAC inhibition (nM)	MCF7 inhibition (nM)
Largazole (2.18)	572 ± 29	5 ± 1
Largazole thiol (2.178)	0.043 ± 0.026	277 ± 130
Bn analogue 2.182	17.2 ± 0.05	377 ± 62
Glycine analogue 2.179	0.17 ± 0.35	2458 ± 1135
β-alanine analogue 2.180	3.15 ± 0.07	>10,000
Aib analogue 2.181	0.99 ± 0.07	5902 ± 1698

Table 2.2 : HDAC inhibition and growth inhibition of largazole and related analogues

Our results show that some modifications in the structure of largazole are possible and tolerated. From the data, we observed that the lipophilicity is a very important factor in cell assay. In fact, incorporating a phenylalanine residue increased the activity in cells and simplification of the valine residue with glycine or β-alanine decreased this activity. In the Aib and β-alanine analogues, the rigidity of the macrocycle is also modified and this might explain the difference in activity.

We were interested in the pharmacokinetic properties of largazole and related analogues and decided to investigate their stability in the presence of mouse liver homogenate. These studies were carried out by Prof Paul Loadman and Jason Lyle from the University of Bradford. Largazole was found to be unstable, with a half-life of less than 5 min, undergoing rapid thioester hydrolysis to largazole thiol (Table 2.3). Largazole thiol itself is relatively stable in liver homogenate with a half-life of 51 min at 37 ℃. The glycine analogue **2.179** has similar stability with a half-life of 32 min. A major metabolite of unknown structure is formed in the case of largazole thiol **2.178** and the glycine analogue **2.179**. The β-alanine and Aib analogues are highly unstable with a half-life of less than 5 min with a complex metabolism which could not be elucidated.

Compound	Half life (min)
Largazole (2.18)	<5
Largazole thiol (2.178)	51
Glycine analogue 2.179	32
β-alanine analogue 2.180	<5
AIB analogue 2.181	<5

Table 2.3 : Half-life of largazole and related analogues

This study showed that the thioester prodrug is metabolised too quickly and the disulfide depsipeptides are more stable than largazole. Studies to find a more stable thioester would be necessary for future *in vivo* applications of largazole or related thioesters.

2.4. SYNTHESIS AND BIOLOGICAL ACTIVITY OF THAILANDEPSINS AND BURKHOLDACS

2.4.1. Synthesis of thailandepsins

2.4.1.1. First structures of thailandepsins

The first structures of thailandepsins A, B and C were published by Cheng and co-workers (Figure 2.35).[83] The structures were elucidated by study of the biosynthesis gene cluster of thailandepsins but no stereochemistry was assigned. Thailandepsins A and B were found to possess an unusual hemiaminal function only rarely encountered in cyclic peptide natural products (for example, tyrocidine A).[127] Thailandepsin C contains an imine function resulting from the loss of water from the hemiaminal **2.22**.

2.21: R = CH₃
2.22: R = H

2.23
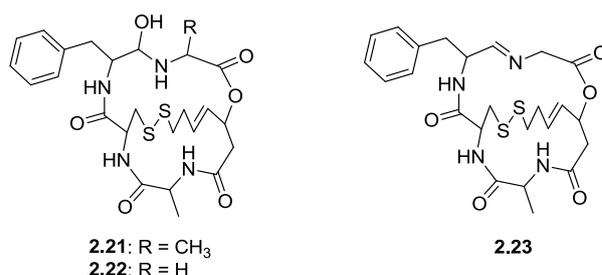
Figure 2.35 : Structures of thailandepsins A (2.21), B (2.22) and C (2.23)

We predicted the stereochemistry by analogy to the other depsipeptides assuming that the stereochemistry of the β-hydroxy acid would be the same as in the other depsipeptides. We thought that D-cysteine, D-alanine and D-phenylalanine are the most plausible stereochemistry for thailandepsins by analogy to spiruchostatins and FK228 (Figure 2.36).

2.195: R = CH₃
2.196: R = H
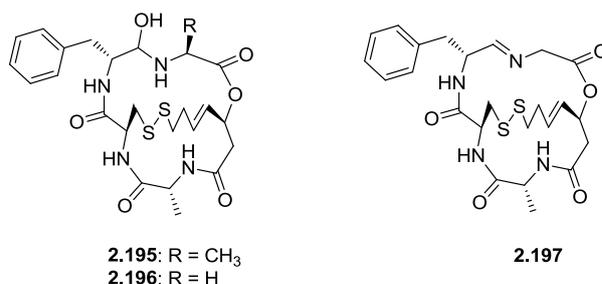
2.197

Figure 2.36 : Suggested stereochemistry of thailandepsins A (2.195), B (2.196) and C (2.197)

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Investigation into the total synthesis of thailandepsins reveals a starting point from the β-hydroxy acid derivative and several amino acids such as alanine, glycine, cysteine and phenylalanine. This is highlighted in the retrosynthetic analysis of thailandepsins A and B (Scheme 2.45). Thailandepsin C can be obtained from thailandepsin B.
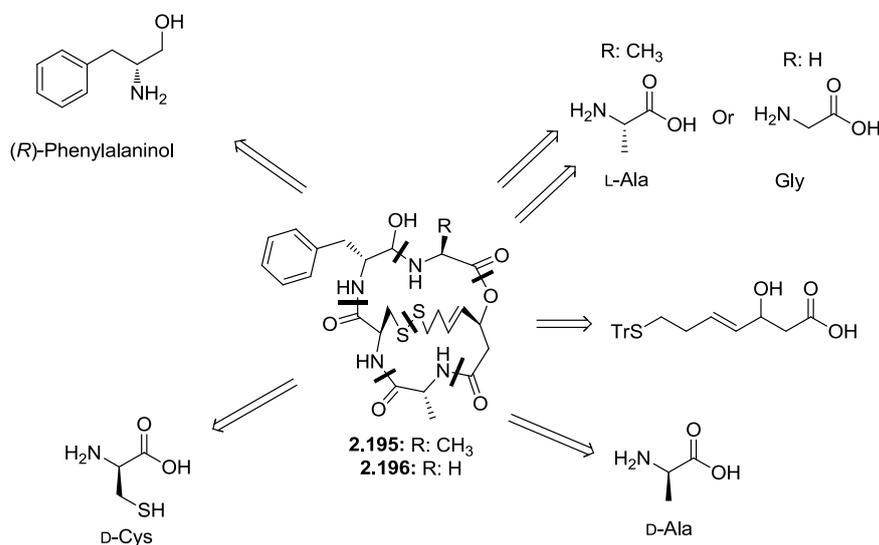
Scheme 2.45 : Retrosynthetic analysis of thailandepsins A (2.195) and B (2.196)

Thailandepsins can be prepared by a convergent synthesis from fragment **2.198** and the β-hydroxy acid derivative **2.199** or **2.200** (Scheme 2.46).
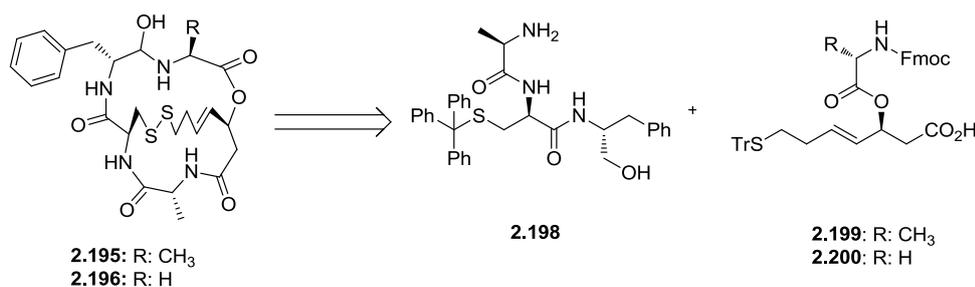
Scheme 2.46 : Retrosynthetic analysis of thailandepsins A (2.195) and B (2.196)

Our first target was the synthesis of the amine fragment **2.198** (Scheme 2.47). Coupling of Fmoc-D-Cys(Tr)-OH and (R)-phenylalaninol, using PyBOP and HOBt as coupling reagents and N-methylmorpholine as a base, led to the formation of **2.201** without the need for protection of the alcohol.[128] Removal of the Fmoc group yielded **2.202** which

was then coupled to Fmoc-D-Ala-OH. The desired fragment **2.198** was obtained after Fmoc deprotection of **2.203**.
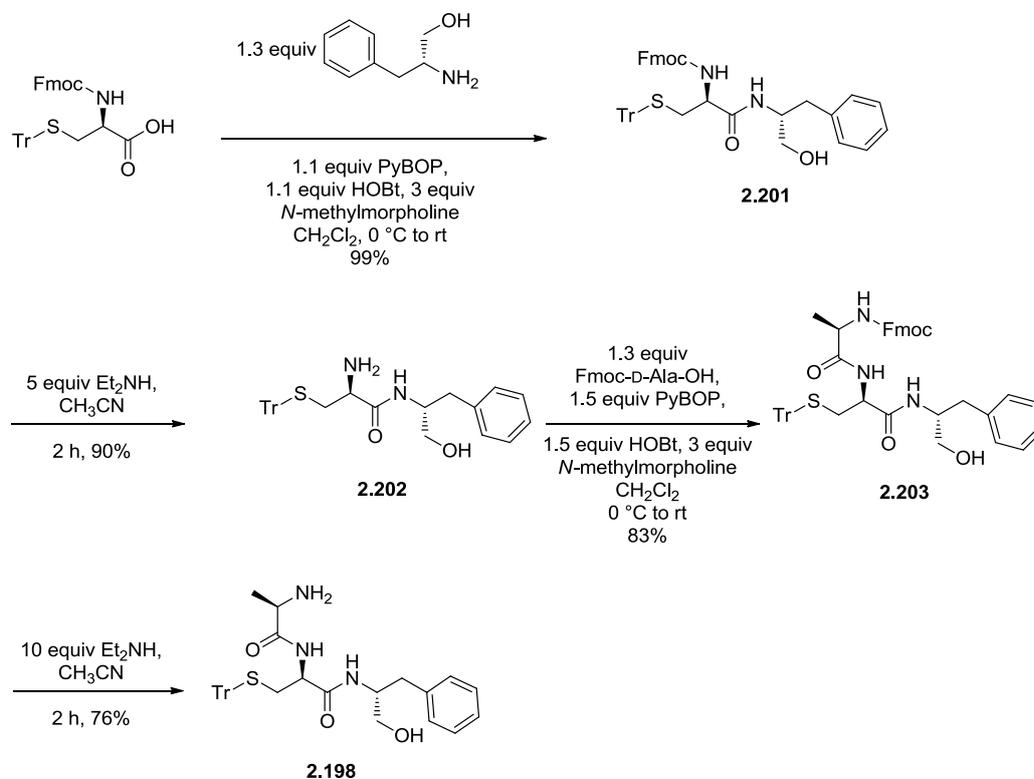
Scheme 2.47 : Synthesis of fragment 2.198

Fragment **2.204** had already been used in the synthesis of the glycine largazole analogue. The treatment of **2.204** with TFA gave an excellent yield with no deprotection of the trityl group (Scheme 2.48).

Scheme 2.48 : Synthesis of the carboxylic acid 2.200

The next step in the synthesis was to couple the two fragments **2.200** and **2.198** and to then form the disulfide bond prior to the challenging cyclisation.

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We worked on the model amide **2.201** in order to find suitable conditions for the oxidation of the alcohol and Fmoc deprotection (Scheme 2.49). Two routes are possible: oxidation followed by Fmoc deprotection or Fmoc removal and then oxidation.
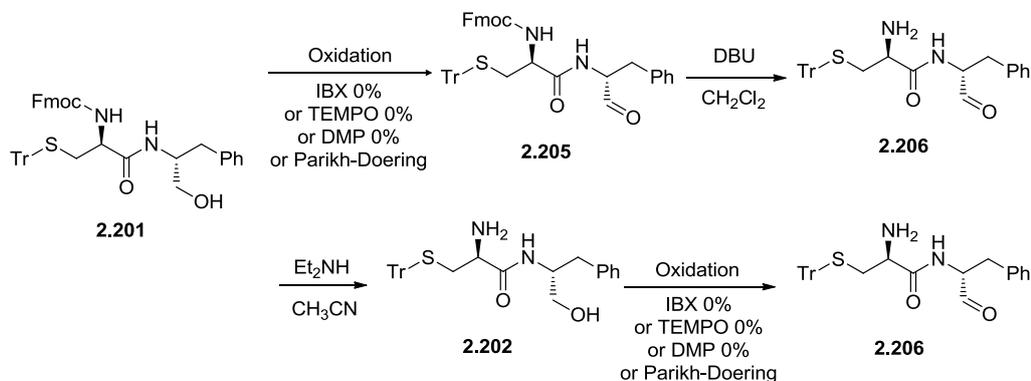
Scheme 2.49 : Attempts to obtain compound 2.206 from model amide 2.201

We first tried different oxidation conditions on substrate **2.201** including IBX, TEMPO, Dess-Martin periodinane and Parikh-Doering.[129-132] IBX, TEMPO and Dess-Martin oxidation were unsuccessful, however the Parikh-Doering conditions allowed us to convert the hydroxyl function into the aldehyde. Fmoc deprotection with a non-nucleophilic base, such as DBU, is necessary due to the presence of the aldehyde. In the alternate pathway, the Fmoc group was removed with diethylamine and the primary alcohol oxidised with Parikh-Doering conditions. Both pathways seemed to lead to the formation of a cyclic compound which we proposed was the 6-membered ring containing an imine function. We would prefer to remove the Fmoc group before oxidation of the alcohol as the free amine will not cause problems in the oxidation step.

The amine **2.198** was coupled to the β-hydroxy acid derivative **2.200**, using the same coupling conditions as previously reported, to afford **2.207** (Scheme 2.50). An oxidative disulfide bond formation was then performed using iodine and MeOH in CH_2Cl_2 to give **2.208**.[78] Firstly, Fmoc was removed using diethylamine and the alcohol **2.209** was then oxidised to the aldehyde. No trace of product was found by NMR or mass spectrometric analysis. We then attempted the oxidation of the hydroxyl group using Parikh-Doering conditions followed by the Fmoc deprotection using diethylamine but were again unsuccessful. One of the major problems encountered in this synthesis is the solubility of the compounds. Whilst mass spectrometric analysis shows the formation of the

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compounds **2.207**, **2.208**, **2.209** and **2.210**, NMR characterisation is problematic as the peaks are not well defined.
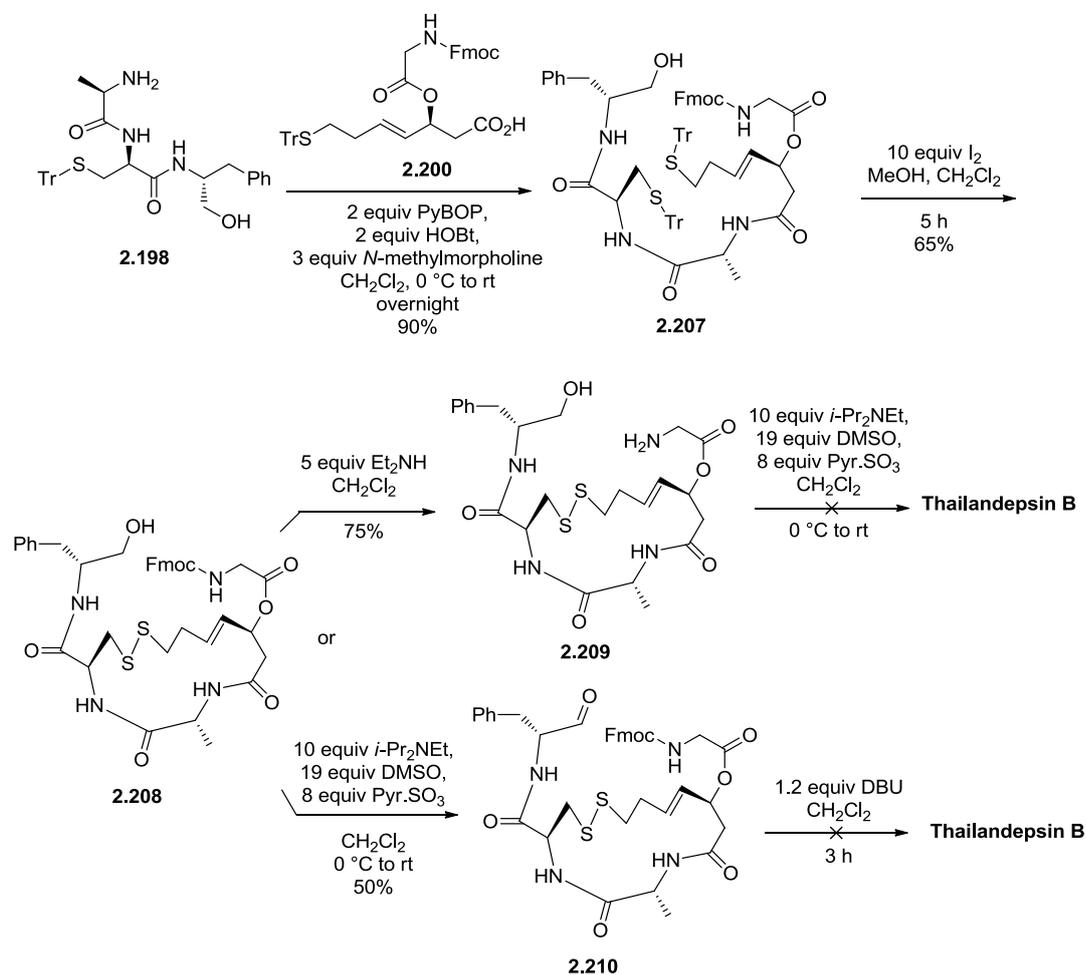
Scheme 2.50 : Attempts to synthesise thailandepsin B

We proposed that a way of solving the oxidation step would be to oxidise the alcohol **2.203**, and then protect as an acetal using Noyori's conditions, earlier in the synthesis.[133] After the protection the reaction sequence is similar to that described previously with the last step being deprotection of the acetal group of **2.216** (Scheme 2.51).
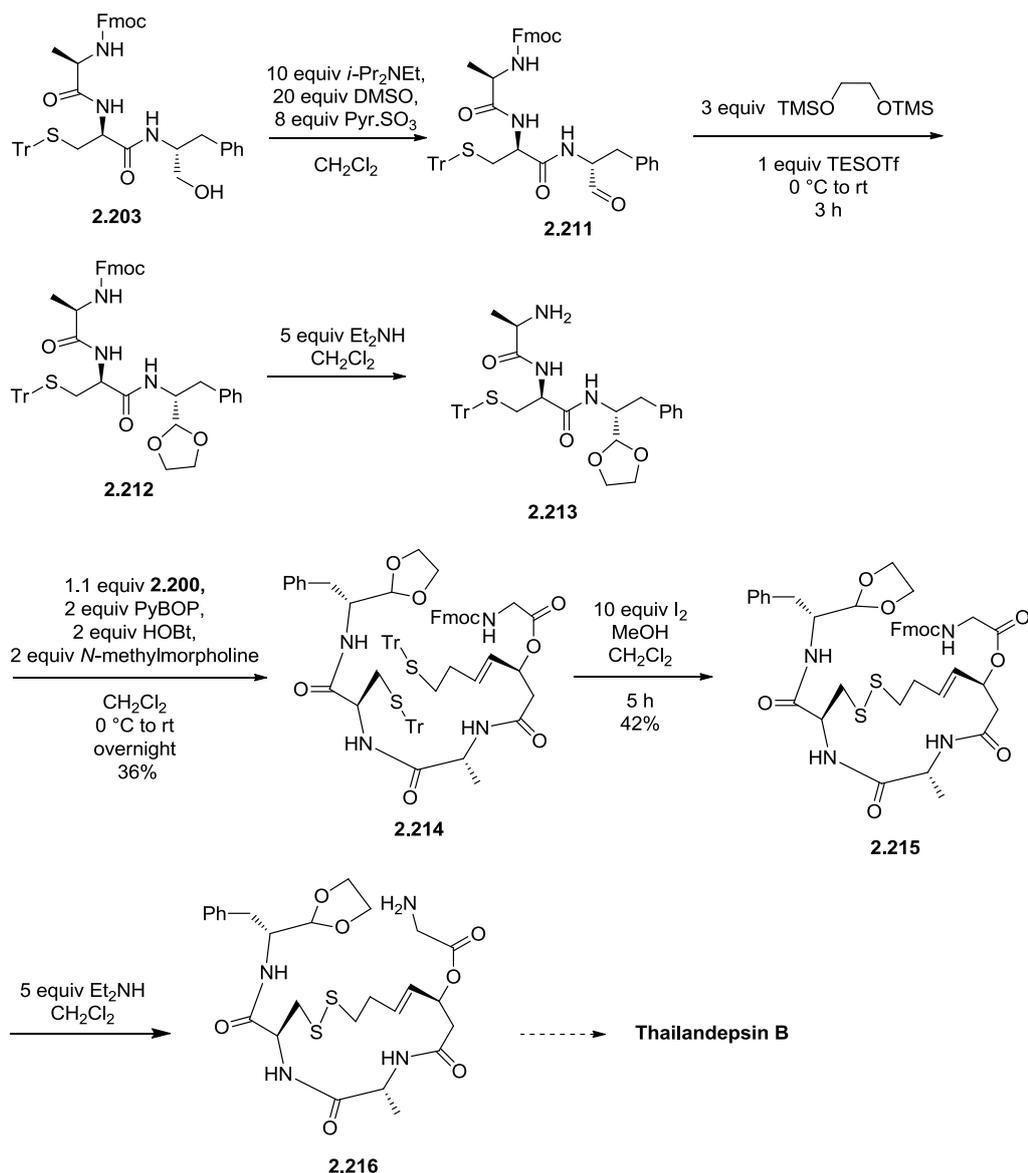
Scheme 2.51 : Attempts to synthesise thailandepsin via aldehyde protection

Unfortunately, we encountered a different problem in this sequence with epimerisation, detected by TLC and NMR spectroscopy, occurring during the protection step of the aldehyde. Separation of these diastereomers was very difficult by flash chromatography. We tried to protect the aldehyde at a lower temperature (−78 °C) but only starting material was recovered. The synthesis was continued using the mixture of the two diastereomers but due to low yields the synthesis could not be finished. Whilst researching alternative literature procedures for the protection of the aldehyde, which would avoid epimerisation (ethylene glycol, pyridinium *p*-toluenesulfonate, toluene), we realised that there were mistakes in the structures published by Cheng.[134] The molecular weights and structures that Cheng published in his patent do not match the

data deduced from the structures (Table 2.4).[83] We contacted Cheng and although he acknowledged that the structures in the patent were incorrect, he refused to share additional information about the natural products.

Compound	Formula and Mw deduced from structures	Formula and Mw given on the patent[83]
Thailandepsin A	Formula: $C_{25}H_{34}N_4O_6S_2$ Mw: 550.69 g/mol	Formula: $C_{25}H_{31}N_4O_6S_2$ Mw: 547.68 g/mol
Thailandepsin B	Formula: $C_{24}H_{32}N_4O_6S_2$ Mw: 536.66 g/mol	Formula: $C_{24}H_{29}N_4O_6S_2$ Mw: 533.65 g/mol
Thailandepsin C	Formula: $C_{24}H_{30}N_4O_5S_2$ Mw: 518.65 g/mol	Formula: $C_{24}H_{27}N_4O_5S_2$ Mw: 515.63 g/mol

Table 2.4 : Comparative table of reported[83] and corrected formula and molecular weight (Mw) for the different thailandepsins

2.4.1.2.   Revised structures of thailandepsins

In a later presentation to the NCI, Cheng reported revised structures for thailandepsins A and B (Figure 2.37). There are only two thailandepsins and not three as he postulated earlier. Their structures, which were elucidated by biosynthesis, are very similar to the spiruchostatins. The stereochemistry was predicted to be L-Met/Nle, D-Cys and L-Ile.
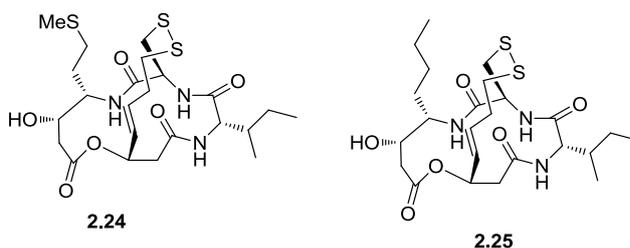
Figure 2.37 : Revised structures of thailandepsins A (2.24) and B (2.25)

Although the structures were not published in the literature but only viewed in a presentation, synthesizing these compounds is still of interest as they are analogues of the potent HDAC inhibitor, depsipeptide spiruchostatin. We decided to prepare thailandepsin A, which contains the methionine residue, and believe that the most likely

stereochemistry would be the D- amino acids by analogy to spiruchostatins. We chose to prepare thailandepsin A over B because of the methionine residue, as we believe that the sulfur containing compound is more interesting than the carbon chain compound. Nevertheless, there is uncertainty regarding the β-chiral centre of the isoleucine residue as during the biosynthesis of the natural product one or two of the chiral centres of isoleucine could be epimerised. To resolve this issue, both diastereomers of D-isoleucine would need to be incorporated in the synthesis. The first compound we prepared contains the β-hydroxy acid, D-methionine statine derivative, D-cysteine and D-isoleucine components (Scheme 2.52).
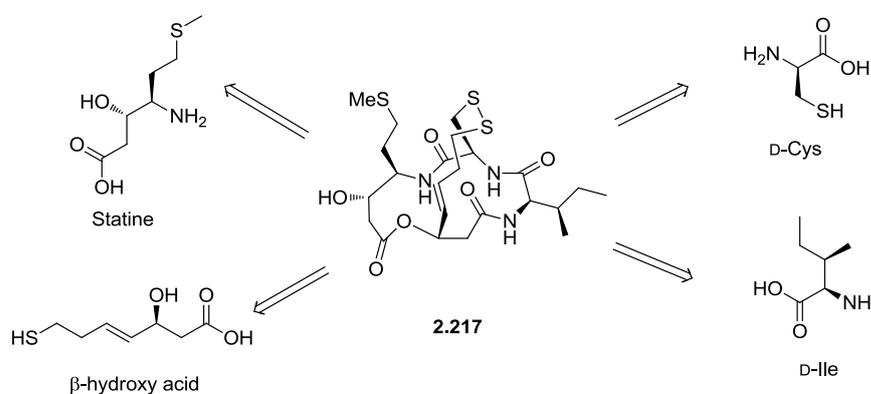
Scheme 2.52 : Retrosynthesis of thailandepsin A (2.217) with our suggested stereochemistry

Our synthesis route will be inspired by the synthesis of spiruchostatin, previously undertaken by our group, with some modifications.[78] We decided to use an allyl protecting group on the statine to avoid deprotection of the methyl ester and its reprotection with Tce.[135] We will prepare compound **2.218** and couple it to the β-hydroxy acid derivative **2.219** (Scheme 2.53). After allyl deprotection, the macrolactonisation will be performed using Shiina reagent which has been shown to give the best yield for this cyclisation.[90] The disulfide bond formation will be the last step of the synthesis.
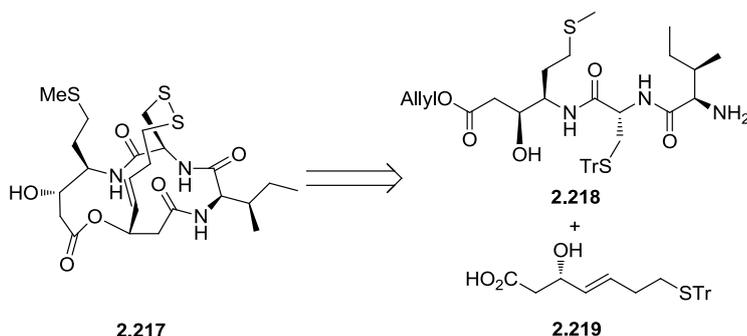
Scheme 2.53 : Retrosynthesis of thailandepsin A (2.217) with our suggested stereochemistry

The statine **2.222** was obtained by Claisen condensation of Boc-D-Met-OH pentafluorophenyl ester **2.220** with allyl acetate, followed by diastereoselective reduction of **2.221** (Scheme 2.54).[106] It was observed that the reaction time of the reduction step is very important. A longer reaction time of 135 min resulted in the additional reduction of the allyl ester with a reaction yield of 6%; with a shorter reaction time of 50 min the yield increased to 81%. The Boc group was then removed by treatment with TFA and the free amine was coupled to Fmoc-D-Cys(Tr)-OH to give **2.223**. After Fmoc deprotection of **2.223**, the free amine was coupled with Fmoc-D-Ile-OH to afford the fragment **2.224**.
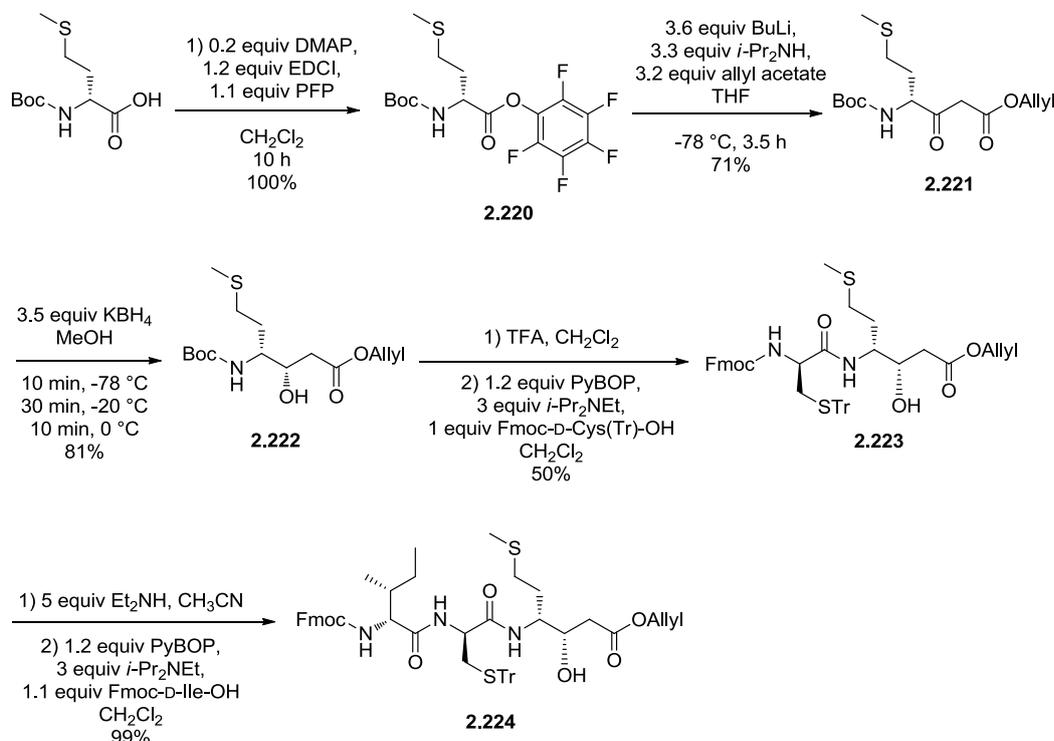
Scheme 2.54 : Synthesis of the tetrapeptide fragment 2.224

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The β-hydroxy acid previously used in the synthesis of largazole was hydrolysed in order to obtain the carboxylic acid **2.219** (Scheme 2.55).
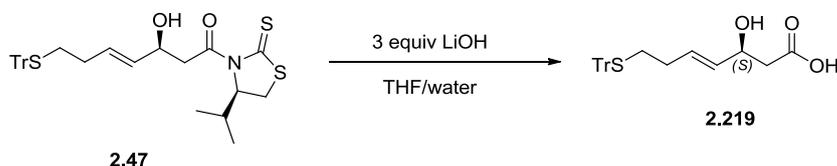
Scheme 2.55 : Synthesis of 2.219 by hydrolysis of 2.47

Fragment **2.224** is Fmoc deprotected and coupled to **2.219**. Removal of the allyl group by palladium-catalysed reaction with morpholine provided the deprotected linear precursor which then underwent macrolactonisation to give **2.226** (Scheme 2.56). We decided to use 2-methyl-6-nitrobenzoic anhydride (MNBA) as the reagent for the macrocyclisation as it gave the best yield for the macrocyclisation of spiruchostatin A, as reported by Doi and Takahashi.[90] An oxidative disulfide bond formation was then performed using iodine and MeOH in CH$_2$Cl$_2$ to afford **2.217**.
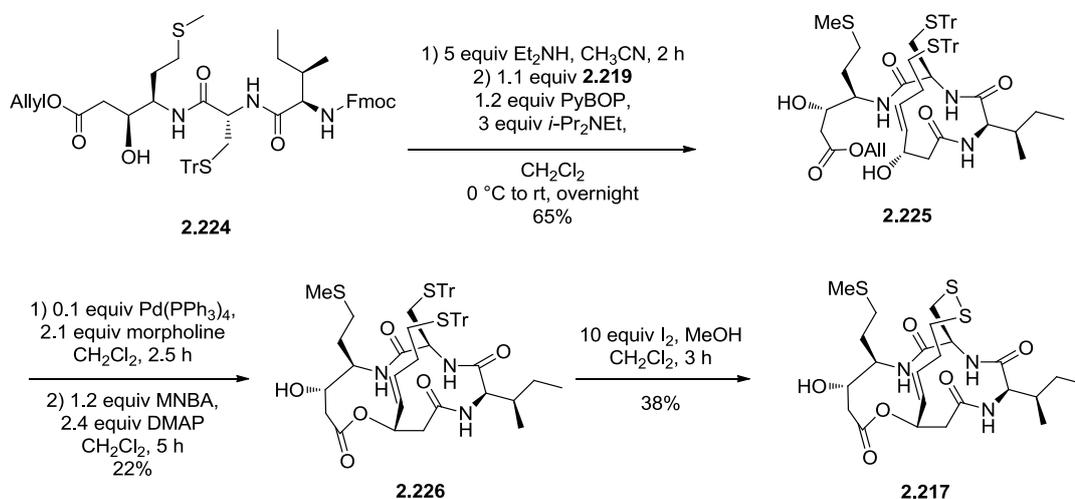
Scheme 2.56 : Synthesis of thailandepsin A (2.217)

No further information was available to us to compare the compound made with the natural product. We asked Cheng once again for further details but with no success. We planned nevertheless to test the biological activity of this spiruchostatin analogue.

2.4.2. Elucidation of the stereochemistry of burkholdacs

2.4.2.1. Synthesis of Brady's proposed diastereomer (L-Ile, D-Cys and L-Met)

Later on the structures of burkholdacs A and B, isolated by Brady and co-workers, were published and found to be surprisingly similar to Cheng's revised structures of the thailandepsins (Figure 2.38).[84] We started to think that the natural products isolated by Cheng and Brady are the same compounds as they were all isolated from the same bacterial strain. The structures of the burkholdacs were elucidated by spectroscopic analysis and analysis of the biosynthetic gene cluster.

2.26 R:H Burkholdac A
2.27 R:Me Burkholdac B
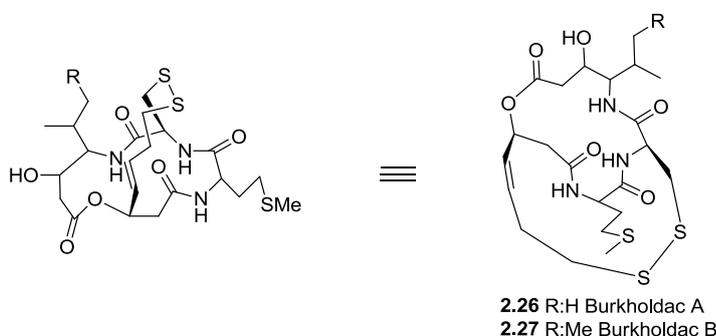
Figure 2.38 : Structures of burkholdacs

We decided to prepare burkholdac A, containing the methionine and the isoleucine, as it is an isomeric compound to the previously synthesised thailandepsin A. In fact, the only difference between the two compounds is that the position of the methionine and isoleucine are inverted. According to the biosynthesis of burkholdacs, Brady predicted the stereochemistry of the natural product to be L-Ile, D-Cys and L-Met. We are able to prepare this compound from the β-hydroxy acid, L-isoleucine statine derivative, D-cysteine and L-methionine (Scheme 2.57).
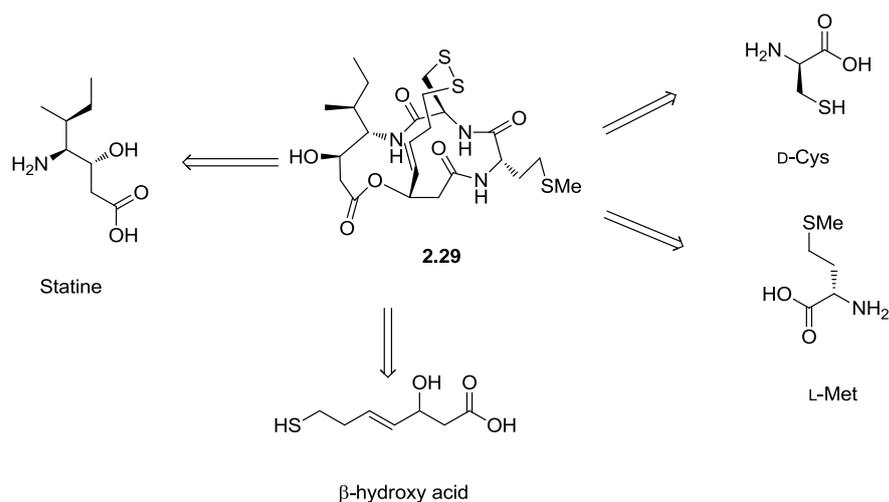
Scheme 2.57 : Retrosynthesis of burkholdac B (2.29) with Brady's predicted stereochemistry

We prepared the L-isoleucine statine derivative **2.228** in a similar manner to the previously described method. Subsequent couplings with Fmoc-D-Cys(Tr)-OH, Fmoc-D-Met-OH and the β-hydroxy acid afforded compound **2.231** (Scheme 2.58). Allyl deprotection followed by macrolactonisation and disulfide bond formation yielded Brady's proposed diastereomer **2.29**.
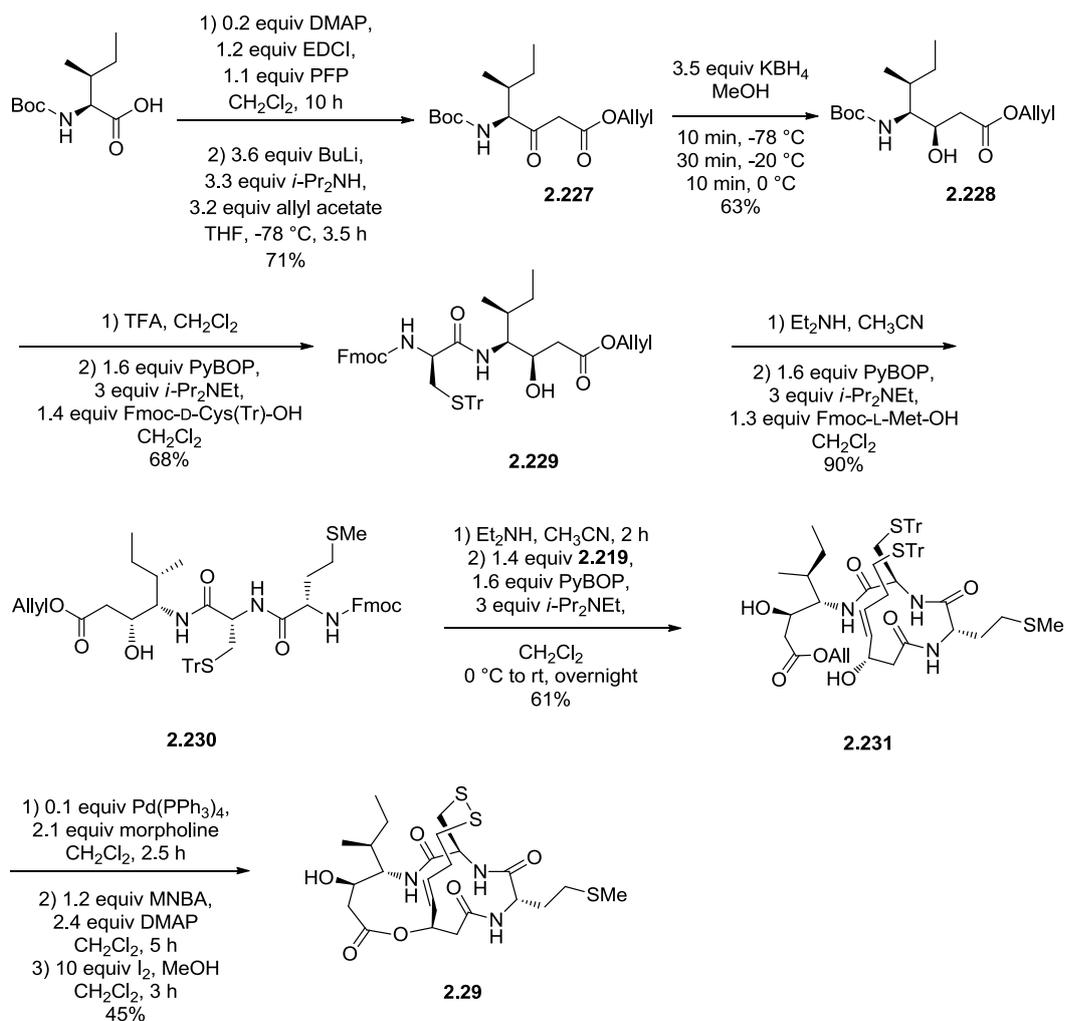
Scheme 2.58 : Synthesis of Brady's predicted diastereomer 2.29 (L-Ile, D-Cys and L-Met)

Comparison of the ^{1}H NMR and ^{13}C NMR spectra of the synthetic compound with the natural product shows that the stereochemistry assigned by Brady is incorrect as several differences are observed (Figures 2.39 and 2.40).[84]
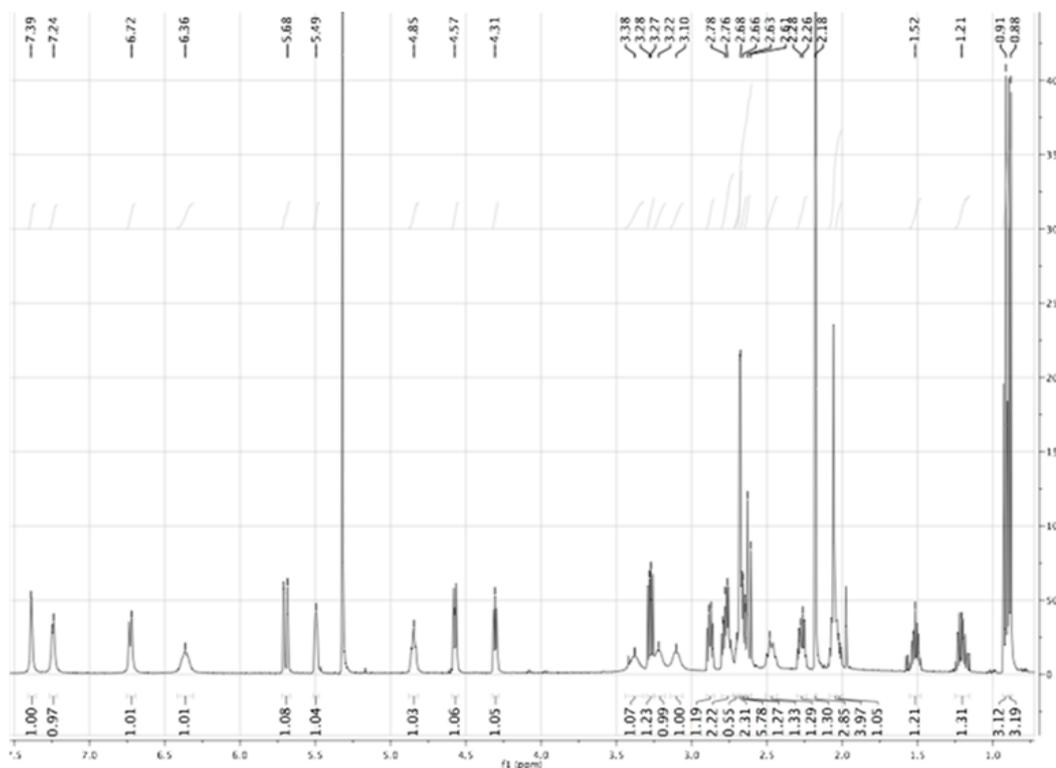
Figure 2.39 : **^{1}H NMR spectra of burkholdac B published by Brady**[84]** in CD**$_2$**Cl**$_2$** (600 MHz)** "Reprinted with permission from Biggins, J. B.; Gleber, C. D.; Brady, S. F. *Org. Lett.* **2011**, *13*, 1536-1539, copyright (2011) American Chemical Society."
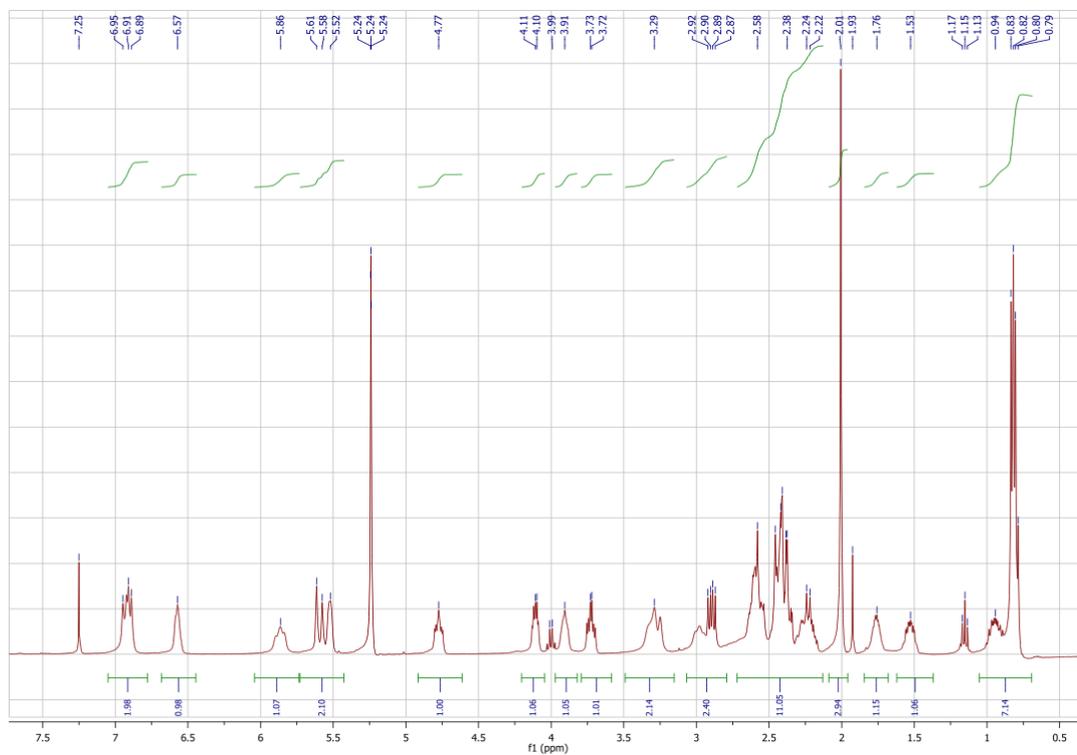
Figure 2.40 : **^{1}H NMR spectra of Brady's proposed diastereomer 2.29 in CD**$_2$**Cl**$_2$** (400 MHz)**

By analogy to spiruchostatin, we decided to prepare the diastereomer with all the amino acids in the D-series. The statine is likely to be the *syn* diastereomer but the same ambiguity resides for the stereochemistry of the isoleucine, which could be D- or D-*allo*- depending on the degree of epimerisation in the biosynthesis.

2.4.2.2. Synthesis of the diastereomer 2.237 (D-Ile, D-Cys and D-Met)

The diastereomer **2.237** is prepared as described previously, starting from D-isoleucine, D-cysteine and D-methionine (Scheme 2.59).
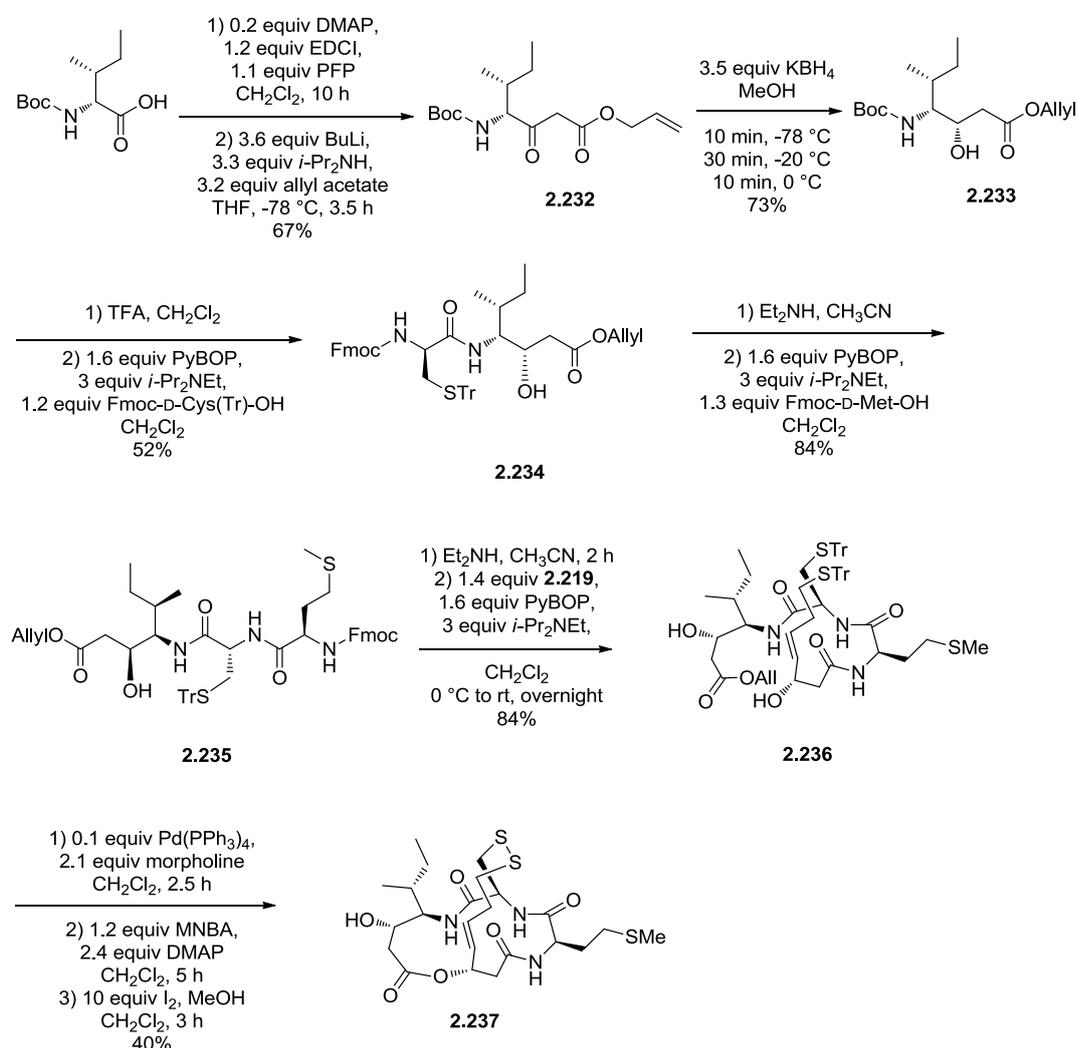
Scheme 2.59 : Synthesis of the diastereomer 2.237 (D-Ile, D-Cys and D-Met)

The ^{1}H NMR spectra of this diastereomer is very similar to that of the natural product (Figures 2.39 and 2.41).[84] We can see a difference in the chemical shifts of the

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isoleucine residue signals which suggests that the stereochemistry of the isoleucine is probably not D- but D-*allo*, as present in spiruchostatin B.
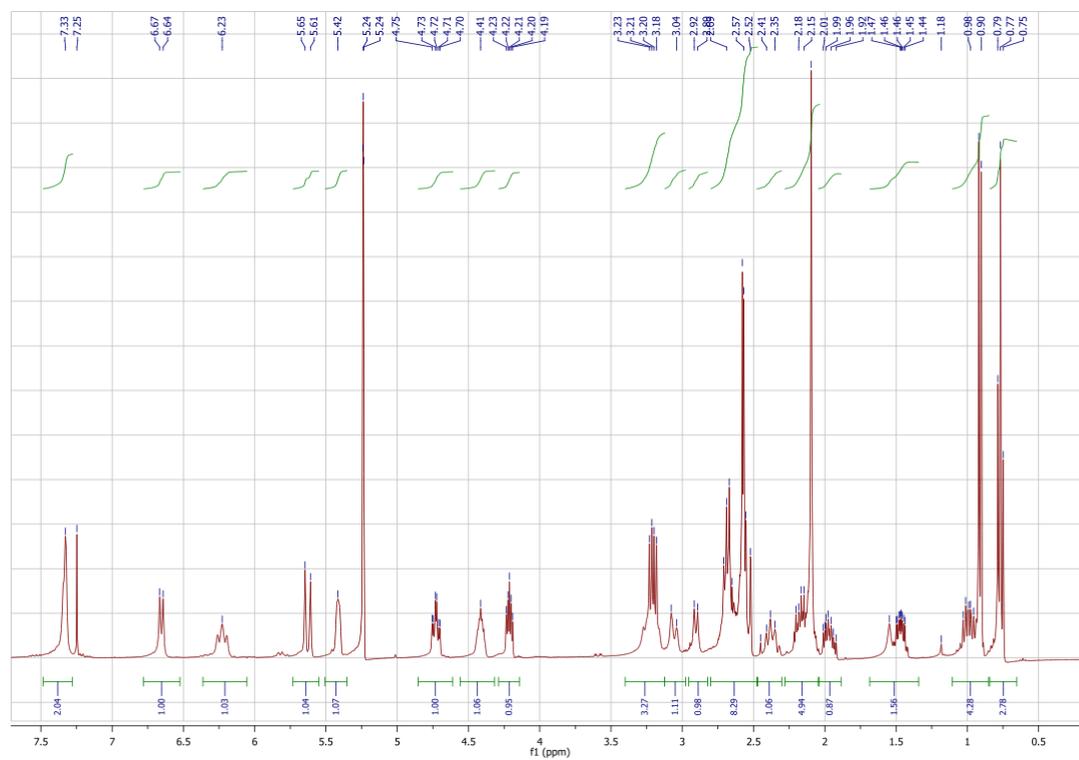
Figure 2.41 : ^{1}H NMR spectra of diastereomer 2.237 (D-Ile, D-Cys and D-Met) in CD$_2$Cl$_2$ (400 MHz)

2.4.2.3. Synthesis of burkholdac B (D-allo-Ile, D-Cys and D-Met)

The prior synthesis is this time repeated with D-*allo*-isoleucine, D-cysteine and D-methionine residues to obtain the diastereomer **2.243** (Scheme 2.60).
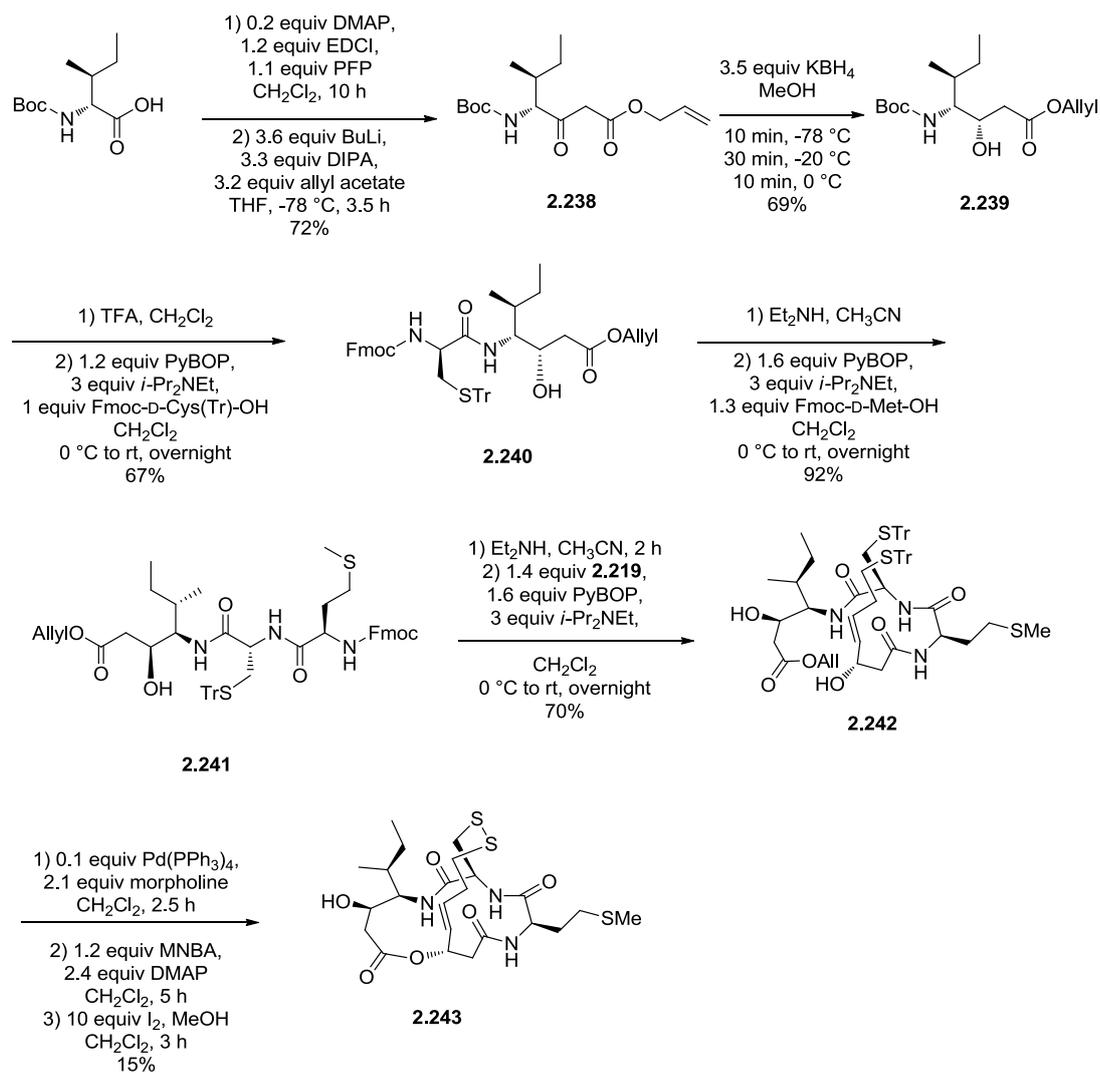
Scheme 2.60 : Synthesis of the burkholdac B (D-*allo*-Ile, D-Cys and D-Met)

The ^{1}H and ^{13}C NMR spectra matched perfectly with that reported in the literature for the natural product (Figures 2.39 and 2.42).[84] Thus, the stereochemistry of the natural product is D-*allo*-isoleucine, D-cysteine and D-methionine. We successfully assigned the stereochemistry of burkholdac B by synthesis.[136]
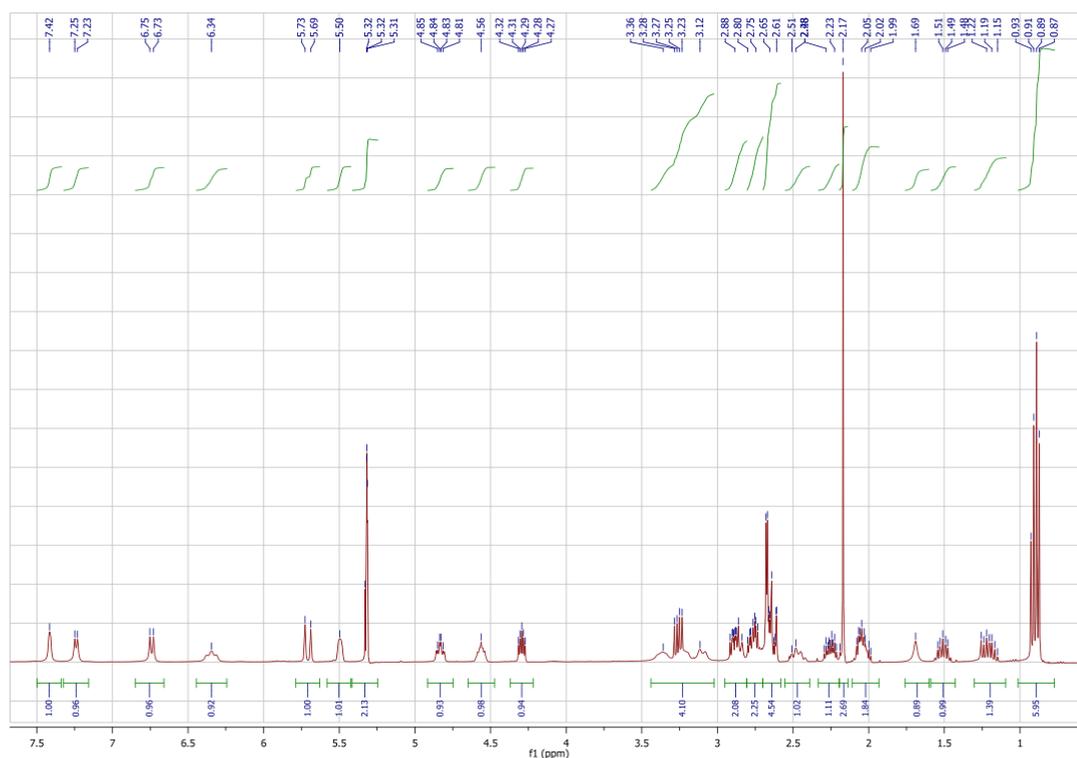
Figure 2.42 : ^{1}H NMR spectra of diastereomer (D-allo-Ile, D-Cys and D-Met) in CD$_2$Cl$_2$ (400 MHz)

No optical rotation value was reported for burkholdac B in Brady's paper. The NMR assignment is the only available information and matched with that of the last diastereomer. Collected ^{1}H and ^{13}C NMR data for the three diastereomers **2.29**, **2.237** and **2.243** were compared to that reported in the literature (Figure 2.43 and Table 2.5). This comparison has confirmed the fact that the correct diastereomer possesses D-*allo*-Ile, D-Cys and D-Met stereochemistry. We can observe larger chemical shift differences (Δδ) for the other diastereomers, particularly for compound **2.29**.
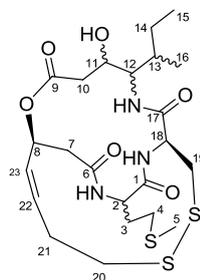
Figure 2.43 : Atom numbering used for burkholdac B

#	¹H Δδ for 2.29	¹³C Δδ for 2.29	¹H Δδ for 2.237	¹³C Δδ for 2.237	¹H Δδ for 2.243	¹³C Δδ for 2.243
1	/	0.1	/	0.1	/	0.2
2	-0.32	-3	-0.01	0.2	-0.01	0
3	0.28	-1.5	0.02	0.2	0.01	0.2
	0.21	/	-0.04	/	-0.01	/
4	0.01	-0.1	0.04	0.1	-0.01	0.1
	-0.11		-0.08	/	-0.02	/
5	-0.09	-0.5	0.05	1.2	-0.01	0.1
NH	-0.74	/	0.02	/	0.02	/
6	/	-1.2	/	0.3	/	0.2
7	0.36	-0.5	0.08	-0.1	0.03	-0.1
	-0.3	/	0.01	/	-0.02	/
8	0.11	-1.4	0.01	0.3	0.01	0.2
9	/	-0.7	/	0.2	/	0.1
10	-0.2	1.4	0.02	0.3	-0.03	0.2
11	-0.38	1.1	-0.07	1.5	-0.01	0.1
12	0.92	-1.8	-0.18	0.8	0.01	0
13	-0.22	-0.7	0.17	-0.4	-0.02	0.1
14	-0.19	-4.1	-0.13	-1	-0.01	0.2
	0.09	/	0.04	/	-0.01	/
15	-0.02	0.3	-0.06	-0.7	0	0.1
16	0	1.1	0.11	0.2	-0.01	0.2
NH	-0.24	/	0.17	/	0	/
OH	/	/	/	/	/	/
17	/	1	/	0	/	0.2
18	0.01	1.9	-0.03	0.4	0	0.2
19	-0.04	-0.9	0.04	0	0	0.2
	0	/	-0.09	/	-0.02	/
NH	0.27	/	0.01	/	0.01	/
20	-0.09	1.4	-0.06	-0.1	0	0.1
	0.15	/	0.06	/	0.03	/
21	-0.39	-1.4	0	0	-0.05	0.1
	0.01	/	0	/	0.01	/
22	-0.45	-1.5	-0.08	0.1	-0.04	0.1
23	-0.01	1	0.03	0.4	0.03	0.2

Table 2.5 : Difference between collected ¹H and ¹³C NMR data for the three diastereomers 2.29, 2.237 and 2.243 with that[84] of reported in the literature (values in ppm are written in red if ¹H Δδ > 0.1 ppm and if ¹³C Δδ > 1 ppm)

Cheng published later on the isolation of thailandepsin A which is the equivalent of burkholdac B.[85] Optical rotation was available and values collected and reported are close ($[\alpha]^{25}_D$ collected -23.3 (*c* 0.08, CH$_3$CN), $[\alpha]^{24}_D$ reported -22.8 (*c* 1.00, CH$_3$CN)).[85]

Only one epimerase is present in the biosynthesis gene cluster of burkholdac B, but surprisingly a total of three chiral centres are epimerised. We believe that the single epimerase present might be responsible for the multiple epimerisations. It is also possible that D-amino acid residues are incorporated in the biosynthesis. Either way, our results show that looking at the biosynthesis gene cluster is not completely reliable for the assignment of stereochemistry in peptide natural products. The synthesis of the compounds is extremely important to confirm the structures and their stereochemistry.

2.4.3. Biological results for thailandepsin A, burkholdac B and other diastereomers

We were now in a position to compare the biological activity of these four compounds: Cheng's revised structure of thailandepsin A (**2.217**), Brady's burkholdac B diastereomer **2.29**, the diastereomer with all the amino acids with D-stereochemistry **2.237**, which is an epimer of burkholdac B (*epi*-burkholdac B), and burkholdac B (**2.243**).
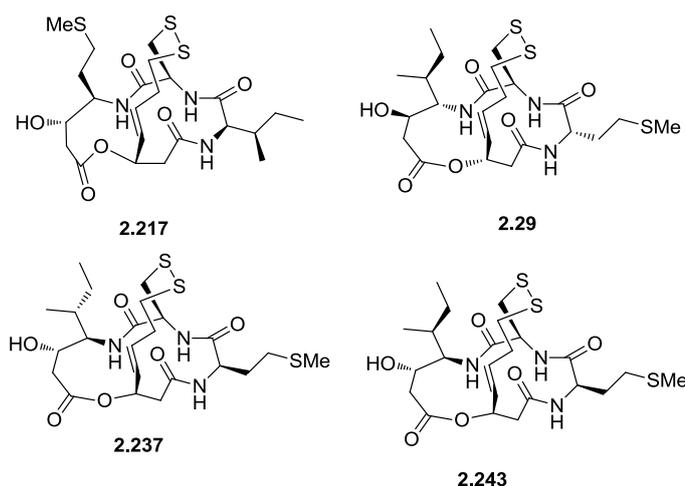
Figure 2.44 : Structures of the different synthesised compounds

Alison Donlevy, at the Cancer Research UK centre in Southampton, tested the four compounds in the Fluor-de-Lys HDAC enzyme assay and growth inhibition against the cancer cell line MCF7, such as performed for largazole and related analogues (Table 2.6).[136] The Brady diastereomer with two L-amino acids appeared to be the least potent inhibitor in both assays, which suggests that L-amino acids are unfavourable for the HDAC inhibition activity. Thailandepsin A, *epi*-burkholdac B and burkholdac B are all nanomolar HDAC inhibitors in both assays. The potency of burkholdac B and its epimer, *epi*-burkholdac B, in cell assay towards the cancer cell line MCF7 is particularly remarkable (subnanomolar). Burkholdac B is the most potent of all the depsipeptides in cell assay with an increase of 13-fold compared to FK228 which had been, until now, the best depsipeptide and a clinically approved drug.

Compound	HDAC inhibition (nM)	MCF7 inhibition (nM)
FK228 (2.15)	24 ± 4	0.8 ± 0.2
Spiruchostatin A (2.16)	5.3 ± 3.3	5.7 ± 0.7
Largazole (2.18)	572 ± 29	5 ± 1
Largazole thiol 2.182	0.04 ± 0.3	277 ± 130
2.217	8.3 ± 2.4	10.5 ± 1.8
2.29	3312 ± 2472	410 ± 82
2.237 (*epi*-burkholdac B)	3.1	0.25 ± 0.05
2.243 (burkholdac B)	5.0 ± 3.0	0.06 ± 0.04

Table 2.6 : HDAC inhibition and cell growth inhibition for the different isomers prepared

The main difference between burkholdac and the other depsipetides is the bigger and more lipophilic methionine amino acid. We believe that the methionine is responsible for the high potency of burkholdac B in cell assay. The poorer potency in the enzyme assay could be explained by the degradation of the product in the enzyme assay.

2.5. CONCLUSION

The total synthesis of largazole was achieved by a convergent synthesis. Largazole thiol exhibited remarkable activity in the enzyme assay (picomolar). Unfortunately, the cell assay results for largazole were disappointing when compared to its outstanding enzyme activity. The convergent strategy used for largazole synthesis allowed us to prepare four analogues in order to study the structure-activity relationships of largazole. All the modifications made to the compounds were tolerated and we determined that simplification of largazole is possible with conservation of the biological activity. A correlation between lipophilicity and growth inhibition activity was also observed. The stability of largazole as a prodrug has to be improved.

The thailandepsins/burkholdacs story illustrates the importance of synthesis in structure elucidation. Elucidation of compounds by study of biosynthesis pathways can lead to errors in the structures. In fact, the first reported structures of thailandepsins were completely wrong, which led to us spending some time trying to form an unusual hemiaminal function which was not present in the molecule. We then worked on the second revised structures of thailandepsins and discovered that they were also incorrect. The compound prepared was however a spiruchostatin analogue and worth testing for biological activity. The structures of burkholdacs were then published with a predicted stereochemistry from the biosynthesis and their structures confirmed by MS and NMR. We decided to prepare burkholdac B with their predicted stereochemistry, however discovered that it was not correct. After preparation of two other diastereomers, we elucidated the stereochemistry of burkholdac B to be as in spiruchostatin B (D-*allo*-Ile, D-Cys and D-Met). Burkholdac B showed excellent cell growth inhibition activity. The L-series amino acids appeared unfavourable in HDAC inhibition activity for burkholdacs whereas the D-series gave better results. The natural product gave the best results out of all the compounds prepared.

At the time of writing, largazole thiol is the most potent among this class of depsipeptide natural products in HDAC enzyme inhibition. Meanwhile, burkholdac B is the most potent depsipeptide in terms of cell growth inhibition.

3. HISTONE DEMETHYLASE INHIBITORS

3.1. INTRODUCTION

As discussed previously, acetylation is an important histone post-translational modification that influences the state of the chromatin but it is not the only modification to occur; methylation is also an essential key element. Histone methylation regulates diverse chromatin-templated processes including transcription. This modification is catalysed by histone methyltransferases (HMTs) which use *S*-adenosylmethionine (SAM) as a methyl group donor.[137] The major methylation sites on histone tails are the basic amino acid chains of lysine and arginine residues with methylation catalysed by histone lysine methyltransferases (HKMTs) and histone arginine methyltransferases (HRMTs) respectively. Lysine residues can be mono-, di-, or trimethylated on the ε-nitrogen, and arginine residues are mono- or dimethylated on the guanidinium group.[138-140] Histone acetylation only occurs on lysine residues, whereas histone methylation can take place on both lysine and arginine residues. Methylation is a distinctive post-translational modification as it can occur in both DNA (C-5 position of cytosine that precedes guanines, CpG) and histone proteins.[141]

3.1.1. Lysine-specific demethylase 1 (LSD1)

Lysine methylation was viewed as a permanent epigenetic mark until recently, most likely because of the thermodynamic stability of the bond N-CH$_3$. The discovery of the first histone lysine-specific demethylase 1 (LSD1), also called KDM1 (or AOF2 or BHC110), showed that methylation is also a reversible process; a dynamic protein modification like all the other known histone modifications.[142] LSD1 demethylates mainly mono- and dimethylated lysine 4 and 9 on histone 3 (H3K4 and H3K9). This enzyme belongs to the amine oxidase family of flavin adenine dinucleotide (FAD)-dependent enzymes. By reducing FAD to FADH$_2$, LSD1 oxidises the methyl ε-amine of the methylated lysine **3.1** to generate the corresponding imine **3.2** which is subsequently hydrolysed to produce a carbinolamine **3.3** (Scheme 3.1).[142] This unstable species **3.3** degrades, releasing formaldehyde **3.5** and the demethylated lysine **3.4**. This reaction

results in a hydride transfer with reduction of FAD to $FADH_2$ that is regenerated to its oxidised form by molecular oxygen producing hydrogen peroxide. This mechanism explains why LSD1 is not able to demethylate trimethylated lysine residues as a lone pair on the nitrogen is required for electron transfer in order to form the imine intermediate **3.2**.
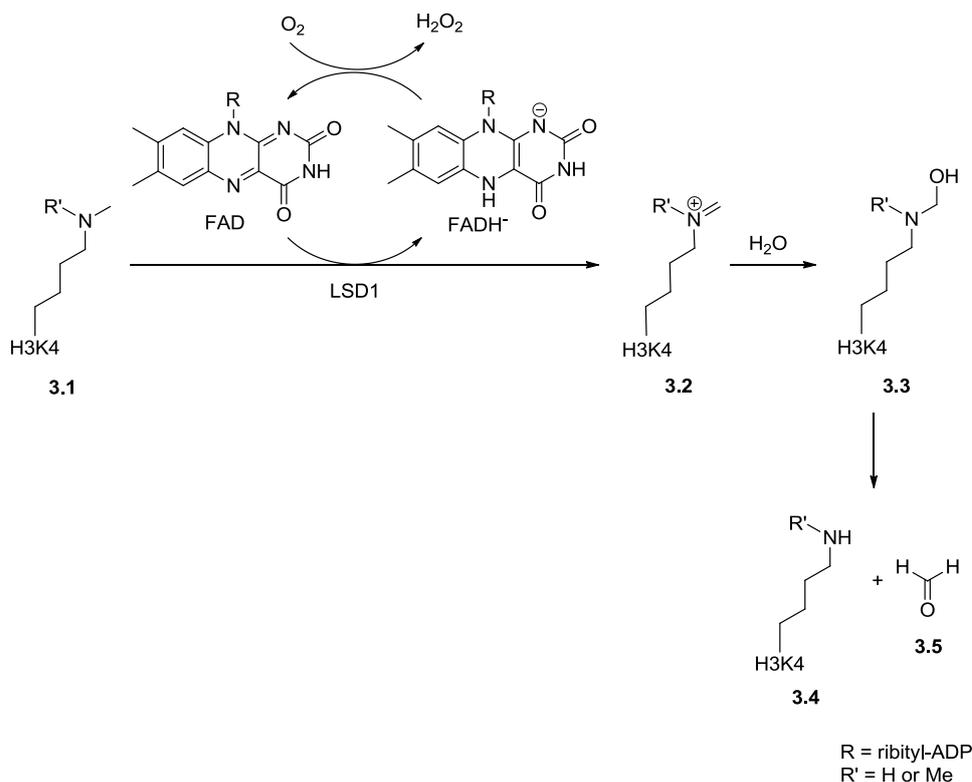
Scheme 3.1 : Mechanism of demethylation by LSD1 on H3K4

The mechanism of the oxidative conversion of the amine to the imine is however controversial and may involve either hydride transfer or single electron mechanisms.[143]

LSD1 is a protein of around 100 kDa and contains three domains: SWIRM domain, amine oxidase domain and the tower domain.[142,144] The amine oxidase domain of LSD1 shares sequence and structural similarities with the catalytic domain of the amine oxidases. LSD1 often forms a complex with the corepressor protein (CoREST). The X-ray crystal structure of the LSD1-CoREST complex has been resolved (Figure 3.1) and shows that the tower domain of LSD1 binds to CoREST.[145-147]
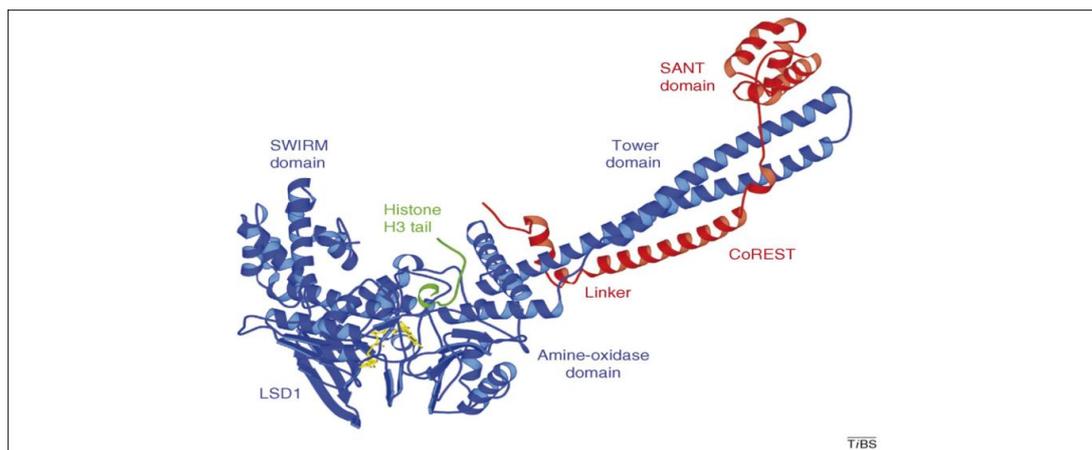
Figure 3.1 : X-Ray structure of the complex LSD1 (in blue) and CoREST (red)[147] "Reprinted from Trends in Biochemical Sciences, *33*, Forneris, F.; Binda, C.; Battaglioli E.; Mattevi A., LSD1: oxidative chemistry for multifaceted functions in chromatin regulation, 181-189 Copyright (2008), with permission from Elsevier."

3.1.1.1. Transcription

Lysine methylation has different effects that are dependent on which residue is methylated and the degree of modifications. It can activate or repress gene transcription; for example, methylation on H3K9 or on H3K27 is a transcriptionally repressive modification whereas on H3K4 it is a transcriptionally activating modification.[148]

LSD1 can also be a transcriptional repressor or activator depending on its binding partner. LSD1 is a transcriptional repressor when it catalyses the demethylation of H3K4.[148] The complex LSD1-CoREST is found binding HDAC1 or HDAC2 forming complexes which are transcriptional repressors.[149] By itself, LSD1 is able to remove methyl on H3K4 but it cannot demethylate H3K4 in the nucleosome; in order to do so, it requires the corepressor CoREST.[146,149] NMR spectroscopy shows that the CoREST SANT2 domain binds to DNA and this binding was established to be crucial for the demethylation of nucleosomal H3K4.[146] LSD1 is also a transcriptional activator in the presence of androgen receptor (AR) as the complex removes methyl groups from H3K9.[150]

3.1.1.2. LSD2

LSD2 also called KDM2 (or KDM1b or AOF1) is the only known mammalian homologue of LSD1.[151] To date, the LSD family consists of LSD1 and LSD2; they both share a similar catalytic domain (around 31% homology) but differ in the *N*-terminal

region.[152] Like LSD1, LSD2 can only demethylate mono- and dimethylated H3K4. LSD2 does not bind to CoREST and is believed to possess different biological functions compared to LSD1. LSD2 is able to demethylate nucleosomal H3K4 by itself unlike LSD1 which requires CoREST to do so.[152]

3.1.1.3. Cancer

Aberrant expression of LSD1 has been detected in several types of cancer, with over-expression being observed in prostate cancer.[150,153,154] LSD1 is also highly expressed in neuroblastoma, breast and bladder cancers.[155] This suggests that the inhibition of LSD is a potential target for chemotherapy.

According to a statistical study led by Cancer Research UK, prostate cancer is the second leading cause of cancer mortality for men in the UK after lung cancer.[156] The androgen receptor (AR), a nuclear hormone and transcription factor, is a therapeutic target in prostate cancer. An experiment carried out by Metzger showed that the LSD1-AR complex is responsible for the inhibition of tumour growth.[150] In prostate cancer cell lines, small interfering RNA (siRNA) induces LSD1 knockdown and therefore reduces the LSD1-AR complex formation. The demethylation of the repressing histone marks, mono- and dimethyl H3K9, by the LSD1-AR complex is no longer possible. This leads to a decrease of the AR mediated transcription and cell proliferation is observed. This experiment confirmed that the LSD1-AR complex allows the derepression of the AR and promotes gene activation.[150,154] Inhibition of LSD1 by pargyline was also shown to block the demethylation of H3K9 by the complexation of LSD1 and the AR.[150,154] Over-expression of LSD1 in prostate tumours is a predictive mark for aggressive tumours and correlates significantly with relapse during therapy.[150,154]

Over-expression of the tumour suppressor p53 is also a sign of relapse in prostate cancer.[18] LSD1 can control the tumour suppressor activity of p53 by directly demethylating a specific p53 lysine (Lys370me2 and Lys370me1).[157] Therefore, the transcriptional activation mediated by p53 is repressed and p53-promoted apoptosis is inhibited.[157]

It is possible that LSD1 and HDACs cooperate to repress the transcription of genes. Inhibitors of LSD1 exhibit antitumor activities and it might be possible to use them in

combination with HDAC inhibitors.[65,158] In a recent study, treatment of breast cancer cells with zinc-dependent HDAC inhibitors (class I and II) reduced the activity of LSD1, thus leading to an increase of the LSD1 substrate H3K4me2 known as a transcriptionally active modification.[65] On the other hand, they observed that inhibition of LSD1 resulted in an enhanced level of AcH3K9 also known as a mark of gene activation.[65] Simultaneous treatment with HDAC and LSD1 inhibitors led to the growth inhibition of breast cancer cells.[65]

3.1.2. Inhibitors of LSD1

As LSD1 belongs to the family of flavin adenine dinucleotide (FAD)-dependent amine oxidases, certain inhibitors of monoamine oxidases (MAOs), which belong to the same family, are also capable of inhibiting LSD1.[159] MAOs are responsible for the deamination of neurotransmitters such as serotonin or dopamine. According to Shiekhattar and McCafferty, tranylcypromine presents the highest inhibition activity towards LSD1 when compared to other MAO inhibitors such as nialamide, phenelzine, deprenyl, chlorgyline and pargyline.[138,159] So far, only a few LSD1 inhibitors have been described in the literature: tranylcypromine, pargyline, phenelzine and polyamines (Figure 3.2).
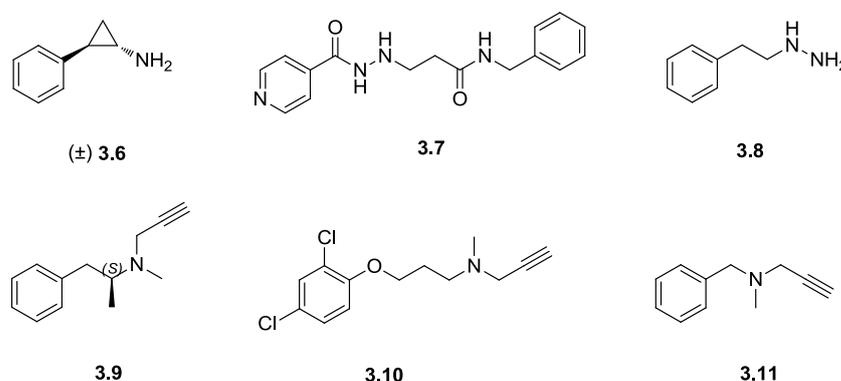
Figure 3.2 : Structures of tranylcypromine (3.6), nialamide (3.7), phenelzine (3.8), deprenyl (3.9), chlorgyline (3.10) and pargyline (3.11)

3.1.2.1. Pargyline and propargyl derivatives

Pargyline (**3.11**) is a known MAO inhibitor and was first proposed to inhibit LSD1 by Metzger *et al.*[150] Cole and co-workers designed a propargyl-Lys-derivatised peptide

3.12 as a potent LSD1 inactivator.[160,161] The inhibitor irreversibly inactivates LSD1; the mechanism was elucidated by MS, NMR and absorbance spectrometric analysis (Scheme 3.2).[161] The amine is oxidised to the propargylic iminium ion which undergoes Michael addition with N^5 of the flavin.
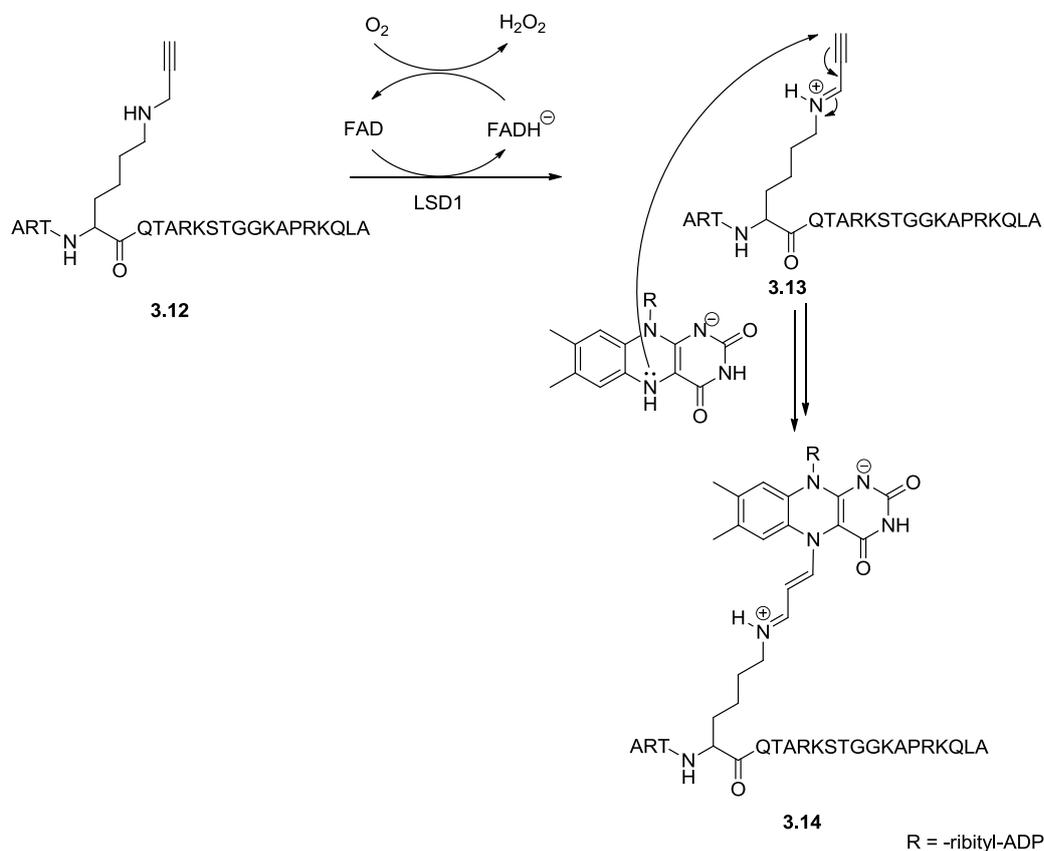
Scheme 3.2 : Mechanism of inactivation of LSD1 by 3.12

3.1.2.2. Tranylcypromine

Tranylcypromine (**3.6**, PCPA or *trans*-2-phenylcyclopropylamine or Parnate) is a MAO inhibitor, a clinically used antidepressant, and has also been shown to inhibit LSD1. The value of the IC_{50} found in the literature for the LSD1 inhibition by tranylcypromine, varies depending on the assay performed (micromolar order). Tranylcypromine is accepted as a substrate by LSD1. This is followed by rapid opening of the cyclopropyl ring and formation of a covalent adduct with the cofactor FAD **3.15** (Scheme 3.3).[162] The FAD-PCPA adduct **3.19** is a five-membered ring. Tranylcypromine is an irreversible inhibitor with the active part functionalities being the cyclopropyl ring and the amine group.

152
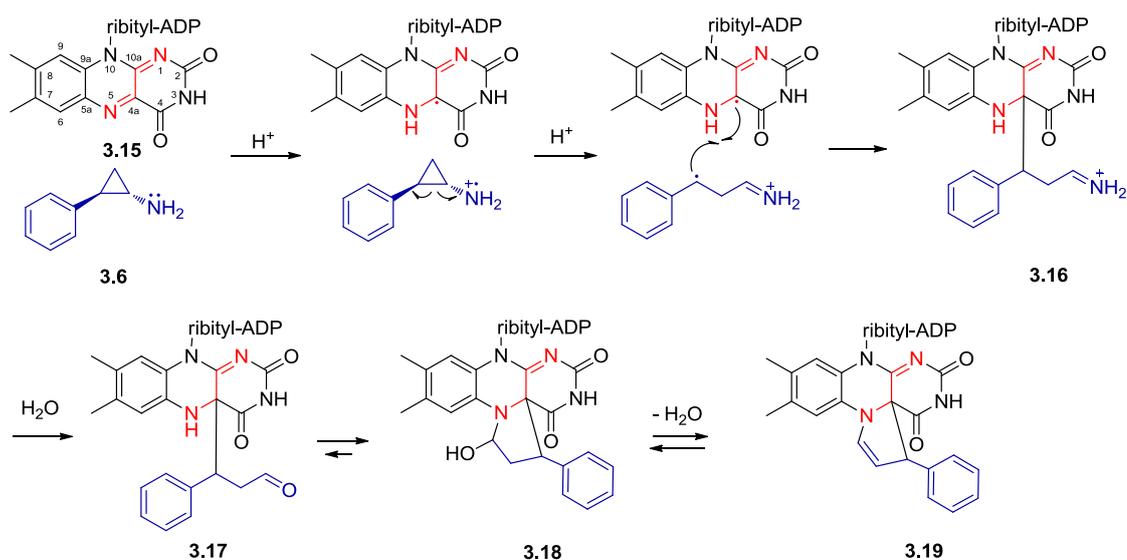
Scheme 3.3 : Mechanism of inactivation of LSD1 by tranylcypromine

The crystal structure of LSD1 inhibited by tranylcypromine was first solved by Yu *et al*.[162] It provided evidence supporting the mechanism of LSD1 inactivation by tranylcypromine and the formation of the FAD-PCPA adduct **3.19**. Yokoyama and co-workers solved this complex with an improved resolution and suggested that the covalent adduct **3.19** is the major adduct formed but also observed the N^5 adduct **3.20** (Figure 3.3).[163]
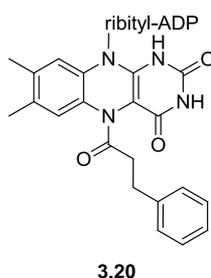
Figure 3.3 : Structure of the N^5 adduct (3.20)

The mechanism of inactivation of LSD1 by tranylcypromine is different to that of the MAO inhibition (Scheme 3.4). In fact, the FAD-PCPA adduct obtained in MAO inactivation is not the same as **3.19**, which is formed in LSD1 inactivation. The covalent adduct FAD-PCPA, formed in the inactivation of MAO B, is believed to be the aldehyde **3.21**. Depending on the opening of the cyclopropane another adduct can be

obtained.[138,162] The site of modification of MAO by tranylcypromine has not yet been identified without ambiguities.
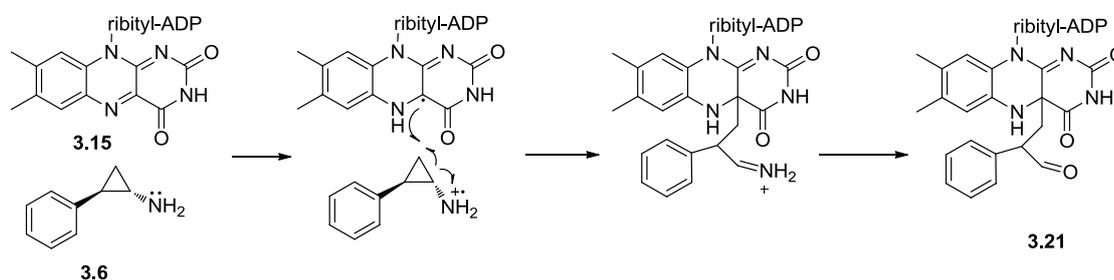
Scheme 3.4 : Mechanism of inactivation of MAOB by tranylcypromine

According to Yu *et al.*, the phenyl group of the FAD-PCPA adduct **3.19** is located in a large hydrophobic pocket in the active site of LSD1.[162] The model showed that the cavity space is not completely filled (Figure 3.4).[162] There is potential that tranylcypromine analogues with hydrophobic substituents on the phenyl ring might be more potent inhibitors of LSD1.
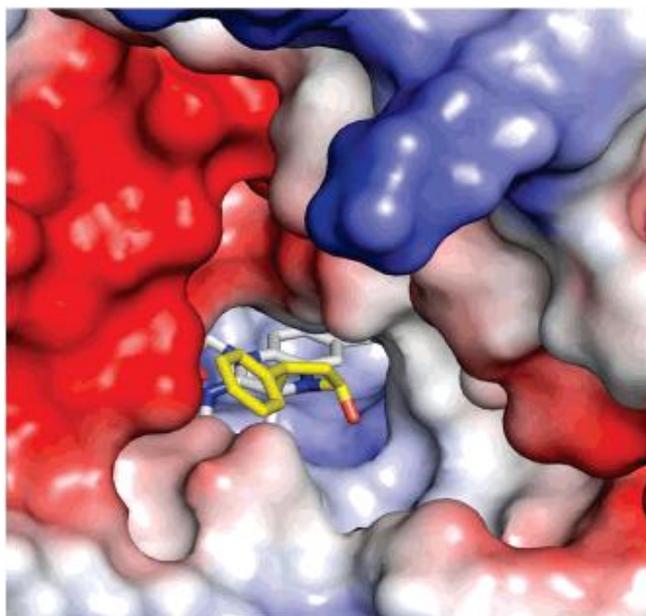
Figure 3.4 : FAD-PCPA adduct in LSD1[162] "Reprinted with permission from Yang, M. *et al.*. *Biochemistry* **2007**, *46*, 8058-8065, copyright (2007) American Chemical Society."

McCafferty *et al.* believed that substituents on the *para*-position should make the analogues more selective towards LSD1 than MAO.[164]

Tranylcypromine, used as a drug in the treatment of depression, is administered as the racemate. It appeared that the (–)-enantiomer had the same therapeutic effect as the racemate with fewer side effects.[165] One of the two enantiomers should also be more appropriate in the inhibition of LSD1.

Mai *et al.* synthesised the enantiomers (+) and (–)-tranylcypromine.[166] The difference in LSD1 inhibition activity found for the two distinct enantiomers was small, with the (+)-enantiomer found to be the most potent inhibitor. Crystallographic studies showed that the FAD-PCPA adducts in LSD1 differed for the two enantiomers.

During the course of this work, several groups reported the preparation of tranylcypromine analogues.

McCafferty's tranylcypromine analogues (Figure 3.5) were prepared by two different cyclopropanation methods: either cyclopropanation of *trans*-cinnamate with a diazo compound or Michael-initiated ring closure with a sulfur ylide.[164] The compounds were tested against LSD1, MAOA and MAOB and inhibition kinetics were reported. The *para*-bromo- derivative was the only analogue with greater LSD1 inhibition compared to tranylcypromine, while the analogues with trifluoromethyl- and methoxy-substituents exhibited a decreased inhibition. The thienyl-, naphthyl- and biphenyl-compounds were found to be inactive.
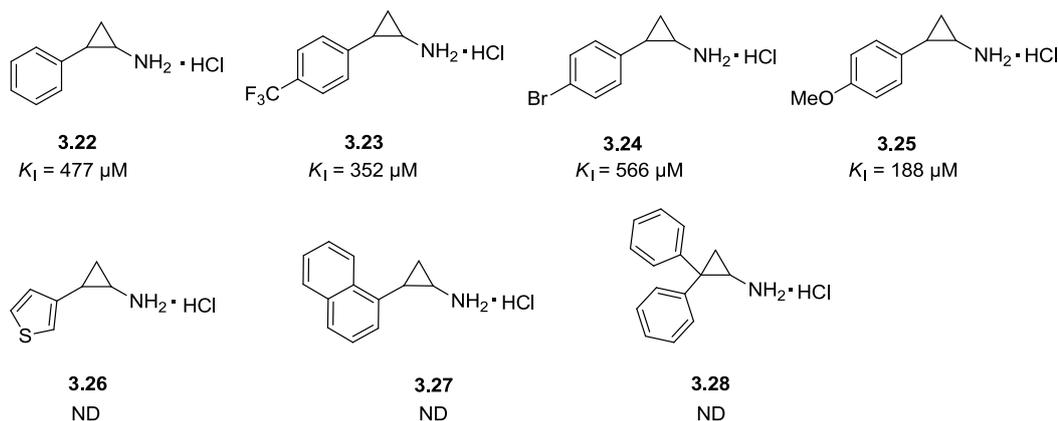
Figure 3.5 : Tranylcypromine analogues prepared by McCafferty

In 2009, Miyata published tranylcypromine-lysine hybrid analogues **3.29** and **3.30** (Figure 3.6).[167] They designed these small molecules by comparison of the X-ray crystal

structures of FAD-PCPA adduct **3.19** and the FAD-*N*-propargyl lysine peptide adduct **3.14**. The cyclopropanation was performed by Michael-initiated ring closure with the sulfur ylide on *trans* cinnamate derivatives. Amino acid side chains were linked by an ether bond on the aromatic ring at the *meta* or *para* positions. Benzylamino and benzoyl groups were used in order to increase hydrophobic interactions with residues in the active site. These compounds are active in enzyme and cell growth inhibition assay with the *para* compound **3.30** showing slightly better biological activity than the *meta* derivative **3.29**.

3.6

IC$_{50}$ = 32 μM

3.29

IC$_{50}$ = 2.5 μM

3.30

IC$_{50}$ = 1.9 μM
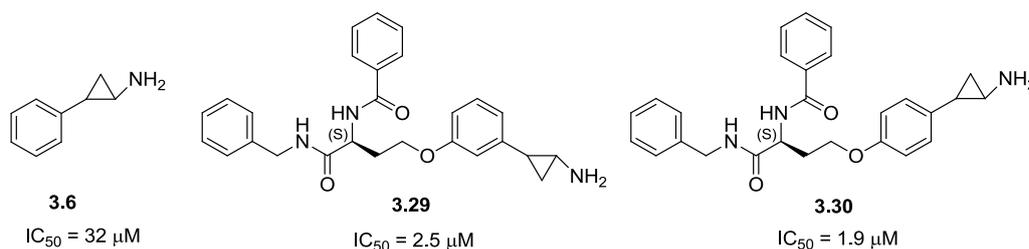
Figure 3.6 : Tranylcypromine analogues prepared by Miyata

In 2010, Mai *et al.* meanwhile prepared a series of tranylcypromine analogues in order to increase the selectivity of the analogues towards LSD1 versus MAO (Figure 3.7).[166] They performed the cyclopropanation of styrene using diazoester with copper triflate. Their compounds are similar to that reported by Myata but instead of ether linkage, amide linkage is employed to incorporate the side chain containing phenyl rings.[167] Good inhibition activity and selectivity were obtained for the linear and branched analogues.

3.22

IC$_{50}$ = 271 µM

3.31

IC$_{50}$ = 1.1 µM

3.32

IC$_{50}$ = 1.9 µM

3.33

IC$_{50}$ = 1.3 µM
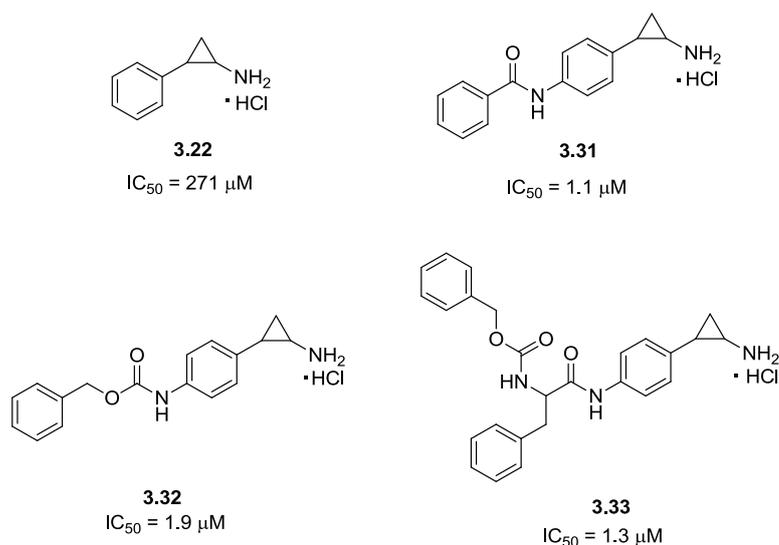
Figure 3.7 : Selection of tranylcypromine analogues prepared by Mai

In 2010, Yokoyama published some fluoro- tranylcypromine analogues (Figure 3.8).[168] They performed the cyclopropanation of styrene using a diazoester and rhodium catalyst, followed by epimerisation with sodium hydroxide solution in ethanol. They first made compound **3.34** and realised that the fluoro- groups were beneficial for LSD1 inhibition activity. From the crystal structure analysis of FAD-**3.34** in LSD1, they discovered that a phenyl ring in *ortho* position of the cyclopropyl group would enhance the biological activity. Some modifications were performed on the phenyl ring in the *ortho* position and gave interesting LSD1 inhibitors (**3.36**, **3.37** and **3.38**).
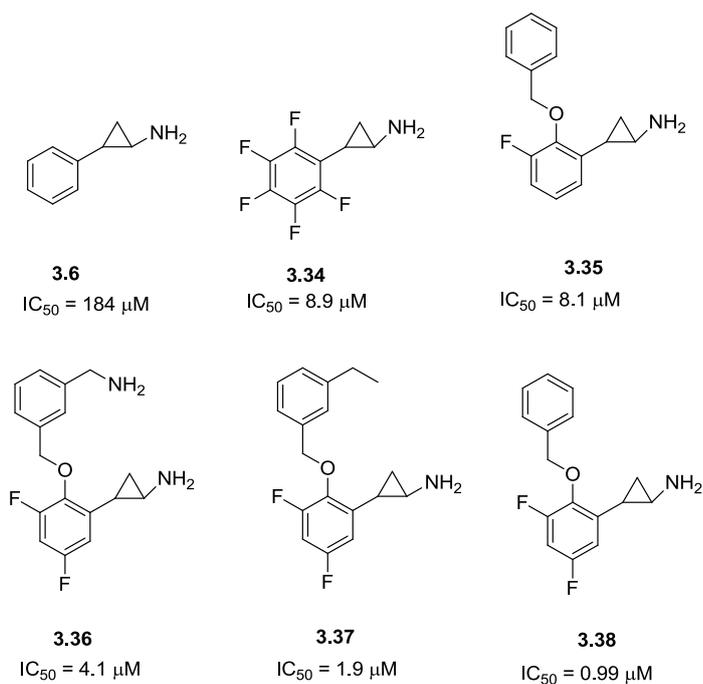
Figure 3.8 : Selection of tranylcypromine analogues prepared by Yokoyama

It is important to highlight that the IC_{50} of the analogues prepared by the different groups are not comparable due to differences in assay conditions. The IC_{50} of tranylcypromine is the reference required to compare the compounds.

3.1.2.3. Peptide derivatives and phenelzine

The study led by Cole and co-workers was a comparison of peptides versus small molecules.[169] The chlorovinyl-containing compounds **3.39** and **3.40** and the hydrazine peptide derivative **3.41** presented respectively similar and greater LSD1 inhibition activity than the propargyl- peptide derivative **3.12** (Figure 3.9).
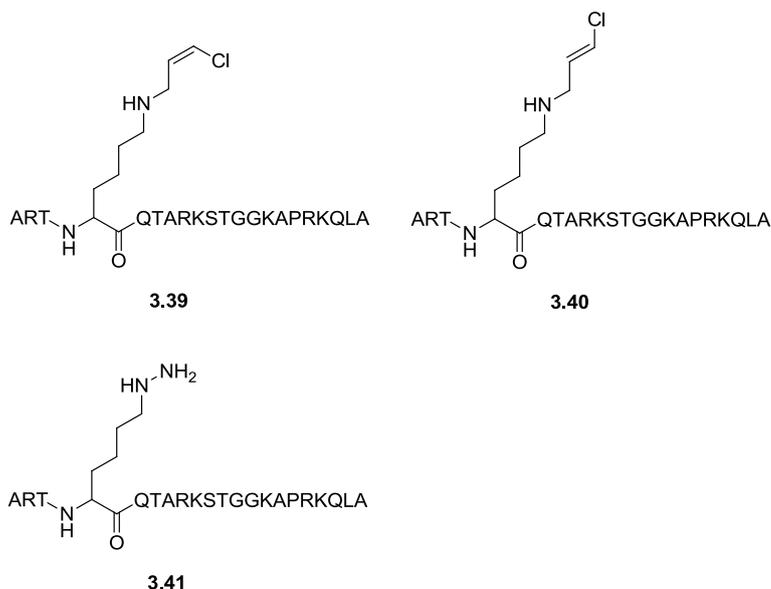
Figure 3.9 : Structures of peptide derivatives

They stated that phenelzine (**3.8**) is a more potent LSD1 inhibitor than previously reported.[169] According to their research, this inhibitor is 35-fold more potent than tranylcypromine in enzyme assay. The mechanism of LSD1 inactivation by phenelzine is proposed to involve oxidations to afford **3.43** via the formation of the diazene **3.42** (Scheme 3.5). Nucleophilic attack of the flavin on the electrophilic carbon of **3.43** formed the covalent adduct **3.44** after loss of nitrogen.
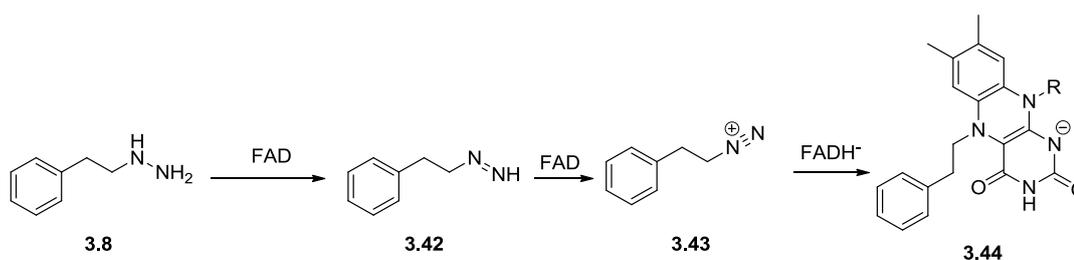
Scheme 3.5 : Proposed mechanism of LSD1 inactivation by phenelzine

They also tested this compound in cell assay and determined that it blocked the demethylation of H3K4Me in cells.[169]

3.1.2.4. Polyamines

Some polyamines were found to exhibit LSD1 inhibition, such as biguanide and bisguanidine polyamine analogues (Figure 3.10).[170] These polyamine analogues are LSD1 inhibitors with an IC$_{50}$ lower than 2.5 µM. They are able to re-express several aberrantly silenced tumour suppressor genes.[170,171] These biguanide and bisguanidine polyamine analogues were shown to reactivate aberrantly silenced genes in colon cancer.[170] A later study also showed that **3.46** was also able to alter gene expression in ER-negative human breast cancer cell lines.[172]
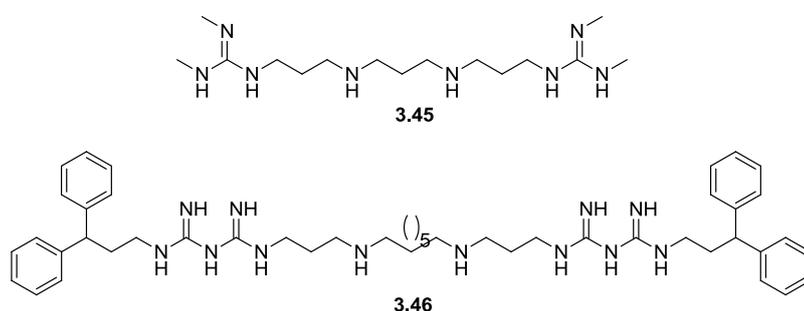
Figure 3.10 : Structures of biguanide 3.45 and bisguanidine 3.46 inhibitors of LSD1

3.1.3. Jumonji-containing histone demethylases

Other histone demethylases recently discovered include Jumonji-containing histone demethylases (JHDMs).[143] The demethylase activity of these enzymes requires Fe(II) and α-ketoglutarate as cofactors. The Jumonji family of iron(II)-α-ketoglutarate-dependent demethylases are able to demethylate different sites on a lysine substrate with different degrees of methylation.[143] Unlike LSD1, these enzymes are able to demethylate trimethyl- lysine. This demethylation proceeds with a different mechanism than that of LSDs: hydroxylation of the methyl group followed by elimination of formaldehyde (Scheme 3.6).
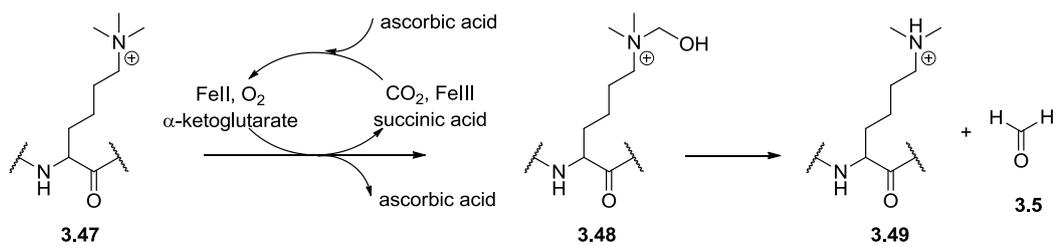
Scheme 3.6 : Demethylation of trimethyl-lysine by the Jumonji proteins

3.2. AIMS

We focused on LSD1 enzyme and envisaged preparing inhibitors of LSD1 as they are potential antitumor therapeutic agents.

We planned to study the SAR of tranylcypromine. As a large hydrophobic pocket was clearly observed in the crystal structure of FAD-PCPA in LSD1, analogues of tranylcypromine with substituents on the phenyl ring were interesting targets. We were also planning to set up the LSD1 assay in order to be able to test the compounds ourselves.

We also wanted to prepare some phenelzine analogues and investigate their biological activity as Cole *et al.* mentioned the potency of phenelzine in inhibiting LSD1 but no phenelzine analogues were evaluated.[169]

3.3. SYNTHESIS OF TRANYLCYPROMINE ANALOGUES

Different strategies are conceivable to prepare analogues of tranylcypromine. As cyclopropane rings are found in a variety of natural products and biologically active compounds, methods to construct those rings are highly developed in racemic and asymmetric series.[173] Several cyclopropanation methods are reported in the literature such as cyclopropanation of styrene derivatives or *trans*-cinnamates with diazo compounds, or Simmons-Smith cyclopropanation of *trans*-cinnamates.[173,174] Action of the sulfur ylide initiated by Michael addition on *trans*-cinnamate is also a possible way to prepare cyclopropane rings.[175]

As the transition-metal catalysed cyclopropanation of olefins, using diazo compounds as a carbene source, is a major and convenient method for the preparation of cyclopropanes, we chose to perform the cyclopropanation on terminal activated olefins using this strategy.

We first decided to prepare derivatives of tranylcypromine bearing substituents, such as halogens, aryl or alkyls groups, on the *para* position of the phenyl ring. The retrosynthetic analysis of the target molecules **3.50** indicates they will be obtained from styrene derivatives **3.52** via formation of the intermediate esters **3.51** (Scheme 3.7).

R = X, Ar or Alkyl
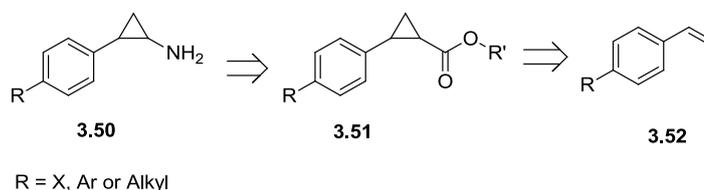
Scheme 3.7 : Retrosynthetic analysis of tranylcypromine derivatives

3.3.1. First strategy in racemic series

Firstly, cyclopropanation was performed in racemic series (Scheme 3.8). The reaction of ethyl diazoacetate in the presence of *para*-fluoro- or *para*-methoxy- styrene **3.53**, using rhodium catalysis at reflux in toluene, afforded a mixture of *trans*- and *cis*-ethyl cyclopropanecarboxylates **3.54** (63/37 for *para*-fluoro-; 75/25 for *para*-methoxy-compound).[176] An epimerisation using sodium methoxide as a base gave the thermodynamic diastereomer, *trans*-ester and the *trans*-acid **3.55** as major compound.[176]

Then, the hydrolysis of this *trans*-ester by action of lithium hydroxide in a mixture of methanol and water gave the carboxylic acid **3.55**. Treatment with diphenylphosphoryl azide and triethylamine at reflux in *tert*-butanol gave the carbamate **3.56** with a yield ranging from 22 to 53%.[176] The Curtius rearrangement is a relatively slow step as it required 48 h. The reaction is carried out in *tert*-butanol which scavenged the isocyanate intermediate to lead to the carbamate **3.56**. Subsequent acidic treatment should provide the amine **3.57**.
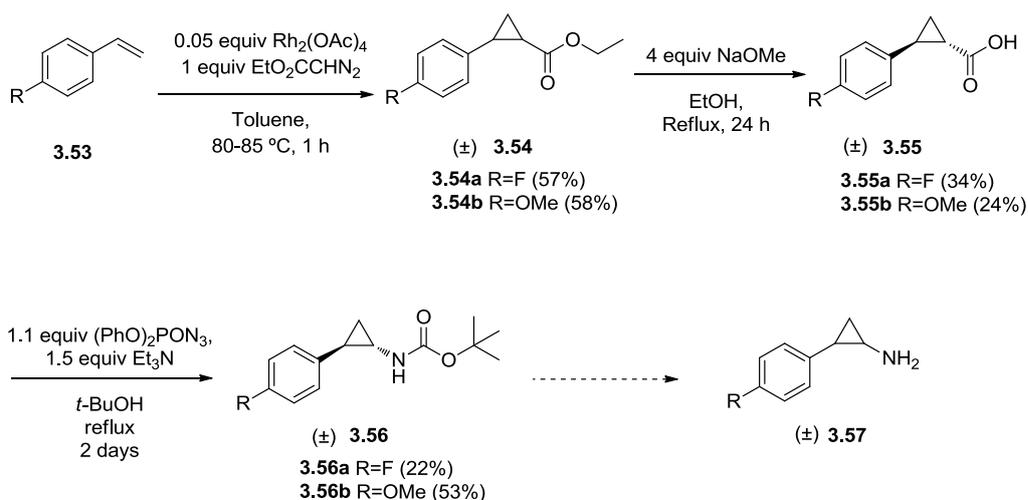
Scheme 3.8 : Attempts to prepare tranylcypromine analogues via rhodium catalysed cyclopropanation

Problems were encountered during the epimerisation which was not found to be reproducible. Many trials were performed to repeat the epimerisation but all unsuccessfully: a mixture of *cis*- and *trans*-ester and -acid was obtained. Hydrolysis of the ester took place during the work-up and the quantity of ester obtained was low. The source of sodium methoxide bottle and the concentration of the reaction were changed. In addition the reaction was performed under anhydrous conditions. We decided to change the strategy in order to avoid the epimerisation step. We should have changed the base and use sodium hydroxide solution in ethanol as Yokoyama did for the synthesis of his fluoro- tranylcypromine analogues.

3.3.2. Second strategy using an asymmetric cyclopropanation

As we believed that one of the *trans* enantiomers might be a better LSD1 inhibitor, we decided to attempt to prepare analogues using an asymmetric strategy. The most active enantiomer for MAO inhibition is the (−)-tranylcypromine with the (1*S*, 2*R*) stereochemistry. We decided to prepare the same enantiomer in the belief that the homologous LSD1 enzyme would exhibit the same stereochemical preference.

A cationic copper complex, formed in situ from chiral bis(oxazolines) **3.58** and copper triflate, was used as catalyst to perform the asymmetric cyclopropanation on the monosubstituted olefin **3.53** with an achiral diazo ester (*tert*-butyl diazoacetate) at ambient temperature (Scheme 3.9).[164,177] The products were isolated as *trans/cis* mixtures of the cyclopropyl esters (83/17 for the fluoro- derivative). The *trans* isomer **3.59** (1*S*, 2*S*) is the major compound and its absolute configuration was deduced by analogy from previous work. We used *tert*-butyl diazoacetate instead of ethyl diazoacetate in order to obtain higher enantioselectivity. The *trans-tert*-butyl cyclopropanecarboxylate **3.59** was then hydrolysed using trifluoroacetic acid and triethylsilane in dichloromethane.[178] The carboxylic acid obtained **3.60** was converted into a carbamate **3.61** by Curtius rearrangement and finally HCl gas was employed to remove the Boc protecting group.[176] The fluoro- and methoxy- derivatives were prepared.
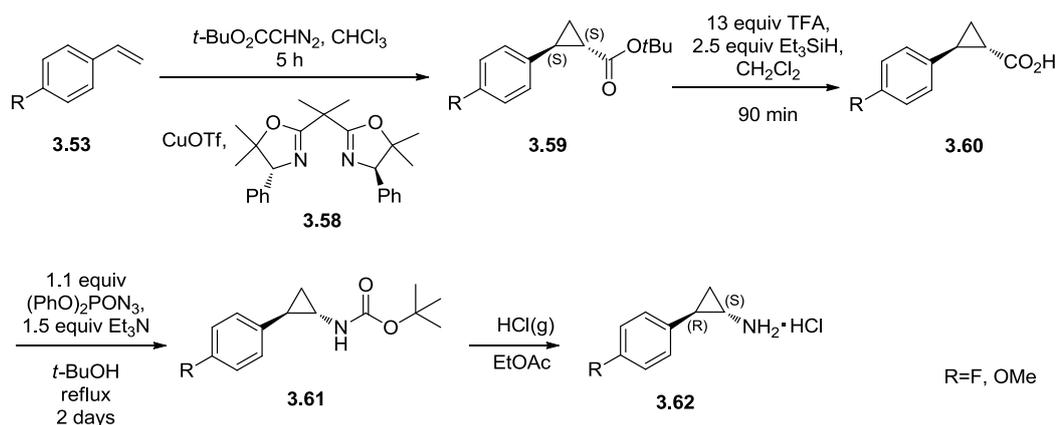
Scheme 3.9 : Asymmetric synthesis of tranylcypromine analogues with (1*S*, 2*R*) stereochemistry

Resolution of the tranylcypromine racemic mixture

A resolution of the racemic mixture of tranylcypromine was carried out by forming diastereomeric tartrates (Scheme 3.10).[165] Recrystallisation allowed the isolation of one diastereomeric tartrate and therefore the corresponding enantiomer after hydrolysis (*e.g.* **3.63** with L-tartaric acid).

3.63
(-)-tranylcypromine
$K_{i(inact)}$ 28.1 µM

(+)-tartaric
acid resolution

3.6
(±)-tranylcypromine
$K_{i(inact)}$ 25.0 µM

(-)-tartaric
acid resolution

3.64
(+)-tranylcypromine
$K_{i(inact)}$ 26.6 µM
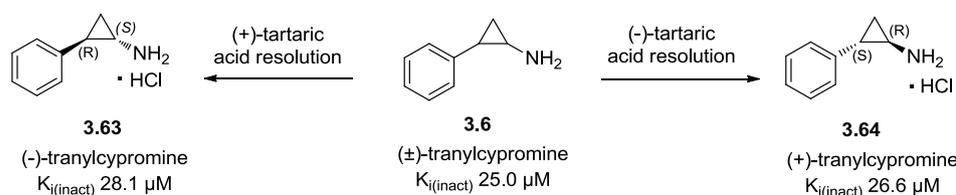
Scheme 3.10 : Resolution of (±) racemic tranylcypromine

The LSD1 enzyme assay of the racemic mixture of tranylcypromine and the two enantiomers was performed. The assay will be described in detail at a later stage. The racemic mixture gave the best enzyme assay result with 25 µM for the Ki. The Ki of (+)-enantiomer was 26.6 µM and the (−)-enantiomer was 28.1 µM. Based on these results, we decided to prepare the (+)-enantiomer as it showed slightly better activity than the (−)-enantiomer. Asymmetric cyclopropanation on styrene derivatives **3.65** was performed using *tert*-butyl diazoacetate at ambient temperature and a catalyst formed in situ from chiral bis(oxazolines) **3.66** and copper triflate (Scheme 3.11). We used the same procedure described earlier, but used ligand **3.66**, with the opposite stereochemistry to that of ligand **3.58**, in order to obtain the (1*R*, 2*S*) tranylcypromine analogue instead of the (1*S*, 2*R*) as described in scheme 3.9. Using this pathway, we prepared several analogues in four steps: *para*-fluoro-, *para*-methoxy-, *para*-bromo-, *meta*-bromo- and *ortho*-bromo- tranylcypromine derivatives. Four of them were prepared by Christopher Hodgkinson, an undergraduate student under my supervision. Cu(OTf)$_2$ instead of CuOTf was used by mistake for the synthesis of all the derivatives however, as Cu(OTf)$_2$ must be reduced to CuOTf during the reaction, the cyclopropanation worked quite well. This mistake may explain the wide range of yield obtained for the different analogues (30-77%).
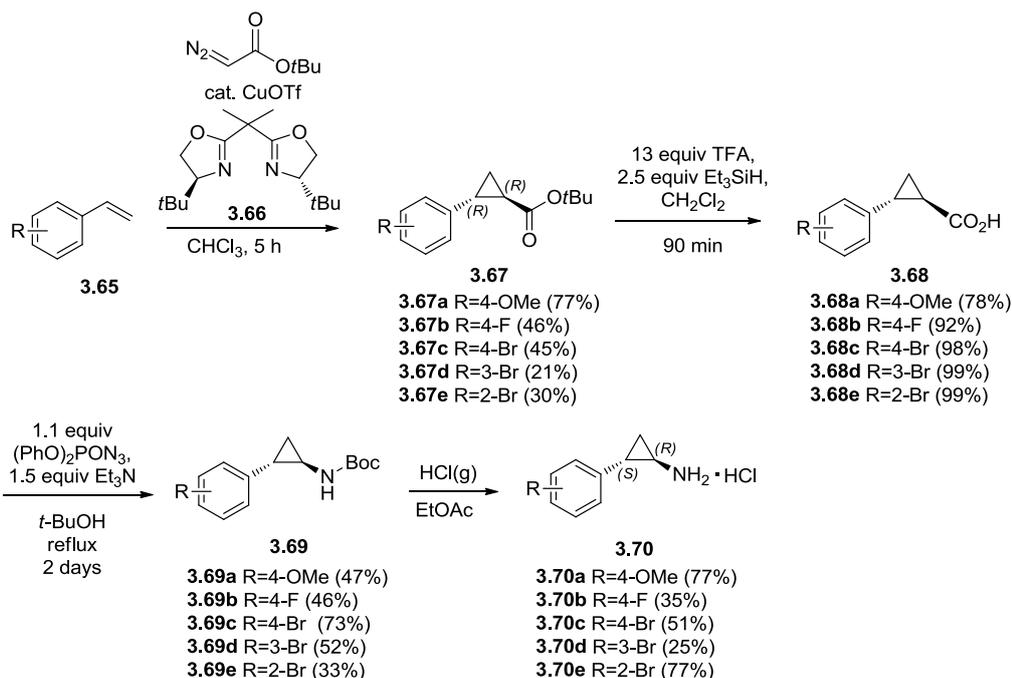
Scheme 3.11 : Asymmetric synthesis of tranylcypromine analogues with (1R, 2S) stereochemistry

In addition to being interested in the bromo- derivatives LSD1 inhibition activity (**3.70c-e**), we chose the bromo substituent in order to be able to prepare additional analogues, by diverse palladium catalysed crosscoupling reactions, with ease. Suzuki couplings were performed on the bromo- carbamate derivatives in order to incorporate phenyl groups. Initial work on the Suzuki was performed by a student but he was unable to obtain the purified carbamates (**3.69c-e**), due to reactions not going to completion and difficulty separating the product from the starting material, during his placement. By increasing the number of equivalents of the reagents we were able to force the reactions to completion and successfully prepare the derivatives **3.69f-h**. The final step is the Boc removal (Scheme 3.12). Suzuki coupling enabled us to prepare the *para-*, *meta-* and *ortho-* phenyl tranylcypromine derivatives (**3.70f-h**) in a total of five steps.
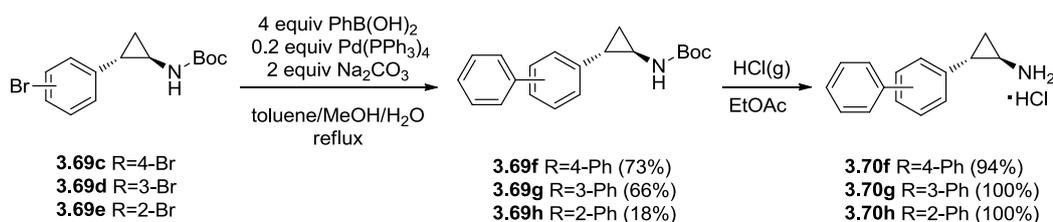
Scheme 3.12 : Synthesis of the biphenyl tranylcypromine analogues via Suzuki coupling

We attempted to prepare the furyl-, thienyl- and pyridyl- analogues (Figure 3.11).
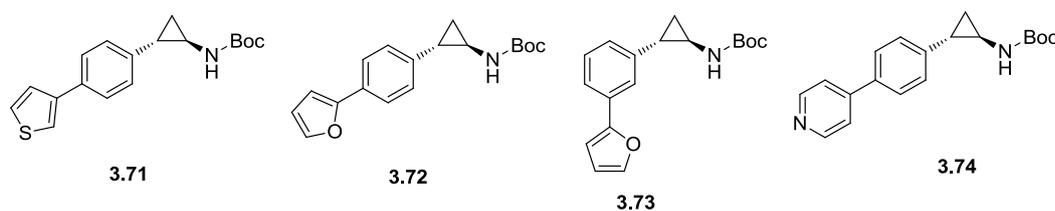
Figure 3.11 : Bicyclic compound carbamates

We successfully obtained the *meta-* and *para-* furyl- derivatives along with the *para-* pyridyl- compound but did not manage to obtain the thienyl- compound. HCl deprotection was unsuccessful and no product was obtained, probably due to sensitivity of the furan and pyridine rings to acidic conditions. No further work was carried out for these compounds. Different Boc deprotection methods could be tried in the future as the biological activity of these compounds is potentially interesting.

We are going to test the tranylcypromine analogues prepared as LSD1 inhibitors (Figure 3.12).
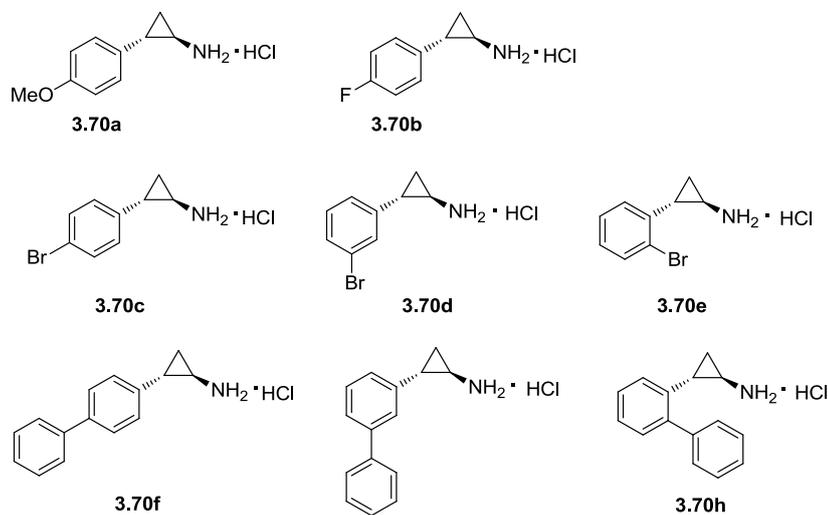
Figure 3.12 : Structures of the prepared tranylcypromine analogues (3.70a-h)

3.4. BIOLOGY TESTING OF TRANYLCYPROMINE ANALOGUES

3.4.1. Expression and purification of full length human LSD1

Dr Patrick Duriez, a collaborator at the Cancer Research UK centre in Southampton, performed all the work to prepare the enzyme.

3.4.1.1. Cloning of LSD1 in pET15b and protein expression principles

The human recombinant protein LSD1 was produced in bacteria using molecular biology techniques. First, the DNA coding for the LSD1 protein was inserted in the plasmid pET15b to create the construct pET15b-LSD1 (generous gift from Dr. Yang Shi, Harvard Medical School, Boston). A plasmid is a small circular DNA that can replicate independently of the chromosomal DNA once incorporated into bacteria. Among other features, the plasmid carries an antibiotic resistant gene that allows the selection of the bacteria that contain the plasmid. The bacterial strain used was *E. coli* BL21 CodonPlus-RIPL.

The pET plasmids have been engineered to allow the production of protein upon the addition of a chemical inducer, IPTG (isopropyl-β-D-thiogalactopyranoside), to the bacterial culture. The construct pET15b-LSD1 also includes a stretch of 6 histidine residues at the *N*-terminus of the LSD1 protein for purification purposes.

3.4.1.2. Protein expression

The induction of the expression of the protein LSD1 was carried out by adding IPTG to exponentially growing bacteria. Following the protein induction, the bacteria were centrifuged. The supernatant was removed and the bacteria pellet was kept in the freezer.

3.4.1.3. Protein extraction

The bacteria pellet was lysed in a mixture of Tris-HCl, NaCl, Triton X100, glycerol, DNase I and $MgCl_2$ in the presence of a cocktail of protease inhibitors to prevent the

degradation of the protein of interest by bacterial enzymes. The detergent Triton X100 helps the lysis of the bacteria while the DNase I is an enzyme that breaks down the DNA which is released during the lysis of the bacteria and will make the sample very viscous otherwise. The supernatant containing the soluble proteins was kept and will be purified.

3.4.1.4. Protein purification

The expressed protein His_6-LSD1 was subjected to successive chromatography steps due to the presence of contaminant proteins to obtain a protein which was as pure as possible. The first chromatography separation relied on the binding of the His_6 tag present at the *N*-terminus of the protein on a Nickel column. The second purification step used a gel filtration, or size exclusion column to separate LSD1 from other proteins according to its molecular weight. Finally, the last chromatography used an anion exchanger (Q-sepharose column) to separate LSD1 from other proteins according to its isoelectric point or pI (pI of LSD1 ~ 6). The fractions containing LSD1 were combined, aliquoted and stored at −80 ºC. This purified enzyme will be used in the enzyme assay.

3.4.2. Mass spectrometric analysis

This analysis was performed by Dr P. Duriez with the help of Dr B. Zeidan and Prof P. Townsend.

Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) was used to control the demethylation activity of LSD1 on the substrate. This method employs chromatographic surfaces coupled to a time-of-flight mass spectrometry.[179] In SELDI-TOF, samples are spotted on ProteinChip® arrays (surface modified with a chemical functionality) then, the matrix that absorbs laser energy (energy absorbing molecule or EAM) is added and co-crystallised with the sample. The mass-to-charge ratios are deduced from the time required by the ionised proteins to reach the detector.

The enzyme was incubated with the peptide fragment of H3K4me2: ARTK(me2)QTARKSTGGKAPRKQLA. SELDI-TOF mass spectrometric analysis of the substrate presented a peak with a molecular weight equal to 2283 (Figure 3.13). The mass spectrometer presented a calibration error responsible for an additional 5 units in

all the detected mass. SELDI-TOF mass spectrometric analysis of the reaction mixture showed the peaks of the methylated (MW 2269) and non-methylated substrate (MW 2255). Addition of tranylcypromine resulted in the enhancement of the peak corresponding to the dimethyllysine (MW 2283). This experiment confirmed the fact that the prepared enzyme is able to demethylate H3K4 and that tranylcypromine is an inhibitor of LSD1.
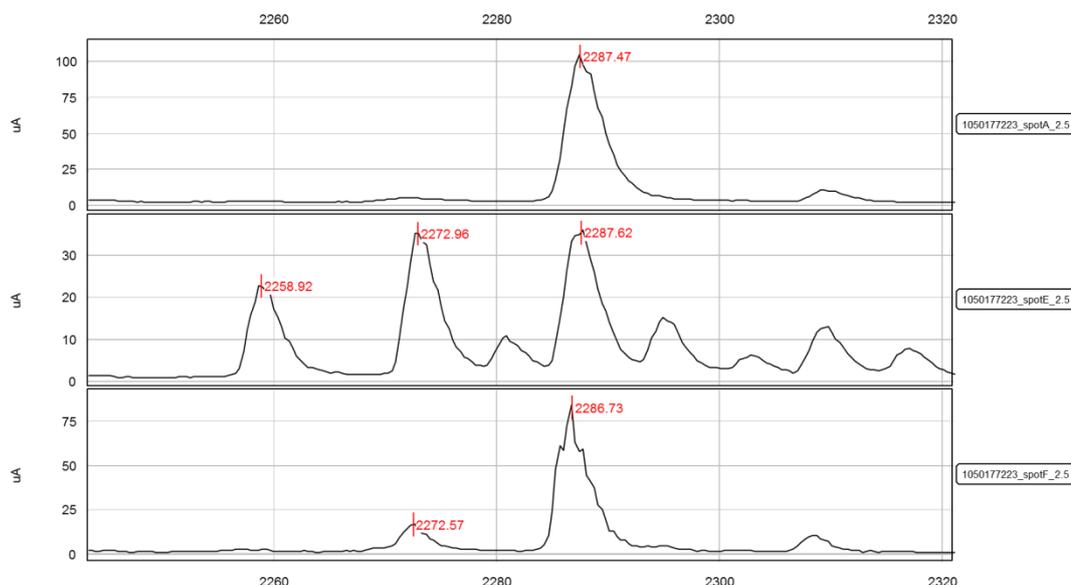
Figure 3.13 : Mass spectrometric analysis of LSD1 activity with the substrate H3K4me2: top graph MS of peptide alone, middle graph: peptide with LSD1 and bottom graph: peptide with LSD1 and tranylcypromine

3.4.3. Fluorescence based LSD1 enzyme assay

Our collaborator Dr Patrick Duriez first attempted to use a colorimetric assay in which the formaldehyde is converted by formaldehyde dehydrogenase to formic acid with the reduction of NAD^+ to NADH. The latter is detected spectrophotometrically at 340 nM. Unfortunately, this method did not detect any enzyme activity in our hands.

We then decided to detect hydrogen peroxide, the other by-product formed during the demethylation of lysine by LSD1. For the enzymatic determination of hydrogen peroxide, we decided to use a method based on the peroxidase catalysed oxidation of *N*-acetyl-3,7-dihydroxyphenoxazine **3.75** (Amplex® Red) to the fluorescent product resorufin **3.76** (Scheme 3.13).[180] Amplex® Red, a colourless and nonfluorescent

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derivative of dihydroresorufin, is oxidised to the fluorescent resorufin. This highly sensitive method gave reliable and reproducible results.
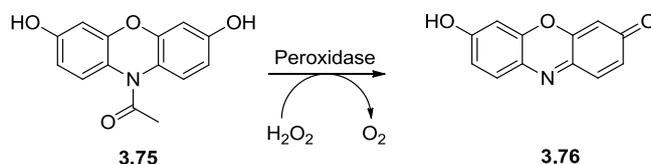
Scheme 3.13 : Oxidation of Amplex® Red (3.75) to resorufin (3.76) by hydrogen peroxide and HRP

From that point, I performed the enzyme assay after receiving help and explanations from Patrick Duriez, our co-worker.

The experiments were performed in a 96-well microplate. LSD1 was incubated with the commercial peptide substrate which consists of the first 21 amino acids of human histone 3 dimethylated on lysine 4 ARTK(me$_2$)QTARKSTGGKAPRKQLA for 20 min. Amplex® Red and horseradish peroxidise (HRP) were then added in order to detect subsequent hydrogen peroxide formation. After 30 min, Amplex® Red stop reagent was added in order to stop the reaction. It is essential to stop the reaction to measure the fluorescence after a certain time period for all the experiments as the fluorescence is time-dependent. The fluorescence was read on a microplate reader with excitation at 530 nm wavelength and emission at 590 nm wavelength. The value read represents the relative fluorescence unit (RFU).

For determining the effect of the inhibitors on the enzyme, the analogues were pre-incubated with LSD1 for 10 min prior to performing the assay.

In order to find the optimum conditions, we had to look into the enzyme kinetics and to determine the Michaelis constant K_m of the enzyme.[181] For three fixed concentrations of the enzyme, the concentration of the substrate was varied and the assay was carried out (Figure 3.14).
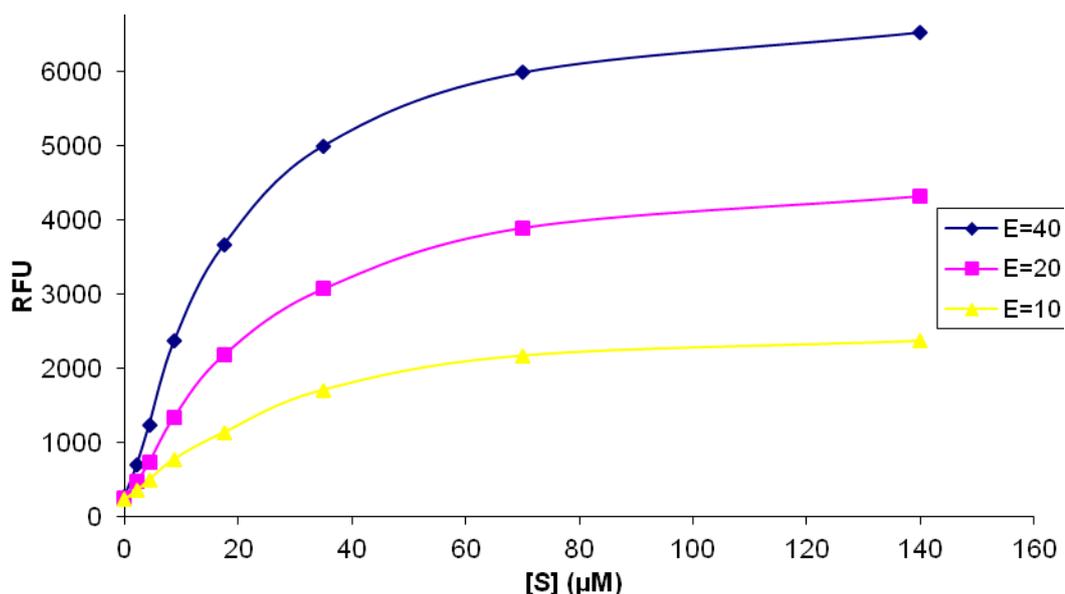
Figure 3.14 : Effect of substrate concentration on the rate of an enzyme catalysed reaction for three different concentrations of the enzyme

Linear transformation of the Michaelis-Menten equation allowed us to determine the value of K_m (Figure 3.15). The determination of the K_m could have been obtained from the first graph but higher accuracy is obtained with the Lineweaver-Burk plot.
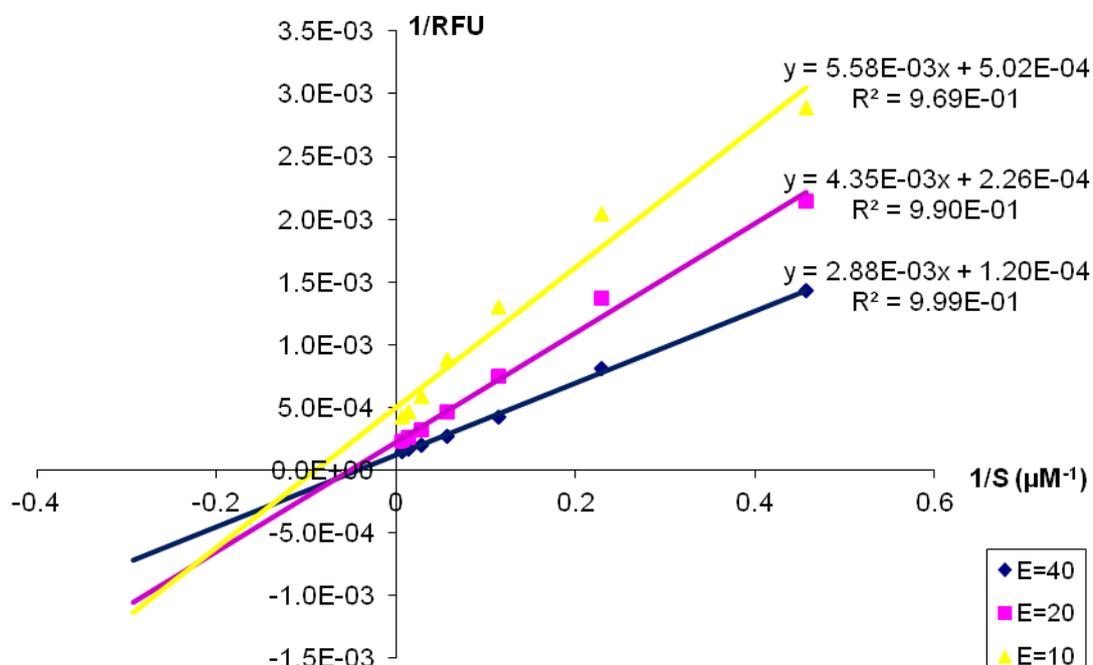
Figure 3.15 : Lineweaver-Burk plot: effect of substrate concentration on the initial rate of an enzyme-catalysed reaction

To determine the K_m, we decided to use the two lines obtained for the concentration of the enzyme equal to 20 and 40 ng/µL, as there is a point which is an outlier in the line obtained for the concentration equal to 10 ng/µL. The lines have an intercept of $-1/K_m$ on the 1/[S] axis. K_m was found to be around 21 µM for the peptide substrate.

We would usually perform a screening of the analogues we prepared at a certain concentration (25 µM or 50 µM).

If the analogues presented a greater activity than tranylcypromine a dose-response experiment would be performed. The Ki was obtained from five to eight concentrations points performed in duplicate or triplicate, fitted by a representative sigmoidal dose response curve (e.g. figure 3.16 for racemic tranylcypromine and for both enantiomers). The LSD1 inhibition profile experiment was then repeated once or twice for each compound in order to obtain the $K_{i(inact)}$ for the inhibitors (Table 3.1).
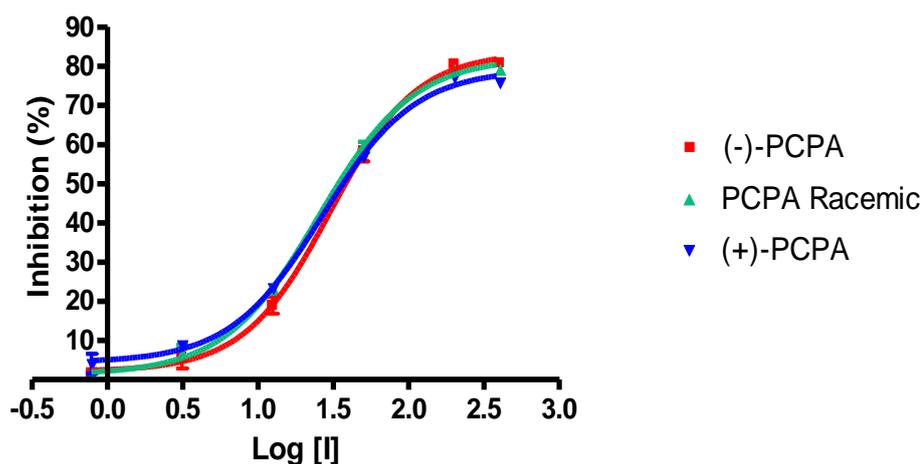
Figure 3.16 : Dose response experiments for racemic tranylcypromine, (+)-tranylcypromine and (–)-tranylcypromine

The 4-methoxy-, 4-fluoro- and 4-bromo- analogues (**3.70a**-**c**) were made in order to study the influence on the LSD1 inhibition activity of the electronic effects on the substituents in the phenyl ring. The bromo- analogue **3.70c** was found to be a greater LSD1 inhibitor than tranylcypromine with a $K_{i(inact)}$ of 3.7 µM, whereas the methoxy- and fluoro- analogues (**3.70a** and **3.70b**) were weaker LSD1 inhibitors with respective $K_{i(inact)}$ of 41.8 and 79.6 µM. We can see that strong electron-withdrawing and electron-donating substituents on the phenyl ring are unfavourable for the LSD1 activity. The encouraging result of the bromo- derivative led us to prepare the *meta*- and *ortho*-

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bromo- analogues (**3.70d** and **3.70e**). These compounds were also more active than tranylcypromine, displaying $K_{i(inact)}$ of 8.9 μM for the *meta-* derivative **3.70d** and 11.7 μM for the *ortho-* analogue **3.70e**, the *para-* analogue **3.70c** being the most potent LSD1 inhibitor. It is likely that the *para-* bromo- analogue is the best inhibitor because the bromo- group in *para* position has a better fit within the hydrophobic pocket. The *para-* phenyl analogue **3.70f** was also a greater LSD1 inhibitor than tranylcypromine whereas the *meta-* and *ortho-* phenyl derivatives (**3.70g** and **3.70h**) were poorer inhibitors in enzyme assay.

3.4.4. LNCaP cell growth inhibition assay

As explained earlier, LSD1 is able to form a complex with the AR and to demethylate the repressive marks of mono- and dimethyllysine 9 on histone 3. The LSD1 inhibitors are able to block this demethylation. We tested our tranylcypromine analogues against the LNCaP prostate adenocarcinoma cell line. A six-day dose response was performed by our collaborators for each derivative in order to obtain IC_{50} (Table 3.1).

Tranylcypromine showed very poor results in cell growth inhibition (IC_{50} 174 mM). The methoxy- and fluoro- compounds (**3.70a** and **3.70b**) were, as expected, poor inhibitors of the cell line whilst the methoxy- compound **3.70a** was better than the fluoro- analogue **3.70b** in accordance with the enzyme assay result. The *para-* and *meta-* bromo- analogues (**3.70c** and **3.70d**) were much more potent than tranylcypromine with respective IC_{50} of 111 and 129 μM (1000 fold compared to tranylcypromine). The *ortho-* bromo- compound **3.70e** showed a good activity in enzyme assay but was relatively poor in cell growth inhibition. We are not able to explain the weak result of the *ortho-* bromo- analogue **3.70e** in cell assay. The phenyl analogues (**3.70f**-**h**) were all quite potent inhibitors of the cell line LNCaP whereas in enzyme assay only the *para-* phenyl analogue **3.70f** showed interesting results. We believed that the cell assay result is highly correlated to the permeability of the compounds. The lipophilicity is responsible for the cell permeability of the compounds, thus lipophilic compounds, such as the phenyl analogues, are able to penetrate the membrane and cause growth inhibition (Figure 3.17).

3.77
CLogP: 1.397

3.78
CLogP: 1.621

3.79
CLogP: 3.366

3.80
CLogP: 2.341
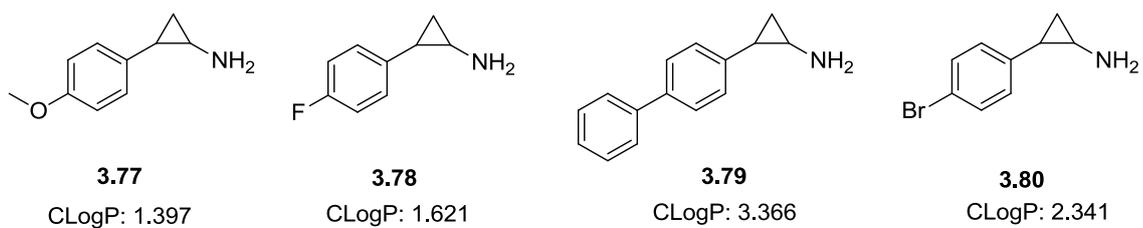
Figure 3.17 : Predicted lipophilicity of the different analogues 3.77, 3.78, 3.79 and 3.80 using ChemDraw

Compound	Structure	LSD1 inhibition $K_{i(inact)}$ (μM)	LNCaP inhibition IC_{50} (μM)
(±)-Tranylcypromine		25.0 ± 9.5	>100,000
(+)-Tranylcypromine		26.6 ± 12.2	>100,000
(−)-Tranylcypromine		28.1 ± 12.9	>100,000
3.70a		41.8 ± 1.0	1706 ± 120
3.70b		79.6 ± 1.0	>100,000
3.70c		3.7 ± 0.5	111 ± 103
3.70d		8.9 ± 3.2	129 ± 3
3.70e		11.7 ± 0.9	5869 ± 470
3.70f		6.8 ± 0.3	110 ± 31
3.70g		>100	226 ± 72
3.70h		>100	181 ± 6
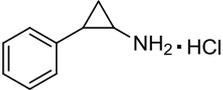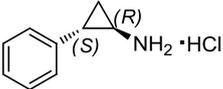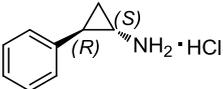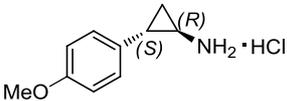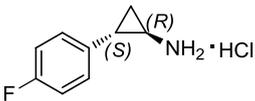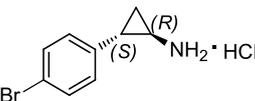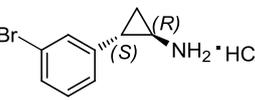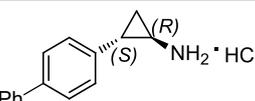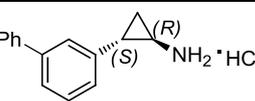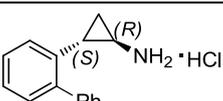
Table 3.1 :Activity of the tranylcypromine derivatives in LSD1 enzyme assay and LNCaP growth inhibition

3.4.5. PSA expression

Another experiment was performed by our collaborators at the Cancer Research UK centre in Southampton: Dr Simon Crabb, Dr Annette Hayden and Rosemary Bulleid. The AR induces prostate-specific antigen (PSA) gene expression, and our collaborators are looking at the influence of the inhibitors on PSA expression (Figures 3.18 and 3.19). The experiment was done with different concentrations of the compounds which makes the comparison of the compounds relatively difficult. They determined that pargyline **3.11**, the *para-* phenyl and the *para-* bromo- analogues (**3.70g**, **3.70f** and **3.70c**) are the most potent inhibitors of PSA expression with the most promising compounds being the *para-* phenyl and the *para-* bromo- derivatives (**3.70f** and **3.70c**). Further testing of these two compounds is ongoing.
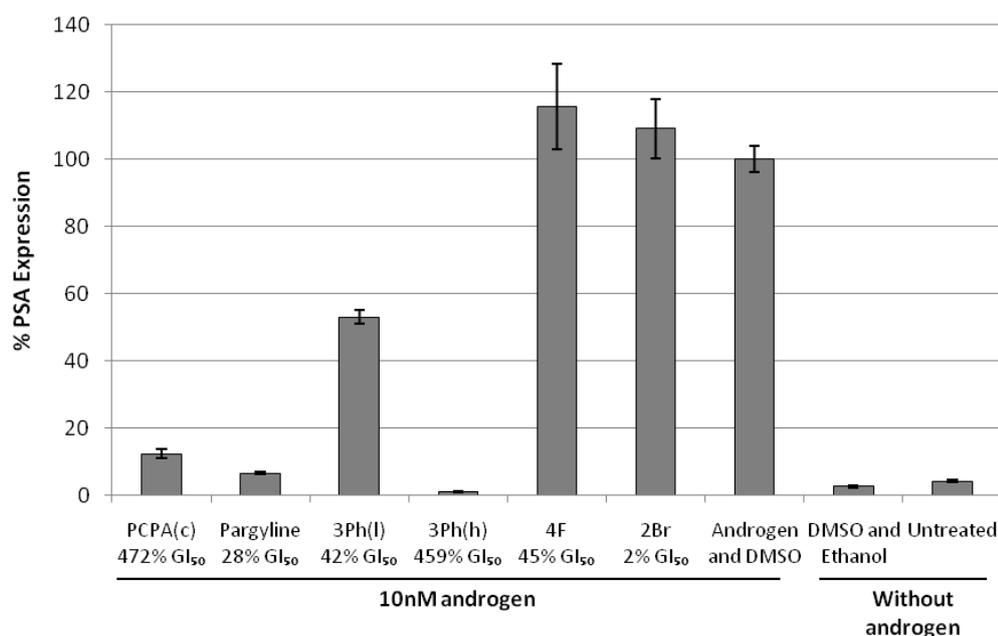
Figure 3.18 : PSA expression following treatment with the different analogues
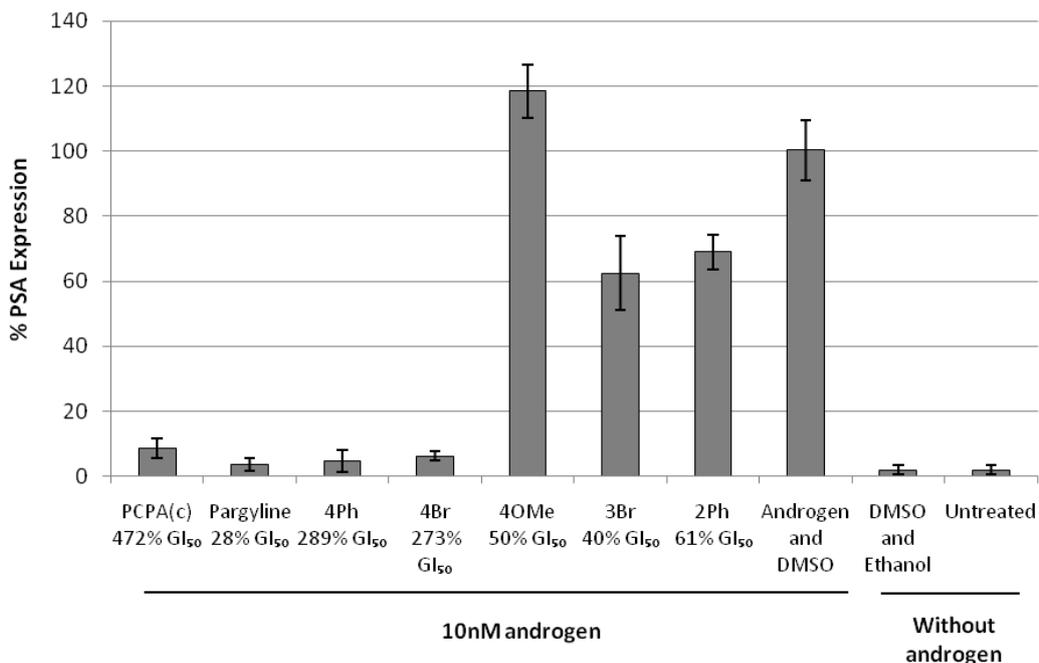
Figure 3.19 : PSA expression following treatment with different analogues

3.4.6. Screening of compounds

3.4.6.1. Screening of Taiwanese compounds

We performed a screening of tranylcypromine analogue MAO inhibitors that we obtained, from Weir-Torn Jiaang, Yu-Sheng Chao and Ting-Yueh Tsai from Taiwan, and performed the dose-response experiment of the analogues with greater inhibition activity than tranylcypromine (Table 3.2). We are trying to understand the SAR of tranylcypromine and discovered some interesting compounds. Chloro- groups in either the *meta-* or *para-* position gave, in both cases, active inhibitors (**3.81**, **3.82** and **3.83**). Donors group in the *meta*-position, such as -OCF$_3$ and –OEt, gave good LSD1 inhibition activity. The phenyl ring was replaced by a methyl thiazole ring and this analogue **3.85** is a good LSD1 inhibitor.
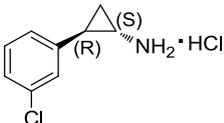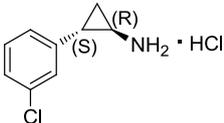
Compound	Structure	LSD inhibition $K_{i(inact)}$ (µM)
3.81		2.38 ± 1.5
3.82		6.30 ± 2.2
3.83		6.68 ± 3.4
3.84		11.31 ± 2.9
3.85		13.39 ± 2.5
3.86		16.73 ± 5.8
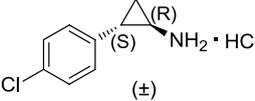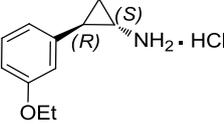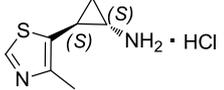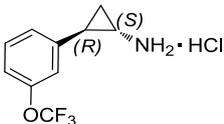
Table 3.2 : $K_{i(inact)}$ table of potent Taiwanese compounds

Only three compounds from the screening were not tested and this was because their inhibition activity was much weaker than tranylcypromine (Figure 3.20). The phenyl ring replaced by 3,5-disubstituted-1,2,4-oxadiazole gave a poor LSD1 inhibitor (**3.87**). The scree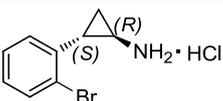ning also showed us that substituting both *ortho* positions with chlorine groups is unfavourable for the biology activity of the compounds, as well as when there are two donor groups (methoxy) on the *meta* positions.
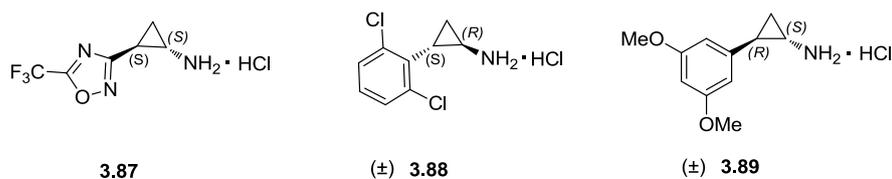
Figure 3.20 : Analogues less active than tranylcypromine in LSD1 enzyme assay

3.4.6.2. Screening of German compounds

A) LSD1 enzyme assay

We tested eighteen fluorinated phenylcyclopropylamines obtained from Professor Günter Haufe from the University of Münster. They were first tested in the enzyme assay at a single concentration and amongst them, four compounds were more potent than tranylcypromine. The compounds displaying higher activity were then tested in the enzyme assay at different concentrations to obtain $K_{i(inact)}$ from dose-response curves (Table 3.3). Compound **3.90** with F_5S- substituent in *para* position is the best analogue obtained so far from all the analogues tested ($K_{i(inact)}$ lower than 1 μM). Compound **3.91** with two phenyl rings attached to the cyclopropyl ring and derivative **3.92** containing F_3C- group in *para* position also showed good activity ($K_{i(inact)}$ around 2 μM). The chloro- compound **3.93** showed good activity, even though the stereochemistry was different to the other analogues (*trans* vs. *cis* for the previous ones).
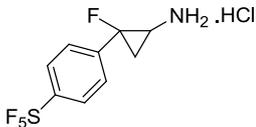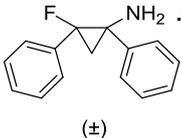
Compound	Structure	LSD inhibition $K_{i(inact)}$ (μM)
3.90		0.77 ± 0.2
3.91		2.06 ± 0.8
3.92		2.13 ± 0.2
3.93		6.70 ± 0.5
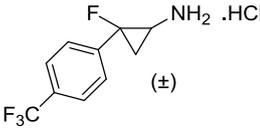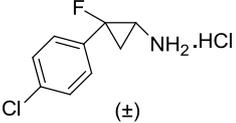
Table 3.3 : $K_{i(inact)}$ values of the potent fluorinated phenylcyclopropylamine analogues

Several fluorinated phenylcyclopropylamines were less active than tranylcypromine (Figure 3.21). Compounds **3.94** and **3.95**, differing from tranylcypromine by the fluoro group in the cyclopropyl ring, are less active than tranylcypromine. The compounds **3.96**, **3.97**, **3.98** and **3.99**, with a strong donor group (-F and -OMe), are not very active

as has also been observed for our analogues. We were not surprised to see that derivatives **3.100** and **3.101**, with extra carbons between the amino group and the cyclopropyl ring, are not active as this part is responsible for the LSD1 inhibition. *Trans* compounds **3.102** and **3.103**, with -CF$_3$ and F$_5$S-, and *cis* compound **3.104**, with the two phenyl substituents on the cyclopropyl ring, are less active than tranylcypromine and their respective *cis* isomers **3.92**, **3.90** and **3.91**. The *cis* derivative **3.105**, with the chloro- group, is less active than tranylcypromine and its *trans* isomer **3.93**. *Cis* and *trans* compounds **3.106** and **3.107**, with a methyl substituent in the 4- position on the aromatic ring, are less active than tranylcypromine.
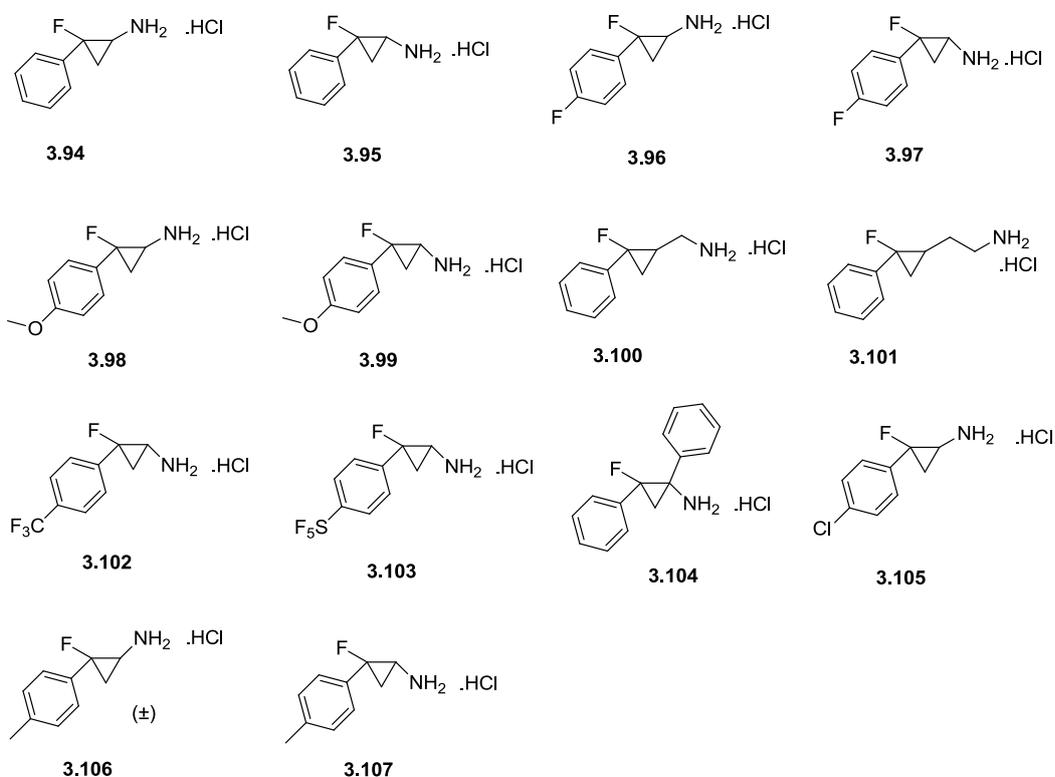
Figure 3.21 : Fluorinated phenylcyclopropylamine analogues less active than tranylcypromine in LSD1 enzyme assay

B) LNCaP cell growth inhibition assay

The compounds are very potent in cell growth inhibition (Table 3.4).

Compound	LNCaP inhibition IC_{50} (μM)
Tranylcypromine	1132 ± 72
3.90	6.67 ± 4.5
3.91	1.53 ± 0.4
3.92	23.23 ± 12.5
3.93	2.76 ± 0.3

Table 3.4 : Activity of the fluorinated phenylcyclopropylamines in LNCaP growth inhibition

C) PSA expression

Tranylcypromine and pargyline reduced significantly PSA expression while the German compounds **3.93** (equivalent to GC6), **3.90** (equivalent to GC8) and **3.91** (equivalent to GC14) failed to show reduction in PSA expression even at high concentrations (Figure 3.22). Compound **3.92** (equivalent to GC11) showed minimal reduction of PSA expression. It is difficult to explain why these compounds are very active in the enzyme and the cell assay but not able to reduce PSA expression. It is possible that the fluoro-group on the cyclopropyl ring is involved in other biological process.
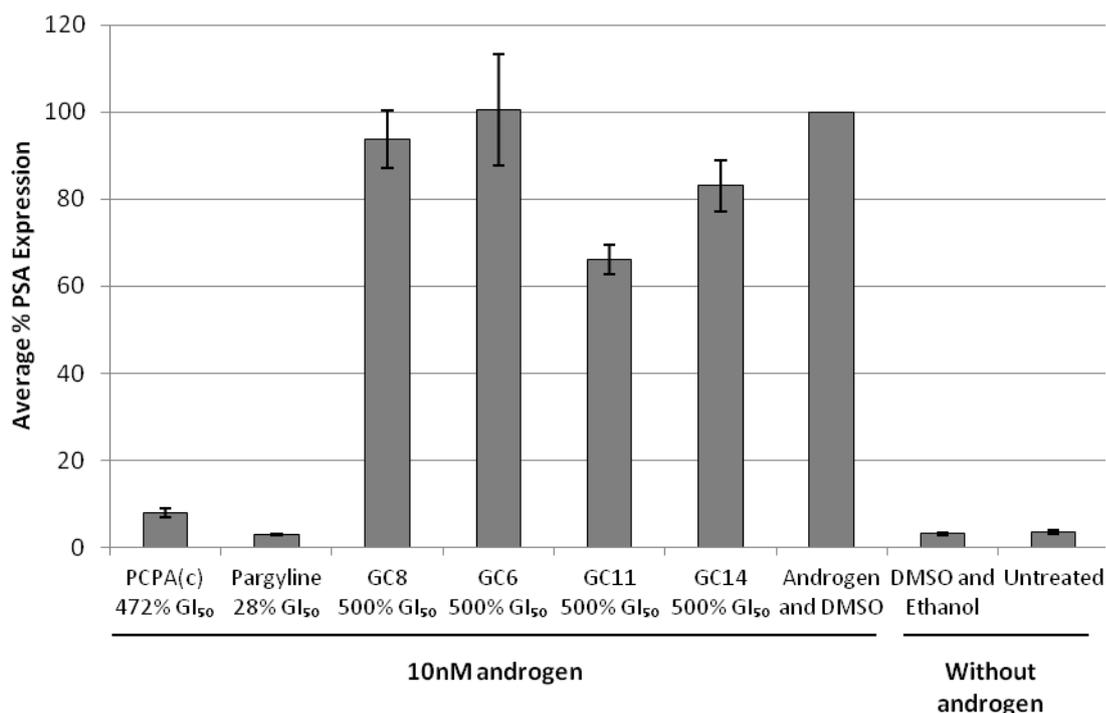
Figure 3.22 : PSA expression following treatment with the fluorinated phenylcyclopropylamines

These four compounds displayed potent activity in enzyme assay and in cell growth inhibition. Nevertheless they were not able to reduce PSA expression.

The facts that derivatives **3.94** and **3.95** are less potent than tranylcypromine and that poor results are also obtained for PSA expression, has encouraged us to prepare analogues without the fluoro group on the cyclopropyl ring. Thus, we are planning to prepare the tranylcypromine derivative **3.108** with F_5S- in *para* position and the *cis* and *trans* derivatives **3.109** and **3.110** with the two phenyl substituents on the cyclopropyl ring. We have already tested compound **3.83**, with the chloro- substituents in *para* position, and McCafferty tested the compound **3.23** with -CF_3 in *para* position.
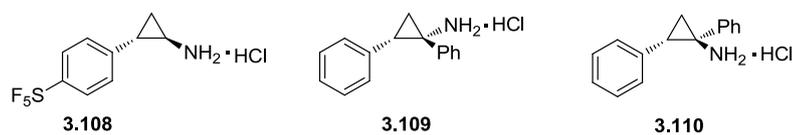
Figure 3.23 : Tranylcypromine derivatives to prepare in the future

3.5. SYNTHESIS OF PHENELZINE ANALOGUES

3.5.1. Route 1

We were planning to prepare some analogues of phenelzine as it has been mentioned that phenelzine was more potent than tranylcypromine in enzyme assay.[169] They also showed that phenelzine was an inhibitor of LSD1 in cells. We decided to prepare the *para-* bromo- and phenyl- phenelzine derivatives due to the encouraging biological activity results we obtained for the two corresponding tranylcypromine derivatives (**3.70c** and **3.70f**).

First, we decided to prepare the phenelzine analogues via formation of the tosylate derivative **3.112** obtained from the carboxylic acid **3.113** (Scheme 3.14).

R: Br or Ph
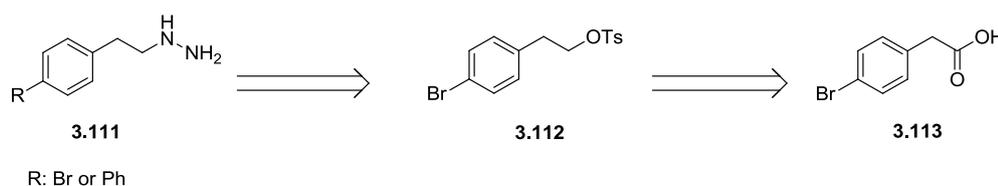
Scheme 3.14 : Retrosynthetic analysis of *para-* bromo- and phenyl- phenelzine derivatives

The commercial 4-bromophenylacetic acid **3.113** was reduced to the alcohol **3.114** followed by protection of the hydroxyl group with a tosyl group. We then attempted to displace the tosylate with Boc hydrazine but were unsuccessful. The final product **3.116** would have been obtained after Boc deprotection. We were planning to perform palladium coupling to insert the phenyl ring followed by Boc deprotection to obtain **3.118**.

As this strategy was unsuccessful we decided to change our method. From previous work performed in our laboratory by former PhD student Sally Radford, we proposed that we could use the reductive alkylation of Boc hydrazine that she developed.[182]
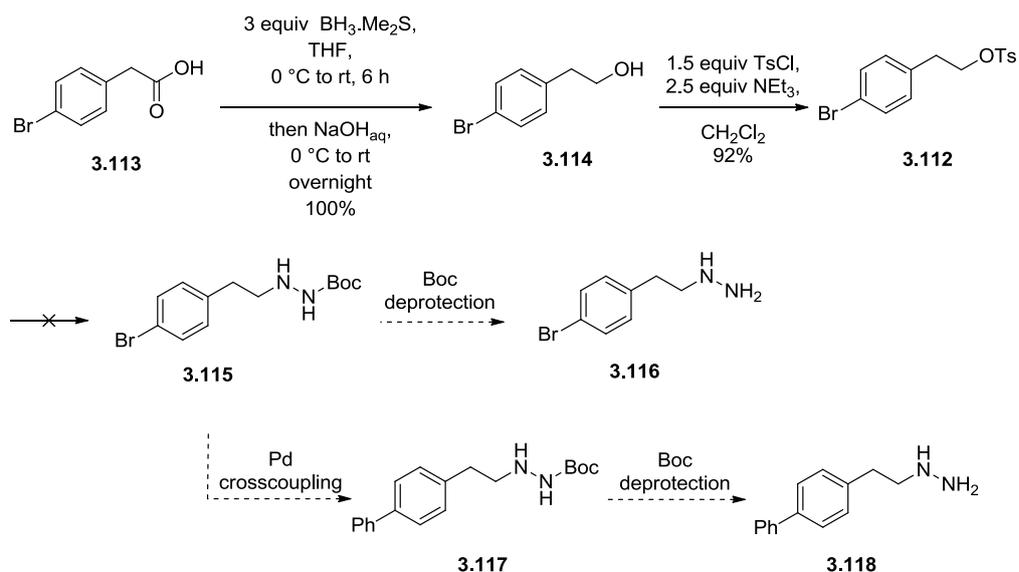
Scheme 3.15 : Attempts to synthesise phenelzine analogues

3.5.2. Route 2

The synthesis of the following phenelzine analogues was performed by two students (Charlotte Poillion and Sebastian Schwenk). We decided to form the aldehyde **3.119** and then condense it with Boc hydrazine to form the hydrazine derivative **3.111**.

R: Br or Ph
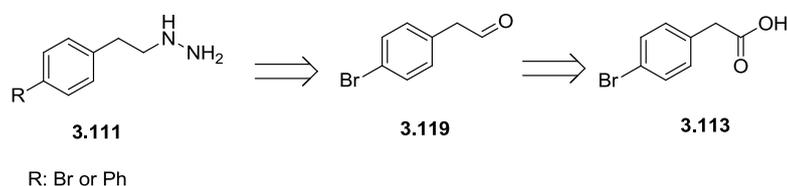
Scheme 3.16 : New retrosynthesis of *para*- bromo- and phenyl- phenelzine derivatives

Oxidization of alcohol **3.114** to the aldehyde **3.119** was performed by Dess-Martin-periodinane in CH_2Cl_2.[183] Washes with solutions of $Na_2S_2O_3$ and $NaHCO_3$ during the work-up are important to obtain a high yield and clean reaction. The aldehyde **3.119** was treated with *tert*-butyl carbazate in dry toluene at 50 °C to form the Boc-protected hydrazone **3.120** in 76%.[182] The hydrazone **3.120** was reduced to the corresponding hydrazine **3.115** using $NaBH(OAc)_3$ in good yield (84%).[184,185] We initially used $NaBH_3CN$ as described by Sally Radford, but decided instead to use $NaBH(OAc)_3$ for safety reasons.[182] The Boc group was successfully removed using HCl in dioxane to afford **3.121**.[186]
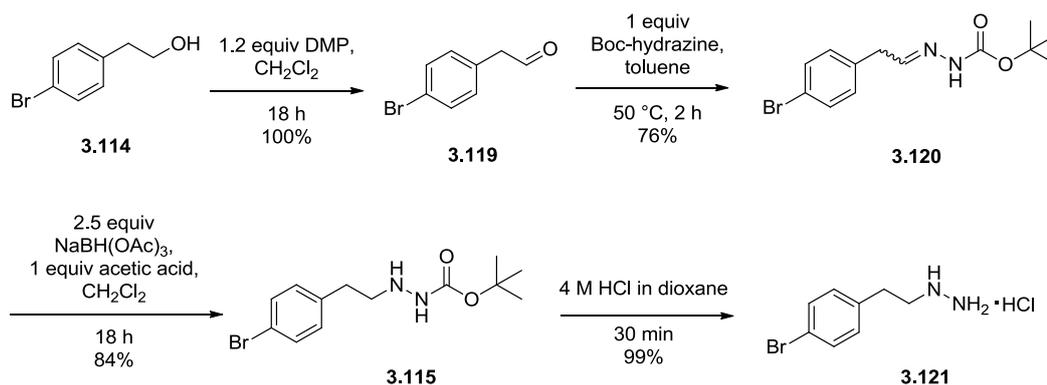
Scheme 3.17 : Synthesis of the bromo- phenelzine derivative

In order to prepare the *para* phenyl phenelzine analogue **3.122** Suzuki coupling was employed. We used the same conditions that were successful for the tranylcypromine analogues (entry 1) but the yield obtained was very low (10%).
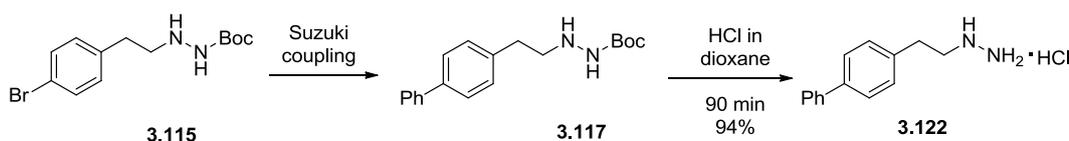
Scheme 3.18: Synthesis of the phenyl- phenelzine derivative

Optimization of the reaction was attempted by changing solvent, base and reaction conditions (Table 3.5). Potassium carbonate and acetonitrile were used (entry 2) and gave the compound **3.117** with low yield 18%.[187] Microwave reaction was tried but with no success (entry 3). The purification of this compound was complicated as several by-products were also formed.

Entry	Chemicals	Solvents	Conditions	Yield (%)
1	4 equiv PhB(OH)$_2$ 0.2 equiv Pd(PPh$_3$)$_4$ 2 equiv Na$_2$CO$_3$	Toluene/MeOH/H$_2$O	120 °C, 18 h	10
2	1 equiv PhB(OH)$_2$ 0.04 equiv Pd(PPh$_3$)$_4$ K$_2$CO$_3$	MeCN/H$_2$O	90 °C, 16 h	18
3	1.2 equiv PhB(OH)$_2$ 0.09 equiv PEPPSI-iPr 2.1 eq. Cs$_2$CO$_3$	Dioxane	120 °C, Microwave 150 psi, 250 W, 40 min	0

Table 3.5 : Optimisation of the Suzuki coupling reaction

Boc removal was performed by treatment with HCl in dioxane to afford **3.122** in 94%. Sebastian Schwenk prepared the bromo- and phenyl- analogues.

3.6. PRELIMINARY STUDIES ON THE BIOLOGY ACTIVITY OF THE PHENELZINE ANALOGUES

Phenelzine sulfate **3.123** was tested in growth inhibition by Annette Hayden and we obtained a potent inhibitor (IC_{50} 121.7 ± 0.1 μM).

LSD1 enzyme assays were performed on the prepared phenelzine analogues **3.121** and **3.122** along with some commercial hydrazine compounds available in our laboratory (**3.123** to **3.127**).
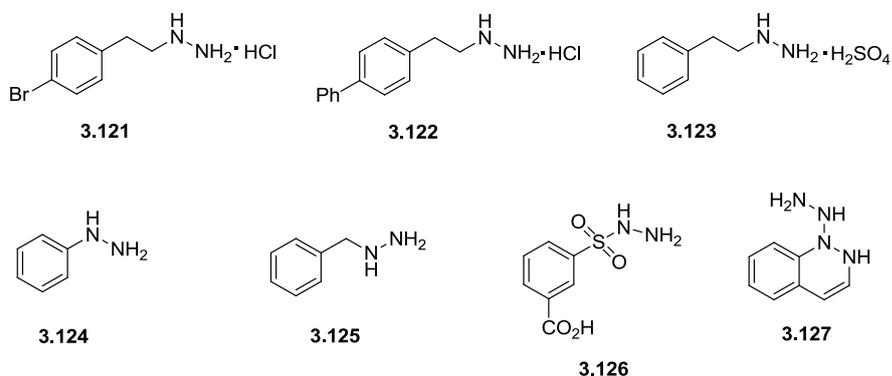
Figure 3.24 : Synthetic phenelzine and commercially available hydrazine derivatives tested

We performed a screening of the inhibitors at high concentration (200 μM instead of 25 or 50 μM) using tranylcypromine as the reference. Unfortunately we discovered that none of the compounds were biologically active against LSD1 while Cole and co-workers obtained a $K_{i(inact)}$ of 17.6 ± 2.8 μM for phenelzine.[169] These results are difficult to explain as Cole *et al.* used the same detection method as us (Amplex® Red coupled assay).[169] We decided to test these compounds in the cell assay and these experiments are currently ongoing.

3.7. CONCLUSION

We decided to prepare the most active enantiomer after studying the LSD1 inhibition activity of both enantiomers after resolution of tranylcypromine racemic mixture. We prepared a series of tranylcypromine analogues in four to five steps using an asymmetric synthesis. We tested the compounds in LSD1 enzyme assay using a fluorometric assay. Cell assay against prostate cancer cell line was then performed. We discovered several tranylcypromine analogues which exhibited greater activity than tranylcypromine itself in enzyme and cell assays. In cell assay, *para-* and *meta-* bromo derivatives are 1000 fold more potent than tranylcypromine. We determined that the size and electronic properties of the substituents are important with substituents in the *para-* and *meta-* positions appearing to be more favourable. It is probable that the substituents in these positions have a better fit within the hydrophobic pocket. Correlation between cell growth inhibition and lipophilicity was observed.

We are planning to prepare other tranylcypromine derivatives. For example, we are going to prepare derivatives starting from *para*-vinylbenzoic acid in order to be able to prepare several inhibitors by simple coupling steps. We are also planning to prepare the derivative with the -SF$_5$ substitution in the *para* position and the derivatives with the two phenyl- groups on the cyclopropyl ring (*cis* and *trans*). Further development of tranylcypromine analogues as LSD1 inhibitors for the treatment of prostate cancer is ongoing. An interesting experiment would be to test the compounds as MAO inhibitors in order to determine their selectivity towards LSD1.

Phenelzine was a potent inhibitor of the cell line LNCaP. Two phenelzine analogues were prepared and tested in LSD1 enzyme assay. Phenelzine and related derivatives were not found to be active as LSD1 inhibitors.

So far, only irreversible LSD1 inhibitors are reported. A reversible LSD1 inhibitor would be a better inhibitor.

4. CONCLUSIONS AND FUTURE WORK

We prepared five different natural product cyclic peptides: sanguinamides A and B, stylissamide A, largazole and burkholdac B. These compounds were all of biological interests.

Sanguinamides and stylissamide A are proline-rich containing cyclopeptides. We planned to prepare these cyclopeptides to evaluate their biological activities. The natural rotamers were not obtained synthetically. We prepared stylissamide A in order to study the translation inhibitory activity of the synthetic compound versus the natural product. The natural and synthetic stylissamide A displayed the same structural characteristics but different biological activity.

Largazole and burkholdacs are cyclic depsipetides presenting histone deacetylase inhibitory activity. Largazole and a series of analogues were prepared in order to study the SAR of this potent HDAC inhibitor. We investigated the metabolic stabilty of largazole and analogues. All the modifications that we made were tolerated. Simplification of largazole is possible with conservation of the biological activity and a correlation between lipophilicity and growth inhibition activity was also observed. Burkholdac B was finally prepared after synthesizing several compounds such as thailandepsins, revised structures of thailandepsins followed by two diastereomers of Burkholdac B. The first set of thailandepsins presented a synthetic challenge as they were containing an unusual cyclic hemiaminal function but we discovered that they were inaccurate structures of the natural products. It was also the case of the revised structures of thailandepsins. The synthesis of the diastereomers of burkholdac B allowed us to elucidate the stereochemistry of burkholdacs which was left unassigned by Brady. Potent HDAC inhibitors were discovered in these two projects. We are planning to synthesise second-generation HDAC inhibitors based on largazole and burkholdacs.

On top of being interested on histone deacetylase inhibitors we were also attracted by histone demethylase inhibitors, especially LSD1 inhibitors. We started our investigation on this area by synthesizing synthetic compounds, analogues of tranylcypromine and phenelzine presenting LSD1 inhibition activity. We were pleased to identify several small molecules with higher activity than tranylcypromine *in vitro* and *in vivo*. We discovered by screening several compounds some possible modifications that could be made in order to continue our investigation on the SAR of tranylcypromine. We also set

up a high-throughput LSD1 assay. Further development of tranylcypromine analogues as LSD1 inhibitors for the treatment of prostate cancer is undergoing.

5. EXPERIMENTAL PROCEDURES

5.1. GENERAL PROCEDURES

Glassware was dried in the oven prior to use if the reaction is water sensitive. The reactions were carried out under argon or nitrogen atmosphere unless otherwise noted. The term *in vacuo* is used to describe the evaporation under vacuum using a rotary evaporator.

All the reagents were purchased from commercial suppliers such as Sigma-Aldrich, Alfa Aesar, Novabiochem. Anhydrous THF, dichloromethane and toluene were freshly distilled under argon from solvent purification system. All other anhydrous solvents were purchased as Aldrich® sure/seal bottles.

The reactions were monitored by TLC using pre-coated aluminium backed sheets of silica coated with 0.14 mm of silica gel 60 with a fluorescence indicator at 254 nm (Merck, Kieselgel 60 F_{254}). The spots were visualised by UV-light (λ 254 nm) and/or stained with cerium-ammonium molybdate or potassium permanganate or ninhydrin followed by heating. Flash chromatographies are performed using silica gel (MN Kieselgel 60, 40-63 μm, 230-400 mesh ASTM).

Optical rotations were determined using polarimeter POLAAR 2001. Solutions of compounds mainly in $CHCl_3$ or CH_3OH were irradiated with D line of sodium lamp (λ 589 nm) and the $[\alpha]_D$ values are given in $deg.cm^3.g^{-1}$. Prior to the measurement of the optical rotation of the compounds, calibration with the solvent used was performed. Some of the optical rotations were taken using polarimeter ADP220 (Bellingham and Stanley). Melting points were obtained using an Electrothermal melting point apparatus. Infrared spectra were collected using a Thermo Nicolet 380 FT-IR spectrometer with a Smart orbit Gate attachment. The software used is Omnic and the values are reported as absorptions as wavelengths in cm^{-1}.

NMR spectra were recorded on a Bruker AV300 spectrometer or a Bruker DPX400 spectrometer at 300 or 400 MHz respectively for ^{1}H and at 75 MHz or 100 MHz for ^{13}C. Spectral data were reprocessed using ACD labs software or MESTRENOVA and referenced to the residual solvent peak ($CDCl_3$, CD_3OD, CD_2Cl_2 or DMSO-*d*6). The ^{1}H NMR data are reported with the chemical shifts in ppm followed by brackets containing

in this order the multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, quin: quintet, sxt: sextet, spt: septet, m: multiplet, br: broad), coupling constants reported in Hz, number of protons (from integration) and the proton assignment if possible (i.e. H-4). The ^{13}C NMR data are reported with chemical shifts followed by the order of carbons (C, CH, CH$_2$ or CH$_3$) and the assignment if possible (i.e. CO for carbonyl or C-6) in parentheses.

Mass spectra were done using ES or EI. Electrospray mass spectra were obtained using a Thermoquest Trace MS and Micromass Platform II single quadrupole mass spectrometer. High resolution mass spectra were collected by the School of Chemistry Mass spectrometry Service of Southampton using a Bruker Apex III FT-ICR mass spectrometer fitted with an Appollo electrospray ionisation source. Some HRMS were acquired by the EPSRC National Mass Spectrometry Service Centre, Swansea.

Intermediate amines or acids prepared for coupling are most of the time not characterised; the deprotection step is checked by TLC or mass spectrometry analysis. Complete characterisation is carried on the amide compound.

5.2. EXPERIMENTAL PROCEDURES FOR CHAPTER 1: PROLINE-CONTAINING CYCLIC PEPTIDE NATURAL PRODUCTS

5.2.1. Sanguinamide A

N-Boc-isoleucinamide (1.38)
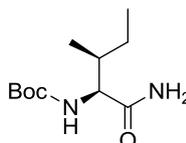
1.38

To a solution of di-*tert* butyl dicarbonate (1.31 g, 6 mmol, 1 equiv) in CH$_2$Cl$_2$ (30 mL) were added L-isoleucinamide hydrochloride (1 g, 6 mmol, 1 equiv) and triethylamine (1.73 mL, 12.4 mmol, 2.1 equiv). The reaction mixture was refluxed for 4 h under argon. Triethylamine salts were extracted twice with water (10 mL). The combined aqueous layers were extracted once with EtOAc. The combined organic layers were dried over MgSO$_4$ and evaporated to provide **1.38** as a white solid (1.38 g, 100%).

^{1}H NMR (400 MHz, CD$_3$OD) δ ppm 3.95 (d, J=6.5 Hz, 1H, CH), 1.90–1.70 (m, 1H, Me-C<u>H</u>-Et), 1.65–1.52 (m, 1H, CH$_2$), 1.48 (s, 9H, CMe$_3$), 1.20 (tt, J=14.2, 7.3 Hz, 1H, CH$_2$), 0.98 (d, J=7.0 Hz, 3H, CH-C<u>H$_3$</u>), 0.95 (t, J=7.5 Hz, 3H, CH$_2$-C<u>H$_3$</u>); **ES$^+$ MS *m/z*** 253 ([M+Na]$^+$).

The spectroscopic data are consistent with that[188] reported in the literature.

N-Boc-isoleucine thioamide (1.39)
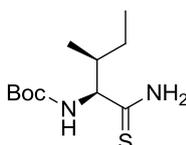
1.39

To a solution of **1.38** (1.36 g, 5.8 mmol, 1 equiv) in dry THF (20 mL) was added Lawesson's reagent (1.43 g, 3.5 mmol, 0.6 equiv) under argon. The reaction mixture

was stirred at 70 °C for 6 h. The solvent was evaporated and the residue purified by flash chromatography using EtOAc/CH$_2$Cl$_2$ (1:9) to give **1.39** as a white solid (730 mg, 50%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.06 (br s, 1H, NH$_2$), 7.66 (br s, 1H, NH$_2$), 5.26 (d, *J*=9.0 Hz, 1H, NH), 4.23 (t, *J*=8.5 Hz, 1H, CH), 2.05–1.86 (m, 1H, Me-C<u>H</u>-Et), 1.75–1.52 (m, 1H, CH$_2$), 1.44 (s, 9H, CM<u>e$_3$</u>), 1.24–1.04 (m, 1H, CH$_2$), 1.04–0.81 (m, 6H, 2CH$_3$); **ES$^+$ MS *m/z*** 269 ([M+Na]$^+$).

The spectroscopic data are consistent with that[189] reported in the literature.

Boc-(Ile)Thz-OMe (1.40)
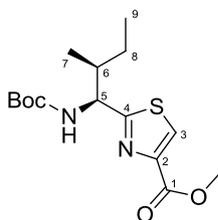
1.40

Method 1

To a solution of **1.39** (620 mg, 2.5 mmol, 1 equiv) in ethanol (10 mL), methyl bromopyruvate (295 µL, 2.8 mmol, 1.1 equiv) and calcium carbonate (136 mg, 1.4 mmol, 0.5 equiv) were added. The reaction mixture was stirred under argon at rt overnight. The reaction mixture was evaporated under reduced pressure and the obtained residue was partitioned between CHCl$_3$ and saturated NaHCO$_3$ solution. The organic layer was then washed once with water. The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford a mixture of diastereomers **1.40** and **1.47** (326 mg, 39%).

Method 2

To a suspension of pulverised KHCO$_3$ (2.37 g, 23.7 mmol, 8 equiv) in a solution of **1.39** (730 mg, 3.0 mmol, 1 equiv) in DME (8 mL), methyl bromopyruvate (946 µL, 8.9 mmol, 3 equiv) was added. The reaction mixture was stirred under argon at rt for 2 h. The suspension was then cooled to 0 °C and a solution of TFAA (1.65 mL, 11.9 mmol,

4 equiv) and pyridine (2.04 mL, 25.2 mmol, 8.5 equiv) in DME was then added. Solvents were evaporated under reduced pressure. The residue was suspended in chloroform and this solution was washed with water. The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 9:1) to afford **1.40** as a colourless oil (308 mg, 32%).

^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.10 (s, 1H, H-3), 5.29 (br s, 1H, NH), 5.03–4.74 (m, 1H, H-5), 3.95 (s, 3H, CO$_2$<u>Me</u>), 2.28–2.06 (m, 1H, H-6), 1.44 (s, 10H, H-8; C<u>Me$_3$</u>), 1.15 (s, 1H, H-8), 1.02–0.79 (m, 6H, H-7; H-9); **ES$^+$ MS *m/z*** (351 [M+Na]$^+$).

The spectroscopic data are consistent with that[190] reported in the literature.
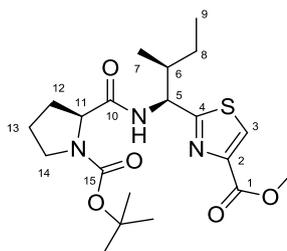
Boc-Pro-(Ile)Thz-OMe (1.42)

1.42

1.40 (365 mg, 1.1 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 2.5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford **1.41**.

To a solution of Boc-L-Pro-OH (390 mg, 1.8 mmol, 1.6 equiv) in CH$_2$Cl$_2$ (10 mL), PyBOP (1.07 g, 1.8 mmol, 1.6 equiv) and Hünig's base (632 μL, 3.6 mmol, 3.2 equiv) were added. The reaction mixture was stirred for 5 min before the free amine **1.41** (253 mg, 1.1 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 8:2) to afford **1.42** as a colourless oil (320 mg, 62%).

[α]$_D^{27}$ –86.9 (*c* 0.55, CH$_3$OH); **IR** 3297, 2970, 2929, 2872, 1724, 1699, 1524, 1474, 1389, 1356, 1242, 1213, 1152, 1123, 1095, 984 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.08 (s, 1H, H-3), 7.89 (br s, 1H, NH), 5.29–5.14 (m, 1H, H-5), 4.48–4.29 (m, 1H, H-11), 3.94 (s, 3H, CO$_2$<u>Me</u>), 3.60–3.22 (m, 2H, H-14), 2.54–2.19 (m, 2H, H-12), 2.05–

1.70 (m, 3H, H-6; H-13), 1.59–1.25 (m, 10H, C$\underline{Me}_3$; H-8), 1.24–1.10 (m, 1H, H-8), 0.99–0.78 (m, 6H, H-7; H-9); ^{13}**C NMR (100 MHz, CDCl$_3$) δ ppm** 173.8 (C), 171.9 (C), 161.9 (C), 156.3 (C), 147.1 (C, C-2), 127.0 (CH, C-3), 80.6 (C, $\underline{C}$Me$_3$), 59.7 (CH, C-5 or C-11), 56.4 (CH, C-5 or C-11), 52.3 (CH$_3$, CO$_2$$\underline{Me}$), 47.1 (CH$_2$, C-14), 39.2 (CH, C-6), 28.3 (3CH$_3$, C$\underline{Me}_3$), 27.2 (CH$_2$, C-8 or C-12 or C-13), 24.7 (CH$_2$, C-8 or C-12 or C-13), 23.9 (CH$_2$, C-8 or C-12 or C-13), 15.8 (CH$_3$, C-7), 11.4 (CH$_3$, C-9); **ES$^+$ MS *m/z*** 448 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{20}$H$_{31}$N$_3$NaO$_5$S (M+Na)$^+$ 448.1877, found 448.1878.

H-Pro-(Ile)Thz-OMe hydrochloride (1.33)

1.33

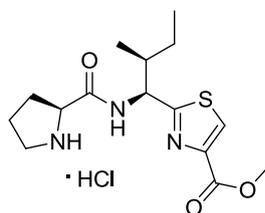**1.42** (164 mg, 0.04 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 300 μL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford **1.33**. The compound was used without further purification.

Methyl 2-((1*S*,2*S*)-2-methyl-1-((*S*)-1-(4-nitrobenzoyl)pyrrolidine-2-carboxami-do)-butyl)-thiazole-4-carboxylate (1.48)
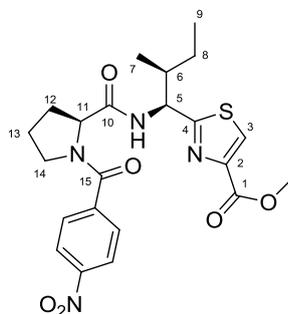
1.48

To a solution of *para*-nitrobenzoic acid (7.1 mg, 42 μmol, 1.1 equiv) in CH$_2$Cl$_2$ (1 mL), Hünig's base (24 μL, 134 μmol, 3.5 equiv), EDCI.HCl (9 mg, 46 μmol, 1.2 equiv),

HOBt (1 mg, 8 μmol, 0.2 equiv) were added at 0 °C. The reaction mixture was stirred for 5 min before **1.33** (12.5 mg, 38 μmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was washed twice with a saturated solution of NH$_4$Cl. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (CH$_2$Cl$_2$/MeOH 98:2) to afford **1.48** as a yellow solid (16 mg, 87%). Crystalline needles were grown from a mixture acetonitrile/water.

[α]$_D^{27}$ –78 (*c* 0.16, CH$_3$OH); **mp** 62–64 °C; **IR** 3305, 2978, 1728, 1687, 1638, 1519, 1433, 1348, 1217 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.32 (d, *J*=8.5 Hz, 2H, H$_{Ar}$), 8.11 (s, 1H, H-3), 7.70 (d, *J*=8.5 Hz, 2H, H$_{Ar}$), 7.53 (d, *J*=7.5 Hz, 1H, NH), 5.29 (dd, *J*=8.5, 6.0 Hz, 1H, H-5), 4.80 (dd, *J*=7.3, 4.8 Hz, 1H, H-11), 3.95 (s, 3H, CO$_2$<u>Me</u>), 3.66–3.50 (m, 1H, H-14), 3.48–3.33 (m, 1H, H-14), 2.51–2.34 (m, 1H, H-12), 2.34–2.20 (m, 1H, H-12 or H-13), 2.19–2.04 (m, 2H, H-12 or H-13), 1.99–1.84 (m, 1H, H-6), 1.58–1.42 (m, 1H, H-8), 1.39–1.12 (m, 1H, H-8), 0.94 (d, *J*=7.0 Hz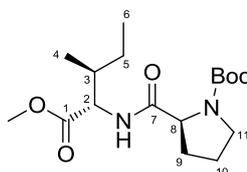, 3H, H-7), 0.89 (t, *J*=7.5 Hz, 3H, H-9); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.1 (C), 170.5 (C), 168.8 (C), 165.6 (C), 148.7 (C), 146.9 (C), 142.0 (C), 128.1 (2CH, H$_{Ar}$), 127.1 (CH, C-3), 123.9 (2CH, H$_{Ar}$), 60.2 (CH, C-5 or C-11), 56.3 (CH, C-5 or C-11), 52.4 (CH$_3$, CO$_2$<u>Me</u>), 50.3 (CH$_2$, C-14), 39.5 (CH, C-6), 27.6 (CH$_2$, C-8 or C-12 or C-13), 25.5 (CH$_2$, C-8 or C-12 or C-13), 24.5 (CH$_2$, C-8 or C-12 or C-13), 15.8 (CH$_3$, C-7), 11.5 (CH$_3$, C-9); **ES$^+$ MS *m/z*** 497 ([M+Na]$^+$); **X-Ray:** X-ray data available in Appendix 6.1.

Boc-Pro-Ile-OMe (1.52)

1.52

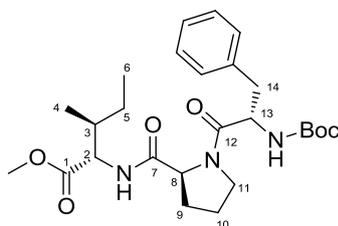To a solution of Boc-L-Pro-OH (539 mg, 2.5 mmol, 1 equiv) in CH$_2$Cl$_2$ (20 mL), EDCI.HCl (720 mg, 3.8 mmol, 1.5 equiv), HOBt (68 mg, 0.5 mmol, 0.2 equiv) and Hünig's base (873 μL, 5.0 mmol, 2 equiv) were added. The reaction mixture was stirred for 5 min before the L-isoleucine methyl ester hydrochloride (500 mg, 2.8 mmol, 1.1 equiv) was added. The reaction mixture was stirred at rt overnight. The reaction mixture

was washed twice with a saturated solution of NH$_4$Cl. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford **1.52** as a colourless oil (850 mg, 99%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.50 (br s, 0.5H, NH), 6.53 (br s, 0.5H, NH), 4.65–4.45 (m, 1H, H-2), 4.43–4.17 (m, 1H, H-8), 3.73 (s, 3H, CO$_2$Me), 3.58–3.24 (m, 2H, H-11), 2.57–1.78 (m, 5H, H-3; H-9; H-10), 1.48 (s, 9H, CMe$_3$), 1.47–1.36 (m, 1H, H-5), 1.24–1.07 (m, 1H, H-5), 0.96–0.82 (m, 6H, H-4; H-6); **ES$^+$ MS *m/z*** 365 ([M+Na]$^+$).

The spectroscopic data are consistent with that[191] reported in the literature.

Boc-Phe-Pro-Ile-OMe (1.54)

1.54

Boc-Pro-Ile-OCH$_3$ **1.52** (939 mg, 2.7 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 8 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford **1.53** (765 mg, 100%).

To a solution of Boc-L-Phe-OH (800 mg, 3.0 mmol, 1.1 equiv) in CH$_2$Cl$_2$ (20 mL), EDCI.HCl (789 mg, 4.1 mmol, 1.5 equiv), HOBt (74 mg, 0.6 mmol, 0.2 equiv) and Hünig's base (1.67 mL, 9.6 mmol, 2 equiv) were added. The reaction mixture was stirred for 5 min before **1.53** previously prepared (765 mg, 2.7 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was washed twice with a saturated solution of NH$_4$Cl. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography using a gradient hexane/EtOAc as eluent (7:3 to 1:1) to afford **1.54** as a colourless oil (Mixture of conformers) (1.02 g, 76%).

[α]$_D$^{27} –21.8 (*c* 0.30, CH$_3$OH); **IR** 3313, 2962, 2925, 2872, 1740, 1691, 1638, 1523, 1499, 1433, 1364, 1249, 1204, 1159, 1057, 1012, 869 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** Major conformer: 7.37–7.13 (m, 5H, H$_{Ar}$), 5.31 (d, *J*=8.8 Hz, 1H, NH),

4.70–4.54 (m, 2H), 4.51 (dd, J=8.0, 4.9 Hz, 1H), 3.74 (s, 3H, CO$_2$<u>Me</u>), 3.62–3.48 (m, 1H, H-11), 3.14 (t, J=9.7 Hz, 1H, H-11), 3.06–2.93 (m, 1H, H-14), 2.92–2.82 (m, 1H, H-14), 2.37–2.24 (m, 1H, H-9), 2.10–1.81 (m, 4H, H-3, H-9; H-10), 1.57–1.42 (m, 1H, H-5), 1.37 (s, 9H, C<u>Me</u>$_3$), 1.28–1.15 (m, 1H, H-5), 1.03–0.85 (m, 6H, H-4; H-6); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** Major conformer: 172.1 (C), 172.0 (C), , 170.4 (C), 155.1 (C), 136.2 (C, C$_{Ar}$), 129.3 (2CH, C$_{Ar}$), 128.5 (2CH, C$_{Ar}$), 126.9 (CH, C$_{Ar}$), 79.7 (C, <u>C</u>Me$_3$), 59.9 (CH, C-2 or C-8 or C-13), 56.8 (CH, C-2 or C-8 or C-13), 53.3 (CH, C-2 or C-8 or C-13), 52.0 (CH$_3$, CO$_2$<u>Me</u>), 47.4 (CH$_2$, C-11), 39.5 (CH$_2$, C-14), 37.6 (CH, C-3), 28.2 (CH$_3$, C<u>Me</u>$_3$), 27.1 (CH$_2$, C-9), 25.2; 25.1 (CH$_2$, C-5, C-10), 15.5 (CH$_3$, C-4), 11.6 (CH$_3$, C-6); **ES$^+$ MS** *m/z* 512 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{26}$H$_{39}$N$_3$NaO$_6$ (M+Na)$^+$ 512.2731, found 512.2730.

Boc-Ala-Phe-Pro-Ile-OMe (1.56)

1.56
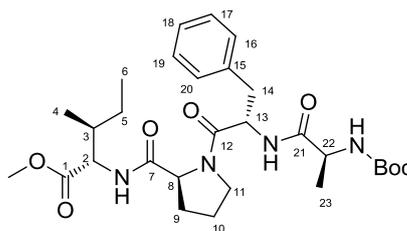
1.54 (943 mg, 1.93 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 6.2 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford **1.55** (813 mg, 99%).

To a solution of Boc-Ala-OH (434 mg, 2.30 mmol, 1.2 equiv) in CH$_2$Cl$_2$ (25 mL), EDCI.HCl (600 mg, 3.13 mmol, 1.6 equiv), HOBt (56.4 mg, 0.42 mmol, 0.2 equiv) and Hünig's base (1.27 mL, 7.30 mmol, 3.8 equiv) were added. The reaction mixture was stirred for 5 min before **1.55** previously prepared (813 mg, 1.93 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was washed twice with a saturated solution of NH$_4$Cl. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography using a gradient hexane/EtOAc as eluent (1:1 to 1:2) to afford **1.56** as a white solid (832 mg, 71%).

$[\alpha]_D^{27}$ –72.4 (*c* 0.29, CH$_3$OH); **mp** 58–60 °C; **IR** 3314, 2966, 2937, 2872, 1748, 1679, 1634, 1528, 1446, 1372, 1250, 1201, 1164 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** Major conformer: 7.41–7.21 (m, 5H, H$_{Ar}$), 7.12 (br s, 1H, NH), 5.13–4.93 (m, 2H), 4.65–4.54 (m, 2H), 4.37–4.19 (m, 1H), 3.80 (s, 3H, CO$_2$<u>Me</u>), 3.65 (td, *J*=8.0, 7.0 Hz, 1H, H-11), 3.28–3.16 (m, 1H, H-11), 3.11 (dd, *J*=13.6, 7.0 Hz, 1H, H-14), 3.00 (dd, *J*=13.6, 6.5 Hz, 1H, H-14), 2.34–2.22 (m, 1H, H-9), 2.14–1.88 (m, 4H, H-3; H-9; H-10), 1.51 (s, 10H, C<u>Me</u>$_3$; H-5), 1.32 (d, *J*=7.0 Hz, 3H, H-23), 1.29–1.23 (m, 1H, H-5), 1.06–0.94 (m, 6H, H-4; H-6); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** Major conformer: 172.2 (C), 172.0 (C), 170.9 (C), 170.7 (C), 155.2 (C), 135.9 (C, C$_{Ar}$), 129.5 (2CH, C$_{Ar}$), 128.5 (2CH, C$_{Ar}$), 127.0 (CH, C$_{Ar}$), 80.0 (C, <u>C</u>Me$_3$), 59.9 (CH), 56.7 (CH), 52.0; 51.9 (CH$_3$; 2CH), 47.5 (CH$_2$, C-11), 39.0 (CH$_2$, C-14), 37.7 (CH, C-3), 28.3 (CH$_3$, C<u>Me</u>$_3$), 27.6 (CH$_2$, C-9), 25.2; 25.0 (CH$_2$, C-5, C-10), 18.7 (CH$_3$, C-23), 15.5 (CH$_3$, C-4), 11.6 (CH$_3$, C-6); **ES$^+$ MS *m/z*** 583 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{29}$H$_{44}$N$_4$NaO$_7$ (M+Na)$^+$ 583.3102, found 583.3096.

Boc-Ala-Phe-Pro-Ile-OH (1.34)
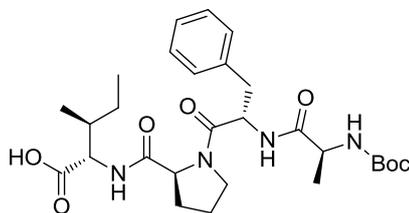
1.34

To a solution of Boc-Ala-Phe-Pro-Ile-OCH$_3$ **1.56** (300 mg, 0.5 mmol, 1 equiv) in 4 mL of a mixture THF/water (3:1) that had been cooled to 0 °C was added LiOH (38.5 mg, 1.6 mmol, 3 equiv) and stirred at 0 °C overnight. The solution was diluted with water (5 mL) and acidified to pH 1–2 with a saturated KHSO$_4$ solution. This aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide **1.34** as a white solid (292 mg, 100%).

Linear precursor 1.58

1.58

To a solution of **1.34** (189 mg, 0.35 mmol, 1.1 equiv) in CH_2Cl_2 (5 mL), PyBOP (242 mg, 0.41 mmol, 1.3 equiv) and Hünig's base (137 μL, 0.79 mmol, 2.5 equiv) were added. The reaction mixture wa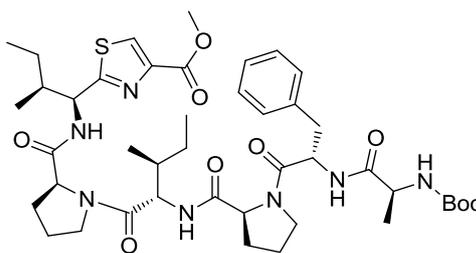s stirred for 5 min before the amine **1.33** previously prepared (103 mg, 0.31 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was concentrated *in vacuo* and the crude material was purified by flash chromatography using a gradient of MeOH in CH_2Cl_2 (0-5%) to afford **1.58** as a white solid with PyBOP by-product (512 mg).

ES$^+$ MS *m/z* 876 ([M+Na]$^+$).

Trans, trans-**Sanguinamide A (1.59)**
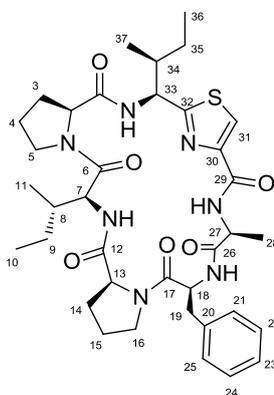
1.59

To a solution of **1.58** (269 mg, 0.3 mmol, 1 equiv) in 8 mL of a mixture THF/water (3:1) that had been cooled to 0 °C was added LiOH (22.6 mg, 0.9 mmol, 3 equiv) and stirred at 0 °C overnight. The solution was diluted with water (5 mL) and acidified to pH 1–2 with a saturated KHSO$_4$ solution. This aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide the carboxylic acid (180 mg, 68%). The

carboxylic acid (180 mg, 0.2 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the linear precursor of sanguinamide A (158 mg, 100%). Acyclic precursor (158 mg, 0.2 mmol, 1 equiv) was dissolved in CH$_3$CN/THF/CH$_2$Cl$_2$ (25 mL) and added dropwise to a vigorously stirred solution of Hünig's base (112 μL, 0.6 mmol, 3 equiv) and HATU (163 mg, 0.4 mmol, 2 equiv) in CH$_3$CN (160 mL). The reaction mixture was allowed to stir overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography with a gradient of MeOH in CH$_2$Cl$_2$ (0-5%) followed by several SCX-3. HPLC was performed to afford **1.59** as colorless oil (4 mg, 3%).

$[\alpha]_D^{24}$ –23 (*c* 0.08, CH$_3$OH); **IR** 3285, 2964, 1646, 1534, 1454 cm^{-1}; **^{1}H NMR (600 MHz, CDCl$_3$) δ ppm** 8.33 (d, *J*=9.4 Hz, 2H, NH), 7.94 (s, 1H, H-31), 7.30–7.25 (m, 2H, H-22; H-24), 7.25–7.18 (m, 1H, H-23), 7.15 (d, *J*=7.6 Hz, 2H, H-21; H-25), 6.52 (d, *J*=5.9 Hz, 1H, NH), 5.06 (t, *J*=9.7 Hz, 1H, H-33), 4.72 (d, *J*=7.6 Hz, 1H, H-7), 4.69–4.65 (m, 2H, H-18; H-27), 4.44 (d, *J*=8.2 Hz, 2H, H-2), 3.85 (t, *J*=7.6 Hz, 1H, H-13), 3.64 (t, *J*=8.5 Hz, 1H, H-16), 3.59 (t, *J*=9.1 Hz, 1H, H-5), 3.54–3.32 (m, 2H, H-5; H-16), 3.17 (dd, *J*=13.8, 5.6 Hz, 1H, H-19), 2.93 (dd, *J*=13.5, 2.3 Hz, 1H, H-19), 2.31–2.23 (m, 1H, H-34), 2.23–2.14 (m, 2H, H-3; H-14), 2.12–1.98 (m, 3H, H-3; H-4; H-15), 1.98–1.67 (m, 3H, H-4; H-14; H-15), 1.57–1.54 (m, 1H, H-8), 1.53 (d, *J*=7.0 Hz, 3H, H-28), 1.49–1.38 (m, 1H, H-35), 1.28 (spt, *J*=7.0 Hz, 1H, H-9), 1.21–1.02 (m, 2H, H-9; H-35), 0.95 (t, *J*=7.6 Hz, 3H, H-10), 0.92 (d, *J*=6.5 Hz, 3H, H-37), 0.86 (t, *J*=7.6 Hz, 3H, H-36), 0.67 (d, *J*=6.5 Hz, 3H, H-11); **^{13}C NMR (150 MHz, CDCl$_3$) δ ppm** 171.4 (C, C-1), 171.3 (C, C-12), 170.9 (C, C-26), 170.63 (C, C-6), 170.58 (C, C-32), 168.9 (C, C-17), 160.8 (C, C-29), 146.6 (C, C-30), 135.3 (C, C-20), 131.1 (2CH, C-21; C-25), 128.0 (2CH, C-22; C-24), 126.7 (CH, C-23), 125.6 (CH, C-31), 62.2 (CH, C-13), 61.4 (CH, C-2), 53.1 (CH, C-33), 53.0 (CH, C-7), 51.3 (CH, C-18), 48.9 (CH, C-27), 47.6 (CH$_2$, C-16), 46.4 (CH$_2$, C-5), 37.7 (CH, C-8), 35.8 (CH$_2$, C-19), 34.1 (CH, C-34), 30.6 (CH$_2$, C-3), 29.8 (CH$_2$, C-14), 27.5 (CH$_2$, C-9), 25.9 (CH$_2$, C-15), 25.3 (CH$_2$, C-35), 24.1 (CH$_2$, C-4), 18.3 (CH$_3$, C-28), 17.0 (CH$_3$, C-37), 13.8 (CH$_3$, C-11), 12.2 (CH$_3$, C-10), 10.0 (CH$_3$, C-36); **ES$^+$ MS *m/z*** 744 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{37}$H$_{51}$N$_7$NaO$_6$S (M+Na)$^+$ 744.3514, found 744.6507, **X-Ray:** X-ray data available in Appendix 6.2.

Boc-Ala-Phe-OMe (1.69)

1.69
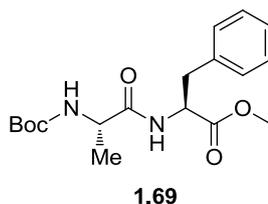
To a solution of Boc-L-Ala-OH (580 mg, 3.1 mmol, 1.1 equiv) in CH_2Cl_2 (20 mL), PyBOP (1.81 g, 3.1 mmol, 1.1 equiv) and Hünig's base (973 µL, 5.6 mmol, 2 equiv) were added. The reaction mixture was stirred for 5 min before the H-Phe-OMe (500 mg, 2.8 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford **1.69** as a white solid (977 mg, 100%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.36–7.19 (m, 3H, H$_{Ar}$), 7.16–7.03 (m, 2H, H$_{Ar}$), 6.50 (d, *J*=7.3 Hz, 1H, NH), 5.06–4.66 (m, 2H, NH; C$\underline{H}$Bn), 4.25–3.99 (m, 1H, C$\underline{H}$Me), 3.72 (s, 3H, CO$_2$Me), 3.22–3.05 (m, 2H, C$\underline{H}_2$Ph), 1.44 (s, 9H, CMe$_3$), 1.32 (d, *J*=7.3 Hz, 3H, Me).

The spectroscopic data are consistent with that[192] reported in the literature.

H-Ala-Phe-OMe hydrochloride (1.70)

1.70
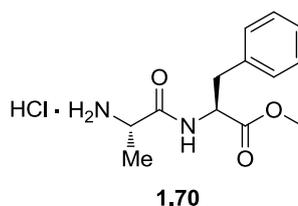
1.69 (100 mg, 0.29 mmol) was treated with HCl in dioxane (4 M, 1 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford **1.70** (81.8 mg, 100%).

Boc-Pro-(Ile)Thz-OH (1.61)
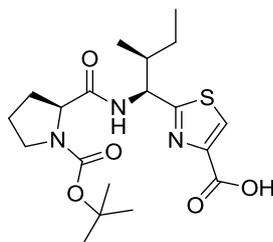
1.61

To compound **1.42** in 8 mL of a mixture THF/water (3:1) at 0 °C was added LiOH (60 mg, 2.5 mmol, 3 equiv) and stirred at 0 °C overnight. The solution was diluted with water (5 mL) and acidified to pH 1–2 with a saturated $KHSO_4$ solution. This aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to provide **1.61** as a white solid (338 mg, 100%).

Boc-Pro-(Ile)Thz-Ala-Phe-OMe (1.65)
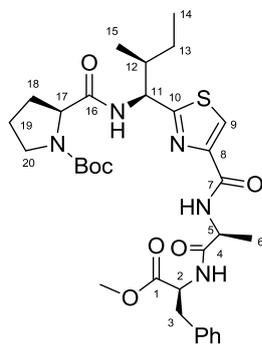
1.65

To a solution of **1.61** (123 mg, 0.30 mmol, 1.05 equiv) in CH_2Cl_2 (8 mL), PyBOP (352 mg, 0.60 mmol, 2.1 equiv), and Hünig's base (149 μL, 0.85 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before **1.70** previously prepared (71 mg, 0.28 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using a gradient hexane/EtOAc as eluent (1:2) to afford **1.65** as a white solid (150 mg, 78%).

$[\alpha]_D^{25}$ 25.4 (*c* 0.11, CH_3OH); **^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ ppm** Major conformer: 7.89 (s, 1H, H-9), 7.05–6.93 (m, 5H, H$_{Ar}$), 4.98 (d, *J*=6.4 Hz, 1H, H-11), 4.60 (t, *J*=6.4 Hz, 1H, H-2), 4.44 (q, *J*=6.8 Hz, 1H, H-5), 4.22–4.10 (m, 1H, H-17), 3.55 (s, 3H, CO$_2$<u>Me</u>), 3.37–3.13 (m, 2H, H-20), 2.99 (dd, *J*=13.8, 5.8 Hz, 1H, H-3), 2.87 (dd,

J=13.9, 7.5 Hz, 1H, H-3), 2.08–1.69 (m, 5H, H-12; H-18; H-19), 1.29 (br d, J=7.2 Hz, 14H, H-6; H-13; CMe$_3$), 0.76 (br d, J=6.8 Hz, 6H, H-14; H-15); **^{13}C NMR (100 MHz, CDCl$_3$/CD$_3$OD) δ ppm** Major conformer: 175.6 (C), 174.4 (C), 173.3 (C), 169.8 (C), 162.7 (C), 156.1 (C), 150.1 (C, C-8), 138.0 (C, C$_{Ar}$), 130.4 (2CH, C$_{Ar}$), 129.6 (2CH, C$_{Ar}$), 128.0 (CH, C$_{Ar}$), 125.4 (CH, C-9), 81.5 (C, $\underline{C}$Me$_3$), 61.2 (CH), 57.4 (CH), 55.4 (CH or CH$_3$), 52.9 (CH or CH$_3$), 50.1 (CH), 47.7 (CH$_2$), 40.6 (CH), 38.4 (CH$_2$), 31.2 (CH$_2$), 28.8 (CH$_3$, $\underline{C}$Me$_3$), 26.2 (CH$_2$), 25.0 (CH$_2$), 18.7 (CH$_3$, C-6), 16.2 (CH$_3$, C-15), 11.7 (CH$_3$, C-14); **ES$^+$ MS m/z** 666 ([M+Na]$^+$); **HRMS (ESI)** m/z calcd. for C$_{32}$H$_{45}$N$_5$NaO$_7$S (M+Na)$^+$ 666.2932, found 666.2930.

Linear precursor 1.64
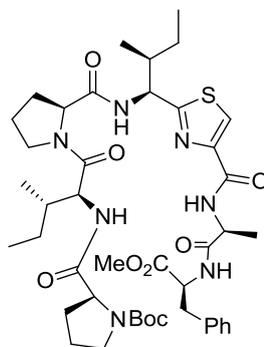
1.64

1.65 (150 mg, 0.24 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the deprotected amine (127 mg, 100%).

To a solution of **1.66** (84 mg, 0.26 mmol, 1.1 equiv) in CH$_2$Cl$_2$ (5 mL), PyBOP (275 mg, 0.47 mmol, 1.8 equiv), and Hünig's base (122 µL, 0.70 mmol, 2.7 equiv) were added. The reaction mixture was stirred for 5 min before the previous deprotected amine (84 mg, 0.26 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc as eluent to afford **1.64** as a white solid with PyBOP by-product (165 mg, 83%).

ES$^+$ MS m/z 876 ([M+Na]$^+$).

Linear precursor 1.60
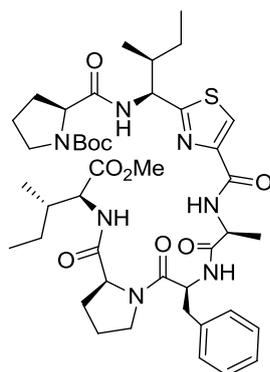
1.60

To a solution of **1.61** (97 mg, 0.24 mmol, 1.3 equiv) in CH_2Cl_2 (10 mL), PyBOP (264 mg, 0.45 mmol, 2.4 equiv), and Hünig's base (117 μL, 0.67 mmol, 3.6 equiv) were added. The reaction mixture was stirred for 5 min before **1.62** (85 mg, 0.19 mmol, 1 equiv) was added. **1.62** was obtained by treatment of **1.56** with HCl in dioxane followed by evaporation. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using a gradient CH_2Cl_2/MeOH as eluent (98:2) to afford **1.60** as a white solid (101 mg, 63%).

ES⁺ MS *m/z* 876 ([M+Na]⁺).

## *Trans, cis*-Sanguinamide A (1.63)
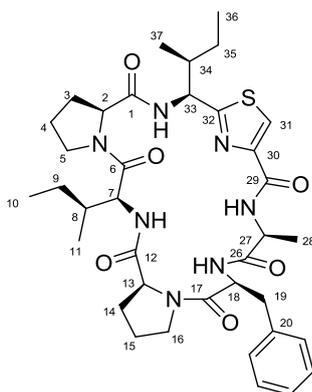
1.63

To a solution of **1.60** (101 mg, 0.12 mmol, 1 equiv) in 8 mL of a mixture THF/water (3:1) that had been cooled to 0 °C was added LiOH (26 mg, 1.1 mmol, 3 equiv) and stirred at 0 °C overnight. The solution was diluted with water (5 mL) and acidified to pH 1–2 with a saturated $KHSO_4$ solution. This aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$

and concentrated *in vacuo* to provide the carboxylic acid (99 mg, 100%). The carboxylic acid was treated with HCl in dioxane (4 M, 5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the linear precursor of sanguinamide A (88 mg, 100%). Deprotected acyclic precursor (87.5 mg, 0.12 mmol, 1 equiv) was dissolved in CH_3CN/THF (25 mL) and added dropwise to a vigorously stirred solution of Hünig's base (62 μL, 0.36 mmol, 3 equiv) and HATU (90 mg, 0.24 mmol, 2 equiv) in CH_3CN (90 mL). The reaction mixture was allowed to stir overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography with EtOAc to afford **1.63** as a white solid (15 mg). HPLC was performed in order to remove HATU by-product to yield **1.63** (2 mg, 2%).

$[\alpha]_D^{23}$ −140 (*c* 0.06, CH_3OH); **^{1}H NMR (600 MHz, CDCl$_3$) δ ppm** 9.24 (d, *J*=7.6 Hz, 1H, NH), 8.78 (d, *J*=7.0 Hz, 1H, NH), 8.13 (d, *J*=8.2 Hz, 1H, NH), 8.01 (s, 1H, H-31), 7.29 (d, *J*=7.6 Hz, 2H, H-22; H-24), 7.27–7.24 (m, 1H, H-23), 7.17 (d, *J*=6.5 Hz, 2H, H-21; H-25), 6.60 (br s, 1H, NH), 5.10 (dd, *J*=7.3, 6.2 Hz, 1H, H-33), 4.77 (ddd, *J*=7.6, 6.5, 6.5 Hz, 1H, H-27), 4.69 (d, *J*=8.2 Hz, 1H, H-2), 4.28 (dd, *J*=10.6, 7.6 Hz, 1H, H-7), 4.25 (ddd, *J*=11.2, 5.3, 1.8 Hz, 1H, H-18), 4.00 (td, *J*=10.1, 6.7 Hz, 1H, H-5), 3.63 (t, *J*=8.2 Hz, 1H, H-5), 3.54 (td, *J*=11.4, 7.6 Hz, 1H, H-16), 3.50–3.44 (m, 1H, H-16), 3.41 (d, *J*=7.6 Hz, 1H, H-13), 3.11 (t, *J*=11.7 Hz, 1H, H-19), 3.02 (dd, *J*=12.3, 5.3 Hz, 1H, H-19), 2.69 (dd, *J*=12.3, 5.9 Hz, 1H, H-3), 2.36–2.21 (m, 1H, H-8), 2.11 (dd, *J*=12.3, 6.5 Hz, 1H, H-14), 2.06–1.98 (m, 1H, H-4), 1.97–1.86 (m, 2H, H-3; H-4), 1.86–1.77 (m, 2H, H-9; H-15), 1.76–1.62 (m, 2H, H-15; H-34), 1.57–1.50 (m, 1H, H-35), 1.48 (d, *J*=6.5 Hz, 3H, H-28), 1.38–1.23 (m, 1H, H-9), 1.23–1.10 (m, 1H, H-35), 1.03 (d, *J*=6.5 Hz, 3H, H-11), 0.99–0.94 (m, 1H, H-14), 0.93 (t, *J*=7.3 Hz, 3H, H-36), 0.92 (t, *J*=8.2 Hz, 3H, H-10), 0.78 (d, *J*=7.0 Hz, 3H, H-37); **^{13}C NMR (150 MHz, CDCl$_3$) δ ppm** 174.4 (C, C-6), 171.4 (C, C-12), 171.3 (C, C-26), 170.7 (C, C-1), 169.9 (C, C-17), 168.2 (C, C-32), 160.5 (C, C-29), 148.5 (C, C-30), 134.7 (C, C-20), 129.4 (2CH, C-21; C-25), 129.1 (2CH, C-22; C-24), 127.7 (CH, C-23), 123.1 (CH, C-31), 61.1 (CH, C-13), 60.9 (CH, C-2), 56.4 (CH, C-7), 56.3 (CH, C-33), 54.6 (CH, C-18), 49.2 (CH, C-27), 48.3 (CH_2, C-5), 46.4 (CH_2, C-16), 41.0 (CH, C-34), 37.8 (CH_2, C-19), 35.5 (CH, C-8), 30.4 (CH_2, C-14), 25.8 (2CH_2, C-3; C-35), 25.5 (CH_2, C-9), 25.0 (CH_2, C-4), 21.9 (CH_2, C-15), 18.0 (CH_3, C-28), 15.2 (CH_3, C-11), 15.1 (CH_3, C-37), 11.5 (CH_3, C-36), 10.4

(CH$_3$, C-10); **ES$^+$ MS *m/z*** 744 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{37}$H$_{52}$N$_7$O$_6$S (M+H)$^+$ 722.3694, found 722.3687.

### *N*-Boc-D-isoleucinamide (1.133)
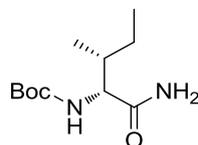
1.133

Boc-D-Ile-OH (500 mg, 2.16 mmol, 1 equiv) was dissolved in CH$_2$Cl$_2$ (5 mL) and HOBt (876 mg, 6.48 mmol, 3 equiv), EDCI (1.24 g, 6.48 mmol, 3 equiv) and Hünig's base (1.5 mL, 8.65 mmol, 4 equiv), were added at 0 °C. After stirring for 5 min, NH$_3$ in dioxane (0.5 M, 43 mL, 21.60 mmol, 10 equiv) was added. The reaction mixture was stirred at rt overnight. HCl solution (0.1 M) was added with cooling to adjust the reaction mixture to pH 4–5. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 times). The combined organic layers were dried and concentrated *in vacuo*. The residue was purified by flash chromatography using CH$_2$Cl$_2$ then 3% MeOH in CH$_2$Cl$_2$ to afford **1.133** as a white solid (361 mg, 73%).

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 3.92 (d, *J*=6.8 Hz, 1H, CH), 1.91–1.66 (m, 1H, C<u>H</u>-Et), 1.63–1.35 (m, 10H, CH$_2$; C<u>Me$_3$</u>), 1.30–1.08 (m, 1H, CH$_2$), 0.97–0.83 (m, 6H, 2Me).

The spectroscopic data are consistent with that[188] reported in the literature.

### *N*-Boc-D-isoleucine thioamide (1.134)
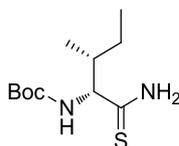
1.134

To a solution of **1.133** (541 mg, 2.35 mmol, 1 equiv) in dry THF (14 mL) was added Lawesson's reagent (570 mg, 1.41 mmol, 0.6 equiv) under argon. The reaction mixture was stirred overnight at rt. Lawesson's reagent (285 mg, 0.7 mmol, 0.3 equiv) was

added and the reaction mixture was stirred overnight at rt. The solvent was evaporated and the residue purified by flash chromatography using EtOAc/CH$_2$Cl$_2$ (1:9) to give **1.134** as a white solid (223 mg, 39%).

^{1}H NMR (400 MHz, CD$_3$OD) δ ppm 4.14 (d, *J*=6.8 Hz, 1H, CH), 1.96-1.76 (m, 1H, C<u>H</u>-Et), 1.72-1.54 (m, 1H, CH$_2$), 1.46 (s, 9H, C<u>Me$_3$</u>), 1.16 (s, 1H, CH$_2$), 0.99-0.87 (m, 6H, 2Me).

The spectroscopic data are consistent with that[189] reported in the literature.

N-Boc-(D-Ile)Thz-OMe (1.72)
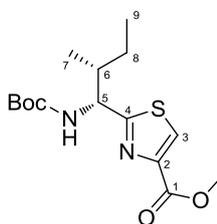
1.72

To a suspension of pulverised KHCO$_3$ (714 mg, 7.13 mmol, 8 equiv) in a solution of **1.134** (219.5 mg, 0.89 mmol, 1 equiv) in DME (2.5 mL), methyl-3-bromopyruvate (484 µL, 2.67 mmol, 3 equiv) was added. The reaction mixture was stirred under argon at rt for 2 h. The suspension was then cooled to 0 °C and a solution of TFAA (496 µL, 3.56 mmol, 4 equiv) and pyridine (612 µL, 7.57 mmol, 8.5 equiv) in DME (2 mL) was then added. Solvents were evaporated under reduced pressure. The residue was suspended in chloroform and this solution was washed with HCl (1 M). The aqueous layer was extracted once with chloroform. The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 9:1) to afford **1.72** as a colourless oil (136 mg, 47%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.02 (s, 1H, H-3), 5.23 (br d, *J*=8.1 Hz, 1H, NH), 4.86 (t, *J*=6.6 Hz, 1H, H-5), 3.87 (s, 3H, CO$_2$<u>Me</u>), 2.25–1.92 (m, 1H, H-6), 1.37 (br s, 10H, H-8; C<u>Me$_3$</u>), 1.20–0.99 (m, 1H, H-8), 0.95-0.69 (m, 6H, H-7; H-9).
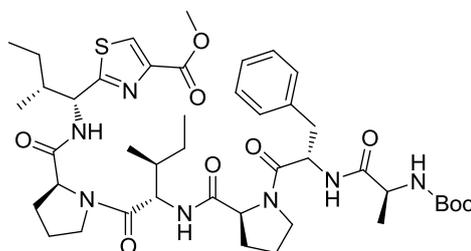
The spectroscopic data are consistent with that[190] reported in the literature.

Boc-Pro-(D-Ile)Thz-OMe (1.135)
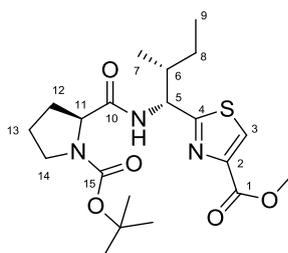
1.135

1.134 (127 mg, 0.39 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 5 mL) for 1.5 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the deprotected amine.

To a solution of Boc-L-Pro-OH (125 mg, 0.58 mmol, 1.5 equiv) in CH$_2$Cl$_2$ (6 mL), PyBOP (343 mg, 0.58 mmol, 1.5 equiv) and Hünig's base (202 µL, 1.16 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before the previous deprotected amine was added. The reaction mixture was stirred at rt overnight. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 8:2) to afford **1.135** as a colourless oil (137 mg, 83%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.07 (s, 1H, H-3), 7.83 (br s, 1H, NH), 5.25 (dd, *J*=8.7, 5.7 Hz, 1H, H-5), 4.51–4.24 (m, 1H, H-11), 3.94 (s, 3H, CO$_2$Me), 3.60–3.30 (m, 2H, H-14), 2.55–2.13 (m, 2H, H-12), 1.97–1.75 (m, 3H, H-13; H-6), 1.47 (br s, 10H, CMe$_3$; H-8), 1.33–1.07 (m, 1H, H-8), 0.96 (d, *J*=6.8 Hz, 3H, H-7), 0.88 (t, *J*=7.3 Hz, 1H, H-9).

Linear precursor 1.74

1.74

1.135 (125 mg, 0.38 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 8 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the deprotected amine **1.73**.

To a solution of **1.34** (156 mg, 0.28 mmol, 1.1 equiv) in CH$_2$Cl$_2$ (8 mL), EDCI.HCl (68 mg, 0.36 mmol, 1.5 equiv), HOBt (6.4 mg, 0.04 mmol, 0.2 equiv) and Hünig's base (124 µL, 0.71 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before the previous deprotected amine **1.73** was added. The reaction mixture was stirred at rt overnight. The reaction mixture was washed twice with a saturated solution of NH$_4$Cl. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography using a gradient of MeOH in CH$_2$Cl$_2$ (0-5%) to afford **1.74** as a colourless oil (162.5 mg, 65%).

ES$^+$ MS *m/z* 876 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{43}$H$_{63}$N$_7$NaO$_9$S (M+Na)$^+$ 876.4300, found 876.4295.

Trans, cis-[D-Ile]-Sanguinamide A (1.75)

1.75

To a solution of **1.74** (145.8 mg, 0.17 mmol, 1 equiv) in 8 mL of a mixture THF/water (3:1) that had been cooled to 0 °C was added LiOH (12.3 mg, 0.51 mmol, 3 equiv) and stirred at 0 °C overnight. The solution was diluted with water and acidified to pH 1–2 with a saturated KHSO$_4$ solution. This aqueous layer was extracted with EtOAc (3 x 7 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide the carboxylic acid (143 mg, 100%). The carboxylic acid (143 mg, 0.17 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and

CHCl$_3$ was performed to afford the linear precursor of [D-Ile]-sanguinamide A (126 mg, 100%). Acyclic precursor (126 mg, 0.17 mmol, 1 equiv) was dissolved in CH$_3$CN/THF (25 mL) and added dropwise to a vigorously stirred solution of Hünig's base (89 μL, 0.51 mmol, 3 equiv) and HATU (130 mg, 0.34 mmol, 2 equiv) in CH$_3$CN (160 mL). The reaction mixture was allowed to stir overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography with a gradient of EtOAc. HPLC was also performed (14 mg, 11%).

^{1}H NMR (600 MHz, DMSO-*d6*) *δ* ppm 8.78 (d, *J*=8.8 Hz, 1H, NH), 8.55 (d, *J*=9.4 Hz, 1H, NH), 8.25 (s, 1H, H-31), 7.78 (d, *J*=8.8 Hz, 1H, NH), 7.71 (d, *J*=8.8 Hz, 1H, NH), 7.31 (d, *J*=7.6 Hz, 2H, H-21; H-25), 7.25 (t, *J*=7.3 Hz, 2H, H-22; H-24), 7.19 (t, *J*=7.0 Hz, 1H, H-23), 5.18 (t, *J*=9.7 Hz, 1H, H-33), 4.68 (dd, *J*=9.1, 2.6 Hz, 1H, H-7), 4.53 (td, *J*=8.8, 3.5 Hz, 1H, H-18), 4.38 (d, *J*=8.2 Hz, 1H, H-2), 4.34–4.25 (m, 2H, H-13; H-27), 3.67 (t, *J*=7.3 Hz, 1H, H-16), 3.59 (t, *J*=8.8 Hz, 1H, H-5), 3.50 (dd, *J*=16.4, 7.6 Hz, 1H, H-5), 3.32 (dd, *J*=15.9, 9.4 Hz, 1H, H-16), 3.01 (dd, *J*=13.5, 2.9 Hz, 1H, H-19), 2.68 (dd, *J*=13.2, 9.7 Hz, 1H, H-19), 2.54 (s, 1H, H-34), 2.29–2.10 (m, 1H, H-4), 2.09–1.88 (m, 5H, H-3; H-4; H-14; H-15; H-35), 1.87–1.78 (m, 2H, H-3; H-14), 1.78–1.68 (m, 2H, H-15; H-35), 1.67–1.58 (m, 1H, H-8), 1.24 (d, *J*=7.6 Hz, 3H, H-28), 1.22–1.11 (m, 1H, H-9), 1.07 (dt, *J*=13.6, 7.0 Hz, 1H, H-9), 0.87 (app q, *J*=7.0 Hz, 6H, H-11; H-37), 0.73 (d, *J*=5.3 Hz, 6H, H-10; H-36); **^{13}C NMR (100 MHz, DMSO-*d6*) *δ* ppm** 171.9 (C, C-32), 171.4 (C, C-12), 171.1 (C, C-26), 171.0 (C, C-1), 169.1 (C, C-6), 168.0 (C, C-17), 160.2 (C, C-29), 148.0 (C, C-30), 137.4 (C, C-20), 129.6 (2CH, C-21; C-25), 127.9 (2CH, C-22; C-24), 126.3 (CH, C-23), 125.7 (CH, C-31), 60.5 (CH, C-13), 59.3 (CH, C-2), 55.0 (CH, C-33), 51.7 (CH, C-18), 51.6 (CH, C-7), 50.1 (CH, C-27), 46.7 (CH$_2$, C-16), 46.3 (CH$_2$, C-5), 40.4 (CH, C-8 or C-34), 36.8 (CH$_2$, C-19), 36.1 (CH, C-8 or C-34), 29.2 (CH$_2$, C-14), 28.7 (CH$_2$, C-3), 26.3 (CH$_2$, C-9 or C-35), 25.5 (CH$_2$, C-15), 25.0 (CH$_2$, C-9 or C-35), 24.5 (CH$_2$, C-4), 17.3 (CH$_3$, C-28), 15.6 (CH$_3$, C-11 or C-37), 14.2 (CH$_3$, C-11 or C-37), 11.7 (CH$_3$, C-10 or C -36), 10.2 (CH$_3$, C-10 or C -36); **ES$^+$ MS *m/z*** 744 ([M+Na]$^+$).

Boc-D-*allo*-Ile-OH (1.136)
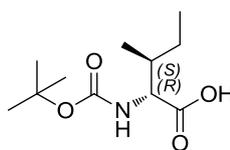
1.136

To a suspension of D-*allo*-isoleucine (500 mg, 3.8 mmol, 1 equiv) in dioxane (8 mL) and NaOH (1 M, 4 mL) was slowly added Boc_2O (915 mg, 4.2 mmol, 1.1 equiv). The reaction mixture was stirred at rt for 5 h and then acidified with a dilute solution of $KHSO_4$. After addition of EtOAc, the organic layer was separated and the aqueous layer extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide **1.136** as a colourless oil (880 mg, 100%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 9.16 (br s, 1H, CO$_2$H), 5.88 (br s, 4/5H, NH), 4.90 (d, *J*=9.0 Hz, 1/5H, NH), 4.34 (dd, *J*=9.2, 3.6 Hz, 4/5H, CH), 4.14 (br s, 1/5H, CH), 1.91 (d, *J*=3.4 Hz, 1H, C<u>H</u>-Et), 1.48–1.33 (m, 10H, CH$_2$; CMe$_3$), 1.28–1.10 (m, 1H, CH$_2$), 1.01–0.74 (m, 6H, 2CH$_3$).

The spectroscopic data are consistent with that[193] reported in the literature.

N-Boc-D-*allo*-isoleucinamide (1.137)
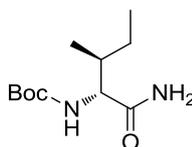
1.137

Boc-D-Ile-OH (1.76 g, 7.6 mmol, 1 equiv) was dissolved in CH_2Cl_2 (5 mL) and HOBt (3.08 g, 22.8 mmol, 3 equiv), EDCI (4.33 g, 22.8 mmol, 3 equiv) and Hünig's base (5.3 mL, 30.4 mmol, 4 equiv), were added at 0 °C. After stirring for 5 min, NH_3 in dioxane (0.5 M, 152 mL, 76.0 mmol, 10 equiv) was added. The reaction mixture was stirred at rt overnight. HCl solution (0.1 M) was added with cooling to adjust the reaction mixture to pH 4–5. The aqueous layer was extracted with CH_2Cl_2 (3 times). The combined organic layers were dried and concentrated *in vacuo*. The residue was purified by flash chromatography using CH_2Cl_2 then 3% MeOH in CH_2Cl_2 to afford **1.137** as a white solid (1.31 g, 85%).

¹H NMR (300 MHz, CD₃OD) δ ppm 4.09 (d, *J*=4.3 Hz, 1H, CH), 1.96–1.77 (m, 1H, C<u>H</u>-Et), 1.46 (s, 10H, CH₂; C<u>Me</u>₃), 1.32–1.09 (m, 1H, CH₂), 0.95 (t, *J*=7.2 Hz, 3H, C<u>H</u>₃-CH₂), 0.88 (d, *J*=6.5 Hz, 3H, C<u>H</u>₃-CH).

The spectroscopic data are consistent with that[194] reported in the literature.

N-Boc-D-*allo*-isoleucine thioamide (1.138)
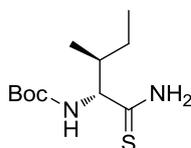
1.138

To a solution of **1.137** (1.27 g, 5.5 mmol, 1 equiv) in dry THF (15 mL) was added Lawesson's reagent (1.34 g, 3.3 mmol, 0.6 equiv) under argon. The reaction mixture was stirred overnight at rt. The solvent was evaporated and the residue purified by flash chromatography using EtOAc/CH₂Cl₂ (1:9) to give **1.138** as a white solid (800 mg, 59%).

¹H NMR (400 MHz, CD₃OD) δ ppm 4.42–4.20 (m, 1H, CH), 2.18–1.95 (m, 1H, C<u>H</u>-Et), 1.47 (br s, 10H, CH₂; C<u>Me</u>₃), 1.37–1.14 (m, 1H, CH₂), 0.97 (t, *J*=7.4 Hz, 3H, C<u>H</u>₃-CH₂), 0.90 (d, *J*=6.3 Hz, 3H, C<u>H</u>₃-CH); **ES⁺ MS *m/z*** 269 ([M+Na]⁺).

N-Boc-(D-*allo*-Ile)Thz-OMe (1.77)
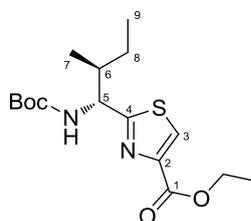
1.77

To a suspension of pulverised KHCO₃ (2.64 g, 26.4 mmol, 8 equiv) in a solution of **1.138** (800 mg, 3.3 mmol, 1 equiv) in DME (9 mL), ethyl-3-bromopyruvate (1.24 mL, 9.9 mmol, 3 equiv) was added. The reaction mixture was stirred under argon at rt for 2 h. The suspension was then cooled to 0 °C and a solution of TFAA (1.83 mL, 13.2 mmol, 4 equiv) and pyridine (2.27 mL, 28.0 mmol, 8.5 equiv) in DME (4.3 mL) was

then added. Solvents were evaporated under reduced pressure. The residue was suspended in chloroform and this solution was washed with HCl (1 M). The aqueous layer was extracted once with chloroform. The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 9:1) to afford **1.77** as a colourless oil (598 mg, 53%).

[α]$_D^{23}$ 11 (*c* 0.99, CH$_3$OH); **IR** 3346, 2968, 1702, 1499, 1366, 1235, 1206, 1164, 1094 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.07 (s, 1H, H-3), 5.24–5.17 (d, *J*=7.1 Hz, 1H, NH), 5.16–4.98 (m, 1H, H-5), 4.42 (q, *J*=7.1 Hz, 2H, CO$_2$C<u>H</u>$_2$-CH$_3$), 2.40–2.13 (m, 1H, H-6), 1.57–1.32 (m, 13H, H-8; CO$_2$CH$_2$-C<u>H</u>$_3$; C<u>Me</u>$_3$), 1.32–1.16 (m, 1H, H-8), 0.96 (t, *J*=7.3 Hz, 3H, H-9), 0.83 (d, *J*=6.5 Hz, 3H, H-7); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 174.2 (C), 161.3 (C), 155.4 (C, CO), 147.5 (C), 126.7 (CH, C-3), 80.0 (C, <u>C</u>Me$_3$), 61.3 (CH$_2$, CO$_2$<u>C</u>H$_2$-CH$_3$), 56.5 (CH, C-5), 39.5 (CH, C-6), 28.2 (3CH$_3$, C<u>Me</u>$_3$), 26.5 (CH$_2$, C-8), 14.3 (CH$_3$), 13.8 (CH$_3$), 11.5 (CH$_3$); **ES$^+$ MS *m/z*** 365 ([M+Na]$^+$).

N-Boc-Pro-(D-*allo*-Ile)Thz-OMe (1.139)
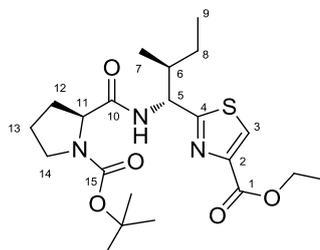
1.139

1.77 (523 mg, 1.53 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 5 mL) for 1.5 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the deprotected amine.

To a solution of Boc-L-Pro-OH (493 mg, 2.3 mmol, 1.5 equiv) in CH$_2$Cl$_2$ (6 mL), PyBOP (1.35 mg, 2.3 mmol, 1.5 equiv) and Hünig's base (800 μL, 4.6 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before the previous amine was added. The reaction mixture was stirred at rt overnight. The organic layer was dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 8:2) to afford **1.139** as a colourless oil (661 mg, 93%).

$[\alpha]_D^{23}$ 16.1 (*c* 0.62, CH$_3$OH); **IR** 2972, 1690, 1479, 1391, 1366, 1235, 1206, 1162, 1121, 1090 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.04 (br s, 1H, H-3), 5.38 (dd, *J*=8.7, 3.4 Hz, 1H, H-5), 4.40 (q, *J*=7.0 Hz, 2H, H-11; CO$_2$C$\underline{\text{H}}_2$CH$_3$), 3.61–3.26 (m, 2H, H-14), 2.52–2.25 (m, 2H, H-12), 1.97–1.80 (m, 3H, H-6; H-13), 1.47 (br s, 9H, C$\underline{\text{Me}}_3$), 1.38 (t, *J*=7.2 Hz, 3H, CO$_2$CH$_2$C$\underline{\text{H}}_3$), 1.34–1.18 (m, 2H, H-8), 0.96 (t, *J*=7.3 Hz, 3H, H-9), 0.83 (d, *J*=6.5 Hz, 3H, H-7); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.6 (C), 172.3 (C), 161.4 (C, CO), 156.2 (C, CO), 147.6 (C, C-2), 126.6 (CH, C-3), 80.8 (C, $\underline{\text{C}}$Me$_3$), 61.4 (CH$_2$, CO$_2$C$\underline{\text{H}}_2$-CH$_3$), 59.8 (CH, C-5 or C-11), 54.9 (CH, C-5 or C-11), 47.2 (CH$_2$, C-14), 39.1 (CH, C-6), 29.7 (CH$_2$), 28.4 (3CH$_3$, C$\underline{\text{Me}}_3$), 27.7 (CH$_2$), 26.7 (CH$_2$), 14.4 (CH$_3$, CO$_2$CH$_2$-$\underline{\text{C}}$H$_3$), 13.6 (CH$_3$, C-7), 11.6 (CH$_3$, C-9); **ES$^+$ MS *m/z*** 462 ([M+Na]$^+$).

Fmoc-Pro-Ile-Cys(*t*Bu)-Ala-Phe-Pro-Ile-OH (1.100)

1.100
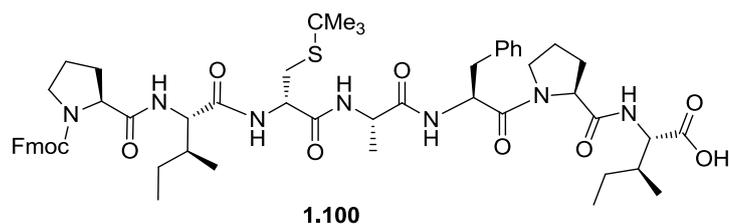
Swelling of the Wang resin

The dry Wang resin (600 mg, loading 1.7 mmol/g) placed in an appropriate SPPS reaction vessel was covered with CH_2Cl_2 and the reaction vessel was shaken for 5 min. The resin was dried by application of vacuum. This operation was repeated twice with CH_2Cl_2 and twice with DMF.

DIC coupling (used for the coupling of the first amino acid)

Fmoc-Ile-OH (3.6 g, 10.2 mmol, 10 equiv) was dissolved in a mixture of CH_2Cl_2 (30 mL) and DMF (1 mL). DIC (790 µL, 5.1 mmol, 5 equiv) was added at 0 °C and the reaction mixture was stirred for 30 min. CH_2Cl_2 was removed by evaporation under reduced pressure. A minimum volume of DMF, CH_2Cl_2 and NMP was added to dissolve the symmetrical anhydride. The solution and DMAP (0.01 equiv) were added to the Wang resin, the reaction vessel was shaken for 1 h. The resin was dried by application of vacuum, DMF was added and the solvent was drained off. This last operation was repeated once with CH_2Cl_2 and then with DMF.

Fmoc deprotection

A solution of 20% piperidine in DMF (v/v) was added to cover the beads. The reaction vessel was shaken for 5 min. The reagent was washed from the resin with DMF. The deprotection step was repeated once for 15 min. The resin was washed several times with DMF.

PyBOP coupling

To a solution of Fmoc protected amino acid (5.1 mmol, 5 equiv) in a mixture of CH_2Cl_2 and DMF, were added PyBOP (2.65 g, 5.1 mmol, 5 equiv) and Hünig's base (1.78 mL, 10.2 mmol, 10 equiv). The solution was added to the resin and the reaction vessel was shaken for 1 h. The reagents were washed from the resin with CH_2Cl_2 (twice) and DMF (twice).

219

Monitoring of the coupling and deprotection steps

The deprotection and coupling steps were monitored by ESI-MS or monitored using a qualitative ninhydrin assay (Kaiser test).

<u>Preparation of the Kaiser test solutions</u>

In a vial, phenol (8 g) was dissolve in absolute ethanol (2 mL). In another vial, potassium cyanide (13 mg) was dissolved in water (20 mL). To pyridine (980 µL), were added aqueous KCN solution (20 µL) followed by phenol solution in ethanol (100 µL) to form the Kaiser solution A. Ninhydrin (1 g) was dissolve in absolute ethanol (20 mL) and thus forming the solution B.

Some dried beads were removed and solution A (20 µL) was added followed by of solution B (40 µL). In the presence of unprotected amine, the solution turns blue in colour (positive Kaiser test) while if no free amine is present the solution will be yellow in colour (negative Kaiser test).

In the case of incomplete couplings, the coupling step was repeated a maximum of two times. The peptide was capped by *N*-acetylation if necessary.

Acetylation of the *N*-terminus

A solution of acetic anhydride (5 drops), pyridine (100 µL) and DMF was added to cover the resin. The reaction vessel was shaken for 15 min. The resin was washed several times with DMF.

Cleavage of the resin

A solution of ethane dithiol (200 µL), anisole (200 µL), TIPS (200 µL), water (200 µL), TFA (3 mL) and CH_2Cl_2 (2 mL) was prepared and added to the resin in a flask. The resin was filtered off and washed twice with TFA. The filtrate was evaporated. Cold diethyl ether was added and the precipitate was filtered to afford **1.100** as a pink solid (605 mg, 59%).

ES⁺ MS *m/z* 1060 ([M+Na]⁺).

Cyclo[Pro-Ile-Cys(*t*Bu)-Ala-Phe-Pro-Ile] (1.101)
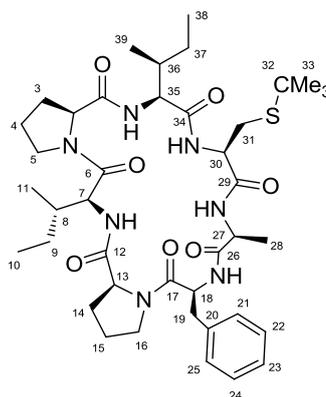
1.101

To heptapeptide **1.100** (200 mg, 0.19 mmol) in DMF (4 mL) was added piperidine (1 mL). The reaction mixture was stirred for 1 h at rt. The solvents were removed by evaporation under reduced pressure to afford the deprotected linear precursor.

Unprotected acyclic precursor (140 mg, 0.17 mmol, 1 equiv) was dissolved in CH_3CN/THF (40 mL) and was added dropwise to a vigorously stirred solution of Hünig's base (88 µL, 0.51 mmol, 3 equiv) and HATU (131 mg, 0.34 mmol, 2 equiv) in CH_3CN (150 mL). The reaction mixture was stirred overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography using as eluent EtOAc then with 2 to 5% MeOH in CH_2Cl_2 to afford **1.101** (120 mg, 87%). HPLC was performed on 60 mg of this product (2 mg, 3%).

^{1}H NMR (600 MHz, CDCl₃) δ ppm 7.86 (br d, *J*=2.9 Hz, 1H, NH), 7.36 (d, *J*=6.7 Hz, 1H, NH), 7.29 (t, *J*=7.0 Hz, 2H, H-22; H-24), 7.24 (t, *J*=7.0 Hz, 1H, H-23), 7.20 (d, *J*=7.0 Hz, 2H, H-21; H-25), 6.10–5.96 (br m, 1H, NH), 4.70 (td, *J*=7.9, 6.2 Hz, 1H, H-30), 4.52 (t, *J*=8.4 Hz, 1H, H-7), 4.30 (quin, *J*=5.1 Hz, 1H, H-18), 4.24 (t, *J*=7.8 Hz, 1H, H-2), 4.16 (br t, *J*=5.9 Hz, 1H, H-35), 4.11–3.96 (m, 2H, H-5; H-27), 3.63 (dt, *J*=9.6, 7.1 Hz, 1H, H-5), 3.54–3.42 (m, 2H, H-16), 3.34 (d, *J*=7.9 Hz, 1H, H-13), 3.18 (dd, *J*=13.1, 5.1 Hz, 1H, H-31), 3.11 (dd, *J*=12.6, 5.0 Hz, 1H, H-19), 2.95 (t, *J*=10.9 Hz, 1H, H-19), 2.79 (dd, *J*=13.1, 8.1 Hz, 1H, H-31), 2.40 (dd, *J*=13.8, 5.9 Hz, 1H, H-3), 2.19–2.06 (m, 2H, H-4; H-36), 2.06–1.89 (m, 4H, H-3; H-4; H-8; H-14), 1.77–1.67 (m, 1H, H-15), 1.63 (d, *J*=7.0 Hz, 3H, H-28), 1.61–1.53 (m, 1H, H-37), 1.46–1.38 (m, 2H, H-9; H-15), 1.33 (s, 9H, CMe₃), 1.16–1.01 (m, 2H, H-9; H-14), 0.97 (d, *J*=6.7 Hz, 3H, H-39), 0.94 (d, *J*=6.7 Hz, 3H, H-11), 0.93–0.91 (m, 1H, H-37),0.91 (t, *J*=7.0 Hz, 3H, H-38), 0.86 (t, *J*=7.3 Hz, 3H, H-10); **^{13}C NMR (150 MHz, CDCl₃) δ ppm** 173.5 (C, C-6),

172.9 (C, C-1), 172.7 (C, C-26), 171.34 (C, C-29), 171.30 (C, C-12), 170.7 (C, C-34), 170.4 (C, C-17), 135.9 (C, C-20), 129.6 (2CH, C-21; C-25), 129.0 (2CH, C-22; C-24), 127.5 (CH, C-23), 62.3 (CH, C-2), 61.1 (CH, C-13), 59.9 (CH, C-35), 55.3 (CH, C-7), 53.9 (CH, C-30), 53.8 (CH, C-18), 53.7 (CH, C-27), 48.5 (CH$_2$, C-5), 46.6 (CH$_2$, C-16), 42.7 (C, C-32), 39.7 (CH$_2$, C-19), 36.6 (CH, C-8), 35.4 (CH, C-36), 31.1 (3CH$_3$, C-33), 31.0 (CH$_2$, C-14), 30.7 (CH$_2$, C-31), 30.0 (CH$_2$, C-3), 25.4 (CH$_2$, C-4), 25.3 (CH$_2$, C-37), 24.9 (CH$_2$, C-9), 22.2 (CH$_2$, C-15), 16.9 (CH$_3$, C-28), 16.5 (CH$_3$, C-39), 16.1 (CH$_3$, C-11), 11.8 (CH$_3$, C-38), 10.9 (CH$_3$, C-10); **ES$^+$ MS** *m/z* 820 ([M+Na]$^+$).

5.2.2. Sanguinamide B

N-Boc-Pro-Val-OMe (1.112)

1.112
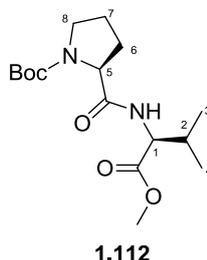
To a solution of Boc-L-Pro-OH (1.55 g, 7.2 mmol, 1.1 equiv) in CH_2Cl_2 (30 mL), EDCI.HCl (1.7 g, 9.0 mmol, 1.5 equiv), HOBt (161 mg, 1.19 mmol, 0.2 equiv) and Hünig's base (3.64 mL, 20.9 mmol, 2 equiv) were added. The reaction mixture was stirred for 5 min before L-Val-OMe hydrochloride (1 g, 6.0 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight. The reaction mixture was washed twice with a saturated solution of NH_4Cl. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography using a gradient hexane/EtOAc as eluent (7:3) to afford **1.112** as a white solid (1.96 g, 100%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm (mixture of conformers) 7.50 (br s, 0.5H, NH), 6.53 (br s, 0.5H, NH), 4.56–4.44 (m, 1H, H-1 or H-5), 4.39–4.24 (m, 1H, H-1 or H-5), 3.73 (s, 3H, CO$_2$<u>Me</u>), 3.54–3.30 (m, 2H, H-8), 2.45–2.30 (m, 0.5H, H-6), 2.21–2.11 (m, 1.5H, H-6), 1.99–1.83 (m, 3H, H-2; H-7), 1.48 (s, 9H, C<u>Me</u>$_3$), 0.91 (t, *J*=7.3 Hz, 6H, H-3; H-4); **ES$^+$ MS *m/z*** 329 ([M+H]$^+$), 351 ([M+Na]$^+$), 392 ([M+Na+CH$_3$CN]$^+$).

The spectroscopic data are consistent with that[195] reported in the literature.

N-Boc-alaninamide (1.115)

1.115
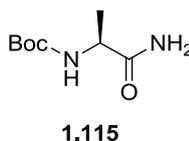
To a solution of di-*tert* butyl dicarbonate (1.75 g, 8.0 mmol, 1 equiv) in CH_2Cl_2 (40 mL) were added L-alaninamide hydrochloride (1 g, 8.0 mmol, 1 equiv) and triethylamine (2.32 mL, 16.6 mmol, 2.3 equiv). The reaction mixture was refluxed for 5 h.

Triethylamine salts were extracted twice with water (10 mL) and the combined aqueous layers were extracted once with EtOAc. The combined organic layers were dried over MgSO$_4$ and evaporated to provide **1.115** as a white solid (926 mg, 61%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 6.23 (br s, 1H, NH$_2$), 5.61 (br s, 1H, NH$_2$), 5.07 (br d, J=6.8 Hz, 1H, NH), 4.38–4.09 (m, 1H, CH), 1.45 (s, 9H, CMe$_3$), 1.38 (d, J=7.2 Hz, 3H, Me); **ES$^+$ MS m/z** 211 ([M+Na+CH$_3$CN]$^+$), 252 ([M+Na+CH$_3$CN]$^+$).

The spectroscopic data are consistent with that[196] reported in the literature.

N-Boc-alanine thioamide (1.116)

1.116
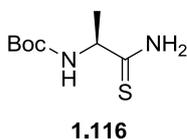
To a solution of *N*-Boc-alaninamide **1.115** (926 mg, 4.92 mmol, 1 equiv) in dry THF (7 mL) was added Lawesson's reagent (1.19 g, 2.95 mmol, 0.6 equiv) under argon. The reaction mixture was stirred at rt overnight. The solvent was evaporated and the residue purified by flash chromatography using EtOAc/CH$_2$Cl$_2$ (1:9) to give **1.116** as a white solid (947 mg, 94%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.09 (br s, 0.5H, NH$_2$), 7.71 (br s, 0.5H, NH$_2$), 5.34 (d, J=7.2 Hz, 1H, NH), 4.53 (quin, J=7.1 Hz, 1H, CH), 1.82 (br s, 1H, SH or NH$_2$), 1.47 (d, J=7.2 Hz, 3H, CH$_3$), 1.44 (s, 9H, CMe$_3$).

The spectroscopic data are consistent with that[196] reported in the literature.

(*S*)-Methyl 2-(1-(*tert*-butoxycarbonylamino)ethyl)thiazole-4-carboxylate (1.117)

1.117
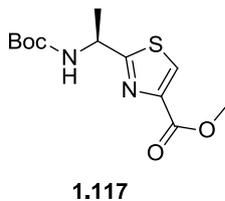
To a suspension of pulverised KHCO$_3$ (3.34 g, 33.4 mmol, 8 equiv) in a solution of *N*-Boc-alanine thioamide **1.116** (853 mg, 4.2 mmol, 1 equiv) in DME (20 mL), methyl

bromopyruvate (1.33 mL, 12.5 mmol, 3 equiv) was added. The reaction mixture was stirred under argon at rt for 2 h. The suspension was then cooled to 0 °C and a solution of TFAA (2.32 mL, 16.7 mmol, 4 equiv) and pyridine (2.87 mL, 35.5 mmol, 8.5 equiv) was then added. The reaction mixture was stirred 30 min at 0 °C and stirred at rt. Solvents were evaporated under reduced pressure. The residue was separated between $CHCl_3$ (100 mL) and HCl solution (1 M, 100 mL). The aqueous layer was extracted with $CHCl_3$ (50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford **1.117** as a yellow oil (940 mg, 79%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.11 (s, 1H, H$_{Ar}$), 5.33–4.97 (m, 2H, NH; CH-Me), 3.95 (s, 3H, CO$_2$<u>Me</u>), 1.63 (d, *J*=7.0 Hz, 3H, CH$_3$), 1.45 (s, 9H, C<u>Me</u>$_3$).

The spectroscopic data are consistent with that[190] reported in the literature.

(*S*)-2-(1-(*Tert*-Butoxycarbonylamino)ethyl)thiazole-4-carboxylic acid (1.118)

1.118
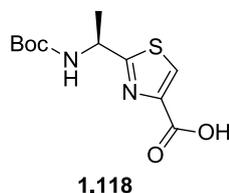
To a solution of **1.117** (933 mg, 3.3 mmol, 1 equiv) in 16 mL of a mixture THF/water (3:1) at 0 °C was added LiOH (234 mg, 9.8 mmol, 3 equiv) and the reaction mixture was stirred at rt overnight. The solution was diluted with water (10 mL) and acidified to pH 1–2 with a saturated $KHSO_4$ solution. The aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to provide the carboxylic acid **1.118** (745 mg, 84%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.22 (s, 1H, H$_{Ar}$), 5.26 (br s, 1H, NH), 5.19–4.96 (br s, 1H, CH-Me), 1.64 (d, *J*=6.8 Hz, 3H, CH$_3$), 1.45 (s, 9H, C<u>Me</u>$_3$).

The spectroscopic data are consistent with that[197] reported in the literature.

(*S*)-Methyl 2-(2-((*S*)-1-(*tert*-butoxycarbonylamino)ethyl)thiazole-4-carboxami-do)-4-methylpentanoate (1.119)
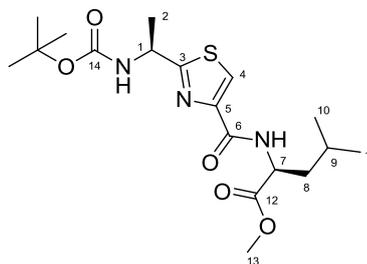
1.119

To a solution of Boc-L-(Ala)Thz-OH (**1.118**) (737 mg, 2.7 mmol, 1 equiv) in CH_2Cl_2 (30 mL), PyBOP (1.97 g, 3.8 mmol, 1.4 equiv) and Hünig's base (1.4 mL, 8.1 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before H-L-Leu-OMe.HCl (541 mg, 3.0 mmol, 1.1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 1:1) to afford **1.119** as a yellow oil (817 mg, 76%).

$[\alpha]_D^{23}$ –20.9 (*c* 1.13, CH_3OH); **IR** 3301, 2966, 2860, 1748, 1712, 1654, 1552, 1515, 1491, 1454, 1356, 1246, 1209, 1160, 1062, 1001 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 8.00 (s, 1H, H-4), 7.63 (d, *J*=8.7 Hz, 1H, NH), 5.20 (br s, 1H, NH), 5.14–5.01 (m, 1H, H-1 or H-7), 4.88–4.71 (m, 1H, H-1 or H-7), 3.76 (s, 3H, H-13), 1.85–1.66 (m, 3H, H-8; H-9), 1.61 (d, *J*=6.8 Hz, 3H, H-2), 1.46 (s, 9H, CMe$_3$), 1.10–0.87 (m, 6H, H-10; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 174.3 (C), 173.3 (C), 160.8 (C), 154.9 (C), 149.2 (C, C-5), 123.5 (CH, C-4), 80.3 (C, <u>C</u>Me$_3$), 52.3 (CH$_3$, C-13), 50.6 (CH, C-1 or C-7), 48.7 (CH, C-1 or C-7), 41.6 (CH$_2$, C-8), 28.3 (CH$_3$, C<u>Me</u>$_3$), 24.9 (CH, C-9), 22.8; 21.8 (2CH$_3$, C-10; C-11), 21.3 (CH$_3$, C-2); **ES$^+$ MS *m/z*** 422 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for $C_{18}H_{29}N_3NaO_5S_1$ (M+Na)$^+$ 422.1720, found 422.1722.

N-Boc-prolinamide (1.120)
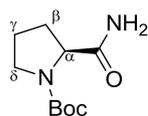
1.120

Boc-Pro-OH (1.08 mg, 5 mmol, 1 equiv) was dissolved in CH_2Cl_2 (15 mL) and HOBt (2 g, 15 mmol, 3 equiv), EDCI.HCl (2.87 g, 15 mmol, 3 equiv) and Hünig's base (3.48 mL, 20 mmol, 4 equiv), were added at 0 °C. After stirring for 5 min, NH$_3$ in dioxane

(0.5 M, 100 mL, 50 mmol, 10 equiv) was added. The reaction mixture was stirred at rt overnight. HCl solution (0.1 M) was added with cooling to adjust the reaction mixture to pH 4–5. The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried and concentrated *in vacuo*. The residue was purified by flash chromatography using 100% of CH_2Cl_2 then 3 to 4% MeOH in CH_2Cl_2 to afford **1.120** as a white solid (1.15 g, 100%).

^{1}H NMR (400 MHz, CDCl$_3$) δ ppm (mixture of rotamers) 6.9 (br s, 0.5H, NH$_2$), 6.05 (br s, 0.5H, NH$_2$), 5.78–5.45 (m, 1H, NH$_2$), 4.45–4.15 (m, 1H, CHα), 3.55–3.30 (m, 2H, CH$_2$δ), 2.55–2.09 (m, 2H, CH$_2$β), 2.02–1.83 (m, 2H, CH$_2$γ), 1.47 (s, 9H, C$\underline{Me}_3$); **ES$^+$ MS *m/z*** 278 ([M+Na+CH$_3$CN]$^+$).

The spectroscopic data are consistent with that[198] reported in the literature.

N-Boc-proline thioamide (1.121)

1.121
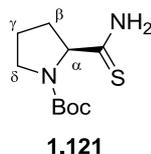
To a solution of *N*-Boc-prolinamide **1.120** (1.19 g, 5.55 mmol, 1 equiv) in dry THF (7 mL) was added Lawesson's reagent (1.35 g, 3.33 mmol, 0.6 equiv) under argon. The reaction mixture was stirred at rt overnight. The solvent was evaporated and the residue purified by flash chromatography using EtOAc/CH_2Cl_2 (1:9) to give **1.121** as a white solid (716 mg, 56%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm (mixture of conformers) 7.50 (br s, 1H, NH$_2$), 4.77–4.54 (m, 1H, CHα), 3.68–3.38 (m, 2H, CH$_2$δ), 2.46–2.18 (m, 2H, CH$_2$β), 2.07–1.80 (m, 2H, CH$_2$γ), 1.60 (s, 1H, SH or NH$_2$), 1.47 (s, 9H, C$\underline{Me}_3$).

The spectroscopic data are consistent with that[199] reported in the literature.

(S)-Methyl 2-(1-(tert-butoxycarbonyl)pyrrolidin-2-yl)thiazole-4-carboxylate (1.122)
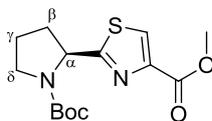
1.122

To a suspension of pulverised KHCO$_3$ (2.47 g, 24.7 mmol, 8 equiv) in a solution of *N*-Boc-alanine thioamide **1.121** (710 mg, 3.1 mmol, 1 equiv) in DME (17 mL), methyl bromopyruvate (984 µL, 9.3 mmol, 3 equiv) was added. The reaction mixture was stirred under argon at rt for 2 h. The suspension was then cooled to 0 °C and a solution of TFAA (1.71 mL, 12.3 mmol, 4 equiv) and pyridine (2.12 mL, 26.2 mmol, 8.5 equiv) was slowly added. The reaction mixture was stirred 30 min at 0 °C and stirred at rt. Solvents were evaporated under reduced pressure. The residue was separated between chloroform (100 mL) and HCl solution (1 M, 100 mL). The aqueous was extracted with CHCl$_3$ (50 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford **1.122** as a yellow oil (560 mg, 58%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm (mixture of conformers) 8.09 (s, 1H, H$_{Ar}$), 5.30–5.16 (m, 1H, CHα), 3.95 (s, 3H, CO$_2$<u>Me</u>), 3.76–3.24 (m, 2H, CH$_2\delta$), 2.71–2.10 (m, 2H, CH$_2\beta$), 2.10–1.81 (m, 2H, CH$_2\gamma$), 1.55–1.28 (m, 9H, C<u>Me$_3$</u>); **ES$^+$ MS *m/z*** 335 ([M+Na]$^+$).

The spectroscopic data are consistent with that[200] reported in the literature.

(S)-2-(1-(*Tert*-Butoxycarbonyl)pyrrolidin-2-yl)thiazole-4-carboxylic acid (1.123)
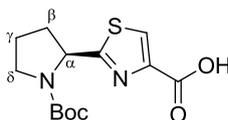
1.123

To a solution of **1.122** (550 mg, 1.76 mmol, 1 equiv) in 8 mL of a mixture THF/water (3:1) that had been cooled to 0 °C was added LiOH (127 mg, 5.28 mmol, 3 equiv) and stirred at 0 °C overnight. The solution was diluted with water (5 mL) and acidified to

pH 1–2 with a saturated KHSO$_4$ solution. This aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide the carboxylic acid **1.123** (493 mg, 94%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm (mixture of conformers) 8.20 (s, 1H, H$_{Ar}$), 5.33–5.15 (m, 1H, CHα), 3.74–3.36 (m, 2H, CH$_2\delta$), 2.51–2.21 (m, 2H, CH$_2\beta$), 2.11–1.82 (m, 2H, CH$_2\gamma$), 1.59–1.28 (m, 9H, CMe$_3$).

The spectroscopic data are consistent with that[201] reported in the literature.

N-Boc-(Pro)Thz-Ser-OMe (1.124)

1.124
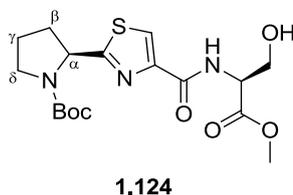
To a solution of Boc-L-(Ile)Thz-OH (**1.123**) (737 mg, 2.7 mmol, 1 equiv) in CH$_2$Cl$_2$ (30 mL), PyBOP (1.97 g, 3.8 mmol, 1.4 equiv) and Hünig's base (1.4 mL, 8.1 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before H-Ser-OMe (541 mg, 3.0 mmol, 1.1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 1:1) to afford **1.124** as a colourless oil (817 mg, 76.4%).

[α]$_D^{23}$ –49.5 (*c* 0.21, CH$_3$OH); **IR** 3391, 2974, 2876, 2357, 2341, 1744, 1695, 1667, 1544, 1491, 1458, 1389, 1209, 1168, 1111 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** (mixture of rotamers) 8.13 (br d, *J*=16.5 Hz, 1H, NH), 8.03 (s, 1H, H$_{Ar}$), 5.30–4.97 (m, 1H, CHα), 4.90–4.73 (m, 1H, <u>CH</u>-CH$_2$OH), 4.18–3.96 (m, 2H, CH$_2$OH), 3.82 (s, 3H, CO$_2$Me), 3.70–3.37 (m, 2H, CH$_2\delta$), 2.47–2.11 (m, 2H, CH$_2\beta$), 2.08–1.79 (m, 2H, CH$_2\gamma$), 1.60–1.25 (m, 9H, CMe$_3$); **ES$^+$ MS *m/z*** 422 ([M+Na]$^+$).

(*S*)-Methyl 2-(2-(1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl)thiazol-4-yl)oxazole-4-carboxylate (1.126)
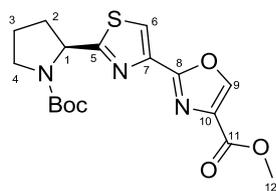
1.126

To a solution of *N*-Boc-(Pro)Thz-Ser-OMe (**1.124**) (100 mg, 0.25 mmol, 1 equiv) in dry CH_2Cl_2, DAST (40 µL, 0.30 mmol, 1.2 equiv) was added at −78 °C under argon. After stirring the reaction mixture for 1 h at −78 °C, anhydrous K_2CO_3 (52.0 mg, 0.38 mmol, 1.5 equiv) was added in one portion and the mixture was warmed to rt. The reaction mixture was poured into saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the oxazoline intermediate **1.125** (95 mg, 100%).

To **1.125** (95 mg, 0.24 mmol, 1 equiv) in CH_2Cl_2 (4 mL), DBU (41 µL, 0.27 mmol, 1.2 equiv) was added dropwise followed by $BrCCl_3$ (32 µL, 0.32 mmol, 1.4 equiv) at −10 °C. The mixture was stirred overnight at rt. The mixture was washed with saturated NH_4Cl (2 x 5 mL), and the combined aqueous layers were extracted once with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 3:1 to 1:1) to afford **1.126** as a white solid (58 mg, 64%).

$[\alpha]_D^{23}$ −89.2 (*c* 0.17, CH_3OH); **mp** 146–148 °C; **IR** 3130, 2983, 2946, 1732, 1704, 1589, 1565, 1401, 1315, 1176, 1156, 1115, 1005, 952 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** (mixture of conformers) 8.26 (s, 1H, H-6), 8.04 (s, 1H, H-9), 5.40–5.09 (m, 1H, H-1), 3.91 (s, 3H, H-12), 3.72–3.37 (m, 2H, H-4), 2.45–2.16 (m, 2H, H-2), 2.06–1.80 (m, 2H, H-3), 1.51–1.21 (m, 9H, CMe$_3$); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** (mixture of conformers) 177.8 (C), 161.4 (C), 157.7 (C), 154.0 (C), 143.5 (CH, C-9), 142.3 (C, C-7), 134.2 (C, C-10), 120.8 (CH, C-6), 80.4 (C, CMe$_3$), 59.5 (CH, C-1), 52.1 (CH$_3$, C-12), 46.6 (CH$_2$, C-4), 34.1 (CH$_2$, C-2), 28.1 (CH$_3$, CMe$_3$), 23.1 (CH$_2$, C-3).

Methyl 2-(2-((*S*)-1-((*S*)-2-(2-((*S*)-1-(*tert*-butoxycarbonylamino)ethyl)thiazole-4-carboxamido)-4-methylpentanoyl)pyrrolidin-2-yl)thiazol-4-yl)oxazole-4-carboxylate (1.128)
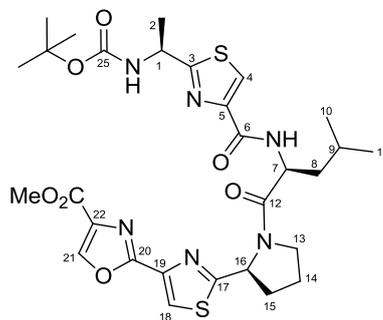
1.128

To *N*-Boc-(Ala)Thz-Leu-OMe (**1.119**) (380 mg, 0.95 mmol, 1 equiv) in 8 mL of THF/water (3:1) at 0 °C was added LiOH (68 mg, 2.85 mmol, 3 equiv). The reaction mixture was stirred at rt overnight and was diluted with water and acidified to pH 1–2 with a saturated $KHSO_4$ solution. This aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to provide the carboxylic acid (357 mg, 97%).

1.126 (243.7 mg, 0.64 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 7 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine as hydrochloride salt **1.127** (203 mg, 100%).

To a solution of the previous carboxylic acid (84 mg, 0.26 mmol, 1.1 equiv) in CH_2Cl_2 (20 mL), PyBOP (501.4 mg, 0.96 mmol, 1.5 equiv), and Hünig's base (336 μL, 1.93 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before the amine **1.127** (203 mg, 0.64 mmol, 1 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:1 to 7:3) as eluent to afford **1.128** as a colorless oil (280 mg, 67%).

$[\alpha]_D^{23}$ −39.7 (*c* 0.21, CH_3OH); **IR** 2957, 1709, 1641, 1536, 1440, 1250, 1167, 824; **^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD) δ ppm** (Mixture of conformers) 8.32–8.17 (m, 1H, H_{Ar}), 8.03 (s, 1H, H_{Ar}), 7.94 (d, *J*=7.6 Hz, 1H, H_{Ar}), 5.55–5.29 (m, 1H, H-16), 5.04–4.80 (m, 2H, H-1; H-7), 3.95–3.82 (m, 4H, CO_2Me; H-13), 3.81–3.53 (m, 1H, H-13), 2.41–2.21 (m, 2H, H-15), 2.20–2.04 (m, 2H, H-14), 1.75-1.60 (m, 2H, H-8), 1.59–1.45 (m, 4H, H-2; H-9), 1.35 (br s, 9H, C<u>Me</u>$_3$), 0.98–0.79 (m, 6H, H-10; H-11); **ES$^+$ MS *m/z*** 669

([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{29}$H$_{38}$N$_6$NaO$_7$S$_2$ (M+Na)$^+$ 669.2136, found 669.2132.

(*S*)-Methyl2-((*S*)-1-(2-(2-((*S*)-1-((*S*)-2-(2-((*S*)-1-(*tert*-butoxycarbonylamino)-ethyl)thiazole   -4-carboxamido)-4-methylpentanoyl)pyrrolidin-2-yl)thiazol-4-yl)-oxazole-4-carbonyl) pyrrolidine -2-carboxamido)-3-methylbutanoate (1.129)
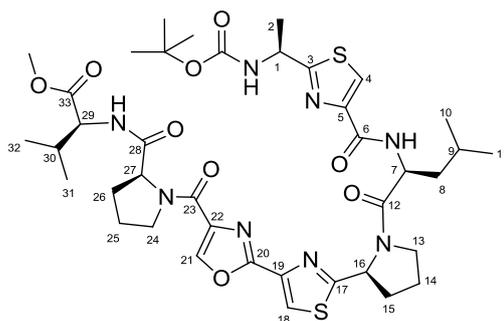
1.129

To **1.128** (277 mg, 0.43 mmol, 1 equiv) in 8 mL of THF/water (3:1) was added LiOH (31 mg, 1.28 mmol, 3 equiv) at 0 °C and stirred at rt overnight. The solution was diluted with water and acidified to pH 1–2 with a saturated KHSO$_4$ solution. This aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide the carboxylic acid (271 mg, 100%).

The dipeptide Boc-Pro-Val-OMe (**1.112**) (500 mg, 2.19 mmol, 1 equiv) was treated with HCl in dioxane (4 M, 5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the free amine as hydrochloride salt **1.113** (403 mg, 100%).

To a solution of carboxylic acid (271 mg, 0.43 mmol, 1 equiv) in CH$_2$Cl$_2$ (5 mL), PyBOP (334 mg, 0.64 mmol, 1.5 equiv), and Hünig's base (224 μL, 1.28 mmol, 3 equiv) were added. The reaction mixture was stirred for 5 min before the amine **1.113** (170 mg, 0.64 mmol, 1.5 equiv) was added. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc as eluent to afford **1.129** as a white solid (209 mg, 58%).

ES$^+$ MS *m/z* 865 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{39}$H$_{54}$N$_8$NaO$_9$S$_2$ (M+Na)$^+$ 865.3347, found 865.3345.

Trans, trans-Sanguinamide B (1.130)
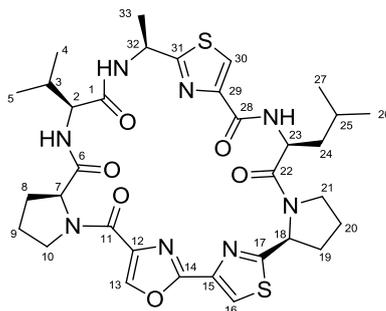
1.130

To a solution of **1.129** (189 mg, 0.22 mmol, 1 equiv) in 4 mL of a mixture THF/water (3:1) that had been cooled to 0 °C was added LiOH (54 mg, 2.20 mmol, 10 equiv) and stirred at 0 °C overnight. The solution was diluted with water and acidified to pH 1–2 with a saturated $KHSO_4$ solution. This aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to provide the carboxylic acid. The carboxylic acid was treated with HCl in dioxane (4 M, 5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the linear precursor of sanguinamide B (171.5 mg, 100%). Acyclic precursor (171 mg, 0.22 mmol, 1 equiv) was dissolved in CH_3CN/THF (100 mL) and added dropwise to a vigorously stirred solution of Hünig's base (117 µL, 0.67 mmol, 3 equiv) and HATU (170 mg, 0.45 mmol, 2 equiv) in CH_3CN (170 mL). The reaction mixture was allowed to stir overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography with 3% MeOH in CH_2Cl_2 to afford sanguinamide B and an impurity. HPLC was performed to afford *trans, trans*-sanguinamide B (**1.130**) (4 mg, 3%).

^{1}H NMR (600 MHz, CDCl₃) δ ppm 8.37 (s, 1H, H-13), 7.92 (s, 1H, H-30), 7.43 (s, 1H, H-16), 7.24 (d, *J*=8.2 Hz, 1H, NH), 7.02 (d, *J*=10.9 Hz, 1H, NH), 6.27 (d, *J*=10.6 Hz, 1H, NH), 5.43 (d, *J*=7.0 Hz, 1H, H-18), 5.13–4.97 (m, 2H, H-23; H-32), 4.78 (dd, *J*=10.6, 2.6 Hz, 1H, H-2), 4.72 (dd, *J*=8.7, 1.3 Hz, 1H, H-7), 3.91–3.74 (m, 4H, H-10; H-21), 2.88 (dq, *J*=6.8, 4.1 Hz, 1H, H-3), 2.36–2.20 (m, 3H, H-8; H-19), 2.20–2.06 (m, 1H, H-9), 2.06–1.98 (m, 1H, H-9), 1.98–1.88 (m, 3H, H-19; H-20; H-24), 1.83–1.71 (m, 1H, H-20), 1.69–1.51 (m, 2H, H-24; H-25), 1.08–0.95 (m, 9H, H-5; H-26; H-33), 0.92 (d, *J*=6.5 Hz, 3H, H-27), 0.86 (d, *J*=6.7 Hz, 3H, H-4); **^{13}C NMR (100 MHz, CDCl₃) δ ppm** 173.6 (C, C-6), 172.8 (C, C-17), 171.02 (C, C-31), 170.96 (C, C-1), 168.9 (C, C-

22), 162.1 (C, C-11), 160.0 (C, C-28), 157.7 (C, C-14), 146.7 (C, C-29), 143.7 (CH, C-13), 140.7 (C, C-15), 136.7 (C, C-12), 125.7 (CH, C-30), 122.6 (CH, C-16), 64.3 (CH, C-7), 59.0 (CH, C-18), 56.9 (CH, C-2), 49.1 (CH, C-23), 48.1 (CH$_2$, C-10), 46.7 (CH$_2$, C-21), 45.9 (CH, C-32), 41.6 (CH$_2$, C-24), 35.8 (CH$_2$, C-19), 32.0 (CH$_2$, C-8), 28.4 (CH, C-3), 24.4 (CH, C-25), 23.5 (CH$_3$, C-27), 22.7 (CH$_2$, C-9), 22.3 (CH$_3$, C-26), 20.3 (CH$_2$, C-20), 19.9 (CH$_3$, C-5), 16.5 (CH$_3$, C-4), 16.3 (CH$_3$, C-33); **ES$^+$ MS** *m/z* 711 ([M+H]$^+$), 733 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{33}$H$_{42}$N$_8$NaO$_6$S$_2$ (M+Na)$^+$ 733.2561, found 733.2582.

5.2.3. Stylissamide A

Protected stylissamide A (1.132)
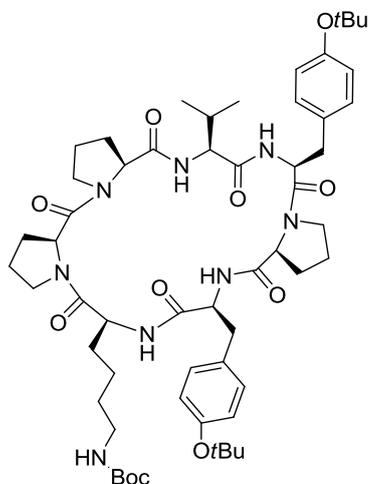
1.132

Acyclic precursor **1.131** (98.7 mg, 0.09 mmol, 1 equiv) was then taken up in CH_2Cl_2 (20 mL) and added dropwise to a vigorously stirred solution of Hünig's base (97 µL, 0.56 mmol, 6 equiv), HOBt (25 mg, 0.19 mmol, 2 equiv) and HATU (71 mg, 0.19 mmol, 2 equiv) in CH_3CN (65 mL). The reaction mixture was allowed to stir overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography (5 to 10% MeOH in CH_2Cl_2) to afford **1.132** as a pale yellow solid (60 mg, 62%).

ES$^+$ MS *m/z*: 1079 ([M+Na]$^+$).

Stylissamide A (1.20)

1.20
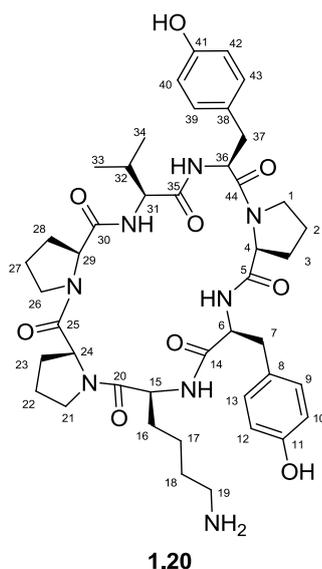
1.132 (50 mg, 47 μmol) was dissolved in a mixture of TFA (3 mL), triisopropylsilane (100 μL) and water (100 μL) at 0 °C. The mixture was stirred for 3 h, before being concentrated. The solid was triturated with ether (25 mg). HPLC was performed to obtain a compound as pure as possible and afforded **1.20** (10 mg, 25%).

^{1}H NMR (600 MHz, DMSO-*d6*) δ ppm 7.83 (br s, 2H, NH$_2$), 7.73–7.69 (d, 1H, NH), 7.62 (d, *J*=8.1 Hz, 1H, NH), 7.33–7.29 (m, 1H, NH), 7.25 (d, *J*=9.5 Hz, 1H, NH), 7.06 (d, *J*=7.7 Hz, 2H, H-9; H-13), 6.99 (d, *J*=8.1 Hz, 2H, H-39; H-43), 6.64 (d, *J*=7.7 Hz, 2H, H-40; H-42), 6.63 (d, *J*=7.7 Hz, 1H, H-10; H-12), 4.90 (t, *J*=10.8 Hz, 1H, H-36), 4.41 (d, *J*=7.0 Hz, 2H, H-15; H-29), 4.36–4.27 (m, 2H, H-6; H-24), 3.97 (t, *J*=8.1 Hz, 1H, H-4), 3.79–3.60 (m, 3H, H-1; H-31), 3.47–3.38 (m, 3H, H-21; H-26), 3.33 (dd, *J*=15.6, 6.8 Hz, 1H, H-21), 3.27 (d, *J*=12.8 Hz, 1H, H-37), 3.17 (d, *J*=12.1 Hz, 1H, H-7), 2.86 (t, *J*=12.6 Hz, 1H, H-7), 2.81–2.71 (m, 2H, H-19), 2.38 (t, *J*=12.1 Hz, 1H, H-37), 2.19–2.11 (m, 2H, H-23; H-28), 2.10–1.97 (m, 4H, H-2; H-3; H-28), 1.96–1.69 (m, 7H, H-16; H-22; H-23; H-27; H-32), 1.63–1.43 (m, 5H, H-3; H-16; H-18; H-27), 1.37–1.26 (m, 2H, H-17), 0.77 (d, *J*=6.2 Hz, 3H, H-33 or H-34), 0.43 (d, *J*=6.2 Hz, 3H, H-33 or H-34); **ES[+] MS *m/z*:** 845 ([M+H][+]), 867 ([M+Na][+]).

The spectroscopic data are consistent with that[28] reported in the literature.

5.3. EXPERIMENTAL DATA FOR CHAPTER 2 : HISTONE DEACETYLASE INHIBITORS

5.3.1. Experimental procedures for largazole and analogues

D-Valinol (2.144)
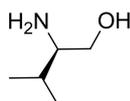
2.144

To a solution of sodium borohydride (7.76 g, 205 mmol, 2.4 equiv) in THF (200 mL) was added in one portion D-valine (10 g, 86 mmol, 1 equiv). The reaction mixture was cooled to 0 °C and a solution of iodine (21.7 g, 86 mmol, 1 equiv) in THF (55 mL) was added dropwise resulting in the formation of hydrogen. After the hydrogen formation stopped, the reaction mixture was refluxed overnight, then, cooled to rt, and neutralised by cautious addition of methanol until the mixture became clear. After stirring 30 min, the solvent was removed *in vacuo* affording a white paste which was dissolved by addition of KOH solution (20%, 150 mL). The solution was stirred for 4 h and extracted with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to form **2.144** as a white solid (7.7 g, 87%).

¹H NMR (300 MHz, CDCl₃) δ ppm 3.65 (dd, *J*=10.6, 4.0 Hz, 1H, C$\underline{H}_2$OH), 3.29 (dd, *J*=10.6, 8.8 Hz, 1H, C$\underline{H}_2$OH), 2.56 (ddd, *J*=8.8, 6.2, 4.0 Hz, 1H, C$\underline{H}$NH₂), 1.98 (br s, 3H, NH₂, OH), 1.58 (qd, *J*=13.5, 6.7 Hz, 1H, C$\underline{H}$(CH₃)₂), 0.92 (app dd, *J*=6.8, 4.6 Hz, 6H, C$\underline{H_3}$).

The spectroscopic data are consistent with that[115] reported in the literature.

(*R*)-4-Isopropylthiazolidine-2-thione (2.145)
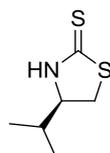
2.145

To a stirred solution of D-valinol **2.144** (7.7 g, 75 mmol, 1.0 equiv) in potassium hydroxide solution (1 M, 380 mL), carbon disulfide (23 mL, 380 mmol, 5.0 equiv) was added. The reaction mixture was stirred at 100 °C for 24 h. After cooling to rt, the reaction mixture was extracted with CH_2Cl_2 (2 x 300 mL). The combined organic layers were dried over $MgSO_4$ and then evaporated under reduced pressure to afford **2.145** as a yellow solid (11.2 g, 93%).

¹H NMR (300 MHz, CDCl₃) δ ppm 7.61 (br s, 1H, N<u>H</u>), 4.10–4.00 (m, 1H, C<u>H</u>NH), 3.52 (dd, *J*=11.1, 8.1 Hz, 1H, C<u>H</u>₂), 3.34 (dd, *J*=11.1, 8.5 Hz, 1H, C<u>H</u>₂), 1.98 (octet, *J*=6.8 Hz, 1H, C<u>H</u>(CH₃)₂), 1.05 (d, *J*=6.6 Hz, 3H, C<u>H</u>₃), 1.01 (d, *J*=6.6 Hz, 3H, C<u>H</u>₃).

The spectroscopic data are consistent with that[116] reported in the literature.

(*R*)-1-(4-Isopropyl-2-thioxothiazolidin-3-yl)ethanone (2.46)

2.46
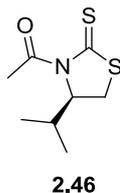
To a suspension of 60% NaH (3.05 g, 76.4 mmol, 1.1 equiv) in dry THF (65 mL) was added a solution of **2.145** (11.2 g, 69.4 mmol, 1 equiv) in THF (65 mL). The mixture was stirred at 0 °C for 10 min. Acetyl chloride (5.43 mL, 76.4 mmol, 1.1 equiv) was added to the reaction mixture which was stirred at 0 °C to rt overnight. HCl solution (5%) was added and the aqueous layer was extracted with EtOAc (3 x 300 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 5:95) to give **2.46** as a yellow oil (12.6 g, 89%).

¹H NMR (300 MHz, CDCl₃) δ ppm 5.16 (ddd, *J*=7.7, 6.2, 1.1 Hz, 1H, C<u>H</u>NH), 3.51 (dd, *J*=11.5, 8.1 Hz, 1H, C<u>H</u>₂), 3.03 (dd, *J*=11.7, 1.1 Hz, 1H, C<u>H</u>₂), 2.78 (s, 3H, C<u>H</u>₃), 2.38 (octet, *J*=6.8 Hz, 1H, C<u>H</u>(CH₃)₂), 1.07 (d, *J*=6.8 Hz, 3H, C<u>H</u>₃), 0.99 (d, *J*=7.2 Hz, 3H, C<u>H</u>₃).

The spectroscopic data are consistent with that[117] reported in the literature.

(*E*)-5-(Tritylthio)pent-2-enal (2.38)
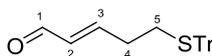
2.38

To a solution of triphenylmethanethiol (3.5 g, 12.7 mmol, 1 equiv) in CH_2Cl_2 (70 mL) was added NEt_3 (2.5 mL, 18.1 mmol, 1.43 equiv) followed by acrolein (1.20 mL, 17.7 mmol, 1.4 equiv) at rt under argon with stirring. After 2 h, the reaction mixture was concentrated *in vacuo* and the solid was dried.

The crude intermediate and formylmethene triphenylphosphorane (3.85 g, 12.7 mmol, 1 equiv) were dissolved in toluene (70 mL). The reaction mixture was heated at 80 °C overnight. The reaction was allowed to reach rt and pass through a silica plug (eluent EtOAc/hexane 1:4). The solvent was then removed and the residue purified by flash chromatography (5-10% EtOAc in hexane) to give **2.38** as a beige solid (3.0 g, 65%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 9.41 (d, *J*=7.5 Hz, 1H, H-1), 7.50–7.11 (m, 15H, H$_{Ar}$), 6.60 (dt, *J*=15.7, 6.3 Hz, 1H, H-3), 5.96 (dd, *J*=15.8, 7.9 Hz, 1H, H-2), 2.38–2.22 (m, 4H, H-4; H-5).

The spectroscopic data are consistent with that[88] reported in the literature.

(3*S*)-Hydroxy-1-(4*R*-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4*E*-en-1-one (2.47)
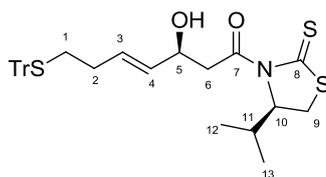
2.47

To a stirred solution of the acetylated Nagao chiral auxiliary **2.46** (2.73 g, 13.4 mmol, 1.7 equiv) in CH_2Cl_2 (110 mL) at 0 °C was added $TiCl_4$ (1.67 mL, 15.2 mmol, 1.9 equiv). The reaction mixture was stirred for 5 min, cooled to −78 °C before the addition of Hünig's base (2.65 ml, 15.2 mmol, 1.9 equiv) and stirred for 2 h. The aldehyde **2.38** (2.92 g, 8.1 mmol, 1 equiv) in CH_2Cl_2 (17 mL) was added dropwise and the reaction mixture stirred for 30 min. Saturated NH_4Cl (100 mL) was added to the reaction mixture which was diluted with CH_2Cl_2 (60 mL) and allowed to reach rt. The aqueous layer was extracted with CH_2Cl_2 (3 x 60 mL), washed with saturated NaCl (50 mL) and

dried weith Na$_2$SO$_4$. The solvent was then removed and the residue purified by flash chromatography (10-25% EtOAc in hexane) to give the major isomer **2.47** as a yellow solid (1.9 g, 42%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.49–7.13 (m, 15H, H$_{Ar}$), 5.69–5.53 (m, 1H, H-3), 5.47 (dd, J=15.6, 6.0 Hz, 1H, H-4), 5.14 (t, J=6.8 Hz, 1H, H-10), 4.68–4.50 (m, 1H, H-5), 3.57 (dd, J=17.6, 3.0 Hz, 1H, H-6), 3.48 (dd, J=11.5, 8.0 Hz, 1H, H-9), 3.29 (dd, J=17.6, 8.5 Hz, 1H, H-6), 3.01 (d, J=11.5 Hz, 1H, H-9), 2.74 (br s, 1H, OH), 2.36 (dq, J=13.4, 6.8 Hz, 1H, H-11), 2.22 (t, J=7.5 Hz, 2H, H-1), 2.10 (q, J=7.0 Hz, 2H, H-2), 1.06 (d, J=6.5 Hz, 3H, H-12 or H-13), 0.98 (d, J=7.0 Hz, 3H, H-12 or H-13).

The spectroscopic data are consistent with that[78] reported in the literature.

(3S,4E)-3-Hydroxy-7-[(triphenylmethyl)thio]-4-heptenoic acid (2-trimethyl-silyl)-ethyl ester (2.85)

2.85
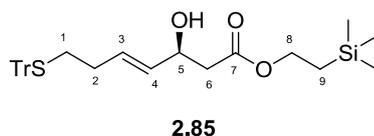
Compound **2.47** (800 mg, 1.42 mmol, 1 equiv) was dissolved in CH$_2$Cl$_2$ (20 mL) and 2-trimethylsilyethanol (2.04 mL, 14.24 mmol, 10 equiv) was added, followed by DMAP (17.4 mg, 0.14 mmol, 0.1 equiv). The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude was purified by flash chromatography (EtOAc/hexane 2:8) to give **2.85** as a clear oil (384 mg, 52%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.56–7.16 (m, 15H, H$_{Ar}$), 5.73–5.50 (m, 1H, H-3), 5.50–5.30 (m, 1H, H-4), 4.60–4.35 (m, 1H, H-5), 4.29–4.07 (m, 2H, H-8), 2.56–2.36 (m, 2H, H-6), 2.31–2.15 (m, 2H, H-1), 2.08 (q, J=6.9 Hz, 2H, H-2), 1.11–0.85 (m, 2H, H-9), 0.05 (s, 9H, Si$\underline{Me}_3$).

The spectroscopic data are consistent with that[42] reported in the literature.

(*S*,*E*)-2-(Trimethylsilyl)ethyl3-((*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonyl-amino)-3-methylbutanoyloxy)-7-(tritylthio)hept-4-enoate (2.86)
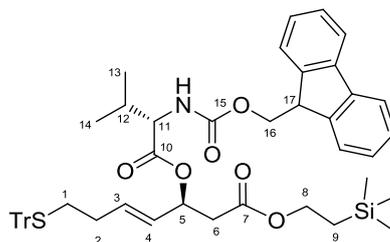
2.86

Compound **2.85** (156 mg, 0.30 mmol, 1 equiv) and *N*-Fmoc-L-valine (112 mg, 0.33 mmol, 1.1 equiv) were dissolved in CH_2Cl_2 (5 mL). The reaction was cooled to 0° C. DCC (74 mg, 0.36 mmol, 1.2 equiv) and DMAP (4 mg, 0.03 mmol, 0.1 equiv) were added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude was purified by flash chromatography (EtOAc/hexane 5:95) to give **2.86** as a white solid (238 mg, 94%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.78 (d, *J*=7.7 Hz, 2H, H$_{Ar}$), 7.61 (d, *J*=6.6 Hz, 2H, H$_{Ar}$), 7.52–7.17 (m, 19H, H$_{Ar}$), 5.89–5.56 (m, 2H), 5.48–5.13 (m, 2H), 4.47–4.08 (m, 6H, H-8; H-11; H-16; H-17), 2.78–2.50 (m, 2H, H-6), 2.31–1.92 (m, 5H, H-1; H-2; H-12), 1.06–0.68 (m, 8H, H-9; H-13; H-14), 0.03 (s, 9H, SiMe$_3$); **ES$^+$ MS *m/z*** 862 ([M+Na]$^+$).

The spectroscopic data are consistent with that[42] reported in the literature.

Allyl carbamoylmethylcarbamate (2.153)
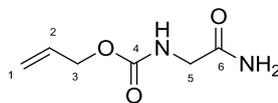
2.153

To a solution of glycinamide hydrochloride (3 g, 27.1 mmol, 1 equiv) in THF (65 mL) at rt was added Hünig's base (23.6 mL, 135.7 mmol, 5 equiv), allylchloroformate (8.7 mL, 81.4 mmol, 3 equiv), and DMAP (332 mg, 2.7 mmol, 0.1 equiv). The reaction mixture was stirred for 3 h at rt and diluted with ethyl acetate. The organic layer was washed with $KHSO_4$ solution (0.05 M), saturated $NaHCO_3$ solution and brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*.

The crude material was purified by flash chromatography using 0-10% MeOH in CH$_2$Cl$_2$ with to afford **2.153** as a white solid (1.65 g, 37%).

mp 104–106 ºC; **IR** 3334, 3198, 1678, 1652, 1550, 1546, 1300, 1270 cm^{-1}; **^{1}H NMR (300 MHz, CD$_3$OD) δ ppm** 5.98 (ddd, J=22.7, 10.6, 5.5 Hz, 1H, H-2), 5.35 (dq, J=17.3, 1.8 Hz, 1H, H-1), 5.22 (dq, J=10.5, 1.4 Hz, 1H, H-1), 4.59 (br d, J=5.5 Hz, 2H, H-3), 3.79 (s, 2H, H-6); **^{13}C NMR (75 MHz, CD$_3$OD) δ ppm** 175.2 (C, CO), 159.0 (C, CO), 134.4 (CH, C-2), 117.8 (CH$_2$, C-1), 66.9 (CH$_2$, C-3), 44.6 (CH$_2$, C-5); **ES$^+$ MS** *m/z* 181 ([M+Na]$^+$).

Allyl thiocarbamoylmethylcarbamate (2.154)

2.154
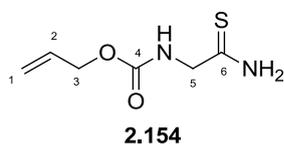
To a solution of **2.153** (1.65 g, 10.5 mmol, 1 equiv) in toluene (25 mL) was added the Lawesson's reagent (2.5 g, 6.3 mmol, 0.6 equiv). The reaction mixture was refluxed for 24 h. The reaction mixture was purified by flash chromatography using 0-3% MeOH in CH$_2$Cl$_2$ to afford **2.154** as a yellow solid (1.44 g, 79%).

mp 78–80 ºC; **IR** 3330, 3160, 2926, 1690, 1610, 1527, 1448, 1399, 1361, 1251, 1149, 1047, 990, 945 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 7.87 (br s, 2H, NH$_2$), 6.04–5.81 (m, 1H, H-2), 5.71 (br s, 1H, NH), 5.33 (dd, J=17.2, 1.1 Hz,1H, H-1), 5.25 (dd, J=10.2, 1.1 Hz, 1H, H-1), 4.61 (d, J=5.9 Hz, 2H, H-3), 4.21 (d, J=5.9 Hz, 2H, H-5); **^{13}C NMR (75 MHz, CDCl$_3$) δ ppm** 204.4 (C, C=S), 156.7 (C, C=O), 132.1 (CH, C-2), 118.3 (CH$_2$, C-1), 66.4 (CH$_2$, C-3), 51.4 (CH$_2$, C-5); **ES$^+$ MS** *m/z* 197 ([M+Na]$^+$).

Allyl (4-(methoxycarbonyl)thiazol-2-yl)methylcarbamate (2.155)

2.155
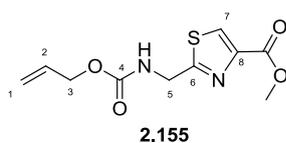
To a solution of **2.154** (2.1 g, 12.16 mmol, 1 equiv) in ethanol (28 mL), methyl bromopyruvate (1.42 mL, 3.15 mmol, 1.1 equiv) and calcium carbonate (657 mg, 1.55

mmol, 0.5 equiv) were added. The reaction mixture was stirred under argon at rt overnight. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was partitioned between chloroform and saturated $NaHCO_3$ solution. The organic layer was then washed once with water. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford **2.155** as a yellow oil (2 g, 64%).

IR 3330, 3115, 2952, 1712, 1523, 1440, 1240, 1142, 1092, 987, 926 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 8.15 (s, 1H, H-7), 6.03–5.84 (m, 1H, H-2), 6.02–5.54 (br s, 1H, NH), 5.32 (d, *J*=16.6 Hz, 1H, H-1), 5.24 (dd, *J*=10.5, 1.0 Hz, 1H, H-1), 4.72 (d, *J*=6.5 Hz, 2H, H-5), 4.63 (d, *J*=5.5 Hz, 2H, H-3), 3.96 (s, 3H, Me); **^{13}C NMR (75 MHz, CDCl$_3$) δ ppm** 169.3 (C), 161.3 (C), 155.9 (C), 146.1 (C, C-8), 132.1 (CH, C-2), 127.8 (CH, C-7), 117.7 (CH$_2$, C-1), 65.8 (CH$_2$, C-3), 52.1 (CH$_3$, Me), 42.3 (CH$_2$, C-5); **ES$^+$ MS *m/z*** 279 ([M+Na]$^+$).

Allyl (4-(carboxylic acid)thiazol-2-yl)methylcarbamate (2.156)

2.156
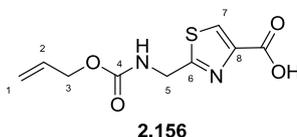
To a solution of **2.155** (2 g, 7.8 mmol, 1 equiv) in 40 mL of a mixture THF/water (3:1) was added LiOH (560 mg, 23.4 mmol, 3 equiv) at 0 °C. The reaction mixture was stirred at rt overnight. The solution was diluted with water and acidified to pH 1–2 with a saturated KHSO$_4$. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to provide **2.156** as a yellow solid (1.5 g, 79%).

mp 137–138 °C; **IR** 3312, 3119, 2922, 1712, 1516, 1251, 1110, 1039, 983, 934 cm^{-1}; **^{1}H NMR (300 MHz, CD$_3$OD) δ ppm** 8.29 (s, 1H, H-7), 6.34–6.12 (m, 1H, H-2), 5.32 (d, *J*=17.2 Hz, 1H, H-1), 5.20 (d, *J*=10.6 Hz, 1H, H-1), 4.65–4.44 (m, 4H, H-3; H-5); **^{13}C NMR (75 MHz, CD$_3$OD) δ ppm** 173.2 (C, CO$_2$H), 164.0 (C), 159.8 (C), 148.3 (C, C-8), 134.3 (CH, C-2), 129.5 (CH, C-7), 117.9 (CH$_2$, C-1), 67.0 (CH$_2$, C-3), 43.5 (CH$_2$, C-5); **ES$^-$ MS *m/z*** 242 ([M-H]$^-$).

(*R*)-2-Amino-2-methyl-3-(tritylthio)propanoic acid (2.158)

2.158
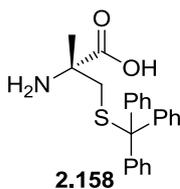
A mixture of (*R*)-2-methylcysteine hydrochloride (100 mg, 0.58 mmol, 1 equiv) and glacial acetic acid (1.8 mL) was warmed at 60 °C. Triphenylmethanol (152 mg, 0.58 mmol, 1 equiv) was added at rt and the temperature is raised again to 60 °C before boron trifluoride diethyl etherate (152 mg, 0.58 mmol) was added. The reaction mixture was heated at 80 °C for 30 min and stirred at rt for 45 min. To the reaction mixture, EtOH (800 μL), water (300 μL) and sodium acetate (16 mg) were added. Water was added and the reaction mixture was cooled. The precipitated was filtered and washed with diethyl ether to provide **2.158** as a white solid (124 mg, 96%).

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.52–7.17 (m, 15H, H$_{Ar}$), 2.72 (d, *J*=12.1 Hz, 1H, C$\underline{H}_2$), 2.48 (d, *J*=12.1 Hz, 1H, C$\underline{H}_2$), 1.40 (s, 3H, Me); **ES$^+$ MS *m/z*** 243 ([CPh$_3$]$^+$), 400 ([M+Na]$^+$).

The spectroscopic data are consistent with that[202] reported in the literature.

(*R*)-2-Amino-2-methyl 3-tritylsulfanyl-propionic acid methyl ester (2.159)

2.159
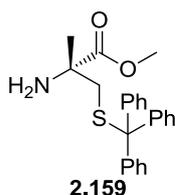
To a solution of thionyl chloride (93 μL, 1.27 mmol, 5 equiv) in dry methanol (5mL), compound **2.158** (96 mg, 0.25 mmol, 1 equiv) was added at 0 °C. This solution was refluxed overnight under argon. The reaction mixture was concentrated *in vacuo* and the crude material purified by flash chromatography (hexane/EtOAc 1:1) to afford **2.159** as a white solid (67 mg, 67%).

¹H NMR (300 MHz, CDCl₃) δ ppm 7.43–7.05 (m, 15H, H$_{Ar}$), 3.69 (s, 3H, CO$_2$<u>Me</u>), 2.95 (d, *J*=12.1 Hz, 1H, C<u>H</u>$_2$), 2.54 (d, *J*=12.1 Hz, 1H, C<u>H</u>$_2$), 1.96 (br s, 2H, NH$_2$), 1.62 (s, 3H, Me); **ES⁺ MS *m/z*** 243 ([CPh₃]⁺), 413 ([M+Na]⁺).

The spectroscopic data are consistent with that[203] reported in the literature.

(*R*)-Methyl 2-(2-((allyloxycarbonylamino)methyl)thiazole-4-carboxamido)-2-methyl-3-(tritylthio)propanoate (2.160)
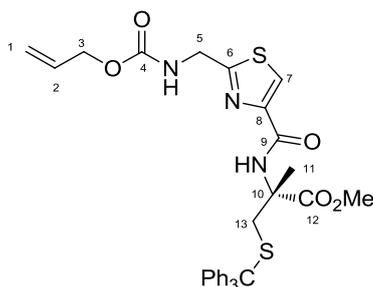
2.160

To a solution of **2.156** (283 mg, 1.17 mmol, 1.1 equiv) in MeCN (17 mL) cooled at 0 °C, PyBOP (688 mg, 1.17 mmol, 1.1 equiv) and Hünig's base (462 μL, 2.65 mmol, 2.5 equiv) were added. After 10 min, **2.159** (400 mg, 1.06 mmol, 1 equiv) in solution in CH$_2$Cl$_2$ (17 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 1:1) to afford **2.160** as a white solid (650 mg, 100%).

$[\alpha]_D^{26}$ –4.3 (*c* 0.22, CHCl₃); **mp** 76–80 °C; **IR** 3361, 2949, 2930, 1735, 1675, 1538, 1493, 1448, 1251 cm⁻¹; **¹H NMR (300 MHz, CDCl₃) δ ppm** 8.04 (s, 1H, H-7), 7.94 (s, 1H, NH), 7.48–7.13 (m, 15H, H$_{Ar}$), 6.03–5.84 (m, 1H, H-2), 5.55 (br t, *J*=5.7 Hz, 1H, NH), 5.33 (dd, *J*=17.0, 0.6 Hz, 1H, H-1), 5.28–5.20 (m, 1H, H-1), 4.68 (d, *J*=6.2 Hz, 2H, H-5), 4.64 (dt, *J*=5.6, 1.4 Hz, 2H, H-3), 3.71 (s, 3H, CO$_2$<u>Me</u>), 3.06 (d, *J*=11.8 Hz, 1H, H-13), 2.89 (d, *J*=11.8 Hz, 1H, H-13), 1.51 (s, 3H, H-11); **¹³C NMR (75 MHz, CDCl₃) δ ppm** 173.1 (C, CO), 168.2 (C), 159.9 (C), 156.1 (C), 149.7 (C, C-8), 144.5 (3C, C$_{Ar}$), 132.4 (CH, C-2), 129.6 (6CH, C$_{Ar}$), 127.9 (6CH, C$_{Ar}$), 126.7 (3CH, C$_{Ar}$), 123.9 (CH, C-7), 118.1 (CH$_2$, C-1), 66.5 (C, <u>C</u>Ph₃), 66.2 (CH$_2$, C-3), 59.2 (C, C-10), 52.9 (CH₃, CO$_2$<u>Me</u>), 42.6 (CH$_2$, C-5), 37.9 (CH$_2$, C-13), 23.1 (CH₃, C-11); **ES⁺ MS *m/z*** 638 ([M+Na]⁺).

Allyl (4-carbamoylthiazol-2-yl)methylcarbamate (2.163)
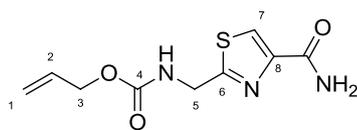
2.163

Carboxylic acid **2.156** (200 mg, 0.83 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 and HOBt (335 mg, 2.48 mmol, 3 equiv), EDCI.HCl (475 mg, 2.48 mmol, 3 equiv) and Hünig's base (575 µL, 3.30 mmol, 4 equiv) were added. After stirring for 10 min, NH_3 in dioxane (0.5 M, 16.5 mL, 8.26 mmol, 4 equiv) was added. After stirring at rt overnight, HCl solution (0.1 M) was added with cooling to adjust the reaction mixture to pH 4–5. The aqueous layer was extracted 3 times with CH_2Cl_2. The combined organic layers were dried and concentrated *in vacuo*. The residue was purified by flash chromatography using 5% MeOH in CH_2Cl_2 to afford **2.163** as a white solid (144 mg, 72%).

mp 128–130 °C; **IR** 3323, 3100, 2930, 1724, 1701, 1652, 1520, 1414, 1278, 1244, 1172, 979 cm^{-1}; **^{1}H NMR (300 MHz, CD$_3$OD/CDCl$_3$)** δ **ppm** 8.44 (s, 1H, H-7), 6.44 (br s, 1H, NH), 5.96–5.77 (m, 1H, H-2), 5.28 (dd, *J*=16.1, 1.1 Hz, 1H, H-1), 5.18 (dd, *J*=10.4, 0.4 Hz, 1H, H-1), 4.61–4.54 (m, 4H, H-3; H-5), 2.75 (br s, 2H, NH$_2$); **^{13}C NMR (75 MHz, CD$_3$OD/CDCl$_3$)** δ **ppm** 169.0 (C), 163.4 (C), 156.4 (C), 148.9 (C), 132.3 (CH, C-2), 124.8 (CH, C-7), 117.9 (CH$_2$, C-1), 66.0 (CH$_2$, C-3), 42.3 (CH$_2$, C-5); **ES$^+$ MS *m/z*** 264 ([M+Na]$^+$).

Allyl (4-cyanothiazol-2-yl)methylcarbamate (2.164)
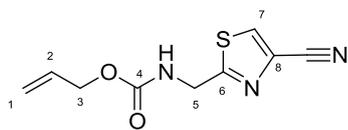
2.164

Amide **2.163** (370 mg, 1.53 mmol, 1 equiv) was dissolved in CH_2Cl_2 (8 mL). Triethylamine (291 µL, 2.08 mmol, 2.1 equiv) was added to the reaction mixture at 0 °C following by the addition of TFAA (145 µL, 1.04 mmol, 1.05 equiv). The reaction mixture was stirred at rt overnight, the solvent removed and the crude mixture purified by flash chromatography using EtOAc/hexane (3:7) to afford **2.164** as a yellow solid (318 mg, 93%).

mp 46–48 °C; **IR** 3327, 3107, 2945, 1712, 1527, 1247, 1153, 1123, 990, 941 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$)** δ **ppm** 7.97 (s, 1H, H-7), 6.02–5.84 (m, 1H, H-2), 5.59 (br s, 1H, NH), 5.33 (dt, J=16.4, 1.0 Hz, 1H, H-1), 5.25 (dq, J=10.4, 1.3 Hz, 1H, H-1), 4.69 (d, J=6.3 Hz, 2H, H-5), 4.63 (dt, J=5.7, 1.4 Hz, 2H, H-3); **^{13}C NMR (75 MHz, CDCl$_3$)** δ **ppm** 170.5 (C), 156.2 (C), 132.2 (CH, C-2 or C-7), 130.9 (CH, C-2 or C-7), 126.5 (C, C-8), 118.3 (CH$_2$, C-1), 113.7 (C, CN), 66.3 (CH$_2$, C-3), 42.5 (CH$_2$, C-5); **ES$^+$ MS m/z** 246 ([M+Na]$^+$).

(4*R*)-4-Methyl-2'-({[(prop-2-en-1-yloxy)carbonyl]aminomethyl)-4,5-dihydro-2,4'-bi-1,3-thiazole-4-carboxylic acid (2.165)
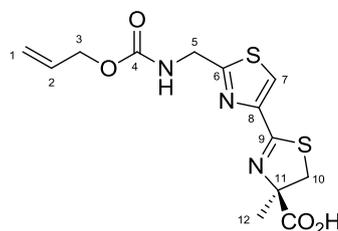
2.165

To a solution of nitrile **2.164** (142 mg, 0.63 mmol, 1 equiv) and (*R*)-methylcysteine hydrochloride (207 mg, 1.21 mmol, 1.9 equiv) in MeOH (7.9 mL), were added solid NaHCO$_3$ (266 mg, 3.17 mmol, 5 equiv) and phosphate buffer solution pH = 6 (0.1 M, 4 mL). The reaction mixture was heated 2 h at 70 °C. To the cold reaction mixture were added water (10 mL) and saturated NaHCO$_3$ solution (5 mL). The mixture was washed with EtOAc (2 x 10 mL). The aqueous layer was acidified (pH = 1–2) with KHSO$_4$ solution and extracted 3 times with EtOAc. The combined organic layers were dried and concentrated *in vacuo* to afford **2.165** as a white solid (208 mg, 96%).

mp 48–50 °C; **IR** 3312, 3058, 2930, 1724, 1523, 1417, 1274, 896 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$)** δ **ppm** 7.98 (s, 1H, H-7), 6.00–5.87 (m, 1H, H-2), 5.84 (br s, 1H, NH), 5.32 (d, J=17.3 Hz, 1H, H-1), 5.23 (dt, J=10.4, 0.7 Hz, 1H, H-1), 4.68 (d, J=6.3 Hz, 2H, H-3 or H-5), 4.62 (d, J=5.9 Hz, 2H, H-3 or H-5), 3.90 (d, J=11.5 Hz, 1H, H-10), 3.33 (d, J=11.5 Hz, 1H, H-10), 1.68 (s, 3H, Me); **^{13}C NMR (75 MHz, CDCl$_3$)** δ **ppm** 174.5 (C, CO$_2$H), 168.3 (C), 163.5 (C), 155.3 (C), 146.8 (C), 131.4 (CH, C-2), 122.1 (CH, C-7), 117.2 (CH$_2$, C-1), 83.2 (C, C-11), 65.2 (CH$_2$, C-3), 41.5 (CH$_2$, C-5 or C-10), 40.2 (CH$_2$, C-5 or C-10), 23.2 (CH$_3$, Me); **ES$^+$ MS m/z** 364 ([M+Na]$^+$).

(3*S*,4*E*)-2-(Trimethylsilyl)ethyl-3-[(*S*)-2-((*R*)-2-[2-[(Allyloxycarbonyl)methyl]-thiazol-4-yl]-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyl-oxy]-7-(tritylthio) hept-4-enoate (2.166)

2.166
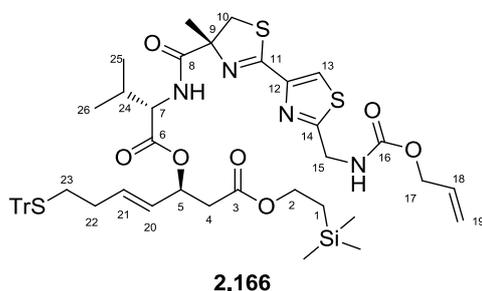
Compound **2.86** was dissolved in MeCN and diethylamine was added to the reaction mixture. After stirring for 2 h, the reaction mixture was concentrated *in vacuo*. The free amine was dried under high vacuum.

To a solution of **2.165** (113 mg, 0.33 mmol, 1.1 equiv) in MeCN (5 mL) cooled at 0 °C, PyBOP (212 mg, 0.36 mmol, 1.1 equiv) and Hünig's base (157 µL, 0.90 mmol, 3 equiv) were added. After 15 min, the previously deprotected amine (185 mg, 0.30 mmol, 1 equiv) in solution in CH_2Cl_2 (5 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane, gradient 9:1 to 5:5) to afford **2.166** as a colourless oil (220 mg, 78%).

$[\alpha]_D^{26}$ –30.1 (*c* 0.42, CHCl₃); **IR** 3383, 2956, 2926, 1731, 1675, 1512, 1247, 1176, 979, 869, 835 cm⁻¹; **¹H NMR (300 MHz, CDCl₃) δ ppm** 7.92 (d, *J*=1.1 Hz, 1H, H-13), 7.44–7.34 (m, 6H, H$_{Ar}$), 7.32–7.13 (m, 9H, H$_{Ar}$), 6.00–5.86 (m, 1H, H-2), 5.77–5.58 (m, 2H, H-5; H-21), 5.47 (br s, 1H, NH), 5.42–5.33 (m, 2H, H-1), 5.25 (dq, *J*=10.3, 1.2 Hz, 1H, H-20), 4.67 (dd, *J*=19.4, 5.9 Hz, 4H, H-15; H-17), 4.50 (dd, *J*=9.0, 4.6 Hz, 1H, H-7), 4.23–4.11 (m, 2H, H-2), 3.79 (dd, *J*=11.5, 1.1 Hz, 1H, H-10), 3.33 (dd, *J*=11.5, 1.1 Hz, 1H, H-10), 2.70 (dd, *J*=15.7, 7.7 Hz, 1H, H-4), 2.56 (dd, *J*=15.7, 5.5 Hz, 1H, H-4), 2.24–1.99 (m, 5H, H-22; H-23; H-24), 1.59 (s, 3H, Me), 1.03–0.92 (m, 2H, H-1), 0.83 (dt, *J*=6.9, 0.5 Hz, 3H, H-25 or H-26), 0.76 (d, *J*=6.9 Hz, 3H, H-25 or H-26), 0.04 (s, 9H, Si$\underline{Me}_3$); **¹³C NMR (75 MHz, CDCl₃) δ ppm** 174.4 (C), 170.5 (C), 170.3 (C), 169.6 (C), 163.0 (C), 156.1 (C), 148.8 (C), 144.8 (3C, C$_{Ar}$), 133.9 (CH, C-18 or C-21), 132.4 (CH, C-18 or C-21), 129.5 (6CH, C$_{Ar}$), 127.8 (CH, C-20), 127.8 (6CH, C$_{Ar}$), 126.6 (3CH, C$_{Ar}$), 121.4 (CH, C-13), 118.1 (CH₂, C-19), 85.2 (C, C-9), 71.8 (CH, C-5), 66.6 (C, $\underline{C}$Ph₃), 66.2 (CH₂), 63.1 (CH₂), 56.8 (CH, C-7), 42.6 (CH₂), 41.5 (CH₂), 39.7 (CH₂),

31.3 (CH$_2$), 31.2 (CH, C-24), 31.1 (CH$_2$), 24.7 (CH$_3$, Me), 19.0 (CH$_3$, C-25 or C-26), 17.5 (CH$_3$, C-25 or C-26), 17.3 (CH$_2$, C-1), -1.5 (3CH$_3$, SiMe$_3$); **ES$^+$ MS *m/z*** 964 ([M+Na]$^+$).

N-Boc-glycinamide (2.171)
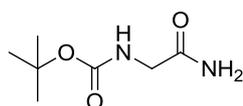
2.171

To a solution of di-*tert* butyl dicarbonate (21.7 g, 100 mmol, 1 equiv) in CH$_2$Cl$_2$ (500 mL) were added glycinamide hydrochloride (11 g, 100 mmol, 1 equiv) and triethylamine (29 mL, 207 mmol, 2.1 equiv). The reaction mixture was refluxed for 3 h. Triethylamine salts were extracted twice with water (100 mL) and the organic layer, dried over MgSO$_4$, was evaporated to provide **2.171** as a white solid (8.15 g, 47%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 6.16 (br s, 1H, NH$_2$), 5.73 (br s, 1H, NH$_2$), 5.24 (br s, 1H, NH), 3.82 (s, 2H, CH$_2$), 1.46 (s, 9H, CMe$_3$); **ES$^+$ MS *m/z*** 197 ([M+Na]$^+$).

The spectroscopic data are consistent with that[204] reported in the literature.

N-Boc-glycine thioamide (2.149)
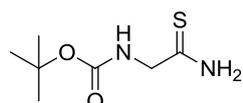
2.149

To a solution of *N*-Boc-glycinamide (2.5 g, 14.3 mmol, 1 equiv) in anhydrous THF (20 mL) was added Lawesson's reagent (3.5 g, 8.6 mmol, 1 equiv) under argon. The reaction mixture was stirred at rt overnight. The solvent was evaporated and the residue purified by flash chromatography using EtOAc/CH$_2$Cl$_2$ (1:9) to give **2.149** as a white solid (1.4 g, 51%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.89 (br s, 1H, NH$_2$), 7.63 (br s, 1H, NH$_2$), 5.32 (br s, 1H, NH), 4.16 (d, *J*=4.9 Hz, 2H, CH$_2$), 1.47 (s, 9H, CMe$_3$); **ES$^+$ MS *m/z*** 213 ([M+Na]$^+$).

The spectroscopic data are consistent with that[204] reported in the literature.

Methyl 2-Boc-aminomethyl-thiazole-4-carboxylate (2.151)

2.151
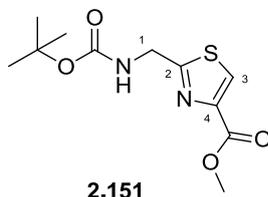
To a solution of **2.149** (666 mg, 3.5 mmol, 1 equiv) in ethanol (10 mL), methyl bromopyruvate (410 μL, 3.9 mmol, 1.1 equiv) and calcium carbonate (189 mg, 1.9 mmol, 0.5 equiv) were added. The reaction mixture was stirred under argon at rt overnight. The reaction mixture was evaporated under reduced pressure. The residue was partitioned between CHCl$_3$ and saturated NaHCO$_3$ solution. The organic layer was then washed once with water. The combined organic layers were dried over MgSO$_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 7:3) to afford **2.151** as a yellow solid (585 mg, 56%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.14 (s, 1H, H-3), 5.31 (br s, 1H, NH), 4.65 (d, *J*=6.3 Hz, 2H, H-1), 3.95 (s, 3H, Me), 1.47 (s, 9H, C<u>Me$_3$</u>); **ES$^+$ MS *m/z*** 295 ([M+Na]$^+$).

The spectroscopic data are consistent with that[205] reported in the literature.

2-Boc-aminomethyl-thiazole-4-carboxylic acid (2.147)

2.147
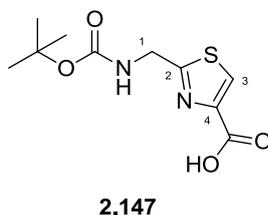
To a solution of **2.151** (575 mg, 2.11 mmol, 1 equiv) in 20 mL of THF/water (3:1) was added LiOH (152 mg, 6.33 mmol, 3 equiv) at 0 °C. The reaction mixture was left at rt overnight. The solution was diluted with water (6 mL) and acidified to pH 1–2 with saturated KHSO$_4$ solution. The aqueous layer was extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide **2.147** as a yellow solid (545 mg, 100%).

^{1}**H NMR (300 MHz, CD$_3$OD) δ ppm** 8.17 (s, 1H, H-3), 4.63 (s, 2H, H-1), 1.46 (s, 9H, CMe$_3$); **ES$^+$ MS** *m/z* 281 ([M+Na]$^+$).

The spectroscopic data are consistent with that[204] reported in the literature.

Boc-2-aminomethylthiazole-4-carboxamide (2.176)

2.176
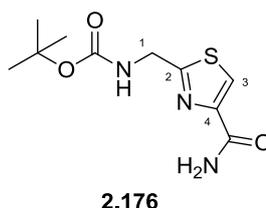
Carboxylic acid **2.147** (705 mg, 2.6 mmol, 1 equiv) was dissolved in anhydrous CH$_2$Cl$_2$ (10 mL) and THF (4 mL). HOBt (1.05 g, 7.8 mmol, 3 equiv), EDCI.HCl (1.5 g, 7.8 mmol, 3 equiv) and Hünig's base (1.8 mL, 10.4 mmol, 4 equiv) were added. After stirring for 10 min, NH$_3$ in dioxane (0.5 M, 52 mL, 26.0 mmol, 10 equiv) was then added. The reaction mixture was stirred overnight and HCl (0.1 M) was added with cooling to adjust the pH to 4–5. The aqueous layer was extracted 3 times with CH$_2$Cl$_2$. The combined organic layers were dried and concentrated *in vacuo*. The residue was purified by flash chromatography using 5% MeOH in CH$_2$Cl$_2$ to afford **2.176** as a white solid (583 mg, 88%).

^{1}**H NMR (300 MHz, CD$_3$OD/CDCl$_3$) δ ppm** 8.10 (s, 1H, H-3), 7.11 (br s, 1H, NH), 5.69 (br s, 1H, NH), 5.24 (br s, 1H, NH), 4.59 (s, 2H, H-1), 1.47 (s, 9H, CMe$_3$).

The spectroscopic data are consistent with that[102] reported in the literature.

2-(*N*-Boc-aminomethyl)thiazole-4-carbonitrile (2.100)

2.100
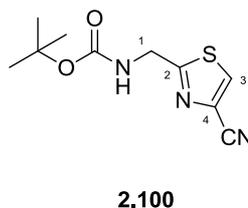
Amide **2.176** (583 mg, 2.42 mmol, 1 equiv) was dissolved in anhydrous CH$_2$Cl$_2$ (15 mL). Triethylamine (707 µL, 5.07 mmol, 2.1 equiv) was added to the reaction mixture

at 0 °C followed by TFAA (353 μL, 2.54 mmol, 1.05 equiv). The reaction mixture was stirred overnight, the solvent removed and the crude product purified by flash chromatography using EtOAc/hexane (3:7) to afford **2.100** as a yellow solid (478 mg, 83%).

¹H NMR (300 MHz, CDCl₃) δ ppm 7.95 (s, 1H, H-3), 5.31 (br s, 1H, NH), 4.62 (d, *J*=6.0 Hz, 2H, H-1), 1.48 (s, 9H, C<u>Me₃</u>).

The spectroscopic data are consistent with that[102] reported in the literature.

Methyl 2-*tert*-butyl-1,3-thiazolidine-4-carboxylate (2.173a) and (2.173b)
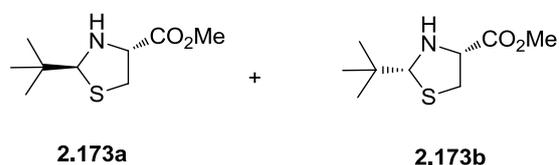
A mixture of (*R*)-cysteine methyl ester hydrochloride (8.6 g, 50 mmol, 1 equiv), pivaldehyde (10.9 mL, 100 mmol, 2 equiv) and triethylamine (7.7 mL, 55 mmol, 1.1 equiv) in pentane (80 mL) was refluxed for 9 h with continual removal of water using a Dean-Stark trap. The mixture was cooled and the resulting suspension was filtered. The residue was washed with ether and the filtrate was concentrated *in vacuo* to give **2.173a-b** as a mixture of (2*R*, 4*R*) and (2*S*, 4*R*) diastereomers 1.9:1 (10.74 g, 100%).

¹H NMR (300 MHz, CDCl₃) δ ppm Major isomer 4.46 (s, 1H, C<u>H</u>CMe₃), 3.81 (dd, *J*=9.7, 6.6 Hz, 1H, C<u>H</u>CO₂Me), 3.78 (s, 3H, CO₂<u>Me</u>), 3.26 (dd, *J*=10.2, 6.8 Hz, 1H, C<u>H</u>₂), 2.68 (t, *J*=9.9 Hz, 1H, C<u>H</u>₂), 1.07 (s, 9H, CMe₃); minor isomer 4.53 (s, 1H, C<u>H</u>CMe₃), 4.14 (t, *J*=6.0 Hz, 1H, C<u>H</u>CO₂Me), 3.76 (s, 3H, CO₂<u>Me</u>), 3.15–2.97 (m, 2H, CH₂), 0.98 (s, 9H, CMe₃).

The spectroscopic data are consistent with that[124] reported in the literature.

(2*R*,4*R*)-Methyl 2-*tert*-butyl-1,3-thiazolidine-3-formyl-4-carboxylate (2.174)
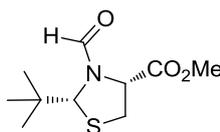
2.174

Acetic anhydride (15 mL, 162.9 mmol, 3.1 equiv) was added dropwise to a solution of formic acid (50 mL), diastereomers **2.173** (10.74 g, 52.8 mmol, 1 equiv) and sodium formate (4.02 g, 59.1 mmol, 1.1 equiv) at 0-5 °C. The solution was stirred at rt overnight. The solvents were removed and the residue was cautiously neutralised with NaHCO$_3$ solution and extracted with ether. The combined ether extracts were dried and evaporated *in vacuo* to afford a white solid which after recrystallisation from petroleum ether gave the thiazolidine **2.174** as white crystals (6.7:1 mixture of conformers) (9.7 g, 79%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm Major conformer 8.36 (s, 1H, CHO), 4.90 (t, *J*=8.7 Hz, 1H, C<u>H</u>CO$_2$Me), 4.75 (s, 1H, C<u>H</u>CMe$_3$), 3.78 (s, 3H, CO$_2$<u>Me</u>), 3.35–3.26 (m, 2H, CH$_2$), 1.04 (s, 9H, C<u>Me</u>$_3$).

The spectroscopic data are consistent with that[125] reported in the literature.

(2*R*,4*R*)-Methyl 2-*tert*-butyl-1,3-thiazolidine-3-formyl-4-methyl-4-carboxylate (2.175)
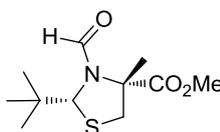
2.175

A solution of BuLi in hexanes (1.6 M, 12.2 mL, 19.5 mmol, 1.05 equiv) was added dropwise to a stirred solution of diisopropylamine (3.9 mL, 27.8 mmol, 1.5 equiv) in dry THF (86 mL) at –90 °C under argon. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) (13 mL, 107.5 mmol, 5.8 equiv) was added in one portion, and the mixture was then stirred at –90 °C for 1 h. A solution of **2.174** (4.3 g, 18.6 mmol, 1 equiv) in dry THF (5 mL) was added over 15 min at –95 °C. The resulting solution was stirred for 45 min at –90 °C and iodomethane (1.4 mL, 22.3 mmol, 1.2 equiv) was then slowly added over 5 min. The reaction mixture was stirred for 2 h at –90 °C and then

warmed to rt. Solvents were removed resulting an oily residue. Brine (200 mL) and ether (300 mL) were added to the oil and then the layers were separated. The separated aqueous layer was extracted with ether (3 x 300 mL), and the combined organic layers were dried and evaporated *in vacuo* to leave an oil. The resulting oil was purified by flash chromatography using 5-10% EtOAc in light petroleum ether as eluent to afford **2.175** as a mixture of conformers 2.3:1 (2.3 g, 51%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm Major conformer 8.29 (s, 1H, CHO), 4.67 (s, 1H, CH), 3.78 (s, 3H, OMe), 3.33 (d, J=11.5 Hz, 1H, CH$_2$), 2.73 (d, J=11.6 Hz, 1H, CH$_2$), 1.77 (s, 3H, Me), 1.08 (s, 9H, CMe$_3$); minor conformer 8.42 (s, 1H, CHO), 5.31 (s, 1H, CH), 3.83 (s, 3H, OMe), 3.65 (d, J=12.2 Hz, 1H, CH$_2$), 2.87 (d, J=12.3 Hz, 1H, CH$_2$), 1.79 (s, 3H, Me), 0.97 (s, 9H, CMe$_3$).

The spectroscopic data are consistent with that[125] reported in the literature.

(*R*)-2-Methyl cysteine hydrochloride (2.157)
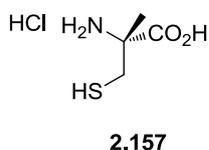
2.157

Hydrochlorydric acid (5 M, 35.5 mL) was added to **2.175** (2.3 g, 9.4 mmol) and the solution was then refluxed under argon for 3 days. The solution was washed with EtOAc (3 x 20 mL) and then the aqueous layer was concentrated *in vacuo* to afford **2.157** as a yellow oil (1.3 g, 81%).

^{1}H NMR (300 MHz, D$_2$O) δ ppm 3.16 (d, J=15.0 Hz, 1H, CH$_2$), 2.87 (d, J=15.1 Hz, 1H, CH$_2$), 1.58 (s, 3H, Me).

The spectroscopic data are consistent with that[125] reported in the literature.

(*R*)-2-(2-((*tert*-Butoxycarbonylamino)methyl)thiazol-4-yl)-4-methyl-4,5-dihydro-thiazole-4-carboxylic acid (2.109)
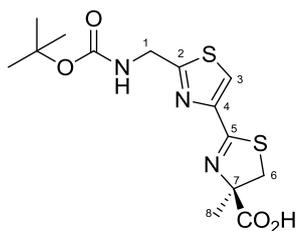
2.109

To a solution of nitrile **2.100** (445 mg, 1.9 mmol, 1 equiv) and (*R*)-methylcysteine hydrochloride (**2.157**) (606 mg, 3.5 mmol, 1.9 equiv) in MeOH (25 mL), were added solid NaHCO$_3$ (781 mg, 9.3 mmol, 5 equiv) and phosphate buffer solution pH = 6 (0.1 M, 12.7 mL). The reaction mixture was heated for 2 h at 70 °C. To the cold reaction mixture were added water (10 mL) and saturated NaHCO$_3$ solution (5 mL). The mixture was washed twice with EtOAc (10 mL). The aqueous layer was acidified (pH = 1–2) with KHSO$_4$ solution and extracted 3 times with EtOAc. The combined organic layers were dried and concentrated *in vacuo* to afford **2.109** as a white solid (663 mg, 99%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 8.01 (s, 1H, H-3), 5.33 (br s, 1H, NH), 4.67–4.59 (m, 2H, H-1), 3.89 (d, *J*=11.7 Hz, 1H, H-6), 3.38 (d, *J*=11.7 Hz, 1H, H-6), 1.69 (s, 3H, H-8), 1.48 (s, 9H, CMe$_3$); **ES$^+$ MS *m/z*** 373 ([M+NH$_4$]$^+$).

The spectroscopic data are consistent with that[102] reported in the literature.

(3*S*,4*E*)-2-(Trimethylsilyl)ethyl-3-[(*S*)-2-((*R*)-2-[2-[(*tert*-butoxycarbonyl)methyl]-thiazol-4-yl]-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyl-oxy]-7-(tritylthio) hept-4-enoate (2.177)
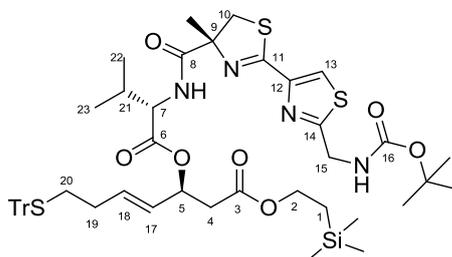
2.177

Amine **2.86** was prepared by deprotection of the Fmoc derivative: the Fmoc carbamate (422 mg, 0.50 mmol, 1 equiv) was dissolved in anhydrous acetonitrile (4 mL) and diethylamine (260 μl, 2.51 mmol, 5 equiv) was added. After stirring for 2 h, the reaction

mixture was concentrated *in vacuo* and the free amine purified by flash chromatography (EtOAc/hexane 1:1).

To a solution of thiazole-thiazoline compound **2.109** (191 mg, 0.54 mmol, 1.1 equiv) in anhydrous CH_2Cl_2 (10 mL) cooled at 0 °C, were added PyBOP (345 mg, 0.58 mmol, 1.1 equiv) and Hünig's base (254 µL, 1.46 mmol, 3 equiv). After 10 min, amine previously deprotected (300 mg, 0.49 mmol, 1 equiv) in solution in CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 3:7) to afford **2.177** as a white solid (351 mg, 77%).

^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.95 (s, 1H, H-13), 7.44–7.33 (m, 6H, H$_{Ar}$), 7.33–7.12 (m, 9H, H$_{Ar}$), 5.74–5.55 (m, 2H, H-5; H-18), 5.38 (dd, J=15.6, 7.5 Hz, 1H, H-17), 5.25 (br s, 1H, NH), 4.63 (d, J=6.0 Hz, 2H, H-15), 4.48 (dd, J=9.0, 5.0 Hz, 1H, H-7), 4.25–4.06 (m, 2H, H-2), 3.80 (d, J=11.5 Hz, 1H, H-10), 3.33 (d, J=11.5 Hz, 1H, H-10), 2.69 (dd, J=15.8, 7.8 Hz, 1H, H-4), 2.56 (dd, J=15.6, 5.5, Hz, 1H, H-4), 2.24–1.96 (m, 5H, H-19; H-20; H-21), 1.59 (s, 3H, Me), 1.48 (s, 9H, CMe$_3$), 1.01–0.93 (m, 2H, H-1), 0.83 (d, J=7.0 Hz, 3H, H-22 or H-23), 0.76 (d, J=7.0 Hz, 3H, H-22 or H-23), 0.04 (s, 9H, SiMe$_3$); **ES$^+$ MS *m/z*** 979 ([M+Na]$^+$).

The spectroscopic data are consistent with that[96] reported in the literature.

S-Trityl macrocycle 2.168
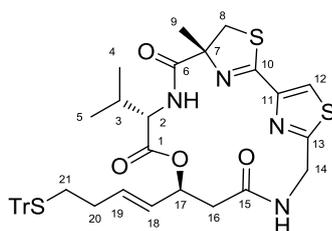
2.168

To a solution of the acyclic precursor **2.177** (338 mg, 0.35 mmol) in CH_2Cl_2 (17 mL) was added TFA (3.4 mL) at 0 ºC. The reaction mixture was warmed to rt and stirred overnight. The reaction mixture was concentrated *in vacuo* and then coevaporated with toluene to remove residual TFA. The crude amino acid (267 mg, 0.35 mmol, 1 equiv) was then dissolved in CH_2Cl_2 (20 mL) and added dropwise to a vigorously stirred solution of Hünig's base (369 µL, 2.12 mmol, 6 equiv), HOBt (95 mg, 0.71 mmol, 2

equiv) and HATU (269 mg, 0.71 mmol, 2 equiv) in anhydrous acetonitrile (250 mL). The reaction mixture was stirred overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 10:1 then 100% EtOAc) to afford **2.168** as a pale yellow solid (170 mg, 65%).

^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD) δ ppm 7.80 (s, 1H, H-12), 7.51–7.14 (m, 15H, H$_{Ar}$), 7.11 (d, *J*=9.5 Hz, 1H, NH), 5.69 (dt, *J*=15.4, 6.6 Hz, 1H, H-19), 5.58 (t, *J*=7.5 Hz, 1H, H-17), 5.39 (dd, *J*=15.6, 7.0 Hz, 1H, H-18), 5.19 (d, *J*=17.6 Hz, 1H, H-14), 4.55 (dd, *J*=9.3, 3.3 Hz, 1H, H-2), 4.17 (d, *J*=17.6 Hz, 1H, H-14), 4.00 (d, *J*=11.5 Hz, 1H, H-8), 3.30 (d, *J*=11.5 Hz, 1H, H-8), 2.90 (dd, *J*=16.3, 10.8 Hz, 1H, H-16), 2.59 (dd, *J*=16.1, 2.5 Hz, 1H, H-16), 2.26–2.14 (m, 2H, H-21), 2.14–1.96 (m, 3H, H-3; H-20), 1.84 (s, 3H, H-9), 0.67 (d *J*=7.0 Hz, 3H, H-4 or H-5), 0.45 (d, *J*=6.5 Hz, 3H, H-4 or H-5); **ES$^+$ MS *m/z*** 761 ([M+Na]$^+$).

The spectroscopic data are consistent with that[96] reported in the literature.

Largazole thiol (2.178)
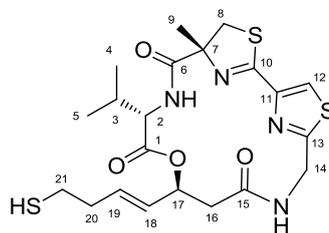
2.178

Macrocycle **2.168** (77.2 mg, 0.10 mmol, 1 equiv) was dissolved in anhydrous CH$_2$Cl$_2$ (13 mL) and cooled to 0 ℃. The mixture was treated with Et$_3$SiH (33 µL, 0.21 mmol, 2 equiv) followed by TFA (515 µL, 6.93 mmol, 69 equiv). The reaction mixture was warmed to rt and stirred for 2 h, concentrated *in vacuo* and purified by flash chromatography with EtOAc as eluent to provide largazole thiol (**2.178**) as a white solid (41.2 mg, 79%).

^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.77 (s, 1H, H-12), 7.19 (d, *J*=9.5 Hz, 1H, NH), 6.50 (d, *J*=7.0 Hz, 1H, NH), 5.92–5.77 (m, 1H, H-19), 5.69 (t, *J*=7.0 Hz, 1H, H-17), 5.55 (dd, *J*=15.6, 7.0 Hz, 1H, H-18), 5.28 (dd, *J*=17.6, 9.5 Hz, 1H, H-14), 4.61 (dd, *J*=9.5, 3.5 Hz, 1H, H-2), 4.28 (dd, *J*=17.6, 3.0 Hz, 1H, H-14), 4.05 (d, *J*=11.0 Hz, 1H,

H-8), 3.29 (d, *J*=11.0 Hz, 1H, H-8), 2.87 (dd, *J*=16.6, 10.0 Hz, 1H, H-16), 2.71 (dd, *J*=16.3, 2.8 Hz, 1H, H-16), 2.57 (q, *J*=7.4 Hz, 2H, H-21), 2.45–2.29 (m, 2H, H-20), 2.19–2.02 (m, 1H, H-3), 1.87 (s, 3H, H-9), 1.43 (t, *J*=7.8 Hz, 1H, SH), 0.70 (d, *J*=6.5 Hz, 3H, H-4 or H-5), 0.54 (d, *J*=6.5 Hz, 3H, H-4 or H-5); **ES⁺ MS *m/z*** 519 ([M+Na]⁺).

The spectroscopic data are consistent with that[96] reported in the literature.

Largazole (2.18)
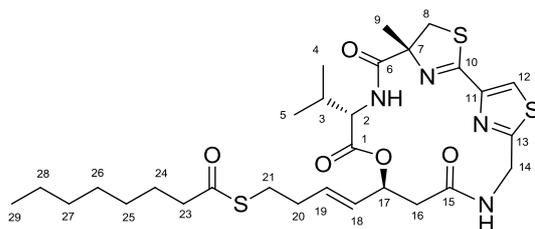
2.18

To largazole thiol (20.6 mg, 42 μmol, 1 equiv) in CH_2Cl_2 (4 mL), was added Et_3N (12 μL, 83 μmol, 2 equiv) followed by octanoyl chloride (35.5 μL, 207 μmol, 5 equiv) at 0 ℃. The reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was then quenched with CH_3OH (5 mL) at 0 ℃, concentrated *in vacuo* and purified by flash chromatography using EtOAc as eluent to provide largazole (**2.18**) as a clear oil (16 mg, 62%).

¹H NMR (400 MHz, CDCl₃) δ ppm 7.77 (s, 1H, H-12), 7.17 (d, *J*=9.0 Hz, 1H, NH), 6.44 (dd, *J*=9.3, 2.8 Hz, 1H, NH), 5.90–5.77 (m, 1H, H-19), 5.72–5.64 (m, 1H, H-17), 5.52 (dd, *J*=15.6, 7.0 Hz, 1H, H-18), 5.29 (dd, *J*=17.8, 9.3 Hz, 1H, H-14), 4.61 (dd, *J*=9.3, 3.3 Hz, 1H, H-2), 4.28 (dd, *J*=17.6, 3.0 Hz, 1H, H-14), 4.05 (d, *J*=11.5 Hz, 1H, H-8), 3.28 (d, *J*=11.5 Hz, 1H, H-8), 2.91 (t, *J*=7.5 Hz, 2H, H-21), 2.84 (dd, *J*=16.6, 10.5 Hz, 1 H, H-16), 2.69 (dd, *J*=16.1, 2.5 Hz, 1H, H-16), 2.54 (t, *J*=7.5 Hz, 2H, H-23), 2.32 (q, *J*=6.5 Hz, 2H, H-20), 2.11 (td, *J*=6.8, 3.5 Hz, 1H, H-3), 1.88 (s, 3H, H-9), 1.71–1.55 (m, 2H, H-24), 1.37–1.19 (m, 8H, H-25; H-26; H-27; H-28), 0.88 (t, *J*=6.8 Hz, 3H, H-29), 0.69 (d, *J*=7.0 Hz, 3H, H-4 or H-5), 0.53 (d, *J*=7.0 Hz, 3H, H-4 or H-5); **¹³C NMR (100 MHz, CDCl₃) δ ppm** 199.4 (C, C-22), 173.5 (C, C-6), 169.4 (C, C-15), 168.8 (C, C-1), 167.9 (C, C-13), 164.3 (C, C-10), 147.4 (C, C-11), 132.7 (CH, C-19), 128.4 (CH, C-18), 124.2 (CH, C-12), 84.4 (C, C-7), 72.0 (CH, C-17), 57.8 (CH, C-2), 44.1 (CH₂, C-23), 43.3 (CH₂, C-8), 41.1 (CH₂, C-14), 40.5 (CH₂, C-16), 34.2 (CH, C-3), 32.3 (CH₂,

C-20), 31.6 (CH$_2$, C-27), 29.0; 28.9 (2CH$_2$, C-25;C-26), 27.9 (CH$_2$, C-21), 25.6 (CH$_2$, C-24), 24.1 (CH$_2$, C-9), 22.6 (CH$_2$, C-28), 18.8 (CH$_3$, C-4 or C-5), 16.7 (CH$_3$, C-4 or C-5), 14.0 (CH$_3$, C-29); **ES$^+$ MS *m/z*** 645 ([M+Na]$^+$), 686 ([M+Na+CH$_3$CN]$^+$).

The spectroscopic data are consistent with that[80] reported in the literature.

Synthesis of largazole analogues

(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-(2-((((9*H*-fluoren-9-yl)methoxy)carbonylamino)-acetoxy)-7-(tritylthio)hept-4-enoate (2.184)
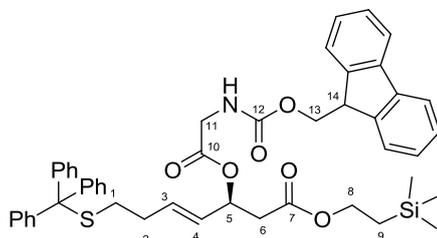
2.184

The protected β-hydroxy ester **2.85** (200 mg, 0.39 mmol, 1 equiv) and *N*-Fmoc-glycine (126 mg, 0.42 mmol, 1.1 equiv) were dissolved in anhydrous CH_2Cl_2 (7 mL). DCC (96 mg, 0.46 mmol, 1.2 equiv) and DMAP (4.7 mg, 0.04 mmol, 0.1 equiv) were added to the reaction mixture cooled to 0 °C. The reaction mixture was stirred overnight. The precipitate was filtered and solvents were evaporated. The crude was purified by flash chromatography (EtOAc/hexane 5:95) to give **2.184** as a colourless oil (180 mg, 59%).

$[\alpha]_D^{23}$ −9.9 (*c* 0.54, CHCl$_3$); **IR** 3368, 2964, 2918, 2843, 1735, 1520, 1448, 1247, 1172, 1051, 858, 839 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.78 (d, *J*=7.5 Hz, 2H, H$_{Ar}$), 7.60 (d, *J*=7.0 Hz, 2H, H$_{Ar}$), 7.46–7.15 (m, 19H, H$_{Ar}$), 5.76–5.57 (m, 2H, H-3; H-5), 5.39 (dd, *J*=15.6, 7.0 Hz, 1H, H-4), 5.24 (br s, 1H, NH), 4.39 (d, *J*=7.0 Hz, 2H, H-13), 4.24 (t, *J*=7.0 Hz, 1H, H-14), 4.16 (t, *J*=8.5 Hz, 2H, H-8), 3.96 (d, *J*=5.5 Hz, 2H, H-11), 2.67 (dd, *J*=15.8, 8.3 Hz, 1H, H-6), 2.55 (dd, *J*=15.8, 5.3 Hz, 1H, H-6), 2.20 (t, *J*=7.5 Hz, 2H, H-1), 2.08 (t, *J*=7.0 Hz, 2H, H-2), 0.97 (t, *J*=8.5 Hz, 2H, H-9), 0.04 (s, 9H, SiMe$_3$); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 169.7 (C, CO), 168.9 (C, CO), 156.1 (C, CO), 144.8 (3C, C$_{Ar}$), 143.8 (2C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 133.6 (CH, C-3), 129.6 (6CH, C$_{Ar}$), 127.9 (6CH, C$_{Ar}$), 127.7 (2CH, C$_{Ar}$), 127.6 (CH, C-4), 127.1 (2CH, C$_{Ar}$), 126.6 (3CH, C$_{Ar}$), 125.1 (2CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 71.9 (CH, C-5), 67.2 (CH$_2$, C-13), 66.6 (C, $\underline{C}$Ph$_3$), 63.1 (CH$_2$, C-8), 47.1 (CH, C-14), 42.8 (CH$_2$, C-11), 39.6 (CH$_2$, C-6), 31.3; 31.1 (2CH$_2$, C-1; C-2), 17.3 (CH$_2$, C-9), −1.5 (3CH$_3$, SiMe$_3$); **ES$^+$ MS *m/z*** 820 ([M+Na]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{48}$H$_{51}$NNaO$_6$SSi (M+Na)$^+$ 820.3099, found 820.3090.

(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-(2-((*R*)-2-(2-((*tert*-butoxycarbonylamino)methyl)-thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxamido)acetoxy)-7-(tritylthio)hept-4-enoate (2.186)

2.186

Carbamate **2.184** (170 mg, 0.21 mmol, 1 equiv) was deprotected by stirring for 2 h in anhydrous acetonitrile (5 mL) and diethylamine (110 μl, 1.07 mmol, 5 equiv) followed by concentration *in vacuo*. To a solution of **2.109** (84 mg, 0.24 mmol, 1.1 equiv) in anhydrous CH$_2$Cl$_2$ (5 mL) cooled at 0 °C, PyBOP (151 mg, 0.26 mmol, 1.2 equiv) and Hünig's base (112 μL, 0.64 mmol, 3 equiv) were added. After 10 min, the previous deprotected amine (123 mg, 0.21 mmol, 1 equiv) in solution in anhydrous CH$_2$Cl$_2$ (5 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 3:7) to afford **2.186** as a colourless oil (153 mg, 78%).

[α]$_D^{24}$ –40.3 (*c* 0.27, CHCl$_3$); **IR** 3342, 2971, 2952, 1727, 1671, 1599, 1512, 1444, 1365, 1244, 1168, 858, 835 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.91 (s, 1H, H-13), 7.47–7.34 (m, 6H, H$_{Ar}$), 7.34–7.12 (m, 9H, H$_{Ar}$), 5.73–5.54 (m, 2H, H-5; H-18), 5.36 (dd, *J*=15.6, 7.0 Hz, 1H, H-17), 5.26 (br s, 1H, NH), 4.64 (d, *J*=6.0 Hz, 2H, H-15), 4.22–4.09 (m, 2H, H-2), 4.02 (dd, *J*=7.5, 5.5 Hz, 2H, H-7), 3.76 (d, *J*=11.5 Hz, 1H, H-10), 3.34 (d, *J*=11.5 Hz, 1H, H-10), 2.66 (dd, *J*=15.8, 8.3 Hz, 1H, H-4), 2.53 (dd, *J*=15.8, 5.3 Hz, 1H, H-4), 2.18 (t, *J*=7.0 Hz, 2H, H-20), 2.12–1.96 (m, 2H, H-19), 1.60 (s, 3H, Me), 1.48 (s, 9H, CMe$_3$), 1.07–0.88 (m, 2H, H-1), 0.04 (s, 9H, SiMe$_3$); **ES$^+$ MS** *m/z* 937 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{47}$H$_{58}$N$_4$NaO$_7$S$_3$Si (M+Na)$^+$ 937.3129, found 937.3136.

(5*R*,11*S*)-5-Methyl-11-((*E*)-4-(tritylthio)but-1-en-1-yl)-10-oxa-3,17-dithia-7,14,19,20-tetraazatricyclo[14.2.1.12,5]icosa-1(18),2(20),16(19)-triene-6,9,13-trione (2.188)
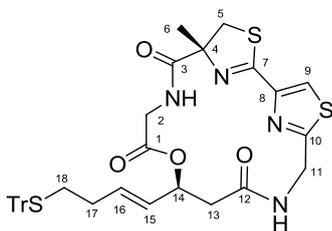

2.188

Acyclic precursor **2.186** (138 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (7 mL), cooled to 0 ºC and treated with TFA (1.5 mL). The reaction mixture was warmed to rt and stirred overnight. The reaction mixture was concentrated *in vacuo* and then coevaporated with toluene to remove residual TFA. The crude amino acid (108 mg, 0.15 mmol, 1 equiv) was taken up in anhydrous CH_2Cl_2 (13 mL) and added dropwise to a vigorously stirred solution of Hünig's base (158 μL, 0.90 mmol, 6 equiv), HOBt (41 mg, 0.30 mmol, 2 equiv) and HATU (115 mg, 0.30 mmol, 2 equiv) in anhydrous acetonitrile (110 mL). The reaction mixture was stirred overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 10:1 then 100% EtOAc) to afford **2.188** as a pale yellow solid (55.4 mg, 53%).

$[\alpha]_D^{24}$ –51.2 (*c* 0.22, CH_3OH); **mp** 136–138 ºC; **IR** 3628, 2923, 1734, 1670, 1541, 1507, 1262, 840 cm^{-1}; **^{1}H NMR (400 MHz, CD$_3$OD) δ ppm** 8.02 (s, 1H, H-9), 7.40–7.29 (m, 6H, H$_{Ar}$), 7.29–7.10 (m, 9H, H$_{Ar}$), 5.74–5.53 (m, 2H, H-14; H-16), 5.41 (dd, *J*=15.6, 7.0 Hz, 1H, H-15), 4.97 (d, *J*=17.6 Hz, 1H, H-11), 4.34 (d, *J*=17.6 Hz, 1H, H-11), 4.06 (d, *J*=18.1 Hz, 1H, H-2), 3.97 (d, *J*=11.5 Hz, 1H, H-5), 3.73 (d, *J*=18.1 Hz, 1H, H-2), 3.31 (d, *J*=11.5 Hz, 1H, H-5), 2.97 (dd, *J*=17.3, 11.3 Hz, 1H, H-13), 2.63 (dd, *J*=17.1, 2.0 Hz, 1H, H-13), 2.17 (t, *J*=6.0 Hz, 2H, H-18), 2.02 (q, *J*=6.5 Hz, 2H, H-17), 1.74 (s, 3H, H-6); **^{13}C NMR (100 MHz, CD$_3$OD) δ ppm** 176.2 (C, CO), 172.8 (C, CO), 169.7 (C, CO), 168.0 (C, C-10), 166.8 (C, C-7), 148.4 (C, C-8), 146.5 (3C, C$_{Ar}$), 134.4 (CH, C-16), 130.9 (6CH, C$_{Ar}$), 129.5 (CH, C-15), 129.0 (6CH, C$_{Ar}$), 127.9 (3CH, C$_{Ar}$), 126.7 (CH, C-9), 85.4 (C, C-4), 73.8 (CH, C-14), 67.9 (C, $\underline{C}$Ph$_3$), 44.6 (CH$_2$), 42.7 (CH$_2$), 42.3 (CH$_2$), 40.2 (CH$_2$), 32.5 (CH$_2$), 32.4 (CH$_2$), 25.5 (CH$_3$, C-6); **ES$^+$ MS *m/z*** 719 ([M+Na]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{37}$H$_{36}$N$_4$NaO$_4$S$_3$ (M+Na)$^+$ 719.1791, found 719.1795.

(5R,11S)-11-((E)-4-Mercaptobut-1-en-1-yl)-5-methyl-10-oxa-3,17-dithia-7,14,19,20-tetra-azatricyclo[14.2.1.1²,⁵]icosa-1(18),2(20),16(19)-triene-6,9,13-trione (2.179)
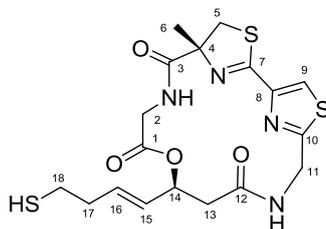
2.179

S-Trityl macrocycle **2.188** (45.3 mg, 65 µmol, 1 equiv) was dissolved in CH_2Cl_2 (8 mL) and cooled to 0 °C and successively treated with Et_3SiH (21 µL, 130 µmol, 2 equiv) and TFA (320 µL, 6.93 mmol, 64 equiv). The reaction mixture was warmed to rt and stirred for 2 h, before being concentrated and purified by flash chromatography with EtOAc as eluent to provide **2.179** as a white solid (6 mg, 20%).

$[\alpha]_D^{24}$ +9.7 (*c* 0.24, CH_3OH); **mp** 102–104 °C; **IR** 3380, 2964, 2930, 2843, 1750, 1678, 1591, 1512, 1262, 1187, 1043 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.74 (s, 1H, H-9), 7.09 (br s, 1H, NH), 6.41 (br s, 1H, NH), 5.94–5.71 (m, 2H, H-14; H-16), 5.52 (dd, *J*=15.5, 7.3 Hz, 1H, H-15), 5.23 (dd, *J*=17.4, 8.9 Hz, 1H, H-11), 4.27 (dd, *J*=17.3, 4.0 Hz, 1H, H-11), 4.21–4.07 (m, 2H, H-2; H-5), 3.88 (dd, *J*=18.8, 3.1 Hz, 1H, H-2), 3.25 (d, *J*=11.3 Hz, 1H, H-5), 2.92 (dd, *J*=16.7, 11.0 Hz, 1H, H-13), 2.69 (d, *J*=15.2 Hz, 1H, H-13), 2.58 (q, *J*=7.4 Hz, 2H, H-18), 2.37 (q, *J*=6.7 Hz, 2H, H-17), 1.84 (s, 3H, H-6), 1.39 (t, *J*=7.8 Hz, 1H, SH); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 173.8 (C, CO), 169.5 (C, CO), 167.7 (C, CO), 167.5 (C, C-10), 166.2 (C, C-7), 135.4 (C, C-8), 133.1 (CH, C-16), 128.8 (CH, C-15), 124.6 (CH, C-9), 84.3 (C, C-4), 72.5 (CH, C-14), 43.7 (CH$_2$), 42.2 (CH$_2$), 41.3 (CH$_2$), 40.2 (CH$_2$), 36.2 (CH$_2$), 25.3 (CH$_3$, C-6), 23.8 (CH$_2$); **ES$^+$ MS** *m/z* 477 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for $C_{18}H_{22}N_4NaO_4S_3$ (M+Na)$^+$ 477.0695, found 477.0702.

(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-(3-(((9H-fluoren-9-yl)methoxy) carbonylamino)-propa-noy-loxy)-7-(tritylthio)hept-4-enoate (2.185)
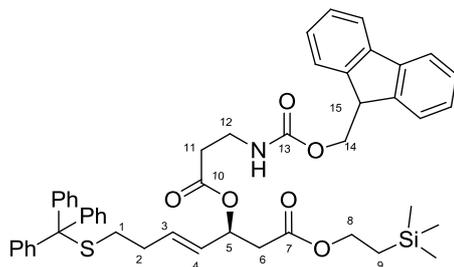
2.185

The protected β-hydroxy ester **2.85** (208 mg, 0.40 mmol, 1 equiv) and *N*-Fmoc-β-alanine (137 mg, 0.44 mmol, 1.1 equiv) were dissolved in anhydrous CH_2Cl_2 (8 mL), cooled to 0º C and DCC (99 mg, 0.48 mmol, 1.2 equiv) and DMAP (4.9 mg, 0.04 mmol, 0.1 equiv) were then added. The reaction mixture was stirred overnight. The precipitate was filtered and solvents were evaporated. The crude was purified by flash chromatography (EtOAc/hexane 5:95) to give **2.185** as a colourless oil (207 mg, 64%).

$[\alpha]_D^{23}$ –9.9 (*c* 0.56, CHCl$_3$); **IR** 3376, 3058, 2949, 1724, 1504, 1455, 1244, 1164, 1081, 1066, 1036, 990, 975, 862, 835 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.75 (d, *J*=7.5 Hz, 2H, H$_{Ar}$), 7.59 (d, *J*=7.0 Hz, 2H, H$_{Ar}$), 7.52–7.34 (m, 19H, H$_{Ar}$), 5.70–5.53 (m, 2H, H-3; H-5), 5.48 (br s, 1H, NH), 5.38 (dd, *J*=15.6, 7.0 Hz, 1H, H-4), 4.34 (d, *J*=7.0 Hz, 2H, H-14), 4.28–4.07 (m, 3H, H-8; H-15), 3.50–3.40 (m, 2H, H-12), 2.69–2.46 (m, 4H, H-6; H-11), 2.19 (t, *J*=7.0 Hz, 2H, H-1), 2.07 (t, *J*=6.5 Hz, 2H, H-2), 0.95 (t, *J*=8.5 Hz, 2H, H-9), 0.01 (s, 9H, SiMe$_3$); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 171.2 (C, CO), 170.1 (C, CO), 156.3 (C, CO), 144.8 (3C, C$_{Ar}$), 144.0 (2C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 133.1 (CH, C-3), 129.6 (6CH, C$_{Ar}$), 128.0 (CH, C-4), 127.9 (6CH, C$_{Ar}$), 127.7 (2CH, C$_{Ar}$), 127.0 (2CH, C$_{Ar}$), 126.6 (3CH, C$_{Ar}$), 125.1 (2CH, C$_{Ar}$), 119.9 (2CH, C$_{Ar}$), 70.9 (CH, C-5), 66.8 (CH$_2$, C-14), 66.6 (C, <u>C</u>Ph$_3$), 63.1 (CH$_2$, C-8), 47.2 (CH, C-15), 39.7 (CH$_2$, C-6), 36.7 (CH$_2$, C-12), 34.8 (CH$_2$, C-11), 31.3, 31.2 (2CH$_2$, C-1; C-2), 17.3 (CH$_2$, C-9), –1.5 (3CH$_3$, SiMe$_3$); **ES$^+$ MS *m/z*** 834 ([M+Na]$^+$). **HRMS (ESI) *m/z*** calcd. for C$_{49}$H$_{53}$NNaO$_6$SSi (M+Na)$^+$ 834.3255, found 834.3270.

(*S*,*E*)-2-(Trimethylsilyl)ethyl   3-(3-((*R*)-2-(2-((*tert*-butoxycarbonylamino)methyl)-thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxamido)propanoyloxy)-7-(tritylthio)hept-4-enoate (2.187)

2.187

Carbamate **2.185** (185 mg, 0.23 mmol, 1 equiv) was dissolved in anhydrous acetonitrile (5 mL) and diethylamine (118 μl, 1.14 mmol, 5 equiv) was added to the reaction mixture. After stirring for 2 h, the reaction mixture was concentrated *in vacuo*.

To a solution of **2.109** (90 mg, 0.25 mmol, 1.1 equiv) in anhydrous CH_2Cl_2 (5 mL) cooled at 0 °C, PyBOP (162 mg, 0.28 mmol, 1.2 equiv) and Hünig's base (120 μL, 0.69 mmol, 3 equiv) were added. After 15 min, the previous deprotected amine (135 mg, 0.23 mmol, 1 equiv) in anhydrous CH_2Cl_2 (5 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 3:7) to afford **2.187** as a colourless oil (175 mg, 82%).

$[\alpha]_D^{25}$ −39.8 (*c* 0.61, CHCl$_3$); **IR** 3361, 2967, 2941, 2926, 1724, 1659, 1520, 1440, 1368, 1259, 1168, 1032, 866, 835 cm^{-1}; **¹H NMR (300 MHz, CDCl₃)** *δ* **ppm** 7.97 (br s, 1H, H-14), 7.39 (d, *J*=7.5 Hz, 6H, H$_{Ar}$), 7.33–7.12 (m, 9H, H$_{Ar}$), 5.57 (dt, *J*=14.6, 7.5 Hz, 2H, H-5; H-19), 5.35 (dd, *J*=15.1, 7.0 Hz, 1H, H-18), 5.28 (br s, 1H, NH), 4.63 (d, *J*=5.5 Hz, 2H, H-16), 4.21–4.06 (m, 2H, H-2), 3.76 (d, *J*=11.5 Hz, 1H, H-11), 3.59–3.43 (m, 2H, H-8), 3.33 (d, *J*=11.5 Hz, 1H, H-11), 2.62 (dd, *J*=15.8, 7.8 Hz, 1H, H-4), 2.51 (dt, *J*=10.3, 5.5 Hz, 3H, H-4; H-7), 2.18 (t, *J*=7.5 Hz, 2H, H-21), 2.08–1.95 (m, 2H, H-20), 1.57 (s, 3H, Me), 1.48 (s, 9H, C*Me*₃), 1.04–0.87 (m, 2H, H-1), 0.03 (s, 9H, SiMe₃); **¹³C NMR (100 MHz, CDCl₃)** *δ* **ppm** 174.5 (C), 170.8 (C), 169.9 (2C), 165.5 (C), 154.3 (C), 144.8 (3C, C$_{Ar}$), 132.9 (CH, C-19), 129.6 (6CH, C$_{Ar}$), 128.1 (CH, C-18), 127.9 (6CH, C$_{Ar}$), 126.6 (3CH, C$_{Ar}$), 122.1 (CH, C-14), 85.0 (C, C-10), 70.8 (CH, C-5), 66.6 (C, <u>C</u>Ph₃), 63.0 (CH₂), 42.3 (CH₂), 41.3 (CH₂), 39.7 (CH₂), 34.9 (CH₂), 34.2 (CH₂), 31.3 (CH₂), 31.2 (CH₂), 28.3 (3CH₃, C*Me*₃), 24.8 (CH₃, Me), 17.3 (CH₂, C-1), −1.5

(3CH$_3$, SiMe$_3$); **ES$^+$ MS** *m/z* 951 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{48}$H$_{60}$N$_4$NaO$_7$S$_3$Si (M+Na)$^+$ 951.3286, found 951.3287.

(5R,12S)-5-Methyl-12-((E)-4-(tritylthio)but-1-en-1-yl)-11-oxa-3,18-dithia-7,15,20,21-tetraazatricyclo[15.2.1.1^{2,5}]henicosa-1(19),2(21),17(20)-triene-6,10,14-trione (2.189)
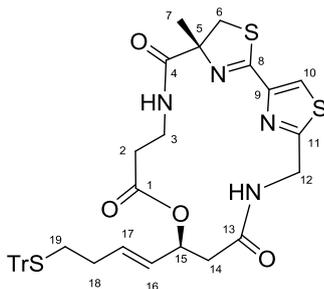
2.189

Acyclic precursor **2.187** (152 mg, 0.16 mmol) was dissolved in CH$_2$Cl$_2$ (7.6 mL), cooled to 0 ºC and treated with TFA (1.6 mL). The reaction mixture was warmed to rt, stirred overnight, concentrated *in vacuo* and then coevaporated with toluene to remove residual TFA. The crude amino acid (120 mg, 0.17 mmol, 1 equiv) was then taken up in anhydrous CH$_2$Cl$_2$ (14 mL) and added dropwise to a vigorously stirred solution of Hünig's base (172 μL, 0.99 mmol, 6 equiv), HOBt (45 mg, 0.33 mmol, 2 equiv) and HATU (125 mg, 0.33 mmol, 2 equiv) in anhydrous acetonitrile (120 mL). The reaction mixture was stirred overnight, concentrated *in vacuo* and the crude material was purified by flash chromatography (hexane/EtOAc 10:1 then 100% EtOAc) to afford **2.189** as a colourless oil (20 mg, 17%).

[α]$_D^{24}$ +37.5 (*c* 0.01, CH$_3$OH); **IR** 2960, 2926, 2850, 1739, 1671, 1463, 1266, 1085, 1028, 805 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.71 (s, 1H, H-10), 7.36–7.16 (m, 15H, H$_{Ar}$), 6.96 (br s, 1H, NH), 6.09 (dd, *J*=15.6, 7.5 Hz, 1H), 5.73–5.59 (m, 1H), 5.55 (td, *J*=7.2, 3.7 Hz, 1H), 4.99 (dd, *J*=16.8, 7.8 Hz, 1H, H-12), 4.03 (d, *J*=11.5 Hz, 1H, H-6), 3.94 (dd, *J*=17.1, 4.0 Hz, 1H, H-12), 3.49 (d, *J*=4.0 Hz, 2H, H-3), 3.33 (d, *J*=11.5 Hz, 1H, H-6), 2.78–2.61 (m, 2H, H-2 or H-14), 2.56–2.42 (m, 2H, H-2 or H-14), 2.39–2.22 (m, 2H, H-19), 2.18–1.95 (m, 2H, H-18), 1.68 (s, 3H, H-7);

^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 174.1 (C, CO), 171.5 (C, CO), 169.7 (C, CO), 167.5 (C, C-11), 162.0 (C, C-8), 148.0 (C, C-9), 144.7 (3C, C$_{Ar}$), 132.0 (CH, C-17),

129.9 (CH, C-16), 129.6 (6CH, C_{Ar}), 127.9 (6CH, C_{Ar}), 126.7 (3CH, C_{Ar}), 124.1 (CH, C-10), 84.6 (C, C-5), 73.0 (CH, C-15), 66.9 (C, $\underline{C}Ph_3$), 42.7 (CH_2), 42.3 (CH_2), 40.9 (CH_2), 35.4 (CH_2), 33.8 (CH_2), 31.4 (CH_2), 31.3 (CH_2), 25.5 (CH_3, C-7); **ES$^+$ MS** *m/z* 733 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for $C_{38}H_{38}N_4NaO_4S_3$ (M+Na)$^+$ 733.1947, found 733.1947.

(5*R*,12*S*)-12-((*E*)-4-Mercaptobut-1-en-1-yl)-5-methyl-11-oxa-3,18-dithia-7,15,20,21-tetra-azatricyclo[15.2.1.12,5]henicosa-1(19),2(21),17(20)-triene-6,10,14-trione (2.180)
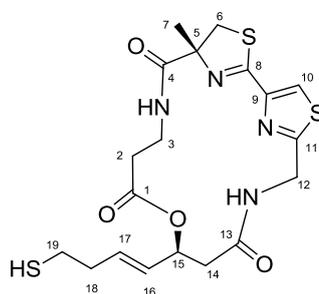
2.180

S-Trityl macrocycle **2.189** (16.1 mg, 23 μmol, 1 equiv) was dissolved in CH_2Cl_2 (5.5 mL) and cooled to 0 °C. The mixture was successively treated with Et_3SiH (10 μL, 130 μmol, 2.7 equiv) and TFA (320 μL, 6.93 mmol, 64 equiv). The reaction mixture was warmed to rt and stirred for 2 h, before being concentrated and purified by flash chromatography with EtOAc as eluent to provide **2.180** as a white solid (9 mg, 85%).

$[\alpha]_D^{24}$ +1.6 (*c* 0.36, CH_3OH); **mp** 80–82 °C; **IR** 3353, 2926, 2854, 1739, 1671, 1542, 1179, 1039 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 7.78 (br s, 1H, NH), 7.65 (s, 1H, H-10), 7.19 (br s, 1H, NH), 5.65 (t, *J*=5.0 Hz, 2H, H-16; H-17), 5.51–5.34 (m, 1H, H-15), 3.92 (d, *J*=11.5 Hz, 1H, H-6), 3.39 (q, *J*=5.0 Hz, 2H, H-3), 3.24 (d, *J*=11.5 Hz, 1H, H-6), 2.72 (dd, *J*=15.8, 6.8 Hz, 1H, H-14), 2.58–2.34 (m, 5H, H-2; H-14; H-19), 2.34–2.15 (m, 2H, H-18), 1.65 (s, 3H, H-7); **^{13}C NMR (100 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 174.4 (C, CO), 171.3 (C, CO), 170.8 (C, CO), 167.8 (C, C-11), 163.3 (C, C-8), 147.5 (C, C-9), 131.2 (CH, C-17), 129.9 (CH, C-16), 124.1 (CH, C-10), 84.1 (C, C-5), 72.6 (CH, C-15), 42.1 (CH_2), 41.9 (CH_2), 40.5 (CH_2), 35.9 (CH_2), 34.8 (CH_2), 33.1 (CH_2), 25.1 (CH_3, C-7), 23.4 (CH_2); **ES$^+$ MS** *m/z* 491 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for $C_{19}H_{24}N_4NaO_4S_3$ (M+Na)$^+$ 491.0852, found 491.0850.

Methyl 2-(2-((*tert*-butoxycarbonylamino)methyl)thiazole-4-carboxamido)-2-methylpropanoate (2.190)
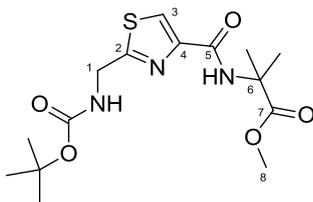
2.190

To a solution of **2.147** (200 mg, 0.77 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) cooled at 0 °C, PyBOP (549 mg, 0.93 mmol, 1.1 equiv) and Hünig's base (405 µL, 2.33 mmol, 3 equiv) were added. After 10 min, 2-aminoisobutyric acid methyl ester hydrochloride (108 mg, 0.70 mmol, 1 equiv) in solution in CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 3:7) to afford **2.190** as a white solid (244 mg, 97%).

mp 91.7–93.6 ℃; **IR** 3338, 2986, 2933, 1746, 1712, 1667, 1538, 1448, 1368, 1285, 1247, 1149 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 8.00 (s, 1H, H-3), 7.74 (br s, 1H, NH), 5.28 (br s, 1H, NH), 4.60 (d, *J*=5.9 Hz, 2H, H-1), 3.77 (s, 3H, H-8), 1.67 (s, 6H, C<u>Me</u>$_2$), 1.48 (s, 9H, C<u>Me</u>$_3$); **^{13}C NMR (75 MHz, CDCl$_3$) δ ppm** 174.7 (C, CO), 169.2 (C), 160.2 (C), 150.0 (C), 149.8 (C), 123.6 (CH, C-3), 80.5 (C, <u>C</u>Me$_3$), 56.4 (C, C-6), 52.7 (CH$_3$, C-8), 42.3 (CH$_2$, C-1), 28.3 (3CH$_3$, C<u>Me</u>$_3$), 25.0 (2CH$_3$, C<u>Me</u>$_2$); **ES$^+$ MS *m/z*** 380 ([M+Na]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{15}$H$_{23}$N$_3$NaO$_5$S (M+Na)$^+$ 380.1251, found 380.1249.

2-(2-((*tert*-Butoxycarbonylamino)methyl)thiazole-4-carboxamido)-2-methyl-propanoic acid (2.191)
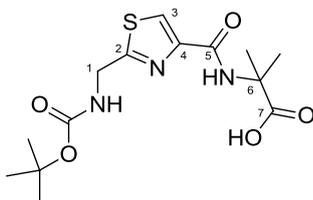
2.191

To a solution of **2.190** (242 mg, 0.68 mmol, 1 equiv) in 8 mL of THF/water (3:1) at 0 °C was added LiOH (49 mg, 2.03 mmol, 3 equiv). The reaction mixture was stirred at rt

overnight. The solution was diluted with water (3 mL) and acidified to pH 1–2 with a saturated KHSO$_4$ solution. This aqueous layer was extracted twice with EtOAc (10 mL). The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo* to provide **2.191** as a yellow solid (225 mg, 96%).

mp 171–173 °C; **IR** 3342, 2930, 2850, 2363, 2340, 1712, 1656, 1542, 1455, 1376, 1278, 1251, 1160 cm^{-1}; **^{1}H NMR (400 MHz, CD$_3$OD) δ ppm** 8.10 (s, 1H, H-3) 4.55 (s, 2H, H-1) 1.66 (s, 6H, C$\underline{\text{Me}}_2$) 1.50 (s, 9H, C$\underline{\text{Me}}_3$); **^{13}C NMR (100 MHz, CD$_3$OD) δ ppm** 177.8 (C, CO), 173.0 (C), 162.7 (C), 158.5 (C), 150.9 (C), 124.9 (CH, C-3), 81.1 (C, $\underline{\text{C}}$Me$_3$), 57.7 (C, C-6), 43.3 (CH$_2$, C-1), 28.8 (3CH$_3$, C$\underline{\text{Me}}_3$), 25.3 (2CH$_3$, C$\underline{\text{Me}}_2$); **ES$^+$ MS *m/z*** 366 ([M+Na]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{14}$H$_{21}$N$_3$NaO$_5$S (M+Na)$^+$ 366.1094, found 366.1094.

(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-((*S*)-2-(2-(2-((*tert*-butoxycarbonylamino)methyl)-thiazole-4-carboxamido)-2-methylpropanamido)-3-methylbutanoyloxy)-7-(tritylthio)hept-4-enoate (2.192)
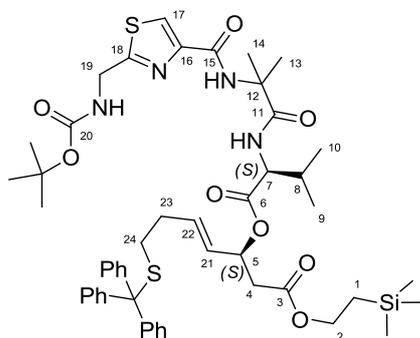
2.192

Carbamate **2.86** (371 mg, 0.44 mmol) was dissolved in anhydrous acetonitrile (4 mL) and diethylamine (229 µl, 2.21 mmol). After stirring for 2 h, the reaction mixture was concentrated *in vacuo*. The free amine was purified by flash chromatography (EtOAc/hexane 1:1).

To a solution of **2.191** (86.2 mg, 0.25 mmol, 1.1 equiv) in anhydrous CH$_2$Cl$_2$ (5 mL) cooled to 0 °C, PyBOP (162 mg, 0.27 mmol, 1.2 equiv) and Hünig's base (119 µL, 0.69 mmol, 3 equiv) were added. After 10 min, the previous deprotected amine (141 mg, 0.23 mmol, 1 equiv) in solution in CH$_2$Cl$_2$ (5 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was

purified by flash chromatography (EtOAc/hexane 3:7) to afford **2.192** as a white solid (134 mg, 62%).

$[\alpha]_D^{25}$ +5.4 (*c* 0.26, CHCl$_3$); **mp** 61–63 °C; **IR** 3338, 2960, 2926, 1731, 1682, 1535, 1497, 1444, 1391, 1365, 1251, 1172, 1028 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.10 (s, 1H, H-17), 7.81 (s, 1H, NH), 7.54–7.40 (m, 6H, H$_{Ar}$), 7.40–7.22 (m, 9H, H$_{Ar}$), 7.19 (d, *J*=8.5 Hz, 1H, NH), 5.82–5.57 (m, 2H, H-5; H-22), 5.41 (dd, *J*=15.6, 7.5 Hz, 1H, H-21), 5.34 (br s, 1H, NH), 4.66 (d, *J*=5.5 Hz, 2H, H-19), 4.58 (dd, *J*=8.5, 4.0 Hz, 1H, H-7), 4.30–4.09 (m, 2H, H-2), 2.72 (dd, *J*=15.8, 7.8 Hz, 1H, H-4), 2.58 (dd, *J*=15.6, 6.0 Hz, 1H, H-4), 2.35–2.15 (m, 3H, H-8; H-24), 2.09 (q, *J*=6.5 Hz, 2H, H-23), 1.73 (s, 3H, H-13 or H-14), 1.76 (s, 3H, H-13 or H-14), 1.55 (s, 9H, CMe$_3$), 1.09–0.79 (m, 8H, H-1; H-9; H-10), 0.10 (s, 9H, SiMe$_3$); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 173.9 (C), 170.7 (C), 169.6 (C), 169.4 (C), 160.7 (C), 155.6 (C), 149.8 (C), 144.8 (3C, C$_{Ar}$), 133.7 (CH, C-22), 129.5 (6CH, C$_{Ar}$), 127.83 (6CH, C$_{Ar}$), 127.78 (CH, C-21), 126.6 (3CH, C$_{Ar}$), 123.8 (CH, C-17), 80.5 (C, C-12), 71.6 (CH, C-5), 66.6 (C, $\underline{C}$Ph$_3$), 63.1 (CH$_2$), 57.6 (C, C-12), 57.1 (CH, C-7), 42.3 (CH$_2$), 39.7 (CH$_2$), 31.3 (CH, C-8), 31.1 (2CH$_2$), 28.3 (3CH$_3$, CMe$_3$), 25.8 (CH$_3$, C-13 or C-14), 25.0 (CH$_3$, C-13 or C-14), 19.0 (CH$_3$, C-9 or C-10), 17.4 (CH$_3$, C-9 or C-10), 17.3 (CH$_2$, C-1), –1.5 (3CH$_3$, SiMe$_3$); **ES$^+$ MS *m/z*** 965 ([M+Na]$^+$). **HRMS (ESI) *m/z*** calcd. for C$_{50}$H$_{66}$N$_4$NaO$_8$S$_2$Si (M+Na)$^+$ 965.3984, found 965.3964.

(7*S*,10*S*,*E*)-7-Isopropyl-4,4-dimethyl-10-(4-(tritylthio)but-1-enyl)-9-oxa-16-thia-3,6,13,18-tetraaza-bicyclo[13.2.1]octadec-1(17)-ene-2,5,8,12-tetraone (2.193)

2.193
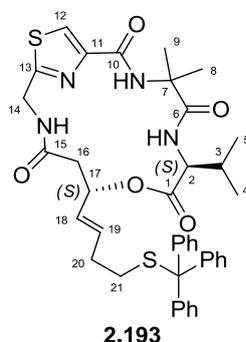
Acyclic precursor **2.192** (218 mg, 0.23 mmol) was dissolved in CH$_2$Cl$_2$ (11 mL), cooled to 0 °C and treated with TFA (2.3 mL). The reaction mixture was warmed to rt, stirred overnight, concentrated *in vacuo* and then coevaporated with toluene to remove residual

TFA. The crude amino acid (172 mg, 0.23 mmol, 1 equiv) was then taken up in CH_2Cl_2 (20 mL) and added dropwise to a vigorously stirred solution of Hünig's base (242 μL, 1.39 mmol, 6 equiv), HOBt (63 mg, 0.46 mmol, 2 equiv) and HATU (176 mg, 0.46 mmol, 2 equiv) in acetonitrile (165 mL). The reaction mixture was stirred overnight, and then concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/ EtOAc 10:1 to 100% EtOAc) to afford **2.193** as a pale yellow solid (108 mg, 64%).

$[\alpha]_D^{25}$ +32.3 (*c* 0.34, CHCl$_3$); **mp** 132–134 ºC; **IR** 3364, 2967, 2926, 2854, 1739, 1678, 1542, 1493, 1444, 1255, 1179, 839 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 8.32 (s, 1H, H-12), 8.05 (d, *J*=9.0 Hz, 1H, NH), 7.87 (d, *J*=2.5 Hz, 1H, NH), 7.64–7.37 (m, 15H, H$_{Ar}$), 6.00–5.82 (m, 2H, H-17; H-19), 5.75 (dd, *J*=15.6, 7.0 Hz, 1H, H-18), 5.26 (d, *J*=17.6 Hz, 1H, H-14), 4.78 (dd, *J*=9.3, 4.8 Hz, 1H, H-2), 4.47 (d, *J*=17.1 Hz, 1H, H-14), 3.06 (dd, *J*=17.1, 9.5 Hz, 1H, H-16), 2.90 (d, *J*=16.1 Hz, 1H, H-16), 2.60–2.40 (m, 3H, H-3 or H-20; H-21), 2.40–2.17 (m, 2H, H-3 or H-20), 2.12 (s, 3H, H-8 or H-9), 1.86 (s, 3H, H-8 or H-9), 1.14 (d, *J*=7.0 Hz, 3H, H-4 or H-5), 0.97 (d, *J*=7.0 Hz, 3H, H-4 or H-5); **^{13}C NMR (100 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 175.9 (C), 171.8 (C), 170.7 (C), 167.9 (C), 163.1 (C), 149.9 (C, C-11), 145.5 (3C, C$_{Ar}$), 133.7 (CH, C-19), 130.3 (6CH, C$_{Ar}$), 128.9 (CH, C-18), 128.6 (6CH, C$_{Ar}$), 127.4 (3CH, C$_{Ar}$), 124.0 (CH, C-12), 72.7 (CH, C-17), 67.5 (C, $\underline{C}$Ph$_3$), 60.0 (C, C-7), 58.4 (CH, C-2), 41.1 (CH$_2$, C-14), 40.8 (CH$_2$, C-16), 32.0; 31.9 (CH$_2$, C-20; C-21), 31.8 (CH, C-3), 25.6 (CH$_3$, C-8 or C-9), 25.3 (CH$_3$, C-8 or C-9), 19.8 (CH$_3$, C-4 or C-5), 17.5 (CH$_3$, C-4 or C-5); **ES$^+$ MS** *m/z* 747 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{40}$H$_{44}$N$_4$NaO$_5$S$_2$ (M+Na)$^+$ 747.2645, found 747.2641.

(7S,10S,E)-7-Isopropyl-10-(4-mercaptobut-1-enyl)-4,4-dimethyl-9-oxa-16-thia-3,6,13,18-tetraaza-bicyclo[13.2.1]octadec-1(17)-ene-2,5,8,12-tetraone (2.181)
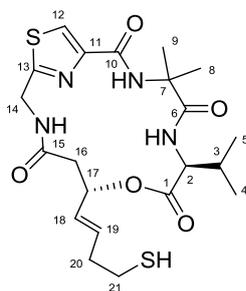
2.181

S-Trityl macrocycle **2.193** (73.1 mg, 0.10 mmol, 1 equiv) was dissolved in CH_2Cl_2 (12 mL) and cooled to 0 ℃. The mixture was successively treated with Et_3SiH (32 µL, 0.20 mmol, 2 equiv) and TFA (495 µL, 6.93 mmol, 61 equiv). The reaction mixture was warmed to rt and stirred for 2 h, before being concentrated and purified by flash chromatography with EtOAc as eluent to provide **2.181** as a white solid (6 mg, 12%).

$[\alpha]_D^{25}$ +61.3 (*c* 0.24, CHCl$_3$); **mp** 56–58 ℃; **IR** 3334, 2960, 2926, 2854, 1734, 1678, 1542, 1463, 1383, 1256, 974 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.03 (s, 1H, H-12), 7.65 (br s, 1H, NH), 6.60–6.39 (m, 2H, NH), 5.84–5.70 (m, 3H, H-17; H-18 H-19), 5.16 (dd, *J*=17.3, 7.8 Hz, 1H, H-14), 4.64 (dd, *J*=9.5, 4.0 Hz, 1H, H-2), 4.37 (dd, *J*=17.6, 4.0 Hz, 1H, H-14), 2.86–2.62 (m, 2H, H-16), 2.62–2.46 (m, 2H, H-21), 2.46–2.21 (m, 3H, H-3; H-20), 1.90 (s, 3H, H-8 or H-9), 1.62 (s, 3H, H-8 or H-9), 1.35 (t, *J*=7.5 Hz, 1H, SH), 0.91 (d, *J*=6.5 Hz, 3H, H-4 or H-5), 0.72 (d, *J*=7.0 Hz, 3H, H-4 or H-5); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 174.4 (C), 170.0 (C), 168.9 (C), 166.9 (C), 161.0 (C), 149.5 (C, C-11), 132.1 (CH, C-19), 129.0 (CH, C-18), 123.3 (CH, C-12), 71.8 (CH, C-17), 59.6 (C, C-7), 57.4 (CH, C-2), 41.3 (CH$_2$, C-14), 40.8 (CH$_2$, C-16), 36.0 (CH$_2$, C-20), 31.3 (CH, C-3), 25.8 (2CH$_3$, C-8; C-9), 23.8 (CH$_2$, C-21), 19.1 (CH$_3$, C-4 or C-5), 16.8 (CH$_3$, C-4 or C-5); **ES$^+$ MS *m/z*** 505 ([M+Na]$^+$); **HRMS (ESI) *m/z*** calcd. for $C_{21}H_{30}N_4NaO_5S_2$ (M+Na)$^+$ 505.1550, found 505.1551.

Boc-valine-largazole analogue 2.194

2.194
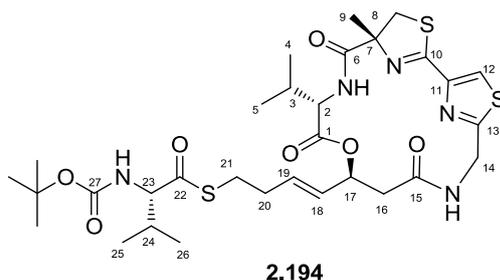
To a solution of Boc-L-Valine (20 mg, 0.09 mmol, 2 equiv) in CH_2Cl_2 (2 mL) cooled at 0 °C, PyBOP (53 mg, 0.09 mmol, 2 equiv) and Hünig's base (16 µL, 0.09 mmol, 2 equiv) were added. After 15 min, largazole thiol **2.178** (22.3 mg, 0.05 mmol, 1 equiv) in solution in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was stirred at rt overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 1:3) to afford **2.194** as a white solid (31 mg, 100%).

$[\alpha]_D^{24}$ +11.2 (*c* 0.23, MeOH); **mp** 132–134 ºC; **IR** 3361, 3300, 2971, 2930, 2850, 1720, 1682, 1508, 1376, 1262, 1172, 1024, 839 cm^{-1}; **^{1}H NMR (400 MHz, CD$_3$OD) δ ppm** 8.18 (s, 1H, H-12), 7.35 (d, *J*=9.5 Hz, 1H, NH), 5.94–5.79 (m, 1H, H-19), 5.73–5.58 (m, 2H, H-17; H-18), 5.17 (d, *J*=17.6 Hz, 1H, H-14), 4.55 (dd, *J*=9.3, 3.3 Hz, 1H, H-2), 4.41 (d, *J*=17.6 Hz, 1H, H-14), 4.09 (d, *J*=6.0 Hz, 1H, H-23), 3.98 (d, *J*=11.5 Hz, 1H, H-8), 3.44 (d, *J*=11.5 Hz, 1H, H-8), 3.06 (dd, *J*=16.6, 10.5 Hz, 1H, H-16), 2.96 (t, *J*=7.0 Hz, 2H, H-21), 2.75 (dd, *J*=16.3, 2.3 Hz, 1H, H-16), 2.36 (q, *J*=7.0 Hz, 2H, H-20), 2.23 (sxt, *J*=6.5 Hz, 1H, H-3 or H-24), 2.14 (quind, *J*=7.0, 3.5 Hz, 1H, H-3 or H-24), 1.87 (s, 3H, H-9), 1.51 (s, 9H, C<u>Me</u>$_3$), 1.00 (d, *J*=6.5 Hz, 3H), 0.95 (d, *J*=7.0 Hz, 3H), 0.75 (d, *J*=6.5 Hz, 3H), 0.55 (d, *J*=7.0 Hz, 3H); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 183.2 (C, CO), 176.1 (C, CO), 172.4 (C, CO), 170.4 (C), 170.1 (C), 168.2 (C), 58.4 (C), 148.3 (C, C-11), 133.9 (CH, C-19), 130.1 (CH, C-18), 126.9 (CH, C-12), 81.0; 79.6 (2C, C-7; C<u>Me</u>$_3$), 74.1 (CH, C-17), 67.7 (CH, C-23), 59.2 (CH, C-2), 43.8 (CH$_2$, C-8), 42.0 (CH$_2$, C-14 or C-16), 40.7 (CH$_2$, C-14 or C-16), 35.6 (CH, C-3), 33.4 (CH$_2$, C-20), 31.9 (CH, C-24), 28.92 (3CH$_3$, CMe$_3$), 28.87 (CH$_2$, C-21), 24.5 (CH$_3$, C-9), 19.9 (CH$_3$), 19.8 (CH$_3$), 18.2 (CH$_3$), 17.2 (CH$_3$); **ES$^+$ MS *m/z*** 718 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for $C_{31}H_{45}N_5NaO_7S_3$ (M+Na)$^+$ 718.2373, found 718.2369.

Valine-largazole analogue 2.183

2.183
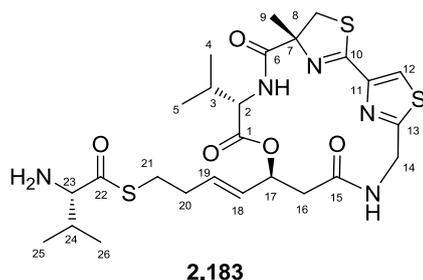
To a solution of **2.194** (28.7 mg, 0.04 mmol, 1 equiv) in CH_2Cl_2 (2 mL), was added TFA (1 mL) at 0 °C. The reaction mixture was stirred for 1 h, before being concentrated and purified by flash chromatography using CH_2Cl_2/hexane (1:1) to CH_2Cl_2/MeOH/NH$_3$ 96:3:1 as eluent to provide **2.183** as a white solid (18 mg, 73%).

$[\alpha]_D^{24}$ + 41.9 (*c* 0.17, MeOH); **mp** 76–78 °C; **IR** 3380, 2967, 2926, 2877, 1735, 1682, 1591, 1535, 1504, 1414, 1262, 1194, 1176, 1039, 975 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 8.18 (s, 1H, H-12), 7.34 (d, *J*=9.0 Hz, 1H, NH), 5.92–5.77 (m, 1H, H-19), 5.72–5.59 (m, 2H, H-17; H-18), 5.15 (d, *J*=17.6 Hz, 1 H, H-14), 4.61–4.50 (m, 1H, H-2), 4.42 (d, *J*=17.6 Hz, 1H, H-14), 3.97 (d, *J*=11.5 Hz, 1H, H-8), 3.53–3.40 (m, 2H, H-8; H-23), 3.13–2.94 (m, 3H, H-16; H-21), 2.74 (dd, *J*=16.6, 2.5 Hz, 1H, H-16), 2.45–2.31 (m, 2H, H-20), 2.20–2.07 (m, 2H, H-3; H-24), 1.97 (s, 2H, NH$_2$), 1.87 (s, 3H, H-9), 1.04 (d, *J*=7.0 Hz, 3H), 0.96 (d, *J*=6.5 Hz, 3H), 0.74 (d, *J*=7.0 Hz, 3H), 0.54 (d, *J*=6.5 Hz, 3H); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 176.1 (C, CO), 176.0 (C, CO), 172.4 (C, CO), 170.4 (C), 170.1 (C), 168.2 (C), 148.3 (C), 133.6 (CH, C-19), 130.3 (CH, C-18), 126.9 (CH, C-12), 85.3 (C, C-7), 74.1 (CH, C-17), 68.0 (CH, C-23), 59.1 (CH, C-2), 43.8 (CH$_2$, C-8), 41.9 (CH$_2$, C-14 or C-16), 40.7 (CH$_2$, C-14 or C-16), 35.6 (CH, C-3), 33.5 (CH$_2$, C-20), 33.4 (CH, C-24), 28.9 (CH$_2$, C-21), 24.5 (CH$_3$, C-9), 19.8 (2CH$_3$), 17.6 (CH$_3$), 17.2 (CH$_3$); **ES$^+$ MS *m/z*** 596 ([M+H]$^+$), 618 ([M+Na]$^+$).

5.3.2. Experimental procedures for thailandepsins and burkholdacs

5.3.2.1. Towards the synthesis of first structures of thailandepsins

(9H-Fluoren-9-yl)methyl (*S*)-1-((*R*)-1-hydroxy-3-phenylpropan-2-ylamino)-1-oxo-3-(tritylthio)propan-2-ylcarbamate (2.201)
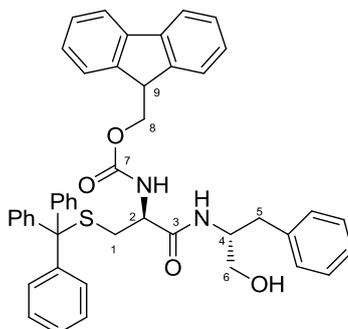
2.201

To Fmoc-D-Cys(Tr)-OH (500 mg, 0.9 mmol, 1 equiv) in CH_2Cl_2 (12 mL), *N*-methylmorpholine (282 μL, 2.6 mmol, 3 equiv), PyBOP (554 mg, 0.939 mmol, 1.1 equiv) and HOBt (127 mg, 0.9 mmol, 1.1 equiv) were added at 0 °C under argon. (*R*)-Phenylalaninol (173 mg, 1.1 mmol, 1.34 equiv) was added after 5 min. The reaction mixture was stirred at rt overnight and then concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 1:1) to afford **2.201** as a white solid (607 mg, 99%).

$[\alpha]_D^{25}$ +2.5 (*c* 0.17, CHCl$_3$); **mp** 88–90 °C; **IR** 3300, 3058, 3020, 2964, 1705, 1656, 1523, 1493, 1448, 1262, 1032, 911 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.90 (t, *J*=6.5 Hz, 2H, H$_{Ar}$), 7.70 (d, *J*=6.5 Hz, 2H, H$_{Ar}$), 7.59–7.24 (m, 25H, H$_{Ar}$, NH), 6.05 (d, *J*=8.0 Hz, 1H, NH), 5.00 (d, *J*=7.0 Hz, 1H), 4.52 (d, *J*=6.5 Hz, 2H, H-8), 4.32 (t, *J*=6.5 Hz, 1H, H-9), 4.25–4.15 (m, 1H), 3.85-3.73 (m, 2H), 3.64 (dd, *J*=11.0, 5.0 Hz, 1H), 2.96 (dd, *J*=13.6, 7.0 Hz, 1H, H-5), 2.89 (dd, *J*=14.1, 7.5 Hz, 1H, H-5), 2.77 (dd, *J*=13.6, 7.5 Hz, 1H, H-1), 2.70 (dd, *J*=13.1, 5.5 Hz, 1H, H-1); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 170.3 (C, C=O), 155.9 (C, C=O), 144.3 (3C, C$_{Ar}$), 143.6 (2C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 137.3 (C, C$_{Ar}$), 129.5 (6CH, C$_{Ar}$), 129.1 (2CH, C$_{Ar}$), 128.6 (2CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 127.0 (3CH, C$_{Ar}$), 126.6 (CH, C$_{Ar}$), 125.0 (2CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 67.4 (C, $\underline{C}$Ph$_3$), 66.9 (CH$_2$, C-6 or C-8), 63.6 (CH$_2$, C-6 or C-8), 54.3 (CH, C-2 or C-4), 53.1 (CH, C-2 or C-4), 47.1 (CH, C-9), 36.7

(CH$_2$, C-5), 33.6 (CH$_2$, C-1); **ES$^+$ MS** *m/z* 741 ([M+Na]$^+$); **HRMS** *m/z* calcd. for C$_{46}$H$_{42}$N$_2$NaO$_4$S (M+Na)$^+$ 741.2757, found 741.2743.

(*S*)-2-Amino-*N*-((*R*)-1-hydroxy-3-phenylpropan-2-yl)-3-(tritylthio)propanamide (2.202)

2.202
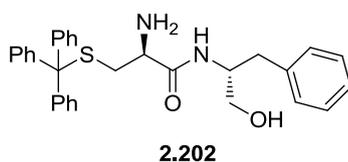
To a solution of the **2.201** (596 mg, 0.83 mmol, 1 equiv) in acetonitrile (10 mL), diethylamine (430 µL, 4.15 mmol, 5 equiv) was added. After stirring for 2 h at rt, the reaction mixture was concentrated. The crude material was purified by flash chromatography (hexane/EtOAc 9:1 to 1:1 to 100% EtOAc) to afford **2.202** as a white solid (397 mg, 90%).

(9H-Fluoren-9-yl)methyl (*R*)-1-((*S*)-1-((*R*)-1-hydroxy-3-phenylpropan-2-ylamino)-1-oxo-3-(tritylthio)propan-2-ylamino)-1-oxopropan-2-ylcarbamate (2.203)

2.203
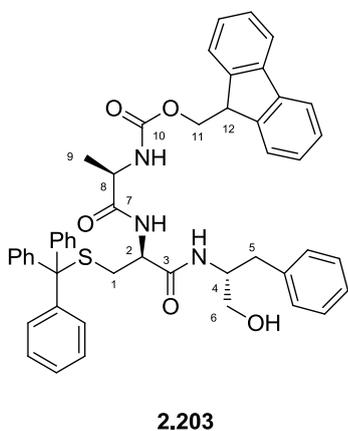
To Fmoc-D-alanine (383 mg, 1.23 mmol, 1.3 equiv) in CH$_2$Cl$_2$ (13 mL), *N*-methylmorpholine (303 µL, 2.76 mmol, 3 equiv), PyBOP (814 mg, 1.38 mmol, 1.5 equiv) and HOBt (186 mg, 1.38 mmol, 1.5 equiv) were added at 0°C under argon. Compound **2.202** (456 mg, 0.92 mmol, 1 equiv) was added after 5 min. The reaction mixture was stirred at rt overnight and then concentrated *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 1:1) to afford **2.203** as a white solid (724 mg, 99%).

276

$[\alpha]_D^{24}$ –25.1 (*c* 0.49, MeOH); **mp** 82–84 °C; **IR** 3383, 3296, 3058, 3032, 2922, 1671, 1512, 1444, 1300, 1247, 1077, 1036, 847 cm^{-1}; **^{1}H NMR (400 MHz, CD$_3$OD) δ ppm** 7.97–7.13 (m, 28H, H$_{Ar}$), 4.41–4.26 (m, 2H), 4.23–4.03 (m, 4H), 3.54 (dd, *J*=5.0, 3.5 Hz, 2H, H-6), 2.93 (dd, *J*=13.7, 6.1 Hz, 1H, H-5), 2.78 (dd, *J*=13.6, 7.9 Hz, 1H, H-5), 2.72–2.61 (m, 1H, H-1), 2.57 (dd, *J*=12.5, 5.3 Hz, 1H, H-1), 1.37 (d, *J*=7.0 Hz, 2H, H-9); **^{13}C NMR (100 MHz, CD$_3$OD) δ ppm** 175.5 (C, C=O), 171.9 (C, C=O), 158.8 (C, C=O), 145.9 (3C, C$_{Ar}$), 145.3 (2C, C$_{Ar}$), 142.7 (2C, C$_{Ar}$), 139.6 (C, C$_{Ar}$), 130.7 (6CH, C$_{Ar}$), 130.3 (2CH, C$_{Ar}$), 129.5 (2CH, C$_{Ar}$), 129.1 (6CH, C$_{Ar}$), 128.9 (2CH, C$_{Ar}$), 128.3 (2CH, C$_{Ar}$), 128.0 (3CH, C$_{Ar}$), 127.4 (CH, C$_{Ar}$), 126.3 (2CH, C$_{Ar}$), 121.0 (2CH, C$_{Ar}$), 68.4 (CH$_2$, C-11), 68.1 (C, CPh$_3$), 64.0 (CH$_2$, C-6), 54.5; 54.4; 54.3; 52.7 (4CH, C-2; C-4; C-8; C-12), 37.8 (CH$_2$, C-5), 34.5 (CH$_2$, C-1), 17.8 (CH$_3$, C-9); **ES$^+$ MS *m/z*** 812 ([M+Na]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{49}$H$_{47}$N$_3$NaO$_5$S (M+Na)$^+$ 812.3129, found 812.3120.

(*S*)-2-((*R*)-2-Aminopropanamido)-*N*-((*R*)-1-hydroxy-3-phenylpropan-2-yl)-3-(tritylthio)-propanamide (2.198)

2.198
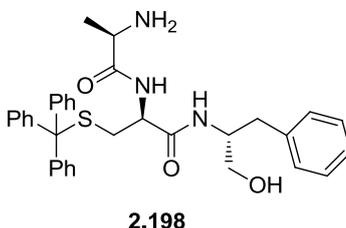
To a solution of the **2.203** (787 mg, 1 mmol, 1 equiv) in a mixture acetonitrile/CH$_2$Cl$_2$ (20:5 mL), diethylamine (515 µL, 10 mmol, 10 equiv) was added. After stirring for 2 h at rt, the reaction mixture was concentrated. The crude material was purified by flash chromatography (hexane/EtOAc 9:1 to 1:1 to 100% EtOAc) to afford **2.198** as a white solid (413 mg, 73%).

5.3.2.2. Total synthesis of Cheng's revised thailandepsin A

(*R*)-Allyl 4-(*tert*-butoxycarbonylamino)-6-(methylthio)-3-oxohexanoate (2.221)

2.221
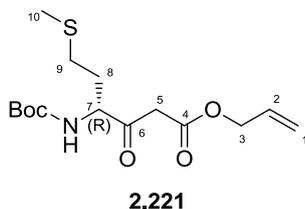
To a solution of Boc-D-Met-OH (1 g, 4.0 mmol, 1 equiv) in CH_2Cl_2 (20 mL), were added DMAP (98 mg, 0.8 mmol, 0.2 equiv), pentafluorophenol (812 mg, 4.4 mmol, 1.1 equiv) and EDCI.HCl (923 mg, 4.8 mmol, 1.2 equiv). The reaction mixture was stirred at rt for 4 h. The layers were separated after addition of HCl (1 M) to the reaction mixture. The organic layer was washed with saturated $NaHCO_3$ solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting white solid was placed under high vacuum.

To a solution of diisopropylamine (1.85 mL, 12.9 mmol, 3.3 equiv) in THF (10 mL), were added dropwise at −78 °C a solution of BuLi in hexanes (1.6 M, 9 mL, 14.1 mmol, 3.6 equiv) followed by allylacetate (1.37 mL, 12.4 mmol, 3.2 equiv). The reaction was stirred for 30 min at −78 °C and a solution in THF (15 mL) of the intermediate ester of Boc-D-Met-OH was added. After stirring at −78 °C for 3 h, the reaction mixture was quenched with HCl solution (1 M, 25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (25 mL) and then with saturated brine (25 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **2.221** as a yellow oil (918.5 mg, 71%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 6.08–5.77 (m, 1H, H-2), 5.46–5.10 (m, 3H, NH, H-1), 4.76–4.56 (m, 2H, H-3), 4.56–4.38 (m, 1H, H-7), 3.63 (app dd, *J*=16.2, 3.0 Hz, 2H, H-5), 2.54 (t, *J*=7.2 Hz, 2H, H-9), 2.32–2.19 (m, 1H, H-8), 2.10 (s, 3H, H-10), 1.87 (dt, *J*=14.2, 7.0 Hz, 1H, H-8), 1.45 (s, 9H, CMe$_3$).

The spectroscopic data are consistent with that[206] reported in the literature.

(3S,4R)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-6-(methylthio)hexanoate (2.222)

2.222
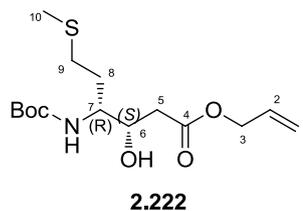
To a solution of **2.221** (887.7 mg, 2.68 mmol, 1 equiv) in anhydrous MeOH (10 mL) at –78 °C, was added KBH_4 in portions (506 mg, 9.37 mmol, 3.5 equiv). The reaction was stirred for 10 min at –78 °C, warmed to –20 °C for 30 min and to 0 °C for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (2:8) to provide **2.222** as a white solid (719 mg, 81%).

^{1}H NMR (300 MHz, CDCl₃) δ ppm 6.08–5.77 (m, 1H, H-2), 5.52–5.19 (m, 2H, H-1), 4.77 (d, *J*=7.7 Hz, 1H, NH), 4.68–4.59 (m, 2H, H-3), 4.17–3.97 (m, 1H, H-6), 3.80–3.59 (m, 1H, H-7), 3.39 (br s, 1H, OH), 2.74–2.44 (m, 4H, H-5; H-9), 2.11 (s, 3H, H-10), 1.91 (m, 1H, H-8), 1.80–1.57 (m, 1H, H-8), 1.45 (s, 9H, CMe₃).

The spectroscopic data are consistent with that[206] reported in the literature.

(3S,4R)-Allyl 4-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(trityl-thio)-propanamido)-3-hydroxy-6-(methylthio)hexanoate (2.223)

2.223
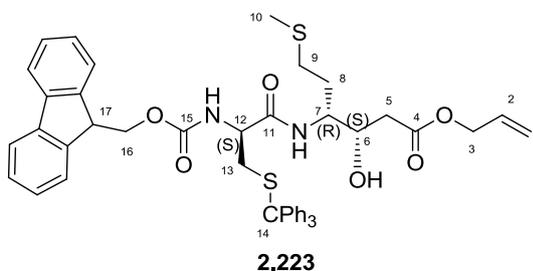
Compound **2.222** (150 mg, 0.24 mmol, 1 equiv) was treated with TFA (3.9 mL) in CH_2Cl_2 (10 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and

coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine (495 mg, 100%).

To a solution of Fmoc-D-Cys(Tr)-OH (726 mg, 2.12 mmol, 1 equiv) in CH_2Cl_2 (30 mL) cooled at 0 °C, PyBOP (1.3 g, 2.50 mmol, 1.2 equiv), and Hünig's base (1.3 mL, 7.43 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine (495 mg, 2.12 mmol, 1 equiv) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (3:7) as eluent to give **2.223** as a colourless oil (830 mg, 50%).

$[\alpha]_D^{28}$ −2 (*c* 0.1, CH_3OH); **IR** 3318, 3060, 2925, 2901, 1712, 1654, 1524, 1446, 1250, 1176, 1037 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.87–7.67 (m, 2H, H$_{Ar}$), 7.57 (d, *J*=7.2 Hz, 2H, H$_{Ar}$), 7.50–7.37 (m, 8H, H$_{Ar}$), 7.36–7.14 (m, 11H, H$_{Ar}$), 6.14 (d, *J*=8.5 Hz, 1H, NH), 5.98–5.73 (m, 1H, H-2), 5.38–5.09 (m, 2H, H-1), 4.95 (d, *J*=7.0 Hz, 1H, NH), 4.58 (d, *J*=5.6 Hz, 2H, H-3), 4.41 (d, *J*=6.0 Hz, 2H, H-16), 4.26–4.16 (m, 1H, H-17), 4.05–3.89 (m, 2H), 3.89–3.68 (m, 1H), 3.32 (d, *J*=3.9 Hz, 1H, OH), 2.82–2.68 (m, 1H, H-13), 2.65–2.36 (m, 5H, H-5; H-9; H-13), 2.01 (s, 3H, H-10), 1.97–1.80 (m, 1H, H-8), 1.78–1.61 (m, 1H, H-8); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.2 (C, CO), 170.1 (C, CO), 158.9 (C, CO), 144.2 (3C, C$_{Ar}$), 143.7 (C, C$_{Ar}$), 143.6 (C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 131.7 (CH, C-2), 129.5 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 127.0 (3CH, C$_{Ar}$), 124.9 (2CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 118.7 (CH$_2$, C-1), 70.0 (CH, C-6), 67.4 (C, CPh$_3$), 66.0 (CH$_2$, C-3 or C-16), 65.5 (CH$_2$, C-3 or C-16), 54.3 (CH, C-7 or C-12), 52.7 (CH, C-7 or C-12), 47.1 (CH, C-17), 38.1 (CH$_2$, C-5), 33.6 (CH$_2$, C-13), 30.6 (CH$_2$, C-8 or C-9), 28.6 (CH$_2$, C-8 or C-9), 15.5 (CH$_3$, C-10); **ES$^+$ MS** *m/z* 823 ([M+Na]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for C$_{29}$H$_{44}$N$_4$NaO$_7$ (M+Na)$^+$ 823.2846, found 823.2849.

(5*R*,8*S*,11*R*,12*S*)-Allyl 5-((*R*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-11-(2-(methylthio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetra-decan-14-oate (2.224)
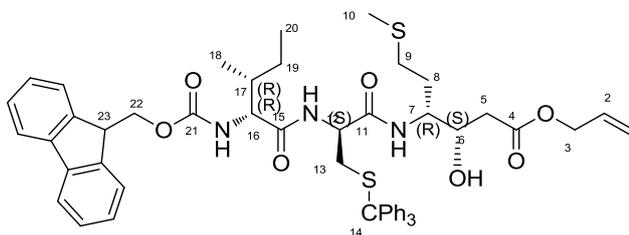
2.224

Compound **2.223** (325 mg, 0.41 mmol, 1 equiv) was treated with diethylamine (1 mL) in CH_3CN (10 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine (235 mg, 100%).

To a solution of Fmoc-D-Ile-OH (158 mg, 0.45 mmol, 1.1 equiv) in CH_2Cl_2 (30 mL) cooled at 0 °C, PyBOP (254 g, 0.49 mmol, 1.2 equiv), and Hünig's base (213 µL, 1.22 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine (235 mg, 0.41 mmol, 1 equiv) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:4 to 3:7) as eluent to afford **2.224** as a white solid (265 mg, 99%).

$[\alpha]_D^{23}$ +33.9 (*c* 0.20, CH_3OH); **mp** 60–62 °C; **IR** 3288, 3056, 3036, 2966, 2962, 2921, 1707, 1646, 1536, 1446, 1234, 1172, 1033, 841 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 7.79 (d, *J*=7.5 Hz, 2H, H$_{Ar}$), 7.59 (d, *J*=7.5 Hz, 2H, H$_{Ar}$), 7.56 (d, *J*=7.5 Hz, 8H, H$_{Ar}$), 7.47–7.17 (m, 11H, H$_{Ar}$), 6.04–5.79 (m, 1H, H-2), 5.31 (dd, *J*=17.1, 1.5 Hz, 1H, H-1), 5.23 (dd, *J*= 10.3, 1.3 Hz, 1H, H-1), 4.60 (dd, *J*=5.5, 1.0 Hz, 2H, H-3), 4.52 (dd, *J*=10.5, 6.5 Hz, 1H, H-22), 4.30 (dd, *J*=10.0, 6.5 Hz, 1H, H-22), 4.20–3.88 (m, 5H, H-6; H-7; H-12; H-16; H-23), 2.78 (dd, *J*=12.3, 6.3 Hz, 1H, H-13), 2.66–2.38 (m, 5H, H-5; H-9; H-13), 2.05 (s, 3H, H-10), 2.01–1.92 (m, 1H, H-8), 1.88–1.83 (m, 2H, H-8; H-17), 1.53–1.32 (m, 1H, H-19), 1.17–1.00 (m, 1H, H-19), 0.98–0.81 (m, 6H, H-18; H-20); **^{13}C NMR (100 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 172.2 (C, CO), 171.5 (C, CO), 170.0 (C, CO), 156.9 (C, CO), 144.1 (3C, C$_{Ar}$), 143.7 (C, C$_{Ar}$), 143.4 (C, C$_{Ar}$), 141.2 (2C, C$_{Ar}$), 131.7 (CH, C-2), 129.3 (6CH, C$_{Ar}$), 128.0 (6CH, C$_{Ar}$), 127.7 (2CH, C$_{Ar}$), 127.0 (2CH, C$_{Ar}$), 126.8 (3CH, C$_{Ar}$), 124.9 (CH, C$_{Ar}$), 124.8 (CH, C$_{Ar}$),

119.9 (2CH, C_{Ar}), 118.4 (CH_2, C-1), 70.0 (CH, C-6), 67.0 (C; CH_2, $\underline{C}Ph_3$; C-22), 65.4 (CH_2, C-3), 60.0 (CH), 52.9 (CH), 52.4 (CH), 47.0 (CH, C-23), 38.1 (CH_2), 36.9 (CH), 32.9 (CH_2), 30.5 (CH_2), 29.0 (CH_2), 24.7 (CH_2, C-19), 15.4; 15.3 ($2CH_3$, C-10; C-18), 11.3 (CH_3, C-20); **ES$^+$ MS m/z** 936 ([M+Na]$^+$ 100%); **HRMS (ESI)** m/z calcd. for $C_{29}H_{44}N_4NaO_7$ (M+Na)$^+$ 936.3687, found 936.3704.

(*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enoic acid (2.219)
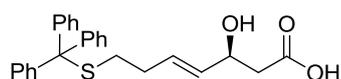
2.219

To a solution of compound **2.47** (937 mg, 1.7 mmol, 1 equiv) in a mixture of THF/water (30 mL:10 mL) was added LiOH (120 mg, 5.0 mmol, 3 equiv). The reaction was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (2:8) then 100% EtOAc as eluent to afford **2.219** as yellow oil (374 mg, 55%).

(7*S*,11*R*,14*R*,17*R*,18*R*,E)-Allyl 11-((*R*)-*sec*-butyl)-7,18-dihydroxy-17-(2-(methyl-thio)-ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-((tritylthio)methyl)-2-thia-10,13,16-triazanonadec-5-en-19-oate (2.225)
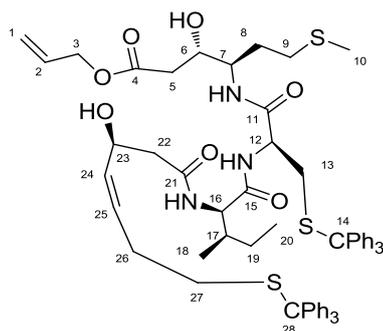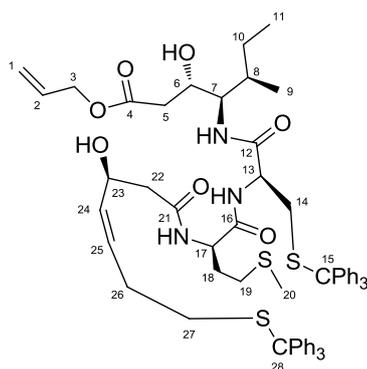

2.225

The tetrapeptide fragment **2.224** (264 mg, 0.29 mmol, 1 equiv) was treated with diethylamine (1 mL) in CH_3CN (10 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine (200 mg, 100%).

To a solution of the β-hydroxy acid **2.219** (130 mg, 0.32 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) cooled at 0 °C, PyBOP (180 mg, 0.35 mmol, 1.2 equiv) and Hünig's base (151 μL, 0.87 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine (200 mg, 0.29 mmol, 1 equiv) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:1) as eluent to provide **2.225** as a white solid (206 mg, 65%).

$[\alpha]_D^{23}$ +40.5 (*c* 0.127, CH_3OH). **mp** 58–60 °C; **IR** 3273, 3052, 2958, 2929, 1740, 1630, 1531, 1487, 1446, 1180, 854 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 7.47–7.17 (m, 30H, H$_{Ar}$), 5.97–5.74 (m, 1H, H-2), 5.59–5.45 (m, 1H, H-25), 5.44–5.34 (m, 1H, H-24), 5.34–5.26 (m, 1H, H-1), 5.21 (dd, *J*=10.3, 1.3 Hz, 1H, H-1), 4.58 (d, *J*=5.5 Hz, 2H, H-3), 4.35 (q, *J*=6.2 Hz, 1H, H-23), 4.14 (dd, *J*=7.5, 5.5 Hz, 1H, H-12), 4.11–4.07 (m, 1H, H-16), 4.07–3.97 (m, 1H, H-6 or H-7), 3.89 (ddd, *J*=10.5, 5.0, 3.0 Hz, 1H, H-6 or H-7), 2.66–2.36 (m, 7H, H-5; H-9; H-13), 2.33 (d, *J*=6.5 Hz, 2H, H-22), 2.26–2.16 (m, 2H, H-27), 2.09 (q, *J*=7.4 Hz, 2H, H-26), 2.00 (s, 3H, H-10), 1. 97–1.83 (m, 2H, H-8; H-17), 1.76–1.64 (m, 1H, H-8), 1.51–1.32 (m, 1H, H-19), 1.15 (ddd, *J*=13.6, 9.5, 7.0 Hz, 1H, H-19), 0.98–0.82 (m, 6H, H-18; H-20); **^{13}C NMR (100 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 173.1 (C, CO), 172.1 (C, CO), 171.6 (C, CO), 170.4 (C, CO), 144.7 (3C, C$_{Ar}$), 144.1 (3C, C$_{Ar}$), 132.4 (CH), 131.7 (CH), 130.0 (CH), 129.4 (6CH, C$_{Ar}$), 129.3 (6CH, C$_{Ar}$), 127.9 (6CH, C$_{Ar}$), 127.7 (6CH, C$_{Ar}$), 126.8 (3CH, C$_{Ar}$), 126.5 (3CH, C$_{Ar}$), 118.2 (CH$_2$, C-1), 70.1 (CH), 69.3 (CH), 66.8 (C, $\underline{C}$Ph$_3$), 66.5 (C, $\underline{C}$Ph$_3$), 65.4 (CH$_2$, C-3), 59.2 (CH), 52.9 (CH), 52.6 (CH), 43.2 (CH$_2$), 38.2 (CH$_2$), 35.9 (CH), 32.9 (CH$_2$), 31.3 (CH$_2$), 31.1 (CH$_2$), 30.4 (CH$_2$), 28.9 (CH$_2$), 24.6 (CH$_2$, C-19), 15.6; 15.1 (CH$_3$, C-10; C-18), 11.4 (CH$_3$, C-20); **ES$^+$ MS *m/z*** 1115 ([M+Na]$^+$ 100%).

Thailandepsin A (2.217)
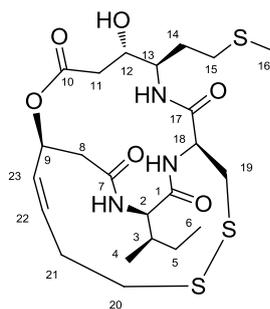
2.217

To a solution of **2.225** (186 mg, 0.17 mmol, 1 equiv) in methanol, were added morpholine (32 μL, 0.36 mmol, 2.1 equiv) and Pd(PPh$_3$)$_4$ (19.7 mg, 17 μmol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH$_2$Cl$_2$ followed by a gradient of 5% then 10% MeOH in CH$_2$Cl$_2$) to afford the carboxylic acid as a yellow solid (162 mg, 90%).

A solution of the previous carboxylic acid (161.8 mg, 0.15 mmol, 1 equiv) in CH$_2$Cl$_2$ (115 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (63.5 mg, 0.18 mmol, 1.2 equiv) in CH$_2$Cl$_2$ (30 mL) containing DMAP (45 mg, 0.37 mmol, 2.4 equiv) at room temperature over 3.5 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH$_2$Cl$_2$/MeOH 1:0 to 98:2) to give the protected dithiol **2.226** (37.9 mg, 24%) as a white amorphous solid.

To a vigorously stirring solution of I$_2$ (93 mg, 0.37 mmol, 10 equiv) in CH$_2$Cl$_2$/MeOH (70 mL:40 mL) was added the protected dithiol (38 mg, 37 μmol, 1 equiv) in MeOH/CH$_2$Cl$_2$ (7 mL:4 mL) dropwise over 2.5 h and stirred for a further 30 min. Na$_2$S$_2$O$_3$ solution (0.01 M, 20 mL) was added, followed by brine (8 mL) and the aqueous phase extracted twice with EtOAc (30 mL). The combined organic layers were dried over MgSO$_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/ CH$_2$Cl$_2$ 1:1 to MeOH/ CH$_2$Cl$_2$ 1:99 then 2:98) to give **2.217** as a white solid (7.7 mg, 38%).

$[\alpha]_D^{32}$ –30.7 (*c* 0.29, CH$_3$OH); **mp** 86–88 °C; **IR** 3383, 3322, 2966, 2925, 1728, 1654, 1536, 1515, 1270, 1160 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$)** *δ* **ppm** 7.57 (d, *J*=7.0 Hz, 1H, NH), 6.74 (d, *J*=9.0 Hz, 1H, NH), 6.51 (t, *J*=13.1 Hz, 1H, H-22), 5.88 (d, *J*=4.1 Hz, 1H, NH), 5.66 (dt, *J*=15.3, 1.6 Hz, 1H, H-23), 5.48 (dd, *J*=3.8, 1.9 Hz, 1H, H-9), 4.91 (td, *J*=8.4, 3.3 Hz, 1H, H-18), 4.55–4.31 (m, 1H, H-12), 4.18 (dd, *J*=5.6, 4.5 Hz, 1H, H-

284

2), 3.60–3.38 (m, 2H, H-8; H-19), 3.25–3.05 (m, 2H, H-13; H-19), 2.85–2.31 (m, 9H, H-8; H-11; H-15; H-20; H-21), 2.10 (s, 3H, H-16), 2.09–1.96 (m, 1H, H-3), 1.70–1.56 (m, 3H, H-5; H-14), 1.36–1.16 (m, 1H, H-5), 1.06 (d, J=6.9 Hz, 3H, H-4), 0.99 (t, J=7.3 Hz, 3H, H-6); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 171.4 (C, CO), 171.2 (C, CO), 169.9 (C, CO), 169.0 (C, CO), 133.9 (CH, C-22), 128.1 (CH, C-23), 70.6 (CH), 70.5 (CH), 61.2 (CH), 56.9 (CH), 54.5 (CH), 42.3 (CH$_2$), 40.8 (CH$_2$), 40.4 (CH$_2$), 38.8 (CH$_2$), 35.7 (CH, C-3), 33.6 (CH$_2$), 31.2 (CH$_2$), 29.3 (CH$_2$), 26.1 (CH$_2$), 16.1 (CH$_3$), 15.4 (CH$_3$), 11.4 (CH$_3$, C-6); **ES$^+$ MS m/z** 570 ([M+Na]$^+$ 100%); **HRMS (ESI)** m/z calcd. for C$_{23}$H$_{37}$N$_3$NaO$_6$S$_3$ (M+Na)$^+$ 570.1737, found 570.1745.

5.3.2.3. Total synthesis of the Brady's proposed diastereomer

(4S,5S)-Allyl 4-($tert$-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (2.227)
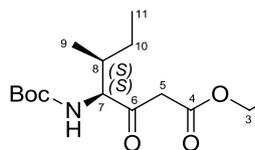
2.227

To a solution of Boc-L-Ile-OH (949 mg, 4.0 mmol, 1 equiv) in CH$_2$Cl$_2$ (15 mL), were added DMAP (96.5 mg, 0.8 mmol, 0.2 equiv), pentafluorophenol (800 mg, 4.4 mmol, 1.1 equiv) and EDCI (909 mg, 4.7 mmol, 1.2 equiv). The reaction mixture was stirred at rt for 4 h. The layers were separated after addition of HCl (1 M) to the reaction mixture. The organic layer was washed with saturated NaHCO$_3$ solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting white solid was placed under high vacuum.

To a solution of diisopropylamine (1.83 mL, 13.0 mmol, 3.3 equiv) in THF (4.5 mL), was added dropwise a solution of BuLi in hexanes (2.5 M, 5.7 mL, 14.2 mmol, 3.6 equiv) followed by allylacetate (1.35 mL, 12.5 mmol, 3.2 equiv) at –78 °C. The reaction was stirred at –78 °C for 30 min and a solution in THF (13 mL) of the intermediate ester of Boc-L-Ile-OH was added. After stirring at –78 °C for 3 h, the reaction mixture was quenched with HCl solution (1 M). The layers were separated and the aqueous layer was extracted with EtOAc (3 times). The combined organic layers were washed with saturated NaHCO$_3$ solution and then with saturated brine, dried over MgSO$_4$, and

concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **2.227** as a colourless oil (875 mg, 70%).

$[\alpha]_D^{23}$ −31.9 (*c* 0.42, CH$_3$OH); **IR** 3347, 2967, 2933, 1751, 1702, 1517, 1457, 1363, 1318, 1246, 1160, 1013, 990 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 6.06–5.84 (m, 1H, H-2), 5.34 (dq, *J*=17.2, 1.5 Hz, 1H, H-1), 5.26 (dd, *J*=10.6, 1.0 Hz, 1H, H-1), 5.05 (d, *J*=8.1 Hz, 1H, NH), 4.64 (d, *J*=5.6 Hz, 2H, H-3), 4.33 (dd, *J*=8.6, 4.5 Hz, 1H, H-7), 3.68–3.52 (m, 2H, H-5), 2.09–1.88 (m, 1H, H-8), 1.45 (s, 9H, C<u>Me</u>$_3$), 1.41–1.24 (m, 1H, H-10), 1.17–1.03 (m, 1H, H-10), 0.99 (d, *J*=7.1 Hz, 3H, H-9), 0.94–0.87 (m, 3H, H-11).

(3*R*,4*S*,5*S*)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (2.228)

2.228
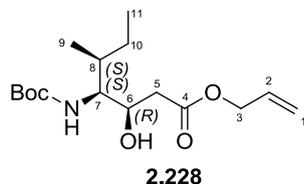
To a solution of **2.227** (467 mg, 1.5 mmol, 1 equiv) in anhydrous MeOH (5 mL) at −78 °C, was added KBH$_4$ in portions (281 mg, 5.2 mmol, 3.5 equiv). The reaction was stirred for 10 min at −78 °C, warmed to −20 °C for 30 min and to 0 °C for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO$_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to provide **2.228** as a colourless oil (295 mg, 63%).

^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 6.07–5.81 (m, 1H, H-2), 5.34 (dd, *J*=17.2, 1.5 Hz, 1H, H-1), 5.26 (d, *J*=10.6 Hz, 1H, H-1), 4.63 (d, *J*=6.1 Hz, 2H, H-3), 4.40 (d, *J*=9.6 Hz, 1H, NH), 4.13–3.93 (m, 1H, H-6), 3.66–3.48 (m, 1H, H-7), 3.24 (br d, *J*=4.5 Hz, 1H, OH), 2.62 (dd, *J*=16.2, 2.0 Hz, 1H, H-5), 2.56–2.41 (m, 1H, H-5), 1.93–1.68 (m, 1H, H-8), 1.68–1.51 (m, 1H, H-10), 1.45 (s, 9H, H-14), 1.12–0.84 (m, 7H, H-9; H-10; H-11).

The spectroscopic data are consistent with that[206] reported in the literature.

(3R,4S,5S)-Allyl 4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(trityl-thio)-propanamido)-3-hydroxy-5-methylheptanoate (2.229)

2.229
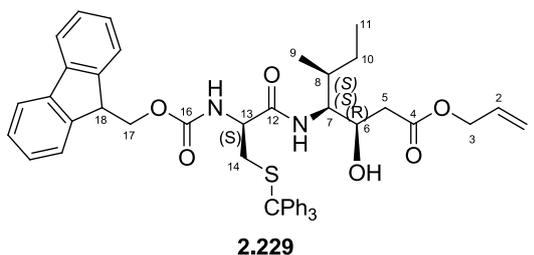
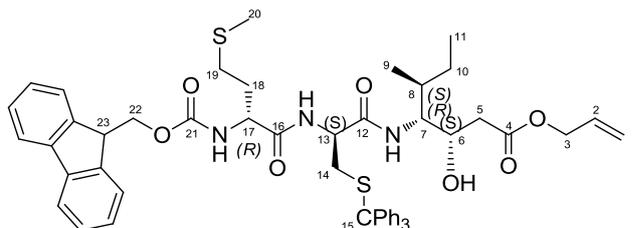Compound **2.228** (282 mg, 0.89 mmol, 1 equiv) was treated with TFA (2 mL) in CH_2Cl_2 (5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine.

To a solution of Fmoc-D-Cys(Tr)-OH (428 mg, 1.25 mmol, 1.4 equiv) in CH_2Cl_2 (6 mL) cooled at 0 °C, PyBOP (744 mg, 1.43 mmol, 1.6 equiv), and Hünig's base (467 µL, 2.68 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine in CH_2Cl_2 (2 mL) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) as eluent to give **2.229** as a colourless oil (477 mg, 68%).

$[\alpha]_D^{23}$ +8.6 (*c* 0.14, CH_3OH); **mp** 48–50 °C; **IR** 3407, 3326, 3056, 2962, 2929, 2868, 1716, 1659, 1524, 1446, 1315, 1250, 1172, 1042, 988, 903 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.78 (dd, *J*=7.3, 5.3 Hz, 2H, H$_{Ar}$), 7.58 (t, *J*=6.1 Hz, 2H, H$_{Ar}$), 7.52–7.37 (m, 8H, H$_{Ar}$), 7.35–7.16 (m, 11H, H$_{Ar}$), 5.98–5.73 (m, 2H, H-2; NH), 5.25 (dd, *J*=17.4, 1.3 Hz, 1H, H-1), 5.16 (dd, *J*=10.1, 1.0 Hz, 1H, H-1), 5.03 (d, *J*=7.1 Hz, 1H, NH), 4.54 (dd, *J*=5.8, 1.3 Hz, 2H, H-3), 4.40 (t, *J*=5.8 Hz, 2H, H-17), 4.21 (t, *J*=6.6 Hz, 1H, H-18), 4.09–3.97 (m, 1H, H-6), 3.94–3.71 (m, 2H, H-7; H-13), 2.80 (dd, *J*=13.1, 7.1 Hz, 1H, H-14), 2.64–2.48 (m, 2H, H-5; H-14), 2.43 (dd, *J*=9.1 Hz, 1H, H-5), 1.88–1.68 (m, 1H, H-8), 1.63–1.41 (m, 1H, H-10), 1.00–0.73 (m, 7H, H-9; H-10; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.6 (C, CO), 170.7 (C, CO), 156.1 (C, CO), 144.3 (3C, C$_{Ar}$), 143.7 (C, C$_{Ar}$), 143.6 (C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 131.8 (CH, C-2), 129.5 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 127.0 (3CH, C$_{Ar}$), 125.0 (2CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 118.5 (CH$_2$, C-1), 68.6 (CH, C-6), 67.4 (C, $\underline{C}$Ph$_3$), 67.2 (CH$_2$, C-3 or C-17), 65.4 (CH$_2$, C-3 or C-17), 57.7 (CH, C-7 or C-13), 54.4 (CH, C-7 or C-13), 47.0 (CH, C-18), 38.0 (CH$_2$, C-5), 34.5 (CH, C-8), 33.5 (CH$_2$, C-14), 23.5 (CH$_2$, C-10),

16.3 (CH$_3$, C-9), 11.5 (CH$_3$, C-11); **ES$^+$ MS m/z** 805 ([M+Na]$^+$ 100%); **HRMS (ESI)** m/z calcd. for C$_{48}$H$_{51}$N$_2$O$_6$S$_1$ (M+H)$^+$ 783.3462, found 783.3461.

(5S,8S,11S,12R)-Allyl 11-((S)-sec-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-5-(2-(methyl-thio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetra-decan-14-oate (2.230)
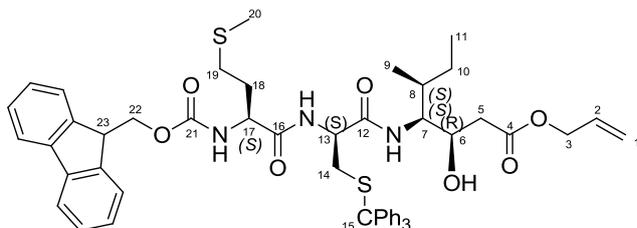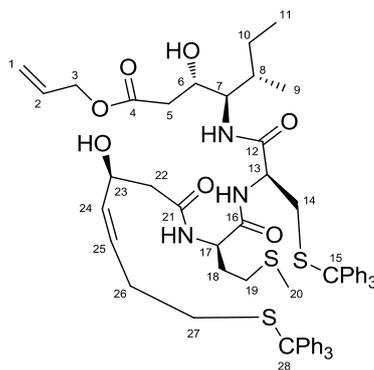

2.230

Compound **2.229** (470 mg, 0.6 mmol, 1 equiv) was treated with diethylamine (2.3 mL) in CH$_3$CN (11.6 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the free amine.

To a solution of Fmoc-L-Met-OH (290 mg, 0.8 mmol, 1.3 equiv) in CH$_2$Cl$_2$ (5 mL) cooled at 0 °C, PyBOP (500 g, 1.0 mmol, 1.6 equiv), and Hünig's base (314 μL, 1.8 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added in CH$_2$Cl$_2$ (1 mL). The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (2:8 then 3:7 then 4:6) as eluent to afford **2.230** as a colourless oil (495 mg, 90%).

[α]$_D^{23}$ −1.2 (c 0.35, CH$_3$OH); **IR** 3305, 3060, 2958, 2921, 1712, 1650, 1520, 1438, 1250, 1172, 1050 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 7.77 (d, J=7.2 Hz, 2H, H$_{Ar}$), 7.62–7.49 (m, 2H, H$_{Ar}$), 7.49–7.12 (m, 19H, H$_{Ar}$), 6.53 (d, J=7.5 Hz, 1H, NH), 6.23 (d, J=9.8 Hz, 1H, NH), 5.82 (tdd, J=16.9, 10.9, 5.5 Hz, 1H, H-2), 5.55 (d, J=7.2 Hz, 1H, NH), 5.24 (d, J=17.3 Hz, 1H, H-1), 5.16 (d, J=10.2 Hz, 1H, H-1), 4.52 (d, J=5.7 Hz, 2H, H-3), 4.47–4.23 (m, 2H, H-22), 4.20–4.02 (m, 3H, H-6; H-17; H-23), 4.02–3.91 (m, 1H, H-13), 3.91–3.77 (m, 1H, H-7), 3.26 (d, J=5.3 Hz, 1H, OH), 2.81 (dd, J=12.8, 7.5 Hz, 1H, H-14), 2.69–2.31 (m, 5H, H-5; H-14; H-19), 2.17–1.98 (m, 4H, H-18; H-20), 1.97–1.81 (m, 1H, H-18), 1.75–1.59 (m, 1H, H-8), 1.59–1.39 (m, 1H, H-10), 1.04–0.72 (m, 7H, H-9; H-10; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.4

288

(C, CO), 171.4 (C, CO), 170.3 (C, CO), 156.3 (C, CO), 144.3 (3C, C_{Ar}), 143.6 (C, C_{Ar}), 143.5 (C, C_{Ar}), 141.3 (2C, C_{Ar}), 131.9 (CH, C-2), 129.5 (6CH, C_{Ar}), 128.1 (6CH, C_{Ar}), 127.8 (2CH, C_{Ar}), 127.1 (2CH, C_{Ar}), 126.9 (3CH, C_{Ar}), 125.0 (CH, C_{Ar}), 124.9 (CH, C_{Ar}), 120.0 (2CH, C_{Ar}), 118.5 (CH_2, C-1), 68.7 (CH, C-6), 67.2 (C, $\underline{C}Ph_3$), 67.1 (CH_2, C-22), 65.4 (CH_2, C-3), 57.8 (CH, C-7), 54.0 (CH, C-17), 52.6 (CH, C-13), 47.1 (CH, C-23), 37.7 (CH_2, C-5), 34.7 (CH, C-8), 32.9 (CH_2, C-14), 30.4; 30.1 (2CH_2, C-18; C-19), 24.0 (CH_2, C-10), 16.1 (CH_3, C-9), 15.2 (CH_3, C-20), 11.3 (CH_3, C-11); **ES$^+$ MS** *m/z* 936 ([M+Na]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for $C_{53}H_{60}N_3O_7S_2$ (M+H)$^+$ 914.3867, found 914.3867.

(7S,11S,14R,17S,18R,E)-Allyl 17-((S)-*sec*-butyl)-7,18-dihydroxy-11-(2-(methyl-thio)-ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-((tritylthio)methyl)-2-thia-10,13,16-triazanonadec-5-en-19-oate (2.231)


2.231
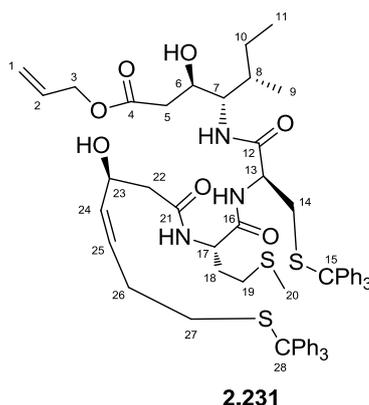
Compound **2.230** (478 mg, 0.53 mmol, 1 equiv) was treated with diethylamine (2 mL) in CH_3CN (10 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine.

To a solution of the β-hydroxy acid **2.219** (298 mg, 0.73 mmol, 1.4 equiv) in CH_2Cl_2 (10 mL) cooled at 0 °C, PyBOP (435 mg, 0.84 mmol, 1.6 equiv), and Hünig's base (274 μL, 1.57 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (4:6 then 1:1) as eluent to provide **2.231** as a white solid (347 mg, 61%).

[α]$_D^{23}$ +2.1 (*c* 0.26, CH_3OH); **mp** 130-132 °C; **IR** 3395, 3056, 2966, 2933, 1728, 1654, 1536, 1487, 1438, 1270, 1180, 1025, 976, 849 cm^{-1}; **^{1}H NMR (400 MHz,**

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CDCl$_3$/CD$_3$OD) δ ppm 7.61–7.04 (m, 30H, H$_{Ar}$), 5.97–5.76 (m, 1H, H-2), 5.60–5.45 (m, 1H, H-25), 5.39 (t, J=6.1 Hz, 1H, H-24), 5.28 (d, J=17.2 Hz, 1H, H-1), 5.19 (d, J=10.6 Hz, 1H, H-1), 4.56 (d, J=5.6 Hz, 2H, H-3), 4.39–4.29 (m, 1H, H-23), 4.24 (t, J=7.1 Hz, 1H, H-17), 4.18–4.02 (m, 2H, H-6; H-13), 3.87–3.70 (m, 1H, H-7), 2.64–2.16 (m, 10H, H-5; H-14; H-19; H-22; H-27), 2.16–1.98 (m, 6H, H-18; H-20; H-26), 1.92 (td, J=14.4, 7.5 Hz, 1H, H-18), 1.59 (q, J=7.6 Hz, 1H, H-8), 1.54–1.40 (m, 1H, H-10), 1.02–0.73 (m, 7H, H-9; H-10; H-11); **^{13}C NMR (100 MHz, CDCl$_3$/CD$_3$OD) δ ppm** 172.40 (C, CO), 172.36 (C, CO), 171.9 (C, CO), 171.0 (C, CO), 144.7 (3C, C$_{Ar}$), 144.2 (3C, C$_{Ar}$), 132.4 (CH), 131.7 (CH), 129.5 (CH), 129.4 (6CH, C$_{Ar}$), 129.3 (6CH, C$_{Ar}$), 127.8 (6CH, C$_{Ar}$), 127.7 (6CH, C$_{Ar}$), 126.7 (3CH, C$_{Ar}$), 126.4 (3CH, C$_{Ar}$), 118.1 (CH$_2$, C-1), 68.6 (CH, C-23), 68.4 (CH, C-6), 66.8 (C, CPh$_3$), 66.4 (C, CPh$_3$), 65.3 (CH$_2$, C-3), 57.7 (CH, C-7), 52.8 (CH, C-17), 52.5 (CH, C-13), 42.6 (CH$_2$), 40.0 (CH$_2$), 37.3 (CH$_2$), 34.5 (CH, C-8), 32.9 (CH$_2$), 31.3 (CH$_2$), 31.1 (CH$_2$), 29.9 (CH$_2$), 24.1 (CH$_2$, C-10), 15.8 (CH$_3$, C-9), 14.9 (CH$_3$, C-20), 10.8 (CH$_3$, C-11); **ES$^+$ MS m/z** 1115 ([M+Na]$^+$ 100%).

(1S,5S,6S,9R,20S,E)-6-((S)-sec-Butyl)-5-hydroxy-20-(2-(methylthio)ethyl)-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (2.29)
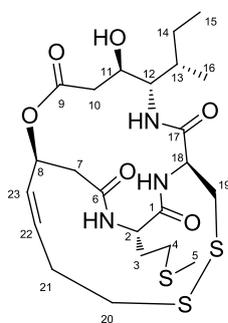
2.29

To a solution of **2.231** (326 mg, 0.30 mmol, 1 equiv) in methanol (10 mL), were added morpoline (55 µL, 0.63 mmol, 2.1 equiv) and Pd(PPh$_3$)$_4$ (35 mg, 30 µmol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH$_2$Cl$_2$ then 5% of MeOH in CH$_2$Cl$_2$ with formic acid) to afford the carboxylic acid as a yellow solid (315 mg, 100%).

A solution of the previous carboxylic acid (315 mg, 0.30 mmol, 1 equiv) in CH$_2$Cl$_2$ (250 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (124 mg, 0.36 mmol, 1.2 equiv) in CH$_2$Cl$_2$ (60 mL) containing

DMAP (88 mg, 0.72 mmol, 2.4 equiv) at room temperature over 4 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 99:1) to give the protected dithiol (224 mg, 68%) as a yellow solid.

To a vigorously stirring solution of I_2 (528 mg, 2.10 mmol, 10 equiv) in CH_2Cl_2/MeOH (390 mL:39 mL) was added the protected dithiol (215 mg, 0.21 mmol, 1 equiv) in MeOH/CH_2Cl_2 (220 mL:22 mL) dropwise over 2.5 h and stirred for a further 30 min. $Na_2S_2O_3$ (50 mL) was added, followed by brine (5 mL) and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/CH_2Cl_2 1:1 then CH_2Cl_2 then MeOH/CH_2Cl_2 1:99 to 2:98) to give **2.29** as a white solid (74 mg, 65%).

$[\alpha]_D^{32}$ −139.1 (*c* 0.40, CH_3OH); **mp** 148–150 °C; **IR** 3375, 3305, 2962, 2917, 1736, 1663, 1552, 1438, 1295, 1238, 1168 cm^{-1}; **^{1}H NMR (400 MHz, CD$_2$Cl$_2$) δ ppm ^{1}H NMR (400 MHz, CD$_2$Cl$_2$) δ ppm** 7.07–6.94 (m, 2H, NH), 6.65 (br s, 1H, NH), 5.94 (t, *J*=11.1 Hz, 1H, H-22), 5.67 (d, *J*=15.2 Hz, 1H, H-23), 5.67–5.64 (m, 1H, H-8), 4.91–4.79 (m, 1H, H-18), 4.19 (td, *J*=8.6, 4.3 Hz, 1H, H-11), 4.04–3.91 (m, 1H, H-2), 3.80 (dt, *J*=9.5, 4.3 Hz, 1H, H-12), 3.49–3.30 (m, 2H, H-19; H-20), 3.15–3.03 (m, 1H, H-19), 2.98 (dd, *J*=13.1, 7.1 Hz, 1H, H-7), 2.81–2.40 (m, 9H, OH; H-3; H-4; H-7; H-10; H-20; H-21), 2.40–2.22 (m, 2H, H-3; H-21), 2.09 (s, 3H, H-5), 1.92–1.77 (m, 1H, H-13), 1.61 (dtd, *J*=21.2, 7.5, 3.5 Hz, 1H, H-14), 1.02 (ddd, *J*=13.8, 6.7, 3.3 Hz, 1H, H-14), 0.95–0.77 (m, 6H, H-15; H-16); **^{13}C NMR (100 MHz, CD$_2$Cl$_2$) δ ppm** 171.6 (C, CO), 170.6 (C, CO), 170.4 (C, CO), 170.2 (C, CO), 132.1 (CH, C-22), 130.4 (CH, C-23), 69.7 (2CH, C-8; C-11), 60.3 (CH, C-12), 56.8 (CH, C-18), 54.8 (CH, C-2), 42.9 41.4, 41.0 (4CH$_2$, C-7; C-10; C-19; C-20), 36.0 (CH, C-13), 32.5 (CH$_2$, C-21), 31.5 (CH$_2$, C-4), 27.1 (CH$_2$, C-3), 23.4 (CH$_2$, C-14), 16.7 (CH$_3$, C-16), 15.2 (CH$_3$, C-5), 12.1 (CH$_3$, C-15); **HRMS (ESI)** *m/z* calcd. for $C_{23}H_{38}N_3O_6S_3$ (M+H)$^+$ 548.1917, found 548.1901.

5.3.2.4. Total synthesis of *epi*-burkholdac B

(4*R*,5*R*)-Allyl 4-(*tert*-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (2.232)
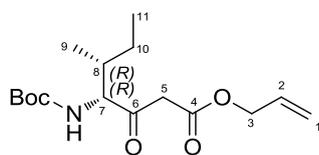
2.232

To a solution of Boc-D-Ile-OH (527 mg, 2.5 mmol, 1 equiv) in CH_2Cl_2 (10 mL), were added DMAP (61 mg, 0.5 mmol, 0.2 equiv), pentafluorophenol (507 mg, 2.8 mmol, 1.1 equiv) and EDCI (577 mg, 3.0 mmol, 1.2 equiv). The reaction mixture was stirred at rt for 4 h. The layers were separated after addition of HCl (1 M) to the reaction mixture. The organic layer was washed with saturated $NaHCO_3$ solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting white solid was placed under high vacuum.

To a solution of diisopropylamine (1.06 mL, 7.5 mmol, 3.3 equiv) in THF (2.5 mL), were added dropwise a solution of BuLi in hexanes (2.5 M, 3.3 mL, 8.2 mmol, 3.6 equiv) followed by allylacetate (779 μL, 7.2 mmol, 3.2 equiv) at −78 °C. The reaction was stirred at −78 °C for 30 min and a solution in THF (7.5 mL) of the intermediate ester of Boc-D-Ile-OH was added. After stirring at −78 °C for 3 h, the reaction mixture was quenched with HCl solution (1 M). The layers were separated and the aqueous layer was extracted with EtOAc (3 times). The combined organic layers were washed with saturated $NaHCO_3$ solution and then with saturated brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **2.232** as a colourless oil (480.3 mg, 67%).

$[\alpha]_D^{28}$ +42.7 (*c* 0.08, CH_3OH); **IR** 3342, 2966, 2925, 1753, 1704, 1503, 1458, 1368, 1307, 1246, 1160, 984, 935 cm^{-1}; **¹H NMR (400 MHz, CDCl₃) δ ppm** 6.06–5.81 (m, 1H, H-2), 5.34 (dd, *J*=17.2, 1.5 Hz, 1H, H-1), 5.26 (dd, *J*=10.1, 1.0 Hz, 1H, H-1), 5.03 (br d, *J*=8.1 Hz, 1H, NH), 4.64 (d, *J*=6.1 Hz, 2H, H-3), 4.33 (dd, *J*=8.6, 4.5 Hz, 1H, H-7), 3.67–3.52 (m, 2H, H-5), 2.07–1.88 (m, 1H, H-8), 1.45 (s, 9H, C*Me₃*), 1.41–1.27 (m, 1H, H-10), 1.16–1.04 (m, 1H, H-10), 1.00 (d, *J*=6.6 Hz, 3H, H-9), 0.96–0.85 (m, 3H, H-11); **¹³C NMR (100 MHz, CDCl₃) δ ppm** 202.2 (C, CO), 166.4 (C, CO), 155.7 (C, CO), 131.5 (CH, C-2), 118.8 (CH₂, C-1), 80.0 (C, *C*Me₃), 66.0 (CH₂, C-3), 64.3 (CH, C-7), 47.2 (CH₂, C-5), 36.3 (CH, C-8), 28.3 (3CH₃, C*Me₃*), 24.1 (CH₂, C-10), 16.0

(CH$_3$, C-9), 11.6 (CH$_3$, C-11); **ES$^+$ MS *m/z*** 336 ([M+Na]$^+$ 100%), 377 ([M+Na+CH$_3$CN]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for C$_{16}$H$_{27}$NNaO$_5$ (M+Na)$^+$ 336.1781, found 336.1781.

(3*S*,4*R*,5*R*)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (2.233)

2.233
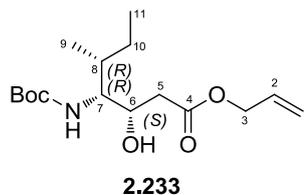
To a solution of **2.232** (465 mg, 1.49 mmol, 1 equiv) in anhydrous MeOH (5 mL) at −78 °C, was added KBH$_4$ in portions (281 mg, 5.22 mmol, 3.5 equiv). The reaction was stirred for 10 min at −78 °C, warmed to −20 °C for 30 min and to 0 °C for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO$_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) to provide **2.233** as a white solid (342 mg, 73%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 6.05–5.78 (m, 1H, H-2), 5.34 (dd, *J*=17.3, 1.5 Hz, 1H, H-1), 5.26 (d, *J*=10.5 Hz, 1H, H-1), 4.63 (d, *J*=5.7 Hz, 2H, H-3), 4.41 (d, *J*=10.2 Hz, 1H, NH), 4.12–3.95 (m, 1H, H-6), 3.69–3.48 (m, 1H, H-7), 3.26 (d, *J*=4.9 Hz, 1H, OH), 2.62 (dd, *J*=3.0 Hz, 1H, H-5), 2.49 (dd, *J*=9.0 Hz, 1H, H-5), 1.88–1.67 (m, 1H, H-8), 1.61–1.50 (m, 1H, H-10), 1.45 (s, 9H, CMe$_3$), 1.11–0.83 (m, 7H, H-9; H-10; H-11); **ES$^+$ MS *m/z*** 338 ([M+Na]$^+$ 100%), 379 ([M+Na+CH$_3$CN]$^+$ 100%).

The spectroscopic data are consistent with that[206] reported in the literature.

(3S,4R,5R)-Allyl 4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(trityl thio)-propanamido)-3-hydroxy-5-methylheptanoate (2.234)

2.234
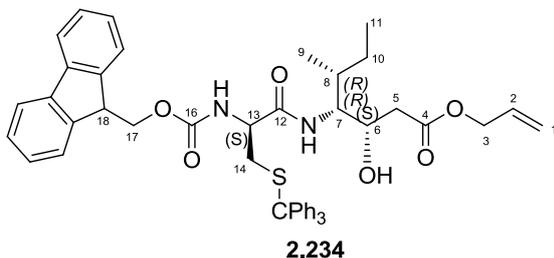
Compound **2.233** (336 mg, 1.07 mmol, 1 equiv) was treated with TFA (2 mL) in CH_2Cl_2 (5 mL) for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and $CHCl_3$ was performed to afford the free amine.

To a solution of Fmoc-D-Cys(Tr)-OH (401 mg, 1.17 mmol, 1 equiv) in CH_2Cl_2 (4 mL) at 0 °C, PyBOP (665 mg, 1.28 mmol, 1.2 equiv), and Hünig's base (557 µL, 3.19 mmol, 3 equiv) were added. After 10 min, the previous deprotected amine in CH_2Cl_2 (2 mL) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) as eluent to give **2.234** as a white solid (432.3 mg, 52%).

$[\alpha]_D^{28}$ +0.6 (*c* 0.47, CH_3OH); **mp** 60–62 °C; **IR** 3314, 3052, 2966, 1708, 1659, 1520, 1446, 1258, 1172, 1033 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.76 (t, *J*=7.3 Hz, 2H, H$_{Ar}$), 7.56 (t, *J*=7.6 Hz, 2H, H$_{Ar}$), 7.53–7.36 (m, 8H, H$_{Ar}$), 7.36–7.10 (m, 11H, H$_{Ar}$), 5.82 (br d, *J*=9.6 Hz, 2H, H-2; NH), 5.29 (d, *J*=16.7 Hz, 1H, H-1), 5.21 (d, *J*=10.6 Hz, 1H, H-1), 4.93 (d, *J*=7.6 Hz, 1H, NH), 4.56 (d, *J*=5.1 Hz, 2H, H-3), 4.49–4.30 (m, 2H, H-17), 4.30–4.14 (m, 1H, H-18), 4.05–3.90 (m, 1H, H-6), 3.90–3.77 (m, 1H, H-7), 3.73 (q, *J*=6.4 Hz, 1H, H-13), 3.25 (br s, 1H, OH), 2.77–2.59 (m, 2H, H-14), 2.53 (d, *J*=16.2 Hz, 1H, H-5), 2.40 (dd, *J*=16.4, 9.3 Hz, 1H, H-5), 1.87–1.70 (m, 1H, H-8), 1.58–1.45 (m, 1H, H-10), 1.00–0.75 (m, 7H, H-9; H-10; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.6 (C, CO), 170.6 (C, CO), 156.1 (C, CO), 144.3 (3C, C$_{Ar}$), 143.58 (C, C$_{Ar}$), 143.56 (C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 131.8 (CH, C-2), 129.5 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 126.9 (3CH, C$_{Ar}$), 124.92 (CH, C$_{Ar}$), 124.86 (CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 118.5 (CH$_2$, C-1), 68.5 (CH, C-6), 67.4 (C, $\underline{C}$Ph$_3$), 67.0 (CH$_2$, C-3 or C-17), 65.4 (CH$_2$, C-3 or C-17), 57.5 (CH, C-7), 54.2 (CH, C-13), 47.0 (CH, C-18), 38.1 (CH$_2$, C-5), 34.3 (CH, C-8), 33.2 (CH$_2$, C-14), 23.3 (CH$_2$, C-10), 16.2 (CH$_3$,

C-9), 11.6 (CH$_3$, C-11); **ES$^+$ MS** *m/z* 805 ([M+Na]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for C$_{48}$H$_{50}$N$_2$NaO$_6$S (M+Na)$^+$ 805.3282, found 805.3280.

(5R,8S,11R,12S)-Allyl 11-((R)-sec-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-5-(2-(methyl-thio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetradecan-14-oate (2.235)
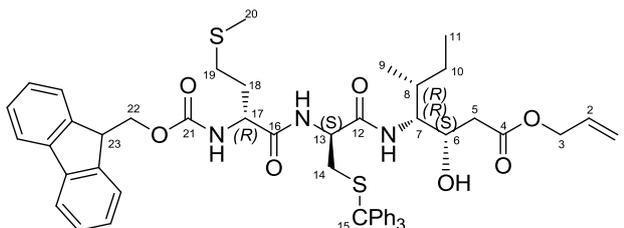
2.235

Compound **2.234** (405 mg, 0.52 mmol, 1 equiv) was treated with diethylamine (2 mL) in CH$_3$CN (10 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the free amine.

To a solution of Fmoc-D-Met-OH (250 mg, 0.67 mmol, 1.3 equiv) in CH$_2$Cl$_2$ (5 mL) cooled at 0 °C, PyBOP (431 g, 0.83 mmol, 1.6 equiv), and Hünig's base (271 µL, 1.55 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added in CH$_2$Cl$_2$ (1 mL). The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/Petroleum ether (2:8 then 3:7 then 4:6) as eluent to afford **2.235** as a white foam (398 mg, 84%).

[α]$_D^{23}$ +22.9 (*c* 0.29, CH$_3$OH); **mp** 144–146 °C; **IR** 3293, 3060, 2962, 2925, 1708, 1650, 1524, 1438, 1266, 1221, 1172, 1025 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.78 (d, *J*=7.6 Hz, 2H, H$_{Ar}$), 7.54 (dd, *J*=7.6, 3.5 Hz, 2H, H$_{Ar}$), 7.49–7.36 (m, 8H, H$_{Ar}$), 7.36–7.06 (m, 11H, H$_{Ar}$), 6.49 (d, *J*=6.1 Hz, 1H, NH), 6.09 (d, *J*=9.1 Hz, 1H, NH), 5.97–5.77 (m, 1H, H-2), 5.58 (d, *J*=6.1 Hz, 1H, NH), 5.30 (dd, *J*=17.2, 1.5 Hz, 1H, H-1), 5.21 (dd, *J*=10.4, 1.3 Hz, 1H, H-1), 4.70–4.50 (m, 2H, H-3), 4.43 (dd, *J*=10.4, 7.3 Hz, 1H, H-22), 4.37–3.79 (m, 6H, H-6; H-13; H-17; H-22; H-23), 3.93–3.79 (m, 1H, H-7), 3.24 (br s, 1H, OH), 2.86 (dd, *J*=12.6, 6.1 Hz, 1H, H-14), 2.61–2.34 (m, 5H, H-5; H-14; H-19), 2.18–1.94 (m, 4H, H-18; H-20), 1.94–1.73 (m, 2H, H-8; H-18), 1.47–1.63 (m, 1H, H-10), 1.06–0.77 (m, 7H, H-9; H-10; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ**

ppm 172.7 (C, CO), 171.0 (C, CO), 170.1 (C, CO), 156.2 (C, CO), 144.2 (3C, C$_{Ar}$), 143.7 (C, C$_{Ar}$), 143.4 (C, C$_{Ar}$), 141.33 (C, C$_{Ar}$), 141.29 (C, C$_{Ar}$), 131.9 (CH, C-2), 129.4 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 126.9 (3CH, C$_{Ar}$), 124.9 (2CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 118.3 (CH$_2$, C-1), 68.5 (CH, C-6), 67.22 (C, $\underline{C}$Ph$_3$), 67.19 (CH$_2$, C-22), 65.3 (CH$_2$, C-3), 57.7 (CH, C-7), 54.6 (CH, C-17), 52.8 (CH, C-13), 47.0 (CH, C-23), 38.0 (CH$_2$, C-5), 34.4 (CH, C-8), 32.9 (CH$_2$, C-14), 31.0 (CH$_2$, C-18), 30.2 (CH$_2$, C-19), 23.6 (CH$_2$, C-10), 16.2 (CH$_3$, C-9), 15.3 (CH$_3$, C-20), 11.6 (CH$_3$, C-11); **ES$^+$ MS** *m/z* 936 ([M+Na]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for C$_{53}$H$_{59}$N$_3$NaO$_7$S$_2$ (M+Na)$^+$ 936.3687, found 936.3679.

(7*R*,11*R*,14*S*,17*R*,18*S*,*Z*)-Allyl 17-*sec*-butyl-7,18-dihydroxy-11-(2-(methylthio)-ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-(tritylthiomethyl)-2-thia-10,13,16-triazaicos-5-en-20-oate (2.236)

2.236

2.235 (374 mg, 0.41 mmol, 1 equiv) was treated with diethylamine (2 mL) in CH$_3$CN (10 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the free amine.

To a solution of the β-hydroxy acid **2.219** (233 mg, 0.57 mmol, 1.4 equiv) in CH$_2$Cl$_2$ (10 mL) cooled at 0 °C, PyBOP (340 mg, 0.65 mmol, 1.6 equiv), and Hünig's base (214 µL, 1.23 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (4:6 then 1:1) as eluent to provide **2.236** as a white solid (375 mg, 84%).

[α]$_D^{23}$ +15.3 (*c* 0.32, CH$_3$OH); **mp** 84–86 °C; **IR** 3286, 3011, 2957, 2931, 1726, 1639, 1527, 1489, 1438, 1214, 1176, 1035, 974, 914 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$)** *δ*

ppm 7.55–7.12 (m, 31H, NH, H_{Ar}), 6.68 (d, J=7.1 Hz, 1H, NH), 6.14 (d, J=9.6 Hz, 1H, NH), 6.01–5.77 (m, 1H, H-2), 5.53–5.42 (m, 1H, H-25), 5.42–5.25 (m, 2H, H-1; H-24), 5.25–5.13 (m, 1H, H-1), 4.57 (dd, J=5.6, 1.5 Hz, 2H, H-3), 4.49–4.23 (m, 2H, H-17; H-23), 4.20–3.99 (m, 2H, H-6; H-13), 3.85 (dt, J=9.6, 6.3 Hz, 1H, H-7), 3.29 (d, J=4.5 Hz, 1H, OH), 2.92 (br s, 1H, OH), 2.74–2.62 (m, 1H, H-14), 2.60–2.16 (m, 9H, H-5; H-14; H-19; H-22; H-27), 2.15–2.02 (m, 6H, H-18; H-20; H-26), 2.02–1.89 (m, 1H, H-18), 1.87–1.68 (m, 1H, H-8), 1.64–1.46 (m, 1H, H-10), 1.05–0.79 (m, 7H, H-9; H-10; H-11); ^{13}**C NMR (100 MHz, CDCl$_3$)** δ **ppm** 172.6 (C, CO), 172.0 (C, CO), 171.0 (C, CO), 170.5 (C, CO), 144.8 (3C, C_{Ar}), 144.2 (3C, C_{Ar}), 132.3 (CH, C-25), 132.0 (CH, C-24), 130.4 (CH, C-2), 129.5 (6CH, C_{Ar}), 129.4 (6CH, C_{Ar}), 128.1 (6CH, C_{Ar}), 127.9 (6CH, C_{Ar}), 126.9 (3CH, C_{Ar}), 126.6 (3CH, C_{Ar}), 118.3 (CH$_2$, C-1), 69.9 (CH, C-23), 68.7 (CH, C-6), 66.9 (C, CPh$_3$), 66.6 (C, CPh$_3$), 65.3 (CH$_2$, C-3), 57.8 (CH, C-7), 53.9 (CH, C-17), 52.9 (CH, C-13), 44.1 (CH$_2$), 37.9 (CH$_2$), 34.5 (CH, C-8), 33.1 (CH$_2$), 31.3 (CH$_2$), 31.2 (CH$_2$), 30.5 (CH$_2$), 30.1 (CH$_2$), 23.9 (CH$_2$, C-10), 16.2 (CH$_3$, C-9), 15.4 (CH$_3$, C-20), 11.5 (CH$_3$, C-11); **ES$^+$ MS m/z** 1115 ([M+Na]$^+$ 100%).

Epi-Burkholdac B (2.237)
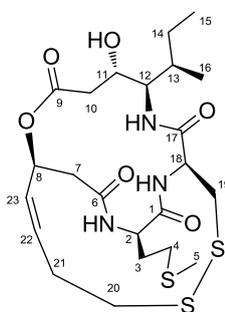
2.237

To a solution of **2.236** (345.7 mg, 0.32 mmol, 1 equiv) in methanol (10 mL), were added morpoline (58 μL, 0.66 mmol, 2.1 equiv) and Pd(PPh$_3$)$_4$ (37 mg, 31.6 μmol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH$_2$Cl$_2$ then 5% of MeOH in CH$_2$Cl$_2$ with formic acid) to afford the carboxylic acid as a yellow solid (333 mg, 100%).

A solution of the previous carboxylic acid (333 mg, 0.32 mmol, 1 equiv) in CH$_2$Cl$_2$ (250 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (131 mg, 0.38 mmol, 1.2 equiv) in CH$_2$Cl$_2$ (60 mL) containing

DMAP (93 mg, 0.76 mmol, 2.4 equiv) at room temperature over 5 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 99:1) to give the protected dithiol (224 mg, 68%) as a yellow solid.

To a vigorously stirring solution of I_2 (471 mg, 1.90 mmol, 10 equiv) in CH_2Cl_2/MeOH (350 mL: 35 mL) was added the protected dithiol (192 mg, 0.19 mmol, 1 equiv) in MeOH/CH_2Cl_2 (200 mL:20 mL) dropwise over 2.5 h and stirred for a further 30 min. $Na_2S_2O_3$ (50 mL) was added, followed by brine (5 mL) and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/CH_2Cl_2 1:1 then CH_2Cl_2 then MeOH/CH_2Cl_2 1:99 to 2:98) to give **2.237** as a white solid (60 mg, 59%).

$[\alpha]_D^{23}$ –45 (*c* 0.26, CH_3OH); **mp** 108–110 °C; **IR** 3324, 2960, 2918, 1724, 1653, 1529, 1431, 1261, 1160, 1047, 1020, 979 cm^{-1}; **^{1}H NMR (400 MHz, CD_2Cl_2) δ ppm** 7.41 (br s, 2H, NH), 6.74 (d, *J*=9.1 Hz, 1H, NH), 6.31 (t, *J*=13.1 Hz, 1H, H-22), 5.71 (d, *J*=15.7 Hz, 1H, H-23), 5.59–5.43 (m, 1H, H-8), 4.81 (dt, *J*=9.1, 3.5 Hz, 1H, H-18), 4.57–4.40 (m, 1H, H-11), 4.30 (td, *J*=8.5, 4.6 Hz, 1H, H-2), 3.44–3.21 (m, 3H, H-7; H-19; H-20), 3.14 (d, *J*=15.2 Hz, 1H, H-19), 2.99 (d, *J*=10.1 Hz, 1H, OH), 2.86–2.56 (m, 8H, H-4; H-7; H-10; H-12; H-20; H-21), 2.56–2.37 (m, 1H, H-21), 2.32–2.14 (m, 5H, H-3; H-5; H-13), 2.10–1.96 (m, 1H, H-3), 1.68–1.49 (m, 1H, H-14), 1.12–1.03 (m, 1H, H-14), 1.00 (d, *J*=6.6 Hz, 3H, H-16), 0.85 (t, *J*=7.6 Hz, 3H, H-15); **^{13}C NMR (100 MHz, CD_2Cl_2) δ ppm** 172.5 (C, C-9), 171.7 (C, C-6), 170.6 (C, C-1), 169.4 (C, C-17), 133.7 (CH, C-22), 129.8 (CH, C-23), 71.4 (CH, C-8), 70.1 (CH, C-11), 62.9 (CH, C-12), 58.0 (CH, C-2), 55.3 (CH, C-18), 41.9 (CH_2, C-19), 41.4 (2CH_2, C-7; C-20), 40.3 (CH_2, C-10), 36.3 (CH, C-13), 33.9 (CH_2, C-21), 31.7 (CH_2, C-4), 28.8 (CH_2, C-3), 26.5 (CH_2, C-14), 16.9 (CH_3, C-16), 15.8 (CH_3, C-5), 11.1 (CH_3, C-15); **ES$^+$ MS *m/z*** 570 ([M+Na]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for $C_{23}H_{37}N_3NaO_6S$ (M+Na)$^+$ 570.1737, found 570.1738.

5.3.2.5. Total synthesis of burkholdac B

(4R,5S)-Allyl 4-(*tert*-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (2.238)
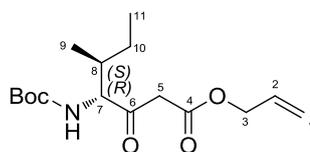
2.238

To a solution of Boc-D-*allo*-Ile-OH (870 mg, 3.8 mmol, 1 equiv) in anhydrous CH_2Cl_2 (15 mL), were added DMAP (92 mg, 0.8 mmol, 0.2 equiv), pentafluorophenol (762 mg, 4.1 mmol, 1.1 equiv) and EDCI (865 mg, 4.5 mmol, 1.2 equiv). The reaction mixture was stirred at rt overnight. The layers were separated after addition of HCl (1 M) to the reaction mixture. The organic layer was washed with saturated $NaHCO_3$ solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting colourless oil was placed under high vacuum.

To a solution of diisopropylamine (1.75 mL, 12.4 mmol, 3.3 equiv) in anhydrous THF (4.5 mL), were added dropwise a solution of BuLi in hexanes (2.5 M, 5.4 mL, 9.4 mmol, 3.6 equiv) followed by allylacetate (1.3 mL, 12.0 mmol, 3.2 equiv) at −78 °C. The reaction was stirred at −78 °C for 30 min and a solution in anhydrous THF (13 mL) of the intermediate ester of Boc-D-*allo*-Ile-OH was added. After stirring at −78 °C for 3 h, the reaction mixture was quenched with HCl solution (1 M). The layers were separated and the aqueous layer was extracted with EtOAc (3 times). The combined organic layers were washed with saturated $NaHCO_3$ solution and then with saturated brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **2.238** as a colourless oil (851 mg, 72%).

$[\alpha]_D^{23}$ +13.5 (*c* 0.28, CH_3OH); **IR** 3363, 2970, 2921, 2876, 1753, 1704, 1511, 1462, 1368, 1238, 1156, 993 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 6.06–5.80 (m, 1H, H-2), 5.34 (dd, *J*=17.2, 1.5 Hz, 1H, H-1), 5.26 (dd, *J*=10.6, 1.0 Hz, 1H, H-1), 5.05 (br d, *J*=9.1 Hz, 1H, NH), 4.64 (d, *J*=5.6 Hz, 2H, H-3), 4.48 (dd, *J*=9.1, 3.0 Hz, 1H, H-7), 3.57 (s, 2H, H-5), 2.07–1.89 (m, 1H, H-8), 1.45 (s, 9H, CMe$_3$), 1.28 (dquin, *J*=14.0, 7.2 Hz, 1H, H-10), 0.97 (t, *J*=7.3 Hz, 3H, H-11), 0.94–0.82 (m, 1H, H-10), 0.79 (d, *J*=7.1 Hz, 3H, H-9); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 202.2 (C, CO), 166.4 (C, CO), 155.9 (C, CO), 131.5 (CH, C-2), 118.9 (CH$_2$, C-1), 80.1 (C, $\underline{C}$Me$_3$), 66.1 (CH$_2$, C-3), 62.7

(CH, C-7), 46.7 (CH$_2$, C-5), 35.9 (CH, C-8), 28.3 (3CH$_3$, C$\underline{\text{Me}}_3$), 26.8 (CH$_2$, C-10), 13.8 (CH$_3$, C-9), 11.8 (CH$_3$, C-11).

(3*S*,4*R*,5*S*)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (2.239)

2.239
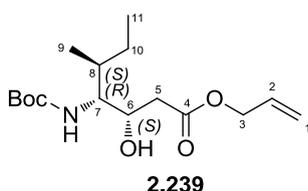
To a solution of **2.238** (819 mg, 2.6 mmol, 1 equiv) in anhydrous MeOH (9 mL) at −78 °C, was added KBH$_4$ in portions (493 mg, 9.1 mmol, 3.5 equiv). The reaction was stirred for 10 min at −78 °C, warmed to −20 °C for 30 min and to 0 °C for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 40 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO$_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) to provide **2.239** as a colourless oil (571.6 mg, 69%).

$[\alpha]_D^{23}$ −1.2 (*c* 0.52, CH$_3$OH); **IR** 3436, 3367, 2966, 2933, 2872, 1691, 1516, 1458, 1393, 1360, 1242, 1156, 1070, 980, 931 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 6.05–5.82 (m, 1H, H-2), 5.33 (dd, *J*=17.2, 1.0 Hz, 1H, H-1), 5.25 (d, *J*=10.6 Hz, 1H, H-1), 4.62 (d, *J*=5.6 Hz, 2H, H-3), 4.43 (br d, *J*=10.1 Hz, 1H, NH), 3.93 (dt, *J*=8.5, 2.3 Hz, 1H, H-6), 3.71–3.54 (m, 1H, H-7), 3.23 (br s, 1H, OH), 2.66 (dd, *J*=17.2, 3.0 Hz, H-5), 2.59–2.36 (m, 1H, H-5), 2.02–1.80 (m, 1H, H-8), 1.44 (s, 9H, CMe$_3$), 1.41–1.31 (m, 1H, H-10), 1.29–1.11 (m, 1H, H-10), 0.92 (t, *J*=7.6 Hz, 3H, H-11), 0.86 (d, *J*=6.6 Hz, 3H, H-9); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 173.0 (C, C-4), 156.2 (C, CO), 131.8 (CH, C-2), 118.6 (CH$_2$, C-1), 79.5 (C, $\underline{\text{C}}$Me$_3$), 69.1 (CH, C-6), 65.4 (CH$_2$, C-3), 56.6 (CH, C-7), 38.5 (CH$_2$, C-5), 33.9 (CH, C-8), 28.3 (3CH$_3$, C$\underline{\text{Me}}_3$), 27.0 (CH$_2$, C-10), 13.2 (CH$_3$, C-9), 11.7 (CH$_3$, C-11); **ES$^+$ MS *m/z*** 338 ([M+Na]$^+$ 100%), 379 ([M+Na+CH$_3$CN]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for C$_{16}$H$_{30}$NO$_5$ (M+H)$^+$ 316.2118 found 316.2122.

(3S,4R,5S)-Allyl 4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(tritylthio)-propanamido)-3-hydroxy-5-methylheptanoate (2.240)
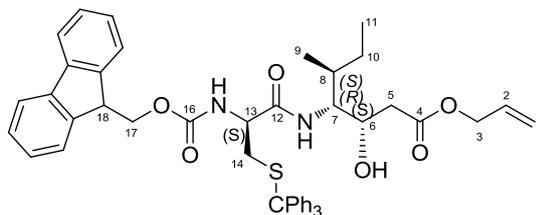
2.240

Compound **2.239** (547.6 mg, 1.74 mmol, 1 equiv) was treated with TFA (4 mL) in CH$_2$Cl$_2$ (10 mL) for 3 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the free amine.

To a solution of Fmoc-D-Cys(Tr)-OH (832 mg, 2.40 mmol, 1 equiv) in CH$_2$Cl$_2$ (8 mL) cooled at 0 °C, PyBOP (1.45 g, 2.78 mmol, 1.2 equiv), and Hünig's base (908 µL, 5.20 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine in CH$_2$Cl$_2$ (4 mL) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) as eluent to give **2.240** as a white foam (907 mg, 67%).

$[\alpha]_D^{24}$ +8.3 (*c* 0.45, CH$_3$OH); **mp** 44–46 °C; **IR** 3403, 3322, 3060, 2958, 1708, 1667, 1511, 1450, 1258, 1172, 1033, 988 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.75 (t, *J*=6.8 Hz, 2H, H$_{Ar}$), 7.54 (t, *J*=8.3 Hz, 2H, H$_{Ar}$), 7.49–7.34 (m, 8H, H$_{Ar}$), 7.33–7.13 (m, 11H, H$_{Ar}$), 5.97–5.72 (m, 2H, H-2; NH), 5.28 (d, *J*=17.2 Hz, 1H, H-1), 5.20 (d, *J*=10.6 Hz, 1H, H-1), 4.93 (d, *J*=7.6 Hz, 1H, NH), 4.64–4.48 (m, 2H, H-3), 4.48–4.30 (m, 2H, H-17), 4.18 (t, *J*=6.6 Hz, 1H, H-18), 4.00–3.81 (m, 2H, H-6; H-7), 3.70 (q, *J*=6.4 Hz, 1H, H-13), 3.22 (d, *J*=4.5 Hz, 1H, OH), 2.76–2.51 (m, 3H, H-5; H-14), 2.43 (dd, *J*=8.6 Hz, 1H, H-5), 1.89 (qd, *J*=6.9, 3.0 Hz, 1H, H-8), 1.19 (sxt, *J*=6.9 Hz, 1H, H-10), 1.07 (spt, *J*=7.2 Hz, 1H, H-10), 0.91–0.66 (m, 6H, H-9; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.9 (C, CO), 170.3 (C, CO), 156.1 (C, CO), 144.3 (3C, C$_{Ar}$), 143.6 (C, C$_{Ar}$), 143.5 (C, C$_{Ar}$), 141.3 (2C, C$_{Ar}$), 131.7 (CH, C-2), 129.5 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 126.9 (3CH, C$_{Ar}$), 124.91 (CH, C$_{Ar}$), 124.86 (CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 118.5 (CH$_2$, C-1), 68.5 (CH, C-6), 67.4 (C, $\underline{C}$Ph$_3$), 67.0 (CH$_2$, C-17), 65.4 (CH$_2$, C-3), 55.1 (CH, C-7), 54.2 (CH, C-13), 47.0 (CH, C-18), 38.3 (CH$_2$, C-5), 33.6 (CH, C-8), 33.2 (CH$_2$, C-14), 27.0 (CH$_2$, C-10), 13.2 (CH$_3$, C-9),

11.6 (CH₃, C-11); **ES⁺ MS *m/z*** 805 ([M+Na]⁺ 100%); **HRMS (ESI)** *m/z* calcd. for C₄₈H₅₁N₂O₆S₁ (M+H)⁺ 783.3462, found 783.3457.

(5*R*,8*S*,11*R*,12*S*)-Allyl   11-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-5-(2-(methyl-thio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetradecan-14-oate (2.241)

2.241

Compound **2.240** (617.5 mg, 0.79 mmol, 1 equiv) was treated with diethylamine (3 mL) in CH₃CN (15 mL) for 2.5 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine.

To a solution of Fmoc-D-Met-OH (381 mg, 1.03 mmol, 1.3 equiv) in CH₂Cl₂ (10 mL) cooled at 0 °C, PyBOP (657 mg, 1.26 mmol, 1.6 equiv), and Hünig's base (413 µL, 2.37 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added in CH₂Cl₂ (5 mL). The reaction mixture was stirred overnight and then concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/Petroleum ether (2:8 then 3:7 then 4:6) as eluent to afford **2.241** as a white foam (661.7 mg, 92%).

[α]$_D^{23}$ +28.1 (*c* 0.42, CH₃OH); **mp** 66–68 °C; **IR** 3309, 3060, 2962, 2925, 2868, 1699, 1650, 1524, 1442, 1238, 1172, 1037, 907 cm⁻¹; **¹H NMR (400 MHz, CDCl₃) δ ppm** 7.87 (d, *J*=7.6 Hz, 2H, H$_{Ar}$), 7.64 (dd, *J*=7.3, 3.8 Hz, 2H, H$_{Ar}$), 7.58–7.45 (m, 8H, H$_{Ar}$), 7.45–7.19 (m, 11H, H$_{Ar}$), 6.56 (d, *J*=6.6 Hz, 1H, NH), 6.22–6.06 (m, 1H, NH), 6.06–5.86 (m, 1H, H-2), 5.63 (d, *J*=6.6 Hz, 1H, NH), 5.39 (dd, *J*=17.2, 1.5 Hz, 1H, H-1), 5.30 (dd, *J*=10.4, 1.3Hz, 1H, H-1), 4.76–4.58 (m, 2H, H-3), 4.55–4.45 (m, 1H, H-22), 4.45–4.36 (m, 1H, H-22), 4.36–4.19 (m, 2H, H-17; H-23), 4.11–3.96 (m, 3H, H-6; H-7; H-13), 3.34 (d, *J*=4.0 Hz, 1H, OH), 2.92 (dd, *J*=12.6, 6.6 Hz, 1H, H-14), 2.75–2.45 (m, 5H, H-5; H-14; H-19), 2.24–2.06 (m, 4H, H-18; H-20), 2.06–1.86 (m, 2H, H-8; H-18), 1.46–1.29 (m, 1H, H-10), 1.29–1.14 (m, 1H, H-10), 1.04–0.84 (m, 6H, H-9; H-11); **¹³C**

NMR (100 MHz, CDCl$_3$) δ ppm 173.0 (C, CO), 171.0 (C, CO), 169.8 (C, CO), 156.2 (C, CO), 144.2 (3C, C$_{Ar}$), 143.7 (C, C$_{Ar}$), 143.5 (C, C$_{Ar}$), 141.33 (C, C$_{Ar}$), 141.29 (C, C$_{Ar}$), 131.8 (CH, C-2), 129.4 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.8 (2CH, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 126.9 (3CH, C$_{Ar}$), 124.9 (2CH, C$_{Ar}$), 120.0 (2CH, C$_{Ar}$), 118.4 (CH$_2$, C-1), 68.5 (CH, C-6), 67.21 (C, $\underline{C}$Ph$_3$), 67.17 (CH$_2$, C-22), 65.3 (CH$_2$, C-3), 55.4 (CH, C-7), 54.4 (CH, C-17), 52.8 (CH, C-13), 47.1 (CH, C-23), 38.3 (CH$_2$, C-5), 33.9 (CH, C-8), 32.9 (CH$_2$, C-14), 31.2 (CH$_2$, C-18), 30.2 (CH$_2$, C-19), 27.0 (CH$_2$, C-10), 15.3 (CH$_3$, C-20), 13.4 (CH$_3$, C-9), 11.7 (CH$_3$, C-11); **ES$^+$ MS *m/z*** 936 ([M+Na]$^+$ 100%); **HRMS (ESI)** *m/z* calcd. for C$_{53}$H$_{60}$N$_3$O$_7$S$_2$ (M+H)$^+$ 914.3867, found 914.3863.

(7*S*,11*R*,14*R*,17*R*,18*R*,E)-Allyl 17-((*S*)-*sec*-butyl)-7,18-dihydroxy-11-(2-(methyl--thio)-ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-((tritylthio)methyl)-2-thia-10,13,16-triazanona-dec-5-en-19-oate (2.242)

2.242

2.241 (601 mg, 0.66 mmol, 1 equiv) was treated with diethylamine (3.2 mL) in CH$_3$CN (15 mL) for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH$_3$CN and CHCl$_3$ was performed to afford the free amine.

To a solution of the β-hydroxy acid **2.219** (374 mg, 0.92 mmol, 1.4 equiv) in anhydrous CH$_2$Cl$_2$ (18 mL) cooled at 0 °C, PyBOP (547 mg, 1.05 mmol, 1.6 equiv) and Hünig's base (344 µL, 1.97 mmol, 3 equiv) were added. After 10 min, the previous deprotected amine was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (4:6 then 1:1) as eluent to provide **2.242** as a white solid (503 mg, 70%).

[α]$_D^{23}$ +19.4 (*c* 0.41, CH$_3$OH); **mp** 134–136 °C; **IR** 3273, 3077, 3056, 2958, 2909, 1720, 1630, 1544, 1483, 1442, 1385, 1250, 1176, 1074, 1033, 980, 903 cm^{-1}; **^{1}H NMR**

(400 MHz, CDCl$_3$) δ ppm 7.53–7.33 (m, 12H, H$_{Ar}$), 7.33–7.03 (m, 19H, NH; H$_{Ar}$), 6.57 (d, J=7.6 Hz, 1H, NH), 5.98 (d, J=9.6 Hz, 1H, NH), 5.91–5.75 (m, 1H, H-2), 5.51–5.39 (m, 1H, H-25), 5.39–5.22 (m, 2H, H-1; H-24), 5.18 (dd, J=10.4, 1.3 Hz, 1H, H-1), 4.54 (dd, J=5.3, 3.8 Hz, 2H, H-3), 4.47–4.26 (m, 2H, H-17; H-23), 4.05–3.77 (m, 3H, H-6; H-7; H-13), 3.27 (d, J=4.5 Hz, 1H, OH), 2.95 (d, J=3.0 Hz, 1H, OH), 2.71–2.12 (m, 10H, H-5; H-14; H-19; H-22; H-27), 2.12–1.97 (m, 6H, H-18; H-20; H-26), 1.97–1.79 (m, 2H, H-8; H-18), 1.23 (dq, J=13.9, 7.0 Hz, 1H, H-10), 1.10 (spt, J=7.4 Hz, 1H, H-10), 0.93–0.63 (m, 6H, H-9; H-11); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 172.9 (C, CO), 171.8 (C, CO), 171.0 (C, CO), 170.2 (C, CO), 144.8 (3C, C$_{Ar}$), 144.2 (3C, C$_{Ar}$), 132.3 (CH), 131.9 (CH), 130.4 (CH), 129.6 (6CH, C$_{Ar}$), 129.4 (6CH, C$_{Ar}$), 128.1 (6CH, C$_{Ar}$), 127.9 (6CH, C$_{Ar}$), 126.9 (3CH, C$_{Ar}$), 126.6 (3CH, C$_{Ar}$), 118.3 (CH$_2$, C-1), 69.9 (CH, C-23), 68.5 (CH, C-6), 67.0 (C, CPh$_3$), 66.6 (C, CPh$_3$), 65.3 (CH$_2$, C-3), 55.5 (CH, C-7), 53.6 (CH, C-17), 52.9 (CH, C-13), 44.0 (CH$_2$), 38.3 (CH$_2$), 33.9 (CH, C-8), 33.2 (CH$_2$), 31.3 (CH$_2$), 31.2 (CH$_2$), 30.4 (CH$_2$), 30.2 (CH$_2$), 27.0 (CH$_2$, C-10), 15.4 (CH$_3$, C-20), 13.6 (CH$_3$, C-9), 11.7 (CH$_3$, C-11); **ES$^+$ MS *m/z*** 1115 ([M+Na]$^+$ 100%).

Burkholdac B (2.243)
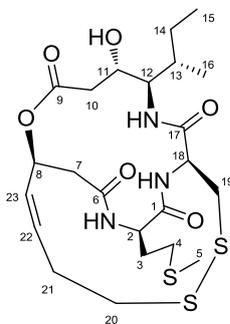
2.243

To a solution of **2.242** (473 mg, 0.43 mmol, 1 equiv) in anhydrous methanol (12 mL), were added morpoline (80 µL, 0.91 mmol, 2.1 equiv) and Pd(PPh$_3$)$_4$ (50 mg, 43 µmol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH$_2$Cl$_2$ then 5% of MeOH in CH$_2$Cl$_2$ with formic acid) to afford the carboxylic acid as a yellow solid (456 mg, 100%).

A solution of the previous carboxylic acid (456 mg, 0.43 mmol, 1 equiv) in CH$_2$Cl$_2$ (343 mL) was added dropwise to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (179 mg, 0.52 mmol, 1.2 equiv) in anhydrous CH$_2$Cl$_2$ (82 mL)

containing DMAP (127 mg, 1.04 mmol, 2.4 equiv) at room temperature over 5 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 99:1 to 98:2) to give the protected dithiol (170 mg, 38%) as a yellow solid.

To a vigorously stirring solution of I_2 (417 mg, 1.64 mmol, 10 equiv) in anhydrous CH_2Cl_2/MeOH (310 mL:31 mL) was added the protected dithiol (170 mg, 0.16 mmol, 1 equiv) in anhydrous CH_2Cl_2/MeOH (177 mL:18 mL) dropwise over 2.5 h and stirred for a further 30 min. $Na_2S_2O_3$ solution (50 mL) was added, followed by brine (5 mL) and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/ CH_2Cl_2 1:1 then CH_2Cl_2 then MeOH/CH_2Cl_2 1:99 to 2:98) to give burkholdac B (**2.243**) as a white solid (36 mg, 39%).

$[\alpha]_D^{32}$ −62.6 (*c* 0.34, CH_3OH), $[\alpha]_D^{25}$ −23.3 (*c* 0.08, CH_3CN), [lit.[85] $[\alpha]_D^{24}$ −22.8 (*c* 1.00, CH_3CN)]; **mp** 110–112 °C; **IR** 3334, 2962, 2925, 1728, 1650, 1520, 1430, 1274, 1160, 980 cm^{-1}; **^{1}H NMR (400 MHz, CD_2Cl_2)** δ **ppm** 7.41 (br s, 1H, NH), 7.24 (d, *J*=7.1 Hz, 1H, NH), 6.74 (d, *J*=9.6 Hz, 1H, NH), 6.45–6.26 (m, 1H, H-22), 5.71 (dt, *J*=15.7, 2.0 Hz, 1H, H-23), 5.55–5.44 (m, 1H, H-8), 4.84 (td, *J*=8.8, 3.5 Hz, 1H, H-18), 4.64–4.47 (m, 1H, H-11), 4.30 (td, *J*=8.7, 4.5 Hz, 1H, H-2), 3.46–3.31 (m, 1H, H-19), 3.26 (dd, *J*=13.1, 7.1 Hz, 2H, H-7; H-20), 3.10 (d, *J*=12.6 Hz, 1H, H-19), 2.94–2.82 (m, 2H, OH; H-12), 2.82–2.59 (m, 7H, H-4; H-7; H-10; H-20; H-21), 2.56–2.40 (m, 1H, H-21), 2.34–2.20 (m, 1H, H-3), 2.17 (s, 3H, H-5), 2.10–1.97 (m, 2H, H-3; H-13), 1.59–1.42 (m, 1H, H-14), 1.29–1.15 (m, 1H, H-14), 0.91 (t, *J*=7.6 Hz, 3H, H-15), 0.88 (d, *J*=6.6 Hz, 3H, H-16); **^{13}C NMR (100 MHz, CD_2Cl_2)** δ **ppm** 172.4 (C, C-9), 171.6 (C, C-6), 170.7 (C, C-1), 169.6 (C, C-17), 133.6 (CH, C-22), 129.7 (CH, C-23), 71.3 (CH, C-8), 68.7 (CH, C-11), 62.1 (CH, C-12), 57.9 (CH, C-2), 55.1 (CH, C-18), 42.1 (CH_2, C-19), 41.6; 41.4 (2CH_2,C-7; C-20), 40.1 (CH_2, C-10), 36.8 (CH, C-13), 34.0 (CH_2, C-21), 31.7 (CH_2, C-4), 28.8 (CH_2, C-3), 27.7 (CH_2, C-14), 15.8 (2CH_3, C-5; C-16), 11.9 (CH_3, C-15); **HRMS (ESI)** *m/z* calcd. for $C_{23}H_{41}N_4O_6S_3$ (M+NH$_4$)$^+$ 565.2183, found 565.2170.

The spectroscopic data are consistent with that[207] reported in the literature.

5.4. EXPERIMENTAL DATA FOR CHAPTER 3: HISTONE DEMETHYLASE INHIBITORS

5.4.1. Tranylcypromine analogues

5.4.1.1. Synthesis of tranylcypromine analogues

(–)-Tranylcypromine (3.63)
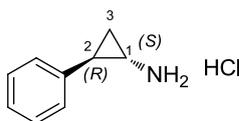
3.63

L-(+)-tartaric acid (193 mg, 1.28 mmol, 1 equiv) was dissolved in EtOH and (±)-tranylcypromine **3.6** (171 mg, 1.28 mmol, 1 equiv) was added. The reaction mixture was stirred 15 min at 0 °C and 30 min at rt. The salts precipitated after a few minutes. The reaction mixture was cooled and the diastereomeric tartrates were filtered, dried under high vacuum to provide a white solid (280 mg). Two recrystallisations using a mixture of iPrOH/water (3:1) were performed to afford 19.6 mg of a crystalline product (ee 89%).

$[\alpha]_D^{29}$ –26.8 (c 0.26, H$_2$O) [lit.[165] $[\alpha]_D^{25}$ –30.1 (c 1, H$_2$O)].

(–)-Tranylcypromine (+)-tartrate (19.6 mg) was taken up in water and the solution was made basic to pH 12 with KOH (60%) and the solution was extracted with ether. The combined ether extracts were washed with water, dried and concentrated. Water was added to the crude material and HCl (1 M) was added. The resulting salt was filtered, washed with diethyl ether to afford **3.63** as a white solid (6 mg, 6%).

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.43–7.02 (m, 5H, H$_{Ar}$), 2.85 (ddd, J=7.8, 3.7, 3.6 Hz, 1H, H-1), 2.40 (ddd, J=10.0, 6.5, 3.7 Hz, 1H, H-2), 1.43 (ddd, J=10.0, 6.7, 4.6 Hz, 1H, H-3), 1.33 (dt, J=8.0, 6.6 Hz, 1H, H-3); **^{13}C NMR (75 MHz, CD$_3$OD) δ ppm** 139.9 (C, C$_{Ar}$), 129.8 (CH, C$_{Ar}$), 128.0 (CH, C$_{Ar}$), 127.5 (CH, C$_{Ar}$), 32.1 (CH, C-1), 22.7 (CH, C-2), 13.9 (CH$_2$, C-3).

The spectroscopic data are consistent with that[57] reported in the literature.

(+)-Tranylcypromine (3.64)

3.64
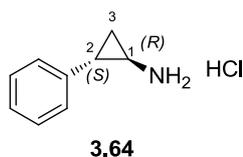
D-(−)-tartaric acid (175 mg, 1.16 mmol, 1 equiv) was dissolved in EtOH and (±)-tranylcypromine **3.6** (155 mg, 1.16 mmol, 1 equiv) was added. The reaction mixture was stirred 5 min at 0 °C and 30 min at rt. The salts precipitated after a few minutes. The mixture was cooled and the diastereomeric tartrates were filtered, dried under high vacuum to provide a white solid (246 mg). Two recrystallisations using a mixture of *i*PrOH/water (3:1) were performed to provide 43 mg of a white crystals product (ee 72%).

$[\alpha]_D^{24}$ +21.7 (*c* 0.30, H$_2$O) [lit.[165] $[\alpha]_D^{25}$ +30.1 (*c* 1, H$_2$O)].

(+)-tranylcypromine (−)-tartrate (43 mg) was taken up in water and the solution was made basic to pH 12 with KOH (60%) and the solution was extracted with ether. The combined ether extracts were washed with water, dried and concentrated. Water was added to the crude material and HCl (1 M) was added. The resulting salt was filtered, washed with diethyl ether to afford **3.64** as a white solid (11 mg, 11%).

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.38–7.10 (m, 5H, H$_{Ar}$), 2.85 (ddd, *J*=7.8, 3.7, 3.6 Hz, 1H, H-1), 2.39 (ddd, *J*=10.2, 6.6, 3.7 Hz, 1H, H-2), 1.43 (ddd, *J*=10.2, 7.0, 4.4 Hz, 1H, H-3), 1.34 (dt, *J*=7.8, 6.7 Hz, 1H, H-3); **^{13}C NMR (75 MHz, CD$_3$OD) δ ppm** 139.9 (C, C$_{Ar}$), 129.8 (CH, C$_{Ar}$), 128.0 (CH, C$_{Ar}$), 127.5 (CH, C$_{Ar}$), 32.1 (CH, C-1), 22.7 (CH, C-2), 13.9 (CH$_2$, C-3).

The spectroscopic data are consistent with that[57] reported in the literature.

Styrene asymmetric cyclopropanation to 3.67a-3.67e

(*S*,*S*)-2,2'-Isopropylidene-bis(4-*tert*-butyl-2-oxazoline) (0.01 equiv) and copper (II) trifluoromethanesulfonate (0.01 equiv) were dissolved in chloroform and stirred for 45 min under argon. Styrene derivatives **3.65** (5 equiv) and *tert*-butyl diazoacetate (1 equiv) were then added and the reaction mixture was stirred for 5 h, followed by

concentration *in vacuo* and purification of the crude material by flash chromatography using CH_2Cl_2/hexane (2:8) to provide the pure *trans*-diastereoisomers **3.67**.

(R,R)-tert-Butyl 2-(4-methoxyphenyl)cyclopropanecarboxylate (3.67a)

3.67a
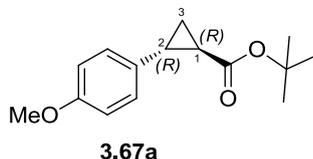
$[\alpha]^{23}_D$ −192.2 (*c* 0.24, $CHCl_3$), [lit.[208] $[\alpha]^{24}_D$ −241.4 (*c* 1.0, $CHCl_3$)]; **¹H NMR (400 MHz, $CDCl_3$) δ ppm** 7.03 (d, *J*=8.5 Hz, 2H, H_{Ar}), 6.83 (d, *J*=9.0 Hz, 2H, H_{Ar}), 3.79 (s, 3H, OMe), 2.47–2.34 (m, 1H, H-2), 1.76 (dt, *J*=8.7, 4.6 Hz, 1H, H-1), 1.52–1.50 (m, 1H, H-3), 1.48 (s, 9H, C$\underline{Me_3}$), 1.18 (ddd, *J*=8.0, 6.5, 4.5 Hz, 1H, H-3); **ES⁺ MS *m/z*** 303 ([M+Na+MeOH]⁺); **Yield** 77%.

The spectroscopic data are consistent with that[209] reported in the literature.

(R,R)-tert-Butyl 2-(4-fluorophenyl)cyclopropanecarboxylate (3.67b)

3.67b
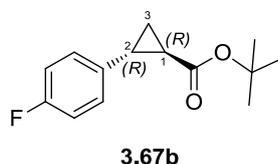
$[\alpha]^{25}_D$ −213.9 (*c* 0.44, $CHCl_3$), [lit.[209] $[\alpha]^{20}_D$ −182 (*c* 0.64, $CHCl_3$)]; **¹H NMR (300 MHz, $CDCl_3$) δ ppm** 7.10–7.02 (m, 2H, H_{Ar}), 7.01–6.93 (m, 2H, H_{Ar}), 2.43 (ddd, *J*=9.2, 6.5, 4.1 Hz, 1H, H-2), 1.78 (ddd, *J*=8.5, 5.3, 4.2 Hz, 1H, H-1), 1.52 (dd, *J*=9.5, 4.4 Hz, 1H, H-3), 1.48 (s, 9H, C$\underline{Me_3}$), 1.19 (ddd, *J*=8.4, 6.4, 4.5 Hz, 1H, H-3); **Yield** 46%.

The spectroscopic data are consistent with that[209] reported in the literature.

(R,R)-tert-Butyl 2-(4-bromophenyl)cyclopropanecarboxylate (3.67c)

3.67c
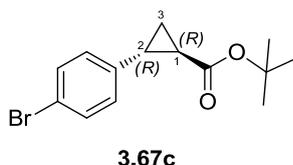
$[\alpha]^{25}_D$ –196.1 (*c* 0.41, CHCl$_3$), [lit. [209] $[\alpha]^{24}_D$ –216.7 (*c* 1.00, CHCl$_3$)]; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 7.10–7.02 (br d, *J*=8.5 Hz, 2H, H$_{Ar}$), 6.70 (br d, *J*=8.2 Hz, 2H, H$_{Ar}$), 2.40 (ddd, *J*=9.2, 6.3, 4.1 Hz, 1H, H-2), 1.80 (ddd, *J*=8.4, 5.4, 4.2 Hz, 1H, H-1), 1.54 (td, *J*=4.6, 0.8 Hz, 1H, H-3), 1.47 (s, 9H, CMe$_3$), 1.20 (ddd, *J*=8.5, 6.4, 4.5 Hz, 1H, H-3); **Yield** 45%.

The spectroscopic data are consistent with that[208] reported in the literature.

(*R,R*)-*tert*-Butyl 2-(3-bromophenyl)cyclopropanecarboxylate (3.67d)
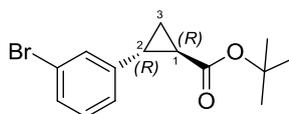
3.67d

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.33 (dq, *J*=7.7, 1.0 Hz, 1H, H$_{Ar}$), 7.23 (t, *J*=1.8 Hz, 1H, H$_{Ar}$), 7.14 (t, *J*=7.9 Hz, 1H, H$_{Ar}$), 7.03 (dt, *J*=7.8, 1.4 Hz, 1H, H$_{Ar}$), 2.41 (ddd, *J*=9.1, 6.2, 4.0 Hz, 1H, H-2), 1.83 (ddd, *J*=8.4, 5.3, 4.2 Hz, 1H, H-1), 1.61–1.50 (m, 1H, H-3), 1.48 (s, 9H, CMe$_3$), 1.22 (ddd, *J*=8.5, 6.3, 4.6 Hz, 1H, H-3); **Yield** 21%.

The spectroscopic data are consistent with that[210] reported in the literature.

(*R,R*)-*tert*-Butyl 2-(2-bromophenyl)cyclopropanecarboxylate (3.67e)
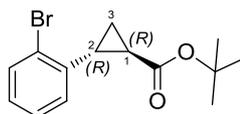
3.67e

$[\alpha]^{25}_D$ –66.3 (*c* 0.27, CH$_3$OH); Colourless oil; **IR** 3060, 1716, 1442 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 7.57 (dd, *J*=7.9, 1.3 Hz, 1H, H$_{Ar}$), 7.23 (td, *J*=7.5, 1.3 Hz, 1H, H$_{Ar}$), 7.09 (td, *J*=7.7, 1.8 Hz, 1H, H$_{Ar}$), 7.01 (dd, *J*=7.6, 1.5 Hz, 1H, H$_{Ar}$), 2.64 (ddd, *J*=9.0, 6.7, 4.5 Hz, 1H, H-2), 1.69 (dt, *J*=8.6, 5.1 Hz, 1H, H-1), 1.56 (dt, *J*=9.2, 4.7 Hz, 1H, H-3), 1.50 (s, 9H, CMe$_3$), 1.29 (ddd, *J*=8.4, 6.7, 4.6 Hz, 1H, H-3); **^{13}C NMR (75 MHz, CDCl$_3$) δ ppm** 172.4 (C, CO), 139.4 (C, C$_{Ar}$), 132.6 (CH, C$_{Ar}$), 128.0 (CH, C$_{Ar}$), 127.4 (CH, C$_{Ar}$), 127.3 (CH, C$_{Ar}$), 126.4 (C, C$_{Ar}$), 80.6 (C, CMe$_3$), 28.2 (3CH$_3$, CMe$_3$), 26.8 (CH, C-2), 24.3 (CH, C-1), 15.1 (CH$_2$, C-3); **EI MS *m/z*** 240, 242 ([M–tBu]$^+$); **Yield** 30%.

Ester hydrolysis of 3.67 to 3.68

The carboxylate esters **3.67** (1 equiv), TFA (13 equiv) and triethylsilane (2.5 equiv) were taken up in CH_2Cl_2. The reaction mixture was stirred for 90 min followed by concentration *in vacuo.* The crude material was used with no further purification or purified by flash chromatography using CH_2Cl_2 as eluent if required to provide **3.68**.

(R,R)-2-(4-Methoxyphenyl)cyclopropane carboxylic acid (3.68a)

3.68a
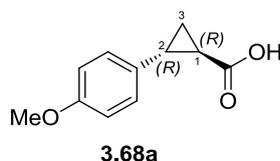
^{1}H NMR (300 MHz, CDCl₃) *δ* **ppm** 7.05 (d, *J*=8.7 Hz, 2H, H$_{Ar}$), 6.84 (d, *J*=8.7 Hz, 2H, H$_{Ar}$), 3.80 (s, 3H, OMe), 2.61–2.41 (m, 1H, H-2), 1.84 (dt, *J*=8.6, 4.6 Hz, 1H, H-1), 1.63 (dt, *J*=9.4, 4.8 Hz, 1H, H-3), 1.41–1.15 (m, 1H, H-3); **Yield** 78%.

The spectroscopic data are consistent with that[176] reported in the literature.

(R,R)-2-(4-Fluorophenyl)cyclopropane carboxylic acid (3.68b)

3.68b
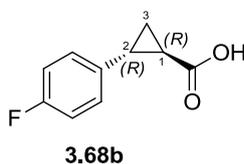
^{1}H NMR (300 MHz, CDCl₃) *δ* **ppm** 10.76 (br s, 1H, CO$_2$H), 7.32–7.20 (m, 2H, H$_{Ar}$), 7.20–7.08 (m, 2H, H$_{Ar}$), 2.62 (ddd, *J*=9.3, 6.6, 4.2 Hz, 1H, H-2), 1.88 (ddd, *J*=9.1, 5.1, 4.0 Hz, 1H, H-1), 1.68 (dt, *J*=9.4, 4.9 Hz, 1H, H-3), 1.39 (ddd, *J*=8.4, 6.7, 4.7 Hz, 1H, H-3); **Yield** 92%.

The spectroscopic data are consistent with that[176] reported in the literature.

(R,R)-2-(4-Bromophenyl)cyclopropanecarboxylic acid (3.68c)

3.68c
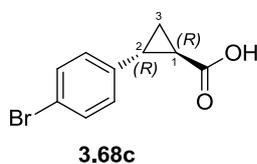
¹H NMR (300 MHz, CDCl₃) δ ppm 7.42 (br d, J=8.4 Hz, 2H, H$_{Ar}$), 6.99 (br d, J=8.4 Hz, 2H, H$_{Ar}$), 2.57 (ddd, J=9.2, 6.6, 4.0 Hz, 1H, H-2), 1.88 (ddd, J=8.5, 5.2, 4.2 Hz, 1H, H-1), 1.68 (dt, J=9.4, 5.0 Hz, 1H, H-3), 1.38 (ddd, J=8.4, 6.6, 4.8 Hz, 1H, H-3); **Yield** 98%.

The spectroscopic data are consistent with that[211] reported in the literature.

(*R,R*)-2-(3-Bromophenyl)cyclopropanecarboxylic acid (3.68d)
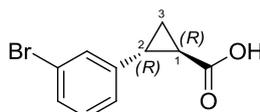
3.68d

¹H NMR (300 MHz, CDCl₃) δ ppm 7.40–7.33 (m, 1H, H$_{Ar}$), 7.28–7.25 (m, 1H, H$_{Ar}$), 7.17 (t, J=7.7 Hz, 1H, H$_{Ar}$), 7.05 (dt, J=7.8, 1.4 Hz, 1H, H$_{Ar}$), 2.58 (ddd, J=9.2, 6.6, 4.0 Hz, 1H, H-2), 1.91 (ddd, J=8.5, 5.2, 4.2 Hz, 1H, H-1), 1.68 (dt, J=9.9, 5.1 Hz, 1H, H-3), 1.41 (ddd, J=8.4, 6.8, 4.6 Hz, 1H, H-3); **Yield** 99%.

The spectroscopic data are consistent with that[212] reported in the literature.

(*R,R*)-2-(2-Bromophenyl)cyclopropanecarboxylic acid (3.68e)
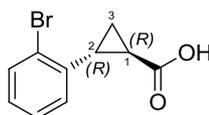
3.68e

[α]²⁵$_D$ –76.4 (*c* 0.29, CHCl₃), [lit.[212] (*S,S*)-enantiomer [α]$_D$ +109.9 (*c* 0.77, CHCl₃)]; **¹H NMR (300 MHz, CDCl₃) δ ppm** 7.59 (dd, J=7.9, 1.3 Hz, 1H, H$_{Ar}$), 7.24 (td, J=7.7, 1.1 Hz, 1H, H$_{Ar}$), 7.12 (td, J=7.6, 1.7 Hz, 1H, H$_{Ar}$), 7.05 (dd, J=7.7, 1.5 Hz, 1H, H$_{Ar}$), 2.80 (ddd, J=9.2, 7.0, 4.4 Hz, 1H, H-2), 1.83 (dt, J=8.4, 5.1 Hz, 1H, H-1), 1.71 (dt, J=9.2, 4.7 Hz, 1H, H-3), 1.44 (ddd, J=8.4, 7.0, 4.8 Hz, 1H, H-3); **Yield** 99%.

The spectroscopic data are consistent with that[213] reported in the literature.

Curtius rearrangement of 3.68 to 3.69

The acids **3.68** (1 equiv), diphenylphosphoryl azide (1.1 equiv) and triethylamine (1.5 equiv) were combined in *tert*-butanol under argon and heated at reflux for 48 h. The mixture was diluted with EtOAc and saturated Na_2CO_3 solution. The organic layer was separated and the aqueous layer was extracted with EtOAc. The organics layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/hexane 2:8) to provide the desired carbamates **3.69**.

(1*R*,2*S*)-*tert*-Butyl 2-(4-methoxyphenyl)cyclopropylcarbamate (3.69a)

3.69a
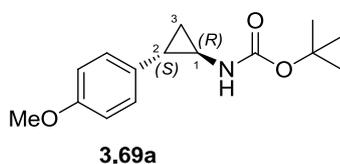
¹H NMR (300 MHz, CDCl₃) δ ppm 7.15–7.05 (m, 2H, H$_{Ar}$), 6.90–6.76 (m, 2H, H$_{Ar}$), 4.91 (br s, 1H, NH), 3.78 (s, 3H, OMe), 2.70–2.64 (m, 1H, H-1), 2.05–1.95 (m, 1H, H-2), 1.48 (s, 9H, C$\underline{Me}_3$), 1.16–1.03 (m, 2H, H-3); **Yield** 47%.

The spectroscopic data are consistent with that[176] reported in the literature.

(1*R*,2*S*)-*tert*-Butyl 2-(4-fluorophenyl)cyclopropylcarbamate (3.69b)

3.69b
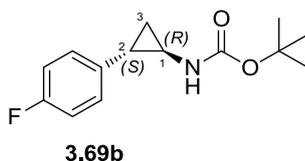
¹H NMR (300 MHz, CDCl₃) δ ppm 7.15–7.11 (m, 2H, H$_{Ar}$), 7.01–6.88 (m, 2H, H$_{Ar}$), 4.83 (br s, 1H, NH), 2.76–2.60 (m, 1H, H-1), 2.12–1.98 (m, 1H, H-2), 1.46 (s, 9H, C$\underline{Me}_3$), 1.19–1.04 (m, 2H, H-3); **Yield** 46%.

The spectroscopic data are consistent with that reported in the literature.

(1*R*,2*S*)-*tert*-Butyl 2-(4-bromophenyl)cyclopropylcarbamate (3.69c)

3.69c
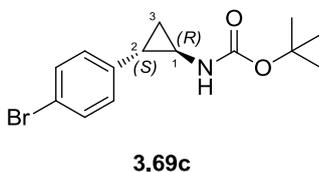
^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.38 (br d, *J*=8.3 Hz, 2H, H$_{Ar}$), 7.03 (br d, *J*=8.3 Hz, 2H, H$_{Ar}$), 4.82 (br s, 1H, NH), 2.73–2.64 (m, 1H, H-1), 2.09–1.94 (m, 1H, H-2), 1.46 (s, 9H, CM̲e$_3$), 1.19–1.08 (m, 2H, H-3); **ES$^+$ MS *m/z*** 334, 336 ([M+Na]$^+$); **Yield** 73%.

The spectroscopic data are consistent with that[214] reported in the literature.

(1*R*,2*S*)-*tert*-Butyl 2-(3-bromophenyl)cyclopropylcarbamate (3.69d)
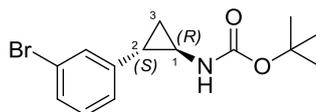
3.69d

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.43–7.37 (m, 1H, H$_{Ar}$), 7.31 (m, 1H, H$_{Ar}$), 7.13 (t, *J*=7.8 Hz, 1H, H$_{Ar}$), 7.10–7.04 (m, 1H, H$_{Ar}$), 4.82 (br s, 1H, NH), 2.77–2.69 (m, 1H, H-1), 2.02 (td, *J*=7.8, 3.0 Hz, 1H, H-2), 1.46 (s, 9H, CM̲e$_3$), 1.20–1.13 (m, 2H, H-3); **ES$^+$ MS *m/z*** 334, 336 ([M+Na]$^+$); **Yield** 52%.

The spectroscopic data are consistent with that[215] reported in the literature.

(1*R*,2*S*)-*tert*-Butyl 2-(2-bromophenyl)cyclopropylcarbamate (3.69e)
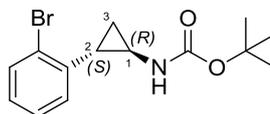
3.69e

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.64–7.53 (m, 1H, H$_{Ar}$), 7.36–7.22 (m, 1H, H$_{Ar}$), 7.18–7.04 (m, 2H, H$_{Ar}$), 5.05 (br s, 1H, NH), 2.90–2.65 (dt, *J*=7.9, 4.1 Hz, 1H, H-1), 2.25 (ddd, *J*=9.8, 6.4, 3.4 Hz, 1H, H-2), 1.52 (s, 9H, CM̲e$_3$), 1.40–1.14 (m, 2H, H-3); **ES$^+$ MS *m/z*** 334, 336 ([M+Na]$^+$); **Yield** 33%.

The spectroscopic data are consistent with that[216] reported in the literature.

Suzuki coupling of 3.69c-e to 3.69f-h

To a solution of the aryl bromide in a mixture of toluene/methanol/water (80:18:2) were added tetrakis(triphenylphosphine)palladium (0.2 equiv), phenylboronic acid or 4-

pyridylboronic acid (4 equiv), and Na_2CO_3 (2 equiv). The reaction mixture was refluxed overnight. The mixture was then diluted with EtOAc, washed with water and brine and purified by flash chromatography (EtOAc/hexane 5:95) to provide the biphenyl product.

(1*R*,2*S*)-*tert*-Butyl-2-(biphenyl-4-yl)cyclopropylcarbamate (3.69f)
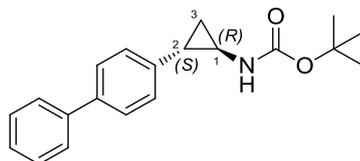
3.69f

$[\alpha]^{23}_D$ –99.4 (*c* 0.25, CHCl$_3$); **mp** 98–100 °C; **IR** 3338, 1687, 1523, 1483 cm^{-1}; **^{1}H NMR (300 MHz, CDCl$_3$) δ ppm** 7.61–7.48 (m, 4H, H$_{Ar}$), 7.44 (t, *J*=7.5 Hz, 2H, H$_{Ar}$), 7.35 (d, *J*=7.4 Hz, 1H, H$_{Ar}$), 7.21 (d, *J*=8.1 Hz, 2H, H$_{Ar}$), 4.88 (br s, 1H, NH), 2.85–2.72 (m, 1H, H-1), 2.09 (ddd, *J*=9.3, 6.4, 3.1 Hz, 1H, H-2), 1.48 (s, 9H, CMe$_3$), 1.33–1.08 (m, 2H, H-3); **^{13}C NMR (75 MHz, CDCl$_3$) δ ppm** 156.3 (C, CO), 141.0 (C, C$_{Ar}$), 139.9 (C, C$_{Ar}$), 139.0 (C, C$_{Ar}$), 128.7 (2CH, C$_{Ar}$), 127.0 (3CH, C$_{Ar}$), 126.9 (2CH, C$_{Ar}$), 126.8 (2CH, C$_{Ar}$), 79.7 (C, CMe$_3$), 32.6 (CH, C-1), 28.4 (3CH$_3$, CMe$_3$), 24.8 (CH, C-2), 16.4 (CH$_2$, C-3); **ES$^+$ MS *m/z*** 332 ([M+Na]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{20}$H$_{23}$NNaO$_2$ (M+Na)$^+$ 332.1621, found 332.1616; **Yield** 73%.

(1*R*,2*S*)-*tert*-Butyl-2-(biphenyl-3-yl)cyclopropylcarbamate (3.69g)
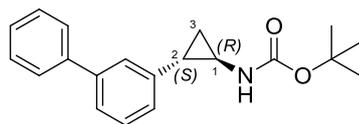
3.69g

$[\alpha]^{25}_D$ –50.8 (*c* 0.52, CH$_3$OH); **mp** 68–70 °C; **IR** 3330, 1687, 1511, 1483 cm^{-1}; **^{1}H NMR (400 MHz, CDCl$_3$) δ ppm** 7.67–7.52 (m, 2H, H$_{Ar}$), 7.50–7.39 (m, 3H, H$_{Ar}$), 7.38–7.31 (m, 3H, H$_{Ar}$), 7.13 (d, *J*=7.6 Hz, 1H, H$_{Ar}$), 4.87 (br s, 1H, NH), 2.89–2.73 (m, 1H, H-1), 2.12 (ddd, *J*=9.5, 6.4, 3.3 Hz, 1H, H-2), 1.48 (s, 9H, CMe$_3$), 1.32–1.13 (m, 2H, H-3); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 156.2 (C, CO), 141.3 (C, C$_{Ar}$), 141.2 (2C, C$_{Ar}$), 128.73 (CH, C$_{Ar}$), 128.69 (2CH, C$_{Ar}$), 127.23 (CH, C$_{Ar}$), 127.19 (2CH, C$_{Ar}$), 125.4 (CH, C$_{Ar}$), 125.3 (CH, C$_{Ar}$), 125.0 (CH, C$_{Ar}$), 79.7 (C, CMe$_3$), 32.6 (CH, C-1), 28.4 (3CH$_3$, CMe$_3$), 25.1 (CH, C-2), 16.6 (CH$_2$, C-3); **ES$^+$ MS *m/z*** 373

([M+Na+CH$_3$CN]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{20}$H$_{23}$NNaO$_2$ (M+Na)$^+$ 332.1621, found 332.1628; **Yield** 66%.

(1*R*,2*S*)-*tert*-Butyl-2-(biphenyl-2-yl)cyclopropylcarbamate (3.69h)
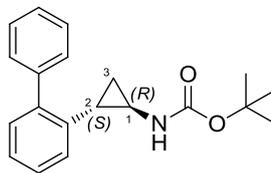
3.69h

mp 64–66 °C; **IR** 3338, 1696, 1480 cm^{-1}; ^{1}**H NMR (400 MHz, CDCl$_3$)** *δ* **ppm** 7.45 (d, *J*=5.1 Hz, 4H, H$_{Ar}$), 7.38 (dq, *J*=8.8, 4.3 Hz, 1H, H$_{Ar}$), 7.33–7.20 (m, 3H, H$_{Ar}$), 7.04 (d, *J*=7.1 Hz, 1H, H$_{Ar}$), 4.56 (br s, 1H, NH), 2.87–2.62 (m, 1H, H-1), 2.00 (ddd, *J*=9.9, 6.3, 3.0 Hz, 1H, H-2), 1.46 (s, 9H, C$\underline{Me}_3$), 1.15 (q, *J*=6.1 Hz, 1H, H-3), 1.08–0.98 (m, 1H, H-3); ^{13}**C NMR (100 MHz, CDCl$_3$)** *δ* **ppm** 156.2 (C, CO), 142.5 (C, C$_{Ar}$), 141.6 (C, C$_{Ar}$), 137.8 (C, C$_{Ar}$), 129.7 (2CH, C$_{Ar}$), 129.6 (2CH, C$_{Ar}$), 128.0 (CH, C$_{Ar}$), 127.5 (CH, C$_{Ar}$), 126.9 (CH, C$_{Ar}$), 125.9 (CH, C$_{Ar}$), 125.0 (CH, C$_{Ar}$), 79.5 (C, $\underline{C}$Me$_3$), 33.2 (CH, C-1), 28.3 (3CH$_3$, C$\underline{Me}_3$), 23.3 (CH, C-2), 17.0 (CH$_2$, C-3); **ES$^+$ MS** *m/z* 373 ([M+Na+CH$_3$CN]$^+$); **HRMS (ESI)** *m/z* calcd. for C$_{20}$H$_{23}$NNaO$_2$ (M+Na)$^+$ 332.1621, found 332.1626; **Yield** 18%.

***tert*-Butyl ((1*R*,2*S*)-2-(4-(pyridin-4-yl)phenyl)cyclopropyl)carbamate (3.74)**
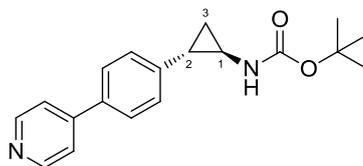
3.74

^{1}**H NMR (300 MHz, CDCl$_3$)** *δ* **ppm** 8.63 (d, *J*=5.3 Hz, 2H, H$_{Ar}$), 7.54 (br d, *J*=8.3 Hz, 2H, H$_{Ar}$), 7.47 (br d, *J*=6.4 Hz, 2H, H$_{Ar}$), 7.24 (br d, *J*=8.3 Hz, 2H, H$_{Ar}$), 5.01 (br s, 1H, NH), 2.82–2.72 (m, 1H, H-1), 2.15–2.06 (m, 1H, H-2), 1.46 (s, 9H, C$\underline{Me}_3$), 1.32–1.10 (m, 2H, H-3); ^{13}**C NMR (100 MHz, CDCl$_3$)** *δ* **ppm** 156.3 (C), 150.1 (2CH, C$_{Ar}$), 148.0 (C, C$_{Ar}$), 142.1 (C, C$_{Ar}$), 135.7 (C, C$_{Ar}$), 127.1 (2CH, C$_{Ar}$), 126.9 (2CH, C$_{Ar}$), 121.3

(2CH, C$_{Ar}$), 79.7 (C, <u>C</u>Me$_3$), 32.8 (CH, C-1), 28.4 (3C, C<u>Me$_3$</u>), 25.0 (CH, C-2), 16.4 (CH$_2$, C-3); **Yield** 43%.

Suzuki coupling of furyl derivatives (3.72 and 3.73)

3.69c or **3.69d** (1 equiv), Pd(PPh$_3$)$_4$ (0.16 equiv) and 2- or 3- furanylboronic acid (4 equiv) were dissolved in DME (4 mL), and the mixture was degassed for 1 min and stirred for 10 min at rt. To the mixture was added K$_2$CO$_3$ solution (2 M). The mixture was degassed again for 1 min and stirred at 85 °C for 2 days in a sealed tube. The resulting mixture was cooled to rt and poured into a mixture of HCl (0.1 N)/EtOAc (1:1). After partition, the organic layer was washed with water, filtered, and concentrated. The residue was purified by flash chromatography using 2.5-5% EtOAc in hexane to afford **3.72** or **3.73** as a yellow solid.

tert-**Butyl ((1*R*,2*S*)-2-(4-(furan-2-yl)phenyl)cyclopropyl)carbamate (3.72)**
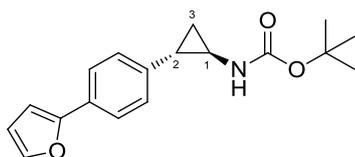
3.72

^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.57 (d, *J*=8.5 Hz, 2H, H$_{Ar}$), 7.45 (d, *J*=1.0 Hz, 1H, H$_{Ar}$), 7.15 (d, *J*=8.0 Hz, 2H, H$_{Ar}$), 6.60 (d, *J*=3.0 Hz, 1H, H$_{Ar}$), 6.46 (dd, *J*=3.3, 1.8 Hz, 1H, H$_{Ar}$), 4.85 (br s, 1H, NH), 2.90–2.62 (m, 1H, H-1), 2.05 (ddd, *J*=9.5, 6.5, 3.5 Hz, 1H, H-2), 1.47 (s, 9H, C<u>Me$_3$</u>), 1.22–1.12 (m, 2H, H-3); **^{13}C NMR (100 MHz, CDCl$_3$) δ ppm** 156.3 (C), 154.0 (C), 141.8 (CH, C$_{Ar}$), 140.0 (C, C$_{Ar}$), 128.8 (C, C$_{Ar}$), 126.7 (2CH, C$_{Ar}$), 123.8 (2CH, C$_{Ar}$), 111.6 (CH, C$_{Ar}$), 104.4 (CH, C$_{Ar}$), 79.7 (C, <u>C</u>Me$_3$), 32.6 (CH, C-1), 28.4 (3CH$_3$, C<u>Me$_3$</u>), 25.0 (CH, C-2), 16.4 (CH$_2$, C-3). **Yield** 21%.

tert-**Butyl ((1*R*,2*S*)-2-(3-(furan-2-yl)phenyl)cyclopropyl)carbamate (3.73)**
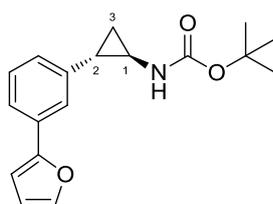
3.73

¹H NMR (400 MHz, CDCl₃) δ ppm 7.55–7.38 (m, 3H, H$_{Ar}$), 7.27 (td, *J*=7.6, 1.3 Hz, 1H, H$_{Ar}$), 7.04 (d, *J*=7.5 Hz, 1H, H$_{Ar}$), 6.66–6.62 (m, 1H, H$_{Ar}$), 6.46 (dt, *J*=3.4, 1.7 Hz, 1H, H$_{Ar}$), 4.85 (br s, 1H, NH), 2.84–2.75 (m, 1H, H-1), 2.15–1.98 (m, 1H, H-2), 1.47 (s, 9H, C**Me₃**), 1.29–1.08 (m, 2H, H-3); **¹³C NMR (100 MHz, CDCl₃) δ ppm** 156.3 (C), 153.9 (C), 142.0 (CH, C$_{Ar}$), 141.2 (C, C$_{Ar}$), 130.9 (C, C$_{Ar}$), 128.7 (CH, C$_{Ar}$), 125.6 (CH, C$_{Ar}$), 121.7; 121.6 (2CH, C$_{Ar}$), 111.6 (CH, C$_{Ar}$), 105.0 (CH, C$_{Ar}$), 79.7(C, CMe₃), 32.5 (CH, C-1), 28.4 (3CH₃, CMe₃), 25.0 (CH, C-2), 16.5 (CH₂, C-3); **Yield** 24%.

Boc deprotection of 3.69 to 3.70

Carbamate derivative **3.69** was taken up in anhydrous EtOAc cooled at 0 °C and HCl$_{(g)}$ was bubbled into the solution for 40-90 min. The reaction was monitored by TLC. The resulting HCl salt **3.70** was filtered and washed with diethyl ether.

(1*R*,2*S*)-2-(4-Methoxyphenyl)cyclopropanamine hydrochloride (3.70a)

3.70a
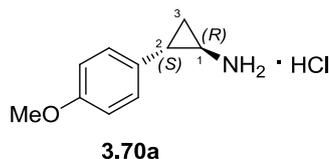
¹H NMR (300 MHz, CD₃OD) δ ppm 7.09 (d, *J*=8.8 Hz, 2H, H$_{Ar}$), 6.85 (d, *J*=8.4 Hz, 2H, H$_{Ar}$), 3.75 (s, 3H, OMe), 2.76 (dt, *J*=7.7, 3.7 Hz, 1H, H-1), 2.34 (ddd, *J*=10.2, 6.5, 3.5 Hz, 1H, H-2), 1.37 (ddd, *J*=10.4, 6.4, 4.4 Hz, 1H, H-3), 1.25 (q, *J*=6.6 Hz, 1H, H-3); **HRMS (ESI) *m/z*** calcd. for C₁₀H₁₄NO (M+H)⁺ 164.1070, found 164.1074; **Yield** 77%. The spectroscopic data are consistent with that[176] reported in the literature.

(1*R*,2*S*)-2-(4-Fluorophenyl)cyclopropanamine hydrochloride (3.70b)

3.70b
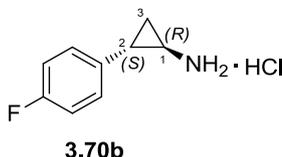
¹H NMR (300 MHz, CD₃OD) δ ppm 7.25–7.14 (m, 2H, H$_{Ar}$), 7.10–6.96 (m, 2H, H$_{Ar}$), 2.89–2.65 (m, 1H, H-1), 2.39 (ddd, *J*=10.1, 6.5, 3.6 Hz, 1H, H-2), 1.42 (ddd, *J*=10.2, 6.7, 4.4 Hz, 1H, H-3), 1.30 (dt, *J*=7.8, 6.7 Hz, 1H, H-3); **ES⁺ MS *m/z*** 193 ([M+H+CH₃CN]⁺); **HRMS (ESI) *m/z*** calcd. for C₉H₁₁FN (M+H)⁺ 152.0870, found 152.0870; **Yield** 35%.

The spectroscopic data are consistent with that[176] reported in the literature.

(1*R*,2*S*)-2-(4-Bromophenyl)cyclopropanamine hydrochloride (3.70c)

3.70c
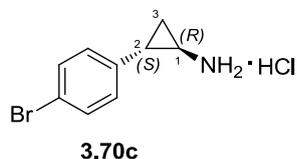
^{1}H NMR (400 MHz, CD$_3$OD) δ ppm 7.51 (d, *J*=8.5 Hz, 2H, H$_{Ar}$), 7.16 (d, *J*=8.5 Hz, 2H, H$_{Ar}$), 2.90 (quin, *J*=7.9 Hz, 1H, H-1), 2.42 (ddd, *J*=10.3, 6.5, 3.8 Hz, 1H, H-2), 1.49 (ddd, *J*=10.3, 6.8, 4.5 Hz, 1H, H-3), 1.39 (q, *J*=7.0 Hz, 1H, H-3); **ES$^+$ MS *m/z*** 253, 255 ([M+H+CH$_3$CN]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_9$H$_{11}$BrN (M+H)$^+$ 212.0069, found 212.0073; **Yield** 51%.

The spectroscopic data are consistent with that[215] reported in the literature.

(1*R*,2*S*)-2-(3-Bromophenyl)cyclopropanamine hydrochloride (3.70d)

3.70d
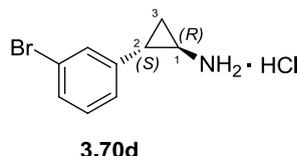
^{1}H NMR (400 MHz, CD$_3$OD) δ ppm 7.48–7.40 (m, 2H, H$_{Ar}$), 7.29 (t, *J*=7.8 Hz, 1H), 7.23–7.17 (m, 1H, H$_{Ar}$), 2.93 (quin, *J*=8.0 Hz, 1H, H-1), 2.42 (ddd, *J*=10.0, 6.5, 3.5 Hz, 1H, H-2), 1.49 (ddd, *J*=10.3, 6.3, 4.5 Hz, 1H, H-3), 1.41 (q, *J*=7.0 Hz, 1H, H-3); **ES$^+$ MS *m/z*** 253, 255 ([M+H+CH$_3$CN]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_9$H$_{11}$BrN (M+H)$^+$ 212.0069, found 212.0072; **Yield** 25%.

The spectroscopic data are consistent with that[215] reported in the literature.

(1*R*,2*S*)-2-(2-Bromophenyl)cyclopropanamine hydrochloride (3.70e)

3.70e
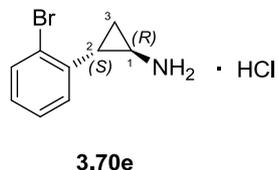
^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.63 (d, *J*=7.9 Hz, 1H, H$_{Ar}$), 7.34 (t, *J*=7.5 Hz, 1H, H$_{Ar}$), 7.18 (dd, *J*=16.2, 7.5 Hz, 2H, H$_{Ar}$), 2.96–2.79 (m, 1H, H-1), 2.73–2.53 (m, 1H, H-2), 1.62–1.35 (m, 2H, H-3); **Yield** 77%.

The spectroscopic data are consistent with that[216] reported in the literature.

(1R,2S)-2-(Biphenyl-4-yl)cyclopropanamine hydrochloride (3.70f)
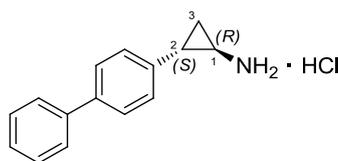
3.70f

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.70–7.53 (m, 4H, H$_{Ar}$), 7.44 (t, *J*=7.5 Hz, 2H, H$_{Ar}$), 7.39–7.21 (m, 3H, H$_{Ar}$), 2.91 (quin, *J*=4.1 Hz, 1H, H-1), 2.44 (ddd, *J*=10.1, 6.7, 3.6 Hz, 1H, H-2), 1.54–1.33 (m, 2H, H-3); **ES$^+$ MS *m/z*** 251 ([M+H+CH$_3$CN]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{15}$H$_{16}$N (M+H)$^+$ 210.1277, found 210.1282; **Yield** 94%.

The spectroscopic data are consistent with that[217] reported in the literature.

(1R,2S)-2-(Biphenyl-3-yl)cyclopropanamine hydrochloride (3.70g)
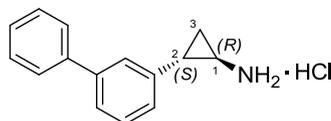
3.70g

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.73–7.56 (m, 2H, H$_{Ar}$), 7.56–7.32 (m, 6H, H$_{Ar}$), 7.18 (br d, *J*=7.5 Hz, 1H, H$_{Ar}$), 2.95 (quin, *J*=7.7 Hz, 1H, H-1), 2.50 (ddd, *J*=10.1, 6.7, 3.6 Hz, 1H, H-2), 1.58–1.38 (m, 2H, H-3); **ES$^+$ MS *m/z*** 210 ([M+H]$^+$), 251 ([M+H+CH$_3$CN]$^+$); **HRMS (ESI) *m/z*** calcd. for C$_{15}$H$_{16}$N (M+H)$^+$ 210.1277, found 210.1281; **Yield** 100%.

The spectroscopic data are consistent with that[217] reported in the literature.

(1R,2S)-2-(Biphenyl-2-yl)cyclopropanamine hydrochloride (3.70h)
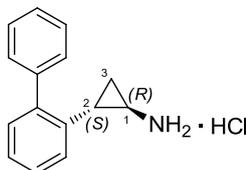
3.70h

[α]$^{25}_D$ −15.3 (*c* 0.07, CH$_3$OH); **mp** 52–54 °C; **IR** 1597, 1487, 1454 cm^{-1}; **^{1}H NMR (400 MHz, CD$_3$OD) δ ppm** 7.56–7.26 (m, 8H, H$_{Ar}$), 7.12–6.98 (m, 1H, H$_{Ar}$), 3.03–2.87 (m, 1H, H-1), 2.39 (td, *J*=8.6, 3.5 Hz, 1H, H-2), 1.07–1.45 (m, 2H, H-3); **^{13}C NMR (100 MHz, CD$_3$OD) δ ppm** 143.9 (C, C$_{Ar}$), 142.5 (C, C$_{Ar}$), 136.7 (C, C$_{Ar}$), 130.9 (CH, C$_{Ar}$), 130.5 (2CH, C$_{Ar}$), 129.5 (2CH, C$_{Ar}$), 128.9 (CH, C$_{Ar}$), 128.3 (CH, C$_{Ar}$), 127.7 (CH, C$_{Ar}$),

125.4 (CH, C_{Ar}), 32.3 (CH, C-1), 21.0 (CH, C-2), 14.8 (CH_2, C-3); **ES$^+$ MS *m/z*** 210 ([M+H]$^+$), 251 ([M+H+CH$_3$CN]$^+$); **HRMS (ESI) *m/z*** calcd. for $C_{15}H_{16}N$ (M+H)$^+$ 210.1277, found 210.1279; **Yield** 100%.

5.4.1.2. Biological testing of tranylcypromine analogues

Expression and purification of full length human LSD1 (performed by Dr P. Duriez)

The plasmid pET15b-His-tagged full length human LSD1 (provided by Fei Lan in Dr. Yang Shi's laboratory) was expressed in *E. Coli* BL21 RIPL Codon Plus (DE3) cells (Stratagene). The protein expression was induced with IPTG (0.1 mM) for 3 h at rt. The bacteria were centrifuged at 6,500 rpm for 10 min at 4 ℃. The supernatant was removed and the bacteria pellet was frozen down at –20 ℃. The bacterial pellet was lysed (40 mM Tris–HCl pH 8.0, 300 mM NaCl, 0.2% Triton X100, 5% glycerol, 10 µg/ml DNase I and 10 mM MgCl$_2$ in the presence of protease inhibitors), sonicated on ice and centrifuged at 15,000 rpm for 20 min at 4 ℃. The supernatant was used to purify LSD1 recombinant protein using an AKTA Prime FPLC system. Three chromatography steps (Nickel, gel filtration and Q-sepharose columns) were successively performed and the purified protein was stored in 20 mM Tris–HCl pH 8.0, 300 mM NaCl and 5% glycerol at –80 ℃.

Mass spectrometric LSD1 enzyme assay (performed by Dr P. Duriez with the help of Dr B. Zeidan and Prof P. Townsend)

In a total volume of 50 µL, 50 µM of tranylcypromine was incubated alone or in the presence of 2.4 µg of LSD1 at rt for 10 min. The peptide ARTK(me2)QTARKSTGGKAPRKQLA was added at a concentration of 35 µM and the reaction was carried out for an extra 20 min at rt. The samples were kept on ice before mass spectrometric analysis, carried out with NP20 ProteinChip (BioRad) arrays. An NP20 array was pre-rinsed with 5 µL of ultrapure H$_2$O and 3 µL sample was subsequently applied on each spot. The sample-loaded arrays were then incubated for 15 min in a humidity chamber at rt. Then, 1 µL of energy absorbing matrix [EAM; a-cyano-4-hydroxycinnamic acid (CHCA) in 200 µL acetonitrile and 200 µL of 1% TFA] was added to each spot, air dried and reapplied. ProteinChips were read using a PBS II SELDI mass spectrometer (Bio-Rad). Data acquisition was performed using the

following parameters: mass range between 0.5 and 20 kDa with a focus mass of 3 kDa and detector blinding at 0.5 kDa. The laser settings used were: intensity of 3000 nJ and 18 shots, 2 warming shots at an intensity of 3300 nJ, acquisition of one shot every four pixels in a randomised fashion.

Fluorescence based LSD1 enzyme assay

LSD1 enzymatic activity was measured with a synthetic peptide corresponding to the first 21 amino acids of human H3 dimethylated on lysine 4: ARTK(me2)QTARKSTGGKAPRKQLA (PeptideSynthetics). Assays were carried out in a final volume of 100 µL in white 96 microplate wells (Greiner). An aliquot of 1.25 µg of recombinant LSD1 enzyme was added to a 50 µL reaction containing 35 µM of peptide substrate in 50 mM potassium phosphate pH 7.2 and the reaction mixture was incubated at rt for 20 min. 50 µL of Amplex® Red/HRP were added and the reaction mixture kept at rt for another 30 min before quenching by adding 20 µL of Amplex® Red Stop solution (InVitrogen). Fluorescence was measured on a Varioskan Flash microplate reader (Thermo Fisher) (λ excitation = 530 nm and λ emission = 590 nm).

Preparation of Amplex® Red

The following reagents were added to prepare the Amplex® Red/HRP solution required for 10 assays:

- 5 µL of 10 mM Amplex® Red (InVitrogen)
- 10 µL of 10 U/mL HRP (prepared with 2 µL of 1000 U/mL HRP stock solution (Sigma) in 198 µL of 50 mM potassium phosphate buffer)
- 485 µL of 50 mM potassium phosphate buffer.

If the number of assay is higher, the quantities used are increased proportionally. 50 µL of this mix is added in each well.

Reconstitution of Amplex® Red Stop reagent

Amplex® Red Stop reagent contained in one vial (InVitrogen) was dissolved in ethanol (1.45 mL). 1.35 mL of this solution was transferred in a vial and diluted with 1.35 mL of water. The solution was stored in a cold room in the dark.

For assays with inhibitors, the compound was incubated with LSD1 for 10 min prior to the addition of the peptide substrate. The RFU is taken for several concentrations (5 to 8 concentrations). $Ki_{(inact)}$ values are determined from two to three dose–response curves for each compound (Table 5.1). The sigmoidal dose response curves were obtained using GraphPad Prism software.

Compound	Structure	Ki (1) µM	Ki (2) µM	Ki (3) µM	Ki average	STDEV
PCPA racemic	(±)	25.29	34.25	15.31	24.95	9.5
(-)-PCPA		30.06	39.98	14.31	28.12	12.9
(+)-PCPA		26.62	38.07	13.73	26.14	12.2
3.70c		3.82	4.146	3.227	3.73	0.5
3.70d		12.32	8.436	6.077	8.94	3.2
3.70e		11.32	12.75	11.05	11.71	0.9
3.70f		5.18	8.714	9.047	7.65	2.1
3.81		1.29	3.46	/	2.38	1.5
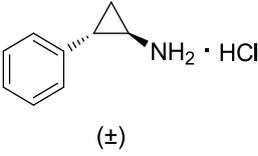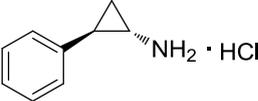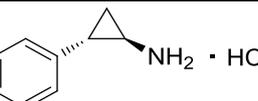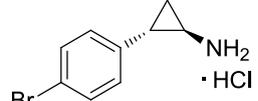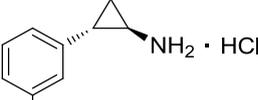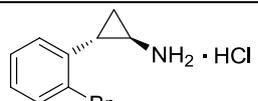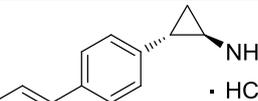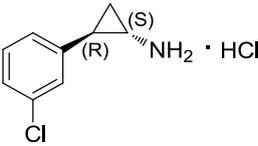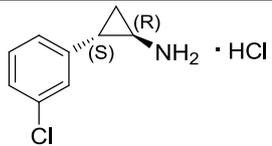
	Structure					
3.82		4.76	7.84	/	6.30	2.2
3.83		4.3	9.06	/	6.68	3.4
3.84		13.38	9.24	/	11.31	2.9
3.85		15.17	11.6	/	13.39	2.5
3.86		20.81	12.65	/	16.73	5.8
3.90		0.62	0.91	/	0.77	0.2
3.91		1.49	2.64	/	2.06	0.8
3.92		2.02	2.25	/	2.13	0.2
3.93		6.31	7.08	/	6.70	0.5
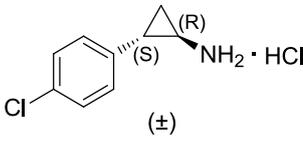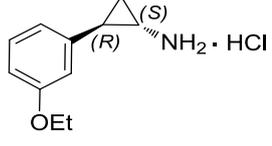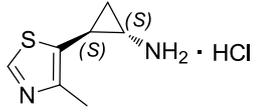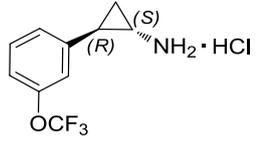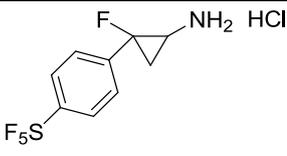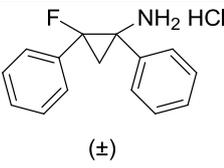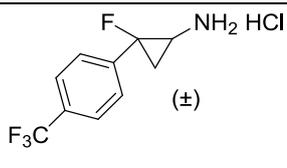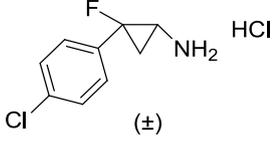
Table 5.1 : Activity of the tranylcypromine derivatives in LSD1 enzyme assay

LNCaP cell growth inhibition assay

This experiment was done by Rosemary Bulleid, Annette Hayden and Simon Crabb.

Tranylcypromine analogues prepared:

Six-day proliferation assay were undertaken using the LNCaP prostate adenocarcinoma cell line to produce IC_{50} values for inhibition. The IC_{50} are obtained from two experiments for each compound (Table 5.2).

Compound	Substitution	IC_{50} (µM)		Mean (µM)	STDEV	SEM
		IC_{50} (1)	IC_{50} (2)			
(±)-Tranylcypromine	/	236200	111800	**174000**	87964.08	**62200**
3.70a	4-OMe	1790	1621	**1705.5**	119.501	**84.5**
3.70b	4-F	189400	297600	**243500**	76508.95	**54100**
3.70c	4-Br	38.63	183.6	**111.115**	102.5093	**72.485**
3.70d	3-Br	126.9	131.4	**129.15**	3.181981	**2.25**
3.70e	2-Br	6201	5537	**5869**	469.5189	**332**
3.70f	4-Ph	88.2	131.7	**109.95**	30.75914	**21.75**
3.70g	3-Ph	298.1	155.1	**226.6**	101.1	**5.75**
3.70h	2-Ph	175.8	187.3	**181.6**	8.1	**71.5**

Table 5.2 : Activity of the tranylcypromine derivatives in LNCaP growth inhibition

Examples of dose response obtained for LNCaP cell growth inhibition assay: (for 4-Ph, 4-F, tranylcypromine, 3-Br, 4-Br and 4-OMe)

First IC$_{50}$ determination:

Dose response: Novel LSD1 inhibitors, 6 day LNCaP assay
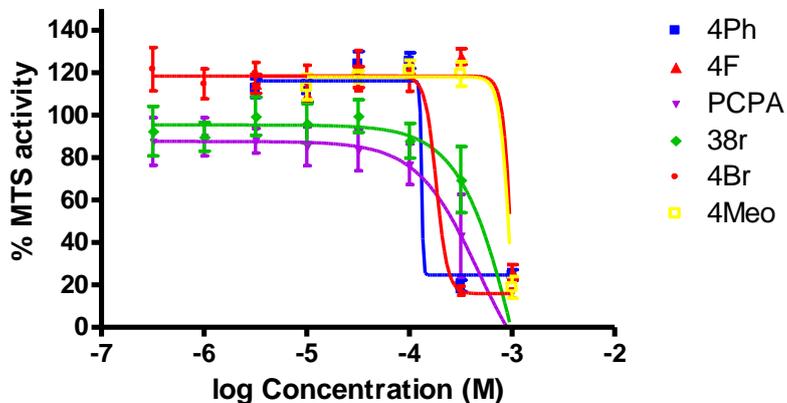
	4Ph	4F	PCPA	38r	4Br	4Meo
BOTTOM	24.69	-163.3	-34.72	-148.8	15.89	-64.56
TOP	116.1	118.4	87.64	95.40	118.5	118.0
LOGEC50	-3.880	-2.965	-3.328	-2.881	-3.736	-3.009
HILLSLOPE	-57.33	-8.997	-1.444	-1.488	-7.870	-8.335
EC50	0.0001317	0.001083	0.0004703	0.001314	0.0001836	0.0009792

Second IC$_{50}$ determination:

LNCAP
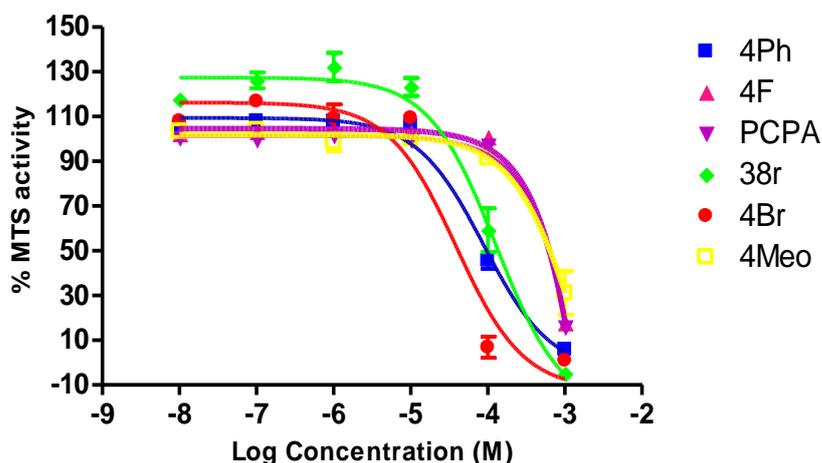
	4Ph	4F	PCPA	38r	4Br	4Meo
BOTTOM	-4.313	-16330	-20100	-22.29	-12.24	-95.63
TOP	109.4	104.6	101.4	127.4	116.3	101.9
LOGEC50	-4.055	-0.7227	-0.6268	-3.896	-4.413	-2.747
EC50	8.820e-005	0.1894	0.2362	0.0001269	3.863e-005	0.001790

German compounds activity in LNCaP growth inhibition:

The IC_{50} are also obtained from two experiments for each compound (Table 5.3).

Compound	IC$_{50}$(µM)		Mean (µM)	SEM
	IC$_{50}$ (1)	IC$_{50}$ (2)		
PCPA	1204	1060	1132	72.00
3.90	11.15	2.18	6.67	4.48
3.91	1.139	1.93	1.53	0.40
3.92	35.67	10.78	23.23	12.45
3.93	3.06	2.46	2.76	0.30

Table 5.3 : Activity of the German compounds in LNCaP growth inhibition

5.4.2. Phenelzine analogues

5.4.2.1. Synthesis of phenelzine analogues

2-(4-Bromophenyl)ethanol (3.114)
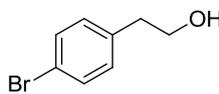
3.114

To a solution of 4-bromophenylacetic acid (1 g, 4.7 mmol, 1 equiv) in THF (10 mL) was added dropwise $BH_3.Me_2S$ (850 µL, 14.0 mmol, 3 equiv) in THF (3 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h, and then warming up at rt for 3 h. To the reaction mixture cooled to 0 °C was added dropwise a solution of sodium hydroxide (3 M). The mixture was stirred at rt overnight. Granulated sodium hydroxide was added to adjust the pH to 11. The aqueous layer was saturated with potassium carbonate and the THF layer was separated. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and evaporated to provide **3.114** as a colourless oil (935 mg, 100%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.44 (d, *J*=8.3 Hz, 2H, H$_{Ar}$), 7.12 (d, *J*=8.3 Hz, 2H, H$_{Ar}$), 3.86 (t, *J*=6.6 Hz, 2H, C$\underline{H}_2$OH), 2.84 (t, *J*=6.6 Hz, 2H, C$\underline{H}_2$-Ph), 1.48 (br s, 1H, OH).

The spectroscopic data are consistent with that[218] reported in the literature.

2-(*para*-Bromophenyl)ethyl tosylate (3.112)
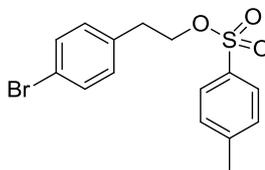
3.112

To a solution of 2-(4-bromophenyl)ethanol (**3.114**) (970 mg, 4.8 mmol, 1 equiv) in CH_2Cl_2 (20 mL) were added triethylamine (1.68 mL, 12.0 mmol, 2.5 equiv) and tosylchloride (1.4 g, 7.2 mmol, 1.5 equiv) at 0 °C. The reaction mixture was stirred at rt overnight. The reaction mixture was washed with a saturated NH_4Cl solution. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude

material was purified by flash chromatography (Hexane/EtOAc 9:1) to afford **3.112** as a white solid (1.57 g, 92%).

¹H NMR (300 MHz, CDCl₃) δ ppm 7.66 (d, J=8.3 Hz, 2H, H$_{Ar}$), 7.35 (d, J=8.3 Hz, 2H, H$_{Ar}$), 7.28 (d, J=7.5 Hz, 2H, H$_{Ar}$), 6.97 (d, J=8.3 Hz, 2H, H$_{Ar}$), 4.21 (t, J=6.6 Hz, 2H, C<u>H</u>$_2$O), 2.91 (t, J=6.6 Hz, 2H, C<u>H</u>$_2$-Ph), 2.45 (s, 3H, Me).

The spectroscopic data are consistent with that[219] reported in the literature.

2-(4-Bromophenyl)acetaldehyde (3.119)
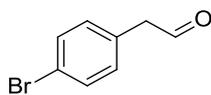
3.119

To a solution of 2-(4-bromophenyl)ethanol **3.114** (0.92 g, 4.57 mmol, 1 equiv) in CH₂Cl₂ (0.33 M, 15 mL) Dess-Martin Periodinane (2.33 g, 5.49 mmol, 1.2 equiv) was added slowly at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 19 h under argon at rt. After adding CH₂Cl₂ (45 mL) and Na₂S₂O₃ solution (1 M, 60 mL) the reaction mixture was stirred for another 10 min. The aqueous layer was extracted with CH₂Cl₂ (2 x 50 mL) and the combined organic layers were then washed with NaHCO₃ solution (5%, 2 x 100 mL) and brine (100 mL). The combined organic layers were dried over MgSO₄ and the solvent was removed *in vacuo* to provide **3.119** as colourless needles (950 mg, 100%).

¹H NMR (300 MHz, CDCl₃) δ ppm 9.74 (t, J=2.3 Hz, 1H, -C<u>H</u>O), 7.56–7.45 (m, 2H, H$_{Ar}$), 7.16–7.03 (m, 2H, H$_{Ar}$), 3.67 (d, J=2.3 Hz, 2H, CH₂).

The spectroscopic data are consistent with that[220] reported in the literature.

tert-Butyl-2-(2-(4-bromophenyl)ethylidene)hydrazinecarboxylate (3.120)
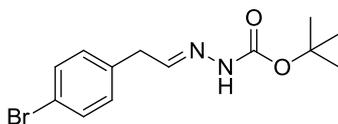
3.120

The aldehyde **3.119** (0.95 g, 4.77 mmol, 1 equiv) was dissolved in anhydrous toluene (16 mL) and *tert*-butyl carbazate (0.63 g, 4.77 mmol, 1 equiv) was added. The solution was stirred for 2 h at 50 °C under argon. Reducing the volume of toluene and cooling overnight resulted in the precipitation of a white solid. The supernatant was removed and the solid washed with cold toluene (2 x 5 mL) to obtain the hydrazone **3.120** as a colourless powder (1.13 g, 76%).

tert-Butyl-2-(4-bromophenethyl)hydrazinecarboxylate (3.115)
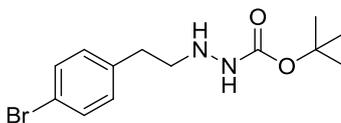
3.115

To a solution of hydrazone **3.120** (0.71 g, 2.27 mmol, 1 equiv) in anhydrous CH_2Cl_2 (8 mL), $NaBH(OAc)_3$ (1.2 g, 5.68 mmol, 2.5 equiv) and anhydrous acetic acid (0.13 mL, 2.27 mmol, 1 equiv) were added. The reaction mixture was allowed to stir for 18 h under argon at rt. NaOH solution (1 M, 10 mL) was added and the solution was stirred for another 20 min. The CH_2Cl_2 layer was separated and the solvent removed *in vacuo*, the crude product was dissolved in NaOH solution (1 M, 10 mL) and extract with Et_2O (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude material was purified by flash chromatography (hexane/EtOAc 9:1) to afford **3.115** as a colourless solid (600 mg, 84%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.38 (d, *J*=8.4 Hz, 2H, H$_{Ar}$), 7.07 (d, *J*=8.4 Hz, 2H, H$_{Ar}$), 2.96 (m, 2H, C$\underline{H}_2$NH), 2.79–2.72 (m, 2H, C$\underline{H}_2$Ar), 1.47 (s, 9H, CMe$_3$); **^{13}C NMR (75 MHz, CDCl$_3$) δ ppm** 155.7 (C, CO), 138.8 (C, C$_{Ar}$), 131.7 (CH, C$_{Ar}$), 130.5 (CH, C$_{Ar}$), 119.8 (C, C$_{Ar}$), 81.9 (C, CMe$_3$), 52.6 (CH$_2$, C$\underline{H}_2$NH), 33.6 (CH$_2$, C$\underline{H}_2$Ar), 28.3 (3CH$_3$, C$\underline{Me}_3$).

2-(4-Bromophenethyl)hydrazinium chloride (3.121)
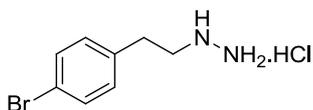
3.121

The Boc-protected hydrazine **3.115** (23.2 mg, 0.07 mmol, 1 equiv) was dissolved in a solution of HCl in dioxane (4 M, 1.2 mL). After 30 min of stirring the solvent was evaporated and was coevaporated with toluene, MeCN and twice with $CHCl_3$. The unprotected hydrazine chloride salt **3.121** was obtained as a yellow solid (18.3 mg, 99%).

^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 7.49–7.37 (m, 2H, H$_{Ar}$), 7.24–7.07 (m, 2H, H$_{Ar}$), 3.27 (dt, *J*=3.2, 1.8 Hz, 2H, C$\underline{H}_2$NH), 2.91 (d, *J*=7.9 Hz, 2H, C$\underline{H}_2$Ar).

The spectroscopic data are consistent with that[221] reported in the literature.

tert-Butyl-2-(2-(4-biphenyl)acetyl)hydrazinecarboxylate (3.117)
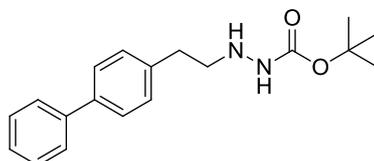
3.117

The arylbromide **3.115** (100 mg, 0.32 mmol, 1 equiv) was dissolved in MeCN (0.3 M, 2 mL) then phenylboronic acid (67 mg, 0.32 mmol, 1 equiv) and of K_2CO_3 solution (0.5 M, 2 mL) were added. The reaction mixture was heated to reflux for 10 min. After adding Pd(PPh$_3$)$_4$ (14.7 mg, 0.01 mmol, 0.04 equiv) the dispersion was refluxed for another 16 h. The reaction mixture was cooled to rt, filtered and the filtrate diluted with HCl solution (1 M, 20 mL) and extracted with CH_2Cl_2 (2 x 20 mL) and EtOAc (2 x 20 mL). The organic layer was washed with brine (2 x 10 mL), dried with $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (hexane/EtOAc 9:1) to give the biphenyl compound **3.117** as a yellow solid (17.4 mg, 18%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.60–7.24 (m, 9H, H$_{Ar}$), 3.02 (m, 2H, C$\underline{H}_2$NH), 2.92–2.80 (m, 2H, C$\underline{H}_2$Ar), 1.53–1.40 (s, 9H, CM$\underline{e}_3$); **^{13}C NMR (75 MHz, CDCl$_3$) δ**

ppm 140.0 (C, C_{Ar}), 138.0 (C, C_{Ar}), 130.4 (CH, C_{Ar}), 129.5 (CH, C_{Ar}), 128.2 (CH, C_{Ar}), 127.7 (CH, C_{Ar}), 126.0 (CH, C_{Ar}), 79.0 (C, CMe_3), 58.5 (CH_2, $C\underline{H}_2NH$), 32.3 (CH_2, $C\underline{H}_2Ar$), 27.4 ($3CH_3$, $C\underline{Me}_3$).

2-(4-Diphenethyl)hydrazinium chloride (3.122)
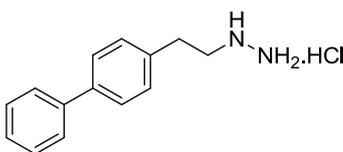
3.122

The Boc-protected biphenyl compound **3.117** (15 mg, 0.05 mmol, 1 equiv) was dissolved in a solution of HCl in dioxane (4 M, 1 mL) and stirred at rt for 90 min. After removing the solvent *in vacuo*, the remaining solid was washed with Et_2O. The hydrochloride salt **3.122** was obtained as a yellow solid (11.3 mg, 94%).

^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 7.64–7.21 (m, 9H, H_{Ar}), 3.28 (m, 2H, $C\underline{H}_2NH$), 3.04 (m, 2H, $C\underline{H}_2Ar$).

The spectroscopic data are consistent with that[222] reported in the literature

5.4.2.2. Biological testing of phenelzine

LNCaP cell growth inhibition assay

Six-day proliferation assay was performed using the LNCaP prostate adenocarcinoma cell line to give the IC_{50} for the inhibition by phenelzine sulfate.

Compound	IC_{50} (μM)		mean (μM)	SEM
	IC_{50} (1)	IC_{50} (2)		
Phenelzine sulfate (**3.123**)	121.8	121.6	**121.7**	**0.1**

Table 5.4 : Activity of phenelzine in LNCaP growth inhibition

6. APPENDIX

6.1. X-RAY CRYSTAL STRUCTURE DATA FOR 1.48

Table 1. Crystal data and structure refinement details.

Identification code	**2010sot0230**
Empirical formula	$C_{22}H_{26}N_4O_6S$
Formula weight	474.53
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	$a = 10.8208(8)$ Å
	$b = 10.2781(8)$ Å $\beta = 110.432(4)°$
	$c = 10.8216(8)$ Å
Volume	1127.83(15) Å^3
Z	2
Density (calculated)	1.397 Mg / m^3
Absorption coefficient	0.191 mm^{-1}
$F(000)$	500
Crystal	Lath; Colourless
Crystal size	$0.30 \times 0.12 \times 0.03$ mm^3
θ range for data collection	$3.03 - 25.03°$
Index ranges	$-12 \leq h \leq 11, -12 \leq k \leq 12, -11 \leq l \leq 12$
Reflections collected	9619
Independent reflections	2108 [$R_{int} = 0.0658$]
Completeness to $\theta = 25.03°$	99.4%
Absorption correction	Semi−empirical from equivalents
Max. and min. transmission	0.9943 and 0.9450
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2108 / 7 / 298
Goodness-of-fit on F^2	1.204
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1 = 0.1047, wR2 = 0.2662$
R indices (all data)	$R1 = 0.1118, wR2 = 0.2706$
Absolute structure parameter	Not reliably determined
Largest diff. peak and hole	1.352 and -0.467 e Å^{-3}
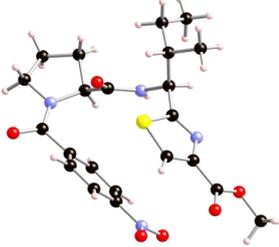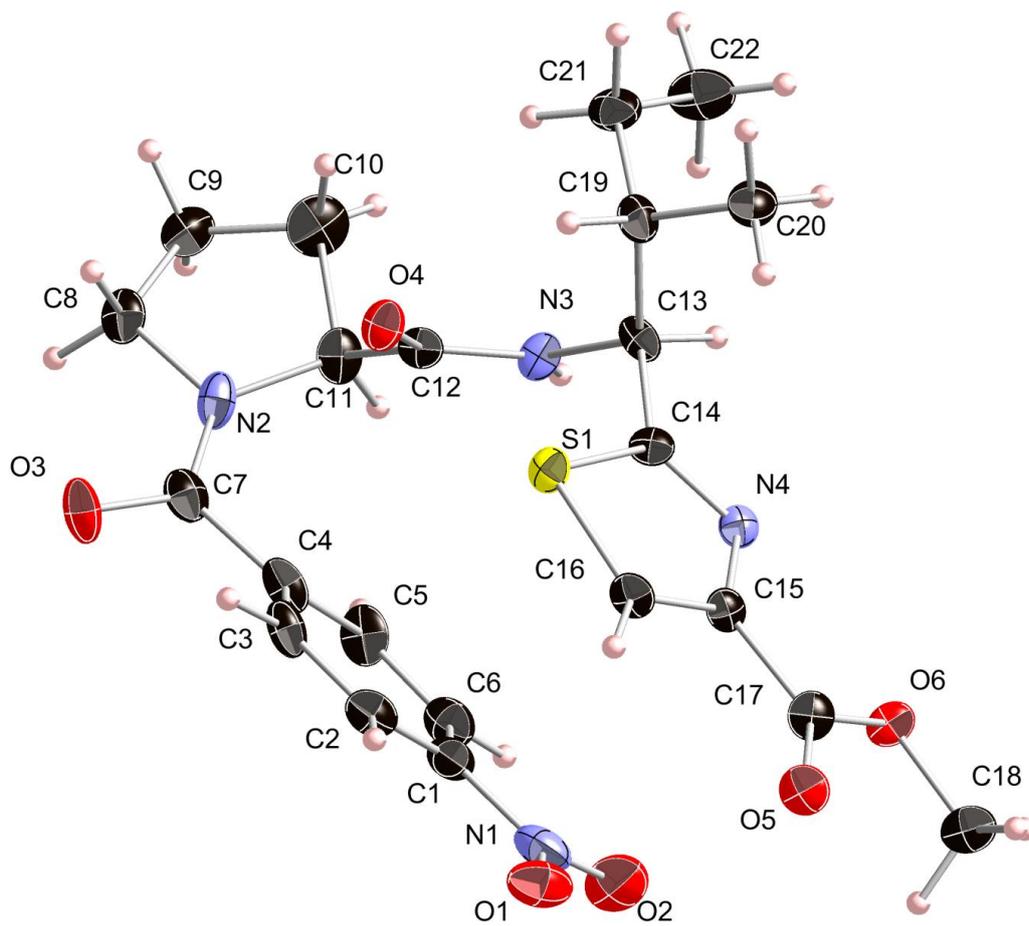
Thermal ellipsoids are drawn at the 35% probability level

6.2. X-RAY CRYSTAL STRUCTURE DATA FOR 1.59

Table 1. Crystal data and structure refinement details.

Identification code	**2010sot0301 (HB6/15)**
Empirical formula	$C_{37}H_{51}N_7O_6S$, $4(H_2O)$
Formula weight	793.97
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	$a = 7.6044(3)$ Å
	$b = 12.4594(6)$ Å $\beta = 95.416(3)°$
	$c = 22.9801(11)$ Å
Volume	2167.56(17) Å^3
Z	2
Density (calculated)	1.217 Mg / m^3
Absorption coefficient	0.134 mm^{-1}
$F(000)$	852
Crystal	Rod; Colourless
Crystal size	$0.30 \times 0.10 \times 0.08$ mm^3
θ range for data collection	$2.91 - 27.48°$
Index ranges	$-9 \le h \le 9, -16 \le k \le 16, -29 \le l \le 29$
Reflections collected	25060
Independent reflections	5171 [$R_{int} = 0.0761$]
Completeness to $\theta = 27.48°$	99.7%
Absorption correction	Semi−empirical from equivalents
Max. and min. transmission	0.9893 and 0.9608
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5171 / 25 / 528
Goodness-of-fit on F^2	1.181
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1 = 0.1121$, $wR2 = 0.2526$
R indices (all data)	$R1 = 0.1444$, $wR2 = 0.2731$
Absolute structure parameter	$-10(10)$
Largest diff. peak and hole	0.770 and -0.368 e Å^{-3}
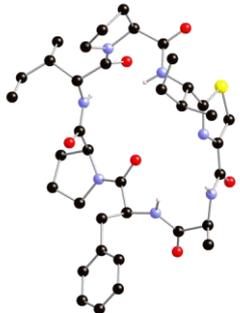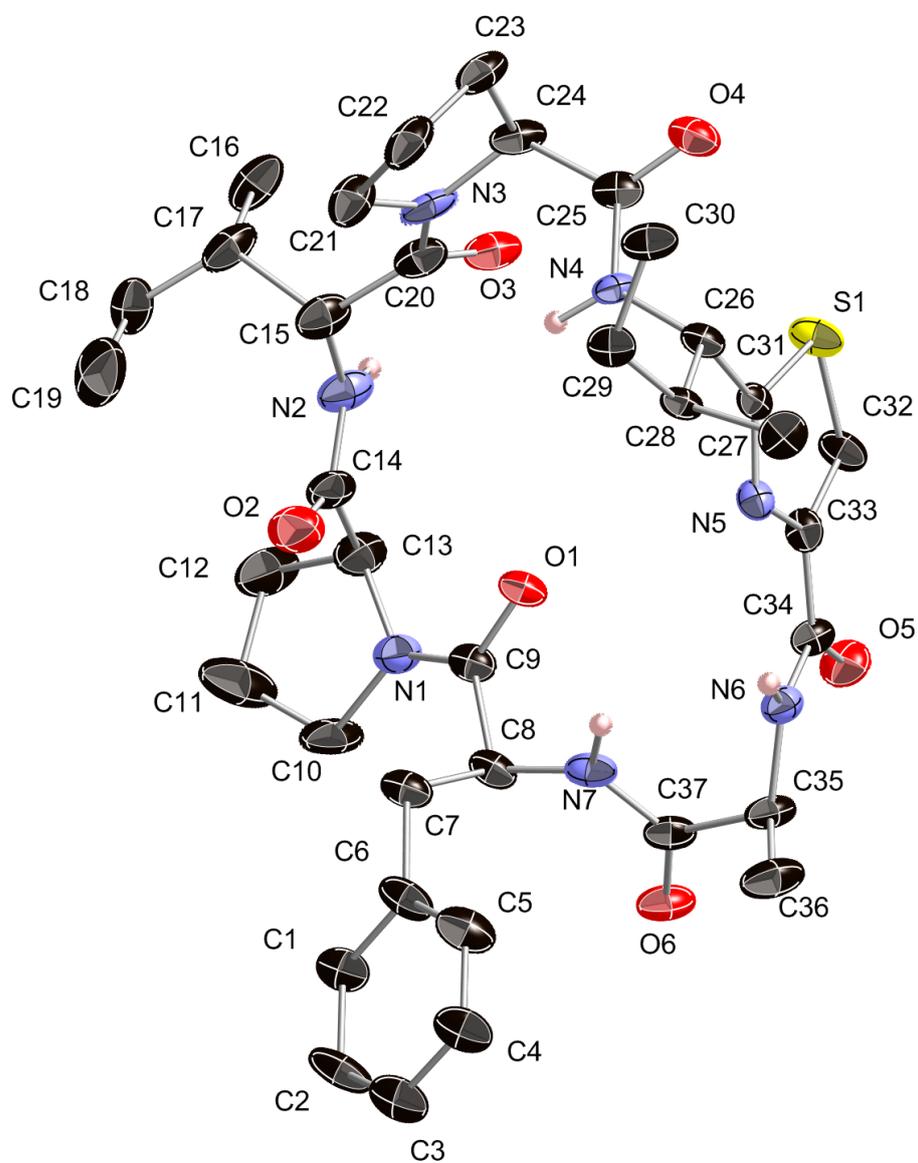
Thermal ellipsoids drawn at the 35% probability level, solvent water and selected hydrogens omitted for clarity.

6.3. NMR SPECTRA

^{1}H NMR spectrum of 1.38

HB5-91.esp

1.38
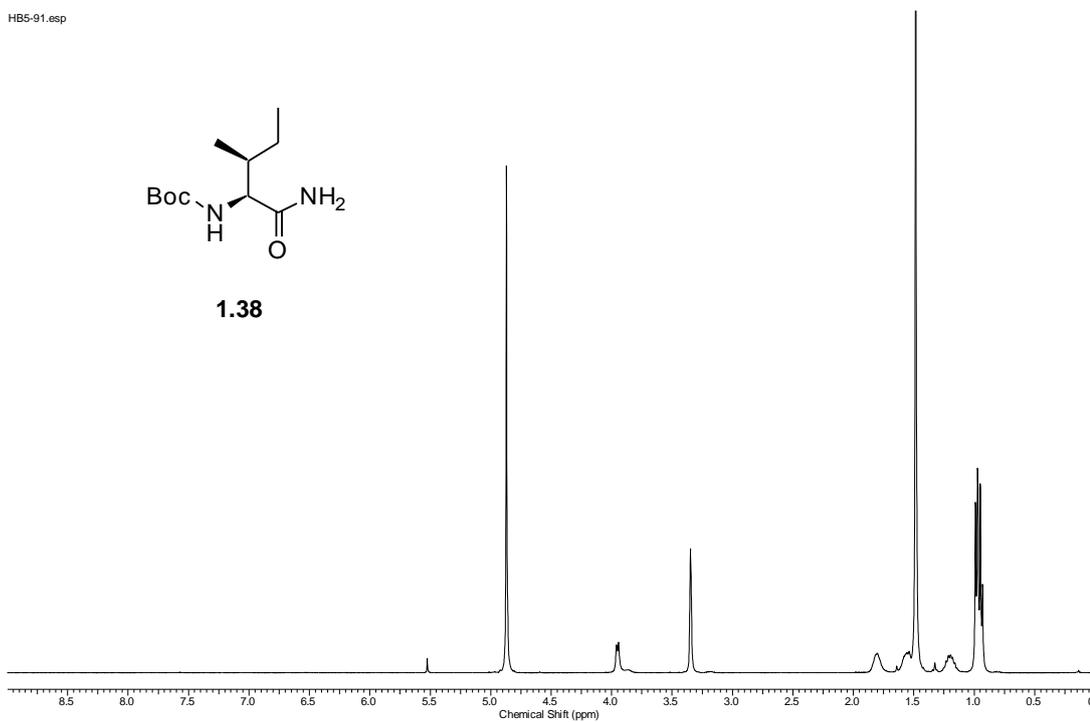
^{1}H NMR spectrum of 1.39

HB5-92.esp


1.39
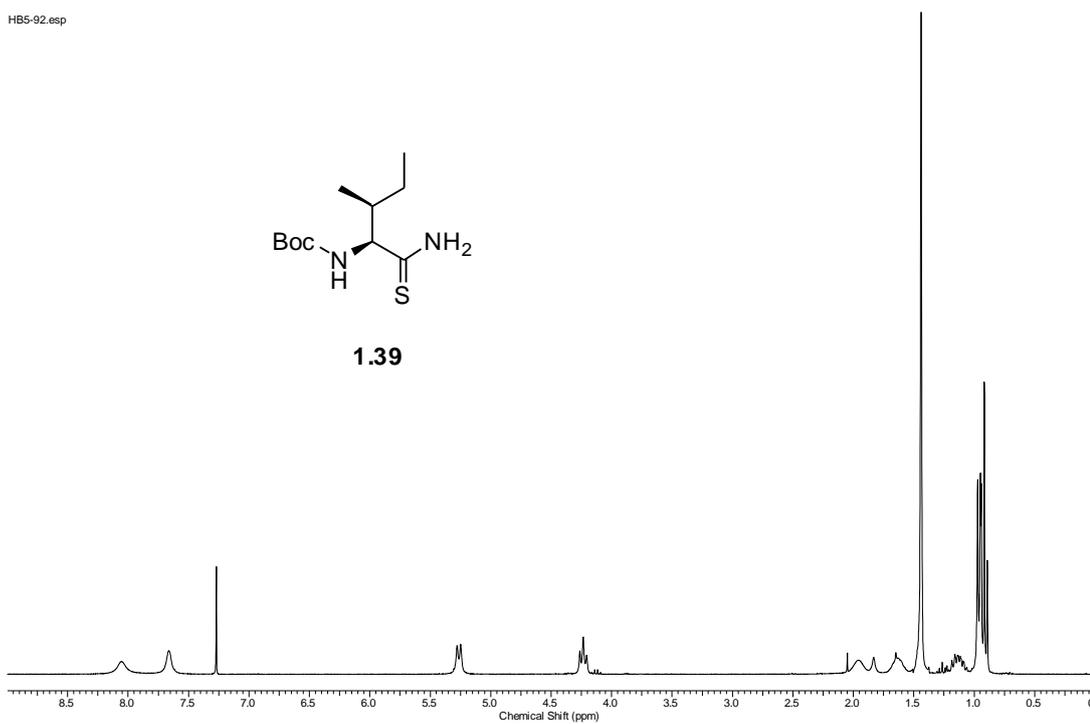
^{1}H NMR spectrum of 1.40

HB6-50.esp

1.40

Chemical Shift (ppm)
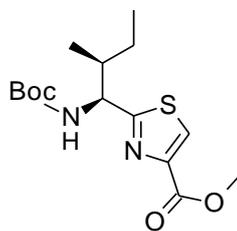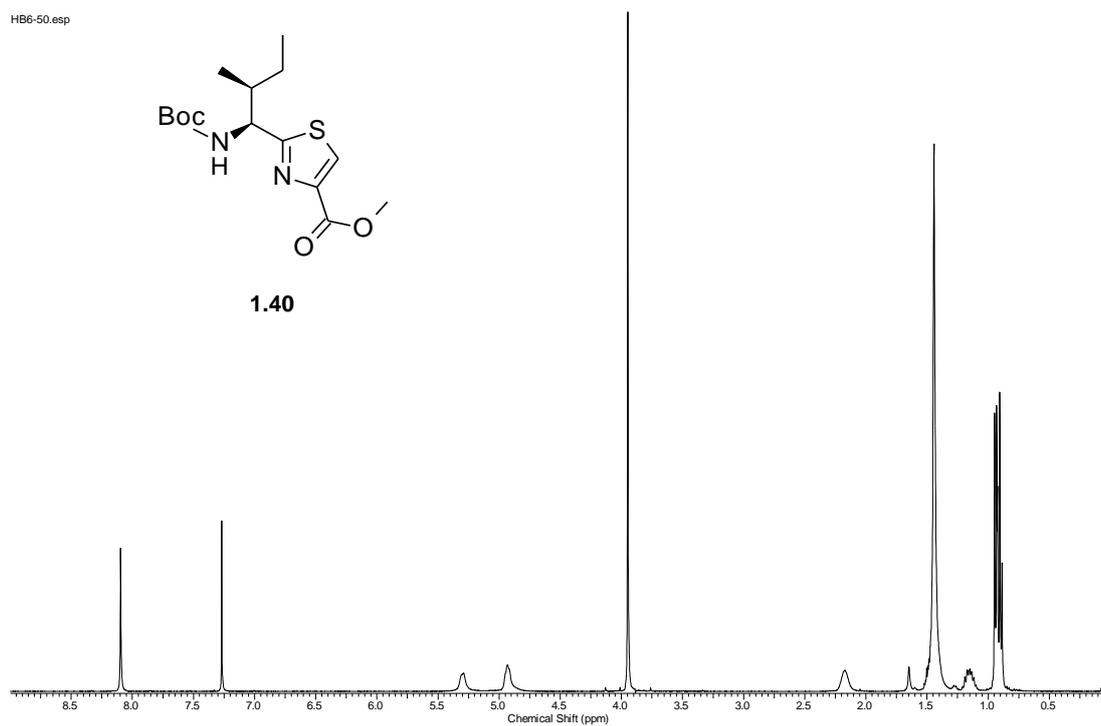
^{1}H and ^{13}C NMR spectra of 1.42

HB6-51.esp


1.42

13C-HB6-51.esp

1.42
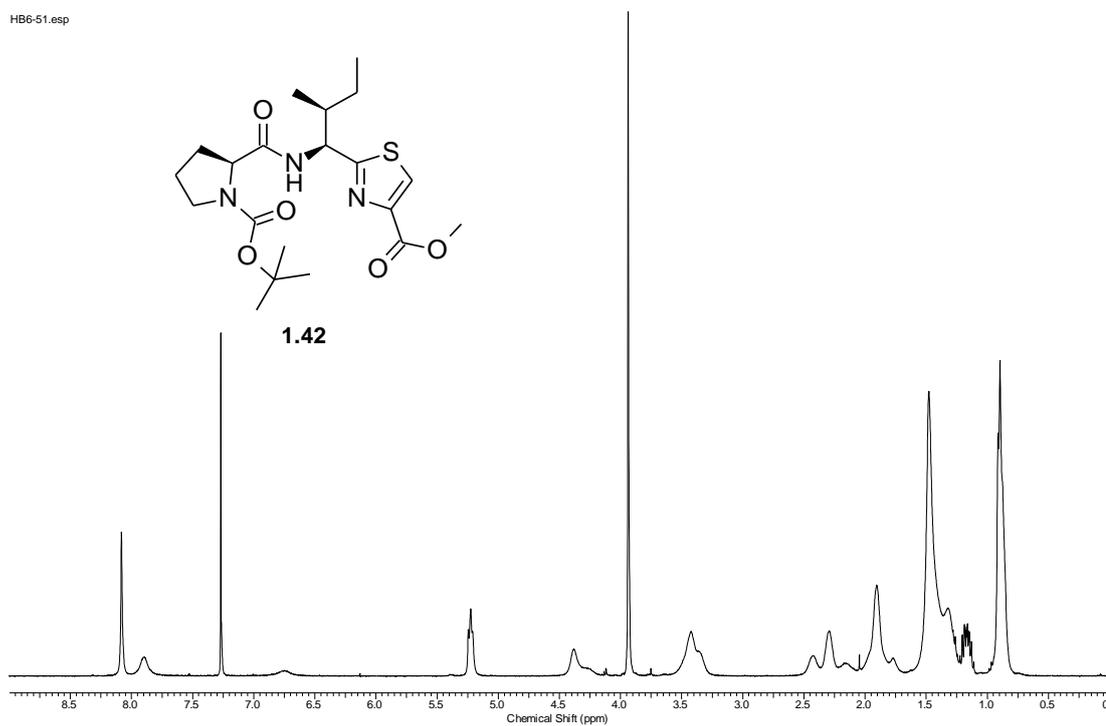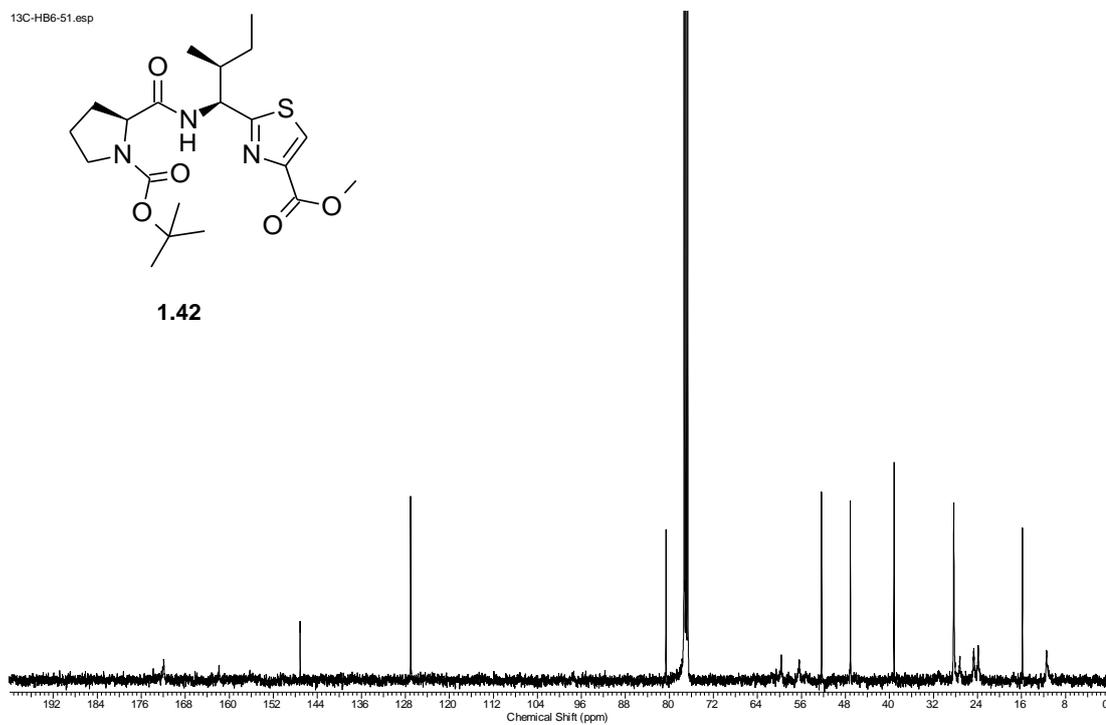
¹H and ¹³C NMR spectra of 1.48

HB6-24.esp

1.48

Chemical Shift (ppm)

13C_HB6-24.esp

1.48

Chemical Shift (ppm)
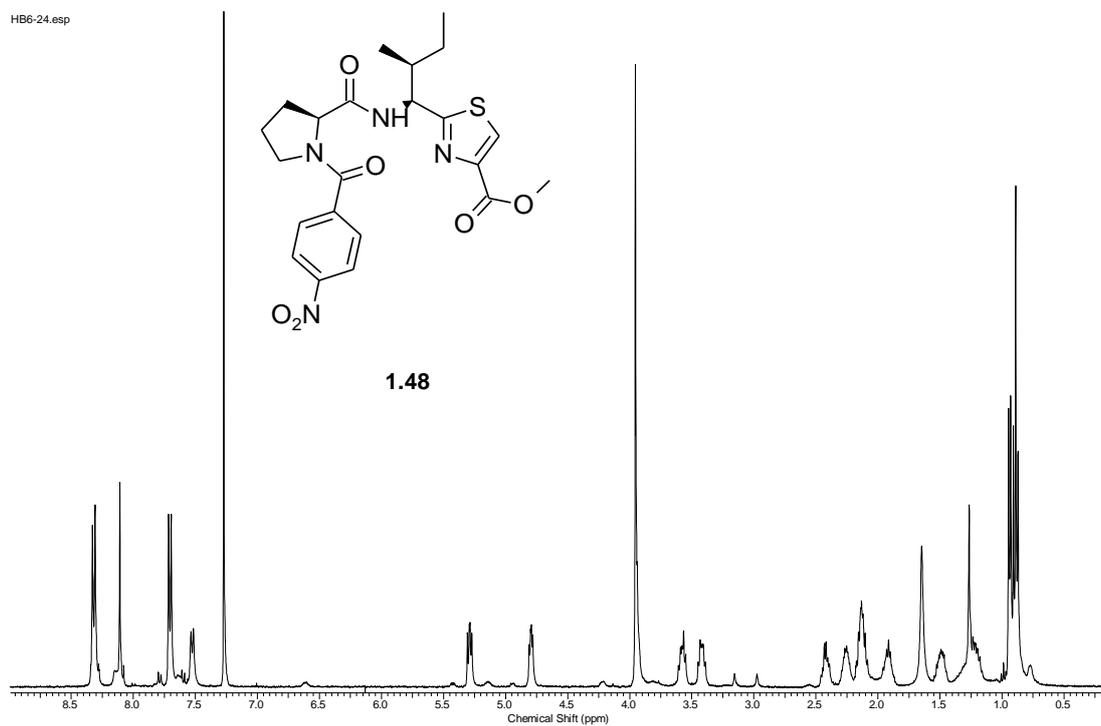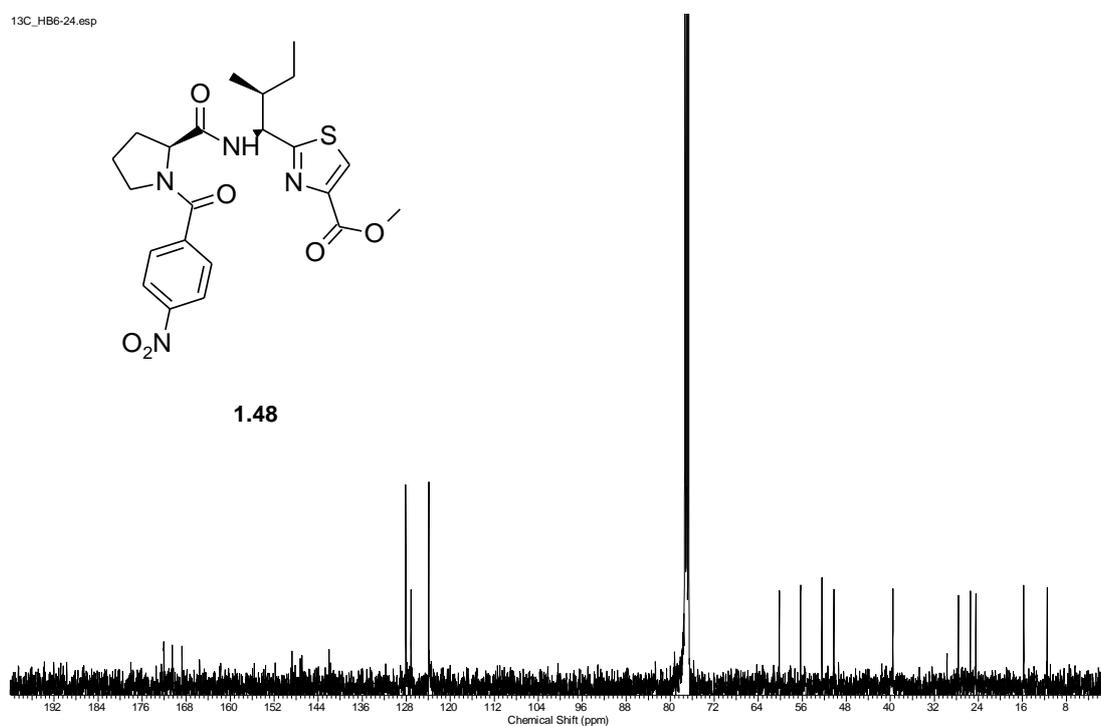
¹H NMR spectrum of 1.52

HB5-96.esp

1.52

Chemical Shift (ppm)
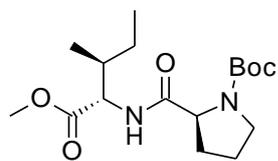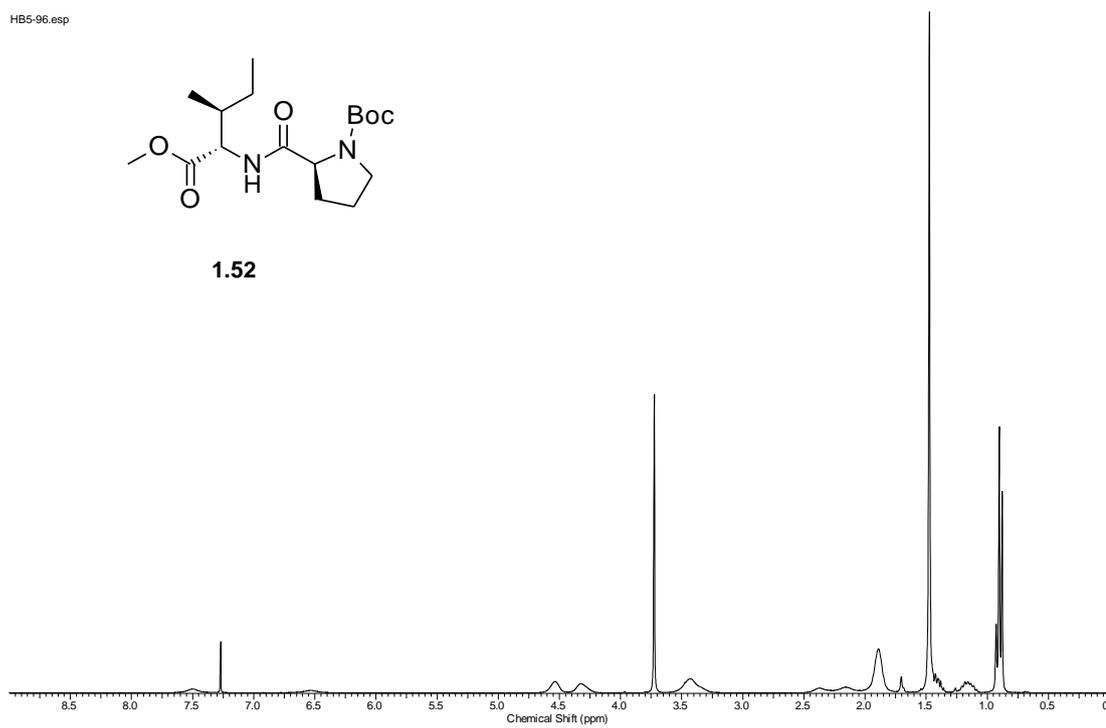
^{1}H and ^{13}C NMR spectra of 1.54

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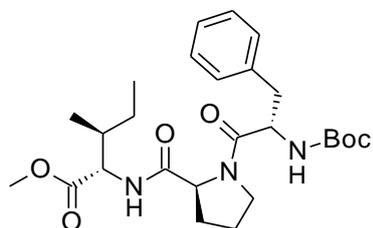
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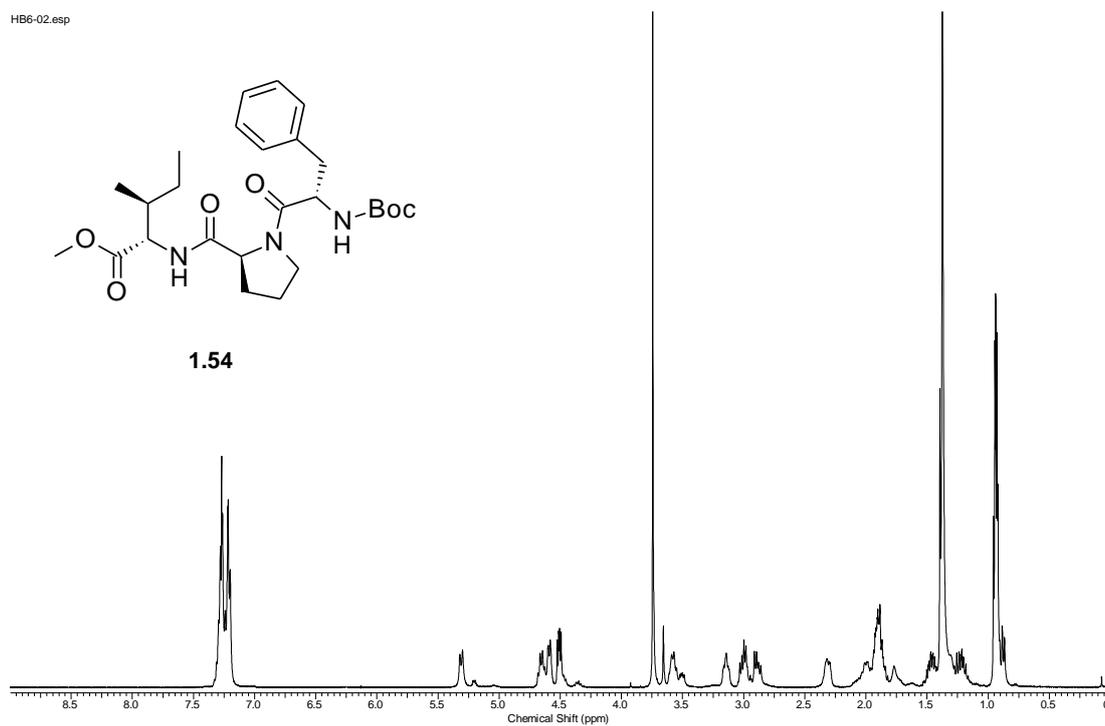
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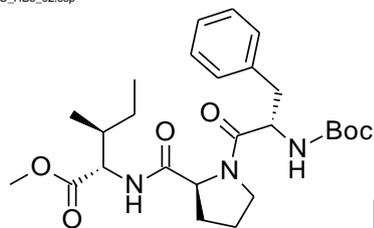
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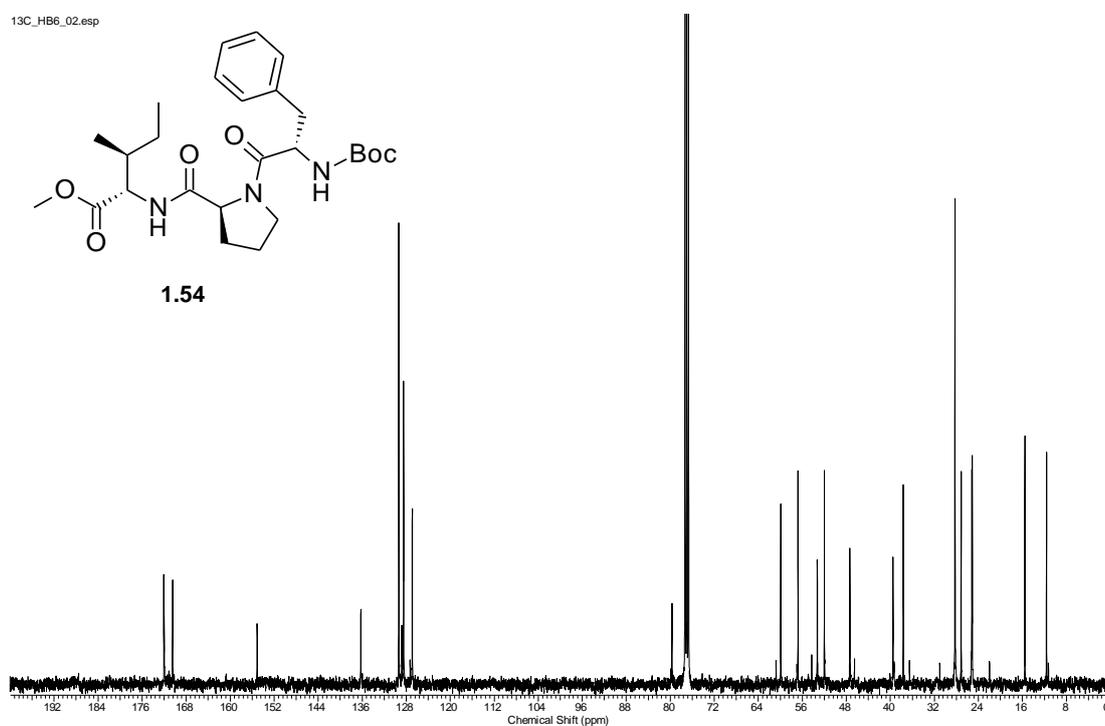
^{1}H and ^{13}C NMR spectra of 1.56

HB6-05.esp

1.56

Chemical Shift (ppm)

13C-HB6-05.esp

1.56

Chemical Shift (ppm)
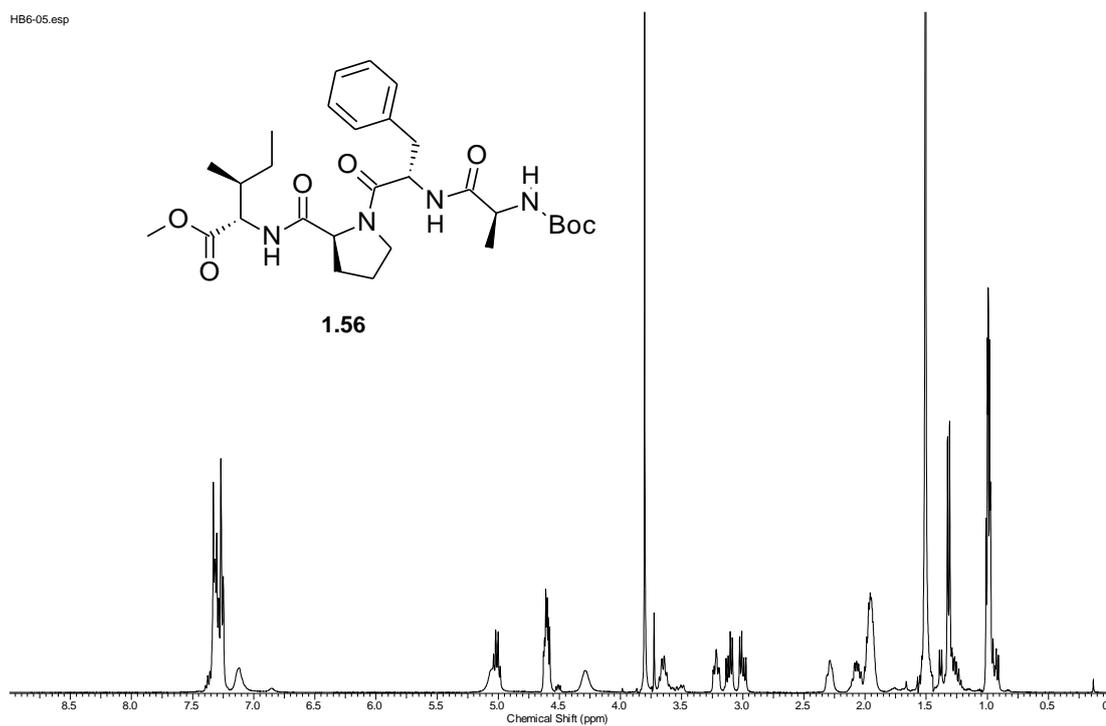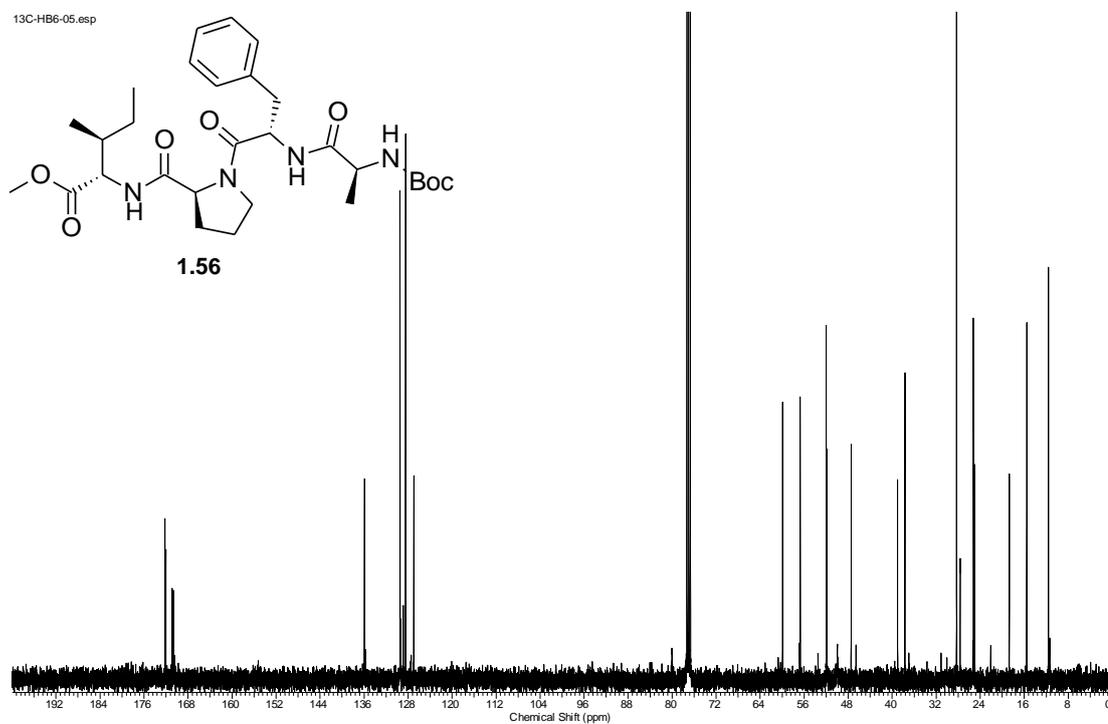
¹H NMR spectrum of 1.59

SOTON_HB6_15_MINUS10DEG_1H

1.59
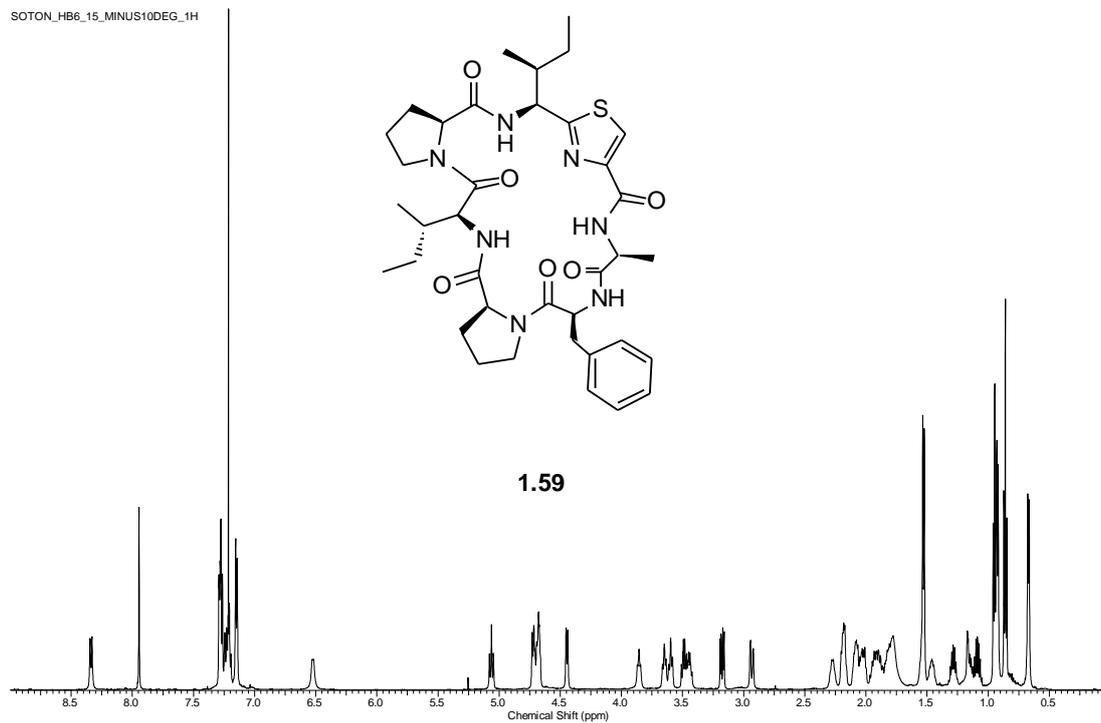
¹H NMR spectrum of 1.69

HB6-49 ALA_PHE.ESP

1.69

^{1}H and ^{13}C NMR spectra of 1.65

^{1}H NMR spectrum of 1.63

1.63
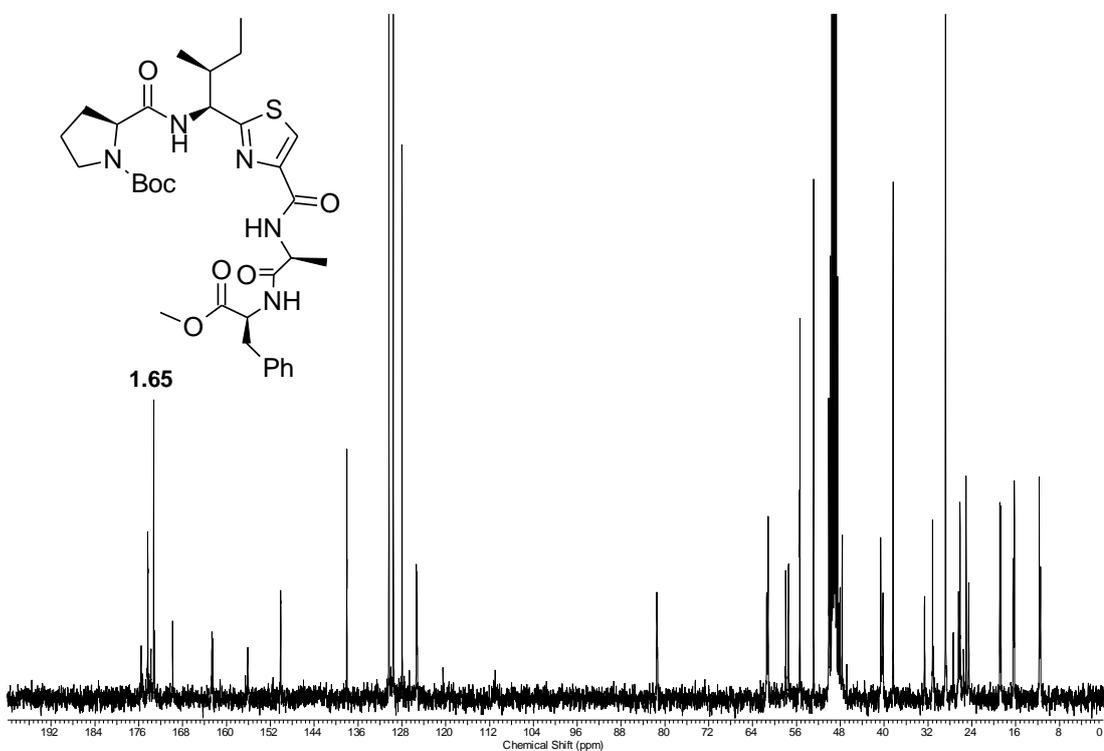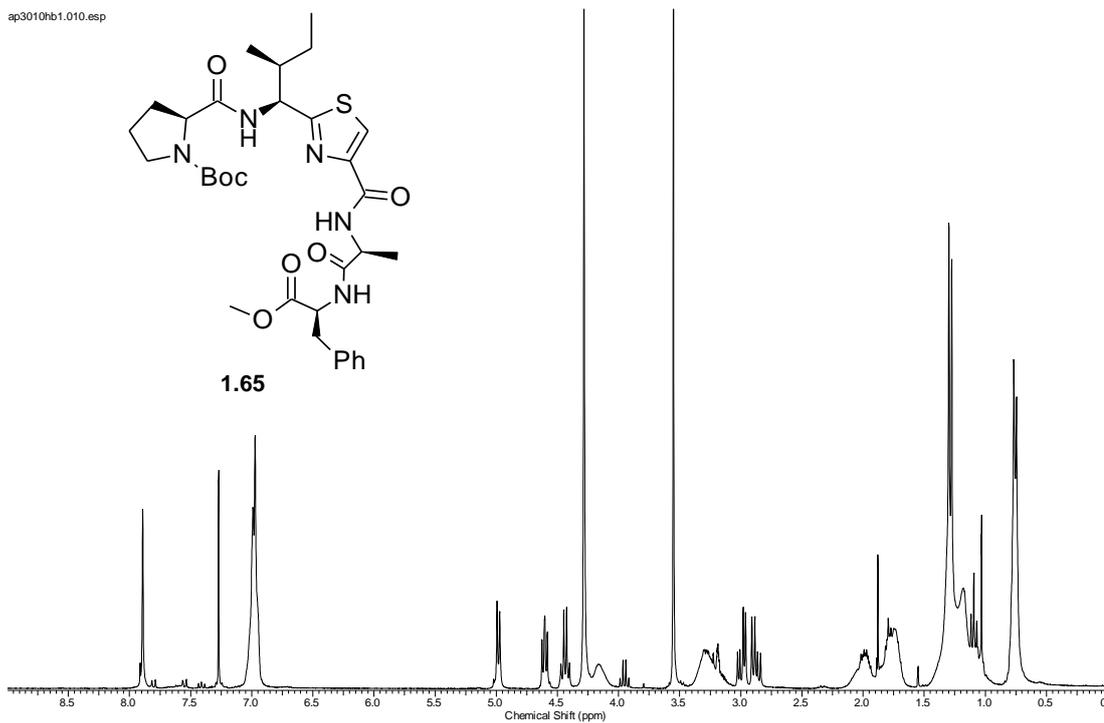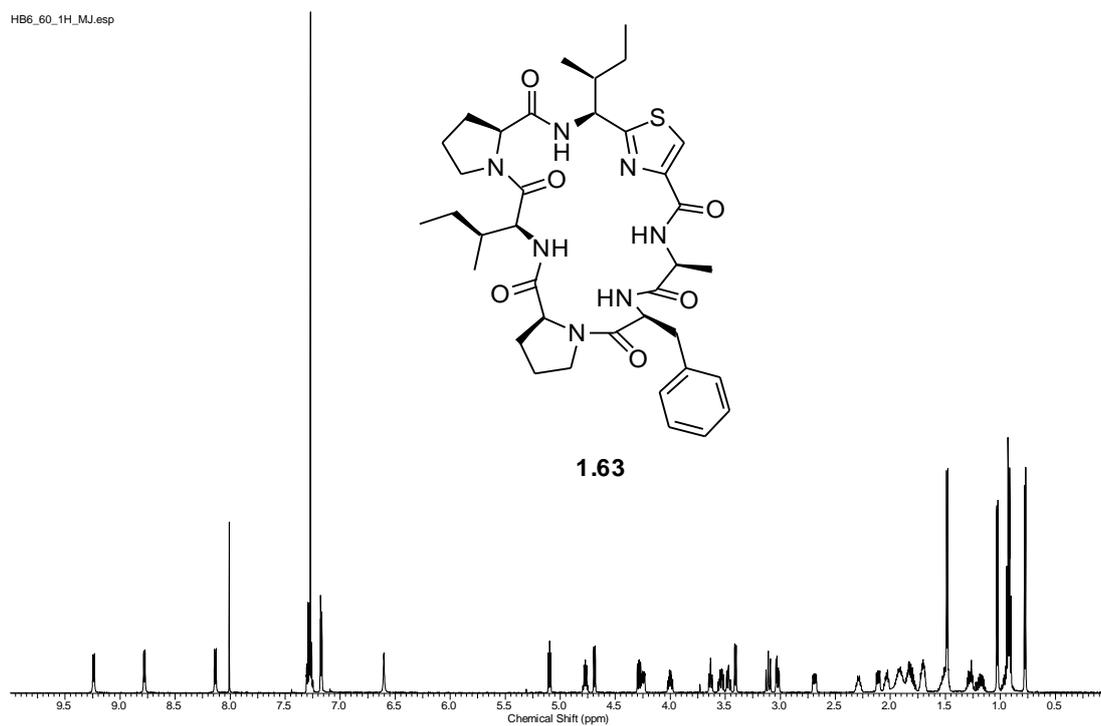
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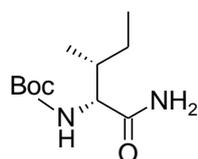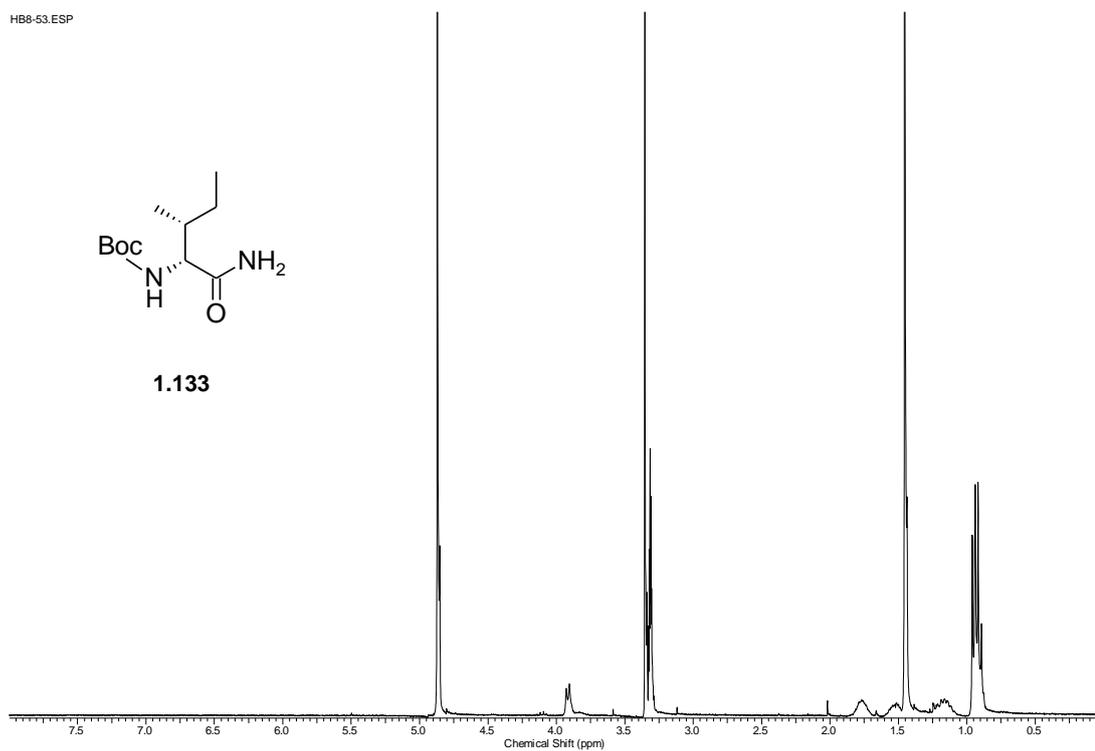
^{1}H NMR spectrum of 1.134
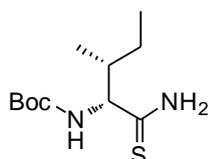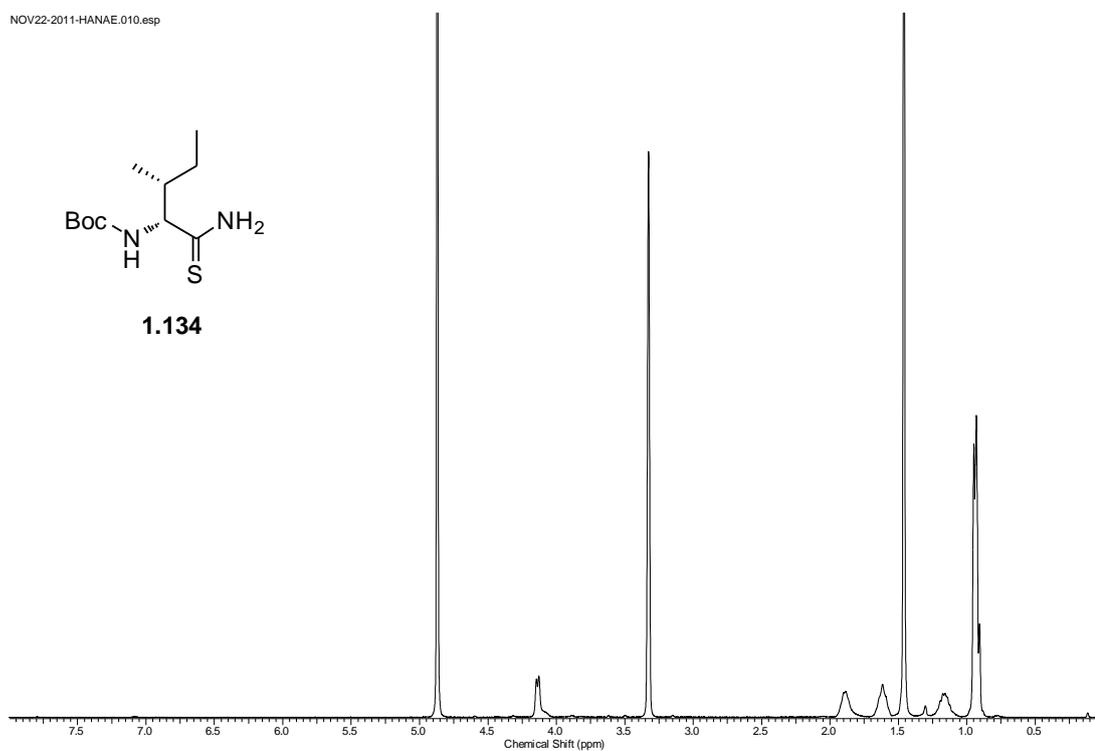
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ma0211hb2.010.esp
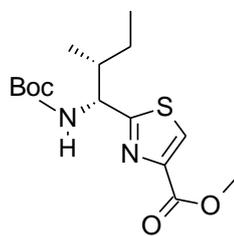
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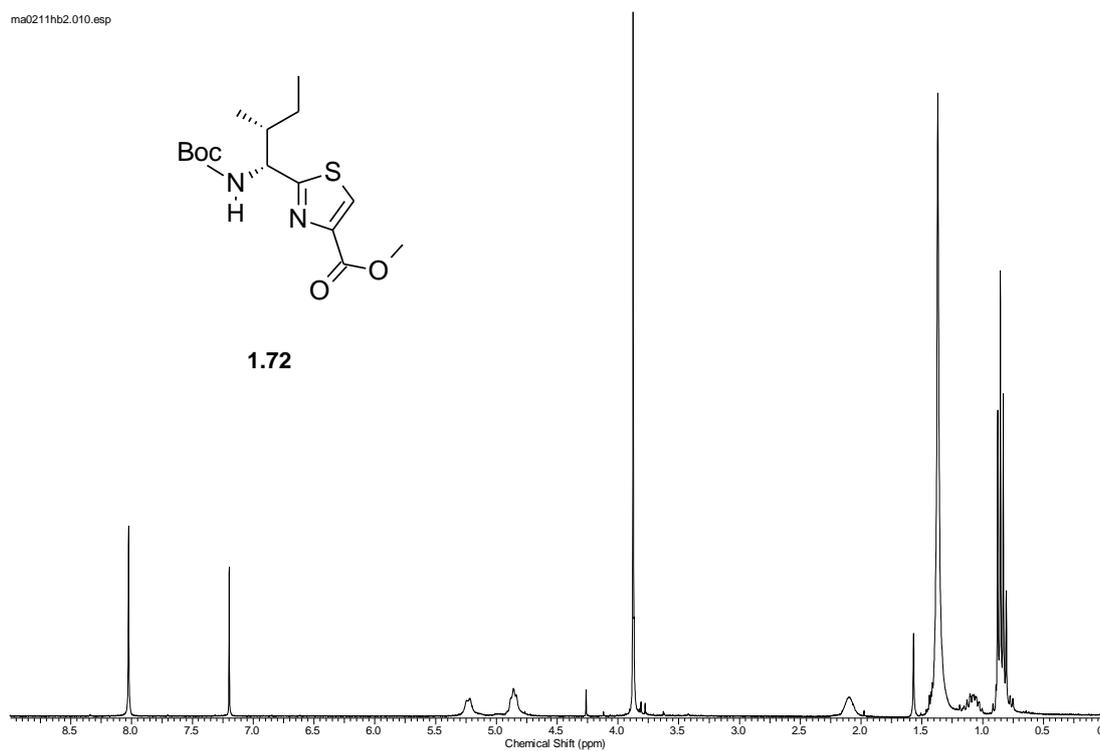
^{1}H NMR spectrum of 1.135

HB8-57.ESP
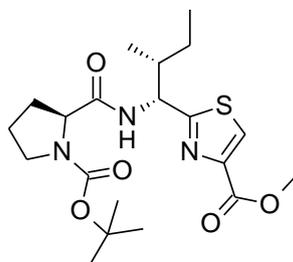
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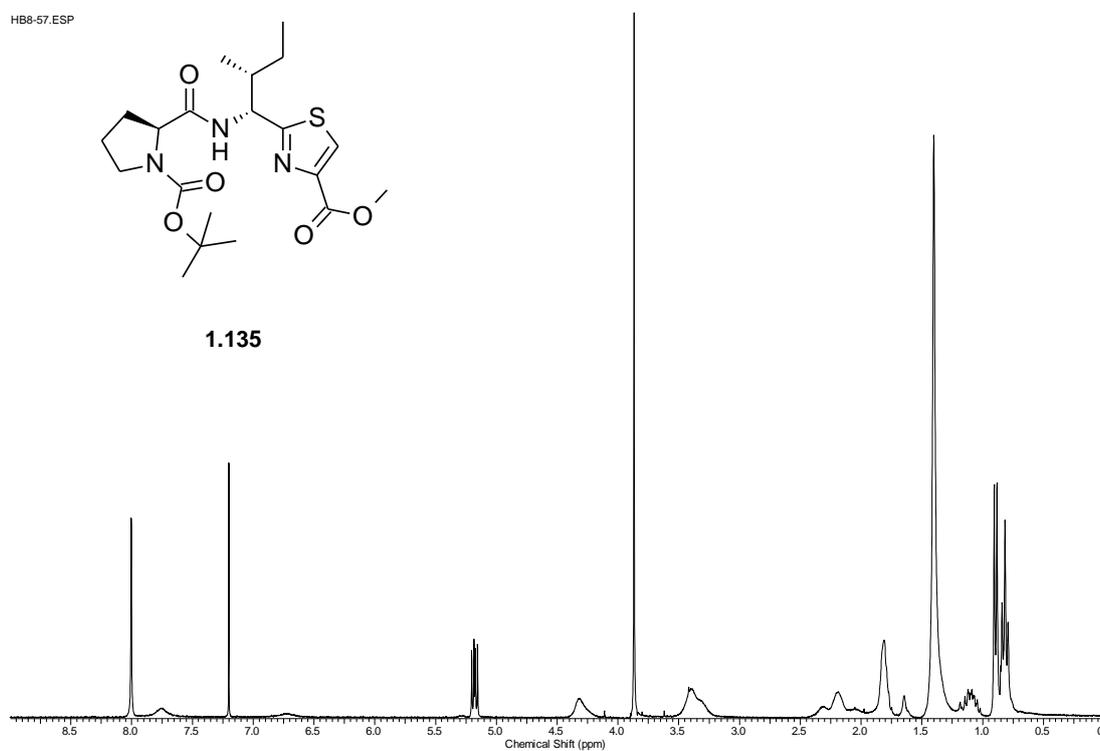
¹H NMR spectrum of 1.75

1.75
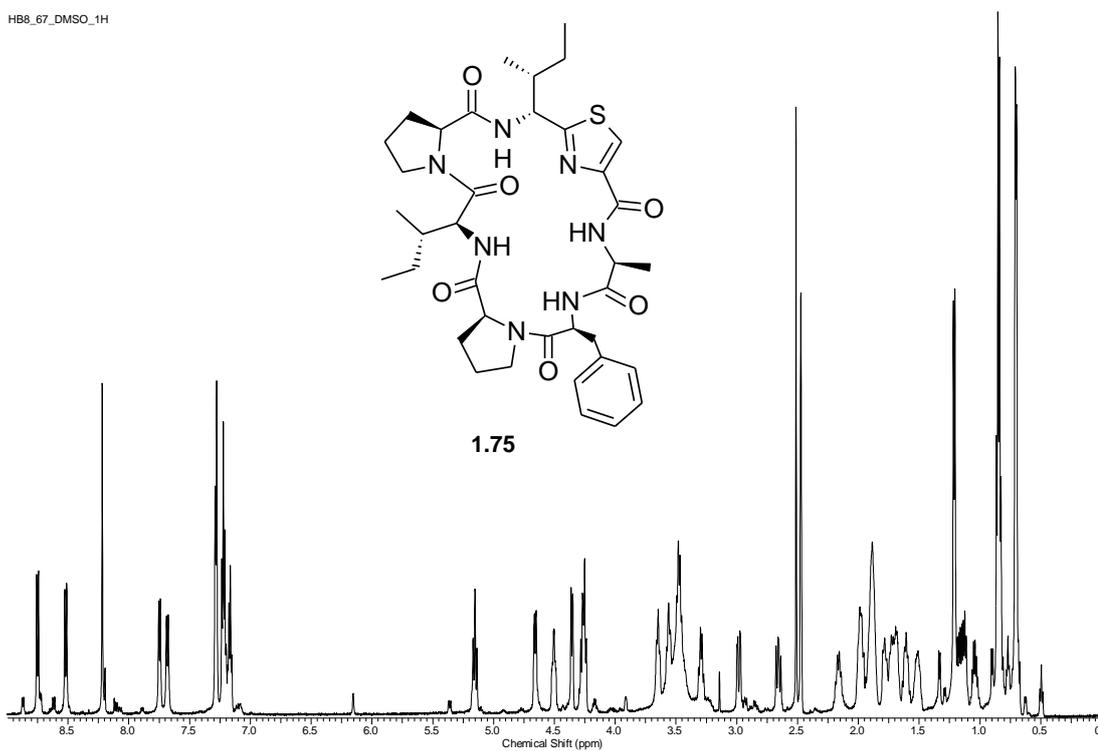
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AUG18-2011-HANAE.010.esp

1.137
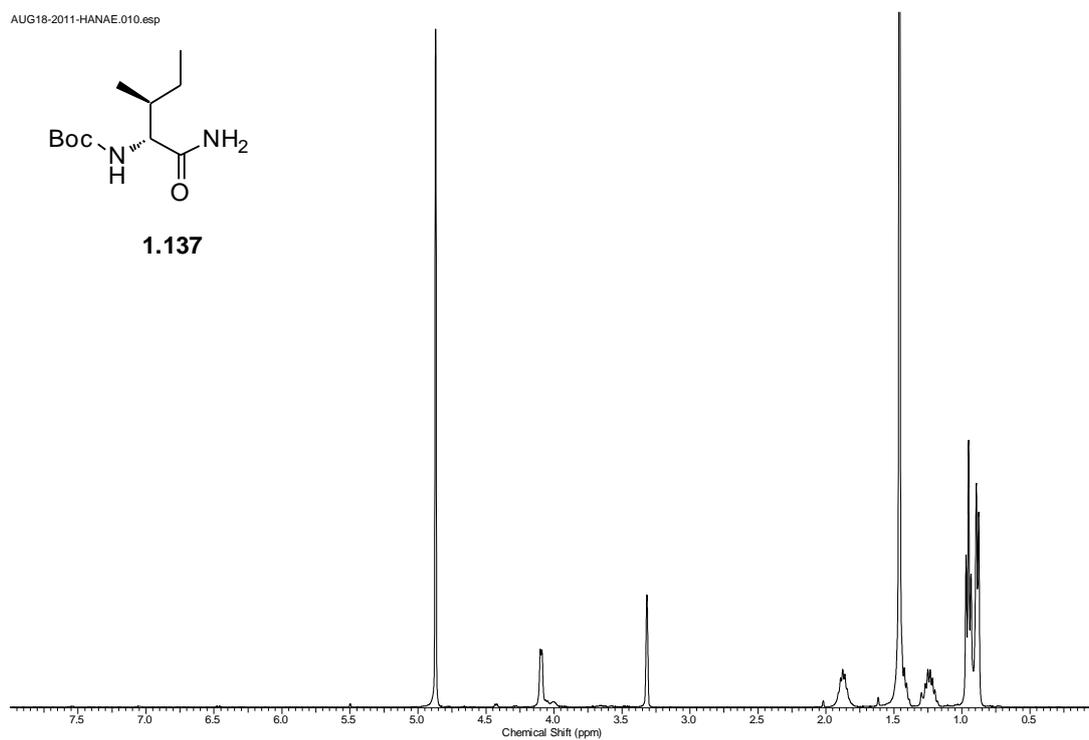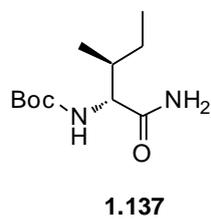
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NOV25-2011-HANAE.020.esp

1.138
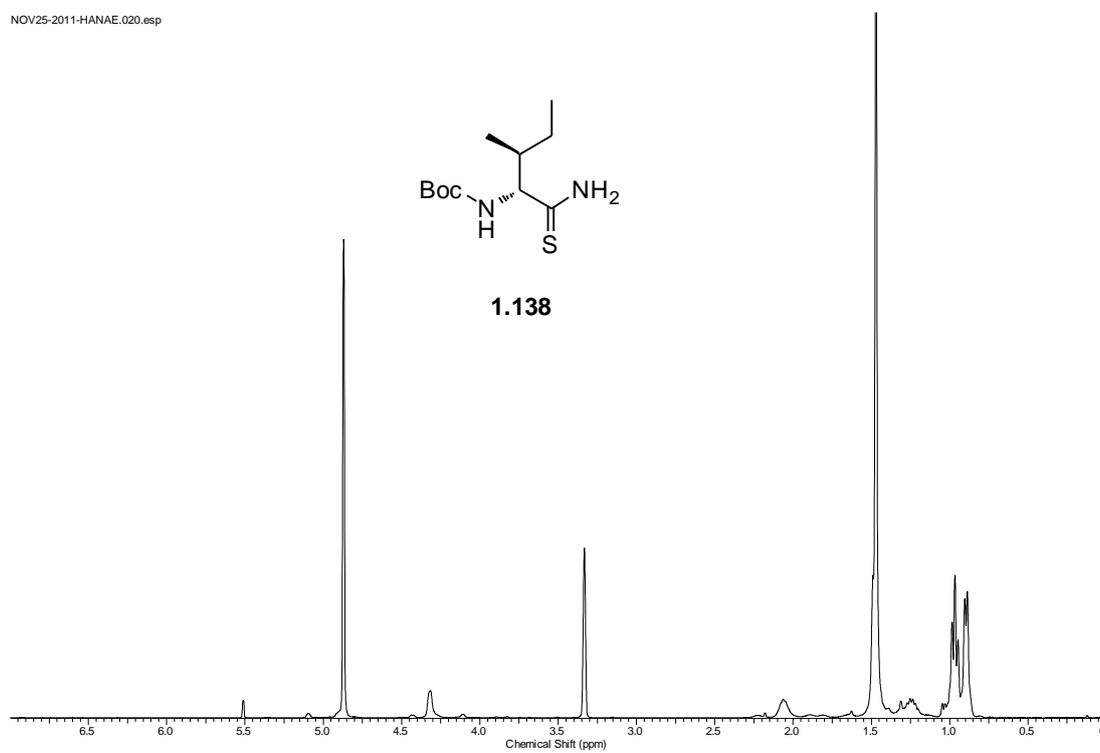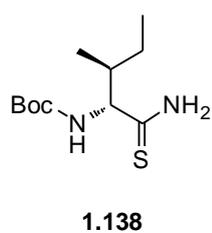
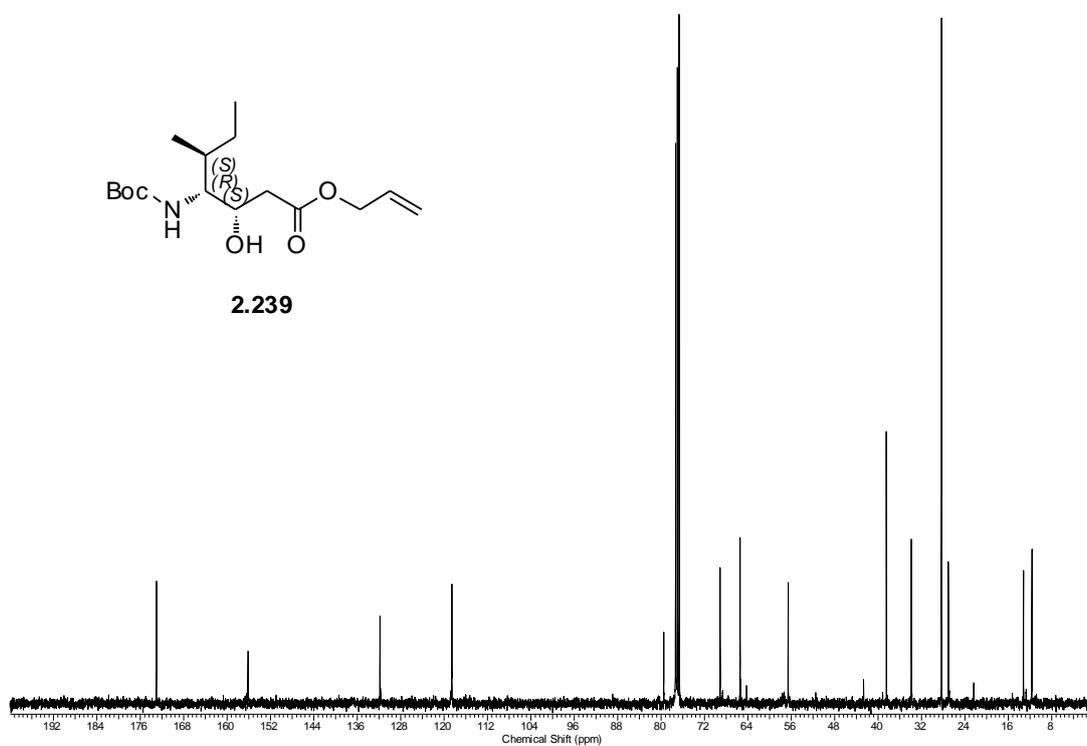# ^{1}H and ^{13}C NMR spectra of 1.77

Nov22-2011-Hanae.020.esp

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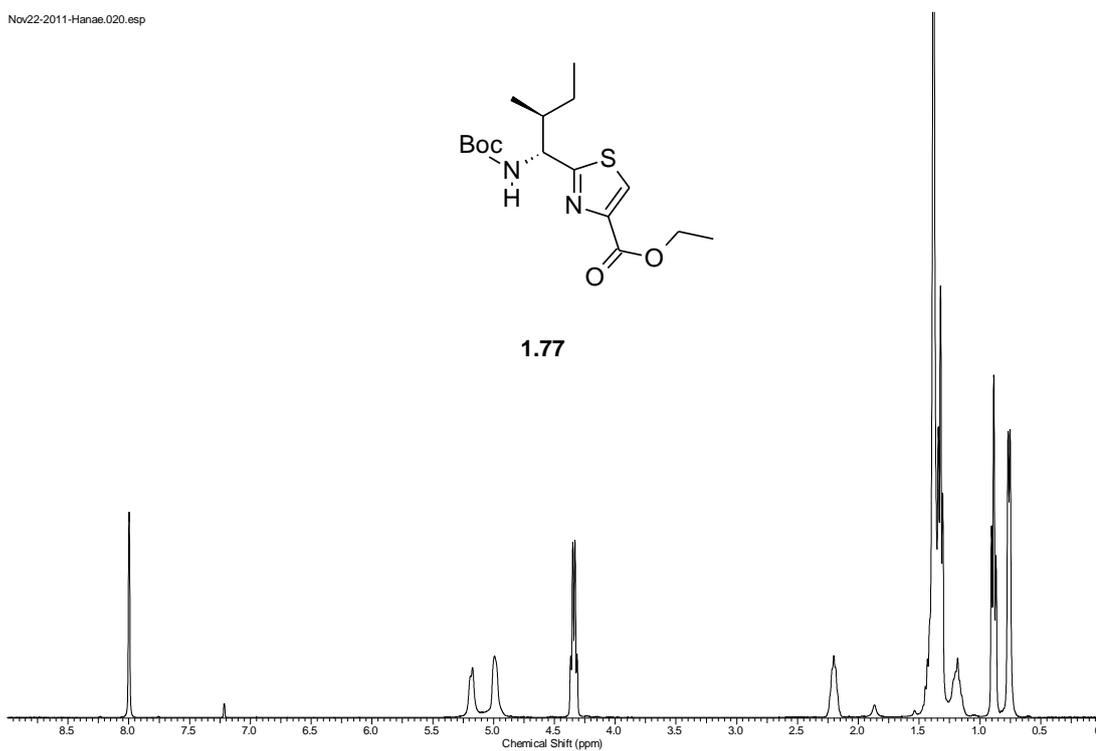
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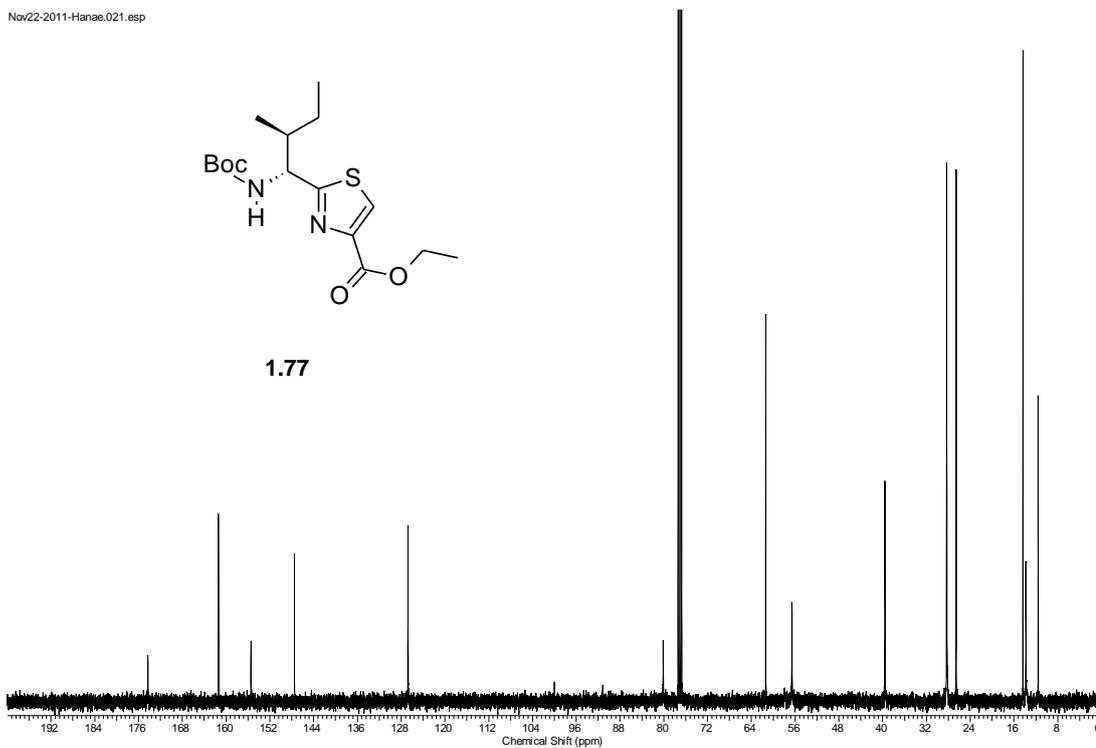
^{1}H and ^{13}C NMR spectra of 1.139

1.139

1.139
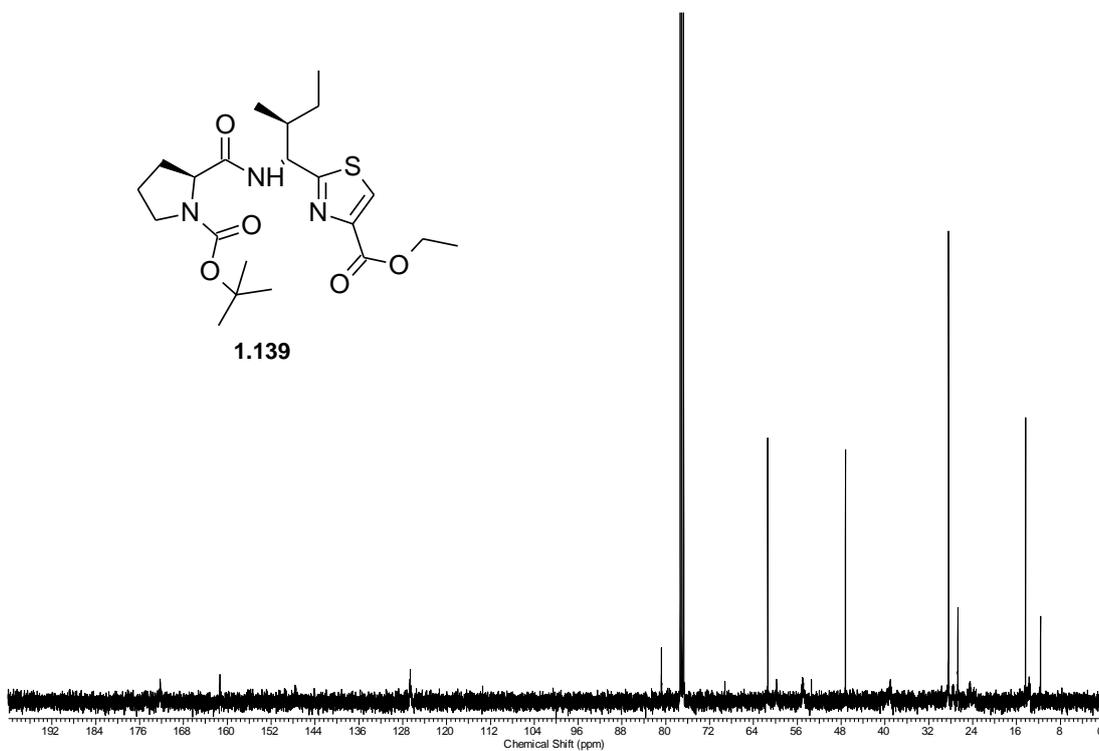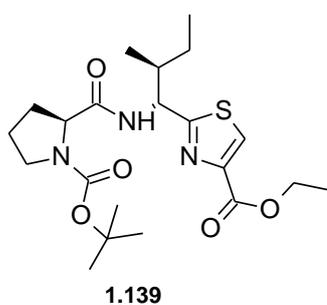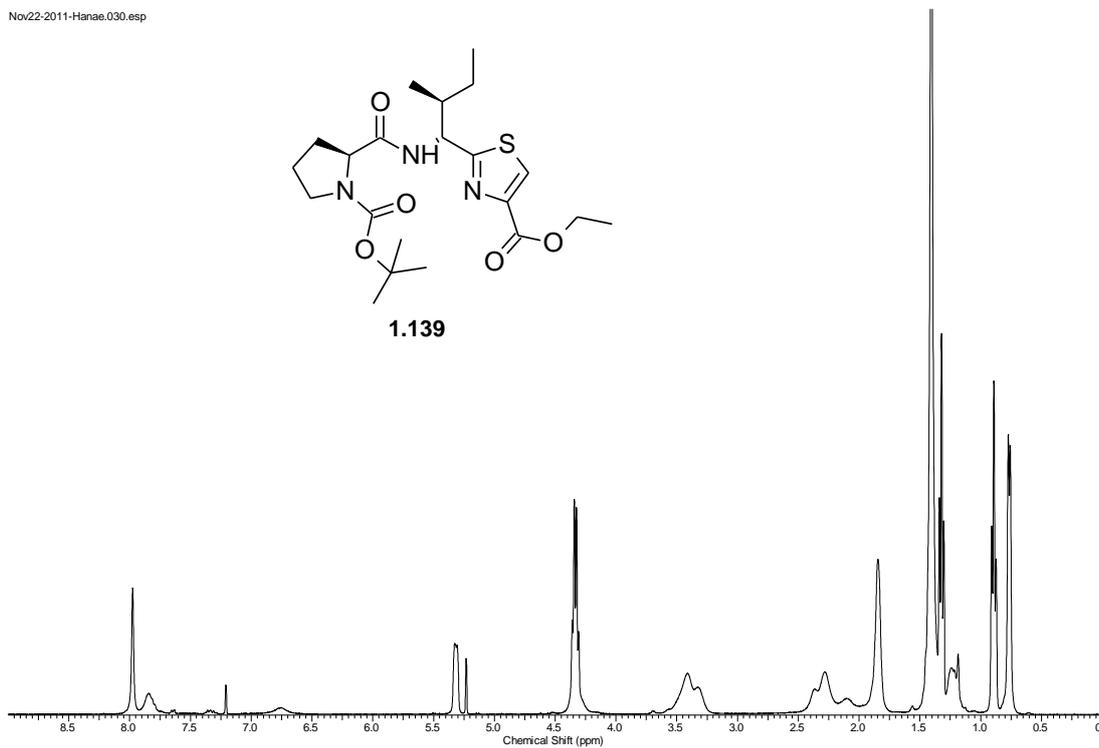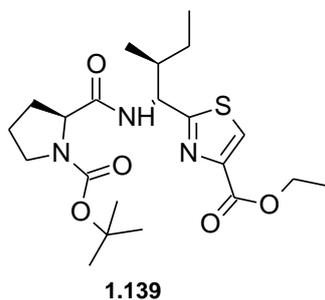
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1.101
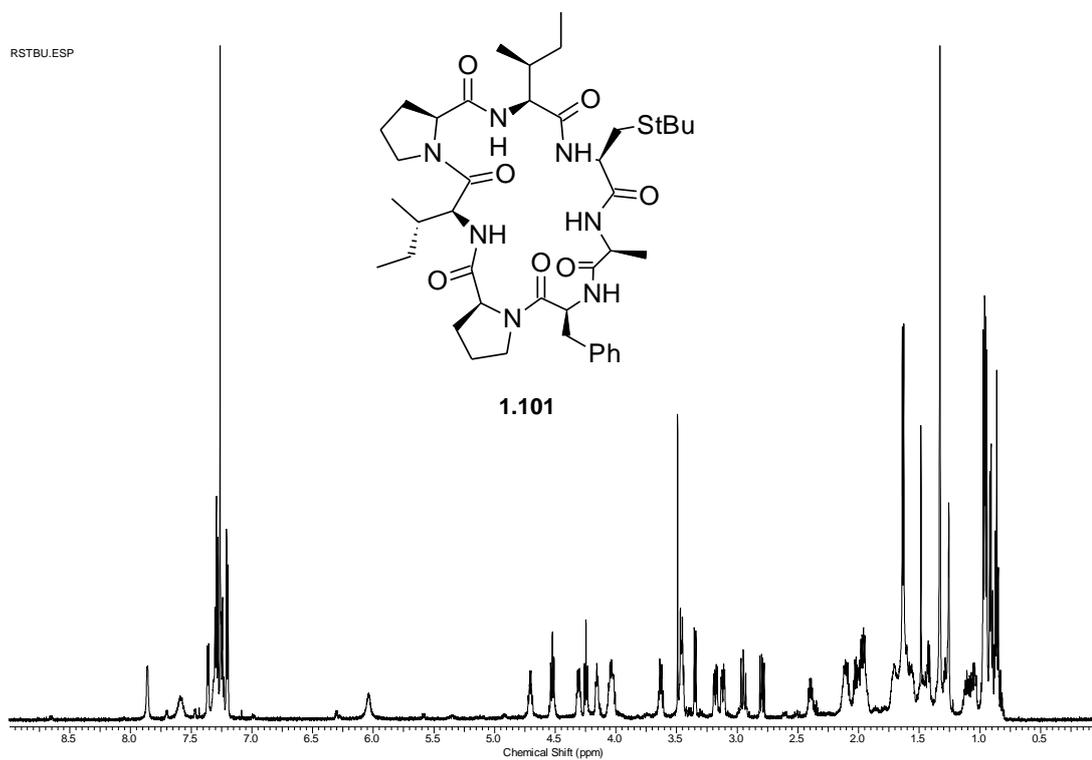
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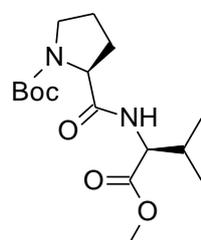
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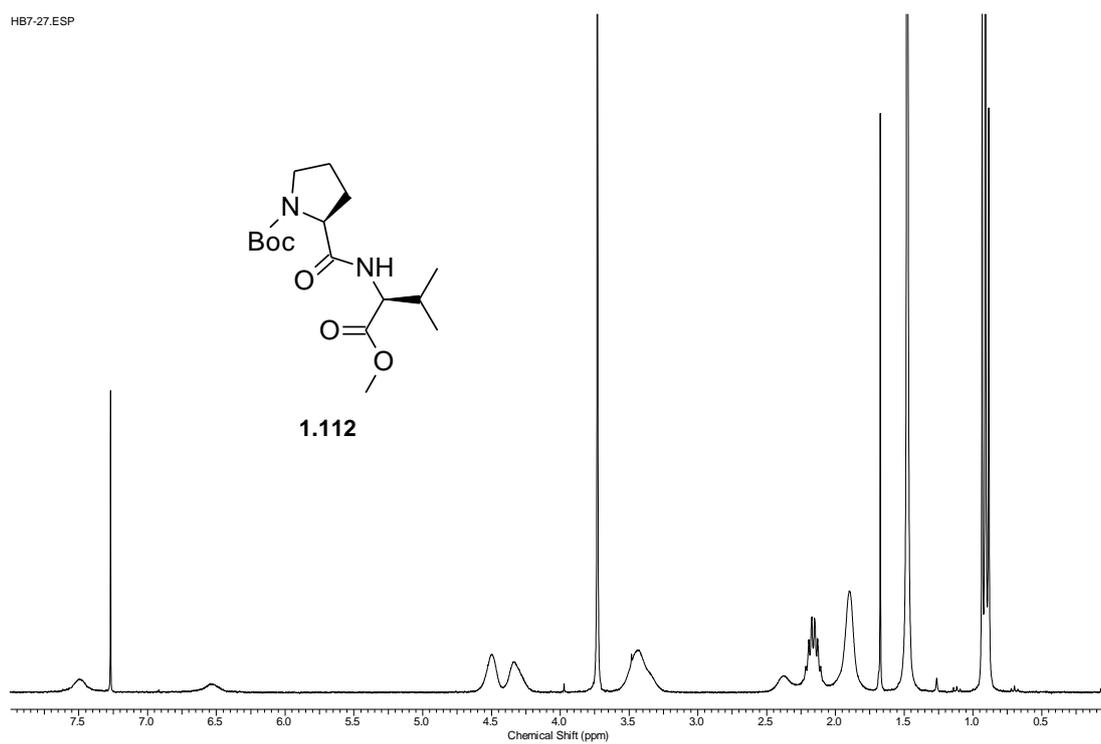
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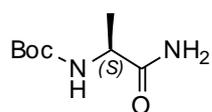
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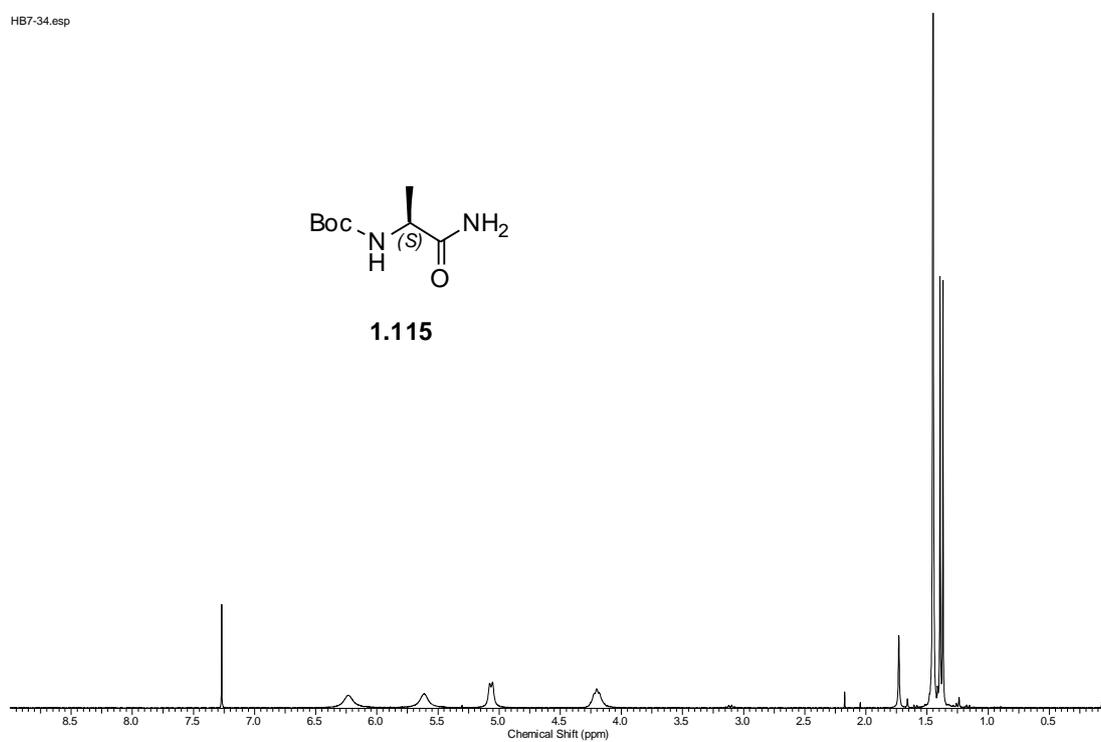
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HB7-36.esp

1.116

Chemical Shift (ppm)
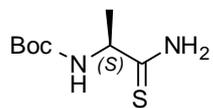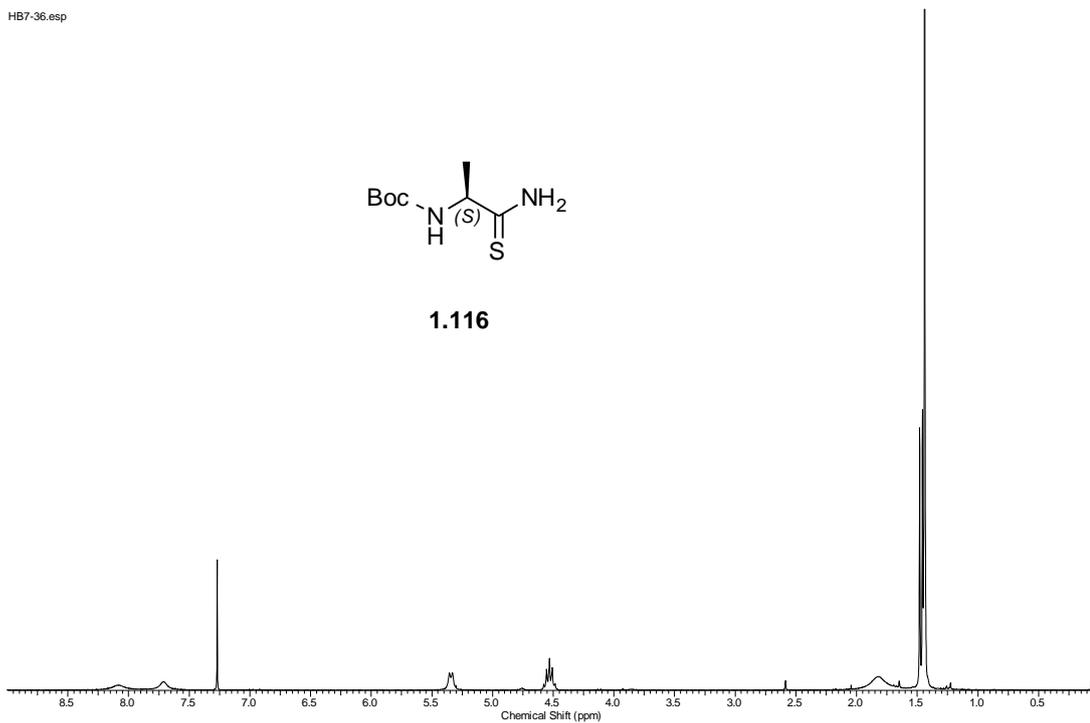
^{1}H NMR spectrum of 1.117

HB7-37.esp

1.117

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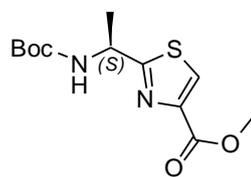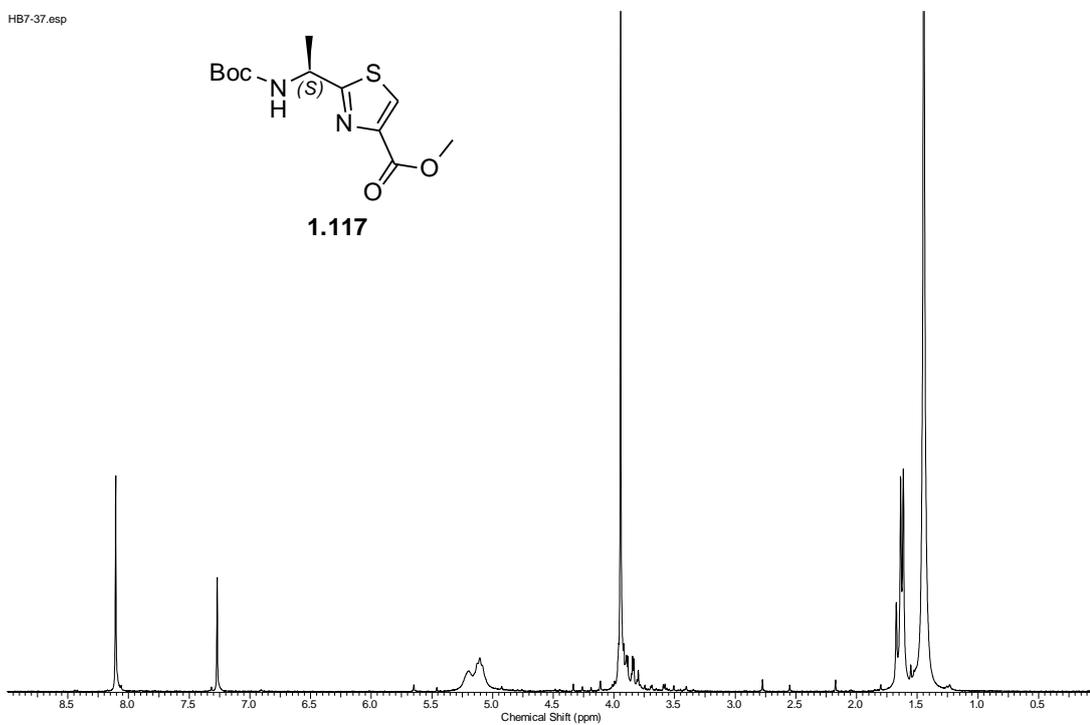
¹H and ¹³C NMR spectra of 1.119
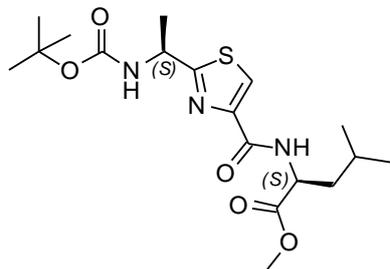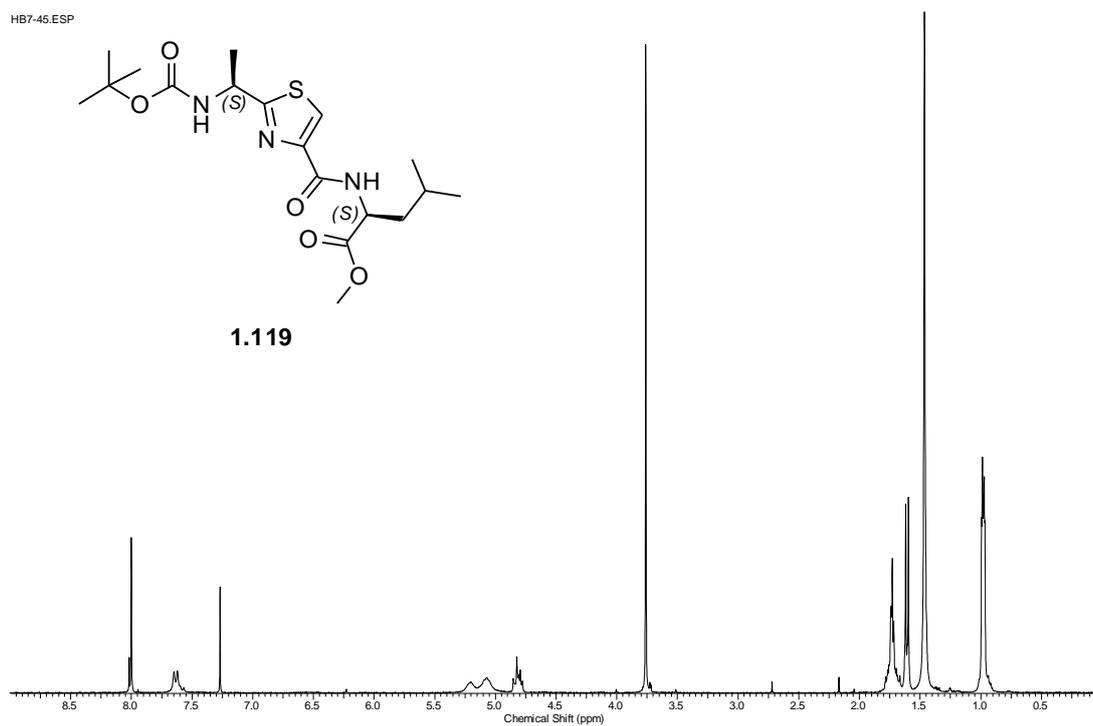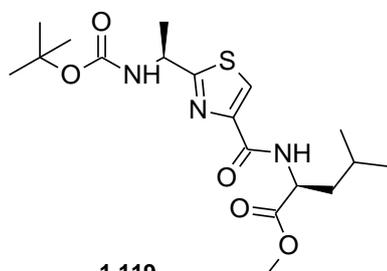
1.119

1.119
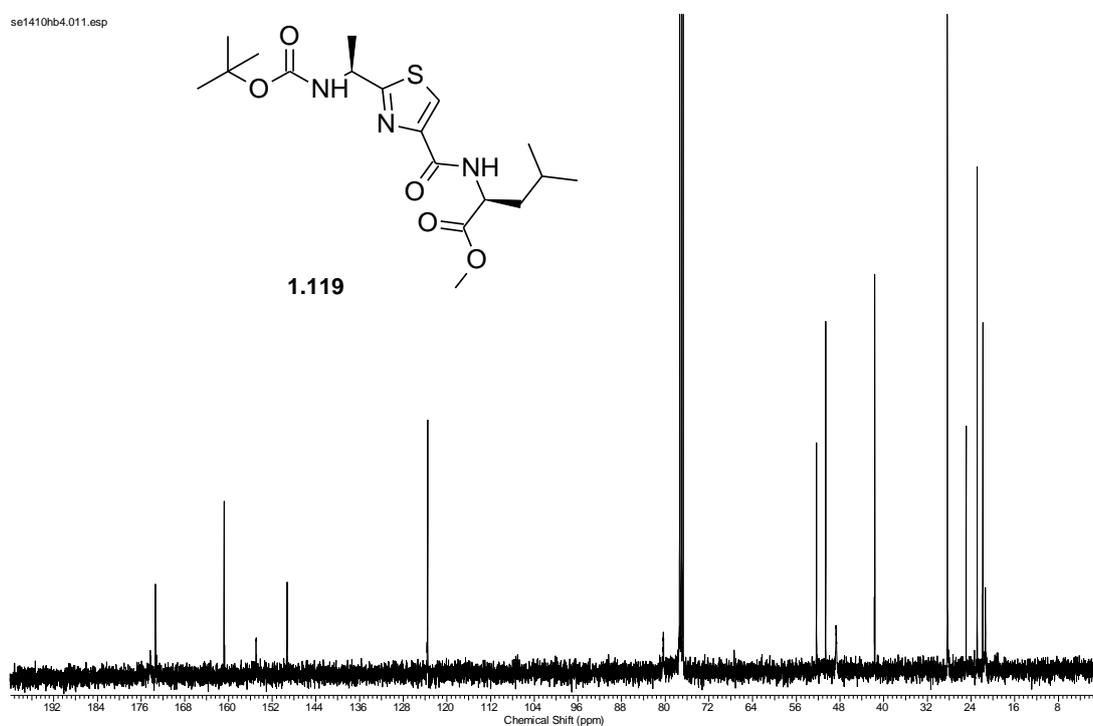
¹H NMR spectrum of 1.124

HB7-39-C2.esp

1.124

Chemical Shift (ppm)
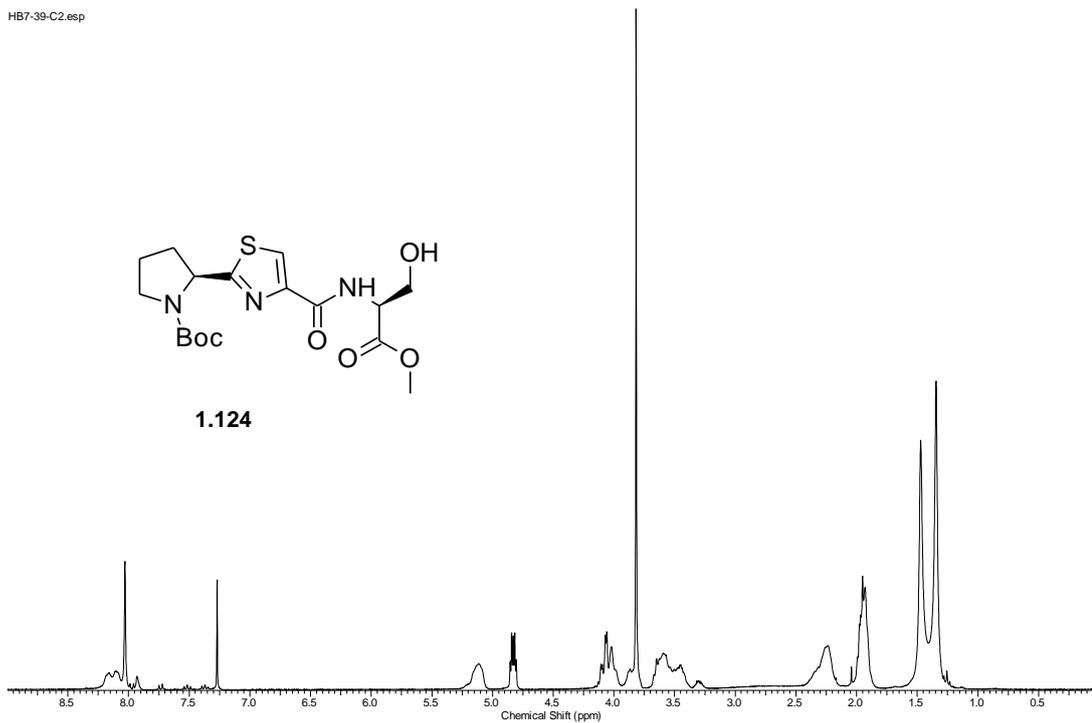
^{1}H and ^{13}C NMR spectra of 1.126

HB7-49.esp
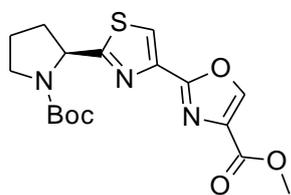
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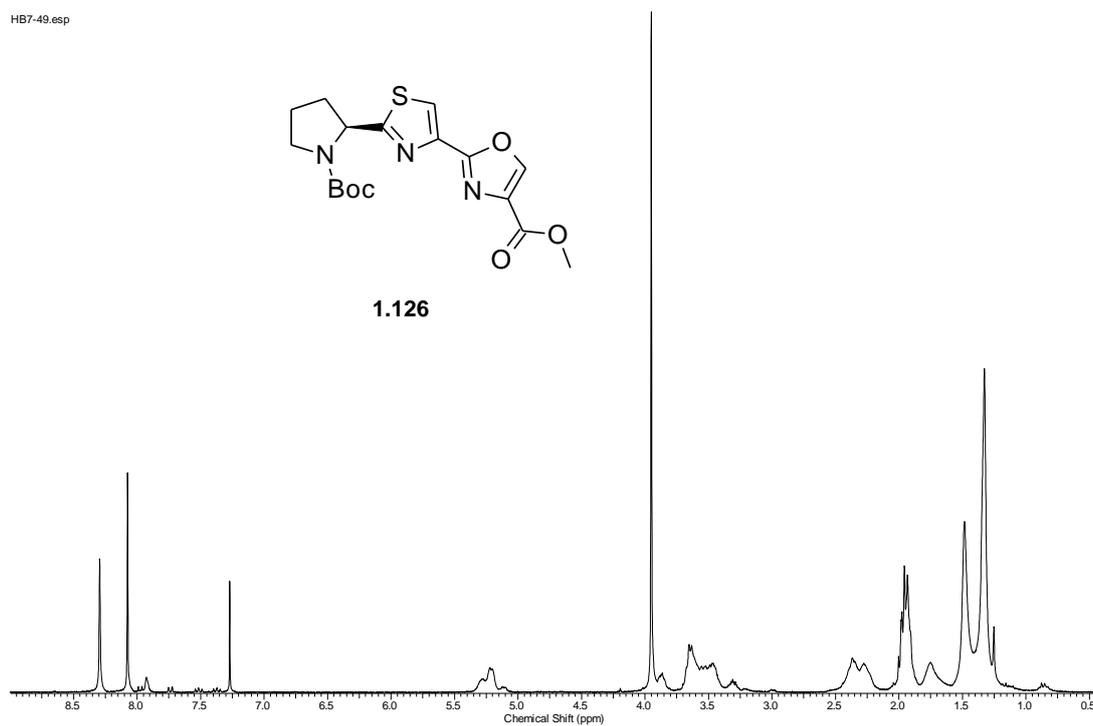
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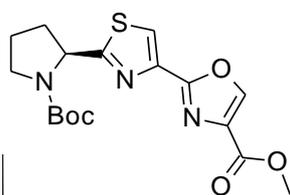
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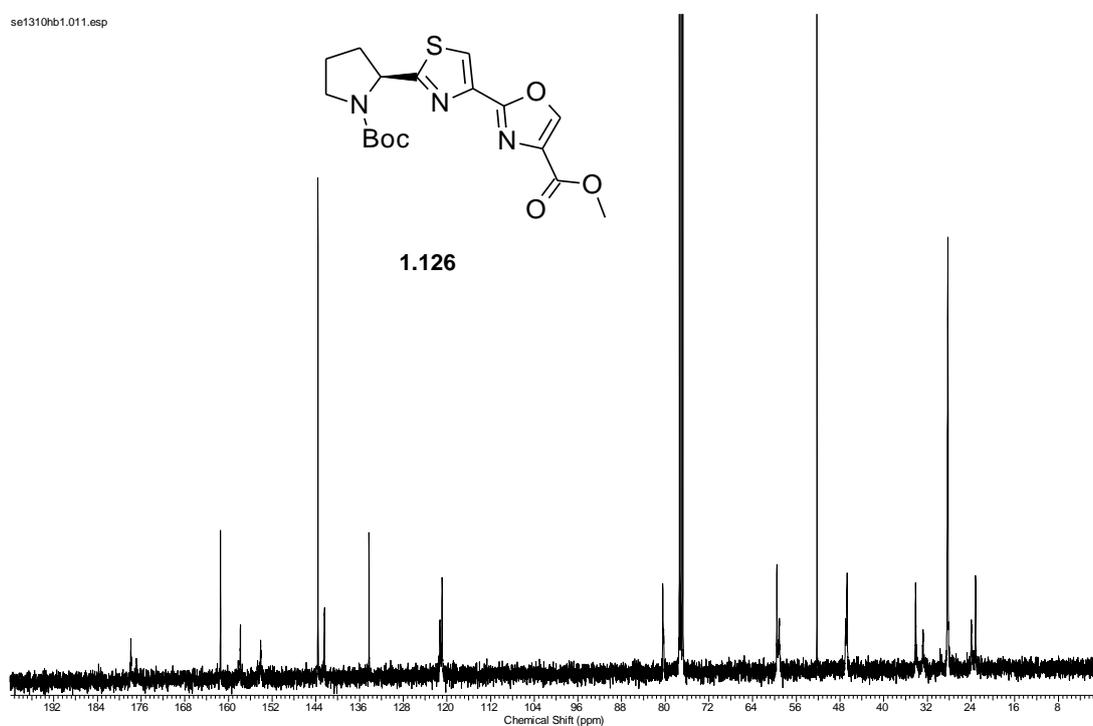
^{1}H NMR spectrum of 1.128

se2710hb2.010.esp

1.128
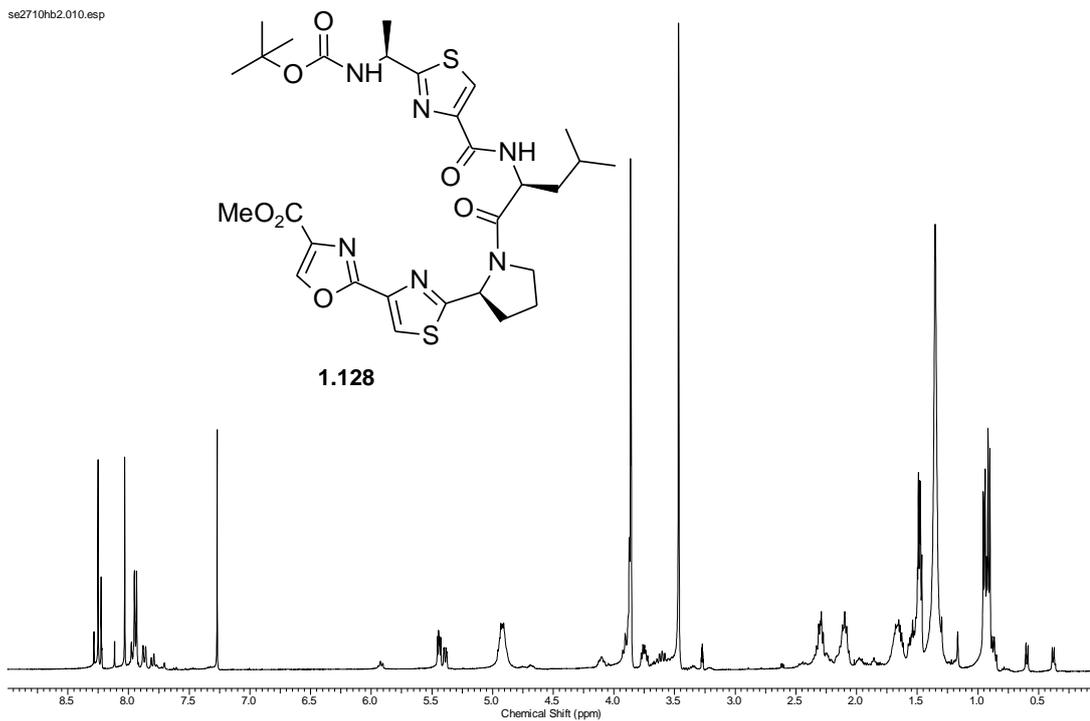
^{1}H NMR spectrum of 1.130

HB7_88_38.jcamp

1.130
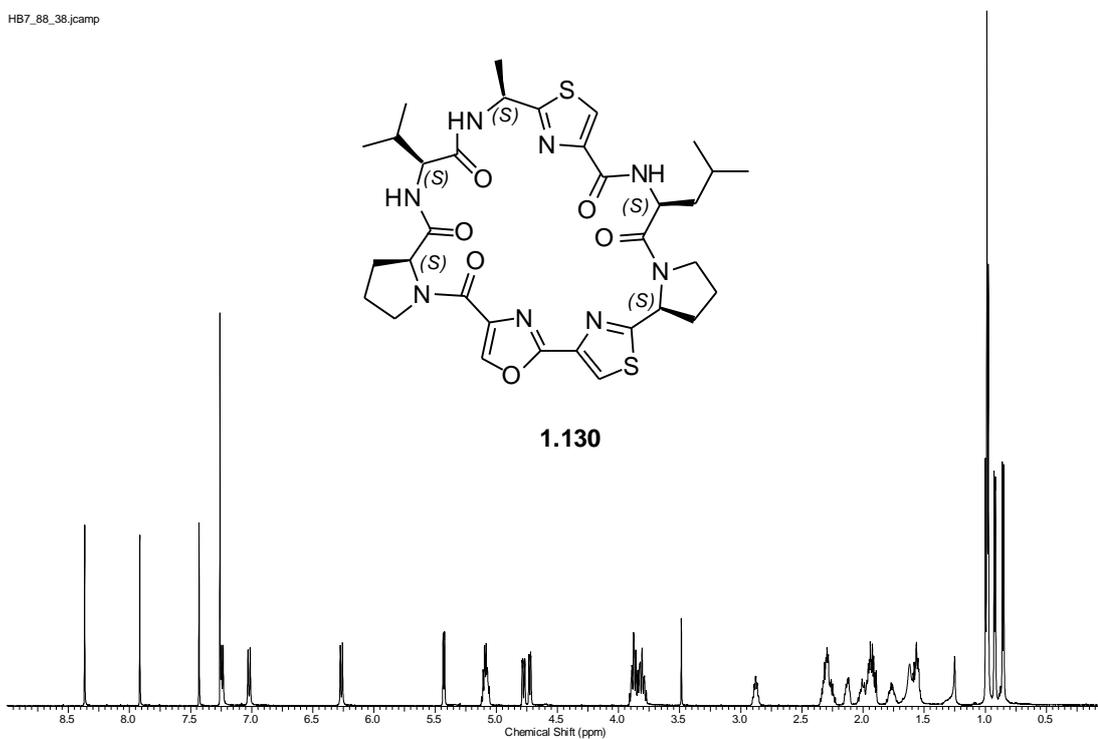
^{1}H NMR spectrum of 1.20

SSAPROTON8_20_10

1.20
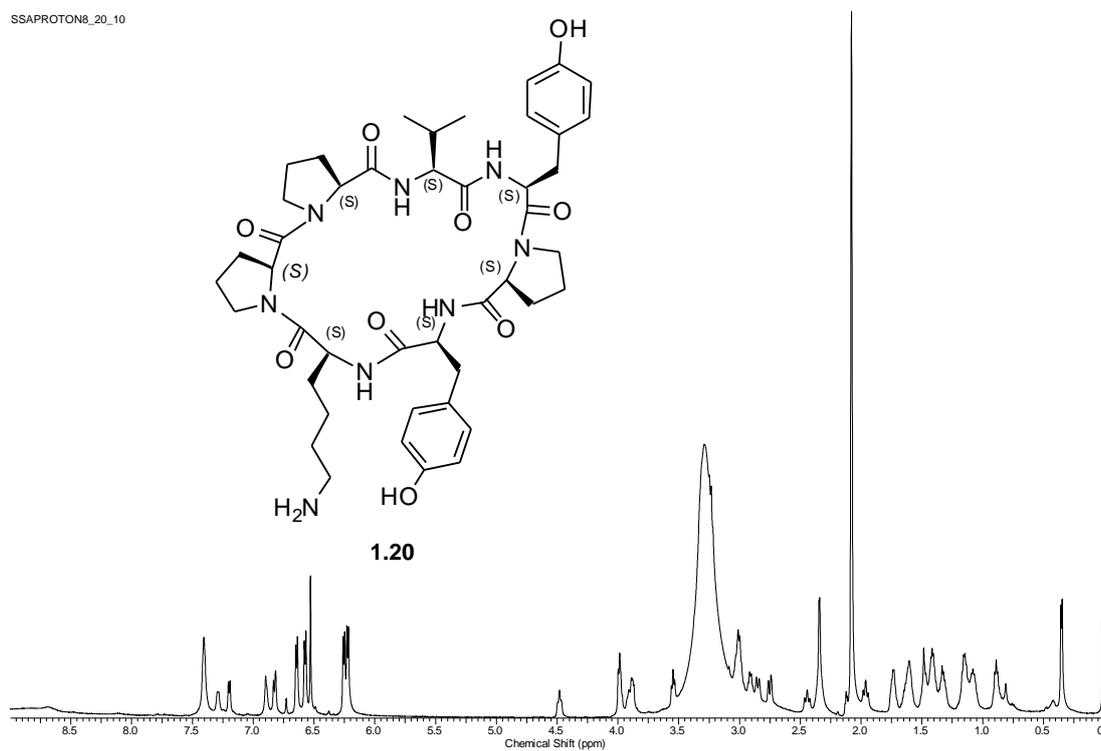
¹H NMR spectrum of 2.144

HB4_94.esp
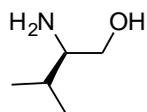
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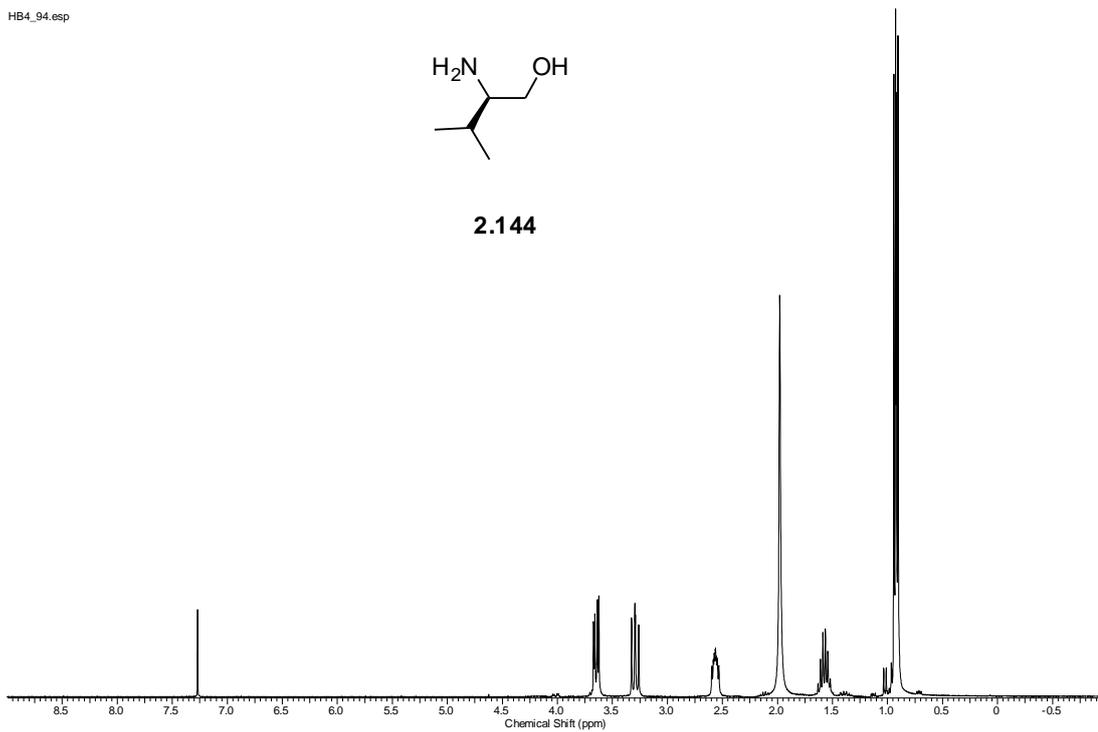
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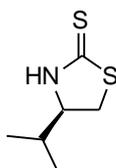
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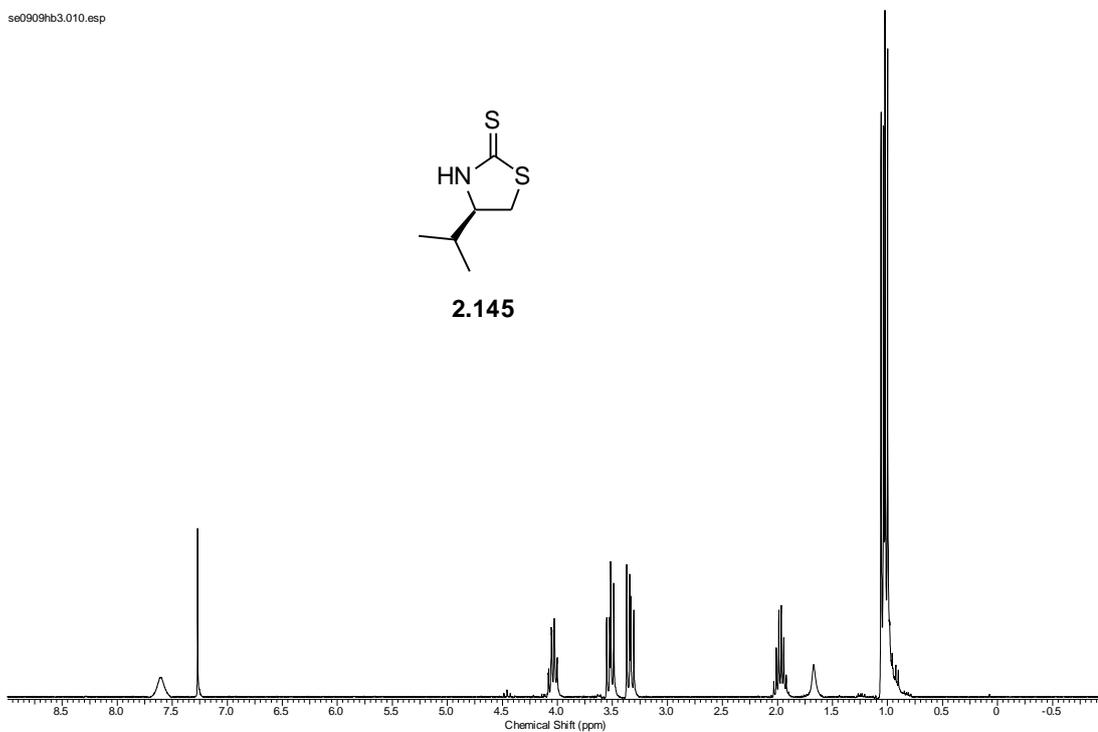
^{1}H NMR spectrum of 2.46

se1109hb2.010.esp

2.46
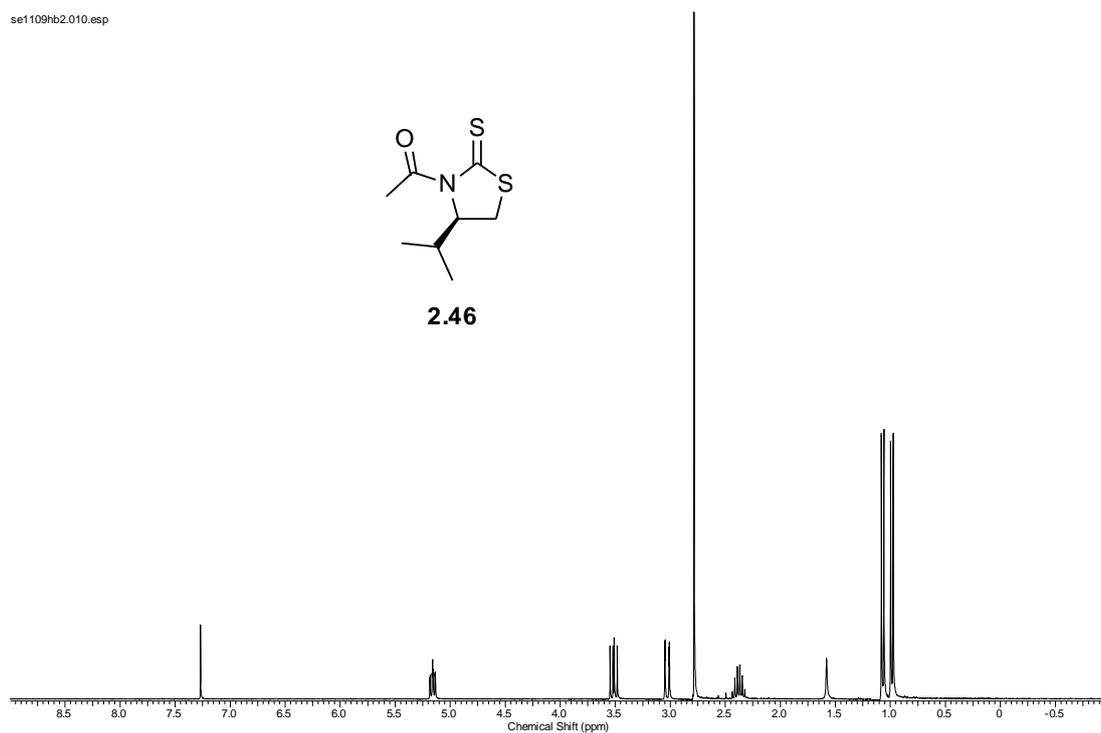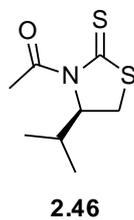
^{1}H NMR spectrum of 2.38

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2.38

¹H NMR spectrum of 2.47
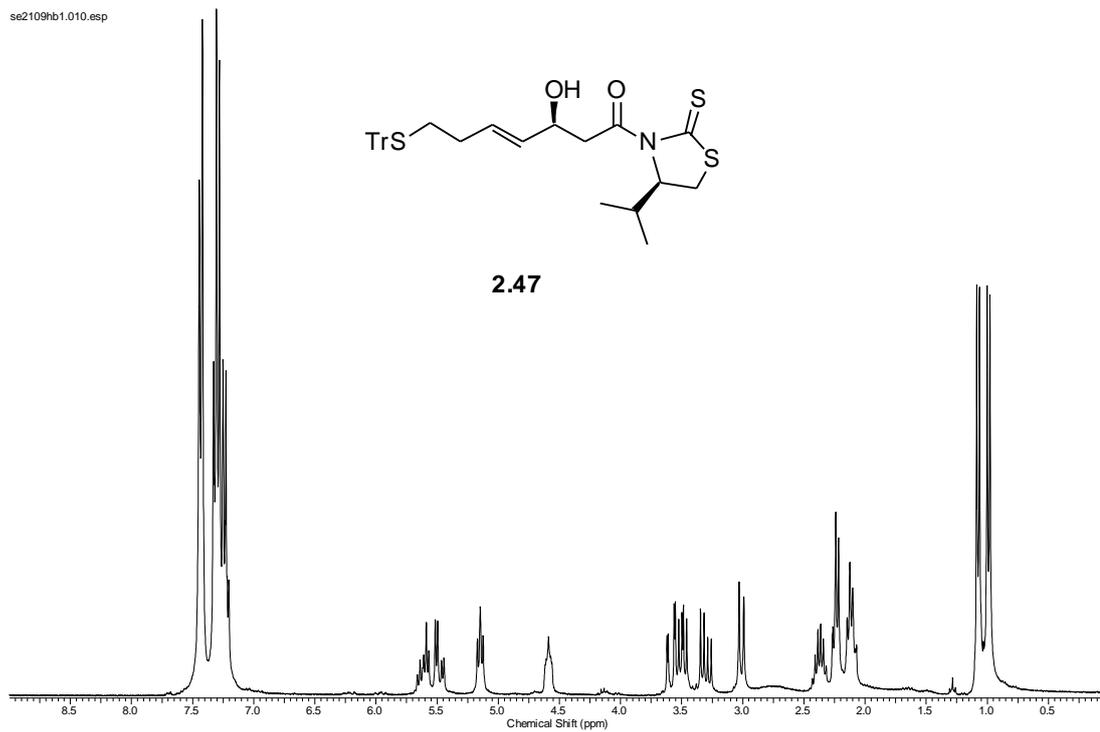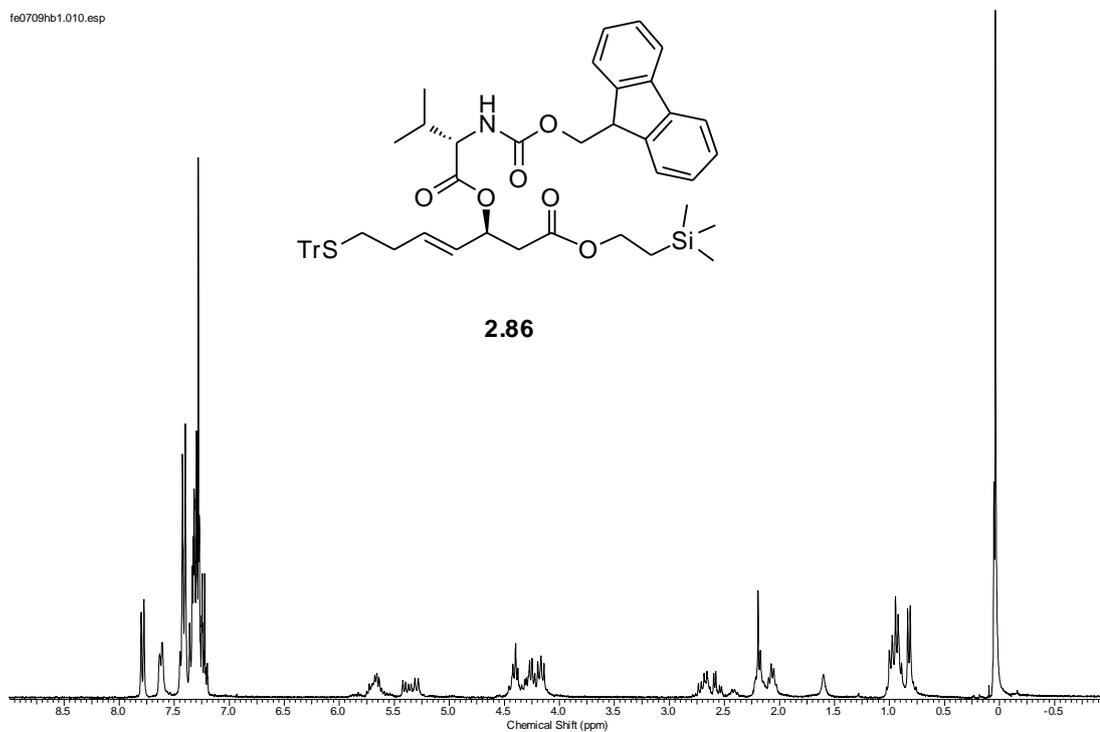
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2.47

¹H NMR spectrum of 2.86

fe0709hb1.010.esp

2.86

^{1}H and ^{13}C NMR spectra of 2.153

^{1}H and ^{13}C NMR spectra of 2.154

ju0608hb2.010.esp

2.154

Chemical Shift (ppm)

ju2008hb2.011.esp

2.154

Chemical Shift (ppm)
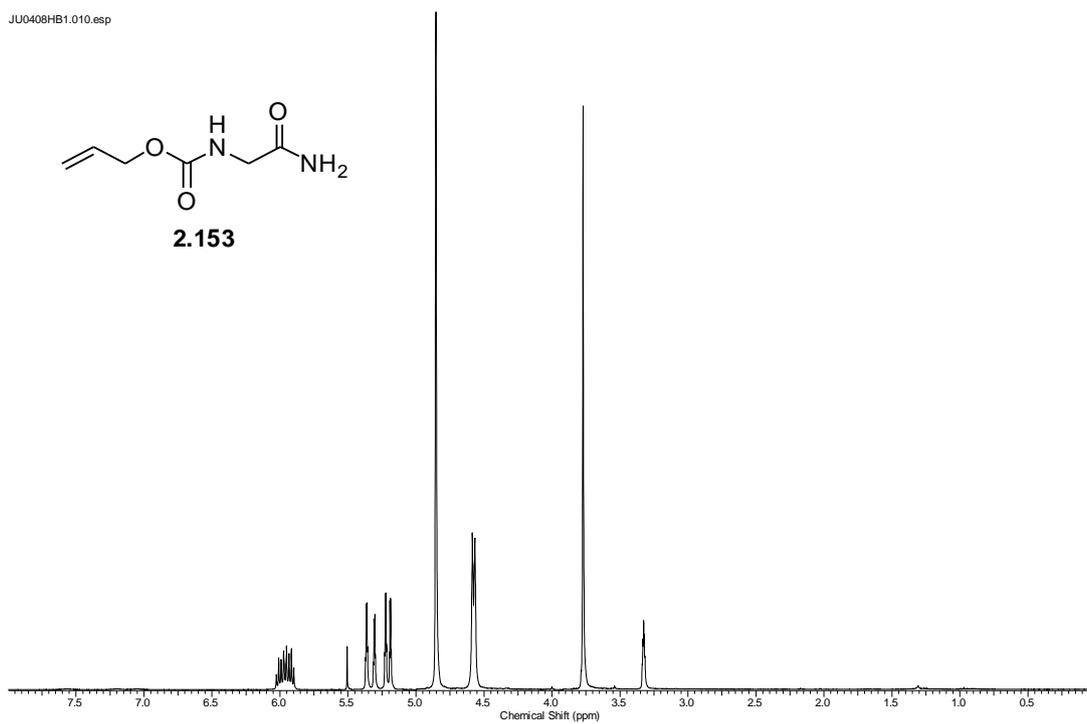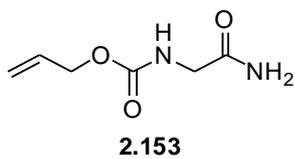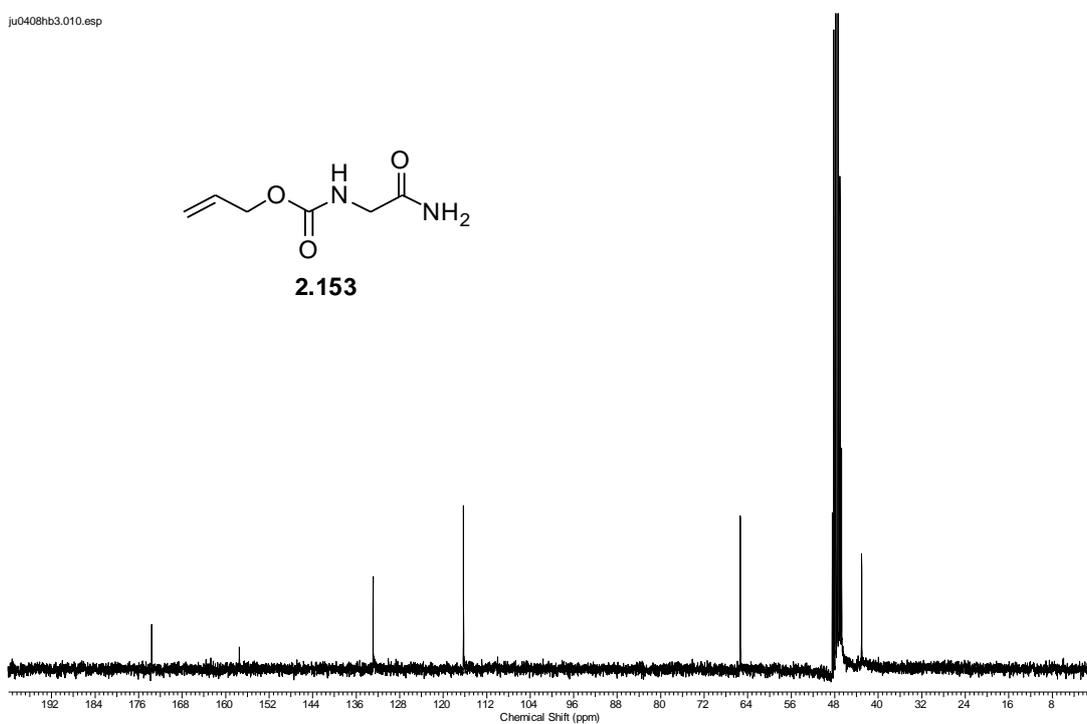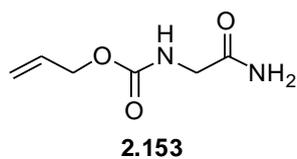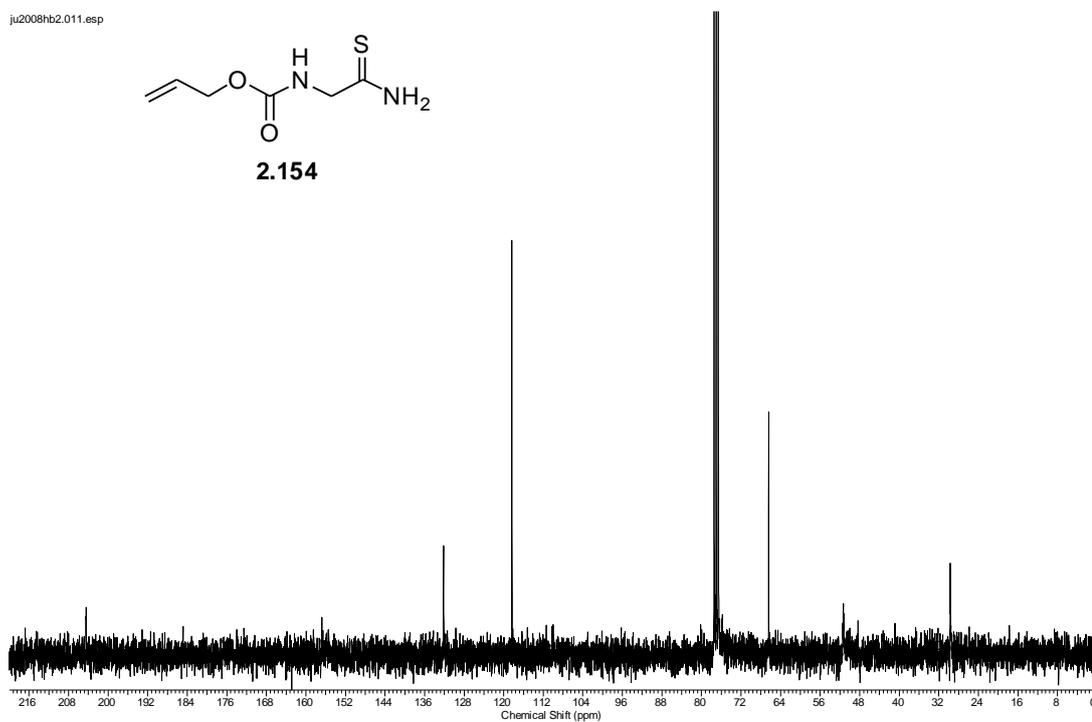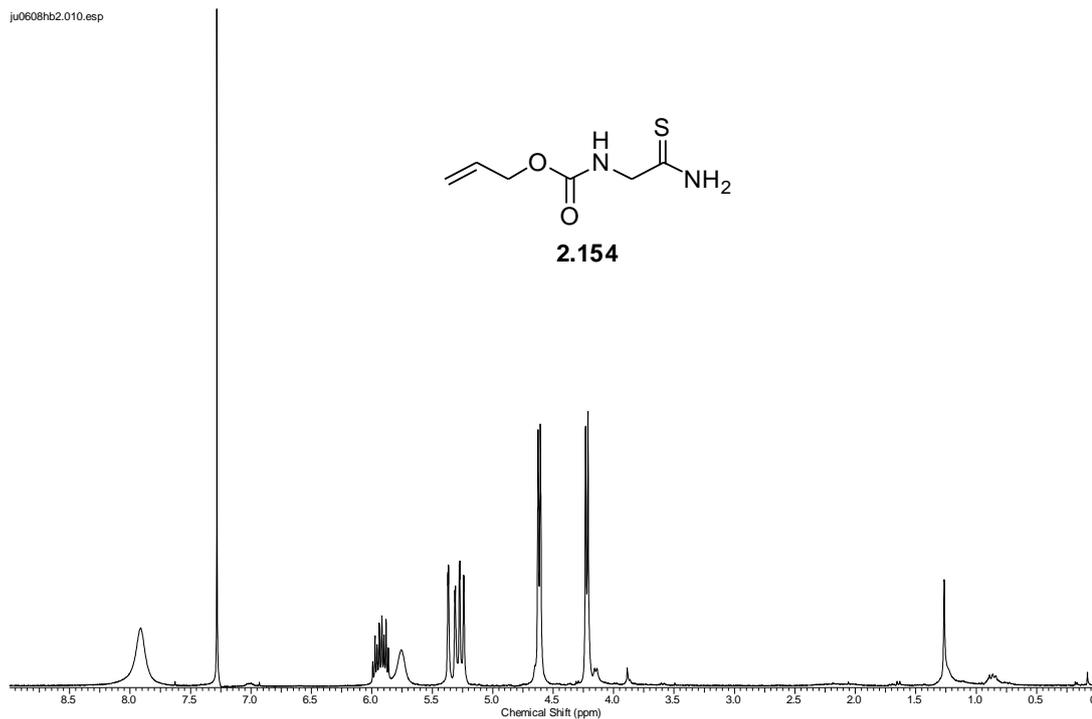
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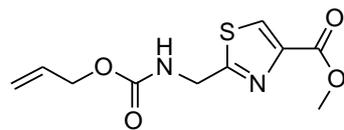
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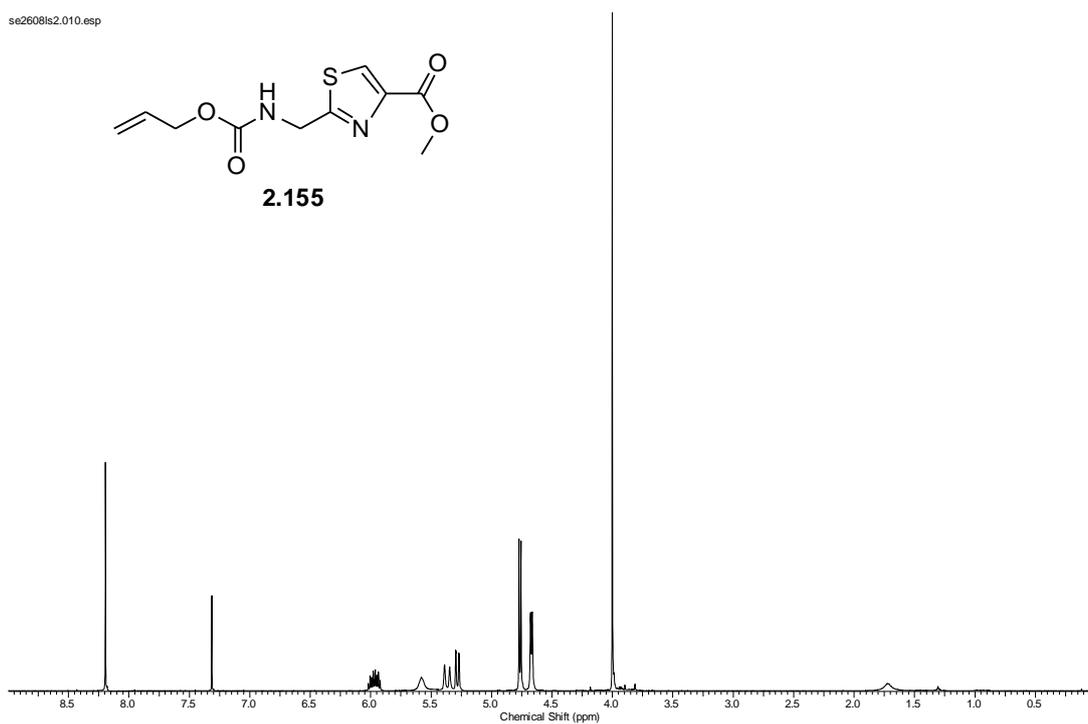
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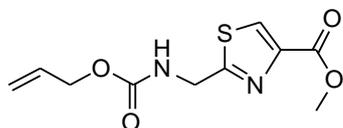
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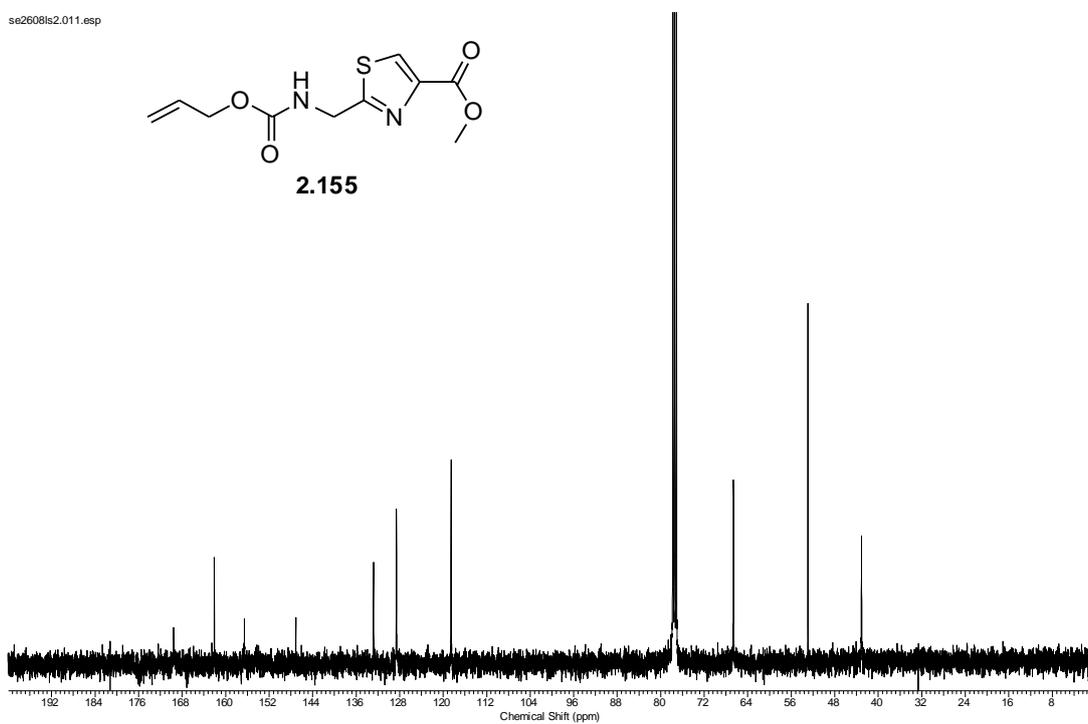
^{1}H and ^{13}C NMR spectra of 2.156

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2.156

se0308hb1.011.esp

2.156

^{1}H NMR spectrum of 2.158
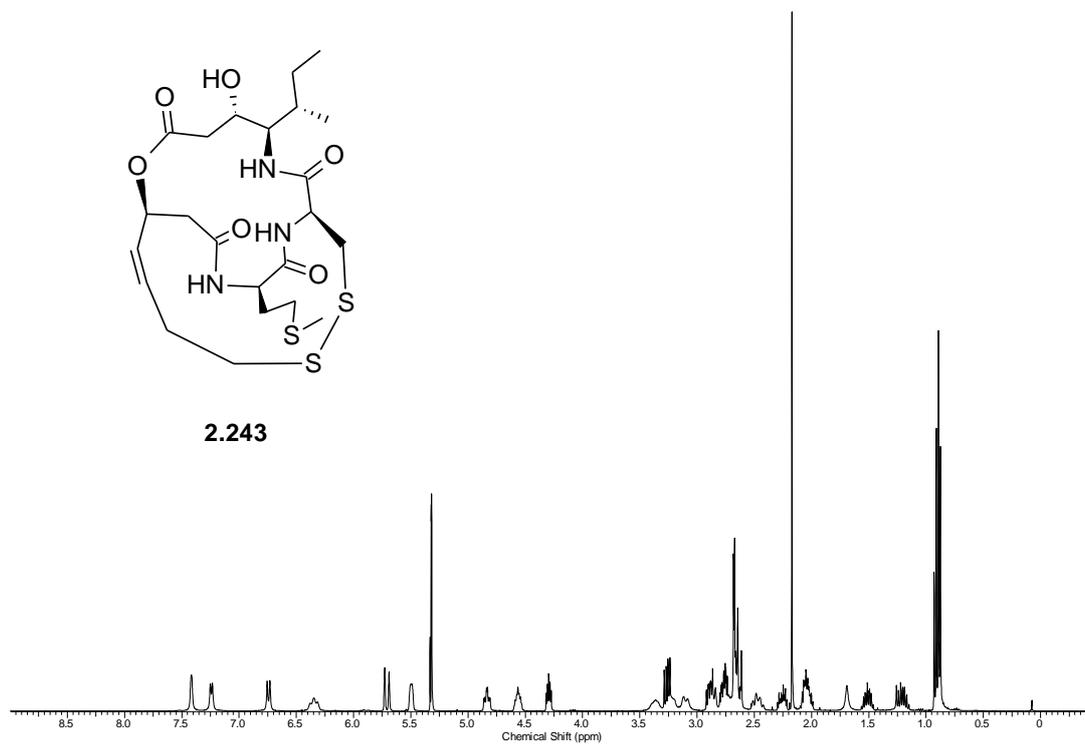
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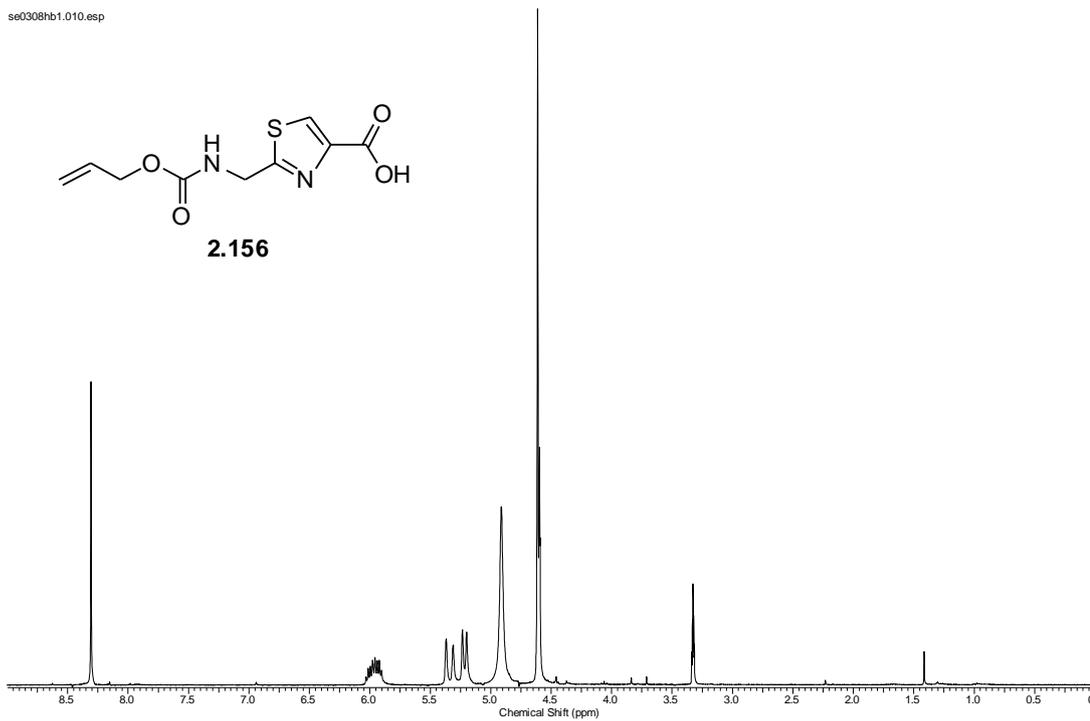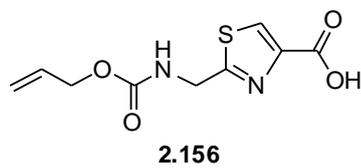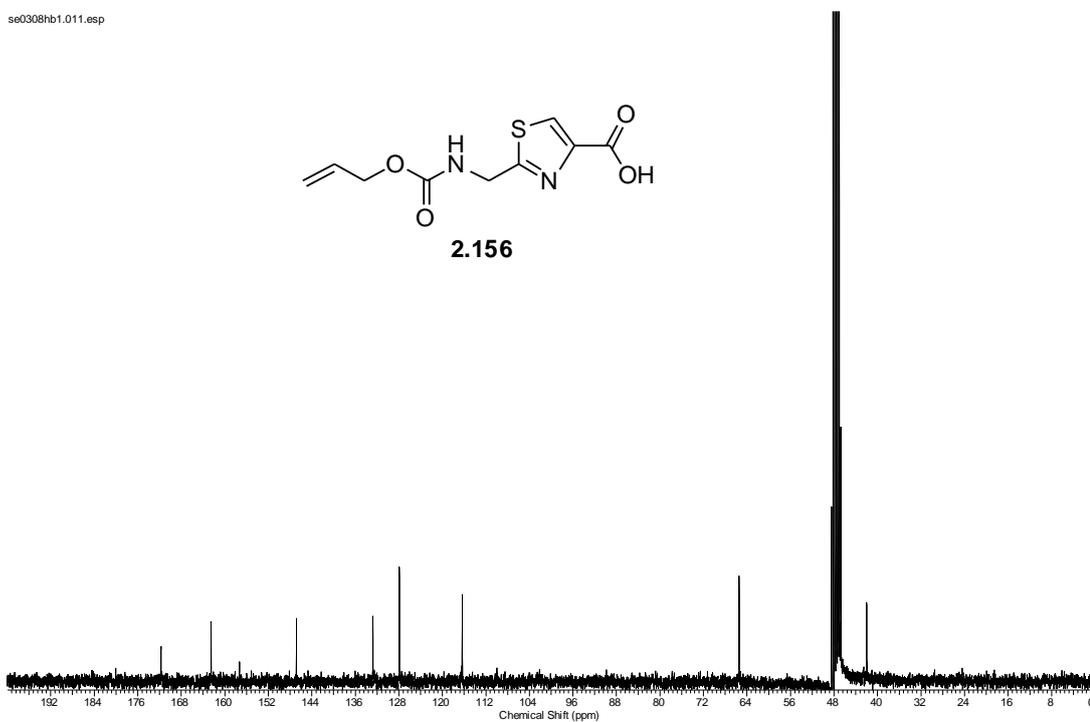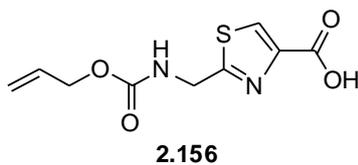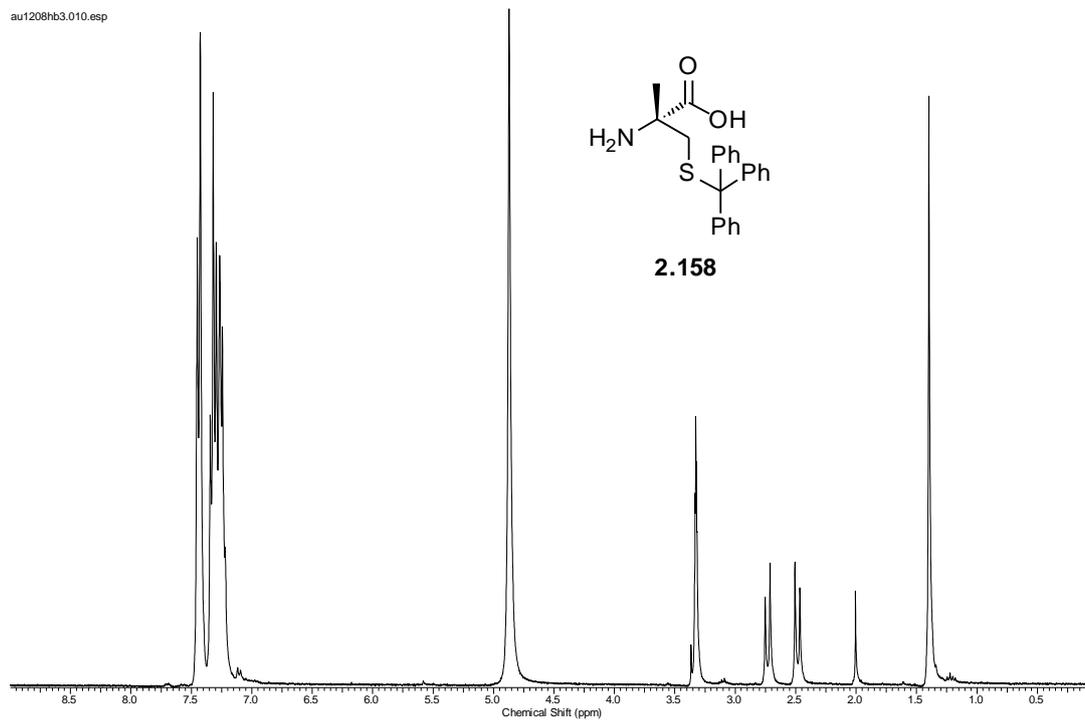
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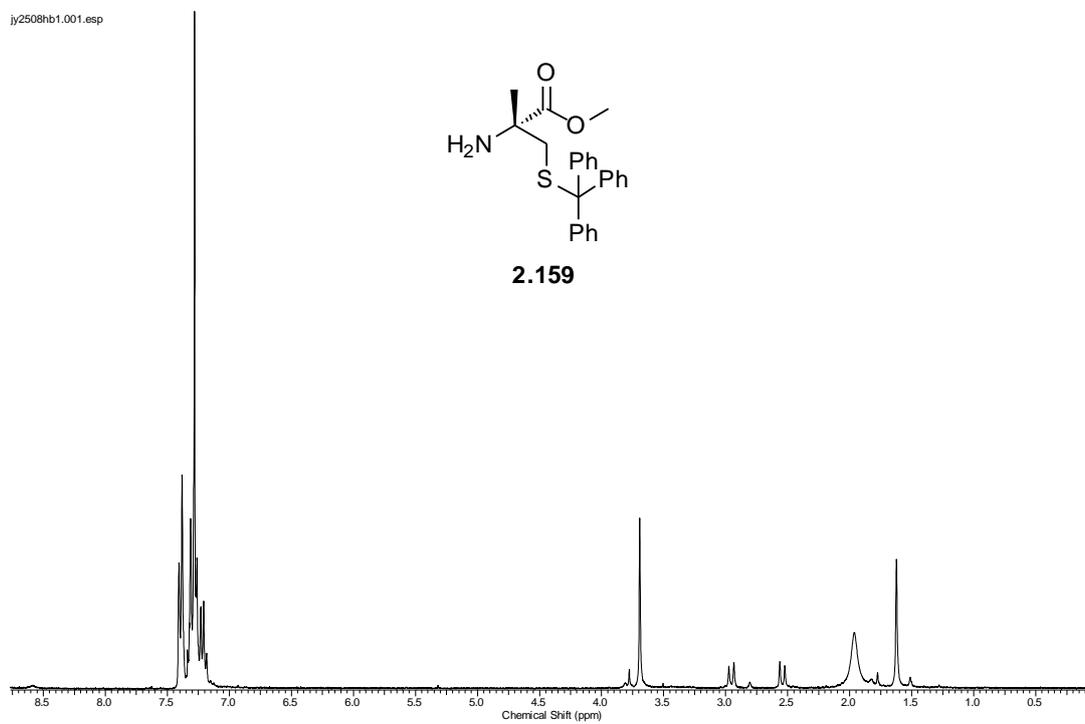
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2.160

se0608hb2.011.esp

2.160
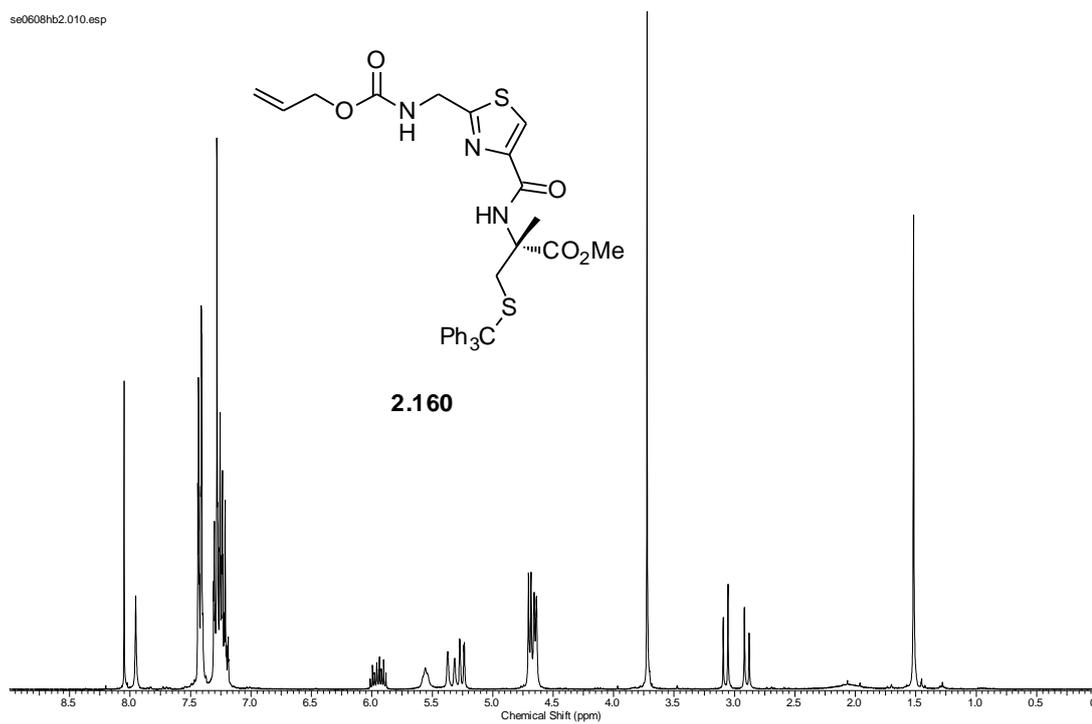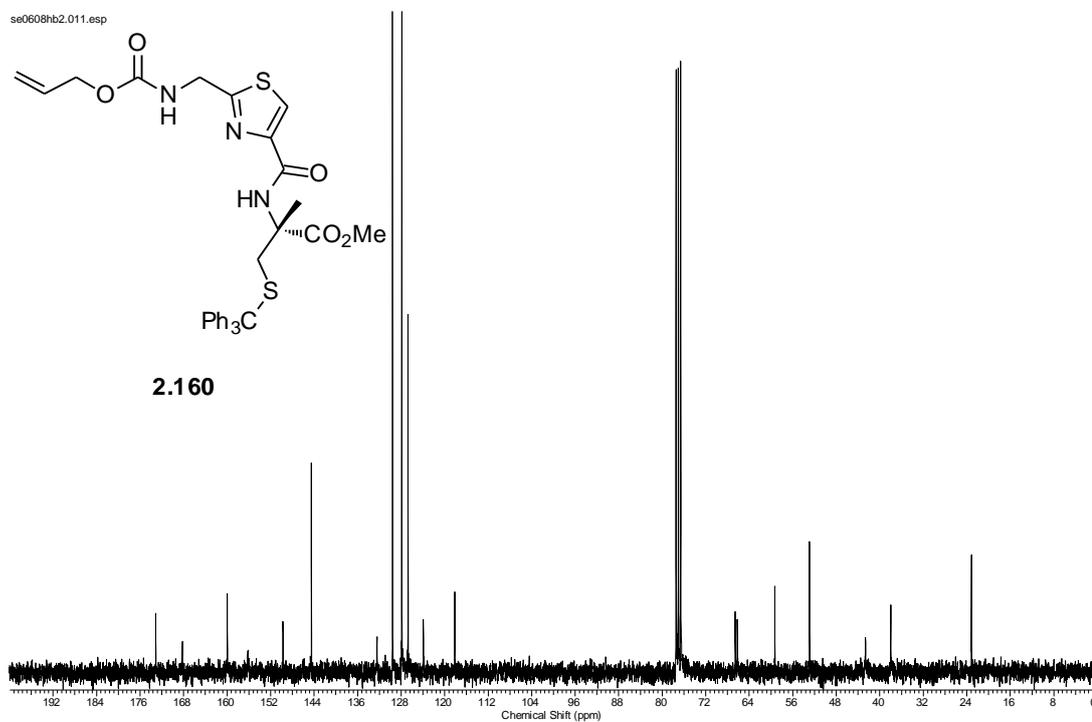
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2.163

dc1408ls4.011.esp

2.163
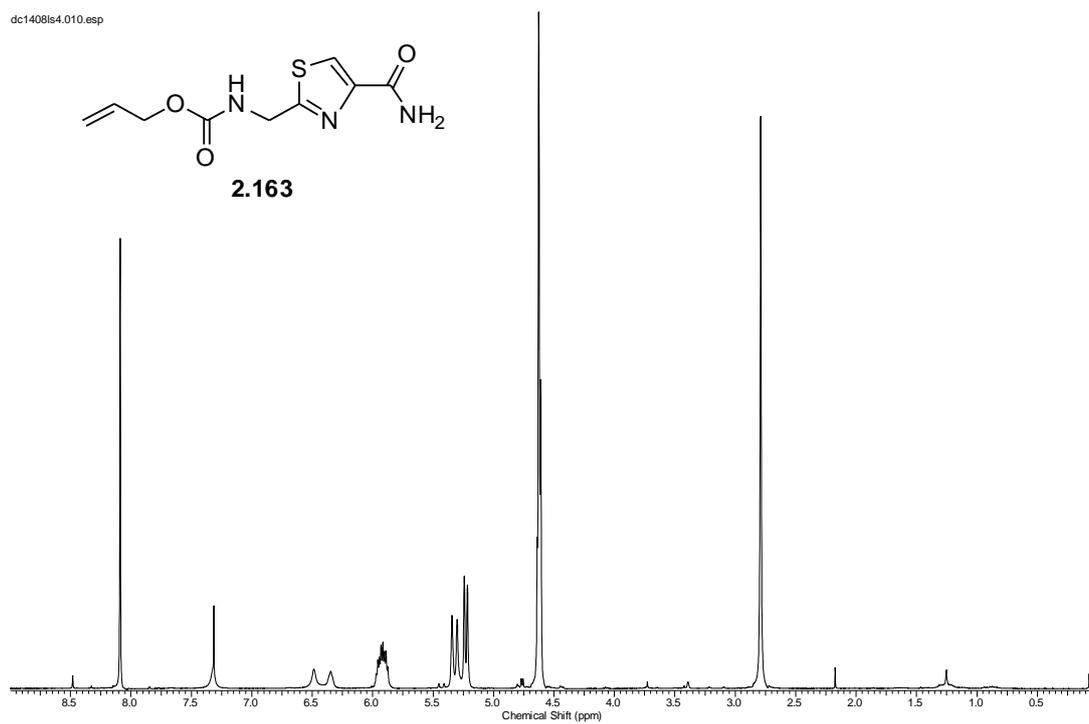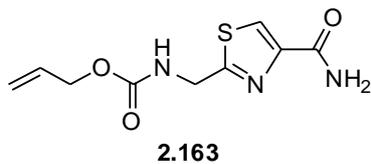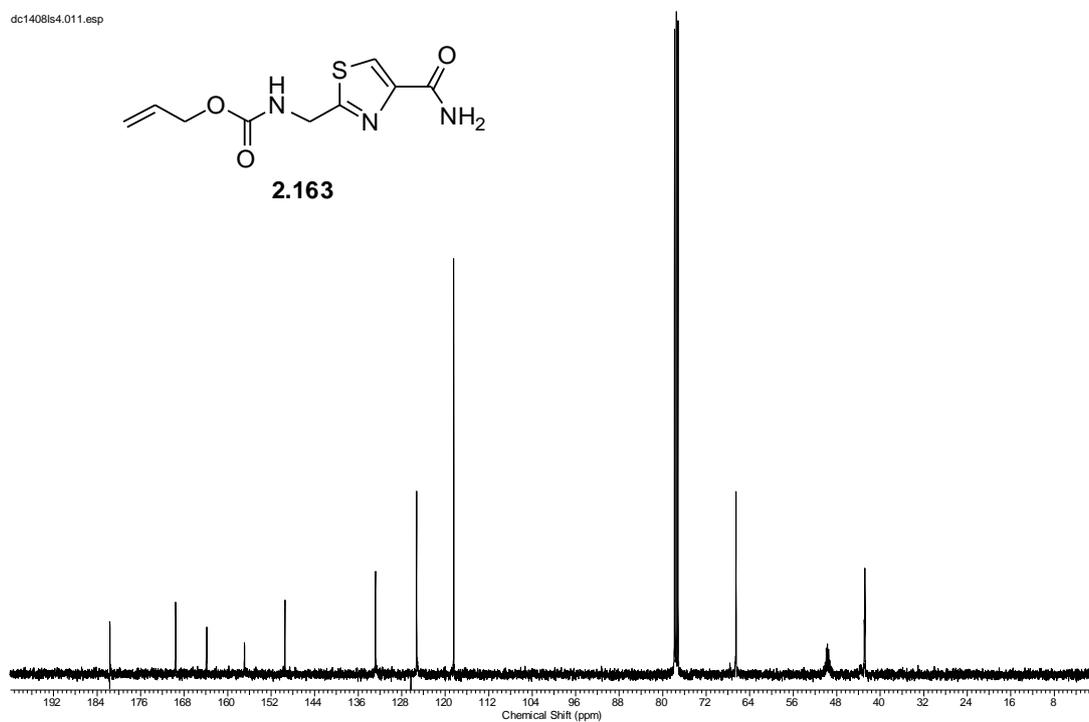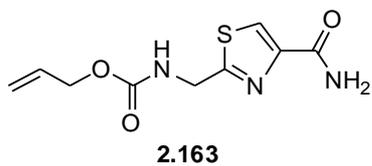
^{1}H and ^{13}C NMR spectra of 2.164
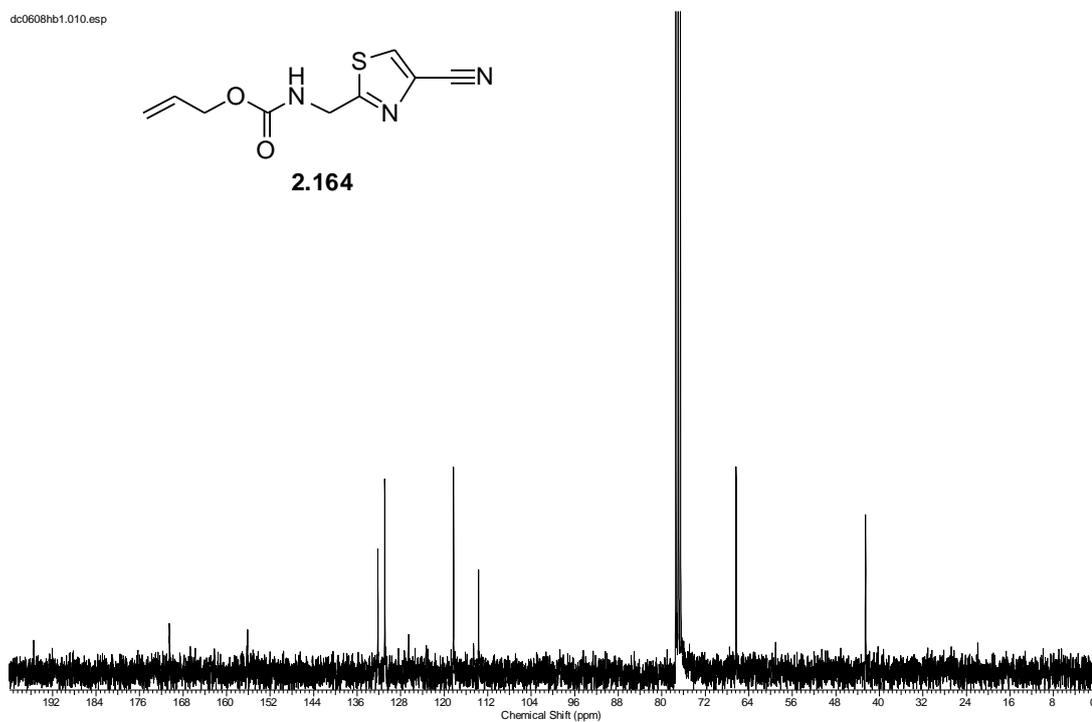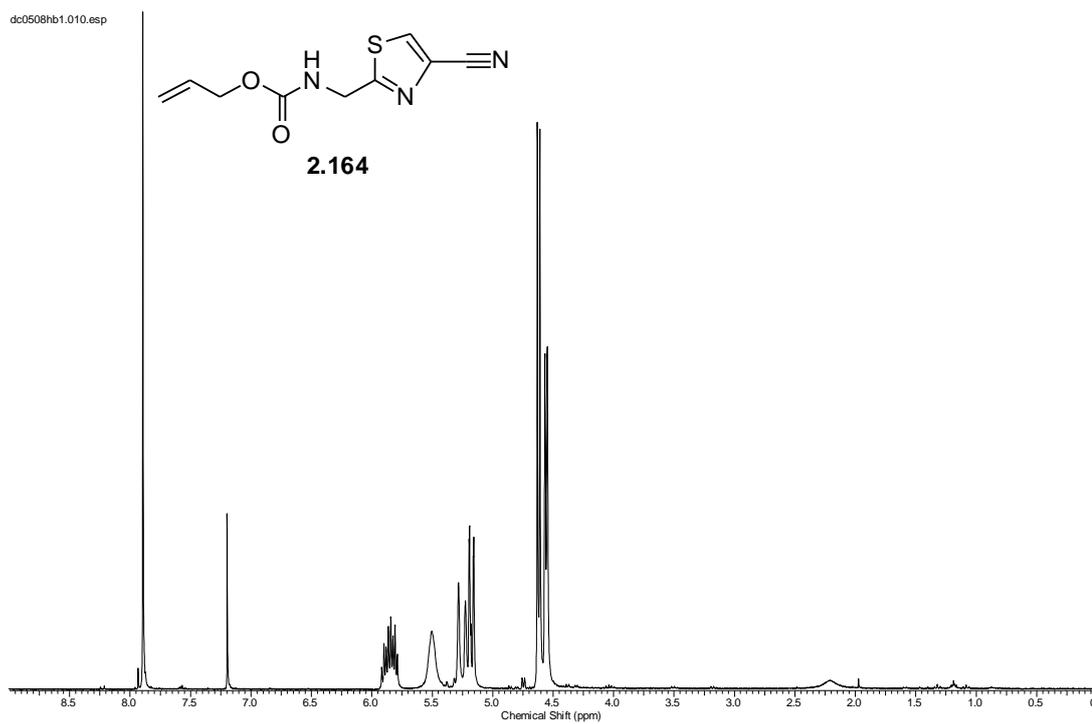
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2.165

Chemical Shift (ppm)
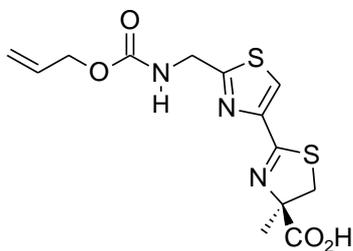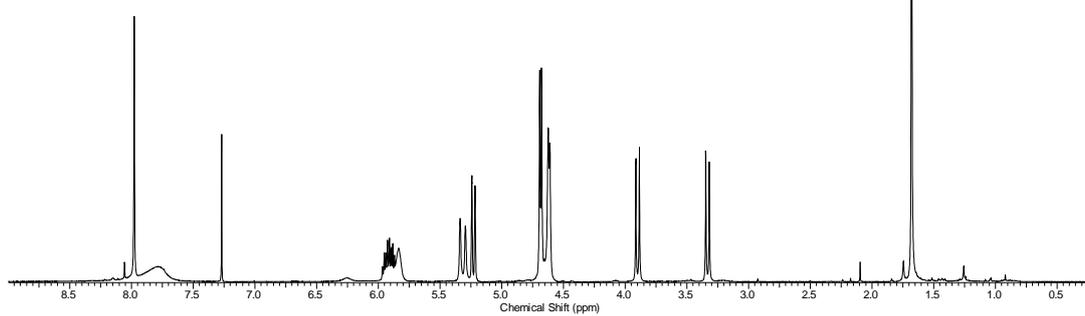
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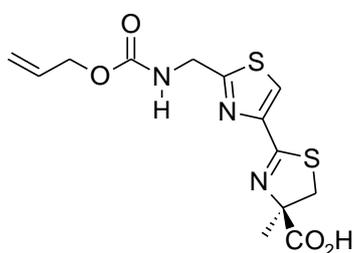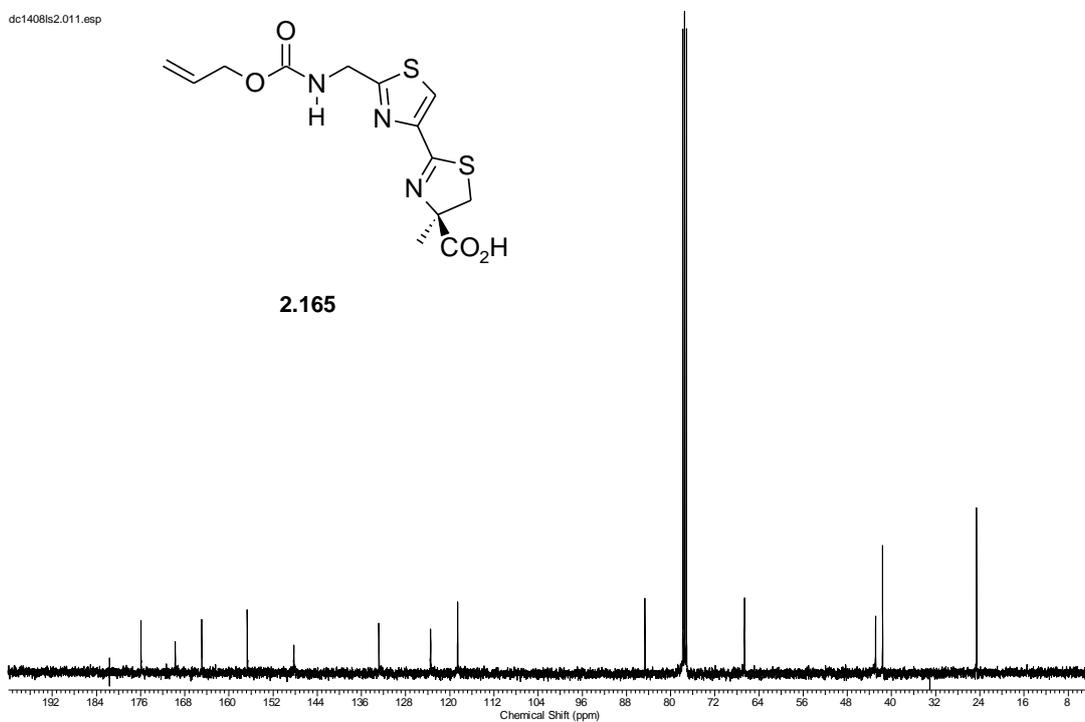
^{1}H and ^{13}C NMR spectra of 2.166

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2.166

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2.166
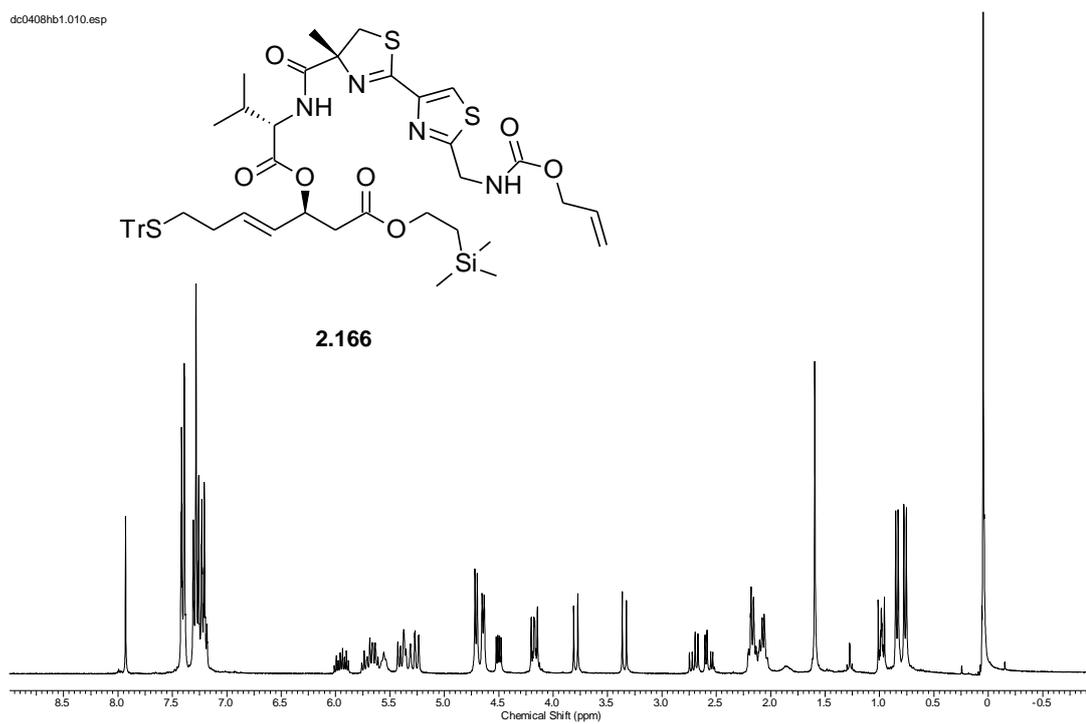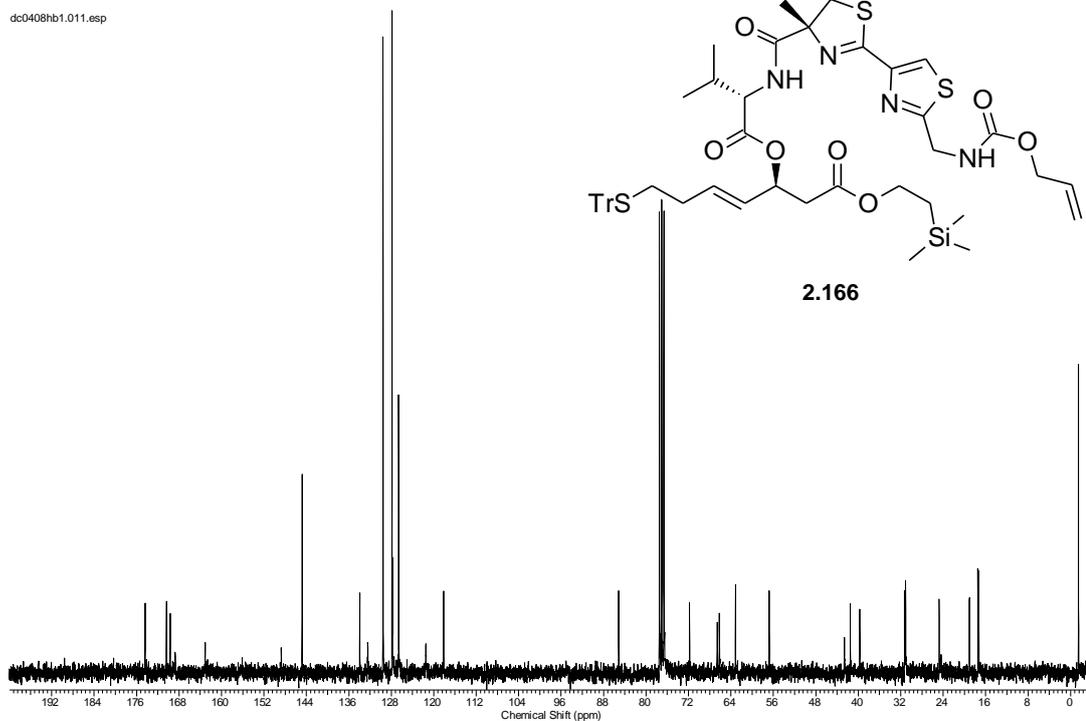
^{1}H NMR spectrum of 2.171

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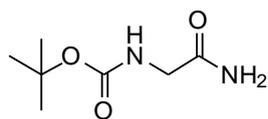
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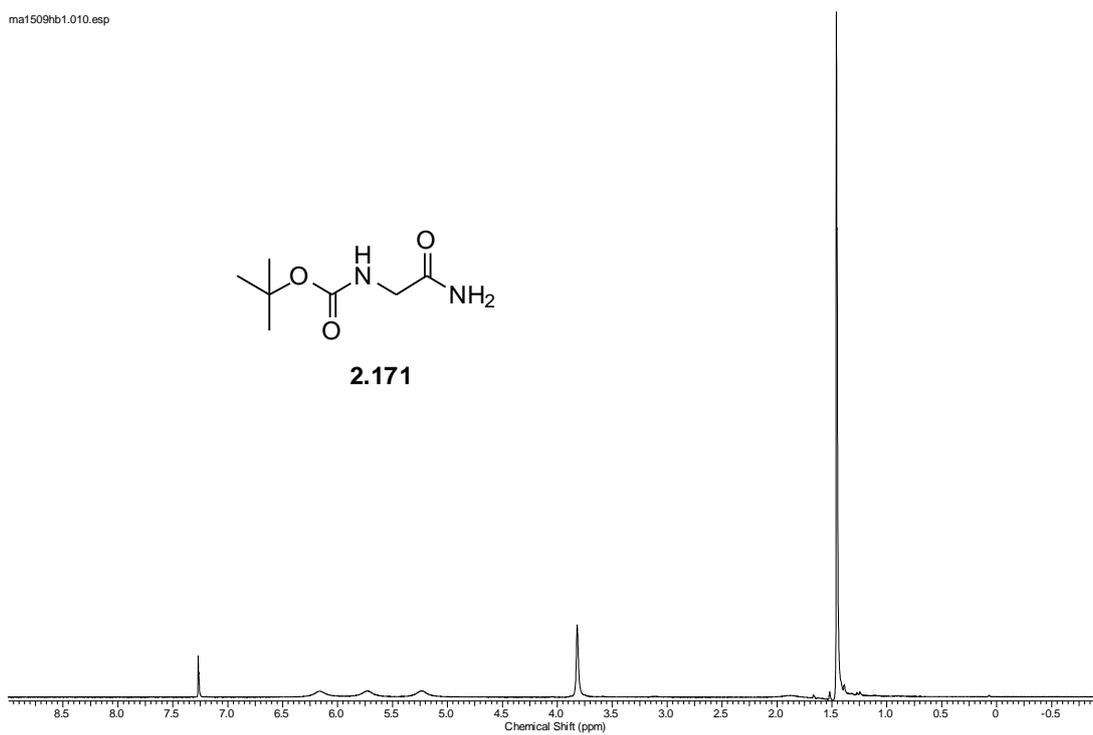
^{1}H NMR spectrum of 2.149

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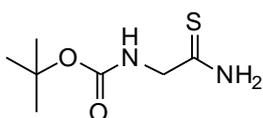
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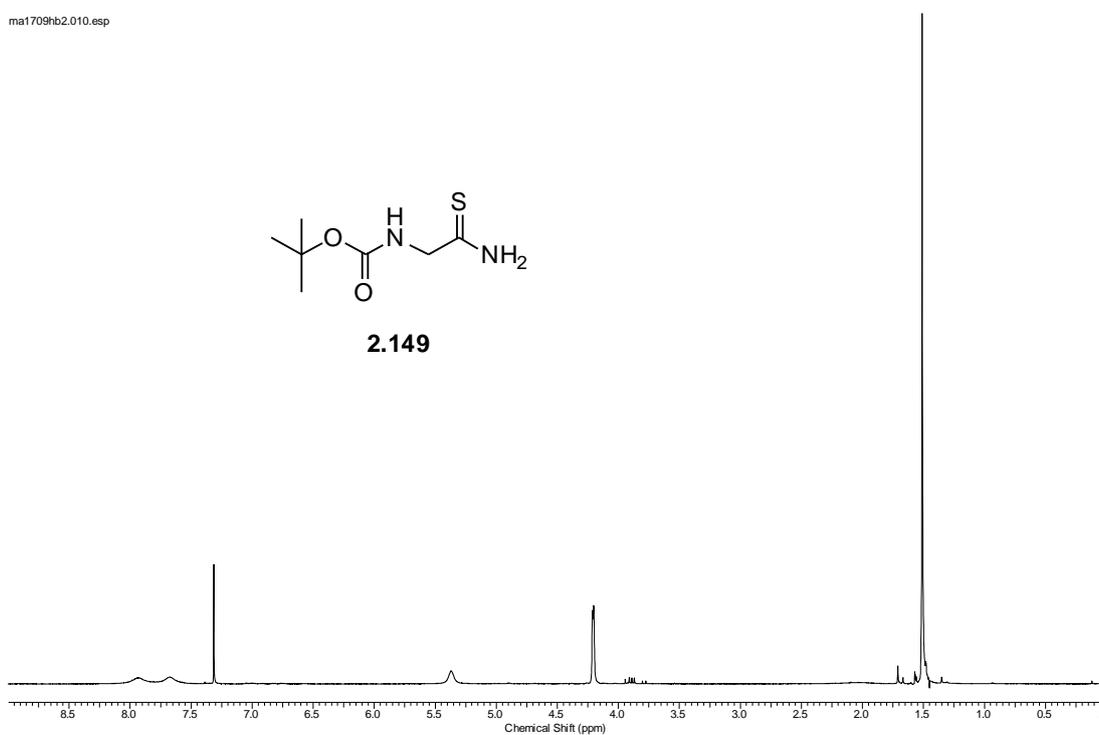
^{1}H NMR spectrum of 2.151

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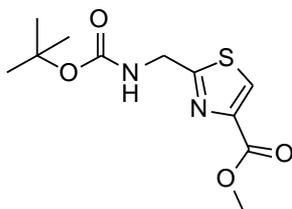
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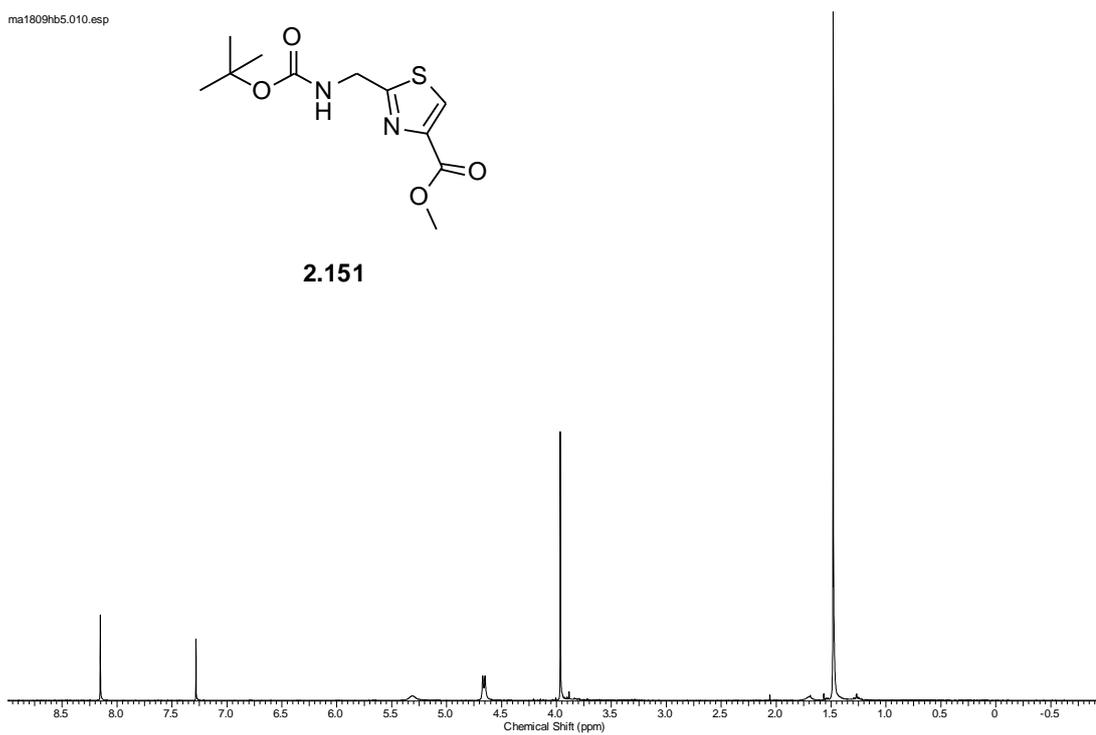
^{1}H NMR spectrum of 2.147

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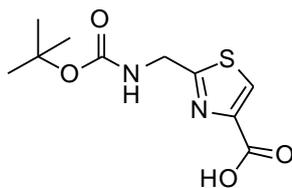
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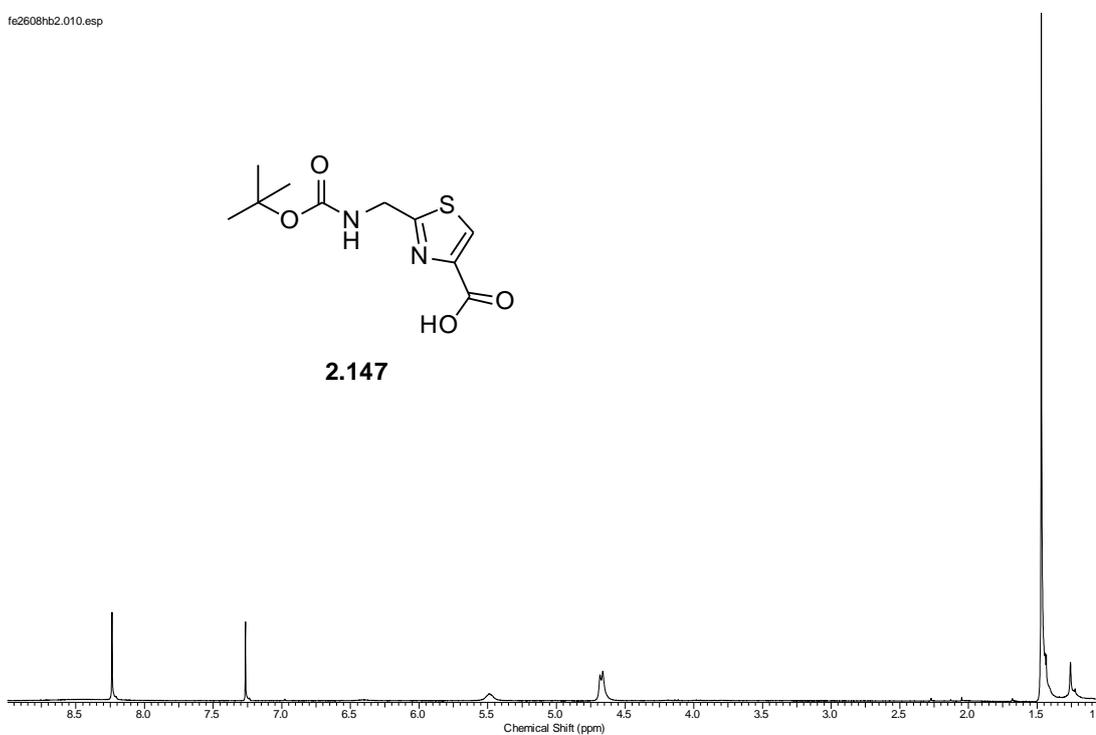
¹H NMR spectrum of 2.176

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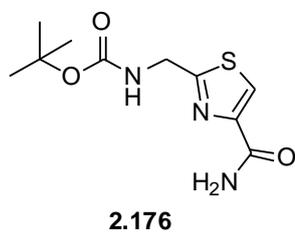
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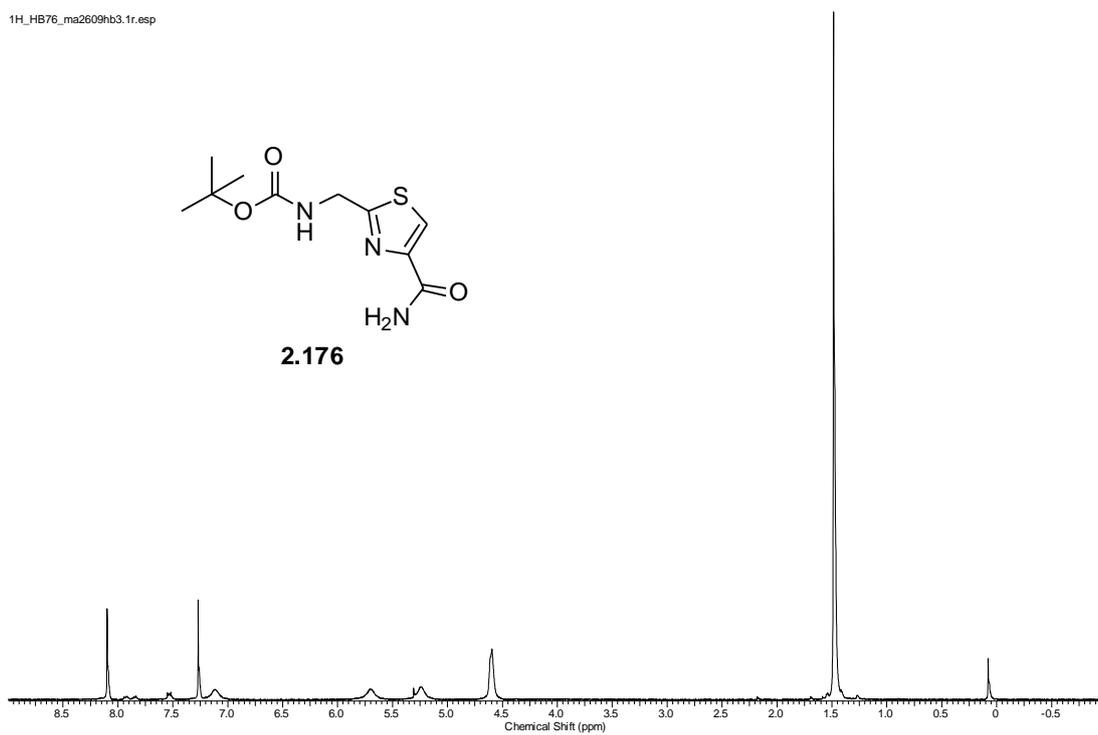
¹H NMR spectrum of 2.100

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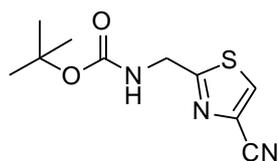
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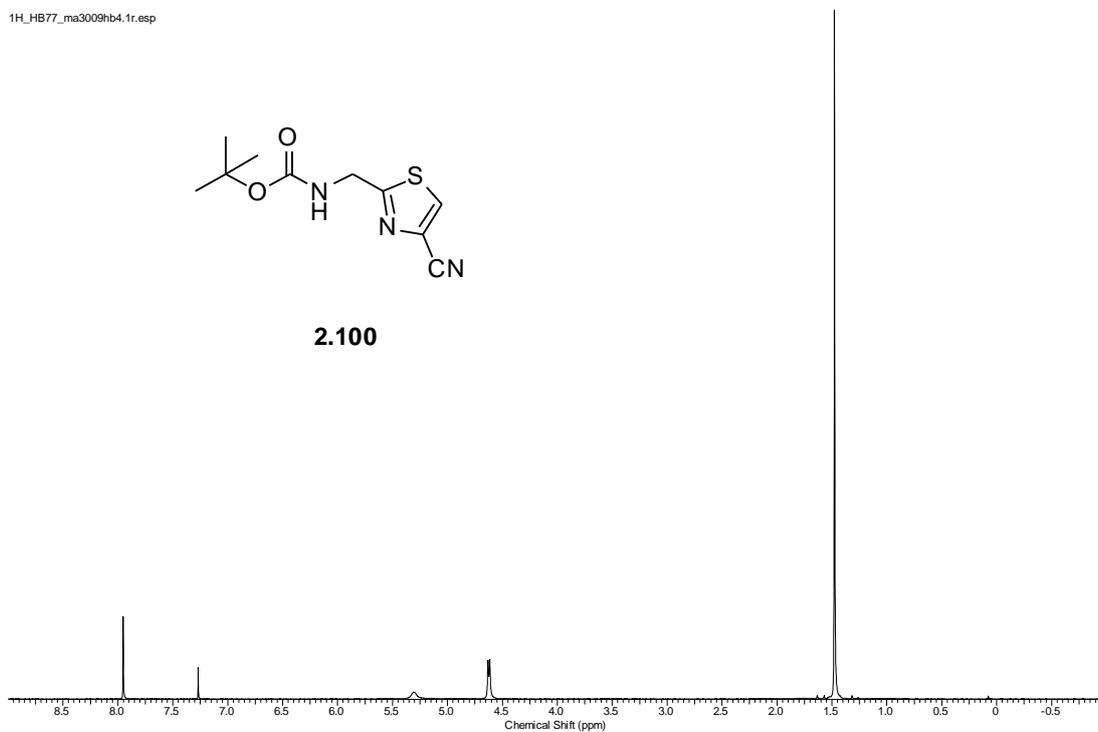
¹H NMR spectrum of 2.173

my1508hb3.010.esp

2.173a + **2.173b**
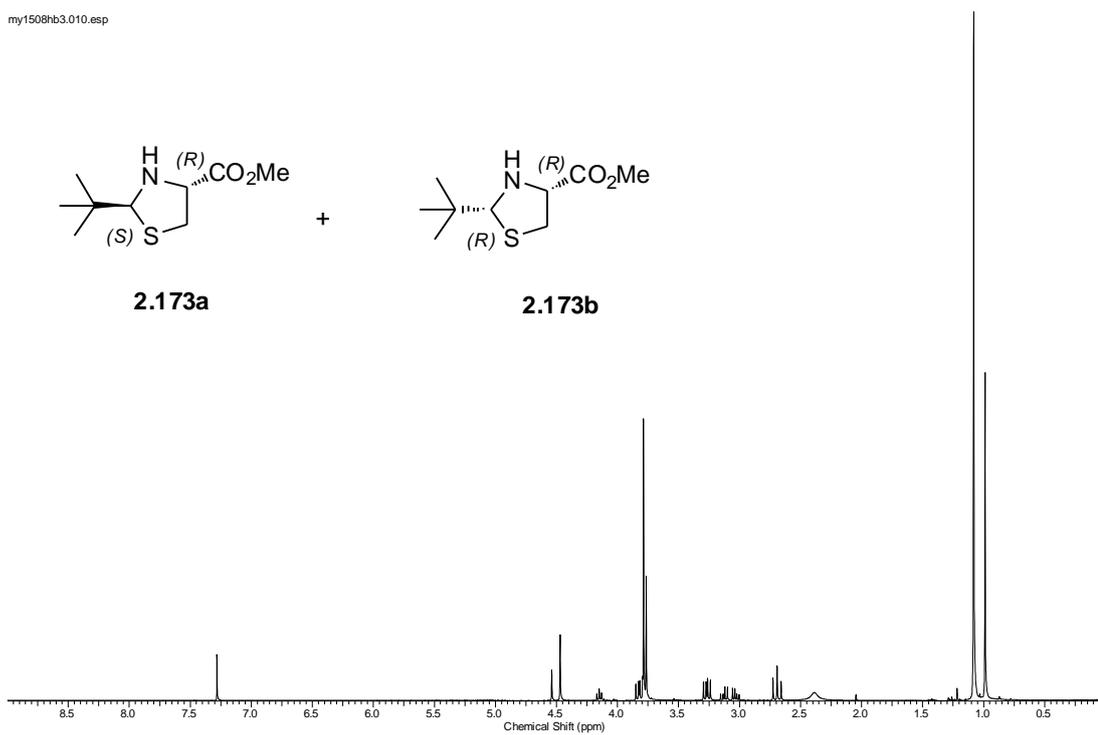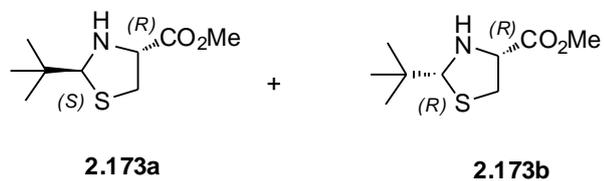
¹H NMR spectrum of 2.174

oc2708hb2.010.esp

2.174
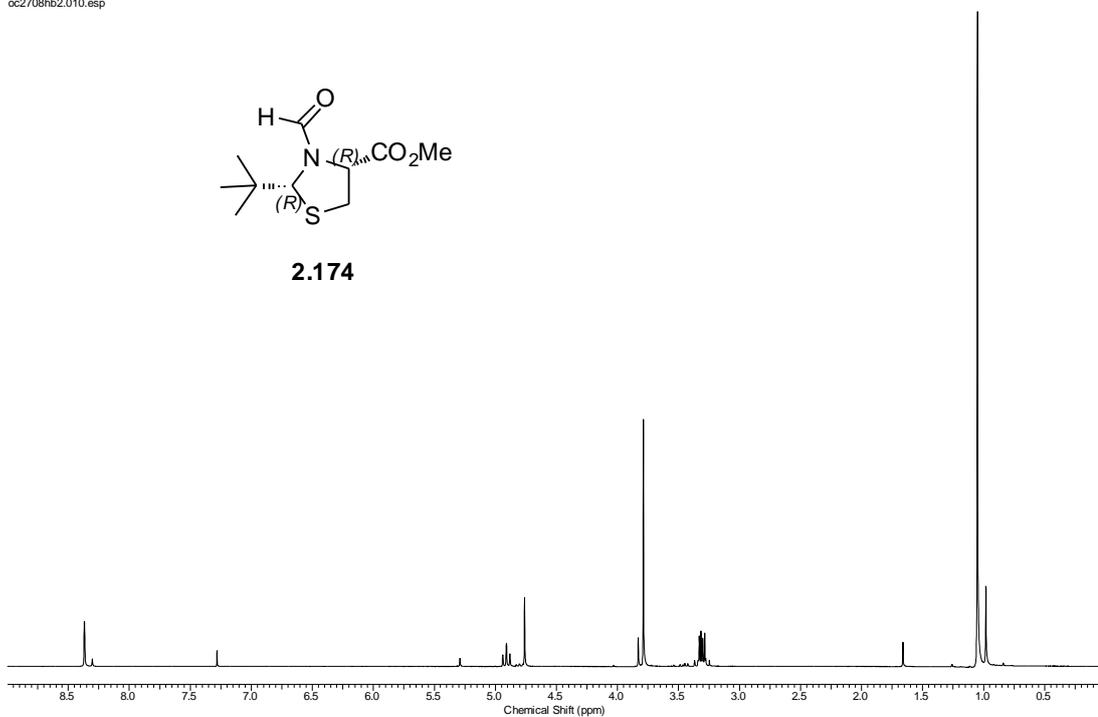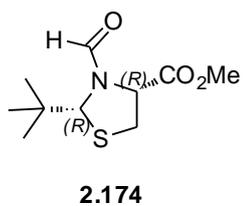
^{1}H NMR spectrum of 2.175

nv0908hb1.010.esp

2.175
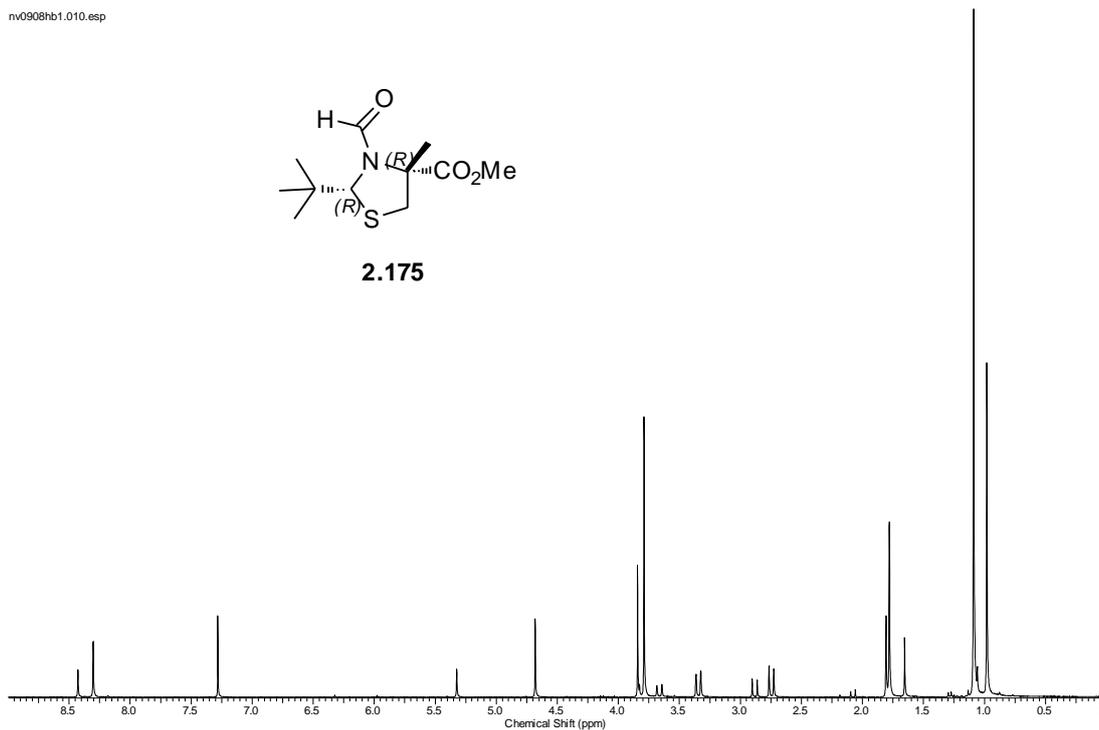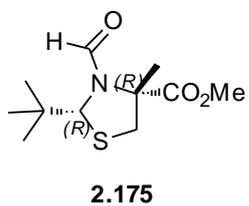
^{1}H NMR spectrum of 2.109

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2.109
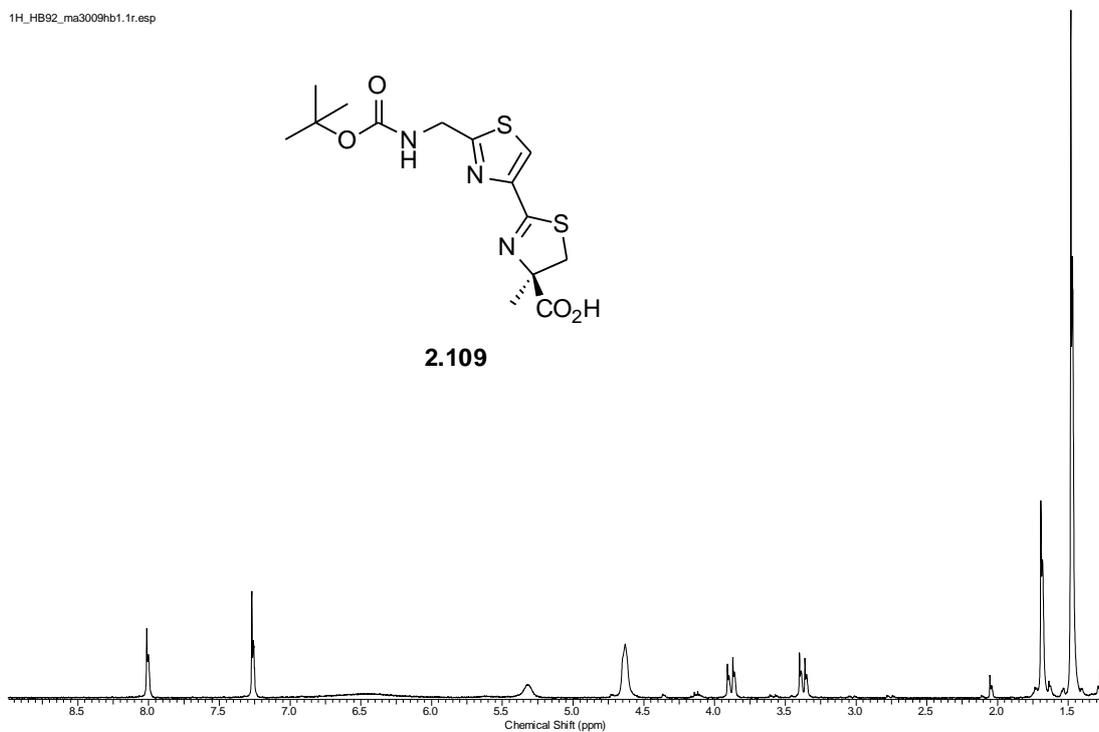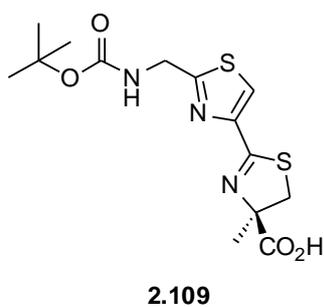
^{1}H NMR spectrum of 2.177

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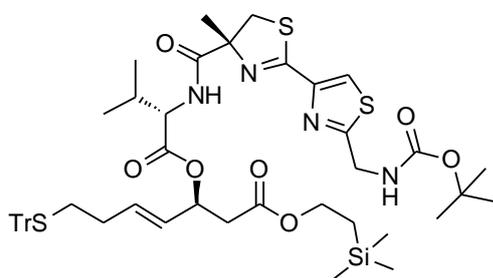
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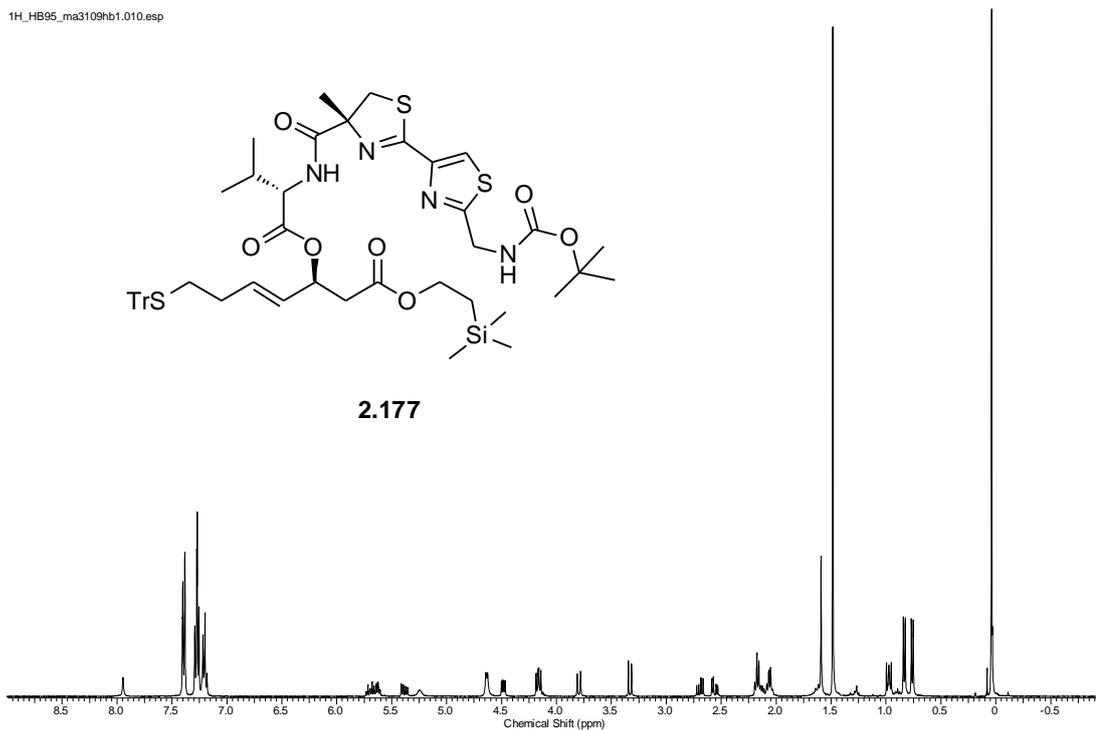
^{1}H NMR spectrum of 2.168

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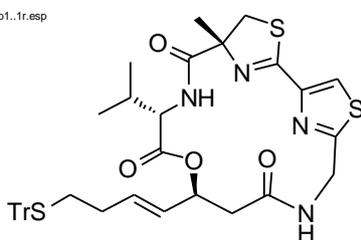
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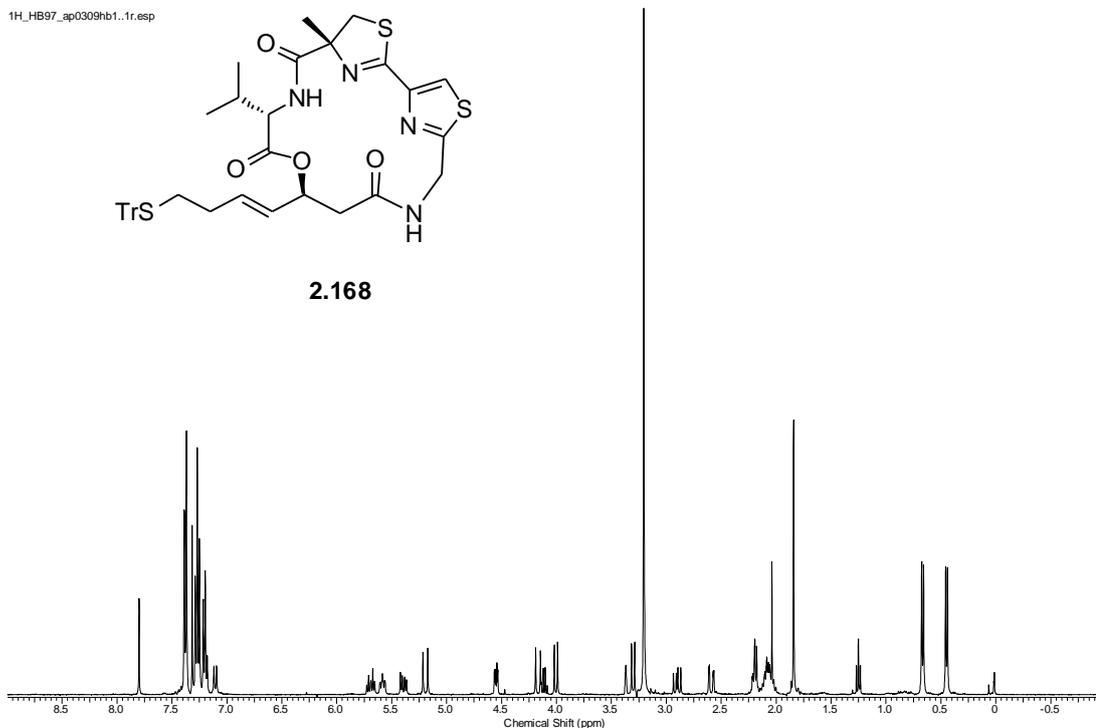
^{1}H NMR spectrum of 2.178

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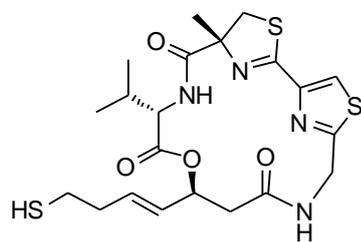
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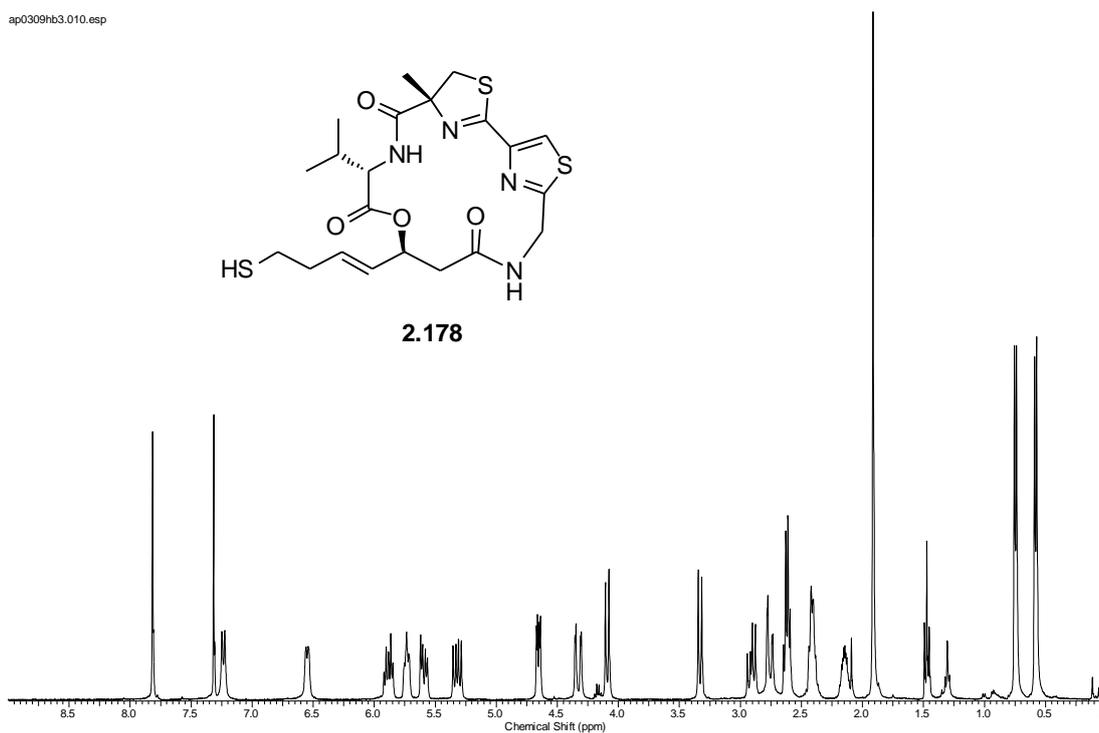
^{1}H spectrum of 2.18

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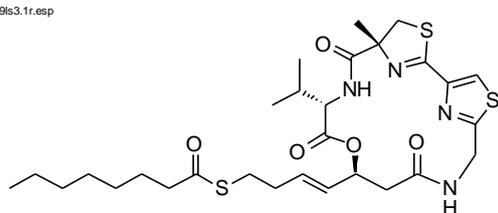
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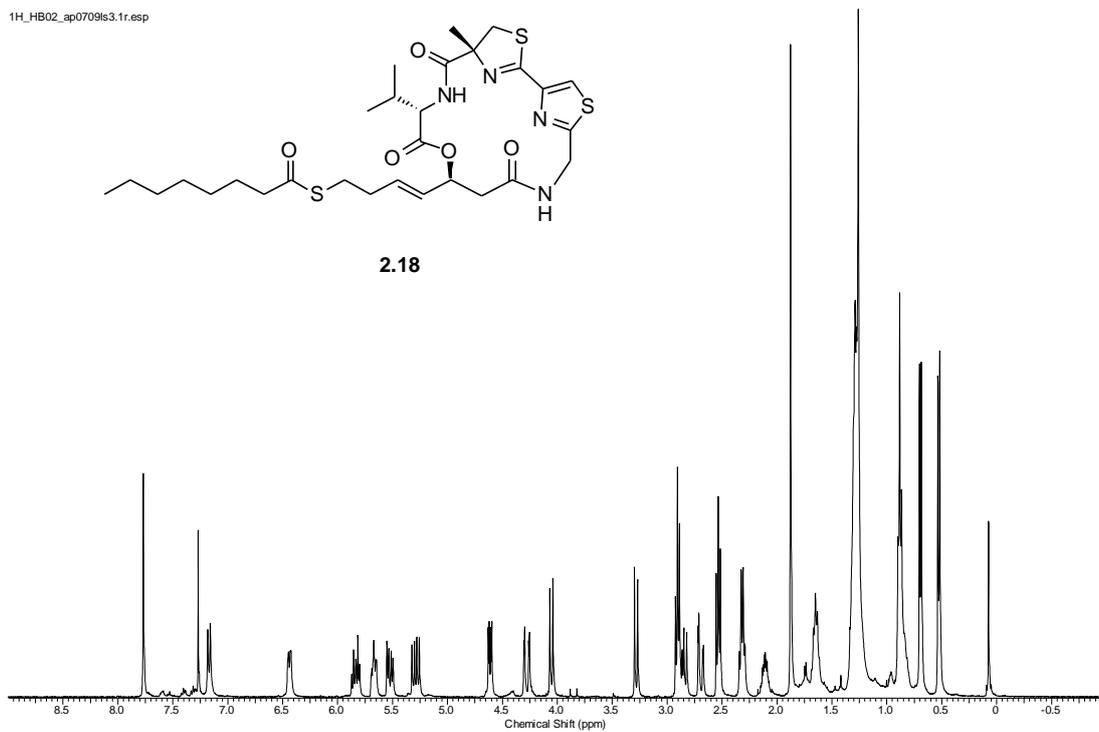
^{1}H and ^{13}C NMR spectra of 2.184
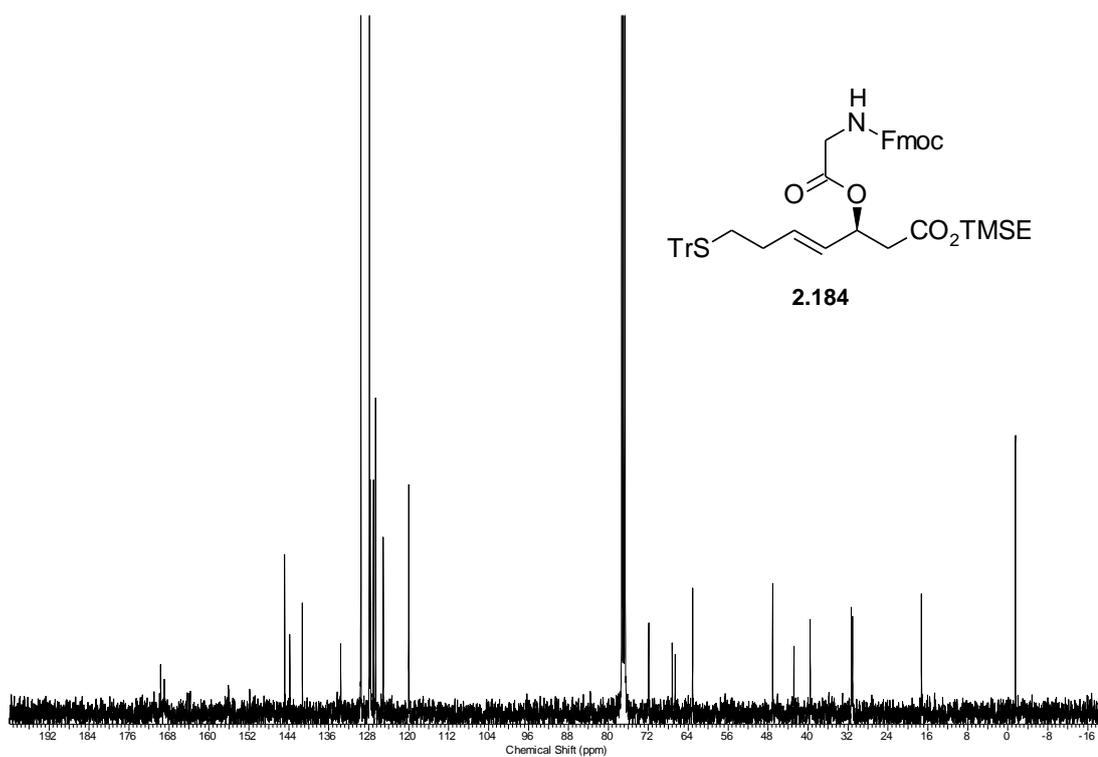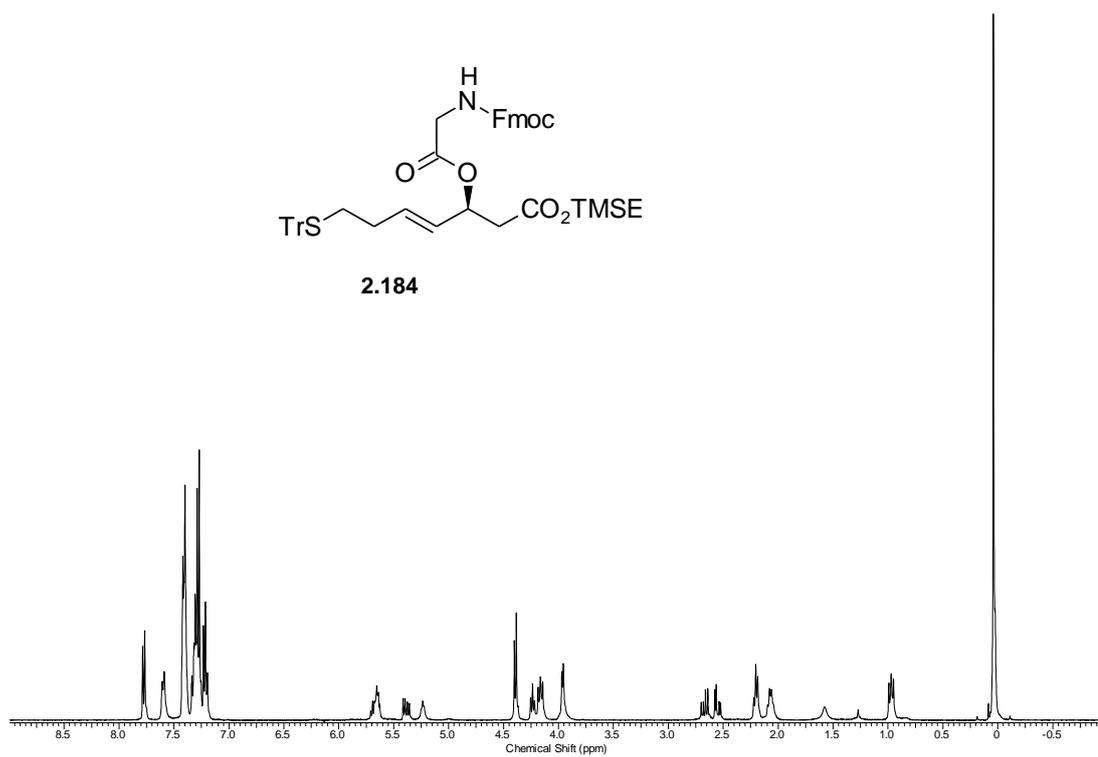
¹H NMR spectrum of 2.186

2.186
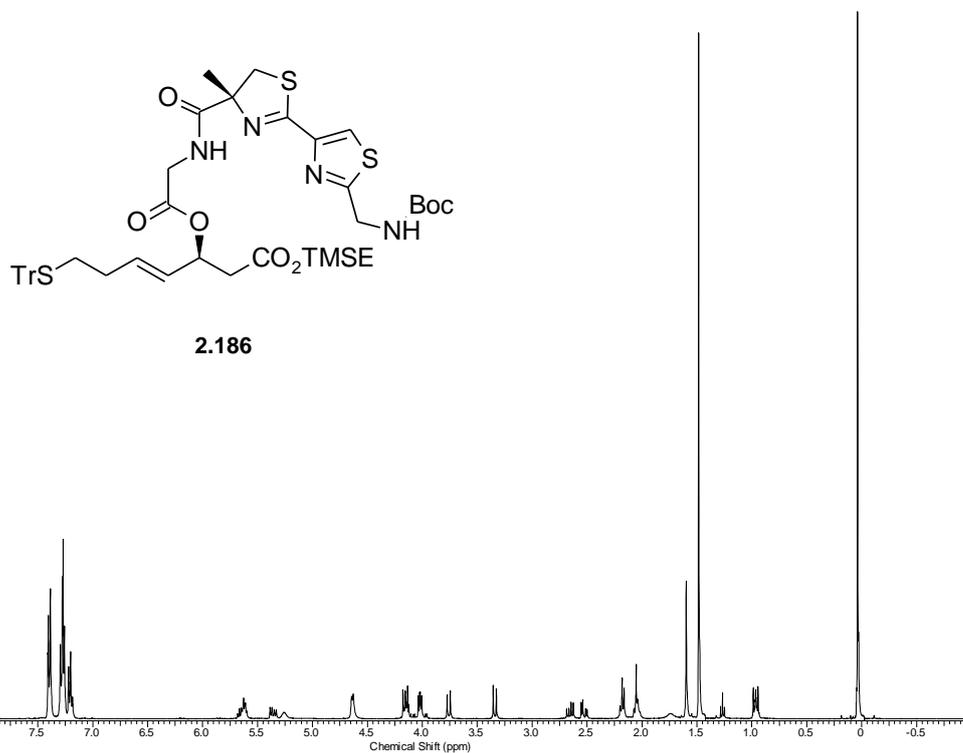
^{1}H and ^{13}C NMR spectra of 2.188

2.188

2.188
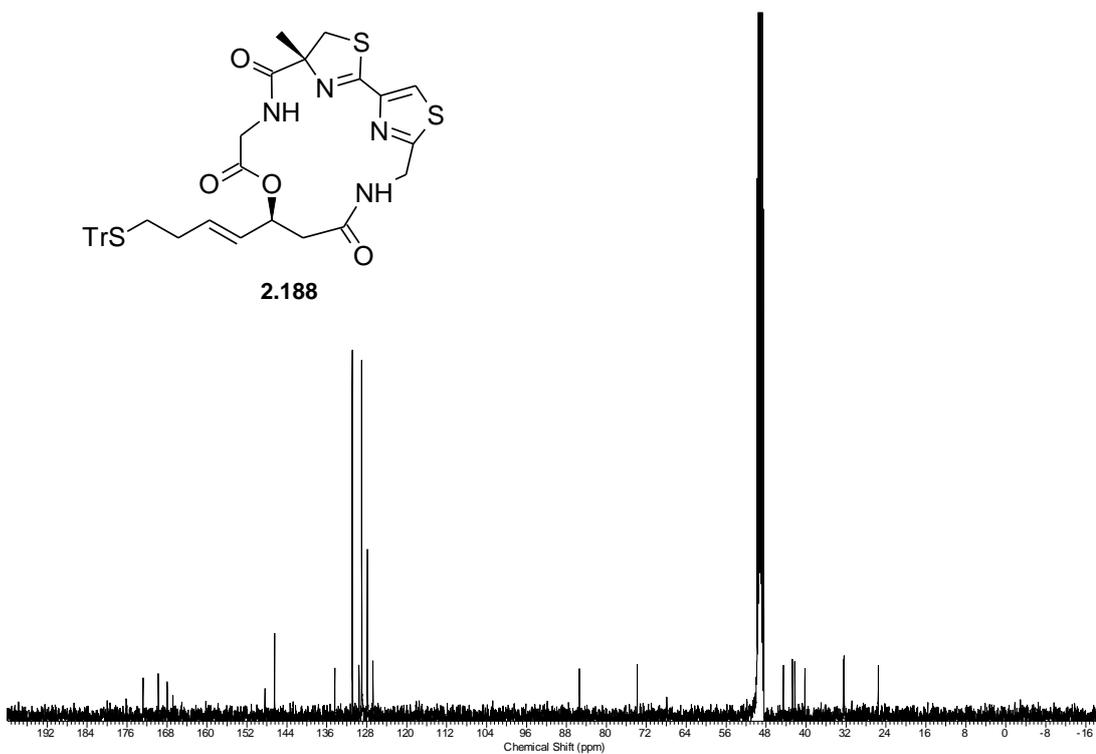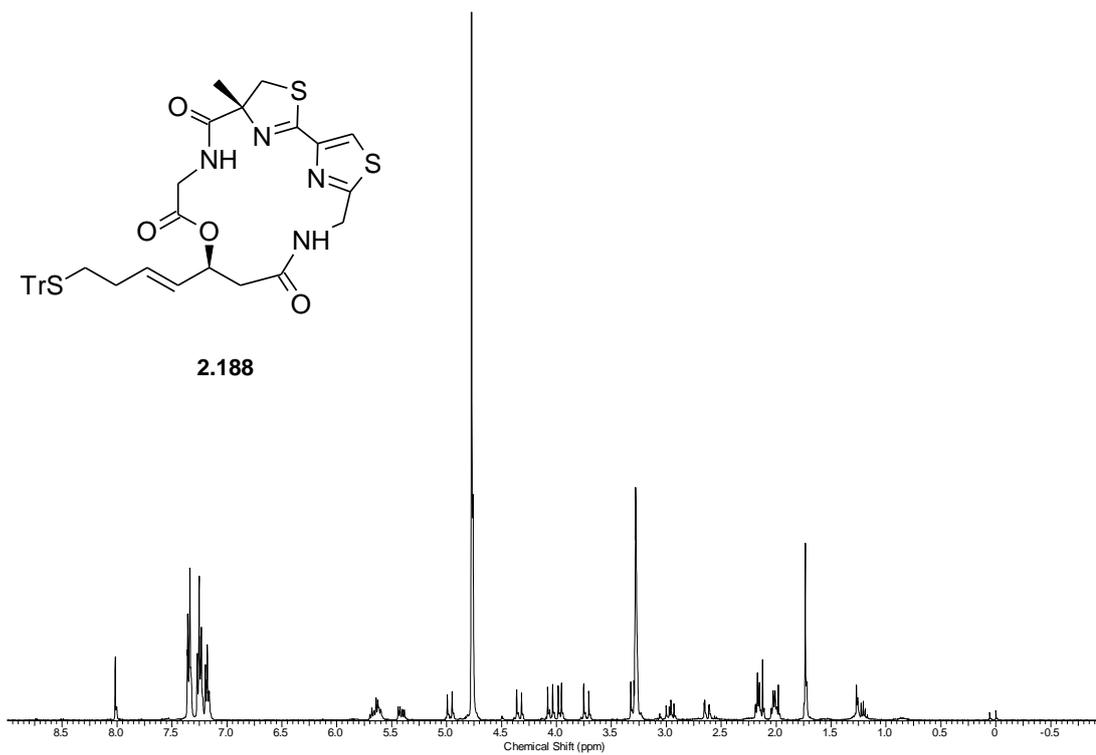
^{1}H and ^{13}C NMR spectra of 2.179

2.179

2.179
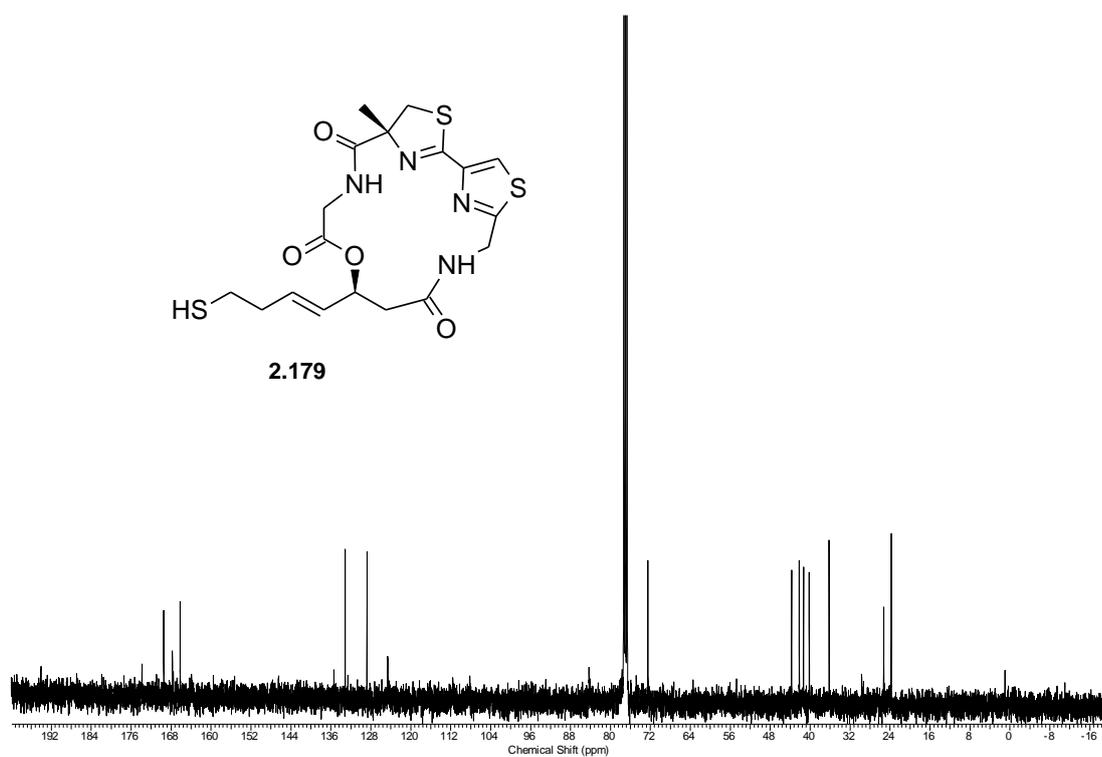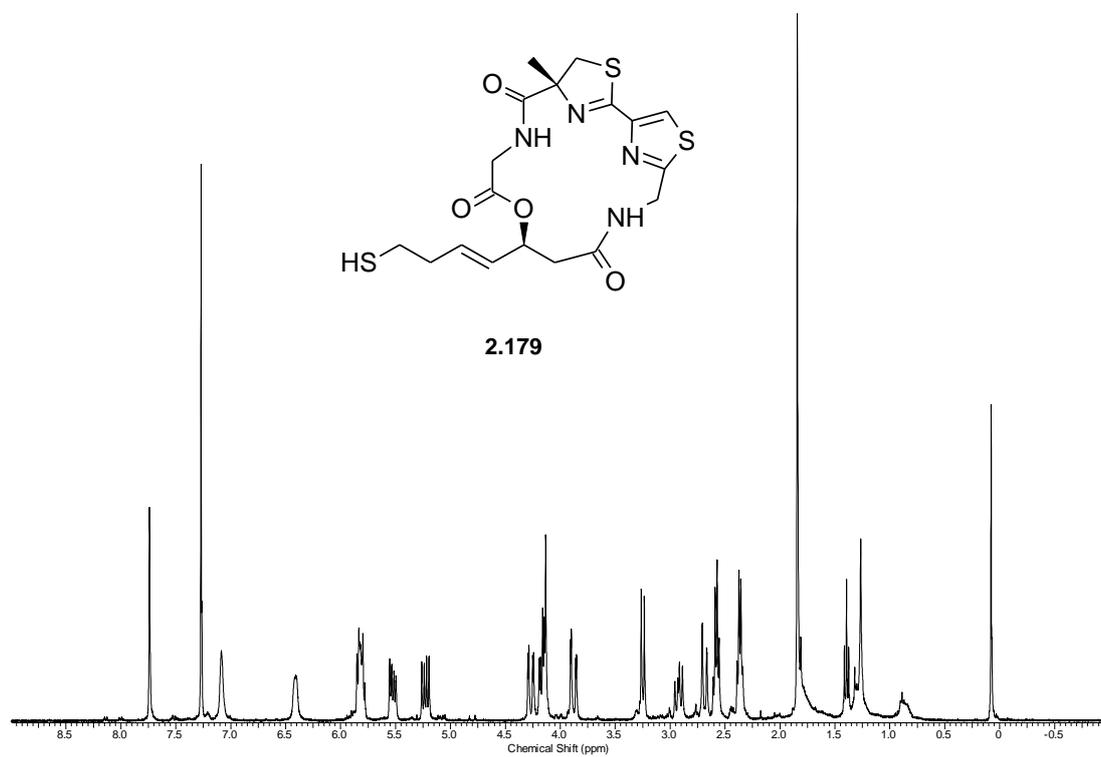
^{1}H and ^{13}C NMR spectra of 2.185

2.185

2.185

^{1}H and ^{13}C NMR spectra of 2.187

2.187

2.187

^{1}H and ^{13}C NMR spectra of 2.189

2.189

Chemical Shift (ppm)

2.189

Chemical Shift (ppm)
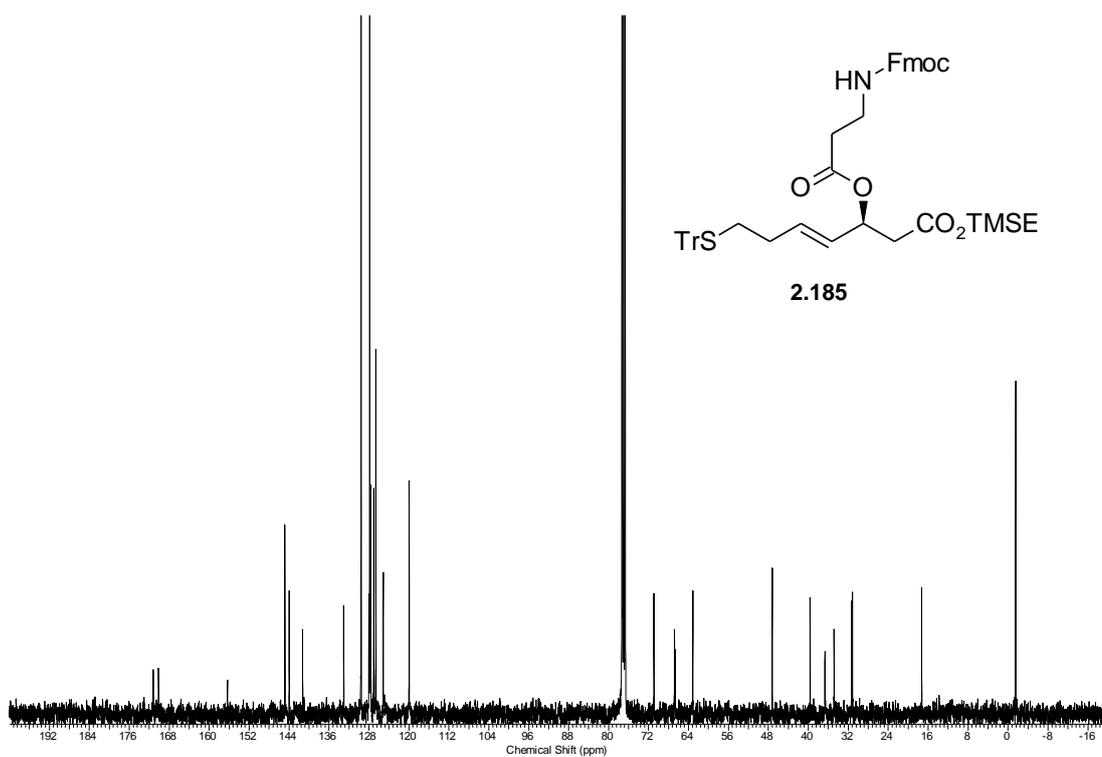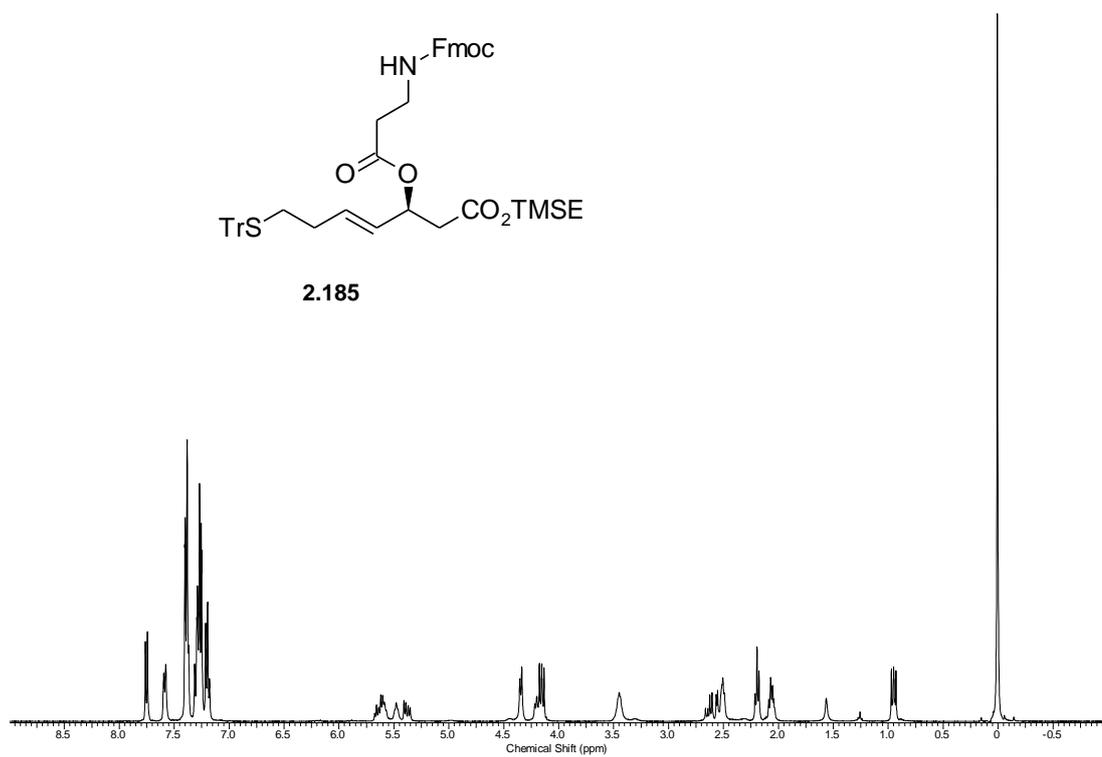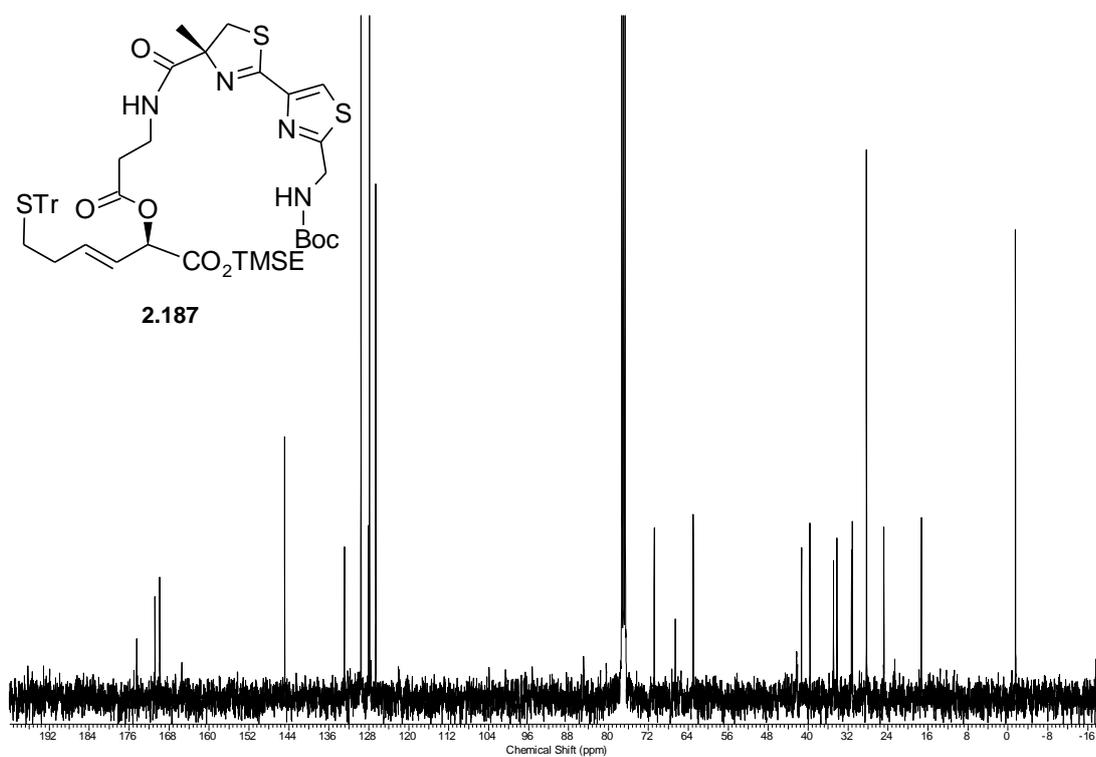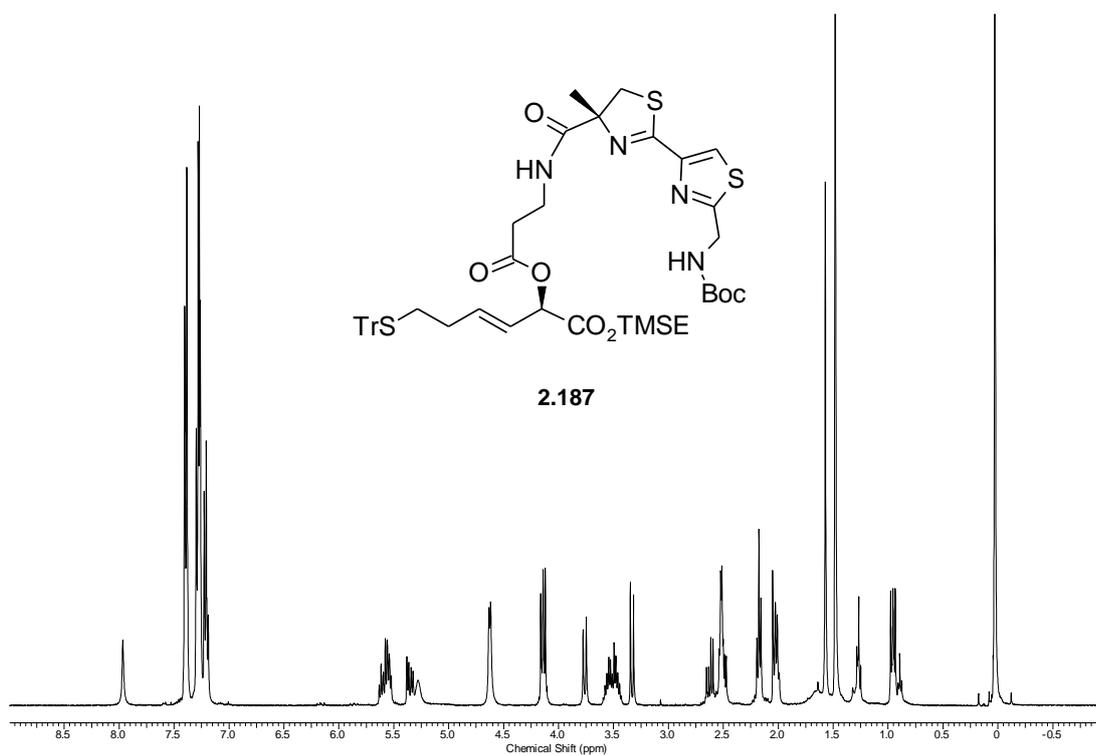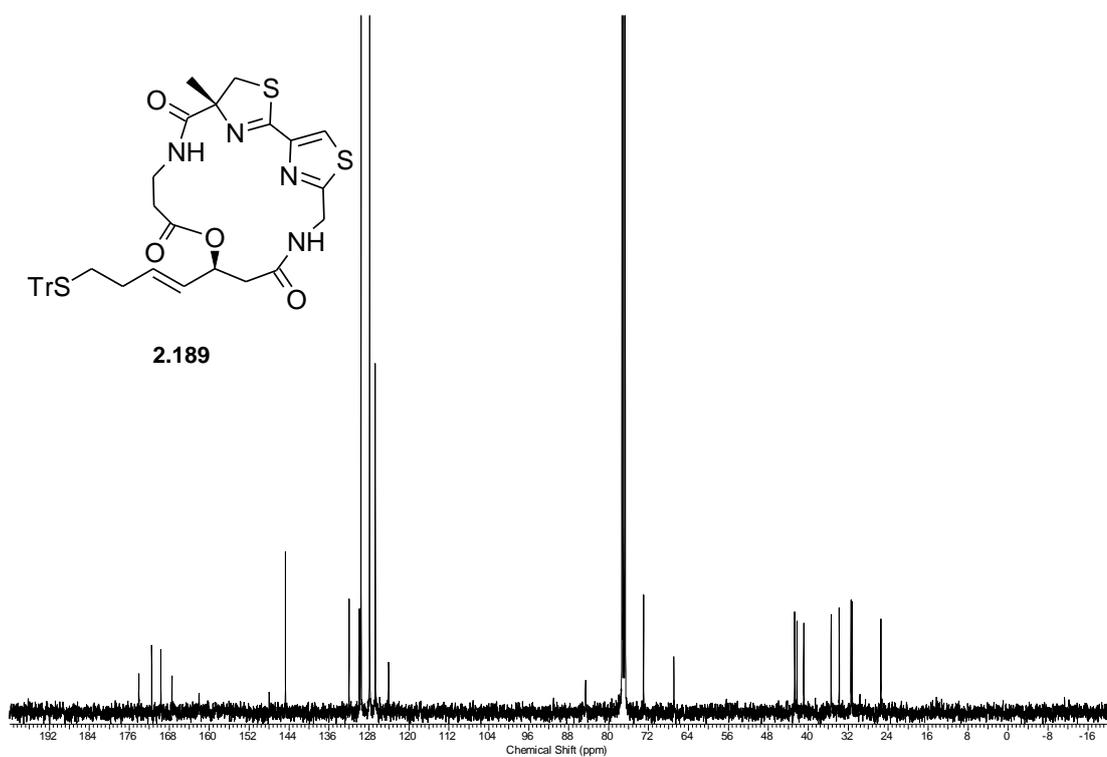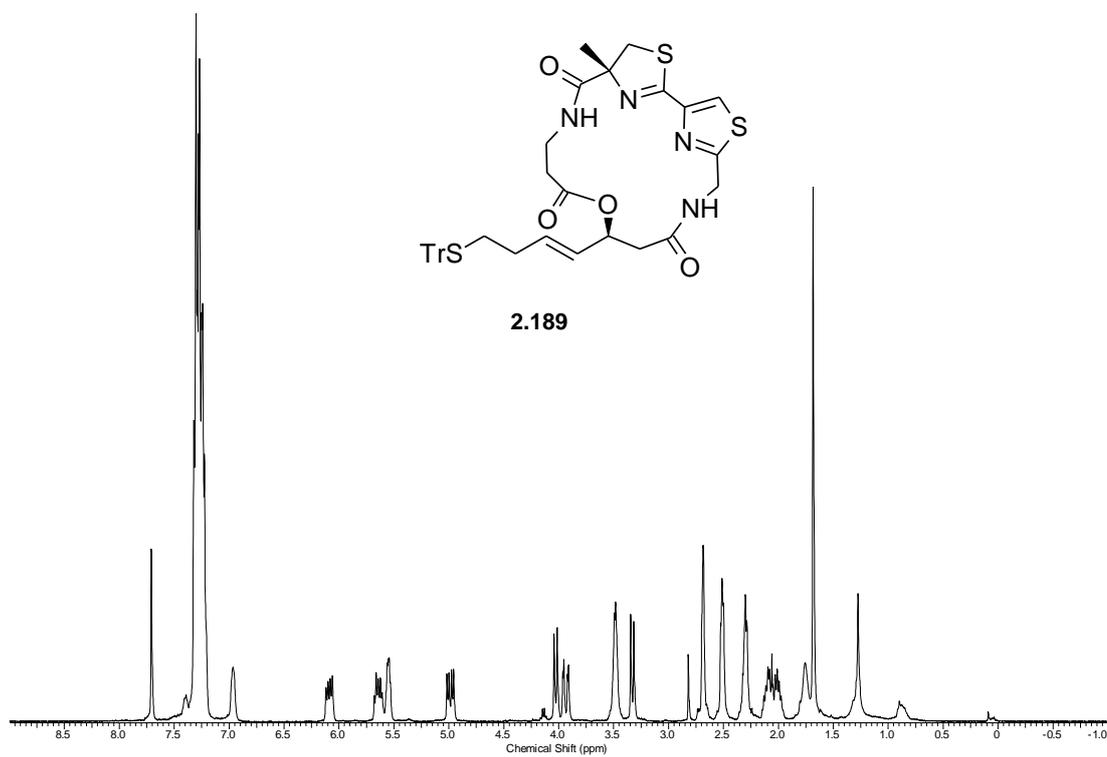
^{1}H and ^{13}C NMR spectra of 2.180
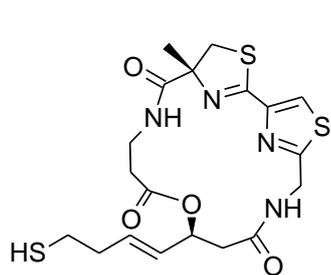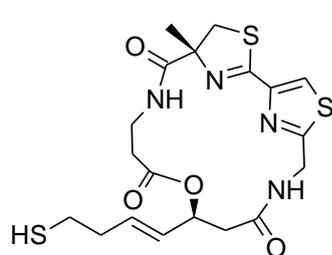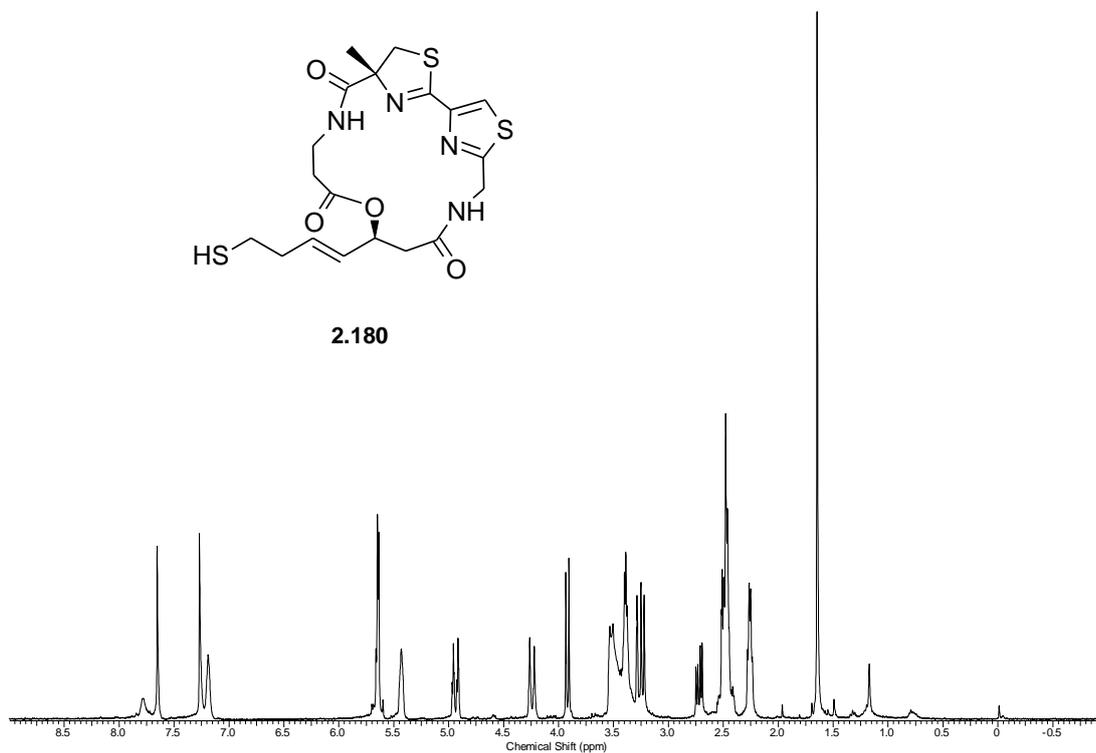
2.180

2.180
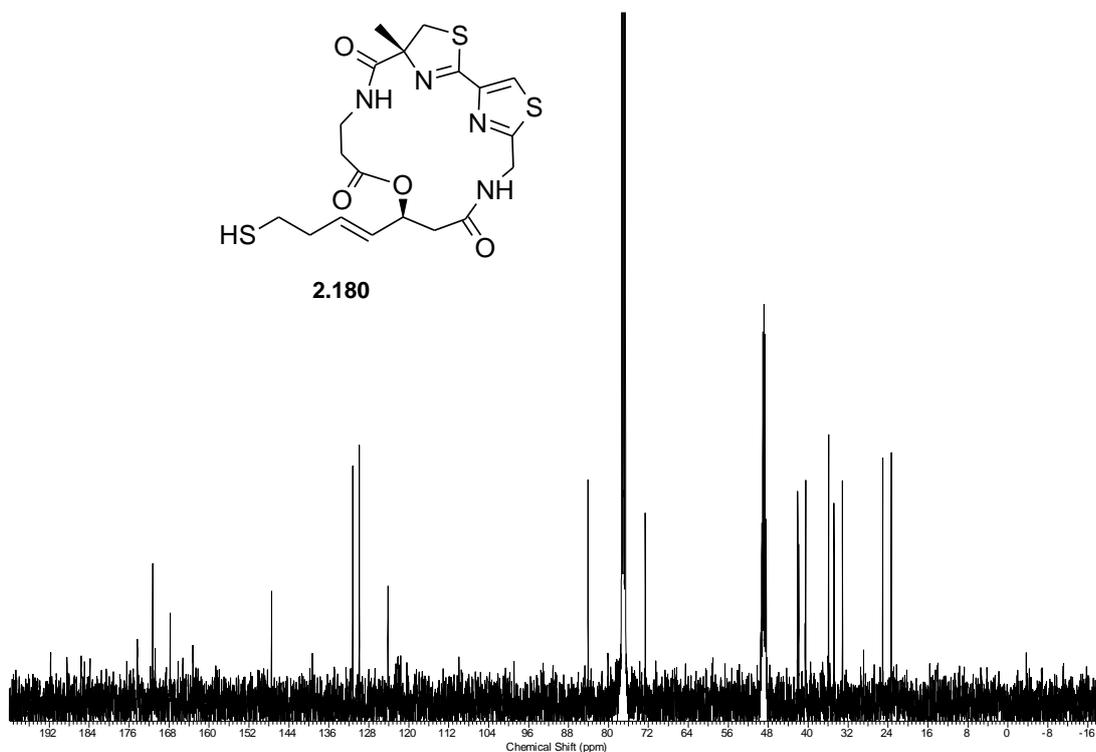
^{1}H and ^{13}C NMR spectra of 2.190

2.190

2.190
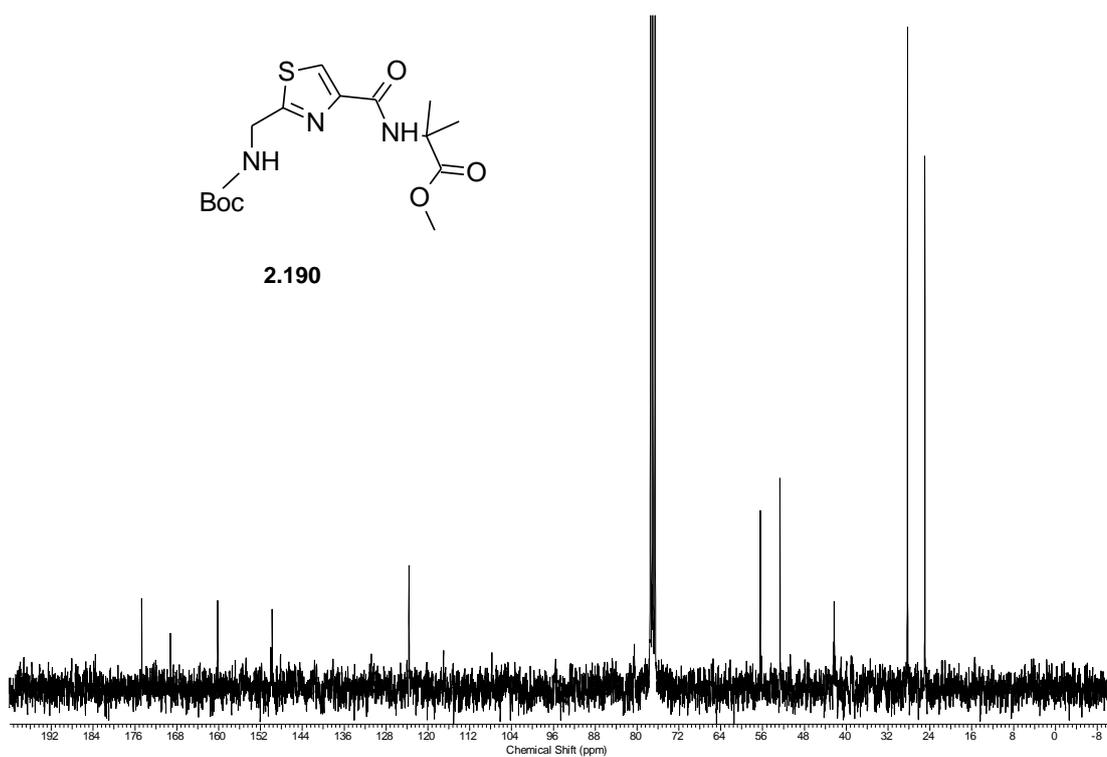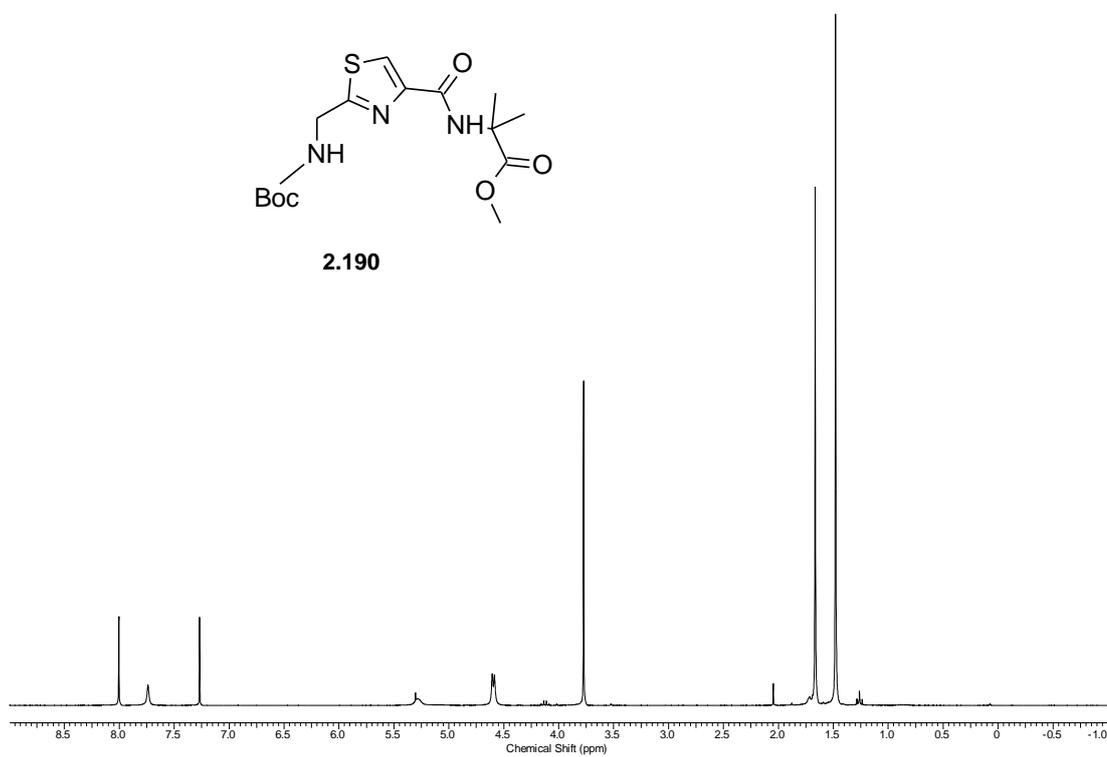
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2.191

2.191
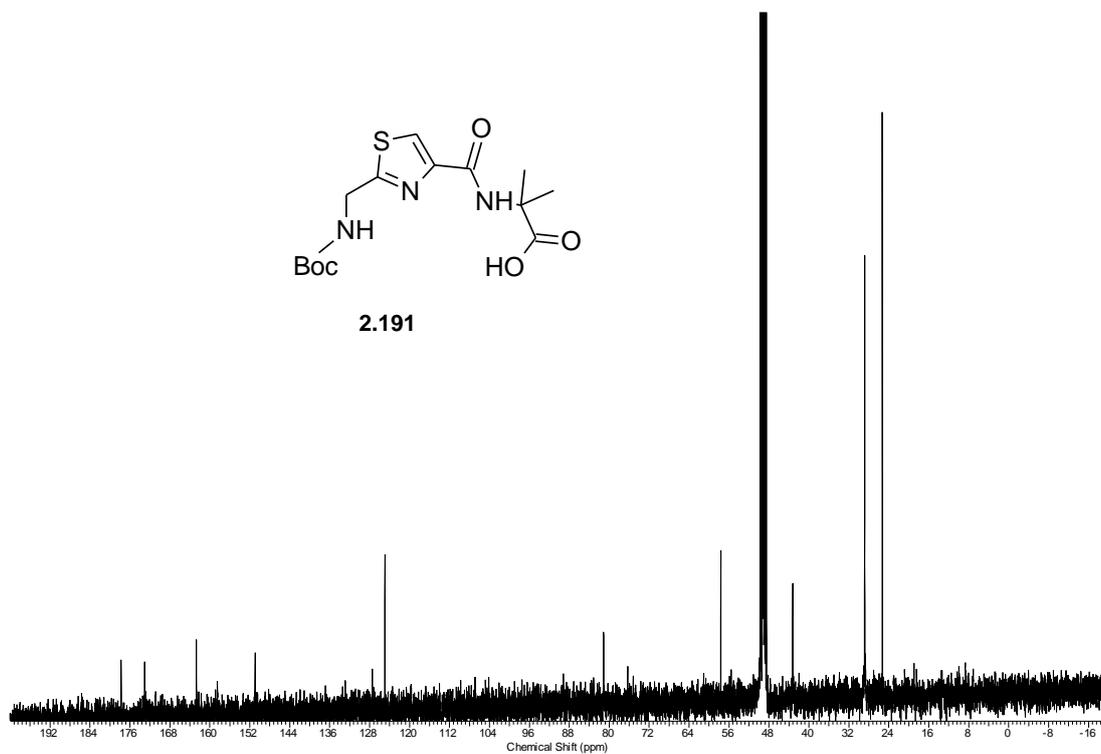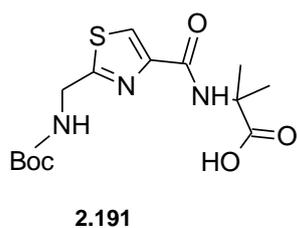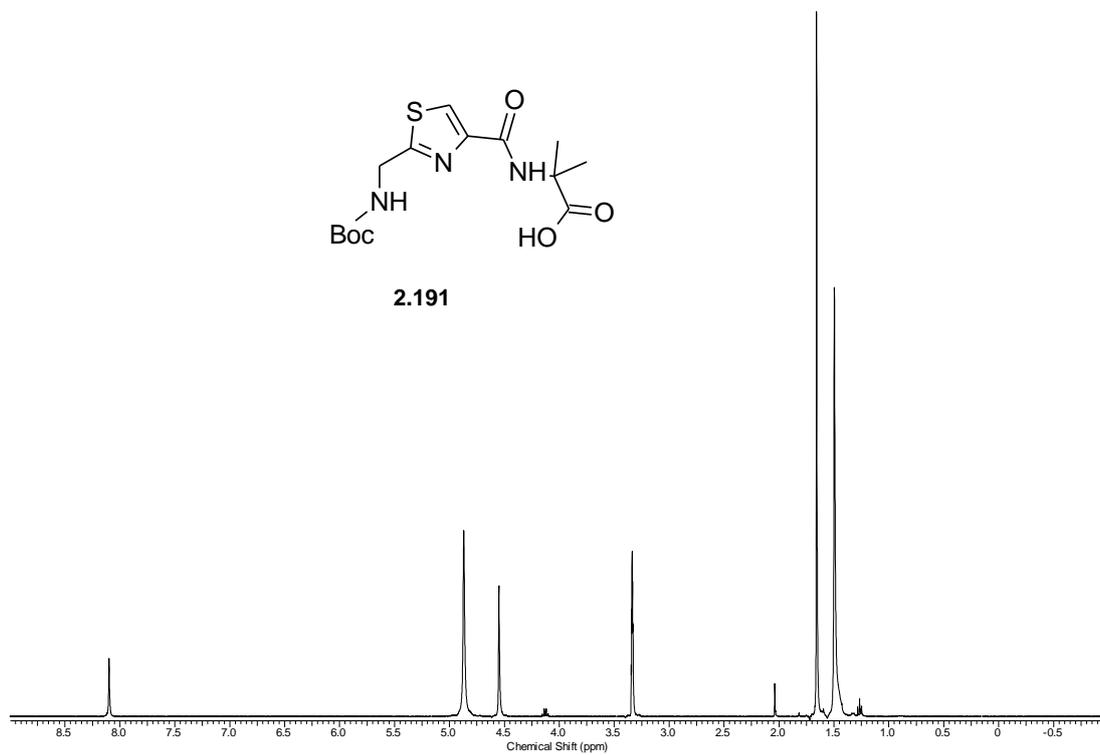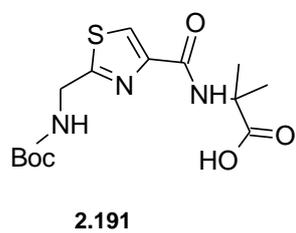
^{1}H and ^{13}C NMR spectra of 2.192

2.192

2.192
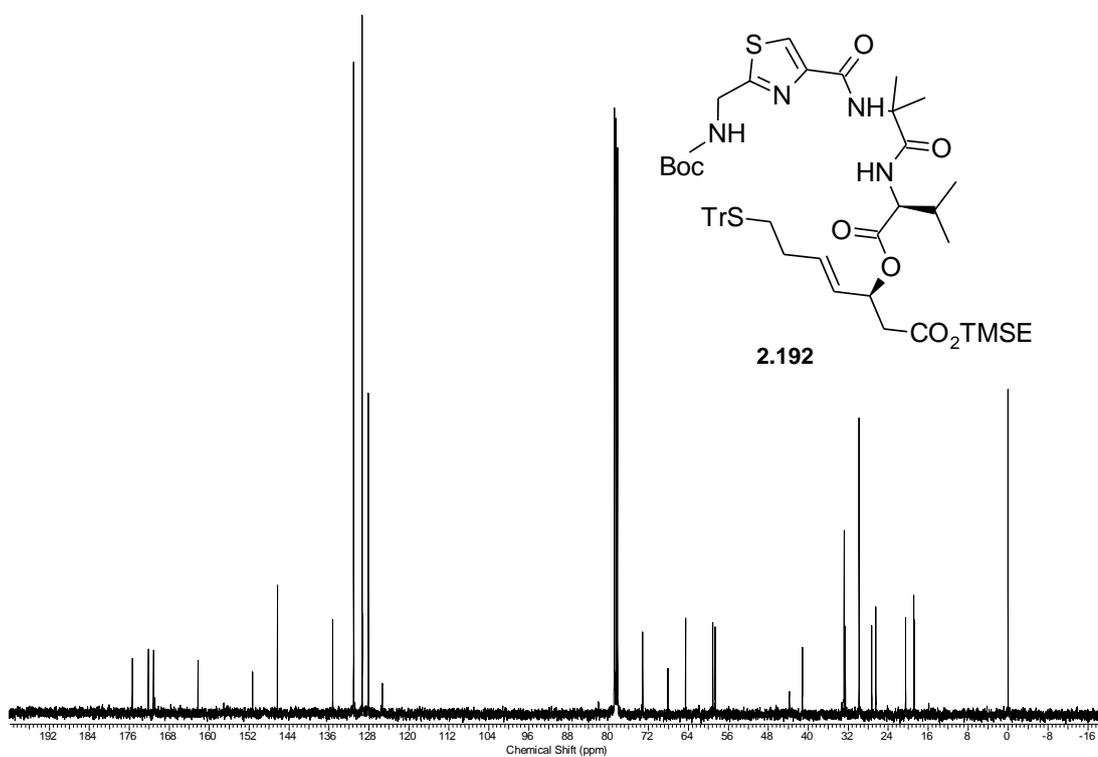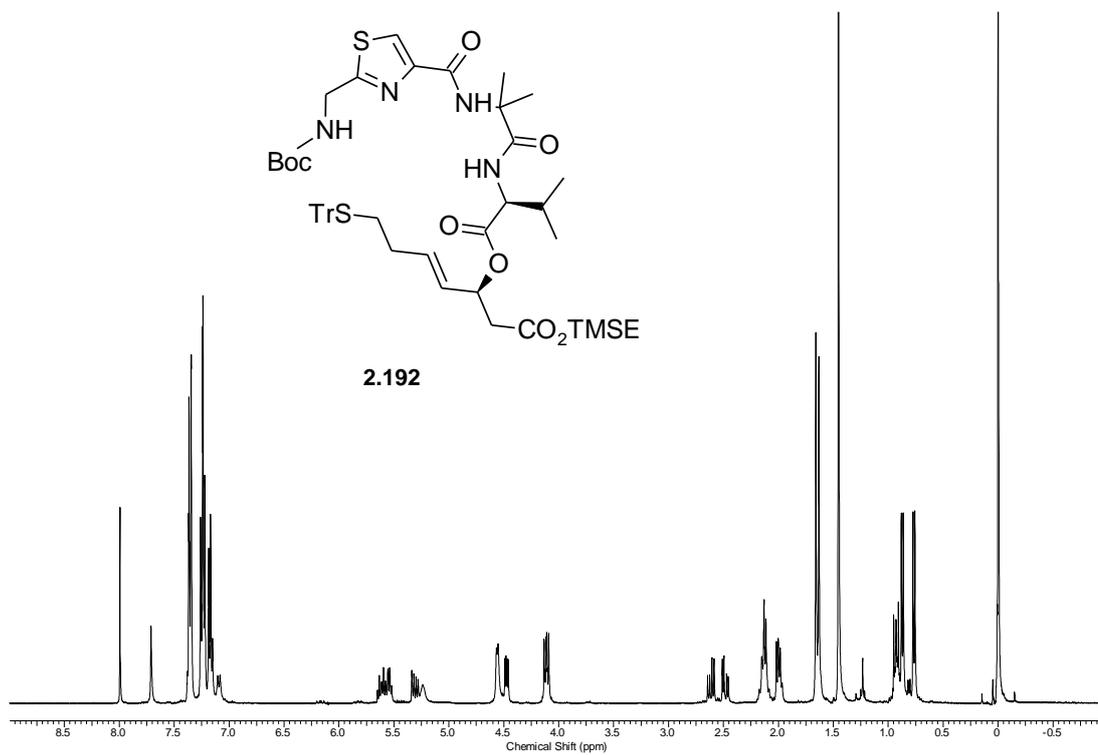
^{1}H and ^{13}C NMR spectra of 2.193

2.193

2.193
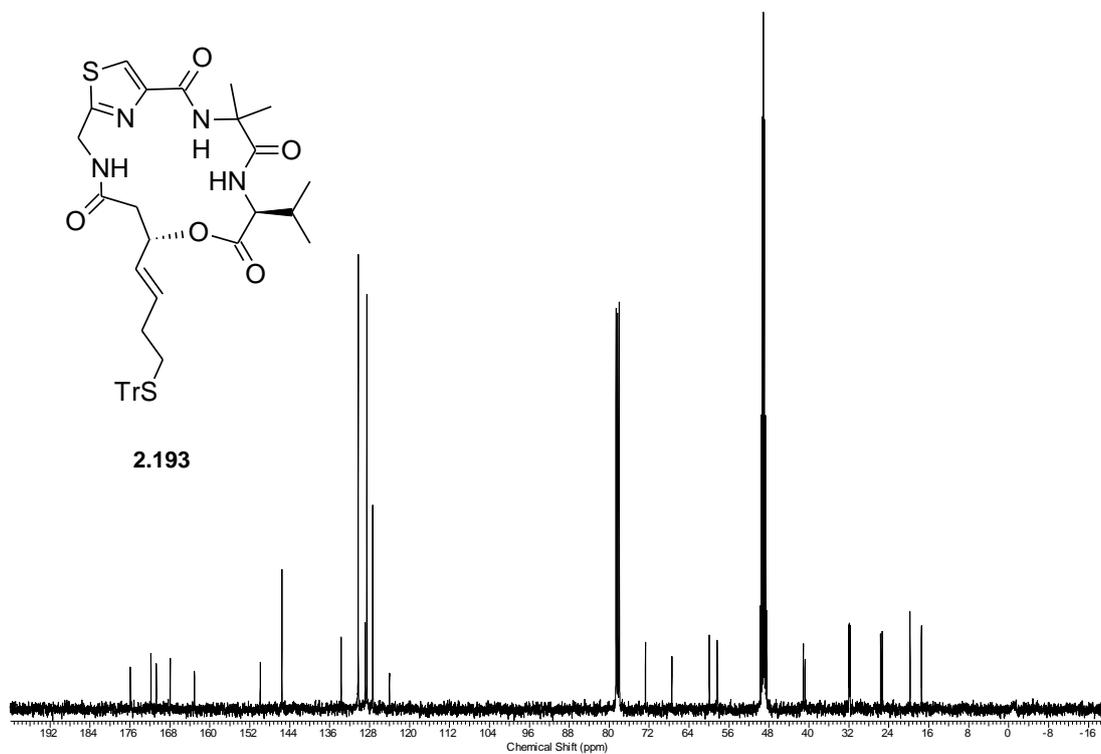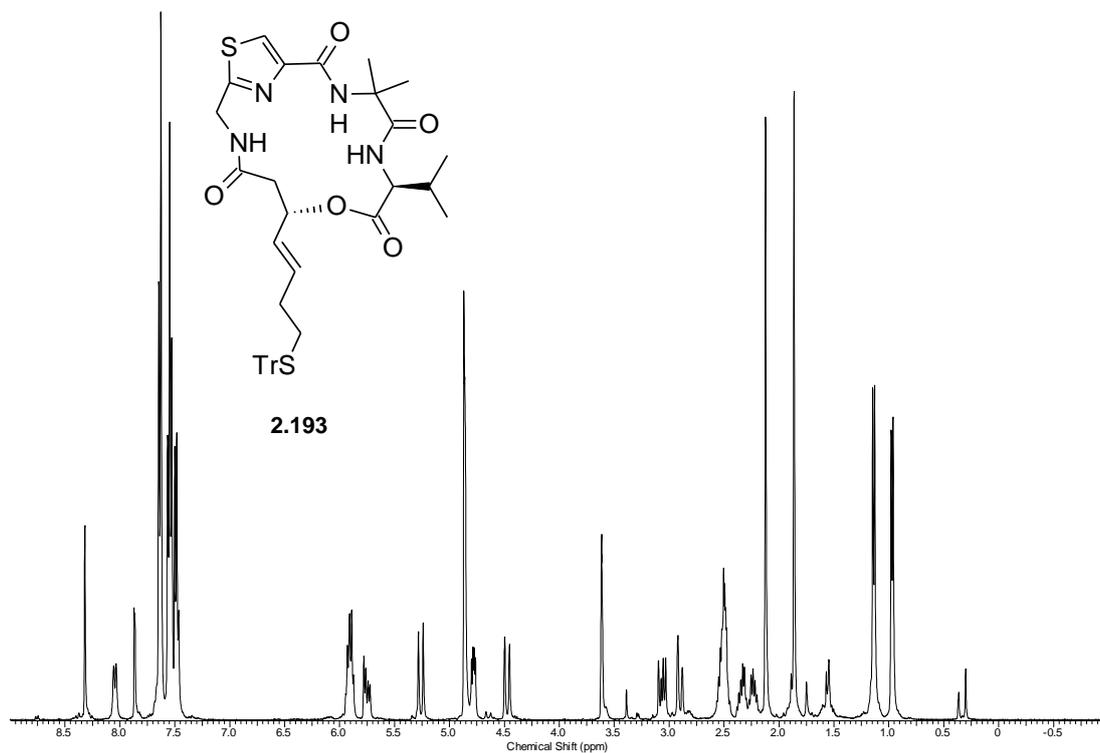
^{1}H and ^{13}C NMR spectra of 2.181

2.181

2.181
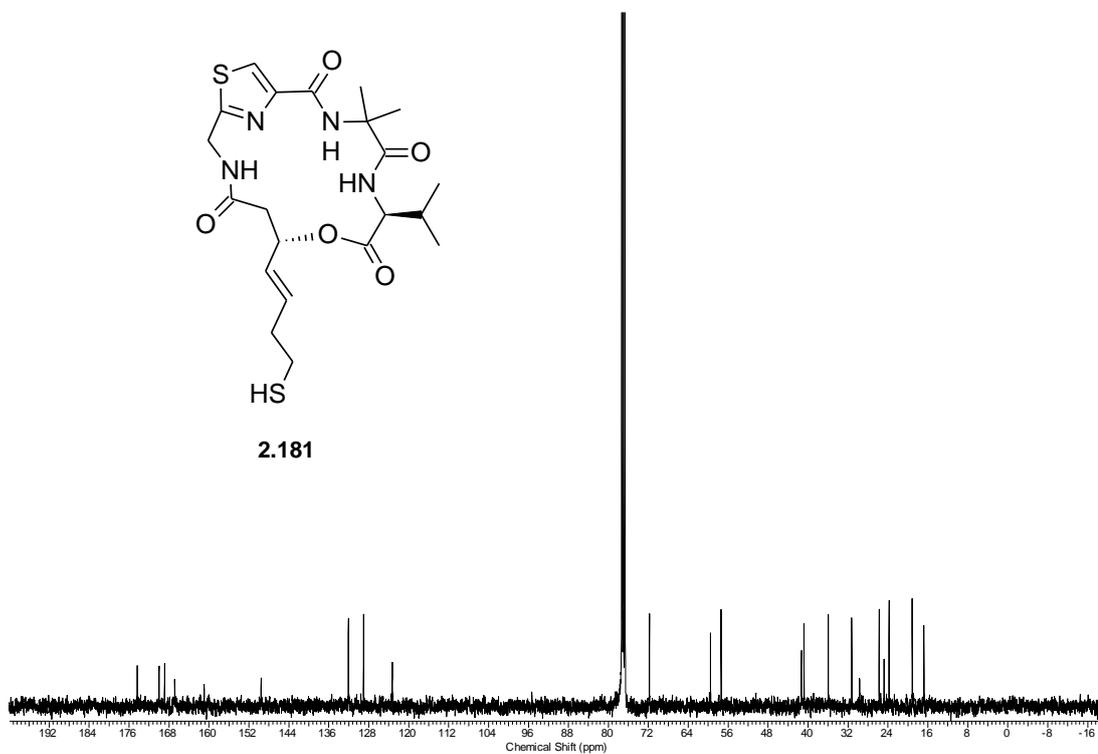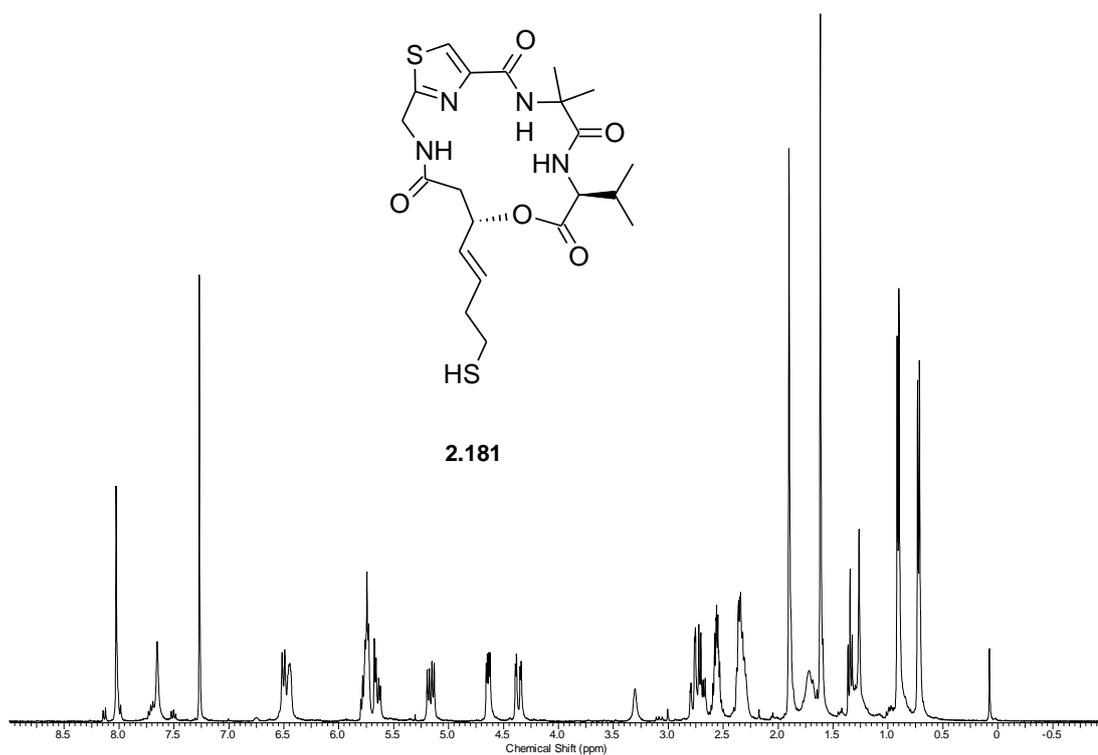
^{1}H and ^{13}C NMR spectra of 2.194

2.194

2.194
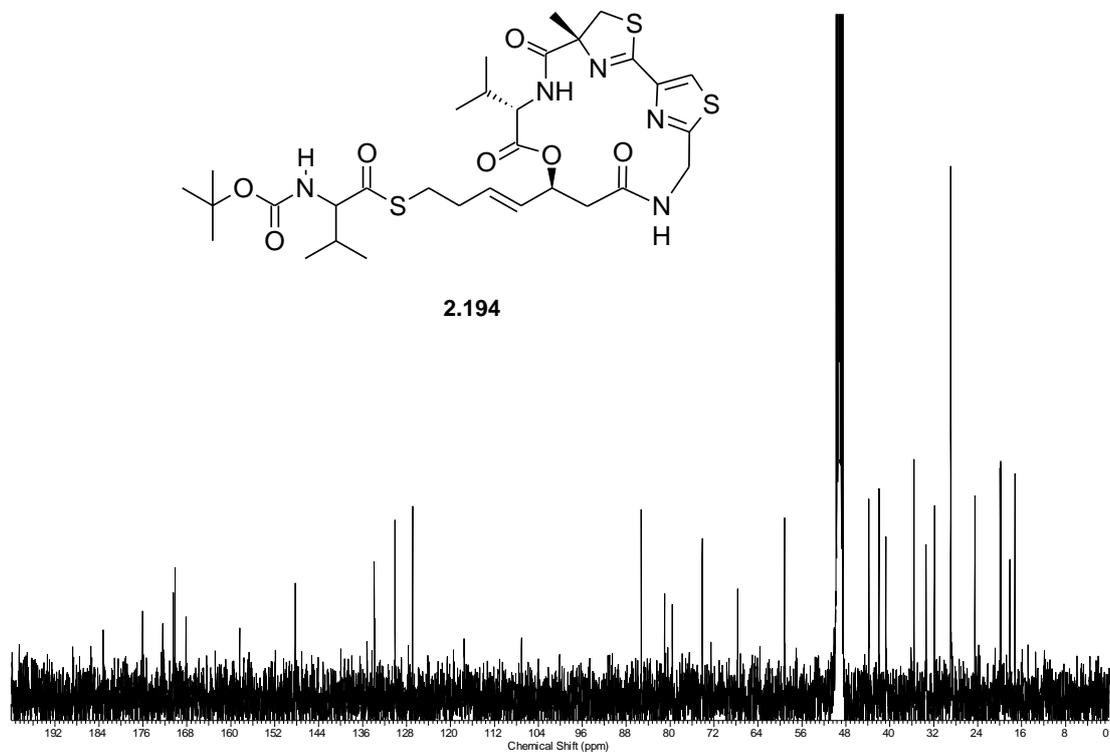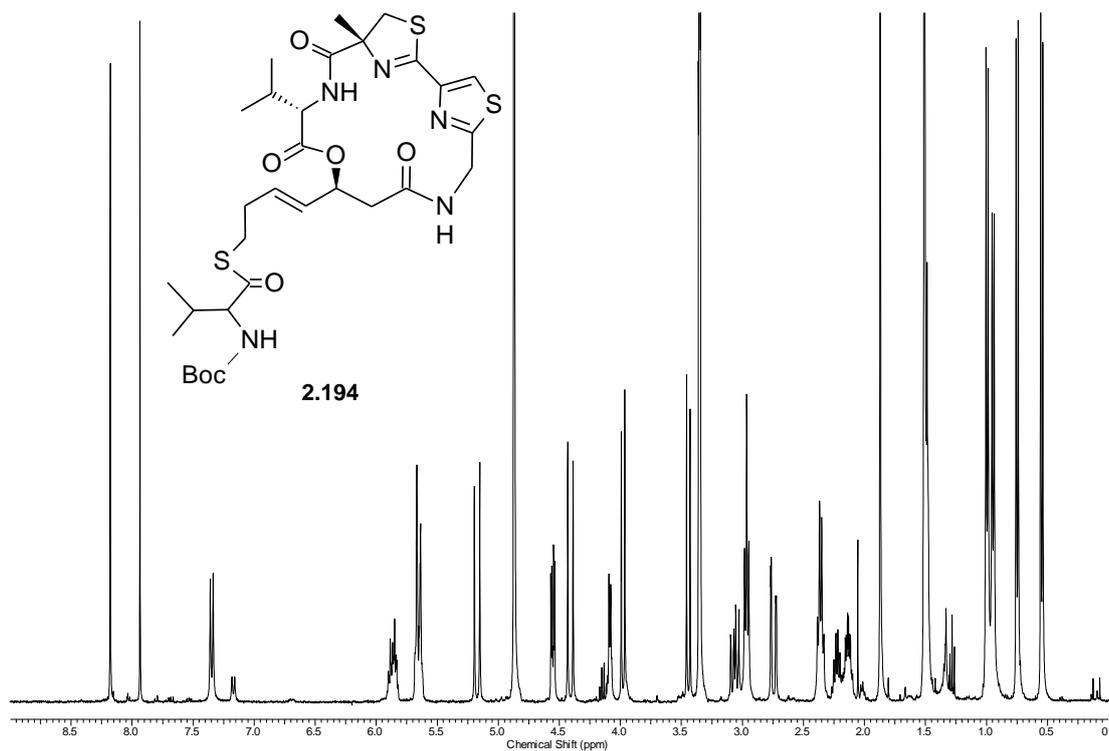
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2.183

2.183
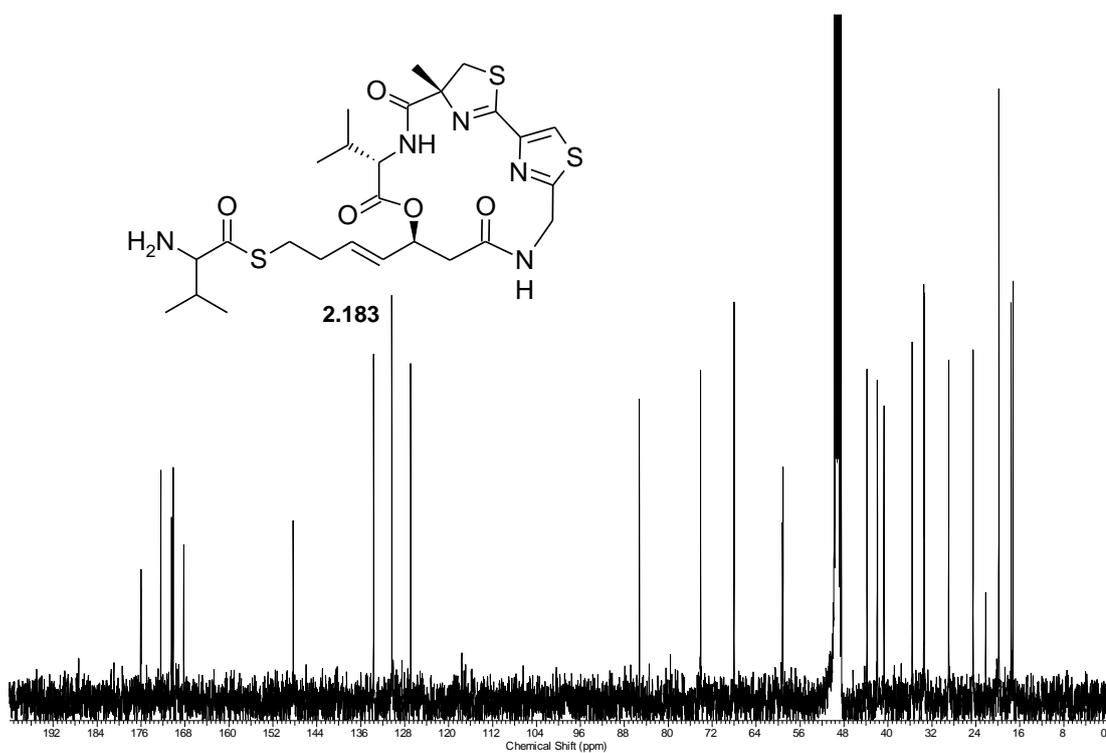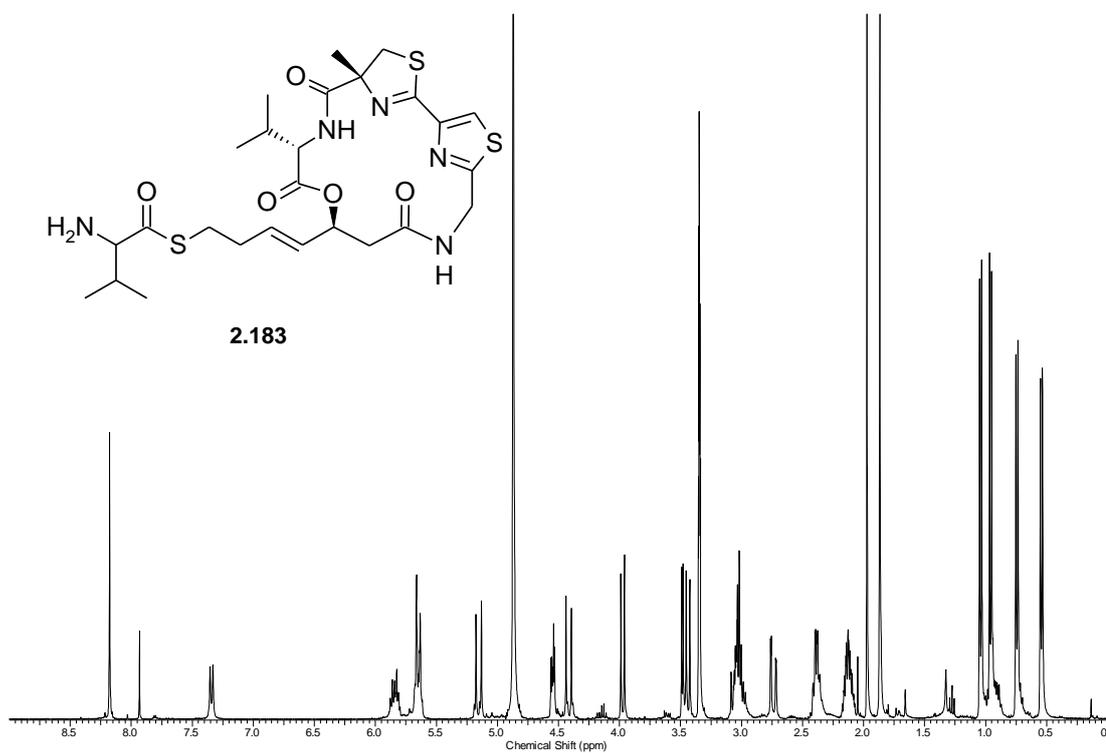
^{1}H and ^{13}C NMR spectra of 2.201

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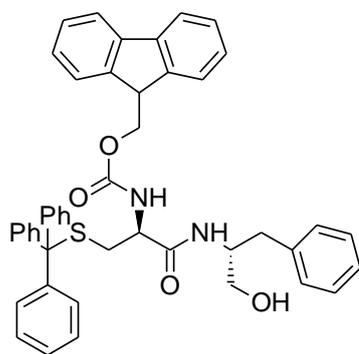
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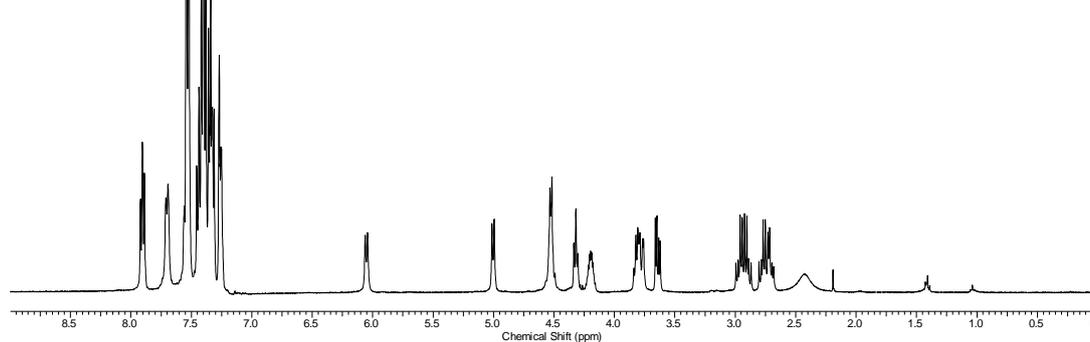
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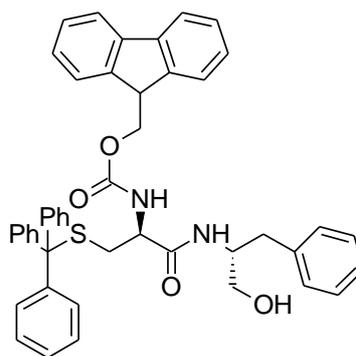
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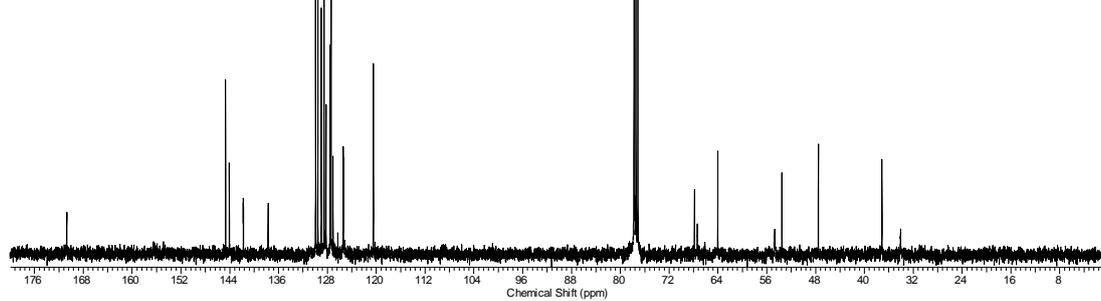
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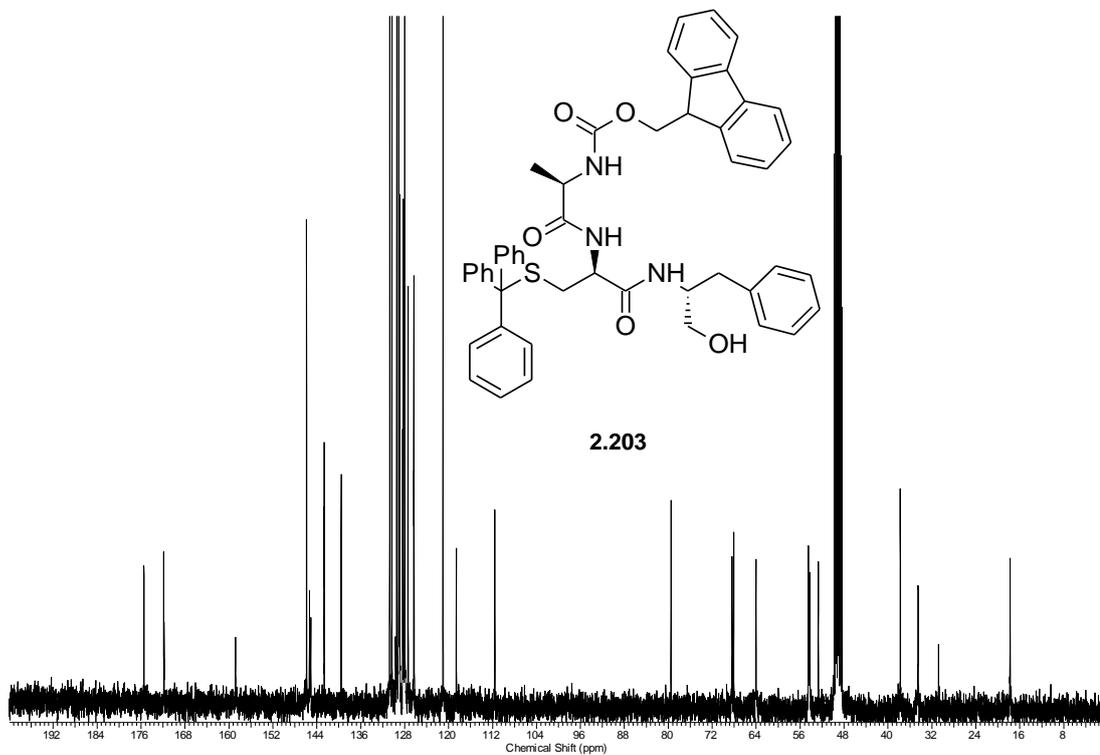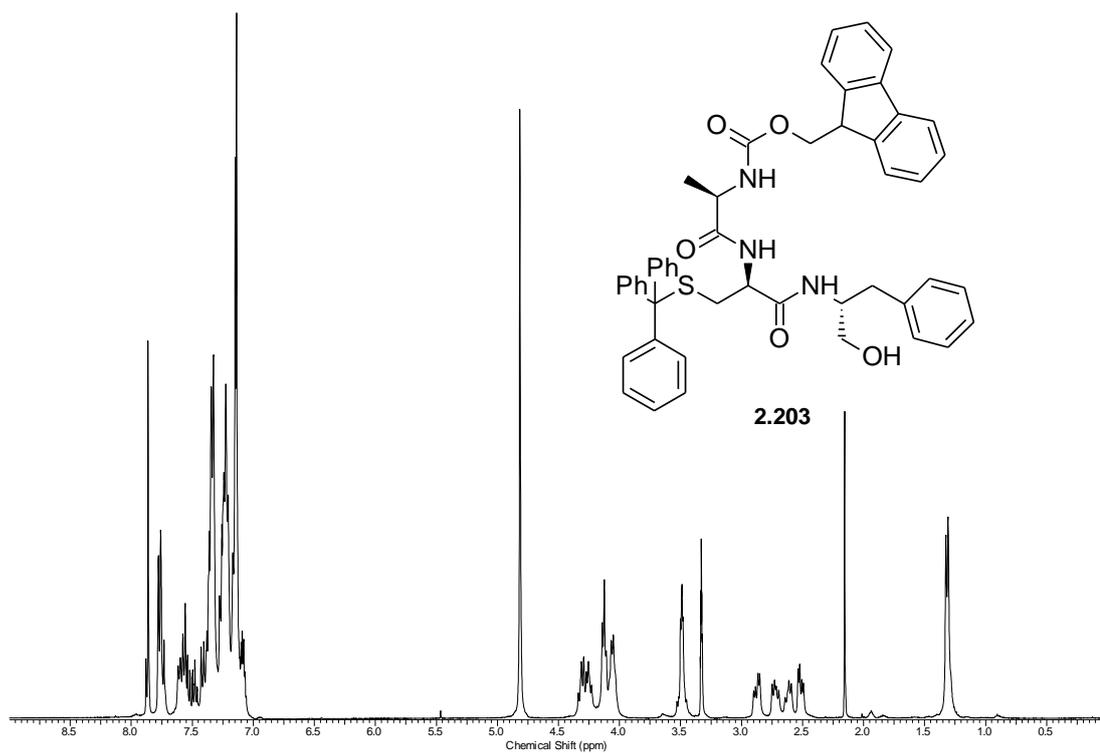
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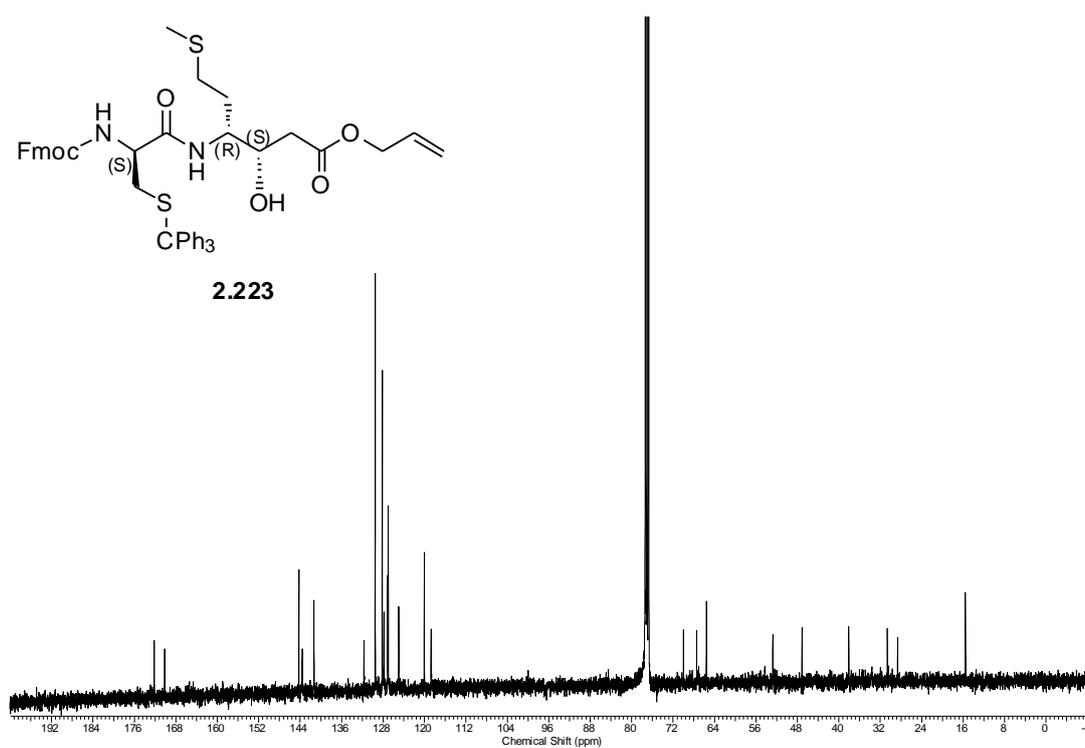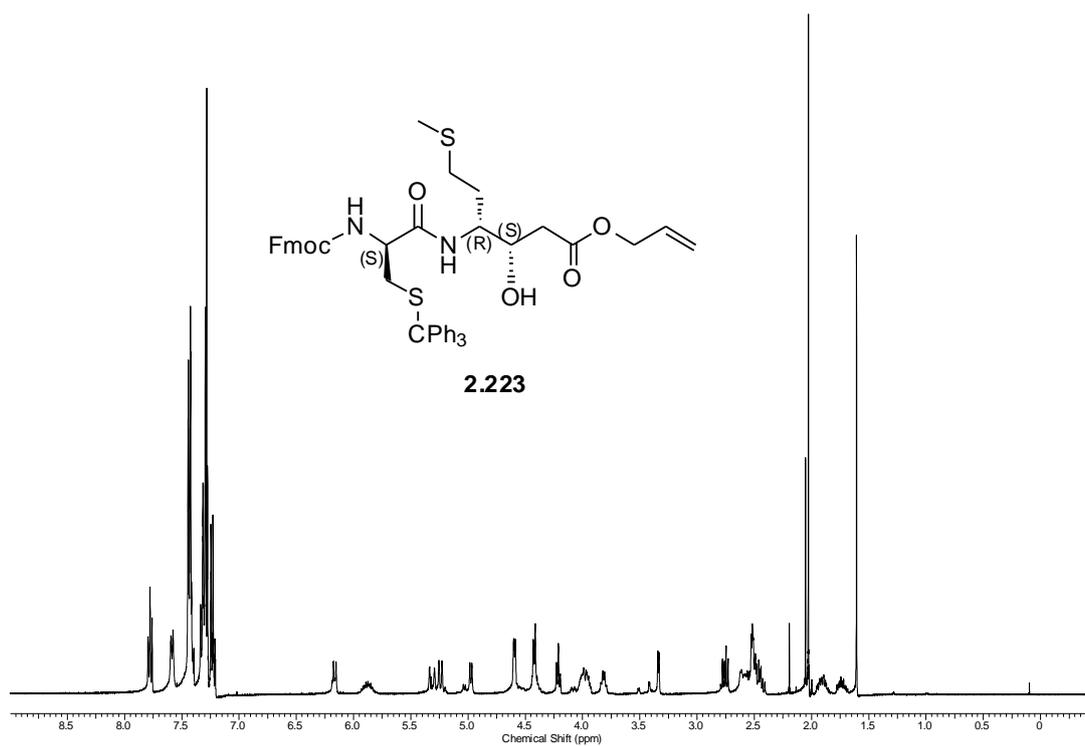
2.223

2.223

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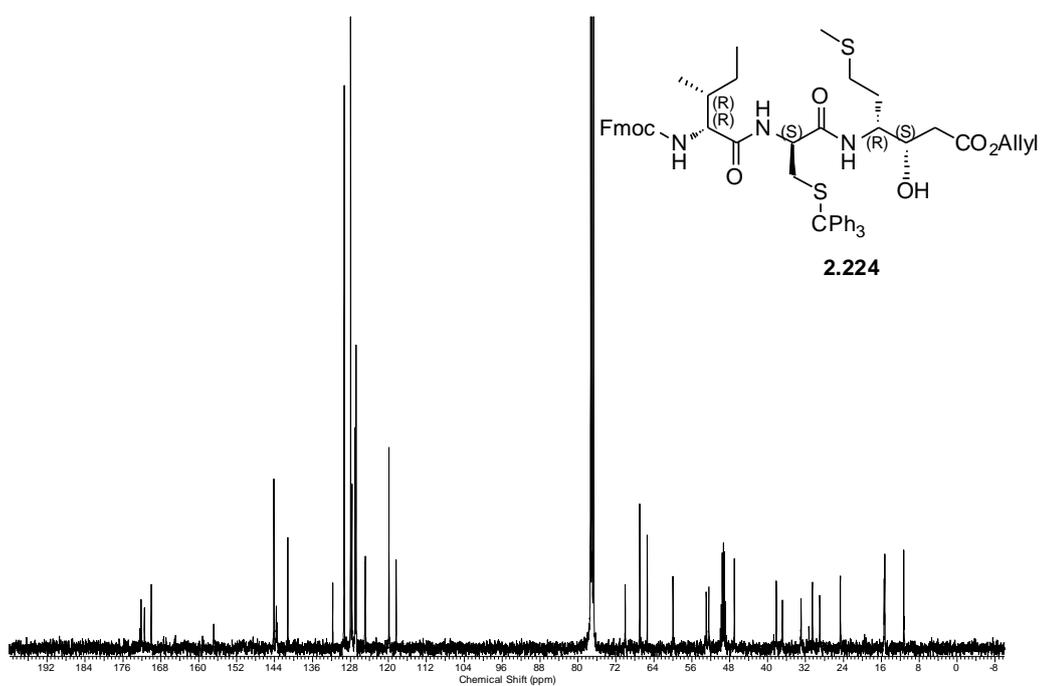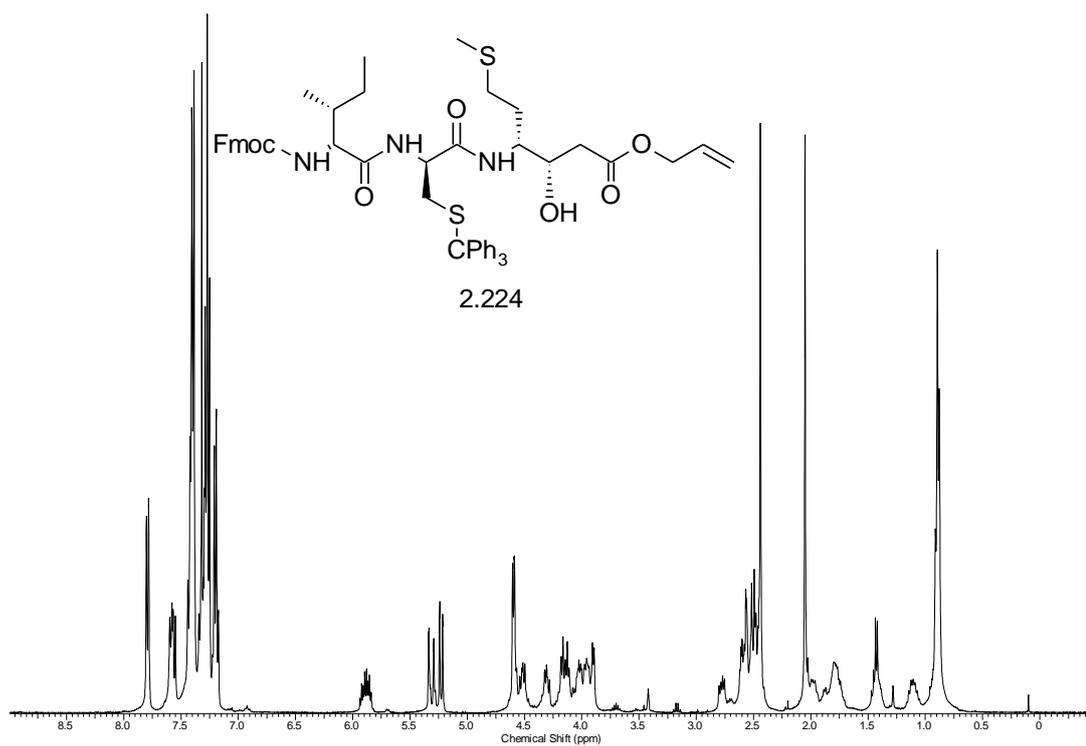
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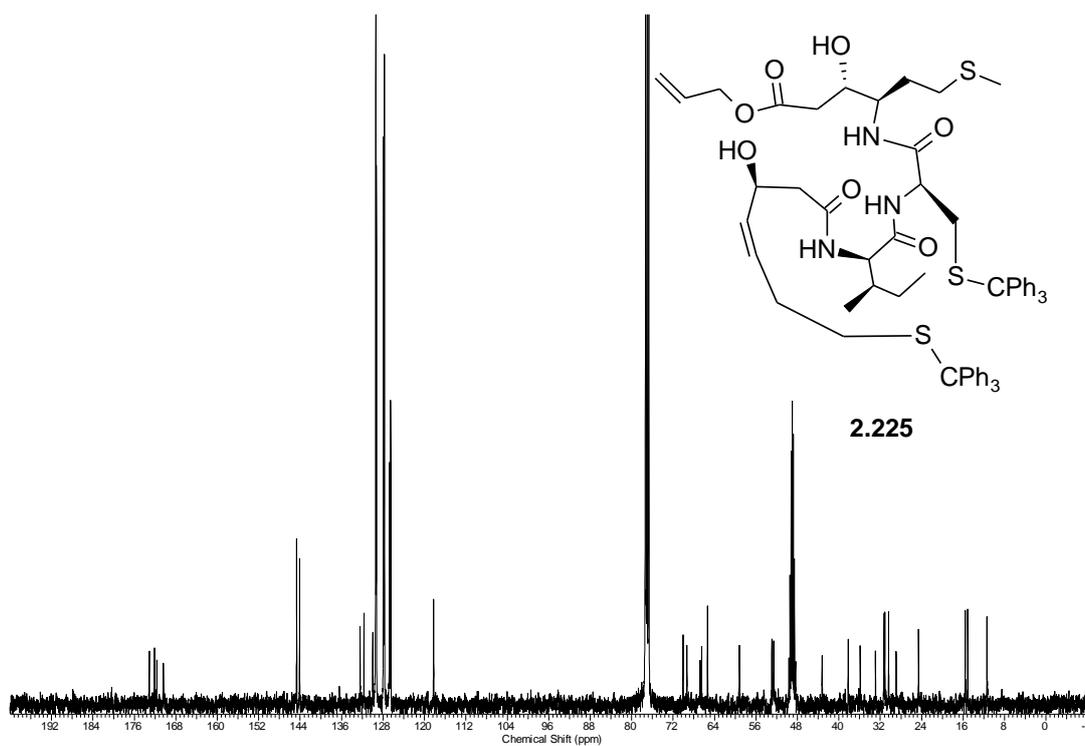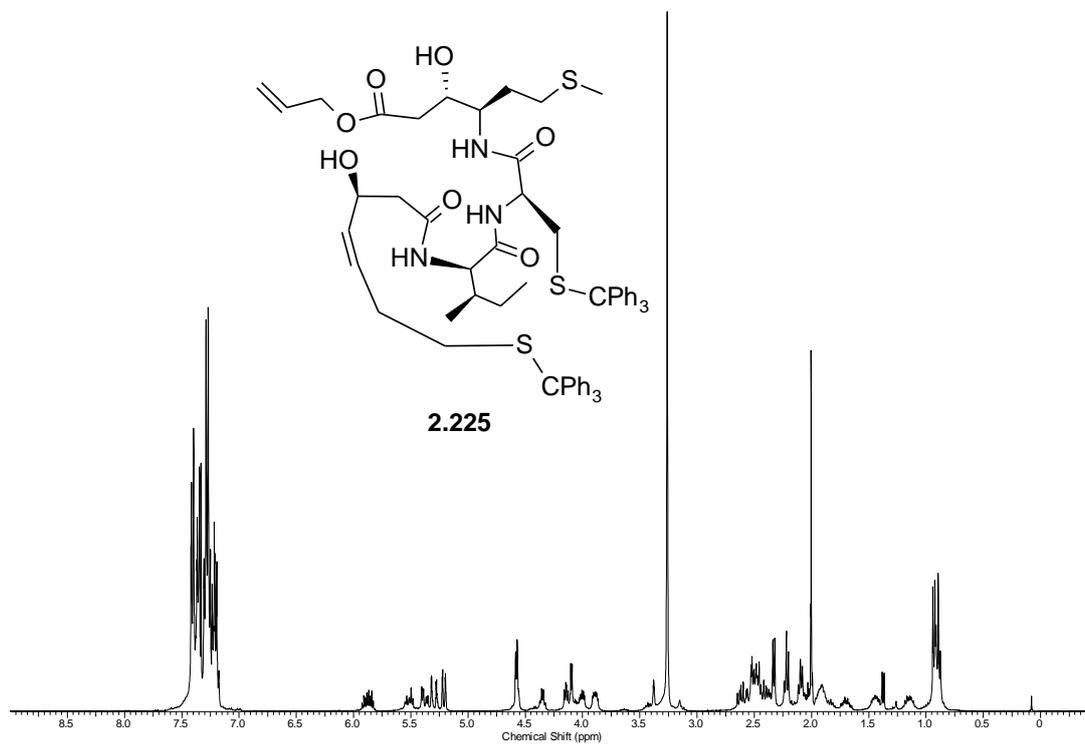
^{1}H and ^{13}C NMR spectra of 2.217

2.217

2.217
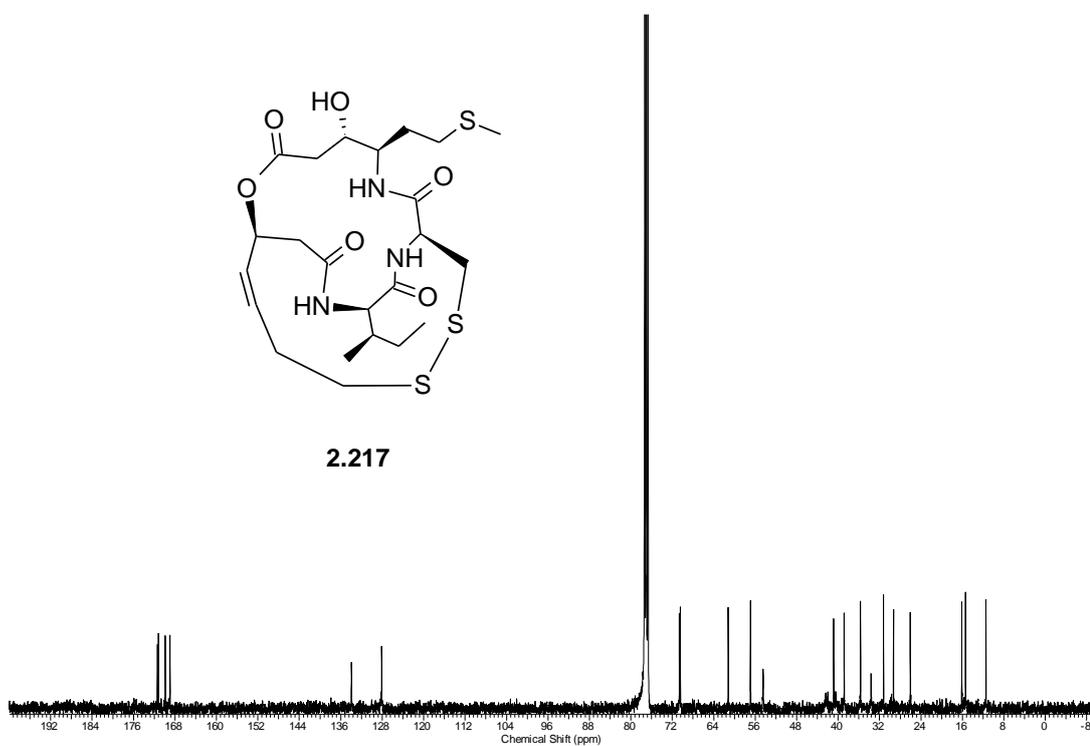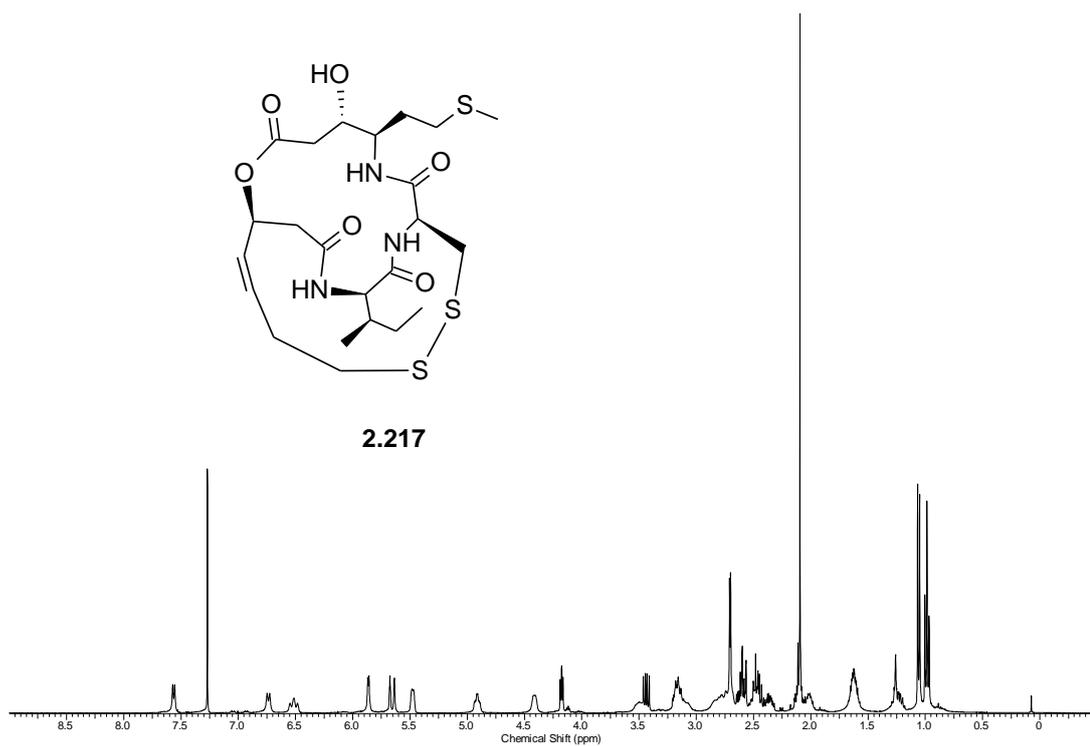
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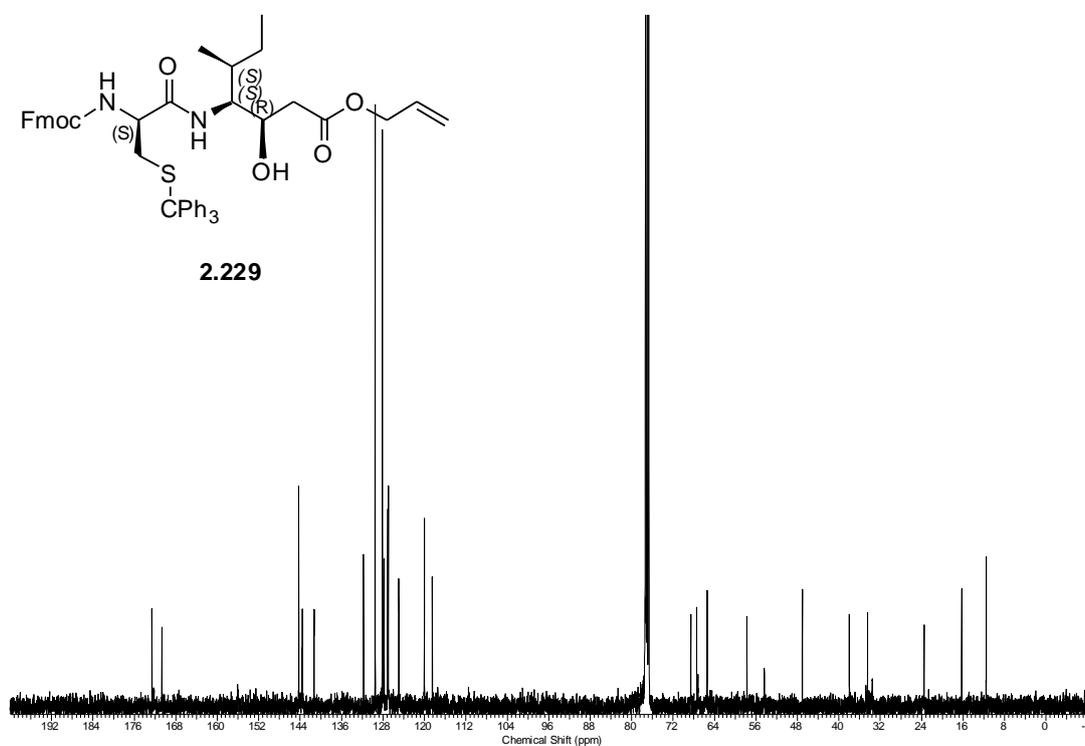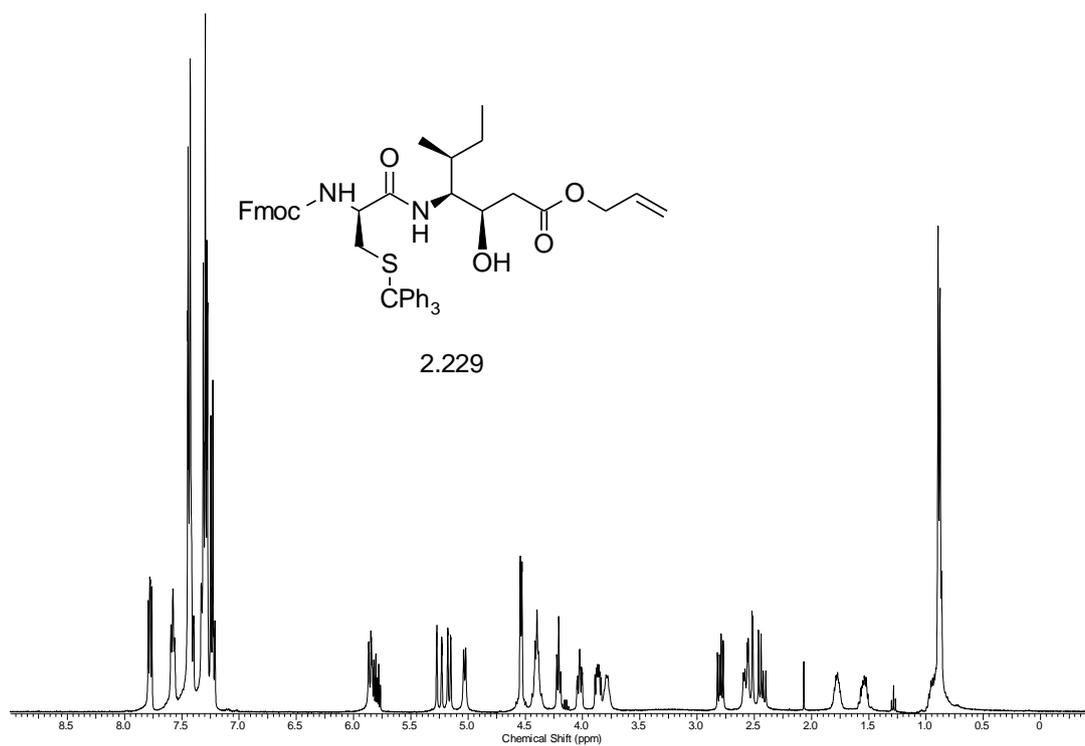
^{1}H and ^{13}C NMR spectra of 2.230

2.230

2.230
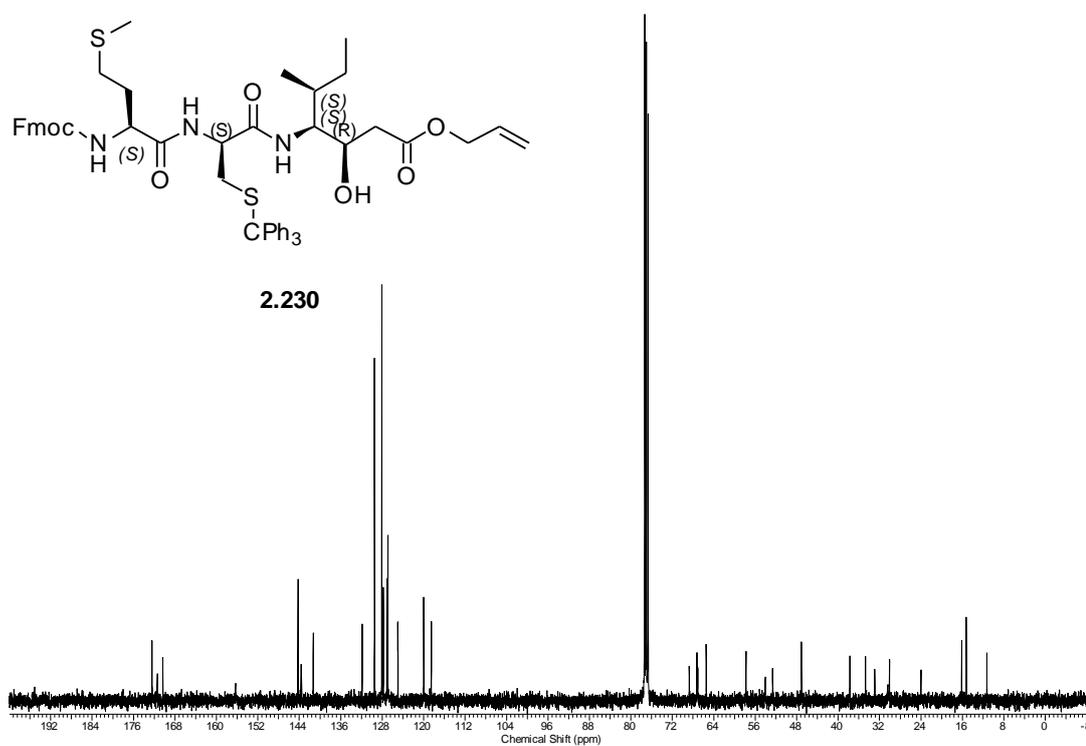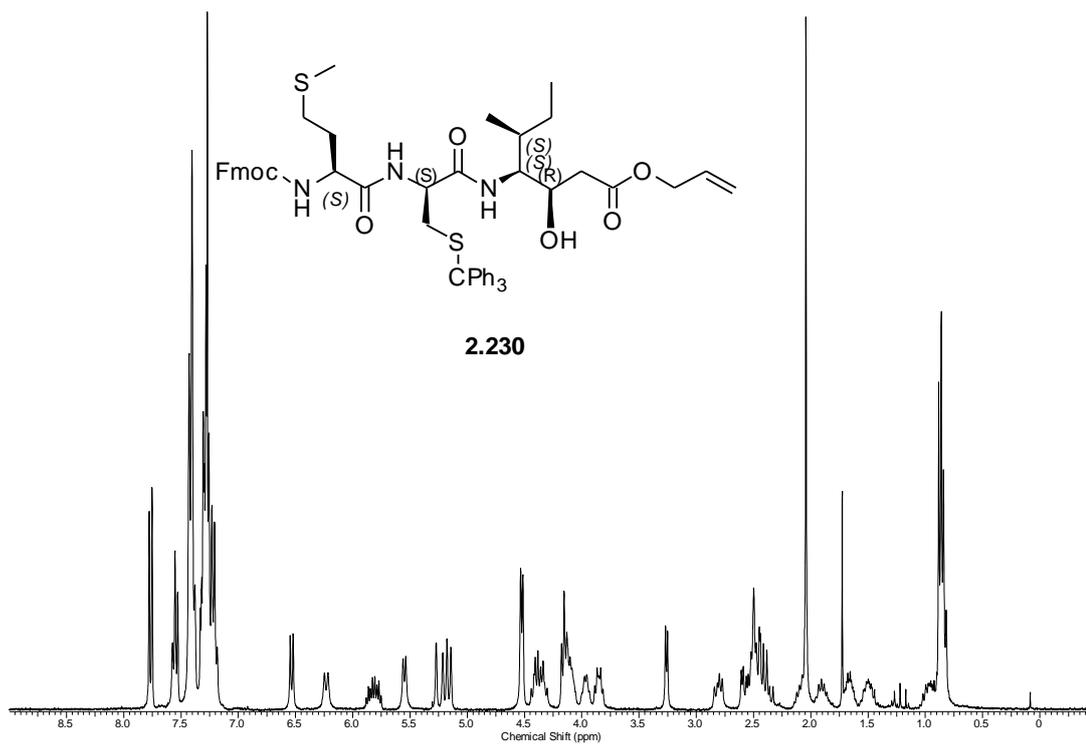
^{1}H and ^{13}C NMR spectra of 2.231

2.231

2.231
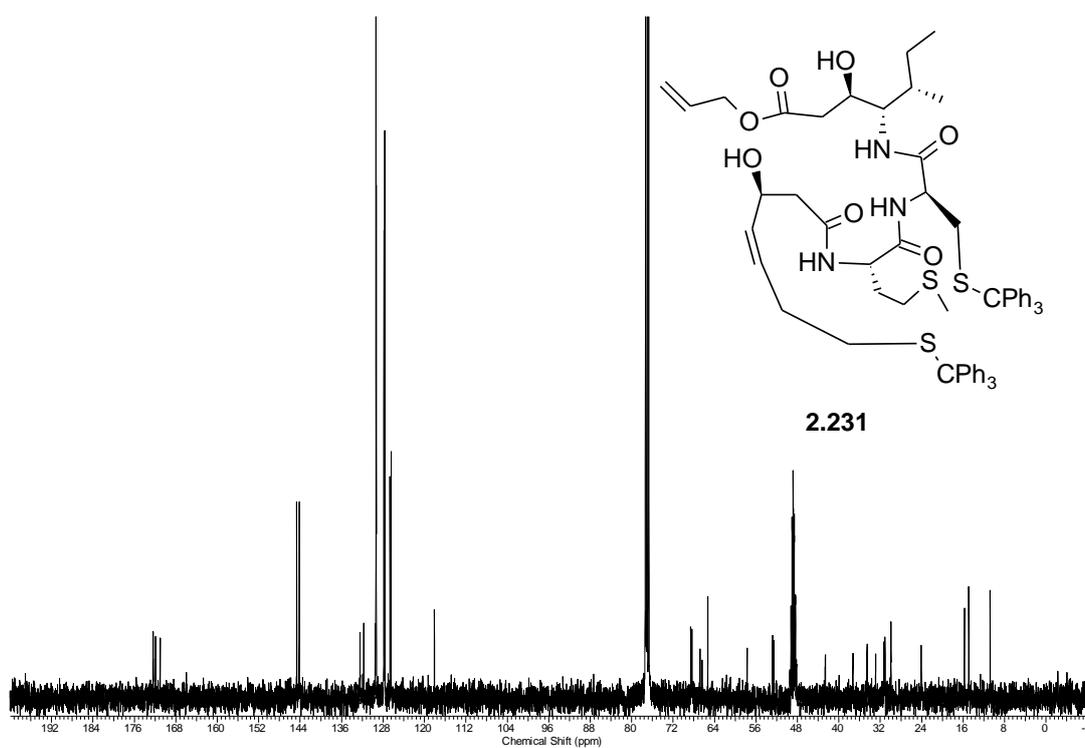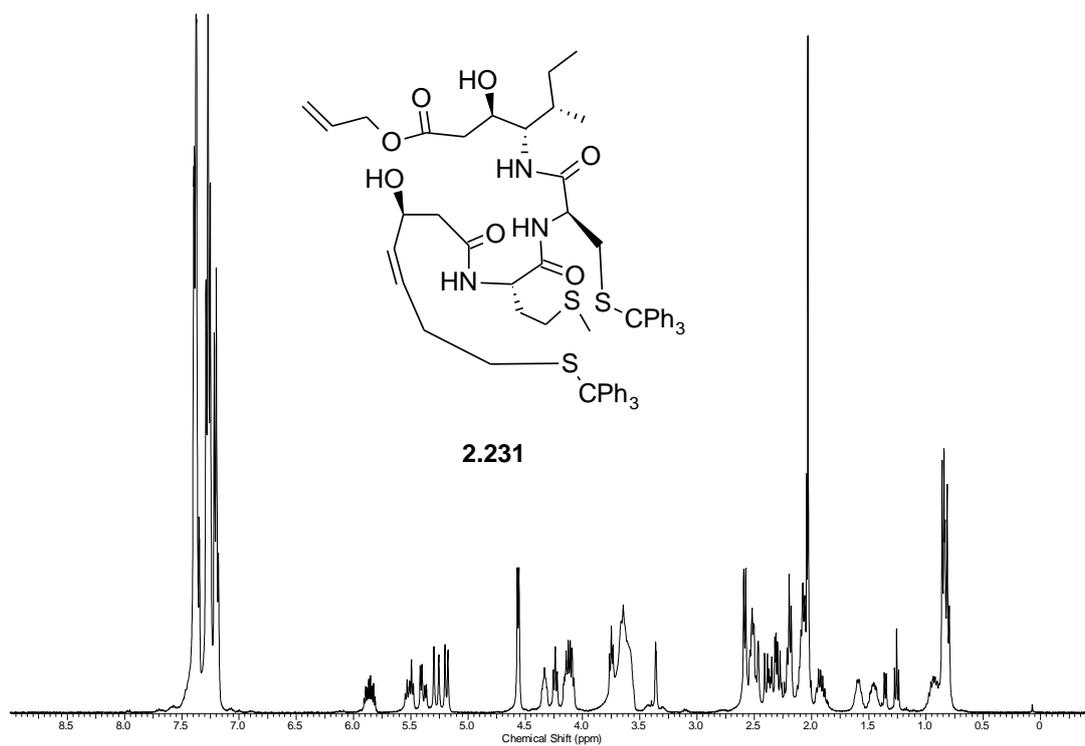
^{1}H and ^{13}C NMR spectra of 2.29

2.29

2.29
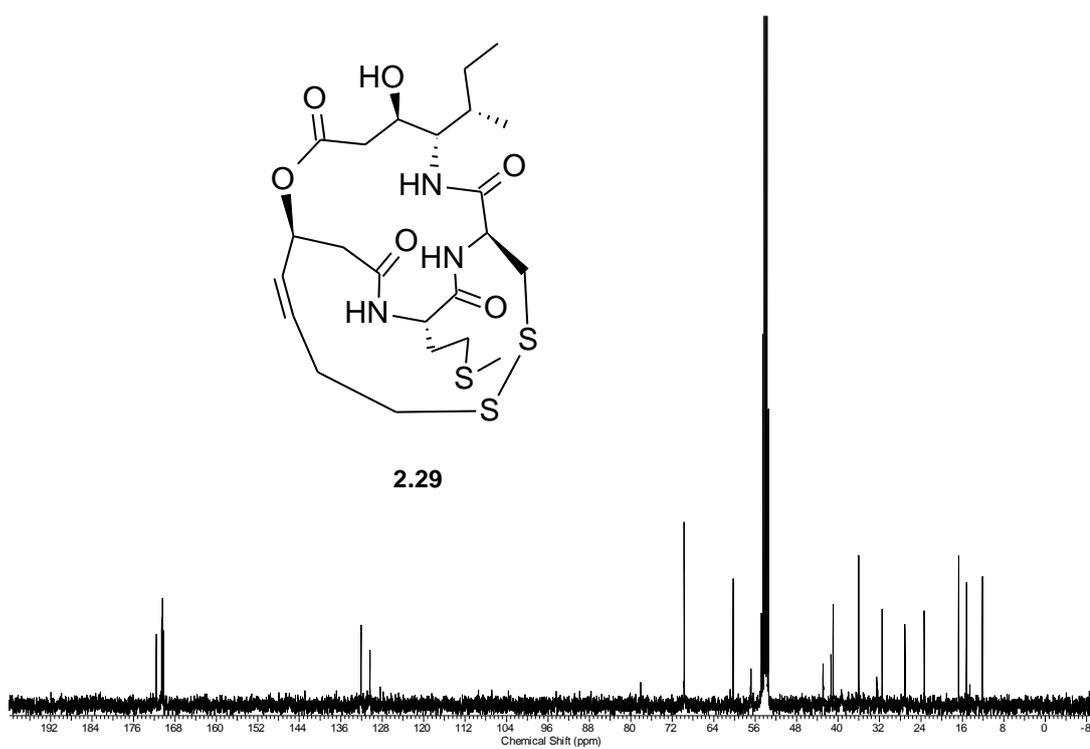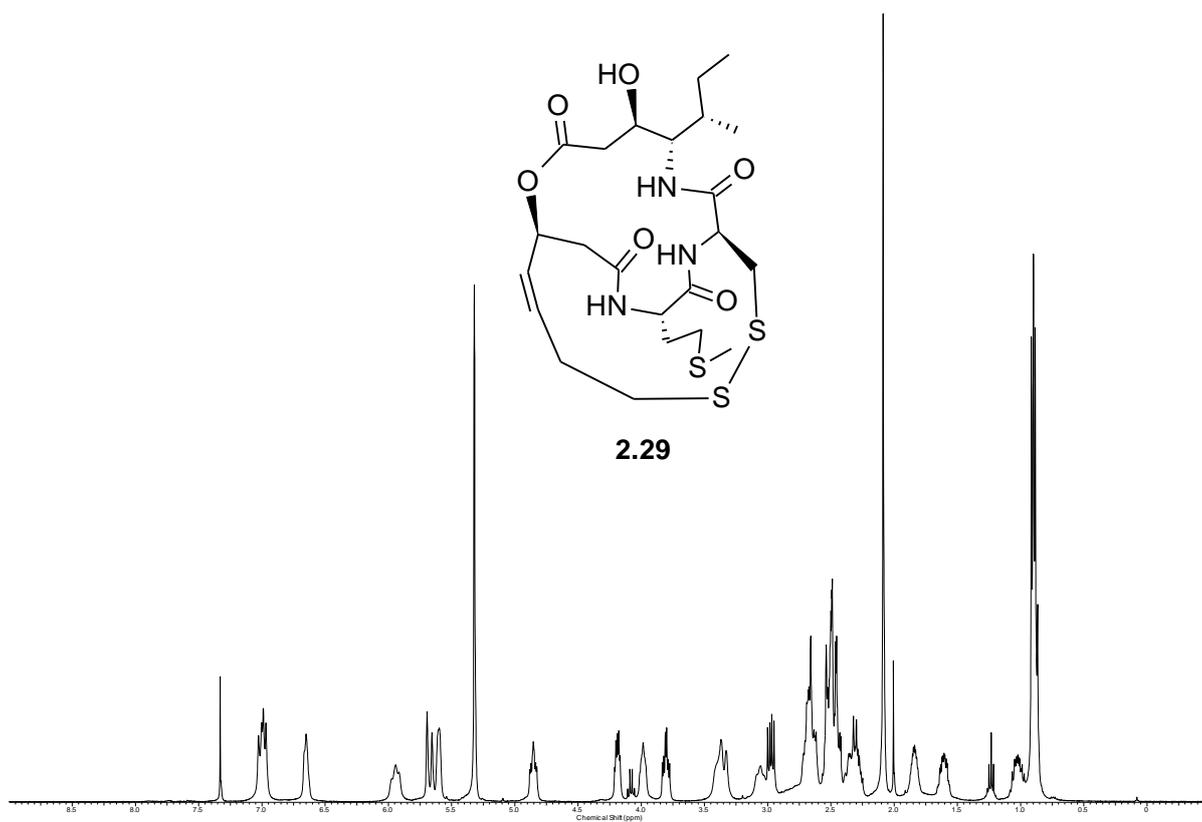
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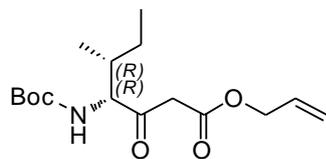
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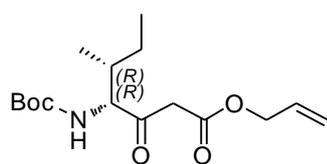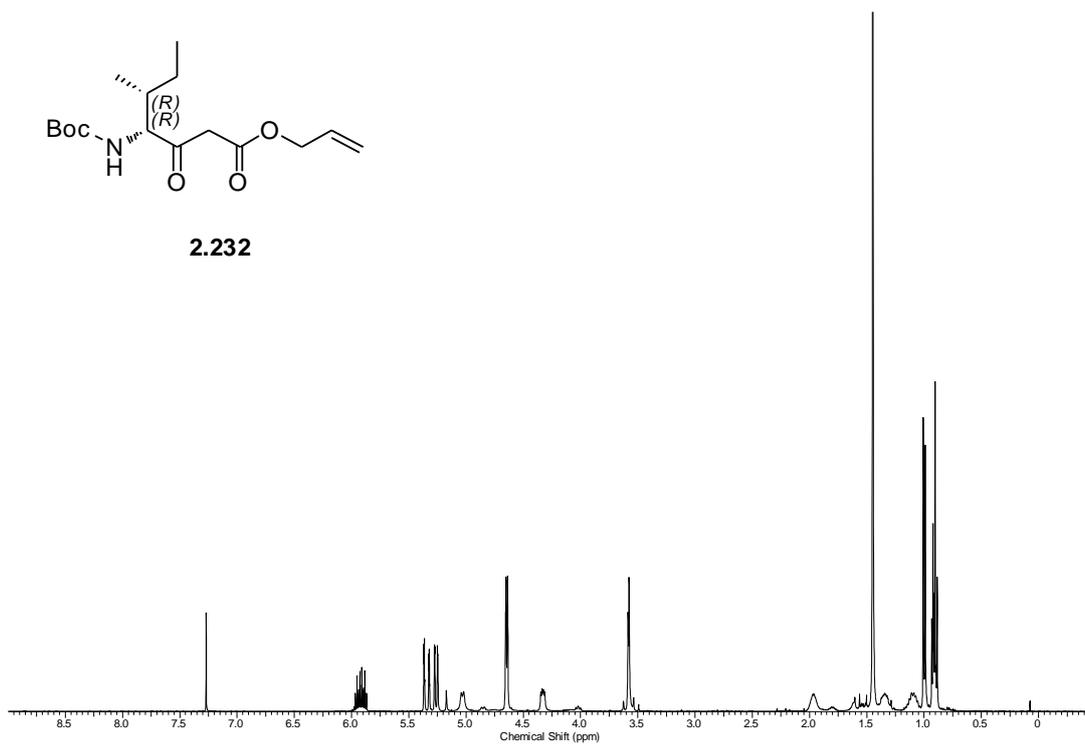
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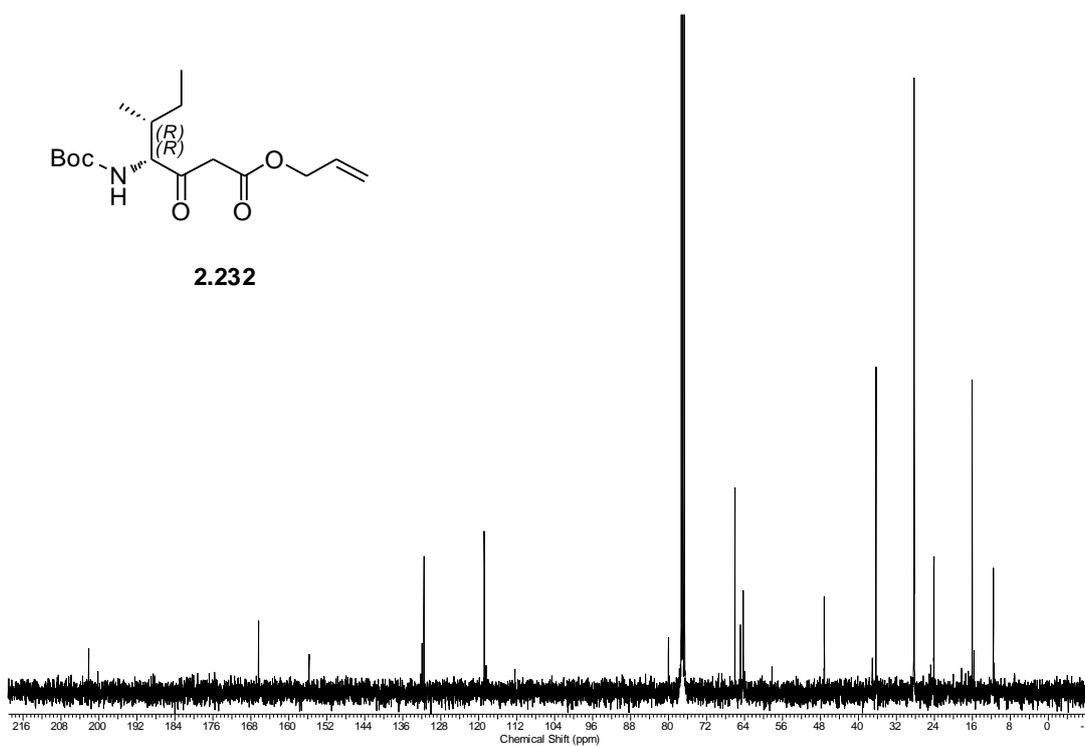
^{1}H and ^{13}C NMR spectra of 2.234

2.234

2.234
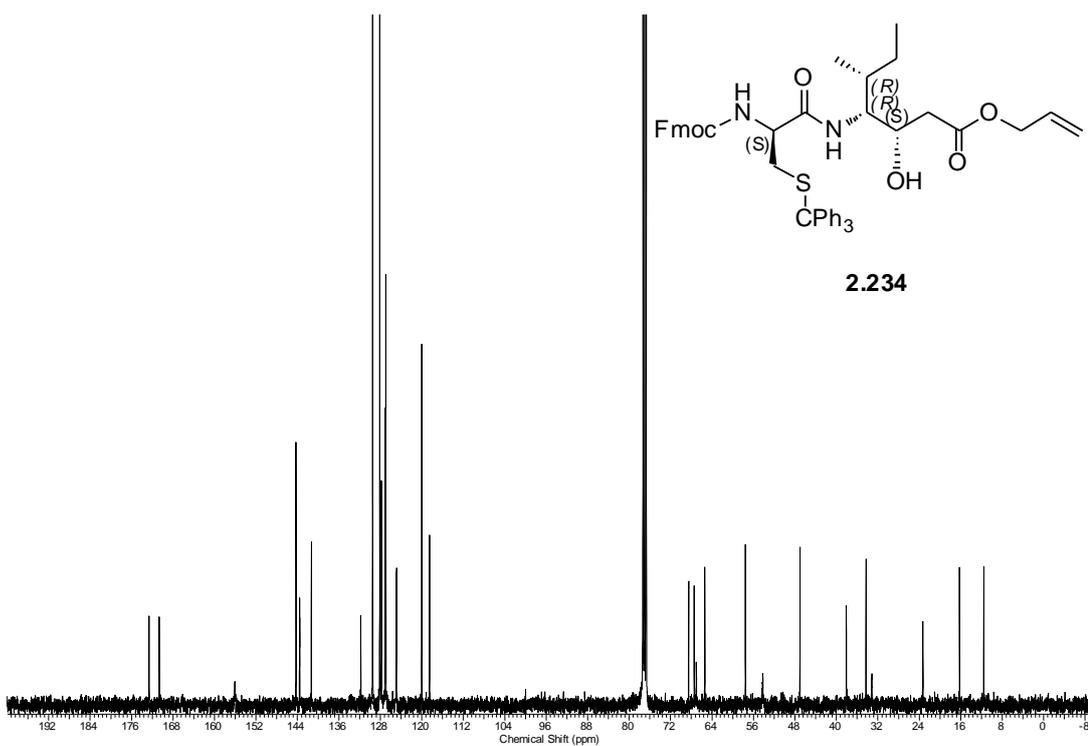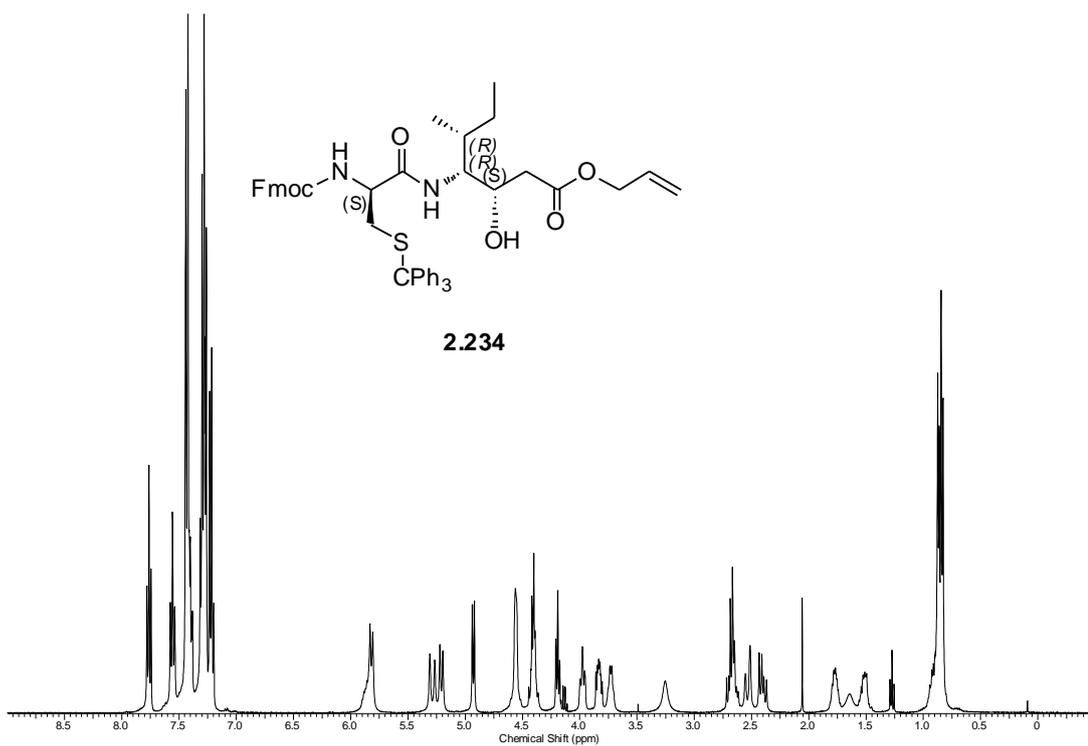
^{1}H and ^{13}C NMR spectra of 2.235

2.235

2.235
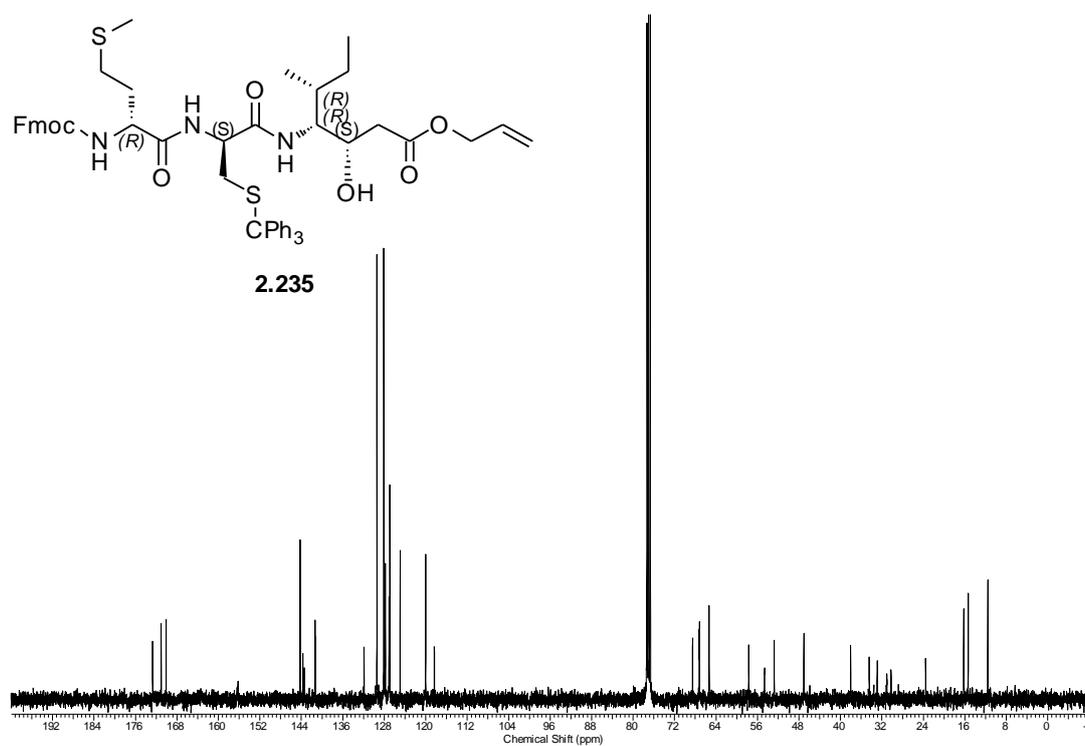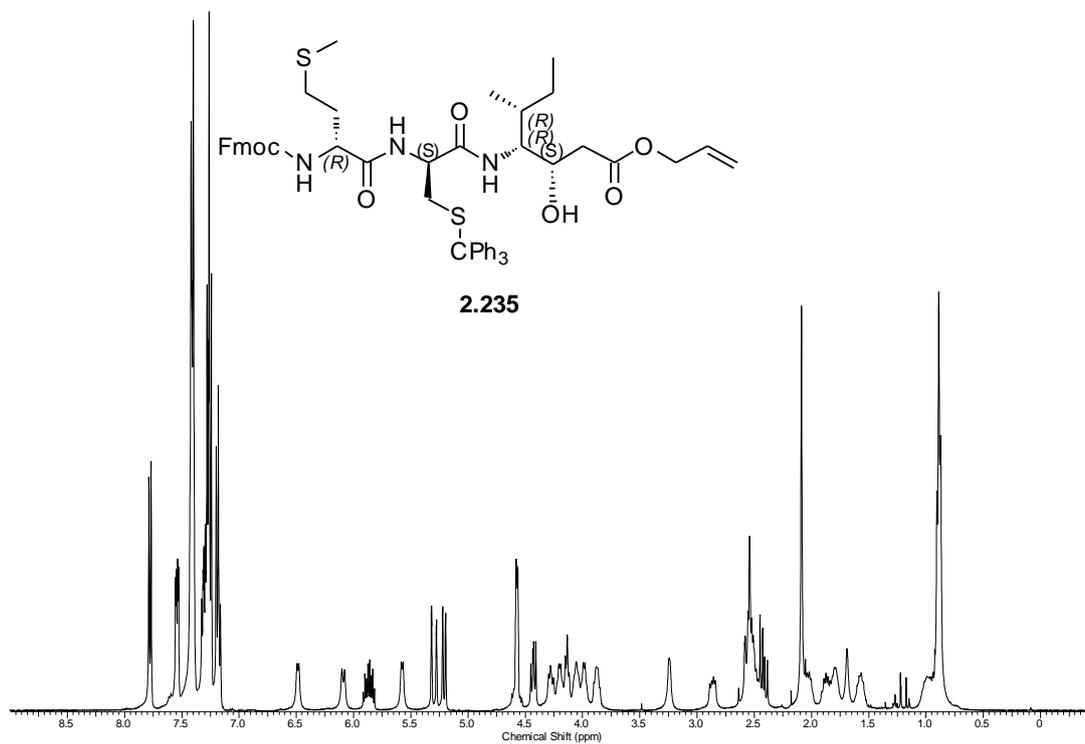
^{1}H and ^{13}C NMR spectra of 2.236

2.236

2.236
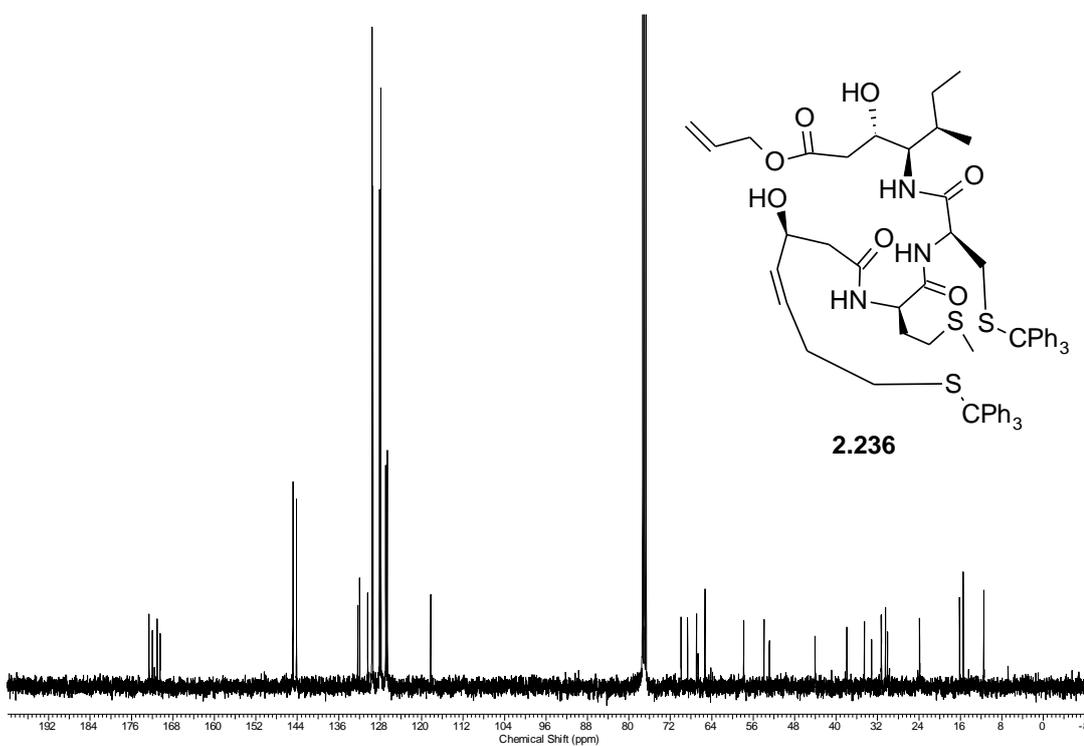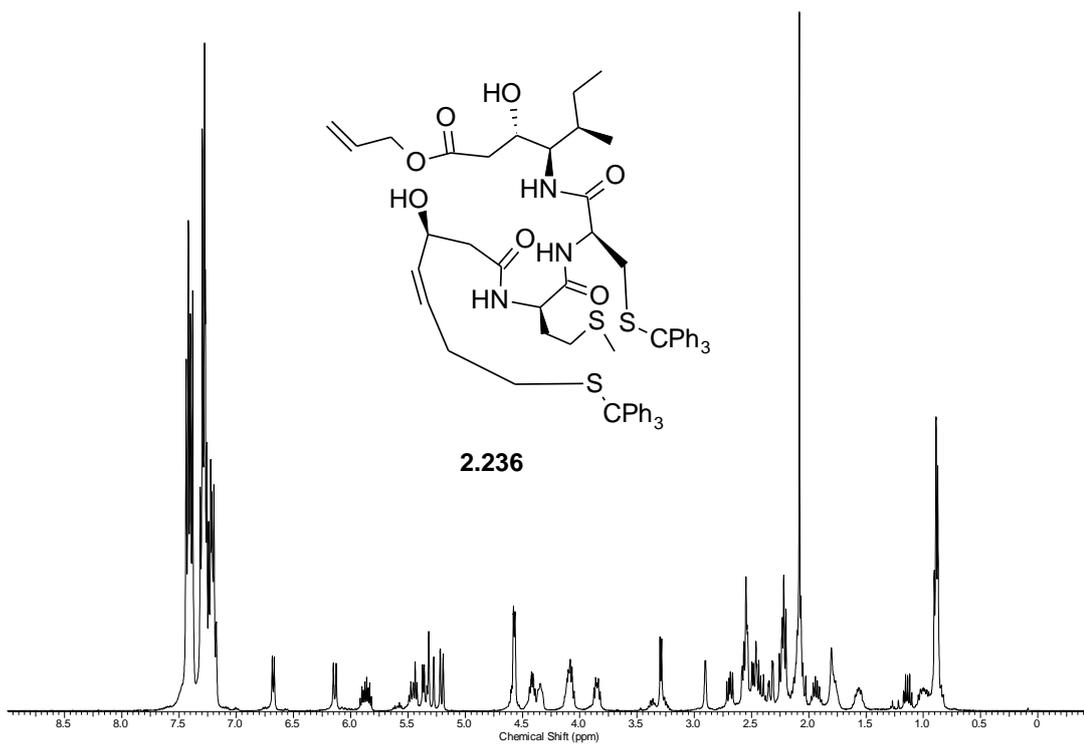
^{1}H and ^{13}C NMR spectra of 2.237

2.237

2.237
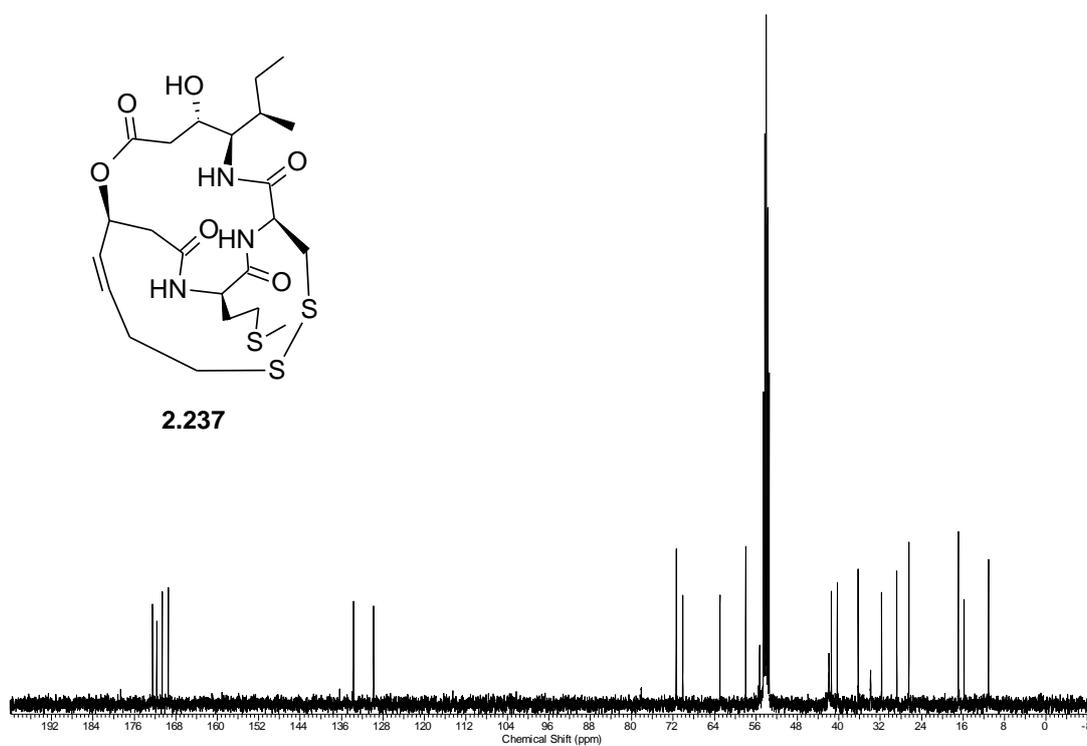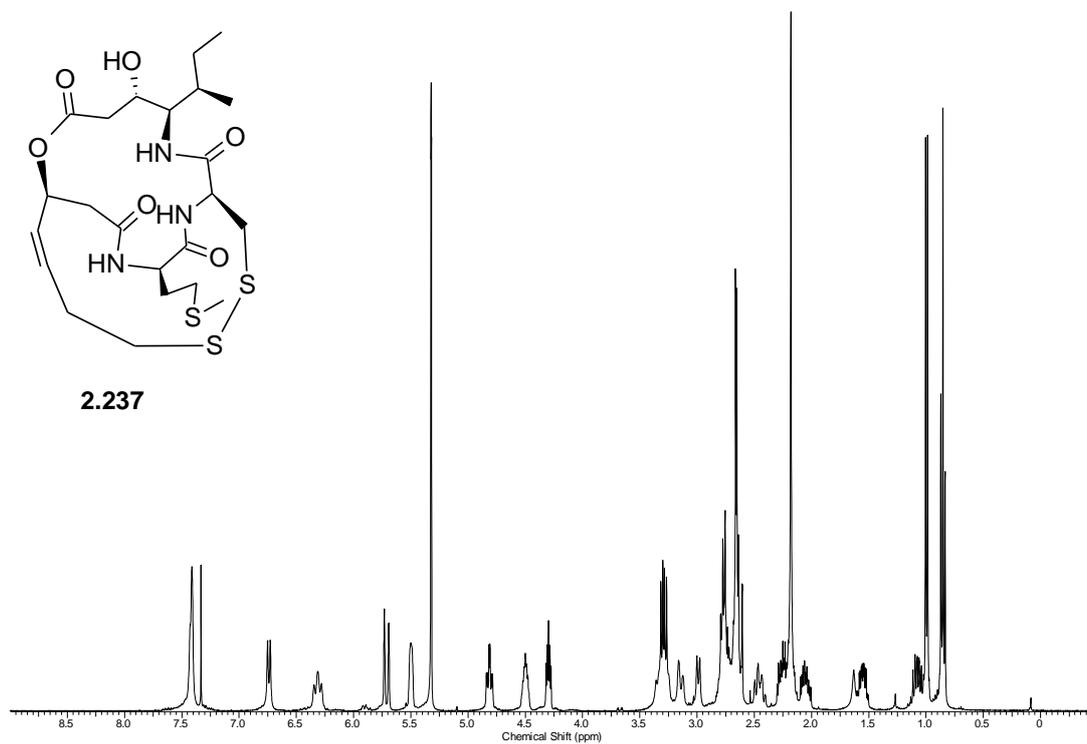
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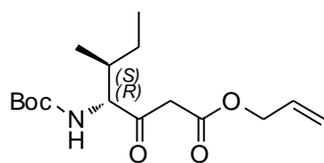
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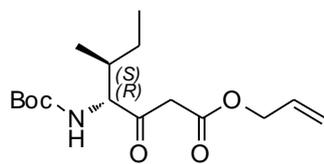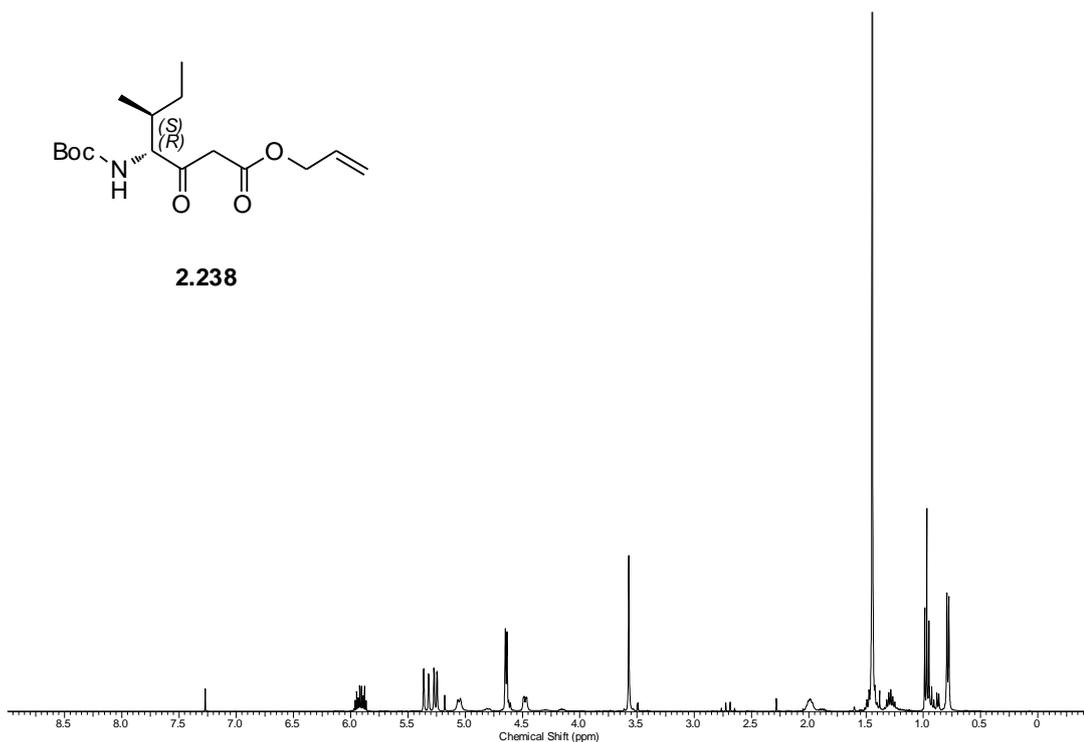
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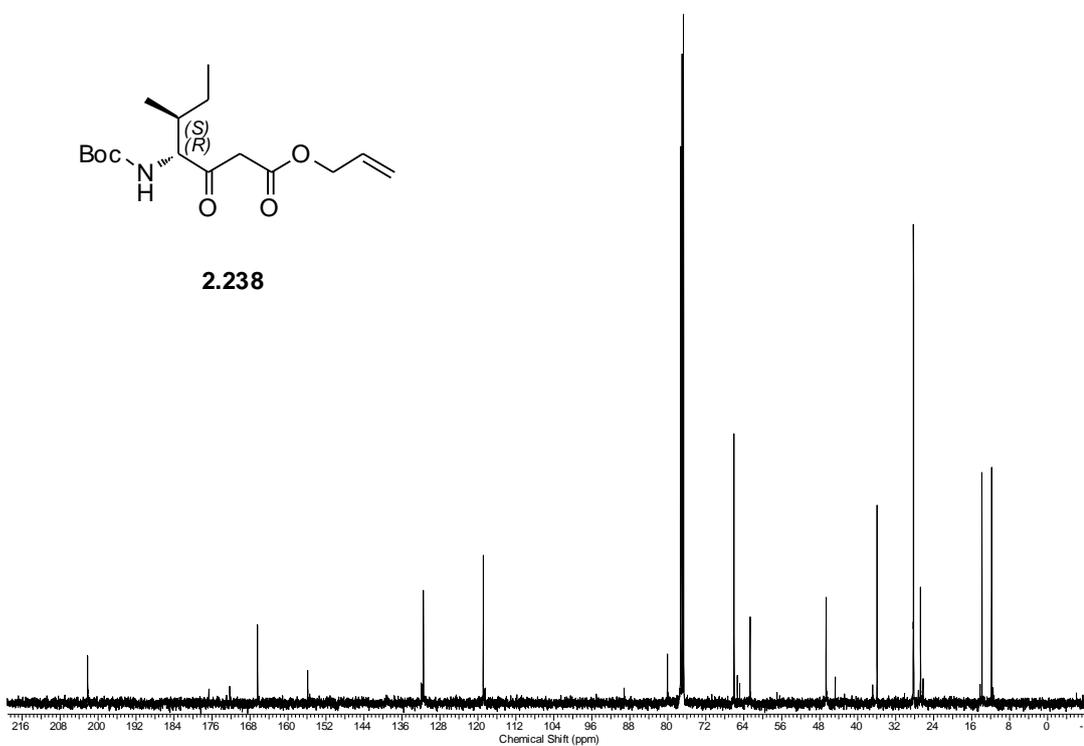
¹H and ¹³C NMR spectra of 2.239

2.239

2.239
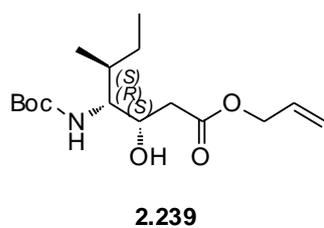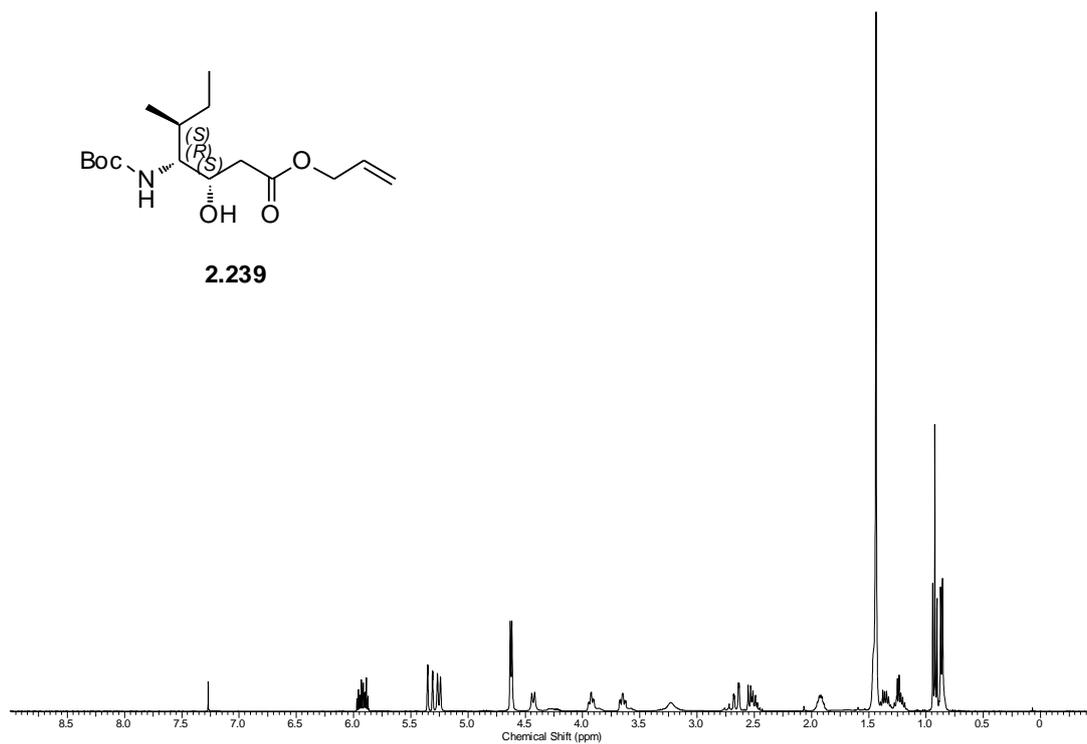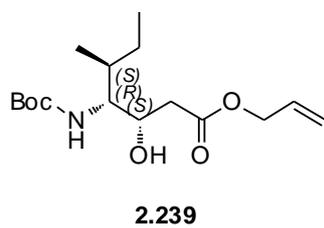
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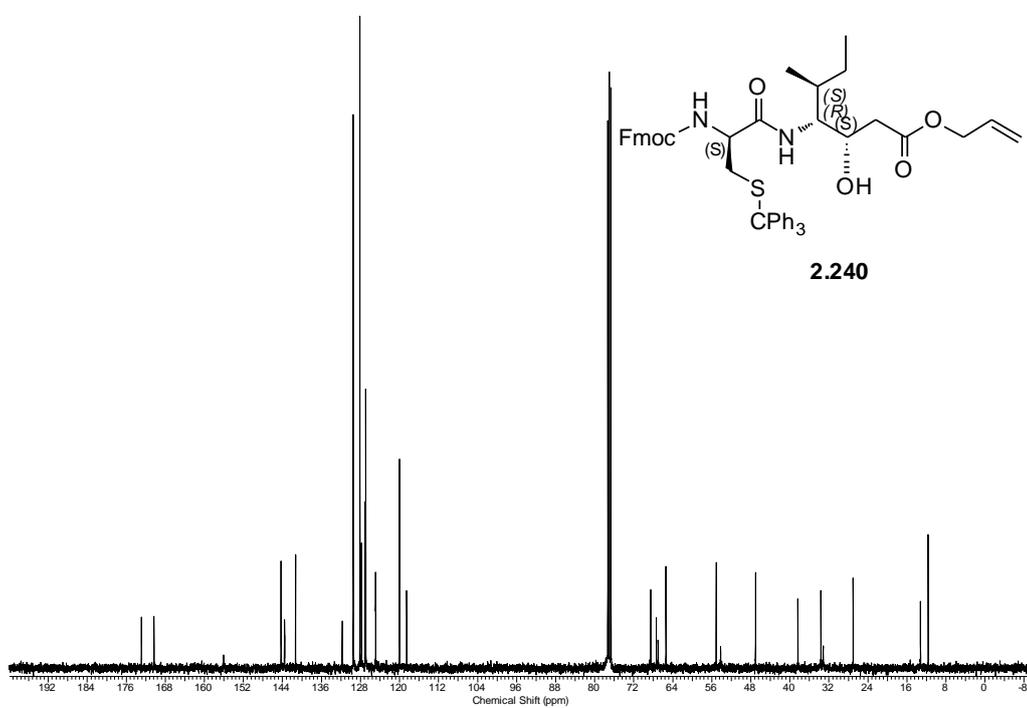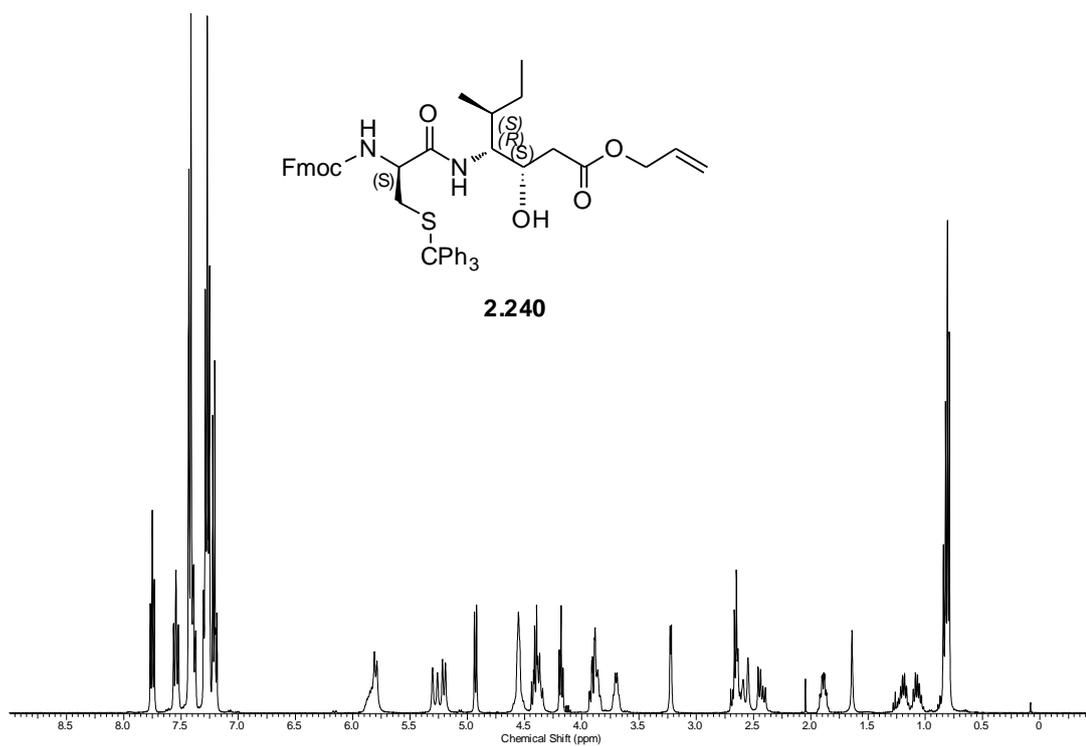
^{1}H and ^{13}C NMR spectra of 2.241

2.241

2.241
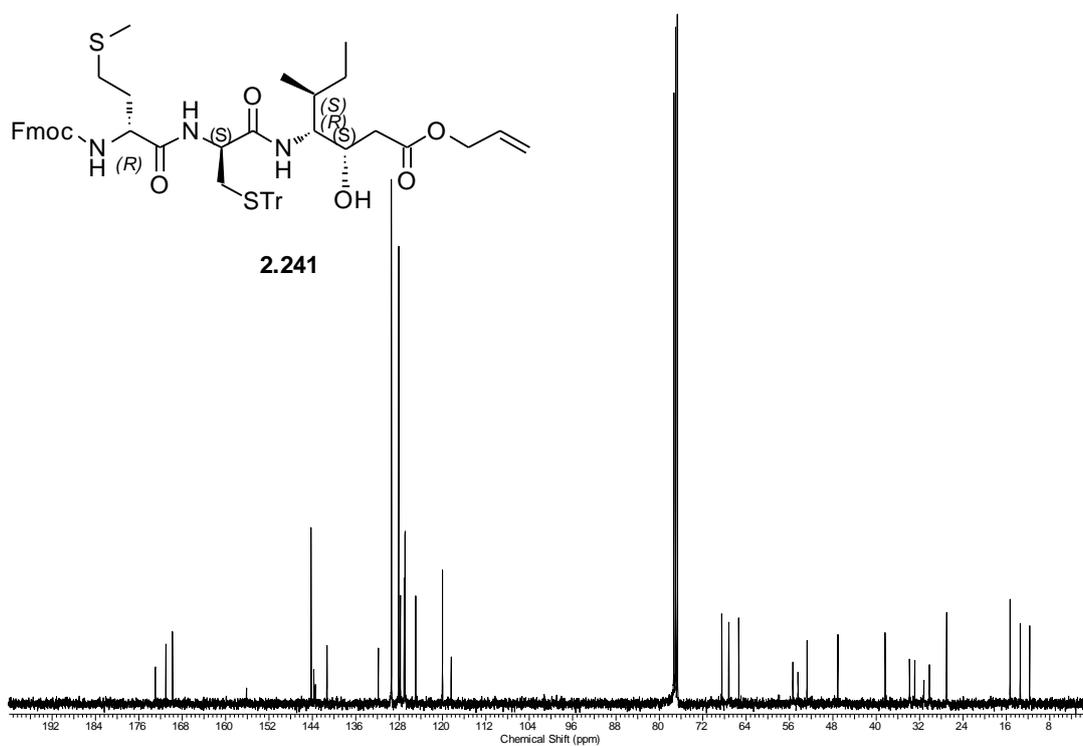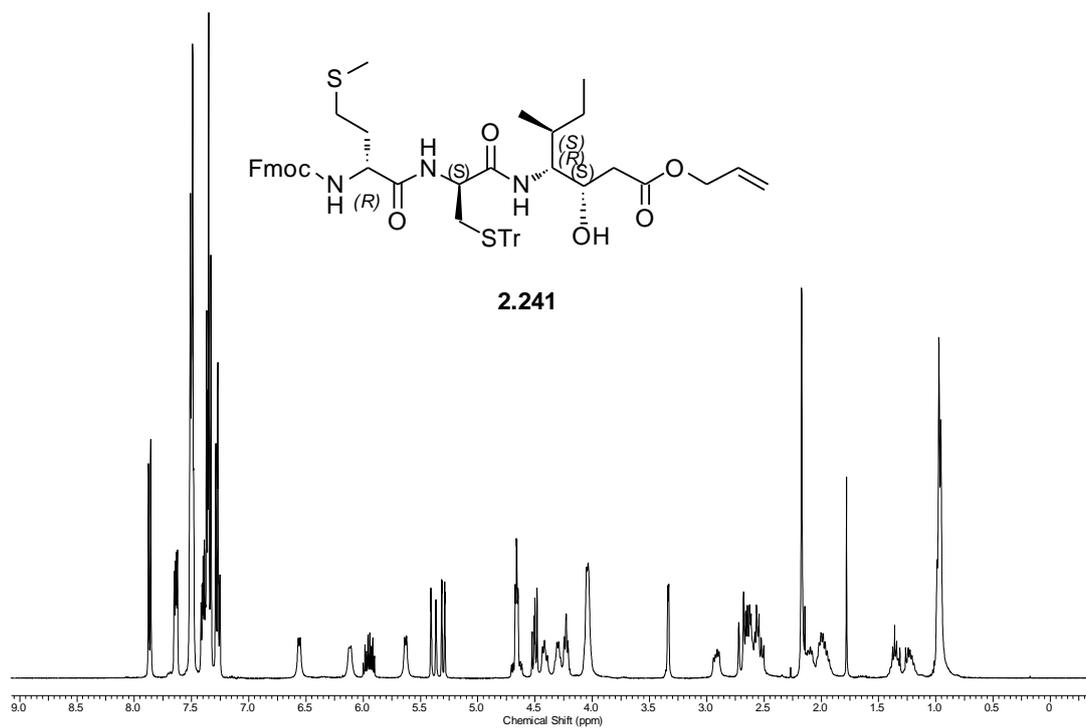
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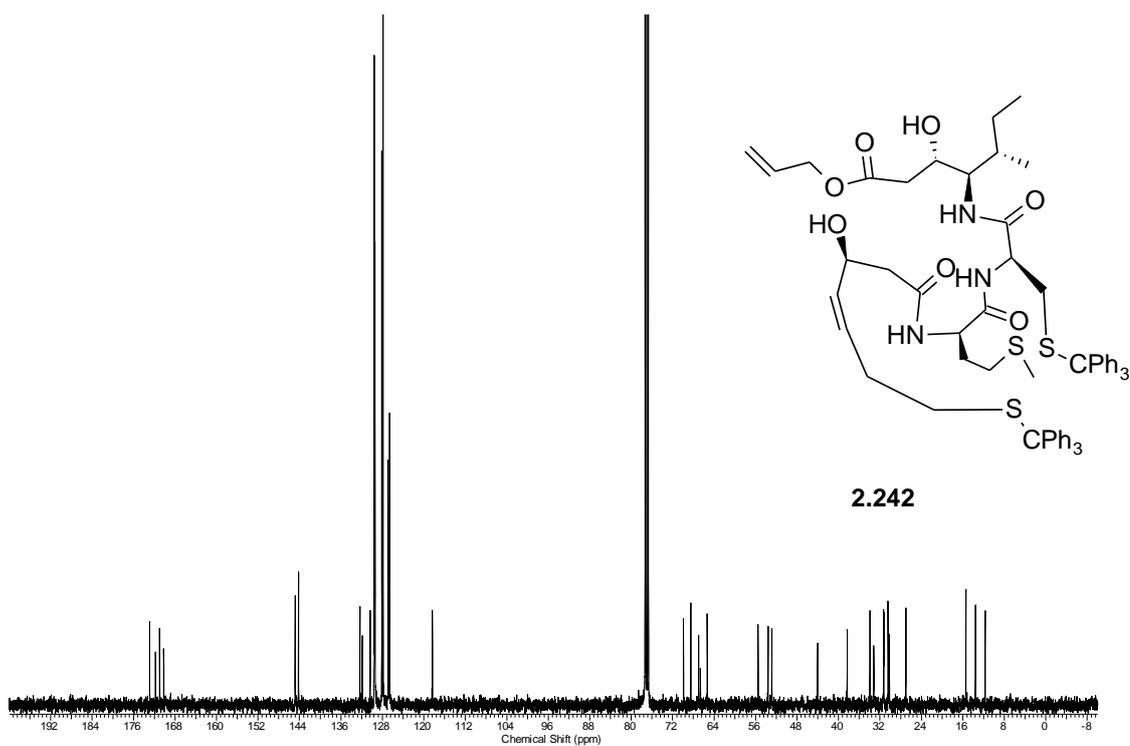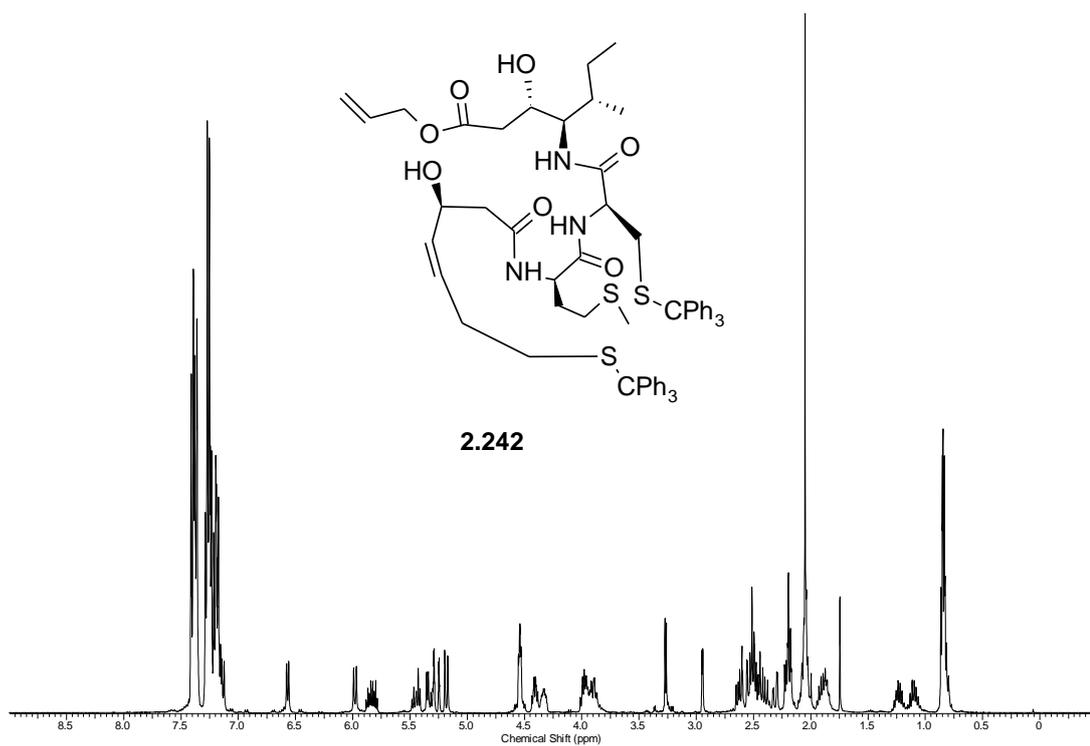
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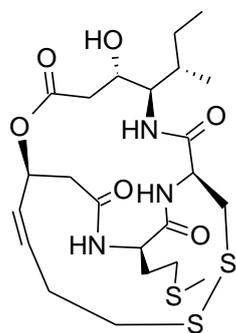
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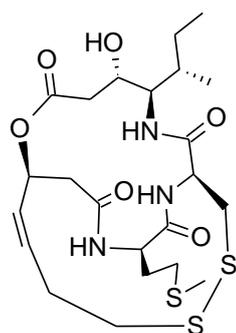
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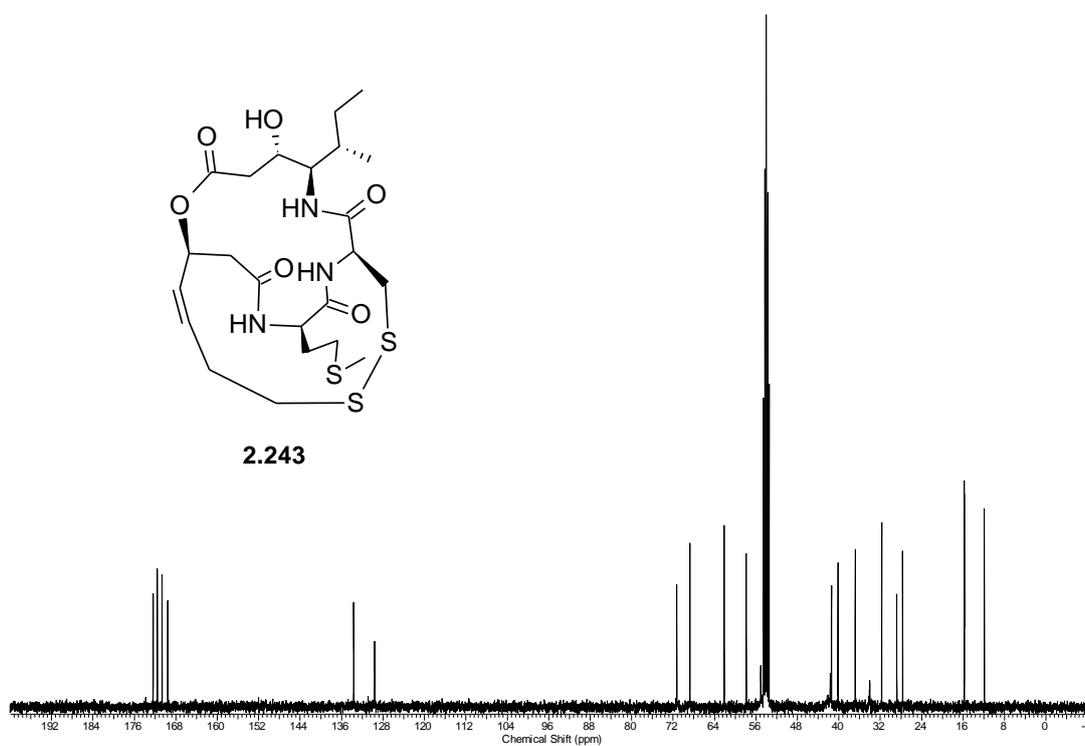
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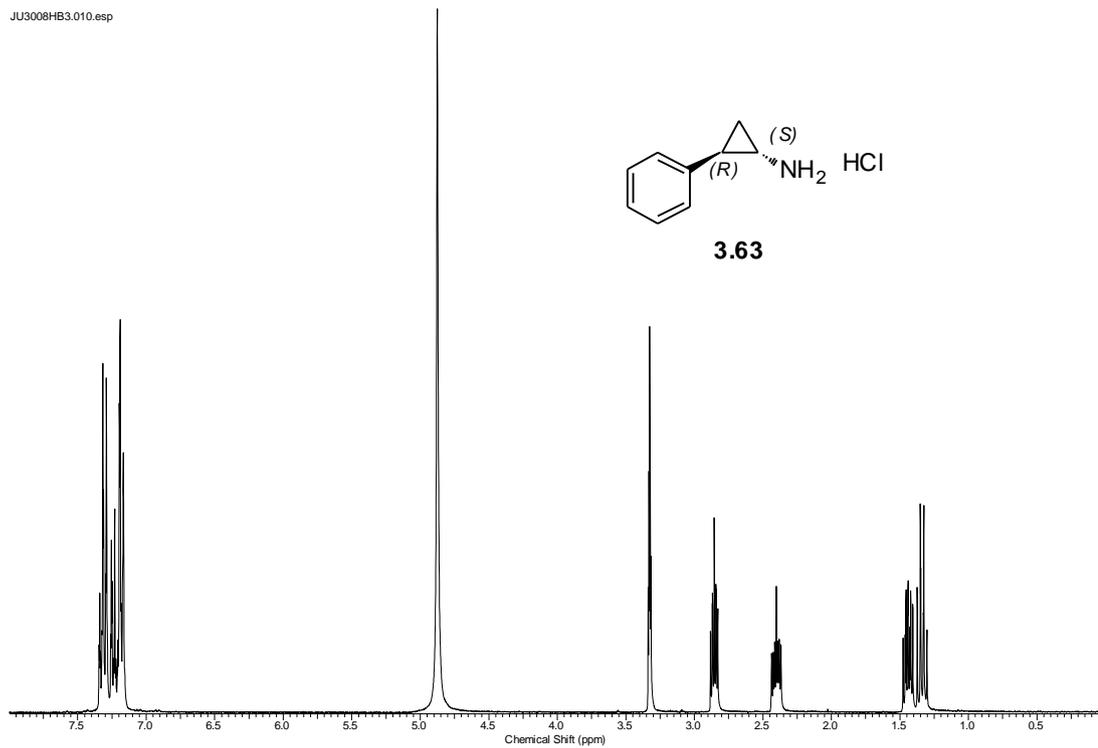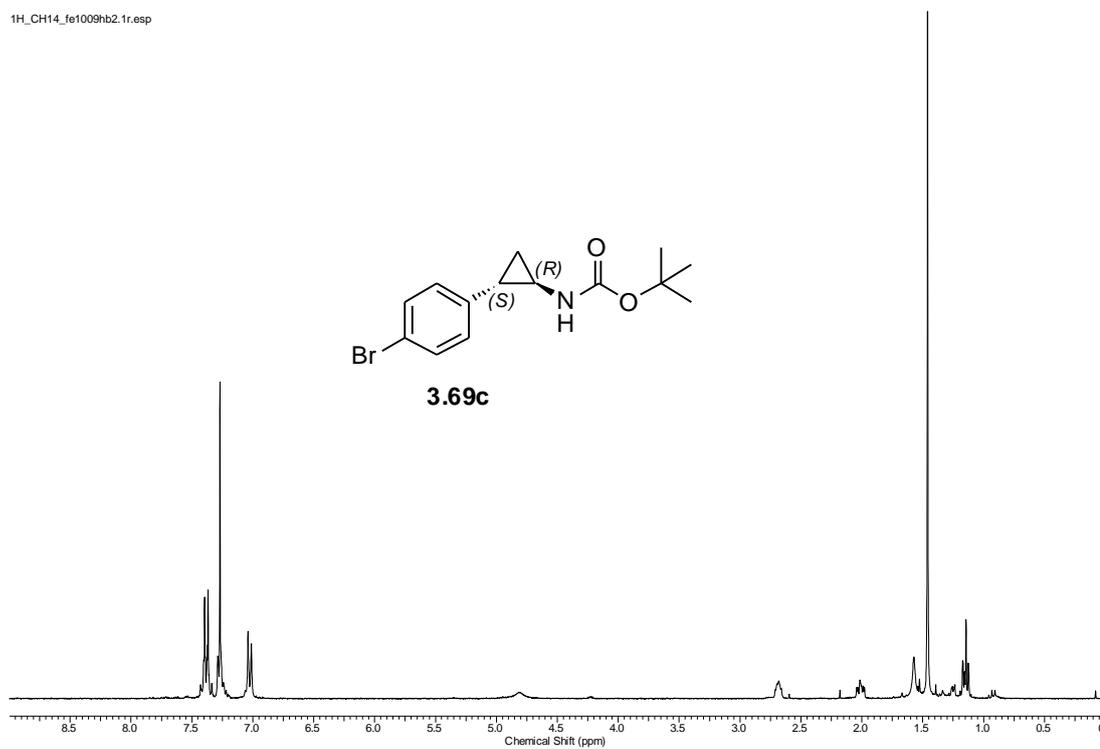
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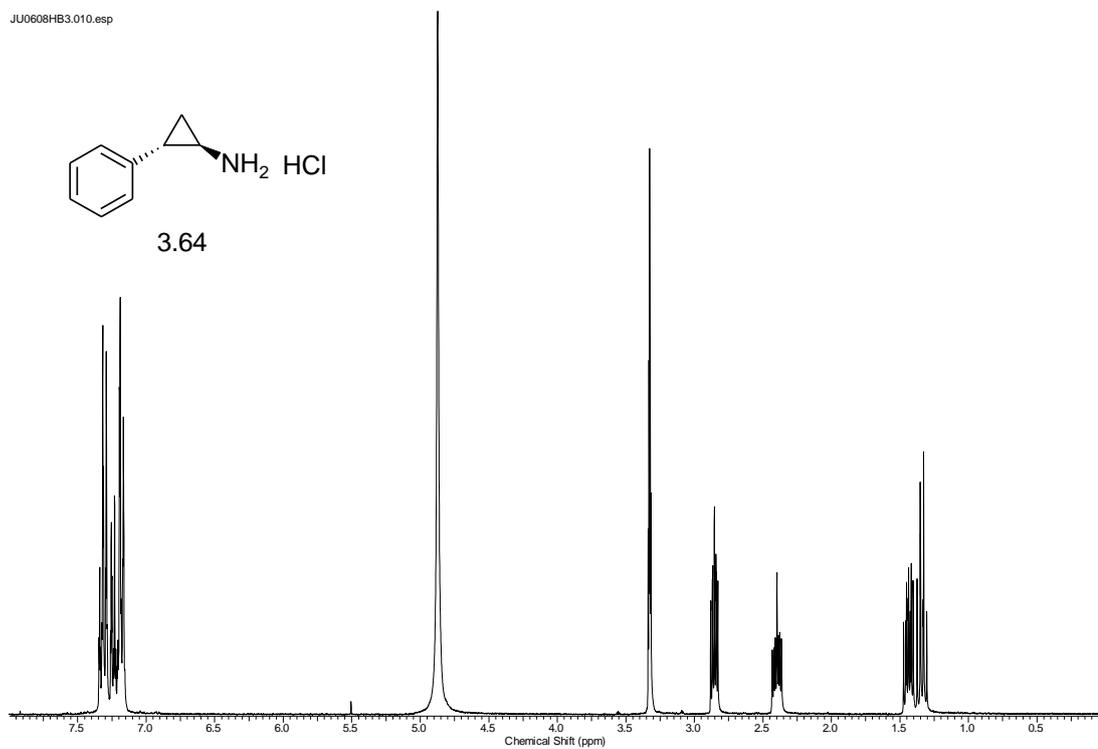
^{1}H NMR spectrum of 3.67a

3.67a
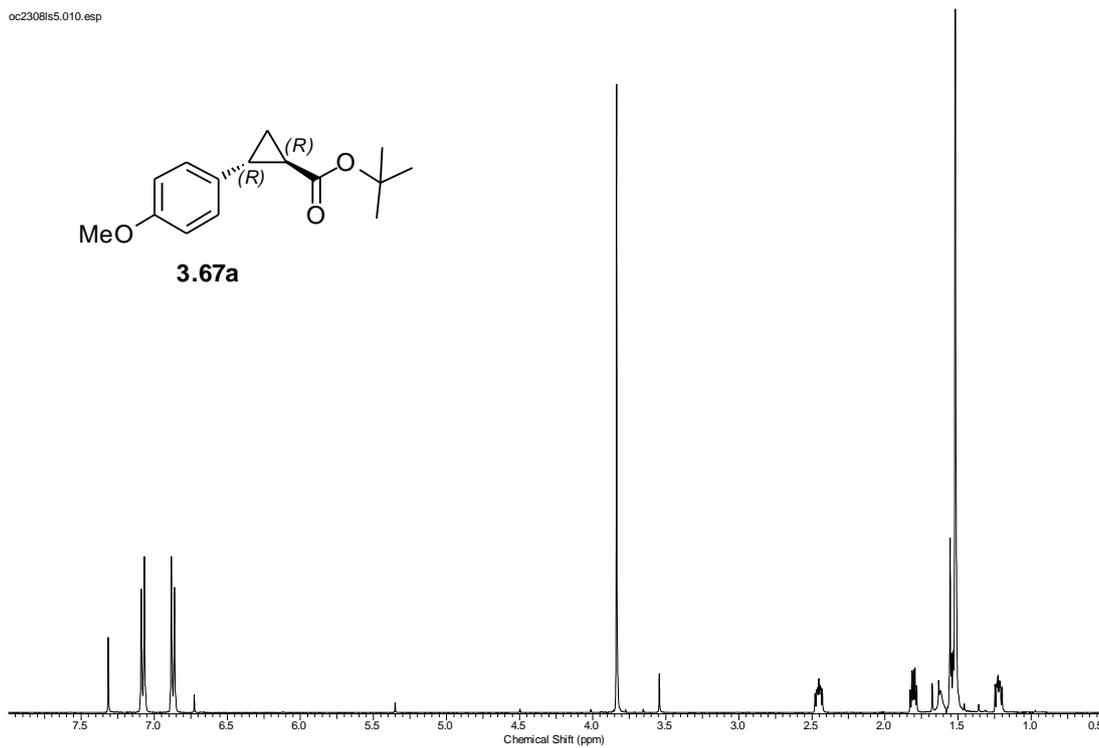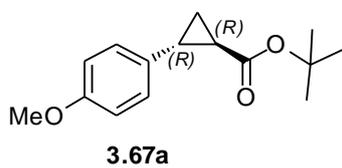
^{1}H NMR spectrum of 3.67b

3.67b
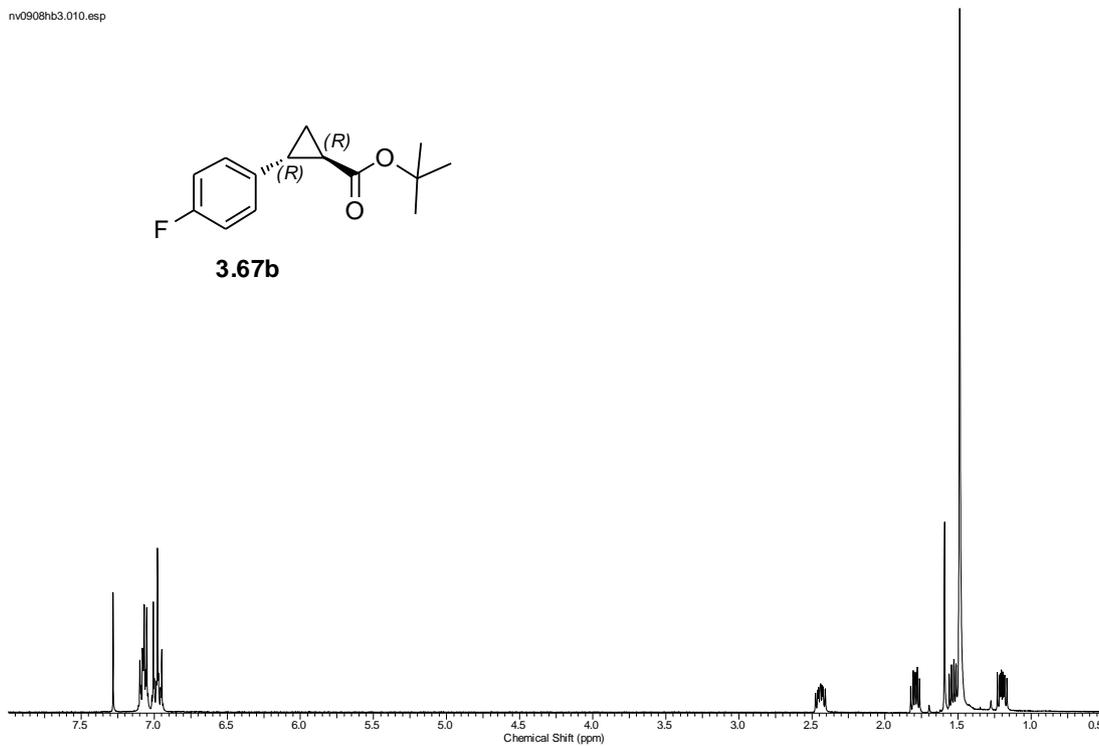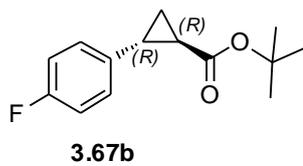
¹H NMR spectrum of 3.67c

MY2010HB3.010.esp

3.67c
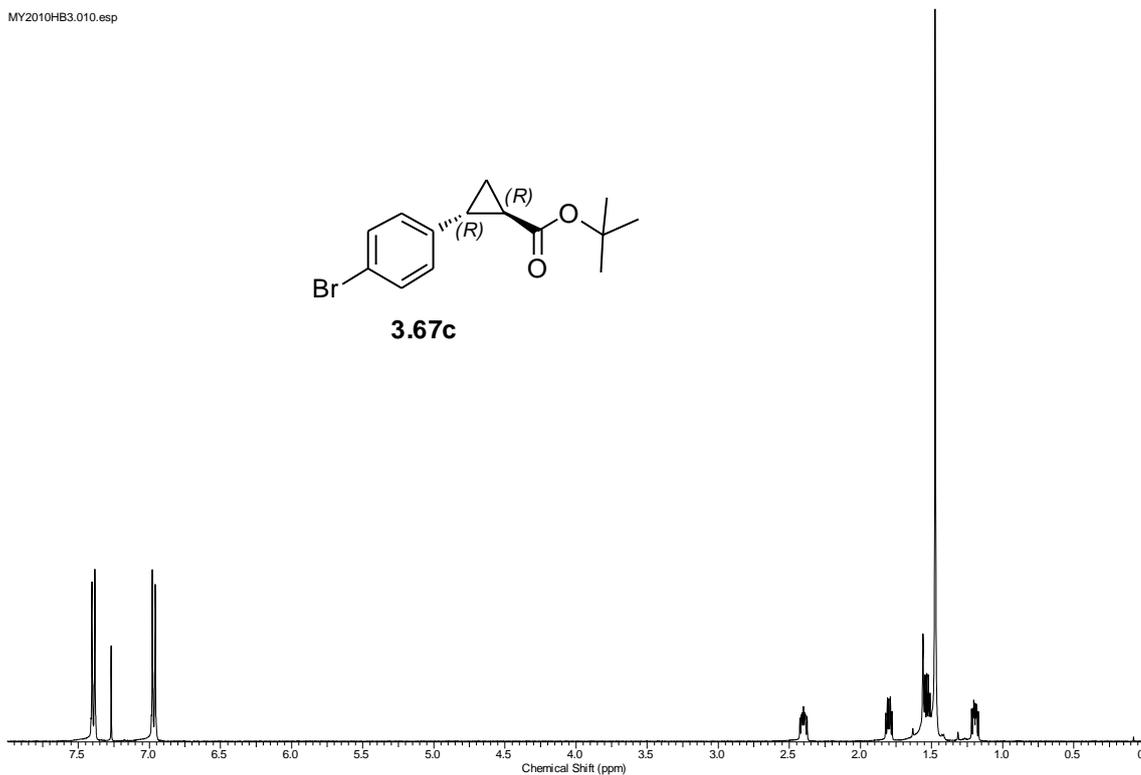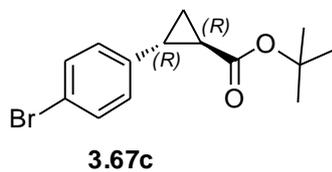
¹H NMR spectrum of 3.67d

1H_CH15_dc0508hb6.1r.esp

3.67d
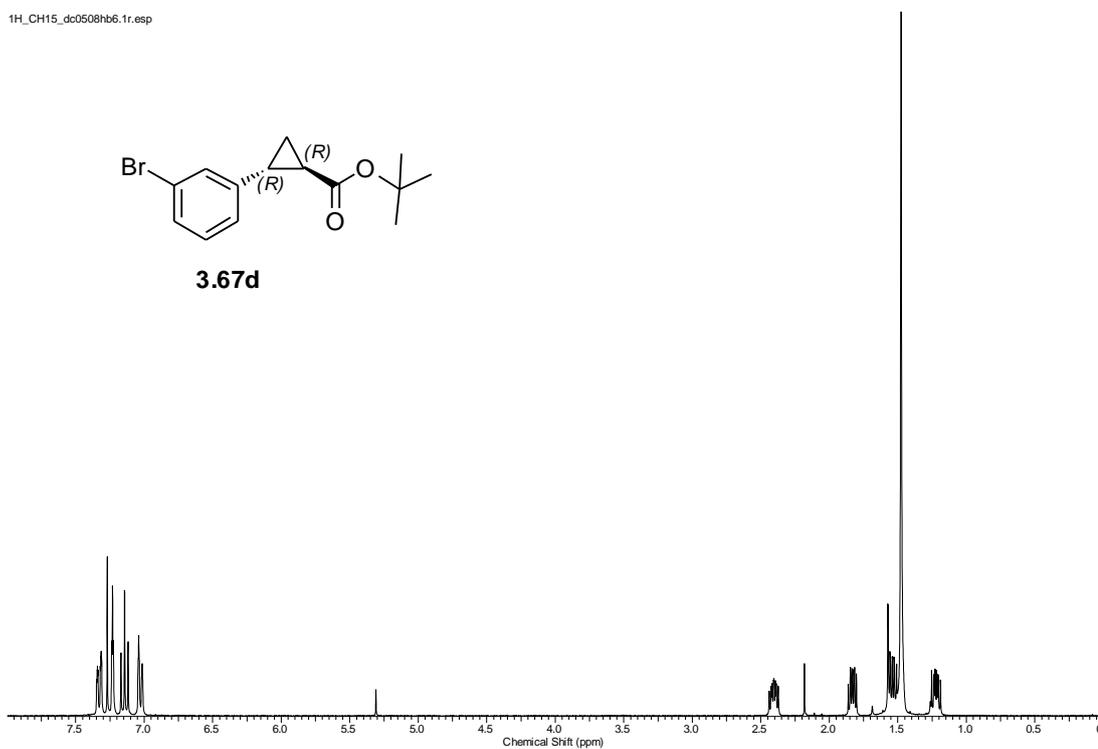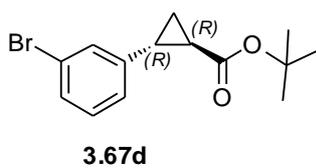
¹H and ¹³C NMR spectra of 3.67e
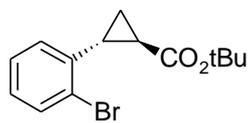
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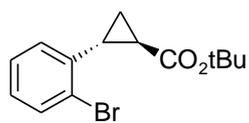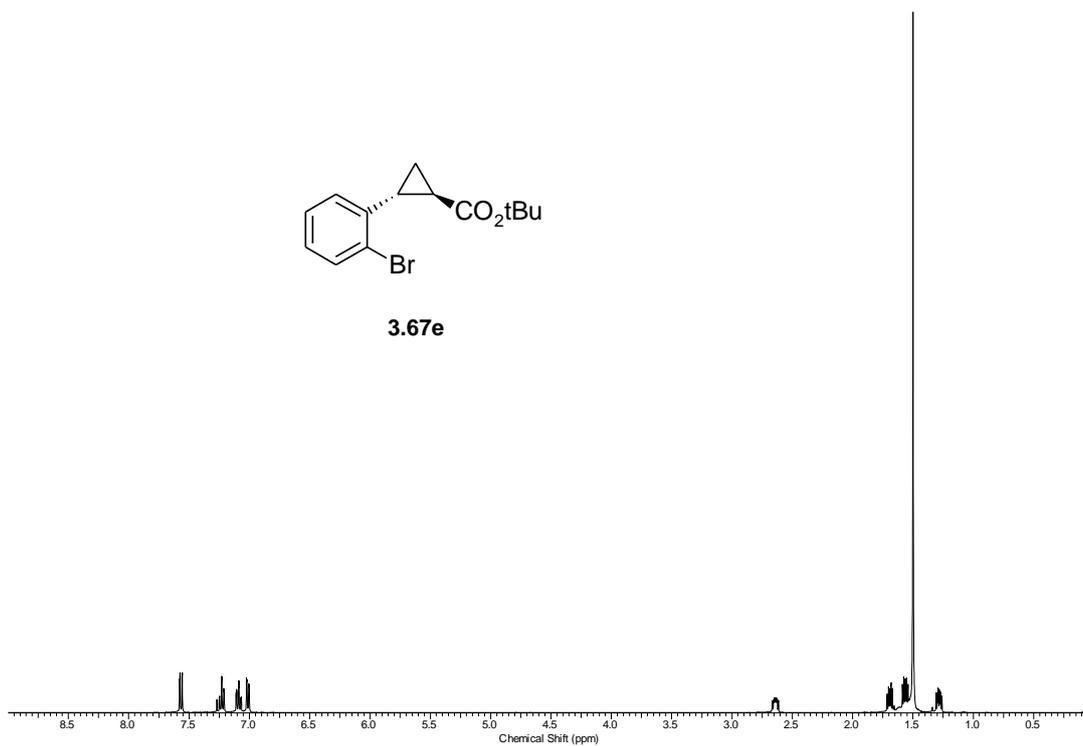
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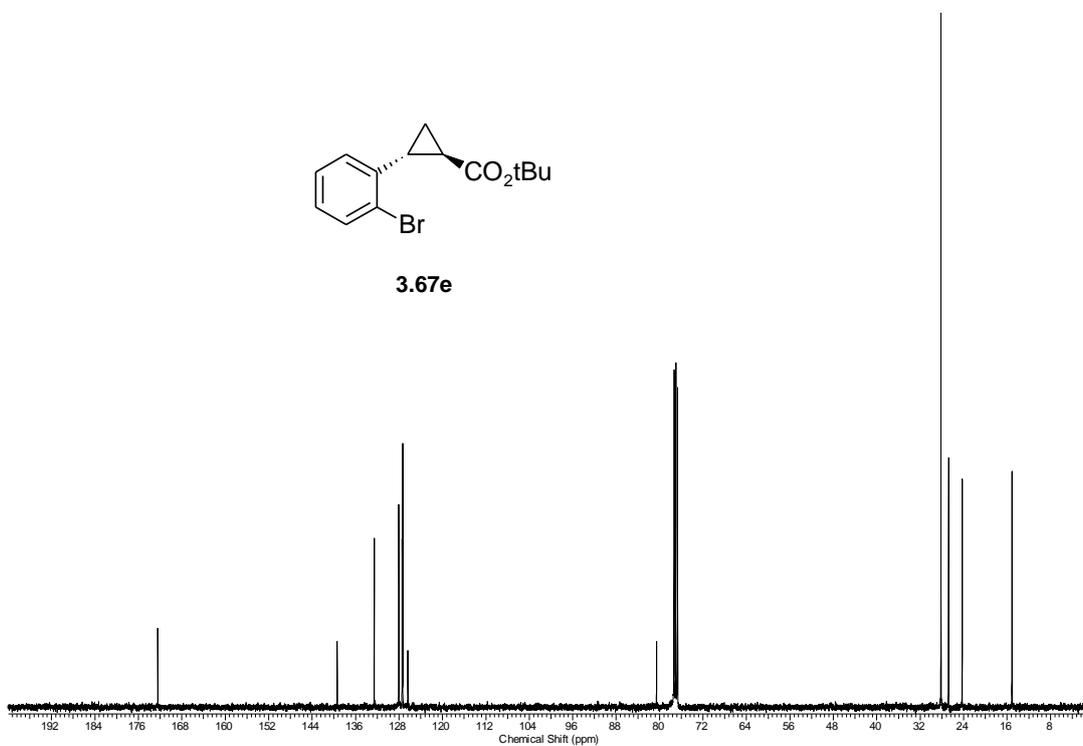
^{1}H NMR spectrum of 3.68a

oc2908hb2.010.esp

3.68a
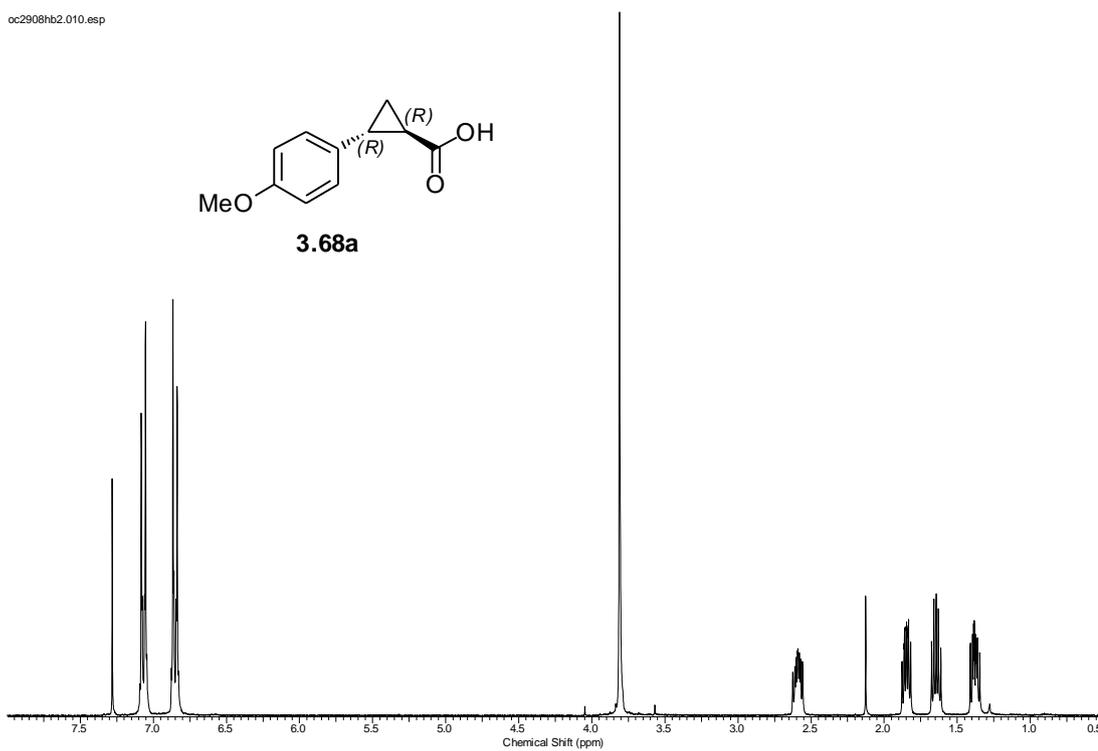
^{1}H NMR spectrum of 3.68b

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3.68b

^{1}H NMR spectrum of 3.68c

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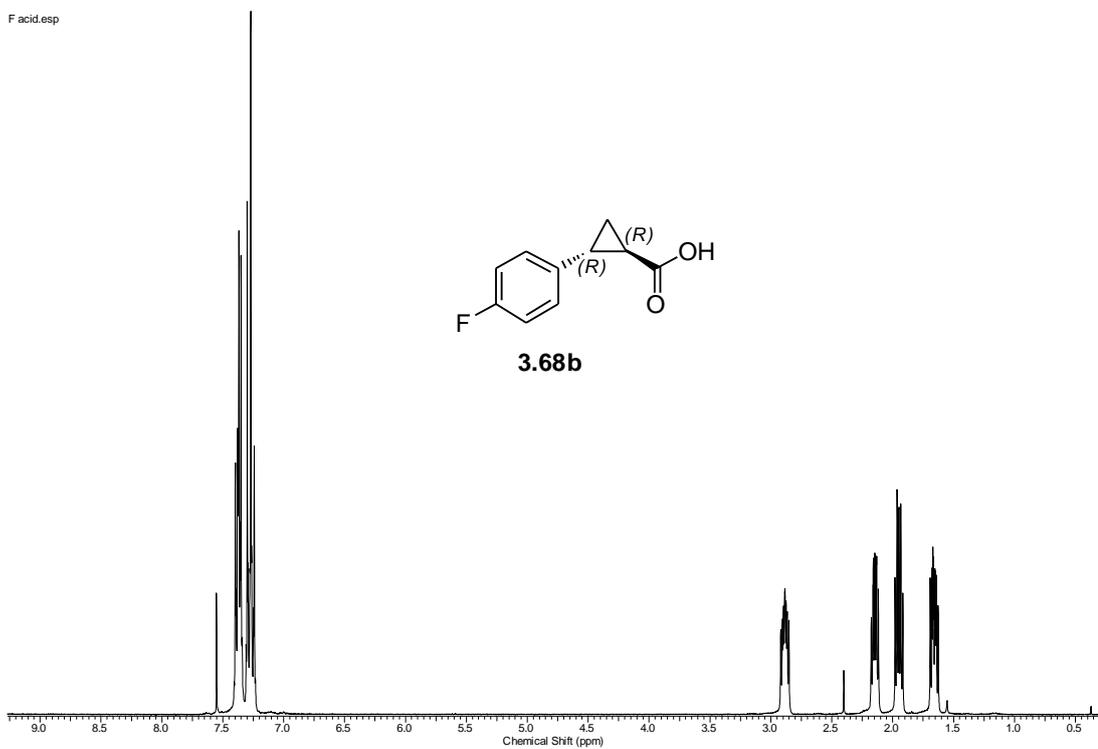
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^{1}H NMR spectrum of 3.68d

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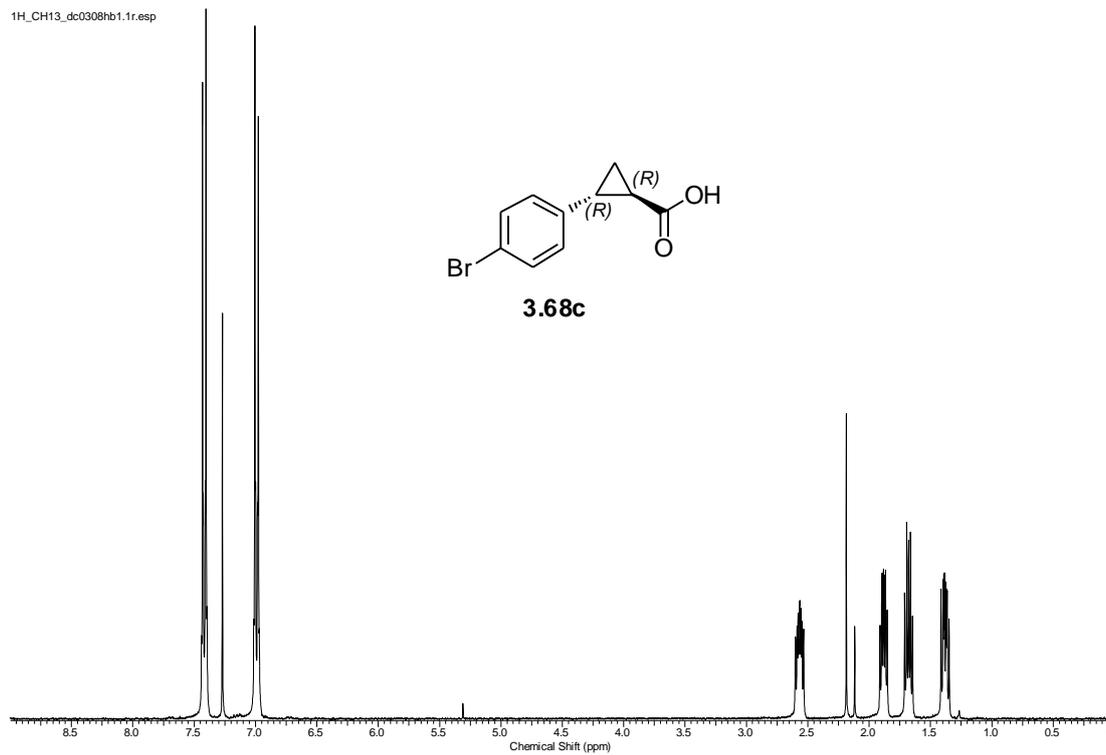
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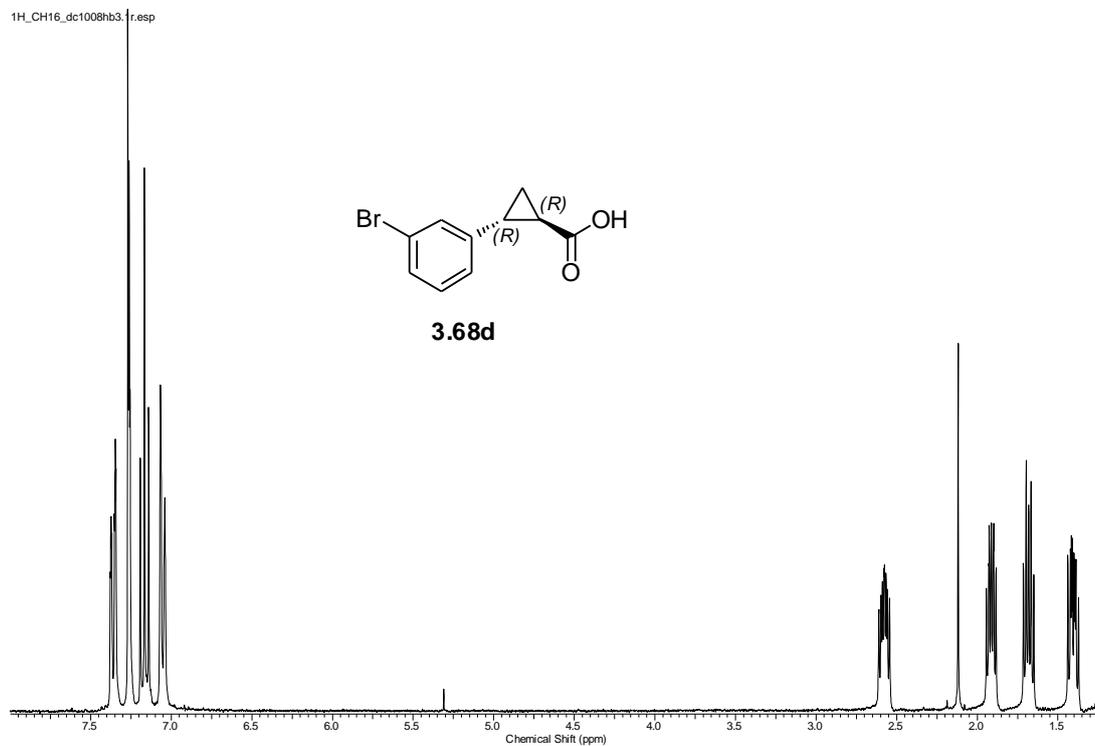
¹H NMR spectrum of 3.68e

CH27.esp

3.68e

¹H NMR spectrum of 3.69c

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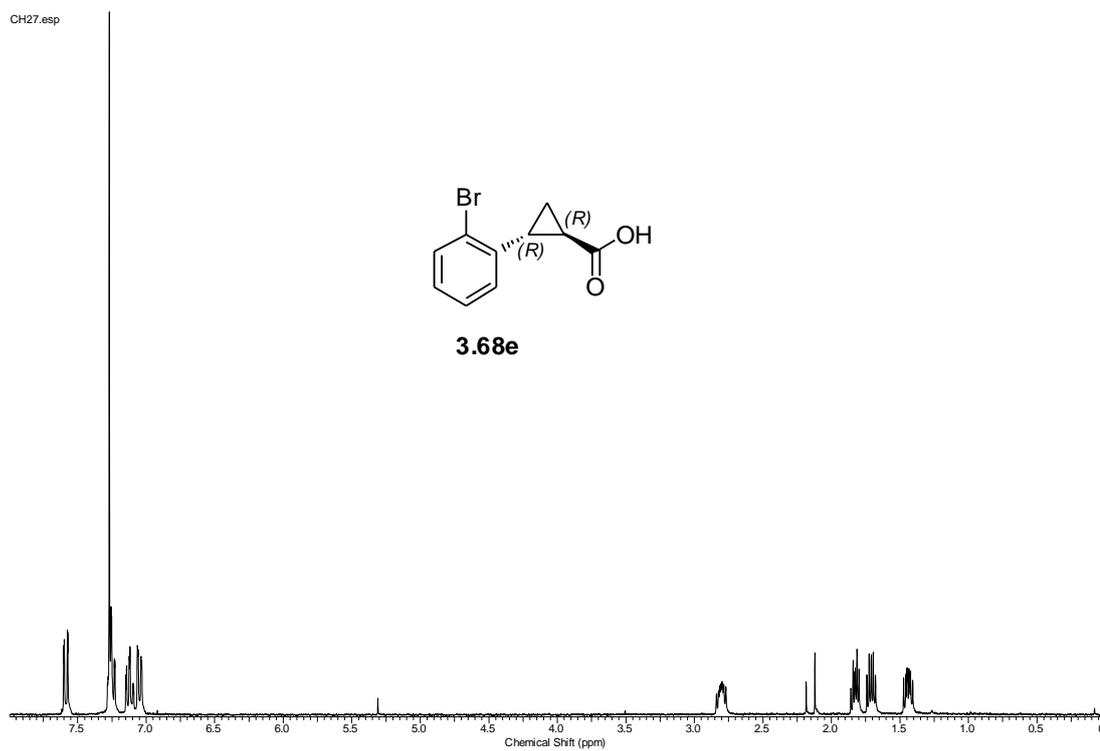
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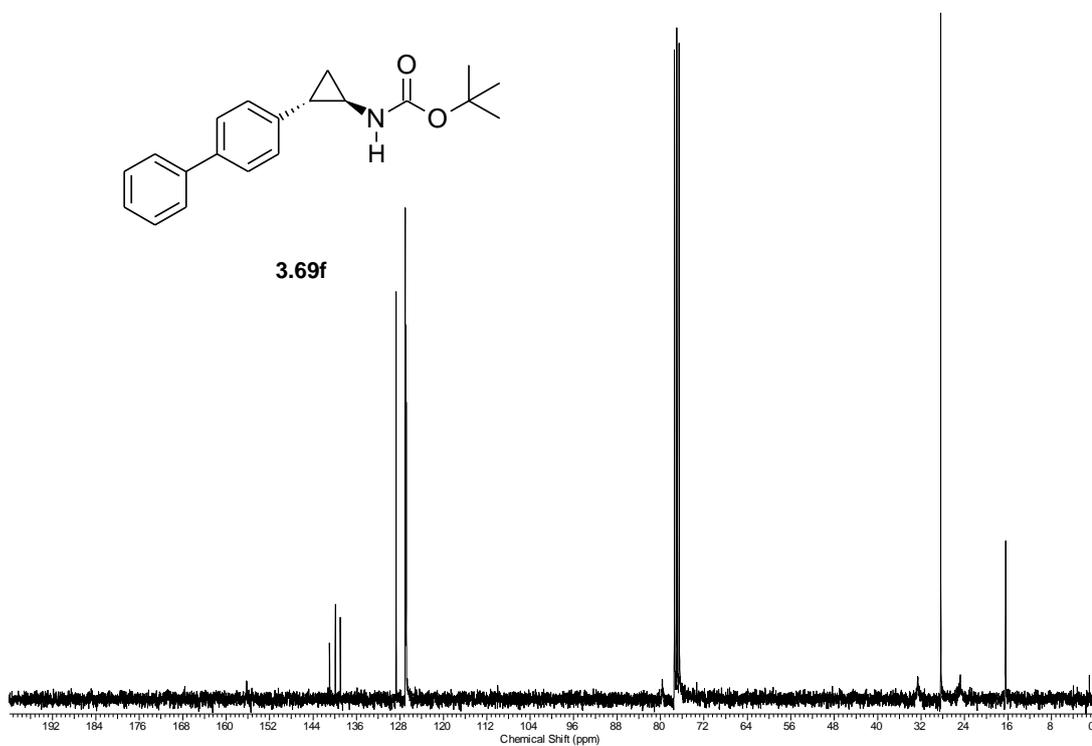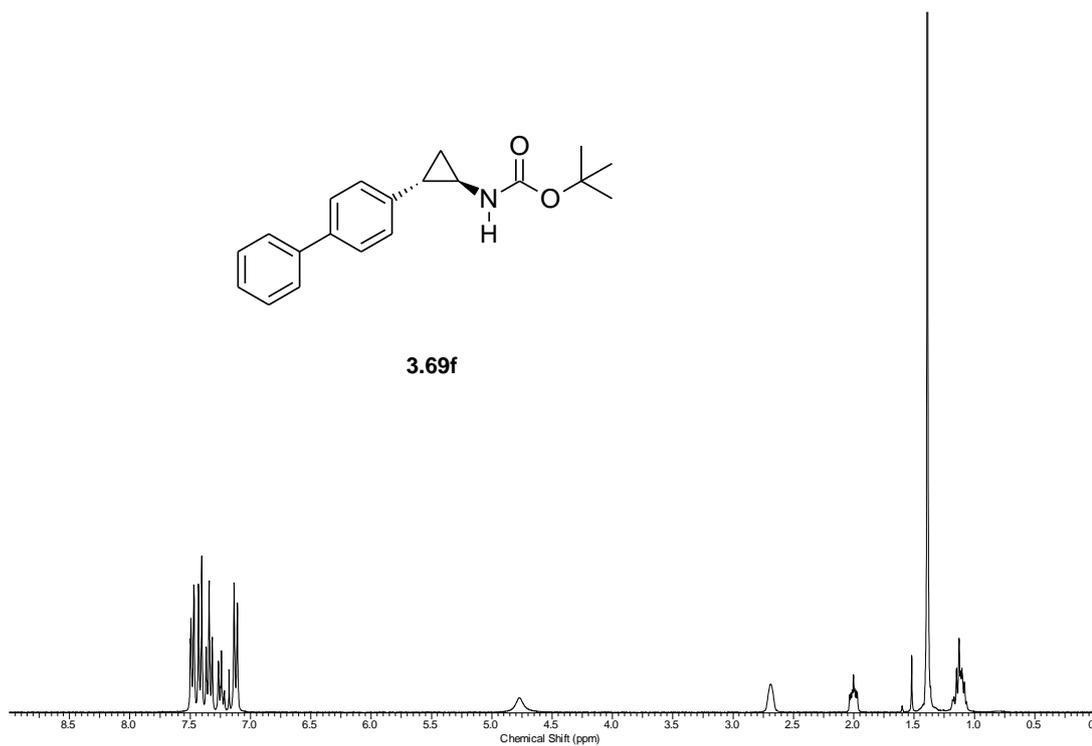# ^{1}H NMR spectrum of 3.69d

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3.69d
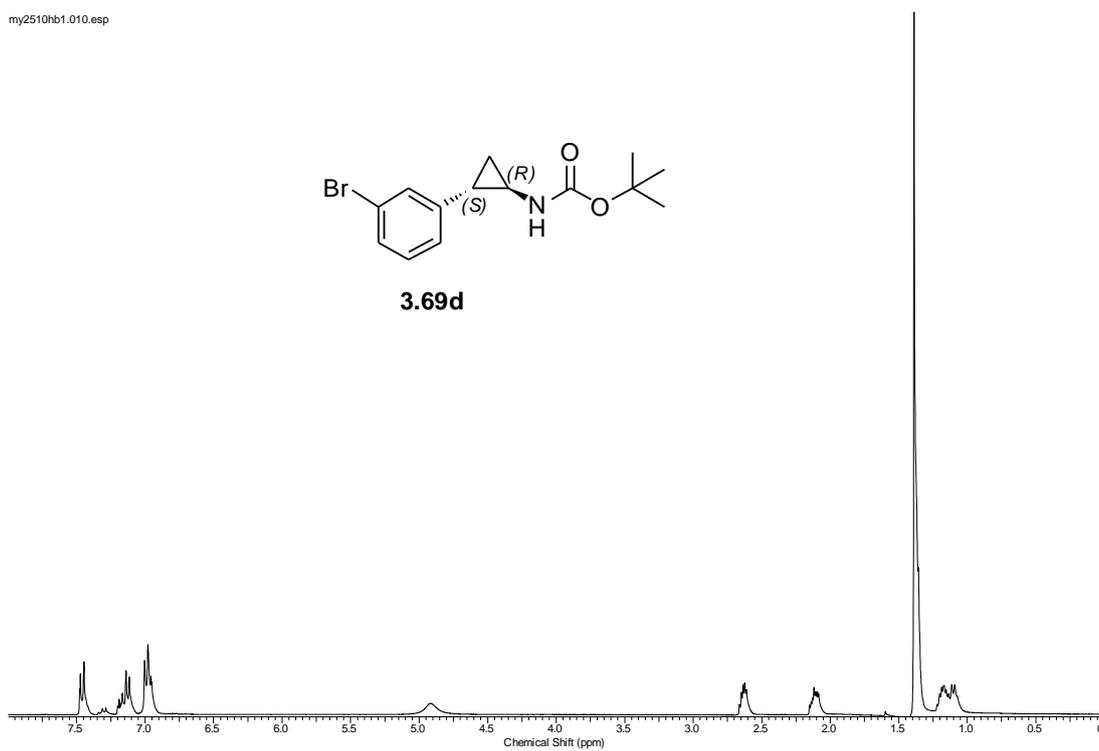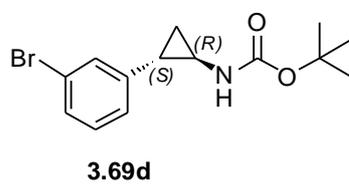
^{1}H NMR spectrum of 3.69e

1H_HB6_80.ESP

3.69e
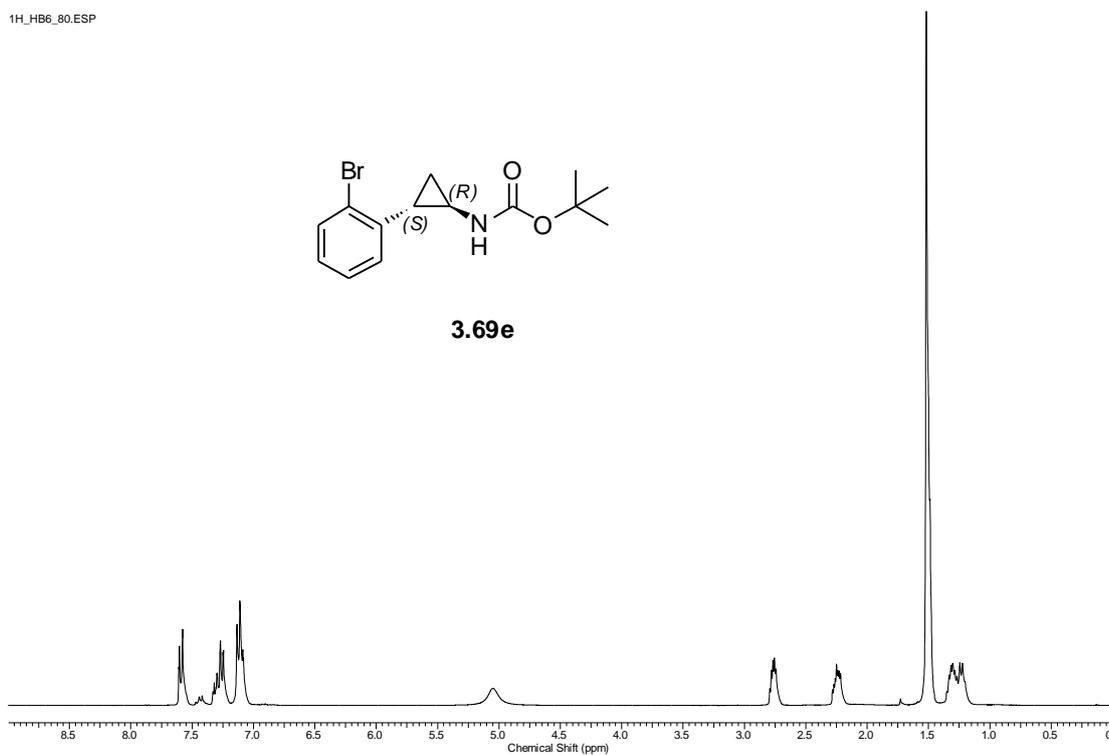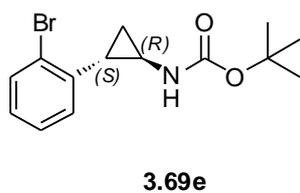
^{1}H and ^{13}C NMR spectra of 3.69f

3.69f

3.69f

¹H and ¹³C NMR spectra of 3.69g
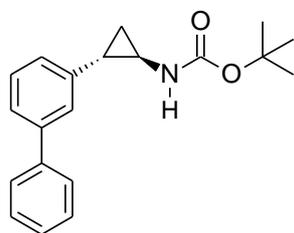
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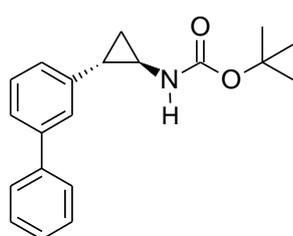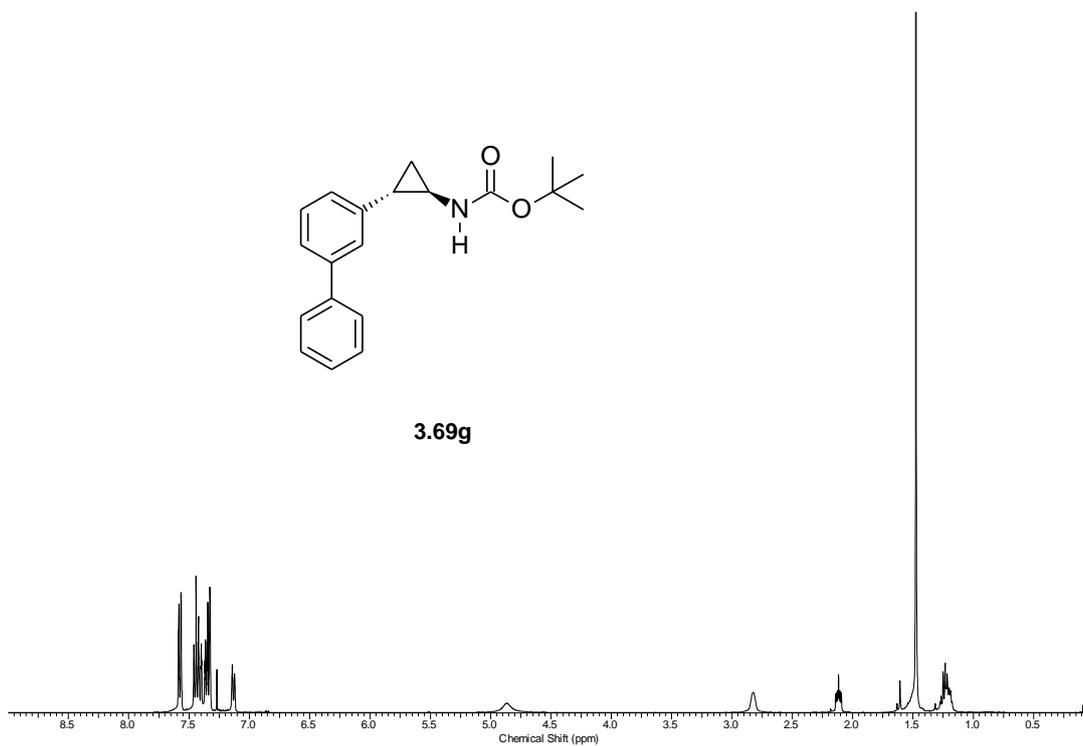
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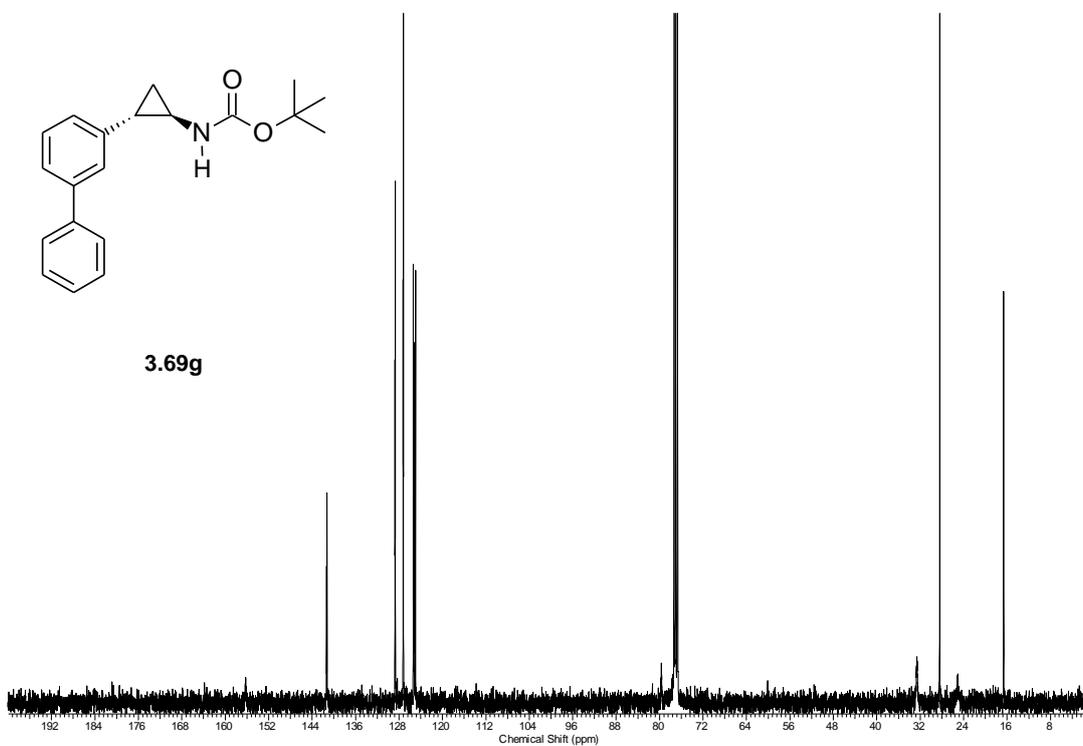
^{1}H and ^{13}C NMR spectra of 3.69h

3.69h

3.69h
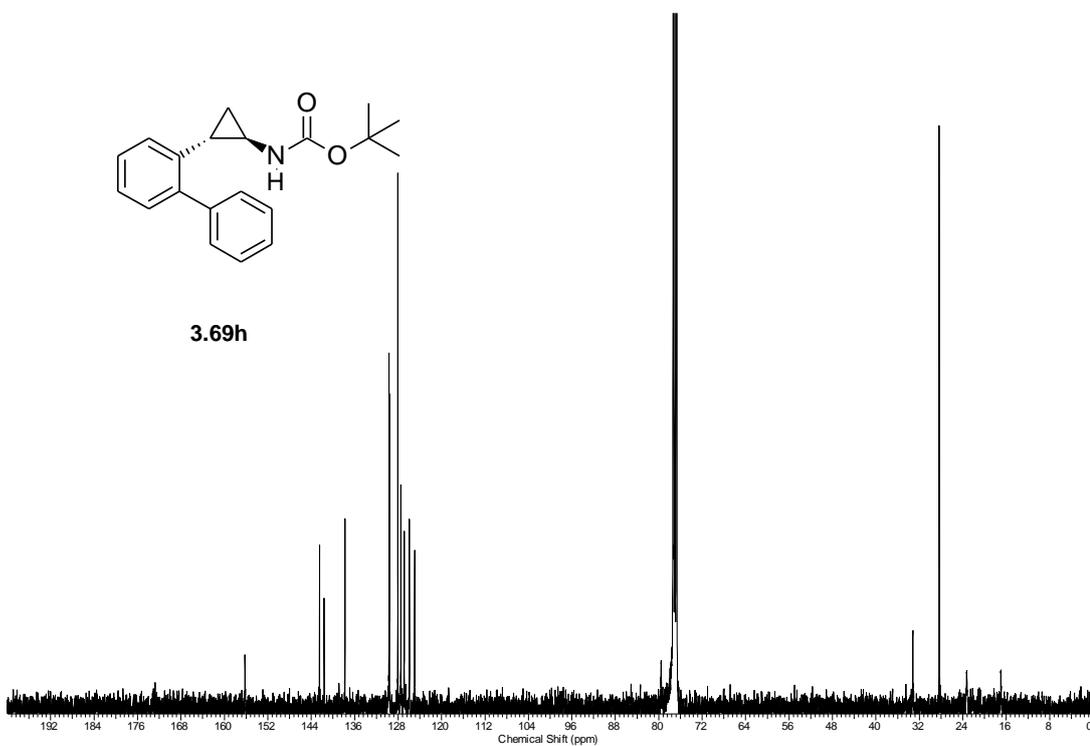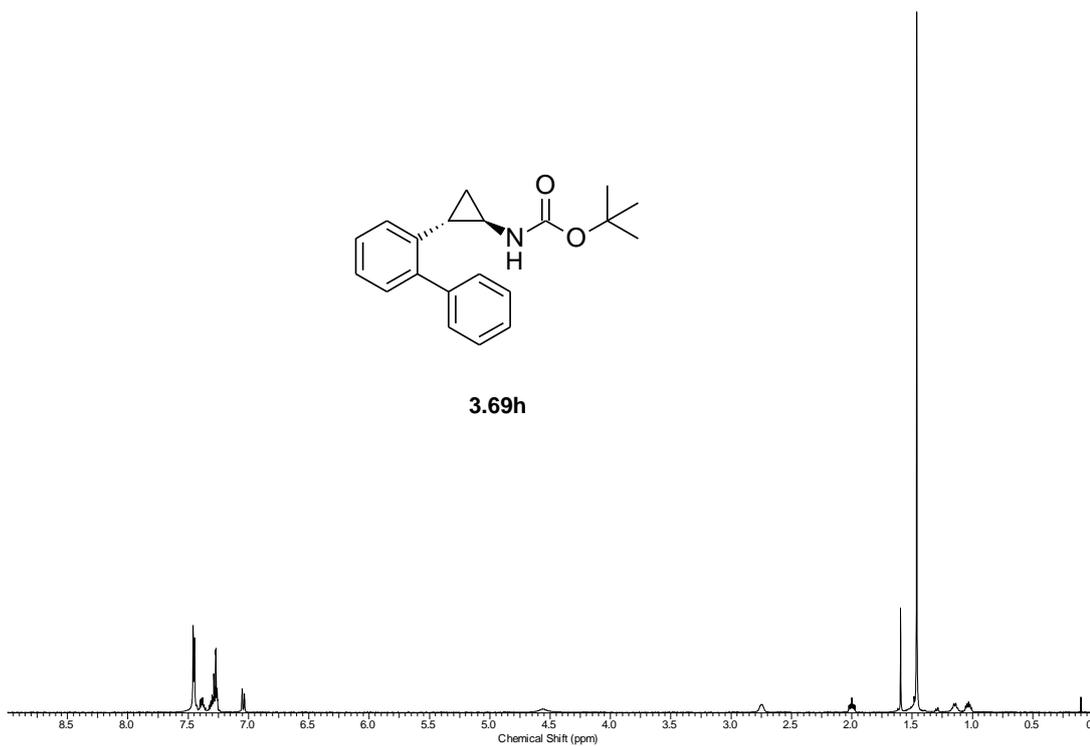
^{1}H and ^{13}C NMR spectra of 3.73

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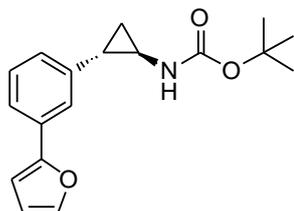
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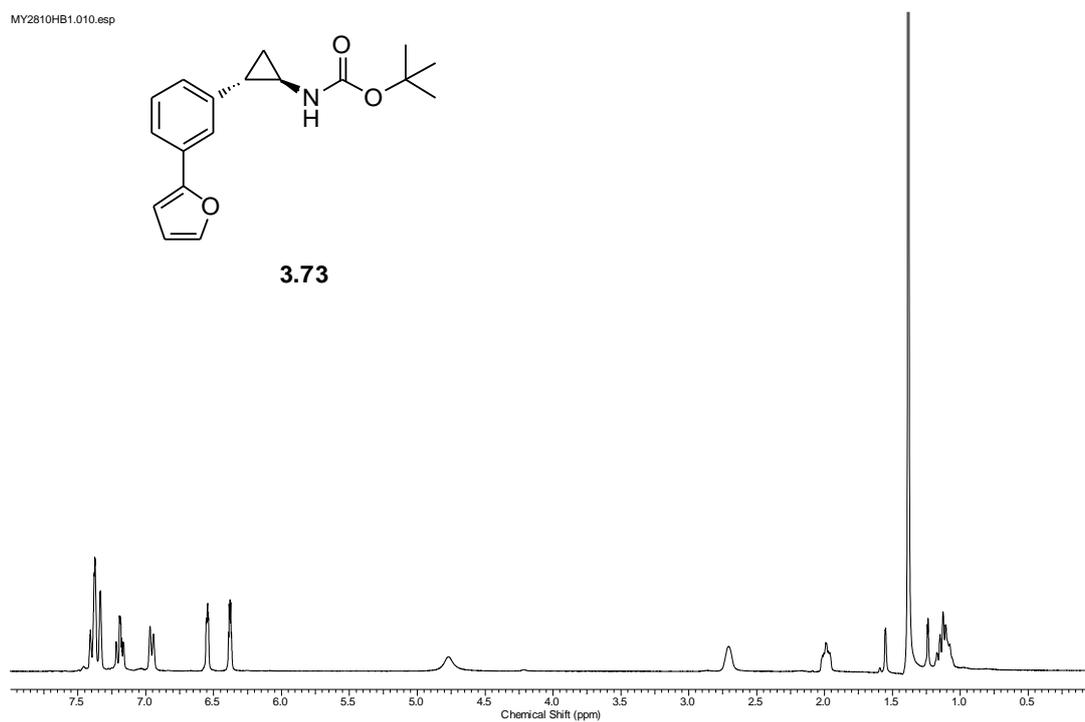
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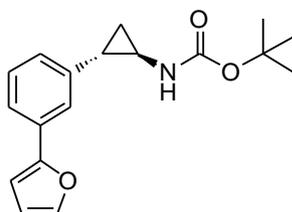
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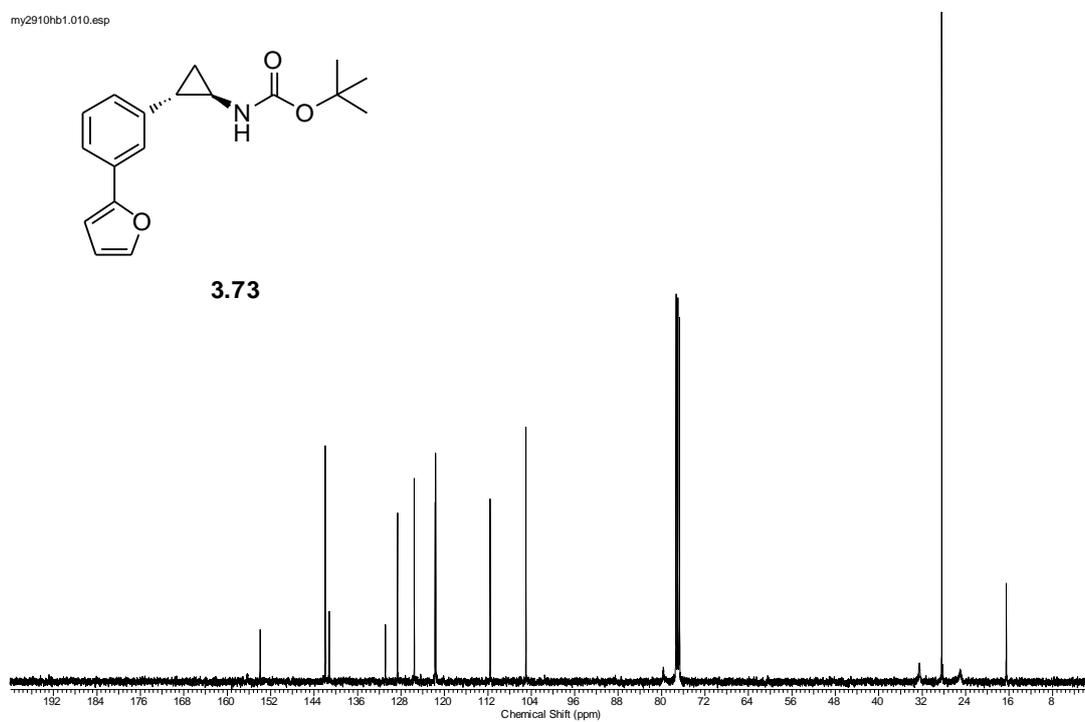
^{1}H and ^{13}C NMR spectra of 3.72

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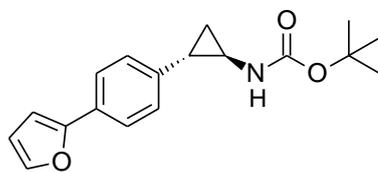
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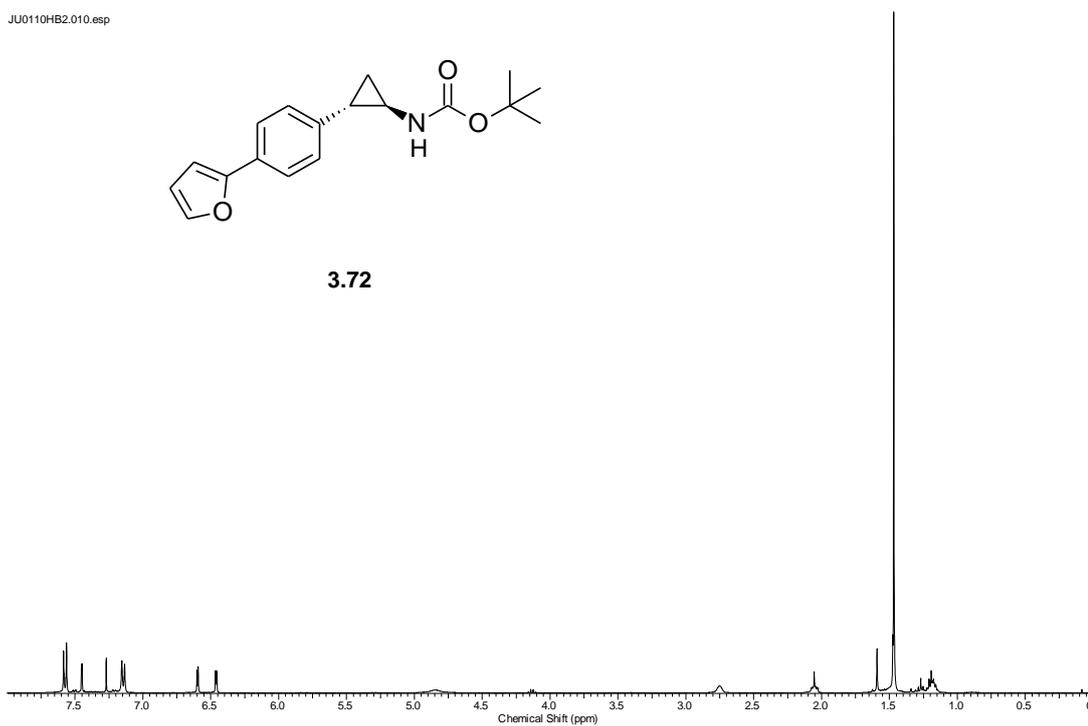
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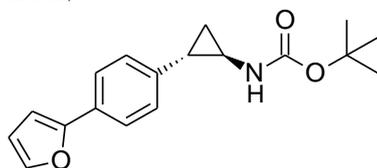
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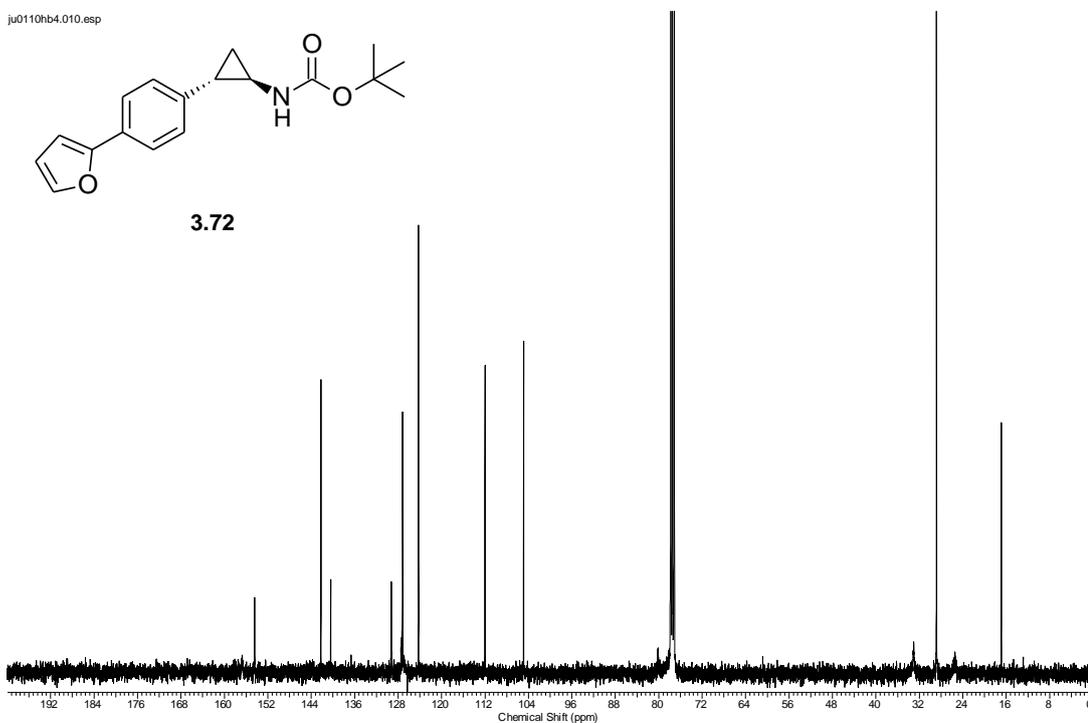
^{1}H and ^{13}C NMR spectra of 3.74

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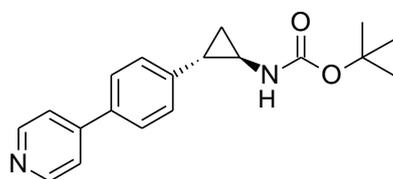
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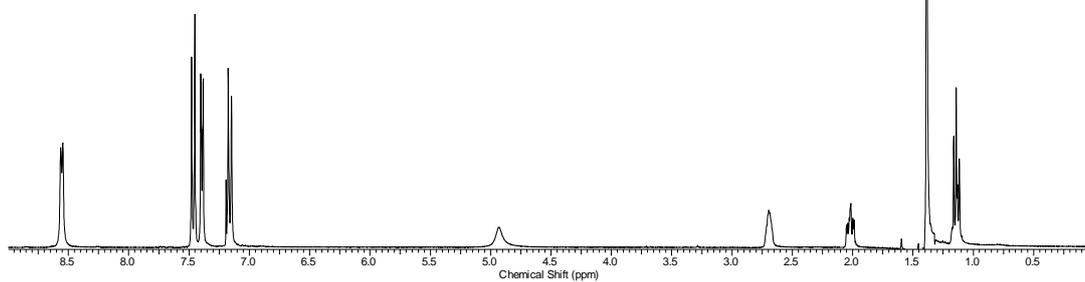
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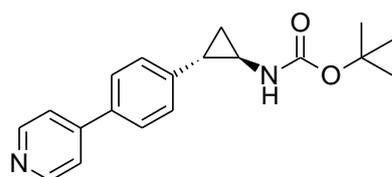
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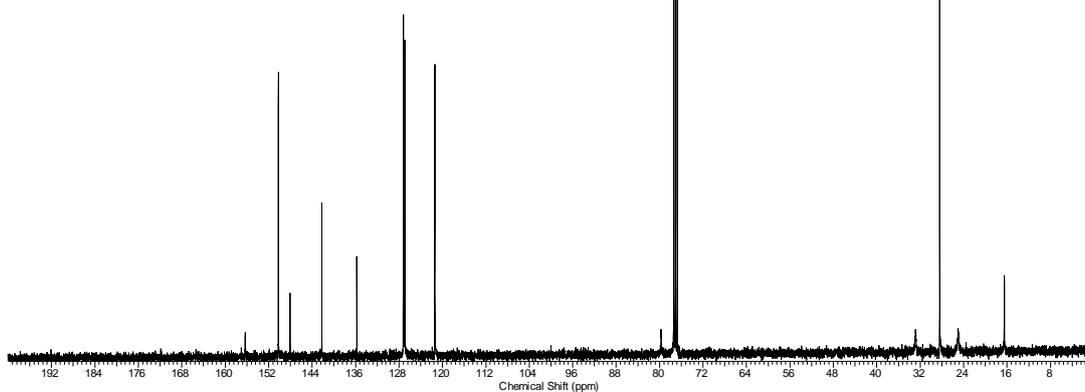
^{1}H NMR spectrum of 3.70a

nv2408hb1.010.esp

3.70a
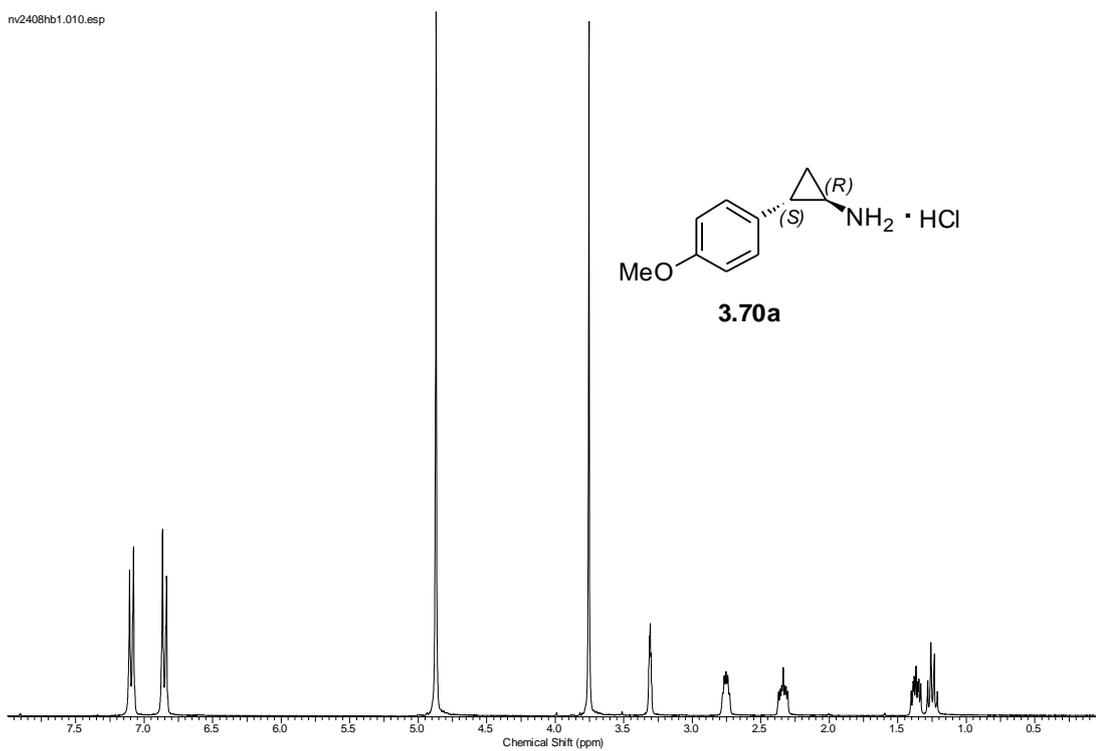
^{1}H NMR spectrum of 3.70b

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3.70b
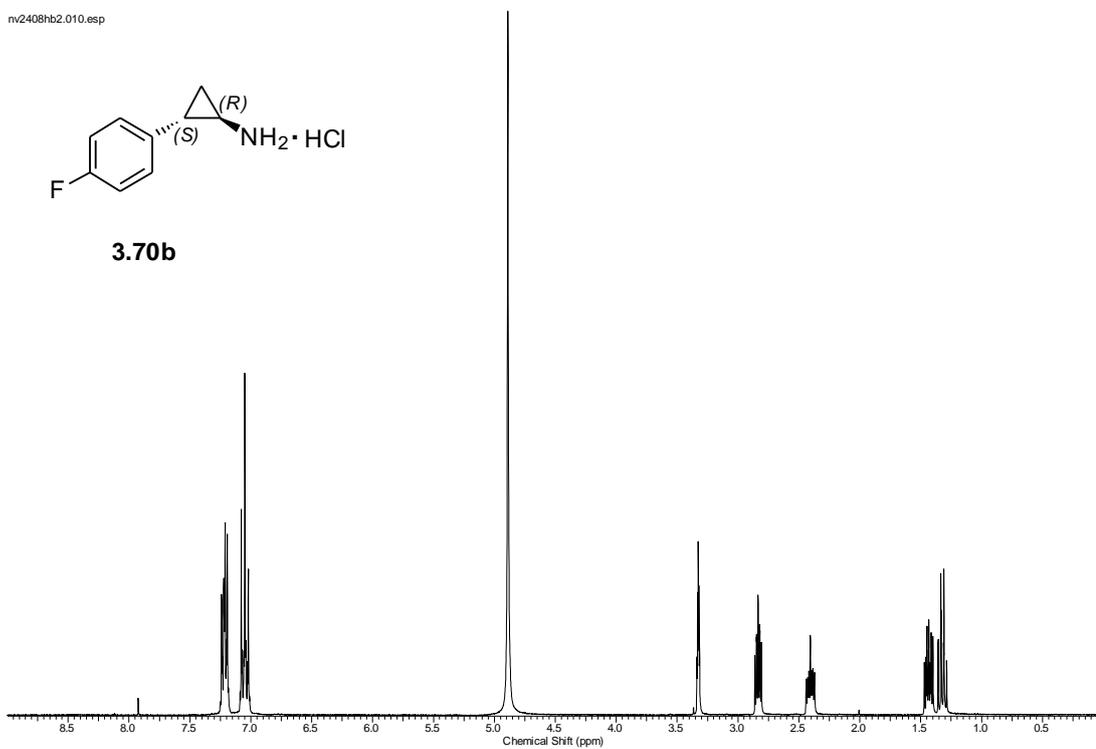
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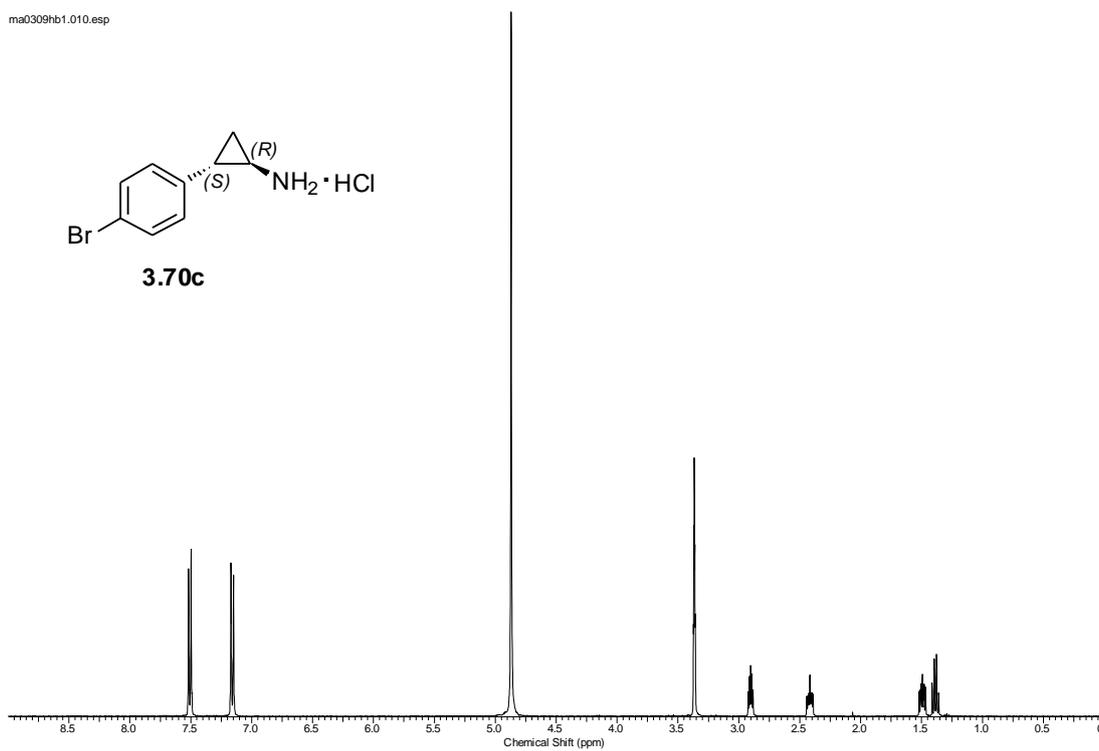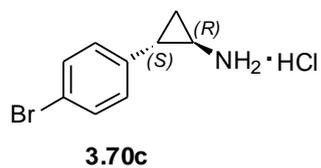
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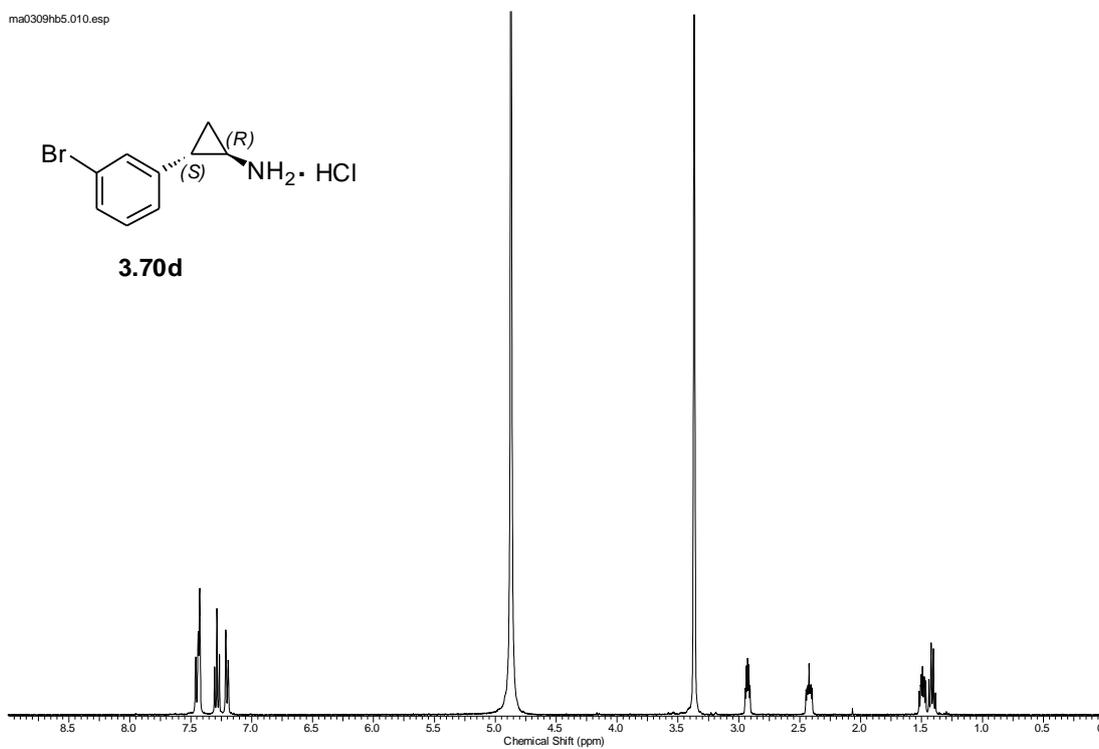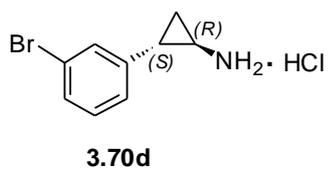
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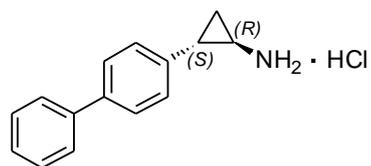
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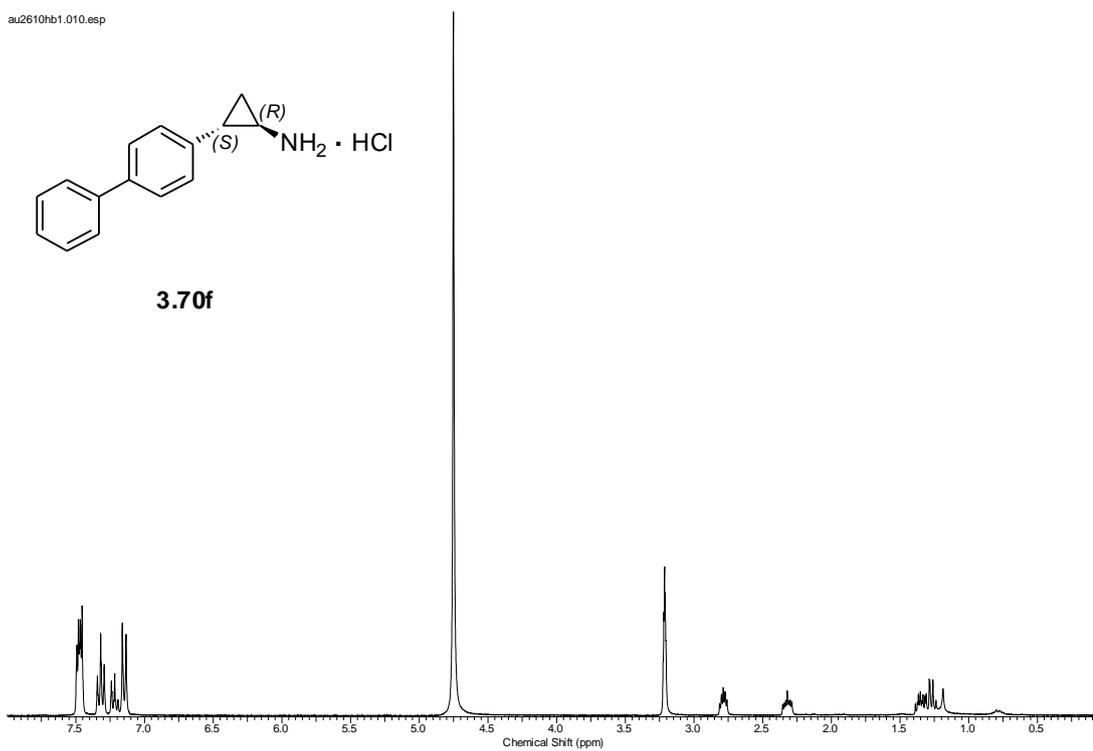
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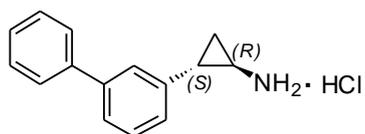
3.70g
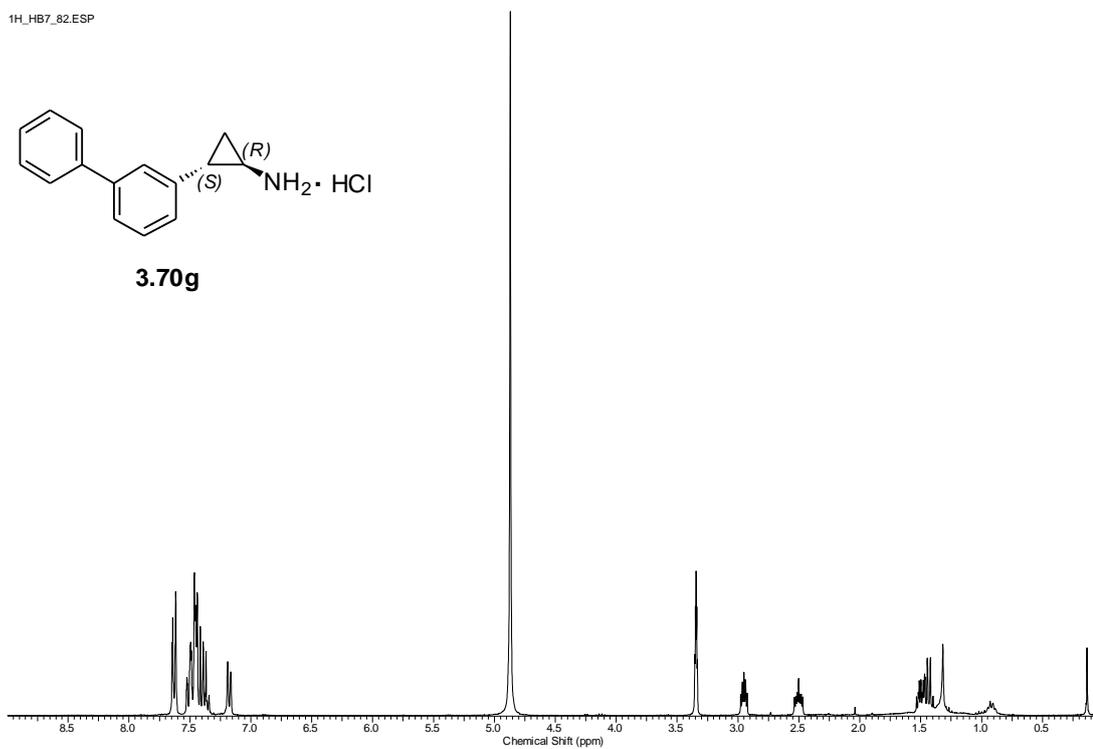
^{1}H and ^{13}C NMR spectra of 3.70h

3.70h

3.70h
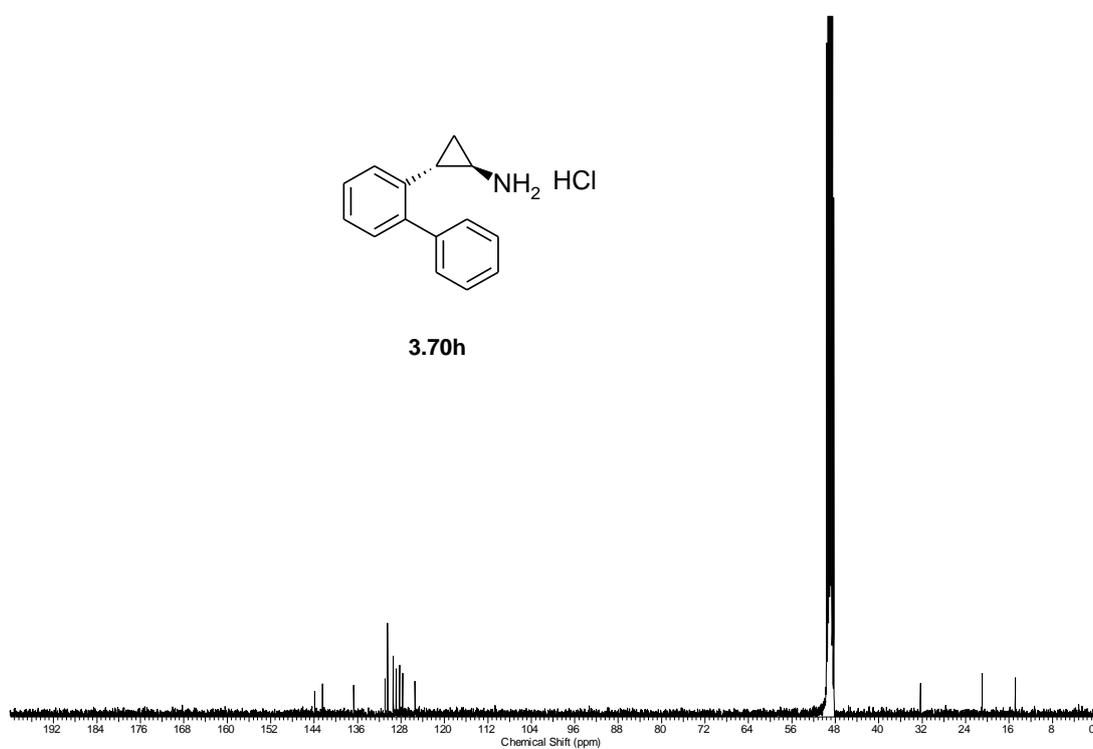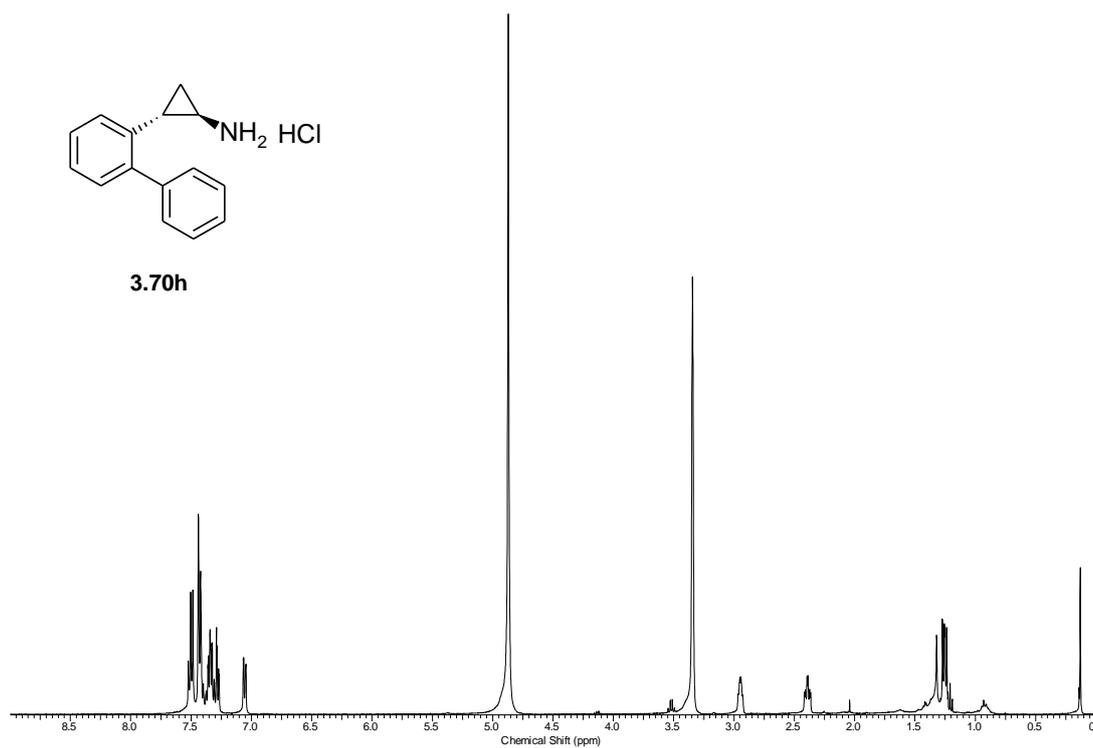
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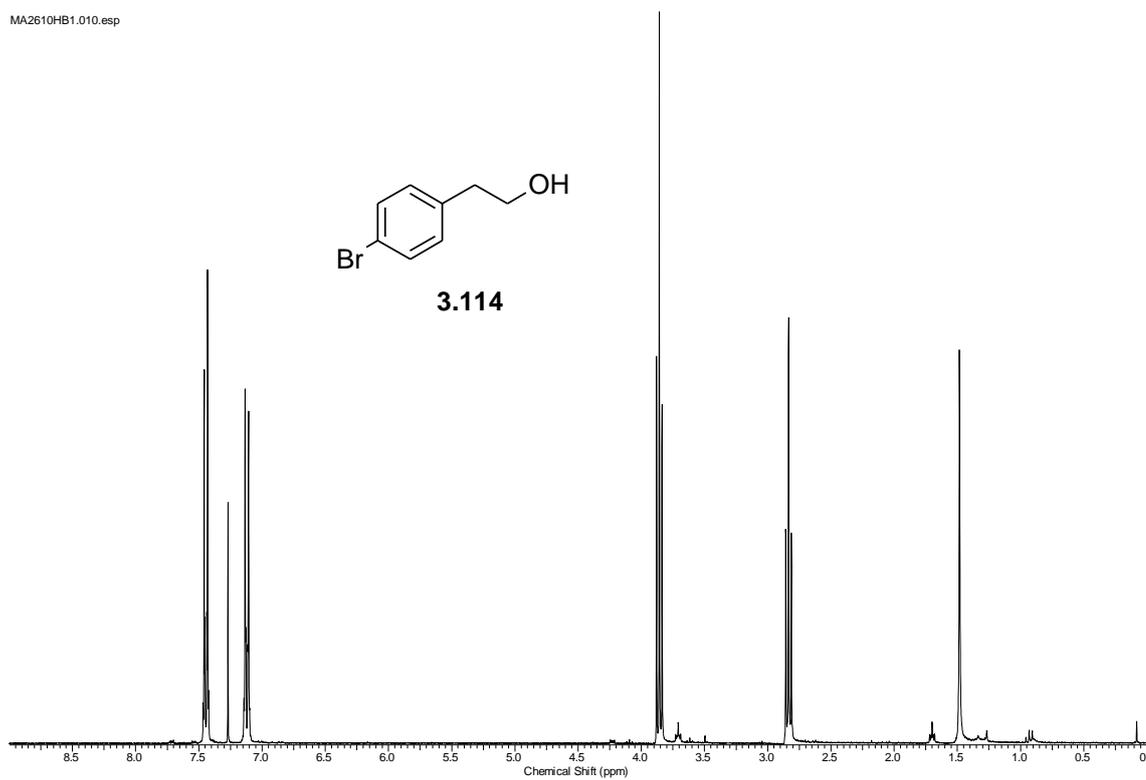
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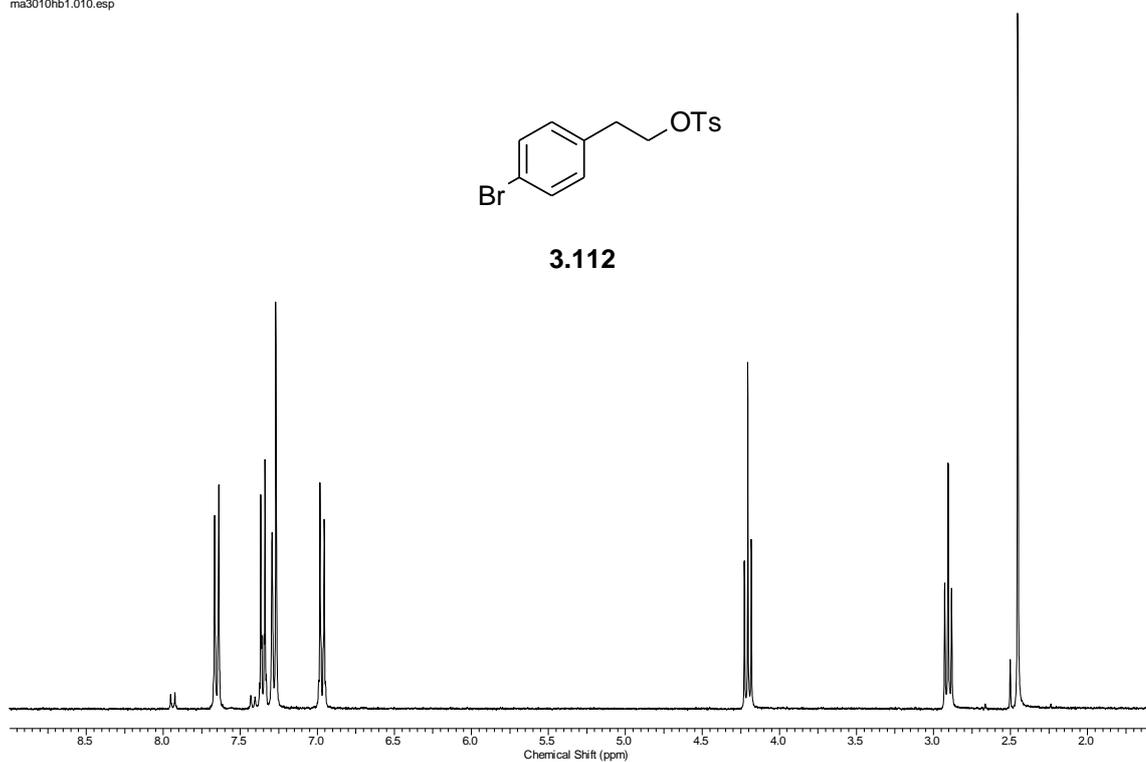
¹H NMR spectrum of 3.115

SS4.4.010.esp

3.115

Chemical Shift (ppm)
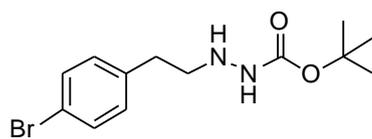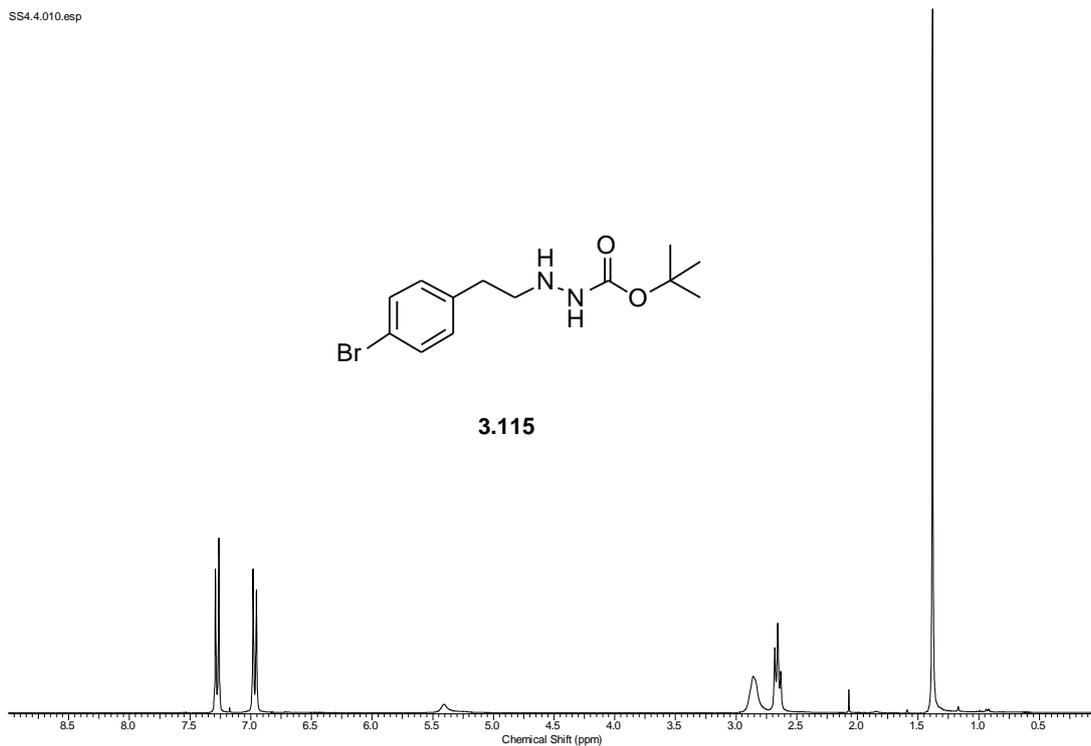
REFERENCES

(1)   Baker, D. D.; Chu, M.; Oza, U.; Rajgarhia, V. *Nat. Prod. Rep.* **2007**, *24*, 1225-1244.

(2)   Ortholand, J.-Y.; Ganesan, A. *Curr. Opin. Chem. Biol.* **2004**, *8*, 271-280.

(3)   Ganesan, A. *Curr. Opin. Chem. Biol.* **2008**, *12*, 306-317.

(4)   Goodman, J.; Walsh, V. *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-Cancer Drug*; Cambridge University Press., 2001.

(5)   Garcia-Carbonero, R.; Supko, J. G.; Maki, R. G.; Manola, J.; Ryan, D. P.; Harmon, D.; Puchalski, T. A.; Goss, G.; Seiden, M. V.; Waxman, A.; Quigley, M. T.; Lopez, T.; Sancho, M. A.; Jimeno, J.; Guzman, C.; Demetri, G. D. *J. Clin. Oncol.* **2005**, *23*, 5484-5492.

(6)   Laupacis, A.; Keown, P. A.; Ulan, R. A.; McKenzie, N.; Stiller, C. R. *Can Med Assoc J.* **1982**, *126*, 1041-1046.

(7)   Lipinski, C. A. *Drug Discovery Today: Technologies* **2004**, *1*, 337-341.

(8)   Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827–10852.

(9)   Cox, C.; Lectka, T. *Acc. Chem. Res.* **2000**, *33*, 849-858.

(10)   Wipf, P. *Chem. Rev. (Washington, DC, U.S.)* **1995**, *95*, 2115-2134.

(11)   Wedemeyer, W. J.; Welker, E.; Scheraga, H. A. *Biochemistry* **2002**, *41*, 14637-14644.

(12)   Vitagliano, L.; Rita, B.; Antonio, M.; Lelio, M.; Adriana, Z. *Protein Sci.* **2001**, *10*, 2627-2632. .

(13)   Siemion, I. Z.; Wieland, T.; Pook, K.-H. *Angew. Chem., Int. Ed.* **1975**, *14*, 702-703.

(14)   Napolitano, A.; Rodriquez, M.; Bruno, I.; Marzocco, S.; Autore, G.; Riccio, R.; Gomez-Paloma, L. *Tetrahedron* **2003**, *59*, 10203-10211.

(15)   Pettit, G. R.; Xu, J.-P.; Cichacz, Z. A.; Williams, M. D.; Chapuis, J.-C.; Cerny, R. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2677-2682.

(16)   Pettit, G. R.; Xu, J.-p.; Dorsaz, A.-C.; Williams, M. D.; Boyd, M. R.; Cerny, R. L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1339-1344.

(17)   Pettit, G. R.; Tan, R.; Williams, M. D.; Tackett, L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2869-2874.

(18)   Tabudravu, J. N.; Jaspars, M.; Morris, L. A.; Kettenes-van den Bosch, J. J.; Smith, N. *J. Org. Chem.* **2002**, *67*, 8593-8601.

(19)   Mechnich, O.; Kessler, H. *Lett. Pept. Sci.* **1997**, *4*, 21-28.

(20)   Pettit, G. R.; Rhodes, M. R.; Tan, R. *J. Nat. Prod.* **1999**, *62*, 409-414.

(21)   Pettit, G. R.; Toki, B. E.; Xu, J.-P.; Brune, D. C. *J. Nat. Prod.* **2000**, *63*, 22-28.

(22)   Napolitano, A.; Bruno, I.; Riccio, R.; Gomez-Paloma, L. *Tetrahedron* **2005**, *61*, 6808-6815.

(23)   Pettit, G. R.; Lippert, J. W.; Taylor, S. R.; Tan, R.; Williams, M. D. *J. Nat. Prod.* **2001**, *64*, 883-891.

(24)   Napolitano, A.; Bruno, I.; Rovero, P.; Lucas, R.; Peris, M. P.; Gomez-Paloma, L.; Riccio, R. *Tetrahedron* **2001**, *57*, 6249-6255.

(25)   Tabudravu, J. N.; Morris, L. A.; Kettenes-van, d. B. J. J.; Jaspars, M. *Tetrahedron* **2002**, *58*, 7863-7868.

(26)   Randazzo, A.; Dal, P. F.; Orru, S.; Debitus, C.; Roussakis, C.; Pucci, P.; Gomez-Paloma, L. *Eur. J. Org. Chem.* **1998**, *11*, 2659-2665.

(27)   Brennan, M. R.; Costello, C. E.; Maleknia, S. D.; Pettit, G. R.; Erickson, K. L. *J. Nat. Prod.* **2008**, *71*, 453-456.

(28)   Schmidt, G.; Grube, A.; Köch, M. *Eur. J. Org. Chem.* **2007**, *24*, 4103-4110.

(29)   Tan, L. T.; Williamson, R. T.; Gerwick, W. H.; Watts, K. S.; McGough, K.; Jacobs, R. *J. Org. Chem.* **2000**, *65*, 419-425.

(30)   Yokokawa, F.; Sameshima, H.; In, Y.; Minoura, K.; Ishida, T.; Shioiri, T. *Tetrahedron* **2002**, *58*, 8127-8143.

(31)   McKeever, B.; Pattenden, G. *Tetrahedron* **2003**, *59*, 2701-2712.

(32)   Dalisay, D. S.; Rogers, E. W.; Edison, A. S.; Molinski, T. F. *J. Nat. Prod* **2009**, *72*, 732-738.

(33)   Aihara, K.; Kano, Y.; Shiokawa, S.; Sasaki, T.; Setsu, F.; Sambongi, Y.; Ishii, M.; Tohyama, K.; Ida, T.; Tamura, A.; Atsumi, K.; Iwamatsu, K. *Bioorg. Med. Chem.* **2003**, *11*, 3475-3485.

(34)   Bredenkamp, M. W.; Holzapfel, C. W.; Snyman, R. M.; Wynand, J. Z. *Synth. Commun.* **1992**, *21*, 3029-3039.

(35)   Bredenkamp, M. W.; Holzapfel, C. W.; Wynand, J. Z. *Synth. Commun.* **1990**, *20*, 2235-2249.

(36)   Anthoni, U.; Christensen, D.; Christophersen, C.; Nielsen, P. H. *Acta Chem. Scand.* **1995**, *49*, 203-206.

(37)   Baeza, J. L.; Gerona-Navarro, G.; Perez de Vega, J.; Garcia-Lopez, M. T.; Gonzalez-Muniz, R.; Martin-Martinez, M. *J. Org. Chem.* **2008**, *73*, 1704-1715.

(38)   Hayashi, T.; Asai, T.; Ogoshi, H. *Tetrahedron Lett.* **1997**, *38*, 3039-3042.

(39)   Thomas, K. M.; Naduthambi, D.; Zondlo, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 2216-2217.

(40)   Umezawa, N.; Matsumoto, N.; Iwama, S.; Kato, N.; Higuchi, T. *Bioorg. Med. Chem.* **2010**, *18*, 6340-6350.

(41)   Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; S., S. B. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378-1382.

(42)   Wen, S.; Packham, G.; Ganesan, A. *J. Org. Chem.* **2008**, *73*, 9353-9361.

(43)   White, P. D.; Weng, C. C. *Fmoc solid phase peptide synthesis: a practical approach*; Oxford University Press: Oxford, 2000.

(44)   You, S.; Hossein, R.; Kelly, J. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 83-85.

(45)   Doi, T.; Yoshida, M.; Shin-ya, K.; Takahashi, T. *Org. Lett.* **2006**, *8*, 4165-4167.

(46)   Kedrowski, B. L.; Heathcock, C. H. *Heterocycles* **2002**, *58*, 601-634.

(47)   Green, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley-Interscience: New York, 2007.

(48)   Błaszczyk, A.; Elbinga, M.; Mayor, M. *Org. Biomol. Chem.* **2004**, *2*, 2722-2724.

(49)   Jonczyk, A.; Felding-Habermann, B.; Diefenbach, B.; Rippmann, F., (Merck Patent GmbH, Germany.), DE4336758A1, 1995.

(50)   Sakakura, A.; Kondo, R.; Umemura, S.; Ishihara, K. *Tetrahedron* **2009**, *65*, 2102-2109.

(51)   Li, G.; Warner, P. M.; Jebaratnam, D. J. *J. Org. Chem.* **1996**, *61*, 778-780.

(52)   Deber, H. M.; Torchiat, D. A.; Wong, S. C. K.; Blout, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1825-1829

(53)   Herald, D. L.; Cascarano, G. L.; Pettit, G. R.; Srirangam, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 6962-6973.

(54)   Tabudravu, J.; Morris, L. A.; Kettenes-van den Boschb, J. J.; Jaspars, M. *Tetrahedron Lett.* **2001**, *42*, 9273-9276.

(55) Kochanowska, A. J. Active Metabolites from Marine and Terrestrial Organisms, Dissertation, The University of Mississippi, 2009.

(56) Biel, M.; Wascholowski, V.; Giannis, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 3186-3216.

(57) Csuk, R.; Schabel, M. J.; von Scholz, Y. *Tetrahedron: Asymmetry* **1996**, *7*, 6505-3512.

(58) Handel, A. E.; Ebers, G. C.; Ramagopalan, S. V. *Trends Mol. Med.* **2010**, *16*, 7-16.

(59) Yang, X.-J.; Seto, E. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 206-218.

(60) Nakagawa, T.; Guarante, L. *J. Cell Sci.* **2011**, *124*, 833-838.

(61) Imai, S.-i.; Guarente, L. *Trends Pharmacol. Sci.* **2010**, *31*, 212-220.

(62) Rikiishi, H. *J. Biomed. Biotechnol.* **2011**, *2011*.

(63) Kim, H.; Bae, S. *Am. J. Transl. Res.* **2011**, *3*, 166-179.

(64) Ropero, S.; Esteller, M. *Molecular Oncology* **2007**, *1*, 19-25.

(65) Huang, Y.; Vasilatos, S. N.; Boric, L.; Shaw, P. G.; Davidson, N. E. *Breast Cancer Res. Treat.* **2011**, published online.

(66) Newkirk, T. L.; Bowers, A. A.; Williams, R. M. *Nat. Prod. Rep.* **2009**, *26*, 1293-1320.

(67) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174-17179.

(68) Duvic, M.; Talpur, R.; Ni, X.; Zhang, C. i.; Hazarika, P.; Kelly, C.; Chiao, J. H.; Reilly, J. F.; Ricker, J. L.; Richon, V. M.; Frankel, S. R. *Blood* **2007**, *109*, 31-39.

(69) Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature (London, U.K.)* **1999**, *401*, 188-193.

(70) Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097-5116.

(71) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Mosley, R. T.; Dombrowski, A. W.; Bills, G. F.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, M. A. *J. Org. Chem.* **2002**, *67*, 815-825.

(72) Nakao, Y.; Yoshida, S.; Matsunaga, S.; Shindoh, N.; Terada, Y.; Nagai, K.; Yamashita, J. K.; Ganesan, A.; van, S. R. W. M.; Fusetani, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 7553-7557.

(73) Wen, S.; Carey, K. L.; Nakao, Y.; Fusetani, N.; Packham, G.; Ganesan, A. *Org. Lett.* **2007**, *9*, 1105-1108.

(74) Shigematsu, N.; Ueda, H.; Takase, S.; Tanaka, H.; Yamamoto, K.; Tada, T. *J. Antibiot.* **1994**, *47*, 311-314.

(75) Ueda, H.; Nakajima, H.; Hori, Y.; Fujita, T.; Nishimura, M.; Goto, T.; Okuhara, M. *J. Antibiot.* **1994**, *47*, 301-310.

(76) Fujisawa Pharmaceutical Co. Ltd., J. K. T. K., JP, 03141296, 1991.

(77) Masuoka, Y.; Nagai, A.; Shin-ya, K.; Furihata, K.; Nagai, K.; Suzuki, K. i.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **2001**, *42*, 41-44.

(78) Yurek-George, A.; Habens, F.; Brimmell, M.; Packham, G.; Ganesan, A. *J. Am. Chem. Soc.* **2004**, *126*, 1030-1031.

(79) Takizawa, T.; Watanabe, K.; Narita, K.; Oguchi, T.; Abe, H.; Katoh, T. *Chem. Commun. (Cambridge, U. K.)* **2008**, 1677-1679.

(80) Taori, K.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 1806-1807.

(81) Cheng, Y.-Q.; Yang, M.; Matter, A. M. *Appl. Environ. Microbiol.* **2007**, *73*, 3460-3469.

(82) Narita, K.; Kikuchi, T.; Watanabe, K.; Takizawa, T.; Oguchi, T.; Kudo, K.; Matsuhara, K.; Abe, H.; Yamori, T.; Yoshida, M.; Katoh, T. *Chem. --Eur. J.* **2009**, *15*, 11174-11186.

(83) Cheng, Y.-Q., World patent 098199A2, 2008.

(84) Biggins, J. B.; Gleber, C. D.; Brady, S. F. *Org. Lett.* **2011**, *13*, 1536-1539.

(85) Wang, C.; Henkes, L. M.; Doughty, L. B.; He, M.; Wang, D.; Meyer-Almes, F.-J.; Cheng, Y.-Q. *J. Nat. Prod.* **2011**.

(86) Li, K. W.; Wu, J.; Xing, W.; Simon, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 7237-7238.

(87) Greshock, T. J.; Johns, D. M.; Noguchi, Y.; Williams, R. M. *Org. Lett.* **2008**, *10*, 613-616.

(88) Chen, Y.; Gambs, C.; Abe, Y.; Wentworth, P., Jr.; Janda, K. D. *J. Org. Chem.* **2003**, *68*, 8902-8905.

(89) Calandra, N. A.; Cheng, Y. L.; Kocak, K. A.; Miller, J. S. *Org. Lett.* **2009**, *11*, 1971-1974.

(90) Doi, T.; Iijima, Y.; Shin-Ya, K.; Ganesan, A.; Takahashi, T. *Tetrahedron Lett.* **2006**, *47*, 1177-1180.

(91) Iijima, Y.; Munakata, A.; Shin-ya, K.; Ganesan, A.; Doi, T.; Takahashi, T. *Tetrahedron Lett.* **2009**, *50*, 2970-2972.

(92) Fuse, S.; Okada, K.; Iijima, Y.; Munakata, A.; Machida, K.; Takahashi, T.; Takagi, M.; Shin-ya, K.; Doi, T. *Org. Biomol. Chem.* **2011**, *9*, 3825-3833.

(93) Guz, N. R.; Phillips, A. J. *Org. Lett.* **2002**, *4*, 2253-2256.

(94) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E. *Tetrahedron Lett.* **1987**, *28*, 39-42.

(95) Xiao, Q.; Wang, L.-P.; Jiao, X.-Z.; Liu, X.-Y.; Wu, Q.; Xie, P. *J. Asian Nat. Prod. Res.* **2010**, *12*, 940-949.

(96) Bowers, A.; West, N.; Taunton, J.; Schreiber, S. L.; Bradner, J. E.; Williams, R. M. *J. Am. Chem. Soc.* **2008**, *130*, 11219-11222.

(97) Ren, Q.; Dai, L.; Zhang, H.; Tan, W.; Xu, Z.; Ye, T. *Synlett* **2008**, 2379-2383.

(98) Ying, Y.; Liu, Y.; Byeon, S. R.; Kim, H.; Luesch, H.; Hong, J. *Org. Lett.* **2008**, *10*, 4021-4024.

(99) Matsumura, K.; Hashiguchi, S.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1997**, *119*, 8738-8739.

(100) Ghosh, A. K.; Kulkarni, S. *Org. Lett.* **2008**, *10*, 3907-3909.

(101) Nasveschuk, C. G.; Ungermannova, D.; Liu, X.; Phillips, A. J. *Org. Lett.* **2008**, *10*, 3595-3598.

(102) Seiser, T.; Kamena, F.; Cramer, N. *Angew. Chem., Int. Ed.* **2008**, *47*, 6483-6485.

(103) Souto, J. A.; Vaz, E.; Lepore, I.; Poppler, A.-C.; Franci, G.; Alvarez, R.; Altucci, L.; de Lera, A. R. *J. Med. Chem.* **2010**, *53*, 4654-4667.

(104) Wang, B.; Forsyth, C. J. *Synthesis* **2009**, 2873-2880.

(105) Ying, Y.; Taori, K.; Kim, H.; Hong, J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 8455-8459.

(106) Liang, B.; Richard, D. J.; Portonovo, P. S.; Joullie, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 4469-4474.

(107) Luo, J.-M.; Dai, C.-F.; Lin, S.-Y.; Huang, P.-Q. *Chem. --Asian J.* **2009**, *4*, 328-335.

(108) Konno, H.; Kubo, K.; Makabe, H.; Toshiro, E.; Hinoda, N.; Nosaka, K.; Akaji, K. *Tetrahedron* **2007**, *63*, 9502-9513.

(109) DeRoy, P. L.; Charette, A. B. *Org. Lett.* **2003**, *5*, 4163-4165.

(110) Numajiri, Y.; Takahashi, T.; Takagi, M.; Shin-ya, K.; Doi, T. *Synlett* **2008**, 2483-2486.

(111) Zeng, X.; Yin, B.; Hu, Z.; Liao, C.; Liu, J.; Li, S.; Li, Z.; Nicklaus, M. C.; Zhou, G.; Jiang, S. *Org. Lett.* **2010**, *12*, 1368-1371.

(112) Yurek-George, A.; Cecil, A. R. L.; Mo, A. H. K.; Wen, S.; Rogers, H.; Habens, F.; Maeda, S.; Yoshida, M.; Packham, G.; Ganesan, A. *J. Med. Chem.* **2007**, *50*, 5720-5726.

(113) Bowers, A. A.; West, N.; Newkirk, T. L.; Troutman-Youngman, A. E.; Schreiber, S. L.; Wiest, O.; Bradner, J. E.; Williams, R. M. *Org. Lett.* **2009**, *11*, 1301-1304.

(114) Bowers, A. A.; Greshock, T. J.; West, N.; Estiu, G.; Schreiber, S. L.; Wiest, O.; Williams, R. M.; Bradner, J. E. *J. Am. Chem. Soc.* **2009**, *131*, 2900-2905.

(115) McKennon, M. J.; Meyers, A. I.; Drauz, K.; Schwarm, M. *J. Org. Chem.* **1993**, *58*, 3568-3571.

(116) Delaunay, D.; Toupet, L.; Le Corre, M. *J. Org. Chem.* **1995**, *60*, 6604-6607.

(117) Nagao, Y.; Dai, W.; Ochiai, M.; Tsukagoshi, S.; Fujita, E. *J. Org. Chem.* **1990**, *55*, 1148-1156.

(118) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Estrada, A. A.; Lee, S. H.; Nevalainen, M. *J. Am. Chem. Soc.* **2005**, *127*, 11176-11183.

(119) Hollosi, M.; Majer, Z.; Zewdu, M.; Ruff, F.; Kajtar, M.; Kover, K. E. *Tetrahedron* **1988**, *44*, 195-202.

(120) Hiskey, R. G.; Adams, J. B. *J. Org. Chem.* **1965**, *30*, 1340.

(121) Rudolph, J.; Theis, H.; Hanke, R.; Endermann, R.; Johannsen, L.; Geschke, F. *J. Med. Chem.* **2001**, *44*, 619-626.

(122) Bergeron, R. J.; Wiegand, J.; McManis, J. S.; Weimar, W. R.; Park, J.-H.; Eiler-McManis, E.; Bergeron, J.; Brittenham, G. M. *J. Med. Chem.* **2005**, *48*, 821-831.

(123) Houssin, R.; Lohez, M.; Bernier, J.; Henichart, J. *J. Org. Chem.* **1985**, *50*, 2787-2788.

(124) Calmes, M.; Escale, F.; Paolini, F. *Tetrahedron: Asymmetry* **1997**, *8*, 3691-3697.

(125) Pattenden, G.; Thorn, S. M.; Jones, M. F. *Tetrahedron* **1993**, *49*, 2131-2138.

(126) Benelkebir, H.; Marie, S.; Hayden, A. L.; Lyle, J.; Loadman, P. M.; Crabb, S. J.; Packham, G.; Ganesan, A. *Bioorg. Med. Chem.* **2011**, *19*, 3650-3658.

(127) Enck, S.; Kopp, F.; Marahiel, M. A.; Geyer, A. *Org. Biomol. Chem.* **2010**, *8*, 559-563.

(128) Chatterjee, S., US5,852,007, 1997.

(129) Chen, J. J.; Aduda, V. *Synth. Commun.* **2007**, *37*, 3493-3499.

(130) Vugts, D. J.; Veum, L.; al-Mafraji, K.; Lemmens, R.; Schmitz, R. F.; de Kanter, F. J. J.; Groen, M. B.; Hanefeld, U.; Orru, R. V. A. *Eur. J. Org. Chem.* **2006**, *7*, 1672-1677.

(131) Silverman, R. B.; Haitao, J. I.; Lawton, G. R., US021119, 2007.

(132) Mukherjee, S.; Lee, D. *Org. Lett.* **2009**, *11*, 2916-2919.

(133) Tsunoda, T.; Suzuki, M.; Noyori, R. *Tetrahedron Lett.* **1980**, *21*, 1357-1358.

(134) Nakamura, M.; Miyashita, H.; Yamaguchi, M.; Shirasaki, Y.; Nakamura, Y.; Inoue, J. *Bioorg. Med. Chem.* **2003**, *11*, 5449-5460.

(135) Ganesan, A.; Packham, G. K.; Cecil, A. R. L., World patent 062201, 2008.

(136) Benelkebir, H.; Donlevy, A. M.; Packham, G.; Ganesan, A. *Org. Lett.* **2011**, *13*, 6334–6337.

(137) Paik, W. K.; Kim, S. *Science (Washington, DC, U.S.)* **1971**, *174*, 114-119.

(138) Schmidt, D. M. Z.; McCafferty, D. G. *Biochemistry* **2007**, *46*, 4408-4416.

(139) Bannister, A. J.; Kouzarides, T. *Methods Enzymol.* **2004**, *376*, 269-288.

(140) Bedford, M. T.; Richard, S. *Mol. Cell* **2005**, *18*, 263-272.

(141) Szyf, M. *Annu. Rev. Pharmacol. Toxicol.* **2009**, *49*, 243-263.

(142) Shi, Y.; Lan, F.; Matson, C.; Mulligan, P.; Whetstine, J. R.; Cole, P. A.; Casero, R. A.; Shi, Y. *Cell (Cambridge, MA, U.S.)* **2004**, *119*, 941-953.

(143) Culhane, J.; Cole, P. *Curr. Opin. Chem. Biol.* **2007**, *11*, 561-568.

(144) Shi, Y.; Whetstine, J. R. *Mol. Cell* **2007**, *25*, 1-14.

(145) Chen, Y.; Yang, Y.; Wang, F.; Wan, K.; Yamane, K.; Zhang, Y.; Lei, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13956-13961.

(146) Yang, M.; Gocke, C. B.; Luo, X.; Borek, D.; Tomchick, D. R.; Machius, M.; Otwinowski, Z.; Yu, H. *Mol. Cell* **2006**, *23*, 377-387.

(147) Forneris, F.; Binda, C.; Battaglioli, E.; Mattevi, A. *Trends Biochem. Sci.* **2008**, *33*, 181-189.

(148) Bannister, A. J.; Kouzarides, T. *Nature (London, U.K.)* **2005**, *436*, 1103-1106.

(149) Lee, M. G.; Wynder, C.; Cooch, N.; Shiekhattar, R. *Nature (London, U.K.)* **2005**, *437*, 432-435.

(150) Metzger, E.; Wissmann, M.; Yin, N.; Mueller, J. M.; Schneider, R.; Peters, A. H. F. M.; Guenther, T.; Buettner, R.; Schuele, R. *Nature (London, U.K.)* **2005**, *437*, 436-439.

(151) Karytinos, A.; Forneris, F.; Profumo, A.; Ciossani, G.; Battaglioli, E.; Binda, C.; Mattevi, A. *J. Biol. Chem.* **2009**, *284*, 17775-17782.

(152) Fang, R.; Barbera, A. J.; Xu, Y.-F.; Rutenberg, M.; Leonor, T.; Bi, Q.; Lan, F.; Mei, P.-C.; Yuan, G.-C.; Lian, C.; Peng, J.-M.; Cheng, D.-M.; Sui, G.-C.; Kaiser, U. B.; Shi, Y.; Shi, Y.-J. G. *Mol. Cell* **2010**, *39*, 222-233.

(153) Hirst, M.; Marra, M. A. *Int. J. Biochem. Cell Biol.* **2009**, *41*, 136-146.

(154) Kahl, P.; Gullotti, L.; Heukamp, L. C.; Wolf, S.; Friedrichs, N.; Vorreuther, R.; Solleder, G.; Bastian, P. J.; Ellinger, J.; Metzger, E.; Schuele, R.; Buettner, R. *Cancer Res.* **2006**, *66*, 11341-11347.

(155) Wang, Y.; Zhang, H.; Chen, Y.; Sun, Y.; Yang, F.; Yu, W.; Liang, J.; Sun, L.; Yang, X.; Shi, L.; Li, R.; Li, Y.; Zhang, Y.; Li, Q.; Yi, X.; Shang, Y. *Cell (Cambridge, MA, U. S.)* **2009**, *138*, 660-672.

(156) CancerResearchUK *News and Resources Centre CancerStats. Prostate cancer.*, http://info.cancerresearchuk.org/cancerstats/, 2008.

(157) Huang, J.; Sengupta, R.; Espejo, A. B.; Lee, M. G.; Dorsey, J. A.; Richter, M.; Opravil, S.; Shiekhattar, R.; Bedford, M. T.; Jenuwein, T.; Berger, S. L. *Nature (London, U.K.)* **2007**, *449*, 105-108.

(158) Lee, M. G.; Wynder, C.; Bochar, D. A.; Hakimi, M.-A.; Cooch, N.; Shiekhattar, R. *Mol. Cell Biol.* **2006**, *26*, 6395-6402.

(159) Lee, M. G.; Wynder, C.; Schmidt, D. M.; McCafferty, D. G.; Shiekhattar, R. *Chem. Biol.* **2006**, *13*, 563-567.

(160) Culhane, J. C.; Szewczuk, L. M.; Liu, X.; Da, G.; Marmorstein, R.; Cole, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 4536-4537.

(161) Szewczuk, L. M.; Culhane, J. C.; Yang, M.; Majumdar, A.; Yu, H.; Cole, P. A. *Biochemistry* **2007**, *46*, 6892-6902.

(162) Yang, M.; Culhane, J. C.; Szewczuk, L. M.; Jalili, P.; Ball, H. L.; Machius, M.; Cole, P. A.; Yu, H. *Biochemistry* **2007**, *46*, 8058-8065.

(163) Mimasu, S.; Sengoku, T.; Fukuzawa, S.; Umehara, T.; Yokoyama, S. *Biochem. Biophys. Res. Commun.* **2008**, *366*, 15-22.

442

(164) Gooden, D. M.; Schmidt, D. M. Z.; Pollock, J. A.; Kabadi, A. M.; McCafferty, D. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3047-3051.

(165) Rajadhyaksha, V. J., (Nelson Research and Development Co.), US patent 4016204, 1977.

(166) Binda, C.; Valente, S.; Romanenghi, M.; Pilotto, S.; Cirilli, R.; Karytinos, A.; Ciossani, G.; Botrugno, O. A.; Forneris, F.; Tardugno, M.; Edmondson, D. E.; Minucci, S.; Mattevi, A.; Mai, A. *J. Am. Chem. Soc.* **2010**, *132*, 6827-6833.

(167) Ueda, R.; Suzuki, T.; Mino, K.; Tsumoto, H.; Nakagawa, H.; Hasegawa, M.; Sasaki, R.; Mizukami, T.; Miyata, N. *J. Am. Chem. Soc.* **2009**, *131*, 17536-17537.

(168) Mimasu, S.; Umezawa, N.; Sato, S.; Higuchi, T.; Umehara, T.; Yokoyama, S. *Biochemistry* **2010**, *49*, 6494-6503.

(169) Culhane, J. C.; Wang, D.; Yen, P. M.; Cole, P. A. *J. Am. Chem. Soc.* **2010**, *132*, 3164-3176.

(170) Huang, Y.; Greene, E.; Stewart, T. M.; Goodwin, A. C.; Baylin, S. B.; Woster, P. M.; Casero, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8023-8028.

(171) Huang, Y.; Stewart, T. M.; Wu, Y.; Baylin, S. B.; Marton, L. J.; Perkins, B.; Jones, R. J.; Woster, P. M.; Casero, R. A., Jr. *Clin. Cancer Res.* **2009**, *15*, 7217-7228.

(172) Zhu, Q.; Huang, Y.; Marton, L.; Woster, P.; Davidson, N.; Casero, R. *Amino Acids* **2011**, 1-12.

(173) Lebel, H.; Marcoux, J.-F.; Molinaro, C.; Charette, A. B. *Chem. Rev. (Washington, DC, U. S.)* **2003**, *103*, 977-1050.

(174) Arvidsson, L. E.; Johansson, A. M.; Hacksell, U.; Nilsson, J. L. G.; Svensson, K.; Hjorth, S.; Magnusson, T.; Carlsson, A.; Lindberg, P. *J. Med. Chem.* **1988**, *31*, 92-99.

(175) Kaiser, C.; Trost, B. M.; Beeson, J.; Weinstock, J. *J. Org. Chem.* **1965**, *30*, 3972-3975.

(176) Pryde, D. C.; Cook, A. S.; Burring, D. J.; Jones, L. H.; Foll, S.; Platts, M. Y.; Sanderson, V.; Corless, M.; Stobie, A.; Middleton, D. S.; Foster, L.; Barker, L.; Van, D. G. P.; Stacey, P.; Kohl, C.; Coggon, S.; Beaumont, K. *Bioorg. Med. Chem.* **2007**, *15*, 142-159.

(177) Evans, D. A.; Woerpel, K. A.; Hinman, M. M.; Faul, M. M. *J. Am. Chem. Soc.* **1991**, *113*, 726-728.

(178) Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron Lett.* **1992**, *33*, 5441-5444.

(179) Vorderwuelbecke, S.; Cleverley, S.; Weinberger, S. R.; Wiesner, A. *Nat. Methods* **2005**, *2*, 393-395.

(180) Zhou, M.; Diwu, Z.; Panchuk-Voloshina, N.; Haugland, R. P. *Anal. Biochem.* **1997**, *253*, 162-168.

(181) Wilson, K.; J., W. *Biochemistry and molecular biology*; 6th ed., 2005.

(182) Radford, S. K. The synthesis of 1,2,4-triazine-3,6-diones and the structure-based design and synthesis of HIV-1 protease inhibitors, University of Southampton 2007.

(183) Silverman, R. B.; Ji, H.; Lawton, G. R., (Northwestern University, USA .), World patent 042353A1, 2008.

(184) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849-3862.

(185) Abdel-Magid, A. F.; Maryanoff, C. A.; Carson, K. G. *Tetrahedron Lett.* **1990**, *31*, 5595-5598.

(186) Han, G.; Tamaki, M.; Hruby, V. J. *J. Pept. Res.* **2001**, *58*, 338-341.

(187) Teng, M.; Zhu, J.; Johnson, M. D.; Chen, P.; Kornmann, J.; Chen, E.; Blasina, A.; Register, J.; Anderes, K.; Rogers, C.; Deng, Y.; Ninkovic, S.; Grant, S.; Hu, Q.; Lundgren, K.; Peng, Z.; Kania, R. S. *J. Med. Chem.* **2007**, *50*, 5253-5256.

(188) Toumi, M.; Couty, F.; Evano, G. *J. Org. Chem.* **2008**, *73*, 1270-1281.

(189) Houssin, R.; Lohez, M.; Bernier, J.; Henichart, J. *J. Org. Chem.* **1985**, *50*, 2787-2788.

(190) Hamada, Y.; Shibata, M.; Sugiura, T.; Kato, S.; Shioiri, T. *J. Org. Chem.* **1987**, *52*, 1252-1255.

(191) Mohan, R.; Chou, Y.; Bihovsky, R.; Lumma, W. C.; Erhardt, P. W.; Shaw, K. J. *J. Med. Chem.* **1991**, *34*, 2402-2410.

(192) Hardee, D. J.; Kovalchuke, L.; Lambert, T. H. *J. Am. Chem. Soc.* **2010**, *132*, 5002-5003.

(193) Kogen, H.; Kiho, T.; Nakayama, M.; Furukawa, Y.; Kinoshita, T.; Inukai, M. *J. Am. Chem. Soc.* **2000**, *122*, 10214-10215.

(194) Katoh, O.; Uragaki, T.; Nakamura, T., (Mitsubishi Rayon Co., Ltd., Japan), World patent 087819A1, 2001.

(195) Miller, J. J.; Sigman, M. S. *J. Am. Chem. Soc.* **2007**, *129*, 2752-2753.

(196) Kigoshi, H.; Yamada, S. *Tetrahedron* **1999**, *55*, 12301-12308.

(197) Shin, C.; Okumura, K.; Ito, A.; Nakamura, Y. *Chem. Lett.* **1994**, *7*, 1301-1304.

(198) Lawandi, J.; Toumieux, S.; Seyer, V.; Campbell, P.; Thielges, S.; Juillerat-Jenneret, L.; Moitessier, N. *J. Med. Chem.* **2009**, *52*, 6672-6684.

(199) Kruszynski, M. P. *Pol. J. Chem.* **1986**, *60*, 95-105.

(200) Yokokawa, F.; Sameshima, H.; In, Y.; Minoura, K.; Ishida, T.; Shioiri, T. *Tetrahedron* **2002**, *58*, 8127-8144.

(201) Jayaprakash, S.; Pattenden, G.; Viljoen, M. S.; Wilson, C. *Tetrahedron* **2003**, *59*, 6637-6646.

(202) Singh, S.; Rao, S. J.; Pennington, M. W. *J. Org. Chem.* **2004**, *69*, 4551-4554.

(203) Hara, S.; Makino, K.; Hamada, Y. *Tetrahedron Lett.* **2006**, *47*, 1081-1085.

(204) Moody, C. J.; Bagley, M. C. *J. Chem. Soc., Perkin Trans.* **1998**, 601-607.

(205) Lenore, M. M.; Bi-Huang, H., US161007 A1, 2006.

(206) Preciado, A.; Williams, P. G. *J. Org. Chem.* **2008**, *73*, 9228-9234.

(207) Biggins, J. B.; Gleber, C. D.; Brady, S. F. *Org. Lett.* **2011**, *13*, 1536-1539.

(208) Ito, J.-i.; Ujiie, S.; Nishiyama, H. *Chem. --Eur. J.* **2010**, *16*, 4986-4990.

(209) Lyle, M. P. A.; Wilson, P. D. *Org. Lett.* **2004**, *6*, 855-858.

(210) Buerli, R.; Haughan, A. F.; Mack, S. R.; Perry, B. G.; Raphy, G., World patent 52448, 2010.

(211) Liu, H.; Black, L. A.; Bennani, Y. L.; Cowart, M. D., World patent 150010, 2007.

(212) Dube, D.; Gallant, M.; Lacombe, P.; Aspiotis, R.; Dube, L.; Girard, Y.; Macdonald, D. , World patent 48374, 2004.

(213) Kozikowski, A.; Kurome, T.; Setola, V.; Roth, B., World patent 25144, 2007.

(214) Andreotti, D.; Arista, L.; Cardullo, F.; Spada, S.; Thewlis, K. M.; Ward, S. E., World patent 87169, 2006.

(215) Pannala, M.; Kher, S.; Wilson, N.; Gaudette, J.; Sircar, I.; Zhang, S.; Bakhirev, A.; Yang, G.; Yuen, P.; Gorcsan, F.; Sakurai, N.; Barbosa, M.; Cheng, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5978-5982.

(216) Vallgarda, J.; Appelberg, U.; Arvidsson, L.; Hjorth, S.; Svensson, B. E.; Hacksell, U. *J. Med. Chem.* **1996**, *39*, 1485-1493.

(217) Guile, S.; Springthorpe, B., World patent 5143, 1999.

(218) Glover, S. A.; Rowbottom, C. A.; Scott, A. P.; Schoonraad, J. L. *Tetrahedron* **1990**, *46*, 7247-7262.

(219) Barnett, C. J.; Wilson, T. M.; Wendel, S. R.; Winningham, M. J.; Deeter, J. B. *J. Org. Chem.* **1994**, *59*, 7038-7045.

(220) Ito, K.; Tanaka, H. *Chem. Pharm. Bull.* **1977**, *25*, 1732-1739.

(221) Bream, J. B.; Picard, C. W.; White, T. G., CH 530964 A 19730115, 1973.

(222) Anderson, F. E.; Kaminsky, D.; Dubnick, B.; Klutchko, S. R.; Cetenko, W. A.; Gylys, J.; Hart, J. A. *J. Med. Pharm. Chem.* **1962**, *51*, 221-230