UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

School of Chemistry

TOTAL SYNTHESIS OF SPIRUCHOSTATIN A AND STRUCTURAL ANALOGUES

by

Alexander Yurek-George

Thesis for the Degree of Doctor of Philosophy

March 2004

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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Spiruchostatin A is a recently isolated bicyclic depsipeptide from the protobacterium Pseudomonas sp. by Shin-ya et al. It bears a resemblance to FK228, a potent inhibitor of histone deacetylase (HDACs). HDACs inhibitors are currently of great interest as anticancer agents, and examples have now advanced to phase II clinical trials. The total synthesis of spiruchostatin A and the structural analogues was accomplished. This work unambiguously confirms the structure of spiruchostatin A. Biological testing shows spiruchostatin A to be a potent HDAC inhibitor. The synthetic route is suitable for the preparation of novel spiruchostatin and FK228 analogues.

Contents

Preface

The research described in this thesis was carried out under the supervision of Dr A. Ganesan at the University of Southampton between October 2000 and October 2003. No part of this thesis has previously been submitted for a degree.

Acknowledgements

I would like to thank my supervisor, Dr Ganesan and my advisor Dr D. C. Harrowven for their support, advice and encouragement. I would also like to thank my family and all members of the research group, past and present, especially, Elizabeth, Helen, Jefferson, Jennifer, Sally and Paul and for all their help.

Abbreviations

Ac	acetyl
b	broad
BINOL	1,1'-bi-2-naphthol
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
BOP	benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
Cbz	benzyloxycarbonyl
d	doublet
δ	chemical shift in parts per million
DABCO	1,4-diazabicyclo[2.2.2]octane
DCC	dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DDT	dithiothreitol
DIAD	diisopropyl azodicarboxylate
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
EDAC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et	ethyl
$Eu(hfc)_3$	tris[3-(heptafluoropropylthyloxymethylene)-(+)-camphorate]
Fmoc-Cl	9-fluorenylmethyl chloroformate
h	hour
HBTU	*O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HDAC	histone deacetylase
HPLC	high performance liquid chromatography
hν	ultraviolet irradiation

IPTT	isopropyl-1,3-thiozolidine-2-thione
IR	infrared
J	coupling constant
LDA	lithium diisopropylethylamine
m	multiplet
Me	methyl
m/z	mass to charge ratio
m-CPBA	*m*-chloroperoxybenzoic acid
mp	melting point
MS	mass spectrometry
MEM	2-methoxyethoxymethyl
NBS	*N*-bromosuccinimide
NMI	*N*-methyl-imidazole
NMR	nuclear magnetic resonance
MSNT	1-(2-mesitylene-sulfonyl)-3-nitro-1,2,4-triazole
NOBIN	2-hydroxy-2'-amino-1,1'-binaphthyl
PFP	pentafluorophenyl
Ph	phenyl
PMB	4-methoxybenzyl
PPA	polyphosphoric acid
PPTS	pyridinium *p*-toluene sulfonate
Pr	propyl
PTSA	*p*-toluenesulfonic acid
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
q	quartet
rt	room temperature
sat.	saturated
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl

TBDPS	*tert*-butyldiphenylsilyl
Tce	trichloroethyl
TES	triethylsilyl
Tf	tiflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSI	trimethylsilyl iodide
Ts	*p*-toluenesulfonyl
TSA	trichostatin A
Trt	triphenylmethyl

Chapter 1: Introduction

1.1 Histone deacetylase (HDAC) inhibitors

Globally, cancer is the second largest cause of death after cardiac disease, affecting nearly 1 in 3 people. There has yet to be found a single therapeutically effective, selective treatment for cancer. Recent developments in understanding the cancer cell cycle, particularly the relationship with chromatin control, are providing opportunities for developing a new range of cancer drugs. Chromatin is a proteinaceous material mainly composed of histones which package with DNA to form nucleosomes. They play an important role in the cell cycle, tightly packaging DNA around histone proteins during the rest phase of the cell cycle. This interaction is partially mediated by attraction between negatively charged DNA and protonated lysine residues on histones. This is regulated by reversible acetylation of the histone lysine residues on the ε-amino groups of specific internal lysine residues. The histone terminal tails have an important regulatory role and are modified by a series of enzymes. These modifications include phosphorylation, acetylation and methylation. The enzyme histone deacetylase removes these acetyl groups. Histone deacetylase inhibitors increase histone acetylation arresting the growth of healthy and cancerous cells. Conversely histone acetyl transferases (HATs) acetylate these lysine amino-terminal tails of histones.[1,2]

The core histones H2A, H2B, H3 and H4 (Figure 1A) on the N-terminal tail region of chromatin are acetylated and deactetylated by the action of HAT and HDAC respectively. 146 base pairs of DNA are wrapped around this octamer of core histones. The core histones have a similar structure that consists of a basic N-terminal domain, a central histone-fold domain responsible for histone-histone and histone-DNA interactions and a C-terminal tail. The crystal structure of the nucleosome shows that the N-terminal tails emanate from the nucleosome in all directions (Figure 1B). Reversible acetylation occurs on specific lysines that are located in the N-terminal tail domains of the core histones (Figure 1A). With the exception of H2A, the core histones are acetylated at four or five sites; thus a nucleosome has the potential to acetylate at 28 or more sites.[1,3,4]

The acetylation of the histone tails disrupts higher-order chromatin folding[5] and promotes the solubility of chromatin at physiological ionic strength.[6] It has been shown that the nucleosomes need only be 46% acetylated for the DNA to unfold and stimulation of transcription by RNA polymerase III to occur.[7] Acetylation of core histone tails removes the latent positive charge and thus interferes with interactions with proteins and/or latent negatively charged DNA, thereby destabilizing higher-order chromatin organization, opening up the chromatin and potentially activating to transcription (Figure 1C).[8,9]

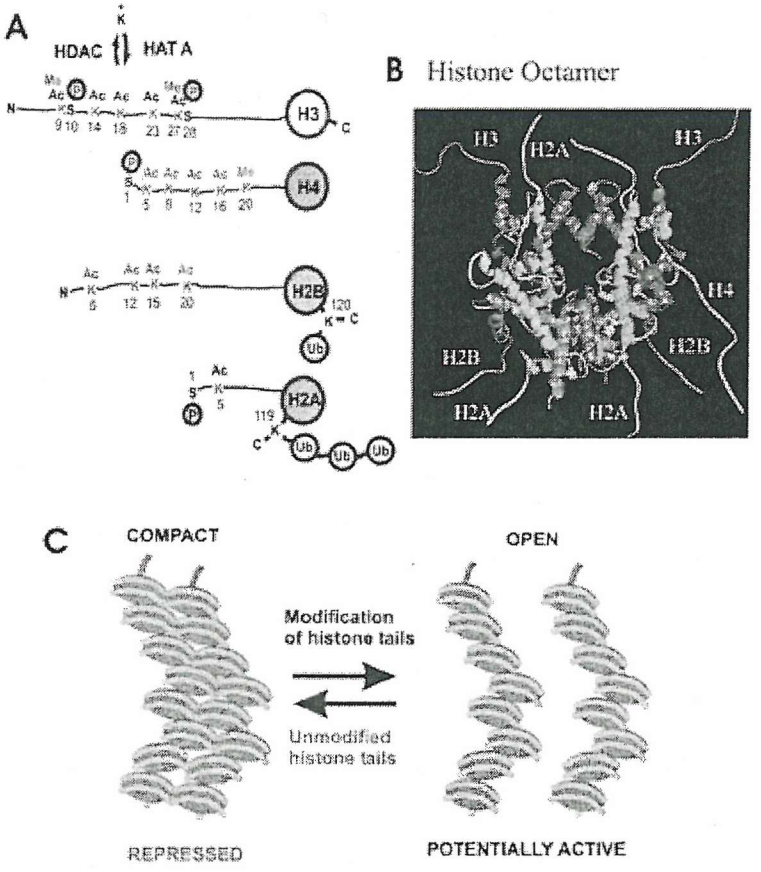


Figure 1. **(A)** Structures of the core histones H2A, H2B, H3 and H4 (K = lys residue, S = serine residue, N and C = N and C terminus respectively) and the sites of postsynthetic modification are indicated. Modifications shown are acetylation (Ac), phosphorylation (P), ubiquitination (Ub) and methylation (Me). **(B)** Crystal structure of the nucleosome. (**C**) Chromatin fibers bearing unmodified tails interact; however, these interactions are disfavored when the tails are modified.[3]

There are two distinct protein families with HDAC activity, the classical zinc-dependent HDAC family and the Sir2 family of NAD^+-dependent HDACs. Members of the classical HDAC family fall into two different phylogenetic classes, namely class I and class II.[10] Class I display homology in their active sites (HDACs 1, 2, 3 and 8); Class II having some homology at both the N-terminal regulatory domain and the C-terminal catalytic domain (HDACs 4, 5, 7, and 9) or just the class II catalytic domain (HDACs 6 and 10). HDAC11 contains homology to both the catalytic sites of Class I and II. Class III is the conserved nicotinamide adenine dinucleotide-dependent Sir2 family of deacetylases.[11]

There are a large number of histone deacetylase (HDAC) inhibitors in early clinical trials (Figure 2) and they represent a promising new therapeutic approach to cancer treatment by intervening in the cell cycle. All of these potential cancer drugs are still in clinical trials and yet to be completely proven as to their effectiveness.

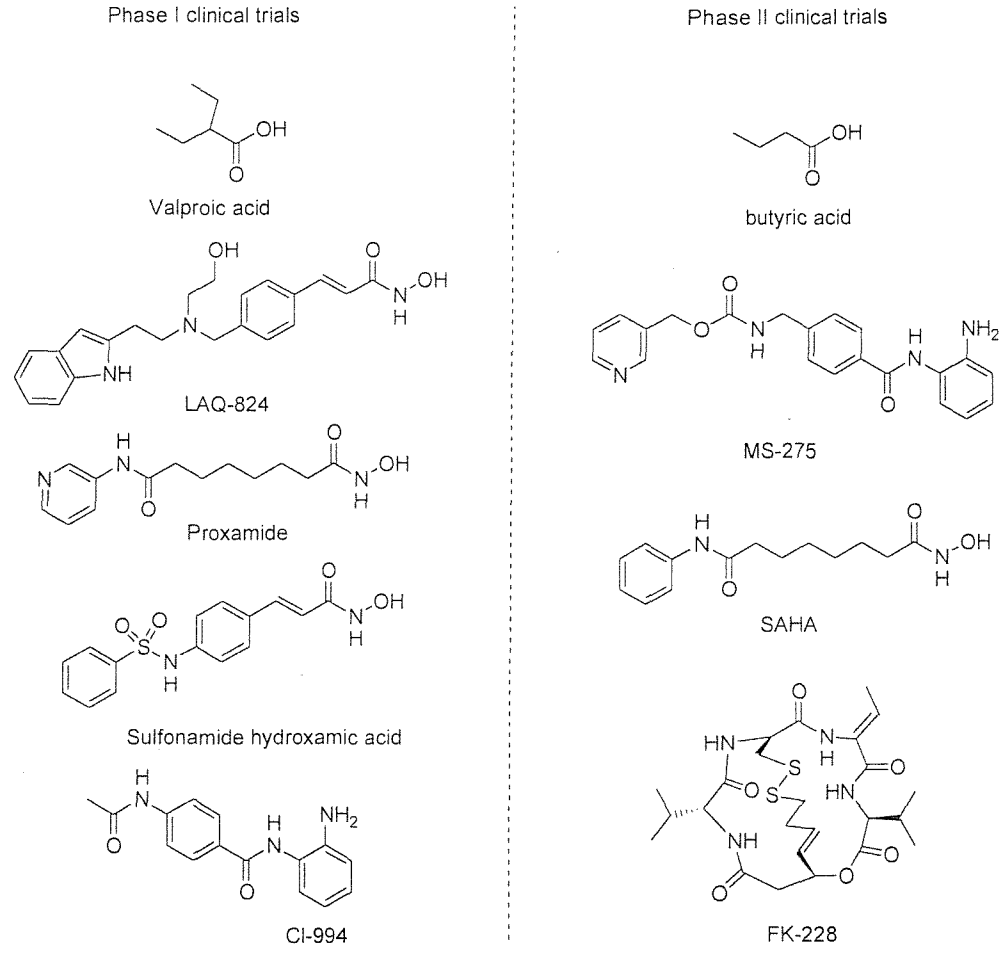
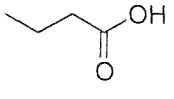


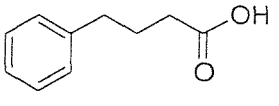
Figure 2. Histone deacetylase inhibitors currently in clinical trials

HDAC inhibitors have been shown to bind directly to the HDAC active site and thereby block substrate access resulting in the accumulation of acetylated histones. HDAC inhibitors induce cancer cell growth arrest, differentiation and apoptosis. Inhibitors include trichostatin and other hydroxamic acids (SAHA), trapoxins, apicidins (selective against *Plasmodium* HDACs) and depsipeptides which show selective activity towards class I HDACS with low activity against class II HDACS.

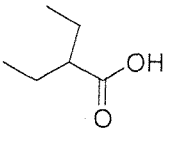
HDAC inhibitors can be categorised into the following structural classes: the simplest being small aliphatic acids, benzamides, electrophilic ketones, to the more complex cyclic depsipeptides. The simplest compound, butyrate, is a short-chain fatty acid derived from bacterial metabolism of dietary fiber in the colon. Butyrate was thought to be important for proper epithelial cell regulation, but was also found to have an antiproliferative and differentiation-inducing activity on various human colon carcinoma cells, normal cells, and neoplastic cells. These HDAC inhibitors induce differentiation, inhibit cell proliferation, and induce apoptosis of tumor cells in cultures and animal models and are emerging as a new class of potential therapeutic agents for the treatment of solid and hematologic malignancies.[2,12]



butyric acid



Phenylbutyric acid



Valproic acid

Figure 3. Histone deacetylase inhibitors: Aliphatic acids.

The aliphatic acids (Figure 3), the least potent class of HDAC inhibitors (active only at millimolar levels), include butyrate,[3] valproic acid[13] (IC_{50} for HDAC1 = 0.4 mM) and phenyl butyrate. The carboxylate class of inhibitors are generally of poor bioavailability and have rapid metabolic degradation. This coupled to poor enzymatic inhibitory activity makes them less attractive drug candidates.

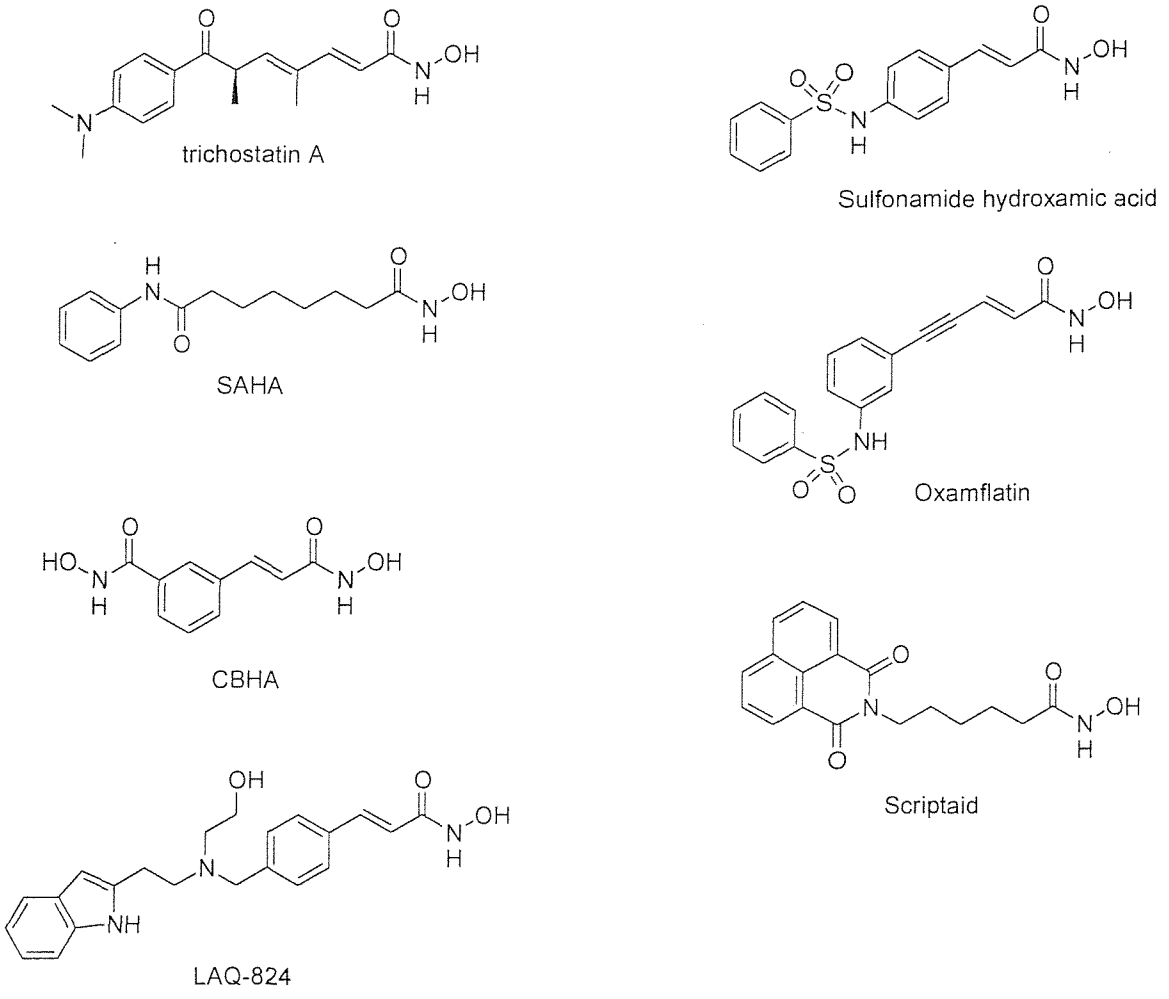


Figure 4. Histone deacetylase inhibitors: Hydroxamates.

Hydroxamic acid HDAC inhibitors are to date the most extensively studied. It is from this class of inhibitors that the model of the pharmacophore at the HDAC active site was hypothesized: the zinc metal binding domain (the hydroxamate functionality), a spacer/linker, the midsection which is generally hydrophobic, saturated or unsaturated and

a hydrophobic surface recognition domain (often aromatic). Trichostatin A (TSA) was the first natural product hydroxamate from *Streptomyces* to be discovered that inhibited HDACs directly.[14] SAHA which is a synthetic analogue of TSA was found to be an inhibitor (at nanomolar concentrations).[15] Cinnamic acid bishydroxamic acid (CBHA) has been shown to be a potent HDAC inhibitor[16] and several CBHA derivatives have been described, including LAQ-824 and sulfonamide hydroxamic acids[17,18]. Other hydroxamates of interest include oxamflatin[19] and scriptaid[20] (Figure 4).

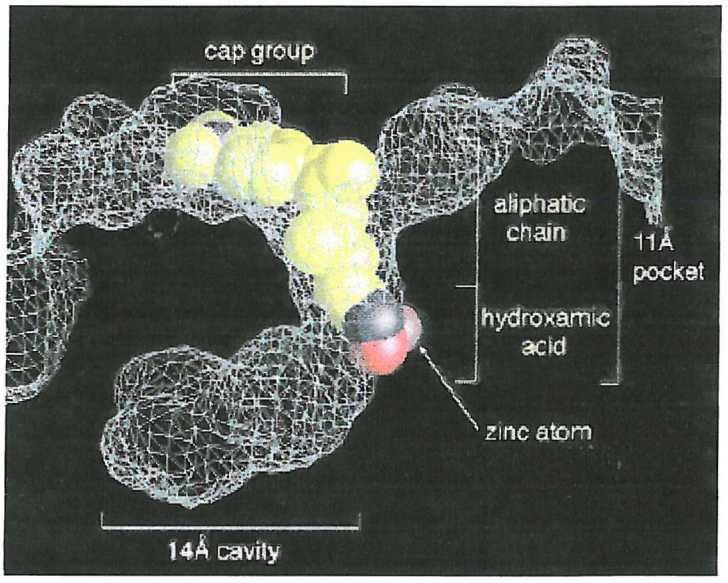


Figure 5. Space-filling representation of TSA in the active-site pocket.[21]

The interaction of TSA with the HDAC active site has been studied by X-ray crystallography. This shows contact to residues at the rim, walls and bottom of the pocket. Space-filling representation of TSA in the active-site pocket shows the hydroxamic acid group, most of the aliphatic chain and part of the dimethylamino-phenyl group of TSA are contained within the pocket (60% of TSA's surface area). Cocrystallization of a HDAC-like protein with HDAC inhibitors, such as TSA, demonstrated that these inhibitors mimic the substrate and that chelation of the zinc in the catalytic pocket by the hydroxamic acid group is the main mechanism of inhibition.[21]

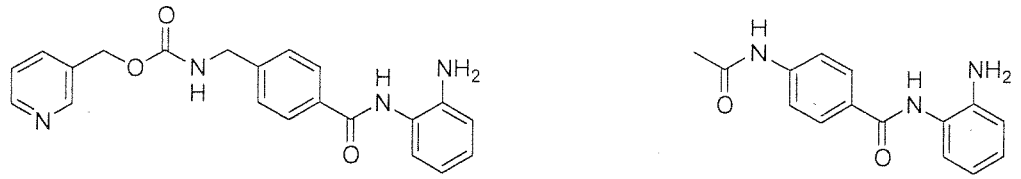


Figure 6. Histone deacetylase inhibitors: Benzamides.

The benzamide class, including MS-275 and CI-994, is generally less potent than the corresponding hydroxamates and cyclic tetrapeptides.[22] CBHA-derived benzamides have about the same potency as other benzamides[18] (Figure 6).

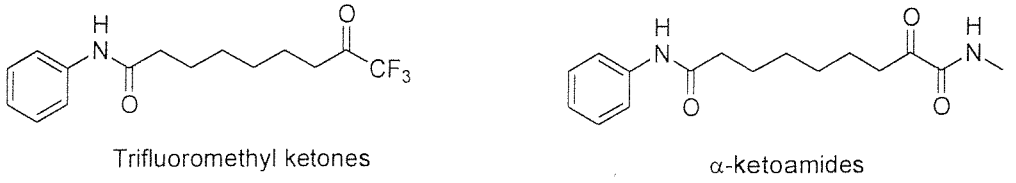
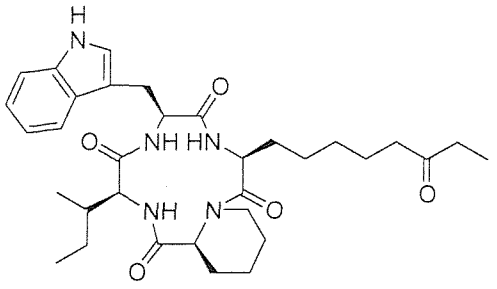
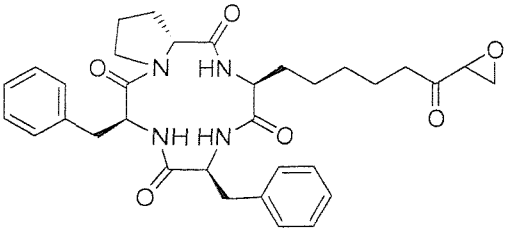


Figure 7. Histone deacetylase inhibitors: Electrophilic ketones.

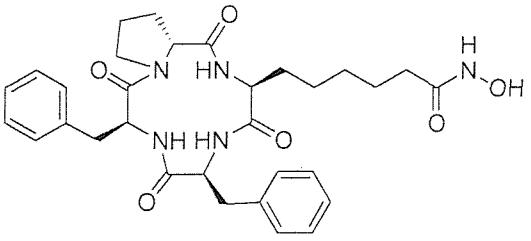
The electrophilic ketones form a new class of HDAC inhibitors. These agents include various trifluoromethyl ketones and α-ketoamides[23] and these agents, like the benzamides, possess HDAC inhibitory activity at micromolar levels (Figure 7).



Apicidin



Trapoxin B



TPX-HA analogue (CHAP)

Figure 8. Histone deacetylase inhibitors: Cyclic tetrapeptides.

Cyclic tetrapeptides, which are among the most structurally complex class of HDAC inhibitors, include apicidins[24] and the CHAPs (cyclic hydroxamic acid-containing peptides)[25] and are active at nanomolar levels. Their utility in the treatment of disease remains unproven. These compounds contain an electrophilic ketone or epoxyketone side-chain that mimics the substrate (Figure 8).

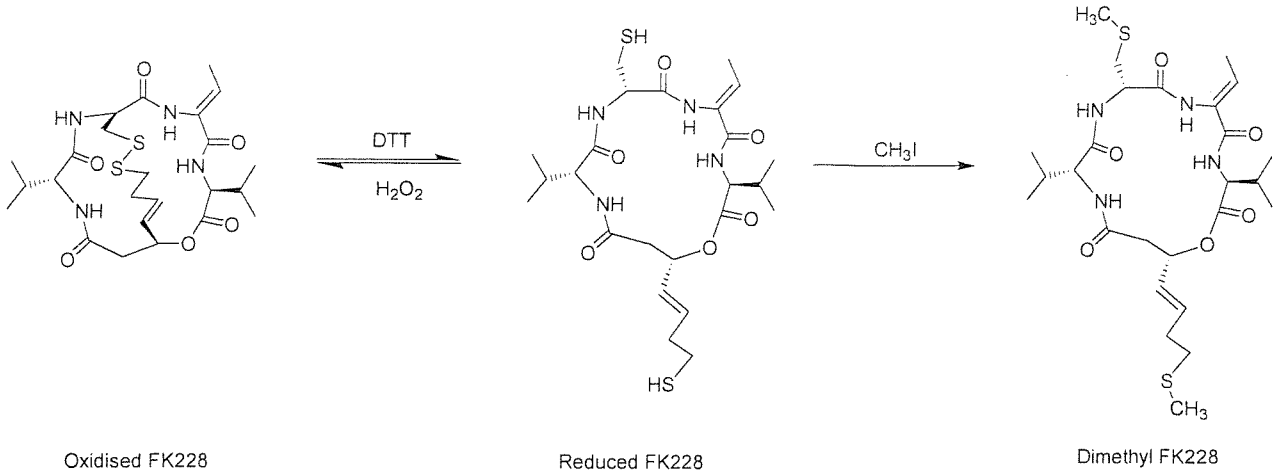


Figure 9. Oxidised, reduced and dimethyl FK228.

FK228 is a bicyclic depsipeptide produced by a broth culture of *Chromobacterium violaceum* No. 968. FK228 is a potent HDAC inhibitor and shows potent *in vivo* antitumor activity against both human tumour xenografts and murine tumours. Mechanistic studies show that FK228 is converted to its active, reduced form (redFK) by cellular reducing activity. RedFK possesses a functional sulfhydryl group capable of interacting with the zinc in the active-site pocket. Because FK228 is much more stable than redFK in medium and serum, it was proposed that FK228 is a natural prodrug, which is activated after incorporation into the cells.[26]

Reduction of the intramolecular disulfide bond in FK228 by dithiothreitol (DDT) *in vitro* greatly enhanced its inhibitory activity. *In vivo* this occurs due to the cellular reducing activity involving glutathione. Experimentally the importance of the reduced form of FK228 being the active drug was shown by methylation of the reduced thiols and also permanent oxidation with H_2O_2, both of which resulted in loss of activity *in vitro* against HDACs (Figure 9). Computer modelling suggests that one of the sulfhydryl groups of the reduced form of FK228 (redFK) interacts with the active-site zinc, preventing the access of the substrate. HDAC1 and HDAC2 were more strongly inhibited by redFK than HDAC4 and HDAC6. RedFK was less active than FK228 in inhibiting *in vivo* HDAC activity, due to rapid inactivation in medium and serum. Thus, FK228 serves as a stable prodrug to inhibit class I enzymes and is activated by reduction after uptake into the cells. The

glutathione-mediated activation also implicates its clinical usefulness for counteracting glutathione-mediated drug resistance in chemotherapy (Figure 10).

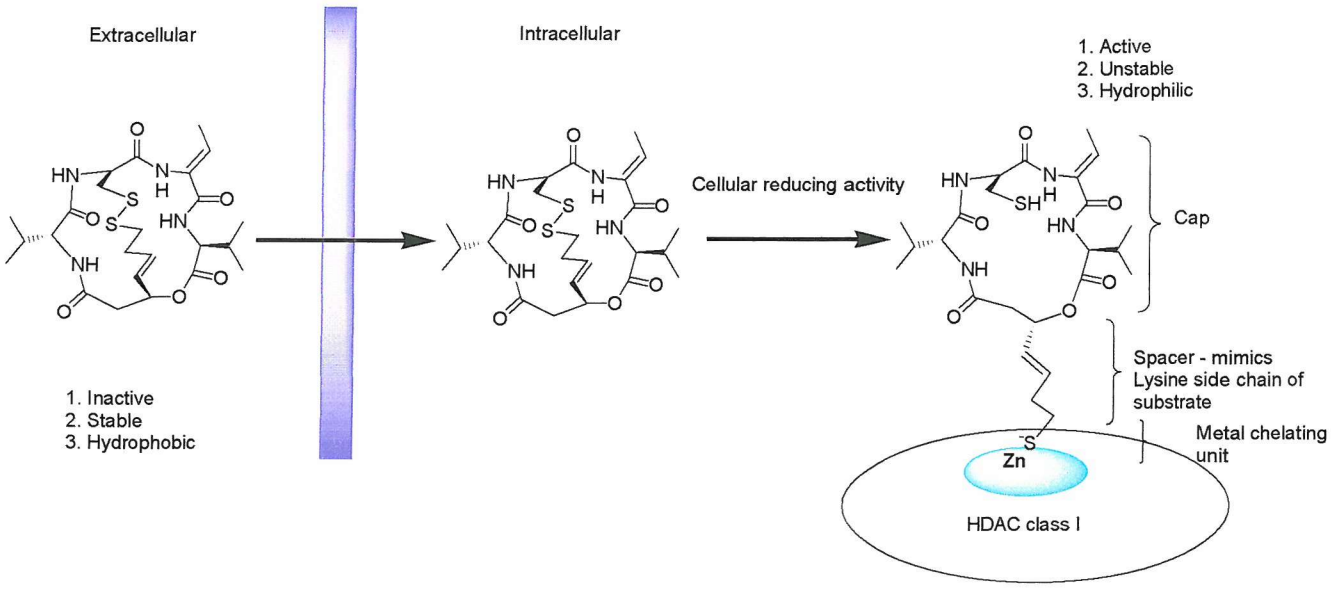
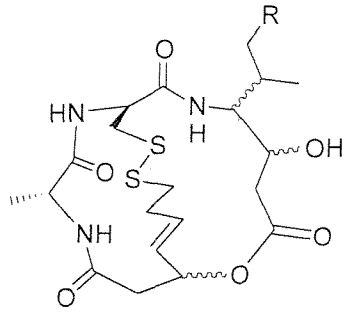


Figure 10. A model for inhibition of HDAC by FK228.[26]

Spiruchostatin A and B (Figure 11) are recently isolated bicyclic depsipeptides from the protobacterium *Pseudomonas sp.* by Shin-ya *et al.*[27] Through biological assays spiruchostatin A and B had been shown to have gene expression enhancement properties on TGF-β induced gene expression. The structural similarity to FK228 suggested that spiruchostatins are HDAC inhibitors. For these reasons spiruchostatin A is an attractive target for total synthesis. Furthermore it could be used for the preparation of unnatural analogues with improved selectivity. This approach may be an avenue by which improved agents are identified and specific diseases can be targeted. The absolute stereochemistry at several stereocentres had not been assigned in the initial publication.



R = H, spiruchostatin A
R = CH_3, spiruchostatin B

Figure 11. Spiruchostatins A and B.

1.2 The total synthesis of FK228

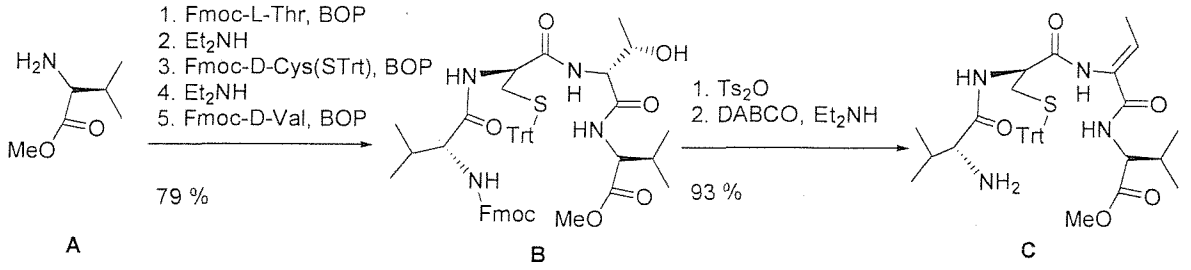


Figure 12. Synthesis of FK228 depsipeptide subunit.

The total synthesis of FK228 by Simon *et al.*[28] remains the only one for this class of depsipeptide. FK228 isolated from *Chromobacterium violaceum* (No. 968), although similar to spiruchostatin A, lacks the added complexity of the statine subunit. Nevertheless Simon's work provided us with an invaluable guide for our own synthesis. The peptide portion **C** was assembled by standard peptide synthesis methods, L-valine methyl ester being coupled sequentially with *N*-Fmoc protected amino acids L-threonine, D-cysteine-(*S*-triphenylmethyl) and D-valine using the BOP ((benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) coupling protocol followed by Fmoc removal using Et_2NH. At this stage the L-threonine residue was converted into *Z*-dehydrobutyrine through activation as a tosylate which underwent elimination upon treatment with DABCO/Et_2NH (Figure 12).

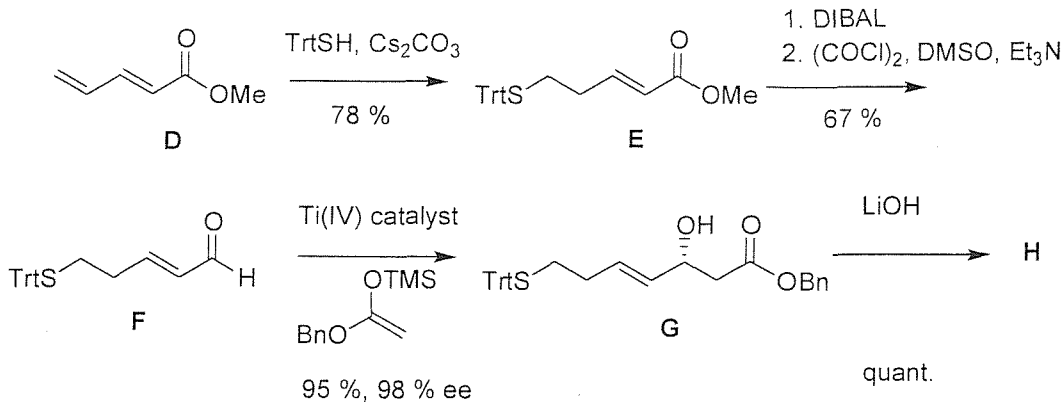


Figure 13. Synthesis of the thiol-containing β-hydroxy acid.

The thiol-containing β-hydroxy acid **H** was synthesized using the Carreira asymmetric acetate aldol reaction of **F** followed by LiOH-mediated hydrolysis. The Carreira aldol involved Ti(IV)-catalyzed addition of *O*-benzyl, *O*-TMS ketene acetal to aldehyde **F** in the presence of the ligand derived from (*S*)-(-)-hydroxy-2'-amino-1,1'-binaphthyl (NOBIN). The aldol substrate **F** was prepared by conjugate addition of cesium triphenylmethyl thiolate anion to methyl 2,4-pentadienoate **D** in the presence of Cs_2CO_3, yielding the α,β-unsaturated ester **E**. DIBAL reduction to the primary alcohol followed by Swern oxidation gave the α,β-unsaturated aldehyde **F** (Figure 13).

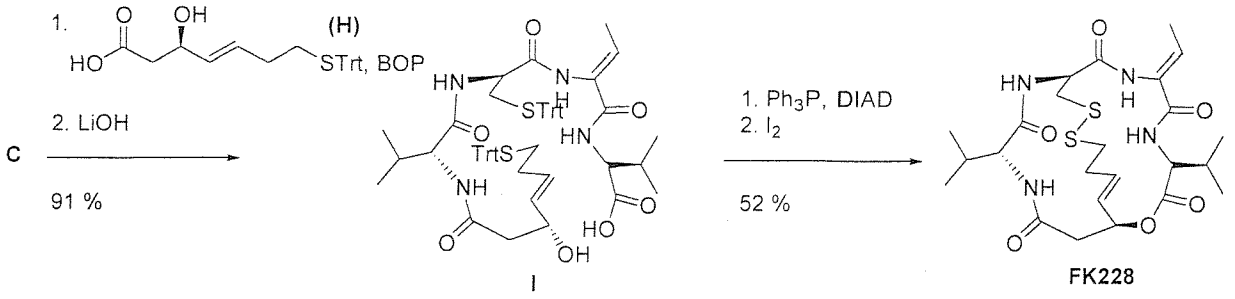


Figure 14. Synthesis of FK228.

Coupling of the hydroxy acid **H** to the tetrapeptide **C** followed by methyl ester hydrolysis gave **I**. Simon *et al.* were unable to perform the macrolactonisation using standard Yamaguchi conditions. Instead Mitsunobu cyclization of the hydroxy acid **I** with DIAD and PPh_3 afforded the desired lactone. Addition of TsOH was critical for suppressing elimination of the activated allylic alcohol. Oxidation of the bis(*S*-triphenylmethyl)lactone with iodine in dilute MeOH solution provided the natural product FK228 (Figure 14).

1.3 The total synthesis of FR-901375

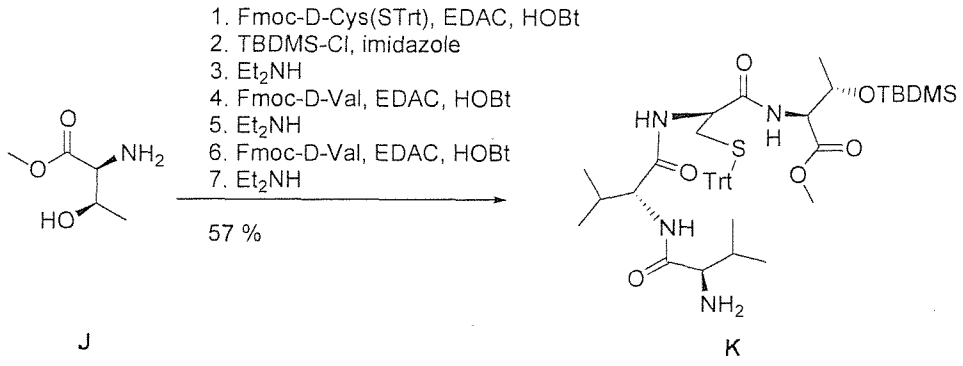


Figure 15. Synthesis of FR-901375 tetrapeptide.

The total synthesis of FR-901375 was reported by Janda *et al.*[29] after we had completed our experimental work on the synthesis of spiruchostatin A and structural analogues. Isolated from the fermentation broth of *Pseudomonas chloroaphis* (No. 2522), FR-901375 although similar to spiruchostatin A and FK228, lacks the presence of a statine subunit unique to our class of depsipeptide and contains L-threonine rather than the *Z*-dehydrobutyrine present in FK228. The peptide portion **K** was assembled by standard peptide synthesis methods starting from L-threonine methyl ester and coupled sequentially to *N*-Fmoc protected amino acids D-cysteine-(*S*-triphenylmethyl), the alcohol protected as TBDMS and the dipeptide then coupled to D-valine twice. As with the synthesis of Simon *et al.*[28] the lactone was constructed from the acyclic precursor **N** via a Mitsunobu macrolactonization reaction.

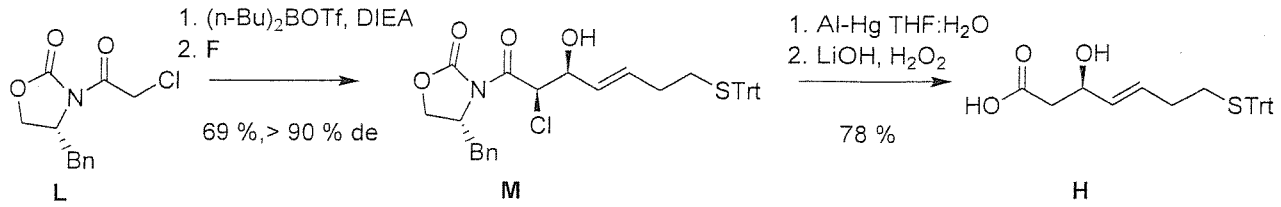


Figure 16. Janda's asymmetric synthesis of β-hydroxy acid.

The hydroxy acid synthon **H** was synthesized using an Evans auxiliary-based aldolization of haloacetyloxazolidinones as a masked chiral acetate enolate equivalent. The asymmetric aldol reaction between the dibutylboron enolate derivative of chloroacetyl-oxazolidinone **L** and aldehyde **F** provided the desired chlorohydrin **M** with good diastereoselectivity. The subsequent dechlorination of **M** was accomplished in the presence of the acid labile *S*-trityl protecting group with Al-Hg and subsequent hydrolysis of the chiral auxiliary with $LiOH/H_2O_2$, furnished **H** (Figure 16).

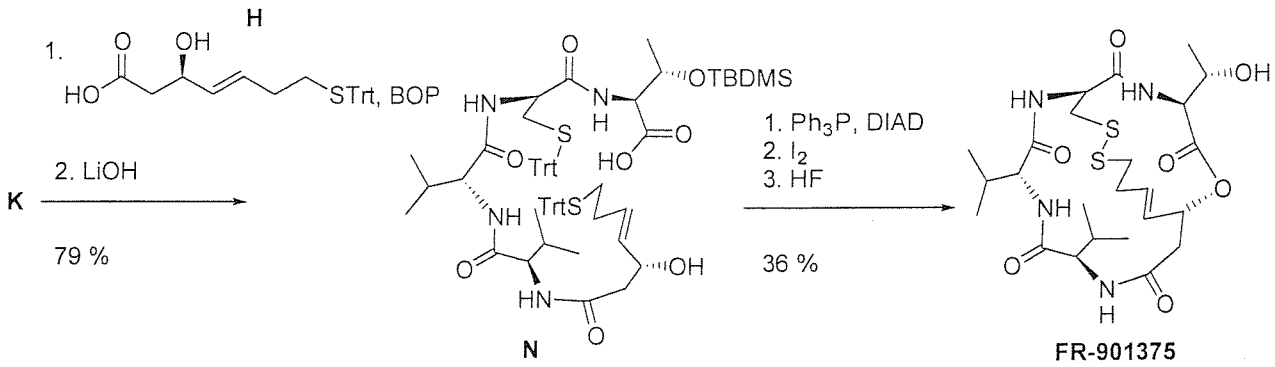
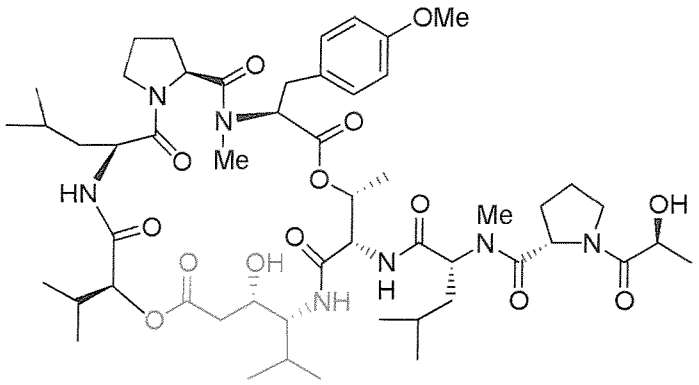


Figure 17. Synthesis of FR-901375, late stage assembly.

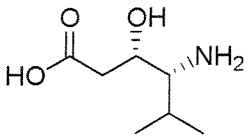
The coupling of **K** and **H** with BOP reagent followed by basic hydrolysis of the methyl ester afforded the hydroxy acid **N.** Mitsunobu macrolactonization followed by oxidative deprotection of the bis(*S*-triphenyl)lactone with iodine in dilute MeOH solution and HF/CH_3CN gave the natural product FR-901375 (Figure 17).

1.4 Natural products containing the statine subunit.

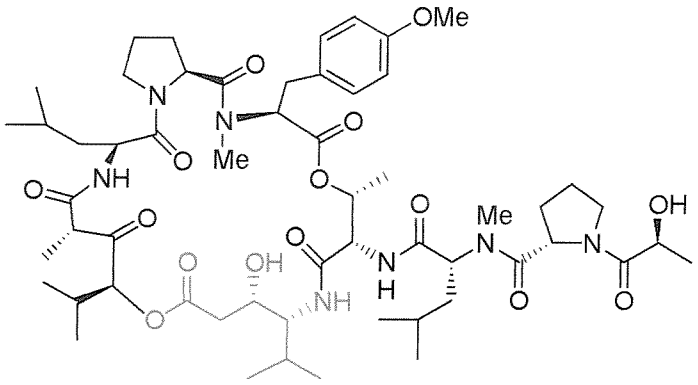
The natural products spiruchostatin A and B differ from FK228 and FR-901375 by the presence of the 4-amino-3-hydroxy-5-methylhexanoic subunit (statine), and 4-amino-3-hydroxy-5-methylheptanoic subunit (isostatine), which contains two stereo-centres at C-3 and C-4 carbons. Natural products that contain the statine subunit include the tamandarins and didemnins (Figure 18).



Tamandarin B



Statine subunit



Didemnin B

Figure 18. Statine containing cyclic depsipeptides: (-)-Tamandarin B and Didemnin B

1.5 The synthesis of spiruchostatin A

Only small quantities of the spiruchostatins have ever been extracted. A facile synthetic procedure could potentially be a high yielding source of the natural product. Given the successful clinical progression of FK228, spiruchostatin A is an attractive target for total synthesis with two additional hurdles. Firstly, the stereochemistry of the β-hydroxy acid and statine subunit were not established. We reasoned that the former was (*S*), as in FK228, and the statine a *syn* diastereomer, given its prevalence in peptide natural products. These conjectures might have required syntheses of multiple diastereomers before attaining the correct structure. Fortunately, the statine stereochemistry was later assigned as (3*S*,4*R*) during the course of our work by Shinya *et al.* The second synthetic challenge relates to the statine, a unit prone to problems such as β-elimination, protecting group migration, and intramolecular cyclization. The statine subunit sets the spiruchostatins apart from FK228 and FR901375 and adds another level of complexity in protecting group compatibility with the rest of the molecule.

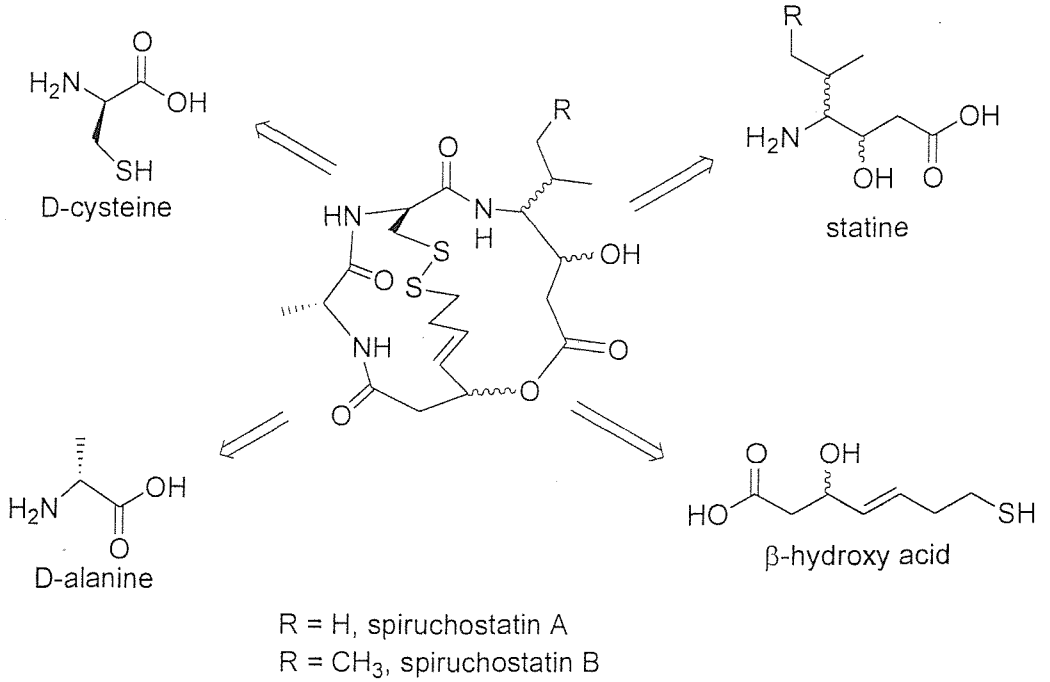


Figure 19. Retrosynthesis of spiruchostatin A.

By retrosynthetic analysis the bicyclic depsipeptide spiruchostatin A can be fragmented (Figure 19) into four substructures by cleavage of the five heteroatom-heteroatom bonds. These are two unnatural amino acids D(*R*)-alanine and D(*S*)-cysteine, a (3*S*,4*R*)-4-amino-3-hydroxy-5-methylhexanoic acid (statine) unit and a 3-hydroxy-7-mercapto-4-heptenoic acid unit. The absolute stereochemistry of the latter acid has not as yet been assigned by Shinya *et al.*

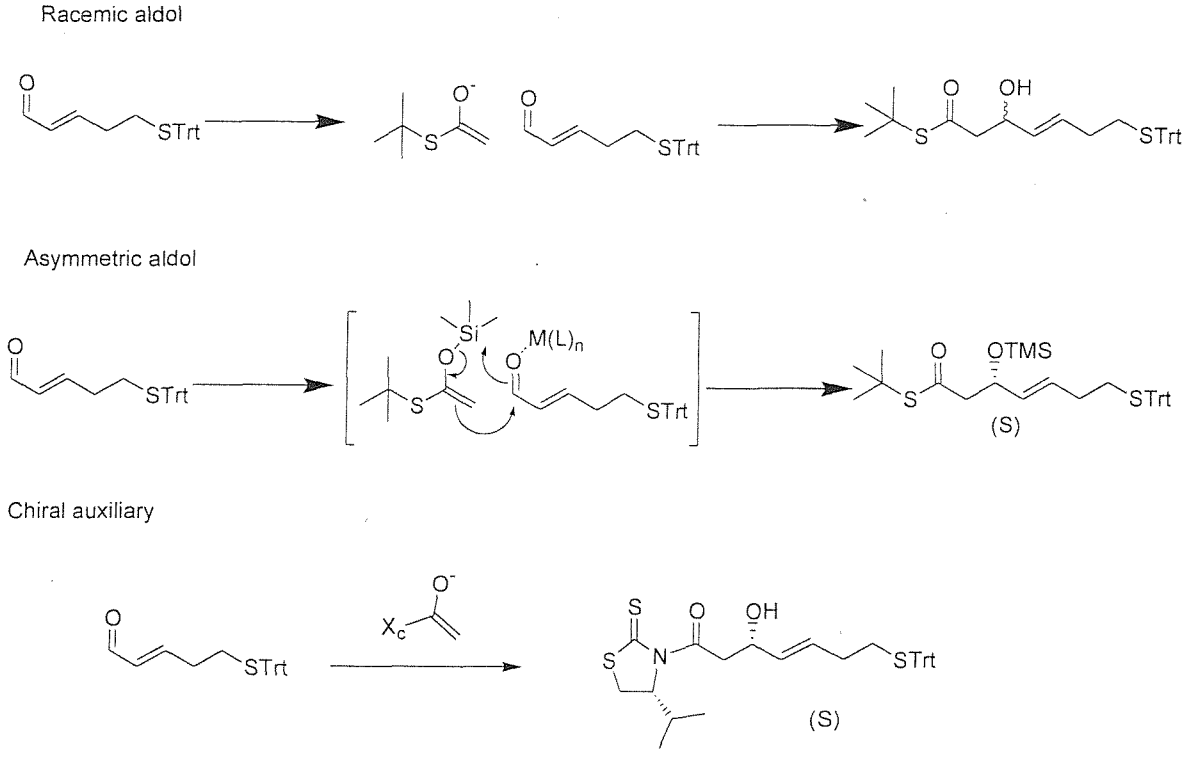


Figure 20. Possible routes to the formation of the 3-hydroxy-7-mercapto-4-heptenoic acid sub-unit.

For the 3-hydroxy-7-mercapto-4-heptenoic acid subunit Simon *et al.* in their synthesis of FK228 utilised the Carreira Lewis acid catalyst NOBIN with Ti(O-*i*-Pr)$_4$.[30] The NOBIN reagent is not however commercially available and its synthesis requires several steps.[31] Because of this and the fact that the stereocentre has not as yet been assigned, we decided to initially try alternative methods of synthesis. Both stereoisomers at the 3 position can be synthesised either asymmetrically via a Mukaiyama aldol reaction[32] with the chiral Lewis acid developed by Keck prepared from (*R*) or (*S*) 1,1-Bi-2-naphthol (BINOL) with Ti(O-*i*-Pr)$_4$, or utilising a chiral auxiliary.[33] An alternative procedure would be to synthesise both

isomers through a racemic aldol reaction, and then on coupling with the peptide sub-unit it may be possible to separate the diastereoisomers by chromatography (Figure 20).[34] BINOL is commercially available and reported to work with a wide range of simple allylic and aliphatic aldehydes to afford the desired hydroxy ester with TMS thioketene acetal often in quite high yield and ee. NOBIN is a multidentate ligand and the overall yield and asymmetric induction using this ligand with TMS enolates derived from methyl, ethyl and benzyl acetates is reported to be very high by Carreira.

Aldol type reactions employing the Nagao auxiliary, 3-acetyl-(4)-IPTT (isopropyl-1,3-thiozolidine-2-thione) with α,β-unsaturated aldehydes with $SnOTf_2$ as Lewis acid is reported to give the desired product in a diastereoselective manner. These can then be readily separated by chromatography. Other Lewis acids can also be used in the reaction such as $TiCl_4$ by Vilarrasa.[33]

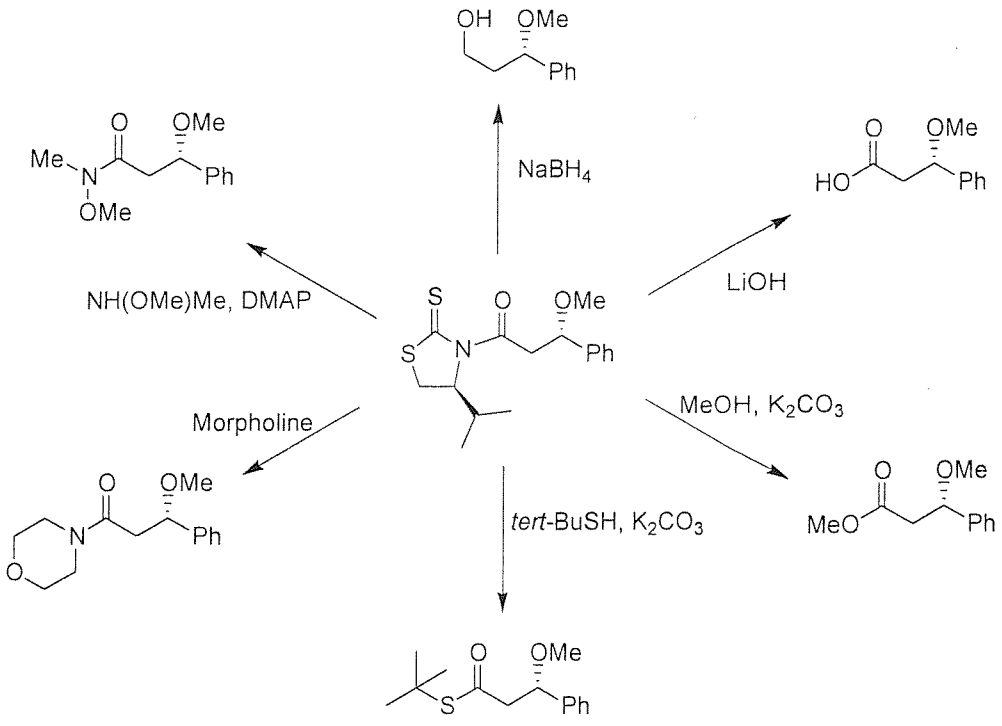


Figure 21. Thiazolidinethione transformations.

The chiral thiazolidine-2-thione auxiliary is an activated amide and can be interconverted into a wide range of derivatives; alcohol, carboxylic acid, methyl ester, thioester, morpholine amide and Weinreb amide under a variety of mild conditions (Figure 21).[35] This allows the possibility for us to convert our proposed 3-hydroxy-7-mercapto-4-heptenoic amide auxiliary derivative directly to the desired intermolecular amide or a intramolecular lactamization to form the cyclic depsipeptide (Figure 22).

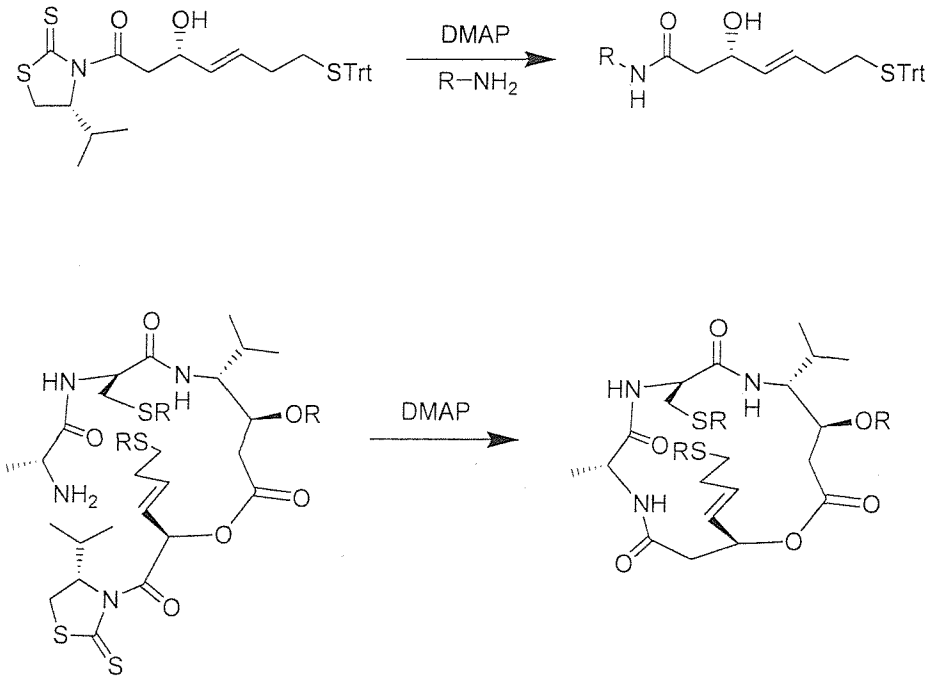


Figure 22. Possible routes to amide formation with the 3-hydroxy-7-mercapto-4-heptenoic acid sub-unit.

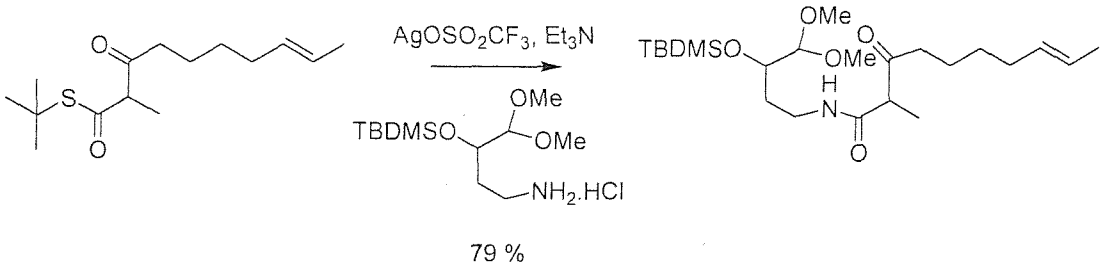


Figure 23. Ag^{+}-mediated thioester coupling.

Thioesters can be activated towards nucleophilic attack with Lewis acids, such as in the case of Clive *et al.*[36] towards the synthesis of brevioxime involving Ag^{+}-mediated coupling with a β-keto thioester (Figure 23).

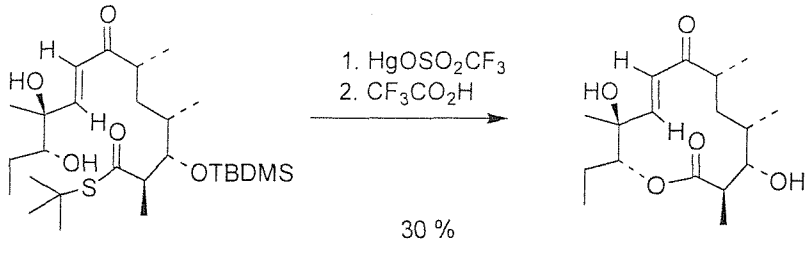


Figure 24. $HgOSO_2CF_3$ mediated macrolactonization.

Macrolactonization was achieved with mercuric trifluoroacetate in the case of the synthesis of the macrolide antibiotic methymycin (Figure 24).[37]

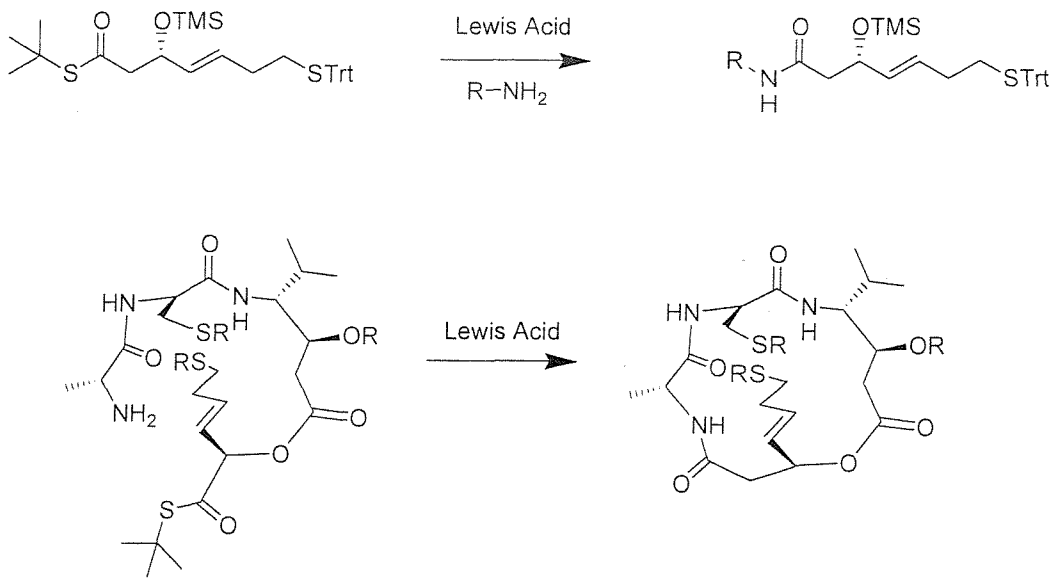


Figure 25. Proposed linear peptide coupling or macrolactonization utilizing Lewis acid to activate the thioester.

In our case for the synthesis of spiruchostatin A we can envisage using this methodology to form either the linear depsipeptide or macrolactamization (Figure 25).

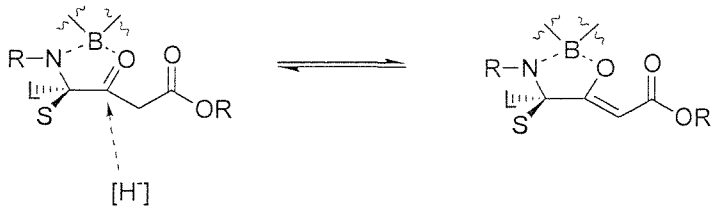


Figure 26. Cyclic transition state models of β-keto ester reduction with borohydride.

Stereoselective reduction of the statine precursor β-keto esters using borohydride reagents was shown to give the *erythro* product as the predominant isomer, typically in good yield. KBH_4 being of lower reactivity gave the best selectivity, possibly due to greater chance for chelation of the borohydride to form the cyclic transition state (Figure 26) with stereospecific reduction rather than reduction of the keto group in the acyclic form.[38] Homogenous asymmetric hydrogenation of prochiral β-keto esters using BINAP-Ru(II) complexes gave a stereocontrolled route to the *threo* statine series with high de (Figure 27).[39]

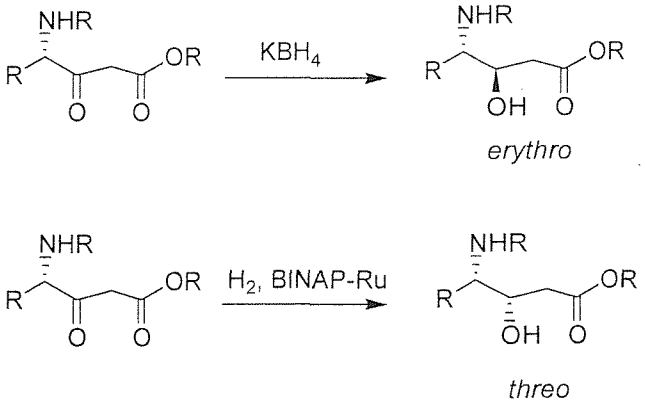


Figure 27. Routes to *threo* and *erythro* statines

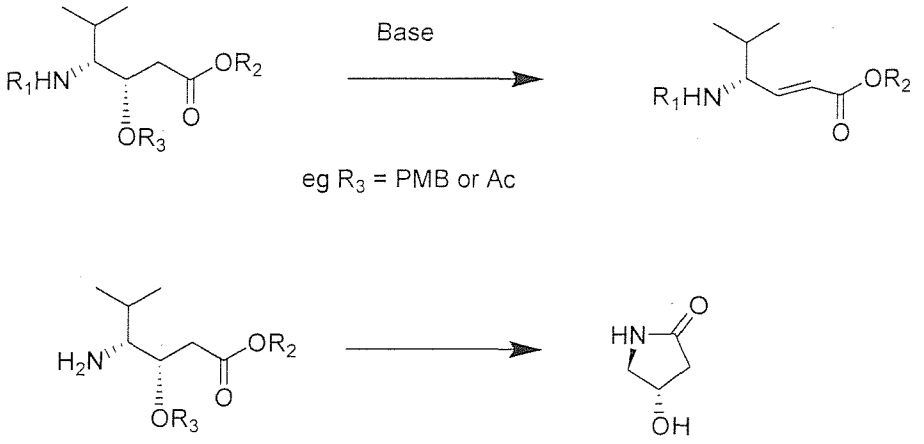


Figure 28. α,β-Elimination and cyclization of statine.

β-Hydroxy-acids are known to be susceptible to side reactions. Under certain basic conditions α,β-elimination is observed,[40] while cyclization of statines gives the five membered γ-lactam. The unwanted lactamization of statines, even when the nitrogen is protected with carbamates has been observed,[41] while spontaneous lactamization is also known (Figure 28).[42]

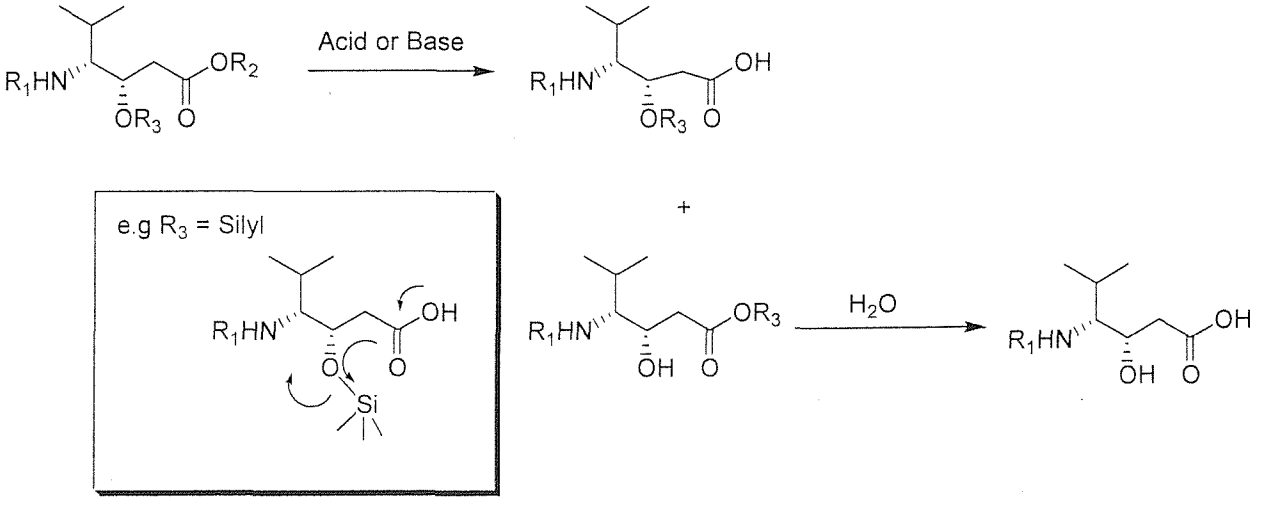


Figure 29. Proposed silyl migration of statine.

Silyl migration is well known with alcohols, occuring to a less sterically encumbered alcohol under acidic and basic conditions. With the statine subunit when the acid is unprotected migration from the alcohol to the acid can occur under either acidic or basic conditions. Migration can be limited with bulky silyl protecting groups such as TIPS (Triisopropylsilyl) and TBDPS (*tert*-butyldiphenylsilyl) (Figure 29).

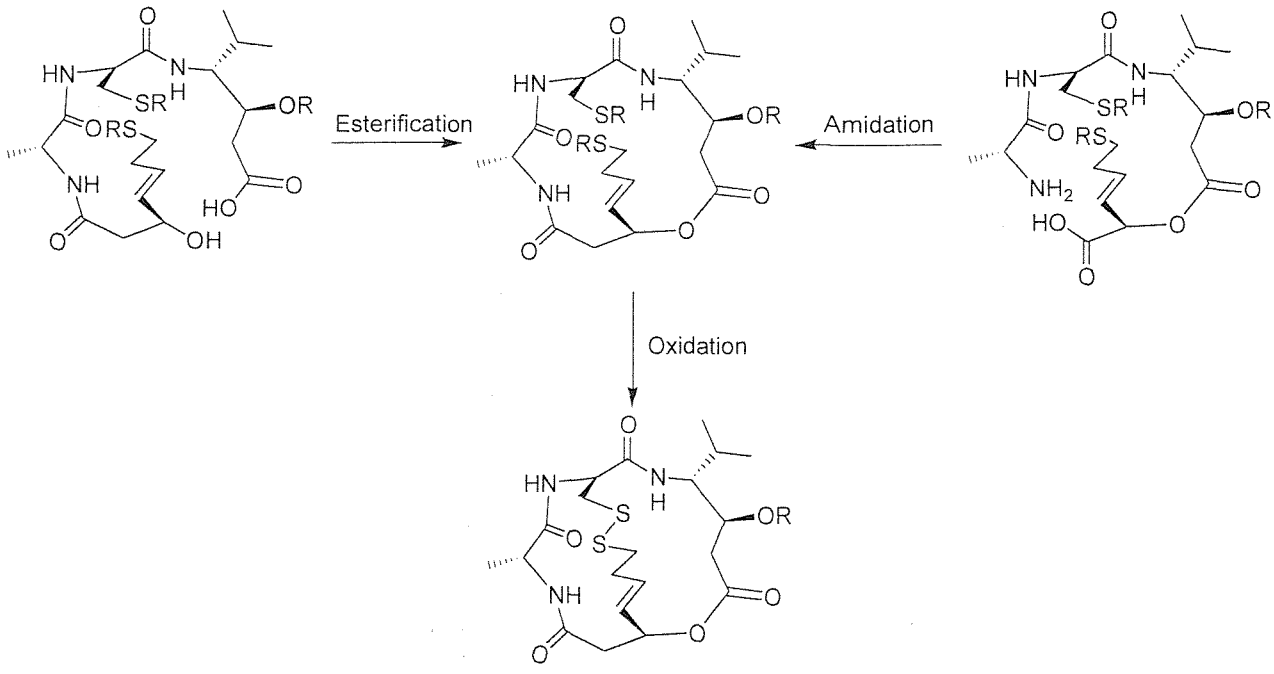


Figure 30. Alternative approaches to cyclization; macrolactonisation or macrolactamization.

On coupling together of the fragments described from the retrosynthesis, two possibilities for cyclization are macrolactonization or macrolactamization to give the cyclic depsipeptide. Macrolactamization can be envisaged using either an activated ester on the acid (thioester or thiazolidine thione) or alternatively standard phosphonium and uronium salt-based peptide coupling conditions. The alternative macrolactonization using Mitsunobu conditions was used by Simon and Janda (Figure 30).[28]

Oxidation of the free or protected thiols can be attempted at any stage after coupling together of both thiol containing fragments and should be successful if the depsipeptide is able to reach the desired conformation. Standard peptide thiol to disulfide oxidizing reagents include DMSO, I_2, O_2, or H_2O_2.

1.6 The synthesis of a structural mimic of spiruchostatin A

The synthesis of a structural mimic of spiruchostatin A where the β-hydroxy acid functionality of the natural product is replaced by the amino acids glycine and D-valine (Figure 31) is to investigate the effect of modification of the structure on the biological activity. This would allow future libraries of similar compounds with simpler structure that are accessible more easily synthetically. Possibilities for further analogues can substitute a range of amino acids for those of the tetrapeptide, maintaining the cysteine residue which is important for the intramolecular disulfide formation.

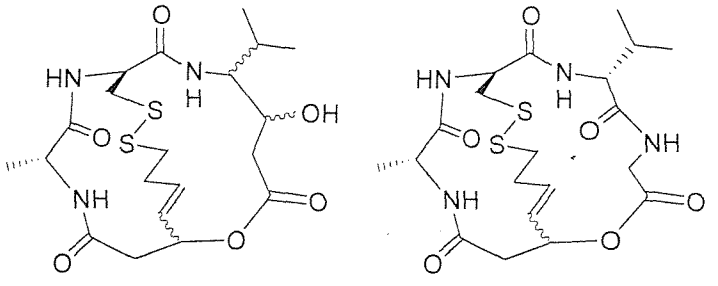
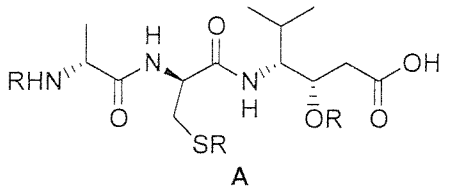
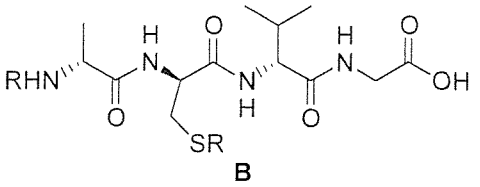


Figure 31. Spiruchostatin A and structural mimic.



A



B

Figure 32. Substitution of subunit A with B to simplify synthesis and investigate biological activity.

The retrosynthesis of the unnatural analogue (Figure 33) remains essentially the same to that proposed for the synthesis of spiruchostatin A. The bicyclic depsipeptide is broken down to the two components **B** and **C**; tetrapeptide fragment and the 3-hydroxy-7-mercapto-4-heptenoic acid respectively. Without the alcohol functionality of the statine, protecting group compatibility issues such as α,β-elimination and cyclization of statine are avoided. The synthesis of spiruchostatin A analogues should thus be simplified.

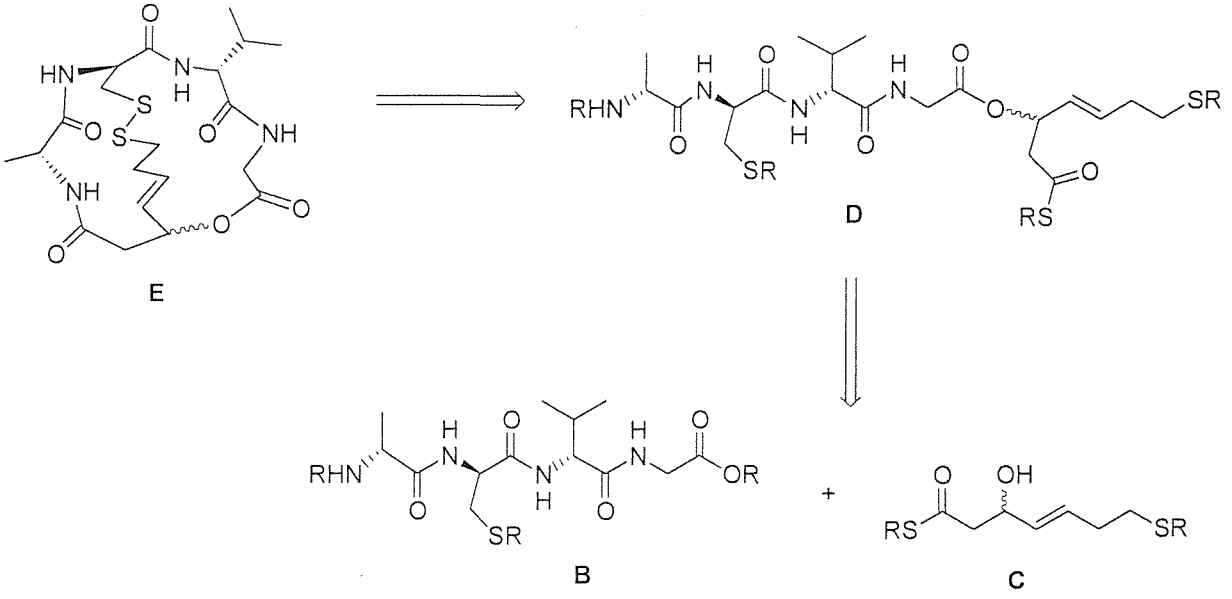


Figure 33. Retrosynthesis of the spiruchostatin A structural analogue.

1.7 References

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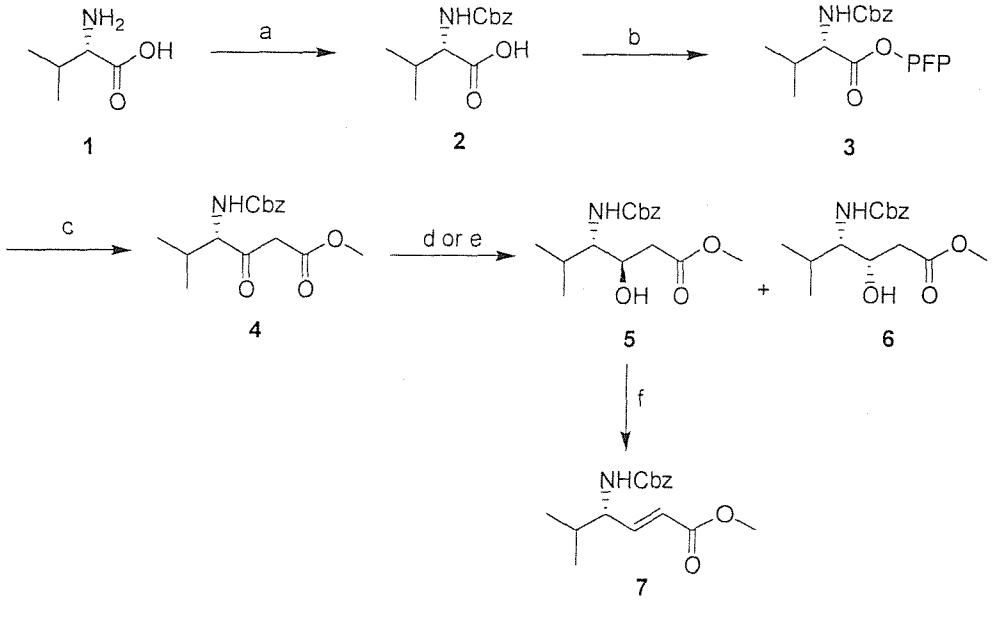
Chapter 2: The Total Synthesis of Spiruchostatin A

Spiruchostatin A was the original synthetic target of our work. This bicyclic peptide differing from the previously synthesised FK228 by the presence of a statine subunit in the molecule posing the synthetic challenges which include avoiding elimination, protecting group migration and intramolecular cyclization. At the commencement of our synthesis there were also several unassigned stereocentres; the β-hydroxy acid which we assumed to be the (*S*)-configuration as in FK228. This we later confirmed by synthesis of the epimer of the natural product. The statine diastereoisomer was also unassigned and this was harder to predict, in fact leading us initially to the synthesis of the opposite configuration. The configuration was assigned during the course of our work as the (3*S*,4*R*). A protecting group strategy was embarked upon that was compatible with those of the greater molecule and that could be carried through to the point at which they are no longer required and could be removed in a selective and controlled manner.

2.1 The synthesis of the (3*R*,4*S*)-statine

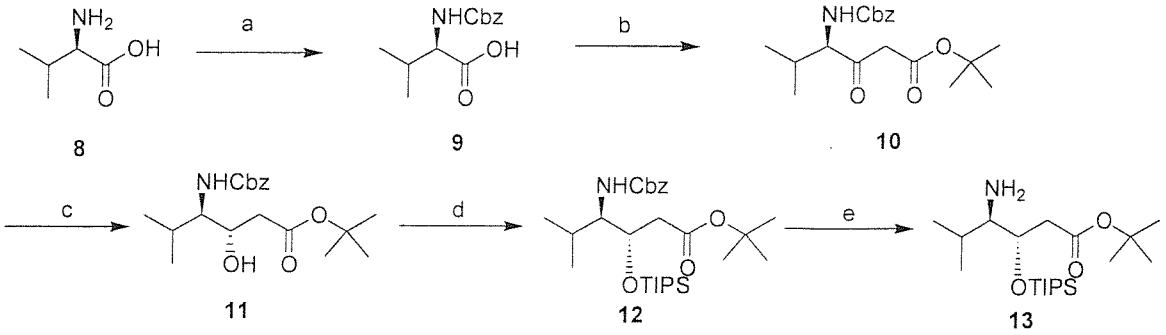


Scheme 1. Reagents and conditions: **(a)** Cbz-Cl, 5% $NaHCO_3$, rt 20 h (85%). **(b)** Pentafluorophenol, EDAC·HCl, DMAP, CH_2Cl_2, 0 °C 0.5 h then rt 4 h (99%). **(c)** $LiCH_2CO_2CH_3$, THF, -78 °C 45 min (24%). **(d)** KBH_4, MeOH, -78 °C to 0 °C 50 min (**5**, 68%). **(e)** $NaBH_4$, THF:MeOH (98:2), -78 °C 2 h (**5**, 61%; **6**, 23%). **(f)** 3 equiv. Ph_3P, 3 equiv. p-NO_2PhCO_2H, 3 eq. DIAD, THF, 0 °C 20 h (64%).

The synthesis of compounds **3**, **4** and **5** was carried out by a modification of the method of Joullié *et al.*[1] L(*S*)-Valine **1** was protected with the benzyloxycarbonyl (Cbz) group to give **2** by the method of Toyooka *et al.*[2] With the amine functionality protected the acid functionality was activated by conversion into a pentafluorophenyl (PFP) ester **3**, followed by condensation with the lithium enolate of methyl acetate forming the β-ketoester **4**. The enolate was prepared from methyl acetate with LDA. Unfortunately, the major product formed from this reaction was not the reported β-ketoester but what we believe to be the methyl ester of *N*-Cbz-L-valine, with the desired product being isolated in a far lower yield. This may have been due to a Claisen self condensation of the methyl acetate occurring during enolate formation.

The obtained β-keto ester **4** was then selectively reduced with KBH_4, followed by crystallisation to afford the desired 3*R*,4*S* alcohol **5** (by NMR a small amount of the 3*S*,4*S* isomer is formed during the reaction). In Shin-ya's original isolation, the relative stereochemistry of the statine unit was undetermined. By comparison of the NMR data of spiruchostatin A with our synthetic statine, it is possible that we would be in a position to settle this issue. Having made the anti diastereoisomer **5**, we now needed a sample of the syn diastereoisomer **6**. First a Mitsunobu inversion was attempted on **5**. However, this resulted in elimination of the alcohol to form **7**. A less stereoselective reduction of the β-keto ester was achieved following the method by Rich *et al.*[3] using $NaBH_4$ to afford both isomers (in a ratio 73:27 3*R*,4*S* to 3*S*,4*S* respectively) which were then readily separated by flash column chromatography. Comparison of the NMR data of the two isomers with the spectroscopic data reported for spiruchostatin A proved inconclusive. Through contact made with Professor Shin-ya it was found that the stereochemistry had since been established to be the 3*S*,4*R* isomer, the absolute stereochemistry of this sub unit being: (3*S*,4*R*)-4-amino-3-hydroxy-5-methylhexanoic acid. With this information in hand, the synthesis of the correct enantiomer commencing with D(*R*)-valine follows (Scheme 2).

2.2 The synthesis of the (3*S*,4*R*)-statine



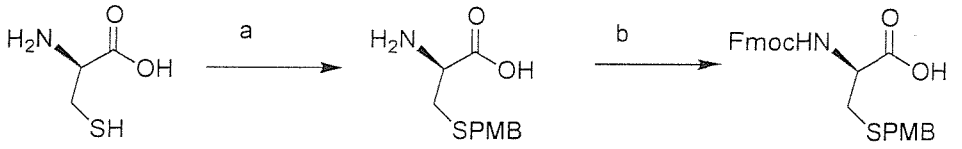
Scheme 2. Reagents and conditions: **(a)** Cbz-Cl, 5% $NaHCO_3$, rt 20 h (85%). **(b)** (i) PFPOH, EDAC·HCl, DMAP, CH_2Cl_2, 0 °C 0.5 h then 20 °C 4 h (ii) $LiCH_2CO_2(CCH_3)_3$, THF, -78 °C 45 min (84%). **(c)** KBH_4, MeOH, -78 °C to 0 °C 50 min (82%). **(d)** TIPSOTf, 2,6-lutidine, CH_2Cl_2, rt 3 h (77%). **(e)** H_2, Pd/C, MeOH/EtOAc 1:1, rt 18 h (95%).

Synthesis of the correct diastereoisomer once again followed the general method of Joullié *et al.*[1] except that a *tert*-butyl ester was used rather than the methyl ester. The *tert*-butyl ester is less likely to undergo ring closure on deprotection of the amine (step e) due to increased steric encumbrance.

D(*R*)-Valine **8** was protected with the benzyloxycarbonyl (Cbz) group to give **9**. With the amine functionality protected, the acid was activated by conversion into a pentafluorophenyl ester and the crude product condensed with the lithium enolate of *tert*-butyl acetate (prepared from *tert*-butyl acetate with LDA) forming the β-ketoester (**10**). The β-keto ester was then selectively reduced with KBH_4, followed by crystallisation to afford the desired alcohol diastereoisomer 3*S*,4*R* **11**. In comparison to the methyl ester, reduction of the β-ketone for the *tert*-butyl ester provided the desired product in higher yield.

The alcohol functionality was protected as the triisopropylsilyl ether (TIPS) (**12**), *via* addition of the respective triflate to the alcohol in 2,6-lutidine. The amine functionality could then be unmasked through a catalytic hydrogenation on Pd/C to furnish the free amine **13** (Scheme 2).

2.3 The synthesis of (2*S*)-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionic acid

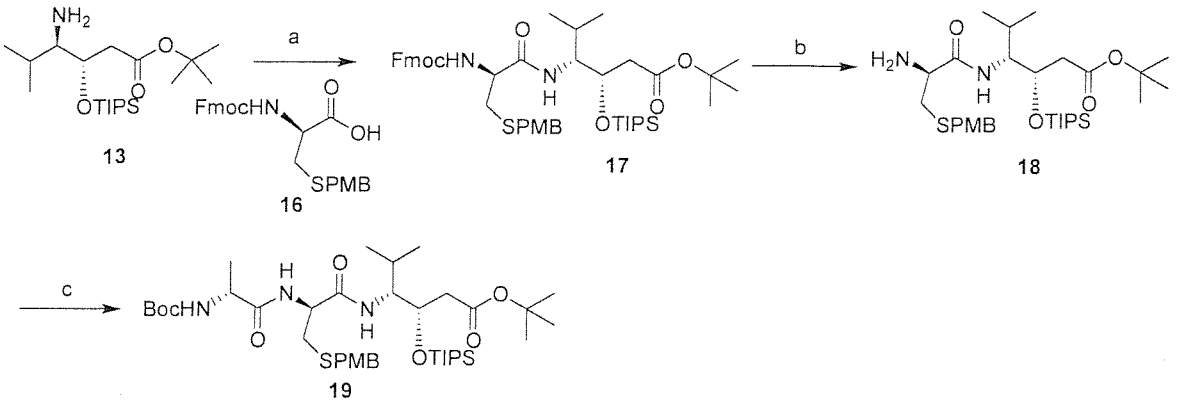


14 **15** **16**

Scheme 3. Reagents and conditions: **(a)** $pMeOC_6H_4CHO$, 1:1 TFA/CH_2Cl_2, 20 °C 1.5 h then Et_3SiH at 0 °C then rt 16 h (91%). **(b)** Fmoc-Cl, 19:1 pyridine/TMS-Cl, 0 °C 3 h (90%).

We initially chose to use the PMB (4-Methoxybenzyl) group as protection on the cysteine thiol moiety; deprotection would then be carried out by oxidation with DDQ. This would be compatible with the acidic conditions to be used to hydrolyse the *tert*-butyl ester. D(*S*)-cysteine **14** was protected on the thiol functionality with the PMB group following the procedure of Richter *et al.*[4] 4-Methoxybenzaldehyde was added to D(*S*)-cysteine in TFA/CH_2Cl_2 to form a thiazolidine. Subsequent treatment with triethylsilane provides the thioether **15** in good yield. Protection of the amine functionality as its Fmoc derivative **16** was achieved following the method of Nambiar *et al.*[5] The PMB protected amino acid was protected on the amino functionality using Fmoc-Cl in a solution of pyridine/TMS-Cl (19:1), (Scheme 3).

2.4 Coupling of peptides to the statine subunit

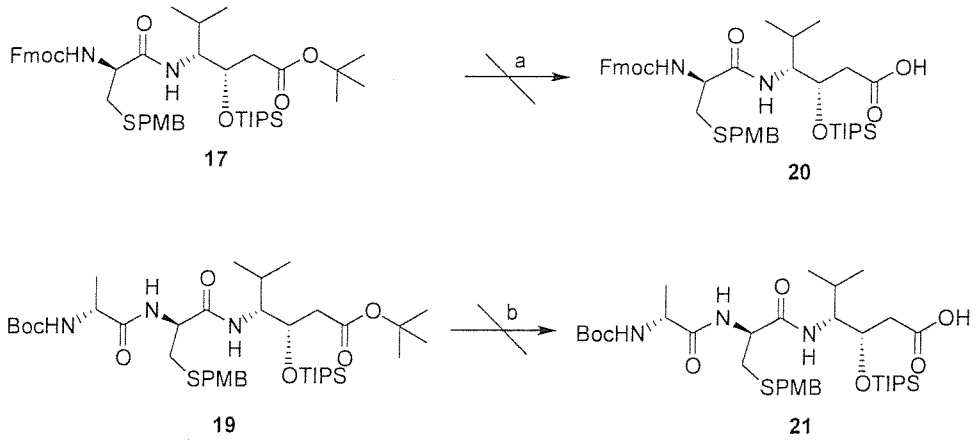


Scheme 4. Reagents and conditions: **(a)** PyBOP, DIEA, CH_3CN, 20 °C 1 h (78%). **(b)** Et_2NH CH_3CN, 20 °C 3 h (97%). **(c)** PyBOP, DIEA, Boc-D-alanine CH_3CN, 20 °C 0.5 h (89%).

The statine free amine **13** and protected cysteine derived acid **16** were coupled together with the reagent PyBOP following general amino acid coupling conditions to successfully furnish the desired amide **17** in reasonable yield. The structure of **17** was confirmed by H-H and H-C NMR correlation spectroscopy. Deprotection of the peptide **17** with diethylamine in acetonitrile gave the free amine **18**. D(*R*)-alanine was then coupled to the amine **18** once again using the PyBOP reagent to give **19** (Scheme 4).

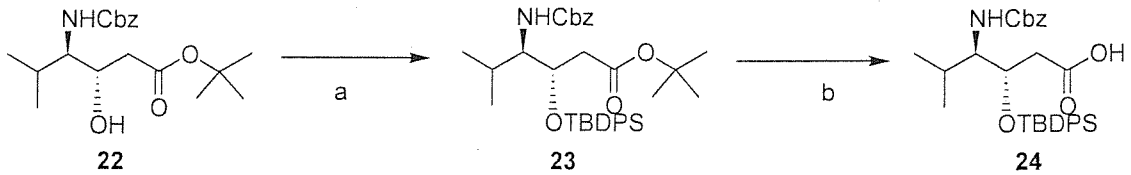
Selective deprotection of the *tert*-butyl ester of **19** failed to give the desired free acid. We believed that it would be possible to selectively hydrolyse the ester functionality in the presence of the TIPS group, this was not however the case. Low concentrations of both HCl and TFA resulted in deprotection of both the TIPS and ester. Heating **17** in TMSOTf[6] also resulted in deprotection of both functionalities. Alternative methods involving hydroxide, both aqueous NaOH in THF/MeOH/H_2O[7] and KOBu^t/H_2O[8] did not effect saponification. Selective deprotection using $CeCl_3$ with NaI[9] possibly resulted in deprotection of the acid labile protecting groups of **19**. Preliminary ^{1}H NMR data showed the loss of both the Boc and TIPS groups. We next turned our attention to acid hydrolysis of the *tert*-butyl ester of **17** (Scheme 5).

2.5 Attempted saponification of the statine *tert*-butyl ester



Scheme 5. Reagents and conditions: **(a)** NaOH, THF/MeOH/H_2O or $KOBu^t/H_2O$ or $CeCl_3$, NaI. **(b)** HCl/CH_2Cl_2 or TFA/CH_2Cl_2 or TMSOTf, CH_2Cl_2, 50 °C.

We next investigated substituting the TBDPS (*tert*-butyldiphenylsilyl ether) protecting group for the TIPS alcohol protecting group as it would be less labile and less susceptible to the possibility of migration.

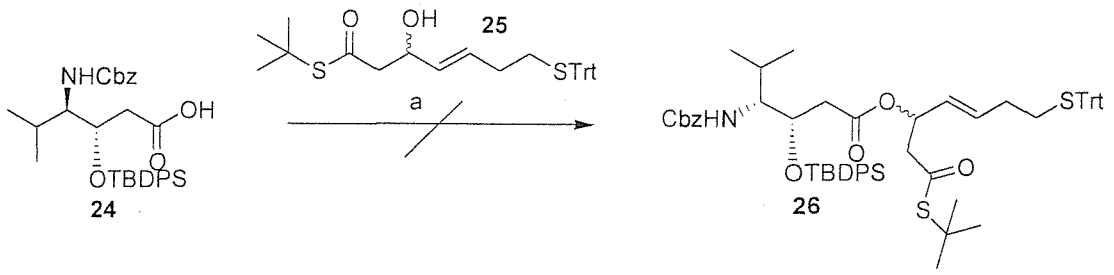


Scheme 6. Reagents and conditions: **(a)** TBDPS-Cl, DMSO, imidazole, 30 h (72%). **(b)** $5\%TFA/CH_2Cl_2$, 0 °C 0.5 h (69%).

The statine hydroxyl **22** was protected with TBDPS group using an excess of reagents TBDPS-Cl (5 eq.) and imidazole (5.1 eq.) over 30 hours by the method of Thomas *et al.*[10] Purification of the product **23** was difficult due to similar physical properties of the desired product to the TBDPS-OH side-product on silica. Deprotection of the *tert*-butyl ester of **23** to give the free acid **24** TFA/CH_2Cl_2[11] resulted in formation of the desired product along

with the undesired by-products of silyl migration: the free alcohol and TBDPS protected acid which were difficult to separate by chromatography. The ratio of desired product compared to by-products was however higher than when the alcohol-protecting group was TIPS (Scheme 6).

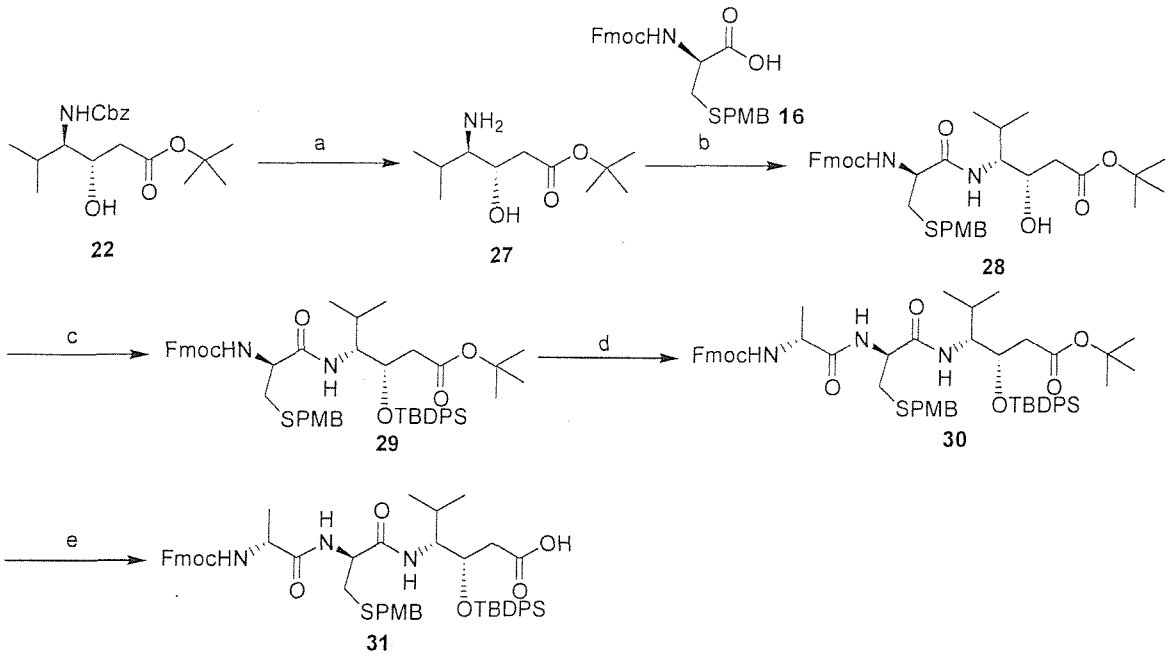
2.6 Attempted esterification of the statine and β-hydroxy thioester



Scheme 7. Reagents and conditions: **(a)** EDAC.HCl, DMAP, CH_2Cl_2, 0 °C.

A carbodiimide coupling was attempted in formation of the ester bond between the statine acid **24** and alcohol **25** (whose synthesis is detailed in chapter 3) using an adaptation of the method of Joullié *et al.*[1] using EDAC and DMAP in dichloromethane. The water-soluble urea by-product can then be removed on work up, which makes analysis of the crude product easier as there are less components present. After chromatography a small amount of product with characteristic NMR to that of the desired product was observed, however due to the limited amount of product obtained further purification proved inconclusive (Scheme 7).

2.7 Coupling of peptides to the statine subunit

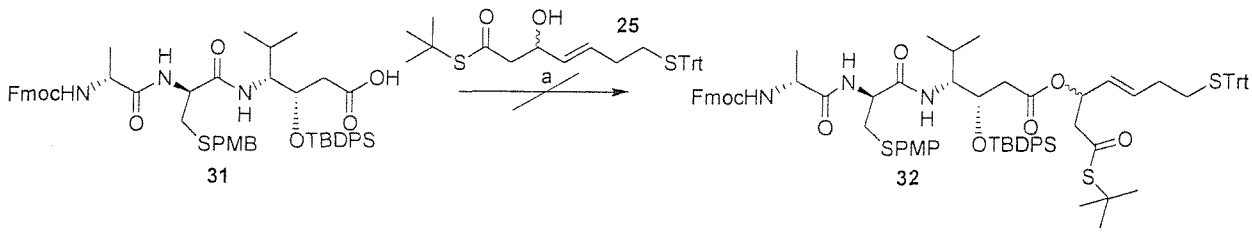


Scheme 8. Reagents and conditions: **(a)** H_2, Pd/C, MeOH/EtOAc 1:1, rt 18 h (95%). **(b)** PyBOP, DIEA, CH_3CN, 20 °C 1 h (78%). **(c)** TBDPS-Cl, DMF, 30 h (71%). **(d)** Et_2NH, CH_3CN, 20 °C 3 h then PyBOP, DIEA, Fmoc-D-alanine CH_3CN, 20 °C 3 h (86%). **(e)** TFA, CH_2Cl_2, 0 °C 0.5 h (58%).

Due to the problems associated with purification of the TBDPS protected alcohol **23** we decided to attempt to remove the Cbz protecting group without first protecting the alcohol. The amine functionality of **22** was unmasked through a catalytic hydrogenation on Pd/C to furnish the free amino-alcohol **27**. The statine amino-alcohol was then coupled to the protected cysteine derived acid **16** with the reagent PyBOP following general amino acid coupling conditions to successfully furnish the desired amide **28** in good yield. The unprotected alcohol did not appear to adversely affect the reaction as would be expected due to the increased reactivity of the amine. The hydroxyl was then protected with TBDPS group using a large excess of reagents TBDPS-Cl (10 eq.) and imidazole (10.5 eq.) over 18 hours to give **29**. The protected alcohol **29** was then easily separated from the excess reagents through chromatography. De-protection of the peptide **29** with Et_2NH in acetonitrile gave the free amine. After removal of the Et_2NH under reduced pressure the

crude product was directly coupled to Fmoc protected D(*R*)-alanine once again using the PyBOP reagent to give **30** in overall good yield. Deprotection of the *tert*-butyl ester of **30** to give the free acid **31** as previously resulted in formation of the desired product along with the undesired by-products of silyl migration: the free alcohol, TBDPS protected acid and hydroxy acid (Scheme 8).

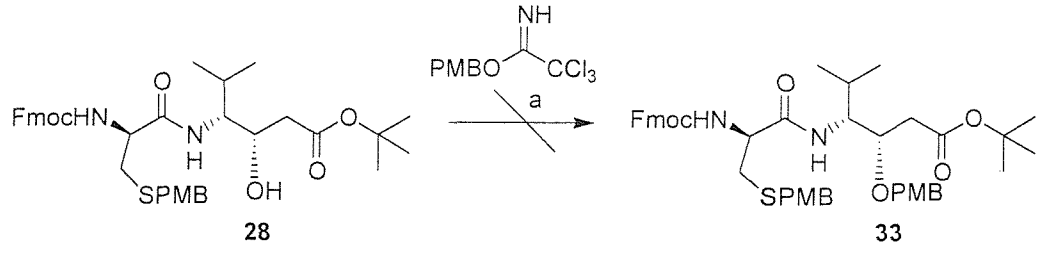
2.8 Attempted esterification of the statine and β-hydroxy thioester



Scheme 9. Reagents and conditions: (**a**) DCC, HOBt, CH_2Cl_2, rt or DCC, DMAP, CH_2Cl_2, rt

Coupling together of the acid **31** with the alcohol **25** were attempted using the carbodiimide DCC initially in the presence of HOBt and then on failure of this method with DMAP.[12] In both cases only the starting alcohol was recovered. It is quite possible that the acid **31** is undergoing degradation on the silica or that the reagents used in esterification result in loss of the Fmoc protecting group (Scheme 9).

2.9 Attempted PMB protection of the statine alcohol

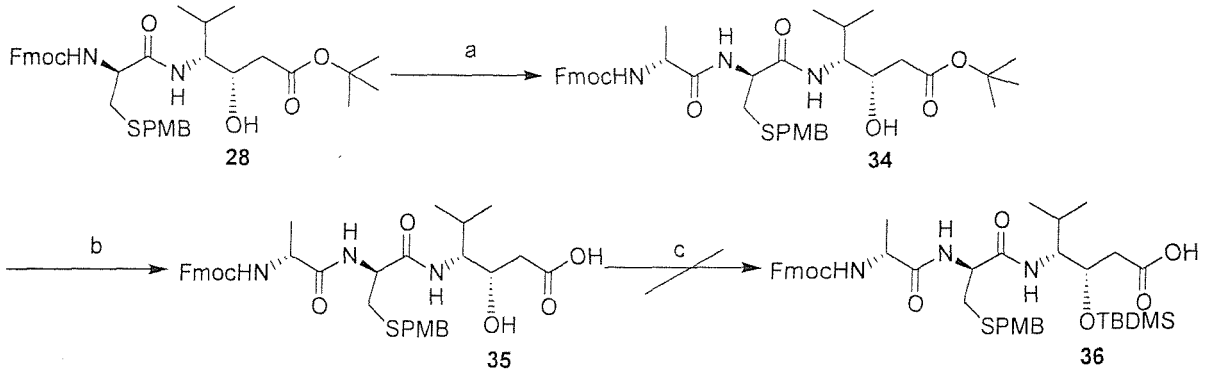


Scheme 10. Reagents and conditions: (**a**) $PMBOCNHCCl_3$, $BF_3.OEt_2$, CH_2Cl_2, 18 h.

Attempts to place a PMB group on the hydroxyl via the conditions of Danishefsky *et al.*[13] initially failed and further experiments discontinued in the light of a report by Thomas *et al.*[10] (Scheme 10). Thomas investigated several protecting groups on a β-hydroxy ester, attempts to de-protect a PMB and SEM group on the hydroxyl resulted in formation of the α,β-unsaturated acid as the major product. TBDPS proved to be the most successful in their synthesis however it was observed that deprotection of the TBDPS was accomplished with greater ease than would be expected.

2.10 Attempted one step statine carboxyl deprotection with concomitant hydroxyl protection

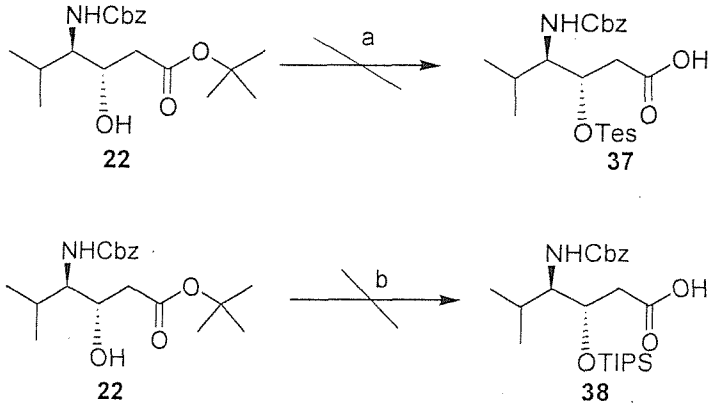
With the continued problem of removing the *tert*-butyl acid protecting group without also deprotecting the β-hydroxy alcohol or elimination or lactamization, we synthesised the β-hydroxy acid without protecting the alcohol. We hoped to later protect up the alcohol functionality once the *tert*-butyl ester had been hydrolysed. With the previously synthesised alcohol **28** deprotection with Et_2NH afforded the amino-alcohol which was followed directly by a PyBOP coupling with Fmoc-D-alanine to form **34**. Removal of the *tert*-butyl ester[11] with TFA/CH_2Cl_2 provided the hydroxy acid **35**. Attempted protection of the hydroxyl functionality with the TBDMS protecting group[14] however failed to discernibly give the correct product **36** (Scheme 11).



Scheme 11. Reagents and conditions: **(a)** Et_2NH, CH_3CN, 3 h then PyBOP, DIEA, Fmoc-D-alanine, CH_3CN, 3 h (66%). **(b)** TFA, CH_2Cl_2, 1 h (64%). **(c)** TBDMS-Cl, imidazole, DMF, 60 h.

We next turned our attention to the simpler statine subunit **22** to attempt a one step protection of the alcohol whilst simultaneously de-protecting the acid *tert*-butyl ester functionality. Firstly using a modification of the method of Danishefsky[15] at 0 °C, TES-OTf and 2,6-lutidine were added to the hydroxy ester in CH_2Cl_2. Analysis by TLC gave exclusively a compound of a higher Rf than that of the starting material which on characterisation we believed had formed the α,β-elimination product brought about by base catalysed elimination, as a mixture of other inseparable products.

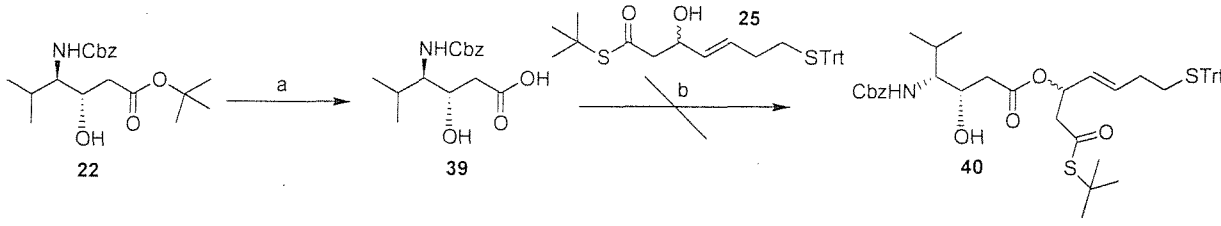
Using an excess of TIPS-OTf it was hoped that the triflic acid produced on formation of the protected alcohol would go on to de-protect the *tert*-butyl ester to give the desired free acid **38** without elimination due to absence of base. A multi-component mixture was produced from which the acid **38** could not be identified (Scheme 12).



Scheme 12. Reagents and conditions: (**a**) TES-OTf. CH_2Cl_2, 2,6-lutidine, 0 °C 18 h. (**b**) TIPS-OTf, CH_2Cl_2, 1 h.

2.11 Coupling of the β-hydroxy ester to the statine subunit without protection of the statine alcohol

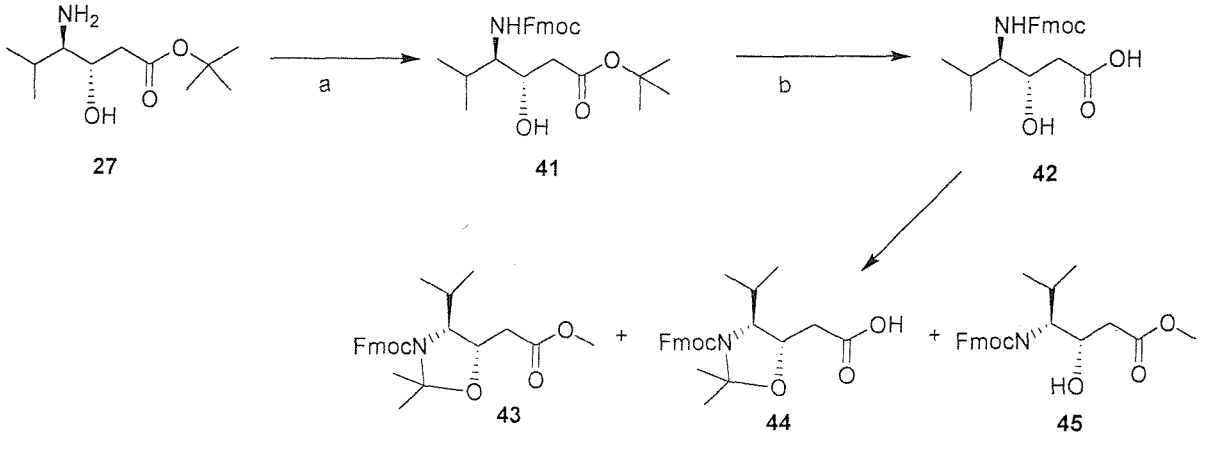
Hydrolysis of the *tert*-butyl ester of **22** followed by coupling to alcohol **25** was attempted using the reagent 1-(2-mesitylene-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) with the base *N*-methyl-imidazole (NMI). On solid phase esterifications of unreactive alcohols, MSNT/NMI mediated couplings have been shown to be high yielding.[16] A small amount of the desired ester **40** was obtained as an inseparable mixture. With prolonged reaction times in an attempt to increase the yield of the desired product, an inseparable mixture of products resulted.



Scheme 13. Reagents and conditions: **(a)** 5%TFA/CH_2Cl_2, 0 °C 0.5 h (quantitative, crude). **(b)** MSNT, NMI, CH_2Cl_2, 18 h.

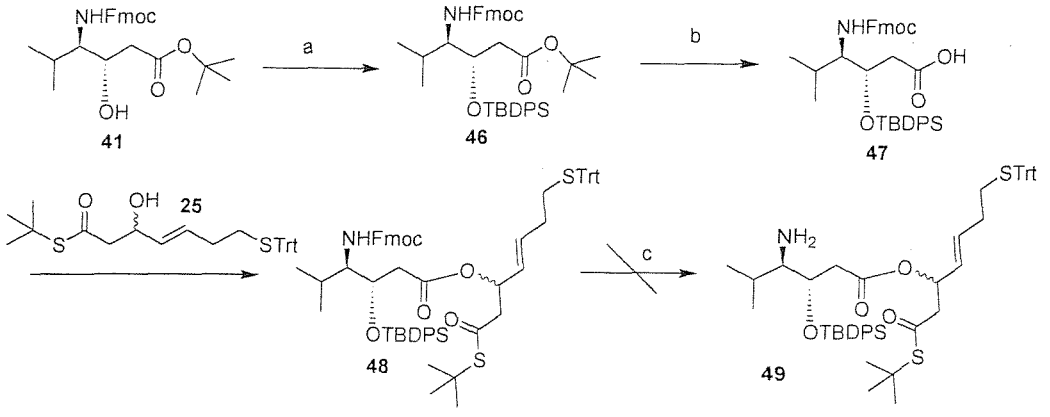
2.12 Acetonide protection of the statine unprotected hydoxyacid.

The use of an acetonide protecting group on the amine and alcohol functionalities was hoped to prevent the occurrence of self-lactamization and would remove steric bulk from the acid. Amino alcohol **27** was protected using Fmoc-Cl[17] and then the *tert*-butyl ester hydrolysed to give the hydroxy acid.[18] Attempted conversion of the hydroxy acid into the dimethyloxazolidine (isopropylidene) with 2,2-dimethoxypropane (DMP)[19] in the presence of *p*-toluene sulfonic acid (PTSA) catalyst resulted in the formation of the methyl ester **45** in addition to the desired protection **44** and both methylation and acetonide protection **43**. Milder reaction conditions (less equivalents of DMP and lower temperature) still favoured methylation. Using anhydrous acetone alone[20] or toluene[21] to effect acetonide formation failed. In the presence of $ZnCl_2$ the desired product was observed by TLC. However on attempted column chromatography on silica gel the desired product decomposed, the predominant product was the starting material (Scheme 14).



Scheme 14. Reagents and conditions: **(a)** Fmoc-Cl, Dioxane/H_2O, $NaHCO_3$, 2.5 h (84%). **(b)** TFA, CH_2Cl_2, 1 h (74%).

2.13 Coupling of the β-hydroxy ester to the statine subunit.

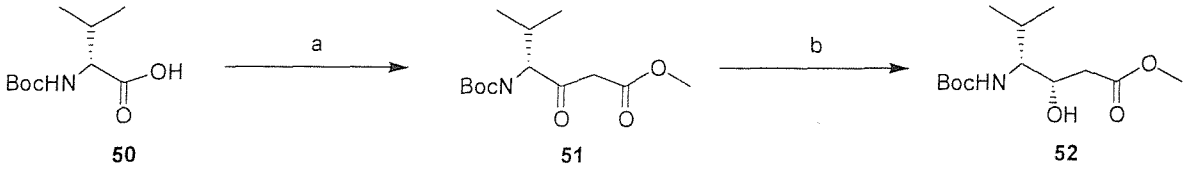


Scheme 15. Reagents and conditions: **(a)** TBDPS-Cl, DMF, 30 h (81%). **(b)** TFA, CH_2Cl_2, Et_3SiH, 3 h (58%). **(c)** DDC/DMAP, DMF 15 min, then **10**, 18 h (58%). **(d)** Et_2NH, CH_3CN.

The hydroxyl of statine **41** was protected with the TBDPS group to give **46**. As previously experienced it was simpler to not purify compound **46** due to the identical elution times by HPLC/chromatography with the excess TBDPS alcohol. On saponification the acid is far more polar and so can easily be separated. The *tert*-butyl ester functionality of the fully protected statine **46** was deprotected with TFA/CH_2Cl_2 and Et_3SiH. Coupling of the hydroxy acid **47** with the alcohol **25** using DCC/DMAP in DMF gave compound **48** and both diastereoisomers where separable by chromatography. Attempted deprotection of **48** using Et_2NH as previously did not give the desired compound **49** (Scheme 15).

2.14 The synthesis of the (3*S*,4*R*)-statine

We then turned our attention back to the use of the methyl ester for statine acid protection in place of the *tert*-butyl ester. Activation of the carboxyl group of Boc-D-valine as the pentafluorophenyl ester and condensing with methyl lithioacetate afforded the β-keto ester **51** (Scheme 16). Stereospecific reduction to statine **52** with KBH_4, generating the desired 3*S*,4*R* isomer as the major product by the method of Joullié *et al.*[1] which could easily be isolated by chromatography. The stereochemical outcome of this reduction is well documented.[3,22,23]



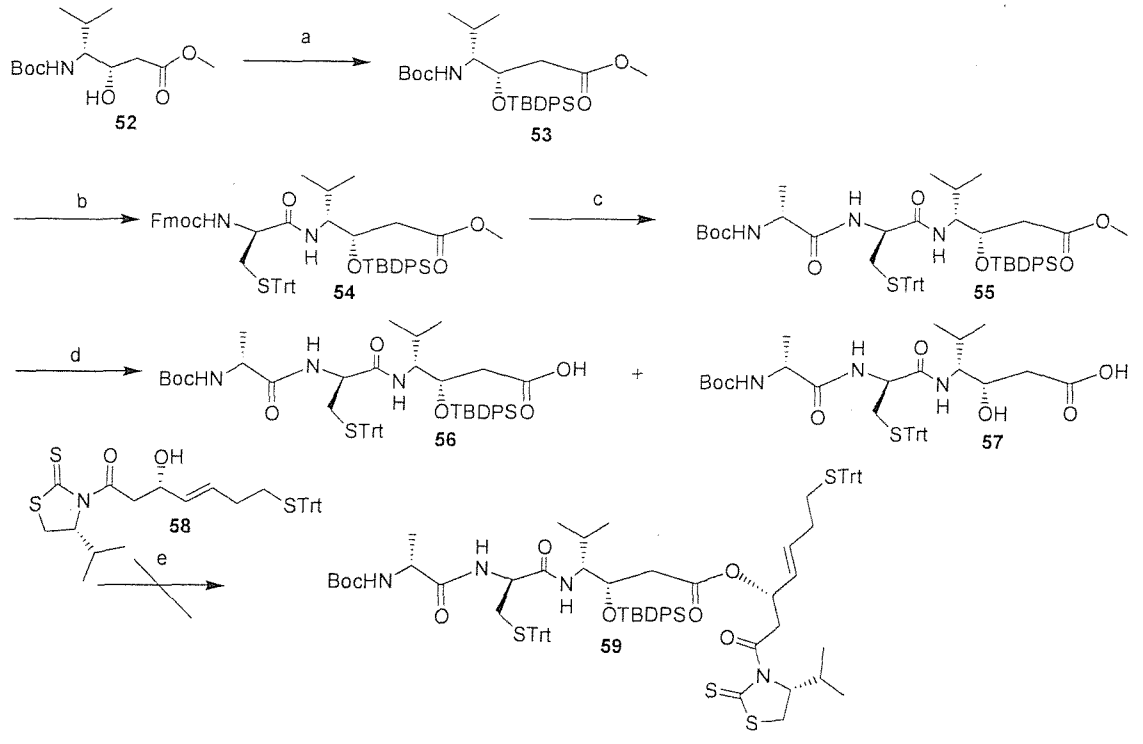
50 **51** **52**

Scheme 16. Reagents and conditions: **(a)** (i) PFPOH, EDAC·HCl, DMAP, CH_2Cl_2, 0 °C 0.5 h then 20 °C 4 h (ii) $LiCH_2CO_2CH_3$, THF, -78 °C 45 min (66%). **(b)** KBH_4, MeOH, -78 °C to 0 °C 50 min (70%).

2.15 Coupling of peptides to the statine subunit and attempted esterification with the β-hydroxy thiazolidinethione

Once again using the TBDPS protecting group on the alcohol, this time in conjuction with a methyl ester protecting the statine carboxyl. The bulky TBDPS group was considered as being the most sterically encumbered and so least likely to migrate. Using excess TBDPS-Cl at a high concentration in DMF yielded the desired silyl protected alcohol **53** after several days stirring at room temperature. Removal of the Boc protecting group selectively in the presence of the TBDPS group followed by successive coupling to Fmoc-(*S*Trt)-D-cysteine to give **54**. Deprotection with Et_2NH/CH_3CN, and coupling to Boc-D-alanine gave the peptide **55**. Saponification of the methyl ester appeared to give the desired acid **56** as the predominant product as a mixture with compound **57** resulting from

loss of the TBDPS protecting group. Attempts at purification by chromatography were not successful due to possible silyl migration to the acid functionality followed by degradation to the acid. Attempted coupling of the crude acid to alcohol **58** failed to give the desired ester **59** (Scheme 17).

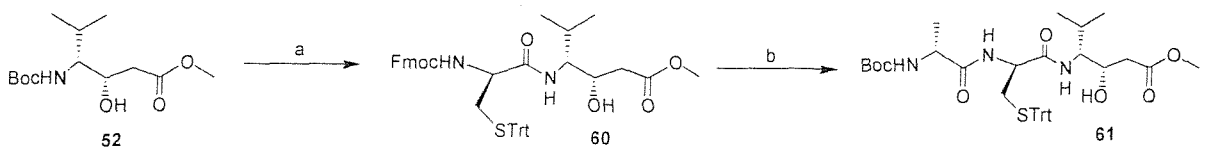


Scheme 17. Reagents and conditions: **(a)** TBDPS-Cl, imidazole, DMF, 20 °C 18 h (91%). **(b)** TFA, CH_2Cl_2, 0 °C 2.5 h then Fmoc-(*S*Trt)-D-cysteine, PyBOP, DIEA, CH_3CN, 20 °C 1 h (81%). **(c)** Et_2NH, CH_3CN, 20 °C 3 h then PyBOP, DIEA, Boc-D-alanine, CH_2Cl_2, 20 °C 3 h (72%). **(d)** LiOH, H_2O:THF, 18 h (47%). **(e)** DDC/DMAP, DCM 15 min, then **58**.

2.16 Investigation into a suitable protection strategy for the statine hydroxyl that is compatible with the adjacent methyl ester saponification

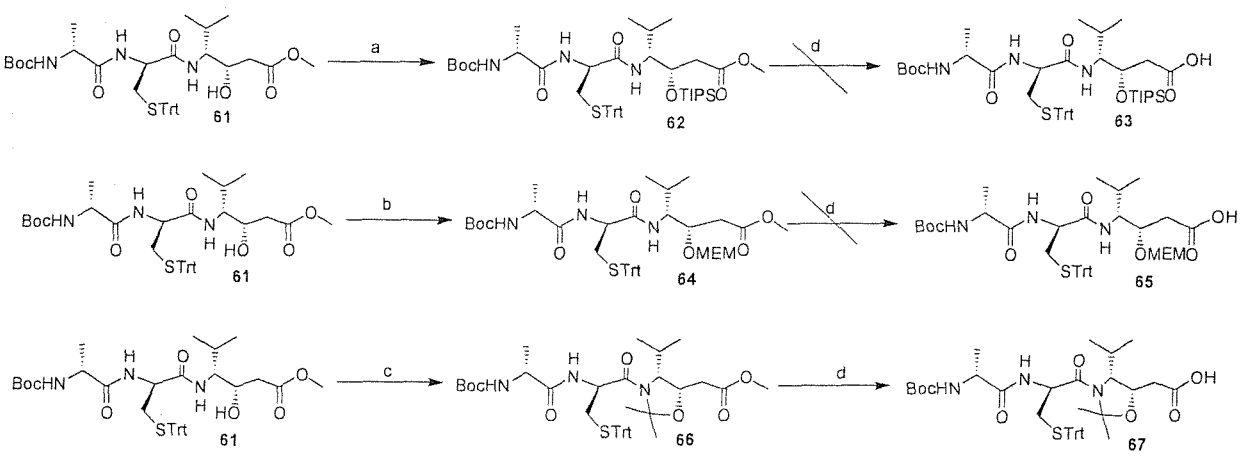
With the lack of success in finding suitable protection for the statine moiety the peptide **61** was synthesised and a variety of alcohol protected compounds were subjected to hydrolysis conditions to investigate which was most stable. Deprotection of the Boc protecting group of **52** with standard acidic conditions and subsequent couplings to the protected amino acid Fmoc-(*S*Trt)-D-cysteine gave **60**, deprotection with Et_2NH/CH_3CN

and coupling to Boc-D-alanine gave the peptide **61** with the free alcohol functionality (Scheme 18).

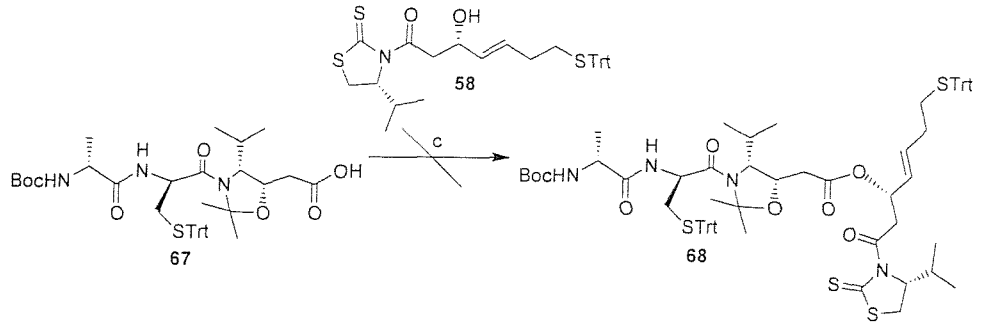


Scheme 18. Reagents and conditions: **(a)** (i) TFA, CH_2Cl_2, 0 °C 0.5 h (82%). (ii) PyBOP, DIEA, Fmoc-(*S*Trt)-D-cysteine, CH_3CN, 20 °C 20 min (82%). **(b)** Et_2NH, CH_3CN, 20 °C 3 h then Boc-D-alanine, PyBOP, DIEA, CH_3CN, 20 °C 1 h (78%).

In parallel, the free alcohol was protected with the TIPS silyl protecting group **62**, the MEM ether **64** and cyclic acetonide **66**. Subjecting these compounds each to standard methyl ester hydrolysis conditions (LiOH in H_2O/THF) afforded the desired acid cleanly only in the case of the acetonide product **66** affording acid **67** in good yield 92% (Scheme 19). This was a very pleasing result, the problem of the β-hydroxyl protection of the statine had apparently been resolved.



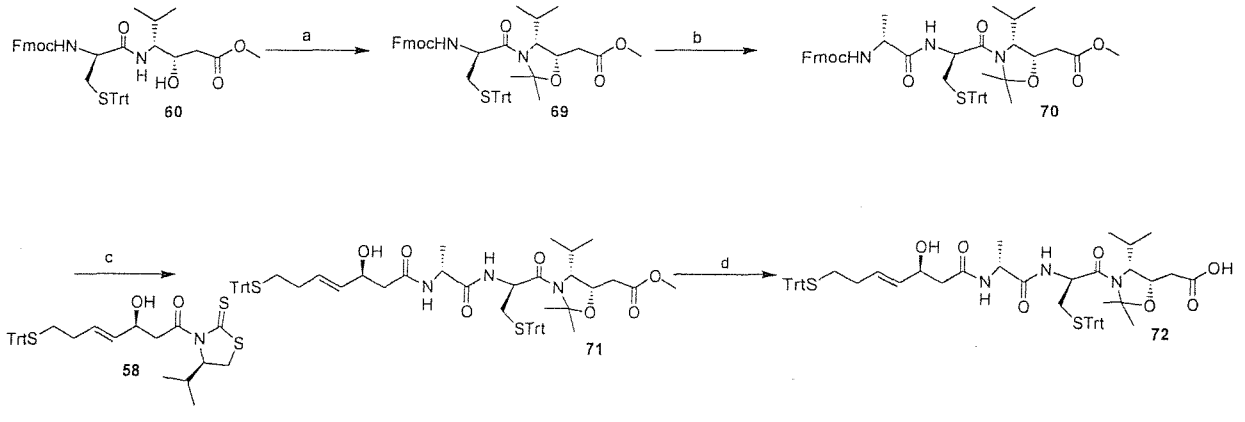
Scheme 19. Reagents and conditions: **(a)** TIPS-OTf, 2,6-lutidine, CH_2Cl_2, rt 3 h (82%). **(b)** MEM-Cl, DIEA, CH_2Cl_2, rt 18 h (71%). **(c)** PTSA, DMP, benzene, reflux 8 h (84%). **(d)** LiOH, H_2O:THF, 0 °C, 18 h (0%, **63**), (0%, **65**), (92%, **67**).



Scheme 20. Reagents and conditions: **(a)** DCC/DMAP, CH_2Cl_2, 15 min, then **58**.

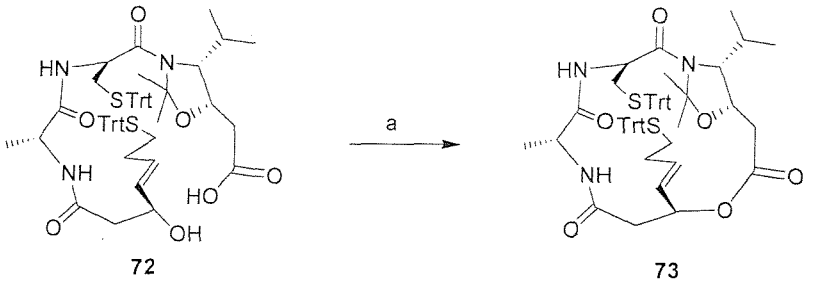
2.17 The use of an acetonide as protecting group for the statine hydroxyl and an attempted synthesis of spiruchostatin A

The acetonide protecting group for the statine hydroxyl and amine functionalities yields a product that would not be prone to any sort of migration to the acid, and may in fact 'free up' the ester to saponification, being of low steric bulk. Saponification of the methyl ester of the acetonide protected peptide yielded the desired acid as the only observable product **67**, cleanly and in high yield. Attempted coupling of this acid to the alcohol functionality of the β-hydroxy thiazolidinethione **58** (preparation discussed in the next chapter) did not yield the desired product **68**, a large mixture of products was observed by NMR (Scheme 20).



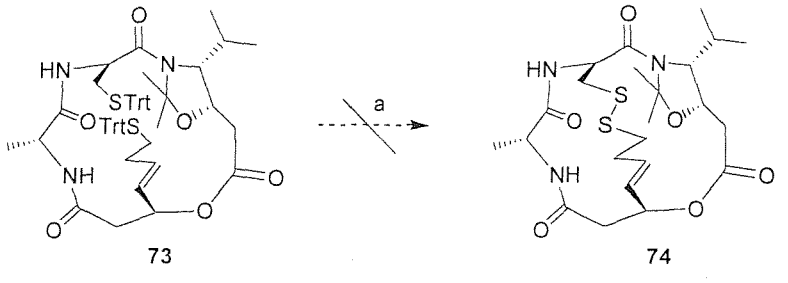
Scheme 21. Reagents and conditions: **(a)** PTSA, DMP, benzene, reflux 8 h (84%). **(b)** Et_2NH, CH_3CN, 20 °C 3 h then PyBOP, DIEA, Fmoc-D-alanine CH_3CN, 20 °C 3 h (86%). **(c)** DMAP, CH_2Cl_2, DIEA, 0 °C then 20 °C 7 h (78%). **(d)** LiOH, H_2O:THF, 5 h (90%).

The clean saponification of the methyl ester with the acetonide protected product gave us encouragement with this protecting group on our statine alcohol functionality. Turning our attention to a final lactonisation approach compound **60** was acetonide protected with DMP/PTSA to give **69**. Fmoc deprotection of **69** followed by coupling to Fmoc-D-alanine using PyBop coupling conditions gave peptide **70**. Removal of the Fmoc protecting group of **70** with Et_2NH gave the free amine which was then coupled to the activated aldol thiazolidinethione **58** with catalytic DMAP affording the protected linear precursor to the natural product **71**. As expected saponification of the methyl ester of **71** with LiOH cleanly gave the free seco-acid **72** (Scheme 21).



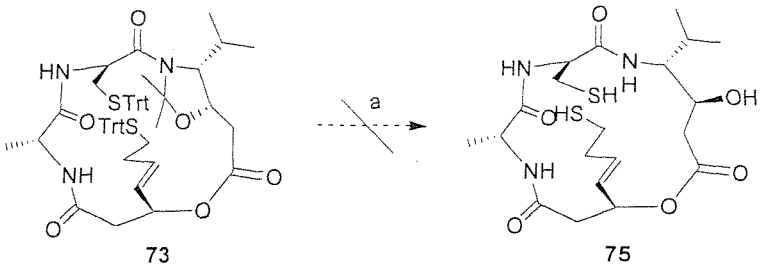
Scheme 22. Reagents and conditions: **(a)** (i) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, THF 0 °C then 20 °C 1 h. (ii) DMAP, Toluene, 50 °C 4 h (72%).

Macrolactonisation of seco-acid **72** afforded **73** in good yield 72% as the major product using the Yamaguchi method.[24-26,27] Addition of the hydroxy acid **72** to the activating agent 2,4,6-$Cl_3C_6H_2COCl$, and Et_3N which was then added dropwise over 2 hours to a vigorously stirring solution of DMAP (0.005 M with respect to the hydroxy acid) at 50 °C (Scheme 22).



Scheme 23. Reagents and conditions: **(a)** I_2, 10% $MeOH/CH_2Cl_2$, rt.

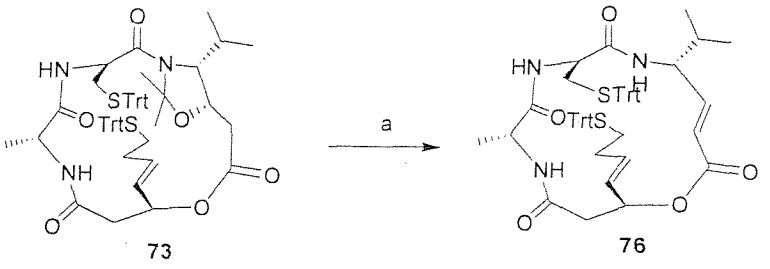
Oxidation of the trityl protected thiol **73** to the cyclic disulfide **74** was attempted using various concentrations of iodine at high dilution[28] however none of the desired disulfide was observed (Scheme 23).



73 75

Scheme 24. Reagents and conditions: **(a)** various H^+/solvent or H^+/H_2O or $TFA/CH_2Cl_2/Et_3SiH$.

All attempts at removal of the acetonide protecting group failed using a variety of conditions which would be expected to remove the acetonide ($pTsOH/CH_3OH$[29] AcOH/HCl,[30] $AcOH/H_2O$[30] $HCl/MeOH/H_2O$[20]) and possibly also the trityl groups. With prolonged reaction times only loss of trityl or degradation of the molecule was observed. Global deprotection of both trityl and acetonide was attempted in a variety of strongly acidic conditions such as TFA/MeOH 9:1[19] and TFA/CH_2Cl_2.[31,32] Selective deprotection of acetonides has been accomplished using Lewis acids. Attempts at the selective deprotection of **73** using a variety of Lewis acids, $BF_3.AcOH$,[33] ferric chloride adsorbed on silica gel,[34] $SnCl_2$ and BCl_3 in CH_2Cl_2,[35] in all cases led to either the deprotection of the trityl protecting groups or a mixture of inseparable products (Scheme 24).

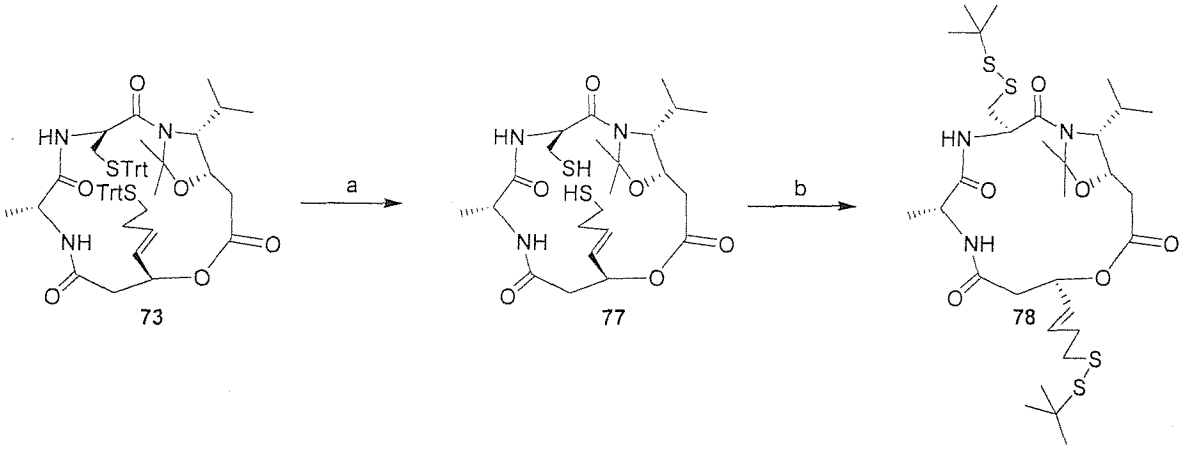


73 76

Scheme 25. Reagents and conditions: **(a)** TBAF, THF, rt 3 h (87%).

Treatment of the acetonide protected cyclic peptide **73** with TBAF in THF gave the elimination product **76** in high yield (87%). Attempts to oxidise the protected dithiol to give the intramolecular disulfide using various concentrations of iodine at high dilution[28] failed (Scheme 25).

2.18 Conversion of the acetonide protected spiruchostatin A into a mixed disulfide prodrug



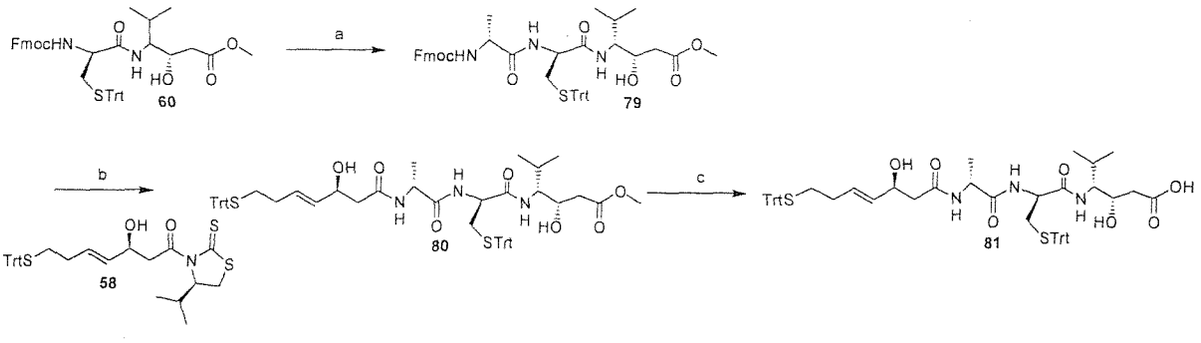
73 77 78

Scheme 26. Reagents and conditions: **(a)** TFA, Et_3SiH, CH_2Cl_2, 0 °C 15 min (90%). **(b)** ^tBuSH, Et_3N, O_2, CH_2Cl_2, rt 10 days (54%).

Due to the inability to form the intramolecular disulfide for either compound **73** or the free dithiol **77**, we attempted formation of a mixed disulfide as a potential pro-drug HDAC inhibitor. Conversion to the reduced form would occur in vivo from glutathione reducing activity. Sulfur containing cyclic peptides as dimers and mixed disulfide hybrids were recently synthesised based on the CHAP 31 (cyclic tetrapeptide) scaffold with a sulfhydryl zinc binding moiety based on the mechanism of FK228 activity formation of a mixed

disulfide as with the cyclic tetrapeptides.[24] These hybrid molecules showed potent activity and good selectivity for HDAC-1. The free dithiol **77** was subjected to excess *tert*-butylthiol, triethylamine under an oxygen atmosphere for 10 days[36] to give the mixed disulfide **78** (Scheme 26).

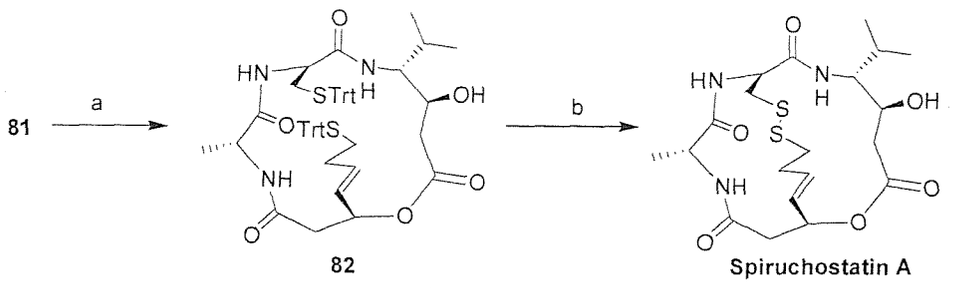
2.19 Synthesis of spiruchostatin A without statine hydroxyl protection



Scheme 27. Reagents and conditions: **(a)** (i) 5% Et_2NH, CH_3CN, 20 °C 3 h. (ii) Fmoc-D-alanine PyBOP, DIEA, CH_3CN, 20 °C 1 h (67%). **(b)** DMAP, CH_2Cl_2, DIEA, 0 °C then 20 °C 7 h (63%). **(c)** LiOH, H_2O:THF, 5 h (93%).

Attempting the synthesis of spiruchostatin A without protection of the statine β-hydroxy alcohol was performed starting with the previously synthesised peptide **60**. Removal of the Fmoc protecting group from **60** with Et_2NH in acetonitrile and coupling with Fmoc-D-Alanine by the PyBop protocol gave the peptide **79**. Removal of the Fmoc protecting group from **79** with Et_2NH and coupling the crude amine with activated ester **58** in the presence of a catalytic amount of DMAP furnished ester **80**. Saponification of the methyl ester of the diol **80** with LiOH in THF/H_2O afforded the desired hydroxy acid **81** (Scheme 27). Macrolactonisation using the Yamaguchi method[24-27] proved difficult affording a multitude of minor products and a single major product, assumed to be the macrocycle **82.** The yield for the macrolactonisation was low and the macrocycle obtained impure, co-eluting in a variety of solvent mixtures with an unknown major impurity. The crude product was therefore taken onto the next step, treating with I_2 in MeOH at high dilution[28]

oxidised the trityl protected thiols to the disulfide affording the natural product spiruchostatin A (Scheme 28).

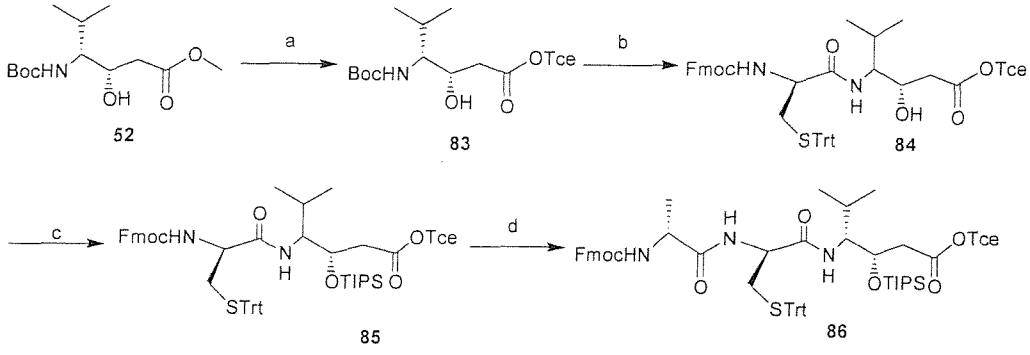


Scheme 28. Reagents and conditions: **(a)** (i) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, THF 0 °C then 20 °C 1 h. (ii) DMAP, Toluene, 50 °C 4 h (20%). **(b)** I_2, 10% MeOH/CH_2Cl_2, 20 mins (84%).

2.20 Synthesis of spiruchostatin A with a neutral trichoroethyl ester in conjunction with TIPS alcohol statine protection strategy

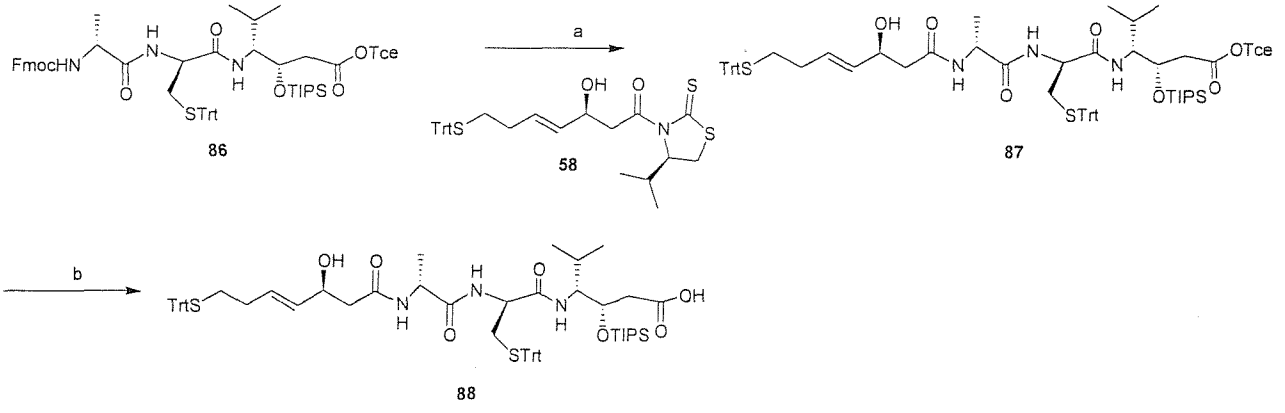
With a sample of spiruchostatin A now in hand, we sought to still find a compatible series of protecting groups so as to afford the desired natural product without the numerous by-products associated with having the free hydroxyl causing unwanted reactions in the macrolactonization. Acid labile silyl group protection of the statine hydroxyl is convenient and compatible with our synthetic strategy however in strongly basic conditions required for saponification of the adjacent methyl ester silyl migration to the acid and eventual degradation to the hydroxy acid was observed. The use of a trichloroethyl ether which can be removed under near neutral pH 6.5-7.0 conditions was expected to minimise silyl migration. The 3*S*,4*R* statine isomer **52** formed as previously by a modification of the method of Joullié *et al.*[1] with the stereoselective reduction of the β-keto ester is well documented.[3,22,23] Saponification followed by re-esterification with excess trichloroethanol gave **83**. Removal of the Boc protecting group from **83** with TFA followed by coupling to Fmoc-(*S*Trt)-D-cysteine with the PyBop protocol gave **84**. The alcohol functionality was protected with TIPS group by TIPS-OTf, 2,6-lutidine to give **85**.

Removal of the Fmoc protecting group with Et_2NH and coupling with Fmoc-D-alanine by the PyBop protocol gave the peptide **86** (Scheme 29).

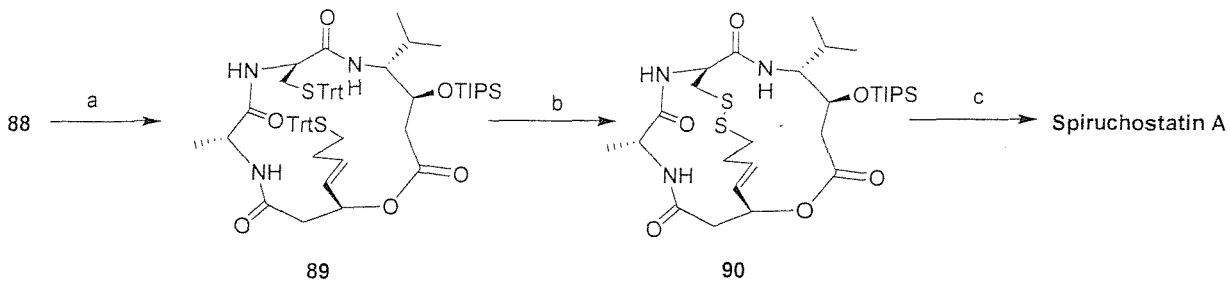


Scheme 29. Reagents and conditions: **(a)** (i) LiOH, 4:1 THF/H_20, 1 h 0 °C; (ii) TceOH, DCC, DMAP, CH_2Cl_2, 0 °C to rt 18 h (95%). **(b)** (i) TFA, CH_2Cl_2, 3 h, (ii) PyBOP, DIEA, Fmoc-(*S*Trt)-D-cysteine, CH_3CN, 20 °C 20 min (74%). **(c)** TIPS-OTf, 2,6-lutidine, CH_2Cl_2, rt 3 h (93%). **(d)** (i) 5% Et_2NH, CH_3CN, 20 °C 3 h. (ii) Fmoc-D-alanine, PyBOP, DIEA, CH_3CN, 20 °C 1 h (82%).

The aldol product **58** containing the thiazolidinethione functionality mildly activates towards nucleophilic substitution, cleavage and reduction.[37,38] Removal of the Fmoc protecting group from **86** with Et_2NH and coupling the crude amine with activated ester **58** in the presence of a catalytic amount of DMAP smoothly furnished ester **87**. Selective cleavage of the Tce group[14] with Zn/NH_4OAc pH 6.5-7.0 afforded the desired hydroxy acid **88** in an acceptable yield, near neutral conditions being important to minimise silyl group migration to the acid which we observed under basic and acid media (Scheme 30).



Scheme 30. Reagents and conditions: **(a)** (i) 5% Et_2NH CH_3CN, 20 °C 5 h. (ii) DMAP, CH_2Cl_2, 0 °C then 20 °C 7 h (84%). **(b)** Zn/NH_4OAc, pH 6.5-7.0, 20 °C 5 h (71%).

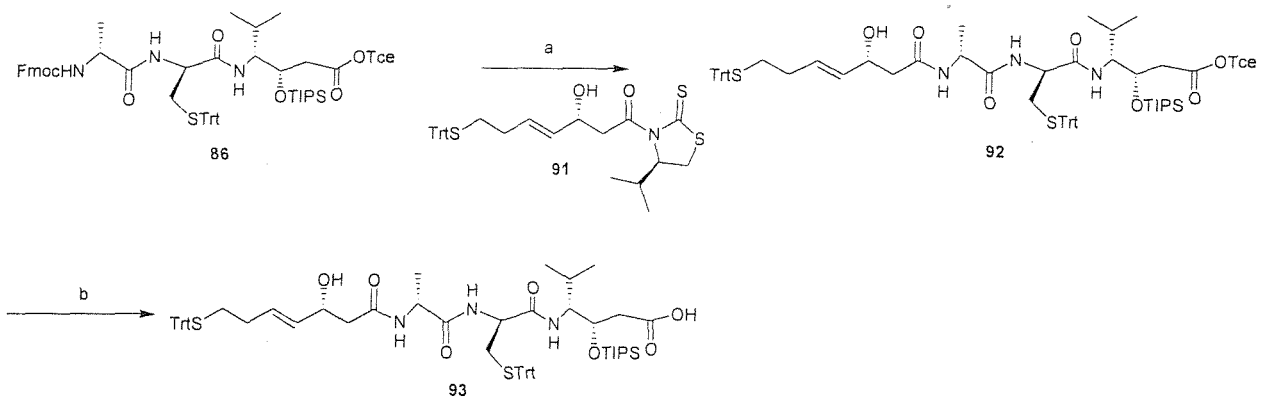


Scheme 31. Reagents and conditions: **(a)** (i) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, THF 0 °C then 20 °C 1 h. (ii) DMAP, Toluene, 50 °C 4 h (53%). **(b)** I_2, 10% MeOH/CH_2Cl_2, 20 mins (84%). **(c)** HCl, EtOAc, -30 °C to 0 °C 3 hours (77%).

Macrolactonisation of seco-acid **88** afforded **89** in reasonable yield for this type of peptide containing system using the Yamaguchi method.[24-27] Oxidation of the trityl protected thiols to the cyclic disulfide **90** proceeded in good yield using 12 equivalents of iodine at high dilution.[28] Finally deprotection of the TIPS group using HCl(g) in EtOAc afforded spiruchostatin A (Scheme 31). The ^1H NMR spectra was very similar to that reported in the literature.[39] Differences were observed for the labile protons from the NH alanine and statine hydroxyl. This was shown by addition of CD_3OD to the sample in $CDCl_3$ causing a large shift of these protons (1% CD_3OD: ^1H alanine from 5.86 to 6.82 ppm and decrease in integral value, ^1H hydroxyl from 2.94 ppm to completely exchanged).

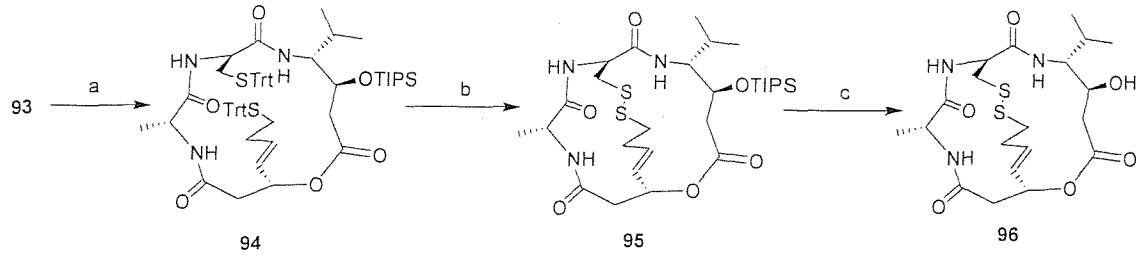
2.21 The synthesis of epi-spiruchostatin A

Following the protocol for that of the synthesis of spiruchostatin A with the peptide **86** and the corresponding epimer which is the (*R*)-isomer of the activated hydroxy ester **91**.



Scheme 32. Reagents and conditions: **(a)** (i) 5% Et_2NH CH_3CN, 20 °C 5 h. (ii) DMAP, CH_2Cl_2, 0 °C then 20 °C 7 h (46%). **(b)** Zn/NH_4OAc , THF 20 °C 5 h (72%).

Removal of the Fmoc protecting group from **86** with Et_2NH and coupling the crude amine with activated ester **91** in the presence of catalytic DMAP smoothly furnished ester **92.** Selective cleavage of the Tce group with Zn/NH_4OAc pH 6.5-7 afforded the desired hydroxy acid **93** (Scheme 32).



Scheme 33. Reagents and conditions **(a)** (i) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N CH_3CN, THF 0 °C then 20 °C 1 h. (ii) DMAP, toluene, 50 °C 4 h (51%). **(b)** I_2, 10% MeOH/CH_2Cl_2, 20 mins (58%). **(c)** HCl, EtOAc, -30 °C to 0 °C 3 hours (76 %).

Macrolactonisation of the seco-acid **93** afforded **94** using the Yamaguchi method.[24-27] Oxidation of the trityl protected thiols to the cyclic disulfide **95** proceeded in good yield using 12 equivalents of iodine at high dilution.[28] Finally deprotection of the TIPS group using HCl(g) in EtOAc afforded epi-spiruchostatin A **96** (Scheme 33).

2.22 Summary

A journey into the discovery of compatible protection of precursors to spiruchostatin A, led us ultimately to the first reported synthesis of the natural product, its epimer at the previously unassigned stereocentre and a mixed disulfide acetonide derived analogue. Our synthesis unambiguously confirms the complete structure of spiruchostatin A. A noteworthy feature is the dual role of the Nagao auxiliary[40] as a chiral auxiliary for accomplishing acetate aldols and as an acylating agent.

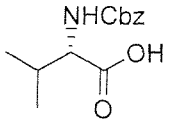
The biological results were very pleasing. Spiruchostatin A inhibited the growth of breast cancer cells with an IC_{50} of approximately 10 nM, compared to 100 nM for the HDAC inhibitor trichostatin A. Epi-spiruchostatin A was essentially inactive at 10 μM, highlighting the importance of (*S*) stereochemistry in the β-hydroxy acid for favorable interactions with residues around the rim of HDAC active sites. This observation parallels[41] trichostatin A, where the unnatural enantiomer is a significantly less active HDAC inhibitor.

2.23 Experimental

General Methods

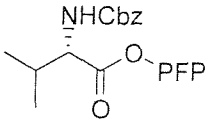
Glassware was oven dried before use. Reactions were carried out under dry nitrogen or argon atmosphere unless otherwise stated. For air sensitive reactions the apparatus was alternatively evacuated with an oil pump and filled with argon three times. All chemicals were obtained from commercial suppliers and used without further purification except where stated. CH_2Cl_2 and MeOH were distilled from CaH_2. THF was distilled over Na wire and benzophenone. Toluene was distilled from sodium immediately before use. TLC was carried out on precoated plates: analytical (Merck; Kieselgel 60 F_{254}, aluminium backed), spots visualised with UV light and phosphomolybdic acid solution or $KMnO_4$ solution. Column chromatography was performed with silica (Apollo Scientific; 40-63 micron) unless stated otherwise. Infrared spectra (IR) were recorded neat with maxima (ν_{max}) on a Nicolet 400 FT-IR fitted with a Thunderdome HATR Ge crystal or NaCl. Mass spectra were recorded on a Navigator open access Electrospray. ^{1}H-NMR spectra were recorded with chemical shift reported in parts per million relative to tetramethylsilane (δ_H = 0.00 ppm) or residual $CHCl_3$ (δ_H = 7.27 ppm). ^{13}C NMR spectra were recorded with chemical shift reported in parts per million relative to tetramethylsilane (δ_C = 0.00 ppm) or $CDCl_3$ (δ_C = 77.15 ppm). Optical rotations were recorded on an Optical Activity POLAR 2001. Melting points are uncorrected.

(*S*)-2-Benzyloxycarbonylamino-3-methyl-butyric acid (2)



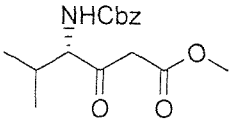
To a stirred solution of L-valine (20.0 g, 171.0 mmol) in 5% $NaHCO_3$ solution (800 mL) was added benzyl chloroformate (24.4 mL, 171.0 mmol). After stirring for 20 hours at room temperature the solution was washed with ether, the aqueous layer was then acidified with 20% HCl to pH 5, extracted with CH_2Cl_2,(4 x 500 mL) dried and concentrated to give **2** as a white solid (36.5 g, 85%): mp 50-51 °C (lit.[2] mp 47.5-50 °C); $[\alpha]^{22}_D$ = +15.2 (c 0.50, $CHCl_3$). IR ν_{max} 3410, 1721, 1653 cm^{-1}; ^1H NMR 300 MHz 7.37 (m, 5H), 5.34 (d, *J* = 9.0 Hz, 1H), 5.13 (s, 2H), 4.36 (dd, *J* = 9.0 Hz, 4.5 Hz, 1H), 2.24 (m, 1H), 1.08 (d, *J* = 6.7 Hz, 3H), δ 0.94 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR 75 MHz 177.1, 156.6, 136.2, 128.7, 128.4, 128.3, 67.4, 59.0, 31.2, 19.2, 17.5; MS *m/z* 525.4 $(2M + Na)^+$, 798.6 (3M + 2Na). The material was spectroscopically identical to that reported in the literature.[2]

(*S*)-2-Benzyloxycarbonylamino-3-methyl-butyric acid pentafluorophenyl ester (3)



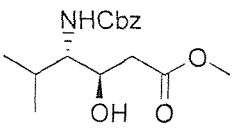
To a solution of acid **2** (35.34 g, 133.0 mmol) in anhydrous CH_2Cl_2 (400 mL) at 0 °C, was sequentially added pentafluorophenol (PFPOH) (25.74 g, 140.0 mmol), EDAC•HCl (30.64 g, 160.0 mmol) and DMAP (3.25 g, 27.0 mmol). The reaction mixture was stirred at 0 °C for half an hour, and then at rt for 4 hours. The reaction mixture was diluted with CH_2Cl_2 (400 mL) and the organic layer washed sequentially with 10% HCl (100 mL), 5% $NaHCO_3$ (100 mL) and saturated NaCl (100 mL) solutions. The organic layer was dried (Na_2SO_4), filtered and concentrated to give a slightly yellow oil which was used without further purification (55.1 g, 99%): IR ν_{max} 3348, 1788, 1721 cm^{-1}; ^1H NMR 300 MHz 7.38 (m, 5H) 5.32 (d, J = 7.8 Hz, 1H), 5.17 (s, 2H), 4.70 (m, 1H), 2.37 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H), 1.04 (d, J = 6.9 Hz, 3H); ^{19}F NMR 282 MHz: 10.2, 4.8, 0.1.

(*S*)-4-Benzyloxycarbonylamino-5-methyl-3-oxo-hexanoic acid methyl ester (4)



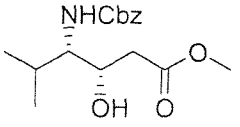
To a stirred solution of LDA (492.0 mmol in 250 mL anhydrous THF) at -78 °C was added methyl acetate (39.2 mL, 492.0 mmol) *via* syringe. After stirring for 1 hour, the enolate was added to a stirred solution of **3** (55.1 g, 132 mmol) in THF (250 mL), at -78 °C. The reaction mixture was stirred for 45 minutes at the same temperature, and then carefully quenched with saturated aqueous NH_4Cl (500 mL) at –78 °C. After warming to room temperature, the THF was removed on a rotary evaporator and the resulting aqueous solution was extracted with CH_2Cl_2 (3 x 500 mL). The combined organic layers were then washed with 10% aqueous HCl (400 mL), 5% aqueous $NaHCO_3$ (400 mL) and saturated aqueous NaCl (400 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to afford a yellow oil which was purified by flash chromatography (eluent 5-25% EtOAc/hexane) to give the β–keto ester **4** as a slightly yellow oil (9.82 g, 24%): $[\alpha]^{22}_D$ +22.5 (c 0.50, $CHCl_3$); IR ν_{max} 3353, 1716, 1627 cm^{-1}; ^1H NMR 300 MHz 7.34 (m, 5H), 5.46 (d, J = 12.0 Hz, 1H), 5.10 (s, 2H), 4.42 (dd, J = 4.4 Hz, 1H), 3.71 (s, 3H), 3.56 (s, 1H), 3.34 (s, 1H), 2.24 (m, 1H), 1.22 (d, J = 6.8 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H); ^{13}C NMR 75 MHz 201.8, 167.2, 156.6, 136.3, 128.7, 128.4, 128.3, 67.3, 64.9, 52.6, 47.0, 19.5, 16.7; (spectrum contains EtOAc and CH_2Cl_2), MS *m/z*: 330.2 $(M + Na)^+$.

(3*R*,4*S*)-4-Benzyloxycarbonylamino-3-hydroxy-hexanoic acid methyl ester (5)



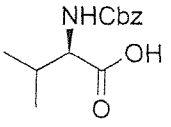
To a stirred solution of the β–keto ester **4** (2.79 g, 8.7 mmol), in anhydrous MeOH (30 mL) at –78 °C was added potassium borohydride (1.64 g, 30.5 mmol) in portions. The reaction mixture was stirred at –78 °C for 10 min, warmed to –20 °C for 30 min, and then to 0 °C for 10 min. The reaction mixture was quenched by the dropwise addition of glacial acetic acid until the aqueous layer was neutral to litmus (not pH <6). The resulting solution was concentrated *in vacuo*, dissolved in EtOAc:H_2O (1:1, 100 mL) and separated. The organic phase was washed with saturated aqueous NaCl (30 mL), then dried (Na_2SO_4), filtered, and concentrated to give the crude product **5** as a colourless oil (2.41 g, 86%). Crystallization of the crude oil with ether/hexanes afforded pure **5** as a white crystalline solid (1.89 g, 68%): mp 83-84 °C; $[\alpha]^{22}_D$ +9.8 (c 0.50, $CHCl_3$); IR ν_{max} 3418, 3308, 1701; MS *m/z* 332.1 cm^{-1}; ^1H NMR 300 MHz 7.36 (m, 5H), 5.11 (s, 2H), 4.75 (d, J = 9.7 Hz, 1H), 3.96 (m, 1H), 3.71 (s, 3H), 3.65 (m, 1H), 3.51 (m, 1H), 3.30 (d, J = 3.7 Hz, 1H), 2.62 (dd, J = 16.6 Hz, 2.9 Hz, 1H), 2.50 (dd, J = 16.6 Hz, 9.0 Hz, 1H), 2.15 (m, 1H), 0.96 (d, J = 6.8 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H); ^{13}C NMR 75 MHz: 173.7, 157.1, 136.5, 128.7, 128.4, 128.3, 69.2, 67.2, 59.5, 52.1, 38.3, 27.6, 20.3, 16.3; MS *m/z* (M+Na), 373.1 $(M + NH_4 + Na)^+$, 641.0 $(2M + Na)^+$.

(3*S*,4*S*)-4-Benzyloxycarbonylamino-3-hydroxy-hexanoic acid methyl ester (6)



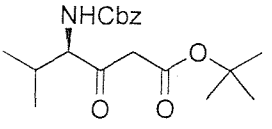
To a stirred solution of the β–keto ester **4** (1.0 g, 3.26 mmol) in 98/2 THF:MeOH (30 mL) cooled to -78 °C, was added sodium borohydride (0.164 g, 4.16 mmol) in a single portion. The reaction mixture was stirred at -78 °C for 2 hours, and then poured onto ice cold 1 N HCl (40 mL) covered with diethyl ether (20 mL). The aqueous phase was extracted with diethyl ether (2 x 30 mL), dried with $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (eluent 5-25% EtOAc/hexane) to give the 3*R*,4*S* diastereomer **5** as a white solid (0.61 g, 61%) and the (3*S*,4*S*) diastereomer **6** as a colourless oil (0.23 g, 23%): $[\alpha]^{22}_{D}$ -35.5 (c 0.50, $CHCl_3$); IR ν_{max} 3435, 3372, 1712, 1694 cm^{-1}; ^1H NMR 300 MHz 7.37 (m, 5H), 5.15 (m, 1H) 5.11 (s, 2H), 4.28 (d, J = 9.5 Hz, 1H), 3.71 (s, 3H), 3.32 (m, 1H), 3.25 (m, 1H), 2.70-2.53 (m, 1H), 2.46 (dd, J = 16.5 Hz, 2.9 Hz, 1H), 1.89 (m, 1H), 1.00 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H); ^{13}C NMR 75 MHz: 174.1, 157.0, 136.7, 128.7, 128.3, 128.1, 67.0, 66.9, 60.4, 52.1, 44.8, 38.9, 30.5, 19.9, 19.7; MS *m/z* 332.2 $(M+Na)^+$, 641.5 $(2M + Na)^+$.

(2*R*)-Benzyloxycarbonylamino-3-methyl-butyric acid (9)



To a stirred solution of D-Valine **8**, (20.0 g, 171.0 mmol) in 5% $NaHCO_3$ solution (800 mL) was added benzyl chloroformate (24.4 mL, 171.0 mmol). The reaction mixture was stirred for 20 hours at room temperature and then washed with ether. The aqueous layer was then acidified with 20% HCl to pH 5, extracted with CH_2Cl_2 (4 x 500 mL), dried and the solvent removed to give a white solid (36.6 g, 85 %): mp 51-52 °C; $[\alpha]^{22}_D$ -16.8 (c 0.50, $CHCl_3$); IR ν_{max} 3408, 1724, 1649 cm^{-1}; ^1H NMR 300 MHz 7.37 (m, 5H), 5.34 (d, *J* = 9.0 Hz, 1H), 5.13 (s, 2H), 4.36 (dd, *J* = 9.0 Hz, 4.5 Hz, 1H), 2.24 (m, 1H), 1.08 (d, *J* = 6.7 Hz, 3H), δ 0.94 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR 75 MHz: 177.1, 156.5, 136.2, 128.7, 67.3, 59.0, 31.2, 19.1, 17.5; MS *m/z* 525.4 $(2M + Na)^+$. The material was spectroscopically identical to that reported in the literature.[2]

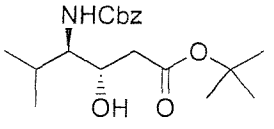
(*R*)-4-Benzyloxycarbonylamino-5-methyl-3-oxo-hexanoic acid *tert*-butyl ester (10)



To a solution of acid **9** (3.53 g, 14.1 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C, was sequentially added pentafluorophenol (PFPOH) (2.72 g, 14.84 mmol), EDAC•HCl (3.25 g, 16.96 mmol) and DMAP (0.35 g, 2.86 mmol). The reaction mixture was stirred at 0 °C for half an hour, and then at 20 °C for 4 hours. The reaction mixture was diluted with CH_2Cl_2 (400 mL), washed with 10% HCl (100 mL), 5% $NaHCO_3$ (100 mL) and saturated NaCl (100 mL) solutions. The organic layer was dried (Na_2SO_4), filtered and concentrated to give the crude PFP ester as a slightly yellowed oil (5.65 g).

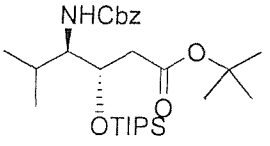
To a stirred solution of LDA (49.2 mmol in 25 mL anhydrous THF) at -78 °C was added *tert*-butyl acetate (5.70 g, 49.2. mmol) *via* syringe. After stirring for 1 hour, the enolate as added to a stirred solution of the PFP ester (5.65 g, 13.5 mmol) in THF (25 mL), at -78 °C. The reaction mixture was stirred for a further 45 minutes at -78 °C, and then carefully quenched with saturated aqueous NH_4Cl (50 mL) at -78 °C. After warming to room temperature, the THF was removed on a rotary evaporator and the resulting aqueous solution was extracted with CH_2Cl_2 (3 x 100 mL), washed with 10% HCl (100 mL), 5% $NaHCO_3$ (100 mL) and saturated NaCl (100 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 5-25% EtOAc/hexanes) to obtain the β–keto ester **10** as a colourless oil (4.11 g, 84%): $[\alpha]^{22}_D$ -28.3 (c 0.50, $CHCl_3$); IR ν_{max} 3330, 1705, 1515 cm^{-1}; ^1H NMR 300 MHz 7.32(m, 5H), 5.62 (d, J = 8.9 Hz, 1H), 5.08 (s, 2H), 4.46 (dd, J = 9.2 Hz, J = 4.1 Hz, 1H), 3.42 (s, 2H), 2.24 (m, 1H), 1.43 (s, 9H), 1.00 (d, J = 7.0 Hz, 3H), 0.78 (d, J = 6.6 Hz, 3H); ^{13}C NMR 75 MHz: 203.3, 166.8, 157.4, 137.1, 129.2, 129.1, 129.0, 82.6, 67.3, 65.1, 48.5, 31.7, 19.8, 16.5; MS *m/z* 372.3 $(M+Na)^+$, 721.6 (2M + Na).

(3*S*,4*R*)-4-Benzyloxycarbonylamino-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (11)



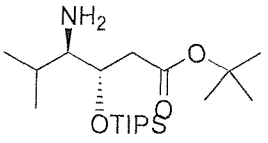
To a solution of the β–keto ester **10** (2.0 g, 5.7 mmol), in anhydrous MeOH (30 mL) at -78 °C was added potassium borohydride (1.1 g, 19.9 mmol) in portions. The reaction mixture was stirred at -78 °C for 10 min, warmed to rt for 30 min, and then to 0 °C for 10 min. Glacial acetic acid was added dropwise until the aqueous layer was neutral to litmus (not pH < 6). The resulting solution was concentrated *in vacuo*, dissolved in EtOAc:H_2O (1:1, 100 mL) and separated. The organic phase was washed with sat. NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated. Crystallization of the residue with ether/hexanes to give **11** as white needles (1.63 g, 82 %): mp = 63-64 °C; $[\alpha]^{22}_D$ -15.6 (c 0.50, $CHCl_3$); IR ν_{max} 3474, 3332, 1711, 1692, 1528 cm^{-1}; ^1H NMR 300 MHz 7.36 (m, 5H), 5.11 (s, 2H), 4.75 (d, J = 9.9 Hz, 1H), 3.90 (m, 1H), 3.59 (m, 1H), 3.44 (m, 1H), 2.62 (dd, J = 16.5 Hz, 2.6 Hz, 1H), 2.40 (dd, J = 16.6 Hz, 9.0 Hz, 1H), 2.21 (m, 1H), 1.47 (s, 9H), 0.95 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ^{13}C NMR 75 MHz 174.2, 172.8, 156.9, 136.5, 128.6, 128.3, 128.2, 81.6, 69.1, 67.0, 59.3, 39.3, 28.2, 27.4, 20.3, 16.1; MS *m/z* 725.4 $(2M + Na)^+$; Anal. Calcd. for $C_{19}H_{29}NO_5$: C, 64.93; H, 8.32; N, 3.99; O, 22.76, Found C, 64.96; H, 8.61; N, 4.02.

(3*S*,4*R*)-4-Benzyloxycarbonylamino-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (12)



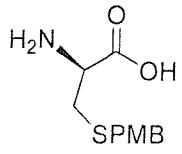
To a solution of the alcohol **11** (1.0 g, 2.85 mmol) in CH_2Cl_2 (12mL) at 0 °C was added 2,6-lutidine (0.85 mL, 7.13 mmol), followed by the dropwise addition of triisopropylsilyl triflate (1.14 mL, 4.25 mmol). The reaction mixture was stirred at 0 °C for 30 min, then at rt for 2 hours, diluted with CH_2Cl_2 (80 mL), washed with 10% HCl (40 mL), 5% $NaHCO_3$ (40 mL) and sat. NaCl (40 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 10-35% EtOAc/hexanes) to give **12** as a colourless oil (1.11 g, 77 %): $[\alpha]^{22}_D$ +8.4 (c 0.50, $CHCl_3$); IR ν_{max} 3282, 1724, 1698, 1681 cm^{-1}; ^1H NMR 300 MHz 7.35 (m, 5H), 5.11 (s, 2H), 5.02 (d, J = 10.7 Hz, 1H), 4.35 (m, 1H), 3.65 (m, 1H), 2.52 (m, 1H), 2.06 (m, 1H), 1.43 (s, 9H), 1.08 (s, 18H), 1.02 (s, 3H), 0.94 (t, J = 6.9 Hz, 6H); ^{13}C NMR 75 MHz 170.9, 156.8, 136.9, 128.6, 128.1, 81.1, 70.4, 66.7, 60.5, 41.4, 28.4, 28.1, 20.9, 18.2, 17.9, 17.4, 12.8; MS *m/z* 530.3 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{49}NO_5Si$: C, 66.23; H, 9.73; N, 2.76; O, 15.75; Si, 5.53, Found C, 66.01; H, 9.91; N, 2.77.

(3*S*,4*R*)-4-Amino-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (13)



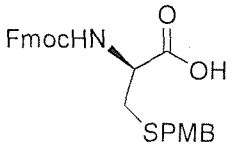
To a solution of Cbz protected amine **12** (750 mg, 1.48 mmol) in MeOH/EtOAc 1:1 (40 mL) was added 5% Pd/C (150 mg). The vessel was purged with argon, then filled with an atmosphere of H_2 and stirred at rt for 18 hours. The slurry was filtered through diatomaceous earth, dried (Na_2SO_4), filtered, and concentrated to give the amine **13** as a colourless oil (521 mg, 95%): $[\alpha]^{22}_D$ +6.4 (c 0.50, $CHCl_3$); IR ν_{max} 3283, 1725, 1697 cm^{-1}; ^1H NMR 400 MHz 4.47 (m, 1H), 2.58 (dd, J = 8.9 Hz, 3.6 Hz, 1H), 2.50 (dd, J = 16.0 Hz, 4.4 Hz, 1H), 2.37 (dd, J = 16.0 Hz, 6.4 Hz, 1H), 1.44 (s, 13H), 1.08 (s, 18H), 0.98 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H); ^{13}C NMR 75 MHz 171.8, 80.6, 70.8, 63.0, 38.4, 30.6, 28.2, 20.2, 20.0, 19.9, 18.4, 18.1, 12.7; MS *m/z* 374.3 $(M + H)^+$.

(*S*)-2-Amino-3-(4-methoxy-benzylsulfanyl)-propionic acid (15)



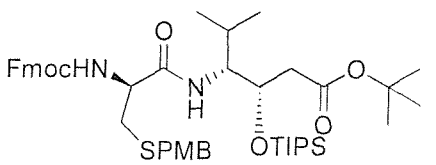
To a stirred solution of D-cysteine **14** (1.5 g, 8.5 mmol) in 1:1 TFA/CH_2Cl_2 (40 mL) was added 4-methoxybenzaldehyde (1.13 mL, 9.4 mL) dropwise. After stirring at rt for 90 minutes, the reaction mixture was cooled to 0 °C, Et_3SiH (2.7 ml, 17 mmol) added dropwise, stirred at rt for a further 16 hours, MeOH (40 mL) was added, the reaction mixture poured onto water (100 mL)/CH_2Cl_2 (80 mL), and re-extracted with water (80 mL). The combined aqueous layers were evaporated to dryness. The crude product was dissolved in MeOH/H_2O (80 mL) brought to pH 6 with $NaHCO_3$ and re-crystallized from MeOH/H_2O (20 mL) to give **15** as white crystalline plates (1.86 g, 91%): mp = 209-210 °C; IR ν_{max} 2908, 1606, 1573 cm^{-1}; ^1H NMR 300 MHz (CD_3OD) 7.29 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 3.77 (s, 5H), 3.64 (dd, J = 8.8 Hz, 4.0 Hz, 1H), 3.04 (dd, J = 14.7 Hz, 4.0 Hz, 1H), 2.83 (dd, J = 14.7 Hz, 8.4 Hz, 1H); MS *m/z* 264.1 $(M + Na)^+$.

(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionic acid (16)



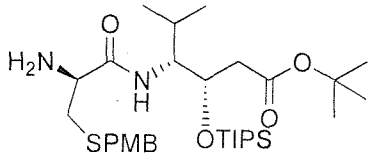
To a stirred solution of the PMB protected cysteine **15** (1.5 g, 6.2 mmol) in 19:1 pyridine/TMS-Cl (100 mL) at 0 °C was added Fmoc-Cl (1.76 g, 6.87 mmol). After stirring at rt for 3 hours, the solvent was removed and the residue dissolved in ether, separated into $NaHCO_3$ (80 mL), washed with ether (2 x 40 mL), acidified to pH~6 (1 M HCl) and extracted with CH_2Cl_2 (2 x 50 mL). The organic layer was washed with sat. NaCl (40 mL), dried ($MgSO_4$), solvent removed to give **16** as a yellow glass (2.59 g, 90%): mp = 47-50 °C; $[\alpha]^{22}_D$ -7.0 (c 0.50, $CHCl_3$); IR ν_{max} 1702, 1607 cm^{-1}; ^1H NMR 400 MHz 7.79 (d, J = 7.0 Hz, 2H), 7.63 (m, 2H), 7.42 (m, 2H), 7.34 (m, 2H), 7.22 (d, J = 7.5 Hz, 2H), 6.85 (d, J = 7.5 Hz, 2H), 5.70 (s, 1H), 4.63 (s, 1H), 4.45 (d, J = 6.5 Hz, 2H), 4.26 (s, 1H), 3.78 (s, 3H), 3.71 (s, 2H), 2.95 (m, 2H) (all peaks broad); ^{13}C NMR 100 MHz 175.5, 158.9, 156.2, 143.9, 143.7, 141.4, 130.2, 129.5, 127.9, 127.2, 125.2, 120.1, 114.2, 113.8, 67.5, 55.3, 53.7, 47.2, 36.2, 33.3; MS *m/z* 498 $(M - H + HCl)^-$ 924.8 $(2M - H)^-$.

(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (17)



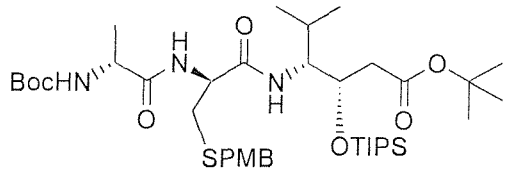
To a stirred solution of the amine **13** (0.45 g, 1.34 mmol) in CH_3CN (20 mL) was added the acid **16** (0.93 g, 2.01 mmol), PyBOP (1.05 g, 2.01 mmol) DIEA (0.70 mL). After stirring at rt for 1 hour, the solvent was removed and the residue was purified by flash chromatography (eluent 10-35% EtOAc/hexanes) to give **17** as a white glass (0.86 g, 78%): $[\alpha]^{22}_D$ +13.2 (c 0.50, $CHCl_3$); IR ν_{max} 3313, 1721, 1661 cm^{-1}; ^1H NMR 400 MHz 7.76 (d, J = 7.6 Hz, 2H), 7.60 (t, J = 7.5 Hz, 2H), 7.39 (t, J = 7.6 Hz, 2H), 7.28 (m, 4H), 6.82 (d, J = 7.0 Hz, 2H), 6.69 (t, J = 10.0 Hz,1H), 5.76 and 5.55 (s, br, 1H), 4.42 (m, 1H), 4.33 (m, 1H), 4.33 (m, 1H), 4.22 (t, J = 7.0 Hz, 1H), 3.96 (m, 1H), 3.75 (s, 4H), 2.91 (dd, J = 13.5 Hz, 6 Hz, 1H), 2.80 (m, 1H), 2.76 (dd, J = 14.0 Hz, 7.0 Hz, 1H), 2.60-2.53 (m, 2H), 2.03-1.90 (m, 1H), 1.45 (s, 9H), 1.06 (s, 21H), 0.92 (t, J = 8.0 Hz, 6H); ^{13}C NMR 75 MHz 174.3, 171.4, 171.0, 170.3, 170.0, 158.9, 156.2, 155.8, 143.9, 141.4, 130.3, 130.2, 130.0, 127.9, 127.3, 125.3, 120.2, 114.1, 81.5, 70.0, 67.4, 58.7, 55.4, 54.6, 47.3, 41.2, 36.3, 36.0, 34.4, 33.9, 29.0, 28.9, 28.2, 20.9, 18.3, 17.9, 13.0; MS *m/z* 841.3 $(M + Na)^+$; Anal. Calcd for $C_{46}H_{66}N_2O_7SSi$: C, 67.44; H, 8.12; N, 3.42; O, 13.67; S, 3.91; Si, 3.43, Found C, 67.14; H, 8.18; N, 3.45.

(3*S*,4*R*)-4-[(*S*)-2-Amino-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (18)



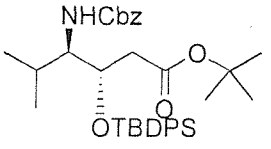
To a stirred solution of **17** (100 mg, 0.122 mmol) in CH_3CN (2.5 mL) at rt, was added Et_2NH (125 μL). After stirring for 3 hours at rt the reaction mixture was diluted with hexane (30 mL), the solvent removed and the residue purified by flash chromatography (eluent 1-5% MeOH/CH_2Cl_2) to give **18** as a colourless oil (79.2 mg, 97%): IR ν_{max} 3365, 1725, 1671 cm^{-1}; ^1H NMR 400 MHz 7.54 (d, J = 10.5 Hz, 1H), 7.17 (d, J = 8.6 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 4.26 (m, 1H), 3.81 (m, 1H), 3.61 (s, 3H), 3.40 (s, 2H), 3.38 (dd, J = 8.5 Hz, 3.5 Hz, 1H), 2.93 (dd, J = 14.1 Hz, 3.5 Hz, 1H), 2.62 (dd, J = 13.6 Hz, 8.5 Hz, 1H), 2.51-2.39 (m, 3H), 2.02-1.97 (m, 1H), 1.37 (s, 9H), 0.99 (s, 21H), 0.84 (t, J = 8.0 Hz, 6H); ^{13}C NMR 100 MHz 171.2, 168.8, 156.8, 128.3, 128.1, 112.1, 79.0, 68.6, 56.0, 55.9, 53.4, 52.5, 52.3, 39.8, 35.6, 33.9, 26.4, 26.2, 19.1, 19.0, 16.3, 15.6, 10.9; MS *m/z* 597.6 $(M + H)^+$.

(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-butoxycarbonylamino-propionylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (19)



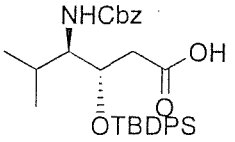
To a stirred solution of the amine **18** (79 mg, 0.132 mmol) in CH_3CN (2 mL) at rt was added Boc-D-alanine (34 mg, 0.18 mmol), PyBOP (92 mg, 0.18 mmol) and DIEA (62 μL, 0.36 mmol). After stirring at rt for 0.5 hour the solvent was removed and the residue was purified by flash chromatography (eluent 10-35% EtOAc/hexanes) to give **19** as a colourless oil (90.2 mg, 89%); IR ν_{max} 3287, 1643, 1510 cm^{-1}; ^1H NMR 400 MHz 7.19 (d, J = 8.0 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.76 (m, 1H), 6.53 (d, J = 9.5 Hz, 2H), 4.86 (m, 1H), 4.41 (m, 1H), 4.30 (m, 2H), 4.06 (m, 1H), 3.84 (m, 1H), 3.71 (s, 3H), 3.64 (s, 2H), 2.83 (m, 1H), 2.69 (dd, J = 13.6 Hz, 3.3 Hz, 1H), 2.50 (dd, J = 15.6 Hz, 6.5 Hz, 1H), 2.40 (dd, J = 15.6 Hz, 4.5 Hz, 1H), 1.82 (m, 2H), 1.38 (s, 9H), 1.37 (s, 9H), 1.26 (d, 0.84 J = 6.5 Hz, 3H) 0.99 (s, 18H), 0.93 (s, 3H), 0.8 (t, J = 5.5 Hz, 6H); ^{13}C NMR 100 MHz 172.8, 171.1, 170.1, 158.9, 130.3, 114.1, 81.1, 80.4, 69.8, 59.2, 59.1, 55.4, 52.8, 52.6, 50.4, 41.1, 41.0, 36.2, 34.0, 33.3, 29.0, 28.4, 28.2, 20.7, 19.0, 18.3, 12.8; MS *m/z* 768.3 $(M + H)^+$.

(3*S*,4*R*)-4-Benzyloxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid *tert*-butyl ester (23)



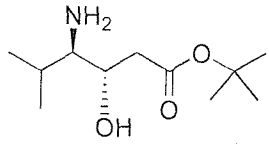
To a solution of the alcohol **22** (300 mg, 0.85 mmol) in DMF (2.0 mL) at 0 °C was added imidazole (347.3 mg, 5.1 mmol) followed by *tert*-butyldiphenylsilyl chloride (1.1 ml, 4.25 mmol). The reaction mixture was allowed to warm to rt and stirred for 30 hours then diluted with *hexanes* (20 mL), washed with sat. NaCl (10 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 5-10% EtOAc/hexanes) to give **23** as a colourless oil (358 mg, 72%); IR ν_{max} 3400, 1724, 1708 cm^{-1}; ^1H NMR 400 MHz 7.80-7.73 (m, 5H), 7.51-7.39 (m, 10H), 5.14 (d, J = 4.0 Hz, 2H), 5.03 (d, J = 10.6 Hz, 1H), 4.21 (m, 1H), 3.70 (m, 1H), 2.50 (m, 2H), 2.09 (m, 1H), 1.35 (s, 9H), 1.35 (s, 9H), 1.11 (s, 9H), 0.87 (d, J = 6.5 Hz, 3H), 0.79 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100MHz 170.7, 156.8, 136.9, 136.1, 135.4, 134.9, 133.9, 133.0, 129.8, 129.7, 129.1, 128.5, 128.3, 128.2, 128.0, 127.8, 127.7, 125.4, 80.9, 70.8, 70.6, 67.0, 66.6, 60.8, 28.4, 28.0, 27.1, 26.7, 21.6, 20.5, 19.4, 19.1, 16.8, 14.3; MS *m/z* 612 $(M + Na)^+$, 1201 $(2M + Na)^+$.

(3*S*,4*R*)-4-Benzyloxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid (24)



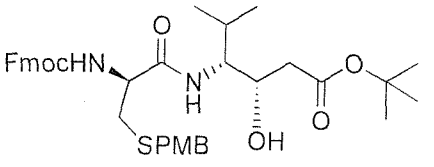
To a stirred solution of **23** (60 mg, 0.10 mmol) in CH_2Cl_2 (1.9 mL) at 0 °C was added TFA (100 μl). After stirring for 30 minutes, EtOAc (20 mL) was added followed by the dropwise addition of $NaHCO_3$ until bubbling ceased. The aqueous layer was extracted with EtOAc (3 x 10 mL) and combined organic extracts washed with $NaHCO_3$ (15 mL), H_2O (15 mL), sat. NH_4Cl (15 mL), sat. NaCl (15 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 3-5% MeOH/CH_2Cl_2) to give **24** as an impure colourless oil (37 mg, 69%): IR ν_{max} 3246, 1701, 1664 cm^{-1}; ^1H NMR 400 MHz 7.63-7.50 (m, 5H), 7.28-7.22 (m, 10H), 6.83 (d, J = 11 Hz, 2H), 5.12 (d, J = 12.5 Hz, 2H), 4.96 (m, 2H), 4.04 (m, 1H), 3.62-3.47 (m, 1H), 2.43 (m, 1H), 2.32 (m, 2H), 2.07 (m, 1H), 1.19 (m, 1H), 0.96 (s, 9H), 0.73 (m, 3H), 0.87 (d, J = 6.5 Hz, 1H), 0.79 (d, J = 7.0 Hz, 2H); ^{13}C NMR 100 MHz 175.1, 174.4, 158.8, 156.8, 136.5, 136.2, 134.1, 132.9, 130.1, 130.0, 129.9, 129.8, 127.9, 127.7, 71.5, 71.0, 66.9, 63.0, 61.1, 40.8, 39.5, 31.7, 28.2, 27.9, 27.5, 25.9, 22.8, 20.4, 16.2, 14.5; MS *m/z* 646.2 $(M + TFA - H)^-$.

(3*S*,4*R*)-4-Amino-3-hydroxy-5-methyl-hexanoic *tert*-butyl ester (27)



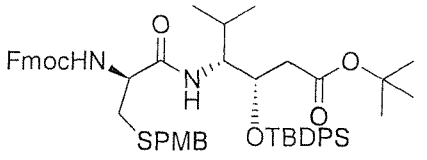
To a solution of Cbz protected amine **22** (1.0 g, 2.85 mmol) in MeOH/EtOAc 1:1 (40 mL) was added 5 % Pd/C (150 mg). The vessel was purged with argon, then filled with an atmosphere of H_2 and stirred at rt for 18 hours. The slurry was filtered through diatomaceous earth washing with MeOH/EtOAc 1:1 (20 mL), dried (Na_2SO_4), filtered, and concentrated to give the amine **27** as a colourless oil (571 mg, 92 %): $[\alpha]^{22}_D$ + 42.4 (c 0.50, $CHCl_3$). IR ν_{max} 3354, 2962, 2931, 1649 cm^{-1}; ^1H NMR 300 MHz 3.98 (m, 1H), 2.54-2.33 (m, 3H), 1.68 (m, 1H), 1.47 (s, 9H), 0.93 (t, J = 6.2 Hz, 6H); ^{13}C NMR 75 MHz 174.3, 81.8, 69.7, 61.0, 37.7, 30.1, 28.4, 20.1, 18.3; MS *m/z* 218 $(M + H)^+$.

(3*S*,4*R*)-4-[*(S)*-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (28)



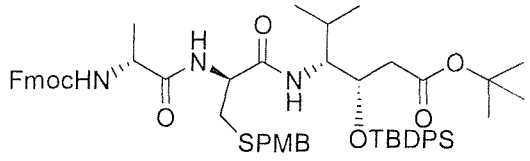
To a stirred solution of the amine **27** (0.45 g, 2.07 mmol) in CH_3CN (20 mL) was added the protected cysteine acid (0.96 g, 2.07 mmol), PyBOP (1.08 g, 2.07 mmol) and DIEA (0.72 mL). After stirring at rt for 1 hour, the solvent was removed and the residue was purified by flash chromatography (eluent 15-40% EtOAc/hexanes) to give **28** as a white glass (0.86 g, 78 %): mp = 58-59 °C; $[\alpha]^{22}_D$ +25 (c 0.50, $CHCl_3$); IR ν_{max} 3311, 1701, 1655, 1510 cm^{-1}; ^1H NMR 400 MHz 7.69 (d, J = 7.6 Hz, 2H), 7.50 (d, J = 7.0 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.23 (t, J = 7.0 Hz, 2H), 7.18 (d, J = 7.6 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.99 (s, br, 1H), 5.50 (s, br, 1H), 4.34 (d, J = 6.5 Hz, 2H), 4.14 (t, J = 7.0 Hz, 2H), 3.78 (m, 1H), 3.69 (s, 4H), 2.79 (m, 1H), 2.68 (dd, J = 13.6 Hz, J = 7.5 Hz, 1H), 2.54 (m, br, 1H), 2.43-2.25 (m, 3H), 2.05 (m, 1H), 1.37 (s, 9H), 0.79 (t, J = 8.0 Hz, 6H); ^{13}C NMR 100 MHz 172.7, 170.6, 159.0, 156.1, 143.8, 143.7, 141.4, 130.2, 127.9, 127.2, 125.2, 120.2, 114.3, 114.2, 81.7, 68.9, 67.4, 57.6, 55.4, 54.6, 47.2, 39.3, 36.4, 33.9, 28.2, 27.5, 20.5, 20.4, 18.7, 17.4, 16.5; MS *m/z* 663.1 $(M + H)^+$;

(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-(9*H*-fluoren-9-ylme thoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-hexanoic acid *tert*-butyl ester (29)



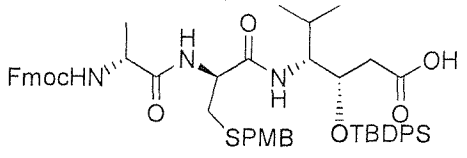
To a solution of the alcohol **28** (100 mg, 0.151 mmol) in DMF (0.5 mL) at 0 °C was added imidazole (266.5 mg, 1.59 mmol) followed by *tert*-butyldiphenylsilyl chloride (392 μL, 1.51 mmol). The reaction mixture was allowed to warm to rt and stirred for 18 hours then diluted with hexanes (20 mL), washed with sat. NaCl (10 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 5-15% EtOAc/hexanes) to give **29** as a colourless solid (1.11 g, 77 %): mp = 60-61 °C; $[\alpha]^{22}_D$ +16 (c 0.50, $CHCl_3$); IR ν_{max} 3318, 1719, 1665, 1509 cm^{-1}; ^1H NMR 400 MHz 7.70-7.50 (m, 7H), 7.35-7.28 (m, 7H), 7.24-7.14 (m, 4H), 6.72 (d, J = 8.5 Hz, 2H), 6.55 (d, J = 10.0 Hz, 1H), 5.61 and 5.42 (d, br, 1H) 4.36-4.30 (m, 2H), 4.15 (t, J = 6.8 Hz, 2H), 3.84 (m, 1H), 3.67 (s, 3H), 3.60 (s, 2H), 2.74, (m, 2H), 2.67 (m, 1H), 2.42 (dd, J = 15.8 Hz, J = 7 Hz, 1H), 2.33 (dd, J = 15.6 Hz, J = 4 Hz, 1H), 1.80 (m, 1H), 1.56 (s, 2H), 1.24 (s, 9H), 0.95 (s, 9H), 0.70 (m, 1H), 0.63 (m, 6H); ^{13}C NMR 100 MHz 170.9, 170.3, 158.9, 143.9, 141.4, 136.2, 136.1, 133.8, 133.0, 130.3, 130.2, 130.1, 129.9, 127.7, 127.2, 125.3, 120.1, 114.2, 81.1, 70.4, 67.4, 58.8, 55.4, 54.7, 47.3, 40.7, 36.4, 33.8, 28.9, 28.8, 28.1, 27.1, 20.5, 19.4, 17.6; MS *m/z* 901 $(M + H)^+$.

(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-[(*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-hexanoic acid *tert*-butyl ester (30)



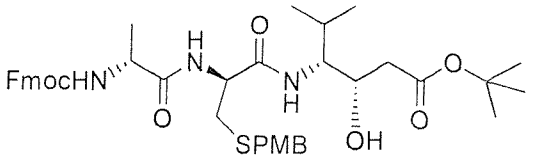
To a stirred solution of **29** (400 mg, 0.44 mmol) in CH_3CN (10 mL) at rt was added Et_2NH (500 μl). After stirring for 3 hours, the reaction mixture was diluted with *n*-heptane (100 ml) and solvent removed to give the free amine. The residue was dissolved in CH_3CN (10 mL) to which was sequentially added Fmoc-D-alanine (274 mg, 0.88 mmol), PyBOP (458 mg, 0.88 mmol) and DIEA (307.3 μL, 1.74 mmol). After stirring at rt for 3 hours the solvent was removed and the residue was purified by flash chromatography (eluent 20-30% EtOAc/hexanes) to give **30** as a white solid (375 mg, 86%): mp = 60-61 °C; $[\alpha]^{22}_D$ +25.6 (c 0.50, $CHCl_3$); IR ν_{max} 3283, 1719, 1701, 1645, 1510 cm^{-1}; ^1H NMR 400 MHz 7.80-7.60 (m, 7H), 7.45-7.31 (m, 9H), 7.23 (d, J = 8.0 Hz, 2H), 6.83 (d, J = 8.0 Hz, 2H), 6.72-6.65 (m, 2H), 5.35 (s, 1H), 5.33 (s, br, 1H), 4.44 (m, 4H) 4.26 (t, J = 6.5 Hz, 3H), 4.20 (m, 1H), 3.95 (m, 2H), 3.77 (s, 3H), 3.69 (s, 2H), 2.89, (m, 1H), 2.80 (dd, J = 14.0 Hz, J = 6.5 Hz, 1H) 2.55 (dd, J = 16.1 Hz, J = 7.0 Hz, 1H), 2.48 (dd, J = 15.6 Hz, J = 4.0 Hz, 1H) 1.90 (s, 3H), 1.40 (s, 9H), 1.08 (s, 9H), 0.72 (m, 6H); ^{13}C NMR 100 MHz 170.9, 170.1, 158.9, 141.5, 136.2, 136.1 133.1, 130.2, 130.0, 129.9, 127.9, 127.7, 127.2, 125.2, 120.1, 114.2, 81.1, 70.3, 67.2, 59.0, 55.3, 52.8, 47.3, 40.7, 36.3, 33.6, 28.8, 28.1, 27.1, 20.4, 19.4, 17.5; MS *m/z* 972.4 $(M + H)^+$, 994.4 $(M + Na)^+$.

(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-[(*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-hexanoic acid (31)



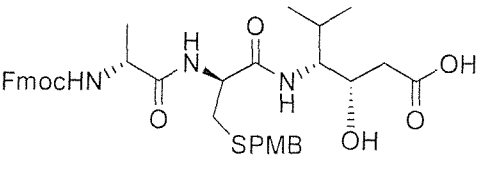
To a stirred solution of **30** (120 mg, 0.122 mmol) in CH_2Cl_2 (4.75 mL) at 0 °C was added TFA (250 µl). After stirring for 30 minutes, $NaHCO_3$ (2.0 mL) and EtOAc (40 mL) was added followed by the dropwise addition of $NaHCO_3$ until bubbling ceased. The aqueous layer was extracted with EtOAc (3 x 20 mL) and combined organic extracts washed with $NaHCO_3$ (30 mL), H_2O (30 mL), sat. NH_4Cl (25 mL), sat. NaCl (25 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 3-5% MeOH/CH_2Cl_2) to give **31** as a colourless oil (65 mg, 58%): IR ν_{max} 3280, 1702, 1636 cm^{-1}; ^1H NMR 400 MHz 7.68-7.45 (m, 8H), 7.32-7.18 (m, 10H), 7.02 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 8.5 Hz, 2H), 6.27 (s, br, 1H), 5.33 (s, br, 1H), 5.2 (s, 1H), 4.41-4.28 (m, 4H), 4.09-4.01 (m, 4H), 3.91 (m, 2H), 3.61 (s, 3H), 3.48 (s, 2H), 2.85, (m, 2H), 2.61 (m, 2H), 2.44 (m, 2H), 1.95 (s, 2H), 1.20 (m, 3H), 0.93 (s, 9H), 0.65 (d, J = 6.0 Hz, 3H), 0.48 (d, J = 6.0 Hz, 3H); ^{13}C NMR 100 MHz 173.1, 171.3, 170.4, 158.8, 156.4, 143.8, 141.4, 136.1, 133.7, 133.0, 130.2, 130.0, 129.9, 128.0, 127.9, 127.7, 127.3, 127.2, 125.1, 120.1, 114.1, 70.3, 67.4, 60.5, 59.4, 55.3, 53.0, 50.8, 47.2, 39.5, 36.1, 33.1, 30.9, 28.0, 27.1, 26.1, 25.8, 24.7, 21.1, 20.4, 19.3, 18.6, 18.3, 16.2, 14.3; MS *m/z* 1027.9 $(M + TFA - 2H)^{2-}$, 994.4 $(2M + TFA - 2H)^{2-}$.

(3*S*,4*R*)-4-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (34)



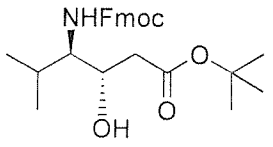
To a stirred solution of **28** (240 mg, 0.44 mmol) in CH_3CN (10 mL) was added Et_2NH (500 μl). After stirring for 3 h the reaction mixture was diluted with *n*-heptane (100 mL) and solvent removed to give the free amine. The residue was dissolved in CH_3CN (5 mL) to which was sequentially added Fmoc-D-alanine (124 mg, 0.398 mmol), PyBOP (207.1 mg, 0.398 mmol) and DIEA (159 μL, 0.91 mmol). After stirring for 2 hours the solvent was removed and the residue was purified by flash chromatography (eluent 30-50% EtOAc/hexanes) to give **34** as a white solid (175 mg, 66 %): mp = 57-58 °C; $[\alpha]^{22}_D$ +26.6 (c 0.50, $CHCl_3$); IR ν_{max} 3298, 1700, 1643 cm^{-1}; ^1H NMR 400 MHz 7.77 (d, J = 7.5 Hz, 2H), 7.60 (t, J = 8.0 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (d, J = 7.5 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 6.77 (d, J = 8.0 Hz, 3H), 6.34 (d, J = 8.5 Hz, 1H), 5.43 (m, 3H), 4.21 (t, J = 6.5 Hz, 1H), 4.13 (m, 1H), 3.96 (m, 1H), 3.84 (m, 1H), 3.73 (s, 3H), 3.64 (s, 2H), 3.03, (m, 1H), 2.71 (dd, J = 13.6 Hz, J = 7.0 Hz, 1H), 2.47 (dd, J = 16.5 Hz, J = 2.5 Hz, 1H), 2.34 (dd, J = 16.6 Hz, J = 9.5 Hz, 1H) 2.07 (m, 1H), 2.04 (s, 3H), 1.44 (s, 9H), 1.33 (d, 2H), 1.25 (m, 1H), 0.87 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 172.7(C), 172.4(C), 170.4(C), 158.8(C), 156.2(C), 143.7(C), 141.3(C), 130.2(CH), 130.1(CH), 127.8(CH), 127.2(CH), 124.9(CH), 120.1(CH), 114.1(CH), 81.4(CH), 68.8(CH), 67.1(CH_2), 57.7(CH), 55.2(CH_3), 52.5(CH), 51.1(CH), 47.0(CH), 38.8(CH_2), 36.2(CH_2), 33.1(CH_2), 28.1(CH_3), 27.5(CH), 20.2(CH_3), 18.1(CH_3), 16.9(CH_3); MS *m/z* 734.3 $(M + H)^+$.

(3*S*,4*R*)-4-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-hydroxy-5-methyl-hexanoic acid (35)



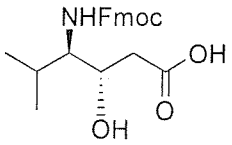
To a stirred solution of **34** (100 mg, 0.136 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added TFA (500 μl). After 60 minutes the solvent was removed and the residue purified by flash chromatography (eluent 1-10% MeOH/CH_2Cl_2) to give **35** as a colourless oil (59 mg, 64 %): $[\alpha]^{22}_D$ +28.3 (c 0.50, $CHCl_3$); IR ν_{max} 3285, 1769, 1632 cm^{-1}; ^1H NMR 400 MHz 7.76 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 6.0 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (d, J = 7.5 Hz, 2H), 7.13 (m, 3H), 6.74 (d, J = 8.0 Hz, 2H), 6.50 (d, J = 9.5 Hz, 1H), 5.36 (br, s, 1H), 4.43 (m, 3H), 4.17 (m, 2H), 4.01 (br, s, 1H), 3.88 (s, br, 1H), 3.70 (s, 3H), 3.60 (s, 2H), 3.01 (d, J = 9.0 Hz, 1H), 2.71 (dd, J = 13.6 Hz, J = 6.5 Hz, 1H), 2.61 (d, J = 14.1 Hz, 1H), 2.49 (dd, J = 16.1 Hz, J = 8.0 Hz, 1H), 2.08 (s, br, 1H), 1.29 (d, J = 7.0 Hz, 2H), 1.25 (s, 1H), 0.85 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 175.3(C), 173.2(C), 171.2(C), 158.9(C), 156.6(C), 143.7(C), 141.5(C), 130.2(CH), 127.9(CH), 127.3(CH), 125.0(CH), 120.2(CH), 114.2(CH), 68.8(CH), 67.4(CH_2), 58.4(CH), 55.3(CH_3), 53.0(CH), 51.4(CH), 47.1(CH), 37.7(CH_2), 36.3(CH_2), 33.1(CH_2), 27.8(CH), 20.2(CH_3), 18.3(CH), 17.1(CH_3); MS *m/z* 790.2 (M + TFA – H)$^-$.

(3*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (41)



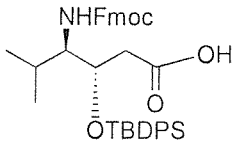
To a stirred solution of **27** (500 mg, 2.3 mmol) in 1:1 dioxane/water (40 mL) was added $NaHCO_3$ (840 mg, 10.14 mmol) and Fmoc-Cl (569 mg, 2.3 mmol). After stirring for 2.5 h, 10% aq $NaHCO_3$ (30 mL) was added and the product extracted with EtOAc (4 x 50 mL) and dried with $MgSO_4$. The solvent was then removed and the residue purified by flash chromatography (eluent 10-25% EtOAc/hexanes) to give **41** as a white solid (850 mg, 84 %): mp = 64-65 °C; $[\alpha]^{22}_D$ -7.6 (c 0.50, $CHCl_3$); IR ν_{max} 3335, 1701 cm^{-1}; ^1H NMR 400 MHz δ (d_6-DMSO) 7.89 (d, J = 7.5 Hz, 2H), 7.73 (t, J = 7.0 Hz, 2H), 7.41 (m, 2H), 7.31 (t, J = 7.0 Hz, 2H), 6.90 (d, J = 7.0 Hz, 1H), 4.84 (d, J = 7.0 Hz, 1H), 4.33 (d, J = 7.0 Hz, 2H), 4.22 (t, J = 7.0 Hz, 1H), 3.79 (m, 1H), 3.29 (m, 1H), 2.51 (s, 1H), 2.32 (dd, J = 15.5 Hz, J = 2.4 Hz, 1H), 2.04 (m, 2H), 1.42 (s, 9H), 0.80 (dd, J = 7.0 Hz, J = 2.5 Hz, 6H); ^{13}C NMR 100 MHz δ; 172.7(C), 156.8(C), 144.0(C), 143.8(C), 141.4(C), 127.7(CH), 127.1(CH), 125.0(CH), 120.0(CH), 81.5(C), 69.0(CH), 66.5(CH_2), 59.2(CH), 47.4(CH), 39.3(CH_2), 28.1(CH_3), 27.4(CH), 27.2(CH), 20.3(CH_3), 16.1(CH_3); MS *m/z* 462.0 $(M + Na)^+$, 900.8 $(2M + Na)^+$.

(3*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-5-methyl-hexanoic acid (42)



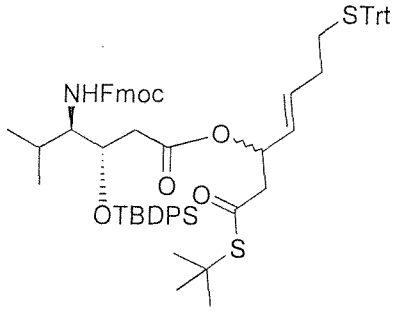
To a stirred solution of **41** (200 mg, 0.136 mmol) in CH_2Cl_2 (750 μl) at 0 °C was added TFA (680 μl). After stirring for 1 h, the solvent was removed. The residue was triturated with Et_2O (1 mL) and washed with ice cold ether (1 mL) to give **42** as a colourless oil (125 mg, 72 %): $[\alpha]^{22}_D$ -8.2 (c 0.50, $CHCl_3$); IR ν_{max} 3401, 2361, 2255, 1648 cm^{-1}; ^1H NMR 400 MHz (d_6-DMSO) 7.89 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (d, J = 7.5 Hz, 2H), 7.02 (d, J = 10.0 Hz, 2H), 4.22 (t, J = 6.5 Hz, 1H), 3.82 (t, J = 8.5 Hz, 1H), 2.51 (s, 1H), 2.38 (d, J = 13.1, 1H), 2.07, (m, 3H), 0.81 (dd, J = 6.5, J = 2.8 Hz, 3H); ^{13}C NMR 100 MHz 176.7(C), 157.5(C), 143.9(C), 143.8(C), 141.5(C), 127.9(CH), 127.2(CH), 125.1(CH), 125.0(CH), 120.2(CH), 69.1(CH), 68.8(CH_2), 59.7(CH), 47.5(CH), 37.9(CH_2), 27.7(CH), 20.2(CH_3), 16.7(CH_3); MS *m/z* 496.1 (M + TFA – H)$^-$.

3*S*-(*tert*-Butyl-diphenyl-silanyloxy)-4*R*-(9*H*-fluoren-9-ylmethoxy-carbonylamino)-5-methyl-hexanoic acid (47)



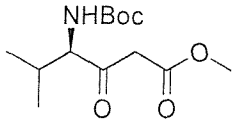
To a stirred solution of **47** (200 mg, 0.30 mmol) in CH_2Cl_2 (3.5 mL) at 0 °C was added Et_3SiH (119 μl) and TFA (500 μL). After stirring for 4 h, the solution was concentrated. The residue was triturated with Et_2O (1 mL) and washed with ice cold ether (1 mL) to give **17** as a white solid (108 mg, 58 %): mp = 119-120 °C; IR ν_{max} 3250, 1708, 1656 cm^{-1}; ^1H NMR 300 MHz 7.83 (d, *J* = 6.5 Hz, 2H), 7.80-7.30 (m, 16H), 6.92 (d, *J* = 9.0 Hz, 2H), 4.52 (d, *J* = 7.3 Hz, 2H), 4.28 (t, *J* = 4.7 Hz, 1H), 3.99 (t, *J* = 9.19 Hz, 1H), 3.26 (t, *J* = 9.6 Hz, 1H), 2.30 (d, *J* = 14.0 Hz, 1H), 2.09-1.94 (m, 2H), 1.11 (s, 9H), 0.64 (d, *J* = 6.6 Hz, 3H), 0.28 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR 75 MHz δ; 174.7(C), 158.8(C), 144.2(C), 144.1(C), 141.7(C), 141.5(C), 136.2(CH) 134.0(C), 132.9(C), 129.9(CH), 127.8(CH), 127.2(CH), 125.0(CH), 120.2 (CH), 71.3(CH), 67.4 (CH_2), 63.0(CH), 61.0(C), 47.3(CH), 40.3(CH), 39.9(CH_2), 27.2(CH_3), 20.3(CH_3), 19.5(C), 16.2(CH), 14.0(CH_3); MS *m/z* 734.1 $(M + TFA - H)^-$

(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-(9*H*-fluoren-9-ylmethoxycarbonylamino)-5-methyl-hexanoic acid (*E*)-1-*tert*-butylsulfanylcarbonylmethyl-5-tritylsulfanyl-pent-2-enyl ester (48)



To a stirred solution of **47** (100 mg, 0.161 mmol) in DMF (1.5 mL) was added DCC (52 mg, 0.242 mmol) and DMAP (6.1 mg, 0.242 mmol). After stirring at rt for 15 minutes, the alcohol **25** (71 mg, 0.145 mmol) was added and the reaction mixture stirred for 18 h. The solvent was removed under vacuum and the residue dissolved up in ether (10 mL) and the solid precipitate filtered off. The solvent was removed and the residue chromatographed using 10-30% EtOAc/hexanes to give **48** as a colourless oil (85.7 mg, 54%): IR ν_{max} 3275, 3065, 1727, 1633 cm^{-1}; ^1H NMR 400 MHz 7.74 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 7.0 Hz, 2H), 7.62 (d, J = 6.5 Hz, 2H), 7.57 (m, 2H), 7.39-7.16 (m, 25H), 5.45 (dt, J = 15.1, J = 6.5 Hz, 1H), 5.31 (m, 1H), 5.19 (dd, J = 15.6, 7.02 Hz, 1H), 4.90 (d, J = 10.0 Hz, 1H), 4.46-4.14 (m, 5H), 3.60 (m, 1H), 2.59 (dd, J = 14.6, 6.5 Hz, 1H), 2.46 (m, 2H), 2.13 (m, 2H), 1.97 (m, 2H), 1.33 (s, 9H), 1.01 (s, 9H), 0.74 (d, J = 6.5 Hz, 3H), 0.63 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 195.7(C), 169.9(C), 156.6(C), 144.9(C), 144.2(C), 144.0(C), 141.3(C), 136.0(CH), 135.9(CH), 133.6(CH), 133.0(C), 132.8(CH), 129.9(CH), 129.8(CH), 129.6(CH), 127.9(CH), 127.7(CH), 127.6(CH), 127.0(CH), 126.6(CH), 125.2(CH), 119.9 (CH), 71.1(CH), 70.5(CH), 66.6(CH_2), 60.6(CH), 48.6(CH_2), 48.3(CH), 47.4(CH), 41.7(CH), 39.9(CH_2), 31.5(CH_2), 31.1(CH_2), 29.7(CH_3), 28.2(CH), 27.0(CH_3), 20.4(CH) 19.3(C), 16.7(CH); MS *m/z* 1116.0 $(M + Na)^+$.

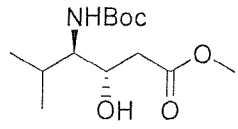
(*R*)-4-*tert*-Butoxycarbonylamino-5-methyl-3-oxo-hexanoic acid methyl ester (51)



To a solution of Boc-D-valine **50** (2.89 g, 13.3 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C, was sequentially added pentafluorophenol (2.58 g, 14.0 mmol), EDAC•HCl (3.06 g, 16.0 mmol) and DMAP (0.32 g, 2.7 mmol). The reaction mixture was stirred at 0 °C for half an hour, and then at 20 °C for 4 hours. The reaction mixture was diluted with CH_2Cl_2 (400 mL), washed with 10% HCl (100 mL), 5% $NaHCO_3$ (100 mL), and saturated NaCl (100 mL) solutions. The organic layer was dried (Na_2SO_4), filtered and concentrated to give the crude PFP ester as a slightly yellowed oil.

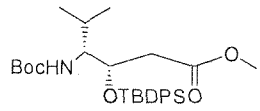
To a stirred solution of LDA (42.9 mmol in 25.0 mL anhydrous THF) at -78 °C was added methyl acetate (3.92 mL, 42.9. mmol) *via* syringe. After stirring for 1 hour, the enolate was added to a stirred solution of the PFP ester in THF (25 mL) at -78 °C. The reaction mixture was stirred for a further 45 minutes at -78 °C, and then carefully quenched with saturated aqueous NH_4Cl (50.0 mL) at -78 °C. After warming to room temperature, the THF was removed on a rotary evaporator and the resulting aqueous solution was extracted with CH_2Cl_2 (3 x 100 mL), washed with 10% HCl (100 mL), 5% $NaHCO_3$ (100 mL) and saturated NaCl solutions (100 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 5-25% EtOAc/hexanes) to obtain the β–keto ester **51** as a colourless oil (2.38 g, 66%): $[\alpha]^{22}_D$ -26.5°(c 0.50, $CHCl_3$); IR ν_{max} 3366.2, 1753.4, 1706.1, 1502.7 cm^{-1}; ^1H NMR 400 MHz 5.06 (m, 1H), 4.31 (m, 1H), 3.74 (s, 3H), 3.56 (s, 2H), 2.24 (m, 1H), 1.44 (s, 9H), 1.01 (d, J = 7.0 Hz, 3H), 0.92 (app. t, J = 7.0 Hz, isopropyl rotomer), 0.83 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 202.2 (C), 167.3 (C), 156.0 (C), 80.2 (CH), 64.5 (C), 52.5 (CH_3), 51.4 (CH) 47.1 (CH_2), 29.6 (CH_3), 28.5 (CH), 19.8 (CH_3), 16.9 (CH_3); MS *m/z* 274.3 $(M+H)^+$, 291.3 $(M+NH_4)^+$, 296.3 $(M+Na)^+$, 312.3 $(M+K)^+$, 569.5 $(2M+Na)^+$.

(3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-methyl-hexanoic acid methyl ester (52)



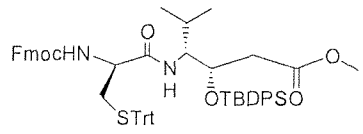
To a solution of the β–keto ester **51** (2.38 g, 8.7 mmol), in anhydrous MeOH (30 mL) at -78 °C was added potassium borohydride (1.64 g, 30.5 mmol) in portions. The reaction mixture was stirred at -78 °C for 10 min, warmed to rt for 30 min, and then to 0 °C for 10 min. Glacial acetic acid was added dropwise until the aqueous layer was neutral to litmus (not pH < 6). The resulting solution was concentrated *in vacuo*, dissolved in EtOAc:H_2O (1:1, 100 mL) and separated. The organic phase was washed with sat. NaCl (30 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent 15-25% EtOAc/hexanes) to give **52** as a white solid (1.68 g, 70%): mp = 65-66 °C; $[\alpha]^{22}_D$ -8.4 (c 0.50, $CHCl_3$); IR ν_{max} 3446.6, 3375.7, 1715.5, 1502.7 cm^{-1}; ^1H NMR 400 MHz 4.44 (d, J = 9.6 Hz, 1H), 3.93 (s, 1H), 3.71 (s, 3H), 3.53 (m, 1H), 3.27 (d, J = 4.5 Hz, 1H), 2.60 (d, J = 16.6 Hz, 1H), 2.48 (dd, J = 16.5 Hz, 9.0 Hz, 1H), 2.12 (m, 1H), 1.44 (s, 9H), 0.95 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 173.7(C), 156.5(C), 79.7(C), 69.4(CH), 59.0(CH), 51.9(CH_3), 38.4(CH_2), 28.5(CH_3), 27.7(CH), 20.2(CH_3), 16.4(CH_3); MS *m/z* 276.2 $(M+H)^+$ 568 $(2M+NH_4)^+$. Anal. Calcd for $C_{14}H_{24}Cl_3NO_5$: C, 56.71; H, 9.15; N, 5.08. Found C, 56.67; H, 9.39; N, 5.00;

(3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid methyl ester (53)



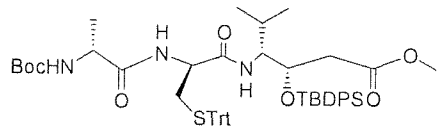
To a stirred solution of the alcohol **52** (0.5 g, 1.82 mmol) in DMF (5 mL) at rt was added imidazole (0.74 g, 10.9 mmol) and TBDPS-Cl (2.4 mL, 9.1 mmol) and stirred at rt for 18 hours. Water (15 mL) was added and the reaction mixture was extracted with ether (3 x 50 mL). The combined organic phases were concentrated and the residue was purified by flash chromatography (eluent 10% EtOAc/hexanes) to give **53** as a glass (0.85 g, 91%): mp = 95-96 °C; $[\alpha]^{22}_{D}$ +3.0 (c 0.50, $CHCl_3$); IR ν_{max} 1737.4, 1732.5, 1500.7, 1461.9 cm^{-1}; ^1H NMR 400 MHz 7.72 (d, J = 6.5 Hz, 2H), 7.62 (d, J = 6.5 Hz, 2H), 7.44-7.34 (m, 6H), 4.38 (d, J = 6.0 Hz, 1H), 4.18 (m, 1H), 3.57 (m, 1H), 3.34 (s, 3H), 2.51 (d, J = 6.0 Hz, 2H), 2.00 (m, 1H), 1.40 (s, 9H), 1.04 (s, 9H), 0.79 (d, J = 6.5 Hz, 3H), 0.61 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 172.0, 156.1, 136.2, 136.0, 134.9, 134.1, 130.3, 130.0, 129.7, 127.8, 127.6, 79.0, 70.9, 51.6, 39.8, 28.5, 27.1, 20.4, 19.4, 16.2; MS *m/z* 536.4 $(M + Na)^+$.

(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-(9*H*-fluoren-9-ylme thoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-5-methyl-hexanoic acid methyl ester (54)



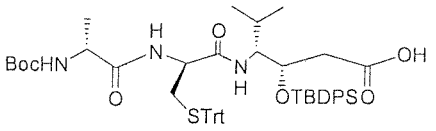
To a stirred solution of **53** (0.80 g, 1.55 mmol) in CH_2Cl_2 (14 mL) at 0 °C was added TFA (4 mL). After stirring for 2.5 h, the solution was concentrated and the TFA azeotroped with Et_2O (2 x 25 mL). To a stirred solution of Fmoc-(*S*Trt)-D-cysteine (908 mg, 1.55 mmol), PyBOP (887.3 mg, 1.77 mmol) and DIEA (948 μL, 5.43 mmol) in CH_3CN (50 mL) was added the crude amine. After stirring at rt for 1 hour the solvent was removed and the residue was purified by flash chromatography (eluent 5-25% EtOAc/hexanes) to give **54** as a glass (1.23 g, 81%): mp = 97-98 °C; $[\alpha]^{22}_D$ -0.8 (c 0.50, $CHCl_3$); IR ν_{max} 1735.8, 1675.7, 1511.5, 1491.5, 1466.6 cm^{-1}; ^1H NMR 400 MHz 7.71 (m, 3H), 7.56 (m, 3H), 7.42-7.16 (m, 27H), 5.96 (d, J = 10.5 Hz, 1H), 4.84 (d, J = 8.0 Hz, 1H), 4.35 (d, J = 6.5 Hz, 2H), 4.14 (m, 2H), 3.83 (m, 1H), 3.75 (m, 1H), 3.31 (s, 3H), 2.63 (m, 2H), 2.45 (d, J = 3.5 Hz, 2H), 1.87 (m, 1H), 0.99 (s, 9H), 0.63 (d, J = 6.5 Hz, 3H), 0.49 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 172.0, 170.3, 144.6, 143.9, 143.8 141.4, 136.3, 136.0, 133.9, 133.0, 130.0, 128.2, 127.8, 127.6, 127.0, 125.1, 120.1, 70.1, 67.5, 60.5, 58.9, 54.3, 51.6, 47.2, 39.3, 33.5, 28.2, 27.1, 20.3, 19.4, 16.5, 14.3; MS *m/z* 1003.7 $(M + Na)^+$.

(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid methyl ester (55)



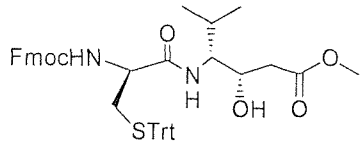
To a stirred solution of **54** (600 mg, 0.611 mmol) in CH_3CN (20 mL) at rt, was added Et_2NH (1.0 mL). After stirring for 3 h at rt the reaction mixture was diluted with heptane (50 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of Boc-D-alanine (115.6 mg, 0.92 mmol) in CH_2Cl_2 (20 mL), PyBOP (477 mg, 0.92 mmol) and DIEA (267 μL, 1.53 mmol) was added the crude amine. After stirring at rt for 3 hours the solvent was removed and the residue purified by flash chromatography (eluent 20-30% EtOAc/hexanes) to give **55** as a glass (407 mg, 72%): mp = 93-94 °C; $[\alpha]^{22}_D$ +4.1 (c 0.50, $CHCl_3$); IR ν_{max} 3311, 1737, 1651, 1491, 1444 cm^{-1}; ^1H NMR 400 MHz 7.74 (d, J = 7.0 Hz, 2H), 7.60 (d, J = 7.0 Hz, 2H), 7.42-7.20 (m, 23H), 6.30 (s, 1H), 6.14 (s, 1H), 4.81 (s, 1H), 4.20 (m, 1H), 4.01 (m, 1H), 3.84 (m, 1H), 3.33 (s, 3H), 2.90 (m, 1H), 2.48 (d, J = 5.5 Hz, 1H), 2.47 (m, 1H), 1.82 (m, 1H), 1.35 (s, 9H), 1.25 (d, J = 7.0 Hz, 3H), 1.00 (s, 9H), 0.64 (d, J = 6.5 Hz, 3H), 0.52 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 181.4, 172.4, 172.2, 169.9, 144.6, 136.3, 136.1, 134.1, 133.2, 129.9, 129.7, 128.2, 127.7, 127.5, 127.0, 80.5, 70.2, 67.3, 59.2, 52.4, 51.6, 39.2, 32.9, 28.4, 27.1, 20.2, 19.4, 18.4, 16.8; MS *m/z* 930.3 $(M + H)^+$, 952.2 $(M + Na)^+$.

(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid (56)



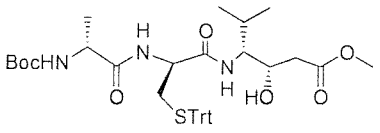
To a stirred solution of **55** (350 mg, 0.376 mmol) in 4:1 THF/H_2O (4.0 mL) at 0 °C was added LiOH (18.1 mg, 0.75 mmol). After stirring for 18 hours, the reaction mixture was diluted with H_2O (15 ml), acidified to pH 4-5 with 2M $KHSO_4$ and extracted with EtOAc (3 x 30 ml). The organic layer was washed with sat. NaCl (20 mL), dried (Na_2SO_4), filtered, and concentrated to give a white solid which was triturated with ether, ether decanted to give **56** as a white solid (160 mg, 47%): $[\alpha]^{22}_D$ +3.3 (c 0.50, $CHCl_3$); IR ν_{max} 3442, 3373, 1725, 1701, 1502, 1473 cm^{-1}; ^1H NMR 400 MHz 7.69 (d, J = 7.5 Hz, 2H), 7.64 (d, J = 7.5 Hz, 2H), 7.62-7.17 (m, 23H), 6.57 (s, 1H), 6.16 (d, J = 6.5 Hz, 1H), 4.86 (s, 1H), 4.13 (m, 2H), 4.04 (m, 1H), 3.93 (m, 1H), 2.99 (m, 1H), 2.44 (m, 3H), 1.94 (m, 1H), 1.34 (s, 9H), 1.25 (d, J = 7.0 Hz, 3H), 1.04 (s, 9H), 0.70 (d, J = 6.5 Hz, 3H), 0.54 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 173.1, 170.1, 155.8, 144.5, 136.2, 133.8, 133.2, 130.0, 129.8, 128.2, 128.0, 127.8, 127.0, 70.3, 67.2, 60.5, 59.5, 52.8, 39.3, 33.3, 28.4, 27.1, 20.2, 19.4, 16.3, 14.3; MS *m/z* 1028.0 $(M + TFA - H)^-$.

(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-5-methyl-hexanoic acid methyl ester (60)



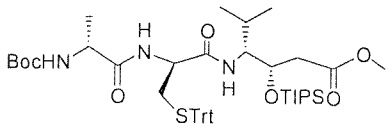
To a stirred solution of **52** (1.0 g, 3.63 mmol) in CH_2Cl_2 (24 mL) at 0 °C was added TFA (6 mL). After stirring for 2.5 h, the solution was concentrated and the TFA chased with Et_2O (2 x 25 mL). To a stirred solution of Fmoc-(*S*Trt)-D-cysteine (746 mg, 1.27 mmol), PyBOP (2.24 g, 4.3 mmol) DIEA (770 μL, 12.6 mmol) was added the crude amine. After stirring at rt for 1 h, the solvent was removed and the residue was purified by flash chromatography (eluent 20-50 % EtOAc/hexanes) to give **60** as a white glass (1.79 g, 67 %): mp = 89-90 °C; $[\alpha]^{22}_D$ +6.1 (c 0.50, $CHCl_3$); IR ν_{max} 3313, 1701, 1661, 1526, 1445 cm^{-1}; ^1H NMR 400 MHz 7.75 (t, *J* = 7.0 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.38 (m, 8H), 7.24 (m, 11H), 5.81 (d, *J* = 9.5 Hz, 1H), 4.97 (d, *J* = 7.0 Hz, 1H), 4.38 (m, 2H), 4.17 (d, *J* = 6.5 Hz, 1H), 3.87 (m, 1H), 3.77 (m, 1H), 3.70 (d, *J* = 6.6 Hz, 1H), 3.61 (s, 3H), 3.26 (s, 1H), 2.65 (d, *J* = 6.5 Hz, 1H), 2.53 (d, *J* = 16.0 Hz, 1H), 2.37 (dd, *J* = 16.5, 9.0 Hz, 1H), 2.07 (m, 1H), 0.81 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 173.6, 170.8, 156.2, 144.4, 143.7, 141.4, 129.6, 128.2, 127.9, 127.2, 127.1, 125.0, 120.1, 68.9, 67.5, 67.2, 57.6, 54.4, 51.9, 47.2, 38.2, 33.3, 27.4, 20.2, 16.4; MS *m/z* 760.3 $(M + Na)^+$.

(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tri tylsulfanyl-propionylamino]-3-hydroxy-5-methyl-hexanoic acid methyl ester (61)



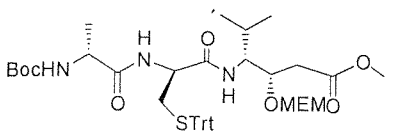
To a stirred solution of **60** (500 mg, 0.674 mmol) in CH_3CN (20 mL) at rt was added Et_2NH (1.0 mL). After stirring for 3 h at rt the reaction mixture was diluted with heptane (50 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of the amine in CH_3CN (15 mL) at rt was added Boc-D-alanine (127.5 mg, 0.674 mmol), PyBOP (350.7 mg, 0.674 mmol) and DIEA (294 μL, 1.69 mmol). After stirring at rt for 3 hours the solvent was removed and the residue was purified by flash chromatography (eluent 40-60 % EtOAc/hexanes) to give **61** as a white glass (364 mg, 78 %); $[\alpha]^{22}_D$ -8.0 (c 0.50, $CHCl_3$); IR ν_{max} 3311, 1719, 1650, 1493, 1444 cm^{-1}; ^1H NMR 400 MHz 7.41 (m, 6H), 7.29 (m, 6H), 7.24 (m, 3H), 6.56 (m, 1H), 6.52 (d, J = 9.0 Hz, 1H), 4.83 (d, J = 4.0 Hz, 1H), 4.10 (m, 1H), 3.92 (m, 1H), 3.87 (m, 1H), 3.64 (s, 3H), 3.17 (d, J = 9.0 Hz, 1H), 3.16 (s, br, 1H), 2.50 (d, J = 15.5 Hz, 1H), 2.41 (d, J = 10.0 Hz, 1H), 2.35 (m, 1H), 1.93 (m, 1H), 1.30 (s, 9H), 1.37 (m, 3H), 0.93 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 173.8, 172.9, 170.9, 144.4, 129.6, 128.3, 127.1, 81.2, 69.0, 67.5, 58.7, 52.8, 52.0, 51.5, 37.1, 32.7, 28.3, 28.2, 20.0, 18.3, 17.8; MS *m/z* 714.43 $(M + Na)^+$.

(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tri tylsulfanyl-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid methyl ester (62)



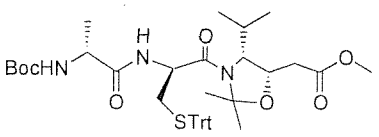
To a solution of the alcohol **61** (100 mg, 0.145 mmol) in CH_2Cl_2 (4mL) at 0 °C was added 2,6-lutidine (43 μl, 0.36 mmol), followed by the dropwise addition of triisopropylsilyl triflate (58 μl, 0.22 mmol). The reaction mixture was stirred at 0 °C for 30 min, then at rt for 2 hours, diluted with CH_2Cl_2 (30 mL), washed with 10% HCl (10 mL), 5% $NaHCO_3$ (10 mL) and sat. NaCl (10 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 25-35% EtOAc/hexanes) to give **62** as a white glass (101 mg, 82%); IR ν_{max} 3311, 1737, 1657, 1492 cm^{-1}; ^1H NMR 400 MHz 7.42 (m, 6H), 7.27 (m, 6H), 7.21 (m, 3H), 6.35 (m, 1H), 4.83 (d, J = 5.5 Hz, 1H), 4.39 (m, 1H), 4.15 (m, 1H), 4.03 (m, 1H), 3.87 (m, 1H), 3.62 (s, 3H), 2.99 (m, 1H), 2.59 (dd, J = 16.0, 5.5 Hz, 1H), 2.47 (dd, J = 16.0, 6.0 Hz, 1H), 2.45 (m, 1H), 2.35 (m, 1H), 1.86 (m, 1H), 1.36 (s, 9H), 1.28 (d, J = 7.0 Hz, 3H), 1.04 (m, 21H), 0.98 (m, 1H), 0.93 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 172.4, 169.8, 154.4, 144.6, 129.7, 128.1, 126.9, 120.2, 67.6, 67.3, 59.4, 52.4, 51.8, 50.7, 39.4, 32.7, 28.9, 28.6, 24.5, 20.4, 18.9, 18.2, 17.9, 17.7, 17.1, 14.4, 13.1, 12.1; MS *m/z* 848.3 $(M + H)^+$.

(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tri tylsulfanyl-propionylamino]-3-(2-methoxy-ethoxymethoxy)-5-methyl-hexanoic acid methyl ester (64)



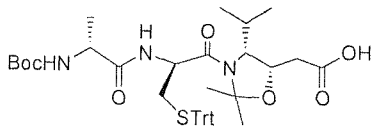
To a stirred solution of the alcohol **61** (100 mg, 0.145 mmol) in CH_2Cl_2 (5 mL) at rt was added MEM-Cl (22 μL, 0.181 mmol) and DIEA (32 μL, 0.181 mmol). After stirring at rt for 18 hours the reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with 5% $NaHCO_3$ (10 mL), sat. citric acid (10 mL), and sat. NaCl (10 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 25-35 % EtOAc/hexanes) to give **64** as a glass (80 mg, 71 %); IR ν_{max} 3301, 1737, 1649, 1524, 1492 cm^{-1}; ^1H NMR 400 MHz 7.41 (m, 6H), 7.29 (m, 6H), 7.21 (m, 3H), 6.59 (m, 1H), 6.52 (m, 1H), 4.86 (m, 2H), 4.74 (d, J = 7.0 Hz, 1H), 4.69 (d, J = 7.0 Hz, 1H), 4.18 (m, 1H), 4.10 (m, 1H), 3.93 (m, 1H), 3.70 (m, 2H), 3.66 (s, 3H), 3.35 (m, 2H), 3.38 (s, 3H), 3.03 (s, br, 1H), 2.52 (d, J = 9.0 Hz, 2H), 2.39 (m, 1H), 1.84 (m, 1H), 1.34 (s, 9H), 1.30 (m, 3H), 0.92 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 172.2, 169.8, 144.6, 129.7, 128.2, 127.0, 80.6, 71.9, 67.6, 67.2, 59.1, 56.4, 52.3, 51.8, 50.8, 33.3, 28.6, 28.4, 20.5, 18.2; MS *m/z* 797.3 $(M + NH_4)^+$.

{(4*R*,5*S*)-3-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionyl]-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl}-acetic acid methyl ester (66)



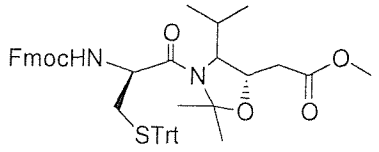
To a stirring solution of the peptide **61** (400 mg, 0.578 mmol) and dimethoxypropane (1.4 mL, 11.6 mmol) in benzene (40 mL) was added PTSA (12.2 mg, 0.064 mmol). The reaction mixture was refluxed in a soxhlet containing 4Å molecular sieves for 1.5 hours. The solvent was reduced to 4 mL, diluted with ether (40 mL), washed with 10% HCl (20 mL), 5% $NaHCO_3$ (20 mL) and saturated NaCl (20 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 30-50% EtOAc/hexanes) to give **66** as a white glass (343 mg, 81%): mp = 111-113 °C; $[\alpha]^{22}_D$ +46.9 (c 0.50, $CHCl_3$); IR ν_{max} 3289, 2940, 1739, 1716, 1678, 1644, 1492, 1444 cm^{-1}; ^1H NMR 400 MHz 7.40 (m, 6 H), 7.26 (m, 6H), 7.20 (m, 3H), 6.23 (d, J = 9.1 Hz, 1H), 4.93 (m, 1H), 4.69 (m, 1H), 4.43 (m, 1H), 4.17 (m, 1H), 4.11 (m, 1H), 3.71 (s, 3H), 2.66 (m, 3H), 2.51 (dd, J = 13.0, 5.0 Hz, 1H), 1.85 (m, 1H), 1.54 (s, 3H), 1.46 (s, 3H), 1.42 (s, 9H), 1.29 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H), 0.85 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 172.4, 170.8, 168.2, 144.6, 129.7, 128.1, 126.9, 94.5, 73.8, 67.1, 63.7, 52.1, 50.9, 34.6, 33.4, 29.1, 28.4, 25.7, 23.0, 20.7, 20.2; MS *m/z* 754.4 $(M + Na)^+$.

{(4*R*,5*S*)-3-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tr itylsulfanyl-propionyl]-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl}-acetic acid (67)



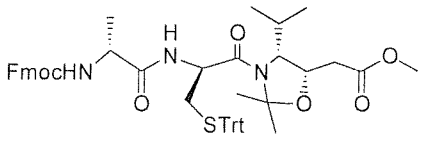
To a stirred solution of **66** (50 mg, 0.068 mmol) in 4:1 THF/H_2O (1.0 mL) at 0 °C was added LiOH (3.26 mg, 0.14 mmol) and stirred for 18 hours. The reaction mixture was diluted with H_2O (5 ml) acidified to pH 4-5 with 2M $KHSO_4$ and extracted with EtOAc (3 x 10 ml), the organic layer washed with sat. NaCl (5 mL), dried (Na_2SO_4), filtered, and concentrated to give a white solid which was triturated with ether (5 mL) to give **67** as a white solid (45 mg, 92%): $[\alpha]^{22}_D$ +67.0 (c 0.50, $CHCl_3$); IR ν_{max} 3300.4, 3060.1, 2978.3, 2934.8, 1714.0, 1666.9, 1642.3, 1605.8, 1492.8, 1444.2 cm^{-1}; ^1H NMR 400 MHz 7.39 (m, 6 H), 7.28 (m, 6H), 7.21 (m, 3H), 6.60 (d, J = 8.5 Hz, 1H), 5.33 (m, 1H), 4.64 (m, 1H), 4.45 (m, 1H), 4.22 (m, 1H), 4.11 (m, 1H), 2.72-2.63 (m, 3H), 2.49 (dd, J = 12.5, 4.5 Hz, 1H), 1.87 (m, 1H), 1.54 (s, 3H), 1.44 (s, 3H), 1.40 (s, 9H), 1.26 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 173.4, 168.4, 155.4, 144.6, 129.7, 128.1, 127.0, 94.7, 73.8, 67.2, 63.8, 51.2, 34.6, 33.1, 29.3, 28.5, 25.7, 23.0, 20.6, 20.3, 18.8; MS *m/z* 716.3 $(M - H)^-$.

{(4*R*,5*S*)-3-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionyl]-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl}-acetic acid methyl ester (69)



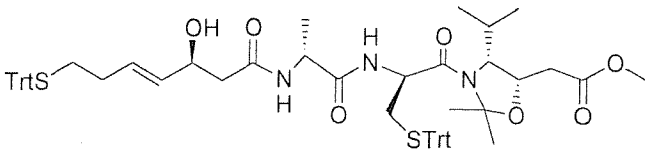
To a stirring solution of the peptide **60** (2.0 g, 2.69 mmol) and dimethoxypropane (6.59 mL, 53.8 mmol) in benzene (200 mL) was added PTSA (52.2 mg, 0.269 mmol). The reaction mixture was refluxed in a soxhlet containing 4Å molecular sieves for 1.5 hours. The solvent was reduced to 20 mL, diluted with ether (200 mL), washed with 10% HCl (60 mL), 5% $NaHCO_3$ (60 mL) and saturated NaCl (60 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 30-50% EtOAc/hexanes) to give **69** as a white glass (1.77 g, 84%): mp = 107-109 °C; $[\alpha]^{22}_D$ +48.0 (c 0.50, $CHCl_3$); IR ν_{max} 3278, 3016, 1713, 1631, 1509 cm^{-1}; ^1H NMR 400 MHz 7.74 (m, 2 H), 7.56 (t, J = 7.5 Hz, 2H), 7.35 (m, 8H), 7.20 (m, 12H), 4.91 (d, 9.5 Hz, 1H), 4.41 (m, 4H), 4.20 (dt, J = 10.5, 5.0 Hz, 2H), 3.69 (s, 3H), 2.68 (d, J = 7.6 Hz, 2H), 2.62 (dd, J = 12.0, 9.0 Hz, 1H), 2.49 (dd, J = 12.5, 4.0 Hz, 1H), 1.84 (m, 1H), 1.69 (s, 3H), 1.69 (s, 3H), 1.51 (s, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 170.8, 168.6, 155.9, 144.6, 144.5, 143.8, 141.5, 129.7, 128.1, 127.9, 127.2, 126.9, 125.1, 120.1, 94.5, 73.8, 67.2, 63.7, 52.6, 52.1, 47.2, 34.6, 33.8, 29.2, 25.7, 23.2, 20.7, 20.2; MS *m/z* 805.3 $(M + Na)^+$.

((4*R*,5*S*)-3-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-tritylsulfanyl-propionyl}-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl)-acetic acid methyl ester (70)



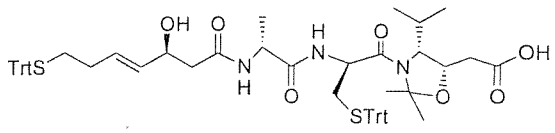
To a stirred solution of **69** (200 mg, 0.256 mmol) in CH_3CN (20 mL) at rt was added Et_2NH (1.0 mL). After stirring for 3 h at rt the reaction mixture was diluted with heptane (50 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of Fmoc-D-alanine (120 mg, 0.384 mmol) in CH_2Cl_2 (10 mL), PyBOP (160 mg, 0.307 mmol) and DIEA (112 μL, 0.64 mmol) was added the crude amine. After stirring at rt for 3 hours, the solvent was removed and the residue was purified by flash chromatography (eluent 25-30% EtOAc/hexanes) to give **70** as a white glass (188 mg, 86%): mp = 109-110 °C; $[\alpha]^{22}_D$ +54.0 (c 0.50, $CHCl_3$); IR ν_{max} 3311, 3016, 1737, 1644, 1502 cm^{-1}; ^1H NMR 400 MHz 7.68 (d, J = 7.5 Hz, 2H), 7.49 (dd, J = 17.0, 7.0 Hz, 2H), 7.32 (m, 8H), 7.18 (m, 8H), 7.10 (m, 3H), 5.60 (m, 1H), 5.22 (m, 1H), 4.60 (m, 1H), 4.32 (m, 3H), 4.08 (m, 3H), 3.62 (s, 3H), 2.59 (m, 3H), 2.45 (dd, J = 12.5, 4.5 Hz, 1H), 1.78 (m, 1H), 1.62 (m, 1H), 1.48 (s, 3H), 1.38 (s, 3H), 1.18 (m, 2H), 0.92 (d, J = 6.5 Hz, 3H), 0.78 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 171.9, 170.8, 168.1, 155.8, 144.6, 144.5, 144.0, 143.8, 141.4, 129.7, 128.1, 127.8, 127.2, 126.9, 125.2, 120.1, 94.5, 73.8, 67.2, 63.7, 52.1, 51.0, 50.5, 47.3, 34.6, 33.3, 29.1, 25.7, 23.0, 21.8, 20.2, 19.2; MS *m/z* 871.5 $(M + NH_4)^+$.

((4*R*,5*S*)-3-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionyl}-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl)-acetic acid methyl ester (71)



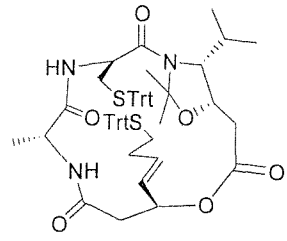
To a stirred solution of **70** (989.6 mg, 1.57 mmol) in CH_3CN (100 mL) at rt was added Et_2NH (5.0 mL). After stirring for 5 h at rt the reaction mixture was diluted with heptane (1.0 mL), filtered, solvent removed, and concentrated to give the crude amine as a colourless oil. To a stirred solution of the crude amine in CH_2Cl_2 (50 ml) was added alcohol (800 mg, 1.42 mmol) and DMAP (17.3 mg, 0.014 mmol) at 0 °C. After stirring at rt for 5 hours, the solvent was removed and the residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes) to give **71** as a white glass (1.15 g, 78%): mp = 104-105 °C; $[\alpha]^{22}_D$ +44.0 (c 0.50, $CHCl_3$); IR ν_{max} 3301, 3013, 1739, 1632, 1492, 1492 cm^{-1}; ^1H NMR 400 MHz 7.37 (m, 12H), 7.28 (m, 12H), 7.20 (m, 6H), 5.90 (d, J = 7.5 Hz, 1H), 5.53 (dt, J = 15.0 Hz, 6.0 Hz, 1H), 5.38 (dd, J = 15.0 Hz, 5.5 Hz, 1H), 4.71 (m, 1H), 4.60 (m, 1H), 4.39 (t, J = 7.5 Hz, 1H), 4.20 (t, J = 4.5 Hz, 1H), 3.71 (s, 3H), 2.68 (m, 2H), 2.61 (m, 1H), 2.42 (dd, J = 12.5 Hz, 5.0 Hz, 1H), 2.31 (dd, J = 13.0 Hz, 2.5 Hz, 1H), 2.31 (m, 3H), 2.20 (m, 3H), 2.09 (m, 3H), 1.86 (m, 1H), 1.75 (s, 1H), 1.51 (s, 3H), 1.49 (s, 3H), 1.36 (d, J = 7.0 Hz, 3H), 1.00 (d, J = 6.5 Hz, 3H), 0.82 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 171.8, 171.2, 170.6, 168.6, 145.0, 144.7, 132.9, 129.7, 129.6, 128.1, 128.0, 127.0, 126.9, 126.8, 94.6, 73.8, 70.2, 66.9, 66.8, 63.8, 52.1, 50.8, 59.7, 44.4, 34.7, 33.4, 31.6, 31.4, 29.1, 25.6, 23.1, 20.6, 20.2, 20.2, 17.8; MS *m/z* 1049.3 $(M+ NH_4)^+$.

((4*R*,5*S*)-3-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionyl}-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl)-acetic acid (72)



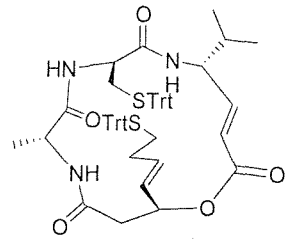
To a stirred solution of **71** (500 mg, 0.48 mmol) in 4:1 THF/H_2O (5.0 mL) at 0 °C was added LiOH (23 mg, 0.97 mmol). After stirring for 5 hours, the reaction mixture was diluted with H_2O (30 ml), acidified to pH 4-5 with 2M $KHSO_4$, extracted with EtOAc (3 x 30 ml), the organic layer washed with sat. NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated to give a yellow oil which was triturated with ether to give **72** as a white solid (451 mg, 91 %): mp = 107 °C; $[\alpha]^{22}_D$ +36.8 (c 0.50, $CHCl_3$); IR ν_{max} 3314, 3057, 3015, 1713, 1629, 1527, 1490 cm^{-1}; ^1H NMR 400 MHz 7.40 (m, 12H), 7.26 (m, 12H), 7.20 (m, 6H), 6.52 (d, J = 7.0 Hz, 1H), 5.52 (dt, J = 15.0 Hz, 6.5 Hz, 1H), 5.38 (dd, J = 15.0 Hz, 5.5 Hz, 1H), 4.66 (m, 1H), 4.44 (m, 1H), 4.37 (m, 2H), 4.21 (m, 1H), 2.64 (m, 3H), 2.44 (dd, J = 12.5 Hz, 5.0 Hz, 1H), 2.35 (m, 1H), 2.21 (m, 3H), 2.07 (m, 3H), 1.84 (m, 1H), 1.50 (s, 3H), 1.44 (s, 3H), 1.31 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.80 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 172.8, 172.0, 168.5, 145.0, 144.6, 132.8, 129.7, 129.5, 128.3, 128.1, 128.0, 127.0, 126.7, 94.7, 73.9, 69.9, 67.1, 66.7, 63.9, 51.0, 49.8, 43.9, 33.2, 31.6, 31.5, 29.1, 25.6, 23.0, 20.6, 20.3, 17.8; MS *m/z* 1130.2 $(M - H^+ + CF_3CO_2H)^-$.

(3*R*,6*R*,10*S*,14*S*,17*R*)-17-Isopropyl-6,16,16-trimethyl-10-((*E*)-4-tritylsulfanyl-but-1-enyl)-3-tritylsulfanylmethyl-11,15-dioxa-1,4,7-triaza-icyclo[12.2.1]heptadecane-2,5,8,12-tetraone (73)



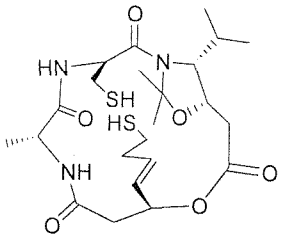
To a stirred solution of the hydroxy acid **72** (150 mg, 0.147 mmol) in THF (3.0 mL) at 0 °C was added Et_3N (123 μl, 0.88mmol) and 2,4,6-trichlorobenzoyl chloride (13 μl, 0.08 mmol). After stirring at room temperature for 1 hour the reaction mixture was diluted with toluene 10 mL and then added dropwise to a vigorously stirring solution of DMAP (87.12 mg, 0.713 mmol) in toluene (29.4 mL, 0.005 M with respect to the hydroxy acid) at 75 °C over 2 hours and then stirred at the same temperature for a further 2 hours. The reaction mixture was allowed to cool to rt then washed with 5% $NaHCO_3$ (15 mL), sat. citric acid (15 mL), and sat. NaCl (15 mL) solutions (each back extracted with EtOAc 15 mL). The combined organic phase was dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-60% EtOAc/hexanes) to give **73** as a white glass (106.2 mg, 72%): mp = 238 °C; $[\alpha]^{22}_D$ +62.2 (c 0.50, $CHCl_3$); IR ν_{max} 3224, 3055, 3029, 2981, 2933, 1744, 1648, 1544, 1489 cm^{-1}; ^1H NMR 400 MHz 7.39 (m, 12 H), 7.27 (m, 12H), 7.20 (m, 6H), 6.61 (d, J = 6.0 Hz, 1H), 5.83 (d, J = 7.0 Hz, 1H), 5.61 (m, 2H), 5.31 (dd, J = 15.0 Hz, 6.5 Hz, 1H), 4.19 (m, 2H), 3.57 (t, J = 7.0 Hz, 1H), 3.14 (m, 1H), 3.03 (dd, J = 13.5, 5.5 Hz, 1H), 2.89 (dd, J = 14.5, 8.5 Hz, 1H), 2.74 (d, J = 15.0 Hz, 1H), 2.51 (m, 1H), 2.47 (s, 1H), 2.38 (dd, J = 15.5, 11.0 Hz, 1H), 2.18 (t, J = 6.5 Hz, 1H), 2.06 (t, J = 6.0 Hz, 1H), 1.72 (m, 1H), 1.53 (s, 6H), 1.27 (d, J = 6.5 Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.80 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 170.7, 169.3, 168.3, 165.8, 144.9, 133.25, 129.8, 129.7, 128.1, 128.0, 127.8, 126.9, 126.8, 94.8, 74.6, 71.3, 67.4, 66.8, 62.1, 55.4, 49.2, 40.9, 35.7, 32.9, 31.4, 31.2, 28.2, 26.1, 23.4, 21.4, 20.3, 16.6; MS *m/z* 1017.1 $(M+NH_4)^+$.

(*E*)-(2*S*,6*R*,9*S*,12*R*)-12-Isopropyl-6-methyl-2-((*E*)-4-tritylsulfanyl-but-1 -enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadec-13-ene-4,7,10,15-tetraone (76)



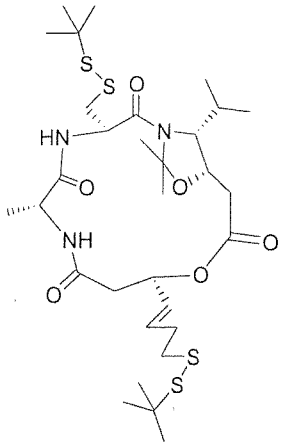
To a stirred solution of the acetonide **73** (45 mg, 0.045 mmol) in THF (1.0 mL) at 0 °C was added 1M tetrabutylammonium fluoride in CH_2Cl_2 (1.0 mL, 0.9 mmol). After stirring at room temperature for 1.5 hours, sat. NH_4Cl (1 mL) was added. The reaction mixture was extracted with EtOAc (3 x 5 mL), washed with sat. NaCl (10 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-60% EtOAc/hexanes) to give **76** as a white glass (37 mg, 87%): $[\alpha]^{22}_D$ +14.4 (c 0.50, $CHCl_3$); IR ν_{max} 3286, 3085, 3062, 3030, 1722, 1665, 1651, 1530, 1491 cm^{-1}; ^1H NMR 400 MHz 7.46 (m, 2H), 7.34 (m, 12 H), 7.20 (m, 12H), 7.12 (m, 6H), 6.31 (dd, J = 15.5, 9.0 Hz, 1H), 6.03 (d, J = 8.5 Hz, 1H), 5.56 (m, 2H), 5.30 (m, 1H), 5.27 (dd, J = 15.5 Hz, 6.0 Hz, 1H), 4.16 (t, J = 7.0 Hz, 1H), 3.93 (m, 1H), 3.05 (m, 1H), 2.69 (m, 2H), 2.37 (m, 1H), 2.27 (m, 1H), 2.10 (t, J = 6.0 Hz, 2H), 1.96 (m, 2H), 1.64 (m, 1H), 1.16 (d, J = 6.5 Hz, 3H), 0.82 (d, J = 6.5 Hz, 3H), 0.77 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 175.2, 169.9, 168.7, 164.3, 145.6, 145.0, 144.4, 132.5, 129.7, 129.6, 128.5, 128.2, 128.0, 127.0, 122.8, 72.0, 67.2, 66.7, 59.4, 57.1, 48.6, 42.5, 31.5, 31.7, 31.3, 30.3, 19.2, 19.0; MS *m/z* 959.6 $(M+NH_4)^+$.

(3*S*,6*R*,10*S*,14*S*,17*R*)-17-Isopropyl-10-((*E*)-4-mercapto-but-1-enyl)-3-mercaptomethyl-6,16,16-trimethyl-11,15-dioxa-1,4,7-triaza-bicyclo[12.2.1]heptadecane-2,5,8,12-tetraone (77)



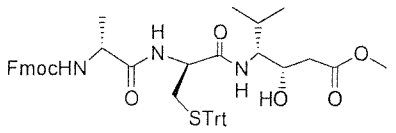
To a stirring solution of **73** (50 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) at 0 °C under Ar was added TFA (0.2 mL) then Et_3SiH (36 μL, 0.22 mmol). After stirring for 15 minutes, the solvent was removed under vacuum, the remaining TFA azeotroped with ether and the residue was purified by flash chromatography (2-10% MeOH/CH_2Cl_2) to give **77** as a white solid (3.4 mg, 90%): mp = 251 °C; $[\alpha]^{22}_D$ + 5.6 (c 0.50, $CHCl_3$); IR ν_{max} 3263, 3027, 1740.6, 1645, 1534 cm^{-1}; ^1H NMR 400 MHz (5% CD_3OD, $CDCl_3$) 7.30 (m, 1H), 7.21 (m, 1H), 7.12 (d, J = 7.5 Hz, 1H), 5.75 (m, 2H), 5.51 (m, 1H), 4.32 (m, 2H), 4.13 (m, 1H), 3.73 (m, 1H), 3.06 (m, 1H), 2.78 (m, 3H), 2.57 (m, 5H), 2.37 (m, 2H), 1.91 (m, 1H), 1.62 (s, 3H), 1.60 (s, 3H), 1.31 (d, J = 7.0 Hz, 3H), 1.03 (t, J = 6.0 Hz, 6H); ^{13}C NMR 100 MHz (5% CD_3OD, $CDCl_3$) 172.4, 169.7, 168.4, 166.2, 132.7, 129.5, 128.8, 128.3, 126.3, 94.5, 74.7, 71.6, 62.5, 56.7, 40.4, 36.2, 35.5, 27.9, 25.8, 23.3, 21.3, 20.3, 16.7; MS *m/z* 516.2 $(M+H)^+$.

(3*S*,6*R*,10*S*,14*S*,17*R*)-10-((*E*)-4-*tert*-Butyldisulfanyl-but-1-enyl)-3-*tert*-butyldisulfanylmethyl-17-isopropyl-6,16,16-trimethyl-11,15-dioxa-1,4,7-triaza-bicyclo[12.2.1]heptadecane-2,5,8,12-tetraone (78)



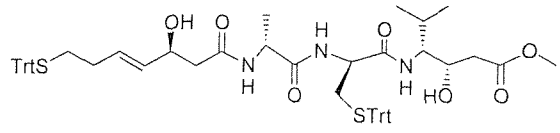
To a stirring solution of **77** (10 mg, 0.019 mmol) in CH_2Cl_2 (1 mL) at rt was added *tert*-butylthiol (211 μL, 1.9 mmol) then Et_3N (30 μL, 0.19 mmol) and stirred for 10 days under O_2. The solvent was removed under vacuum, and the residue was purified by flash chromatography (2-10% MeOH/CH_2Cl_2) to give **78** as a white glass (7.1 mg, 54%): IR ν_{max} 3256, 1743, 1646, 1537, 1415 cm^{-1}; ^1H NMR 400 MHz 6.98 (d, J = 6.5 Hz, 1H), 5.84 (d, J = 6.5 Hz, 1H), 5.79 (t, J = 7.0 Hz, 1H), 5.69 (m, 1H), 5.50 (dd, J = 15.5, 7.0 Hz, 1H), 4.32 (m, 3H), 3.81 (m, 1H), 3.24 (dd, J = 14.0, 4.5 Hz, 1H), 3.14 (dd, J = 13.5, 8.5 Hz, 1H), 2.82 (m, 1H), 2.73 (t, J = 7.0 Hz, 2H), 2.61 (m, 1H), 2.53 (m, 1H), 2.43(m, 1H), 1.94 (m, 1H), 1.62 (s, 6H), 1.36 (d, J = 7.5 Hz, 3H), 1.32 (s, 18H), 1.07 (d, J = 6.5 Hz, 3H), 1.01 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 171.1, 169.5, 168.3, 166.3, 133.0, 128.3, 94.8, 74.8, 71.4, 62.6, 54.7, 50.1, 48.5, 48.0, 42.0, 40.9, 39.7, 35.6, 32.0, 30.0, 27.9, 26.1, 23.6, 21.9, 20.3, 16.7; HRMS *m/z* 714.2709 $(M + Na)^+$ expected 714.2716.

(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propiony lamino]-3-tritylsulfanyl-propionylamino}-3-hydroxy-5-methyl-hexanoic acid methyl ester (79)



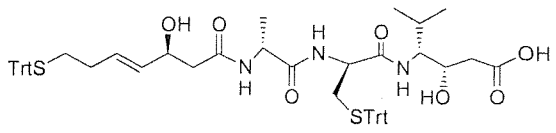
To a stirred solution of **60** (500 mg, 0.674 mmol) in CH_3CN (20 mL) at rt was added Et_2NH (1.0 mL). After stirring for 3 h at rt the reaction mixture was diluted with heptane (50 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of Fmoc-D-alanine (209.8 mg, 0.674 mmol) in CH_2Cl_2 (20 mL), PyBOP (350.75 mg, 0.674 mmol) and DIEA (294 μL, 1.68 mmol) was added the crude amine and stirred at rt for 2 hours, the solvent was removed and the residue was purified by flash chromatography (eluent 30-60% EtOAc/hexanes) to give **79** as a white glass (367.5 mg, 67%): mp = 121-122 °C; $[\alpha]^{22}_D$ +8.7 (c 0.50, $CHCl_3$); IR ν_{max} 3309, 1705, 1650, 1515, 1446 cm^{-1}; ^1H NMR 400 MHz 7.76 (d, J = 7.5 Hz, 2H), 7.50 (m, 2H), 7.39 (m, 8H), 7.31-7.14 (m, 11H), 6.37 (s, 1H), 6.28 (d, J = 8.5 Hz, 1H), 5.17 (s, 1H), 4.34 (m, 2H), 4.05 (m, 4H), 3.83 (s, 1H), 3.63 (s, 3H), 2.93 (m, 1H), 2.83 (s, br, 1H), 2.56 (d, J = 16.5 Hz, 1H), 2.50 (dd, J = 12.5, 5.0 Hz, 1H), 2.39 (dd, J = 16.5, 9.5 Hz, 1H), 2.09 (m, 1 H), 1.29 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 173.8, 172.3, 170.5, 144.4, 143.9, 141.5, 141.4, 129.5, 128.2, 128.0, 127.9, 127.2, 127.0, 125.0, 120.2, 68.8, 67.4, 58.1, 52.8, 51.9, 51.3, 47.2, 37.9, 32.9, 27.7, 20.3, 18.3, 17.0; MS *m/z* 836.2 $(M + Na)^+$.

(3*S*,4*R*)-3-Hydroxy-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-hexanoic acid methyl ester (80)



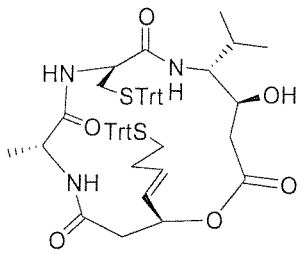
To a stirred solution of **79** (460 mg, 0.45 mmol) in CH_3CN (25 mL) at rt was added Et_2NH (1.25 mL). After stirring for 4 h at rt the reaction mixture was diluted with heptane (50 mL), solvent removed, filtered and concentrated to give the crude amine as a colourless oil. To a stirred solution of the crude amine in CH_2Cl_2 (15 ml) was added alcohol **58** (253.0 mg, 0.45 mmol) and DMAP (5.5 mg, 0.045 mmol) at 0 °C. After stirring at rt for 8 hours, the solvent was removed and the residue was purified by flash chromatography (eluent 25-40% EtOAc/hexanes) to give **80** as a glass (277 mg, 63%): mp = 123 °C; $[\alpha]^{22}_D$ +17.0 (c 0.50, $CHCl_3$); IR ν_{max} 3283, 3059, 2970, 2359, 2327, 1738, 1652, 1620, 1535, 1488, 1444 cm^{-1}; ^1H NMR 400 MHz 7.40-7.18 (m, 30H), 6.71 (d, J = 7.5 Hz, 1H), 5.49 (dt, 15.5, 6.0 Hz, 1H), 5.38 (dd, 15.0, 6.0 Hz, 1H), 4.31 (m, 1H), 4.26 (m, 1H), 3.97 (m, 2H), 3.72 (m, 1H), 3.64 (s, 3H), 2.60 (m, 1H), 2.55 (d, J = 16.5 Hz, 2H), 2.36 (dd, J = 16.0, 10.0 Hz, 1H), 2.23 (m, 1H), 2.09 (m, 4H), 2.07 (m, 3H), 1.31 (d, J = 7.0 Hz, 3H), 1.26 (m, 1H), 0.85 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 173.4, 173.1, 172.3, 171.1, 144.8, 144.3, 132.7, 129.7, 129.5, 128.0, 127.8, 126.8, 126.6, 69.5, 68.4, 67.0, 66.6, 60.6, 58.2, 52.8, 51.7, 43.5, 38.4, 32.7, 31.5, 31.4, 31.2, 27.7, 22.6, 19.8, 17.2, 16.3, 14.0; MS *m/z* 992.4 $(M + H)^+$, 1014.5 $(M + Na)^+$.

(3*S*,4*R*)-3-Hydroxy-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-hexanoic acid (81)



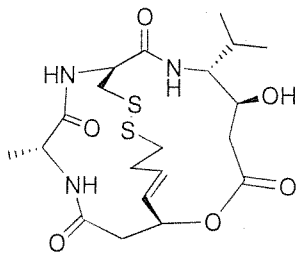
To a stirred solution of **80** (130 mg, 0.133 mmol) in 4:1 THF/H_2O (2.0 mL) at 0 °C was added LiOH (6.30 mg, 0.27 mmol). After stirring for 1 hour the reaction mixture was diluted with H_2O (5 ml) acidified to pH 4-5 with 2M $KHSO_4$ and extracted with EtOAc (3 x 10 ml). The organic layer was washed with sat. NaCl (5 mL), dried (Na_2SO_4), filtered, and concentrated to give a white solid which was triturated with ether, to give the crude dihydroxyacid **81** as a white solid (118 mg, 93%) which was used directly in the next step.

(2*S*,6*R*,9*S*,12*R*,13*S*)-13-Hydroxy-12-isopropyl-6-methyl-2-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,10,15-tetraone (82)



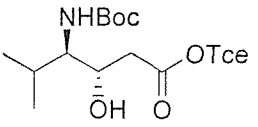
To a stirred solution of the hydroxy acid **81** (45.0 mg, 0.047 mmol) in THF (1.0 mL) at 0 °C was added $^{i}Pr_2NEt$ (49 µl, 0.28 mmol) and 2,4,6-trichlorobenzoyl chloride (15 µl, 0.60 mmol). After stirring at room temperature for 2 hours, the reaction mixture was diluted with toluene (10 mL) and then added dropwise to a vigorously stirring solution of DMAP (17.0 mg, 0.14 mmol) in toluene (25mL) at 70 °C over 2 hours. After stirring at the same temperature for a further 2 hours the reaction mixture was allowed to cool to rt then washed with 5% $NaHCO_3$ (4 mL), sat. citric acid (4 mL), and sat. NaCl (4 mL) solutions, each back extracted with EtOAc(3 x 4 mL). The combined organic phase was dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes) to give **82** as an impure off white solid (9 mg, 20%) which was used directly in the next step.

Spiruchostatin A



To a vigorously stirring solution of I_2 (19.3 mg, 0.076 mmol) in MeOH (25 mL) was added the protected dithiol **82** (18 mg, 0.019 mmol) in 1:1 MeOH/CH_2Cl_2 (5 mL) dropwise over 10 minutes. After stirring for a further 10 minutes, 0.01 N $Na_2S_3O_2$ (25 mL) was added and the organic phase extracted with CH_2Cl_2 (4 x 25 mL). The combined organic extract was washed with sat. NaCl (30 mL) solution, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes then 5% MeOH/CH_2Cl_2) to give **spiruchostatin A** as a white solid (4.0 mg, 45%): $[\alpha]^{22}_D$ -61.1 (c 0.14, MeOH), Lit -63.6[39] (c 0.14, MeOH); IR ν_{max} 3375, 3332, 1732, 1650, 1520 cm^{-1}; ^1H NMR 400 MHz ($CDCl_3$) δ; 7.39 (d, *J* = 7.1 Hz, 1H), 6.71 (d, *J* = 9.1 Hz, 1H), 6.41 (t, *J* = 12.6 Hz, 1H), 5.86 (s, 1H), 5.66 (d, *J* = 15.2 Hz, 1H), 5.51 (s, 1H), 4.94 (m, 1H), 4.59 (m, 1H), 4.29 (m, 1H), 3.38 (dd, *J* = 13.1 Hz, 6.1 Hz, 2H), 3.24 (m, 1H), 3.16 (m, 1H), 2.94 (d, *J* = 10.5 Hz, 1H), 2.75 (m, 3H), 2.74 (d, *J* = 4.0 Hz, 2H), 2.57 (d, *J* = 13.1 Hz, 1H), 2.45 (m, 1H), 2.41 (m, 1H), 1.52 (d, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR 100 MHz δ; 172.0, 171.0, 170.9, 170.7, 169.2, 133.8, 128.6, 70.6, 69.5, 64.0, 63.9, 54.4, 54.2, 52.4, 40.9, 39.8, 33.5, 29.8, 20.8, 19.8, 16.9; HR MS *m/z* 496.1556 $(M + Na)^+$ expected 496.1546.

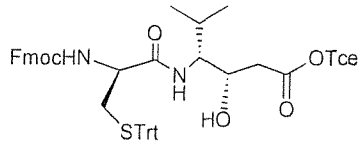
(3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-methyl-hexanoic acid 2,2,2-trichloro-ethyl ester (83)



To a stirred solution of **52** (500 mg, 0.142 mmol) in 4:1 THF/H_2O (20 mL) at 0 °C was added LiOH (87 mg, 3.63 mmol). After stirring for 2 hours at rt, the reaction mixture was diluted down with H_2O (20 ml) acidified to pH 4-5 with 2M $KHSO_4$, extracted with EtOAc (4 x 50 ml), the organic layer washed with sat. NaCl (25 mL), dried (Na_2SO_4), filtered, and concentrated to give a white foam (475 mg, quantitative) which was used directly in the next step.

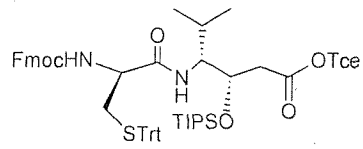
To a stirred solution of the hydroxy acid (400 mg, 1.53 mmol) in CH_2Cl_2 (50 mL) was added $HOCH_2CCl_3$ (2.2 ml, 22.95 mmol), DCC (1.30g, 9.55 mmol) and DMAP (23 mg, 0.191 mmol) at 0 °C. After stirring at rt for 18 hours the reaction mixture was diluted with CH_2Cl_2 (50 mL) washed with water (25 mL), 10% HCl (25 mL), 5% $NaHCO_3$ (25mL) and sat. NaCl (25 mL) solutions, dried (Na_2SO_4), filtered, solvent removed and excess $HOCH_2CCl_3$ azeotroped with toluene (2 x 50 mL). The residue was purified by flash chromatography (eluent 15-25% EtOAc/hexanes) to give **83** as a white solid (569 mg, 95%): mp = 92-93 °C; $[\alpha]^{22}_D$ -6.5 (c 0.50, $CHCl_3$); IR ν_{max} 3371, 1751, 1690, 1507 cm^{-1}; ^1H NMR 400 MHz 7.27 (q, J = 11.5 Hz, 2H), 4.46 (d, 1H), 4.03 (m, 1H), 3.56 (m, 1H), 3.11 (d, J = 5.0 Hz, 1H), 2.74 (d, J = 6.0 Hz, 1H), 2.40 (dd, J = 16.5 Hz, 8.6 Hz, 1H), 2.10 (m, 1H), 1.45 (s, 9H), 0.96 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 171.4 (C), 156.7 (C), 94.9 (C), 80.0 (C), 74.2 (CH_2), 69.4 (CH), 59.2 (CH), 38.5 (CH_2), 28.5 (CH_3), 27.9 (CH), 20.2 (CH_3), 16.7 (CH_3); HRMS *m/z* 414.0618 $(2M + Na)^+$ expected 414.0612; Anal. Calcd for $C_{14}H_{24}Cl_3NO_5$: C, 42.82; H, 6.16; N, 3.57. Found C, 42.72; H, 6.27; N, 3.41.

(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-5-methyl-hexanoic acid 2,2,2-trichloro-ethyl ester (84)



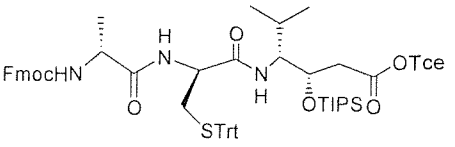
To a stirred solution of **83** (500 mg, 1.27 mmol) in CH_2Cl_2 (16 mL) at 0 °C was added TFA (4 mL). After stirring for 3h, the solution was concentrated and the TFA chased with Et_2O (2 x 20 mL). To a stirred solution of Fmoc-(*S*Trt)-D-cysteine (746 mg, 1.27 mmol), PyBOP (793 mg, 1.5 mmol) DIEA (770 μL, 4.45 mmol) was added the crude amine. After stirring at rt for 20 minutes the solvent was removed and the residue was purified by flash chromatography (eluent 20-40% EtOAc/hexanes) to give **84** as a white glass (808.5 mg, 74%): mp = 83-84 °C; $[\alpha]^{22}_D$ +6.1 (c 0.50, $CHCl_3$); IR ν_{max} 3318, 3060.7, 3019, 1702, 1665, 1524 cm^{-1}; ^1H NMR 400 MHz 7.72 (m, 2H), 7.52 (m, 2H), 7.38 (m, 8H), 7.25 (m, 8H), 7.20 (m, 3H), 5.85 (d, J = 9.5 Hz, 1H), 4.90 (d, J = 7.5 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.67 (d, J = 11.5 Hz, 1H), 4.39 (m, 2H), 4.17 (t, J = 6.5 Hz, 1H), 3.97 (m, 1H), 3.79 (m, 1H), 3.65 (m, 1H), 3.03 (s, 1H), 2.66 (m, 3H), 2.53 (dd, J = 16.5 Hz, 9.0 Hz 1H), 2.06 (m, 1H), 0.82 (t, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 171.2, 171.0, 144.4, 143.7, 143.7, 143.7, 141.5, 129.7, 128.3, 128.0, 127.2, 127.1, 125.1, 125.0, 120.1, 94.8, 74.1, 68.9, 67.6, 67.2, 57.7, 54.4, 47.2, 38.4, 33.2, 27.5, 20.2, 16.5, 14.3; MS *m/z* 881 $(M + Na)^+$.

(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester (85)



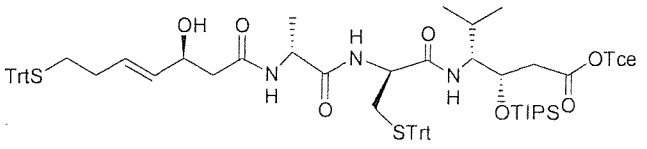
To a solution of the alcohol **84** (200 mg, 0.23 mmol) in CH_2Cl_2 (5mL) at 0 °C was added 2,6-lutidine (0.85 mL, 1.39 mmol), followed by the dropwise addition of triisopropylsilyl triflate (250 µl, 0.93 mmol). The reaction mixture was stirred at 0 °C for 30 min, then at rt for 4 hours, diluted with CH_2Cl_2 (20 mL), washed with 10% HCl (10 mL), 5% $NaHCO_3$ (10 mL) and sat. NaCl (10 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 10-20% EtOAc/hexanes) to give **85** as a white glass (219 mg, 93%): mp = 71-73 °C; $[\alpha]^{22}_D$ +7.3 (c 0.50, $CHCl_3$); IR ν_{max} 3336, 2945, 2927, 2867, 1752, 1678, 1511, 1448 cm^{-1}; ^1H NMR 400 MHz 7.72 (m, 2H), 7.54 (m, 2H), 7.37 (m, 8H), 7.20 (m, 11H), 6.13 (d, J = 10.0 Hz, 1H), 4.84 (d, J = 8.0 Hz, 1H), 4.72 (d, J = 12.0 Hz, 1H), 4.59 (d, J = 11.5 Hz, 1H), 4.37 (m, 3H), 4.19 (t, J = 6.5 Hz, 1H), 3.85 (m, 2H), 2.76-2.61 (m, 4H), 1.97 (m, 1H), 1.04 (s, 18H), 0.96 (m, 3H), 0.83 (t, J = 6.0 Hz, 6H); ^{13}C NMR 100 MHz 170.5, 170.1, 170.0, 156.3, 144.6, 143.8, 143.7, 141.4, 129,7, 128.2, 127.9, 127.2, 127.0, 125.1, 120.1, 94.9, 74.4, 69.6, 69.4, 67.5, 67.3, 59.0, 54.3, 47.2, 39.9, 33.3, 28.4, 20.6, 19.0, 18.3, 18.2, 17.9, 17.8, 17.2, 17.1, 14.4, 13.0, 12.8, 12.5; MS *m/z* 1015.5 $(M+H)^+$.

(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propiony lamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilany loxy-hexanoic acid 2,2,2-trichloro-ethyl ester (86)



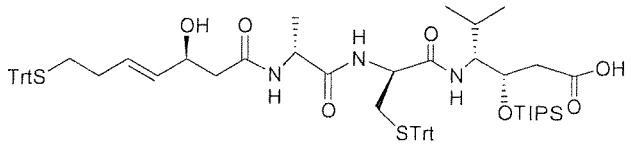
To a stirred solution of **85** (200 mg, 0.197 mmol) in CH_3CN (20 mL) at rt, was added Et_2NH (1.0 mL). After stirring for 3 h at rt the reaction mixture was diluted with heptane (50 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of Fmoc-D-alanine (80 mg, 0.26 mmol) in CH_2Cl_2 (10 mL) was added PyBOP (133.3 mg, 0.26 mmol, DIEA (163 μL, 0.591 mmol) and the crude amine and the reaction mixture stirred at rt for 2 hours. The solvent was removed and the residue was purified by flash chromatography (eluent 25-30% EtOAc/hexanes) to give **86** as a white glass (174.1 mg, 82%): mp = 110-111 °C; $[\alpha]^{22}_D$ +16.7 (c 0.50, $CHCl_3$); IR ν_{max} 3287, 3057, 1755, 1704, 1673, 1647, 1536 cm^{-1}; ^1H NMR 400 MHz 7.75 (d, J = 7.5 Hz, 2H), 7.53 (d, J = 7.5 Hz, 2H), 7.41 (m, 8H), 7.29-7.15 (m, 11H), 6.23 (d, J = 8.8 Hz, 1H), 6.11 (m, 1H), 5.17 (s, 1H), 4.74 (d, J = 11.5 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.40 (m, 3H), 4.13 (m, 3H), 3.89 (m, 1H), 2.87 (m, 1H), 2.73 (dd, J = 16.0 Hz, 6.5 Hz, 1H), 2.63 (dd, J = 16.0 Hz, 5.1 Hz, 1H), 2.55 (dd, J = 13.1 Hz, 5.0 Hz, 1H), 1.98 (m, 1H), 1.28 (s, 3H), 1.05 (s, 18H), 0.98 (m, 3H), 0.85 (s, 6H); ^{13}C NMR 100 MHz 172.3, 170.2, 170.0, 156.0, 144.6, 144.0, 143.8, 141.5, 141.4, 129.6, 128.2, 127.9, 127.2, 127.0, 125.1, 120.1, 95.0, 74.3, 69.6, 67.3, 67.2, 59.3, 52.5, 50.6, 47.3, 39.9, 32.6, 28.4, 20.6, 18.7, 18.3, 17.2, 12.9; MS *m/z* 1086.4 (M+H), 1086.4 $(M + Na)^+$; Anal. Calcd for $C_{58}H_{70}Cl_3N_3O_7SSi$: C, 64.05; H, 6.49; N, 3.86. Found C, 64.06; H, 6.55; N, 3.69.

(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester (87)



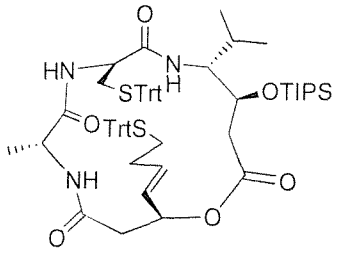
To a stirred solution of **86** (200 mg, 0.183 mmol) in CH_3CN (20 mL) at rt was added Et_2NH (1.0 mL). After stirring for 5 h at rt the reaction mixture was diluted with heptane (50 mL), solvent removed and concentrated to give the crude amine as a colourless oil. To a stirred solution of the crude amine in CH_2Cl_2 (6 ml) was added alcohol **58** (94.0 mg, 0.165 mmol) and DMAP (2.2 mg, 0.0183 mmol) at 0 °C. After stirring at rt for 7 hours, the solvent was removed and the residue was purified by flash chromatography (eluent 25-30% EtOAc/hexanes) to give **87** as a white glass (176 mg, 84%): mp = 170-171 °C; $[\alpha]^{22}_D$ +2.3 (c 0.50, $CHCl_3$); IR ν_{max} 3289, 1756, 1633, 1551 cm^{-1}; ^1H NMR 400 MHz 7.41 (m, 12H), 7.25 (m, 12H), 7.20 (m, 6H), 6.86 (d, J = 8.6 Hz, 1H), 6.16 (d, J = 10.5 Hz, 1H), 6.10 (d, J = 7.0 Hz, 1H), 5.46 (dt, J = 15.5 Hz, 6.0 Hz, 1H), 5.33 (dd, J = 15.6 Hz, 6.1 Hz, 1H), 4.72 (d, J = 10.2 Hz, 1H), 4.61 (d, J = 10.2 Hz, 1H), 4.42 (m, 1H), 4.34 (m, 2H), 4.08 (m, 1H), 3.86 (m, 1H), 3.16 (s, 1H), 2.76-2.60 (m, 3H), 2.52 (dd, J = 12.6 Hz, 5.5 Hz, 1H), 2.30 (dd, J = 14.0 Hz, 3.0 Hz, 1H), 2.20 (m, 3H), 2.06 (m, 2H), 1.93 (m, 1H), 1.31 (d, J = 7.0 Hz, 3H), 1.04 (s, 18H), 0.98 (m, 3H), 0.85 (t, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 172.3, 171.5, 170.2, 145.0, 144.5, 144.4, 132.6, 130.1, 129.7, 129.6, 128.2, 128.1, 128.0, 127.0, 126.8, 94.6, 77.5, 77.4, 77.2, 76.8, 74.3, 69.8, 69.5, 67.1, 66.8, 59.4, 52.5, 49.5, 44.0, 39.6, 33.0, 31.5, 31.4, 28.6, 20.5, 18.3, 17.8, 17.4, 12.9; MS *m/z* 1283.5 $(M\{Cl_{35}+Cl_{35}+Cl_{37}\} + NH_4)^+$, 1288.7 $(M\{Cl_{35}+Cl_{35}+Cl_{37}\} + Na)^+$.

(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid (88)



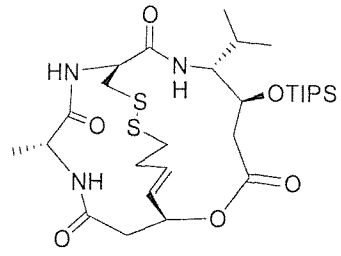
To a stirred solution of **87** (100 mg, 0.079 mmol) in THF (3 mL) at rt was added Zn (51.4 mg, 0.79 mmol) followed by the dropwise addition of NH_4Ac (0.6 mL). After stirring for 5 h at rt, sat. NaCl (2 mL) and EtOAc (2 mL) was added. The organic phase was decanted with EtOAc (5 x 4 mL), combined, dried, the solvent removed, and the residue was purified by flash chromatography (eluent 40-60% EtOAc/hexanes) to give **88** as a white glass (64 mg, 71%): mp = 147-148 °C; $[\alpha]^{22}_D$ +2.5 (c 0.50, $CHCl_3$); IR ν_{max} 3287, 2944, 1710, 1639, 1528, 1447 cm^{-1}; ^1H NMR 400 MHz ($CDCl_3$) δ; 7.32 (m, 12H), 7.18 (m, 12H), 7.12 (m, 6H), 6.84 (d, J = 8.5 Hz, 1H), 6.45 (d, J = 8.0 Hz, 1H), 6.28 (d, J = 10.0 Hz, 1H), 5.39 (dt, J = 15.5 Hz, 6.5 Hz, 1H), 5.26 (dd, J = 15.5 Hz, 6.0 Hz, 1H), 4.37 (t, J = 7.5 Hz, 1H), 4.31 (m, 1H), 4.23 (m, 1H), 4.14 (m, 2H), 4.08 (m, 1H), 3.77 (m, 1H), 2.82 (m, 1H), 2.48-2.37 (m, 3H), 2.52 (d, J = 13.5 Hz, 1H), 2.13 (m, 3H), 1.99 (m, 3H), 1.26 (d, J = 7.1 Hz, 3H), 1.19 (s, 18H), 0.97 (m, 3H), 0.77 (t, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 173.9, 172.8, 172.1, 170.1, 145.0, 144.5, 132.3, 130.2, 129.7, 129.6, 128.2, 128.0, 127.1, 126.8, 69.9, 69.5, 67.2, 66.8, 60.3, 53.2, 49.5, 44.4, 39.9, 33.4, 31.5, 31.4, 29.8, 28.5, 20.5, 18.3, 17.9, 17.7, 17.1, 12.8; MS *m/z* 1246.2 $(M - H^+ + CF_3CO_2H)^-$.

(2*S*,6*R*,9*S*,12*R*,13*S*)-12-Isopropyl-6-methyl-13-triisopropylsilanyloxy-2-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,10,15-tetraone (89)



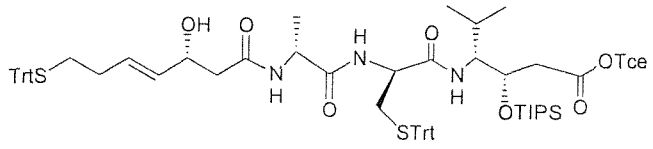
To a stirred solution of the hydroxy acid **88** (60.0 mg, 0.053 mmol) in THF (1.0 mL) at 0 °C was added Et_3N (11 µl, 0.08mmol) and 2,4,6-trichlorobenzoyl chloride (13 µl, 0.08 mmol). After stirring at room temperature for 2 hours, the reaction mixture was diluted with toluene (10 mL) and then added dropwise to a vigorously stirring solution of DMAP (6.72 mg, 0.053 mmol) in toluene (26.5 mL, 0.002 M with respect to the hydroxy acid) at 50 °C over 2 hours. After stirring at the same temperature for a further 2 hours, the reaction mixture was allowed to cool to rt then washed with 5% $NaHCO_3$ (10 mL), sat. citric acid (10 mL), and sat. NaCl (10 mL) solutions, each back extracted with EtOAc (10 mL). The combined organic phase was dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 30-50% EtOAc/hexanes) to give **89** as a white glass (31 mg, 53%): $[\alpha]^{22}_D$ -3.3 (c 0.50, $CHCl_3$); IR ν_{max} 3304, 3057, 1663, 1644 1543, 1491 cm^{-1}; ^1H NMR 400 MHz 7.39 (m, 12 H), 7.26 (m, 12H), 7.20 (m, 6H), 6.67 (d, J = 8.0 Hz, 1H), 6.58 (d, J = 10.5 Hz, 1H), 6.24 (s, 1H), 5.59 (m, 2H), 5.28 (dd, J = 15.5 Hz, 7.0 Hz, 1H), 4.35-4.26 (m, 1H), 4.07 (t, J = 7.0 Hz, 1H), 3.69 (t, J = 9.5 Hz, 1H), 3.42 (t, J = 12.6 Hz, 1H), 3.24 (s, 1H), 2.77 (dd, J = 12.0, 3.5 Hz, 1H), 2.49-2.16 (m, 4H), 2.07-2.05 (m, 3H), 2.04-2.00 (m, 2H), 1.36 (d, J = 7.2 Hz, 3H), 1.02 (s, 18H), 0.96 (m, 3H) 0.85 (t, J = 5.5 Hz, 6H); ^{13}C NMR 100 MHz 171.3(C), 170.5(C), 170.0(C), 169.9(C), 144.9(C), 144.8(C), 133.1(CH), 129.1(CH) 128.3(CH), 126.9(CH), 126.8(CH), 71.2, 69.1, 67.0, 66.8, 60.5, 56.4, 50.3, 43.0, 42.2, 32.1, 31.5, 31.2, 28.0, 27.9, 20.9, 19.1, 18.3(CH_3), 17.9, 17.2, 15.8, 15.7, 14.6, 13.2(CH_2), 13.1(CH_3), 12.9; MS *m/z* 1134.7 $(M+NH_4)^+$.

(*E*)-(1*S*,5*S*,6*R*,9*S*,20*R*)-6-Isopropyl-20-methyl-5-triisopropylsilanyloxy-2-oxa-11,12-dithia-7,19,22-triaza-bicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (90)



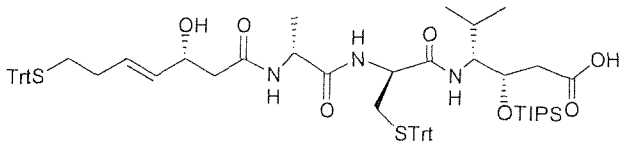
To a vigorously stirring solution of I_2 (68 mg, 0.269 mmol) in 10% MeOH/CH_2Cl_2 (67.2 mL) was added the protected dithiol **89** (25 mg, 0.022 mmol) in 10% MeOH/CH_2Cl_2 (22.4 mL) dropwise over 10 minutes. After stirring for 10 minutes, 0.01 N $Na_2S_3O_2$ (50 mL) was added and the organic phase extracted with CH_2Cl_2 (4 x 60 mL). The combined organic extract was washed with sat. NaCl (80 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes) to give **90** as a white solid (11.5 mg, 82%): $[\alpha]^{22}_D$ +36.0 (c 0.50, $CHCl_3$); IR ν_{max} 3322, 1745, 1543 cm^{-1}; ^1H NMR 400 MHz ($CDCl_3$) δ; 7.07 (m, 1H), 6.63 (d, J = 9.5 Hz, 1H), 6.34 (m, 1H), 5.80 (s, 1H), 5.57 (t, J = 15.5 Hz, 2H), 4.94 (m, 2H), 4.22 (m, 1H), 3.48 (m, 1H), 3.12-2.90 (m, 4H), 2.64 (m, 4H), 2.50 (m, 2H), 2.22 (m, 1H), 1.44 (d, J = 7.5 Hz, 3H), 1.05 (s, 18H), 1.00 (m, 3H) 0.92 (d, J = 6.0 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz δ; 171.1, 171.0, 169.4, 169.1, 133.1, 129.1, 69.3, 69.1, 68.0, 67.8, 63.5, 63.3, 53.4, 52.2, 41.2, 41.0, 40.1, 29.4, 21.4, 18.3, 17.4, 17.0, 14.7, 13.2, 13.1; HRMS *m/z* 652.2883 $(M + Na)^+$ expected 652.2881;

(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*R*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester (92)



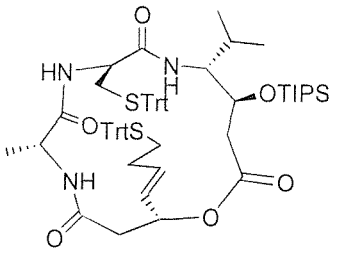
To a stirred solution of **86** (189 mg, 0.173 mmol) in CH_3CN (20 mL) at rt was added Et_2NH (1.0 mL). After stirring for 5 h at rt the reaction mixture was diluted with heptane (50 mL), CH_2Cl_2 (20 ml) added, filtered, and concentrated to give the crude amine as a colourless oil. To a stirred solution of the crude amine in CH_2Cl_2 (6 ml) was added alcohol **90** (89.0 mg, 0.156 mmol) and DMAP (2.1 mg, 0.0173 mmol) at 0 °C and then stirred at rt for 7 hours. The solvent was removed and the residue was purified by flash chromatography (eluent 25-30% EtOAc/hexanes) to give **92** as a white glass (89 mg, 46%): mp = 168-169 °C; IR ν_{max} 3311, 2960, 2867, 1755, 1640, 1536 cm^{-1}; $[\alpha]^{22}_D$ +6.3 (c 0.50, $CHCl_3$); ^1H NMR 400 MHz 7.41 (m, 12H), 7.25 (m, 12H), 7.19 (m, 6H), 6.31 (t, J = 6.5 Hz, 2H), 6.18 (d, J = 10.5 Hz, 1H), 5.48 (dt, J = 15.0 Hz, 6.5 Hz, 1H), 5.35 (dd, J = 15.0 Hz, 6.0 Hz, 1H), 4.74 (d, J = 10.2 Hz, 1H), 4.62 (d, J = 10.2 Hz, 1H), 4.40 (m, 1H), 4.34 (m, 2H), 4.04 (m, 1H), 3.86 (m, 1H), 3.21 (s, 1H), 2.81-2.54 (m, 4H), 2.30 (m, 2H), 2.20 (t, J = 7.5 Hz, 2H), 2.07 (t, J = 7.0 Hz, 2H), 1.95 (m, 1H), 1.27 (d, J = 7.0 Hz, 3H), 1.04 (s, 18H), 0.98 (m, 3H), 0.83 (t, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 172.2, 171.7, 170.2, 170.0, 145.0, 144.6, 144.4, 132.3, 130.4, 129.7, 129.6, 128.2, 128.0, 127.1, 126.7, 94.9, 69.5, 69.2, 67.3, 66.7, 59.3, 52.6, 48.8, 42.7, 39.8, 32.8, 31.6, 31.4, 28.5, 20.5, 19.0, 18.3, 18.2, 17.9, 17.8, 17.23, 17.2, 17.0, 14.5, 13.1, 13.0, 12.9; MS *m/z* 1283.5 $(M\{Cl_{35}+Cl_{35}+Cl_{37}\} + NH_4)^+$, 1288.4 $(M\{Cl_{35}+Cl_{35}+Cl_{37}\} + Na)^+$.

(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*R*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid (93)



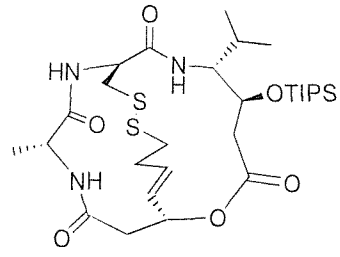
To a stirred solution of **92** (85 mg, 0.067 mmol) in THF (2 mL) at rt was added Zn (43.5 mg, 0.67 mmol) followed by the dropwise addition of NH_4Ac (0.5 mL). After stirring for 5 h at rt, sat. NaCl (1 mL) and EtOAc (1 mL) was added and the organic phase decanted with EtOAc (5 x 2 mL). The combined organic layers were dried and the solvent was removed and the residue was purified by flash chromatography (eluent 40-60% EtOAc/hexanes) to give **93** as a white glass (54 mg, 72%): mp = 144-145 °C; $[\alpha]^{22}_D$ +6.8 (c 0.50, $CHCl_3$); IR ν_{max} 3290, 1711, 1636, 1536, 1490 cm^{-1}; ^1H NMR 400 MHz 7.31 (m, 12H), 7.16 (m, 12H), 7.10 (m, 6H), 6.75 (m, 1H), 6.65 (m, 1H), 6.19 (d, J = 10.5 Hz, 1H), 5.42 (dt, J = 15.0 Hz, 7.0 Hz, 1H), 5.28 (dd, J = 15.5 Hz, 6.5 Hz, 1H), 4.31 (m, 2H), 4.20 (m, 1H), 4.15 (m, 1H), 4.08 (m, 1H), 3.78 (m, 1H), 4.08 (m, 1H), 3.78 (m, 1H), 2.78 (m, 1H), 2.45 (m, 3H), 2.25 (m, 2H), 2.12 (m, 2H), 1.98 (m, 3H), 1.22 (d, J = 7.0 Hz, 3H), 0.97 (s, 18H), 0.91 (s, 3H), 0.74 (d, J = 6.5 Hz, 6H); ^{13}C NMR 100 MHz 173.7, 172.9, 172.0 170.0, 145.0, 144.5, 132.2, 130.3, 129.7, 129.6, 128.2, 128.0, 127.0, 126.8, 70.0, 69.2, 67.2, 66.7, 59.6, 53.4, 49.3, 43.0, 40.0, 33.3, 31.6, 31.4, 28.1, 20.6, 19.0, 18.4, 18.3, 18.2, 18.0, 17.9, 17.2, 16.8, 14.4, 13.1, 13.0, 12.8; MS *m/z* 1246.2 $(M - H^+ + CF_3CO_2H)^-$.

(2*R*,6*R*,9*S*,12*R*,13*S*)-12-Isopropyl-6-methyl-13-triisopropylsilanyloxy-2-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,10,15-tetraone (94)



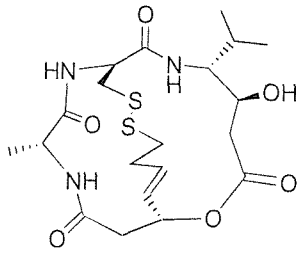
To a stirred solution of the hydroxy acid **93** (45.0 mg, 0.040 mmol) in THF (1.0 mL) at 0 °C was added Et_3N (7.2 µl, 0.052 mmol) and 2,4,6-trichlorobenzoyl chloride (8 µl, 0.052 mmol). After stirring at room temperature for 2 hours the reaction mixture was diluted with toluene (10 mL) and then added dropwise to a vigorously stirring solution of DMAP (5.07 mg, 0.04 mmol) in toluene (20 mL, 0.002 M with respect to the hydroxy acid) at 50 °C over 2 hours. After stirring at the same temperature for a further 2 hours the reaction mixture was allowed to cool to rt, then washed with 5% $NaHCO_3$ (8 mL), sat. citric acid (8 mL), and sat. NaCl (8 mL) solutions each back extracted with EtOAc (8 mL). The combined organic phase was dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 30-50% EtOAc/hexanes) to give **94** as a white glass (22.6 mg, 51%): $[\alpha]^{22}_D$ +15.0 (c 0.50, $CHCl_3$); IR ν_{max} 3291, 3045, 1730, 1649, 1531, 1489 cm^{-1}; ^1H NMR 400 MHz 7.39 (m, 12 H), 7.26 (m, 12H), 7.22 (m, 6H), 7.11 (d, J = 10.5 Hz, 1H), 6.81 (d, J = 7.0 Hz, 1H), 6.35 (d, J = 8.0 Hz, 1H), 5.53 (m, 2H), 5.34 (dd, J = 15.6 Hz, J = 6.0 Hz, 1H), 4.31 (t, J = 7.5 Hz, 1H), 4.25 (t, J = 6.5 Hz, 1H), 3.68 (m, 1H), 3.26 (m, 2H), 2.60-2.54 (m, 3H), 2.37 (d, J = 16.5 Hz, 2H), 2.19 (t, J = 7.0 Hz, 2H), 2.10 (m, 1H), 2.04 (m, 2H), 1.32 (d, J = 7.0 Hz, 3H), 0.92 (s, 18H), 0.90 (m, 3H), 0.81 (d, J = 7.5 Hz, 3H), 0.77 (d, J = 7.5 Hz, 3H); ^{13}C NMR 100 MHz 172.9, 172.7, 171.3, 169.6, 144.9, 144.6, 132.7, 129.7, 128.2, 128.0, 127.0, 126.8, 70.0, 68.3, 67.1, 66.9, 60.0, 58.0, 48.9, 42.2, 40.9, 32.4, 31.4, 31.3, 28.1, 20.9, 18.2, 17.9, 17.8, 17.1, 16.1, 15.5, 13.0, 12.8; MS *m/z* 1138.6 $(M + Na)^+$.

(*E*)-(1*R*,5*S*,6*R*,9*S*,20*R*)-6-Isopropyl-20-methyl-5-triisopropylsilanyloxy-2-oxa-11,12-dithia-7,19,22-triaza-bicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (95)



To a vigorously stirring solution of I_2 (55.4 mg, 0.22 mmol) in 10% MeOH/CH_2Cl_2 (54.4 mL) was added the protected dithiol **94** (20 mg, 0.018 mmol) in 10% MeOH/CH_2Cl_2 (22.4 mL) dropwise over 10 minutes. After stirring for a further 10 minutes, 0.01 N $Na_2S_3O_2$ (40 mL) was added and the aqueous phase extracted with CH_2Cl_2 (4 x 40 mL). The combined organic extract was washed with sat. NaCl (60 mL), dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes) to give **95** as a white solid (6.5 mg, 58%): $[\alpha]^{22}_D$ +22.0 (c 0.50, $CHCl_3$); IR ν_{max} 3404, 3289, 1743, 1664, 1547 cm^{-1}; ^1H NMR 400 MHz 7.26 (m, 1H), 6.67 (m, 1H), 6.59 (m, 1H), 6.42 (m, 1H), 5.80 (d, J = 15.5 Hz, 1H), 5.58 (m, 1H), 4.58 (m, 1H), 4.37 (m, 1H), 3.90 (t, J = 4.0 Hz, 3H), 3.76 (m, 1H), 3.66 (t, J = 8.5 Hz, 1H), 3.59 (m, 1H), 3.15 (m, 1H), 2.61 (m, 1H), 2.55 (m, 1H), 2.51 (m, 1H), 2.37 (m, 1H), 2.27 (m, 2H), 2.08 (t, J = 13.0 Hz, 1H), 1.45 (m, 1H), 1.21 (m, 2H), 1.12 (s, 18H), 1.07 (m, 3H), 0.95 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H); HRMS *m/z* 652.2878 $(M + Na)^+$ expected 652.2881.

Epi-Spiruchostatin A (96)



To a stirring solution of **95** (6 mg, 0.0095 mmol) at -30 °C in EtOAc under Ar was added HCl(g) over 30 minutes to saturation. The reaction mixture was then allowed to warm to 0°C and stirred at this temperature for 3 hours. The solution was partially degassed with Ar, and solvent removed. The residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes then 5% MeOH/CH_2Cl_2) to give **96** as a white solid (3.4 mg, 76%): $[\alpha]^{22}_D$ + 19.3; IR ν_{max} 3402, 3281, 1692, 1637, 1548 cm^{-1}; ^1H NMR 400 MHz 7.36 (d, J = 8.5 Hz, 1H), 6.71 (s, 1H), 6.39 (m, 1H), 5.81 (d, J = 15.5 Hz, 1H), 5.54 (s, 1H), 4.58 (s, 1H), 4.18 (m, 1H), 3.86 (m, 1H), 3.73 (m, 1H), 3.64 (m, 1H), 3.56 (m, 1H), 3.13 (q, J = 7.0 Hz, 1H), 2.60 (dd, J = 13.0 Hz, 5.0 Hz, 1H), 2.49 (d, J = 13.5 Hz, 1H), 2.44 (m, 1H), 2.3-2.18 (m, 3H), 2.14-2.04 (m, 3H), 1.53 (d, J = 7.0 Hz, 3H), 0.97 (d, J = 6.5 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H); HRMS *m/z* 496.1549 $(M + Na)^+$ expected 496.1546.

2.24 References

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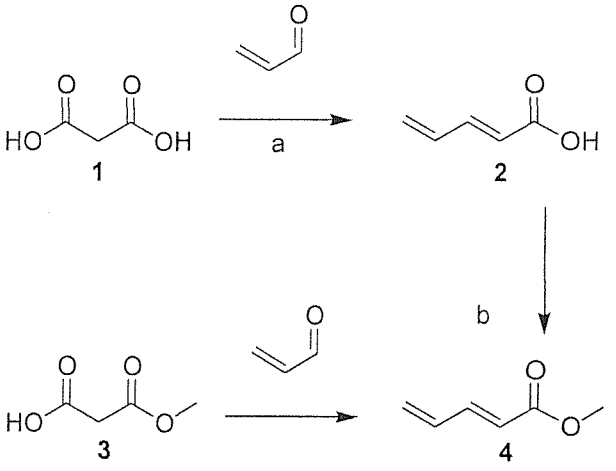
Chapter 3: Synthesis of the 3-hydroxy-7-mercapto-4-heptenoic acid subunit.

The 3-hydroxy-7-mercapto-4-heptenoic acid subunit is present throughout the bicyclic depsipeptide family. The subunit was previously synthesised by Simon *et al.*[1], we sought to both duplicate their synthesis as well as synthesise an ester that was primed to nucleophilic attack firstly through a *tert*-butyl thioester and secondly by a thiazolidinethione.

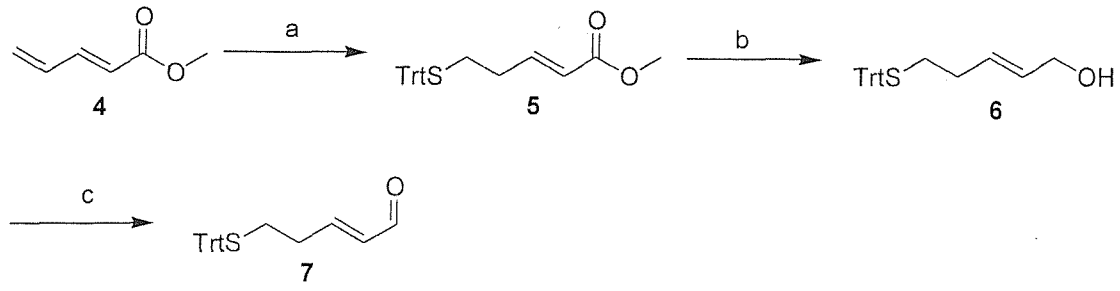
3.1 Synthesis of penta-2,4-dienoic acid methyl ester



Scheme 1. Reagents and conditions: **(a)** $CH_2(CO_2H)_2$, acrolein, pyridine, 70 °C 1 h (51%). **(b)** cH_2SO_4, MeOH, 20 °C 20 h (31%). **(c)** $HO_2CCH_2CO_2CH_3$, acrolein, pyridine, 50 °C 18 h (78%).

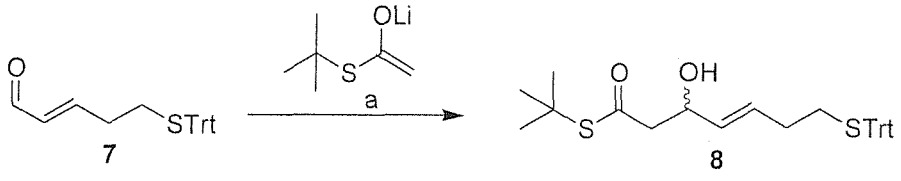
Penta-2,4-dienoic acid methyl ester **4** was initially prepared via the Knoevenagel[2] condensation of malonic acid **1** with acrolein followed by methylation of the corresponding pentadienoic acid **2** with methanol in the presence of acid catalysis. This method however proved very inefficient, with a very low yield over the two steps. This was possibly due to the instability of the dienoic acid as the diene is prone to thermal Diels-Alder reactions as well as polymerisation under the acidic conditions. A more direct and higher yielding synthesis of **4** was accomplished with monomethyl malonic acid **3** and acrolein following the method of Waegell *et al.*[3] (Scheme 1).

3.2 Synthesis of (*E*)-5-tritylsulfanyl-pent-2-enal



Scheme 2. Reagents and conditions: **(a)** TrtSH, $CsCO_3$, THF, 0 °C then 20 °C 20 h (84%). **(b)** 2 equiv. DIBAL-H, CH_2Cl_2, -78 °C 3 h (85%). **(c)** (i) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C 30 min (ii) **26**, Et_3N, -78 °C 30 min then -30 °C 4 h (69%).

Penta-2,4-dienoic acid methyl ester **4** was converted to (*E*)-5-mercapto[*S*-triphenylmethyl]-2-pentenoic acid methyl ester **5**, by treatment with triphenylmethane thiol and cesium carbonate. Reduction with diisobutylaluminium hydride (DIBAL-H) gave the alcohol **6** following the method of Simon *et al.*[1] As stated in their experimental procedure, a small quantity of the β,γ-alkene is formed. It is not necessary to separate the isomers because the β,γ-alkene is isomerised in the next step. The alcohol was oxidized up to the α,β-unsaturated aldehyde **7** through a Swern type oxidation using DMSO activated by oxalyl chloride (Scheme 2).

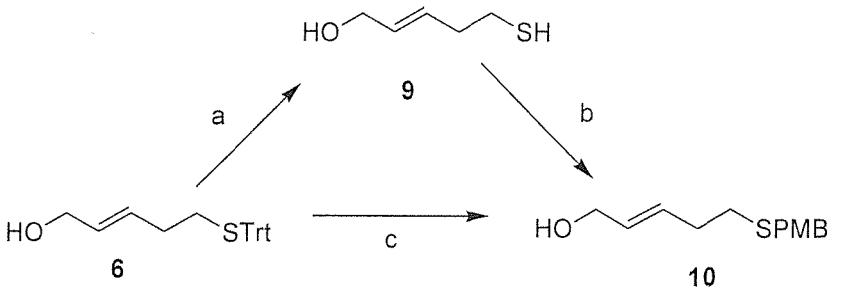


Scheme 3. Reagents and conditions: **(a)** 10 eq. $LiCH_2COS(CCH_3)_3$, THF, -78 °C 1 h (84%).

A racemic aldol reaction was performed on the aldehyde **7** with the lithium enolate of *S*-*tert*-butylthioacetate to give the unsaturated β-hydroxy acid **8** simply and effectively

(Scheme 3). On coupling with the peptide sub-unit it may then be possible to separate the diastereoisomers by chromatography. This approach does have the major disadvantage that half of the resulting coupled product would be the incorrect stereochemistry, however at the onset of our work the correct stereochemistry of the unsaturated β-hydroxy ester was not known.

3.3 De-protection and re-protection of (*E*)-5-mercapto-2-pentenol with PMB protecting group

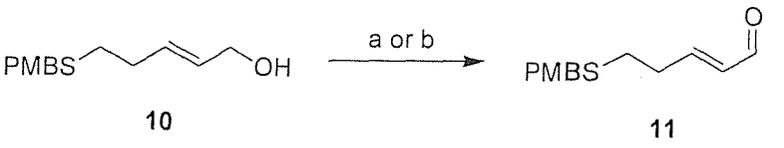


Scheme 4. Reagents and conditions: **(a)** 1.5:8.5 TFA/CH_2Cl_2, Et_3SiH, rt 30 min. **(b)** PMB-Cl, rt 1 h (37%). **(c)** (i) 1.5:8.5 TFA/CH_2Cl_2, 1.1 equiv. Et_3SiH, 20 °C 30 min. (ii) PMB-Cl, rt 1 h (53%).

The substitution of the trityl protecting group with that of PMB was desirable in our initial synthetic strategy in the synthesis of spiruchostatin A. Deprotection of the trityl group of **6** in TFA/CH_2Cl_2 with the cation scavenger Et_3SiH by the method of Pandey *et al.*[4] gave the free thiol **9**. Subsequent re-protection to give the PMB protected thiol **10**, proceeded in poor overall yield due to the volatility of the free thiol, which was lost on attempted removal of solvent and more importantly triethylsilane. The free thiol was protected with PMB-Cl in TFA/CH_2Cl_2 following the procedure of Richter *et al.*[5] This was problematic due to the handling of the free thiol and also not being able to separate the crude product from the higher boiling triethylsilane that could possibly act to scavenge the 4-methoxybenzyl cation. A direct one-pot substitution of protecting groups was achieved by the acid deprotection of the trityl protecting group immediately followed by addition of

PMB-Cl affording the desired PMB protected thiol in reasonable yield (53%) (Scheme 4). It was not possible to improve on this substitution of protecting groups and the following oxidation also proved inefficient (Scheme 5), we therefore decided to continue the synthesis utilising the trityl protected thiol **6**.

3.4 Oxidation of the PMB protected (*E*)-5-mercapto-2-pentenol

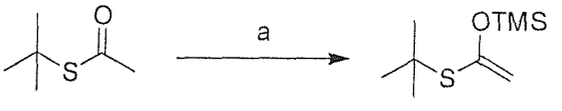


10 11

Scheme 5. Reagents and conditions: **(a)** Dess-Martin periodinane, CH_2Cl_2, 2.5 h (28%). **(b)** (i) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C 30 min (ii) **10**, Et_3N, -78 °C 30 min then -30 °C 4 h (14%).

Oxidation of the PMB protected unsaturated thio alcohol **10** was achieved through both a Swern and Dess-Martin periodinane oxidations. The yield however was poor and so formation of the α,β-unsaturated PMB protected thio alcohol was abandoned (Scheme 5).

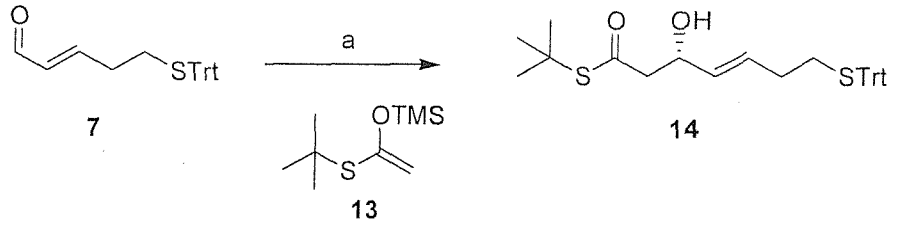
3.5 Asymmetric synthesis of (*E*)-(*S*)-3-hydroxy-7-tritylsulfanyl-hept-4-enethioic acid *S*-*tert*-butyl ester



12 13

Scheme 6. Reagents and conditions: **(a)** (i) LDA, *S*-*tert*-butylthioacetate, THF, -78 °C 1 h. (ii) TMS-Cl, -78 °C then rt 1h (73%).

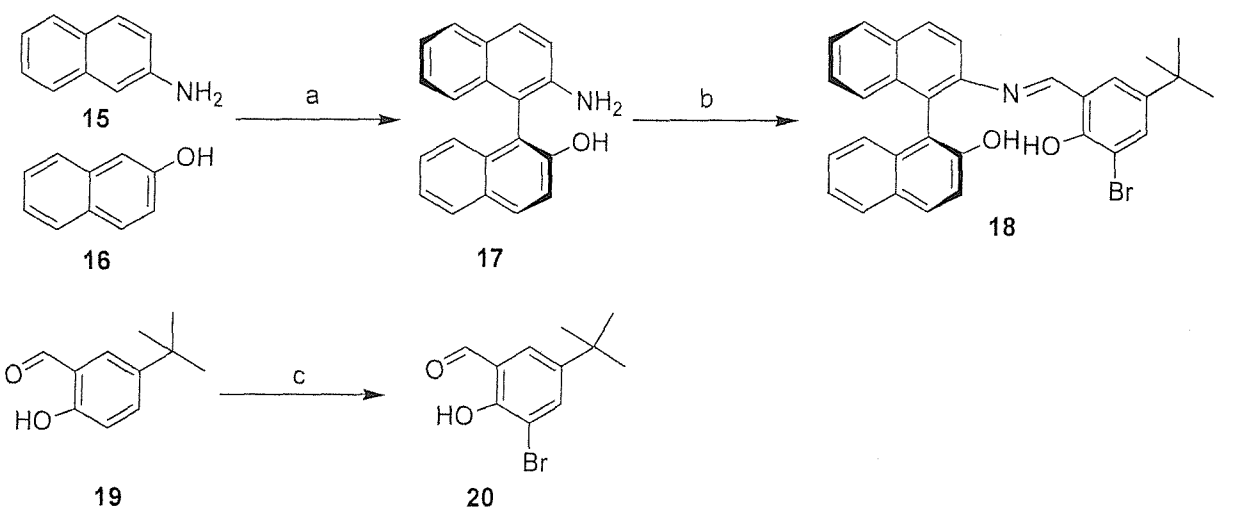
We reasoned that the β-hydroxy acid of spiruchostatin A was the (*S*) stereoisomer, as in FK228, and therefore sought an asymmetric approach to the *S*-stereoisomer of 3-hydroxy-7-tritylsulfanyl-hept-4-enethioic acid. *S*-*tert*-butylthioacetate **12** was converted into the TMS-thioketene acetal **13** by addition to LDA and then addition of TMS-Cl to capture the enolate as the TMS-thioketene acetal following the procedure of Evans *et al.*[6] (Scheme 6).



Scheme 7. Reagents and conditions: **(a)** (i) (*S*)-(-)-1,1-bi-2-naphthol, Ti(O-*i*-Pr)$_4$, 4Å sieves, O(CH_2CH_3)$_2$, 35 °C 1 h. (ii) **7**, rt 5 min. then **13**, at -78 °C then -30 °C 36 h (46%).

Asymmetric synthesis of the 3-hydroxy-7-mercapto-4-heptenoic acid sub-unit via a Mukaiyama aldol reaction was attempted under Keck conditions[7] with the chiral Lewis acid prepared from (*R*) and (*S*)-1,1-bi-2-naphthol (BINOL) and Ti(O-*i*-Pr)$_4$. The catalyst was prepared by heating (*S*)-BINOL and oven-dried powdered 4Å sieves at reflux for 1 hour in ether. Subjecting the aldehyde **7** to the chiral Lewis acid catalyst and then addition of the TMS-thioketene acetal **13** was attempted in a variety of solvents: ether, toluene and THF, with varying reaction times. No reaction was observed when carried out in toluene. In THF the reaction furnished only 14% of the desired product, even with extended reaction times. In ether with 2.8 equivalents of the TMS-thioketene acetal the desired aldol product **14** and the TMS protected alcohol were obtained. Treatment of the crude reaction mixture with TBAF furnished (*E*)-(*S*)-3-hydroxy-7-tritylsulfanyl-hept-4-enethioic acid S-*tert*-butyl ester **14** in 46% yield. The enantiomeric excess of the reaction was determined by using the chiral NMR shift reagent europium tris[3-(heptafluoropropylthyloxymethylene)-(+)-camphorate] {Eu(hfc)$_3$}. 0.5 equivalents of the chiral shift reagent was found to show significant splitting of the protons from the *tert*-butylthiol group. The results showed that the reaction had relatively low enantiomeric purity, 61% ee under our optimised conditions (Scheme 7).

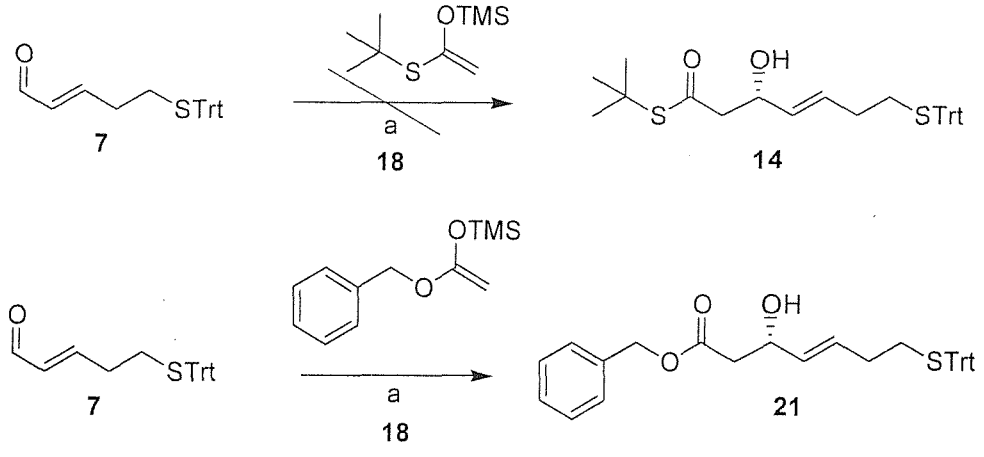
Trying to improve the synthesise of the *S*-stereoisomer of the 3-hydroxy-7-mercapto-4-heptenoic acid subunit, good asymmetric induction has been reported by both Keck and Carreira with Mukaiyama aldol reaction on aldehydes using Ti(IV) ligand complexes derived from binaphthyl ligands. Previously the Keck Ti(O-*i*-Pr)$_4$, (*S*)-(-)-1,1-bi-2-naphthol (BINOL) catalyst[7] afforded the desired product in a relatively low yield and ee (46% yield, 61% ee). The Carreira type tridentate ligand derived from Ti(O-*i*-Pr)$_4$, (*S*)-(-)-2-amino-2-hydroxy-1,1-binaphthyl (NOBIN) 3-bromo-5-*tert*-butylsalicaldehyde and 3,5-di-*tert*-butylsalicylic acid catalyst[8], was synthesised in an attempt increase the ee and overall yield.



Scheme 8. Reagents and conditions: **(a)** (i) $CuCl_2.2H_2O$, $^iPrOH_4$, α-methylbenzylamine, 20 h. (ii) $CuCl_2.2H_2O$, $(CH_3)_2CO$, (8*S*,9*R*)-(-)-*N*-benzylcinchonidium chloride, reflux, 4 h (41%). **(b) 20**, CH_3CH_2OH, reflux, 24 h (68%). **(c)** (i) CH_3CO_2H, CH_3CO_2Na, Br_2, 50 °C, 14 h (71%).

NOBIN (2,2'-Diamino-1,1'-binaphthyl) **18** was synthesised by a cross coupling of 2-naphthylamine **15** and 2-naphthol **16** using a $CuCl_2$-α-methylbenzylamine complex.[9] The crude reaction mixture contains an excess of the *S*-(-) isomer complexed with the $CuCl_2$-α-methylbenzylamine, which was filtered, decomposed with conc. HCl, and through a series of kinetic re-crystallisations afforded the desired isomer in 26% yield, 46% ee. Further re-crystallisations did not improve the ee of the reaction. Correspondence with the author indicated that without a seed crystal of 99+% ee further improvement was difficult. Taking the crude reaction mixture and following a procedure by Ding and Mikami[10]

optical resolution was possible by complexation with (8*S*,9*R*)-(-)-*N*-benzylcinchonidium chloride. Refluxing crude **17** with the alkaloid in acetone for 4 hours, filtration gave the (*S*)-(-) isomer in the mother liquor, the *R*-(+) isomer was obtained by decomposition of the cinchonidium complex with conc. HCl to give the desired isomer in overall 41 % yield with > 98% ee. Reaction of the *S*-(-) isomer of NOBIN with aldehyde **20** (synthesised from the commercially available des-bromo compound **19** with Br_2 in acetic acid) in ethanol at reflux gave imine **18**.[8]



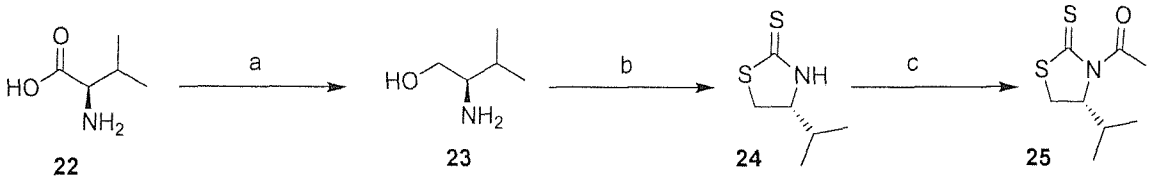
Scheme 9. Reagents and conditions: **(a)** (i) NOBIN, toluene, Ti(O-*i*-Pr)$_4$ 3,5-di-*tert*-butylsalicylic acid catalyst 1 h then **13**, TMS enolate, 4 °C 36 h. (ii) TBAF, THF, 5 min.

Using the TMS-thioketene acetal in the Mukaiyama aldol reaction with the NOBIN catalyst none of the desired product **14** was observed. A variety of conditions were employed following procedures of Carreira[8] and Simon.[1] The presence of isoproponal (from the Ti(IV) complex) and water in substoichiometric quantities has been reported by Carreira to almost totally prevent the reaction taking place. The original literature method requires azeotropic removal of isopropanol with toluene, reported to not be consistent and convenient. Therefore we followed more recent reaction conditions[11] whereby TMS-Cl and Et_3N are added to the reaction mixture after formation of the multidentate ligand, to remove the freed isopropanol. These modified reaction conditions also failed to give the desired product in our hands. Using the literature procedure for the formation of **18** using benzyl ketene acetal afforded the desired compound albeit in a low yield 32%. The optical

rotation matched that of the literature compound[8] indicating the desired stereochemistry was achieved (Scheme 9). Due to these difficulties with enantioselective acetate aldol reactions, we next turned to a chiral auxiliary based approach.

3.6 Asymmetric synthesis of (*E*)-(*S*)-3-hydroxy-1-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4-en-1-one

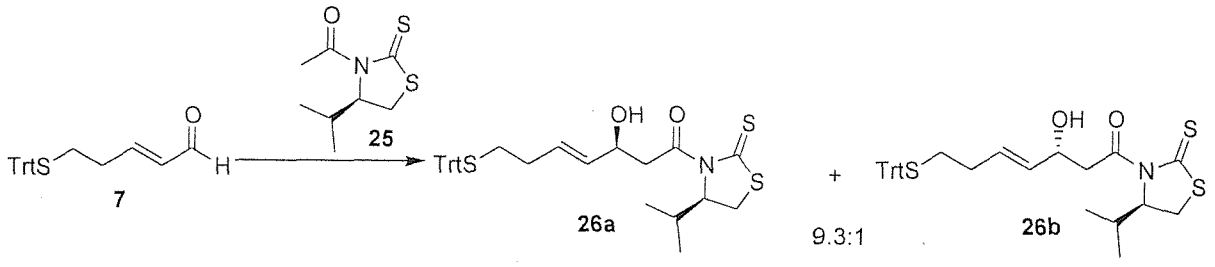
The acylated Nagao auxiliary was synthesised from commercially available D-Valine[12,13] **22** by reduction with $LiBH_4$, in the presence of TMS-Cl/Et_3N[12] to give valinol **23**. Cyclisation with CS_2 in refluxing 1M KOH[13]afforded **24** which was then acylated with acetyl chloride and Et_3N to give **25** which over 3 steps afforded the chiral auxiliary in 53% yield (Scheme 10).



22 **23** **24** **25**

Scheme 10. Reagents and conditions: **(a)** $LiBH_4$, TMS-Cl/Et_3N, THF 0 °C to rt 18 h (78%). **(b)** CS_2, 1M KOH, reflux 18 h (74%). **(c)**, Ac-Cl, Et_3N, CH_2Cl_2, 0 °C then rt 8 h (92%).

Formation of the activated ester **26a** was realised by asymmetric aldol condensation of unsaturated mercapto-aldehyde **7** with Nagao auxiliary **25** derived from D-Valine. Reaction of the Nagao auxiliary with both fresh and aged $SnOTf_2$[14] did not afford any of the desired compound **26A.** We then used $TiCl_4$ as reported by Vilarrasa *et al.*[15] who also found no reaction taking place with $SnOTf_2$ unless it was freshly prepared and used immediately. A series of reactions was performed which showed that the yield of the desired diastereoisomer is very sensitive to amounts of reagents deviating from a 1:1 equivalent of acid to base with either excess Lewis acid or excess base resulting in both lower yields and stereochemical control of the reaction.

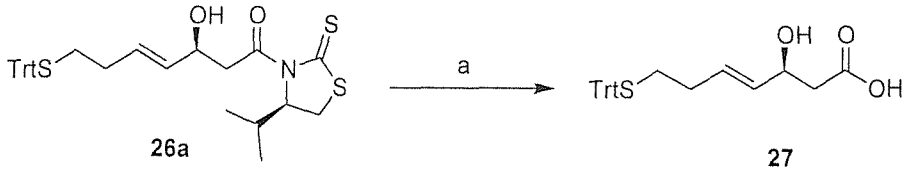


Scheme 11. Reagents and conditions: **(a)** Nagao auxiliary, $TiCl_4$, DIEA, CH_2Cl_2, -40 °C 2 h then aldehyde -78 °C 0.5 h (84%, 80% de).

Using conditions suggested by Vilarrasa *et al.*[15] gave **26** in good overall yield (84%) and *de* (80%) of the desired isomer **26a**. The major diastereoisomer formed was based on results in the literature with α,β-unsaturated aldehydes. The (*R*)-isomer of the thiazolidinethione favours the formation of the (*S*)-isomer of the alcohol.[14] It was possible to separate the minor isomer **26b** easily by chromatography. This compound was then useful in the synthesis of an epimer of spiruchostatin A and in the absolute assignment of configuration of the natural product (Scheme 11).

3.7 Confirmation of stereocentres

Conversion of the aldol product **26a** into the literature[1] hydroxyl acid **27** with NaOH confirmed the correct stereochemistry by comparison of the optical rotation which was identical to the literature value $[\alpha]^{22}_D$ -5.0 (c 2.0, $CHCl_3$), (Scheme 12).



Scheme 12. Reagents and conditions: **(a)** LiOH, 4:1 THF/H_20, 5 h 0 °C (73%).

3.8 Summary

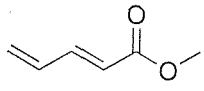
The 3-hydroxy-7-mercapto-4-heptenoic acid subunit was synthesised stereoselectively and in good yield as a thiazolidinethione through an aldol reaction of the Nagao auxiliary with Lewis acid $TiCl_4$. Separating the minor isomer from that of the major diastereomer was easily accomplished by chromatography. The minor stereoisomer was then useful in the synthesis of an epimer of spiruchostatin A and in the elucidation of the absolute configuration of the natural product, as detailed in the previous chapter.

3.9 Experimental

General Methods

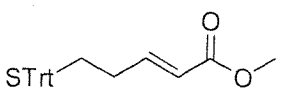
See section 2.23

(*E*)-Penta-2,4-dienoic acid methyl ester (4)



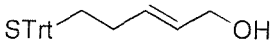
The synthesis of **4** followed the procedure of Waegell *et al.* To a stirred solution of monomethyl malonic acid (30.0 g, 0.25 moles) in pyridine (51.0 mL) was rapidly added acrolein (0.169 moles, 12.6 mL) and DMAP (1.56 g, 12.2 mmol) in succession. After stirring at 50 °C for 18 hours the reaction mixture was poured onto water (170 mL) and extracted with ether (3 x 150 mL), washed with 15 % HCl (3 x 80 mL), and concentrated under reduced pressure. The deep orange oil was distilled using Kugelrohr distillation at 24 mbar, 58 °C, to give **4** as a colourless oil (14.9 g, 78%): IR ν_{max} 1715, 1643 cm^{-1}; ^{1}H NMR 300 MHz 7.24 (dd, J = 15.2, 11.0 Hz, 1H), 6.43 (dt, J = 17.0 Hz, 10.5 Hz, 1H), 5.91 (d, J = 15.0 Hz, 1H), 5.61 (d, J = 17 Hz, 1H), 5.56 (d, J = 10.2 Hz, 1H), 3.72 (s, 1H); ^{13}C NMR 75 MHz 167.43, 145.1, 139.8, 125.8, 121.8, 51.7. The material was spectroscopically identical to that reported in the literature.[3]

(*E*)-5-mercapto[*S*-triphenylmethyl]-2-pentenoic acid, methyl ester (5)



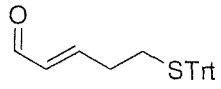
The synthesis of **5** followed the procedure of Simon *et al.* To a stirred solution of methyl 2,4-pentadienoate **4** (10.0 g, 89.3 mmol) in THF (150 mL), was added triphenylmethane thiol (25.7 g, 93.2 mmol, 1.2 equiv.) and Cs_2CO_3 (30.3 g, 92.8 mmol, 1.2 equiv.) at 0 °C. The mixture was stirred under a nitrogen atmosphere at rt for 72 hours. The reaction was then quenched by the addition of saturated aqueous NaCl (180 mL) and extracted with EtOAc (3 x 400 mL). The organic extracts were concentrated *in vacuo* and the residue purified by flash chromatography (eluent 0-20% EtOAc/hexane) to give the α,β unsaturated ester **5** as the predominant isomer (28.4 g, 84 % yield): IR ν_{max} 3056, 3026, 2359, 1722 cm^{-1}; ^1H NMR 300 MHz 7.45-7.24 (m, 15H), 6.80 (dt, J = 15.7 Hz, 6.9 Hz, 1H), 5.74 (dt, J = 15.6 Hz, 1.4 Hz, 1H), 3.73 (s, 3H), 2.31 (m, 2H), 2.15 (m, 2H); ^{13}C NMR 75 MHz: 166.9, 147.1, 144.8, 129.8, 128.1, 126.9, 122.2, 67.0, 51.7, 31.5, 30.4; ES *m/z* 383.2 $(M + Na)^+$. The material was spectroscopically identical to that reported in the literature.[1]

(*E*)-5-mercapto-[*S*-triphenylmethyl]-2-pentenol (6)



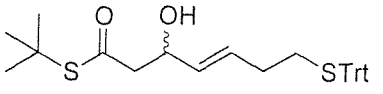
The synthesis of **7** followed the procedure of Simon *et al.* To a stirred solution of the methyl ester **5** (21.0 g, 54 mmol) in CH_2Cl_2 (200 mL) at -78 °C was added DIBAL-H (1.5 M solution in CH_2Cl_2 (72.0 mL, 108 mmol, 2 equiv.), and the solution was stirred under a nitrogen atmosphere at -78 °C for 3 hours. The reaction was allowed to warm to -10 °C and then quenched by the addition of saturated aqueous sodium potassium tartrate (100 mL). The mixture was extracted with CH_2Cl_2 (3 x 500 mL). The solvent was removed and the residue purified by flash chromatography (eluent 20-40% EtOAc/hexane) to give the alcohol **6** (16.4 g, 85% yield) as a crystalline solid: mp 88-90 °C (lit mp 87-90 °C); IR ν_{max} 3452, 3026, 2359, 2329, 1739 cm^{-1}; ^1H NMR 300 MHz 7.37 (m, 15H), 5.57 (m, 2H), 4.04 (m, 2H), 2.27 (m, 2H), 2.13 (m, 2H); ^{13}C NMR 75 MHz 145.0, 130.7, 130.4, 129.8, 127.9, 126.8, 66.8, 63.5, 31.7, 31.5; ES *m/z* 411.2 $(M + Na)^+$. The material was spectroscopically identical to that reported in the literature.[1]

(*E*)-5-Tritylsulfanyl-pent-2-enal (7)



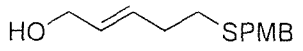
The synthesis of **7** followed the procedure of Simon *et al.* To a stirred solution of oxalyl chloride (0.9 mL, 10.3 mmol) in CH_2Cl_2 (30 mL) at -78 °C was added DMSO (1.5 mL, 20.6 mmol). After stirring under argon for 30 minutes, the alcohol **6** (3.1 g, 8.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the reaction mixture stirred for a further 30 minutes at -78 °C. Et_3N (2.9 mL, 20.6 mmol) was added, the reaction mixture allowed to warm to -30 °C and stirred at this temperature for a further 4 hours. Sat. NaCl (50 mL) was then added and extracted with EtOAc (3 x 50 mL), the solvent removed and the residue purified by flash chromatography (eluent 15% EtOAc/hexane) to give **7** as a crystalline solid (2.14 g, 69 %): mp 144-145 °C (lit mp 142-144 °C); IR ν_{max} 2357, 1737, 1683 cm^{-1}; ^1H NMR 300 MHz 9.44 (d, J = 9.0 Hz, 1H), 7.44 (d, J = 7.3 Hz, 6H), 7.34-7.21 (m, 9H), 6.64 (dt, J = 15.5 Hz, 6.2 Hz, 1H), 5.99 (dd, J = 15.8 Hz, 8.1 Hz, 1H), 2.39-2.30 (m, 4H); ^{13}C NMR 75 MHz 195.12, 175.2, 156.9, 145.6, 134.6, 130.5, 128.9, 127.7, 67.5, 32.0, 30.3; MS *m/z* 381.2 $(M + Na)^+$. The material was spectroscopically identical to that reported in the literature.[1]

(*E*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enethioic acid *S-tert*-butyl ester (8)



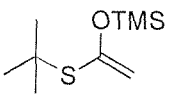
To a stirred solution of LDA (13.95 mmol in 50 mL anhydrous THF) at -78 °C was added *S-tert*-butylthioacetate (1.99 mL, 13.95 mmol) *via* syringe. After stirring at the same temperature for 1 hour, the enolate was then added to a stirred solution of the aldehyde **7** (1.0 g, 2.79 mmol) in THF (100 mL), at -78 °C. The reaction mixture was stirred for 45 minutes more at the same temperature, and then carefully quenched with saturated aqueous NH_4Cl (50 mL). After warming to room temperature, the THF was removed on a rotary evaporator and the resulting aqueous solution was extracted with CH_2Cl_2 (4 x 50 mL). The combined organic layers were then washed with 10% (60 mL) aqueous HCl (60 mL), 5% aqueous $NaHCO_3$ (60 mL) and saturated aqueous NaCl (60 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (eluent 20% EtOAc/hexane) to give **14** as a colourless oil (1.15 g, 83%): IR ν_{max} 3455, 3057, 3029, 1677, 1594 cm^{-1}; ^1H NMR 400 MHz 7.32 (d, J = 7.5 Hz, 6H), 7.22-7.11 (m, 9H), 5.48 (dt, J = 15.1 Hz, 7.0 Hz, 1H), 5.30 (dd, J = 15.1 Hz, 6.5 Hz, 1H), 4.37 (q, J = 6.0 Hz, 1H), 2.54 (d, J = 6.0 Hz, 2H), 2.13 (t, J = 7.5 Hz, 2H), 1.98 (dd, J = 14.6 Hz, 7.0 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR 100 MHz 199.9, 145.0, 131.9, 130.3, 129.7, 128.0, 126.7, 69.4, 66.7, 50.9, 48.7, 31.6, 29.9; MS *m/z* 513.2 $(M + Na)^+$, 1003.2 $(2M + Na)^+$.

(*E*)-5-(4-Methoxy-benzylsulfanyl)-pent-2-en-1-ol (10)



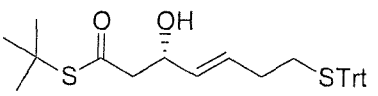
To a stirred solution of (2*E*)-5-mercapto-[*S*-triphenylmethyl]-2-penten-1-ol **6** (1.0 g, 2.78 mmol) in 1.5:8.5 TFA/ CH_2Cl_2 (10 mL) was added Et_3SiH (0.49 mL, 3.06 mmol). After stirring for 30 minutes at rt the reaction mixture was cooled to 0 °C and 4-methoxybenzylchloride (1.5 mL, 11.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 30 minutes. The reaction mixture was stirred at rt for 1 hour, methanol (10 mL) added and then concentrated. The residue was dissolved up in CH_2Cl_2 (100 mL) washed with 5% $NaHCO_3$ (50 mL) and saturated NaCl (50 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by chromatography on basic alumina (eluent 1-5% MeOH/CH_2Cl_2) to give **10** as a colourless oil (0.35 g, 53%): IR ν_{max} 3376, 2833, 1608 cm^{-1}; ^1H NMR 300 MHz 7.23 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 5.67 (m, 2H), 4.09 (m, 2H), 3.80 (s, 3H), 3.68 (s, 2H), 2.49 (t, J = 7.0 Hz, 3H), 2.29 (m, 2H), 1.61 (s, br, 1H); ^{13}C NMR 75 MHz; 158. 7, 130.7, 130.6, 130.4, 130.0, 63.6, 35.8, 32.1, 30.8; MS *m/z* 261.2 $(M + Na)^+$.

(1-*tert*-Butylsulfanyl-vinyloxy)-trimethyl-silane (13)



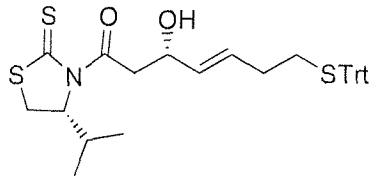
To a stirred solution of diisopropylamine (5.88 mL, 41.7 mmol) in THF (25 mL) was added BuLi (16.32 mL, 2.5 M solution in hexane, 40.8 mmol) at -78 °C followed by dropwise addition of *S-tert*-butylthioacetate (5.0 g, 0.038 M). The reaction mixture was stirred at -78 °C for 1 hour, and TMS-Cl (5.25 mL, 41.6 mmol) added via syringe at -78 °C. After stirring at rt for a further 1 hour, the reaction mixture was washed with 10% HCl (20 mL), back extracted with hexanes (2 x 50 mL), washed with $CuSO_4$ (0.5 M, 50 mL), sat. NaCl (50 mL), dried ($MgSO_4$) and concentrated. The residue was distilled to give **13** as colourless oil. (5.73 g, 73%): IR ν_{max} 2357, 1738, 1592 cm^{-1}; ^1H NMR 400 MHz 4.53 (s, 1H), 4.43 (s, 1H), 1.23 (s, 9H), 0.10 (s, 9H); ^{13}C NMR 100 MHz 153.0, 102.6, 45.6, 31.5, 0.2; The material was spectroscopically identical to that reported in the literature.[6]

(*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enethioic acid *S*-*tert*-butyl ester (14)



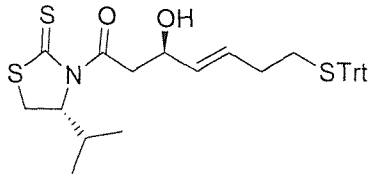
A mixture of *S*-(-)-1,1-bi-2-naphthol (16.05 mg, 0.056 mmol), Ti(O-*i*-Pr)$_4$ (15.9 mg, 0.056 mmol) and oven dried powdered 4Å sieves (100 mg) was heated at reflux for 1 hour in ether (2 mL). The reaction mixture was cooled to room temperature before adding the aldehyde **7** (100 mg, 0.28 mmol). After stirring for 5 minutes at the same temperature, the reaction mixture was cooled to -78 °C before dropwise addition of the TMS-thioketene acetal (160 mg, 0.784 mmol). The reaction mixture was placed in a -20 °C freezer without stirring for 36 hours, pH 7 buffer (2.0 mL) was then added. After stirring for 15 minutes the mixture was filtered through celite, extracted with ether, and the solvent removed to give a crude mixture of the title product and TMS protected alcohol. The crude product was dissolved in ether (5 mL) and treated with 1M TBAF (0.28 mL, 0.28 mmol) and stirred for 5 minutes. The reaction mixture was then quenched with sat. NH_4Cl (2 mL), washed with $NaHCO_3$ (5 mL) and brine (5 mL), the aqueous wash extracted with EtOAc (2 x 5 mL). The organic phase was concentrated and the residue purified by flash chromatography (eluent 20% EtOAc/hexane) to give **14** as a colourless oil (63.1 mg, 46%, 61%ee using 0.5 eq. Eu(hfc)$_3$): $[\alpha]^{22}_D$ -3.6 (c 0.50, $CHCl_3$); IR ν_{max} 3455, 3057, 3029, 1677, 1594 cm^{-1}; ^{1}H NMR 400 MHz 7.32 (d, J = 7.5 Hz, 6H), 7.22-7.11 (m, 9H), 5.48 (dt, J = 15.1 Hz, 7.0 Hz, 1H), 5.30 (dd, J = 15.1 Hz, 6.5 Hz, 1H), 4.37 (q, J = 6.0 Hz, 1H), 2.54 (d, J = 6.0 Hz, 2H), 2.13 (t, J = 7.5 Hz, 2H), 1.98 (dd, J = 14.6 Hz, 7.0 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR 100 MHz 199.9, 145.0, 131.9, 130.3, 129.7, 128.0, 126.7, 69.4, 66.7, 50.9, 48.7, 31.6, 29.9; MS *m/z* 513.2 $(M + Na)^+$, 1003.2 $(2M + Na)^+$.

(*E*)-(*S*)-3-Hydroxy-1-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4-en-1-one (26a)



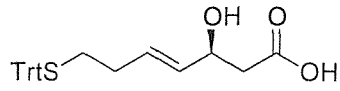
To a stirred solution of the chiral auxiliary **11** (964 mg, 4.74 mmol) in CH_2Cl_2 (38 mL) at 0 °C was added $TiCl_4$ (572 µl, 5.22 mmol). After stirring for 5 minutes the solution was cooled to -78 °C before the addition of DIEA (907 µl, 5.21 mmol) and stirring for 2 hours. The aldehyde **7** (1.0 g, 2.79 mmol) in CH_2Cl_2 (2.6 mL) was added dropwise and the reaction mixture stirred for 30 minutes. Sat. NH_4Cl (25 mL) was added the reaction mixture diluted with CH_2Cl_2 (20 mL), allowed to attain rt, extracted with CH_2Cl_2 (3 x 50 mL), washed with sat. NaCl (50 mL) and dried with Na_2SO_4. The solvent was then removed and the residue purified by flash chromatography (eluent 25-35% EtOAc/hexanes) to give the major isomer **26a** as a yellow oil (1.19 g, 76%, 80% de): $[\alpha]^{22}_D$ -1.45 (c 0.50, $CHCl_3$); IR ν_{max} 2360, 1691 cm^{-1}; ^1H NMR 400 MHz ($CDCl_3$) δ; 7.41 (d, J = 1.5 Hz, 6 H), 7.27 (t, J = 1.5 Hz, 6H), 7.19 (t, J = 2.5 Hz, 3H), 5.60 (dt, J = 15.0 Hz, 6.5 Hz, 1H), 5.45 (dd, J = 15.0 Hz, 5.5 Hz, 1H), 5.11 (t, J = 7.5 Hz, 1H), 4.56 (s, 1H), 3.54 (dd, J = 17.1 Hz, 3.0 Hz, 1H), 3.45 (dd, J = 11.0, J = 7.5 Hz, 1H), 3.25 (q, J = 8.5 Hz, 1H), 2.97 (d, J = 11.5 Hz, 1H), 2.76 (d, J = 4.5 Hz, 1H), 2.33 (m, 1H), 2.21 (t, J = 7.0 Hz, 2H), 2.08 (q, J = 7.5 Hz, 2H), 1.05 (d, J = 6.5 Hz, 3H), 0.95 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz δ; 203.0(C), 172.5(C), 145.0(C), 132.1(C), 130.0(CH), 129.7(CH), 127.9(CH), 126.7(CH), 71.5(CH), 68.6(CH), 66.7(C), 45.3(CH_2), 31.5(CH_2), 30.9(CH_2), 30.7(CH), 21.1(CH_2), 19.2(CH_3), 17.8(CH_3); HRMS *m/z* 584.1722 $(M + Na)^+$ expected 584.1722; Anal. Calcd for $C_{58}H_{70}Cl_3N_3O_7SSi$: C, 68.41; H, 6.28; N, 2.49; S, 17.12. Found C, 68.09; H, 6.29; N, 2.46, S; 16.83.

(*E*)-(*R*)-3-Hydroxy-1-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4-en-1-one (26b)



Minor isomer **26b** as a yellow oil (125 mg, 8%): $[\alpha]^{22}_{D}$ +4.5 (c 0.50, $CHCl_3$); IR ν_{max} 2360, 1691 cm^{-1}; ^1H NMR 400 MHz ($CDCl_3$) δ; 7.39 (d, *J* = 1.5 Hz, 6 H), 7.25 (t, *J* = 1.5 Hz, 6H), 7.20 (t, *J* = 2.5 Hz, 3H), 5.58 (dt, *J* = 15.5 Hz, 6.0 Hz, 1H), 5.44 (dd, *J* = 15.5Hz, 6.0 Hz, 1H), 5.14 (t, *J* = 7.0 Hz, 1H), 4.58 (s, 1H), 3.57-3.44 (m, 2H), 3.34 (dd, *J* = 17 Hz, 3.5 Hz, 1H), 3.11 (d, *J* = 8.5 Hz, 1H), 2.99 (d, *J* = 11.5 Hz, 1H), 2.35 (sextet, *J* = 7.0 Hz, 1H), 2.22 (t, *J* = 7.0 Hz, 2H), 2.11 (m, 2H), 1.05 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR 100 MHz δ; 203.0(C), 173.0(C), 145.0(C), 132.1(C), 130.2(CH), 129.7(CH) 128.0(CH), 126.7(CH), 71.5(CH), 68.9(CH), 66.7(C), 45.2(CH_2), 31.6(CH_2), 31.5(CH_2), 30.9(CH), 30.7(CH_2), 19.2(CH_3), 17.9(CH_3); MS *m/z* 584.1722 $(M + Na)^+$.

(*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoic acid (27)



To a stirred solution of **26a** (20 mg, 0.035 mmol) in 4:1 THF/H_2O (2.0 mL) at 0 °C was added LiOH (6.8 mg, 0.283). After stirring for 1 hour, the reaction mixture was diluted down with H_2O (20 ml) acidified to pH 4-5 with 2M $KHSO_4$, and extracted with EtOAc (3 x 20 ml). The organic layer washed with sat. NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent 50-80% EtOAc/hexanes) to give **27** as a white solid (11.7 mg, 73 %): mp = 124-126 °C; $[\alpha]^{22}_D$ = -5.0 (c 2.0, CH_2Cl_2); which was spectroscopically identical to that reported in the literature.[1]

3.10 References

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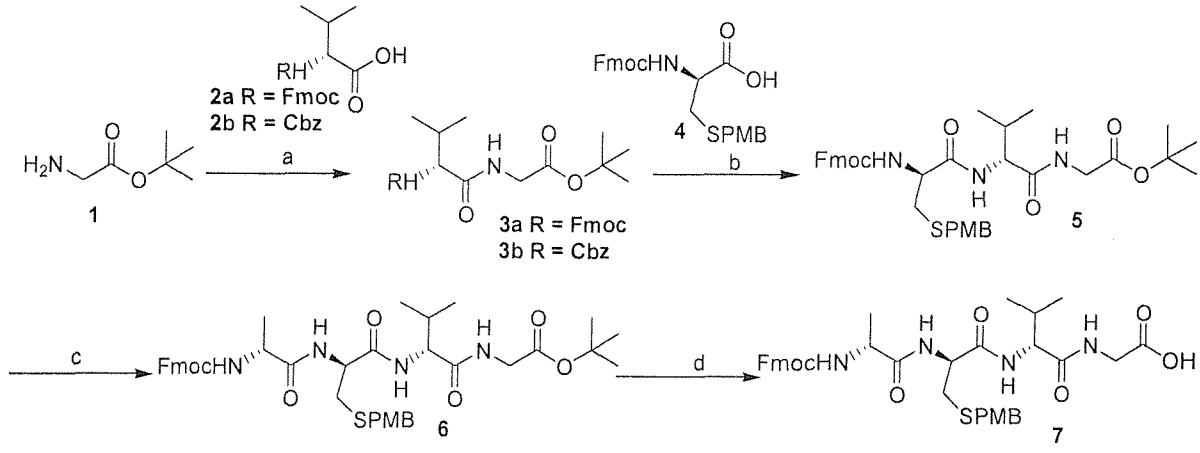
Chapter 4: The Synthesis of an Analogue of Spiruchostatin A

A simplified analogue of spiruchostatin A, substituting the statine subunit by the amino acids glycine and D-valine was designed. The synthesis follows that of spiruchostatin A however without the statine subunit the synthesis should be simplified. FK228 has reached phase III clinical trials as an anticancer agent.[1,2] Spiruchostatin A has also recently entered clinical trials. Although both show high levels of activity at nanomolar concentrations they are unlikely to be optimized by nature for potency or selectivity against human HDACs. It is therefore feasible that unnatural analogues may be synthesised with greater selectivity and activity against HDACs.

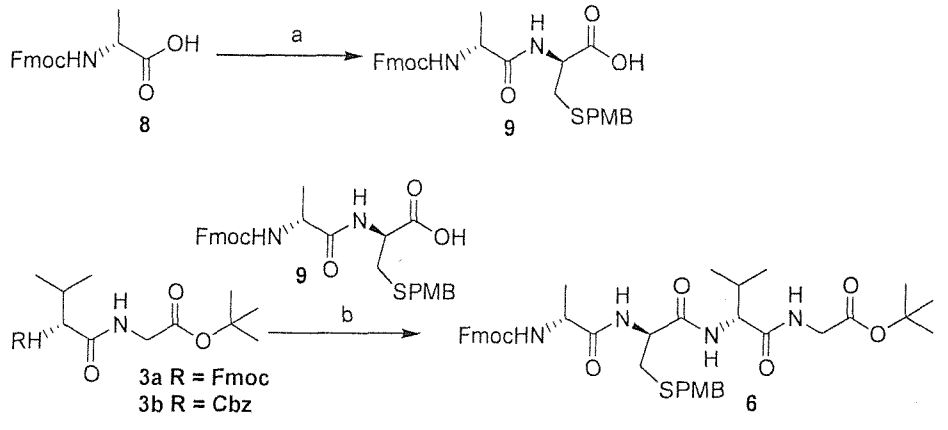
4.1 The synthesis of a tetrapeptide analogue of spiruchostatin A



Scheme 1. Reagents and conditions: **(a) 2**, EDAC•HCl, HOBt, DIEA, CH_2Cl_2, 4 h (77%). **(b) 3a,** Et_2NH, CH_3CN, 3 h or **3b** 5% Pd/C, H_2, MeOH/EtOAc, 18 h then EDAC•HCl, HOBt, DIEA, **4**, CH_3CN, 4 h (82%). **(c) 5,** Et_2NH, CH_3CN, 3 h then Fmoc-D-alanine, EDAC•HCl, HOBt, DIEA, CH_3CN, 4 h (66%). **(d)** TFA, CH_2Cl_2, Et_3SiH, 3 h (84%).

The *tert*-butyl ester of glycine **1** was coupled to either Fmoc **2a** or Cbz **2b** protected D-valine using the water soluble carbodiimide EDAC/HOBt (1-(3-dimethylaminopropyl)-3-ethylcarboiimide/1-hydroxybenzotriazole hydrate) following a generalised procedure by Sheehan *et al*[3]. The water soluble urea formed as a by-product can then easily be removed

during aqueous workup. The products obtained **3a** and **3b** were white solids and re-crystallised using acetonitrile. Removal of the amine protecting groups, Cbz and Fmoc, was achieved with H_2/Pd-C and Et_2NH respectively. The amine was then coupled to the protected cysteine **4**, using the EDAC/HOBt protocol. Analysis of compound **5** by NMR indicated that ~25% racemisation had taken place. Removal of the impurity was accomplished on the next step. Peptide **5** was deprotected with Et_2NH in acetonitrile and the resulting amine coupled to Fmoc-D-alanine was again achieved with EDAC/HOBt protocol. Sonication of the highly insoluble product **6** in MeOH gave predominantly the desired diastereoisomer. Deprotection of the *tert*-butyl ester in TFA/CH_2Cl_2 with Et_3SiH[4] gave the acid **7** (Scheme 1).

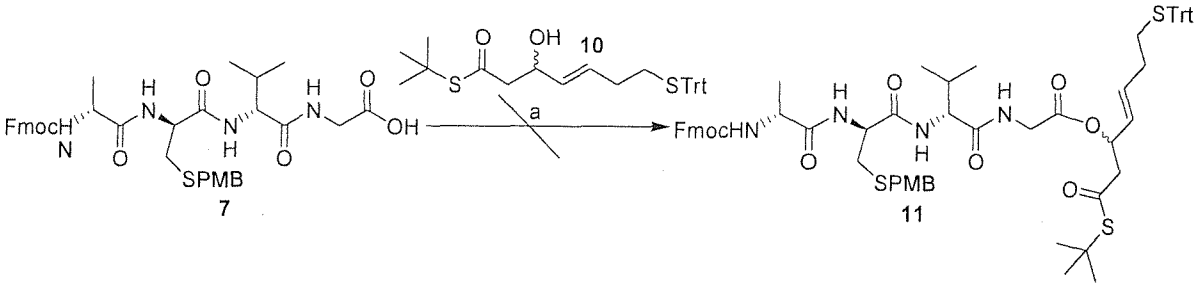


Scheme 2. Reagents and conditions: **(a)** Fmoc-D-alanine, $SOCl_2$, CH_2Cl_2, 0.5 h then PMB-D-cysteine, 1:1 5% Na_2CO_3/CH_2Cl_2, 10 minutes (23%). **(b) 3a,** Et_2NH, CH_3CN, 3 h or **3b** 5% Pd/C, H_2, MeOH/EtOAc, 18 h then EDAC•HCl, HOBt, DIEA, **9**, CH_3CN, 4 h (58%).

An alternative route to the tetrapetide **6** involved joining together the two dipeptides **3** and **9** with EDAC/HOBt in a convergent synthesis. The acid **9** was synthesised from the acid chloride of Fmoc-D-alanine with PMB protected D-cysteine[5] (Scheme 2).

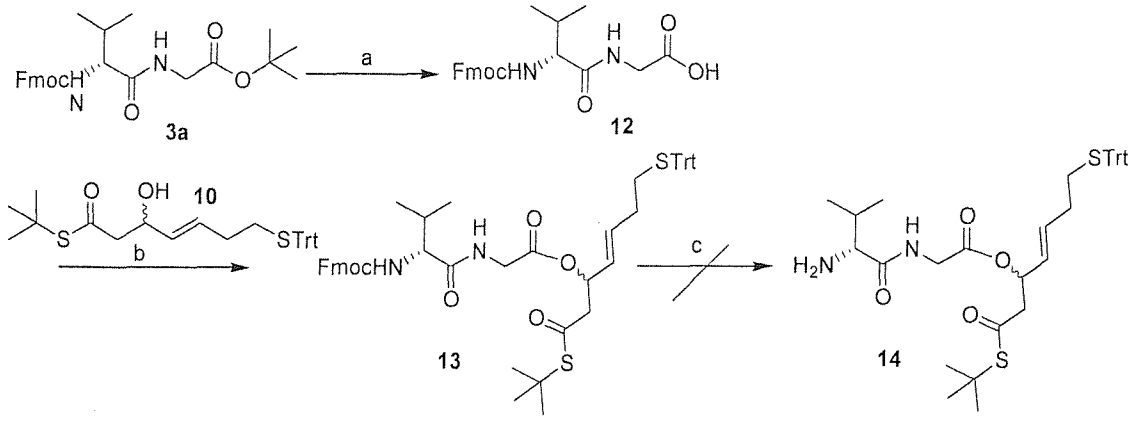
4.2 Attempted coupling of the tetrapeptide to the β-hydroxyacid subunit

Attempted coupling of the acid **7** to the alcohol **10** did not afford the desired ester **11**. The general ester coupling conditions DCC/DMAP (1,3-Dicyclohexylcarbodiimide/4-Dimethylaminopyridine) and EDAC/DMAP have so far been attempted in DMF and toluene[6] (Scheme 3).



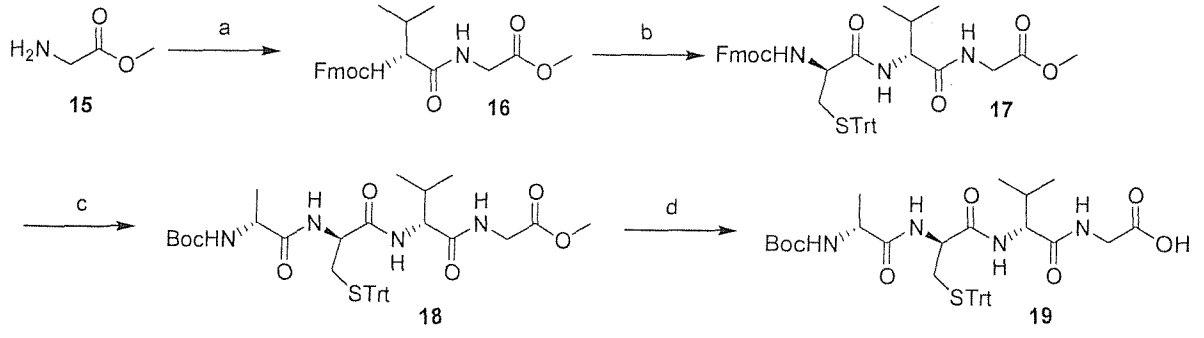
Scheme 3. Reagents and conditions: **(a)** DCC/DMAP or EDAC/DMAP, DMF or toluene.

We then tried coupling the acid to the simple dipeptide **12**, formed from deprotection of ester **3a.** Coupling to the alcohol **10,** afforded the desired ester **13**, using DCC/DMAP in DMF. Attempted deprotection of **13** using Et_2NH in CH_3CN did not afford the desired amine **14**, various bases are known to cause degradation of thioesters[7] and the unsaturated β-hydroxy ester may also be prone to base assisted α,β-elimination[8] (Scheme 4).



Scheme 4. Reagents and conditions: **(a)** TFA, CH_2Cl_2, Et_3SiH, 3 h (86%). **(b)** DDC/DMAP, DMF 15 min, then **10**, 18 h (62%). **(c)** Et_2NH, CH_3CN, 3 h.

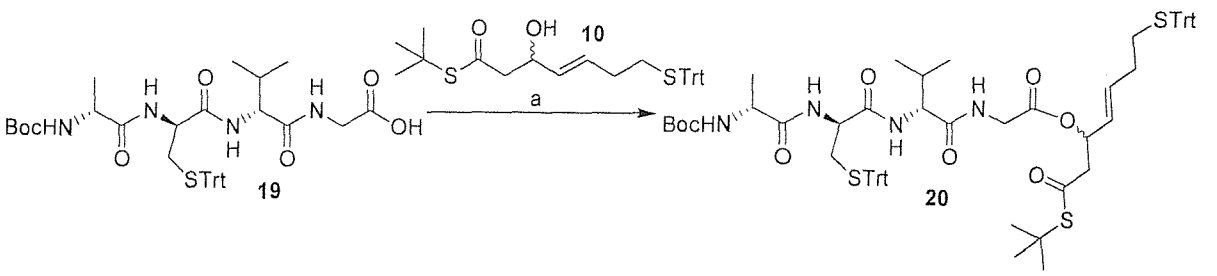
4.3 The synthesis of a tetrapeptide analogue of spiruchostatin A – a different protecting group strategy



Scheme 5. Reagents and conditions: **(a)** Fmoc-D-valine, EDAC•HCl, HOBt, DIEA, CH_2Cl_2, 4 h (86%). **(b)** Et_2NH, CH_3CN, 3 h then Fmoc-(*S*Trt)-D-cysteine EDAC•HCl, HOBt, DIEA, CH_3CN, 4 h (79%). **(c)** Et_2NH, CH_3CN, 3 h then Boc-D-alanine, PyBop, DIEA, CH_3CN, 4 h (84%). **(d)** LiOH, 4:1 THF/H_2O, 1 h 0 °C (92%).

It was evident that the Fmoc protection of the terminal amino group of our tetrapeptide was incompatible with our synthetic strategy, on coupling to the β-hydroxy alcohol attempts to use base on the molecule gave the elimination product. The methyl ester of glycine **15** was coupled to Fmoc protected D-valine using the water soluble carbodiimide EDAC/HOBt following a generalised procedure by Sheehan *et al.*[3] to give peptide **16**. Removal of the Fmoc protecting group with Et_2NH followed by repetition of the EDAC/HOBt protocol gave the tripeptide **17**, PyBop coupling to Boc-D-alanaine gave the tetrapeptide **18**. Deprotection of the methyl ester with LiOH in 4:1 THF/H_2O gave the acid **19** (Scheme 5).

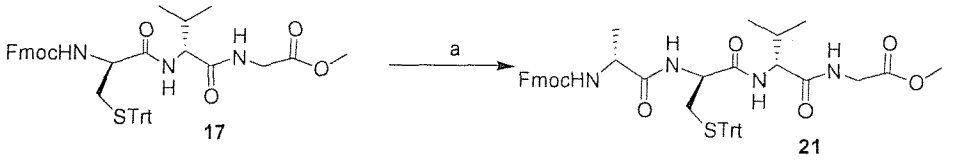
4.4 Coupling of the tetrapeptide acid to the β-hydroxyacid subunit alcohol via an esterification



Scheme 6. Reagents and conditions: **(a)** DCC/DMAP CH_2Cl_2, 0 °C-rt, 18 h (81%).

Coupling of the acid **19** to the alcohol **10** successfully afforded ester **20**. The general ester coupling conditions[6] DCC/DMAP (1,3-Dicyclohexylcarbodiimide/4-Dimethylaminopyridine) proved successful to give the ester in good yield 81% (Scheme 6). A high yield was only possible by using an excess of the acid. Due to the reaction being relatively slow in comparison to an amide bond forming reaction a competing reaction is often observed in esterification reactions.[9] The initially generated *O*-acylisourea can collapse to generate the much less reactive *N*-acylurea by intramolecular acyl transfer. This compound was found by MS and NMR studies and made purification of the desired ester problematic due to co-elution. It was not possible at this stage to separate the diastereoisomers. Various bases are known to cause degradation of thioesters[7] and the unsaturated β-hydroxy ester may also be prone to base assisted α,β-elimination.[8] These problems were overcome by using excess of the acid (1.4 eq.) and carrying out the reaction at lower temperatures.

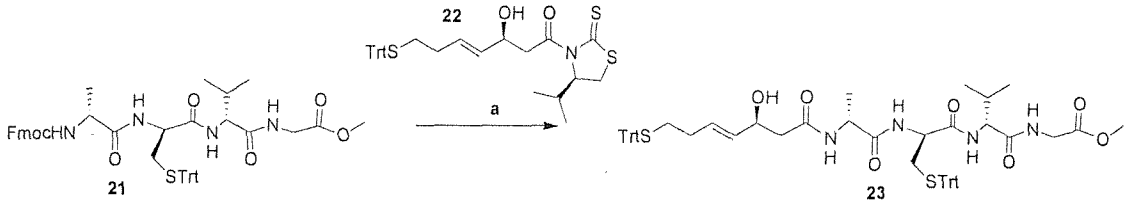
4.5 The synthesis of a tetrapeptide analogue of spiruchostatin A



Scheme 7. Reagents and conditions: **(a)** Et_2NH, CH_3CN, 3 h then Fmoc-D-alanine, PyBop, DIEA, CH_3CN, 18 h (81%).

The tetrapeptide **21** was synthesised from previously synthesised tripeptide **17.** Removal of the Fmoc protecting group with Et_2NH followed by the PyBop protocol with Fmoc-D-alanine gave **21** (Scheme 7).

4.6 Coupling of the tetrapeptide amine to the β-hydroxyacid subunit through an activated thiazolidinethione

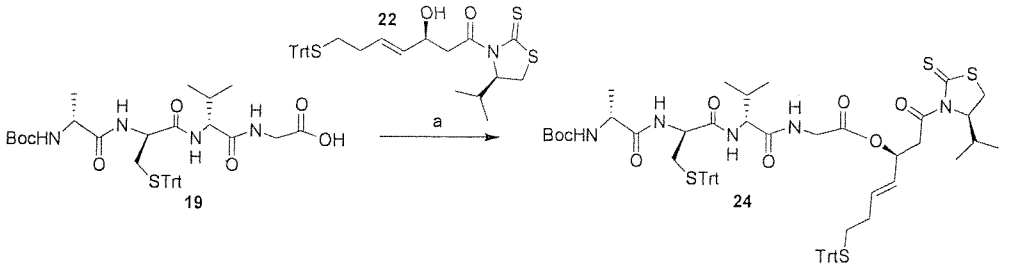


Scheme 8. Reagents and conditions: **(a)** (i) 5% Et_2NH CH_3CN, 20 °C 6 h. (ii) DMAP, CH_2Cl_2, 0 °C then 20 °C 7 h (73%).

Removal of the Fmoc protecting group from **21** with Et_2NH and coupling the crude amine with the thiazolidinethione **22** which mildly activates towards nucleophilic substitution, in the presence of catalytic DMAP smoothly furnished ester **23** (Scheme 8).

4.7 Coupling of the tetrapeptide to the β-hydroxyacid thiazolidinethione via an aldol reaction

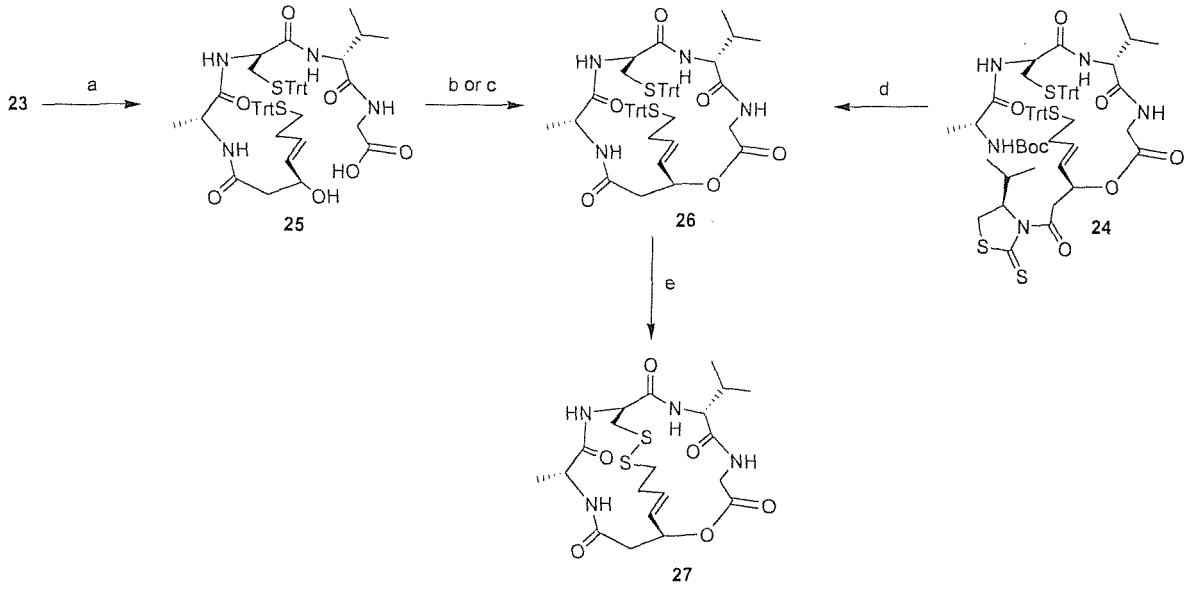
Acid **19** was coupled to alcohol **22** using standard esterification conditions giving ester **24** (Scheme 9). The general ester coupling conditions DCC/DMAP proved successful however due to possible attack on the activated ester which occurs in the presence of DMAP the yield was only 50% (a small percentage of the Nagao auxiliary was recovered from the reaction). Excess acid is used in the coupling once again due to *N*-acylurea formation. Further investigation into esterification conditions include the possibility of forming an activated ester on the acid without the need for using DMAP.



Scheme 9. Reagents and conditions: **(a)** DCC/DMAP CH_2Cl_2, 0 °C to rt, 18 h (50%).

4.8 Macrolactonisation and macrolactamization to form the cyclic depsipeptide followed by intramolecular disulfide formation

Formation of the cyclic depsipeptide **26** was achieved by saponification of **23** to hydroxyacid **25** followed by macrolactonization,[10-13] or macrolactamization by deprotection of the Boc protected amine of **24** to give the free amine which then cyclised in the presence of catalytic DMAP. The cyclisation step was not high yielding in forming either the lactone or lactam. The best yields were achieved using standard peptide esterification conditions under high dilution. However, this method was also problematic in purification with a DCC *N*-acylurea adduct proving difficult to separate from the desired product. Oxidation of the trityl protected thiols to the cyclic disulfide was achieved using four equivalents of iodine at high dilution[14] to give the unnatural analogue **27** (Scheme 10).



Scheme 10. Reagents and conditions **(a)** (i) LiOH, 4:1 THF/H_2O, 0 °C 1 h (91%). **(b)** (i) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N CH_3CN, THF 0 °C then 20 °C 1 h. (ii) DMAP, toluene, 50 °C 4 h (11%) or **(c)** DCC/DMAP CH_2Cl_2, 0 °C-rt, 18 h (36%). **(d)** (i) TFA, TrtOH, CH_2Cl_2, rt 5 min (ii) DMAP, DIEA, CH_2Cl_2, (12%). **(e)** I_2, MeOH, 20 min (67%).

4.9 Summary

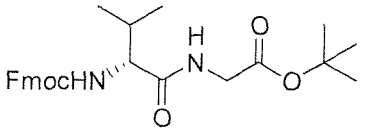
An analogue of spiruchostatin A was successfully synthesised substituting the statine subunit by commercially available amino acids. The analogue showed high activity and selectivity for HDACs however the activity was lower than that of the natural product. This paves the way for the synthesise of a large number of analogous compounds to the cyclic depsipeptides, in order to optimise activity and selectivity for HDACs.

4.10 Experimental

General Methods

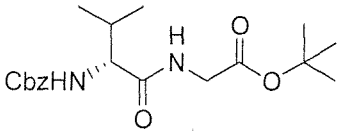
See section 2.23

(*R*)-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-acetic acid *tert*-butyl ester (3a)



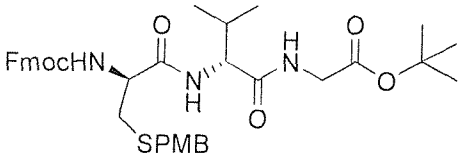
To a stirred solution of Fmoc protected D-valine **2a** (2.59 g, 7.60 mmol) in CH_2Cl_2 (50 mL) was added EDAC•HCl (2.19 g, 11.43 mmol), HOBt (1.50g, 11.43mmol) and DIEA (4.0 mL, 22.86 mmol). After stirring at rt for 15 minutes, the amine **1** (1.0 g, 7.62 mmol) was then added and the reaction mixture stirred for 4 h, diluted with CH_2Cl_2 (50 mL) washed with water (25 mL) 10% HCl (25 mL), 5% $NaHCO_3$ (25mL) and sat. NaCl (25 mL) solutions, dried (Na_2SO_4), filtered, and concentrated to give an off white solid which was re-crystallised from CH_3CN (20 mL) to give **3a** as a white solid (2.41 g, 77%): mp = 159-161 °C; $[\alpha]^{22}_D$ -7.50 (c 0.50, $CHCl_3$); IR ν_{max} 3290, 1721, 1687, 1650, 1534 cm^{-1}; ^1H NMR 400 MHz (DMSO-d_6) 8.39 (t, *J* = 6.0 Hz, 1H), 8.00 (d, *J* = 7.5 Hz, 2H), 7.86 (t, *J* = 5.5 Hz, 2H), 7.53 (t, *J* = 7.0 Hz, 3H), 7.43 (dt, *J* = 7.5, *J* = 3.0 Hz, 2H), 4.35 (m, 3H), 4.0 (t, *J* = 8.5 Hz, 1H), 3.91 (dd, *J* = 17.6, *J* = 6.0 Hz, 1H), 3.78 (dd, *J* = 17.6, *J* = 6.0 Hz, 1H) 2.10 (m, 1H), 1.51 (s, 9H), 1.01 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR 100 MHz (DMSO-d_6) 170.5(C), 167.7(C), 155.0(C), 142.7(C), 142.6(C), 139.6(C) 127.9(CH), 126.5(CH), 125.9(CH), 124.3(CH), 118.9(CH), 79.4(C), 64.6(CH_2), 58.9(CH), 45.5(CH), 40.3(CH_2), 29.2 (CH), 26.6(CH_3), 18.1(CH_3), 17.1(CH_3); MS *m/z* 475.0 $(M + Na)^+$.

(*R*)-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-acetic acid *tert*-butyl ester (3b)



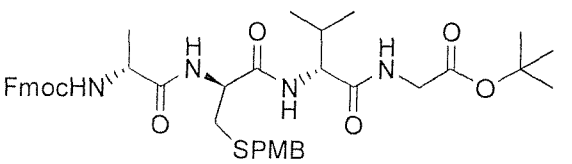
To a stirred solution of the amine **1** (0.75 g, 5.7 mmol) in CH_3CN (50 mL) was added the acid **2b** (3.56 g, 6.8 mmol), PyBOP (3.56 g, 6.8 mmol) and DIEA (2.48 mL, 14.25 mmol). After stirring at rt for 2 h, the solvent was removed, the residue dissolved up in CH_2Cl_2 (60 mL) washed with water (20 mL) 10% HCl (20 mL), 5% $NaHCO_3$ (20mL) and sat. NaCl (20 mL) solutions, dried (Na_2SO_4), filtered, and concentrated to give an off white solid which was re-crystallised from CH_3CN (10 mL) to give **3b** as a white solid (1.50 g, 72%): mp = 146-148 °C; $[\alpha]^{22}_D$ -7.62 (c 0.50, $CHCl_3$); IR ν_{max} 3284, 1740, 1694, 1650, 1535 cm^{-1}; ^1H NMR 400 MHz 8.36 (t, J = 5.5 Hz, 1H), 7.48-7.36 (m, 5H), 5.14 (s, 2H), 4.0 (t, J = 8.5 Hz, 1H), 3.88 (dd, J = 17.1, J = 6.0 Hz, 1H), 3.78 (dd, J = 17.2, J = 6.0 Hz, 1H) 3.42 (s, 1H), 2.09 (m, 1H), 1.51 (s, 9H), 0.99 (dd, J = 13.6, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 172.0(C), 169.3(C), 156.6(C), 137.5(C), 128.8(CH), 128.2(CH), 125.9(CH), 81.0(C), 65.8(CH_2), 60.5(CH), 55.4(C), 41.8(CH_2), 30.7 (CH), 28.2(CH_3), 19.7(CH_3), 18.6(CH_3); MS *m/z* 387.3 $(M + Na)^+$, 751.3 $(2M + Na)^+$.

{(*R*)-2-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid *tert*-butyl ester (5)



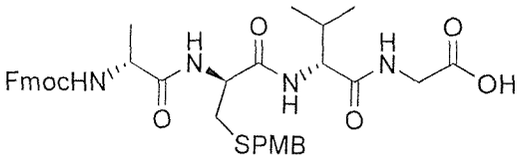
To a solution of Cbz protected amine **3b** (1.0 g, 2.74 mmol) in MeOH/EtOAc 1:1 (25 mL) was added 5 % Pd/C (150 mg). The vessel was purged with argon, then filled with an atmosphere of H_2 and stirred at rt for 18 h. The slurry was filtered through diatomaceous earth, dried (Na_2SO_4), filtered, and concentrated to give the amine as colourless oil (0.61 g, 96 %) and used directly in the next step. To a stirred solution of acid **4** (299 mg, 0.65 mmol) in CH_2Cl_2 (15 mL) was added EDAC•HCl (287.6 mg, 0.65 mmol), HOBt (85 mg, 0.65 mmol) and DIEA (149 μL, 1.08 mmol). After stirring at rt for 15 minutes, the amine (100 mg, 0.43 mmol) was then added and the reaction mixture stirred for 4 h, diluted with CH_2Cl_2 (20 mL) washed with water (15 mL) 10% HCl (15 mL), 5% $NaHCO_3$ (15mL) and sat. NaCl (15 mL) solutions, dried (Na_2SO_4), filtered, and concentrated to give a off white solid which was re-crystallised from CH_3CN (20 mL) to give **5** as a white solid (238.3 mg, 82%): mp = 156-158 °C; IR ν_{max} 3284, 1692, 1639, 1510 cm^{-1}; $[\alpha]^{22}_D$ -2.60 (c 0.50, $CHCl_3$); ^1H NMR 400 MHz 7.75 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.0 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.26 (m, 2H), 6.83 (d, J = 8.5 Hz, 2H), 6.75-6.48 (m, 2H), 5.66 (m, 1H), 4.40 (d, J = 7.5, 2H), 4.33 (t, J = 8.0 Hz, 1H), 4.22 (t, J = 8.0 Hz, 2H), 3.90 (m, 2H), 3.80 (s, 3H), 3.71 (s, 2H), 2.86-2.76 (m, 2H), 2.23 (m, 1H), 1.67 (s, 1H), 1.44 (s, 9H), 0.93 (dd, J = 13.6, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 170.4(C), 168.6(C), 158.9(C), 156.0(C), 143.8(C), 143.7(C), 141.3(C), 141.3(C), 130.1(CH), 129.9(CH), 129.7(CH), 127.8(CH) 127.1(CH), 125.1(CH), 120.0(CH), 114.1(CH), 82.3(C), 67.4(CH_2), 58.6(CH), 55.3(CH), 54.4(C), 47.1(CH), 42.0(CH_2), 36.2(CH_2), 33.9(CH_2), 30.8(CH), 30.5(CH), 28.0(CH_3), 19.3(CH_3), 17.8(CH_3); MS *m/z* 698.3 $(M + Na)^+$, 1373.7 $(2M + Na)^+$.

{(*R*)-2-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid *tert*-butyl ester (6)



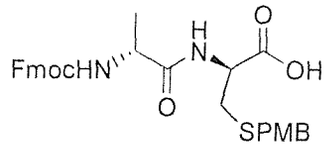
To a stirred solution of **5** (400 mg, 0.59 mmol) in CH_3CN (25 mL) at rt, was added Et_2NH (1.75 mL). After stirring for 3 h at rt the reaction mixture was diluted with hexane (100 mL) and concentrated to give the amine as a colourless oil. To a stirred solution of Fmoc-D-alanine (247 mg, 0.79 mmol) in CH_2Cl_2 (25 mL) was added EDAC•HCl (151.4 mg, 0.79 mmol), HOBt (106.7 mg, 0.79 mmol) and DIEA (220 μL, 1.6 mmol). After stirring at rt for 15 minutes, the amine (240 mg, 0.53 mmol) was then added and the reaction mixture stirred for 4 h, diluted with CH_2Cl_2 (20 mL) washed with water (15 mL) 10% HCl (15 mL), 5% $NaHCO_3$ (15mL) and sat. NaCl (15 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed to give a off white solid which was purified by sonication in MeOH (5 mL) to give **6** as a white solid (261 mg, 66%): mp = 193-195 °C; IR ν_{max} 3283, 1636, 1539, 1512 cm^{-1}; ^1H NMR 400 MHz (DMSO-d_6) 8.27 (t, J = 7.5 Hz, 1H), 8.27 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 7.6 Hz, 2H), 7.81 (d, J = 9.0 Hz, 1H), 7.72 (t, J = 10.0 Hz, 2H), 7.58 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 8.0 Hz, 2H), 4.56 (m, 1H), 4.21 (m, 4H), 4.11 (t, J = 7.0 Hz, 1H), 3.76 (dd, J = 17.1, J = 6.0 Hz, 1H), 3.69 (m, 4H), 2.75 (dd, J = 13.6, J = 5.5 Hz, 1H), 2.58 (dd, J = 13.6, J = 8.0 Hz, 1H), 1.99 (m, 1H), 1.39 (s, 9H), 1.23 (d, J = 6.6 Hz, 3H), 0.85 (t, J = 7.5 Hz, 6H); ^{13}C NMR 100 MHz (DMSO-d_6) 173.2(C), 171.5(C), 170.6(C), 169.3(C), 158.7(C), 156.3(C), 144.5(C), 141.3(C), 130.7(CH), 128.3(CH), 127.7(CH), 125.9(CH) 120.7(CH), 114.3(CH), 81.2(C), 66.3(CH_2), 58.0(CH), 55.6(CH), 53.0(CH), 50.7(CH), 47.3(CH), 40.8(CH_2), 35.3(CH_2), 33.3(CH_2), 31.4(CH_2), 28.3 (CH_3), 19.7(CH_3), 18.8(CH_3), 18.5(CH_3); MS *m/z* 768.9 $(M + Na)^+$, 1515.7 $(2M + Na)^+$.

{(*R*)-2-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid ester (7)



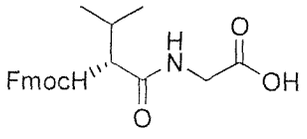
To a stirred solution of **6** (100 mg, 0.133 mmol) in CH_2Cl_2 (1.75 mL) at 0 °C was added Et_3SiH (38 μl) and TFA (0.25 mL). After stirring for 3 h, the reaction mixture was then concentrated. The residue was triturated with Et_2O (1 mL) and washed with ice cold ether (1 mL) to give **7** as a white solid (77.1 mg, 84%): mp = 193-195 °C; IR ν_{max} 3279, 1634, 1535, 1510 cm^{-1}; ^1H NMR 400 MHz (DMSO-d_6) 8.35 (t, *J* = 7.5 Hz, 1H), 8.25 (d, *J* = 7.5 Hz, 1H), 8.00 (d, *J* = 7.6 Hz, 2H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.83 (t, *J* = 10.0 Hz, 2H), 7.68 (d, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 2H), 7.44 (t, *J* = 7.5 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 4.67 (d, *J* = 6.5 Hz, 1H), 4.34 (m, 4H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.80 (m, 5H), 2.87 (dd, *J* = 13.6, *J* = 5.5 Hz, 1H), 2.72 (dd, *J* = 13.6, *J* = 8.0 Hz, 1H), 2.10 (m, 1H), 1.34 (d, *J* = 6.6 Hz, 3H), 0.96 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR 100 MHz (DMSO-d_6) 172.2(C), 171.6(C), 169.3(C), 158.7(C), 156.8(C), 144.5(C), 141.3(C), 130.7(CH), 128.3(CH), 127.7(CH), 125.9(CH) 120.7(CH), 114.3(CH), 81.2(C), 66.3(CH_2), 58.0(CH), 55.6(CH), 52.6(CH), 50.2(CH), 47.3(CH), 40.4(CH_2), 35.3(CH_2), 33.7(CH_2), 31.4(CH_2), 19.7(CH_3), 18.8(CH_3), 18.5(CH_3); MS *m/z* 803.1 (M + TFA – H)$^-$.

(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionic acid (9)



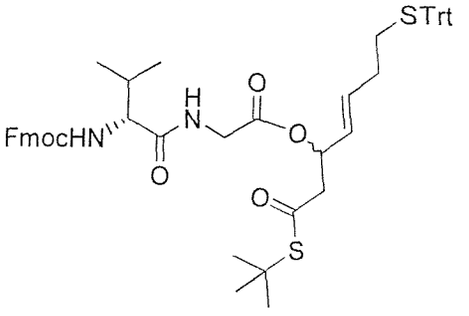
To a stirred solution of Fmoc-D-alanine (1.0 g, 3.21 mmol) in CH_2Cl_2 (5 mL) was added $SOCl_2$ (2 mL). After stirring at reflux for 0.5 h, the solvent was removed, CH_2Cl_2 (20 mL) added and removed under reduced pressure 3 times to give a yellow oil, which was crystallised from ether/hexanes, washed with hexanes, and dried over silica gel under vacuum. To a mixed phase solution of (*S*PMB)-D-cysteine (1.0 mg, 4.15 mmol) in 1:1 5% $Na_2CO_3/CHCl_3$ (25 mL) was added the crude acid chloride (3.21 mmol). After stirring for 10 minutes, the reaction mixture was diluted with CH_2Cl_2 (20 mL), acidified with 1 M HCl to pH 5, separated with CH_2Cl_2 (2 x 25 mL), washed with sat. NaCl (20 mL), filtered, dried and concentrated. Re-crystallised from CH_3CN (10 mL) to give the acid **9** as a white solid (395 mg, 23%): mp = 152-154 °C; IR ν_{max} 3289, 1691, 1643, 1510 cm^{-1}; ^1H NMR 400 MHz 12.89 (s, br, 1H), 8.25 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 7.5 Hz, 2H), 7.78 (t, J = 7.5 Hz, 2H), 7.64 (d, J = 8.0 Hz, 2H), 7.49 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 7.0 Hz, 2H), 7.26 (d, J = 8.5 Hz, 2H), 6,88 (d, J = 8.5 Hz, 2H), 4.49 (m, 1H), 4.28 (m, 4H), 3.74 (m, 4H) 2.85 (dd, J = 13.6, J = 5.0 Hz, 1H), 2.76 (dd, J = 14.1, J = 8.0 Hz, 1H), 1.29 (d, J = 7.0, 3H); ^{13}C NMR 100 MHz 173.1(C), 172.5(C), 158.6(C), 156.1(C), 144.4(C), 144.2(C), 141.2(C), 130.5(CH), 128.1(CH), 127.5(CH), 125.8(CH) 125.7(CH), 120.5(CH), 114.2(CH), 66.1(CH_2), 55.4(CH), 52.5(CH), 50.3(CH), 47.1(CH), 35.3(CH_2), 32.6(CH_2), 18.7(CH_3); MS *m/z* 646.8 $(M + TFA - H)^-$, 1066.7 $(2M - H)^-$.

[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-acetic acid (12)



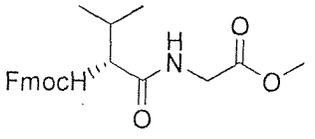
To a stirred solution of **3a** (200 mg, 0.266 mmol) in CH_2Cl_2 (3.5 mL) at 0 °C was added Et_3SiH (76 μl) and TFA (500 μL). After stirring for 4 h, the solution was concentrated. The residue was triturated with Et_2O (2 mL) and washed with ice cold ether (2 mL) to give **12** as a white solid (156 mg, 86%): mp = 195-197 °C; IR ν_{max} 3285, 1718, 1682, 1656, 1644, 1535 cm^{-1}; ^1H NMR 400 MHz (DMSO-d_6) 12.62 (s, br, 1H), 8.34 (t, J = 5.5 Hz, 1H), 7.80 (d, J = 7.5 Hz, 2H), 7.87 (t, J = 7.0 Hz, 2H), 7.53 (t, J = 7.5 Hz, 2H), 7.46-7.41 (m, 2H), 4.36 (m, 3H), 4.01 (t, J = 8.5 Hz, 1H), 3.94 (dd, J = 17.6, J = 5.5 Hz, 1H), 3.83 (dd, J = 17.6, J = 5.5 Hz, 1H) 3.50 (m, 1H), 2.11(m, 1H), 1.00 (t, J = 8.5 Hz, 6H); ^{13}C NMR 100 MHz (DMSO-d_6) 171.7(C), 171.2(C), 156.2(C), 143.9(C), 140.8(C), 127.7(CH), 127.1(CH), 125.5(CH), 120.2(CH), 65.8(CH_2), 60.1(CH), 46.7(CH), 40.7(CH_2), 30.4 (CH), 19.3(CH_3), 18.3(CH_3); MS *m/z* 475.0 $(M + Na)^+$.

[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-ac etic acid (*E*)-1-*tert*-butylsulfanylcarbonylmethyl-5-tritylsulfanyl-pent-2-enyl ester (13)



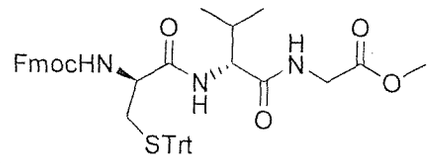
To a stirred solution of **12** (100mg, 0.252 mmol) in DMF (1.5 mL) was added DCC (52 mg, 0.252 mmol) DMAP (6.1 mg, 0.05 mmol). After stirring at rt for 15 minutes, the alcohol **10** (62 mg, 0.126 mmol) was then added and the reaction mixture stirred for 18 h. The solvent was removed under vacuum and the residue dissolved up in ether (4 mL), the solid precipitate filtered off, solvent removed and the residue chromatographed using 10-30% EtOAc/hexanes to give **14** as a colourless oil (67 mg, 62%), (It was not possible to separate the diastereoisomers at this stage): IR ν_{max} 3059, 1719, 1663, 1630, 1596 cm^{-1}; ^1H NMR 400 MHz 7.76 (d, J = 6.4 Hz, 2H), 7.46 (d, J = 6.5 Hz, 2H), 7.38-7.18 (m, 19H), 6.23 (s, br, 1H), 5.61 (dd, J = 13.6, J = 7.0 Hz, 2H), 5.31 (dd, J = 15.6, J = 7.0 Hz, 2H), 4.42 (t, J = 8.5 Hz, 2H), 4.0 (t, J = 6.5 Hz, 1H), 4.02 (m, 2H), 2.78 (dd, J = 14.0, J = 8.0 Hz, 1H), 2.65 (dd, J = 15.1, J = 5.0 Hz, 1H) 2.18 (t, J = 7.0 Hz, 3H), 2.03 (m, 2H), 1.58 (s, 2H), 1.41 (s, 9H), 0.91 (m, 6H); ^{13}C NMR 100 MHz 196.3(C), 168.8(C), 145.2(C), 144.3(C), 141.7(C), 134.2(CH) 130.0(CH), 128.3(CH), 128.1(CH), 127.7 (CH), 127.5(CH), 125.5(CH), 120.4(CH), 72.6(C), 67.4(CH_2), 48.9(CH_2), 47.7(CH), 41.7(CH), 31.8(CH_2) 31.5(CH_2), 30.1(CH_3), 19.6(CH_3), 18.2(CH) 14.5(C); MS *m/z* 508.9 (M + TFA – H)$^-$, 790.7 (2M – H)$^-$.

[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-acetic acid methyl ester (16)



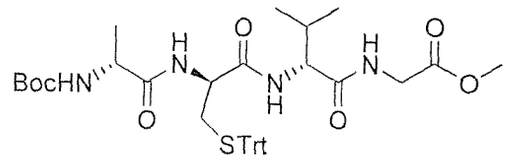
To a stirred solution of Fmoc protected D-valine (2.70 g, 7.96 mmol) in CH_2Cl_2 (50 mL) was added EDAC•HCl (1.83 g, 9.55 mmol), HOBt (1.30g, 9.55mmol) and DIEA (3.8 mL, 27.86 mmol). After stirring at rt for 15 minutes, glycine methyl ester (1.0 g, 7.96 mmol) was then added and the reaction mixture stirred for 4 h, diluted with CH_2Cl_2 (50 mL) washed with water (25 mL), 10% HCl (25 mL), 5% $NaHCO_3$ (25 mL) and sat. NaCl (25 mL) solutions, dried (Na_2SO_4), filtered, and concentrated to give an off white solid which was re-crystallised from CH_3CN (10 mL) to give **16** as a white solid (2.81 g, 86%): mp = 148-150 °C; $[\alpha]^{22}_D$ -7.32 (c 0.50, $CHCl_3$); $[\alpha]^{22}_D$ -7.32 (c 0.50, $CHCl_3$); IR ν_{max} 3287, 1750, 1690, 1649, 1535 cm^{-1}; ^1H NMR 400 MHz 7.75 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.28 (m, 2H), 6.54 (s, 1H), 5.44 (s, 1H), 7.43 -4.38 (m, 2H), 4.21 (t, J = 7.0 Hz, 1H), 4.01-3.96 (m, 3H) 3.72 (s, 3H), 2.16 (m, 1H), 0.96 (t, J = 9.0 Hz, 6H); ^{13}C NMR 100 MHz 171.7(C), 170.1(C), 156.6(C), 143.9(C), 141.4(C), 127.8(CH), 127.2(CH), 125.2(CH), 120.1(CH), 67.2(CH_2), 60.4(CH), 52.5(CH), 47.3(CH_3), 41.2 (CH_2), 31.2(CH), 19.3(CH_3), 17.9(CH); MS *m/z* 432.9 $(M + Na)^+$, 842.8 $(2M + Na)^+$.

{(*R*)-2-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(tritylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid methyl ester (17)



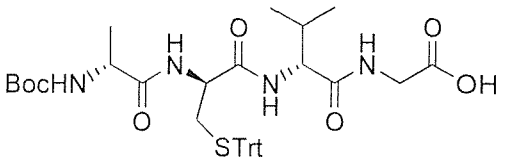
To a stirred solution of **16** (1.0 g, 2.44 mmol) in CH_3CN (48.5 mL) at rt, was added Et_2NH (2.5 mL). After stirring for 3 h at rt the reaction mixture was diluted with hexane (100 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of Fmoc-(*S*Trt)-D-cysteine (1.70g, 2.9 mmol) in CH_3CN (25 mL) was added EDAC•HCl (561.0 mg, 2.93 mmol), HOBt (396 mg, 2.93 mmol) and DIEA (1.31 ml, 7.32 mmol). After stirring at rt for 15 minutes, the crude amine was then added and the reaction mixture stirred for 4 h, solvent removed and the residue dissolved up in CH_2Cl_2 (100 mL), washed with water (25 mL), 10% HCl (25 mL), 5% $NaHCO_3$ (25 mL) and sat. NaCl (25 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed to give a off white solid which was re-crystallised from CH_3CN to give **17** as a white solid (1.46 g, 79%): $[\alpha]^{22}_D$ -2.35 (c 0.50, $CHCl_3$); IR ν_{max} 3267, 1646, 1543 cm^{-1}; ^1H NMR 400 MHz 7.76 (t, J = 7.0 Hz, 2H), 7.54 (d, J = 6.5 Hz, 2H), 7.39-7.21 (m 19H), 6.87 (s, 1H), 6.23 (d, J = 8.0, 2H), 5.04 (d, J = 7.0, 1H), 4.37 (d, J = 7.0, 2H), 4.29 (dd, J = 8.6, J = 5.0, 1H), 4.17 (t, J = 6.5, 1H), 3.96 (m, 1H), 3.72 (dd, , J = 18.1, J = 5.0, 1H), 3.65 (s, 3H), 3.60 (m, 1H), 2.70 (d, J = 6.5, 2H), 2.30 (m, 1H), 1.70 (s, 1H), 0.87 (dd, J = 13.0, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz 170.6(C), 170.5(C), 170.1(C), 156.2(C), 144.3(C), 143.8(C), 143.7(C) 141.4(C), 129.6(CH), 128.4(CH), 128.0(CH), 127.8(CH) 127.2(CH), 125.1(CH), 120.2(CH), 67.6(CH), 67.2(CH_2), 58.4(CH), 54.3(CH), 52.4(CH_3), 47.1(CH), 40.9(CH_2), 33.6(CH_2), 30.0(CH), 19.4 (CH_3), 17.4(CH_3); MS *m/z* 777.8 $(M + Na)^+$. Anal. Calcd for $C_{45}H_{45}N_3O_6S$: 71.50; H, 6.00; N, 5.56. Found C, 71.42; H, 5.99; N, 5.55.

{(*R*)-2-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-trityl sulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid methyl ester (18)



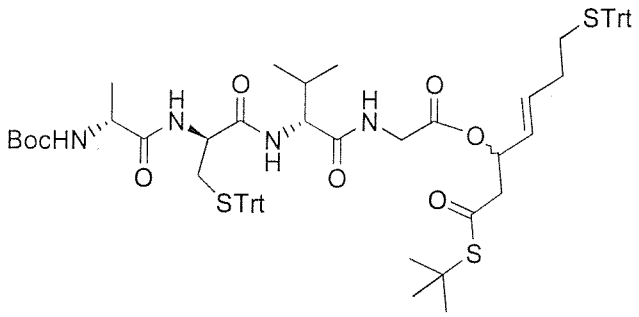
To a stirred solution of **17** (500 mg, 0.66 mmol) in CH_3CN (10 mL) at rt was added Et_2NH (500 μl). After stirring for 3 hours, the reaction mixture was diluted down with n-heptane (100 ml) and solvent removed to give the free amine. The residue was dissolved in CH_3CN (10 mL) to which were sequentially added Boc-D-alanine (187 mg, 0.99 mmol), PyBOP (515 mg, 0.99 mmol) and DIEA (461 μL, 2.64 mmol). After stirring at rt for 3 hours solvent was removed to give a off white solid which was recrystallised by sonication in MeOH (5 mL) to give **18** as a white solid (393.1 mg, 84%): $[\alpha]^{22}_D$ -5.35 (c 0.50, $CHCl_3$); IR ν_{max} 3385, 3277, 1723, 1636, 1510 cm^{-1}; ^1H NMR 400 MHz (DMSO-d_6) 8.48 (s, 1H), 8.06 (d, J = 7.5 Hz, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.46-7.33 (m, 15H), (m 19H), 7.13 (d, J = 7.5, 1H), 4.43 (m, 1H), 4.30 (t, J = 7.0, 1H), 4.05 (t, J = 7.0, 1H), 3.95 (dd, J = 17.6, J = 6.0, 1H), 3.89 (dd, J = 17.6, J = 6.0, 1H), 3.42 (s, 3H), 2.48 (d, J = 6.0, 2H), 2.05 (m, 1H), 1.47 (s,9H), 1.26 (d, J = 7.0, 3H), 0.91 (dd, J = 16.0, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz (DMSO-d_6) 170.4(C), 169.5(C), 168.8(C), 143.7(C), 128.5(CH), 127.5(CH), 126.2(CH) 77.6(C), 65.2(C), 56.7(CH), 51.1(CH_3), 40.8(CH_2), 30.4(CH), 27.6(CH), 18.4 (CH_3), 17.4(CH_3), 17.2(CH_3); MS *m/z* 727.0 $(M + Na)^+$, 1430.9 $(2M + Na)^+$.

{(*R*)-2-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-trityl sulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid (19)



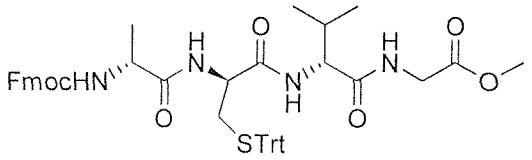
To a stirred solution of **18** (100 mg, 0.142 mmol) in 4:1 THF/H_2O (2.0 mL) at 0 °C was added LiOH (6.8 mg, 0.283). After stirring for 1 hour, the reaction mixture was diluted down with H_2O (20 ml) acidified to pH 4-5 with 2M $KHSO_4$ and extracted with EtOAc (3 x 20 ml). The organic layer was washed with sat. NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated to give an off white solid which was washed with ether (5 mL) to give **19** as a white solid (95 mg, 97%) which was used crude directly in the next step. mp = 252-254 °C: $[\alpha]^{22}_D$ -5.15 (c 0.50, $CHCl_3$); IR ν_{max} 3262, 1711, 1696, 1630, 1545 cm^{-1}; NMR 400 MHz (DMSO-d_6) 12.46 (s, br, 1H), 8.23 (s, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.62 (d, J = 9.0 Hz, 1H), 7.34-7.22 (m, 15H), 7.00 (d, J = 7.0 Hz, 1H), 4.30 (m, 1H), 4.18 (t, J = 6.0 Hz, 1H), 3.92 (m, 1H), 3.75 (dd, J = 17.5 Hz, 6.0 Hz, 1H), 3.67 (dd, J = 17.5 Hz, 6.0 Hz, 1H), 2.36 (d, J = 6.5 Hz, 2H), 1.93 (m, 1H), 1.35 (s, 9H), 1.15 (d, J = 7.0 Hz, 3H), 0.80 (d, J = 7.0 Hz, 3H), 0.77 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz (DMSO-d_6) 173.5, 171.4, 170.2, 155.9, 145.2, 130.0, 129.0, 127.6, 79.0, 66.7, 58.2, 52.4, 50.7, 49.5, 34.3, 31.9, 29.0, 20.0, 18.9, 18.6; MS *m/z* 713.5 $(M + Na)^+$.

{(*R*)-2-[(*S*)-2-((*R*)-2*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid 1-*tert*-butylsulfanylcarbonylmethyl-5-tritylsulfanyl-pent-2-enyl ester (20)



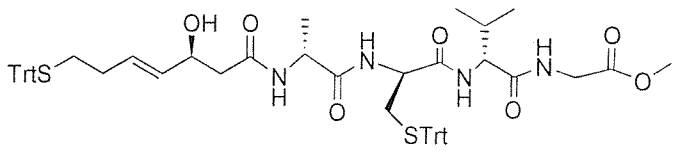
To a stirred solution of **19** (85 mg, 0.123 mmol), **10** (43 mg, 0.088mmol) and DMAP (4 mg, 0.033 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added a solution of DCC (52 mg, 0.242 mmol) in CH_2Cl_2 (0.1 mL). After stirring for 2 h at 0 °C, then 16 h at 6 °C, the reaction mixture was allowed to reach rt. The solid precipitate was filtered off, and the supernatant washed with CH_2Cl_2 (20 mL). The organic layer was washed with water (10 mL), 5% $KHSO_4$ (10 mL), 5% $NaHCO_3$ (10 mL) and sat. NaCl (10 mL) solutions, dried (Na_2SO_4), filtered, and concentrated to give **20** as a colourless oil (82 mg, 80%): $[\alpha]^{22}_D$ -6.35 (c 0.50, $CHCl_3$); IR ν_{max} 3271, 1748, 1676, 1629 cm^{-1}; ^1H NMR 400 MHz 7.35-7.11 (m, 34 H), 6.77 (s 1H), 6.60 (s 1H), 6.49 (s 1H), 5.53-5.43 (m, 2H), 5.23 (dd, J = 15.0, J = 6.0 Hz, 2H), 4.77 (m, 1H), 4.27 (m, 1H), 3.94 (m, 4H), 3.68 (m, 2H), 2.96 (m, 1H), 2.71 (dd, J = 15.0, J = 7.5, 1H), 2.54 (dd, J = 17.6, J = 6.0, 1H), 2.34 (m, 1H), 2.26 (m, 1H), 2.10 (m, 2H), 1.94 (m, 2H), 1.61 (s,3H), 1.32 (s,9H), 1.26 (s, 9H), 1.19 (m, 3H), 0.86 (t, J = 8.0, 3H); ^{13}C NMR 100 MHz 196.0(C), 173.3(C), 171.1(C), 170.0(C), 168.5(C), 145.0(C), 144.4(C), 133.3(CH), 129.7(CH), 129.6(CH) 128.3(CH), 128.0(CH), 127.1(CH), 126.7(CH), 81.0(CH), 71.9(CH), 67.5(C), 66.8(C), 58.9(CH), 52.9(CH), 51.0(CH), 48.8(CH), 41.3(CH_2), 34.1(CH_2), 32.9(CH), 31.6(CH), 31.2(CH), 29.8 (CH_3), 28.3 (CH_3), 25.7 (C), 25.1 (C), 18.1(CH_3), 17.5(CH_3), 17.4(CH_3); MS *m/z* 1185.3 $(M + Na)^+$.

((*R*)-2-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-3-methyl-butyrylamino)-acetic acid methyl ester (21)



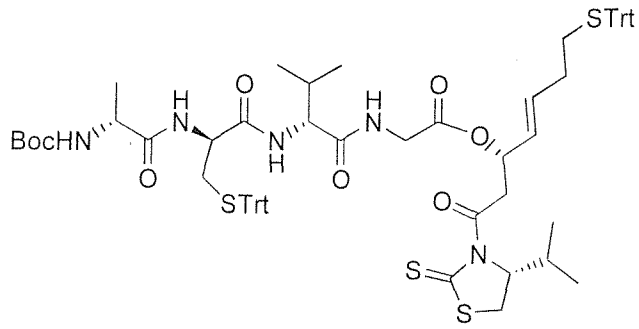
To a stirred solution of **17** (400 mg, 0.53 mmol) in CH_3CN (30 mL) at rt was added Et_2NH (1.5 mL). After stirring for 3 h at rt the reaction mixture was diluted with heptane (60 mL) and concentrated to give the crude amine as a colourless oil. To a stirred solution of Fmoc-D-alanine (198 mg, 0.636 mmol) in CH_3CN (25 mL) was added EDAC•HCl (122.0 mg, 0.634 mmol), HOBt (86 mg, 0.636 mmol) and DIEA (502 μl, 1.91 mmol). After stirring at rt for 15 minutes the crude amine was then added and the reaction mixture stirred for 18 h, solvent removed and the residue dissolved up in CH_2Cl_2 (50 mL), washed with water (15 mL) 10% HCl (15 mL), 5% $NaHCO_3$ (15 mL) and sat. NaCl (15 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed to give a off white solid which was recrystallised from CH_3CN (5 mL) to give **21** as a white solid (670 mg, 81%): mp = 195-197 °C; $[\alpha]^{22}_D$ +5.1 (c 0.50, $CHCl_3$); IR ν_{max} 3267, 3054, 1744, 1706, 1635, 1531.1 cm^{-1}; ^1H NMR 400 MHz 7.75 (d, J = 7.5 Hz, 2H), 7.52 (d, J = 7.0 Hz, 2H), 7.39 (m, 7H), 7.26-7.15 (m, 13H), 6.79 (s, 1H), 6.62 (s, 1H), 5.47 (s, 1H), 4.43-4.28 (m, 4H), 4.13 (m, 1H), 4.01 (m, 1H), 3.88 (s, 2H), 3.63 (s, 3H), 2.81 (m, 1H), 2.52 (m, 1H), 2.26, (m, 1H), 1.30 (d, J = 6.0 Hz, 3H), 0.94 (d, J = 6.0 Hz, 3H), 0.89 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 172.7, 171.1, 170.2, 156.1, 144.4, 143.9, 143.8, 141.4, 129.6, 128.3, 127.9, 127.2, 127.1, 125.1, 120.1, 58.5, 52.7, 52.3, 50.8, 47.2, 41.0, 33.5, 30.4, 19.3, 19.0, 17.7; MS *m/z* 849.2 $(M + Na)^+$, 865.1 $(M + K)^+$.

((*R*)-2-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoyla mino)-propionylamino]-3-tritylsulfanyl-propionylamino}-3-methyl-butyry lamino)-acetic acid methyl ester (23)



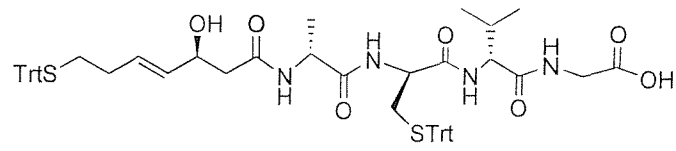
To a stirred solution of **21** (137 mg, 0.166 mmol) in CH_3CN (10 mL) at rt was added Et_2NH (0.5 mL). After stirring for 5 h at rt the reaction mixture was diluted with heptane (30 mL) and solvent removed, CH_2Cl_2 (10 ml) added, filtered and concentrated to give the crude amine as a colourless oil. To a stirred solution of the crude amine in CH_2Cl_2 (4 ml) was added **22** (84.0 mg, 0.149 mmol) and DMAP (2.2 mg, 0.0176 mmol) at 0 °C. After stirring at rt for 8 hours, the solvent was removed and the residue was purified by flash chromatography (eluent 30-40% EtOAc/hexanes) to give **23** as a white glass (127 mg, 85%): mp = 191-193 °C; $[\alpha]^{22}_D$ -18.0 (c 0.50, $CHCl_3$); IR ν_{max} 3272, 3064, 1758, 1692, 1621 cm^{-1}; ^1H NMR 400 MHz (5% $CD_3OD/CDCl_3$) 7.40 (m, 12H), 7.25 (m, 12H), 7.20 (m, 6H), 6.96 and 6.88 (labile NH, d, J = 8.0 Hz, 1H), 5.49 (dt, J = 15.0 Hz, 6.5 Hz, 1H), 5.37 (dd, J = 15.5 Hz, 6.0 Hz, 1H), 4.32 (m, 2H), 4.22 (d, J = 6.0 Hz, 1H), 3.98 (t, J = 7.0 Hz, 1H), 3.92 (d, J = 18.1 Hz, 1H), 3.72 (d, J = 17.6 Hz, 1H), 3.67 (s, 3H), 2.64 (dd, J = 13.0 Hz, 7.5 Hz, 1H), 2.58 (dd, J = 13.0 Hz, 7.5 Hz, 1H), 2.56-2.18 (m, 9H), 2.11 (q, J = 6.5 Hz, 2H), 1.31 (d, J = 7.5 Hz, 3H), 0.91 (t, J = 7.0 Hz, 6H); ^{13}C NMR 100 MHz (5% $CD_3OD/CDCl_3$) 173.0, 172.0, 171.3, 170.5, 170.2, 144.9, 144.2, 132.8, 129.9, 129.7, 129.6, 129.5, 128.2, 128.1, 127.9, 127.1, 126.7, 69.6, 67.1, 66.7, 58.9, 58.8, 52.7, 52.2, 50.0, 49.8, 49.6, 49.5, 49.4, 49.1, 43.7, 40.9, 40.8, 33.1, 31.5, 31.3, 30.0, 19.2, 17.5; MS *m/z* 1050.4 $(M + 2Na)^+$.

{(*R*)-[(*S*)-22-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid 1*S*-[2-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-2-oxo-ethyl]-5-tritylsulfanyl-pent-2-enyl ester (24)



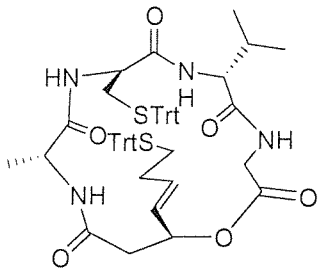
To a stirred solution of **19** (75 mg, 0.109 mmol) and **22** (51.0 mg, 0.088 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added a solution of DCC (28.2 mg, 0.137 mmol) in CH_2Cl_2 (0.1 mL) dropwise and stirred for 1h at 0 °C before addition of DMAP (2.8 mg, 0.023 mmol) then stirred for 16 h at 6 °C. The reaction mixture was allowed to reach rt before the solid precipitate was filtered off, washed with CH_2Cl_2 (20 mL). The filtrate was washed with water (10 mL), 5% $KHSO_4$ (10 mL), 5% $NaHCO_3$ (10 mL) and sat. NaCl (10 mL) solutions, dried (Na_2SO_4), filtered, and concentrated to give **24** as a yellow oil (56 mg, 50%): $[\alpha]^{22}_D$ -5.35 (c 0.50, $CHCl_3$); IR ν_{max} 3278, 2341, 1746, 1698, 1637 cm^{-1}; ^1H NMR 400 MHz 7.43-7.19 (m, 30 H), 6.82 (s, 1H), 6.67 (s, 1H), 6.53 (s 1H), 5.72 (m, 1H), 5.62 (dt, J = 15.0 Hz, 6.5 Hz, 1H), 5.37 (dd, J = 15.0 Hz, 7.0 Hz, 1H), 5.10 (t, J = 7.0 Hz, 1H), 4.79 (s, 1H), 4.30 (m, 1H), 4.04-3.97 (m, 2H), 3.90 (dd, J = 17.5 Hz, 5.5 Hz, 1H), 3.83 (dd, J = 17.5 Hz, 5.5 Hz, 1H), 3.60-3.55 (m, 2H), 3.46 (dd, J = 17.0, J = 4.0 Hz, 1H), 3.06 (m, 1H), 2.96 (d, J = 11.0 Hz, 1H), 2.41-2.29 (m, 3H), 2.17 (t, J = 7.0 Hz, 2H), 2.03 (m, 2H), 1.32 (s, 9H), 1.26 (d, J = 7.0 Hz, 3H), 1.02-0.89 (m, 12H); ^{13}C NMR 100 MHz 203.3(C), 173.3(C), 170.0(C), 169.8(C), 168.5(C), 145.0(C), 144.4(C), 133.0(C), 129.7(CH), 129.6(CH) 128.3(CH), 128.2(CH), 128.0(CH), 127.2(CH), 126.7(CH), 81.1(C), 71.9(CH), 71.4(CH), 67.4(C), 66.8(C), 59.0(CH), 52.9(CH), 51.2(CH), 43.0(CH_2), 41.4(CH_2), 31.6(CH_2), 31.3(CH_2), 30.9(CH_2), 29.8 (C), 28.3 (CH_3), 19.4 (C), 19.2(C), 19.0(CH_3), 18.5(CH_3), 18.0(CH_3), 17.9(CH_3), 17.5(CH_3); MS *m/z* 1256.8 (M + Na)$^+$.

((*R*)-2-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoyla mino)-propionylamino]-3-tritylsulfanyl-propionylamino}-3-methyl-butyry lamino)-acetic acid (25)



To a stirred solution of **23** (220 mg, 0.222 mmol) in 4:1 THF/H_2O (4.0 mL) at 0 °C was added LiOH (11 mg, 0.440 mmol). After stirring for 1 hour, the reaction mixture was diluted with H_2O (30 ml), acidified to pH 4-5 with 2M $KHSO_4$, and extracted with EtOAc (3 x 30 mL). The organic layer was washed with sat. NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated to give a white solid which was triturated with ether, to give **25** as a white solid (199.5 mg 91%) which was used crude directly in the next step. mp = 191-193 °C; $[\alpha]^{22}_D$ -18.5 (c 0.50, $CHCl_3$); IR ν_{max} 3413, 1711, 1678, 1630, 1451 cm^{-1}; ^1H NMR 400 MHz (5%CD_3OD/$CDCl_3$) 7.30 (m, 12H), 7.16 (m, 12H), 7.13 (m, 6H), 5.43 (dt, J = 15.5 Hz, 6.0 Hz, 1H), 5.30 (dd, J = 15.5 Hz, 6.0 Hz, 1H), 4.27 (m, 2H), 4.21 (m, 1H), 3.99 (m, 1H), 3.75 (s, 2H), 3.77 (m, br, 6H), 2.55 (dd, J = 12.5 Hz, 6.5 Hz, 1H), 2.44 (dd, J = 12.5 Hz, 7.5 Hz, 1H), 2.21 (m, 2H), 2.12 (m, 3H), 2.02 (m, 2H), 1.23 (d, J = 7.0 Hz, 3H), 0.83 (d, J = 7.0 Hz, 3H), 0.80 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz (5%CD_3OD/$CDCl_3$) 173.0, 172.1, 171.6, 171.5, 170.5, 144.9, 144.3, 132.7, 129.8, 129.6, 129.5, 128.2, 127.9, 127.0, 126.7, 69.5, 67.1, 66.7, 58.7, 52.7, 43.5, 40.9, 33.2, 31.5, 31.3, 30.3, 19.2, 19.1, 17.6; MS *m/z* 1013.2 $(M + Na)^+$.

(6*R*,9*S*,12*R*,16*S*)-6-Isopropyl-12-methyl-16-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-4,7,10,13-tetraaza-cyclohexadecane-2,5,8,11,14-pentaone (26)



Macrolactonisation

Method A DCC/DMAP

To a stirred solution of the hydroxyl acid **25** (100 mg, 0.102 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added a solution of DCC (28.2 mg, 0.137 mmol) in CH_2Cl_2 (0.5 mL) dropwise. After stirring for 30 minutes at 0 °C a solution of DMAP (2.5 mg, 0.0213 mmol) in CH_2Cl_2 (51 mL) was added, then stirred for 16 h at room temperature. The solid precipitate was filtered off, and the filtrate washed with CH_2Cl_2 (5 mL). The organic layer was washed with water (10 mL), 5% $KHSO_4$ (10 mL), 5% $NaHCO_3$ (10 mL) and sat. NaCl (10 mL) solutions, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent 30-50% EtOAc/hexanes) to give **26** as a white glass (37 mg, 38%).

Method B Yamaguchi

To a stirred solution of the hydroxy acid **25** (90 mg, 0.091 mmol) in THF (1.0 mL) at 0 °C was added Et_3N (30 µl, 0.22 mmol) and 2,4,6-trichlorobenzoyl chloride (35 µl, 0.22 mmol). After stirring for 30 minutes the reaction mixture was diluted with toluene (10 mL) and then added dropwise to a vigorously stirring solution of DMAP (11.1 mg, 0.091 mmol) in toluene (45.5 mL) at room temperature and stirred for 2 hours. The reaction mixture was then washed with 5% $NaHCO_3$ (10 mL), 15% citric acid (10 mL), and sat. NaCl (10 mL) solutions, each back extracted with EtOAc (20 mL). The combined organic

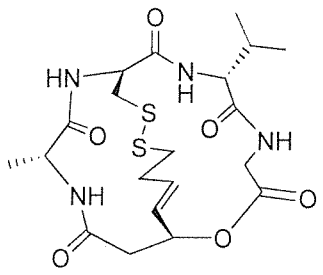
phase was dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 50-70% EtOAc/hexanes) to give **26** as a white glass (9.7 mg, 11 %):

Macrolactamisation

To a stirred solution of triphenylmethyl alcohol (100mg, 0.384 mmol) in 10% TFA/CH_2Cl_2 (5 mL) was added **25** (50 mg, 0.041 mmol). After stirring for 5 minutes, the solvent was removed and the triphenylmethyl alcohol triturated with ether (4 mL), filtered and solvent removed to give the crude amine as a colourless oil.

The amine was dissolved in CH_2Cl_2 (50 mL), to which was added DIEA (14 μL, 0.082 mmol) and DMAP (1.0 mg, 0.0082 mmol) and stirred at room temperature for 2 hours. The solvent was removed and the residue was purified by flash chromatography (eluent 1-6% MeOH/CH_2Cl_2) to give **26** as a white glass (4.8 mg, 12%): $[\alpha]^{22}_D$ -7.0 (c 0.50, $CHCl_3$); IR ν_{max} 1735, 1659, 1526 cm^{-1}; ^1H NMR 400 MHz 7.42-7.38 (m, 12H), 7.29-7.20 (m 18H), 7.08 (m, 1H), 6.85 (m, 1H), 5.60 (dt, J = 14.1 Hz, 7.0 Hz, 1H), 5.35 (m, 2H), 4.54 (d, J = 17 Hz, 1H), 4.41 (d, J = 4.5 Hz, 1H), 3.90 (m, 1H), 3.67 (m, 1H), 3.43 (d, J = 17.1 Hz, 1H), 3.08 (m, 1H), 2.60-2.51 (m, 3H), 2.35 (m, 1), 2.20 (t, J = 7.5 Hz, 2H), 2.06 (m, 2H), 1.39 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H), 0.89 (d, J = 7.0 Hz, 3H); ^{13}C NMR 100 MHz 74.0, 171.1 170.8, 170.0, 169.0, 144.9, 144.3, 133.3, 129.7, 129.5, 128.3, 128.0, 127.1, 126.8, 72.4, 7.3, 66.8, 58.2, 56.2, 50.2, 49.9, 49.7, 41.6, 32.4, 31.3, 31.2, 29.8, 28.7, 19.7, 17.2, 16.3; MS *m/z* 995.6 $(M + Na)^+$.

(*E*)-(1*S*,7*R*,10*S*,21*R*)-7-Isopropyl-21-methyl-2-oxa-12,13-dithia-5,8,20,23-tetraaza-bicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentaone (27)



To a vigorously stirring solution of I_2 (37.1 mg, 0.146 mmol) in MeOH (50 mL) was added the protected dithiol **26** (35 mg, 0.0365 mmol) in MeOH (20 mL) dropwise over 5 minutes. After stirring for a further 10 minutes, 0.2M ascorbate in 0.2M citric acid buffer (4 mL) was added and the organic phase concentrated, 1:1 EtOAc:NaCl (20 mL) was added, extracted with EtOAc (5 x 25 mL), the combined organic extract was dried (Na_2SO_4), filtered, and solvent removed. The residue was purified by flash chromatography (eluent 1-6% MeOH/$CHCl_3$) to give **27** as a white solid (11.8 mg, 67%): $[\alpha]^{22}_D$ -98.0 (c 0.50, $CHCl_3$); IR ν_{max} 3300, 2477, 1734, 1659, 1526, 1446 cm^{-1}; ^1H NMR 400 MHz 7.41 (d, J = 6.0 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H), 6.37 (s, 1H), 5.94 (dt, J = 16.5, 7.5 Hz, 1H), 5.78-5.71 (m, 1H), 4.86 (m, 1H), 4.26 (d, J = 7.5 Hz, 1H), 4.17 (d, J = 14.0 Hz, 1H), 4.08 (d, J = 14.0 Hz, 1H), 3.44 (dd, J = 14.5 Hz, 10.0 Hz, 1H), 3.22 (d, J = 10.0 Hz, 1H), 2.88-2.56 (m, 8H), 1.49 (d, J = 7.5 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 173.2, 171.9, 170.9, 170.1, 168.3, 129.8, 70.2, 64.4, 55.9, 51.7, 41.7, 38.2, 37.9, 35.7, 31.0, 26.9, 20.2, 19.7, 15.4; HRMS *m/z* 509.1495 $(M + Na)^+$ expected 509.1499.

4.11 References

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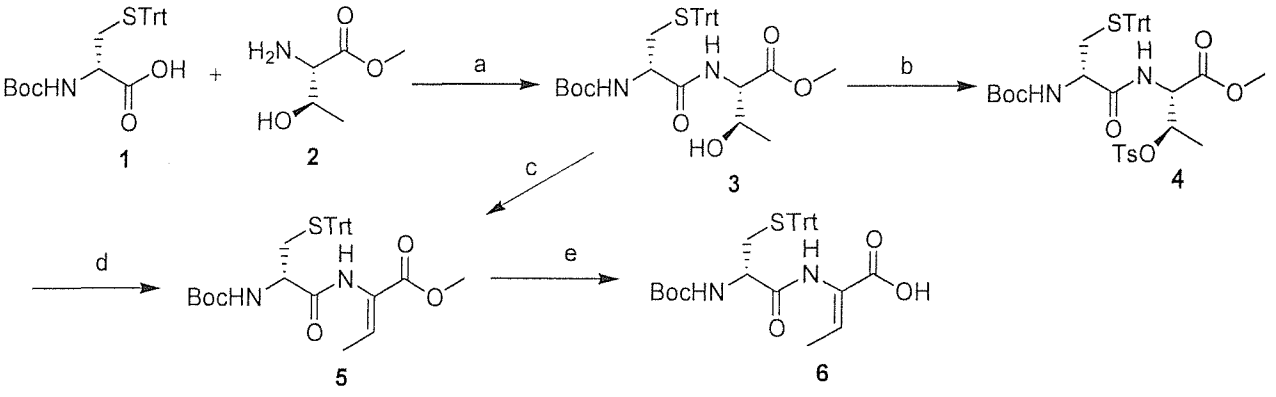
Chapter 5: Towards the Synthesis of FK228 on Solid Phase

The synthesis of this small family of bicyclic depsipeptide was attempted on solid phase utilizing Kenner's acylsulfonamide "Safety-Catch" solid-phase supported linker, whereby an initial sulfonamide is formed between the solid phase linker acylsulfonamide and a carboxylic functionality from the depsipeptide. The linear depsipeptide is then built onto the solid support. Cyclization is then achieved by activation to nucleophilic cleavage. Alkylation of the sulfonamide with concomitant displacement by the free amine of the linear peptide furnishes the desired cyclic product.[1,2] The synthetic target in this case was the bicylic depsipeptide FK228. The unique property of the "safety-catch" resin that makes it appealing to our synthesis of FK228 is its stability to both strongly acidic and basic conditions.[3] An acid labile protecting group strategy is required due to the high probability of elimination of the β-hydroxy ester present throughout this group of cyclic depsipeptides.

5.1 The synthesis of the dehydroamino acid subunit

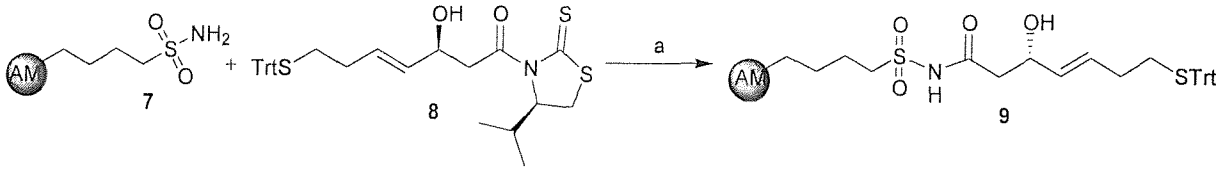


Scheme 1. Reagents and conditions: **(a) 1**, EDAC•HCl, HOBt, DIEA, CH_2Cl_2, 15 min then **2**, rt 4 h (83%). **(b)** TsCl, pyridine, -10 °C then 0 °C 8 h (66%). **(c)** TsCl, Et_3N, DMAP, CH_2Cl_2, rt 18 h (87%). **(d)** DABCO, Et_2NH, CH_2Cl_2, rt 8 h (67%). **(e)** LiOH, THF/H_2O, 0 °C to rt 18 h (96%).

The dipeptide portion containing cysteine and the threonine derived *Z*-dehydrobutyrine was assembled by EDAC.HCl/HOBt coupling conditions of L-threonine methyl ester **2**

with Boc-(*S*Trt)-D-cysteine **1**. Elimination of the hydroxyl was first attempted by a two step process, conversion first to the tosylate **4** and then elimation of the activated alcohol with DABCO/Et_2NH to give **5** by the method used in the synthesis of FK228 by Simon *et al.*[4] It proved however more efficacious to undergo the activation/elimination in one step. The L-threonine residue was converted into *Z*-dehydrobutyrine in one pot with two equivalents each of TsCl, Et_3N, DMAP in good yield 87%. The methyl ester was saponified using LiOH in THF/H_2O to give **6** (Scheme 1).

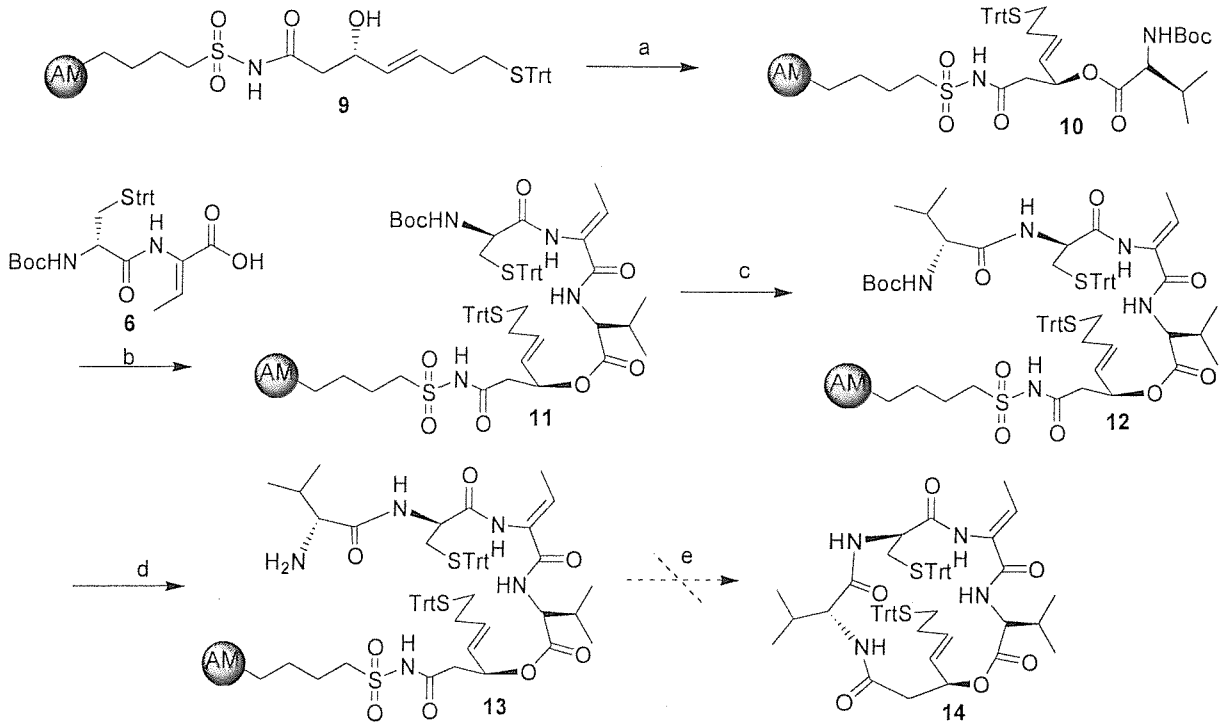
5.2 Coupling of the thiazolidinethione to the solid support



Scheme 2. Reagents and conditions: **(a)** DMAP, CH_2Cl_2, rt 4 days.

The sulfonamide resin was coupled to the aldol product thiazolidinethione **8**, which is activated towards nucleophilic substitution. Addition of catalytic DMAP to a solution of the resin **7** with **8** gave the resin bound product **9**. Completion of the reaction being shown by decolouration of the previously yellow solution, the displaced thiazolidinethione moiety appearing by TLC and the starting material used up (Scheme 2).

5.3 Attempted synthesis of FK228 on the solid support; coupling of the dehydroamino acid and cyclisation



Scheme 3. Reagents and conditions: **(a)** DIC, HOBt, DMAP, CH_2Cl_2, rt 3 days. **(b) (i)** TFA/TrtOH, rt 30 min. **(ii)** DIC, HOBt, $^iPr_2EtN$, CH_2Cl_2, rt 3 days. **(c) (i)** TFA/TrtOH, rt 30 min. **(ii)** DIC, HOBt, $^iPr_2EtN$, CH_2Cl_2, rt 3 days. **(d)** TFA/TrtOH, rt 30 min. **(e)** ICH_2CN, NMP, $^iPr_2EtN$, rt 18 h (dark).

On the solid support was sequentially added Boc-L-valine methyl ester followed by the depsipeptide consisting of Z-dehydrobutyrine and Boc-(*S*Trt)-D-cysteine, followed by Boc-D-valine. The DIC/HOBt coupling protocol was used for each coupling followed by Boc removal using TFA/TrtOH. The presence of the trityl alcohol was necessary to prevent loss of the trityl protecting group from the thiols. To test for the loss of the free amine on the resin after each coupling the ninhydrin test was used. After each coupling the resin was sequentially washed with 3 x THF, then hexanes, CH_2Cl_2 then methanol, THF then hexanes and CH_2Cl_2 then ether. Cyclization was attempted using iodoacetonitrile and Hunig's base in NMP. The reaction failed to release the desired cyclic product (Scheme 3).

5.4 Summary

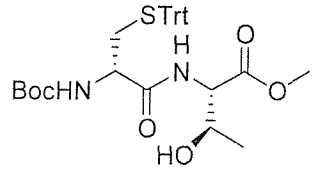
Preliminary results in the use of a "Safety-Catch" solid-phase supported linker in the synthesis of FK228 was not successful. The β-hydroxy of the unsaturated 3-hydroxy-7-mercapto-4-heptenoic acid subunit is prone to elimination. This is one possibility for the failure of this method. Time was limited at this stage of our work and it was not possible to study further the lack of success in finding the desired product from the solid support.

5.5 Experimental

General Methods

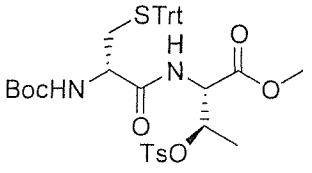
See section 2.23

(2*S*,3*R*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino)-3-hydroxy-butyric acid methyl ester (3)



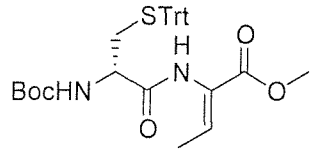
To a stirred solution of Boc-D-cysteine (3.83 g, 8.25 mmol) in CH_2Cl_2 (100 mL), was added EDAC•HCl (1.73 g, 9.08 mmol), HOBt (1.11 g, 8.25 mmol) and DIEA (7.1 ml, 27.2 mmol). After stirring at rt for 15 minutes, L-serine methyl ester (1.10 g, 8.25 mmol) was then added and the reaction mixture stirred for 4 h, diluted with CH_2Cl_2 (200 mL) washed with water, 10% HCl (80 mL), 5% $NaHCO_3$ (80 mL) and sat. NaCl (80 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed to give a off white solid which was recrystallised from CH_3CN (40 mL) to give **3** as a white solid (3.96 g, 83%): $[\alpha]^{22}_D$ -5.5 (c 0.50, $CHCl_3$); mp = 162-164 °C; IR ν_{max} 3474, 3372, 3317, 1735, 1686, 1650, 1502 cm^{-1}; ^1H NMR 400 MHz 7.42 (m, 6H), 7.28 (m, 6H), 7.22 (m, 3H), 6.73 (m, 1H), 4.84 (d, J = 6.5 Hz, 1H), 4.50 (dd, J = 8.5, 2.5 Hz, 1H), 4.26 (m, 1H), 3.92 (m, 1H), 3.71 (m, 3H), 2.67 (m, 1H), 2.61 (dd, J = 12.5, 5.0 Hz, 1H), 2.20 (m, 1H), 1.42 (s, 9H), 1.16 (d, J = 6.0 Hz, 3H); ^{13}C NMR 100 MHz 171.1, 170.9, 144.5, 129.7, 128.2, 127.1, 80.6, 68.3, 67.4, 57.3, 52.6, 33.8, 28.4, 20.1; MS *m/z* 601.5 $(M + Na)^+$.

(2*S*,3*R*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino)-3-(toluene-4-sulfonyloxy)-butyric acid methyl ester (4)



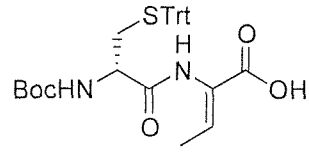
To a stirred solution of **3** (1.0 g, 1.90 mmol) in pyridine (10 mL) at -10 °C was added TsCl (362 mg, 1.90 mmol), in pyridine (4 mL). After stirring at 0 °C for 8 hours, the precipitate was filtered off and solvent removed. The residue was dissolved up in EtOAc (80 mL), washed with 10% $CuSO_4$ (40 mL), 5% $NaHCO_3$ (40 mL), 15% citric acid (40 mL) and sat. NaCl (40 mL) solutions, dried (Na_2SO_4), filtered, and solvent removed and the residue was purified by flash chromatography (eluent 30-40 % ether/hexanes) to give **4** as a white glass (836 mg, 66%): mp = 171-173 °C; $[\alpha]^{22}_D$ -3.5 (c 0.50, $CHCl_3$); IR ν_{max} 3376, 3320, 1751, 1681, 1597 cm^{-1}; ^1H NMR 400 MHz 7.71 (d, J = 8.0 Hz, 2H), 7.35-7.20 (m, 11H), 6.62 (m, 1H), 5.12 (m, 1H), 4.76 (m, 1H), 4.66 (dd, J = 9.5, 2.0 Hz, 1H), 3.83 (m, 1H), 3.57 (s, 3H), 2.65 (m, 1H), 2.59 (dd, J = 13.0, 5.5 Hz, 1H), 2.44 (s, 3H), 1.40 (s, 9H), 1.22 (d, J = 6.5 Hz, 3H); ^{13}C NMR 100 MHz 171.1, 168.8, 145.1, 144.4, 133.9, 129.9, 129.7, 129.5, 128.5, 128.3, 128.0, 127.1, 80.6, 78.6, 67.5, 55.9, 52.8, 33.6, 28.4, 21.8, 18.0, 14.3; MS *m/z* 755.4 $(M + Na)^+$.

(*Z*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino) -but-2-enoic acid methyl ester (5)



To a stirred solution of **3** (0.5 g, 0.86 mmol) in CH_2Cl_2 (10 mL) at -10 °C was added TsCl (362 mg, 1.90 mmol), DMAP (32 mg, 0.190 mmol), and Et_3N (364 μL, 2.59 mmol) at 0 °C then rt overnight. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with 5% $NaHCO_3$ (20 mL), 15% citric acid (20 mL) and sat. NaCl (20 mL) solutions, dried ($MgSO_4$), filtered, and solvent removed and the residue was purified by flash chromatography (eluent 20-30 % EtOAc/hexanes) to give **5** as a white solid (421 mg, 87%); mp = 163-165 °C; $[\alpha]^{22}_D$ +7.2 (c 0.50, $CHCl_3$); IR ν_{max} 3313, 3014, 1719, 1681, 1491 cm^{-1}; ^1H NMR 400 MHz 7.44 (m, 7H), 7.28 (m, 6H), 7.22 (m, 3H), 6.77 (q, J = 7.0 Hz, 1H), 4.82 (m, 1H), 3.92 (m, 1H), 3.72 (s, 3H), 2.74 (m, 1H), 2.59 (dd, J = 13.0, 5.5 Hz, 1H), 1.71 (d, J = 7.5 Hz, 3H), 1.43 (s, 9H); ^{13}C NMR 100 MHz 168.2, 164.6, 144.5, 134.5, 129.7, 128.2, 127.0, 125.8, 67.4, 53.9, 52.3, 33.6, 28.4, 14.7; MS *m/z* 583.3 $(M + Na)^+$.

(*Z*)-2-((S)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino) -but-2-enoic acid (6)



To a stirred solution of **5** (400 mg, 0.714 mmol) in 4:1 THF/H_2O (10 mL) at 0 °C was added LiOH (34.3 mg, 1.43 mmol). After stirring for 18 hours, the reaction mixture was diluted with H_2O (30 ml), acidified to pH 4-5 with 2M $KHSO_4$, and extracted with EtOAc (3 x 30 mL). The organic layer was washed with sat. NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated to give a white solid which was triturated with ether (20 mL) to give **6** as a white solid (375 mg, 96%); mp = 101-103 °C; $[\alpha]^{22}_D$ - 1.5 (c 0.50, $CHCl_3$); IR ν_{max} 3290, 3013, 1689, 1492 cm^{-1}; ^1H NMR 400 MHz 7.44 (m, 1H), 7.35 (m, 6H), 7.20 (m, 6H), 7.13 (m, 3H), 6.80 (q, J = 7.5 Hz, 1H), 4.86 (m, 1H), 3.91 (m, 1H), 2.63 (m, 1H), 2.53 (m, 1H), 1.67 (d, J = 7.0 Hz, 3H), 1.34 (s, 9H); ^{13}C NMR 100 MHz δ; 169.0, 168.0, 155.8, 144.5, 136.5, 129.7, 128.2, 127.0, 125.4, 80.9, 67.4, 53.9, 52.4, 33.7, 28.4, 15.1; MS *m/z* 545 $(M - H)^-$.

5.6 References

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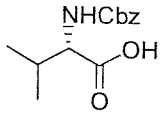
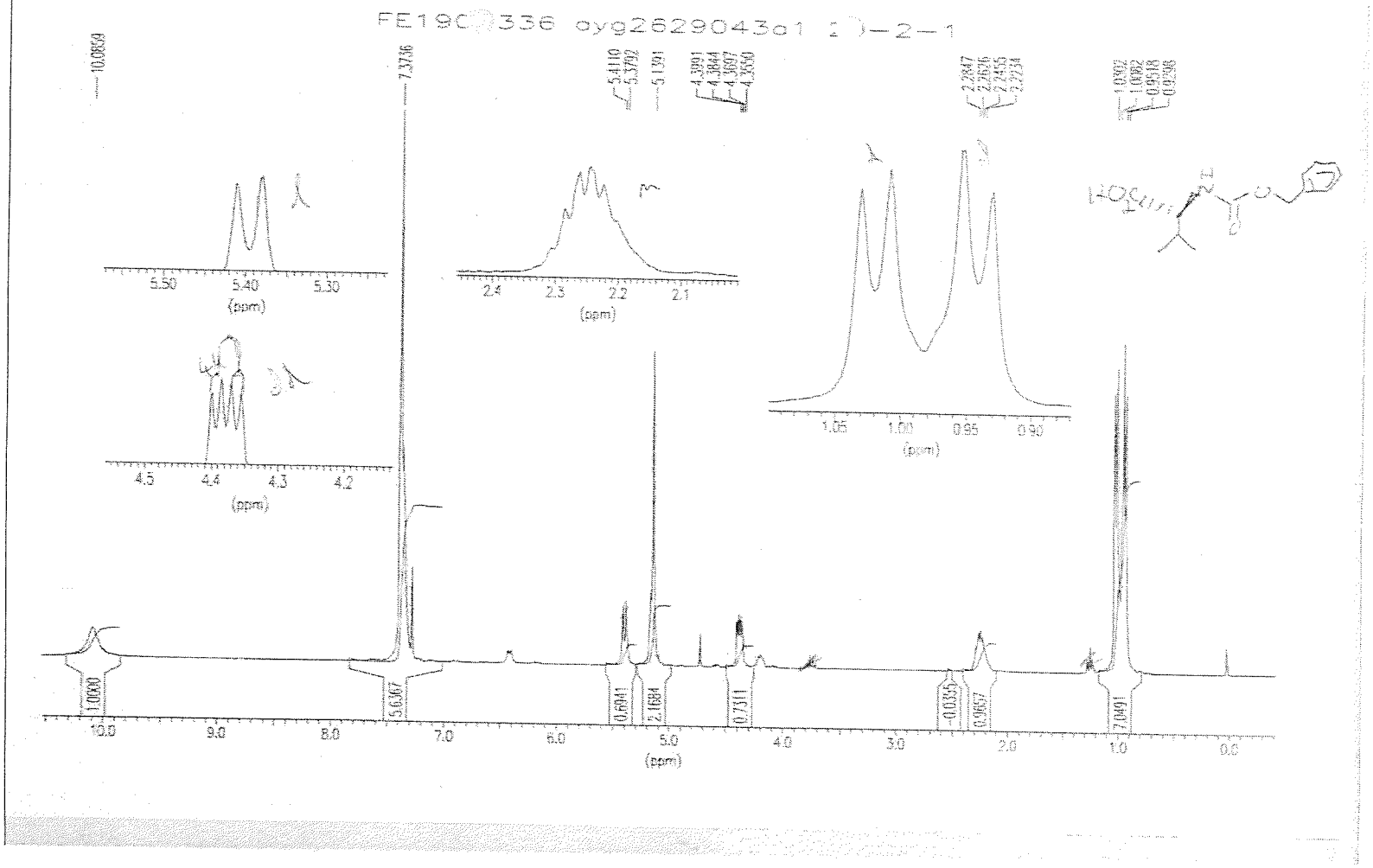
Appendix

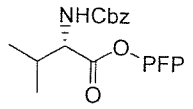
^{1}H NMR Spectra for compounds in Chapter 2

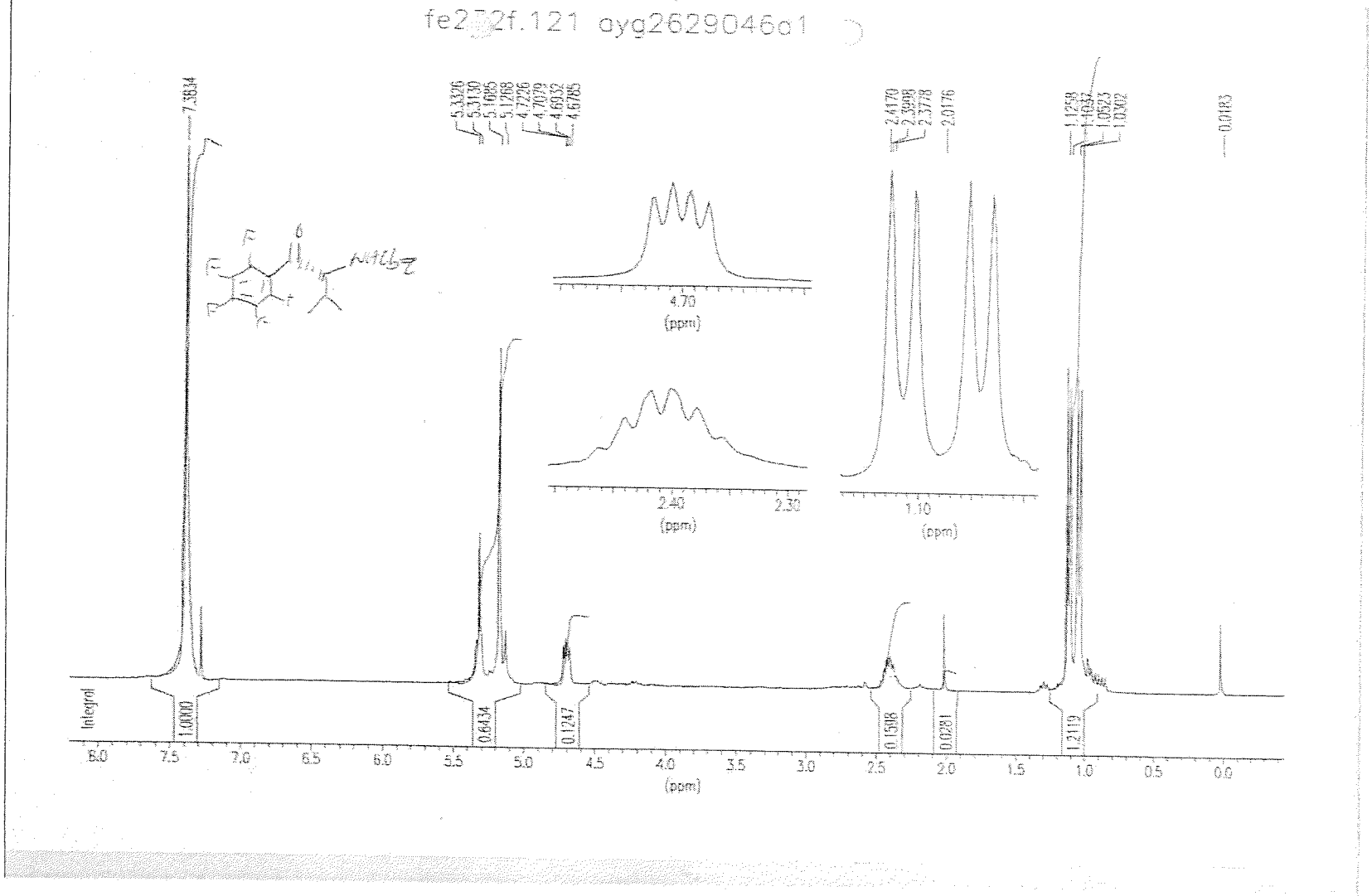
(*S*)-2-Benzyloxycarbonylamino-3-methyl-butyric acid (2)



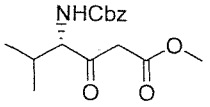


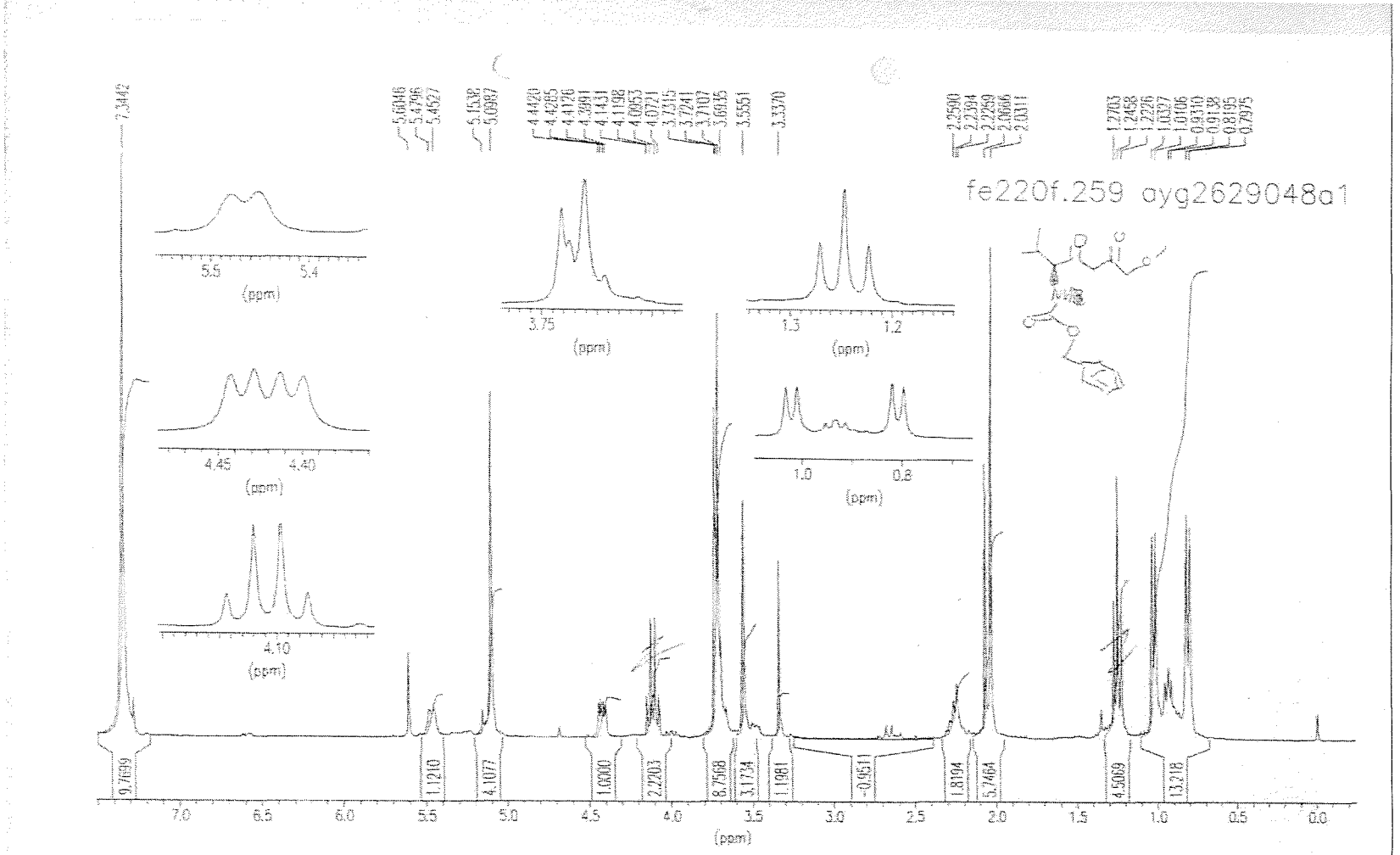
(*S*)-2-Benzyloxycarbonylamino-3-methyl-butyric acid pentafluorophenyl ester (3)



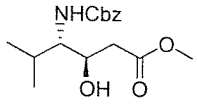


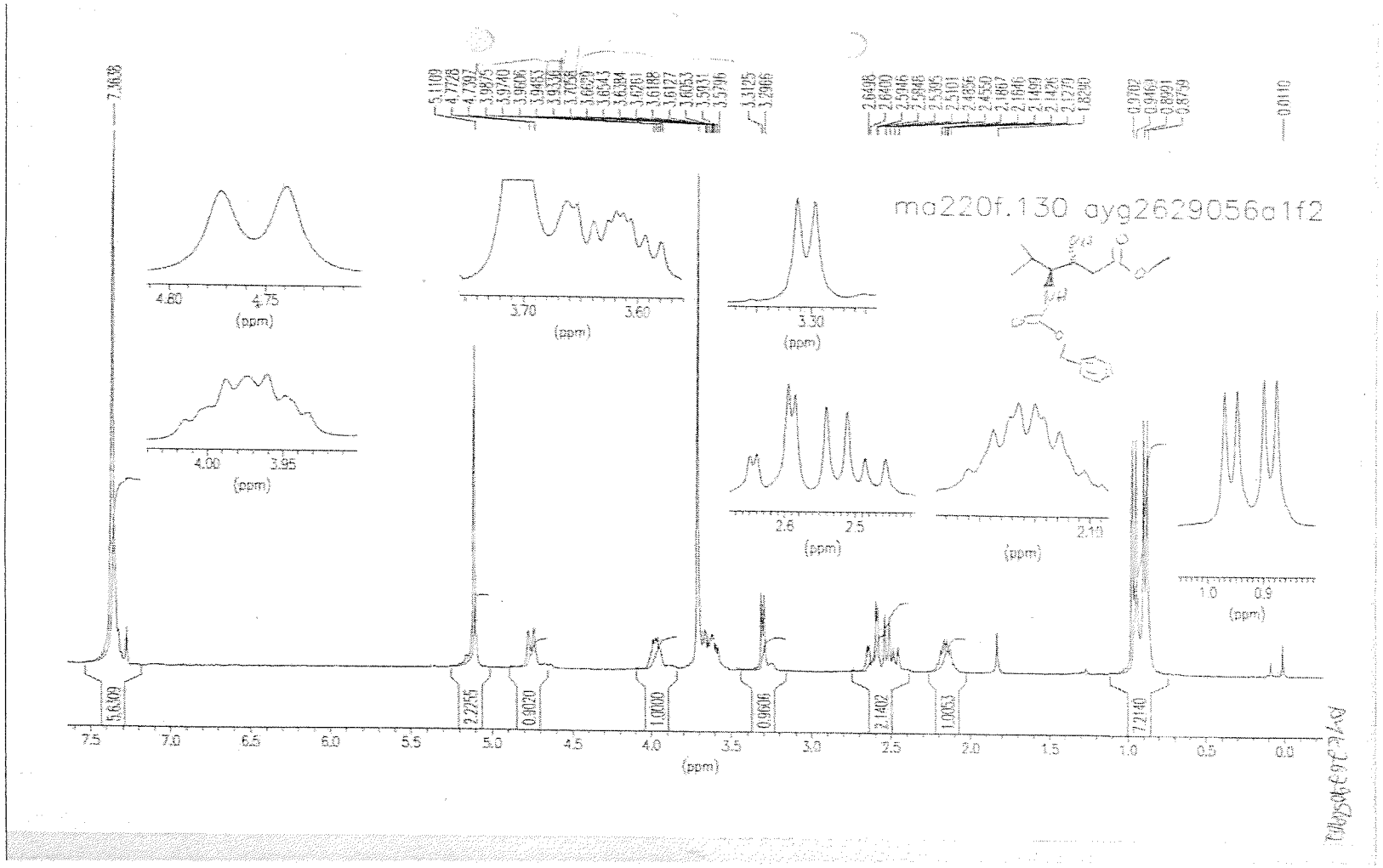
(*S*)-4-Benzyloxycarbonylamino-5-methyl-3-oxo-hexanoic acid methyl ester (4)



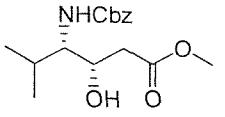


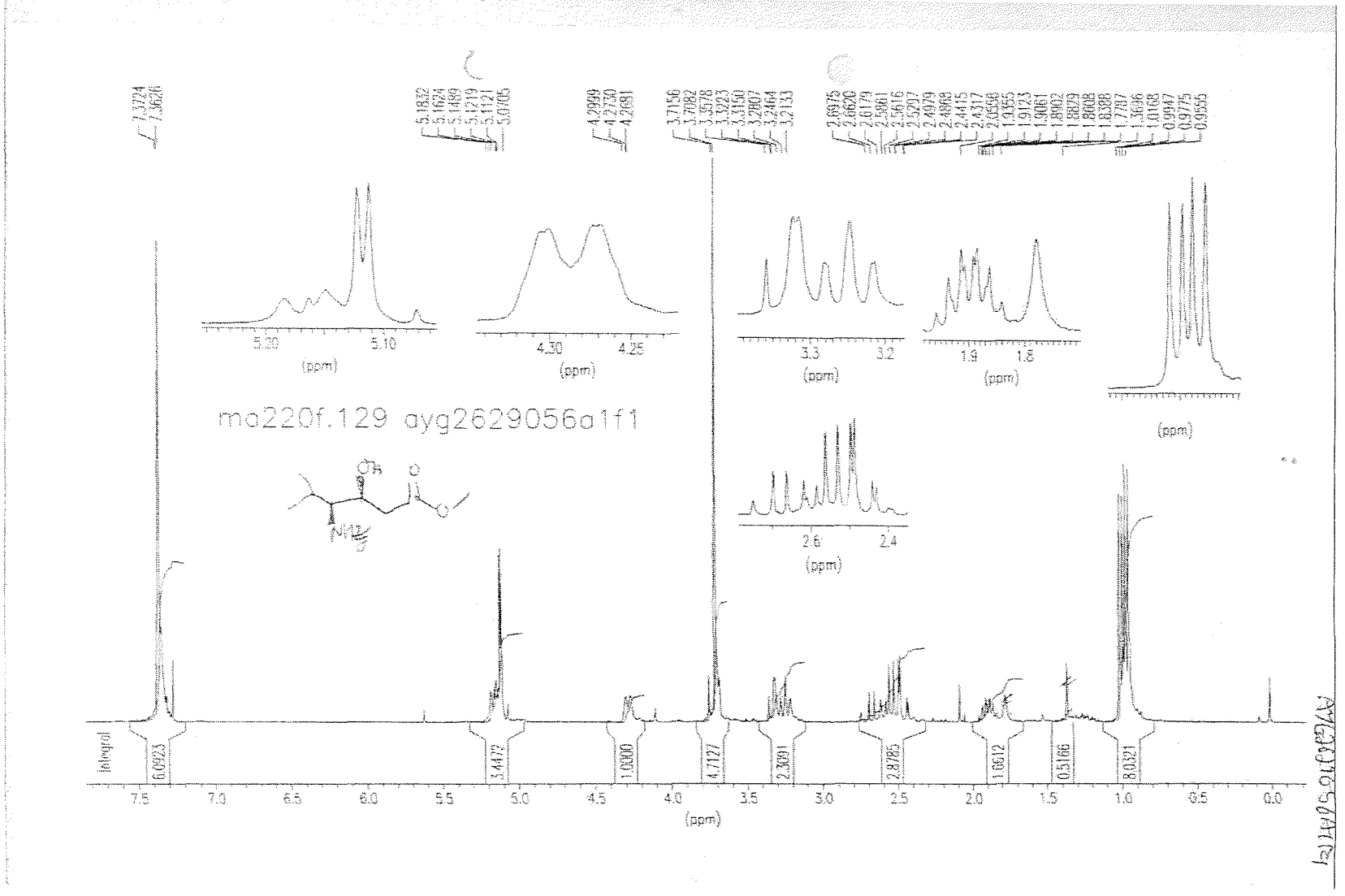
(3*R*,4*S*)-4-Benzyloxycarbonylamino-(3)-hydroxy-hexanoic acid methyl ester (5)



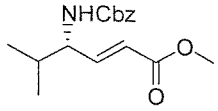


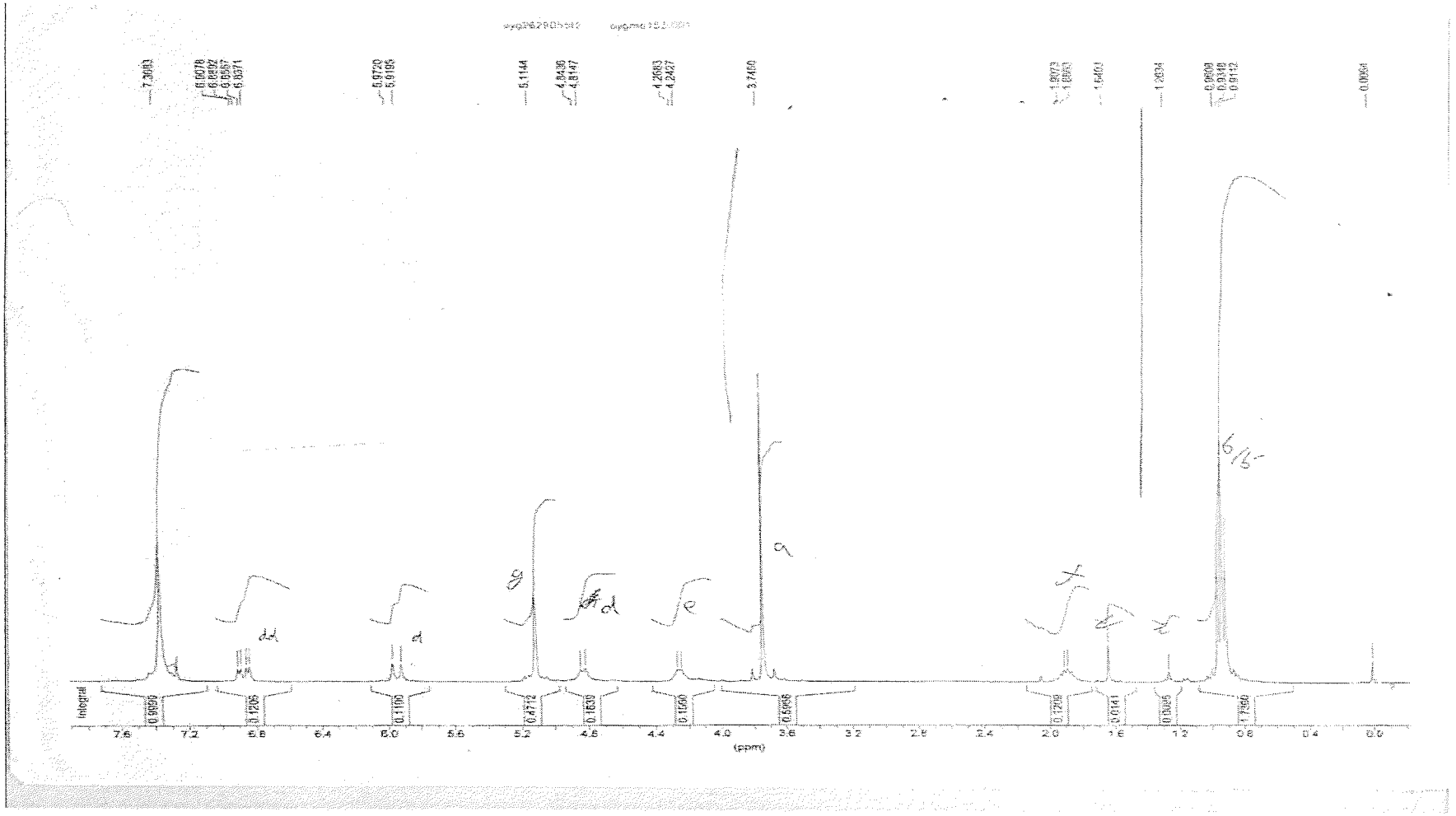
(3*S*,4*S*)-4-Benzyloxycarbonylamino-(3)-hydroxy-hexanoic acid methyl ester (6)

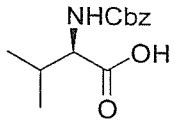




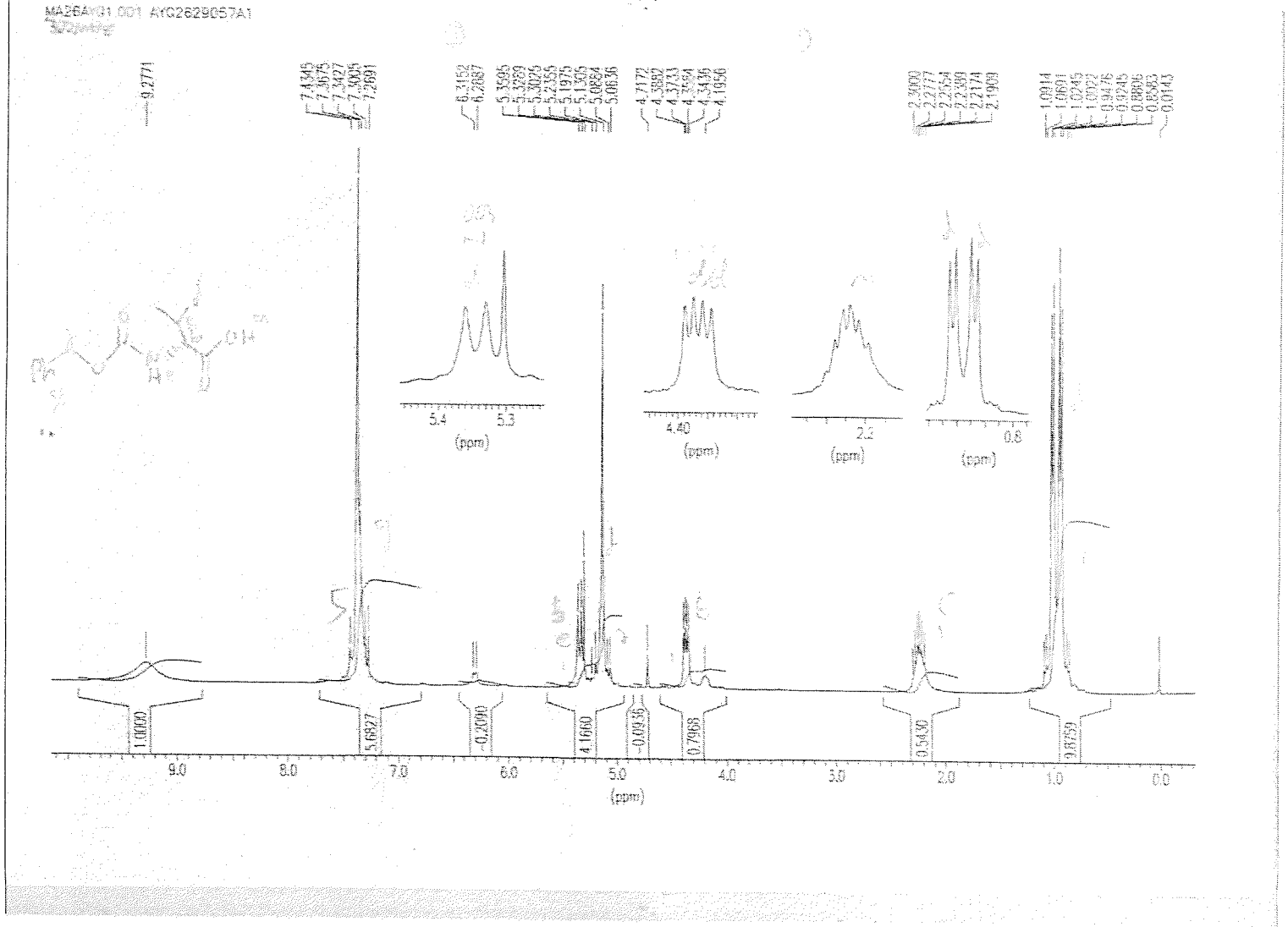
(*E*)-(*S*)-4-Benzyloxycarbonylamino-5-methyl-hex-2-enoic acid methyl ester (7)



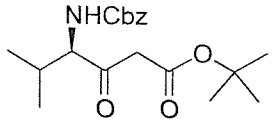


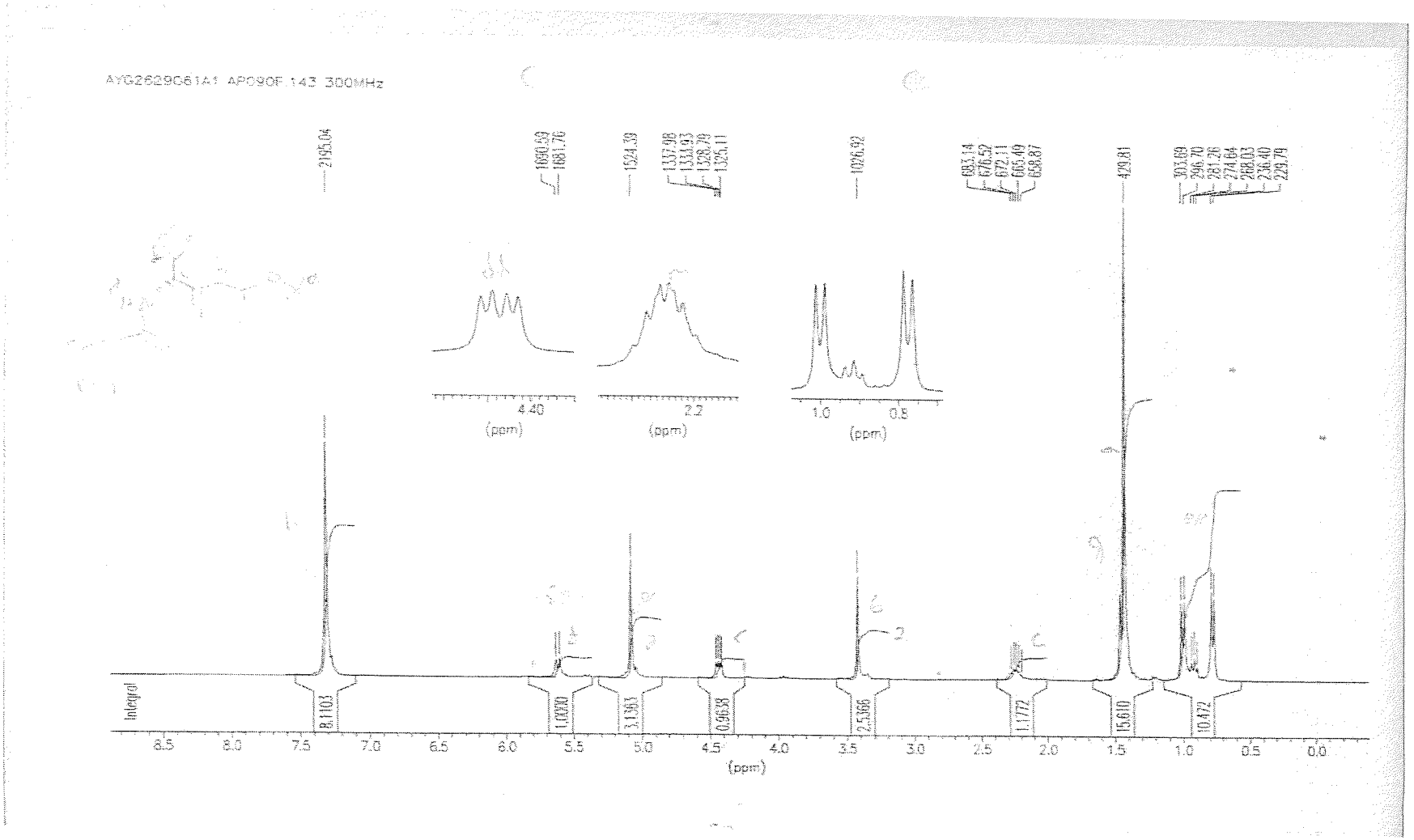


(*R*)-2-Benzyloxycarbonylamino-3-methyl-butyric acid (9)

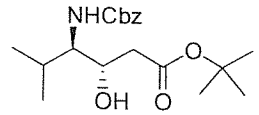


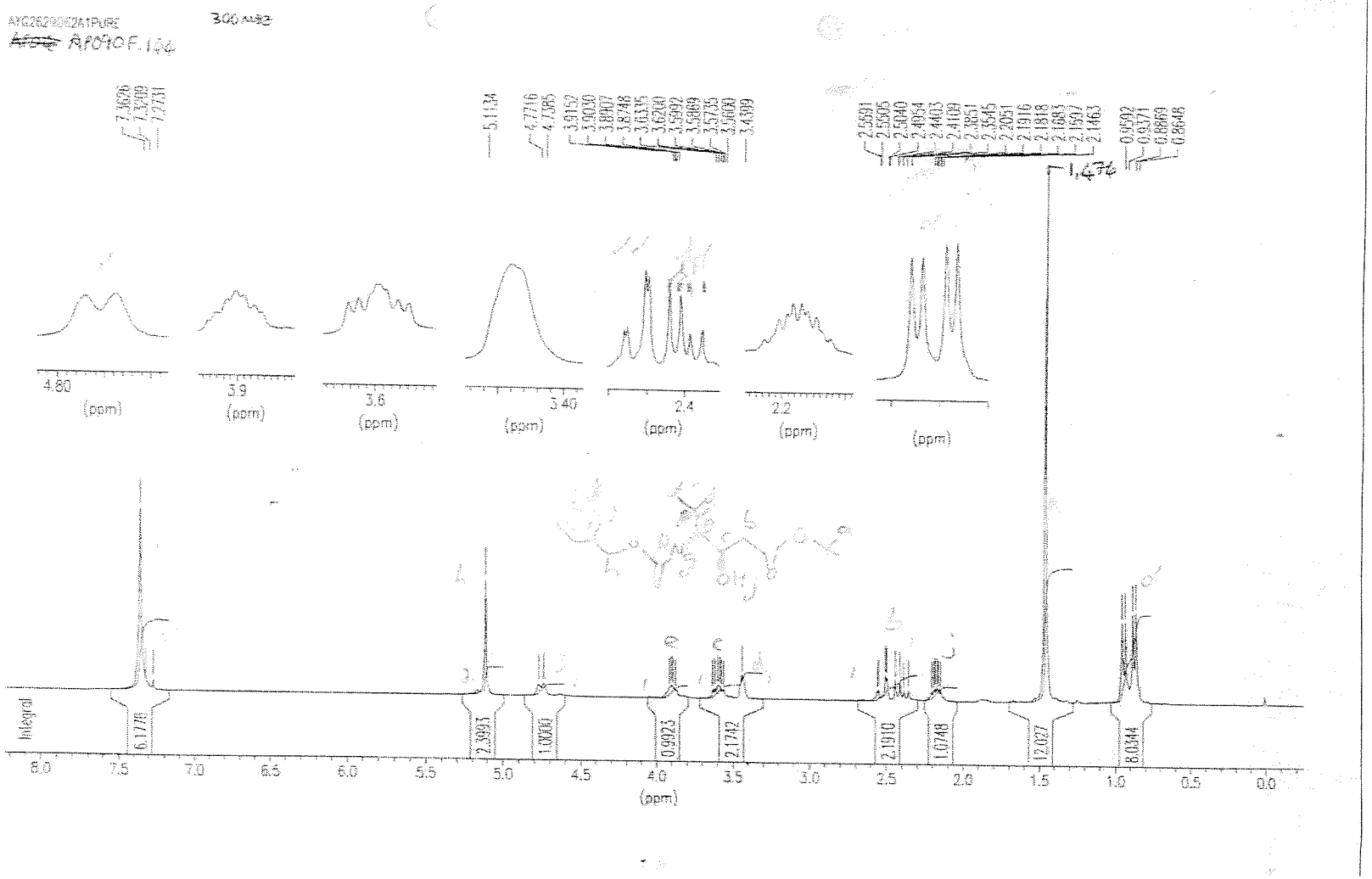
(*R*)-4-Benzyloxycarbonylamino-5-methyl-3-oxo-hexanoic acid *tert*-butyl ester (10)



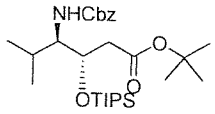


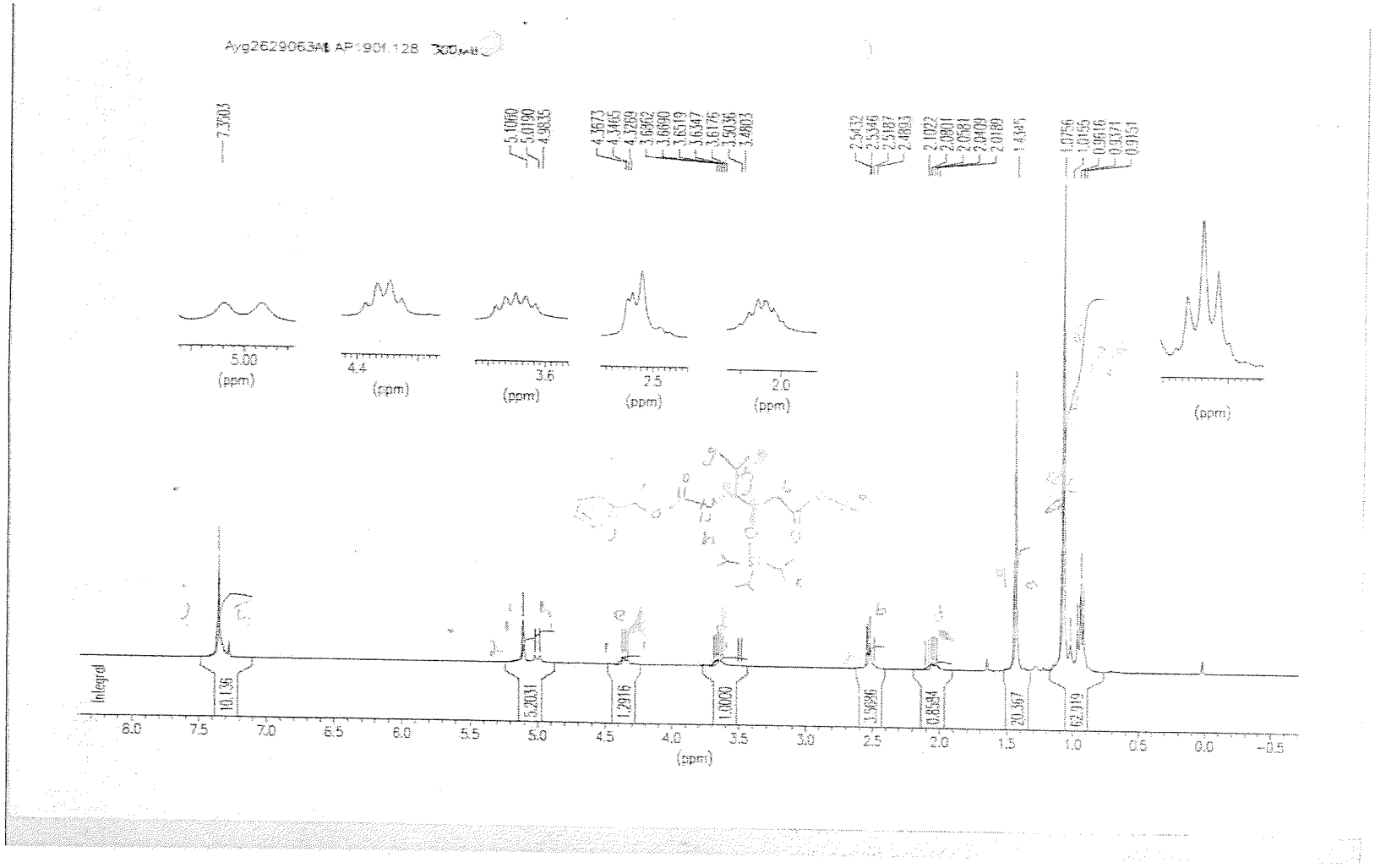
(3*S*,4*R*)-4-Benzyloxycarbonylamino-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (11)



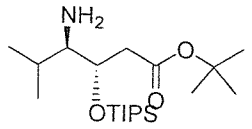


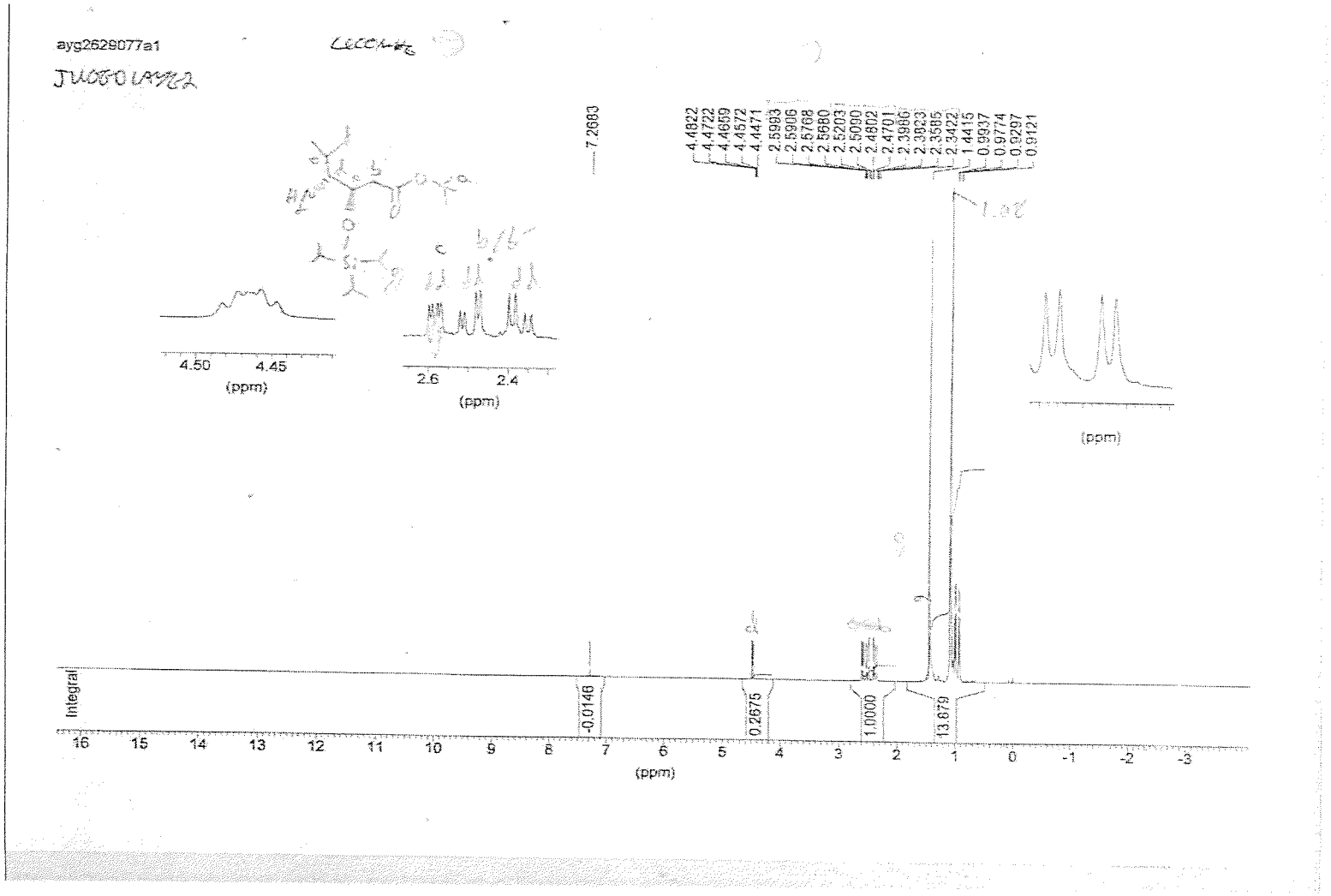
(3*S*,4*R*)-4-Benzyloxycarbonylamino-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (12)



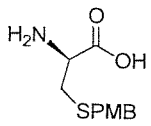


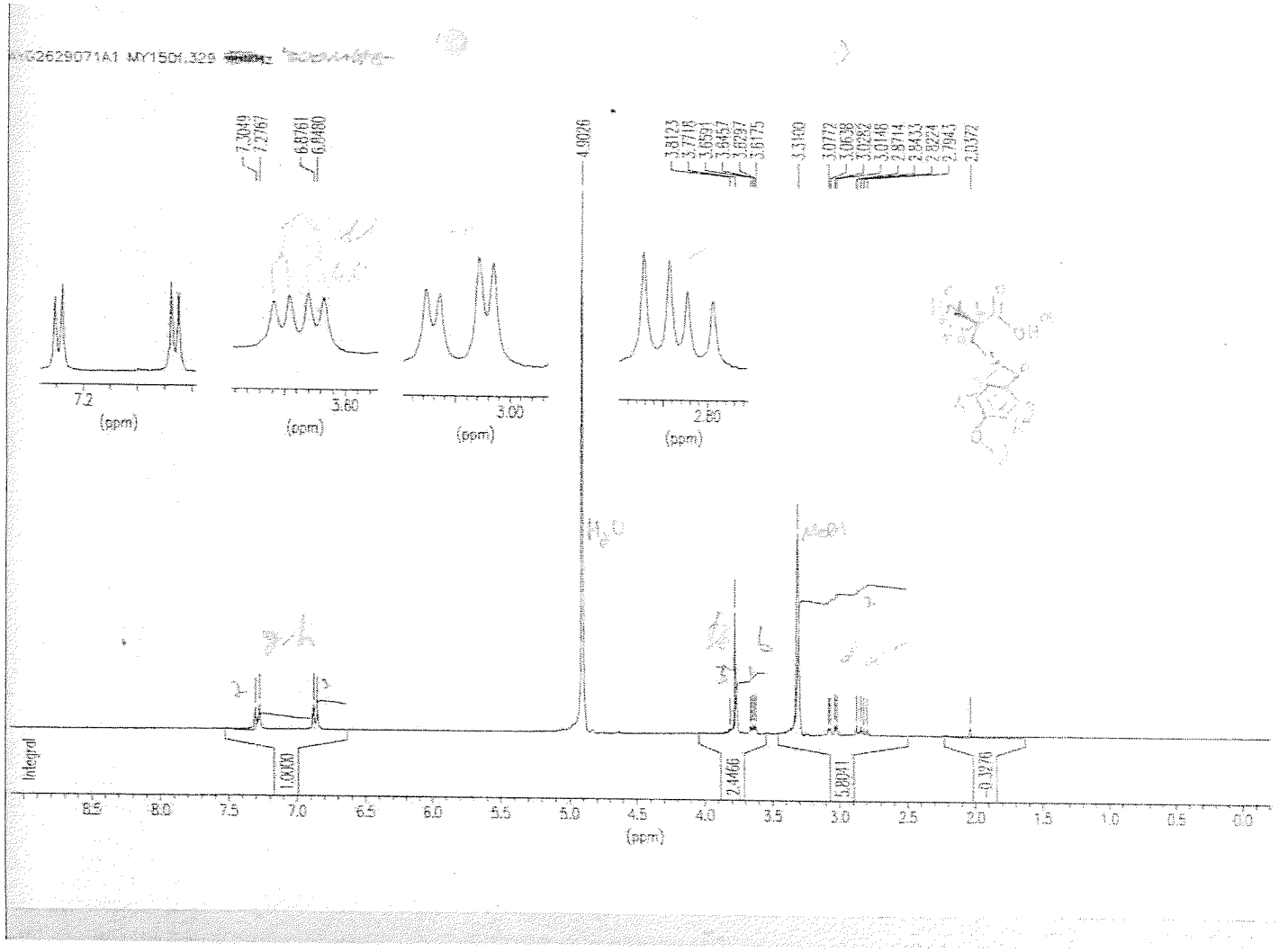
(3*S*,4*R*)-4-Amino-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (13)

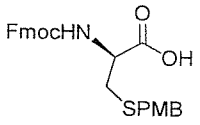




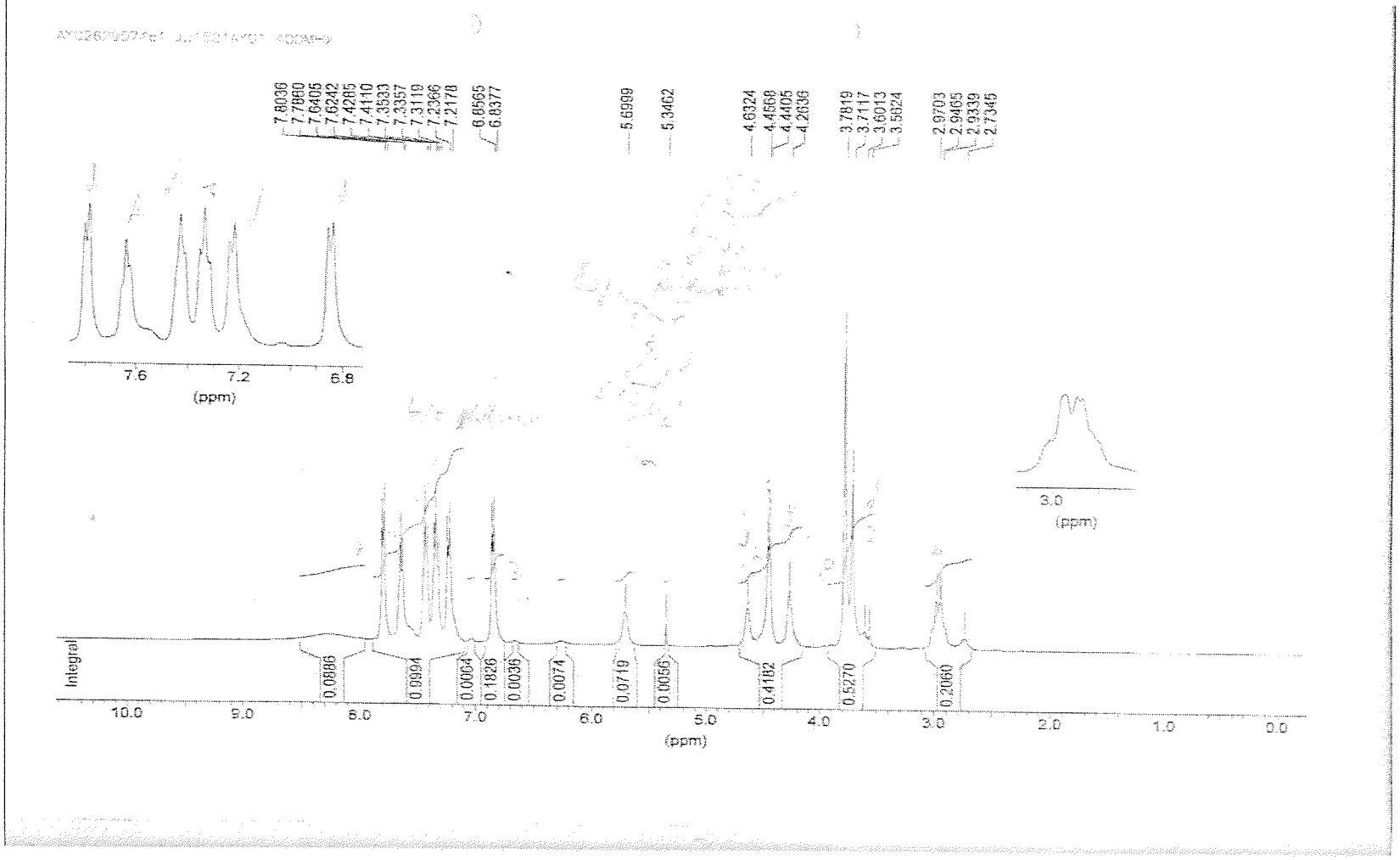
(*S*)-2-Amino-3-(4-methoxy-benzylsulfanyl)-propionic acid (15)

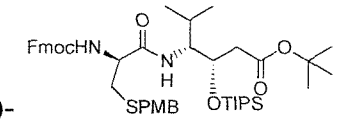




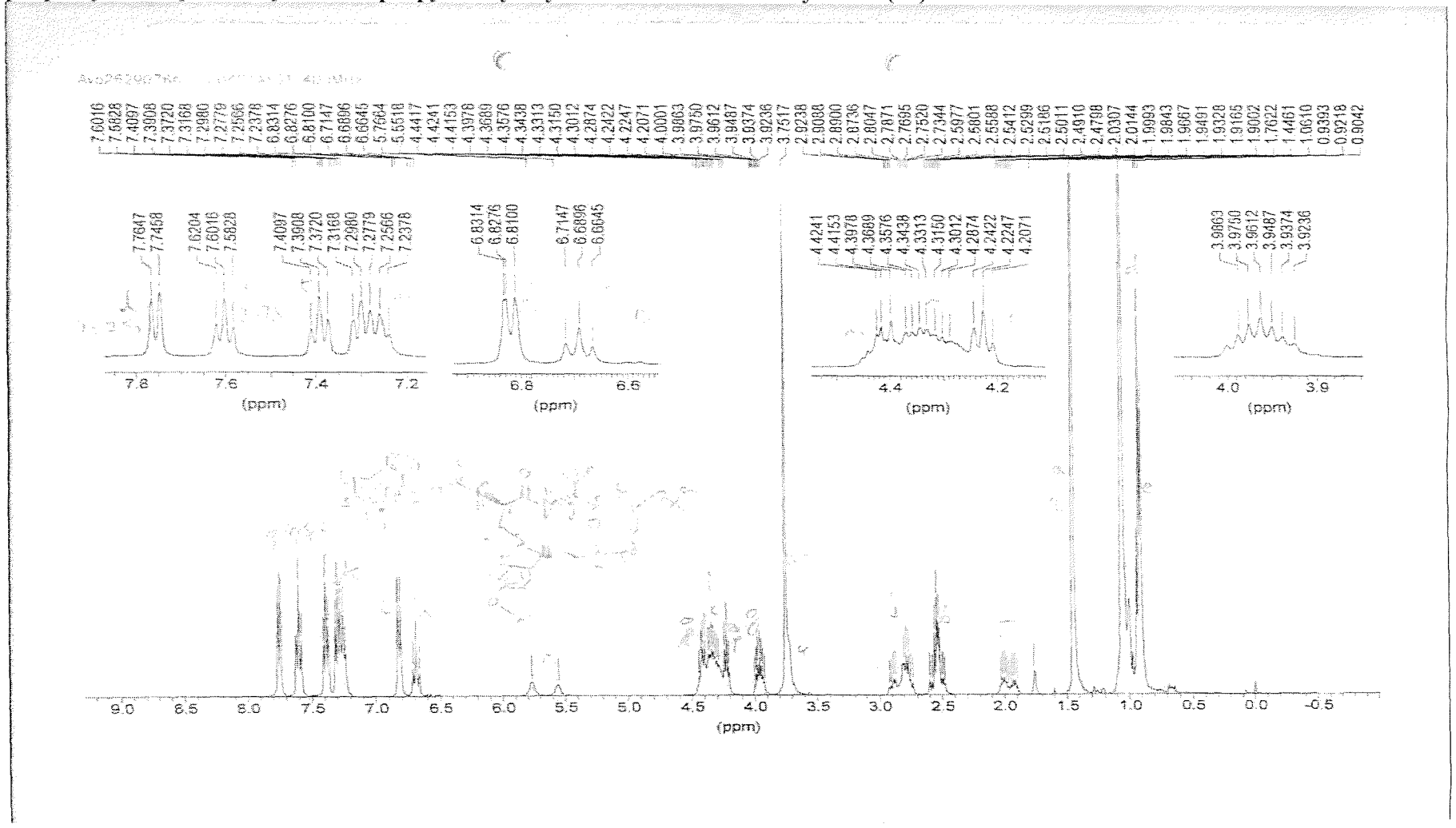


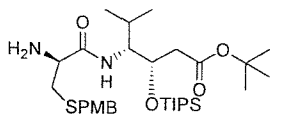
(*S*)- 2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionic acid (16)



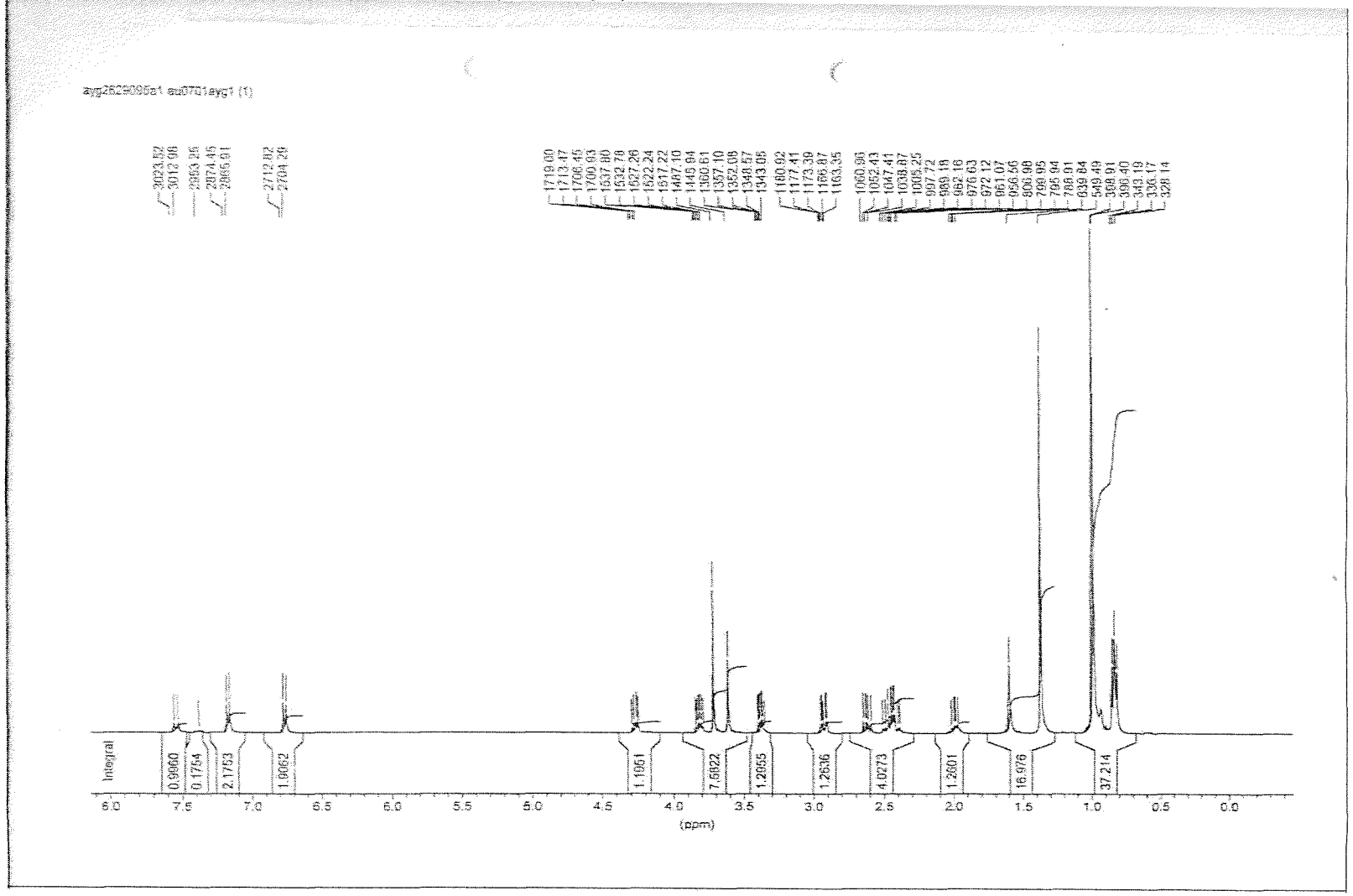


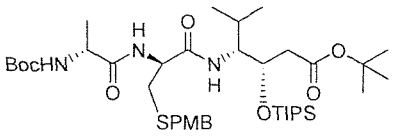
(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (17)



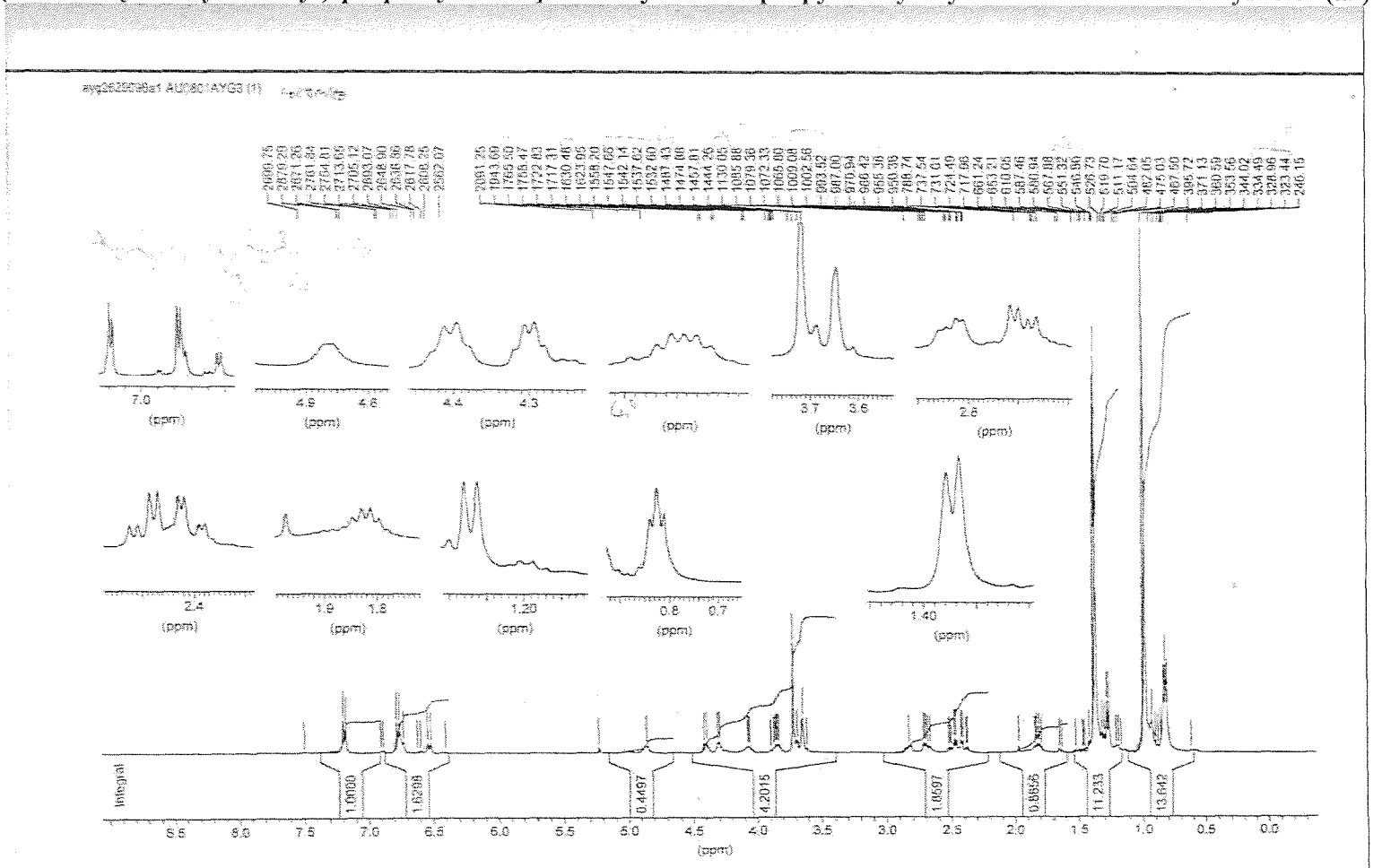


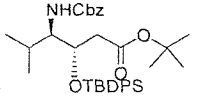
(3*S*,4*R*)-4-[(*S*)-2-Amino-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (18)



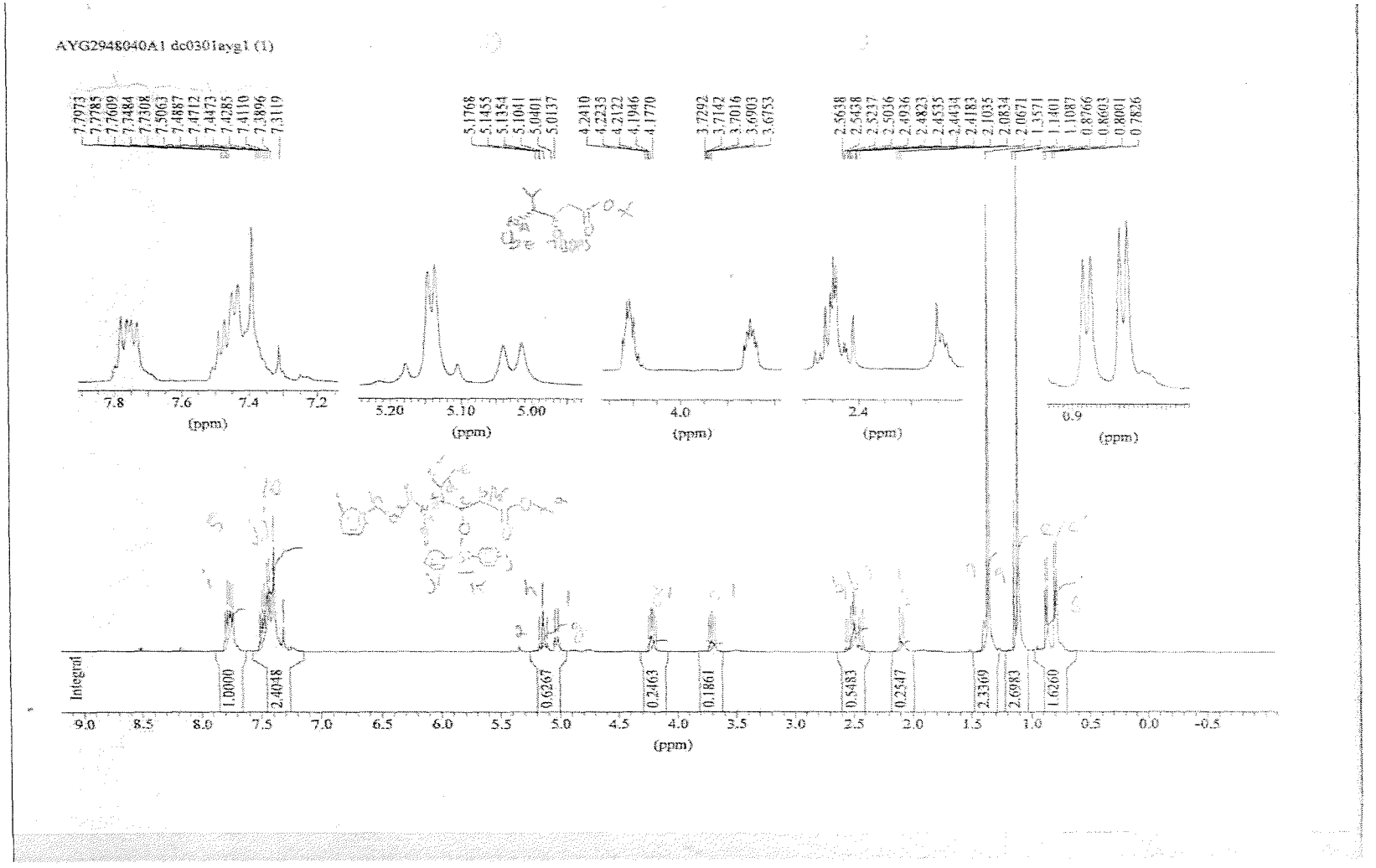


(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-butoxycarbonylamino-propionylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid *tert*-butyl ester (19)

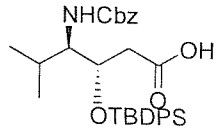


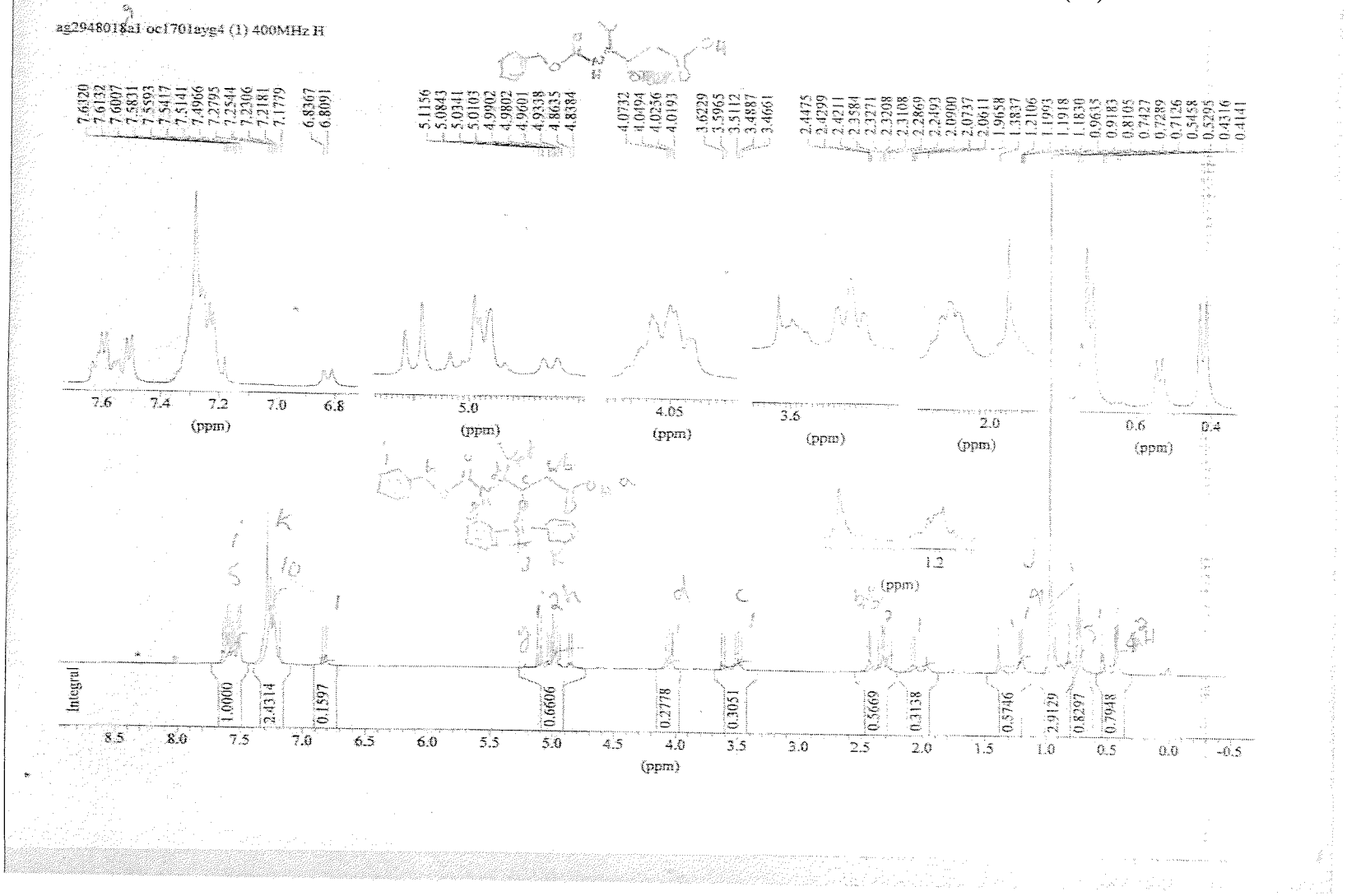


(3*S*,4*R*)-4-Benzyloxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid *tert*-butyl ester (23)

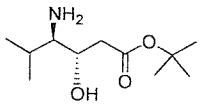


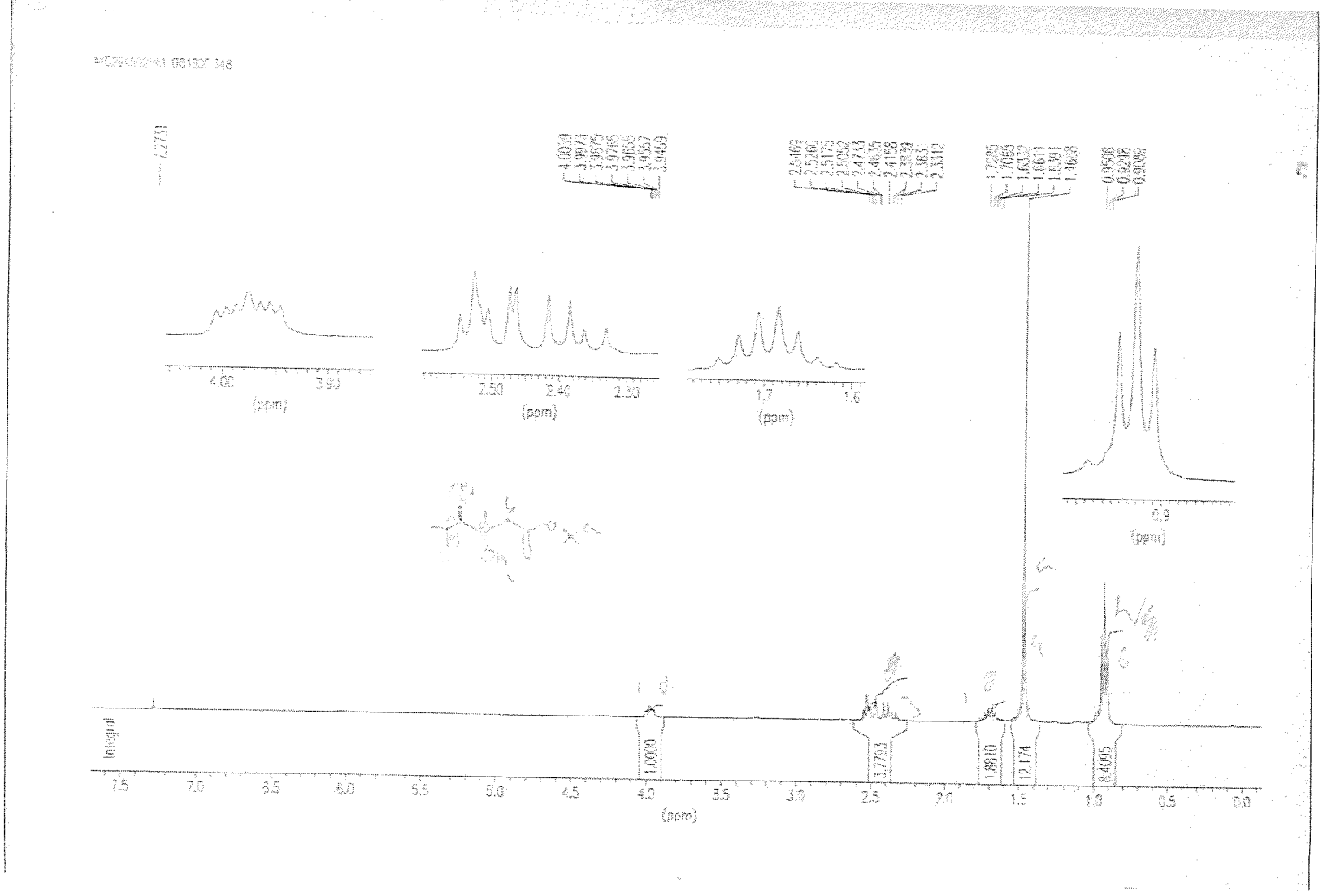
(3*S*,4*R*)-4-Benzyloxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid (24)

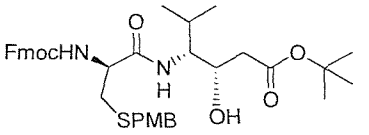




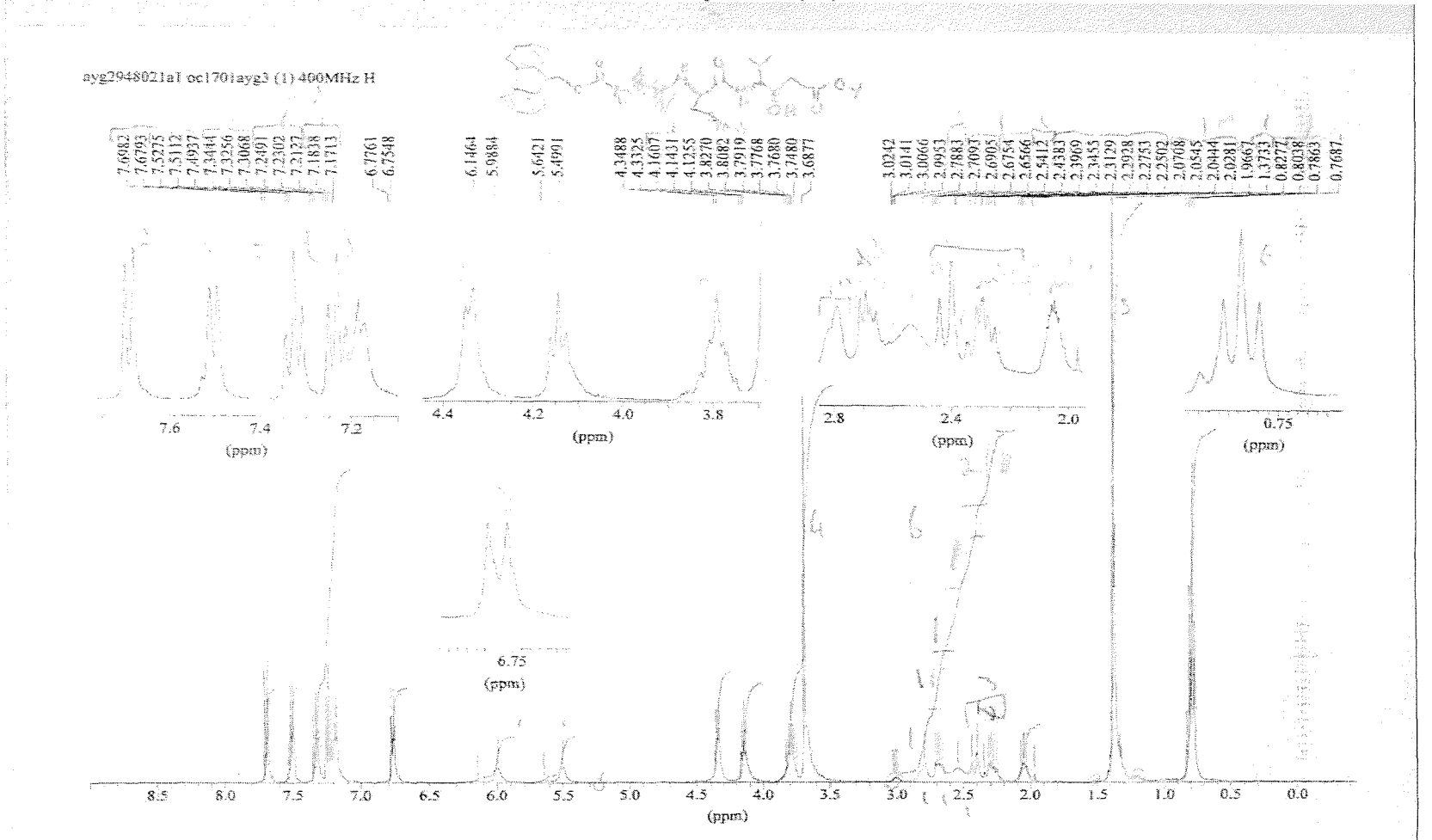
(3*S*,4*R*)-4-Amino-3-hydroxy-5-methyl-hexanoic *tert*-butyl ester (27)

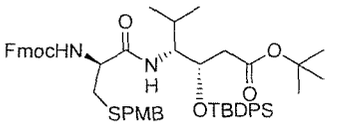




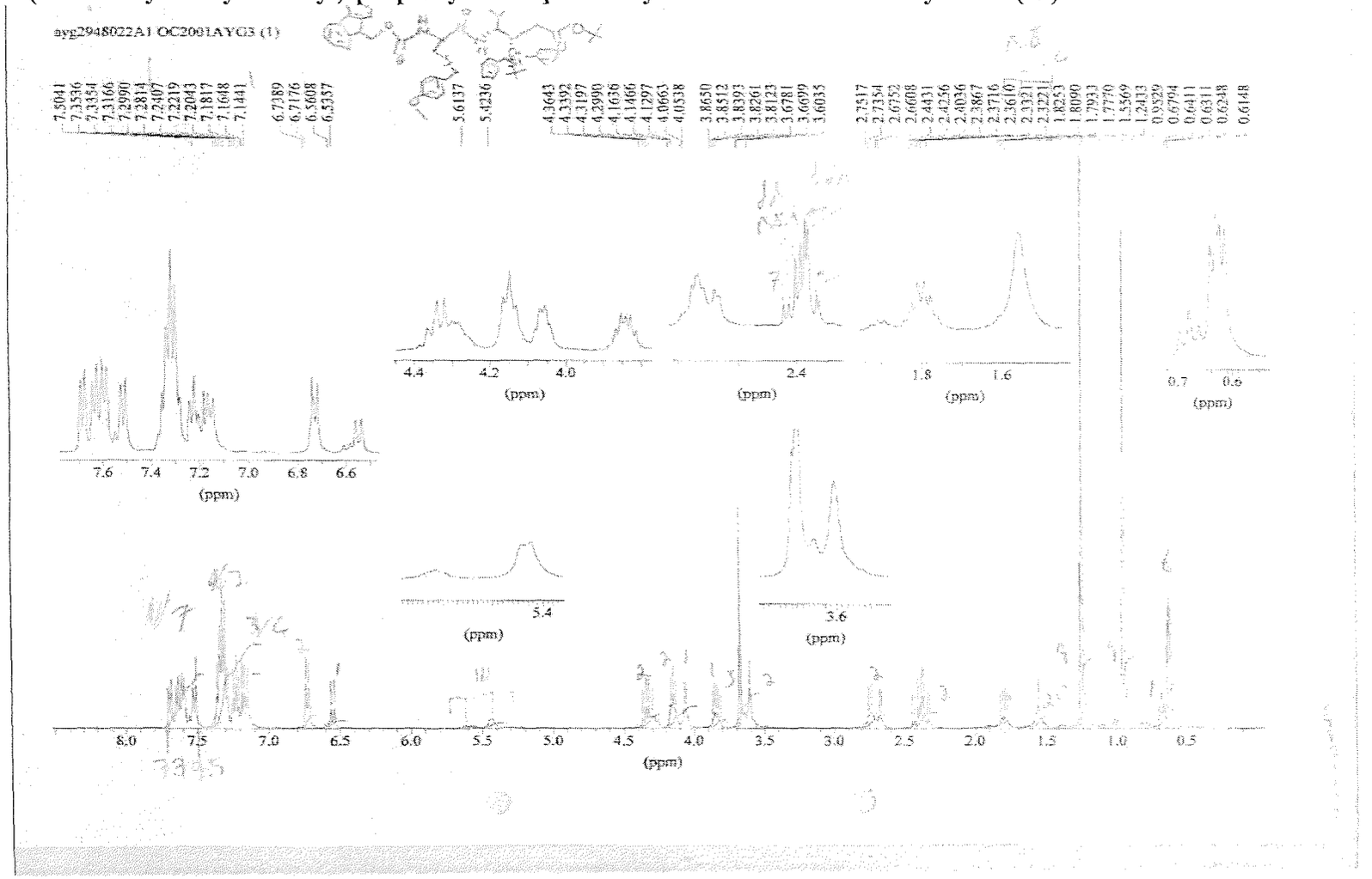


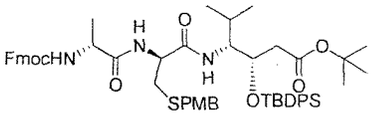
(3*S*,4*R*)-4-[*(S)*-2-(*9H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (28)



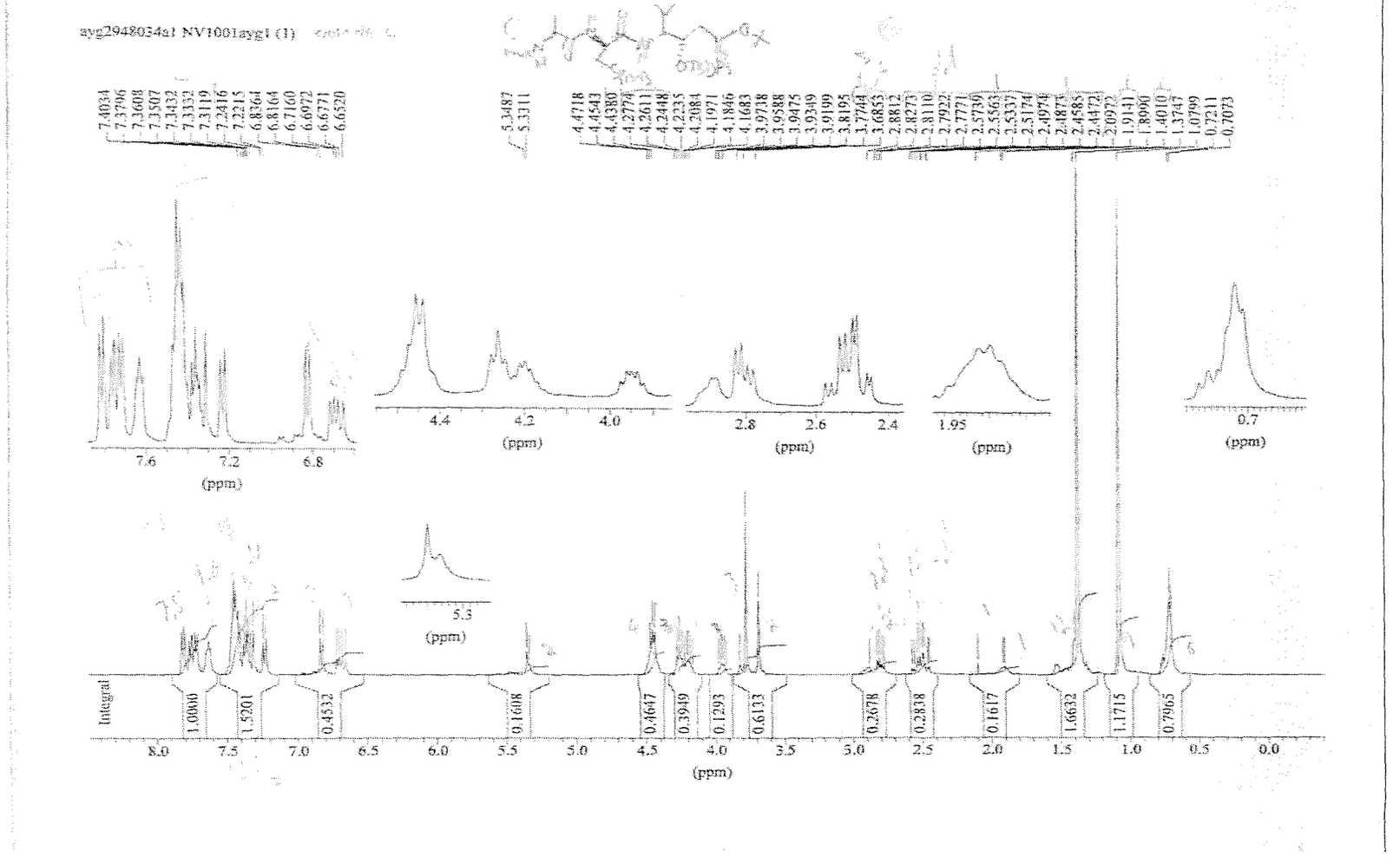


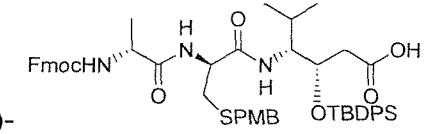
(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-hexanoic acid *tert*-butyl ester (29)



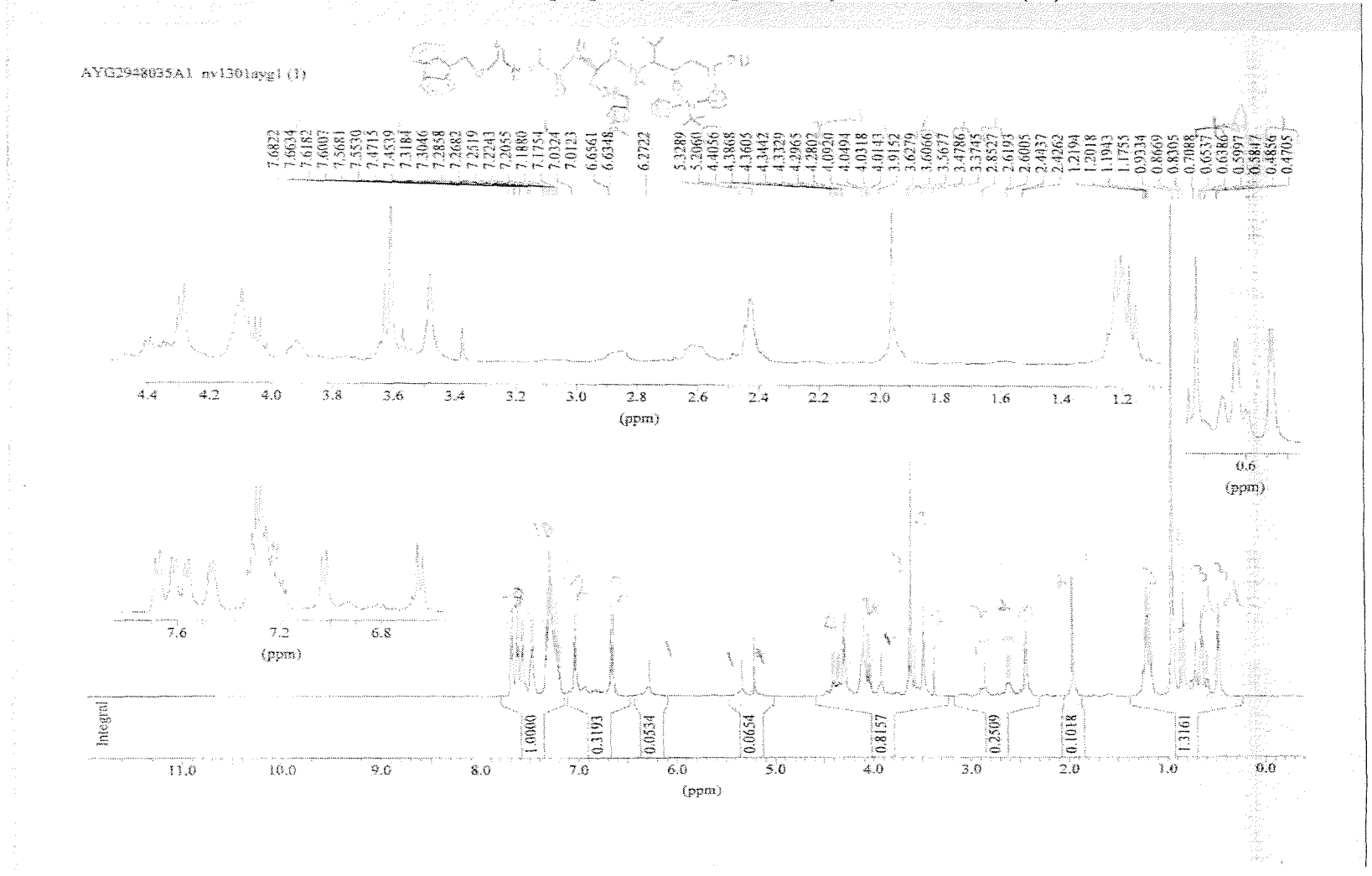


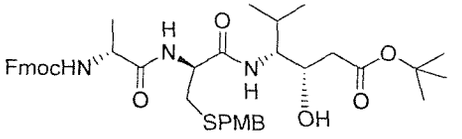
(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-[(*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-hexanoic acid *tert*-butyl ester (30)



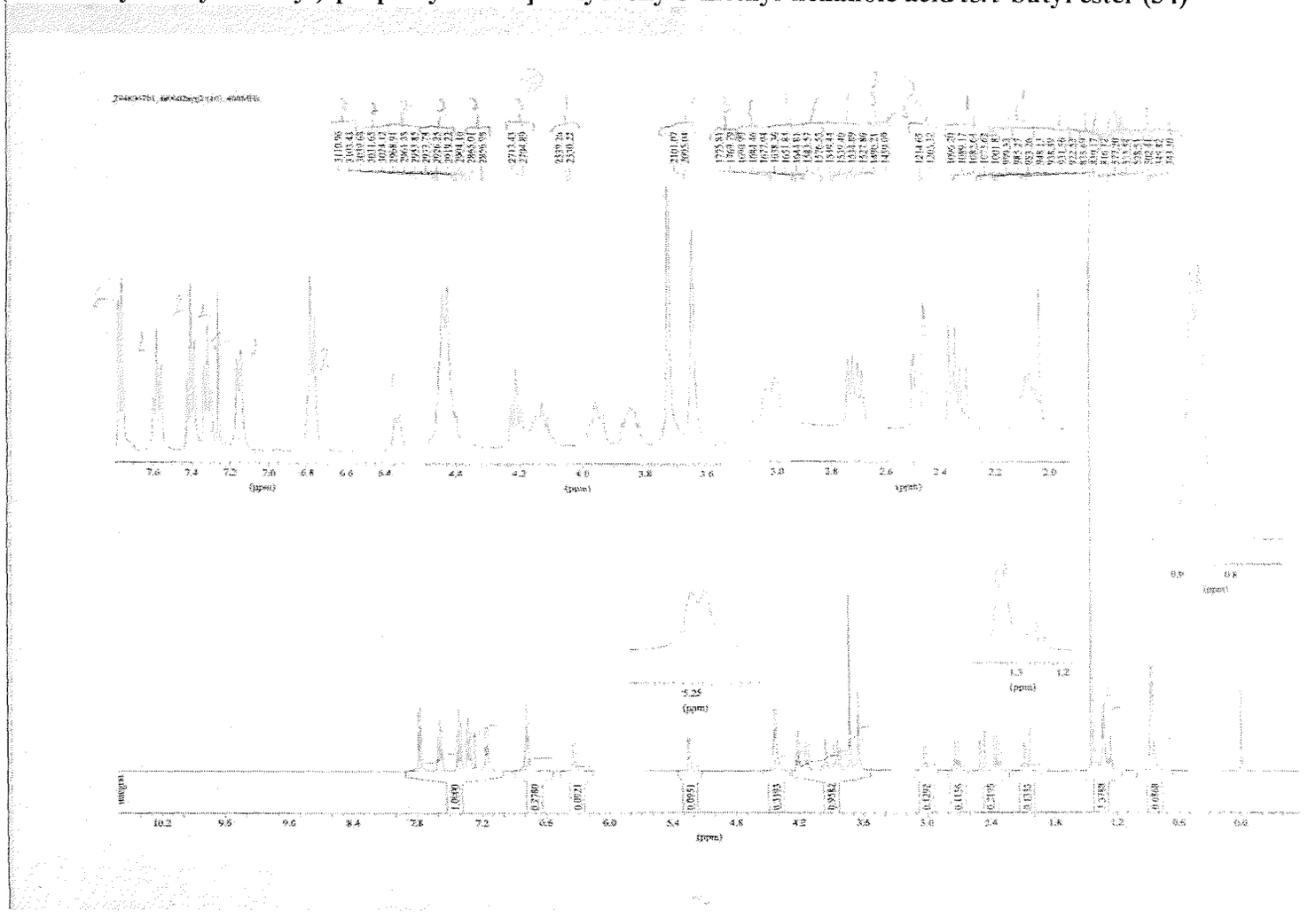


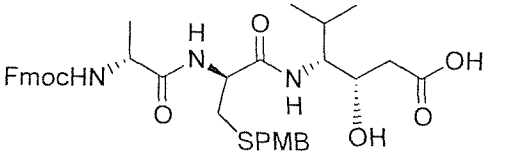
(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-[(*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-5-methyl-hexanoic acid (31)



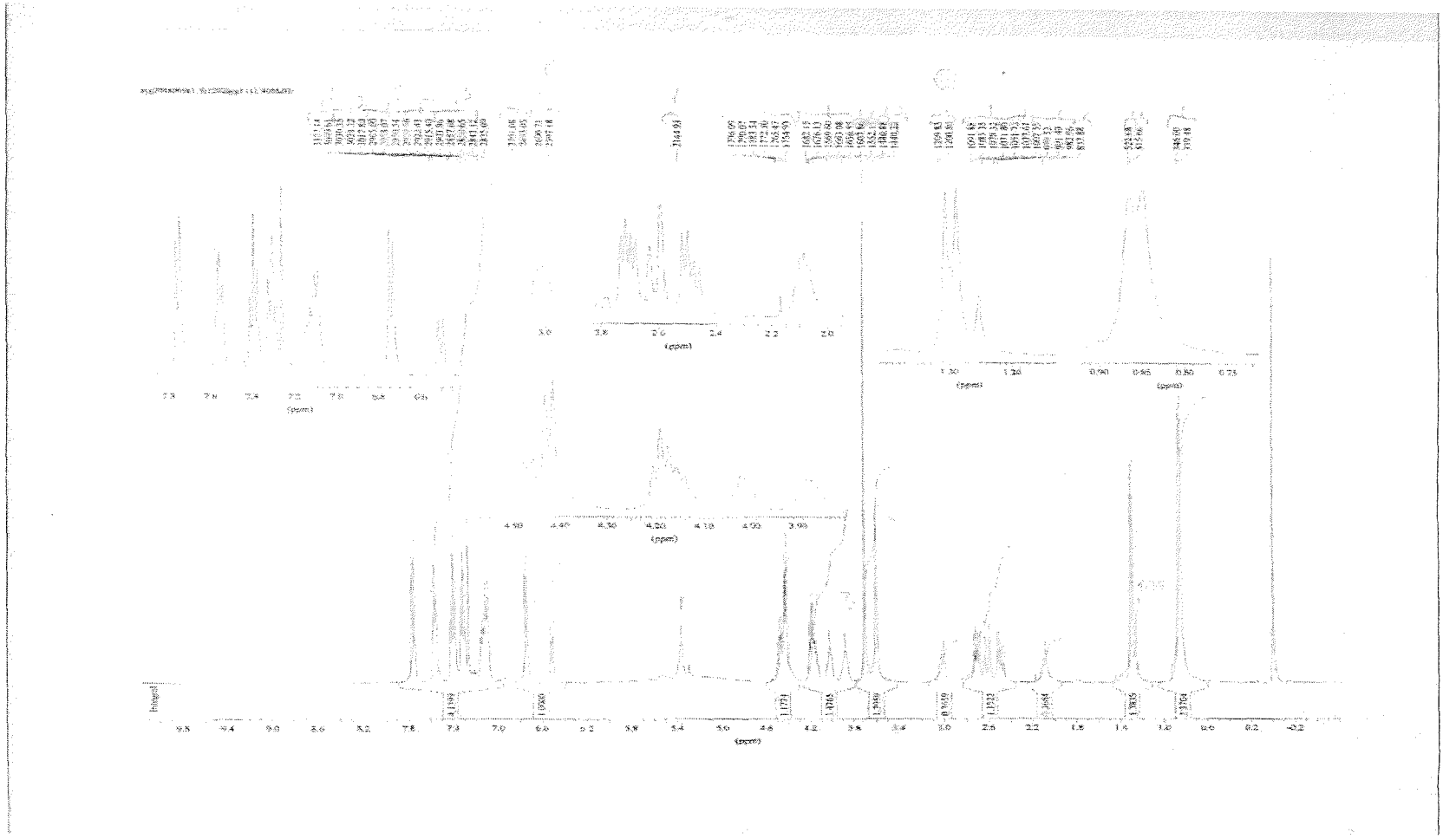


(3*S*,4*R*)-4-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (34)

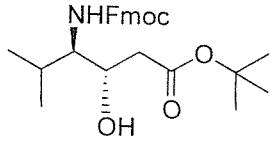


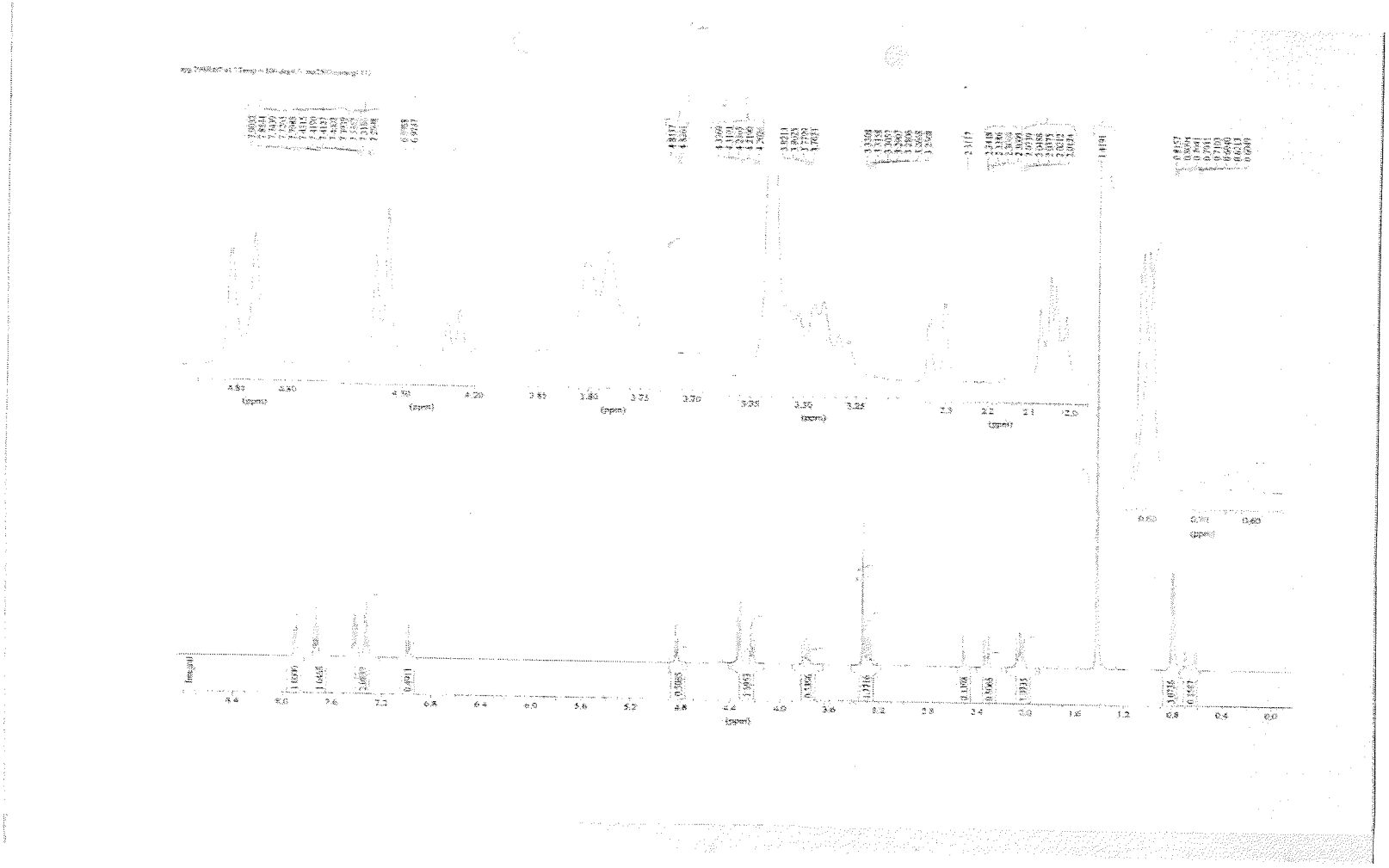


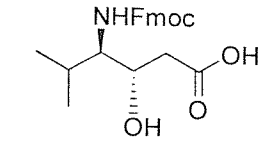
(3*S*,4*R*)-4-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonyl amino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-hydroxy-5-methyl-hexanoic acid (35)



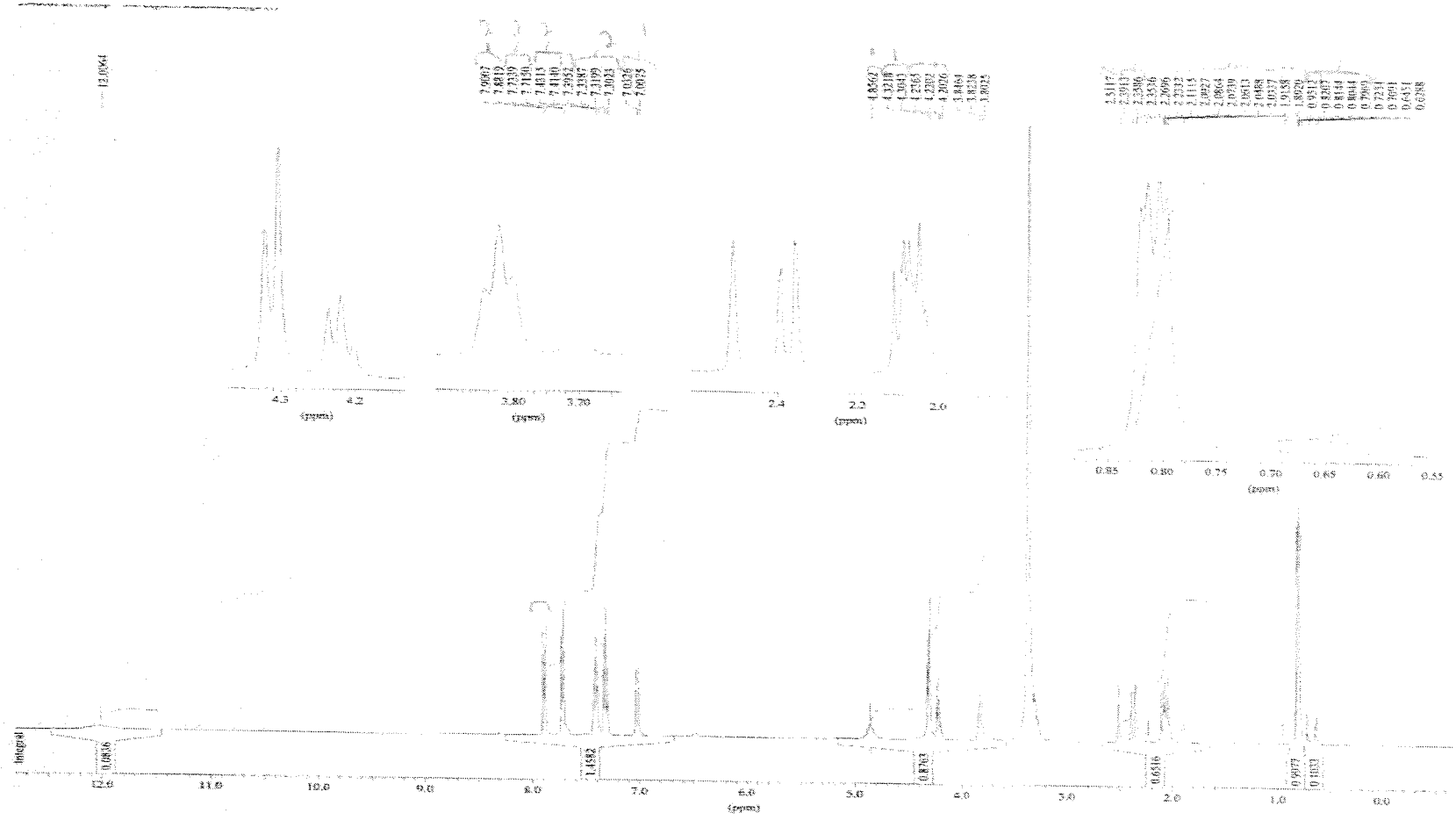
(3*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-5-methyl-hexanoic acid *tert*-butyl ester (41)

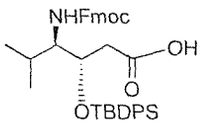




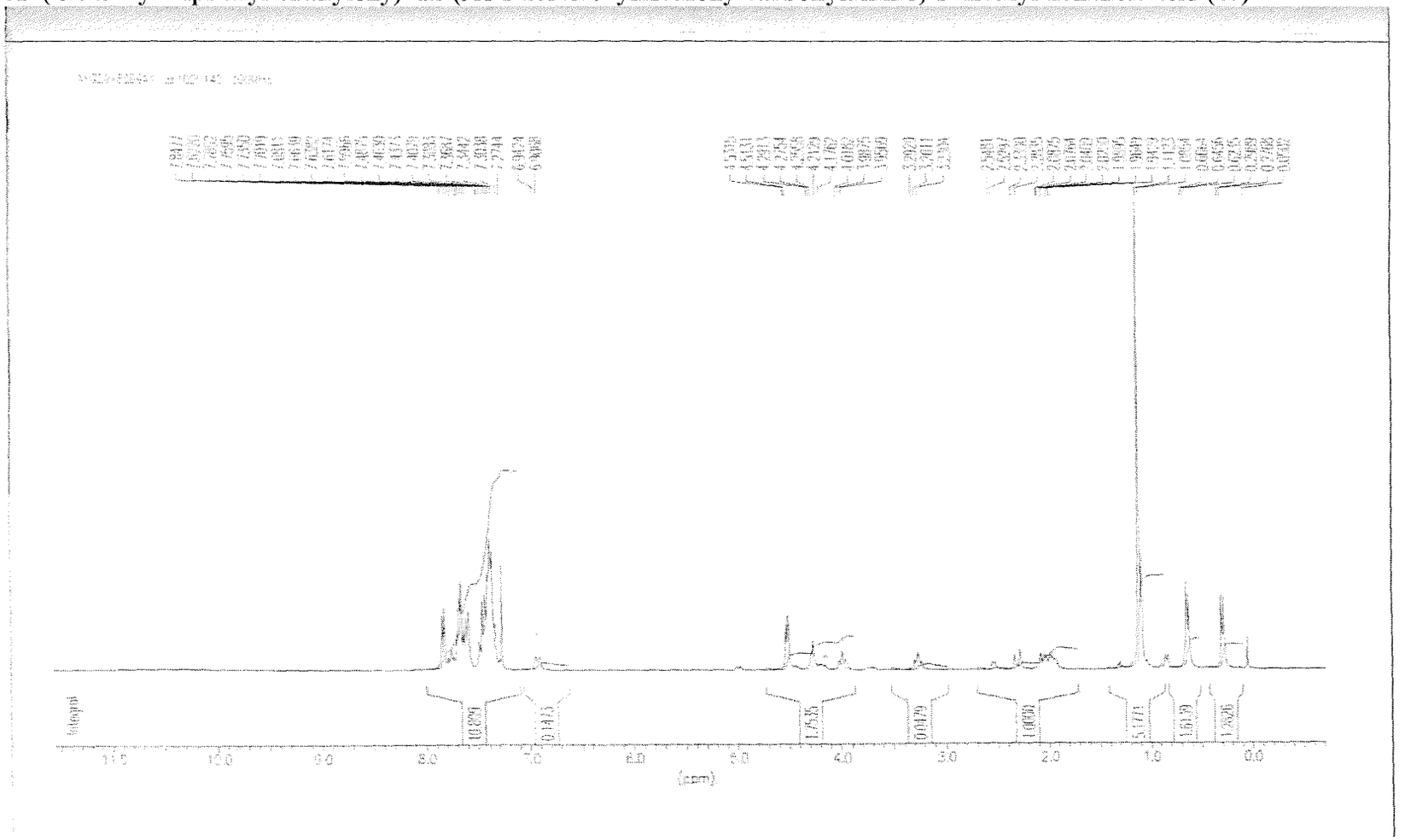


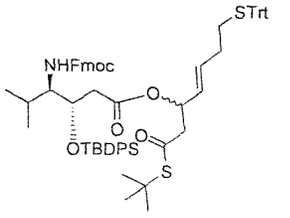
(3*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-5-methyl-hexanoic acid (42)



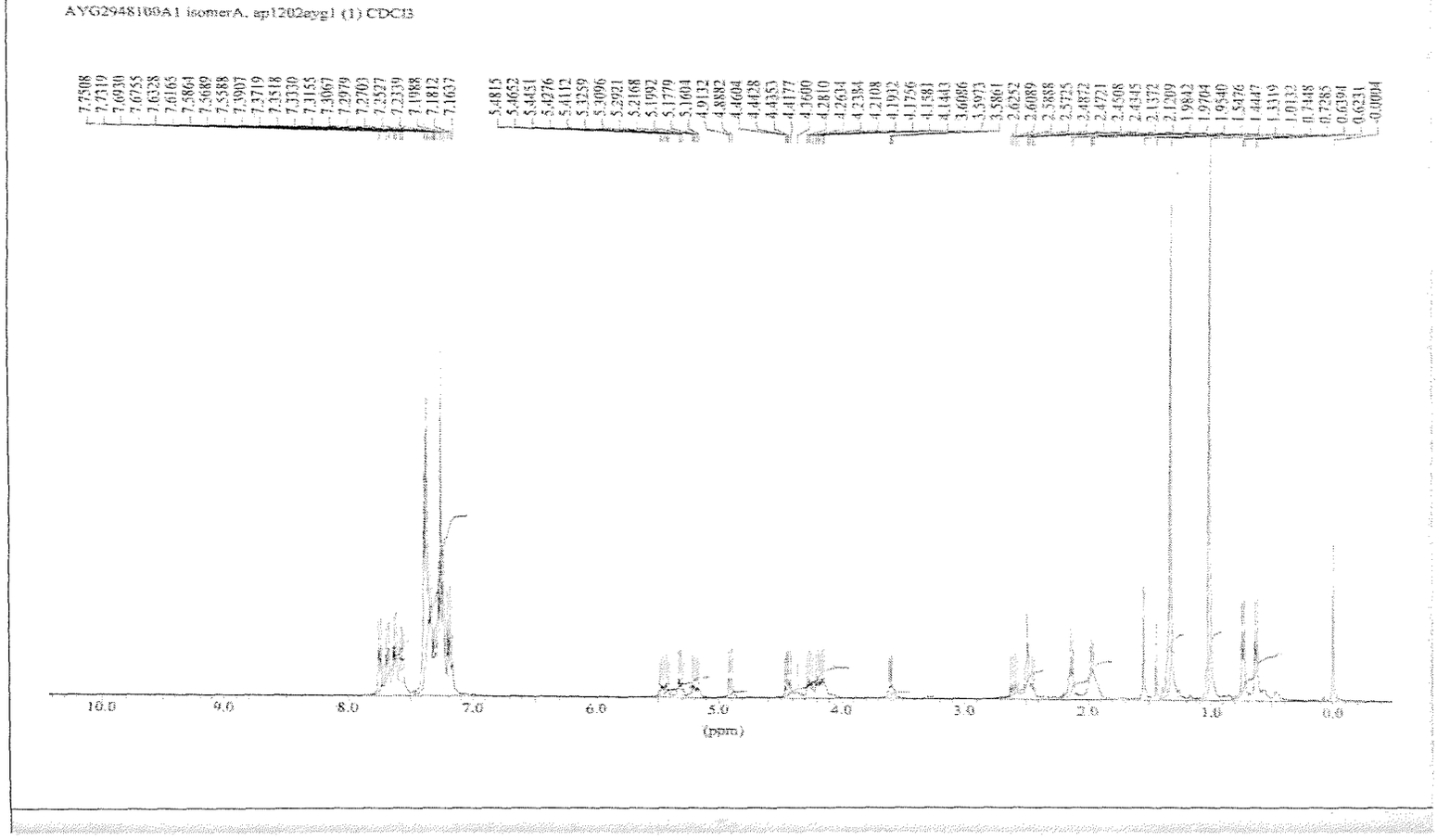


3*S*-(*tert*-Butyl-diphenyl-silanyloxy)-4*R*-(9*H*-fluoren-9-ylmethoxy-carbonylamino)-5-methyl-hexanoic acid (47)

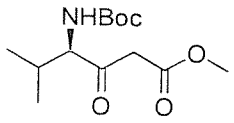


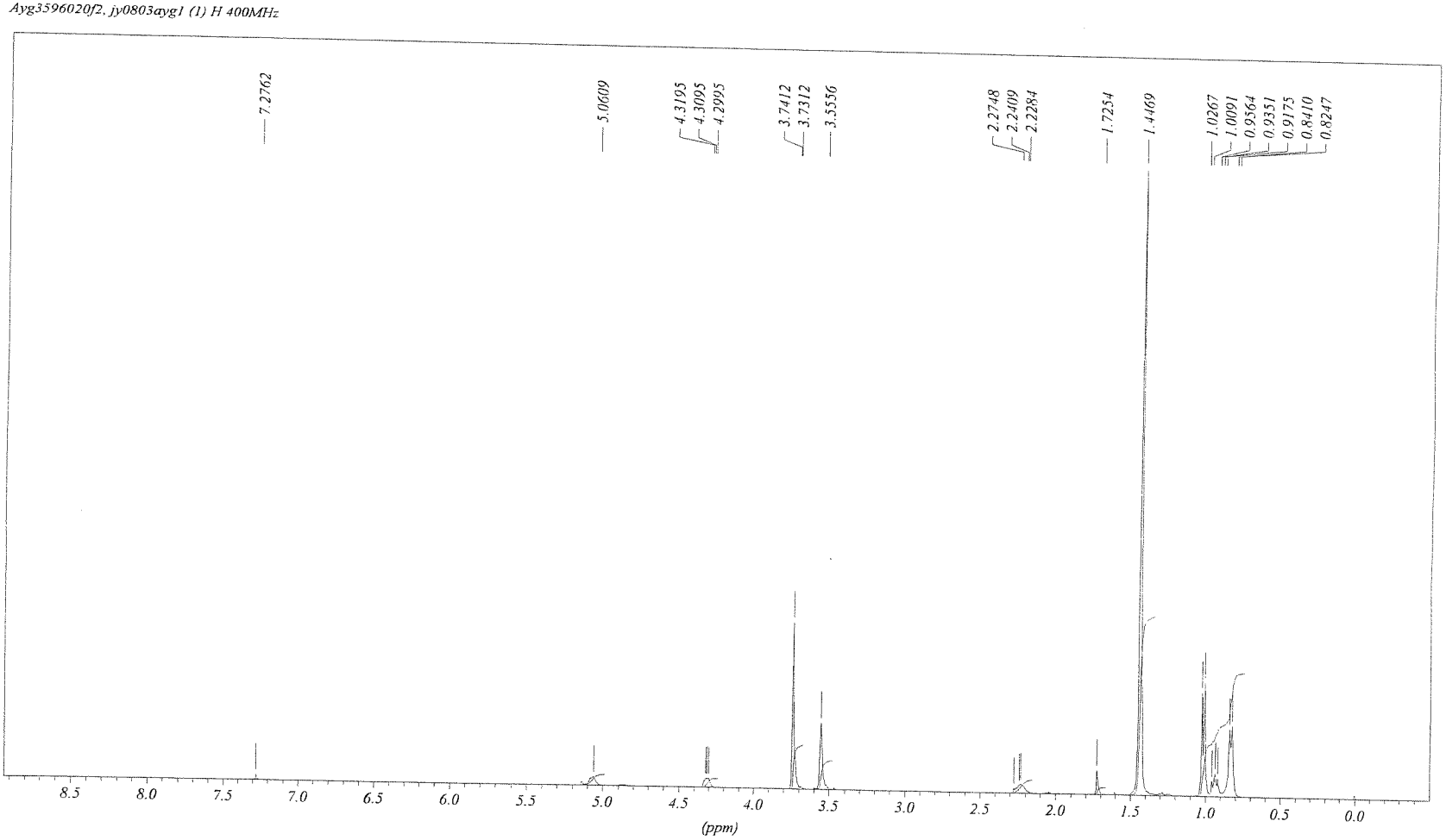


(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-(9*H*-fluoren-9-ylmethoxycarbonylamino)-5-methyl-hexanoic acid (*E*)-1-*tert*-butylsulfanylcarbonylmethyl-5-tritylsulfanyl-pent-2-enyl ester (48)

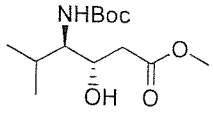


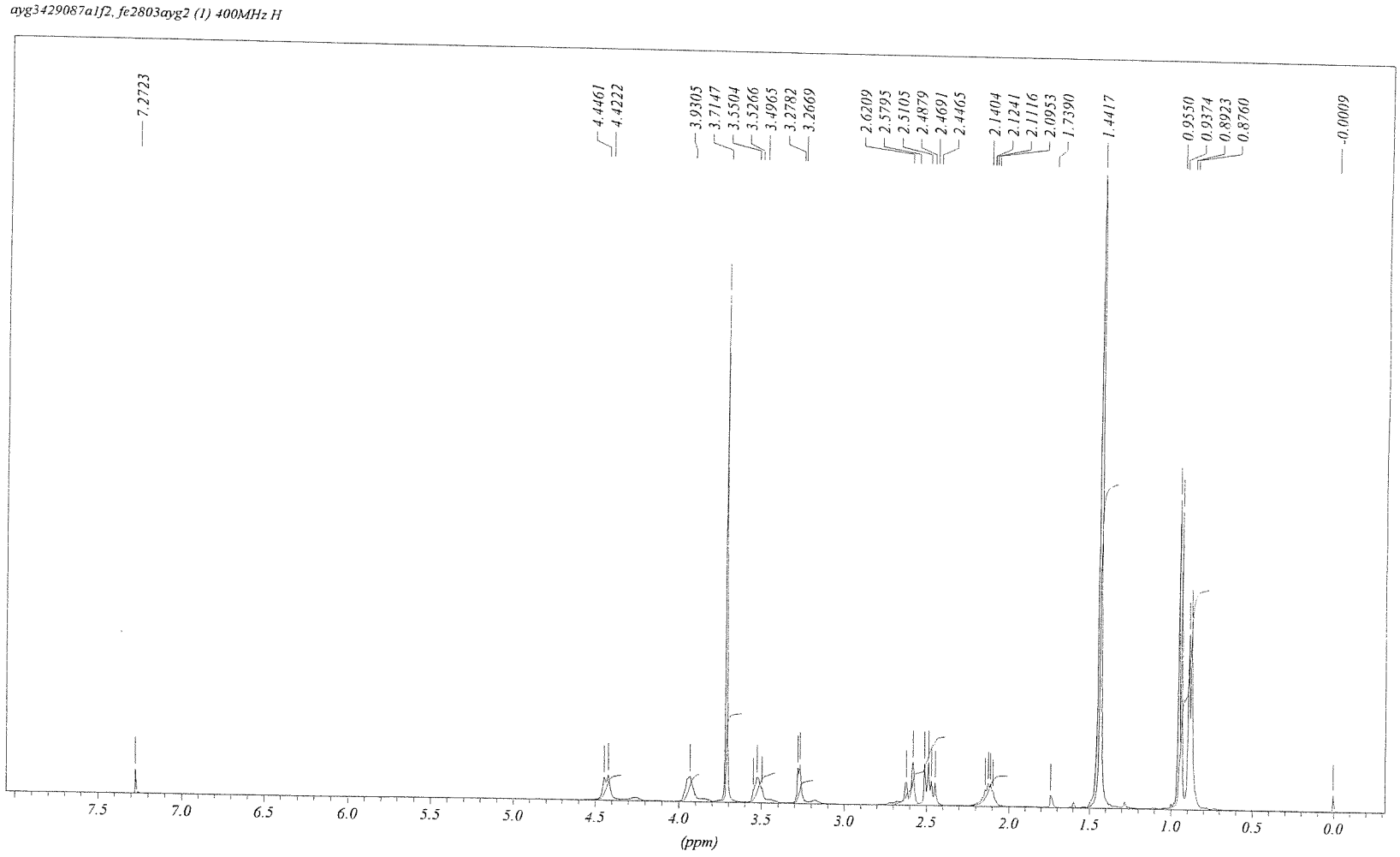
(*R*)-4-*tert*-Butoxycarbonylamino-5-methyl-3-oxo-hexanoic acid methyl ester (51)

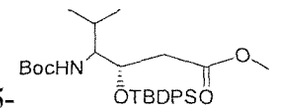




(3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-methyl-hexanoic acid methyl ester (52)

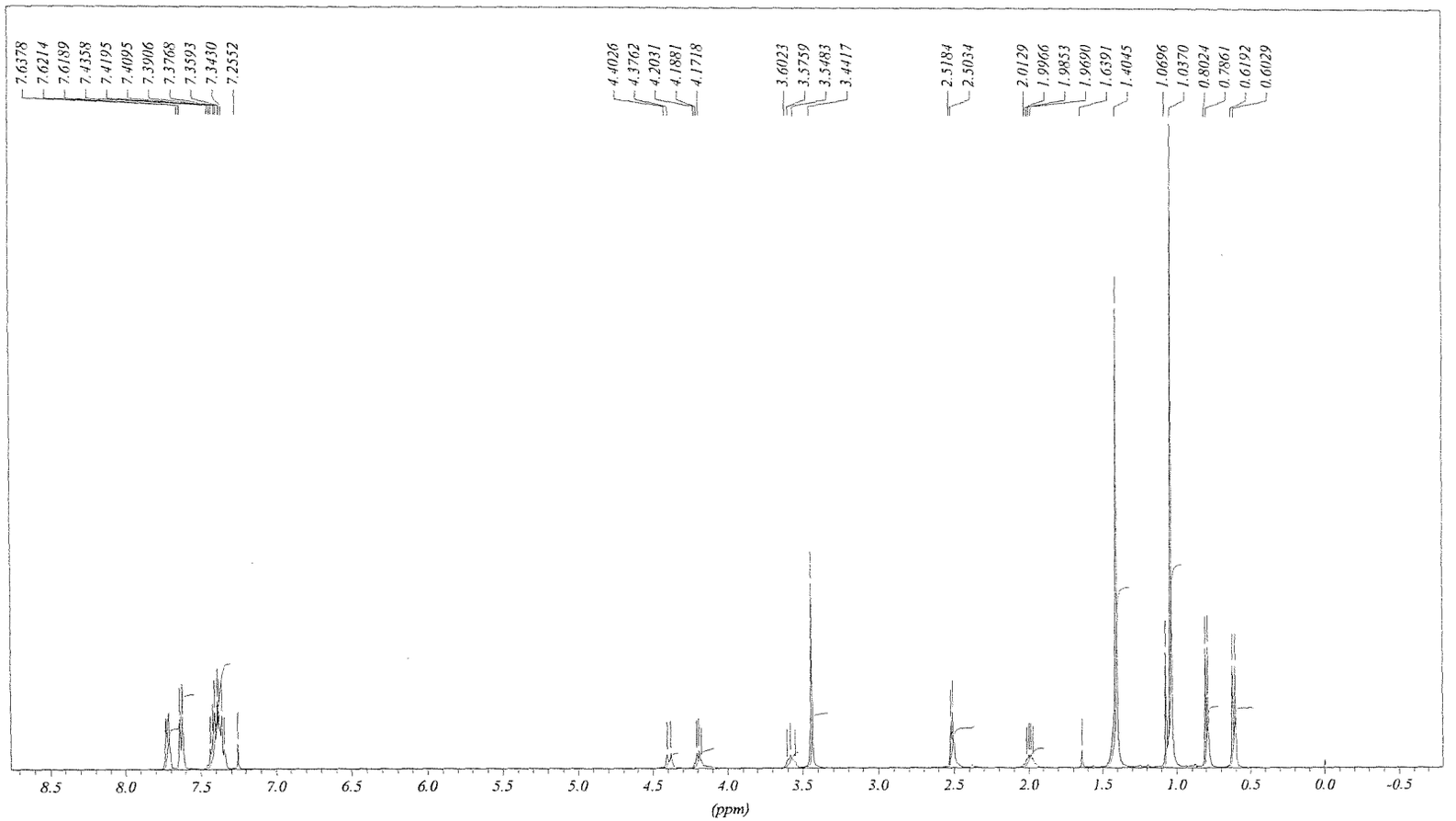


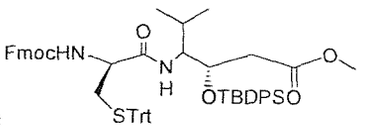




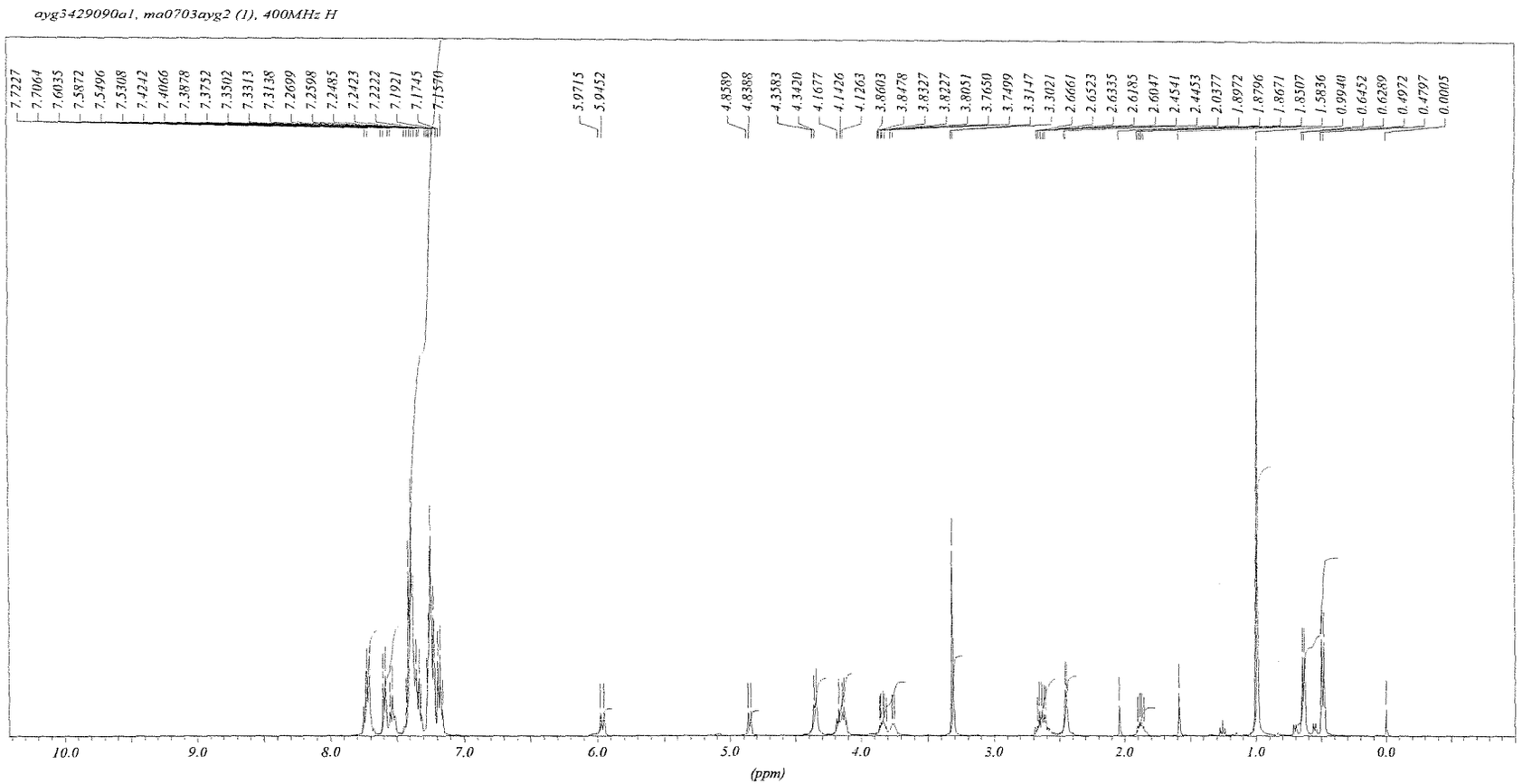
(3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid methyl ester (53)

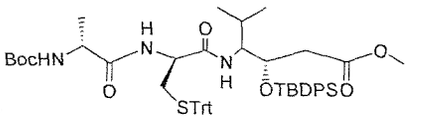
ayg3429088a1, ma0703ayg1 (1) 400MHz H





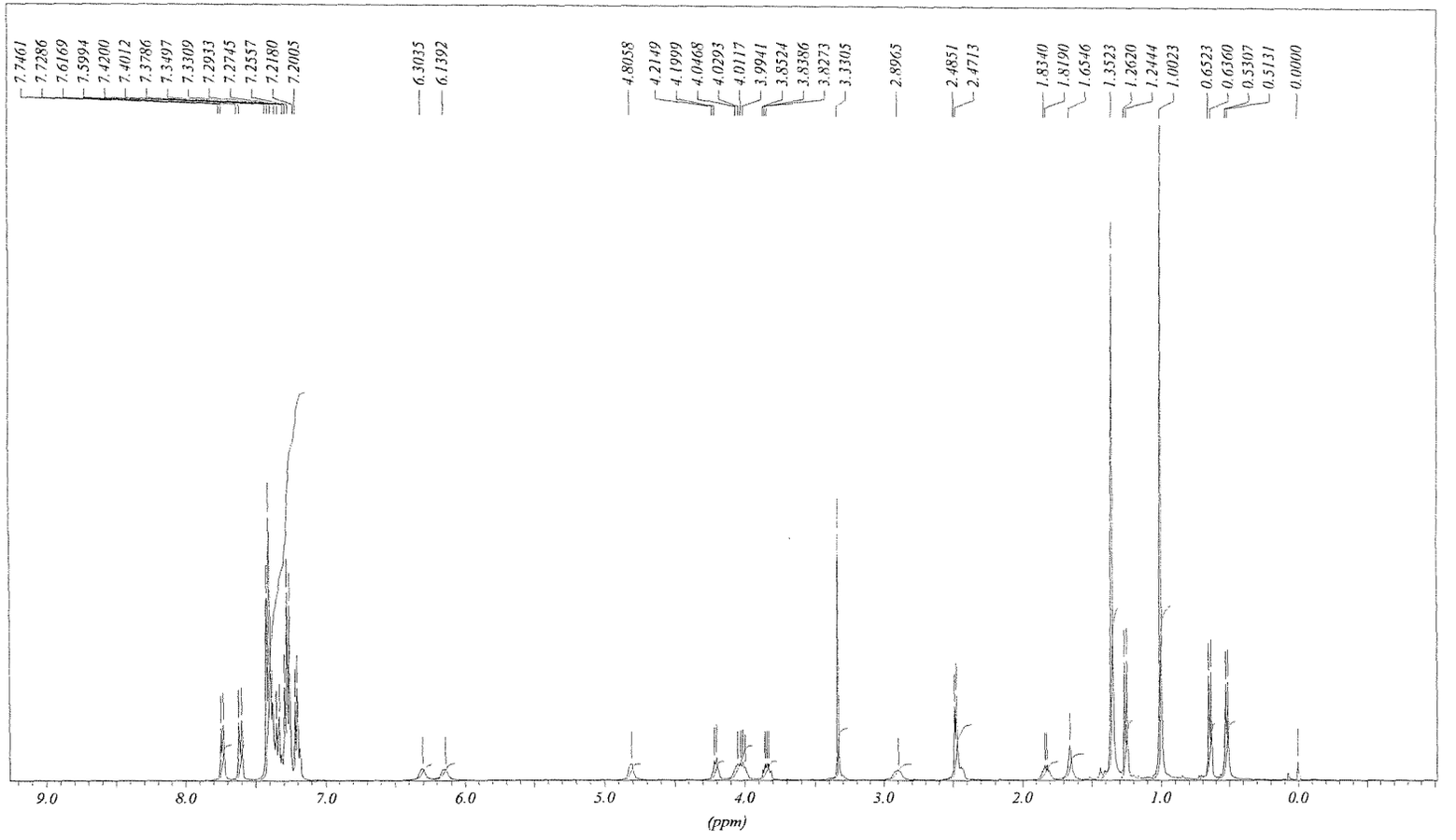
(3*S*,4*R*)-3-(*tert*-Butyl-diphenyl-silanyloxy)-4-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-5-methyl-hexanoic acid methyl ester (54)

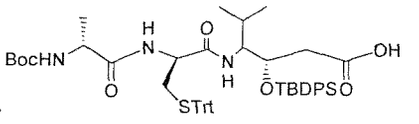




(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid methyl ester (55)

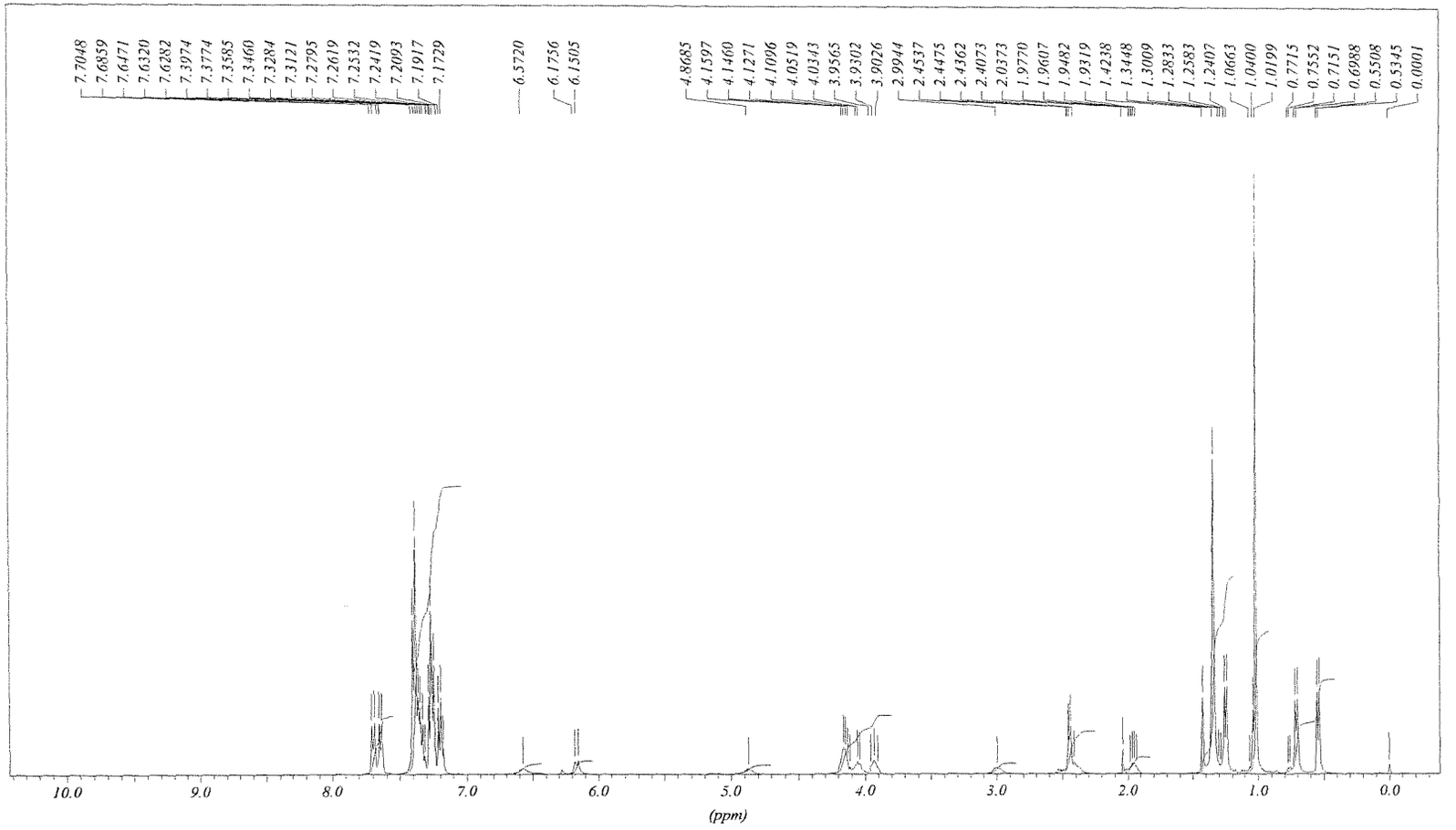
ayg3429092a1, ja3104ayg1 (1) 400MHz H

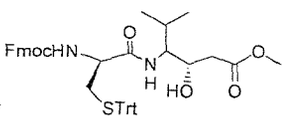




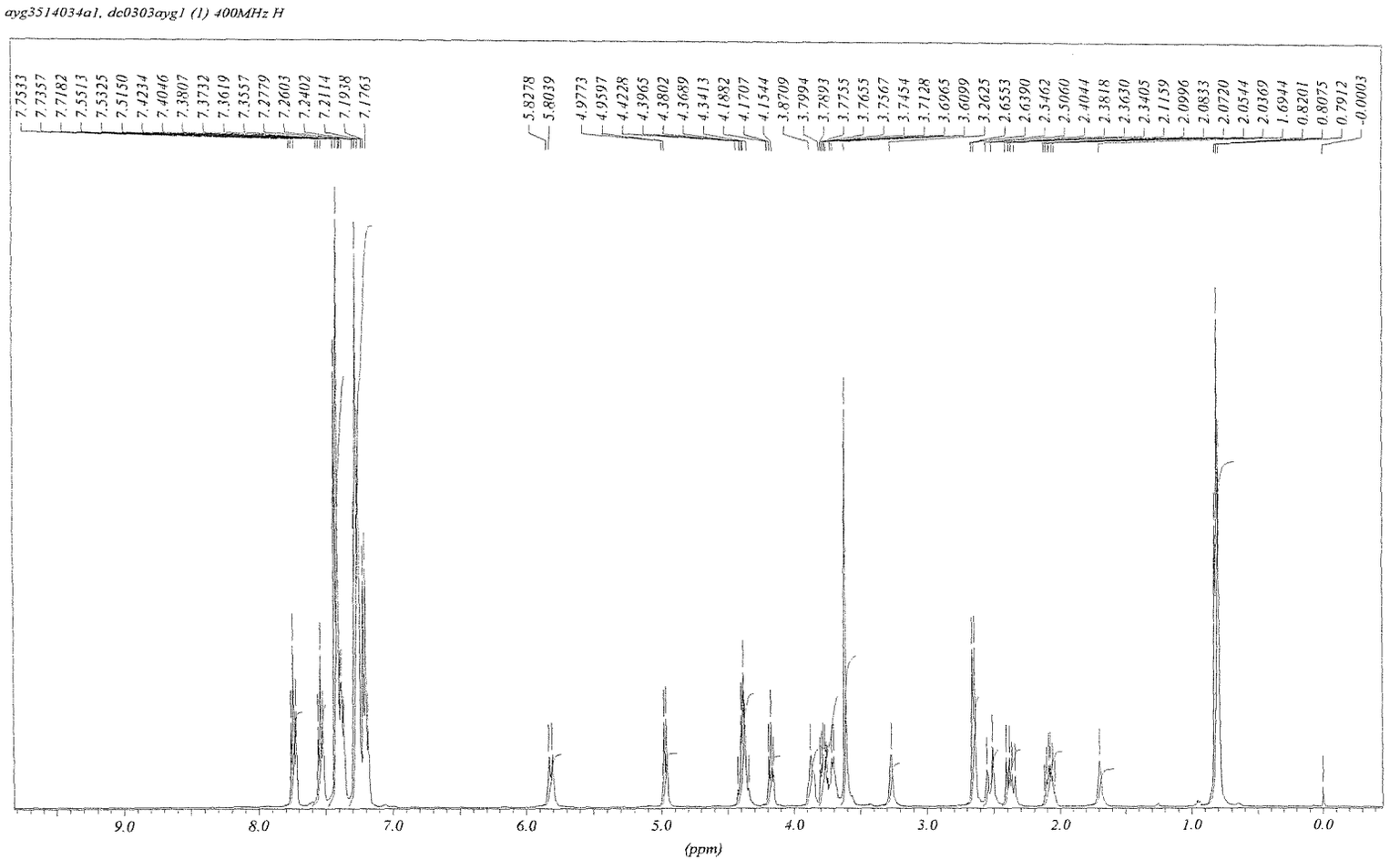
(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-(*tert*-butyl-diphenyl-silanyloxy)-5-methyl-hexanoic acid (56)

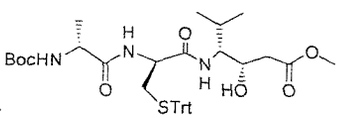
ayg3429094a1, ma1003ayg1 (1) 400MHz, H



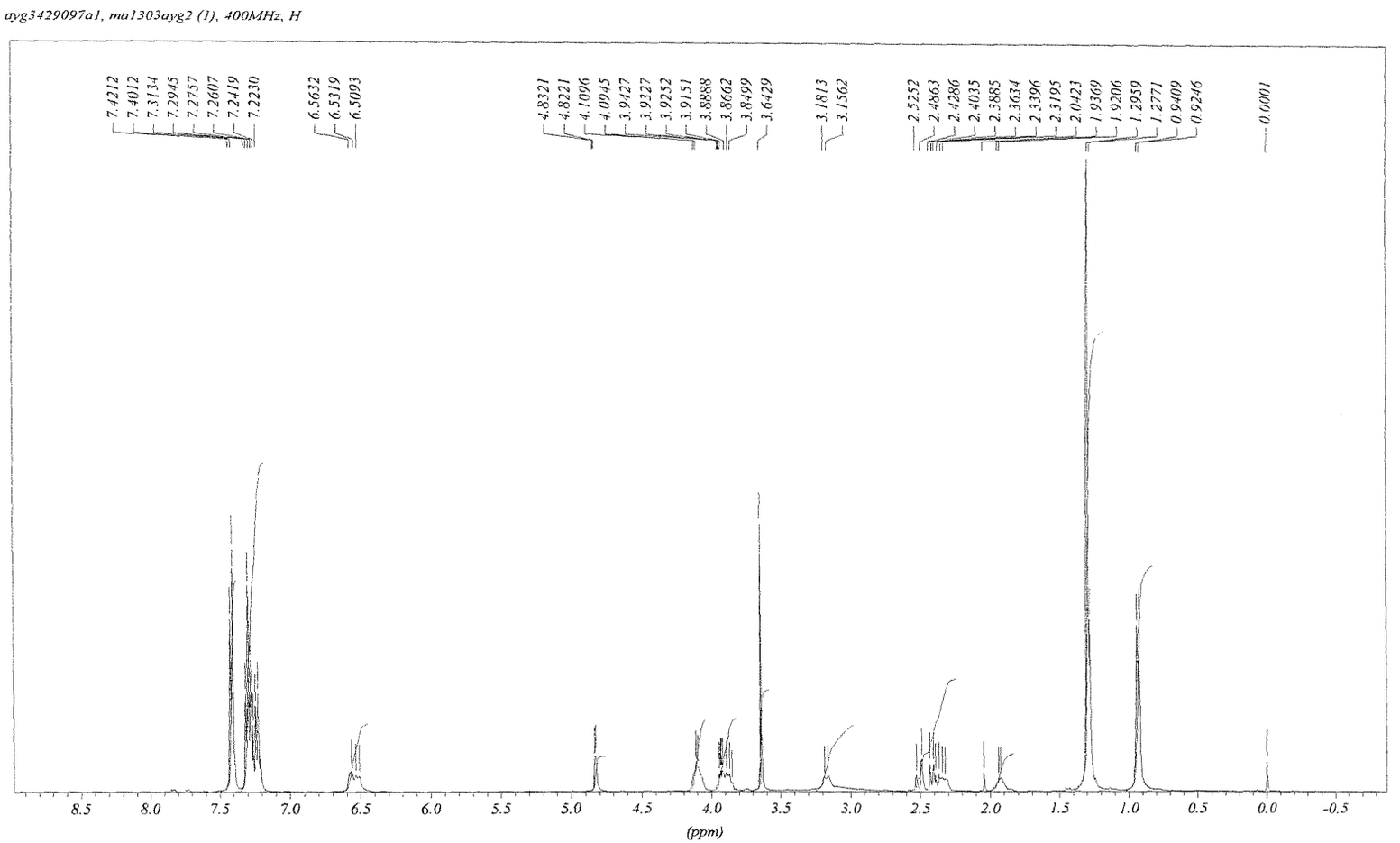


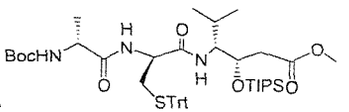
(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-5-methyl-hexanoic acid methyl ester (60)





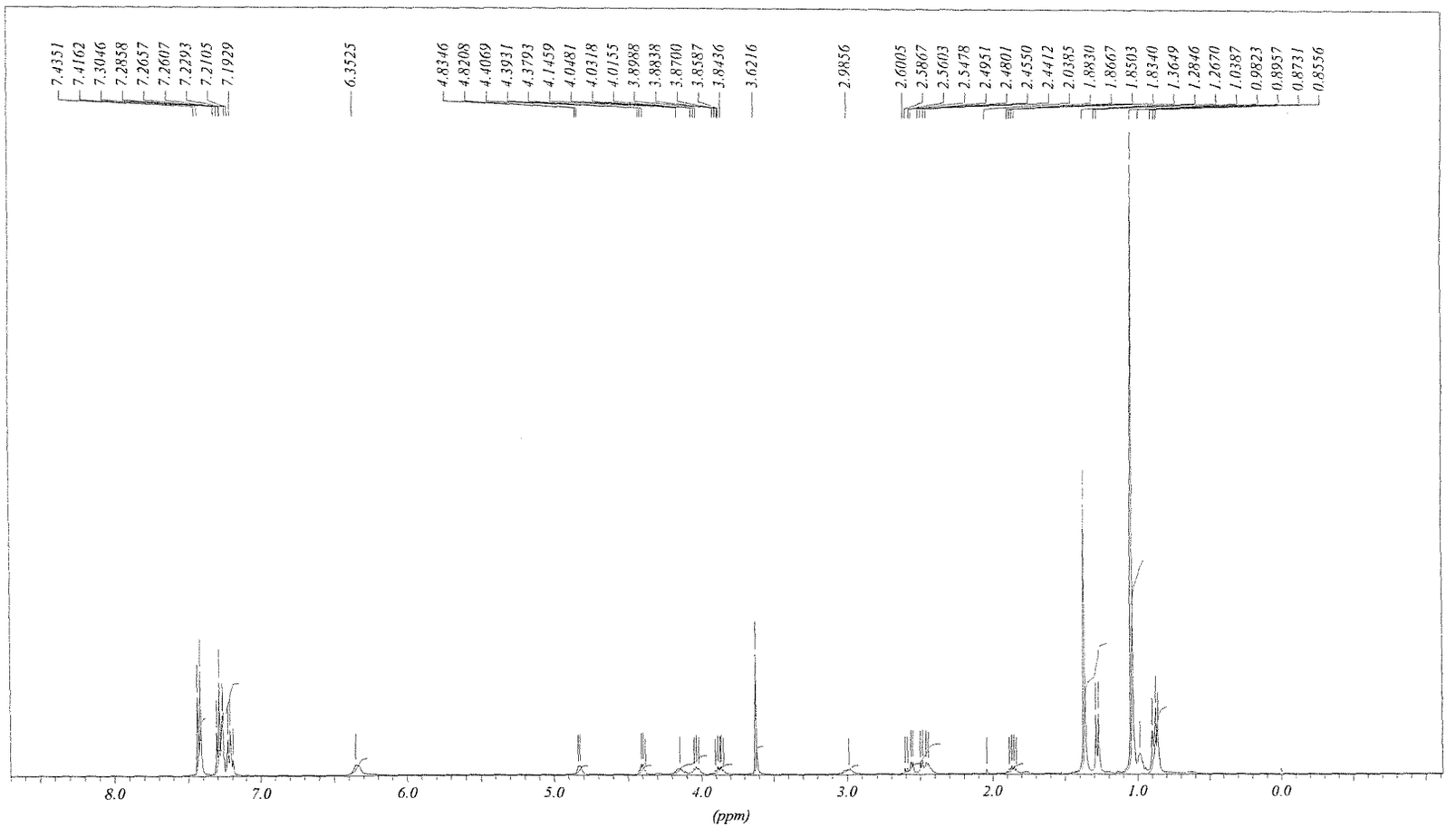
(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-5-methyl-hexanoic acid methyl ester (61)

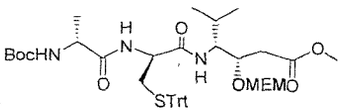




(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid methyl ester (62)

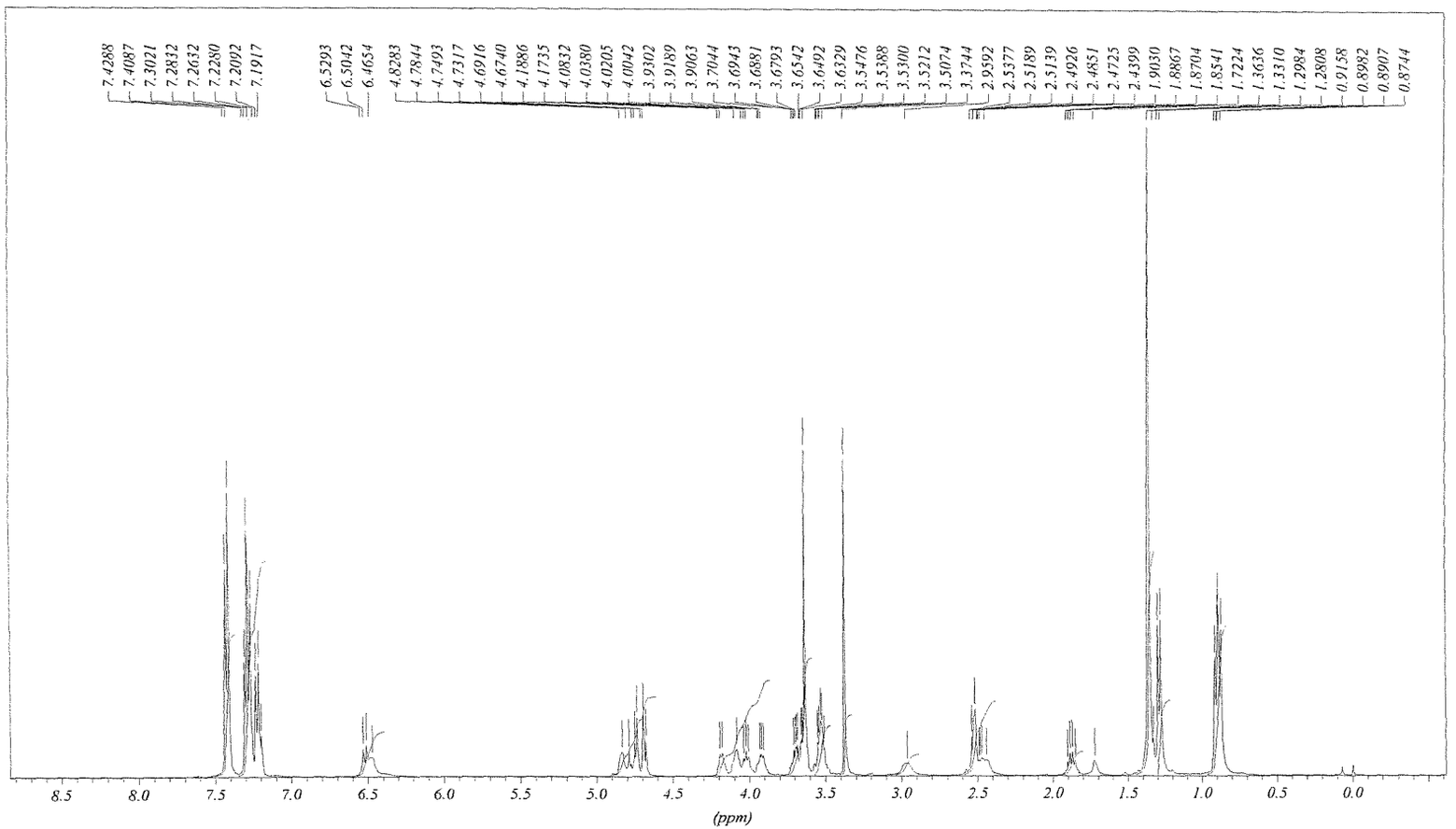
ayg3514001a1, ma1403ayg2 (1) 400MHz H

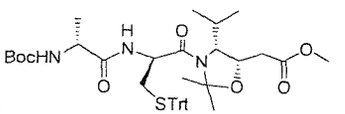




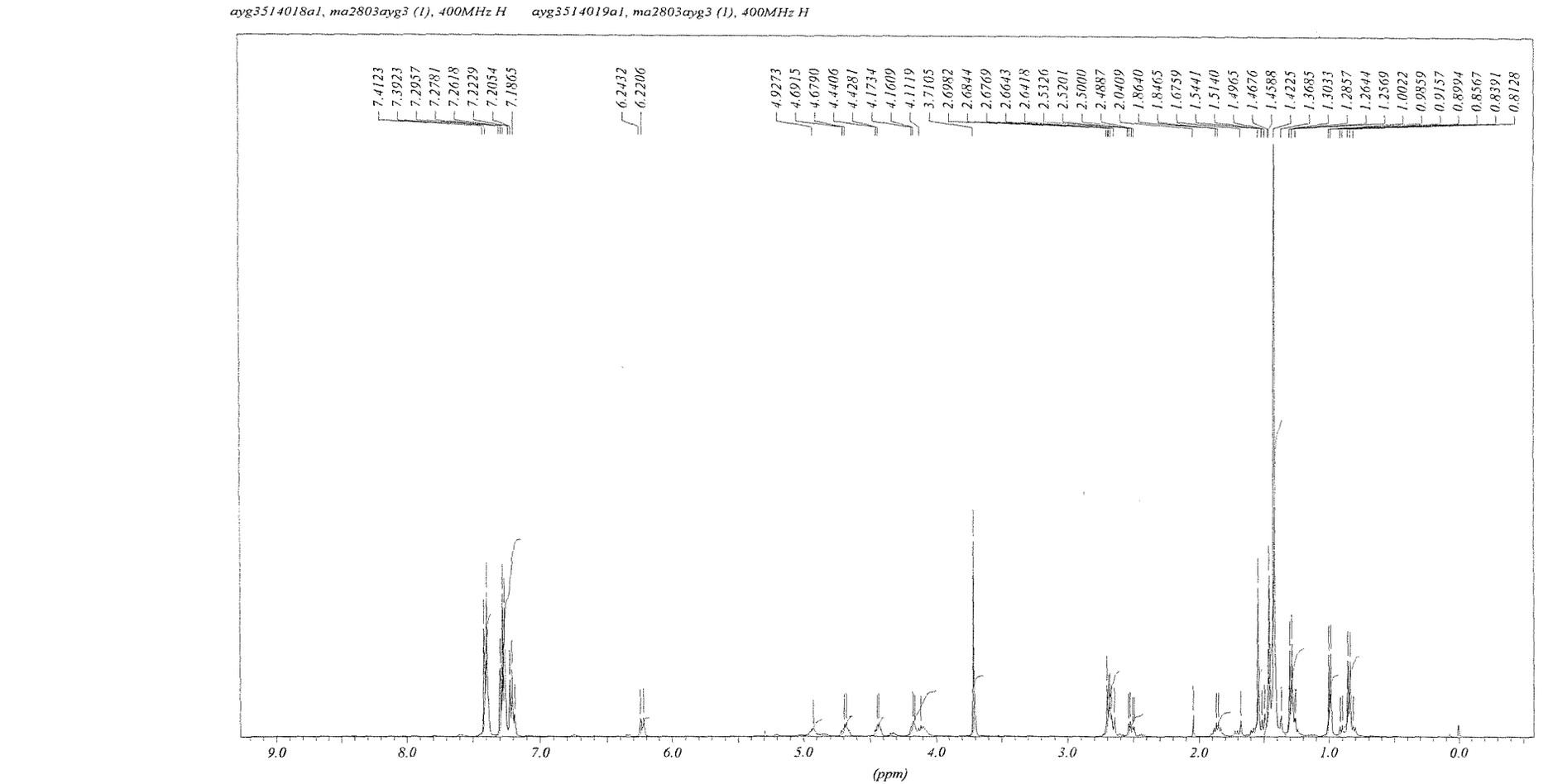
(3*S*,4*R*)-4-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-(2-methoxy-ethoxymethoxy)-5-methyl-hexanoic acid methyl ester (64)

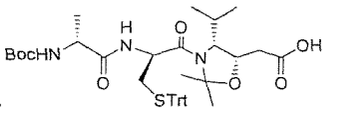
ayg3429100a1, Fe2304ayg2 (1) 400MHz H



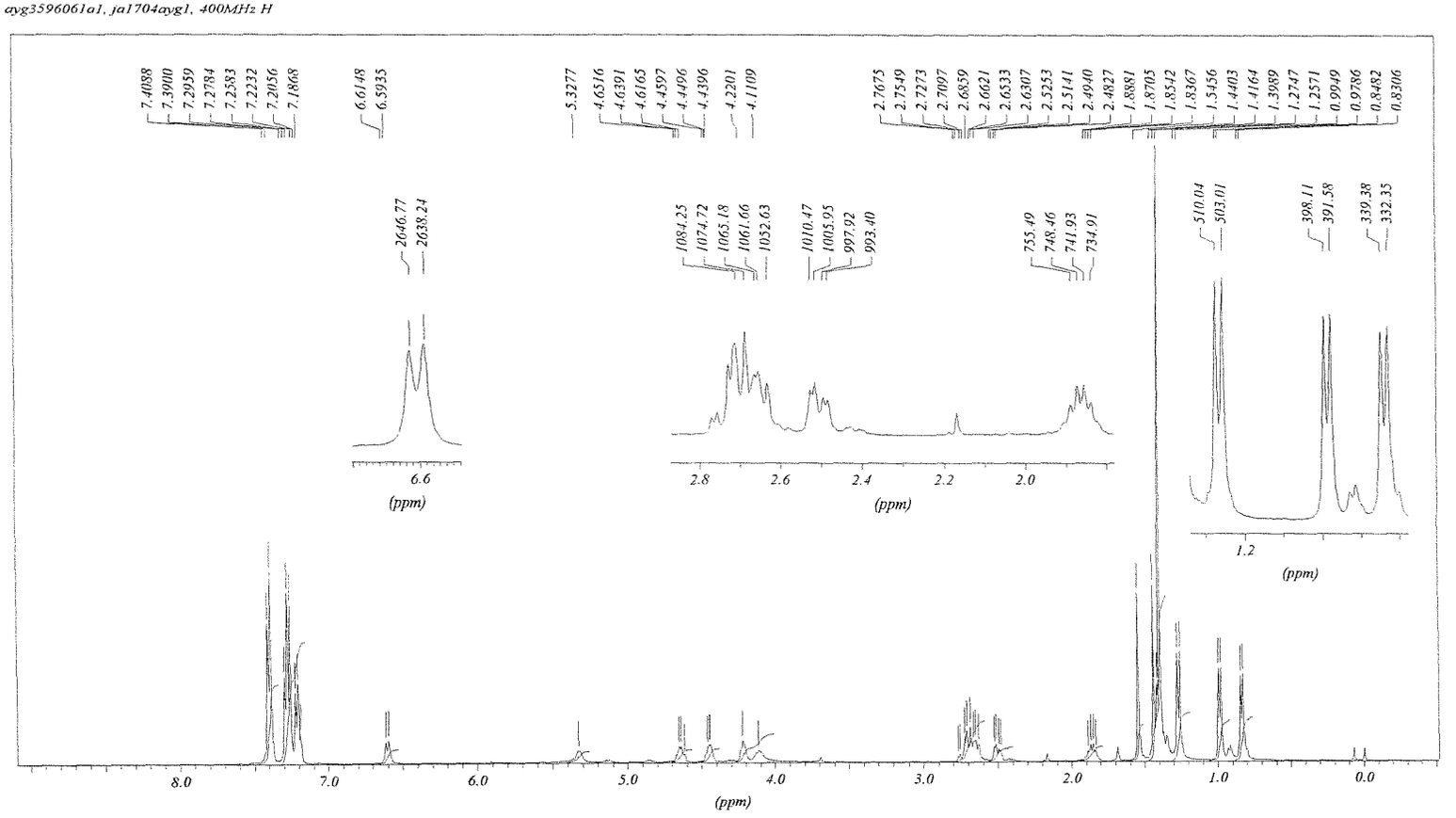


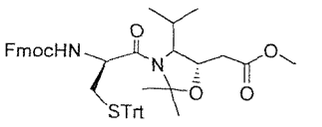
{(4*R*,5*S*)-3-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionyl]-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl}-acetic acid methyl ester (66)



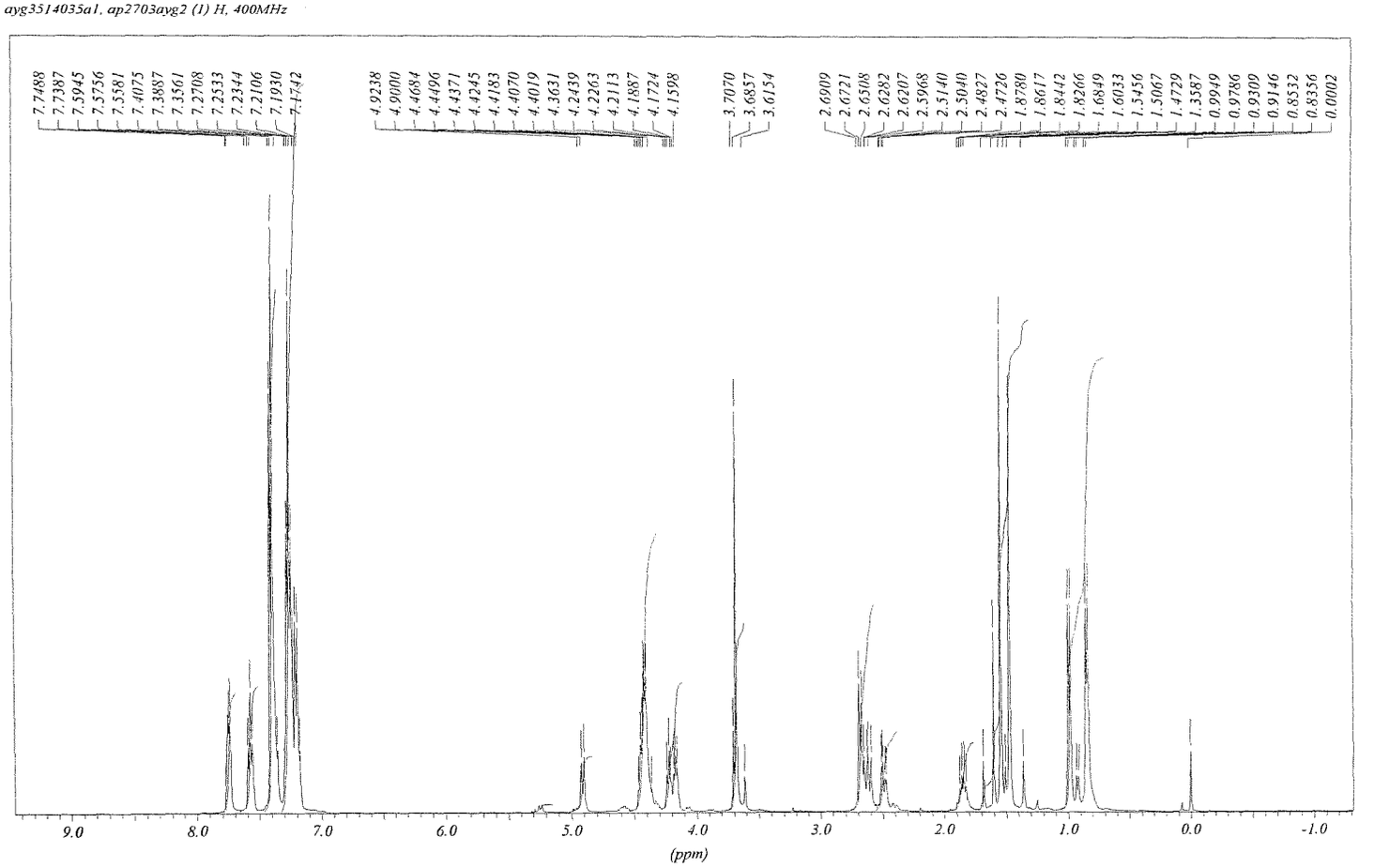


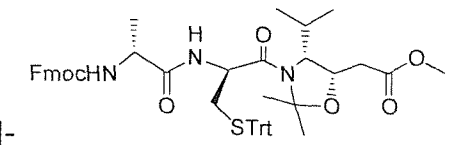
{(4*R*,5*S*)-3-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionyl]-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl}-acetic acid (67)



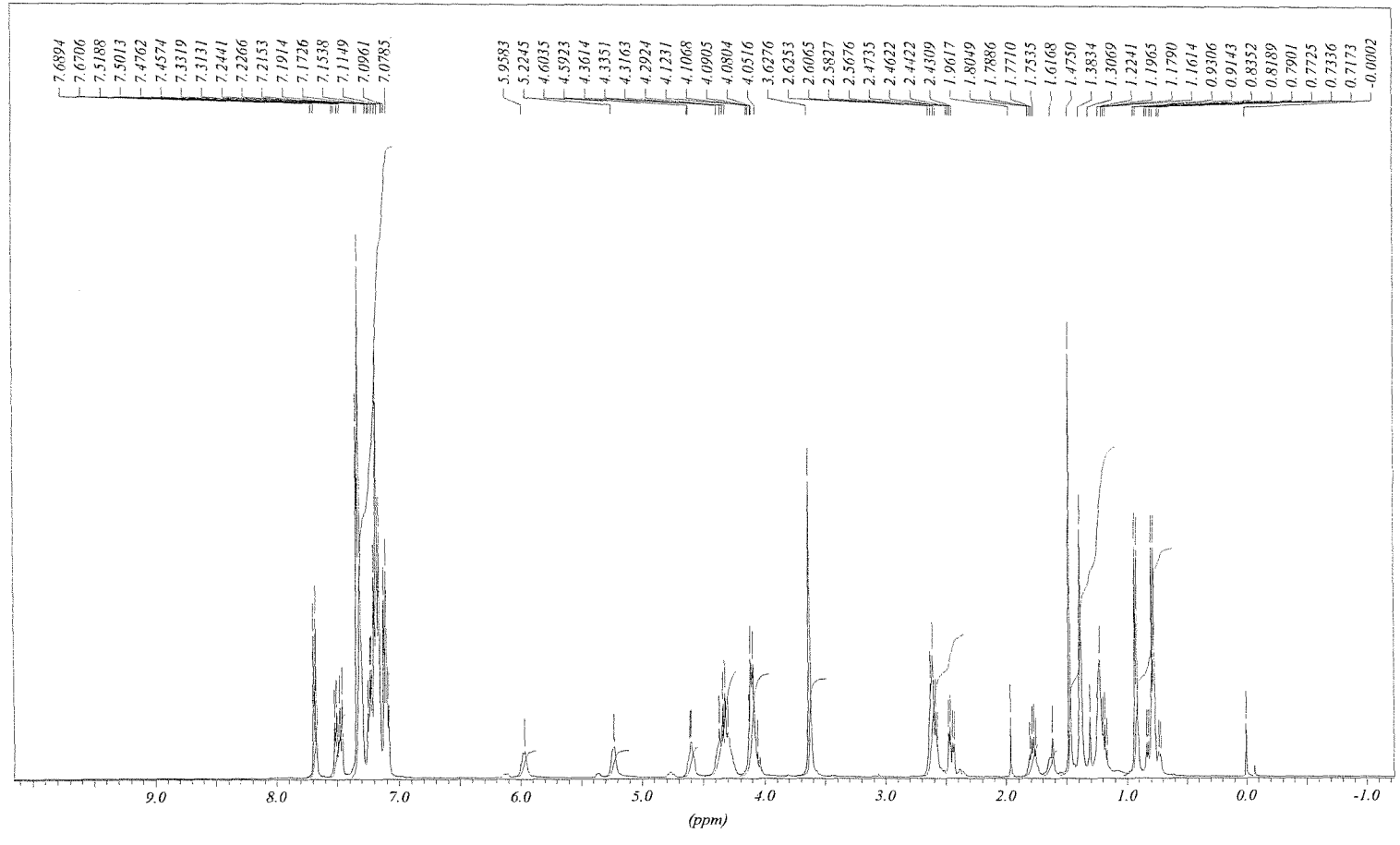


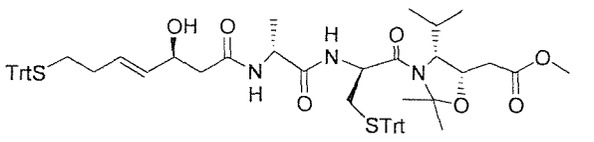
{(4*R*,5*S*)-3-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionyl]-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl}-acetic acid methyl ester (69)



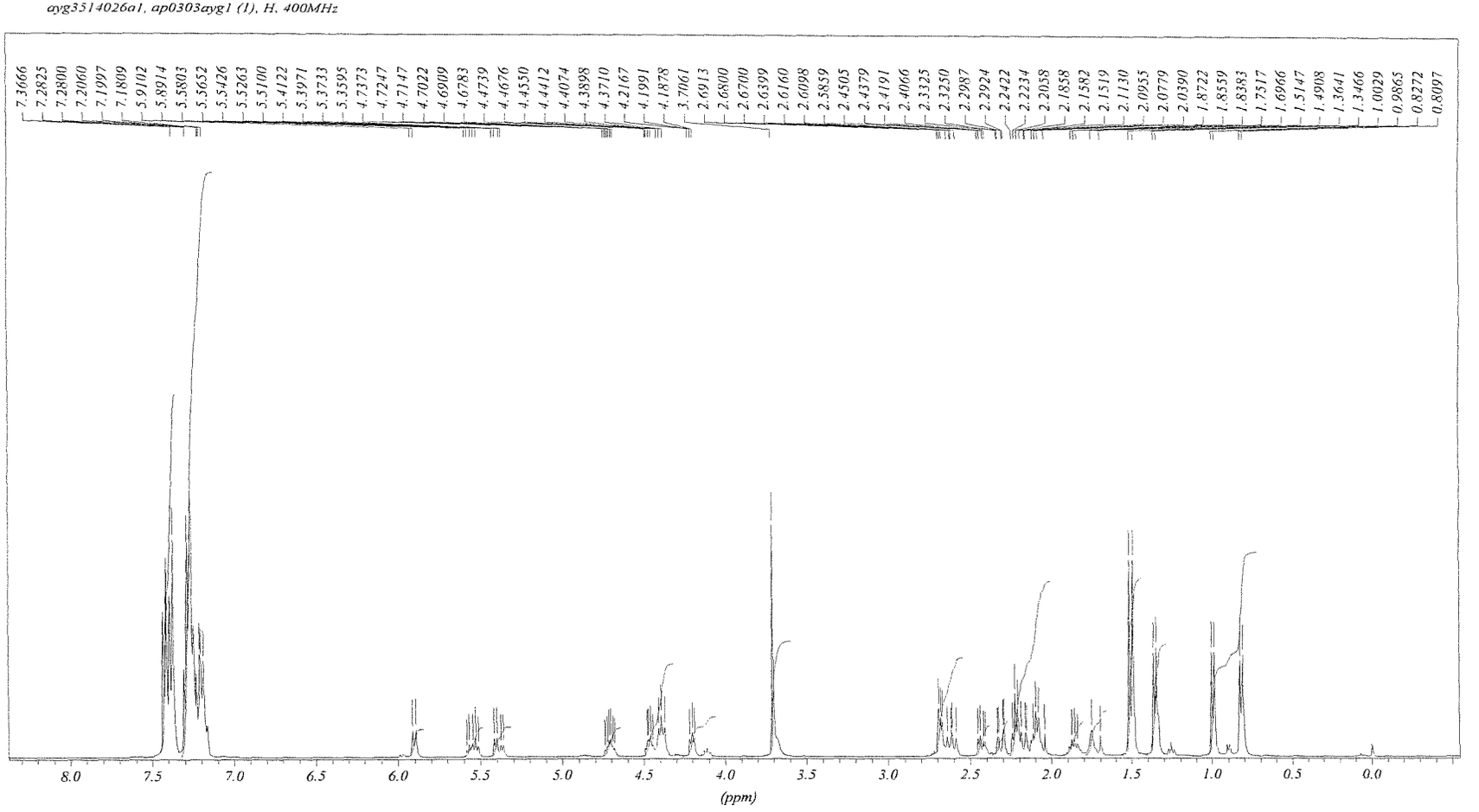


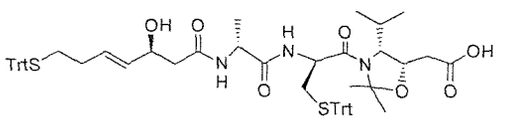
((4*R*,5*S*)-3-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-tritylsulfanyl-propionyl}-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl)-acetic acid methyl ester (70)



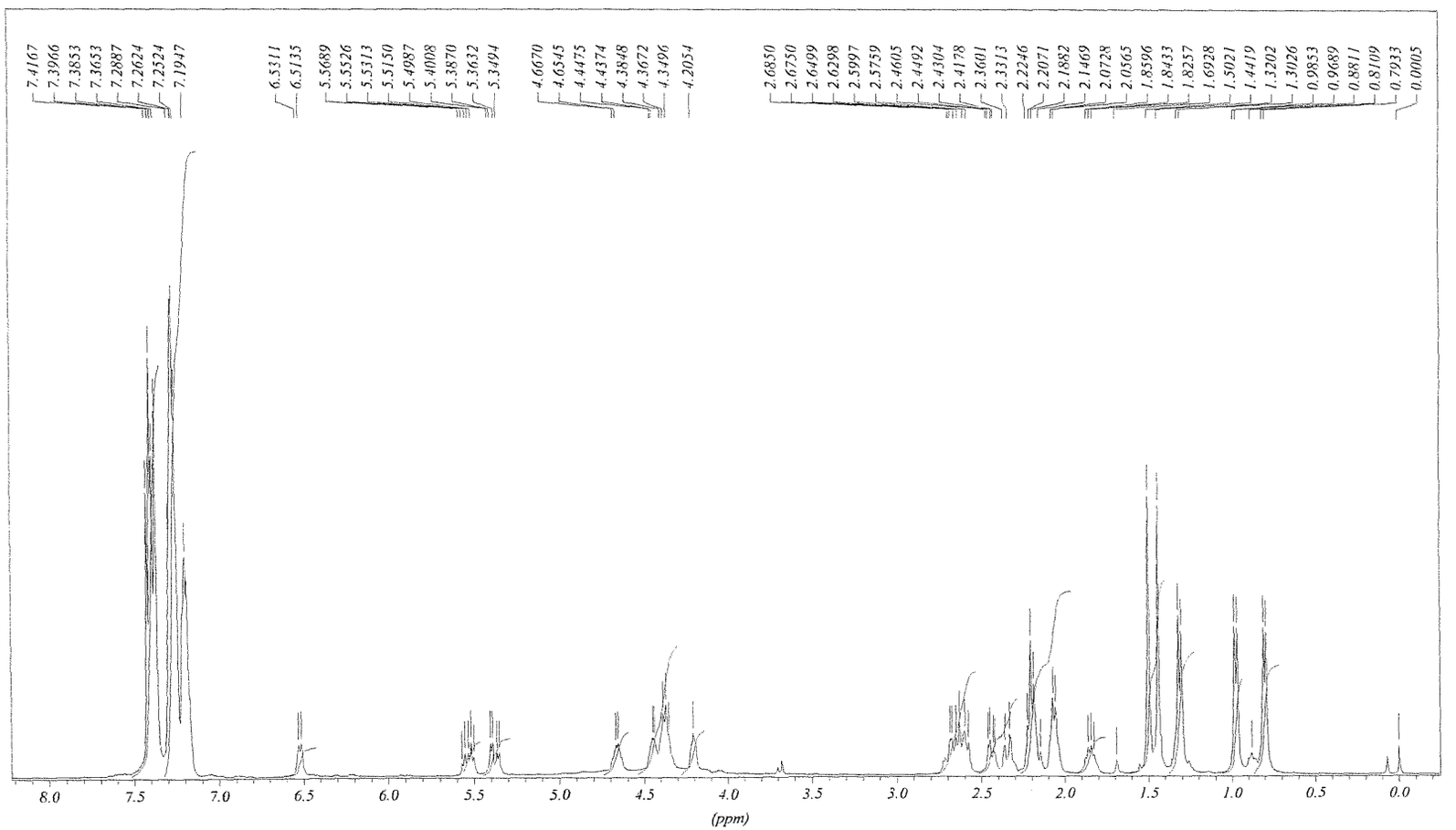


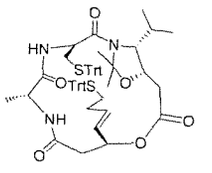
((4*R*,5*S*)-3-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionyl}-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl)-acetic acid methyl ester (71)



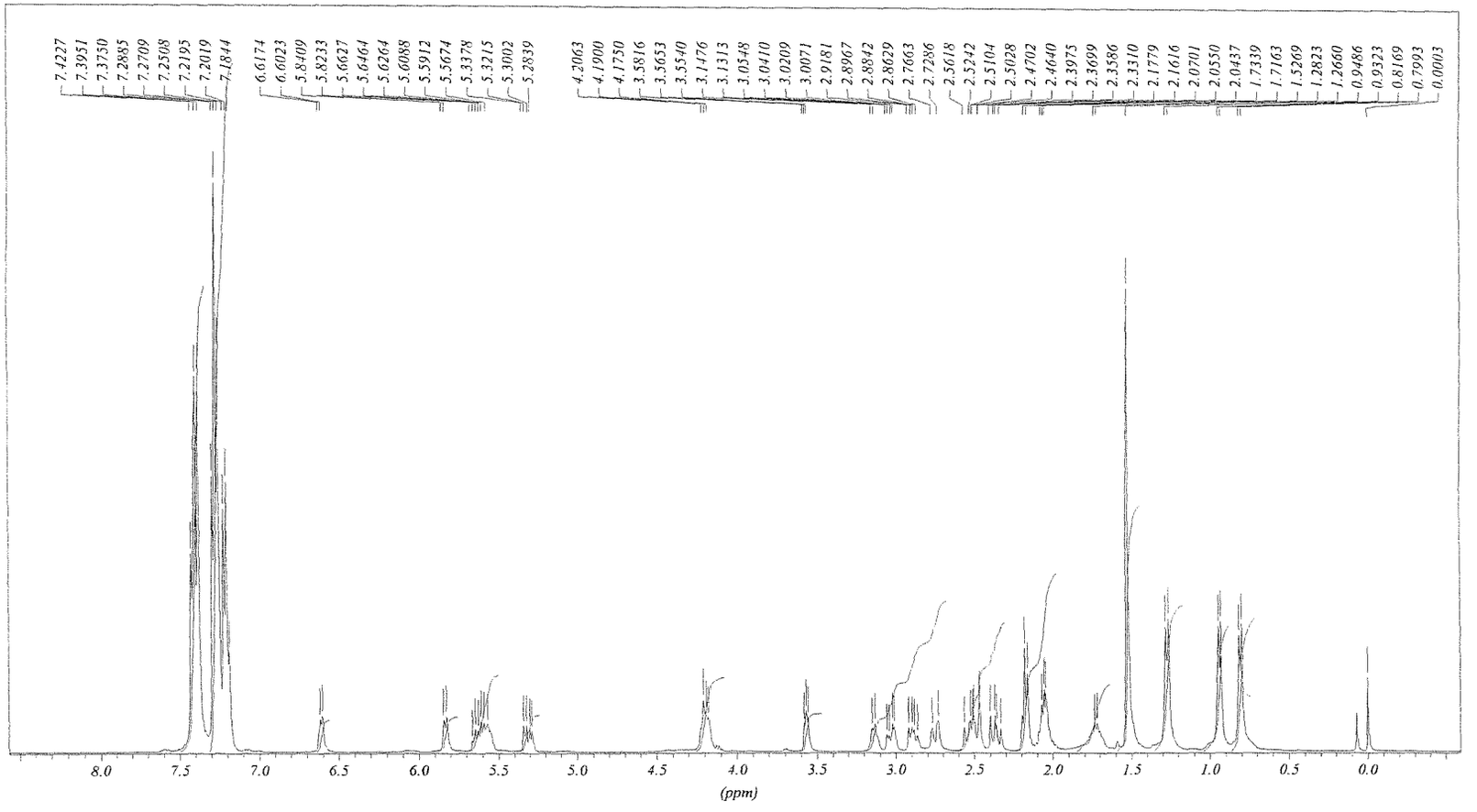


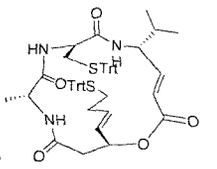
((4*R*,5*S*)-3-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionyl}-4-isopropyl-2,2-dimethyl-oxazolidin-5-yl)-acetic acid (72)





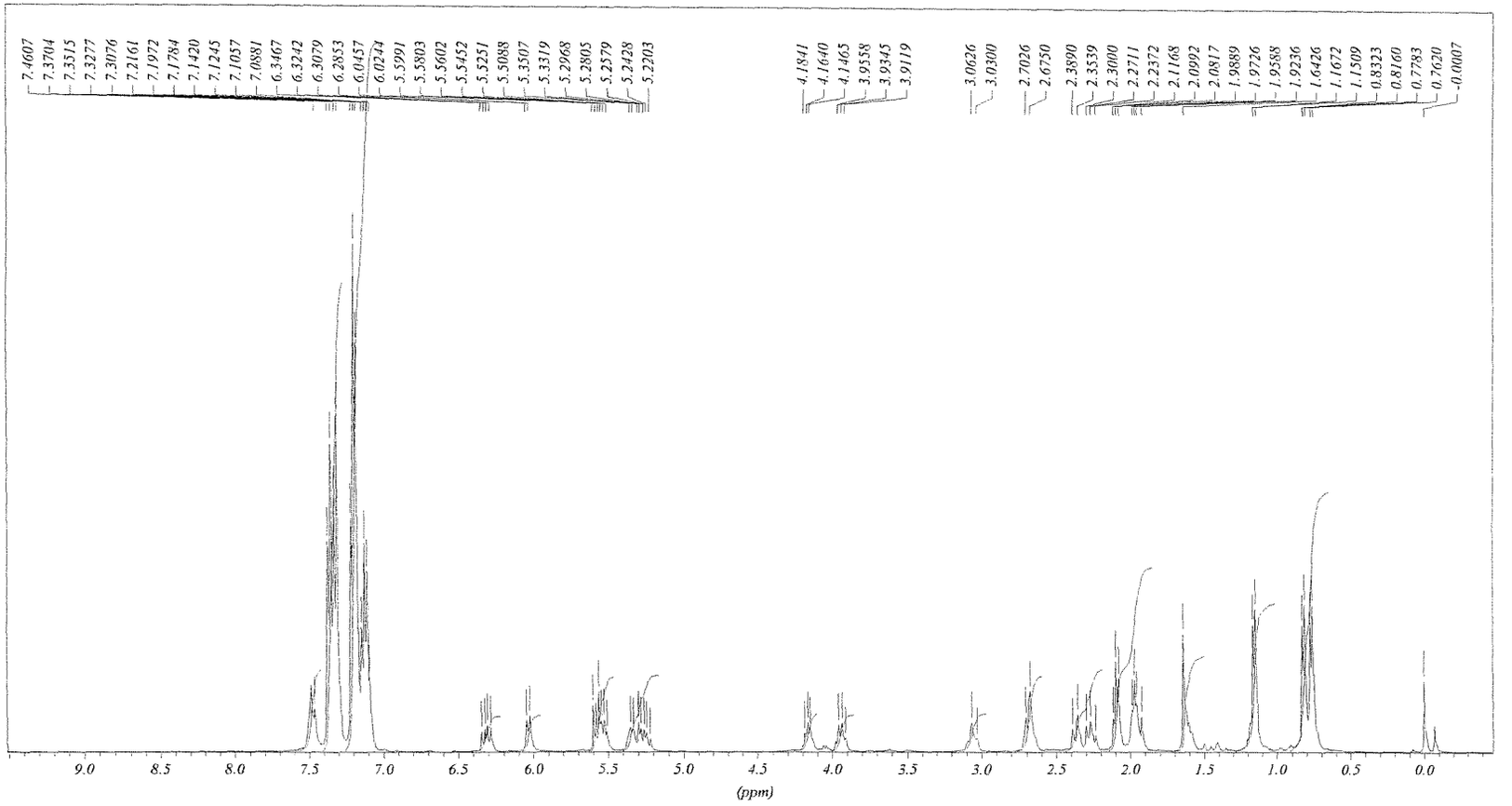
(3*R*,6*R*,10*S*,14*S*,17*R*)-17-Isopropyl-6,16,16-trimethyl-10-((*E*)-4-tritylsulfanyl-but-1-enyl)-3-tritylsulfanylmethyl-11,15-dioxa-1,4,7-triaza-icyclo[12.2.1]heptadecane-2,5,8,12-tetraone (73)

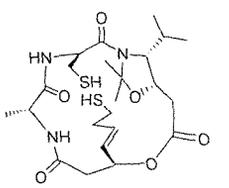




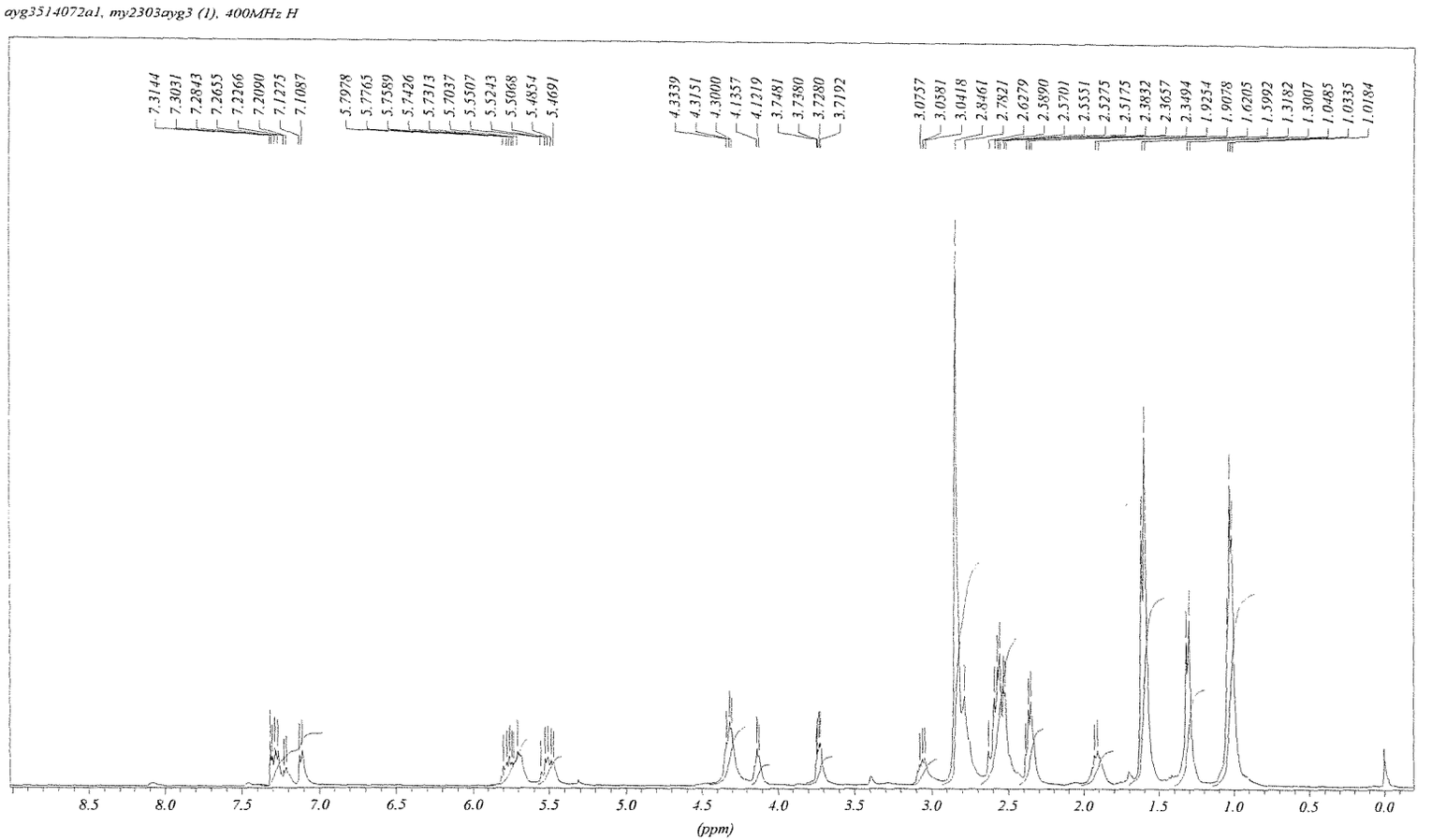
(*E*)-(2*S*,6*R*,9*S*,12*R*)-12-Isopropyl-6-methyl-2-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadec-13-ene-4,7,10,15-tetraone (76)

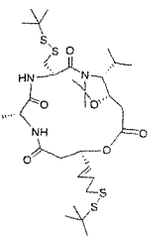
cyg3514063a1, my1103cyg2 (1), 400MHz H



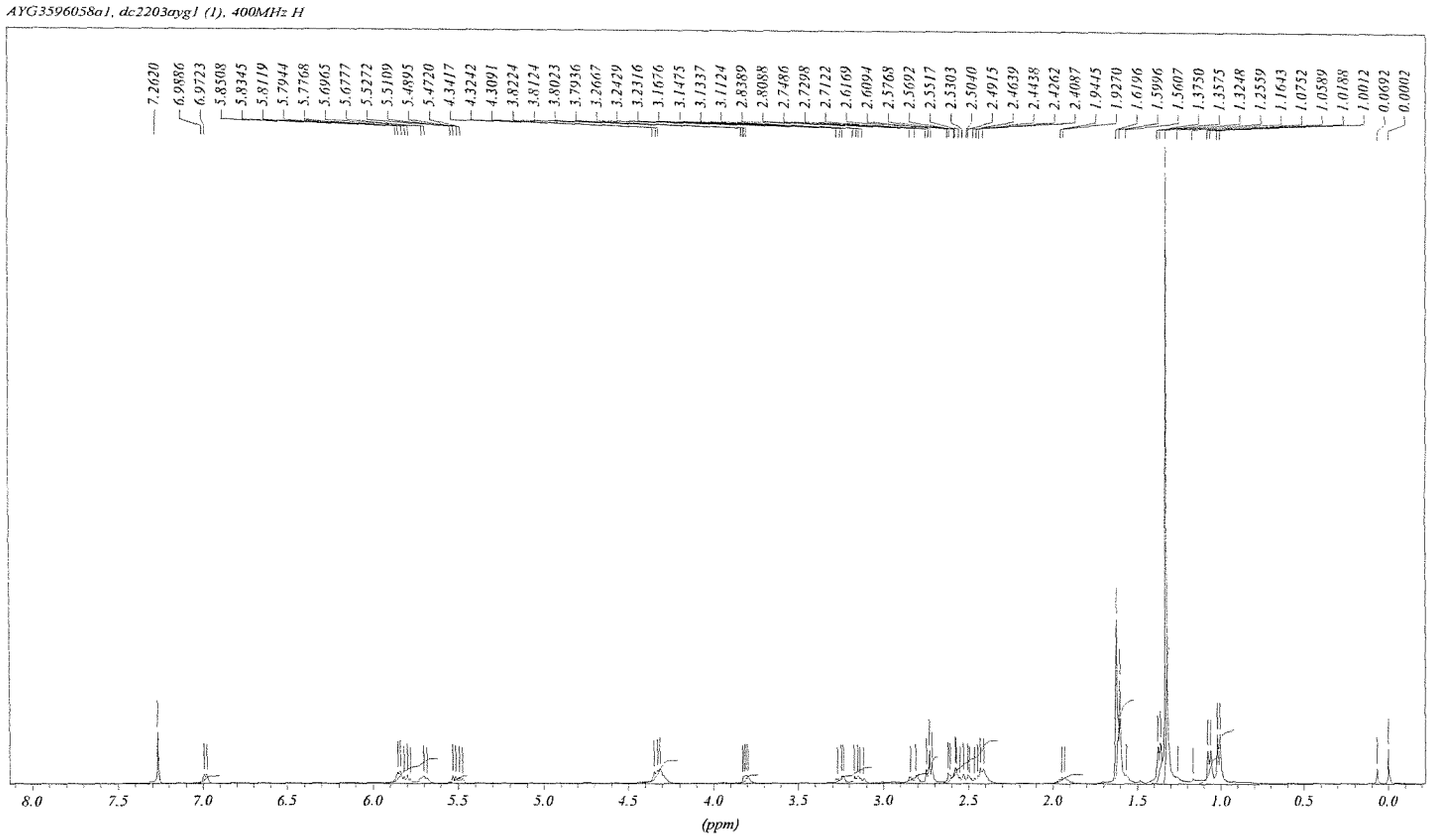


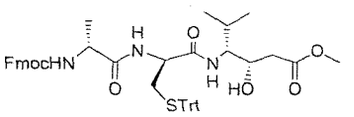
(3*S*,6*R*,10*S*,14*S*,17*R*)-17-Isopropyl-10-((*E*)-4-mercapto-but-1-enyl)-3-ercaptomethyl-6,16,16-trimethyl-11,15-dioxa-1,4,7-triaza-bicyclo[12.2.1]heptadecane-2,5,8,12-tetraone (77)



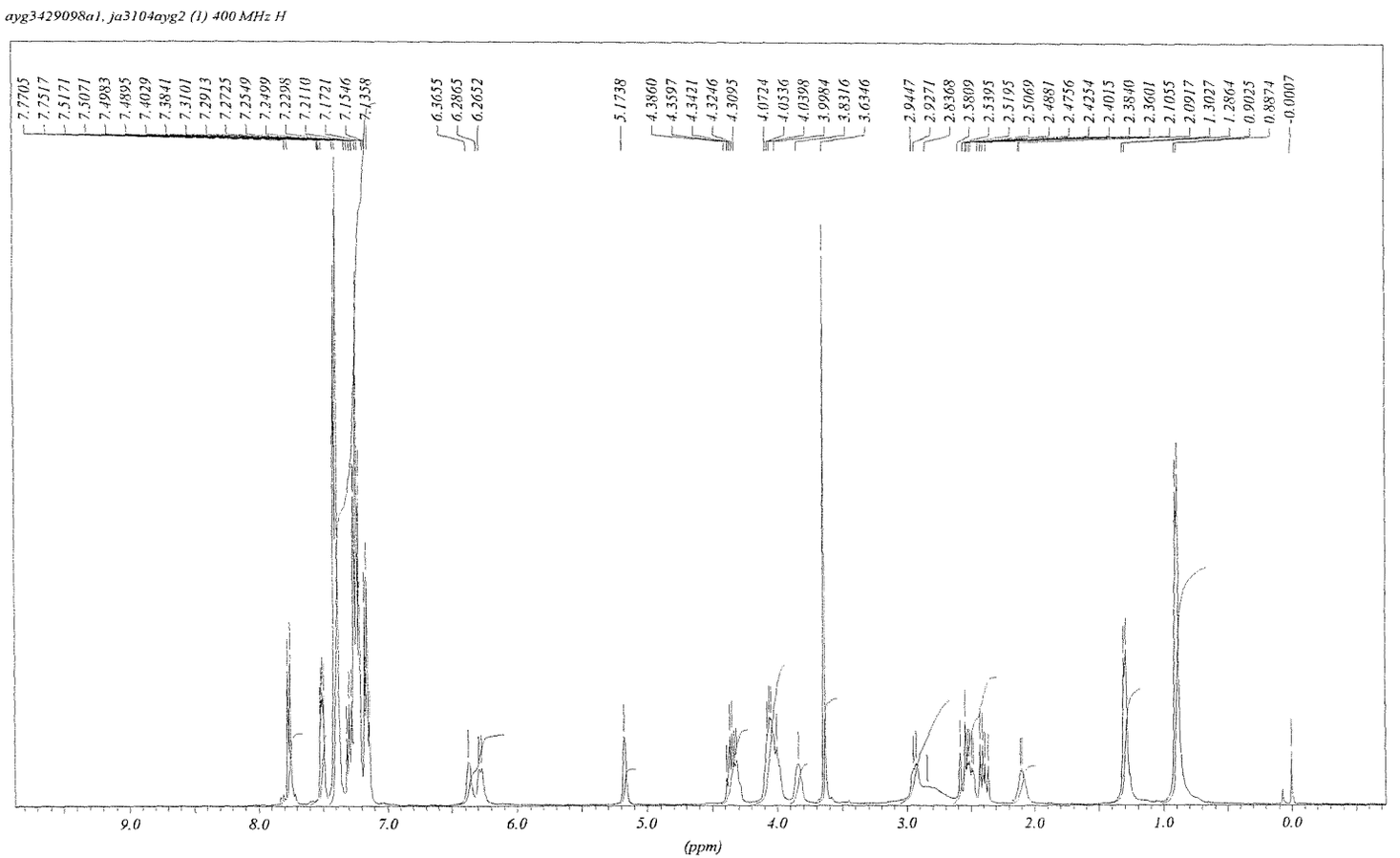


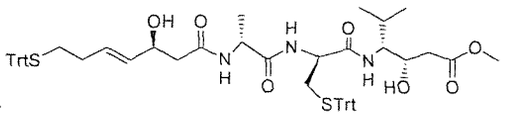
(3*S*,6*R*,10*S*,14*S*,17*R*)-10-((*E*)-4-*tert*-Butyldisulfanyl-but-1-enyl)-3-*tert*-butyldisulfanylmethyl-17-isopropyl-6,16,16-trimethyl-11,15-dioxa-1,4,7-triaza-bicyclo[12.2.1]heptadecane-2,5,8,12-tetraone (78)



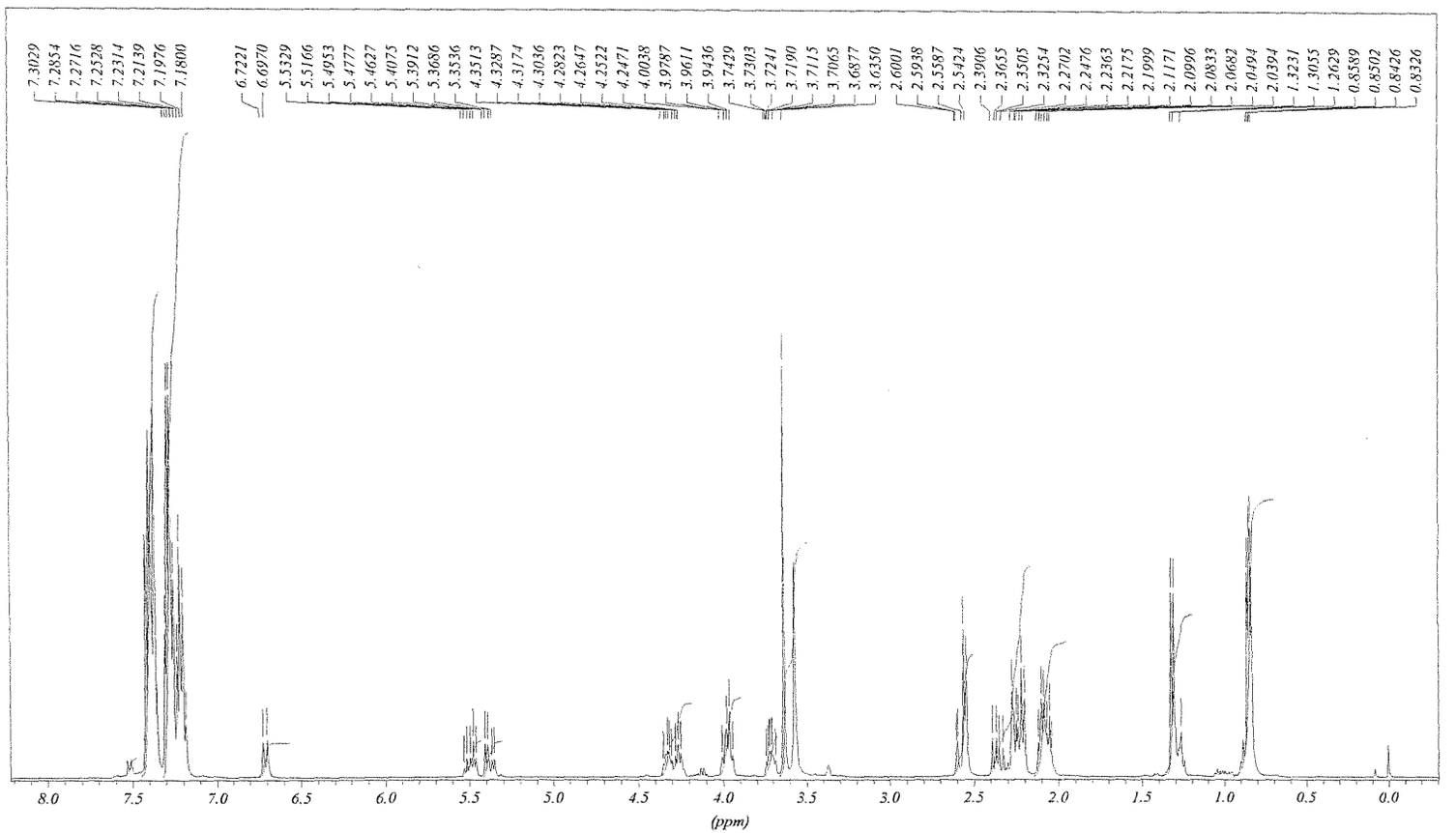


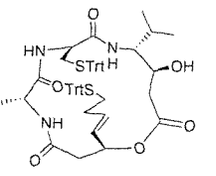
(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propiony lamino]-3-tritylsulfanyl-propionylamino}-3-hydroxy-5-methyl-hexanoic acid methyl ester (79)



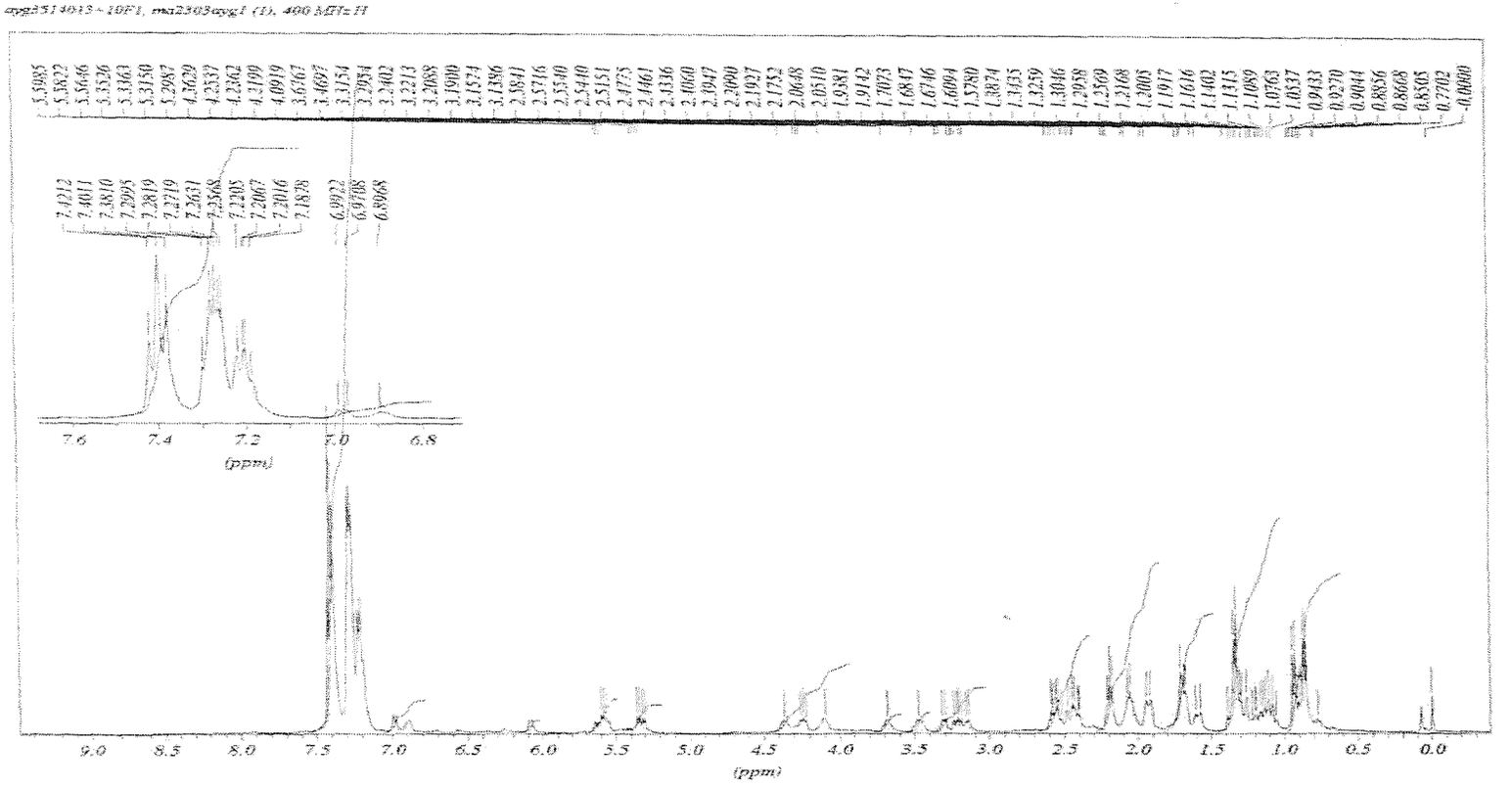


(3*S*,4*R*)-3-Hydroxy-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-hexanoic acid methyl ester (80)

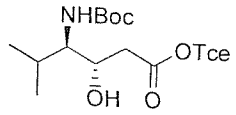


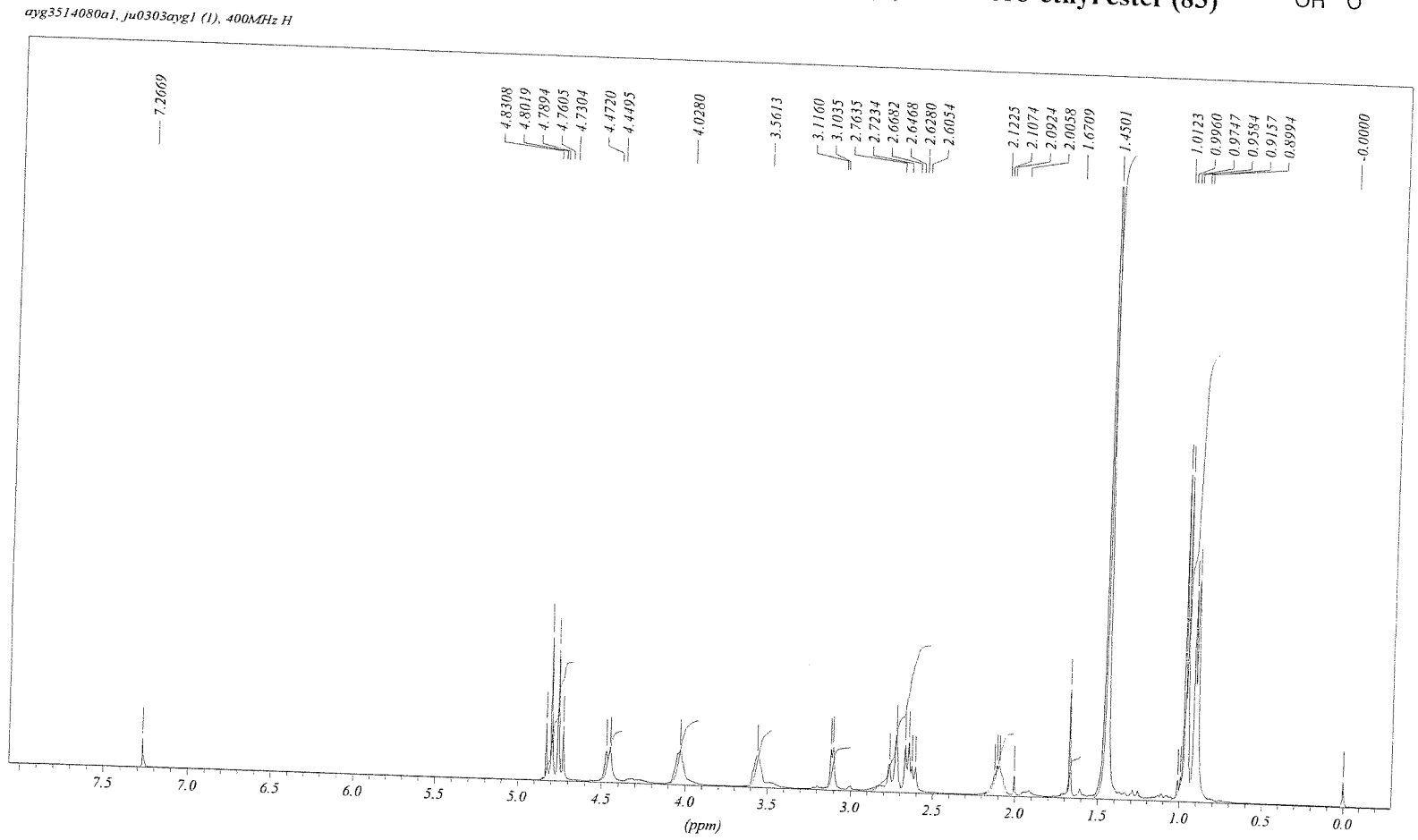


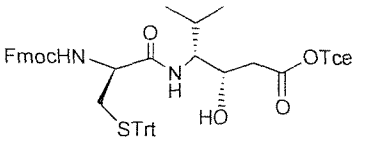
(2*S*,6*R*,9*S*,12*R*,13*S*)-13-Hydroxy-12-isopropyl-6-methyl-2-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,10,15-tetraone (82)



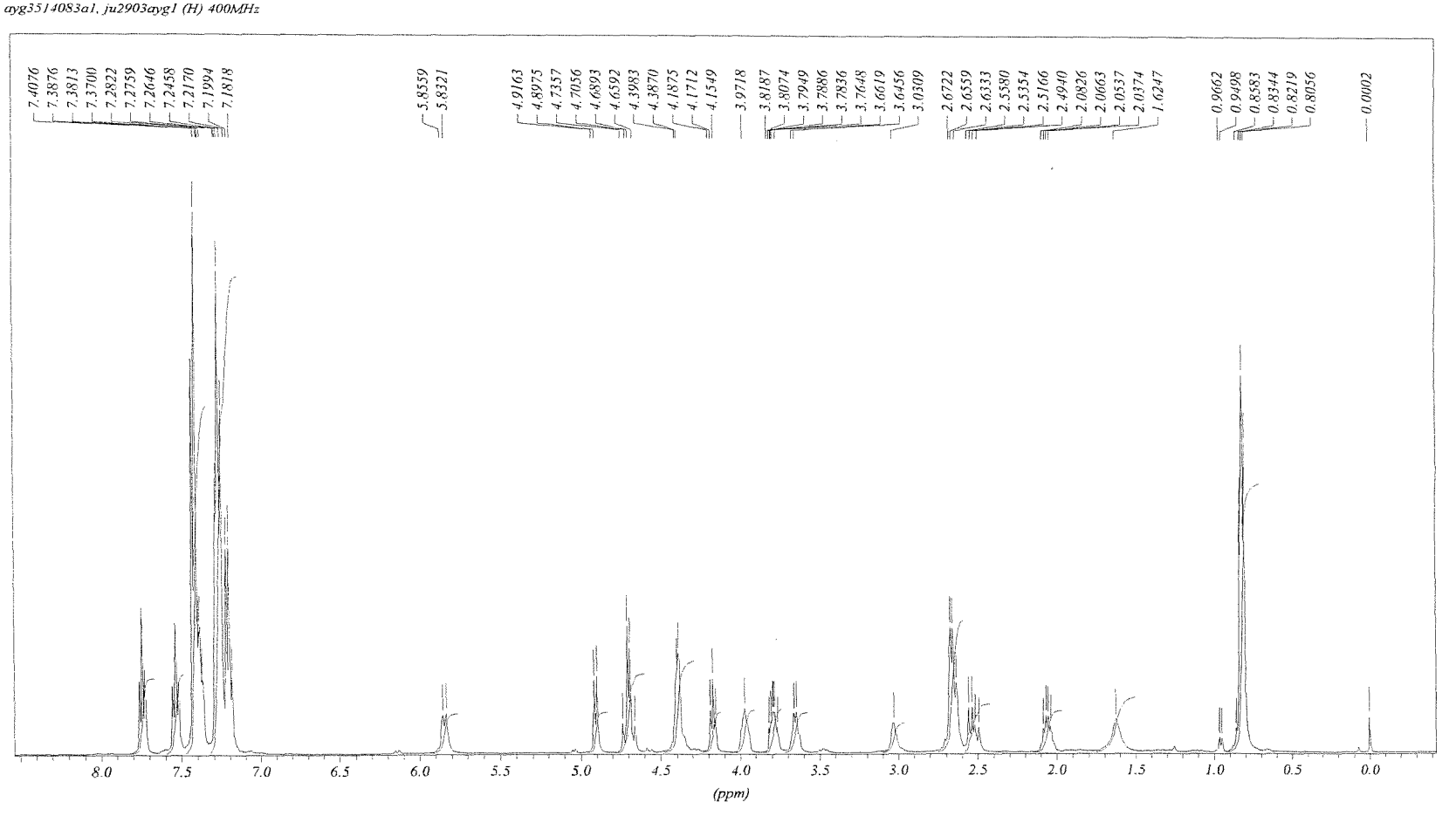
(3S,4R)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-methyl-hexanoic acid 2,2,2-trichloro-ethyl ester (83)

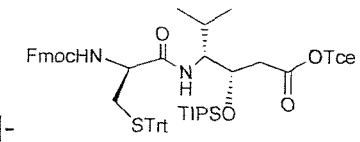






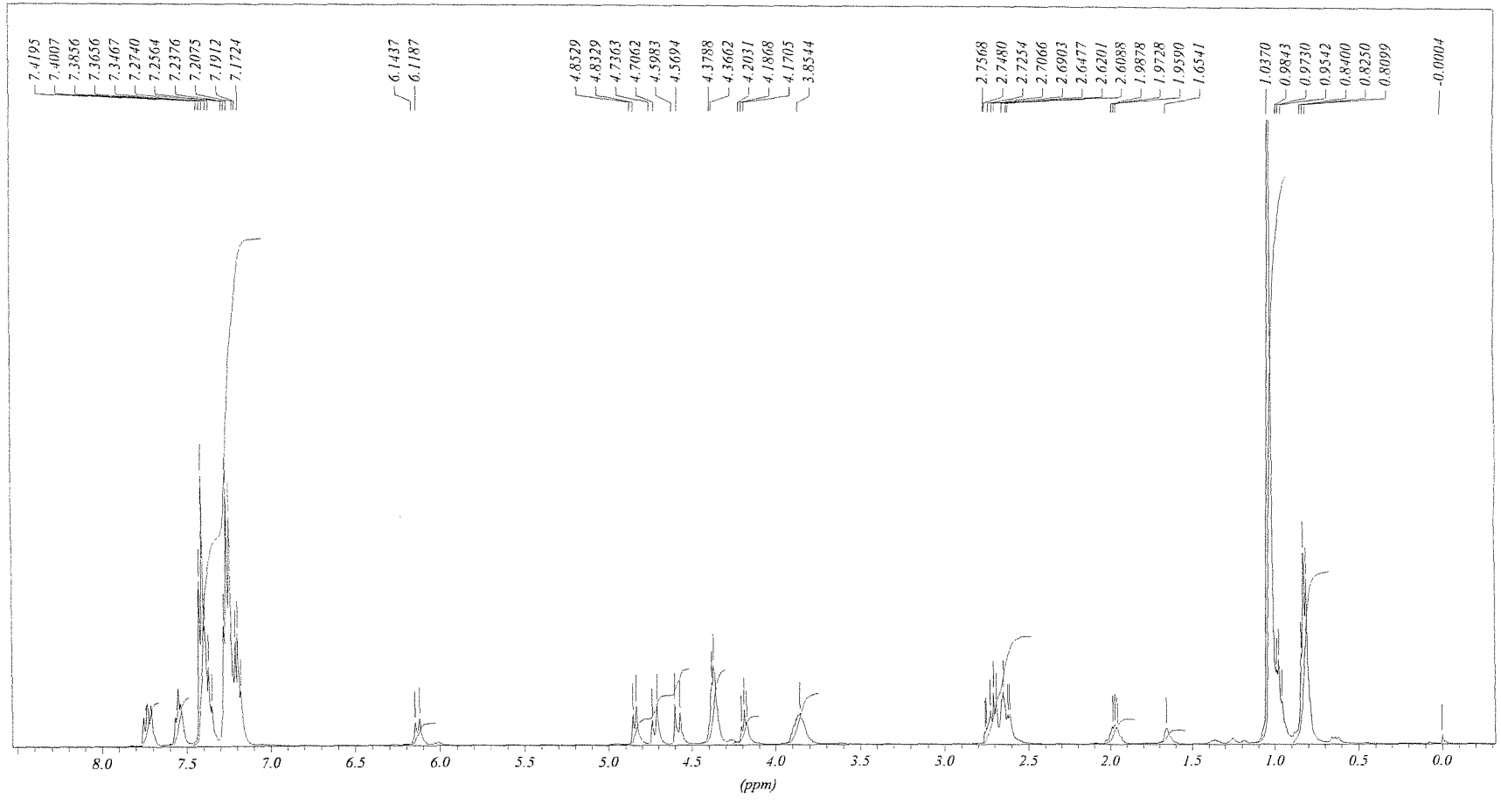
(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-5-methyl-hexanoic acid 2,2,2-trichloro-ethyl ester (84)

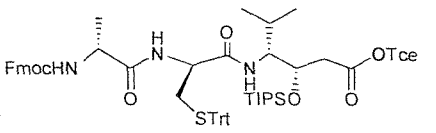




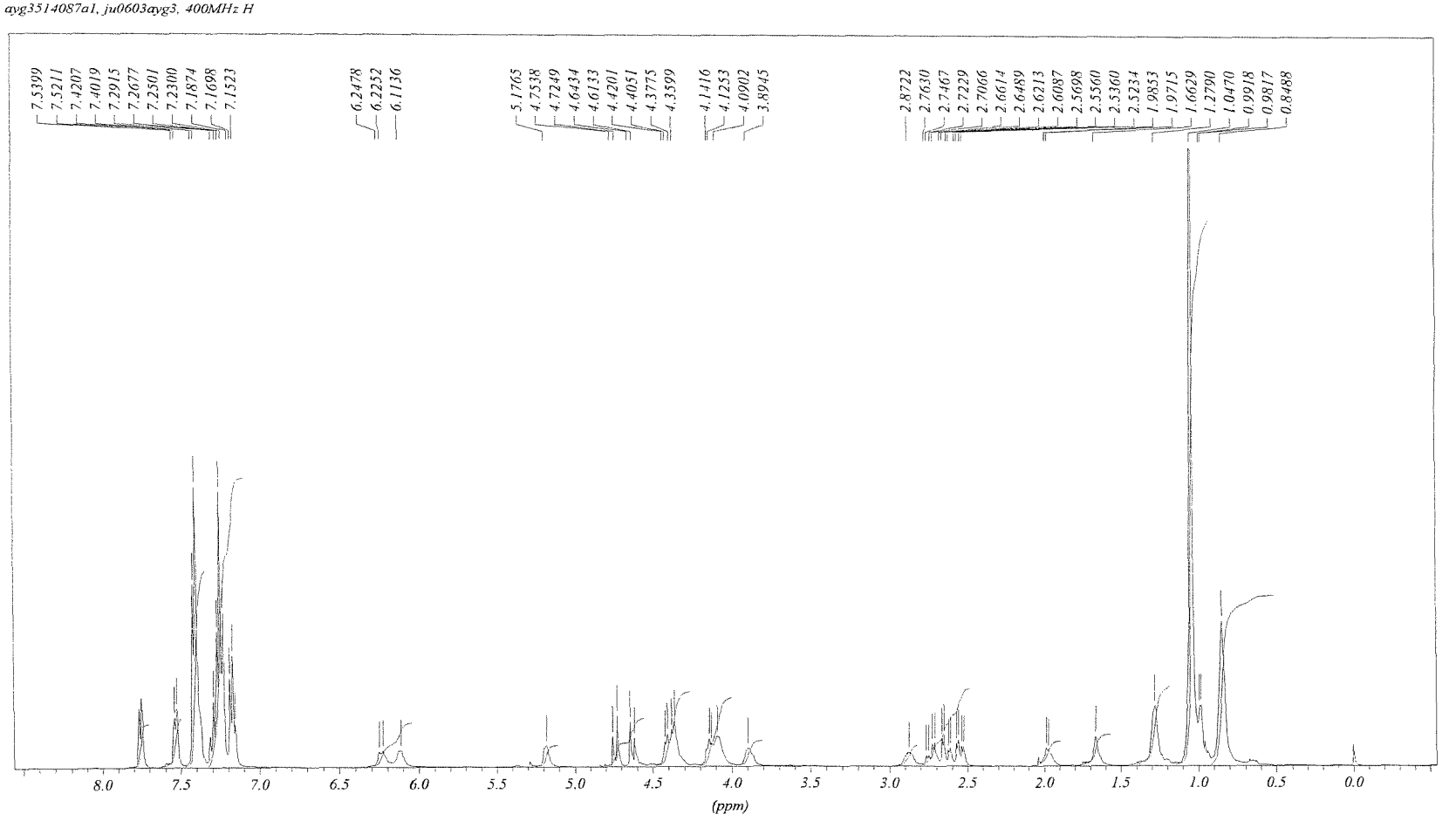
(3*S*,4*R*)-4-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester (85)

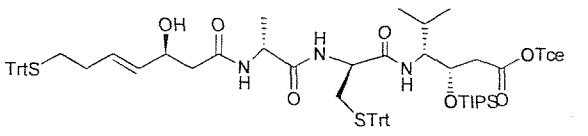
ayg3514084a1, ju0503ayg4, H, 400MHz



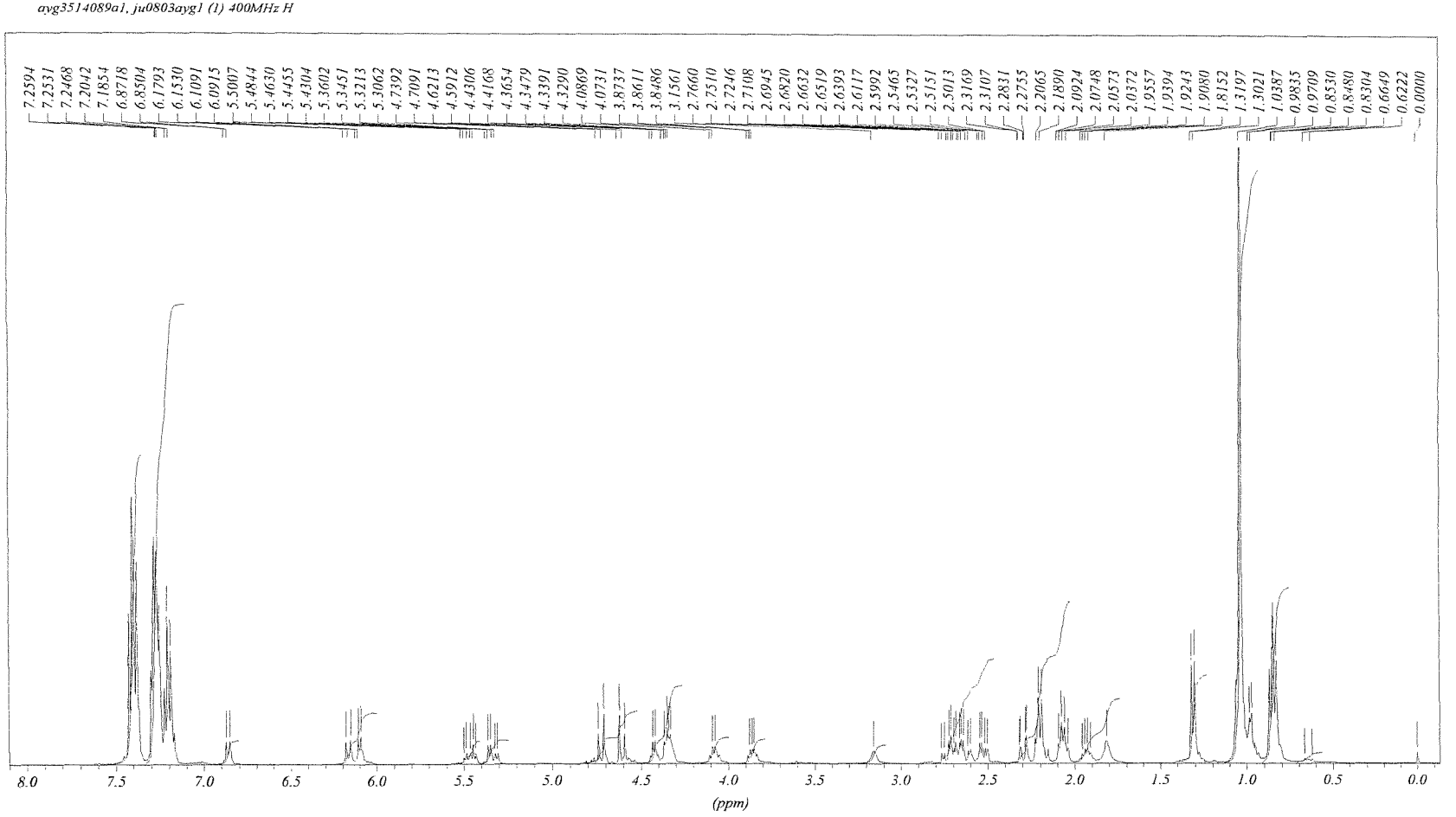


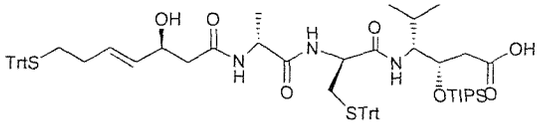
(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester (86)



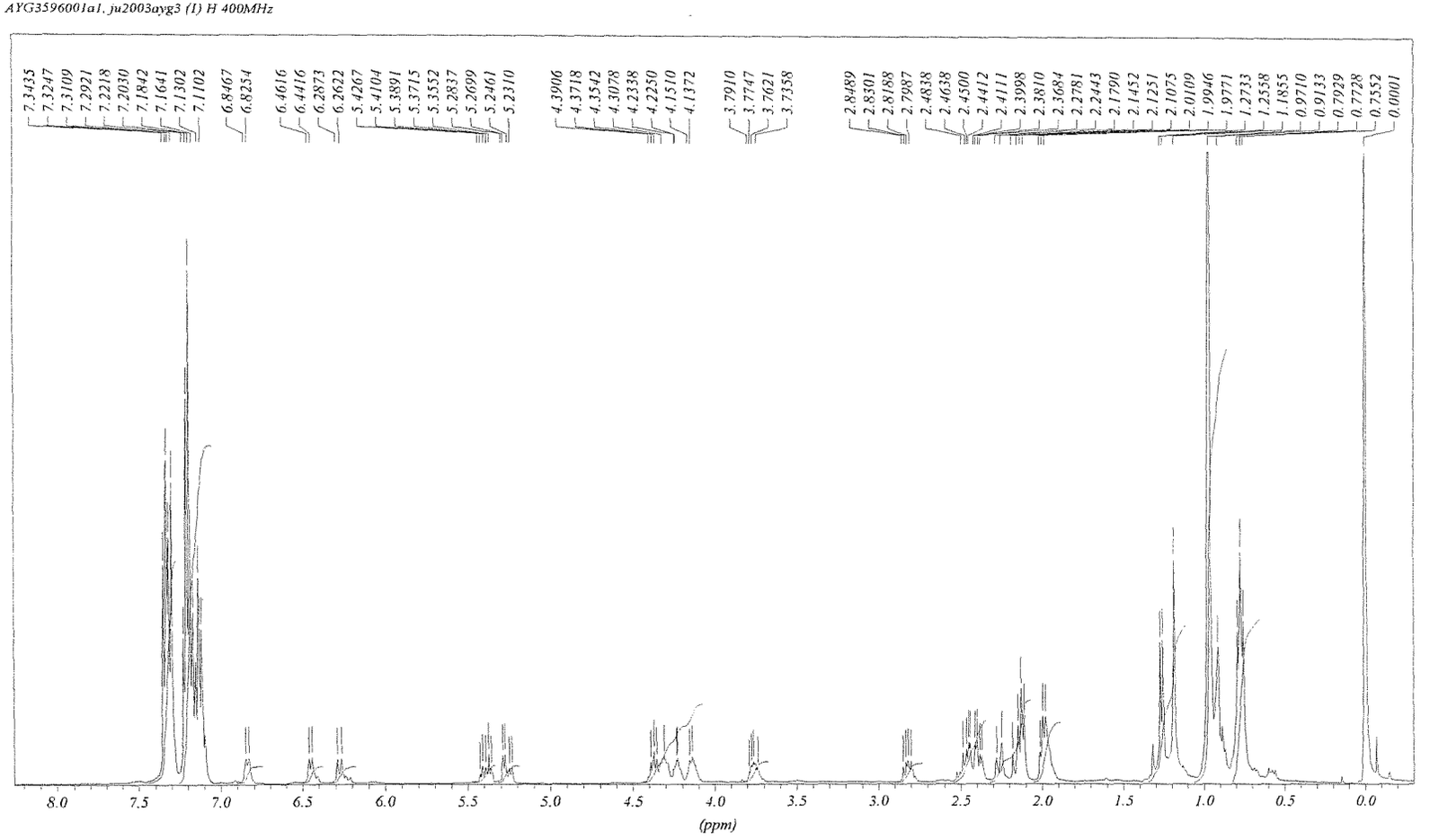


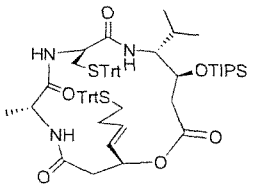
(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino] -3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester(87)



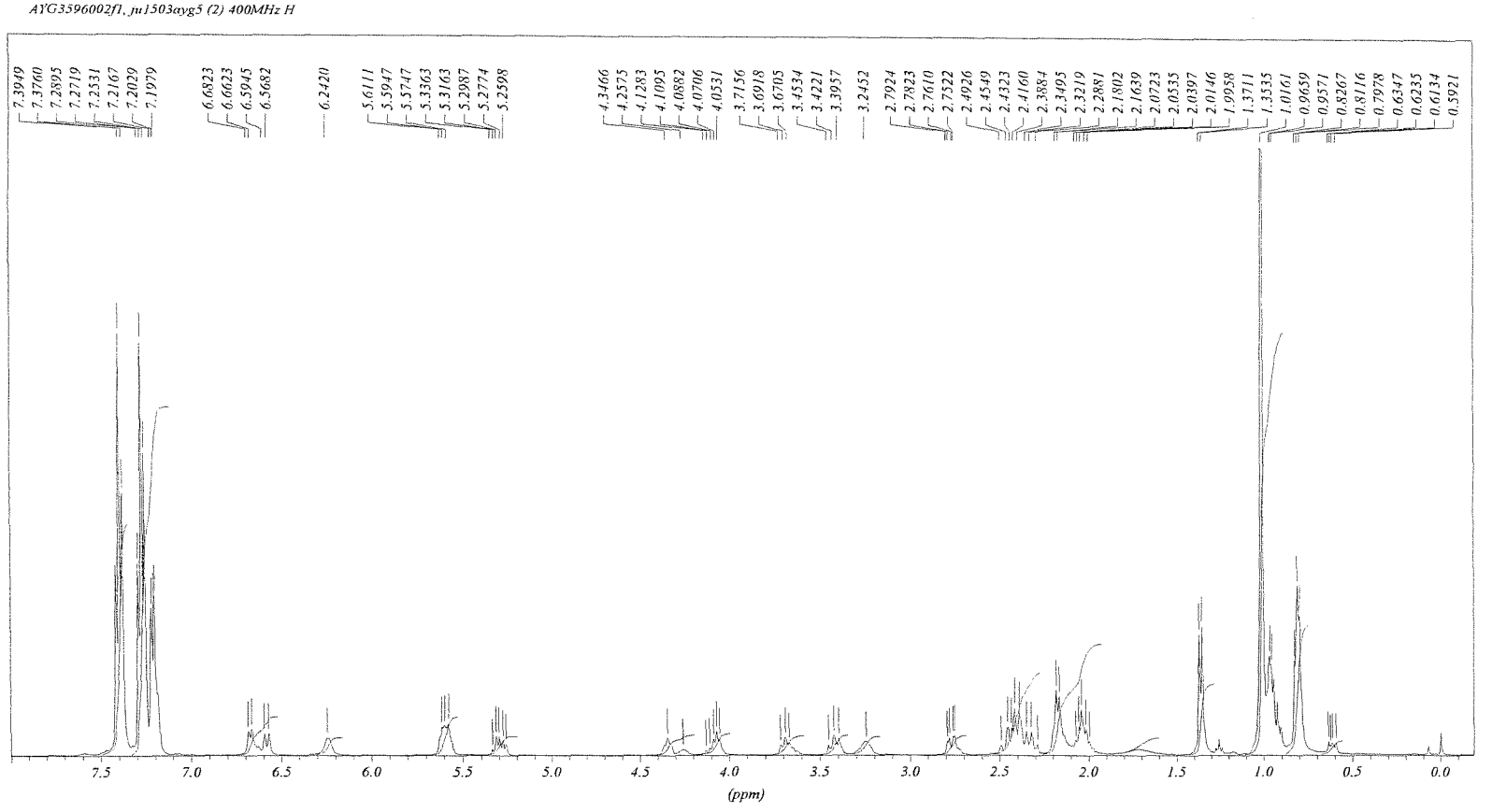


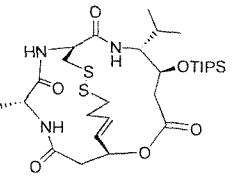
(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid (88)



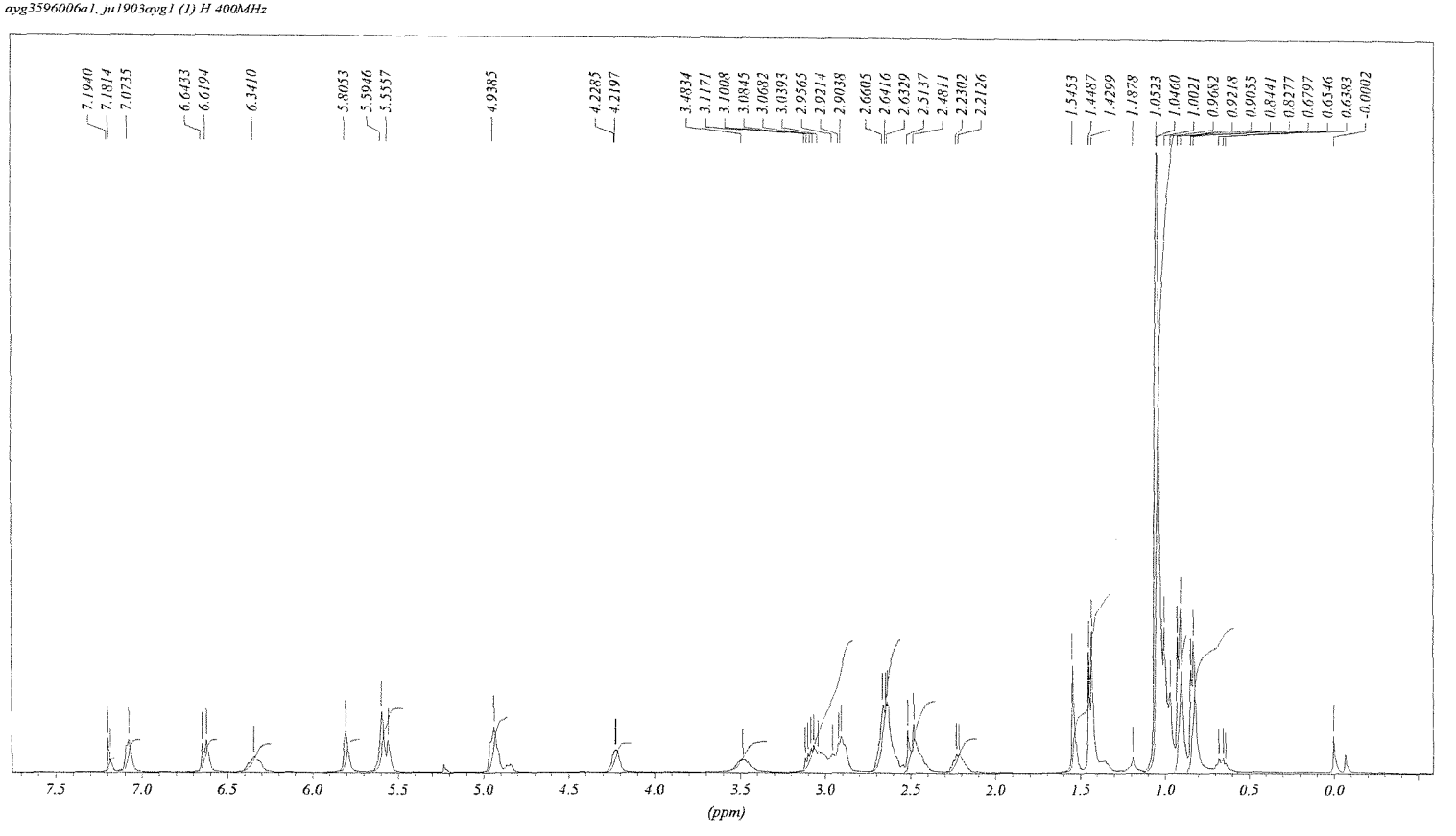


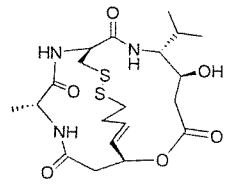
(2*S*,6*R*,9*S*,12*R*,13*S*)-12-Isopropyl-6-methyl-13-triisopropylsilanyloxy-2- ((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,10,15-tetraone (89)





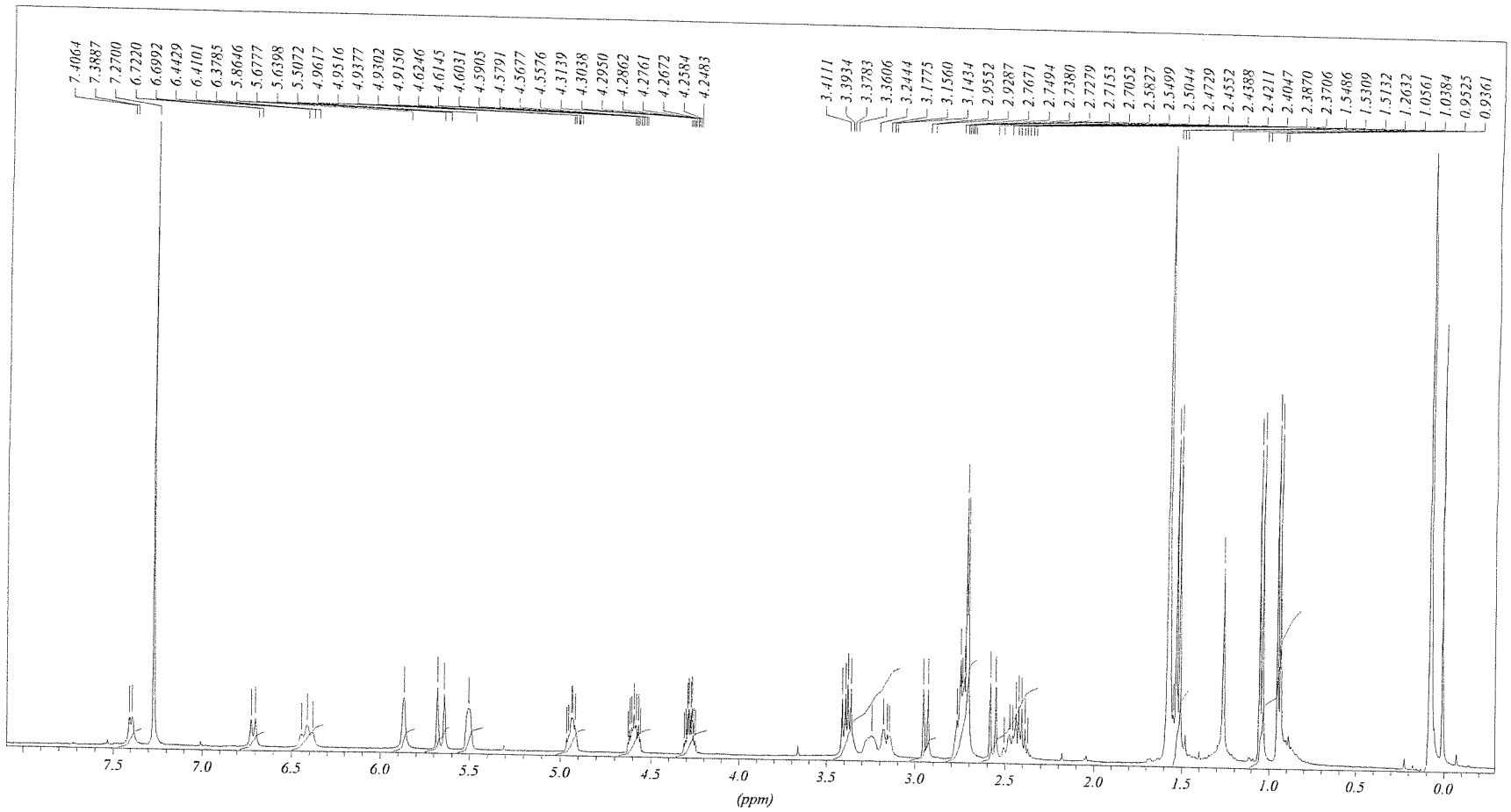
(*E*)-(1*S*,5*S*,6*R*,9*S*,20*R*)-6-Isopropyl-20-methyl-5-triisopropylsilanyloxy-2-oxa-11,12-dithia-7,19,22-triaza-bicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (90)



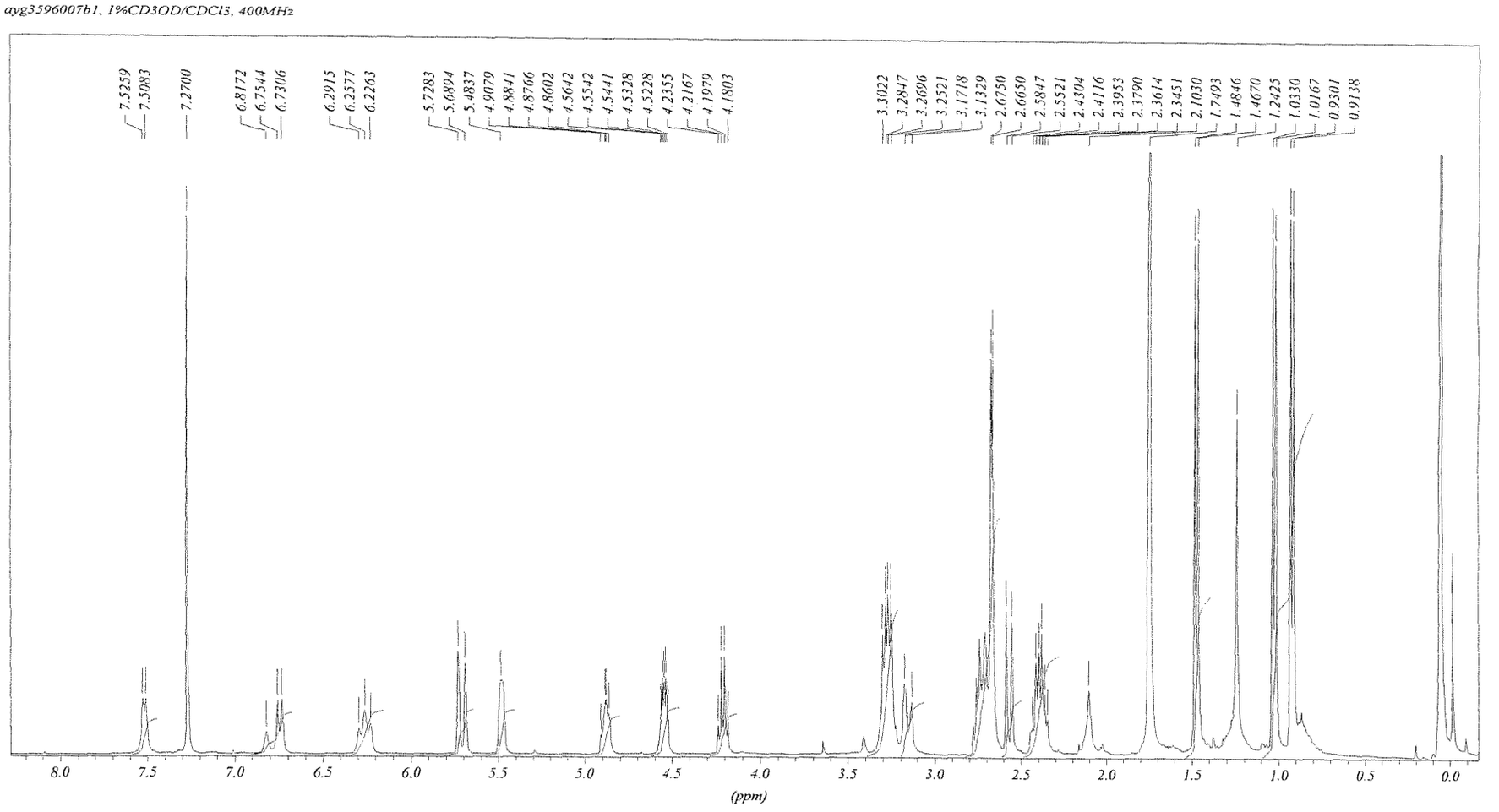


Spiruchostatin A

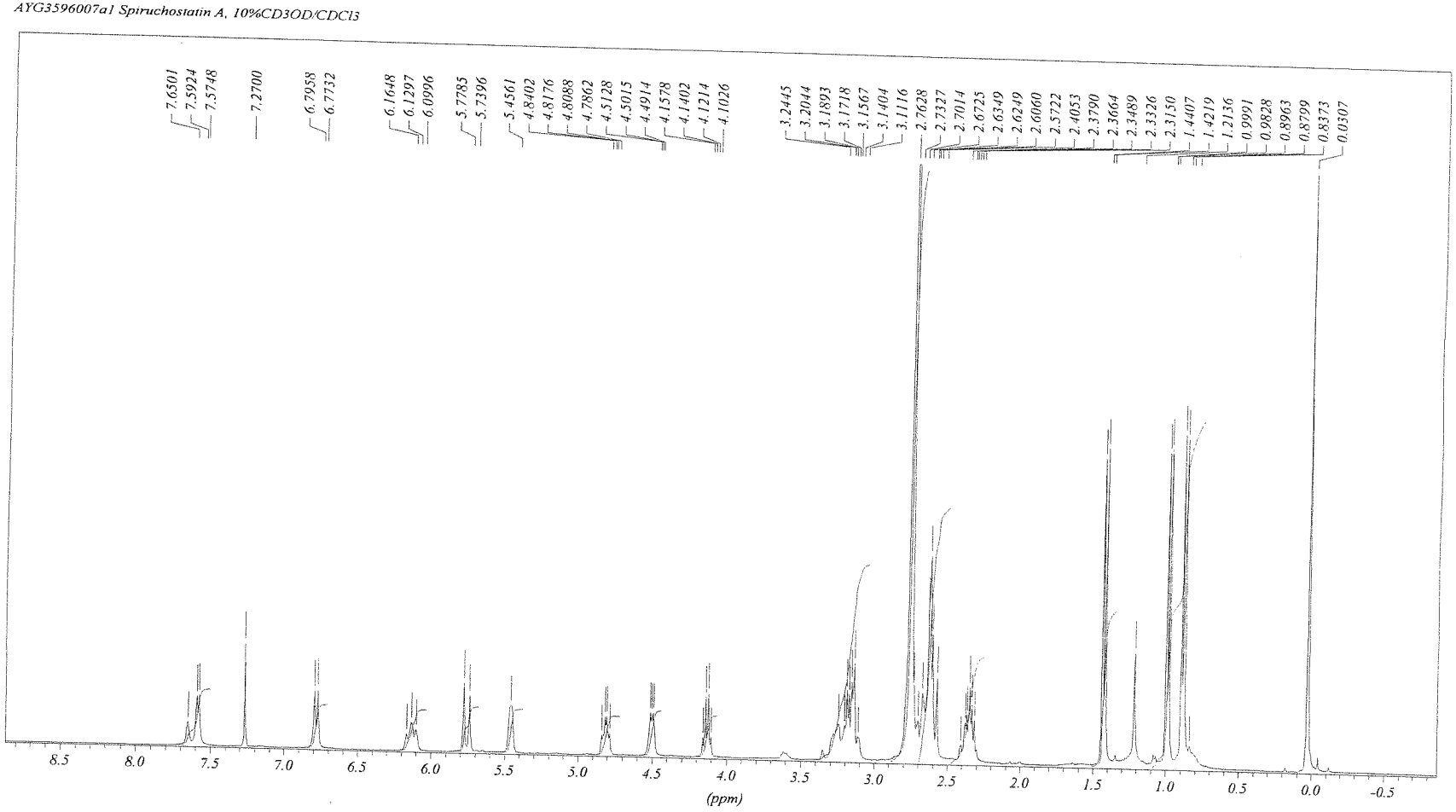
AYG3596007b1 Temp = 27degC -- njw□ CDCl3

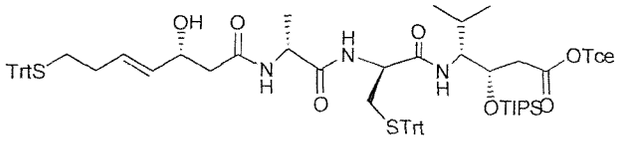


Spiruchostatin A 1% $CD_3OD/CDCl_3$

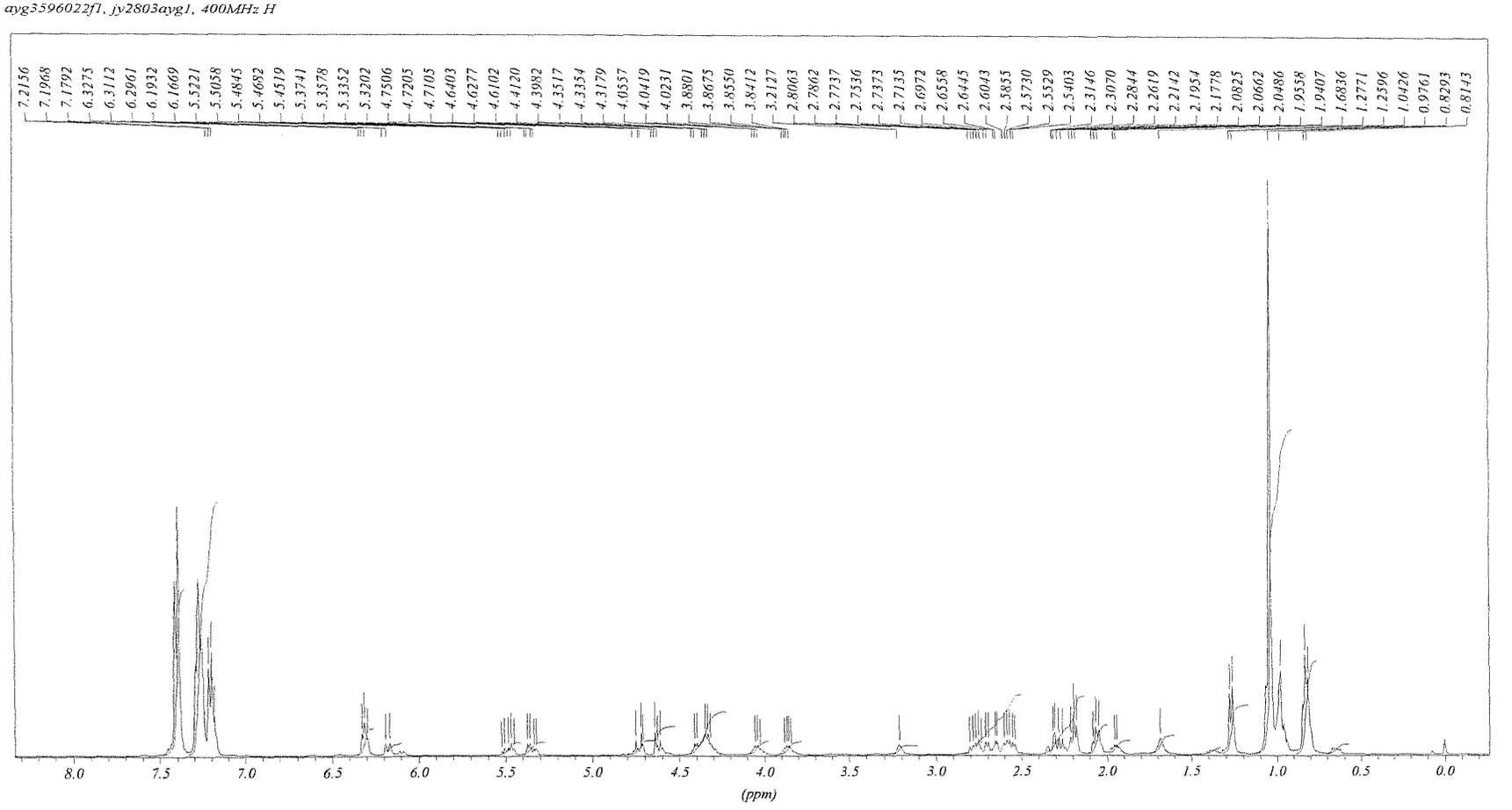


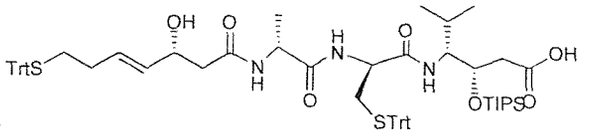
Spiruchostatin A 10% $CD_3OD/CDCl_3$





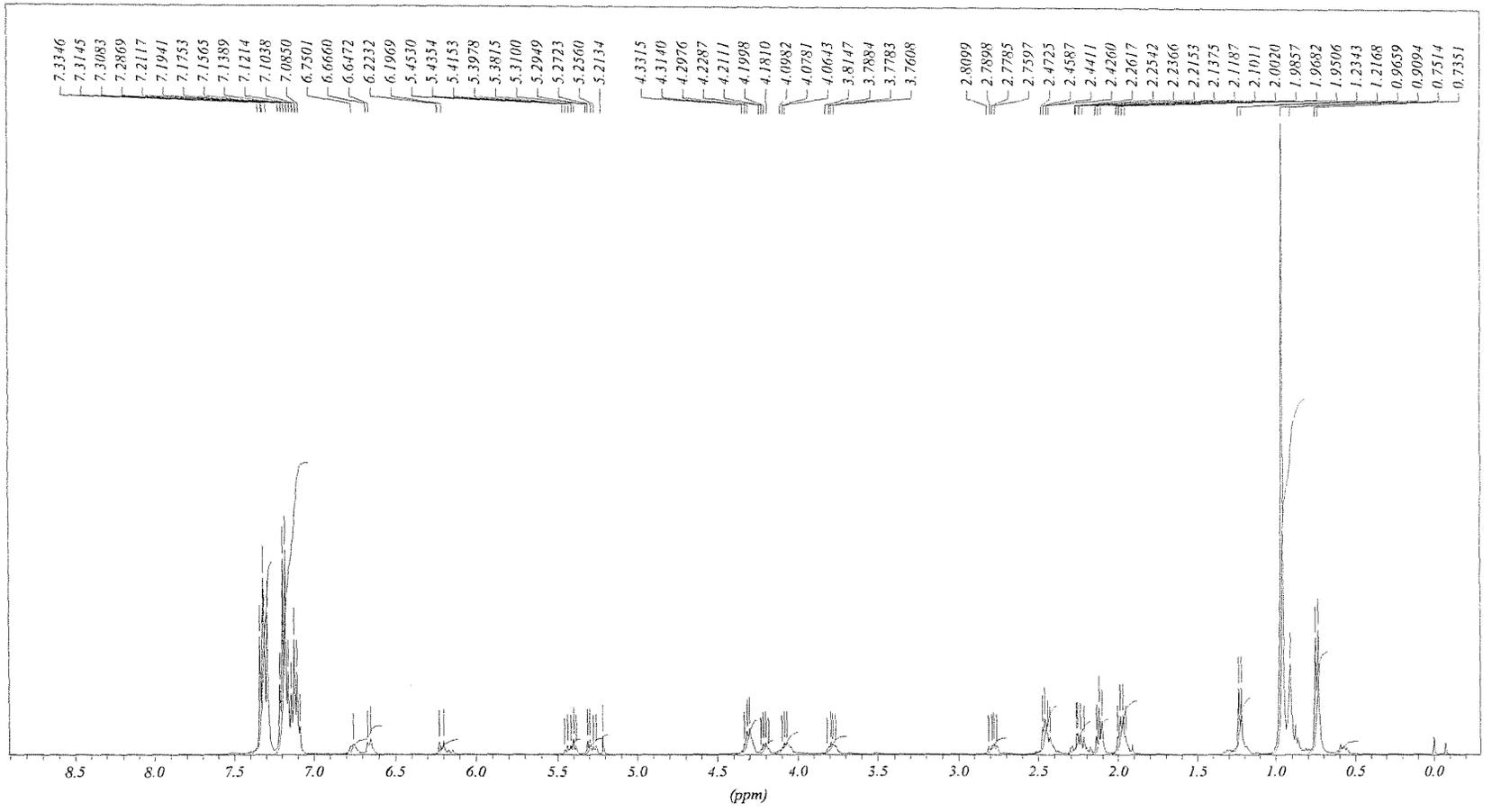
(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*R*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid 2,2,2-trichloro-ethyl ester (92)

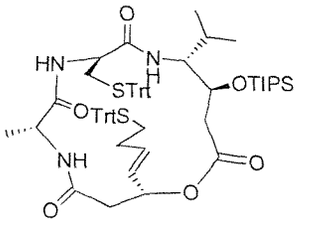




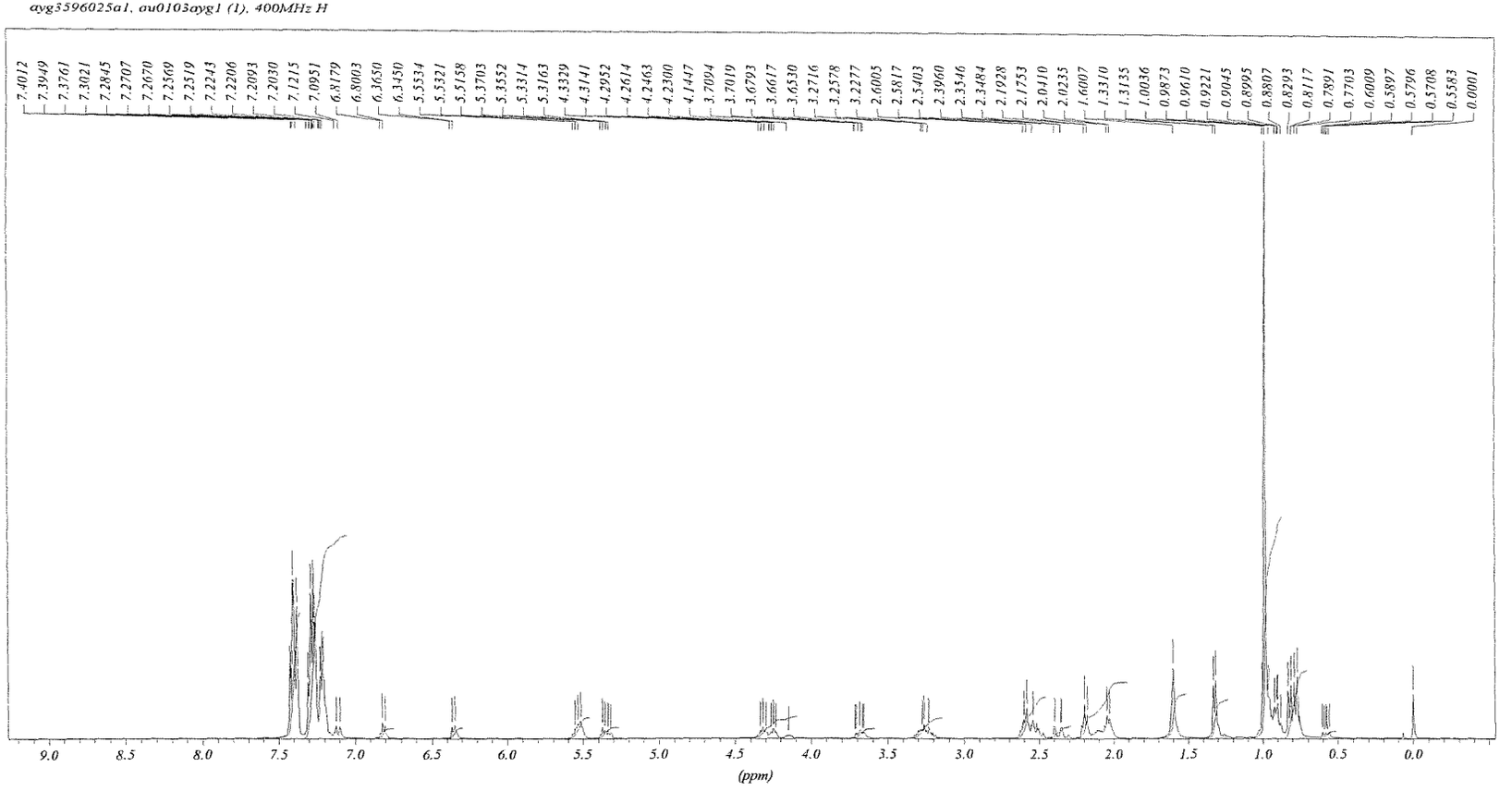
(3*S*,4*R*)-4-{(*S*)-2-[(*R*)-2-((*E*)-(*R*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-5-methyl-3-triisopropylsilanyloxy-hexanoic acid (93)

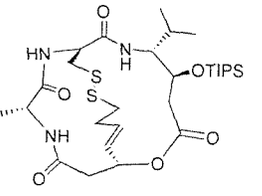
AYG3596025a1, jy3103ayg2 (1), 400MHz H



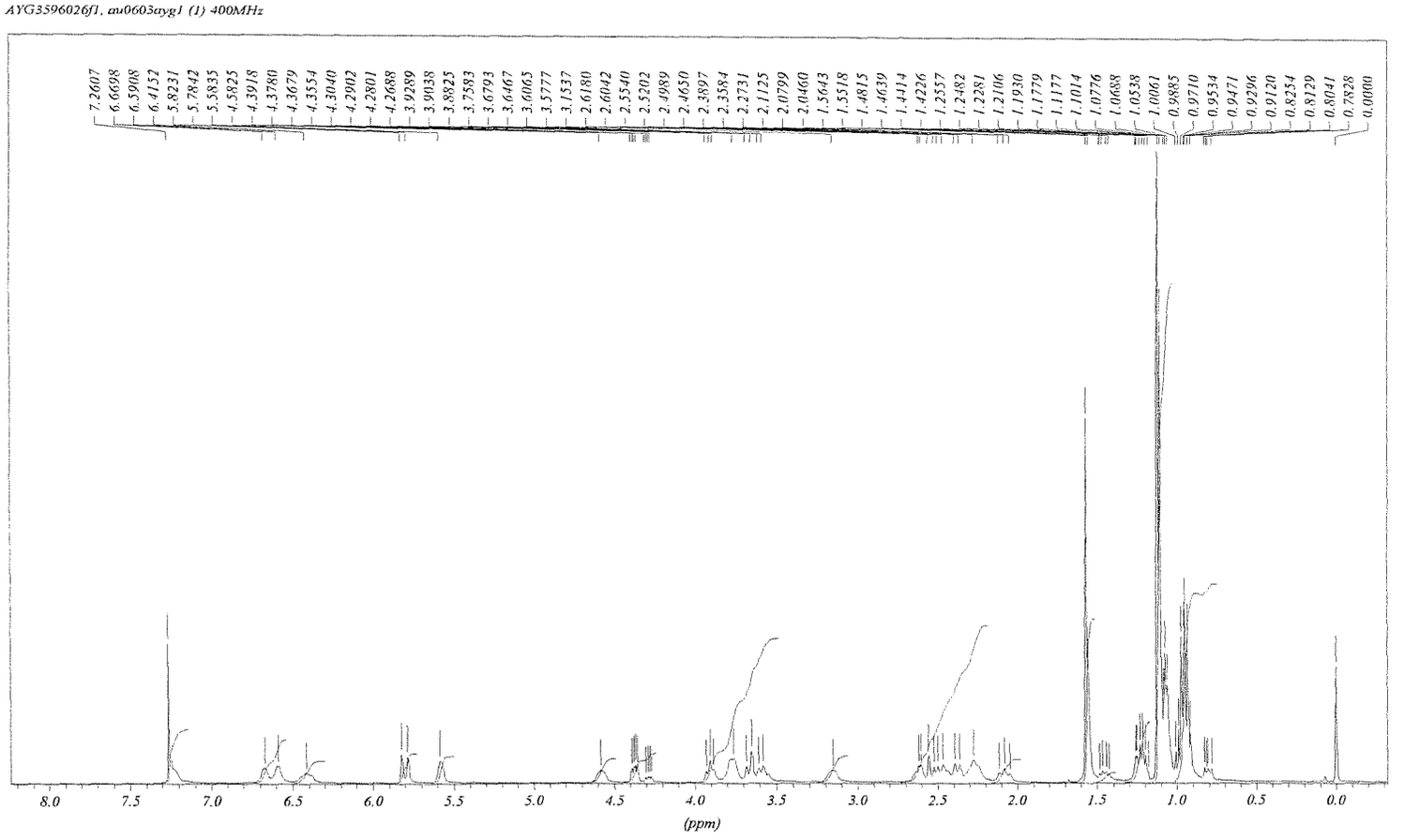


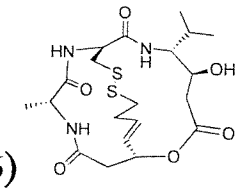
(2*R*,6*R*,9*S*,12*R*,13*S*)-12-Isopropyl-6-methyl-13-triisopropylsilanyloxy-2-((*E*)-4-tritylsulfanyl-but-1-enyl) -9-tritylsulfanylmethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,10,15-tetraone (94)





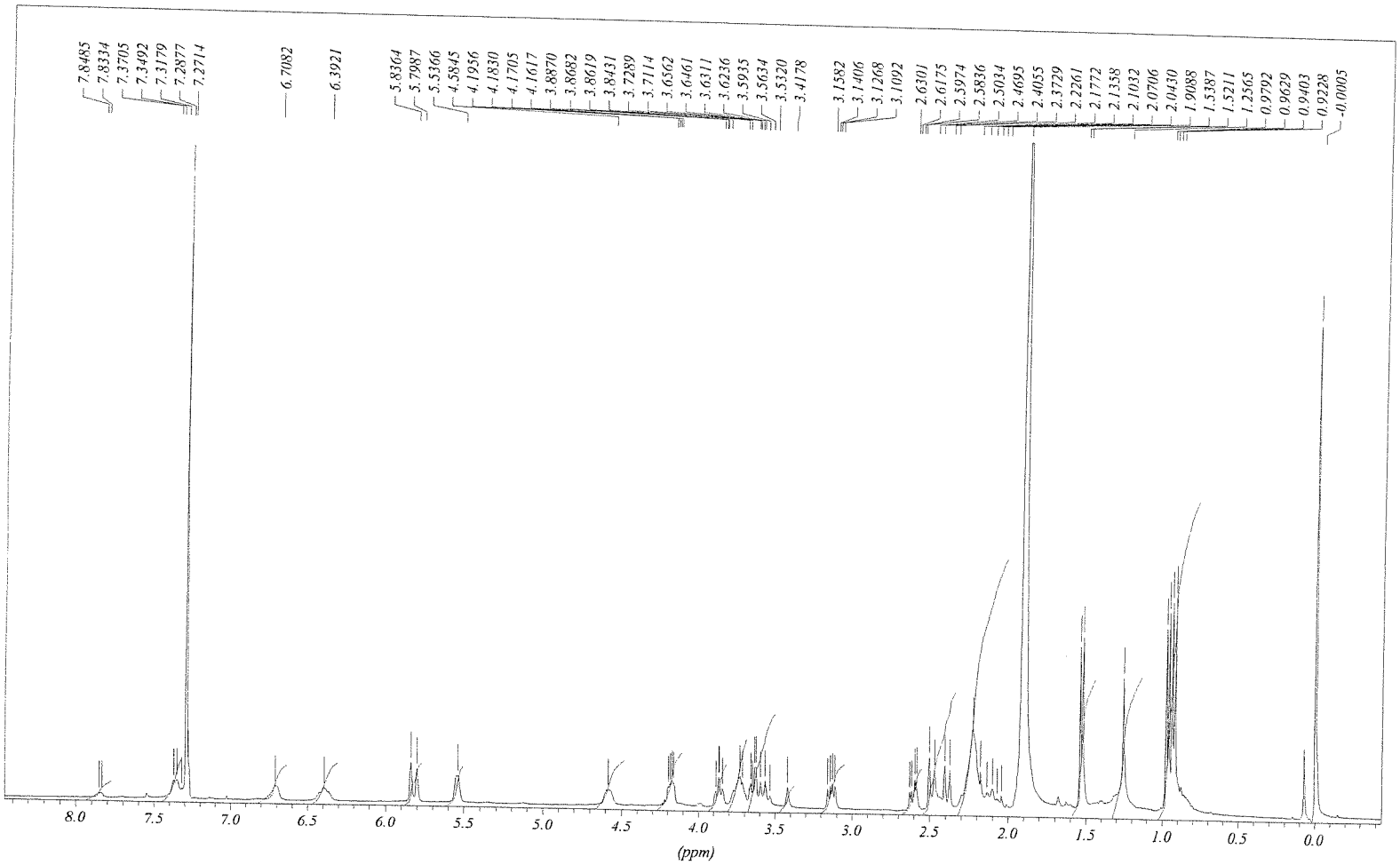
(*E*)-(1*R*,5*S*,6*R*,9*S*,20*R*)-6-Isopropyl-20-methyl-5-triisopropylsilanyloxy-2-oxa-11,12-dithia-7,19,22-triaza-bicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (95)





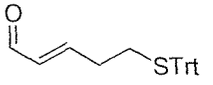
Epi-Spiruchostatin A (96)

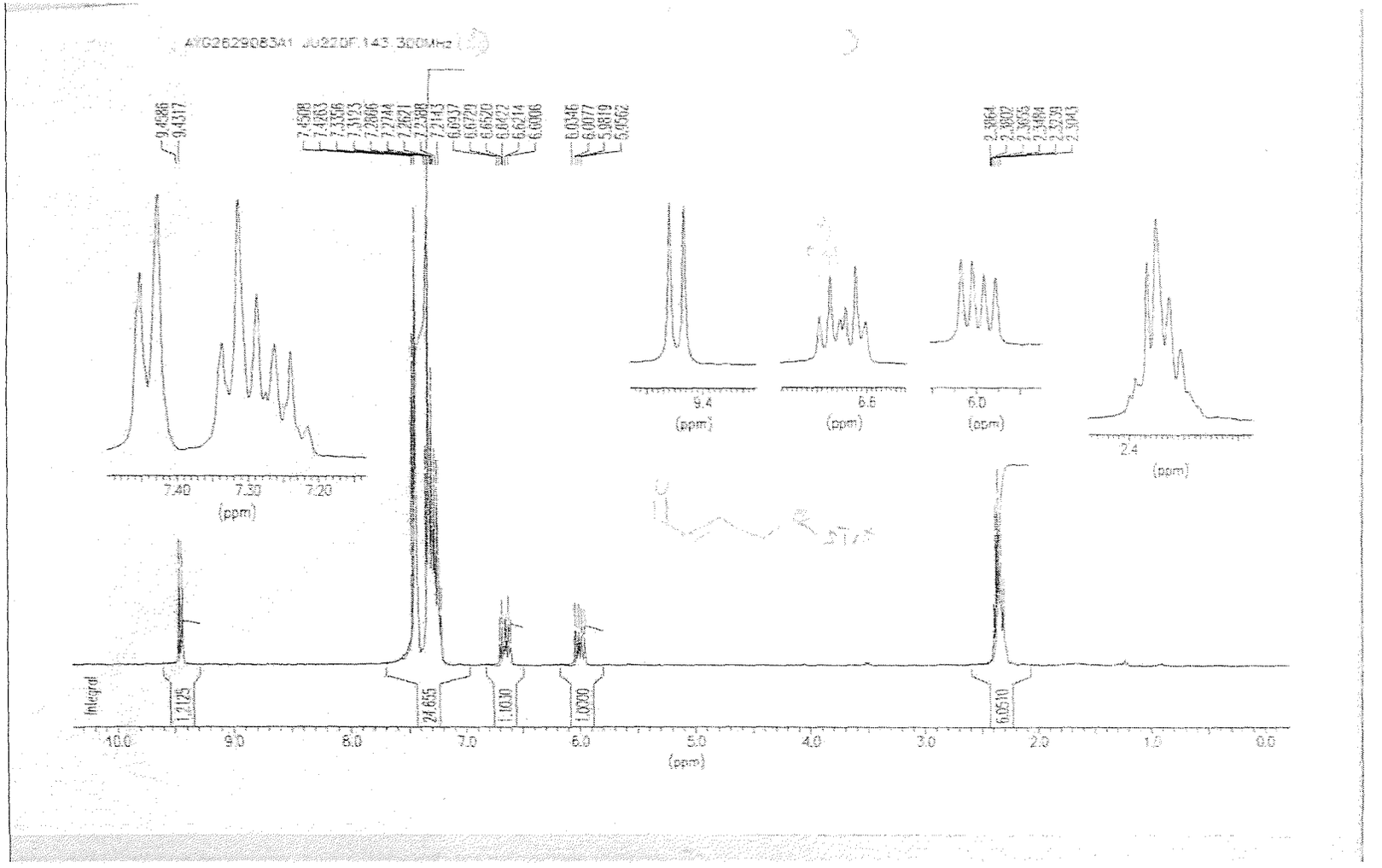
AYG3596027a2, au1203ayg2 (1), 400MHz H Epispiruchostatin A



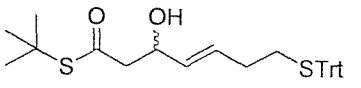
^{1}H NMR Spectra for compounds in Chapter 3

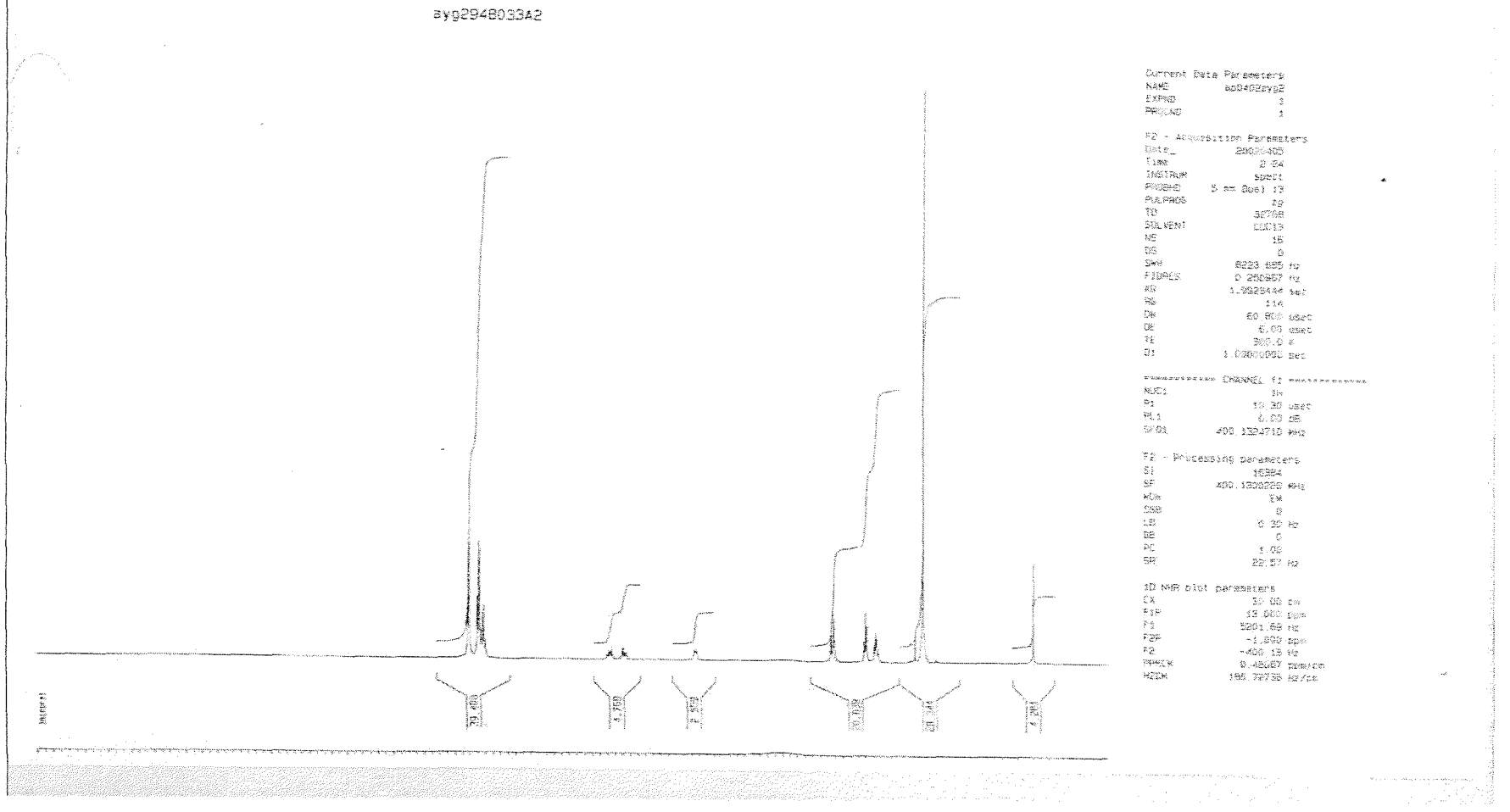
(*E*)-5-Tritylsulfanyl-pent-2-enal (7)



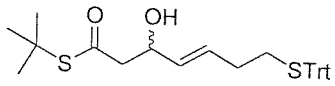


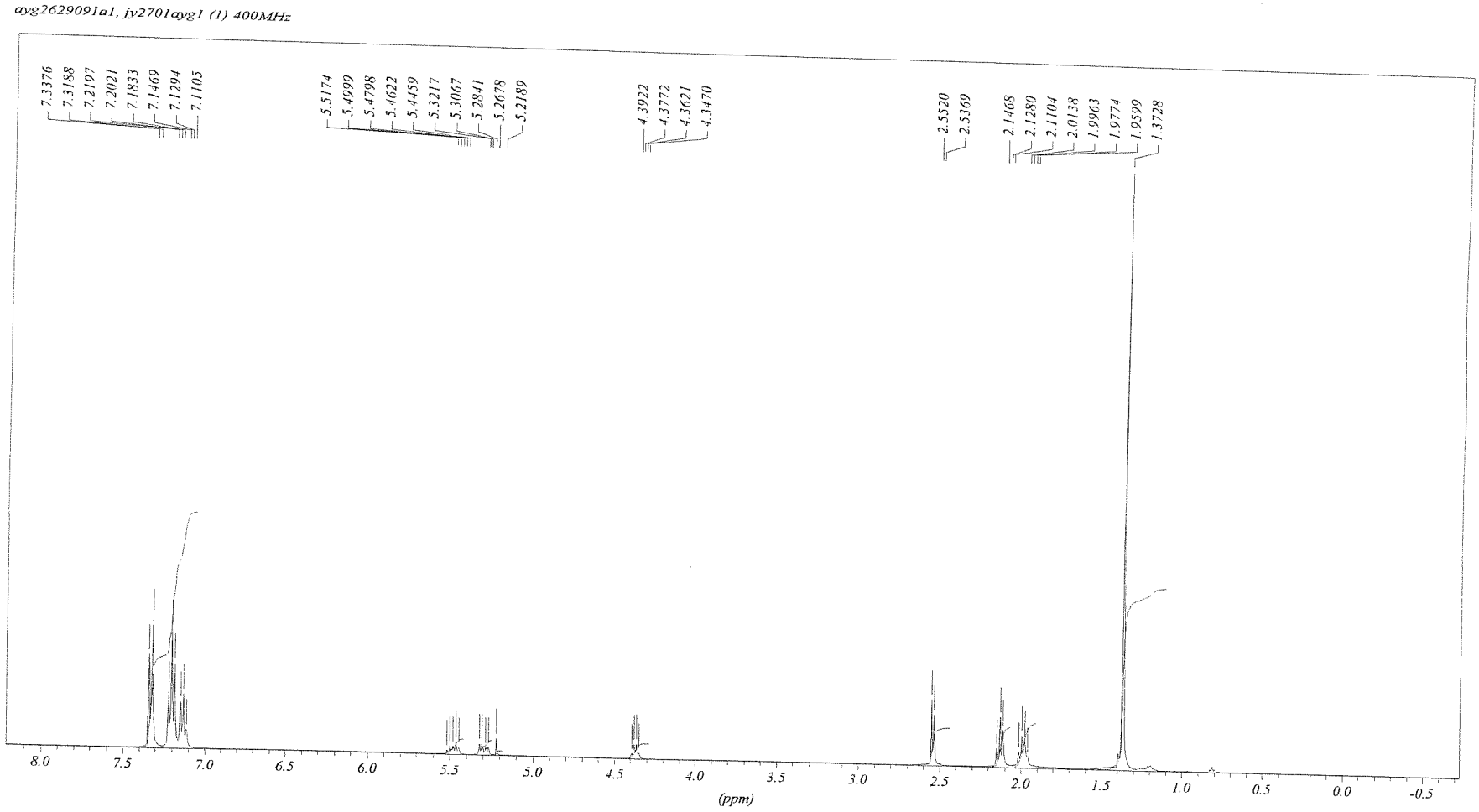
3-Hydroxy-7-tritylsulfanyl-hept-4-enethioic acid S-*tert*-butyl ester (8)



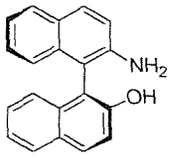


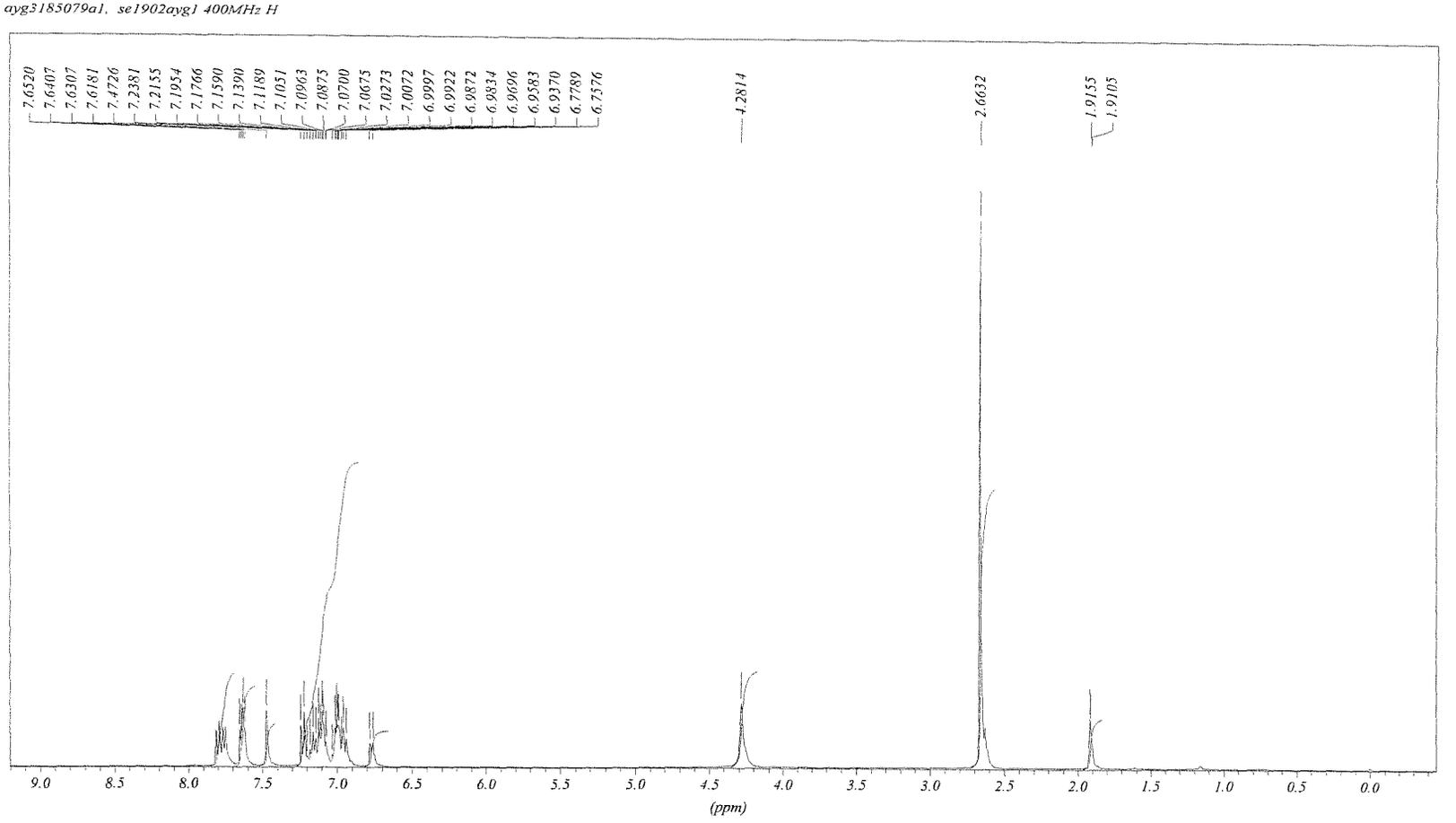
(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enethioic acid S-*tert*-butyl ester (14)



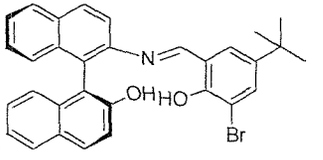


S-(-)-2-amino-2-hydroxy-1,1-binaphthyl (17)

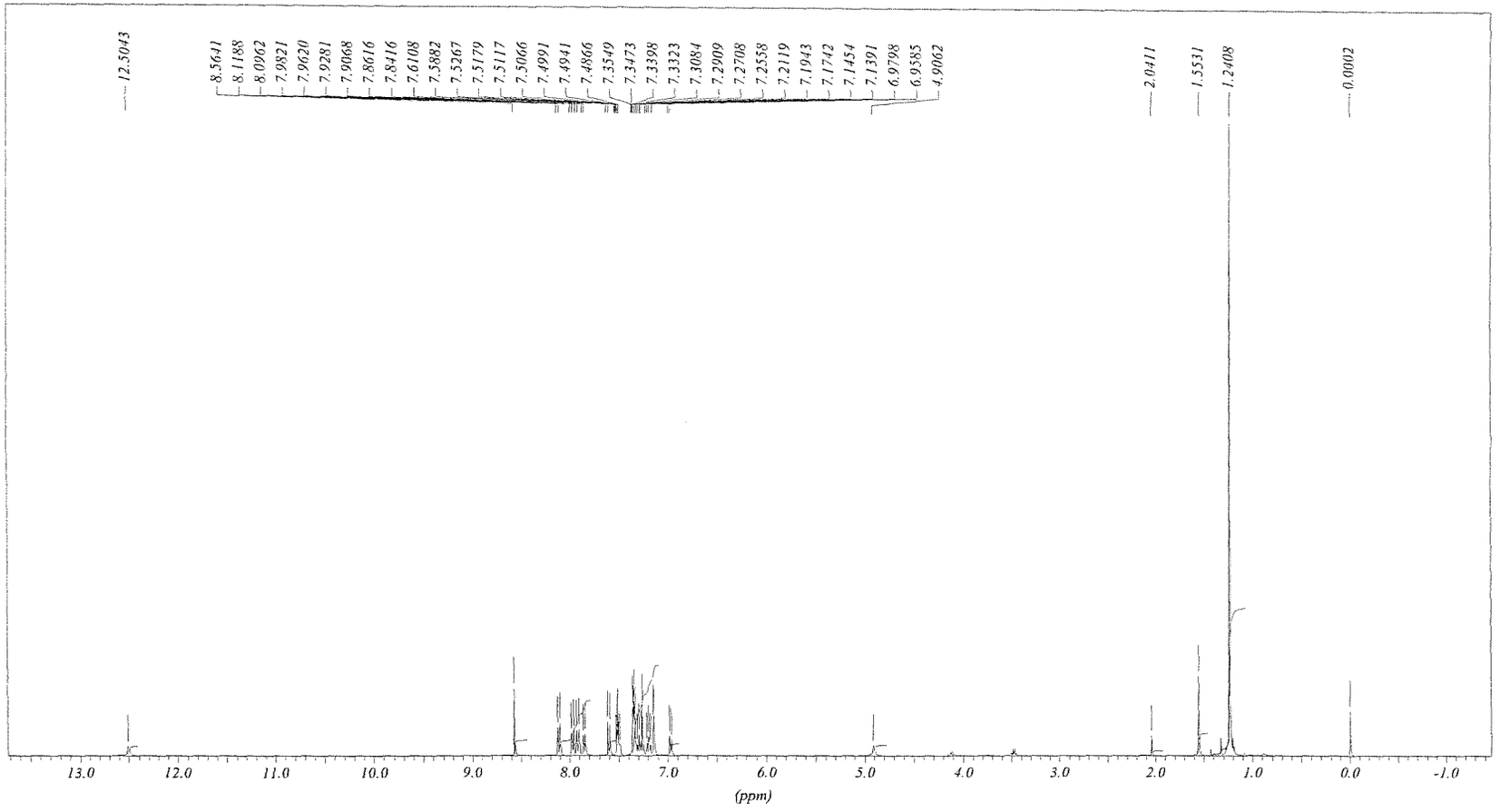




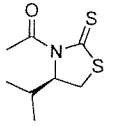
(S)-2'-{[1-(3-Bromo-5-tert-butyl-2-hydroxy-phenyl)-meth-(E)-ylidene]-amino}-[1,1']binaphthalenyl-2-ol (18)



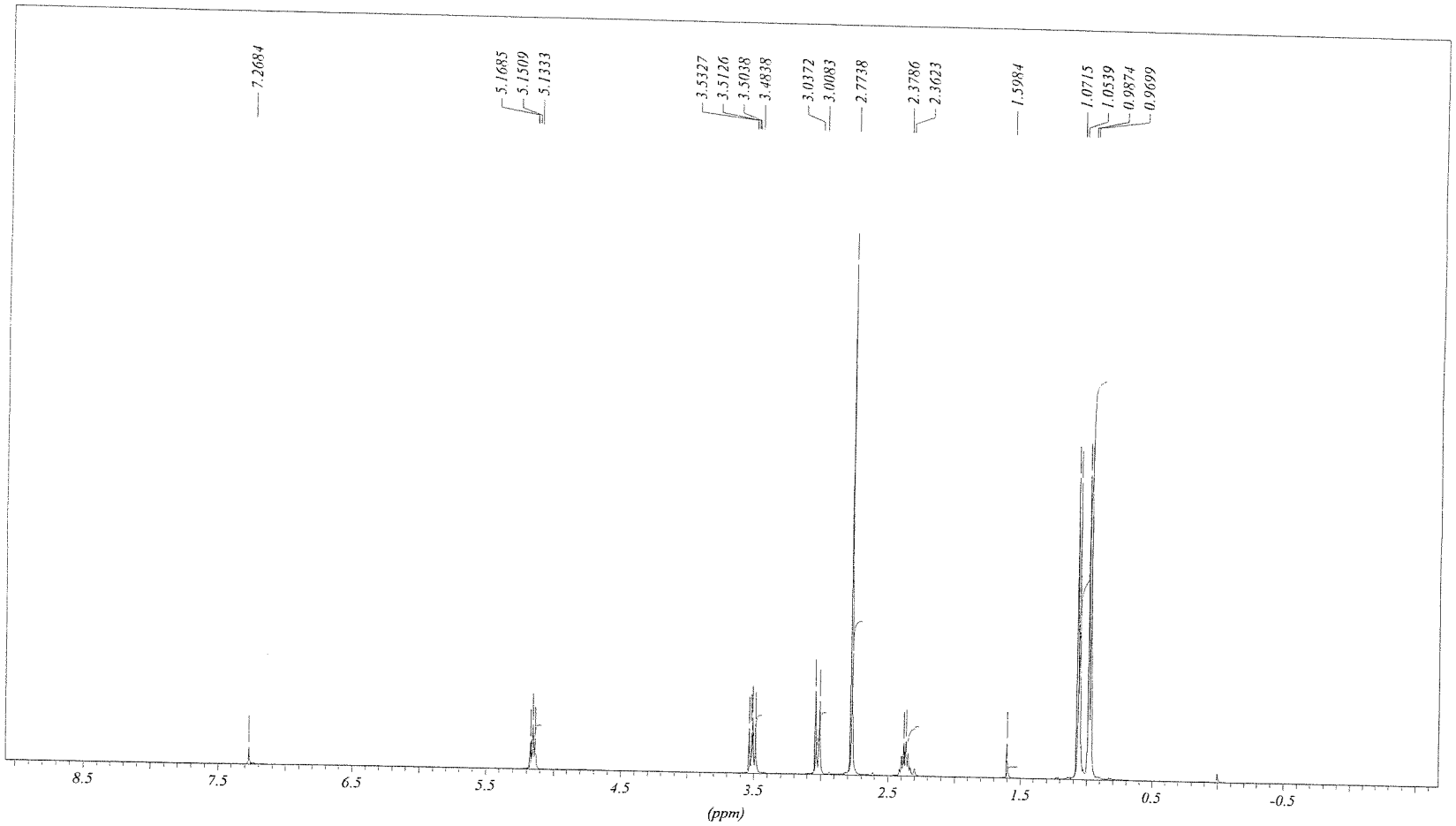
ayg3185086a1, 0c2102ayg1 H 400MHz

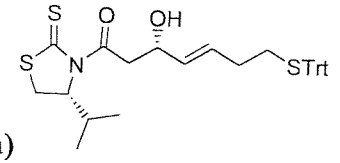


1-((*R*)-4-Isopropyl-2-thioxo-thiazolidin-3-yl)-ethanone (25)

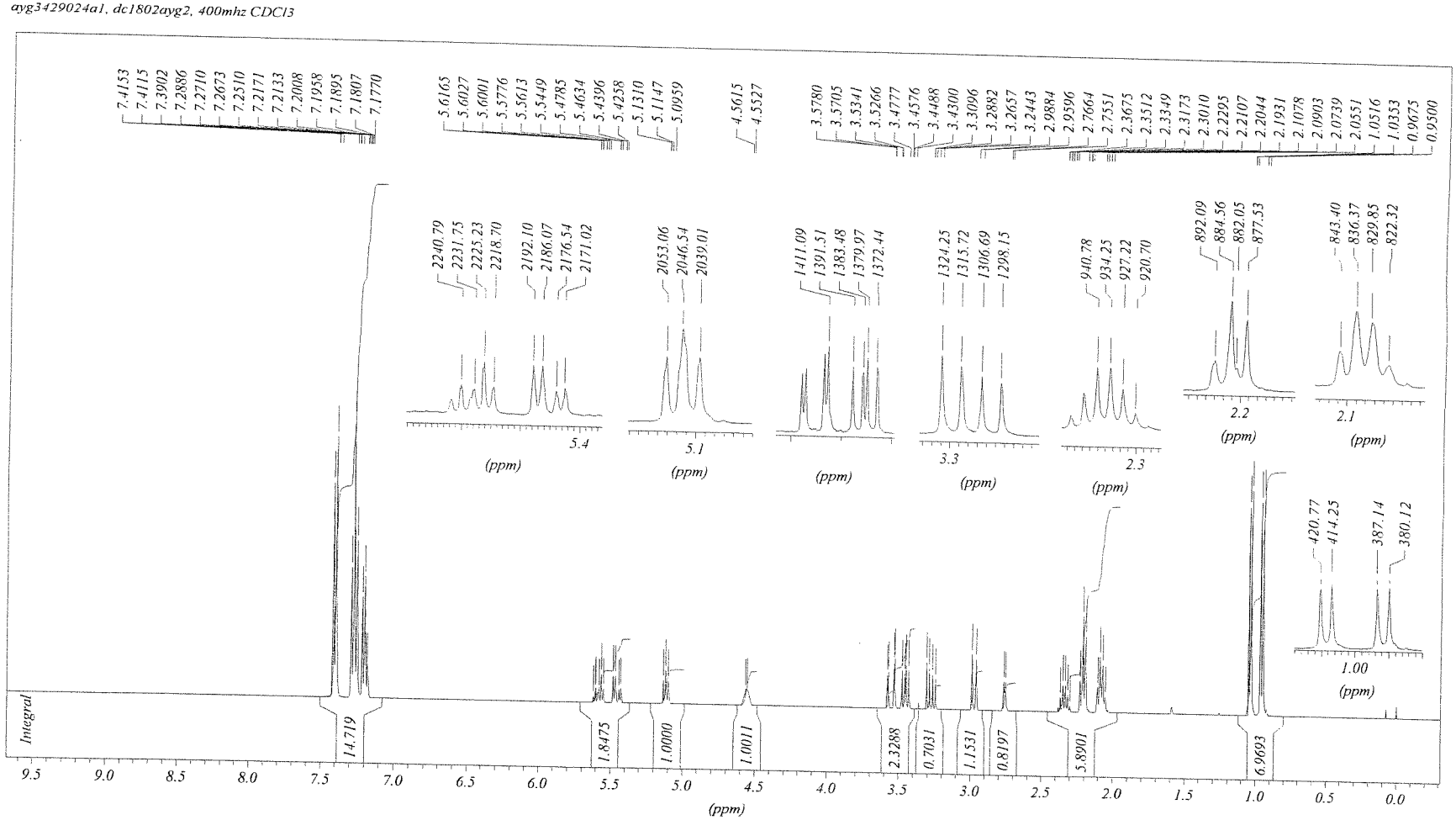


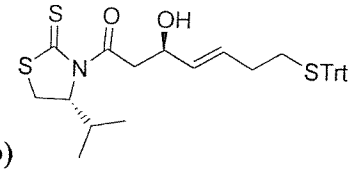
ayg3429010a1, nv3002ayg2, 400MHz H



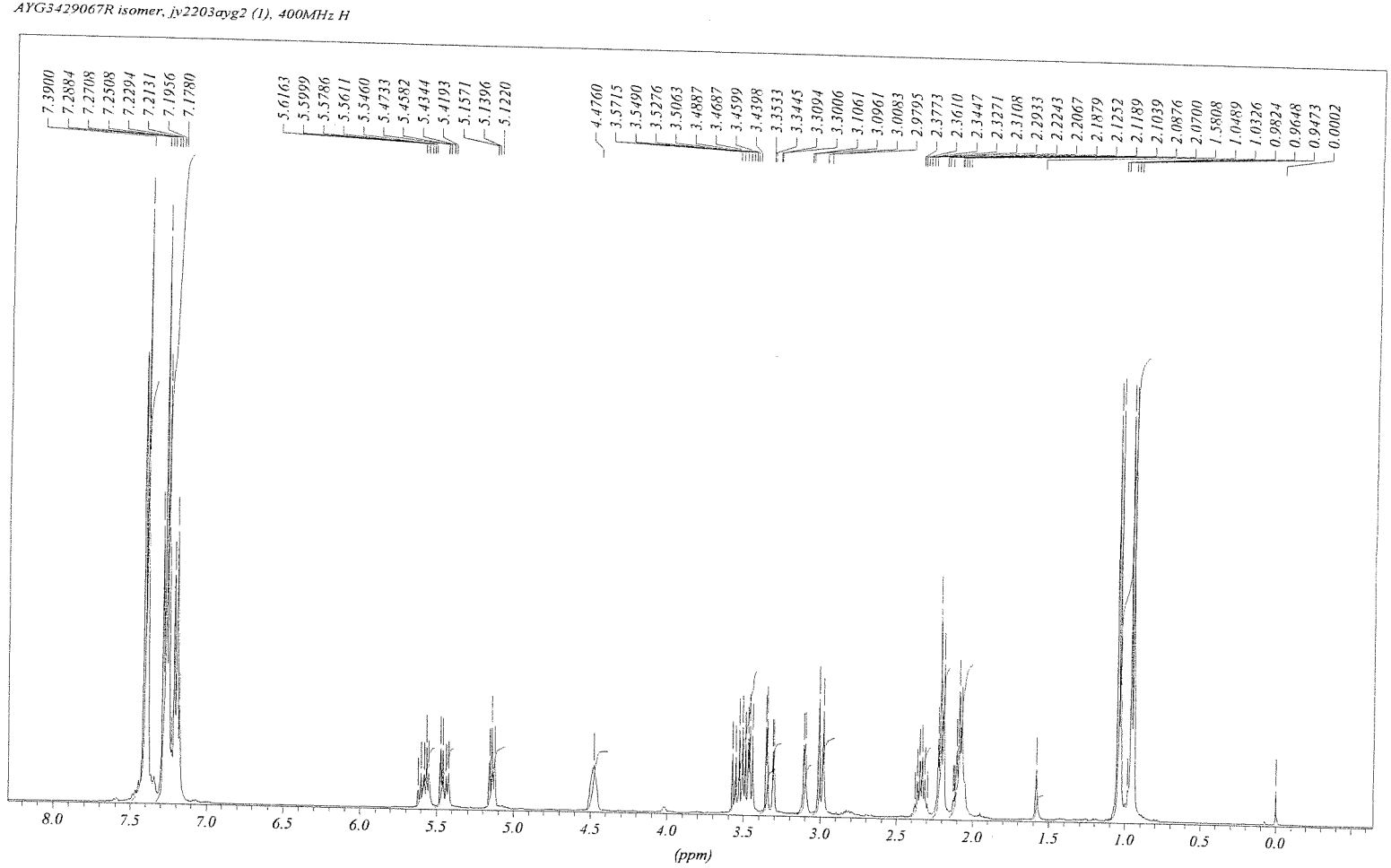


(*E*)-(*S*)-3-Hydroxy-1-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4-en-1-one (26a)

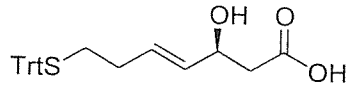


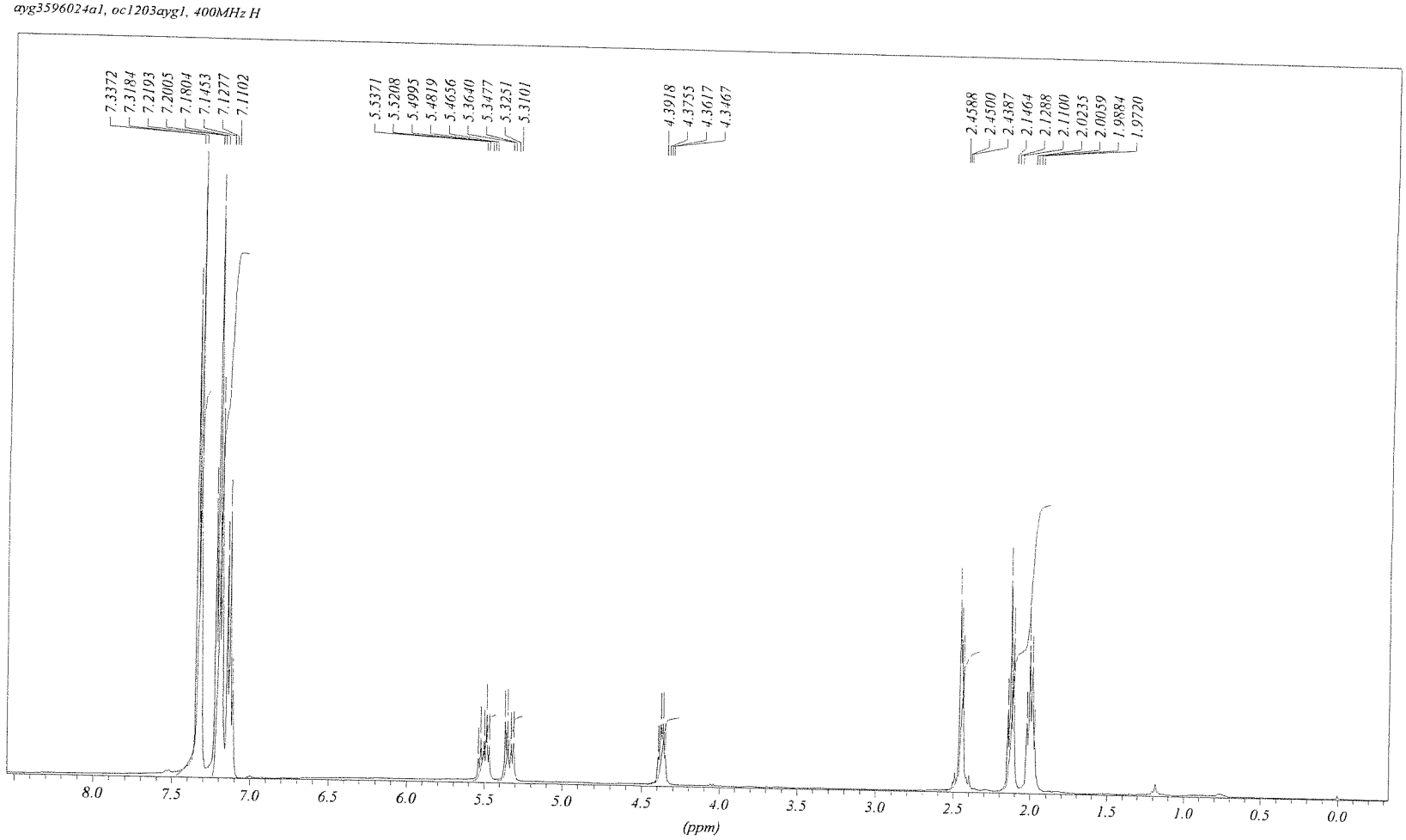


(*E*)-(*R*)-3-Hydroxy-1-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-7-tritylsulfanyl-hept-4-en-1-one (26b)



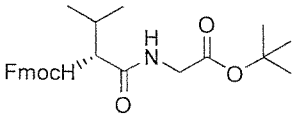
(*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4enoic acid (27)

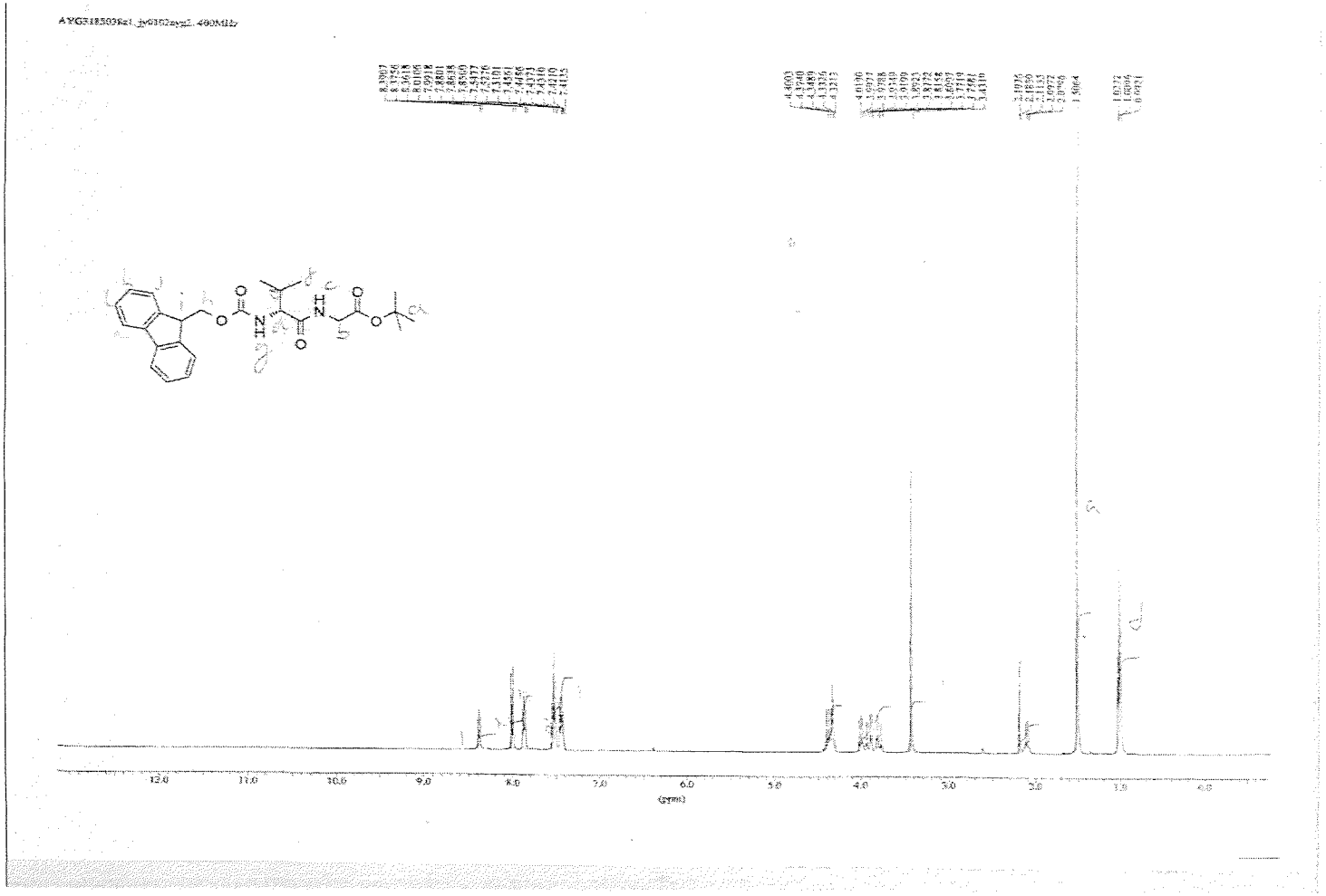




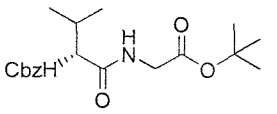
^{1}H NMR Spectra for compounds in Chapter 4

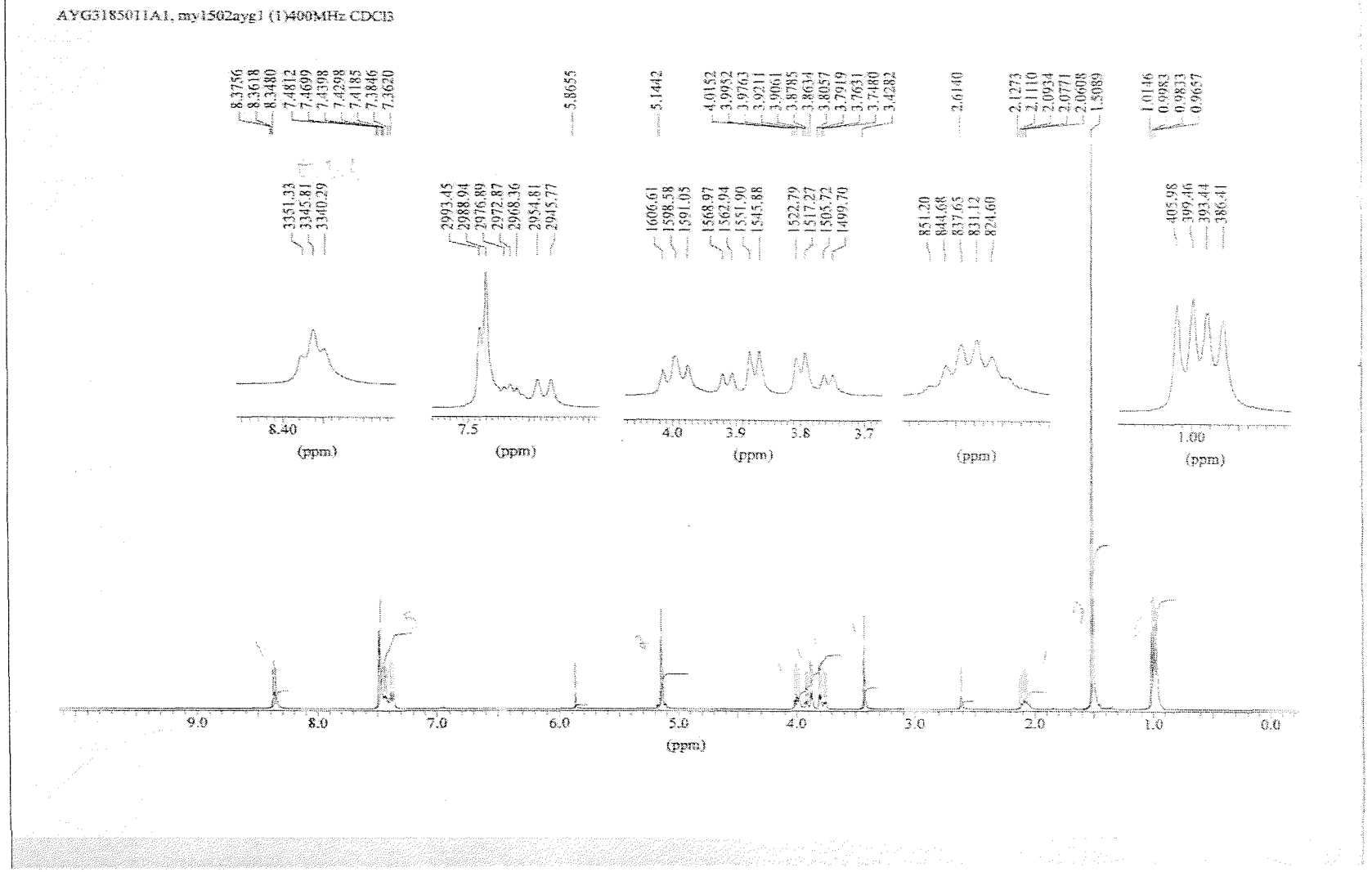
(2*R*)-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-acetic acid *tert*-butyl ester (3a)

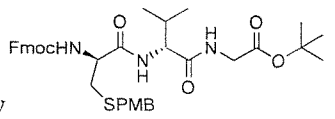




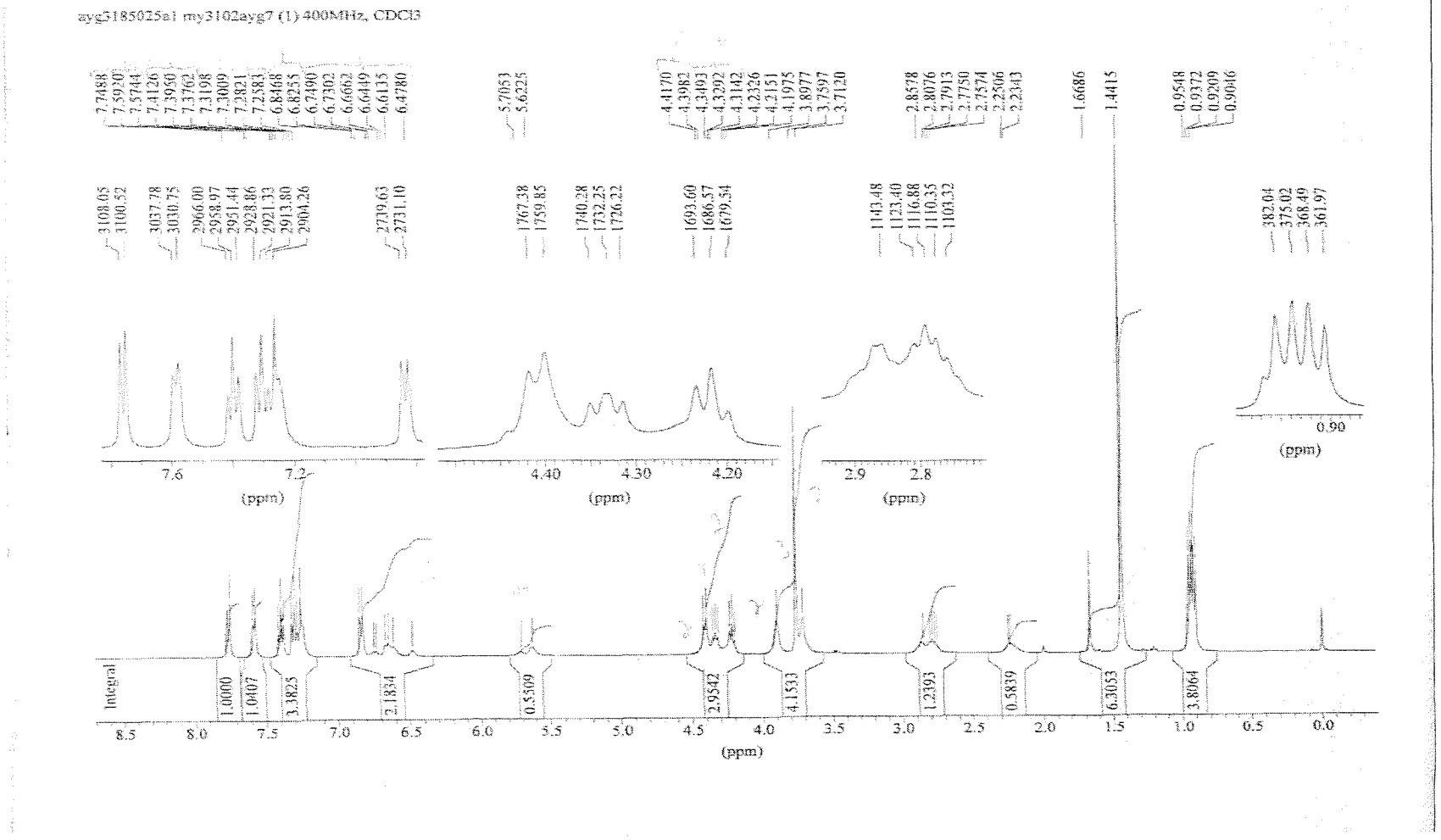
(*R*)-(2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-acetic acid *tert*-butyl ester (3b)

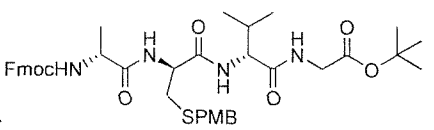




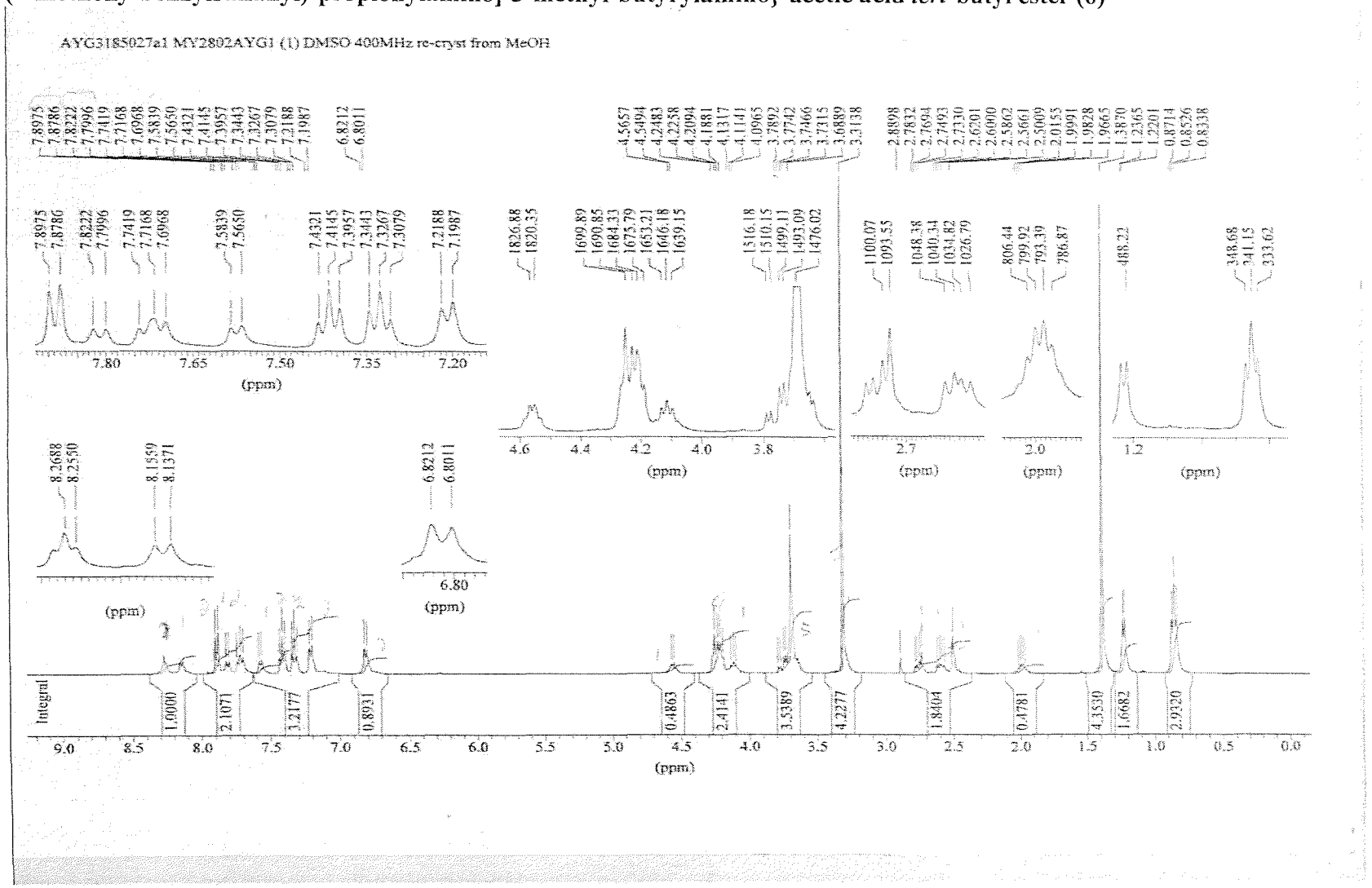


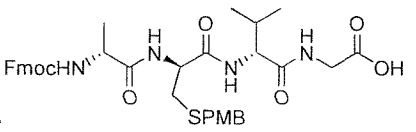
{(*R*)-2-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid *tert*-butyl ester (5)



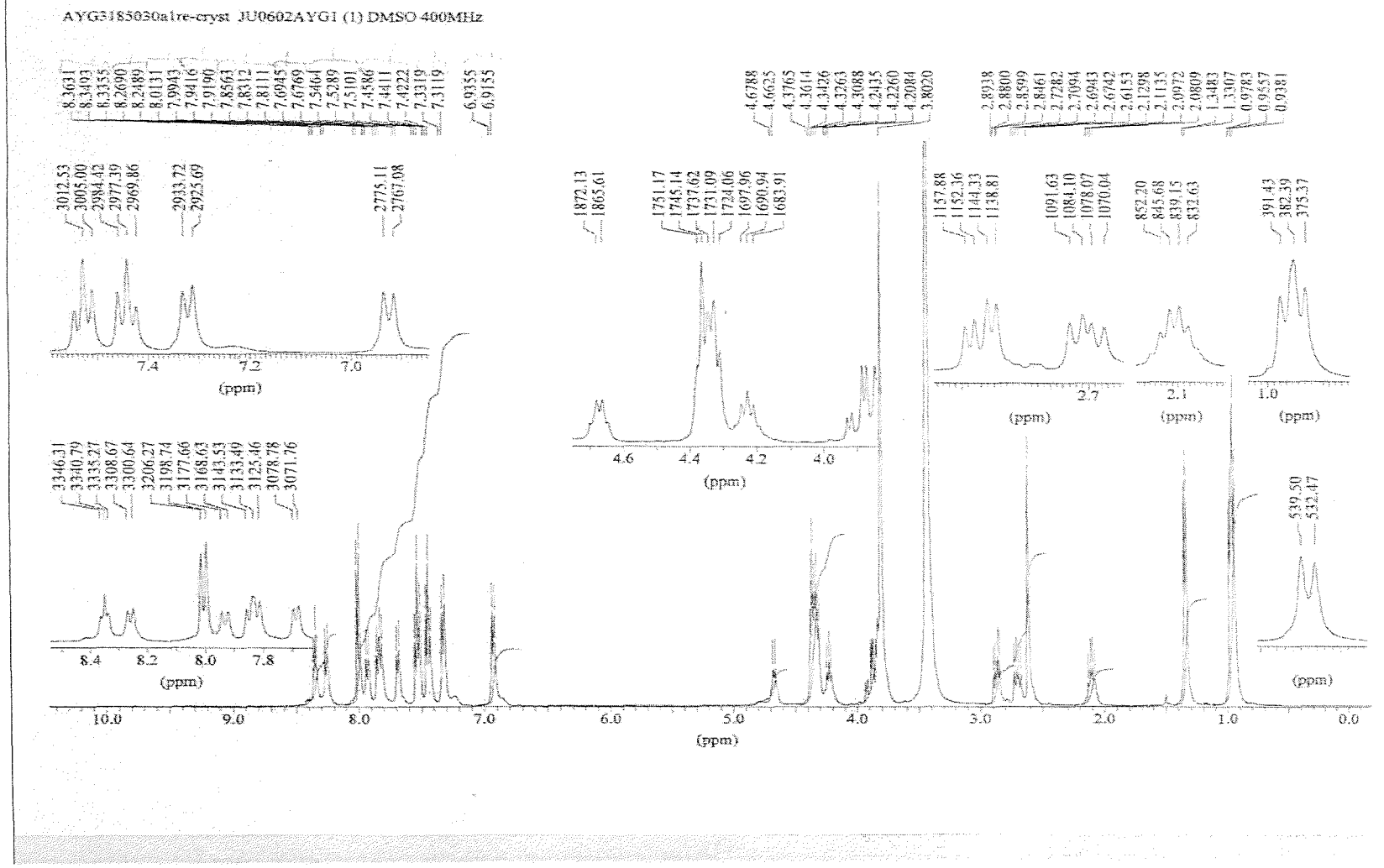


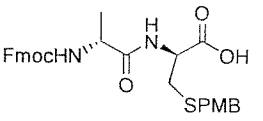
{(*R*)-2-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid *tert*-butyl ester (6)



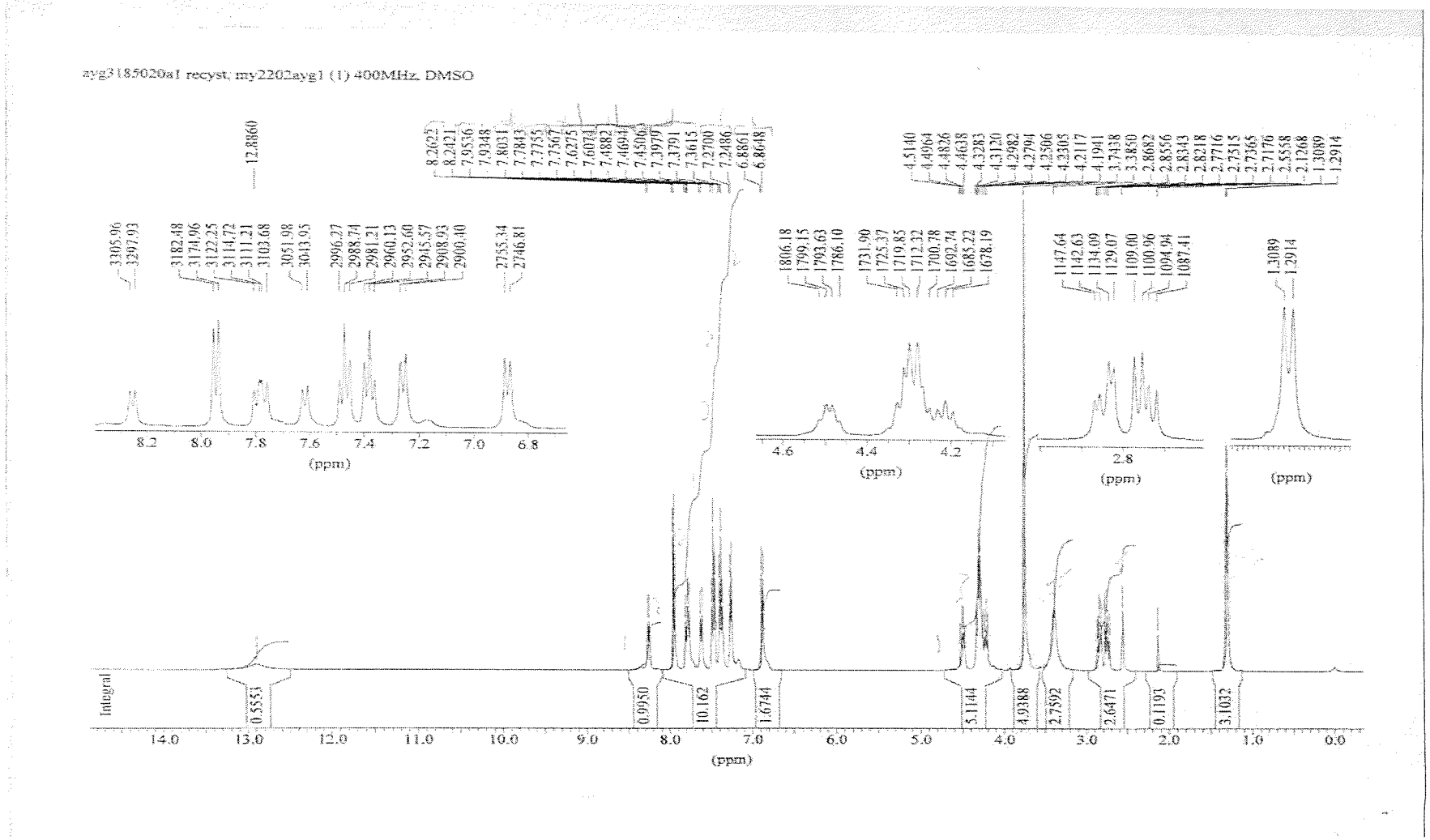


{(*R*)-2-[(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid ester (7)

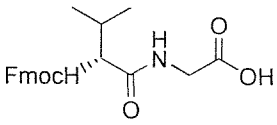


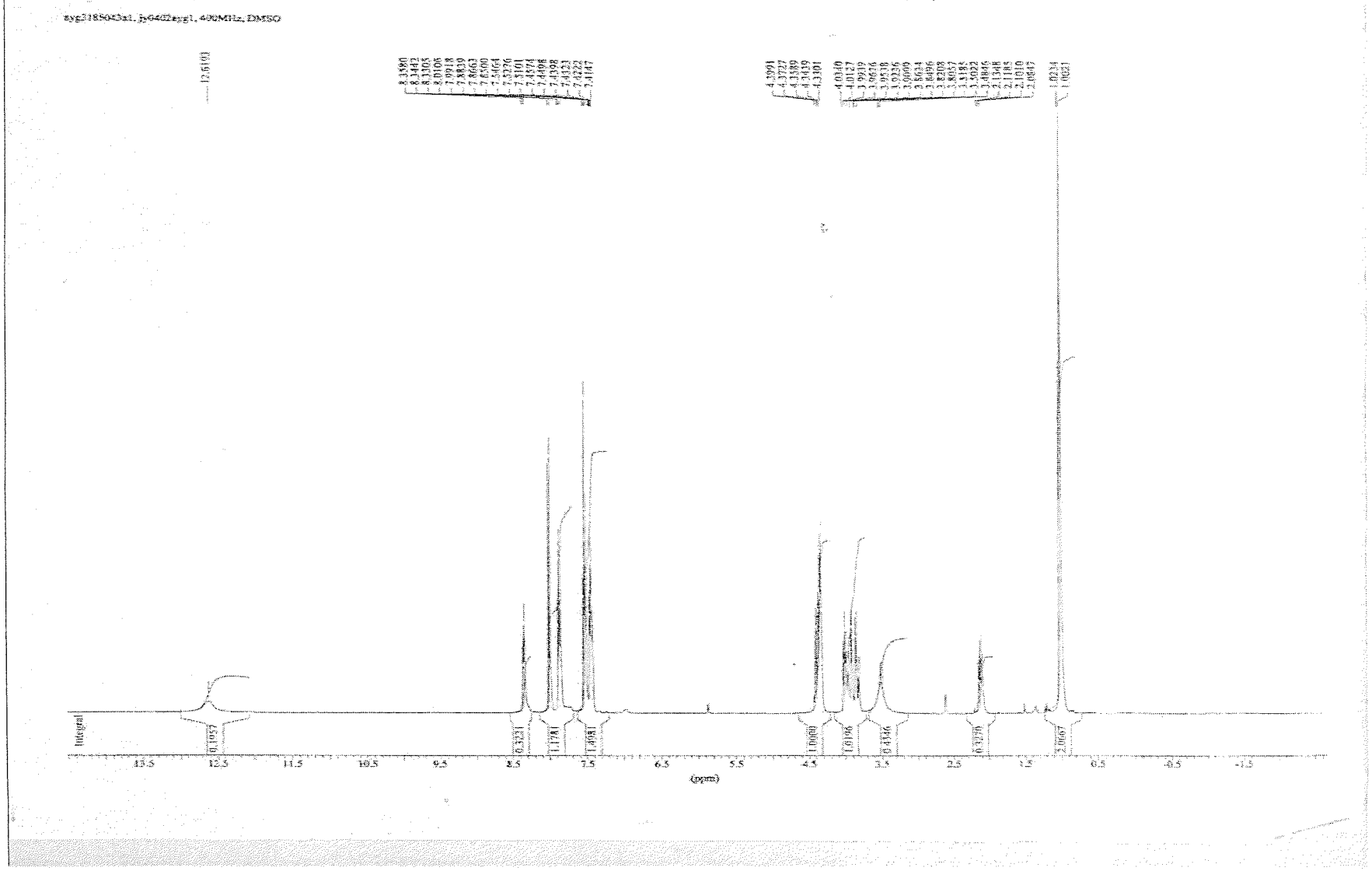


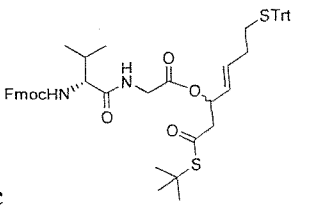
(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-(4-methoxy-benzylsulfanyl)-propionic acid (9)



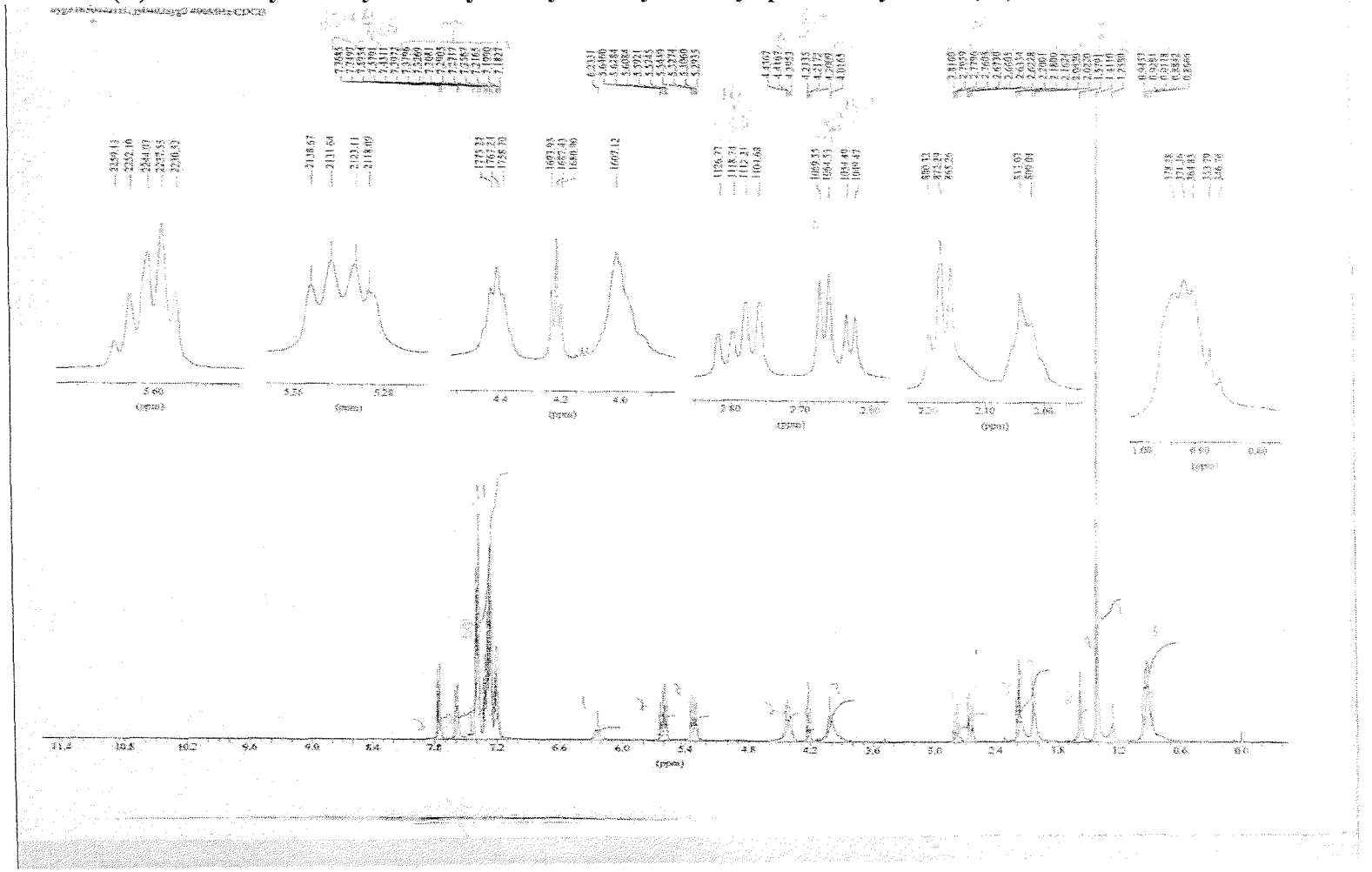
[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-acetic acid (12)

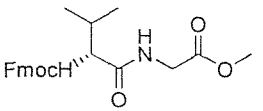




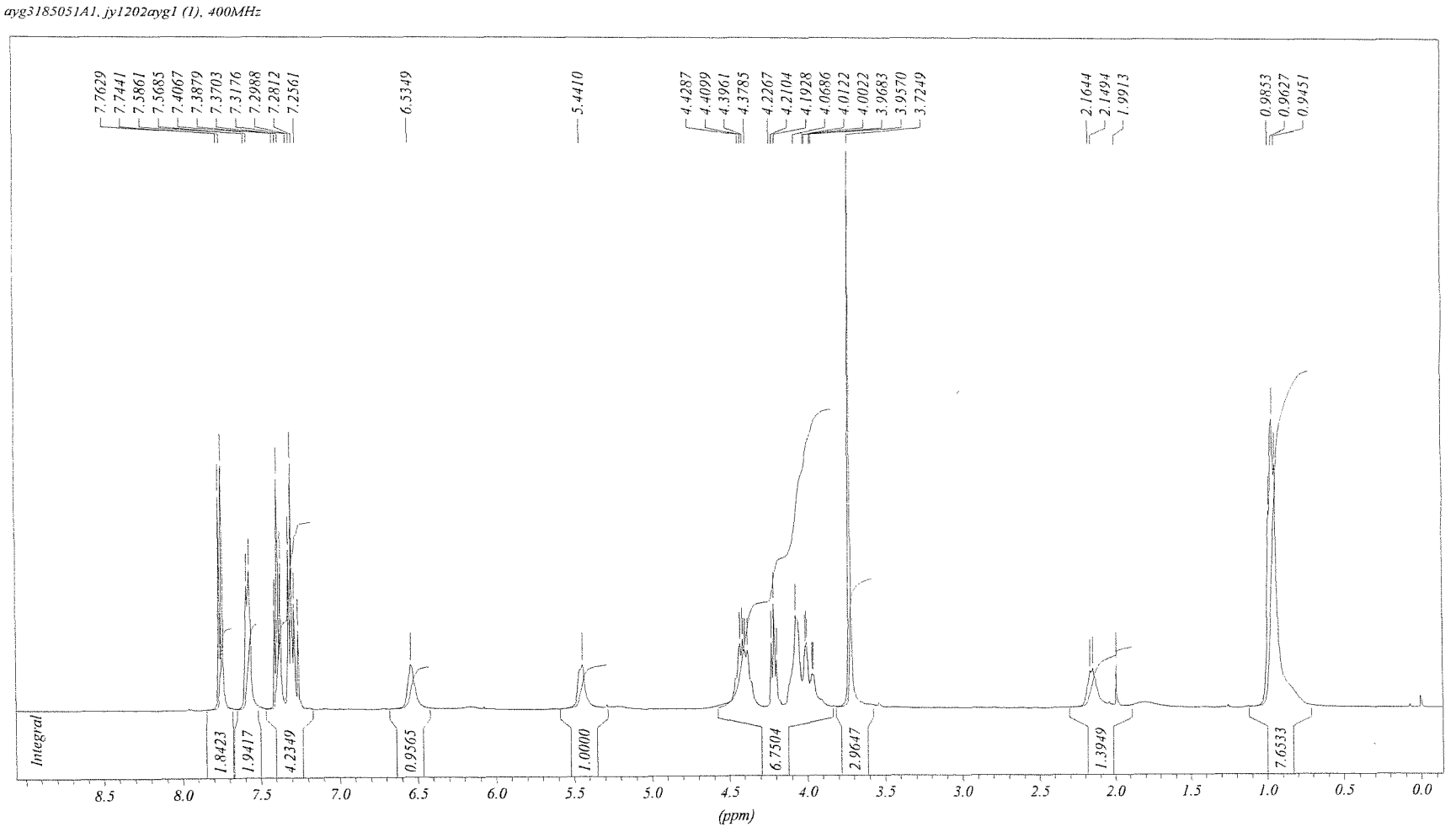


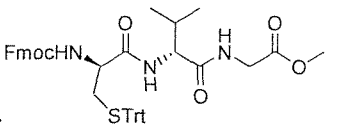
[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-ac etic acid (*E*)-1-*tert*-butylsulfanylcarbonylmethyl-5-tritylsulfanyl-pent-2-enyl ester (13)





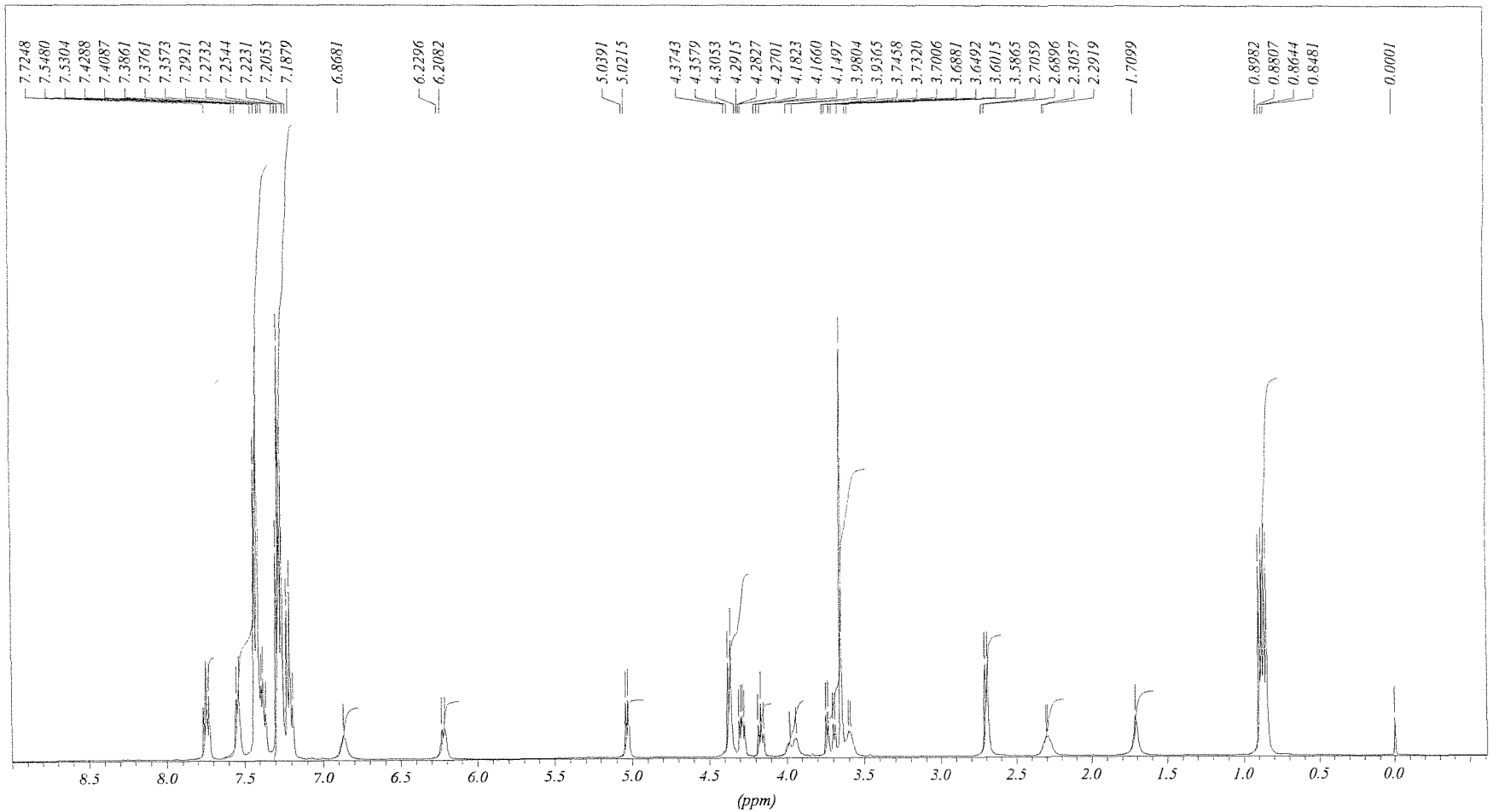
[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino] -acetic acid methyl ester (16)

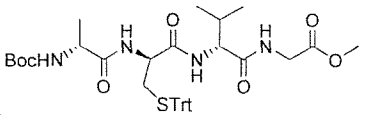




{(*R*)-2-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(tritylsulfanyl)-propionylamino]-3-methyl-butyrylamino}-acetic acid methyl ester (17)

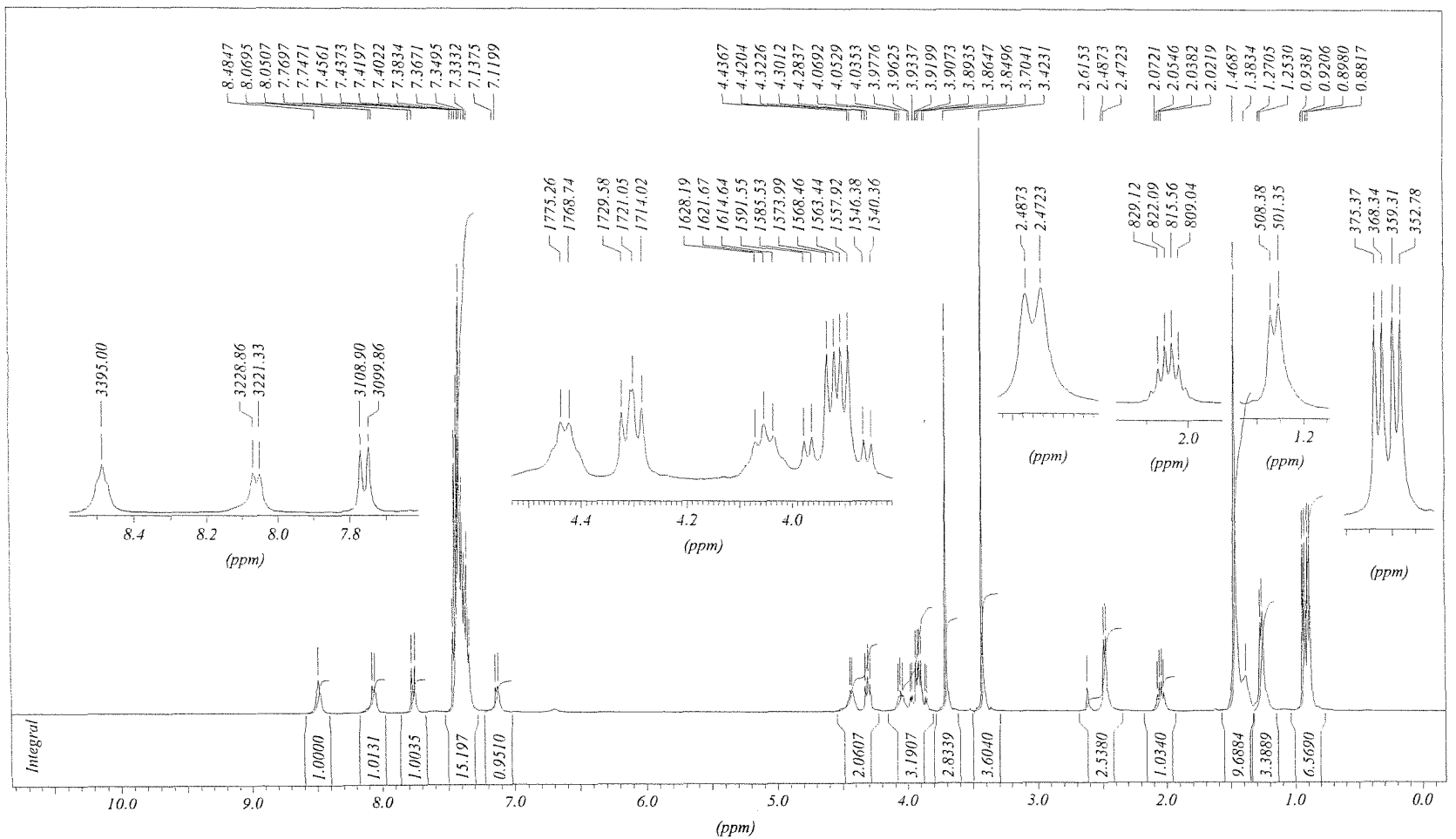
ayg3429034a1, jy1403ayg1, 400MHz H

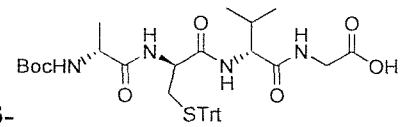




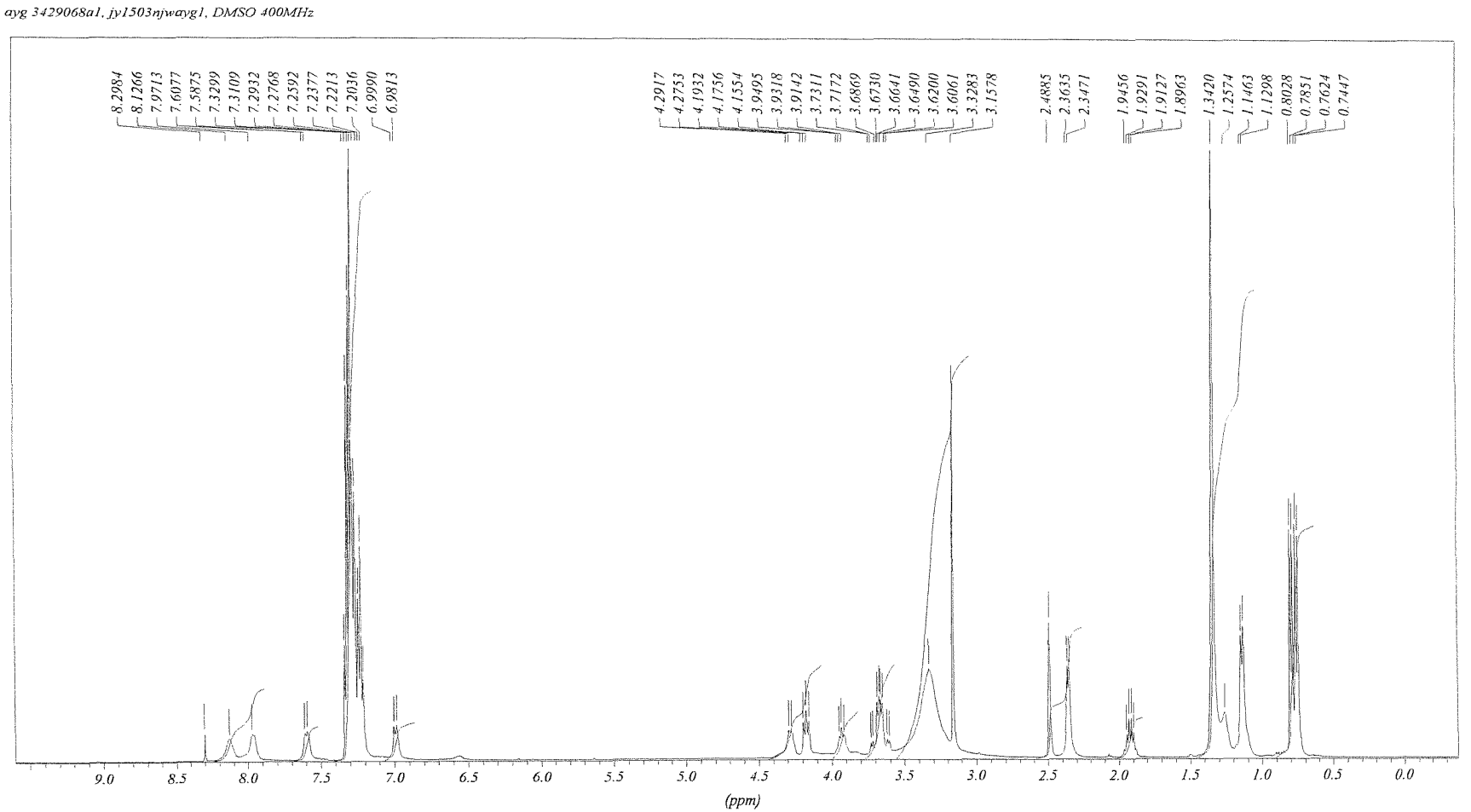
{(*R*)-2-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-trityl sulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid methyl ester (18)

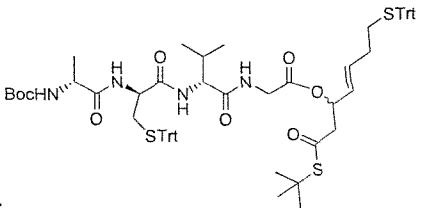
AYG3185076A1re-crystl, jy1602aygl (1), 400MHz



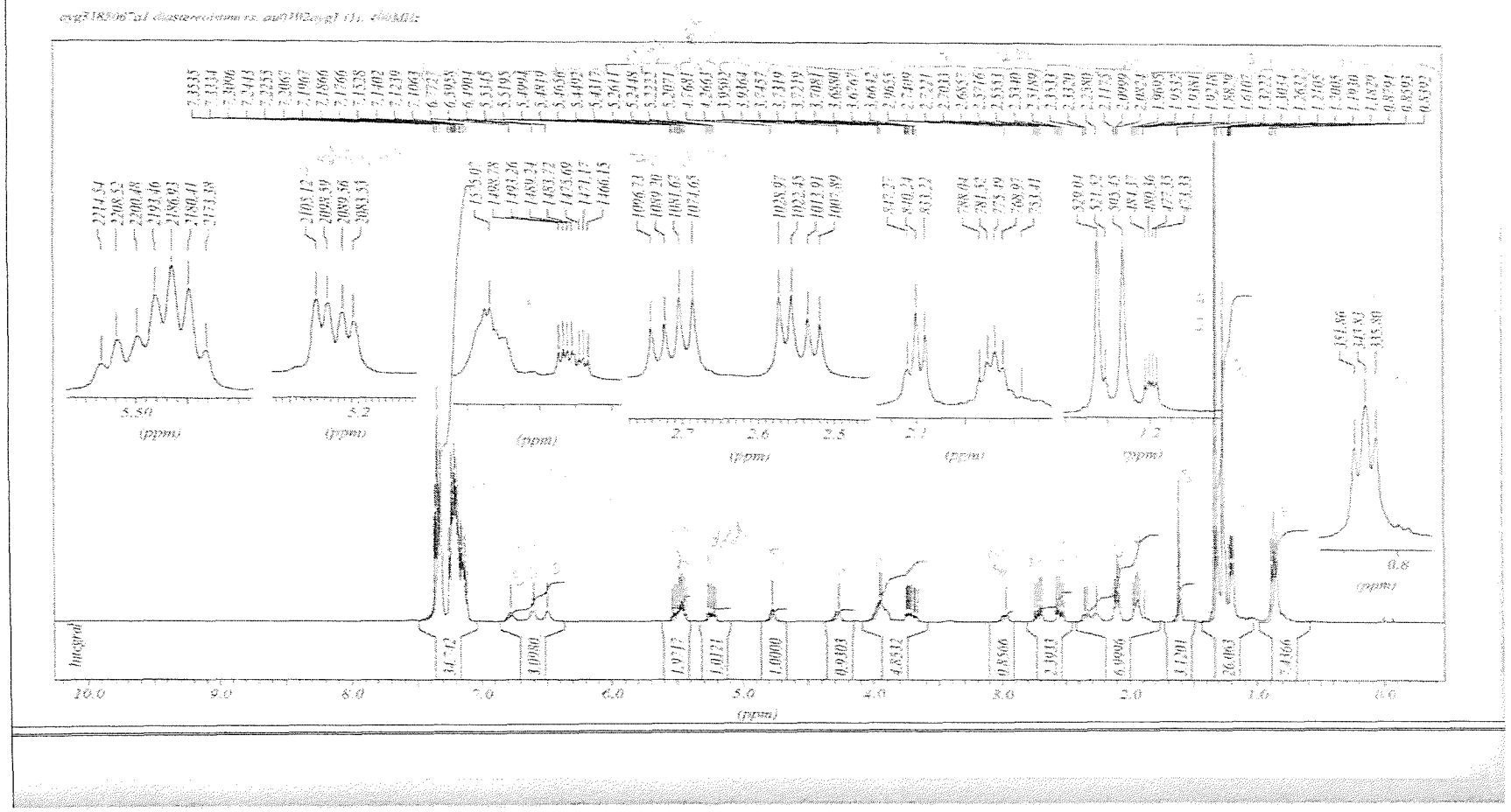


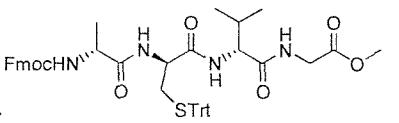
{(*R*)-2-[(*S*)-2-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid (19)





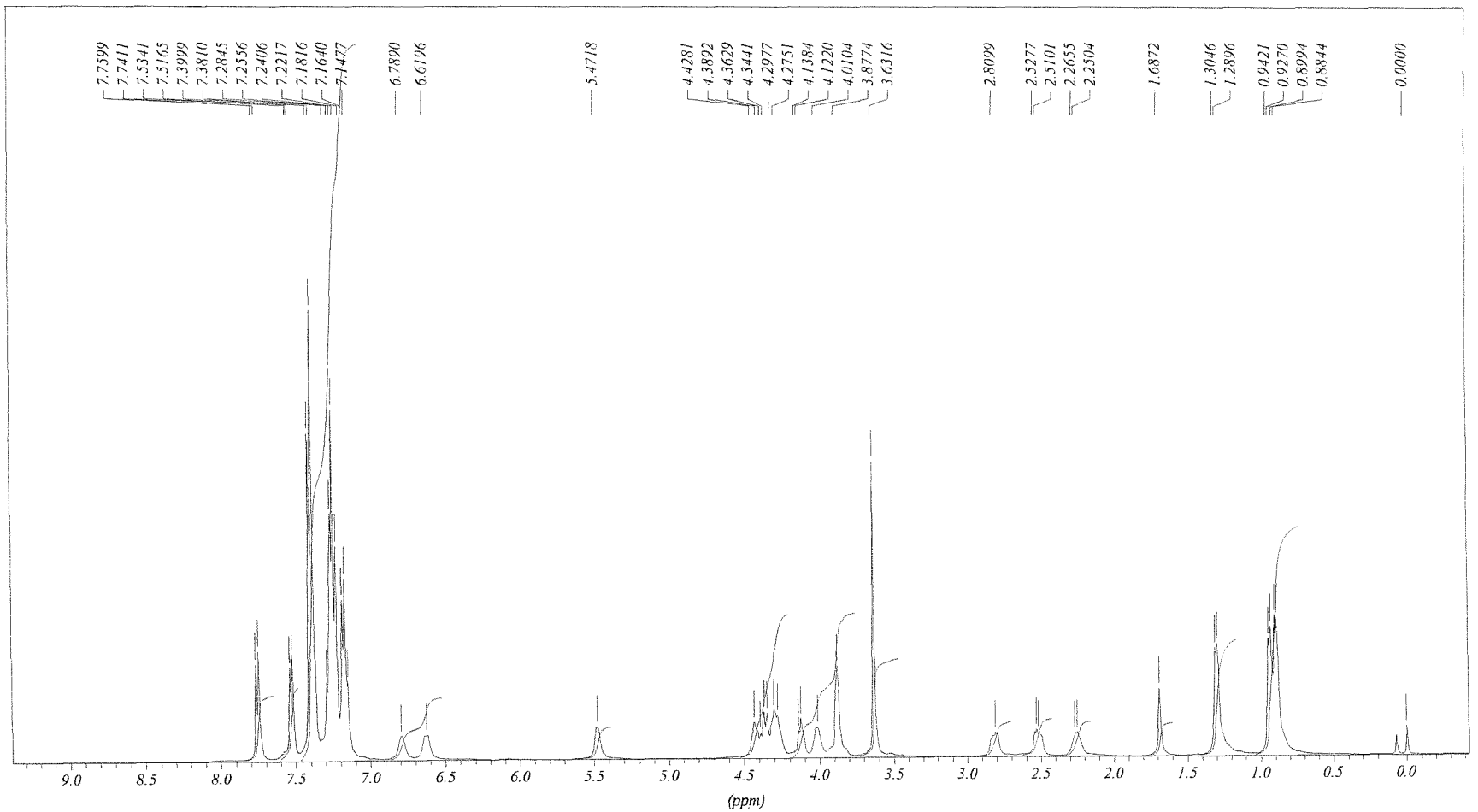
{(*R*)-2-[(*S*)-2-((*R*)-2*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid 1-*tert*-butylsulfanylcarbonylmethyl-5-tritylsulfanyl-pent-2-enyl ester (20)

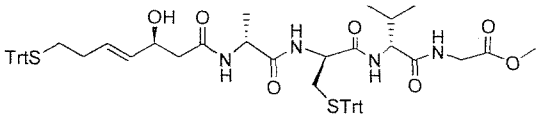




((*R*)-2-{(*S*)-2-[(*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-tritylsulfanyl-propionylamino}-3-methyl-butyrylamino)-acetic acid methyl ester (21)

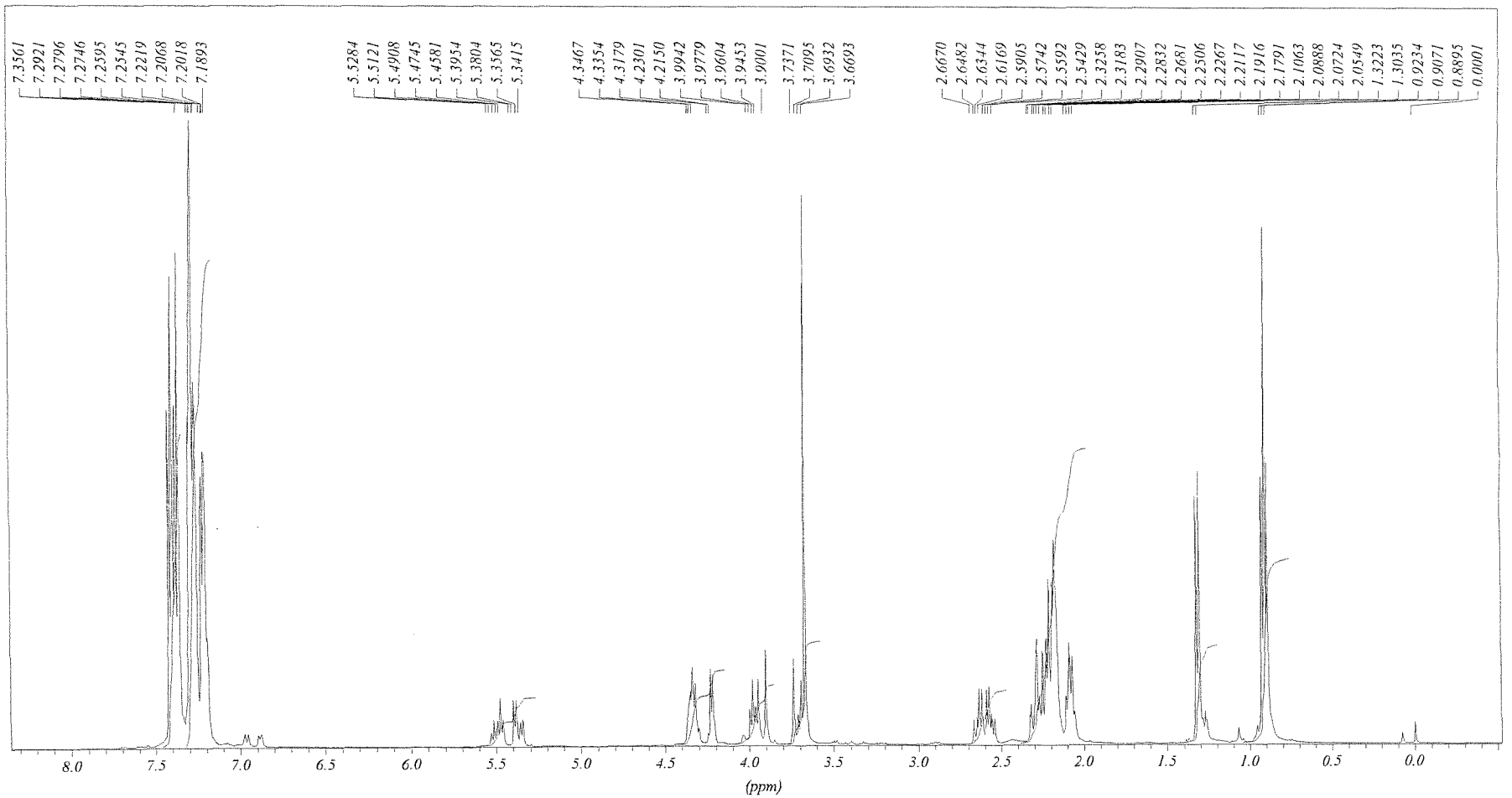
ayg3596013a1□y0203ayg1 (1) H 400MHz

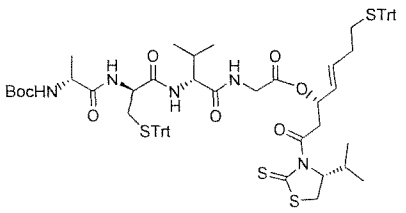




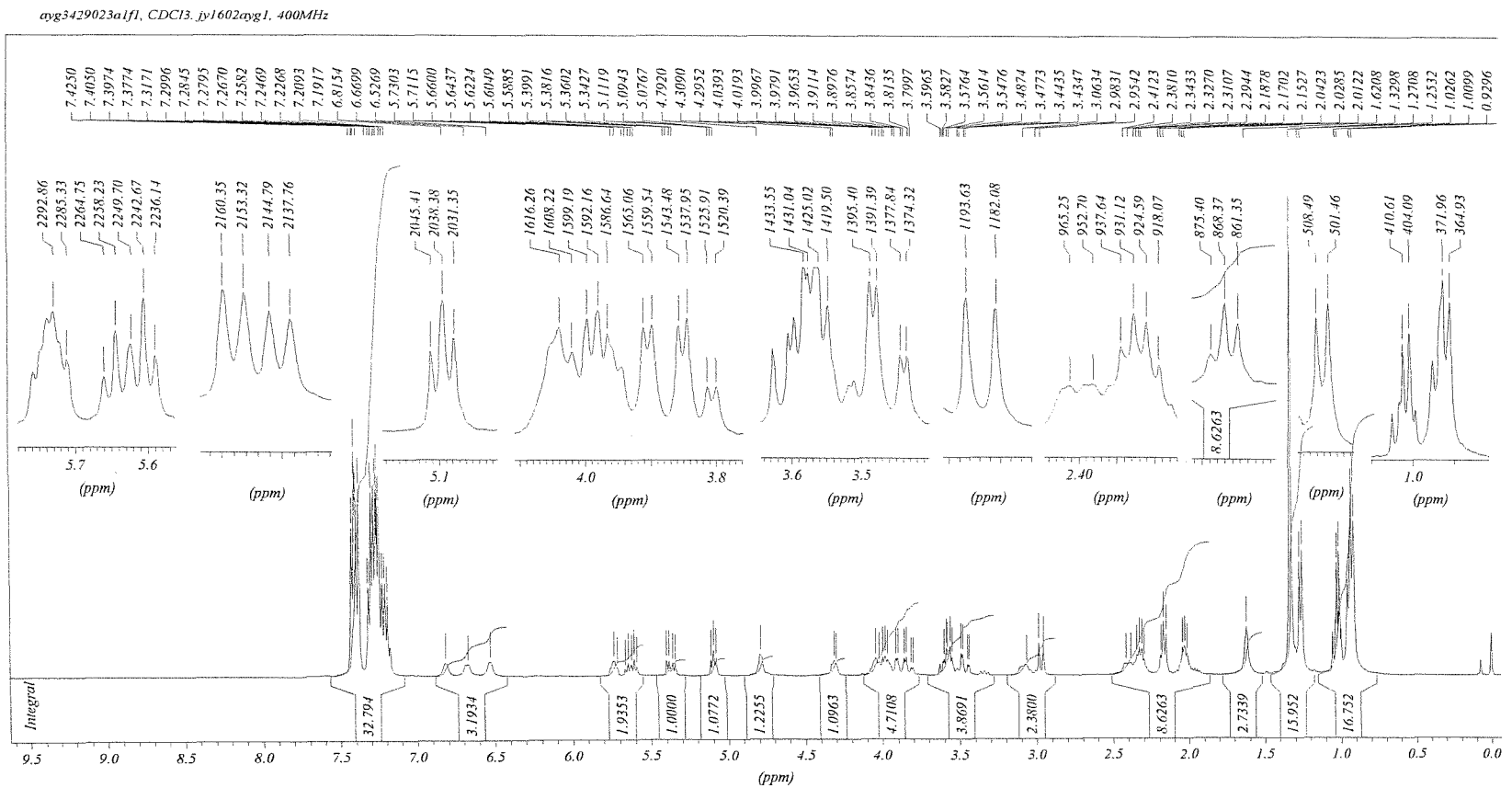
((*R*)-2-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-propionylamino]- 3-tritylsulfanyl-propionylamino}-3-methyl-butyrylamino)-acetic acid methyl ester (23)

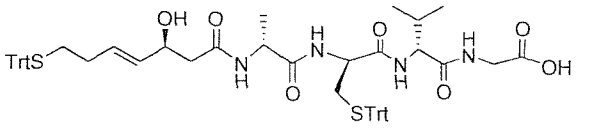
ayg3596015a1, jy0303ayg1 (1), 400 MHz, H



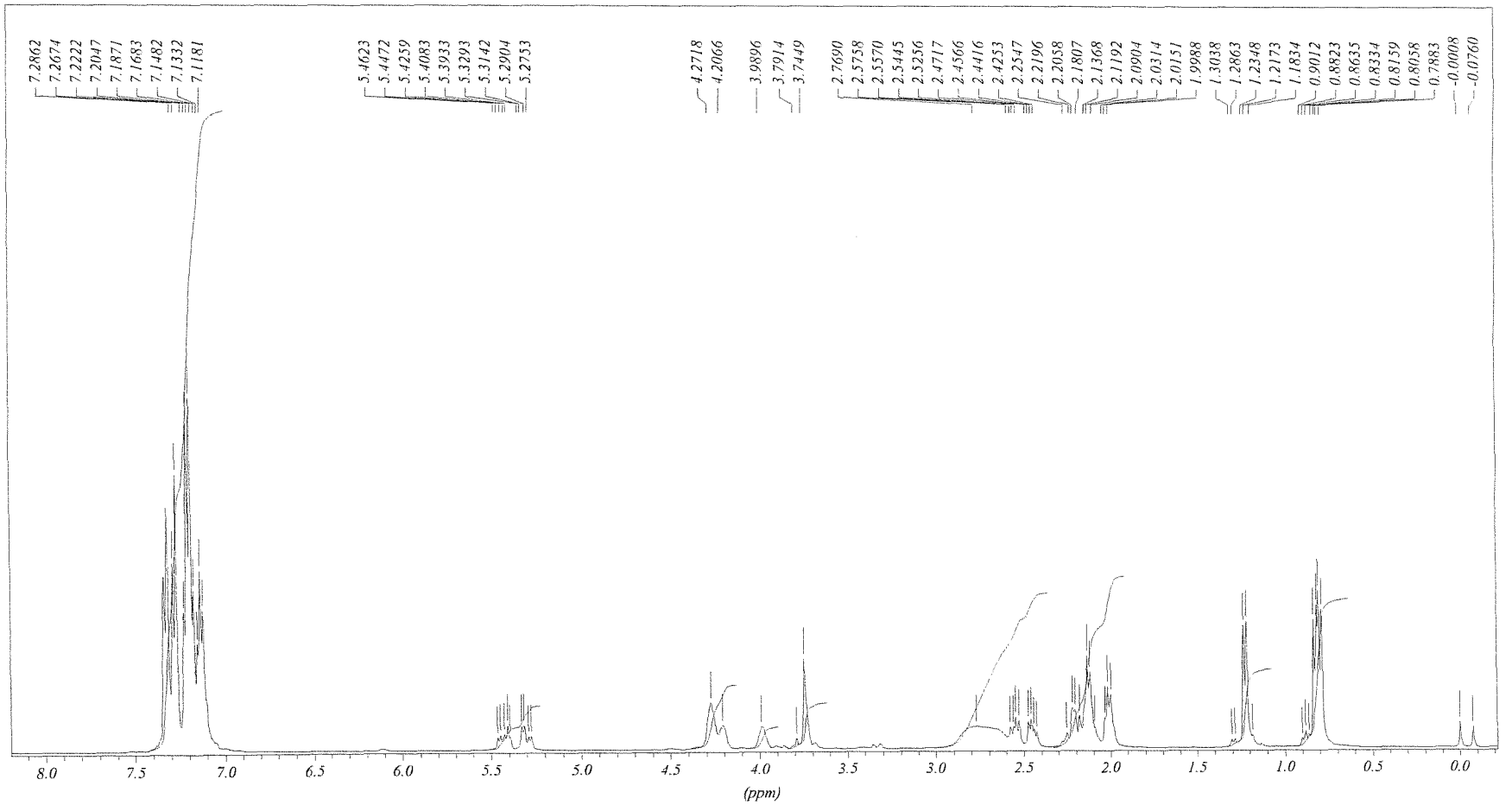


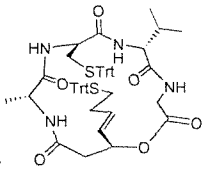
{(*R*)-[(*S*)-22-((*R*)-2-*tert*-Butoxycarbonylamino-propionylamino)-3-tritylsulfanyl-propionylamino]-3-methyl-butyrylamino}-acetic acid 1*S*-[2-((*R*)-4-isopropyl-2-thioxo-thiazolidin-3-yl)-2-oxo-ethyl]-5-tritylsulfanyl-pent-2-enyl ester (24)



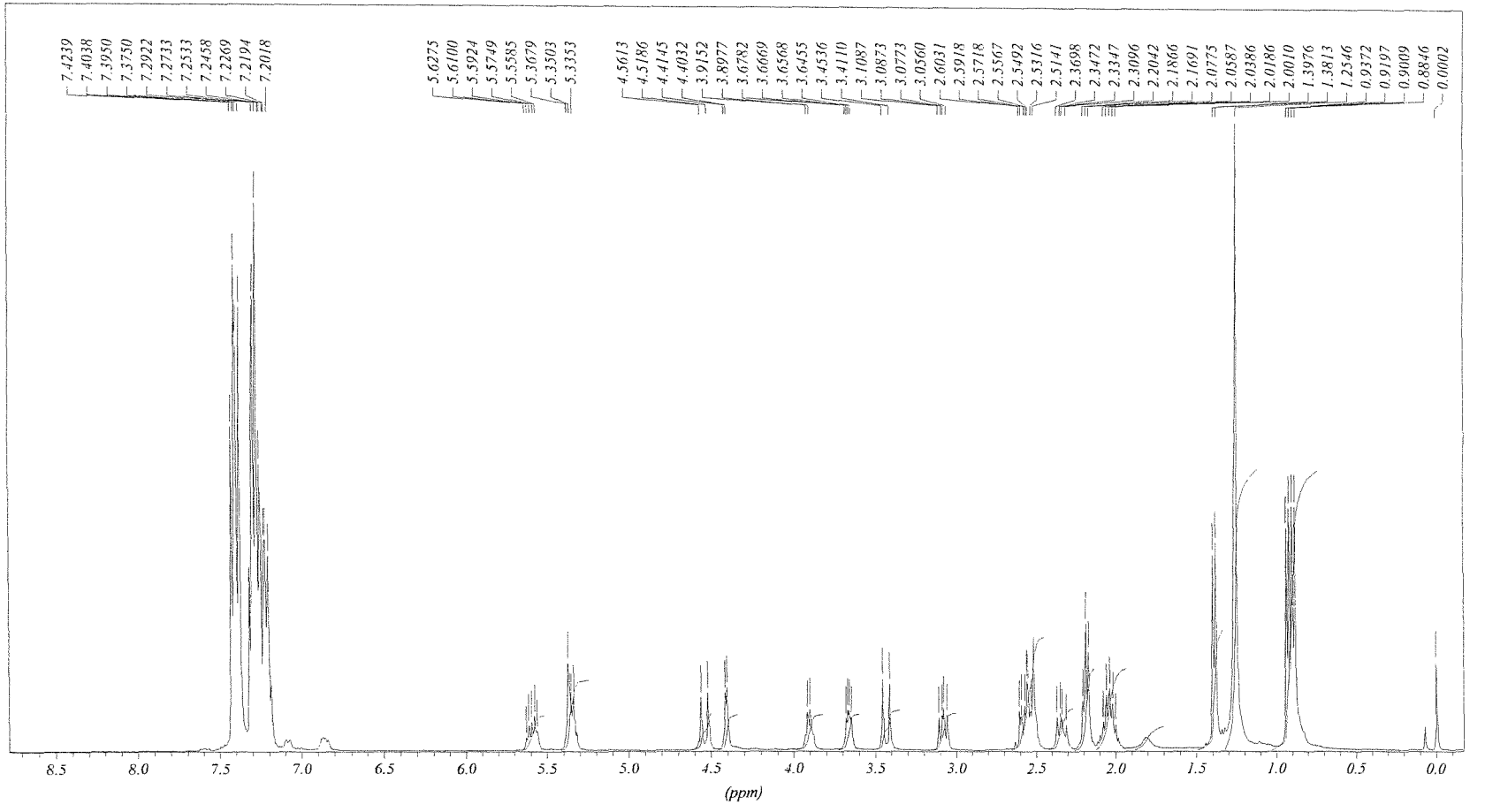


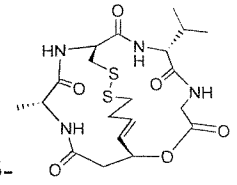
((*R*)-2-{(*S*)-2-[(*R*)-2-((*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoyla mino)-propionylamino]-3-tritylsulfanyl-propionylamino}-3-methyl-butyrylamino)-acetic acid (25)



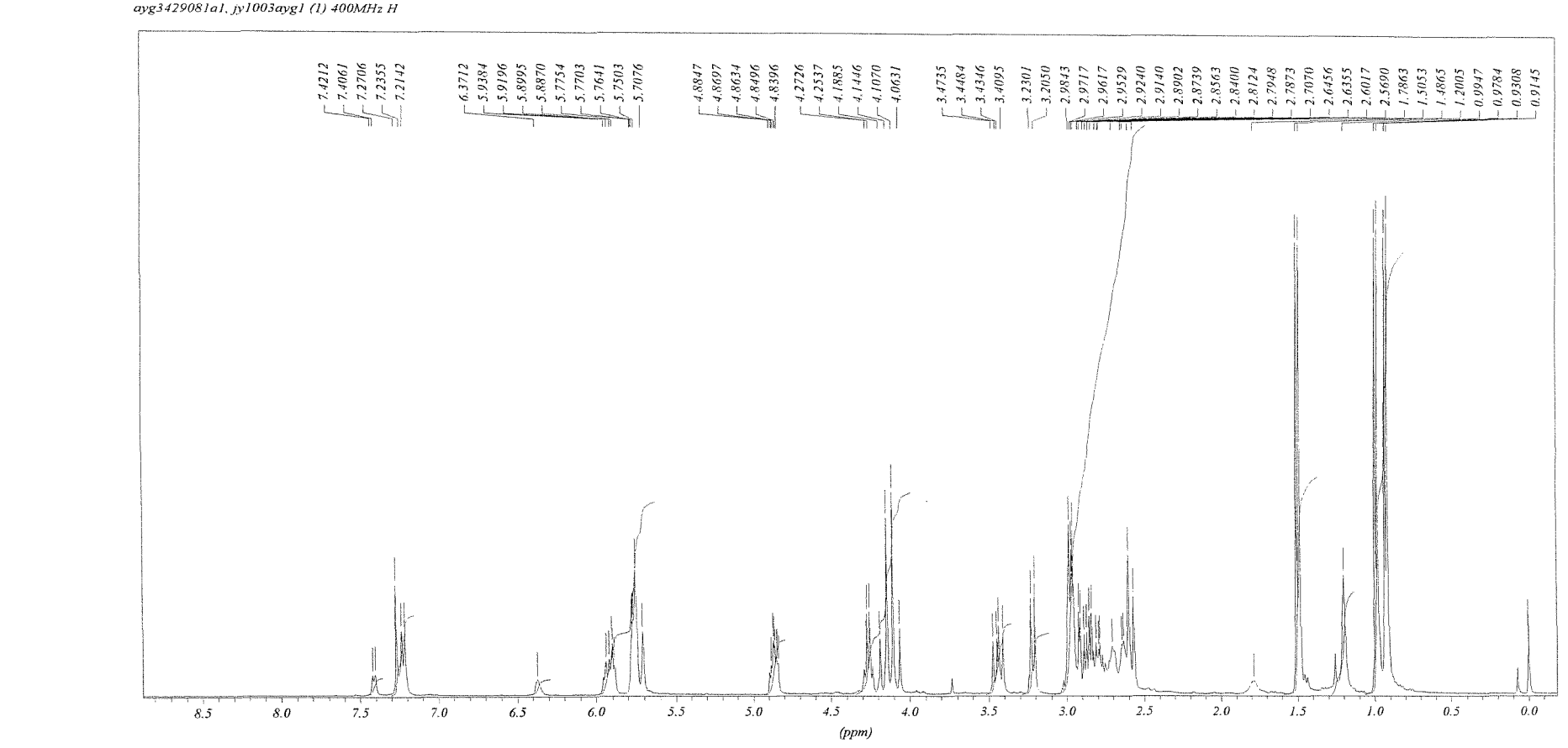


(6*R*,9*S*,12*R*,16*S*)-6-Isopropyl-12-methyl-16-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-4,7,10,13-tetraaza-cyclohexadecane-2,5,8,11,14-pentaone (26)



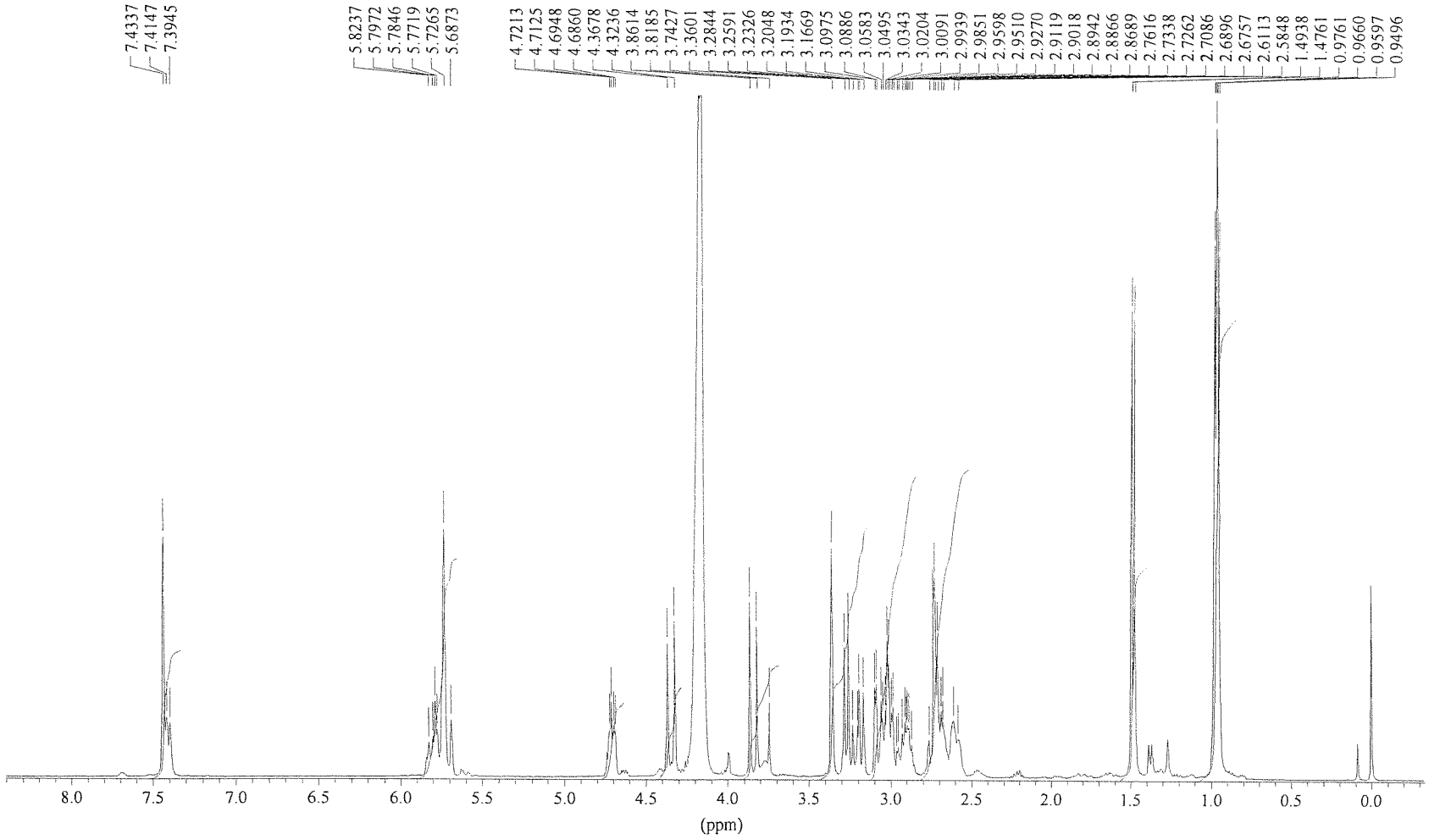


(*E*)-(1*S*,7*R*,10*S*,21*R*)-7-Isopropyl-21-methyl-2-oxa-12,13-dithia-5,8,20,23-tetraaza-bicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentaone (27)

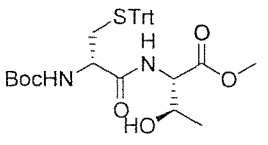


5%$CD_3OD/CDCl_3$

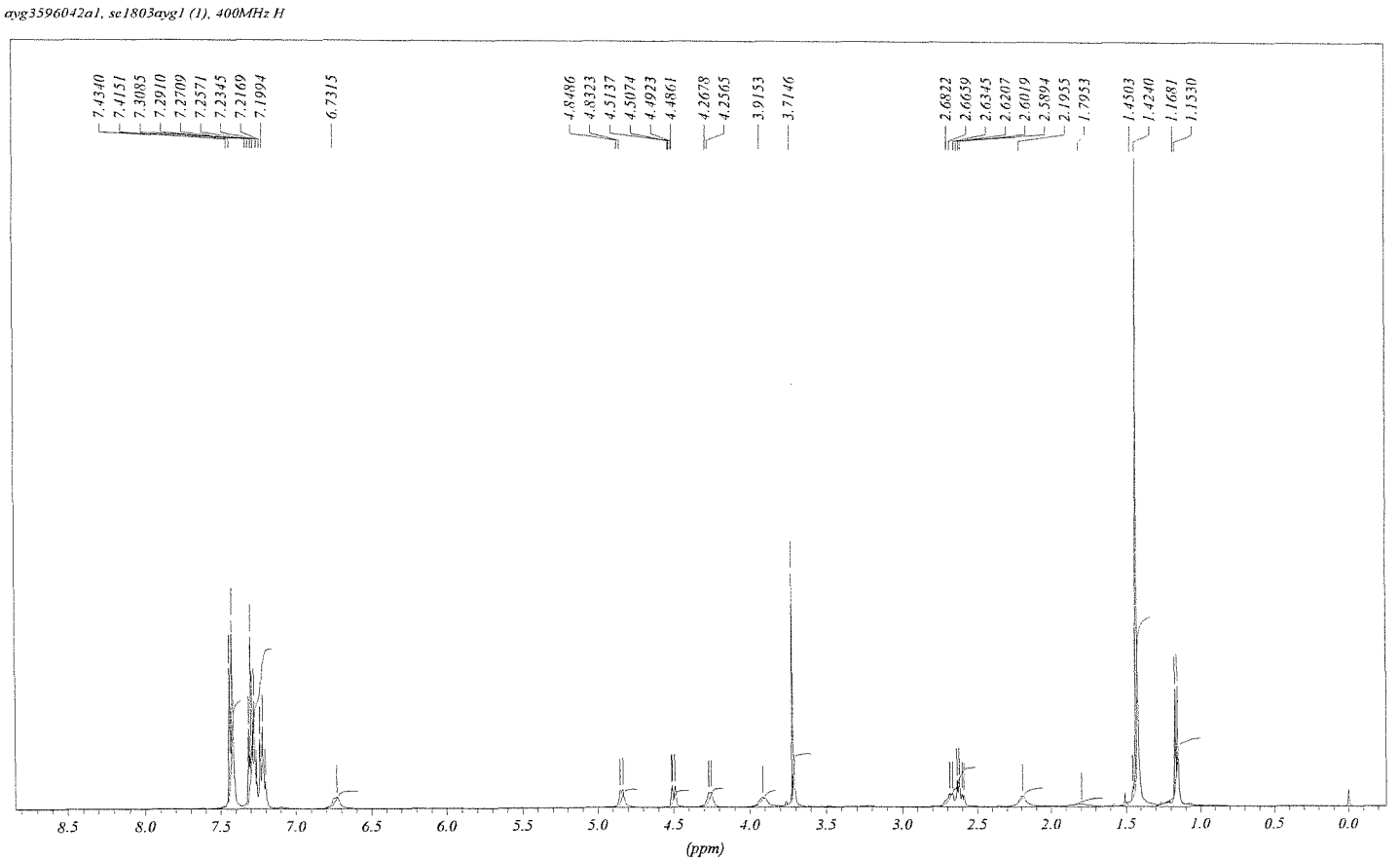
ayg 3429081a1, jy1503njwayg2 (1) 400MHz H, 5% CD3OD/CDCl3

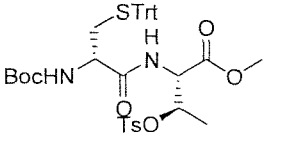


^{1}H NMR Spectra for compounds in Chapter 5



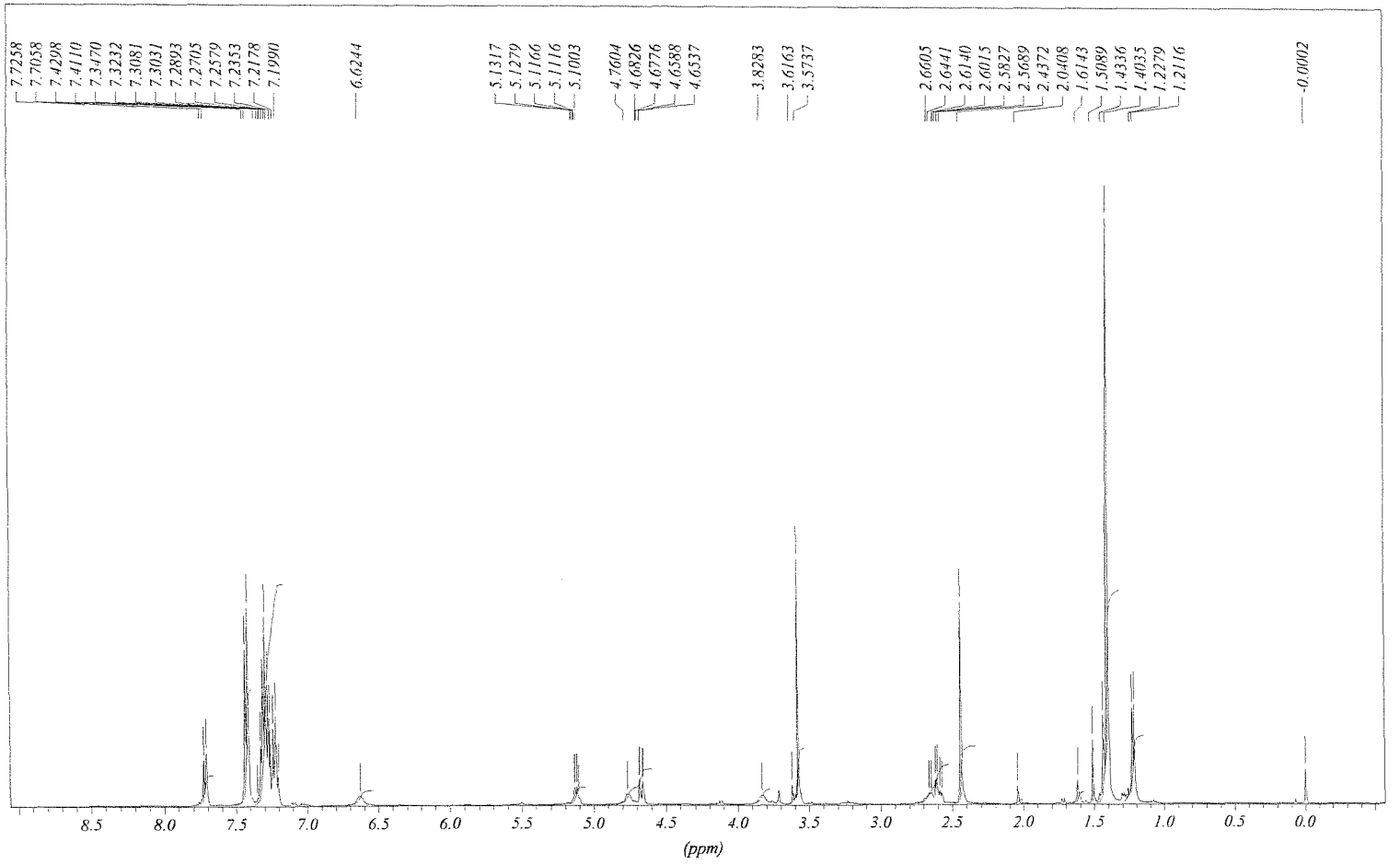
(2*S*,3*R*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino)-3-hydroxy-butyric acid methyl ester (3)

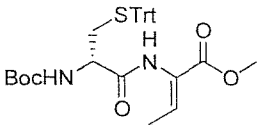




(2*S*,3*R*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino)-3-(toluene-4-sulfonyloxy)-butyric acid methyl ester (4)

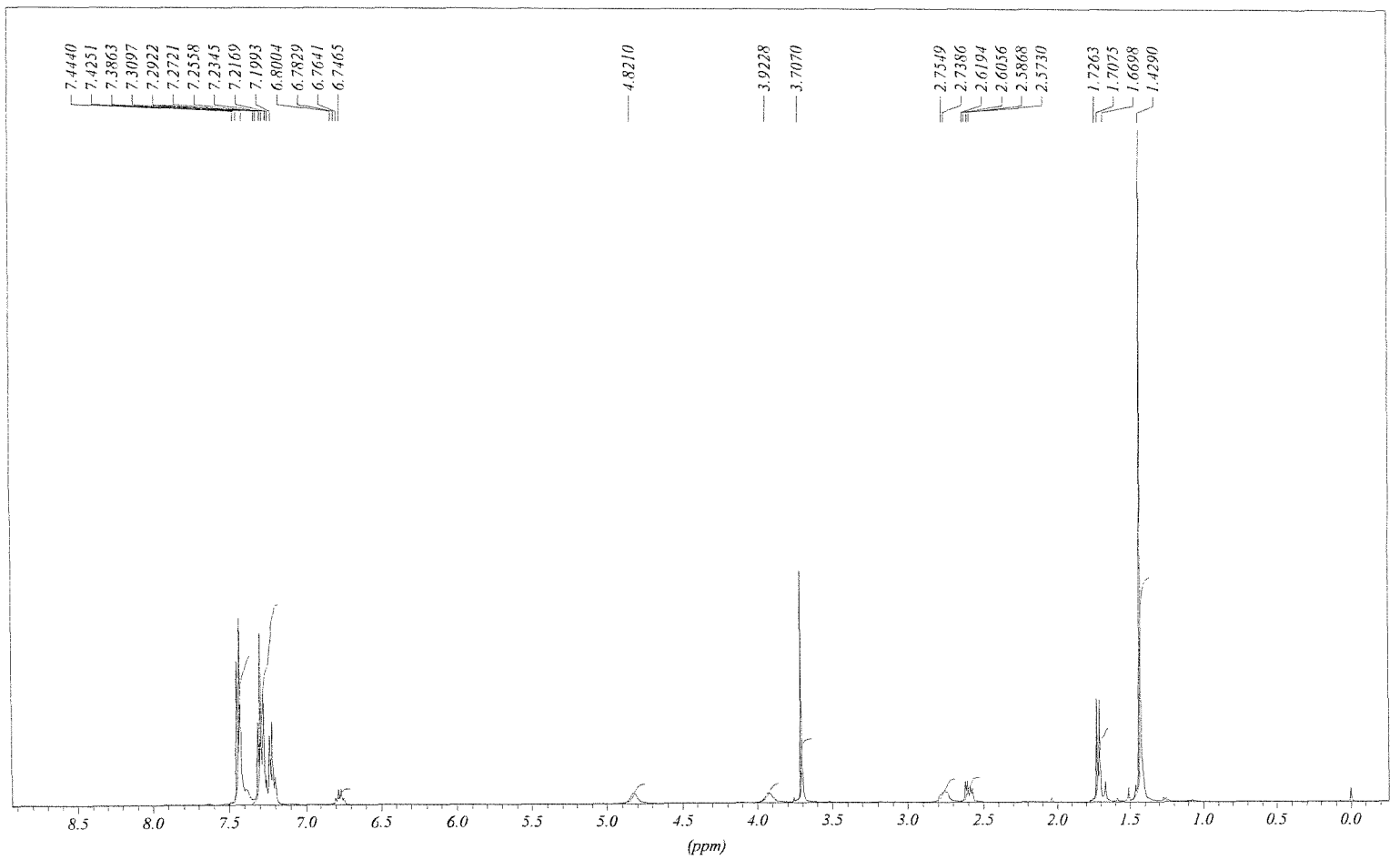
ayg3596043a1, Se2303ayg1 (1), 400MHz H

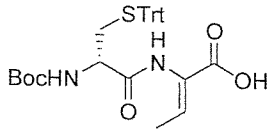




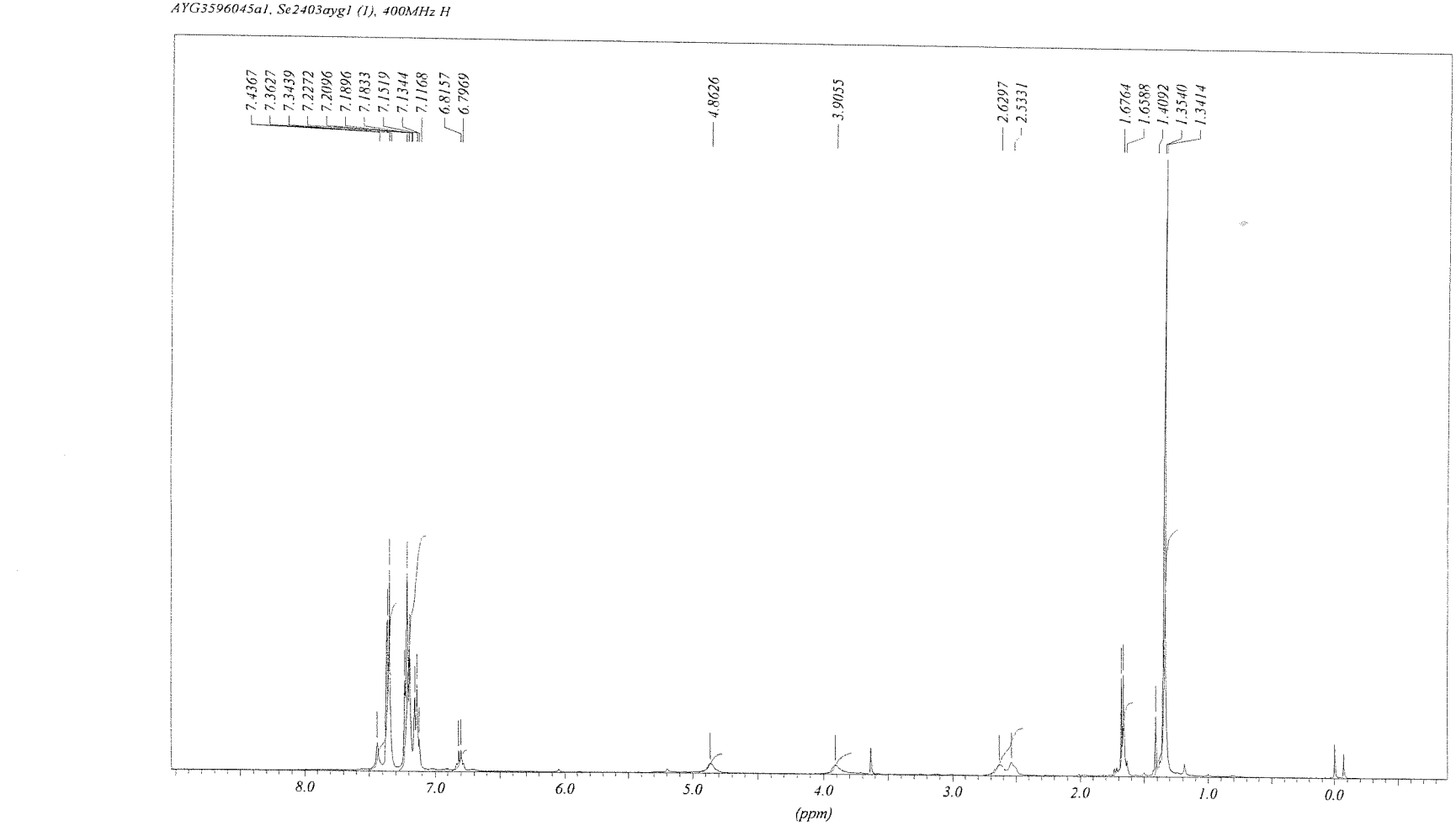
(*Z*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino) -but-2-enoic acid methyl ester (5)

AYG3596044a1, Se2203ayg1 (1), 400MHz H





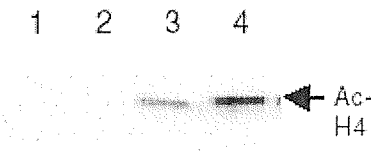
(*Z*)-2-((S)-2-*tert*-Butoxycarbonylamino-3-tritylsulfanyl-propionylamino) -but-2-enoic acid (6)



Biological data

Biological screening results comparing the activity of spiruchostatin A and epi-spiruchostatin A against the HDAC inhibitor trichostatin A (TSA).

Effects of compounds on histone acetylation



Legend. Accumulation of acetylated histone H4 in MCF7 breast cancer cells. 1, untreated cells; 2, solvent control (DMSO); 3, TSA (100 nM); 4, Spiruchostatin A (100 nM).

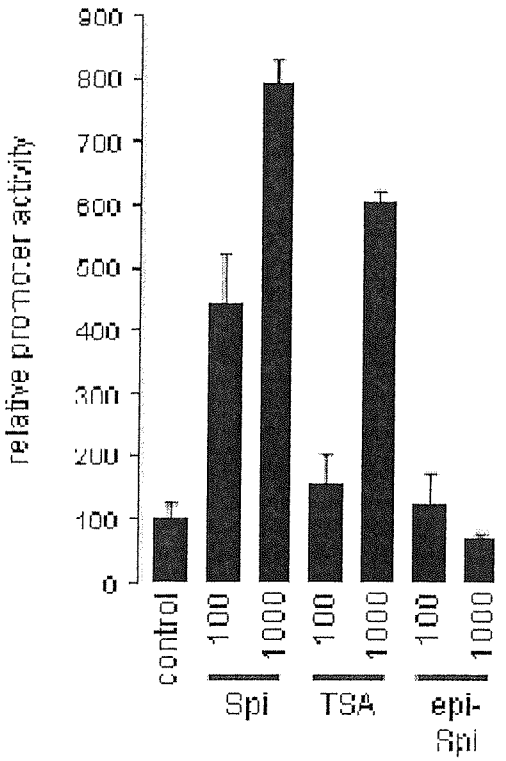
Effects of the compounds on histone acetylation were determined by immunoblotting. MCF7 cells were treated with compounds or solvent control (DMSO) for 16 hours. Cells were collected by scraping and centrifugation, washed in PBS and lysed in protein sample buffer supplemented with dithiothreitol (New England Biolabs). Genomic DNA was sheared by brief sonication and the sample was heated to 95 °C for 5 minutes before being resolved by SDS-polyacrylamide gel electrophoresis. Immunoblotting was performed as previously described (*Packham et al., Mammalian cells express two differently localized Bag-1 isoforms generated by alternative translation initiation. Biochem J. 1997 328:807-13*) using a rabbit anti-acetylated histone H4 antibody (Upstate Biotech). Acteylated histone H4 was undetectable in control MCF7 cells, or cells exposed to solvent alone as a control, but was abundant in cells exposed to trichostatin A or spiruchostatin A.

Effects of compounds on promoter activity

We also analysed the effects of compounds on activity of the p21waf1/cip1 promoter which has previously been demonstrated to be activated in cells treated with HDAC

inhibitors (*Huang L, Sowa Y, Sakai T, Pardee AB, Activation of the p21WAF1/CIP1 promoter independent of p53 by the histone deacetylase inhibitor suberoylanilide hydroxamic acid*

(SAHA) through the Sp1 sites. Oncogene. 2000 19:5712-9). MCF7 cells were transfected with a p21 promoter luciferase construct (pWWP) and a beta-galactosidase expression construct to correct for variation in transfection efficiencies. Transfections were performed using the TransFast reagent (Promega) according to the manufacturer's instructions. The cells were allowed to recover overnight before being treated with compounds. After 24 hours, cells were harvested and luciferase and beta-galactosidase activity determined using standard assays. The p21waf1/cip1 promoter was maximally activated 6 to 7-fold in cells treated wih 1 μM spiruchostatin A or TSA, but not in cells treated with *epi*-Spiruchostatin. At 100 nM, only Spiruchostatin A significantly increased p21 promoter activity.



Legend. MCF7 cells were transfected with a p21-luc reporter construct and allowed to recover overnight. Cells were treated with the indicated concentrations (nM) of compounds or left untreated as a control. After 24 hours, luciferase activity was

determined. Luciferase values were normalised to beta-galactosidase activity to control for variation in transfection efficiency and the relative luciferase activity of control cells set at 100%. DMSO (solvent control) had no significant effect on p21 promoter actvity. Values are derived from duplicate transfections.

Growth inhibition assays

MCF7 human breast cancer cells were obtained from the European Collection of Animal Cell Cultures and maintained in Dulbecco's Modified Eagle's Medium containing glutamine and supplemented with 10% (v/v) fetal calf serum and antibiotics. The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega) was used to determine the effects of compounds on cell growth according to the manufacturer's instructions. Briefly, MCF7 cells (5,000 per well of a 96 well plate) were incubated in the presence or absence of test compound in a total volume of 100 µl for 48 hours. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and phenazine methosulfate (PMS) reagents were added in a total volume of 20 µl and the cells were incubated at 37 °C. Absorbance was measured at 490nm. The concentration of compound required to inhibit the growth of MCF7 cells by 50% (IC50) was determined. Spiruchostatin A and trichostatin A (Calbiochem) inhibited the growth of cells with an IC_{50} of approximately 10 nM and 100 nM, respectively, whereas *epi*-spiruchostatin A was essentially inactive at the highest tested concentration, 10 µM.