TOWARDS THE SYNTHESIS OF HISTONE DEACETYLASE INHIBITORS BASED ON THE DEPSIPEPTIDE FK228

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

SCHOOL OF ENGINEERING, SCIENCE AND MATHEMATICS

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Histones are proteins that are used as a scaffold for DNA to wrap around and on these proteins are lysines. Histone Deacetylases (HDACs) are metalloenzymes that remove acetyl groups from lysines on histones and thus cause genetic material to wrap tighter which can stop transcription. If the genes that can regulate tumour activity are affected by this method, then these are rendered inactive. In cancer patients, it can be seen that the amount of HDACs are higher than normal then in a normal person and this has created the need to understand these and find products that can inhibit these.

The search for inhibitors have led to such products as SAHA, FK228 and spiruchostatins (both of the latter are depsipeptides with disulfide bridges) and by researching depsipeptides such as FK228, more understanding can be made in how these compounds work. Depsipeptides such as FK228 consist of from three components, a zinc binding thiol, which is the active site, a 'cap', which is the macrocycle and a linker that connects the two. This research shows how attempts at modifying key components, such as the linker length and saturation and the 'cap' structure of the depsipeptides have been made.

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Chapter 1

1.1 Introduction

A hallmark of cellular pathologies such as neoplastic transformation is that the normal control of differentiation, cell cycle, and apoptosis becomes deranged and this abnormal phenotype is due to altered patterns of protein expression, which in turn come from genetic abnormalities. An area of particular interest in basic and clinical research are the epigenetic control mechanisms of DNA packaging as a means of gene expression[1].

In eukaryotes, DNA is tightly compacted into an ordered structure of chromatin comprising histones, non-histone proteins and DNA (Fig. 1).

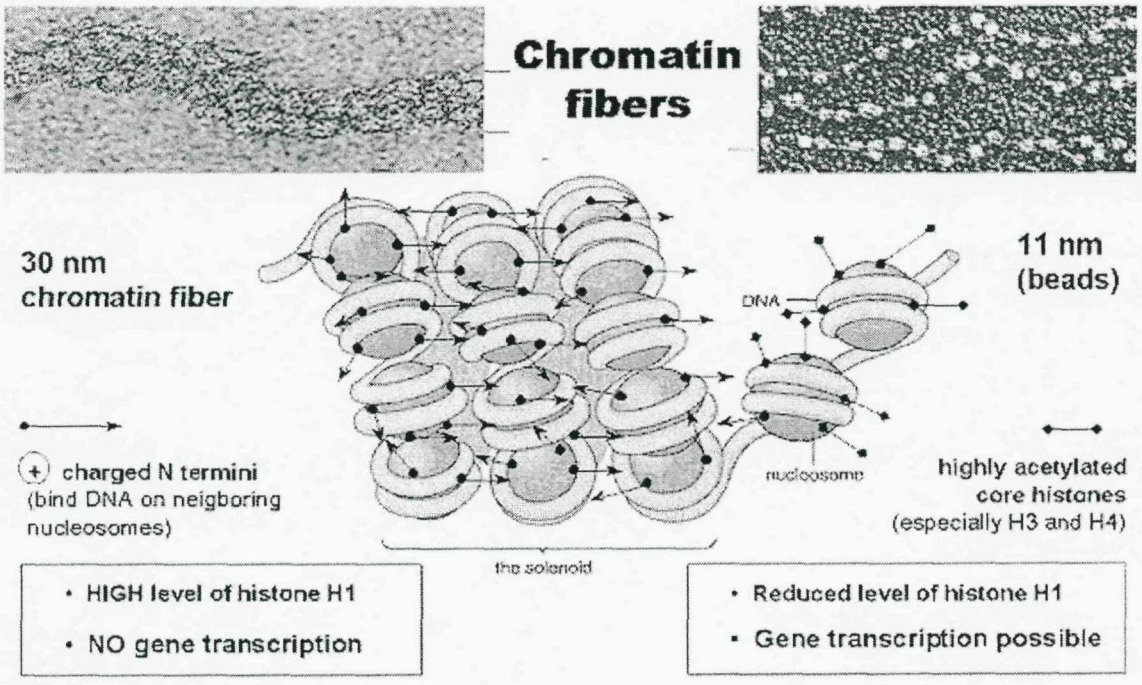


Fig. 1: A diagram of chromatin.

These are ordered in a way that the basic repeating unit is 146 base pairs of DNA wrapped left handed twice around a core of proteins. To these macromolecules a number of modifications can be applied for example, DNA is primarily methylated at CpG residues which appears to be a gene silencing mechanism[2]. Histones are globular proteins

with long N-tails making up 25% of their structure and are susceptible to a number of post-translational modifications including methylation of lysine and arginine, acetylation of lysine, phosphorylation of serine, ubiquitinylation of lysine and poly-ADPribosylation of glutamic acid residues.

Histone acetyltransferases (HATs) are enzymes that mediate the transfer of an acetyl group from acetyl-coenzyme A (CoA) to the ε-amino group of lysine residues (Scheme 1).

Scheme 1: Mechanism of acetylation of a lysine residue by acetyl-CoA and HATs.

At physiological pH, lysine residues are normally protonated and therefore positively charged, however when the residues are acetylated by HATs, then the amino group becomes a neutral amide group. As a result of this dramatic change in charge, the affinity between the positively charged histones and the negatively charged phosphodiester backbone is weakened, allowing complex proteins and transcriptional factors to bind to DNA and relax the tightly wound structure. Apart from allowing transcription to occur, with some histones, the acetylation of some lysine residues appears to enhance gene expression. As with most physiological mechanisms, there are also enzymes that perform the reverse reaction and these hydrolysing enzymes are called histone deacetylases (HDACs). These catalyse the removal the acetyl groups on the acetylated lysine residues and cause the affinity between histones and the phopshodiester backbone to increase, thereby causing the DNA to be more tightly wound and in a more transcriptionally silenced state.

HDACs are a group of enzymes that catalyse the hydrolysis of acetyl-lysine residues in proteins and while these are important for the process in which histones modulate the chromatin structure, reversible lysine acetylation is not limited to just histones. It rivals phosphorylation as a general mechanism of cellular signal transduction with non-histone proteins[3] both nuclear and cytoplasmic being known substrates for HATs and HDACs.

There are more then a dozen HDAC enzymes in the human body[4] of which there are two major classes. Class I and class II HDAC enzymes comprising HDAC 1-11 in the human genome show a common mechanistic feature in that they are metalloenzymes with a highly conserved catalytic domain of 390 amino acids containing a zinc atom. HDAC 1,2,3 and 8 belong to class I and the rest except HDAC 11 being class II (HDAC 11 shows common characteristics of both class I and II). There is a class III, however these are not metalloenzymes and thus will not be further mentioned.

Although the first mammalian HDACs were first characterised a decade ago, these enzymes have been already been identified as being attractive therapeutic targets[5] for a number of diseases that include cancer, inflammation and neurodegenerative.

A hallmark of primary tumours[6] is altered acetylation patterns and the best evidence for the importance of HDACs in cancer comes from studies of small HDACs molecule inhibitors from in vitro to tumour models and clinical trials. Despite the potential for HDAC inhibitors to affect activity in healthy cells, early clinical studies have shown that HDAC inhibitors are well tolerated in the human body[7].

Apart from cancer research, HDAC inhibitors also show promising results in mouse and *Drosophila* models for the treatment of neurodegenerative ailments such as Parkinson's and Huntington's disease[8]. In vitro studies have also shown that in mice, cardiac hypertrophy and aberrant HDAC activity are linked, while mouse knockouts of class II HDACs 5 and 9 lead to hypertrophic phenotype, showing that non-selective HDAC inhibitors are not suitable. HDAC inhibitors are also showing results in models for other therapeutic areas and infectious diseases.

1.2 Structures of HDAC inhibitors

There are four structural classes of inhibitors, but all are generally substrate mimics of the acetyl-lysine side group with a zinc binding group (Fig. 2).

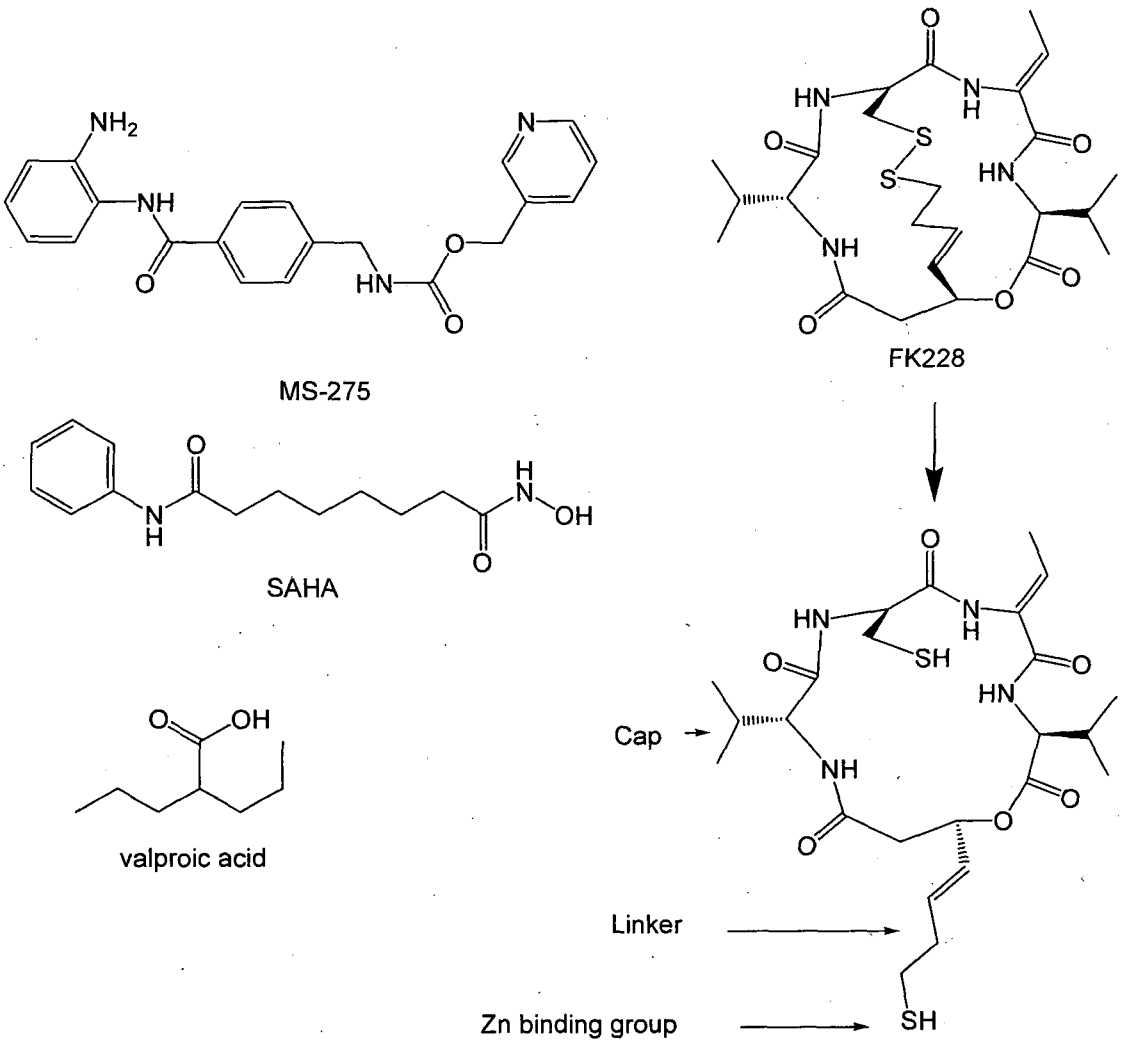


Fig. 2: The four structural classes of HDAC inhibitors.

With these different structural classes, a varying degree of inhibition can be seen. Short chain aliphatic fatty acids are generally weak, micromolar inhibitors with a short half-life *in vivo*. Linear hydroxamic acids such as SAHA are inhibitors at the nanomolar level due to a superior zinc binding group. Benzamides are similar to the hydroxamic acids in potentcy. The last class of inhibitors are also the most potent among clinical candidates.

These are depsipeptides that work as a prodrug that upon intracellular reduction of the disulphide releases a free thiol that acts as a zinc binding group.

With HDAC inhibitors, the minimal pharmacophore is a zinc binding group, an aliphatic linker and a 'cap' that extends beyond the substrate binding channel in the enzyme. The hydroxamic acids illustrate this principle with a small cap and a strong zinc binding group. However the hydroxamic acids show little selectivity between HDACs due to the binding being largely in the highly conserved substrate channel. The depsipeptides show higher selectivity between HDACs mainly because the weaker zinc binding group is compensated by the binding interactions of the large 'cap' and the 'rim' of the enzyme outside the active site.

FK228 is shown to be active in tumour xenograft animal models and to have similar effects to other known HDAC inhibitors. However superficially, FK228 does not resemble classic HDAC inhibitors, but one can deduce in the reducing environment of the cell, the disulphide is reduced to free thiols thereby fitting the classical models by having generated the zinc binding group with a linker. Experiments performed[9] have provided evidence to prove this hypothesis. Against partially purified HDAC 1 and 2, FK228 becomes significantly more active when the reduced dithiol is used or when reducing agent such as DDT is used in conjunction with FK228. Also seen is the loss of activity when the oxidising agent H_2O_2 is used and a thiomethyl resulting from the alkylation of FK228 also proved inactive. The data proved also that the FK228 was more potent towards the class I HDACs 1 and 2 then class II HDACs 4 and 6 revealing that the structural interactions outside on the 'rim' of the enzyme enable selectivity between isoforms.

Another depsipeptide natural product discovered is the spiruchostatins. These are structurally similar to FK228 and so were likely to be HDAC inhibitors (Fig. 3)

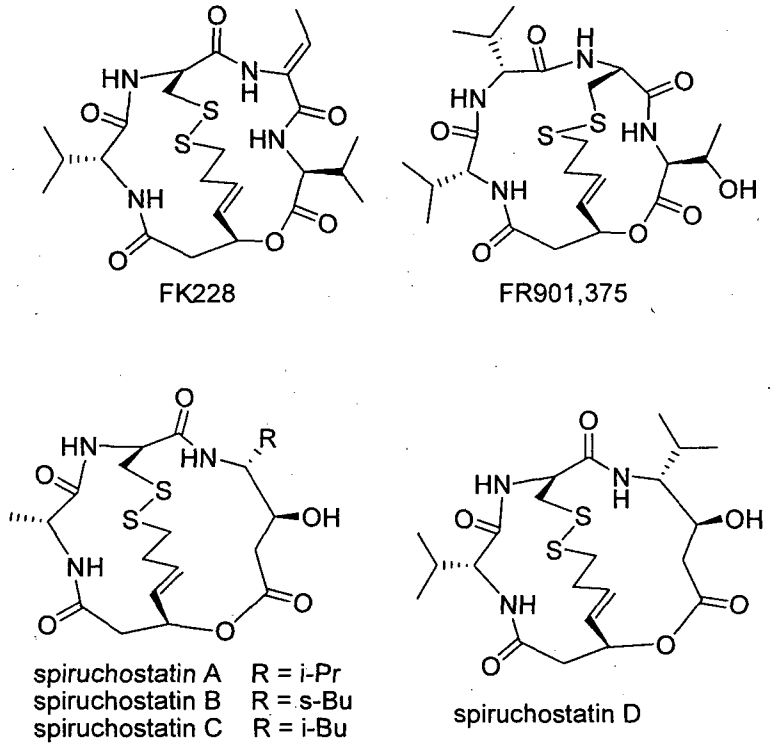


Fig. 3: Diagram showing the different depsipeptide natural product HDAC inhibitors.

The spiruchostatins were first isolated based on their ability to regulate gene expression but due to the similarity to FK228 in structure, the spiruchostatins were used in HDAC model systems and showed potency at the same level as FK228. Evidence has shown that, like FK228, spiruchostatins also undergo reduction of the disulphide bond in order to become active and also proved similar in the ability to be selective between isoforms of HDACs, with activity against the class I HDAC 1 being 500 times greater then the class II HDAC 6. These results show that FK228 and spiruchostatins have similar characteristics and mechanisms as HDAC inhibitors[9].

When side-by-side studies to compare the growth inhibitory activity between the depsipeptides and the other classes of HDAC inhibitors were performed, the results showed that FK228 and the spiruchostatins are potent inhibitors with sub-nanomolar/low-nanomolar potency in cell growth inhibition assays. This is more potent then the hydroxamic acids that are in clinical trials, which gave high-nanomolar IC_{50} values in these cells (Fig. 4). Similar results were generated from other comparisons using various tumour cells[10].

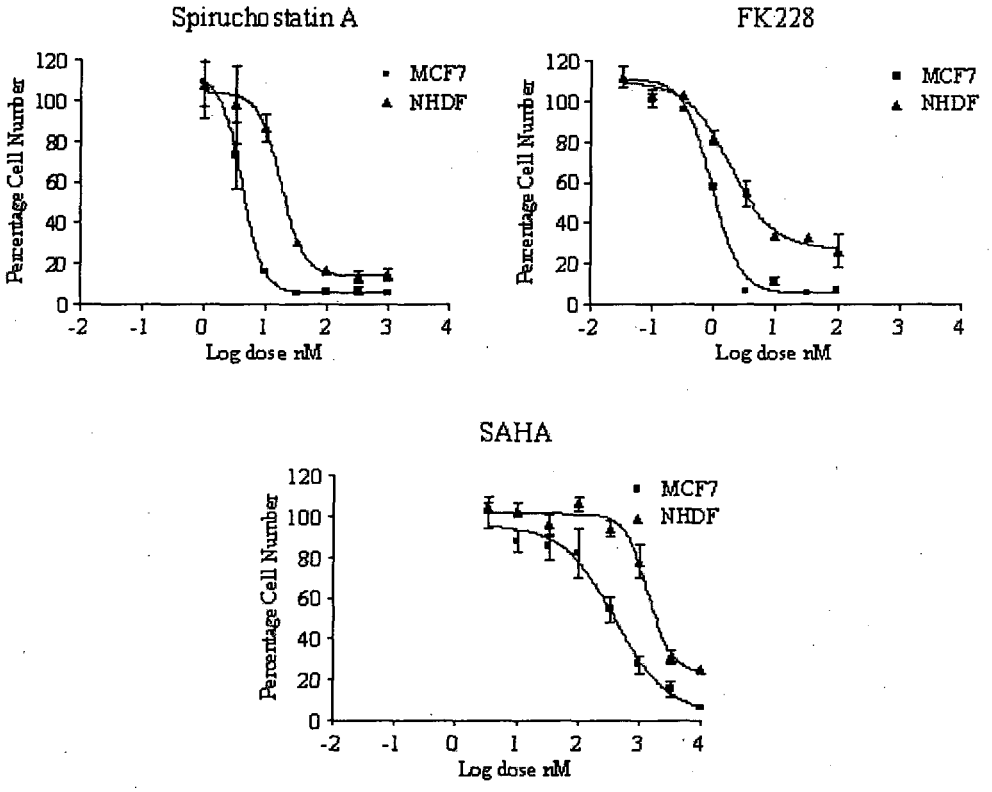


Fig. 4: Growth inhibition curves of HDAC inhibitors in MCF7 and NHDF (normal human dermal fibroblast) cell lines. Cells were treated with inhibitor and relative cell growth determined six days later.[10]

The interactions between the 'cap' and the 'rim' of the enzyme outside the active site with depsipeptides offer plenty of opportunity for modification in order to increase potency and selectivity. When altered, the interactions alter the activity of the analogue and results from numerous analogues of show that activity can be modulated by altering the 'cap'.

1.3 The synthesis of FK228

FK228 can be synthesised from two fragments; a tetrapeptide and a β-hydroxy acid (Fig. 5).

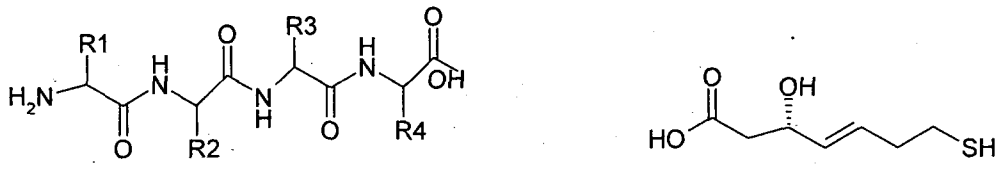
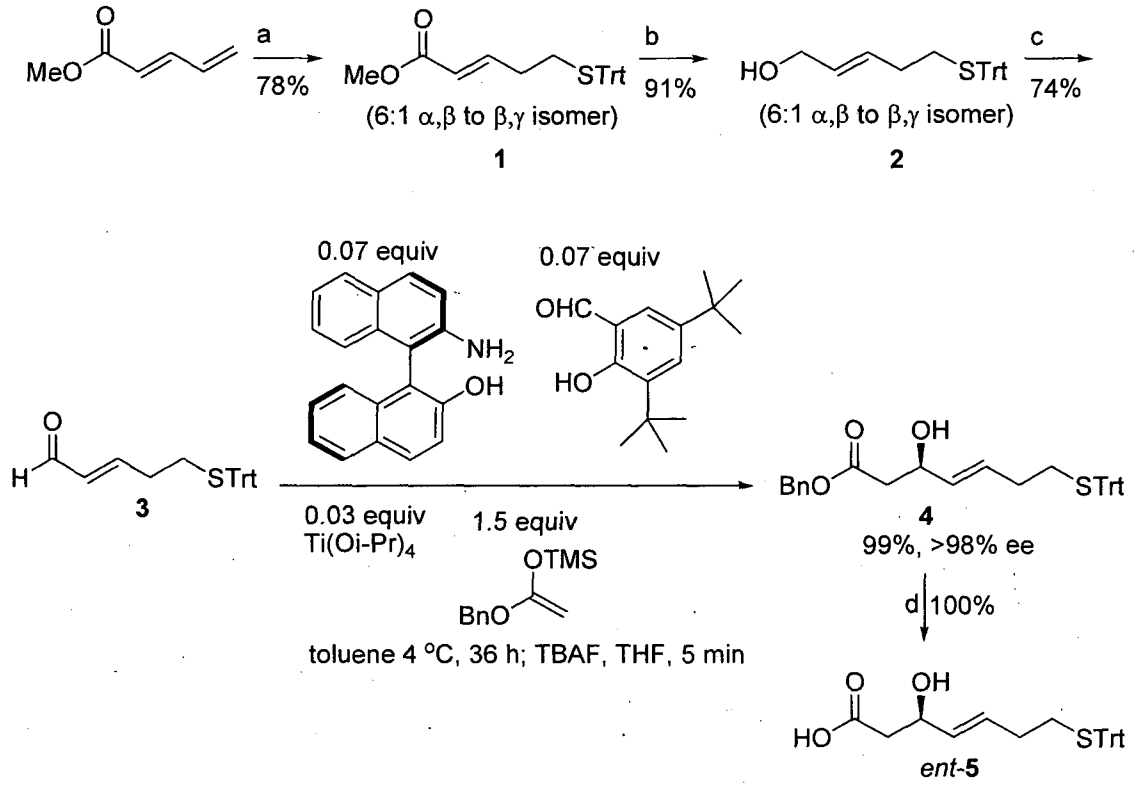


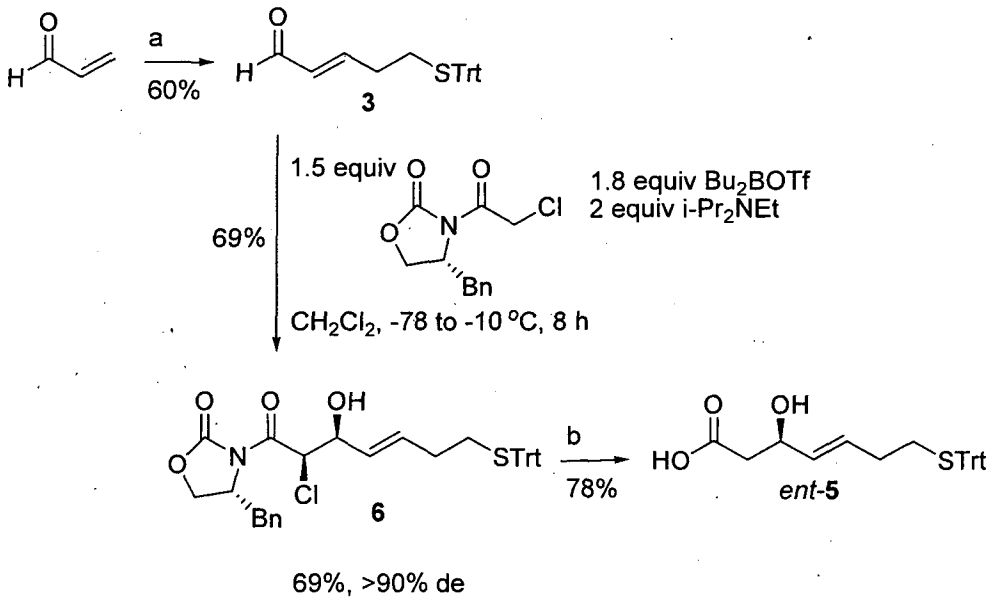
Fig. 5: A generic tetrapeptide and β-hydroxy acid

There are two routes to forming the β-hydroxy acid with the Simon's synthesis of FK228[11] first used. This method used methyl pentadieonate and trityl thiol to form the 1,5 conjugate addition product **1**. This produced a mixture of α,β- and β,γ-unsaturated isomers which are reduced to the alcohol **2** and then oxidised to form the α,β-unsaturated aldehyde **3**. This underwent an asymmetric aldol reaction under Carreira's conditions and then a hydrolysis (Scheme 2) to form the β-hydroxy acid **5**.



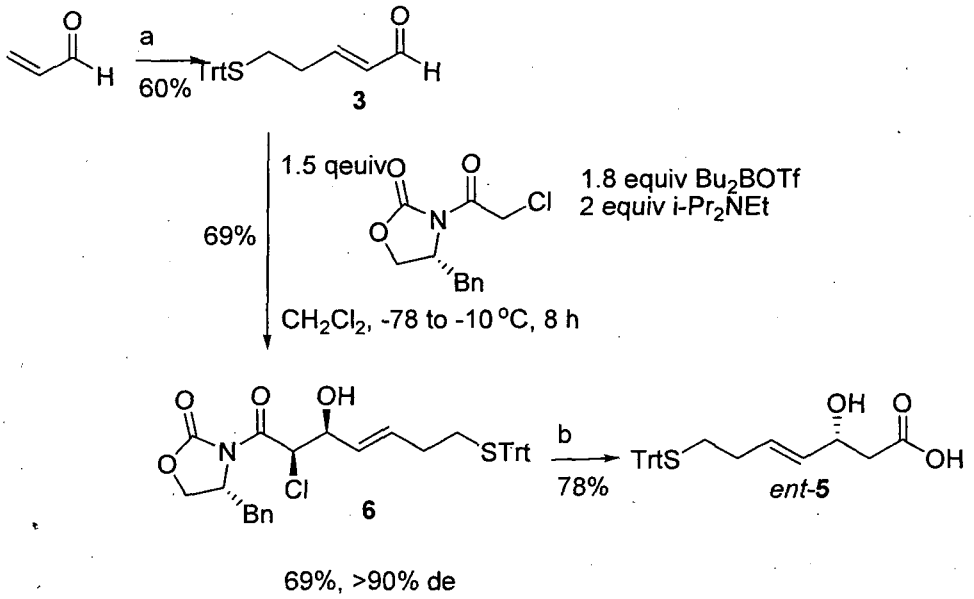
Scheme 2: Simon's route to the β-hydroxy acid. Reagents and conditions: a) 1.2 equiv TrtSH, 1.2 equiv Cs_2CO_3, THF, 20 h. b) 2 equiv DIBAL, CH_2Cl_2, -78 °C, 3 h. c) 1.2 equiv $(COCl)_2$, 2.4 equiv DMSO, CH_2Cl_2, -78 °C, 30 min; 2.4 equiv Et_3N, -30 °C, 4 h. d) 10 equiv LiOH, MeOH, 3 h.

The second route was the Wentworth-Janda synthesis[12] of a different depsipeptide where a shorter route is achieved by first a conjugate addition of acrolein and then a Wittig reaction to form the aldehyde **3**. This then underwent an aldol reaction with the Evans auxillary to form **6** and was then hydrolysed to form the β-hydroxy acid **5** (scheme 3).



Scheme 3: The Wentworth-Janda route to the β-hydroxy acid. Reagents and conditions: a) i. 0.7 equiv TrtSH, 0.7 equiv Et_3N, CH_2Cl_2, 1 h; ii. Ph_3P=CH-CHO. benzene, reflux 7 h. b) i. Al amalgam, aq THF, 0 °C, 2 h; ii. aq $LiOH/H_2O_2$ in THF, 1 h.

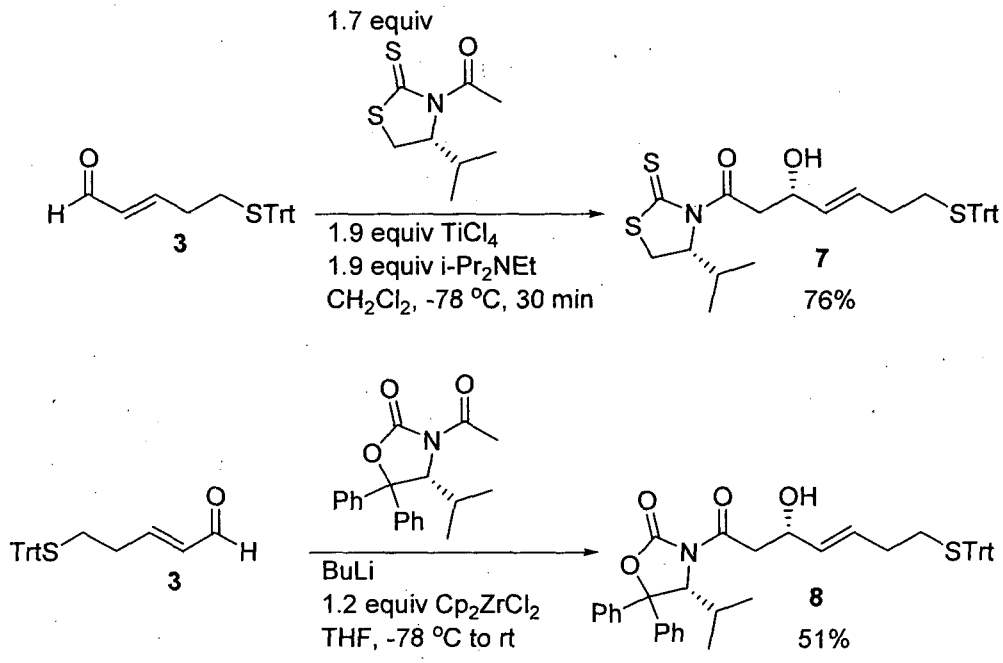
Although the Wentworth-Janda route is shorter then the Simon's route, the enantioselectivity was less then the product achieved by the Simon's route, therefore alternatives were found, where a chloroacetate was attached to the Evans auxiliary. The chloride acts as a 'dummy' substitute to ensure high diastereoselectivity in the aldol product, then the chloride can be reduced and the auxiliary removed to give the β-hydroxy acid **5** (Scheme 4).



Scheme 4: Scheme showing how the dummy substitute is used in the formation of the β-hydroxy acid.

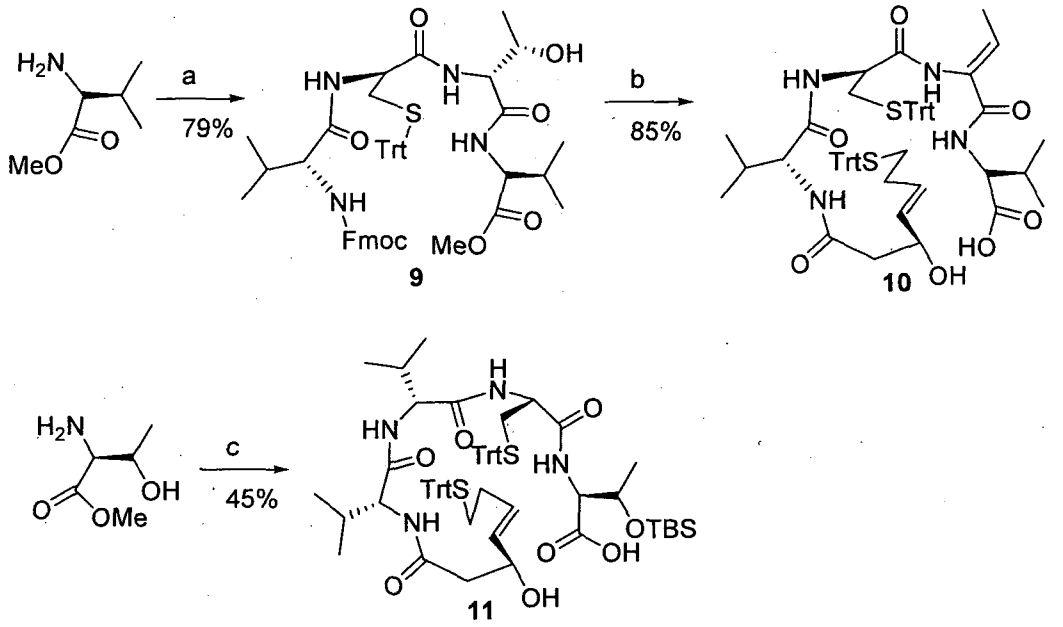
In the synthesis of spiruchostatin A[13] by Ganesan, the Nagao auxiliary was used instead of the Evans auxiliary, resulting in higher enantioselectivity (Scheme 5).

In the Doi-Takahashi synthesis[14] of spiruchostatin A, the acetate aldol was performed with the Seebach quaternary oxzolidinone chiral auxiliary, with the best diastereoselectivity observed with the transmetallation of the lithium enolate to zirconium. The product is then hydrolysed to form the free acid **5** which is then used for coupling.



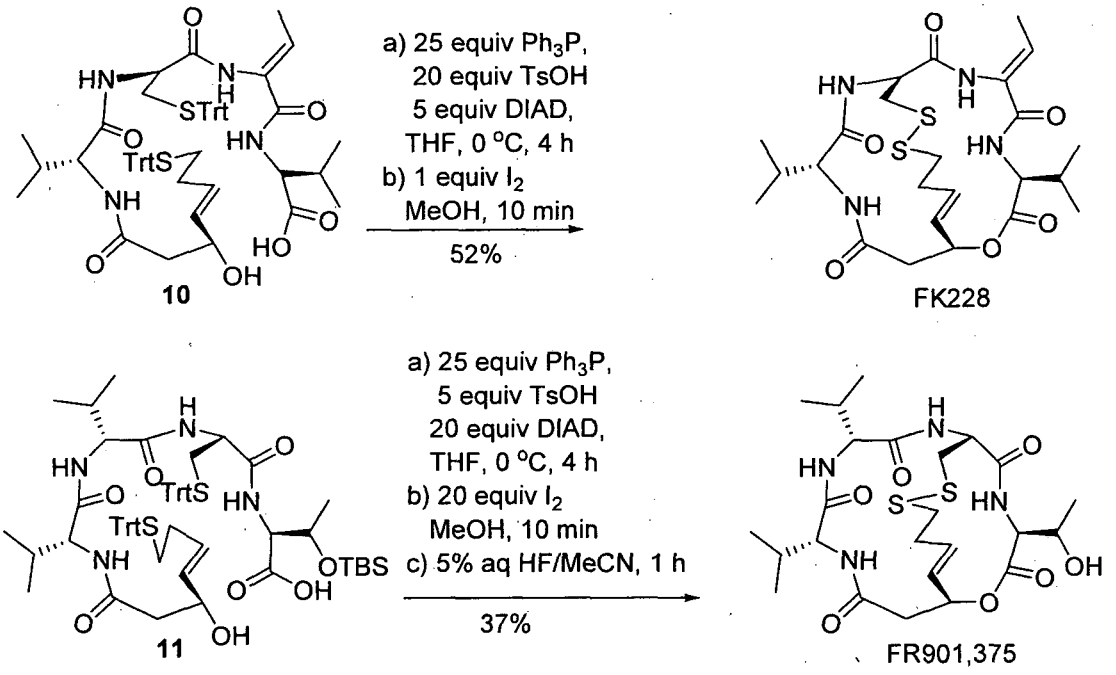
Scheme 5: The Ganesan and Doi-Takahashi procedures for enantioselective acetate aldol reactions with aldehyde.

In all the syntheses of the depsipeptide, the macrocycle is formed from the peptide and alcohol from the β-hydroxy acid by macrocyclisation. The Simon's synthesis of FK228 explored the possibility of activating the carboxylic acid **10** for intramolecular attack by the alcohol (Scheme 6). However this proved unsuccessful and so the possibility of making the alcohol into a group susceptible to attack from the carboxylic acid was explored.



Scheme 6: Simon's and Wentworth-Janda's routes to a linear *seco*-hydroxy acid. Reagents and conditions: a) i. 1 equiv Fmoc-L-Thr-OH, 1.5 equiv BOP, 3 equiv i-Pr_2NEt, MeCN, 30 min; ii. 5% Et_2NH/MeCN, 3 h; 1.1 equiv Fmoc-D-Cys(STrt)-OH, 1.1 equiv BOP, 2.5 equiv i-Pr_2NEt, MeCN, 30 min; iii. 5% Et_2NH/MeCN, 3 h; iv. 1.1 equiv Fmoc-D-Val-OH, 1.6 equiv BOP, 6 equiv i-Pr_2NEt, MeCN, 30 min. b) i. 3 equiv Ts_2O, pyridine, 0 °C, 20 min; ii. 10 equiv DABCO, MeCN, 2 h; 5% Et_2NH/MeCN, 22 h; iii. 1 equiv acid *ent*-**5**, 1.5 equiv BOP, 3 equiv i-Pr_2NEt, MeCN/CH_2Cl_2, 30 min. iv. 2 equiv LiOH, aq THF, 0 °C, 3.5 h. c) i. 1 equiv Fmoc-D-Cys(STrt)-OH, 1.2 equiv EDC, 1.2 equiv HOBt, DMF/CH_2Cl_2, 20 h; ii. 1.3 equiv TBSCl, 1.3 equiv imidazole, DMF, 20 h; iii. 50% Et_2NH/CH_2Cl_2, 0 °C, 3 h; iv. 1.1 equiv Fmoc-D-Val-OH, 1.3 equiv EDC, 1.4 equiv HOBt, DMF/CH_2Cl_2, 20 h; v. 50% Et_2NH/CH_2Cl_2, 0 °C, 4 h; vi. 1.1 equiv Fmoc-D-Val-OH, 1.3 equiv EDC, 1.4 equiv HOBt, 20 h; vii. 38% Et_2NH/CH_2Cl_2, 0 °C, 3 h, rt 3 h; viii. 1 equiv acid *ent*-**5**, 1.5 equiv BOP, 3 equiv i-Pr_2NEt, MeCN/CH_2Cl_2, 1 h. ix. LiOH, aq THF, 16 h.

Under carefully controlled Mitsunobu conditions (Scheme 7) the macrocyclisation can be achieved with the same chirality as the natural product. The last step involved another cyclisation to form the disulphide bridge and is achieved by iodine oxidation to complete the total synthesis of FK228.

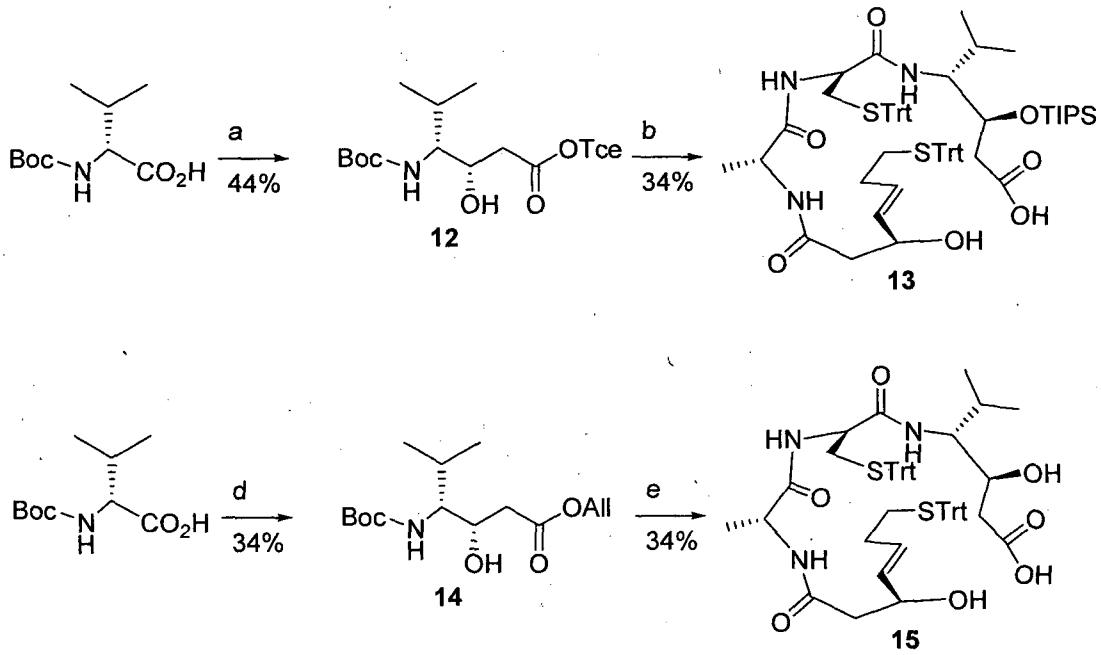


Scheme 7: Completion of the total syntheses of FK228 and FR901,375 by Mitsunobu macrolactonization.

1.4 The Synthesis of Spiruchostatin A

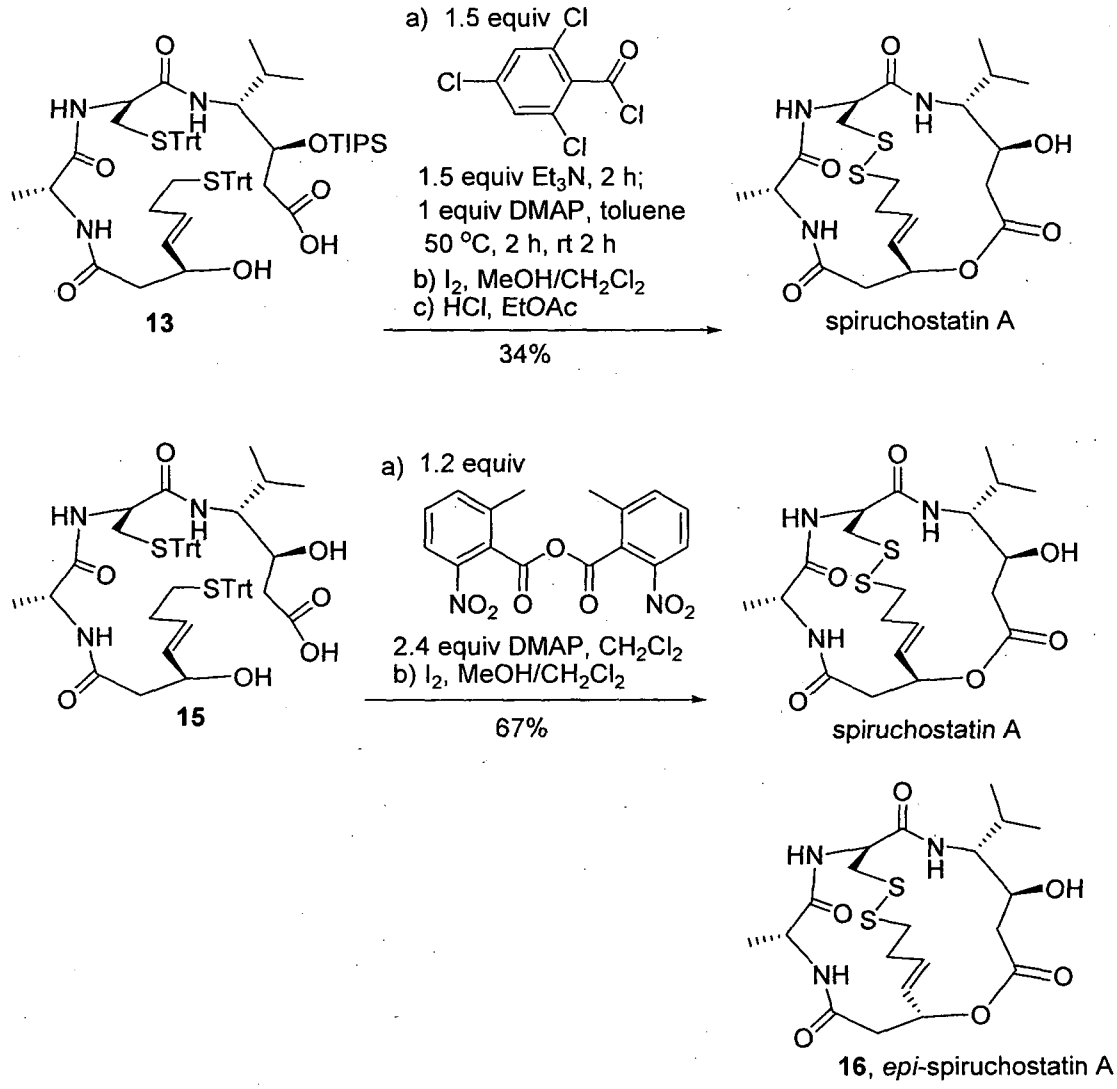
Spiruchostain A is a can be synthesised using the same method as FK228 except the macrocyclisation can be carried out by activating the carboxylic acid. Using the Yamaguchi method proved promising, and when the second alcohol was protected, the product was formed in good yield. A mechanism for this observation had suggested that the activating species is the symmetrical anhydride and can be replaced by simpler benzoic acids[15].

To complete the synthesis of spiruchostatin A, the synthesis follows the Simon's procedure of using iodine oxidation to form the disulphide bridge (Scheme 8).



Scheme 8: The Ganesan and Doi-Takahashi syntheses of spiruchostatin A *seco*-acids. Reagents and conditions: a) i. 1.1 equiv PfpOH, 1.2 equiv EDC·HCl, 0.2 equiv DMAP, CH_2Cl_2, 0 °C, 30 min, rt, 4 h; ii. 3.2 equiv $LiCH_2CO_2CH_3$, THF, -78 °C, 45 min; iii. 3.5 equiv KBH_4, MeOH, -78 °C to 0 °C, 50 min; iv. 26 equiv LiOH, 4:1 THF/H_20, 0 °C, 2 h; v. 15 equiv TceOH, 6.2 equiv DCC, 0.12 equiv DMAP, CH_2Cl_2, 0 °C to rt, 18 h. b) i. 20% TFA/CH_2Cl_2, 3 h; ii. 1 equiv Fmoc D-Cys(STrt)-OH, 1.2 equiv PyBOP, 3.5 equiv i-Pr_2NEt, CH_3CN, 20 min; iii. 4 equiv TIPSOTf, 6 equiv 2,6-lutidine, CH_2Cl_2, 3 h; iv. 5% Et_2NH/CH_3CN, 3 h; v. 1.3 equiv Fmoc D-Ala-OH, 1.3 equiv PyBOP, 3 equiv i-Pr_2NEt, CH_3CN, 1 h; vi. 5% Et_2NH/CH_3CN, 5 h; vii. 0.9 equiv 7, 0.1 equiv DMAP, CH_2Cl_2, 0 °C, then rt, 7 h; viii. 10 equiv Zn, NH_4OAc/THF, 5 h. c) i. im_2CO, $(EtO_2CCH_2CO_2)_2Mg$, THF; ii. $NaBH_4$, THF/MeOH; iii. LiOH, aq THF; iv. allyl bromide, K_2CO_3. d) i. HCl, EtOAc; ii. Fmoc-D-Cys(STrt)-OH, EDC, HOBt, i-Pr_2NEt, iii, Et_2NH; iv. Fmoc-D-Ala-OH, EDC, HOBt, i-Pr_2NEt; v. acid **5**, PyBOP, i-Pr_2NEt; vi. $Pd(PPh_3)_4$, morpholine, MeOH.

In the Doi-Takahashi synthesis of spiruchostatin A, in which the additional alcohol remains unprotected, the Shiina procedure was used. This shows that macrocyclisation can be achieved via carbonyl activation with milder conditions and not just by alcohol activation as in the Simon's procedure (Scheme 9). This method has lead to the synthesis of numerous unnatural analogues of FK228.



Scheme 9: Final stages in the Ganesan and Doi-Takahashi total syntheses of spiruchostatin A, and the structure of spiruchostatin A epimer.

1.5 Previous work on an analogue of FK228

Previous work with an analogue of FK228 has shown that the disulphide is not required as long as there is a zinc binding thiol at the end of the linker to inhibit the enzyme and this is done by replacing the cysteine at the R3 (Fig. 5). This monothiol was a potent HDAC inhibitor, but inactive in cell growth inhibition.

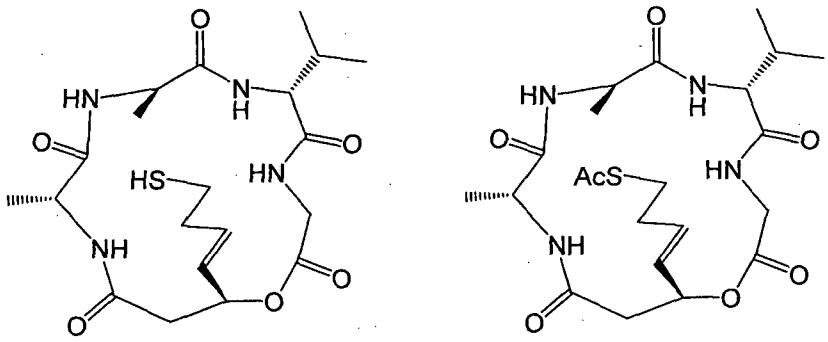


Fig. 5: The monothiol and thiol acetate produced from previous work on FK228.

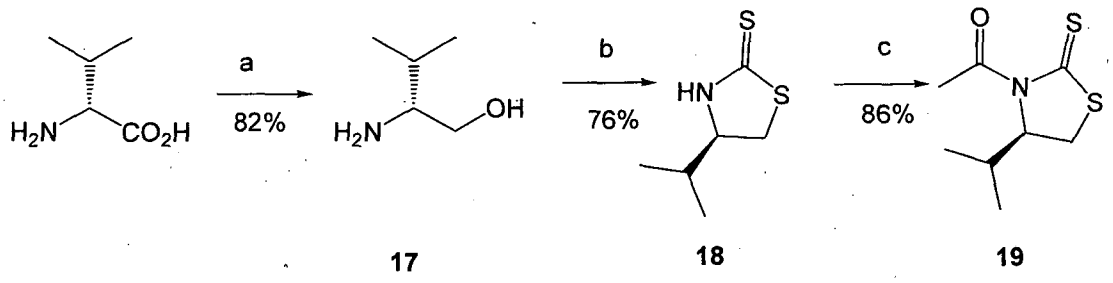
The function of the disulphide appears to act as a prodrug allowing the compound to pass through the cell walls, as the monothiol analogue synthesised was unable to pass through into cells.

This research focuses on the total synthesis of analogues of FK228, however the research focuses not on the groups that bind to the 'rim' of the enzyme but major structural changes such as changing the linker length and saturation.

Chapter 2

2.1 Synthesis of the analogue of FK228 with a shortened, saturated linker

The β-hydroxy acid can be synthesised by an enantioselective aldol reaction using an acylated chiral auxiliary **19**[16] (Scheme 10).



Scheme 10: Reaction scheme showing the formation of the amide **19**. Reagents and conditions: a) 2.4 equiv $NaBH_4$, 1.0 equiv I_2, THF, 70 °C, 18h. b) 5.0 equiv CS_2, 1M $KOH_{(aq)}$, 100 °C, 16h. c) 1.2 equiv AcCl, 1.6 equiv NEt_3, CH_2Cl_2, 8h.

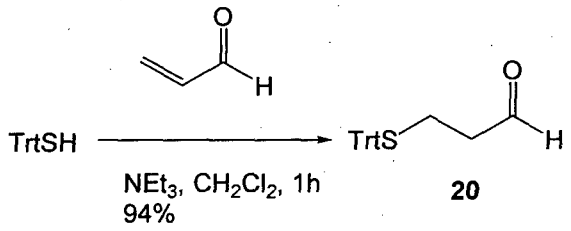
The acylated auxiliary was synthesised in three steps. The first step was the reduction of D-valine to D-valinol[17] using sodium borohydride and iodine. This generates the alcohol **17** in yield of 82%.

The second step to form the auxiliary **18** was to form the 5-membered ring by refluxing the alcohol **17** with carbon disulphide with yield of 76%.

The thiazolidine **18** was chosen to be used for this synthesis because it provided greater diastereoselectivity in the aldol reaction of 10:1 ratio while the Evans auxiliary and the oxothiazolidine do not provide as much diastereoselectivity.

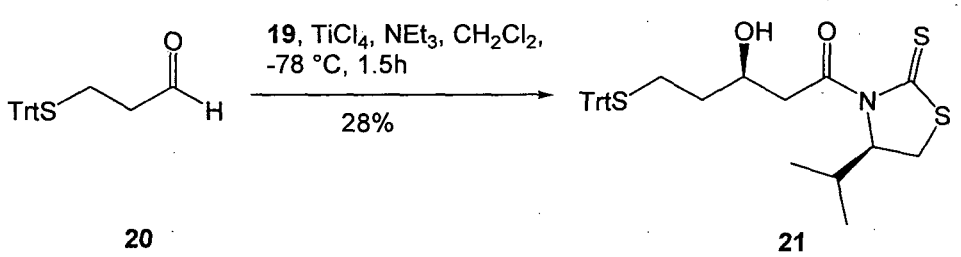
The final step of the synthesis of the auxiliary was a simple acetylation of the thiazolidine **18**[18] to form the amide **19** in yield of 86%.

The aldehyde **20** was made simply from the reaction between acrolein and triphenylmethyl thiol[19] (TrtSH), resulting in yield of 94% (Scheme 11).



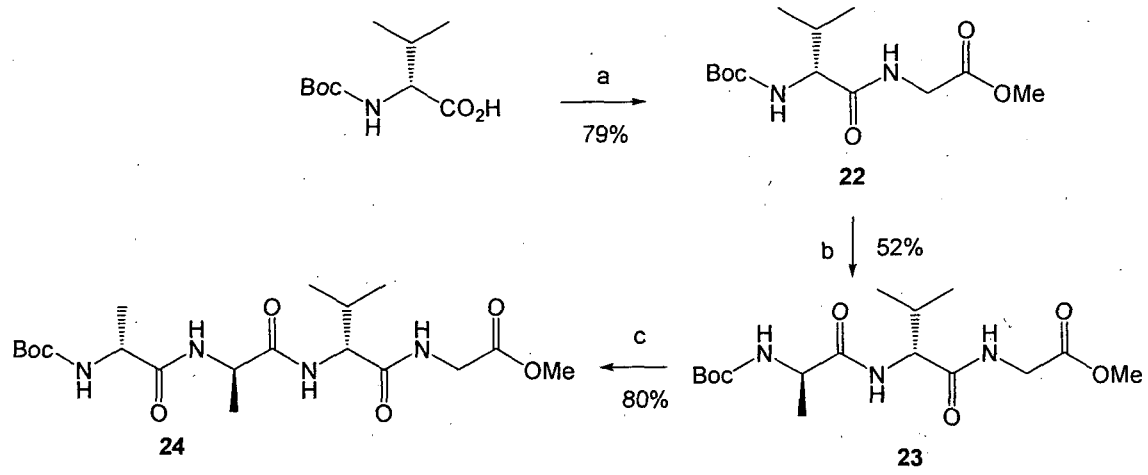
Scheme 11: Reaction scheme of the synthesis of the aldehyde.

From here the aldehyde **20** was coupled with the amide **19** using titanium (IV) chloride[20] to form the protected β-hydroxy acid **21** (Scheme 12).



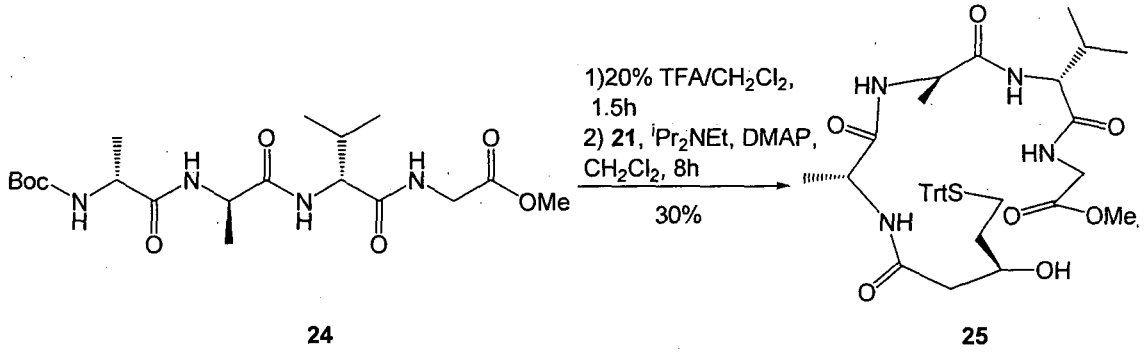
Scheme 12: Reaction scheme showing the synthesis of the protected β-hydroxy acid.

With the β-hydroxy acid **21** completed, the next step was the synthesis of the tetrapeptide **24**. This was synthesised using standard procedures and reagents[21] of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDAC) and 1-hydrobenzotriazole (HOBt), providing yields above 50% (Scheme 13).



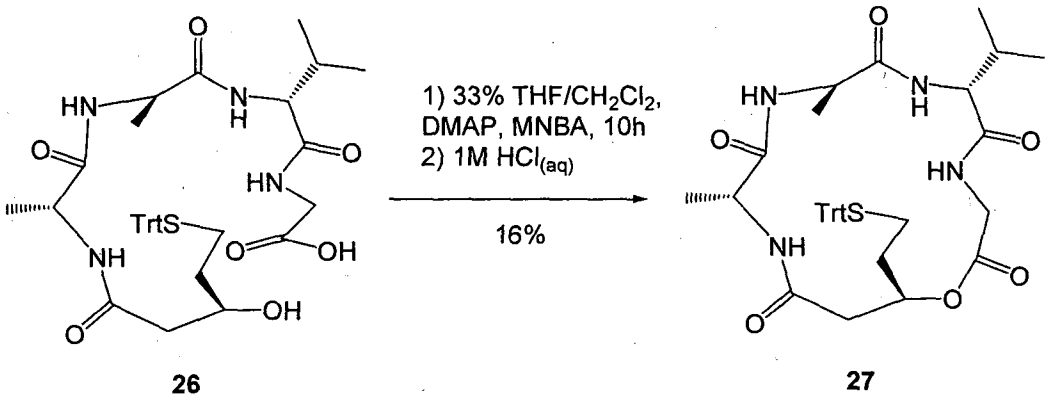
Scheme 13: Reaction scheme showing the synthesis of the tetrapeptide **24**. Reagents and conditions: a) 1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^{i}Pr_2NEt$, 1.1 equiv glycine methyl ester hydrochloride, CH_2Cl_2, 3h. b) i) 20% TFA/CH_2Cl_2, 1.5h. ii) 1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^{i}Pr_2NEt$, 1.1 equiv Boc-D-alanine, CH_2Cl_2, 3h. c) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^{i}Pr_2NEt$, 1.1 equiv Boc-D-alanine, CH_2Cl_2, 3h.

The tetrapeptide **24** was then coupled with the protected β-hydroxy acid **21** to form the linear product **25** (Scheme 14). The yield seems poor in comparison to synthesis of other analogues of FK228.



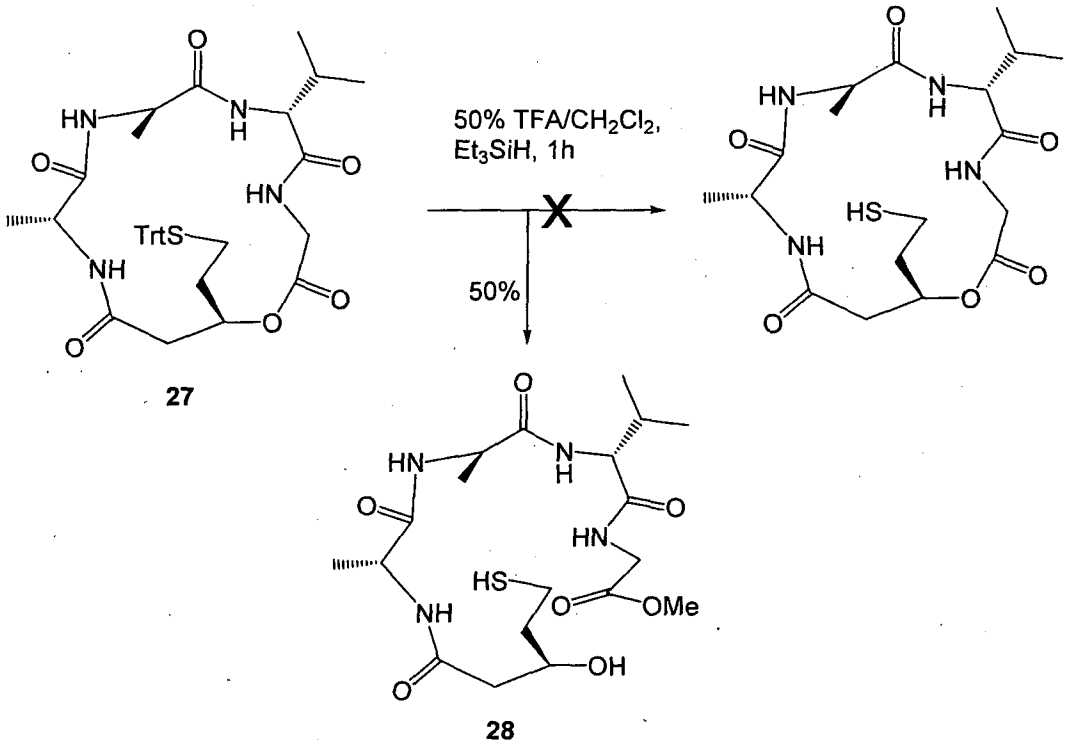
Scheme 14: Reaction scheme of the coupling between the two key components.

The linear product **25** was then hydrolysed with lithium hydroxide to form the free acid **26**. The crude acid was then macrocyclised using 4-dimethylaminopyridine (DMAP) and 2-methyl-6-nitrobenzoic anhydride (MNBA)[20] (Scheme 15).



Scheme 15: Reaction scheme showing the macrocylisation to form the depsipeptide.

The last step required the deprotection of the trityl group using TFA and a scavenger (Scheme 16).



Scheme 16: Reaction scheme showing the deprotection of the depsipeptide.

However after deprotecting the thiol **27**, we did not obtain the desired product but the linear product **28**. A possible mechanism for these results was that since the linker was shorter and without any unsaturation compared to FK228, the thiol had to react with the

ester first to form a thioester. This then broke down when methanol was added during purification to form the linear product **28**.

This had shown that the linker was too short and flexible to use in making an analogue and further consideration was needed when changing the linker length.

Chapter 3

Synthesis of an analogue using a pentapeptide

FK228 is composed of a tetrapeptide and β-hydroxy acid. We proposed the replacement of the β-hydroxy acid with a β-amino acid. Doing this would cause the 'cap' to change from a depsipeptide to a cyclic peptide and would prove interesting as an amide bond is more stable then an ester bond and this change might also affect inhibition of HDACs. Also with these molecules, the linker and zinc binding group would be different as there would be an amide bond mimicking the double bond and the zinc binding group could be anything from a thiol to a carboxylic acid (Fig. 6).

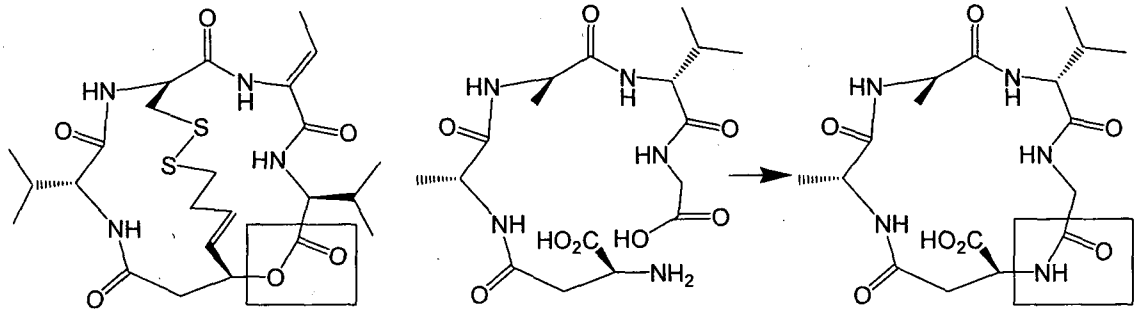
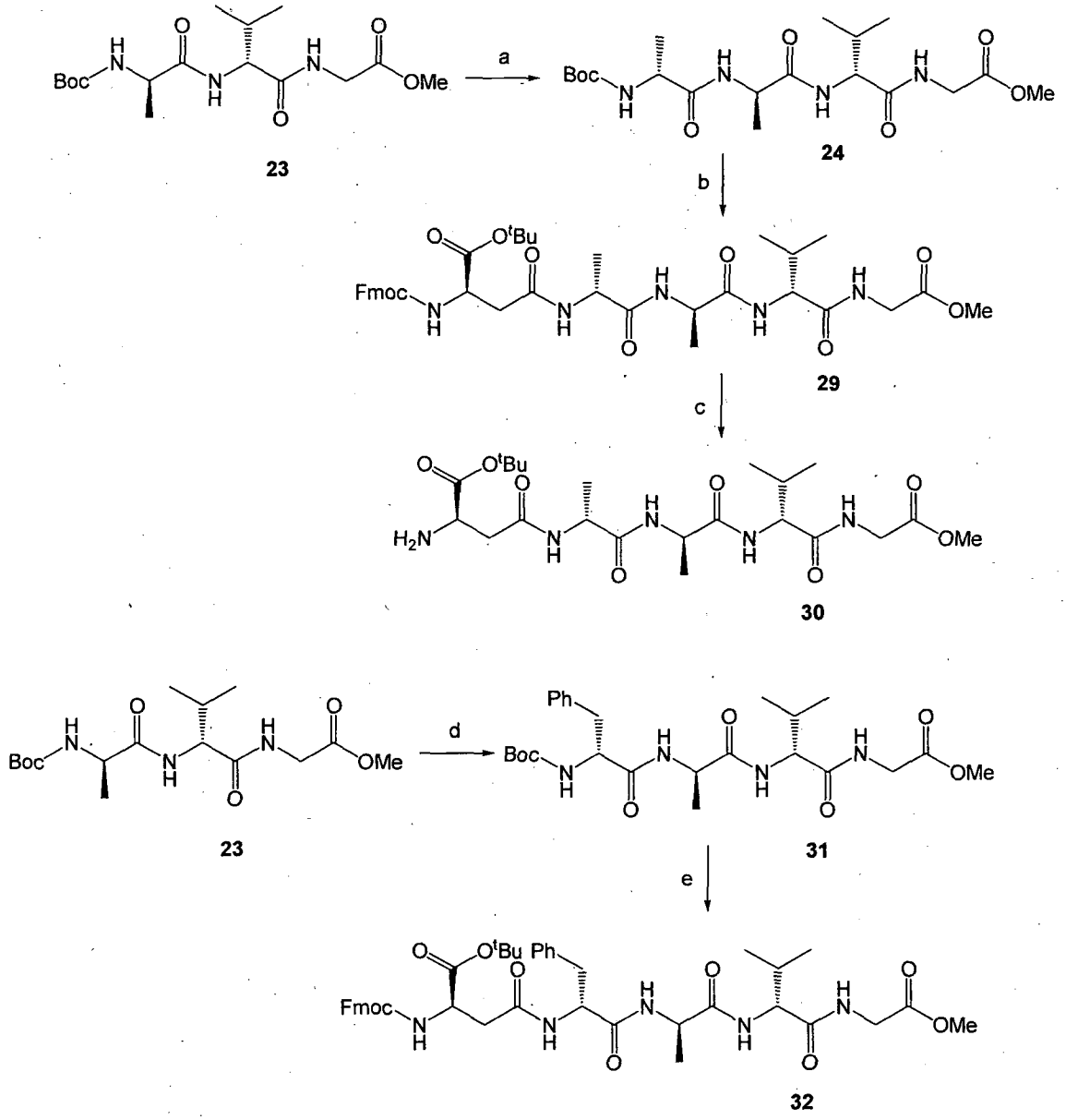


Fig. 6: FK228 (left), the proposed pentapeptide before cyclisation (centre) and after cyclisation (right).The most notable difference in the depsipeptide would be the change from an ester to an amide and is highlighted by a box.

Initial research on synthesising the β-amino acid using homologation of an α-amino acid and trimethylsilane-diazomethane[17] have resulted in failure using several different methods. An alternative β-amino acid had to be used and we chose aspartic acid, but with the α-carboxylic acid protected, allowing the other carboxylic acid to be used as a β-amino acid.

The tripeptide **23** was re-synthesised and from this was synthesised the tetrapeptides **24** and **31** and the pentaptides **29** and **32** were synthesised (Scheme 17). As the molecules grew in size and polarity, it became increasingly more difficult to obtain a good yield

from these couplings and eventually, there was insufficient material to continue with the synthesis.

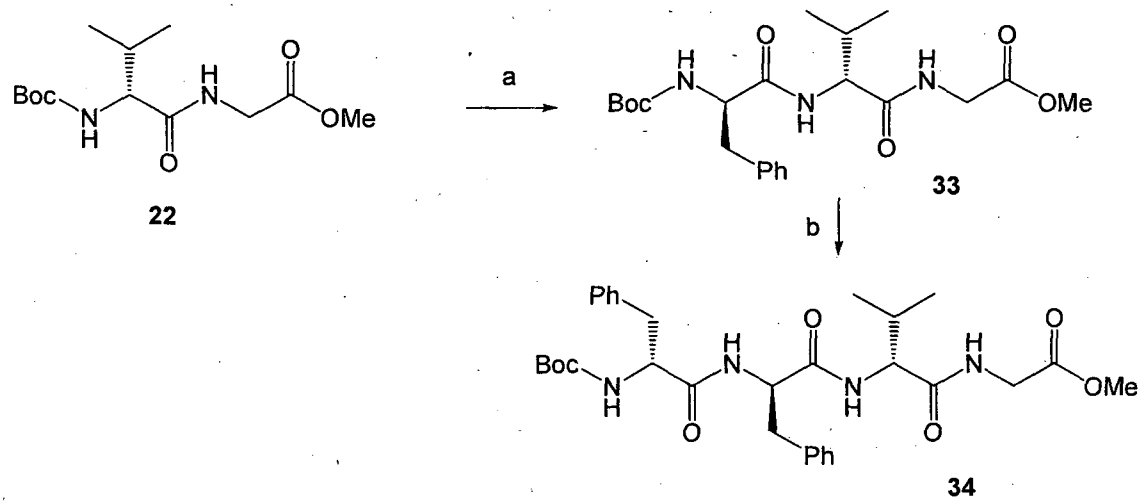


Scheme 17: Reaction scheme showing the initial synthesis of the pentapeptides[19] **29** and **32**. Reagents and conditions: a) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^i Pr_2NEt$, 1.1 equiv Boc-D-alanine, CH_2Cl_2, 3h. b) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^i Pr_2NEt$, 1.1 equiv Fmoc-L-aspartic acid O^{t}Bu, CH_2Cl_2, 3h. c) 20% piperidine/$CHCl_3$, 4h. d) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^i Pr_2NEt$, 1.1 equiv Boc-D-phenylalanine, CH_2Cl_2, 3h. e) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^i Pr_2NEt$, 1.1 equiv Fmoc-L-aspartic acid O^{t}Bu, CH_2Cl_2, 3h.

The peptides was re-synthesised using with Benzotriazol-1-yloxyl tripyrrolidinophosphonium hexafluorophosphate (PyBOP), a more potent activating agent, rather then EDAC to ensure a better yield of usually greater then 80%.

After the tetrapeptide **24** was coupled to the β-aspartic acid to form the pentapetide **29** and the Fmoc was removed to generate the amine **30**. However after hydrolyzing the methyl ester, the product could not be extracted from the aqueous layer.

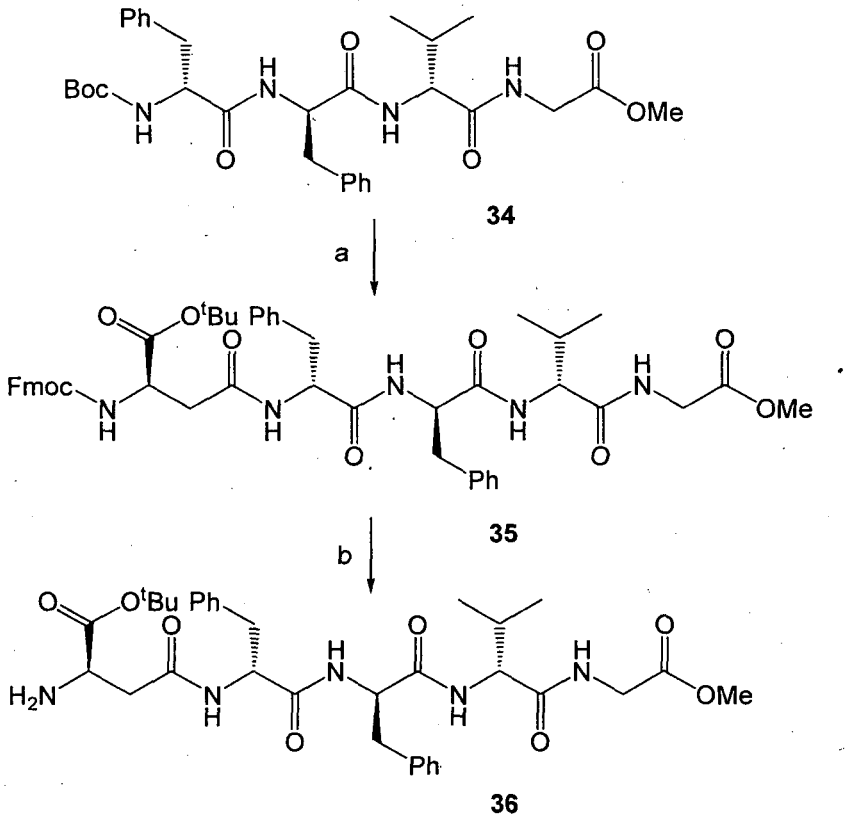
The attempt was to make the tetrapeptide less polar by making the groups more hydrophobic and so the idea was to modify the alanine residues in the tetrapeptide into phenylalanine residues (Scheme 18).



Scheme 18: Reaction scheme of the synthesis of the tetrapeptide **33**, with the alanines of tetrapeptide **24** replaced with phenylalanines. Reagents and conditions: a) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 PyBOP, 3.5 equiv $^iPr_2Net$, 1.1 equiv Boc-D-phenylalanine, CH_2Cl_2, 3h. b) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 equiv EDAC, 1.2 equiv HOBt, 3.5 equiv $^iPr_2Net$, 1.1 equiv Boc-D-phenylalanine, CH_2Cl_2, 3h.

The same procedure was used to make the pentapeptide **35** from the tetrapeptide **34** and after deprotecting the pentapeptide **35** to the free amine **36** (Scheme 19), the methyl ester was once again hydrolysed, however after several extractions; only a small amount of product was retrieved. A solution to this problem for future work could be to use a

protecting group that does not require any aqueous solutions to deprotect the carboxylic acid.

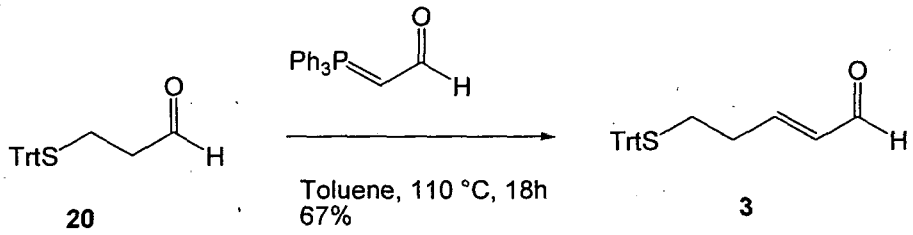


Scheme 19: Reaction scheme showing synthesis of a more hydrophilic pentapeptide **36**. Reagents and conditions: a) i) 20% TFA/CH_2Cl_2, 1.5h. ii)1.2 PyBOP, 3.5 equiv $^iPr_2NEt$, 1.1 equiv Fmoc-L-aspartic acid, CH_2Cl_2, 3h. b) 20% Piperidine/CH_2Cl_2, 4h.

Chapter 4

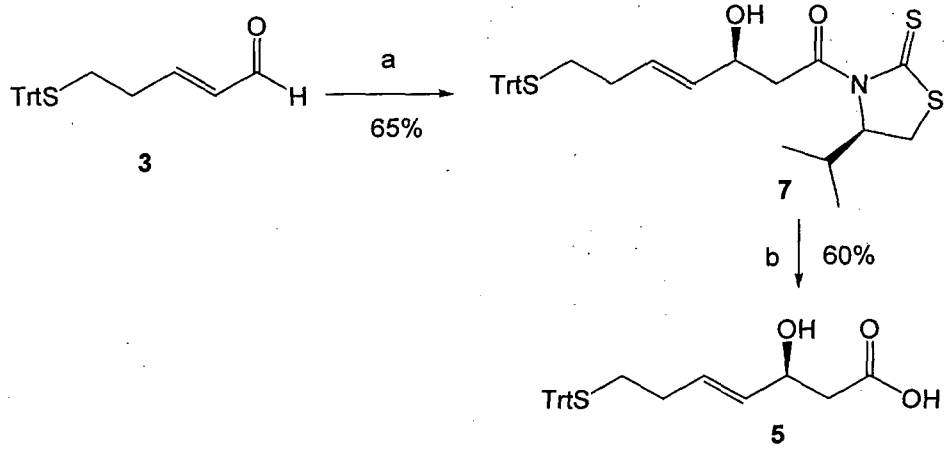
Synthesis of an analogue of FK228

This analogue was synthesised whilst developing the pentapeptide **36** and was prepared from the tetrapeptide **34** and β-hydroxy acid fragment **5**, generated after aldehyde **20** had been reacted with a Wittig reagent to form the aldehyde **3** (Scheme 20).



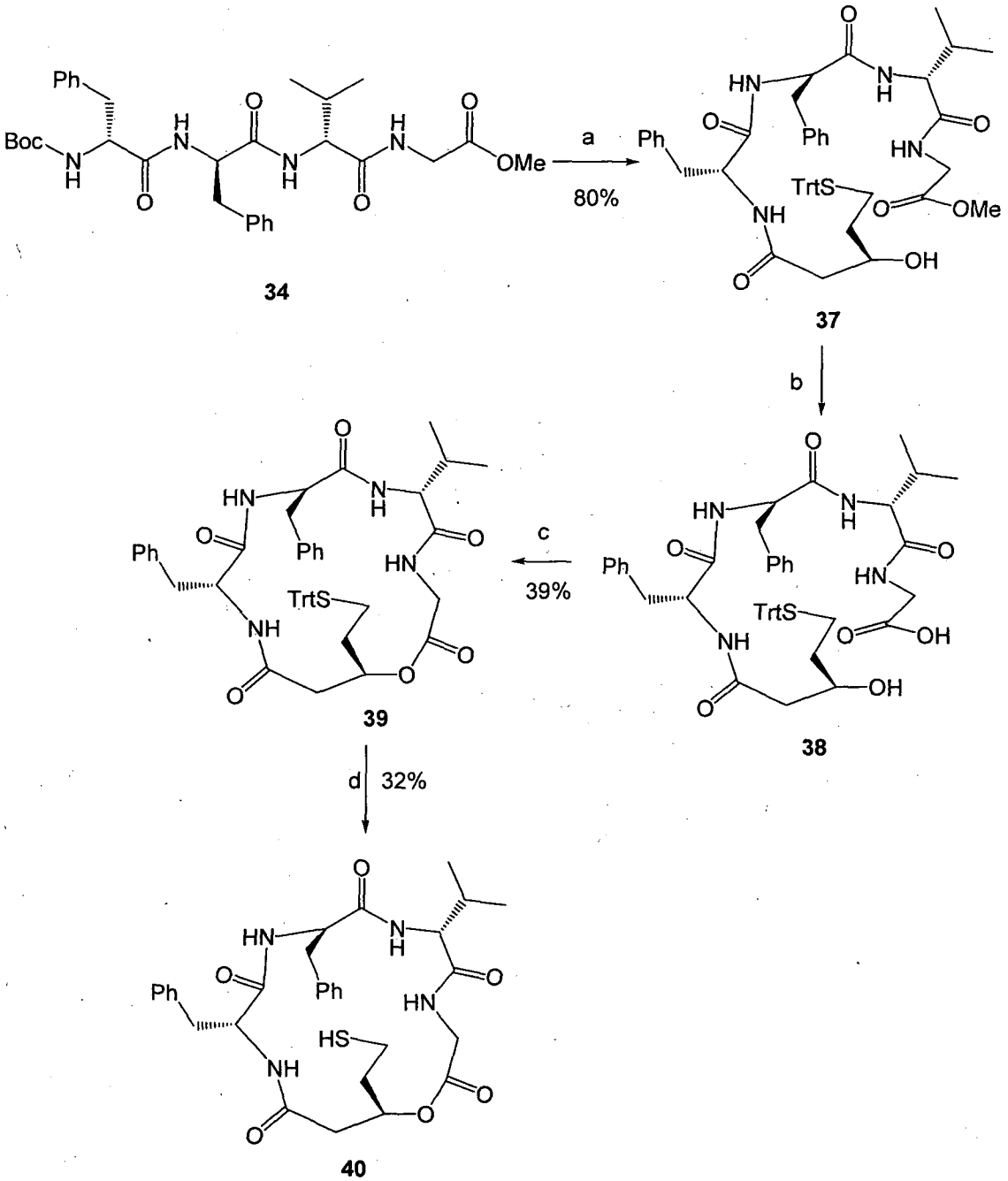
Scheme 20: Reaction scheme showing the Wittig reaction.

Aldehyde **3** was subjected to an aldol reaction with the acylated chiral auxillary to form the amide **7**. This was then was converted into the acid **5** as previous work has indicated that the coupling between the tetrapeptide with the acid **5** generates a higher yield than the coupling of the tetrapeptide with amide **7** (Scheme 21).



Scheme 21: Synthesis of the β-hydroxy acid **5**. Reagents and conditions: a) 1.7 equiv **19**, 1.8 equiv $TiCl_4$, 1.8 equiv NEt_3, CH_2Cl_2, -78 °C, 1.5h. b) 3.0 equiv LiOH, 25% H_2O/THF, 0 °C, 1h.

Analogue **40** was then synthesised using the same procedure as the synthesis of **28** (Scheme 22).



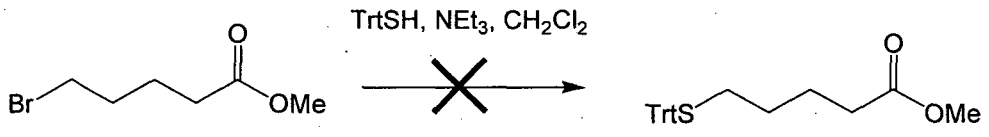
Scheme 22: The synthesis of monothiol analogue **40**. Reagents and conditions: a) i) 20% TFA/CH_2Cl_2, 1.5h. ii) 0.7 equiv PyBOP, 2.3 equiv $^iPr_2NEt$, 0.7 equiv **5**, CH_2Cl_2, 3h. b) i) 20% THF/H_2O, 0 °C, 2h. ii) 1M $KHSO_{4(aq)}$. c) i) 33% THF/CH_2Cl_2, 2.2 equiv DMAP, 1.1 equiv MNBA, 10h. ii) 1M $HCl_{(aq)}$. d) 50% TFA/CH_2Cl_2, 2.0 equiv Et_3SiH, 1h.

Chapter 5

Synthesis of a saturated linker

FK228 contains a double bond in the linker and we are interested in producing a saturated linker. The shortest route would be to hydrogenate with a palladium catalyst to remove the double bond either in the final compound or at the earlier β-hydroxy acid. Attempts at hydrogenating the double bond in either case have both ended in failure with the starting material being recovered and this could have been due to the sulphur poisoning the palladium catalyst.

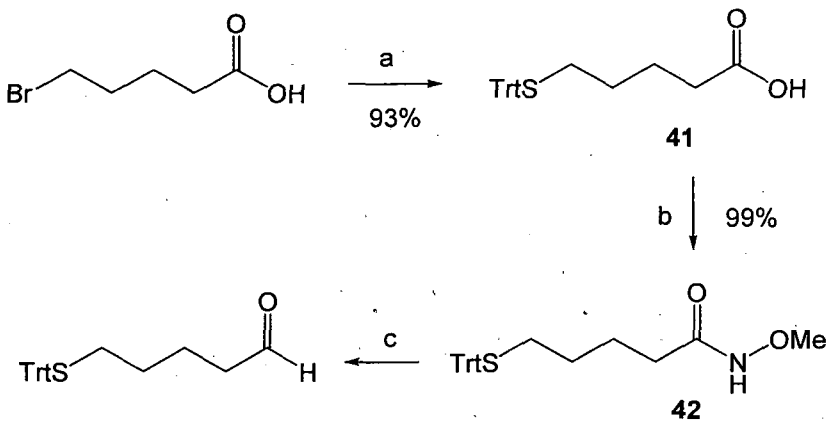
Therefore the alternative was to synthesise a saturated linker. An attempt at synthesising the saturated linker began with the reaction between triphenylmethylthiol and 5-bromovalerate methyl ester. (Scheme 23) However the resulting product from this reaction was a methyl ester that was too unstable and decomposed quickly.



Scheme 23: Reaction scheme showing the failure of the first attempt at synthesising the saturated linker.

With this unsuccessful reaction, an alternative route to the desired aldehyde described in a patent by Gilon and Chaim[22] was attempted (Scheme 24). The first step involved the reaction of the 5-bromovaleric acid with TrtSH resulting in the carboxylic acid **41** in good yield. The next step of the Gilon and Chaim procedure was to react the carboxylic acid **41** with *N,O* dimethylhydroxylamine to synthesise the Weinreb amide **42** and this was again done in good yields of 99%.

The final step required the Weinreb amide to be reduced to the aldehyde using $LiAlH_4$; however the product was of poor quality and little yield with the impurities hard to remove.

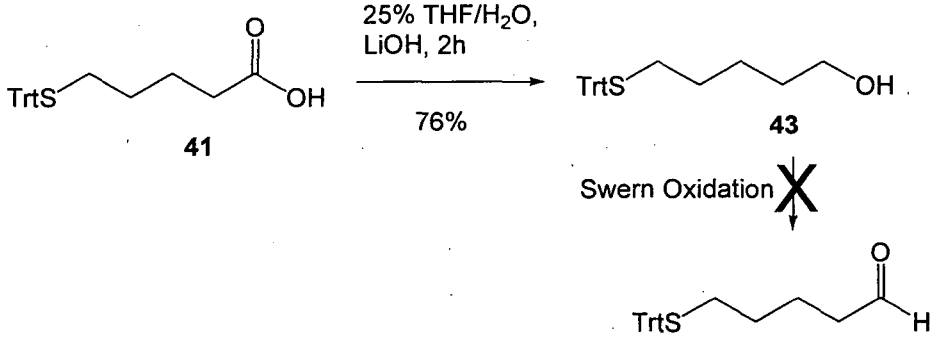


Scheme 24: Reaction scheme showing the attempt at synthesising the saturated linker by going through a Weinreb amide intermediate. Reagents and conditions: a) 1.0 equiv TrtSH, 2.2 equiv NaH, THF, 4h. b) 1.1 equiv HN(OMe)Me, 3.0 equiv $^{i}Pr_2NEt$, 1.1 equiv PyBOP, THF, 4h. c) 2.0 equiv $LiAlH_4$, THF, 0 °C, 2h.

As the yield was poor it was thought that this could have been due to the strength of the hydride used, so diisobutyl aluminium hydride (DIBAL), a milder hydride, was used for the final step but this also yielded little product. The byproduct produced from this reaction was isolated and had it had been deduced that the aliphatic chain had cyclised.

Therefore the reaction was repeated with 6-bromohexanoic acid hoping that the larger ring size formed from cyclisation would be less favoured then generating the product. However this also yielded little results.

The original method of making the β-hydroxy acid was to use a Swern oxidation and so the next attempt at making the saturated linker used this method. The acid **41** w as reduced into the alcohol **43** using excess $LiAlH_4$ at room temperature with a yield of 76% however the Swern oxidation of the alcohol **43** failed (Scheme 25).



Scheme 25: Reaction scheme showing the attempt to synthesise the saturated linker by over reduction and oxidation.

Chapter 6

Summary and future work

The total synthesis of an analogue of FK228 had been achieved and this had shown activity of 156nM in enzyme assays, which was not as active as FK228 or the other monothiols. However this showed that activity can be greatly altered by changing a single group in a position of the peptide chain.

Any future work regarding the pentapeptide would be done using a different protecting group from the methyl ester, as it is at that stage that the most difficulty occurs. By using a group that does not require water as a solvent means that the pentapeptide can be easier purified for the cyclisation. An example would be to use an allyl ester instead of a methyl ester, as then all that is needed is a palladium catalyst to remove the protecting group.

For any future work regarding the synthesising the saturated linker, a suggestion would be to initially develop a linker with a group that can be exchanged for a thiol later in the synthesis as a way to prevent cyclisation. An alternative is to use a different zinc binding group such as an acid. An example of this would be use 5-chloropentanal and attempt to synthesis the macrocycle and then replace the chloride with the thiol at the end.

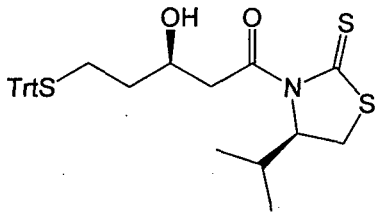
Also as future work an interesting concept would be to extend the length of the linker whilst retaining the double bond. This would be interesting to see how changing the linker length would affect the inhibition of HDACs.

Chapter 7

Experimental

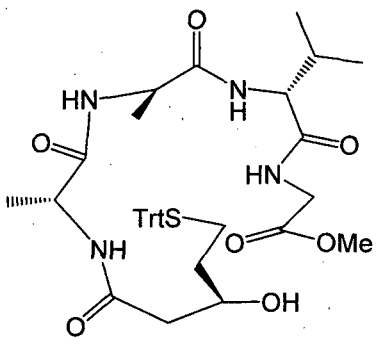
All chemicals and general reagents have been purchased from commercial suppliers and without any further purification carried out unless stated. Anhydrous CH_2Cl_2 was obtained from distillation with calcium hydride as the drying agent and anhydrous THF was obtained from distillation of THF with sodium wire and benzophenone as an indicator. All other anhydrous solvents were obtained from Aldrich Sure-Seal® bottles. Analytical TLC was taken from precoated aluminium plates with normal phase Merck 60 F_{254} silica plates and visualisation carried out by shortwave UV or staining with ninhydrin solution or ceric ammonium molybdate and flash chromatography was done with silica (Fisher, 37-70 micron). Melting points were taken on a hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR were done on a Bruker AM spectrometer with the results given for ^{1}H NMR in 300 Hz or 400 Hz and ^{13}C NMR in 75 Hz or 100 Hz respectively. Characteristic splitting patterns due to spin-spin coupling are expressed as s = singlet, br. s = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartet, t = triplet, q = quartet and m = multiplet with the coupling constants J given in Hertz. Low resolution mass spectra were obtained with a Micromass® platform single quadrupole mass spectrometer and the results generated are all electrospray positive results unless stated otherwise. Thin film infrared spectra were obtained from an Perkin Elmer spectrometer with Golden Gate© attachment and all values generated for infrared spectra are in cm^{-1}.

Formation of 3-hydroxy-1-(4-isopropyl-2-thioxo-thiazolidin-3-yl)-5-tritylsulfanyl-pentan-1-one (21)



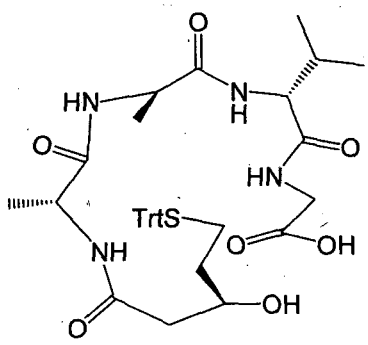
To a stirred solution of **19**[20] (1.25 g, 6.15 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 °C was added titanium (IV) chloride (0.69 mL, 6.31 mmol) under inert atmosphere. To the red solution was added $^{i}Pr_2NEt$ (1.10 ml, 6.31 mmol) turning the solution black and this was stirred for 1.5 h at -78 °C. A solution of **20**[19] (1.12 g, 3.37 mmol) in anhydrous CH_2Cl_2 (20 mL) was added to the mixture and stirred for 1 h at -78°C. Sat. $NH_4Cl_{(aq)}$ (20 mL) was added slowly to the solution and the mixture was allowed to warm to RT, to which was then added distilled water (40 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 25 mL). The organic layers were combined and washed with sat. $brine_{(aq)}$ (60 mL), separated and dried (Na_2SO_4). The solvent was concentrated *in vacuo* and the oil purified by flash chromatography (10% EtOAc/Hexane) to yield a yellow viscous oil **21** (0.50g, 28%). $[\alpha]_D^{25}$ = -44.7. IR υ_{max} 3538, 2962, 2925, 1687 cm^{-1}. ^1H NMR (300 Hz $CDCl_3$)δ 7.14-7.45 (m, 15H, Ph), 5.10 (t, 1H, J = 6.6 Hz, CH), 4.08 (m, 1H, CH), 3.39-3.52 (m, 2H, CH_2), 2.94-3.07 (m, 2H, CH_2), 2.64 (br. s, 1H, CH), 2.31 (t, 2H, J = 7.4 Hz, CH_2), 1.39-1.70 (m, 3H, CH, CH_2), 0.93-1.06 (dd, 6H, J = 6.0 Hz, 9.0 Hz, Me) ppm. ^{13}C NMR (75 Hz $CDCl_3$) δ 202.9, 172.8, 144.9, 129.6, 127.9, 126.6, 71.3, 66.9, 66.7, 45.1, 35.2, 30.9, 30.6, 28.2, 19.1, 17.8 ppm; *m/z* = 558.3 ($M+Na^+$).

Formation of (2-{2-[2-(3-hydroxy-5-tritylsulfanyl-pentanoylamino)-propionylamino]-propionylamino}-3-methyl-butyrylamino)-acetic acid methylester (25)



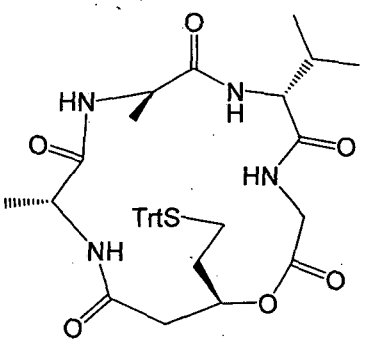
Compound **24**[20] (150 mg, 0.35 mmol) was deprotected in 20% volume TFA/anhydrous CH_2Cl_2 (5 mL) and stirred for 1.5 h at RT under inert atmosphere. The solution was diluted with hexane (10 mL), the solvent removed *in vacuo* and the oil diluted with toluene (10 ml). The solvent was then removed *in vacuo* to give a solid. To a solution of the solid in 10% volume anhydrous THF/CH_2Cl_2 (5 mL) was added $^{i}Pr_2NEt$ (0.30 mL, 1.74 mmol) and stirred for 0.5 h at 0 °C. Then to the mixture was added **21** (263 mg, 0.49 mmol) and DMAP (5 mg, 0.04 mmol) at 0 °C, then the reaction mixture was allowed to warm to RT and stirred for 8 h. The solvent was removed *in vacuo* and the solid purified by flash chromatography (5% MeOH/CH_2Cl_2) to yield a white solid **25** (75 mg, 30%): $[\alpha]_D^{27}$ = +8.72. IR υ_{max} 3277, 1626, 1537 cm^{-1}. ^1H NMR (400 Hz $CDCl_3$/MeOD) δ 7.68 (d, 1H, J = 6.9 Hz, Ph), 7.55 (t, 1H, J = 5.5 Hz, Ph), 7.14-7.44 (m, 13H, Ph), 4.29-4.39 (m, 2H, CH), 4.20 (dd, 1H, J = 8.7 Hz, 6.7 Hz, CH), 3.97 (dd, 2H, J = 7.8 Hz, 5.6 Hz, CH_2), 3.92 (d, 1H, J = 4.0, CH), 3.72 (s, 3H, Me), 2.27 (t, 2H, J = 7.5 Hz, CH_2), 2.05-2.21 (m, 3H, CH, CH_2), 1.57 (dt, 1H, J = 14.2 Hz, 7.2 Hz, CH), 1.39-1.51 (m, 1H, CH), 1.34 (dd, 6H, J = 7.2, 1.8, 2 x Me), 0.93 (t, 6H, J = 6.7, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD) δ 172.9, 172.6, 172.0, 169.7, 144.3, 129.1, 127.4, 126.2, 67.6, 66.2, 58.3, 58.2, 51.7, 42.6, 40.6, 40.5, 35.7, 30.1, 27.5, 18.5, 17.2, 16.9, 16.7 ppm; *m/z* = 727.4 ($M+Na^+$).

Formation of (2-{2-[2-(3-hydroxy-5-tritylsulfanyl-pentanoylamino)-propionylamino]-propionylamino}-3-methyl-butyrylamino)-acetic acid (26)



To a solution of **25** (60 mg, 85 μmol) in 25% volume THF / distilled water (1.5 mL) was added lithium hydroxide (6 mg, 256 μmol). After stirring for 2h at 0 °C, the solution was diluted with distilled water (2 mL), acidified to pH 1 with 1M $KHSO_{4(aq)}$ and extracted with EtOAc (3 x 30 mL). The solution was washed with sat. brine (10 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a grey solid **26** (62 mg, >100%) that was used without further purification. *m/z* (ES-) = 689.4 ($M\text{-}H^+$).

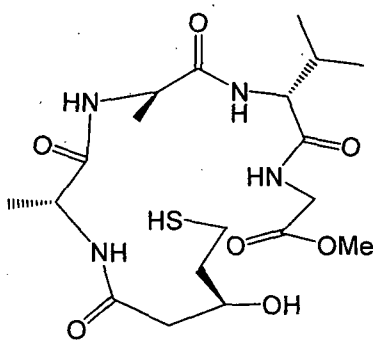
Formation of 6-isopropyl-9,12-dimethyl-16-(2-tritylsulfanyl-ethyl)-1-oxa-4,7,10,13-tetraaza-cyclohexadecane-2,5,8,11,14-pentaone (27)



To a solution of **26** (59 mg (maximum pure yield), 85 μmol) in 33% volume anhydrous THF/CH_2Cl_2 (210 mL) and DMF (4 mL) was added dropwise to a solution of MNBA (32 mg, 94 μmol) and DMAP (23 mg, 188 μmol) in anhydrous CH_2Cl_2 (20 mL) and over 2 h under inert atmosphere at RT. The yellow solution was stirred for a further 8 h at RT. After which time 1M $HCl_{(aq)}$ (80 mL) was added, the layers separated and then the organic layer was washed with sat. $NaHCO_{3(aq)}$ (50 mL) and sat. brine (50 mL). The

organic layers were dried (Na_2SO_4) and the solvent removed *in vacuo*, to which the crude product was then purified by flash chromatography (2% MeOH/CH_2Cl_2) to yield a white solid **27** (9 mg, 16%): $[\alpha]_D^{27}$ = -1.20. IR υ_{max} 3305, 2962, 2929, 2031, 2061, 1742, 1655, 1533 cm^{-1}. ^1H NMR (400 Hz $CDCl_3$/MeOD) δ 7.15-7.41 (m, 15H, Ph), 5.00-5.08 (m, 1H, CH), 4.21-4.31 (m, 2H, CH_2), 4.07 (q, 1H, J = 7.0 Hz, CH), 3.52-3.67 (m, 1H, CH), 3.10-3.16 (m, 1H, CH), 2.43 (dd, 1H, J = 13.1 Hz, 6.4 Hz, CH), 2.28-2.36 (m, 1H, CH), 2.21 (d, 1H, J = 8.9 Hz, CH), 2.16 (d, 1H, J = 8.9 Hz, CH), 1.83 (ddd, 1H, J = 6.4 Hz, 3.6 Hz, 3.3 Hz, CH), 1.69-1.78 (m, 1H, CH), 1.57 (d, 3H, J = 7.4, Me), 1.35 (d, 3H, J = 7.2, Me), 0.86-0.98 (m, 6H, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD) δ 174.9, 174.4, 172.2, 170.9, 169.6, 144.9, 129.8, 128.2, 127.0, 71.8, 67.2, 59.1, 46.5, 41.6, 40.3, 33.7, 29.4, 27.8, 26.7, 19.6, 17.5, 16.8, 16.6 ppm. *m/z* = 695.5 ($M+Na^+$).

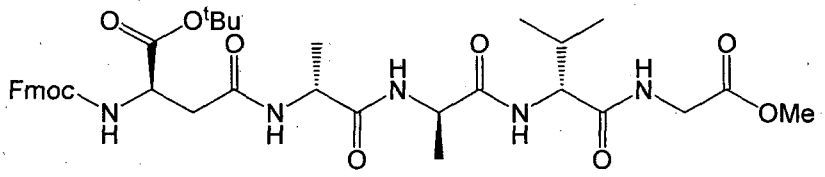
Formation of (2-{2-[2-(3-hydroxy-5-mercapto-pentanoylamino)-propionylamino]-propionylamino}-3-methyl-butyrylamino)-acetic acid methyl ester (28)



To a solution of **27** (9 mg, 13.4 μmol) in 50% volume TFA/anhydrous CH_2Cl_2 (4 mL) was added Et_3SiH (4.3 μL, 26.8 μmol) and the reaction mixture was stirred at RT for 1 h under inert atmosphere. The solvent was then concentrated *in vacuo* and toluene (10 mL) was added and removed. The solid was purified by flash chromatography (1-5% MeOH / CH_2Cl_2) to generate an grey solid **28** (3 mg, 50%): ^1H NMR (400 Hz $CDCl_3$/MeOD) δ 4.24 (dd, 2H, J = 7.2 Hz, CH_2), 4.04-4.15 (m, 2H, CH_2), 3.98 (m., 2H, CH), 3.71 (s, 3H, Me), 2.52-2.67 (m, 3H, CH_2, CH), 2.35-2.42 (m, 2H, CH), 2.04 (dt, 1H, J = 13.9 Hz, 7.0 Hz, CH), 1.82 (dt, 1H, J = 14.1 Hz, 7.0 Hz, CH), 1.70 (td, 1H, J = 10.8 Hz, 7.7 Hz, CH), 1.35 (t, 6H, J = 7.22 Hz, 2 x Me), 0.93 (d, 6H, J = 6.8 Hz, 2 x Me) ppm. ^{13}C NMR (100

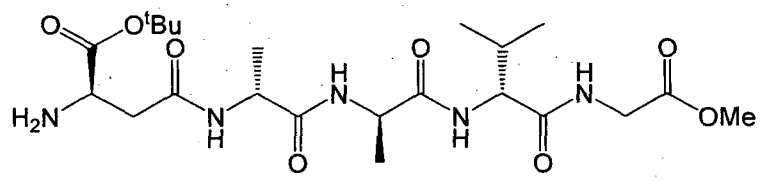
Hz $CDCl_3$/MeOD) δ 174.3, 173.9, 172.6, 172.5, 170.5, 67.5, 59.5, 52.4, 50.3, 42.7, 41.0, 40.8, 30.3, 29.6, 20.5, 20.4, 18.8, 18.3, 16.6 ppm. *m/z* = 485.5 ($M+Na^+$).

Formation of 2-(9H-fluoren-9-ylmethoxycarbonylamino)-*N*-(1-{1-[1-(methoxycarbonylmethyl-carbamoyl)-2-methyl-propylcarbamoyl]-ethylcarbamoyl}-ethyl)-succinamic acid tert-butyl ester (29).



Compound **24**[20] (250 mg, 0.58 mmol) was stirred in 20% volume TFA/anhydrous CH_2Cl_2 (10 mL) under inert atmosphere for 1.5 h. The solvent was concentrated *in vacuo*, diluted with toluene (2 x 10 mL) and then the solvent removed *in vacuo* to generate a solid. Meanwhile to a solution of Fmoc-L-aspartic acid-O^{t}Bu (286 mg, 0.70 mmol), PyBOP (363 mg, 0.70 mmol) in anhydrous CH_2Cl_2 (20 mL) that was cooled to 0 °C was added $^i Pr_2NEt$ (300 μL, 1.74 mmol) and the reaction mixture was stirred for 0.5 h. The resultant amine of **24** was then added to the reaction mixture and the reaction was continued at RT for 4 h. The solvent was removed *in vacuo* to generate a white solid **29** (175 mg). The resultant solid was used without further purification. *m/z* = 756.8 ($M+Na^+$)

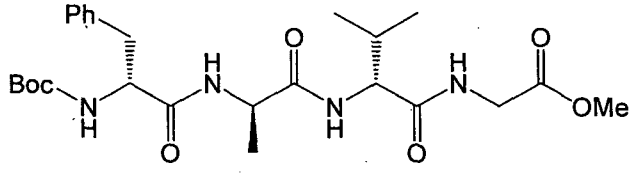
Formation of 2-amino-*N*-(1-{1-[1-(methoxycarbonylmethyl-carbamoyl)-2-methyl-propylcarbamoyl]-ethylcarbamoyl}-ethyl)-succinamic acid tert-butyl ester (30).



Compound **29** (175 mg, 0.24 mmol) was stirred in 20% volume piperidine / $CHCl_3$ (5 mL) and the reaction mixture was stirred at RT under inert atmosphere for 4 h. The

solvent was removed *in vacuo* and the solid purified by flash chromatography (0-5% MeOH/CH_2Cl_2) to yield a white solid **30** (102 mg, 85%): Decomp. ≥225 °C; $[\alpha]_D^{27}$ = +35.9 (*c* 1.30, MeOH/$CHCl_3$ 1:9). IR υ_{max} 3281, 2967, 1731, 1690, 1622, 1527 cm^{-1}. ^1H NMR (400 Hz $CDCl_3$/MeOD) δ 4.50-4.71 (m, 2H, CH_2), 4.38 (d, 1H, J = 6.9 Hz, CH), 3.89-4.09 (m, 2H, CH), 3.69 (s, 3H, Me), 3.65 (dd, 1H, J = 8.9 Hz, 3.9 Hz, CH), 2.63 (dd, 1H, J = 14.9 Hz, 4.0 Hz, CH), 2.50 (d, 1H, J = 9.0 Hz, CH_2), 2.13 (dq, 1H, J = 13.7 Hz, 6.8 Hz, CH), 1.41 (s, 9H, ^{t}Bu), 1.33 (dd, 6H, J = 6.9 Hz, 5.7 Hz, 2 x Me), 0.94 (dd, 6H, J = 9.2 Hz, 6.8 Hz, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD) δ 173.7, 172.8, 172.6, 171.8, 170.9, 170.1, 81.8, 50.4, 58.3, 52.0, 41.0, 40.9, 39.6, 30.9, 2 x 27.8, 18.9, 18.4, 18.2, 17.9 ppm. *m/z* 502.6 ($M+H^+$).

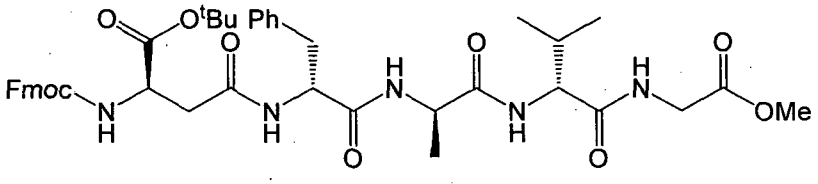
Formation of {2-[2-(2-tert-butoxycarbonylamino-1-methylene-3-phenyl-propylamino)-propionylamino]-3-methyl-butyrylamino}-acetic acid methyl ester (31).



A solution of **23**[20] (256 mg, 0.71 mmol) was dissolved in 20% volume TFA/anhydrous CH_2Cl_2 (5 mL) at RT under inert atmosphere and stirred for 1.5 h. The solvent was removed *in vacuo* to generate a residue from which TFA was removed via the azeotropic removal of toluene (2 x 10 mL). Then a solution of Boc-D-Phenylalanine-OH (208 mg, 0.78 mmol), EDAC.HCl (164 mg, 0.86 mmol) and HOBT.H_2O (116 mg, 0.86 mmol) in anhydrous CH_2Cl_2 (10 mL), at 0 °C was added ^{i}Pr$_2$NEt (430 μL, 2.50 mmol) and the reaction mixture was stirred for 0.5 h. To the solution was then added the resultant amine of **23** and the solution allowed stir at RT for 4 h. The reaction mixture was then washed with distilled water (20 mL), the organic layer separated and then washed with sat. $NH_4Cl_{(aq)}$ (20 mL), sat. $NaHCO_{3(aq)}$ solution (20 mL) and sat. brine (20 mL). The organic layers were dried ($MgSO_4$). The solvent was removed *in vacuo* to generate a transparent

film. The film was then purified by flash chromatography (0-5% MeOH/CH_2Cl_2) to yield a white solid **31** (224 mg, 62%): M.p. 219-221 °C. $[\alpha]_D^{27}$ = +30.9 (*c* 0.95, MeOH/$CHCl_3$ 1:9). IR υ_{max} 3270, 2960, 1754, 1693, 1663, 1637, 1527 cm^{-1}. ^1H NMR (400 Hz $CDCl_3$/MeOD) δ 7.42 (d, 1H, J = 8.2 Hz, NH), 7.12-7.33 (m, 5H, Ph), 4.35-4.49 (m, 2H, CH), 4.25 (t, 1H, J = 6.3 Hz, CH), 3.85-4.12 (m, 2H, CH_2), 3.08 (dd, 1H, J = 13.9 Hz, 5.5 Hz, CH), 2.99 (s, 3H, Me), 2.87-2.97 (m, 1H, CH), 2.14 (dq, 1H, J = 13.6 Hz, 6.8 Hz, CH), 1.36 (s, 9H, Boc), 1.33 (d, 3H, J = 7.0 Hz, Me), 0.95 (dd, 6H, J = 10.0 Hz, 6.8 Hz, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD)δ 172.5, 172.5, 171.8, 171.7, 170.0, 136.4, 129.2, 128.4, 126.8, 58.5, 55.3, 52.1, 41.0, 40.8, 38.3, 30.5, 28.0, 19.0, 17.8 ppm. *m/z* 529.3 ($M+Na^+$).

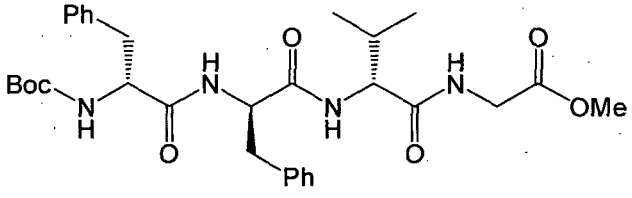
Formation of 2-(9H-fluoren-9-ylmethoxycarbonylamino)-*N*-(1-{1-[1-(methoxycarbonylmethyl-carbamoyl)-2-methyl-propylcarbamoyl]-ethylcarbamoyl}-2-phenyl-ethyl)-succinamic acid tert-butyl ester (32).



A solution of **31** (224 mg, 0.44 mmol) was stirred in 20% volume TFA/anhydrous CH_2Cl_2 (10 mL) at RT under inert atmosphere for 1.5 h. After completion, the solvent was removed *in vacuo* to generate a residue from which TFA was removed via the azeotropic removal of toluene (2 x 10 mL). Then to a solution of Fmoc-L-aspartic acid-O^{t}Bu (263 mg, 0.64 mmol), EDAC.HCl (134 mg, 0.70 mmol) and HOBt.H_2O (94.0 mg, 0.70 mmol) in anhydrous CH_2Cl_2 (10 mL), at 0 °C was added $^iPr_2NEt$ (350 μL, 2.03 mmol) and the reaction mixture was stirred for 0.5 h. To the solution was then added the resultant amine of **31** and the solution allowed to stir at RT for 4 h. Then the reaction mixture was washed with distilled water (20 mL), the layers separated and then washed with sat. $NH_4Cl_{(aq)}$ (20 mL), sat. $NaHCO_{3(aq)}$ (20 mL) and sat. brine (20 mL). The organic layers were then dried ($MgSO_4$) and then the solvent was removed *in vacuo* to generate a

transparent film. The film was then purified by flash chromatography (0-5% $MeOH/CH_2Cl_2$) to yield a white solid **32** (90 mg, 25%): M.p. 198-201 °C; $[\alpha]_D^{27}$ = +27.2 (*c* 1.20, $MeOH/CHCl_3$ 1:9). IR υ_{max} 3277, 2967, 1712, 1629, 1527 cm^{-1}. ^1H NMR (400 Hz $CDCl_3$/MeOD) δ 7.08-7.79 (m, 13H, Fmoc, Ph), 4.68-4.83 (m, 1H, CH), 4.32-4.53 (m, 3H, CH, CH_2), 4.22-4.32 (m, 2H, CH_2), 4.18 (t, 1H, J = 6.7 Hz, CH), 3.98-4.12 (m, 1H, CH), 3.85-3.96 (m, 1H, CH), 3.69 (s, 3H, Me), 3.33-3.43 (m, 1H, CH), 3.13-3.32 (m, 2H, CH_2), 3.07 (dd, 1H, J = 13.9 Hz, 5.6 Hz, CH), 2.88-2.98 (m, 1H, CH), 2.73-2.81 (m, 1H, CH), 2.59-2.70 (m, 1H, CH), 2.07 (dq, 1H, J = 13.4 Hz, 6.6 Hz, CH), 1.40 (s, 9H, O^{t}Bu), 1.29 (d, 3H, J = 6.9 Hz, Me), 0.84-1.02 (dd, 6H, J = 4.0 Hz, 8.0 Hz, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD) δ 172.4, 171.7, 171.1, 170.2, 170.0, 156.3, 143.7, 143.7, 143.7, 143.6, 141.1, 136.2, 129.1, 128.3, 127.6, 126.9, 126.8, 124.9, 119.8, 82.3, 67.0, 58.3, 53.9, 52.0, 51.3, 47.0, 40.8, 38.1, 37.4, 30.7, 27.6, 18.9, 17.9 ppm. *m/z* 822.5 ($M+Na^+$).

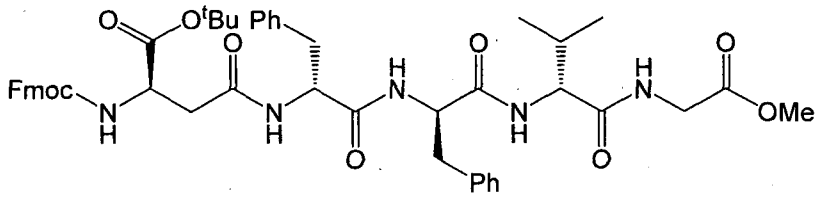
Formation of (1-{1-[2-methyl-1-(2-oxo-propylcarbamoyl)-propylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenyl-ethyl)-carbamic acid tert-butyl ester (34).



A solution of **33**[20] (2.49 g, 5.72 mmol) was stirred in 20% volume TFA/anhydrous CH_2Cl_2 (25 mL) at RT under inert atmosphere for 1.5 h. The solvent was removed *in vacuo* to generate a residue from which TFA was removed via the azeotropic removal of toluene (2 x 40 mL). Then to a solution of Boc-D-Phenylalanine-OH (1.82 g, 6.86 mmol), PyBOP (3.57 g, 6.86 mmol) in anhydrous CH_2Cl_2 (30 mL), at 0 °C was added $^i Pr_2NEt$ (3.5 mL, 20.0 mmol) and the reaction mixture was stirred for 0.5 h. Then to the solution was added the resultant amine of **33** and the solution was allowed to stir at RT for 4 h. The reaction mixture was washed with distilled water (50 mL), the layers

separated and then washed with sat. $NH_4Cl_{(aq)}$ (50 mL), sat. $NaHCO_{3(aq)}$ (50 mL) and sat. brine (50 mL). The organic layers were dried ($MgSO_4$) and then the solvent was removed *in vacuo* to generate a transparent film. The film was then purified by flash chromatography (1% MeOH/CH_2Cl_2) to yield a white solid **34** (2.98 g, 90%): M.p. 187-190 °C. ^{1}H NMR δ 7.03-7.30 (m, 10H, 2 x Ph), 4.57-4.66 (m, 1H, N-H), 4.32 (br s, 1H, N-H), 3.89 (m, 2H, CH_2), 3.70 (s, 3H, Me), 3.12 (q, 2H, J = 7.4 Hz, CH), 2.97 (d, 2H, J = 4.0 Hz, CH), 2.12 (dq, 1H, J = 13.5 Hz, 6.7 Hz, CH), 1.30 (s, 9H, Boc), 0.86 (dd, 6H, J = 10.1 Hz, 6.8 Hz, 2 x Me) ppm. ^{13}C NMR δ 172.0, 171.9, 171.4, 171.1, 170.0, 136.2, 136.0, 129.2, 129.1, 128.4, 126.8, 80.2, 58.6, 55.5, 54.9, 54.5, 54.4, 52.1, 43.0, 40.8, 38.0, 37.7, 30.2, 28.0, 12.4 ppm. *m/z* 605.7 ($M+Na^+$).

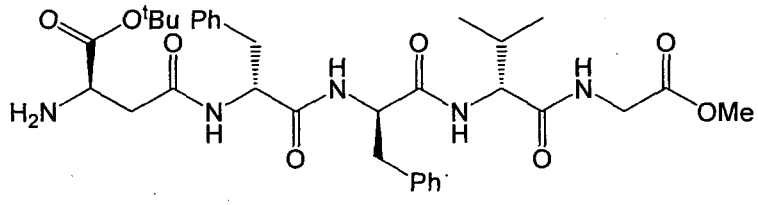
Formation of 2-methylamino-*N*-(1-{1-[2-methyl-1-(2-oxo-propylcarbamoyl)-propylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenyl-ethyl)-succinamic acid tert-butyl ester (35).



A solution of **34** (428 mg, 0.74 mmol) was stirred in 20% volume TFA/anhydrous CH_2Cl_2 (15 mL) at RT under inert atmosphere for 1.5 h. The solvent was removed *in vacuo* to generate a residue from which TFA was removed via the azeotropic removal of toluene (2 x 20 mL). Then to a solution of Fmoc-L-aspartic acid-O^{t}Bu (200 mg, 0.49 mmol), PyBOP (253 mg, 0.49 mmol) in anhydrous CH_2Cl_2 (10 mL), at 0 °C was added ^{i}Pr$_2$NEt (0.30 mL, 1.72 mmol) and the reaction mixture was stirred for 0.5 h. To the solution was then added the resultant amine of **34** dissolved in 75% volume anhydrous CH_2Cl_2/MeOH (5 mL) and the reaction mixture was allowed to stir at RT for 4 h. The reaction mixture was washed with distilled water (50 mL), the layers separated and then the organic layer was washed with sat. $NH_4Cl_{(aq)}$ (50 mL), sat. $NaHCO_{3(aq)}$ (50 mL) and sat. brine (50 mL). The organic layers were then dried ($MgSO_4$) and then the solvent was

removed *in vacuo* to generate a white solid. The solid was then purified by flash chromatography (1% MeOH/CH_2Cl_2) to yield a white solid **35** (179 mg, 42%): M.p. 202-207 °C; ^{1}H NMR δ 6.97-7.73 (m, 20H, Fmoc, 2 x Ph), 4.41-4.58 (m, 2H, CH), 4.25-4.34 (m, 1H, CH), 4.15-4.25 (m, 1H, CH), 4.03-4.14 (m, 2H, CH_2), 3.80 (s, 2H, CH_2), 3.26 (dt, 1H, J = 3.3 Hz, 1.6 Hz, CH), 2.91-3.02 (m, 2H, CH_2), 2.79-2.88 (m, 1H, CH), 2.74 (dd, 1H, J = 13.9 Hz, 7.8 Hz, CH), 2.60-2.69 (m, 1H, CH), 2.45-2.57 (m, 1H, CH), 1.99 (dq, 1H, J = 13.6 Hz, 6.8 Hz, CH), 1.33 (s, 9H, ^{t}Bu), 0.80 (dd, 6H, J = 10.8 Hz, 6.9 Hz, 2 x Me) ppm. ^{13}C NMR δ 171.5, 171.4, 171.0, 170.4, 170.1, 170.0, 165.8, 148.0, 143.5, 141.0, 136.4, 136.2, 136.1, 129.0, 128.9, 2 x 128.2, 127.5, 126.9, 124.8, 119.7, 82.3, 68.3, 68.1, 58.3, 54.6, 54.0, 52.0, 51.2, 46.9, 40.7. 37.5, 30.3, 2 x 27.5, 18.8, 17.5 ppm. *m/z* 899.4 ($M+Na^+$).

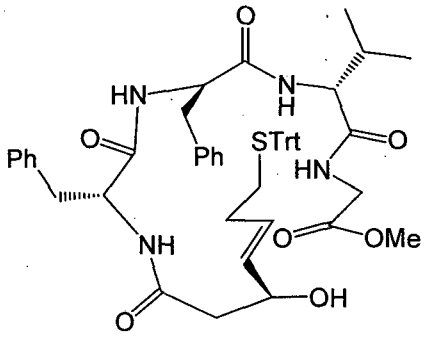
Formation of 2-amino-*N*-(1-{1-[2-methyl-1-(2-oxo-propylcarbamoyl)-propylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenyl-ethyl)-succinamic acid tert-butyl ester (36).



Compound **35** (159 mg, 0.18 mmol) was deprotected in 20% volume piperidine / CH_2Cl_2 (40 mL) and the reaction mixture was stirred at RT under inert pressure for 4 h. The solvent was removed *in vacuo* and the solid purified by flash chromatography (1-5% MeOH/CH_2Cl_2) to yield a transparent solid **36** (95 mg, 80%): Decomp. ≥154 °C; ^{1}H NMR δ 6.98-7.60 (m, 10H, 2 x Ph), 4.73-4.84 (m, 2H, 2 x CH), 4.37 (dd, 1H, J = 8.5 Hz, 6.9 Hz, CH), 4.00 (d, 2H, J = 5.6 Hz, CH_2), 3.75 (s, 3H, Me), 3.47 (dd, 1H, J = 10.3 Hz, 3.4 Hz, CH), 2.96-3.13 (m, 4H, 2 x CH_2), 2.52 (dd, 1H, J = 14.8 Hz, 3.4 Hz, CH), 2.15-2.27 (m, 2H, CH_2), 1.45 (s, 9H, ^{t}Bu), 0.93 (dd, 6H, J = 12.9 Hz, 6.8 Hz, 2 x Me) ppm. ^{13}C NMR δ 173.5, 171.3, 171.2, 171.0, 170.6, 170.2, 136.5, 136.2, 129.3, 128.6, 128.5, 127.1,

126.9, 126.9, 82.0, 62.4, 58.9, 54.6, 53.2, 52.2, 41.1, 40.1, 37.7, 37.4, 30.3, 28.0, 19.2, 18.0 ppm. *m/z* 676.6 ($M+Na^+$).

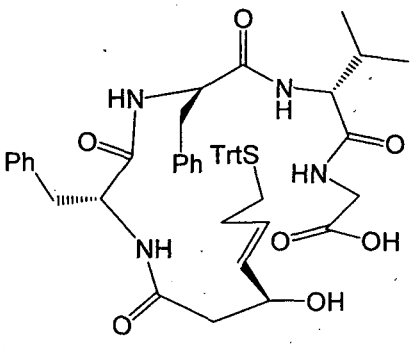
Formation of (2-{2-[2-(3-hydroxy-7-tritylsulfanyl-hept-4-enoylamino)-3-phenyl-propionylamino]-3-phenyl-propionylamino}-3-methyl-butyrylamino)-acetic acid methyl ester (37).



A solution of **34** (210 mg, 0.36 mmol) was stirred in 20% volume TFA/anhydrous CH_2Cl_2 (15 mL) at RT under inert atmosphere for 1.5 h. The solvent was removed *in vacuo* to generate a residue from which TFA was removed via the azeotropic removal of toluene (2 x 20 mL). Then to a solution of **5**[16] (100 mg, 0.24 mmol), PyBOP (124 mg, 0.24 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C was added $^iPr_2NEt$ (146 μL, 0.84 mmol) and the reaction mixture was stirred for 0.5 h. To the solution was then added the resultant amine of **34** dissolved in 75% volume anhydrous CH_2Cl_2/MeOH (5 mL) and the reaction mixture was allowed to stir at RT for 4 h. The reaction mixture was washed and with distilled water (50 mL) and the layers separated and then washed with sat. $NH_4Cl_{(aq)}$ (50 mL), sat. $NaHCO_{3(aq)}$ (50 mL) and sat. brine (50 mL). The organic layers were then dried ($MgSO_4$) and then the solvent was removed *in vacuo* to generate a solid. The solid was then purified by flash chromatography (1-2% MeOH/CH_2Cl_2) to yield a pale yellow solid **37** (170 mg, 80%): M.p. 85-87 °C. ^1H NMR δ 7.09-7.44 (m, 25H, 5 x Ph), 5.44-5.60 (m, 1H, CH), 5.27-5.40 (m, 1H, CH), 4.67-4.96 (m, 2H, CH_2), 4.33 (dd, 1H, J = 8.4 Hz, 6.2 Hz, CH), 3.98 (d, 2H, J = 5.5 Hz, CH), 3.73 (s, 3H, Me), 3.22 (m, 1H, CH), 3.16 (d, 1H, J = 5.1 Hz, CH), 3.01-3.13 (m, 2H, CH_2) 2.99 (d, 1H, J = 6.4 Hz, CH), 2.55 (dd, 1H, J = 14.0 Hz, 9.4 Hz, CH), 2.21-2.37 (m, 1H, CH), 2.19 (d, 2H, J = 5.7 Hz, CH_2), 2.00-

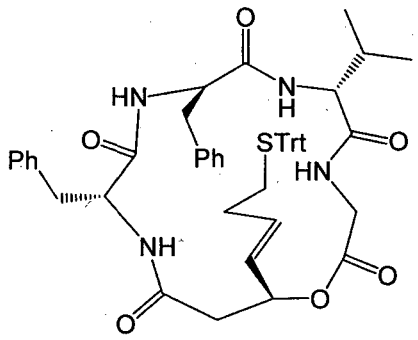
2.12 (m, 1H, CH), 1.82-1.92 (m, 1H, CH), 1.43 (d, 2H, J = 6.6 Hz, CH_2), 0.89 (dd, 6H, J = 12.1 Hz, 6.7 Hz, 2 x Me) ppm. ^{13}C NMR δ 171.2, 171.0, 170.2, 170.1, 169.9, 144.8, 136.5, 2 x 129.5, 129.4, 129.3, 2 x 128.7. 128.6, 2 x 127.8, 2 x 127.0, 126.6, 58.4, 56.0, 55.5, 54.5, 52.3, 52.3, 41.1, 37.7, 30.4, 29.9, 30.4, 29.9, 19.1, 17.7, 12.5 ppm. *m/z* 906.0 ($M+Na^+$).

Formation of (2-{2-[2-(3-hydroxy-5-tritylsulfanyl-pentanoylamino)-3-phenyl-propionylamino]-3-phenyl-propionylamino}-3-methyl-butyrylamino)-acetic acid (38)



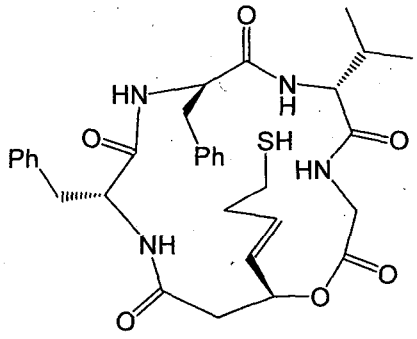
To a solution of **37** (170 mg, 0.20 mmol) in 25% volume THF / distilled water (2 mL) was added lithium hydroxide (14 mg, 0.60 mmol). After stirring for 2h at 0 °C, the solution was diluted with distilled water (4 mL), acidified to pH 1 with 1M $KHSO_{4(aq)}$ and extracted with EtOAc (3 x 30 mL). The solution was washed with sat. $brine_{(aq)}$ (10 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a grey solid **39** (170 mg, >100%) that was used without further purification. *m/z* (ES-) = 851.4 ($M-H^+$).

Formation of 9,12-dibenzyl-6-isopropyl-16-(4-tritylsulfanyl-but-1-enyl)-1-oxa-4,7,10,13-tetraaza-cyclohexadecane-2,5,8,11,14-pentaone (39)



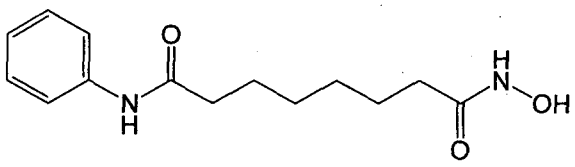
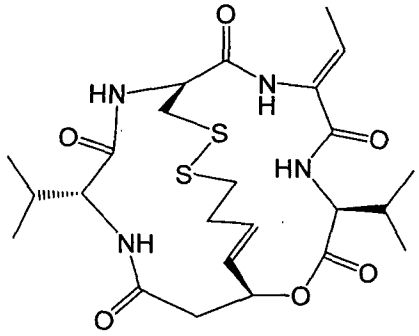
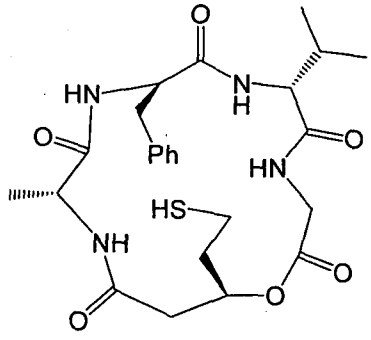
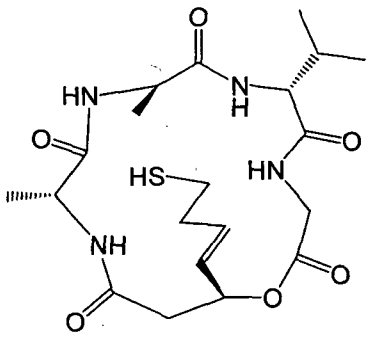
To a solution of **38** (150 mg (maximum pure yield), 0.17 mmol) in anhydrous THF (40 mL)/anhydrous CH_2Cl_2 (200 mL) was added dropwise to a solution of MNBA (64 mg, 0.19 mmol) and DMAP (46 mg, 0.37 mmol) in anhydrous CH_2Cl_2 (40 mL) and the reaction mixture was stirred over a period of 3 h under inert atmosphere at RT. After completing the addition, the reaction mixture was further stirred for a further 8 h at RT. Then the reaction mixture was quenched by the addition of 1M $HCl_{(aq)}$ (80 mL), the layers separated and then the organic layer was washed with sat. $NaHCO_{3(aq)}$ (50 mL) and sat. brine (50 mL). The organic layers were dried (Na_2SO_4) and then the solvent was removed *in vacuo*, to which the solid was then purified by flash chromatography (1-2% MeOH / CH_2Cl_2) to yield a yellow solid **39** (57 mg, 39%): M.p. 128-134 °C. ^{1}H NMR (400 Hz $CDCl_3$ / MeOD) δ 6.92-7.37 (m, 25H, 5 x Ph), 5.48 (dd, 1H, J = 14.1 Hz, 7.2 Hz, CH), 5.12-5.48 (m, 1H, CH), 4.13-4.27 (m, 2H, CH_2), 4.07 (m, 1H, CH), 3.79-3.99 (m, 1H, CH), 3.34-3.50 (m, 1H, CH), 3.11-3.28 (m, 4H, CH_2), 2.63-2.88 (m, 2H, CH_2), 2.07 (d, 2H, J = 6.9 Hz, CH_2), 1.94 (m, 2H, 2 x CH), 0.75-0.93 (ddd, 6H, J = 44.0 Hz, 24.0 Hz, 8.0 Hz, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD) δ 180.3, 172.7, 171.2, 170.1, 168.2, 144.3, 144.2, 136.5, 136.0, 132.3, 2 x 129.0, 128.4, 128.2, 128.0, 2 x 127.3, 126.4, 126.1, 126.1, 72.0, 70.9, 66.1, 59.3, 58.3, 41.2, 35.9, 35.4, 30.7, 30.6, 28.8, 18.7, 16.3 ppm. *m/z* = 874.0 ($M+Na^+$).

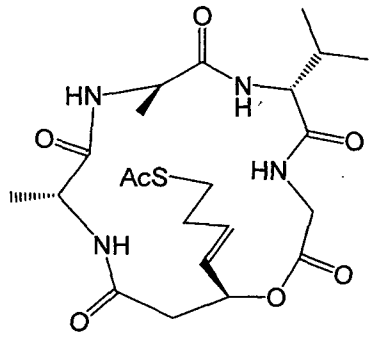
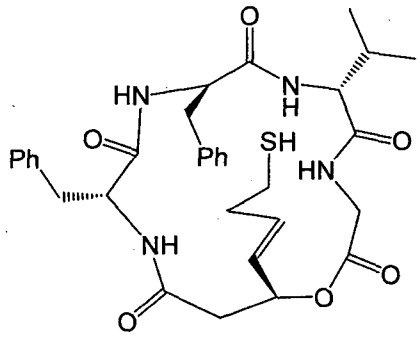
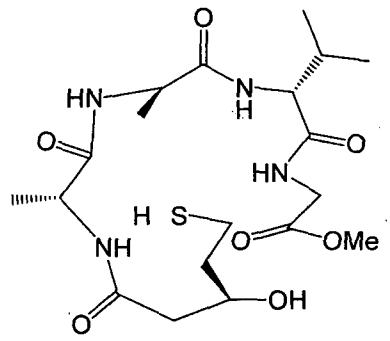
Formation of 9,12-dibenzyl-6-isopropyl-16-(4-mercapto-but-1-enyl)-1-oxa-4,7,10,13-tetraaza-cyclohexadecane-2,5,8,11,14-pentaone (40)



To a solution of **39** (35 mg, 41.1 μmol) in 50% volume TFA/anhydrous CH_2Cl_2 (4 mL) was added Et_3SiH (13 μL, 82.3 μmol) and the reaction mixture was stirred at RT for 1 h under inert atmosphere. The solvent was then concentrated *in vacuo* and toluene (10 mL) was added and removed. The solid was purified by flash chromatography (1-5% MeOH / CH_2Cl_2) to generate an orange solid **40** (8 mg, 32%): M.p. 192-196 °C. ^{1}H NMR (400 Hz $CDCl_3$ / MeOD) δ 6.95-7.21 (m, 10H, 2 x Ph), 5.58 (t, 1H, J = 7.0 Hz, CH), 5.27-5.44 (m, 1H, CH), 4.27 (d, 1H, J = 5.0 Hz, CH), 4.16 (ddd, 2H, J = 15.4 Hz, 10.1 Hz, 5.7 Hz, CH_2), 3.95 (dd, 1H, J = 10.2 Hz, 5.7 Hz, CH), 3.42 (dd, 2H, J = 17.1 Hz, 7.6 Hz, CH_2), 3.27-3.30 (m, 1H, CH), 3.25 (d, 2H, J = 1.5 Hz, CH_2), 3.14 (dt, 1H, J = 13.9 Hz, 5.5 Hz, CH) 2.77-2.83 (m, 1H, CH), 2.69-2.76 (m, 1H, CH), 2.44 (t, 2H, J = 7.0 Hz, CH_2), 2.39 (d, 1H, J = 7.7 Hz, CH), 2.33 (d, 1H, J = 7.2 Hz, CH), 2.28 (d, 1H, J = 6.5 Hz, CH), 2.19-2.25 (m, 2H, CH_2), 1.87-1.96 (m, 1H, CH), 1.62-178 (m, 1H, CH), 0.88 (ddd, 6H, J = 15.9 Hz, 8.9 Hz, 7.0 Hz, 2 x Me) ppm. ^{13}C NMR (100 Hz $CDCl_3$/MeOD) δ 180.6, 172.7, 171.4, 170.3, 168.6, 136.7, 136.2, 132.0, 2 x 128.7, 128.7, 128.6, 128.5, 128.4, 126.8, 72.3, 59.8, 54.9, 54.8, 41.4, 40.5, 39.9, 38.5, 29.4, 28.9, 23.3, 19.8, 19.2 ppm. *m/z* = 631.7 ($M+Na^+$).

Biological Data

Compound	Enzyme inhibition (nM)
SAHA 	287.0
FK228  FK228	15.5
KA037[20] 	47.0
Monothiol[20] 	7.0

Monothiol acetate[20] 	>80
Compound **40** 	156.9
Compound **28** 	8000

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AM4733/14

1H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 7.14 - 7.45 (15 H, m), 5.10 (1 H, t, *J*=6.59 Hz), 4.08 (1 H, br. s.), 3.39 - 3.52 (2 H, m), 2.94 - 3.07 (2 H, m), 2.64 (1 H, br. s.), 2.31 (2 H, t, *J*=7.36 Hz), 1.39 - 1.70 (3 H, m), 0.93 - 1.06 (6 H, m)

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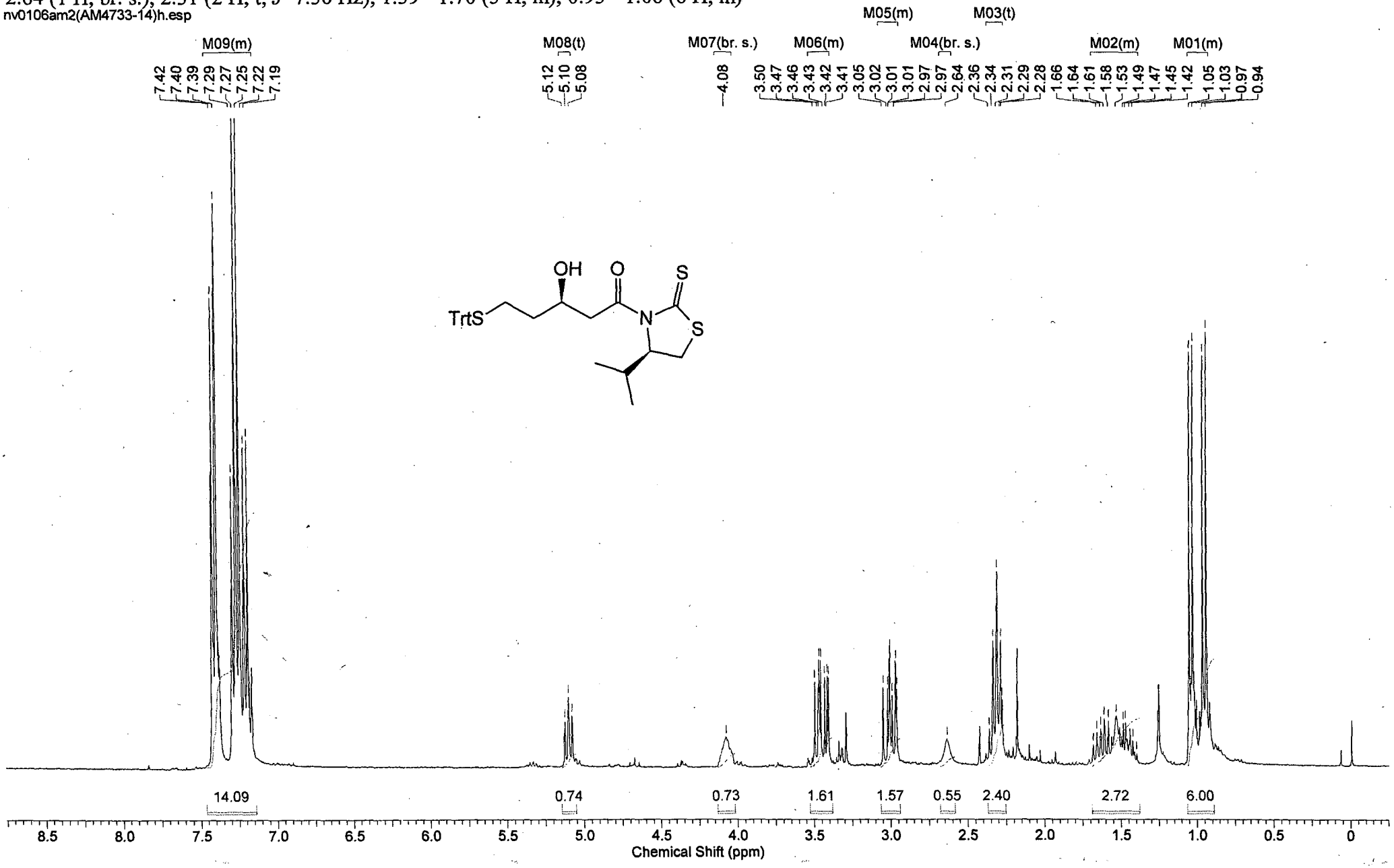

AM4733/14

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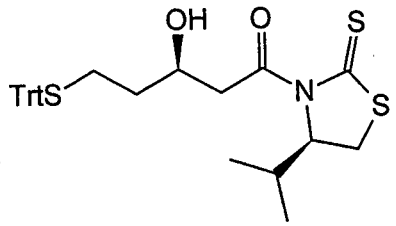
28.18
19.06
17.81



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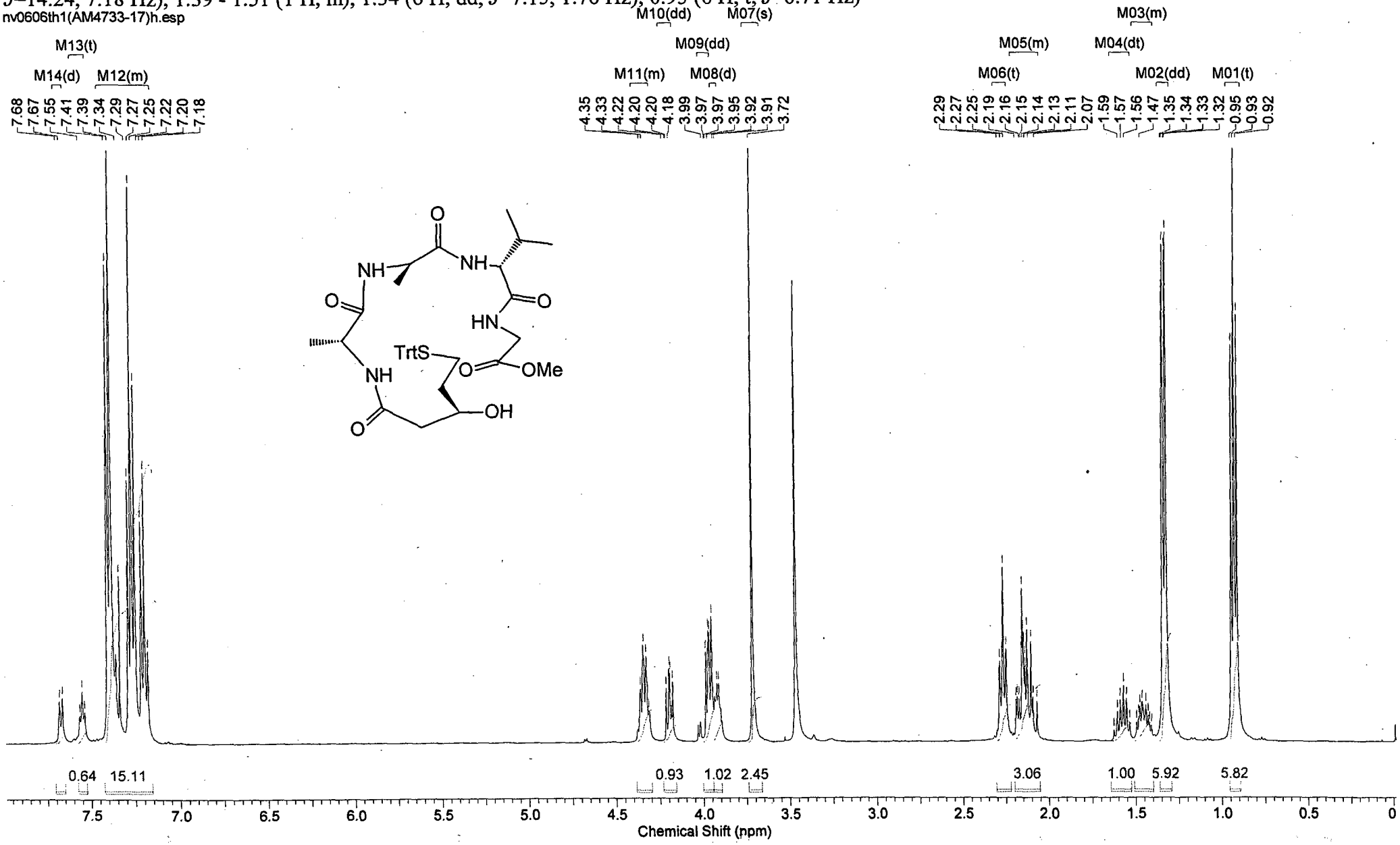
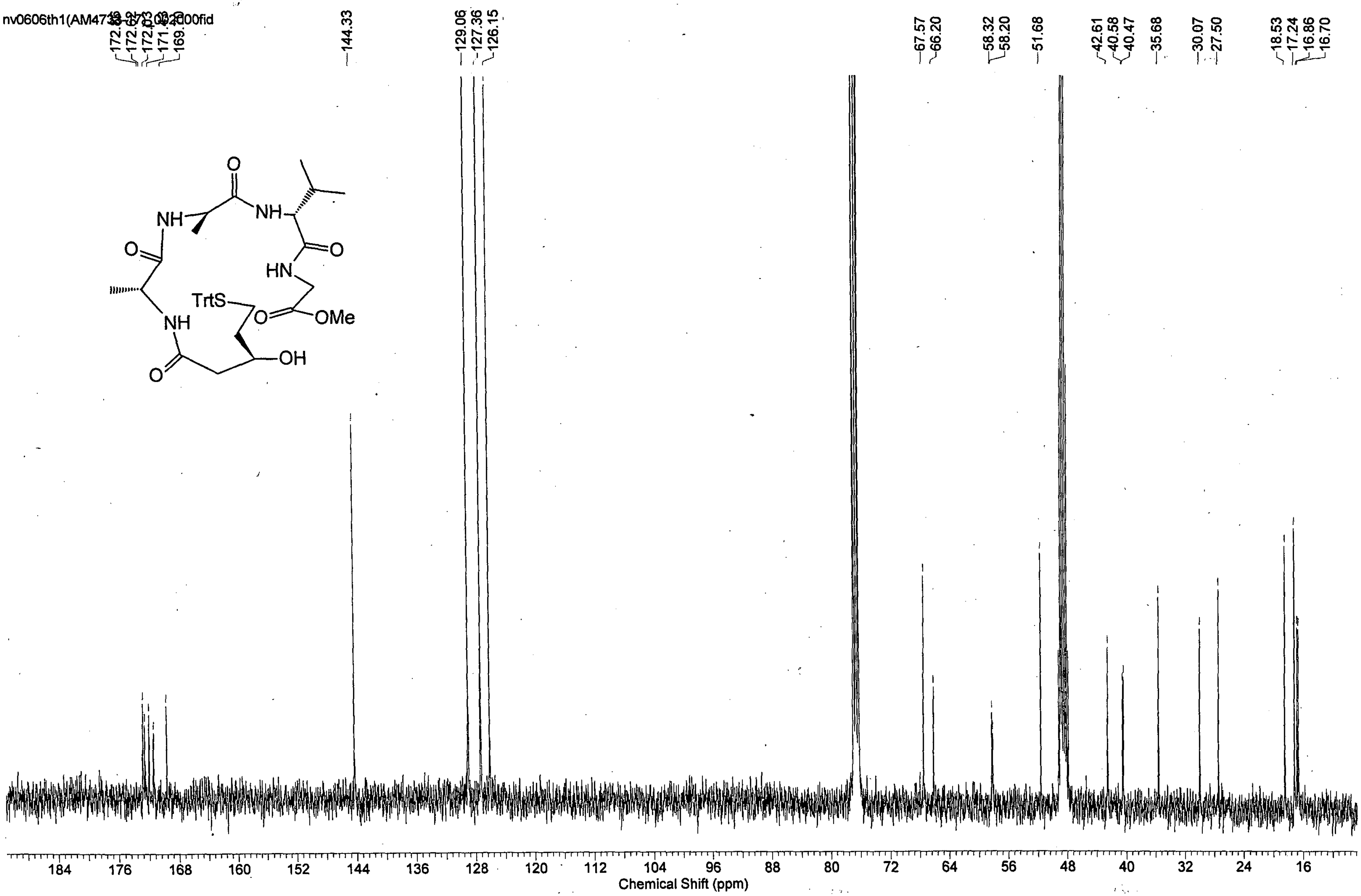
Chemical Shift (ppm)

AM4733/17

1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.68 (1 H, d, *J*=6.90 Hz), 7.55 (1 H, t, *J*=5.52 Hz), 7.14 - 7.44 (15 H, m), 4.29 - 4.39 (2 H, m), 4.20 (1 H, dd, *J*=8.72, 6.71 Hz), 3.97 (2 H, dd, *J*=7.84, 5.58 Hz), 3.92 (1 H, d, *J*=4.02 Hz), 3.72 (3 H, s), 2.27 (2 H, t, *J*=7.53 Hz), 2.05 - 2.21 (3 H, m), 1.57 (1 H, dt, *J*=14.24, 7.18 Hz), 1.39 - 1.51 (1 H, m), 1.34 (6 H, dd, *J*=7.15, 1.76 Hz), 0.93 (6 H, t, *J*=6.71 Hz)



AM4733/17



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42.61
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18.53
17.24
16.86
16.70
TrtS
NH
HN
OMe
OH
184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16
Chemical Shift (ppm)

AM4733/22

1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.15 - 7.41 (15 H, m), 5.00 - 5.08 (1 H, m), 4.21 - 4.31 (2 H, m), 4.07 (1 H, q, *J*=7.03 Hz), 3.52 - 3.67 (1 H, m), 3.10 - 3.16 (1 H, m), 2.43 (1 H, dd, *J*=13.05, 6.40 Hz), 2.28 - 2.36 (1 H, m), 2.21 (1 H, d, *J*=8.91 Hz), 2.16 (2 H, t, *J*=7.28 Hz), 1.83 (1 H, ddd, *J*=6.40, 3.58, 3.33 Hz), 1.69 - 1.78 (1 H, m), 1.57 (3 H, d, *J*=7.40 Hz), 1.35 (3 H, d, *J*=7.15 Hz), 0.86 - 0.98 (6 H, m)

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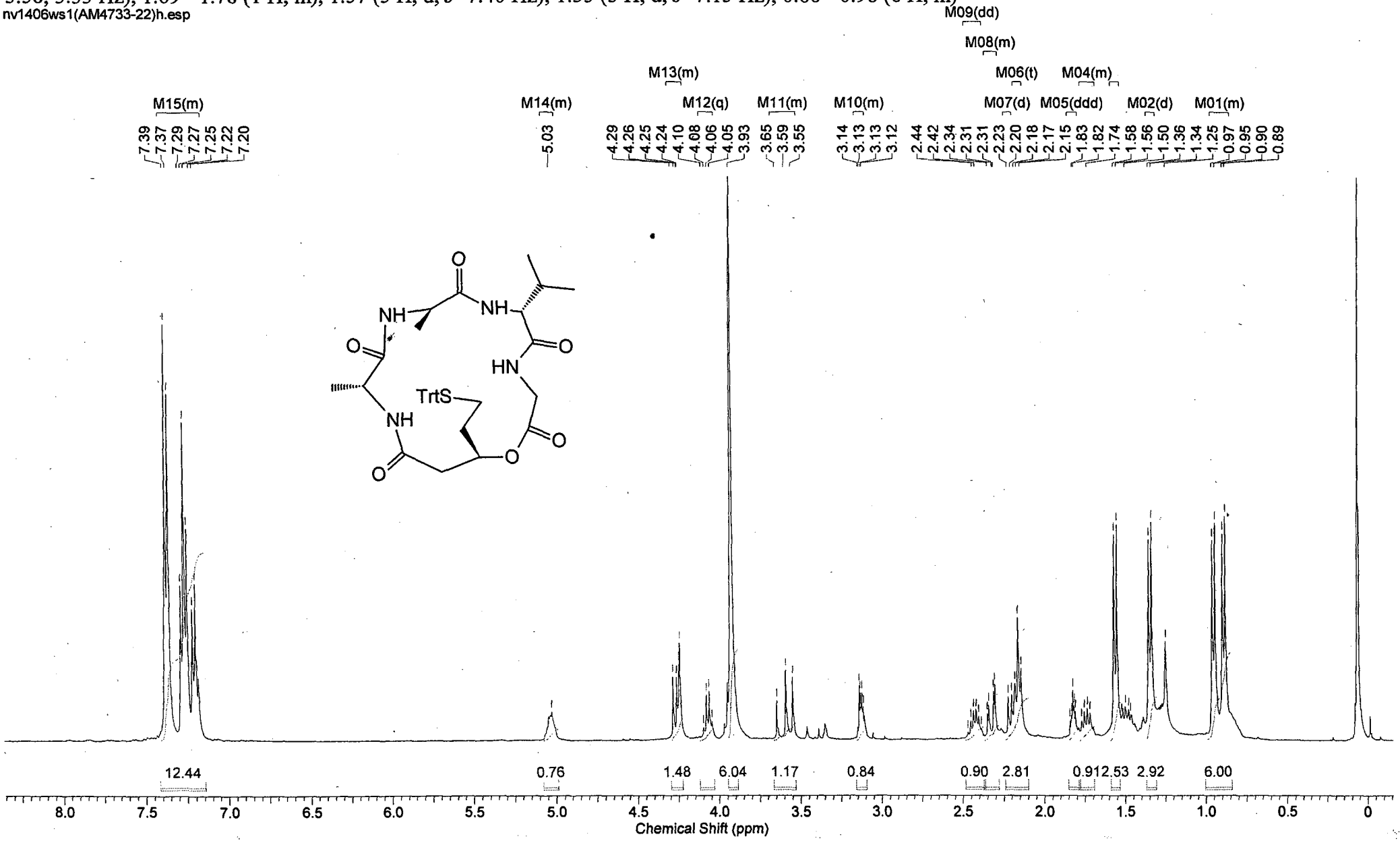

AM4733/22

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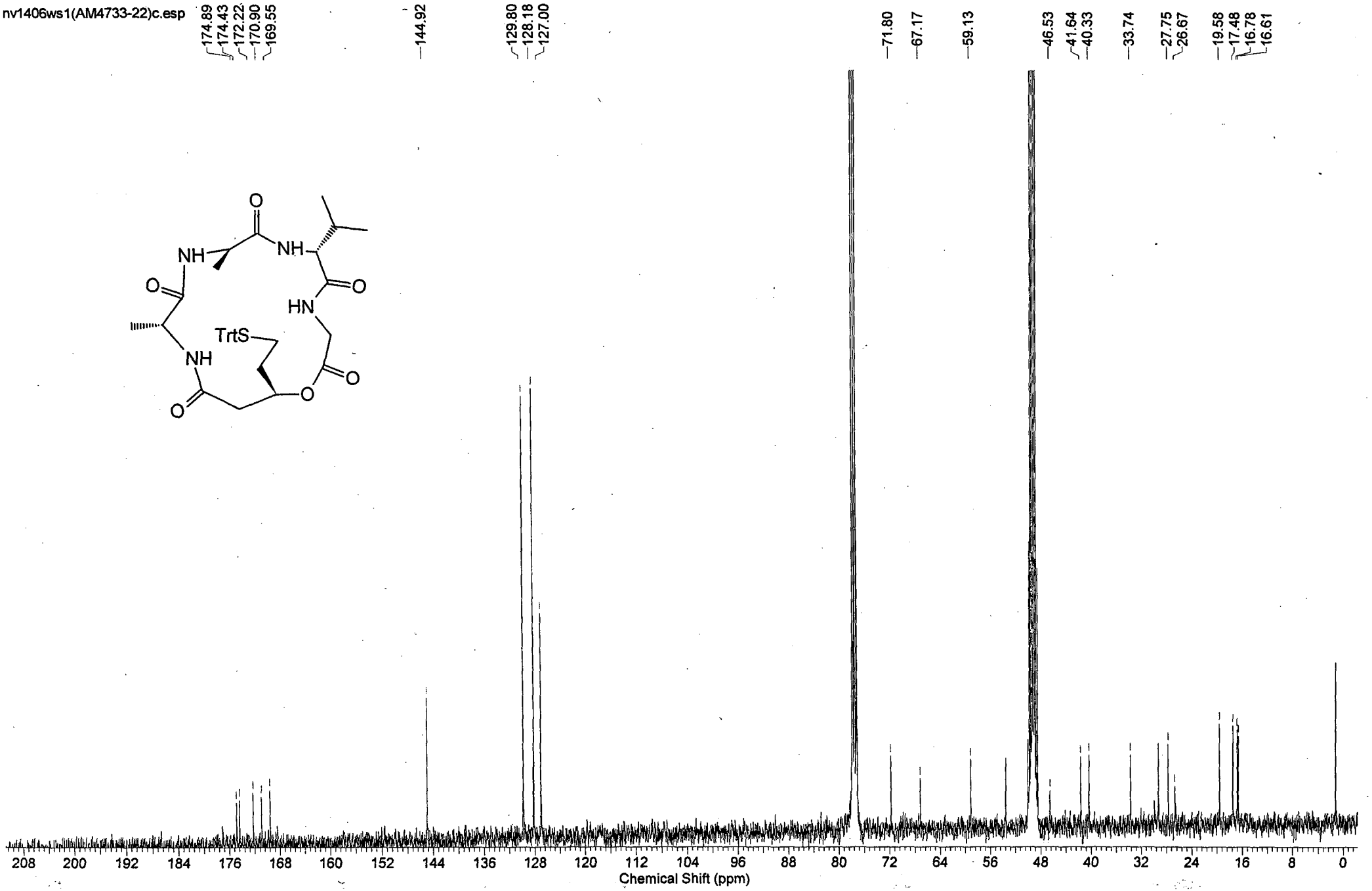
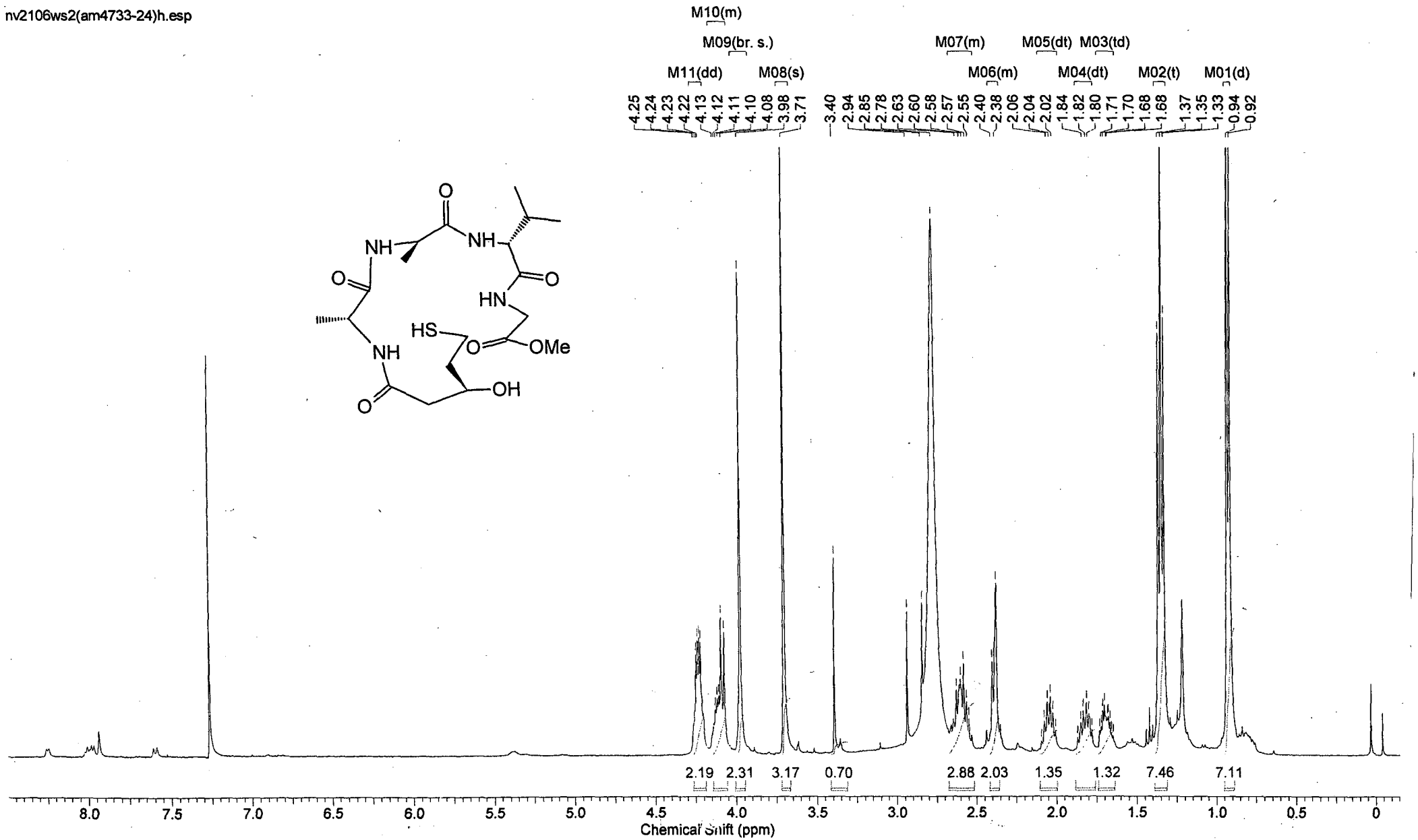
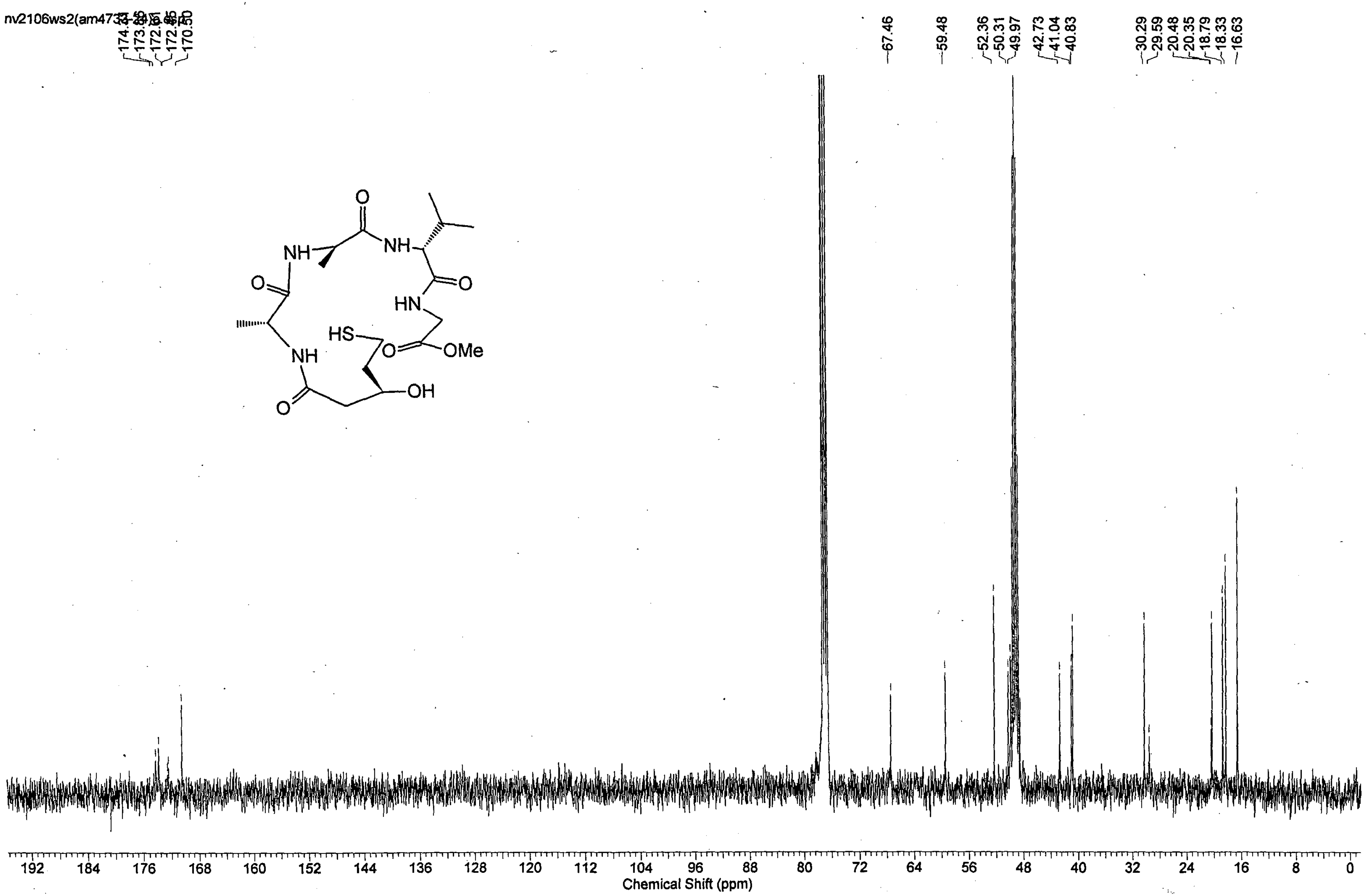


AM4733/24

1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 4.24 (2 H, dd, *J*=7.22, 3.58 Hz), 4.04 - 4.15 (2 H, m), 3.98 (2 H, br. s.), 3.71 (3 H, s), 2.52 - 2.67 (3 H, m), 2.35 - 2.42 (2 H, m), 2.04 (1 H, dt, *J*=13.93, 6.96 Hz), 1.82 (1 H, dt, *J*=14.05, 7.03 Hz), 1.70 (1 H, td, *J*=10.82, 7.72 Hz), 1.35 (6 H, t, *J*=7.22 Hz), 0.93 (6 H, d, *J*=6.78 Hz)



AM4733/24



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HN
O
HS
O
OMe
NH
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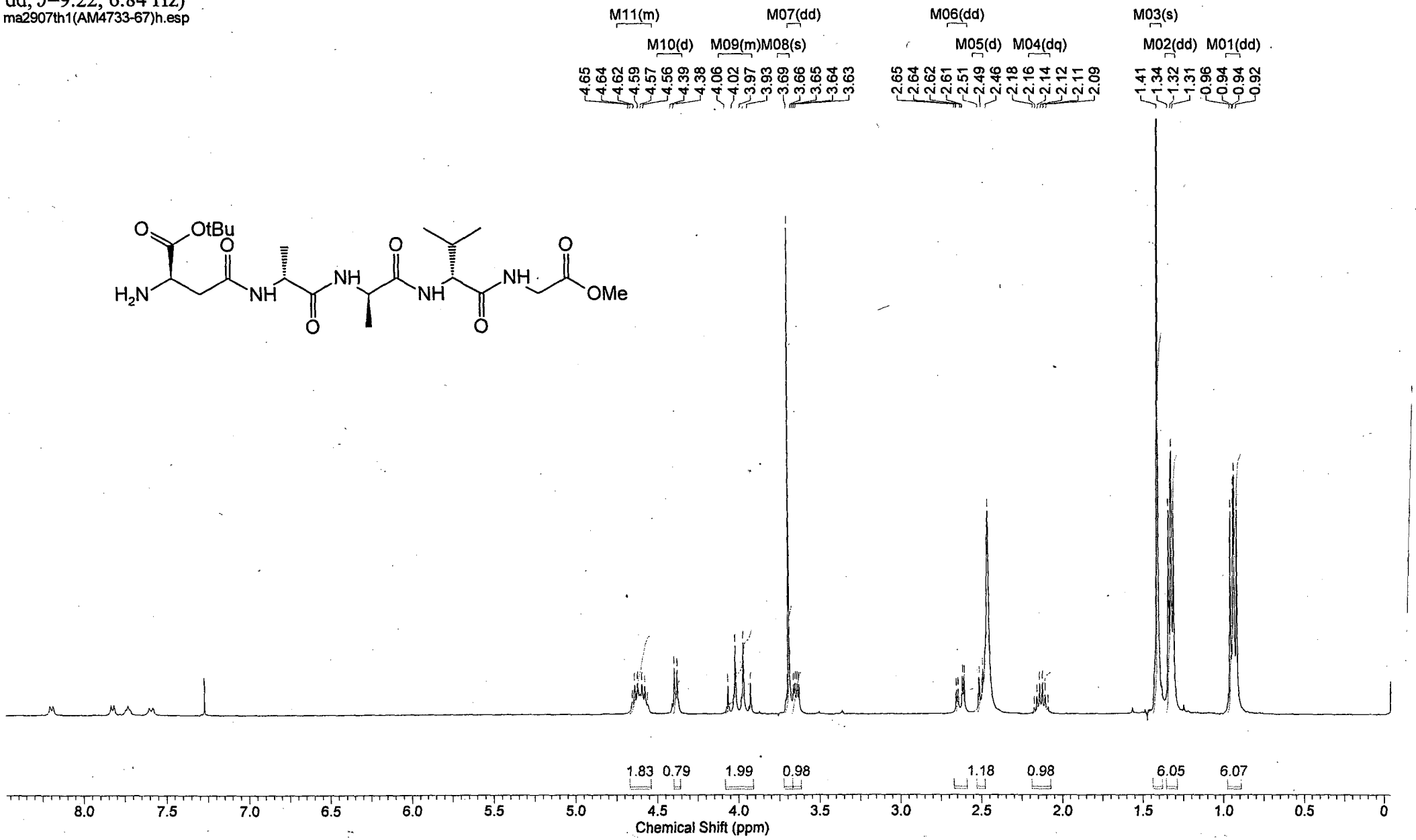
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)

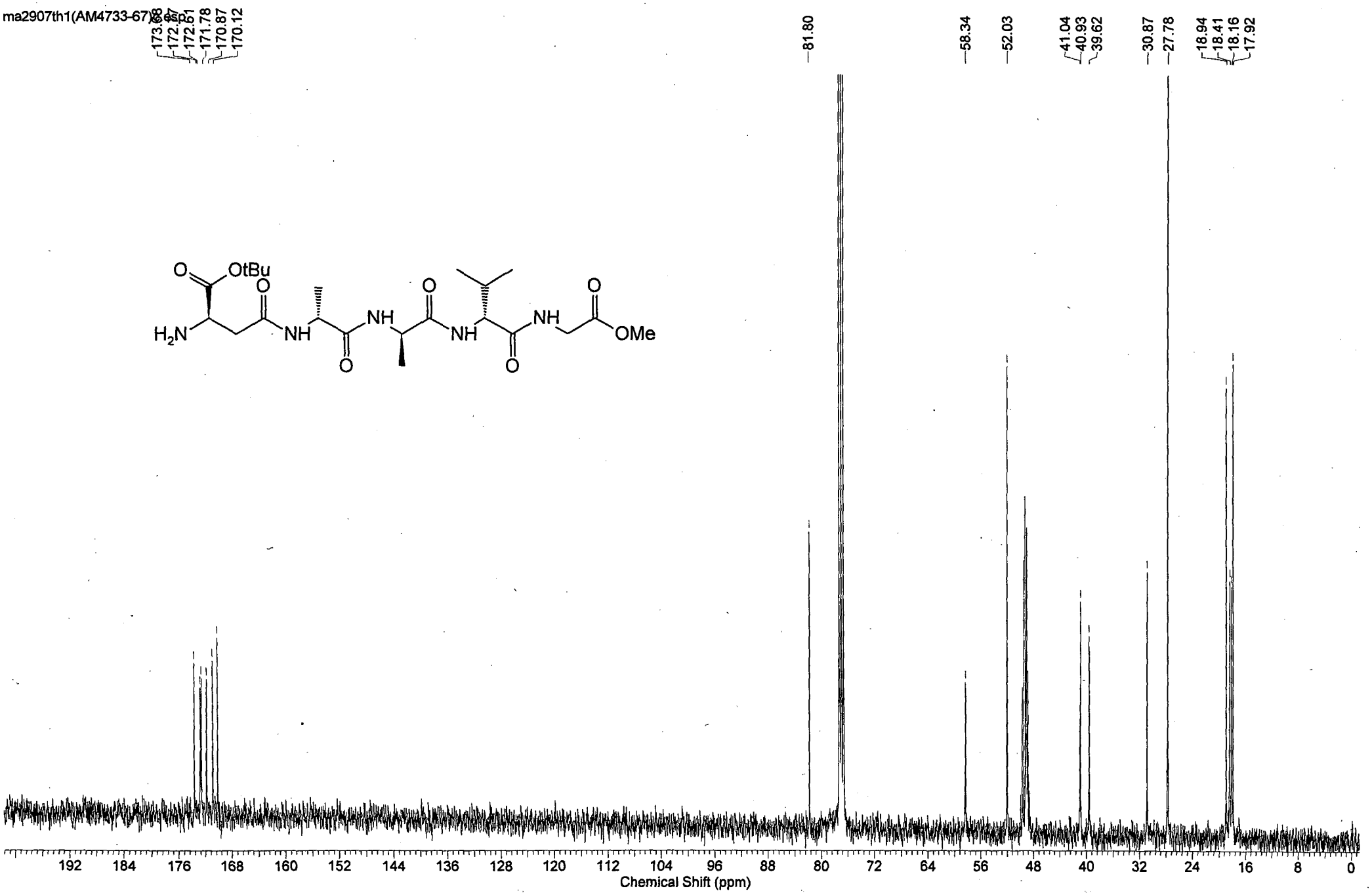
AM4733/67

1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 4.50 - 4.71 (2 H, m), 4.38 (1 H, d, *J*=6.90 Hz), 3.89 - 4.09 (2 H, m), 3.69 (3 H, s), 3.65 (1 H, dd, *J*=8.91, 3.89 Hz), 2.63 (1 H, dd, *J*=14.87, 3.95 Hz), 2.50 (1 H, d, *J*=9.03 Hz), 2.13 (1 H, dq, *J*=13.65, 6.79 Hz), 1.41 (9 H, s), 1.33 (6 H, dd, *J*=6.90, 5.65 Hz), 0.94 (6 H, dd, *J*=9.22, 6.84 Hz)

ma2907th1(AM4733-67)h.esp

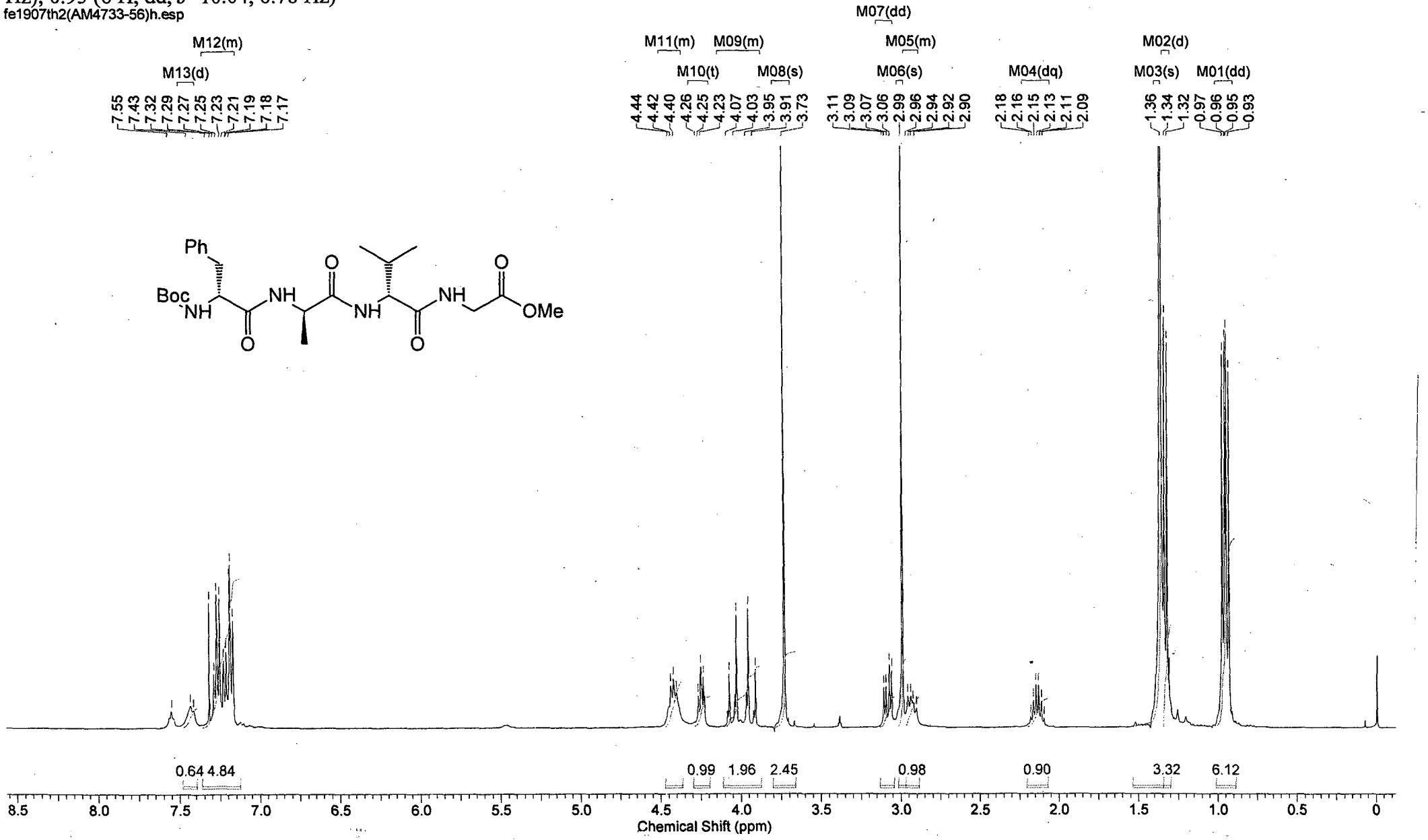


AM4733/67


ma2907th1(AM4733-67)8.esp
173.62
172.47
172.61
171.78
170.87
170.12
81.80
58.34
52.03
41.04
40.93
39.62
30.87
27.78
18.94
18.41
18.16
17.92
O
OtBu
O
H2N
NH
NH
NH
O
O
O
O
OMe
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)

AM4733/56

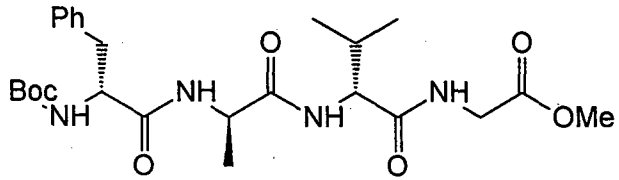
1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.42 (1 H, d, *J*=8.16 Hz), 7.12 - 7.33 (5 H, m), 4.35 - 4.49 (2 H, m), 4.25 (1 H, t, *J*=6.34 Hz), 3.85 - 4.12 (2 H, m), 3.73 (3 H, s), 3.08 (1 H, dd, *J*=13.93, 5.52 Hz), 2.99 (2 H, s), 2.87 - 2.97 (1 H, m), 2.14 (1 H, dq, *J*=13.55, 6.78 Hz), 1.36 (9 H, s), 1.33 (3 H, d, *J*=7.03 Hz), 0.95 (6 H, dd, *J*=10.04, 6.78 Hz)



AM4733/56

fe1907th2(AM4733-56)c.esp

172.54
172.46
171.78
171.66
170.01
136.36
129.16
128.37
126.75
80.13
58.52
58.43
55.26
52.07
40.95
40.83
38.25
30.52
28.03
18.95
17.82



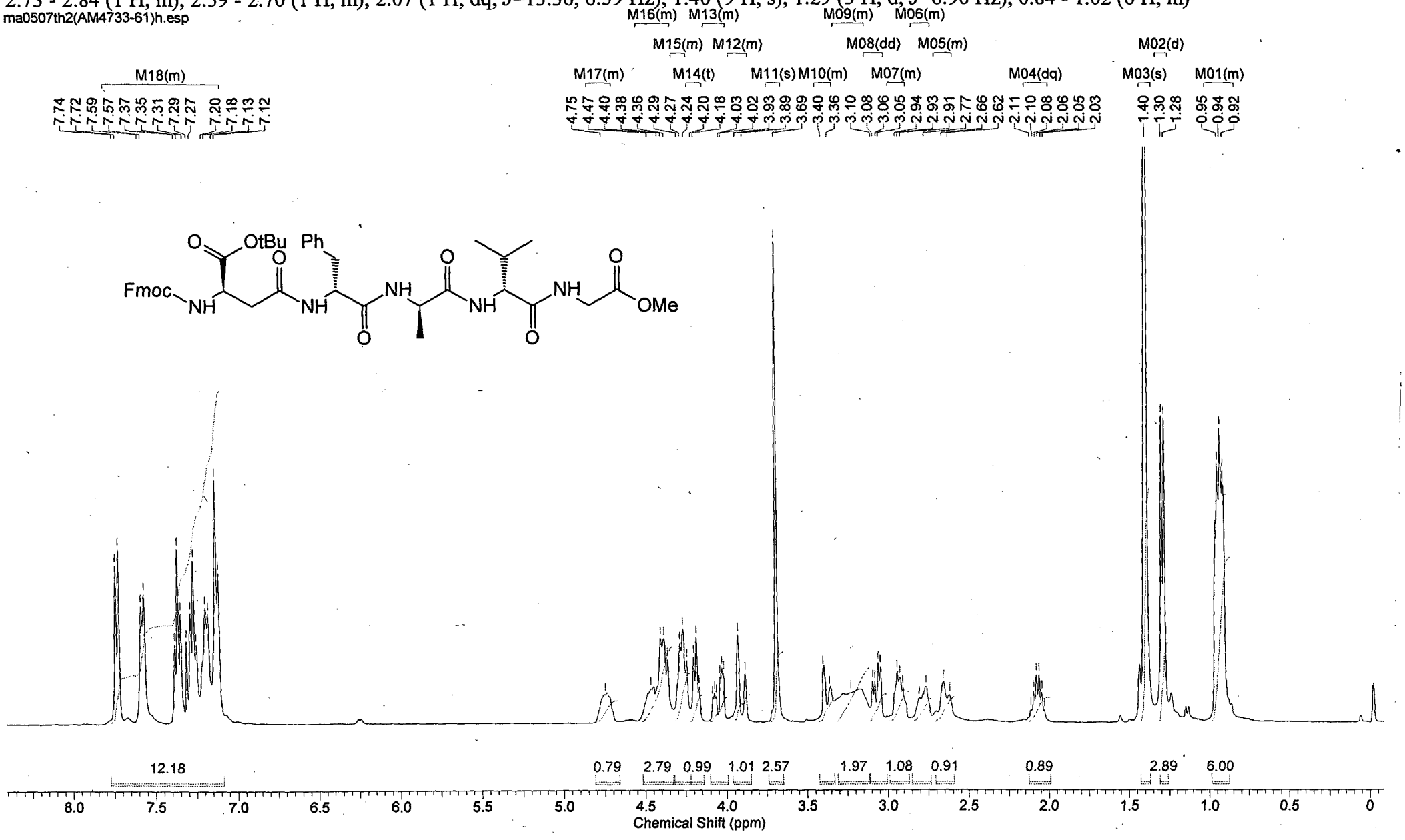
200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0

Chemical Shift (ppm)

AM4733/61

1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.08 - 7.79 (13 H, m), 4.68 - 4.83 (1 H, m), 4.32 - 4.53 (3 H, m), 4.22 - 4.32 (2 H, m), 4.18 (1 H, t, *J*=6.71 Hz), 3.98 - 4.12 (1 H, m), 3.85 - 3.96 (1 H, m), 3.69 (3 H, s), 3.33 - 3.43 (1 H, m), 3.13 - 3.32 (2 H, m), 3.07 (1 H, dd, *J*=13.87, 5.58 Hz), 2.88 - 2.98 (1 H, m), 2.73 - 2.84 (1 H, m), 2.59 - 2.70 (1 H, m), 2.07 (1 H, dq, *J*=13.36, 6.59 Hz), 1.40 (9 H, s), 1.29 (3 H, d, *J*=6.90 Hz), 0.84 - 1.02 (6 H, m)

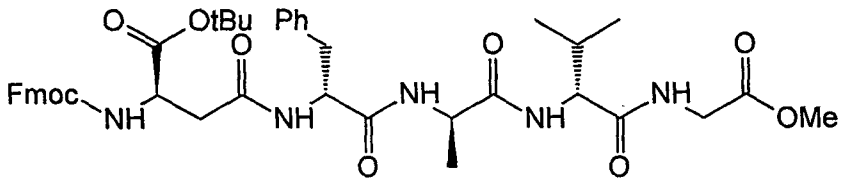
ma0507th2(AM4733-61)h.esp



AM4733/61

ma0507th2(AM4733-61)c.esp

172.40
171.72
171.05
170.16
170.04
156.25
143.69
143.58
141.10
136.16
129.05
128.28
127.57
126.92
126.75
124.91
119.79
82.28
66.99
58.26
53.92
52.02
51.26
46.96
40.82
38.14
37.42
30.74
27.61
18.88
17.91



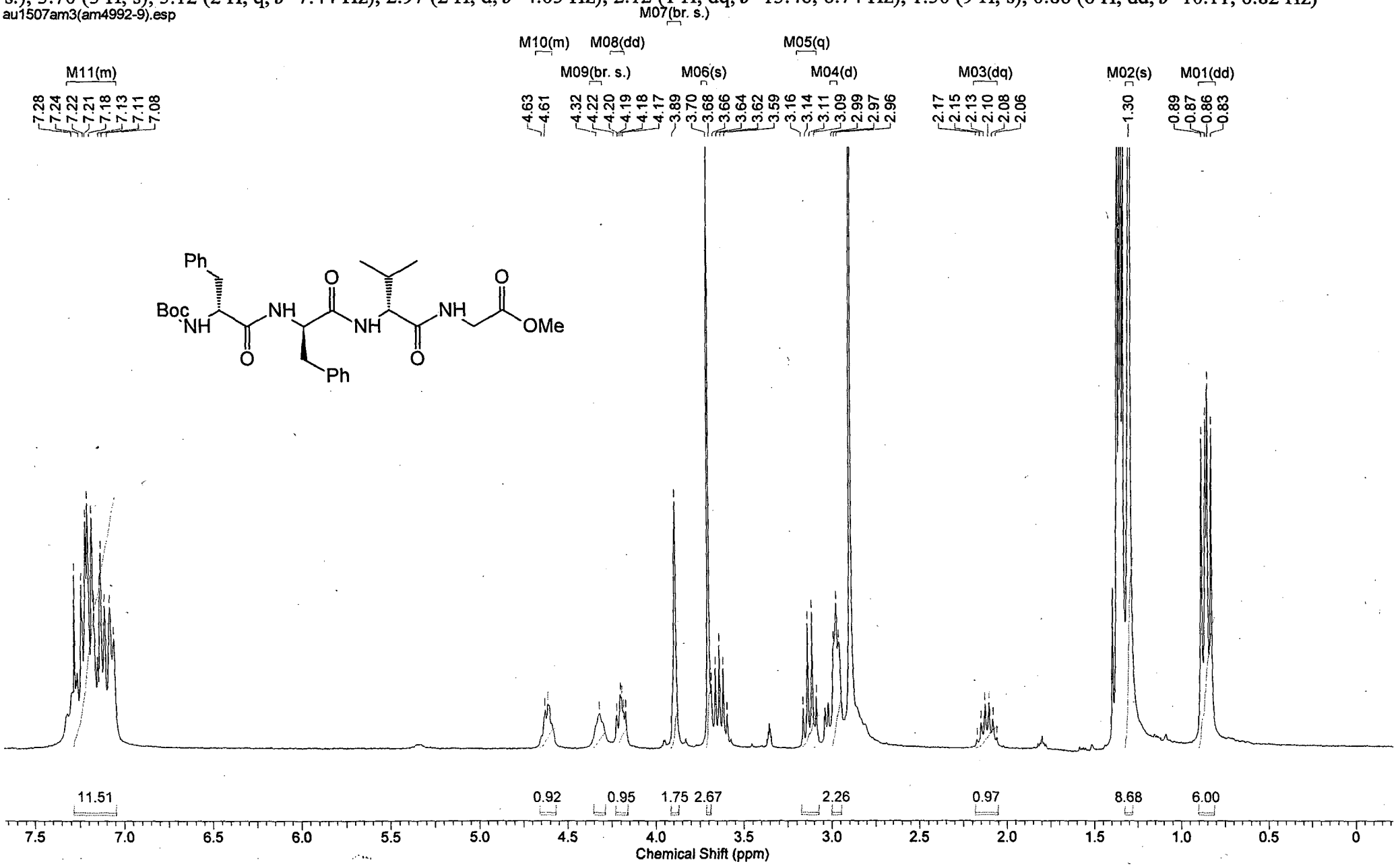
200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0

Chemical Shift (ppm)

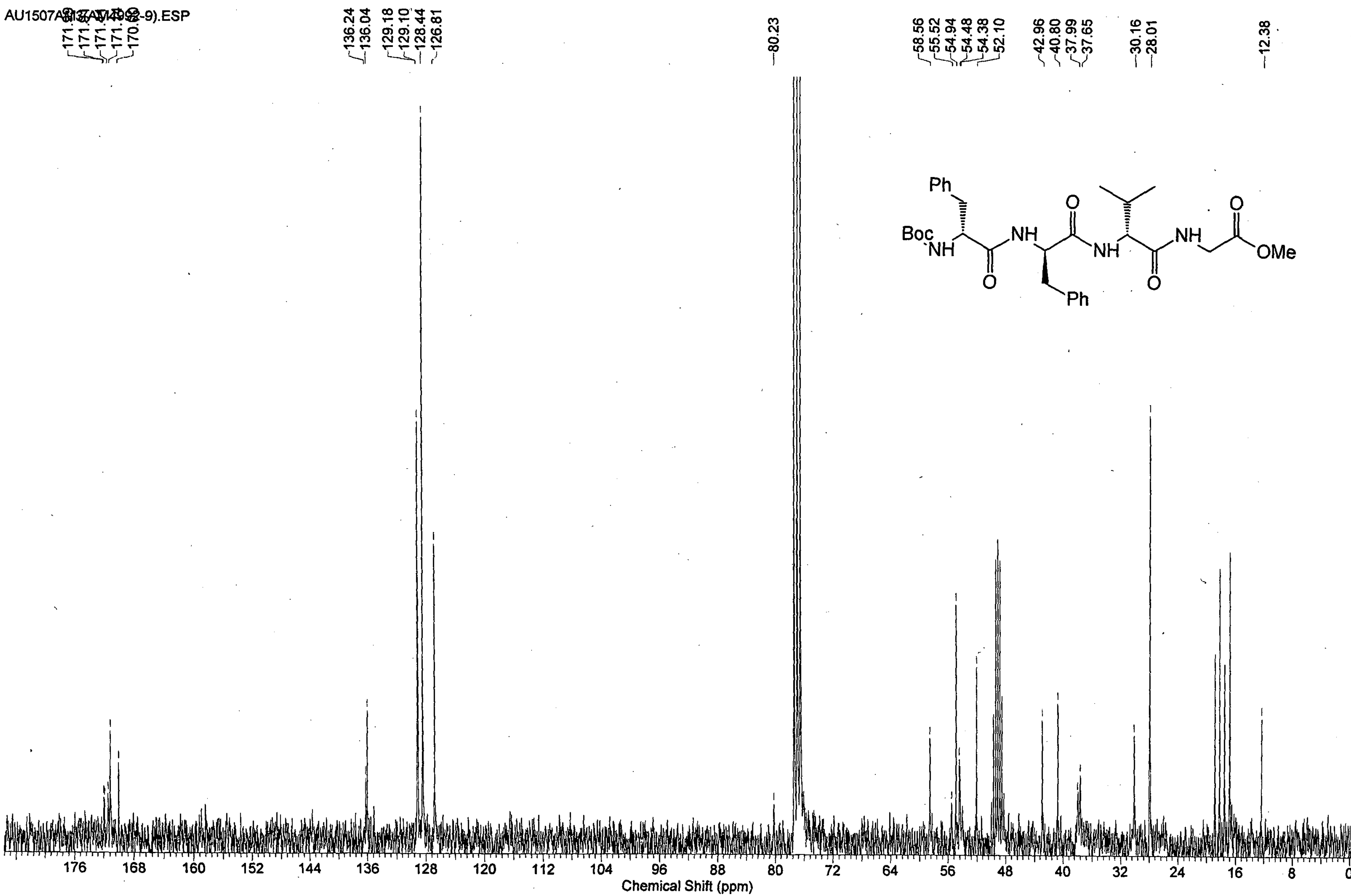
AM4992/9

1H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 7.03 - 7.30 (14 H, m), 4.57 - 4.66 (1 H, m), 4.32 (1 H, br. s.), 4.20 (1 H, dd, *J*=8.51, 6.59 Hz), 3.89 (2 H, br. s.), 3.70 (3 H, s), 3.12 (2 H, q, *J*=7.44 Hz), 2.97 (2 H, d, *J*=4.03 Hz), 2.12 (1 H, dq, *J*=13.46, 6.74 Hz), 1.30 (9 H, s), 0.86 (6 H, dd, *J*=10.11, 6.82 Hz)

au1507am3(am4992-9).esp



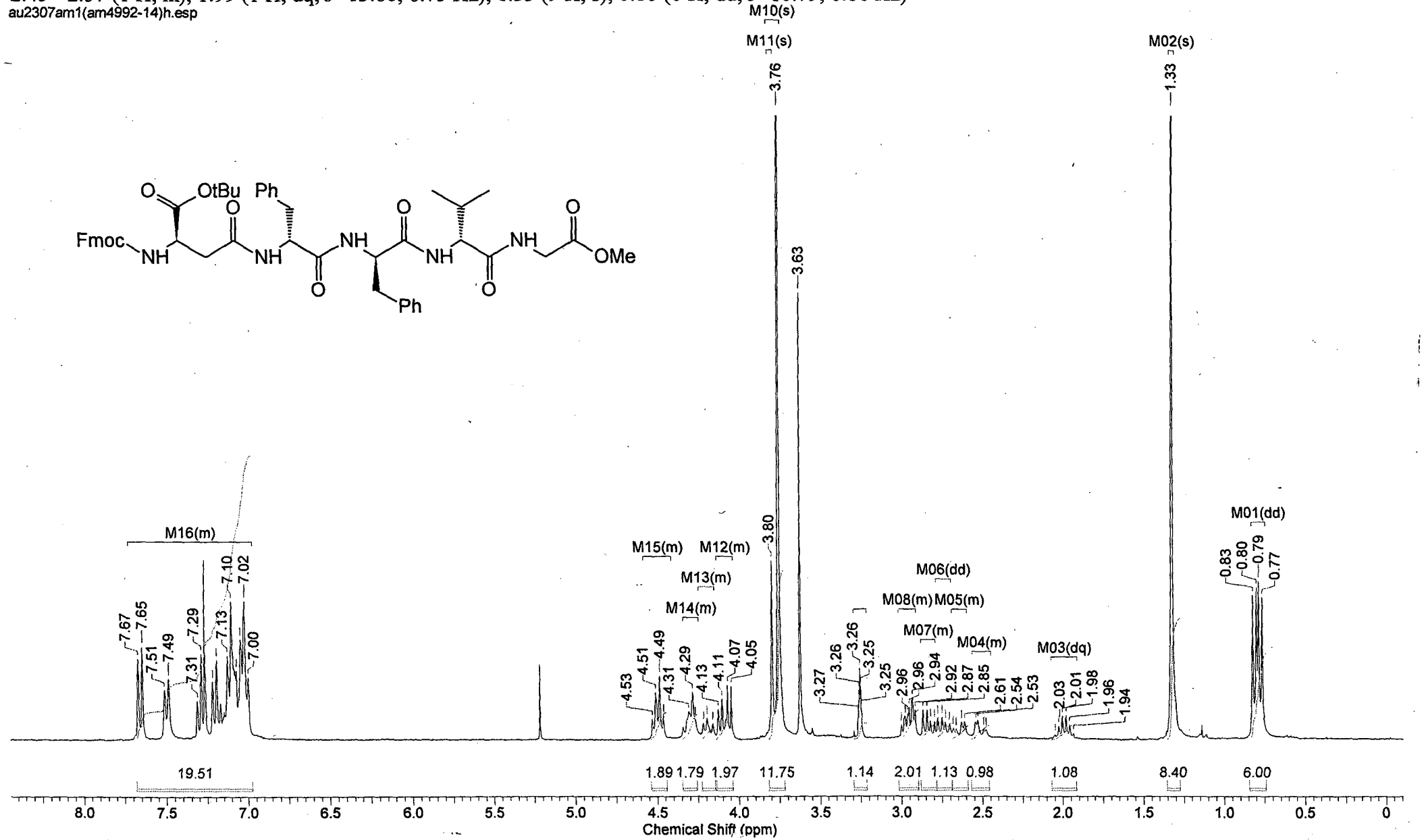
AM4992/9


AU1507A ... -9).ESP
136.24
136.04
129.18
129.10
128.44
126.81
80.23
58.56
55.52
54.94
54.48
54.38
52.10
42.96
40.80
37.99
37.65
30.16
28.01
12.38
Ph
Boc
NH
O
NH
NH
O
O
NH
O
OMe
Ph
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

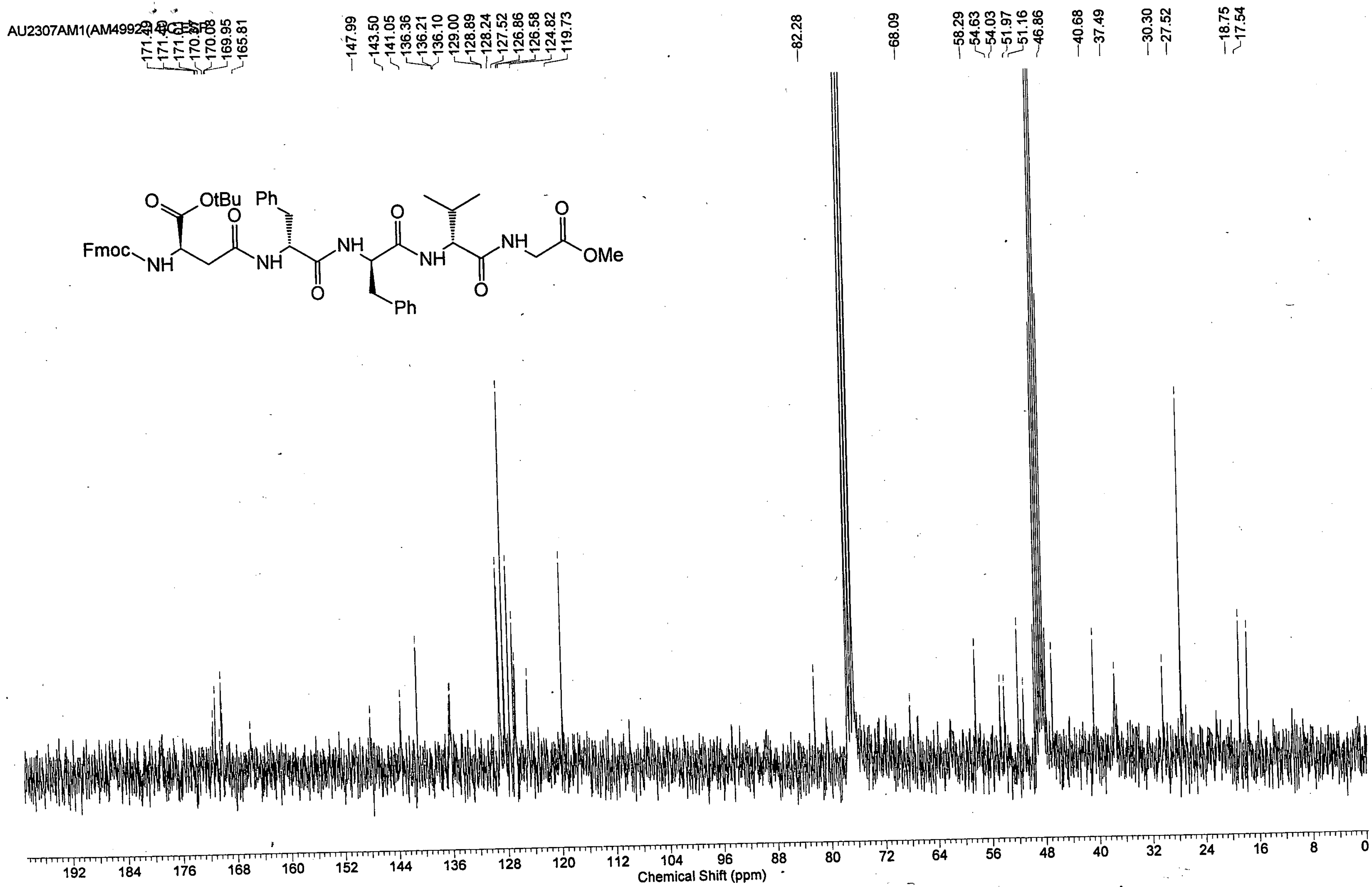
AM4992/14

1H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 6.97 - 7.73 (23 H, m), 4.41 - 4.58 (2 H, m), 4.25 - 4.34 (1 H, m), 4.15 - 4.25 (1 H, m), 4.03 - 4.14 (2 H, m), 3.80 (2 H, s), 3.76 (11 H, s), 3.26 (1 H, dt, *J*=3.25, 1.58 Hz), 2.91 - 3.02 (2 H, m), 2.79 - 2.88 (1 H, m), 2.74 (1 H, dd, *J*=13.86, 7.82 Hz), 2.60 - 2.69 (1 H, m), 2.45 - 2.57 (1 H, m), 1.99 (1 H, dq, *J*=13.60, 6.75 Hz), 1.33 (9 H, s), 0.80 (6 H, dd, *J*=10.79, 6.86 Hz)

au2307am1(am4992-14)h.esp

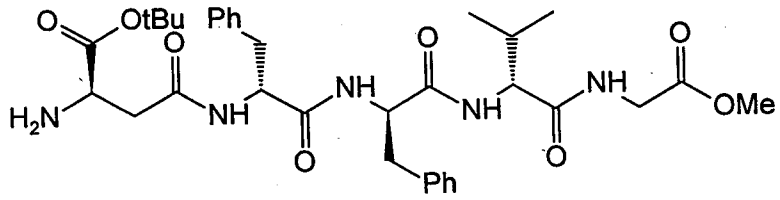


AM4992/14

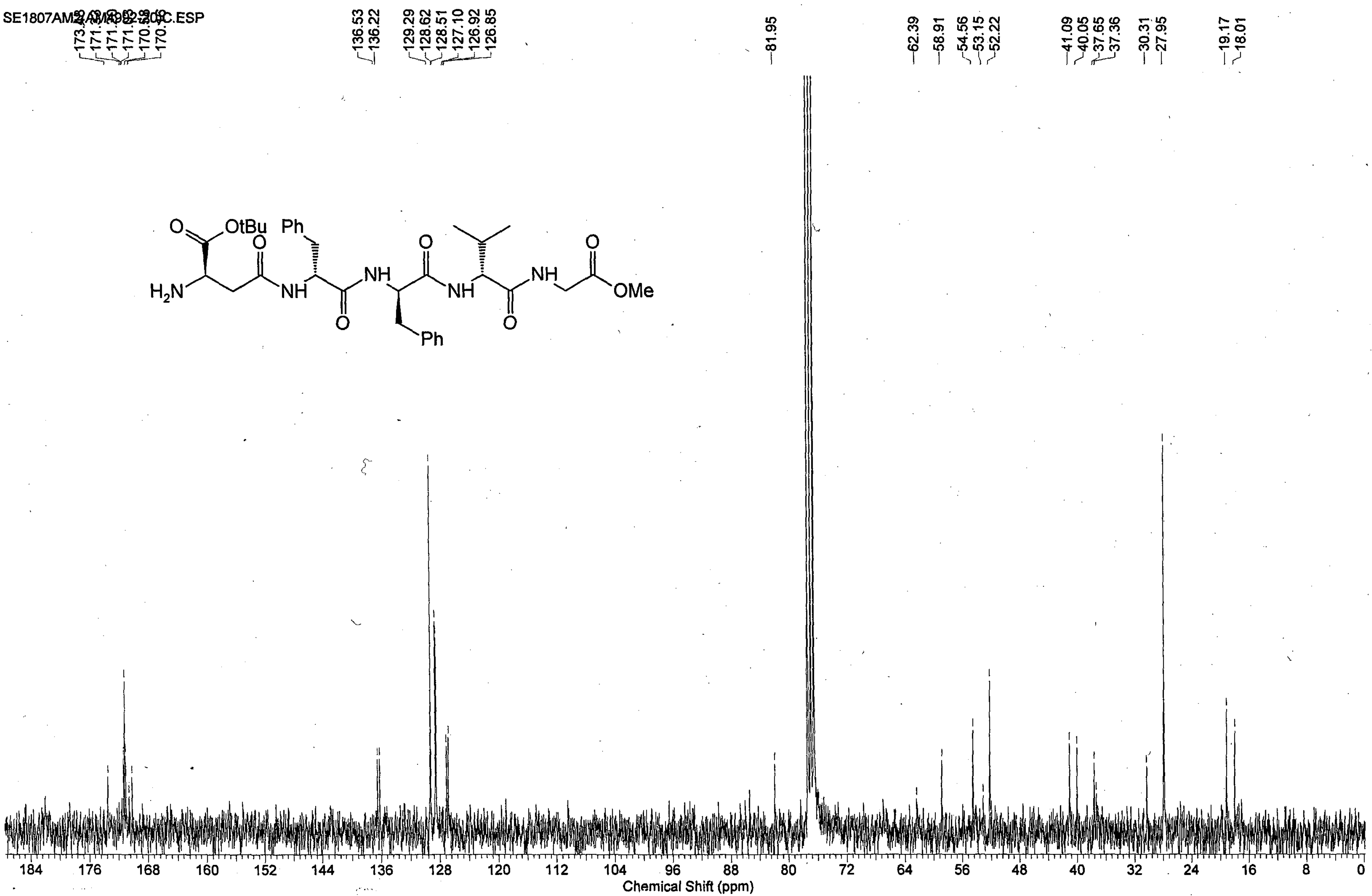

AU2307AM1(AM4992
170.37
170.08
169.95
165.81
147.99
143.50
141.05
136.36
136.21
136.10
129.00
128.89
128.24
127.52
126.86
126.58
124.82
119.73
82.28
68.09
58.29
54.63
54.03
51.97
51.16
46.86
40.68
37.49
30.30
27.52
18.75
17.54
Fmoc
NH
O
OtBu
O
NH
Ph
O
NH
Ph
O
NH
O
NH
O
OMe
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

AM4992/20

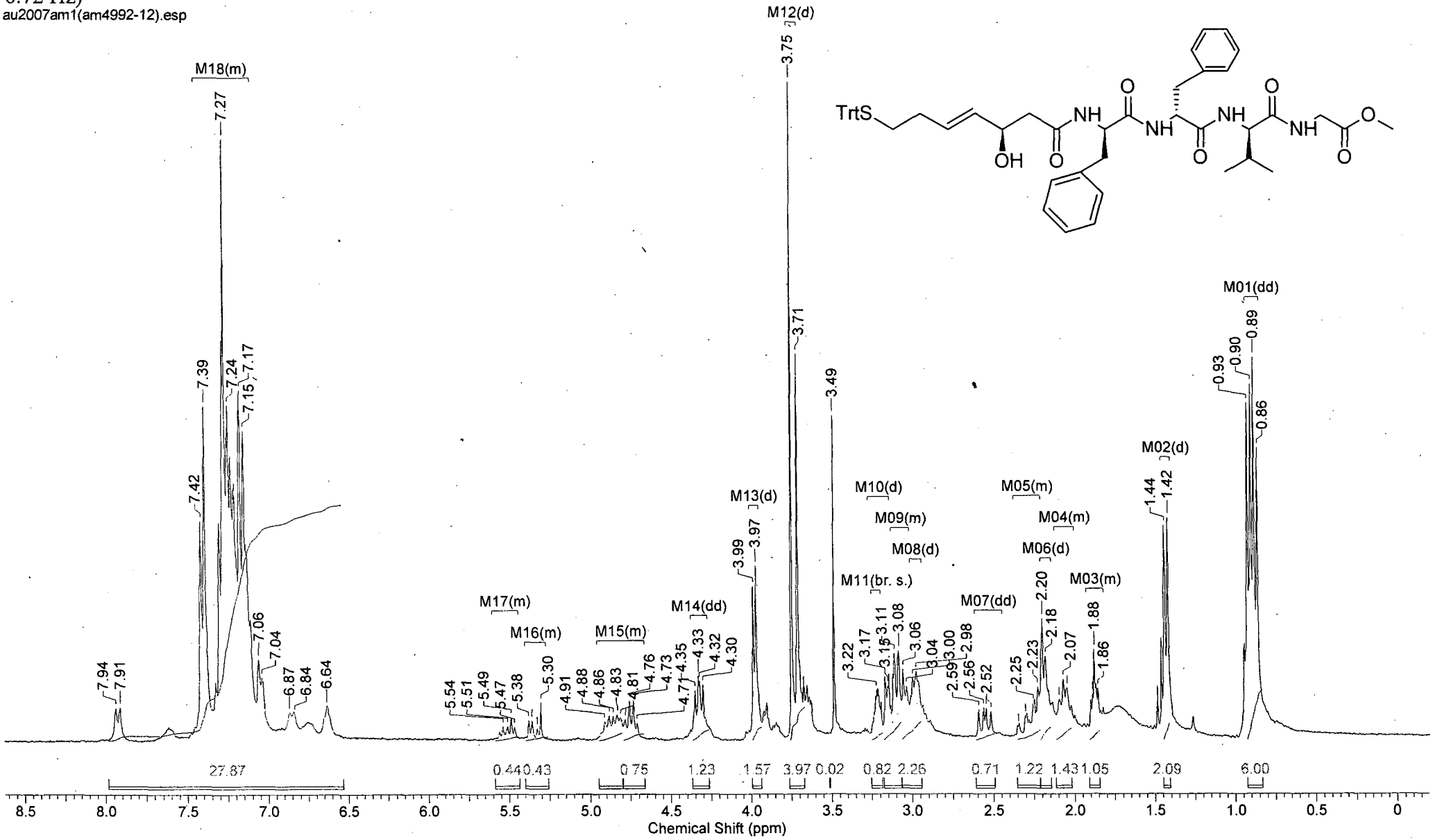
se1807am2(am4992-20)c.esp



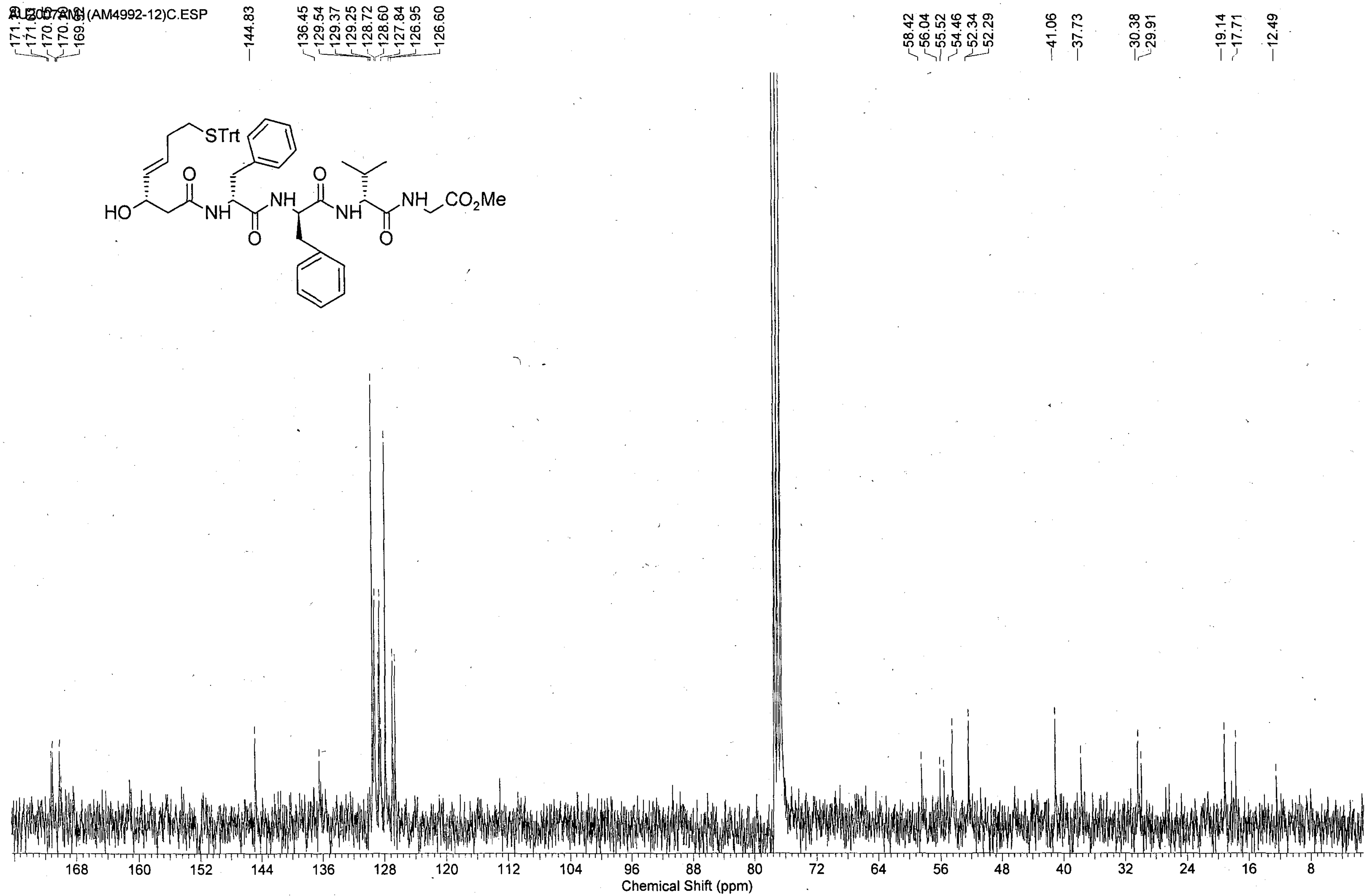
AM4992/20


SE1807AM2/AM4992-20C.ESP
136.53
136.22
129.29
128.62
128.51
127.10
126.92
126.85
81.95
62.39
58.91
54.56
53.15
52.22
41.09
40.05
37.65
37.36
30.31
27.95
19.17
18.01
OtBu
Ph
O
H2N
NH
NH
NH
NH
OMe
Ph
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

1H NMR (300 MHz, CHLOROFORM-*d*) δ ppm 7.09 - 7.44 (23 H, m), 5.44 - 5.60 (1 H, m), 5.27 - 5.40 (1 H, m), 4.67 - 4.96 (2 H, m), 4.33 (1 H, dd, *J*=8.42, 6.22 Hz), 3.98 (2 H, d, *J*=5.49 Hz), 3.73 (4 H, d), 3.22 (1 H, br. s.), 3.16 (1 H, d, *J*=5.12 Hz), 3.01 - 3.13 (2 H, m), 2.99 (1 H, d, *J*=6.40 Hz), 2.55 (1 H, dd, *J*=13.95, 9.38 Hz), 2.21 - 2.37 (1 H, m), 2.19 (2 H, d, *J*=5.67 Hz), 2.00 - 2.12 (1 H, m), 1.82 - 1.92 (1 H, m), 1.43 (2 H, d, *J*=6.59 Hz), 0.89 (6 H, dd, *J*=12.12, 6.72 Hz)



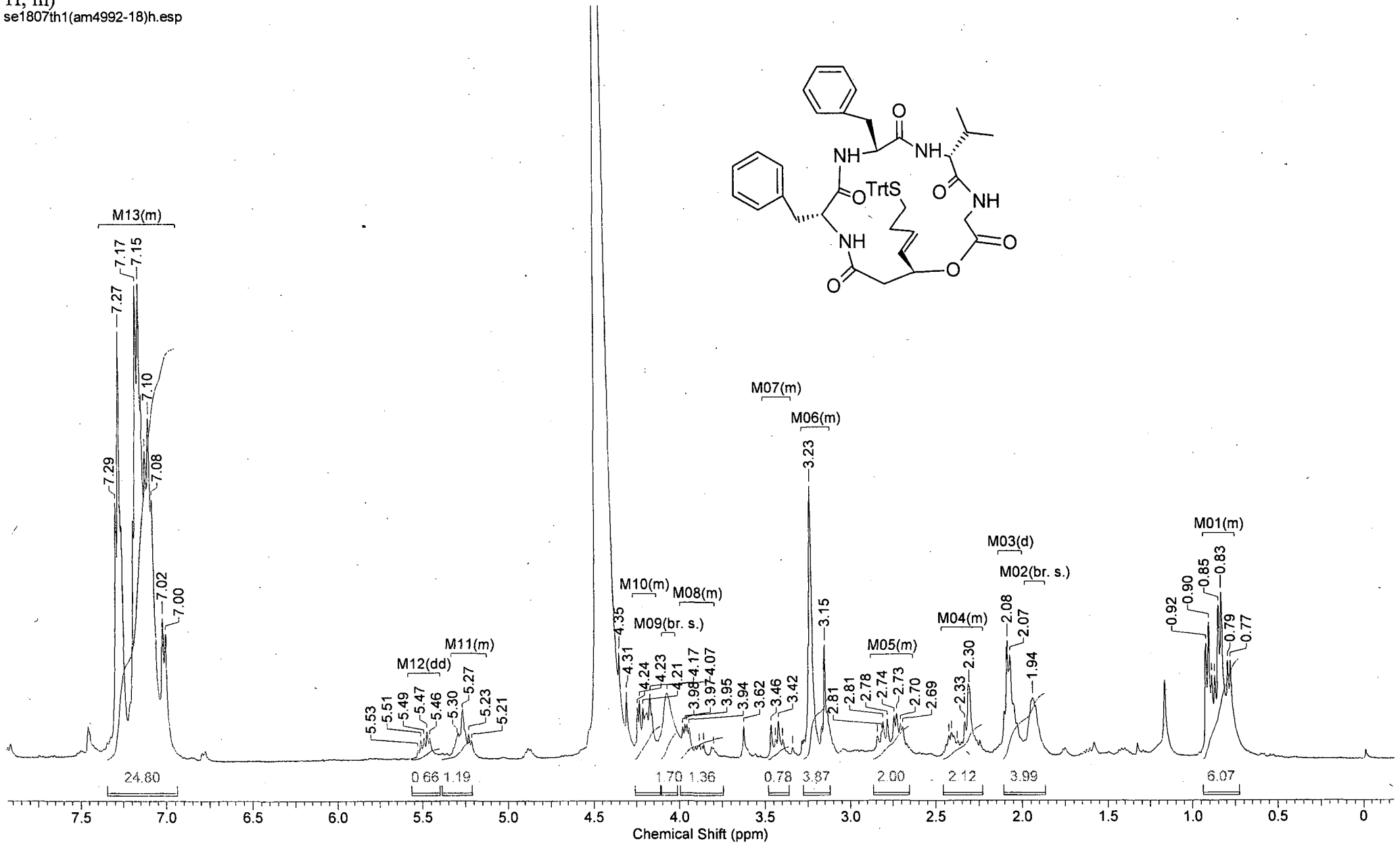
AM4992/12



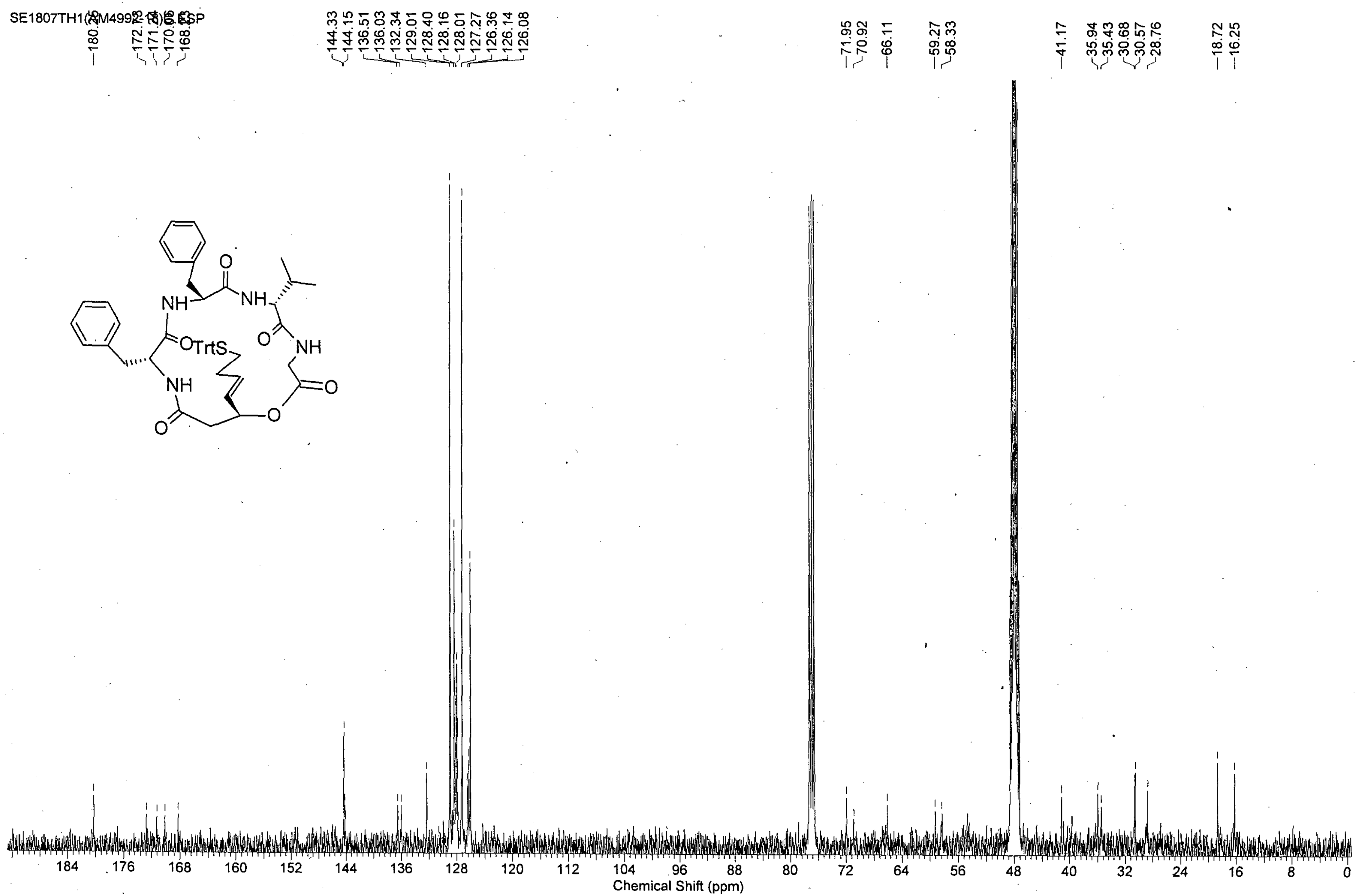
(AM4992-12)C.ESP
171.
171.
170.
170.
169.
144.83
136.45
129.54
129.37
129.25
128.72
128.60
127.84
126.95
126.60
58.42
56.04
55.52
54.46
52.34
52.29
41.06
37.73
30.38
29.91
19.14
17.71
12.49
STrt
HO
NH
NH
NH
NH
O
CO_2Me
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
Chemical Shift (ppm)

1H NMR (400 MHz, MeOH) δ ppm 6.92 - 7.37 (25 H, m), 5.48 (1 H, dd, *J*=14.12, 7.22 Hz), 5.12 - 5.33 (1 H, m), 4.13 - 4.27 (2 H, m), 4.07 (1 H, br. s.), 3.79 - 3.99 (1 H, m), 3.34 - 3.50 (1 H, m), 3.11 - 3.28 (4 H, m), 2.63 - 2.88 (2 H, m), 2.23 - 2.47 (2 H, m), 2.07 (2 H, d, *J*=6.90 Hz), 1.94 (2 H, br. s.), 0.75 - 0.93 (6 H, m)

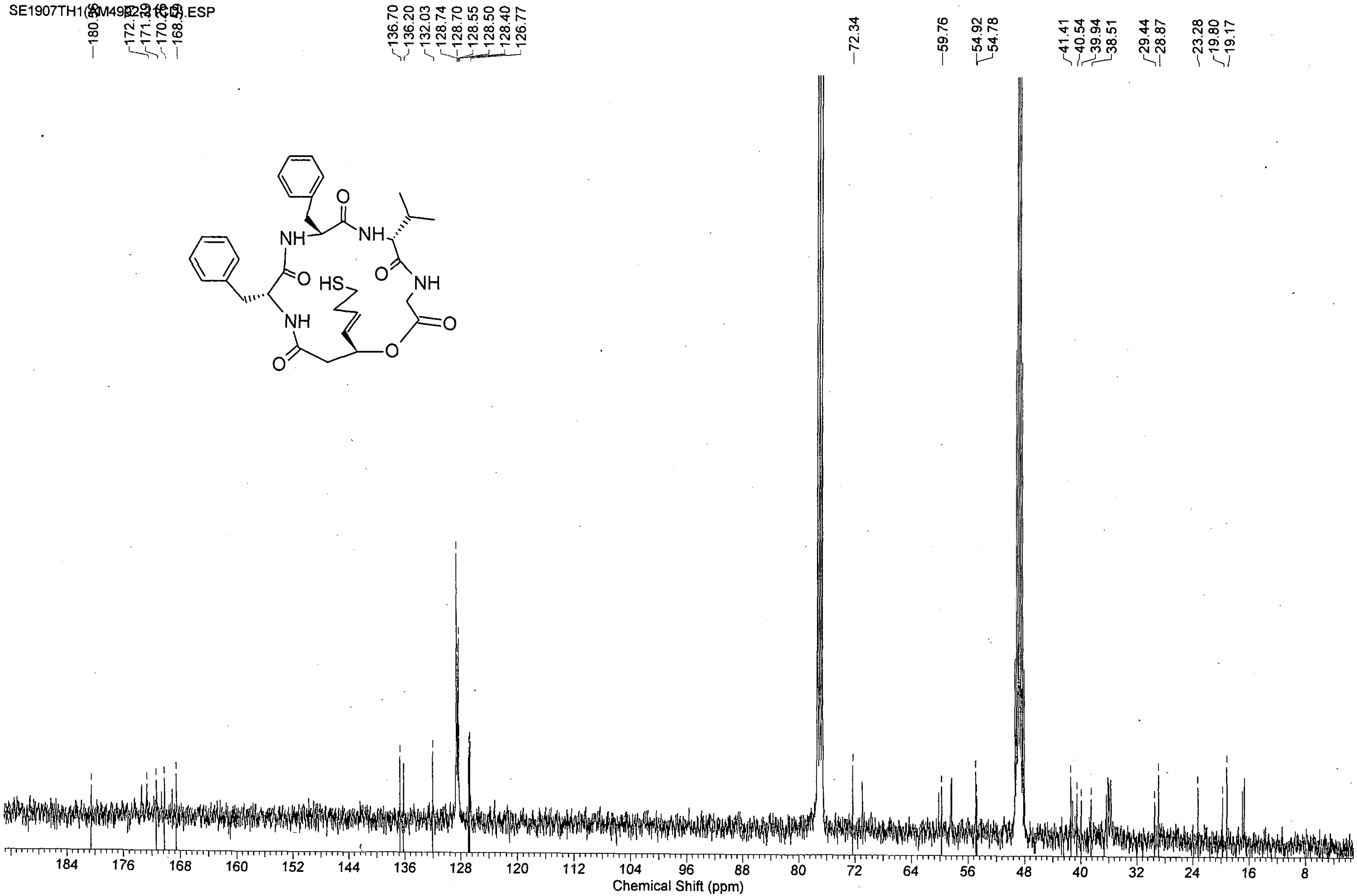
se1807th1(am4992-18)h.esp



AM4992/18


SE1807TH1(AM4992-18)C.ESP
180.
172.
171.
170.
168.
144.33
144.15
136.51
136.03
132.34
129.01
128.40
128.16
128.01
127.27
126.36
126.14
126.08
71.95
70.92
66.11
59.27
58.33
41.17
35.94
35.43
30.68
30.57
28.76
18.72
16.25
NH
NH
O
O
NH
OTrtS
NH
O
O
O
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

AM4992/21


SE1907TH1(AM4992-21)(CD).ESP
180.
172.
171.
170.
168.
136.70
136.20
132.03
128.74
128.70
128.55
128.50
128.40
126.77
72.34
59.76
54.92
54.78
41.41
40.54
39.94
38.51
29.44
28.87
23.28
19.80
19.17
HS
NH
O
184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8
Chemical Shift (ppm)

AM4992/21

1H NMR (400 MHz, MeOH) δ ppm 6.95 - 7.21 (10 H, m), 5.58 (1 H, t, *J*=6.96 Hz), 5.27 - 5.44 (1 H, m), 4.39 (1 H, d, *J*=17.19 Hz), 4.27 (1 H, d, *J*=5.02 Hz), 4.16 (2 H, ddd, *J*=15.43, 10.10, 5.71 Hz), 3.95 (1 H, dd, *J*=10.16, 5.27 Hz), 3.89 (1 H, dd, *J*=10.10, 5.71 Hz), 3.42 (2 H, dd, *J*=17.13, 7.59 Hz), 3.27 - 3.30 (1 H, m), 3.25 (2 H, d, *J*=1.51 Hz), 3.14 (1 H, dt, *J*=13.93, 5.52 Hz), 2.77 - 2.83 (1 H, m), 2.69 - 2.76 (1 H, m), 2.44 (2 H, t, *J*=6.96 Hz), 2.39 (1 H, d, *J*=7.65 Hz), 2.33 (1 H, d, *J*=7.15 Hz), 2.28 (1 H, d, *J*=6.53 Hz), 2.19 - 2.25 (2 H, m), 1.87 - 1.96 (1 H, m), 1.62 - 1.78 (1 H, m), 0.88 (6 H, ddd, *J*=15.90, 8.88, 6.96 Hz)

se2007th2(am4992-21h)_001000fid