약학박사학위논문

Conformationally-Inspired Total Syntheses of Ohmyungsamycins A and B and Structural Revision of Ohmyungsamycin B

천연물의 입체구조에 기반한 ohmyungsamycin A 와 B 의 전합성 및 ohmyungsamycin B 의 구조 교정 연구

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Abstract

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Cyclic peptide natural products have received special attention in drug discovery because they exhibit not only high serum stability and target specificity but also a variety of biological activities against 'undruggable' target which cannot be controlled by the conventional small molecules such as protein-protein interactions (PPIs). Ohmyungsamycins A and B, which are novel cyclodepsipeptide natural products, show potent antiproliferative and anti-tuberculosis activities with the unprecedent mechanism. Focusing on their unique structure and the possibility of development as an anti-tuberculosis drug, we accomplished the first total synthesis of ohmyungsamycins. The proposed structure of ohmyungsamycin B was revised based on its synthesis. The cyclic core of the ohmyungsamycins was shown to be responsible for the excellent anti-tuberculosis activity, and ohmyungsamycin variants with truncated chains were evaluated for their biological activity.

Macrocyclization of ohmyungsamycins was predicted to be hampered due to the congestion with bulky amino acids and *N*-methyl amides in the 31-membered ring. To overcome these hurdles, we designed the synthetic route to induce the cyclization precursor into bent conformation as much as possible by exploiting the information about three dimensional structure of the natural products. Indeed, we observed the β-turn in the turn inducing sequence via intensive NMR studies in the solution state, which could support our synthetic strategy.

Keywords: Cyclodepsipeptide, Natural Products, Ohmyngsamycins, Total Synthesis, Cyclization, Structural Revision, Peptide Conformation, Hydrogen-Deuterium Exchange, Anti-Tuberculosis, NMR spectroscopy

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Abbreviations

Ac: Acetyl

aq : Aqueous

Bn: Benzyl

Boc: *t*-Butyloxycarbonyl

Cbz: Carboxybenzyl

COSY: Correlation spectroscopy

DABCO: 1,4-Diazabicyclo[2.2.2]octane

DEPBT: 3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one

DIBAL: Diisobutylaluminum hydride

DIPEA: *N,N'*-Diisopropylethylamine

DMAP: *N,N*-dimethylaminopyridine

DMF: *N,N*-dimethylformamide

DMSO: Dimethylsulfoxide

EtOAc: Ethyl acetate

EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EWG : Electron withdrawing group

Fmoc: Fluorenylmethyloxycarbonyl

Hag: Homoallylglycine

HATU: 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate

HMBC: Heteronuclear multiple-bond correlation spectroscopy

HOAt: 1-Hydroxyazabenzotriazole

HPLC: High-Performance Liquid Chromatography

HR-MS: High resolution mass

HSQC: Heteronuclear single-quantum correlation spectroscopy

Hz : Herts

IR : Infrared

LC/MS: Liquid chromatography-mass spectrometry

NMO: *N*-methylmorpholine *N*-oxide

NMR: Nuclear magnetic resonance

NOESY: Nuclear Overhauser effect spectroscopy

Oxyma: Ethyl (hydroxyimino)cyanoacetate

PPIs: Protein-Protein interactions

PyBOP: (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

ROESY: Rotating frame nuclear Overhauser effect spectroscopy

TBAF: Tetra-*n*-butylammonium fluoride

TB: Tuberculosis

TBS: *tert*-Butyldimethylsilyl

TEA: Triethylamine

TFA: Trifluoroacetic acid

THF: Tetrahydrofuran

TLC: Thin layer chromatography

TMS: Trimethylsilyl

TMSE: 2-(trimethylsilyl)ethyl

TOCSY: Total correlation spectroscopy

TOF: Time of flight

TS: Transition state

I. Introduction

1. Cyclic Peptides

Macrocyclic natural products have received special attention in the drug discovery.[1,2] Owing to their larger size and structural complexity compared with the classical small molecule drugs within the Rule of 5 boundary, macrocyclic compounds exhibit not only high potency and specificity but also regulatory ability to the traditionally undruggable targets such as protein-protein interactions (PPIs; Figure 1).[3–6] Nonribosomal cyclic peptides are major class of macrocyclic compounds possessing a variety of biological activities, mainly originated from marine resource.[7] A number of naturally occurring cyclic peptides are decorated with D-amino acids, *N*-methylated amino acids and structurally unique building blocks, which improves their ADME properties including serum stability and cell permeability.[8] However, as structural complexity of cyclic peptides increase, synthetic

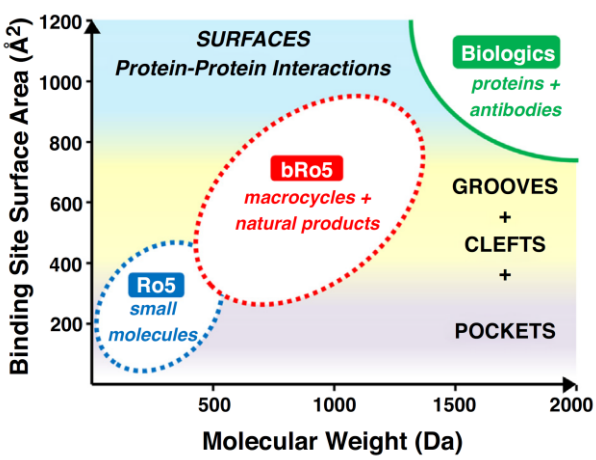


Figure 1. The pharmaceutical opportunity for macrocyclic drugs to target larger biological binding sites not accessible to conventional small molecules[5]

chemists must overcome a number of hurdles such as preparation of non-proteinogenic amino acid, *N*-methyl amide formation and final macrocyclization. Although some leading achievements of complex cyclic peptide total synthesis are presented, synthetic strategies of unconquered cyclic peptides are still compoundwise and should be carefully planned. Moreover, since the physicochemical properties of the cyclic peptides are intrinsically poor in terms of the Rule of 5 parameter, a structural modification of bioactive cyclic peptides to improve oral bioavailability is an actively ongoing research area.[9–16]

1-1. Strategy for Orally Bioavailable Cyclic Peptide

An orally bioavailable drug must overcome multiple physiological barriers to reach systemic circulation, including gastrointestinal (GI) membranes, proteolysis, and first-pass hepatic metabolism (Figure 2). These barriers are particularly daunting for the oral delivery of peptides due to their poor passive membrane permeability and their susceptibility to

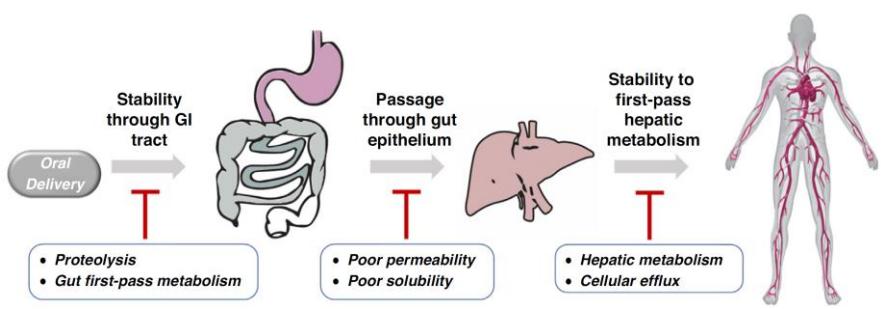


Figure 2. Barriers to systemic oral bioavailability of peptides[5]

proteolysis by peptidases in the GI tract. For this reason, peptides are traditionally have been considered as poor candidates for oral administration.

Once orally administered, the drug must cross the enterocyte layer to reach blood vessels. Penetration from one side of the cell membrane to the other side is referred to as cell permeability, and involves two possible pathways: the paracellular pathway, in which the drug passes through tight junctions between enterocytes; and the transcellular pathway, in which the drug passes through enterocytes (Figure 3). Transcellular transport involves either active transport, which is mediated by transporters embedded in the cell membrane, or passive transport, in which the drug diffuses across the lipid membrane.

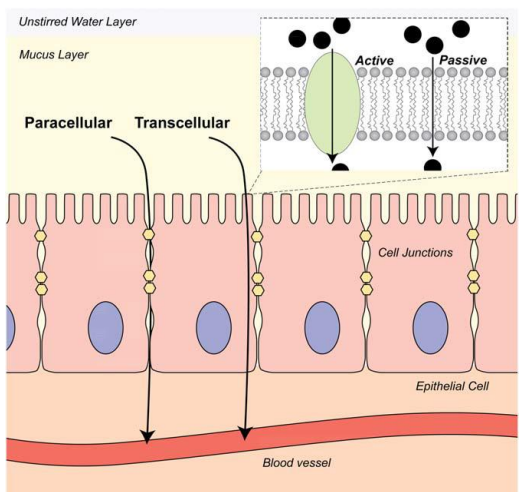


Figure 3. Cell permeability and transport pathways[16]

1-1-1. Hydrogen bond

As peptides transition across a lipid bilayer, they move from an aqueous environment with a high dielectric constant to the bilayer core which has a low dielectric constant (Figure 4). In the aqueous environment, the hydrogen bond acceptors and donors of the peptide participate in hydrogen bonds with solvent water molecules, unless they are shielded from the solvent because of the local conformation or are involved in internal hydrogen bonds. These solvent interactions are disrupted upon entering the bilayer, leading to an energetic penalty that disfavors permeability.[17] Based on this premise, any peptide modification that reduces solvent interactions[10,14,15,18,19] should enhance peptide permeability (Figure 4A).

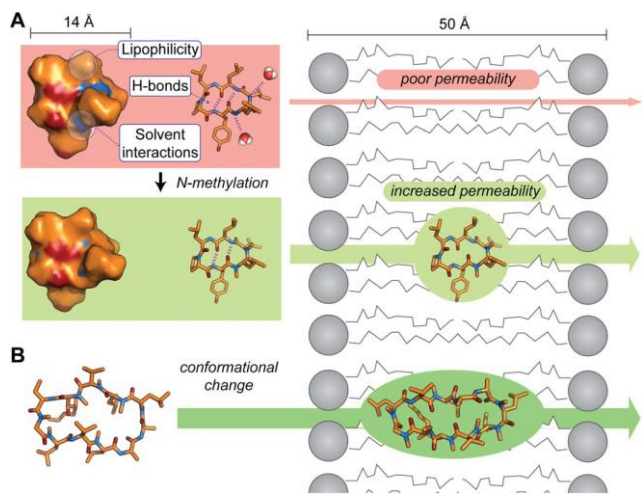


Figure 4. Strategies for improved oral bioavailability of cyclic peptides[16]

1-1-2. Lipophilicity

Cyclic peptides require a degree of lipophilicity to cross a membrane – they can be neither too lipophobic nor too lipophilic. Peptides that are too lipophobic do not readily insert into the membrane, and therefore cannot cross to the other side, whereas peptides that are too lipophilic may insert too readily into the membrane, overly favoring the membrane bound state and resulting in poor overall transport.

1-1-3. Flexibility

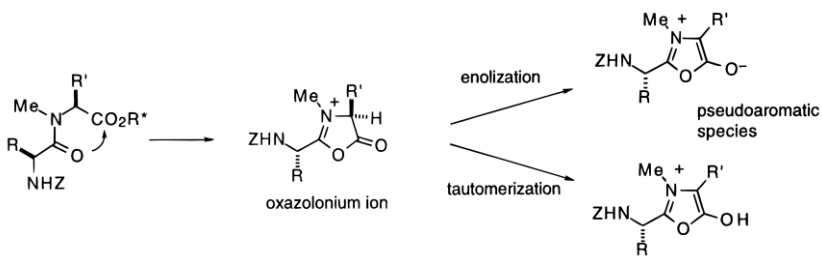
Solvent dependent conformational polymorphism of some membrane permeable peptides, such as cyclosporine A, led to the conformational hypothesis, which speculates that peptides that can shape-shift[20,21] can negotiate into alternative energy pathways that are more favorable for permeability (Figure 4B).[22,23] In chloroform, a solvent which mimics the environment of the lipid bilayer core,[12] cyclosporine A enters a conformational space that is different to that in an aqueous environment but one that is more compatible with its current environment by hiding most of its hydrogen bond donors and acceptors in internal hydrogen bonds.[24]

2. Hurdles in Cyclic Peptide Synthesis

2-1. *N*-Methyl amide formation[25]

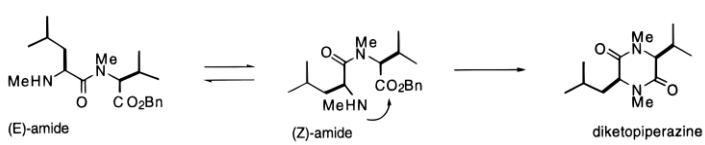
Efficient amide bond formation with *N*-methyl amino acids can be challenging, because racemization and 2,5-diketopiperazine (DKP) formation are common side reactions. Due to slow coupling rates, oxazolone or oxazolonium ion formation[26] is more prevalent when

acylating *N*-methylamines than primary amines. Abstraction of α-proton in the oxazolone spices leads to generate stable pseudoaromatic structure, and subsequent oxazolone ring opening followed by coupling with *N*-methylamine afford epimerized polypeptide (Scheme 1).



Scheme 1. Epimerization mechanism through oxazolone formation[25]

For dipeptide esters containing *N*-methyl or prolyl-type amide linkages, chain extension in the *N*-terminal direction can be hampered by spontaneous 2,5-diketopiperazine formation due to increased population of *cis*-amide (Scheme 2).[27–29]

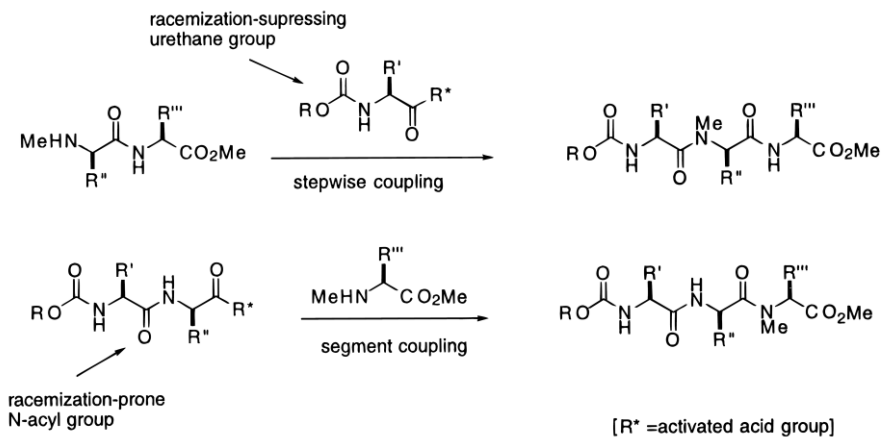


Scheme 2. Diketopiperazine formation[25]

2-2. Segment coupling[25]

To maximize convergence in complex polypeptide synthesis, segment coupling, which is coupling between *N*-terminal amine of a peptide and activated *C*-terminal of another peptide,

is inevitable in most case. Such segment couplings are much more prone to epimerize the product comparing with stepwise coupling, via oxazolones that form readily during the activation of *N*-acyl amino acids. Epimerization can be reduced in some cases through the judicious choice of peptide bond disconnections or appropriate coupling condition.



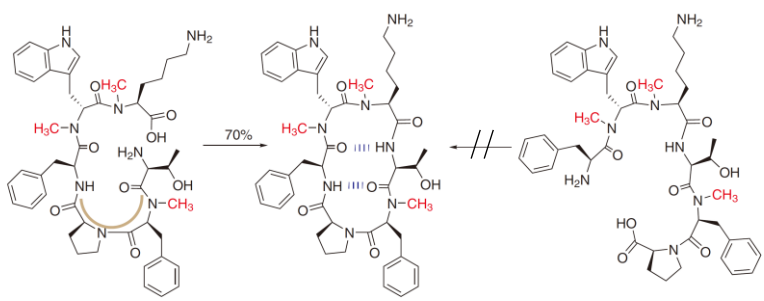
Scheme 3. Segment coupling[25]

2-3. Macrocyclization[25,30]

Macrocyclization reactions have always been key step in the cyclic peptide syntheses. The inefficiency in bringing *C*- and *N*-terminal together often leads to prolonged reaction times and side-reactions, such as cyclodimerization and epimerization. In addition, compounds that have an unfavorable conformation often cannot be cyclized at all, whereas even large, pre-organized linear molecules cyclize in high yields. Therefore, the ring disconnection for the macrocyclization must be chosen carefully.

2-3-1. Conformation-directed macrocyclization[31]

The success of macrocyclization depends on the ability of a linear precursor to conformationally pre-organize its both ends closer. Peptide secondary structures are induced by a multitude of directional changes and folding patterns stabilized by certain elements inducing hydrogen bonds across the chains, the so-called turn units.[32–36] Intramolecular hydrogen bonding can lead to one conformation being favored over another, thereby enabling the two cyclization sites to achieve productive proximity. Generally for target molecules displaying hydrogen-bonding interactions, the incorporation of a turn unit into a middle of linear precursor is the obvious choice in order to obtain a conformationally pre-organized substrate (Scheme 4).



Scheme 4. Crucial role of turn inducing unit for macrocyclization[37]

3. Ohmyungsamycins A, B and Ecumicin

In 2012, Oh and co-workers discovered novel cyclodepsipeptides ohmyungsamycins A (**1**) and B (**2**) from *Streptomyces* genus strain collected from Jeju island, South Korea.[38] Structurally, both natural products share the same 31-membered macrocyclic core consists of ten L-amino acids including two non-proteinogenic (4MeO-L-Trp[4], L-βHyPhe[2]) and four

N-methylated amino acids. In addition, the L-Val[11]-L-Val[12] dipeptide side chain is appended to the common macrocycle, and only terminal *N*-methylation patterns distinguish the each

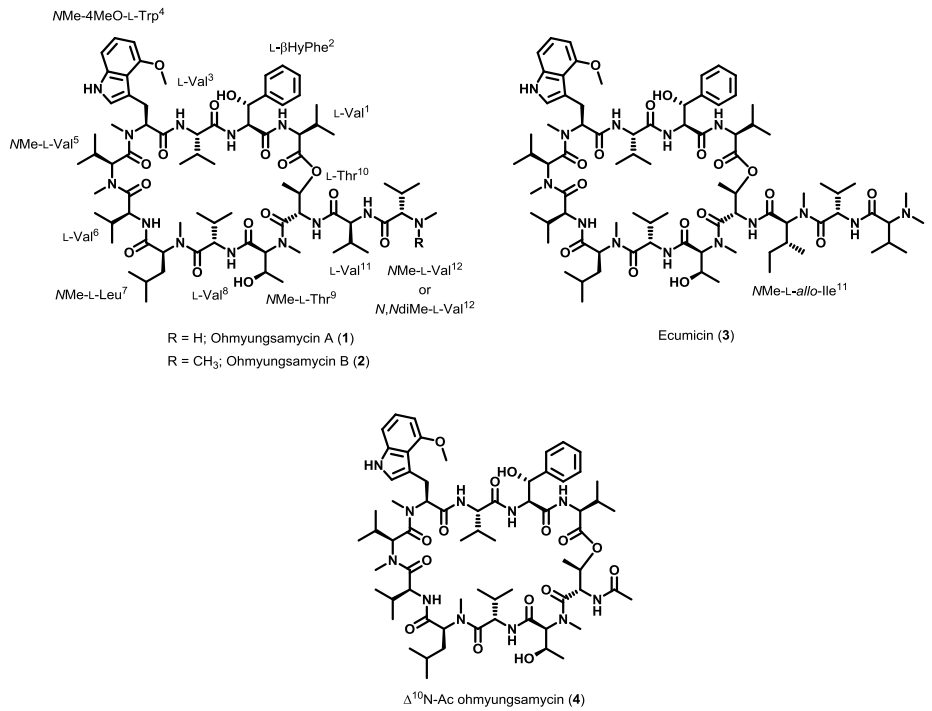


Figure 5. Chemical structures of ohmyungsamycin A, B, ecumicin and side chain-truncated analogue

natural products (Figure 3). Primary screening of the biological activities of the ohmyungsamycins revealed potent cytotoxicity against various cancer cell lines and antibacterial activities with a narrow spectrum.[38] Two years later, ecumicin (**3**) was reported by Pauli and co-workers as an *anti*-tuberculosis (TB) lead compound via high-throughput

screening of 65,000 actinomycete extracts.[39] This tridecacyclicpeptide is a congener of ohmyungsamycins which shares the same cyclic core, and one non-ribosomal amino acid unit (*N*Me-L-*allo*-Ile[11]) is inserted into the side chain (Figure 5). The potent *anti*-TB activity against *Mycobacterium tuberculosis* (*M. tb*) including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strain as well as inactive *M. tb* was attributed to inhibition of mycobacterial ClpC1 ATPase complex,[40] which is essential for maintaining protein homeostasis in *M. tb*.[41] Very recently, it was disclosed that ohmyungsamycins also exhibit strong anti-TB activity by promoting host cell autophagy,[42] which is innate immune system targeting intracellular bacteria,[43] via AMP-activated protein kinase pathway activation. Judging from the structural similarity of three natural products, it is presumed that they exhibit anti-TB activity through the same mode of actions; direct killing activity and stimulation of host defenses against *M. tb*. To explore their potential as novel arsenal to combat against drug-resistant TB by the collaboration of dual mechanisms, thus, establishment of synthetic route for this lariat structures is an essential process. In addition, to elucidate the core of the ohmyungsamycins that is responsible for the biological activities, we designed and synthesized a side chain-truncated ohmyungsamycin analogue.

II. Results and Discussion

1. Total Syntheses of Ohmyungsamycins A and B

Peptide macrocyclization strongly depends on the sequence of cyclization precursor. Generally, macrocyclization of peptides affords the desired product with high yield if the cyclization site is not sterically encumbered by *N*-methyl or bulky amino acids.[25,30] In addition, performing cyclization between two terminus with opposite stereochemistry can exhibit beneficial effect.[30,44–46] Unfortunately, the core structure of ohmyungsamycins A and B are highly congested with bulky amino acids: five valine, a leucine, a 4MeO-trytophane

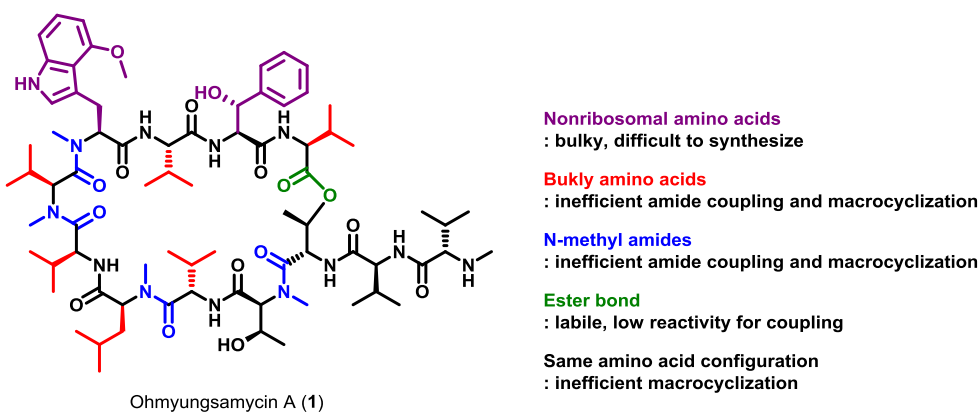


Figure 6. Plausible challenges in ohmyungsamycins synthesis

and a hydroxyl phenylalanine as well as four *N*-methyl amino acids (Figure 6). Structures of ohmyungsamycins with only L-form amino acids may also limit the key macrocyclization.

To overcome this potential challenge in ohmyungsamycins syntheses, we decided to exploit the three-dimensional structural information of ecumicin which was previously reported.

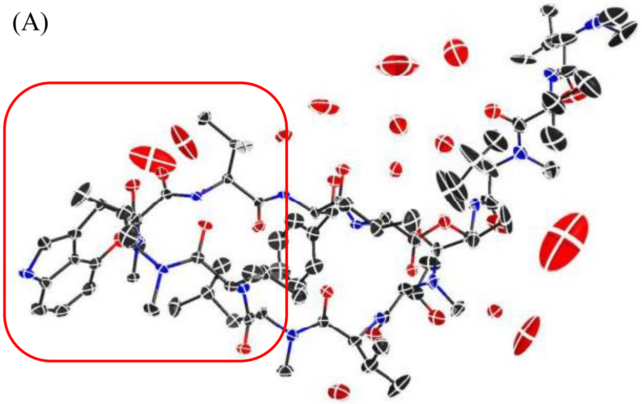
1-1. Solid-state conformation of ecumicin

The X-ray crystallographic structure of ecumicin shows that four interstrand hydrogen bond and two n to π* interaction[47] stabilize twisted β-sheet (Figure 7).[39] Based on the existence of contiguous *N*-methyl amide known as one of the turn inducing unit and synergetic stabilization of the two hydrogen bonds and two n to π* interactions, we considered the four amino acid sequence from Val^3 to Val^6 as a potential turn inducing sequence (Figure 7), which may induce an overall bent conformation in the linear cyclization precursor.

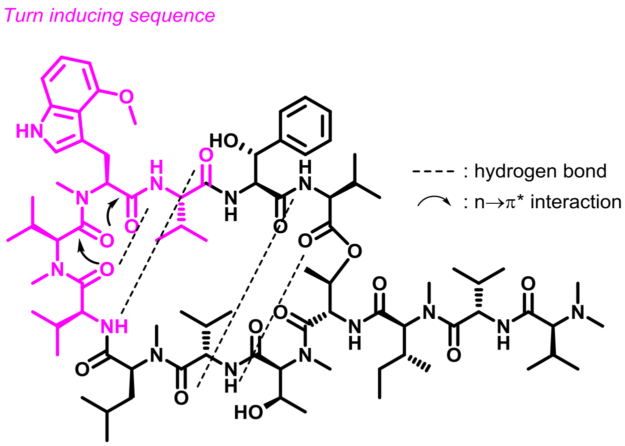
1-2. Retrosynthesis

Our retrosynthetic analysis is outlined in Figure 8. Firstly, we envisioned that terminal dimethylamine moiety of ohmyungsamycin B (**2**) could be converted from monomethylamine of ohmyungsamycin A (**1**) by chemoselective methylation. Next, we paid special attention to the macrocyclization site selection. Since the *B*-factors acquired from ecumicin crystal structure gave us the insight of structural features of which the cyclic core is highly ordered whereas the side chain residue is not. Based on the structural similarity between ecumicin and ohmyungsamycins, we considered that trivial difference of side chains

(A)



(B)



Ecumicin (**3**)

Figure 7. (A) X-ray chrystallographic structure of ecumicin; (B) Conformation-stabilizing factors and position of potential turn inducing sequence in ecumicin.

in ohmyungsamycins could not affect to the three dimensional conformation of the common cyclic core. Therefore, we anticipated that the conformational character of the cyclic core could be utilized in the syntheses of ohmyungsamycins. To exploit plausible bent structure of linear peptide, we decided to locate cyclization site at the opposite side from the potential turn inducing sequence as much as possible. Backbone ester (Site A) and tertiary amide (Site B) were precluded due to the low reactivity in macrocyclization, and secondary amide between Val^8 and Thr^9 (Site C) were also considered as unfavorable site because of steric hindrance caused by inevitable protection at Thr^9 hydroxyl group (described at next paragraph). Thus, we potentially selected secondary amide between Val^1 and $\beta HyPhe^2$ as the most suitable ring-closing site.

To maximize efficiency of the fragments assembly, all of the coupling sites were disconnected to generate primary amines, highlighted as green (Figure 8). Although direct attachment of side chain onto the prepared macrocyclic core would be ideal for further modification, we planned to execute side chain diversification during the southern part synthesis, due to the risk of intramolecular O to N acyl shift.[48,49] We envisioned that backbone ester could be readily linked at the last stage of southern fragment synthesis by using the smallest unit, *N*-protected valine as counterpart with no concerns of epimerization. Protecting group manipulation was carried out in the way of maximizing orthogonality by using *N*-terminal Boc group and *C*-terminal Allyl or 2-(trimethylsilyl)ethyl (TMSE) group. Notably, the third level of protection on the side chain nucleophile of some amino acids component would be necessary only at the Thr^9 hydroxyl group to differentiate esterification site.

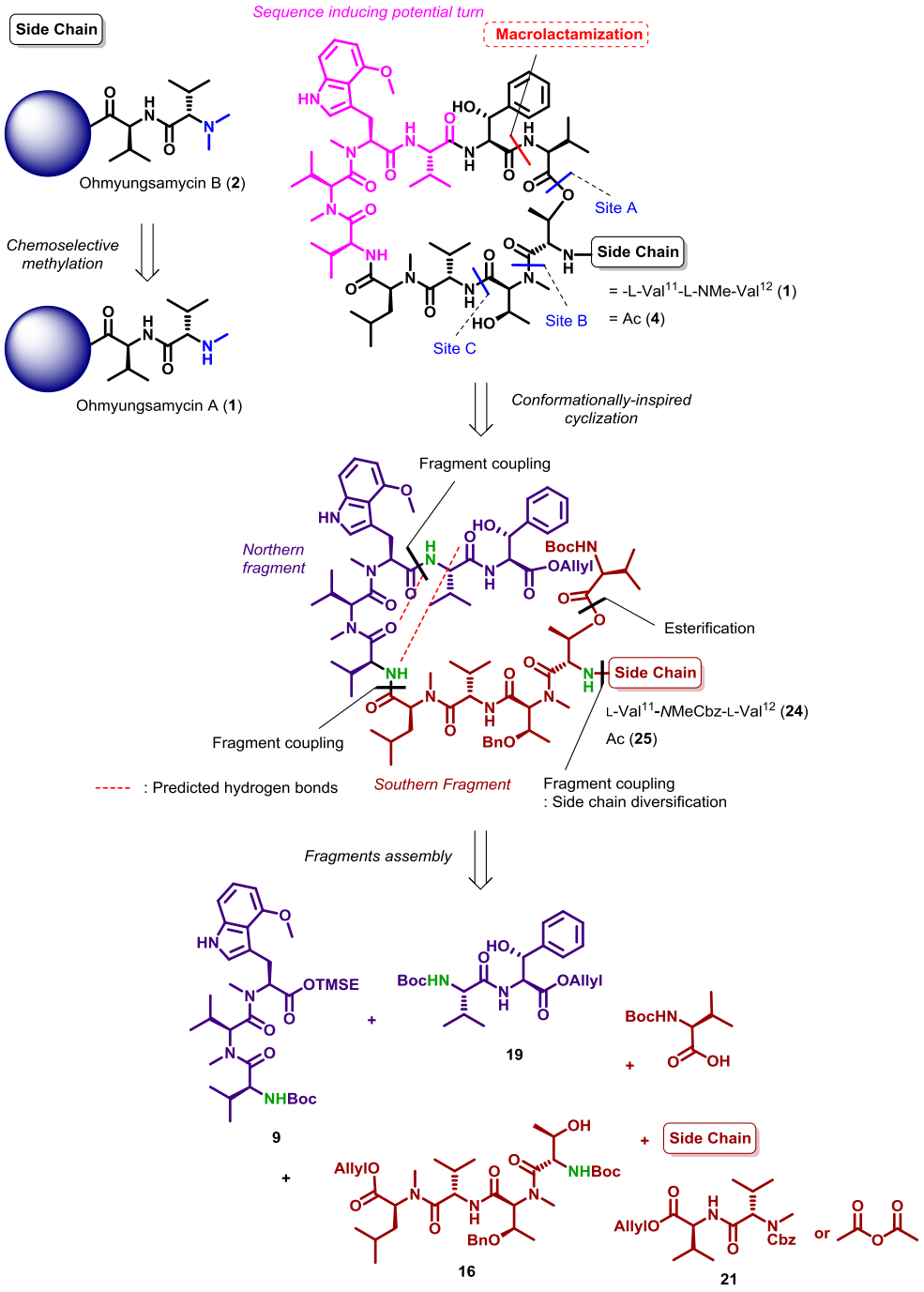
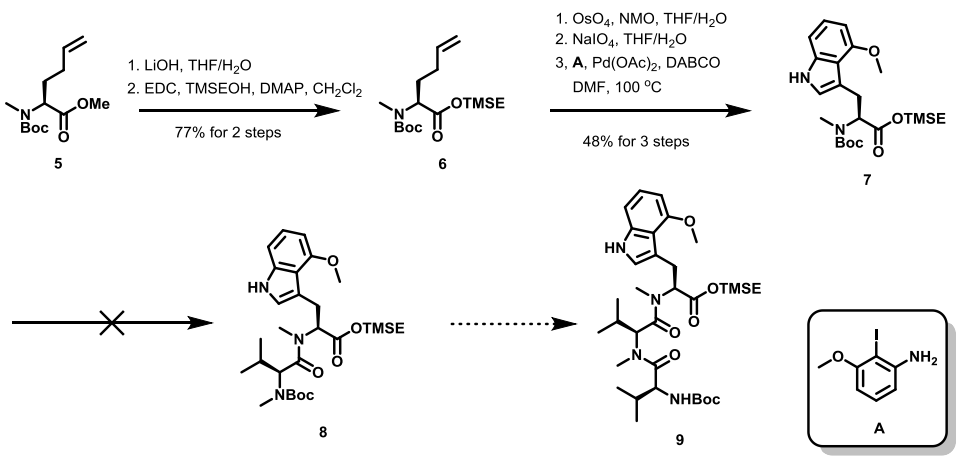


Figure 8. Retrosynthetic analysis of ohmyungsamycins

1-3. Synthesis of tripeptide 9

Our initial attempt of synthesis of 4MeO-L-Trp containing tripeptide **9**, the most sterically encumbered fragment in approach toward the target macrocycle, is depicted in Scheme 5. To address the C4-methoxy substituted indole ring, we decided to use the palladium-catalyzed annulation between *o*-iodoaniline and aldehyde originally developed by Chen group[50] and improved by Zhu[51,52] and Maier group.[53] Our initial reaction was hydrolysis of the known homoallyl glycine methyl ester **5**,[53] followed by re-esterification of resulting acid to the 2-(trimethylsilyl)ethyl (TMSE) ester **6** (77% for 2 steps). Due to the possibility of DKP formation at the second amidation step, we speculated that the TMSE ester, which is more bulky and removable under mild condition, would be more suitable protecting group than methyl ester to slow down the rate of this side reaction. Sequential dihydroxylation and

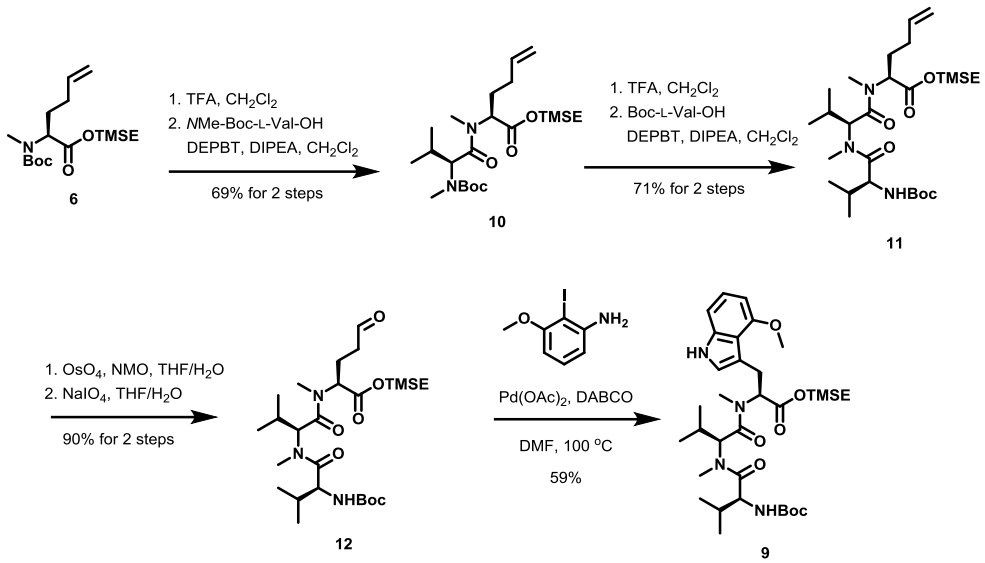


Scheme 5. The first trial of tripeptide 9 synthesis

oxidative cleavage of the terminal olefin of **6** followed by palladium acetate catalyzed annulation of the resulting aldehyde afforded tryptophane derivative **7** possessing the 4MeO-indole moiety in 48% yield. Unfortunately, the 4MeO-Trp intermediate **7** did not undergo amidation with *N*-Me valine, which is most likely due to high steric hindrance of each amido acids.

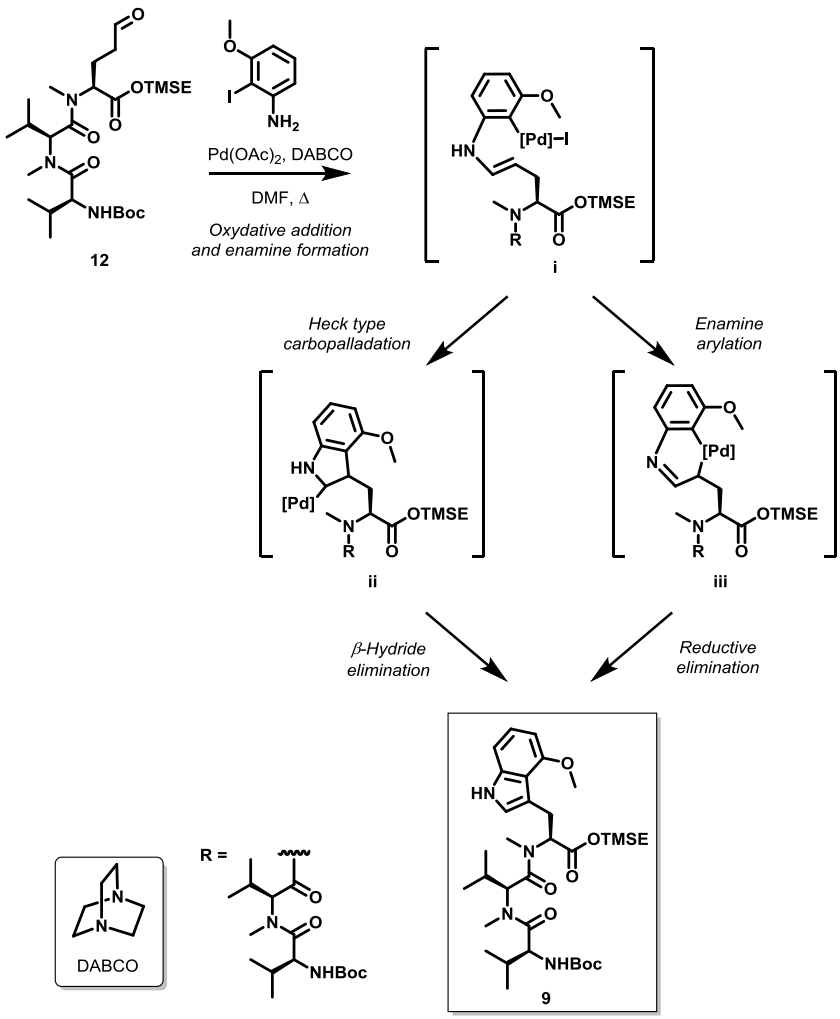
To overcome this problem, we decided to elongate tripeptide chain first then construct the bulky indole ring as shown in scheme 6. Removal of the the *N*-Boc group of **6** under TFA in CH_2Cl_2 followed by condensation with Boc-*N*Me-L-Val-OH (DEPBT, DIPEA and CH_2Cl_2) gave dipeptide **10** successfully in 71% yield for two steps. Second removal of the *N*-Boc group of **10** under TFA in CH_2Cl_2 followed by immediate condensation with Boc-L-Val-OH (DEPBT, DIPEA, CH_2Cl_2) provided tripeptide **11** in 69% yield for two steps with undetectable DKP formation. Dihydroxylation of the terminal olefin followed by oxidative cleavage generated aldehyde **12** (90% for 2 steps), and then, palladium acetate catalyzed annulation of 4MeO indole ring afforded tripeptide **9** in 59% yield. Probably due to the *cis-trans* isomerism by influence of the consecutive *N*-methyl amide linkage as well as the steric hindrance of the bulky amino acids, severe rotameric mixture of **9** were observed by ^{1}H-NMR spectroscopy, which were even observed under the elevated temperature and thin-layer chromatography.

Plausible mechanisms for the palladium-catalyzed indolization are depicted in Scheme 7. The reaction commence with the condensation of the aldehyde and *o*-iodoaniline to afford the corresponding enamine **i**, and the oxidative addition of Pd(0) catalyst simultaneously takes place in the carbon-iodine bond of the enamine. Then, the reaction may procedeed via



Scheme 6. The second trial of tripeptide 9 synthesis

two pathways: (1) intramolecular Heck-type carbopalladation followed by β-hydride elimination to generate the functionalized indole ring; and (2) neucleophilic attack of the enamine to the palladium electrophile providing 6-membered palladacycle **iii** followed by reductive elimination to complete the desired indole ring. To date, although palladium-catalyzed indolization has been applied to the mono-amino acids precursor to afford tryptophane derivatives, we exploited this reaction at the stage of the polypeptide synthesis and successfully achieved to provide indole ring containing polypeptide in sufficient yield without side reaction.

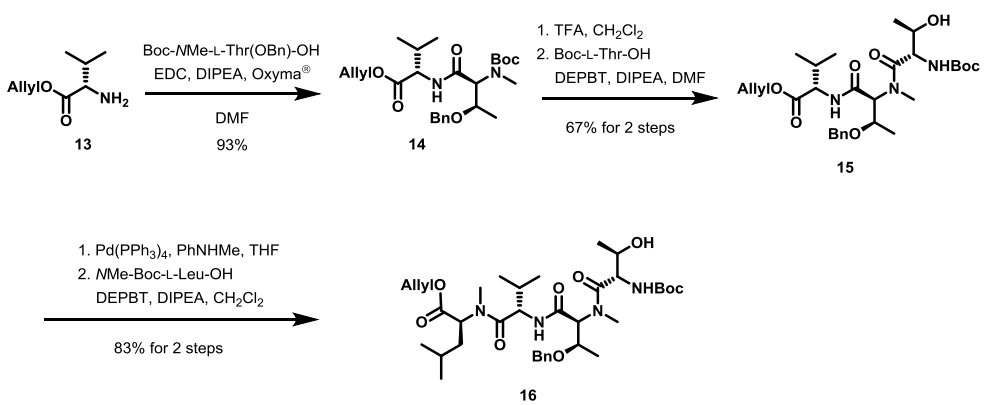


Scheme 7. Plausible mechanisms of palladium-catalyzed indolization

1-4. Synthesis of tetrapeptide 16

With the tripeptide fragment **9** in hand, we turned our attention to the synthesis of tetrapeptide **16** shown as Scheme 8. To avoid the possibility of DKP formation when the elongation was initiated from the C-terminal *N*Me-Leu[7] residue, we planned to connect the

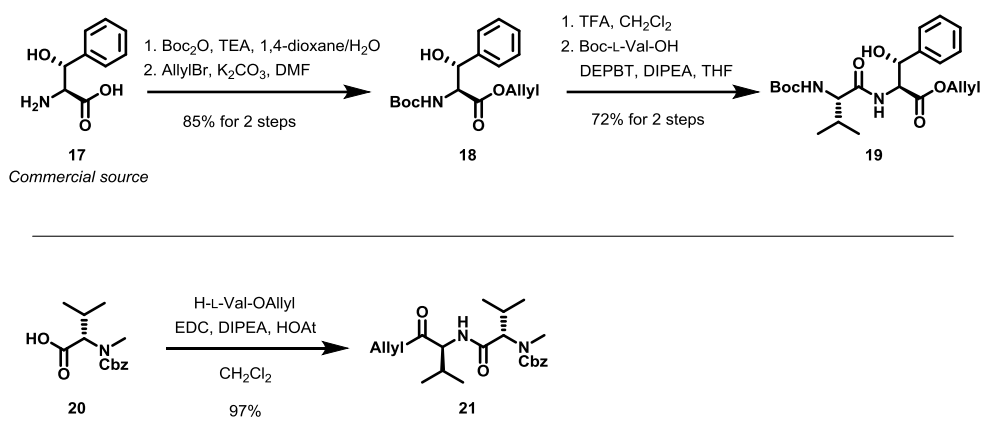
problematic *N*-methyl amide linkage between Leu[7] and Val[8] after the completion of the other tripeptide formation. Condensation between **13** and Boc-*N*Me-L-Thr(OBn)-OH under optimized condition (EDC, DIPEA, Oxyma in DMF) afforded dipeptide **14** in 93% yield. Removal of the *N*-Boc protecting group of **14** with TFA in CH_2Cl_2 solution followed by coupling with Boc-L-Thr-OH (HATU, DIPEA, HOAt in DMF) provided tripeptide **15** in 68% yield for two steps. After removal of allyl group of **15** in the presence of $Pd(PPh_3)_4$ and *N*-methylaniline in THF liberated corresponding free acid, reverse condensation with *N*Me-L-Leu-OAllyl was achieved by using DEPBT reagent to provide **16** as a single diastereomer (85% yield for two steps). It is noteworthy that the possible epimerization at the C_α position of Val[8] via oxazolone formation was efficiently suppressed by the DEPBT mediated coupling condition.[54,55]



Scheme 8. Synthesis of tetrapeptide 16

1-5. Synthesis of dipeptides 19 and 21

After the densely *N*-methyl amide containing building blocks **9** and **16** were successfully in hand, the syntheses of remaining dipeptide fragment **19** and **21** were smoothly proceeded (Scheme 9). At first, synthesis of another building block **19** consist of the northern fragment was initiated from the protection of commercially available non-ribosomal amino acid, L-β-hydroxy phenylalanine. Then, Boc-deprotection of **18** followed by coupling with Boc-L-Val-OH afforded the dipeptide **19** in 72% for two steps. Dipeptide **21** which would become a side chain of ohmyungsamycins was obtained via amide coupling between two L-valine derivatives in almost quantitative yield.



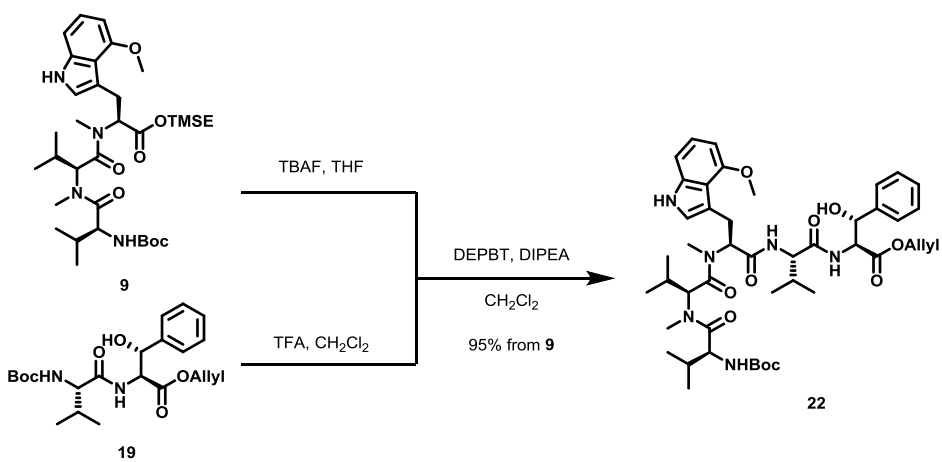
Scheme 9. Synthesis of dipeptides 19 and 21

1-6. Synthesis of northern fragment 22

With all of the small building blocks (**9**, **16**, **19** and **21**) in hand, we turned out our attention to the synthesis of northern fragment **22**. The treatment of tripeptide **9** with TBAF in THF

and dipeptide **19** with TFA in CH_2Cl_2 afforded free acid and amine, respectively, and coupling of both compounds (DEPBT, DIPEA, CH_2Cl_2) proceeded well to provide northern pentapeptide **22** in 95% yield from tripeptide **6** without epimerized product (Scheme 10).

Surprisingly, despite of its larger molecule size, ^{1}H- and ^{13}C-NMR spectra of the northern pentapeptide **22** was more simplified compared with tripeptide **9**, especially at the *N*-methyl amide region (Figure 9). This observation obviously indicates that every rotamers existed in



Scheme 10. Synthesis of northern fragment 22

the tripeptide **9** became a single conformational isomer as the additional dipeptide chain was introduced. We speculated that this conformation-stabilizing event might be induced via generation of certain hydrogen bond framework of **22**. Particularly, the completion of the potential turn inducing sequence may induce the stabilized bent conformation of **22** with the same β-turn existed in the X-ray crystallographic structure of ecumicin. To investigate the actual conformation of the key northern fragment **22**, we decided to exploit the NMR

spectroscopic technique which is one of the most powerful method to comprehend the molecular conformation in solution.

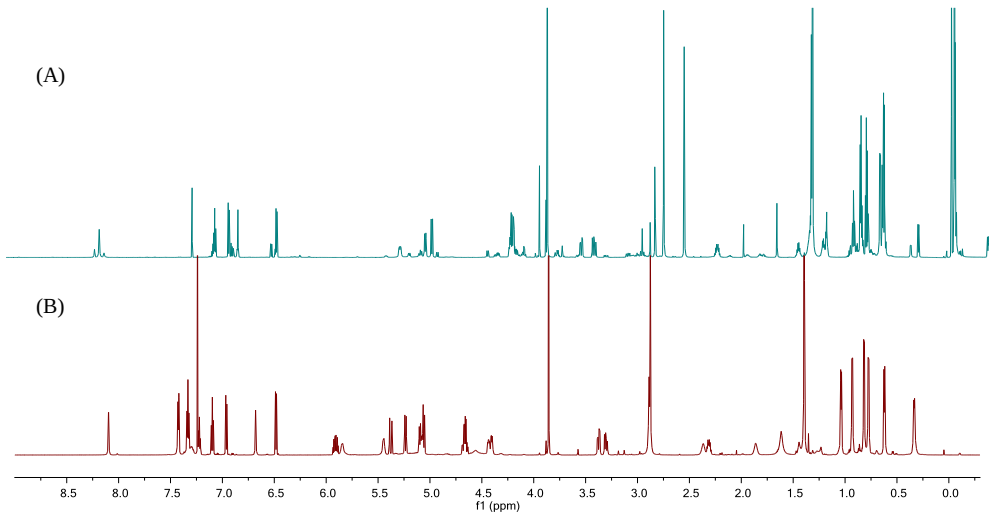


Figure 9. ^{1}H-NMR spectra of tripeptide 9 (A) and northern pentapeptide 22 (B)

1-7. Conformational study of the northern fragment 22 in chloroform solution

Hydrogen bonding is a fundamental feature in maintaining structured folding of proteins and synthetic foldamers.[56] Hydrogen-deuterium exchange is an analytical technique that has been used to correlate hydrogen bond strength with the rate of chemical exchange of the participating hydrogen to deuterium. Additionally, it provides a method to elucidate the separate roles of both hydrogen bond donors and hydrogen bond acceptors.[57] Therefore, we anticipated that the measurement of hydrogen-deuterium exchange rate for the amide and carbamate protons of tripeptide **9** and pentapeptide **22** (Figure 10) could provide a clue to the hydrogen bonding networks in **22** as well as a relative strength of each hydrogen bond.

Chloroform-d, which could not act as a hydrogen bond donor or acceptor, was selected as a base solvent to effectively induce the intramolecular hydrogen bonding.

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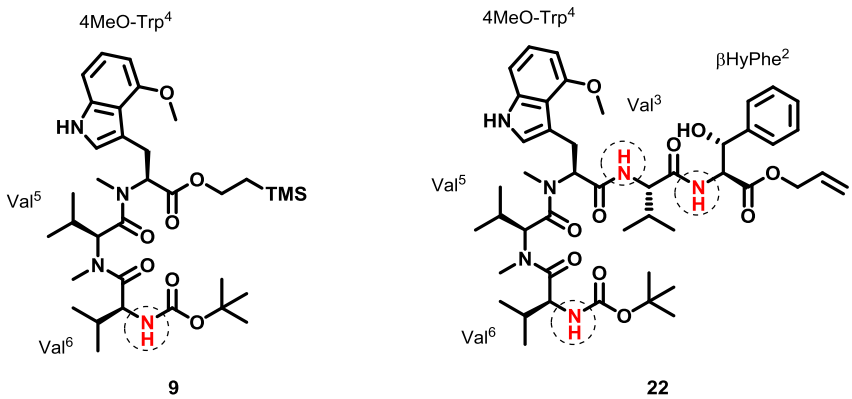


Figure 10. Chemical structures of tripeptide 9 and pentapeptide 22 reside in turning site. Exchangeable protons of interest are highlighted with dashed circle.

Before hydrogen-deuterium exchange experiment, initial integration of proton peak was recorded using a solution of each compounds (10 mmol) in 10% $CD_3OH/CDCl_3$ which does not contain an exchangeable deuterium. In addition, chemical shifts of each exchangeable protons of interest were assigned by a set of 2D NMR experiment such as COSY, e-HSQC, HMBC and TOCSY in the same solvent system. This sample was recovered and fully dried under high vacuum condition over a day. To initiate exchange, each compound was dissolved in 10% $CD_3OD/CDCl_3$ and spectra were immediately recorded 12 times at 10, 20, 30, 40, 80, 120, 150, 180, 270, 330 and 1400 minutes. Overlay of the obtained ^{1}H-NMR set for each

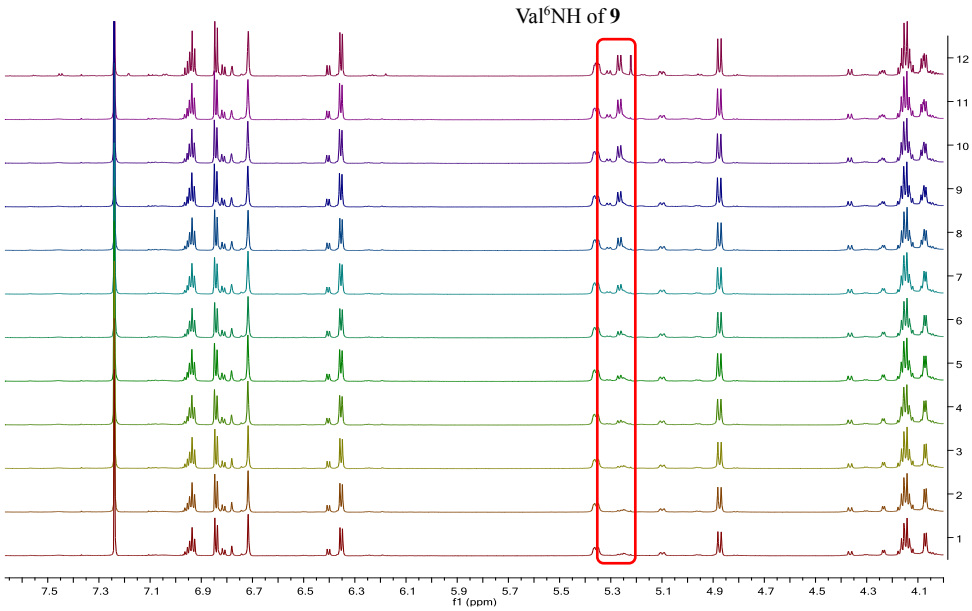


Figure 11. Stacked ^{1}H-NMR spectra of 9 in 10% $CD_3OD/CHCl_3$ solution at 10 mM

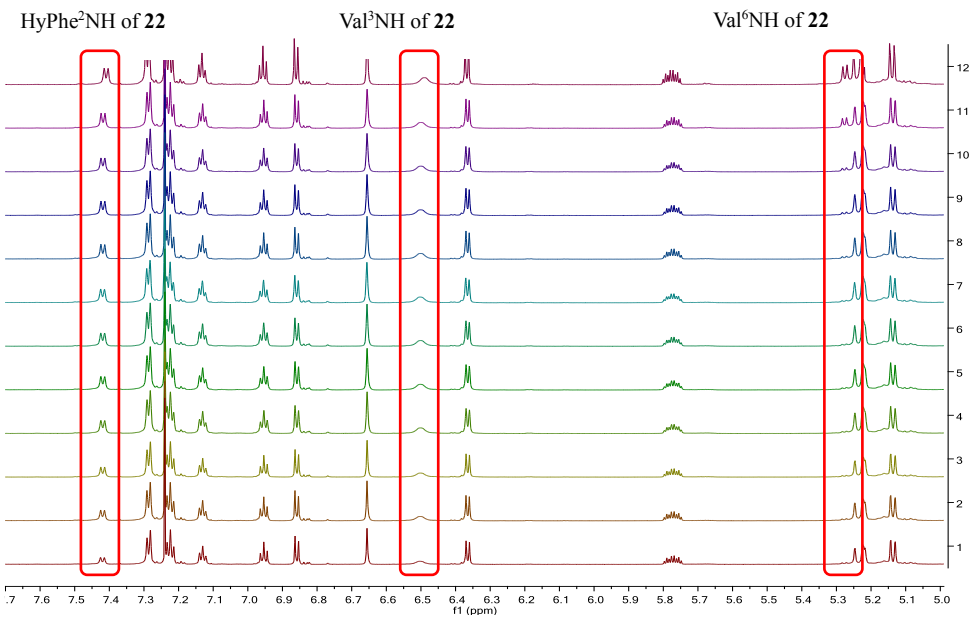


Figure 12. Stacked ^{1}H-NMR spectra of 22 in 10% $CD_3OD/CHCl_3$ solution at 10 mM

compounds clearly disclosed that amide and carbamate protons of the tripeptide **9** and pentapeptide **22** disappeared at different rates as shown in Figure 11 and Figure 12.

The integration of the exchanging amide and carbamate signal was calibrated to a non-exchanging reference peak. The relative integration of exchangeable peaks of interest was plotted to compare the relative deuterium exchange rate of each exchangeable protons at a glance (Figure 13). First, much slower exchange rate of βHyPhe^2 and Val^3 amide protons than Val^6 carbamate proton of **22** indicate that amide protons are not exposed to the solvent.

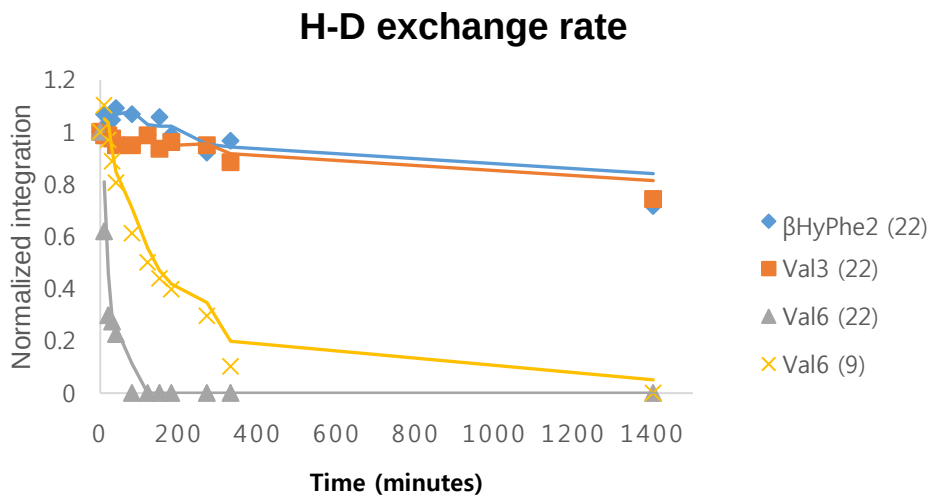


Figure 13. Time-dependent relative integration of each proton peak of 9 and 22 compared to initial integration, which indicates the rate of deuterium exchange

This result demonstrates that βHyPhe^2 and Val^3 amide protons serve as hydrogen bonding donors. In contrast, faster exchange rate of Val^6 carbamate proton of **22** than **9** demonstrates that carbonyl group of Boc-carbamate become hydrogen bonding acceptor as synthetic

intermediate is elongated from **9** to **22**. To sum, the extension of peptide chain from tripeptide **9** to pentapeptide **22** induce the conformation-stabilizing hydrogen bond framework, where

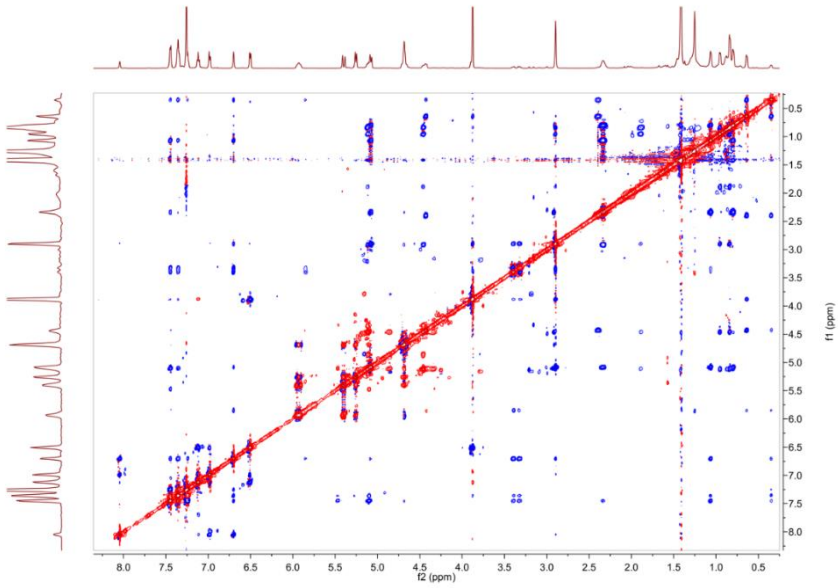


Figure 14. ROESY spectrum of northern pentapeptide 22

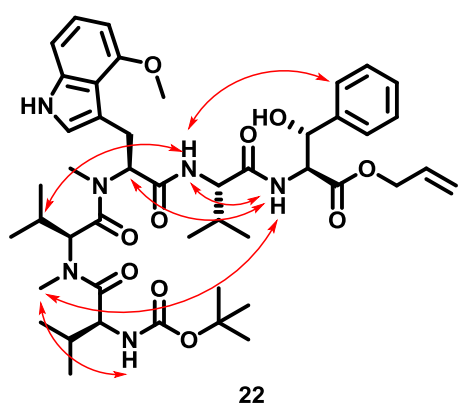


Figure 15. Crucial NOE of northern fragment 22 utilized structure calculation.

the both amide proton on βHyPhe[2] and Val[3] serve as hydrogen bond donor and the carbamate carbonyl group of Boc protecting group serves as hydrogen bond acceptor. Moreover, to identify the residues of **22** in the spatial proximity, we collected the crucial NOE cross-peaks by using ROESY experiment (Figure 14 and 15).

The three-dimensional structures of northern fragment **22** in $CDCl_3$ solution were calculated with 21 distance restraints identified from the NOE measurement and 2 hydrogen bonding restraints identified from the hydrogen-deuterium exchange experiment. Initial 40 structures with standard amino acids were generated with NMR fold module in YASARA software.[58–60] Side chain of selected 4 structures harboring β-turn were modified in UCSF Chimera.[61,62] Final coordinates were minimized with amberff14sb force field using UCSF Chimera. Structure validation was performed with Molprobity,[63] and all coordinates were analyzed and displayed with UCSF Chimera.

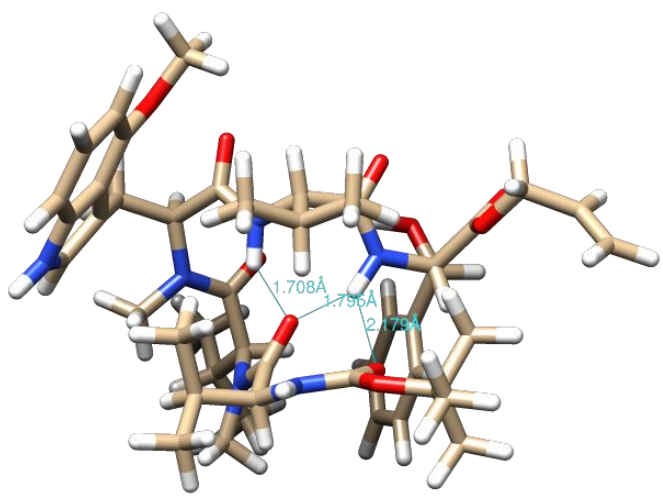


Figure 16. 3D structure of northern fragment 22 in $CDCl_3$ solution

The result of molecular modeling displayed that the northern fragment **22** exist in bent conformation stabilized by β-turn forming hydrogen bond at the same position with the crystal structure of ecumicin (Figure 16 and 17). Second hydrogen bond contributing to the β-hairpin conformation was not detected, probably due to the presence of carbamate protecting group instead of amide chain, which exhibit different electronic nature with amide functional group.

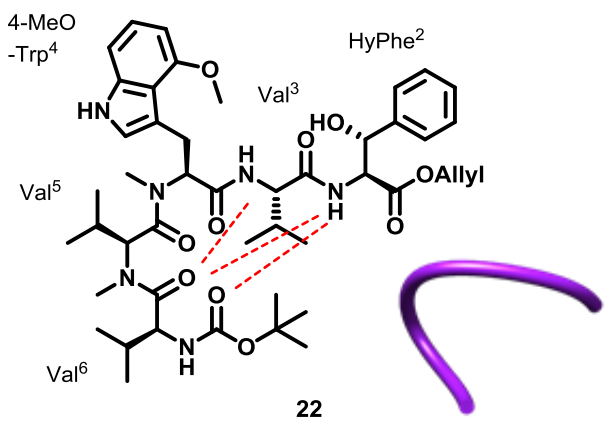
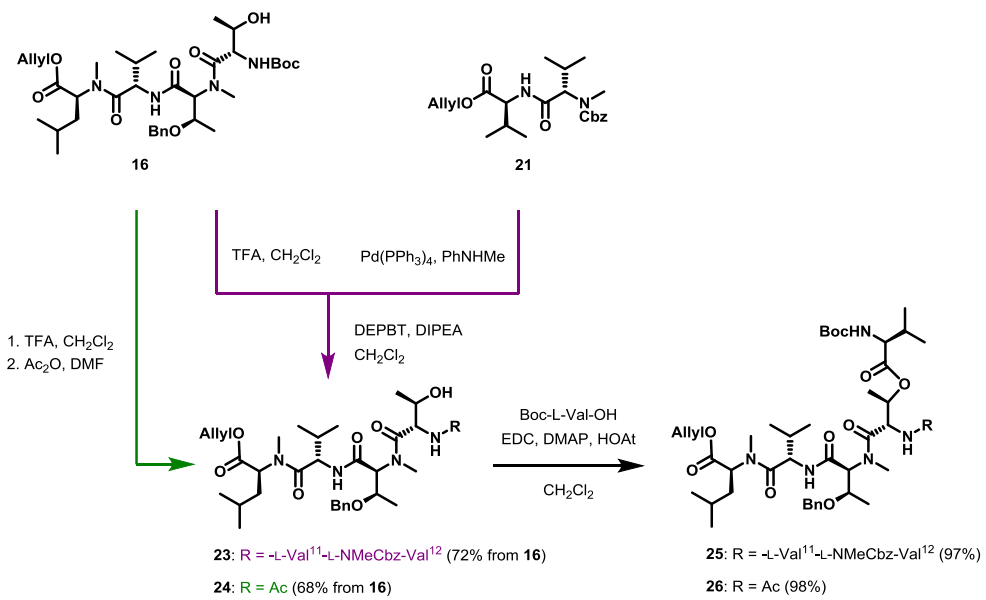


Figure 17. Identified hydrogen bond framework and distinct bent conformation depicted in ribbon model of pentapeptide 22

1-8. Synthesis of southern fragments

The structural diversification of southern fragments was commenced by introducing different side chain at the Thr^{10} position (Scheme 11). After *N*-terminus of common tetrapeptidic intermediate **16** was unmasked by the action of TFA in CH_2Cl_2, dipeptidic acid liberated from **21** was assembled to amine via DEPBT mediated amidation to provide

hexapeptide **23** (75% from **16**). Independently, acetyl group, the simplest side chain, was introduced to the free amine liberated from **16** by treatment of Ac_2O in DMF (68% from **16**). The backbone ester formations of **25** and **26** were smoothly achieved under EDC, DMAP, HOAt in CH_2Cl_2 with Boc-L-Val-OH counterpart in almost quantitative yields (97% for **25**, 98% for **26**).

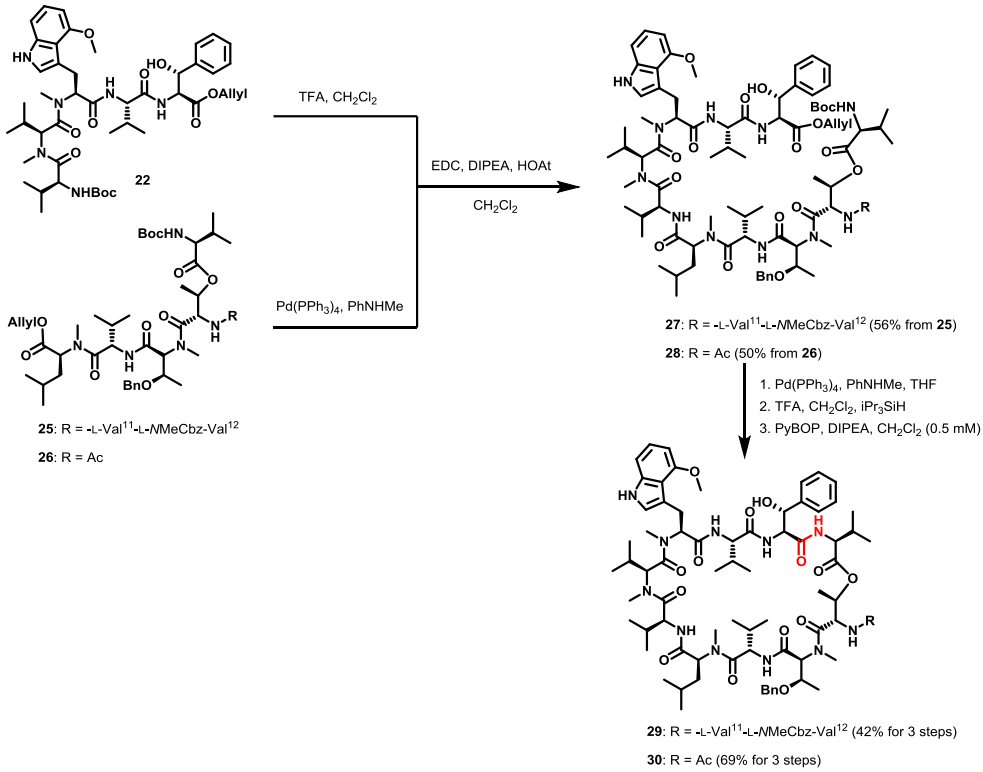


Scheme 11. Syntheses of southern fragments 25 and 26

1-9. Fragments assembly and macrolactamization.

At the stage of final fragment assembly to afford full-length linear depsipeptide, we suffered from rapid epimerization at the Leu^7-C_α position in case of dodecapeptide **27** synthesis as shown in Scheme 12. Having examined multiple conditions, we concluded that portionwise addition of EDC into the mixture of substrates, DIPEA and HOAt in CH_2Cl_2 at

0 °C was the best result to give **27** in 56% as isolation yield (3.2:1, **27**/*epi*-**27**). Decapeptide **28** was prepared by the same condition in moderate yield (50%). With the cyclization precursors in hand, we conducted subsequent exposure of *C*- and *N*- termini followed by key macrocyclization. The allyl ester cleavage of **27** and **28** followed by the careful removal of *N*-Boc protecting groups (1:10 mixture of TFA in CH_2Cl_2, 20 equiv. of *i*Pr_3SiH, 20 min at room temperature) afforded amino-acid TFA salts. Notably, Boc-deprotection should be performed under the large volume of TFA in CH_2Cl_2 mixture in short reaction time to avoid side reactions. And then, we performed the macrolactamization under high-dilution conditions.[64] Although roughly examined in test scale, we observed that the use of CH_2Cl_2 rather than DMF as solvent was a crucial factor of the ring closure. When DMF was used, cyclodimer and even cyclotrimer were produced as a major product regardless of coupling reagent such as EDC, HATU and PyBOP (Table 1). Increased production of the cyclodimer and cyclotrimer is likely due to the diminished population of pre-organized bent form of cyclization precursor, caused by exposure of the intramolecular hydrogen bond donor and acceptor to the high-dielectric solvent.[22] Indeed, the population of desired cyclomonomer was increased by replacement of the reaction solvent to the more lipophilic solvent, dichloromethane. In addition, the slow addition technique of the substrate into the coupling reagent in DMF was beneficial to provide cyclomonomer, but yield was not satisfactory. Thus, after intensive optimization of cyclization condition, we revealed that the combination of the non-polar solvent dichloromethane and the slow addition of the substrate into the solution of PyBOP (5 equiv.) and DIPEA (5 equiv.) at 0.5 mM concentration could afford desired 31-membered macrocycle **29** in 45% yield for 3 steps. In case of simplified analog, macrolactamization was more smoothly proceeded to provide **30** in 69% yield for three steps, presumably due to the reduced steric hindrance.



Scheme 12. Fragment assembly and macrolactamization

Conditions					Results
EDC, HOAt					
HATU	DIPEA	DMF	1 mM	-	Dimer
PyBOP					
		CH_2Cl_2	1 mM	-	Mono+Dimer
PyBOP	DIPEA	DMF	0.5 mM	**Slow add.**	Mono:di = 2:1
		CH_2Cl_2	0.5 mM	**Slow add.**	Monomer, 42%

Table 1. Macrolactamization conditions screening for 29

1-10. Macrocyclization studies at other sites

As mentioned above, we considered that the best cyclization site for the synthesis of ohmyungsamycins is the Val[1]-βHyPhe[2] amide bond, in terms of balance between distance from the turn inducing sequence to the cyclization site and reactivity of cyclization site. In order to support our synthetic strategy, we tested four additional cyclization site (Figure 18). First, we attempted cyclization at the Val[1]-Thr[10] ester bond and the Thr[9]-Thr[10] amide bond, which could be favored because they were most opposite positions to the turn-inducing unit although we concerned the low reactivity of alcohol and *N*-methyl amine moiety. Indeed, they did not underwent cyclization under various reaction conditions. Next, we performed macrolactamization at the Val[6]-Val[7] amide bond, which is closer to the turn-inducing sequence compared to the Val[1]-βHyPhe[2] bond, may have less beneficial effects by the turn-inducing unit. Interestingly, cyclization at the Val[6]-Val[7] amide bond under the optimized

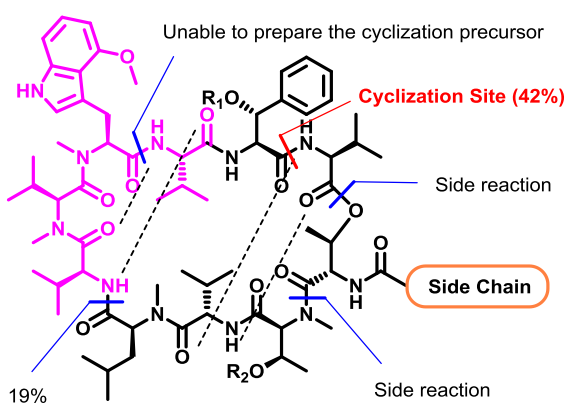
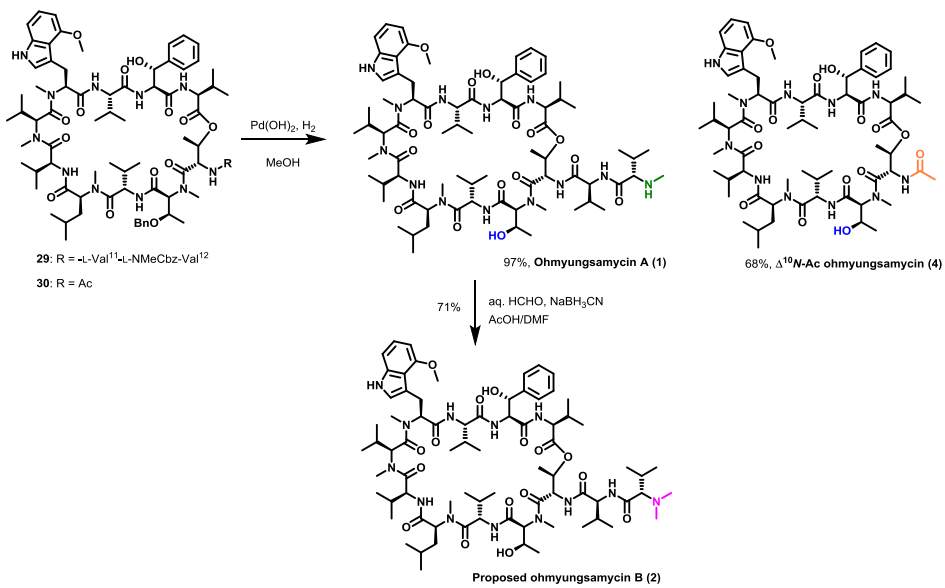


Figure 18. Summary of macrocyclization studies at other sites

reaction conditions proceeded although the yield was quite low (19%) compared to that (42%) of the cyclization at the Val[1]-βHyPhe[2] bond. These results seems to imply the position effects on the cyclization as anticipated. Further examination for the cyclization at the Val[3]-Trp[4] amide bonds, which might have least beneficial effects of plausible bent conformation because the bond is included in the turn-inducing unit, was not successful. Unfortunately, we were not able to prepare the cyclization precursor due to instability of the fragments for the precursor and/or reluctance to amide formation of the precursor fragments.

1-11. Completion of syntheses



Scheme 13. Completion of syntheses

Finally, global deprotection of **29** via $Pd(OH)_2$ mediated hydrogenolysis produced ohmyungsamycin A (**1**) in 97% yield after purification by flash column chromatography (Scheme 13). As expected, all spectroscopic data were exactly matched with the reported one (Figure 19). In addition, the side chain-truncated ohmyungsamycin (**4**) was also obtained using the same deprotection in 68% yield. The structure of the novel cyclodepsipeptide **4** was also confirmed by careful analysis of the spectral data. Importantly, the $J_{H\text{-}H}$ coupling constants and major through-space correlation measured by the ROESY experiment were consistent with the reported data for ohmyungsamycin A (**1**), supporting the role of the cyclic core in maintaining a stable 3D structure regardless of the side chain (Figure 20).

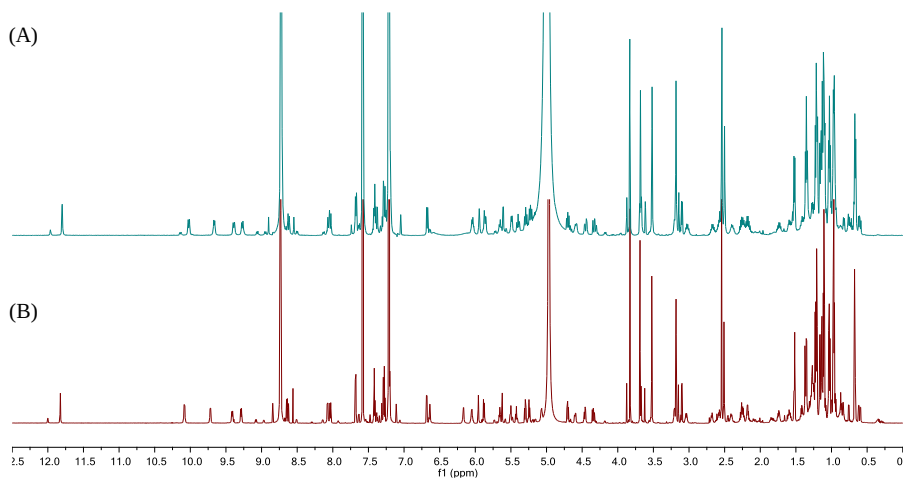


Figure 19. ^{1}H NMR comparison of natural ohmyungsamycin A (A) and synthetic ohmyungsamycin A (1; B)

For the synthesis of proposed structure of ohmyungsamycin B (**2**), a new methyl substituent was directly introduced onto the terminal amine of **1** via reductive methylation[65,66] in 71% yield. Addition of single methyl substituent onto the ohmyungsamycin A (**1**) was identified via high-resolution mass spectroscopy. Unexpectedly, other spectral data of the synthetic ohmyungsamycin B (**2**) were not identical to those of natural ohmyungsamycin B. In particular, the ^{1}H- and ^{13}C-NMR spectra corresponding to the side chain were quite different. To elucidate this structural discrepancy, we try to correct the ambiguous position of addtional methyl substituent of ohmyungsamycin B by intensive NMR assignment.

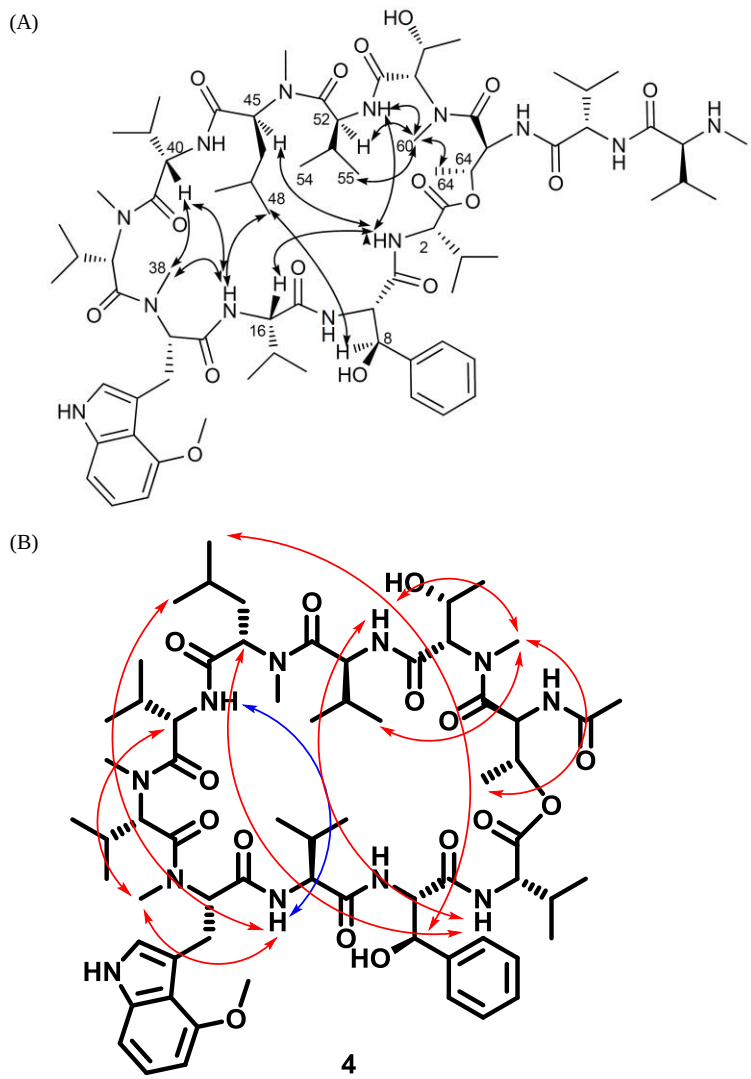


Figure 20. Comparison of key NOE correlations between ohmyungsamycin A[38] (A) and side chain-truncated ohmyungamycin (B).

2. Structural Revision of Ohmyungsamycin B

2-1. Comparison of NMR spectra for natural and synthetic ohmyungsamycin B

After a sample of natural ohmyungsamycin B was kindly provided from professor Dong-Chan Oh, we reinvestigate the actual structure of it. As depicted in Figure 21, chemical shift difference between natural and synthetic ohmyungsamycin B mainly existed at the side chain region. This result indicates that structural ambiguity of ohmyungsamycin B located on the side chain. To investigate the influence of additional methyl substituent introduced from ohmyungsamycin A, we compared chemical shift for the ohmyungsamycin A with those to the synthetic (Figure 22A and 22B) and natural ohmyungsamycin B (Figure 22C and 22D).

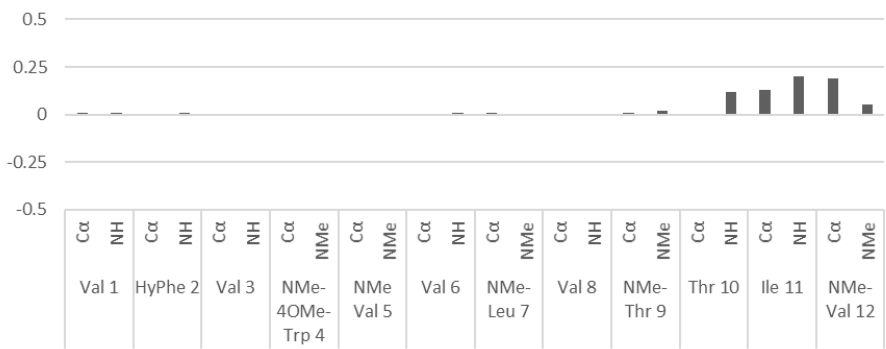


Figure 21. Comparison of ^{1}H-NMR chemical shift between natural and synthetic ohmyungsamycin B

In case of synthetic ohmyungsamycin B (**2**), the additional methyl substituent on the terminal amine affected to the NMR chemical shift across the side chain. Whereas, the methyl

substituent of natural ohmyungsamycin A slightly affected to the chemical shift only on the 11th amino acid. Consequently, the ambiguous position of additional methyl substituent should be positioned at the 11th position in form of aliphatic chain, not on the terminal amine.

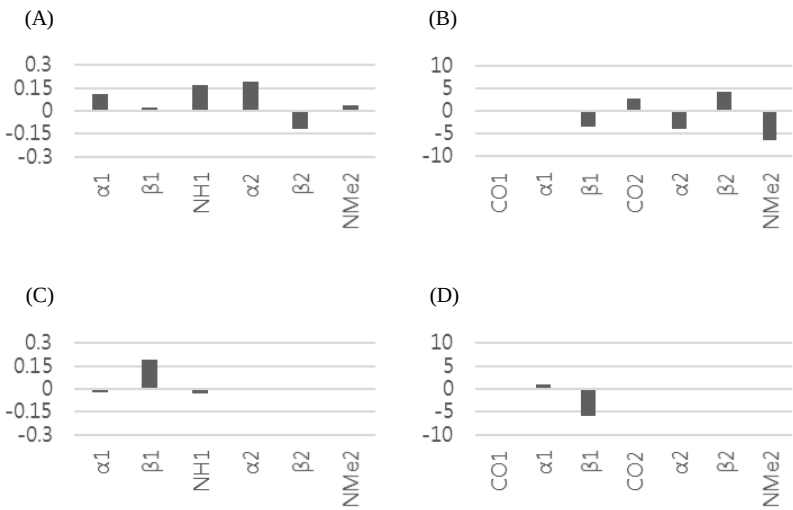


Figure 22. NMR comparison between ohmyungsamycin A and natural (A and B) or synthetic ohmyungsamycin B (C and D)

2-2. Structural assignment of 11th amino acid

To determine the exact structure of 11th amino acid, we performed intensive 1D and 2D NMR studies including COSY and e-HSQC experiments. First, the e-HSQC spectrum of natural ohmyungsamycin B showed that unassigned signals of methylene group (δ_H 1.68 and 1.26) and terminal methyl group (δ_H 0.72) certainly exist (Figure 23). Interpreting ^{1}H-^{1}H coupling signals between δ_H 5.27/δ_H 2.07 (1H, CH), and δ_H 2.07/δ_H 0.96 (3H, CH_3), NH-CH-CH-CH_3 partial structure could be deducted. In addition, existence of a strong COSY correlation of δ_H 2.07/δ_H 1.26 (1H) and a weak COSY correlation of δ_H 2.07/δ_H 1.68 (1H)

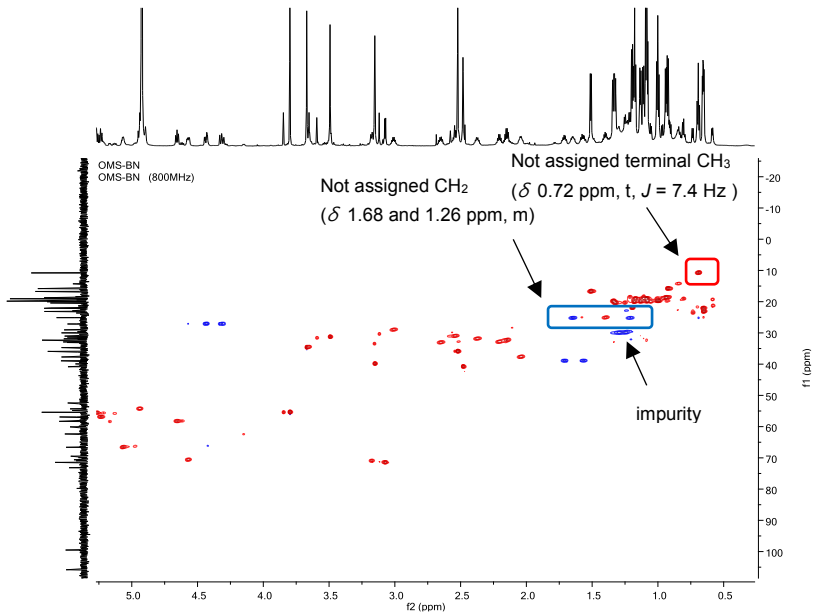


Figure 23. Expanded e-HSQC region of natural ohmyungsamycin B

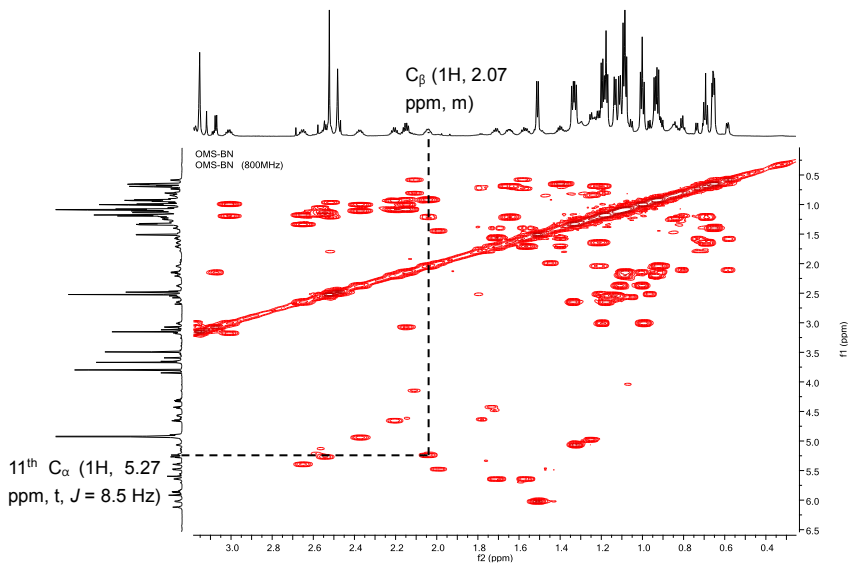


Figure 24. Expanded COSY region of natural ohmyungsamycin B

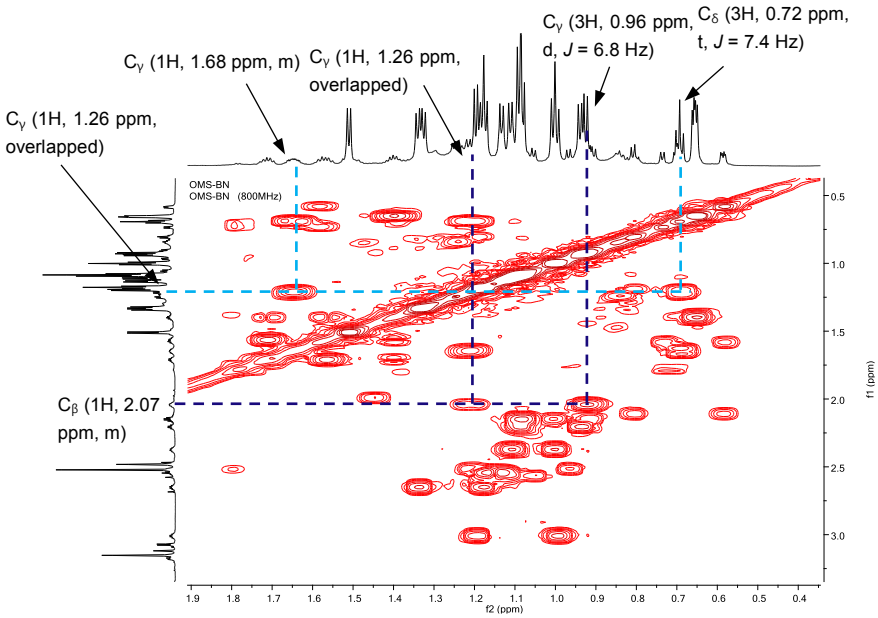


Figure 25. Expanded COSY region of natural ohmyungsamycin B

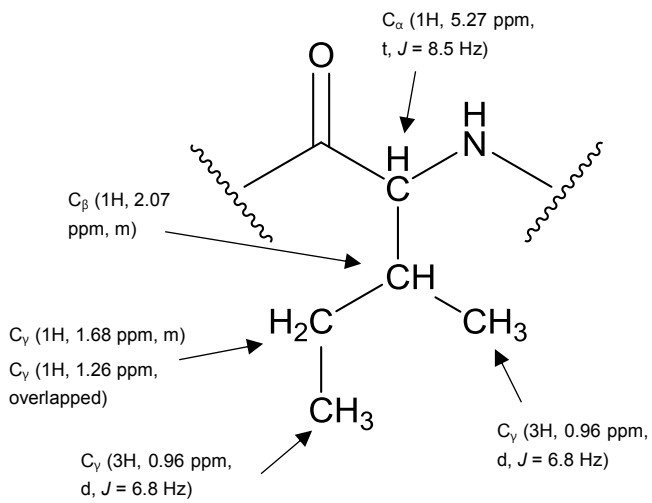
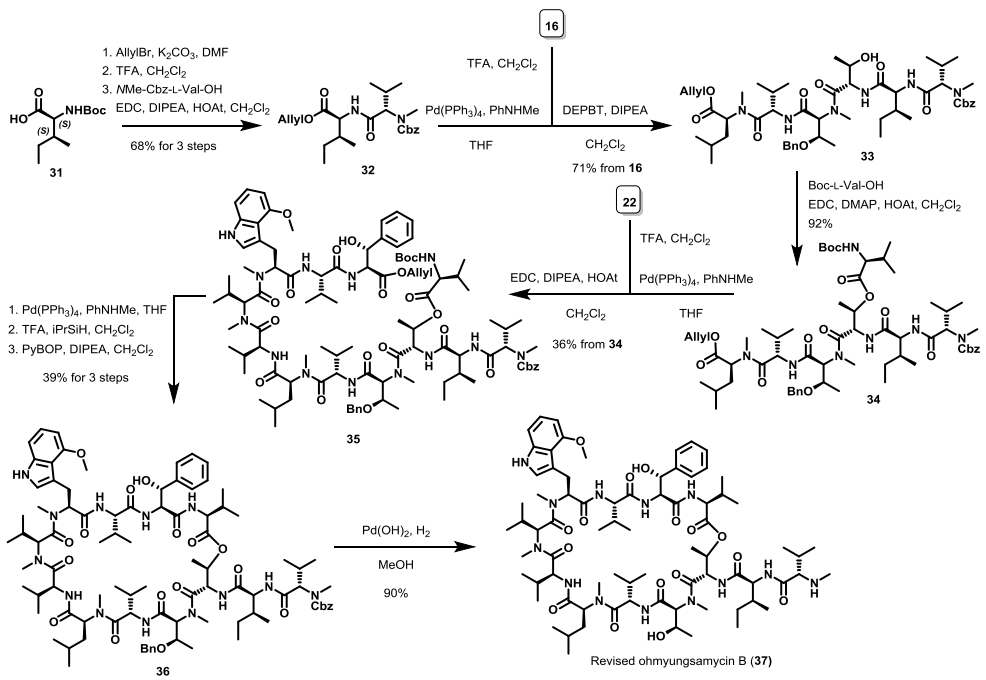


Figure 26. Structural analysis for the 11th amino acid

indicated that the C-3 (δ_H 2.07) of this unit linked to the methylene group (δ_H 1.68 and 1.26). The protons (δ_H 1.68 and 1.26) of the methylene displayed a COSY correlation with the triplet methyl group at δ_H 0.72. Therefore, this amino acid unit was identified as isoleucine unit (Figure 24-26). Among the four possible diastereomers of isoleucine, we selected the naturally abundant L-Ile and utilized to the revised ohmyungsamycin B synthesis.

2-3. Synthesis of revised ohmyungsamycin B



Scheme 14. Synthesis of revised ohmyungsamycin B 37

We embarked on the total synthesis of the revised structure of ohmyungsamycin B, starting from Boc-protected L-isoleucine. Following the established procedure, the revised ohmyungsamycin B (**37**) was provided in 6% overall yield (Scheme 14). To our delight, the

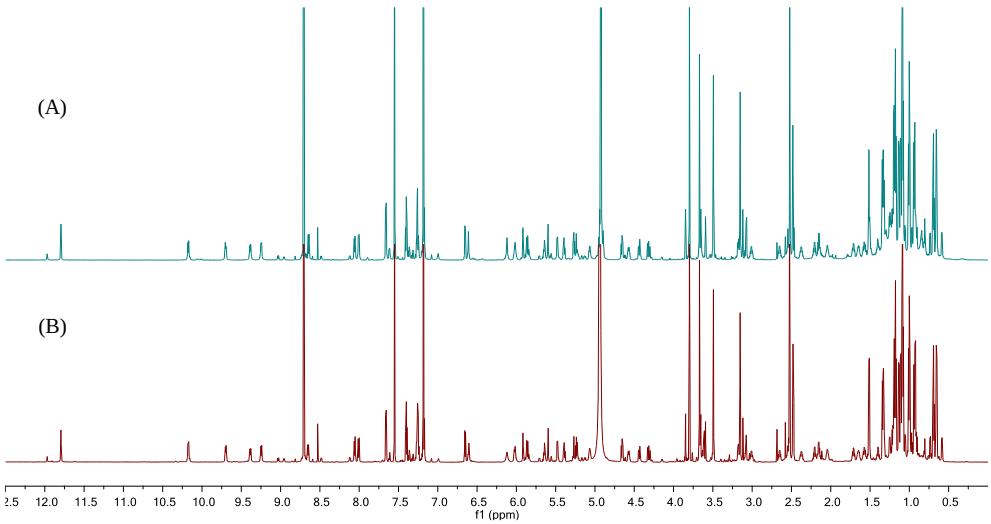


Figure 27. ^{1}H NMR comparison of natural ohmyungsamycin B (A) and revised ohmyungsamycin A (37; B)

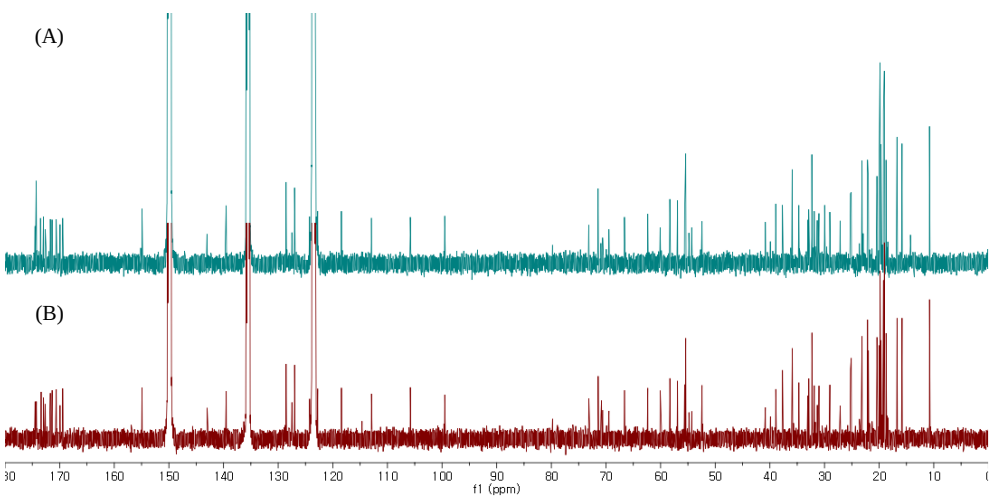


Figure 28. ^{13}C NMR comparison of natural ohmyungsamycin B (A) and revised ohmyungsamycin A (37; B)

spectral data of this synthetic ohmyungsamycin B perfectly matched the reported data (Figure 27 and 28).

2-4. Confirmation of absolute configuration of the isoleucine unit included in natural ohmyungsamycin B.

Then, we further analyzed the absolute configuration of isoleucines in natural ohmyungsamycin B via LC/MS assisted manner. Because derivatization of Ile with Marfey's reagent could not provide HPLC retention times long enough to determine the absolute stereochemistry of β-position of Ile, we used another chiral derivatizing reagent, 2,3,4,6-tetra-*O*-acetyl-*β*-d-glucopyranosyl isothiocyanate (GITC).[67] The HPLC analysis of GITC derivatives of Ile in natural ohmyungsamycin B and authetic L-Ile, L-*allo*-Ile, D-Ile and D-*allo*-Ile revealed that ohmyungsamycin B possesses L-Ile (Figure 29), confirming our synthesis of revised ohmyungsamycin B is unequivocally correct.

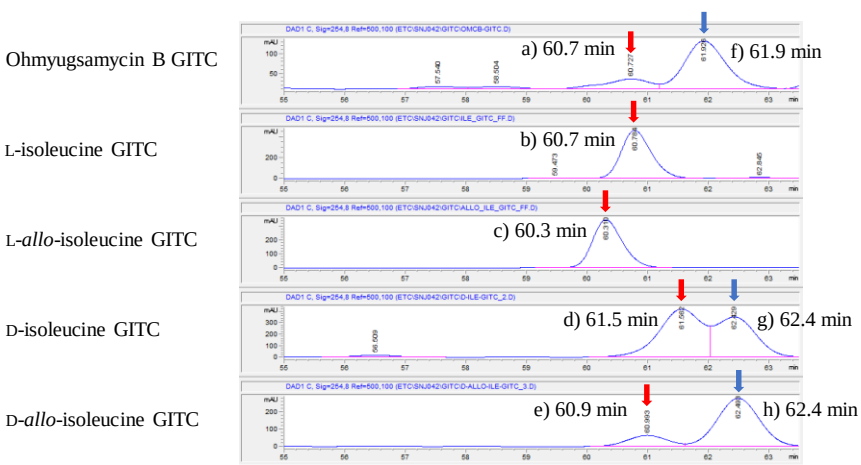


Figure 29. LC/MS analysis of the GITC derivative of the isoleucine unit in natural ohmyungsamycin B and the four diastereomers of isoleucines (f, g, h: unidentified byproduct)

3. Anti-TB Activity Evaluation of Synthetic Ohmyungsamycins

The anti-TB activities (MIC_{50}, nM) of all obtained compounds (**1**, **2**, **4** and **37**) were tested with natural ohmyungsamycins and ethambutol as positive controls (Table 2). As expected, synthetic ohmyungsamycin A (**1**) presented nearly the same activity (33 nM) with reported data (57 nM). Interestingly, both proposed (**2**) and revised ohmyungsamycin B (**37**) exhibited similar potency despite 2~3 fold less than that of **1**. This result indicates that the trivial alteration of structural features of side chain caused by *N*-methyl group relocation (**2** to **37**) cannot largely affect affinity against biological target. Also noteworthy, the anti-TB activity of side chain truncated analog **4** was decreased 10 and 20 fold (740 nM) relative to ohmyungsamycin A, but still retained sufficient potency compared to the first line anti-TB drug, ethambutol (3100 nM). To sum up, these data suggest that the crucial role of the cyclic core for anti-TB activity as well as the significant beneficial effects of the side chain.

Compound	MIC_{50} (nM)
Ohmyungsamycin A (**1**)	33
Ohmyungsamycin B; proposed (**2**)	65
Ohmyungsamycin B; revised (**37**)	108
Δ^{10}N-Ac ohmyungsamycin (**4**)	740
Ethambutol	3100

Table 2. Anti-TB activities of the ohmyungsamycins and structurally relevant cyclopeptides

III. Conclusion

We have achieved the first total synthesis of ohmyungsamycin A and ohmyungsamycin B via conformationally-inspired solution phase peptide synthesis in a convergent manner. The key macrocyclization strategy for the syntheses of the ohmyungamycins was inspired by the conformation of the cyclic core. Turn inducing effect of key intermediate, which was anticipated from the 3D structure of natural product, was identified via intensive NMR experiment and molecular modeling. In addition, proposed structure of ohmyungsamycin B was revised from our synthetic endeavor. Finally, synthesis of the side chain-truncated ohmyungsamycin A enabled the elucidation of the core of ohmyungsamycins, which is responsible for the excellent anti-TB activities. Our convergent syntheses of the ohmyungsamycins can be widely utilized by synthetic and medicinal chemists.

IV. Experimental

General experimental

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and Et_2O were distilled from sodium benzophenone ketyl. Dichloromethane, chloroform, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 °C. Air and moisture sensitive reactions were performed under argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Optical rotations were measured with JASCO P-2000 digital polarimeter at ambient temperature using cylindrical cell of 10 mm or 100 mm pathlength. Infrared spectra were recorded on a JASCO FT-IR-4200 spectrometer. High resolution mass spectra were obtained with JEOL JMS-700 instrument and Agilent Q TOF 6530. 1H and 13C NMR spectra were recorded using JEOL JNM-ECA-600, BRUKER AVANCE-500, and BRUKER AVANCE-800. Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$, ^1H δ 7.24, ^{13}C δ 77.0; pyridine-d_5, ^1H δ 7.58, ^{13}C δ 135.5). ^1H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; p, pentet; m, multiplet and/or multiple resonances; br, broad signal), coupling constant in hertz (Hz) and number of protons. High performance liquid chromatography (HPLC) experiments were performed with Waters 1525 binary pump and Waters 2489 UV/Visible detector at 35 °C.

Experimental procedure

Boc-*N*Me-L-Hag-OTMSE (6) To a solution of Boc-*N*Me-L-Hag-OMe[53] **5** (7.4 g, 28.58 mmol) in THF/H_2O (1:1, 144 mL) was added lithium hydroxide (9.6 g, 228.74 mmol) at 0 °C. After stirring overnight at the same temperature, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, acidified with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 7.0 g (100%) of crude acid as a clear oil. The acid was used in the next reaction without further purification. To a solution of the above acid (7.0 g. 28.58 mmol), EDC (8.2 g, 42.87 mmol) and DMAP (349 mg, 2.86 mmol) in CH_2Cl_2 (57 mL) was added 2-(trimethylsilyl)ethanol (6.2 mL, 42.87 mmol) via syringe pump over 1 h at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/hexane = 1:8) to afford 7.6 g (77%) of TMSE ester **6** as a clear oil. $[\alpha]^{20}_D$ -15.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$, 1:1 mixture of rotamers) δ 5.81-5.75 (m, 1H), 5.04-5.00 (m, 2H), 4.70 (dd, *J* = 10.6, 4.3 Hz, 0.5H), 4.38 (dd, *J* = 10.1, 3.5 Hz, 0.5H), 4.20-4.14 (m, 2H), 2.80 (s, 1.5H), 2.76 (s, 1.5H), 2.11-1.97 (m, 3H), 1.83-1.72 (m, 1H), 1.44 (s, 4.5H), 1.41 (s, 4.5H), 0.97 (t, *J* = 8.3 Hz, 2H), 0.02 (s, 4.5H), 0.01 (s, 4.5H); ^{13}C NMR (200 MHz, $CDCl_3$, 1:1 mixture of rotamers) δ 172.1, 171.9, 156.2, 155.7, 137.2, 137.1, 115.7, 115.5, 80.2, 79.9, 63.3, 63.3, 58.6, 57.4, 31.0, 30.6, 30.3, 30.1, 28.4, 28.4, 28.3, 28.1, 17.4, 17.4, -1.5.; HRMS (FAB+) calcd for $C_{17}H_{34}NO_4Si$ ($M+H^+$) 344.2257, found 344.2252; IR (thin film, neat) ν_{max} 2955, 1739, 1700, 1392, 1367, 1251, 1178, 1149, 861, 838, 772 cm^{-1}

2-(trimethylsilyl)ethyl (*S*)-2-((tert-butoxycarbonyl)(methyl)amino)-5-oxopentanoate (S1) To a stirred solution of the Boc-*N*Me-l-Hag-OTMSE **6** (189 mg, 0.55 mmol) and 4-methylmorpholine *N*-oxide (194 mg, 1.65 mmol) in THF/H_2O (3:1, 6 mL) was added OsO_4 (0.1 M in toluene, 0.55 mL, 0.06 mmol) dropwise at 0 °C. After stirring for 3 h at the same temperature, the reaction mixture was quenched with 1*N* $Na_2S_2O_3$ and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford crude diol as a brown oil. The diol was directly used in the next step without further purification. To a solution of the above diol (0.55 mmol) in THF/H_2O (3:1, 6 mL) was added $NaIO_4$ (353 mg, 1.65 mmol) at 0 °C. After stirring for 30 min at the same temperature, the reaction mixture was filtered through a pad of Celite and extracted with Et_2O. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:5) to afford 181 mg (95% for 2 steps) of aldehyde **S1** as a yellow oil. $[\alpha]^{20}_D$ -28.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$) δ 9.75 (s, 0.5H), 9.73 (s, 0.5H), 4.62 (dd, *J* = 10.3, 5.0 Hz, 0.5H), 4.36 (dd, *J* = 9.6, 4.7 Hz, 0.5H), 4.23-4.11 (m, 2H), 2.76 (s, 1.5H), 2.72 (s, 1.5H), 2.56-2.38 (m, 2H), 2.31-2.22 (m, 1H), 2.05-1.90 (m, 1H), 1.43 (s, 4.5H), 1.39 (s, 4.5H), 0.96 (t, *J* = 8.3 Hz, 2H), 0.00 (s, 4.5H), 0.00 (s, 4.5H).; ^{13}C NMR (200 MHz, $CDCl_3$) δ 201.0, 200.8, 171.2, 171.1, 156.2, 155.4, 80.5, 80.2, 63.5, 63.5, 58.7, 57.7, 40.5, 40.1, 31.6, 31.1, 28.3, 28.1, 21.6, 21.3, 17.4, 17.3, -1.6.; HRMS (FAB+) calcd for $C_{16}H_{32}NO_5Si$ ($M+H^+$) 346.2047, found 346.2050; IR (thin film, neat) ν_{max} 2955, 2899, 1737, 1698, 1392, 1251, 1174, 1147, 861, 839 cm^{-1}

Boc-*N*Me-4MeO-L-Trp-OTMSE (7) The 2-iodo-3-methoxyaniline[68,69] (176 mg, 0.71 mmol), DABCO (148 mg, 1.32 mmol) and aldehyde **S1** (151 mg, 0.44 mmol) were dissolved in DMF (1.5 mL). The solution was degassed by bubbling Ar gas in a vacuum for 20 min. Then, $Pd(OAc)_2$ (15 mg, 0.07 mmol) was added and the flask was immersed into a preheated oil bath (100 °C). After stirring for 16 h at the same temperature under dark circumstance, the reaction mixture was cooled to room temperature and extracted with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (Acetone/Hexane = 1:4) to afford 101 mg (51%) of tirpeptide Boc-*N*Me-4MeO-L-Trp-OTMSE **7** as a brown oil. $[\alpha]^{20}_{D}$ -36.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$, 7:3 mixture of rotamers) δ 8.24 (s, 0.7H), 8.12 (s, 0.3H), 7.04 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.87 (s, 0.3H), 6.79 (s, 0.7H), 6.45 (d, *J* = 7.7 Hz, 1H), 5.02-4.91 (m, 1H), 4.29-4.20 (m, 2H), 3.89 (s, 3H), 3.60 (dd, *J* = 14.4, 4.1 Hz, 0.7H), 3.56 (dd, *J* = 15.0, 4.8 Hz, 0.3H), 3.36 (dd, *J* = 14.8, 10.6 Hz, 0.3H), 3.06 (dd, *J* = 14.3, 11.0 Hz, 0.7H), 2.76 (s, 2.1H), 2.72 (s, 0.9H), 1.38 (s, 2.7H), 1.07 (s, 6.3H), 1.02 (t, *J* = 8.3 Hz, 2H), 0.04 (s, 6.3H), 0.01 (s, 2.7H).; ^{13}C NMR (200 MHz, $CDCl_3$, 7:3 mixture of rotamers) δ 172.3, 172.0, 155.9, 155.6, 154.7, 154.5, 138.1, 137.9, 122.7, 122.5, 121.6, 121.3, 117.4, 117.3, 112.2, 112.0, 104.6, 104.4, 99.3, 99.2, 79.5, 79.5, 77.2, 77.2, 77.0, 76.8, 63.1, 63.1, 60.9, 60.0, 55.0, 55.0, 32.5, 31.8, 28.3, 27.8, 26.8, 26.4, 17.3, 17.3, -1.5, -1.5.; HRMS (FAB+) calcd for $C_{23}H_{36}N_2O_5Si$ (M^+) 448.2392, found 448.2394; IR (thin film, neat) ν_{max} 3313, 2953, 1736, 1676, 1509, 1438, 1366, 1254, 1171, 1091, 860, 839, 733 cm^{-1}

Boc-*N*Me-L-Val-*N*Me-L-Hag-OTMSE (10) To a solution of Boc-*N*Me-L-Hag-OTMSE **6** (7.6 g, 22.15 mmol) in CH_2Cl_2 (60 mL) was added TFA (15 mL) dropwise at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 4.6 g (85%) of free amine as a yellow liquid. The amine was used in the next step without further purification. To a solution of the above amine (4.6 g, 18.92 mmol), Boc-*N*Me-L-Val-OH[70] (6.4 g, 27.69 mmol) and DIPEA (6.6 mL, 37.84 mmol) in CH_2Cl_2 (60 mL) was added DEPBT (8.5 g, 28.38 mmol) at 0 °C. After stirring for 2 days at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:9) to give 7.0 g (81%) of dipeptide **10** as a colorless oil. $[\alpha]^{20}_D$ -102.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$, 5:2:2:1 mixture of rotamers) δ 5.81-5.71 (m, 1H), 5.09-4.92 (m, 2.7H), 4.82 (dd, *J* = 10.9, 4.3 Hz, 0.2H), 4.67 (d, *J* = 10.8 Hz, 0.5H), 4.61 (dd, *J* = 11.3, 4.1 Hz, 0.1H), 4.51 (d, *J* = 10.5 Hz, 0.2H), 4.43 (d, *J* = 10.6 Hz, 0.2H), 4.27 (d, *J* = 10.4 Hz, 0.1H), 4.17-4.04 (m, 2H), 2.99 (s, 1.5H), 2.95 (s, 0.6H), 2.81 (s, 0.3H), 2.75 (s, 0.6H), 2.74 (s, 1.5H), 2.73 (s, 0.6H), 2.63 (s, 0.6H), 2.58 (s, 0.3H), 2.41-2.24 (m, 1H), 2.16-1.86 (m, 3H), 1.80-1.72 (m, 1H), 1.45 (s, 1.8H), 1.42 (s, 0.9H), 1.42 (s, 4.5H), 1.38 (s, 1.8H), 1.01-0.77 (m, 8H), 0.00 (m, 9H).; ^{13}C NMR (200 MHz, $CDCl_3$, 5:2:2:1 mixture of rotamers) δ 171.7, 171.3, 171.1, 171.0, 170.9, 170.8, 170.3, 156.3, 155.7, 155.2, 154.3, 137.1, 136.8, 136.8, 136.7, 116.7, 115.8, 115.8, 115.6, 80.4, 80.3, 79.8, 79.7, 64.0, 63.7, 63.5, 63.4, 61.5, 61.1, 59.8, 59.5, 58.7, 58.0, 56.4, 56.3, 31.8, 31.4, 30.1, 30.0, 29.9, 29.9, 29.4, 29.2, 29.2, 29.1, 28.9, 28.7, 28.4, 28.4, 28.3, 28.3, 28.3, 28.2, 27.7, 27.6, 27.5, 27.4, 27.2, 27.0, 20.4, 20.0, 19.8, 19.5, 18.3, 18.2, 18.1, 17.6, 17.5, 17.5, 17.4, -1.6, -1.7.;

HRMS (FAB+) calcd for $C_{23}H_{45}N_2O_5Si$ ($M+H^+$) 457.3098, found 457.3102; IR (thin film, neat) ν_{max} 2961, 1738, 1689, 1656, 1391, 1367, 1307, 1252, 1176, 1158, 861, 838 cm^{-1}

Boc-L-Val-*N*Me-L-Val-*N*Me-L-Hag-OTMSE (11) To a solution of Boc-*N*Me-L-Val-*N*Me-L-Hag-OTMSE **10** (5.6 g, 12.17 mmol) in CH_2Cl_2 (48 mL) was added TFA (12 mL) dropwise at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 4.1 g (95%) of free amine as a yellow liquid. The free amine was immediately used in the next step without further purification. To a solution of the above amine (4.1 g, 11.57 mmol), Boc-L-Val-OH (3.8 g, 17.49 mmol), DIPEA (6.0 mL, 34.71 mmol) and HOAt (1.6 g, 11.57 mmol) in CH_2Cl_2 (23 mL) was added EDC (6.7 g, 34.71 mmol) in three portions over a period of 30 min at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:6) to give 4.8 g (75%) of tripeptide **11** as a colorless oil. $[\alpha]^{20}_D$ -110.9 (*c* = 1.0, $CHCl_3$); ^1H NMR (800 MHz, $CDCl_3$, a mixture of rotamers) Major rotamer δ 5.88-5.68 (m, 1H), 5.20 (d, *J* = 10.8 Hz, 1H), 5.11 (d, *J* = 9.6 Hz, 1H), 5.05 (dd, *J* = 10.9, 4.7 Hz, 1H), 5.03-4.92 (m, 2H), 4.44-4.34 (m, 1H), 4.17-4.01 (m, 2H), 3.07 (s, 3H), 3.00 (s, 3H), 2.35-2.28 (m, 1H), 2.07-2.01 (m, 1H), 1.93-1.85 (m, 3H), 1.77-1.73 (m, 1H), 1.39 (s, 9H), 0.97-0.73 (m, 14H), 0.00 (s, 9H).; ^{13}C NMR (200 MHz, $CDCl_3$, a mixture of rotamers) Major rotamer δ 173.4, 171.3, 171.1, 155.9, 136.9, 115.6, 79.6, 63.6, 57.8, 56.2, 55.5, 31.8, 31.0, 30.4, 30.0, 28.2, 27.5, 27.3, 19.5, 19.2, 18.2, 17.6, 17.4, -1.6.; HRMS (FAB+) calcd for

$C_{28}H_{54}N_3O_6Si$ ($M+H^+$) 556.3782, found 556.3774; IR (thin film, neat) ν_{max} 3329, 2962, 1736, 1711, 1640, 1490, 1252, 1175, 861, 839, 772 cm^{-1}

2-(trimethylsilyl)ethyl (6*S*,9*S*,12*S*)-6,9-diisopropyl-2,2,8,11-tetramethyl-4,7,10-trioxo-12-(3-oxopropyl)-3-oxa-5,8,11-triazatridecan-13-oate (12) To a stirred solution of the Boc-L-Val-*N*Me-L-Val-*N*Me-L-Hag-OTMSE **11** (1.8 g, 3.30 mmol) and 4-methylmorpholine N-oxide (1.2 g, 9.87 mmol) in THF/H_2O (3:1, 33 mL) was added OsO_4 (0.1 M in toluene, 3.3 mL, 0.33 mmol) dropwise at 0 °C. After stirring for 5h at the same temperature, the reaction mixture was quenched with 1*N* $Na_2S_2O_3$ and extracted with Et_2O. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford crude diol as a yellow oil. The diol was directly used in the next step without further purification. To a solution of the above diol (3.3 mmol) in THF/H_2O (3:1, 33 mL) was added $NaIO_4$ (2.1 g, 9.87 mmol) at 0 °C. After stirring for 30 min at the same temperature, the reaction mixture was filtered through a pad of Celite and extracted with Et_2O. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:1.5) to afford 1.7 g (90% for 2 steps) of aldehyde **12** as a colorless foam. $[\alpha]^{20}_D$ -102.4 (*c* = 2.0, $CHCl_3$); ^1H NMR (800 MHz, $CDCl_3$, a mixture of rotamers) Major rotamer δ 9.68 (s, 1H), 5.14 (d, *J* = 10.9 Hz, 1H), 5.11 (d, *J* = 9.5 Hz, 1H), 4.94 (dd, *J* = 10.1, 4.7 Hz, 1H), 4.39 (dd, *J* = 9.5, 6.9 Hz, 1H), 4.17-4.13 (m, 2H), 3.08 (s, 3H), 3.00 (s, 3H), 2.46-2.24 (m, 4H), 1.97-1.87 (m, 2H), 1.38 (s, 9H), 0.97-0.74 (m, 14H), 0.00 (s, 9H).; ^{13}C NMR (200 MHz, $CDCl_3$, a mixture of rotamers) Major rotamer δ 200.4, 173.6, 171.5, 170.3, 155.9, 79.6, 63.8, 57.9, 56.6, 55.5, 40.2, 32.3, 30.9, 30.5, 28.2, 27.4, 20.6, 19.5, 19.2, 18.3, 17.6, 17.4, -1.6.; HRMS (FAB+) calcd for $C_{27}H_{52}N_3O_7Si$ ($M+H^+$) 558.3575, found

558.3571; IR (thin film, neat) ν_{max} 3329, 2963, 1734, 1639, 1490, 1252, 1175, 861, 839, 772 cm^{-1}

Boc-L-Val-*N*Me-L-Val-*N*Me-4MeO-L-Trp-OTMSE (9) The 2-iodo-3-methoxyaniline (2.3 g, 9.12 mmol), DABCO (2.3 g, 20.88 mmol) and aldehyde **12** (3.9 g, 6.96 mmol) were dissolved in DMF (20 mL). The solution was degassed by bubbling Ar gas in a vacuum for 20 min. Then, $Pd(OAc)_2$ (234 mg, 1.04 mmol) was added and the flask was immersed into a preheated oil bath (100 °C). After stirring for 12 h at the same temperature under dark circumstance, the reaction mixture was cooled to room temperature and extracted with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:2) to afford 2.7 g (59%) of tirpeptide **9** as a brown solid. $[\alpha]^{20}_D$ -110.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, 3:1 mixture of rotamers) δ 11.95 (s, 0.7H), 11.89 (s, 0.3H), 8.04 (m, 1H), 7.33 (d, *J* = 2.0 Hz, 0.3H), 7.29 (s, 0.7H), 7.25-7.18 (m, 2H), 6.68 (d, *J* = 7.5 Hz, 0.3H), 6.62 (d, *J* = 7.6 Hz, 0.7H), 6.07 (dd, *J* = 11.4, 4.5 Hz, 0.7H), 5.88 (dd, *J* = 11.4, 3.6 Hz, 0.3H), 5.48 (d, *J* = 10.6 Hz, 0.7H), 5.02 (d, *J* = 10.4 Hz, 0.3H), 4.71 (dd, *J* = 9.5, 7.7 Hz, 0.3H), 4.62 (dd, *J* = 9.2, 7.8 Hz, 0.7H), 4.49-4.45 (m, 0.3H), 4.41-4.29 (m, 2H), 4.12 (s, 0.7H), 4.09 (dd, *J* = 15.2, 4.0 Hz, 0.7H), 3.92 (s, 2.3H), 3.86 (dd, *J* = 14.9, 11.6 Hz, 0.7H), 3.49 (dd, *J* = 14.4, 11.4 Hz, 0.3H), 3.25 (s, 0.7H), 3.23 (s, 0.7H), 3.21 (s, 2.3H), 2.91 (s, 2.3H) 2.55-2.49 (m, 0.7H), 2.40-2.36 (m, 0.3H), 2.35-2.31 (m, 0.3H), 2.14-2.08 (m, 0.7H), 1.48 (s, 9H), 1.16 (d, *J* = 6.7 Hz, 0.7H), 1.15 (d, *J* = 6.8 Hz, 0.7H), 1.03 (d, *J* = 6.4 Hz, 2.3H), 0.98 (d, *J* = 6.4 Hz, 2.3H), 0.97 (d, *J* = 6.3 Hz, 2.3H), 0.86 (d, *J* = 6.8 Hz, 2.3H), 0.84 (t, *J* = 7.2 Hz, 2H), 0.60 (d, *J* = 6.7 Hz, 0.7H), 0.11 (d, *J* = 6.5 Hz, 0.7H), 0.08 (s,

2.3H), 0.02 (s, 6.7H).; ^{13}C NMR (200 MHz, Pyridine-d_5, 3:1 mixture of rotamers) δ 173.1, 172.6, 171.8, 171.6, 171.0, 170.5, 156.9, 156.8, 155.4, 155.1, 139.5, 139.4, 122.9, 122.8, 122.8, 118.1, 118.1, 111.5, 111.0, 105.6, 105.3, 99.7, 99.2, 78.6, 78.6, 63.8, 63.3, 62.1, 59.6, 58.8, 57.7, 56.5, 55.5, 55.2, 33.1, 31.7, 30.8, 30.7, 30.6, 30.2, 29.8, 28.4, 27.6, 27.5, 27.4, 27.0, 26.8, 22.8, 20.4, 20.2, 20.0, 18.2, 18.1, 18.0, 18.0, 17.6, 17.5, -1.6, -1.6.; HRMS (FAB+) calcd for $C_{34}H_{56}N_4O_7Si$ (M^+) 660.3918, found 660.3931; IR (thin film, neat) ν_{max} 3327, 2962, 1715, 1634, 1509, 1366, 1254, 1173, 1090, 839, 755 cm^{-1}

Boc-*N*Me-L-Thr(OBn)-L-Val-OAllyl (14) To a solution of Boc-*N*Me-L-Thr(OBn)-OH[71] **13** (2.0 g, 6.25 mmol), H-L-Val-OAllyl (1.8 g, 11.70 mmol), DIPEA (3.3 mL, 18.74 mmol) and Oxyma (888 mg, 6.25 mmol) in DMF (63 mL) was added EDC (2.9 g, 18.74 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with Et_2O. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:3) to give 2.7 g (93%) of dipeptide **14** as a colorless oil. $[\alpha]^{20}_D$ -22.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$, 2:1 mixture of rotamers) δ 7.28 (br, 4H), 7.23 (br, 1H), 6.86 (d, *J* = 5.1 Hz, 0.7H), 6.51 (d, *J* = 6.8 Hz, 0.3H), 5.87 (dq, *J* = 10.8, 5.8 Hz, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.22 (d, *J* = 10.0 Hz, 1H), 4.63-4.56 (m, 3H), 4.51 (abq, *J* = 11.8 Hz, 2H), 4.51 (dd, *J* = 8.2, 4.6 Hz, 1H), 4.26-4.22 (m, 0.3H), 4.21-4.17 (m, 0.7H), 2.93 (s, 1H), 2.85 (s, 2H), 2.21-2.13 (m, 1H), 1.45 (s, 9H), 1.25 (d, *J* = 6.0 Hz, 3H), 0.89 (d, *J* = 6.7 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H).; ^{13}C NMR (200 MHz, $CHCl_3$, 2:1 mixture of rotamers) δ 171.2, 169.6, 169.3, 156.8, 155.8, 138.5, 138.3, 131.6, 131.5, 128.2, 127.5, 127.4, 119.0, 118.8, 80.9, 80.3, 77.2, 73.5, 72.5, 71.4, 70.7, 65.8, 65.7,

64.1, 63.4, 56.9, 32.3, 32.2, 31.3, 31.0, 28.3, 19.0, 17.6, 17.5, 16.7.; HRMS (FAB+) calcd for $C_{25}H_{39}N_2O_6$ ($M+H^+$) 463.2808, found 463.2821; IR (thin film, neat) ν_{max} 3342, 2974, 1742, 1686, 1219, 1150, 772 cm^{-1}

Boc-L-Thr-*N*Me-L-Thr(OBn)-L-Val-OAllyl (15) To a solution of Boc-*N*Me-L-Thr(OBn)-L-Val-OAllyl **14** (1.5 g, 3.26 mmol) in CH_2Cl_2 (8 mL) was added TFA (2 mL) dropwise at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 1.2 g (100%) of amine as a yellow oil. The free amine was used in the next step without further purification. To a solution of the above amine (1.2 g, 3.26 mmol), Boc-L-Thr-OH (1.7 g, 7.84 mmol), DIPEA (1.7 mL, 9.78 mmol) and HOAt (444 mg, 3.26 mmol) in DMF (10 mL) was added HATU (3.7 g, 9.78 mmol) at 0 °C. After stirring for 13 h at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with Et_2O. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:3) to give 1.2 g (67%) of tripeptide **15** as a yellow oil. $[\alpha]^{20}_D$ -47.3 (*c* = 1.0, $CHCl_3$); ^1H NMR (800 MHz, $CDCl_3$, a mixture of rotamers) Major rotamer δ 7.79 (d, *J* = 8.3 Hz, 1H), 7.32-7.17 (m, 5H), 5.93-5.83 (m, 1H), 5.64 (d, *J* = 7.3 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.4 Hz, 1H), 5.21 (ddd, *J* = 10.4, 2.4, 1.2 Hz, 1H), 4.83 (d, *J* = 10.0 Hz, 1H), 4.64-4.57 (m, 2H), 4.56 (d, *J* = 11.0 Hz, 1H), 4.41 (dd, *J* = 8.3, 6.1 Hz, 1H), 4.37 (s, 1H), 4.26 (d, *J* = 11.1 Hz, 1H), 4.22 (d, *J* = 7.4 Hz, 1H), 3.92 (dq, *J* = 11.8, 5.9 Hz, 1H), 3.63 (q, *J* = 6.4 Hz, 1H), 2.75 (s, 3H), 2.11-2.05 (m, 1H), 1.40 (s, 9H), 1.26 (d, *J* = 5.9 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.0 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H).; ^{13}C

NMR (200 MHz, $CHCl_3$, a mixture of rotamers) Major rotamer δ 175.3, 170.9, 167.7, 156.9, 137.4, 131.7, 128.5, 128.5, 128.1, 118.7, 80.4, 71.3, 70.3, 66.6, 65.6, 64.8, 57.8, 51.3, 30.8, 29.0, 28.3, 19.1, 18.8, 18.4, 16.7.; HRMS (ESI+) calcd for $C_{29}H_{45}N_3NaO_8$ ($M+Na^+$) 586.3099, found 586.3082; IR (thin film, neat) ν_{max} 3328, 2976, 1681, 1524, 1219, 1168, 772 cm^{-1}

H-*N*Me-L-Leu-OAllyl (S4) To a solution of Boc-*N*Me-L-Leu-OH[4] **S2** (5.3 g, 21.60 mmol) and potassium carbonate (4.0 g, 32.40 mmol) in DMF (50 mL) was added allyl bromide (2.2 mL, 25.92 mmol) at room temperature. After stirring for 6 h at the same temperature, the reaction mixture was quenched with H_2O and extracted with Et_2O. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:10) to afford 5.4 g (87%) of Boc-*N*Me-L-Leu-OAllyl **S3** as a colorless oil. $[\alpha]^{20}_D$ -26.0 (*c* = 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, 1:1 mixture of rotamers) δ 5.99-5.75 (m, 1H), 5.27 (d, *J* = 17.1 Hz, 1H), 5.19 (t, *J* = 9.5 Hz, 1H), 4.85 (t, *J* = 7.9 Hz, 0.5H), 4.56 (s, 2.5H), 2.77 (s, 1.5H), 2.74 (s, 1.5H), 1.75-1.57 (m, 2H), 1.55-1.48 (m, 1H), 1.42 (s, 4.5H), 1.41 (s, 4.5H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H).; ^{13}C NMR (125 MHz, $CHCl_3$, 1:1 mixture of rotamers) δ 172.2, 171.9, 156.2, 155.6, 131.9, 131.8, 118.2, 118.0, 80.2, 79.8, 65.4, 65.3, 57.2, 56.0, 37.9, 37.5, 30.4, 30.4, 28.3, 24.9, 24.6, 23.2, 21.3, 21.1.; HRMS (ESI+) calcd for $C_{15}H_{28}NO_4$ ($M+H^+$) 286.2018, found 286.2013; IR (thin film, neat) ν_{max} 2961, 1745, 1698, 1392, 1367, 1325, 1153, 773 cm^{-1}

To a solution of Boc-*N*Me-L-Leu-OAllyl **S3** (3.0 g, 10.51 mmol) in CH_2Cl_2 (40 mL) was added TFA (10 mL) dropwise at 0 °C. After stirring for 5 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined

organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 1.4 g (74%) of H-*N*Me-L-Leu-OAllyl **S4** as a colorless oil. The free amine was used in the next step without further purification.

Boc-L-Thr-*N*Me-L-Thr(OBn)-L-Val-*N*Me- L-Leu-OAllyl (16) To a solution of Boc-L-Thr-*N*Me-L-Thr(OBn)-L-Val-OAllyl **15** (4.0 g, 7.14 mmol) and $Pd(PPh_3)_4$ (400 mg, 0.36 mmol) in dry THF (24 mL) was added *N*-methylaniline (1.6 mL, 14.28 mmol) at room temperature. After stirring for 1 h, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 9:1) to afford 3.6 g (96%) of the acid as a yellow oil. To a solution of the above acid (2.4 g, 4.60 mmol), H-*N*Me-L-Leu-OAllyl **S4** (1.7 g, 9.18 mmol), DIPEA (2.4 mL, 13.81 mmol) in CH_2Cl_2 (46 mL) was added DEPBT (2.8 g, 9.20 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/Hexane = 1:2) to give 2.7 g (83%) of tetrapeptide **16** as a white foam. $[\alpha]^{20}_D$ –53.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$, a mixture of rotamers) Major rotamer δ 7.93 (d, *J* = 8.6 Hz, 1H), 7.35-7.16 (m, 5H), 5.84 (ddd, *J* = 10.4, 9.9, 5.2 Hz, 1H), 5.62 (d, *J* = 7.3 Hz, 1H), 5.29-5.25 (m, 1H), 5.21-5.19 (m, 1H), 4.95-4.89 (m, 1H), 4.80 (d, *J* = 10.1 Hz, 1H), 4.58-4.53 (m, 3H), 4.38 (s, 1H), 4.26 (d, *J* = 11.1 Hz, 1H), 4.21 (d, *J* = 7.3 Hz, 1H), 3.89 (dd, *J* = 10.1, 5.9 Hz, 1H), 3.63 (q, *J* = 6.4 Hz, 1H), 3.30 (s, 1H), 3.05 (s, 2H), 2.99 (s, 1H), 2.75 (s, 2H), 2.13-2.07 (m, 1H), 1.75-1.62 (m, 3H), 1.41 (s, 9H), 1.20 (d,

J = 5.9 Hz, 3H), 0.98-0.78 (m, 15H).; ^{13}C NMR (200 MHz, $CHCl_3$, a mixture of rotamers) Major rotamer δ 175.4, 172.6, 171.5, 167.6, 156.8, 137.3, 131.7, 128.5, 128.2, 128.1, 127.6, 118.8, 80.6, 71.3, 70.2, 66.6, 65.8, 64.7, 54.8, 54.3, 51.2, 37.0, 31.2, 30.5, 28.8, 28.3, 24.8, 23.4, 21.4, 19.1, 18.8, 18.8, 16.4.; HRMS (FAB+) calcd for $C_{36}H_{59}N_4O_9$ ($M+H^+$) 691.4282, found 691.4280; IR (thin film, neat) ν_{max} 3317, 2972, 1740, 1632, 1496, 1219, 1171, 772 cm^{-1}

Boc-L-βHyPhe-OAllyl (18) To a solution of H-L-βHyPhe-OH **17** (700 mg, 3.86 mmol) and trimethylamine (0.7 mL, 5.02 mmol) in 1,4-dioxane/H_2O (2:1, 12 mL) was added Boc anhydride (1.2 mL, 5.02 mmol) dropwise at 0 °C. After stirring overnight, the reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$. The aqueous layer was neutralized with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 1.1 g (100%) of the crude Boc-protected βHyPhe as a white foam. The Boc-protected amino acid was used in the next step without further purification. To a solution of Boc-L-βHyPhe-OH (1.1 g, 3.86 mmol) and potassium carbonate (800 mg, 5.79 mmol) in DMF (13 mL) was added allyl bromide (0.4 mL, 4.63 mmol) at room temperature. After stirring overnight at the same temperature, the reaction mixture was diluted with Et_2O and washed with brine. The combined organic layer was dried over MgSO4 and concentrated in vacuo. The residue was purified by flash column chromatography on a silica gel (EtOAc/Hexane = 1:5) to afford 1.1 g (85% for 2 steps) of the Boc-L-βHyPhe-OAllyl **18** as a colorless oil. $[\alpha]^{20}_D$ –16.7 (c = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.35 (d, J = 7.4 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.26 (t, J = 6.9 Hz, 1H), 5.90-5.80 (m, 1H), 5.33 (d, J = 8.3 Hz, 1H), 5.30 (d, J = 17.3 Hz, 1H), 5.22 (d, J = 10.2 Hz, 2H), 4.62 (s, 2H), 4.53 (d, J = 7.1 Hz, 1H), 2.84 (s, 1H), 1.31 (s, 9H).; ^{13}C NMR (200 MHz, $CHCl_3$) δ 170.6, 155.6, 139.8, 131.5, 128.3, 128.0, 126.0, 118.8, 80.0, 73.9, 66.2, 59.5, 28.1.;

HRMS (FAB+) calcd for $C_{17}H_{24}NO_5$ ($M+H^+$) 322.1654, found 322.1645; IR (thin film, neat) ν_{max} 3438, 2979, 1720, 1697, 1503, 1368, 1220, 1164, 1058, 772, 707 cm^{-1}

Boc-L-Val-L-βHyPhe-OAllyl (19) To a solution of Boc-L-βHyPhe-OAllyl **18** (60 mg, 0.19 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (0.3 mL) dropwise at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was directly concentrated *in vacuo*. The residue was used in the next step without further purification. To a solution of the above amine salt (0.19 mmol), Boc-L-Val-OH (45 mg, 0.22 mmol) and DIPEA (0.07 mL, 0.37 mmol) in dry THF (2 mL) was added DEPBT (112 mg, 0.37 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/Hexane = 1:2) to give 57 mg (72% for 2 steps) of dipeptide **19** as a white solid. $[\alpha]^{20}_D$ –30.6 (*c* = 2.0, $CHCl_3$); ^1H NMR (800 MHz, $CDCl_3$) δ 7.34 (d, *J* = 7.4 Hz, 2H), 7.27 (t, *J* = 7.6 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 5.86-5.77 (m, 1H), 5.28-5.26 (m, 2H), 5.19 (d, *J* = 10.4 Hz, 1H), 4.97 (d, *J* = 8.5 Hz, 1H), 4.87 (d, *J* = 5.9 Hz, 1H), 4.65-4.54 (m, 2H), 3.92 (s, 1H), 3.85 (t, *J* = 7.5 Hz, 1H), 1.98-1.92 (m, 1H), 1.37 (s, 9H), 0.86 (d, *J* = 5.8 Hz, 3H), 0.80 (d, *J* = 5.8 Hz, 3H).; ^{13}C NMR (200 MHz, $CHCl_3$) δ 171.9, 169.9, 155.8, 139.7, 131.4, 128.3, 127.9, 126.0, 118.8, 79.9, 73.6, 66.2, 59.8, 58.1, 30.9, 28.2, 19.0, 17.8.; HRMS (FAB+) calcd for $C_{22}H_{33}N_2O_6$ ($M+H^+$) 421.2339, found 421.2352; IR (thin film, neat) ν_{max} 3341, 2974, 1742, 1661, 1524, 1220, 1173, 772 cm^{-1}

Pentapeptide (22) To a solution of tripeptide **9** (360 mg, 0.55 mmol) in dry THF (2 mL) was added TBAF (1 M in THF, 1.1 mL, 1.10 mmol) dropwise at 0 °C. After stirring for 5 h at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 16:1 to 9:1) to give 290 mg (95%) of the acid as a dark brown solid. To a solution of dipeptide **17** (150 mg, 0.36 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.5 mL) dropwise at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 118 mg (100%) of corresponding amine as a yellow oil. The free amine was used in the next step without further purification. To a solution of the acid (98 mg 0.18 mmol), the crude amine (118 mg, 0.36 mmol), DIPEA (0.1 mL, 0.52 mmol) and HOAt (24 mg, 0.18 mmol) in CH_2Cl_2 (1.5 mL) was added EDC (100 mg, 0.52 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/Hexane = 1:1) to give 144 mg (95%) of pentapeptide **22** as a pale yellow solid. Cα signal of both ^1H and ^{13}C on 4MeO-Trp[4] were not detected and confirmed by COSY 2D NMR experiment. $[\alpha]^{20}_D$ –169.1 (*c* = 2.0, $CHCl_3$); ^1H NMR (800 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.42 (d, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 7.7 Hz, 2H), 7.30 (br, 1H), 7.22 (t, *J* = 7.3 Hz, 1H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 6.68 (s, 1H), 6.48 (d, *J* = 7.8 Hz, 1H), 5.93-5.88 (m, 1H), 5.84 (d, *J* = 5.7 Hz, 1H), 5.45 (d, *J* = 5.6 Hz, 1H), 5.38 (dq, *J* = 17.2, 1.4 Hz, 1H), 5.24 (dq, *J* = 10.5, 1.2 Hz, 1H), 5.10 (d, *J* = 9.5 Hz, 1H), 5.07 (dd, *J* = 9.2, 2.4 Hz, 1H), 5.06 (d, *J* = 10.9 Hz, 1H), 4.70-4.63 (m, 2H), 4.43 (dd, *J* = 9.1, 5.2 Hz, 1H), 4.40 (dd, *J* = 9.2, 3.4 Hz, 1H), 3.86 (s, 3H), 3.38 (dd, *J* = 13.6, 6.5 Hz, 1H), 3.30 (dd, *J* =

13.5, 8.8 Hz, 1H), 2.89 (bs, 3H), 2.88 (s, 3H), 2.37 (bs, 1H), 2.34-2.28 (m, 1H), 1.89-1.84 (m, 1H), 1.39 (s, 9H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.7 Hz, 3H), 0.77 (d, *J* = 6.7 Hz, 3H), 0.62 (d, *J* = 6.8 Hz, 3H), 0.33 (d, *J* = 6.2 Hz, 3H).; ^{13}C NMR (200 MHz, $CHCl_3$) δ 172.9, 171.2, 170.8, 170.5, 169.3, 156.0, 154.0, 140.7, 138.3, 131.8, 128.3, 127.4, 125.5, 123.7, 121.5, 118.3, 116.5, 111.2, 104.9, 99.9, 79.5, 73.7, 66.0, 58.9, 58.2, 57.3, 55.3, 55.2, 30.7, 30.6, 28.3, 26.3, 25.6, 20.0, 19.9, 19.2, 17.8, 16.9, 16.2.; HRMS (FAB+) calcd for $C_{46}H_{66}N_6O_{10}$ (M^+) 862.4840, found 862.4839; IR (thin film, neat) ν_{max} 3340, 2966, 1631, 1509, 1366, 1257, 1219, 1173, 1090, 771 cm^{-1}

Cbz-*N*Me-L-Val-L-Val-OAllyl (21) To a solution of H-L-Val-OAllyl (458 mg, 2.91 mmol), Cbz-*N*Me-L-Val-OH[72] **20** (1.0 g, 3.77 mmol), DIPEA (1.5 mL, 8.73 mmol), HOAt (395 mg, 2.91 mmol) in CH_2Cl_2 (15 mL) was added EDC (1.7 g, 8.73 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/Hexane = 1:5) to give 1.1 g (97% from **20**) of dipeptide **21** as a colorless oil. $[\alpha]^{20}_D$ –88.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 6.46 (d, *J* = 8.1 Hz, 0.8H), 5.94 (d, *J* = 6.1 Hz, 0.2H), 5.87 (dq, *J* = 11.0, 5.8 Hz, 1H), 5.31 (ddd, *J* = 17.2, 2.8, 1.4 Hz, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 5.14 (abq, *J* = 12.3 Hz, 2H), 4.65-4.55 (m, 2H), 4.49 (dd, *J* = 8.9, 4.9 Hz, 1H), 4.11 (d, *J* = 11.2 Hz, 1H), 2.87 (s, 3H), 2.30-2.23 (m, 1H), 2.15-2.10 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H), 0.78 (d, *J* = 6.9 Hz, 3H).; ^{13}C NMR (200 MHz, $CHCl_3$) δ 171.1, 170.0, 157.5, 136.5, 131.6, 128.5, 128.0, 127.7, 118.8, 67.5, 65.7, 65.4, 56.7, 30.9, 29.8, 25.8, 19.5, 18.9,

18.6, 17.4.; HRMS (FAB+) calcd for $C_{22}H_{33}N_2O_5$ ($M+H^+$) 405.2389, found 405.2397; IR (thin film, neat) ν_{max} 3344, 2965, 1742, 1673, 1219, 1160, 772 cm^{-1}

Hexapeptide (23) To a solution of tetrapeptide **16** (454 mg, 0.66 mmol) in CH_2Cl_2 (4 mL) was added TFA (1 mL) dropwise at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 375 mg (95%) of amine as a white foam. The free amine was used in the next step without further purification. To a solution of dipeptide **21** (345 mg, 0.85 mmol) and $Pd(PPh_3)_4$ (99 mg, 0.09 mmol) in dry THF (8.5 mL) was added *N*-methylaniline (0.2 mL, 1.71 mmol) at room temperature. After stirring for 2 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 9:1) to afford 303 mg (98%) of the acid as a yellow oil. To a solution of the crude amine (375 mg, 0.63 mmol), the acid (303 mg 0.83 mmol) and DIPEA (0.2 mL, 1.27 mmol) in CH_2Cl_2 (6 mL) was added DEPBT (380 mg, 1.27 mmol) at 0 °C. After stirring 5 h at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/Hexane = 1:1) to give 445 mg (75%) of hexapeptide **23** as a white solid. $[\alpha]^{20}_D$ -66.3 (*c* = 1.0, $CHCl_3$); ^1H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 9.40 (d, *J* = 8.5 Hz, 1H), 9.36 (d, *J* = 8.5 Hz, 1H), 9.27 (d, *J* = 8.5 Hz, 1H), 7.51-7.42 (m, 3H), 7.41-7.32 (m, 5H), 7.32-7.26 (m, 2H), 6.42 (d, *J* = 3.2 Hz, 1H), 6.00-5.92

(m, 1H), 5.91 (d, J = 7.4 Hz, 1H), 5.77 (dd, J = 8.1, 5.8 Hz, 1H), 5.74 (dd, J = 9.8, 6.0 Hz, 1H), 5.44 (t, J = 9.4 Hz, 1H), 5.39-5.29 (m, 2H), 5.19 (dd, J = 10.4, 1.1 Hz, 1H), 5.09 (t, J = 8.5 Hz, 1H), 4.90 (d, J = 11.2 Hz, 1H), 4.73-4.70 (m, 1H), 4.70-4.66 (m, 2H), 4.63-4.60 (m, 1H), 4.58 (d, J = 11.8 Hz, 1H), 4.52 (p, J = 6.3 Hz, 1H), 4.43 (d, J = 11.4 Hz, 1H), 3.68 (s, 3H), 3.23 (s, 3H), 3.21 (s, 3H), 2.45-2.30 (m, 3H), 1.88-1.82 (m, 2H), 1.64-1.58 (m, 1H), 1.35 (d, J = 6.2 Hz, 3H), 1.09 (d, J = 6.7 Hz, 3H), 1.06 (t, J = 6.5 Hz, 6H), 1.00 (d, J = 6.5 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.81 (d, J = 6.5 Hz, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.5, 172.8, 172.2, 171.6, 169.6, 157.5, 139.4, 137.7, 132.6, 128.9, 128.8, 128.7, 128.7, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.7, 118.4, 73.5, 71.2, 70.9, 68.7, 67.3, 65.8, 64.9, 64.8, 61.2, 59.2, 55.0, 54.9, 54.8, 37.3, 33.4, 31.5, 31.5, 31.1, 30.9, 30.1, 27.4, 24.9, 23.5, 23.4, 21.5, 20.1, 19.9, 19.9, 19.7, 19.5, 19.3, 19.3, 19.2, 19.1, 18.8, 18.7, 18.7, 16.7.; HRMS (ESI+) calcd for $C_{50}H_{77}N_6O_{11}$ ($M+H^+$) 937.5645, found 937.5623; IR (thin film, neat) ν_{max} 3315, 2963, 2873, 1739, 1651, 1537, 1469, 989, 753, 698 cm^{-1}

Tetrapeptide (24) To a solution of tetrapeptide **16** (256 mg, 0.37 mmol) in CH_2Cl_2 (1.6 mL) was added TFA (0.4 mL) dropwise at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 211 mg (97%) of amine as a white foam. The free amine was used in the next step without further purification. To a solution of the crude amine in DMF (3.5 mL) was added acetic anhydride (0.07 mL, 0.72 mmol) at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was quenched with H_2O and extracted with EtOAc. The combined organic layer was dried

over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (Acetone/Hexane = 2:3) to give 159 mg (70%) of tetrapeptide **24** as a white solid. $[\alpha]^{20}_D$ –69.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, 3:2 mixture of rotamers) δ 9.46 (d, *J* = 8.0 Hz, 0.4H), 9.41 (d, *J* = 8.5 Hz, 0.6H), 8.90 (d, *J* = 8.7 Hz, 0.6H), 8.76 (d, *J* = 8.0 Hz, 0.4H), 7.47 (d, *J* = 7.4 Hz, 1.2H), 7.39-7.36 (m, 2H), 7.34 (t, *J* = 7.6 Hz, 0.8H), 7.29-7.25 (m, 1H), 6.48 (s, 0.6H), 5.98-5.90 (m, 1.4H), 5.86 (d, *J* = 8.0 Hz, 0.6H), 5.79 (dd, *J* = 11.3, 4.6 Hz, 0.4H), 5.75 (dd, *J* = 10.6, 5.3 Hz, 0.6H), 5.71 (dd, *J* = 8.7, 5.5 Hz, 0.6H), 5.46 (d, *J* = 9.7 Hz, 0.4H), 5.36-5.29 (m, 1.4H), 5.21-5.17 (m, 1H), 5.05 (t, *J* = 8.6 Hz, 0.6H), 4.95-4.92 (m, 0.4H), 4.68 (d, *J* = 5.5 Hz, 2H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.61-4.57 (m, 0.6H), 4.51 (d, *J* = 11.5 Hz, 0.6H), 4.49-4.44 (m, 1H), 4.43 (d, *J* = 11.6 Hz, 0.4H), 4.30-4.25 (m, 0.4H), 3.68 (s, 1.8H), 3.25 (s, 1.8H), 3.23 (s, 1.2H), 3.18 (s, 1.2H), 2.50-2.41 (m, 0.4H), 2.38-2.32 (m, 0.6H), 2.17 (s, 1.2H), 2.09 (s, 1.8H), 1.94-1.81 (m, 2H), 1.70-1.60 (m, 1H), 1.54 (d, *J* = 6.3 Hz, 1.8H), 1.36 (d, *J* = 5.9 Hz, 1.2H), 1.33 (d, *J* = 6.2 Hz, 1.8H), 1.30 (d, *J* = 6.4 Hz, 1.2H), 1.11 (d, *J* = 6.8 Hz, 1.2H), 1.09 (d, *J* = 6.8 Hz, 1.8H), 1.07 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.5 Hz, 1.2H), 0.95 (d, *J* = 6.5 Hz, 1.8H), 0.91 (d, *J* = 6.7 Hz, 1.2H), 0.88 (d, *J* = 6.7 Hz, 1.8H).; ^{13}C NMR (200 MHz, Pyridine-d_5, 3:2 mixture of rotamers) δ 174.2, 173.6, 173.2, 173.0, 171.9, 171.7, 171.6, 169.9, 169.7, 168.4, 139.3, 138.7, 132.6, 132.6, 128.7, 128.7, 128.6, 128.1, 128.0, 127.7, 118.4, 118.4, 73.3, 71.2, 71.1, 70.9, 68.8, 67.6, 65.8, 65.8, 65.1, 61.1, 55.2, 55.1, 54.9, 54.8, 54.1, 37.3, 37.3, 33.2, 31.5, 31.4, 31.1, 30.8, 29.5, 24.9, 24.8, 23.5, 23.4, 22.9, 22.7, 21.6, 21.5, 20.4, 20.0, 19.4, 19.3, 18.8, 18.5, 16.6, 16.6.; HRMS (FAB+) calcd for $C_{33}H_{53}N_4O_8$ ($M+H^+$) 633.3863, found 633.3852; IR (thin film, neat) ν_{max} 3314, 2962, 1740, 1626, 1535, 1219, 772 cm^{-1}

Heptapeptide (25) To a solution of hexapeptide **23** (419 mg, 0.45 mmol), Boc-L-Val-OH (194 mg, 0.89 mmol), DMAP (165 mg, 1.34 mmol) and HOAt (61 mg, 0.45 mmol) in CH_2Cl_2 (5 mL) was added EDC (259 mg, 1.34 mmol) at room temperature. After stirring 3 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:2) to give 494 mg (97%) of heptapeptide **25** as a white solid. $[\alpha]^{20}_D$ -55.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 9.89 (d, *J* = 8.3 Hz, 1H), 9.68 (d, *J* = 8.2 Hz, 1H), 9.19 (d, *J* = 8.7 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.48-7.41 (m, 4H), 7.40-7.31 (m, 4H), 7.31-7.24 (m, 2H), 6.01-5.90 (m, 1H), 5.89-5.72 (m, 4H), 5.39-5.29 (m, 3H), 5.19 (dd, *J* = 10.4, 1.1 Hz, 1H), 5.05 (t, *J* = 8.5 Hz, 1H), 4.89 (d, *J* = 11.2 Hz, 1H), 4.74-4.66 (m, 3H), 4.66-4.57 (m, 2H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.44-4.37 (m, 1H), 3.64 (s, 3H), 3.24 (s, 3H), 3.23 (s, 3H), 2.54-2.44 (m, 1H), 2.42-2.31 (m, 2H), 2.30-2.17 (m, 1H), 1.93-1.82 (m, 2H), 1.67-1.59 (m, 1H), 1.56 (d, *J* = 6.1 Hz, 3H), 1.50 (s, 9H), 1.35 (d, *J* = 6.1 Hz, 3H), 1.14-1.08 (m, 12H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 6.7 Hz, 6H), 0.86 (d, *J* = 6.4 Hz, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.6, 172.4, 172.4, 172.1, 171.6, 171.3, 171.3, 171.2, 169.7, 157.5, 156.6, 139.4, 139.2, 137.7, 132.7, 132.6, 132.6, 129.2, 129.1, 128.9, 128.8, 128.7, 128.7, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.7, 127.7, 118.4, 118.2, 78.7, 78.6, 73.0, 71.5, 71.4, 70.8, 70.5, 67.3, 67.2, 65.8, 65.6, 64.9, 64.8, 64.7, 61.1, 59.8, 59.4, 58.6, 55.6, 55.5, 55.2, 55.0, 54.8, 54.8, 52.6, 52.6, 37.5, 37.3, 37.3, 33.8, 32.9, 32.2, 31.5, 31.5, 31.4, 31.4, 31.3, 31.2, 31.1, 31.1, 31.0, 30.9, 30.3, 30.2, 28.5, 27.4, 25.1, 24.9, 23.5, 23.5, 23.4, 21.5, 21.2, 20.0, 19.8, 19.8, 19.7, 19.6, 19.5, 19.5, 19.4, 19.4, 19.3, 19.2, 19.1, 18.9, 18.7, 18.7, 18.4, 18.0, 17.9, 17.6, 17.3, 17.1, 17.0, 16.7, 16.6.;

HRMS (ESI+) calcd for $C_{60}H_{93}N_7NaO_{14}$ ($M+Na^+$) 1158.6673, found 1158.6638; IR (thin film, neat) ν_{max} 3319, 2964, 2933, 1741, 1633, 1498, 1368, 1161, 754, 697 cm^{-1}

Pentapeptide (26) To a solution of tetrapeptide **24** (117 mg, 0.19 mmol), Boc-L-Val-OH (80 mg, 0.37 mmol), DMAP (68 mg, 0.56 mmol) and HOAt (25 mg, 0.19 mmol) in CH_2Cl_2 (2 mL) was added EDC (106 mg, 0.56 mmol) at room temperature. After stirring 2 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (Acetone/Hexane = 1:3 to 1:2) to give 144 mg (98%) of pentapeptide **26** as a white solid. $[\alpha]^{20}_D$ –54.0 (*c* = 1.0, $CHCl_3$); ^1H NMR (800 MHz, Pyridine-d_5, 3:2 mixture of rotamers) δ 10.21 (d, *J* = 9.0 Hz, 0.4H), 9.77 (d, *J* = 8.3 Hz, 0.6H), 9.52 (d, *J* = 9.0 Hz, 0.6H), 8.35 (d, *J* = 8.2 Hz, 0.4H), 8.06 (d, *J* = 8.6 Hz, 0.4H), 8.03 (d, *J* = 8.7 Hz, 0.6H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 7.4 Hz, 1H), 7.37-7.32 (m, 2H), 7.27 (t, *J* = 7.3 Hz, 0.4H), 7.24 (t, *J* = 7.4 Hz, 0.6H), 5.99-5.88 (m, 1.4H), 5.86-5.74 (m, 3H), 5.67 (t, *J* = 9.0 Hz, 0.4H), 5.40 (d, *J* = 9.5 Hz, 0.4H), 5.33 (ddd, *J* = 17.2, 3.0, 1.4 Hz, 1H), 5.19 (dd, *J* = 10.4, 1.3 Hz, 1H), 5.02 (t, *J* = 8.6 Hz, 0.6H), 4.99 (t, *J* = 8.1 Hz, 0.4H), 4.70-4.66 (m, 2H), 4.65 (d, *J* = 11.7 Hz, 0.4H), 4.60 (d, *J* = 11.5 Hz, 0.6H), 4.55 (dd, *J* = 8.6, 6.1 Hz, 0.6H), 4.49 (dd, *J* = 8.5, 6.2 Hz, 0.4H), 4.46 (d, *J* = 11.1 Hz, 1H), 4.40-4.36 (m, 0.6H), 4.31-4.27 (m, 0.4H), 3.66 (s, 1.8H), 3.25 (s, 1.8H), 3.20 (s, 1.2H), 3.20 (s, 1.2H), 2.42-2.32 (m, 1.5H), 2.31 (s, 1.2H), 2.25-2.20 (m, 0.5H), 2.18 (s, 1.8H), 1.92-1.81 (m, 2H), 1.67-1.59 (m, 1H), 1.49 (s, 5.4H), 1.49-1.48 (m, 1.8H), 1.48 (s, 3.6H), 1.39 (d, *J* = 5.9 Hz, 1.2H), 1.33 (d, *J* = 6.1 Hz, 1.8H), 1.29 (d, *J* = 6.3 Hz, 1.2H), 1.11 (d, *J* = 6.7 Hz, 1.8H), 1.10 (d, *J* = 6.6 Hz, 1.8H), 1.06 (d, *J* = 6.7 Hz, 3H), 1.04

(d, J = 6.8 Hz, 3H), 1.01 (d, J = 7.2 Hz, 1.2H), 1.00 (d, J = 7.8 Hz, 1.2H), 0.97 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.7 Hz, 1.8H), 0.90 (d, J = 6.6 Hz, 1.2H).; ^{13}C NMR (200 MHz, Pyridine-d_5, 3:2 mixture of rotamers) δ 173.6, 172.8, 172.3, 172.3, 171.7, 171.6, 171.6, 171.5, 171.5, 170.0, 169.7, 168.4, 156.8, 139.1, 138.5, 132.6, 132.6, 128.9, 128.9, 128.7, 128.7, 128.1, 128.1, 128.0, 128.0, 127.7, 118.5, 118.4, 78.7, 72.8, 71.7, 71.7, 71.1, 71.1, 70.4, 65.8, 65.8, 65.0, 61.0, 60.3, 60.3, 55.1, 54.9, 54.9, 54.8, 53.3, 53.2, 37.3, 37.3, 32.6, 31.4, 31.4, 31.1, 31.0, 31.0, 30.9, 29.8, 28.5, 24.9, 24.9, 23.5, 23.4, 23.4, 22.9, 22.9, 21.5, 21.5, 19.4, 19.4, 19.4, 18.8, 18.3, 18.3, 18.3, 17.9, 17.4, 16.6, 16.6.; HRMS (FAB+) calcd for $C_{43}H_{70}N_5O_{11}$ (M+H$^+$) 832.5072, found 832.5084; IR (thin film, neat) ν_{max} 3308, 2966, 1742, 1718, 1679, 1630, 1534, 1368, 1219, 1162, 772 cm^{-1}

Linear dodecapeptide (27) To a solution of pentapeptide **22** (350 mg, 0.41 mmol) in CH_2Cl_2 (3.2 mL) was added TFA (0.8 mL) dropwise at 0 °C. After stirring for 30 min at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 308 mg (99%) of amine as a brown solid. The free amine was used in the next step without further purification. To a solution of heptapeptide **25** (299 mg, 0.26 mmol) and $Pd(PPh_3)_4$ (15 mg, 0.01 mmol) in dry THF (2.5 mL) was added *N*-methylaniline (0.06 mL, 0.53 mmol) at room temperature. After stirring for 1 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 19:1 to 9:1) to afford 287 mg (100%) of the acid as a yellow solid. To a solution of the crude amine (308 mg, 0.40 mmol),

the acid (287 mg 0.26 mmol), HOAt (71 mg, 0.52 mmol) and DIPEA (0.09 mL, 0.52 mmol) in CH_2Cl_2 (1 mL) was added EDC (100 mg, 0.52 mmol) in two portions over a period of 30 min at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3 to 1:1) to give 269 mg (56%) of linear dodecapeptide **27** as a white solid. $[\alpha]^{20}_D$ -74.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamer) Major rotamer δ 11.93 (s, 1H), 9.90 (d, *J* = 7.8 Hz, 1H), 9.54-9.48 (m, 2H), 9.30 (d, *J* = 8.9 Hz, 1H), 9.17 (d, *J* = 8.5 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.94 (d, *J* = 3.9 Hz, 1H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.50-7.43 (m, 5H), 7.41-7.33 (m, 6H), 7.33-7.24 (m, 4H), 7.16 (t, *J* = 7.8 Hz, 1H), 6.55 (d, *J* = 7.8 Hz, 1H), 6.25 (dd, *J* = 10.9, 4.6 Hz, 1H), 5.94-5.88 (m, 1H), 5.88-5.84 (m, 2H), 5.82 (d, *J* = 7.8 Hz, 1H), 5.80-5.78 (m, 1H), 5.78-5.75 (m, 1H), 5.56-5.53 (m, 1H), 5.51 (d, *J* = 10.4 Hz, 1H), 5.38-5.30 (m, 4H), 5.19 (dd, *J* = 8.9, 7.2 Hz, 1H), 5.15 (dd, *J* = 10.5, 1.2 Hz, 2H), 4.91-4.88 (m, 1H), 4.88-4.85 (m, 1H), 4.75-4.68 (m, 3H), 4.67- 4.61 (m, 2H), 4.54 (d, *J* = 11.9 Hz, 1H), 4.44-4.41 (m, 1H), 4.02 (dd, *J* = 15.4, 11.5 Hz, 1H), 3.83 (s, 3H), 3.73 (dd, *J* = 15.4, 4.2 Hz, 1H), 3.66 (s, 3H), 3.45 (s, 3H), 3.38 (s, 3H), 3.23 (s, 3H), 2.80 (s, 3H), 2.58-2.52 (m, 1H), 2.50-2.44 (m, 2H), 2.41-2.34 (m, 3H), 2.29-2.24 (m, 1H), 2.05-2.00 (m, 1H), 1.80-1.75 (m, 1H), 1.59-1.55 (m, 4H), 1.50 (s, 9H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.13-1.00 (m, 22H), 0.99-0.79 (m, 26H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.4, 172.5, 172.4, 172.1, 171.8, 171.4, 171.2, 171.0, 169.6, 157.5, 156.6, 155.1, 142.6, 139.3, 139.2, 137.7, 132.6, 129.1, 128.8, 128.7, 128.7, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.0, 122.7, 122.3, 118.1, 118.1, 111.4, 105.5, 99.6, 78.7, 73.5, 73.2, 71.5, 70.7, 67.3, 65.8, 64.9, 61.1, 59.9, 59.7, 59.3, 58.6, 58.1, 55.2, 55.2, 54.8, 54.5, 52.6, 38.4, 33.1, 32.1, 31.5, 31.3, 31.3, 31.3, 30.3, 30.1,

29.8, 28.5, 27.6, 27.4, 26.3, 25.0, 23.3, 23.0, 22.0, 20.4, 20.0, 19.8, 19.7, 19.6, 19.6, 19.3, 19.2, 18.7, 18.6, 18.4, 18.1, 18.1, 18.0, 17.9, 17.6, 16.0, 16.6.; HRMS (ESI+) calcd for $C_{98}H_{145}N_{13}NaO_{21}$ ($M+Na^+$) 1864.0602, found 1864.0577; IR (thin film, neat) ν_{max} 3313, 2963, 1628, 1509, 1219, 772 cm^{-1}

Linear decapeptide (28) To a solution of pentapeptide **22** (396 mg, 0.46 mmol) in CH_2Cl_2 (3.6mL) was added TFA (0.9 mL) dropwise at 0 °C. After stirring for 30 min at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 313 mg (89%) of amine as a brown solid. The free amine was used in the next step without further purification. To a solution of pentapeptide **26** (333 mg, 0.40 mmol) and $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) in dry THF (4 mL) was added *N*-methylaniline (0.09 mL, 0.80 mmol) at room temperature. After stirring for 30 min at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 16:1 to 9:1) to afford 300 mg (95%) of the acid as a yellow solid. To a solution of the crude amine (313 mg, 0.41 mmol), the acid (300 mg 0.38 mmol), HOAt (52 mg, 0.38 mmol) and DIPEA (0.20 mL, 1.14 mmol) in CH_2Cl_2 (3.8 mL) was added EDC (219 mg, 1.14 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3 to 1:1) to give 309 mg (53%) of linear decapeptide **28** as a white

solid. $[\alpha]^{20}_{D}$ -142.4 (*c* = 1, $CHCl_3$); ^1H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.90 (s, 1H), 10.25 (d, *J* = 8.9 Hz, 0.5H), 9.57 (d, *J* = 8.4 Hz, 0.5H), 9.54 (d, *J* = 9.1 Hz, 0.5H), 9.49 (d, *J* = 8.8 Hz, 1H), 9.38 (d, *J* = 8.8 Hz, 0.5H), 9.27 (d, *J* = 8.8 Hz, 0.5H), 8.27 (d, *J* = 8.2 Hz, 0.5H), 8.08 (d, *J* = 8.6 Hz, 0.5H), 8.04 (d, *J* = 8.6 Hz, 0.5H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.38 (t, *J* = 7.7 Hz, 3H),7.35-7.30 (m, *J* = 10.7, 7.7 Hz, 3H), 7.28-7.22 (m, 2H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.17-7.13 (m, 1H), 6.54 (d, *J* = 7.7 Hz, 1H), 6.24 (dd, *J* = 11.0, 4.4 Hz, 1H), 5.96-5.86 (m, 1H), 5.84 (s, 1H), 5.81 (d, *J* = 8.6 Hz, 1H), 5.79-5.75 (m, 2H), 5.71-5.65 (m, 1H), 5.53 (dd, *J* = 8.8, 3.3 Hz, 1H), 5.50 (dd, *J* = 10.4, 2.4 Hz, 1H), 5.35 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.17 (dd, *J* = 8.9, 7.3 Hz, 1H), 5.14 (dd, *J* = 10.5, 1.3 Hz, 1H), 5.11 (t, *J* = 8.5 Hz, 1H), 4.87-4.83 (m, 1H), 4.74-4.68 (m, 2H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.56 (dd, *J* = 8.5, 6.2 Hz, 1H), 4.47 (d, *J* = 11.5 Hz, 1H), 4.41-4.37 (m, 1H), 4.01 (ddd, *J* = 14.6, 11.5, 2.8 Hz, 1H), 3.83 (s, 3H), 3.72 (dd, *J* = 15.4, 4.3 Hz, 1H), 3.67 (s, 3H), 3.45 (s, 3H), 3.36 (s, 3H), 3.20 (s, 1H), 2.79 (s, 3H), 2.58-2.51 (m, 1H), 2.41-2.30 (m, 3H), 2.18 (s, 3H), 2.05-1.98 (m, 1H), 1.86-1.67 (m, 2H), 1.60-1.53 (m, 1H), 1.50 (s, 9H), 1.32 (d, *J* = 6.0 Hz, 1.5H), 1.29 (d, *J* = 6.3 Hz, 1.5H), 1.11 (d, *J* = 6.8 Hz, 1.5H), 1.10 (d, *J* = 6.7 Hz, 1.5H), 1.08-1.00 (m, 21H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.86 (dd, *J* = 6.6, 2.4 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.81 (t, *J* = 6.6 Hz, 3H), 0.75 (t, *J* = 7.0 Hz, 3H).;^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.3, 172.5, 172.3, 171.9, 171.7, 171.7, 171.6, 171.5, 171.2, 171.0, 171.0, 170.0, 169.6, 168.3, 156.8, 155.1, 142.6, 139.3, 139.1, 138.5, 132.6, 128.9, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.0, 122.7, 122.3, 118.1, 118.1, 111.4, 105.5, 99.6, 79.7, 78.7, 78.7, 73.4, 72.9, 71.7, 71.6, 71.2, 71.1, 70.6, 65.8, 61.1, 60.2, 59.7, 59.3, 58.0, 55.2, 55.1, 55.0, 54.8, 54.4, 54.4, 53.3, 53.2, 38.3, 38.3, 32.7, 32.0, 31.7, 31.3, 31.2, 31.2, 31.0, 30.9, 30.2, 29.8, 28.4, 27.5, 26.3, 25.0, 25.0, 23.1, 23.1, 22.9, 22.9, 22.8, 21.9, 21.8, 20.4, 19.8, 19.7,

19.6, 19.6, 19.6, 19.4, 19.4, 18.6, 18.3, 18.3, 18.3, 18.2, 18.2, 18.2, 18.1, 18.0, 18.0, 17.9, 17.4, 16.6, 16.6, 14.2.; HRMS (ESI+) calcd for $C_{81}H_{122}N_{11}O_{18}$ ($M+H^+$) 1536.8964, found 1536.8890; IR (thin film, neat) ν_{max} 3318, 2964, 2360, 2341, 1629, 1509, 1219, 1091, 772 cm^{-1}

Cyclic dodecapeptide (29) To a solution of linear dodecapeptide **27** (269 mg, 0.15 mmol) and $Pd(PPh_3)_4$ (17 mg, 0.02 mmol) in dry THF (1.5 mL) was added *N*-methylaniline (0.03 mL, 0.80 mmol) at room temperature. After stirring for 2 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 9:1 to 6:1) to afford 246 mg (93%) of the acid as a yellow solid. To a solution of the above acid (115 mg, 0.06 mmol) in CH_2Cl_2 (80 mL) was subsequently added TFA (8 mL) and triisopropylsilane (0.3 mL, 1.27 mmol) at room temperature. After stirring for 20 min at room temperature, the reaction mixture was concentrated *in vacuo* to afford TFA salt as a white powder. This cyclization precursor was used in the next step without further purification. To a solution of PyBOP (167 mg, 0.32 mmol) and DIPEA (0.06 mL, 0.32 mmol) in CH_2Cl_2 (68 mL) was added a solution of the cyclization precursor (0.06 mmol) in CH_2Cl_2 (60 mL) via syringe pump over a period of 10 h at room temperature. After stirring for 3 days at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3) to give 49 mg (45% for 2 steps) of cyclic dodecapeptide **29** as a

white powder. $[\alpha]^{20}_{D}$ -69.0 (*c* = 1, $CHCl_3$); ^1H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.81 (d, *J* = 1.6 Hz, 1H), 10.16 (d, *J* = 8.8 Hz, 1H), 9.66 (d, *J* = 6.7 Hz, 1H), 9.43 (d, *J* = 8.6 Hz, 1H), 9.40 (d, *J* = 9.4 Hz, 1H), 9.33 (d, *J* = 9.2 Hz, 1H), 8.04-8.00 (m, 2H), 7.66 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 7.3 Hz, 2H), 7.48-7.43 (m, 4H), 7.43-7.39 (m, 3H), 7.38-7.33 (m, 1H), 7.33-7.26 (m, 5H), 7.19 (s, 1H), 6.68 (d, *J* = 7.2 Hz, 1H), 6.56 (s, 1H), 6.09 (d, *J* = 6.1 Hz, 1H), 5.95 (s, 1H), 5.87 (d, *J* = 7.7 Hz, 1H), 5.72 (d, *J* = 3.6 Hz, 1H), 5.62 (t, *J* = 7.5 Hz, 1H), 5.50 (d, *J* = 6.7 Hz, 1H), 5.42 (t, *J* = 9.2 Hz, 1H), 5.35 (s, 2H), 5.26 (t, *J* = 8.3 Hz, 1H), 5.08 (t, *J* = 8.1 Hz, 1H), 4.98-4.92 (m, 2H), 4.80-4.69 (m, 3H), 4.68-4.62 (m, 1H), 4.59 (dd, *J* = 11.0, 4.8 Hz, 1H), 4.46 (dd, *J* = 13.5, 4.6 Hz, 1H), 4.34 (dd, *J* = 13.4, 11.3 Hz, 1H), 3.83 (s, 3H), 3.58 (s, 3H), 3.54 (s, 3H), 3.24 (s, 3H), 3.20 (d, *J* = 8.0 Hz, 1H), 3.17 (s, 3H), 3.07-3.00 (m, 1H), 2.71-2.64 (m, 1H), 2.62-2.56 (m, 1H), 2.50 (s, 3H), 2.47-2.38 (m, 2H), 2.28-2.19 (m, 2H), 1.77-1.72 (m, 1H), 1.61-1.53 (m, 4H), 1.43-1.39 (m, 1H), 1.36 (d, *J* = 6.6 Hz, 3H), 1.22 (t, *J* = 6.3 Hz, 6H), 1.17 (t, *J* = 5.9 Hz, 6H), 1.14 (d, *J* = 5.8 Hz, 6H), 1.09 (d, *J* = 6.7 Hz, 3H), 1.04 (d, *J* = 7.1 Hz, 3H), 1.02 (d, *J* = 7.4 Hz, 3H), 0.98-0.92 (m, 6H), 0.90 (d, *J* = 6.7 Hz, 3H), 0.88-0.81 (m, 6H), 0.65 (d, *J* = 6.5 Hz, 6H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 174.5, 174.5, 173.7, 173.2, 172.9, 172.8, 171.7, 171.6, 171.5, 169.9, 169.7, 169.4, 157.5, 154.9, 143.0, 139.9, 139.5, 137.7, 128.9, 128.7, 128.6, 128.3, 128.2, 127.9, 127.9, 127.6, 127.5, 126.9, 124.2, 122.8, 118.4, 112.9, 105.8, 99.5, 74.9, 73.2, 72.5, 70.9, 70.6, 69.4, 67.3, 64.8, 62.3, 61.8, 59.9, 58.7, 58.4, 58.2, 55.9, 55.4, 54.8, 54.3, 52.7, 41.1, 40.8, 39.9, 38.9, 34.5, 33.1, 32.9, 31.9, 31.7, 31.4, 31.3, 30.9, 30.1, 29.0, 27.7, 27.1, 25.0, 23.1, 22.8, 22.0, 20.0, 19.8, 19.8, 19.7, 19.6, 19.6, 19.5, 19.4, 19.3, 19.2, 19.4, 19.0, 18.8, 17.0, 16.7, 14.2.; HRMS (ESI+) calcd for $C_{90}H_{131}N_{13}NaO_{18}$ ($M+Na^+$) 1704.9627, found 1704.9627; IR (thin film, neat) ν_{max} 3307, 2963, 2931, 2873, 1652, 1644, 1634, 1505, 1469, 1455, 752, 698 cm^{-1}

Cyclic decapeptide (30) To a solution of linear decapeptide **28** (93 mg, 0.06 mmol) and $Pd(PPh_3)_4$ (4 mg, 0.003 mmol) in dry THF (1.2 mL) was added *N*-methylaniline (0.01 mL, 0.12 mmol) at room temperature. After stirring for 1 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH =16:1 to 5:1) to afford 88 mg (98%) of the acid as a yellow solid. To a solution of the above acid (96 mg, 0.06 mmol) in CH_2Cl_2 (60 mL) was subsequently added TFA (6 mL) and triisopropylsilane (0.3 mL, 1.28 mmol) at room temperature. After stirring for 20 min at room temperature, the reaction mixture was concentrated *in vacuo* to afford TFA salt as a white powder. This cyclization precursor was used in the next step without further purification. To a solution of PyBOP (167 mg, 0.32 mmol) and DIPEA (0.06 mL, 0.32 mmol) in CH_2Cl_2 (73 mL) was added a solution of the cyclization precursor (0.06 mmol) in CH_2Cl_2 (55 mL) and DMF (3 mL) via syringe pump over a period of 10 h at room temperature. After stirring for additional 3 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 3:4 to 1:1) to give 62 mg (70% for 2 steps) of cyclic decapeptide **30** as a white powder. $[\alpha]^{20}_D$ -33.4 (*c* = 1, MeOH); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.82 (s, 1H), 9.63 (d, *J* = 6.8 Hz, 1H), 9.40 (d, *J* = 9.0 Hz, 2H), 9.25 (d, *J* = 9.0 Hz, 1H), 8.02 (d, *J* = 9.5 Hz, 1H), 7.99 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 2H), 7.50-7.43 (m, 4H), 7.42-7.38 (m, *J* = 7.7, 3.0 Hz, 2H), 7.33-7.25 (m, 4H), 7.19 (d, *J* = 1.7 Hz, 1H), 6.69 (s, 1H), 6.68 (d, *J* = 7.4 Hz, 1H), 6.07 (dd, *J* = 6.3, 2.1 Hz, 1H), 6.03 (s, 1H), 5.75 (dd, *J* = 9.1, 2.3 Hz, 1H), 5.68 (d, *J* = 4.4 Hz, 1H), 5.58 (d, *J* = 7.8 Hz, 1H), 5.51

(d, J = 6.0 Hz, 1H), 5.41 (t, J = 9.0 Hz, 1H), 5.25 (t, J = 8.6 Hz, 1H), 5.16 (dd, J = 7.6, 3.5 Hz, 1H), 4.97 (t, J = 9.3 Hz, 1H), 4.73 (t, J = 8.9 Hz, 1H), 4.71-4.62 (m, 3H), 4.60-4.54 (m, 1H), 4.45 (dd, J = 13.5, 4.5 Hz, 1H), 4.32 (dd, J = 13.4, 11.3 Hz, 1H), 3.83 (s, 3H), 3.55 (s, 3H), 3.54 (s, 3H), 3.18 (s, 3H), 2.73-2.55 (m, 3H), 2.49 (s, 3H), 2.45-2.40 (m, 1H), 2.28-2.22 (m, 1H), 2.11 (s, 3H), 1.80-1.73 (m, 2H), 1.58-1.54 (m, 1H), 1.49 (d, J = 6.4 Hz, 3H), 1.38-1.32 (m, 3H), 1.22 (dd, J = 12.8, 6.7 Hz, 9H), 1.18 (d, J = 7.2 Hz, 3H), 1.17 (d, J = 7.1 Hz, 3H), 1.15 (d, J = 6.7 Hz, 3H), 1.15 (d, J = 5.7 Hz, 3H), 1.04 (d, J = 6.7 Hz, 3H), 1.02 (d, J = 7.1 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H), 0.63 (d, J = 6.5 Hz, 3H), 0.60 (d, J = 6.6 Hz, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 174.5, 173.8, 172.9, 171.8, 171.6, 170.6, 169.9, 169.6, 169.6, 169.4, 154.9, 143.2, 139.8, 139.5, 128.7, 128.6, 127.7, 127.7, 127.6, 127.4, 126.8, 124.2, 122.8, 118.4, 112.9, 105.8, 99.5, 74.5, 72.9, 72.1, 70.6, 69.5, 62.3, 60.0, 58.8, 58.5, 58.2, 55.9, 55.4, 54.3, 52.9, 47.0, 46.6, 46.6, 45.4, 38.9, 34.0, 33.2, 33.0, 31.9, 31.4, 31.0, 30.5, 29.0, 27.1, 26.1, 25.0, 24.6, 23.9, 23.1, 22.6, 22.4, 21.9, 21.7, 20.0, 19.9, 19.7, 19.7, 19.4, 19.4, 19.3, 19.3, 19.1, 19.0, 18.9, 18.7, 17.1, 16.8, 13.6.; HRMS (ESI+) calcd for $C_{73}H_{108}N_{11}O_{15}$ ($M+H^+$) 1378.8021, found 1378.7992; IR (thin film, neat) ν_{max} 3297, 2962, 2929, 1633, 1509, 1256, 848, 769 cm^{-1}

Ohmyungsamycin A (1) To a solution of cyclic dodecapeptide **29** (11 mg, 0.0065 mmol) in MeOH (1 mL) was added 20% palladium hydroxide on carbon (11 mg) and the reaction vial was thoroughly sealed. After stirring for 14 h under H_2 atmosphere, the reaction mixture was filtered through a pad of Celite The filtered solution was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 10:1) to afford 9.2 mg (97%) of ohmyungsamycin A as a white powder. $[\alpha]^{20}_D$ –51.9 (*c* = 0.2, MeOH); ^{1}H NMR (800 MHz, Pyridine-d_5) See Table below.; ^{13}C NMR (200 MHz, Pyridine-d_5) See Table below.; HRMS (FAB+) calcd for $C_{75}H_{119}N_{13}NaO_{16}$ ($M+Na^+$) 1480.8795, found 1480.8806; IR (thin film, neat) ν_{max} 3309, 2961, 1632, 1508, 1201 cm^{-1}

		Natural ohmyungsamycin A (**1**; 500 MHz)		Synthetic ohmyungsamycin A (**1**; 800 MHz)		Δ*δ*, ppm (Natural **1** – Synthetic **1**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
Val[1]	1	-	174.3	-	174.3	-	0.0
	2	4.70, dd, 8.8, 8.3	58.3	4.70, dd, 8.7, 8.3	58.3	0.00	0.0
	3	2.23, m	32.9	2.23, m	32.9	0.00	0.0
	4	1.12, m	19.2	1.13, d, 6.9	19.2	-0.01	0.0
	5	0.97, m	18.6	0.97, d, 6.7	18.6	0.00	0.0
	NH	9.27, d, 9.2	-	9.28, d, 9.2	-	-0.01	-
HyPhe[2]	1	-	172.7	-	172.7	-	0.0
	2	5.49, dd, 7.2, 2.5	60.0	5.50, dd, 7.2, 2.7	60.1	-0.01	-0.1
	3	5.95, d, 2.1	73.1	5.96, s	73.1	-0.01	0.0
	4	-	143.0	-	143.0	-	0.0
	5	7.67, d, 7.5	127.0	7.68, d, 7.5	127.0	-0.01	0.0
	6	7.67, d, 7.5	127.0	7.68, d, 7.5	127.0	-0.01	0.0
	7	7.41, t, 7.4	128.5	7.42, t, 7.7	128.6	-0.01	-0.1
	8	7.41, t, 7.4	128.5	7.42, t, 7.7	128.6	-0.01	-0.1
	9	7.27, d, 7.5	127.5	7.27, d, 7.5	127.5	0.00	0.0
	NH	9.67, d, 7.1	-	9.71, d, 7.0	-	-0.04	-
	OH	-	-	6.64, s[a]	-	-	-
Val[3]	1	-	174.5	-	174.5	-	0.0

		Natural ohmyungsamycin A (**1**; 500 MHz)		Synthetic ohmyungsamycin A (**1**; 800 MHz)		Δδ, ppm (Natural **1** – Synthetic **1**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	2	5.40, t, 9.2	58.3	5.42, t, 9.3	58.3	-0.02	0.0
	3	2.66, m	33.1	2.69, m	33.1	-0.03	0.0
	4	1.36, d, 7.0	19.8	1.37, d, 6.7	19.8	-0.01	0.0
	5	1.20, d, 6.6	20.0	1.20, d, 6.7	20.0	0.00	0.0
	NH	8.03, d, 9.9	-	8.03, d, 9.8	-	0.00	-
NMe-4MeO-Trp[4]	1	-	169.9	-	169.9	-	0.0
	2	4.59, dd, 10.9, 4.3	70.6	4.60, dd, 10.7, 4.6	70.6	-0.01	0.0
	3a	4.45, dd, 13.5, 4.4	27.1	4.46, dd, 13.3, 4.8	27.1	-0.01	0.0
	3b	4.32, dd, 13.3, 11.5		4.34, dd, 13.4, 11.3		-0.02	
	4	-	112.9	-	112.9	-	0.0
	5	7.19, m	124.2	7.20, d, 1.8	124.2	-0.01	0.0
	6	-	139.5	-	139.5	-	0.0
	7	7.30, m	105.8	7.30, m	105.8	0.00	0.0
	8	7.27, m	122.8	7.27, m	122.8	0.00	0.0
	9	6.68, d, 7.3	99.5	6.68, d, 6.8	99.5	0.00	0.0
	10	-	154.9	-	154.9	-	0.0
	11	-	118.4	-	118.4	-	0.0
	OMe	3.83, s	55.4	3.83, s	55.4	0.00	0.0
	NMe	2.50, s	40.8	2.51, s	40.8	-0.01	0.0
	NH	11.80, d, 1.5	-	11.82, d, 1.6	-	-0.02	-
NMe-Val[5]	1	-	169.4	-	169.4	-	0.0
	2	3.20, overlapped	70.9	3.21, d, 7.9	70.9	-0.01	0.0
	3	3.02, m	29.0	3.04, m	29.0	-0.02	0.0
	4	1.22, d, 6.7	22.0	1.22, d, 6.5	22.0	0.00	0.0
	5	1.02, d, 6.3	19.7	1.02, d, 7.4	19.7	0.00	0.0
	NMe	3.18, s	39.9	3.18, s	39.9	0.00	0.0
Val[6]	1	-	172.9	-	172.9	-	0.0
	2	4.96, overlapped	54.3	4.96, m	54.3	0.00	0.0
	3	2.40, m	31.9	2.40, m	31.9	0.00	0.0
	4	1.14, m	19.0	1.14, d, 6.8	19.1	0.00	-0.1
	5	1.03, d, 6.0	19.7	1.03, d, 7.0	19.7	0.00	0.0
	NH	9.38, d, 9.3	-	9.41, d, 9.0	-	-0.03	-
NMe-Leu[7]	1	-	171.7	-	171.7	-	0.0
	2	5.65, t, 7.5	54.8	5.66, t, 7.5	54.8	-0.01	0.0
	3a	1.74, m	38.9	1.74, m	38.9	0.00	0.0

		Natural ohmyungsamycin A (**1**; 500 MHz)		Synthetic ohmyungsamycin A (**1**; 800 MHz)		$\Delta\delta$, ppm (Natural **1** – Synthetic **1**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	3b	1.59, m		1.59, m		0.00	
	4	1.42, m	25.1	1.42, m	25.1	0.00	0.0
	5	0.67, d, 5.8	23.1	0.67, d, 6.6	23.1	0.00	0.0
	6	0.67, d, 5.8	22.1	0.67, d, 6.6	22.1	0.00	0.0
	NMe	3.52, s	31.3	3.52, s	31.3	0.00	0.0
Val[8]	1	-	173.5	-	173.5	-	0.0
	2	5.29, t, 8.2	55.6	5.30, t, 8.2	55.6	-0.01	0.0
	3	2.57, m	31.0	2.57, m	31.0	0.00	0.0
	4	1.20, d, 6.6	19.0	1.20, d, 6.7	19.1	0.00	-0.1
	5	1.16, d, 7.0	19.7	1.16, d, 6.8	19.7	0.00	0.0
	NH	8.06, d, 9.1	-	8.07, d, 8.9	-	-0.01	-
NMe-Thr[9]	1	-	170.6	-	170.6	-	0.0
	2	5.61, d, 3.7	62.4	5.62, d, 3.7	62.4	-0.01	0.0
	3	5.06, overlapped	66.5	5.07, m	66.5	-0.01	0.0
	4	1.34, d, 6.5	20.5	1.35, d, 6.4	20.5	-0.01	0.0
	NMe	3.68, s	34.6	3.69, s	34.6	-0.01	0.0
	OH	-	-	6.16, d, 3.8[a]	-	-	-
Thr[10]	1	-	171.4	-	171.4	-	0.0
	2	5.86, dd, 9.2, 2.3	52.5	5.88, dd, 9.2, 2.5	52.5	-0.02	0.0
	3	6.04, qd, 6.5, 2.4	69.4	6.05, qd, 6.5, 2.6	69.4	-0.01	0.0
	4	1.52, d, 6.5	16.7	1.52, d, 6.5	16.7	0.00	0.0
	NH	10.02, d, 9.0	-	10.08, d, 8.9	-	-0.06	-
Val[11]	1	-	173.2	-	173.2	-	0.0
	2	5.22, dd, 8.8, 7.6	57.9	5.25, dd, 9.1, 7.2	57.9	-0.03	0.0
	3	2.26, m	31.8	2.26, m	31.8	0.00	0.0
	4	0.96, m	19.8	0.97, d, 6.9	19.8	-0.01	0.0
	5	0.96, m	18.8	0.96, d, 6.9	18.8	0.00	0.0
	NH	8.62, d, 9.2	-	8.64, d, 9.2	-	-0.02	-
NMe-Val[12]	1	-	174.2	-	174.3	-	-0.1
	2	3.10, d, 5.8	71.4	3.10, d, 5.8	71.5	0.00	-0.1
	3	2.18, m	32.2	2.18, m	32.3	0.00	-0.1
	4	1.09, m	19.0	1.10, d, 7.2	19.1	-0.01	-0.1
	5	1.09, m	19.0	1.10, d, 7.2	19.1	-0.01	-0.1
	NMe	2.54, s	35.9	2.54, s	35.9	0.00	0.0

[a] Assigned by COSY NMR.

Table S1. Comparison of ^{1}H and ^{13}C NMR spectral data of natural and synthetic ohmyungsamycin A (1)

Δ^{10}*N*-Ac ohmyungsamycin (4) To a solution of cyclic decapeptide **30** (110 mg, 0.08 mmol) in MeOH (5 mL) was added 20% palladium hydroxide on carbon (110 mg) and the reaction vial was thoroughly sealed. After stirring for 2 h under H_2 atmosphere, the reaction mixture was filtered through a pad of Celite The filtered solution was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 16:1) to afford 70 mg (68%) of Δ^{10}N-Ac ohmyungsamycin as a white powder. $[\alpha]^{20}_{D}$ –91.3 (*c* = 1, MeOH); ^{1}H NMR (800 MHz, Pyridine-d_5) See Table below.; ^{13}C NMR (200 MHz, Pyridine-d_5) See Table below.; HRMS (ESI+) calcd for $C_{66}H_{101}N_{11}NaO_{15}$ ($M+Na^{+}$) 1310.7371, found 1310.7391; IR (thin film, neat) ν_{max} 3308, 2960, 1630, 1508, 1199, 1088 cm^{-1}

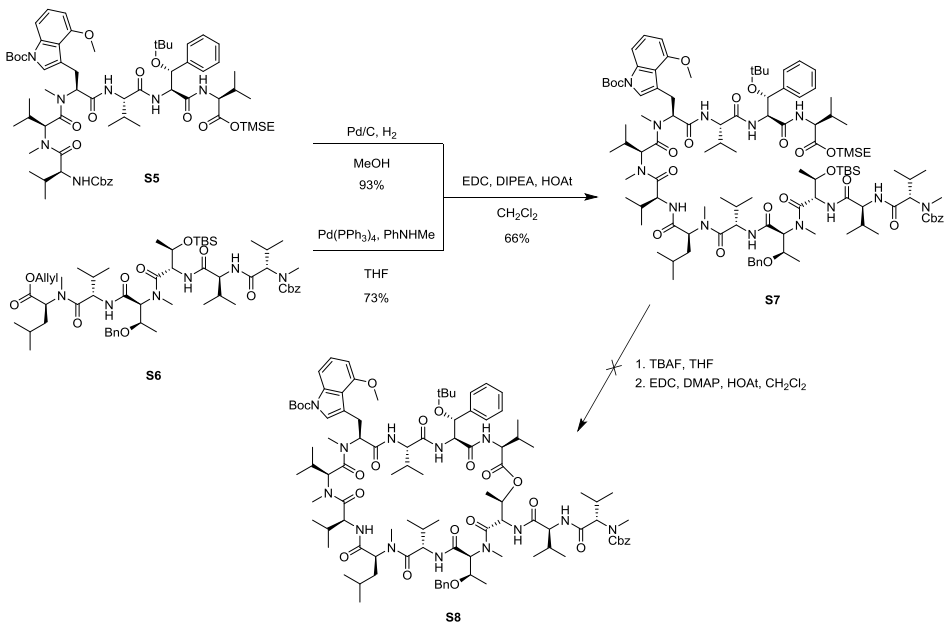
		Synthetic ohmyungsamycin A (**1**; 800 MHz)		Δ^{10}*N*-Ac ohmyungsamycin (**4**; 800 MHz)		$\Delta\delta$, ppm (**1** - **4**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
Val[1]	1	-	174.3	-	174.4	-	-0.1
	2	4.70, dd, 8.7, 8.3	58.3	4.73, t, 8.7	58.4	-0.03	-0.1
	3	2.23, m	32.9	2.26, m	33.0	-0.03	-0.1
	4	1.13, d, 6.9	19.2	1.17, d, 6.7	19.3	-0.04	-0.1
	5	0.97, d, 6.7	18.6	0.99, d, 6.7	18.9	-0.02	-0.3
	NH	9.28, d, 9.2	-	9.35, d, 8.9	-	-0.07	-
HyPhe[2]	1	-	172.7	-	172.8	-	-0.1
	2	5.50, dd, 7.2, 2.7	60.1	5.51, d, 7.5	60.1	-0.01	0.0
	3	5.96, s	73.1	6.01, s	72.9	-0.05	0.2
	4	-	143.0		143.0	-	0.0
	5	7.68, d, 7.5	127.0	7.66, d, 7.6	127.0	0.02	0.0
	6	7.68, d, 7.5	127.0	7.66, d, 7.6	127.0	0.02	0.0
	7	7.42, t, 7.7	128.6	7.42, t, 7.6	128.6	0.00	0.0
	8	7.42, t, 7.7	128.6	7.42, t, 7.6	128.6	0.00	0.0

		Synthetic ohmyungsamycin A (**1**; 800 MHz)		Δ^{10}*N*-Ac ohmyungsamycin (**4**; 800 MHz)		$\Delta\delta$, ppm (**1** - **4**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	9	7.27, d, 7.5	127.5	7.27, d, 7.5	127.5	0.00	0.0
	NH	9.71, d, 7.0	-	9.67, d, 6.2	-	0.04	-
	OH	6.64, s	-	6.70, overlapped	-	-0.06	-
Val[3]	1	-	174.5	-	174.5	-	0.0
	2	5.42, t, 9.3	58.3	5.41, t, 9.2	58.2	0.01	0.1
	3	2.69, m	33.1	2.66, m	33.1	0.03	0.0
	4	1.37, d, 6.7	19.8	1.36, d, 6.6	19.8	0.01	0.0
	5	1.20, d, 6.7	20.0	1.22, overlapped	19.8	-0.02	0.2
	NH	8.03, d, 9.8	-	8.02, d, 9.7	-	0.01	-
NMe-MeO-Trp[4]	1	-	169.9	-	169.9	-	0.0
	2	4.60, dd, 10.7, 4.6	70.6	4.60, dd, 10.3, 3.8	70.6	0.00	0.0
	3a	4.46, dd, 13.3, 4.8	27.1	4.46, dd, 13.5, 4.6	27.1	0.00	0.0
	3b	4.34, dd, 13.4, 11.3		4.33, dd, 13.1, 11.5		0.01	
	4	-	112.9	-	112.9	-	0.0
	5	7.20, d, 1.8	124.2	7.19, d, 1.5	124.2	0.01	0.0
	6	-	139.5	-	139.5	-	0.0
	7	7.30, m	105.8	7.29, m	105.8	0.01	0.0
	8	7.27, m	122.8	7.27, m	122.8	0.00	0.0
	9	6.68, d, 6.8	99.5	6.68, d, 6.6	99.5	0.00	0.0
	10	-	154.9	-	154.9	-	0.0
	11	-	118.4	-	118.4	-	0.0
	OMe	3.83, s	55.4	3.83, s	55.4	0.00	0.0
	NMe	2.51, s	40.8	2.50, s	40.8	0.01	0.0
	NH	11.82, d, 1.6	-	11.81, d, 1.3	-	0.01	-
NMe-Val[5]	1	-	169.4	-	169.4	-	0.0
	2	3.21, d, 7.9	70.9	3.20, overlapped	70.9	0.01	0.0
	3	3.04, m	29.0	3.04, m	29.0	0.00	0.0
	4	1.22, d, 6.5	22.0	1.22, d, 6.8	21.9	0.00	0.1
	5	1.02, d, 7.4	19.7	1.02, d, 7.0	19.8	0.00	-0.1
	NMe	3.18, s	39.9	3.18, s	39.9	0.00	0.0
Val[6]	1	-	172.9	-	172.9	-	0.0
	2	4.96, m	54.3	4.97, overlapped	54.3	-0.01	0.0
	3	2.40, m	31.9	2.42, m	31.9	-0.02	0.0
	4	1.14, d, 6.8	19.1	1.15, d, 6.7	18.9	-0.01	0.2
	5	1.03, d, 7.0	19.7	1.04, d, 6.7	19.7	-0.01	0.0

		Synthetic ohmyungsamycin A (**1**; 800 MHz)		Δ^{10}*N*-Ac ohmyungsamycin (**4**; 800 MHz)		$\Delta\delta$, ppm (**1** - **4**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	NH	9.41, d, 9.0	-	9.41, d, 9.1	-	0.00	-
NMe-Leu[7]	1	-	171.7	-	171.6	-	0.1
	2	5.66, t, 7.5	54.8	5.61, overlapped	54.9	0.05	-0.1
	3a	1.74, m	38.9	1.76, m	38.9	-0.02	0.0
	3b	1.59, m		1.56, m		0.03	
	4	1.42, m	25.1	1.39, m	25.0	0.03	0.1
	5	0.67, d, 6.6	23.1	0.65, d, 6.5	23.1	0.02	0.0
	6	0.67, d, 6.6	22.1	0.63, d, 6.6	19.3	0.04	0.1
	NMe	3.52, s	31.3	3.53, s	31.4	0.0	-0.1
Val[8]	1	-	173.5	-	173.7	-	-0.2
	2	5.30, t, 8.2	55.6	5.27, t, 8.4	55.7	0.03	-0.1
	3	2.57, m	31.0	2.61, m	31.1	-0.04	-0.1
	4	1.20, d, 6.7	19.1	1.22, overlapped	19.3	-0.02	-0.2
	5	1.16, d, 6.8	19.7	1.17, d, 6.9	19.8	-0.01	-0.1
	NH	8.07, d, 8.9	-	8.05, d, 8.7	-	0.02	-
NMe-Thr[9]	1	-	170.6	-	170.5	-	0.1
	2	5.62, d, 3.7	62.4	5.61, overlapped	62.6	0.01	-0.2
	3	5.07, m	66.5	5.02, m	66.3	0.05	0.2
	4	1.35, d, 6.4	20.5	1.32, d, 6.3	20.8	0.03	-0.3
	NMe	3.69, s	34.6	3.68, s	34.4	0.01	0.2
	OH	6.16, d, 3.8	-	6.17, br	-	-0.01	-
Thr[10]	1		171.4	-	171.7	-	-0.3
	2	5.88, dd, 9.2, 2.5	52.5	5.79, dd, 9.0, 2.0	52.8	0.03	-0.3
	3	6.05, qd, 6.5, 2.6	69.4	6.06, d, 4.7	69.5	-0.01	-0.1
	4	1.52, d, 6.5	16.7	1.51, d, 6.5	16.9	0.01	-0.2
	NH	10.08, d, 8.9	-	9.27, d, 9.0	-	0.81	-
Ac	1	-	-	-	170.6	-	-
	2	-	-	2.09, s	22.6	-	-

Table S2. Comparison of ^{1}H and ^{13}C NMR spectral data for the cyclic core of ohmyungsamycin A (1) and Δ^{10}*N*-Ac ohmyungsamycin (4).

Macrocyclizations at other sites

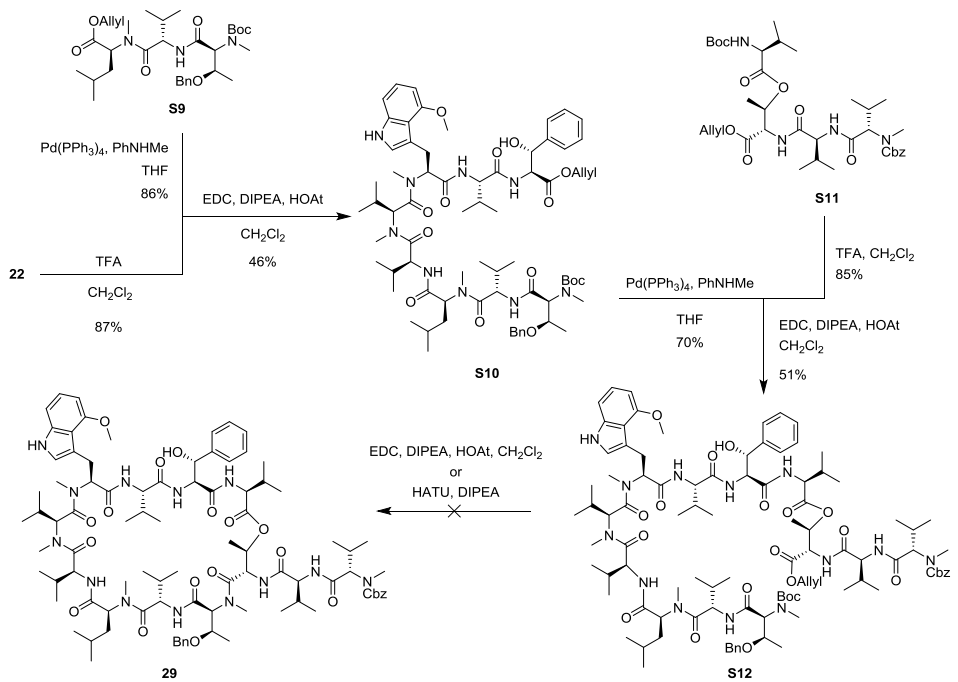


Scheme S1. Attempted macrolactonization between Val[1] and Thr[10]

Linear dodecapeptide (S7) To a solution of hexapeptide **S5** (70 mg, 0.06 mmol) in MeOH (0.6 mL) was added 5% palladium on carbon (35 mg) and the reaction vial was thoroughly sealed. After stirring for 1 h under H_2 atmosphere, the reaction mixture was filtered through a pad of Celite. The filtered solution was concentrated *in vacuo* to afford 58 mg (93%) of free amine as white solid. The free amine was used in the next step without further purification. To a solution of hexapeptide **S6** (106 mg, 0.10 mmol) and $Pd(PPh_3)_4$ (12 mg, 0.01 mmol) in dry THF (1 mL) was added *N*-methylaniline (0.02 mL, 0.20 mmol) at room temperature. After stirring for 2 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and

concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 15:1) to afford 74 mg (73%) of a free acid as a pale yellow oil. To a solution of above crude amine (48 mg, 0.05 mmol), above acid (57 mg, 0.06 mmol), HOAt (12 mg, 0.09 mmol), and DIPEA (0.02 mL, 0.14 mmol) in CH_2Cl_2 (1 mL) was added EDC (26 mg, 0.14 mmol) at -10 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (acetone/hexane = 1:3) to give 61 mg (66%) of linear dodecapeptide **S7** as white solid. $[\alpha]^{20}_D$ -60.0 (*c* = 0.2, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 9.48 (d, *J* = 8.5 Hz, 1H), 9.45 (d, *J* = 8.9 Hz, 1H), 9.42 (d, *J* = 8.6 Hz, 1H), 9.23 (d, *J* = 8.6 Hz, 1H), 9.07 (d, *J* = 9.1 Hz, 1H), 9.00 (d, *J* = 8.7 Hz, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 7.3 Hz, 3H), 7.45 (t, *J* = 8.2 Hz, 5H), 7.38-7.31 (m, 9H), 7.11 (s, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.31 (bs, 1H), 5.95 (d, *J* = 8.1 Hz, 1H), 5.77 (dd, *J* = 9.0, 6.8 Hz, 1H), 5.64 (dd, *J* = 8.5, 5.8 Hz, 1H), 5.60 (d, *J* = 10.6 Hz, 1H), 5.47 (dd, *J* = 8.5, 5.5 Hz, 1H), 5.41-5.31 (m, 3H), 5.23 (dd, *J* = 8.7, 7.4 Hz, 1H), 5.14 (t, *J* = 8.3 Hz, 1H), 5.09-5.07 (m, 1H), 4.94-4.87 (m, 3H), 4.64 (d, *J* = 11.9 Hz, 1H), 4.51 (d, *J* = 11.9 Hz, 2H), 4.42-4.31 (m, 4H), 4.12-4.05 (m, 1H), 3.79 (s, 3H), 3.63 (s, 3H), 3.44 (s, 3H), 3.39 (s, 3H), 3.20 (s, 3H), 3.13 (s, 3H), 2.65-2.59 (m, 1H), 2.47-2.43 (m, 2H), 2.40-2.34 (m, 2H), 2.31-2.23 (m, 2H), 2.03-1.99 (m, 1H), 1.82-1.74 (m, 2H), 1.64 (s, 9H), 1.50 (d, *J* = 6.1 Hz, 3H), 1.32 (d, *J* = 6.0 Hz, 3H), 1.18 (s, 9H), 1.15-1.02 (m, 23H), 0.99 (s, 6H), 1.01-0.93 (m, 14H), 0.91-0.85 (m, 10H), 0.79 (d, *J* = 6.7 Hz, 3H), 0.74 (d, *J* = 6.5 Hz, 3H), 0.04 (s, 15H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.3, 172.9, 172.1, 172.0, 171.8, 171.3, 171.0, 170.0, 169.8, 157.5, 154.8, 142.1, 139.2, 137.7, 136.0, 128.8, 128.8, 128.7, 128.6, 128.4, 128.2, 128.2, 128.0, 127.9, 127.7, 127.6, 120.1, 117.2,

108.8, 104.0, 83.8, 75.6, 74.4, 73.3, 70.5, 67.3, 65.0, 63.4, 60.7, 59.6, 59.0, 58.4, 58.2, 58.0, 55.4, 55.4, 55.1, 55.0, 54.4, 38.3, 33.0, 32.3, 31.9, 31.8, 31.3, 31.3, 30.4, 30.2, 30.1, 28.6, 28.5, 28.0, 28.0, 27.9, 27.6, 27.2, 26.3, 26.1, 25.0, 23.0, 22.0, 20.8, 20.2, 19.9, 19.7, 19.7, 19.6, 19.3, 19.2, 19.1, 18.8, 18.5, 18.4, 18.3, 18.2, 18.2, 18.1, 17.6, 16.6, -1.7, -4.4, -4.7.; HRMS (ESI+) calcd for $C_{110}H_{175}N_{13}NaO_{21}Si_2$ ($M+Na^+$) 2093.2456, found 2093.2495.; IR (thin film, neat) ν_{max} 3311, 2963, 1656, 1628, 1496, 1261, 749.



Scheme S2. Attempted macrolactamization between Thr^9 and Thr^{10}

Octapeptide (S10) To a solution of pentapeptide **22** (200 mg, 0.23 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.2 mL) dropwise at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The

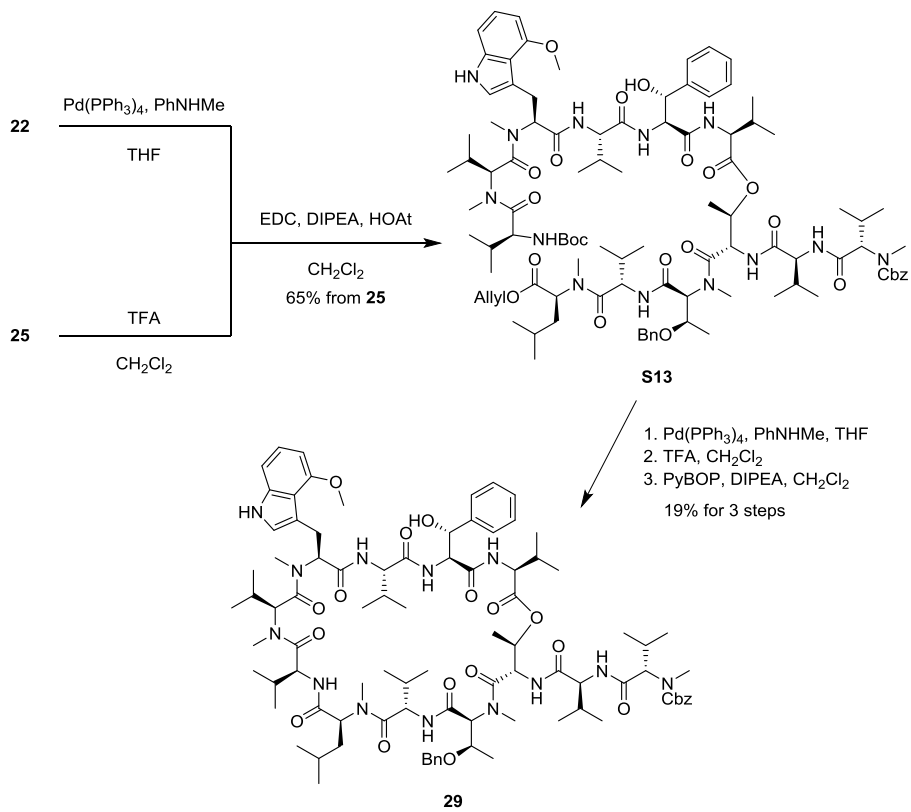
combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 153 mg (87%) of amine as brown solid. This free amine was used in the next step without further purification. To a solution of tripeptide **S9** (170 mg, 0.29 mmol) and $Pd(PPh_3)_4$ (33 mg, 0.03 mmol) in dry THF (1.5 mL) was added *N*-methylaniline (0.06 mL, 0.58 mmol) at room temperature. After stirring for 3 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 16:1) to afford 139 mg (86%) of the acid as yellow solid. To a solution of above crude amine (153 mg, 0.20 mmol), above acid (139 mg, 0.25 mmol), HOAt (27 mg, 0.20 mmol), and DIPEA (0.1 mL, 0.60 mmol) in CH_2Cl_2 (2 mL) was added EDC (75 mg, 0.60 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 1:2) to give 149 mg (46%) of linear octapeptide **S10** as white solid. $[\alpha]^{20}_D$ -141.9 (*c* = 1, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.90 (s, 1H), 9.48 (d, *J* = 8.7 Hz, 1H), 9.29 (d, *J* = 8.2 Hz, 1H), 9.25 (d, *J* = 8.9 Hz, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.48 (td, *J* = 7.5, 1.1 Hz, 1H), 7.42 (td, *J* = 7.7, 2.8 Hz, 3H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.32 (dd, *J* = 13.3, 6.0 Hz, 2H), 7.25 (t, *J* = 7.2 Hz, 2H), 7.22 (t, *J* = 7.4 Hz, 2H), 7.08 (s, 1H), 6.52 (d, *J* = 7.7 Hz, 1H), 6.22 (dd, *J* = 11.1, 4.5 Hz, 1H), 5.87 (ddd, *J* = 16.2, 10.7, 5.4 Hz, 1H), 5.82 (s, 1H), 5.74- 5.71 (m, 1H), 5.51 (dd, *J* = 8.8, 3.3 Hz, 1H), 5.47 (d, *J* = 10.4 Hz, 1H), 5.34-5.31 (m, 2H), 5.15 (dd, *J* = 8.9, 7.2 Hz, 1H), 5.11 (d, *J* = 10.5 Hz, 1H), 4.85-4.82 (m, 1H), 4.72-4.65 (m, 3H), 4.63 (d, *J* = 11.6 Hz, 1H), 4.47 (d, *J* = 11.6 Hz, 1H), 3.99 (dd, *J* = 15.4, 11.4 Hz, 1H), 3.80 (s, 3H), 3.70 (dd, *J* = 15.6, 4.6 Hz, 1H), 3.43 (s, 3H), 3.34 (s, 3H), 3.24 (s, 3H),

2.76 (s, 3H), 2.54-2.48 (m, 1H), 2.39-2.31 (m, 2H), 1.78-1.70 (m, 2H), 1.57-1.51 (m, 1H), 1.47 (s, 9H), 1.30 (d, J = 6.0 Hz, 3H), 1.12-1.00 (m, 18H), 0.86 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H), 0.77 (d, J = 6.5 Hz, 3H), 0.72 (d, J = 6.4 Hz, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.3, 172.6, 172.4, 171.8, 171.3, 171.1, 171.1, 170.4, 162.4, 156.9, 155.1, 142.6, 139.4, 139.3, 139.3, 132.6, 128.6, 128.6, 128.5, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.0, 122.7, 122.4, 118.2, 118.1, 111.4, 105.5, 99.7, 79.6, 73.5, 73.3, 70.6, 65.9, 62.8, 59.7, 59.3, 58.1, 57.8, 55.2, 55.1, 54.8, 54.5, 38.3, 35.7, 32.1, 31.8, 31.3, 31.3, 30.3, 29.8, 28.4, 28.3, 27.6, 26.3, 25.0, 23.1, 21.9, 20.4, 19.8, 19.8, 19.7, 18.5, 18.5, 18.4, 18.1, 18.0, 16.8.; HRMS (ESI+) calcd for $C_{70}H_{103}N_9NaO_{14}$ ($M+Na^+$) 1316.7517, found 1316.7484.; IR (thin film, neat) ν_{max} 3317, 2963, 1668, 1631, 1510, 1258, 752.

Linear dodecapeptide (S12) To a solution of tetrapeptide **S11** (31 mg, 0.04 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.2 mL) dropwise at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 19 mg (70%) of free amine as a clear oil. This free amine was used in the next step without further purification. To a solution of octapeptide **S10** (35 mg, 0.03 mmol) and $Pd(PPh_3)_4$ (4 mg, 0.003 mmol) in dry THF (0.3 mL) was added *N*-methylaniline (0.01 mL, 0.05 mmol) at room temperature. After stirring for 50 min at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 9:1) to afford 29 mg

(85%) of the acid as pale yellow solid. To a solution of above crude amine (19 mg, 0.03 mmol), above acid (29 mg, 0.02 mmol), HOAt (3 mg, 0.02 mmol), and DIPEA (0.01 mL, 0.07 mmol) in CH_2Cl_2 (0.3 mL) was added EDC (13 mg, 0.07 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3) to give 22 mg (51%) of linear dodecapeptide **S12** as a white solid. $[\alpha]^{20}_D$ -113.2 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.90 (s, 1H), 9.90 (d, *J* = 9.4 Hz, 1H), 9.52 (d, *J* = 8.5 Hz, 1H), 9.27 (d, *J* = 8.5 Hz, 1H), 9.24 (d, *J* = 8.8 Hz, 1H), 8.86 (d, *J* = 8.1 Hz, 1H), 8.53 (d, *J* = 7.7 Hz, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.45-7.39 (m, 5H), 7.37-7.22 (m, 11H), 7.12 (t, *J* = 7.9 Hz, 1H), 7.08 (s, 1H), 6.51 (d, *J* = 7.8 Hz, 1H), 6.31 (dd, *J* = 11.4, 4.5 Hz, 1H), 6.01-5.94 (m, 1H), 5.93- 5.86 (m, 2H), 5.74-5.69 (m, 1H), 5.53 (d, *J* = 9.6 Hz, 1H), 5.49 (dd, *J* = 8.8, 3.9 Hz, 1H), 5.47 (d, *J* = 10.5 Hz, 1H), 5.37 (d, *J* = 17.4 Hz, 1H), 5.32 (dd, *J* = 16.8, 11.3 Hz, 3H), 5.22 (d, *J* = 10.5 Hz, 1H), 5.00-4.96 (m, 1H), 4.94-4.89 (m, 1H), 4.86 (d, *J* = 11.1 Hz, 1H), 4.84-4.81 (m, 1H), 4.77 (ddd, *J* = 33.2, 13.2, 5.9 Hz, 3H), 4.63 (d, *J* = 11.5 Hz, 1H), 4.54-4.51 (m, 1H), 4.47 (t, *J* = 13.0 Hz, 1H), 4.38- 4.34 (m, 1H), 4.03-3.98 (m, 1H), 3.83 (s, 3H), 3.77-3.71 (m, 1H), 3.42 (s, 3H), 3.32 (s, 3H), 3.23 (s, 3H), 3.15 (s, 3H), 2.73 (s, 3H), 2.53-2.47 (m, 1H), 2.40-2.28 (m, 6H), 2.02-1.94 (m, 2H), 1.77-1.69 (m, 2H), 1.53 (d, *J* = 6.3 Hz, 3H), 1.46 (s, 9H), 1.29 (d, *J* = 5.9 Hz, 3H), 1.11-0.95 (m, 25H), 0.94-0.74 (m, 23H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.5, 173.3, 172.6, 172.1, 172.1, 171.8, 171.6, 171.3, 171.1, 170.9, 170.4, 170.0, 162.4, 157.4, 156.9, 155.1, 142.6, 139.4, 139.3, 139.3, 137.7, 132.5, 128.9, 128.6, 128.5, 128.3, 128.2, 127.9, 127.7, 127.7, 127.1, 122.8, 122.3, 119.0, 118.0, 111.3, 105.5, 99.7, 79.7, 73.3, 72.7, 72.0, 70.6, 67.3, 66.4, 64.7,

62.8, 59.9, 59.2, 59.2, 59.0, 58.2, 57.7, 56.0, 55.2, 55.1, 54.8, 54.5, 38.3, 35.7, 31.8, 31.7, 31.5, 31.3, 31.3, 31.2, 31.0, 30.9, 30.8, 30.7, 30.5, 30.3, 30.1, 29.8, 28.4, 28.3, 27.6, 27.6, 26.3, 25.0, 23.1, 21.9, 20.5, 19.8, 19.7, 19.7, 19.5, 19.2, 19.1, 18.5, 18.1, 18.1, 18.0, 17.9, 16.8, 16.7, 14.2.; HRMS (ESI+) calcd for $C_{98}H_{145}N_{13}O_{21}$ (M^+) 1841.0704, found 1841.0674.; IR (thin film, neat) ν_{max} 3321, 2964, 1742, 1669, 1511, 1258, 753.



Scheme S3. Macrolactamization between Val[6] and Leu[7]

Dodecapeptide (S13) To a solution of heptapeptide **25** (34 mg, 0.03 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.2 mL) dropwise at 0 °C. After stirring for 40 min at room temperature, the reaction mixture was concentrated *in vacuo* to afford TFA salt as white solid. This amine salt was used in the next step without further purification. To a solution of pentapeptide **22** (50 mg, 0.06 mmol) and $Pd(PPh_3)_4$ (7 mg, 0.006 mmol) in dry THF (1 mL) was added *N*-methylaniline (0.01 mL, 0.12 mmol) at room temperature. After stirring for 40 min at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 6:1) to afford 43 mg (90%) of the acid as ellow solid. To a solution of above amine salt (0.03 mmol), above (43 mg, 0.05 mmol), HOAt (4 mg, 0.03 mmol), and DIPEA (0.02 mL, 0.09 mmol) in CH_2Cl_2 (1 mL) was added EDC (17 mg, 0.09 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 1:2) to give 36 mg (65%) of linear dodecapeptide **S13** as white solid. $[\alpha]^{20}_D$ -82.4 (*c* = 1, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.88 (s, 1H), 9.66 (d, *J* = 8.0 Hz, 1H), 9.47 (d, *J* = 8.0 Hz, 1H), 9.20 (d, *J* = 8.8 Hz, 1H), 9.11 (d, *J* = 7.9 Hz, 1H), 8.87 (d, *J* = 8.1 Hz, 1H), 8.02 (d, *J* = 8.6 Hz, 1H), 7.99 (d, *J* = 9.4 Hz, 1H), 7.74-7.66 (m, 2H), 7.49-7.41 (m, 5H), 7.40-7.32 (m, 6H), 7.27-7.22 (m, 4H), 6.53 (d, *J* = 7.5 Hz, 1H), 6.30 (dd, *J* = 11.4, 4.5 Hz, 1H), 5.96-5.88 (m, 1H), 5.86-5.79 (m, 4H), 5.75 (dd, *J* = 10.4, 5.4 Hz, 1H), 5.73-5.71 (m, 1H), 5.57-5.53 (m, 1H), 5.47 (t, *J* = 10.4 Hz, 1H), 5.36 (dd, *J* = 15.0, 8.1 Hz, 2H), 5.30 (dd, *J* = 17.3, 1.4 Hz, 1H), 5.16 (dd, *J* = 10.4, 1.0 Hz, 1H), 5.12-5.07 (m, 1H), 5.02 (d, *J* = 4.5 Hz, 1H), 4.74-4.67 (m, 1H), 4.66 (d, *J* = 4.4

Hz, 1H), 4.63 (d, J = 12.0 Hz, 1H), 4.51 (dd, J = 14.5, 7.8 Hz, 2H), 4.40-4.34 (m, 1H), 4.10-3.99 (m, 1H), 3.82 (s, 3H), 3.74 (dd, J = 15.4, 4.3 Hz, 1H), 3.62 (s, 3H), 3.34 (s, 3H), 3.22 (s, 3H), 3.21 (s, 3H), 2.72 (s, 3H), 2.53-2.47 (m, 1H), 2.47-2.41 (m, 2H), 2.38-2.34 (m, 2H), 2.32-2.26 (m, 2H), 1.96-1.90 (m, 1H), 1.84-1.79 (m, 1H), 1.57-1.53 (m, 4H), 1.45 (s, 9H), 1.32 (d, J = 6.0 Hz, 3H), 1.12-1.02 (m, 15H), 1.00-0.93 (m, 17H), 0.93-0.80 (m, 16H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.7, 173.0, 172.4, 172.2, 171.7, 171.7, 171.6, 171.4, 171.4, 171.3, 169.7, 157.5, 156.9, 155.2, 142.5, 139.3, 139.2, 137.7, 132.6, 129.1, 128.9, 128.8, 128.8, 128.7, 128.5, 128.2, 128.1, 127.9, 127.7, 127.3, 122.8, 122.3, 118.5, 118.4, 118.1, 111.5, 105.5, 99.7, 79.8, 78.6, 73.3, 73.0, 71.9, 70.6, 67.3, 65.8, 64.9, 61.1, 59.8, 59.3, 58.6, 58.4, 58.3, 57.6, 56.5, 55.4, 55.3, 55.2, 54.9, 37.3, 33.0, 32.0, 31.5, 31.1, 30.5, 30.2, 30.2, 29.7, 28.4, 27.6, 27.4, 26.3, 24.8, 23.4, 21.5, 20.5, 20.0, 19.8, 19.8, 19.7, 19.5, 19.4, 19.2, 18.9, 18.6, 18.3, 18.2, 18.2, 18.1, 17.8, 16.8, 16.7.; HRMS (ESI+) calcd for $C_{98}H_{145}N_{13}NaO_{21}$ ($M+Na^+$) 1864.0570, found 1864.0602.; IR (thin film, neat) ν_{max} 3318, 2964, 1740, 1650, 1511, 1219, 770.

Cyclic Dodecapeptide (29) To a solution of linear dodecapeptide **S13** (22 mg, 0.01 mmol) and $Pd(PPh_3)_4$ (1 mg, 0.001 mmol) in dry THF (0.5 mL) was added *N*-methylaniline (0.003 mL, 0.02 mmol) at room temperature. After stirring for 1 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 16:1) to afford 17 mg (79%) of the free acid as yellow solid. To a solution of above acid (8 mg, 0.004 mmol) in CH_2Cl_2 (10 mL) was added TFA (1 mL) and triisopropylsilane (0.02 mL, 0.09 mmol) at room

temperature. After stirring for 20 min at room temperature, the reaction mixture was concentrated *in vacuo* to afford a TFA salt as white powder. This cyclization precursor was used in the next step without further purification. To a solution of PyBOP (11 mg, 0.02 mmol) and DIPEA (0.004 mL, 0.02 mmol) in CH_2Cl_2 (3 mL) was added a solution of the cyclization precursor (0.004 mmol) in CH_2Cl_2 (6 mL) via syringe pumping over a period of 5 h at room temperature. After stirring for 1 days at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3) to give 1.8 mg (24% for 2 steps) of cyclic dodecapeptide **29** as white powder.

Proposed ohmyungsamycin B (2) To a solution of **1** (7 mg, 0.005 mmol) and formaldehyde (37% in H_2O, 5 μL) in AcOH/DMF (1:10, 0.55 mL) was added sodium cyanoborohydride (3 mg, 0.048 mmol) at room temperature. After stirring for 1 h 30 min at the same temperature, the reaction mixture was quenched with aqueous $NaHCO_3$ and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 9:1) to afford 5 mg (71%) of proposed structure of ohmyungsamycin B as a white powder. $[\alpha]^{20}_D$ –52.6 (*c* = 0.2, MeOH); ^{1}H NMR (800 MHz, Pyridine-d_5) See Table below.; ^{13}C NMR (200 MHz, Pyridine-d_5) See Table below.; HRMS (ESI+) calcd for $C_{76}H_{122}N_{13}O_{16}$ ($M+H^+$) 1472.9127, found 1472.9146; IR (thin film, neat) ν_{max} 3295, 2959, 2926, 2851, 1638, 1508, 1199 cm^{-1}

		Natural ohmyungsamycin B (800 MHz)		Proposed ohmyungsamycin B (**2**; 800 MHz)		Δδ, ppm (Natural – Proposed **2**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
Val[1]	1	-	174.3	-	174.3	-	0.0
	2	4.69, t, 8.4	58.3	4.68, t, 8.6	58.3	0.01	0.0
	3	2.24, m	32.9	2.24, m	32.9	0.00	0.0
	4	1.12, d, 7.2	19.1	1.12, d, 6.8	19.1	0.00	0.0
	5	0.97, d, 6.7	18.7	0.97, d, 7.3	18.8	0.00	-0.1
	NH	9.28, d, 9.2	-	9.27, d, 9.1	-	0.01	-
HyPhe[2]	1	-	172.6	-	172.7	-	-0.1
	2	5.51, dd, 7.1, 2.7	60.1	5.51, dd, 7.2, 2.6	60.1	0.00	0.0
	3	5.95, s	73.1	5.95, s	73.2	0.00	-0.1
	4	-	143.0	-	143.0	-	0.0
	5	7.69, d, 7.6	127.0	7.68, d, 7.5	127.0	0.01	0.0
	6	7.69, d, 7.6	127.0	7.68, d, 7.5	127.0	0.01	0.0
	7	7.43, t, 7.7	128.6	7.42, t, 7.7	128.6	0.01	0.0
	8	7.43, t, 7.7	128.6	7.42, t, 7.7	128.6	0.01	0.0
	9	7.29, m	127.5	7.27, d, 7.5	127.5	0.02	0.0

		Natural ohmyungsamycin B (800 MHz)		Proposed ohmyungsamycin B (**2**; 800 MHz)		$\Delta\delta$, ppm (Natural – Proposed **2**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	NH	9.73, d, 7.0	-	9.72, d, 7.0	-	0.01	-
	OH	6.64, s	-	6.66, s	-	-0.02	-
Val[3]	1	-	174.5	-	174.5	-	0.0
	2	5.42, t, 9.2	58.3	5.42, t, 9.2	58.3	0.00	0.0
	3	2.68, m	33.1	2.67, m	33.1	0.01	0.0
	4	1.37, d, 6.7	19.8	1.37, d, 6.6	19.8	0.00	0.0
	5	1.20, d, 6.7	19.9	1.20, d, 6.5	20.0	0.00	-0.1
	NH	8.04, d, 9.7	-	8.04, d, 9.7	-	0.00	-
NMe-4MeO-Trp[4]	1	-	169.9	-	169.9	-	0.0
	2	4.60, dd, 10.9, 4.6	70.6	4.60, dd, 10.9, 4.7	70.6	0.00	0.0
	3a	4.47, dd, 13.6, 4.6	27.1	4.47, dd, 13.5, 4.7	27.1	0.00	0.0
	3b	4.35, dd, 13.3, 11.4		4.34, dd, 13.4, 11.3		0.01	
	4	-	112.9	-	112.9	-	0.0
	5	7.20, d, 1.5	124.2	7.20, d, 1.9	124.2	0.00	0.0
	6	-	139.5	-	139.5	-	0.0
	7	7.29, m	105.8	7.29, m	105.8	0.00	0.0
	8	7.27, m	122.8	7.27, m	122.8	0.00	0.0
	9	6.68, d, 6.6	99.5	6.68, dd, 7.1, 0.7	99.5	0.00	0.0
	10	-	154.9	-	154.9	-	0.0
	11	-	118.4	-	118.4	-	0.0
	OMe	3.83, s	55.4	3.83, s	55.4	0.00	0.0
	NMe	2.51, s	40.8	2.51, s	40.8	0.00	0.0
	NH	11.82, d, 1.0	-	11.82, d, 1.4	-	0.00	-
NMe-Val[5]	1	-	169.4	-	169.4	-	0.0
	2	3.21, d, 8.2	70.9	3.21, d, 8.0	70.9	0.00	0.0
	3	3.04, m	29.0	3.04, m	29.0	0.00	0.0
	4	1.23, d, 6.5	22.0	1.23, d, 6.5	22.0	0.00	0.0
	5	1.03, d, 7.6	19.7	1.03, d, 7.6	19.7	0.00	0.0
	NMe	3.18, s	39.9	3.18, s	39.9	0.00	0.0
Val[6]	1	-	173.0	-	172.9	-	-0.1
	2	4.96, overlapped	54.3	4.96, overlapped	54.3	0.00	0.0
	3	2.41, m	31.9	2.41, m	31.9	0.00	0.0
	4	1.14, d, 6.7	19.1	1.14, d, 6.7	19.1	0.00	0.0
	5	1.04, d, 7.1	19.7	1.04, d, 6.9	19.7	0.00	0.0
	NH	9.42, d, 9.4	-	9.41, d, 9.3	-	0.01	-

		Natural ohmyungsamycin B (800 MHz)		Proposed ohmyungsamycin B (**2**; 800 MHz)		$\Delta\delta$, ppm (Natural – Proposed **2**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
NMe-Leu[7]	1	-	171.7	-	171.7	-	0.0
	2	5.67, t, 7.5	54.8	5.66, t, 7.4	54.8	0.01	0.0
	3a	1.74, m	38.9	1.74, m	38.9	0.00	0.0
	3b	1.60, m		1.59, m		0.01	
	4	1.43, m	25.1	1.42, m	25.1	0.01	0.0
	5	0.68, d, 6.4	23.1	0.68, d, 6.5	23.1	0.00	0.0
	6	0.69, d, 6.4	22.1	0.68, d, 6.5	22.1	0.01	0.0
	NMe	3.52, s	31.3	3.52, s	31.4	0.00	-0.1
Val[8]	1	-	173.5	-	173.5	-	0.0
	2	5.30, t, 8.2	55.6	5.30, t, 8.2	55.6	0.00	0.0
	3	2.57, m	31.0	2.57, m	31.0	0.00	0.0
	4	1.20, d, 6.7	19.2	1.20, d, 6.5	19.2	0.00	0.0
	5	1.16, d, 6.8	19.8	1.16, d, 6.8	19.8	0.00	0.0
	NH	8.09, d, 8.8	-	8.09, d, 8.7	-	0.00	-
NMe-Thr[9]	1	-	170.6	-	170.6	-	0.0
	2	5.63, d, 3.4	62.4	5.62, d, 3.6	62.4	0.01	0.0
	3	5.10, m	66.6	5.06, m	66.5	0.04	0.1
	4	1.35, d, 6.4	20.4	1.33, d, 6.4	20.5	0.02	-0.1
	NMe	3.70, s	34.7	3.68, s	34.6	0.02	0.1
	OH	6.15, d, 4.1	-	6.14, d, 4.3	-	0.01	-
Thr[10]	1	-	171.4	-	171.4	-	0.0
	2	5.89, dd, 9.1, 2.6	52.4	5.89, dd, 9.2, 2.6	52.4	0.00	0.0
	3	6.05, qd, 6.4, 2.5	69.5	6.05, qd, 6.4, 2.5	69.5	0.00	0.0
	4	1.54, d, 6.5	16.7	1.56, d, 6.5	16.7	-0.02	0.0
	NH	10.20, d, 9.1	-	10.08, d, 9.1	-	0.12	-
Val[11]	1	-	173.4	-	173.3	-	0.1
	2	5.27, t, 8.5	56.9[a]	5.14, t, 8.3	58.2	0.13	-1.3
	3	2.07, m	37.7[b]	2.24, m	35.2	-0.17	2.5
	4	0.96, d, 6.8	15.8[c]	0.96, d, 7.1	19.7	0.00	-3.9
	5	Not found	Not found	1.00, d, 6.7	19.0	-	-
	NH	8.67, d, 9.3	-	8.47, d, 8.8	-	0.20	-
NMe-Val[12]	1	-	174.3	-	171.5	-	2.8
	2	3.10, d, 5.9	71.4	2.91, d, 8.9	75.4	0.19	-4
	3	2.18, m	32.3	2.30, m	28.0	-0.12	4.3
	4	1.11, d, 7.1	19.1	1.09, d, 6.6	19.3	0.02	-0.2

		Natural ohmyungsamycin B (800 MHz)		Proposed ohmyungsamycin B (**2**; 800 MHz)		$\Delta\delta$, ppm (Natural – Proposed **2**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	5	1.11, d, 7.1	19.1	1.09, d, 6.5	20.2	0.02	-1.1
	NMe	2.55, s	35.9	2.50,s	42.4	0.05	-6.5
	NMe	Not found	Not found	2.50,s	42.4	-	-
Not assigned[d]	CH_2	1.68, m	25.2	-	-	-	-
		1.26, overlapped		-	-	-	-
	CH_3	0.72, t, 7.4	10.8	-	-	-	-

[a] Originally assigned as 57.9, [b] Originally assigned as 27.6, [c] Originally assigned as 19.8,

[d]Identified by COSY and e-HSQC NMR data of natural ohmyungsamycin B sample

Table S3. Comparison of ^{1}H and ^{13}C NMR spectral data of natural and proposed ohmyungsamycin B (2)

Structural revision of ohmyungsamycin B

Boc-L-Ile-OAllyl (S14) To a solution of Boc-L-Ile-OH **31** (400 mg, 1.73 mmol) and potassium carbonate (359 mg, 2.60 mmol) in DMF (9 mL) was added allyl bromide (0.2 mL, 2.08 mmol) at room temperature. After stirring 2 h at the same temperature, the reaction mixture was diluted with Et_2O and washed with brine. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on a silica gel (EtOAc/Hexane = 1:5) to afford 386 mg (82%) of the Boc-L-Ile-OAllyl **S14** as a colorless oil. $[\alpha]^{20}_D$ 10.2 (*c* = 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.85 (ddd, *J* = 22.8, 10.9, 5.8 Hz, 1H), 5.28 (dd, *J* = 17.1, 1.2 Hz, 1H), 5.19 (d, *J* = 10.4 Hz, 1H), 5.01 (d, *J* = 8.4 Hz, 1H), 4.57 (qd, *J* = 13.2, 5.8 Hz, 2H), 4.23 (dd, *J* = 8.7, 4.7 Hz, 1H), 1.82 (br, 1H), 1.38 (s, 10H), 1.17-1.05 (m, 1H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H).; ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0, 155.5, 131.7, 118.6, 79.6, 65.5, 57.8, 38.0, 28.2, 24.9, 15.4, 11.5.; HRMS (FAB+) calcd for $C_{14}H_{26}NO_4$ ($M+H^+$) 272.1862, found 272.1858; IR (thin film, neat) ν_{max} 3381, 2969, 2935, 1717, 1502, 1366, 1250 cm^{-1}

Cbz-*N*Me-L-Val-L-Ile-OAllyl (32) To a solution of Boc-L-Ile-OAllyl **S14** (386 mg, 1.42 mmol) in CH_2Cl_2 (6 mL) was added TFA (1.5 mL) dropwise at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was directly concentrated *in vacuo*. The residue was used in the next step without further purification. To a solution of the above amine salt (1.42 mmol), Cbz-*N*Me-L-Val-OH **20** (452 mg, 1.70 mmol), DIPEA (0.7 mL, 4.26 mmol) and HOAt (193 mg, 1.42 mmol) in CH_2Cl_2 (14 mL) was added EDC (544 mg, 2.84 mmol) at 0 °C. After stirring overnight at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$,

dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/Hexane = 1:5) to give 494 mg (83% for 2 steps) of dipeptide **32** as a colorless oil. $[\alpha]^{20}_D$ -71.9 (*c* = 1, $CHCl_3$); ^1H NMR (800 MHz, $CDCl_3$, 4:1 mixture of rotamers) δ 7.38-7.25 (m, 5H), 6.49 (d, *J* = 7.9 Hz, 0.8H), 5.97 (d, *J* = 5.9 Hz, 0.2H), 5.87 (dq, *J* = 10.9, 5.8 Hz, 1H), 5.30 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 5.09 (abq, *J* = 12.4 Hz, 2H), 4.59 (ddd, *J* = 35.8, 13.2, 5.8 Hz, 2H), 4.52 (dd, *J* = 8.7, 4.9 Hz, 1H), 4.10 (d, *J* = 11.2 Hz, 0.8H), 4.00 (d, *J* = 10.2 Hz, 0.2H), 2.87 (s, 3H), 2.30-2.22 (m, 1H), 1.87-1.83 (m, 1H), 1.36-1.29 (m, 1H), 1.10-1.03 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.80 (d, *J* = 6.9 Hz, 3H).; ^{13}C NMR (200 MHz, $CDCl_3$) δ 171.1, 169.9, 157.5, 136.5, 131.6, 128.5, 128.0, 127.6, 118.8, 67.5, 65.6, 65.4, 56.2, 37.4, 29.8, 25.9, 24.9, 19.5, 18.6, 15.4, 11.5.; HRMS (FAB+) calcd for $C_{23}H_{35}N_2O_5$ ($M+H^+$) 419.2546, found 419.2558; IR (thin film, neat) ν_{max} 3353, 2965, 1743, 1673, 1533, 1457, 1299, 1161, 984 cm^{-1}

Hexapeptide (33) To a solution of tetrapeptide **16** (158 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.5 mL) dropwise at 0 °C. After stirring for 3 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 375 mg (95%) of amine as a white foam. The free amine was used in the next step without further purification. To a solution of dipeptide **32** (345 mg, 0.85 mmol) and $Pd(PPh_3)_4$ (99 mg, 0.09 mmol) in dry THF (8.5 mL) was added *N*-methylaniline (0.2 mL, 1.71 mmol) at room temperature. After stirring for 2 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$

and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 9:1) to afford 303 mg (98%) of the acid as a yellow oil. To a solution of the crude amine (375 mg, 0.63 mmol), the acid (303 mg 0.83 mmol) and DIPEA (0.2 mL, 1.27 mmol) in CH_2Cl_2 (6 mL) was added DEPBT (380 mg, 1.27 mmol) at 0 °C. After stirring 5 h at room temperature, the reaction mixture was quenched with 1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:5) to give 163 mg (75%) of hexapeptide **33** as a white solid. $[\alpha]^{20}_D$ -69.4 (*c* = 1, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 9.52 (d, *J* = 8.5 Hz, 1H), 9.40 (d, *J* = 8.6 Hz, 1H), 9.25 (d, *J* = 8.5 Hz, 1H), 7.52-7.42 (m, 4H), 7.41-7.32 (m, 4H), 7.32-7.26 (m, 2H), 6.37 (d, *J* = 3.3 Hz, 1H), 6.01-5.92 (m, 1H), 5.91 (d, *J* = 7.2 Hz, 1H), 5.79 (dd, *J* = 8.3, 5.8 Hz, 1H), 5.74 (dd, *J* = 9.7, 6.2 Hz, 1H), 5.45 (t, *J* = 11.2 Hz, 1H), 5.38-5.30 (m, 2H), 5.19 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.09 (t, *J* = 8.5 Hz, 1H), 4.90 (d, *J* = 11.1 Hz, 1H), 4.72 (t, *J* = 4.7 Hz, 1H), 4.69-4.67 (m, 2H), 4.63-4.60 (m, 1H), 4.59 (d, *J* = 11.8 Hz, 1H), 4.53 (p, *J* = 6.3 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 3.69 (s, 3H), 3.23 (s, 3H), 3.22 (s, 3H), 2.43- 2.37 (m, 1H), 2.36-2.30 (m, 1H), 2.21-2.14 (m, 1H), 1.89-1.81 (m, 2H), 1.76-1.64 (m,2H), 1.64-1.59 (m, 1H), 1.35 (d, *J* = 6.2 Hz, 3H), 1.09 (d, *J* = 6.7 Hz, 3H), 1.08-1.05 (m, 3H), 0.98 (dd, *J* = 6.6, 2.3 Hz, 3H), 0.96 (d, *J* = 7.4 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H), 0.68 (t, *J* = 6.9 Hz, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.5, 172.8, 172.4, 171.6, 171.6, 169.6, 157.4, 139.4, 138.6, 137.7, 132.7, 132.6, 128.9, 128.8, 128.7, 128.7, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 118.4, 73.6, 71.2, 71.0, 68.8, 67.3, 67.2, 67.2, 65.8, 64.8, 64.8, 61.2, 58.1, 55.0, 54.9, 54.7, 37.3, 36.8, 33.5, 31.5, 31.5, 31.1, 30.9, 30.1, 30.0, 27.5, 25.3, 24.9, 23.5, 23.4, 21.6, 21.5, 20.1, 19.9, 19.7, 19.5, 19.4,

19.2, 19.2, 18.7, 16.7, 16.6, 16.2, 15.9, 10.7, 10.4.; HRMS (ESI+) calcd for $C_{51}H_{78}N_6NaO_{11}$ ($M+Na^+$) 973.5621, found 973.5645; IR (thin film, neat) ν_{max} 3309, 2962, 2933, 1740, 1647, 1534, 1455, 1299, 1110, 698 cm^{-1}

Heptapeptide (34) To a solution of hexapeptide **33** (142 mg, 0.15 mmol), Boc-L-Val-OH (65 mg, 0.30 mmol), DMAP (55 mg, 0.45 mmol) and HOAt (20 mg, 0.15 mmol) in CH_2Cl_2 (1.5 mL) was added EDC (86 mg, 0.45 mmol) at room temperature. After stirring 2 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:2) to give 159 mg (92%) of heptapeptide **34** as a white solid. $[\alpha]^{20}_D$ -43.1 (c = 1.0, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 9.93 (d, J = 8.2 Hz, 1H), 9.65 (d, J = 8.1 Hz, 1H), 9.33 (d, J = 8.6 Hz, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.51-7.42 (m, 4H), 7.41-7.33 (m, 4H), 7.32-7.25 (m, 2H), 6.02-5.90 (m, 1H), 5.88-5.73 (m, 4H), 5.40-5.30 (m, 3H), 5.19 (d, J = 10.4 Hz, 1H), 5.06 (t, J = 8.4 Hz, 1H), 4.91-4.87 (m, 1H), 4.71 (d, J = 9.3 Hz, 1H), 4.69 (d, J = 5.4 Hz, 2H), 4.66 (d, J = 11.9 Hz, 1H), 4.63 (d, J = 5.5 Hz, 1H), 4.54 (d, J = 11.8 Hz, 1H), 4.43-4.38 (m, 1H), 3.65 (s, 3H), 3.25 (s, 3H), 3.24 (s, 3H), 2.54-2.43 (m, 1H), 2.42-2.34 (m, 2H), 2.13-2.07 (m, 1H), 1.89-1.83 (m, 2H), 1.74-1.60 (m, 2H), 1.58 (d, J = 6.0 Hz, 3H), 1.50 (s, 9H), 1.36 (d, J = 6.0 Hz, 3H), 1.28-1.21 (m, 1H), 1.12 (d, J = 6.5 Hz, 3H), 1.10 (d, J = 6.6 Hz, 3H), 1.09 (d, J = 6.9 Hz, 3H), 1.03-1.00 (m, 6H), 0.97 (d, J = 6.5 Hz, 3H), 0.91-0.89 (m, 6H), 0.87 (d, J = 6.4 Hz, 3H), 0.64 (t, J = 7.4 Hz, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.6, 173.4, 172.6, 172.1, 171.7, 171.6, 171.3, 171.2, 169.7, 157.5, 156.6, 139.2, 138.6, 137.7, 132.7,

132.6, 129.2, 128.9, 128.8, 128.7, 128.7, 128.3, 128.2, 128.1, 127.9, 127.7, 118.4, 78.6, 78.6, 73.1, 71.5, 71.2, 70.8, 70.6, 67.3, 67.2, 65.8, 64.9, 64.7, 61.1, 59.7, 59.3, 57.6, 55.2, 54.8, 54.7, 52.7, 52.5, 37.3, 37.2, 37.2, 34.3, 33.0, 31.5, 31.4, 31.3, 31.2, 31.1, 30.9, 30.2, 28.5, 27.7, 27.5, 25.3, 24.9, 23.5, 23.5, 21.5, 19.8, 19.6, 19.6, 19.4, 19.3, 19.2, 19.1, 18.9, 18.0, 17.6, 17.6, 16.9, 16.7, 16.1, 15.8, 15.8, 10.7, 10.2.; HRMS (ESI+) calcd for $C_{61}H_{95}N_7NaO_{14}$ ($M+Na^+$) 1172.6829, found 1172.6845; IR (thin film, neat) ν_{max} 3319, 2964, 2874, 1741, 1632, 1531, 1498, 1219, 1161, 772 cm^{-1}

Linear dodecapeptide (35) To a solution of pentapeptide **22** (65 mg, 0.08 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.2 mL) dropwise at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford 57 mg (99%) of amine as a brown solid. The free amine was used in the next step without further purification. To a solution of heptapeptide **34** (79 mg, 0.07 mmol) and $Pd(PPh_3)_4$ (8 mg, 0.01 mmol) in dry THF (1 mL) was added *N*-methylaniline (0.02 mL, 0.14 mmol) at room temperature. After stirring for 1 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 16:1 to 9:1) to afford 69 mg (90%) of the acid as a yellow solid. To a solution of the crude amine (57 mg, 0.07 mmol), the acid (69 mg 0.06 mmol), HOAt (8 mg, 0.06 mmol) and DIPEA (0.03 mL, 0.19 mmol) in CH_2Cl_2 (1 mL) was added EDC (36 mg, 0.19 mmol) in two portions over a period of 20 min at 0 °C. After stirring for 2 h at room temperature, the reaction mixture was quenched with

1*N* HCl, extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3) to give 46 mg (40%) of linear dodecapeptide **35** as a white solid. $[\alpha]^{20}_D$ -122.1 (*c* = 1, $CHCl_3$); ^{1}H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.93 (s, 1H), 9.94 (d, *J* = 8.0 Hz, 1H), 9.52 (d, *J* = 8.6 Hz, 1H), 9.48 (d, *J* = 8.3 Hz, 1H), 9.33 (d, *J* = 8.6 Hz, 1H), 9.31 (d, *J* = 9.0 Hz, 1H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.94 (d, *J* = 3.5 Hz, 1H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.49-7.42 (m, 5H), 7.41-7.33 (m, 6H), 7.32-7.23 (m, 4H), 7.16 (s, 1H), 6.55 (d, *J* = 7.7 Hz, 1H), 6.25 (d, *J* = 6.0 Hz, 1H), 5.94-5.81 (m, 4H), 5.80-5.75 (m, 2H), 5.55 (dd, *J* = 8.7, 3.5 Hz, 1H), 5.51 (d, *J* = 10.4 Hz, 1H), 5.41-5.30 (m, 4H), 5.20-5.17 (m, 1H), 5.16-5.12 (m, 2H), 4.92-4.89 (m, 1H), 4.88-4.85 (m, 1H), 4.75-4.67 (m, 3H), 4.66 (d, *J* = 11.4 Hz, 2H), 4.57-4.54 (m, 1H), 4.44-4.42 (m, 1H), 4.02 (dd, *J* = 15.2, 11.7 Hz, 1H), 3.83 (s, 3H), 3.73 (dd, *J* = 15.1, 4.0 Hz, 1H), 3.67 (s, 3H), 3.46 (s, 3H), 3.38 (s, 3H), 3.26 (s, 3H), 2.80 (s, 3H), 2.60-2.44 (m, 3H), 2.42-2.34 (m, 2H), 2.13-2.06 (m, 1H), 2.06-1.99 (m, 1H), 1.77 (dd, *J* = 17.7, 11.2 Hz, 1H), 1.67 (d, *J* = 15.1 Hz, 1H), 1.63-1.54 (m, 4H), 1.50 (s, 9H), 1.34 (d, *J* = 5.8 Hz, 3H), 1.27-1.19 (m, 2H), 1.15-0.99 (m, 21H), 0.97-0.79 (m, 21H), 0.76 (d, *J* = 6.4 Hz, 3H), 0.66-0.64 (m, 3H).; ^{13}C NMR (200 MHz, Pyridine-d_5, a mixture of rotamers) δ 173.4, 173.4, 172.6, 172.5, 172.4, 172.1, 171.8, 171.5, 171.3, 171.2, 171.0, 169.7, 157.5, 156.6, 155.1, 142.6, 139.3, 139.2, 137.7, 132.6, 132.5, 129.1, 128.9, 128.7, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.7, 127.0, 122.7, 122.3, 118.1, 118.0, 111.4, 105.5, 99.7, 78.6, 78.6, 73.5, 73.4, 71.5, 70.8, 67.3, 65.8, 64.9, 64.7, 61.1, 59.8, 59.7, 59.3, 59.3, 58.1, 57.8, 57.6, 55.2, 55.2, 54.8, 54.5, 52.6, 38.4, 37.2, 33.2, 32.1, 31.5, 31.3, 31.3, 31.3, 30.3, 30.2, 30.0, 29.8, 28.5, 27.6, 27.5, 26.3, 25.3, 25.0, 23.7, 23.3, 23.0, 22.0, 21.7, 20.4, 19.9, 19.8, 19.8, 19.7, 19.6, 19.6, 19.5, 19.4, 19.3, 19.2, 18.7, 18.4, 18.1, 18.0, 18.0, 17.9, 17.6, 17.6,

17.0, 16.9, 16.7, 16.2, 15.8, 10.7, 10.2.; HRMS (ESI+) calcd for $C_{99}H_{147}N_{13}NaO_{21}$ ($M+Na^+$) 1877.0727, found 1877.0766; IR (thin film, neat) ν_{max} 3316, 2963, 1629, 1509, 1257, 1161, 1092, 753 cm^{-1}

Cyclic dodecapeptide (36) To a solution of linear dodecapeptide **35** (39 mg, 0.02 mmol) and $Pd(PPh_3)_4$ (2 mg, 0.002 mmol) in dry THF (1 mL) was added *N*-methylaniline (0.004 mL, 0.04 mmol) at room temperature. After stirring for 30 min at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on a short pad of silica gel (CH_2Cl_2/MeOH = 9:1 to 6:1) to afford 37 mg (97%) of the acid as a yellow solid. To a solution of the above acid (37 mg, 0.02 mmol) in CH_2Cl_2 (30 mL) was subsequently added TFA (3 mL) and triisopropylsilane (0.08 mL, 0.41 mmol) at room temperature. After stirring for 20 min at room temperature, the reaction mixture was concentrated *in vacuo* to afford TFA salt as a white powder. This cyclization precursor was used in the next step without further purification. To a solution of PyBOP (52 mg, 0.10 mmol) and DIPEA (0.02 mL, 0.10 mmol) in CH_2Cl_2 (10 mL) was added a solution of the cyclization precursor (0.02 mmol) in CH_2Cl_2 (30 mL) via syringe pump over a period of 10 h at room temperature. After stirring for 20 h at the same temperature, the reaction mixture was quenched with 1*N* HCl and extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (Acetone/Hexane = 2:3) to give 14 mg (41% for 2 steps) of cyclic dodecapeptide **36** as a white powder. $[\alpha]^{20}_D$ -12.63 (c = 1, $CHCl_3$); ^1H NMR (800 MHz, Pyridine-d_5, a mixture of rotamers) Major rotamer δ 11.79 (s, 1H), 10.24

(d, J = 8.5 Hz, 1H), 9.63 (d, J = 6.4 Hz, 1H), 9.50 (d, J = 8.5 Hz, 1H), 9.38 (d, J = 9.5 Hz, 1H), 9.30 (d, J = 9.1 Hz, 1H), 8.02-7.98 (m, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 7.3 Hz, 2H), 7.46-7.41 (m, 4H), 7.38 (t, J = 6.0 Hz, 2H), 7.35-7.23 (m, 7H), 7.08 (s, 1H), 6.65 (d, J = 7.2 Hz, 1H), 6.53 (s, 1H), 6.08-6.02 (s, 1H), 5.92 (s, 1H), 5.86 (d, J = 9.3 Hz, 1H), 5.84 (s, 1H), 5.70 (d, J = 3.1 Hz, 1H), 5.60 (t, J = 7.5 Hz, 1H), 5.48 (d, J = 5.8 Hz, 1H), 5.39 (t, J = 9.2 Hz, 1H), 5.32 (d, J = 3.0 Hz, 1H), 5.23 (t, J = 8.3 Hz, 1H), 5.08-5.05 (m, 1H), 4.78-4.69 (m, 4H), 4.66 (d, J = 11.7 Hz, 1H), 4.61 (t, J = 8.9 Hz, 1H), 4.56 (dd, J = 10.9, 4.7 Hz, 1H), 4.43 (dd, J = 13.4, 4.5 Hz, 1H), 4.31 (dd, J = 13.4, 11.4 Hz, 1H), 3.80 (s, 3H), 3.57 (s, 3H), 3.51 (s, 3H), 3.23 (s, 3H), 3.17 (d, J = 8.2 Hz, 1H), 3.15 (s, 3H), 3.04-2.97 (m, 1H), 2.68-2.61 (m, 1H), 2.59-2.53 (m, 1H), 2.47 (s, 3H), 2.45-2.41 (m, 1H), 2.40-2.34 (m, 1H), 2.22-2.16 (m, 1H), 2.06-2.02 (m, 1H), 1.74-1.69 (m, 1H), 1.64-1.60 (m, 1H), 1.56 (d, J = 6.2 Hz, 4H), 1.41-1.36 (m, 1H), 1.33 (d, J = 6.6 Hz, 3H), 1.28-1.24 (m, 1H), 1.19 (t, J = 7.1 Hz, 9H), 1.14 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 6.9 Hz, 3H), 1.10 (d, J = 8.9 Hz, 3H), 1.08-1.06 (m, 3H), 1.00 (d, J = 7.2 Hz, 3H), 0.99 (d, J = 8.5 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.6 Hz, 3H), 0.81 (d, J = 7.2 Hz, 3H), 0.62 (d, J = 6.4 Hz, 6H), 0.59 (t, J = 6.3 Hz, 3H).; ^{13}C NMR (200 MHz, $CHCl_3$, a mixture of rotamers) δ 174.5, 174.5, 173.7, 173.3, 172.9, 172.8, 171.7, 171.6, 171.4, 170.6, 169.9, 169.4, 157.4, 154.9, 143.0, 139.9, 139.5, 137.8, 128.9, 128.7, 128.6, 128.2, 127.9, 127.6, 127.5, 126.9, 124.2, 122.8, 118.4, 112.1, 105.8, 99.5, 74.9, 74.5, 73.2, 72.5, 70.9, 70.6, 69.4, 67.2, 64.8, 62.2, 60.3, 59.9, 58.4, 58.2, 57.5, 55.9, 55.4, 54.8, 52.6, 40.8, 39.9, 38.9, 36.9, 34.5, 33.1, 32.9, 31.9, 31.4, 30.9, 30.1, 30.0, 29.0, 27.7, 27.1, 25.4, 25.0, 23.6, 23.1, 22.0, 22.0, 20.8, 20.0, 19.8, 19.8, 19.7, 19.6, 19.4, 19.3, 19.2, 19.1, 18.8, 18.4, 16.9, 16.7, 15.7, 14.2, 10.6, 9.2.; HRMS (ESI+) calcd for $C_{91}H_{133}N_{13}NaO_{18}$ ($M+Na^+$) 1718.9784, found 1718.9792; IR (thin film, neat) ν_{max} 3308, 2963, 2926, 1648, 1508, 1260, 1219, 771 cm^{-1}

Revised ohmyungsamycin B (37) To a solution of cyclic dodecapeptide **36** (4.6 mg, 0.003 mmol) in MeOH (1 mL) was added 20% palladium hydroxide on carbon (10 mg) and the reaction vial was thoroughly sealed. After stirring for 2 h under H_2 atmosphere, the reaction mixture was filtered through a pad of Celite. The filtered solution was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 10:1) to afford 4.1 mg (90%) of revised structure of ohmyungsamycin B **37** as a white powder. $[\alpha]^{20}_D$ –33.4 (*c* = 1, MeOH); ^{1}H NMR (800 MHz, $CDCl_3$) See Table below.; ^{13}C NMR (200 MHz, $CHCl_3$) See Table below.; HRMS (ESI+) calcd for $C_{76}H_{122}N_{13}O_{16}$ $(M+H)^+$ 1472.9127, found 1472.9119; IR (thin film, neat) ν_{max} 3308, 2960, 1640, 1566, 1199 cm^{-1}

		Natural ohmyungsamycin B (800 MHz)		Revised ohmyungsamycin B (**37**; 800 MHz)		Δδ, ppm (Natural – Revised **37**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
Val[1]	1	-	174.3	-	174.3	-	0.0
	2	4.69, t, 8.4	58.3	4.68, t, 8.4	58.3	0.01	0.0
	3	2.24, m	32.9	2.23, m	32.9	0.01	0.0
	4	1.12, d, 7.2	19.1	1.12, d, 7.2	19.1	0.00	0.0
	5	0.97, d, 6.7	18.7	0.97, d, 6.8	18.7	0.00	0.0
	NH	9.28, d, 9.2	-	9.28, d, 9.2	-	0.00	-
HyPhe[2]	1	-	172.6	-	172.6	-	0.0
	2	5.51, dd, 7.1, 2.7	60.1	5.51, dd, 7.2, 2.8	60.1	0.00	0.0
	3	5.95, s	73.1	5.95, s	73.1	0.00	0.0
	4	-	143.0	-	143.0	-	0.0
	5	7.69, d, 7.6	127.0	7.69, d, 7.6	127.0	0.00	0.0
	6	7.69, d, 7.6	127.0	7.69, d, 7.6	127.0	0.00	0.0
	7	7.43, t, 7.7	128.6	7.43, t, 7.7	128.6	0.00	0.0
	8	7.43, t, 7.7	128.6	7.43, t, 7.7	128.6	0.00	0.0
	9	7.29, m	127.5	7.29, m	127.5	0.00	0.0
	NH	9.73, d, 7.0	-	9.73, d, 7.1	-	0.00	-
	OH	6.64, s	-	6.63, s	-	0.01	-
Val[3]	1	-	174.5	-	174.5	-	0.0
	2	5.42, t, 9.2	58.3	5.42, t, 9.3	58.3	0.00	0.0

		Natural ohmyungsamycin B (800 MHz)		Revised ohmyungsamycin B (**37**; 800 MHz)		$\Delta\delta$, ppm (Natural – Revised **37**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	3	2.68, m	33.1	2.68, m	33.1	0.00	0.0
	4	1.37, d, 6.7	19.8	1.37, d, 6.7	19.8	0.00	0.0
	5	1.20, d, 6.7	19.9	1.20, d, 6.6	19.9	0.00	0.0
	NH	8.04, d, 9.7	-	8.04, d, 9.7	-	0.00	-
NMe-4MeO-Trp[4]	1	-	169.9	-	169.9	-	0.0
	2	4.60, dd, 10.9, 4.6	70.6	4.60, dd, 10.8, 4.7	70.6	0.00	0.0
	3a	4.47, dd, 13.6, 4.6	27.1	4.46, dd, 13.5, 4.6	27.1	0.01	0.0
	3b	4.35, dd, 13.3, 11.4		4.34, dd, 13.4, 11.4		0.01	
	4	-	112.9	-	112.9	-	0.0
	5	7.20, d, 1.5	124.2	7.20, d, 1.7	124.2	0.00	0.0
	6	-	139.5	-	139.5	-	0.0
	7	7.29, m	105.8	7.29, m	105.8	0.00	0.0
	8	7.27, m	122.8	7.27, m	122.8	0.00	0.0
	9	6.68, d, 6.6	99.5	6.68, d, 6.8	99.5	0.00	0.0
	10	-	154.9	-	154.9	-	0.0
	11	-	118.4	-	118.4	-	0.0
	OMe	3.83, s	55.4	3.83, s	55.4	0.00	0.0
	NMe	2.51, s	40.8	2.51, s	40.8	0.00	0.0
	NH	11.82, d, 1.0	-	11.83, d, 1.5	-	-0.01	-
NMe-Val[5]	1	-	169.4	-	169.4	-	0.0
	2	3.21, d, 8.2	70.9	3.21, d, 8.0	70.9	0.00	0.0
	3	3.04, m	29.0	3.04, m	29.0	0.00	0.0
	4	1.23, d, 6.5	22.0	1.23, d, 6.5	22.0	0.00	0.0
	5	1.03, d, 7.6	19.7	1.03, d, 7.9	19.7	0.00	0.0
	NMe	3.18, s	39.9	3.18, s	39.9	0.00	0.0
Val[6]	1	-	173.0	-	173.0	-	0.0
	2	4.96, overlapped	54.3	4.96, overlapped	54.3	0.00	0.0
	3	2.41, m	31.9	2.40, m	31.9	0.01	0.0
	4	1.14, d, 6.7	19.1	1.14, d, 6.7	19.1	0.00	0.0
	5	1.04, d, 7.1	19.7	1.03, d, 7.1	19.7	0.01	0.0
	NH	9.42, d, 9.4	-	9.41, d, 9.4	-	0.01	-
NMe-Leu[7]	1	-	171.7	-	171.7	-	0.0
	2	5.67, t, 7.5	54.8	5.67, t, 7.5	54.8	0.00	0.0
	3a	1.74, m	38.9	1.74, m	38.9	0.00	0.0
	3b	1.60, m		1.60, m		0.00	

		Natural ohmyungsamycin B (800 MHz)		Revised ohmyungsamycin B (**37**; 800 MHz)		$\Delta\delta$, ppm (Natural – Revised **37**)	
		^{1}H	^{13}C	^{1}H	^{13}C	ΔH	ΔC
	4	1.43, m	25.1	1.43, m	25.1	0.00	0.0
	5	0.68, d, 6.4	23.1	0.68, d, 6.5	23.1	0.00	0.0
	6	0.69, d, 6.4	22.1	0.69, d, 6.5	22.1	0.00	0.0
	NMe	3.52, s	31.3	3.52, s	31.3	0.00	0.0
Val[8]	1	-	173.5	-	173.5	-	0.0
	2	5.30, t, 8.2	55.6	5.30, t, 8.2	55.6	0.00	0.0
	3	2.57, m	31.0	2.57, m	31.0	0.00	0.0
	4	1.20, d, 6.7	19.2	1.21, d, 6.6	19.2	0.00	0.0
	5	1.16, d, 6.8	19.8	1.16, d, 6.8	19.8	0.00	0.0
	NH	8.09, d, 8.8	-	8.09, d, 8.7	-	0.00	-
NMe-Thr[9]	1	-	170.6	-	170.6	-	0.0
	2	5.63, d, 3.4	62.4	5.63, d, 3.5	62.3	0.00	0.1
	3	5.10, m	66.6	5.10, m	66.6	0.00	0.0
	4	1.35, d, 6.4	20.4	1.35, d, 6.4	20.4	0.00	0.0
	NMe	3.70, s	34.7	3.70, s	34.7	0.00	0.0
	OH	6.15, d, 4.1	-	6.15, d, br	-	-	-
Thr[10]	1	-	171.4	-	171.4	-	0.0
	2	5.89, dd, 9.1, 2.6	52.4	5.89, dd, 9.2, 2.6	52.4	0.00	0.0
	3	6.05, qd, 6.4, 2.5	69.5	6.05, m	69.5	0.00	0.0
	4	1.54, d, 6.5	16.7	1.54, d, 6.5	16.7	0.00	0.0
	NH	10.20, d, 9.1	-	10.20, d, 9.2	-	0.00	-
Ile[11]	1	-	173.4	-	173.4	-	0.0
	2	5.27, t, 8.5	56.9	5.27, t, 8.5	56.9	0.00	0.0
	3	2.07, m	37.7	2.08, m	37.7	-0.01	0.0
	4	0.96, d, 6.8	15.8	0.95, d, 6.8	15.8	0.01	0.0
	5a	1.68, m	25.2	1.68, m	25.2	0.00	0.0
	5b	1.26, overlapped		1.26, overlapped		0.00	
	6	0.72, t, 7.4	10.8	0.72, t, 7.4	10.8	0.00	0.0
	NH	8.67, d, 9.3	-	8.68, d, 9.2	-	-0.01	-
NMe-Val[12]	1	-	174.3	-	174.3	-	0.0
	2	3.10, d, 5.9	71.4	3.11, d, 5.9	71.4	-0.01	0.0
	3	2.18, m	32.3	2.18, m	32.3	0.00	0.0
	4	1.11, d, 7.1	19.1	1.12, d, 6.6	19.0	-0.01	0.1
	5	1.11, d, 7.1	19.1	1.11, d, 7.1	19.0	0.00	0.1
	NMe	2.55, s	35.9	2.55, s	35.9	0.00	0.0

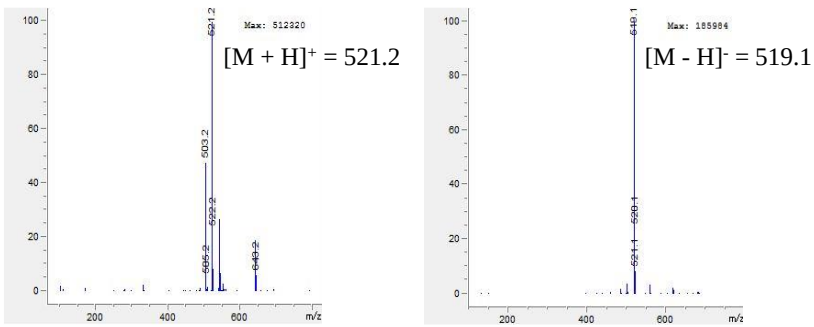
Table S4. Comparison of ^{1}H and ^{13}C NMR spectral data of natural and revised ohmyungsamycin B (37)

Determination of absolute configuration of the β-carbon of isoleucine unit included in natural ohmyungsamycin B.

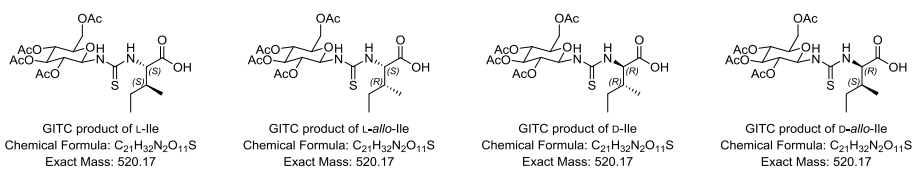
Using 1 mL of 6 *N* HCl, 1 mg of natural ohmyungsamycin B was subjected to acid hydrolysis at 115 °C for 1 h to obtain free amino acid units. The hydrolysate was cooled by using an ice bath (0 °C) for 5 min and the HCl solution was evaporated *in vacuo*, rapidly. For eliminating residual HCl in the reaction mixture, 1 mL of water was added to the vial and vaporized under low pressure for three times. The acid hydrolysate mixture was lyophilized for 24 h and dissolved with 100 μL of trimethylamine solution. Subsequently, 100 μL of 1% 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC) in acetone solution was added to the vial containing hydrolysate and chemical derivatization was processed at room temperature (25 °C).[11] After 15 min, 100 μL of 5% acetic acid solution was added for quenching the reaction. 20 μL of aliquot was analyzed by LC/MS under a reversed-phase gradient solvent system (column: Phenomenex®, Gemini, 250 X 4.6 mm, C_{18}, 5 μm; flow rate: 0.25 mL/min; UV detection: 254 nm; 35% to 50% acetonitrile/water with 0.1% trifluoroacetic acid over 80 min). The GITC derivative of isoleucine residue from natural ohmyungsamycin B was detected by ESIMS data at 60.7 min with this analysis condition (Figure S10C). For comparative analysis, GITC derivatives of commercially available authentic L-isoleucine (CAS No. 73-32-5, Sigma-Aldrich), L-*allo*-isoleucine (CAS No. 1509-34-8, Sigma), D-leucine (CAS No. 319-78-8, Sigma), and D-*allo*-isoleucine (CAS No. 1509-35-9, Aldrich) were prepared and analyzed by the same procedure described above. Each GITC derivative of authentic L-isoleucine, L-*allo*-isoleucine, D-isoleucine, and D-*allo*-isoleucine eluted at the retention times of 60.7, 60.3, 61.5, and 60.9 min, respectively (Figure S10C). The GITC derivative of the isoleucine residue included in natural ohmyungsamycin B eluted at 60.7 min, thus determining this unit as L-isoleucine. In addition, the GITC reaction product from

natural ohmyungsamycin B was co-injected to LC/MS with the GITC derivative of authentic L-isoleucine or L-*allo*-isoleucine. The GITC adduct of isoleucine from ohmyungsamycin B eluted together with authentic L-isoleucine, which confirmed that the isoleucine unit involved in natural ohmhyungsamycin B is L-isoleucine.

(A)



(B)



(C)

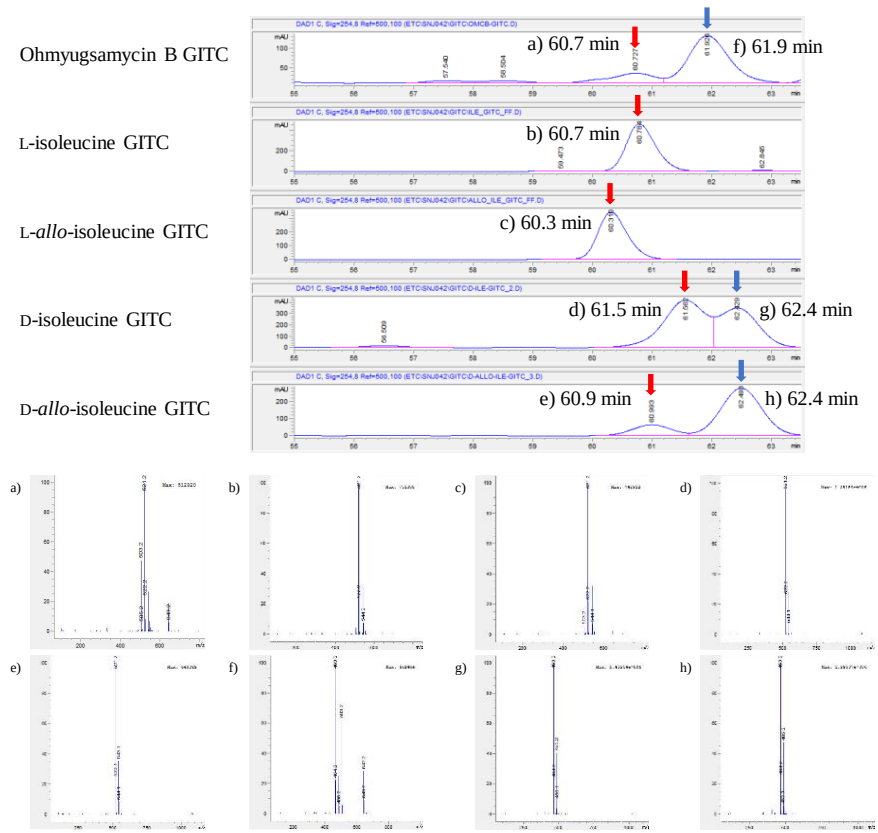
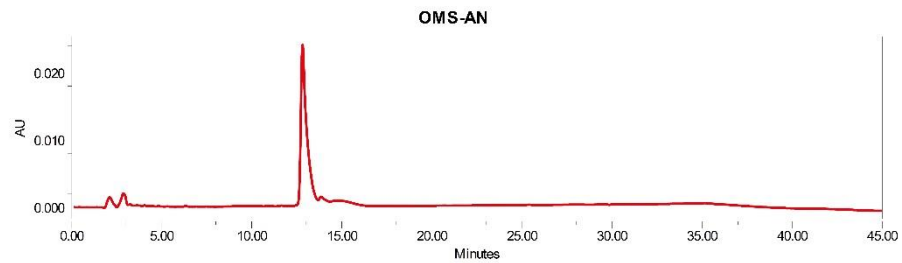


Figure S1. GITC analysis of ohmyungsamycin B (A) Positive/negative ESIMS data of the peak at 60.7 min in the LC/MS analysis of natural ohmyungsamycin B GITC mixture. (B) The structures of GITC products of four diastereomers of isoleucine (C) LC/MS analysis of the GITC derivative of the isoleucine unit in natural ohmyungsamycin B and the four diastereomers of isoleucines; positive-mode ESI mass spectrum of the GITC product of a) isoleucine from natural ohmyungsamycin B, b) L-Ile, c) L-*allo*-Ile, d) D-Ile, e) D-*allo*-Ile. These GITC products commonly displayed the ion $[M+H]^+$ at *m*/*z* 521. f-h) unidentified by-products in the GITC reactions with the ion at *m*/*z* 483, which is not related with Ile.

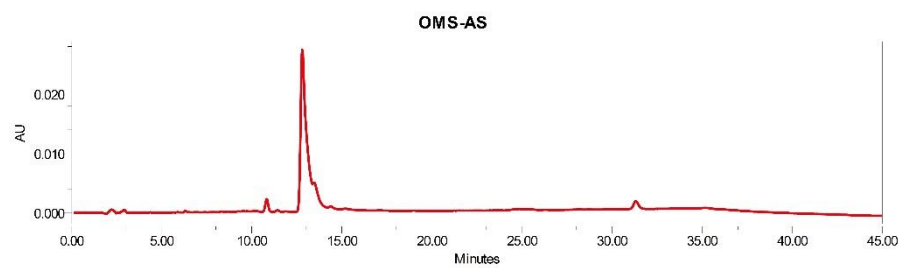
HPLC Analysis for Final Compounds

All chromatograms were obtained via following conditions; Column, Agilent Pursuit XRs 100Å C18, 4.6 x 250 mm, 5 μm; detection, UV 280 nm; flow rate 1.0 mL/min; Mobile phase, A: MeCN with 0.1% TFA, B: H_2O with 0.1% TFA; Gradient, 50% of A to 75% of A in 30 min, 75% of A for additional 15 min

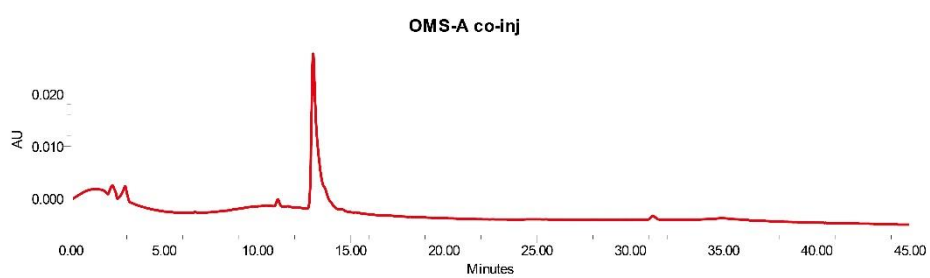
Natural ohmyungsamycin A



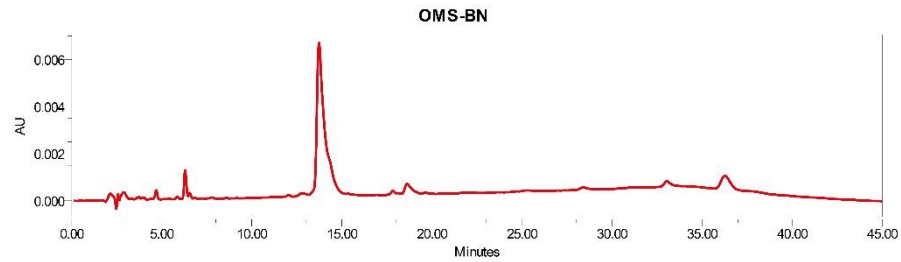
Synthetic ohmyungsamycin A



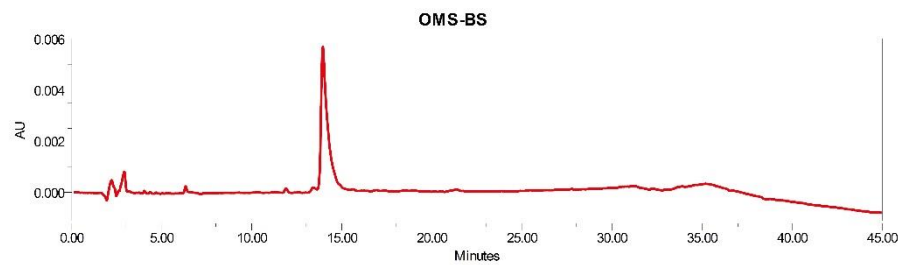
Co-injection of natural and synthetic ohmyungsamycin A



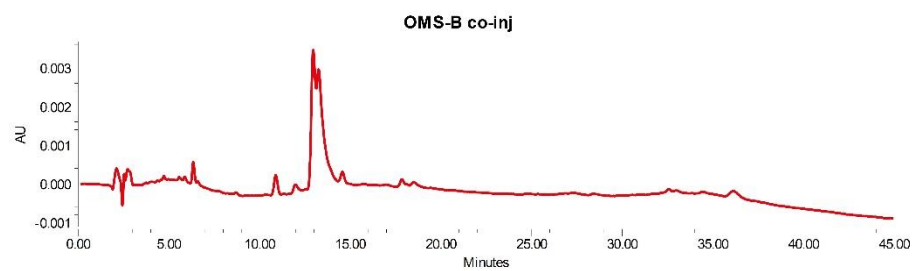
Natural ohmyungsamycin B



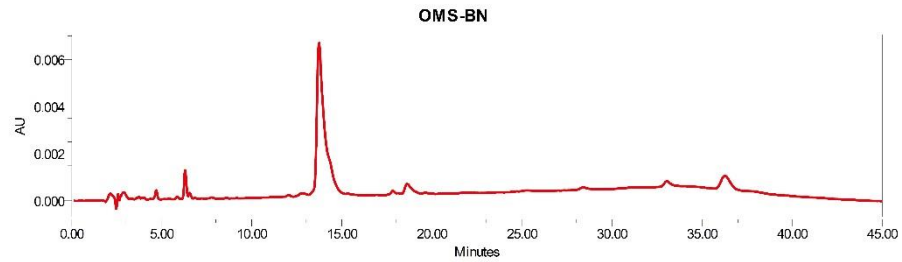
Proposed ohmyungsamycin B (2)



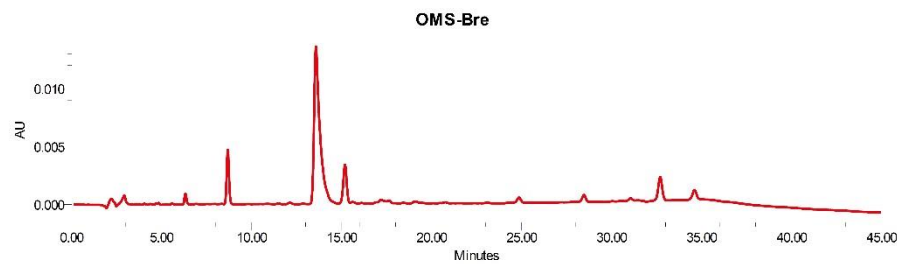
Co-injection of natural and proposed ohmyungsamycin B (2)



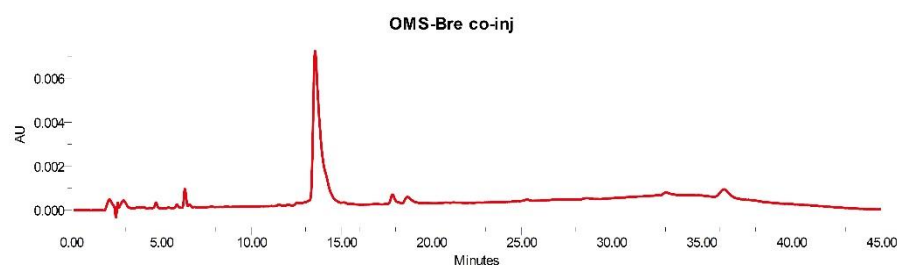
Natural ohmyungsamycin B



Revised ohmyungsamycin B (28)



Co-injection of natural and revised ohmyungsamycin B (28)



Determining the MICs by resazurin microtiter assay (REMA)

The REMA was performed as described previously to determine the MICs of ohmyungsamycin A (**1**), proposed ohmyungsamycin B (**2**), revised ohmyungsamycin B (**28**), Δ^{10}*N*-Ac ohmyungsamycin (**4**) and ethambutol (E4630, from Sigma, St. Louis, MO, USA) against H37Rv.[73] Briefly, a 100 μl inoculum was used to inoculate each well of the plate, and two-fold serial dilutions of each test compound were prepared in 96-well plates in triplicate. An inoculum at an optical density 600 of 0.005 was prepared by diluting mid-log cultures and then added to each well. Growth controls containing no drug and a sterile control were also prepared in each experiment. Plates were incubated at 37 °C for 5 days, and 40 μL 0.025% resazurin (Sigma, R7017) solution were then added to each well. After an overnight incubation, the fluorescence of the resazurin metabolite resorufin was determined by excitation at 560 nm and emission at 590 nm using the Synergy H1 microplate reader (Biotechnology Inc, Dallas, TX, USA). The MIC50 (the MIC required to inhibit the growth of 50% of the organism) was determined using GraphPad Prism 5.0 software.

V. References

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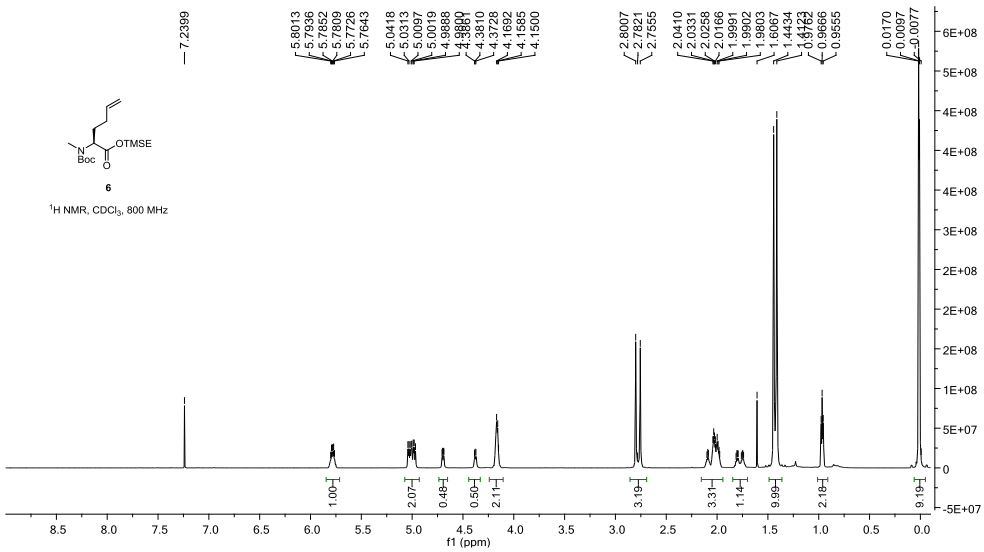
VI. Appendix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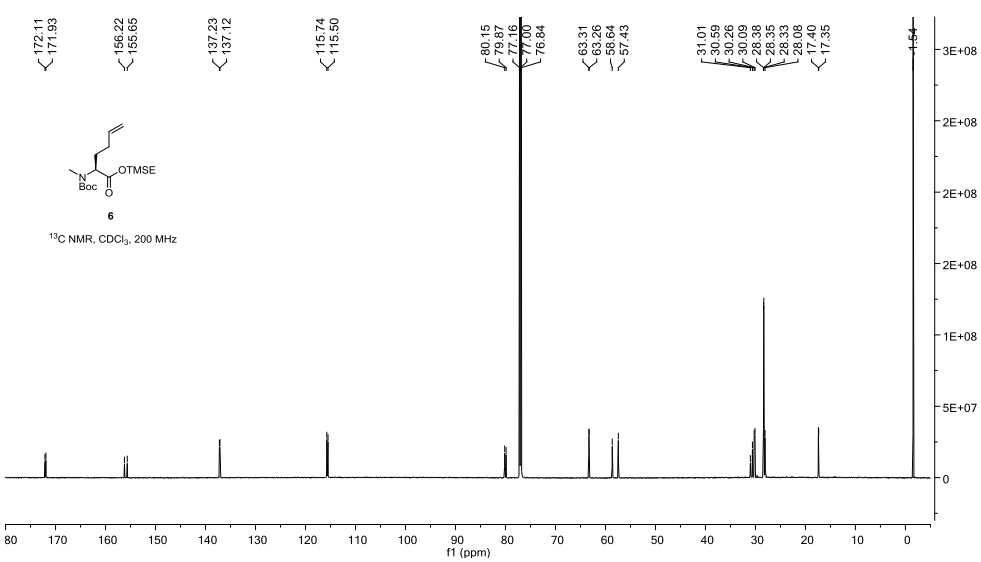
5.0019
4.9888
4.3810
4.3728
4.1692
4.1585
4.1500
2.8007
2.7821
2.7555
2.0410
2.0331
2.0258
2.0166
1.9991
1.9902
1.9803
1.6067
1.4434
0.9666
0.9555
0.0170
0.0097
0.0077
OTMSE
Boc
6
1H NMR, CDCl3, 800 MHz
1.00
2.07
0.48
0.50
2.11
3.19
3.31
1.14
9.99
2.18
9.19
f1 (ppm)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137.12
115.74
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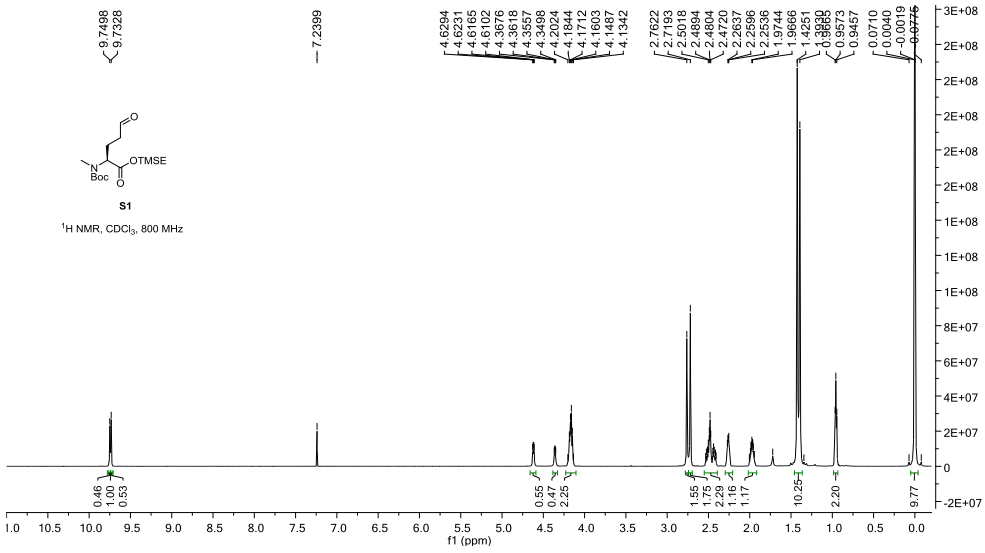
79.87
77.16
77.00
76.84
63.31
63.26
58.64
57.43
31.01
30.59
30.26
30.09
28.38
28.35
28.33
28.08
17.40
17.35
1.54
OTMSE
Boc
6
13C NMR, CDCl3, 200 MHz
f1 (ppm)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4.3498
4.2024
4.1844
4.1712
4.1603
4.1487
4.1342
2.7622
2.7193
2.5018
2.4894
2.4804
2.4720
2.2637
2.2596
2.2536
1.9744
1.9666
1.4251
0.9573
0.9457
0.0710
0.0040
-0.0019
-0.0776
OTMSE
Boc
S1
1H NMR, CDCl3, 800 MHz
0.46
1.00
0.53
0.55
0.47
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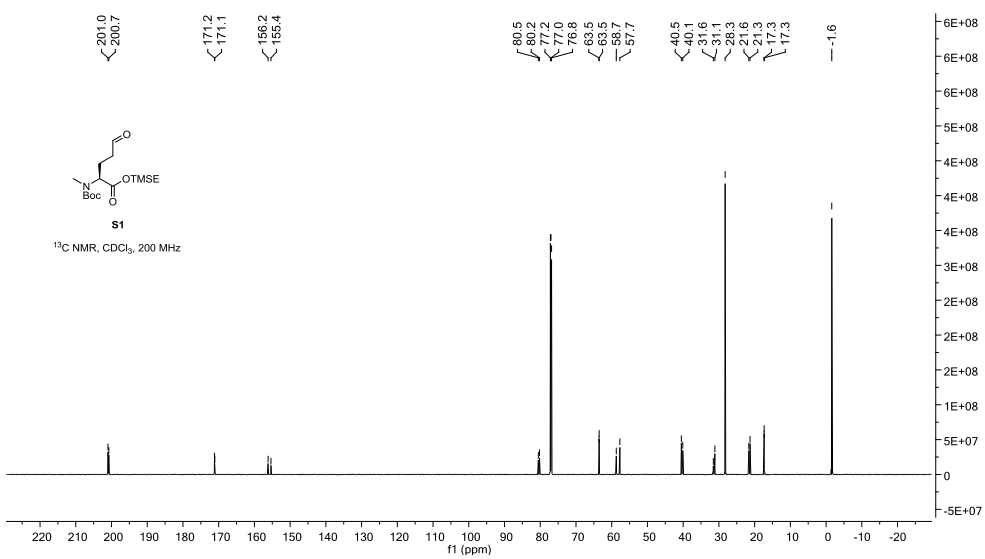
1.55
1.75
2.29
1.16
1.17
10.25
2.20
9.77
f1 (ppm)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200.7
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155.4
80.5
80.2
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77.0
76.8
63.5
63.5
58.7
57.7
40.5
40.1
31.6
31.1
28.3
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17.3
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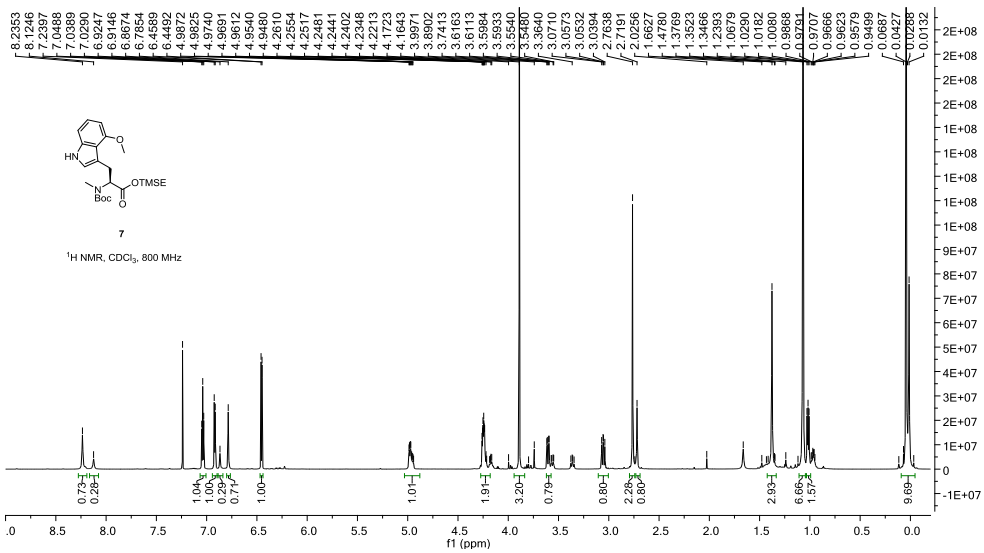
OTMSE
Boc
S1
13C NMR, CDCl3, 200 MHz
f1 (ppm)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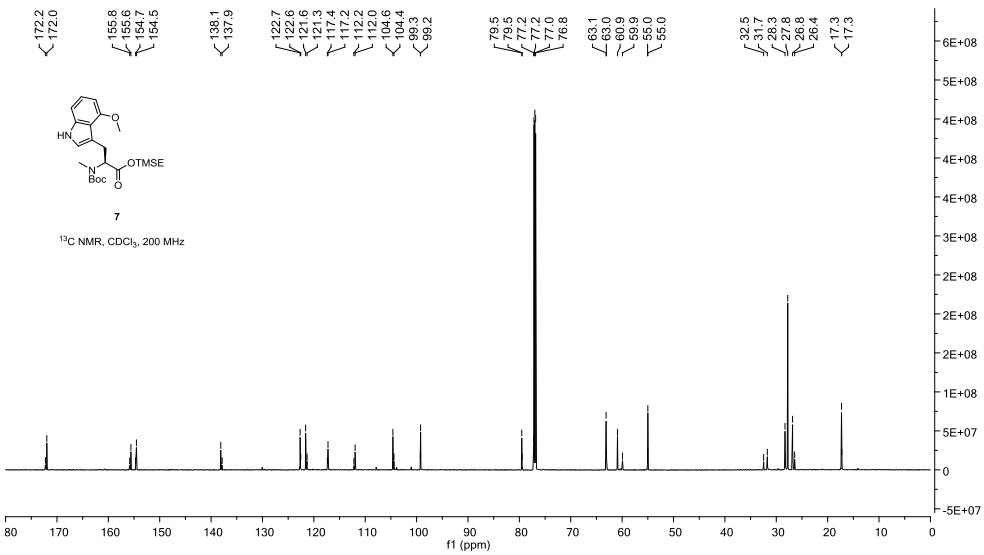
f1 (ppm)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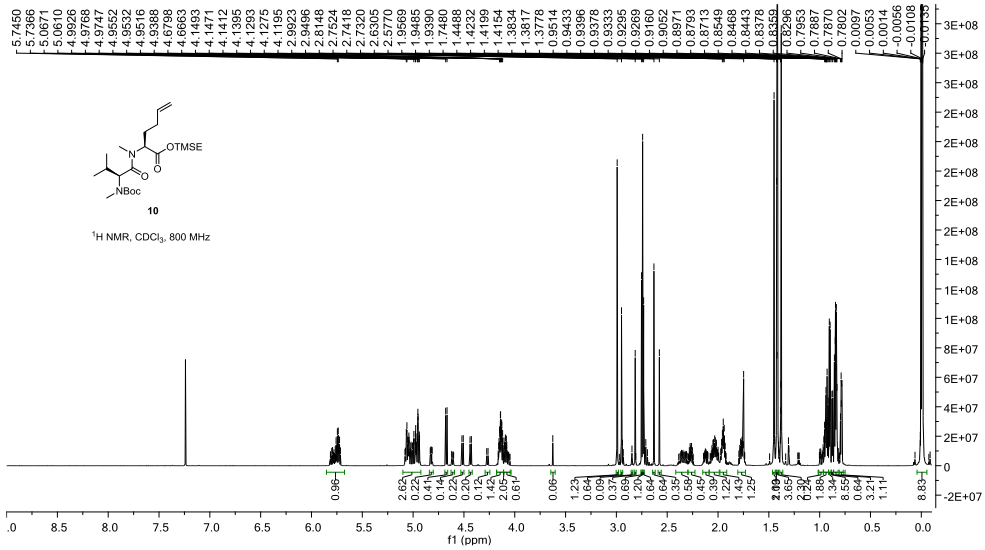
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10
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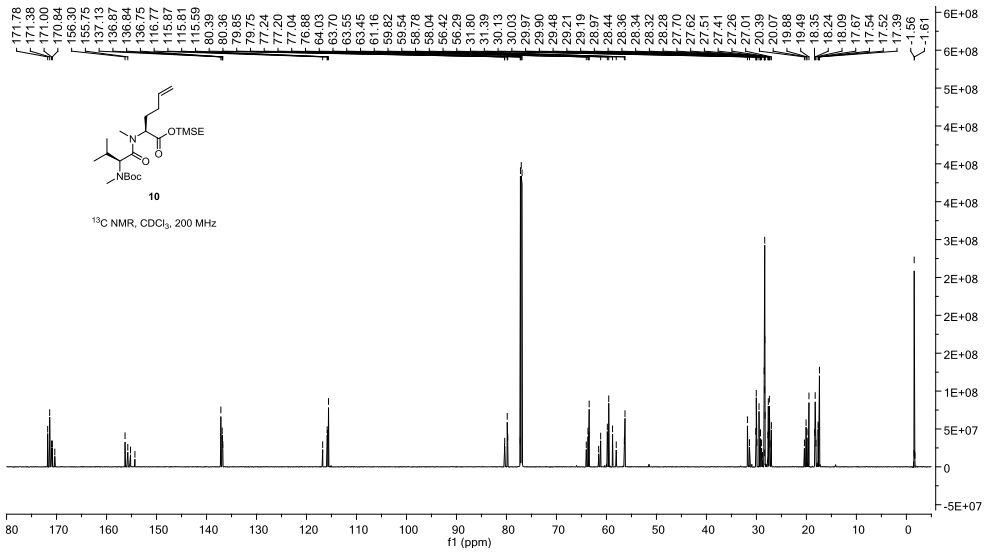
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O
N
10
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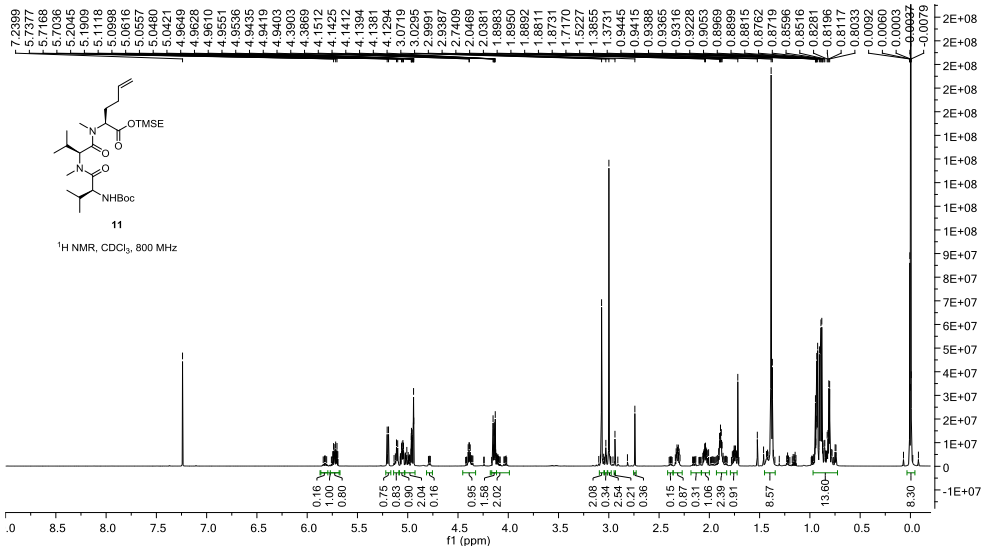
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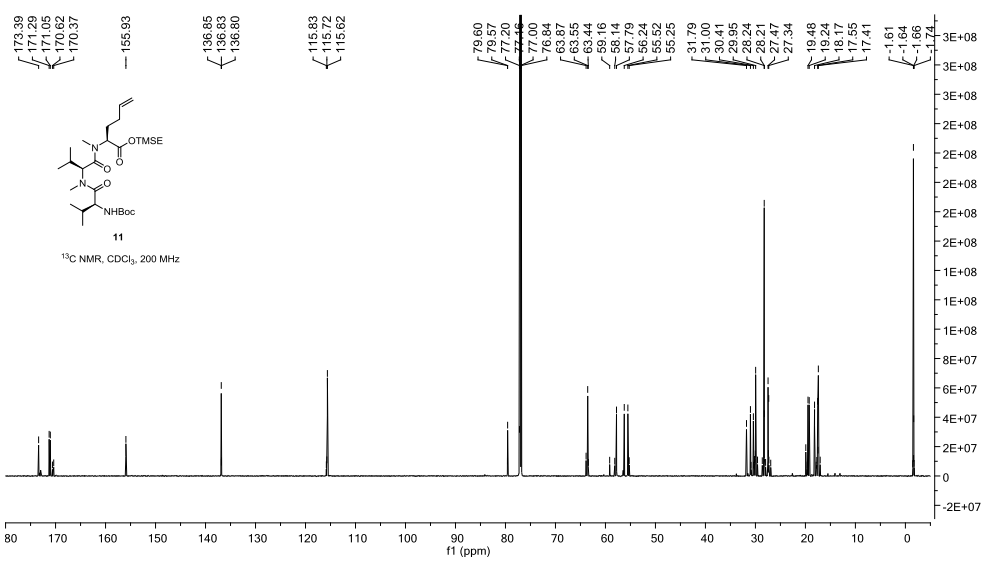
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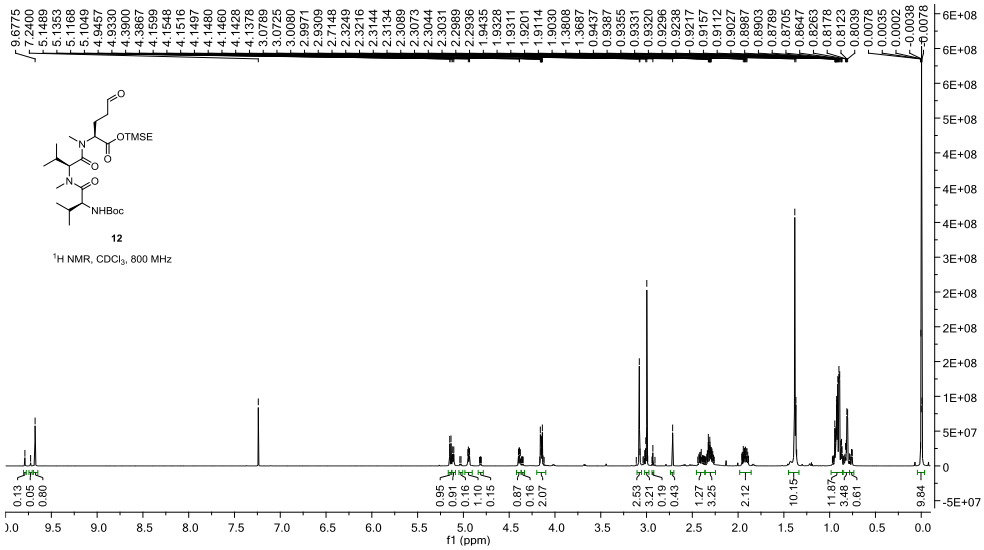
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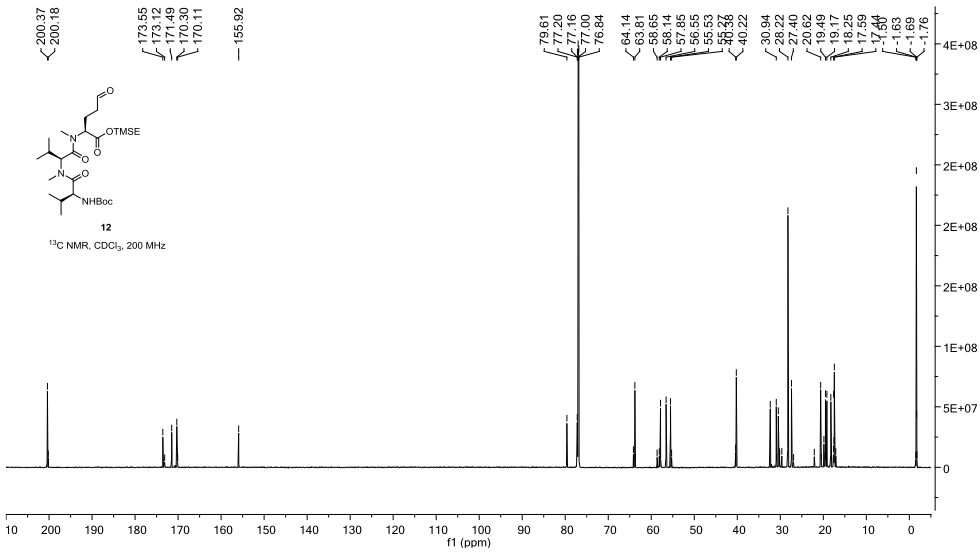
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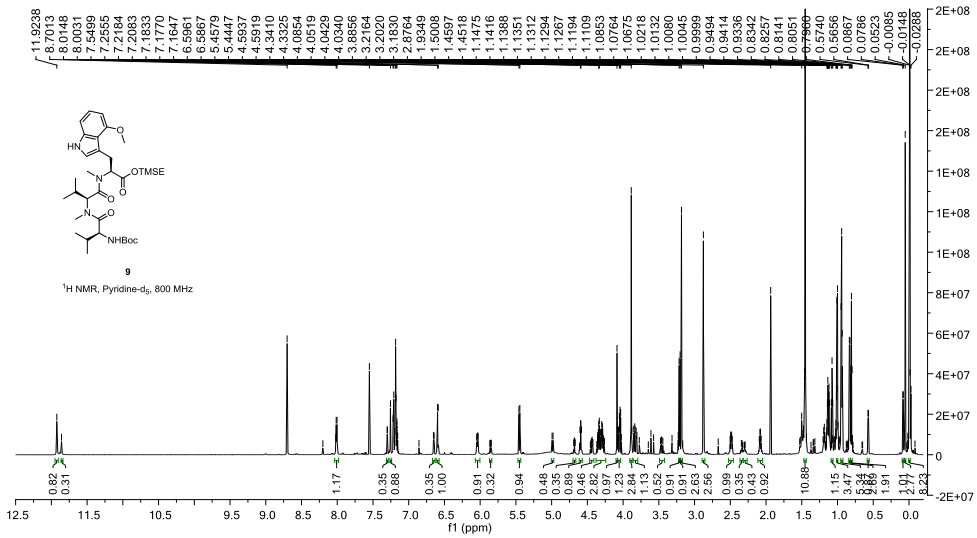
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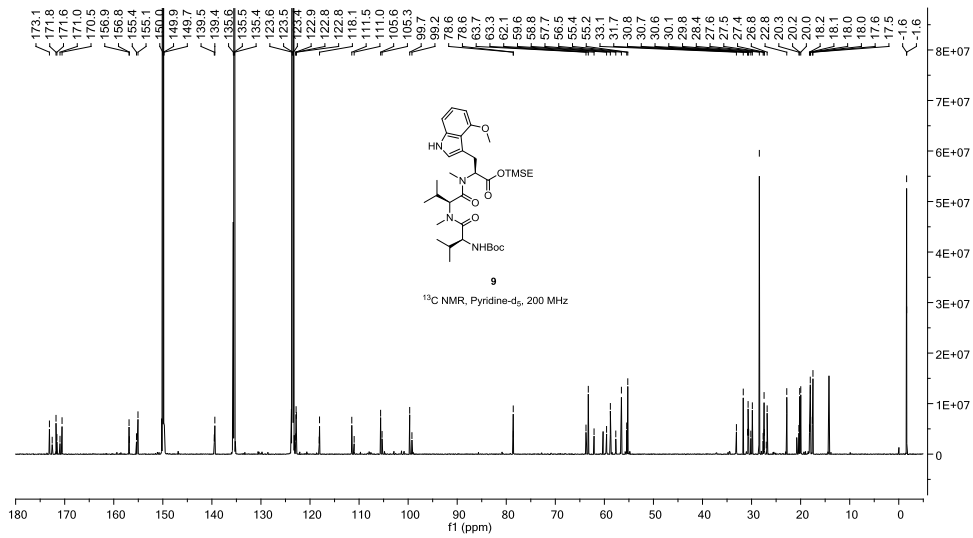
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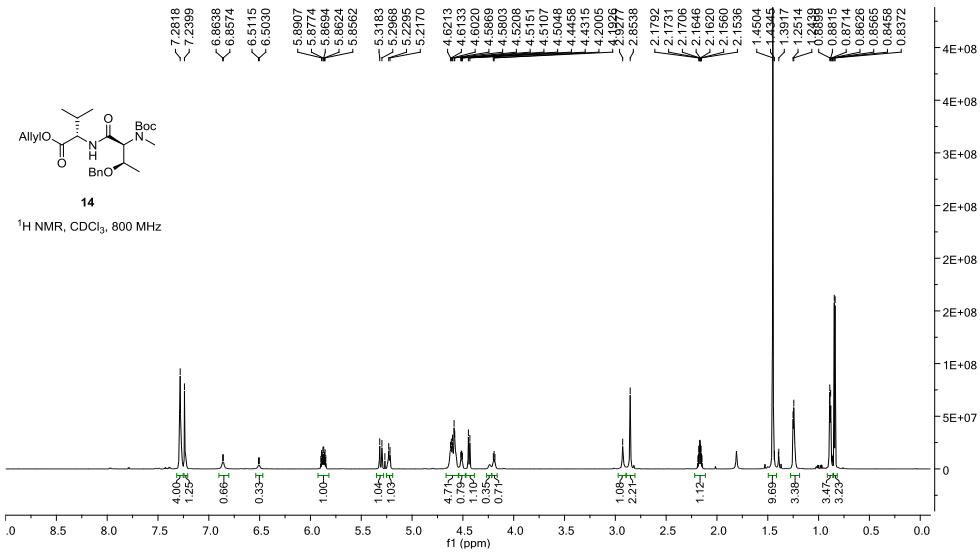
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Boc
N
H
BnO
14
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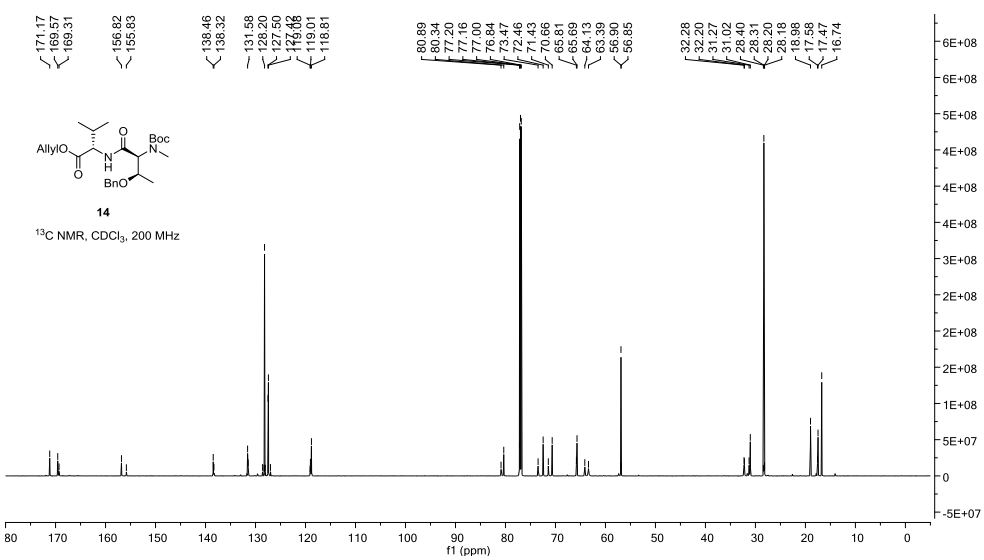
f1 (ppm)


AllylO
Boc
N
H
BnO
14
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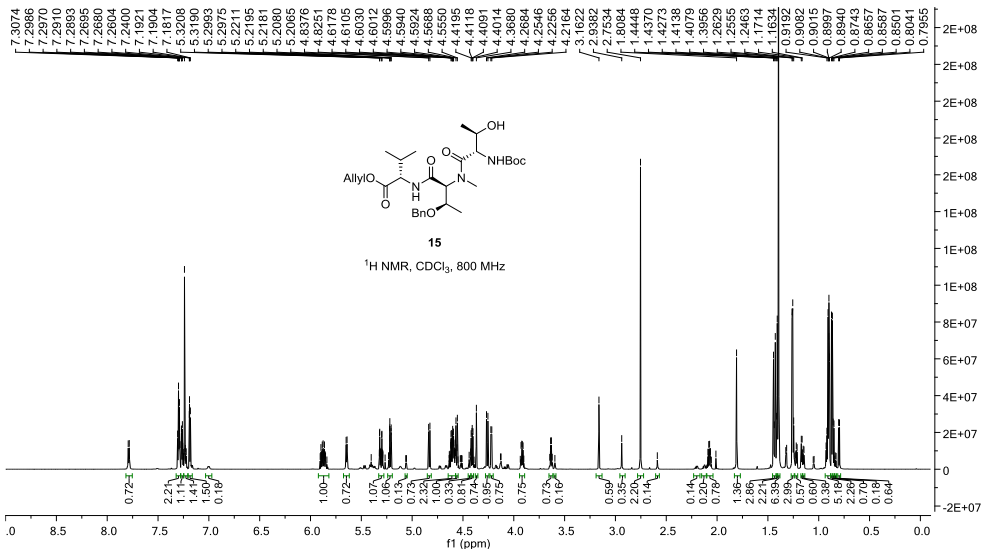
f1 (ppm)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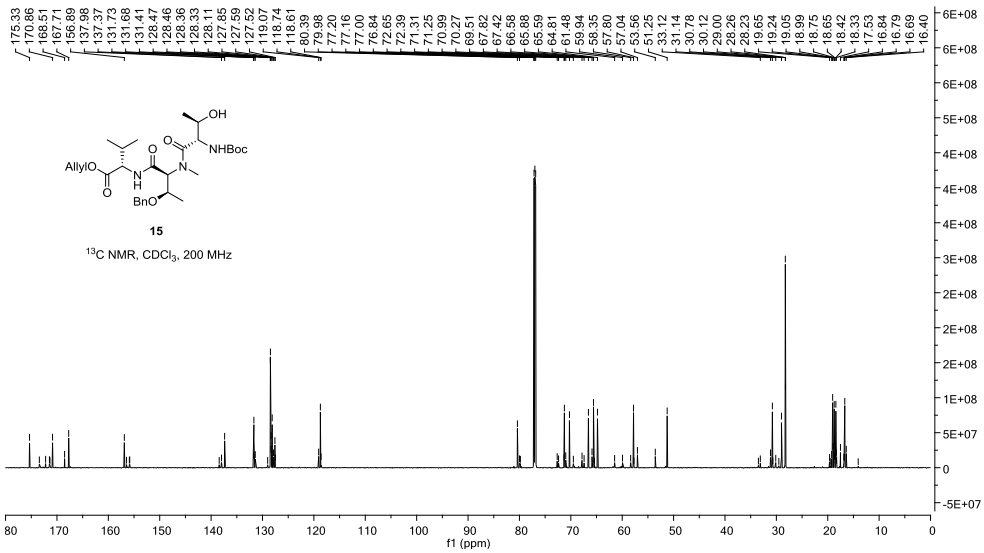
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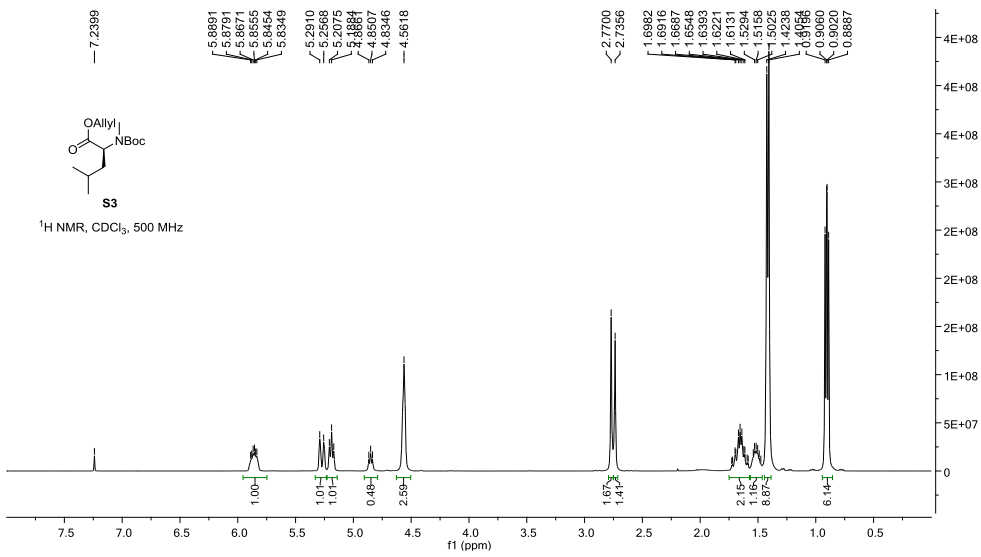
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0.9020
0.8887
OAllyl
NBoc
O
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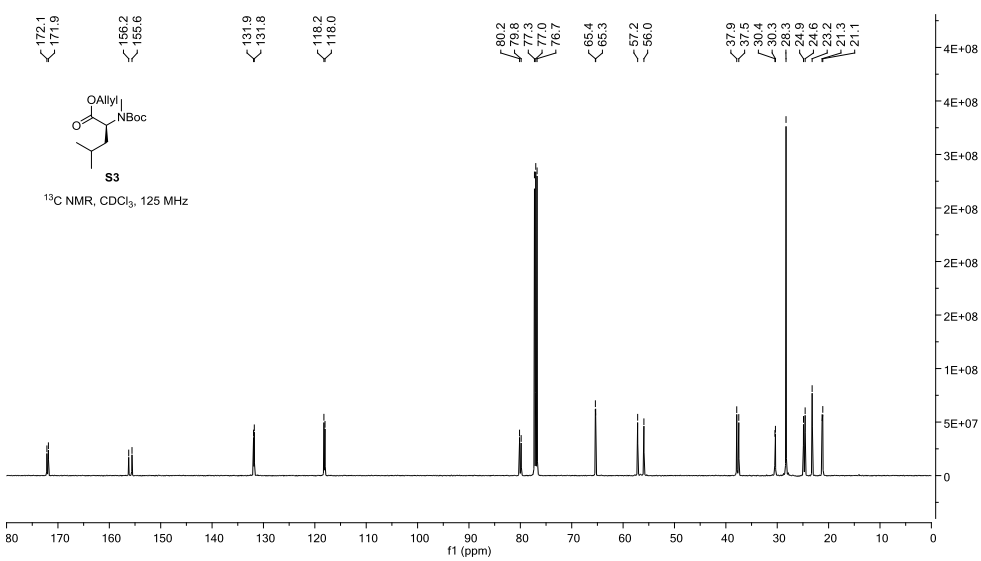
6.14
f1 (ppm)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37.5
30.4
30.3
28.3
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NBoc
O
S3
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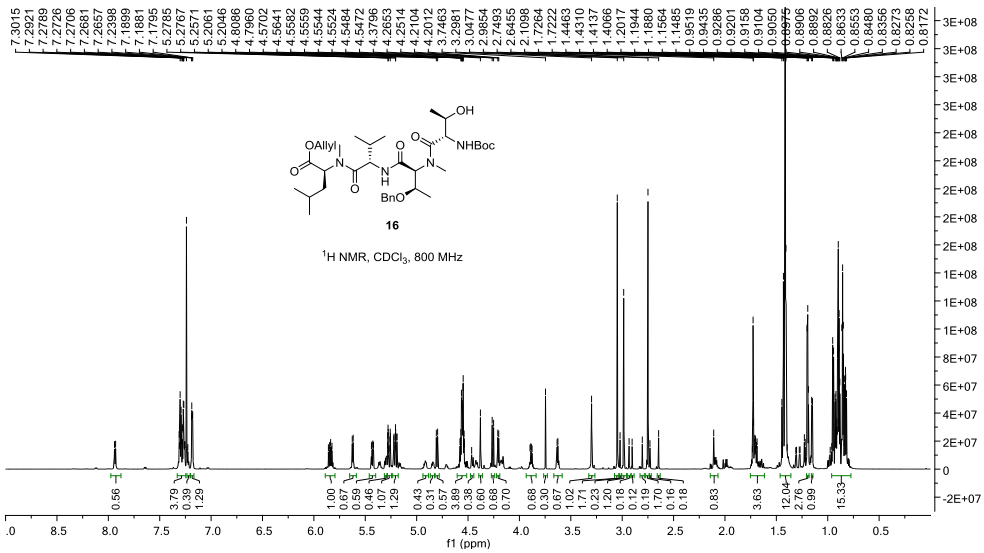
f1 (ppm)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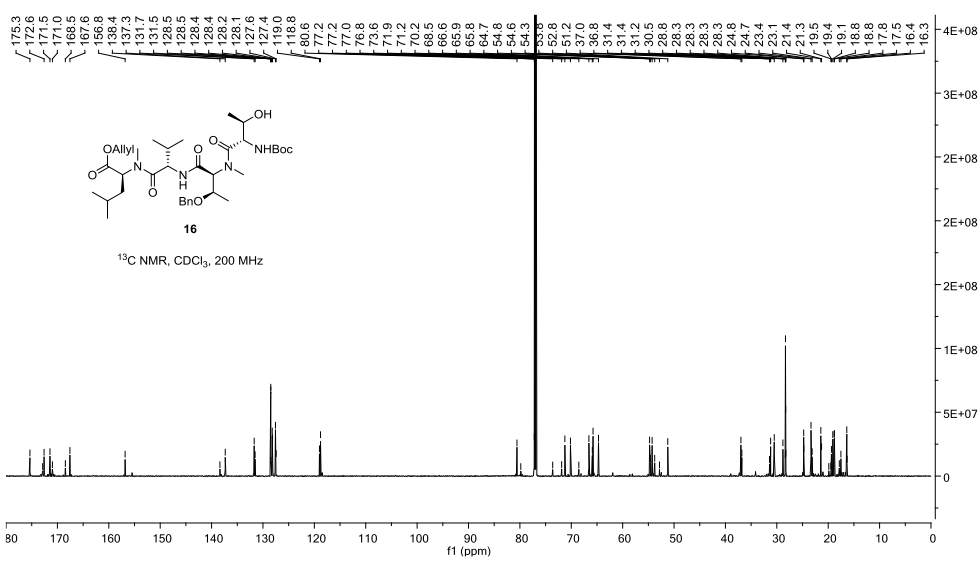
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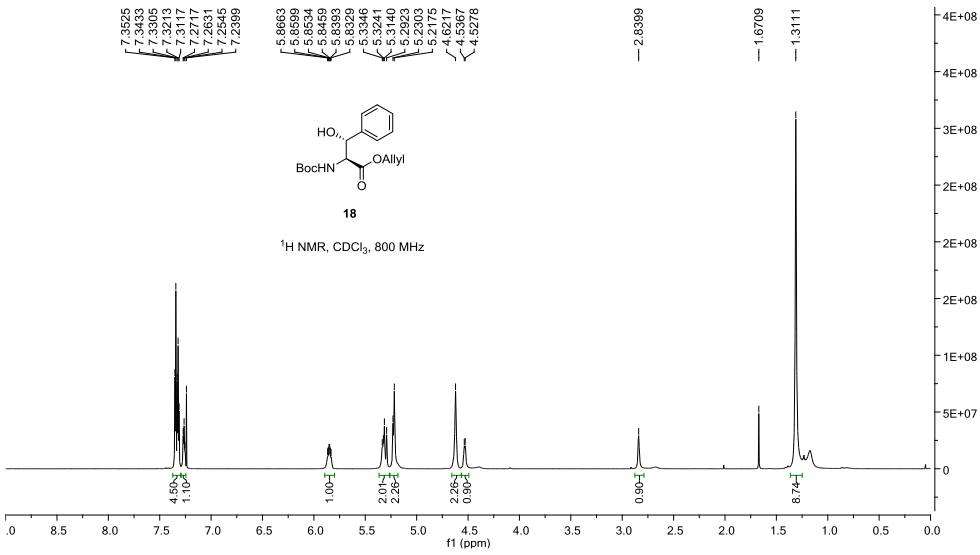
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5.3140
5.2993
5.2303
5.2175
4.6217
4.5367
4.5278
2.8399
1.6709
1.3111
HO
BocHN
OAllyl
O
18
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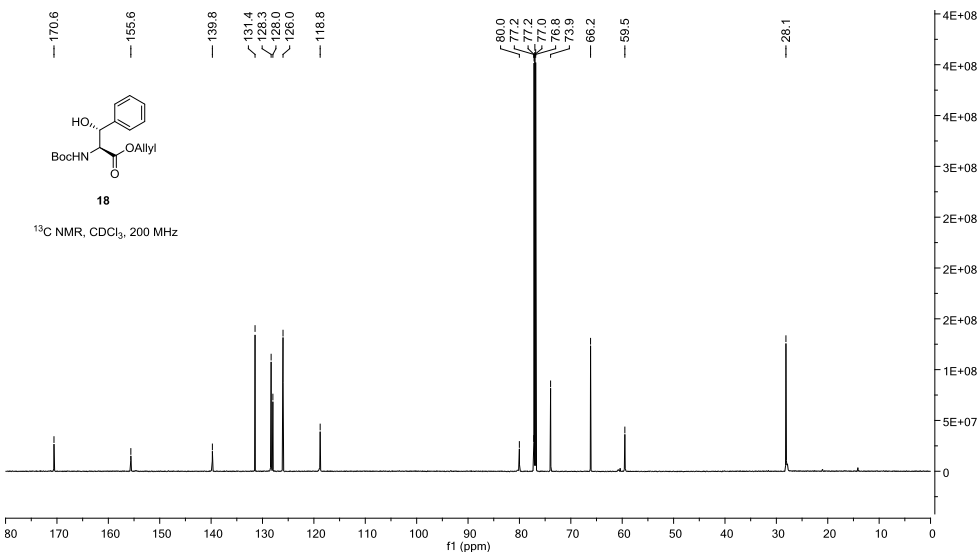
8.74
f1 (ppm)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76.8
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O
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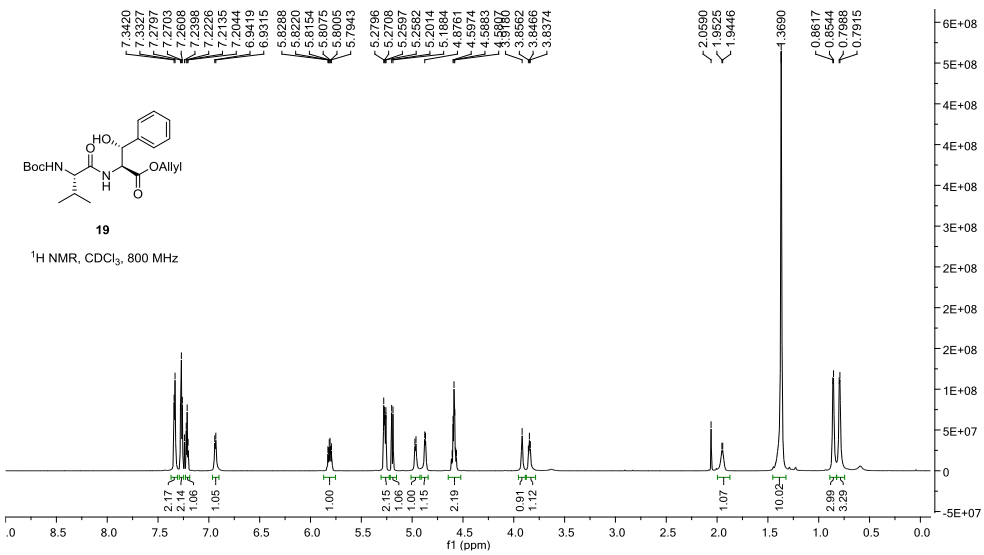
f1 (ppm)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1.3690
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0.8544
0.7988
0.7915
HO
O
BocHN
N
H
OAllyl
O
19
1H NMR, CDCl3, 800 MHz
2.17
2.14
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1.00
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10.02
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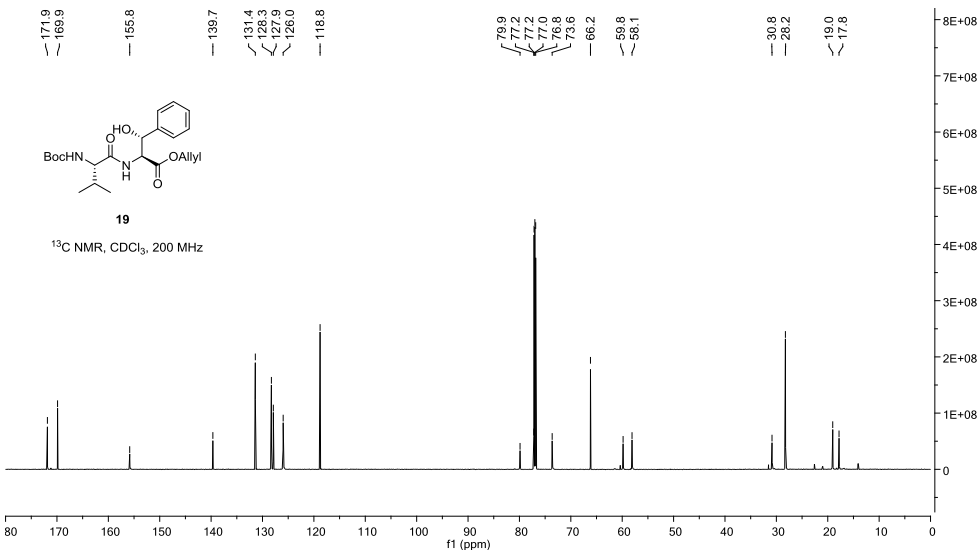
3.29
f1 (ppm)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73.6
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59.8
58.1
30.8
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HO
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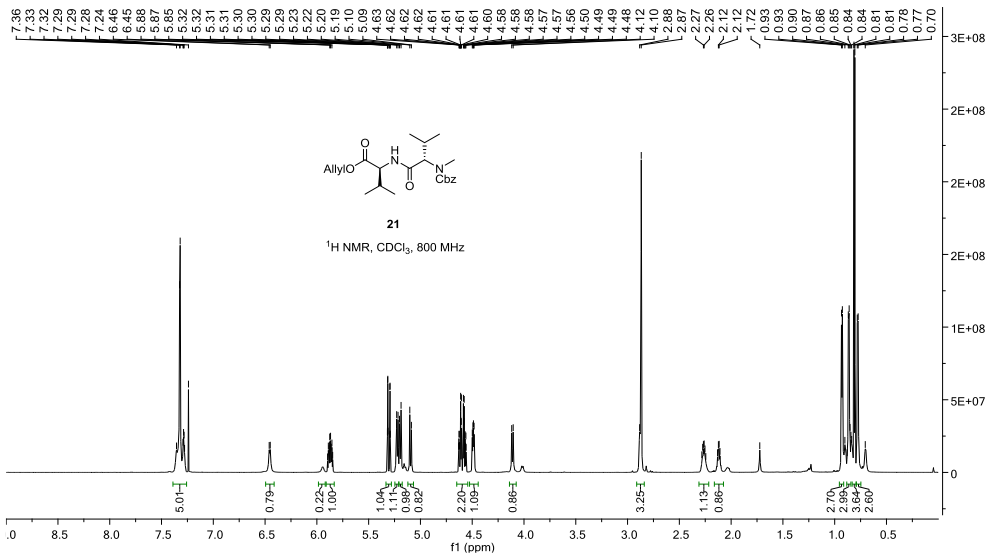
f1 (ppm)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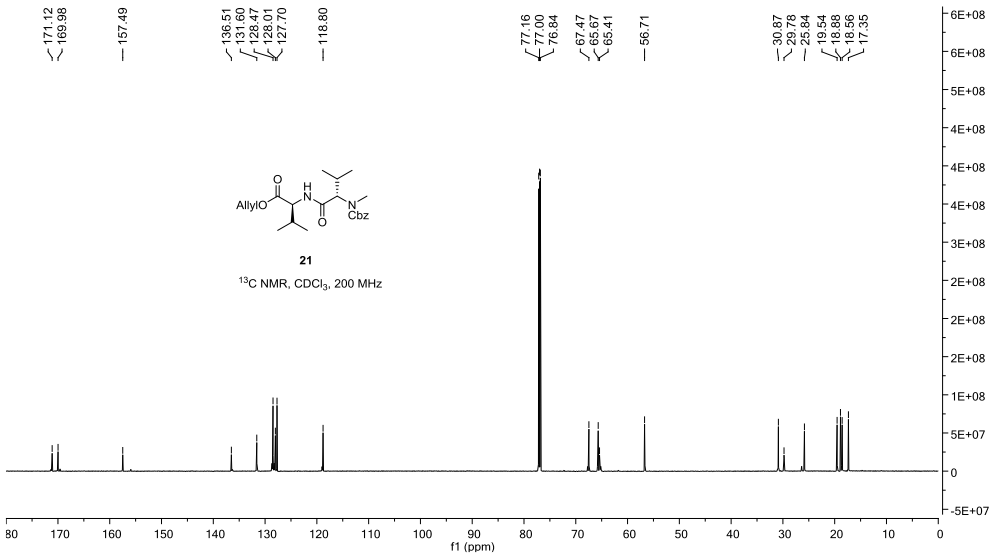
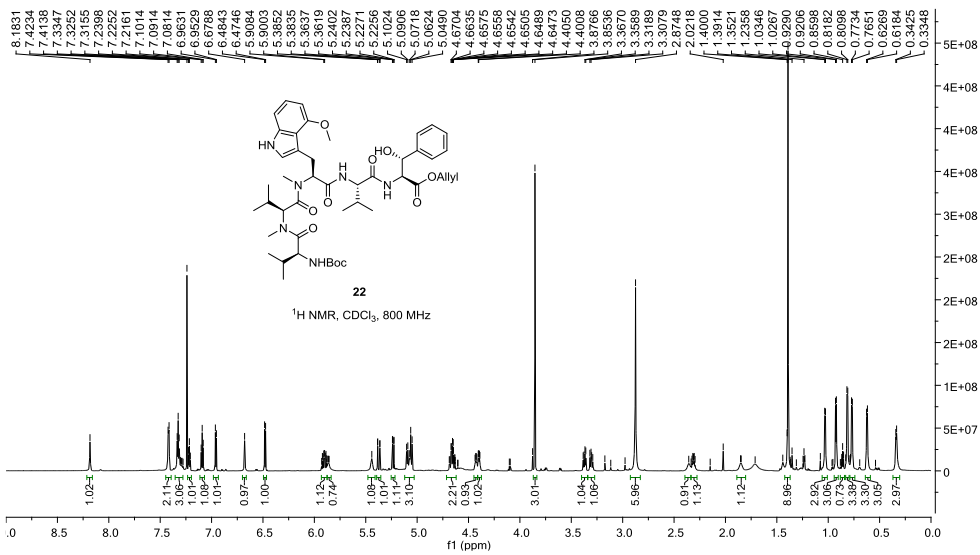
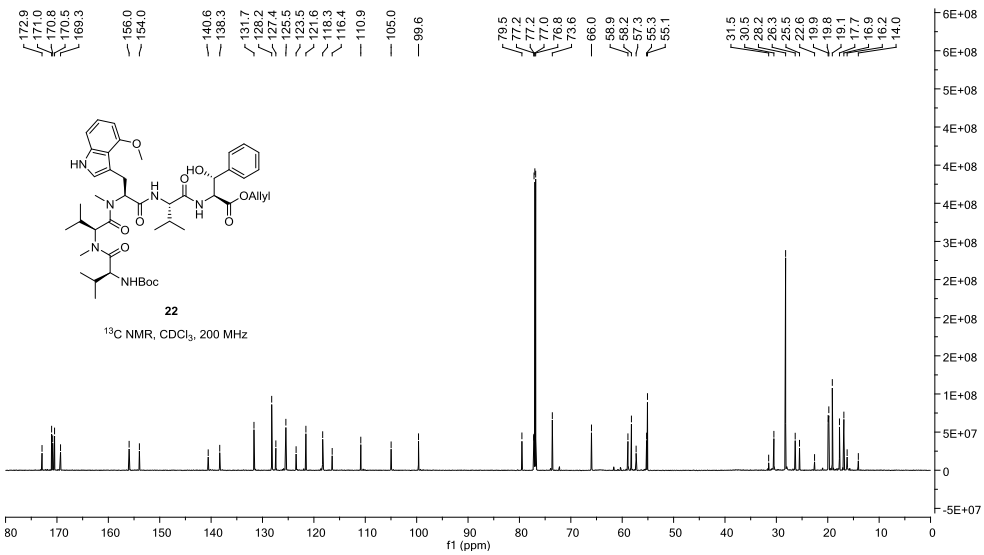
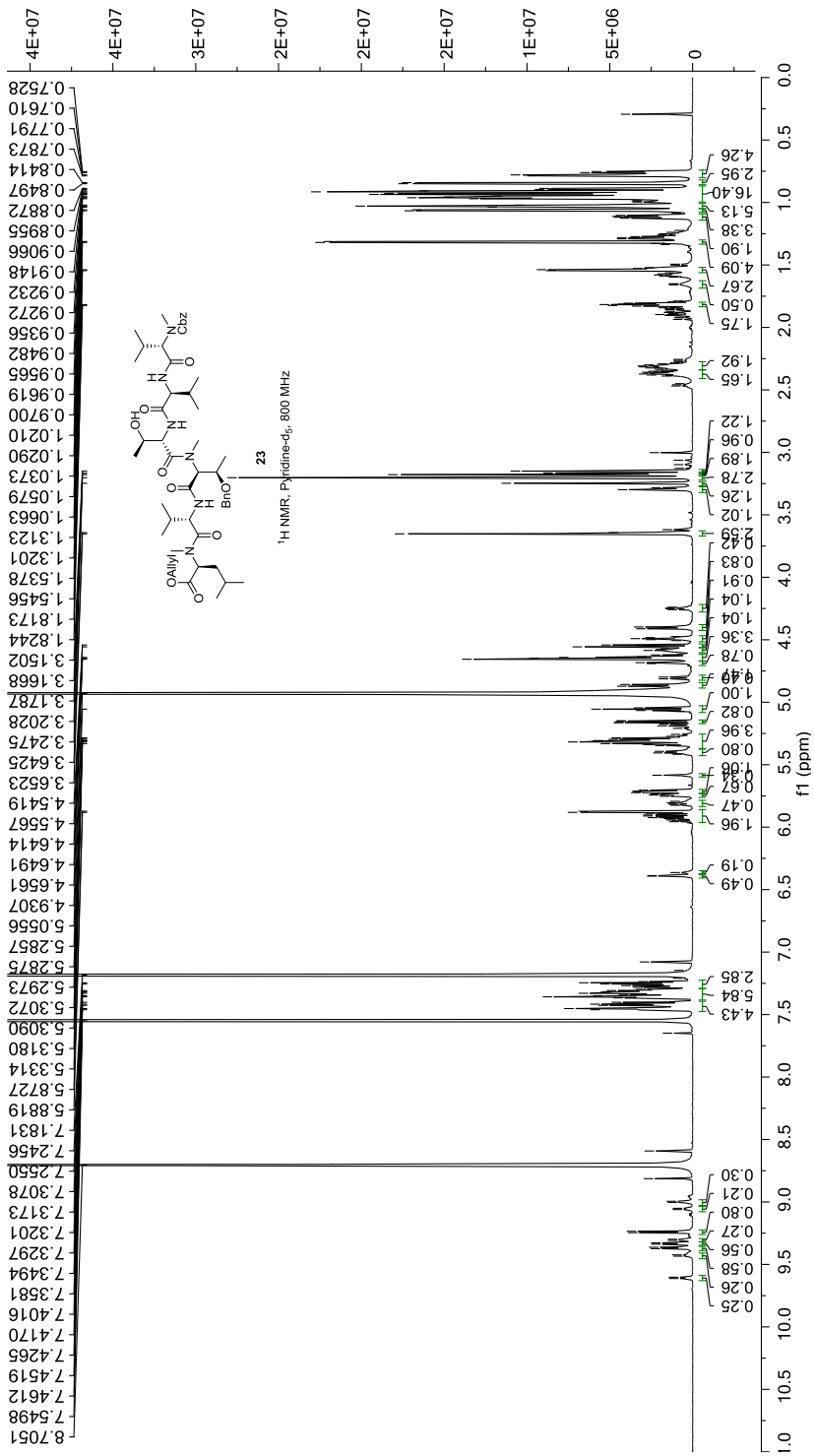
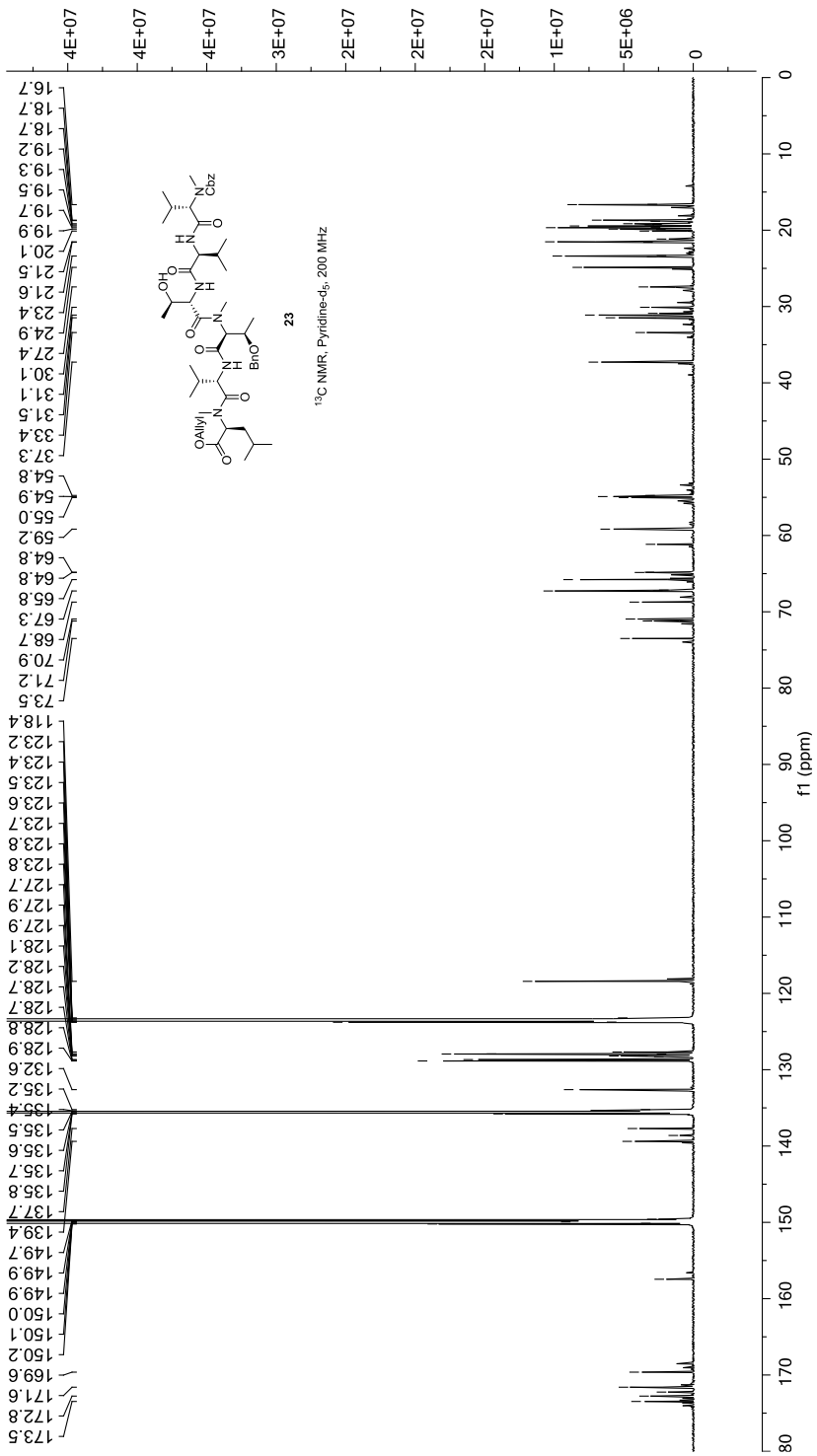
^{1}H NMR, CDCl$_3$, 800 MHz
f1 (ppm)

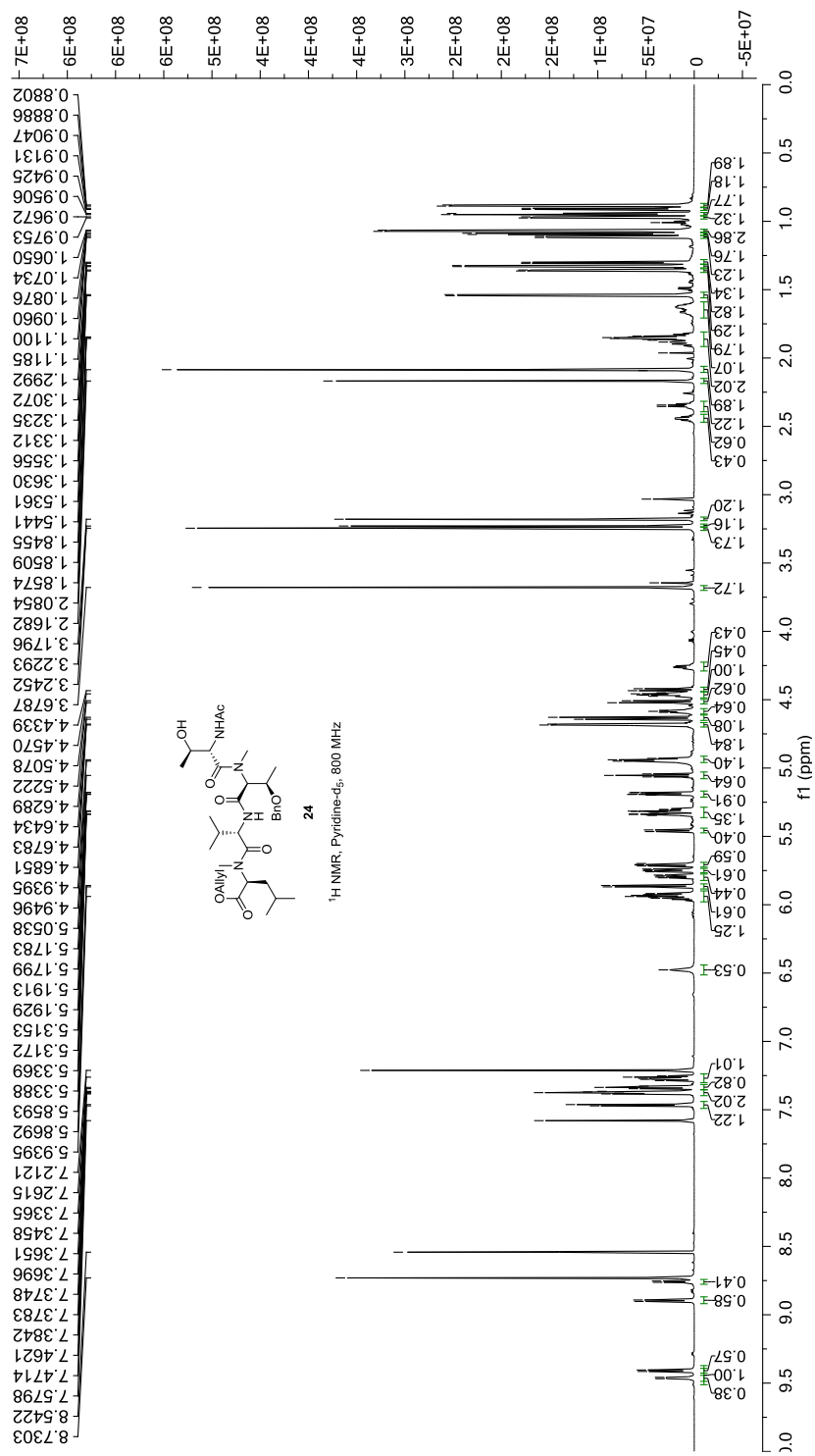

AllylO
Cbz
21
^{13}C NMR, CDCl$_3$, 200 MHz
f1 (ppm)

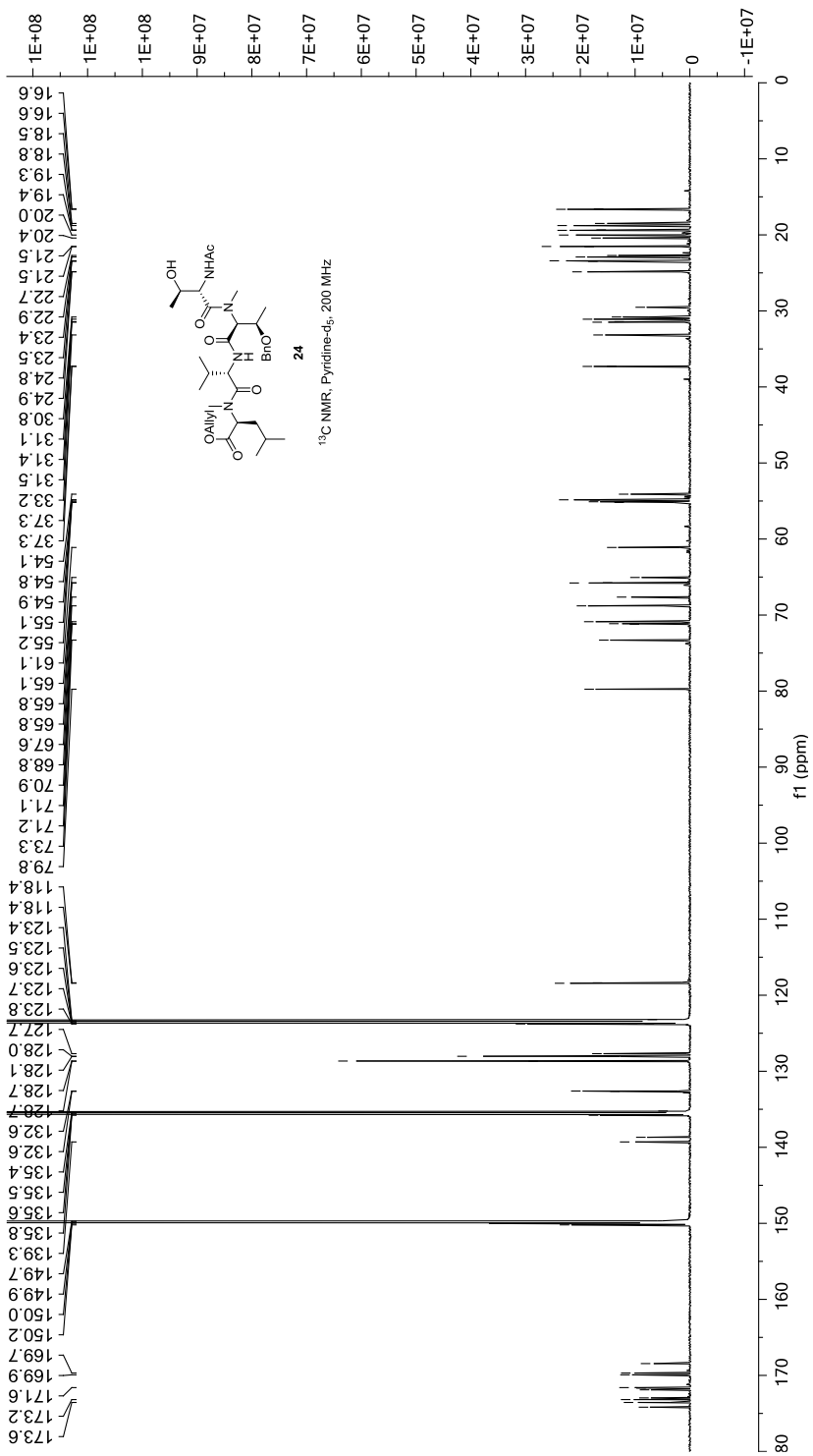

22
^{1}H NMR, $CDCl_3$, 800 MHz
f1 (ppm)

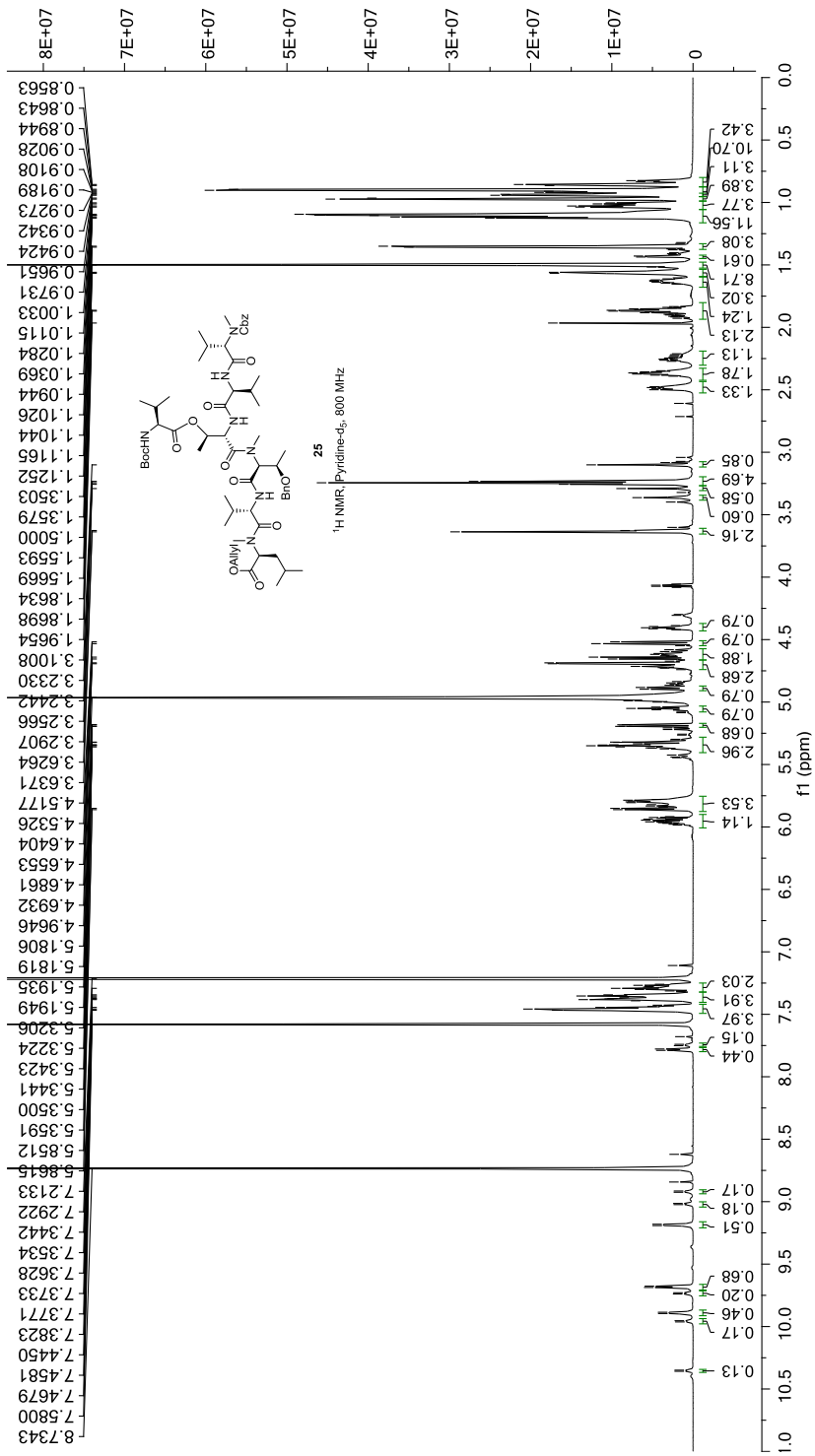

22
^{13}C NMR, $CDCl_3$, 200 MHz
f1 (ppm)

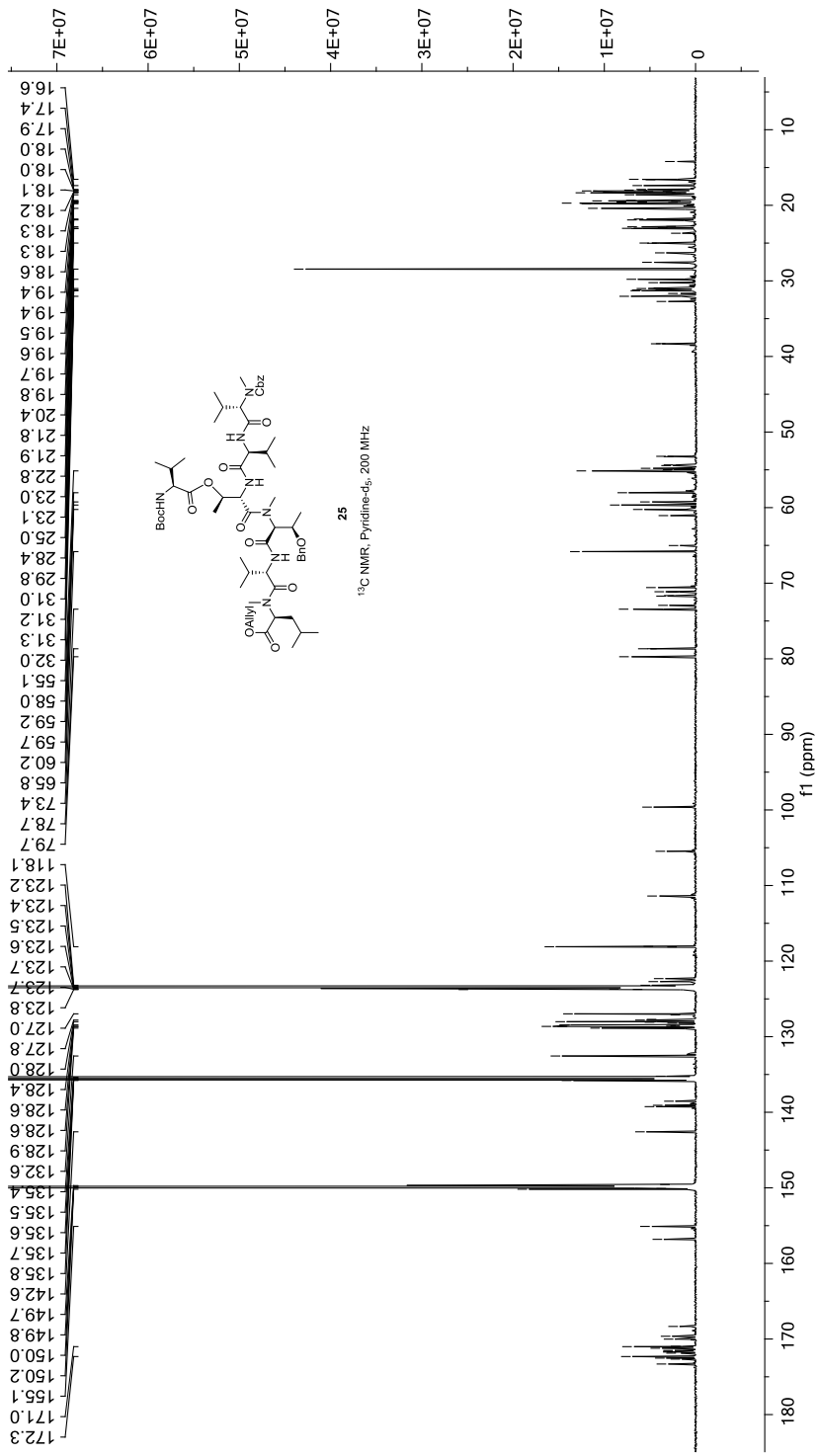

23
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)


23
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

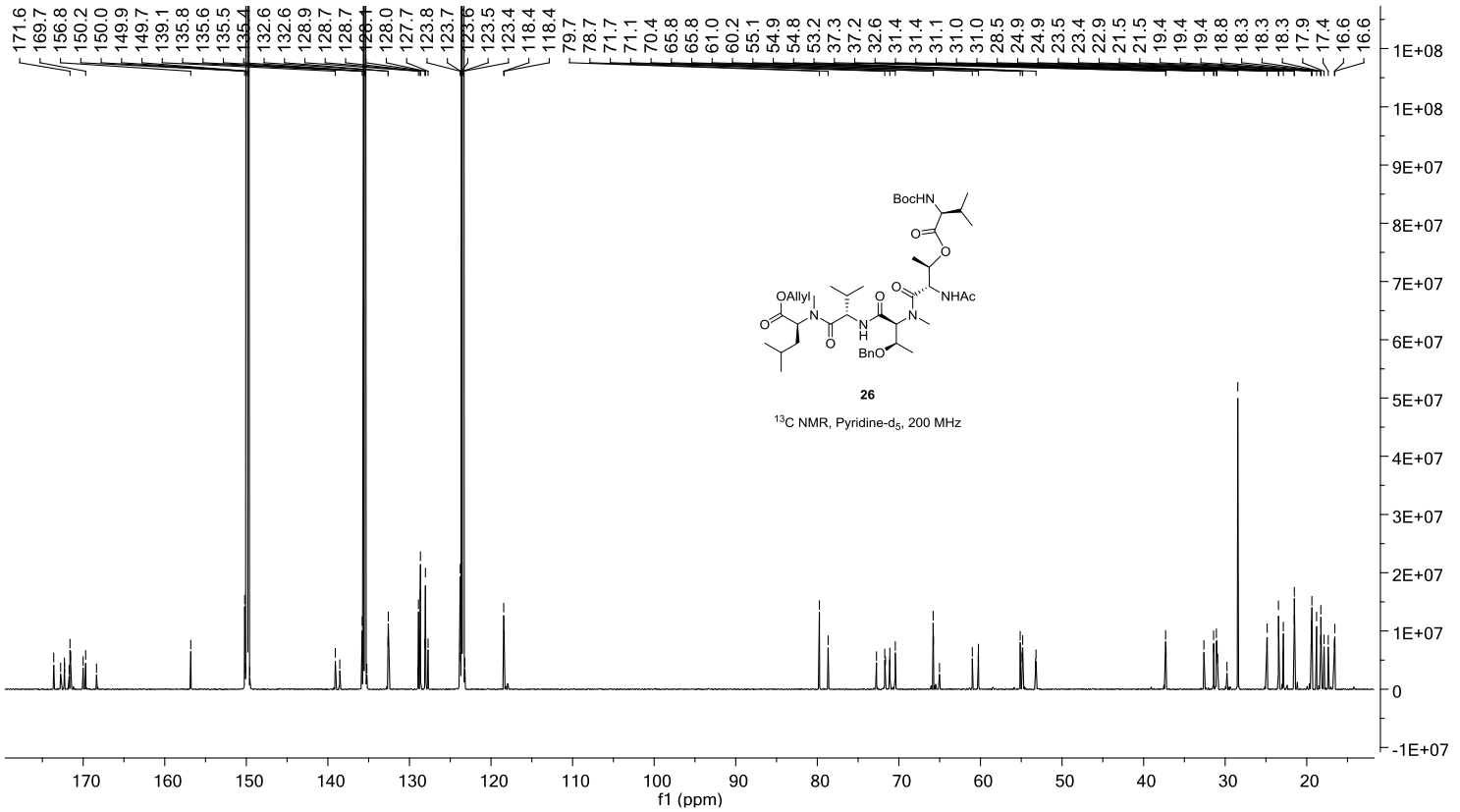

24
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

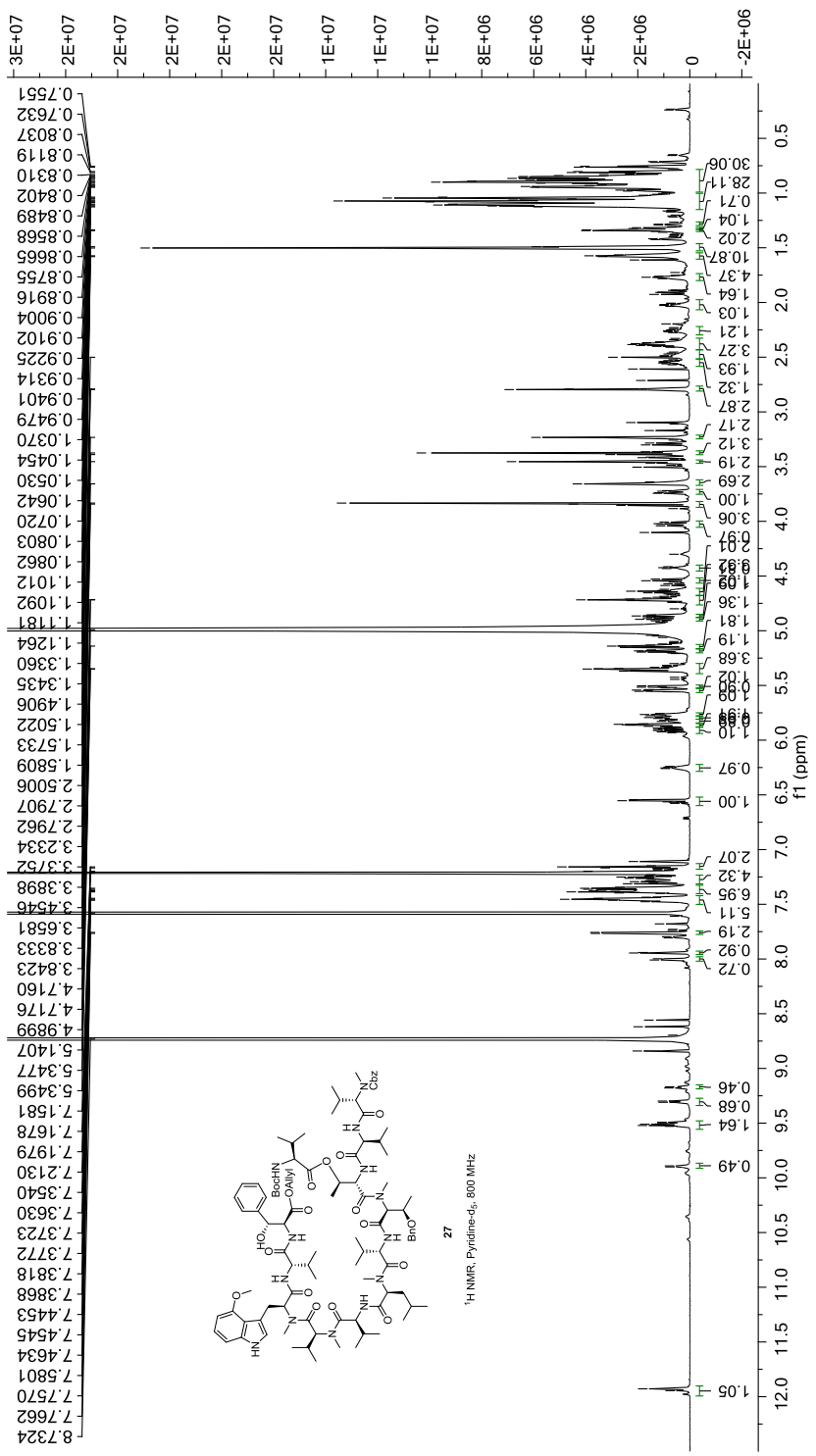

24
^{13}C NMR, Pyridine-d$_5$, 200 MHz
f1 (ppm)

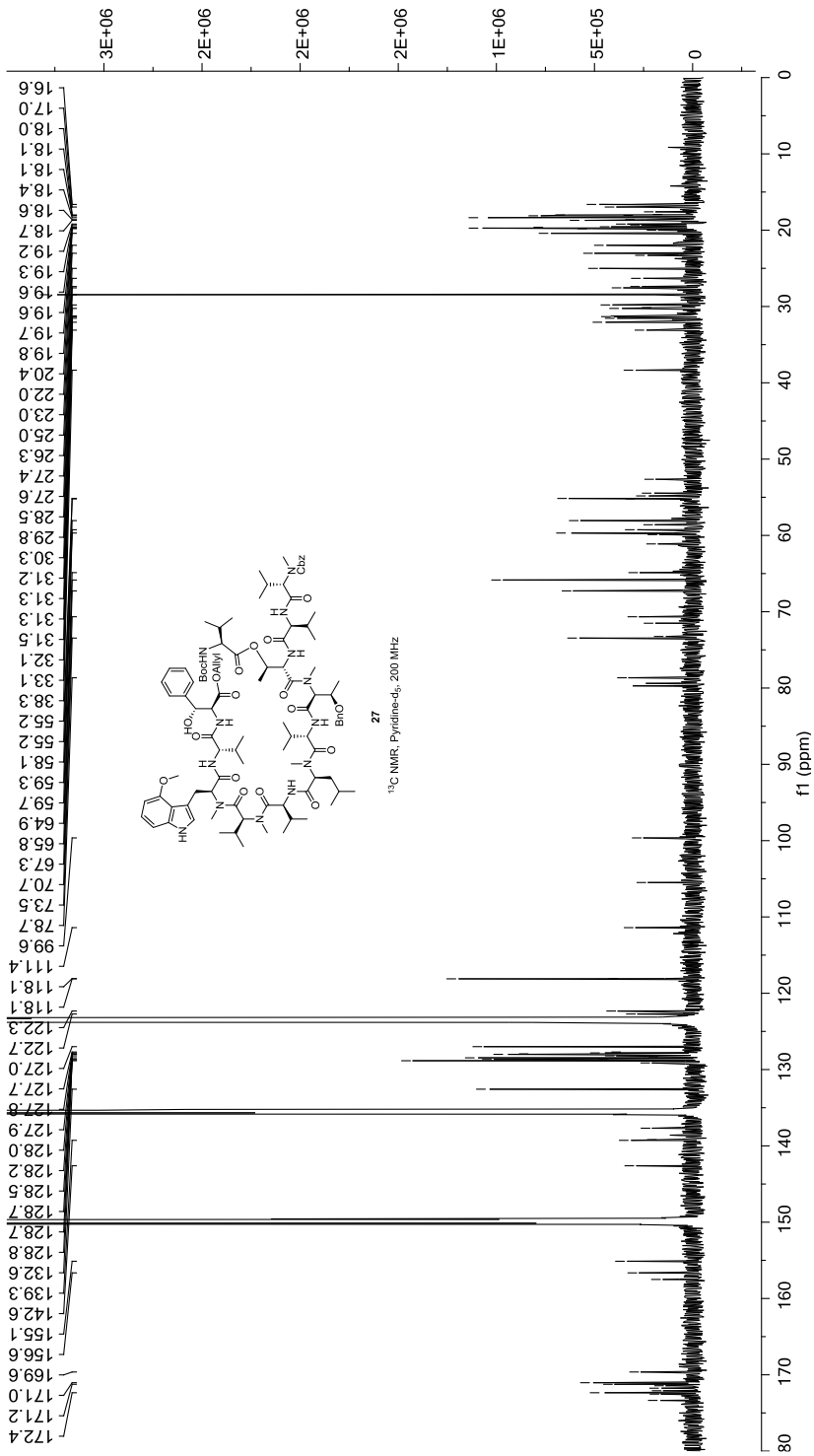

25
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

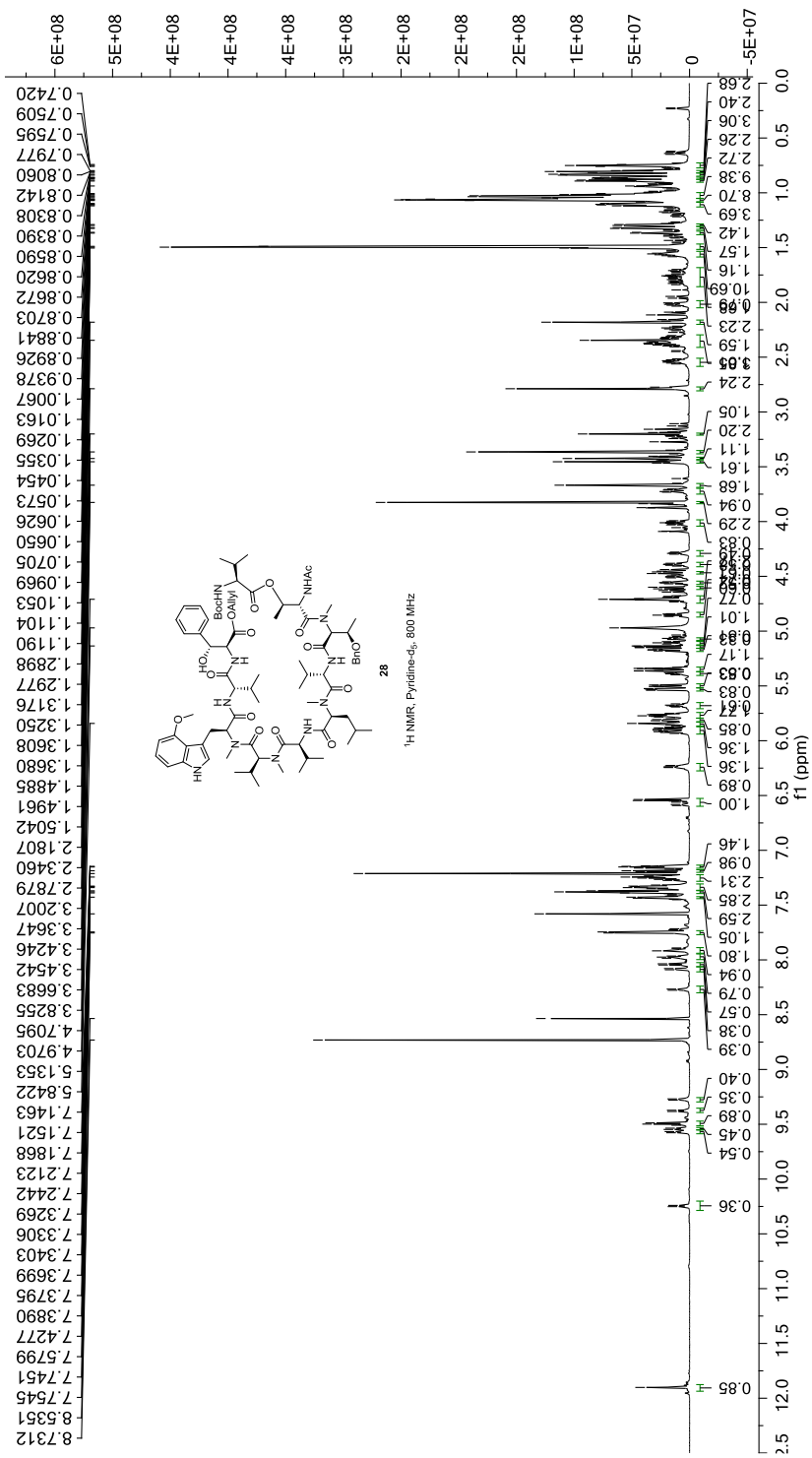

25
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

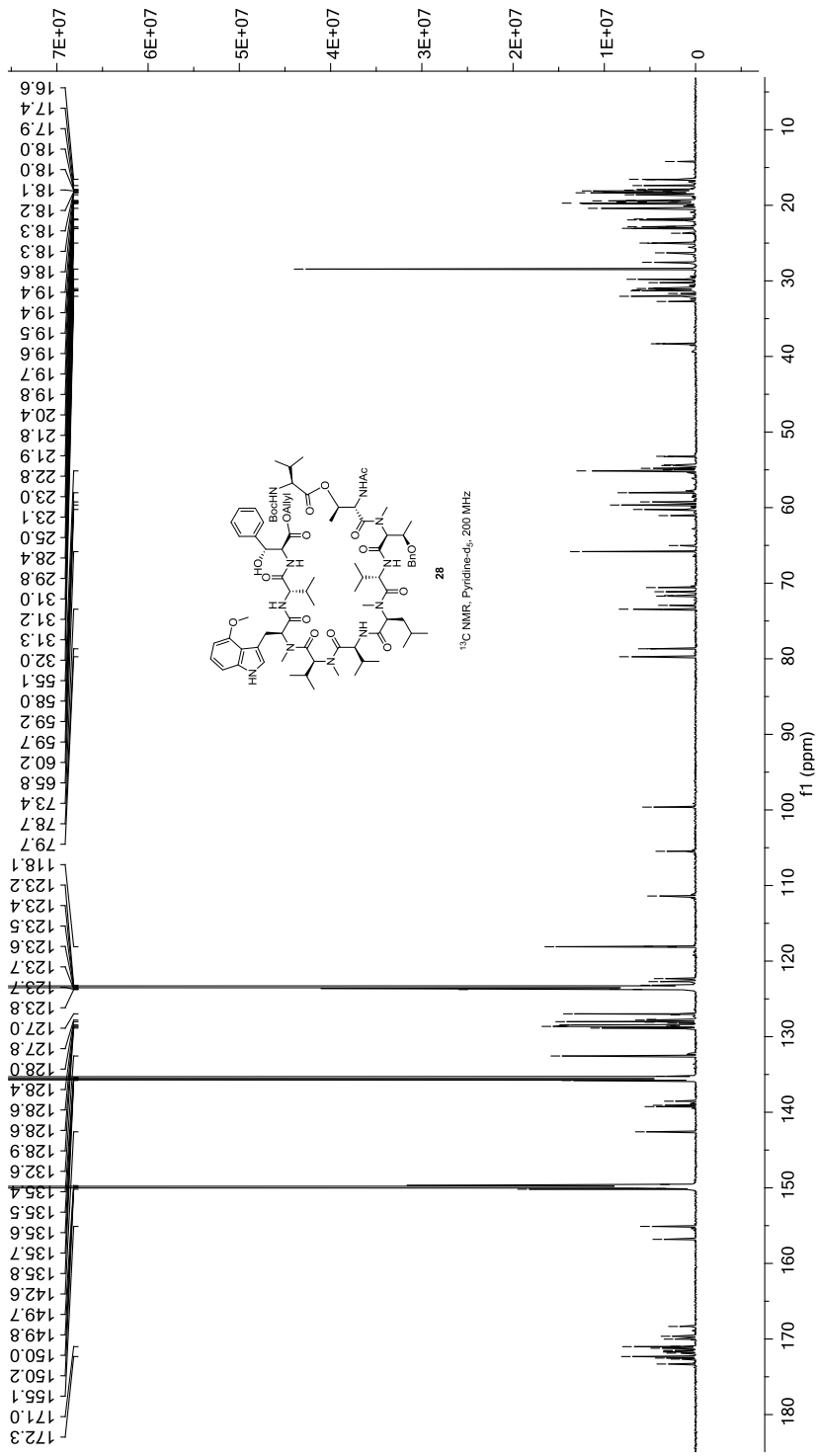
BocHN
NHAc
BnO
OAllyl
26
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

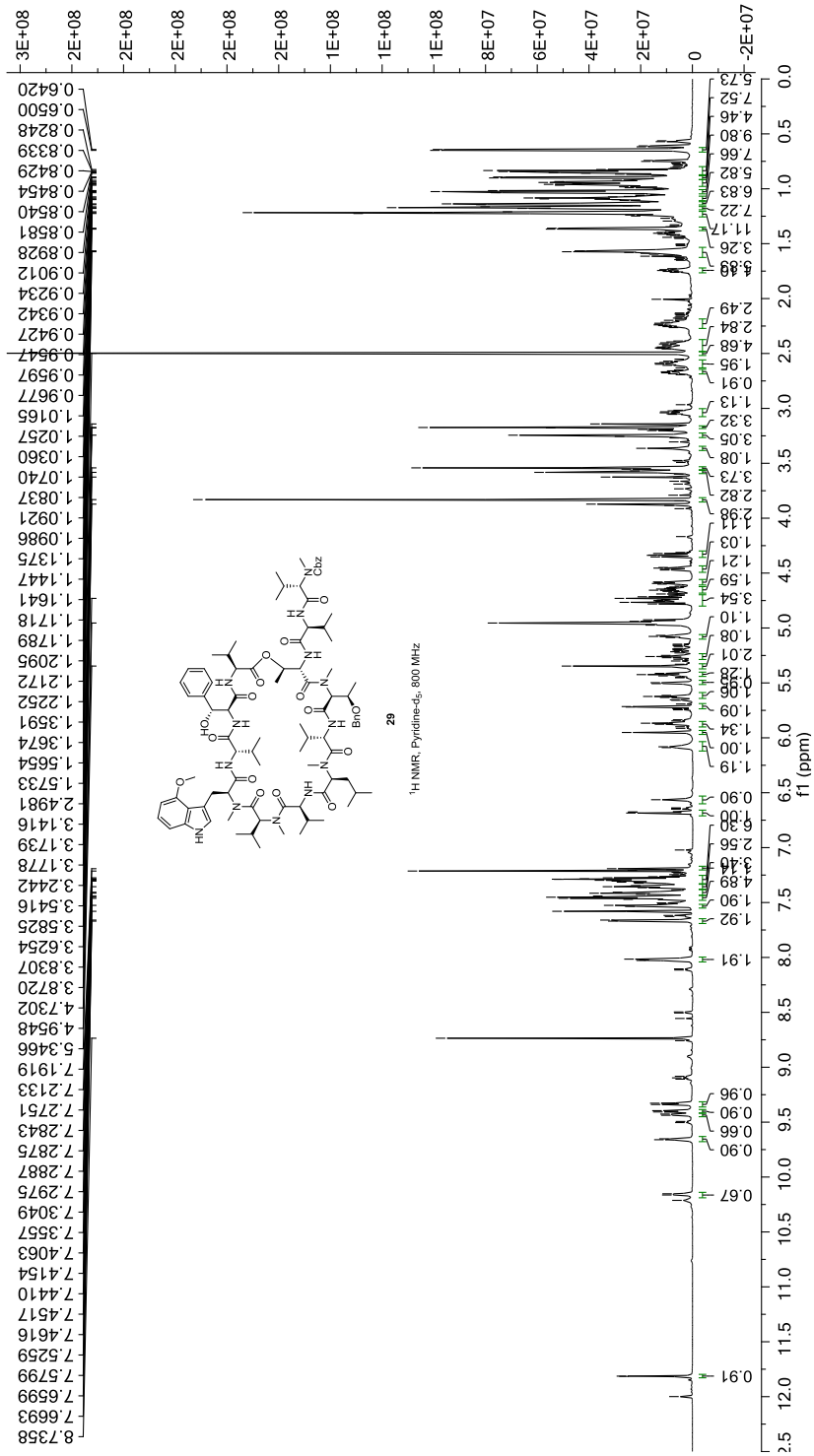

171.6
169.7
156.8
150.2
150.0
149.9
149.7
139.1
135.8
135.6
135.5
135.4
132.6
132.6
128.9
128.7
128.7
128.1
128.0
127.7
123.8
123.7
123.6
123.5
123.4
118.4
118.4
79.7
78.7
71.7
71.1
70.4
65.8
65.8
61.0
60.2
55.1
54.9
54.8
53.2
37.3
37.2
32.6
31.4
31.4
31.1
31.0
31.0
28.5
24.9
24.9
23.5
23.4
22.9
21.5
21.5
19.4
19.4
19.4
18.8
18.3
18.3
18.3
17.9
17.4
16.6
16.6
BocHN
OAllyl
NHAc
BnO
26
13C NMR, Pyridine-d5, 200 MHz
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

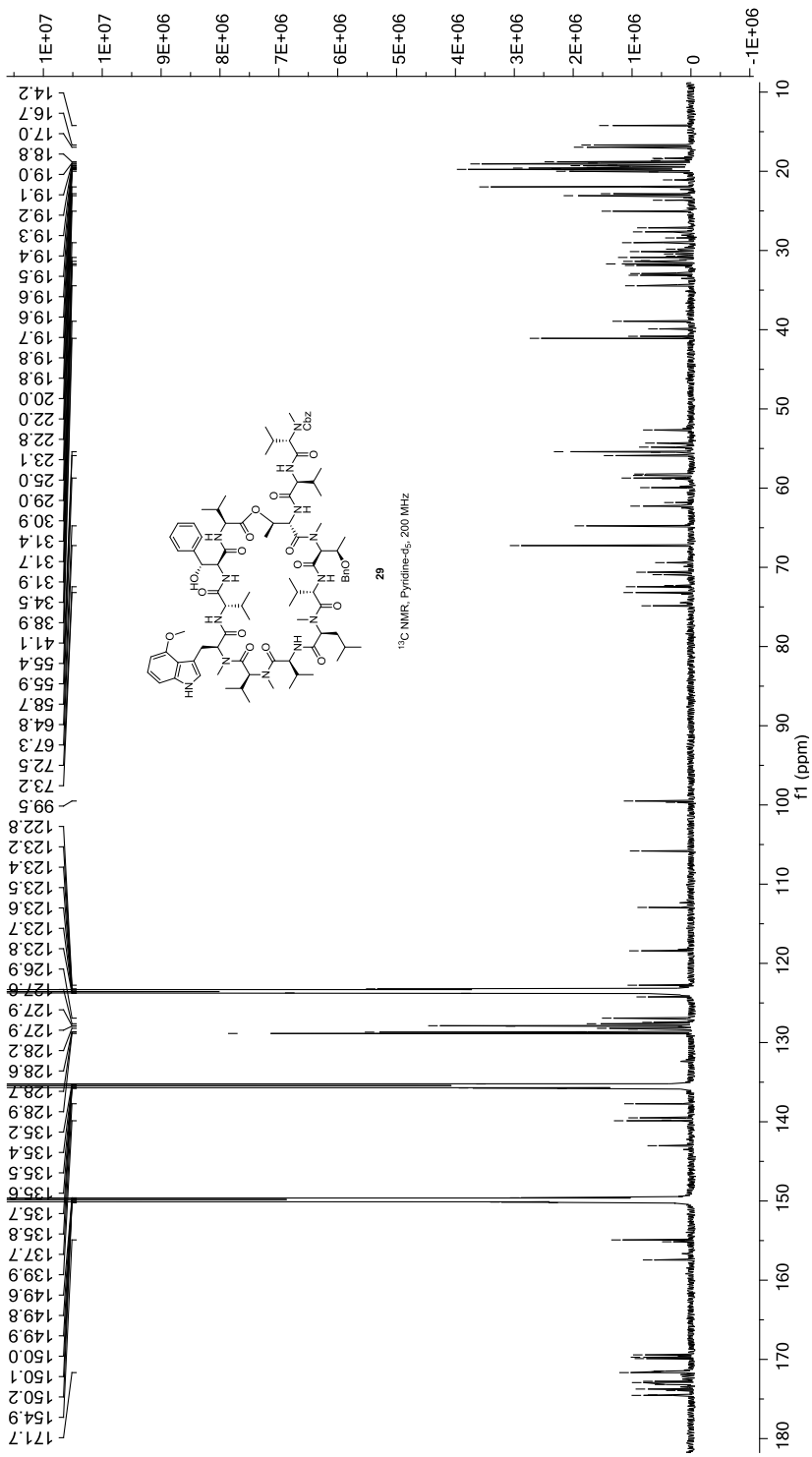

27
^{1}H NMR, Pyridine-d$_5$, 800 MHz
f1 (ppm)

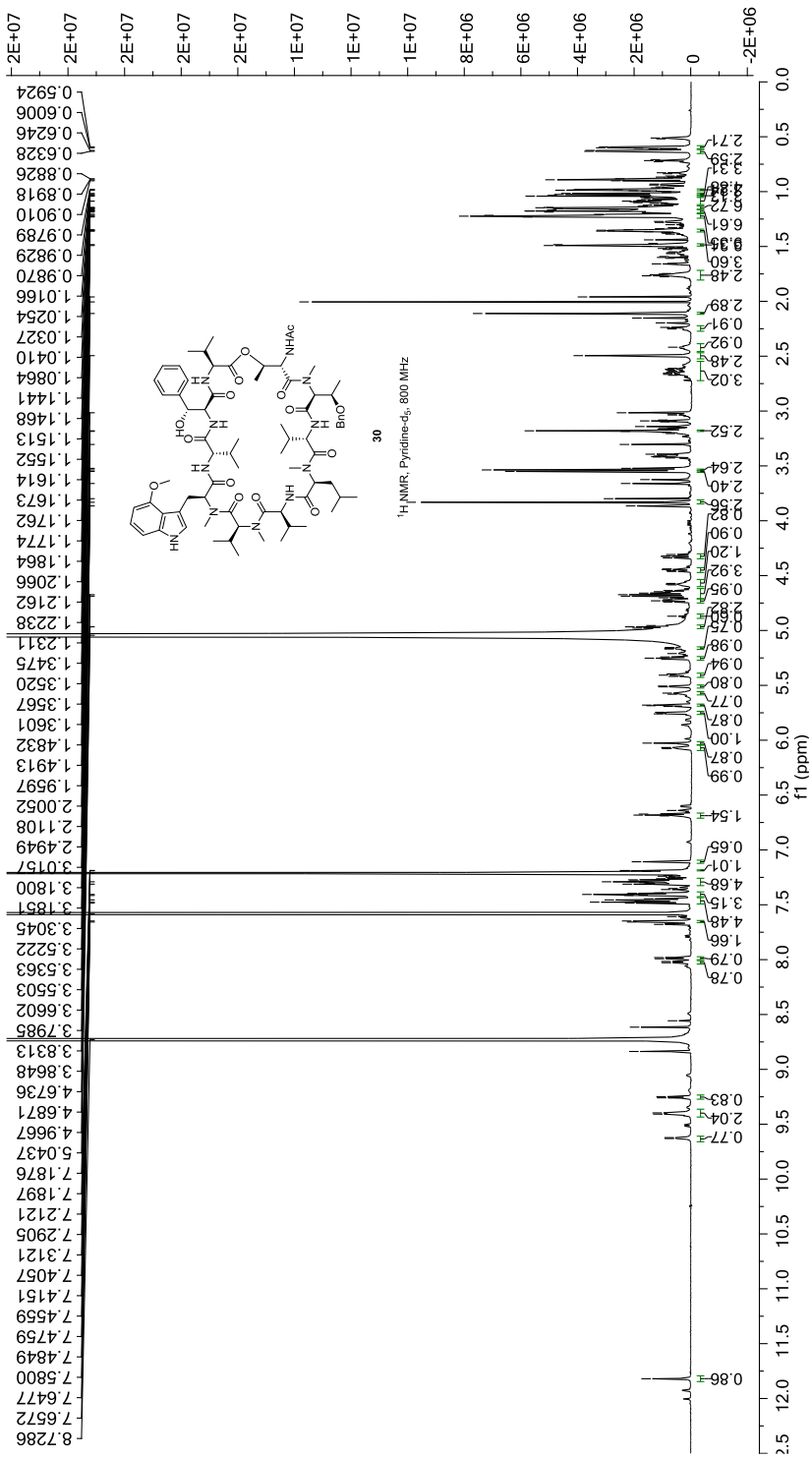

27
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

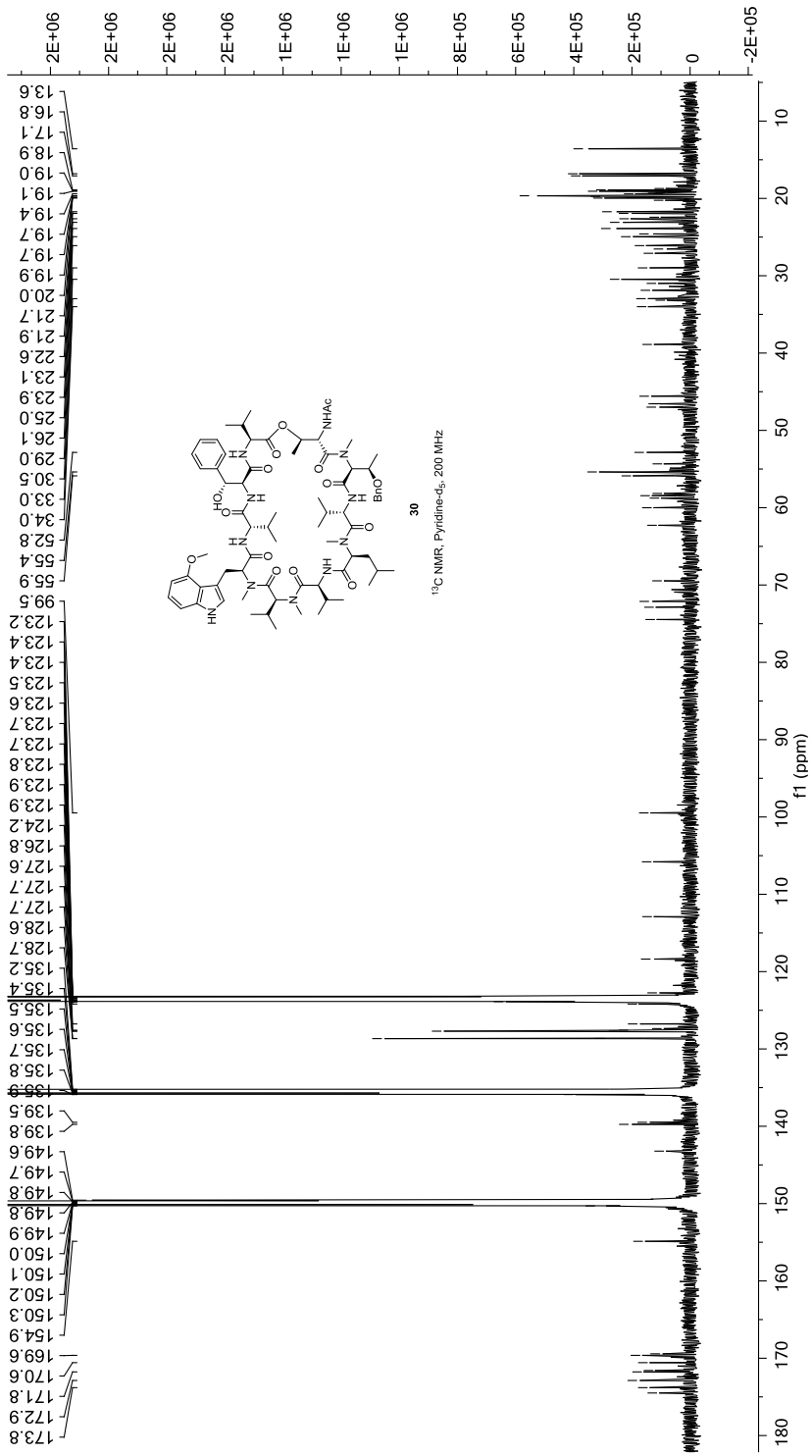

BocHN
OAllyl
NHAc
BnO
28
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

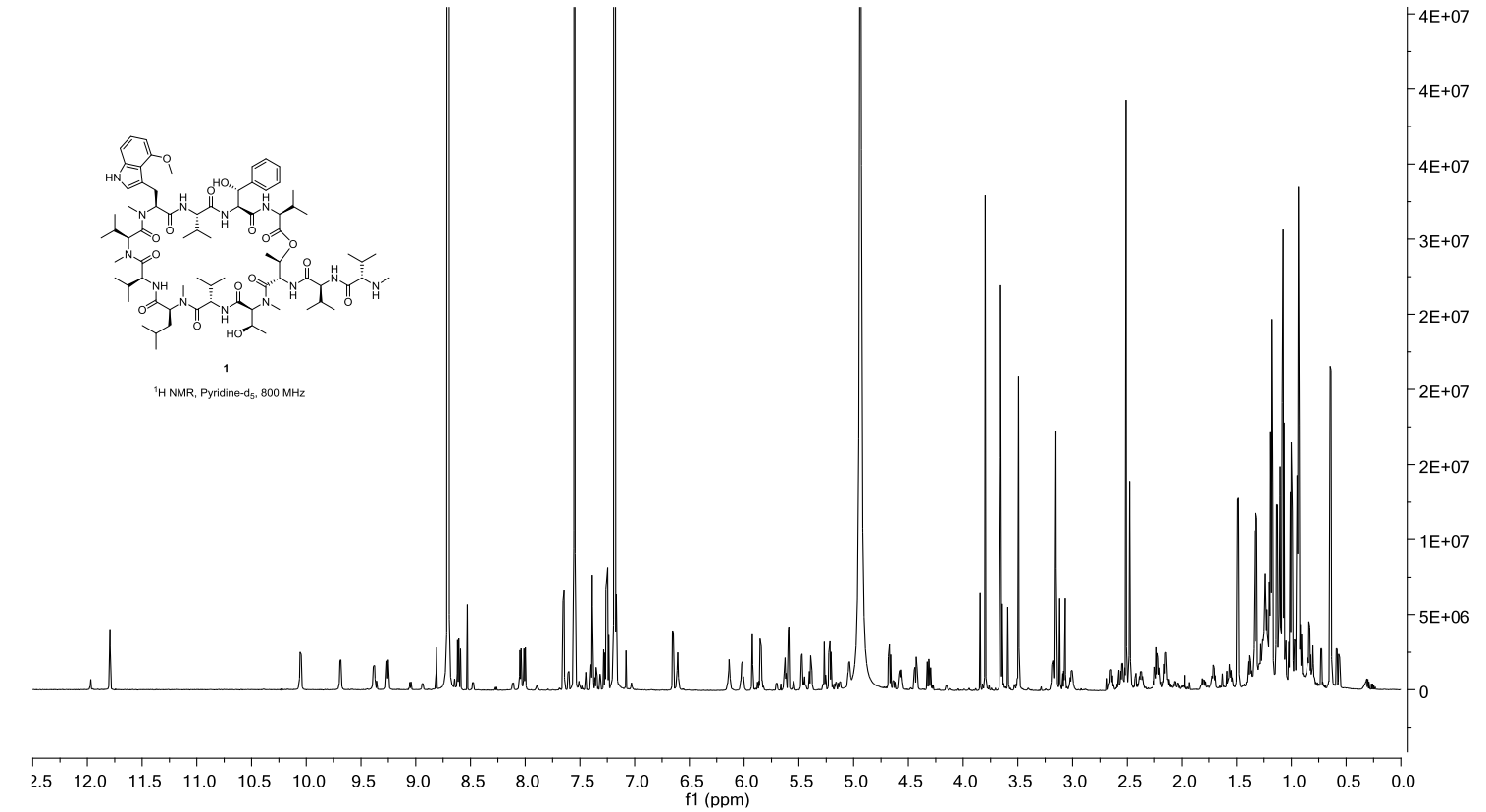

28
^{13}C NMR, Pyridine-d$_5$, 200 MHz
f1 (ppm)

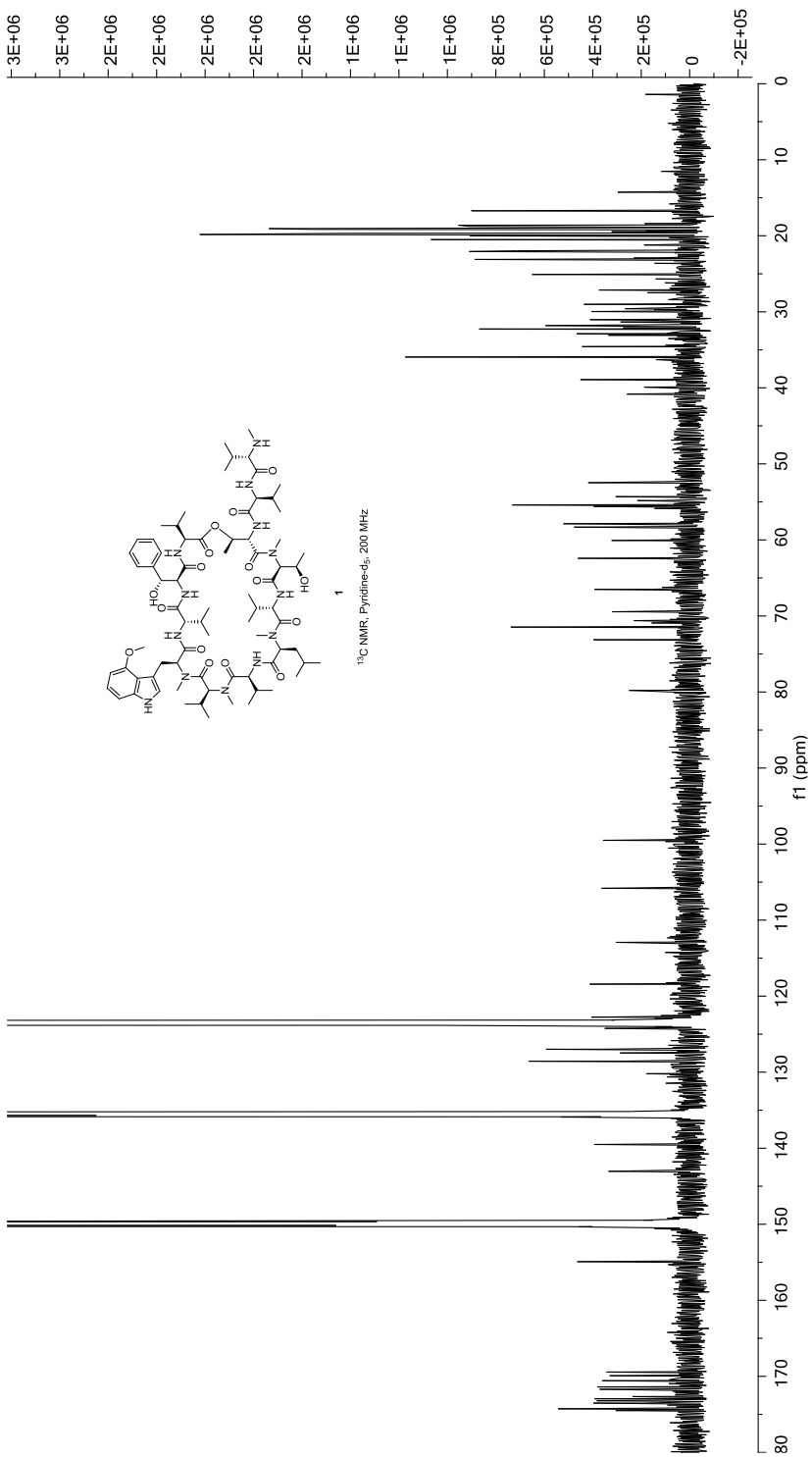

29
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

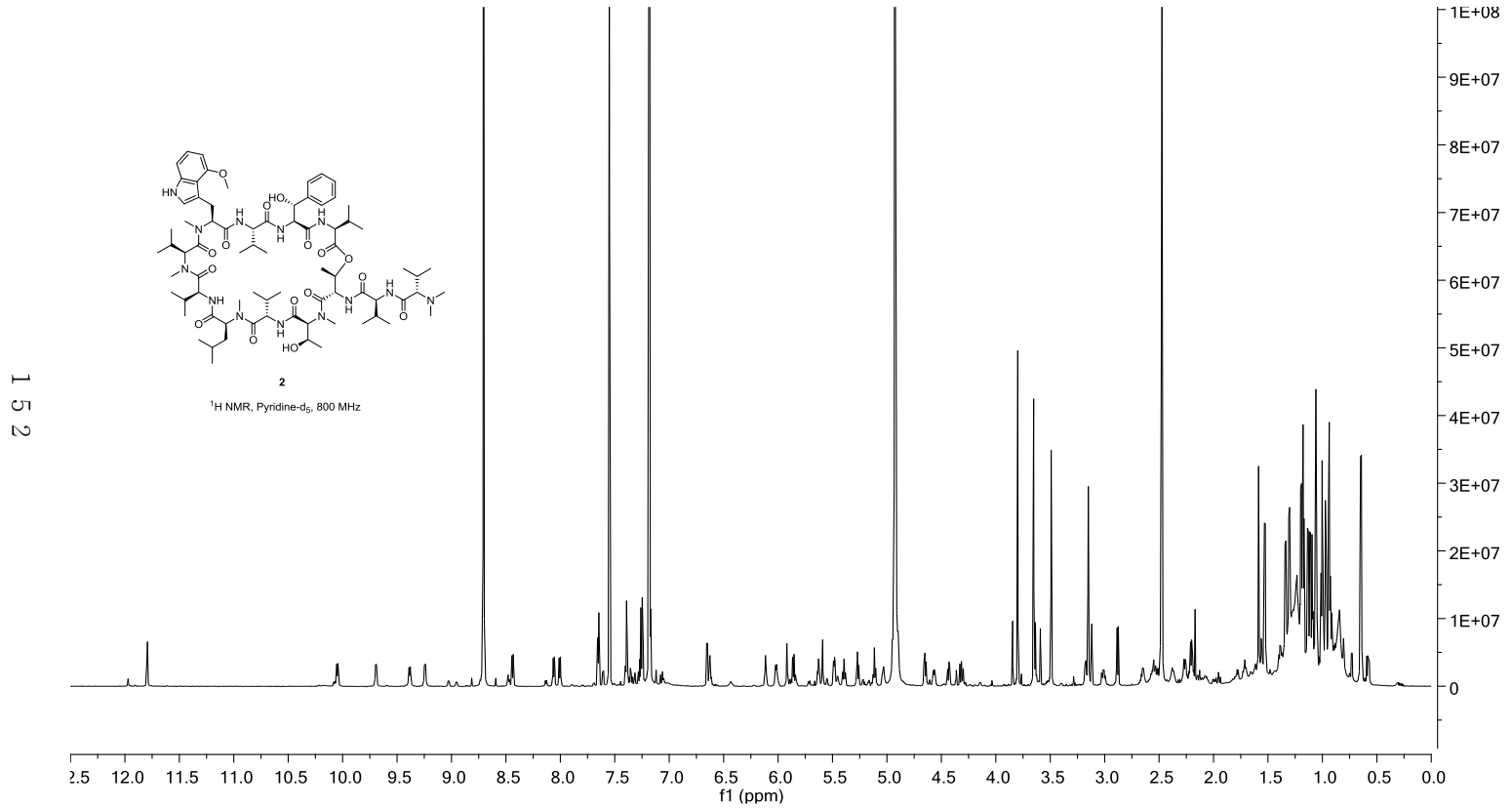

29
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)


30
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

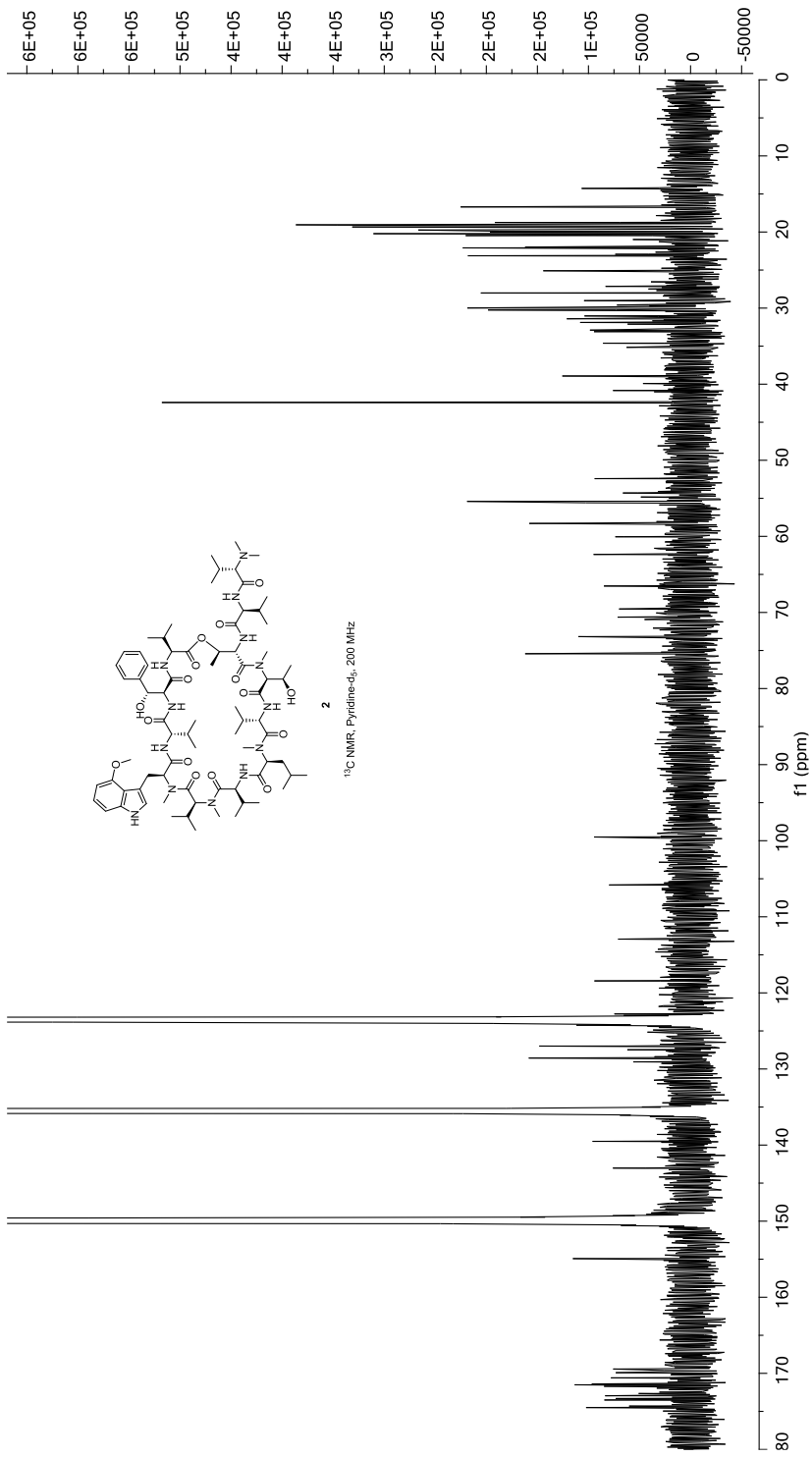

30
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)


1
^{1}H NMR, Pyridine-d_5, 800 MHz
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
2.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

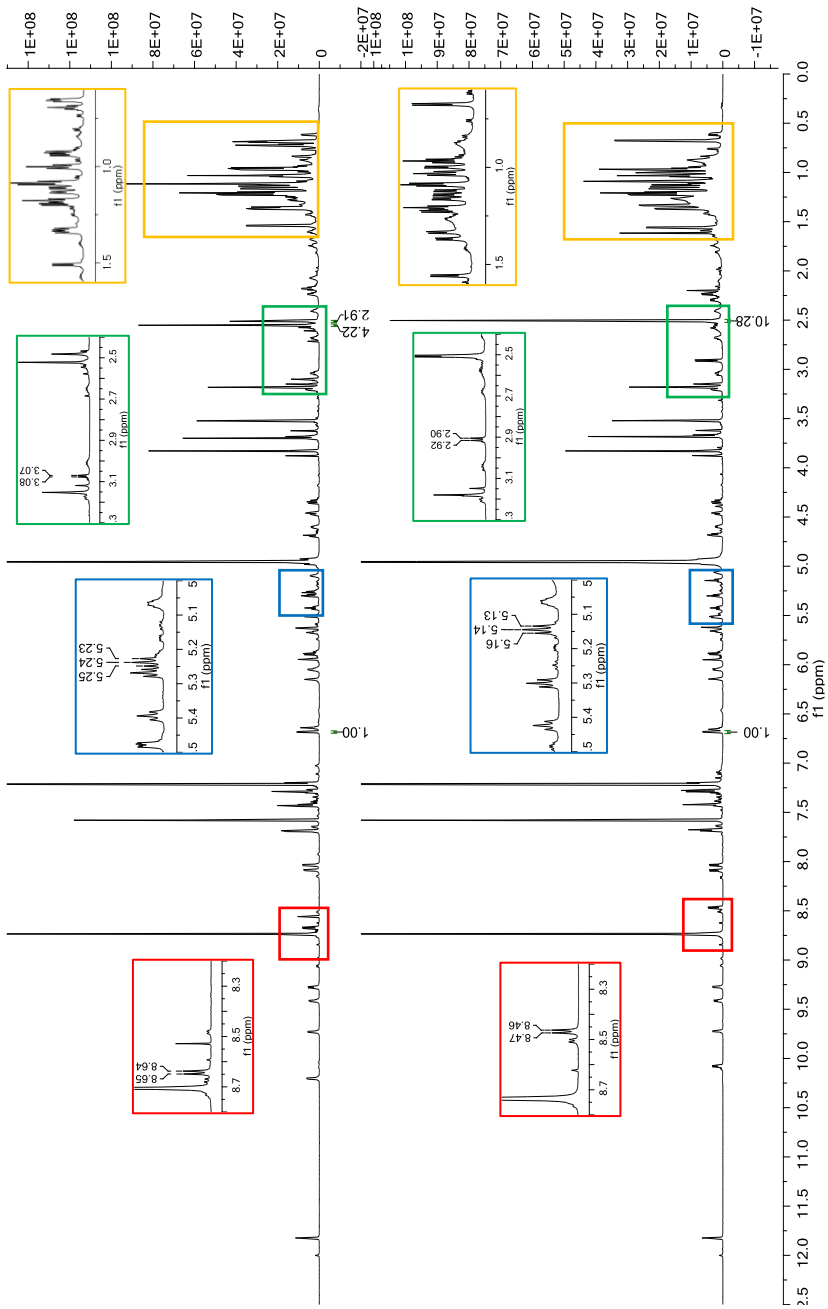

3E+06
3E+06
2E+06
2E+06
2E+06
2E+06
2E+06
1E+06
1E+06
1E+06
8E+05
6E+05
4E+05
2E+05
0
-2E+05
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)
1
^{13}C NMR, Pyridine-d_5, 200 MHz


2
1H NMR, Pyridine-d5, 800 MHz
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

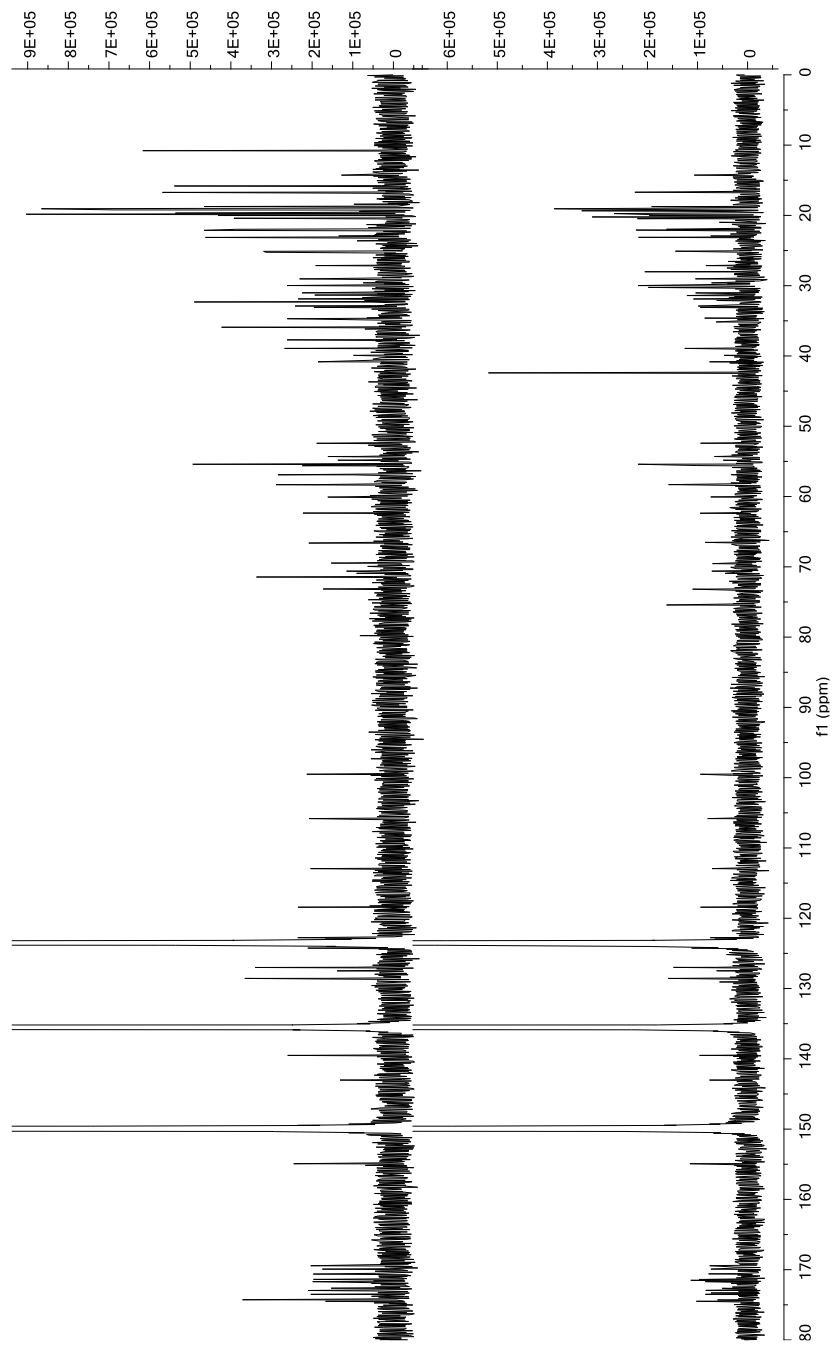
1 5 2

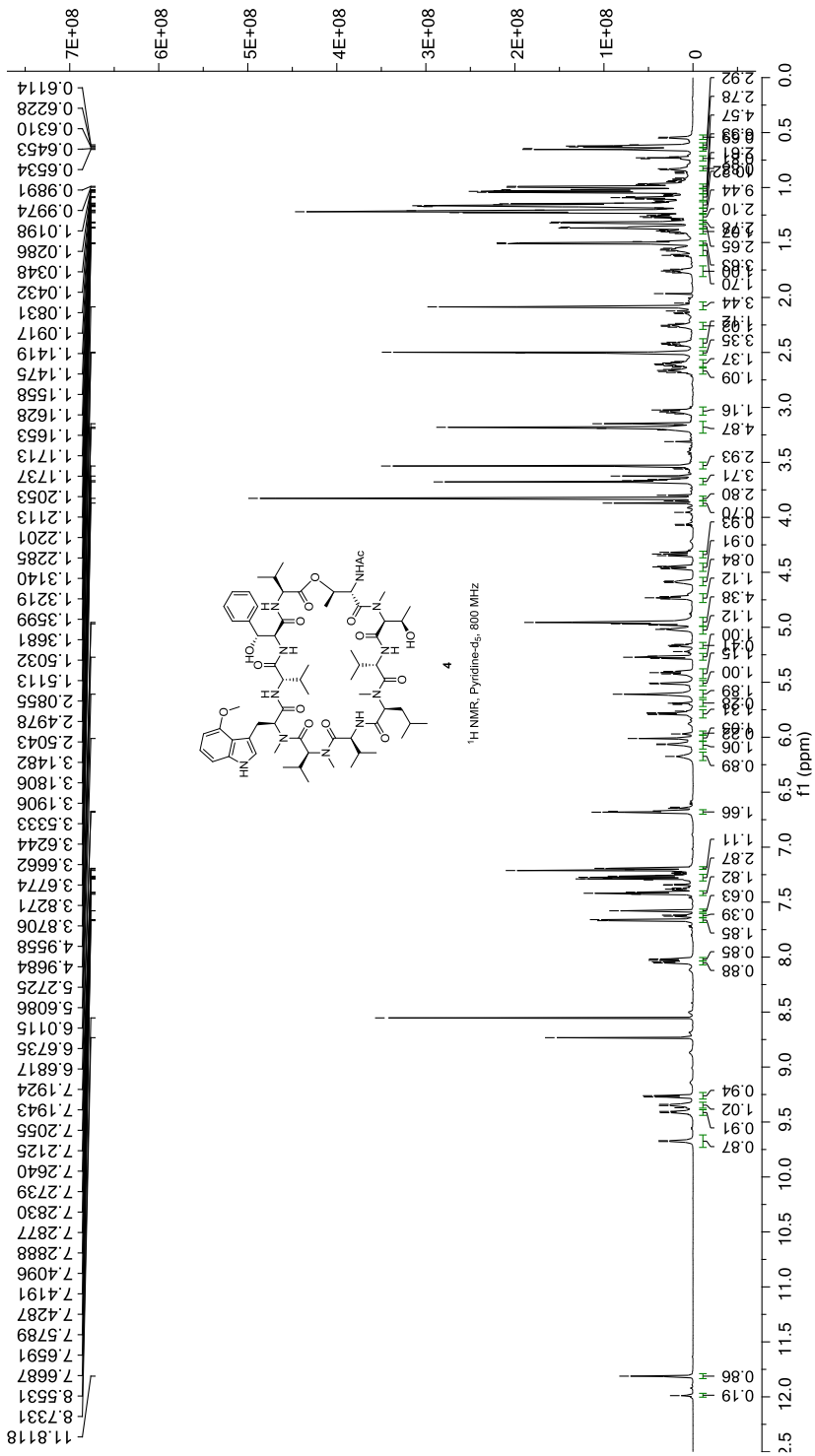

6E+05
6E+05
6E+05
5E+05
4E+05
4E+05
4E+05
3E+05
2E+05
2E+05
2E+05
1E+05
50000
0
-50000
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
80
f1 (ppm)
2
^{13}C NMR, Pyridine-d_5, 200 MHz

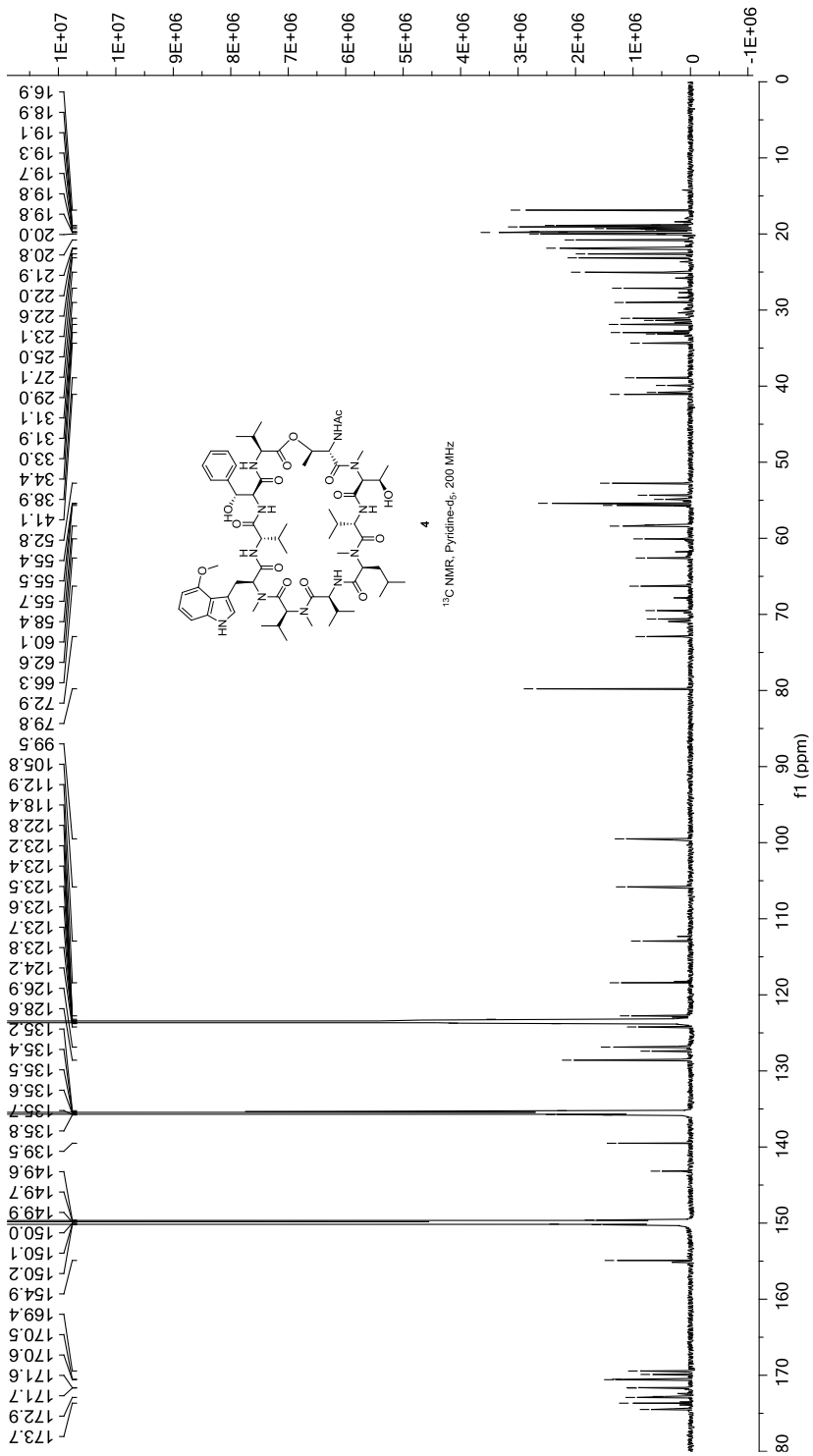
Comparison of the ^{1}H NMR-spectra for the natural ohmyungsamycin B and the proposed ohmyungsamycin B (**2**)



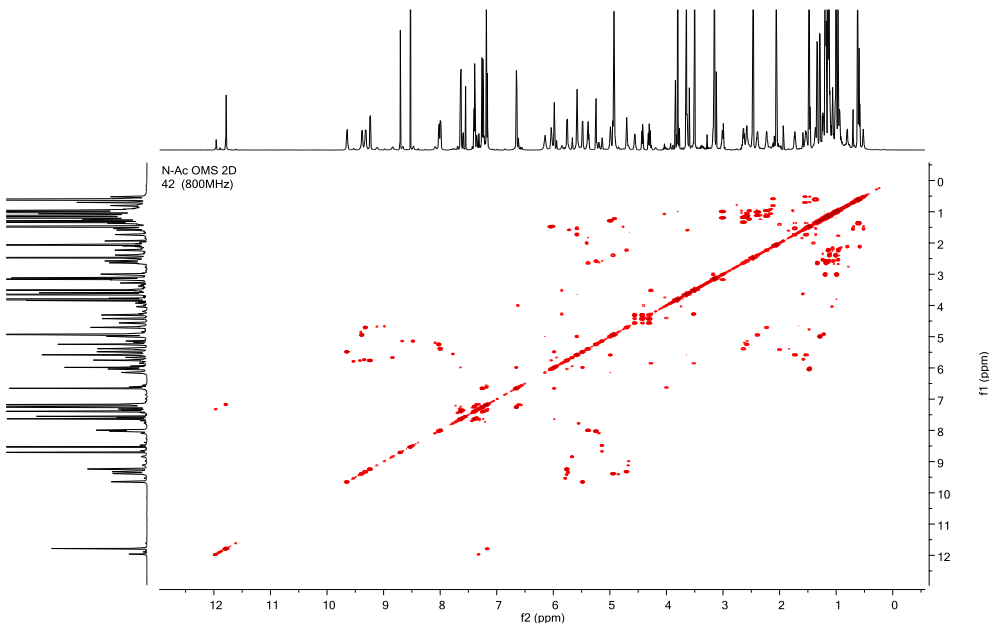
Comparison of the ^{13}C NMR spectra for the natural ohmyungsamycin B and the proposed ohmyungsamycin B (**2**)



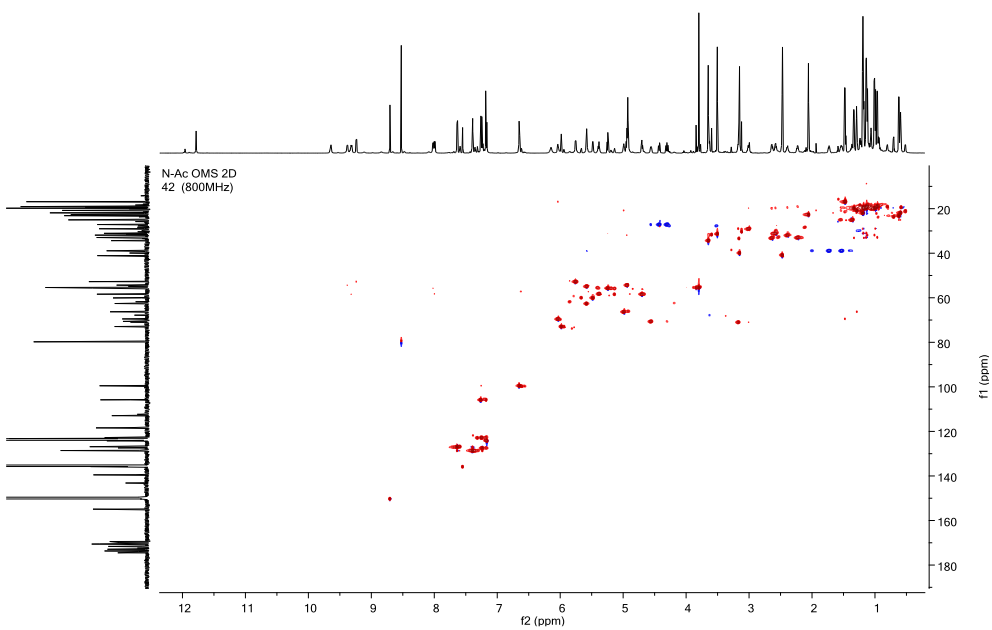

4
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)


4
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

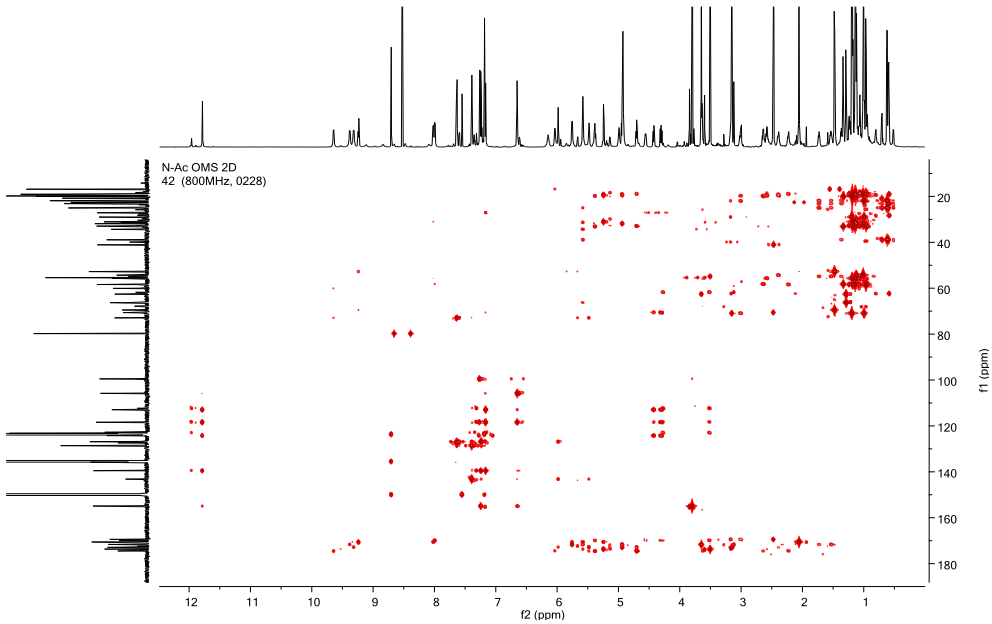
▼ COSY of **4**



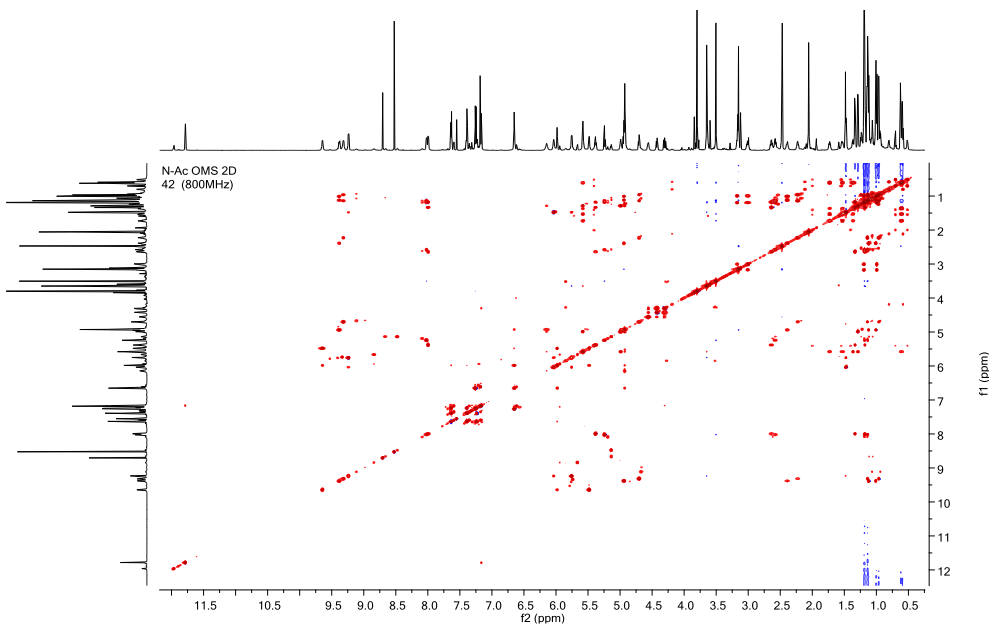
▼ e-HSQC of **4**



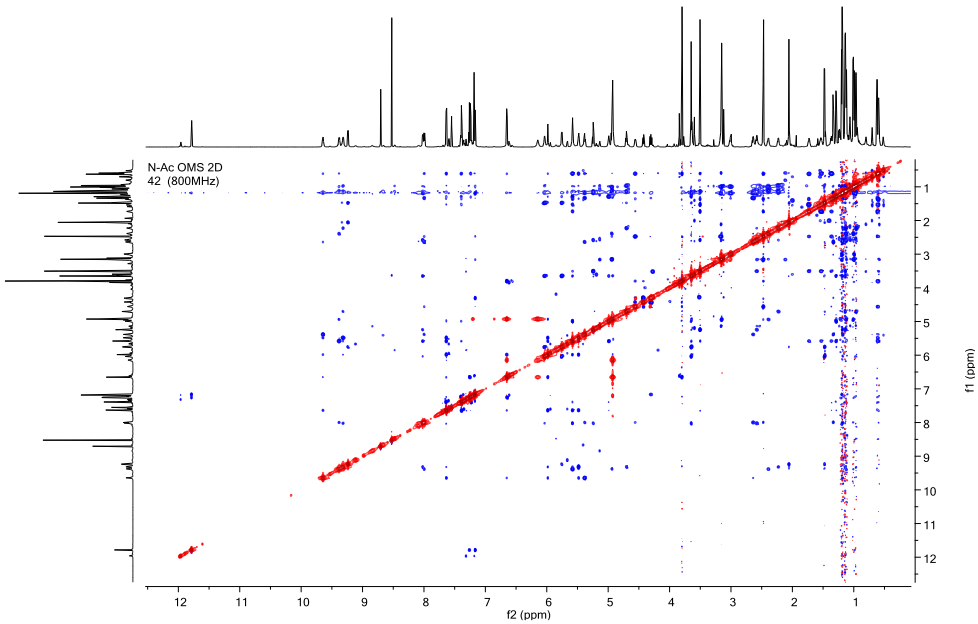
▼ HMBC of **4**

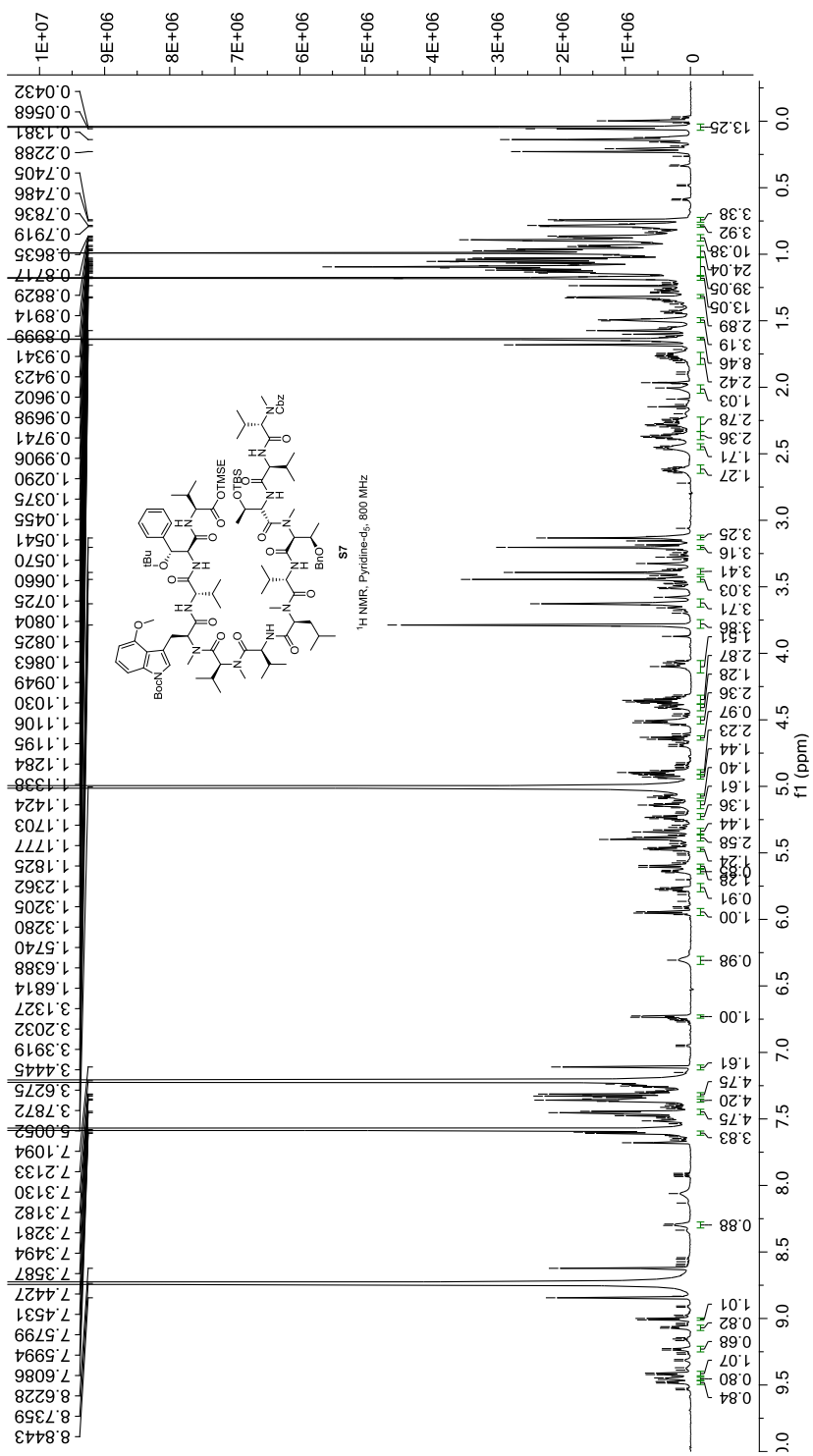


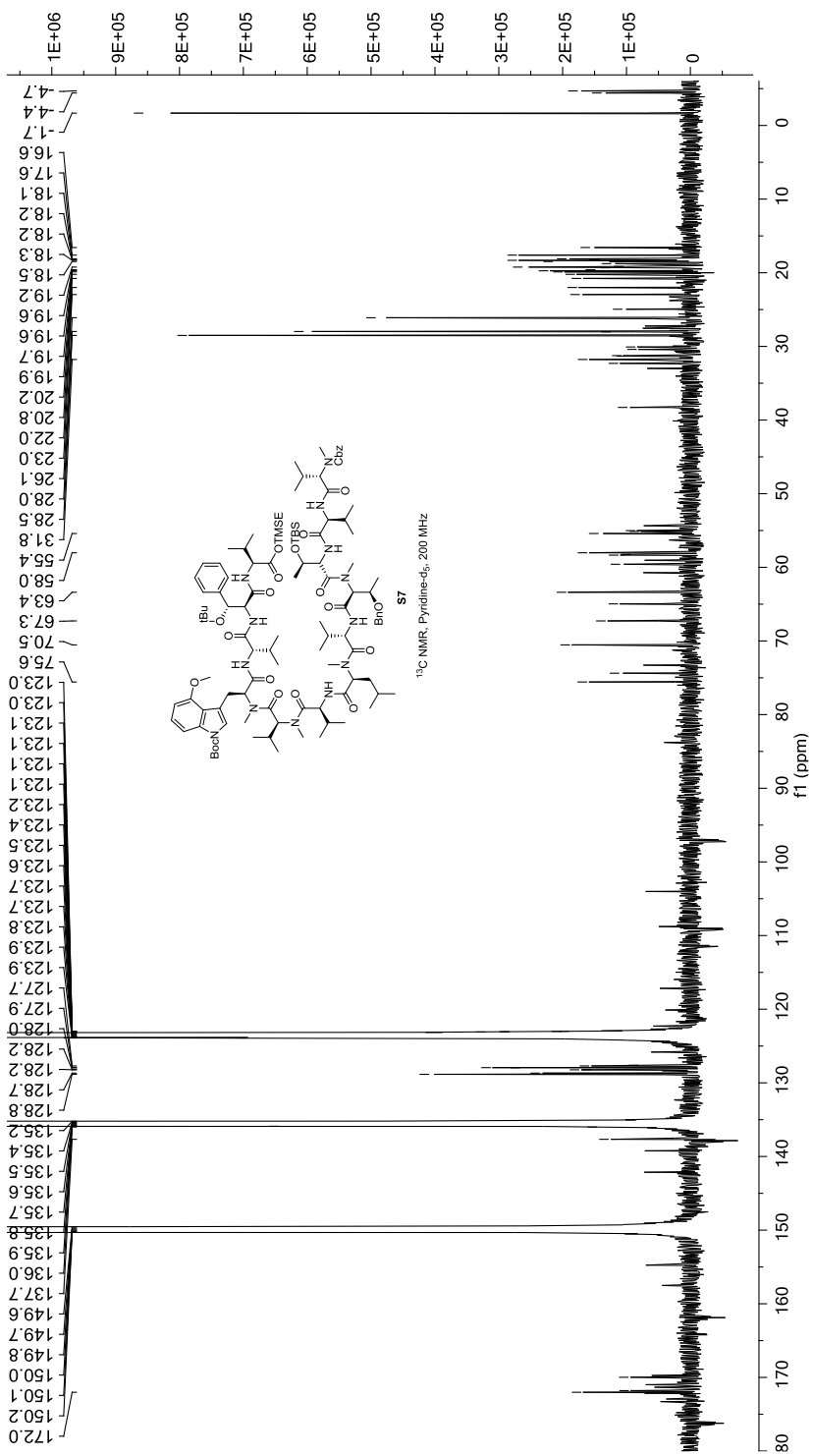
▼ TOCSY of **4**

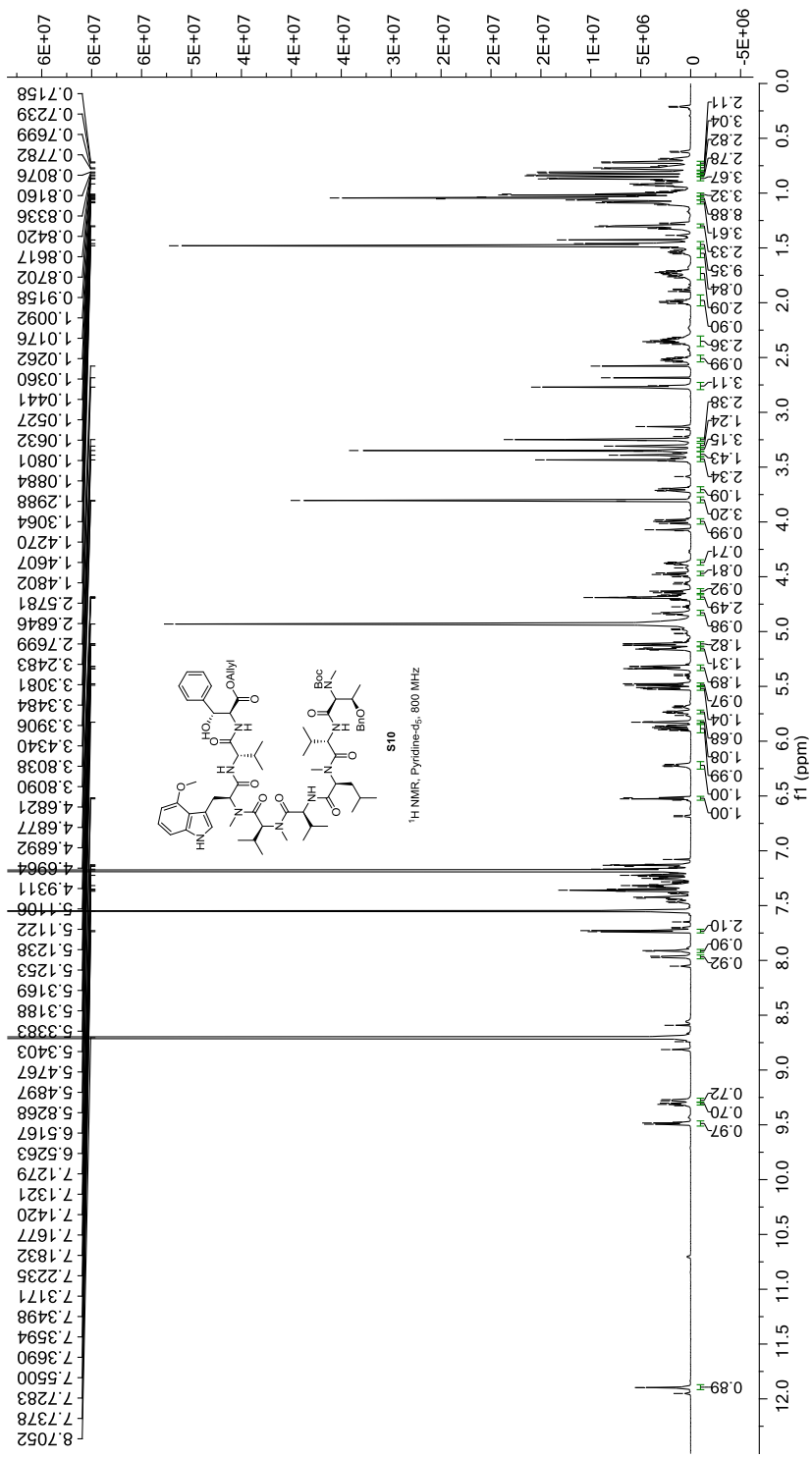


▼ ROESY of **4**

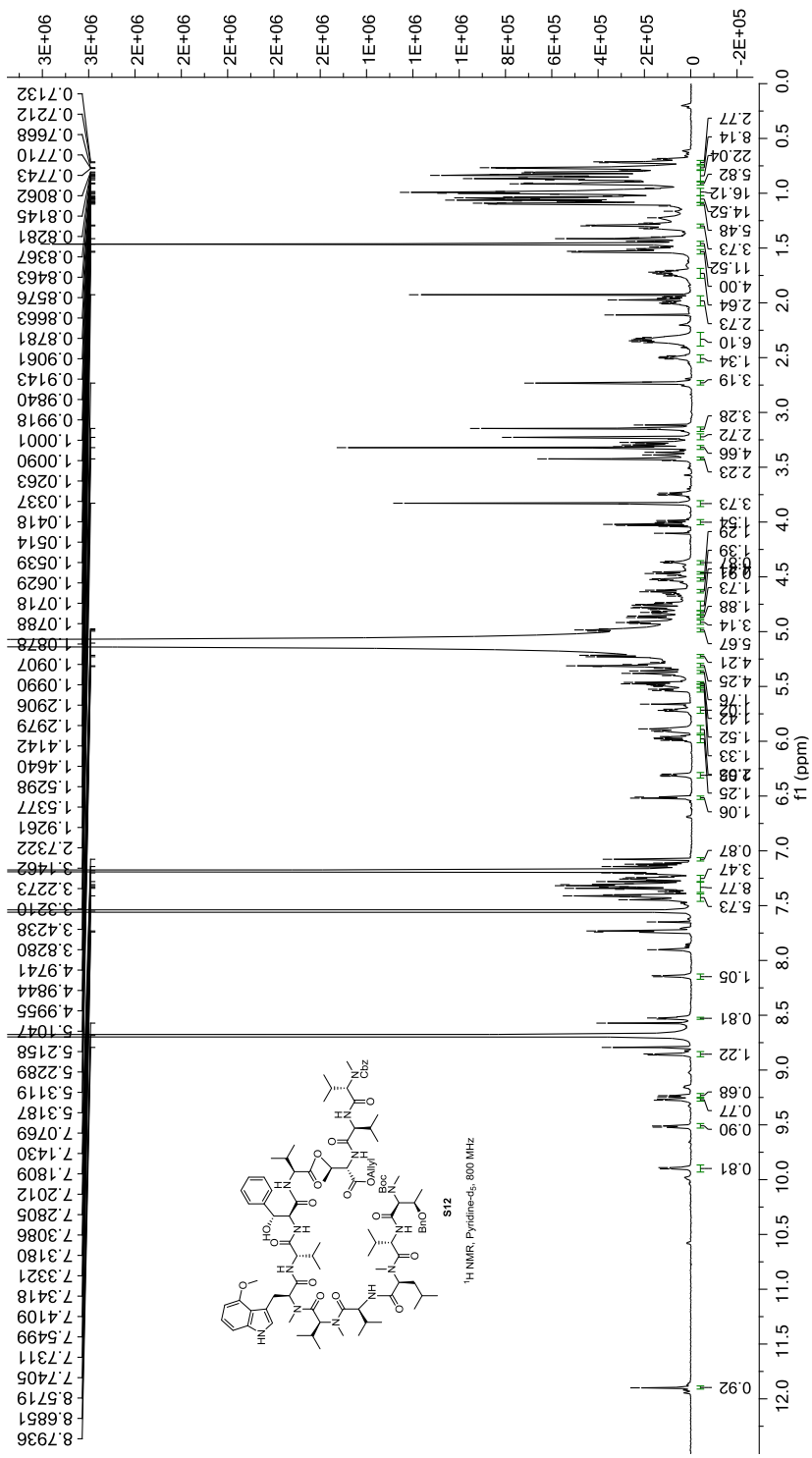


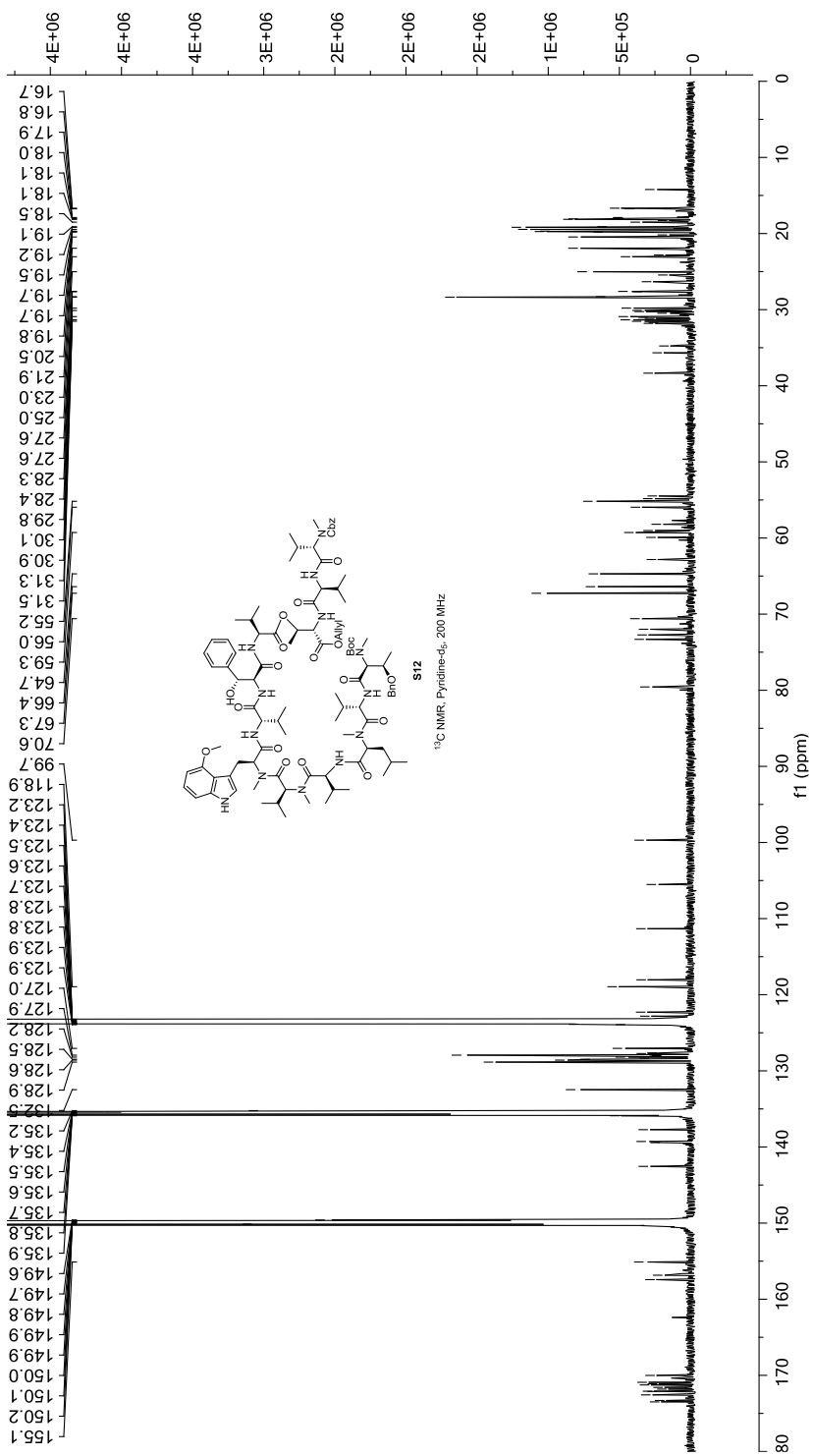

S7
^{1}H NMR, Pyridine-d$_5$, 800 MHz
f1 (ppm)

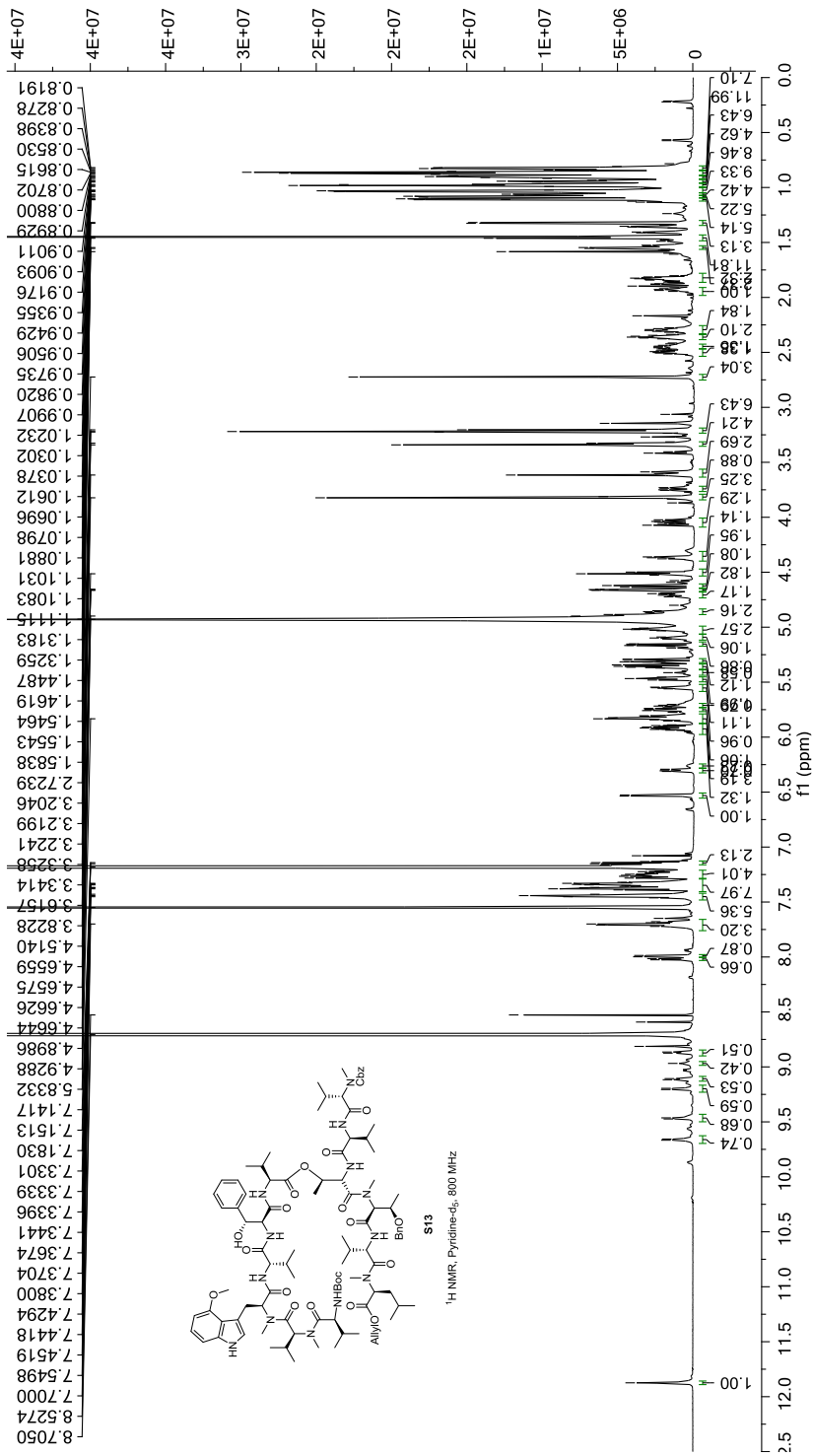

S7
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

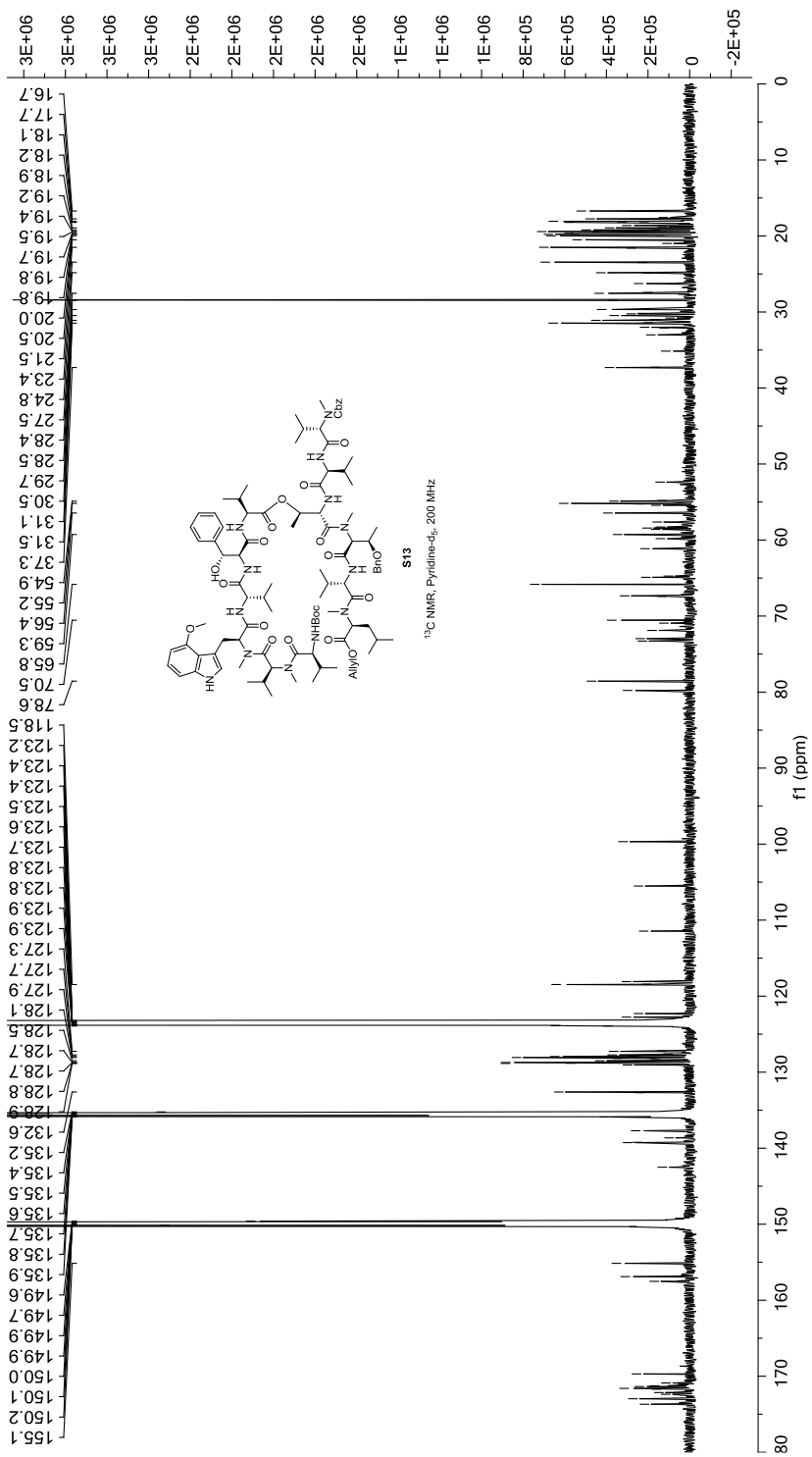

S10
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

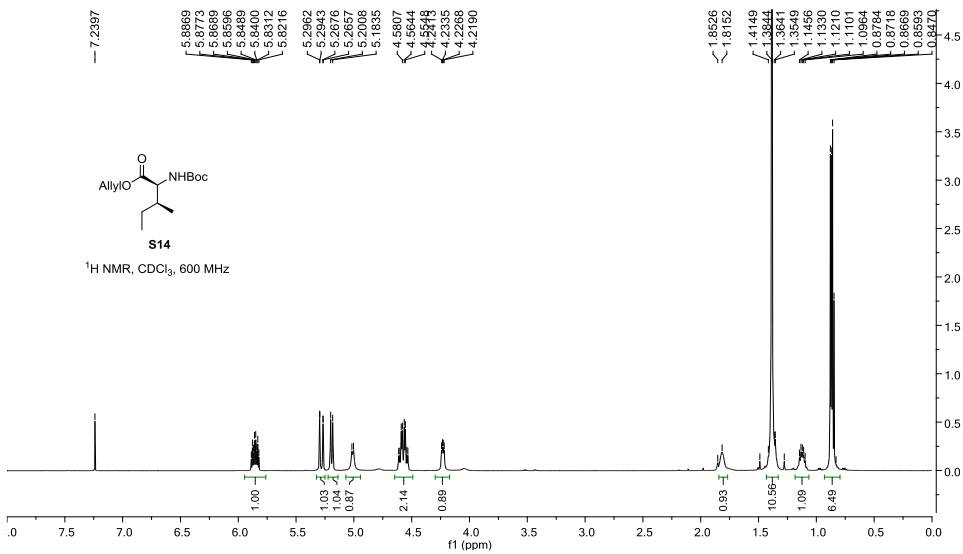
S10
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

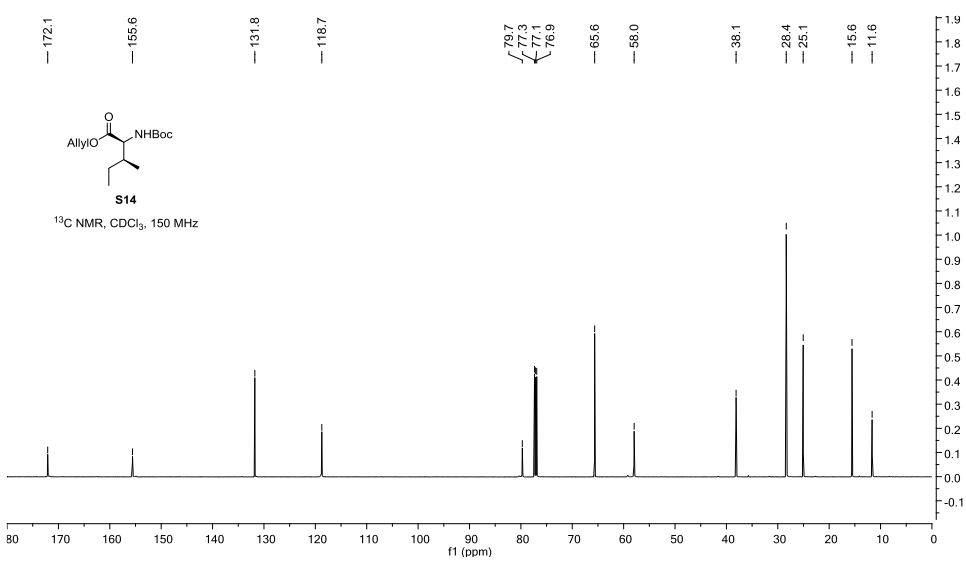

S12
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

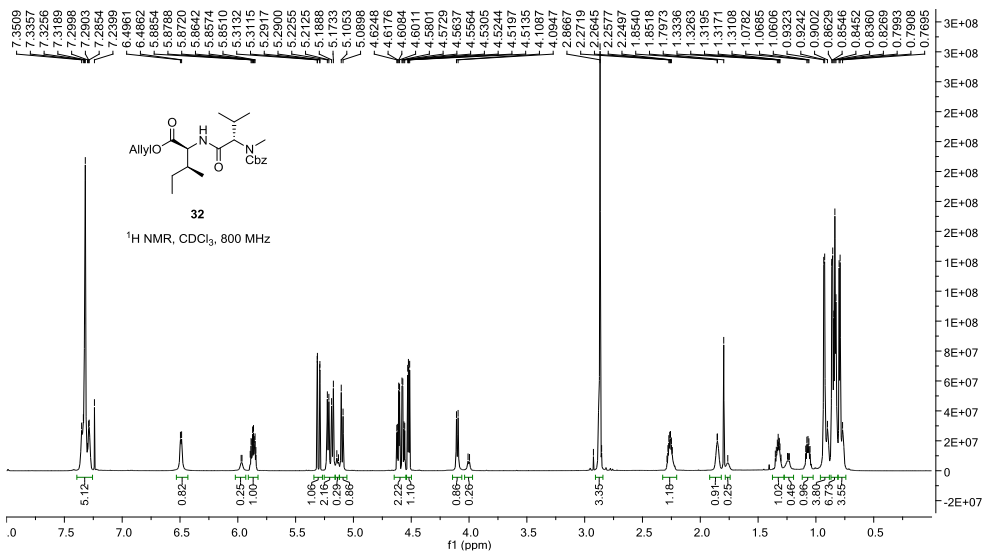

S12
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

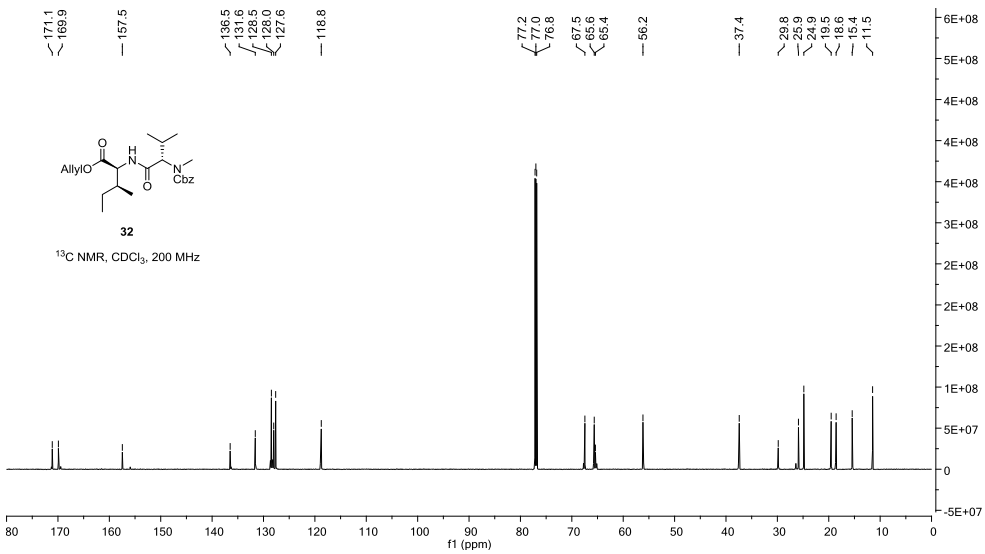

S13
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

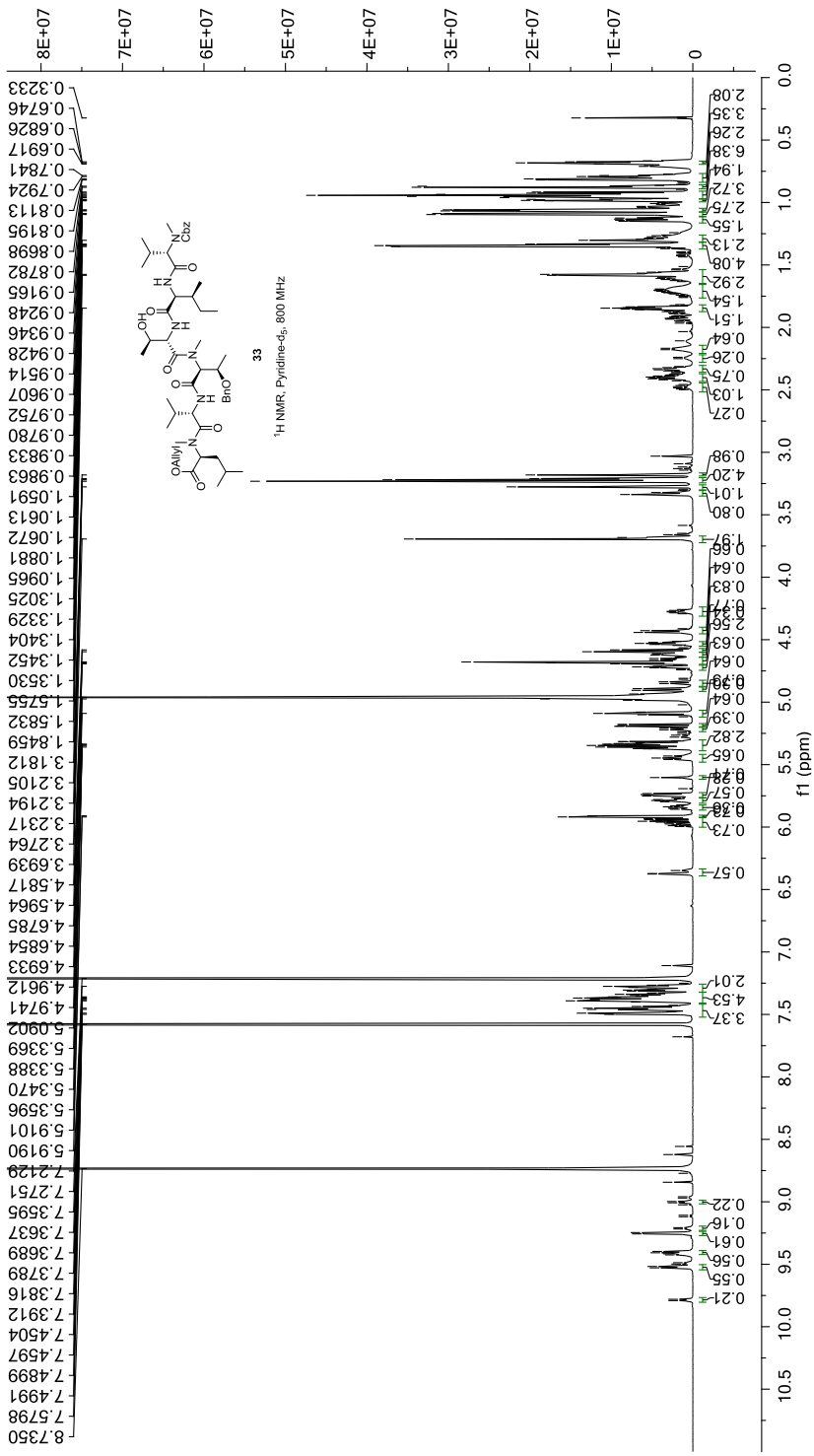

S13
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

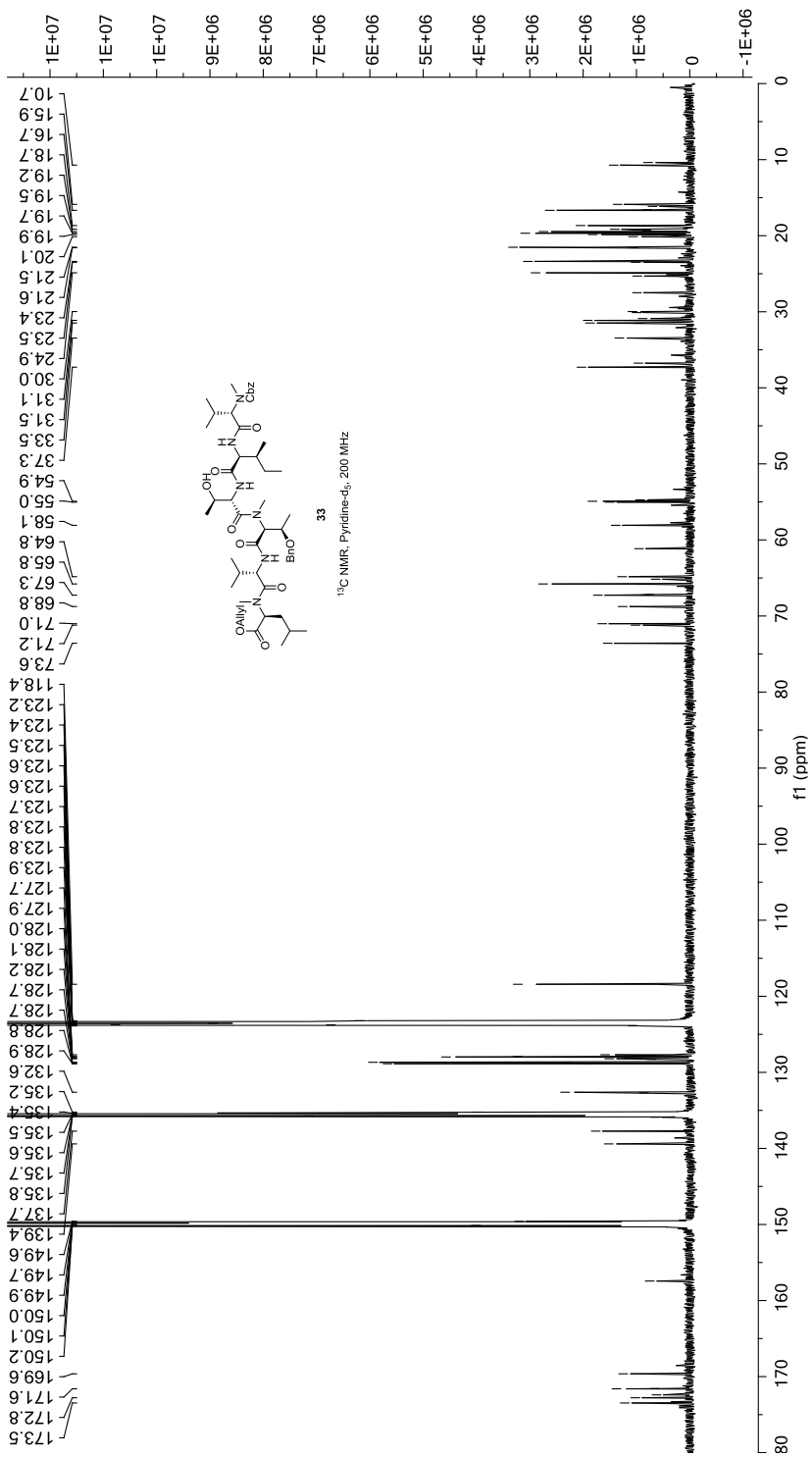

7.2397
5.8869
5.8773
5.8689
5.8596
5.8489
5.8400
5.8312
5.8216
5.2962
5.2943
5.2676
5.2657
5.2008
5.1835
4.5807
4.5644
4.5518
4.5348
4.2335
4.2268
4.2190
1.8526
1.8152
1.4149
1.3844
1.3641
1.3549
1.1456
1.1330
1.1210
1.1101
1.0964
0.8784
0.8718
0.8669
0.8593
0.8470
AllylO
O
NHBoc
S14
1H NMR, CDCl3, 600 MHz
1.00
1.03
1.04
0.87
2.14
0.89
0.93
10.56
1.09
6.49
f1 (ppm)

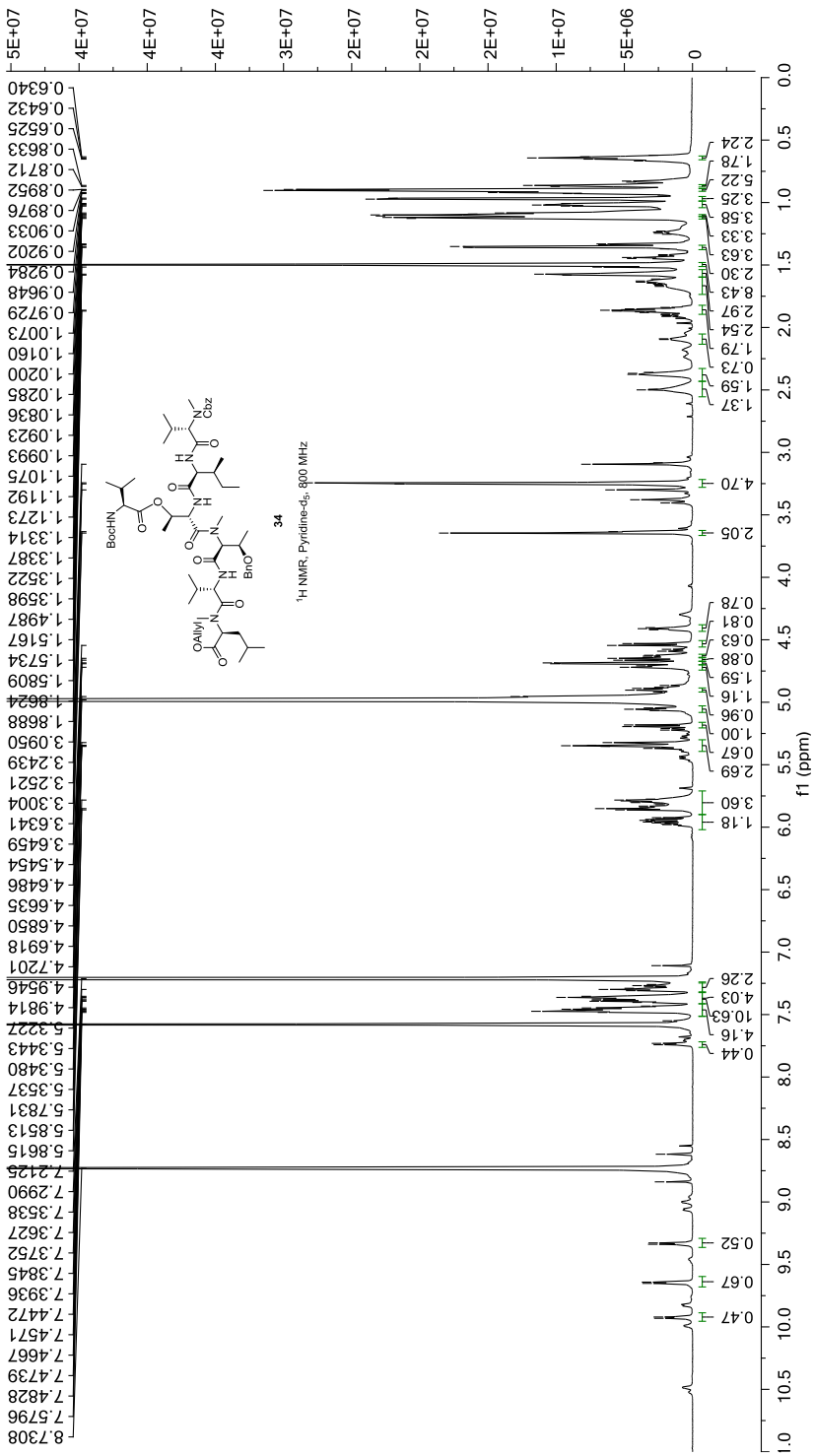

172.1
155.6
131.8
118.7
79.7
77.3
77.1
76.9
65.6
58.0
38.1
28.4
25.1
15.6
11.6
AllylO
O
NHBoc
S14
13C NMR, CDCl3, 150 MHz
f1 (ppm)

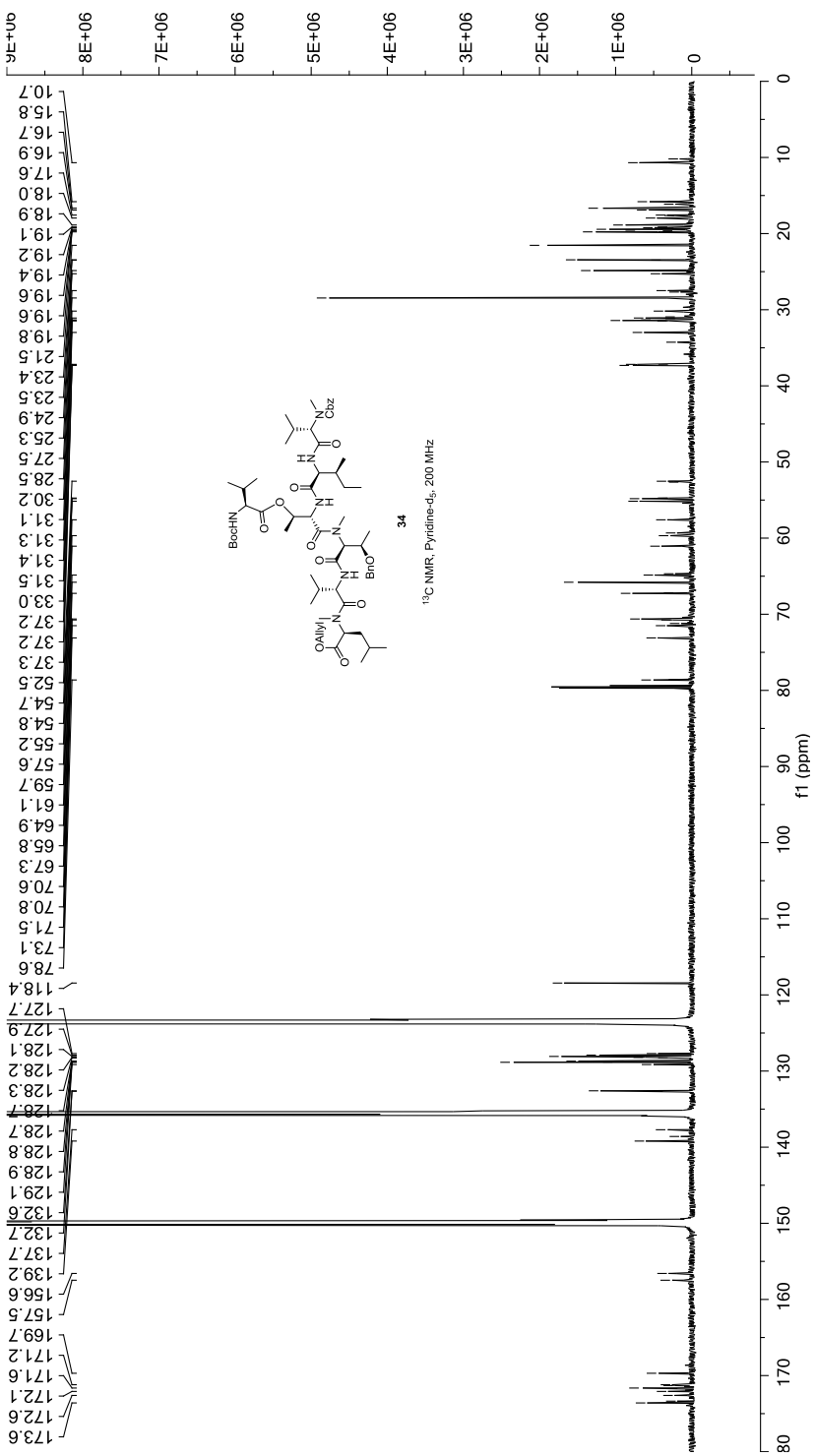

AllylO
Cbz
32
^{1}H NMR, CDCl$_3$, 800 MHz
f1 (ppm)

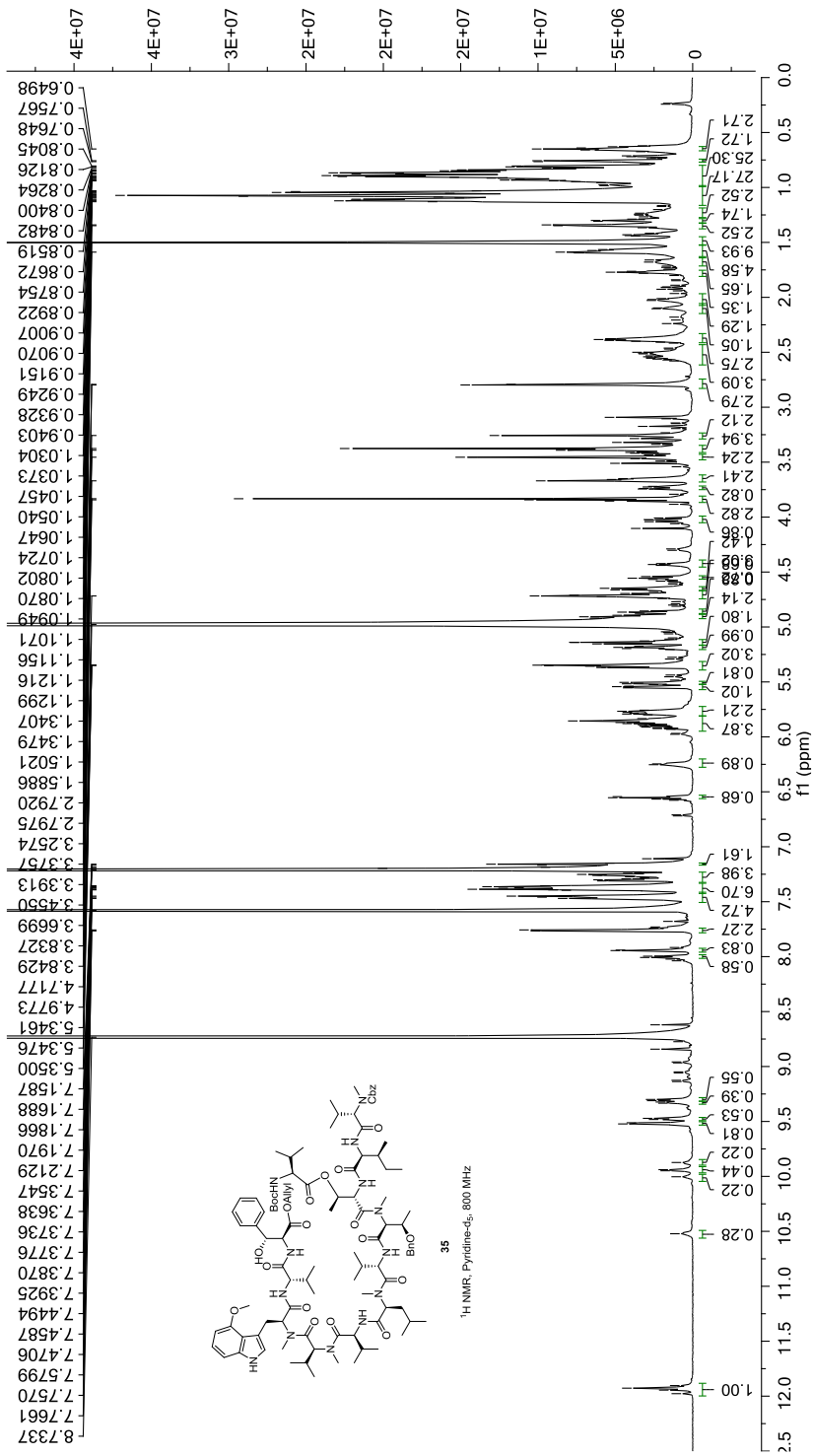

AllylO
Cbz
32
^{13}C NMR, CDCl$_3$, 200 MHz
f1 (ppm)

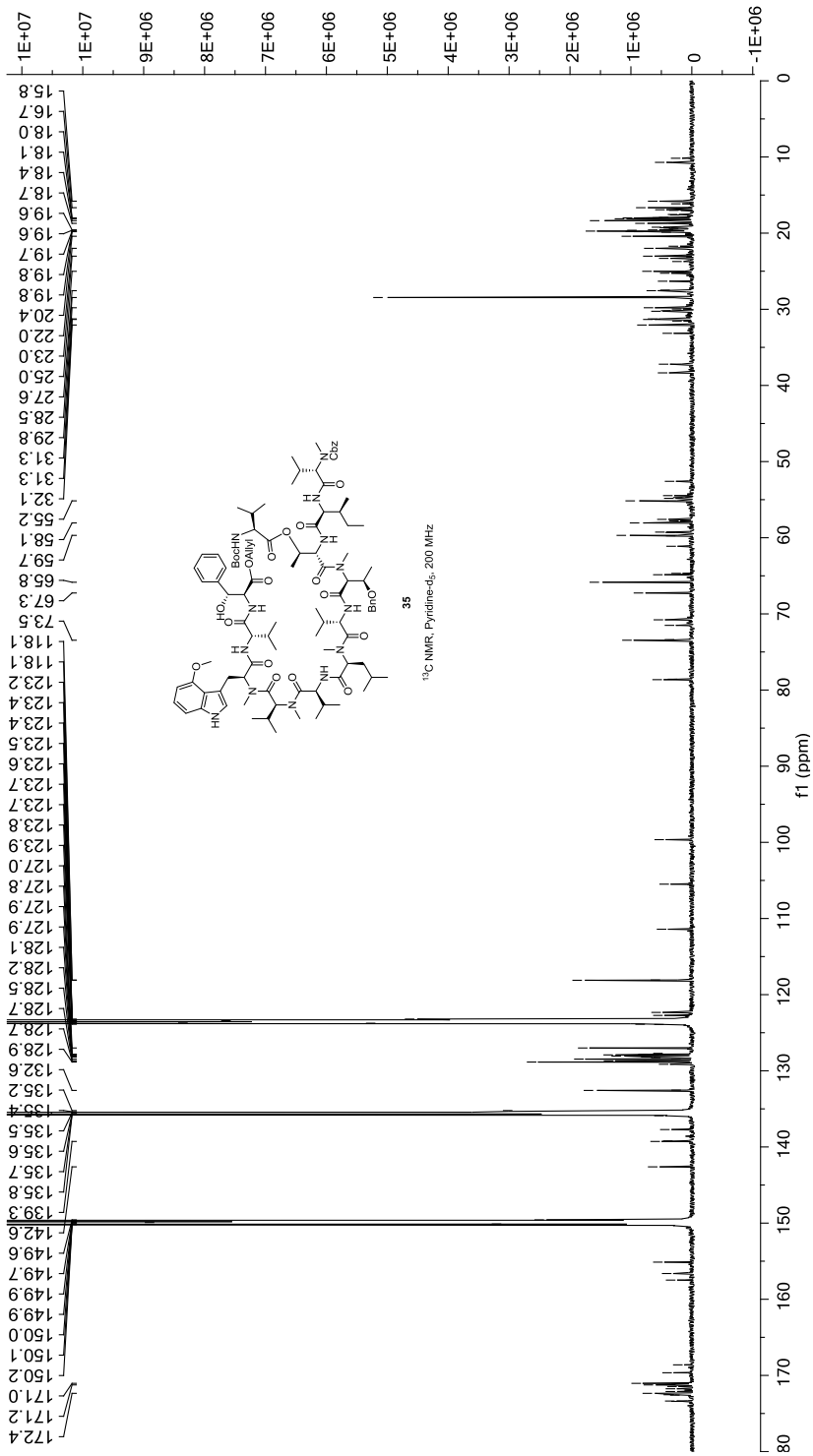

33
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)

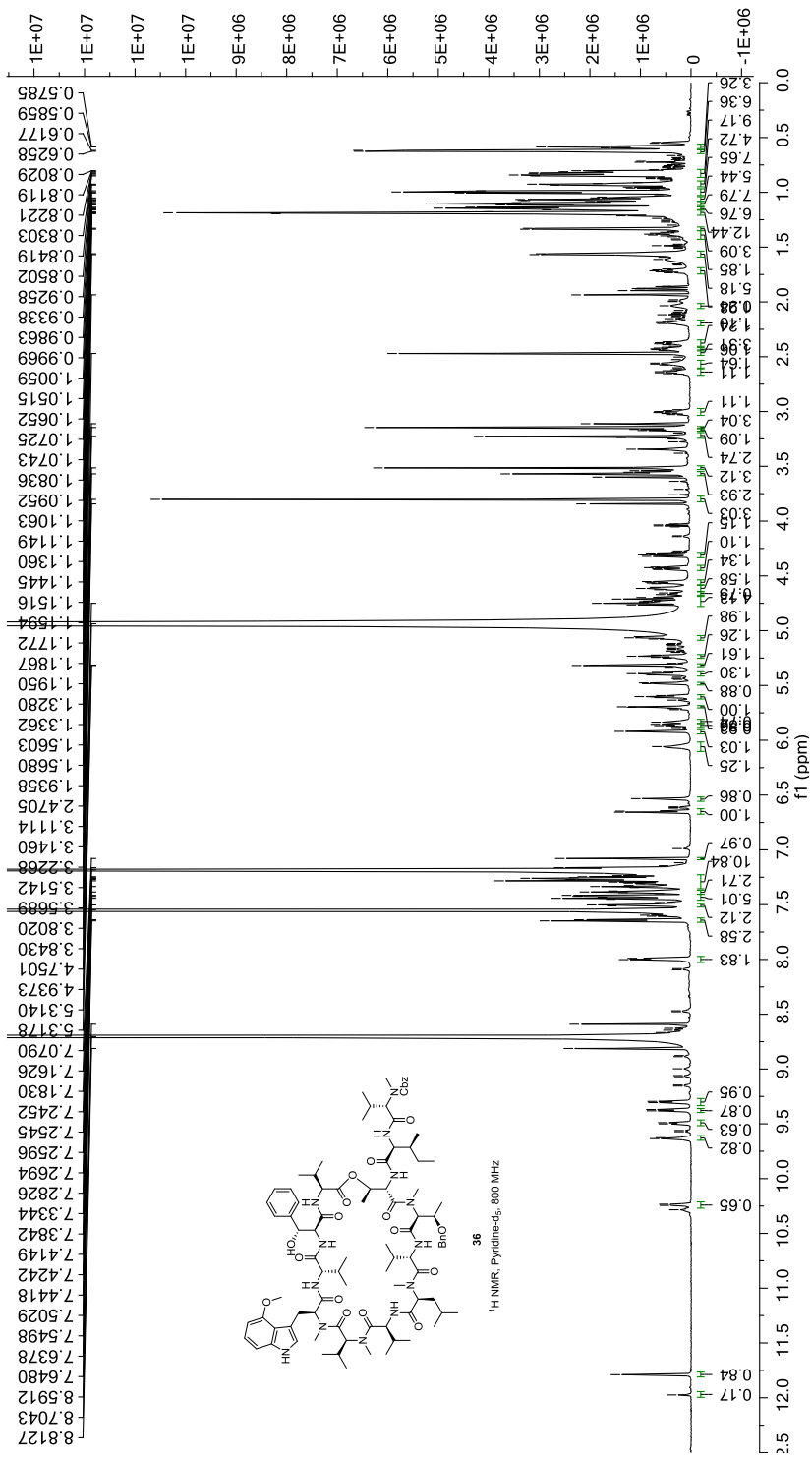

33
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

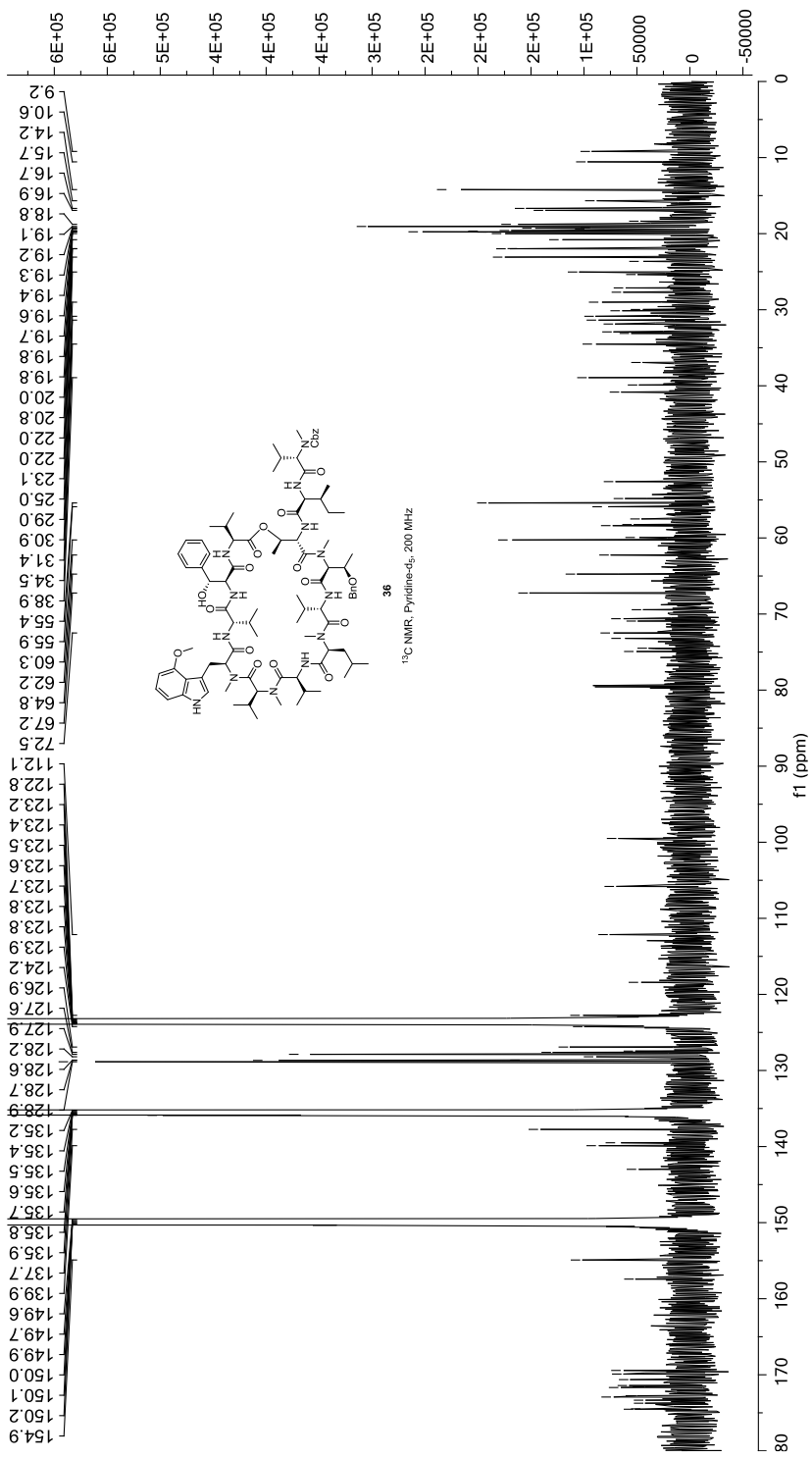

34
^{1}H NMR, Pyridine-d$_5$, 800 MHz
f1 (ppm)


34
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

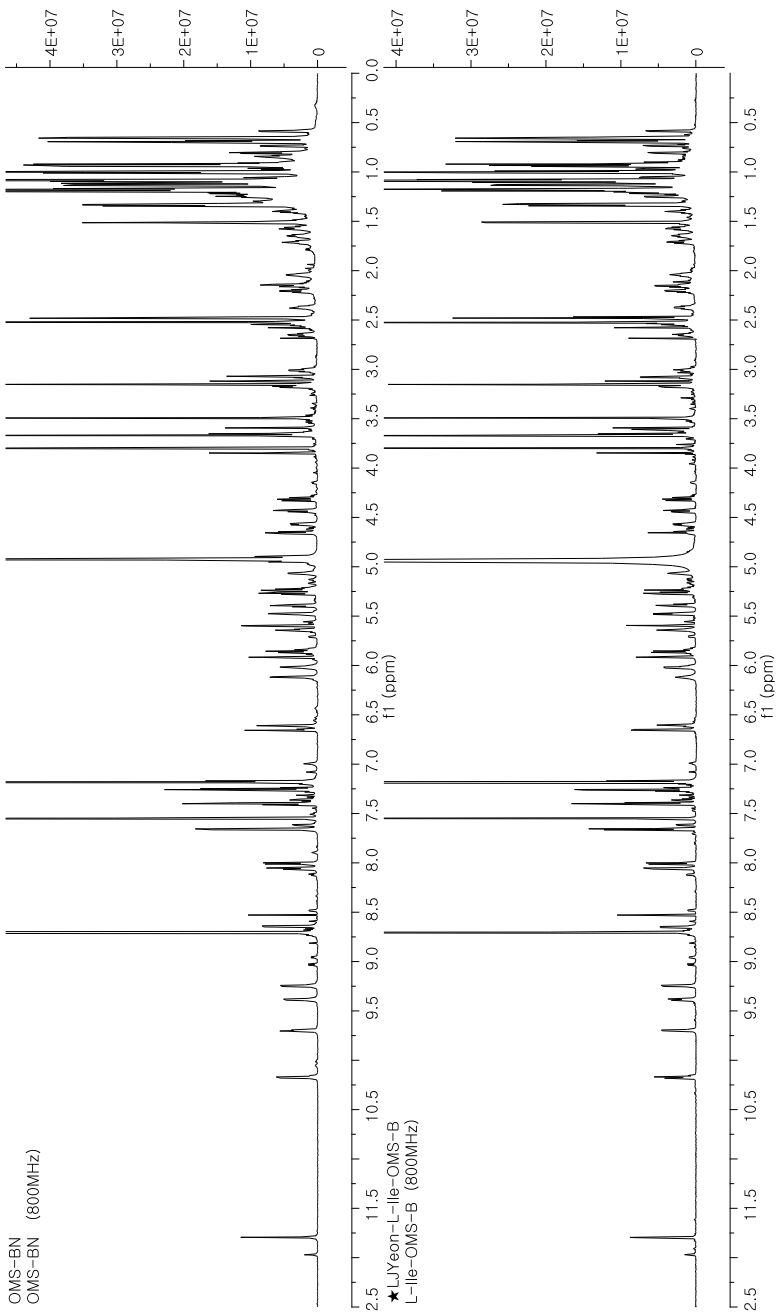

35
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)


35
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

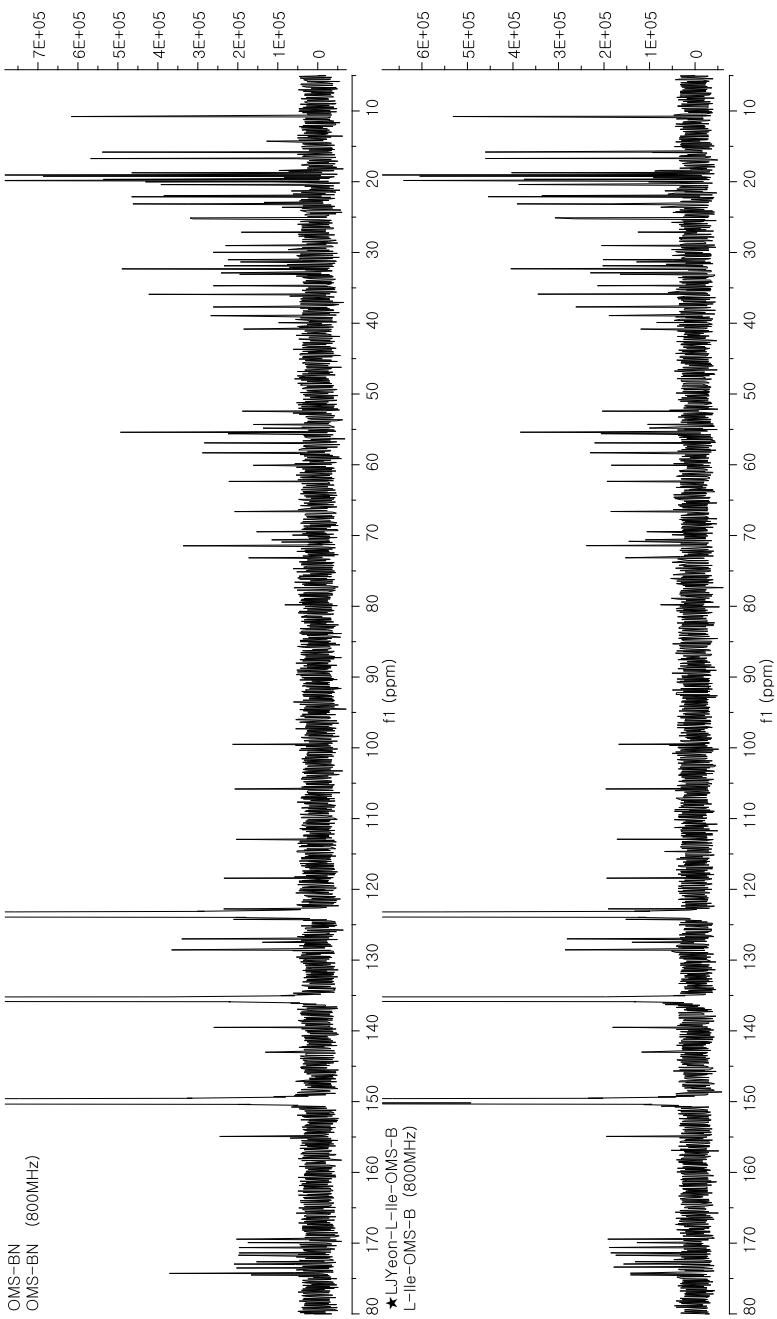

36
^{1}H NMR, Pyridine-d_5, 800 MHz
f1 (ppm)


36
^{13}C NMR, Pyridine-d_5, 200 MHz
f1 (ppm)

Comparison of the ^{1}H NMR spectra for the natural ohmyungsamycin B and the structure-revised ohmyungsamycin B (**37**)



Comparison of the ^{13}C NMR spectra for the natural ohmyungsamycin B and the structure-revised ohmyungsamycin B (**37**)



VII. 국문초록

고리형 펩타이드 천연물은 전통적인 저분자 약물이 다루지 못하는 target 에 대해 다양한 생리활성을 나타내면서도 선형 펩타이드에 비해 생체이용률이 높아 약물로서 발전 가능성이 높은 계열의 물질이다. 그 중 ohmyungsamycins A 와 B 는 새로운 cyclodepsipeptide 계열의 천연물로서 항암활성 및 강력한 항결핵 활성이 알려져 있다. 본 연구자는 ohmyungsamycins 의 독특한 구조 및 유망한 항결핵 약물로서의 발전 가능성에 주목하여 ohmyungsamycin A 와 B 의 최초 전합성을 완료했다. 이 과정에서 선행 보고된 ohmyungsamycin B 의 구조를 교정할 수 있었다. 또한 천연에 공통으로 존재하는 cyclic core part 의 항결핵 활성에 있어서의 중요성을 확인하기 위해 side chain 을 절단한 유도체를 설계하고 합성 및 활성 평가를 진행했다.

Ohmyungsamycins 는 bulky 한 잔기의 아미노산과 *N*-메틸 아마이드 골격이 밀집되어 있으므로 고리화 반응에 불리한 구조를 가지고 있다. 이를 극복하기 위해 본 연구자는 천연물의 입체 구조에 관한 정보를 최대한 활용하여, 고리화 반응의 전구체가 turn 을 이루어 최대한 굽은 구조를 가질 것으로 예상되는 방향으로 합성을 설계했다. 실제로, turn 을 유도할 것으로 생각되는 중간체로 NMR 분광학에 기반한 용액상 구조 분석을 진행하여 turn 유도 효과가 있음을 확인했다.

주요어: Cyclodepsipeptide, 천연물, Ohmyngsamycins, 전합성, 고리화 반응, 구조 교정, 펩타이드 구조, 수소-중수소 교환반응, 항결핵 활성, NMR 분광학

학번: 2013-21624