NOVEL METHANOPYRROLIDINE β–AMINO ACIDS

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By
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ABSTRACT

Novel Methanopyrrolidine β-Amino Acids

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Doctoral Advisory Committee Chair: Professor Grant R. Krow

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Methanopyrrolidine-5-carboxylic acids (MetPyr-5-acids), or 5-*syn*-carboxy-2-azabicyclo[2.1.1]hexanes are building blocks for β-peptides that cannot form backbone hydrogen bonds. To introduce functionality to this ring system, 6-*syn*-benzyloxymethyl and 6-*syn*-phenyl substituted derivatives have been prepared by an efficient synthetic procedure. Addition of appropriately substituted allyl amines to 3-butynone, amide protection, and irradiation afford mainly 5-acetyl-2-azabicyclo[2.1.1]hexanes. Haloform oxidation leads to the desired 6-substituted MetPyr-5-acids.

A 1-ethoxycarbonyl-MetPyr-5-acid also was prepared in high yield. Condensation of ally amine with ethyl 2,4-dioxopentanoate afforded ethyl 2-(allylamino)-4-oxopent-2-enoate, and this was protected to give ethyl 2-[allyl(tert-butoxycarbonyl)amino]-4-oxopent-2-enoate. Irradiation afforded 5-*syn*-acetyl-1-ethoxycarbonyl-2-azabicyclo[2.1.1]hexane with high stereoselectivity and oxidation of the acetyl group afforded the desired 1-ethoxycarbonyl-MetPyr-5-acid.

Resolutions of (±)-6-*syn*-benzyloxymethyl-MetPyr-5-acid and (±)-1-ethoxycarbonyl-MetPyr-5-acid were carried out (> 98% ee) by a classical resolution method using (*S*)-(-)-α-methylbenzylamine. The absolute configurations of (1*S*,4*R*,5*R*,6*S*)-(-)-6-benzyloxymethyl-MetPyr-5-acid and (1*R*,4*S*,5*S*)-(+)- 1-ethoxycarbonyl-MetPyr-5-acid were determined by X-ray analysis of their 5-(*S*)-(-)-α-methylbenzylamide.

A prior X-ray analysis of *N*-Boc-$(MetPyr)_4$-CO_2Me indicated all amides to be *trans* oriented with all 5-*syn*-carbonyl groups directed toward Carbon-4 of the methanopyrrolidine. These structures were assigned as T4T4T4T4 or $[T4]_n$ (n = 4). The solution structure was not determined. Homooligomers of (1*S*,4*R*,5*R*)-5-*syn*-carboxy-2-azabicyclo[2.1.1]hexane (MPCA) terminally protected as *N*-Boc methylesters were constructed by EDC/HOBt coupling of terminal ester *N*-deprotected free amine units and *N*-Boc free acid units. To facilitate NMR analysis of the secondary structures of homooligomers, *N*-Boc was replaced by *N*-isobutyryl. NMR experiments indicated that *N*-isobutyryl-$(MetPyr)_n$-CO_2Me, (n = 2, 3, 4) have major favored $[T4]_{n-1}T$ where the orientation of the terminal ester carbonyl could not be determined.

ACKNOWLEDGEMENTS

There are many people who deserve my sincere gratitude for their invaluable help and support in my long journey to pursue my Ph.D. degree.

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CHAPTER 1

INTRODUCTION

Biological functions, such as catalysis and signal transduction, are governed by biopolymers. Proteins, the key biopolymers, achieve the structural complexity required for complex activity via folding. Nucleic acids, too, can achieve a high level of operational complexity by adopting diverse secondary and tertiary structures. The substantial difference of residues between proteins and nucleic acids suggests that the propensity to fold, rather than any specific composition, provide the foundation for sophisticated molecular function. The rapidly expanding study of nonnatural "foldamers" emerges from these considerations.[1-2] Foldamers are oligomers that adopt well-defined conformations. The ability to control molecular shape has allowed creation of foldamers with interesting functions. It has become clear that short oligomers constructed from subunits other than α-amino acids or nucleotides can adopt discrete secondary structures. β-Amino acids represent the smallest step away from α-amino acids in that each subunit has an extra carbon inserted between the α-carbon and nitrogen. β-Amino acids can be subdivided into β^2–, β^3–, $\beta^{2,3}$–amino acids, depending upon the positions of the side chain(s) on the 3-aminoalkanoic acid skeleton (Figure 1).[3] Short β-peptides (constructed from β-amino acids) had been documented to have all three types of regular secondary structure: helix, sheet, and reverse turn, similar to their naturally occurring α-amino acid peptide (constructed from α-amino acids) counterparts.[4-6] Additional to the propensity to form discrete secondary structures, this class of compounds is characterized by another important feature: β-peptides are resistant to all kinds of mammalian proteases and

peptidases.[7-9] These properties warrant closer scrutiny of this class of compounds as peptidomimetics in medicinal chemistry.

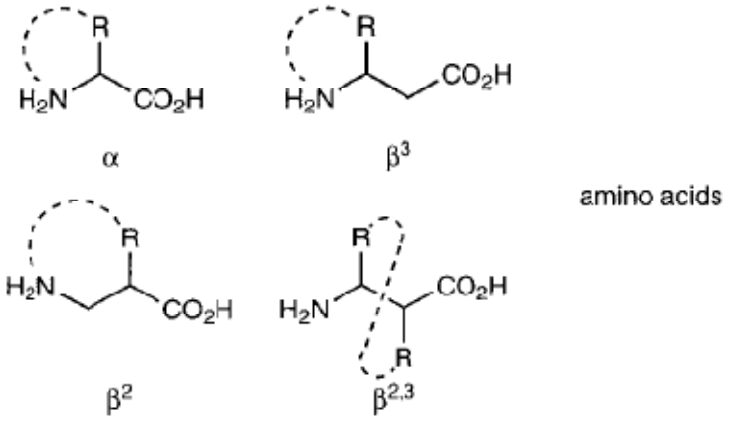


Figure 1. Types of α- and β-amino acids[1]

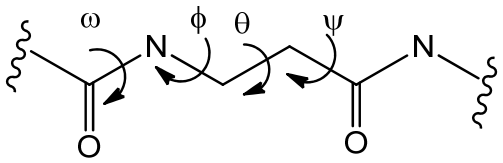


Figure 2. Definition for the torsional angles in β-peptides.[10]

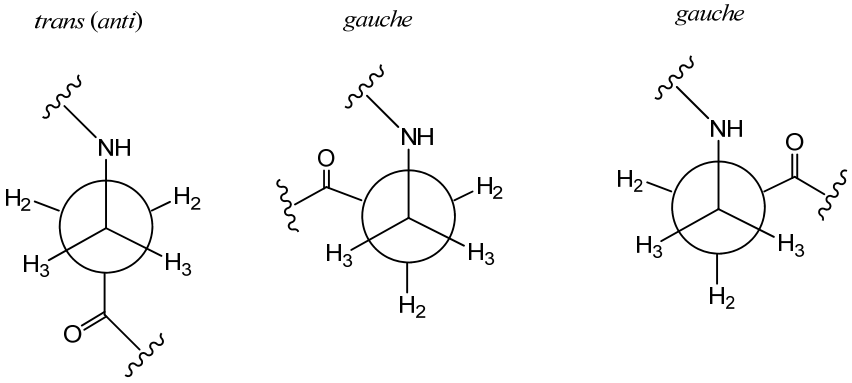


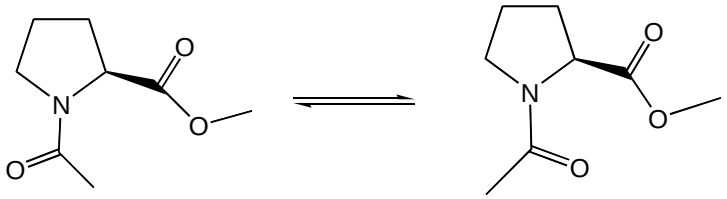
Figure 3. Rotamers of β-alanine regarding the θ dihedral.[10]

The conformations of β–peptides can be analyzed in terms of the main chain torsional angles, which are assigned the angles ω, ϕ, θ, ψ (Figure 2) in the convention of Balaram.[10] The effects of substituents on the conformation of β-amino acids have been the subject of extensive studies.[4,11-13] Folded helical or turn-like conformations of β-peptides require a gauche conformation about the θ torsion angle defined by the C^2-C^3 bond (Figure 3). Gauche-type torsion angles are even more strongly promoted when these substituents are included in a cyclohexane or cyclopentane ring, as in *trans*-2-aminocyclohexanecarboxylic acid (ACHC),[14] *trans*-2-aminocyclopentanecarboxylic acid (ACPC), [15] or *trans*-3-amino-pyrrolidine-4-carboxylic acid (APC).[16] Within the past decade, several research groups have described unnatural peptide oligomers, especially β-peptides and mixed α/β-peptides, with interesting conformational properties.[9,17] Those efforts have revealed that success in function-oriented design can depend critically upon access to multiple foldamer scaffolds, each of which provides a distinctive way to orient sets of side chains in space. Those efforts also suggest that control over oligomer folding could lead to new types of molecules with useful biological properties. For example, Gellman and co-workers designed a series of globally amphiphilic 14-helical β-peptides, showed antifungal activity and selectivity while other 14-helical β-peptides not globally amphiphilic (scrambled 14-helical β-peptides) are completely inactive.[14c]

In proteins, regular secondary structures are defined largely in terms of H-bonding between sites embedded in the polymeric backbone. However, it should be noted that H-bonding is not necessarily the only, or even the major, driving force for observed

secondary structures. Dipolar forces or the intrinsic conformational preferences of subunits of the protein backbone, i.e. the pyrrolidine ring in proline, could be other important factors.

Proline has two prominent attributes that are unique among the proteinogenic amino acids: only proline is a secondary amine, and only proline has a saturated ring.[18] These attributes make proline residues a key determinant of protein structures.[19, 20] As a secondary amine, proline has a much greater propensity than other natural amino acids to form *cis* (that is, *E*) peptide bonds (Scheme 1).[21,22] Xxx-Pro linkages are therefore tertiary amides, which cannot serve as hydrogen bond donors. Despite the lack of internal hydrogen bonding, oligomers and polymers of L-proline adopt discrete secondary structures because proline's ring structure makes it more conformationally restricted than the other amino acids.



cis-Amide *trans*-Amide

SCHEME 1

There are two stable secondary structures of polyproline helices (Figure 3): polyproline I (PPI) and polyproline II (PPII), which differ in their amide conformations.[23] PPI is a right-handed helix containing all *cis* peptide bonds and backbone dihedral angles

of (ϕ,ψ,ω) = (-75°, 160°, 0°). The PPI helix is compact, having a helical pitch of 5.6Å/turn and 3.3 residues/turn. In comparison, PPII is a left-handed helix with all *trans* peptide bonds and backbone dihedral angles of (ϕ,ψ,ω) = (-75°, 145°, 180°). The PPII helix is extended, having a helical pitch of 9.3Å/turn and 3.0 residues/turn. The PPI helix is favored in organic solvents, such as *n*-propanol, whereas the PPII helix dominates in aqueous solution.[24-26]

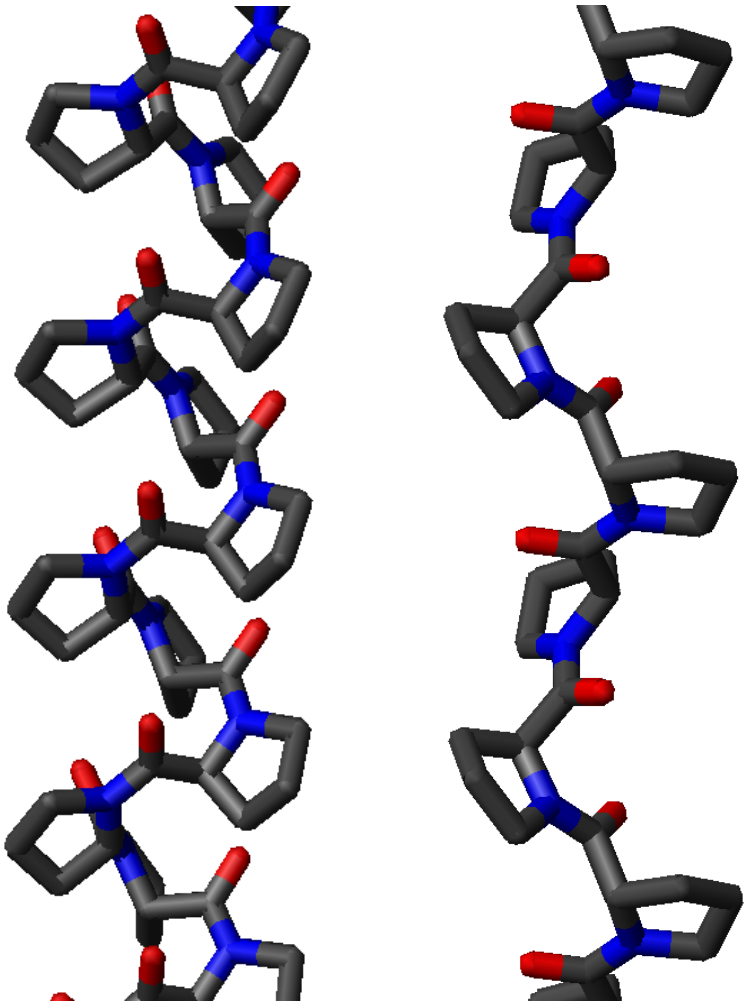
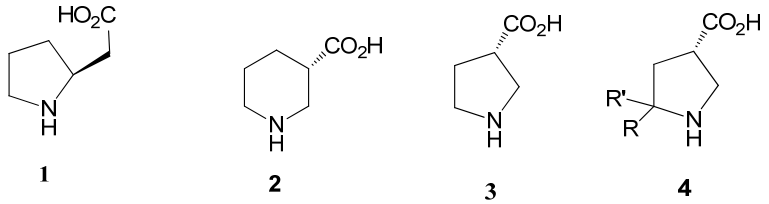


Figure 4. Side view of a polyproline I helix (left) and a polyproline II helix (right)

Seebach and co-workers described the synthesis and conformational studies of oligomers constructed from homo-proline β-amino acids: (S)-pyrrolidine-2- acetic acid [(S)-β^2] **1** and (S)-piperidine-3-carboxylic acid [(S)-β^3] **2** (Scheme 2).[27] The CD spectra of the all (S)-β^2- and the all (S)-β^3-containing β-peptides display novel and intensive CD patterns which may be indicative of a secondary structure.

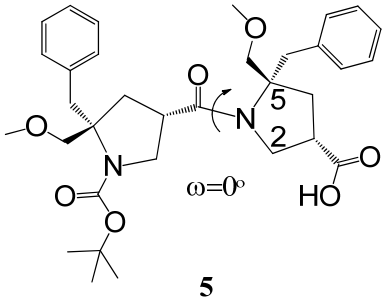


1 **2** **3** **4**

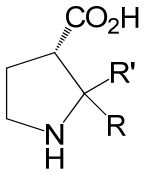
SCHEME 2

Gellman and co-workers studied the oligo-tertiary amides constructed from (S)-pyrrolidine-3-carboxylic acid **3** (P-3-C) (Scheme 3), a cyclic β-amino acid.[28] The preliminary evidence for ordered folding of (P-3-C) homoologomers suggests that the conformational analogy between conventional α-peptides and β-peptides extended beyond hydrogen-bonded secondary structures to non-hydrogen-bonded structures such as the PPII helix. In order to induce a secondary structural preference at the amide bond of a β-peptides, the same group also described 2D NMR and CD conformational studies of homooligomers based on 5,5-disubstituted P-3-C compounds **4**. Distinct conformational preferences were suggested, but not identified with certainty.[29] It is expected that β-amino acid **3** residues will possess higher conformational flexibility than proline residues because the tertiary amide group is one more carbon away from the carboxylate. ^{13}C NMR studies of β-peptides prepared from 5-monosubstituted

pyrrolidine-3-carboxic acid (R or R' as H on C_5 of compound **4** indicate multiple rotameric states).[29]



5



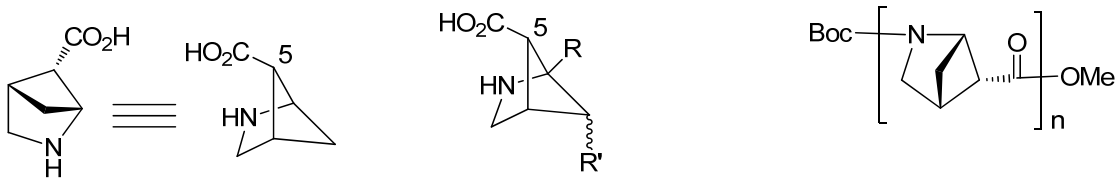
6

SCHEME 3

A stronger rotamer bias is observed for β-peptides derived from 5,5-disubstituted **4**. Both NOEs and X-ray crystal structure of compound **5** show that the amide linkage adopts the expected *cis* conformation, which is defined as the amide C=O pointing toward the disubstituted C_5 position.[29] Accordingly, 2,2-disubstituted **6** could induce a *trans* conformation of the *N*-terminal amide as the major rotamer. However, because of the difficulty of introducing functionality at the hindered C_2-position, synthesis of related β-peptides was unsuccessful.[29] The goal of preparing solely pyrrolidine-based β-peptides of defined secondary structure has not been achieved.

An alternative to forming pyrrolidine-based backbones that fold into predictable shapes is replacement of 2,2-disubstituted pyrrolidines **6** with a bridged MetPyr **7** (Scheme 4). The methylene bridge may control the amide conformation by limiting rotations of ω and ψ (Figure 5), which in turn may play a role in folding of oligomers of **7**. Functionalized groups on methylene (**8a**, R = H) and bridgehead (**8b**, R' = H) oriented

in defined positions in space, because of the rigidity of the core scaffold, will result in a conformation defined structural motif for diversity generation and bioactivity screening.



7 **8** **9**

8a R = H; R'=alkyl, aryl

8b R = alkyl, aryl; R'=H

8c R = alkyl, aryl; R' = alkyl, aryl

(-)-**9a** n = 1
(-)-**9b** n = 2
(-)-**9c** n = 3
(-)-**9d** n = 4
(-)-**9e** n = 6
(-)-**9f** n = 8

SCHEME 4

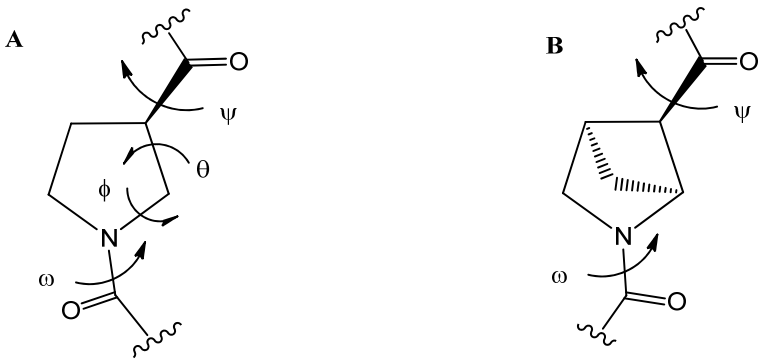


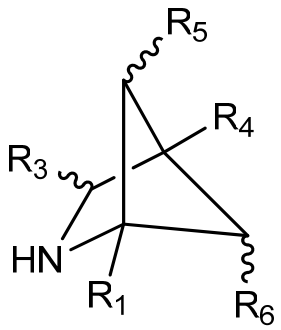
Figure 5. (A). Torsional angles in β-proline. (B). Torsional angles in β-methanopyrrolidine.

Dr. Nian Liu from our group succeeded in synthesizing a series of homooligomers *N*-Boc-(MetPyr)$_n$-CO_2Me **9** (n = 1, 2, 4, 6, 8) from MetPyr **7**.[30] The circular dichroism (CD) spectra of the oligomers clearly show that an ordered conformation starts to form on tetramer (-)-**9d** (n = 4 in **9**) and enhances for longer oligomers: hexamer **9e** and octamer **9f**. The X-ray analysis of the tetramer (-)-**9d** indicated that the oligomer has a T4T4T4T4 or $[T4]_4$ structure [from N to C, the *N*-Boc carbonyl bond is *trans* with ω = 171.9°, the carbonyl of unit 1 is directed toward H_4 with ψ = 45.73°; the *nth* carbonyl bond (amide, *n* = 2, 3, 4) is *trans* with ω = 171.4~176.6°, the carbonyl of unit n (unit 4 is an ester carbonyl) is directed toward H_4 with ψ = 55.79~86.17°].[30] However, a distinct solution conformation was not identified. We will continue to investigate the solution conformation of oligomers **9** from MetPyr **7** in Chapter 4.

During the past decade combinatorial chemistry has become a synthetic tool to complement rational design in the development of molecules with useful biological properties.[31,32] One approach to molecular libraries is to utilize a rigid scaffold with functionality displayed in three dimensions.[31,33,34] In this light the multifunctional azabicyclo[2.1.1]hexane structure **10** shown in Scheme 5 present unique conformationally defined structural motifs for diversity generation.

The 2-azabicyclo[2.1.1]hexane derivatives as in Scheme 5 can also be viewed as pyrrolidine analogues bridged between C_2 and C_4. Pyrrolidines are common to many biologically significant molecules. In the search for selective bioactive molecules one useful strategy is to incorporate this key pharmacophoric entity into a less flexible

structure. The 2-azabicyclo[2.1.1]hexane -- a 2,4-methanopyrrolidine -- is one example of a constrained model for pyrrolidines. There is increasing interest in heterocylces related to **10** in drug discovery.[35-40]



10

R_1, R_3,= H, allyl, aryl, carboxyl, methano-R

R_4, R_5, R_6=H, halo, amino, hydroxyl, fluoro,
alyl, aryl, carboxyl, methano-R,
amino-R, hydroxyl-R

SCHEME 5

In a broader perspective, discovery of practical methods for introduction of useful (hydrophilic and lipophilic) functionalities (-R) on rings in methanopyrrolidine β–amino acids will be our first effort. Because of conformational constraints, and even in the absence of hydrogen bonding interactions, β-peptides constructed from methanopyrrolidine β–amino acids are expected to fold into organized secondary

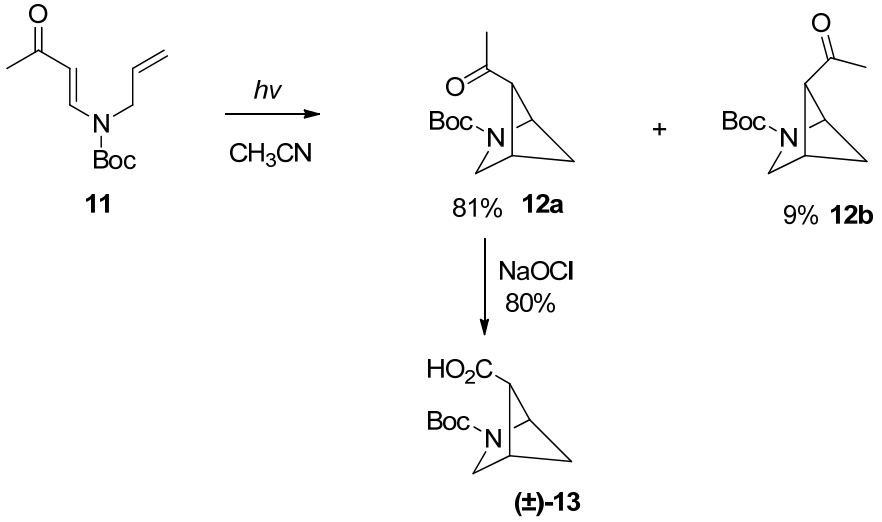
structures.[30] This will expose biologically useful functional groups with selected spatial orientations. Identification of foldamer secondary structures will enable us to understand the rationale behind the folding patterns in oligomers of methanopyrrolidine β–amino acids. Appropriately functionalized β–amino acids foldamers may have useful biological applications.[9] They also are expected to be resistant to enzyme degradation. This is the first step in the ultimate creation of a new type of metanoproline-based β–peptide that will inhibit specific protein-protein interactions.

CHAPTER 2

CHEMISTRY OF C_6-SUBSTITUTED METHANOPYRROLIDINE β-AMINO ACIDS

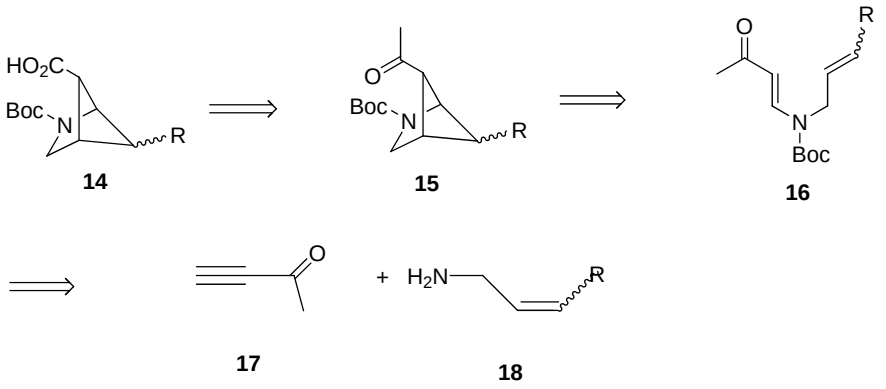
2.1 Introduction

According to the literature, there are mainly three different routes to synthesize 2-azabicyclo[2.1.1]hexanes, which include the rearrangement from 2-azabicyclo[2.2.0]hex-5-enes,[41,42] intramolecular [2+2] photochemical cycloaddition,[43] and nucleophilic ring closure of cyclobutanes.[44] Winkler and coworkers studied extensively the synthesis of C_5-acetyl-C_6-H (substituted)-2-azabicyclo[2.1.1]hexanes by employing [2+2] photochemical cycloaddition.[43a, 43b, 43c] Applying the method, we have developed a convenient route to prepare racemic 5-*syn*-acid (±)-**13** stereoselectively in multigram scale (Scheme 6).[45]



SCHEME 6

2.2 Retrosynthesis of C_6-substituted methanopyrrolidine β-amino acids



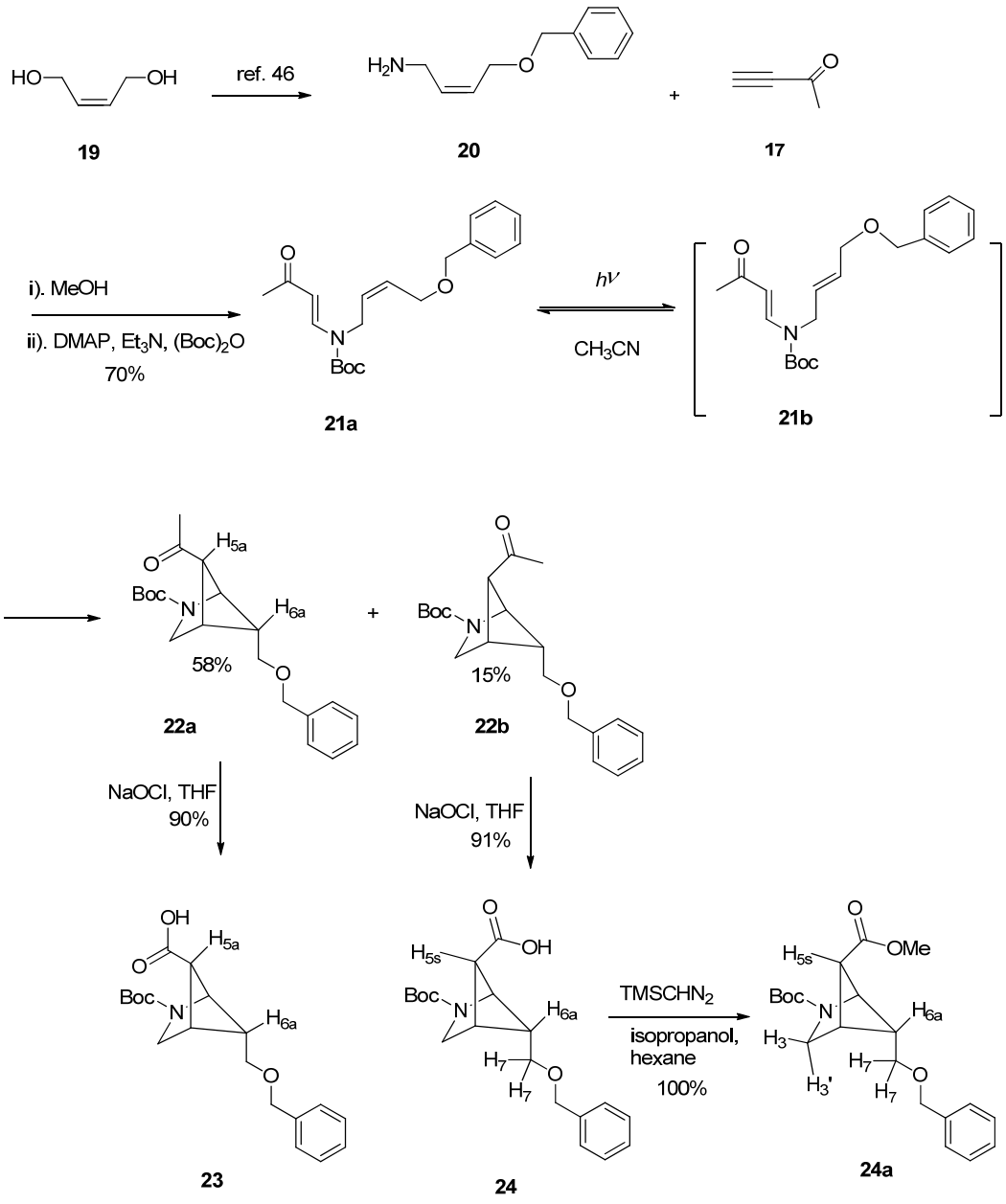
SCHEME 7

The synthesis of 6-substituted methanopyrrolidine β-amino acids **14** can be derived from the 5-acetyl **15** by oxidation. The compound **15** could be prepared by [2+2] photochemical cycloaddition of diene **16**, which could be prepared from the amine **18** and alkyn **17** (Scheme 7).[43b, 43c, 43f]

2.3 Synthesis of C_6-benzyloxymethyl methanopyrrolidine β-amino acids

cis-Allylamine **20** was synthesized from *cis*-diol **19** following a literature procedure (Scheme 8).[46] The photosubstrate **21a** was made in 70% yield by addition of allylamine **20** to commercial 3-butynone **17** and subsequent reaction with di-*tert*-butyl carbonate in the presence of DMAP and Et_3N. Irradiation of dienone **21a** yielded ketones **22a** and **22b**.[43b, 43c, 43f] Carefully chromatographic separation of the reaction mixture after the irradiation of **21a** revealed the formation of the thermodynamically more stable *trans*-alkene **21b.** Irradiation of **21b** affords almost the same yield of ketones **22a** and **22b** as

the irradiation of **21a**. During this work, Winkler and co-worker reported the similar observation during their irradiation reaction. [43c]

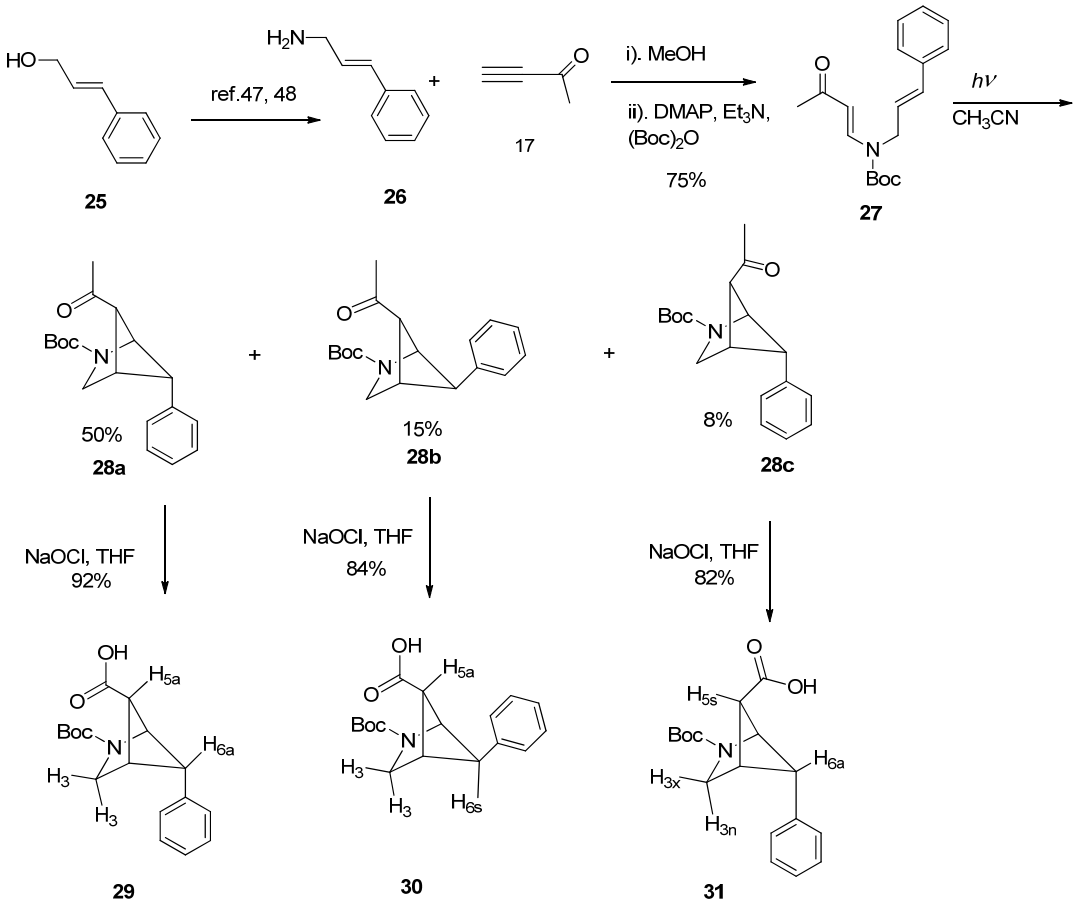


SCHEME 8

The 5-*syn*, 6-*syn* stereochemistry is assigned to the major product **22a** by the absence of long-range coupling between H_{5a} proton at δ 2.53 and H_{6a} proton at δ 2.15 and the small couplings of H_{5a} with the H_1 proton at δ 4.68 and 4.56 (J = 1.7 Hz) and with H_{6a} (J = 1.7 Hz). The NOE between H_{5a} and H_{6a} further confirms the structure. Attempted oxidation of ketone **22a** using the aqueous sodium hypochlorite (bleach, 4~7% Cl_2), which worked well for **12a** in THF at 40 °C, did not afford the desired acid **23**.[45] After many trials, aqueous sodium hypochlorite (Aldrich, 10~15% Cl_2) was used instead and the desired acid **23** was made with high yield (90%) at room temperature in 5 minutes. The 5-*syn*, 6-*syn* stereochemistry could be assigned to the acid **23** on the basis of the NOE between H_{5a} proton at δ 2.58 and H_{6a} proton at δ 2.13. The stereochemistry of acid **23** was further supported by X-ray of amide (-)-**34** (section **2.7**).[30]

The 5-*anti*, 6-*syn* stereochemistry could be assigned to the minor product **22b** from NOE studies of its derivative ester **24a**. Minor ketone **22b** was also oxidized in high yield (91%) to afford acid **24**, which was converted to its methyl ester **24a** by reaction with trimethylsilyldiazomethane.[45] The NOE between H_6 proton at δ 2.81 with the OMe protons at δ 3.72 and the CH_2O protons at δ 3.27, and the NOE between the H_{5s} proton at δ 2.56 and the H_3 proton at δ 3.29 confirmed the 5-*anti*, 6-*syn* stereochemistry of the ester **24a**.

2.4 Synthesis of C_6-phenyl-methanopyrrolidine β-amino acids



SCHEME 9

trans-Allylamine **26** was synthesized from alcohol **25** following a literature procedure (Scheme 9).[47,48] The photo substrate **27** was made by addition of allylamine **26** to commercial keto-alkyne **17** and subsequent reaction with di-*tert*-butyl carbonate in the presence of DMAP and Et_3N.[43f] The irradiation of dienone **27** yielded ketones **28a, 28b** and **28c** as in Scheme 9 (**28c** is not pure enough for good spectra after many

chromatography trials. The stereochemistry of **28c** is assigned based on the analysis of its oxidized products acid **31**).

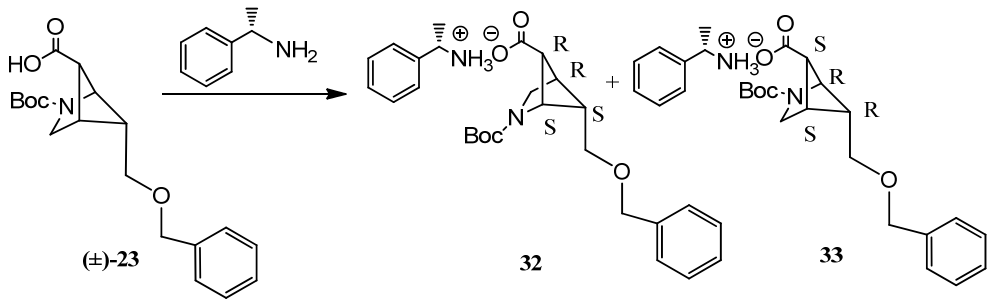
The 5-*syn*, 6-*syn* stereochemistry can be assigned to the major product **28a** by the absence of long-range W-plan coupling between H_{5a} proton at δ 2.73 and H_{6a} proton at δ 3.16. The H_1 proton at δ 5.12 and 4.98 shows the expected small vicinal coupling (J = 1.8 Hz) with H_{5a} and the small vicinal coupling (J = 1.8 Hz) with H_{6a}. Ketone **28a** can be oxidized to afford **29** in high yield (92%) by using aqueous sodium hypochlorite (Aldrich, 10~15% Cl_2). The 5-*syn*, 6-*syn* stereochemistry can be further confirmed by the NOE between C_6-Ph with H_{3n} proton at δ 2.88, 2.83 and H_6 proton at δ 2.85.

The ketone **28b** was oxidized to afford acid **30**. The 5-*syn*, 6-*anti* stereochemistry could be assigned to the product **30** by the NOE between C_6-Ph with to H_4 proton at δ 3.36 and 3.34, H_{5a} proton at δ 3.11 and H_{6s} δ 3.18. Surprisingly**,** the impure ketone **28c** was also oxidized by using aqueous sodium hypochlorite (Aldrich, 10~15% Cl_2) to afford the oxidized acid **31** without further purification. The 5-*anti*, 6-*syn* stereochemistry could be assigned to **31** on the basis of the NOE between C_6-Ph and H_{3n} proton at δ 3.08, 3.0 and no NOE between the H_6 proton at δ 3.89 and 3.86 with the H_5 proton at δ 2.80 and 2.77.

2.5 Resolution of racemic acid (±)-23

To build oligomers, enantiopure acid monomers have to be resolved from their racemic mixtures. The classical resolution method is to form diastereomeric salts between racemic acids and chiral amines and then to selectively crystallize a single diastereomeric salt to effect separation. Even though resolution is an efficient way to enrich enantiomers, a successful execution depends on suitable resolution reagents and solvents, recovery-efficient crystallization/re-crystallization conditions, and reliable analytical methods to monitor the enantiomeric purity.

(*S*)-(-)-α-Methylbenzylamine was selected as the resolution reagent because it is readily available. The diastereomeric salts from (*S*)-(-)-α-methylbenzylamine and racemic acid (±)-**23** are shown in Scheme 10. Crystallization and recrystallization efforts, initially carried out on small scale (10 mg) in order to obtain rapid results using multiple solvent systems, are shown in Table 1.



SCHEME 10

TABLE 1. Screening of Small-Scale Crystallization/Recrystallizations of Diastereomeric Salts from Acid Mixture (±)-**23** and (*S*)-(-)-α-Methylbenzylamine

Entry	Crystallization/Recrystallization Solvent(s)	Properties of Salts Solvent Systems
1	Ethyl Acetate	- Salts precipitate out at room temperature - Completely dissolves in solution at 60 °C
2	Acetone	- Salts precipitate out at room temperature - Completely dissolves in solution at 60 °C
3	1:1 Ethyl Acetate: Hexane	- Salts precipitate out at room temperature - Partially soluble at 45 °C
4	1:1 Hexane: Ether	- Insoluble at room temperature and higher temperature
5	Methanol	- Soluble at room temperature - No crystals formed at 4 °C
6	95 % Ethanol/ 5 % water	- Soluble at room temperature - No solid formed after scratching, seeding, or cooling.
7	100 % Ethanol	- Soluble at room temperature - No solid formed after scratching, seeding, or cooling.
8	De-ionized Water	- Few salts precipitate out at room temperature - Insoluble at higher temperature
9	Tetrahydrofuran (THF)	- Salts precipitate out at room temperature - Completely dissolves at 60 °C.

Observations on salt formations and solubility profiles suggested that ethyl acetate, acetone and tetrahydrofuran could be ideal solvents for resolution. A second round of experiments screened the recrystallization of diastereomeric salts in these three solvents. The results are summarized in Table 2.

TABLE 2. Results of Recrystallization of Diastereomeric Salts from Acid Mixture (±)-**23** and (*S*)-(-)-α-Methylbenzylamine in Three Different Solvents

Entry	Solvent	Yield of Salt	de (%)
1	EtOAc	57 %	34.2
2	Acetone	30 %	79
3	THF	18 %	12

We quickly ruled out the possibility of EtOAc and THF as suitable solvents for resolution. Based upon reasonable recovery yields of salt, acetone was the candidate for resolution. To find out the enantiomeric purity of resolved acid from acetone, a reliable chiral analytical method needed to be developed.

2.6 HPLC analysis of amides (-)-34 and (+)-35

High-performance liquid chromatography (HPLC) has advanced considerably in the past decades and has become a useful method for determining compound purity. Particularly in the pharmaceutical industry, HPLC is the most popular instrumental technique for research and development of drugs. Directly adding the coupling reagents (EDC, HOBt, Et_3N) to small quantity samples (10 mg) in dichloromethane from the

crystallization, with the help of HPLC (**APPENDIX B**) and NMR, we can easily identify the ratio of the two enantiomers (Scheme 11).

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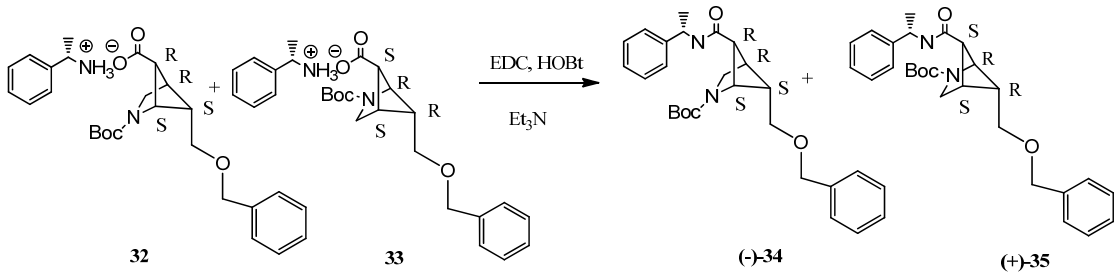


SCHEME 11

The optical purity of resolved acids is of crucial importance in preparing oligomers in order to avoid formation of diastereomers in the coupling steps. We desired to avoid extensive purification steps and loss of materials. To check optical purities, we devised an HPLC analytical method with two wavelengths (**APPENDIX B**). Upon the development of the analytical method, the resolution was closely monitored to separate the two enantiomers. Initial crystallization of diastereomeric salts from racemic acid (±)-**23** and (*S*)-(-)-α-methylbenzylamine in acetone yielded (-)-acid in 79% ee of purity and one recrystallization of the salts in acetone enriched (-)-acid in greater than 98% ee with an overall 32% yield. Acid was freed from the diastereomeric salt by ethyl acetate extraction from acidic aqueous solution and the optical rotation, $[\alpha]_D$, of the free acid was –10.7 (20 °C, c = 2.34, chloroform).

Since (*R*)-(+)-α-methylbenzylamine is also commercially available, the other enantiomer, acid (+)-**23** was separated from the above mother-liquor residue following the same resolution procedure (38% yield with around 96% ee). Repeating the operation, the two enantiomers were separated in high chiral purity.

2.7 Determination of absolute configuration of resolved acid (-)-23

Slow evaporation of the solution of amide (-)-**34** in THF/hexane provided short needle-like crystals which were of sufficient quality for X-ray analysis. The structure of (-)-**34** was determined by X-ray crystallography (Figure 6). Since the methylbenzyl amine has an (*S*)-configuration, the absolute configuration of acid (-)-**23** was assigned as (1*S*,4*R*,5*R,*6*S*). Accordingly, the enantiomer, acid (+)-**23**, has absolute configuration of (1*R*,4*S*,5*S,*6*R*) (Scheme 12).

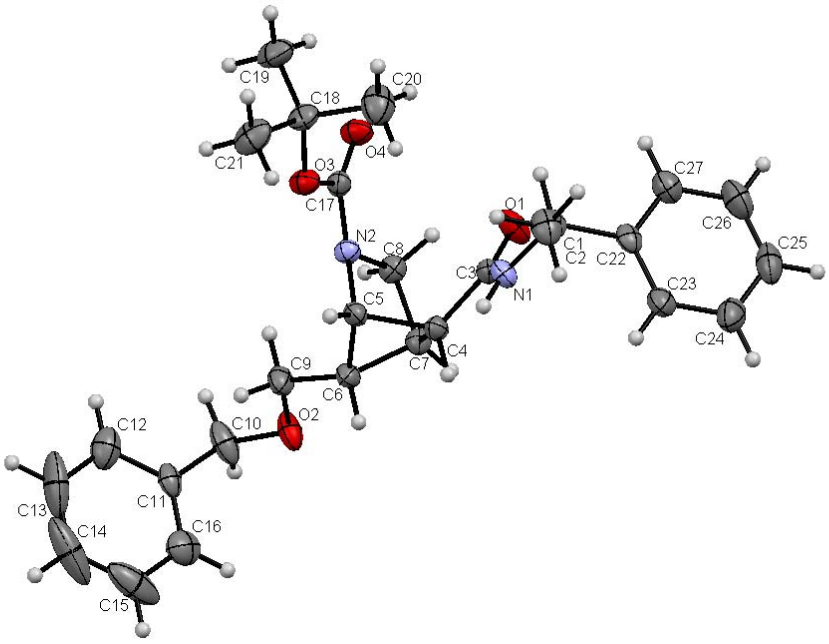
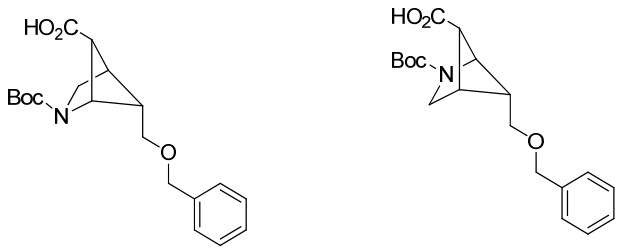


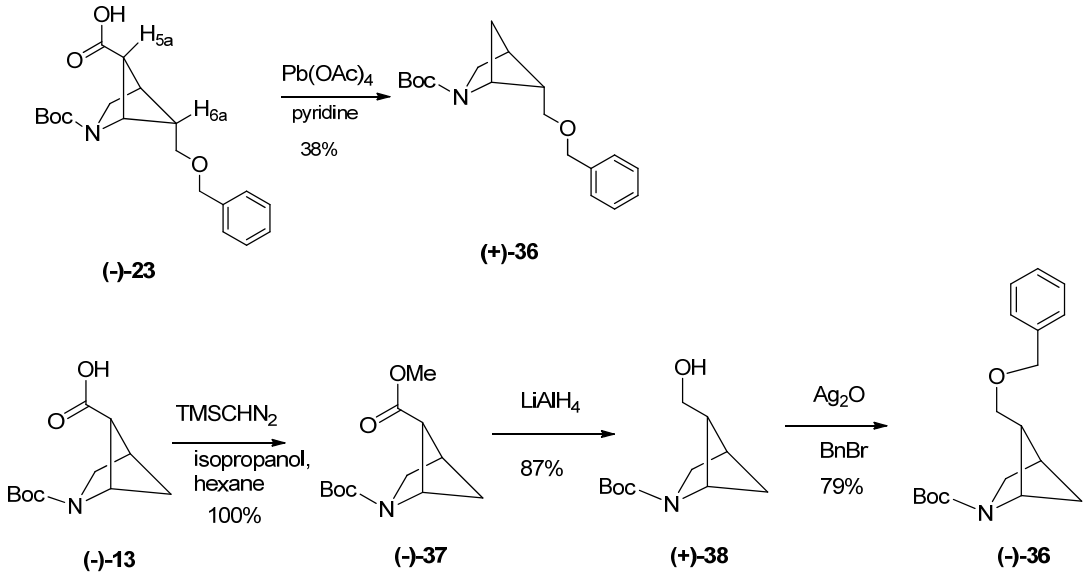
Figure 6. X-ray crystal structure of amide (-)-**34**



(1*S*,4*R*,5*R*,6*S*)-(-)-**23** (1*R*,4*S*,5*S*,6*R*)-(+)-**23**

SCHEME 12

While waiting for the growth of the crystal, we designed another way to determine the absolute configuration of acid (-)-**23**. The acid (-)-**23** reacts with $Pb(OAc)_4$ in cyclohexane to give (+)-**36**.[45] Compound (-)-**36** can also be made from enantiomerically pure (1*S*,4*R*,5*R*)-(-)-**13**.[30] The acid (-)-**13** first was converted to ester (-)-**37**. The ester (-)-**37** was then reduced by LAH to alcohol (+)-**38**, which was protected as its benzyl ether (-)-**36** (Scheme 13). Therefore, the (-)-**23** acid has the absolute configuration of (1*S*,4*R*,5*R*,6*S*).



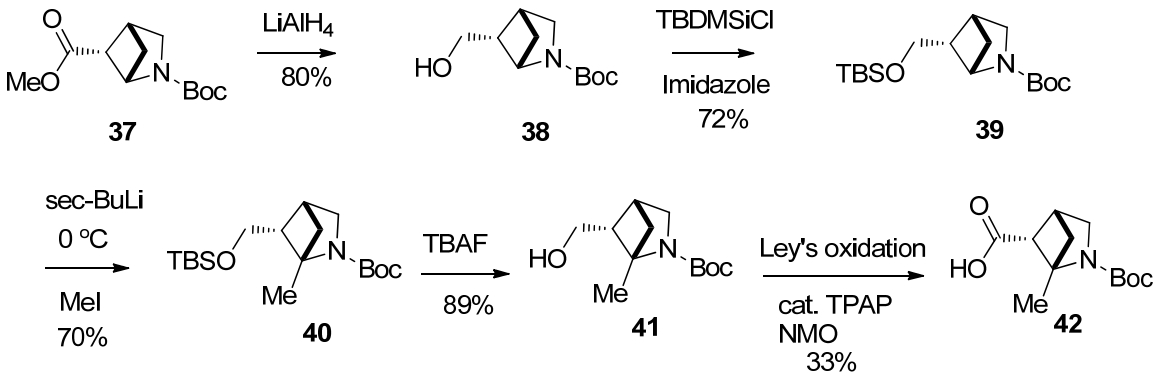
SCHEME 13

CHAPTER 3

SYNTHESIS OF C_1-SUBSTITUTED METHANOPYRROLIDINE β-AMINO ACIDS

3.1 Introduction

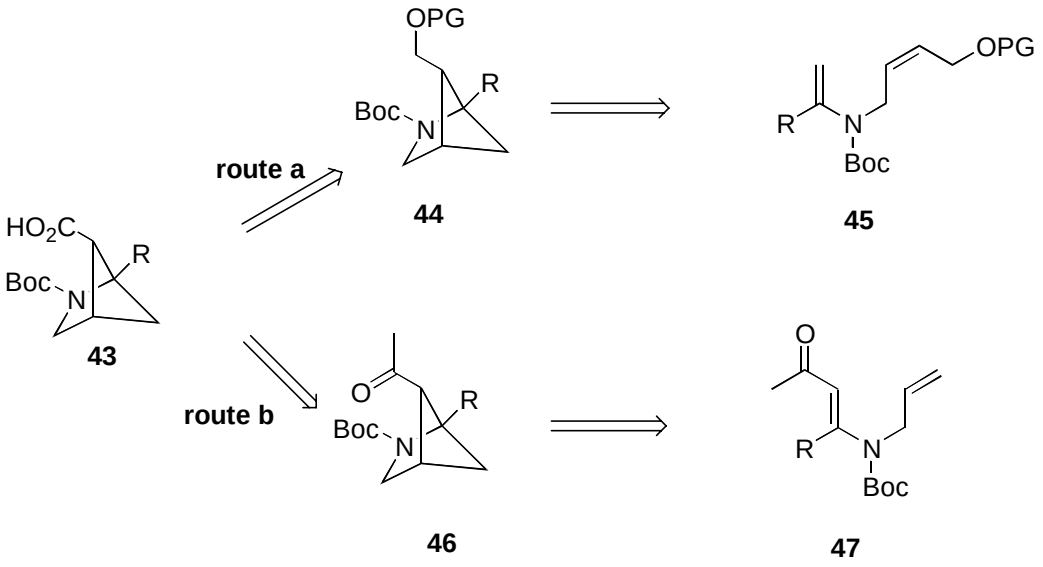
As mentioned in Chapter 1, C_1-substituted MetPyr β-peptide oligomers may give more ordered secondary structures. Dr. Deepa Rapolu and Dr. Nian Liu in our group have developed a synthetic route to prepared 1-R-MetPyr-β-carboxylic acids (Scheme 14). Before introducing the C_1-Me substituent, the enantiomerically pure ester **37** was reduced by LAH to alcohol **38**, which was then protected as its *t*-butyl-dimethylsilyl ether **39**. The C_1-Me of **40** was selectively introduced following Beak's procedure at 0 ^oC with *sec*-BuLi/MeI.[49] After TBAF deprotection, the primary alcohol **41** was mildly oxidized to the carboxylic acid **42** by *Ley's* oxidation (catalytic TPAP/NMO) to avoid decomposition.[50]



SCHEME 14

The route is not applicable for multi-gram synthesis because of the difficulty in scaling by Beak's procedure for α-substitution. A more efficient new route is desired if we want to use the monomers in peptides.

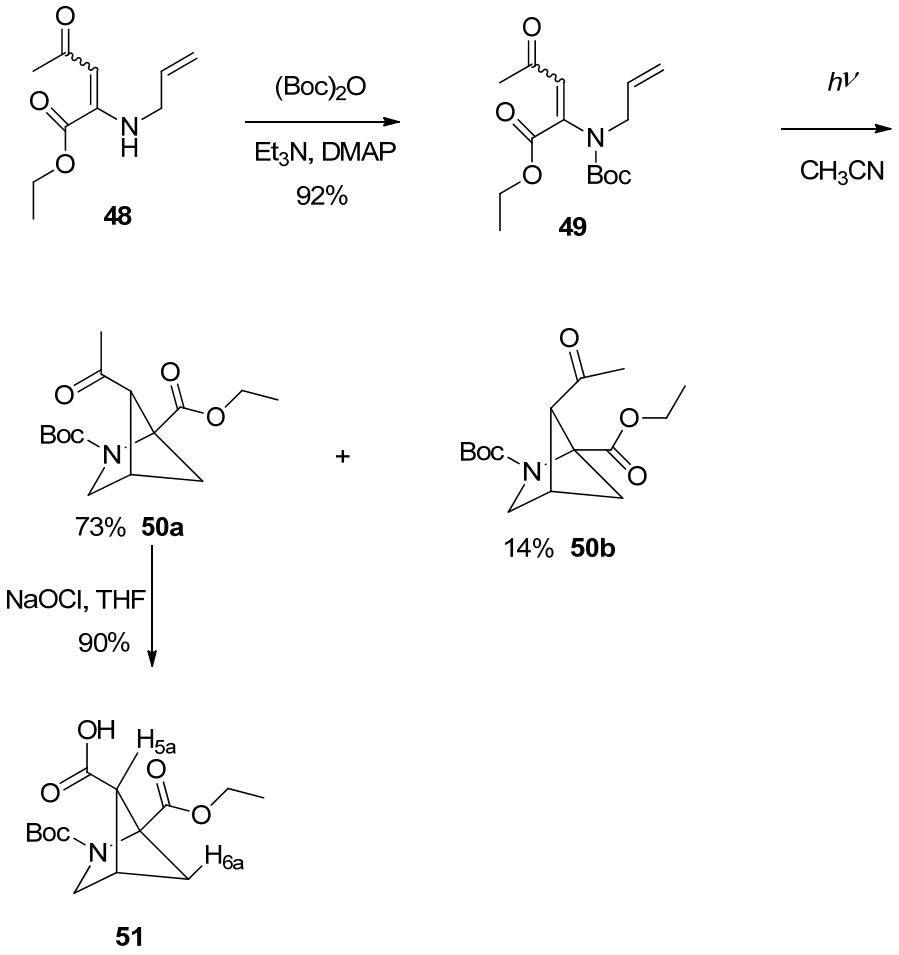
3.2 Retrosynthesis of C_1-substituted methanopyrrolidine β-amino acids



SCHEME 15

The synthesis of 1-substituted methanopyrrolidine β-amino acids **43** can be derived from either **44** (route a) or 5-acetyl **46** by oxidation (route b). The compound **44** could be prepared by [2+2] photochemical cycloaddition of diene **45**. The compound **45** could be prepared by [2+2] photochemical cycloaddition of dienone **47**. Between these two routes, we chose route **b** for the synthesis since it is more convenient by using the allyamine (Scheme 15).

3.3 Synthesis of C_1-ethoxycarbonyl- methanopyrrolidine β-amino acids



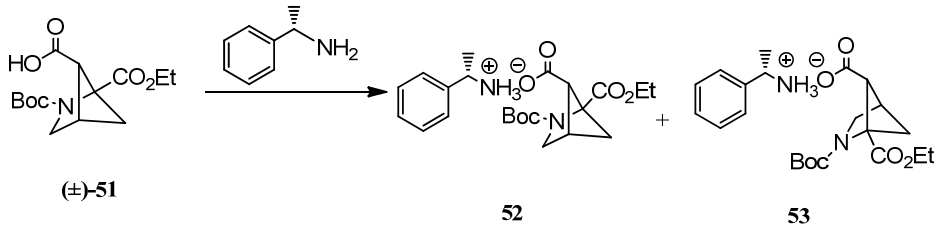
SCHEME 16

Diene **48** was made by reaction of allyl amine with ethyl 2,4-dioxopentanoate (75%).[51] Subsequent reaction of **48** with di-*tert*-butyl carbonate in the presence of DMAP and Et_3N gave **49**. Irradiation of the dienone **49** yielded **50a** and **50b**. The 5-*syn*-acetyl stereochemistry for **50a** is assigned by the absence of long-range coupling between H_{5a} proton at δ 2.79 and H_{6s} proton at δ 1.75. In the 5-*anti*-acetyl isomer **50b**, H_{5s} proton at δ

3.02 is a doublet (J = 8.0 Hz), long range W-plan coupling to H_{6s} proton at δ 2.65. Ketone **50a** was oxidized to afford acid **51** as in Scheme 16.

3.4 Resolution of racemic acid (±)-51

Again, (*S*)-(-)-α-methylbenzylamine was selected as the resolution reagent. The diastereomeric salts from (*S*)-(-)-α-methylbenzylamine and racemic acid **(±)-51** are shown in (Scheme 17). Crystallization and recrystallization efforts, initially carried out on small scale (15 mg) in order to obtain rapid results using multiple solvent systems, are shown in Table 3.



(±)-51 52 53

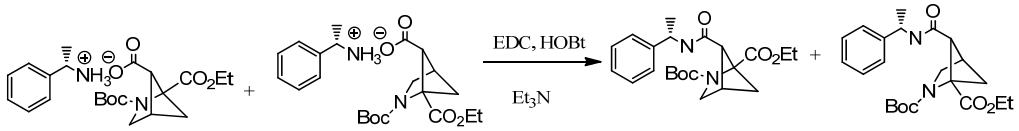
SCHEME 17

TABLE 3. Screening of Small-Scale Crystallization/Recrystallizations of Diastereomeric Salts from Acid Mixture **(±)- 51** and (*S*)-(-)-α-Methylbenzylamine

Entry	Crystallization/Recrystallization Solvent(s)	Properties of Salts Solvent Systems
1	Ethyl Acetate	- Salts precipitate out at room temperature - Completely dissolves in solution at 60 °C
2	Acetone	- Few salts precipitate out at room temperature
3	THF	- Few salts precipitate out at room temperature

We quickly ruled out the possibility of acetone and THF as suitable solvents for resolution. Based upon reasonable recovery yields of salt, ethyl acetate was the candidate for resolution. To find out the enantiomeric purity of resolved acid from ethyl acetate, HPLC was applied to analysis the amides (+)-**54** and (-)-**55**. The coupling reagents (EDC, HOBt, Et_3N) were directly added to a small quantity of the samples from the crystallization. With the help of HPLC (**APPENDIX B**), we easily identified the ratio of the two enantiomers (+)-**54** and (-)-**55** (Scheme 18).

3.5 HPLC analysis of amides (+)-54 and (-)-55



52 53 (+)-54 (-)-55

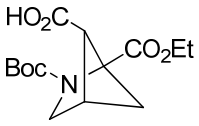
SCHEME 18

Initial crystallization of the diastereomeric salts from racemic acid (±)-**51** and (*S*)-(-)-α-methylbenzylamine in ethyl acetate yielded (+)-acid in 75% ee of purity and one recrystallization of the salts in ethyl acetate enriched (+)-acid in greater than 99% ee with an overall 32.5% yield (**APPENDIX B**). Acid was freed from the diastereomeric salt by EtOAc extraction from acidic aqueous solution and the optical rotation, $[\alpha]_D$, of the free acid was 92.7 (20 °C, c = 0.73, chloroform).

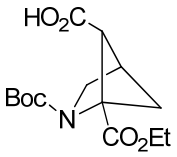
Since (*R*)-(+)-α-methylbenzylamine is also commercially available, the other enantiomer, acid (-)-**51** can be separated from the above mother-liquor residue following the same resolution procedure (35% yield with around 97% ee). Repeating the operation, the two enantiomers were separated in high chiral purity.

3.6 Determination of absolute configuration of resolved acid (+)-51

Slow evaporation of the solution of salt **52** from acid **(+)-51** and (*S*)-(-)-α-methylbenzylamine in THF/hexane provided crystals. The structure of **52** was determined by X-ray crystallography (Figure 7). Since the methylbenzyl amine has an (*S*)-configuration, the absolute configuration of acid (+)-**51** was assigned as (1*R*,4*S*,5*S*). Accordingly, the enantiomer, acid (-)-**51**, has absolute configuration of (1*S*,4*R*,5*R*) (Scheme 19).



(1*R*,4*S*,5*S*)-(+)-**51**



(1*S*,4*R*,5*R*)-(-)-**51**

SCHEME 19

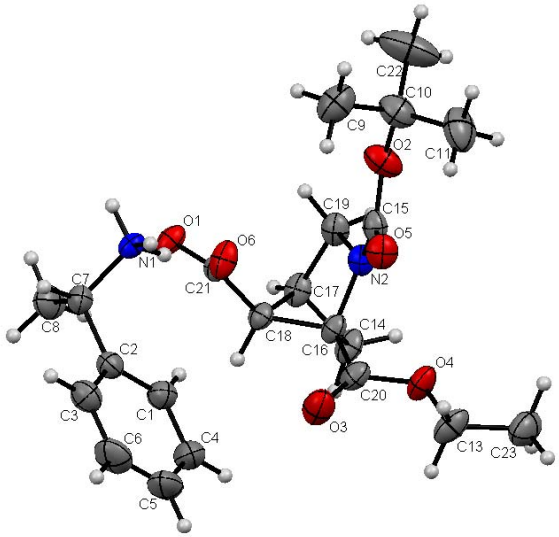
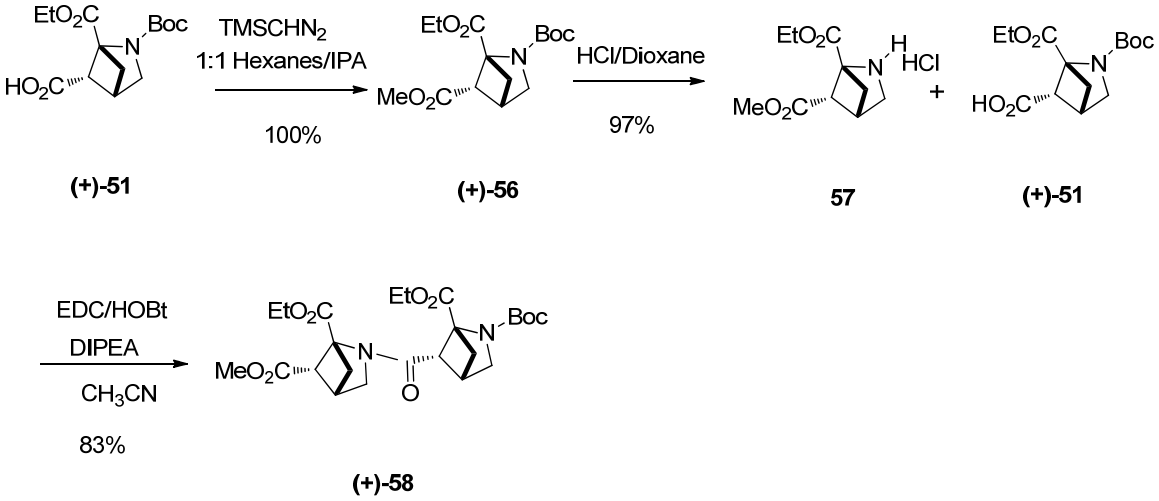


Figure 7. X-ray crystal structure of salt **52** from acid **(+)-51** and (*S*)-(-)-α-methylbenzylamine

3.7 Preparation of dimer (+)-58



SCHEME 20

Acid (+)-**51** was converted to its methyl ester (+)-**56** by reaction with trimethylsilyldiazomethane as shown in Scheme 20. Subsequent *N*-debocylation using HCl/dioxane generated the free amine HCl salt **57**. The free amine was generated from the acid salt **57** *in situ* during subsequent coupling reactions by using the organic base diisopropylethyl amine (DIPEA, or Hunig's base). Coupling in the presence of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC) and 1-hydroxybenzotriazole (HOBt) in acetonitrile yielded 83% of dimer (+)-**58**.

CHAPTER 4

CHARACTERIZATION OF OLIGOMERS

4.1 Introduction

Molecular modeling methods are now routinely used to investigate the structure, dynamics, and thermodynamics of inorganic, biological, and polymeric systems.[52] Molecular mechanics (MM) model uses Newtonian mechanics to model molecular systems. MM models are attractive for the description of molecular equilibrium geometry and conformation. The prototypical MM application is energy minimization. The potential energy of all systems in MM is calculated using force fields. That is, the force field is used as an optimization criterion and the (local) minimum searched by an appropriate algorithm. The main aim of optimization methods is to find the lowest energy conformation of a molecule or to identify a set of low-energy conformers that are in equilibrium with each other. MM can also provide important dynamic parameters, such as energy barriers between different conformers or steepness of a potential energy surface around a local minimum. MM is perhaps the only practical technique for searching conformation space for any but the simplest molecules or for systems with more than a few degrees of conformational freedom. Austin Model 1, or AM1, is a semi-empirical method for the quantum calculation of molecular electronic structure in computational chemistry. It is based on the neglect of differential diatomic overlap integral approximation. Specifically, it is a generalization of the modified neglect of differential diatomic overlap approximation. AM1 may in some cases be suitable for identifying conformational minima and for determining the geometries of these minima. The results

of AM1 calculations are sometimes used as the starting points for parameterizations of force fields in molecular modeling.

Molecular modeling software, Spartan®, is employed to simulate the conformations of methanopyrrolidine β-oligomers. Preliminary MM/AM1 calculations of homooligomers from **7** provided two different secondary structures with *trans*-amide preference. Both indicate some ordered folding behavior. The repeated order is easily noted in nonamers as shown in Figure 8 and Figure 9. The modeling of energy-minimized oligomers indicates that all linkage amides adopt *trans* conformations. Molecular mechanics geometry optimizations of homooligomers from **7** with carbonyl groups oriented to C_4 resulted in a strong preference for $[T4]_n$ (See Page 9) conformations as shown in Figure 8. Molecular mechanics geometry optimizations of homooligomers from **7** with carbonyl groups oriented to C_1 resulted in a strong preference for $[T1]_n$ [from N to C, the *N*-Boc carbonyl bond is *trans*, the carbonyl of unit 1 is directed toward H_1; the *nth* carbonyl bond (amide) is *trans*, the carbonyl of unit n (the last unit is the ester carbonyl) is directed toward H_1] conformations as shown in Figure 9. (Molecular modeling calculation was performed by Dr. Phillip E. Sonnet)

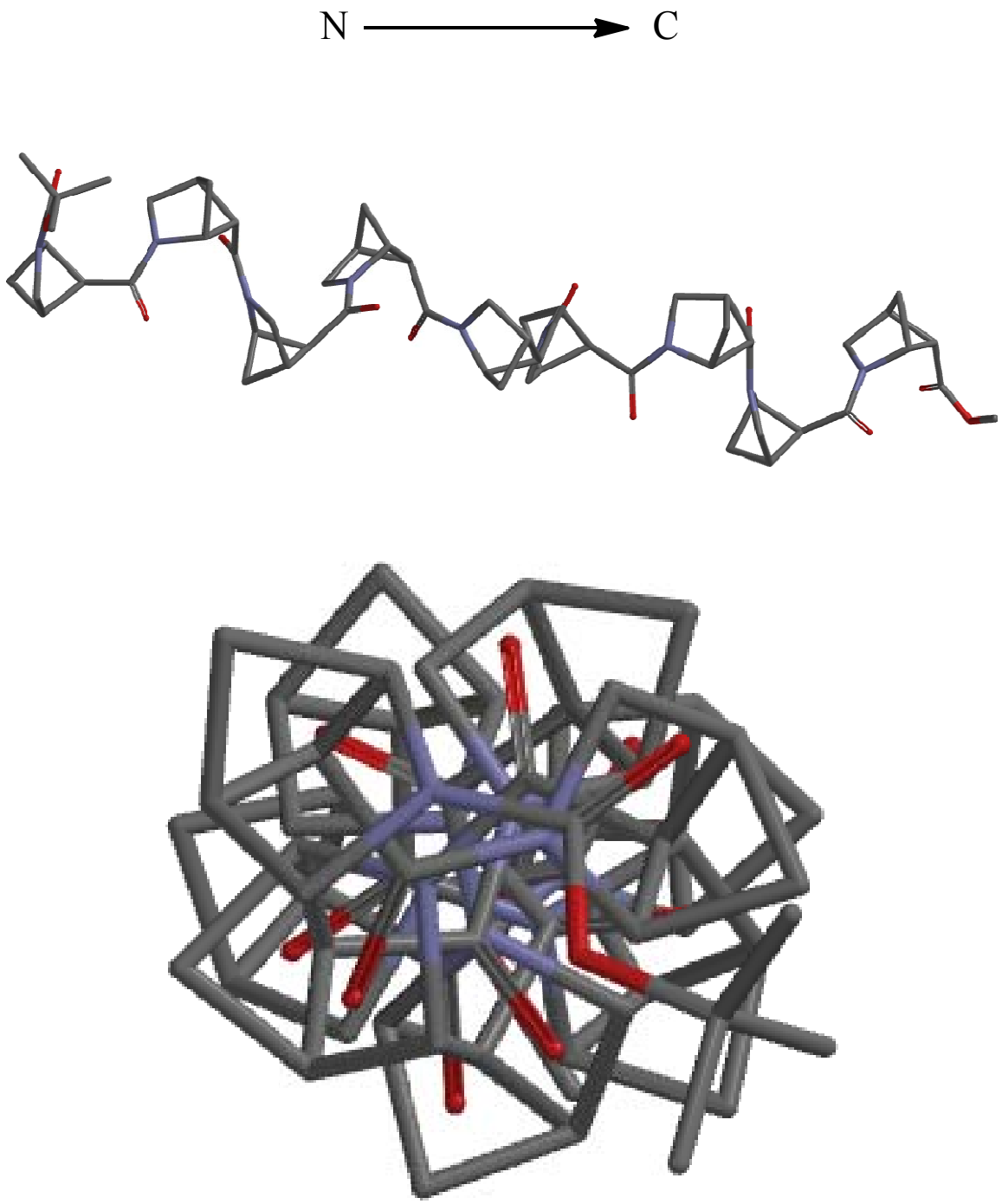


Figure 8. A calculated energy minima structure for a $[T4]_9$ oligomers of (1*S*,4*R*,5*R*)-(-)-1-H-MetPyr-5-carboxylic acids **7**

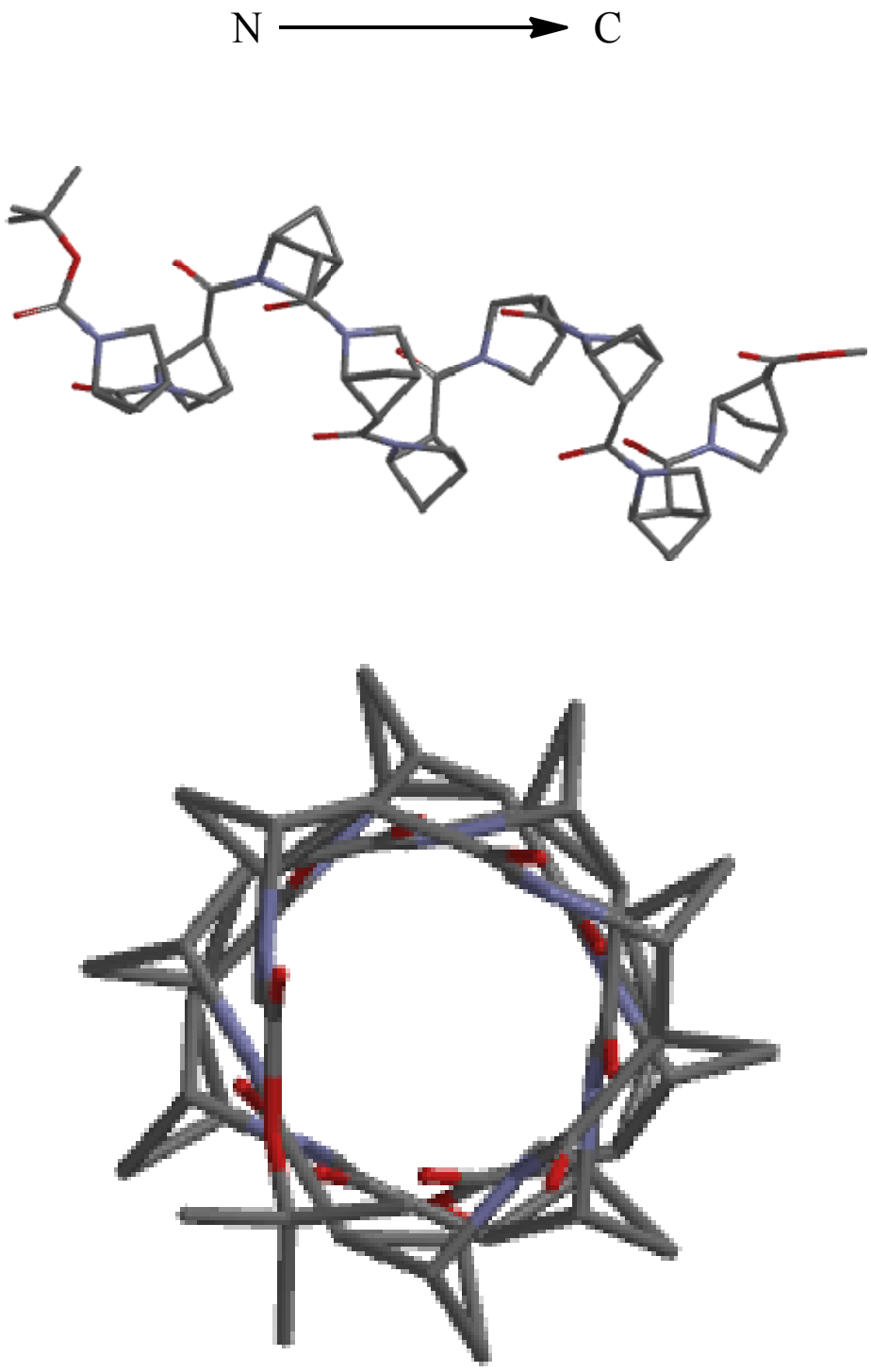
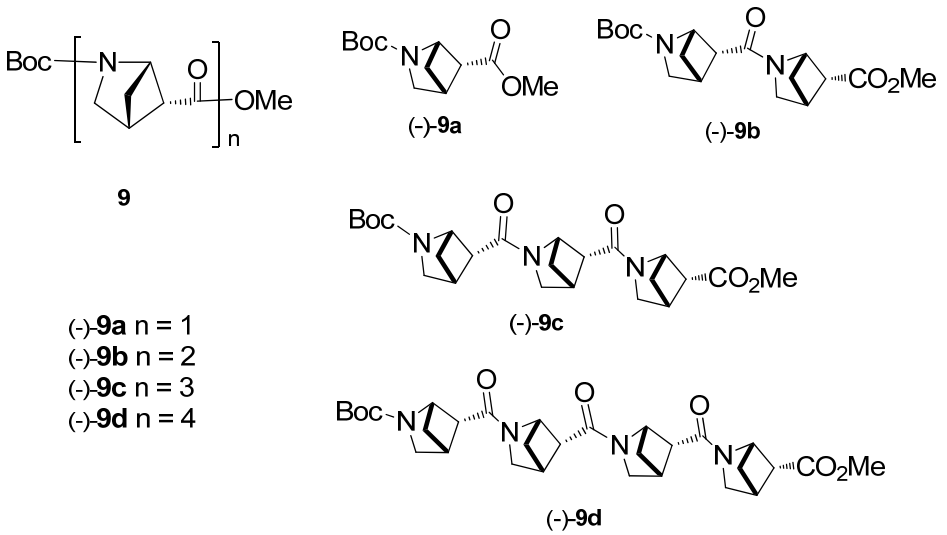


Figure 9. A calculated energy minima structure for a $[T1]_9$ oligomers of (1*S*,4*R*,5*R*)-(-)-1-H-MetPyr-5-carboxylic acids **7**

Dr. Nian Liu from our group succeeded to get crystals of tetramer (-)-**9d** as in Scheme 21 after slow evaporation of the solution in CH_2Cl_2/THF/*n*-heptane, as the monolinic space group C_2, which contains 1 equivalent of CH_2Cl_2 and ½ equivalent of H_2O as solvates (not shown in the X-ray crystal structure) (Figure 10).



SCHEME 21

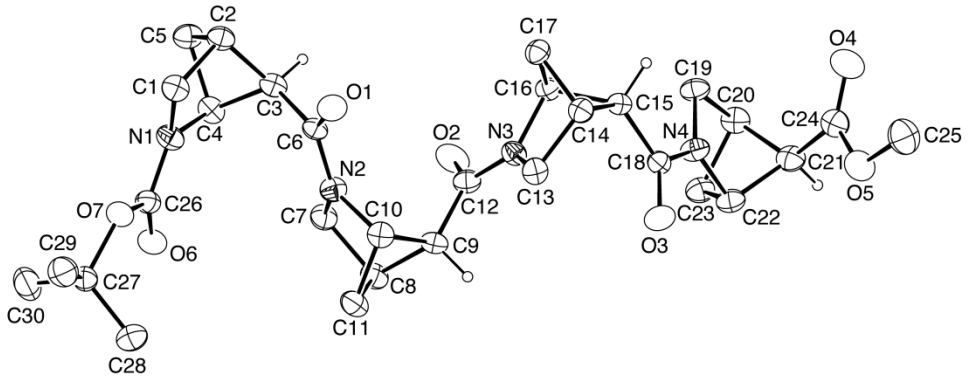


Figure 10. X-ray crystal structure of (-)-**9d** (Copy from Dr. Nian Liu's thesis[30])

The crystal structure of tetramer (-)-**9d** showed that the tertiary carbamate and three tertiary amides are oriented in a *trans* conformation, which confirms the design hypothesis that the methylene bridge can force the amide bond to orient toward the bridgehead C_1 without an additional C_1 substituent. However, the folding pattern results of the crystal structure are inconclusive due to the presence of water hydrogen bonded to the O_2-carbonyl oxygens between neighboring tetrameric units. Dr. Nian Liu also attempted to grow crystals under an anhydrous environment, but the small needle-like crystals that formed were not suitable for X-ray crystallographic studies.

Dr. Matthew Shoulders with Professor Ron Raines at the University of Wisconsin used Dr. Nian Liu's samples perform CD spectroscopy studies of homooligomers and determine the ability of oligomers to adopt an ordered secondary structure. The overlapping of CD spectra from monomer to octamer in methanol clearly shows an ordered conformation is enhanced for longer oligomers because both the intensity of the maximum increases with increasing oligomer length, and the shift to lower wavelength (Figure 11).

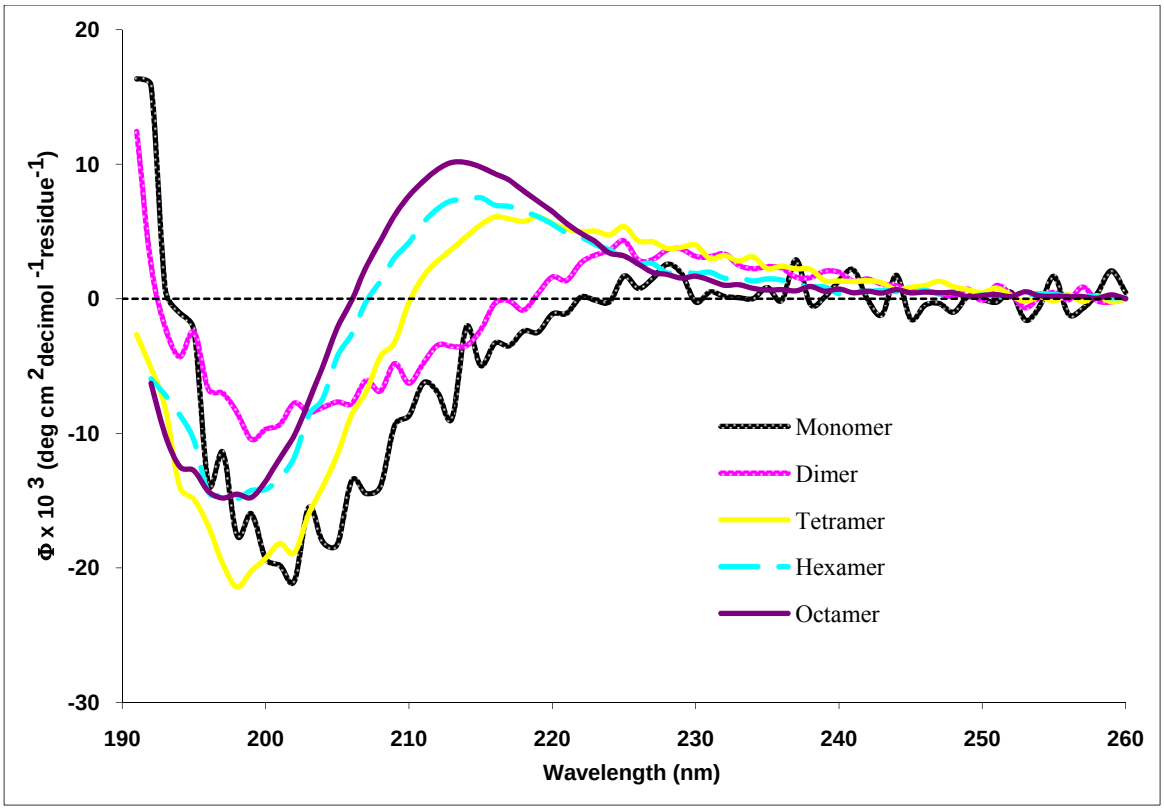


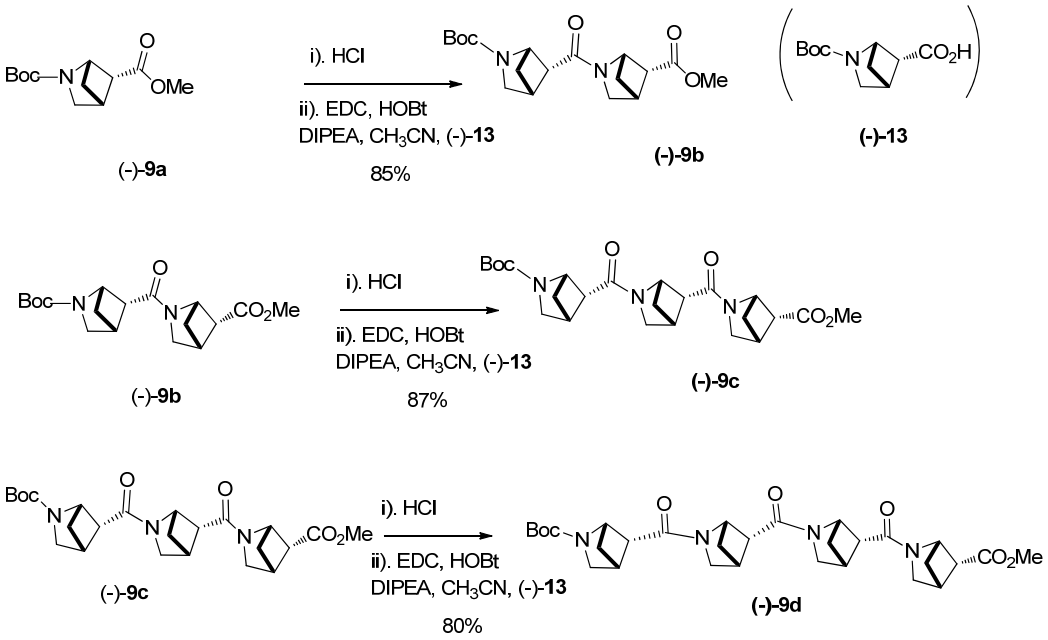
Figure 11. CD spectra of monomer to octamer 100 μM in MeOH (Measurements performed by Dr. Matthew Shoulders, University of Wisconsin, Madison. Copy from Dr. Nian Liu's thesis[30])

The CD data shows there is a secondary structure in these peptides in methanol. Ordered CD signal begins to appear at the tetramer length and the magnitude of the CD curve/oligomer unit is strongest with the octamer. The CD pattern and wavelength of oligomers are different from those reported in literature.[27,28,53,54] Due to lack of similar oligomers as standard references, the exact secondary structure of this series of homooligomers in solution remains unknown.

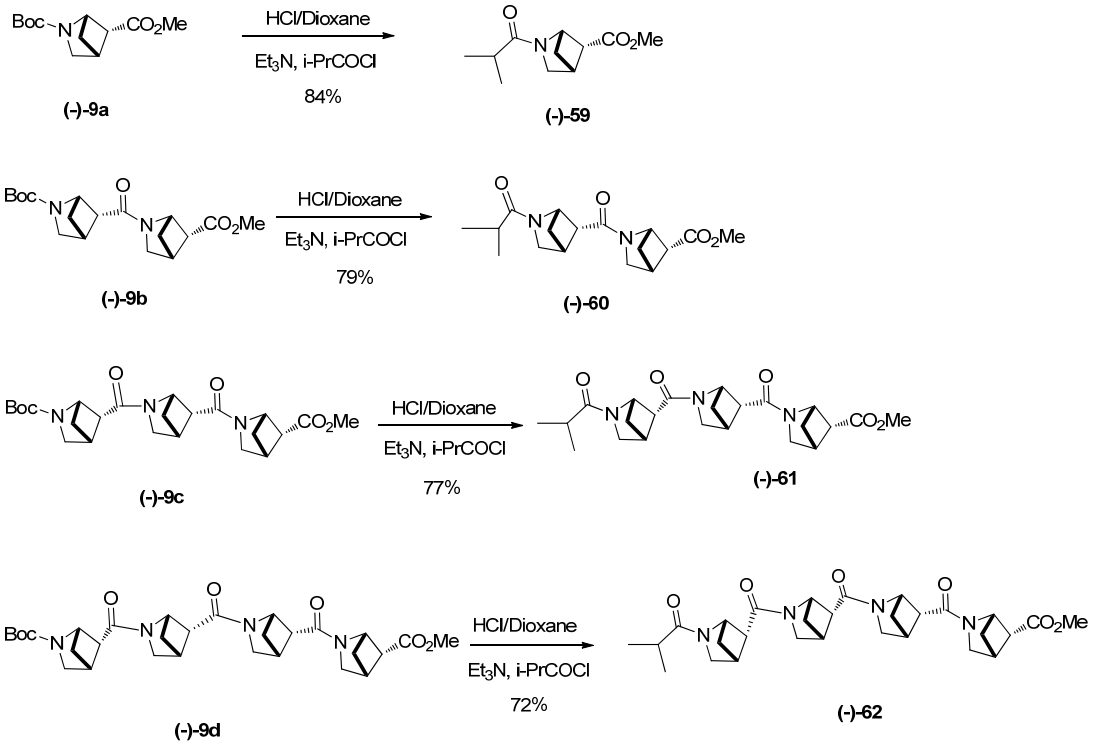
Even though X-ray crystallography gives atomically resolved data of peptides, it can only reveal conformations of proteins or peptides in a solid state. Due to the existence of multiple crystal forms and the presence of solvates, X-ray spectroscopy often leads to mistaken assignments of a secondary structure in solution.[55] Also conformational analysis based on CD data must be regarded as tentative.[56] On the other hand, NMR data can provide insights on folding at specific positions along the β-peptides. Therefore, two-dimensional NMR was applied to examine monomer (-)-**9a,** dimer (-)-**9b**, trimer (-)-**9c** and tetramer (-)-**9d (**Scheme 21).

4.2 Preparation of *N*-isobutyryl-monomer (-)-59, dimer (-)-60, trimer (-)-61 and tetramer (-)-62

Following Dr. Nian Liu's synthetic route, the Boc protected monomer (-)-**9a,** dimer (-)-**9b**, trimer (-)-**9c** and tetramer (-)-**9d** were synthesized (Scheme 22).[30] The proton resonances of these Boc protected short peptides were not well resolved due to multiple rotameric states of the amide. The corresponding *N*-isobutyryl-monomer (-)-**59**, dimer (-)-**60**, trimer (-)-**61** and tetramer (-)-**62** were made by *N*-debocylation using HCl/dioxane and subsequent reaction with isobutyryl chloride in the presence of Et_3N (Scheme 23).



SCHEME 22



SCHEME 23

4.3 Selective NOE and High resolution two-dimensional NMR

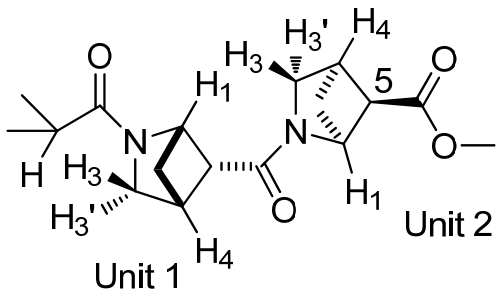
To test the possibility of bias for the amide bond (ω), MetPyr acid (-)-**13** was converted by Me_3SiCHN_2 into its methyl ester (-)-**9a**. This was converted to the *N*-isobutyryl amide (-)-**59** via the aminoester with HCl/dioxane (Scheme 23). NMR analysis indicated two H_1 resonances in a 1:1 ratio consistent with a mixture of the *cis/trans* amides (**APPENDIX A**).

Multiple conformations for dimer (-)-**9b** are possible (Scheme 23). For each of two *N*(sp^2)-*C*(sp^2) and two *C*(sp^3)-*C*(sp^2) bonds there are, respectively, two and three different staggered conformations. In theory there are at least 36 potential local minima. Despite this, the ^1H NMR spectrum of dimer (-)-**9b** indicated two major downfield H_1 resonances. In an effort to clarify the spectral analysis the terminal *N*-Boc group of (-)-**9b** was replaced with an *N*-isobutyryl substituent to give (-)-**60**. Expansion of the downfield H_1 region indicated ten H_1 resonances that individually integrate for at least 3% of the total and that together account for 95% of the H_1 peaks. But, two major H_1 resonances together represent 62% of the H_1 peaks.

The key NMR resonances associated with the major conformation of dimer (-)-**60** are shown in Table 4. The data from Selective NOE experiment (SELNOGP) shows that the isopropyl C<u>H</u>Me_2 is near the methylene $H_{3,3'}$ protons (two protons on Carbon-3) of unit 1. The C_3 carbon associated with these $H_{3,3'}$ protons (by HSQC) is coupled to H_1 of this first unit (by HMBC). Most importantly, the H_1 proton of the first unit has an NOE to the $H_{3,3'}$ protons of the second unit, H(1,i)-H(3,i+1). The H_4 resonances for units 1 and 2 also could be identified by their long range W-plan coupling with H_1 protons for each

unit. We designate this preferred structure as the T4T4 structure (See page 9) with the final ester conformation not determined by NMR (Table 4).

TABLE 4. Key Major NMR Resonances for Dimer (-)-**60**



proton	unit 1 δ	unit 2 δ	Carbon	unit 1 δ	unit 2 δ
CH	2.52[a]	-		31.8	
H_1	5.10[b,c]	4.88[d]	C_1	60.0	60.7
H_3	3.81	3.75	C_3	47.7	46.2
$H_{3'}$	3.37	3.70			
H_4	3.18	3.04	C_4	40.3	41.1

(a) NOE to $H_{3,3'}$ of unit 1. (b) Coupled to C_3 of unit 1(HMBC).

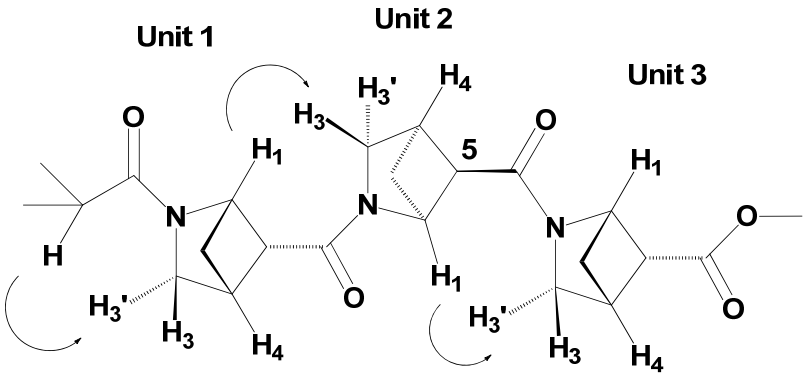
(c) NOE to $H_{3,3'}$ of unit 2. (d) Coupled to C_3 of unit 2.

* Selective NOE and 2D NMR experiments were performed on sample (-)-**60** by Matthew Sender and Dr. Charles DeBrosse.[57]

The *N*-isobutyryl-trimer (-)-**61** exhibited three major H_1 peaks that by integration represent 66% of one major conformation. NMR analysis indicated a *trans*-isobutyryl

group and H_1-$H_{3,3'}$ NOE interactions for H(1,i)-H(3,i+1) and H(1,i+1)-H(3,i+2). This is consistent with a T4T4T4 structure (See page 9), although the ester conformation was not determined (Table 5).

TABLE 5. Major NOEs between Sequentially Nonadjacent Residues Observed for (-)-**61** in $CDCl_3$



proton	unit 1 δ	unit 2 δ	unit3 δ
CH	2.51		
H_1	5.08	4.97	4.88
H_3	3.78[a]	3.76[b]	3.71[c]
$H_{3'}$	3.37	3.72	3.62

(a) NOE with CH; (b) NOE with H_1 in unit 1 at δ 5.08;

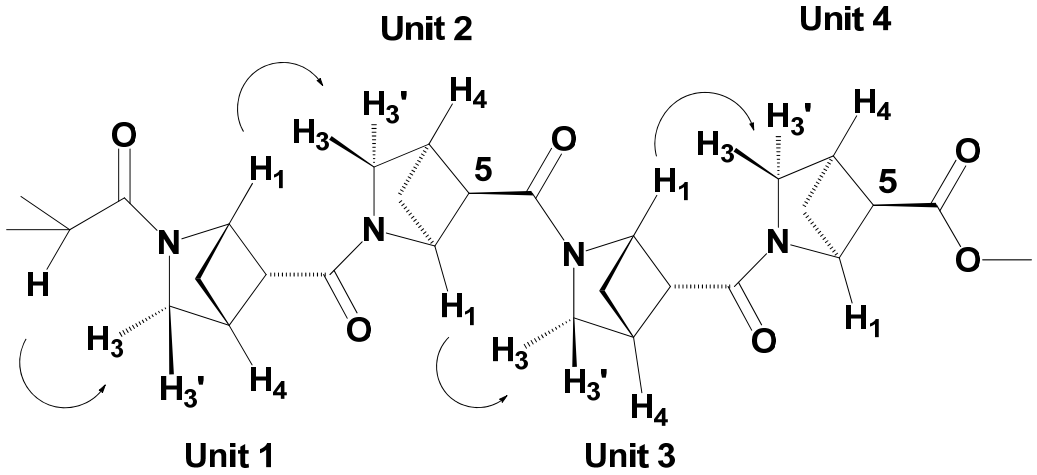
(c) NOE with H_1 in unit 2 at δ 4.97.

* Selective NOE and 2D NMR experiments were performed on sample (-)-**61** by Matthew Sender and Dr. Charles DeBrosse.[57]

The *N*-Boc-tetramer (-)-**9d** formed a crystalline solid suitable for X-ray analysis (Figure 7). Although a water molecule attached to the C_5-carbonyl oxygen (O_2) of unit 2 disrupts the ordered structural arrangement predicted by calculations, the tetramer does have the T4T4T4T4 (See page 9) arrangement that places each carbonyl, including the terminal ester group, so that the alternate dipoles point in opposite directions. In solution, the *N*-Boc-tetramer (-)-**9d** exhibited four major H_1 peaks that represent 72% of a major conformation. Selective NOE analysis of tetramer (-)-**62** (**APPENDIX C**), as for the dimer (-)-**60** and trimer (-)-**61**, is consistent with a T4T4T4T4 conformation with the final ester conformation not determined by NMR (Table 6).[57]

Molecular mechanics geometry optimizations of (-)-**62** indicated a strong preference for $[T4]_n$ conformations. Importantly, in the calculations H_1 of the *nth* residue was in close proximity to $H_{3,3'}$ of the (n+1) residue, and this is consistent with the NOE observations (Table 7). Furthermore, the calculations of torsional angles of ω [O-C (carbonyl group)-N-C_3] in *N*-Boc-tetramer (-)-**9d** (166.98~171.43°) and in *N*-isobutyryl tetramer (-)-**62** (161.61~171.41°) are close to the result from crystal structure of (-)-**9d** (171.4~176.6°) and close to 180° (trans conformation, **APPENDIX D**). The calculations of torsional angles of ψ [C_1-C_5-C(carbonyl group)-N] in *N*-Boc-tetramer (-)-**9d** (56.28~63.96°) and in *N*-isobutyryl tetramer (-)-**62** (59.09~64.49°) are close to the result from crystal structure of (-)-**9d** (45.73~86.17°, around 65±20°, **APPENDIX E**).

TABLE 6. Major NOEs between Sequentially Nonadjacent Residues Observed for (-)-**62** in $CDCl_3$



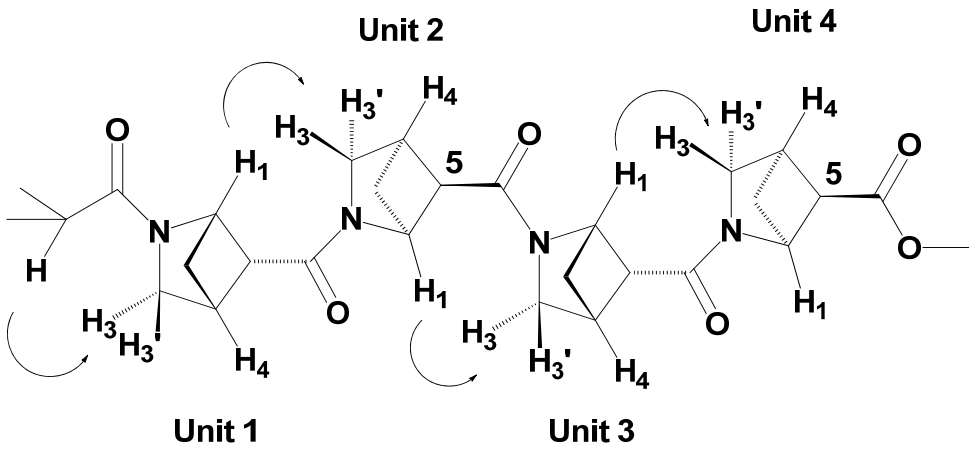
proton	Unit 1 δ	Unit 2 δ	Unit3 δ	Unit4 δ
CH	2.52			
H_1	5.08	4.95	4.97	4.87
H_3	3.77[a]	3.71[b]	3.74[c]	3.70[d]
$H_{3'}$	3.35[a]	3.68[b]	3.57[c]	3.58[d]

(a) NOE with CH; (b) NOE with H_1 in unit 1 at δ 5.08;

(c) NOE with H_1 in unit 2 at δ 4.95; (d) NOE with H_1 in unit 3 at δ 4.97.

*Selective NOE and 2D NMR experiments were performed on sample (-)-**62** by Matthew Sender and Dr. Charles DeBrosse.[57]

TABLE 7. Spartan MMFF Minimized $[T4]_4$ Conformer of Tetramer (-)-**62**

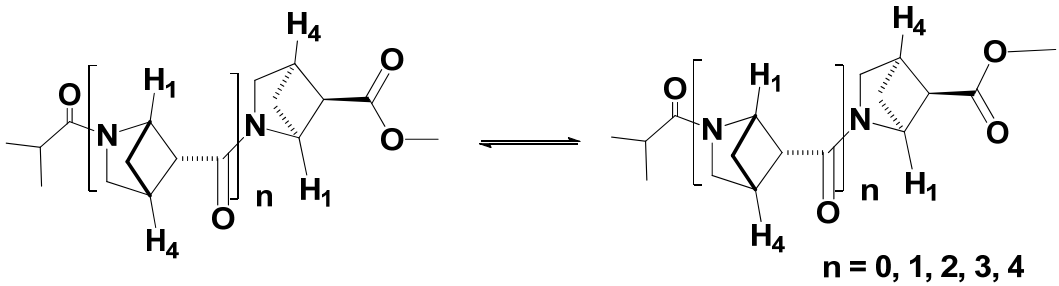


Unit	Distance (Å) H_1 to H_3 ($H_{3'}$) n ⟶ n+1	Distance (Å) H_1 to H_3 n+1 ⟶ n	Torsion angles (°) C_n=O to C_{n+1}=O	Distance (Å) $\underline{C}_n$=O to $\underline{C}_{n+1}$=O
Iso-butyryl	2.236 (2.929)			
1	2.386 (3.005)	4.734	142.91	3.652
2	2.393 (2.967)	4.732	145.97	3.738
3	2.372 (3.010)	4.705	144.46	3.649
4 (CO_2Me)		4.718	145.89	3.772

*n is the Unit n (n = 1, 2, 3, 4); H_3 and $H_{3'}$ are two protons on Carbon-3

The ester conformation (underline 4) could not be determined by NMR. Calculations (Spartan MMFF) indicate [T4]nT4 to be favored over [T4]nT1 for all examples (n=0-4) as in Table 8.

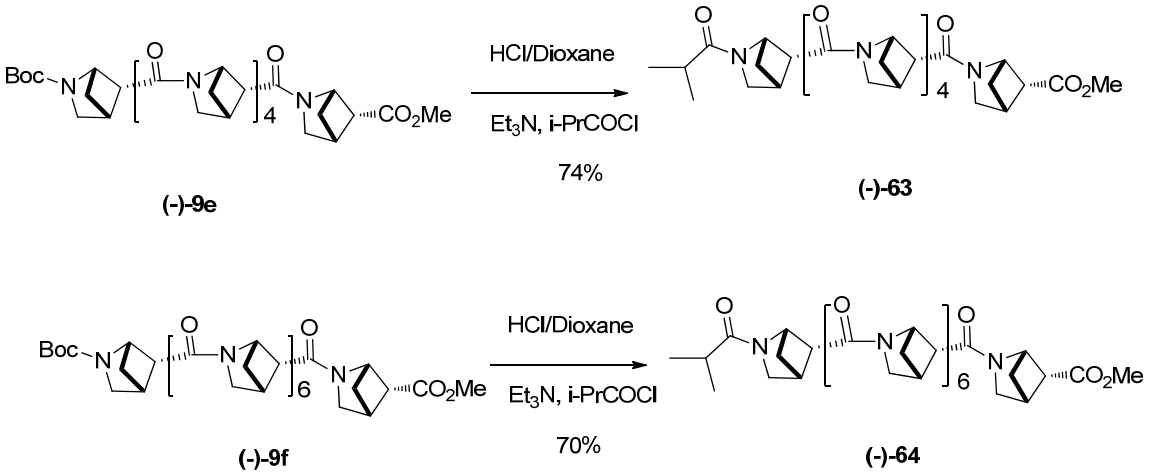
TABLE 8. Spartan MMFF Energy Calculation of [T4]nT4 *versus* [T4]nT1



	Energy (kcal/mol)	
n	[T4]n+1	[T4]nT1
0 [Monomer (-)-**59**]	5.0255	6.6780
1 [Dimer (-)-**60**]	12.1379	13.5775
2 [Trimer (-)-**61**]	19.5693	21.0869
3 [Tetramer (-)-**62**]	34.7165	38.5385
4 (Pentamer)	42.1349	45.9484

In solution, the *N*-Boc-hexamer (-)-**9e** (Dr. Nian Liu's sample)[30] exhibited six major H_1 peaks (down field) that represent 65% of a major conformation, and the *N*-Boc-

octamer (-)-**9f** (Dr. Nian Liu's sample)[30] exhibited eight major H_1 peaks (down field) that represent 63% of a major conformation. The *N*-isobutyryl-hexamer (-)-**63** and *N*-isobutyryl-octamer (-)-**64** were made for NMR analysis by *N*-debocylation using HCl/dioxane and subsequent reaction with isobutyryl chloride in the presence of Et_3N (Scheme 24). The *N*-isobutyryl-hexamer (-)-**63** exhibited six major H_1 peaks (down field) that represent 70% of a major conformation and *N*-isobutyryl-octamer (-)-**64** exhibited eight major H_1 peaks (down field) that represent 68% of a major conformation. The result is consistent with the analysis of the dimer, trimer and tetramer.



SCHEME 24

4.4 Conclusion and future plans

The X-ray analysis of *N*-Boc-$(MetPyr)_4$-CO_2Me (-)-**9d**, the CD data of *N*-Boc-$(MetPyr)_n$-CO_2Me (n = 1, 2, 4, 6, 8) and the 2D NMR analysis of homooligomers of *N*-

isobutyryl-(MetPyr)$_n$-CO_2Me (n = 1-4) indicated that short oligomers constructed from (1*S*,4*R*,5*R*)-(-)-MetPyr-5-acid residues **7** have distinct conformational preferences despite the absence of internal hydrogen bonds. The next step is to prepare C_6 substituted MetPyr acid oligomers with functional groups [modification from acid (+)-**23**] that mimic peptide structures and are amendable to NMR analysis. Introduction of biologically useful groups into C_6 of the monomer residue of the oligomers will most likely maintain the [T4]n structure conformation with functional groups oriented in defined predictable position in space. The preparation and structure determination of C_1 substituted MetPyr acid oligomers will most likely generate a new favored conformer. For example, although *N*-acetyl-1-functional-2-azabicyclo[2.1.1]hexanes such as acid (+)-**51** are restricted to the conformer with the carbonyl directed to the C_1 position,[58] in oligomers of such substrates steric interference will not permit H(1,i)-H(3,i+1) to orient in the $[T4]_n$ conformations observed in the present study.

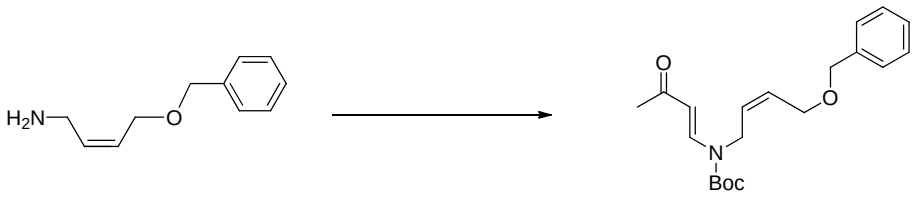
CHAPTER 5

EXPERIMENTAL

General Procedures: Thin layer chromatography was performed on precoated plates of silica gel GF 250 microns (Analtec, Inc.). Column Chromatography was performed on silica gel, Merck grade 60 (230-400 mesh) purchased from Aldrich Chemical Co. High Pressure Liquid Chromatography (HPLC) was performed on Agilent 1100 system equipped with vacuum degasser, quaternary pump, autosampler, a variable-wavelength UV detector, and reverse phase HPLC columns. High-resolution mass spectra were collected at Merck. X-Ray crystallography was performed by Clifton Hamilton and Dr. Michael Zdilla at Department of Chemistry, Temple University. Circular dichroism (CD) was performed by Dr. Matthew Shoulders at the University of Wisconsin, Madison. Optical rotation was recorded by Perkin Elmer Polarimetry Model 341 in 1 dm unit cell with Sodium D line. Chemical shifts in ^{1}H and ^{13}C NMR are reported in parts per million (δ) values and were recorded on a 300, 400 or 500 MHz spectrometer. ^{1}H NMR splitting patterns with observed first-order coupling are designated as singlet (s), doublet (d), triplet (t), or quartet (q). Hydrogen nuclear magnetic resonance chemical shifts are reported in ppm (δ) relative to the line of the $CDCl_3$ singlet (δ 7.26). Carbon nuclear magnetic resonance chemical shifts are reported in ppm (δ) relative to the central line of the $CDCl_3$ triplet (δ 77.0). Coupling constants (J) in ^{1}H NMR spectra are reported in Hertz. One dimensional NOE (Selective NOE: SELNOGP) and Two dimensional NMR experiments (COSY, HMBC, HSQC) were performed by Matthew Sender and Dr. Charles DeBrosse at the Department of Chemistry, Temple University.[57] Only positive

NOEs were observed (signal is larger than three times of noise). Molecular modeling was performed by Dr. Phillip Sonnet. Melting points were recorded on a Uni-melt apparatus and were uncorrected. Reagent chemicals were obtained from commercial suppliers and chemical grade solvents were used without further purification.

***tert*-Butyl ((*Z*)-4-(benzyloxy)but-2-en-1-yl)((*E*)-3-oxobut-1-en-1-yl)carbamate (21a)**

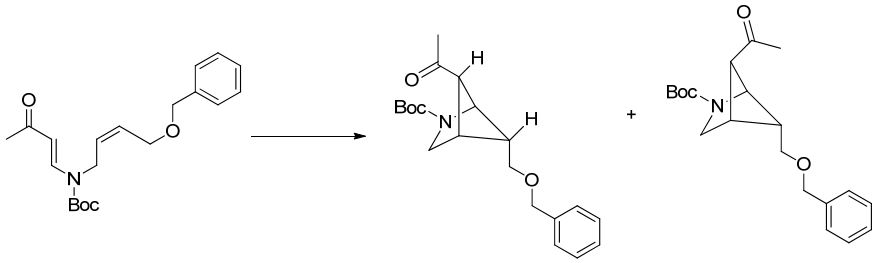


20 **21a**

Following a modified literature procedure,[43f] to a solution of 4-(benzyloxy)-2(*Z*)-methylbut-2-en-1-amine **20**[46] (177 mg, 1.0 mmol) in MeOH (10 mL) was added 3-butyne-2-one (78.3 μL, 1.0 mmol) at 0 °C dropwise. The reaction mixture was stirred for 1 hr at 0 °C, then allowed to room temperature and then continued stirring for 12 h. After starting material disappearance on TLC, solvent was removed *in vacuo* and to the residue in DCM (10.0 ml) at 0 °C was added Et_3N (200 mg, 2.0 mmol), DMAP (10.0 mg) and di-*t*-butyl carbonate (259 mg, 1.2 mmol). The reaction mixture was stirred for 1 hr at 0 °C, then warmed to room temperature, and then continued stirring for 12 h. Solvent was removed *in vacuo* and the residue was chromatographed (gradient, 1:4 ethyl acetate/ hexanes) to afford titled compound **21a** (241 mg, 70%) as an orange color oil. R_f = 0.33 (1:4 ethyl acetate/ hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.10 (d, J = 16.0 Hz, 1H), 7.36~7.26 (m, 5H, Ph), 5.80 (m, 1H), 5.53 (d, J = 16.0 Hz, 1H), 5.43 (m, 1H), 4.55 (s, 2H, CH_2Ph), 4.25 (d, J = 4.0 Hz, 2H), 4.16 (d, J = 4.0 Hz, 2H), 2.20 (s, 3H, Me), 1.52 (s,

9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 197.0, 151.8, 142.0, 137.9, 129.3, 128.4, 127.7, 126.8, 108.2, 83.5, 72.6, 65.9, 42.0, 28.3, 27.9, 27.3. HRMS: *m/z* found 368.1820, calcd. for $C_{20}H_{27}NO_4Na$ (M + Na) 368.1833.

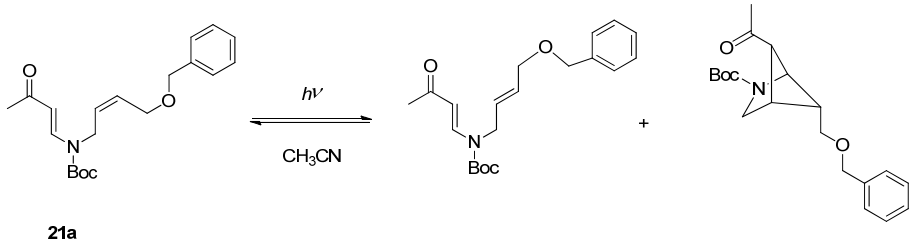
tert-Butyl-5-_syn_-acetyl-6-_syn_-((benzyloxy)methyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (22a) and _tert_-Butyl-5-_anti_-acetyl-6-_syn_-((benzyloxy)methyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (22b)



Following a modified literature procedure,[43f] a solution of **21a** (345 mg, 1.0 mmol) in acetonitrile (100 mL) was bubbled with argon for 20 min and then irradiated with a UV lamp (Hanovia, 450 watts) in a photoreactor at ambient temperature circulated with cooling water for 18 hours until no more starting material was detected by TLC. Acetonitrile was removed *in vacuo,* the mixture was chromatographed (2:1 hexane/ethyl acetate) to afford 200 mg (58%) of **22a** as a colorless oil. R_f = 0.29 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35~7.27 (m, 5H, Ph), 4.68 and 4.56 (2dt, *J* = 6.8, 1.7 Hz, 1H, H_1), 4.45 (m, 2H, CH_2Ph), 3.35 and 3.30 (2d, *J* = 9.4 Hz, 1H, H_3), 3.25 (m, 2H, CH_2O), 3.09 and 3.00 (2d, *J*= 9.4 Hz, 1H, H_3), 3.01 (d, *J* = 6.6 Hz, 1H, H_4), 2.53 (br, 1H, H_5, NOE with to H_6), 2.15 (m,1H, H_6), 2.08 and 2.06 (2s, 3H, CH_3), 1.46 and 1.41 (2s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 206.3, 205.4, 156.2, 155.9, 138.1,

128.5, 128.3, 127.8, 127.7, 127.6, 79.8, 73.5, 64.7, 63.1, 56.1, 44.9, 43.7, 41.6, 28.5, 27.0. HRMS: *m/z* found 368.1844, calcd. for $C_{20}H_{27}NO_4Na$ (M + Na) 368.1833. In addition, 52 mg (15%) of **22b** as oil was also isolated. R_f = 0.31 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.26 (m, 5H, Ph), 4.53 (m, 3H, H_1 and CH_2Ph), 3.66 (d, J = 7.2 Hz, 1H) 3.45~3.15 (m, 3H, OCH_2), 3.11 (m, 1H), 2.93 (br, 1H), 2.64 (m, 1H), 2.06 (s, 3H, $COCH_3$), 1.44 (s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.4, 205.3, 156.9, 138.0, 128.4, 128.3, 127.7, 127.6, 79.7, 73.3, 3.1, 67.13, 65.48, 62.8, 62.2, 55.4, 50.0, 48.2, 42.8, 42.0, 28.4, 27.8. HRMS *m/z* found 346.2115, calcd. for $C_{20}H_{28}NO_4$ (M + H) 346.2013.

***tert*-Butyl ((*E*)-4-(benzyloxy)but-2-en-1-yl)((*E*)-3-oxobut-1-en-1-yl)carbamate (21b)**

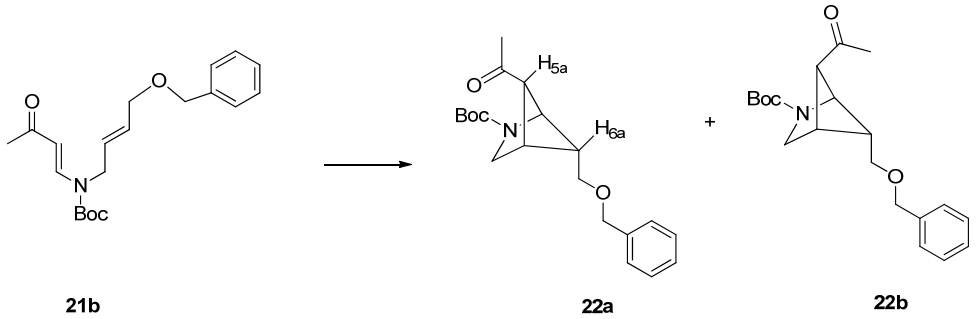


21a **21b** **22a**

A solution of **21a** (1.72 g, 5.0 mmol) in acetonitrile (500 mL) was bubbled with argon for 20 min and then irradiated with a UV lamp (Hanovia, 450 watts) in a photoreactor at ambient temperature circulated with cooling water for around 12 hours. Acetonitrile was removed *in vacuo,* the mixture was chromatographed (2:1 hexane/ethyl acetate) to afford 0.64 g (37 %) of **22a** as an oil. R_f = 0.29 (2:1 hexane/ethyl acetate), and 0.58 g (34%) of **21b** as an oil. R_f = 0.34 (1:4 ethyl acetate/ hexanes). (Note: No effort was made to separate a small amount of **22b** mixed with **22a** in this early experiment.) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.16 (d, J = 16.0 Hz, 1H), 7.39~7.26 (m, 5H, Ph), 5.73 (m,

2H), 5.57 (d, J = 16.0 Hz, 1H), 4.52 (s, 2H, CH_2Ph), 4.20 (d, J = 4.0 Hz, 2H), 4.16 (d, J = 4.0 Hz, 2H), 2.25 (s, 3H, Me), 1.56 (s, 9H).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 197.5, 175.5, 155.7, 152.0. 142.4, 138.2, 129.7, 128.4, 127.7, 125.5, 108.7, 83.5, 72.3, 65.9, 45.7, 28.4, 27.9, 27.1. HRMS: *m/z* found 346.2021, calcd. for $C_{20}H_{28}NO_4$ (M + H) 346.2013.

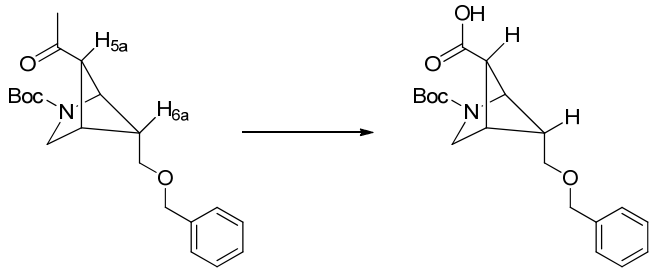
tert-Butyl-5-_syn_-acetyl-6-_syn_-((benzyloxy)methyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (22a) and _tert_-Butyl-5-_anti_-acetyl-6-_syn_-((benzyloxy)methyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (22b)



21b **22a** **22b**

A solution of **21b** (580 mg, 1.68 mmol) in acetonitrile (170 mL) was bubbled with argon for 20 min and then irradiated with a UV lamp (Hanovia, 450 watts) in a photoreactor at ambient temperature circulated with cooling water for 18 hours until no more starting material was detected by TLC. Acetonitrile was removed *in vacuo,* the mixture was chromatographed (2:1 hexane/ethyl acetate) to afford 341 mg (59%) of **22a** as an oil, and 87 mg (15%) of **22b** as an oil. [An independently prepared mixture of **21a**/**21b** from *cis*/*trans* **19**, afforded the same mixture of **22a**/**22b**.--see Page 52]

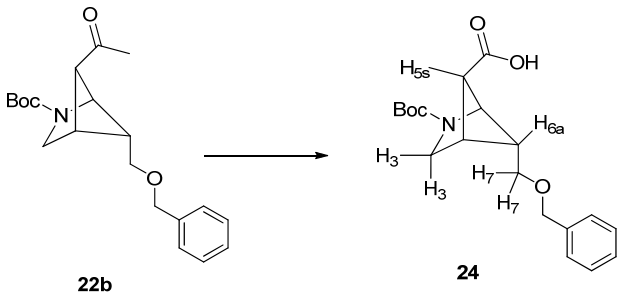
(±)-6-_syn_-((Benzyloxy)methyl)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.1.1]hexane-5-_syn_-carboxylic acid (23)



22a (±)-**23**

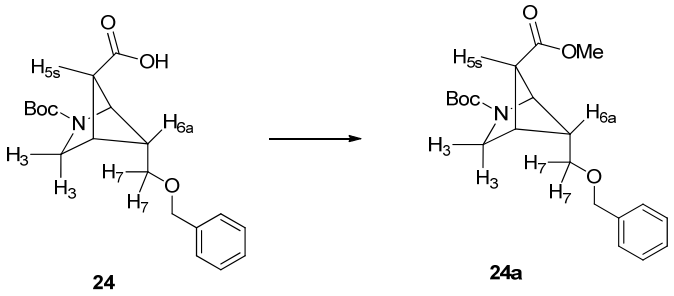
A solution of sodium hypochlorite aqueous (available chlorine is more than 10%, 7 mL) was added to **22a** (345 mg, 1.0 mmol) in THF (4 mL). The reaction mixture was stirred vigorously at room temperature for 5 min. The reaction solution was extracted with methyl *t*-butyl ether (2 x 15 mL) to remove any lipophilic impurities. The aqueous solution was adjusted to pH 3.0 with 1.0 N HCl and extracted with EtOAc (2 x 15 mL). The combined ethyl acetate extractions were washed with water (2 x 10 mL) and brine (10 mL) dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 315 mg (90%) of solid (±)-**23**. Mp = 104-105 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.29~7.19 (m, 5H, Ph), 6.0~4.5 (br, 1H, COOH), 4.53 and 4.43 (2br,1H, H_1) 4.44~4.32 (m, 2H, CH_2Ph), 3.42 (2d, J = 9.3 Hz, 1H, H_3), 3.22~3.11 (m, 2H, OCH_2), 3.06 (2d, J = 9.3 Hz, 1H, H_3), 2.96 (m, 1H, H_4), 2.58 (br, 1H, H_5, NOE with to H_6), 2.13 (m, 1H, H_6), 1.37 (s, 9H, Boc).^{13}C NMR($CDCl_3$, 100 MHz) δ: 176.6, 173.1 172.9, 156.8, 156.5, 137.9, 128.5, 128.5, 127.8, 127.7, 80.1, 80.0, 73.4, 73.3, 64.7, 64.4, 63.1, 62.4, 50.5, 48.5, 47.8, 45.3, 45.2, 43.8, 42.5, 42.3, 28.4, 20.8. HRMS: *m/z* found 370.1625, calcd. for $C_{19}H_{25}NO_5Na$ (M + Na) 370.1633.

6-*syn*-((Benzyloxy)methyl)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.1.1]hexane-5-*anti*-carboxylic acid (24)



A sodium hypochlorite aqueous solution (available chlorine is more than 10%, 7 mL) was added to **22b** (172 mg, 0.5 mmol) in THF (2 mL). The reaction mixture was stirred at room temperature for 5 min. The reaction solution was extracted with methyl *t*-butyl ether (2 x 10 mL) to remove any lipophilic impurities. The aqueous solution was adjusted to pH 3.0 with 1.0 N HCl and extracted with EtOAc (2 x 10 mL). The combined ethyl acetate extractions were washed with water (2 x 10 mL) and brine (10 mL, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 159 mg (91%) of solid **24**. Mp = 116-117 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.0~10.0 (br, 1H, COOH), 7.28~7.19 (m, 5H, Ph), 4.51~4.35 (m, 3H, H_1 and CH_2Ph), 3.24 (m, 4H), 3.01 and 2.99 (2d, J = 6.8 Hz, 1H, H_4), 2.85 (br, 1H,), 2.54 (br,1H), 1.38 (s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 175.3, 156.0, 155.7, 137.9, 126.5, 128.5, 128.3, 127.8, 127.7, 80.3, 73.4, 65.5, 63.9, 62.8, 60.5, 53.7, 46.8, 46.4, 46.1, 43.1, 28.4. HRMS: *m/z* found 370.1621, calcd. for $C_{19}H_{25}NO_5Na$ (M + Na) 370.1633.

6-*syn*-((Benzyloxy)methyl)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.1.1]hexane-5-*anti*-carboxylmethyl ester (24a)



Acid **24** (49 mg, 0.14 mmol) was dissolved in isopropanol (10 mL) and hexane (10 mL) and trimethylsilyldiazomethane (0.075 mL, 0.15 mmol, 2 M in hexanes) was added. The mixture was stirred for 40 min at room temperature. Solvent was removed *in vacuo* to afford 51 mg (100%) of **24a** as colorless oil. R_f = 0.32 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36~7.29 (m, 5H, Ph), 4.53 (br, 1H, H_1), 4.48 and 4.41 (2d, J = 12.0 Hz, 2H, CH_2Ph), 3.72 (s, OMe), 3.32~3.19 (m, 4H), 3.06 and 3.05 (2d, J = 6.6 Hz, 1H, H_4), 2.81 (br, 1H, H_6), 2.54 (br,1H, H_5), 1.45 (s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0, 138.1, 128.4, 127.7, 127.7, 79.9, 73.4, 65.6, 63.8, 53.8, 52.1, 46.8, 46.4, 43.0, 28.4. HRMS: *m/z* found 362.1963, calcd. for $C_{19}H_{25}NO_5Na$ (M + H) 362.1962.

Resolution of (±)-6-*syn*-((Benzyloxy)methyl)-2-(tert-butoxycarbonyl)-2-azabicyclo[2.1.1]hexane-5-*syn*-carboxylic acid (23)

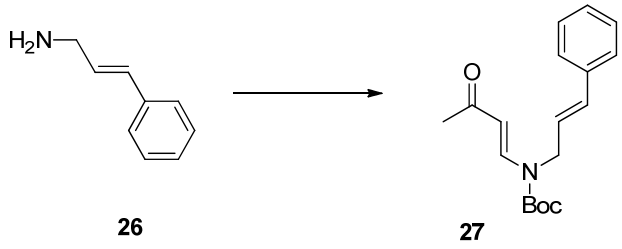
(*S*)-(-)-α-Methylbenzylamine (0.50 g, 4.1 mmol) was slowly added to acid (±)-**23** (1.53 g, 4.4 mmol) in acetone (15.0 mL) solution. After stirring for 30 minutes at room temperature, white slurry was gradually formed. The slurry was stirred for an additional 6 hours at room temperature. The mixture was heated to reflux until all solid was dissolved. The solution was slowly cooled to room temperature and the salt gradually precipitated

out. The salt was collected in a Busch funnel by vacuum filtration. The yield of the first crop was 0.89 g (43%, 1.9 mmol, 79% ee). Mp = 163-167 °C.

The salt (0.89 g, 1.9 mmol) from the last step was taken up in 10.0 mL of acetone. The mixture was heated to reflux until all solid was dissolved. The solution was slowly cooled to room temperature and the salt gradually precipitated out. The slurry was stirred at room temperature for 2 hours and the salt was collected by vacuum filtration. After drying, 0.66 g (1.41 mmol) of salt **32** (98% ee, **APPENDIX B**, Mp = 165-166 °C) was obtained and the yield was 32% based on 5,6-di-*syn*-acid (±)-**23**.

Recrystallized salt (0.66 g, 1.41 mmol) was partitioned between EtOAc (5.0 mL) and water (5.0 mL). The mixture was adjusted to pH 2.5 with 1.0 N HCl. The EtOAc layer was separated, and the aqueous layer was extracted with 5.0 mL of EtOAc. The combined EtOAc solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield free acid (-)-**23** (0.47 g, 1.34 mmol, 30%) as white solid. Mp = 104-105 °C. Optical rotation $[\alpha]_D$ of (-)-acid **23**: -10.7 (20 °C, c= 2.3, chloroform).

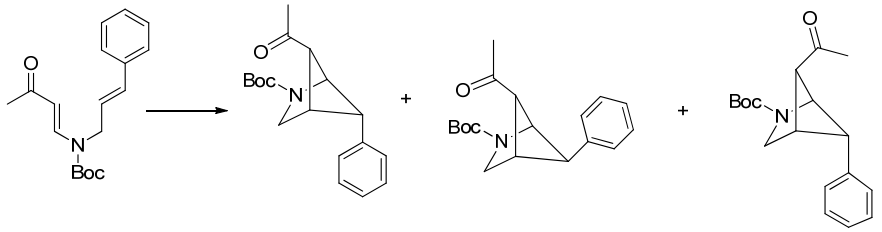
***tert*-Butyl cinnamyl((*E*)-3-oxobut-1-en-1-yl)carbamate (27)**



26 **27**

Following a modified literature procedure,[43f] to a solution of **26** (133 mg, 1.0 mmol) in MeOH (5 mL) was added 3-butyne-2-one (94.0 μL, 1.2 mmol) at 0 ^{o}C dropwise. The reaction mixture was stirred for 1 hr at 0 ^{o}C, then allowed to warm to room temperature and then continued stirring for 12 h. Solvent was removed *in vacuo* and to the residue in DCM (20.0 ml) at 0 ^{o}C was added Et_3N (200 mg, 2.0 mmol), DMAP (10 mg) and di-*tert*-butyldicarbonate (326 mg, 1.5 mmol). The reaction mixture was stirred for 1 hr at 0 ^{o}C, then allowed to warm to room temperature with continued stirring for 12 h. Solvent was removed *in vacuo* and the residue was chromatographed (gradient, 1:4 ethyl acetate/ hexanes) to afford the titled compound (225 mg, 75%) as an orange color oil. R_f = 0.26 (1:4 ethyl acetate/ hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 14.5 Hz, 1H), 7.27 (m, 5H, Ph), 6.46 (d, J = 15.9 Hz, 1H), 6.07 (td, J = 15.9, 5.8 Hz, 1H), 5.60 (d, J = 14.5 Hz, 1H), 4.29 (br d, J = 5.5 Hz, 2H), 2.21 (s, 3H, $COCH_3$), 1.52 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.1, 151.9, 142.2, 136.1, 132.5, 128.5, 127.8, 126.3, 122.2, 108.6, 83.4, 46.2, 27.9, 27.0. HRMS *m/z* found 302.1758, calcd. for $C_{18}H_{24}NO_3$ (M + H) 302.1751.

tert-Butyl-5-_syn_-acetyl-6-_syn_-phenyl-2-azabicyclo[2.1.1]hexane-2-carboxylate (28a), _tert_-Butyl-5-_syn_-acetyl-6-_anti_-phenyl-2-azabicyclo[2.1.1]hexane-2-carboxylate (28b) and _tert_-Butyl-5-_anti_-acetyl-6-_syn_-phenyl-2-azabicyclo[2.1.1]hexane-2-carboxylate (28c)

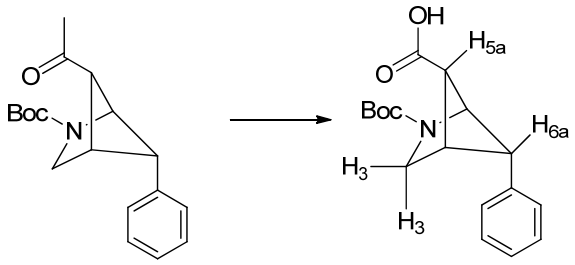


27 28a 28b 28c

Following a modified literature procedure,[43f] a solution of 27 (300 mg, 1.0 mmol) in acetonitrile (100 mL) was bubbled with argon for 20 min and then irradiated with a UV lamp (Hanovia, 450 watts) in a photoreactor at ambient temperature circulated with cooling water for 18 hours until no more starting material was detected by TLC. Acetonitrile was removed *in vacuo*. The mixture was chromatographed (4:1 hexane/ethyl acetate) to afford 150 mg (50%) of **28a** as a colorless oil. R_f = 0.21 (3:1 hexane/ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$δ 7.31-7.03 (m, 5H, Ph), 5.12 and 4.98 (2dt, J = 6.8, 1.8 Hz, 1H, H_1), 3.40 and 3.34 (2dt, J = 6.8, 3.3, 1.0 Hz, 1H, H_4), 3.27 (2d, J = 9.0 Hz, 1H, H_3), 3.16 (br, 1H), 2.85 and 2.76 (2d, J = 9.0 Hz, 1H, H_3), 2.73 (m, 1H), 2.19 and 2.16 (2s, 3H, $COCH_3$), 1.50 and 1.30 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4, 135.9, 128.3, 127.4, 126.6, 79.4, 62.6, 54.7, 48.3, 43.0, 42.5, 28.2, 27.9. HRMS: *m/z* found 324.1581, calcd. for $C_{18}H_{23}NO_3Na$ (M + Na) 324.1570. In addition, 45 mg (15%) of **28b** as colorless oil was also isolated. R_f = 0.35 (3:1 hexane/ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.16 (m, 5H, Ph), 4.81 (2br, 1H, H_1), 3.36 (m, 1H), 3.27 (br, 2H), 2.94 (br, 2H), 2.06 (s, 3H, $COCH_3$), 1.47 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.5, 206.7, 157.9, 136.7, 128.8, 128.0, 127.0, 126.5, 80.2, 79.9, 66.1, 56.2, 53.2, 47.7, 43.6, 30.3, 28.4. HRMS *m/z* found 324.1586, calcd. for $C_{18}H_{23}NO_3Na$ (M + Na) 324.1570. And 24 mg (8%) of **28c** as a colorless oil was also isolated. R_f = 0.24 (3:1 hexane/ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.19 (m, 5H, Ph), 4.09 (m, 1H), 3.85

(m, 1H), 3.50 (m, 1H), 2.94 (m, 1H), 2.57 (m, 1H), 2.30~2.16 (m, 4H), 1.55 (s, 9H, Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.8, 152.9, 133.7, 128.8, 128.6, 126.4, 125.8, 124.8, 80.7, 52.8, 52.4, 47.7, 37.6, 36.5, 30.3, 28.4, 27.9. HRMS *m/z* found 302.1752, calcd. for $C_{18}H_{24}NO_3$ (M + H) 302.1751.

tert-Butyl-5-_syn_-carboxylicacid-6-_syn_-phenyl-2-azabicyclo[2.1.1]hexane-2-carboxylate (29)

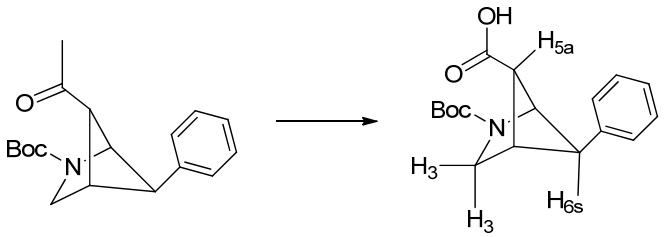


28a **29**

A sodium hypochlorite aqueous solution (available chlorine is more than 10%, 10 mL) was added to **28a** (0.50 g, 1.66 mmol) in THF (4 mL). The reaction mixture was stirred at room temperature for 5 min. The reaction solution was extracted with methyl *t*-butyl ether (2 x 25 mL) to remove any lipophilic impurities. The aqueous solution was adjusted to pH 3.0 with 1.0 N HCl and extracted with EtOAc (2 x 20 mL). The combined ethyl acetate extractions were washed with water (2 x 20 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 0.46 g (92%) of **29** as white solid. Mp = 149-150 °C. ^{1}H NMR (CD_3Cl, 300 MHz) δ 7.31~7.07 (m, 5H, Ph), 6.0~4.0 (br, 1H, COOH), 5.06 and 4.91(2dt, *J* = 6.8, 1.7 Hz, 1H, H_1), 3.46 (2d, *J* = 9.1 Hz,1H, H_3), 3.37 and 3.33 (2dt, *J* = 6.8, 3.2 Hz,1H, H_4), 3.11 (br, 1H, H_5), 2.88 and 2,83 (2d, *J* = 9.2 Hz 1H, H_3), 2.85 (m, 1H, H_6), 1.47 and 1.30 (2s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100

MHz) δ 172.7, 172.4, 156.3, 155.9, 135.9, 135.7, 128.4, 128.4, 127.4, 127.2, 126.8, 126.6, 80.0, 79.9, 63.2, 62.8, 49.2, 48.8, 47.6, 46.8, 43.8, 43.5, 43.4, 42.8, 28.5, 28.3.HRMS: *m/z* found 304.1552, calcd. for $C_{18}H_{24}NO_3$ (M + H) 304.1543.

tert-Butyl-5-_syn_-carboxylicacid-6-_anti_-phenyl-2-azabicyclo[2.1.1]hexane-2-carboxylate (30)

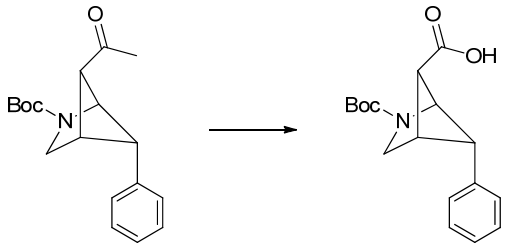


28b **30**

A sodium hypochlorite aqueous solution (available chlorine is more than 10%, 2 mL) was added to **28b** (40 mg, 0.14 mmol) in THF (2 mL). The reaction mixture was stirred at room temperature for 5 min. The reaction solution was extracted with methyl *t*-butyl ether (2 x 5 mL) to remove any lipophilic impurities. The aqueous solution was adjusted to pH 3.0 with 1.0 N HCl and extracted with EtOAc (2 x 5 mL). The combined ethyl acetate extractions were washed with water (2 x 5 mL) and brine (7 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 33 mg (84%) of **30** as white solid. Mp = 135-137 °C. ^{1}H NMR (CD_3Cl, 300 MHz) δ: 7.41~7.26 (m, 5H, Ph, NOE with to H_4, H_5, H_6), 5.0~4.0 (br, 2H, COOH and H_1), 3.76 (d, J = 9.3 Hz, 1H, H_3), 3.50 (d, J = 9.3 Hz, 1H, H_3), 3.36 and 3.34 (2d, J = 6.6 Hz, 1H, H_4), 3.18 (br, 1H, H_5), 3.11 (br, 1H, H_6), 1.57 (s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 156.1, 136.3, 128.8, 127.9, 127.1,

80.3, 54.2, 44.4, 28.5. HRMS: *m/z* found 304.1550, calcd. for $C_{18}H_{24}NO_3$ (M + H) 304.1543.

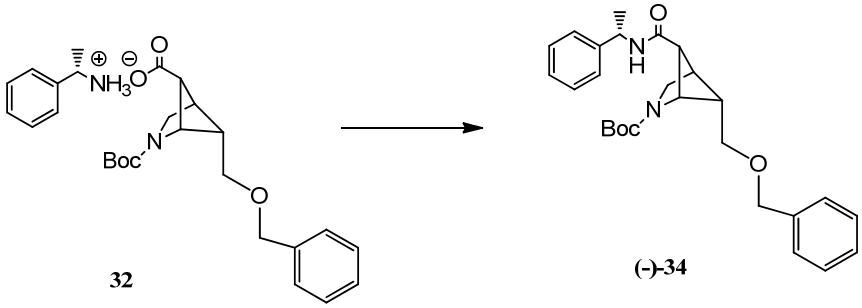
tert-Butyl-5-_syn_-carboxylicacid-6-_anti_-phenyl-2-azabicyclo[2.1.1]hexane-2-carboxylate (31)



28c **31**

A sodium hypochlorite aqueous solution (available chlorine is more than 10%, 2 mL) was added to **28c** (24 mg, 0.08 mmol) in THF (2 mL). The reaction mixture was stirred at room temperature for 5 min. The reaction solution was extracted with methyl *t*-butyl ether (2 x 5 mL) to remove any lipophilic impurities. The aqueous solution was adjusted to pH 3.0 with 1.0 N HCl and extracted with EtOAc (2 x 5 mL). The combined ethyl acetate extractions were washed with water (2 x 5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 21 mg (82%) of **31** as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.32~7.06 (m, 5H, Ph), 5.04 and 4.92 (2d, br, J = 7.0 Hz, 1H, H_1), 3.89 and 3.86 (2s, 1H, H_6), 3.35 (br,1H, H_4), 3.30 and 3.25 (2d, J = 9.3 Hz, 1H, H_{3x}), 3.08 and 3.00 (2d, J = 9.3 Hz, 1H, H_{3n}), 2.80 and 2.77 (2s, 1H, H_5), 1.47 and 1.30 and (2s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 175.4, 175.3, 155.3, 154.9, 136.8, 128.4, 127.4, 127.2, 126.5, 80.2, 79.8, 63.9, 63.0, 52.5, 52.2, 50.7, 50.6, 45.9, 44.6, 44.4, 31.1, 28.5, 28.3. HRMS: *m/z* found 304.1540, calcd. for $C_{18}H_{24}NO_3$ (M + H) 304.1543.

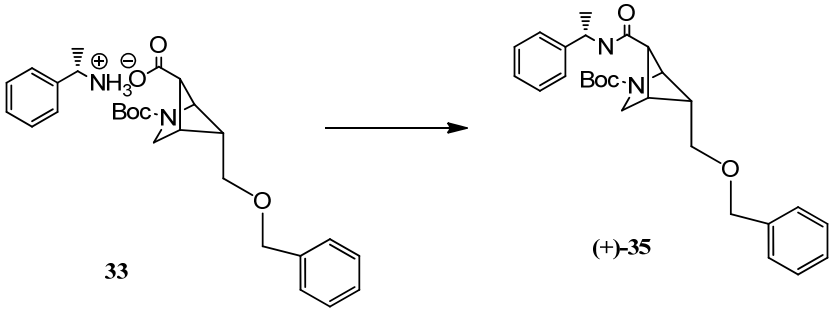
(1*S*,4*R*,5*R*,6*S*)-(-)- *tert*-Butyl 5-((benzyloxy)methyl)-6-(((*S*)-1-phenylethyl)carbamoyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (34)



Following a modified literature procedure, the salt **32** (68 mg, 0.14 mmol, formed from acid (-)-**23** and (*S*)-(-)-α-methylbenzylamine) dissolved in in DMF (5.0 mL). To this solution there was added 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide HCl salt (EDC or EDAC) (30 mg, 0.16 mmol), and 1-hydroxybenzotriazole (HOBt) (19 mg, 0.14 mmol). At room temperature, diisopropylethylamine (DIPEA) (21 mg, 0.16 mmol) was slowly added. The reaction mixture was stirred at room temperature for 1 hour. DMF was removed *in vacuo* and the residue was partitioned between 1.0 N HCl (5.0 mL) and EtOAc (8.0 mL). The bottom acidic aqueous layer was removed and the organic solution was washed with 20% potassium bicarbonate aqueous solution (2 x 5.0 mL). The EtOAc solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give (-)-**34** (56 mg, 86%) as white solid. Mp = 134-135 ^{o}C. R_f = 0.31 (1:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.33 (m, 10H), 6.0 (2d, J = 8.0 Hz, 1H), 5.09 (m, 1H), 4.50 (m, 3H), 3.36~3.23 (m, 3H), 3.10 (m, 2H), 2.53 (br, 1H), 2.18 (br, 1H), 1.50 (m, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.8, 167.6, 156.0, 155.5, 143.0, 142.7, 138.0, 137.7, 128.8, 128.5, 127.8, 127.8, 126.2, 125.9, 80.2, 79.8, 73.6, 73.3, 64.8, 64.7, 62.1, 60.6, 50.4, 50.3, 48.8, 48.5, 45.1, 44.0, 43.8, 43.2, 42.4, 42.2, 28.5, 22.0, 21.6. HRMS: *m/z*

found 451.2606, calcd. for $C_{27}H_{35}N_2O_4$ (M + H) 451.2591. Optical rotation $[\alpha]_D$ of (-)-**34**: -29.5 (20 ^oC, c = 0.64, chloroform).

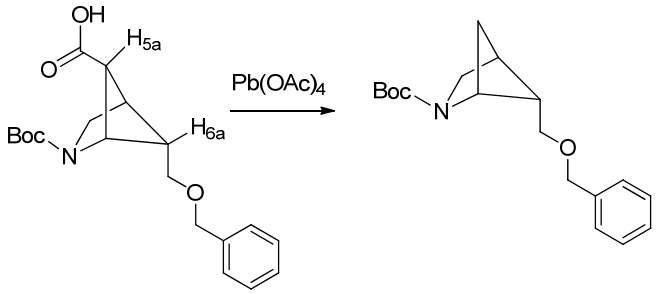
(1*R*,4*S*,5*S*,6*R*)-(+)- *tert*-Butyl 5-((benzyloxy)methyl)-6-(((*S*)-1-phenylethyl)carbamoyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (35)



Following a modified literature procedure, acid (+)-**23** (49 mg, 0.14 mmol) and (*S*)-(-)-α-methylbenzylamine (17 mg, 0.14 mmol) were dissolved in DMF (5.0 mL). To this solution there was added 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide HCl salt (EDC or EDAC) (30 mg, 0.16 mmol), and 1-hydroxybenzotriazole (HOBt) (19 mg, 0.14 mmol). At room temperature, diisopropylethylamine (DIPEA) (21 mg, 0.16 mmol) was slowly added. The reaction mixture was stirred at room temperature for 1 hour. DMF was removed *in vacuo* and the residue was partitioned between 1.0 N HCl (5.0 mL) and EtOAc (8.0 mL). The bottom acidic aqueous layer was removed and the organic solution was washed with 20% potassium bicarbonate aqueous solution (2 x 5.0 mL). The EtOAc solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give **35** (56 mg, 0.12 mmol, 86%) as white solid. Mp = 121-122 °C. R_f = 0.31 (1:1 hexane/ethyl acetate). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.33 (m, 10H), 6.0 (2d, J = 8.0 Hz, 1H), 5.09 (m, 1H), 4.50 (m, 3H), 3.27~3.05 (m, 5H), 2.63 (br, 1H), 2.19 (br, 1H), 1.46 (m, 12H). ^{13}C NMR

($CDCl_3$, 100 MHz) δ: 167.9, 167.7, 156.0, 155.6, 143.3, 142.9, 137.9, 137.9, 128.8, 128.6, 128.5, 127.8, 127.8, 127.1, 125.9, 83.7, 79.9, 79.9, 73.5, 73.4, 64.9, 64.5, 62.2, 60.7, 50.3, 50.0, 48.8, 48.7, 45.1, 44.6, 43.9, 43.2, 42.7, 42.3, 28.5, 28.4, 28.4, 22.3, 22.0. HRMS: *m/z* found 451.2594, calcd. for $C_{27}H_{35}N_2O_4$ (M + H) 451.2591. Optical rotation $[\alpha]_D$ of (+)-**35**: 11.2 (20 °C, c = 0.9, chloroform).

(+)-*tert*-Butyl-5-*syn*-((benzyloxy)methyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (36)

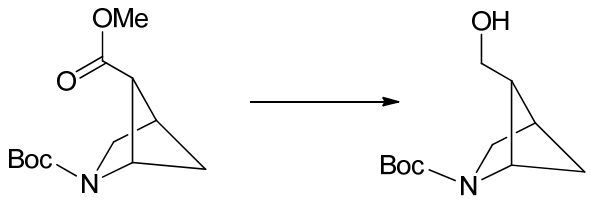


(-)-23 (+)-36

The mixture of acid (-)-**23** (56 mg, 0.16 mmol), lead tetraacetate (117 mg, 0.26 mmol) and pyridine (28 mg, 0.35 mmol) in cyclohexane (10 mL) was refluxed under argon for 16 h. The solid in the reaction mixture was removed by filtration. The filtrate was concentrated and the residue was chromatographed (gradient, 1:4 ethyl acetate/ hexanes) to afford titled compound **(+)-36** (18 mg, 37%) as a colorless oil. R_f = 0.35 (1:4 ethyl acetate/ hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.35 (m, 5H, Ph), 4.60~4.30 (m, 3H, H_1 and CH_2Ph), 3.24 (br, 4H, $2H_3$ and CH_2O), 2.79 (2dt, J = 7.0, 3.02 Hz, 1H, H_4), 2.32 (m, 1H, H_5), 1.74 (m, 1H, H_6), 1.48 (s, 9H, Boc), 1.34 (br, 1H, H_6). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 156.4, 138.3, 128.4, 127.7, 79.2, 73.3, 66.2, 47.9, 45.8, 39.4, 38.4,

28.5. HRMS: *m/z* found 326.1731, calcd. for $C_{18}H_{25}NNaO_3$ (M + Na) 326.1737. Optical rotation $[\alpha]_D$ of (+)-**36**: 13.9 (20 °C, c = 1.1, chloroform).

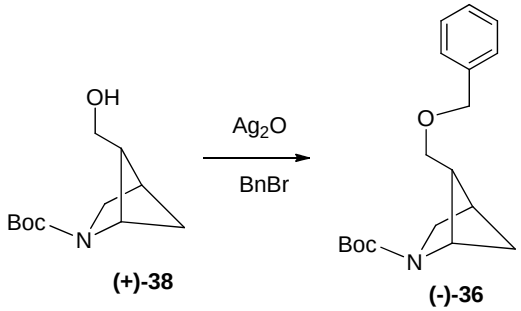
(+)-*tert*-Butyl 5-*syn*-(hydroxymethyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (38)



(-)-37 **(+)-38**

Following Dr. Nian Liu's procedure,[30] at –78 °C, to a solution of (-)-5-*syn*-CO_2Me **37** (130 mg, 0.54 mmol) in dry THF (13.0 mL) was slowly added 1M LAH solution (0.35 mL, 0.35 mmol). The reaction solution was allowed to stir at -78 ^oC for 1 hour. The reaction was brought to room temperature and stirred for additional 2 hours. The reaction was quenched by water (25 μL), followed by 15% sodium hydroxide (25 μL) and water (100 μL). The resulting solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 100 mg (87%) of (+)-**38** as a colorless oil. ^1H NMR (CDCl3, 400 MHz) δ: 4.40 (d, J = 7.2 Hz, 1H, H_1), 3.41 (m, 4H, OCH_2 and $2H_3$), 2.82 (m, 1H, H_4), 2.25 (m, 1H, H_5), 2.00 (br, 1H, OH), 1.80 (d, J = 7.4 Hz, $1H_6$), 1.45 (s, 9H, *t*-butyl), 1.40 (d, J = 7.4 Hz, $1H_6$). ^{13}C NMR (CDCl3, 100 MHz) δ: 157.9, 79.7, 61.2, 60.8, 57.8, 49.6, 46.5, 38.8, 37.7, 28.3. Optical rotation $[\alpha]_D$ of (+)-**38**: 9.5 (20 °C, c = 1.2, chloroform)

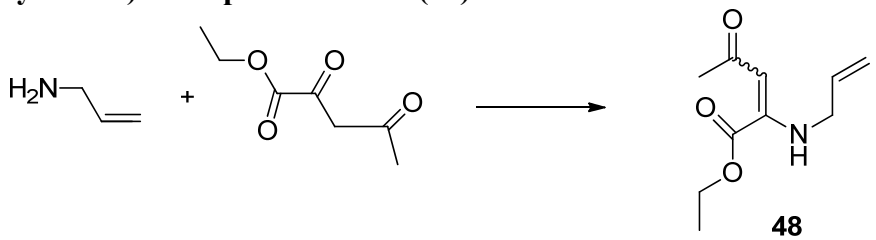
(-)-*tert*-Butyl 5-*syn*-((benzyloxy)methyl)-2-azabicyclo[2.1.1]hexane-2-carboxylate (36)



(+)-38 **(-)-36**

Alcohol (+)-**38** (42 mg, 0.20 mmol) was added to 5 mL CH_2Cl_2 with vigorous stirring. Ag_2O (79 mg, 0.34 mmol) was added in one portion followed by BnBr (43 mg, 0.23 mmol). After 4 h the solid in the reaction mixture was removed by filtration. The filtrate was concentrated and the residue was chromatographed (gradient, 1:4 ethyl acetate/ hexanes) to afford titled compound (-)-**36** (48 mg, 79%) as colorless oil. R_f = 0.35 (1:4 ethyl acetate/ hexanes). Optical rotation $[\alpha]_D$ of (-)-**36**: -14.2 (20 °C, c = 1.9, chloroform).

Ethyl 2-(allylamino)-4-oxopent-2-enoate (48)

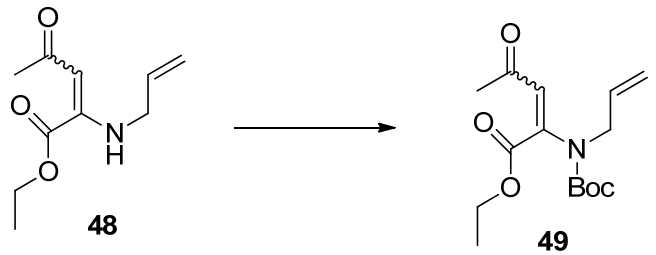


48

A stirred suspension of the 2,4-dioxopentanoate (316 mg, 2.0 mmol) in dry benzene (25 mL) containing acetic acid (1 mL) and allyamine (284 mg, 4.0 mmol) was heated under reflux with azeotropic removal of water using a Dean-Stark apparatus for 2 hours. The cooled mixture was washed with saturated $NaHCO_3$ solution (10 mL). The organic layer was dried and evaporated *in vacuo*. The residue was chromatographed (gradient, 1:10 MeOH/ DCM) to afford titled compound **48** (297 mg, 75%) as a yellow

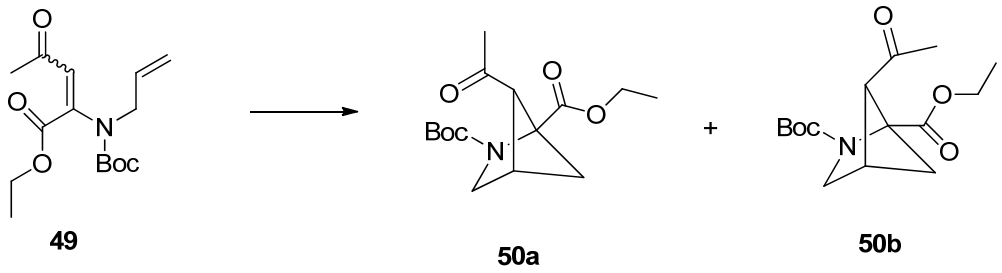
color oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.2 (br, 1H, NH), 5.88 (m, 1H), 5.51 (s, 1H), 5.19 (m, 2H), 4.30 (q, *J* = 7.3 Hz, 2H), 4.02 (m, 2H), 2.14 (s, 3H), 1.36 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 198.3, 163.6, 151.6, 134.6, 116.5, 96.2, 62.0, 46.9, 41.0, 29.7, 14.0. HRMS: *m/z* found 198.1125, calcd. for $C_{10}H_{16}NO_3$ (M + H) 198.1130.

Ethyl 2-(allyl(*tert*-butoxycarbonyl)amino)-4-oxopent-2-enoate (49)



To the amine **48** (173 mg, 0.87 mmol) in DCM (20 mL) with Et_3N (131 mg, 1.3 mmol) and DMAP (106 mg, 0.87 mmol) was added $(Boc)_2O$ (324 mg, 1.5 mmol). The result solution was stirred at room temperature for 12hr. Solvent was removed *in vacuo* and the residue was chromatographed (gradient, 1:3 ethyl acetate/ hexanes) to afford title compound **49** (241 mg, 93%) as an orange color oil. R_f = 0.37 (1:3 ethyl acetate/ hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.93 (s, 1H), 5.85 (m, 1H), 5.27 (m, 2H), 4.31 (q, *J* = 8.0 Hz, 2H), 4.20 (d, *J* = 4.0 Hz, 2H), 2.21 (s, 3H), 1.47 (s, 9H), 1.32 (t, *J* = 8.0 Hz). ^{13}CNMR ($CDCl_3$, 300 MHz) δ: 196.2, 164.3, 151.9, 144.5, 132.1, 116.6, 114.4, 83.5, 62.0, 51.6, 30.6, 27.9, 13.5. HRMS: *m/z* found 320.1467, calcd. for $C_{15}H_{23}NNaO_5$ (M + Na) 320.1468.

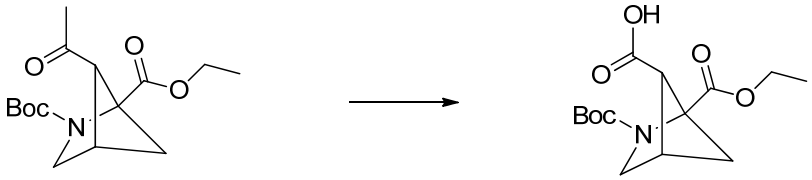
2-*tert*-Butyl-1-ethyl-5-*syn*-acetyl-2-azabicyclo[2.1.1]hexane-1,2-dicarboxylate (50a) and 2-*tert*-Butyl-1-ethyl-5-*anti*-acetyl-2-azabicyclo[2.1.1]hexane-1,2-dicarboxylate (50b)



A solution of **49** (298 mg, 1.0 mmol) in acetonitrile (100 mL) was bubbled with argon for one hour and then irradiated with a UV lamp (Hanovia, 450 watts) in a photoreactor at ambient temperature circulated with cooling water for 18 hours until no more starting material was detected by TLC. Acetonitrile was removed *in vacuo,* the residue was chromatographed on SiO_2 gel (1:1 hexane/ether) to afford 218 mg (73%) of **50a** as colorless oil. R_f = 0.27 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.19 (q, J = 7.1 Hz, 2H, CH_2), 3.79 (d, J = 8.0 Hz, 1H, H_3), 3.32 (d, J = 8.0 Hz, 1H, H_3), 2.91 (d, J = 4.0 Hz, 1H, H_4), 2.79 (2d, J = 4.0 Hz, 1H, H_5), 2.19 (s, 3H, CH_3), 1.82 (ddd, J = 8.0, 3.2, 1.2 Hz, 1H, H_6), 1.75 (dd, J = 8.0, 1.2 Hz, 1H, H_6), 1.43 (s, 9H), 1.34 (t, J = 7.19 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz) δ: 196.2, 164.3, 151.9, 144.5, 132.1, 116.6, 114.4, 83.5, 62.0, 51.6, 30.6, 27.9, 13.5. HRMS: *m/z* found 320.1467, calcd. for $C_{15}H_{23}NNaO_5$ (M + Na) 320.1468. In addition, 39 mg (13%) of **50b** as colorless oil was also isolated. R_f = 0.31 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.17 (q, J = 7.1 Hz, 2H, CH_2), 3.40 (br,2H, $2H_3$), 3.02 (d, J = 8.0 Hz, 1H, H_5), 2.88 (dt, J = 3.5, 1.0 Hz, 1H, H_4), 2.65 (ddd, J = 8.0, 3.52, 1.0 Hz, 1H, H_{6s}) 2.11 (s, 3H, CH_3), 1.65 (t, J = 8.0 Hz, 1H, H_{6a}), 1.36 (s, 9H), 1.22 (t, J = 8.0 Hz, 3H).^{13}C NMR ($CDCl_3$, 100 MHz)

δ: 205.9, 196.8, 167.7, 83.6, 89.8, 73.1, 62.1, 61.2, 56.4, 51.7, 50.2, 42.5, 37.5, 30.7, 30.2, 28.1, 27.9, 14.1, 13.5. HRMS**:** *m/z* found 320.1465, calcd. for $C_{15}H_{23}NNaO_5$ (M + Na) 320.1468.

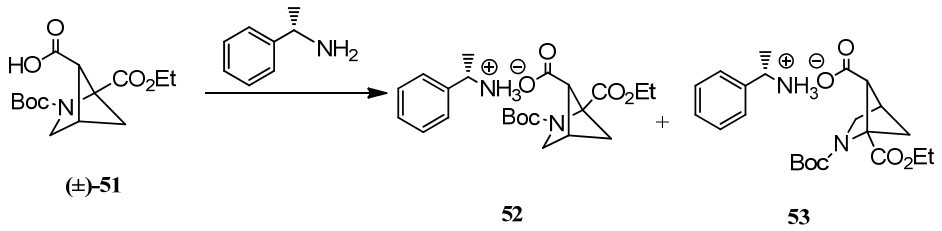
(±)-2-(*tert*-Butoxycarbonyl)-1-(ethoxycarbonyl)-2-azabicyclo[2.1.1]hexane-5-carboxylic acid (51)



50a **(±)-51**

A sodium hypochlorite aqueous solution (available chlorine is more than 10%, 7 mL) was added to **50a** (300 mg, 1.0 mmol) in THF (4 mL). The reaction mixture was stirred at room temperature for 5 min. The reaction solution was extracted with methyl *t*-butyl ether (2 x 15 mL) to remove any lipophilic impurities. The aqueous solution was adjusted to pH 3.0 with 1.0 N HCl and extracted with EtOAc (2 x 15 mL). The combined ethyl acetate extractions were washed with water (2 x 10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give 270 mg (90%) of solid. Mp = 97-99 °C. ^{1}H NMR (CD_3Cl, 300 MHz) δ: 4.35 (q, *J* = 8.0 Hz, 2H), 3.62 (d, *J* = 8.0 Hz, 1H, H_3), 3.45 (d, *J* = 8.0 Hz, 1H, H_3), 3.05 (tt, *J* = 2.7, 0.7 Hz,1H, H_4), 2.92 (d, *J* = 4.0 Hz, 1H, H_5), 1.95 (ddd, *J* = 8.0, 4.0, 2.0 Hz, 1H, H_6), 1.69 (dd, *J* = 8.0, 4.0 Hz, 1H, H_6), 1.38 (s, 9H), 1.30 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.8, 168.8, 155.6, 81.3, 70.5, 62.8, 52.6, 49.3, 40.5, 37.3, 28.2, 13.9. HRMS: *m/z* found 322.1263, calcd. for $C_{14}H_{21}NNaO_6$ (M + Na) 322.1261.

Resolution of (±)-2-(tert-Butoxycarbonyl)-1-(ethoxycarbonyl)-2-azabicyclo[2.1.1]hexane-5-carboxylic acid (51)



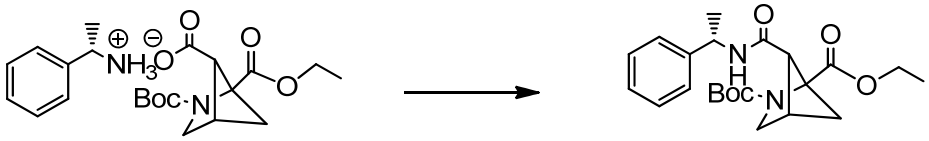
(±)-51 52 53

(*S*)-(-)-α-Methylbenzylamine (0.50 g, 4.1 mmol) was slowly added to acid (±)-**51** (1.31 g, 4.4 mmol) in EtOAc (15.0 mL) solution. After stirring for 30 minutes at room temperature, white slurry was gradually formed. The slurry was stirred for an additional 6 hours at room temperature. The mixture was heated to reflux until all solid was dissolved. The solution was slowly cooled to room temperature and the salt was gradually precipitated out. The salt was collected in a Busch funnel by vacuum filtration. The yield of the first crop was 0.79 g (1.88 mmol or 44%, 72% ee, Mp = 151-154 °C). The salt was taken up in 10.0 mL of EtOAc. The mixture was heated to reflux until all solid was dissolved. The solution was slowly cooled to room temperature and the salt was gradually precipitated out. The slurry was stirred at room temperature for 2 hours and the salt was collected by vacuum filtration. After drying, 0.60 g (1.43 mmol) of salt **52** (100% ee, Mp = 153-154 °C, Appendix B) was obtained and the yield was 32.5% based on acid (±)-**51**.

Recrystallized salt **52** (0.53 g, 1.26 mmol) was partitioned between EtOAc (10.0 mL) and water (10.0 mL). The mixture was adjusted to pH 2.5 with 1.0 N HCl. The EtOAc layer was separated, and the aqueous layer was extracted with 10.0 mL of EtOAc.

The combined EtOAc solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield of free acid (+)-**51 (**0.34 g, 26%) as white solid. Mp = 97-98 ^oC. Optical rotation $[\alpha]_D$ of (+)- **51**: 92.7 (20 ^oC, c = 0.73, chloroform).

(1*R*,4*S*,5*S*)-(+)-2-*tert*-Butyl-4-ethyl-5-(((S)-1-phenylethyl)carbamoyl)-2-azabicyclo[2.1.1]hexane-2,4-dicarboxylate (54)



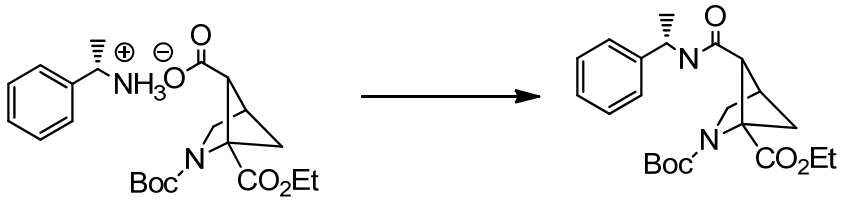
52 **(+)-54**

Following a modified literature procedure, salt **52** (42 mg, 0.10 mmol, formed from acid (+)-**51** with (*S*)-(-)-α-methylbenzylamine) dissolved in DMF (5 mL). To this solution there was added 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide HCl salt (EDC or EDAC) (23 mg, 0.12 mmol), and 1-hydroxybenzotriazole (HOBt) (14 mg, 0.10 mmol). At room temperature, diisopropylethylamine (DIPEA) (16 mg, 0.12 mmol) was slowly added. The reaction mixture was stirred at room temperature for 1 hour. DMF was removed *in vacuo* and the residue was partitioned between 1.0 N HCl (5.0 mL) and EtOAc (8.0 mL). The bottom acidic aqueous layer was removed and the organic solution was washed with 20% potassium bicarbonate aqueous solution (2 x 5.0 mL). The EtOAc solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give (+)-**54** (33 mg, 82%) as white solid. Mp = 162-163 °C. R_f = 0.27 (2:1 hexane/ethyl acetate). ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.75~7.24 (m, 5H), 5.01 (br, 1H), 4.35 (m, 2H), 3.73 (d, J = 12.0 Hz, 1H), 3.43 (d, J = 12.0 Hz, 1H), 3.05 (b, 1H), 2.90 (s, 1H), 1.93 (br, 1H), 1.72 (br, 1H), 1.47 (s, 12H), 1.30 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.4,

167.2, 155.5, 143.6, 128.6, 127.1, 126.3, 126.1, 125.8, 80.4, 70.7, 61.9, 53.4, 49.5, 48.8, 40.7, 36.8, 28.3, 22.4, 13.9. HRMS: *m/z* found 425.2044, calcd. for $C_{22}H_{30}N_2NaO_5$ (M + H) 425.2047. Optical rotation $[\alpha]_D$ of (+)-**54**: 70.1 (20 °C, c = 0.71, chloroform).

.

(1*S*,4*R*,5*R*)-(-)-2-*tert*-Butyl-1-ethyl-5-(((S)-1-phenylethyl)carbamoyl)-2-azabicyclo[2.1.1]hexane-1,2-dicarboxylate (55)

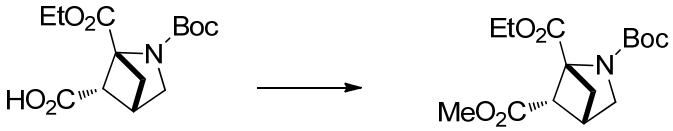


53 **(-)-55**

Following a modified literature procedure, (-)-acid **51** (30 mg, 0.10 mmol) and (*S*)-(-)-α-methylbenzylamine) (12 mg, 0.10 mmol) were dissolved in DMF (5 mL). To this solution there was added 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide HCl salt (EDC or EDAC) (23 mg, 0.12 mmol), and 1-hydroxybenzotriazole (HOBt) (14 mg, 0.10 mmol). At room temperature, diisopropylethylamine (DIPEA) (16 mg, 0.12 mmol) was slowly added. The reaction mixture was stirred at room temperature for 1 hour. DMF was removed *in vacuo* and the residue was partitioned between 1.0 N HCl (5.0 mL) and EtOAc (8.0 mL). The bottom acidic aqueous layer was removed and the organic solution was washed with 20% potassium bicarbonate aqueous solution (2 x 5.0 mL). The EtOAc solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give (-)-**55** (32 mg, 80%) as white solid. Mp = 168-169 °C. R_f = 0.27 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.90 (br, 1H), 7.31 (m, 5H), 5.07 (m, 1H), 4.34 (m, 2H), 3.47 (d, *J* = 8.8 Hz, 1H), 3.37 (d, *J* = 8.8 Hz, 1H), 3.05 (br, 1H), 2.92 (d, *J* = 3.2 Hz, 1H), 1.98

(m, 1H), 1.65 (d, *J* = 7.6 Hz, 1H), 1.49 (d, *J* = 6.8 Hz, 3H), 1.37 (m, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.3, 167.3, 155.8, 143.6, 128.5, 126.9, 125.8, 80.6, 70.51, 61.9, 54.7, 49.6, 48.7, 39.6, 37.2, 28.2, 22.9, 13.9. HRMS: *m/z* found 425.2046, calcd. for $C_{22}H_{30}N_2NaO_5$ (M + H) 425.2047. Optical rotation $[\alpha]_D$ of (-)-**55**: -84.1 (20 °C, c = 0.76, chloroform).

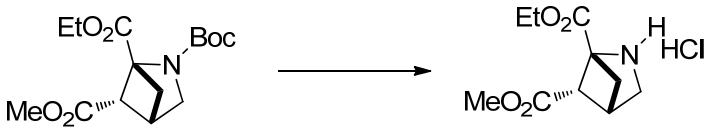
(1*R*,4*S*,5*S*)-(+)-2-*tert*-Butyl-1-ethyl-5-methyl-2-azabicyclo[2.1.1]hexane-1,2,5-tricarboxylate (56)



(+)-51 **(+)-56**

The acid (+)-**51** (84 mg, 0.28 mmol) was dissolved in isopropanol (10 mL) and hexanes (10 mL) and trimethylsilyldiazomethane (0.15 mL, 0.3 mmol, 2 M in hexanes) was added. The mixture was stirred for 40 min at room temperature. Solvent was removed *in vacuo* to afford 88 mg (100 %) of titled compound (+)-**56** as colorless oil. R_f = 0.32 (2:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.25 (q, *J* = 7.8 Hz, 2H), 3.85 (br, 1H, H_3), 3.65 (s, 3H, COOMe), 3.43 (d, *J* = 8.0 Hz, 1H, H_3), 2.95 (m, 2H, H_4 and H_5), 1.95 (m, 1H, H_6), 1.75 (d, *J* = 8.0, 4.0 Hz, 1H, H_6), 1.42 (s, 9H), 1.29 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.6, 167.2, 156.8, 80.6, 72.1, 61.1, 57.9, 51.7, 49.9, 38.8, 38.3, 14.2. HRMS: *m/z* found 336.1421, calcd. for $C_{15}H_{23}NNaO_6$ (M + Na) 336.1418. Optical rotation $[\alpha]^2_D$ of (+)-**56**: 58.0 (20 °C, c = 1.5, chloroform).

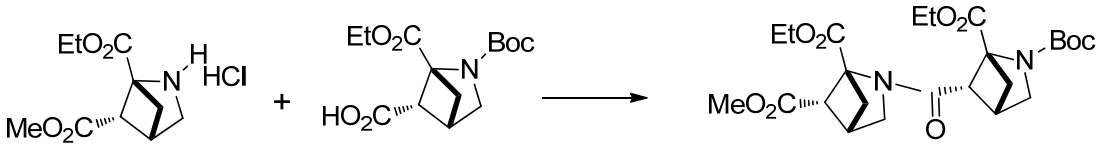
(1*R*,4*S*,5*S*)-1-Ethyl -5-methyl 2-azabicyclo[2.1.1]hexane-1,5-dicarboxylate hydrochloride (57)



(+)-56 **(1*R*,4*S*,5*S*)-57**

5-*syn*-Ester (+)-**56** (150 mg, 0.50 mmol) was dissolved in 4 N HCl/dioxane (2.0 mL) and stirred at room temperature for 2 h. The reaction was monitored by thin layer chromatography (9:1 DCM/MeOH). The solution was concentrated *in vacuo* and further dried under high vacuum to afford 108 mg (97 %) of (1*R*,4*S*,5*S*)-NH HCl salt **57** as white solid. The solid was used directly for next step.

Preparation of dimer (+)-58

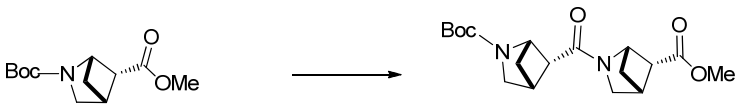


(1*R*,4*S*,5*S*)-57 **(+)-51** **(+)-58**

A solution of DIPEA (62 mg, 0.48 mmol) was added to 100 mL round bottom bottle with (+)-**51** (66 mg, 0.22 mmol), HCl salt **57** (55 mg, 0.22 mmol), EDC (46 mg, 0.24 mmol) and HOBt (30 mg, 0.22 mmol) in acetonitrile (1.5 mL) and EtOAc (0.5 mL) at 0 ^{o}C. The reaction solution was warmed to room temperature and stirred for 1 hour. The reaction mixture was diluted with EtOAc (2.5 mL) and washed with 1 N HCl (2.0 mL) and 20% $KHCO_3$ aqueous solution (2 x 2.0 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford 64 mg (83%) of dimer (+)-**58**

(100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as yellow oil. R_f = 0.28 (1:1 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.20~3.98 (m, 7H), 3.66 (s, 3H, COOMe), 3.41 (d, *J* = 8.2 Hz, 1H), 2.96 and 2.89 (2br, 4H, $2H_4$ and $2H_5$), 1.95 (m, 2H, $2H_6$), 1.75 (m, 2H, $2H_6$), 1.43 (s, 9H), 1.31 and 1.26 (2t, *J* = 8.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.4, 168.1, 166.7, 80.2, 72.4, 71.9, 61.0, 51.7, 48.9, 38.8, 37.7, 28.3, 14.1, 14.0. HRMS: *m/z* found 495.2336, calcd. for $C_{24}H_{34}N_2O_9$ (M + H) 495.2337. Optical rotation $[\alpha]_D$ of (+)-**58**: 88.5 (20 °C, c = 2.0, chloroform).

***N*-Boc-dimer (-)-9b**

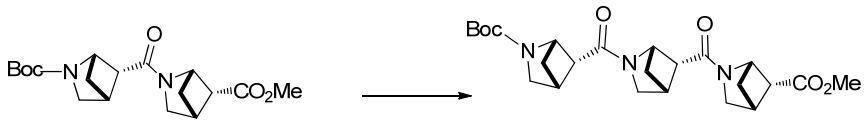


(-)-**9a** (-)-**9b**

Monomer (-)-**9a** (60 mg, 0.25 mmol) was dissolved in 4 N HCl/dioxane (2.0 mL) and stirred at room temperature for 2 h.[30] The reaction was monitored by thin layer chromatography (9:1 DCM/MeOH). The solvent was concentrated *in vacuo*. To the residue in acetonitrile (1.5 mL) and EtOAc (0.5 mL) at 0 °C, DIPEA (65 mg, 0.50 mmol), acid monomer (-)-**13** (55 mg, 0.24 mmol), EDC (50 mg, 0.25 mmol) and HOBt (34 mg, 0.25 mmol) was added. The reaction solution was warmed to room temperature and stirred for 1 hour. The reaction mixture was diluted with EtOAc (2.5 mL) and washed with 1 N HCl (2.0 mL) and 20% $KHCO_3$ aqueous solution (2 x 2.0 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* and the residue was chromatographed (gradient, 1:1 ethyl acetate/ hexanes) to afford 74 mg (85%) the desired dimer (-)-**9b** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as

yellow oil. R_f = 0.21 (1:1 hexane/ethyl acetate). R_f = 0.62 (9:1 DCM/MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.87 (m, 1H), 4.51 (d, *J* = 5.6 Hz, 1H), 3.70 (d, *J* = 7.2 Hz, 1H), 3.60 (d, *J* = 15.2 Hz, 3H), 3.35 (d, *J* = 9.6 Hz, 1H), 3.23 (mbr, 2H), 3.03 (br, 2H), 2.80 (m, 2H), 1.84 (m, 2H), 1.41 (m, 9H), 1.28 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 194.0, 169.4, 167.7, 79.5, 62.3, 51.8, 51.6, 51.0 and 50.8, 48.4, 46.5, 45.8, 40.6 and 40.5, 39.9, 38.1, 37.4, 30.9, 28.5. HRMS *M/Z* ($M+Na^+$) calculated for $C_{16}H_{26}N_2O_5Na$ ($M+Na^+$) 373.1734, observed 373.1740. Optical rotation $[\alpha]_D$ of (-)-**9b**: -28.6 (20 °C, c = 1.3, chloroform).

***N*-Boc-trimer (-)-9c**

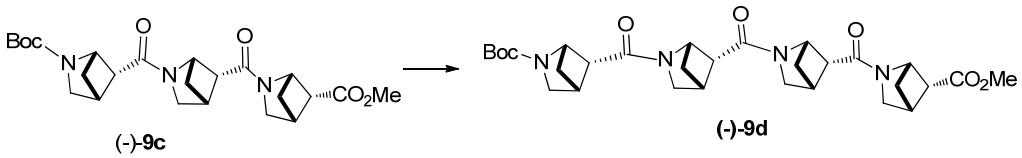


(-)-**9b** (-)-**9c**

Dimer (-)-**9b** (93 mg, 0.24 mmol) was dissolved in 4 N HCl/dioxane (2.0 mL) and stirred at room temperature for 2 h.[30] The reaction was monitored by thin layer chromatography (9:1 DCM/MeOH). The solvent was concentrated *in vacuo*. To the residue in acetonitrile (1.5 mL) and EtOAc (0.5 mL) at 0 ^oC, DIPEA (62 mg, 0.48 mmol), acid monomer (-)-**13** (50 mg, 0.22 mmol), EDC (46 mg, 0.24 mmol) and HOBt (30 mg, 0.22 mmol) was added. The reaction solution was warmed to room temperature and stirred for 1 hour. The reaction mixture was diluted with EtOAc (2.5 mL) and washed with 1 N HCl (2.0 mL) and 20% $KHCO_3$ aqueous solution (2 x 2.0 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* and the residue was chromatographed (gradient, 1:1 ethyl acetate/ hexanes) to afford 88 mg (87%) the desired

trimer (-)-**9c** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as yellow oil. R_f = 0.59 (9:1 DCM/MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.0~4.4 (m, 3H, $3H_1$), 3.80~3.60 (m, 3H, $3H_3$), 3.59, 3.42 (2s, 3H, Me), 3.42~3.02 (m, 3H, H_3), 3.01 (m, 3H, $3H_4$), 2.71 (m, 3H, $3H_5$), 1.75~ 1.68 m, 3H, $3H_6$), 1.38 (s, 9H, Boc), 1.29 (m, 3H, $3H_6$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.3, 168.4, 168.1, 155.9, 155.1, 79.9, 79.1, 62.4, 62.4, 62.1, 61.9, 61.6, 61.2, 60.9, 60.5, 51.9, 51.5, 50.9, 50.8, 50.6, 48.3, 47.7, 47.3, 46.6, 46.4, 46.2, 45.8, 41.8, 41.5, 40.5, 40.4, 40.0, 38.4, 37.9, 37.5, 36.9, 28.6. HRMS: *m/z* found 425.2046, calcd. for $C_{22}H_{30}N_2NaO_5$ (M + H) 425.2047. Optical rotation $[\alpha]_D$ of (-)-**9c**: -32.4 (20 °C, c = 1.1, chloroform).

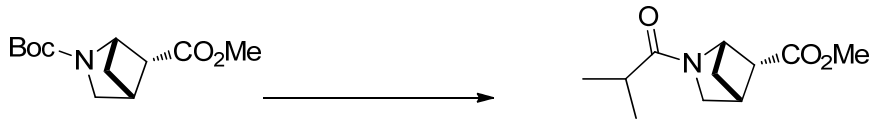
***N*-Boc-tetramer (-)-9d**



Trimer (-)-**9b** (43 mg, 0.1 mmol) was dissolved in 4 N HCl/dioxane (1.0 mL) and stirred at room temperature for 2 h.[30] The reaction was monitored by thin layer chromatography (9:1 DCM/MeOH). The solvent was concentrated *in vacuo*. To the residue in acetonitrile (1.5 mL) and EtOAc (0.5 mL) at 0 ^{o}C, DIPEA (26 mg, 0.2 mmol), acid monomer (-)-**13** (23 mg, 0.1 mmol), EDC (24 mg, 0.11 mmol) and HOBt (15 mg, 0.11 mmol) was added. The reaction solution was warmed to room temperature and stirred for 1 hour. The reaction mixture was diluted with EtOAc (2.5 mL) and washed with 1 N HCl (2.0 mL) and 20% $KHCO_3$ aqueous solution (2 x 2.0 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* and the residue was

chromatographed (gradient, 1:1 ethyl acetate/ hexanes) to afford 72 mg (80%) the desired tetramer (-)-**9d** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as yellow solid. Mp = 215-216 ^{o}C. R_f = 0.54 (9:1 DCM/MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.95 (m, 1H), 4.85 (m, 1H), 4.47 (br, 2H), 3.53 (m, 7H), 2.98 (m, 6H), 2.65 (m, 3H), 1.67 (m, 6H), 1.51 (m, 1H), 1.38 (s, 9H), 1.37 (m 1H), 1.18 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.27, 168.22, 168.12, 168.06, 79.16, 60.89, 60.56, 50.74, 48.25, 47.35, 47.28, 46.46, 41.15, 40.56, 40.46, 40.36, 37.64, 36.46, 28.45. HRMS *M/Z* ($M+Na^+$) calculated for $C_{30}H_{40}N_4O_7Na$ ($M+Na^+$) 591.2790, observed 591.2778. Optical rotation $[\alpha]_D$ of (-)-**9d**: -39.7 (20 °C, c = 1.4, chloroform).

(1*S*,4*R*,5*R*)-(-)-Methyl 2-isobutyryl-2-azabicyclo[2.1.1]hexane-5-carboxylate 59

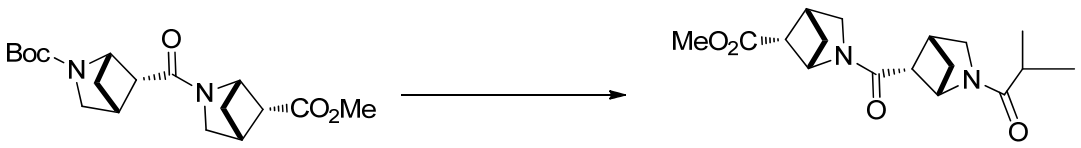


(-)-9a **(-)-59**

Compound (-)-**9a** (40 mg, 0.17 mmol) was dissolved in 4 N HCl/dioxane (1.0 mL) and stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and further dried under high vacuum to remove all solvent. To this residue, DCM (5 mL) was added. The resulting solution was chilled with an ice bath and isobutyryl chloride (35 mg, 0.34 mmol) was added dropwise. The mixture was stirred with an ice bath for 15 min and then at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was chromatographed (gradient, 4:1 ethyl acetate/ hexanes) to afford 29 mg (84%) of amide (-)-**59** (100% pure by SB-C18 column, λ = 245 nm, flow rate = 1.0 mL/min) as a colorless oil. R_f = 0.19 (1:2 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.96,

4.55(2dt, J = 8.0, 2.0 Hz, 1H, H_1), 3.75, 3.47 (2d, J = 8.0 Hz, 1H, H_3), 3.56 (2s, 3H, OMe), 3.35, 3.31 (2d, J = 8.0 Hz, 1H, H_3), 3.09 (br, 1H, H_4), 2.86, 2.77 (2s, 1H, H_5), 2.63, 2.50 (2hep, J = 8.0 Hz, 1H, MeCHMe), 1.85 (2m, 1H, H_6), 1.35, 1.25 (2d, J = 8.0 Hz, 1H, H_6), 1.08 (2t, J = 6.0 Hz, 6H, 2Me).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 175.8, 175.3, 169.4, 169.2, 62.5, 60.4, 51.5, 51.4, 50.8, 48.9, 46.7, 45.8, 40.7, 40.1, 37.9, 37.3, 32.1, 31.9, 29.9, 19.4, 19.2, 18.8, 18.8. HRMS: *m/z* found 212.1281, calcd. for $C_{11}H_{17}NO_3$ (M + H) 212.1281. Optical rotation $[\alpha]_D$ of (-)-**59**: -67.0 (20 °C, c = 1.5, chloroform).

Preparation of dimer (-)-60

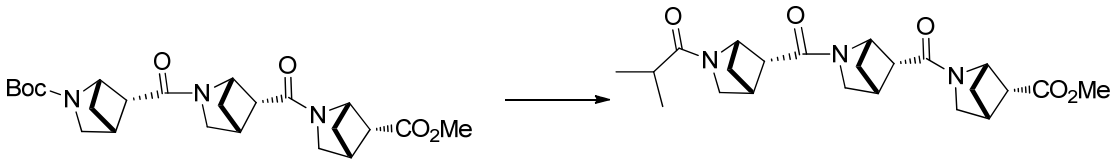


(-)-9b **(-)-60**

Dimer (-)-**9b** (60 mg, 0.17 mmol) was dissolved in 4 N HCl/dioxane (1.0 mL) and stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and further dried under high vacuum to all remove solvent. To this residue, DCM (5 mL) was added. The resulting solution was chilled with an ice bath and isobutyryl chloride (35 mg, 0.34 mmol) was added dropwise. The mixture was stirred with an ice bath for 15 min and then at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was chromatographed (gradient, 4:1 ethyl acetate/ hexanes) to afford 42 mg (79%) of amide (-)-**60** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as a colorless oil. R_f = 0.17 (1:2 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.06~3.89 (12dt, J = 8.0, 2.0 Hz, 2H, $2H_1$), 3.76~3.55 (5d, J = 8.0 Hz, 2H, $2H_3$), 3.53 (s, 3H, OMe), 3.31 (md, J = 8.0 Hz, 2H, $2H_3$), 2.98 (m, 2H, $2H_4$), 2.76, 2.67 (2br, 2H, $2H_5$),

2.47 (hep, 1H, *iso*-H), 1.86~1.70 (m, 2H, $2H_6$), 1.37~1.11 (8d, J = 8.0 Hz, 2H, $2H_6$), 1.06, 0.97 (2d, J = 8.0 Hz, 6H, MeCHMe). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.1, 169.3, 168.1, 168.1, 60.9, 60.1, 51.6, 51.2, 50.7, 48.4, 47.4, 46.5, 41.2, 40.4, 37.7, 36.3, 36.1, 31.9, 19.4, 18.9. HRMS**:** *m/z* found 321.1818, calcd. for $C_{17}H_{24}N_2O_4$ (M + H) 321.1809. Optical rotation $[\alpha]_D$ of (-)-**60**: -68.0 (20 °C, c = 2.0, chloroform).

Preparation of trimer (-)-61

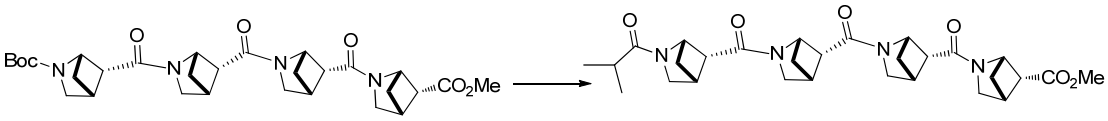


(-)-9c (-)-61

Trimer (-)-**9c** (37 mg, 0.08 mmol) was dissolved in 4 N HCl/dioxane (0.5 mL) and stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and further dried under high vacuum to remove all solvent. To this residue, DCM (5 mL) was added. The resulting solution was chilled with an ice bath and isobutyryl chloride (17 mg, 0.16 mmol) was added dropwise. The mixture was stirred with an ice bath for 15 min and then at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was chromatographed (gradient, 4:1 ethyl acetate/ hexanes) to afford 27 mg (77%) of amide (**-**)**-61** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as a colorless oil. R_f = 0.14 (1:2 hexane/ethyl acetate). ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.12~4.41 (12dt, J = 8.0, 2.0 Hz, 3H, $3H_1$), 3.89~3.52 (8d, J = 8.0 Hz, 3H, $3H_3$), 3.61 (s, 3H, OMe), 3.36 (m, 3H, $3H_3$), 3.19~3.05 (3dt, J = 8.0, 2.0 Hz), 3H, $3H_4$), 2.89~2.77 (m, 3H, $3H_5$), 2.54 (hep, J = 8.0 Hz, 1H, *iso*-H), 1.91~1.76 9m, 3H, $3H_6$), 1.53~1.22 (md, J =

8.0 Hz, 3H, $3H_6$), 1.14 (2d, J = 8.0 Hz, 6H, 2Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.1, 169.5, 169.3, 168.1, 168.1, 60.9, 60.6, 60.1, 51.6, 51.2, 50.7, 48.3, 47.4, 46.5, 41.2, 40.7, 40.4, 37.7, 36.3, 36.0, 32.3, 31.9, 19.7, 19.4, 18.9. HRMS**:** *m/z* found 430.2354, calcd. for $C_{23}H_{31}N_3O_5$ (M + H) 430.2336. Optical rotation $[\alpha]_D$ of (-)-**61**: -54.5 (20 °C, c = 2.0, chloroform).

Preparation of tetramer (-)-62

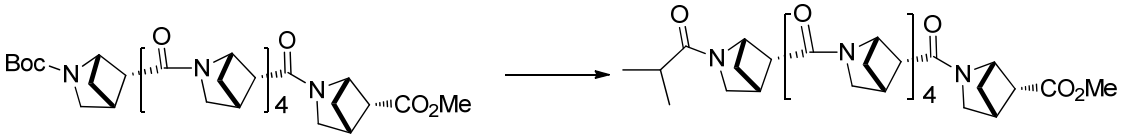


(-)-9d (-)-62

Tetramer (-)-**9d** (46 mg, 0.08 mmol) was dissolved in 4 N HCl/dioxane (0.5 mL) and stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and further dried under high vacuum to remove all solvent. To this residue, DCM (5 mL) was added. The resulting solution was chilled with an ice bath and isobutyryl chloride (17 mg, 0.16 mmol) was added dropwise. The mixture was stirred with an ice bath for 15 min and then at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was chromatographed (gradient, 4:1 ethyl acetate/ hexanes) to afford 31 mg (72%) of amide (**-**)**-62** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as a colorless oil. R_f = 0.18 (1:3 hexane/ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.03~4.32 (mdt, J = 8.0, 2.0 Hz, 4H, $4H_1$), 3.77~3.35 (m, 8H, $8H_3$), 3.52 (s, 3H, OMe), 3.04 (m, 4H, $4H_4$), 2.69 (m, 4H, $4H_5$), 2.46 (hep, J = 8.0 Hz, 1H, *iso*-H), 1.85~1.72 (m, 4H, $4H_6$), 1.33~1.09 (md, J = 8.0 Hz, 6H, $6H_6$), 1.05, 0.97 (2d, J = 8.0 Hz, 6H, 2Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.2, 169.0, 168.0, 60.8, 60.4, 60.1, 51.8, 51.4, 50.9, 50.4,

50.3, 48.1, 47.7, 46.4, 41.0, 40.6, 40.4, 40.3, 37.7 (minor), 37.5, 36.3, 36.2, 35.8, 31.8, 19.2, 18.8. HRMS**:** *m/z* found 539.2893, calcd. for $C_{29}H_{38}N_4O_6$ (M + H) 539.2864. Optical rotation $[\alpha]_D$ of (-)-**62**: -47.2 (20 °C, c = 1.2, chloroform).

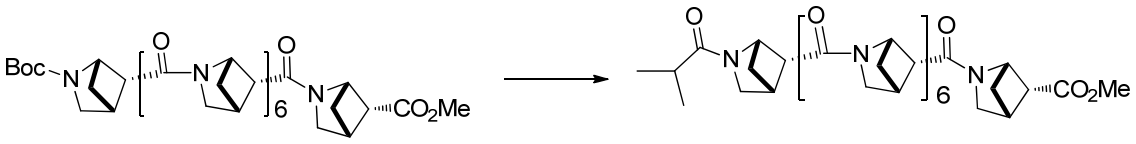
Preparation of hexamer (-)-63



(-)-9e **(-)-63**

N-Boc-Hexamer (-)-**9e** (39 mg, 0.05 mmol, Dr. Nian Liu's sample[30]) was dissolved in 4 N HCl/dioxane (0.4 mL) and stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and further dried under high vacuum to remove all solvent. To this residue, DCM (5 mL) was added. The resulting solution was chilled with an ice bath and isobutyryl chloride (15 mg, 0.14 mmol) was added. The mixture was stirred with an ice bath for 15 min and then at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was chromatographed (gradient, 1:10 MeOH/ CH_2Cl_2) to afford 28 mg (74%) of amide (-)-**62** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as a colorless oil. R_f = 0.48 (1:10 MeOH/ CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.10~4.40 (m, 6H), 3.79~3.22 (m, 12H), 3.61 (s, 3H, OMe), 3.18~3.04 (m, 6H), 2.77 (m, 6H), 2.55 (hep, *J* = 8.0 Hz, 1H, *iso*-H), 1.89~1.72 (m, 6H), 1.39~1.18 (m, 12H), 1.13, 1.04 (2d, *J* = 8.0 Hz, 6H, 2Me). HRMS**:** *m/z* found 757.3895, calcd. for $C_{41}H_{53}N_6O_8$ (M + H) 757.3880. Optical rotation $[\alpha]_D$ of (-)-**63**: -33.7 (20 °C, c = 1.0, chloroform).

Preparation of octamer (-)-64



(-)-9f **(-)-64**

N-Boc-Octamer (-)-**9f** (40 mg, 0.04 mmol, Dr. Nian Liu's sample[30]) was dissolved in 4 N HCl/dioxane (0.4 mL) and stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and further dried under high vacuum to remove all solvent. To this residue, DCM (5 mL) was added. The resulting solution was chilled with an ice bath and isobutyryl chloride (15 mg, 0.14 mmol) was added. The mixture was stirred with an ice bath for 15 min and then at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was chromatographed (gradient, 1:10 MeOH/ CH_2Cl_2) to afford 28 mg (70%) of amide (-)-**64** (100% pure by SB-C18 column, λ = 214 nm, flow rate = 1.0 mL/min) as a colorless oil. R_f = 0.43 (1:10 MeOH/ CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.03~4.31 (m, 8H), 3.71~3.14 (m, 16H), 3.52 (s, 3H, OMe), 3.08~2.97 (m, 8H), 2.72~2.62 (m, 8H), 2.45 (hep, J = 8.0 Hz, 1H, *iso*-H), 1.86~1.63 (m, 8H), 1.37~1.108 (m, 16H), 1.04, 0.96 (2d, J = 8.0 Hz, 6H, 2Me). HRMS**:** *m/z* found 975.4921, calcd. for $C_{53}H_{67}N_8O_{10}$ (M + H) 975.4935. Optical rotation $[\alpha]_D$ of (-)-**64**: -37.4 (20 °C, c = 0.9, chloroform).

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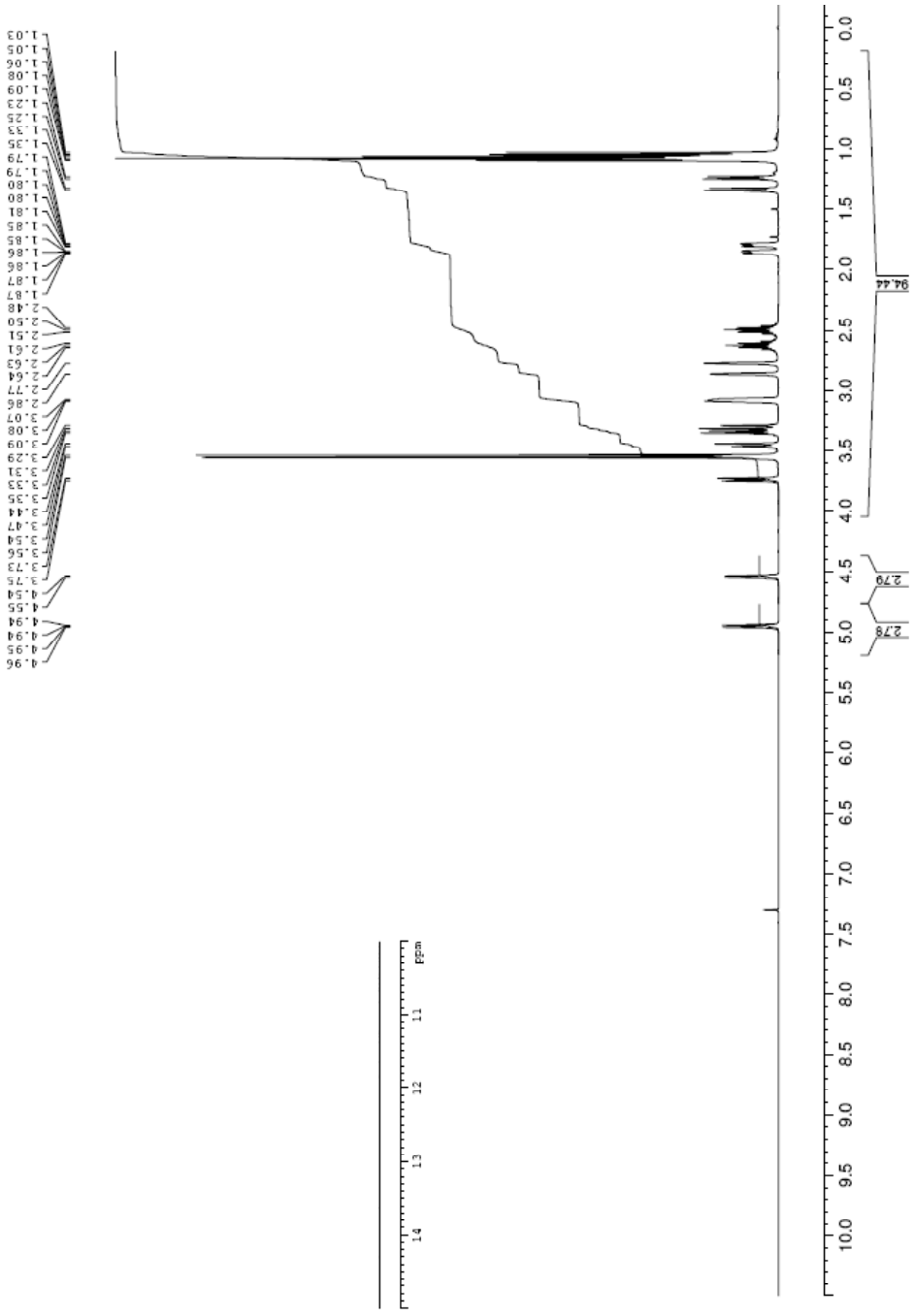
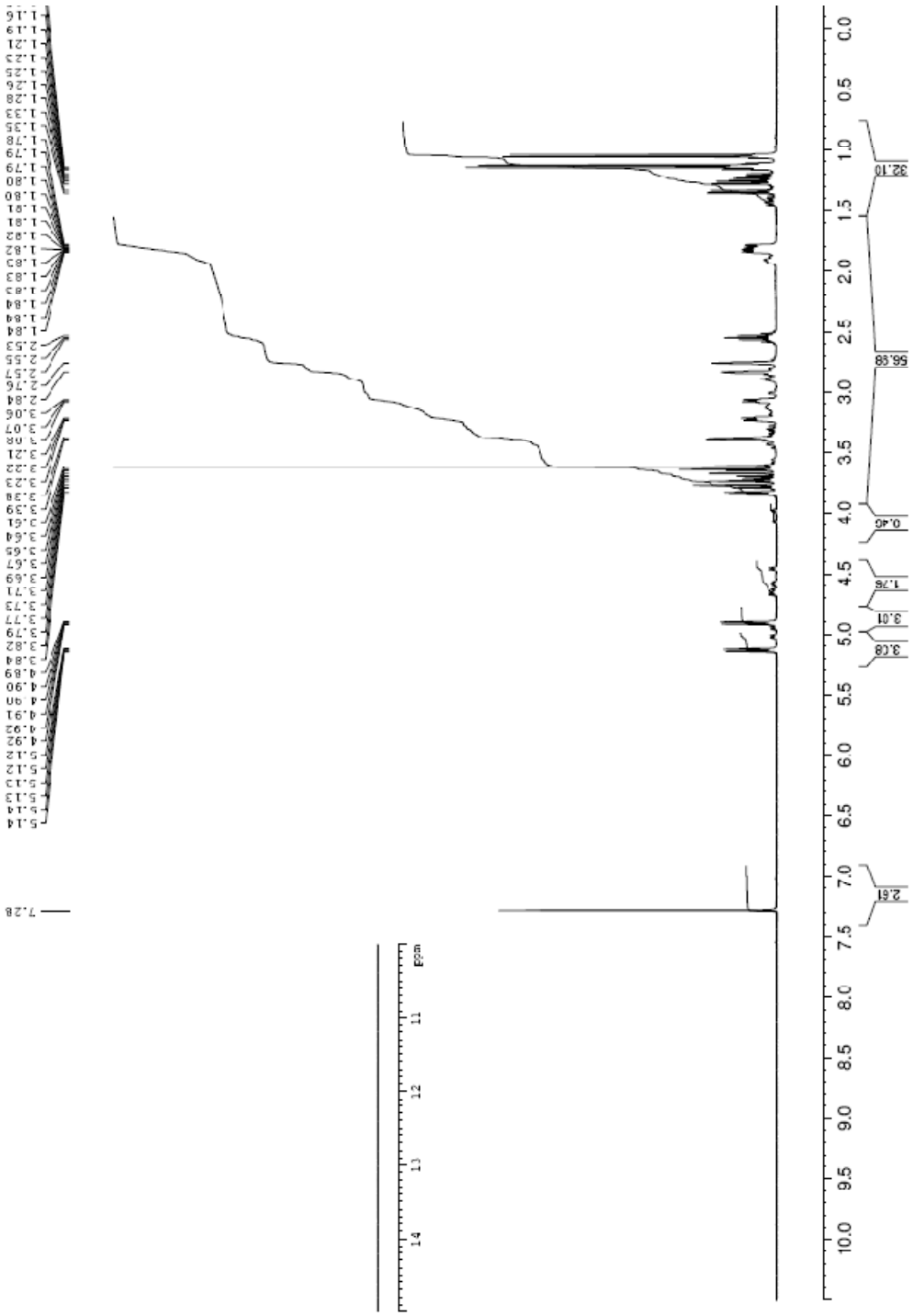
APPENDIX A

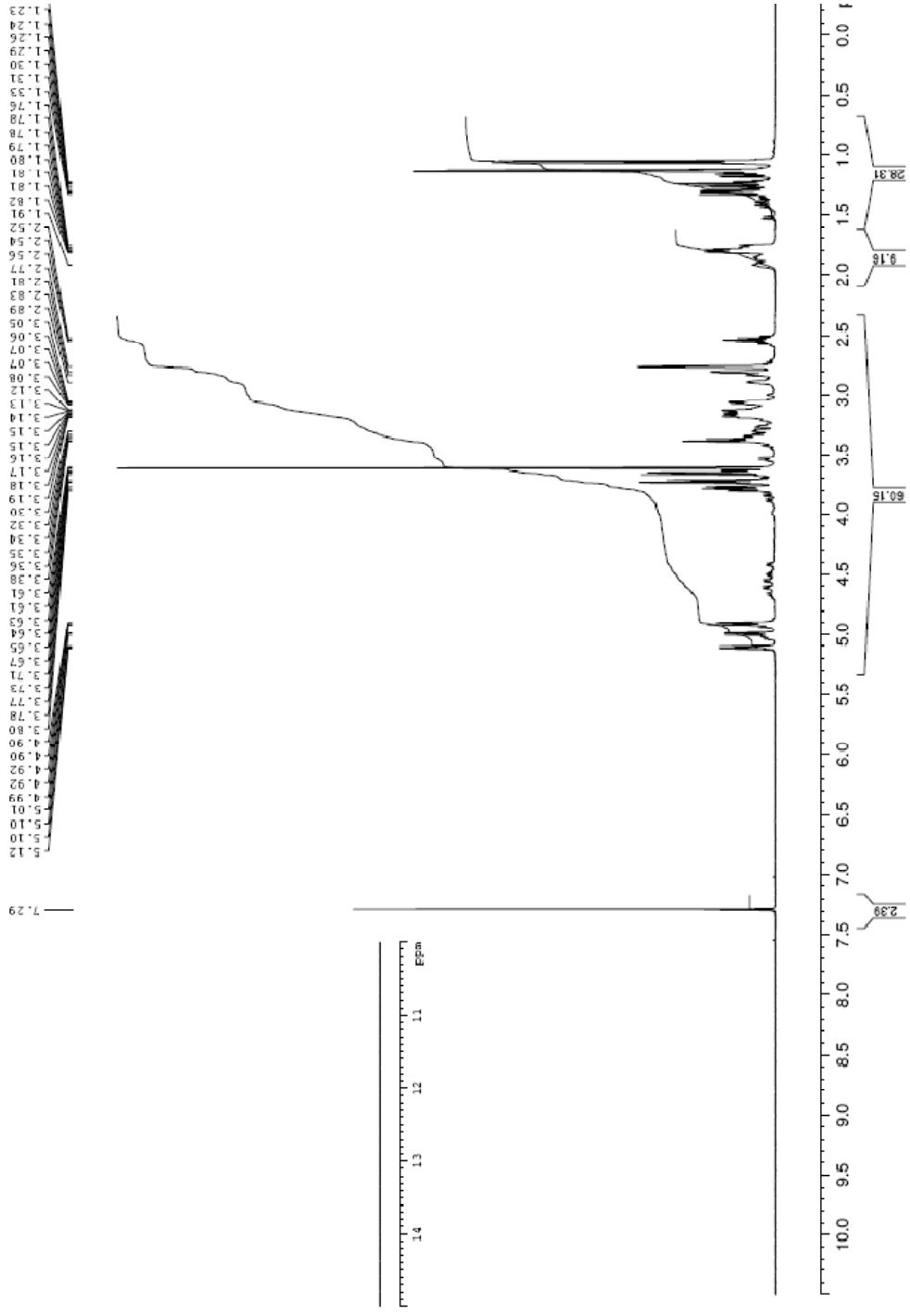
^{1}H NMR Spectra of *N*-Isobutyryl Monomer (-)-**59**



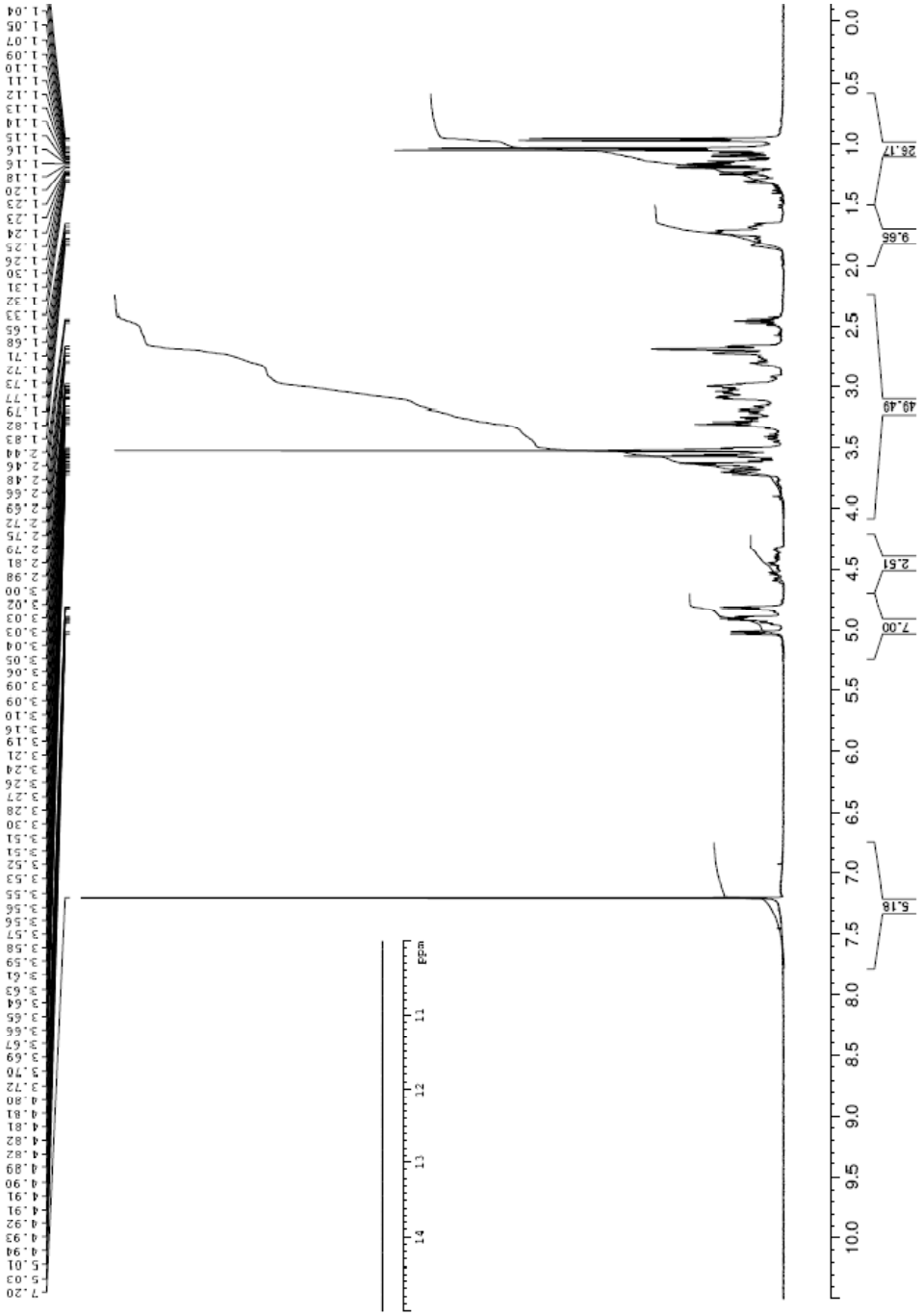
^{1}H NMR Spectra of *N*-Isobutyryl Dimer (-)-**60**



^{1}H NMR Spectra of *N*-Isobutyryl Trimer (-)-**61**



^{1}H NMR Spectra of *N*-Isobutyryl Tetramer (-)-**62**



APPENDIX B

HPLC METHOD DESCRIPTION

Chromatographic Conditions

Column: *Agilent®, SB-C18,1.8 um, 3.0 x 50 mm, Part #USHEH02101.*

Mobile Phase: *A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile*

Flow rate: 1.0 mL/min
Injection volume: 5 uL
Stop time: 9.60 min

Oven Temperature: 60 °C
Detection: 254, 214 nm
Post time: 1.20 min

Sample Preparation

Samples dissolved in methanol.

Timetable

	Time (min)	Solvent B%
1	0.00	30%
2	8.00	65%
3	8.10	95%
4	9.50	95%
5	9.60	30%

Retention Times

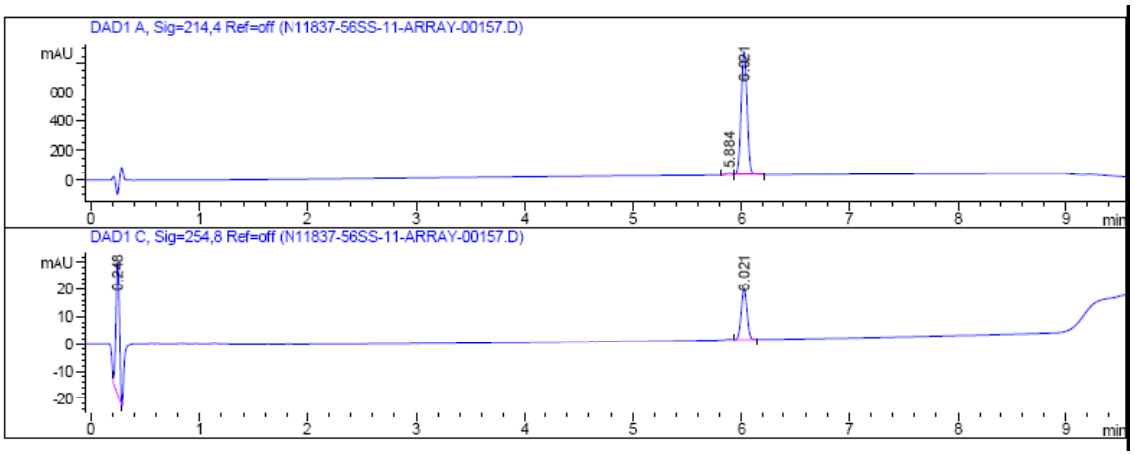
(-)-Amide **34**: ~6.00 minutes

(+)-Amide **35**: ~5.88 minutes

(+)-Amide **54**: ~4.89 minutes

(-)-Amide **55**: ~5.14 minutes

HPLC Analysis of Amide (-)-**34:**



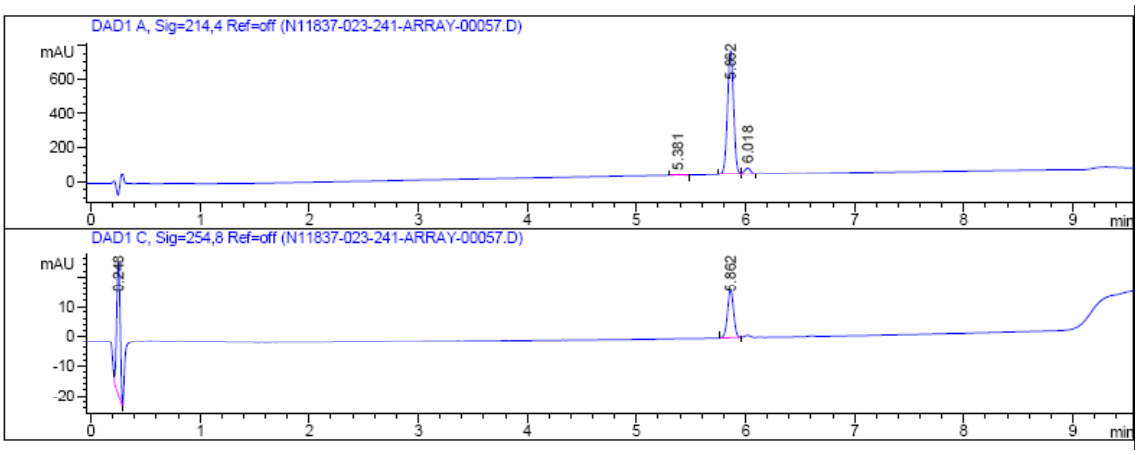
Signal 1: Sig=214 nm:

Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	6.021	3229.00	99.17
2 (+)-Amide **35**	5.884	27.08	0.83
Totals		3256.08	100.00

Signal 2: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	6.021	68.01	100.00
Totals		68.01	100.00

HPLC Analysis of Amide (+)-**35:**



Signal 1: Sig=214 nm:

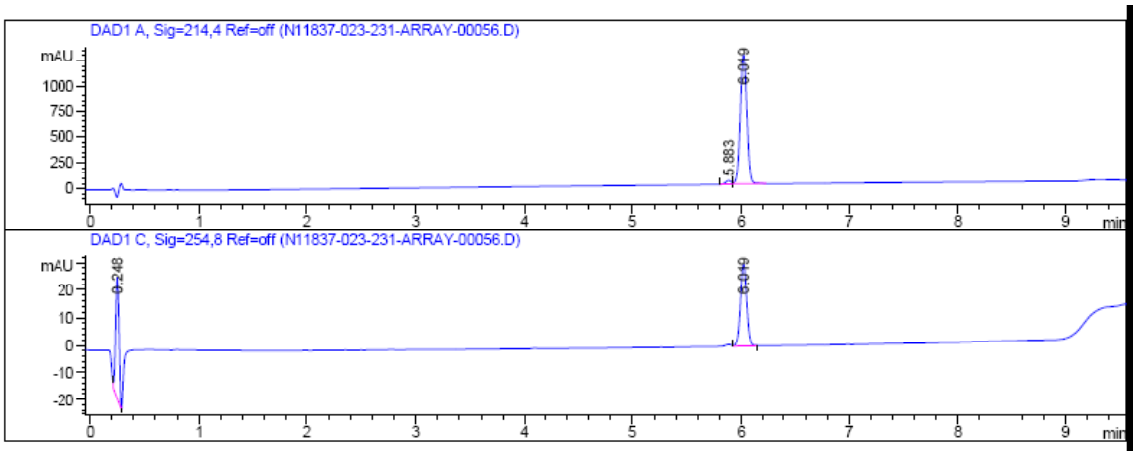
Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	6.018	57.42	1.97
2 (+)-Amide **35**	5.862	2849.65	98.03
Totals		2907.07	100.00

Note: Retention Time = 5.381 (impurity)

Signal 2: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
2 (+)-Amide **35**	6.018	61.95	100.00
Totals		61.95	100.00

HPLC Analysis of Amides (-)-**34** (99%) and (+)-**35** (1%):



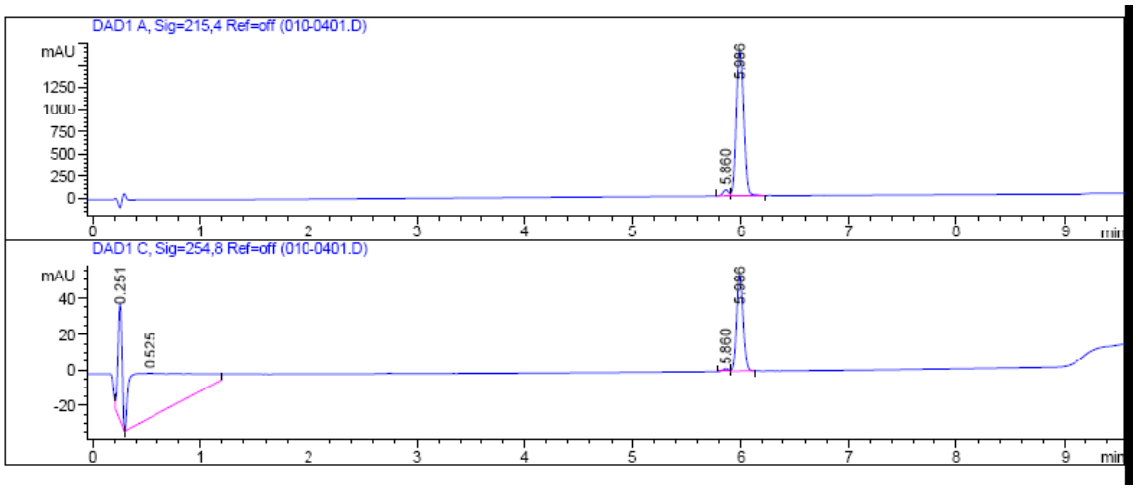
Signal 1: Sig=214 nm:

Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	6.019	5308.06	98.47
2 (+)-Amide **35**	5.883	82.41	1.53
Totals		5390.47	100.00

Signal 2: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	6.019	119.20	100.00
Totals		119.20	100.00

HPLC Analysis of Amides (-)-**34** (97%) and (+)-**35** (3%):



Signal 1: Sig=214 nm:

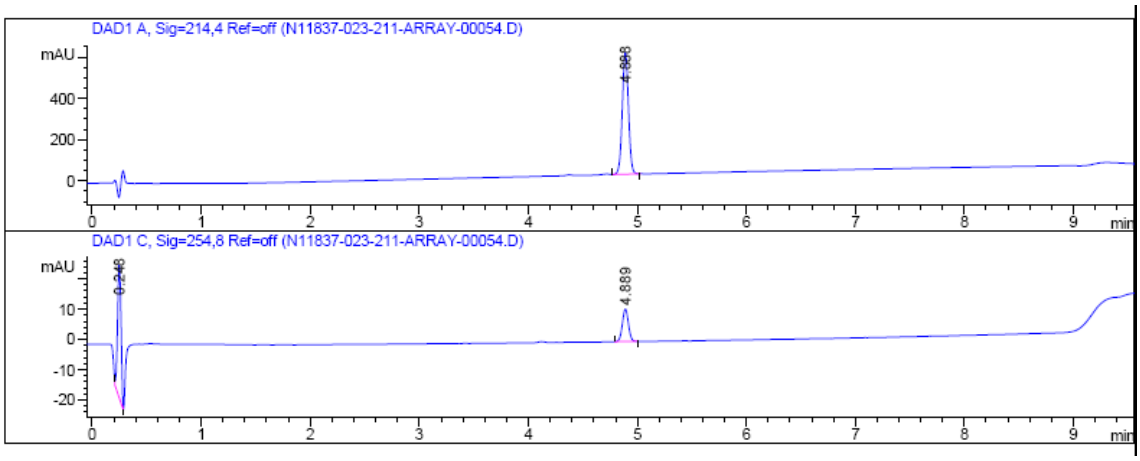
Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	5.986	7869.45	96.85
2 (+)-Amide **35**	5.860	255.88	3.15
Totals		8125.33	100.00

Signal 2: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
1 (-)-Amide **34**	5.986	225.39	97.54
2 (+)-Amide **35**	5.860	5.69	2.46
Totals		231.08	100.00

Conclusion: Signals with λ= 214 nm are more sensitive and can be used to identify the purity of the amide.

HPLC Analysis of Amide (+)-**54**:



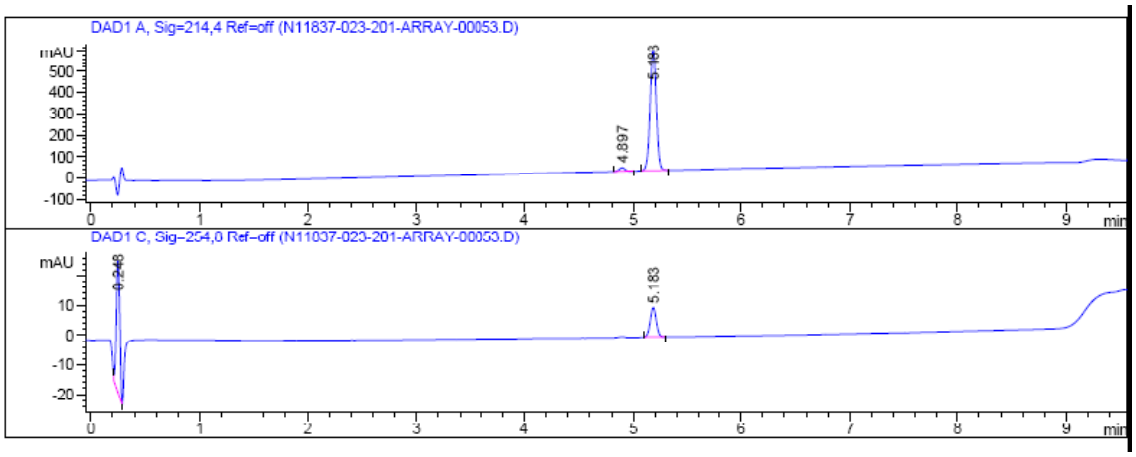
Signal 1: Sig=214 nm:

Pk #	Retention Time	Area	Area Percent
1 (+)-Amide **54**	4.888	2326.00	100.00
Totals		2326.00	100.00

Signal 2: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
1 (+)-Amide **54**	4.888	42.00	100.00
Totals		42.00	100.00

HPLC Analysis of Amide (-)-**55:**



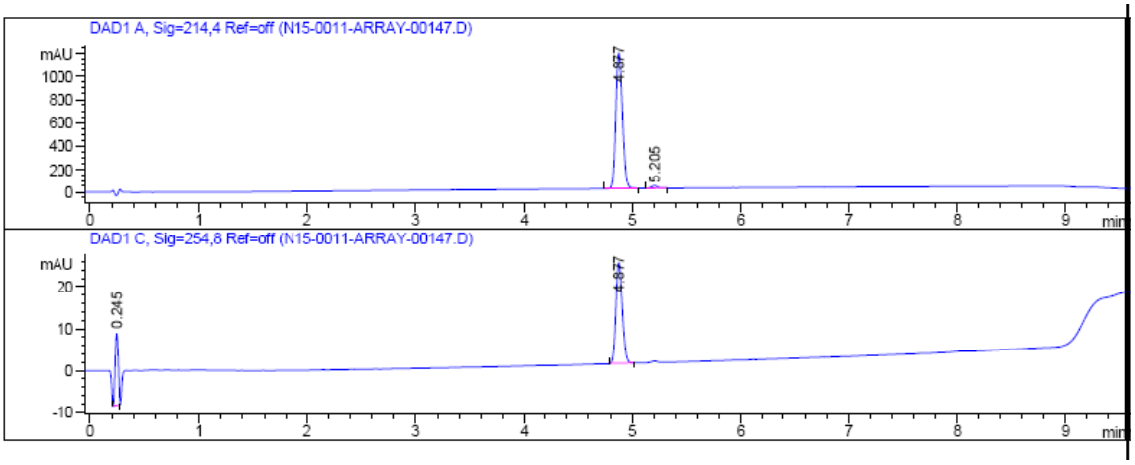
Signal 1: Sig=214 nm:

Pk #	Retention Time	Area	Area Percent
1 (+)-Amide **54**	4.897	35.18	1.54
2 (-)-Amide **55**	5.183	2249.36	98.46
Totals		2284.54	100.00

Signal 1: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
2 (-)-Amide **55**	5.183	38.86	100.00
Totals		38.86	100.00

HPLC Analysis of Amides (+)-**54** (99%) and (-)-**55** (1%):



Signal 1: Sig=214 nm:

Pk #	Retention Time	Area	Area Percent
1 (+)-Amide **54**	4.877	7528.42	98.75
2 (-)-Amide **55**	5.206	95.22	1.25
Totals		7623.64	100.00

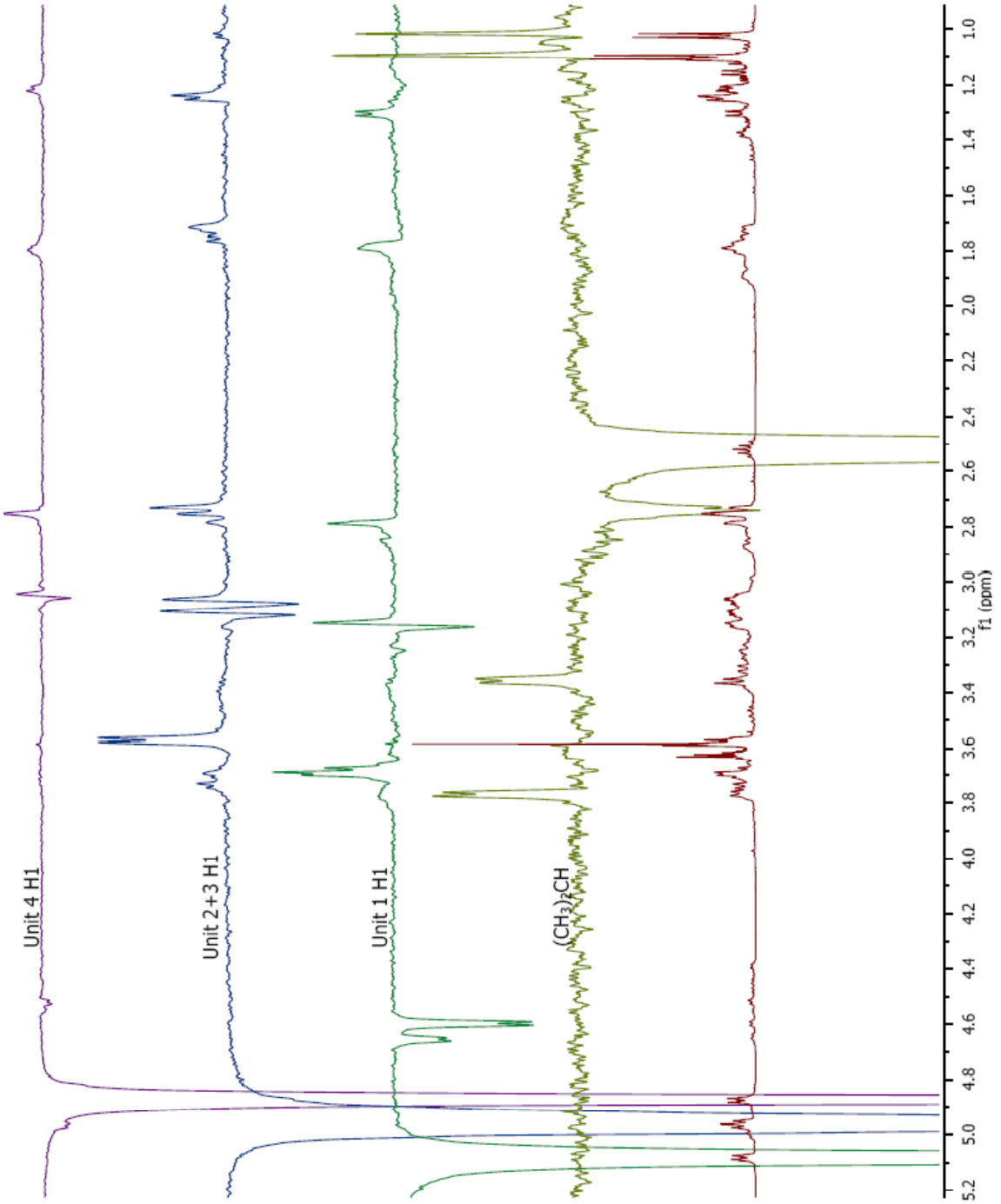
Signal 2: Sig=254 nm:

Pk #	Retention Time	Area	Area Percent
1 (+)-Amide **54**	4.877	165.07	100.00
Totals		165.07	100.00

Conclusion: Signals with λ= 214 nm are more sensitive and can be used to identify the purity of the amide.

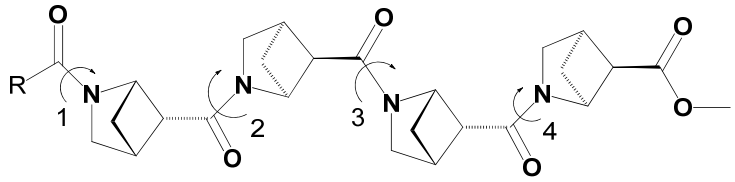
APPENDIX C

Selective NOE Analysis of Tetramer (-)-**62**



APPENDIX D

Spartan MMFF Torsion Angles (ω) Calculation of Tetramer (-)-**9d** and (-)-**62**



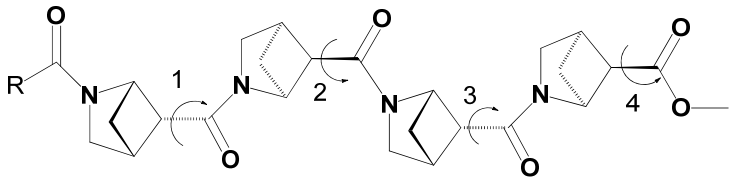
R = *t*-BuO, (-)-**9d**

R = iso-propyl, (-)-**62**

	Spartan MM of (-)-**9d**	X-ray structure of (-)-**9d**	Spartan MM of (-)-**62**
Unit	Torsion angles ω (°) O-C-N-C_3	Torsion angles ω (°) O-C-N-C_3	Torsion angles ω (°) O-C-N-C_3
1	166.98	171.9	161.61
2	167.17	171.4	169.51
3	168.02	176.6	167.77
4	171.43	171.9	171.41

APPENDIX E

Spartan MMFF Torsion Angles (ψ) Calculation of Tetramer (-)-**9d** and (-)-**62**



R = *t*-BuO, (-)-**9d**

R = iso-propyl, (-)-**62**

	Spartan MM of (-)-**9d**	X-ray structure of (-)-**9d**	Spartan MM of (-)-**62**
Unit	Torsion angles ψ (°) C_1-C_5-C(O)-N	Torsion angles ψ (°) C_1-C_5-C(O)-N	Torsion angles ψ (°) C_1-C_5-C(O)-N
1	56.28	45.73	59.09
2	61.63	76.09	58.28
3	62.85	86.17	62.24
4	63.96	55.79	64.49

APPENDIX F

List of Publications

1. Krow, G.; Yuan, J.; **Lin, G.**; Sonnet, P. E. "The Rearrangement Route to 3-CH_2X-2-azabicyclo[2.1.1]hexanes. Substituent Control of Neighboring Group Participation" *Org. Lett*. **2002**, *4*, 1259-1262.

2. Krow, G.; Herzon, S. B.; **Lin, G.**; Qiu, F.; Sonnet, P. E. "Complex-Induced Proximity Effect. Temperature-Dependent Regiochemical Diversity in Lithiation-Electrophilic Substitution Reactions of *N*-Boc-2-Azabicyclo [2.1.1] hexane. 2, 4- and 3, 5-Methanoprolines" *Org. Lett*. **2002**, *4*, 3151-3154.

3. Krow, G.; Lester, W. S.; **Lin, G.**; Fang, Y.; Carroll, P. J. "Chlorosulfonyl Isocyanate Reactions with *N*-(Alkoxycarbonyl)-2-azabicyclo[2.1.1]hex-5-enes. Regiospecific Two Atom Insertion Pathways" *J. Org. Chem*. **2003**, *68*, 1626-1629.

4. Krow, G.; **Lin, G.**; Rapolu, D.; Fang, Y.; Lester, W. S.; Herzon, S. B.; Sonnet, P. E. "The Rearrangement Route to 2-Azabicyclo[2.2.0]hexanes. Solvent Control of Neighboring Group Participation" *J. Org. Chem*. **2003**, *68*, 5292-5299.

5. Krow, G.; **Lin, G.**; Herzon, S. B.; Thomas, A. M.; Moore, K. P.; Huang, Q.; Carroll, P. J. "Convenient Preparations of 2,4-Methanopyrrolidine and 5-Carboxy-2,4-methanopyrrolidines" *J. Org. Chem*. **2003**, *68*, 7562-7564.

6. Krow, G.; **Lin, G.**; Yu, F.; Sonnet, P. E. "A Second-Chance Rearrangement Route to Novel 5(6)-*syn, anti*-Difunctional 2-Azabicyclo[2.1.1]hexanes" *Org. Lett.* **2003**, *5*, 2739-2741.

7. Krow, G. R.; **Lin, G**.; Moore, K. P.; Thomas, A. M.; DeBrosse, C.; Ross, C. W., III; Ramjit, H. G. "Novel Selectfluor and Deoxo-Fluor-Mediated Rearrangements. New 5(6)-Methyl and Phenyl Methanopyrrolidine Alcohols and Fluorides" *Org. Lett.* **2004**, *6*, 1669-1672.

8. Jenkins, C. L.; **Lin, G**.; Duo, J.; Rapolu, D.; Guzei, I. A.; Raines, R. T.; Krow; G. R. "Substituted 2-Azabicyclo[2.1.1]hexanes as Constrained Proline Analogs: Implications for Collagen Stability" *J. Org. Chem*. **2004**, *69*, 5865-5873.

9. Krow, G.; **Lin, G.**; Yu, F. "The Rearrangement Route to 3-Carboxy- and 3-Hydroxymethyl-2-azabicyclo[2.1.1]hexanes 2. 3,5-Methanoprolines" *J. Org. Chem*. **2005**, *70*, 590-595.

10. Krow, G.; Huang, Q.; **Lin, G**.; Centafont, R. A.; Thomas, A. M.; Gandla, D.; DeBrosse, C.; Carroll, P. J. "5-Carboxy-2-azabicyclo[2.1.1]hexanes as Precursors of 5-Halo, Amino, Phenyl, and 2-Methoxycarbonylethyl Methanopyrrolidines*" J. Org. Chem.* **2006**, *71*, 2090-2098.

11. Krow, G.; Gandla, D.; Guo, W.; Centafont, R. A.; **Lin, G**.; DeBrosse, C.; Sonnet, P. E.; Ross, C. W., III; Ramjit, H. G.; Carroll, P. J.; Cannon, K. C. "Neighboring Group Participation in the Additions of Iodonium and Bromonium Ions to *N*-Alkoxycarbonyl-2-azabicyclo[2.2.*n*]alk-5-enes (*n* = 1,2)" *J. Org. Chem*. **2008**, *73*, 2114-2121.

12. Krow, G.; Gandla, D.; Guo, W.; Centafont, R. A.; **Lin, G**.; DeBrosse, C.; Sonnet, P. E.; Ross, C. W., III; Ramjit, H. G.; Cannon, K. C. "Selectfluor as a Nucleofuge in the Reactions of Azabicyclo[*n*.2.1]alkane-Halocarbamic Acid Esters (*n* = 2,3)" *J. Org. Chem*. **2008**, *73*, 2122-2129.

13. Krow, G.; Edupuganti, R.; Gandla, D.; Choudhary, A.; **Lin, G**.; Sonnet, P. E.; DeBrosse, C.; Ross, C. W., III; Cannon, K. C.; Raines, R. "5(6)-*anti*-Substituted-2-azabicyclo[2.1.1]hexanes: A Nucleophilic Displacement Route" *J. Org. Chem*. **2009**, *74*, 8232-8242.