Design and Stereoselective Synthesis of Novel Bicyclic β-Turn Dipeptide Mimetics and cis-4-Substituted Proline Analogues for Peptides and Peptidomimetics

by

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Dedicated to

My wife Xiang Gao

My parents Xingkang Zhang and Quandi Gao
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ABSTRACT

A central goal of modern biology is to develop a detailed, predictive understanding of the relationships of three-dimensional structure and biological function. However, to establish the biologically active conformation is challenging because most small linear peptides are inherently flexible, and at present, our knowledge of 3D structural information of ligand-receptor complexes is very limited. Hence, some strategies have been developed to prepare peptidomimetics with constrained conformations. Both local conformational constraints and global conformational constraints can provide important insights into the structural and topographical basis of biological activity.

A series of novel cis-4-substituted proline analogues were designed and synthesized. Highly stereoselective alkylations at the γ-position of glutamic ester were achieved, followed by reduction, mesylation, and cyclization to afford the proline derivatives in good yields and high diastereoselectivity. These cis-4-substituted proline analogues could be used as conformationally restricted templates in local constrained peptidomimetics.

We also have developed a general and efficient approach for the synthesis of indolizidinone amino acids with stereospecific appendages of side chain functionality at both the C-4 and C-8 positions, which can serve as restricted reverse turn mimetics in global constrained peptidomimetics. Our synthetic reverse turn mimetic targets were designed to serve as surrogates of the dipeptides Phe-Gly and Phe-Arg which contain two important pharmacophore elements in Leu-Enkephalin and melanotropin peptides,
respectively. Introduction of side chain functionality at C-8 was achieved by using β-substituted pyroglutamate as a synthetic precursor which was prepared via Michael addition reaction between a Ni(II) complex of the chiral Schiff base of glycine with (S)-o-[N-(N-benzylprolyl)amino]benzophenone and 3-(trans-enoyl)-oxazolidin-2-one. The side chains at C-4 were introduced by bromination of dehydroamino acid intermediates followed by Suzuki cross-coupling.
CHAPTER 1. DESIGN OF PEPTIDOMIMETICS FROM PEPTIDE LEADS—-CONFORMATIONAL AND TOPOGRAPHICAL CONSIDERATIONS

1.1 Introduction

In 1902, Emil Fischer and Frank Hofmeister revealed that proteins are composed of amino acids, which are linked via "peptide bonds".\(^1\)\(^-\)\(^4\) The progress following this finding was initially slow due to difficulties in synthesis and structure determination of peptides. However, some great advances came in the 1950's. First of all, duVigneaud isolated, determined the structure of, and accomplished the total synthesis of oxytoxin;\(^5\) then, Sanger elucidated the structure of bovine insulin;\(^6\)\(^-\)\(^9\) and the double helix of DNA was discovered, suggesting that there is a connection between nucleotide and peptide sequences.\(^10\)\(^,\)\(^11\) With discovery of more and more biologically active peptides, today it has been well known that peptides exert essential influences on all vital physiological processes via inter- and intracellular communication, and signal transduction mediated through various classes of receptors.\(^12\) As neurotransmitters, neuromodulators, hormones, antibiotics, growth factors, cytokines, antigens, etc., peptides play critical roles in the maintenance of human health, in behavior and in many diseases. Therefore, numerous native peptides and proteins have been isolated and applied as therapeutically useful drugs.

Although numerous native peptides have great potential for medical applications and the fact is that over 50% of all current drugs are peptide-based, the peptides have to be modified to overcome certain problems, such as metabolic stability, selectivity, and
bioavailability, to be suitable therapeutic agents. Thus modified peptide and peptidomimetic research has dramatically advanced during the last two decades.\textsuperscript{13-16} One of the most challenging parts in the research is the rational design of peptidomimetics. The first phase in this approach is to identify the key amino acid residues which are necessary for receptor recognition. This is usually accomplished by single amino acid modification in the peptide, known as alanine & D-amino acid scans.\textsuperscript{17} Once the SAR of the peptide ligand is elucidated, the conformation-activity relationship has to be studied. The three-dimensional arrangement of critical side groups and backbone functionalities can be analyzed via NMR spectroscopy,\textsuperscript{18,19} X-ray crystallography,\textsuperscript{20} circular dichroism measurements,\textsuperscript{21} and computational methods.\textsuperscript{22} The next effort is to find a suitable organic moiety that can replace the peptide scaffold and position the crucial recognition elements correctly. The general scheme of the \textit{de novo} design of peptidomimetics is outlined in Fig. 1-1. As we can see in the scheme, understanding the conformation-activity relationships of biologically active peptides can provide important guidance in the design of peptidomimetics and accelerate the process from native peptides to biologically active peptidomimetics and small molecules.
Figure 1-1. A de novo approach for peptidomimetic design.23
1.2 Conformational and Topographical Features of Peptides — Challenges in the Studies of Biologically Active Conformation

As a key step in the *de novo* approach to peptidomimetics, to establish the biologically active conformation is very challenging. This is partly due to our limited knowledge of the conformations of ligand-receptor complexes. Another major problem we have to overcome is the high degree of flexibility of small linear peptides.

Figure 1-2. Conformations of peptides. (A) Definition of the $\phi$, $\psi$, $\omega$, $\chi$ torsional angles; (B) Newman projection of the three staggered side-chain rotamers in L-amino acids.
As illustrated in Fig. 1-2, each side chain's dihedral angle, referred as chi (χ) space, can adopt three low energy staggered conformations (rotamers), which are referred to as gauche(+), gauche(-) and trans. Therefore, even in a small peptide with four or five residues, these different dispositions of side chain groups will give rise to a great diversity in 3D conformations. Moreover, other torsional angles of the backbone, φ, ψ, and ω, make the topographical issue more complicated. These angles determine the major secondary structures of peptides, such as α-helix, β-sheet, β-turn, and extended conformations (Fig. 1-3), which have been shown to be the energetically preferred backbone conformations for a peptide.24,25

![Figure 1-3. Torsional angles of an α-helix, β-sheet and type I and I’ β-turn conformations.](image)

Studies have shown that both the secondary structural features of peptide and the 3D structure (topography) of the amino acid side chain moieties play critical roles in ligand-receptor recognition events. Thus identification of the side chain rotamer and the secondary structural feature that is present in the superpotent or bioselective ligand can
provide a valuable tool to aid in the development of peptidomimetics with better potencies and selectivities. Some strategies to obtain such information will be discussed next.

1.3 Strategies in the Design of Peptidomimetics

1.3.1. Modification of Amino Acid Side Chains

If conformational flexibility of the side chain groups can be restricted to a greater degree, peptides can provide a more complete evaluation of their biologically active three-dimensional topologies. Usually, the side chain conformation can be controlled in several ways. One general approach is to introduce an alkyl group at the β-position or on the aromatic ring of an amino acid residue. These kinds of modifications can constrain $\chi^1$ and $\chi^2$ angles; on the other hand, they generally do not perturb the backbone conformation drastically, and still allow the peptides to have some degree of flexibility. In a similar way, substitution on the aromatic ring of an aromatic amino acid will limit the conformational flexibility of a peptide to a moderate degree. Furthermore, the introduction of alkyl groups will enhance the lipophilicity and thus help peptide binding to receptors and crossing of membrane barriers.

The Hruby group has been engaged in the asymmetric synthesis of $\chi$-constrained amino acids for over a decade. Fig. 1-4 gives structures of some novel amino acids prepared in the Hruby group. Incorporation of these novel highly constrained amino acids into peptides and studies of such peptidomimetics have provided a very valuable approach to probe the stereochemical requirements of binding pharmacophores for recognition of receptors.
Among natural amino acids, proline is unique with a constrained cyclic system. Proline can be used as a rigid template in design of conformationally constrained peptidomimetics. Substituted proline derivatives are particularly attractive since the substitution can influence not only the conformation of the pyrrolidine ring, but the rate of cis-trans isomerization about the amide bond as well. In Chapter 2, a novel approach to synthesis of 4-substituted prolines will be discussed.
Figure 1-4. \( \chi \)-Constrained amino acids synthesized in the Hruby group.

1.3.2 Modification of the Peptide Backbone

Another strategy in the design of peptidomimetics is peptide backbone modifications which generally refer to the isosteric or isoelectronic exchange of NHCO units in the
peptide chain or introduction of additional groups. Some of the most frequent modifications to the peptide backbone are listed in Fig. 1-5.

Exchange of individual units

\[ \begin{align*}
\text{N} & \quad \begin{array}{c} \text{R} \\ \text{H} \end{array} \quad \begin{array}{c} \text{C} \\ \text{H} \end{array} \quad \begin{array}{c} \text{C} \\ \text{O} \end{array} \\
\text{O} & \quad \begin{array}{c} \text{N} \\ \text{C} \end{array} \quad \begin{array}{c} \text{C} \\ \text{S} \end{array} \\
\text{S} & \quad \begin{array}{c} \text{C-alkyl} \\ \text{H}_2 \end{array}
\end{align*}\]

Extension of peptide chain

\[ \begin{align*}
\text{R} & \quad \begin{array}{c} \text{-NH-X-CH-CO-} \\ \text{X = O, S, CH}_2 \end{array}
\end{align*}\]

Replacement of amide bond

\[\begin{align*}
\text{-CO-NH-} \\
\text{-NH-CO-} & \quad \text{retro-inverso} \\
\text{-CH(OH)-CH}_2 & \quad \text{hydroxyethylene} \\
\text{-CH=CH-} & \quad \text{E-alkene} \\
\text{-CH}_2-\text{CH}_2 & \quad \text{carba}
\end{align*}\]

**Figure 1-5.** The most frequent modifications to the peptide backbone.

The modification to the peptide backbone can also serve to introduce local backbone constraints. For example, \(N\)-alkylation greatly restricts the \(\phi\) torsional angle but eliminates the hydrogen bonding capability of the amide bond. \(N\)-Methyl amino acids have been incorporated into bioactive mimetics of opioid peptides, bradykinin, thyrotropin releasing hormone (THR), angiotensin II, and cholecystokinin (CCK). Other backbone modifications include retro-inverso, reduced amide, thiomethylene,
oxomethylene,\textsuperscript{41} ethylene,\textsuperscript{42,43} thioamide,\textsuperscript{44} olefinic\textsuperscript{45,46} and ketomethylene\textsuperscript{47} analogues and many others, each of which has its own unique stereoelectronic and stereostructural features.

1.3.3 Global Restrictions of Conformation in Peptides and Peptidomimetics

Cyclization of a peptide is another general approach to constrain the conformation by limiting the flexibility of the peptide. In this approach, the amino acid chain groups and backbone moieties that are not important in biological activity are chosen as the sites to construct a cyclic structure.\textsuperscript{48} The cyclization can be formed between side chains through several different types of bonds, such as disulfide,\textsuperscript{49} lactam,\textsuperscript{50} and thioether (Fig. 1-6). Other kinds of cyclic constrain are also possible between side chain and C- or N-terminal or between side chain and backbone nitrogen (Fig. 1-7).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1-6.png}
\caption{Side-chain-to-side-chain cyclizations.}
\end{figure}
As illustrated in Figs. 1-6 and 1-7, this approach allows considerable variability in the design of ring size and ring type. With different sizes and types of cyclic structures, cyclization not only can limit the flexibility of the conformation, but often can induce or stabilize the secondary structure, such as β-turn. Secondary structures (α-helices, β-sheets, and β-turns) play critical roles in biological activities. Many novel types of constraints have been employed to stabilize the secondary structures, especially reverse turns, in peptides and peptidomimetics. This strategy is now universally accepted as a method to design biologically active peptides and peptidomimetics with high potency and improved selectivity. A selection of reverse turn mimicking scaffolds is shown in Fig. 1-8.

**Figure 1-7. Cyclic constrains.**
Figure 1-8. β- and γ-turn mimetics of various types.

As shown in Fig. 1-8, lactam constrains can cause a turn in the peptide backbone, but it is difficult to place functional groups stereoselectively to match the side chains of peptide. From this point of view, the bicyclic thiazolidines are more promising β-turn mimetics since they can be derived from a chiral pool of amino acids and side groups can be introduced stereoselectively onto the scaffold during the synthesis. The synthesis of this type of β-turn mimetic and a novel methodology to introduce functional groups at the specific position on the backbone will be discussed in Chapters 3 and 4.
CHAPTER 2. EFFICIENT AND STEREOSELECTIVE SYNTHESIS OF NOVEL CIS-4-SUBSTITUTED PROLINE ANALOGUES

2.1 Introduction

Among naturally occurring \( \alpha \)-amino acids, proline is the unique one that is cyclic and a secondary amine, providing a conformationally constrained system. The importance of proline is reflected in its presence in many naturally occurring bioactive peptides such as gramicidin\textsuperscript{53,54} and \( \alpha \)-melanotropin,\textsuperscript{55} both of which have important biological activities. The studies of these bioactive peptides have shown that proline exerts great influence on both the structure and function of peptides and proteins.\textsuperscript{56} Peptides and proteins with a proline residue can influence important secondary structures such as a \( \beta \)-turn and an \( \alpha \)-helix.\textsuperscript{57} Due to its special structure, proline has been used as a rigid template to design conformationally constrained amino acids for the study of the interactions of peptide and protein ligands with their receptors/acceptors. When it is incorporated into a peptide or protein, proline can induce a reverse turn which can provide enhanced bioactivity.\textsuperscript{58-60} Such effects on peptide conformations have created a great deal of interest for the design of various substituted proline analogues.\textsuperscript{61,62} Actually, proline and its derivatives have been extensively used in the pharmaceutical industry, such as in angiotensin-converting enzyme (ACE) inhibitors, including Captopril,\textsuperscript{63} Enalapril,\textsuperscript{64} Fosinopril,\textsuperscript{65} and Lisinopril (Fig. 2-1).\textsuperscript{64}
Among these proline analogues, 4-substituted derivatives are particularly attractive since the C4 substituents can influence not only the conformation of the pyrrolidine ring, but the rate of cis-trans isomerization about the amide bond as well.\textsuperscript{66-68} The readily available starting material 4-trans-hydroxyproline has been used as a versatile building block for many biologically important compounds.\textsuperscript{69} In our ongoing melanocyte stimulating hormone (MSH) project, the substitution of histidine with proline in MT-II has generated a potent and selective analogue with agonist activity at the human MC5R.\textsuperscript{70}

To further explore the structure-activity relationship (SAR) of this ligand and its receptors, we have designed novel 4-substituted prolines.
2.2 Background and Designed General Approach to cis-4-Substituted Proline Derivatives

Several methods for the synthesis of 4-substituted prolines have appeared in the literature.\textsuperscript{61,62,71} Recently our and Goodman’s group have reported the synthesis of cis- and trans- 4-substituted proline analogues through hydrogenations of pyrroline intermediates derived from 4-trans-hydroxyproline (Scheme 2-1).\textsuperscript{61,62,66}

\textbf{Scheme 2-1.} Strategies for the asymmetric hydrogenation of pyrroline intermediates.

Meanwhile we have developed asymmetric hydrogenations/Suzuki-couplings for the preparation of a number of novel \( \chi^2 \) constrained amino acids.\textsuperscript{72-75} As an alternative to these methods, herein we would like to disclose an efficient and stereoselective approach to the synthesis of cis-4-substituted prolines. The synthetic strategy for the preparation of the title compounds 1 employs stereoselective alkylation at the \( \gamma \)-position.
of glutamic ester 2, followed by selective reduction, tosylation, and cyclization to obtain cis-4-substituted prolines 1 (Scheme 2-2).

**Scheme 2-2.** Strategy for the preparation of cis-4-substituted proline derivatives 1.

2.3 Stereoselective Synthesis of cis-4-Substituted Proline Derivatives

**Scheme 2-3.** Approach to cis-4-substituted proline derivatives.

*Conditions: (a) MeOH, DCC, 5 hr, 78%; (b) LiHMDS, THF, -78 °C, 30 min, then RBr, 5-6 hr; (c) NaBH₄, MeOH, 16 hr; (d) TsCl, DMAP, Pyridine, 12 hr; (e) NaH, THF, r.t., 3-4 hr.*
The synthesis of cis-4-substituted proline analogues 1 started with commercially available 
(4S)-5-(tert-butoxy)-4-[(tert-butoxycarbonyl)amino]-5-oxopentanoic acid (Scheme 2-3). The carboxylic acid was protected as a tert-butyloxycarbonyl ester, which can be viewed as an orthogonal protection to the ω-methyl ester in 2. Thus, the methyl ester could be selectively reduced without affecting the tert-butyl ester. The free ω carboxyl functional group was converted into a methyl ester 2 using dicyclohexylcarbodiimide (DCC) as an activating agent with methanol in the presence of a catalytic amount of dimethylaminopyridine (DMAP) and triethylamine (TEA) in 76% yield. The resulting compound 2 was used for the alkylations. Alkylation at the C4 position of glutamates has been reported in the literature. The studies indicated that the stereoselectivity depended on the nature of the N-substituents and the esters. Recently Hanessian and co-workers achieved highly stereoselective alkylations with N'-Boc or Cbz and the methyl ester. The stereoselectivity was attributed to a highly coordinated dianionic chair-like transition state (Fig. 2-2).

![Figure 2-2. Transition states in alkylations.](image)

However, substrates with the bigger tert-butyloxycarbonyl ester, as in our case, had not been studied. We rationalized that the size of the ester groups would play an important role in
controlling the stereoselectivity of the alkylations based on the proposed transition state. The large tert-butyl ester would further enhance the diastereoselectivity of the alkylations by stabilization of the chair-like transition state. As expected, only one single diastereomer (anti product) was obtained based upon $^1$H NMR analysis (Scheme 2-3).

**Scheme 2-4.** Selective reduction of $\gamma$-substituted glutamic acid ester.

With the optically pure $\gamma$-substituted alkyl glutamic acid ester 3 in hand, initially we planned to selectively reduce the methyl ester by diisobutylaluminumhydride (DIBAL) at $-78 \, ^\circ\text{C}$ to an aldehyde, which subsequently could undergo reductive amination to give the final products 1. Based on our earlier study, the mono-$N^\alpha$-Boc protected nitrogen interfered with the reduction. Therefore, a second Boc protecting group was introduced by reaction of 3 with di-tert-butyl dicarbonate [(Boc)$_2$O] in the presence of a catalytic amount of DMAP in acetonitrile after chromatography to give the bis-Boc protected methyl ester 6 in over 80% yield (Scheme 2-4). However, the reduction of the methyl ester in 6 using DIBAL at $-78 \, ^\circ\text{C}$ did not give the desired aldehyde 7. Even higher reaction temperatures (room temperature) did not work. In all cases, only the starting materials were recovered. In contrast, our previous study with reduction of a
similar substrate without γ-substituents under the same reaction conditions gave an aldehyde in excellent yield. The presumed reason was steric hindrance in the γ-substituted substrates 6.

Consequently, we modified our synthetic strategy (Scheme 2-3). We proposed the conversion of the methyl ester 3 to an alcohol, which then was transformed into a good leaving group for cyclization. The intramolecular nuclear substitution (cyclization) would provide the target molecules. Reduction of the mono-Boc protected methyl esters 3 with NaBH₄ to give alcohols 4 was achieved in good yields. Then the alcohols were converted into tosylates 5 in high yields. The tosylated intermediates were treated with NaH to give the cyclic proline derivatives 1 in good yields. To test for racemization during these conversions, we selectively deprotected the Boc group in 1a (due to rotamers) to 8, which gave a “clean” NMR (Scheme 2-5). One isomer was observed by NMR indicating that no isomerization occurred.

Scheme 2-5. Deprotection of cis-4-substituted proline derivatives.

This approach also provides important synthetic intermediates to other amino acid derivatives. With compound 3a as an example, the pyroglutamate ester 9, which can be
used as starting material in our dipeptide β-turn mimetic synthesis, was obtained in 3 steps (Scheme 2-6).

Scheme 2-6. Cyclization to pyroglutamic acid ester derivative 9.

2.4 Conclusion

In conclusion, a series of novel cis-4-substituted proline derivatives 1 were efficiently synthesized from readily available starting materials. Highly stereoselective alkylations at the γ-position of the glutamic ester 2 were achieved. The resulting alkylation compounds were transformed to the final products 1 through reduction/tosylation/cyclization in high yields. Future work will aim at the incorporation of these unnatural amino acids into α-MSH peptides and the study of structure-activity relationships of the α-MSH peptides.

2.5 Experimental Section

General. $^1$H and $^{13}$C NMR were performed on DRX-500 spectrometers using TMS and CDCl$_3$ as internal standards. High Resolution Mass Spectra (HRMS) were recorded
on a JOE HX110A instrument in the University of Arizona Mass Spectrum Laboratory. Optical rotations were measured on a JASCO-1020 polarimeter. Commercially available starting materials and reagents were purchased from Aldrich and used as received. THF was distilled from Na and benzophenone.

(S)-(-)-1-tert-Butyl-5-methyl-[2-tert-butoxycarbonyl]amino]pentanedioate (2). To a solution of (4S)-5-(tert-butoxy)-4-[(tert-butoxycarbonyl)amino]-5-oxopentanoic acid (10.0 g, 33 mmol) in 50 mL of CH₂Cl₂ was added DCC (8.9 g, 43.1 mmol) at 0 °C under argon. After 5 min, MeOH (2.7 mL, 26.6 mmol), TEA (6 mL, 43 mmol) and DMAP (400 mg, 3.3 mmol) were added to the above mixture. After stirring 1.5 h at 0 °C and 5 h at rt, the solution was filtered and concentrated under reduced pressure. The residue was redissolved in 500 mL of EtOAc, the organic solution was washed with 1N HCl (100 mL), saturated NaHCO₃ (100 mL) and brine (100 mL), dried over MgSO₄ and concentrated to give crude product as an oil. The crude product was purified by flash column chromatography (hexanes/EtOAc : 6:1) to afford 2 (7.9 g, 76%) as a colorless oil. 

₁H NMR (500 MHz, CDCl₃) δ 1.44 (s, 9H), 1.47 (s, 9H), 1.85-1.98 (m, 1H), 2.14-2.18 (m, 1H), 2.23-2.49 (m, 2H), 4.20 (m, 1H), 5.08 (d, J = 8.1 Hz, 1H).

Procedure A: To a solution of compound 2 in dry THF (6 mL/mmol) was added LiHMDS (2.2 equiv.) at -78 °C under N₂. After 30 min stirring, bromide (3 equiv.) was added at -78 °C. The reaction solution was kept at this temperature for 6 h. The reaction was quenched by H₂O (2 mL) at -78 °C. The organic solution was washed with NH₄Cl solution (10 mL/mmol) and brine (10 mL/mmol), dried over MgSO₄ and evaporated. The crude product was purified by flash column chromatography
Procedure B: To a solution of compound 3 in methanol (10 mL/mmol) was added sodium borohydride (10 equiv.) at rt. The mixture was stirred for 12 h at rt. The solution was washed with NH₄Cl solution (10 mL/mmol) and brine (10 mL/mmol), dried over MgSO₄ and evaporated. The crude product was purified by flash column chromatography (hexanes/EtOAc : 2:1) to give a colorless oil.

Procedure C: To a solution of compound 4 in pyridine (10 mL/mmol) was added toluenesulfonyl chloride (4 eq) and DMAP (0.1 eq). The reaction mixture was stirred at room temperature for 12 h and diluted with EtOAc (30 mL/mmol). The organic solution was washed with 1N HCl (30 mL/mmol, 3×), NaHCO₃ (30 mL/mmol) and brine (30 mL/mmol), dried over MgSO₄ and evaporated. The crude product was purified by flash column chromatography (hexanes/EtOAc : 4:1) to give a colorless oil.

Procedure D: To a solution of compound 5 in THF (10 mL/mmol) was added sodium hydride (1.1 equiv.) at rt under N₂. After 4 h stirring, the reaction was quenched with NH₄Cl solution (5 mL/mmol). The organic solution was washed with NH₄Cl (10 mL/mmol) and brine (10 mL/mmol), dried over MgSO₄ and evaporated. The crude product was purified by flash column chromatography (hexanes/EtOAc : 5:1) to give a colorless oil.

(2S,4R)-(+)-1-tert-Butyl-4-allyl-5-methyl-[(2-tert-butoxycarbonyl)amino] pentanedioate (3a). Procedure A. 81% yield, [α]²³D +14.4 (c 3.66, CHCl₃), ¹H NMR (500 MHz, CDCl₃) δ 5.70-5.61 (1H, m), 5.04-4.98 (2H, m), 4.88 (1H, d, J = 8.5 Hz), 4.16-4.15 (1H, m), 3.62-3.61 (3H, m), 2.56-2.50 (1H, m), 2.32-2.29 (2H, m), 1.90-1.84 (2H, m),
1.41 (9H, s), 1.39 (9H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 175.7, 171.7, 155.6, 134.7, 117.7, 82.2, 79.9, 52.8, 51.8, 42.1, 36.5, 34.2, 28.5, 28.1; HRMS (FAB) calcd for C$_{18}$H$_{32}$NO$_6$ 358.2230, found 358.2230.

(2S,4S)-(−)-1-tert-Butyl-4-allyl-5-hydroxy-[(2-tert-butoxycarbonyl)amino] pentanoate (4a). Procedure B. 60% yield, [α]$^D_{23}$ +10.6 (c 1.74, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 5.78-5.70 (1H, m), 5.19-5.17 (1H, d, $J = 6.2$ Hz), 5.06-5.00 (2H, m), 4.19-4.11 (1H, m), 3.71-3.69 (1H, m), 3.53-3.49 (1H, m), 2.46 (1H, s), 2.13-2.10 (2H, m), 1.88-1.68 (2H, m), 1.65-1.59 (1H, m), 1.44 (9H, s), 1.43 (9H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.3, 155.8, 136.6, 117.0, 82.2, 80.1, 65.2, 52.3, 37.6, 36.1, 35.2, 28.5, 28.2; HRMS (FAB) calcd for C$_{17}$H$_{32}$NO$_5$ 330.2280, found 330.2287.

(2S,4S)-(−)-1-tert-Butyl-4-allyl-5-(toluene-4-sulfonyloxy)-[(2-tert-butoxycarbonyl)amino] pentanoate (5a). Procedure C. 88% yield, [α]$^D_{23}$ +5.3 (c 0.97, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.78 (2H, d, $J = 8.3$ Hz), 7.34 (2H, d, $J = 8.3$ Hz), 5.64-5.56 (1H, m), 5.01-4.96 (2H, m), 4.91 (1H, d, $J = 8.4$ Hz), 4.16-4.15 (1H, m), 3.97-3.90 (2H, m), 2.45 (3H, s), 2.23-2.20 (1H, m), 2.12-2.07 (1H, m), 1.93-1.86 (1H, m), 1.74-1.68 (1H, m), 1.54-1.47 (1H, m), 1.44 (9H, s), 1.43 (9H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.9, 155.6, 145.0, 134.6, 133.0, 130.1, 128.1, 118.2, 82.3, 80.0, 72.3, 52.1, 34.6, 34.3, 34.1, 28.5, 28.1, 21.8; HRMS (FAB) calcd for C$_{24}$H$_{38}$NO$_7$S 484.2369, found 484.2372.

(S)-(−)-4-Allyl-Boc-L-proline tert-butyl ester (1a). Procedure D. 93% yield, [α]$^D_{23}$ −69.3 (c 1.56, CHCl$_3$); $^1$H NMR (rotamers) (500 MHz, CDCl$_3$) δ 5.78-5.73 (1H, m), 5.07-5.01 (2H, m), 4.10(0.7H, t, $J = 8.0$ Hz) (δ 4.15, 0.3H), 3.78 (0.7H, dd, $J_1 = 10.5$ Hz, $J_2 = 6.5$ Hz) (δ 3.65, 0.3H), 3.06-3.00 (1H, m), 2.45-2.38(1H, m), 2.23-2.13 (3H, m),
1.60-1.52 (1H, m), 1.49 (6H, s), 1.47 (6H, s), 1.45 (6H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.5, 172.4, 154.3, 154.0, 136.3, 136.2, 116.5, 116.4, 81.0, 79.9, 79.7, 60.0, 59.9, 52.2, 51.9, 38.2, 37.4, 37.3, 37.2, 36.8, 35.8, 28.6, 28.5, 28.2, 28.1; HRMS (FAB) calcd for C$_{17}$H$_{30}$NO$_4$ 312.2175, found 312.2164.

(2S,4S)-(−)-1-tert-Butyl-4-benzyl-5-methyl-[(2-tert-butoxycarbonyl)amino] pentanedioate (3b). Procedure A. 81% yield, [α]$^{23}_{D}$ −3.5 (c 1.93, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.27-7.24 (2H, m), 7.20-7.14 (3H, m), 4.94 (1H, d, $\nu = 9.0$ Hz), 4.28-4.24 (1H, m), 3.57 (3H, s), 2.91-2.89 (2H, m), 2.81-2.75 (1H, m), 1.96-1.91 (2H, m), 1.44 (18H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 175.7, 171.6, 155.6, 138.7, 129.1, 128.5, 126.7, 82.2, 79.9, 52.7, 51.8, 44.6, 34.8, 34.8, 28.5, 28.1; HRMS (FAB) calcd for C$_{22}$H$_{34}$NO$_6$ 408.2386, found 408.2387.

(2S,4S)-(−)-1-tert-Butyl-4-benzyl-5-hydroxyl-[(2-tert-butoxycarbonyl)amino]-pentanoate (4b). Procedure B. 66% yield, [α]$^{23}_{D}$ +34.7 (c 1.28, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.28-7.25 (2H, m), 7.19-7.17 (3H, m), 5.24 (1H, d, $\nu = 6.8$ Hz), 4.26-4.24 (1H, m), 3.76-3.74 (1H, m), 3.51-3.49 (1H, m), 2.72-2.64 (2H, m), 2.59 (1H, s), 2.01-1.96 (1H, m), 1.74-1.70 (2H, m), 1.45 (9H, s), 1.41 (9H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 172.2, 155.8, 140.4, 129.5, 128.5, 126.2, 82.3, 80.2, 64.3, 52.0, 39.9, 37.8, 35.4, 28.5, 28.2; HRMS (FAB) calcd for C$_{21}$H$_{34}$NO$_5$ 380.2437, found 380.2438.

(2S,4S)-(−)-1-tert-Butyl-4-benzyl-5-(toluene-4-sulfonyloxy)-[(2-tert-butoxycarbonyl)-amino]-pentanoate (5b). Procedure C. 85% yield, [α]$^{23}_{D}$ +1.3 (c 1.72, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.76-7.74 (2H, m), 7.32 (2H, d, $\nu = 8.1$ Hz), 7.19-7.14 (3H, m), 7.03-7.02 (2H, m), 4.94-4.92 (1H, d, $\nu = 8.5$ Hz), 4.29-4.25 (1H, m), 3.88- 3.82 (2H,
(S)-(−)-4-Benzyl-Boc-L-proline tert-butyl ester (1b). Procedure D. 88% yield, \([\alpha]^{23}_{D} = -67.0\) (c 1.65, CHCl₃); \(^1\)H NMR (rotamers) (500 MHz, CDCl₃) \(\delta\) 7.31-7.25 (2H, m), 7.23-7.13 (3H, m), 4.07 (0.7H, t, \(J = 8.0\) Hz) (\(\delta\) 4.12, 0.3H), 3.71 (0.7H, dd, \(J_1 = 10.5\) Hz, \(J_2 = 7.5\) Hz) (\(\delta\) 3.59, 0.3H), 3.13-3.08 (1H, m), 2.75-2.64 (2H, m), 2.45-2.29 (2H, m), 1.67-1.62 (1H, m), 1.47 (6 H, s) (\(\delta\) 1.46, 3 H), 1.42 (6H, s) (\(\delta\) 1.44, 3H); \(^{13}\)C NMR (125 MHz, CDCl₃) \(\delta\) 172.5, 172.4, 154.3, 154.0, 140.3, 140.2, 128.8, 128.7, 128.6, 126.5, 81.1, 80.0, 79.8, 60.0, 52.3, 52.1, 40.5, 39.8, 39.3, 39.2, 37.0, 36.0, 28.6, 28.5, 28.3, 28.2; HRMS (FAB) calcd for C₂₁H₃₂NO₄ 362.2331, found 362.2337.

(S,S)-(-)-1-tert-Butyl-4-(3-phenylallyl)-5-hydroxyl-[(2-tert-butoxycarbonyl)-amino] pentanedioate (3c). Procedure A. 77% yield, \([\alpha]^{23}_{D} = -10.4\) (c 2.35, CHCl₃); \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\) 7.34-7.26 (4H, m), 7.21-7.18 (1H, m), 6.43 (1H, d, \(J = 15.5\) Hz), 6.13-6.07 (1H, m), 4.97 (1H, d, \(J = 8.8\) Hz), 4.26 (1H, dd, \(J_1 = 14.5\) Hz, \(J_2 = 8.7\) Hz), 3.66 (3H, s), 2.69-2.63 (1H, m), 2.52 (2H, t, \(J = 6.9\) Hz), 2.00-1.95 (2H, m), 1.44 (9H, s), 1.43 (9H, s); \(^{13}\)C NMR (125 MHz, CDCl₃) \(\delta\) 175.7, 171.7, 155.6, 137.4, 132.8, 128.7, 128.6, 127.4, 126.4, 126.3, 82.2, 79.9, 52.7, 51.9, 42.4, 35.6, 34.4, 28.5, 28.1, 28.0, 27.9; HRMS (FAB) calcd for C₂₄H₃₆NO₆ 434.2543, found 434.2546.

(2S,4S)-(−)-tert-Butyl-4-(3-phenylallyl)-5-hydroxyl-[(2-tert-butoxycarbonyl)-}
amino]pentanoate (4c). Procedure B. 68% yield, \([\alpha]^{23}_{D} +11.1\) (c 1.53, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.34-7.24 (4H, m), 7.21-7.16 (1H, m), 6.43 (1H, d, \(J = 15.8\) Hz), 6.21-6.15 (1H, m), 5.22 (1H, d, \(J = 7.5\) Hz), 4.26-4.24 (1H, m), 3.78-3.76 (1H, m), 3.60-3.56 (1H, m), 2.52 (1H, br s), 2.30 (2H, t, \(J = 6.8\) Hz), 1.91-1.84 (1H, m), 1.81-1.75 (1H, m), 1.72-1.67 (1H, m), 1.44 (9H, s), 1.43 (9H, s); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 172.3, 155.8, 137.7, 132.3, 128.7, 128.2, 127.2, 126.2, 82.2, 80.1, 65.0, 52.3, 38.1, 35.3, 35.0, 28.5, 28.2; HRMS (FAB) calcd for C\(_{23}\)H\(_{36}\)NO\(_5\) 406.2593, found 406.2582.

\((2S,4S)-(-)-1\text{-}\text{tert-Butyl-4-(3-phenylallyl)-5-(toluene-4-sulfonyloxy)-[(2-\text{tert-butoxycarbonyl)amino}] pentanoate (5c).\) Procedure C. 90% yield, \([\alpha]^{23}_{D} -7.0\) (c 1.27, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.77 (2H, d, \(J = 8.5\) Hz), 7.30-7.19 (7H, m), 6.31 (1H, d, \(J = 16.0\) Hz), 5.97-5.91 (1H, m), 4.92 (1H, d, \(J = 8.5\) Hz), 4.24-4.20 (1H, m), 3.96 (2H, d, \(J = 5.3\) Hz), 2.40 (3H, s), 2.27-2.21 (1H, m), 2.01-1.94 (1H, m), 1.80-1.74 (1H, m), 1.58-1.54 (2H, m), 1.44 (9H, s), 1.43(9H, s); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 172.0, 155.7, 145.1, 137.4, 133.3, 133.0, 130.1, 128.7, 128.2, 127.4, 126.3, 126.2, 82.4, 80.1, 72.3, 52.1, 35.2, 34.4, 33.2, 28.5, 28.2, 21.8; HRMS (FAB) calcd for C\(_{30}\)H\(_{42}\)NO\(_7\)S 560.2682, found 560.2686.

\((S)-(-)-4-(3-\text{Phenyl-allyl})-\text{L-proline tert-butyl ester (1c).}\) Procedure D. 90% yield, \([\alpha]^{23}_{D} -53.6\) (c 1.80, CHCl\(_3\)); \(^1\)H NMR (rotamers) (500 MHz, CDCl\(_3\)) \(\delta\) 7.35-7.26 (4H, m), 7.23-7.19 (1H, m), 6.43-6.39 (1H, m), 6.16-6.10 (1H, m), 4.10 (0.7H, t, \(J = 8.0\) Hz) (\(\delta\) 4.16, 0.3H), 3.80 (0.7H, dd, \(J_1 = 10.5\) Hz, \(J_2 = 6.5\) Hz) (\(\delta\) 3.67, 0.3H), 3.12-3.05 (1H, m), 2.48-2.41 (1H, m), 2.35-2.24 (3H, m), 1.64-1.60(1H, m), 1.47 (6H, s) (\(\delta\) 1.46, 3H), 1.44 (6H, s) (\(\delta\) 1.45, 3H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 172.5, 172.4, 154.3, 154.0,
137.5, 131.8, 131.7, 128.7, 128.6, 128.0, 127.9, 127.4, 127.3, 126.2, 81.1, 80.0, 79.8, 60.0, 59.9, 52.2, 52.0, 38.7, 37.9, 36.8, 36.6, 36.5, 35.9, 28.6, 28.5, 28.2, 28.1; HRMS (FAB) calcd for C\textsubscript{23}H\textsubscript{34}NO\textsubscript{4} 388.2488, found 388.2488.

(2S,4S)-(-)-1-tert-Butyl-4-(4-bromobenzyl)-5-methyl-[(2-tert-butoxycarbonyl)-amino] pentanedioate (3d). Procedure A. 72% yield, \([\alpha]^{23}_D -12.6\) (c 1.79, CHCl\textsubscript{3}); \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 7.38 (2H, d, \(J = 8.2\) Hz), 7.04 (2H, d, \(J = 8.2\) Hz), 4.95 (1H, d, \(J = 8.8\) Hz), 4.28-4.27 (1H, m), 3.57 (3H, s), 2.89-2.83 (2H, m), 2.76-2.73 (1H, m), 1.96-1.90 (2H, m), 1.45 (9H, s), 1.44 (9H, s); \(^{13}\)C NMR (125 MHz, CDCl\textsubscript{3}) \(\delta\) 175.4, 171.6, 155.7, 137.8, 131.7, 131.0, 120.6, 82.5, 80.1, 52.5, 51.9, 44.4, 37.4, 35.1, 28.5, 28.2; HRMS (FAB) calcd for C\textsubscript{22}H\textsubscript{32}BrN\textsubscript{0}6\textsubscript{s} 618.0467, found 618.0445.

(2S,4S)-(+)1-tert-Butyl-4-(4-bromo-benzyl)-5-hydroxy-[(2-tert-butoxycarbonyl)amino] pentanoate (4d). Procedure B. 74% yield, \([\alpha]^{23}_D +24.4\) (c 3.51, CHCl\textsubscript{3}); \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 7.38 (2H, d, \(J = 8.3\) Hz), 7.06 (2H, d, \(J = 8.3\) Hz), 5.28 (1H, d, \(J = 7.4\) Hz), 4.27-4.23 (1H, m), 3.40-3.68 (1H, m), 3.48-3.43 (1H, m), 2.84 (1H, br s), 2.70-2.59 (2H, m), 1.92 (1H, br s), 1.72-1.67 (2H, m), 1.44 (9H, s), 1.42 (9H, s); \(^{13}\)C NMR (125 MHz, CDCl\textsubscript{3}) \(\delta\) 172.1, 155.8, 139.4, 131.5, 131.2, 120.0, 82.3, 80.2, 63.7, 51.9, 39.6, 36.8, 35.2, 28.5, 28.1; HRMS (FAB) calcd for C\textsubscript{21}H\textsubscript{33}BrNO\textsubscript{5} 458.1542, found 458.1526.

(2S,4S)-(--)1-tert-Butyl-4-(4-bromobenzyl)-5-(toluene-4-sulfonyloxy)-[(2-tert-butoxycaronyl)amino] pentanoate (5d). Procedure C. 92% yield, \([\alpha]^{23}_D -5.13\) (c 1.43, CHCl\textsubscript{3}); \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 7.73 (2H, d, \(J = 8.3\) Hz), 7.32 (2H, d, \(J = 8.2\) Hz), 7.28 (2H, d, \(J = 8.3\) Hz), 6.91 (2H, d, \(J = 8.2\) Hz), 4.99 (1H, d, \(J = 8.5\) Hz),
4.30-4.26 (1H, m), 3.83-3.77 (2H, m), 2.86 (1H, dd, J₁ = 13.5 Hz, J₂ = 3.5 Hz), 2.47 (3H, s), 2.01-1.98 (1H, m), 1.79-1.73 (1H, m), 1.57-1.50 (1H, m), 1.45 (18H, s); ¹³C NMR (125 MHz, CDCl₃) δ 171.8, 155.8, 145.1, 137.9, 132.8, 131.6, 131.1, 130.1, 128.1, 120.3, 82.5, 80.1, 71.7, 51.9, 36.9, 35.3, 35.0, 28.5, 28.1, 21.8; HRMS (FAB) calcd for C₂₈H₅₉BrNO₅S 612.1631, found 612.1653.

(S)-(-)-4-(4-Bromobenzyl)-Boc-L-proline tert-butyl ester (1d). Procedure D. 85% yield, [α]²³⁺D = -4.17 (c 1.76, CHCl₃); ¹H NMR (rotamers) (500 MHz, CDCl₃) δ 7.44-7.40 (2H, m), 7.06-7.02 (2H, m), 4.09 (0.7H, t, J = 8.5 Hz) (δ 4.14, 0.3H), 3.71 (0.7H, dd, J₁ = 10.5 Hz, J₂ = 7.5 Hz) (δ 3.59, 0.3H), 3.14-3.08 (IH, m), 2.71-2.63 (2H, m), 2.42-2.32 (2H, m), 1.49 (6H, s) (δ 1.48, 3H), 1.44 (6H, s) (δ 1.46, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 172.4, 172.3, 154.2, 154.0, 139.2, 139.1, 131.8, 130.5, 120.3, 81.2, 80.1, 79.8, 59.9, 59.8, 52.2, 52.0, 40.2, 39.5, 38.7, 38.5, 36.8, 35.8, 28.6, 28.5, 28.2, 28.1; HRMS (FAB) calcd for C₂₁H₃₁BrNO₄ 440.1436, found 440.1436.

(S)-(-)-4-Allyl-L-proline tert-butyl ester (8). A solution of 1a (20 mg, 0.064 mmol) in 1.6 mL CH₂Cl₂ and 0.4 mL TFA was stirred at rt under argon for 30 min. The solution was diluted by CH₂Cl₂ (2 mL) and the organic layer was washed with NaHCO₃ (3 mL), dried over MgSO₄ and concentrated to give a lightly yellow oil with quantitative yield. The crude product was pure enough for characterization. [α]²³⁺D = -29.0 (c 0.3, CHCl₃); ¹H NMR (rotamers) (500 MHz, CDCl₃) δ 5.78-5.69 (IH, m), 5.02-4.95 (2H, m), 3.66 (1H, t, J = 7.8 Hz), 3.03 (1H, dd, J₁ = 10.0 Hz, J₂ = 6.0 Hz), 2.68 (1H, dd, J₁ = 10.0 Hz, J₂ = 7.5 Hz), 2.45 (1H, br s), 2.29-2.26 (1H, m), 2.22-2.15 (1H, s), 2.14-2.02 (2H, m), 1.44 (9H, s), 1.43-1.39 (1H, m); ¹³C NMR (125 MHz, CDCl₃) δ 174.8, 137.2,
116.0, 81.4, 60.6, 52.6, 39.4, 38.1, 36.7, 28.3; HRMS (FAB) calcd for C_{12}H_{22}NO_{2}
212.1651, found 212.1653.

(S)-4-Allyl-5-oxo-Boc-L-proline tert-butyl ester (9). A solution of 3a (205 mg, 0.58 mmol) in 4 mL of CH_{2}Cl_{2} and 1 mL of TFA was stirred at room temperature for 30 min. The solution was diluted with CH_{2}Cl_{2} (5 mL). The organic layer was washed with NaHCO_{3} (2 \times 10 mL), dried over MgSO_{4}, and concentrated to give a light yellow oil. The crude product was redissolved in 5 mL of toluene with TEA (160 \mu L, 1.14 mmol). The solution was heated up to 90 °C for 12 h. After the oil bath was removed, the solvent was removed under reduced pressure. To the solution of the above crude product in 5 mL of acetonitrile was added (Boc)_{2}O (150 mg, 0.69 mmol) and DMAP (7.5 mg, 0.06 mmol). After stirring at rt for 3 h, the solvent was removed under reduced pressure. The residue was redissolved in CH_{2}Cl_{2} (10 mL). The organic layer was washed with brine (10 mL), dried over MgSO_{4}, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexanes/EtOAc : 3:1) to afford 9 (117 mg, 63%) as a colorless oil. ^1H NMR (500 MHz, CDCl_{3}) δ 5.72-5.65 (1H, m), 5.04-5.00 (2H, m), 4.35 (1H, dd, J_{1} = 9.5 Hz, J_{2} =5.5 Hz), 2.61-2.54 (2H, m), 2.43-2.36 (1H, m), 2.21-2.14 (1H, m), 1.69-1.64 (1H, m), 1.46 (9H, s), 1.44 (9H, s); ^13C NMR (125 MHz, CDCl_{3}) δ 174.9, 170.7, 149.6, 135.0, 117.7, 83.5, 82.3, 58.2, 42.3, 35.5, 28.1, 28.0, 26.5; HRMS (FAB) calcd for C_{17}H_{38}NO_{5} 326.1967, found 326.1965.
CHAPTER 3. DESIGN AND SYNTHESIS OF CONFORMATIONALLY
CONSTRAINED REVERSE-TURN PEPTIDOMIMETICS OF
LEU-ENKEPHALIN

3.1 Introduction

In 1975, Hughes and Kosterlitz discovered the opioid pentapeptides
Met(5)-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH) and Leu(5)-enkephalin
(H-Tyr-Gly-Gly-Phe-Leu-OH). Since then, the biology and chemistry of these
endogenous opioid peptides have been extensively studied. Some research results have
shown that these neurotransmitters regulate sensory functions including pain and control
of respiration in the central nervous system by binding to the G-protein coupled μ- and
δ-opioid receptors, respectively. Among these studies, the structure-activity
relationships and the conformation of enkephalin in solution have been specially
interesting, because enkephalin is a noncyclic peptide binding to the same receptor site as
rigid non-peptide opiate agonists. The conformation of enkephalins has been
investigated by using a variety of methods, including NMR, X-ray diffraction,
conformational energy calculations, CD, and Raman.

However, studies that focus on elucidating the bioactive conformation of opioid
peptide ligands have been complicated by the fact that there are four main opioid
receptors (μ, δ, κ, and σ), some of which may also have distinct sub-types.
Although some important pharmacophoric elements have been identified, such as the
relative location and orientation of the aromatic side chains and the relationship of the
N-terminal nitrogen to the phenolic oxygen, the large conformational freedom of enkephalins leads to their nonspecific receptor affinity and thus makes the determination of the bioactive conformation very challenging. On the other hand, the structure determination of the membrane bound opioid receptors is extremely difficult. As discussed in Chapter 1, a general and efficient strategy to study the bioactive conformation of short linear peptides, like enkephalins, is to synthesize the conformationally restricted mimetics and test them in order to obtain some insight into the SAR.

A successful example of this approach is c[DPen²,DPen⁵]enkephalin (DPDPE), a cyclic conformationally and topographically constrained analogue of enkephalin (Fig. 3-1). In this analogue the Gly² and Met⁵ (or Leu⁵) residues were replaced by D-penicillamine (D-Pen, β,β-dimethylcysteine) and formed a 14-membered disulfide ring. DPDPE was found to be highly potent and selective for the δ opioid receptor and to be completely stable to proteolytic breakdown \textit{in vitro} and \textit{in vivo}. Extensive biophysical studies on DPDPE and some of its critical analogues using NMR, molecular dynamics and X-ray crystallography have clearly established the preferred conformation of the 14-membered ring template of this cyclic analogue.
Figure 3-1. Structure of DPDPE.

Although considerable success has been obtained in designing non-peptide peptidomimetics of enkephalins by incorporating some aspects of the overall structural and topographical features into the peptides, like DPDPE, efforts to design in other important features such as the proper topographical relationships of critical side chain groups in $\phi$, $\psi$ and $\chi$ space, and of certain H-bond donating and accepting properties has been difficult. As part of our continuing exploration in this field, we have designed and synthesized novel Leu-enkephalin mimetics.

3.2 Design of Leu-enkephalin Mimetics

Leu-enkephalin has been found to exist predominately in three different conformations: extended, single-bend and double bend (Fig. 3-2). It has been suggested that the single-bend form of enkephalin is representative of the bioactive
conformation at the δ-receptor. DPDPE was designed to mimic the single-bend conformation and its enhanced δ receptor selectivity supported this assumption. Some other peptidomimetics have been also prepared to study this conformation (Fig. 3-3).106,107

Figure 3-2. Schematic drawing of the three enkephalin conformations.
On the other hand, there was speculation that the double-bend conformation may bind at μ-receptor sites. In order to further investigate the bioactive conformation of Leu-enkephalin, we have designed novel double-bend form peptidomimetics of enkephalin (Fig. 3-4).

Figure 3-3. Peptidomimetics of Leu-enkephalin.

Figure 3-4. Reverse turn peptidomimetics of Leu-enkephalin.
One particular interesting approach in design of peptidomimetics is to replace a dipeptide motif which adopts a biologically important conformation in a given natural peptide with a constrained or rigidified counterpart that simulates a so-called reverse turn. It has been found that the double-bend conformation of Leu-enkephalin is adopted in biomimetic media where a γ-turn is centered on Gly(2) and a β-turn is centered on Gly(3)-Phe(4). Thus we designed the reverse turn peptidomimetics of Leu-enkephalin by incorporating a constrained dipeptide mimetic into the peptide to replace dipeptide Tyr(1)-Gly(2) or Gly(3)-Phe(4) (Fig. 3-4).

Ideally, dipeptide mimetics should possess a scaffold with the right conformation and appropriately positioned side chain functionalities in chiral space. The backbone of such β-turn mimetics could then serve as a three-dimensional structural scaffold when the full structures interact with their receptors/acceptors. Indeed, properly placed side chain moieties involved directly in the interaction are critical for biological activities and selectivities between peptide ligands and receptors/acceptors or sub-types receptors. Several dipeptide mimetic systems have been proposed to mimic different types of reverse turns. Among them, the azabicyclo[X.Y.0]alkane amino acids potentially are a particularly important class due to their ability to provide constrained backbone and side-chain conformations (Fig. 3-5).

Figure 3-5. Azabicyclo[X.Y.0]alkane amino acids.
Azabicyclo[X.Y.0]alkane amino acids are restricted dipeptide surrogates that embody the peptide backbone within a bicyclic structure. In the bicyclic framework, three contiguous $\phi_1$, $\psi_1$, and $\omega_1$-dihedral angles are restricted by the structural constraints of the heterocycle (Fig. 3-6). In addition, the outer two $\psi_2$ and $\phi_2$-dihedral angles are restricted by gauche interactions with the ring system. Thus this bicyclic system provides the capacity of constraining five backbone bonds in a row within the dipeptide. Moreover, it has been identified that the relative location and orientation of the aromatic side chains is critical in the bioactivities and selectivities of Leu-enkephalin. Hence, the constraints of $\chi$ angles in azabicycloalkane amino acids can also provide important information for the SAR studies (Fig. 3-6). Based on these advantages, we have chosen azabicycloalkane amino acids as dipeptide mimetics incorporated into Leu-enkephalin in order to study its bioactive conformation (Fig. 3-4). In our attempt to implement this plan, we need to develop a flexible synthetic approach to such dipeptide mimetics.

\[ n=0, 1, 2 \]
\[ X=\text{CH}_2, \text{S, O} \]

**Figure 3-6.** General structure illustrating the dihedral angles constrained by an azabicyclo[X.Y.0]alkane amino acid in a peptide.
3.3 Synthesis of Azabicyclo[X.Y.0]Alkane Amino Acids

Inherent in the synthesis of azabicycloalkane amino acids are three important challenges: stereocontrol, side-chain attachment, and ring size (the three S's: Stereochemistry, Side chains, and Size). The importance of stereochemistry is obvious, since configuration can influence conformations. Thus chiral centers should be introduced with control at the backbone carbons, ring fusion center, and attachment sites of the side-chain appendages. The addition of various functional groups at appropriate points along the azabicycloalkane heterocycle is also critical for mimicry of the nature and the spatial orientation of amino acid side chains. Finally, the approach to a variety of azabicycloalkane ring systems with different sizes is desired, because the size of the heterocycle can bias the peptide conformation. Some synthetic methodologies have been reported to prepare azabicycloalkane amino acids. Examples of the products prepared by these methodologies are listed in Figure 3-7.
Figure 3-7. Azabicycloalkane amino acids.

As listed in Figure 3-7, quite a few successes have been reported in obtaining mimetics which can force or stabilize β-turns. However, little success has been reported in incorporating mimetics for the active site of peptide hormone or neurotransmitter receptors because of the lack of appropriately positioned side-chain groups. As mentioned above, the side-chain functionalities are directly involved in the interaction between the ligand and the receptor and thus are indispensable for the bioactivity, especially for short peptides like Leu-enkephalins. Hence, there is quite a need for methodology to introduce side-chain functionality on the backbone of the mimetics.
In order to prepare the Leu-enkephalin peptidomimetics shown in Figure 3-4, we have developed an efficient approach to the stereoselective synthesis of azabicyclo[4.3.0] (indolizidinone-type) alkane amino acids with appropriate side-chain appendages (Fig. 3-8). This approach can also be further explored to give rise to different size azabicycloalkane ring systems through employment of starting materials of different chain length. We chose 6,5-fused ring system as the first study target because modeling studies showed that the dihedral angles in such systems mimic those angles in natural reverse turn conformations better than other fused ring systems.

\[ \text{Figure 3-8. } \text{Dipeptide mimetics designed for Leu-enkephalin peptidomimetics.} \]

### 3.4 Synthesis of Novel 4-Substituted Unsaturated Indolizidinone Amino Acids

We and other research groups have developed synthetic routes for the preparation of enantiopure indolizidinone type bicyclic lactam systems.\textsuperscript{120-123} However, these approaches suffer from some limitations. For example, the introduction of side chain
functionalities at the C4 position of azabicyclo[X.Y.0] alkane amino acids generally were not accessible, or required a long synthetic sequence;\textsuperscript{124} most methodologies have no way to introduce a phenyl or a p-hydroxylphenyl group, which correspond to the side chains in the amino acids Phe and Tyr, respectively. Moeller and co-workers chose a benzyl group to substitute for a phenyl group. However, their studies showed that the extra methylene group interfered badly in the binding to the TRH-R receptor.\textsuperscript{125} In this section, we report a novel methodology which can allow for the synthesis of 4-phenyl- or p-hydroxylphenyl-substituted saturated and unsaturated indolizidinone amino acids. Such reverse turn mimetics will be used to serve as surrogates of Tyr-Ala dipeptides and be incorporated into peptides.

Some groups have reported on the preparation of indolizidinone type compounds through dehydroamino acid intermediates.\textsuperscript{126,127} Further applications to these methodologies have been successfully developed in our group to introduce side-chain functionalities at the C7 and C8 positions.\textsuperscript{82} Presently, we have extended these methodologies to introduce aryl groups at position 4 through Suzuki cross-couplings. The general retrosynthetic strategy is given in Scheme 3-1. The unsaturated bicyclic lactam system could be approached from a dehydroamino acid intermediate, which can be prepared by Horner-Emmons olefination of a proline aldehyde derivative. Two challenges must be faced in our synthetic approach to these novel targets. The first is the introduction of the side-chain functionality at the C4 position from the precursor dehydroamino acid derivative. Secondly, the major Z dehydroamino acid products from the Horner-Emmons reaction may restrict the cyclization in the following step. We
postulated that bromination of the dehydroamino acid could be a solution to these two problems. Bromination can generate a reactive site for Suzuki cross-coupling and also can provide the geometry required for cyclization. With this methodology, we also can introduce side-chain functionalities at both C4 and C7 or C8 positions using the corresponding chiral pyroglutamic ester derivatives prepared by chemistries previously developed in our laboratory, which will be demonstrated in the next chapter. In this chapter, we will demonstrate the synthetic method with side-chain functionalities at the C4 position.

**Scheme 3-1.** Retrosynthetic analysis.

Our approach to the synthesis of 4-substituted azabicyclo[4.3.0]alkane amino acid derivatives 18a and 19a is illustrated in Scheme 3-2. The readily available (S)-pyroglutamate 10 was reduced to the methoxy aminal 11 by treatment with
Super-Hydride (LiBEt₃H) in THF at -78 °C, and then with methanol in the presence of a catalytic amount of p-TsOH. The crude product 11 was directly subjected to allyltrimethylsilane in the presence of boron trifluoride without further purification. The allylsilane addition to the N-acyliminium compound derived from 11 afforded a 3:1-cis/trans-mixture of proline ester 12. The intermediate 12 underwent osmylation and subsequent oxidation with NaIO₄ to give aldehyde 13. A cis/trans dehydroamino acid mixture 14 was obtained in a 3:1 ratio via Horner-Emmons olefination of 13. The mixture composition was based on ¹H NMR spectra. The assignment of the major product as a cis isomer was achieved according to the literature.¹²⁶
Scheme 3-2. Synthesis of 4-substituted unsaturated indolizidinone amino acid esters.

\[
\begin{align*}
\text{O} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 10 \\
\text{MeO} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 11 \\
\text{COOMe} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 12 \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 13 \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 14 \\
\text{Br} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 15 \text{a} (53\%) \\
\text{Br} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 15 \text{b} \text{ (crude)} \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 16 \text{a: } R = \text{phenyl} (76\%) \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 17 \text{a: } R = 4\text{-methoxyphenyl} (79\%) \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 18 \text{a: } R = \text{phenyl} (91\%) \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 19 \text{a: } R = 4\text{-methoxyphenyl} (89\%) \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 16 \text{b: } R = 4\text{-methoxyphenyl} \text{ (crude)} \\
\text{MeOOC} & \quad \text{N} \quad \text{COOMe} \quad \text{Boc} \quad 17 \text{b: } R = 4\text{-methoxyphenyl} \text{ (two steps: 34\%)}
\end{align*}
\]

*Conditions: (a)(i) Super-Hydride, THF, -78 °C; (ii) p-TsOH (cat.), MeOH; (b) BF₃·Et₂O, Me₂SiCH₂CH=CH₂, three steps: 77%; (c) OsO₄, NaIO₄, THF/H₂O, 4 h; (d) (MeO)₂P(O)CH(NHCBZ)COOCH₃, DBU, DCM, rt, 8 h, two steps: 63%; (e) (i) NBS, CHCl₃, rt, 80 min; (ii) Dabco, CHCl₃, rt, 24 h; (f) RB(OH)₂, Pd(OAc)₂, P(α-tolyI)₃, Na₂CO₃, DME, 80 °C; (g) (i) 20% TFA, DCM, rt, 30 min; (ii) NaHCO₃; (iii) CHCl₃, rt, 24 h.*
The stereoselectivity favoring cis-isomer in the allylation hinges on the facial preference with which an allylsilane attacks the cyclic acyliminium intermediate generated by the BF₃ catalyzed OMe-elimination from aminal 11 (Fig. 3-9). It has been suggested that the mechanistic pathway involved the formation of a BF₃ complex with the ester in which the fluoride acquired sufficient nucleophilicity to attack the trimethylsilyl group and, hence, facilitate the concomitant allyl transfer to the iminium function. Thus it can be suggested that the selective formation of the cis-product is the result of a neighboring group participation of the methyl ester function.

![Figure 3-9. Transition state model for the generation of 12.](image)

Although the β-bromination of dehydroamino acid esters has been well documented, the literature reported inconsistent stereoselectivity, and suggested various intermediates in this reaction, including dibromides, N-bromo compounds, and α-bromoiminium species. In most cases, Z-selectivity was observed in the bromination. Das and co-workers reported a 10:90 E/Z ratio in the bromination of N-acyldehydroalanine derivatives with Br₂ and Et₃N; Danion and co-workers described Z-selectivity in bromination of ethyl 2-(methoxycarbonylamino)cinnamate using NBS and Et₃N; Olsen and co-workers
and Shin and co-workers\textsuperscript{135} also reported $Z$-selective halogenation under similar conditions. On the other hand, some groups have reported $E$-selective bromination using the substrates with a particular substituent attached to the double bond.\textsuperscript{132,136}

Coleman and Carpenter have extensively examined the mechanism of bromination of dehydroamino acid esters.\textsuperscript{136} They isolated $\alpha$-bromoimine as the intermediate produced by reaction of dehydroamino acids with NBS (Scheme 3-3). The two possible ground-state conformers of $\alpha$-bromoimine, A and B, undergo base-promoted tautomerization to give $E$- and $Z$-vinyl bromide, respectively (Scheme 3-4).

Scheme 3-3. Bromination of dehydroamino acids.
Based on the results of molecular mechanics calculations, conformer A is energetic favored and thus E-vinyl bromide is the kinetically favored product. However, this difference in stability of conformer A and B does not account for the observed Z-selectivity in the reaction. Meanwhile, Coleman and Carpenter observed that the mixture of E and Z isomers underwent isomerization in the presence of DABCO and predominately afforded Z-vinyl bromide. They also noticed that other strong but sterically hindered amine bases are inefficient at promoting this isomerization. Based on these observations, they proposed a mechanism for interconversion of the kinetically formed E-vinyl bromide to the thermodynamically more stable Z-isomer, which contains a Michael addition-elimination reaction sequence (Scheme 3-5). This assumption is supported by the fact that DABO, the least hindered and most nucleophilic amine, is the most effective agent promoting the isomerization.
Scheme 3-5. Mechanism of isomerization.

In our case, we treated the dehydroamino acid ester 14 with N-bromosuccinimide (NBS) to produce α-bromoimines, which underwent tautomerization to afford (Z)-β-bromo-α,β-dehydroamino acids 15ab upon treatment with an amine base. We examined various amine bases which could be used in the tautomerization step. The results showed that the use of DABCO instead of Et₃N and DBU as a base improved Z-selectivity and gave the Z-isomer exclusively. This result was consistent with the observations of Coleman and Carpenter. The cis/trans isomers 15ab could be separated at this point by column chromatography eluting with hexanes/ethyl acetate (4/1). However, attempts to purify compound 15b were not successful due to a contamination of the α-bromoimine intermediate in this reaction.

We then investigated the Suzuki cross-coupling of 15a with arylboronic acids. We chose phenylboronic acid and 4-methoxyphenylboronic acid to use in the couplings
because Phe and Tyr are two important amino acid moieties in our α-MSH and δ-opioid studies. Suzuki coupling went smoothly to afford 16a and 17a, in 76 and 79% yields, respectively. Deprotection of Nα-Boc group was performed in 20% TFA in dichloromethane (DCM) at room temperature. The TFA salt was neutralized with NaHCO₃, and the reaction was stirred in chloroform at room temperature for 48 h. Surprisingly the deprotected 16a, 17a underwent cyclization smoothly under this mild condition without base and heat and afforded 18a, 19a in good yields. Crystallization of 19a from EtOAc and X-ray crystallographic analysis confirmed the configuration of the bridgehead proton (Figure 3-10).

![Figure 3-10. The X-ray structure of compound 19a.](image)

On the other hand, we used the crude compound 15b in Suzuki cross-coupling and the resulting crude product 16b was cyclized to afford 17b in 34% overall yield from 15b.
The stereochemistry of 17b was assigned with the use of 1D-NOE experiments (Table 3-1).

Table 3-1. NOE Data for 17b

<table>
<thead>
<tr>
<th>Protons</th>
<th>NOE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₉, H₈α</td>
<td>1.93</td>
</tr>
<tr>
<td>H₉, H₈β</td>
<td>0.39</td>
</tr>
<tr>
<td>H₆, H₇α</td>
<td>n.o.</td>
</tr>
<tr>
<td>H₆, H₈β</td>
<td>0.68</td>
</tr>
<tr>
<td>H₈β, H₇α</td>
<td>0.47</td>
</tr>
<tr>
<td>H₆, H₇β</td>
<td>1.30</td>
</tr>
<tr>
<td>H₈α, H₇α</td>
<td>1.46</td>
</tr>
<tr>
<td>H₈α, H₇β</td>
<td>n.o.</td>
</tr>
<tr>
<td>H₆, H₇α</td>
<td>0.26</td>
</tr>
<tr>
<td>H₅, H₇β</td>
<td>2.01</td>
</tr>
</tbody>
</table>

*NOE not observed.

3.5 Synthesis of Novel 4-Substituted Saturated Indolizidinone Amino Acids

Once the unsaturated bicyclic lactams 18a, 19a were obtained, we investigated the possibility of converting the unsaturated bicyclic structures to the saturated structures by hydrogenation. The literature reported that the hydrogenation of dehydroamino acids in open chain substrates preceded with poor stereoselectivity. However, the restricted conformation in the bicyclic compound provided an asymmetric environment in
hydrogenation. As we expected, compound 18a and 19a were hydrogenated (Pd-C, H₂, 75 psi) to give 20a and 21a exclusively (Scheme 3-6).

**Scheme 3-6. Hydrogenation of 18a and 19a.**

The stereochemistry of 20a was determined based on the NOE data (Table 3-2). In order to further confirm the relative configuration between the C-3 and C-4 protons, we performed a modeling study which showed that the dihedral angles between H₃ and H₄ are 40° and 57° (two conformations) in the *syn* isomer, and -169° in the *anti* isomer. The coupling constants were calculated on the basis of this finding as \( J_{syn} = 4.1 \text{ Hz} \) and \( J_{anti} = 12.5 \text{ Hz} \). The observed coupling constant was 6.9 Hz. Considering the additional fact that *syn* addition has been observed in most metal catalyzed hydrogenations, we drew the conclusion that the relative configuration of H₃ and H₄ in compound 20a is *syn*. 
Table 3-2. NOE Data for 20a

<table>
<thead>
<tr>
<th>Protons</th>
<th>NOE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H_{38} H_{6}</td>
<td>0.35</td>
</tr>
<tr>
<td>H_{5a} H_{6}</td>
<td>1.15</td>
</tr>
<tr>
<td>H_{38} H_{4}</td>
<td>0.50</td>
</tr>
<tr>
<td>H_{5a} H_{4}</td>
<td>1.25</td>
</tr>
<tr>
<td>H_{38} H_{3}</td>
<td>n.o.(^a)</td>
</tr>
<tr>
<td>H_{5a} H_{3}</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^a\) NOE not observed.

We also performed some modeling studies in order to explain the stereoselectivity of hydrogenation. The conformations of 18a and 19a suggested by these studies (Fig. 3-11) were later confirmed by the X-ray structure (Fig. 3-10). The stereoselectivity of this hydrogenation is probably due to the steric hindrance of the axial γ proton adjacent to the olefin group. Although the bottom face approach is sterically hindered by the bridgehead proton, the axial γ proton placed a greater steric effect in this case due to its proximity to the reaction site.
As discussed above, we chose the indolizidinone type system because it is expected to be a good mimetic of a reverse turn conformation. Our modeling studies showed that superposition of the two lowest energy conformations of the compound 20a onto a peptide with various types of β-turn structures (Fig. 3-12) using backbone heavy atoms gave an RMSD of 0.42 Å for the type II' structure for one conformation, and an RMSD of 0.56 Å for the type V' structure for the other conformation. In other words, the backbone conformations of the compound best fit the criteria for type II' and V' β-turn structures.
3.6 Synthesis of Leu-Enkephalin Mimetics

Once the methodologies for preparing bicyclic β-turn dipeptide mimetics were established, we investigated the incorporation of these mimetics into the bioactive peptide, Leu-enkephalin. The Leu-enkephalin mimetic \( 24 \) was synthesized by the usual coupling protocols (Scheme 3-7). Deprotection of \( 19\alpha \) using the LiOH/MeOH system and coupling with tripeptide H-Gly-Phe-Leu-OMe in DMF using PyBOP and HOBT as the coupling reagents gave the protected Leu-enkephalin mimetic \( 22 \). Treatment of \( 22 \) with 1N aqueous LiOH in MeOH released the C-terminal of the peptide and treatment of resulting product \( 23 \) with HBr (30% in AcOH) cleaved the \( N^\alpha \)-Cbz protecting group. However, a problem occurred in the step of purification by HPLC. Free peptidomimetic \( 24 \) was unstable in TFA/H\(_2\)O(1%) and decomposed to yield the corresponding hydroxyl product \( 25 \) (Scheme 3-8).\(^{137,138} \) Therefore, an \( N^\alpha \) protecting group was required for the stability of the peptidomimetic. Although it has been suggested that the cationic amine is necessary to the biological activity of Leu-enkephalin, literature has reported the identification of a \( \mu \)-selective opioid receptor agonist without a cationic amine group.\(^{139} \)

1) LiOH, MeOH
2) DMF, PyBop, HOBt, DIPEA

HBr (30% in AcOH)
In conclusion, we have successfully developed an approach to the synthesis of unsaturated and saturated azabicyclo[4.3.0]alkane amino acids with an aryl side chain at the C4 position. This methodology potentially can be extended to the preparation of more complex molecules, such as 7/5 and 5/5 azabicyclo[X.Y.0]alkane amino acids. The unsaturated azabicyclo[4.3.0]alkane amino acid has been incorporated into the biologically active peptide, Leu-Enkephalin. The study of structure-activity relationships is still under investigation.
3.8 Experimental Section

(2S,5R/S)-1-(tert-Butyloxycarbonyl-5-methoxy)-proline methyl ester (11). To a solution of 10 (2.1 g, 8.63 mmol) in THF (60 mL) was added Super-Hydride (1.0 M in THF, 13 mmol) at -78 °C under Ar. After stirring at -78 °C for 40 min, the reaction mixture was quenched with saturated NaHCO₃ (10 mL) and warmed to 0 °C. Then 30 drops of 30% aqueous H₂O₂ solution was added at this temperature. After the mixture warmed to rt, THF was removed under reduced pressure. The reaction residue was extracted with diethyl ether (3 x 25 mL). The organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under vacuum to give a colorless oil. To the oil in MeOH (50 mL) was added p-TsOH·H₂O (165 mg, 0.86 mmol) and the mixture was stirred at rt overnight. After quenching with saturated NaHCO₃ (12 mL) and removal of the solvent, the reaction mixture was extracted with diethyl ether (3 x 25 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated under vacuum to give crude 11 as a colorless oil (2.03 g, 91%).

Methyl (2S,5R/S)-1-(tert-butyloxycarbonyl)-5-allylprolinate (12). To a solution of crude 11 (2.03 g, 7.83 mmol) in diethyl ether (40 mL) was added allyltrimethylsilane (5.5 mL, 34.6 mmol) and BF₃·Et₂O (1.10 mL, 9.1 mmol) at -40 °C under Ar. The cold bath was removed after stirring at -40 °C for 15 min. The reaction mixture was stirred for an additional 40 min, then quenched with NaHCO₃ (20 mL) and extracted with diethyl ether (3 x 30 mL). The organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under vacuum to afford the crude product as a yellow oil. Purification of the crude product by flash column chromatography (hexanes:EtOAc = 6:1)
gave a cis/trans mixture (3:1) of 12 as a colorless oil (1.78 g, 84%, and 77% overall yield from 10). HRMS (FAB) calcd for C_{14}H_{24}NO_4 (M+H) 270.1705, found 270.1703.

**Methyl (2S,5R/S)-1-(tert-butyloxy carbonyl)-5-(formylmethyl)prolinate (13).** To a solution of 12 (1.76 g, 6.53 mmol) in THF (30 mL) and H_2O (15 mL) was added OsO_4 (80 mg, 0.32 mmol) in the dark. After 5 min, NaIO_4 (3.5 g, 16.4 mmol) was added in small portions in 15 min. The reaction mixture was stirred at rt for 4 hr, then filtered, and washed with MeOH (3 × 10 mL). After removal of the solvent, the residue was redissolved in dichloromethane (30 mL). The organic solution was washed with brine (3 × 30 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to give the crude product 13 as a brown oil (1.55 g, 87%).

**Methyl (2S)-cis/trans-(Z)-1-(tert-butyloxy carbonyl)-5-[(3'-amino-(N-benzyl oxy carbonyl)-3'-methoxycarbonyl)-2'-propenyl]prolinate (14).** To a solution of (MeO)_2P(O)CH(NHCbz)CO_2Me (2.2 g, 6.64 mmol) in dichloromethane (50 mL) was added DBU (990 μL, 6.62 mmol) slowly at rt. After 10 min, to the above solution was added a solution of 13 (1.55 g, 5.71 mmol) in dichloromethane (10 mL) and the reaction mixture was stirred overnight at rt. After removal of the solvent, the residue was redissolved in EtOAc (50 mL), washed with 1N HCl (30 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to afford a brown oil. The crude product was purified by flash column chromatography (hexanes:EtOAc = 3:1) to give a cis/trans (3:1) mixture of Z isomers 14 as a colorless oil (1.95 g, 72%, 63% overall yield from 12). HRMS (FAB) calcd for C_{24}H_{33}N_2O_8 (M+H) 477.2237, found 477.2234.

**Methyl (2S)-cis- and trans-(Z)-1-(tert-Butyloxy carbonyl)-5-[2'-bromo-(3'-amino-
(N-benzyloxy carbonyl)-3'-methoxycarbonyl)-2'-propenyl] prolinate (15ab). To a solution of 14 (240 mg, 0.5 mmol) in chloroform (5 mL) was added NBS (98 mg, 0.55 mmol) at rt. After 80 min, Dabco (85 mg, 0.76 mmol) was added. The reaction mixture was stirred at rt for 24 h. Then the reaction mixture was washed with saturated NH₄Cl (3 × 5 mL) and dried over Na₂SO₄. After removal of the solvent, the reaction residue was subjected to flash column chromatography (Hexanes:EtOAc = 4:1) to afford the cis isomer 15a as a light yellow oil (148 mg, 53% yield from cis/trans mixture 14) and the trans isomer 15b (mixture). 15a. [α]D²³ -10.3 (c 4.39, CHCl₃); ¹H NMR (500 MHz, CDCl₃) (two rotamers) δ 7.36 (brs, 5H), 5.14-5.11 (m, 2H), 4.33-4.29 (m, 1H), 4.23-4.19 (m, 1H), 3.81 (brs, 3H), 3.73 (s, 3H), 3.17-3.12 (m, 1.5H), 2.93 (dd, 0.5H, 7= 14.4, 3.4 Hz), 2.24-2.21 (m, 1H), 2.04-1.86 (m, 3H), 1.48 (s, 4H), 1.40 (s, 5H); ¹³C NMR (125 MHz, CDCl₃) (two rotamers) δ 174.0, 173.8, 163.0, 162.9, 154.0, 153.5, 153.3, 153.2, 135.7, 135.6, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 80.7, 80.5, 68.2, 68.0, 60.3, 59.8, 57.6, 53.0, 52.4, 52.2, 40.0, 39.3, 29.1, 28.7, 28.6, 28.5, 28.4, 28.0; HRMS (FAB) calcd for C₂₄H₃₂BrN₂O₈ (M+H) 555.1342, found 555.1337.

Methyl (25)-CM-(Z)-1-(tert-Butyloxycarbonyl)-5-[2'-phenyl- and 4-methoxyphenyl-(3'-amino-(N-benzyloxy carbonyl)-3'-methoxycarbonyl)-2'-propenyl] prolinate (16a, 17a). To a solution of 15a (175 mg, 0.32 mmol) in DME (2 mL) and degassed water (400 μL) was added phenylboronic acid (80 mg, 0.65 mmol), Na₂CO₃ (70 mg, 0.66 mmol), Pd(OAc)₂ (8 mg, 0.035 mmol) and P(o-tolyl)₃ (10 mg, 0.033 mmol). Then the reaction mixture was stirred at 80 °C overnight. The reaction mixture was passed through a short column containing a bottom 1” layer of silica gel and a top 1”
layer of NaHCO₃ using ethyl acetate as eluent. After removal of the solvent, the crude product was subjected to flash column chromatography (hexanes:EtOAc = 3:1) to afford 16a as a colorless oil (134 mg, 76%). The identical procedure afforded 17a as a colorless oil with a yield of 79%. 16a.  

$$[\alpha]_{D}^{23} = -142.3 \ (c \ 8.72, \ CHCl_{3}); \ \text{H NMR} \ (500 \ MHz, \ CDCl_{3}) \ (\text{rotamers}) \ \delta \ 7.34-7.18 \ (m, \ 10H), \ 5.98 \ (\text{brs, } 1H), \ 5.08 \ (s, \ 2H), \ 4.24 \ (t, \ 0.6H, \ J = 8.0 \ Hz), \ 4.14 \ (t, \ 0.4H, \ J = 8.0 \ Hz), \ 3.84 \ (\text{brs, } 3H), \ 3.74 \ (s, \ 3H), \ 3.70-3.65 \ (m, \ 0.4H), \ 3.57-3.52 \ (m, \ 0.6H), \ 3.18-3.15 \ (m, \ 0.6H), \ 3.14-3.05 \ (m, \ 0.6H), \ 3.04-2.99 \ (m, \ 0.4H), \ 2.91-2.88 \ (m, \ 0.4H), \ 2.19-2.02 \ (m, \ 3H), \ 1.74-1.63 \ (m, \ 1H), \ 1.39 \ (s, \ 4H), \ 1.38 \ (s, \ 5H); \ \text{C NMR} \ (125 \ MHz, \ CDCl_{3}) \ (\text{rotamers}) \ \delta \ 174.2, \ 173.9, \ 165.6, \ 165.4, \ 154.1, \ 154.0, \ 153.9, \ 153.4, \ 137.1, \ 136.8, \ 136.1, \ 136.0, \ 134.5, \ 134.2, \ 129.4, \ 129.3, \ 128.8, \ 128.7, \ 128.6, \ 128.5, \ 128.3, \ 128.2, \ 125.9, \ 125.7, \ 80.2, \ 80.0, \ 67.6, \ 60.5, \ 60.0, \ 56.6, \ 56.5, \ 52.5, \ 52.3, \ 52.1, \ 36.7, \ 35.6, \ 28.9, \ 28.7, \ 28.5, \ 28.3, \ 28.2, \ 27.8; \ \text{HRMS (FAB) calcd for } C_{30}H_{37}N_{2}O_{8} \ (M+H) \ 553.2550, \ \text{found } 553.2554. \ 17a. \ \ [\alpha]_{D}^{23} = -214.1 \ (c \ 2.30, \ CHCl_{3}); \ \text{H NMR} \ (600 \ MHz, \ CDCl_{3}) \ (\text{rotamers}) \ \delta \ 7.36-7.31 \ (m, \ 5H), \ 7.21-7.13 \ (m, \ 2H), \ 6.91-6.88 \ (m, \ 2H), \ 6.00 \ (\text{brs, } 1H), \ 5.09 \ (s, \ 2H), \ 4.25 \ (t, \ 0.5H, \ J = 8.0 \ Hz), \ 4.14 \ (t, \ 0.5H, \ J = 8.0 \ Hz), \ 3.83-3.81 \ (m, \ 6H), \ 3.75 \ (s, \ 3H), \ 3.74-3.70 \ (m, \ 0.5H), \ 3.61-3.57 \ (m, \ 0.5H), \ 3.15-3.11 \ (m, \ 0.5H), \ 3.10-3.04 \ (m, \ 0.5H), \ 3.00-2.98 \ (m, \ 0.5H), \ 2.90-2.88 \ (m, \ 0.5H), \ 2.18-2.06 \ (m, \ 3H), \ 1.71-1.63 \ (m, \ 1H), \ 1.39 \ (s, \ 4H), \ 1.37 \ (s, \ 5H); \ \text{C NMR} \ (150 \ MHz, \ CDCl_{3}) \ (\text{rotamers}) \ \delta \ 174.2, \ 173.9, \ 165.8, \ 165.6, \ 159.8, \ 154.1, \ 153.5, \ 153.3, \ 136.1, \ 134.9, \ 129.7, \ 129.6, \ 129.0, \ 128.7, \ 128.6, \ 128.5, \ 128.4, \ 125.6, \ 125.2, \ 114.8, \ 114.7, \ 80.2, \ 80.0, \ 67.6, \ 60.5, \ 60.0, \ 56.7, \ 56.6, \ 55.5, \ 55.4, \ 52.5, \ 52.3, \ 52.1, \ 36.6, \ 35.5, \ 29.0, \ 28.7, \ 28.5, \ 28.3, \ 28.2, \ 27.7; \ \text{HRMS (FAB) calcd for } C_{31}H_{39}N_{2}O_{9} \ (M+H) \ 583.2656, \ \text{found } 583.2634.$
(6R,9S)-1-Aza-3-N-benzoxy carbonylamino-9-methoxycarbonyl-4-phenyl- and 4-methoxyphenyl-2-oxobicyclo[4.3.0]non-3-enes (18a and 19a). To a solution of 16a (65 mg, 0.12 mmol) in dichloromethane (2.4 mL) was added TFA (0.6 mL) at rt. The mixture was stirred for 30 min, then saturated NaHCO₃ (10 mL) was added. After stirring for 20 min, the organic solution was dried over Na₂SO₄ and concentrated under vacuum. The residue was redissolved in chloroform (5 mL) and stirred at rt for 48 h. After removal of the solvent, the crude product was chromatographed (hexanes:EtOAc = 1:2) to afford 18a as a white solid (45 mg, 91%). The identical procedure afforded 19a as a white solid with a yield of 89%. 18a. mp 169-171 °C; [α]²³ D -24.8 (c 3.53, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.37-7.20 (m, 10H), 6.51 (brs, 1H), 4.99 (d, 1H, J = 12.0 Hz), 4.91 (d, 1H, J = 12.0 Hz), 4.61 (d, 1H, J = 8.5 Hz), 4.07-4.02 (m, 1H), 3.77 (s, 3H), 2.89-2.81 (m, 2H), 2.25-2.19 (m, 2H), 2.17-2.13 (m, 1H), 1.90-1.83 (m, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 172.6, 161.7, 153.7, 138.5, 138.2, 136.4, 128.7, 128.6, 128.4, 128.2, 127.4, 124.5, 67.2, 57.9, 56.2, 52.7, 36.6, 31.7, 29.0; HRMS (FAB) calcd for C₂₄H₂₅N₂O₅ (M+H) 421.1763, found 421.1752. 19a. mp. 170-172 °C; [α]²³ D -19.6 (c 0.94, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 7.34-7.22 (m, 7H), 6.85 (d, 2H, J = 8.6 Hz), 6.52 (brs, 1H), 5.01 (d, 1H, J = 12.3 Hz), 4.94 (d, 1H, J = 12.3 Hz), 4.60 (d, 1H, J = 8.6 Hz), 4.04-4.00 (m, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 2.88-2.77 (m, 2H), 2.24-2.12 (m, 3H), 1.89-1.84 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 172.6, 161.9, 159.6, 153.8, 138.2, 136.5, 130.6, 128.9, 128.5, 128.2, 128.1, 123.7, 114.1, 67.1, 57.8, 56.1, 55.4, 52.6, 36.5, 31.7, 29.0; HRMS (FAB) calcd for C₂₅H₂₇N₂O₆ (M+H) 451.1869, found 451.1862.
bicycle[4.3.0]non-3-ene (17b). Prepared according to the method used for 16a and 18a, starting from mixture 15b, in 34% overall yield from 15b, as a colorless oil. [$\alpha$]$^2_D$ +43.9 (c 3.29, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.34-7.19 (m, 7H), 6.84 (d, 2H, $J$ = 9.0 Hz), 6.69 (brs, 1H), 4.97 (d, 1H, $J$ = 12.5 Hz), 4.88 (d, 1H, $J$ = 12.5 Hz), 4.54 (t, 1H, $J$ = 8.0 Hz), 4.25-4.19 (m, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 2.92 (dd, 1H, $J$ = 17.0, 4.9 Hz), 2.60 (dd, 1H, $J$ = 17.0, 14.1 Hz), 2.47-2.40 (m, 1H), 2.36-2.30 (m, 1H), 2.01-1.93 (m, 1H), 1.77-1.69 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 172.7, 162.0, 159.5, 153.3, 136.5, 136.0, 131.1, 128.7, 128.6, 128.2, 128.1, 123.1, 114.1, 67.1, 58.5, 55.9, 55.4, 52.6, 36.5, 32.9, 28.8; HRMS (FAB) calcd for C$_{25}$H$_{27}$N$_2$O$_6$ (M+H) 451.1869, found 451.1851.

(3S,4R,6R,9S)-1-Aza-3-amino-9-methoxycarbonyl-4-phenyl-2-oxobicyclo[4.3.0]-nonane (20a). A solution of 18a (60 mg, 0.14 mmol) in degassed MeOH (5 mL) with Pd/C (10 wt.%, catalytic amount) was reacted at an initial H$_2$ pressure of 75 psi at rt. After 24 h, the mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (5% MeOH in dichloromethane) to give 20a as colorless oil (34 mg, 84%). [$\alpha$]$^2_D$ -67.8 (c 0.73, CHCl$_3$); $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.35-7.23 (m, 5H), 4.54 (d, 1H, $J$ = 8.9 Hz), 3.81-3.76 (m, 4H), 3.65 (d, 1H, $J$ = 6.9 Hz), 3.51-3.47 (m, 1H), 2.48-2.44 (m, 1H), 2.25-2.17 (m, 2H), 2.13-2.07 (m, 2H), 1.85-1.78 (m, 1H), 1.35 (brs, 2H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 172.5, 172.2, 141.6, 128.9, 128.6, 127.2, 58.4, 57.7, 54.4, 52.6, 44.5, 34.8, 31.9, 29.1; HRMS (FAB) calcd for C$_{16}$H$_{21}$N$_2$O$_3$ (M+H) 289.1552, found 289.1558.

(3S,4R,6R,9S)-1-Aza-3-amino-9-methoxycarbonyl-4-methoxyphenyl-2-oxo-
bicyclo[4.3.0]nonane (21a). In a manner similar to the preparation of 20a, using 19a as starting material gave 21a in 67% yield. 21a. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.22 (d, 2H, $J = 8.6$ Hz), 6.87 (d, 2H, $J = 8.6$ Hz), 4.53 (d, 1H, $J = 8.5$ Hz), 3.84-3.74 (m, 7H), 3.61 (d, 1H, 7.1 Hz), 3.48-3.43 (m, 1H), 2.48-2.43 (1H), 2.25-2.17 (m, 2H), 2.16-2.04 (m, 2H), 1.85-1.77 (m, 1H); HRMS (FAB) calcd for C$_{17}$H$_{23}$N$_2$O$_4$ (M+H) 319.1658, found 319.1648.
CHAPTER 4. STEREOSELECTIVE SYNTHESIS OF 4,8-DISUBSTITUTED AZABICYCLO[4.3.0]NONANE AMINO ACIDS AS PEPTIDOMIMETICS SCAFFOLDS OF MELANOCORTIN RECEPTOR LIGANDS

4.1 Introduction

α-Melanotropin (α-melanocyte stimulating hormone, α-MSH) is a linear tridecapeptide consisting of the amino acid sequence Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH2. It is synthesized in the vertebrate pars intermedia and in the brain.α-MSH is known primarily for its ability to stimulate the integumental melanocyte, in addition to interacting with several other proposed peripheral and central biological systems. To determine the biologically active conformation of α-MSH, numerous analogues have been designed and synthesized. Substitution of Nle (sidechain = CH2CH2CH2CH3) for Met resulted in peptides with more potent biological activity. Inversion of configuration of L-Phe to D-Phe led to the analogue [Nle4,D-Phe7]α-MSH, which is referred as “MT-I” and “NDP-α-MSH”. This analogue showed a substantial increase in potency as well as prolonged activity. The conformationally restricted analogue MT-II possesses potency up to 90 times greater than α-MSH (Figure 4-1). Because cyclization leads to considerable restriction of conformational flexibility of the peptide backbone and, to a lesser degree, the side-chain groups, MT-II lends itself to a more complete evaluation of the biologically active 3D topologies.
To understand further the effects of conformation on biological potency, we would like to design and synthesize novel analogues with conformationally constrained dipeptide mimetics.

4.2 Design of Conformationally Restricted Analogues

Recently the major effort in peptidomimetic research has been to find the organic moieties that can replace the peptide scaffold and position the crucial recognition elements in 3D space correctly. Of particular interest are the constrained scaffolds that not only possess the right conformation but also allow for a wide-ranging display of substitutes. Azabicyclo[4.3.0]alkane amino acids are unique dipeptide β-turn mimetics. As we introduced in previous chapters, these scaffolds have been applied as secondary structure replacement due to their capacity of restricting five backbone bonds in a row within the peptide. Although the studies have shown that indolizidinone systems can mimic β-turn conformation, little success in the stereoselective incorporation of diverse substituents on their backbones has limited their application as peptidomimetic scaffolds. Herein we would like to develop an efficient approach to the synthesis of 4,8-disubstituted azabicyclo[4.3.0]nonane amino acids as peptidomimetic scaffolds of melanocortin receptor ligands.
As part of our ongoing program for the design of novel melanotropin peptide mimetics, we have identified the core bioactive sequence of melanotropin peptides as His-(D/L)Phe-Arg-Trp (Figure 4-2)\textsuperscript{148-151} and found a $\beta$-turn structural feature centered in these core residues.\textsuperscript{152,153} The studies of structure-activity relationships of melanotropin peptides have resulted in the development of various more potent, prolonged acting and enzymatically stable analogues, such MT-I and MT-II, which have been widely used in biological studies. However, these analogues did not show significant selectivity for the different melanocortin receptors. Recently, we reported the first example of a selective and potent antagonist at the hMC3 receptor, MK-9.\textsuperscript{154} Based on our conformational studies of melanotropin peptides and their analogues, we postulated that indolizidinone systems can serve as peptidomimetic scaffolds of the core sequence in melanotropin peptides which not only mimic $\beta$-turn conformation but also place amino acid side-chain functionalities in space correctly (Scheme 4-1).

\begin{align*}
\alpha\text{-MSH} & \quad \text{Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH}_2 \\
\text{MT-I} & \quad \text{Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH}_2 \\
\text{MT-II} & \quad \text{Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH}_2
\end{align*}

\textbf{Figure 4-2.} Core sequence of melanotropin peptides.
For designing the synthetic targets 26, we chose the benzyloxycarbonyl group as a bioisosteric replacement of His because it can be readily introduced from commercially available reagents. The phenyl group as $R_2$ corresponds to the side-chain of Phe. The 2-naphthyl group is also an interesting element to be incorporated here because we found that introducing a Nal residue in place of Phe in MT-II converts this potent agonist to a potent antagonist (SHU9119).$^{155}$ For $R_3$, both guanidine and amine groups are placed at this site since some literature suggested that the guanidine group might not be essential in MC4R agonists when a heteroatomic substituent is present in a proper position. Lastly, tryptamine and phenethylamine are chosen to serve as bioisosteric replacements of tryptophane amide at the $R_4$ position.
4.3 Synthesis of Disubstituted Indolizidinone Amino Acids

4.3.1 Background and General Approach

Our group has developed a strategy to introduce a side-chain group at the C-8 position of indolizidinone amino acids.\(^{82}\) The strategy involves employing \(\beta\)-substituted pyroglutamates as chiral synthetic precursors which could be prepared stereoselectively by methods recently developed in our laboratory.\(^{156-163}\) Stereoselectively introducing allyl groups at the C-5 position of pyroglutamates and their appropriate elaboration could afford dehydroamino acid intermediates, which could undergo asymmetric hydrogenations and cyclizations to afford C8-substituted indolizidinone amino acids. In addition, as discussed in the previous chapter, we recently have developed a novel methodology which could readily prepare C4-substituted unsaturated and saturated indolizidinone amino acid esters.\(^{164}\) The functional groups at C-4 were introduced by bromination of dehydroamino acid intermediates followed by Suzuki coupling. Hydrogenation of the unsaturated bicyclic dehydroamino acid esters provided saturated bicyclic lactams. These successful synthetic results directed our attention to developing a convergent synthetic approach using a combination of these two methods to synthesize peptidomimetics 26. The retrosynthetic analysis of the target analogues 26 is described in Scheme 4-2.
4.3.2 Synthesis of β-Substituted Pyroglutamic Acid Ester.

As the first goal in our studies, a method for introducing an appropriate side-chain group at C-8 was investigated. As discussed above, the side-chain functionality could be introduced at the C8 position by using the pyroglutamic acid ester with a corresponding substituent at the β-position as the starting material. Therefore, a novel β-substituted pyroglutamic acid was the first synthetic target in the approach.
As illustrated in Scheme 4-2, the proposed approach would include diverse reactions. Therefore, it was important to identify the proper protecting group for the amino group in the starting material which would later be functionalized in the final step. This protecting group should be sufficiently robust to survive the projected reactions, and also should be orthogonal to other functionalities and be labile enough to be removed in the final step. Furthermore, based on our earlier studies, it seemed likely that a mono-protected nitrogen would interfere with the aldehyde intermediate. All of these requirements prompted us to choose the phthalimide group to doubly protect the amine group. Aldehyde 28 was prepared in good yield by PCC oxidation of alcohol 27.
following the literature procedure (Scheme 4-3). However, when the reaction was performed on a large scale, the reduced chromium byproduct made workup difficult. Thus we employed the Swern oxidation as a good alternative synthetic method to prepare 28 on a large scale. Wittig olefination of aldehyde 28 with (t-butoxycarbonylmethyl) triphenylphosphonium bromide in the presence of NaOH and triethylamine in a two-phase system of dichloromethane/H2O gave the E-5-N-phthalimido-α,β-unsaturated t-butyl ester 29 in excellent yield. The t-butyl protecting group was removed by treatment with 50% TFA in dichloromethane, and S-4-phenyl-2-oxazolidinone was coupled to the deprotected 29 as the chiral auxiliary for asymmetric functionalization of the β-position in the next step.

Optically pure 4-substituted oxazolidin-2-ones were introduced as chiral auxiliaries by Evans and later were widely used due to their excellent stereocontrolling power in acylation, aldol condensation, and Diels-Alder reactions. According to the literature, successful application of chiral 4-substituted oxazolidin-2-ones in conjugate addition reactions always required the use of a chelating agent (e.g. LiCl, MgClO4, or CuBr) to form the corresponding metal-chelated intermediates, in which the stereocontrolling 4-substituted group could be located in close proximity to the CC double bond so as to efficiently control its facial selectivity (Scheme 4-4). Furthermore, the electrophilicity of the CC double bond is substantially enhanced, as compared with the corresponding esters, due to the fact that an oxazolidin-2-one is a substantially stronger electron-withdrawing substituent than alkoxy groups. Thus 4-substituted oxazolidin-2-ones can not only provide excellent diastereoselectivity, but
also enhanced reactivity in asymmetric Michael addition.

**Scheme 4-4.** Metal-chelated intermediate in Michael addition.

![Diagram of metal-chelated intermediate]

Meanwhile, chiral Ni(II) complex (S)-32 was prepared according to a literature procedure (Scheme 4-5). First the amination of (S)-proline with benzyl chloride was conducted at 40 °C in i-PrOH in the presence of KOH with 78% yield to give N-benzylproline in hydrochloride salt form. Then N-benzylproline was coupled with 2-aminobenzophenone to give the chiral ligand, N-(N-benzylprolyl)-2-aminobenzophenone (BPB, 31). After simple work-up by washing with deionized water, the ligand was used for the next step without further purification. The Ni(II) complex 32 was formed between the chiral ligand and glycine in the presence of NiCl·6H2O under strong base in MeOH at 55 °C for 6 hours.
Scheme 4-5. Preparation of the chiral Ni(II) complex (S)-32.

The presence of the proline stereogenic center in the complex imparts an asymmetric distortion to the rigid polycyclic system of the chelate rings, which brings about a steric shielding of the corresponding enolate on the \( re \) face by the ketimine phenyl group. Accordingly, in the Michael addition, the electrophile approaches preferentially from the \( si \) face to form a new complex with pseudoaxial orientation of the introduced substituent which usually leads to an \( \alpha-(S) \) absolute configuration (Scheme 4-6).
Scheme 4-6. Selective electrophilic attack on the complex enolate.

In agreement with the above explanations, Michael acceptor 30 underwent asymmetric Michael addition with the Ni(II) complex 32 to give a mixture of (2S,3S)-33a and (2R,3S)-33b in a ratio of 9/1 (Scheme 4-7). The stereochemistry of products was assigned based on our detailed knowledge of similar Ni(II) complexes.

Scheme 4-7. Asymmetric Michael addition between 30 and 32.
Hydrolysis of the Ni(II) complex 33a followed by cyclization under the basic condition afforded the corresponding \( \beta \)-substituted pyroglutamic acid (Scheme 4-8). Attempts to purify the amino acid using a Dowex ion-exchange resin column were not successful probably due to a contamination of the uncyclized product. Instead the crude amino acid was protected directly as the \( N^\alpha \)-Boc pyroglutamate 34. Compound 34 was purified by flash column chromatography and the configuration was confirmed by X-ray crystallographic analysis (Figure 4-3).

**Scheme 4-8.** Hydrolysis of Ni(II) complex 33a.

\[
\text{33a} \xrightarrow{\text{a, b, c, d}} \text{34}
\]

\( ^a (a) \text{3N HCl, MeOH}; (b) \text{NH}_4\text{OH}; (c) \text{SO}_2\text{Cl, MeOH}; (d) \text{(Boc)}_2\text{O, DMAP, Acetonitrile} \)
4.3.3 Synthesis of 8-Substituted Azabicyclo[4.3.0]Alkane Amino Acids

The lactam moiety of pyroglutamate usually could be reduced selectively by Super-Hydride (LiBEt₃H).¹⁷⁴ However, in agreement with a previous report,¹⁷⁵ the treatment of 34 with Super-Hydride (LiBEt₃H) also resulted in the reduction of the phthalimide protecting group. Therefore, we chose to use DIBAL-H which the phthalimide group is stable to. The reduction by DIBAL-H followed by treatment with methanol in the presence of a catalytic amount of p-TsOH afforded the methoxyaminal which was directly subjected to allyltrimethylsilane and boron trifluoride in ether without further purification (Scheme 4-9). Consistent with our previous observations, the allylsilane addition to the N-acyliminium intermediate gave exclusively the trans product related to the β-substituent as a result of a neighboring group participation of the methyl
ester and the steric effect of the β-substituent. The optically pure intermediate 35 underwent osmylation and subsequent oxidation with NaIO₄ to afford the aldehyde intermediate. The dehydroamino acid ester 36 was obtained via the Horner-Emmons olefination of the aldehyde intermediate.¹⁷⁶

Scheme 4-9.⁴ Synthesis of dehydroamino acid derivative 36.

\[ \text{34} \xrightarrow{\text{a, b, c}} \text{35} \xrightarrow{\text{d, e}} \text{36} \]

\[ \text{a} (\text{a) DIBAL-H, THF, -78 °C}; \text{b) Ts-OH, MeOH}; \text{c) BF₃·Et₂O, Me₃SiCH₂CH=CH₂, Et₂O, -40 °C}; \text{d) OsO₄, NaIO₄, THF/H₂O}; \text{e) DBU, (MeO)₂P(O)CH(NHCBz)COOCH₃, CH₂Cl₂, rt.} \]
With the important intermediate 36 in hand, we employed asymmetric hydrogenations on this substrate to prepare α-amino acid derivatives 37a and 37b (Scheme 4-10). The Burk's catalysts [Rh(I) (COD) (R,R)- or (S,S)-Et-DuPHOS] OTf were used as the catalyst in hydrogenation with high yields (>90%) and high diastereoselectivity (>96% ee). Deprotection of Nα-Boc group was performed in 20% TFA in dichloromethane at rt. The TFA salt was neutralized with NaHCO₃ and the reaction was stirred in chloroform at rt. The cyclization proceeded smoothly under these mild conditions to give bicyclic lactams 38 and 39 in good yield.

Scheme 4-10. Synthesis of 8-[2-((N-phthalimido)-ethyl] indolizidine amino acid esters.

\[ \text{MeOOC}_a \text{COOMe} \]

\[ \text{CbzHN}_b \]

\[ \text{Boc}_c \]

\[ \text{COOMe}_d \]

\[ \text{MeOOC}_a \text{COOMe} \]

\[ \text{CbzHN}_b \]

\[ \text{Boc}_c \]

\[ \text{COOMe}_d \]

37a

37b

38

39

\(a\) (a) Rh(I)(COD)-(R,R)-Et-DuPHOS, H₂ (75 psi), MeOH; (b) Rh(I)(COD)-(S,S)-Et-DuPHOS, H₂ (75 psi), MeOH; (c) TFA (20% in CH₂Cl₂); (d) CHCl₃, rt, 24 h.
4.3.4 Synthesis of 4,8-Disubstituted Indolizidinone Amino Acid Ester

Having developed the methods to introduce side-chain groups at the C-4 and C-8 positions, respectively, we were able to carry out experiments aimed at preparing peptidomimetics 26 by combining the two methodologies. With the dehydroamino acid ester 36 in hand, we treated it with N-bromosuccinimide (NBS) in chloroform to produce the α-bromoimines (Scheme 4-11). Upon treatment with an amine base, the α-bromoimine intermediates underwent tautomerization to afford the (Z)-β-bromo-α,β-dehydroamino acid 40. Consistent with our previous observation, using DABCO in stead of TEA and DBU in the tautomerization step resulted in the Z isomers exclusively. Suzuki coupling of 40 with phenylboronic acid and 2-naphthaleneboronic acid introduced the phenyl and 2-naphthyl groups at the β-position of the dehydroamino acid. Deprotection of the Nα'-Boc group in 41 and 42 was performed with 20% TFA in dichloromethane at rt. The TFA salt was neutralized with NaHCO₃ solution and the reaction was stirred in chloroform at rt. The cyclization proceeded smoothly to give bicyclic lactam 43 and 44 in good yields after 24 h.
Scheme 4-11. Synthesis of 4,8-disubstituted azabicyclo[4.3.0]alkane amino acids.

\[\text{MeOOC} \quad \text{CbzHN} \quad \text{COOMe} \quad \text{Boc}\]

\[\text{MeOOC} \quad \text{CbzHN} \quad \text{MeOOC} \quad \text{COOMe} \quad \text{Boc}\]

\[\text{CbzHN} \quad \text{MeOOC} \quad \text{COOMe} \quad \text{Boc}\]

\[\text{CbzHN} \quad \text{O} \quad \text{COOMe}\]

\[\text{R}_1 = \text{phenyl}\]

\[\text{R}_1 = 2\text{-naphthyl}\]

\[\text{41; Ri = phenyl}\]

\[\text{42; Ri = 2-naphthyl}\]

\[\text{43; Ri = phenyl}\]

\[\text{44; R}_1 = 2\text{-naphthyl}\]

\(^a\) (a) NBS, CHCl₃; (b) Dabco, CHCl₃; (c) PhB(OH)₂, Pd(OAc)₂, P(α-tolyl)₃, Na₂CO₃, DME, 80 °C; (d) 20% TFA, CH₂Cl₂, rt; (e) CHCl₃, rt, 24 h.

4.3.5. Synthesis of Peptidomimetics of the Core Sequence in Melanotropin Peptides

Deprotection of the phthalimide protecting group in 43 and 44 was accomplished by treatment with hydrazine at rt for 24 h (Scheme 4-12). Work-up and purification of free amine gave poor yields. Thus the crude free amines 45 and 46 were directly guanidinated to give 47 and 48, using the commercial available guanidinating reagent,
Although an initial attempt using DMAP as base failed, the guanidinating reaction with triethylamine afforded 47 and 48 in good yields. The configuration of 47 was confirmed by NOE (Table 4-1). Hydrolysis of the methyl ester group using 1N LiOH and MeOH (1:2) went slowly due to the steric hindrance of the neighboring group. Subsequently, deprotected 47 and 48 were treated with tryptamine or phenethylamine in DMF using BOP and HOBT as the coupling reagent to yield 49-51. On the other hand, hydrazinolysis of 44 followed by protection of amine with Boc afforded 52 (Scheme 4-13). In a similar way, tryptamine was coupled to 52 to afford 53. Compounds 49-51 and 53 were treated with 20% TFA in dichloromethane to remove the Boc group. The deprotected products were purified by HPLC and submitted for biotests.

43: $R_1 = \text{phenyl}$
44: $R_1 = \text{2-naphthyl}$
45: $R_1 = \text{phenyl}$
46: $R_1 = \text{2-naphthyl}$
47: $R_1 = \text{phenyl}$
48: $R_1 = \text{2-naphthyl}$
49: $R_1 = \text{phenyl}; R_2 = \text{tryptamine}$
50: $R_1 = \text{phenyl}; R_2 = \text{phenethylamine}$
51: $R_1 = \text{2-naphthyl}; R_2 = \text{tryptamine}$

*(a) NH$_2$NH$_2$, EtOH, CHCl$_3$; (b) N,N'-Bis(tert-butoxycarbonyl)-N'-triflylguanidine, TEA, CH$_2$Cl$_2$;
(c) LiOH(1N), MeOH; (d) PyBOP, HOBt, DIPEA, R-NH$_2$, CH$_2$Cl$_2$.

Table 4-1. NOE Data for 47

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<th>protons</th>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>$H_6$ $H_9$</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*NOE not observed.
Scheme 4-13. Synthesis of analogue 53.

4.4 Future Work

In our ongoing opioid and α-MSH program, the studies have indicated that the conformational requirements for optimal interaction with different receptors differ in a subtle manner. On the other hand, the configurations of the peptidomimetics influence their conformations. Thus it is very important for us to have the approach to every individual stereoisomer of the peptidomimetics. As discussed in Chapter 3,
hydrogenation of unsaturated indolizidinone amino acid only afforded one stereoisomer due to the steric effect. Therefore, other methods to reduce the double bonds will be investigated (Scheme 4-14). Once other stereoisomers are available, they will be incorporated into the peptidomimetics and provide us with more valuable information.

**Scheme 4-14.** Future work: reduction of double bonds.

4.5 Conclusion

4,8-Disubstituted azabicyclo[4.3.0]alkane amino acid analogues (26) were designed to serve as peptidomimetic scaffolds of the core sequence in melanotropin peptides due to their ability to provide constrained backbone and side-chain conformations. We have developed efficient synthetic methodologies to introduce side-chain functionalities at the C-4 and C-8 positions and also demonstrated the synthesis of analogues 49-51 and 53.
In our approach, the pyroglutamates with appropriate β-substituents were prepared stereoselectively via asymmetric Michael additions between a Ni(II) complex and a chiral oxazolidin-2-one derivative. The alkylation of the β-substituted pyroglutamates followed by osmylation and Horner-Emmons olefination gave the dehydroamino acid ester intermediates in good yield. The aryl side-chain at the C-4 position was introduced via bromination of the dehydroamino acid ester and Suzuki cross-coupling. The cyclization afforded bicyclic lactam structures which were subsequently functionalized and coupled with tryptamine or phenethylamine to provide the target analogues. The biological activities of these analogues are under investigation.

4.6 Experimental Section

3-(N-Phthalimido)propionaldehyde (28). Commercial grade pyridinium chlorochromate (PCC, 21 g, 97.4 mmol) was ground with silica gel (1 wt eq) in a mortar. The resulting free-running light orange solid was suspended in CH\textsubscript{2}Cl\textsubscript{2} (200 mL) at rt and N-(3-hydroxypropyl)phthalimide (10 g, 48.7 mmol) was added in one portion. The resulting brown suspension was stirred for 2 h and filtered through a Büchner funnel packed with Celite, and the granular brown residue was washed with ether (80 mL). The filtrate was concentrated, purified by flash chromatography (hexanes:EtOAc = 1:1), and recrystallized (Hexanes/EtOAc) to give 28 as white crystals (8.1 g, 82%). 28. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \( \delta \) 9.83 (t, 1H, \( J = 1.3 \) Hz), 7.86-7.84 (m, 2H), 7.74-7.72 (m, 2H), 4.04 (t, 2H, \( J = 7.0 \)), 2.90-2.87 (m, 2H); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) \( \delta \) 199.4, 168.0,
134.1, 131.9, 123.3, 42.3, 31.7.

(E)-5-(N-Phthalimido)-pent-2-enoic acid tert-butyl ester (29). To a solution of 28 (500 mg, 2.46 mmol) in CH₂Cl₂ (20 mL) and H₂O (10 mL) was added (tert-butoxycarbonylmethyl)triphenylphosphonium bromide (1.13 g, 2.47 mmol), NaOH (200 mg, 4.92 mmol) and Et₃N (1 mL, 7.38 mmol) at rt. After stirring at rt for 1 h, the organic phase was separated and washed with 1N HCl (30 mL) and saturated aqueous NaHCO₃ (30 mL). The organic phase was dried over Na₂SO₄, filtered, and concentrated to give a crude product, which was purified by flash column chromatography (hexanes:EtOAc = 3:1) to afford pure product 29 as a white solid (704 mg, 95%).

29. mp 91-93 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.85-7.84 (m, 2H), 7.74-7.71 (m, 2H), 6.81 (dt, 1H, J = 15.6, 7.1 Hz), 5.82 (dt, 1H, J = 15.6, 1.5 Hz), 3.81 (t, 2H, J = 7.3 Hz), 2.60-2.55 (m, 2H), 1.46 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 168.0, 165.3, 142.6, 134.0, 132.0, 125.5, 123.3, 80.3, 36.4, 31.0, 28.1; HRMS (FAB) calcd for C₁₇H₂₀NO₄ (M+H) 302.1392, found 302.1405.

(4S,2E)-3-[5-(N-Phthalimido)-1-oxo-2-pentenyl]-4-phenyl-2-oxazolidinone (30). A solution of 29 (2 g, 6.64 mmol) in 50% TFA in dichloromethane (15 mL) was stirred at rt for 2 h. The solution was concentrated, neutralized by saturated aqueous NaHCO₃ and extracted with EtOAc (3 × 30 mL). The organic layers were combined, dried over Na₂SO₄ and evaporated in vacuo to give a crude product. To a solution of the crude product in dry THF (20 mL) were added triethylamine (970 μL, 6.96 mmol) and trimethylacetyl chloride (900 μL, 7.31 mmol) at -78 °C. The mixture was stirred at 0 °C for 1 h and recooled to -78 °C. Meanwhile, the chiral auxiliary,
(S)-4-phenyl-2-oxazolidinone (1.08 g, 6.62 mmol), in THF (40 mL) at -78 °C was treated with the dropwise addition of nBuLi (1.6 M in hexanes, 4.15 mL, 6.64 mmol). This reaction was stirred at -78 °C for 25 min. The preformed mixed anhydride was cannulated into the lithiated chiral auxiliary solution at -78 °C and stirred in an ice bath afterward, allowing the reaction mixture to achieve rt overnight. Water (100 mL) and diethyl ether (50 mL) were added and the organic phase was separated, washed with water (50 mL) and saturated aqueous NaCl (80 mL), dried over Na₂SO₄ and concentrated to give a crude product, which was purified by flash column chromatography (hexanes:EtOAc = 1:1) to afford pure product 30 as a white solid (2.31 g, 89%).

30. mp 139-141 °C; [α]²³ D +65.4 (c 1.14, CHCl₃); H NMR (500 MHz, CDCl₃) δ 7.84-7.82 (m, 2H), 7.73-7.70 (m, 2H), 7.39-7.26 (m, 6H), 7.01 (dt, 1H, J = 15.4, 7.1 Hz), 5.46 (dd, 1H, J = 8.7, 3.9 Hz), 4.68 (t, 1H, J = 8.8 Hz), 4.26 (dd, 1H, J = 8.9, 3.9 Hz), 3.84-3.82 (m, 2H), 2.68-2.64 (m, 2H); C NMR (125 MHz, CDCl₃) δ 168.0, 164.0, 153.5, 146.4, 138.9, 134.0, 132.0, 129.2, 128.7, 125.9, 123.3, 122.5, 69.9, 57.7, 36.3, 31.6; HRMS (FAB) calcd for C₂₂H₁₉N₂O₅ (M+H) 391.1294, found 391.1289.

N-(N-Benzylprolyl)-2-aminobenzophenone (31). To a clear solution of (S)-proline (173 g, 1.5 mol) and potassium hydroxide (252.5 g, 4.5 mol) in 1 L of i-PrOH at 40 °C was added benzyl chloride (207.4 mL, 1.8 mol) dropwise in 3 h. The mixture was stirred at 40 °C overnight, acidified to pH 5-6 with concentrated hydrochloric acid, mixed with 200 mL of chloroform, and allowed to stand overnight. The white precipitate (KCl) was filtered and washed with chloroform thoroughly. The filtrate was evaporated in vacuo to give crude product that was dispersed in acetone and allowed to stand overnight.
Pure product was obtained after filtration and washing with a small amount of cold acetone and dried in vacuum. To a solution of the above product (50 g, 0.207 mol), 2-aminobenzophene (40.8 g, 0.207 mol) and BOP (91.5 g, 0.207 mol) in 300 mL of freshly distilled dichloromethane was added triethylamine (87 mL, 0.621 mol). The mixture was stirred at rt until the 2-aminobenzophene completely disappeared. The reaction was quenched by adding 1N hydrochloric acid with strong agitation. The aqueous layer was separated from the organic layer and washed with methylene chloride (2 × 100 mL). The combined methylene chloride layer was dried over anhydrous MgSO₄ and evaporated in vacuo to afford the crude product which was used in the next step.

(S)-NiGlyBPB (32). The solution of (S)-BPB generated above, NiCl₂-6H₂O (2 eq), potassium hydroxide (7 eq) and glycine (5 eq) in methanol was stirred at 40 °C until the starting ligand (S)-BPB was completely consumed as monitored by TLC (1:1 acetone/hexanes). The mixture was poured into ice water (four times the volume of methanol) with 1% acetic acid. The precipitate was filtered, washed with water, and dried in vacuum to afford the crude product. The crude product was dissolved in a large quantity of chloroform and the precipitate was filtered and washed with chloroform. The filtrate was evaporated in vacuo and dried under vacuum to afford the product. Further recrystallization with acetone/hexanes gave the pure product 32 as a red powder (yield: 75%). 32. mp 219-221 °C; [α]²³ᵇ +2117 (c 0.0145, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 8.29 (d, 1H, J = 8.5 Hz), 8.07 (d, 2H, J = 8.0 Hz), 7.49-7.53 (m, 3H), 7.41-7.44 (m, 2H), 7.30-7.33 (m, 1H), 7.19-7.23 (m, 1H), 7.10 (d, 1H, J = 7.0 Hz), 6.97-6.99 (m,
IH), 6.80 (d, 1H, J = 8.5 Hz), 6.69-6.72 (m, 1H), 4.48 (d, 1H, J = 12.5 Hz), 3.66-3.80 (m, 4H), 3.47 (dd, 1H, J = 11.0, 5.5 Hz), 3.32-3.38 (m, 1H), 2.56-2.59 (m, 1H), 2.40-2.47 (m, 1H), 2.13-2.18 (m, 1H), 2.06-2.10 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) 181.3, 177.3, 171.6, 142.5, 134.6, 133.3, 133.2, 132.1, 129.8, 129.6, 129.3, 129.1, 128.9, 126.2, 125.7, 125.1, 124.2, 120.8, 69.9, 63.1, 61.3, 57.5, 30.7, 23.7. HRMS (FAB) calcd for C$_{27}$H$_{25}$N$_3$NiO$_3$ (M+H) 498.1328, found 498.1328.

Ni(II)-Complex of the Schiff base of (S)-BPB with (2S- and R,3S)-3-[2-(N-phthalimido)-ethyl]-5-[((4S)-3-(4-phenyl-2-oxazolidinonyl)] glutamic acids (33ab). To a suspension of complex (S)-32 (1.2 g, 2.4 mmol) in DMF (8 mL), complex 30 (1 g, 2.56 mmol) was added with stirring. The mixture was stirred at rt for 10 min to get a homogeneous solution and DBU (35 μL, 0.24 mmol) was added dropwise. After 6 min, the reaction was quenched with 5% aqueous acetic acid (10 mL) and the product was extracted with dichloromethane (2 × 20 mL). The combined organic phase was dried over Na$_2$SO$_4$ and concentrated to give a crude product. Diastereomerically pure 33a was recrystallized from ethyl acetate/hexanes as a red solid (1.82 g, 85%). Diastereomerically pure 33b was obtained by flash column chromatography (DCM:acetone = 2:1) as a red oil (200 mg, 9%). 33a. mp 186-188 °C; [α]$^23_D$ +1522 (c 0.05, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.30 (dd, 1H, J = 8.7, 0.8 Hz), 8.02 (d, 2H, J = 7.1 Hz), 7.69-7.65 (m, 2H), 7.59-7.55 (m, 2H), 7.46-7.43 (m, 1H), 7.40-7.37 (m, 2H), 7.35-7.22 (m, 6H), 7.16-7.04 (m, 4H), 6.66 (d, 1H, J = 7.7 Hz), 6.56-6.47 (m, 2H), 5.19 (dd, 1H, J = 8.5, 3.2 Hz), 4.44 (t, 1H, J = 8.6 Hz), 4.37 (d, 1H, J = 12.7 Hz), 4.12 (dd, 1H, J = 8.7, 3.2 Hz), 4.07 (d, 1H, J = 6.2 Hz), 4.00-3.95 (m, 1H), 3.77-3.67 (m, 2H),...
3.50-3.42 (m, 4H), 3.17 (dd, 1H, J = 17.9, 8.3 Hz), 2.96-2.85 (m, 2H), 2.47-2.38 (m 2H), 2.07-2.03 (m, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 180.3, 177.4, 172.3, 170.2, 167.9, 153.5, 142.8, 139.3, 133.8, 133.7, 133.6, 133.5, 132.3, 131.8, 131.4, 129.5, 129.1, 129.0, 128.9, 128.7, 128.6, 128.3, 128.0, 127.0, 125.9, 125.6, 123.1, 123.0, 120.2, 72.0, 70.5, 70.0, 63.3, 57.5, 57.1, 36.7, 36.0, 35.9, 30.3, 29.7, 23.3; HRMS (FAB) calcd for C$_{49}$H$_{44}$N$_5$NiO$_8$ (M+H) 888.2543, found 888.2553.

$^{33b}$ 

$^1$H NMR (500 MHz, CDCl$_3$) δ 8.55 (d, 1H, J = 9.0 Hz), 7.65-7.62 (m, 2H), 7.55-7.49 (m, 4H), 7.44-7.38 (m, 6H), 7.37-7.30 (m, 2H), 7.27-7.25 (m, 2H), 7.18-7.14 (m, 2H), 7.11 (d, 1H, J = 7.4 Hz), 6.75 (d, 1H, J = 7.4 Hz), 6.56 (d, 2H, J = 4.0 Hz), 5.28-5.25 (m, 2H), 4.61 (t, 1H, J = 8.7 Hz), 4.36-4.29 (m, 1H), 4.19 (dd, 1H, J = 8.7, 3.4 Hz), 4.15-4.10 (m, 2H), 3.92-3.76 (m, 4H), 3.21 (dd, 1H, J = 17.4, 8.0 Hz), 2.94 (dd, 1H, J = 17.4, 5.9 Hz), 2.66-2.60 (m, 1H), 2.37-2.29 (m, 2H), 2.06-2.03 (m, 1H), 1.92-1.86 (m, 1H), 1.67-1.57 (m, 2H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 182.5, 177.7, 172.7, 170.2, 168.3, 153.4, 143.1, 139.3, 133.9, 133.8, 133.7, 132.4, 132.3, 131.9, 131.6, 129.5, 129.1, 128.9, 128.8, 128.7, 128.4, 128.2, 126.8, 126.0, 125.5, 123.5, 123.2, 120.4, 72.0, 69.9, 68.4, 60.6, 57.6, 55.8, 37.3, 36.7, 36.6, 31.2, 29.6, 23.6; HRMS (FAB) calcd for C$_{49}$H$_{44}$N$_5$NiO$_8$ (M+H) 888.2543, found 888.2543.

**Methyl (2S,3S)-N$_2$-tert-butoxycarbonyl-3-[2-(N-phthalimido)-ethyl]pyroglutamate (34).** Diastereomerically pure complex $^{33a}$ (7 g, 7.88 mmol) was dissolved in methanol (30 mL) and dichloromethane (10 mL) and added to a 1/1 mixture (50 mL) of 3 N HCl and water dropwise at 70 °C. After decomposition of the complex was completed (disappearance of the orange color), the mixture was evaporated in vacuo,
treated with conc. ammonia and washed with CHCl₃. The aqueous phase was stirred for 2 h before the solvent was evaporated in vacuo. The resultant crude product was dissolved in methanol (60 mL), and thionyl chloride (4.5 mL, 61.8 mmol) was added dropwise at 0 °C. The mixture was allowed to come to rt and stirred overnight. After evaporation, the residue was dissolved in CH₂Cl₂ (50 mL), washed with saturated aqueous NaHCO₃ solution (50 mL) and brine (50 mL), and dried over Na₂SO₄. After filtration and rotary evaporation, the crude ester was dissolved in CH₃CN (15 mL) and di-tert-butyl dicarbonate (1.7 g, 7.79 mmol) and DMAP (50 mg, 0.41 mmol) were added. The mixture was stirred for 5 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (hexanes:EtOAc = 3:2) to give the pure product 34 as a colorless oil (1.77 g, 54%). 34. [α]D²³ +26.4 (c 0.99, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.87-7.84 (m, 2H), 7.76-7.74 (m, 2H), 4.32 (d, 1H, J = 3.8 Hz), 3.80-3.75 (m, 5H), 2.86 (dd, 1H, J = 17.6, 8.8 Hz), 2.36 (dd, 1H, J = 17.6, 4.6 Hz), 2.31-2.27 (m, 1H), 2.09-2.05 (m, 1H), 1.88-1.84 (m, 1H), 1.49 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 172.0, 171.1, 168.1, 149.1, 134.1, 131.9, 123.3, 83.8, 64.2, 52.6, 37.3, 35.4, 33.5, 32.5, 27.8; HRMS (FAB) calcd for C₂₁H₂₅N₂O₇ (M+H) 417.1662, found 417.1675.

**Methyl (2S,3S,5S)-1-(tert-butyloxycarbonyl)-3-[2-(N-phthalimido)-ethyl]-5-allyl-prolinate (35).** To a solution of 34 (220 mg, 0.53 mmol) in dry THF (5 mL) was added DIBAL-H (1M solution in hexanes, 2.1 mL) at -78 °C under Ar. After stirring at -78 °C for 15 min, the reaction was quenched with saturated aqueous NH₄Cl (3 mL) and allowed to come to rt. The solution was passed through a short column of Celite and dried over Na₂SO₄. After removal of the solvent, the residue was dissolved in methanol (4 mL)
and p-TsOH·H₂O (10 mg, 0.05 mmol) was added. After stirring for 3 h, the solution was quenched with saturated NaHCO₃ (4 mL), the solvent was removed, the mixture was extracted with diethyl ether (3 × 8 mL), and the combined organic extracts were dried over Na₂SO₄, filtered and concentrated under vacuum. The residue was dissolved in diethyl ether (4 mL) and allyltrimethylsilane (335 μL, 2.1 mmol) and BF₃·Et₂O (75 μL, 0.59 mmol) were added at −40 °C under Ar. The cold bath was removed after stirring at −40 °C for 15 min. The reaction mixture was stirred for additional 40 min, then quenched with NaHCO₃ (2 mL) and extracted with diethyl ether (3 × 5 mL). The organic extracts were combined, dried over Na₂SO₄, filtered and concentrated under vacuum to afford the crude product. Purification by flash column chromatography (hexanes:EtOAc = 3:1) afforded pure product 35 as a colorless oil (150 mg, 64%). 35. 

[α]ₑ₂ₒ⁺ 24.8 (c 1.84, CHCl₃); \(^1^H \text{NMR (500 MHz, CDCl₃) (two rotamers) δ 7.86-7.83 (m, 2H), 7.74-7.71 (m, 2H), 5.84-5.76 (m, 1H), 5.10 (dd, 1H, } \; J = 17.1, 1.6 \text{ Hz), 5.03 (d, 1H, } \; J = 10.1 \text{ Hz), 4.00 (brs, 0.6H), 3.94-3.90 (m, 0.9H), 3.84 (d, 0.5H, } \; J = 8.6 \text{ Hz), 3.76-3.65 (m, 5H), 2.68-2.66 (m, 0.6H), 2.57-2.54 (m, 0.4H), 2.34-2.30 (m, 1H), 2.23-2.16 (m, 1H), 2.11-2.05 (m, 2H), 1.74-1.66 (m, 2H), 1.46 (s, 4H), 1.40 (s, 5H);} \(^1^C \text{NMR (125 MHz, CDCl₃) (two rotamers) δ 173.3, 173.1, 168.1, 154.0, 153.3, 135.2, 134.0, 132.0, 123.2, 117.1, 80.1, 65.6, 65.1, 57.8, 57.6, 52.2, 52.0, 40.2, 39.3, 39.1, 38.3, 36.2, 35.4, 34.5, 32.2, 32.1, 28.4, 28.2; HRMS (FAB) calcd for C₂₄H₃₁N₂O₆ (M+H) 443.2182, found 443.2177.}

Methyl (2S,3S,5R)-1-(tert-butyloxycarbonyl)-3-[2-(N-phthalimido)-ethyl]-5-[(3-amino-(N-benzylxycarbonyl)-3-methoxycarbonyl)-2-propenyl]-prolinate (36). To a solution of 35 (100 mg, 0.23 mmol) in THF (6 mL) and H₂O (3 mL) was added OsO₄
(cat.) in the dark. After 5 min, NaIO₄ (120 mg, 0.56 mmol) was added. The mixture was stirred at rt for 4 h, filtered, and washed with MeOH (3 × 5 mL). After removal of the solvent, the residue was redissolved in dichloromethane (10 mL). The organic solution was washed with brine (3 × 10 mL), dried over Na₂SO₄, filtered and concentrated under vacuum to give crude aldehyde. Meanwhile, to a solution of (MeO)₂P(O)CH(NHCbz)CO₂Me (75 mg, 0.23 mmol) in dichloromethane (3 mL) was added DBU (34 µL, 0.23 mmol) at rt. After 10 min, to the above solution was added a solution of the crude aldehyde in dichloromethane (2 mL) and the reaction mixture was stirred overnight at rt. After removal of the solvent, the residue was redissolved in EtOAc (5 mL), washed with 1N HCl (5 mL) and brine (5 mL), dried over Na₂SO₄, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography (hexanes:EtOAc = 1:1) to give product 36 as a colorless oil (117 mg, 79%).

36. [α]²⁰ D +49.7 (c 1.84, CHCl₃); ¹H NMR (500 MHz, CDCl₃) (two rotamers) δ 7.83 (brs, 2H), 7.67 (brs, 2H), 7.58-7.29 (m, 5H), 6.65 (brs, 1H), 5.19-5.12 (m, 2H), 4.17-4.10 (m, 1H), 3.91 (d, 0.5H, J = 9.5 Hz), 3.80-3.65 (m, 8.5H), 2.70-2.61 (m, 1H), 2.45-2.42 (m, 0.5H), 2.36-2.33 (m, 1.5H), 2.18-2.05 (m, 2H), 1.83-1.65 (m, 2H), 1.39 (s, 4H), 1.35 (s, 5H); ¹³C NMR (125 MHz, CDCl₃) (two rotamers) δ 173.8, 173.6, 168.3, 165.1, 154.9, 154.8, 153.9, 153.4, 136.3, 134.0, 133.9, 133.4, 131.8, 128.3, 128.2, 127.9, 123.3, 80.6, 80.5, 66.9, 65.5, 65.3, 57.2, 57.0, 52.6, 52.3, 52.0, 40.4, 39.5, 37.0, 36.1, 36.0, 35.8, 34.2, 33.5, 32.2, 32.0, 28.1, 28.0; HRMS (FAB) calcd for C₃₄H₄₀N₃O₁₀ (M+H) 650.2714, found 650.2703.

Methyl (2S,3S,5S)-1-(tert-butyloxy carbonyl)-3-[2-(N-phthalimido)-ethyl]-5-
[(3R)-(3-amino-(N-benzyloxy carbonyl)-3-methoxycarbonyl)-propyl]-prolinate (37a).

A hydrogenation bottle was charged with 36 (90 mg, 0.14 mmol) in degassed methanol (10 mL, HPLC grade) and then purged with argon for 15 min, followed by adding [(R,R)-(COD)-Et-DuPHOS Rh(I)]OTf (10 mole%). After five vacuum/hydrogen cycles, the reaction bottle was pressurized to an initial pressure of 75 psi. The reaction proceeded for 24 h. After evaporation of solvent, the crude product was purified by flash column chromatography (hexanes:EtOAc = 1:1) to afford pure product as a colorless oil (84 mg, 92%). 37a. \([\alpha]_{D}^{23} +24.1 \text{ (c 1.71, CHCl}_3); \) \(^1\)H NMR (500 MHz, CDCl\(_3\)) (two rotamers) \(\delta \) 7.95-7.63 (m, 4H), 7.36-7.28 (m, 5H), 5.89 (d, 0.6H, \(J = 7.8 \text{ Hz}\)), 5.59 (d, 0.4H, \(J = 7.8 \text{ Hz}\)), 5.15-5.10 (m, 2H), 4.35 (brs, 1H), 4.01-3.81 (m, 2H), 3.77-3.65 (m, 8H), 2.29 (brs, 1H), 2.11-2.02 (m, 2H), 1.96-1.63 (m, 5H), 1.49-1.47 (m, 1H), 1.43 (s, 4H), 1.39 (s, 5H); \(^1^3\)C NMR (125 MHz, CDCl\(_3\)) (two rotamers) \(\delta \) 173.2, 173.1, 172.9, 172.7, 168.3, 156.2, 156.0, 153.9, 153.6, 136.4, 136.3, 134.0, 131.8, 128.4, 128.0, 127.9, 123.5, 123.3, 80.2, 66.8, 66.7, 65.3, 64.8, 57.6, 57.3, 54.2, 53.8, 52.2, 52.0, 40.2, 39.4, 36.1, 35.5, 32.3, 32.1, 30.6, 30.3, 29.1, 28.7, 28.3, 28.1; HRMS (FAB) calcd for C\(_{34}\)H\(_{42}\)N\(_3\)O\(_{10}\) (M+H) 652.2870, found 652.2884.

Methyl (2S,3S,5S)-1-(tert-butyloxy carbonyl)-3-[2-(N-phthalimido)-ethyl]-5-[(3S)-(3-amino-(N-benzyloxy carbonyl)-3-methoxycarbonyl)-propyl]-prolinate (37b).

In a manner similar to the preparation of 37a, using [(S,S)-(COD)Et-DuPHOS Rh(I)]OTf as a catalyst gave 37b in 94% yield. 37b. \([\alpha]_{D}^{23} +20.56 \text{ (c 1.49, CHCl}_3); \) \(^1\)H NMR (500 MHz, CDCl\(_3\)) (two rotamers) \(\delta \) 7.84-7.82 (m, 2H), 7.70 (brs, 2H), 7.37-7.28 (m, 5H), 5.56-5.50 (m, 1H), 5.12 (s, 2H), 4.38 (brs, 1H), 4.01-3.84 (m, 2H), 3.75-3.65 (m,
8H), 2.31 (brs, 1H), 2.12-1.90 (m, 3H), 1.79-1.71 (m, 4H), 1.53-1.47 (m, 1H), 1.44 (s, 4H), 1.39 (s, 5H); $^{13}$C NMR (125 MHz, CDCl$_3$) (two rotamers) δ 173.3, 173.2, 172.9, 172.8, 168.1, 156.0, 154.0, 153.5, 136.4, 136.3, 134.0, 131.9, 128.4, 128.0, 127.9, 123.3, 80.3, 80.2, 66.7, 65.3, 64.8, 57.5, 57.2, 53.8, 53.7, 52.2, 52.0, 40.3, 39.5, 36.8, 36.2, 36.0, 32.6, 32.3, 30.6, 29.3, 29.1, 28.3, 28.2; HRMS (FAB) calcd for C$_{34}$H$_{42}$N$_3$O$_{10}$ (M+H) 652.2870, found 652.2877.

$(3R, 6S, 8S, 9S)$-Methyl 2-oxo-3-N-(benzoxycarbonyl)amino-8-[2-(N-phthalimido)ethyl]-1-azabicyclo[4.3.0]nonane-9-carboxylate (38). To a solution of 37a (80 mg, 0.12 mmol) in dichloromethane (2.4 mL) was added TFA (0.6 mL) at rt. The mixture was stirred for 30 min, then saturated NaHCO$_3$ (10 mL) was added. After stirring for 20 min, the organic solution was dried over Na$_2$SO$_4$ and concentrated under vacuum. The residue was redissolved in chloroform (5 mL) and stirred at rt for 48 h. After removal of the solvent, the crude product was chromatographed (hexanes:EtOAc = 1:3) to afford 38 as a colorless oil (50 mg, 80%). 38. [α]$_D$ +20.2 (c 1.16, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.87-7.84 (m, 2H), 7.75-7.71 (m, 2H), 7.36-7.26 (m, 5H), 5.42 (brs, 1H), 5.12-5.06 (m, 2H), 4.17 (s, 1H), 4.08-4.05 (m, 1H), 3.82-3.69 (m, 6H), 2.55 (brs, 1H), 2.29-2.25 (m, 1H), 2.14 (d, 1H, J = 11.5 Hz), 2.05-1.87 (m, 3H), 1.86-1.75 (m, 2H), 1.74-1.64 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.4, 168.2, 168.1, 156.4, 136.3, 134.1, 132.0, 128.5, 128.1, 123.3, 66.8, 64.1, 58.2, 52.5, 52.4, 39.2, 36.4, 36.0, 32.9, 28.7, 27.7; HRMS (FAB) calcd for C$_{28}$H$_{30}$N$_3$O$_7$ (M+H) 520.2084, found 520.2077.

$(3S, 6S, 8S, 9S)$-Methyl 2-oxo-3-N-(benzoxycarbonyl)amino-8-[2-(N-phthalimido)ethyl]-1-azabicyclo[4.3.0]nonane-9-carboxylate (39). In a manner similar to the
preparation of 38, using 37b as starting material gave 39 in 92% yield. 39. [α]23D +24.1 (c 2.25, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.87-7.84 (m, 2H), 7.75-7.72 (m, 2H), 7.35-7.26 (m, 5H), 5.81 (d, 1H, J = 5.0 Hz), 5.11 (s, 2H), 4.27 (s, 1H), 4.24-4.20 (m, 1H), 3.86-3.82 (m, 1H), 3.80-3.73 (m, 2H), 3.71 (s, 3H), 2.55-2.51 (m, 1H), 2.34 (dd, 1H, J = 14.6, 7.3 Hz), 2.18 (dd, 1H, J = 13.0, 6.3 Hz), 2.12-2.09 (m, 1H), 1.93-1.87 (m, 2H), 1.78-1.65 (m, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 171.4, 169.1, 168.1, 156.0, 136.5, 134.1, 132.0, 128.4, 128.0, 127.9, 123.3, 66.7, 63.8, 54.6, 52.4, 50.3, 39.9, 36.5, 35.9, 32.4, 26.9; HRMS (FAB) calcd for C₂₈H₃₀N₃O₇ (M+H) 520.2077, found 520.2077.

Methyl (2S,3S,5R)-1-(tert-butyloxycarbonyl)-3-[2-(N-phthalimido)-ethyl]-5-[2-bromo-(3-amino-(N-benzylxycarbonyl)-3-methoxycarbonyl)-2-propenyl]prolinate (40). To a solution of 36 (405 mg, 0.62 mmol) in chloroform (10 mL) was added NBS (122 mg, 0.69 mmol) at rt. After 80 min, Dabco (140 mg, 1.25 mmol) was added. The mixture was stirred at rt for 24 h, washed with saturated NH₄Cl (3 × 10 mL), and dried over Na₂SO₄. After removal of the solvent, the residue was subjected to flash column chromatography (hexanes:EtOAc = 4:1) to afford 40 in 78% yield as a colorless oil. 40. [α]23D +10.4 (c 1.05 , CHCl₃); ¹H NMR (500 MHz, CDCl₃) (Rotamer) δ 7.85-7.82 (m, 2H), 7.72-7.69 (m, 2H), 7.36-7.27 (m, 5H), 6.61 (brs, 1H), 5.14-5.11 (m, 2H), 4.38-4.35 (m, 0.6H), 4.34-4.28 (m, 0.4H), 3.95-3.84 (m, 1.5H), 3.83-3.62 (m, 7.5H), 3.12-3.10 (m, 1.5H), 2.91-2.87 (m, 0.5H), 2.44-2.41 (m, 1H), 2.24 (brs, 1H), 2.10-2.03 (m, 1H), 1.72-1.66 (m, 2H), 1.47-1.26 (m, 9H); ¹³C NMR (125 MHz, CDCl₃) (rotamers) δ 173.3, 173.1, 168.1, 162.7, 162.5, 153.5, 153.0, 152.9, 152.8, 135.4, 134.1, 134.0, 133.9, 132.1, 132.0, 128.6, 128.5, 128.4, 128.3, 128.2, 123.4, 123.2, 123.1, 80.5, 80.4, 67.9, 67.7, 65.5,
65.0, 57.0, 56.9, 52.7, 52.3, 52.1, 40.3, 39.8, 39.5, 39.2, 36.2, 36.1, 34.4, 33.9, 32.1, 32.0, 28.4, 28.3; HRMS (FAB) calcd for C_{34}H_{39}BrN_{3}O_{10} (M+H) 728.1819, found 728.1804.

Methyl (2S,3S,5R)-1-(tert-butyloxycarbonyl)-3-[2-(N-phthalimido)-ethyl]-5-[2-phenyl- and 2-naphthyl-(3-amino-(N-benzyloxycarbonyl)-3-methoxycarbonyl)-2-propenyl]-prolinate (41 and 42). To a solution of 40 (375 mg, 0.52 mmol) in DME (3 mL) and degassed water (520 μL) were added phenylboronic acid (185 mg, 1.52 mmol), Na_{2}CO_{3} (165 mg, 1.56 mmol), Pd(OAc)_{2} (25 mg, 0.11 mmol) and P(o-tolyl)_{3} (30 mg, 0.10 mmol). The mixture was stirred at 80 °C overnight and passed through a short column containing a bottom 1” layer of silica gel and a top 1” layer of NaHCO_{3} using ethyl acetate as eluent. After removal of the solvent, the crude 41 was subjected to the next step without further purification. The identical procedure starting with 40 afforded 42 as crude product for the next step.

(6R,8S,9S)-1-Aza-3-N-benzoxy carbonylamino-9-methoxycarbonyl-4-phenyl- and 2-naphthyl-8-[2-(N-phthalimido)-ethyl]-2-oxobicyclo[4.3.0]non-3-ene (43 and 44). To a solution of above crude product 41 in dichloromethane (2.4 mL) was added TFA (0.6 mL) at rt. The mixture was stirred for 30 min, then saturated NaHCO_{3} (10 mL) was added. After stirring for 20 min, the organic solution was dried over Na_{2}SO_{4} and concentrated under vacuum. The residue was redissolved in chloroform (5 mL) and stirred at rt for 48 h. After removal of the solvent, the crude product was chromatographed (hexanes:EtOAc = 1:1) to afford 43 as a colorless oil in 61% overall yield from 40. The identical procedure starting with 42 afforded 44 in 63% overall yield from 40.43. [α]^{23}_{D} = -5.7 (c 1.04, CHCl_{3}); ^{1}H NMR (500 MHz, CDCl_{3}) δ 7.88-7.85 (m,
2H), 7.75-7.72 (m, 2H), 7.37-7.33 (m, 4H), 7.31-7.23 (m, 4H), 7.20-7.19 (m, 2H), 6.51 (brs, 1H), 4.97 (d, 1H, J = 12.3 Hz), 4.89 (d, 1H, J = 12.3 Hz), 4.36 (s, 1H), 4.22-4.15 (m, 1H), 3.84-3.72 (m, 5H), 2.90-2.80 (m, 2H), 2.40 (q, 1H, J = 7.0 Hz), 2.19 (dd, 1H, J = 12.8, 5.7 Hz), 2.07-2.02 (m, 1H), 2.01-1.92 (m, 1H), 1.83-1.76 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171.7, 168.2, 161.8, 153.4, 138.2, 137.9, 136.2, 134.1, 132.0, 128.5, 128.4, 128.3, 127.9, 127.2, 124.2, 123.4, 67.0, 63.4, 54.0, 52.6, 39.7, 36.4, 36.0, 35.8, 32.7; HRMS (FAB) calcd for C$_{34}$H$_{32}$N$_3$O$_7$ (M+H) 594.2240, found 594.2222. 44. 

$[\alpha]^{23}_D$ -8.8 (c 2.12, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.90-7.72 (m, 8H), 7.52-7.46 (m, 3H), 7.22-7.16 (m, 3H), 7.09-7.07 (m, 2H), 6.65 (s, 1H), 4.94-4.81 (m, 2H), 4.39 (s, 1H), 4.27-4.22 (m, 1H), 3.85-3.73 (m, 5H), 3.04-2.99 (m, 1H), 2.93-2.87 (m, 1H), 2.43 (dd, 1H, J = 14.6, 7.3 Hz), 2.24-2.20 (m, 1H), 2.10-2.04 (m, 1H), 2.00-1.93 (m, 1H), 1.85-1.80 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171.6, 168.2, 161.8, 153.3, 137.5, 136.1, 135.7, 134.1, 133.1, 132.9, 132.0, 128.5, 128.3, 128.1, 127.9, 127.6, 126.6, 126.5, 126.3, 124.9, 124.4, 123.3, 66.9, 63.4, 54.1, 52.6, 39.8, 36.4, 35.9, 35.8, 32.7; HRMS (FAB) calcd for C$_{38}$H$_{34}$N$_3$O$_7$ (M+H) 644.2397, found 644.2402.

(6R,8S,9S)-1-Aza-3-N-benzoxy carbonylamino-9-methoxy carbonyl-4-phenyl- and 2-naphthyl-8-[2-(N,N'-di-Boc-guanidino)-ethyl]-2-oxobicyclo[4.3.0]non-3-ene (47 and 48). To a solution of 43 (31 mg, 0.052 mmol) in absolute ethanol (2 mL) and chloroform (400 µL) was added anhydrous hydrazine (11 µL, 0.35 mmol) at rt. After stirring at rt for 24 h, the solution was filtered to remove the white precipitate and the filtrate was concentrated in vacuum to afford crude 45. The crude 45 was dissolved in
dichloromethane (2 mL) and TEA (45 μL, 0.33 mmol) and N,N'-bis(tert-butoxycarbonyl)-N'-triflylguanidine (31 mg, 0.079 mmol) were added at rt. The mixture was stirred at rt for 24 h before the solvent was removed in vacuo. The crude product was chromatographed (hexanes:EtOAc = 1:1) to afford 47 as a colorless oil (30.5 mg, 83%). 47. [α]$_D^{23}$ -7.8 (c 2.07, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 11.48 (brs, 1H), 8.43 (brs, 1H), 7.37-7.25 (m, 8H), 7.21-7.19 (m, 2H), 6.48 (brs, 1H), 4.99 (d, 1H, $J$ = 12.3 Hz), 4.91 (d, 1H, $J$ = 12.3 Hz), 4.36 (s, 1H), 4.20-4.13 (m, 1H), 3.78 (s, 3H), 3.66-3.59 (m, 1H), 3.52-3.47 (m, 1H), 2.84 (d, 2H, $J$ = 8.9 Hz), 2.43 (q, 1H, $J$ = 14.7, 7.4 Hz), 2.11-2.07 (m, 1H), 2.05-1.99 (m, 1H), 1.80-1.75 (m, 2H), 1.50 (s, 18H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.7, 163.5, 161.8, 156.2, 153.5, 153.3, 138.3, 138.2, 136.2, 128.5, 128.4, 128.3, 128.0, 127.9, 127.2, 124.3, 83.3, 79.4, 67.0, 62.8, 54.0, 52.6, 39.5, 38.6, 36.4, 36.3, 33.3, 28.3, 28.1, 27.8; HRMS (FAB) calcd for C$_{37}$H$_{48}$N$_5$O$_9$ (M+H) 706.3452, found 706.3445. The identical procedure starting with 46 afforded 48 in 80% overall yield. 48. [α]$_D^{23}$ -16.6 (c 1.14, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 11.51 (s, 1H), 8.44-8.43 (m, 1H), 7.87-7.73 (m, 6H), 7.52-7.47 (m, 3H), 7.24-7.15 (m, 3H), 6.65 (brs, 1H), 4.95-4.85 (m, 2H), 4.39 (s, 1H), 4.22 (brs, 1H), 3.79 (s, 3H), 3.66-3.62 (m, 1H), 3.52-3.48 (m, 1H), 3.01-2.97 (m, 1H), 2.92-2.87 (m, 1H), 2.47-2.43 (m, 1H), 2.13-2.11 (m, 1H), 2.08-2.03 (m, 1H), 1.80-1.75 (m, 2H), 1.51 (s, 9H), 1.50 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.7, 166.6, 163.5, 161.8, 156.2, 153.3, 137.7, 136.1, 135.7, 134.2, 133.1, 132.9, 130.2, 128.3, 128.1, 127.9, 127.6, 126.6, 126.5, 126.3, 124.9, 123.4, 83.2,
79.3, 66.9, 62.8, 54.1, 52.6, 39.6, 38.6, 36.4, 36.3, 33.4, 28.3, 28.0; HRMS (FAB) calcd for C_{41}H_{50}N_{5}O_{9} (M+H) 756.3609, found 756.3611.

(6R,8S,9S)-1-Aza-3-A^-benzoxycarbonylamino-9-methoxycarbonyl-4-2’-naphthyl-8-[2-tert-butoxycarbonylamo-no-ethyl]-2-oxobicyclo[4.3.0]non-3-ene (52). To a solution of 44 (22 mg, 0.034 mmol) in absolute ethanol (2 mL) and chloroform (1 mL) was added anhydrous hydrazine (8 μL, 0.25 mmol). After stirring at rt for 24 h, the solution was filtered to remove the white precipitate and the filtrate was concentrated in vacuum and redissolved in dichloromethane (2 mL). To the solution were added TEA (29 μL, 0.21 mmol) and di-tert-butyl dicarbonate (15 mg, 0.069 mmol). The mixture was stirred at rt for 6 h before the solvent was removed in vacuo. The crude product was chromatographed (hexanes:EtOAc = 1:2) to afford 52 as a colorless oil (18 mg, 86%).

52. [α]^{23}_D -10.7 (c 1.11, CHCl₃), \(^1\)H NMR (500 MHz, CDCl₃) δ 7.82-7.76 (m, 4H), 7.51-7.47 (m, 3H), 7.24-7.10 (m, 5H), 6.61 (brs, 1H), 4.95-4.83 (m, 2H), 4.63 (brs, 1H), 4.35 (s, 1H), 4.24-4.19 (m, 1H), 3.78 (s, 3H), 3.30-3.24 (m, 2H), 3.01-2.96 (m, 1H), 2.92-2.86 (m, 1H), 2.45-2.41 (m, 1H), 2.12-2.02 (m, 2H), 1.70-1.66 (m, 2H), 1.46 (s, 9H); \(^1\)C NMR (125 MHz, CDCl₃) δ 171.8, 161.9, 155.9, 153.4, 137.8, 136.1, 135.7, 133.1, 133.0, 128.4, 128.3, 128.2, 127.9, 127.7, 126.6, 126.5, 126.3, 124.9, 124.4, 79.5, 67.0, 63.1, 54.2, 52.6, 39.6, 38.6, 36.5, 36.2, 34.3, 28.4; HRMS (FAB) calcd for C_{35}H_{40}N_{3}O_{7} (M+H) 614.2866, found 614.2888.

**General procedure of coupling reactions:** The solution of starting material in LiOH
(1N) and methanol (1:2) was stirred at rt for 3 h. After the solution was neutralized by HCl (1N), it was washed with dichloromethane. The organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. To the solution of the residue in dichloromethane was added PyBOP (1.1 eq), HOBt (1.1 eq), DIPEA (3 eq) and amine (2 eq). The mixture was stirred at rt for 2 h before washing with NH$_4$Cl. The organic phase was collected and dried over Na$_2$SO$_4$. After the solvent was removed, the crude product was purified by column chromatography (hexanes:EtOAc = 1:2).

$(6R,8S,9S)$-1-Aza-3-$N$-benzoxycarbonylamino-9-[2-(indol-3-yl)-ethylcarbamoyl]-4-phenyl-8-[2-($N,N'$-di-Boc-guanidino)-ethyl]-2-oxo-bicyclo[4.3.0]non-3-ene \ (49). 

Yield: 68%. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 11.45 (s, 1H), 8.72 (brs, 1H), 8.33 (brs, 1H), 7.60 (d, 1H, $J$ = 7.9 Hz), 7.37-7.27 (m, 9H), 7.24-7.08 (m, 4H), 7.00-6.99 (m, 1H), 6.50-6.46 (m, 2H), 5.00-4.93 (m, 2H), 4.14 (s, 1H), 4.01-3.98 (m, 1H), 3.73-3.70 (m, 1H), 3.50-3.43 (m, 2H), 3.35-3.30 (m, 1H), 3.04-2.95 (m, 2H), 2.68-2.65 (m, 1H), 2.52-2.47 (m, 1H), 2.40-2.37 (m, 1H), 1.94-1.88 (m, 2H), 1.65-1.55 (m, 2H), 1.49 (s, 9H), 1.48 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.0, 163.4, 162.5, 156.3, 153.6, 153.2, 139.3, 138.4, 136.4, 136.1, 128.5, 128.4, 128.3, 128.1, 128.0, 127.1, 127.0, 124.1, 122.9, 121.9, 119.2, 118.7, 112.1, 111.3, 83.4, 79.6, 67.1, 64.7, 54.7, 38.9, 38.8, 36.3, 36.1, 33.1, 29.7, 28.3, 28.1, 24.9; HRMS (FAB) calcd for C$_{46}$H$_{56}$N$_7$O$_8$ (M+H) 834.4190, found 834.4205.

$(6R,8S,9S)$-1-Aza-3-$N$-benzoxycarbonylamino-9-phenethylcarbamoyl]-4-phenyl-
8-[2-(N,N'-di-Boc-guanidino)-ethyl]-2-oxo-bicyclo[4.3.0]non-3-ene (50). Yield: 73%.

\(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 11.47 (s, 1H), 8.38 (brs, 1H), 7.38-7.15 (m, 15H), 6.99 (brs, 1H), 6.50 (brs, 1H), 5.02-4.89 (m, 2H), 4.30 (s, 1H), 4.09-4.05 (m, 1H), 3.68-3.63 (m, 1H), 3.61 (m, 1H), 3.48-3.38 (m, 2H), 2.82-2.77 (m, 3H), 2.71-2.66 (m, 1H), 2.55-2.51 (m, 1H), 2.04-1.99 (m, 1H), 1.97-1.94 (m, 1H), 1.66-1.60 (m, 2H), 1.50 (s, 9H), 1.49 (s, 9H); \(^1\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 170.3, 163.4, 162.6, 156.5, 153.5, 153.3, 139.1, 139.0, 138.1, 138.2, 128.8, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.2, 126.3, 124.3, 83.4, 80.0, 67.0, 64.2, 54.7, 40.7, 38.7, 38.3, 36.3, 36.2, 35.6, 33.0, 28.3, 28.1; HRMS (FAB) calcd for C\(_{44}\)H\(_{55}\)N\(_6\)O\(_8\) (M+H) 795.4081, found 795.4069.

\((6R,8S,9S)-1\text{-Aza-3-N-benzoxy carbonylamino-9-[2-(indol-3-yl)-ethylcarbamoyl]-4-(2-naphthyl)-8-[2-(N,N'-di-Boc-guanidino)-ethyl]-2-oxo-bicyclo[4.3.0]non-3-ene (51). Yield: 63%. \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 11.45 (s, 1H), 8.75 (brs, 1H), 8.34-3.2 (m, 1H), 7.83-7.77 (m, 4H), 7.61-7.33 (m, 5H), 7.27-6.99 (m, 8H), 6.61-6.54 (m, 2H), 4.95-4.86 (m, 2H), 4.17 (s, 1H), 4.07-4.02 (m, 1H), 3.76-3.71 (m, 1H), 3.51-3.45 (m, 2H), 3.36-3.31 (m, 1H), 3.06-2.94 (m, 2H), 2.81-2.77 (m, 1H), 2.57-2.50 (m, 1H), 2.43-2.40 (m, 1H), 1.94-1.91 (m, 2H), 1.66-1.54 (m, 2H), 1.49 (s, 9H), 1.48 (s, 9H); \(^1\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 169.9, 163.4, 162.5, 156.2, 153.6, 153.2, 138.8, 136.4, 136.0, 135.9, 133.1, 132.9, 128.3, 128.1, 128.0, 127.9, 127.7, 127.1, 126.6, 126.4, 126.3, 124.6, 124.3, 122.9, 121.9, 119.2, 118.7, 112.1, 111.3, 83.4, 79.5, 67.1, 64.8, 54.7, 38.9, 38.8, 36.2, 36.1, 33.0, 28.3, 28.0, 24.9; HRMS (FAB) calcd for C\(_{50}\)H\(_{58}\)N\(_7\)O\(_8\) (M+H) 884.4347,
(6R,8S,9S)-1-Aza-3-N-benzoxy carbamylamino-9-[2-(indol-3-yl)-ethyl carbamoyl]-
4-(2-naphthyl)-8-[2-tert-butoxy carbamylamino-ethyl]-2-oxo bicyclo[4.3.0]non-3-ene
(53). Yield: 69%. \( ^1 \)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 8.40 (brs, 1H), 7.84-7.78 (m, 4H),
7.61-7.46 (m, 4H), 7.34-7.04 (m, 8H), 6.99 (brs, 1H), 6.88 (brs, 1H), 6.62 (s, 1H),
4.95-4.88 (m, 2H), 4.77 (brs, 1H), 4.32 (s, 1H), 4.08-4.03 (m, 1H), 3.60-3.58 (m, 2H),
3.32-3.28 (m, 1H), 3.20-3.13 (m, 1H), 3.08-3.03 (m, 1H), 2.97-2.91 (m, 1H), 2.80-2.76
(m, 1H), 2.56-2.50 (m, 2H), 1.90-1.84 (m, 2H), 1.54-1.51 (m, 2H), 1.42 (m, 9H); \( ^{13} \)C
NMR (125 MHz, CDCl\(_3\)) \( \delta \) 170.2, 162.7, 156.3, 153.7, 139.0, 136.4, 136.0, 135.9, 133.1,
133.0, 128.4, 128.1, 128.0, 127.9, 127.7, 127.2, 126.7, 126.4, 126.3, 124.6, 124.4, 122.8,
121.9, 119.2, 118.7, 112.3, 111.3, 79.6, 67.1, 64.2, 54.8, 39.1, 38.7, 38.2, 36.3, 36.2, 34.0,
28.4, 25.0; HRMS (FAB) calcd for C\(_{44}H_{48}N_{5}O_{6}\) (M+H) 742.3605, found 742.3614.
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Compound 5a

Compound 5a
Compound 47