THE DESIGN AND SYNTHESIS OF NOVEL AMINO ACIDS AND THEIR USE IN SYNTHESIS OF β-TURN MIMETICS AND THEIR INCORPORATION INTO BIOLOGICAL ACTIVE PEPTIDES

By

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Xuyuan Gu

entitled The design and synthesis of novel amino acids and their use in synthesis of beta-turn mimetics and their incorporation into biological active peptides

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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Dedicated to

My wife Lili Fan

My daughters Xiaoying Cherie Gu

and Lisa Xiaosha Gu

My son David Xiaofan Gu

For their unselfish support

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ABSTRACT

Peptide ligands represent the most important hormones and neurotransmitters in physiological processes. Although native biologically active peptides have a great potential for medical applications, they often need to be modified to overcome certain inherent problems. A new research area called peptidomimetics has been developed in the last twenty years. The first generation of β-turn mimetics was focused on mimicking the β-turn backbone. In the last decade, many types of bicyclic β-turn dipeptides (BTD) have been design and synthesized. However, these methods do not have straightforward ways to introduce side chain groups on both rings. The introduction of functionalities on BTD, as the second generation of β-turn mimetics, is the major goal of my dissertation.

By retrosynthetic analysis, convergent synthetic methodologies were initiated for [5,5]- and [6,5]-BTDs. Two kinds of nonproteinous amino acids are required in the strategies. One is the β-substituted cysteine derivatives and the other is β-substituted ω-unsaturatured amino acids. The racemic β-vinylphenylalanine was synthesized by using Kazmaier-Claisen rearrangement, and the ω-unsaturated amino acids and β-substituted δ,ε-unsaturated amino acids were synthesized by using Ni(II)-complexes as chiral auxiliaries. Using these starting materials, [5,5]-BTD analogues were synthesized by a five-step strategy. The synthesis of [6,5]-BTDs has to proceed without formation of the 5-membered hemiaminal, which blocks further reaction. A N^{α}-TFA protection group was used in this strategy and finally an efficient methodology was developed to generate the side chain groups into [6,5]-BTD analogues in nine steps.

During the development of these methods, we solved the challenge to synthesize all 16 or 32 of the possible diastereomeric dipeptide mimetics. A novel idea to solve these problems was to synthesize the targeted peptide mimetics by solid phase methods in a combinatorial fashion, as the third generation of β-turn mimetics. We have succeeded in the synthesis of [3,3,0]-BTD^{2,3}-Leu-enkephalins by unconventional solid phase synthesis, and four analogues have been synthesized and purified. This method is ready to expand to other sizes of BTD and to other target peptides with different functionalities.

CHAPTER I.

INTRODUCTION OF PEPTIDE MIMETICS

§ 1.1 Peptide structure and peptidomimetics

Proteins were found to be the primary materials of life system about two hundred years ago. However, their structure remained a mystery until 1902 when E. Fisher and F. Hofmeiser reported that they actually consisted of different amino acids in different combinations.[1-3] These proteinous amino acids are linked together through protein bonds (Figure 1.1). The protein bond is essentially an amide bond formed between the carboxyl group from one amino acid residue and amino group in another amino acid. The short analogues of proteins are called peptides, although there is no clear-cut line between them.

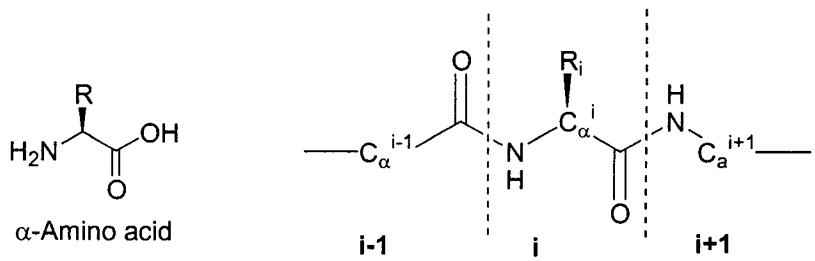


Figure 1.1 General structures of amino acid and peptide

Peptides play a variety of functions in biological systems. They can work as hormones, neurotransmitters, enzymes, enzyme substrates or inhibitors, immune system modulators, growth factors, antibiotics, antigens, cytokine modulators of transcription and translation, or many other effectors. These functions of peptides are known to influence essentially all vital physiological process *via* inter- and intracellular communication and signal transduction mediated through various classes of receptors.[4-6] The biological functions of peptides are determined by their chemical, structural, conformational, topographical and dynamic properties. The conformations of peptides and proteins can be described by their backbone conformation and side chain conformations. The backbone conformations are characterized by three dihedral angles ϕ, ψ, and ω. The side chain conformations are characterized by dihedral angles χ^1, χ^2, etc. (Figure 1.2).

The α-helix, β-sheets, and β-turns are secondary structural motifs found in peptides and proteins which can play a key role in imparting various functions to these biopolymers.[7] To achieve a certain type of secondary backbone conformations, the dihedral angles φ and ψ must be controlled. The ω-angle for peptide bond is generally *trans* except for the peptide bond with proline, which can be either *cis* or *trans*. The delocalization and coplanar arrangement of the three 2p orbitals in O, C, and N atoms make the amide bond have partial double bond character. During the past 30 years, much effort has been directed to develop strategies for the design and synthesis of peptides with specific backbone conformations.[8] By using uniquely constrained amino acids in peptide ligands, receptor selectivities, ligand potencies and the peptide stability

can be dramatically improved.[8,9] The local backbone conformations can be constrained by different ways. Two widely used methods are novel β-substituted amino acids and/or amide bond replacements.[10] The globular structures of peptides can be biased by cyclization through amide bonds and disulfide bonds to limit both backbone and χ spaces.[4,11] Many well-developed strategies have been developed to achieve backbone and global conformation constraints.

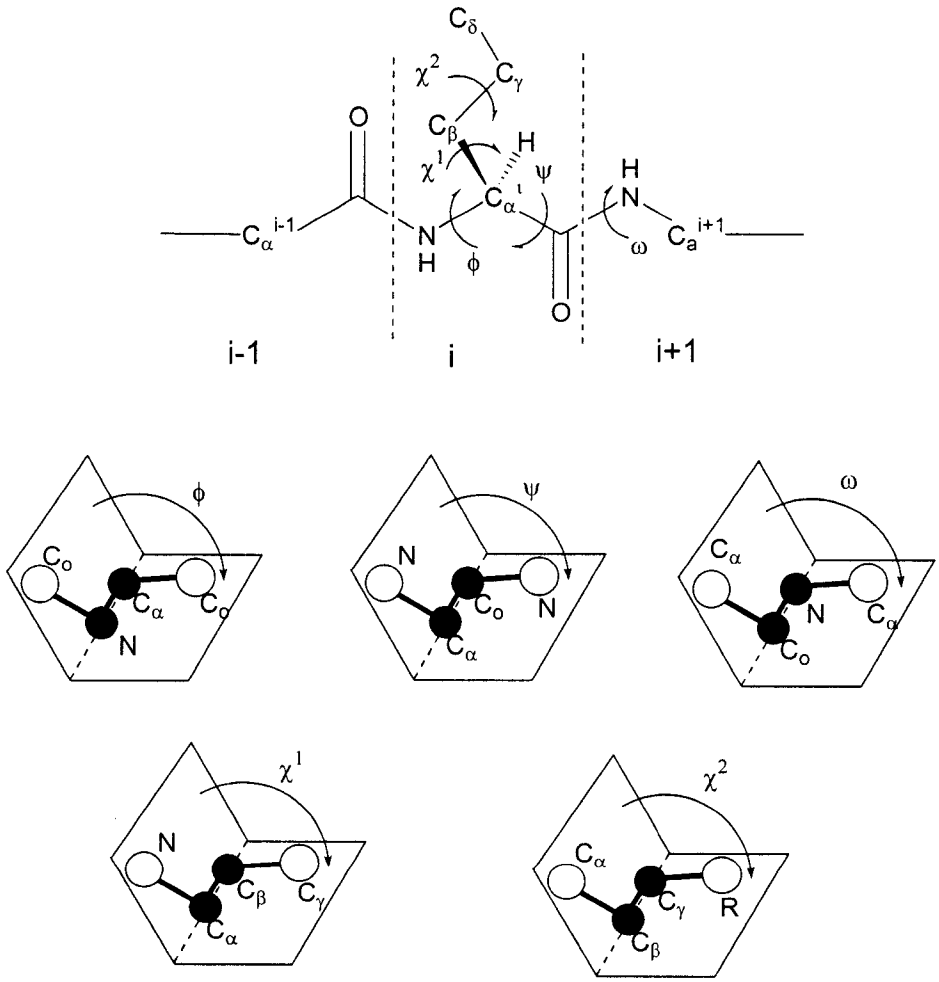


Figure 1.2 Definition of the ϕ, ψ, ω, χ^1 and χ^2 dihedral angles

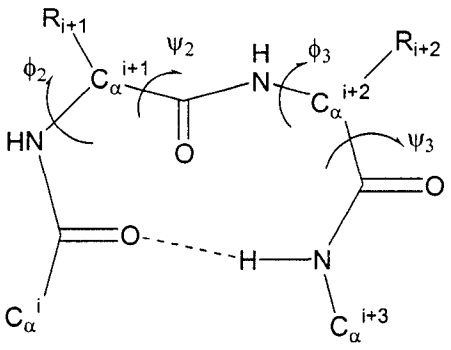
Although native biologically active peptides have a great potential for medical applications, they often need to be modified to overcome certain problems inherent in current drug design and delivery strategies. Conformational constraints play an important role in rational design of peptides and peptidomimetics that can overcome these problems. Some properties which are highly desired but not often present or optimized in the native ligands includes: receptor/acceptor selectivity; high potency; high stability against proteolytic breakdown; and appropriate biodistribution and bioavailability. These issues have been addressed by the design of peptidomimetics. Although people have different definitions for it, I would take the well accepted Hruby definition in 1997:[12]

A peptidomimetics is an organic molecule, such as a peptide analogue or nonpeptide ligand, that interacts with receptor or acceptor in a similar chemical manner as the native peptide/protein ligand to affect the same biochemical (biological) activities. In the parlance of medicinal chemistry, both peptidomimetics and native ligands/proteins should share common pharmacophore elements.

§ 1.2 β-Turn classification and β-turn mimetics

For many biologically active peptides, β-turn are important secondary structural elements which are critical for their biological activities.[13] A β-turn is defined as a tetrapeptide sequence in which the $C\alpha^{i}$-$C\alpha^{i+3}$ distance in a nonhelical region is less than 7 Å. In several versions it is stabilized by a 10 membered hydrogen-bonded ring (Figure 1.3).[13] The β-turn can be classified in many different types according to their ϕ, ψ

dihedral angels.[14] For specific turn, the deviation of these angles should not be larger than 20°.



Turn Type	ϕ_2	ψ_2	ϕ_3	ψ_3
	degrees			
I	-60	-30	-90	0
I'	60	30	90	0
II	-60	120	80	0
II'	60	-120	-80	0
III	-60	-120	-80	0
III'	60	120	80	0
v	-80	80	80	-80
V'	80	-80	-80	80

Figure 1.3 Classification of β-turn

The "secondary structure approach" to the *de novo* design of peptidemimetics is guided by the simple elegance which nature has employed in the molecular architecture of peptide and protein species.[7] Compare to the α-helix and β-extended sheet, β-turns offer the significant synthetic advantage that they are relatively compact and in principle can be more readily mimicked by conformationally constrained or rigidified small organic molecules. Mimicry of peptide backbone by replacement with functional units or addition of secondary-structure-promoting motif, represent effective strategies for conformational restriction of peptides.[4,8,15,16]

In order to mimic different β-turns in peptides, a 'template' is designed at the molecular level by either covalent bonds or non-covalent bonds. In general, β-turn mimetics can be classified as external β-turn mimetics and internal β-turn mimetics

depending on the support which can be either outside or inside of the β-turn (Figure 1.4).[17] In internal β-turn mimetics, for example, this support can be a linker used in cyclization. The advantage of internal β-turn mimetics is the convenience to introduce appropriate side chain groups on the individual amino acid and they can be biased in desired direction due to its flexibility. The disadvantage of this design, however, is from the same character. The χ-space freedom made it impossible to 'see' the precise secondary structure between ligand and receptor interaction.

External β-turn mimetics Internal β-turn mimetics

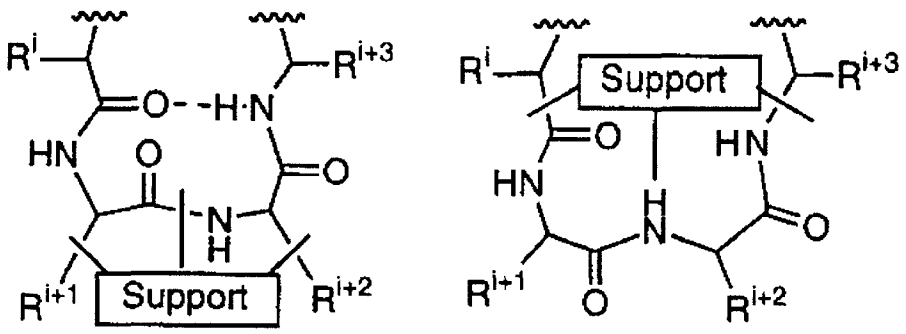


Figure 1.4 External and internal β-turn mimetics

§ 1.3 Design of bicyclic β-turn mimetics

The development of novel β-turn mimetics has drawn significant attention in peptidomimetics research.[18] Our group is one of those pioneer groups working on the external β-turn mimetics. We especially like external bicyclic β-turn dipeptide mimetics because this design not only keeps the backbone unchanged but the side chains unchanged also (Figure 1.5). The beauty of this design is by adding three bonds on the

β-positions and amide nitrogen, both the backbone conformation (ϕ and ψ) and side chain conformations (χ^1 and χ^2) are constrained. It also provides 32 possible diastereomeric isomers which certainly would provide a large topographical library. In this design, the n could be 0, 1, and 2 representing [5,5], [6,5], and [7,5]-bicyclic β-turn dipeptides, respectively. The X might be CH_2, O, and S by different synthetic methodologies.

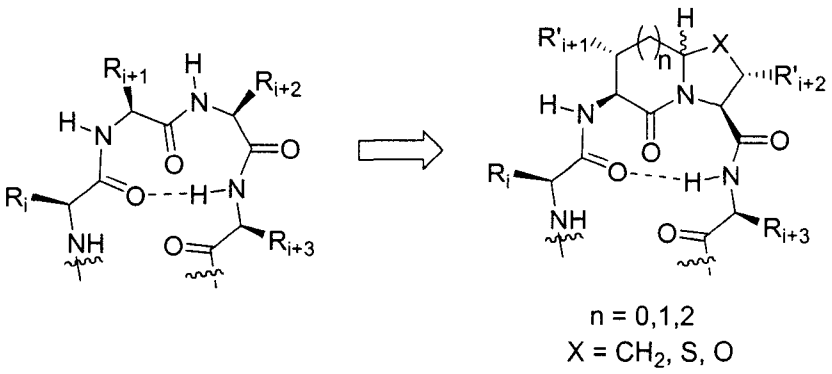


Figure 1.5 Design of external bicyclic β-turn dipeptide

In this area, we are particularly looking for a three stage key and wish this key could open the lock in peptidomimetics design to some extent. The first stage in this key is the first generation of peptidomimetics which are the backbone conformational mimetics. This work has been extensively examined and many groups have proposed and synthesized several types of β-turn mimics (Figure 1.6). The [5,5]-bicyclic dipeptide was used to mimic the type II β-turn, while [6,5]-bicyclic dipeptide is proposed to mimic type II' β-turns.[19,20] A [7,5]-bicyclic dipeptide can represent many

different β-turns depending on the different chiralities on the ring and the different amino acids in the sequence.[21] A *S*-spirolactam was suggested to replace a type II β-turn, while a spirotricycle was thought as the best mimic of a type II β-turn.[19,22] Other types of bicyclic structures have been designed as β-turn mimetics, such as azabicyclic[4,3,0]-alkane for type II' and cyclooctapyrrole for type VI β-turn,[23-25] and tricyclic type II β-turn.[26] Although not all of these designs are exact mimics of β-turns,[27] and not all types of β-turn mimics are available by specific design of small molecules, these previous studies are good enough for me to march on.

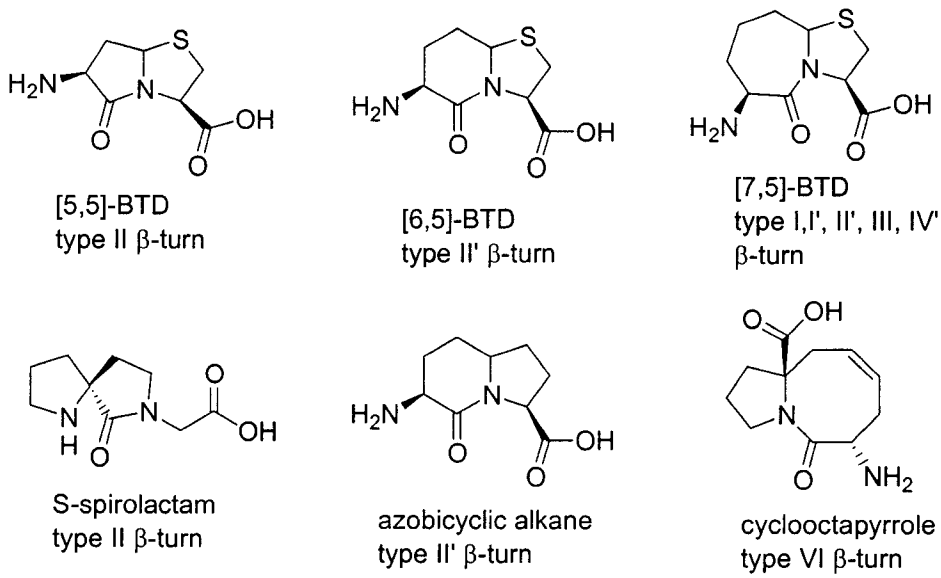


Figure 1.6 Design of bicyclic β-turn mimetic

It should be indicated that all these structure only have designed the backbone conformation, while the side chain groups are not included in these methodologies. It is well established that side chains are extremely important in peptide-receptor

interactions. To successfully design peptidomimetics, the essential amino acid side chain groups of the corresponding peptide pharmacophore need to be positioned on these alternative scaffolds such that the spatial orientation of these critical side chain groups correspond to these in the bioactive conformation of the peptide.[8,15] Therefore the development of an efficient and universal strategy to orient side chain combinations into multiple orientations is needed. So the secondary stage in this key is to develop the possibility of introducing proper side-chain groups or functionalities at proper positions. This is the second generation of peptidomimetics. Several investigations of side chain introduction have been made on azabicyclic [x.y.0] alkane (X = CH_2 in Figure 1.5) by Lubell's group.[28-31] However, without target peptides, their side chain groups are neither specific nor properly positioned. For the thiazolizidinone (X = S in Figure 1.5), the previous strategies were all from aspartic acid and glutamic acid derivatives. The strategy for introduction of side chain groups has not been reported.

The possibility of 32 diastereoisomers is an advantage of this design so that we can have many possibility conformations, and one of them might be very close to the true 3D-structure. On the other hand, it would be a disadvantage for classical organic synthesis. It is a tremendous challenge for the design and synthesis of stereo- and enantio-controlled introduction of a minimum of four asymmetric centers, with different backbone geometries and side chain topographies. This is actually the third stage of the key and the third generation in peptidomimetics. It will require us to develop a robust methodology so that all of these conformers can be synthesized by the same or similar

strategies using systematic or automatic techniques. A combinational chemistry is demanded in this area to satisfy the curiosity of both organic chemists and biologists.

§ 1.4 Retrosynthetic analysis of bicyclic β-turn dipeptides

The design strategy for the synthesis of this bicyclic β-turn dipeptides must include a concise and straightforward way to introduce side chain groups in peptides since side chain groups play critical roles in molecular recognition. The retrosynthetic route for thiazolizidinone is shown in the Figure 1.7. In this design, since the chirality centers can be predetermined in amino acids, the target molecules can have at least 16 different isomers and the constrained side chain groups can be controlled at their χ_1 and χ_2 angles. The configuration of bridgehead hydrogens, however, cannot be specified. Two diastereomers would be expected in different ratio which depends on the different chiralities and the group size at the α and β positions of cysteine derivatives.

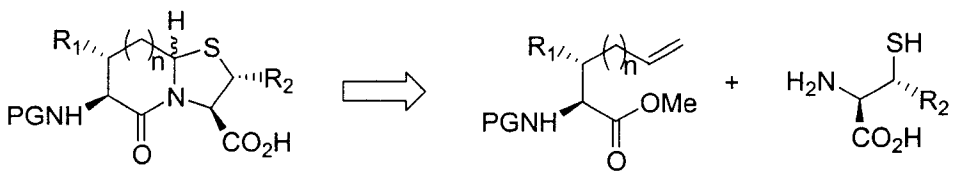


Figure 1.7 Retrosynthesis of bicyclic dipeptide scaffold

The convergent retrosynthetic analysis indicates that this bicyclic dipeptide actually can be synthesized efficiently from two amino acids (Figure 1.7), a β-substituted ω-unsaturated amino acid **A** and a β-substituted cysteine **B**. Our group has developed novel synthetic method for β-cysteine derivatives and both aromatic and aliphatic substituents can be introduced.[32,33] The synthesis of β-substituted amino acid **A**, however, has not been reported. In this dissertation, I will start the synthesis of β-substituted ω-unsaturated amino acids in chapter **II**, followed by a strategy for development of introducing side chains group into [5,5]- and [6,5]-bicyclic β-turn dipeptides in chapter **III** and chapter **IV**, respectively.

Very recently, several types of internal β-turn mimetics have been developed in a combinatorial fashion using solid phase synthesis.[34-37] The convergent retrosynthesis (Figure 1.7) also suggests a possible way that our bicyclic dipeptides can be synthesized on solid phase from the individual amino acids. In this way, a multi-step synthesis of organic compounds which have 5 chiral centers in the molecule becomes practical and simple stepwise procedure. It also became possible to prepare all diastereoisomeric analogues by using different amino acids as starting materials. The design and synthesis of Leu-enkephalin derivatives on solid phase by this novel strategy will be fully discussed in the chapter **V**.

CHAPTER II.

SYNTHESIS OF β-SUBSTITUTED ω-UNSATURATED AMINO ACIDS

§ 2.1 Introduction

From the retrosynthetic analysis in figure 1.7, chapter **I**, I realized that the first thing to do toward the synthesis of thiazolizidinone bicyclic β-turn dipeptides is to develop a synthetic methodology toward β-substituted ω-unsaturated amino acids. The strategies involved in this methodology should be universal enough so that both alphatic and aromatic side chain groups can be introduced. These side chain groups may also include other functionalities and their orthogonal protecting groups. The terminal unsaturated amino acid can either be γ,δ-, δ,ε- and other ω-unsaturated amino acids for [5,5]-, [6,5]- and other size bicyclic dipeptides.

The Hruby group has been active in the syntheses enantiomerically pure β-substituted amino acids in the past decade. Many different side chain groups have been introduced at β-position by using modifies Evans auxiliaries[38-45] and a Ni(II)-complex auxiliary.[46-52] Although these amino acid syntheses introduce different side chain groups at the β-position, the introduction of terminal double bond was not examined. ω-Unsaturated amino acids are of value in terms of their biological importance and their utility as asymmetric synthetic building blocks.[53] The terminal double bond is a precursor in organic synthesis which can be converted to the ω-hydroxy, ω-oxo, ω-

carboxy, ω-epoxy, and ω-amino α-amino acids.[54] They also have been used in cyclization of peptides for secondary structures which were built up by ring closing metathesis.[24,55-57] ω-Unsaturated amino acids are especially important in my project because they can be simply oxidized to aldehydes, which are a precursor of *N,S*-acetals in bicyclic dipeptide formation.

§ 2.2 Large scale synthesis of β-vinyl phenyl aniline

The synthesis of γ,δ-unsaturated amino acids has received much attention in recently years.[58-60] The greatest contribution to synthesis of these amino acids was attributed to Uli Kazmaier, who took advantage of the Claisen rearrangement.[61] This reaction has been expanded to acyclic and cyclic allylic esters,[62] and to peptides.[63,64] Because of his great contribution, the reaction has been called the Kazmaier-Claisen rearrangement since then. According to the Kazmaier, the N^{α}-protected glycine allyl ester would undergo [3,3]-rearrangement in the present of strong bases. The protecting group (PG) in this case must be mono-protected such as Cbz and TFA and it also has to be stable to strong base. The beauty of this reaction is that by using the Lewis acid, $ZnCl_2$, the diastereoselectivity can be increased to >98%. In other words, the enolate formation will give only the *Z*-conformation because of the Zn-coordinated 5-membered ring involved in the transition state (Figure 2.1). The other important fact is that when the *E*-allyl ester is used in this reaction, only *threo*- product will be generated due to the 6-membered chair-like transition state **A** with an equatorial substituent.[65] When *Z*-allyl esters are used in this rearrangement, however, a diastereomeric mixture

will be formed in general.[61,66] Because in this case, a chair like conformation with an axial substituent **B** is competitive with a boat like conformation Transition State **C**. Thus a pair of diastereomers will be generated from transition state **B** and **C**, respectively.

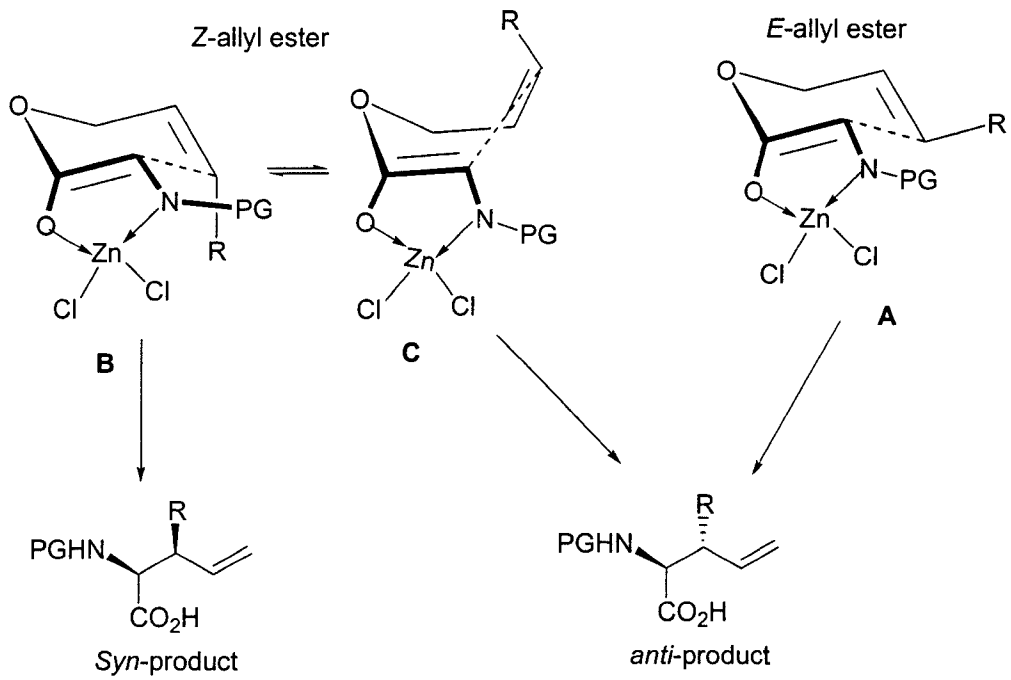
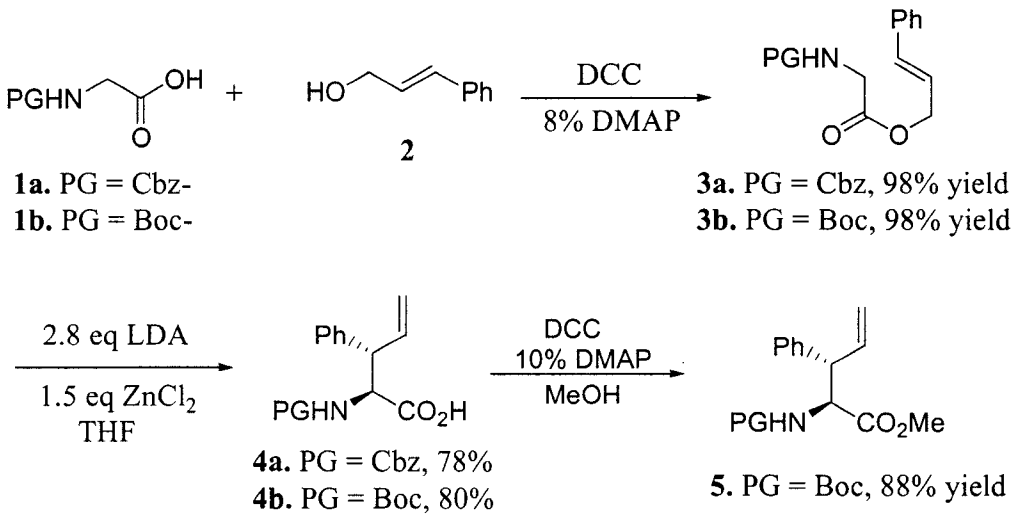


Figure 2.1 *Z*-enolate and TS# in Kazmaier Claisen rearrangement

In order to produce enough material for peptide synthesis, a large-scale Kazmaier-Claisen rearrangement was needed. The first side chain considered was phenyl, as it appears in the β-turn of the α-MSH (α-melanocyte stimulating hormone) core sequence of His-Phe-Arg-Trp.[67,68] Thus the amino acid which needs to be synthesized for a [5,5]-bicyclic dipeptide is the β-phenyl γ,δ-unsaturated amino acid, simply called β-vinyl phenyl alanine. Wei Qiu started this project by using a N^{α}-Cbz protected glycine as a

starting material.[69] The β-vinyl phenylalanine can be synthesized in two steps from N^{α}-protected glycine and allylic alcohol (Scheme 2.1). The ester product **3a** was purified by recrystallization from hexane. The rearrangement under the typical Kazmaier reaction conditions gave the expected product **4a**. The reaction was optimized by refluxing in THF for 1.5 h and the yield was improved to 78%.



Scheme 2.1 Synthesis of β-vinyl phenyl alanine

The N^{α}-Boc protected glycine was chosen in my project because I believe these bicyclic dipeptides would be eventually used in peptide synthesis and N^{α}-Boc chemistry is a standard protecting group used on solid phase peptide synthesis. Starting from N^{α}-Boc protected glycine, esterification with cinnamyl alcohol provided allyl ester **3b** (Scheme 2.1) in excellent yield. A serious side reaction, however, was observed in the Kazmaier-Claisen rearrangement, especially in small scale (200-300 mg) reactions. The

starting material underwent hydrolysis instead of rearrangement to give N^{α}-Boc glycine and cinnamyl alcohol. The side reaction was especially serious when humidity was higher than 50% (such as in Monsoon season) and the yield was usually less than 10%. I thought this side reaction was caused by water contamination of solvent and reagents. However, the reaction was very reliable at about 3 to 15 gram scale to give 64 to 80% yield after flash column chromatograph.

In this Kazmaier Claisen rearrangement, a $ZnCl_2$·THF solution was used instead of solid $ZnCl_2$. The concentration of *n*-BuLi was titrated by 2,5-dimethoxybenzyl alcohol and LDA was prepared at 0°C before it was cannulated into argon protected reaction system at -78°C. 1.5 eq of $ZnCl_2$ Lewis acid and 2.8 eq of LDA was found to be optimal in this reaction. After enolate formation (5 min), the reaction system was then warm up immediately to room temperature by taking away the dry-ice/acetone bath. It should be indicated that the enolate formation is very fast even at -78°C and the rearrangement was thought to happen at a reasonable rate only when the temperature is higher than -20°C.[70-72] Fortunately when the reaction was scaled up (>3 g), only limited amount of ester was hydrolyzed. This reaction was optimized by scaling up the reaction, increasing the amount of LDA, warming up to the room temperature in 30 min, and working up immediately.

The typical Kazmaier-Claisen rearrangement generated β-vinyl phenylalanine **4b** highly diasteroselectively. In all the cases, only one diastereomer was observed by ^1H nmr spectroscopy. The byproduct generated from decomposition of ester **3b** could be isolated by using acid-base extraction. The crude product was purified by liquid

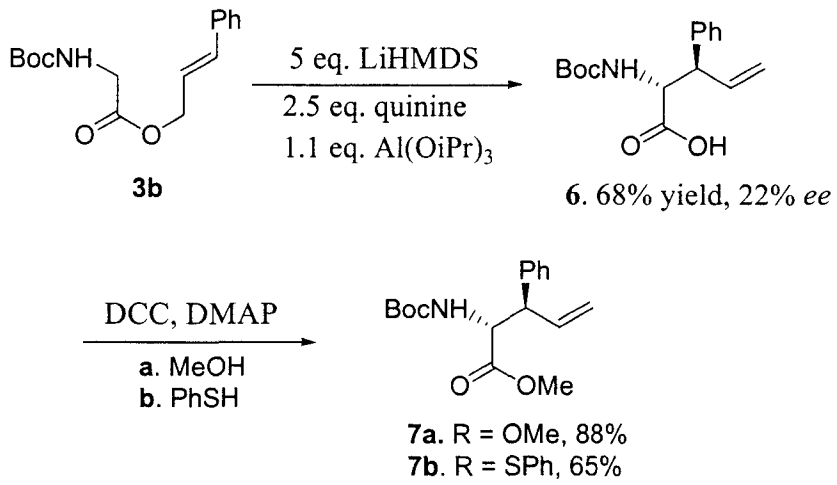
chromatograph. Small amounts of crude material **4b** were transferred to methyl ester **5** by methylation for better purification and characterization (Scheme 2.1).[73]

§ 2.3 Synthesis of enantiomer rich β-vinyl phenylalanine

In the last section, I already demonstrated that the racemic (±)-β-vinyl phenylalanine **4b** can be synthesized by a Kazmaier Claisen rearrangement in large scale (12-15 grams). However, from a synthetic view, starting from a racemic mixture always wastes half of the starting material. As a matter of fact, we would also have to isolate more than two diastereomeric products in the [5,5]-bicyclic dipeptide synthesis (Chapter **III**). One of the methods to obtain this enantiomer rich β-vinyl amino acid product actually has been reported by Kazmaier himself.[74] By using 2.5 eq of quinine as ligand and 1.1 eq of $Al(OiPr)_3$, they were able to synthesize the (2*R*,3*R*)-acid product with 79% *ee*. By using a sterically hindered base, LiHMDS, the yield can be increased up to 98% with 81% e*e*. And the enantiomeric purity can be improved by resolution with α-phenyl ethyl amine. However, his experiment was limited to a very small scale and the percentage of enantiomer excess (*ee*) was based on GLC. The reaction transition state was not discussed in this paper. The only cited paper of his method was reported recently.[75] It was unfortunately that in both of these papers, the authors did not provide detailed experimental information.

Nevertheless, the references were very attractive to me at a time that I succeeded in the large scale synthesis of Kazmaier Claisen rearrangement. The following reaction (Scheme 2.2) was tried according to the reported method. The yield of rearrangement

was 68% without much effort at optimization. The enantiomeric purity was determined by chiral HPLC after transform this acid product to its methyl ester. It is very unfortunate that only 22% *ee* was obtained by comparison of racemic production using chiral HPLC column (Chiralcel OD column 0.46 x 25 cm, Hexane/isopropanol = 98.5/1.5 at a flow rate of 1.0 mL/min. The retention time for two enantiomers: 21.6 min and 25.9 min).

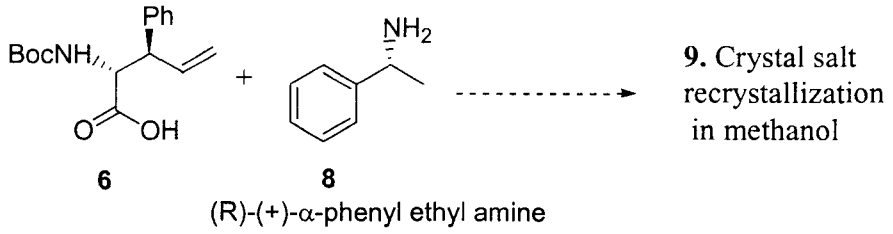


Scheme 2.2 Enantiomer rich Kazmaier-Claisen rearrangement

The UV sensitivity of methyl ester **7a** was relative low compared to the other impurities. We thought this might be the reason why enantiomer purity was lower than we expected. In order to increase product sensitivity in the HPLC detector, acid **6** was transformed to its phenyl thiol ester. However, the %*ee* result was not much different. The real problem, however, might be the reaction conditions. It was reported that the reaction was gradually warmed up from -78°C to room temperature over 12 h,[74] which

was difficult in my case. In fact, the reaction was completed very fast in 5-6 h at -20°C in acetonitrile/dry ice bath.

A resolution method is critical for practical usage. According to Kazmaier's suggestion, this product can be upgraded by using enantiomerically pure α-phenyl ethyl amine to form a diastereomeric salt crystal for resolution. However, since both of above papers did not mention the details of the procedure[74,75], I started with (*R*)-(+)-α-phenyl ethyl amine **8**. The product **6** was mixed with (+)-α-phenylethyl amine **8** and recrystallized in methanol. The isolated crystal salt **9** was recrystallized one more time before it was dissolved in ethyl ether and extracted by 1*N* NaOH aqueous solution, the (+)-α-phenyl ethyl amine was recovered in the organic phase and enantiomer rich acid product was recovered in the aqueous phase. The aqueous solution was acidified to pH = 2 and re-extracted by ether.



Scheme 2.3 Resolution of Kazmaier-Claisen product

It seems to me that the (+)-α-phenyl ethyl amine was the wrong enantiomer used in my experiment because after the product was transferred to its methyl ester, its enantiomer purity was less than 20% according to the chiral HPLC results under the

same analytical conditions. This strategy was eventually given up due to the low yield (10% overall yield after resolution), especially low %*ee* and practically difficult procedure in resolution.

§ 2.4 Ni(II)-Complex and thio-Claisen rearrangement

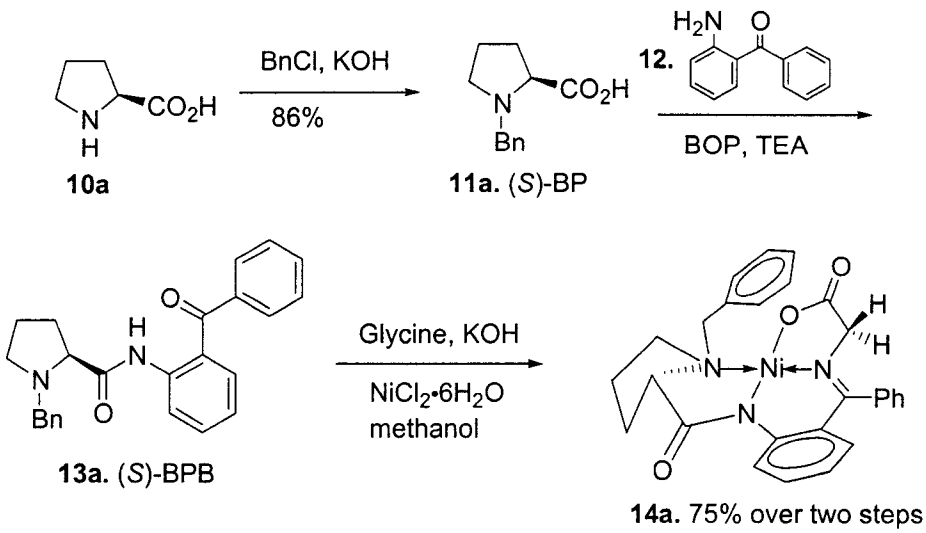
As we discussed in the previous section, there were some problems in the Kazmaier Claisen rearrangement to obtain enantiomer rich products. On the other hand, the asymmetric Claisen rearrangement reported by Corey indicated the possibility of generating a contiguous chiral center at the α,β-positions by using a chiral auxiliary.[76,77] Meyer also reported a successful thio-Claisen rearrangement.[78] In this method, they were able to synthesize enantiomer pure products by using a chiral auxiliary, which can be cleaved later. This method has been successfully used in the natural product synthesis of (-)-trichodiene.[79] Unfortunately, these methods were not applicable to enantiomeric amino acid synthesis. The only chiral auxiliary reported for enantiomer allylglycine synthesis uses (1*S*)-(-)-2,10-camphorsultam analogue.[80] On the other hand, the Ni(II)-complex **14** has been used in amino acid synthesis since its initial development by Belokon *et al.*[81] Our group has worked on it and lots of work has been published. My idea was to sue the Ni(II)–complex as a chiral auxiliary in the Claisen rearrangement.

2.4.1 Synthesis of Ni(II)-complex

The synthesis of the Ni(II)-complex was modified based on the reported method.[47,82] *L*-Proline **10a** was selectively protected by BnCl in 2-propanol (Scheme 2.4). BnBr is not chosen in order to avoid benzyl ester formation at the carboxylic acid terminal. BnCl was diluted by 2-propanol in a dropping funnel so that the addition of BnCl can be controlled in 4 hours which is critical to increase the yield of this reaction. After neutralization, instead of using $CHCl_3$, DCM was used in my case to precipitate the KCl. The inorganic salt was filtrated and the product (*S*)-BP **11a** in DCM solution was evaporated and then the product can be precipitated in acetone. Beilstein Test showed the product **11a** was in a free acid form. Efforts to collect the secondary crop of crystals from the mother liquor failed. The (*S*)-BPB **13a** was generated by coupling with 2-amino benzophenone **12**. Although a strong coupling reagent, BOP, was used in this reaction, the reaction was sluggish and took 40 h at room temperature due to the steric hindrance at amine. Because the (*S*)-BPB could not be purified after work up, the 2-amino benzophenone **12** was used in limited amounts. The Ni(II)-complex **14a** then can be formed at 40°C in 7 eq of KOH methanol solution. If the reaction was slow, more KOH was added until 10 eq so that the reaction can finish in 2 hours after the color changes to brown.

The workup of the Ni(II)-complex depended on the nature of crystal formation and the amount of contamination by impurities. In order to obtain a nice precipitation in acetone and hexane, it is important to be patient to wait for the crude product to completely dry in air before it is dissolved in $CHCl_3$ and filtrated. After crystallization,

the small amount of product in mother liquid can be purified by flash column chromatography. (*R*)-Ni(II)-complex **14b** was also synthesized from the (*R*)-proline **10b** by the same procedure with comparative yield.

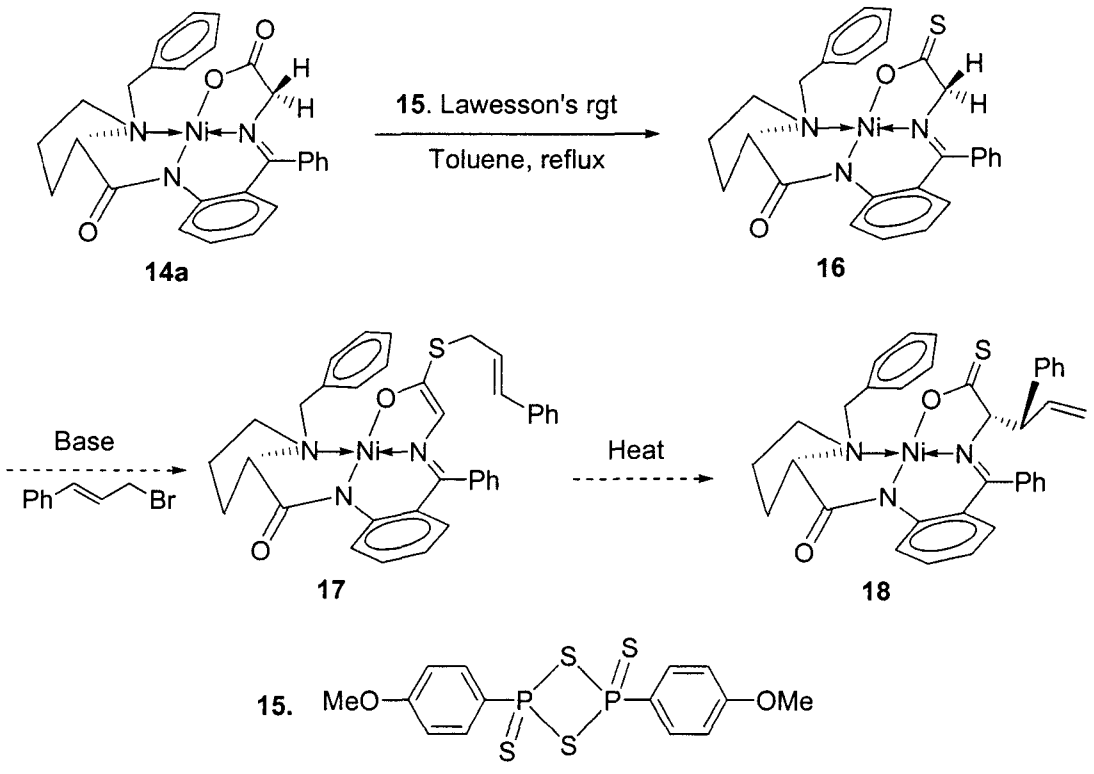


Scheme 2.4 Synthesis of (*S*)-BPB-Gly-Ni(II)-complex

2.4.2 The design of thio-Claisen rearrangement on Ni(II)-complex

The synthesis of the thia-Glycine-Ni(II)-complex and its application for β-substituted γ,δ-unsaturated amino acid is shown in Scheme 2.5. The initial idea was to convert this Ni(II)-complex directly by reacting with Lawesson's reagent.[83,84] It turned out however, the reaction was very slow due to the low reactivity of the ester and the steric hindrance of both starting materials. High temperatures were also tried in refluxing of toluene, but decomposition was obvious in the 3 h reaction. As the result,

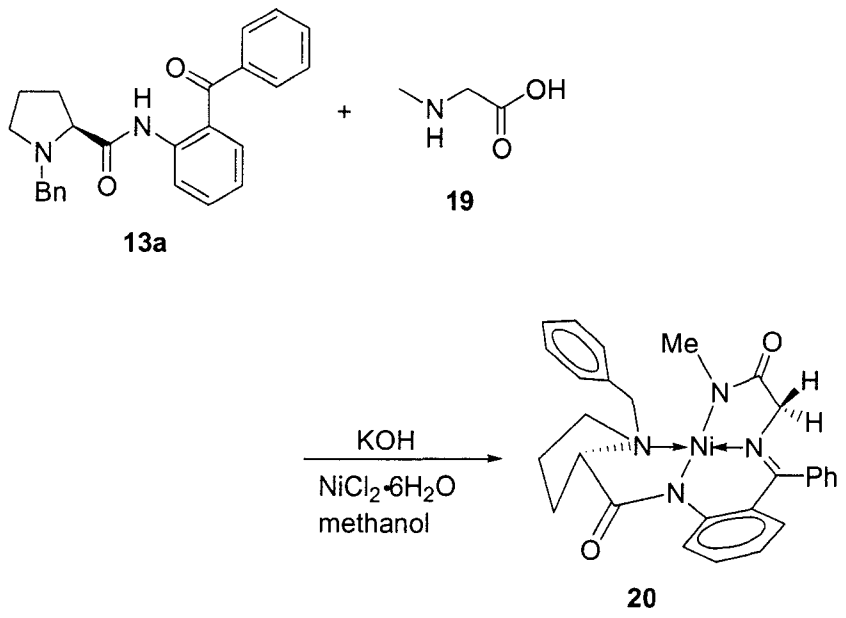
one third of the starting material was decomposed, one third was recovered, and only 34% of product **16** was isolated.



Scheme 2.5 The design of thio-Claisen rearrangement on Ni(II)-complex

By looking at the reactions employing Lawesson's reagent, most of the starting materials are amides instead of ester.[85] The oxygen has less electron donation ability than nitrogen and this is the reason why the above reaction was so slow. A nitrogen-containing precursor *N*-methyl glycine, although expensive, is commercially available. The Ni(II)-complex **20** was synthesized in a similar way (Scheme 2.6). The reaction, however, was slow and the product is massy. Attemps to purify the product mixture by

precipitation in acetone/hexane mixture was failed. As future work, the reaction will be carefully examined and new reagents, such as P_2S_5 and Al_2S_3,[79,86] will be used to synthesize thioester. This new Ni(II)-complex synthesis and its application for β-substituted γ,δ-unsaturated amino acid by [3,3]-sigmatropic rearrangement is under investigation.

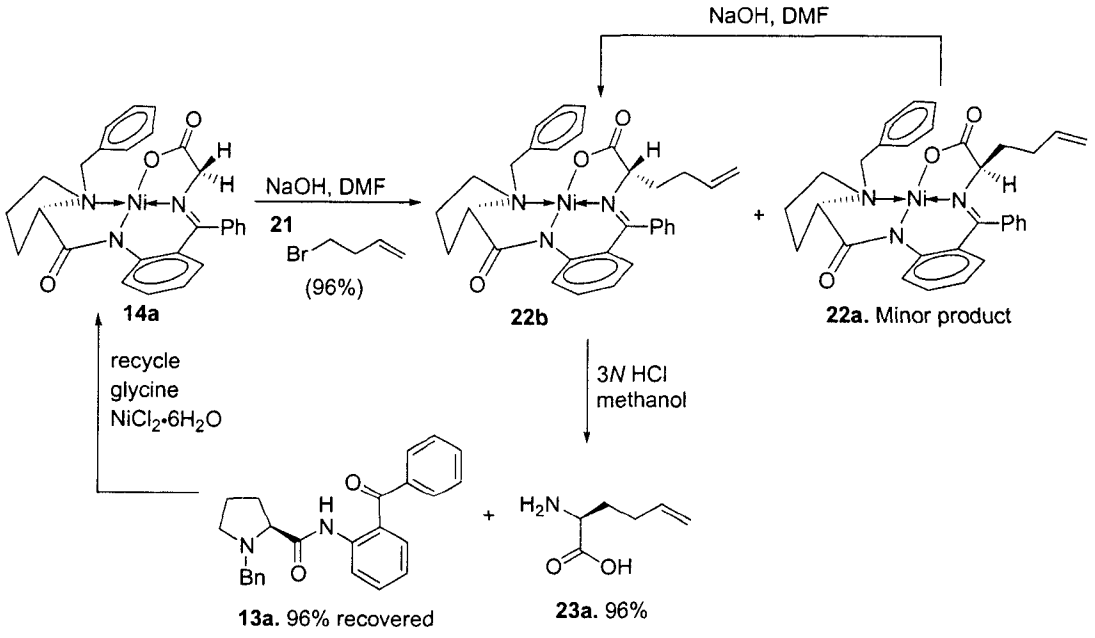


Scheme 2.6. The synthesis of N-methyl-Gly-Ni(II)-complex

§ 2.5 Synthesis of ω-unsaturated amino acids

Amino acid synthesis by alkylation of a Ni(II)-complex in our group was started by Wei Qui, Vadim Soloshonok, and Xuejun Tang.[87-89] The synthesis of 2-amino-pentanic acid *via* Ni(II)-complexes has been reported, though in low yield.[90,91] We have recently modified this reaction and improved the yields to 95-98% (Scheme 2.7). The reaction

was performed in DMF using a large excess of NaOH as base. During the reaction, a green color formed which indicated enolate formation. The color disappeared after addition of 4-bromo-1-butene **21** in 3 minutes at room temperature. The effect of different solvents in this alkylation reaction also has been tried. The Ni(II)-complex has limited solubility in CH_3CN so it is not a good solvent. In methanol, the complex is soluble but the reaction is very slow. After overnight the reaction was only 25% completed by TLC. It was faster in CH_3OH using $NaOCH_3$ as base, but the reaction was not complete in 24 hours. For prolonged reaction times the Ni(II)-complex mainly decomposed under these reaction conditions. DMF is the best solvent because in this homogenous solution, the reaction is very fast and decomposition is limited. In fact, enolate formation was complete in 2 min, and the alkylation reaction only takes 3 min.



Scheme 2.7 The alkylation and decomposition of Ni(II)-complex

In this developed method, NaOH (10 eq) was used as the base and DMF as the solvent, which is different from the typical literature which reported NaH as base and THF as solvent.[90,91] However, this modified method had problems in scale up. The first problem was the contamination of DMF in product which originally was washed away by H_2O after precipitation and filtration of the solvent. This problem was eventually solved by dissolving the product in benzene and washing with brine 4 times. We also tried using DCM as solvent and the solution was washed with brine. However, the wash procedure was not efficient. A second problem is the low efficiency in diastereomers purification by flash column chromatograph. The required silica gel to sample ratio is about 400 to 1. Large scale synthesis became practical by successful use of recrystallization. Fast flash chromatography, however, was usually employed before recrystallization to separate the diastereomeric mixture from impurities which came from the starting material of Ni(II)-complex and its decomposed byproducts in reaction.

The product **22b** (Scheme 2.7) generated from the *si*-face of the glycine enolate was thermodynamically favored and the configuration was assigned as *S(2S)*.[46] We found that this reaction has very good diastereoselectivity with an isomer ratio 2*S*/2*R* = 95/5. The diatereomeric ratio was determined by ^{1}H nmr, particularly according to the most downfield signal, 8.0-8.5 ppm. The Ni(II)-complex products ^{1}H NMR structure were analyzed by COSY and 1D nOe. We found the two most downfield (~8.2 ppm) proton signals are the doublets of H_a and H_b in a ratio of 1 to 2 in all the spectra. In the *S*(2*R*) products, however, the H_a proton signal moves further downfield (~8.5 ppm),

while the H_b proton signal moves toward highfield, and as a result, overlap with other aromatic proton signals.

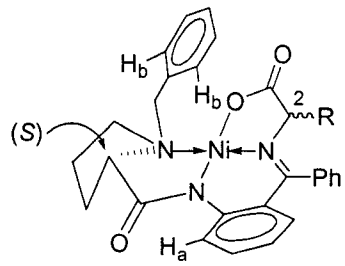


Figure 2.2 The downfield proton H_a and H_b in Ni(II)-complex

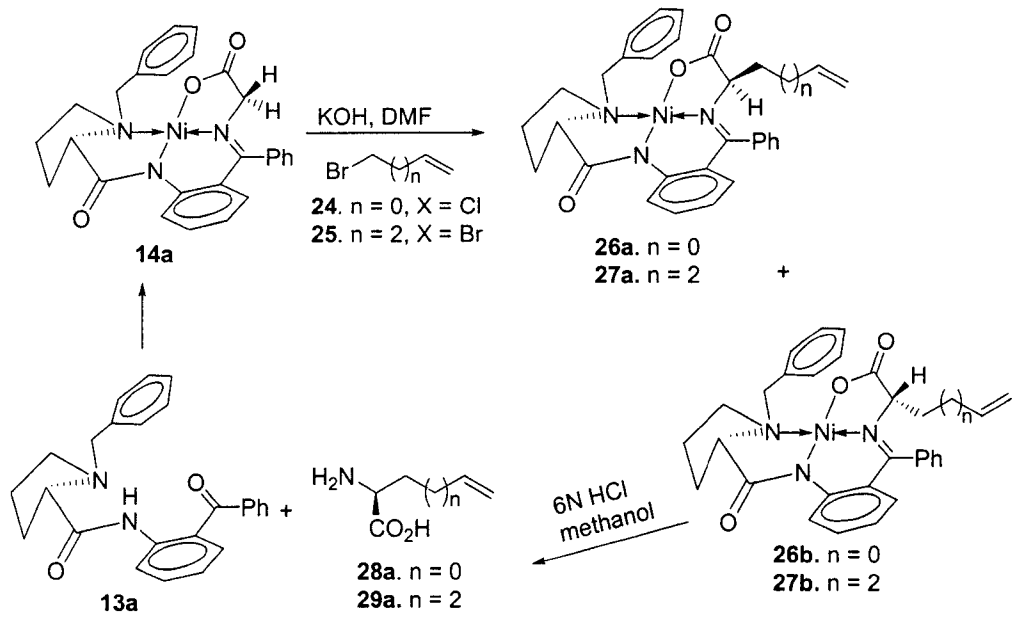
The product can be readily improved to >98% *de* by recrystallization from $CHCl_3$ and ether. The mother liquor mixture was subjected to the same reaction condition so that the *S*(2*R*) was epimerized to *S*(2*S*) major product with the similar ratio as obtained in the reaction. Attempts to characterize the minor product *S*(2*R*) failed due to the difficulty to purify this product even by analytical HPLC silica gel column. The amino acid **23** was generated by decomposition of the Ni(II)-complex with 3*N* HCl in methanol (1 : 1). The disappearance of the reddish color indicated the completeness of hydrolysis. The acid was evaporated in *vacuo* two times and finally neutralized with concentrated $NH_3 \cdot H_2O$. The (*S*)-BPB **13a** was extracted by $CHCl_3$ and 96% of it was recovered. The mixture of amino acid and Ni^{2+} salt was then loaded on an H^+ form ion exchange resin column. The amino acid was washed through with a concentrated aqueous ammonia and water (4 : 1) mixture. The Ni^{2+} salt remained on the column until the column was regenerated with 1*N* HCl solution. The amino acid was collected after

evaporation of H_2O solvent. The residue also can be dissolved in small amounts of water and then dried by lyophilization. In this way, the 2-amino-hexenoic acid **23a** was synthesized in 96% yield.

The alkylation was optimized for temperature and reaction time (Table 2.1). It was found that at low temperatures the reaction took a long time and the kinetic ratio (quenched by HOAc/water solution immediately after the reaction was finished) gives poorer diastereoselectivity. At room temperature, the reaction actually finishes in 3 min and the product ratio did not change with prolonged reaction time. Elevated temperatures make the reaction messy, and the Ni(II)-complex was partially decomposed. We believe epimerization happens very fast at room temperature so that it reaches the thermodynamic ratio.

Table 2.1 The alkylation conditions and isomer ratios

	Reaction time	reaction temperature	isomer ratio(2S/2R)
1	35	-30	92 : 8
2	10	0	91 : 9
3	5	20	95 : 5
4	10	20	95 : 5
5	20	20	95 : 5
6	5	50	inconclusive

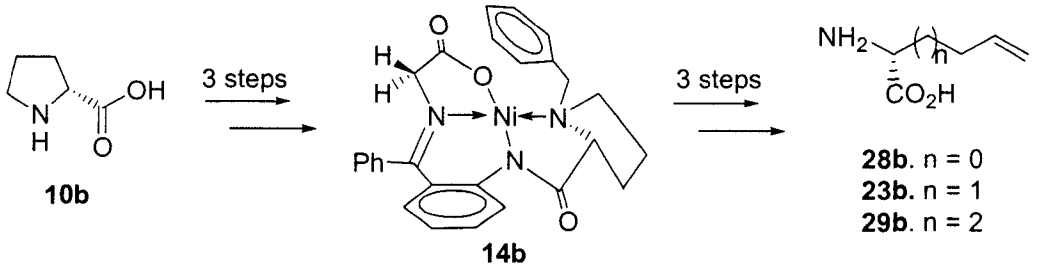


Scheme 2.8. Other alkylation and hydrolysis of Ni(II)-complex

The (*S*)-allylglycine **28a** also can be synthesized by this method (Scheme 2.8). It should be indicated that although both enantiomers of this amino acid are commercially available, the syntheses by using Ni(II)-complex has not been reported. When allylic bromide was used in above strategy under the same reaction condition, however, a messy product was obtained with a byproduct contamination up to 30%. This unexpected result could be attributed to the high reactivity of allylic bromide and its lability at this reaction condition. The byproduct could not be characterized due to the lack of simple purification method. The reaction was also tried at 0°C but no improvement was found. However, when allylic chloride **24** was used at room

temperature, the reaction was very clean and completed in 5 min with 96% yield and 87:13 diastereomeric selectivity.

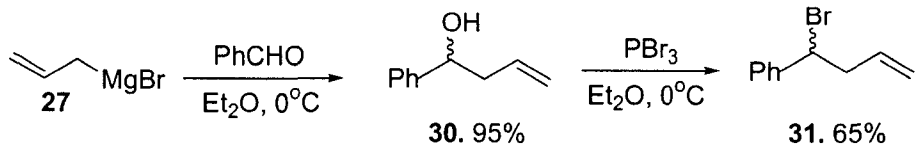
For the [7,5]-BTD inserted peptide synthesis, I also needed 2-amino-hept-6-enyl carboxylic acid, **29a**. 5-Bromo-1-pentene **25** was used as starting material and the reaction was performed as before with 92:8 diastereoselectivity (Scheme 2.8). The diastereomeric ratios were determinated by ^{1}H NMR spectroscopy. In order to avoid double alkylation, the bromide **24** and **25** was used with slightly less than 1 eq. After quick flash liquid chromatograph, the enantiomeric purity of the major isomer *S*(2*S*)-**26b** and **27b** was simply improved by recrystallization form $CHCl_3$ and ether. The mother liquor with enriched minor product mixture can be subjected to the same reaction conditions with 10 equivalents of NaOH for 10 min. The epimerization would transfer most of the minor product to the major isomer and then it could be repurified by recrystallization. In this way, over a 95% yield of product can be obtained. The alkylated products **26b** and **27b** can be hydrolyzed to **28a** and **29a** in excellent yields (Scheme 2.8). The optimized reaction condition was ready to be scaled up to 20 gram scale, and after hydrolysis to generate about 5 gram scale amino acids. (2*R*)-2-Amino-5-hexenoic acid also has been synthesized in a 3 gram scale with (*R*)-proline as starting material in the Ni(II)-complex (Scheme 2.9). The diastereoselectivity, however, dropped to 15 : 1 instead of 20 : 1 in the large scale synthesis. Nevertheless, the purification of the major product by recrystallization could be readily accomplished. The (*R*)-allylglycine and (*R*)-2-amino-hept-6-enyl carboxylic acid were synthesized on a gram scale by exactly the same method.



Scheme 2.9 Synthesis of (*R*)-Ni(II)-complex and *D*-amino acids

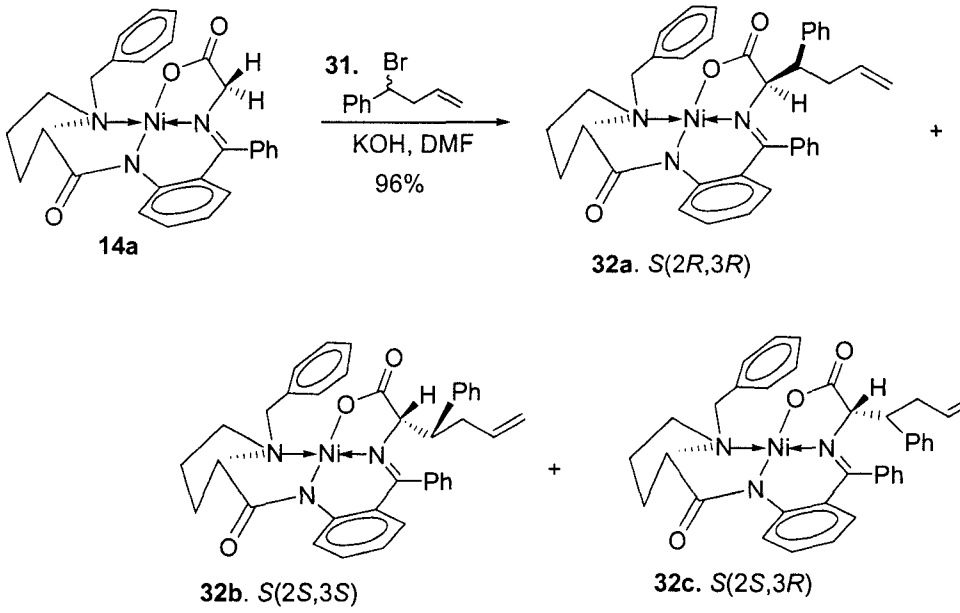
§ 2.6 Alkylation with racemic secondary bromide

If a secondary bromide is used in the alkylation, a β-substituted amino acid will be generated.[47] Secondary bromide **31** can be synthesized in two steps from the commercially available starting material in moderate yield (Scheme 2.8).[92] The preparation was started from benzaldehyde and allyl magnesium bromide. The Grignard reaction was performed in ether at 0°C for 70 min and quenched slowly by 10% aqueous H_2SO_4. The bromination was then accomplished by PBr_3 at 0°C in 45 min. The bromide was purified by flash liquid chromatography. In one experiment, decomposition on the silica gel column was observed after the column was stopped for 1 h.



Scheme 2.10 The synthesis of secondary bromide

The alkylation of secondary bromide on Ni(II) complex, however, generated a mixture of three diastereomers (Scheme 2.11). The reactivity of two bromide enantiomers are very different and high diastereoselectivity can be achieved by using bromide **31** in excess. In practice, 3.0 eq of racemic bromide was used so that the reaction gives high diastereoselectivity. Their relative stereochemistry were assigned by reference to previous work with Ni(II)-alkylated products[47] and were finally confirmed by X-ray crystallography (Section 2.7).



Scheme 2.11. The alkylation of secondary bromide on (*S*)-BPB-Ni(II)-complex

The diastereomeric ratios of these alkylated products are changed when the temperature changed (Table 2.2). At higher temperature (50°C) reaction gave both minor products in higher ratio as well as decomposed Ni(II)-complex. The ratio of

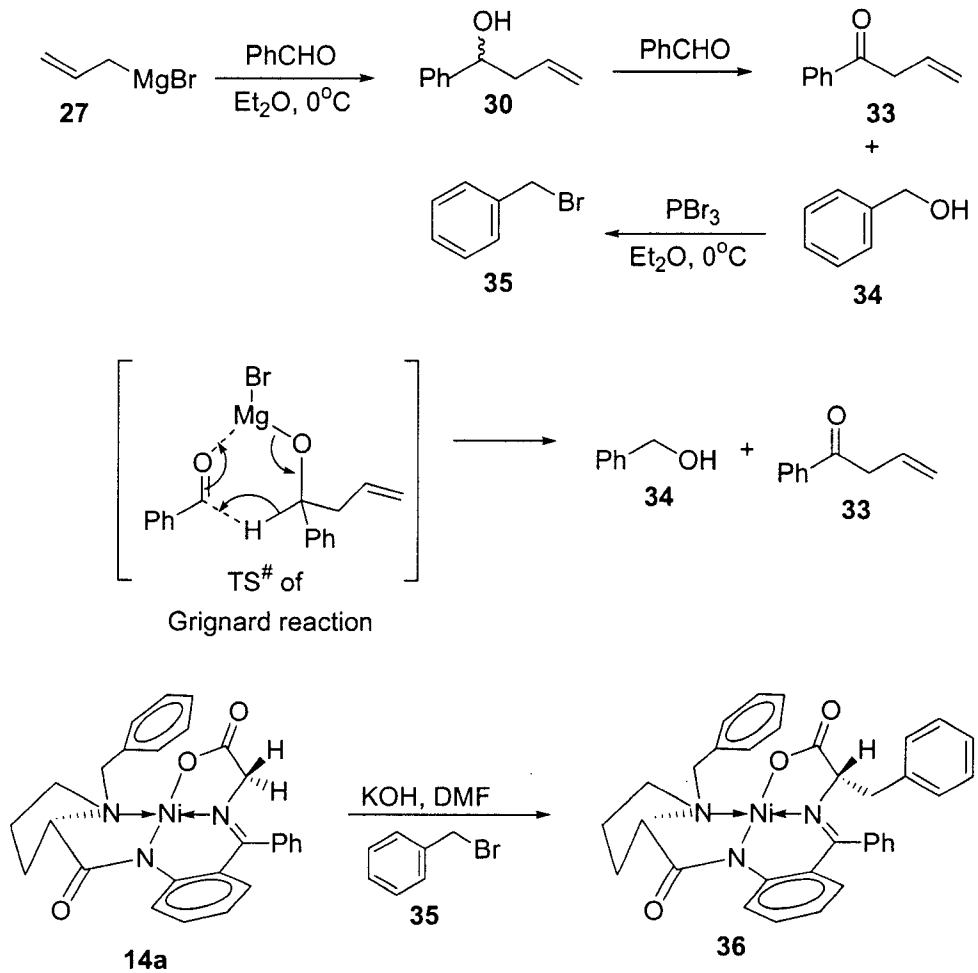
S(2*S*,3*S*) **32b** major and *S*(2*R*,3*R*) **32a** minor can be increased to about 12:1 when the temperature is lowered to -30°C, but in this case the reaction needs 45 min to complete. The minor product *S*(2*S*,3*R*) **32c** is obtained in about 3-5% yield at these reaction conditions. If this alkylation is a typical S_N2 reaction, the major product *S*(2*S*,3*S*) would be generated from the (*R*)-bromide and the *si*-face of Ni(II)-complex enolate. The minor product *S*(2*R*, 3*R*) would be generated from another enantiomeric (*S*)-bromide and the *re*-face of the Ni(II)-complex enolate. In this case, however, they are mismatched and the reaction was much slower. The third product *S*(2*S*,3*R*) could be generated from the (*S*)-bromide and the *si*-face of Ni(II)-complex enolate, again a mismatched reaction. It may also come from the minor product *S*(2*R*,3*R*) **32a** under epimerization, which will be discussed later. Because the racemic starting material was used, the mismatch (*S*)-bromide was left in relative large concentration in the reaction system. These two mismatched minor products cannot be avoided especially in large scale synthesis.

Table 2.2. The alkylation of Ni(II)-Gly-complex

Entry	T,°C	time,min	yield[a]	ratio(**32a/b/c**)
1	50	<5	<80	inconclusive
2	25	<5	98	11/85/4
3	0	15	96	12/85/3
4	-30	45	96	7/90/3

§ 2.7 Purification and X-ray crystallography

The crude alcohol **30** was first used for bromination without purification and only bromide **31** was purified by flash liquid chromatograph in hexane. However, a byproduct **34** contamination (~5 %) in the alcohol synthesis also was transferred to a product **35** after bromination, which can not be purified at the bromide stage (Scheme 2.12). The generation of benzyl alcohol **34** can be explained mechanistically by hydride



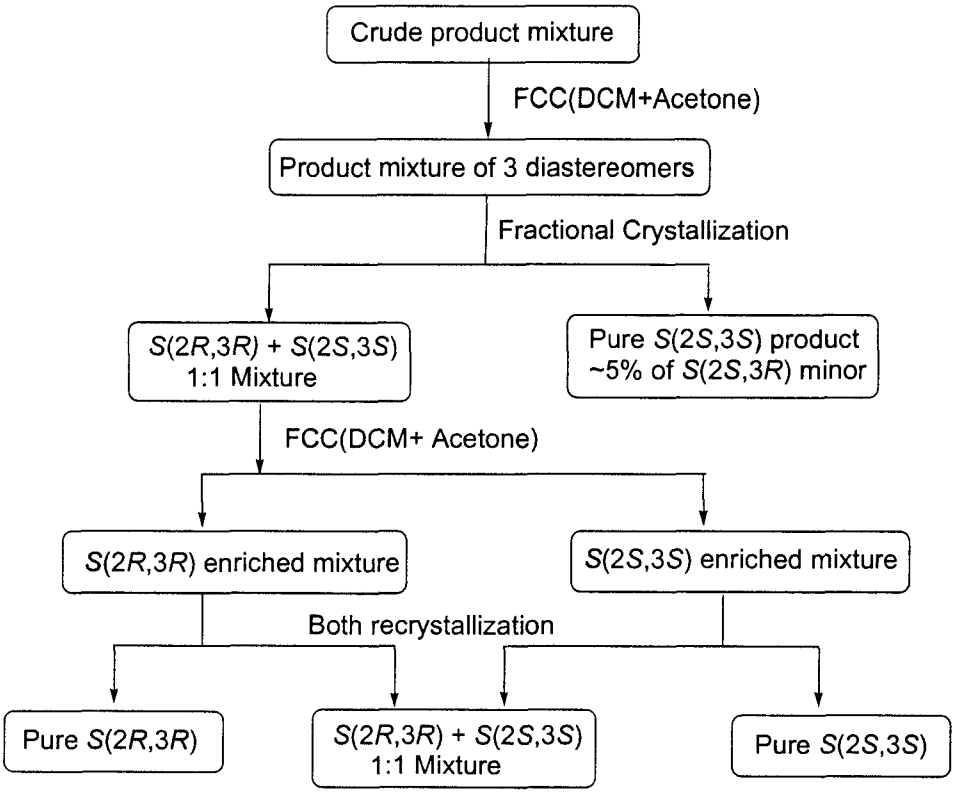
Scheme 2.12 The side reaction in Grignard reaction and its byproduct in alkylation

electronic transfer in a six-membered ring transition state. The byproduct **35** eventually generated a fourth product **36** in the Ni(II)-complex alkylation because the benzyl bromide has higher reactivity toward **14a**. The enolate presence of **36** in the alkylation reaction was up to 15% due to the 3eq of bromide that was used and it cannot be separated by recrystallization. The structure of product **36** was eventually proved by ^{1}H NMR and LRMS. It is fortunate that the byproducts **33** and **34** can be simply removed by flash liquid chromatograph. After using the pure alcohol **30** for the bromination reaction and a pure bromide **31** for the alkylation of the Ni(II)-complex, the product mixture was clean.

After a fast flash column chromatograph, the Ni(II)-alkylation product mixture of **32** was purified by fractional recrystalization. In the process, a 1: 1 mixture of *S*(2*S*,3*S*) and *S*(2*R*,3*R*) crystal and an *S*(2*S*,3*S*) enriched mother liquor was obtained each time (Figure 2.2). The purity of the major product in the mother liquor can be improved until no *S*(2*R*,3*R*) can be detected by ^{1}H NMR. TLC is more sensitive than nmr and could be used to monitor the recrystallizations before nmr. Nevertheless, the *S*(2*S*,3*R*) **32c** minor product (3-5%) which has a similar R_f value as the *S*(2*S*,3*S*) product always coexisted in the mother liquor (R_f = 0.62 for *S*(2*S*,3*S*) and 0.59 for *S*(2*R*,3*R*) in a 1:1 mixture of hexane and acetone). The diasteromeric ratio of recrystallization stopped at about 1:1. Both TLC and nmr show 50% of each isomer in mixture. The crystal mixture was dissolved in DCM and loaded on column and two fractions were obtained. One was the *S*(2*R*,3*R*) enriched mixture, and the other was the *S*(2*S*,3*S*) enriched mixture, both of them can be recrystallized in DCM and ether. Surprisingly, two of them gave the same

1:1 co-crystal *S*(2*S*,3*S*) and *S*(2*R*,3*R*) cocrystal. In this case, however, the first one gave pure *S*(2*R*,3*R*) while the second one gives pure *S*(2*S*,3*S*) in mother liquor.

Figure 2.3 Purification of Ni(II)-Alkylation products



Because of this unexpected and interesting result, a carefully prepared crystal was sent for single X-ray crystallography, and found it is a diastereomeric cocrystal *S*(2*S*, 3*S*) and *S*(2*R*, 3*R*) (Figure 2.3, Next page). The molecules were arranged in crystal in two different layers. One layer is the Gly-Ni(II)-BPB-complex and the secondary layer is the alkylated side-chain. In this layer, we can see a pair of 'racemic' 2-phenyl-4-

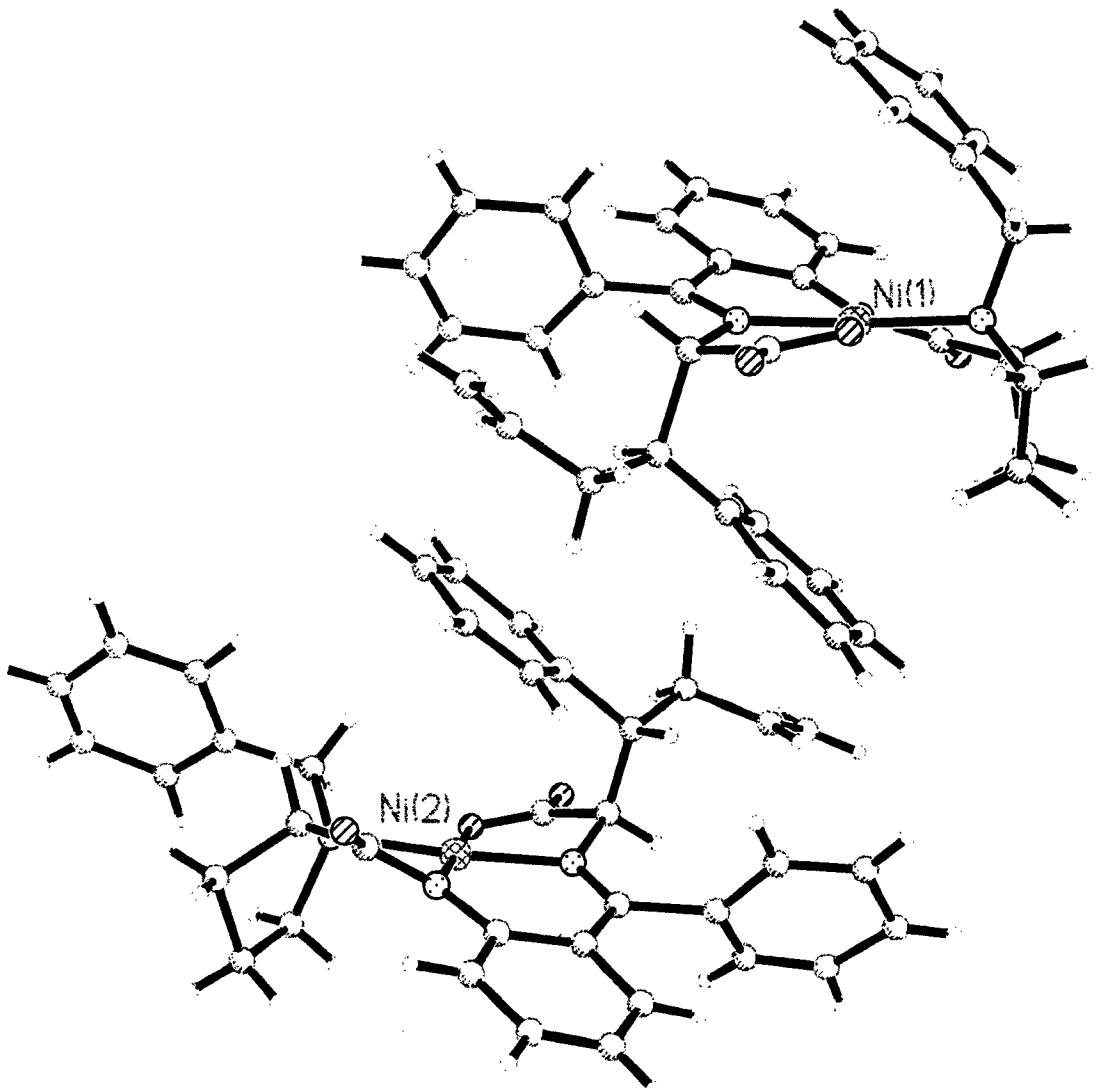
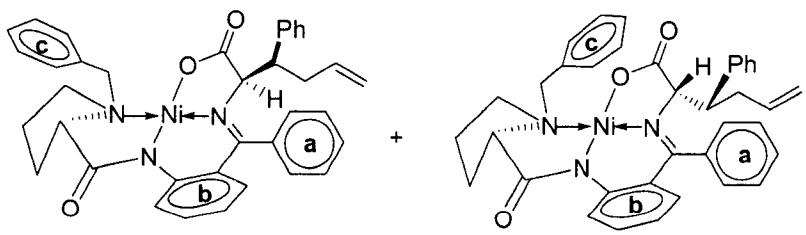


Figure 2.4 Diastereomeric cocrystal of *S*(2*S*, 3*S*) and *S*(2*R*, 3*R*)

butenyl, packing closely due to the van der Waals hydrophobic interaction. However, the distance between the phenyl ring and the terminal alkene gave no indication of strong π-π interactions. The enantiomer pure (*S*)-Gly-Ni(II)-BPB-complex layer was a separate band. In this layer, the phenyl ring **b** is almost parallel to the Ni(II)-complex square plane, while the phenyl ring **a** is perpendicular to the phenyl ring **b**. Phenyl ring **c**, which is different in two isomers, has a tilted angle about 40° compare to the Ni(II)-complex square plane (Figure 2.3). The strong interactions between the two alkylated side chains could be the starting point of crystallization. It explains why 1:1 diastereomeric cocrystal is generated in mixture solution at all the different conditions (Figure 2.2). Overall, the molecule has two 'separated' parts with a single carbon-carbon bond connecting them. This phenomenon is unique in Ni(II)-complex alkylation products. In fact, only a few examples have been reported of diastereomeric cocrystals.[93-96] It also is one of very few examples where two diastereomers cocrystallized due to the intermolecular hydrophobic interaction with no H-bonding or other π-electrons interactions involved.



32a. *S*(2*R*,3*R*) **32b**. *S*(2*S*,3*S*)

Figure 2.5 The diastereomers in co-crystal

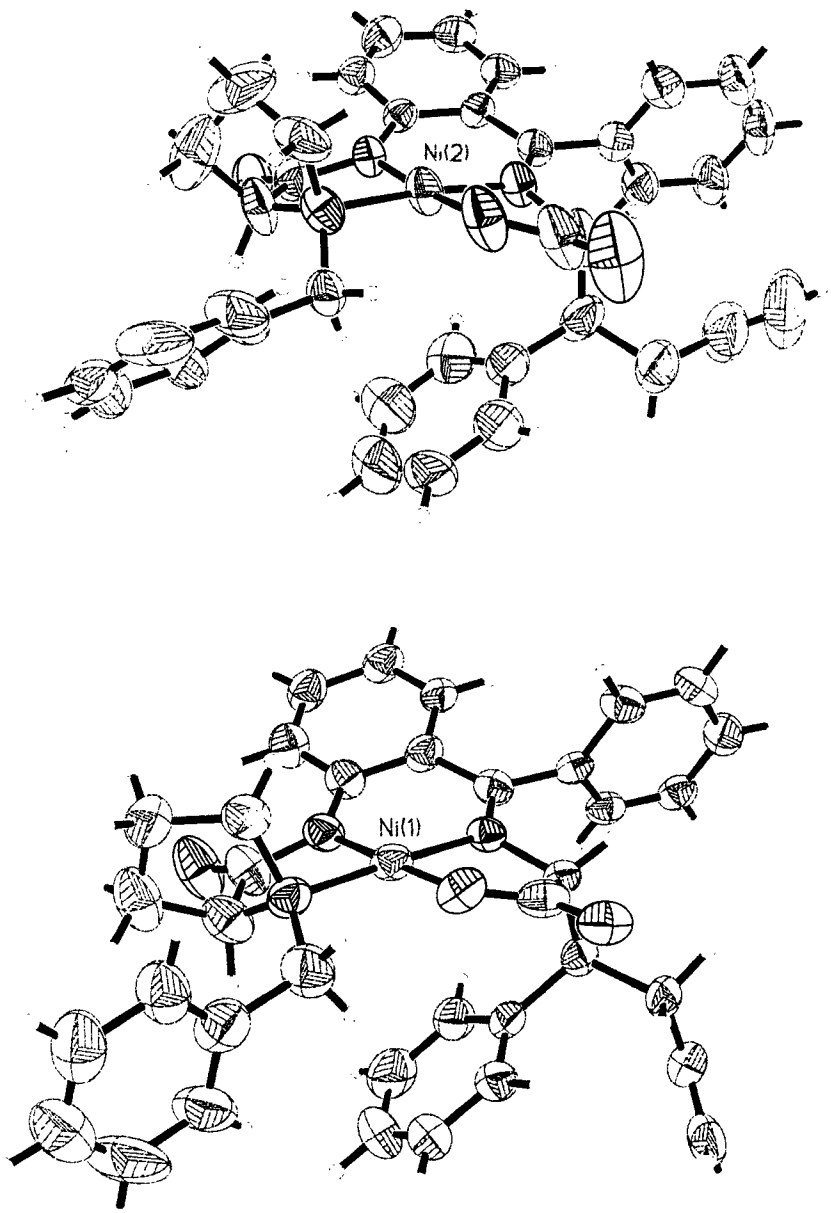
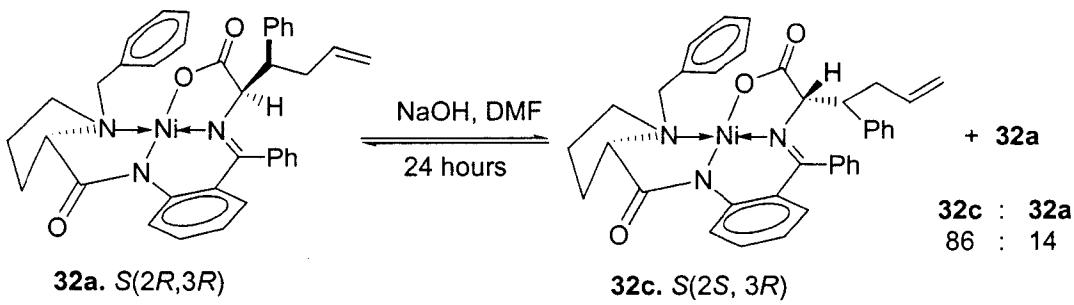


Figure 2.6 Two conformers in pure minor product *S*(2*R*,3*R*).

The stereochemistry of *S*(2*S*,3*S*), the major product, was further confirmed in the [6,5]-bicyclic β-turn dipeptide (Chapter **IV**, Figure 4.3). The minor product *S*(2*R*,3*R*) also was crystallized and its single X-ray structure was obtained (Figure 2.5, next page). The absolute configuration of 2*R* and 3*R* were assigned based on the known chiral center in (*S*)-proline which was used as a starting material in the Ni(II)-complex. It also is interesting to note that there are two different conformers in the unit cell of crystal. Both of them show the benzyl ring **c** was moved away from the top face of Ni(II)-complex square plane. The existence of two conformers for minor product is the first example we found in the X-ray crystal structure of Ni(II)-complex products. The stereoisomer relationship of *S*(2*R*,3*R*) and *S*(2*S*,3*R*) can be further confirmed by epimerization. The assigned *S*(2*R*,3*R*) **32a** minor product was subjected to the same reaction conditions of alkylation but at room temperature for 20 hours. The epimerization give a mixture of *S*(2*R*,3*R*):*S*(2*S*,3*R*) = 16 : 84 from the ^{1}H nmr (Scheme 2.13). The *S*(2*S*,3*R*) product **32c** is the minor product in the alkylation, and could be isolated from the major product by recrystallization and flash liquid chromatograph. In this case, however, it could be isolated from the *S*(2*R*,3*R*) isomer by liquid chromatograph. In fact, this epimerization provides a unique way to obtain this pure minor product which is ready for characterization.



Scheme 2.13 The epimerization of *S*(2*R*,3*R*) to *S*(2*S*,3*R*) product

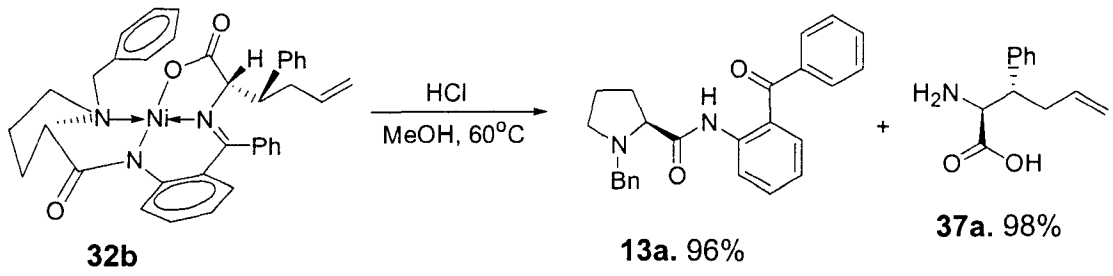
It should be indicated that, however, over 70% of starting material was decomposed in the above condition. The epimerization condition was carefully optimized with different time and the results were shown in Table 2.3. It turns out that over 50% of starting material was transferred in 40 min. The decomposition happened all the time and in about 2 h, more that half material was destroyed. The structure of byproduct is not clear yet. It is a fragment of BPB from the decomposition of Ni(II)-complex. Different solvents and different bases may improve this epimerization. This reaction is under further investigation.

Table 2.3 The epmerization of Ni(II)-alkylation product

Entry	reaction time (min)	starting material *S*(2*R*, 3*R*)	product *S*(2*S*, 3*R*)	byproduct
1	20	74	16	10
2	40	32	42	26
3	120	7	29	64
4	360	7	24	69
5	20 h	5	25	~70

* The percentages were determined by ^{1}H NMR.

In the hydrolysis of alkylated Ni(II)-complex **32b**, the starting material is not very soluble in methanol. A mixture of DCM and methanol (about 2:1) was used and mixture was added by using dropping funnel. The reaction was slow and DCM evaporated during the reaction. The hydrolysis took about 1 h before the solution became totally blue (Scheme 2.14). Following evaporation of the acid and neutralization of the aqueous solution, about 96% of the (*S*)-BPB was extracted into $CHCl_3$, which can be reused in the synthesis of Ni(II)-complex. The aqueous solution was loaded on to a H^+ resin. In one experiment, the column was not completely washed by water before the $NH_3 \cdot H_2O$ and water (4:1) mixture solution was added. As a result, a mixture of amino acid and NH_4Cl was collected after evaporation. In general, the hydrolysis gave quantitative yields of product **37a**. The free amino acids were isolated and collected after lyophilization. The (2*R*,3*R*)-2-amino-3-phenyl-hexanic acid **37b** also was synthesized by using the (*R*)-Ni(II)-complex as a starting material.



32b **13a.** 96% **37a.** 98%

Scheme 2.14 Hydrolysis of alkylated Ni(II)-complex

§ 2.8 Experimental Section

General information

^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 and Bruker DRX-500 MHz NMR spectrometers. The chemical shifts were reported in δ, parts per million (ppm), relative to TMS (δ = 0.00 ppm) as an internal standard. Coupling constants, *J*, were reported in Hertz (Hz) and refer to apparent peak multiplicities and not true coupling constants. Mass spectrometric analyses were conducted by the Mass Spectrometry Facility at the Department of Chemistry of the University of Arizona on a Jeol HX-110A. Optical rotations were measured on a JACSO P1010 polarimeter. THF was distilled from sodium/benzophenone. Dichloromethane was distilled from CaH_2. All the other reagents and solvents, unless otherwise stated, are commercially available and were used as received. Flash column chromatography was performed with 230-400 mesh size silica gel which was purchased from Aldrich Chemical Co. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F_{254}. Melting points

(Mp) are uncorrected and were obtained in open capillaries. All the new compounds were characterized by Mp, $[\alpha]_D$, ^1H, ^{13}C nmr and high resolution mass spectrometry (HRMS), while the known compounds were only characterized by Mp, ^1H and ^{13}C nmr. Unless otherwise stated, all reactions were run under an atmosphere of argon in flame-dried glassware.

***tert*-Butoxycarbonylamino-acetic acid-3-phenyl-allyl ester (3b):** N^{α}-Boc-glycine **1b** (10.0 g, 57.1 mmol) and cinnamyl alcohol **2** (8.05 g, 60.0 mmol) were dissolved in DCM (180 mL) in a 500-mL flask. DCC (12.4 g, 60 mmol) and catalytic amount of DMAP (558 mg, 4.57 mmol) were added at 0°C. Reaction was kept 5 min before warming up to room temperature for overnight reaction. The colorless DCU precipitate was first filtered and the DCM was washed with NH_4Cl, 1*N* NaOH, and brine, successively and finally dried over $MgSO_4$. After the solvent was evaporated, the crude material was purified by flash liquid chromatography to generate 16.3 g (98% yield) colorless liquid product; ^1H (300 MHz, $CDCl_3$), δ 1.45 (9H, s), 3.95 (2H, d, J = 5.7 Hz), 4.80 (2H, dd, J = 1.0, 6.6 Hz), 5.09 (1H, bs), 6.27 (1H, dt, J = 6.6, 15.9 Hz), 6.66 (1H, d, J = 15.9 Hz), 7.26-7.40 (5H, m); ^{13}C (75 MHz, $CDCl_3$), δ 28.2, 42.4, 65.8, 80.0, 122.3, 126.6, 128.2, 128.6, 134.8, 135.9, 155.7, 170.2; HRMS (FAB) MH^+ calcd for $C_{16}H_{22}NO_4$ 292.1594, found 292.1548.

2-*tert*-Butoxycarbonylamino-3-phenyl-pent-4-enoic acid (4b): Diisopropylamine (16.5 mL, 117 mmol) was added to a 30 mL fresh distilled THF in a 250-mL flask. The

solution was cooled down in an ice-water bath before *n*-BuLi in hexane (1.10 M, 96.7 mL) was added slowly. This LDA solution was kept at 0°C for 10 minutes. In another 500-mL flask, ester **3b** was dissolved in distilled THF (80 mL) and $ZnCl_2$ (0.5 M, 106 mL) in THF was added. The solution was then cooled down to –78°C and above prepared LDA was cannulated in 15 min. The reaction was kept at this temperature for 5 minutes before warming up to room temperature in 30 min and was quenched by 1*N* HCl. The THF was then evaporated and the residue was distributed in 800 mL ether and 500 mL NH_4Cl aqueous solution. 1*N* HCl was added to pH = 2. The ether phase was separated and the aqueous phase was extracted by ether (2 x 200 mL). The ether phase was combined and extracted by 1*N* NaOH (3 x 150 mL). The combined basic solution was then acidified with concentrated HCl to pH = 2 again and extracted by EtOAc (3 x 150 mL). After washing with brine, the EtOAc solution was dried over $MgSO_4$ and concentrated by evaporation. The crude material obtained (10.7 g, 98% yield) was purified by flash liquid chromatography (gradient solvent: Hexane : EtOAc = 3 : 1 ~ 1 : 1). The yellowish product then solidified at room temperature slowly and 8.87 g product was obtained (80% yield); Mp: 91-93°C; ^{1}H (300 MHz, $CDCl_3$, rotamer), δ 1.39 (9H, bs), 3.66 (0.3H, brs), 3.86 (0.7H, dd, *J* = 6.6, 7.2 Hz), 4.44 (0.3H, bs), 4.71 (0.7H, dd, *J* = 6.6, 9.0 Hz), 4.87 (0.7H, d, *J* = 9.0 Hz), 5.207 (1H, d, *J* = 12.3 Hz), 5.211 (1H, d, *J* = 15.3 Hz), 5.75 (0.3H, bs), 6.05-6.16 (1H, m), 7.21-7.35 (5H, m), 9.39 (1H, brs), 5.54-5.59 (1H, m), 6.65-6.71 (2H, m), 6.95 (1H, d, *J* = 7.5 Hz), 7.16 (1H, t, *J* = 7.0 Hz); ^{13}C (75 MHz, $CDCl_3$, rotamer), δ 28.2, 51.6(52.6), 57.4(59.3), 80.4(81.6), 117.7, 127.4,

128.1, 128.7, 136.3, 138.5, 155.5, 176.2; HRMS (FAB) MH^+ calcd for $C_{16}H_{22}NO_4$ 292.1549, found 292.1547.

Methyl 2-*tert*-Butoxycarbonylamino-3-phenyl-pent-4-enoate (5): Acid **4b** (5.0 g, 17.2 mmol) was first dissolved in DCM (65 mL) and methanol (2.78 mL, 68.6 mmol). DCC (3.55 g, 17.2 mmol) was added following by DMAP (210 mg, 1.72 mmol). The reaction was kept at room temperature for 5 h before it was stopped by NH_4Cl (150 mL) and extracted with DCM (2 x 50 mL). The DCM solution was washed with brine and dried over $MgSO_4$ before it was concentrated. The colorless product (4.59 g, 88% yield) was obtained after flash column chromatography (hexane : EtOAc = 8 : 1) on silica gel column. Mp: 72-74°C; ^1H (500 MHz, $CDCl_3$), δ 0.87 (9H, s), 2.32 (3H, s), 2.41 (7H, d, J = 5.0 Hz), 2.99 (1H, t, J = 5.0 Hz), 3.09 (1H, d, J = 5.0 Hz), 3.30-3.32 (2H, m), 3.89-3.91 (1H, m); ^{13}C (125 MHz, $CDCl_3$), δ 28.8, 51.9, 52.3, 57.4, 80.0, 117.5, 127.3, 128.1, 128.7, 136.4, 138.8, 155.2, 172.0; HRMS (FAB) MH^+ calcd for $C_{17}H_{24}NO_4$ 306.1705, found 306.1704.

(*S*)-*N*-Benzylproline (BP, 11a): KOH (73.0 g, 1.30 mol) was dissolved in 190 mL *i*-PrOH in a 1000-mL flask. *L*-Proline (50.0 g, 434 mmol) was added and the suspended solution was warmed up to 50°C. BnCl (60 mL, 521 mmol) was premixed with *i*-PrOH (100 mL) in a dropping funnel and the mixture was added to the solution dropwise. The reaction was kept at 50°C and the addition was finished in 4 h and the reaction was kept at this temperature for another 6 h. The reaction was stopped by addition of

concentrated HCl (~100 mL) to pH = 5-6. It was then mixed with DCM (90 mL) and the mixture was allowed to stand overnight. The solution was filtered and the precipitate was washed by DCM. The filtrate was then concentrated and the residue was dissolved in acetone (250 mL). The product precipitated and after standing overnight filtered and washed with cold acetone. The product was dried at room temperature and collected as colorless solid (76.9 g, 86%) and used without further purification. Mp: 205-207°C; ^{1}H (500 MHz, $CDCl_3$), δ 1.90-2.05 (2H, m), 2.24-2.38 (2H, m), 2.94 (1H, q, J = 9.5Hz), 3.67 (1H, ddd, J = 4.0, 7.5, 11.5 Hz), 3.86 (1H, dd, J = 6.5, 9.0 Hz), 4.30 (2H, dd, J_{AB} = 13.0 Hz, J_{AX} = 65.5 Hz), 5.05 (1H, bs), 7.39 (3H, m), 7.46 (2H, dd, J = 3.5, 7.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.0, 28.9, 53.4, 57.8, 67.1, 129.2, 129.5, 130.5, 130.9, 171.2.

***(R)*-*N*-Benzylproline (BP, 11b):** yield 80%, ^{1}H and ^{13}C ($CDCl_3$) spectra are identical to (*S*)-BP (**11a**).

(*S*)-2-[*N*-(*N*'-benzylprolyl)amino]benzophenone (BPB, 13a): (*S*)-*N*-Benzylproline (40 g, 195 mmol) was dissolved in distilled DCM (240 mL) in a 1000-mL flask. 2-aminobenzophenone (34.6 g, 175 mmol), BOP (90.6 g, 205 mmol) was added to the solution before it was cooled to 0°C by an ice-water bath. TEA (81.5 mL, 585 mol) was added and the reaction was allowed to come to room temperature and kept for 40 h. The reaction was quenched by adding saturated aqueous NH_4Cl solution (240 mL). After separation of the organic phase, the aqueous phase was extracted with DCM (2 x 60 mL). The organic phases were combined and washed with brine and dried with anhydrous $MgSO_4$. The solution was concentrated in *vacuo* and the crude sample was

used for the next step without purification. Only a small amount of product was purified by FCC with Hexane-EtOAc (8: 1). Mp: 90-91.5°C; ^{1}H (500 MHz, $CDCl_3$), δ 1.76-1.82 (2H, m), 1.94-1.98 (1H, m), 2.21-2.28 (1H, m), 2.38-2.43 (1H, m), 3.20-3.23 (1H, m), 3.20 (1H, dd, J = 5.0, 10.5 Hz), 3.75 (2H, dd, J_{AB} = 13.0 Hz, J_{AX} = 164.0 Hz), 7.08 (1H, t, J = 7.5 Hz), 7.12-7.15 (3H, m), 7.36-7.38 (2H, m), 7.48-7.55 (4H, m), 7.60 (1H, t, J = 7.5), 7.78 (2H, dd, J = 1.0, 8.5 Hz), 8.56 (1H, d, J = 6.5). ^{13}C (125 MHz, $CDCl_3$), δ 24.1, 30.9, 53.8, 59.8, 68.2, 121.4, 122.1, 125.2, 127.0, 128.1, 128.2, 129.0, 130.0, 132.4, 132.5, 133.3, 138.1, 138.5, 139.1, 174.6, 198.0.

Gly-Ni-(*S*)-BPB (14a): The crude BPB (175 mmol) was dissolved in methanol (360 mL) in a 2000-mL flask together with $NiCl_2 \cdot 6H_2O$ (83.2 g, 350 mmol), glycine (65.7 g, 875 mmol) and KOH (68.6 g, 1.23 mol) were then added. The reaction was kept over 40°C for 2h. The solution was decanted into 3 L of 1% HOAc. It was allowed to stand overnight before filtration and the solid was thoroughly washed with water. The solid was dried completely at room temperature and then was dissolved in $CHCl_3$ and filtered through Celite. The solvent was evaporated and the residue was dissolved in acetone and precipitated in hexane. After filtration and drying at room temperature, a reddish product (56.0 g, 63% from (*S*)-BP) was collected. Mp: 215-217°C; ^{1}H (500 MHz, $CDCl_3$), δ 2.07-2.10 (1H, m), 2.13-2.17 (1H, m), 2.41-2.45 (1H, m), 2.56-2.59 (1H, m), 3.32-3.38 (1H, m), 3.47 (1H, dd, J = 5.5, 10.5 Hz), 3.66-3.80 (4H, m), 4.48 (1H, d, J =12.5 Hz), 6.71 (1H, t, J = 7.5 Hz), 6.80 (1H, d, J = 8.0 Hz), 6.98 (1H, m), 7.10 (1H, d, J = 7.5 Hz), 7.21 (1H, t, J = 7.5 Hz), 7.31 (1H, t, J = 7.0 Hz), 7.43 (2H, t, J = 7.5 Hz),

7.50-7.54 (3H, m), 8.07 (2H, d, J = 7.5 Hz), 8.28 (1H, d, J = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.7, 30.7, 57.5, 61.3, 63.1, 69.9, 120.8, 124.2, 125.2, 125.7, 126.2, 128.9, 129.1, 129.3, 129.6, 129.7, 131.7, 132.2, 133.2, 133.3, 134.6, 142.5, 171.6, 177.3, 181.4.

Gly-Ni-(*R*)-BPB (14b): yield 65%, ^1H and ^{13}C ($CDCl_3$) spectra are identical to Gly-Ni-(*S*)-BPB (**14a**).

Thio-Gly-Ni-(*S*)-BPB (16): Ni(II)-complex **14a** (150 mg, 0.30 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide **15** (Lawesson's reagent, 66.7 mg, 0.165 mmol) were dissolved in benzene (15 mL) in a 50-mL flask. The solution was heated to reflux for 2 hours. After cooling down to room temperature, the solvent was evaporated and product mixture was purified on a silica gel column. A brownish product was obtained (53 mg, 34%). $[\alpha]^{24}_D$ = +816° (*c* 0.336, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.98-2.09 (2H, m), 2.60-2.64 (1H, m), 3.05-3.10 (1H, m), 3.14 (1H, dd, J = 7.0, 10.5 Hz), 3.28 (1H, dd, J = 8.0, 10.0 Hz), 3.42-3.46 (1H, m), 3.73 (1H, d, J = 12.5 Hz), 3.92 (1H, d, J = 19.5 Hz), 4.08 (1H, d, J = 12.5 Hz), 4.12 (1H, d, J = 19.5 Hz), 6.72-6.75 (2H, m), 6.80-6.82 (1H, m), 7.13 (1H, d, J = 5.0 Hz), 7.22 (1H, dt, J = 2.0, 7.5 Hz), 7.28 (1H, t, J = 7.5 Hz), 7.39 (2H, t, J = 7.5 Hz), 7.44-7.49 (3H, m), 7.97 (2H, d, J = 7.5 Hz), 8.13 (1H, d, J = 9.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 22.7, 30.2, 59.6, 64.6, 72.5, 73.4, 120.9, 123.6, 126.1, 126.2, 126.3, 128.9, 129.1, 129.2, 129.4, 129.8, 131,6, 132.3, 132.5, 133.4, 135.5, 142.6, 172.2, 179.0, 206.4; HRMS (FAB) MH^+ calcd for $C_{27}H_{26}N_3NiO_2S$ 514.1099, found 514.1091.

1-Phenyl-3-buten-1-ol (30): In a 500-mL flame-dried flask, 1 *M* allyl magnesium bromide in diethyl ether (100 mL, 100 mmol) was diluted with 140 mL anhydrous ether. The solution was cooled down to 0°C before benzaldehyde (10.1 mL, 100 mmol) was added slowly. It was kept at 0°C for 1 h before being quenched by slowly adding 60 mL 10% of H_2SO_4. The organic phase was then separated and washed by $NaHCO_3$, brine and dried using anhydrous $MgSO_4$. The solution was concentrated in vacuum and the crude material was purified by flash liquid chromatograph. A colorless liquid product was obtained (14.1 g, 95% yield). ^1H (500 MHz, $CDCl_3$), δ 2.12 (1H, s), 2.48-2.52 (2H, m), 4.71 (1H, dd, J = 5.5, 7.5 Hz), 5.11-5.17 (2H, m), 5.75-5.82 (1H, m), 7.24-7.35 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 43.8, 73.3, 118.3, 125.8, 127.5, 128.4, 134.4, 143.8.

1-Bromo-3-butenyl-benzene (31): In a 500-mL flame-dried flask, alcohol **30** (14.1g, 95 mmol) was dissolved in 250 mL anhydrous ether. The solution was then cooled down to 0°C and then PBr_3 (4.75 mL, 150 mmol) was added. The reaction was kept at 0°C until completed in 45 min. It was quenched by 150 mL NH_4Cl. The organic phase was then separated and washed by $NaHCO_3$, brine and dried with $MgSO_4$. The product solution was then concentrated in *vacuo*, and the residue was purified on a silica gel column using hexane. A slight yellowish liquid (11.8 g, 60% yield) was obtained. ^1H (500 MHz, $CDCl_3$), δ 2.95-3.05 (2H, m), 4.96 (1H, t, J = 7.5 Hz), 5.09-5.15 (2H, m), 5.70-5.77 (1H, m), 7.26-7.41 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 44.2, 54.0, 118.1, 127.3, 128.4, 128.7, 134.7, 141.6.

General procedure for alkylation of Ni(II)-complex:

Ni(II)-complex **14a** (1 eq) and ground NaOH (10 eq) were added to a flask which was purged two times with argon. Anhydrous DMF (4 mL/mmol) was added by syringe and the mixture was allowed to react for 5 min at room temperature before bromide **21, 24,** or **25** (0.98 eq) was added in one portion, respectively. The reaction was then kept at room temperature for another 5 min (for bromide **31**, 1-bromo-but-3-enyl-benzene, 3 eq, -30°C, 45 min reaction). The solution was decanted in to an aqueous solution (40 mL/mmol) containing 5% of HOAc. The suspension was extracted with benzene (3 x 20 mL/mmol) and the combined benzene extracts were washed with brine (4 x 40 mL/mmol). The emulsion was diminished by filtration through celite. The solution was dried over anhydrous $MgSO_4$ and concentrated in *vacuo*. The residue was first purified by flash liquid chromatograph, followed by fractional recrystallization in DCM/ether solution.

***(S)*-But-3-enylglycine-Ni-(*S*)-BPB (22b):** 91% yield; Mp: 207-209°C; $[\alpha]^{24}_{D}$ = +4471° (*c* 0.014, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.73 (1H, bs), 2.07-2.12 (1H, m), 2.17-2.21 (1H, m), 2.28-2.31 (1H, m), 2.53-2.58 (1H, m), 2.78 (1H, bs), 3.48-3.57 (2H, m), 3.61 (2H, d, *J* = 12.5 Hz), 3.93 (1H, d, *J* = 6.0 Hz), 4.47 (1H, d, *J* = 12.5 Hz), 4.89 (1H, d, *J* = 10.5 Hz), 4.99 (1H, d, *J* = 17.0 Hz), 5.54-5.59 (1H, m), 6.65-6.71 (2H, m), 6.95 (1H, d, *J* = 7.5 Hz), 7.16 (1H, t, *J* = 7.0 Hz), 7.22 (1H, t, *J* = 7.0), 7.29 (1H, bs), 7.38 (2H, t, *J* = 7.5 Hz), 7.48-7.49 (1H, m), 7.51-7.54 (2H, m), 8.08 (2H, d, *J* = 7.5 Hz), 8.15 (1H, d, *J* = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.7, 29.4, 30.7, 35.0, 57.0, 63.1, 69.9, 70.2, 115.7,

120.7, 123.7, 126.5, 127.3, 127.5, 128.8, 128.89, 128.91, 128.93, 129.7, 131.5, 132.1, 133.2, 136.6, 142.2, 170.4, 179.1, 180.4; HRMS (FAB) MH^+ calcd for $C_{31}H_{31}N_3NiO_3$ 274.0038, found 274.0033.

***(S)*-Allylglycine-Ni-(*S*)-BPB (26b):** 83.5% yield; Mp: 203-205°C; $[\alpha]^{24}_D$ = +1327° (*c* 0.588, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 2.07-2.16 (2H, m), 2.41-2.57 (3H, m), 2.82 (1H, bs), 3.48 (1H, dd, *J* = 6.0, 10.5 Hz), 3.49-3.64 (3H, m), 4.05 (1H, dd, *J* = 4.0, 6.0 Hz), 4.46 (1H, d, *J* = 12.5 Hz), 5.21 (1H, d, *J* = 17.0 Hz), 5.42 (1H, d, *J* = 10.0 Hz), 6.44-6.49 (1H, m Hz), 6.65-6.70 (2H, m), 6.98 (1H, d, *J* = 7.0 Hz), 7.17 (1H, t, *J* = 7.0 Hz), 7.22 (1H, t, *J* = 7.0 Hz), 7.29 (1H, bs), 7.37 (2H, t, *J* = 7.5), 7.49-7.55 (3H, m), 8.07 (2H, d, *J* = 7.0 Hz), 8.20 (1H, d, *J* = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.3, 30.7, 38.4, 56.8, 63.1, 70.3 (2 carbons), 119.7, 120.6, 123.6, 126.4, 127.0, 127.7, 128.79, 128.83, 128.9, 129.7, 131.5, 132.1, 133.1, 133.2, 133.9, 142.4, 170.8, 178.8, 180.3; HRMS (FAB) MH^+ calcd for $C_{30}H_{30}N_3NiO_3$ 538.1641, found 538.1638.

***(R)*-Allylglycine-Ni-(*S*)-BPB (26a):** 12.5% yield; $[\alpha]^{24}_D$ = -545° (*c* 0.545, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.87 (1H, bs), 2.06-2.10 (1H, m), 2.21-2.24 (1H, m), 2.50-2.64 (3H, m), 3.63 (1H, dd, *J* = 4.0, 10.0 Hz), 3.73 (1H, d, *J* = 13.0 Hz), 3.93 (1H, dd, *J* = 3.5, 7.5 Hz), 4.10-4.14 (1H, m), 4.63 (1H, d, *J* = 13.0 Hz), 5.03 (1H, d, *J* = 17.0 Hz), 5.23 (1H, d, *J* = 9.5 Hz), 6.04-6.10 (1H, m), 6.72 (1H, t, *J* = 7.5 Hz), 6.79 (1H, d, *J* = 7.5 Hz), 7.06 (1H, d, *J* = 4.0 Hz), 7.20 (1H, bs), 7.28 (1H, m), 7.44-7.51 (6H, m), 7.93 (2H, d, *J* = 7.0 Hz), 8.5 (1H, d, *J* = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.4, 30.7, 39.3,

58.0, 61.6, 68.9, 70.5, 119.0, 120.7, 123.7, 125.8, 126.9, 128.1, 128.6, 129.0, 129.1, 129.3, 129.7, 131.7, 132.3, 132.5, 133.1, 133.7, 134.2, 142.9, 171.4, 179.1, 182.3; HRMS (FAB) MH^+ calcd for $C_{30}H_{30}N_3NiO_3$ 538.1641, found 538.1644.

***(S)*-Pent-4-enylglycine-Ni-(*S*)-BPB (27b):** 86% yield; Mp: 291-292°C; $[\alpha]^{24}_D$ = +2597° (*c* 0.130, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.65-1.67 (2H, m), 1.91-2.07 (4H, m), 2.14-2.23 (2H, m), 2.50-2.55 (1H, m), 2.76 (1H, bs), 3.47 (1H, dd, *J* = 6.0, 10.0 Hz), 3.52-3.60 (3H, m), 3.91 (1H, d, *J* = 5.0 Hz), 4.44 (1H, d, *J* = 12.5 Hz), 4.95-5.00 (2H, m), 5.70-5.75 (1H, m), 6.62-6.67 (2H, m), 6.92 (1H, d, *J* = 6.5 Hz), 7.14 (1H, t, *J* = 7.0 Hz), 7.19 (1H, t, *J* = 7.0), 7.27 (1H, bs), 7.34 (2H, t, *J* = 7.0 Hz), 7.45-7.50 (3H, m), 8.05 (2H, d, *J* = 7.0 Hz), 8.13 (1H, d, *J* = 8.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.6, 24.6, 30.7, 33.2, 34.8, 56.9, 63.0, 70.2, 70.3, 115.2, 120.7, 123.6, 126.5, 127.1, 127.6, 128.82, 128.85, 128.87, 129.6, 131.5, 132.1, 133.15, 133.17, 136.8,, 137.7, 142.2, 170.3, 179.3, 180.3; HRMS (FAB) MH^+ calcd for $C_{32}H_{34}N_3NiO_3$ 566.1954, found 566.1954.

***(R)*-pent-4-enylglycine-Ni-(*S*)-BPB (27a):** 9% yield; $[\alpha]^{24}_D$ = -692° (*c* 0.364, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.50-1.52 (2H, m), 1.84-1.91 (4H, m), 1.98-1.20 (1H, m), 2.12-2.18 (1H, m), 2.22-2.24 (1H, m), 2.52-2.58 (1H, m), 2.64-2.69(1H, m), 3.59 (1H, d, *J* = 13.0 Hz), 3.67 (1H, d, *J* = 6.0 Hz), 3.79 (1H, d, *J* = 6.5 Hz), 4.21 (1H, t, *J* = 5.0 Hz), 4.48 (1H, d, *J* = 13.0 Hz), 4.93-4.97 (2H, m), 5.63-5.69 (1H, m), 6.72 (1H, t, *J* = 7.5 Hz), 6.76 (1H, d, *J* = 8.0 Hz), 7.00 (1H, d, *J* = 6.5), 7.19 (1H, bs), 7.26 (1H, bs), 7.45-7.50 (6H, m), 7.99 (2H, d, *J* = 7.0 Hz), 8.50 (1H, d, *J* = 9.0 Hz); ^{13}C (125 MHz,

$CDCl_3$), δ 23.3, 24.6, 30.5, 33.2, 35.4, 58.6, 61.5, 69.1, 70.4, 115.1, 120.7, 123.7, 125.9, 126.9, 127.9, 128.6, 129.0, 129.1, 129.6, 131.6, 132.4, 133.5, 133.7, 134.1, 137.9, 142.8, 170.8, 179.7, 182.3; HRMS (FAB) MH^+ calcd for $C_{32}H_{34}N_3NiO_3$ 566.1954, found 566.1954.

(2*S*,3*S*)-(1-Phenyl)-3-butenyl-glycine-Ni(II)-(*S*)-BPB (32b): 86% yield; Mp: 135-137°C; $[\alpha]^{24}_D$ = +2183° (*c* 0.033, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.37-1.45 (1H, m), 1.68-1.76 (1H, m), 1.95 (1H, dt, *J* = 7.0, 11.0 Hz), 2.12-2.23 (2H, m), 2.79 (1H, dt, *J* = 6.0, 11.5 Hz), 3.24 (1H, t, *J* = 8.5 Hz), 3.38 (1H, d, *J* = 12.5 Hz), 4.21 (1H, d, *J* = 12.5 Hz), 4.28 (1H, d, *J* = 3.0 Hz), 4.63 (1H, dd, *J* = 3.0, 9.0 Hz), 4.75-4.86 (1H, m), 4.82 (1H, d, *J* = 3.0 Hz), 6.64-6.69 (2H, m), 7.08 (1H, t, *J* = 7.5 Hz), 7.13 (2H, t, *J* = 13.5 Hz), 7.27 (2H, t, *J* = 7.5 Hz), 7.31 (1H, d, *J* = 7.0 Hz), 7.38 (2H, d, *J* = 7.0 Hz), 7.45-7.60 (6H, m), 8.00 (2H, d, *J* = 7.5 Hz), 8.25 (1H, d, *J* = 9.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.0, 30.7, 36.3, 50.2, 57.4, 63.6, 70.4, 73.2, 117.3, 120.4, 123.1, 126.1, 127.6, 127.7, 128.2, 128.65, 128.71, 128.91, 128.93, 129.3, 129.7, 129.9, 131.5, 132.3, 133.3, 133.5, 134.4, 135.0, 139.9, 143.0, 170.9, 177.4, 180.4; HRMS (FAB) MH^+ calcd for $C_{37}H_{35}N_3NiO_3$ 628.2110, found 628.2122.

(2*R*,3*R*)-(1-Phenyl)-3-butenyl-glycine-Ni(II)-(*S*)-BPB (32a): 7% yield; Mp: 187-189°C; $[\alpha]^{24}_D$ = -2030° (*c* 0.024, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.14-1.18 (1H, m), 1.31-1.35 (1H, m), 1.80-1.84 (1H, m), 2.04-2.10 (1H, m), 2.38-2.42 (1H, m), 2.48-2.56 (2H, m), 2.81 (1H, bs), 3.32-3.34 (1H, m), 3.37 (2H, AB, *J* = 14.0, 35.5 Hz), 3.79 (1H,

m), 4.29 (1H, d, J = 2.5 Hz), 4.63-4.65 (1H, m), 4.85-4.95 (1H, m), 4.88 (1H, d, J = 3.5 Hz), 6.76 (1H, t, J = 7.5 Hz), 6.82 (1H, d, J = 7.5 Hz), 7.13 (1H, d, J = 6.0 Hz), 7.17 (1H, d, J = 5.0 Hz), 7.28-7.34 (5H, m), 7.50 (1H, t, J = 7.0 Hz), 7.53-7.60 (7H, m), 8.44 (1H, d, J = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.7, 31.4, 35.5, 50.9, 55.0, 59.2, 68.6, 73.5, 117.4, 120.7, 123.5, 126.4, 127.6, 127.9, 128.2, 128.66, 128.71, 128.90, 128.93, 129.3, 129.8, 130.5, 131.8, 131.9, 132.6, 133.8, 134.3, 134.9, 141.1, 143.2, 170.7, 176.9, 181.8; HRMS (FAB) MH^+ calcd for $C_{37}H_{35}N_3NiO_3$ 628.2110, found 628.2119.

(2*S*,3*R*)-(1-Phenyl)-3-butenyl-glycine-Ni(II)-(*S*)-BPB (32c): The above purified (2R, 3R)-product (170 mg, 0.271 mmol) and ground NaOH (120 mg, 3.0 mmol) were added to a 10-mL flask. The flask was purged with argon two times and anhydrous DMF (2 mL) was added by syringe. The mixture was kept at room temperature for 24 h before it was quenched by an aqueous acidic solution (20 mL, 5% HOAc). The product mixture was treated as in the general procedure of Ni(II)-complex alkylation, and the product was isolated by flash chromatograph. $[\alpha]^{24}_D$ = +1565° (*c* 0.061, $CHCl_3$); ^{1}H (500 MHz, $CDCl_3$), δ 2.10-2.21 (1H, m), 2.61-2.67 (1H, m), 2.81-2.87 (1H, m), 2.90-2.95 (1H, m), 3.46-3.53 (3H, m), 3.57 (1H, d, J = 12.5 Hz), 3.83-3.92 (1H, m), 4.10 (1H, d, J = 6.0 Hz), 4.45 (1H, d, J = 12.0 Hz), 4.94 (1H, dd, J = 1.0, 10.0 Hz), 5.09 (1H, dd, J = 1.0, 17.0 Hz), 5.54-5.61 (1H, m), 6.17 (1H, d, J = 8.0 Hz), 6.53 (1H, dd, J = 1.5, 8.5 Hz), 6.63 (1H, t, J = 7.5 Hz), 6.72 (2H, d, J = 7.0 Hz), 7..06-7.24 (7H, m), 7.30 (2H, t, J = 7.5 Hz), 7.43-7.46 (2H, m), 8.05 (2H, d, J = 7.0 Hz), 8.21 (1H, d, J = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.4, 30.9, 34.8, 52.1, 56.9, 63.0, 70.7, 75.7, 117.1, 120.6, 123.1,

126.5, 127.0, 127.5, 128.3, 128.6, 128.71, 128.79, 128.81, 128.84, 128.88, 129.5, 131.5, 132.4, 133.1, 133.6, 134.7, 135.7, 138.2, 142.6, 170.7, 177.0, 180.1; HRMS (FAB) MH^+ calcd for $C_{37}H_{35}N_3NiO_3$ 628.2110, found 628.2114.

(*S*)-Benzylgly-Ni-(*S*)-BPB (36), $[\alpha]^{24}_D$ = +1807° (*c* 0.582, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.66 (1H, bs), 1.91-1.95 (1H, m), 2.31-2.32 (3H, m), 2.85 (1H, dd, *J* = 5.5, 14.0 Hz), 3.06-3.12 (2H, m), 3.28-3.31 (1H, m), 3.46 (1H, d, *J* = 12.5 Hz), 4.27-4.30 (2H, m), 6.66 (2H, d, *J* = 3.5 Hz), 6.84 (1H, d, *J* = 7.5 Hz), 7.14-7.20 (4H, m), 7.28-7.31 (3H, m), 7.39-7.43 (4H, m), 7.50-7.56 (2H, m), 8.00 (2H, d, *J* = 2.0, 7.5 Hz), 8.24 (1H, d, *J* = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 23.1, 30.7, 39.7, 57.2, 63.3, 70.3, 71.5, 120.6, 123.4, 126.1, 127.2, 127.4, 127.9, 128.8, 128.9, 129.1, 129.7, 130.6, 131, 5, 132.4, 133.2, 133.5, 134.2, 135.9, 142.9, 171.2, 178.6, 180.3; HRMS (FAB) MH^+ calcd for $C_{34}H_{32}N_3NiO_3$ 588.1797, found 588.1789.

General procedure for hydrolysis of alkylation product of Ni(II)-complex:

Ni(II)-alkylated product (**22b, 26b, 27b,** and **32b**)(1 eq) was dissolved in methanol (3 mL/mmol) and added dropwise into a mixture of HCl (3 *N*, 2 mL/mmol) and methanol (2 mL/mmol) solution at 60°C. The solution turned green (for β-phenyl-substituted product **32b**, 6 *N* HCl at 60°C for 1 h). The methanol-water solution was evaporated and the residue was re-dissolved in water (3 x 5 mL/mmol) and evaporated to remove the HCl. NH_4OH (5 mL/mmol) and water (5 mL/mmol) was added and the mixture was concentrated in *vacuo* to dryness. The residue was then dissolved in water (5 mL/mmol)

and $CHCl_3$ (5 mL/mmol). The organic phase was separated and the water phase was washed with $CHCl_3$ (2 x 5 mL/mmol). The combined organic phase was washed with brine and dried over $MgSO_4$, and then concentrated in *vacuo*. (*S*)-BPB **13a** (about 96%) was recovered. The aqueous phase was evaporated to 10 mL and loaded on an ion-exchange column (DOWEX 50Wx2-100 resin) which was pre-washed with water to neutral pH. The column was eluted by water to pH = 7 and then washed with ammonium hydroxide/water (4/1) until all the amino acid was washed out. The column can be regenerated by 1*N* HCl. The aqueous solution collected from the column was concentrated and the colorless amino acid was collected.

(*S*)-2-Amino-5-hexenoic acid (23a): 96% yield, Mp: >270°C; $[\alpha]^{24}_D$ = +13.1° (*c* 1.30, H_2O); ^1H (500 MHz, DMSO), δ 1.83-1.87 (2H, m), 2.02-2.07 (1H, m), 2.14-2.19 (1H, m), 3.80 (1H, t, *J* = 6.0 Hz), 4.96 (1H, d, *J* = 10.5 Hz), 5.03 (1H, d, *J* = 17.5 Hz), 5.71-5.77 (1H, m); ^{13}C (125 MHz, DMSO), δ 28.7, 29.2, 51.5, 116.3, 137.1, 170.8; HRMS (FAB) MH^+ calcd for $C_6H_{11}NO_2$ 130.0868, found 130.0870.

(*S*)-2-Allylglycine (28a): 96% yield, Mp: >275°C (decomp.); $[\alpha]^{24}_D$ = +21.1° (*c* 1.53, H_2O); ^1H (500 MHz, DMSO), δ 2.46-2.57 (2H, m), 3.69 (1H, t, *J* = 5.5 Hz), 5.13-5.18 (2H, m), 5.62-5.70 (1H, m); ^{13}C (125 MHz, DMSO), δ 35.2, 54.3, 120.8, 131.7, 174.4; HRMS (FAB) MH^+ calcd for $C_5H_{10}NO_2$ 116.0712, found 116.0717.

(*S*)-2-Amino-6-heptenoic acid (29a): 96% yield, Mp: 225°C (decomp); $[\alpha]^{24}_{D}$ = +10.0° (*c* 1.02, H_2O); ^1H (500 MHz, H_2O), δ 1.37-1.42 (2H, m), 1.73-1.80 (2H, m), 2.00-2.04 (2H, q, *J* = 7.0 Hz), 3.64 (1H, t, *J* = 5.5 Hz), 4.93 (1H, d, *J* = 10.0 Hz), 4.98 (1H, d, *J* = 17.0 Hz), 5.76-5.80 (1H, m); ^{13}C (125 MHz, H_2O), δ 23.9, 30.2, 32.9, 55.1, 115.4, 139.0, 175.3; HRMS (FAB) MH^+ calcd for $C_7H_{14}NO_2$ 144.1025, found 144.1021.

(2*S*, 3*S*)-2-Amino-3-phenyl-hexenoic acid (37a): 98% yield, Mp: 132-135°C; $[\alpha]^{24}_{D}$ = +2.98° (*c* 4.3, H_2O); ^1H (500 MHz, D_2O), δ 2.61-2.74 (2H, m), 3.34 (1H, dt, *J* = 5.0, 10.0 Hz), 4.17 (1H, d, *J* = 5.0 Hz), 4.95 (1H, d, *J* = 10.0 Hz), 5.06 (1H, d, *J* = 17.0 Hz), 5.61-5.68 (1H, m), 7.26 (2H, d, *J* = 7.0 Hz), 7.32 (1H, t, *J* = 7.0 Hz), 7.37 (2H, t, *J* = 7.0 Hz); ^{13}C (125 MHz, D_2O), δ 34.2, 45.7, 57.8, 117.6, 128.2, 128.6, 129.1, 135.3, 136.8, 171.1; HRMS (FAB) MH^+ calcd for $C_{12}H_{15}NO_2$ 206.1181, found 206.1184.

CHAPTER III.

SYNTHESIS OF *N,S*-ACETAL [5,5]-BICYCLIC β-TURN DIPEPTIDE

§ 3.1 Introduction

[5,5]-bicyclic dipeptide was a nice mimetic to type-II β-turn.[97] The development of *N,S*-acetal γ-lactam bicyclic dipeptide was first introduced by Nagai and Sato.[98,99] In their strategy, aspartic acid derivatives were used as starting material. Another similar strategy also was reported.[100] Although the syntheses of these [5,5]-bicyclic dipeptide scaffolds have been developed,[101] these methods did not have an efficient way to introduce different side chain groups on both rings stereospecifically.

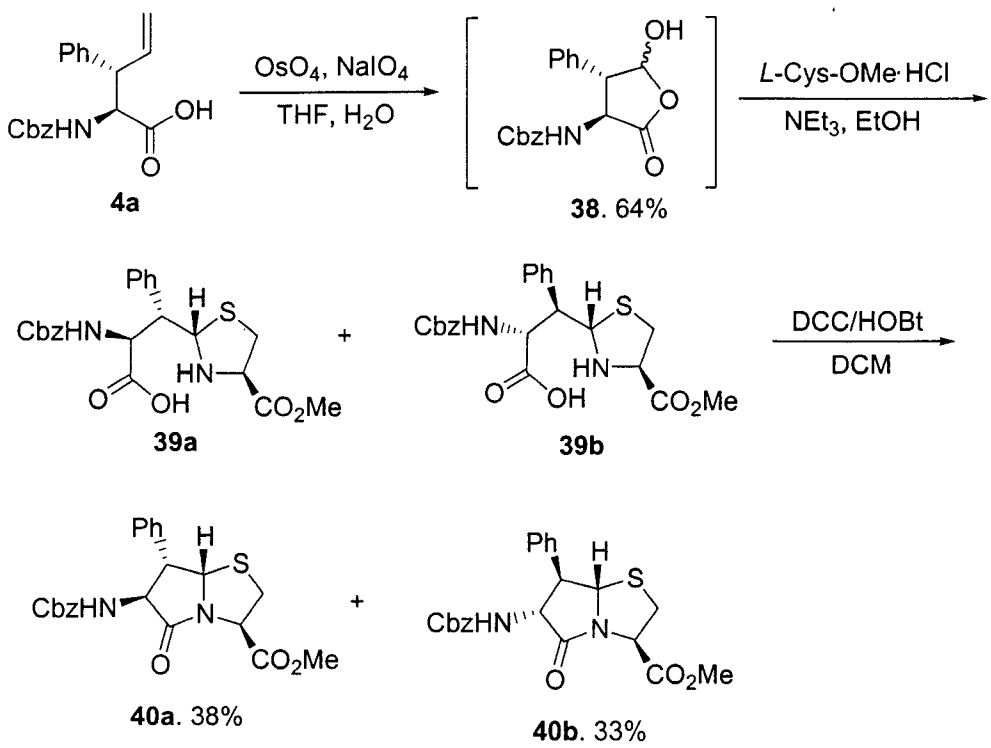
It is well established that side chain groups are extremely important in peptide-receptor interactions.[9,102,103] Though a few successes have been reported in obtaining mimetics which can stabilize β-turns, very little success has been obtained in incorporating such mimetics into target peptides due to the lack of appropriately positioned side chain groups. In the event of molecular recognition, the peptide backbone only serves as a scaffold for the key side chain groups involved in the interaction. To successfully design peptidomimetics, the essential amino acid side chain groups of the corresponding peptide pharmacophore need to be positioned on the alternative scaffold such that the spatial orientation of these critical side chain groups correspond to these in the bioactive conformation of the peptide.[8,15] The side chain

groups' 3D architecture (topography) and stereoelectronic properties provide the critical complementary shape and chemical properties that favors efficient molecular recognition. Therefore the development of a synthetic strategy requires stereocontrolled introduction of a minimum of four asymmetric centers, with different backbone geometry and side chain topography (Scheme 1.6, Chapter **I**).

§ 3.2 Methodology for side-chain substituted [5,5]-bicyclic β-turn mimetics

3.2.1 *N.S*-acetal γ-lactam bicyclic dipeptides

This methodology was developed by Wei Qui in which he took advantage of the Kazmaier-Claisen rearrangement for the synthesis of β-substituted γ,δ-unsaturated amino acids. The β-vinylphenylalanine **4a** thus was synthesized in two steps from commercially available starting materials (Scheme 2.1, Chapter **II**). Osmylation and diol oxidation cleavage gave the γ-hydroxy-γ-lactone **38** (Scheme 3.1). Although intermediate **38** was not purified before further reactions, the existence of this structure was confirmed by transferring **38** into its mono-methyl and tri-methyl derivatives. [69] Condensation of **38** with *L*-cysteine methyl ester in EtOH gave a mixture of monocylic dipeptide **39a** and **39b,** which were cyclized with DCC/HOBt in dichloromethane without further purification. Compounds **40a** and **40b** were separated by flash chromatography. Their stereochemistry was assigned by 1D transient nOe experiments and finally **40b** was confirmed by X-ray diffraction analysis. [69] In this methodology, we had incorporated a phenyl substituent for the first time at the C-3 position on a [5,5]-bicyclic dipeptide.

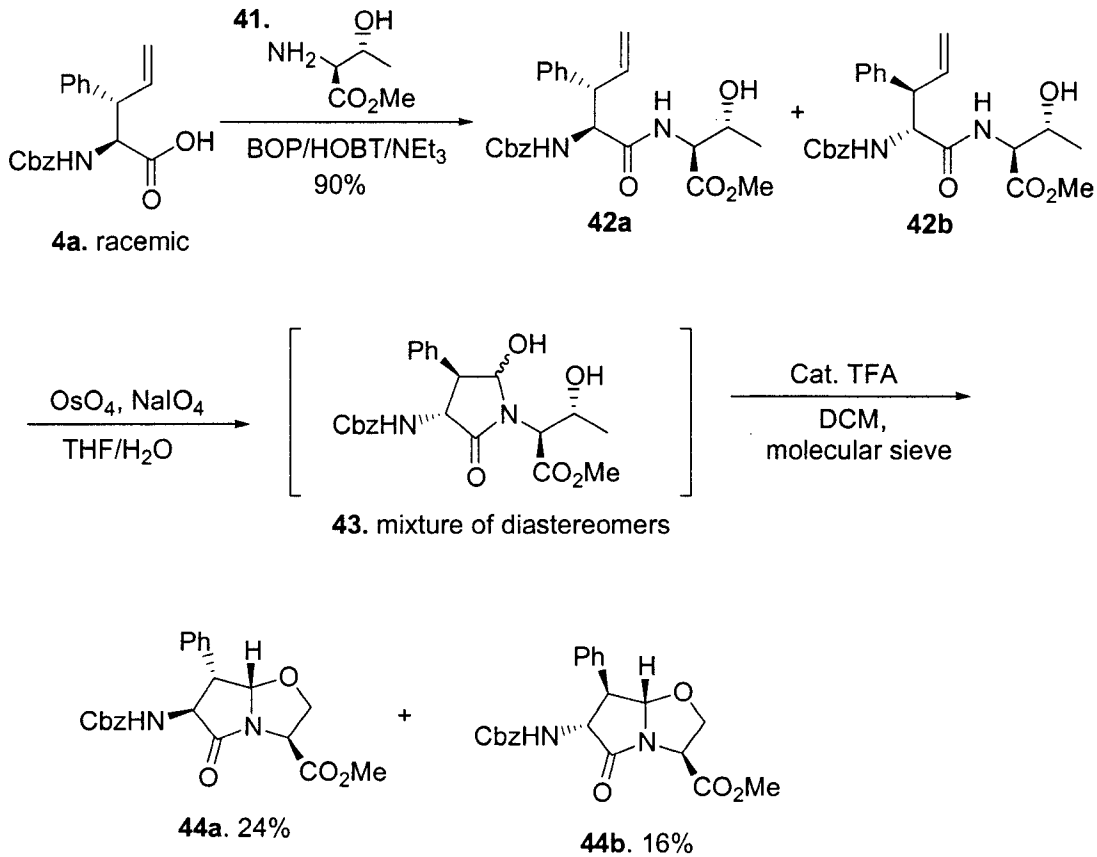


Scheme 3.1 The methodology to synthesize [5,5]-bicyclic dipeptide

3.2.2 *N,O*-acetal [5,5]-bicyclic dipeptide

The synthesis of *N,O*-acetal γ,δ-lactam bicyclic dipeptide also has been reported.[100,104-106] This is a method we tried to introduce a side chain at i+3 residue. The advantage of this method is the use of β-hydroxyl-aminoacids, which are readily available by developed methods.[107-118] The Dipeptide can be coupled by BOP and a pair of diastereomers **42a** and **42b** were formed. Oxidative cleavage gave a mixture of γ-hydroxyl lactams **43**, which were cyclized in dry DCM with a catalytic amount of TFA and 3 Å molecular sieves to give compounds **44a** and **44b**. The formation of the

oxazolidine ring in 3% TFA was tricky and always gave low yield (Scheme 3.2). The reaction was not proceeding very well and the β-elimination product was isolated if TFA was added more than 5%. This method was further investigated by John Ndungu and finally was abandoned due to the difficulty in repeating the literature results.



Scheme 3.2 Synthesis of *N,O*-acetal [5,5]-bicyclic dipeptide

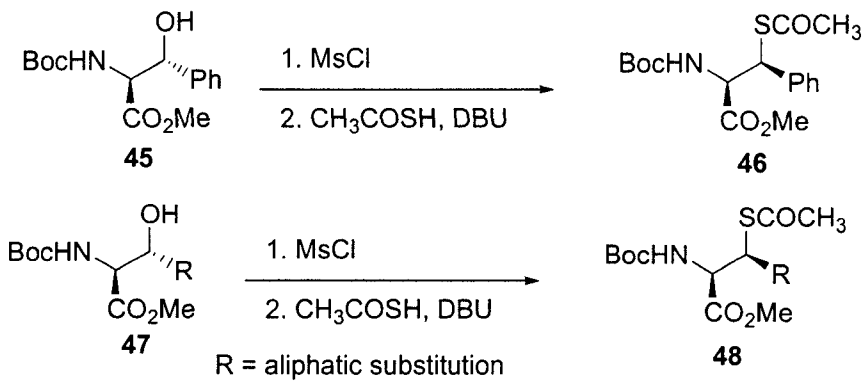
§ 3.3 Synthesis of β-substituted cysteine derivatives

3.3.1 The concept of conversion

For [5,5]-bicyclic β-turn dipeptide synthesis, we already have discussed the importance of introducing side chains functionality. Since we had difficulty developing a *N,O*-acetal bicyclic dipeptide synthesis, it was necessary for us to develop a practical and efficient synthetic method to generate β-substituted cysteine derivatives. β-Substituted cysteine, or β-thio-α-amino acid, not only provides a side chain group at i+3 position, but also provides another chiral center (β-chiral position). β-Substituted cysteine is a building block for disulfide bond formation in cyclic peptides, which in general, is a key contributor to the secondary structures in peptides and proteins.[119,120] Our group has already successfully developed asymmetric syntheses of β-aromatic and aliphatic substituted cysteines by using Sharpless asymmetric epoxidation.[32,33] As a starting point, I am continually working on this project and trying to develop a method which is short enough so that it becomes routine to produce cysteine derivatives on a multi gram scale. This method also must be universal to generate all the four diatereromers which is important if we are systematically studying the conformational and topographical interactions between peptide ligands and receptors.

Although the method to synthesize β-substituted cysteine was very limited, the synthesis of β-hydroxyl amino acid has been accomplished using Ni(II)-complexes.[107-109] It has been reported that β-hydroxyl phenylalanine can be used as starting material to synthesize the β-thio-phenylalanine.[121] So I was thinking if this method actually could be modified and extended to aliphatic amino acids so that all the β-hydroxyl amino

acids could become the precursors of β-thio-amino acids. The concept can be simply shown in Scheme 3.3. Obviously with β-aromatic substituted serine **45**, the β-position is the benzylic position and nucleophilic substitution was relatively easy. In β-aliphatic substituted serine **47** cases, however, this reaction could cause a β-elimination and intramolecular elimination of mesylate in a strong base. Nevertheless, I thought it was worth to try.

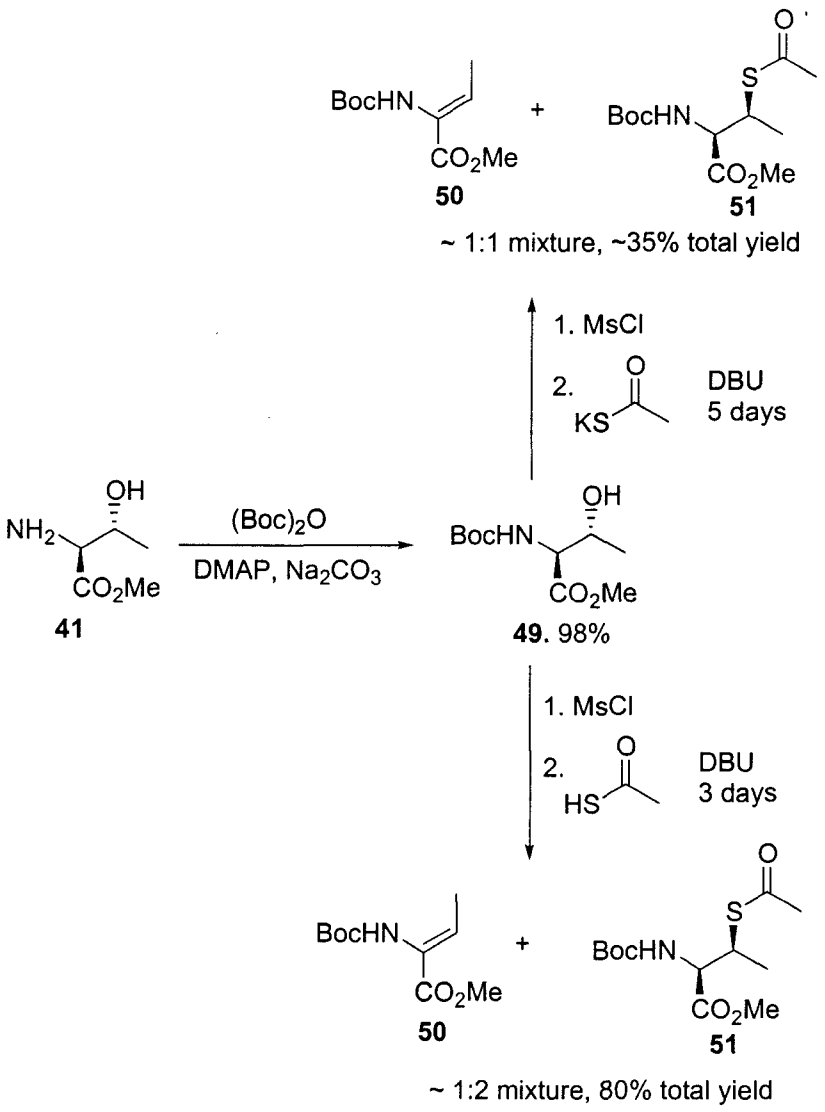


Scheme 3.3 Concept of β-substituted cysteine synthesis from the β-substituted serine

3.3.2 The synthesis and its problems

The investigation of this possibility was started by using threonine, the only commercially available amino acid with β-aliphatic substitution. The reported method was found by transforming hydroxyl to its tosylate and then substituted by thioacetic acid.[122-124] The thio ester was then hydrolyzed in basic solution and the thio was reprotected by BnBr before it was used in peptide synthesis. In our synthesis, threonine was first protected by Boc in 98% yield (Scheme 3.4). The mesylation can be completed

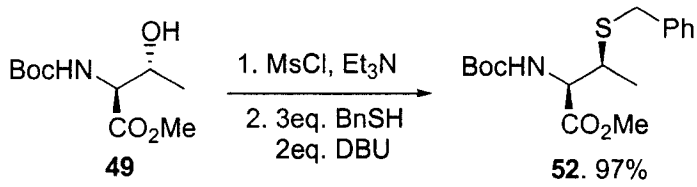
in quantitative yield and purification is not required. The nucleophilic substitution by different reagents turned out to be a problem. This nucleophilic substitution was not complete in our case in 3 and 5 days, respectively, and the products always was contaminated by β-elimination by-product **50** (Scheme 3.4). The basicity of DBU is strong enough to deprotonate the α-H with the help of the β-mesylate elimination. Only the *Z* isomer **50** of this byproduct was isolated, and the stereochemistry was assigned according to the E_2 (not E_{1cb}) mechanism of this reaction. Obviously, nucleophilic substitution was too slow and the β-elimination competed at the same rate. In order to improve this reaction, one of my ideas was to use a better leaving group. $T_{f2}O$ was tried and the triflate formation was as good as mesylation. However, the following S_N2 substitution was messy and only elimination product was isolated in very low yield.



Scheme 3.4 The mesylation of β-hydroxy-amino acids and S_N2 substitution

Another strategy trying to solve the problem was to use a better nucleophile. The mesylate could be substituted by benzyl thiol derivatives in an S_N2 manner. Benzyl mercaptan was first chosen and the reaction completed in 70 min at 0°C after

optimization (Scheme 3.5). The elimination was prevented by using benzyl mercaptans (3 equivalents) in excess and in relatively concentrated DMF solution (> 0.1mol). 2 eq of DBU was used and pre-mixed with benzyl mercaptan in a vial and cool down to 0°C before this mixture was added in one portion into the reaction system. Otherwise, contamination by the elimination byproducts will be present, which was always hard to purify at this stage by flash liquid chromatograph. The excess of benzyl mercaptan can be recovered and be re-used after column purification.



Scheme 3.5 The strategy to synthesize of β-thio-amino acid from threonine

This nucleophilic substitution was further investigated by using different mercaptans (**53**) as nucleophiles under the same reaction conditions as above. The results are summarized in Table 3.1. The reaction results were totally dependent on the structure of these nucleophiles. Acetyl thioacid **53a** and its potassium salt **53b** were not good enough for nucleophilic substitution due to the electron resonance properties on the sulphur. The trimethyl thiol **53f** was bulky under the same reaction conditions so that the elimination product became the major one. The more hindered triphenylmethylthiol **53g** did not have any nucleophilicity in this condition and only elimination product was obtained.

Scheme 3.6 Different nucleophiles in S_N2 substitution

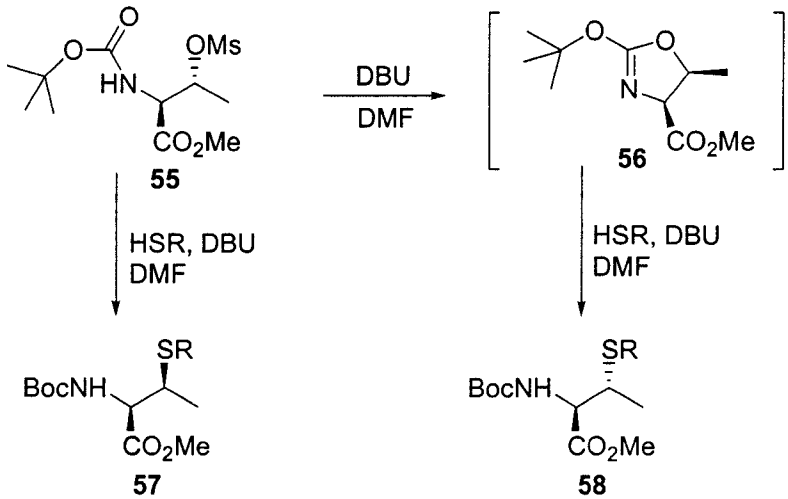
Table 3.1 The nucleophiles and product isomer ratios

(50)	Mercaptan Substrate (**53**)	Product (**54**) Yield [#]		Elimination Byproduct yield[#]
a	$HSCOCH_3$	53%		27%
b	$KSCOCH_3$	~20%		~15%
c	$HSCH_2Ph$	89%	8%	0%
d	$HSCH_2Ph$ OMe-p	87%	10%	0%
e	HSPh	24%	13%	0%
f	$HSC(Me)_3$	9%		60%
g	$HSC(Ph)_3$	0%		80%

[#] All the yield and isomer ratio did not optimized for each specific nucleophile except **53c**

When benzyl thiol **53e** was used as a nucleophile, the products became a mixture of diastereomers which can be separated by silica gel column. It was at this point I noticed that a side-reaction was happened in this reaction and byproduct of diastereomer was generated. The NMR of the diastereomer mixture was mis-interpreted by thinking it was a rotamer. However, careful characterization of the amino acids **54** showed this contamination was around 8-14% when benzylmercaptan and *p*-methoxybenzyl-

mercaptan were used. The diastereomeric mixture could not be purified on silica gel column. This side reaction became serious when phenylmercaptan **53e** was used due to its weaker nucleophilicity. This side reaction could be explained by intramolecular neighboring group participation (Scheme 3.7).



Scheme 3.7 The inter *vs* intra molecular substitution and their products

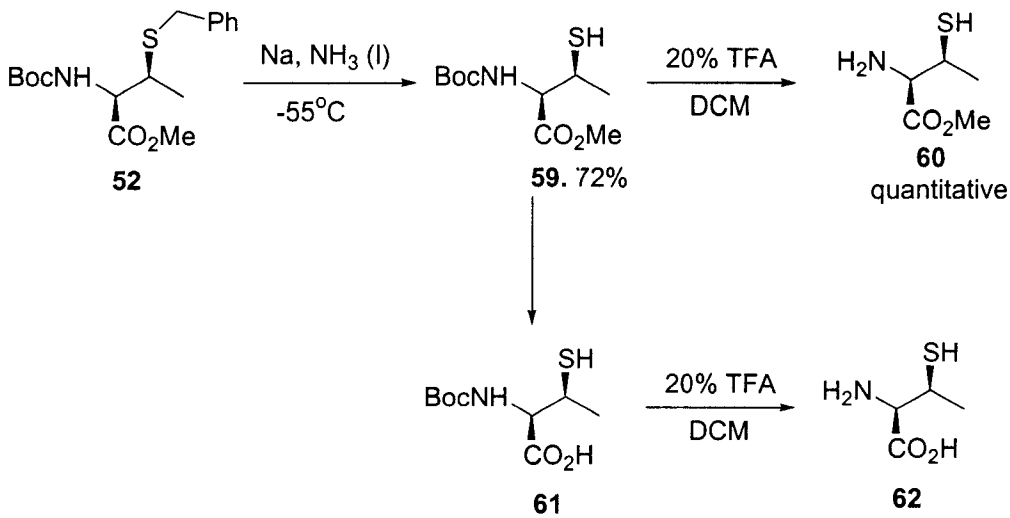
In this reaction, DBU might be strong enough to partly deprotonate the proton on nitrogen which causes 5-membered ring formation by intramolecular substitution (Scheme 3.7). The byproduct **58** could be obtained when mercaptan nucleophile re-attacked the intermediate **56** at β-position, although intermediate **56** was not isolated. Because of this double flip in the reaction, product **58** is a diastereoisomer of product **57**. The stereochemistry of the major product **57** was assigned according to the literature and finally confirmed by nOe on the [6,5]-bicylic dipeptide (Figure 4.3, Chapter **IV**).[123] The possibility of obtaining **58** *via* a Michael addition was eliminated by using isolated

elimination product **50** as a starting material and subjecting it to the same reaction condition with benzyl mercaptan. The Michael addition was found to be very slow and the product did not obviously form in 6 hour at 0°C.

3.3.3 The generation of free β-substituted amino acid

For our peptidomimetics, a free mercaptan and a free amino terminal are required. Deprotection of benzyl thioether was optimized by sodium in liquid ammonia.[125,126] It turns out that 2.8 eq of sodium was the best amount, and that a yield of over 70% can be obtained. Since substrate **52** was hard to transfer, a small amount of distilled THF was used to dissolve **52** before it was added to liquid ammonia in one portion (0.5 mL THF/100 mg **50**). Before adding **52**, a small piece of sodium (3-5 mg) was added to make sure the liquid ammonia was dry (blue point reached). Elimination product **50** was always formed (~10%) during the deprotection, but purification was easily accomplished by flash liquid chromatograph. To compare the reductive cleavage by sodium, Li (16 eq) was used in the presence of 4 eq of EtOH.[127] Compared to sodium, $Li/NH_3(l)$ was easy to handle but the reaction was slow. According to the literature conditions, the reaction actually had to keep the blue point 5 min before being quenched by solid NH_4Cl. The isolated product structure was hard to prove, but the N^{α}-Boc was still present, while both the benzyl ether and methyl ester were deprotected according to its ^{1}H and ^{13}C nmr. Finally the deprotection of N^{α}-Boc group was accomplished by 20% TFA at room temperature in 30 min, and the amino acid **60** was used in bicyclic formation without further purification (Scheme 3.8). In the [6,5]-bicyclic dipeptide, the

required cysteine derivatives **62** should have a free acid, free amino terminal, and free mercaptan. Thus the methyl ester was hydrolyzed in basic solution and the N^{α}-Boc was deprotected by TFA.



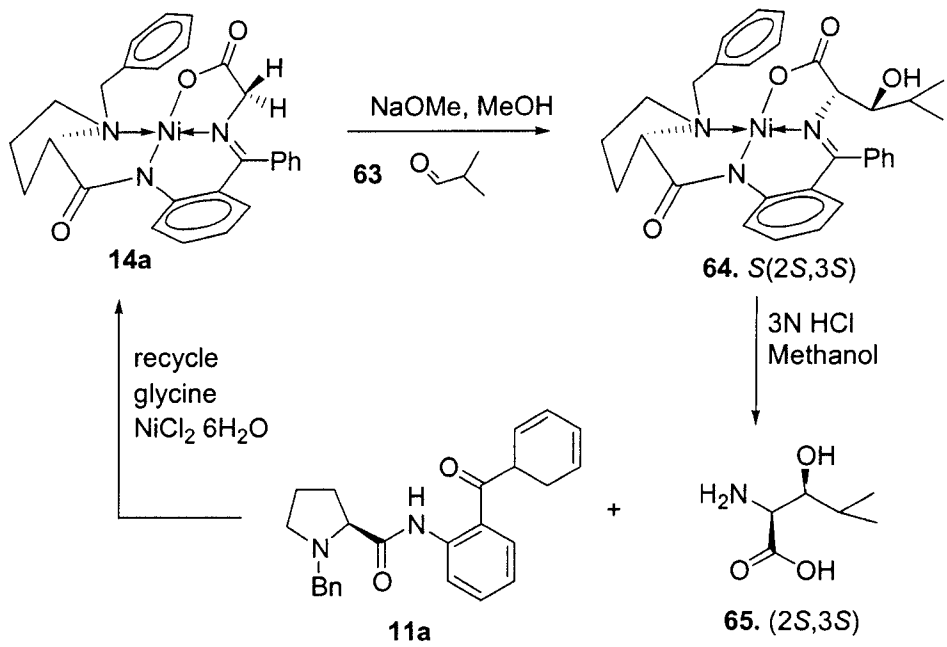
Scheme 3.8 The deprotection of benzyl, Boc, and methyl ester

By using *p*-methoxybenzyl mercaptan (an even better nucleophile), the reaction was completed within 45 min (Scheme 3.6). Although the total yield is about 97%, the reaction was not as clean as the benzyl mercaptan and the product was a diastereomer mixture. The excess of *p*-methoxybenzyl mercaptan was partially oxidized during the reaction, yet it mainly could be recovered. The advantage of using *p*-methoxybenzyl mercaptan is the deprotection by refluxing TFA instead of using sodium in liquid ammonia. The TFA cleavage works efficiently in presence of a scavenger.[128] Other mild

reaction condition to cleave this methoxybenzyl thioester such as $Hg(OAc)_2$ and gas H_2S also was reported to be very efficient in peptide synthesis.[129]

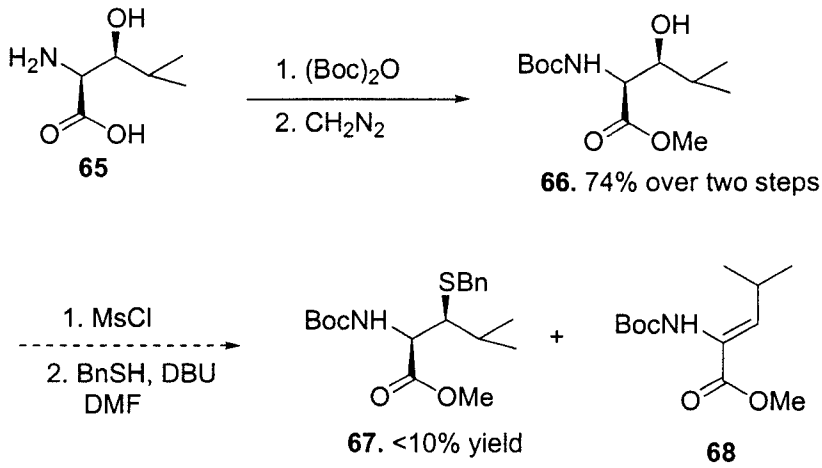
3.3.4 The synthesis of β-hydroxyl and β-thia-leucine

We have demonstrated that a β-substituted cysteine can be obtained from a β-hydroxyl amino acid (Scheme 3.6). The generation of these β-hydroxyl amino acid precursors by using a Ni(II)-complex was a two step strategy.[108] This efficient strategy was illustrated by enantiomeric synthesis of β-hydroxyl leucine (Scheme 3.9). The kinetic product **64,** *S*(2*S*,3*S*), can be generated by aldol reaction on Ni(II)-complex using 1 eq of aldehyde **63** and 2 eq of NaOMe. The reaction should be stopped within 3 min. Otherwise, the major product would be the thermodynamic one, a diastereomer with *S*(2*R*,3*R*) configuration after the reaction was quenched by 5% aqueous acetic acid solution. An acid (kinetic product) *via* an alcohol (thermodynamic product) equilibrium in Ni(II)-complex was discussed in literature.[108] The purification of diastereomeric product **64** was improved by fractional recrystallization in DCM and ether (TLC was used to monitor each step of the recrystallization). ^{1}H NMR spectroscopy show only one isomer before it was hydrolyzed. (Scheme 3.9).



Scheme 3.9 The aldol reaction of Ni(II)-complex and hydrolysis

In our modified strategy in Scheme 3.9, multi gram quantities of β-hydroxyl leucine **65** were synthesized. The protection of the N^{α}-terminal by $(Boc)_2O$ and the carboxyl terminal by diazomethane was completed in two steps in 74% yield (Scheme 3.10). Although the mesylation can be completed as before, the nucleophilic substitution turned out to be impractical. The reaction was very slow and only a 10% yield of desired product was isolated. The major product was the elimination product **68** which was generated as discussed before. Obviously, the steric effect of the isopropyl group prevented the S_N2 substitution of the benzyl mercaptan and β-elimination dominated the reaction. The aziridine formation from **65** following by benzyl mercaptan nucleophilic addition is under investigation.[124]



Scheme 3.10 Protection, mesylation and nucleophilic substitution

§ 3.4 N^{α}-Boc protected [5,5]-bicyclic β-turn dipeptide

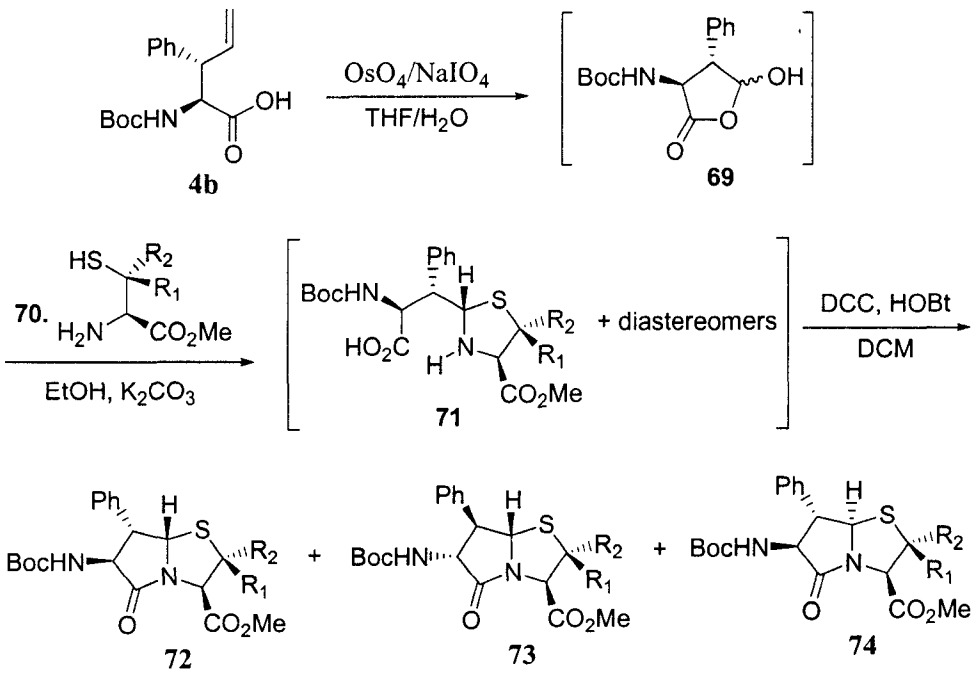
3.4.1 Synthesis of [5,5]-analogues

A convergent method has been developed to synthesize [5,5]-azabicycloalkane dipeptides from N^{α}-Cbz protected β-substituted γ,δ-unsaturated amino acids (Scheme 3.1).[69] However, the methodology developed has not been optimized and the reaction was not clean. The N^{α}-Boc protection was chosen in my project because it was desired to insert these dipeptides into peptide. N^{α}-Boc chemistry is a standard protecting group used in solid phase peptide synthesis.

This project was started from N^{α}-Boc-β-vinyl phenylalanine **4b**, a Kazmaier-Claisen rearrangement product (Scheme 2.1).[130-132] The terminal alkene was transferred to its aldehyde by osmylation followed by $NaIO_4$ oxidative cleavage. This reaction can be performed on a large scale.[133] The lack of an aldehyde-H in the ^1H nmr indicated the

formation of γ-hydroxyl-γ-lactone **69** (Scheme 3.11). After work up, **69** was used directly in reaction with β-mercapto amino acids **70**. The thiazolidine **71** formation was performed under very mild reaction conditions to give products with a 2,5-*trans* relationship, which is preferred to the 2,5-*cis* due to steric effects in all the cases of different β-thio-amino acids. Bicyclization was completed by use of typical amide-coupling reagents such as DCC/HOBT at room temperature to give [5,5]-fused bicyclic γ-lactam dipeptides **72, 73,** and **74**.

Because racemic β-vinyl phenylalanine **4b** and enantiomeric pure β-thio amino acids **70** were used, two or three diastereomers of **72**, **73**, **74** were generated. Fortunately, all these isomers could be isolated by liquid chromatograph on a silica gel column. When *L*-cysteine **70a** was used, the product has three isomers, with ratio about 3:5:2. This result is typically different from the reaction by using N^{α}-Cbz protected starting material, by which only two major products generated. Moreover, the bicyclization from N^{α}-Boc starting material was much cleaner than from N^{α}-Cbz protected starting materials.



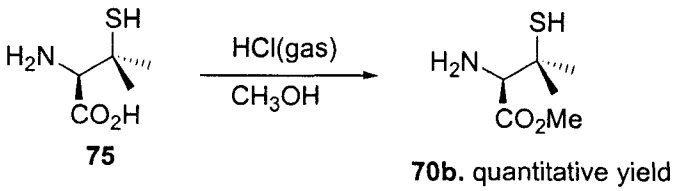
Scheme 3.11 Synthesis of *N,S*-acetal [5,5]-bicyclic dipeptide analogue

Table 3.2 The results of bicyclization of γ-lactam dipeptide

Starting material(**70**)			three-step yield	product ratio		
	R_1	R_2	(%)	**72**	**73**	**74**
a	H	H	77	32	48	20
b	Me	Me	70	30	58	12
c	Me	H	40	50	50	

This strategy toward *N,S*-acetal [5,5]-bicyclic γ-lactam dipeptide analogus has been investigated by using different cysteine derivatives. Penicillamine, another

commercially available β,β-dimethyl substituted cysteine derivative was tried. Although literature reported the direct use of pencillamine with enantiomer pure aldehyde in bicyclic formation the reaction failed in my case.[134] It was transformed to its methyl ester **70b** by bubbling HCl(gas) in CH_3OH solution in quantitative yield (Scheme 3.12).[135] The reaction was not complete in 15 min as reported, but in 45 min with bubbling of gas HCl and then the reaction was kept at room temperature overnight. The bicyclization was preformed by the same strategy shown in Scheme 3.11. However, the adjustment of pH = 5-6 in thiazolidine formation should be indicated by K_2CO_3 to neutralize the excess amount of acid. On the other hand, the bicyclic formation from methyl β-thiol-α-amino-butyrate **70c(60)** only gave two products and the total yield was 40%. It was later understood that deprotection of the N^{α}-Boc without scavenger present caused the low yield of **60** in the previous step (Scheme 3.8). The free mercaptan in this case acted as a scavenger itself. Only **72c, 73c** were found in this reaction which can be explained at the thiazolidine formation stage. The *trans*-relationship is much more favored than the *cis*- due to the strong steric effect (2,5- and 3,5-*trans* relationship).



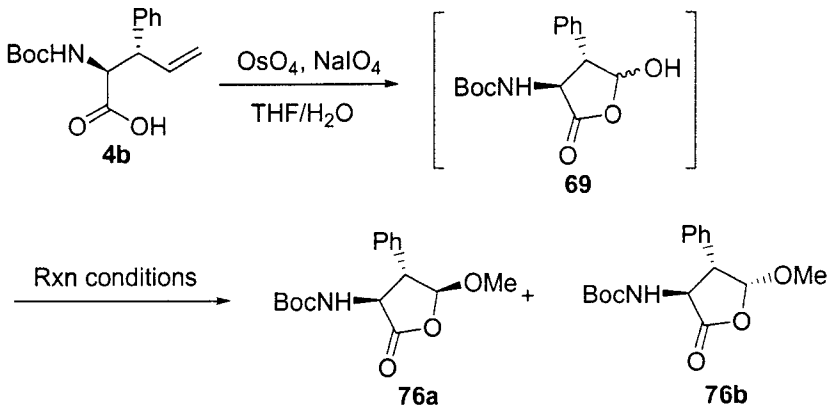
75 **70b.** quantitative yield

Scheme 3.12 Synthesis of penicillamine methyl ester

Other reported methods starting from β-vinyl phenylalanine methyl esters leads to the bicyclic compounds in one step (in refluxing pyridine), or in two steps (monocyclization in EtOH and H_2O following by heating in DMF), are not successful due to the instability of the aldehyde under these reaction conditions.[99,136] The coupling reaction might be improved by using other coupling reagents such as HBTU/HOBt or water soluble EDC instead of DCC and HOBt so that the DCU contamination of product could be avoided.

3.4.2 Intermediate and products characterization

The formation of a γ-hydroxyl-γ-lactone in this case was thought to be important.[137] It stabilized the free aldehyde, which otherwise might undergo epimerization at the β-position and other side reactions. Trying to characterize the aldehyde directly failed due to the formation of γ-hydroxyl-γ-lactone **69** and the rotamer of Boc (Scheme 3.13). The initial idea to use $CH(OMe)_3$ and catalytic amount of sulfuric acid for its acetal formation in my case, however, turns out to be impractical due to the lability of N^{α}-Boc in acidic conditions. According to the literature, the acetal formation of aldehyde also can be completed under basic medium by catalytic amount of Lewis acid.[138-140] However, the reaction failed in my case using $TiCl_4$ and TEA in methanol. The intermediate was finally converted to its acetal under several reaction conditions (Scheme 3.13) in the presence of catalytic amounts of Lewis acids. The results were are in the following scheme. It is unfortunate, however, the products only show one spot on TLC and the separation of these diastereomers became impossible.

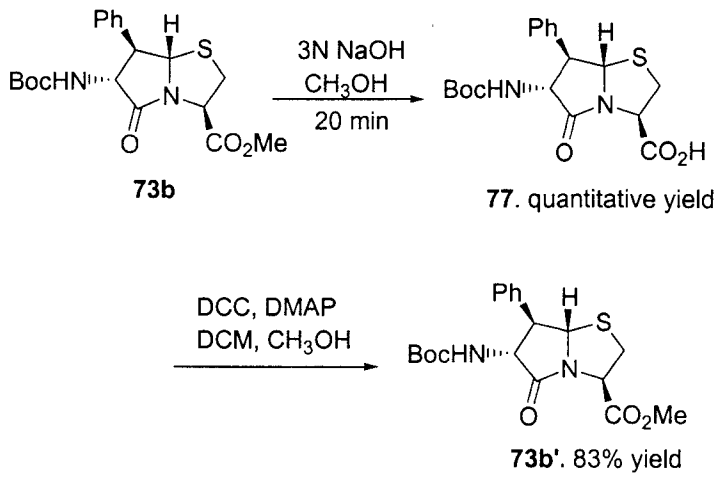


	76a : **76b**	
a. BF_3·OEt 10 mol % in THF, 3 days	3 : 1	50% yield
b. $TiCl_4$·DCM 10 mol% in methanol, 1 hour	2 : 3	50% yield

Scheme 3.13 Osmylation and intermediate acetal formation

By using the Lewis acid BF_3·OEt_2 in THF, the acetal formation was very slow. The reaction took 3 days to complete. On the other hand, the reaction was not complete by using $TiCl_4$·DCM in 3 days. In methanol, reactions can be completed in 1 hour by using $TiCl_4$·DCM and 2 hours by using BF_3·OEt_2. Although the reaction with $TiCl_4$ was faster, the reaction was not as clean as with BF_3·OEt_2. The mixture proved to be a pair of epimers by ^1H nmr instead of rotamers because their ratio was changing with methods but not with nmr detecting temperature. The stereostructure **76a** and **76b** were assumed according to the reaction conditions and its results. Because they are a mixture, it was not worth while to isolate them and to prove their structure.

The deprotection of methyl ester for the bicylic dipeptide also is important because this bicyclic eventually will be used in peptide synthesis. This hydrolysis could be done in methanol by using 3*N* NaOH in 20 mins and no obvious epimerization was observed. The completeness of ester hydrolysis has been confirmed by TLC and ^{1}H nmr. In order to further confirm the hydrolysis, it was re-esterified and the starting material recovered in 83% yield (Scheme 3.14). **73b** and **73b'** did not show any difference by comparing their ^{1}H nmr spectra.



73b

77. quantitative yield

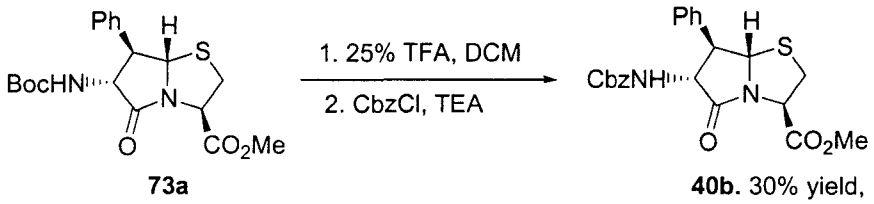
73b'. 83% yield

Scheme 3.14 Hydrolysis of methyl ester and re-esterification

§ 3.5 Stereochemistry of bridge-head hydrogen, X-ray crystallography

The structural identification of the three distereoisomers obtained from *L*-cysteine was investigated. Their tentative structures were shown in Scheme 3.11 in the order of their elution on the column. Their ^{1}H assignments were accomplished by 2D COSY, but the assignment of stereochemistry of these structures failed by ROSY and nOe

technique simply due to the lack of any nOe effect. Attempts to crystallize the minor product **74a** failed due to its liquid state at room temperature. By deprotection of the N^{α}-Boc group and reprotection by Cbz, **73a** has been transferred into its N^{α}-Cbz bicyclic compound which Wei Qiu already synthesized. The X-ray crystal structure of **40b** has been published in our previous paper.[69] By carefully comparing the ^{1}H nmr spectrum, I found the **73a** is identical to the Wei's crystal product **40b**.

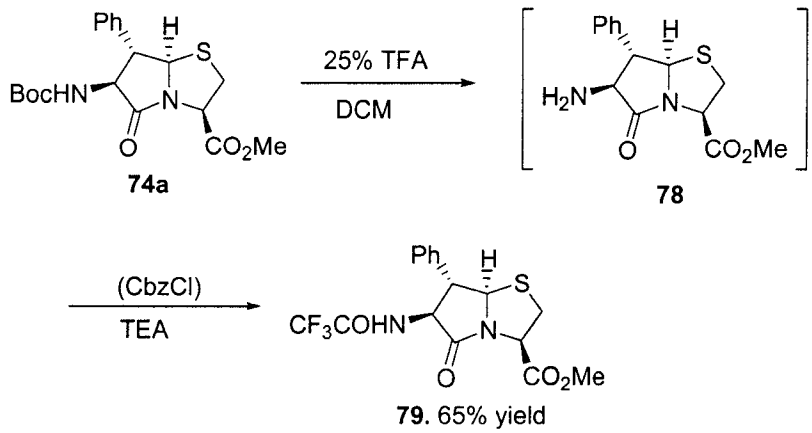


73a **40b.** 30% yield,

Scheme 3.15 Determination of stereochemistry of **73a**

The structure determination was improved dramatically by an unexpected result. The initial idea was to transfer the N^{α}-Boc protected product **74a** to the N^{α}-Cbz protecting group, with the hope that the stereochemistry of this N^{α}-Cbz protected compound can be confirmed by nOe. An unknown compound was obtained by which ^{1}H nmr show very clearly that this compound without having the N^{α}-Cbz protecting group on it (Scheme 3.16). All the hydrogens on the two rings are clearly separated and the *J*-splitting pattern can be analyzed after ^{1}H assignment by COSY. However, the spectrum was difficult to interpret because the *dd*-splitting pattern of the α-H, indicated only one H on the N. It was only after the ^{13}C nmr was obtained that the CF_3CO- was

obviously attached at the end of this terminal amine. The CF_3CO- protection also limits the nucleophilicity of amine and makes the bicyclic compound stable. This unexpected structure was further confirmed by HRMS.



Scheme 3.16 The deprotection of N^{α}-Boc and protection by CF_3CO

The above unexpected reaction results can be understood due to the presence of excess amount of TFA. CbzCl acted as an activating reagent for trifloroacetic acid which was transferred to an acid chloride or acid anhydride. It then reacted with terminal amine to generate product **79** (Scheme 3.16). Another exciting result was this TFA-NH-bicyclic molecule grew big crystals in EtOAc and hexane on standing for five days. The single X-ray diffraction analysis confirmed the stereochemistry previous assigned (Figure 3.1; next page). Besides, the nOe data clearly showed its stereochemistry by radiating α-H, β-H, and acetal-H. All the obtained data are consistent with each other. At this time, we re-checked the nOe for the N^{α}-Boc

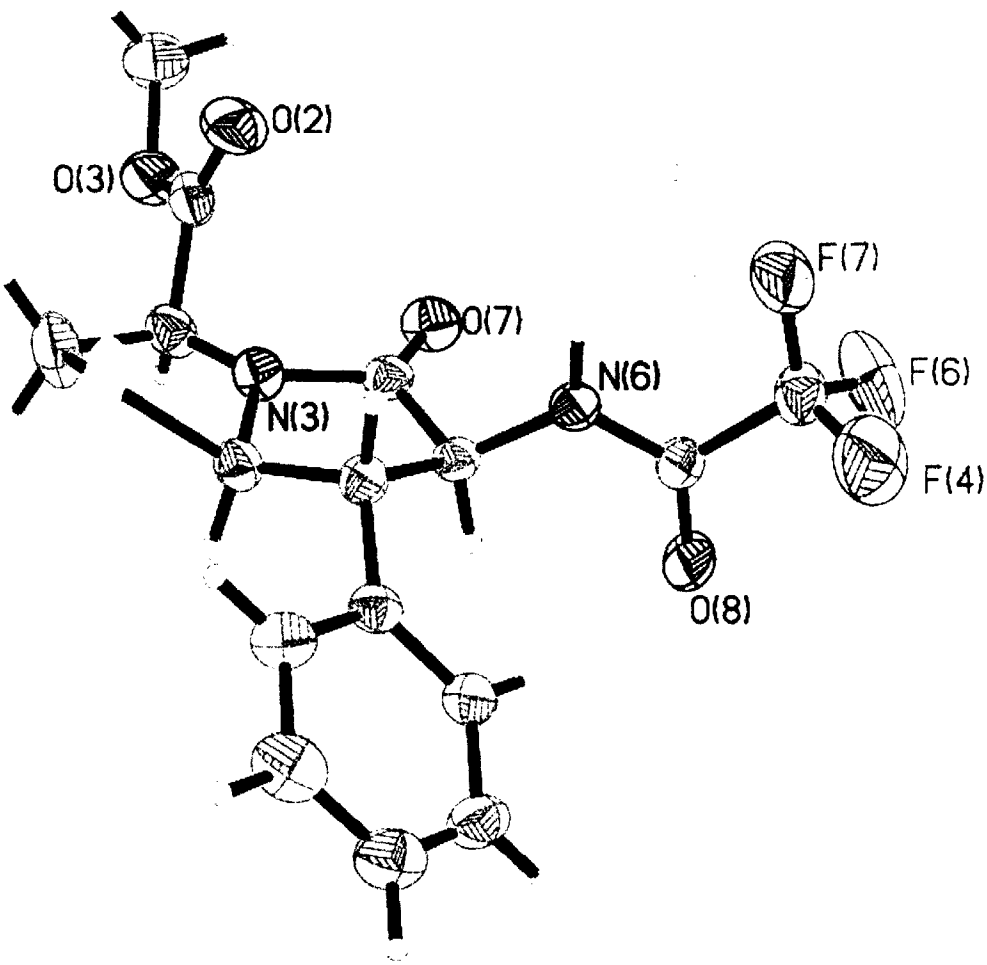


Figure 3.1 X-ray structure of minor bicyclic product

protecting bicyclic compounds **72a**. Once again, there was no nOe obtained on the left 5-membered ring. The only nOe was the α-H and two β-Hs on the thiazolidine ring. We believe that rotamers from the N^{α}-Boc protecting group are the main reason why all the hydrogens on the left ring were broad in the ^{1}H nmr. On the other hand, the sharp peaks and nice splitting pattern on the thiazolidine ring indicated that the N^{α}-Boc does not affect this ring.

Transferring the N^{α}-Boc to an N^{α}-CF_3CO became a novel and unique method for characterizing these bicyclic compounds by nmr technique such as by the *J*-splitting pattern, nOe effects and even the potential possibility for good crystallization for X-ray crystallography. It is fortunate that these TFA-protected bicyclic products can be easily generated by several reagents.[141,142] In future investigation, other functional groups such as guanidine will be introduced by asymmetric synthesis of β-thio-arginine following by bicyclization.

§ 3.6 Experimental Section

General information

^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 and Bruker DRX-500 MHz NMR. The chemical shifts were reported in δ, parts per million (ppm), relative to TMS (δ = 0.00 ppm) as an internal standard. In DMSO, the spectra were referenced to solvent peaks at 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C. In D_2O, ^{1}H chemical shifts were referenced to the HOD peak at 4.67 ppm, and ^{13}C chemical shifts were indirectly

referenced to $CDCl_3$ at 7.26 ppm. Coupling constants, *J*, are reported in Hertz (Hz) and refer to apparent peak multiplicities and not true coupling constants. Mass Spectroscopy analyses were conducted by the Mass spectrometry Facility at the Department of Chemistry of the University of Arizona on a Jeol HX-110A. Optical rotations were measured on a JACSO P1020 polarimeter. THF was distilled from sodium/benzophenone. Dichoromethane was distilled from CaH_2. All the other reagents and solvents, unless otherwise stated, are commercially available and were used as received. Flash liquid chromatograph was performed with 230-400 mesh size silica gel which was purchased from Aldrich Chemical Co. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F_{254}. Melting points (Mp) are uncorrected and were obtained in open capillaries. All the new compounds were characterized by M, $[\alpha]_D$, ^1H, ^{13}C nmr and high resolution mass spectrometry (HRMS), while known compounds were only characterized by Mp, ^1H and ^{13}C nmr. Unless otherwise stated, all reactions were run under an atmosphere of argon in flame-dried glassware.

(2*S*, 3*R*)-2-*tert*-Butoxycarbonylamino-3-hydroxy-butyric acid methyl ester (49): (2*S*, 3*R*)-Threonine **41** (6.0 g, 35.4 mmol) was dissolved in fresh distilled THF (200 mL) in a 500-mL flask at room temperature. *tert*-Butylcarboxyl anhydride (8.49 g, 38.9 mmol) and the catalytic amount of 4-(dimethylamino)-pyridine (DMAP, 240 mg) were added together with sodium bicarbonate (12g). The reaction was kept at room temperature overnight before it was quenched by sodium bicarbonate aqueous solution. THF was evaporated and the residue was diluted in NH_4Cl (100 mL) before it was

extracted with DCM (3 x 50 mL). The combined DCM was washed with brine and dried over $MgSO_4$. After evaporating the solvent, the colorless crude product was purified by flash liquid chromatography. Colorless product (8.15 g, 98% yield) was obtained. $[\alpha]^{24}_D$ = -36.2° (*c* 3.75, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.25 (3H, d, *J* = 6.0 Hz), 1.46 (9H, bs), 2.25 (1H, s), 3.78 (3H, s), 4.26-4.30 (2H, m), 5.35 (1H, d, *J* = 6.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 19.8, 28.2, 52.5, 58.7, 68.1, 80.1, 156.1, 172.0; HRMS (FAB) MH^+ calcd for $C_{10}H_{20}NO_5$ 234.1341, found 234.1343.

Methyl (2*S*,3*S*)-Benzylsufanyl-2-*tert*-butoxycarbonylamino-butyrate (52). Preparation of mesylate: The protected threonine **49** (2.65 g, 11.4 mmol) was dissolved in DCM (80 mL) and triethylamine (3.17 mL, 22.7 mmol) was added. The solution mixture was cooled down to 0°C and then methanesulfonyl chloride (966 μL, 12.5 mmol) was added. The reaction was warmed up to room temperature during 40 min and quenched by saturated aqueous NH_4Cl (100 mL) solution. After the separation, the aqueous phase was extracted with DCM (2 x 30 mL) and the combined DCM was dried over anhydrous $MgSO_4$. It was concentrated and the crude product was used without purification.

In a 50-mL flask, benzyl mercaptan (4.0 mL, 34.2 mmol) was mixted with 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU, 3.4 mL, 22.8 mmol) in DMF (20 mL). The reaction was kept at room temperature for 5 min before it was cooled down to 0°C. The above mesylate was dissolved in DMF (50 mL) in a 250-mL flask and cooled down to 0°C. The above premixed benzyl mercaptan/DBU solution was added in one portion.

The reaction was kept at 0°C for 70 min and then quenched by NH_4Cl (200 mL). The aqueous solution was extracted by ether (3 x 50 mL) and the combined ether phase was washed with brine and dried over anhydrous $MgSO_4$. It was concentrated and purified on silica gel column (Hexane : EtOAc = 16 : 1 ~ 8 : 1). The colorless product (3.86 g, 97% yield) was obtained after evaporating the solvent. $[\alpha]^{24}_D = 0°$ (*c* 2.88, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$), δ 1.33 (3H, d, *J* = 7.0 Hz), 1.48 (9H, bs), 3.29-3.31 (1H, m), 3.73 (3H, s), 3.68-3.77 (2H, m), 4.50 (1H, dd, *J* = 3.0, 9.0 Hz), 5.30 (1H, d, *J* = 9.0 Hz), 7.26-7.36 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 19.5, 28.3, 35.6, 42.3, 52.3, 57.9, 80.2, 137.8, 155.7, 171.4; HRMS (FAB) MH^+ calcd for $C_{17}H_{26}NO_4S$ 340.1583, found 340.1579.

Methyl (2*R*,3*S*)-2-tert-butoxycarbonylamino-3(4-methoxy-benzylsulfanyl)-butyrate (54d): The reaction was performed as for the synthesis of **52**. $[\alpha]^{24}_D = -1.04°$ (*c* 1.41, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$), δ 1.29 (3H, d, *J* = 7.0 Hz), 1.45 (9H, bs), 3.25-3.27 (1H, m), 3.63 (1H, AB, *J* = 13.0 Hz), 3.65 (1H, AB, *J* = 13.0 Hz), 3.74 (3H, s), 3.79 (3H, s), 4.47 (1H, dd, *J* = 3.0, 9.5 Hz), 5.28 (1H, d, *J* = 9.0 Hz), 6.84 (2H, d, *J* = 8.5 Hz), 7.21 (2H, d, *J* = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 19.5, 28.3, 35.0, 42.2, 52.3, 55.3, 57.8, 80.0, 113.9, 129.7, 129.9, 155.7, 158.7, 171.5; HRMS (FAB) MH^+ calcd for $C_{18}H_{28}NO_5S$ 370.1688, found 370.1694.

Methyl (2*S*,3*S*)-2-*tert*-Butoxycarbonylamino-3-mercapto-butyrate (59): A 100-mL three neck flask was connected with a dewer condenser and an argon gas inlet. The third

neck was capped with a rubber septum. Argon was bubbled in for 10 min before the dry ice and acetone was added in the finger condenser. Ammonia (gas) was then condensed and collected into the flask, which was cooled down by dry ice/acetone bath. About 50 mL of liquid ammonia was collected before solid sodium (273 mg, 11.9 mmol) was added. The ammonia solution became blue in 2 min. Substrate **52** (1.44 g, 4.25 mmol) was dissolved in fresh distilled THF (6 mL) and added into the above liquid ammonia solution in one portion. The blue color disappeared in 2 min and the reaction was continued for a further 8 min before solid NH_4Cl (640 mg) was added. The flask was left open to the air at room temperature in the hood. After evaporation of the ammonia, the residue was dissolved in water and extracted with DCM (3 x 30 mL). The combined DCM was dried over anhydrous $MgSO_4$ and evaporated followed by purification on a silica gel column (hexane:EtOAc = 8:1). The colorless product (759 mg, 72% yield) was collected after evaporation of the solvent. $[\alpha]^{24}_D$ = -0.80° (*c* 2.06, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.39 (1H, d, *J* = 7.0 Hz), 1.47 (9H, bs), 1.60 (1H, d, *J* = 6.0 Hz), 3.59-3.61 (1H, m), 3.79 (3H, s), 4.56 (1H, dd, *J* = 7.0, 9.0 Hz), 5.34 (1H, d, *J* = 9.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 21.5, 27.9, 37.2, 52.1, 58.7, 79.5, 155.4, 170.7; HRMS (FAB) MH^+ calcd for $C_{10}H_{11}NO_4S$ 250.1114, found 250.1110.

Methyl (2*R*,3*S*)-2-Amino-3-mercapto-butyrate trifluoroacetic acid (60): (2*R*,3*S*)-Methyl-2-*tert*-butoxycarbonylamino-3-mercapto-butyrate **59** (601.6 mg, 2.42 mmol) was dissolved in DCM (5 mL) and TFA (1.8 mL) was added at room temperature for 20 min reaction. The solvent and TFA acid was evaporated. The residue was dissolved in

H_2O and washed with $CHCl_3$ (5 mL x 3). The aqueous phase was then dried by lyophilization and the product was collected without further purification.

(2*R*,3*S*)-2-Amino-3-mercapto-butyric acid (62): Methyl (2*R*,3*S*)-2-*tert*-butoxycarbonylamino-3-mercapto-butyrate **59** (829.7 mg, 3.34 mmol) was dissolved in 1*N* NaOH in methanol (1:1 mixture). The reaction was kept at room temperature for 6 hours and stopped by adding 1*N* HCl to pH = 2-4. The product was extracted with DCM (3 x 3 mL) and the solvent was evaporated. The residue was dissolved in EtOAc (6 mL) and concentrated HCl (3 mL) together with thioanisole (246 μL, 2.1 mmol). The reaction was kept at room temperature for 30 min. It was diluted by EtOAc (10 mL) and H_2O (10 mL). The organic phase was separated and the aqueous phase was extracted with EtOAc (5 mL x 2). The aqueous phase was dried by lyophilization. The product was used in bicyclization without purification.

Ni(II)-complex of Schiff's base of (*S*)-BPB and (2*S*, 3*S*)-β-i-propylserine (64): The Ni(II)-complex (1.0 g, 2 mmol) was dissolved in methanol (3 mL), NaOMe (2.5 N, 1.6 mL, 4 mmol) was added, and the reaction was kept for 5 min before isobutyraldehyde (364 μL, 4.0 mL) was added in one portion. The reation was kept at room temperature for 3 min before it was quenched by acidic aqueous solution (40 mL, 5% of HOAc). The precipitate was filtered and washed with water and dried in the air. The crude product was purified by recrystallization in DCM and ether. $[\alpha]^{24}_D = +2160^\circ$ (*c* 0.0254, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 0.80 (3H, d, *J* = 6.0 Hz), 1.13 (3H, d, *J* = 6.5 Hz), 1.76

(1H, bs), 2.08 (1H, d, J = 6.0 Hz), 2.16 (1H, bs), 2.52 (1H, d, J = 10.5 Hz), 2.80 (1H, bs), 3.47-3.51 (3H, m), 3.56 (1H, d, J = 12.5 Hz), 3.86 (1H, s), 4.10 (1H, d, J = 5.5 Hz), 4.39 (1H, d, J = 12.5 Hz), 6.65 (2H, d, J = 6.0 Hz), 6.95 (1H, d, J = 12.5 Hz), 7.12-7.18 (2H, m), 7.34 (2H, t, J = 7.0 Hz), 7.43-7.50 (4H, m), 8.07 (2H, d, J = 7.0 Hz), 8.20 (1H, d, J = 8.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 15.7, 21.8, 23.6, 29.9, 30.7, 57.1, 63.3, 70.5, 72.9, 77.4, 120.7, 123.3, 126.4, 127.1, 128.83, 128.86, 128.91, 128.96, 129.8, 131.4, 132.4, 133.3, 133.8, 134.2, 142.5, 172.1, 178.7, 180.2; HRMS (FAB) MH^+ calcd for $C_{31}H_{34}N_3NiO_4$ 570.1903, found 570.1901.

(2*S*,3*S*)-2-Amino-3-hydroxy-4-methyl-pentanoic acid (65): The hydrolysis of Ni(II)-product was performed as the synthesis of **23b** in Chapter **II.** $[\alpha]^{24}_D$ = -1.4° (*c* 3.19, H_2O); ^{1}H (500 MHz, $CDCl_3$), δ 0.87 (3H, d, J = 6.5 Hz), 0.92 (3H, d, J = 6.5 Hz), 1.65-1.69 (1H, m), 3.46 (1H, bs), 3.68 (1H, dd, J = 4.0, 8.0 Hz), 3.74 (1H, d, J = 4.0 Hz); ^{13}C, (125 MHz, $CDCl_3$), δ 17.8, 18.8, 30.6, 57.3, 75.5, 173.8; HRMS (FAB) MH^+-H_2 calcd for $C_6H_{12}NO_3$ 146.0817, found 146.0818.

Methyl (2*S*,3*S*)-2-Butoxycarbonylamino-3-hydroxy-4-methyl-pentanoate (66): The N^{α}-Boc protection and methyl esterification were performed as the synthesis of **110** in Chapter **VI**. $[\alpha]^{24}_D$ = -0.95° (*c* 8.18, $CHCl_3$); ^{1}H (500 MHz, $CDCl_3$), δ 0.97 (3H, d, J = 10.5 Hz), 1.03 (1H, d, J = 6.5 Hz), 1.45 (9H, bs), 1.73-1.78 (1H, m), 2.69(1H, bs), 3.69 (1H, d, J = 8.5 Hz), 3.75 (3H, s), 4.47 (1H, d, J = 9.5 Hz), 5.42 (1H, d, J = 8.5 Hz); ^{13}C

(125 MHz, $CDCl_3$), δ 18.8, 28.2, 30.8, 52.4, 55.7, 77.2, 77.4, 79.9, 156.0, 172.8; HRMS (FAB) MH^+ calcd for $C_{12}H_{24}NO_5$ 262.1654, found 262.1661.

Methyl (*R*)-2-Amino-3-mercapto-3-methyl-butyrate hydrochloric acid (70b): Penicillamine (660 mg, 4.42 mmol) was dissolved in distilled methanol (24 mL) and the HCl (gas) was bubbled into the solution for 45 min. The reaction was then kept at room temperature for overnight before the solvent was evaporated and the product was collected (100% yield). The product was used without purification.

General procedure For osmylation, *N,S*-acetal formation and bicyclization:

The Kazmaier amino acid **4b** (1 eq) was dissolved in a THF/H_2O (2:1, 4 mL/mmol) mixture solution in a flask. The hood lights were turned off and the flask was covered by aluminum foil. Osmium tetroxide (3-5% mmol) was added to the flask. After 5 min, $NaIO_4$ (2.5 eq) was added in small portions over a 15 min period. The reaction was kept at room temperature for 4 h before it was filtered and the THF was evaporated off. The residue was dissolved in EtOAc and saturated NH_4Cl aqueous solution. The aqueous phase was extracted with EtOAc (2 x 10 mL/mmol) and the organic phases were combined, washed with brine, and dried over anhydrous $MgSO_4$. The solution was concentrated in *vacuo* and the crude material was used in the next reaction without further purification.

The above crude sample was dissolved in EtOH (4.3 mL/mmol) and then K_2CO_3 (70 mg/mmol) in H_2O (5 mL/mmol) was added. *L*-Cysteine **70a** (or cysteine derivatives

70b and **70c**) (1 eq) was added. The reaction was kept at room temperature overnight. The EtOH was first evaporated off and then the solution was acidified by NH_4Cl to pH = 6. The aqueous solution was extracted by EtOAc (3 x 10 mL/mmol) and the combined organic phase was washed with brine and dried over anhydrous $MgSO_4$, concentrated in *vacuo* and used without purification.

The above residue was redissolved in DCM (9 mL/mmol), DCC (1eq), and HOBt (1 eq) were added. The reaction was kept at room temperature overnight before it was stopped by NH_4Cl solution. The aqueous solution was extracted by DCM (3 x 20 mL) and the combined organic phase was dried over $MgSO_4$. After evaporation, the product mixture was redissolved in ethyl ether and DCU was precipitated. The concentrated mixture was then purified on the silica gel column (hexane :EtOAc = 3 : 1~ 1 : 1).

Methyl (3*S*, 4*S*, 5*S*, 8*R*)-3-*tert*-Butoxycarbonylamino-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (72a): $[\alpha]^{24}_D$ = -245° (*c* 0.181, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.42 (9H, s), 3.22 (1H, dd, *J* = 5.0, 11.5 Hz), 3.36 (1H, dd, *J* = 8.5, 11.5 Hz), 3.77 (3H, s), 4.16 (1H, bs), 4.74(1H, bs), 5.15 (1H, dd, *J* = 5.0, 8.5 Hz), 5.28 (1H, bs), 5.50 (1H, d, *J* = 4.0 Hz), 7.31-7.40 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 28.2, 35.5, 44.1, 52.8, 54.3, 59.6, 70.1, 80.4, 127.5, 128.0, 128.7, 135.8, 155.2, 170.4, 176.0; HRMS (FAB) MH^+ calcd for $C_{19}H_{25}N_2O_5S$ 393.1484, found 393.1486.

Methyl (3*R*, 4*R*, 5*S*, 8*R*)-3-*tert*-Butoxycarbonylamino-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (73a): $[\alpha]^{24}_D$ = -34.1° (*c* 1.21, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.26 (2H, bs), 1.37 (7H, bs), 3.34 (1H, dd, *J* = 7.0, 11.5 Hz), 3.39 (1H, dd, *J* = 2.5, 11.5 Hz), 3.58 (1H, bs), 3.78 (3H, s), 4.8.2 (1H, dd, *J* = 9.0, 11.5 Hz), 4.92 (1H, bs), 5.13 (1H, d, *J* = 7.5 Hz), 5.31 (1H, dd, *J* = 2.5, 7.0), 7.27-7.40 (5H, m). ^{13}C (125 MHz, $CDCl_3$), δ 28.1 (29.7 rotamer), 34.6, 53.0, 57.6, 58.5, 59.7, 67.7, 80.4, 127.6, 128.0, 129.0, 136.4, 155.0, 169.5, 170.4; HRMS (FAB) MH^+ calcd for $C_{19}H_{25}N_2O_5S$ 393.1484, found 393.1486.

Methyl (3*S*, 4*S*, 5*S*, 8*R*)-3-*tert*-Butoxycarbonylamino-7,7-dimethyl-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (72b): $[\alpha]^{24}_D$ = -208° (*c* 0.357, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.42 (9H, bs), 1.46 (3H, s), 1.57 (3H, s), 3.78 (3H, s), 4.08 (1H, bs), 4.74 (1H, s), 4.79 (1H, s), 5.11 (1H, s), 5.92 (1H, s), 7.27-7.39 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 27.4, 28.2, 32.2, 44.3, 52.2, 54.6, 58.9, 70.2, 70.5, 80.5, 127.6, 127.9, 128.7, 135.9, 155.3, 169.2, 175.5; HRMS (FAB) MH^+ calcd for $C_{21}H_{29}N_2O_5S$ 421.1797, found 421.1814.

Methyl (3*R*, 4*R*, 5*S*, 8R)-3-*tert*-Butoxycarbonylamino-7,7-dimethyl-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (73b): $[\alpha]^{24}_D$ = -31.8° (*c* 4.74, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.37 (9H, bs), 1.46 (3H, s), 1.65 (3H, s), 3.69 (1H, bs), 3.78 (3H, s), 4.71 (1H, s), 4.85 (1H, dd, *J* = 9.0, 11.5 Hz), 4.99 (1H, s), 5.42 (1H, d, *J* =6.5 Hz), 7.27-7.40 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 25.9, 28.1, 31.6, 52.3, 57.4,

57.5, 59.3, 67.7, 68.6, 80.2, 127.5, 127.9, 128.9, 136.3, 155.0, 168.5, 170.2; HRMS (FAB) MH^+ calcd for $C_{21}H_{29}N_2O_4S$ 421.1797, found 421.1814.

Methyl (3*S*, 4*S*, 5*S*, 7*S*, 8*R*)-3-*tert*-Butoxycarbonylamino-7-methyl-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (72c): $[\alpha]^{24}_D$ = -1.10° (*c* 1.42, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.42 (9H, bs), 1.47 (3H, d, *J* = 6.5 Hz), 3.79 (3H, s), 3.83-3.88 (1H, m), 4.08 (1H, s), 4.66 (1H, d, *J* = 5.0 Hz), 4.84 (1H, s), 5.00 (1H, s), 5.59 (1H, d, *J* = 5.5 Hz), 7.31-7.42 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 21.7, 28.2, 44.2, 49.3, 52.8, 54.4, 66.8, 71.1, 80.6, 127.6, 128.0, 128.8, 135.7, 155.2, 170.2, 175.7; HRMS (FAB) MH^+ calcd for $C_{20}H_{27}N_2O_4S$ 407.1641, found 407.1630.

Methyl (3*R*, 4*R*, 5*S*, 7*S*, 8*R*)-3-*tert*-Butoxycarbonylamino-7,7-dimethyl-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (73c): $[\alpha]^{24}_D$ = -1.45° (*c* 1.13, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.41 (9H, bs), 1.55 (1H, d, *J* = 6.5 Hz), 3.71 (1H, bs), 3.98 (1H, dq, *J* = 3.0, 7.0 Hz), 4.84-4.90 (2H, m), 4.95 (1H, bs), 5.22 (1H, d, *J* = 7.5 Hz), 7.32-7.43 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 23.2, 28.1, 33.9, 47.3, 52.9, 58.2, 59.9, 64.8, 68.3, 80.3, 127.5, 128.0, 129.0, 136.4, 154.9, 169.2, 170.4; HRMS (FAB) MH^+ calcd for $C_{20}H_{27}N_2O_4S$ 407.1641, found 407.1657.

Methyl (3*S*, 4*S*, 5*R*, 6*R*)-3-(2,2,2-Trifluoroacetylamino)-4-phenyl-1-aza-2-oxo-6-thiabicyclic [3,3,0] octane-8-carboxylate (79): The minor bicyclic product **74a** (105.7 mg, 0.270 mmol) was dissolved in distilled DCM (3.5 mL), and TFA (880 μL) was

added. The reaction was kept at room temperature for 30 min before the solvents were evaporated off and the residue was redissolved in the DCM (3 mL). Benzyl chloroformate (77 μL, 0.54 mmol) and TEA (225 μL, 1.62 mmol) were added and the reaction was kept at room temperature overnight. The reaction was stopped by NH_4Cl (20 mL) aqueous solution and extracted with DCM (3 x 5 mL). The combined organic phase was dried over anhydrous $MgSO_4$, concentrated, and purified on a silica gel column (hexane : EtOAc = 3 : 1~ 1 : 1). A colorless compound (35 mg, 37%) was collected which can be crystallized in EtOAc and hexane. $[\alpha]^{24}_D$ = -25.0° (*c* 0.558, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 3.44 (1H, dd, *J* = 1.0, 12.5 Hz), 3.71 (1H, dd, *J* = 7.0, 12.5 Hz), 3.80 (1H, dd, *J* = 9.0, 11.5 Hz), 3.85 (3H, s), 4.55 (1H, d, *J* = 6.5 Hz), 5.15 (1H, d, *J* = 9.0 Hz), 5.50 (1H, dd, *J* = 9.0, 12.5 Hz), 7.26 (1H, d, *J* = 3.0 Hz), 7.34-7.41 (5H, m); ^{13}C (125 MHz, $CDCl_3$), δ 38.4, 53.2, 56.6, 58.0, 58.8, 115.6 (q, *J* = 286.1 Hz), 127.1, 128.6, 129.2, 134.3, 157.5 (q, *J* = 37.9 Hz), 168.0, 168.5; HRMS (FAB) MH^+ calcd for $C_{16}H_{16}N_2O_4SF_3$ 389.0783, found 389.0785.

CHAPTER IV.

SYNTHESIS OF *N,S*-ACETAL [6,5]-BICYCLIC β-TURN DIPEPTIDE

§ 4.1 Introduction

According to modeling studies, a [6,5]-bicyclic dipeptide is a nice mimic of type II' β-turns.[20,143,144] The first bicyclic β-turn mimetics were synthesized from glutamic acid derivatives and cysteine by Nagai and co-workers.[98,99] After that, a number of bicyclic β-turn mimetics have been synthesized,[28,145] and used in peptides.[146-148] Though successful, the synthetic methods to prepare these compounds did not provide a straightforward way to introduce side-chain groups on the six membered rings. Thus these simple β-turn dipeptides have not been very useful for improving the activity of small peptides, in which the functionalized β-turn structure itself plays an important role in the peptides for interaction with the receptor site.[21] This is primarily because in these cases individual side chain groups in the natural β-turns are important for interaction with the receptor site, and these side chain groups were not present in the β-turn mimetics.

In chapter **III**, I have demonstrated an efficient method to obtain side chain functionalized [5,5]-bicyclic dipeptides in a 5-step strategy by taking advantage of the Kazmaier-Claisen rearrangement.[149] As shown in the retrosynthetic analysis in Scheme 1.6, Chapter **I**, the two precursors of this target bicyclic scaffold are from the β-

substituted ω-unsaturated amino acids and β-substituted cysteine derivatives. Unforturnately, application of this method to obtain a [6,5]-bicyclic structure was not as straightforward.

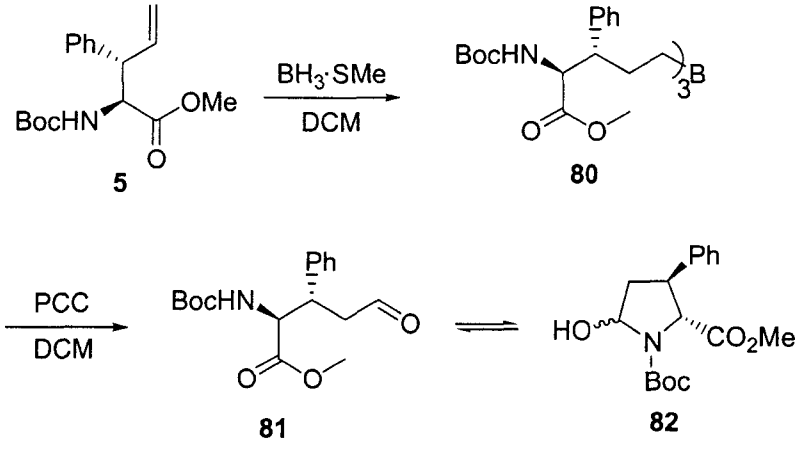
§ 4.2 Methodology development from β-vinyl phenyl alanine

4.2.1 The conversion of terminal alkene to aldehyde

A direct strategy toward this bicyclic β-turn dipeptide was taking advantage of the starting material used in [5,5]-bicyclic dipeptide synthesis, the β-vinyl phenyl alanine, a racemic product synthesized by Kazmaier-Claisen rearrangement in Chapter **II**. Hydroboration on N^{α}-Boc protected free acid **4b** following by basic hydrolysis was an initial idea to start this strategy. However, the reaction only gave very low isolated yield and the literature has reported that the free acid actually could be reduced by $BH_3 \cdot SMe_2$ under similar reaction conditions.[150] The O-B coordination makes it possible that the carboxylate group is reduced to its alcohol. In order to prevent this reduction, the free acid must be protected before hydroboration. Although the methyl ester **5** was obtained in 88% yield from the acid (Scheme 2.1, Chapter **II**), the hydroboration of this terminal alkene **5** turns out to be very complicated (Scheme 4.1).

One of the simplest ways to transform terminal alkene to aldehyde is doing hydroboration following by PCC oxidation.[151,152] One to three equivalents of BH_3 have been tried in DCM and the remaining alkene proton signal in the ^1H nmr spectrum indicated the incompleteness of this reaction. One report shows that coordination of N-B was so strong that the first one mole (3 equivalents of BH_3) was actually consumed in

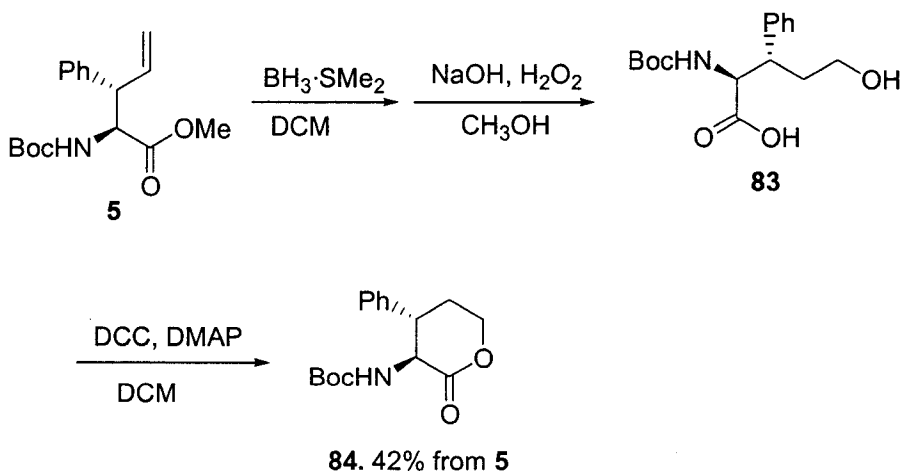
coordination instead of hydroboration.[151] The reaction was finally optimized by checking the ^{1}H nmr of crude product **80**. It turns out that at least 4 equivalents of BH_3 must be used for this reaction, while 5 equivalents were required so that the reaction could be completed in 5 hours (Scheme 4.1). The PCC oxidation of this C-B bond directly to aldehyde **81** has been tried many times under different reaction conditions (Similar reaction conditions and their results will be fully discussed in **4.2.2**). The TLC showed that the products were a mixture. The ^{1}H nmr spectra of both crude and purified products did not show the most downfield aldehyde-H (~9.8 ppm) due to the 5-membered hemiaminal **82** formation.[153-156] By neglecting this hemiaminal possibility, I wasted a lot time on the reaction expecting to see the aldehyde proton signal in the ^{1}H nmr spectrum.



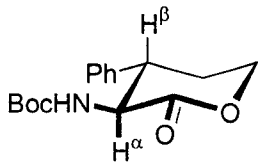
Scheme 4.1 Hydroboration and PCC oxidation of terminal alkene

The hydroboration followed by neutral hydrolysis in $NaBO_3$ solution was also reported.[157] Unfortunately, it turns out to be impractical on my substrate. The yield of

this reaction was always lower than 40% and the reaction was messy, and it could not be scaled up. By classical hydrolysis methods (3*N* NaOH, H_2O_2),[158] the methyl ester **5** was hydrolysized and an acid **83** was generated (Scheme 4.2). TLC indicated that the 6-membered lactone **84** was partly formed after work up with NH_4Cl aqueous solution. The lactonization then can be completed by coupling reagents DCC and DMAP in diluted DCM solution. The *anti*-relationship of the N^{α}-BocHN- and the Ph- substituents in the Kazmaier product **4b** was determined by the ^{3}J-coupling constant (^3J = 8.6 Hz) of H^{α} and H^{β} (Figure 4.1). The 6-membered lactone **85** is in the *pseudo*-chairlike conformation so that both the Phe and the N^{α}-Boc substituents are in the equatorial positions. It is the first time that the relative stereochemistry of a Kazmaier product was determined by a NMR technique. The stereochemical outcome was explained by the chairlike transition state in the Kazmaier Claisen rearrangement in Figure 2.1, Chapter **II**.



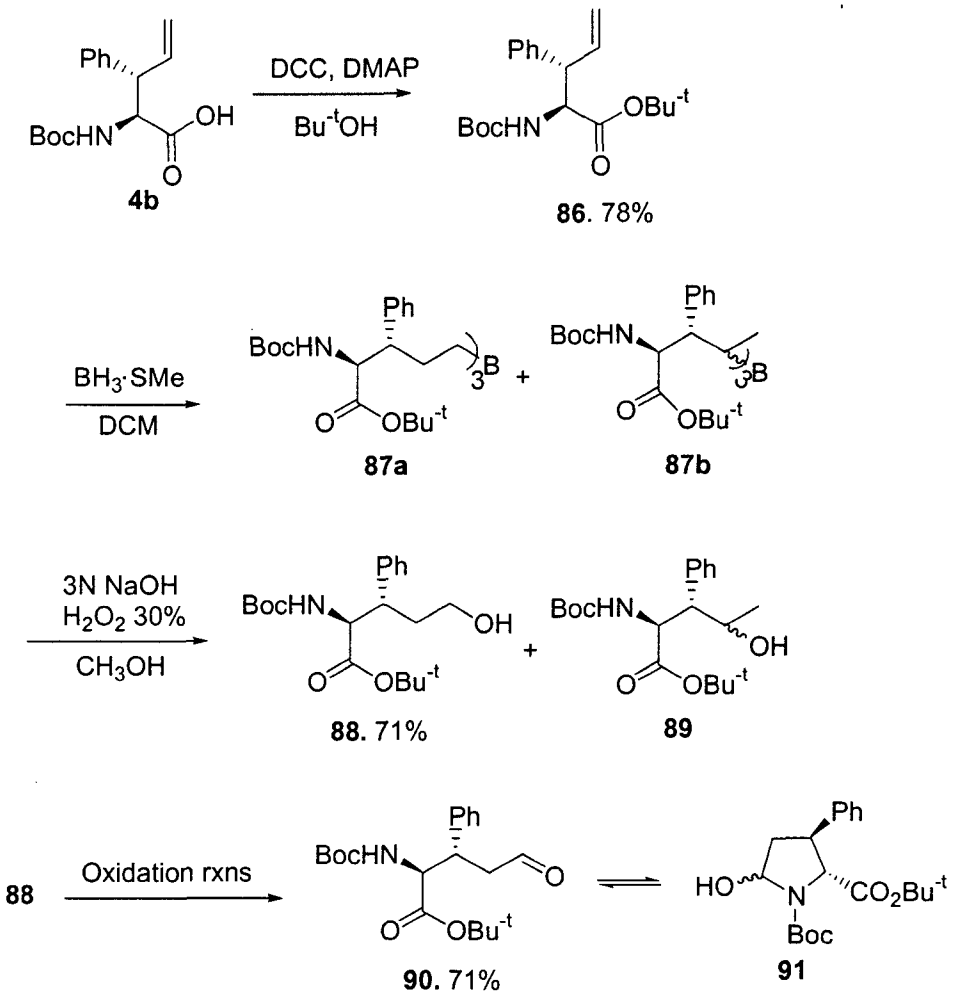
Scheme 4.2 Determination of stereostructure of Kazmaier product



85. $^{3}J_{\alpha,\beta}$ = 8.6 Hz

Figure 4.1 The *pseudo*-chairlike conformation of Kazmaier product

By converting the Kazmaier product **4b** to its *tert*-butyl ester, the ester now can survive the above basic hydrolysis conditions. The *tert*-butyl ester **86** can be synthesized from the acid in 78% yield using the same strategy for the methyl ester (Scheme 4.3). A less polar byproduct in 20% yield was always isolated, yet its structure cannot be determined. The hydroboration (5eq of BH_3 was used) and basic hydrolysis was clean.[158,159] Both the primary and secondary alcohol (**88** and **89**) can be isolated from the column in a ratio of 94 : 6. The same chemoselectivity of this reaction also has been reported.[160-162] The ^{1}H nmr spectra were complicated due to the rotamers of compound **88** and the mixture of diastereoisomer of **89**. The oxidation of this primary alcohol was tried using oxoammonium salt,[163] Dess-Martin reagent,[164-167] PCC-$NaHCO_3$, and Swern oxidation.[168] Unfortunately, none of these reaction conditions provide the aldehyde according to the crude nmr spectra. Once again neglecting of the existence of form **91** wasted a lot of my time.

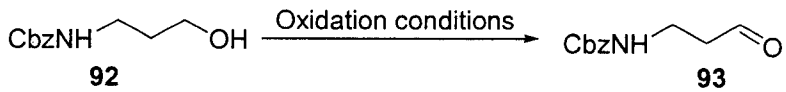


Scheme 4.3 The strategy of hydroboration and oxidation hydrolysis

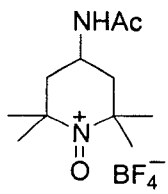
4.2.2 The oxidation of primary alcohol to aldehyde

By thinking that bad techniques could be employed in this primary alcohol oxidation procedure, a model study was designed by using a commercial available starting material **92** (Scheme 4.4). The oxidation by oxoammonium salt **94**[169] and Dess-Martin reagent **95** was only tried several times while PCC oxidation was examined

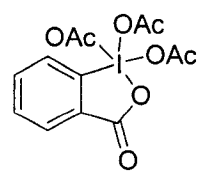
using several conditions (Table 4.1). Some of the results for optimization of the PCC oxidation are shown in the Table 4.2. All the reactions were run for 2-4 hours, monitored by TLC until the reaction stopped or the starting material disappeared. In PCC oxidation, $NaHCO_3$ was added to neutralize the small amount of acid released during the oxidation and to improve the PCC residue physical status in filtration and work up. It turns out that the yield is not related to the amount of PCC used as long as it is in 2 fold excess, but increases as the amount of $NaHCO_3$ increase. Nevertheless, the remaining starting material also increases, and the best reaction conditions do not give more than a 50% yield. The possible coordination between the Cr^{3+} and N requires more than 1 eq PCC,[170-172] but an excess amount of PCC makes the reaction messy. The reaction was also tried by adding PCC in three portions (1 eq every hour for 3 hours) with 100wt% of $NaHCO_3$. The final result was about 38% yield with 32% of staring material recovered. It was later found out that Swern oxidation provided the best yield even before optimization (Table 4.1).



92 **93**



94. Oxoammonium salt



95. Dess-Martin reagent

96. CrO_3·Pyridine
(Collins reagent)

Pydridinium chlorochromate

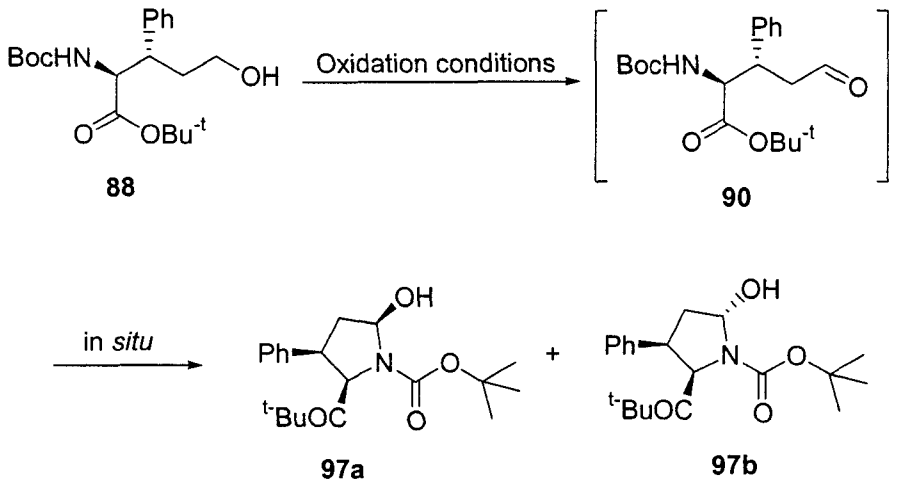
Scheme 4.4 Oxidation of primary alcohol and oxidizing agents

Table 4.1 Comparison of primary alcohol

Reaction agents	yield of purified product **93**
Oxoammonium salt	17%
Dess-Martin reagent	51%
PCC in $NaHCO_3$	50% (40% starting material recovered)
Swern Oxidation	73% without optimization

Table 4.2 The optimization results of PCC oxidation of primary alcohol

	PCC equivalents	$NaHCO_3$ wt%	Yield (starting material recovered)
1	2	0	24%
2	4	10	35%
3	3	20	42% (22%)
4	2	40	41% (22%)
5	2.5	100	50% (40%)
6	4	100	46% (30%)



Scheme 4.5 Oxidation of alcohol and hemiaminal formation

After trying many different methods for primary alcohol oxidation, all these oxidation methods were tried again on my substrate **88** and confirmed the previous results that no aldehyde proton signal in ^{1}H nmr spectroscopy could be found (Scheme 4.5). It was only at this point that I understood the formation of a pair of diastereomers of 5-membered hemiacetals **97a** and **b**. This result can explain why the aldehyde cannot be found on ^{1}H nmr spectrum and why the product cannot be well characterized. To transfer this *N,O*-hemiacetal directly to thiazolidine has been reported only once[146] and I failed several times and finally gave up (The hemiaminal problem will be further discussed in the next section). Double protection of N^{α}- actually is a normal way to avoid this hemiacetal cyclization.[173]

4.2.3 The double protection of N^{α}- and bicyclization

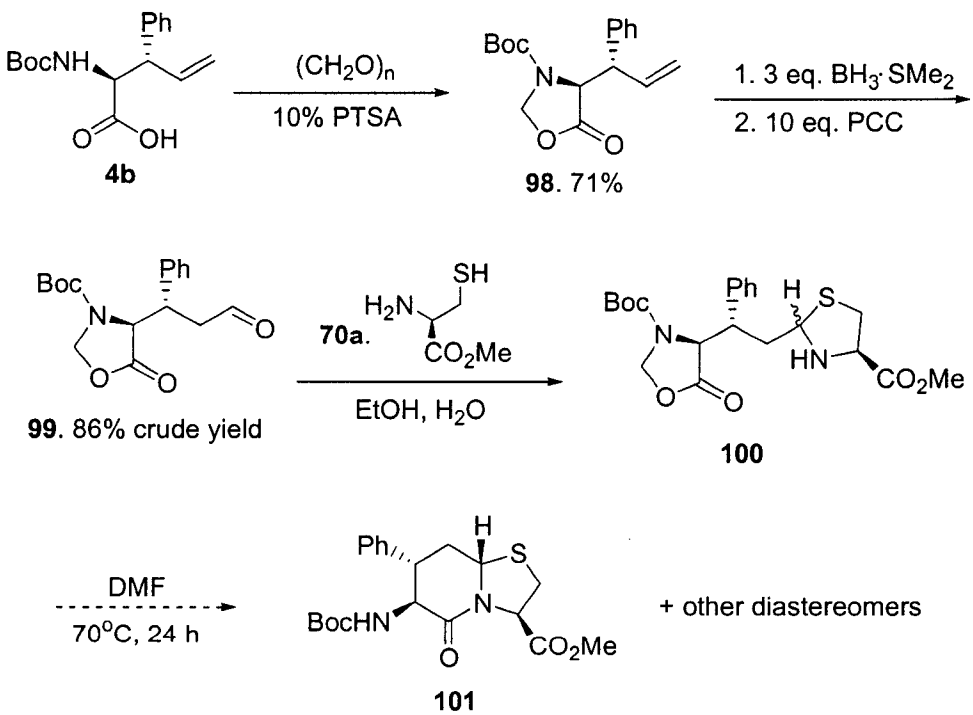
The double Boc protection on **86** failed although this reaction was successful on other amino acids which do not have β-substitution and *t*-butyl esters.[174] Double benzyl protection and N^{α}-Boc plus benzyl protection[175] failed again because the secondary benzyl protection was always slow and incomplete. We finally found out that the formation of an oxazolidinone **98** is easy (Scheme 4.6).[176-180] In this case, protection of the nitrogen and the free acid can be completed in one step. The oxazolidinone formation actually can be optimized up to 71% yield by using 10% of *para*-toluenesulfonic acid and refluxing in benzene in 1 hour. The yield of oxazolidinones **98** was increased by increasing of the amount of PTSA, while the remaining starting material was reacting (Table 4.3). The analysis of the byproduct showed that the terminal double bond was partly destroyed, while the N^{α}-Boc was not affected. It indicated that small amounts of alkene were hydrolyzed in the presence of acid.

Table 4.3 Optimization of oxazolidinones formation.

Experimental #	1	2	3	4	5*	6
PTSA%	1.5	3	4	6	8	10
Product yield **98**(%)	18	56	63	68	62	71
S.M. **4b** recovered(%)	23	28	23	12	12	3

* This experiment failed due to the high humidity

The oxazolidinone protection was useful for both hydroboration and aldehyde formation. The hydroboration became different in this case because N^{α}-terminal was double protected. In fact, only 3 equivalents of $BH_3 \cdot SMe_2$ are required to convert all of the starting material to its borate ester in an overnight reaction (95% conversion by TLC analysis) (Scheme 4.6). The direct oxidation of the C-B bond by PCC also was successful with only a 86% yield crude. The reaction was carefully optimized and the best condition was using 8-10 eq of PCC and 100 wt% of $NaHCO_3$. Unfortunately, the optimized results only were true for the ~80 mg scale reaction. With greater amounts of reactant, this one-pot conversion once again became complicated.

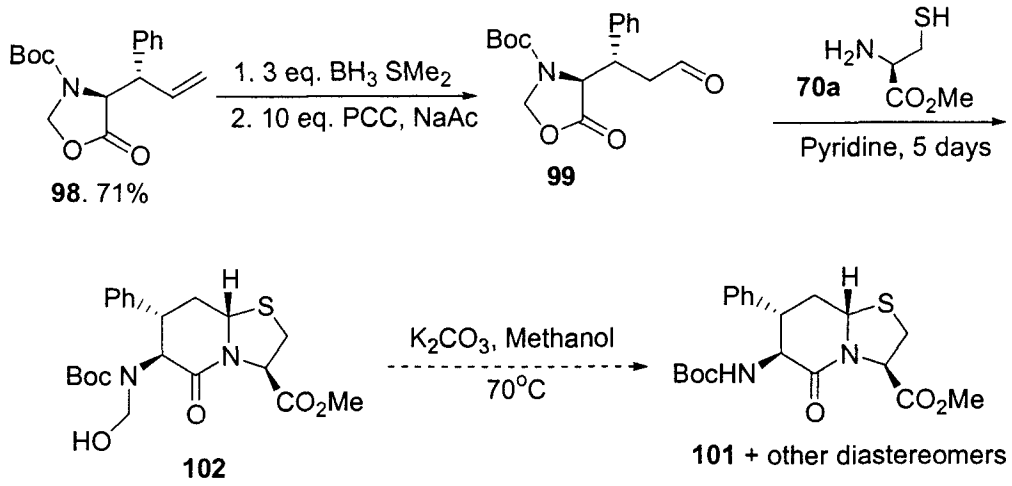


Scheme 4.6 Strategy toward [6,5]-bicyclic dipeptide

Trying to purify the aldehyde **99** on a silica gel column only gave a contaminated product in very low yield. It might be possible to generate the thiazolidine from the aldehyde without purification. Thus crude aldehyde **99** was used under mild reaction condition in EtOH and H_2O to the thiazolidine followed by bicyclization in DMF at 79-90°C (external) for 24 hours (Scheme 4.6).[181] Although the reaction was tried 5 times and three products were isolated, their spectra were not correct for the desired structures. The main problem remaining was the purity of the aldehyde.

Trying to improve the aldehyde formation by PCC in the presence of NaOAc was promising (Scheme 4.7).[182] The crude yield was usually higher than 150% indicating a lots of byproduct contamination. Again, purification by column chromatograph became impractical. The bicyclization was tried several times using the crude aldehyde **99** in various one step strategies (Scheme 4.7). In this case, the *N,S*-thiazolidine and bicyclization can be formed in one-step in pyridine according to the literature.[144] The alcohol fragment in **102** can be cleaved under mild basic conditions in 24 h. Unfortunately, none of them gave conclusive results.

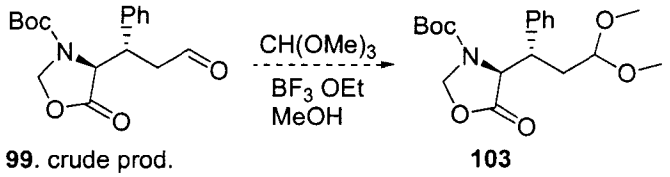
An alternative stepwise method also was tried. That was to transfer the terminal alkene first to its primary alcohol before Swern oxidation. However, a $NaBO_3$ neutral hydrolysis method had to be used in this case since the oxazolidinone cannot survive in basic hydrolysis conditions.[157] The alcohol products were hard to purify and identify. The reaction was messy and the yields were always lower than 15 %.



Scheme 4.7 One-step strategy toward [6,5]-bicyclic dipeptide

4.2.4 Cleaning up the methodology and a short summary

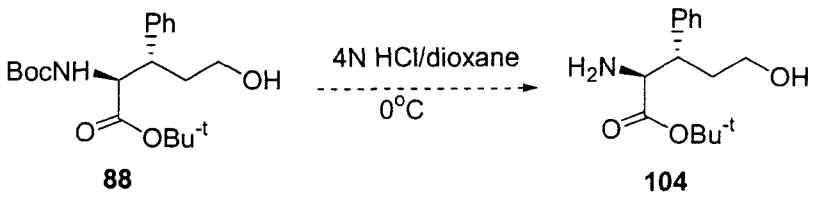
The failure of bicyclization in the above strategies indicated that the purity of the aldehyde is very important in bicyclization. Because it was impossible to purify and characterize the aldehyde **99** directly, the formation of the acetal **103** was tried several times. The reaction was kept at room temperature for 24 hours and TLC did not show any promising product by proton NMR spectrum (Scheme 4.8). Again, the existence of N^{α}-Boc rotamer prevented the further study of this compound and this reaction. Another idea was changing the N^{α}-protection group to others such as benzoyl or TFA. Unfortunately, the deprotecton of Boc by TFA also led to the destruction of oxazolidinone, a reaction which has been reported.[183]



99. crude prod. **103**

Scheme 4.8 Acetal formation from the crude aldehyde

I have indicated that due to the hemiaminal formation the conversion of aldehyde to *N,S*-thiazolidine became impossible (details will be analyzed and further discussed in the next section). The following idea was designed to avoid this problem. Starting from **88** (Scheme 4.9), a product which can be synthesized in large scale, the N^{α}-Boc protecting group was first removed by 4*N* HCl in dioxane (Scheme 4.9),[184-189] so that it could be reprotected by an other protecting group such as TFA. It turned out that deprotection of *tert*-butyl ester happened at the same time. This unexpected result could be explained by the fact that the $RNH_2 \cdot HCl$ salt did not precipitate in this reaction, which was important to avoid further deprotection of *tert*-butyl ester. Besides, the evaporation of dioxane (Bp: 100°C, which needs at least 15 min) usually at room temperature provides extra reaction time to destroy this *t*-butyl ester. A modified reaction condition (eg: reaction at 0°C and quench with $NaHCO_3$ aqueous solution) might have to be developed before this idea would work.



88 104

Scheme 4.9 The selective deprotection of Boc in presence of *t*-butyl ester

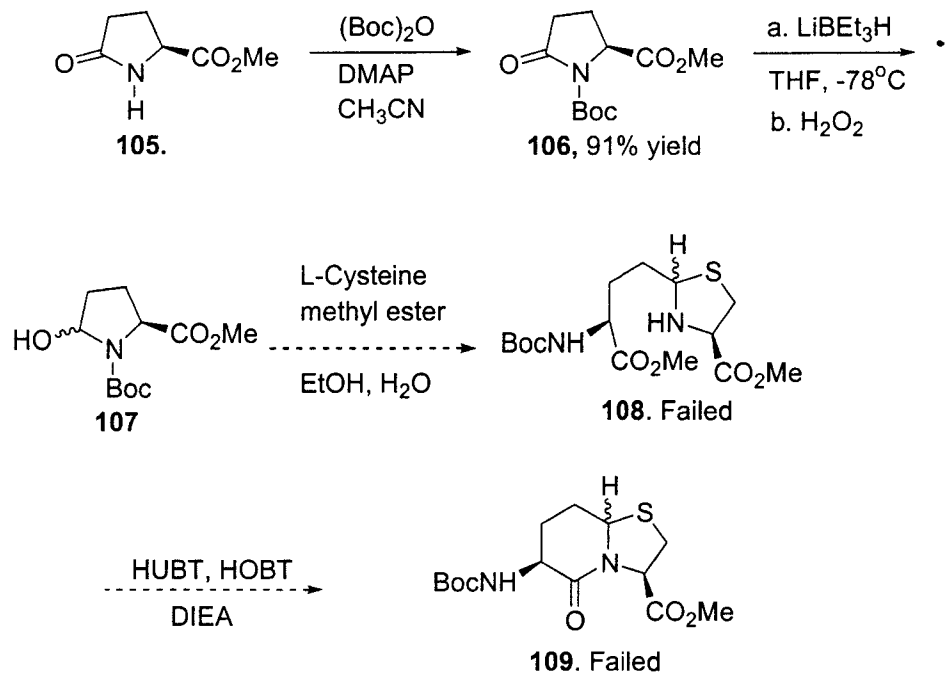
In summary, the orthogonal protection of starting material is a problem in this strategy. The *t*-butyl ester protection is necessary for clean hydroboration, while double protection of N^{α} became very difficult due to the steric problem. The oxazolidinone was good for both N and C terminal protection but is unstable for hydroboration which needs basic conditions. N^{α}-Boc is also troublesome not only due to the existence of rotamers, which makes characterization very difficult, but also the formation of hemiaminal, which is a dead end for this strategy. A racemic starting material which would give four diastereomeric bicyclic products further destroyed my little confidence in this methodology. Finally, this strategy was stopped when a novel strategy became successful.

§ 4.3 Novel methodology for [6,5]-scaffold synthesis

During the above struggles, I was thinking about and trying other methodologies, hoping that a short cut would arise toward the [6,5]-bicyclic dipeptide. According to the retrosynthetic analysis, a δ,ε-unsaturated amino acid also leads to [6,5]-*N,S*-acetal bicyclic dipeptide. At this moment, Xuejun Tang published a paper in Organic Letter.[47] In this work, synthesis of enantiomer pure β-substituted amino acids have been

developed by using Ni(II)-complex and racemic secondary bromides. Although γ,δ-unsaturated amino acids cannot be synthesized by this method due to the S_N2' instead of S_N2 reaction, δ,ε-unsaturated and other ω-unsaturated amino acids can be synthesized from racemic secondary bromides. (The details of this synthesis have been discussed in § 2.4, chapter **II**). In this section, I will focused on the methodological development of the [6,5]-scaffold from δ,ε-unsaturated amino acids.

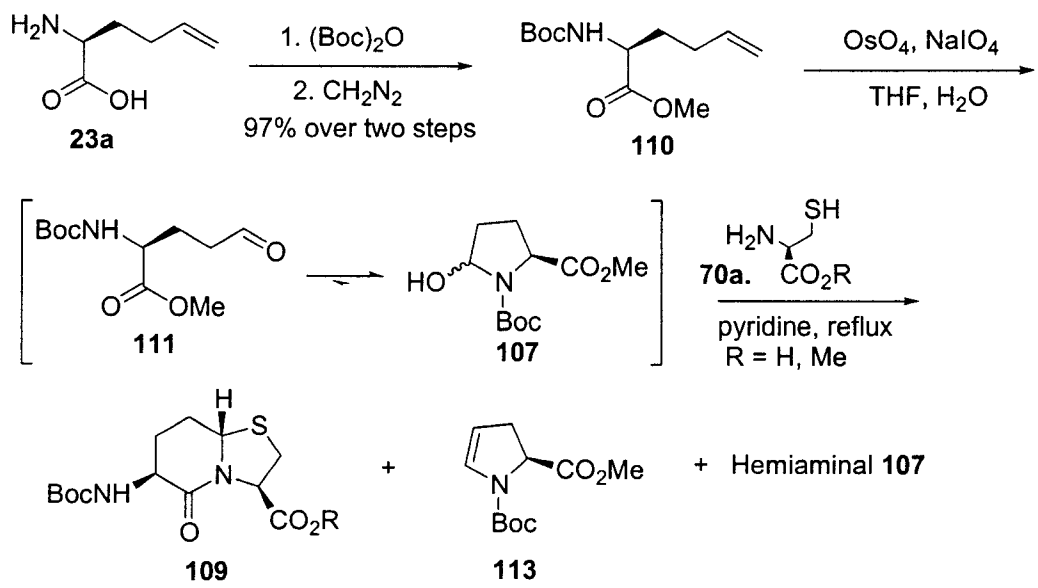
The following strategy was specially designed as a model study to help me understand the real chemistry of hemiaminals. Glutamic acid derivative **105** is a commercially available starting material and N^{α}-Boc protection can be done in 91% yield. The reduction of the amide bond by super hydride gave a hemiaminal **107** in quantitative yield after FCC.[190,191] However the attempt to convert this hemiaminal **107** to the bicyclic product **109** failed (Scheme 4.10). The failure really helped me to further notice why all the [6,5]-methodology developed in the literature used doubly protected amino group before aldehyde formation and bicyclization.



Scheme 4.10 Double check the hemiaminal chemistry

Nevertheless, the first attempt in this [6,5]-scaffold was started from the N^{α}-Boc protected amino acid. The N^{α}-terminal was protected by $(Boc)_2O$ in methanol with catalytic amount of DMAP. The carboxylic acid was esterified by diazomethane in 97% yield over two steps (Scheme 4.11).[192] Osmylation gave the aldehyde **111** which is in equilibrium with 5-membered hemiaminal **107**. This compound is exactly the same as the reduction product **107** in Scheme 4.10. At this moment I realized that the formation of hemiaminal is irreversible if the protection group is a carbamate, such as Boc, Fmoc and Cbz. Actually it has been reported when this amine was protected by amides such as benzoyl, the equilibrium did exist between aldedye and hemiaminal.[193] The ratio of

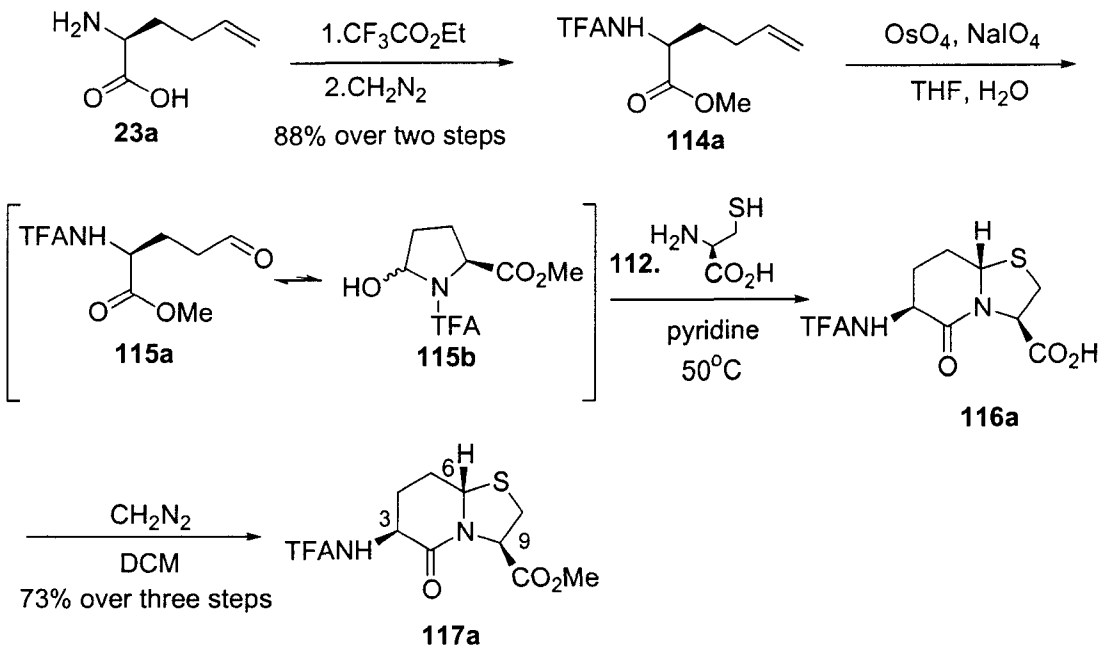
this equilibrium was close to 1:1 but subject to change when the solvent changes. The rotamer also exists at the same time, but it can be diminished as shown by ^{1}H nmr by increasing the temperature to 70°C while the equilibrium does not change. The literature reported the possibility of transforming **107** to a thiazolidine by reacting it with cysteine followed by bicyclization to **109**,[146] the real reason why I stuck with this strategy. To my knowledge, this was the only paper that reported using a mono N^{α}-protected group in [6,5]-*N,S*-acetal-δ-lactam bicyclic formation. However, this transformation was difficult, and in several attempts, the bicyclic product **109** was not obtained, while the elimination product **113** was isolated as a major product together with **107**, the starting material.



Scheme 4.11 Oxidation, thiazolidine formation, and bicyclization

It should be further noted that this hemiaminal **107** is very stable due to the electron donating ability of the oxygen in Boc. The only example to show the aldehyde being trapped by a Horner-Emmons reagent was under kinetic deprotection by using strong base.[153,154] The ^{1}H nmr spectrum in $CDCl_3$ did not show any aldehyde-H.[193] Obviously, in our case pyridine is not strong enough to deprotonate **107** and shift the equilibrium back to **111**. We were not surprise when hydroxyl eliminated and the enecarbamate **113** was isolated, which was a reported reaction in HMPA under elevated temperatures.[194]

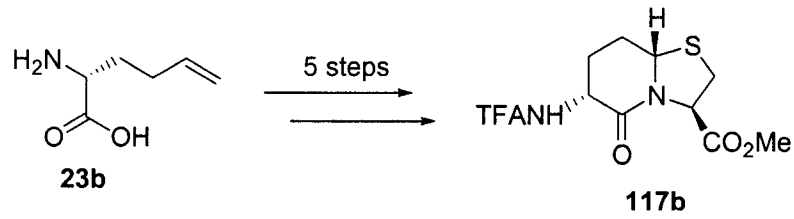
The protection of the N^{α}- once again became a critical problem in this strategy. DiBoc protection was not considered here because later we would need to introduce a β-side chain substitution which would make diBoc protection very difficult. (The oxazolidinone was not considered because the formation and deprotection need extra steps). N^{α}-Trifluoroacetyl was the final choice due to its strong electron withdrawing ability and its ready characterization and easy deprotection.[195] Compound **114a** can be generated from amino acid **23a** in 88% yield over two steps (Scheme 4.12). After osmylation, the chain-ring tautomerism of **115a-115b** actually could be observed in ^{1}H nmr in $CDCl_3$ in a ratio of 5 : 2. The diastereomers **115b** also can be observed in a ratio of 3 : 2. The thiazolidine formation was accomplished at room temperature in 6 h with help of pre-activated 3Å molecular sieves. Finally bicyclization was optimized at 50°C for 4 days.



Scheme 4.12 New strategy toward [6.5]-bicyclic dipeptide

In this strategy, free cysteine **112** with unprotected amino and mercaptan groups is necessary. *L*-Cysteine methyl ester **70a** cannot be used because a cysteine self-oligomerization will consume part of starting material under these reaction conditions. In order to purify and characterize the bicyclic product **116a,** however, it was esterified using diazomethane. Epimerization at position-3 was found to be around 10% under the refluxing pyridine solution conditions in this one flask, three bond formation processes. A byproduct **117b** was obtained in 30% if a two-step strategy (thiazolidine formation in $EtOH/H_2O$ following by DCC/HOBT coupling reaction) was used as in Scheme 3.11 of Chapter **III**. Presumably, this epimerization happened at position-3 due to the stronger acidity of α-H under the electron withdrawing ability of TFA protection group.

Fortunately, the epimerization can be diminished by using milder reaction conditions at 50°C, and the yield was improved to 73%. By starting with (2*R*)-2-amino-5-hexenoic acid, the (3*R*,6*S*,9*R*)-bicyclic product **117b** was synthesized in 5 steps in comparatable yield (Scheme 4.13). The ^{1}H nmr of this compound **117b** was used to compare the byproduct in the two-step strategy and it was found that they are identical. These two products **117a** and **117b** have been purified by HPLC using a silica gel column (IBM Silica 2872053).

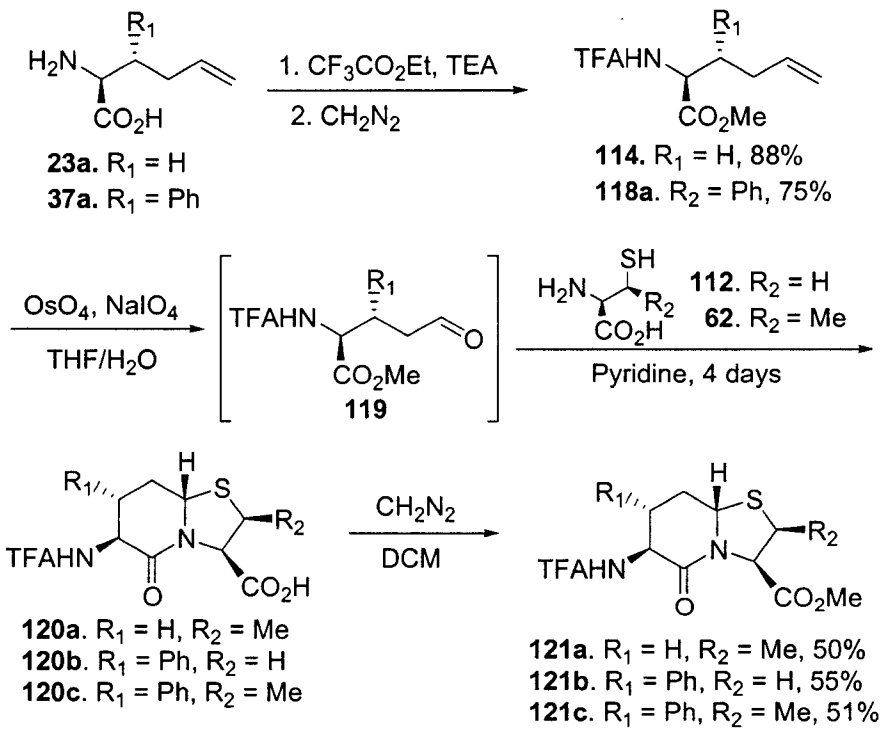


Scheme 4.13 The synthesis of [6,5]-scaffold diastereomers

§ 4.4 Side chain introduction for [6,5]-bicyclic β-turn dipeptide

After the [6,5]-scaffold methodology was developed, the introduction of a side chain group on the 6-membered ring only depended on the availability of β-subsituted δ,ε-unsaturated amino acids. The synthesis of β-phenylsubstituted amino acids was fully discussed in Chapter **II**. The α-amino functional groups were protected using CF_3CO_2Et, while the carboxyl group was converted to its methyl ester (Scheme 4.14). Osmylation and diol oxidation can be completed in 2 h in THF/H_2O. Without further purification, thiazolidine formation was accomplished in 6 h at room temperature using

pyridine in presence of pre-activated 3Å molecular sieves. The bicyclic lactam formation was accomplished in tandem at 50°C in 4 days with no epimerization. For purification and characterization, the bicyclic moieties were converted to the methylated products using diazomethane. The bicyclic products **121a-c** with different side chain groups can be synthesized in 50% yield in 3 steps from the starting materials **23a** and **37a** (Scheme 4.14). The (2*R*,3*R*)-2-amino-3-phenyl-hexonic acid **37b** also has been used in this synthesis by reacting it with cysteine **62**. The bicyclic product **122** (Figure 4.3) was generated in comparable yields. Due to the contamination of byproduct came from the diastereomer of β-methyl-*L*-cysteine, the final products of **121a, c** and **122** have to be purified on HPLC silica gel column (IBM Silica 2872053).



Scheme 4.14 Synthesis of bicyclic dipeptides with different side chain groups

§ 4.5 Stereochemistry of bridgehead Hydrogen, NMR

The [6,5]-scaffold ^{1}H nmr structures synthesized by our novel strategy were determined by use of ^{3}J coupling constants and DQF-COSY. Their stereochemistries have been assigned by nOe (Figure 4.2). The lowest energy conformation of **117b** calculated using *ab initio* methods by Jinfa Ying showed that the H_{β}' is close to the bridgehead H than H_{β} due to the sulfur's upward envelope conformation of thiazolidine. nOe results confirmed this result by comparing it with the structure **117a**. Fortunately, the configuration of the bridgehead H can be assigned without doubt by comparison of the nOe results. The formation of the upward stereochemistry of the bridge-H (*S* configuration) can be explained by its having the thermodynamically more stable 2,5-*trans*-relationship rather than the 2.5-*cis* due to steric effects in thiazolidine formation. About 3% of the putative *cis*-product was isolated, but it was not fully characterized due to the small amount obtained and lack of good purity.

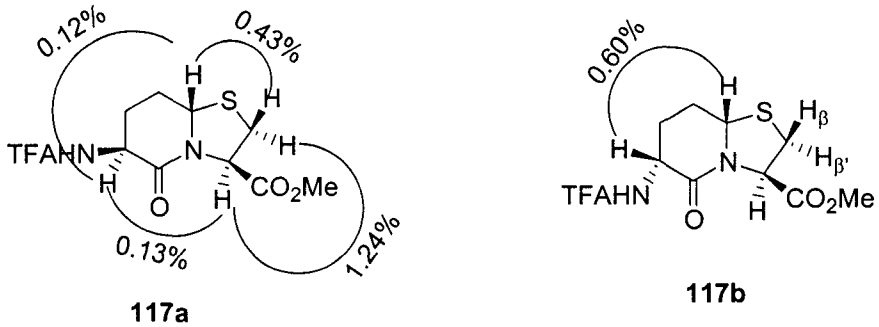


Figure 4.2 Transient nOe observed for bicyclic thiazolidine lactam

The ^{1}H nmr of the side chain groups introduced in the bicyclic structures **121a, b, c** and **122** were assigned by DQF-COSY. The ^{3}J coupling constants of H^{α} and H^{β} in **121b** and **c** are 9.2 Hz, indicated an *anti*-relationship of these two hydrogens (Figure 4.3). This further confirmed the assigned stereochemistry of the major product **37a** and **37b** from the Ni(II)-alkylation. The bridge-head **H** was assigned 'up' (S configuration) for **121a, b, c** and **122**, based on our knowledge of the *N,S*-acetal bicyclic products.[69,149,196] NMR nOe studies also confirmed this bridge-head **H** configuration (Figure 4.3). It is interesting to note that both (*R*) and (*S*) amino acids lead to only one isomer under our reaction conditions, which is different from the [5,5]-bicyclization method and results. This might be explained by suggesting that the formation of the *N,S*-thiazolidine is an equilibrium. The kinetic product isomer with the bridgehead H 'down' gradually transformed to the thermodynamic product isomer with bridgehead H 'up'.

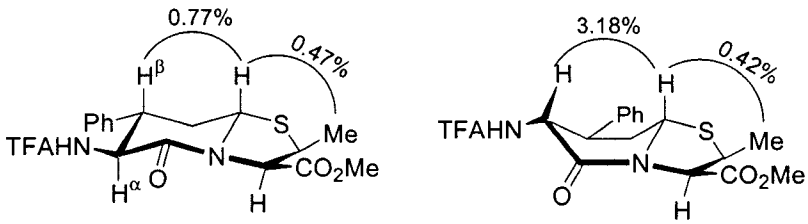


Figure 4.2 NMR coupling constant and nOe studies of bicyclic dipeptide

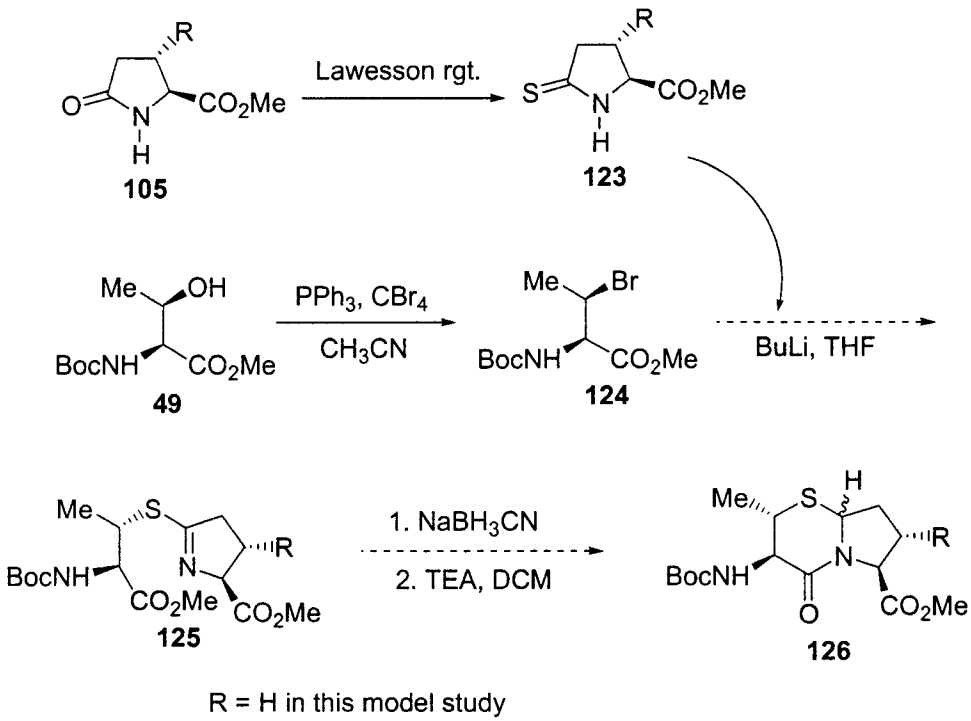
In summary, we have developed a novel and an efficient strategy toward [6,5]-bicyclic dipeptides from β-substituted δ,ε-unsaturated amino acids and β-substituted

cysteine derivatives. In this way two β-side chain groups and five controlled chiral centers have been synthesized in 6 steps. This highly efficient strategy makes it possible to introduce chiral side chain functionalities into these β-turn mimetics, which in turn can be inserted into targeted peptides in a few steps. The novel strategy we have developed was the first example in which a side chain group can be introduced into the six-membered ring. It is also the first report in which the aldehyde (for *N,S*-acetal formation) is generated from the terminal alkene.

§ 4.6 A reversed sequence in [6,5]-methodology, future work

So far we have developed methodologies to introduce side chain groups on [5,5]- and [6,5]-bicyclic β-turn dipeptide. I was thinking of the possibility to develop a similar strategy which can change the order of these two amino acids in the peptide sequence. This alternative method might be important in order to make the developed methodology universally useful. The following strategy was designed for this idea that instead of obtaining bicyclic A-B, B-A amino acid sequence would be incorporated into a peptide. This design was based on the fact that the thio-amide **123** can be easily alkylated on the S with primary and secondary halogens. The thio-amide can be synthesized in one step from glutamic acid derivatives **105**, which can be synthesized from ω-unsaturated amino acids or from the asymmetric Michael addition on Ni(II)-complex, a methodology developed by Chaozhong Cai.[46] β-Bromosubstituted amino acids can be synthesized from β-hydroxyl amino acids, which again can be synthesized from Ni(II)-aldol condensation. Then Lawesson's reaction can transfer amide **105** to

thio-amide **123** in over 90% yield (Scheme 4.15). The intramolecular neighborhood participation and β-elimination made this strategy complicated. The strategy might be modified by using N^{α}-diprotection or aziridine as an electrophile intermediate. The development of this methodology is under investigation.



Scheme 4.15 A new strategy toward B-A bicyclic dipeptide

§ 4.7 Experimental section

General information.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 MHz NMR. The chemical shifts were reported in δ, parts per million (ppm), relative to TMS (δ = 0.00

ppm) as an internal standard. In DMSO, the spectra were referenced to solvent peaks at 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C. In D_2O, ^{1}H chemical shifts were referenced to the HOD peak at 4.67 ppm, and the ^{13}C chemical shifts were indirectly referenced to $CDCl_3$ at 7.26 ppm. Coupling constants, *J*, were reported in Hertz (Hz) and refer to apparent peak multiplicities and not true coupling constants. Mass Spectrometric analyses were conducted by the Mass spectrometry Facility at the Department of Chemistry of the University of Arizona on a Jeol HX-110A. Optical rotations were measured on a JACSO P1020 polarimeter. Dichoromethane was distilled from CaH_2. All the other reagents and solvents, unless otherwise stated, are commercially available and were used as received. FCC was performed with 230-400 mesh size silica gel which was purchased from Aldrich Chemical Co. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F_{254}. Melting points (Mp) are uncorrected and were obtained in open capillaries. All the new compounds were characterized by Mp, $[\alpha]_D$, ^{1}H, ^{13}C nmr and high resolution mass spectrometry (HRMS), while the known compounds were only characterized by Mp, ^{1}H and ^{13}C nmr. Unless otherwise stated, all reactions were run under an atmosphere of argon in flame-dried glassware.

***threo*-3-*tert*-Butoxycarbonylamino-4-phenyl-tetrahydro-pyran-2-one (84)**: Step 1: β-vinyl phenylalanine methyl ester **4b** (200 mg, 0.655 mmol) was dissolved in distilled DCM (2 mL) in a 50-mL flask. Borane-methyl sulfide complex DCM solution (1*M*, 960 μL, 0.960 mmol) was added and the reaction was kept at room temperature for 10 h. Ethanol (2 mL), NaOH (3N, 1.30 mL), and H_2O_2 (30%, 24 uL) were added for an

overnight reaction. The reaction was quenched with NH_4Cl solution and was acidified by 1*N* HCl to pH = 2. The product was extracted with ether (15 mL x 3) and the combined ether solution was washed with brine and dried over $MgSO_4$. The solvent was evaporated and the sample was used in next reaction step without purification.

Step 2: The above residue was dissolved in DCM (15 mL) in 25-mL flask. 1,3-Dicyclohexylcarbodiimide (135 mg, 0.655 mmol) and 4-dimethylaminopyridine (DMAP, 4 mg, 0.0328 mmol) were added. The reaction was kept at room temperature for 2 h before it was quenched with NH_4Cl and extracted with DCM (3 x 15 mL). The combined organic solution was dried over $MgSO_4$. After evaporation the solvent, the product was purified on a silica gel column (Hexane : EtOAc = 3 : 1). The colorless product **84** (80.8 mg, 42% yield over two-step) was obtained. ^1H (500 MHz, $CDCl_3$), δ 1.33 (9H, bs), 2.23-2.32 (2H, m), 3.30 (1H, q, J = 5.5 Hz), 4.34 (1H, t, J = 10.0 Hz), 4.48-4.52 (1H, m), 4.54-4.59 (1H, m), 5.10 (1H, bs), 7.25-7.38 (5H, m). ^{13}C (125 MHz, $CDCl_3$) δ 28.1, 30.8, 43.6, 55.7, 67.6, 80.1, 127.31, 127.33, 128.7, 141.0, 155.4, 171.4; HRMS (FAB) MH^+ calcd for $C_{16}H_{22}NO_4$ 292.1549, found 292.1548.

***Tert*-Butyl *threo*-2-*tert*-Butoxycarbonylamino-3-phenyl-pent-4-enoate (86):** The acid **4b** (2.79 g, 9.58 mmol) and *t*-butyl alcohol (2.66 mL, 28.7 mmol) were first dissolved in DCM (60 mL). 1,3-Dicyclohexylcarbodiimide (2.08 g, 10.0 mmol) was added following by adding 4-dimethylaminopyridine (58.5 mg, 0.48 mmol). The reaction was kept at room temperature for 5 h before it was washed with brine and dried over $MgSO_4$. It was then concentrated and the colorless product **86** (2.35 g, 71% yield) was

obtained after flash liquid chromatography (hexane : EtOAc = 8 : 1). ^{1}H (500 MHz, $CDCl_3$), δ (rotamer): 1.23-1.41 (18H, m), 3.58 (0.5H, t, J = 8.0 Hz), 3.77 (0.5 H, t, J = 7.0 Hz), 4.50 (0.5 H, t, J = 8.5 Hz), 4.56 (0.5H, t, J = 7.0 Hz), 4.89 (0.5H, d, J = 8.5 Hz), 5.04 (0.5H, d, J = 8.5 Hz), 5.12-5.20 (2H, m), 6.02-6.13 (1H, m), 7.23 (2H, d, J = 7.0 Hz), 7.26-7.32 (3H, m). ^{13}C (125 MHz, $CDCl_3$) δ (rotamer): 27.6, 27.9, 28.18, 28.22, 53.4, 53.9, 57.8, 57.9, 79.6, 81.7, 82.0, 117.6, 127.0, 128.31, 128.34, 128.4, 136.5, 136.9, 139.2, 139.5, 155.17, 155.20, 170.3, 170.6; HRMS (FAB) MH^+ calcd for $C_{20}H_{30}NO_4$ 348.2175, found 348.2172.

***Tert*-Butyl *threo*-2-*tert*-Butoxycarbonylamino-5-hydroxy-3-phenyl-pentanoate (88).** The hydroboration reaction was performed using the same procedure as in the synthesis of **84**, step 1. The extracted ether solution was concentrated under vacuum and purified on the silica gel column (hexane : EtOAc = 3 : 1). A colorless product (71% yield) was obtained. ^{1}H nmr, 500 MHz, $CDCl_3$, δ(ppm) (rotamer): 1.24-1.47 (18H, m), 1.96-2.02 (2H, m), 2.14-2.18 (1H, m), 3.09-3.13 (1H, m), 3.41-3.50 (1H, m), 3.65-3.67 (1H, m), 4.46 (1H, bs), 4.96-4.98 (0.3 H, m), 5.20 (0.7 H, bs), 7.22-7.35 (5H, m). ^{13}C, 125 MHz, $CDCl_3$, δ (ppm) (rotamer): 26.1, 26.8, 27.2, 27.8, 28.1, 28.8, 29.8, 34.5, 57.5, 58.6, 60.3, 61.5, 78.0, 79.9, 81.9, 126.54, 127.7, 127.8, 128.1, 128.9, 129.0, 129.4, 139.5, 155.4, 170.6; HRMS (FAB) MH^+ calcd for $C_{20}H_{32}NO_4$ 366.2280, found 366.2274.

3-Benzyloxycarbonylamino-propionaldehyde (93):

Oxidation by oxoammonium salt (94): The starting material **92** (41.9 mg, 0.20 mmol) was first mixed with silica gel (80 mg) in distilled DCM (2 mL). It was stirred for about 5 min before oxoammonium salt **94** was added. The reaction was kept at room temperature 1 day before it was filtered through silica gel and washed with DCM. Colorless product was collected (7.2 mg, 17% yield).

Dess-Martin reaction. The starting material **92** (115 mg, 0.550 mmol) was dissolved in DCM (3 mL) in a 25-mL flask and Dess-Martin reagent (465 mg, 1.10 mmol) was added. The reaction was kept at room temperature for 4 h. A mixture of Et_2O (4 mL), saturated $NaHCO_3$ (3 mL) and saturated $Na_2S_2O_3$ (3 mL) aqueous solution were added to the above mixture until both phases became clear. After separation, the aqueous phase was extracted with ether (2 x 20 mL). The combined ether phase was washed with brine and dried over $MgSO_4$. It was concentrated and colorless product (58 mg, 51%) was collected.

PCC oxidation: The starting material **92** (200 mg, 0.957 mmol) was dissolved in DCM (10 mL) in a 50-mL flask. PCC (515 mg, 2.39 mmol) and $NaHCO_3$ (515 mg) were added and the reaction was kept at room temperature for 2 h. The reaction mixture was then filtered through Celite and washed with DCM. The DCM solution was then evaporated and the product (100 mg, 50%) was collected.

Swern Oxidation. Oxalyl chloride (717 μL, 1.43 mmol) was added to distilled DCM in a 25-mL flask and cooled down to -78°C for 5 min before DMSO (136 μL, 1.91 mmol) was added. Stirring vigorously for 10 min before the starting material **92** (200 mg,

0.957 mmol) in DCM (1 mL) was added dropwisely. The reaction was kept for 15 min before DIEA (666 μL, 3.82 mmol) and H_2O (10 mL) were added. The mixture was warm up to room temperature. The DCM solution was separated and the aqueous solution was extracted with DCM (2 x 10 mL). The combined DCM solution was washed with brine and dried over $MgSO_4$. A colorless product (146 mg, 73% yield) was obtained.

^1H (500 MHz, $CDCl_3$) δ 2.53-2.73 (2H, m), 3.44-3.50 (2H, M), 5.09-5.15 (2h, M), 5.35 (1H, bs), 7.32-7.34 (5H, m), 9.78 (1H, s); ^{13}C (125 MHz, $CDCl_3$) δ 34.2, 36.3, 66.8, 128.0, 128.1, 128.5, 136.3, 156.3, 177.1; HRMS (FAB) MH^+ calcd for $C_{20}H_{30}NO_4$ 348.2175, found 348.2172.

3-*tert*-Butoxycarbonyl-4-(1-phenyl-allyl)-oxazolidin-5-one (98): The Kazmaier product **4b** (1.0 g, 3.43 mmol) was dissolved in benzene (50 mL) in a 100-mL flask. Paraformaldehyde (206 mg) and *para*-toluenesulfonic acid (65 mg, 0.34 mmol) were added. The reaction was refluxed for 1.2 h, and cooled down to room temperature. It was washed with aqueous NH_4Cl and the product was purified on the silica gel column. A colorless product was obtained (735 mg, 71% yield) after evaporation of the solvent. ^1H (500 MHz, $CDCl_3$) δ 1.52 (9H, bs), 4.03 (1H, bs), 4.48 (1H, d, J = 4.5 Hz), 4.56 (1H, bs), 5.24-5.28 (2H, m), 5.38 (1H, bs), 6.32 (1H, bs), 7.24-7.34 (5H, m). ^{13}C, 125 MHz, $CDCl_3$, δ 28.2, 40.0 (m), 51.8, 61.2 (m), 78.3, 118.5, 127.5, 128.5, 128.6, 135.8, 152.4, 171.3; HRMS (FAB) MH^+ calcd for $C_{17}H_{22}NO_4$ 304.1549, found 304.1544.

Methyl (*S*)-1-*tert*-Butoxycarbonyl-5-oxo-pyrrolidine-2-carboxyliate (106): This reaction was performed as for the synthesis of **49** in chapter **III**. It was completed in an overnight reaction with 91% yield. $[\alpha]^{24}_{D}$ = -155° (c 5.51, $CHCl_3$), ^1H (500 MHz, $CDCl_3$), δ 1.48-1.50 (9H, m), 2.01-2.06 (1H, m), 2.30-2.35 (1H, m), 2.46-2.53 (1H, m), 2.60-2.65 (1H, m), 3.77-3.79 (3H, m), 4.61-4.63 (1H, m). ^{13}C (125 MHz, $CDCl_3$), δ 21.4, 27.8, 31.1, 52.5, 58.8, 83.5, 149.2, 171.8, 173.1.

Methyl (*S*)-2-*tert*-butoxycarbonylamino-hex-5-enoate (110): The Boc protection was performed as in the synthesis of **49** in Chapter **III**. The methylesterification was performed as in the synthesis of **114** in this chapter. $[\alpha]^{24}_{D}$ = +3.8° (*c* 2.13, $CHCl_3$); ^1H nmr, 500 MHz, $CDCl_3$, δ(ppm): 1.46 (9H, bs), 1.70-1.76 (1H, m), 1.89-1.93 (1H, m), 2.11-2.14 (2H, m), 3.74 (3H, s), 4.33 (1H, m), 5.00-5.07 (3H, m), 5.75-5.83 (1H, m), ^{13}C, 125 MHz, $CDCl_3$, δ (ppm): 28.1, 29.3, 31.7, 52.0, 52.8, 79.5, 115.5, 136.8, 155.2, 173.1.

General procedure for N^{α}-TFA protection:

The amino acid (1 eq) was suspended in methanol (2 mL/mmol) in a flask and ethyl trifluoroacetate (1.5 eq) and TEA (2 eq) were added. The reaction was kept at room temperature overnight and the starting material was dissolved (for β-substituted amino acid, a 4 day reaction was employed). The product was concentrated in *vacuo* and the crude material was purified by flash liquid chromatograph (Hexane-EtOAc = 3 : 1) to give a colorless product.

(*S*)-2-(2,2,2-trifluoro-acetylamino)-5-hexenoic acid (23a'): 91% yield, Mp: 64-66°C; $[\alpha]^{24}_D$ = +65.8° (*c* 0.856, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.92-1.98 (1H, m), 2.10-2.20 (3H, m), 4.67-4.71 (1H, m), 5.06 (1H, d, *J* = 11.0 Hz), 5.09 (1H, d, *J* = 18.0 Hz), 5.74-5.83 (1H, m), 7.08 (1H, bs), 10.73 (1H, bs); ^{13}C (125 MHz, $CDCl_3$), δ 29.2, 30.6, 115.6 (q, *J* = 1143.0 Hz), 116.7, 135.9, 157.4 (q, *J* = 151.0 Hz), 157.9; HRMS (FAB) MH^+ calcd for $C_8H_{10}F_3NO_3$ 226.0691, found 226.0692.

(*R*)-2-(2,2,2-trifluoro-acetylamino)-5-hexenoic acid (23b'): yield 85%, mp 64.5-66°C, $[\alpha]^{24}_D$ = -65.0° (*c* 1.26, $CHCl_3$); ^1H and ^{13}C ($CDCl_3$) spectra are identical to (*S*)-product.

(2*S*, 3*S*)-2-(2,2,2-Trifluoro-acetylamino)-3-phenyl-5-hexenoic acid (37a'): 77% yield, $[\alpha]^{24}_D$ = +45.3° (*c* 2.41, H_2O); ^1H (500 MHz, $CDCl_3$), δ 2.63-2.68 (1H, m), 2.71-2.76 (1H, m), 3.26 (1H, dt, *J* = 5.5, 7.0 Hz), 4.93 (1H, dd, *J* = 5.5, 8.0 Hz), 5.06 (1H, d, *J* = 10.0 Hz), 5.14 (1H, dd, *J* = 1.0, 17.0 Hz), 5.20 (1H, bs), 5.67-5.75 (1H, m), 6.76 (1H, d, *J* = 8.0 Hz), 7.13 (2H, d, *J* = 7.0 Hz), 7.29-7.35 (3H, m); ^{13}C (125 MHz, $CDCl_3$), δ 35.3, 47.6, 56.1, 115.5 (q, *J* = 286.1 Hz), 118.0, 128.1, 128.9, 134.8, 137.6, 156.5 (q, *J* = 37.8 Hz); HRMS (FAB) MH^+ calcd for $C_{14}H_{14}F_3NO_3$ 302.1004, found 302.1007.

(2*R*, 3*R*)-2-(2,2,2-Trifluoro-acetylamino)-3-phenyl-5-hexenoic acid (37b'): yield 80%, $[\alpha]^{24}_D$ = -45.0° (*c* 2.26, $CHCl_3$); ^1H and ^{13}C ($CDCl_3$) spectra are identical to (*S*)-product.

General procedure for carboxylic methyl ester protection:

Preparation of diazomethane: A mixture of ether (1 mL/mmol) and KOH (40%wt, 0.3 mL/mmol) was prepared in a 20-mL vial and cooled down to 0°C in ice-water bath. *N*-nitrosomethylurea (1.0 eq) was added in small portion as rapidly as it could dissolve with vigorous stirring. After all the solid was added and dissolved, it was cooled down by acetone-dry ice in order to freeze the water phase. The ether phase then was transferred to a 20-mL vial containing a piece of solid KOH. The solution was dried in the refrigerator for 2.5 hours before used.

The amino acid (1 eq) was dissolved in distilled DCM (4 mL/mmol) and cooled down to 0°C and the above diazomethane ether (6-7 eq) solution was added slowly. The reaction was kept at 0°C for 15 min and the solvent was evaporated. The residue was purified by flash liquid chromatograph (Hexane : EtOAc = 8 : 1) to a colorless liquid which was collected after concentration.

Methyl (*S*)-2-(2,2,2-trifluoro-acetylamino)-hex-5-enoate (114a): 97% yield, $[\alpha]^{24}_{D}$ = +52.7° (*c* 1.33, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.85-1.92 (1H, m), 2.03-2.14 (3H, m), 3.81 (3H, s), 4.66 (1H, dt, *J* = 5.0, 7.5 Hz), 5.04 (1H, d, *J* = 6.0 Hz), 5.08 (1H, d, *J* = 18.5 Hz), 5.07-5.80 (1H, m), 6.88 (1H, bs); ^{13}C (125 MHz, $CDCl_3$), δ 29.1, 30.9, 52.2, 52.8, 115.6 (q, *J* = 1144.0 Hz), 116.3, 136.2, 156.8 (q, *J* = 150.0 Hz), 171.3; HRMS (FAB) MH^+ calcd for $C_9H_{12}F_3NO_3$ 240.0848, found 240.0857.

Methyl (*R*)-2-(2,2,2-trifluoro-acetylamino)-hex-5-enoate (114b): yield 98%, $[\alpha]^{24}_{D}$ = -51.7° (*c* 1.58, $CHCl_3$); ^1H and ^{13}C ($CDCl_3$) spectra are identical to (*S*)-product.

Methyl (2*S*,3*S*)-2-(2,2,2-Trifluoro-acetylamino)-3-phenyl-5-hexenoate (118a): 97% yield, $[\alpha]^{24}_D$ = +34.4° (*c* 2.16, H_2O); ^1H (500 MHz, DMSO), δ 2.59-2.70 (2H, m), 3.19 (1H, dt, *J* = 6.0, 8.5 Hz), 3.65 (3H, s), 4.88 (1H, dd, *J* = 6.5, 7.5 Hz), 5.02 (1H, dd, *J* = 1.5, 10.0 Hz), 5.10 (1H, dd, *J* = 1.5, 17.0 Hz), 5.65-5.71 (1H, m), 6.84 (1H, d, *J* = 7.5 Hz), 7.06 (2H, d, *J* = 7.0 Hz), 7.26-7.34 (3H, m); ^{13}C (125 MHz, DMSO), δ 35.5, 48.1, 52.6, 56.6, 115.6 (q, *J* = 286.1 Hz), 117.7, 127.9, 128.1, 128.7, 135.0, 137.9, 156.4 (q, *J* = 37.5 Hz); HRMS (FAB) MH^+ calcd for $C_{15}H_{16}F_3NO_3$ 302.1161, found 316.1148.

Methyl (2*R*,3*R*)-2-(2,2,2-Trifluoro-acetylamino)-3-phenyl-5-hexenoate (118b): yield 98%, $[\alpha]^{24}_D$ = -33.7° (*c* 2.56, $CHCl_3$); ^1H and ^{13}C ($CDCl_3$) spectra are identical to (*S*)-product.

General procedure of Osmylation, *N,S*-acetal formation and bicyclization, methylesterification:

Fully protected amino acid (1 eq) was dissolved in a THF/H_2O (2/1, 4 mL/mmol) mixture in a flask. The hood lights were turned off and the flask was covered by aluminum foil. Osmium tetroxide (5% mmol) was added to the flask. After 5 min, $NaIO_4$ (2.5 eq) was added in small portions over a 15 min period. The reaction was kept at room temperature for 4 h before it was filtered and the THF was evaporated. The residue was dissolved in EtOAc and saturated NH_4Cl aqueous solution. The aqueous phase was extracted with EtOAc (2 x 10 mL/mmol) and the organic phases were combined, washed with brine, and dried over anhydrous $MgSO_4$. The solution was

concentrated in *vacuo* and the crude material was used in the next reaction without further purification.

The above crude sample was dissolved in pyridine (30 mL/mmol) and *L*-cysteine **112** or cysteine derivatives **62** (1.2 eq) was added. 4 Å Molecular sieves (500 mg/mmol) were pre-activated at 200°C for 2 h in *vacuo*. They were cooled down to room temperature in argon and added to the above solution. The reaction was kept at room temperature for 5 h before the temperature was elevated to 50°C and kept for 4 days. The solution was decanted and concentrated in *vacuo*. The residue was dissolved in EtOAc and was washed with 1*N* HCl and saturated $NaHCO_3$ aqueous solution. The organic solution was concentrated before use in the next step without purification.

The residue was dissolved in DCM (5 mL/mmol) and cooled down to 0°C and then diazomethane (6 eq) was added. The reaction was kept for 15 min before it was warmed up to room temperature. It was concentrated and purified by flash liquid chromatograph (Hexane : EtOAc = 3 : 1) to give the product.

Methyl (3*S*, 6*S*, 9*R*)-1-Aza-3-(2,2,2-trifluoroacetylamino)-2-oxo-7-thiabicyclic [4,3,0]-nonane-9-carboxylate (117a): It was concentrated and purified by FCC (Hexane : EtOAc = 3 : 1) to give the product (101.7 mg, 73%). $[\alpha]^{24}_D$ = -151° (*c* 0.67, $CHCl_3$); ^1H (500 MHz, $CDCl_3$) δ 1.86-2.20 (2H, m), 2.41-2.45 (1H, m), 2.72-2.75 (1H, m), 3.19 (1H, dd, *J* = 5.5, 11.5 Hz), 3.41 (1H, dd, *J* = 8.5, 12.0 Hz), 3.80 (3H, s), 4.40-4.44 (1H, m), 4.95 (1H, dd, *J* = 4.0, 10.5 Hz), 5.10 (1H, dd, *J* = 5.5, 8.5 Hz), 7.19 (1H, bs). ^{13}C (125 MHz, $CDCl_3$) δ 26.7, 27.2, 31.9, 51.2, 53.0, 60.5, 62.8, 115.6 (q, *J* =

1144.5 Hz), 157.5 (q, J = 150.0 Hz), 165.6, 170.2; HRMS (FAB) MH^+ calcd for $C_{11}H_{13}F_3N_2O_4S$ 327.0626, found 327.0626.

Methyl (3*R*,6*S*,9*R*)-1-Aza-3-(2,2,2-trifluoroacetylamino)-2-oxo-7-thiabicyclic [4,3,0] nonane-9-carboxylate (117b): The reaction and work up was performed as above and yield was 64%. $[\alpha]^{24}_D$ = -196° (*c* 0.58, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.72-1.80 (1H, m), 2.05-2.10 (1H, m), 2.38-2.45 (1H, m), 2.61-2.67 (1H, m), 3.28 (1H, dd, J = 4.5, 11.5 Hz), 3.33 (1H, dd, J = 7.5, 11.5 Hz), 3.79 (3H, s), 4.40-4.45 (1H, m), 4.99 (1H, t, J = 6.0 Hz), 5.37 (1H, dd, J = 5.0, 7.5 Hz), 7.49 (1H, bs). ^{13}C (125 MHz, $CDCl_3$), δ 23.6, 25.9, 31.7, 50.0, 53.0, 60.5, 60.6, 115.5 (q, J = 1144.0 Hz), 157.0 (q, J = 150.0 Hz), 167.0, 169.7; HRMS (FAB) MH^+ calcd for $C_{11}H_{13}F_3N_2O_4S$ 327.0626, found 327.0618.

Methyl (3*S*, 6*S*, 8S, 9*R*)-3-(2,2,2-Trifluoroacetylamino)-8-methyl-1-aza-2-oxo-7-thiabicyclic [4,3,0] nonane-9-carboxylate (121a): $[\alpha]^{24}_D$ = -50.6° (*c* 1.21, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.54 (1H, d, J = 6.5 Hz), 1.83-1.91 (1H, m), 1.93-2.02 (1H, m), 2.39-2.44 (2H, m), 2.73-2.75(1H, m), 3.71 (1H, p, J = 6.5 Hz), 3.81 (3H, s), 4.43 (1H, dt, J = 6.5, 11.5 Hz), 4.56 (1H, d, J = 4.0, 6.5 Hz), 5.04 (1H, dd, J = 4.5, 11.0 Hz), 7.19 (1H, bs). ^{13}C (125 MHz, $CDCl_3$) δ 20.3, 26.6, 27.3, 44.0, 51.1, 52.9, 63.7, 67.6, 115.6 (q, J = 286.2 Hz), 157.5 (q, J = 37.4 Hz), 165.6, 170.1; HRMS (FAB) MH^+ calcd for $C_{12}H_{15}F_3N_2O_4S$ 341.0783, found 341.0783.

Methyl (3*S*, 4S, 6*S*, 9*R*)-3-(2,2,2-Trifluoroacetylamino)-4-phenyl-1-aza-2-oxo-7-thiabicyclic [4,3,0] nonane-9-carboxylate (121b): $[\alpha]^{24}_{D}$ = +40.9° (*c* 1.28, $CHCl_3$); ^1H (500 MHz, $CDCl_3$) δ 2.37-2.44 (1H, m), 2.80-2.85 (1H, m), 3.39 (1H, p, *J* = 12.5 Hz), 3.51 (1H, dd, *J* = 6.5, 12.5 Hz), 3.89 (3H, s), 4.16 (1H, d, *J* = 9.5 Hz), 4.71 (1H, dd, *J* = 6.0, 9.0 Hz), 5.07 (1H, d, *J* = 6.5 Hz), 5.12 (1H, dd, *J* = 3.0, 12.0 Hz), 6.95 (1H, d, *J* = 6.0), 7.26-7.36 (5H, m). ^{13}C (125 MHz, $CDCl_3$) δ 33.7, 37.8, 42.0, 52.5, 53.1, 60.1, 62.2, 115.2 (q, *J* = 285.9 Hz), 127.7, 128.4, 128.9, 139.4, 156.5 (q, *J* = 37.8 Hz), 166.1, 169.2; HRMS (FAB) MH^+ calcd for $C_{17}H_{17}F_3N_2O_4S$ 403.0939, found 403.0927.

Methyl (3*S*, 4*S*, 6*S*, 8*S*, 9*R*)-3-(2,2,2-Trifluoroacetylamino)-4-phenyl-8-methyl-1-aza-2-oxo-7-thiabicyclic [4,3,0] nonane-9-carboxylate (121c): $[\alpha]^{24}_{D}$ = +59.9° (*c* 1.28, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.58 (3H, d, *J* = 7.0 Hz), 2.37-2.43 (1H, m), 2.77-2.81 (1H, m), 3.85-3.89 (1H, m), 3.87 (3H, s), 4.15 (1H, q, *J* = 9.0 Hz), 4.75-4.78 (2H, m), 5.22 (1H, dd, *J* = 2.5, 12.0 Hz), 6.94 (1H, d, *J* = 5.0 Hz), 7.24-7.34 (5H, m). ^{13}C (125 MHz, $CDCl_3$), δ 22.9, 38.0, 42.0, 44.7, 52.4, 53.0, 58.8, 69.0, 115.2 (q, *J* = 285.8 Hz), 127.7, 128.4, 128.9, 139.4, 156.5 (q, *J* = 38.3 Hz), 166.7, 169.0; HRMS (FAB) MH^+ calcd for $C_{18}H_{19}F_3N_2O_4S$ 417.1096, found 417.1102.

Methyl (3*R*, 4*R*, 6*S*, 8*S*, 9*R*)-3-(2,2,2-Trifluoroacetylamino)-4-phenyl-8-methyl-1-aza-2-oxo-7-thiabicyclic [4,3,0] nonane-9-carboxylate (122): $[\alpha]^{24}_{D}$ = -69.3° (*c* 2.95, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.57 (3H, d, *J* = 7.0 Hz), 2.36-2.43 (1H, m), 2.76-2.81 (1H, m), 3.85-3.91 (1H, m), 3.87 (3H, s), 4.14 (1H, q, *J* = 9.0 Hz), 4.75-4.78 (2H, m),

5.22 (1H, dd, J = 3.0, 11.5 Hz), 6.93 (1H, d, J = 5.5 Hz), 7.24-7.34 (5H, m). ^{13}C, 125 MHz, $CDCl_3$, δ (ppm): 22.9, 38.0, 41.9, 44.7, 52.4, 53.0, 58.8, 69.0, 115.2 (q, J = 285.9 Hz), 127.7, 128.4, 128.9, 139.4, 156.4 (q, J = 37.6 Hz), 166.6, 169.0; HRMS (FAB) MH^+ calcd for $C_{18}H_{19}F_3N_2O_4S$ 417.1096, found 417.1089.

Methyl (*S*)-5-Thioxo-pyrrolidine-2-carboxylate (123): Methyl (*S*)-5-Oxo-pyrrolidine-2-carboxylate **105** (350 mg, 2.45 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide **15** (Lawesson's reagent, 592 mg, 1.46 mmol) were dissolved in benzene (50 mL) in a 100-mL flask. It was heated to reflux for 30 min. After cooling down to room temperature, the solvent was evaporated and product mixture was purified on a silica gel column. A colorless product was obtained (351 mg, 90%). $[\alpha]^{24}_D$ = +3.8° (*c* 2.13, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 2.33-2.39 (1H, m), 2.35-2.61 (1H, m), 2.91-3.02 (2 H, m), 3.80 (3 H, s), 4.55 (1H, dd, J = 6.0, 9.0 Hz), 8.47 (1H, bs); ^{13}C (125 MHz, $CDCl_3$) δ 27.0, 42.6, 52.8, 62.4, 170.5, 206.5.

Methyl (2*S*, 3*S*)-3-Bromo-2-*tert*-butoxycarbonylamino-butyrate (124): Methyl (2*S*,3*R*)-2-*tert*-butoxycarbonylamino-3-hydroxy-butyrate **49** (65.5 mg, 0.281 mmol) was dissolved in acetonitrile (3.0 mL) in a 10-mL flask. The solution was then cooled down to 0°C and triphenylphosphine (81 mg, 0.309 mmol) was added in one portion. Carbon tetrabromide was added (102 mg, 0.309 mmol) in small portions every minute. The reaction was warmed up to room temperature and kept for 24 h before it was concentrated and purified on a silica gel column (hexane : EtOAc = 8 : 1). A colorless

product was collected (54 mg, 65%). $[\alpha]^{24}_{D}$ = +186° (*c* 3.98, $CHCl_3$); ^1H (500 MHz, $CDCl_3$), δ 1.45 (9H, bs), 1.80 (3H, d, *J* = 7.0 Hz), 3.80 (3 H, bs), 4.33-4,35 (1 H, m), 4.53-4.55 (1H, m), 5.41 (1H, d, *J* = 7.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 22.8, 28.2, 49.7, 52.5, 59.3, 80.4, 154.9, 169.3; HRMS (FAB) MH^+ calcd for $C_{10}H_{19}BrNO_4$ 296.0497, found 296.0497.

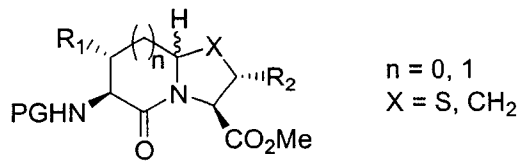
CHAPTER V.

BTD[2,3]-LEU-ENKEPHALIN

SOLID PHASE PEPTIDE SYNTHESIS

§ 5.1 General problems encountered in bicyclic dipeptide β-turn mimetics synthesis

We have developed a synthetic methodology for β-substituted (R_1) γ,δ- and δ,ε-unsaturated amino acids and their application to side chain group introduction for [5,5]- and [6,5]-*N,S*-acetal bicyclic dipeptides (X = S, Figure 5.1),[149,196] and the C-scaffold bicyclic system (X = CH_2) from the glutamic amino acid derivatives.[197,198] Besides, our success in both aromatic and aliphatic β-substituted (R_2) cysteine made it possible to introduce side chain groups in the thiazolidine ring.[32,33] We appeared to be ready to look for the targeted peptides and to start the synthesis of these BTD as a β-turn mimetic in peptides to constrain the secondary conformation and to improve their biological activities.



127

Figure 5.1. The bicyclic dipeptides synthesized in our group

On the other hand, we noticed that although a few groups also were working on the BTD mimetics, their work was limited to the synthesis of the unsubstituted bicyclic peptide moiety. I thought there were some good reasons why these groups limited their research to unsubstituted BTD synthesis. Their synthetic strategies were not good (robust) enough for peptide synthesis. This could either be not good enough in quality (without side chain functionality) or they did not have significant amounts to accomplish the peptide synthesis. In solid phase synthesis, we need at least several hundred milligrams per isomer to start with. Practically it is hard to generate gram scale enantiomer pure functionalized BTD products after multistep synthesis. Another important reason was that these synthetic strategies did not have efficient and systematic way to provide all the possible diastereoisomers, the third generation of peptidomimetics. These BTD inserted peptides with only topographical differences would probably show significant differences in biological activities and eventually give us some details of 3-D structural-activity relationship with the help of NMR conformational study, computer modeling study and X-ray crystallography.

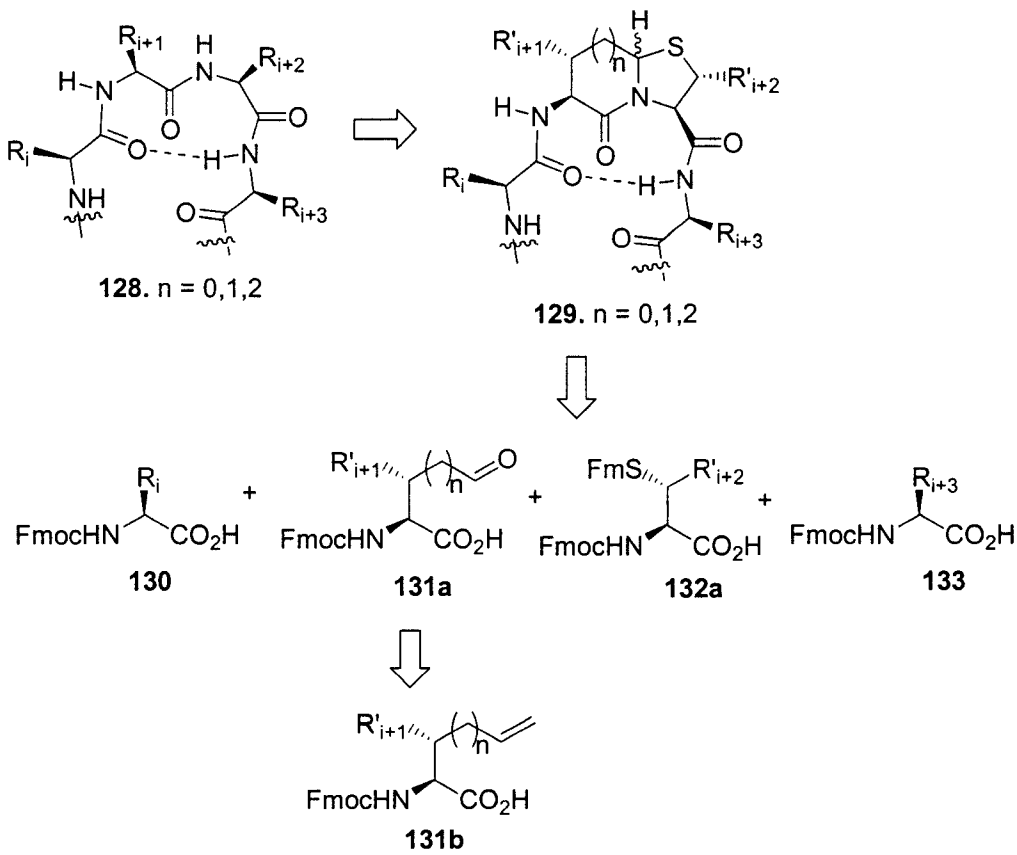
Although we had developed novel strategies for [5,5]- and [6,5]-bicyclic dipeptides with different side chain groups at appropriate positions, we still have the same problems to synthesize all possible chirality isomers in solution phase. Recently, a lot of effort has been focused on the β-turn peptidomimetics and its library build-up.[18,28] Several types of internal β-turn mimetics have been developed,[17,35,36,199-202] some of them have been practically used in search for library generation in combinatorial fashion using solid phase synthesis. On the other hand, external bicyclic β-turn

dipeptide (BTD) is especially interesting because it can control not only the ψ and ϕ dihedral angels on the back bone but also the χ_1 and χ_2 angles for the side chain groups (Scheme 5.1). However, the systematic synthesis of external bicyclic β-turn dipeptide on solid phase supports has not been reported. Because of the lack of an efficient methodology to collecting the structural library, most of work is stopped at bicyclic dipeptide stage. It is demanded for us to develop a practical and systematic methodology which can provide 16 diastereomers (four chiral centers involved) for both [5,5]-, and [6,5]-BTD inserted targeted peptides.

§ 5.2 A novel proposal for BTD inserted SPPS

In the last few years, our group has been working on different sizes of BTD mimetics in order to have a better understanding of the molecular basis of peptide and protein interaction. We like this external BTD inserted peptide design **129** because it keeps the original peptide backbone and side chain group positions unchanged (Scheme 5.1). The structure **129** is only one example among 16 possible diastereomers. BTD also can be used as a dipeptide or inserted into a tetrapeptide or a longer peptide at N, C terminal or in most of the cases in the middle of peptide. We were imagining if the BTD inserted peptide could be synthesized on the solid phase just like the peptides we were routinely making. A retrosynthetic analysis of this concept finds that these BTD inserted peptides actually need two non-standard amino acids (Scheme 5.1). One is the β-substituted ω-aldehyde **131a**, which can be synthesized one step from β-substituted ω-

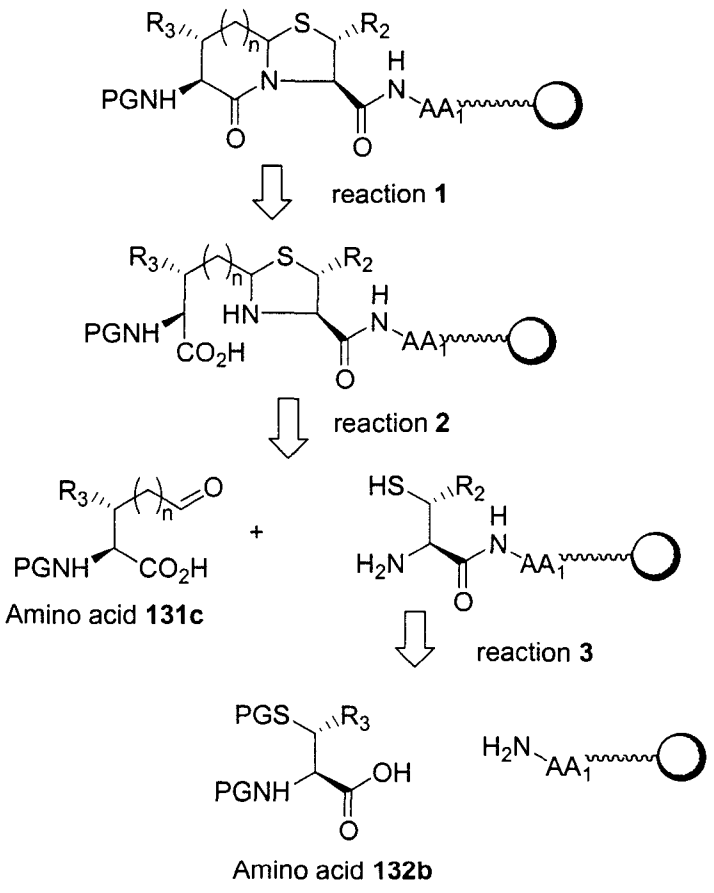
unsaturated amino acid **131b**, and the other is the β-substituted cysteine **132a**. Both of them can be synthesized by the methodology we have recently developed.



Scheme 5.1 The retrosynthetic concept of BTD inserted peptide synthesis

The detailed formation of a BTD by solid phase methods can be further illustrated in the following retrosynthetic analysis (Scheme 5.2). Three nonconventional reactions were involved for the synthesis of bicyclic β-turn dipeptide. The first nonconventional reaction is actually an intramolecular lactamization. The reaction condition should be the same as the conventional coupling reaction conditions. The second reaction, the

intermolecular *N,S*-thiazolidine formation on solid phase have not been reported, although the imine is readily formed between an aldehyde and a primary amine.[203] The third reaction is a deprotection of both N^{α}-Fmoc and S-Fm, which is possible by using high concentration of piperidine or long reaction time.[204] This three-step nonconvensional strategy for BTD on solid phase is totally novel and would open an area in *N,S*-acetal bicyclic formation. This strategy not only makes all chiralities and all



Scheme 5.2 The detailed analysis of BTD formation on solid phase

different sizes of BTD synthesis on solid phase become possible, but also simplifies the purification of the thiazolidine and bicyclic formation.

§ 5.3 Syntheses of nonstandard amino acids

To examine this novel concept we chose the pentapeptide Leu-enkephalin as a targeted peptide (Figure 5.2). Enkephalin is a morphine-like compound and its δ and μ agonist activity has been extensively studied.[205-208] Previous work indicated that although enkephalin itself exists as a random structure in aqueous solution, the existing β-turn conformation at the Gly-Gly[2,3] positions is important for its biological selectivity.[209,210] (3*S*, 6*S*, 9*R*)-[4.3.0]-BTD[2,3]-inserted Leu-enkephalin has been reported on solid phase by conventional method,[211-213] however, one isomer of this peptide could not give the whole picture. I decided to illustrate the above novel strategy by synthesis of Leu-enkephalin analogues. In this synthesis, both non-standard amino acids **131c** and **132b** can be synthesized from commercially available compounds by straight forward methods.

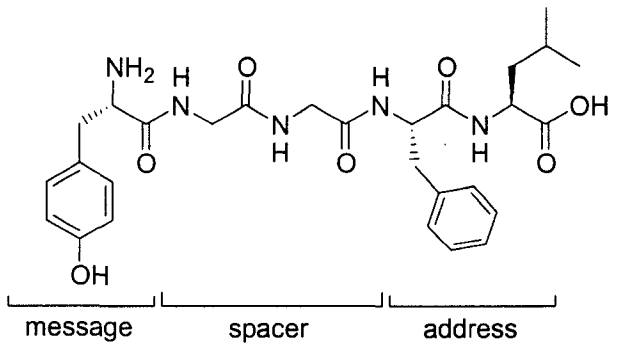
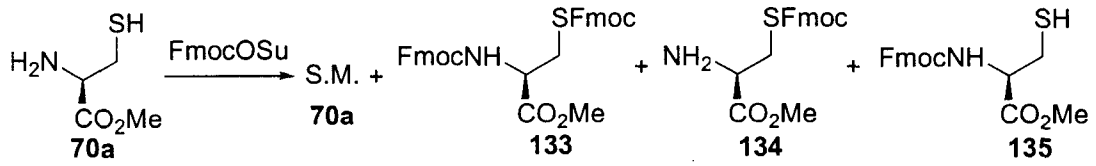
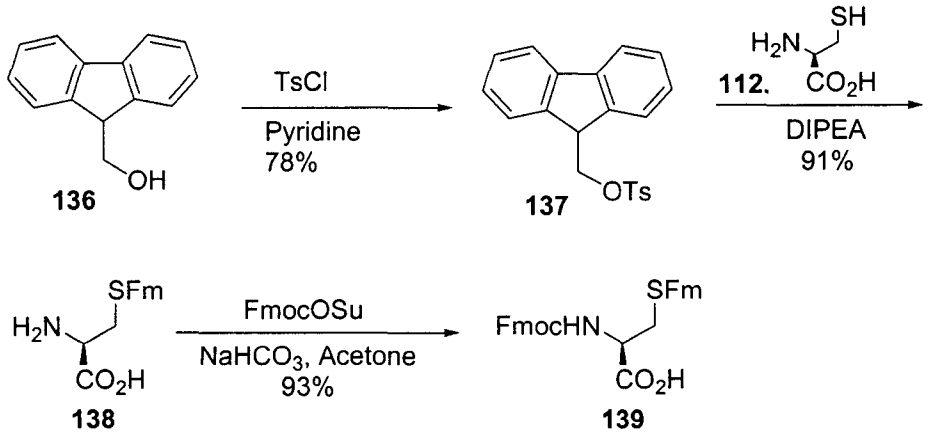


Figure 5. 2 Pentapeptide Leu-enkephalin

The first non-standard amino acid required in solid phase synthesis is cysteine with N^{α}-Fmoc protection and S^{β}-Fm protection groups. S^{β}-Fm protection was chosen so that both protection groups can be deprotected by piperidine simultaneously. My initial strategy to have S^{β}-Fmoc protection failed (Scheme 5.3) because the S-Fmoc protection was too unstable and the protecting reaction of these two groups at one step was messy. The same problem was observed and reported by another group.[214] Fortunately, a reported method for the synthesis of the S-Fm protection group was found.[204,215] According to their report, fluorenylmethyl *p*-toluenesulfonate **137** was a better reagent



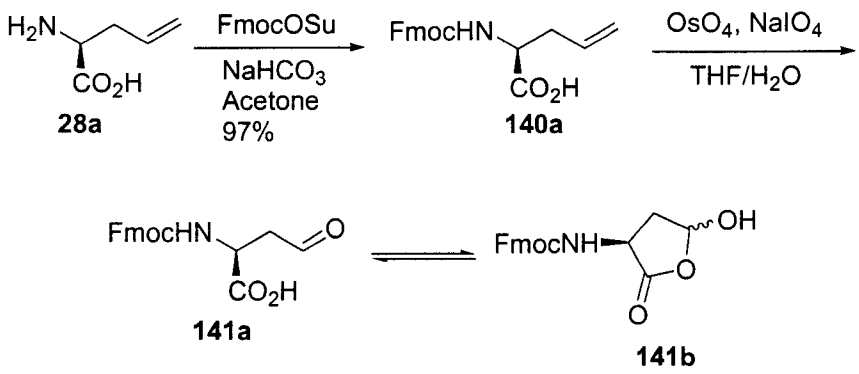
Scheme 5.3 The initial idea to protect N^{α}- and S^{β}- in cysteine



Scheme 5.4 A modified method to synthesize the *N* and *S* protected cysteine

than fluorenylmethyl chloride. It was first synthesized in 78% yield from 9-fluorenemethanol.[216] The precipitated product was stable at room temperature but the color was getting deeper during the storage. (*S*)-9-Fluorenylmethyl-*L*-cysteine **138** was synthesized from **137** in 91% yield in DMF without purification (Scheme 5.4). The recrystallization of **138** in 1*N* HCl was tried but gave only a 68% yield. The final product **139** was synthesized[217] in 93% yield after the product was purified on a column. It should be mentioned that this final product **139** is not soluble in $CHCl_3$ and has very limited solubility in CH_3OH. The product was crystallized after flash liquid chromatograph. It is very fortunate that the product has very good solubility in DMF which is the ideal solvent we need in solid phase synthesis.

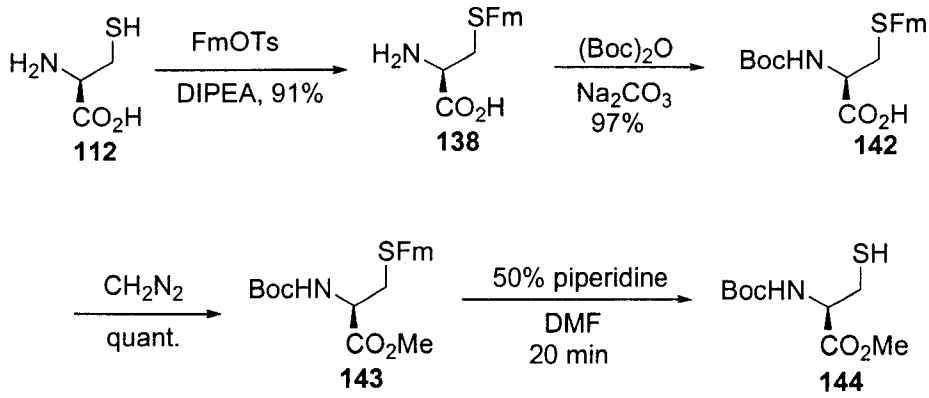
The second amino acid **141** can be synthesized from *L*-allyl amino acid **28a**, which is also commercially available. The N^{α}-Fmoc protection was completed in an acetone and water mixture and the product was used without further purification (Scheme 5.5).[217] The osmylation was performed as before and the aldehyde product **141a** (~75% yield as crude) was used in solid phase synthesis without purification. In fact, the ^1H nmr result show the aldehyde **141a** is formed as the diastereomeric lactone hemiacetal **141b**. This lactone hemiacetal is so stable that after 6 months at room temperature, the ^1H nmr did not show much difference. The beauty of this 5-membered ring formation is it provided a stable structure before reaction, but maintained the reactivity of the γ-aldehyde in the next steps.



Scheme 5.5 The synthesis of γ-aldehyde N^{α}-Fmoc amino acid

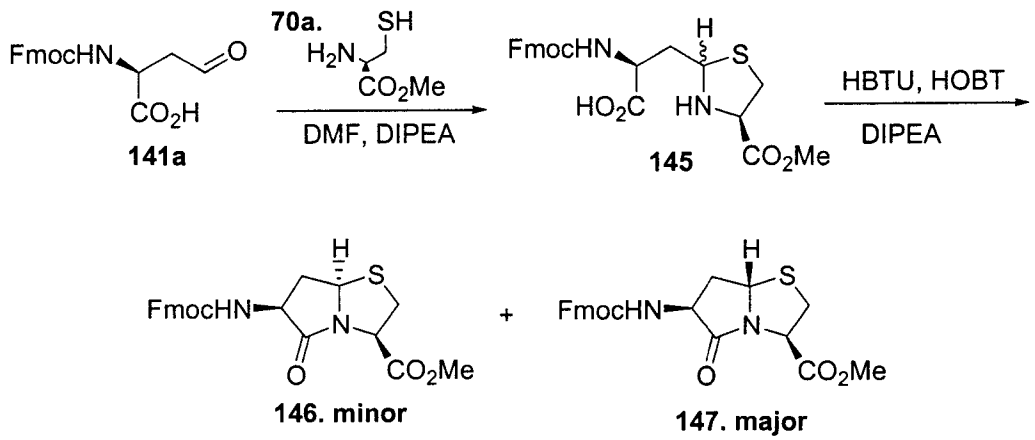
§ 5.4 Optimization of nonstandard reaction in solution phase

The optimization of the deprotection of S^{β}-Fm was specially designed in solution phase so that the reaction can be monitored on TLC (Scheme 5.6). Orthogonal protection group Boc was chosen here so that it would stable in piperidine. This S^{β}-Fm protected amino acid **143** can be synthesized in 3 steps from the free cysteine **112**. By using this starting material **143**, the reaction of S^{β}-Fm deprotection in solution can be monitored on the TLC without the interruption of Fmoc deprotection. It turned out that the deprotection of S^{β}-Fm group by 50% piperidine in DMF can be completed in 20 min.



Scheme 5.6 Optimization of deprotection of S^{β}-Fm in DMF

The thiazolidine formation and bicyclization also were optimized in the solution phase synthesis (Scheme 5.7). We found that *N,S*-acetal formation not only can be accomplished in EtOH and H_2O (1:1 mixture), but also can be completed in DMF with 2 eq of DIPEA within 2 hours. The later condition was again ideal for solid phase synthesis. The intramolecular amide bond formation was completed in 20 min by using HBTU/HOBT coupling reagents and DIEA. Two products were found on TLC and their ^{1}H nmr ratio is 45 : 55. Obviously, two diasteromers were generated due to the bridge-head H. The major product was assigned as [3*S, 5S, 8R*] based on our experience in [5,5]-BTD synthesis. The minor product [3*S, 5R, 8R*] is always more polar than the major product in this [5,5]-BTD. The energy difference, however, is very small because there are no any side chain groups at β and β' position. The diastereomeric ratio (about 1:1) was typically different than the ratio we had in side chain introduced [5,5]-bicyclic formation in Chapter **III**.



Scheme 5.7 Optimization of *N,S*-acetal formation and amide coupling reaction

§ 5.5 The synthesis of [3.3.0]-BTD[2,3]-Leu-enkephalin

After the synthesis of the appropriately protected amino acids, and the optimization of their bicyclic formation in solution, I was ready to start the synthesis of Leu-enkephalin on solid phase by non-conventional methods. In this peptide, a free -NH_2 at the N-terminal and a free carboxylic acid at C-terminal were required for its agonist activity.[218] Considering the slow and always incomplete esterification of the first amino acid on a resin, Wang-resin which already had first amino acid, Leucine, bonded on resin was suggested. In the first try of tripeptide synthesis, the resin was swollen in DMF and DCM for 2 h. After cleaving the tripeptide from the resin, the EIMS of tripeptide was messy, and only a small amount of products was observed. Since most of these MS results can not be simply explained, I decided to study the solid

phase peptide synthesis by careful cleavage from the resin and checking their MS after each coupling of each amino acid residue.

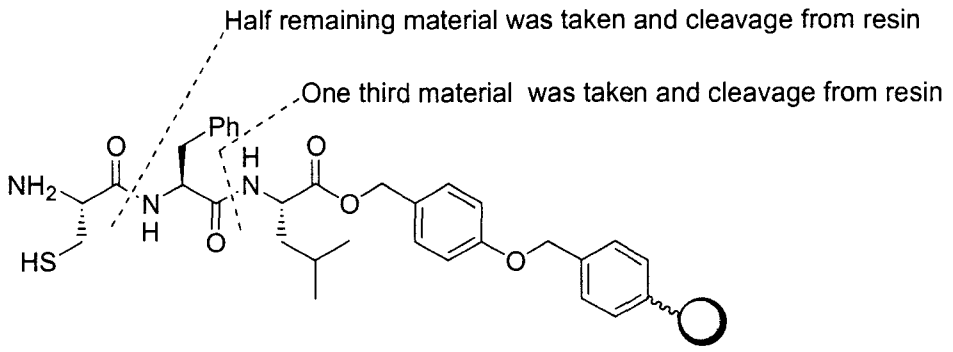


Figure 5.3 The synthesis of tripeptide and the cleavage after each coupling

The synthesis of the tripeptide was restarted. The first amino acid Leucine also was cleaved from resin by using 90% TFA, 5% H_2O and 5% TIPS cocktail solution for 2 hours. The LRMS result shows a fragment of 192.1 with a structure hard to identify by ^1H and ^{13}C NMR. This fragment was found in the monopeptide (Leucine) and also in the dipeptide and tripeptide cleavage. It was assumed the resin did not swell completely and the first coupling was incomplete. This problem was minimized by swelling the resin overnight in DCM/DMF (1/1), extending the phenyalanine coupling reaction to 70°C, and by limiting the cleavage time to 1 hour. In one experiment, the acetylation with 25% acetic anhydride in DCM for 40 min was performed after first coupling. However, since no acetylated fragment was found after cleavage, this step was omitted. Finally no MS = 192.1 peak was found in the crude product mixture. It should be

mentioned that low concentrations of TFA is not recommended in this case because the possible formation of sulfinium trifluoroacetate is desired in this case, which can stabilize the sulfur-containing peptide.

The coupling of N^{α}-Fmoc-Phe-OH and deprotection of the N^{α}-Fmoc group was accomplished by conventional methods and the reactions were monitored by the Kaiser test (Scheme 5.8). Because cysteine is one of the amino acid which can racemized on coupling, a DCM and DMF mixture solution (1/1) was used followed by addition of cysteine derivative (3eq), coupling reagents (3eq) and DIEA (6eq) in order. By using these conditions, no racemized product was found in the final product. The deprotection of N^{α}-Fmoc and S^{β}-Fm was accomplished by using 50% piperidine and 5% TIPS in DMF two times (1 x 5 min and 1 x 25 min). Scavenger TIPS was added in order to guarantee the reaction completion in 30 min. The Kaiser test was negative. [219] The deprotection was employed a second time and the Kaiser test remained negative. It was later understood that the free sulfur took part an intramolecular reaction and an intermediate **148** (Figure 5.4) was formed. This intermediate prevented the formation of the final product which shows the purple color (Ruhemann's purple). Because of this, a blue color would indicate an incompleteness of deprotection S^{β}-Fm on sulfur.

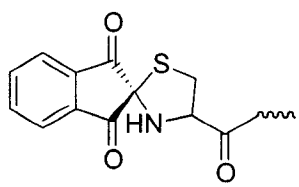


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Figure 5.4 The intermediate in the Kaiser Test

The tripeptide was cleaved from the resin using the same cocktail solution and purified by HPLC. Mainly three products were obtained according to the EIMS. The first peak with MS = 192.1 has been discussed. The second and the third peaks both show $[(2M-2)H]^+$, the dimer of the tripeptide. This result was misconstrued at first thinking that racemization happened during the coupling. However, the second peak was later found as a mixture of monomer and dimer if the sample was sent for MS quickly and checked immediately. The free mercaptan is very easily oxidized and the dimerization happened during the storage and waiting time.

The osmylation and diol oxidation was usually done one day before the peptide synthesis. Four eqs of allylic glycine was used assuming the yield of this reaction is about 75%. The following *N,S*-acetal thiazolidine formation was first tried in EtOH and H_2O (1/1) but the reaction was very slow, although DIEA instead of $NaHCO_3$ would accelerate this reaction. This mixture of solvents is not good because the hydrophobic chain of the peptide partly "cramped" (stuck together) in water containing solution. This reaction was optimized in DMF with 2 eq of DIEA within 2 h. No imcomplete fragment of tripeptide was found in this 5-membered thiazolidine formation.

The 5-membered lactamization was very fast because of its intramolecular reaction. In practice, 1.5 eq of coupling reagent (HBTU and HOBT) and 3 eq of DIPEA were used in this 45 min reaction to ensure completeness. An unexpected difficulty of N^{α}-Fmoc deprotection for the tetrapeptide was found in scale up synthesis (0.5 mmol). The difficulty could be attributed to the concave secondary structure of the peptide after the

BTD formation. In this case, a bridge head H DOWN isomer (the minor isomer with 5R configuration) forced a reversed backbone conformation. As a result, the N^{α}-Fmoc terminal may be close to the peptide backbone which makes the deprotection difficult (steric hindrance). However, when a more concentrated piperidine (40%) in DMF solution was used, the deprotection problem was solved. After deprotection, one half of the remaining material was again taken and cleaved from resin to check bicyclization.

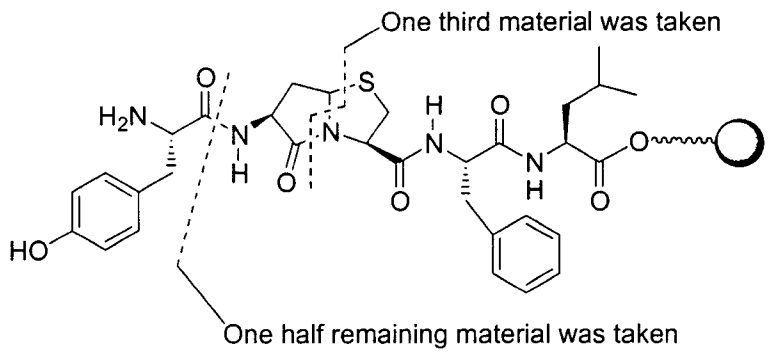
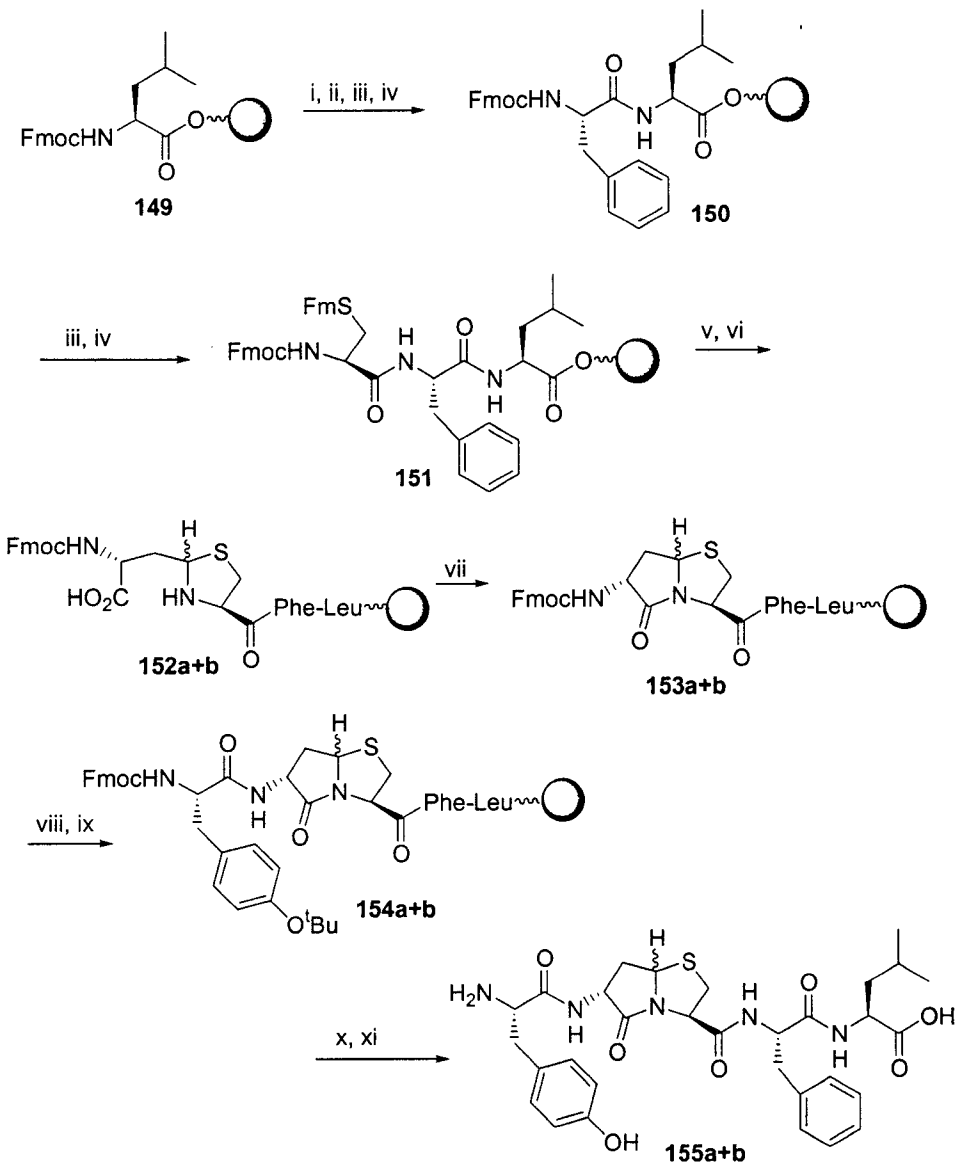


Figure 5.5 The synthesis of tripeptide, tetrapeptide, and pentapeptide

Finally, N^{α}-Fmoc-Tyr-(OBu^{-t})-OH was introduced by conventional synthesis. After deprotection of the Fmoc, the pentapeptide was cleaved from resin by TFA/H_2O/TIPS in ratio of 9.0/0.5/0.5 in 1 hour. It was then filtered and washed. The product was found to be very insoluble in H_2O, but very soluble in ether, and thus the peptide did not precipitate. The TFA was evaporated and the solution was neutralized by ammonium hydroxide. The concentrated sample was sent for LRMS and surprisingly, it was very clean. Only a molecular peak (MH^+ = 626.2) was found and no other significant peak



i. 25%piperidine in DMF; ii. N^{α}-Fmoc-Phe-OH, HBTU, HOBT, DIEA; iii. same to i; iv. N^{α}-Fmoc-Cys-S^{β}-Fm-OH, DCM/DMF, HBTU, HOBT, DIEA; v. 50%piperidine, DMF; 5%TIPS; vi. *L*-N^{α}-Fmoc-2-amino-4-oxo-butyric acid, DIEA, DMF; vii. HBTU, HOBT, DIEA; viii. 40% piperidine in DMF; ix. N^{α}-Fmoc-Tyr-(O^{t}Bu)-OH, HBTU, HOBT, DIEA; x. same to i: xi. 90%TFA, 5%H_2O, 5% TIPS

Scheme 5.8 Total synthesis of (3*S*,5*S*,8*R*)- and (3*S*,5*R*,8*R*)-[3,3,0]-BTD$^{[2,3]}$-Leu-Enkephalin by Fmoc based non-conventional solid phase synthesis.

was observed. It should be indicated that two diastereomeric isomer share this molecular peak. The HPLC result shows a diastereomeric ratio of 47: 53, which is very close to the 45: 55 in the solution phase synthesis results (Scheme 5.7). The total yield of the synthesis, based on the HPLC result of crude sample without precipitation is about 47%. During the synthesis, the Kaiser test was used in every step and all the results are unambiguously clear. In this way, this BTD-inserted pentapeptide was synthesized on solid phase. The detailed reaction conditions are illustrated in Scheme 5.8.

5.6 Purification, characterization and configuration determination, NMR, *etc*

The 11-step synthesis of BTD[2,3]-Leu-enkephalin thus was completed in about 11 hours and two diasteromeric peptides were generated. It should be indicated that the retention time of (3S,5R,8R) and (3R,5S,8R) isomers are very close to each other on reverse column. The purification was improved by using a prep-HPLC with 15% isocratic acetonitrile in aqueous solution (0.1% TFA) following by a semi-Prep HPLC with 23% isocratic elution. The optimized synthetic procedure was used to synthesize the other two diastereomers from *D*-allyl glycine. The total yield of this synthesis is about 42% based on the HPLC result with sample precipitation and the diastereomeric ratio of (3*R*,5*S*,8*R*) to (3*R*,5*R*,8*R*) was 40 : 60. It is very fortunate that the separation of these two analogues was very easy.

The stereochemistry of the bridgehead H of the bicyclic rings in these four diastereomers were assigned by TOCSY and ROSEY. We were fortunate that the bridge-head hydrogens are well separated in D_2O from other protons. In the **155a** (3*S*,5*S*,8*R*) sample, however, the solvent (HOD) peak overlapped the bridge-head H and partly overlapped with two other α-Hs. The solvent peak was moved toward high field by decreasing the experimental temperature to 285 K. In this way, the bridgehead H in this spectrum, like all of the other three, is well separated from other proton signals. In analogue **155b**, no bridge-head H ROE was observed. Because I had all four isomers and their ROE results are all unique, their stereochemistries could be assigned unambiguously.

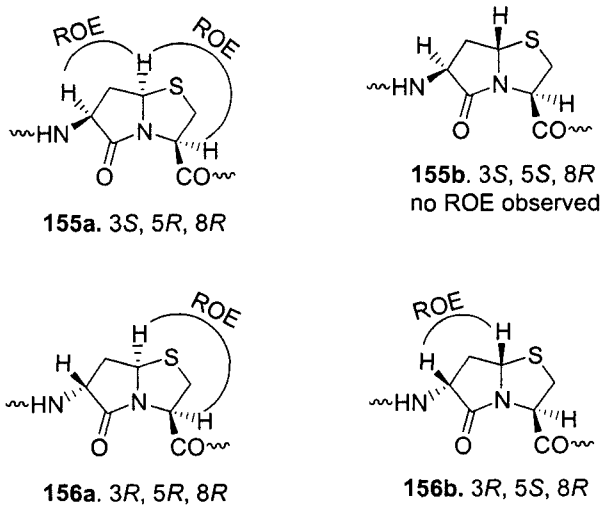


Figure 5.6 ROE results of bridge-head H in leu-enkephalin analogue

All of the above Leu-enkephalin analogues were checked by TLC in five different solvents. These solvents mixture are listed in the order of decreasing their polarity. (A).

1-butanol/HOAc/Pyridine/water = 5/4/1/4; (B). 1-butanol/HOAc/Pyridine/water = 4/1/1/3; (C). EtOAc/HOAc/Pyridine/water = 6/2/2/1; (D). EtOAc/HOAc/Pyridine/water = 8/2/2/1; (E). EtOAc/HOAc/Pyridine/water = 6/1/1/1. Their R_f value was calculated and shown in the Table 5.1. The retention times of peptides also were determined on an analytical reverse-phase column with 10-90% of acetonitrile in 0.1% TFA in H_2O in 40 min. The HPLC k' were calculated and listed in table 5.1.

Table 5.1 The characterization of Leu-enkephalin analogues

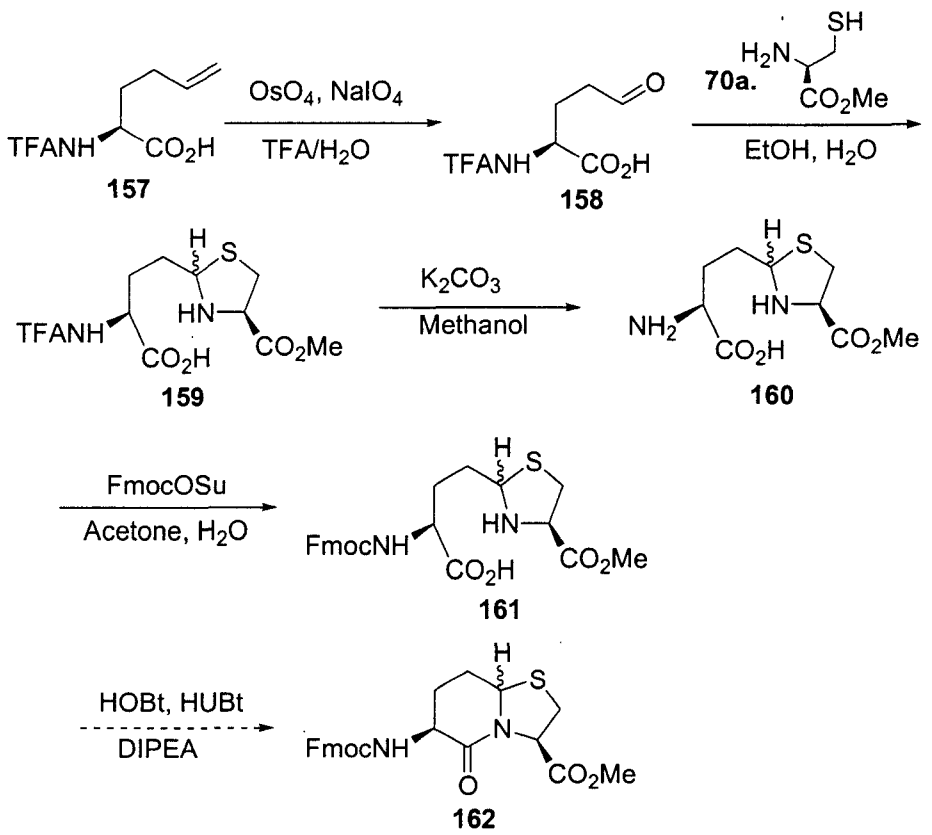
solvents	**155a**	**155b**	**156a**	**156b**
A	0.844	0.862	0.837	0.871
B	0.711	0.720	0.667	0.689
C	0.548	0.645	0.477	0.673
D	0.390	0.434	0.308	0.459
E	0.172	0.213	0.118	0.176
k'*	7.01	7.28	6.50	7.16

* HPLC k' = [(peptide retention time-solvent retention time)/solvent retention time] in a solvent of 10% CH_3CN in 0.1% TFA and a gradient to 90% CH_3CN over 40 min. An analytical Vydac C_{18} column was used with a flow rate of 1 mL/min.

§ 5.7 [4.3.0]-BTD$^{[2,3]}$-Leu-enkephalin synthesis, future work

In the development of [6,5]-BTD inserted peptide synthesis on solid phase supports, it became complicated because neither N^{α}-Boc nor N^{α}-Fmoc can be used as a

protection group for amino acid **130c** due to the formation of a 5-membered hemiaminal formed after osmylation cleavage. Hemiaminals with N^{α}-Boc and N^{α}-Fmoc protected cannot be transferred to their thiazolidines at room temperature due to the electron donating ability of oxygen which has been fully discussed in Chapter **IV**. However, I have developed N^{α}-TFA protected amino acids which were good enough in pursuing the [6,5]-bicyclic dipeptide in solution phase. In these cases, only Fmoc chemistry could be used because the deprotection of TFA is in basic solution. In order to be familiar with all the reaction conditions which would be used in [6,5]-BTD formation and make sure that the above idea is possible on solid phase, the following reaction (Scheme 5.9) has been designed and tried in solution phase.



Scheme 5.9 The [4.3.0]-BTD formation in solution phase

Although *N,S*-thiazolidine can be formed as before, the intramolecular lactamization cannot be done from **159** because of the N^{α}-TFA and the racemization of α-H. It is reported in literature that the deprotection of TFA can be completed in methanol by K_2CO_3 in 6 h.[142,220] Although the reaction can be monitored by TLC, the product could not be worked up because it is soluble in water phase. After evaporation of the solvent methanol, the crude product was used with Fmoc protection followed by coupling. However, no product was obtained in this five step reaction. The main

problem is that none of above intermediate could be isolated and purified. The design was given up after two tries. However, the disadvantage of a solution phase synthesis can be totally avoided on solid phase. The Fmoc protection on N^{α}- can be omitted because solid phase synthesis is in a *pseudo*-diluted solution. It is assumed that no intermolecular coupling reaction would happen and the intramolecular coupling becomes the only possibility. A back up strategy for this [6,5]-BTD synthesis on solid phase is using N^{α}-methyl-N^{α}-Fmoc as starting material. In this way, the terminal aldehyde will become a 6-membered hemiacetal lactone instead of a 5-membered hemiaminal. The syntheses of [6,5]- and [7,5]-BTD[2,3] Leu-enkephalin on Fmoc-Leu-Wang resin are under investigation.

5.8 Structure and biological activity

The synthesized [3.3.0]-BTD[2,3]-Leu-enkephalin analogue samples were tested for binding affinities in competition with [^{3}H]DAMGO (μ) and Deltorphin II (δ) (Table 6.2). All the analogues show low binding affinities, and their IC_{50}s are in the micromolar range. However, comparing the δ and μ binding affinity, we still can observe that they are week δ opioid ligands. One obvious difference is the analogue **156a** with a *D*-amino acid at position-2 is about 20 fold stronger than when **155a** the *L*-amino acids are at this position. The effect of *D*-amino acid at Leu-enkephalin analogues has been discussed before.[221,222] Further investigations with higher concentration of ligands are under the way.

Table 5.2 Binding affinity of four BTD analogues in competition with [^{3}H] DAMGO and Deltorphin II in mouse brain membranes

Peptide isomers	Del II (δ) EC_{50} (μM)	DAMGO (μ)
155a (3*S*,5*S*,8*R*)-analogue	22.5%*	0.6%*
155b (3*S*,5*R*,8*R*)-analogue	~47	0%*
156a (3*R*,5*S*,8*R*)-analogue	2.4	20.6%*
156b (3*R*,5*R*,8*R*)-analogue	33.1% *	2.6%*

*percent of maximum binding achieved with the concentration of 10 μM peptides

The functional assays also show low activity (Table 5.3). They are all weak δ agonist and did not show their maximum activity at 1 μM concentration (the IC_{50}s are not available). Nevertheless, we can see a 2-3 fold active difference in selectivity between δ and μ receptors at this concentration. The analogue with *D*-amino acid at position-2 again shows better activity although overall they are very close. On the other hand, none of these analogues shows antagonist activities.

Table 5.3 Potency of BTD analogues in MVD and GPI/LMMP bioassay*

Peptide isomers	MVD (δ)	GPI/LMMP (μ)
155a (3*S*,5*S*,8*R*)-analogue	14.4%	3.8%
155b (3*S*,5*R*,8*R*)-analogue	10.3%	6.1%
156a (3*R*,5*S*,8*R*)-analogue	15.5%	5.9%
157b (3*R*,5*R*,8*R*)-analogue	11.5%	4.6%

*Percent of maximum effect achieved with the concentration of 1 μM peptide

The weak agonist activity and low binding affinity may result from too constrained peptide conformation. The [3,3,0]-BTD is a very constrained dipeptide, and the backbone conformation is controlled in specific directions, although the side chain groups of Tyr and Phe have some flexibility in space. The bicyclic scaffold itself also may take part in the interactions between the ligands and receptors. Both the steric effect and electrostatic effect of the hydrophobic *N*,*S*-acetal bicyclic dipeptides in receptor binding interaction have not been investigated so far. The overall conformation of [3,3,0]-BTD inserted Leu-enkephalin will be further discussed with the help of modeling studies, NMR, and/or X-ray crystallography. These studies are under investigation.

In conclusion, we have developed a novel strategy which can be used to synthesize external BTD-inserted peptide on solid phase. The synthesis of 4 isomers of [3,3,0]-BTD[2,3]-inserted Leu-enkephalin representing the first example that BTD can be

synthesized on the solid phase. This fast and efficient methodology makes it possible to build up all possible diastereomeric libraries. It would be a unique key in studying structural and biological activity relationships by combining NMR and modeling technique. The method will be general enough for both Boc and Fmoc chemistry in parallel synthesis of all side-chains introduced BTD as far as the relevant amino acids are available.

§ 5.9 Experimental section

General information and materials:

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 MHz and 600 MHz NMR. The chemical shifts were reported in δ, parts per million (ppm), relative to TMS (δ = 0.00 ppm) in $CDCl_3$ as an internal standard. In DMSO, the spectra were referenced to solvent peaks at 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C. In D_2O, ^{1}H chemical shifts were referenced to HOD peak at 4.67 ppm, and ^{13}C chemical shifts were indirectly referenced to $CDCl_3$ at 7.26 ppm. Coupling constants, *J*, were reported in Hertz (Hz) and refer to apparent peak multiplicities and not true coupling constants. Mass spectrometric analyses were conducted by the Mass Spectrometry Facility at the Department of Chemistry of the University of Arizona on a Jeol HX-110A. Optical rotations were measured on a JACSO P1010 polarimeter. Flash liquid chromatography was performed with 230-400 mesh size silica gel which was purchased from Aldrich Chemical Co. Thin-layer chromatography (TLC) was performed with Merck silica gel

60 F_{254} (0.25 mm layer thickness). Melting points (Mp) are uncorrected and were obtained in open capillaries.

All peptides designed in this investigation were synthesized manually by solid phase methods in N_2 atmosphere. N^{α}-Fmoc protected amino acids and Fmoc-Leu-Wang resin were purchased from Nova Biochem (U. S. A.). The sidechain of tyrosine was protected by *t*-butyl. HBTU and HOBt were purchased from Quantum Biotechnologies (Montreal, Canada). *L*-Allylglycine, *L*-cysteine, piperidine, and 9-fluorenemethanol were purchased from Aldrich (Milwaukee, WI). *D*-Allyl glycine was purchased from Sigma (St. Louis, MO); DCM and DMF, and high-pressure liquid chromatography (HPLC) quality acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ) and used without further purification. All the other reagents and solvents, unless otherwise noted, were used as received. The purification of the peptides was achieved using a Hewlett-Packard 1100 Series HPLC fitted with a Hewlett-Packard 1100 Series variable wavelength UV detector for semipreparative HPLC on C_{18}-bonded silica columns (Vydac, Hesperia, CA; Catalog no. 218TP1010). The purity of the peptides was checked by thin-layer chromatography (TLC) in five different solvent systems. The peptides were detected on the TLC plate using iodine vapor. They were also checked by analytical reverse-phase HPLC using Vydac C_{18} column (catalog no. 218TP104) monitored at 220, 254, 280 and 350 nm and by characterization by electrospray mass spectroscopy. In all cases, the purities were greater than 95% as determined by these methods.

Fluorenylmethyl p-toluenesulfonate (137): 9-Fluorenemethanol **136** (19.6 g, 100 mmol) was dissolved in 100 mL $CHCl_3$ in 250-mL flask and cooled down to 0°C by an ice-water bath. Tosyl chloride (22.8 g, 120 mmol) was first dissolved in anhydrous pyridine (16.1 mL, 200 mmol) in a dropping funnel and was added dropwise. Then solution was warmed to room temperature and was kept stirring for 6 hours. The solution was washed with 1*N* HCl (2 x 25 mL), $NaHCO_3$ (2 x 25 mL) and saturated brine (2 x 25 mL) and dried with $MgSO_4$. After filtration the filtrate was evaporated in *vacuo*, and the residue was recrystallized by dissolving in $CHCl_3$, adding hexane to incipient turbidity and allowed to stand at room temperature overnight to give a colorless solid. After filtration and washing by hexane, the crystalline product 27.4 g (78% yield) was collected. Mp. 113-115°C. ^1H (500 MHz, $CDCl_3$), 2.41 (3H, s), 4.23-4.27 (3H, m), 7.27-7.29 (4H, m), 7.38 (2H, t, J = 7.0 Hz), 7.52 (2H, d, J = 8.0 Hz), 7.72 (2H, d, J = 7.5 Hz), 7.75 (2H, d, J = 8.0 Hz); ^{13}C (125 MHz, $CDCl_3$), 21.6, 46.7, 71.8, 120.1, 125.1, 127.2, 127.9, 128.0, 129.8, 132.8, 141.2, 142.4, 144.8.

(*S*)-9-Fluorenylmethyl-*L*-cysteine (138): Fluorenylmethyl p-toluenesulfonate **137** (7.22 g, 20.6 mmol) was first dissolved in anhydrous DMF (72 mL) in 250-mL flask and cooled down to 0°C by an ice-water bath. *L*-Cysteine (2.0 g, 16.5 mmol) and DIPEA (8.42 mL, 49.6 mmol) were added respectively with a formation of white precipite. The suspension was stirred 24 h at room temperature before EtOAc (72 mL) was added. The solid was filtered and washed with cooled EtOAc until no UV active filtrate was detected on TLC. A yellowish product (4.50 g, 91% yield) was collected.

Small amounts of product were recrystallized in 1*N* HCl. Mp. >205°C (decomp), $[\alpha]_D^{24}$ = -50.9° (c 2.01, DMF), ^{1}H (500 MHz, DMSO): 3.00-3.09 (2H, m), 3.21 (2H, d, *J* = 6.0 Hz), 4.17-4.21 (2H, m), 7.32 (2H, t, *J* = 7.5 Hz), 7.39 (2H, t, *J* = 7.5 Hz), 7.75 (2H, d, *J* = 7.5 Hz), 7.86 (2H, d, *J* = 7.5 Hz). ^{13}C (125 MHz, DMSO): 32.5, 36.0, 46.4, 52.1, 120.0, 125.0, 127.0, 127.5, 140.6, 145.7, 169.6.

N^{α}-9-fluorenylmethoxycarbonyl-(S)-9-fluorenylmethyl-*L*-cysteine (139): The (*S*)-9-fluorenylmethyl-*L*-cysteine **138** (4.50 g, 13.4 mmol) was suspended in acetone (120 mL) and water (120 mL), and FmocOSu (4.43 g, 13.1 mmol) was added. $NaHCO_3$ (14.5 g, 53.6 mmol) was added and the reaction was kept at room temperature for two days. The acetone was evaporated and the residue was diluted and acidified with 1*N* HCl to pH = 2~3. The product was extracted with EtOAc (3 x 50 mL) and the combined EtOAc solutions were washed with 50 mL brine and dried over $MgSO_4$. The EtOAc was concentrated in *vacuo* and the crude product was purified by flash liquid chromatograph (gradient solvent, hexane/EtOAc = 8/1 to EtOAc). The pure product was crystallized in a test tube after the column. The product was collected by dissolving in methanol and evaporated to dryness. 6.52 g (93%) of colorless product was obtained. Mp: 105-107°C, $[\alpha]_D^{24}$ = -10.9° (c 1.21, DMF), ^{1}H (500 MHz, DMSO): 2.86 (1H, dd, *J* = 8.0, 13.5 Hz), 2.98-3.13 (3H, m), 4.00-4.06 (1H, m), 4.13 (1H, t, *J* = 6.5 Hz), 4.17-4.28 (3H, m), 7.24-7.30 (4H, m), 7.37 (4H, dt, *J* = 7.5, 18.0 Hz), 7.69 (2H, d, *J* = 7.5 Hz), 7.72 (2H, t, *J* = 7.5 Hz), 7.83 (2H, d, *J* = 7.5 Hz), 7.87 (2H, d, *J* = 7.5 Hz); ^{13}C (125 MHz, DMSO): 33.8, 35.7, 46.5, 46.6, 54.3, 65.8, 120.0, 120.1, 121.2, 124.0,

124.9, 125.0, 125.28, 125.32, 127.0, 127.1, 127.5, 127.7, 129.5, 133.3, 135.4, 140.5, 140.7, 143.8, 143.9, 145.86, 145.89.

N^{α}-9-Fluorenylmethoxycarbonyl-*L*-allylglycine (140a): *L*-allylglycine **28a** (500 mg, 4.34 mmol) was suspended in 1/1 acetone/water solution (80 mL) and cooled down with an ice-water bath. Fmoc-OSu (1.43 g, 4.25 mmol) and $NaHCO_3$ (1.5 g) were added with stirring. The mixture was left stirring at room temperature overnight. TLC (AcOEt-Hexane-AcOH, 25:25:1, R_f = 0.44) showed that the reaction was complete. The reaction mixture was evaporated to dryness and the residue was acidified with 1*N* HCl to pH = 2~3. The mixture was extracted with AcOEt (3 x 25 mL), which was washed with water, dried over $MgSO_4$ and evaporated. The crude Fmoc-Allyl glycine (1.60 g, >100%) was collected without further purification, Mp: 120-122°C, $[\alpha]_D^{24}$ = +6.13° (c = 0.408, $CHCl_3$), ^1H (500 mHz, $CDCl_3$), δ 2.56-2.60 (1H, m), 2.63-2.72 (1H, m), 4.23 (1H, t, *J* = 7.0 Hz), 4.41 (2H, d, *J* = 7.0 Hz), 4.48-4.52 (1H, m), 5.19 (2H, d, *J* = 12.5 Hz), 5.29 (1H, d, *J* = 8.0 Hz), 5.68-5.77 (1H, m), 7.31 (2H, t, *J* = 7.5 Hz), 7.40 (2H, t, *J* = 7.5 Hz), 7.55-7.59 (2H, m), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 36.3, 47.1, 53.0, 67.2, 119.8, 120.0, 125.1, 127.1, 127.7, 131.7, 141.3, 143.7, 155.9, 176.1.

Fmoc-*D*-allylglycine 140b: Crude product (1.46 g, > 100%), Mp: 130-132°C, $[\alpha]_D^{24}$ = -6.23° (c = 0.488, $CHCl_3$), ^1H and ^{13}C ($CDCl_3$) spectra are identical to (*S*)-product.

Methyl 2-*tert*-Butoxycarbonylamino-3-(9*H*-fluoren-9-ylmethylsulfanyl)-propionate (143): The S^{β}-Fm protected amino acid **138** (380 mg, 1.27 mmol) was dissolved in fresh distilled THF (18 mL) in a 100-mL flask. *tert*-Butylcarboxyl anhydride (305 mg, 1.40 mmol) was then added together with K_2CO_3 (380 mg). The reaction was kept at room temperature for 5 h before the THF was evaporated off. The residue was dissolved in water and extracted with DCM and dried over $MgSO_4$. It was then concentrated and the product **142** was used in the next reaction step without purification.

The diazomethane ether solution was prepared as before (see general procedure for carboxylic methyl ester protection in § 4.7 experimental). The above **142** residue was dissolved in distilled DCM (6.5 mL) and the dried diazomethane was added dropwise. The reaction was kept at 0°C for 15 min before the solvent was evaporated and the sample was purified on the silica gel column (hexane : EtOAc = 8 : 1). The colorless product **143** was collected (278 mg, 53% yield) $[\alpha]_D^{24}$ = +7.4° (c = 2.69, $CHCl_3$), ^1H (500 mHz, $CDCl_3$), δ 1.44 (9H, bs), 2.97-3.00 (2H, m), 3.10-3.11 (2H, m), 3.74 (3H, s), 4.09 (1H, d, *J* = 6.5 Hz), 4.56 (1H, s), 5.34 (1H, s), 7.32 (2H, d, *J* = 6.0 Hz), 7.39 (2H, d, *J* = 7.0 Hz), 7.67 (2H, d, *J* = 7.0 Hz), 7.76 (2H, d, *J* = 7.0 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 28.3, 35.5, 37.1, 46.9, 52.5, 53.4, 80.1, 119.9, 120.0, 124.8, 127.0, 127.6, 141.0, 145.69, 145.72, 155.1, 171.5.

The procedure for [3.3.0]-bicyclic dipeptide synthesis in solution phase:

The osmylation product **141a** (0.97 mmol) was dissolved in EtOH and H_2O (1:1 mixture 15 mL). *L*-Cysteine methyl ester **70a** (167 mg, 0.97 mmol) and DIEA (300 μL)

were added. The reaction was kept at room temperature overnight. The EtOH was evaporated and the residue was acidified to pH 4~5 and extracted with DCM (3 x 15 mL). The collected organic phases were combined and dried over $MgSO_4$. It was concentrated and the product mixture **145** was re-dissolved in distilled DCM (25 mL). HUBT (368 mg, 0.97 mmol) and HOBT (135.1 mg, 0.97 mmol) were added. DIEA (390 uL) was added and the reaction was kept at room temperature for 1.5 h. The solution was diluted by water and DCM was separated. The aqueous phase was extracted with DCM (2 x 20 mL) and the combined DCM was dried over $MgSO_4$. The mixture was purified on a silica gel column (hexane : EtOAc = 3 : 1) and two diastereomer products was isolated.

Methyl (3*S*,5*S*,8*R*)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (146): 36% yield, $[\alpha]_D^{24}$ = -69.5° (c = 2.14, $CHCl_3$), ^1H (500 mHz, $CDCl_3$), δ 2.41-2.43 (1H, m), 2.67-2.72 (1H, m), 3.37 (1H, dd, *J* = 3.5, 11.0 Hz), 3.45-3.49 (1H, m), 3.76 (3H, s), 4.21 (1H, t, *J* = 6.5 Hz), 4.42 (3H, bs), 5.05 (1H, bs), 5.16 (1H, d, *J* = 6.0 Hz), 5.52 (1H, bs), 7.30 (2H, t, *J* = 7.5 Hz), 7.39 (2H, t, *J* = 7.5 Hz), 7.58 (2H, d, *J* = 7.5 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 29.9, 37.0, 47.0, 52.1, 52.9, 58.4, 63.9, 67.1, 119.93, 199.94, 125.0, 127.0, 127.7, 141.2, 143.6, 143.7, 156.0, 170.1, 174.7.

Methyl (3*S*,5*R*,8*R*)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-1-aza-2-oxo-6-thiabicyclic [3,3,0]-octane-8-carboxylate (147): 29% yield, Mp. 76.0-77.5°C, $[\alpha]_D^{24}$ = +11.5° (c = 2.46, $CHCl_3$), ^1H (500 mHz, $CDCl_3$), δ 2.17 (1H, q, *J* = 11.5 Hz), 3.09-3.14

(1H, m), 3.40 (1H, d, J = 12.5 Hz), 3.58 (1H, dd, J = 7.5, 13.0 Hz), 3.81 (3H, s), 4.21 (1H, t, J = 7.5 Hz), 4.40 (1H, t, J = 7.5 Hz), 4.78-4.83 (1H, m), 5.07 (1H, t, J = 4.5 Hz), 5.54 (1H, bs), 7.30 (2H, t, J = 7.5 Hz), 7.40 (2H, t, J = 7.5 Hz), 7.60 (2H, dd, J = 4.0, 7.0 Hz), 7.76 (2H, d, J = 7.5 Hz); ^{13}C (125 MHz, $CDCl_3$), δ 38.1, 38.8, 47.0, 52.9, 56.7, 57.1, 63.6, 67.3, 119.9, 125.1, 127.1, 141.2, 143.6, 143.8, 156.1, 168.4, 170.1.

General procedure for 2-(9H-Fluorenyl-9-methoxycarbonylamino)-4-oxo-butyric acid 141a: N^{α}-Fmoc-*L*-allylglycine **140a** (1 eq) was dissolved in THF/H_2O (2/1, 4 mL/mmol) in a flask. The hood lights were turned off and the flask was covered by aluminum foil. Osmium tetroxide (5% mmol) was added to the flask. After 5 min, $NaIO_4$ (2.5 eq) was added in small portions over a 15 min period. The reaction was kept at room temperature for 4 h before it was filtered and the THF was evaporated. The residue was dissolved in EtOAc and saturated NH_4Cl aqueous solution. The aqueous phase was extracted with EtOAc (2 x 10 mL/mmol) and the organic phase was combined, washed with brine, and dried over anhydrous $MgSO_4$. The solution was concentrated in *vacuo* and the crude material was used in peptide synthesis without further purification.

General protocol for synthesis of BTD$^{[2,3]}$-Leu-enkephalin by N^{α}-Fmoc chemistry

The peptides were synthesized manually on a solid phase support under a N_2 atmosphere. A typical washing procedure was performed with DMF (3 x 10 mL x 1 min) and DCM (3 x 10 mL x 1 min). A typical coupling reaction was performed in

DMF (10 mL) with 3 eq of N^{α}-Fmoc Amino acid following by HBTU (3 eq), HOBT (3 eq), and DIEA (6 eq) and the reaction was kept at room temperature for 60 min. A typical deprotection of N^{α}-Fmoc protection group was performed in DMF with 25 % of piperidine (1 x 5 min and 1 x 25 min).

N^{α}-Fmoc-Leu-Wang resin (100-200 mesh, substitution 0.61 mmol/g) was swollen overnight in DMF/DCM (1/1) solution. The resin was washed with a typical procedure and the N^{α}-Fmoc protecting group was removed by deprotection. After washing, the first coupling with N^{α}-Fmoc-Phe-OH was accomplished by a typical coupling procedure in 70 min. At the end of the coupling, the peptide-bonded resin was washed and a Kaiser ninhydrin test was done to check the extent of coupling. After deprotection of N^{α}-Fmoc of dipeptide, the coupling of cysteine was performed in a DCM and DMF (1/1) mixture under typical coupling conditions. The deprotection of the N^{α}-Fmoc and the S^{β}-Fm groups were accomplished using 50% of piperidine and 5% of TIPS in DMF (1 x 5 min and 1 x 25 min). After washing, Kaiser test of tripeptide show a negative result. A premixture of 2-(9-fluorenylmethoxycarbonylamino)-4-oxo-butyric acid **141a** (4 eq used in osmylation) in DMF (10 mL) was added for *N,S*-thiazolidine formation together with 2 eq of DIEA and stirred for 2 h. After washing, a lactamization was accomplished by using HBTU (1.5 eq) and HOBT (1.5 eq), and DIEA (3 eq) in 45 min. After washing, the N^{α}-Fmoc deprotection of tetrapeptide was removed with 40% of piperidine in DMF (1 x 5 min and 1 x 25 min). After washing, the Kaiser test was positive. Finally, the N^{α}-Fmoc-Tyr-(O^{t}Bu)-OH was coupled and N^{α}-Fmoc of pentapeptide was deprotected and the peptide was carefully washed by DMF and DCM.

General procedure for cleavage and deprotection of peptides. A cleavage cocktail solution (20 mL) consisting of TFA/iPr_3SiH/H_2O (9.0/0.5/0.5) was added to the resin-bound protected peptide, placed in two clean glass vials having a Teflon-coated screw cap. The glass vials were capped, and the reaction mixture was agitated for 1 h at room temperature using a mechanical shaker. The solution was then filtered through a Pasteur pipet plugged with some glass wool. The resin was washed with TFA (4-5 mL), and the solution was filtered off. The volume of the combined cleavage cocktail was reduced to 4 mL using a stream of nitrogen gas, and the residue was neutralized by conc. NH_4OH to pH = ~4-6. The crude peptide was diluted in 150 mL diethyl ether and in some cases, one third of product was precipitated out. The organic layer was decanted off after centrifugation of the peptide for 5 min at 12,000 rpm and the precipitate was washed with diethyl ether (3 x 40 mL). The remaining solution mixture was evaporated to dryness.

General procedure for purification of peptides: The crude peptide (both the solid and liquid mixture) was first dissolved in a mixture of acetonitrile (<20%) aqueous solution (TFA, 0.1%) to about 10 mg/mL. The insoluble impurities were removed by passing the solution through a syringe filter (Gelman Laboratory, Acrodisc, 25 mm Syringe Filter with 1 μM Glass Fiber Membrane). The yield and the diasteromeric ratio were determined on the analytical HPLC reverse-phase column by using a 10-50% gradient of acetonitrile in 40 min at 214 nm. The purification of peptide was accomplished on

reverse phase prep-HPLC columns two times. A 15% of acetonitrile (in 0.1 aqueous TFA) isocratic solvent for a preparative Vydac reverse phase column at a flow rate of 9.5 mL/min and a 23% of acetonitrile isocratic solvent for hemi-preparative reversed column (C_{18}-bonded, 300 A; 10 mm x 25 cm) at a flow rate of 3 mL/min were used in purification. The diastereomer synthesized from *D*-allyl glycine are well separated in purification.

(3*S*, 5*R*, 8*R*)-[3,3,0]-BTD$^{[2,3]}$-Leu-enkephalin (155a): $[\alpha]_D^{24}$ = -23.5° (c 0.74, H_2O), ^1H (500 MHz, D_2O), δ 0.72 (3H, d, *J* = 6.0 Hz), 0.78 (3H, d, *J* = 6.5 Hz), 1.44-1.57 (3H, m), 2.14 (1H, q, *J* = 11.5 Hz), 2.73 (1H, dt, *J* = 7.5, 13.0 Hz), 3.00-3.06 (3H, m), 3.08-3.12(2H, m), 3.63 (1H, dd, *J* = 8.0, 12.5 Hz), 4.14-4.19 (3H, m), 4.55 (1H, dd, *J* = 6.5, 8.5 Hz), 4.78 (1H, dd, *J* = 8.5, 10.5 Hz), 4.98 (1H, dd, *J* = 6.0, 9.0 Hz), 6.78 (2H, d, *J* = 8.5 Hz), 7.11 (2H, d, *J* = 8.5 Hz), 7.16-7.20(3H, m), 7.23-7.26 (2H, m); HRMS (FAB) NH^+ calcd for $C_{31}H_{40}N_5O_7S$ 626.2648, found 626.2643.

(3*S*, 5*S*, 8*R*)-[3,3,0]-BTD$^{[2,3]}$-Leu-enkephalin (155b): $[\alpha]_D^{24}$ = -34.1° (c 0.69, H_2O), ^1H (500 MHz, D_2O), δ 0.75 (3H, d, *J* = 5.0 Hz), 0.81 (3H, d, *J* = 5.5 Hz), 1.23 (1H, d, *J* = 6.0 Hz), 1.51-1.60 (2H, m), 2.12 (1H, dt, *J* = 7.0, 14.5 Hz), 2.36 (1H, t, *J* = 11.5 Hz), 2.86-2.90 (2H, m), 3.01-3.05(1H, m), 3.09-3.16 (2H, m), 3.34 (1H, dd, *J* = 8.0, 11.5 Hz), 4.12 (1H, t, *J* = 7.0 Hz), 4.27 (2H, dd, *J* = 7.0, 9.5 Hz), 4.60-4.65 (2H, m), 4.69 (1H, dd, *J* = 6.0, 8.0 Hz), 6.80 (2H, d, *J* = 8.5 Hz), 7.09 (2H, d, *J* = 8.5 Hz), 7.16(2H, d,

J = 7.0 Hz), 7.20-7.25 (3H, m); HRMS (FAB) NH^+ calcd for $C_{31}H_{40}N_5O_7S$ 626.2648, found 626.2649.

(3*R*, 5*R*, 8*R*)-[3,3,0]-BTD$^{[2,3]}$-Leu-enkephalin (156a): $[\alpha]_D^{24}$ = -20.2° (c 0.61, H_2O), ^1H (500 MHz, D_2O), δ 0.74 (3H, d, J = 6.0 Hz), 0.77 (3H, d, J = 6.0 Hz), 1.38-1.55 (3H, m), 1.99 (1H, dd, J = 6.5, 14.0 Hz), 2.37 (1H, dt, J = 9.5, 14.0 Hz), 2.90-3.02 (3H, m), 3.05-3.10 (2H, m), 3.60 (1H, dd, J = 8.0, 12.5 Hz), 4.00 (1H, t, J = 7.5 Hz), 4.16 (1H, d, J = 8.0 Hz), 4.20 (1H, dd, J = 4.0, 9.5 Hz), 4.41 (1H, d, J = 9.5 Hz), 4.57 (1H, t, J = 7.5 Hz), 4.89 (1H, d, J = 7.0 Hz), 6.77(2H, d, J = 8.0 Hz), 7.04 (2H, d, J = 8.0 Hz), 7.17-7.22 (3H, m), 7.25-7.27 (2H, m); HRMS (FAB) NH^+ calcd for $C_{31}H_{40}N_5O_7S$ 626.2648, found 626.2641.

(3*R*, 5*S*, 8*R*)-[3,3,0]-BTD$^{[2,3]}$-Leu-enkephalin (156b): $[\alpha]_D^{24}$ = -72.0° (c 0.58, H_2O), ^1H (500 MHz, D_2O), δ 0.75 (3H, d, J = 6.0 Hz), 0.80 (3H, d, J = 6.0 Hz), 1.47-1.55 (4H, m), 2.68 (1H, dt, J = 6.0, 12.5 Hz), 2.86 (1H, dd, J = 10.0, 13.5 Hz), 2.94 (1H, dd, J = 9.0, 13.5 Hz), 3.02 (1H, dd, J = 5.0, 6.5 Hz), 3.01-3.15 (2H, m), 3.24 (1H, dd, J = 7.5, 11.5 Hz), 4.02 (1H, t, J = 7.5 Hz), 4.25 (1H, bs), 4.59-4.64 (1H, m), 4.73 (1H, t, J = 6.0 Hz), 4.79 (1H, t, J = 10.0 Hz), 6.77 (2H, d, J = 8.0 Hz), 7.05 (2H, d, J = 8.5 Hz), 7.15 (2H, d, J = 7.0 Hz), 7.21 (1H, d, J = 7.0 Hz, 7.25 (2H, d, J = 7.5 Hz); HRMS (FAB) NH^+ calcd for $C_{31}H_{40}N_5O_7S$ 626.2648, found 626.2629.

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APPENDIX: FIRST AUTHOR PUBLICATION IN HRUBY GROUP

1. Synthesis of β-turn mimetics: [5,5]-fused bicyclic γ-lactam dipeptide analogues. X. Gu, W. Qiu, J. Ying, J. M. Ndungu, V. J. Hruby, Peptides: *The wave of the Future, R. A. Houghten and M. Lebl, eds., Am. Peptide Soc., San Diego*, **2001**, 602-603.
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