DEVELOPMENT OF METHODOLOGIES FOR COUPLING AND CYCLIZATION OF PEPTIDES AND SYNTHESIS OF POLYKETIDE FRAGMENTS FOR MODIFIED PEPTIDES

By

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To my family and my friends
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</tr>
<tr>
<td>Å</td>
<td>Angstrom(s)</td>
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<tr>
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<td>Copper(II) sulfate pentahydrate</td>
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<tr>
<td>Cys</td>
<td>Cysteine</td>
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<tr>
<td>δ</td>
<td>Chemical shift in parts per million downfield from tetramethylsilane</td>
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<td>d</td>
<td>Days; Doublet (spectral)</td>
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<tr>
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<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
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</tr>
<tr>
<td>DIPEA</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>D$_2$O</td>
<td>Deuterium oxide</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (stands as an abbreviation for EDAC and EDCI as well)</td>
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From very early on, chemists have identified peptides and proteins as targets for the development of synthetic protocols. New and improved strategies lead to more efficient synthesis of complex peptide targets, opening the way to both new drug candidates and a deeper understanding of the intimate relation between sequence, conformation and properties. Despite recent progress and the arsenal of reagents available, peptide synthesis remains challenging; complex targets and regulatory authority requirements in terms of purity for drugs are continuously stimulating chemists to improve and rethink synthetic approaches. This dissertation addresses the development of synthetic methods and approaches targeting medium-sized cyclic peptides, and coupling of large peptide fragments. A novel approach applying S to N long-range acyl migration to synthesize peptide and peptide analogues and also provided mechanistic evidence for the ligation process are reported. A series of novel S-acyl peptides containing β- and/or γ-amino acid residues were synthesized according to S to N acyl migration protocols. Another challenge of NCL is the slow coupling rate at
proline site. To address this problem, a hydroxyproline ligation strategy utilizing hydroxyproline’s chemoselective bifunctionality was developed.

Ring size is a significant factor in the success of macrolactamization in the synthesis of a cyclic peptide. Ring sizes of 7–15-membered rings are less accessible and can frequently only be synthesized with difficulty, whether using solution phase or polymer-supported strategies. This dissertation describes the development of synthetic methods and approaches targeting medium-sized cyclic peptides including: intramolecular Staudinger ligation, cyclooligomerization, conformationally assisted cyclization.

Among cyclic natural products, cyclodepsipeptide apratoxins are intriguing marine natural products of mixed biogenetic origin. Apratoxins were isolated from cyanobacteria *Lyngbya* spp. (now known as *Moorea bouillonii*) collected in Guam and Palau. Apratoxins were found to deplete cancer cells of several cancer-associated receptor tyrosine kinases by preventing their N-glycosylation, leading to their rapid proteasomal degradation. It has been shown that apratoxin prevents co-translational translocation of proteins destined for the secretory pathway. Part of this dissertation describes the large scale synthesis of polyketide fragment for further synthetic modification and biological studies of apratoxins.
Peptides have found applications that range from catalysis\textsuperscript{1,2} through nanomaterial\textsuperscript{3,4} to drug discovery\textsuperscript{5,6}. Peptides can catalyze enantioselective addition of HCN to benzaldehyde\textsuperscript{7} or asymmetric Strecker amino acid synthesis\textsuperscript{2}. The peptide catalyst was designed to be a small molecule preferred alternative to oxynitrilase, an enzyme that was a known catalyst for the same process\textsuperscript{1}. Peptide derivatives have been utilized as scaffolds for the self-assembly of nanotubes with controllable internal diameter, pore chemistry, and exterior functionality\textsuperscript{4,8}. By modification of the peptide structure, peptide nanotubes have been designed for a wide range of applications from ion channels and antibacterials, to photoactive materials and sensors\textsuperscript{3,4,9}. However, peptides are mainly used in pharmaceutical research (Figure 1-1)\textsuperscript{10,11,12,13} because of their size and relative complexity. Peptides can show greater specificity and potency for biological targets compared to smaller acyclic compounds\textsuperscript{14,15,16}. Peptide scaffolds can modulate more challenging biological targets such as protein-protein interactions or biomolecules that lack well-defined small-molecule binding sites\textsuperscript{16}. Although many peptides have been used as therapeutic agents including: octreotide, calcitonin, cyclosporine A, nisin, polymixin and colistin\textsuperscript{15,16,10}, peptides are underrepresented in clinical use mainly due to difficulties associated with their synthesis.

New and improved strategies lead to more efficient syntheses of complex peptide targets, opening the way to both new drug candidates and a deeper understanding of the relationship between sequence, conformation and properties. Despite recent progress and the arsenal of reagents available, peptide synthesis remains challenging. Complex targets and regulatory authority constraints in terms of drug purity are
continuously stimulating chemists to improve and rethink synthetic approaches. Reducing the number of steps is usually a synonym of better yields and ease of purification, which explains the success of convergent peptide synthesis. My thesis addresses the development of synthetic methods and approaches targeting small and medium-sized cyclic peptides, peptidomimetics, and coupling of large peptide fragments.

Figure 1-1. Top selling peptide drugs
Figure 1-2. Chemoselective ligation and modification strategies for peptides

For coupling of large peptide fragments, native chemical ligation (NCL), has become a widely used, chemoselective technique to synthesize large peptides based on a capture/rearrangement concept (Figure 1-2). The full potential of native chemical ligation was shown in 1994 by Kent and co-workers for the reaction of unprotected thioesters with N-terminal Cys peptides. NCL is nowadays the most widely used chemoselective ligation technique. The impact NCL made on chemistry and biochemistry is illustrated by more than 2000 citations this original article has received so far.

The classical NCL method is limited to peptides possessing an N-terminal cysteine residue. To overcome this requirement, a novel approach was developed
applying S to N long-range acyl migration to synthesize peptide and peptide analogs and also provided mechanistic evidence for the ligation process. A series of novel S-acyl peptides containing β- and/or γ-amino acid residues were synthesized according to original S to N acyl migration protocols.

Another challenge of NCL is the slow coupling rate at a proline site. To address this problem, we developed a hydroxyproline ligation strategy, based on native chemical ligation “capture-rearrangement” principles, utilizing hydroxyproline’s chemoselective bifunctionality.

In the synthesis of cyclic peptides, a peptide can be cyclized in four different ways: head-to-tail (C-terminus to N-terminus), head-to-side chain, side chain-to-tail or side-chain-to-side-chain depending on its functional groups involved (Figure 1-3).

![Figure 1-3. The four possible ways to construct a peptide macrocycle](image)

Cyclic peptides and peptidomimetics have received the most attention in drug discovery, which is explained by the existence of powerful synthetic and biological methods to rapidly put together the required amino acid building blocks. Although, chemical synthesis of cyclic peptides benefits from the availability of relatively
inexpensive orthogonally protected amino acids, many cyclic peptides are notoriously difficult to prepare.

Figure 1-4. Lactamization method for cyclo-peptide ring contractions

Ring size is a significant factor in the success of macrolactamization in the synthesis of a cyclic peptide. Cyclo-peptides of 7–15-members are less accessible and can frequently be difficult to synthesize. They are the main hurdle to the cyclization of peptides to afford 7- to 15-membered rings is the preferential transoid alignment of amide bonds in their acyclic precursors which leads to a preferred extended structure, placing the termini far apart. This dissertation describes the development of synthetic methods and approaches targeting medium-sized cyclic peptides including: intramolecular Staudinger ligation, cyclooligomerization, conformationally assisted macro-cyclization (Figure 1-4). These novel methodologies in combination with
convergent synthetic schemes can provide the key elements for the total chemical synthesis of natural or fully unnatural linear and medium-sized cyclic peptides with yet to be discovered properties.

Among cyclic natural products, the cyclodepsipeptide apratoxins are intriguing marine natural products of mixed biogenetic origin. Apratoxins (Figure 1-5) comprise a family of cyclic depsipeptides isolated from the *Lyngbya* species of cyanobacteria. The first member of the family to be discovered, apratoxin A, was isolated in 2001 by Moore, Paul and co-workers.

![Apratoxin A](image1.png)  
**Apratoxin A**

![Apratoxin E](image2.png)  
**Apratoxin E**

![Apratoxin S4](image3.png)  
**Apratoxin S4**

Figure 1-5. Structure of natural apratoxins A and E and synthetic apratoxin S4

It was isolated from the marine cyanobacterium *Lyngbya majuscula* from Finger's Reef, Apra Harbor, Guam. Structure determination revealed that apratoxin A was composed of discrete polyketide and polypeptide domains, joined via amide and ester linkages. Apratoxin A was found to deplete cancer cells of several cancer-associated receptor tyrosine kinases by preventing their N-glycosylation, leading to their rapid proteasomal degradation. Through rational design, total synthesis of apratoxin S4 (Figure 1-5), hybrid of apratoxins A and E, has been previously achieved with improved antitumor activity and tolerability *in vivo*. The new apratoxin compound was found to be stable during purifications and stability tests. It is important to develop synthetic
approaches to the novel apratoxin derivatives to further study their biological applications. Part of this dissertation describes the multigram synthesis of the major C35-C44 subunit for the preparations of apratoxins for further biology studies.
CHAPTER 2
DEVELOPMENT OF NOVEL STRATEGIES FOR COUPLING OF PEPTIDE FRAGMENTS VIA S TO N LONG-RANGE ACYL MIGRATION

Introduction

Due to the significance and diversity of proteins in living organisms, there has been an increasing amount of interest to study their biochemical activity. Scientists desired a technique that would allow them to alter protein structure by changing the amino acid sequence. Such a technique would provide a means to observe protein function after structural manipulation. During the past few decades, studies of proteins generated by Escherichia coli have been made through techniques of DNA-based molecular biology. Specifically, scientists systematically changed the amino acid sequence of proteins and analyzed how function was affected. Comparisons in function were made to the original protein, and correlated with the specific changes that were made along the polypeptide sequence. Although this molecular biology approach provided useful insight to the chemical structures and functions of proteins, it came with major limitations. This methodology was able to utilize only the twenty genetically encoded amino acids, and modifications to the protein after translation could be difficult to control.

Chemists sought to find a new approach to overcome the limitations of the molecular biology approach. If total chemical synthesis of a protein could be achieved, the manipulation of any amino acid residue along the polypeptide of a protein could be...
carried out with relative ease and high control. In 1963, Merrifeld discovered a technique called solid phase peptide synthesis (SPPS), which greatly facilitated the synthesis of polypeptides and overcame the major shortcomings of previous synthetic-based methodologies (Figure 2-1).\textsuperscript{34,35}

![Diagram of solid phase peptide synthesis]

**LEGEND**
- RESIN
- LINKER
- \(^N\)-protection
- side-chain protecting group
- \(R = \text{OH or modification}
- \(X, Y, Z = \text{amino acid side-chain}

**Figure 2-1. Solid phase peptide synthesis**
Specifically, SPPS initially involves the C-terminus of an amino acid covalently linked to an insoluble polymeric resin. Deprotection of an N-protecting group on the first amino acid residue is then followed by purification, filtration and washing, and the addition of another N-protected amino acid. This process is repeated until the desired amino acid sequence is achieved. In the final step, all protecting groups are removed, and the covalent bond to the insoluble resin is cleaved to yield the desired peptide product.

Decades of optimization have made stepwise SPPS a reliable tool for the preparation of peptides up to around 25 amino acids long\textsuperscript{35,34}, but, yields generally decrease as the length of the polypeptide increases.

In 1992, the concept of chemoselective condensation of unprotected peptides was introduced by Kent and Schnolzer\textsuperscript{21,23}. Specifically, their novel concept used unprotected peptide fragments, each containing a unique, mutually reactive functional group. These two functional groups were designed to react strictly with each other to produce a single polypeptide product. To simplify the total synthesis of proteins through this principle, the conditions to form a peptide bond between the two peptides fragments were removed from consideration. If instead an analog structure at the ligation site was acceptable, a wide variety of known chemistries could easily be applied to covalently link the two peptides (Figure 2-2). This technique was termed native chemical ligation.

![Figure 2-2. Mechanistic pathway of Native Chemical Ligation](image-url)
Primary studies that led to the capture/rearrangement method were reported in 1953 by Wieland, who investigated the chemical properties of amino acid thioesters. In the study, they reported that thiophenol thioesters can undergo intermolecular aminolysis in the presence of amines to give amides. In contrast to these thiophenol thioesters, a glycine thioester of cysteamine could not be synthesized and isolated as such under neutral pH conditions. This observation was attributed to the additional amino group of cystamine located in proximity to the thiol moiety. As a result, a rapid intramolecular S→N shift occurred to yield the corresponding amide. This rearrangement occurred under mild acidic conditions and was accelerated at higher pH.

The previously discussed intramolecular rearrangement was combined with an intermolecular thiol–thioester exchange: specifically, a Val-thiophenol thioester was synthesized and treated with cysteine (Figure 2-3). The highly reactive aryl thioester rapidly exchanged with the thiol moiety of Cys, which represents the capture step. The resulting Val-(S-Cys) thioester subsequently rearranged to form a native Val-Cys dipeptide linked to a native peptide bond (Figure 2-3).

Figure 2-3. Early observation on aminolysis of thioesters by Wieland

Chemical ligation proved to be a breakthrough for the synthesis of many protein molecules and even enzymes. Nowadays, this method has been used to achieve the total synthesis of hundreds of proteins. For example, the complete synthesis of a fully
functional HIV-1 protease synthetic analog was able to be achieved through the 
principles of chemical ligation (Figure 2-4).²¹,²²

![Chemical synthesis diagram]

**Figure 2-4. Total chemical synthesis of native HIV-1 protease via NCL**

Recently, the total synthesis of Erythropoietin, which is a polyglycopeptide 
containing 166 amino acids in the peptide sequence, has been achieved using NCL 
(Figure 2-5).³⁷,³⁸ Erythropoietin is a signaling glycoprotein that controls the fundamental 
process of erythropoiesis, orchestrating the production and maintenance of red blood 
cells. As administrated clinically, erythropoietin has a polypeptide backbone with 
complex dishomogeneity in its carbohydrate domains. The oligosaccharide sectors were 
built by total synthesis and attached stereospecifically to peptidyl fragments of the wild-
type primary sequence, which were obtained by SPPS. The glycopeptidyl constructs 
were joined by chemical ligation.

However, scientists viewed the requirement of having an N-terminal cysteine 
residue in one of the peptide fragment for coupling as a major limitation of NCL since 
cysteine is one of the least common natural amino acid in proteins. Cysteine is a 
relatively rare amino acid (1.3% average content)³⁹ and is not always available in a
terminal position. Moreover, some amino acid-cysteine bonds such as Pro-Cys, Asp-Cys, Glu-Cys and Lys-Cys can be difficult to access by chemical ligation.\textsuperscript{39,40}

Conditions: i) Native chemical ligation; ii) Conversion of thiazolidine into cysteine; iii) Deprotection of the Pac and formyl groups; iv) Deprotection of the Pac group; v) Thioesterification of hydrazide; vi) Desulfurization; vii) Deprotection of the Acm group.

Figure 2-5. Synthesis of homogeneous EPO glycoform \textit{via} NCL
Figure 2-6. NCL with conversion of cysteine residues into other amino acids

To overcome the requirement of a specifically placed cysteine residue one approach is the use of thiol ligation auxiliaries, but unfortunately, removable cysteine mimics can sterically hinder ligation and difficulties can arise at the stage of auxiliary removal (Figure 2-6).
Other methodologies such as sugar assisted ligation\textsuperscript{42} and traceless Staudinger ligation\textsuperscript{43} were developed to provide alternative methods to achieve the total chemical synthesis of native proteins (Figure 2-7).

A useful approach for the synthesis of cysteine containing peptides is isopeptide ligation methodology. Yoshiya \textit{et al.} synthesized S-acyl peptides containing N-terminal cysteine residues; subsequent S→O intramolecular acyl migration then furnished native peptide bonds.\textsuperscript{44}

This dissertation describes the chemical ligation approach using S-acylated cysteine peptides to form native peptides via 11-, 13-, 14-, 15- and 16-membered cyclic transition states via long-range acyl migration. The method allows the synthesis of native peptides from S-acyl isopeptides with a C-terminal cysteine without utilizing auxiliaries.

Our method utilizes non-terminal cysteine residues that could significantly expand the applicability of this isopeptide approach. We describe the first acyl migration in S-acyl isopeptide to form native peptides from non-terminal cysteine residues.

\textbf{Results and Discussion}

\textbf{Microwave-Assisted Chemical Ligation of S-Acyl Peptides Containing Non-Terminal Cysteine Residues}

\textbf{Study of the feasibility of $S\rightarrow N$ acyl migration via an 8-membered compared to a 5-membered cyclic transition state}

The protected dipeptide dimer 2.1.2 was prepared in 79\% yield by mixed anhydride coupling of bis-Boc-cystine 2.1.1 and H-Gly-OCH$_3$ according to a literature procedure. To investigate the feasibility of acyl migration through an 8-membered transition state, the Boc-protected dipeptide dimer 2.1.2 was first reacted with tributylphosphine to afford the dipeptide monomer 2.1.3 in 55\% yield. Subsequent S-
acylation of 2.1.3 with Fmoc-Gly-Bt furnished the mono-isotetrapeptide 2.1.4 in 88% yield. Next, 2.1.4 was Boc-deprotected selectively with methanol saturated with hydrochloric acid gas. The crude HCl salt 2.1.5 was treated with triethylamine to give the corresponding free base which as expected underwent classical S to N acyl migration via a 5-membered transition state to furnish the native tripeptide 2.1.6 in 52% yield (Figure 2.8).

![Chemical ligation of S-acyl dipeptide 2.1.5](image)

**Figure 2.8. Chemical ligation of S-acyl dipeptide 2.1.5**

Attempts to prepare the Fmoc-protected isotetrapeptide 2.1.7 from 2.1.6 failed (Figure 2.8). We therefore deprotected the Boc-protected dipeptide dimer 2.1.2 utilizing saturated hydrochloric acid in methanol to afford the dipeptide dimer 2.1.8 as dihydrochloride (85% yield) followed by mixed anhydride coupling of 2.1.8 with 2 equiv. of Boc-glycine to furnish the novel tripeptide dimer 2.1.9 in 67% yield. Treatment of the disulfide 2.1.9 with tributylphosphine gave the tripeptide monomer 2.1.10 (Scheme 2.9).
S-Acylation of 2.1.10 with Cbz-Ala-Bt in the presence of triethylamine provided the key intermediates 2.1.11 (72% yield) which on deprotection by MeOH-HCl gave the desired isotetrapeptide 2.1.12 in 82% yield (Scheme 2-9).

![Chemical structures](image)

Figure 2-9. Synthesis and attempted chemical ligation of S-acyl tripeptide 2.1.12

We then investigated the chemical ligation of 2.1.12 via an 8-membered cyclic transition state (TS). The isotetrapeptide 2.1.12 was dissolved in a mixture of 0.4 M NaH2PO4/Na2HPO4 buffer (pH = 7.8) and acetonitrile (7:1) and subjected to microwave irradiation for 3 h at 70 °C and 50 W (Scheme 2-3). Interestingly, the HPLC-MS (ESI) analysis of the reaction mixture revealed that the major component was unreacted starting material 2.1.12. The (+)ESI-MSn spectrum of the major product (m/z 455 [M+H]+ ion) was nearly identical to those of 2.1.12. Moreover, the HPLC-MS (ESI) analysis of a mixture of 2.1.12 and the product from the ligation experiment detected only one MW 454 compound, whose characteristics matched those of the mono-
isotetrapeptide 2.1.12. Absence of the S to N acyl migration of 2.1.12 suggests that the S→N acyl migration via an 8-membered cyclic transition state for 2.1.12 is disfavored.

**Study of S to N acyl migration via an 11-membered cyclic transition state**

To study possible chemical ligation via an 11-membered cyclic transition state, we first prepared the N-Boc-protected dipeptides 2.1.15 in 69% yield by peptide coupling reactions of Boc-Gly-Bt with phenylalanine (Phe) in partially aqueous solution in the presence of triethylamine (Figure 2.10). Mixed anhydride couplings of the dipeptide dimer 2.1.8 with 2 equiv. of the dipeptide 2.1.15 furnished the tetrapeptide dimers 2.1.16 in 72% yield (Scheme 2-4). Next, the cleavage of the disulfide 2.1.16 afforded the monomer tetrapeptide 2.1.17 (62%), which was subsequently S-acylated with Cbz-Ala-Bt in the presence of triethylamine. The Boc-protected mono-isopentapeptide 2.1.18 was purified by recrystallization and then isolated in 92% yield. Acid-catalyzed deprotection of Boc group proceeded smoothly to form the desired mono-isopentapeptides 2.1.19 (86%, Figure 2.10).

We investigated S→N acyl migration in compound 2.1.19 via an 11-membered cyclic transition state. The chemical ligation experiment on 2.1.19 was carried out under microwave irradiation at 50 °C and 50 W for 1 h. After workup, HPLC-MS (ESI) analysis of the crude ligation mixture showed that the ligation experiment yielded the expected ligation product 2.1.20a and an intermolecular transacylation product 2.1.20b in ratio of 33:67 (86% combined crude yield) (Figure 2-11).

The product mixture was subsequently purified by semi-preparative HPLC, which allowed the isolation of the desired ligation product 2.1.20a as well as isolation of the
intermolecular transacylation product 2.1.20b in yields of 23% and 52%, respectively. Both products were characterized by analytical HPLC and HRMS.

Figure 2-10. Synthesis of isopentapeptides 2.1.19

Figure 2-11. Chemical ligation of S-acyl mono-isopentapeptides 2.1.19.
The formation of the side product 2.1.20b could be explained as follows. In the first step the intramolecular $S \rightarrow N$ acyl migration provides the desired product 2.1.20a, which is later S-acylated by another equivalent of the starting material 2.1.19 to form the transacylation product 2.1.20b by intermolecular reaction.

**Study of $S \rightarrow N$ acyl migration via a 14-membered cyclic transition state**

The synthesis of starting material 2.1.25 for a possible $S \rightarrow N$ ligation via a 14-membered transition state was accomplished in a five-step procedure starting from the tripeptide dimer 2.1.9. After initial deprotection of 2.1.9 to form 2.1.21, subsequent mixed anhydride coupling of 2.1.21 with Boc-Gly-Phe-OH afforded the pentapeptide dimer 2.1.22 in 74% yield. Cleavage of the disulfide bond in 2.1.22 furnished the monomer 2.1.23 which was S-acylated with Cbz-Ala-Bt to provide the Boc-protected isohexapeptide 2.1.24 (86%). Final deprotection of the Boc group in 2.1.24 afforded the desired starting material 2.1.25 (Figure 2.12).

Chemical ligation from isopeptide 2.1.25 through a 14-membered cyclic transition state was investigated by dissolving 2.1.25 in 0.4 M phosphate buffer (pH = 7.8) and acetonitrile (7:1) and subjecting the mixture to microwave irradiation (50 °C, 50 W, 1h) (Figure 2-13). HPLC-MS (ESI) analysis of the crude ligation product mixture confirmed the major component (57%, Table 2-1) to be the expected ligation product 2.1.26. Again, the intermolecular transacylation product 2.1.27 was formed as side product (Table 2-1). Our results indicate that transition states studied in this present paper are favored in decreasing order 5$\gg$14$\gg$11$\gg$8. These are the first examples of successful isopeptide ligations starting from non-terminal S-acyl peptides and the results strongly
suggest the chemical long-range ligation via 11- and 14-membered cyclic transition states is a promising approach for the synthesis of native peptides.

Figure 2-12. Synthesis of isohexapeptide 2.1.25

Figure 2-13. Chemical ligation of $S$-acyl mono-isohexapeptides 2.1.25.
Table 2-1. Chemical ligation of isopeptides 2.1.25

<table>
<thead>
<tr>
<th>Product</th>
<th>Isolated yield [%]</th>
<th>Purity [%]a</th>
<th>HRMS [M+Na]+ found</th>
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<td>2.1.26</td>
<td>41</td>
<td>&gt;95%</td>
<td>1337.4629b,c</td>
</tr>
</tbody>
</table>

a Purity based on analytical HPLC
b Isolated as disulfide dimer
c HRMS calc. [M+Na]+: 1337.4578

Long-Range Intramolecular $S\rightarrow N$ Acyl Migration: a Study of the Formation of Native Peptide Analogs via 15- and 16-Membered Cyclic Transition States

Demonstration of $S\rightarrow N$ acyl migration in S-acyl tetrapeptide via a 15-membered cyclic transition state.

Unnatural amino acids have been used to prepare peptide analogs with enhanced enzymatic stability, and bioavailability. β-Amino acid residues, which are often encountered in natural products, have been used for obtaining peptidomimetics and for affecting secondary and tertiary structures of synthetic peptides.

To investigate the further applicability of our methodology to access peptide analogs, we studied long range $S\rightarrow N$ acyl migrations of S-acylated cysteine peptides containing β-residues via a 15-membered cyclic transition states, leading to the formation of the corresponding native tetra- and pentapeptide analogs.

For the $S\rightarrow N$ ligation via a 15-membered cyclic transition state, the starting material 2.2.9a was obtained in a six-step procedure starting from the $L$-cystine dimethyl ester dihydrochloride (2.2.1). The protected dipeptide dimer 2.2.2a was prepared in 67% yield from the mixed anhydride coupling of 2.2.1 with Boc-Gly-OH following a literature procedure (Figure 2.14). Deprotection of 2.2.2a using HCl(g) in methanol gave 2.2.3a in 84% yield. The coupling of 2.2.3a with Boc-protected dipeptide Boc-β-Ala-$L$-Leu-OH (2.2.5a) provided the tetrapeptide dimer 2.2.6a in 65% yield, which was subsequently treated with tributylphosphine to afford the tetrapeptide monomer 2.2.7a in
75% yield. \(S\)-Acylation of \(N\)-Boc-protected cysteine tetrapeptide 2.2.8a with Cbz-\(L\)-Ala-Bt in acetonitrile-water (10:1) in the presence of triethylamine gave \(S\)-acyl tetrapeptide 2.2.8a in 86% yield. Finally, compound 2.2.8a was Boc-deprotected using HCl\((g)\) in methanol to give the HCl salt 2.2.9a in 82% yield (Figure 2-14).

The intramolecular \(S\rightarrow N\) acyl migration experiment 2.2.9a→2.2.10 would proceed through a 15-membered-ring transition state. Precedents for successful \(S\rightarrow N\) acyl shift in SAL proceeding (Figure 2-15) through transition states with 15-membered rings encouraged us to pursue this approach.\(^{42}\)
The chemical ligation experiment was carried out at 2 mM concentration under microwave irradiation at 50 °C and 50 W for 1 h at pH = 7.6. HPLC-MS (ESI). Analysis of the crude reaction mixture revealed the presence of two major products in a 35:65 ratio (Table 2-2). The HPLC-MS (ESI) analysis identified the major reaction products as the desired ligation product 2.2.10 and the minor product as the intermolecular transacylation product 2.2.11 (Figure 2.16).

![Proposed 15-membered cyclic transition states](image)

Figure 2-15. Proposed 15-membered cyclic transition states of A) the second-generation Sugar-assisted Ligation (SAL) and (B) the long-range intramolecular acyl-migration.

Compound 2.2.10 was isolated by semi-preparative HPLC and fully characterized by $^1$H and $^{13}$C NMR spectroscopy, HRESI-MS and analytical HPLC (Table 2-3). $^1$H NMR spectra indicated for the formation of the desired ligation product 2.2.10 (appearance of five amide proton signals), while $^{13}$C NMR provided further evidence. Indeed, S-aminoaclytetrapiptide 2.2.9a has four typical amide $^{13}$C signals with chemical shifts ranging from δ 171.5 to 175.4 ppm and a very typical $^{13}$C thioester carbonyl observed at δ 203.2 ppm. In contrast, compound 2.2.10 showed distinct $^{13}$C NMR resonances for five amide bonds at δ 169.1, 170.7, 170.8, 172.4 and 172.5 ppm (Figure 2-17). These results confirmed the S→N migration of Cbz-alanine to the N-terminus.
Table 2-2. Characterization data of ligation product mixtures

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Crude&lt;sup&gt;a&lt;/sup&gt; yield (%)</th>
<th>Product ratio&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>[M+H]&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ligated peptide</td>
<td>Transacylation product</td>
<td>Ligated peptide&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>2.2.9a</td>
<td>92</td>
<td>35, 2.2.10</td>
<td>65, 2.2.11</td>
</tr>
<tr>
<td>2</td>
<td>2.2.9b</td>
<td>94</td>
<td>64, 2.2.12a</td>
<td>36, 2.2.13a</td>
</tr>
<tr>
<td>3</td>
<td>2.2.9c</td>
<td>82</td>
<td>57, 2.2.12b</td>
<td>43, 2.2.13b</td>
</tr>
</tbody>
</table>

<sup>a</sup>The combined crude yield of ligated product was calculated based on the isolated amount of the product mixture ligated peptide:transacylation product according to the following equation: combined crude yield = ([ligated peptide] + 2 x [transacylation product])/[ starting material]. The S-deacylated peptide side products were removed during the work-up.<sup>b</sup> Determined by HPLC-MS semi-quantitative. The area of ion-peak resulting from the sum of the intensities of the [M+H]<sup>c</sup> and [M+Na]<sup>c</sup> ions of each compound was integrated. <sup>c</sup>HPLC-MS (ESI). <sup>d</sup> Analyzed as disulfide dimer.

Table 2-3. Characterization data of the isolated ligation products 2.2.10 and 2.2.12a,b

<table>
<thead>
<tr>
<th>Ligation product</th>
<th>Purity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isolated yield (%)</th>
<th>After semi-preparative HPLC</th>
<th>HRMS [M+Na]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Retention time&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.10</td>
<td>&gt;98%</td>
<td>31</td>
<td>1183.4774</td>
<td>1183.4733</td>
<td>19.42</td>
</tr>
<tr>
<td>2.2.12a</td>
<td>&gt;99%</td>
<td>42</td>
<td>1279.4774</td>
<td>1279.4718</td>
<td>20.89</td>
</tr>
<tr>
<td>2.2.12b</td>
<td>&gt;95%</td>
<td>37</td>
<td>1279.4774</td>
<td>1279.4775</td>
<td>18.64</td>
</tr>
</tbody>
</table>

<sup>a</sup>Purity based on analytical HPLC. <sup>b</sup>Analyzed as disulfide dimer. <sup>c</sup>254 nm, MeOH/H<sub>2</sub>O (65:35), 0.15 mL/min.

**Figure 2-16.** Chemical ligation of S-acyl tetrapeptide 2.2.9a. The ligation compound 2.2.10 is drawn as a monomer for clarity.
Figure 2-17. $^{13}$C NMR carbonyl signals in (A) ligated pentapeptide 2.2.10 and in (B) starting S-acylated tetrapeptide 2.2.9a

To understand the dependence of the substrate concentration on the formation of the ligated product vs. the transacylation product, we compared the initial product ratio 2.2.10:2.2.11 in the ligation reaction of 2.2.9a at pH = 7.1 as a function of concentration of reactant under identical reaction conditions. Interestingly, HPLC-MS (ESI) analysis revealed that the concentration of the substrate does not significantly influence the product ratio. At 2 mM concentration of the substrate 2.2.9a the ligated product vs. the transacylation product ratio was 45:55 while at 0.5 mM and 6 mM concentrations of 2.2.9a the product ratios were 43:57 and 55:45 respectively.
Demonstration of S→N acyl migration in S-acyl tetrapeptides 23b,c each via distinct isomeric 16-membered cyclic transition states

To study the chemical ligation via a 16-membered cyclic transition state, we first prepared the tetrapeptide dimer 2.2.6b in 52% yield by coupling unprotected cystine dipeptide dimer 2.2.3b with Boc-β-Ala-\text{-}L-Phe-OH (2.2.5b). Then, tetrapeptide dimer 2.2.6b was treated with tributylphosphine to release the tetrapeptide monomer 2.2.7b in 68% yield. S-Acylation of N-Boc-protected cysteine tetrapeptide 2.2.7b with Cbz-L-Ala-Bt in a acetonitrile-water mixture (10:1) in the presence of triethylamine afforded S-acyl tetrapeptide 2.2.8b in 88% yield. Boc deprotection of 2.2.8b using HCl(\text{g}) in methanol gave the hydrochloride 2.2.9b in 81% yield (Figure 2-18).

Similarly, 2.2.3b was coupled with Boc-GABA-\text{-}L-Phe-OH (2.2.5c) to afford Boc-protected tetrapeptide dimer 2.2.6c in 57% yield. Cleavage of the disulfide bond in 2.2.6c furnished the monomer 2.2.7c (66%), which was S-acylated with Cbz-L-Ala-Bt to provide the Boc-protected S-acyl tetrapeptide 2.2.8c (92%). Final Boc deprotection afforded the desired amino-unprotected S-acylated tetrapeptide 2.2.9c in 87% yield (Figure 2-18).

We then investigated S→N acyl migration for compounds 2.2.9b and 2.2.9c which proceeds in each case via isomeric 16-membered cyclic transition states. Chemical ligation on 2.2.9b was carried out under microwave irradiation at 50 °C and 50 W for 1 h in NaH₂PO₄/Na₂HPO₄ buffer (1 M, pH 7.6) and acetonitrile (8:1 mixture). HPLC-MS (ESI) analysis of the crude ligation mixture revealed the major component (64%) to be the expected ligation product 2.2.12a (molecular ion of the disulfide dimer [M+H]⁺ m/z: 1257, vs 630 for the [M+H]⁺ molecular ion of the starting S-(Pg-α-aminoacyl)tetrapeptide 2.2.9b). The HPLC-MS (ESI) confirmed that the second major
product is 2.2.13a formed by intermolecular trans-acylation (Table 2-2; Figure 2-19). Separation of 2.2.12a by semi-preparative HPLC allowed isolation of 42% of pure pentapeptide 2.2.12a, which was further characterized by analytical HPLC and HRMS analysis (Table 2-3).

Figure 2-18. Preparation of S-acyl tetrapeptide 2.2.9a
Figure 2-19. Acyl migration of S-acyl tetrapeptides 2.2.12b. The ligation compound 2.2.13a is drawn as a monomer for clarity.

Similarly, the acyl migration experiment of S-acylpeptide 2.2.9c was carried out under the conditions described above. HPLC-MS (ESI) analysis showed the presence of two main products in a 57:43 ratio (Table 2-2, Figure 2-20). As expected, the major product of this experiment was the desired ligation product 2.2.12b. The subsequent separation of 2.2.12b by semi-preparative HPLC provided the purified ligation product 2.2.12b in 37% yield. The product was characterized by HPLC analytical and HRMS (Table 2-3).
Study of pH Dependence on the Ligation Experiment.

Chemical ligation is pH sensitive: ligation usually proceeds rapidly around pH 7 but is rendered less efficient at pH < 5.5.\textsuperscript{52} In addition, in some cases the yields for traceless Staudinger ligation in water increased at higher pH, but decreased drastically at pH ≥ 8.5.\textsuperscript{53} The ratio of the ligation product 2.2.9a versus the intermolecular transacylated compound 2.2.10 was studied under different pH conditions (Table 2-4, Figure 2-21). Chemical ligation experiments on 2.2.9a were carried out under microwave irradiation at 50 °C and 50 W for 1 h in 1M phosphate buffer with pH values ranging from 6.2-8.2. After workup, the crude ligation mixtures were subjected to HPLC-MS (ESI) analysis. The results are summarized in Table 2-4 and Figure 2-9. HPLC-MS (ESI) analysis of the crude ligation mixture at pH = 6.2 showed the presence of the major intermolecular transacylation product 2.2.11 and the desired ligation product 2.2.10 in a 80:20 ratio. When the ligation experiment was conducted at pH = 8.2, HPLC-MS (ESI) analysis identified the same two products: the transacylated product 2.2.11 and the desired ligated product 2.2.10 in a similar 85:15 ratio. In contrast, when ligation was carried out at pH 7.0 and at pH 7.6, HPLC-MS (ESI) showed a significant increase in ligation product 2.2.10 with calculated 2.2.10:2.2.11 ratios of 45:55 and 36:64, respectively. In case of a lower pH, the starting material 2.2.9a exists partially in the unreactive protonated form and the acyl migration reaction is relatively slow, which favors the intermolecular S-acylation of 2.2.10 by the excess of starting material 2.2.9a to form the undesired product 2.2.11. At pH > 8 the thiol group of the ligation product 2.2.10 is partially deprotonated, which again favors the formation of the transacylation product 2.2.11 in a subsequent intermolecular reaction of 2.2.10 with unreacted 2.2.9a. At pH=7.3, a 2.2.10:2.2.11 ratio of 43:57 was obtained. The $S\rightarrow N$ acyl migration in $S$-
acyl tetrapeptide 2.2.9a via a 15-membered cyclic transition state to give 2.2.10 is therefore favored at a pH range from 7.0-7.6.

Table 2-4. Dependence of pH on the product ratio 2.2.10:2.2.11 in the ligation reaction of 2.2.9a

<table>
<thead>
<tr>
<th>Entry</th>
<th>pH</th>
<th>Ligated peptide&lt;sup&gt;b&lt;/sup&gt; (2.2.10)</th>
<th>Transacylation product (2.2.11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.2</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>7.3</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>7.6</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>8.2</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by HPLC-MS semiquantitation. The area of ion-peak resulting from the sum of the intensities of the [M+H]<sup>+</sup> and [M+Na]<sup>+</sup> ions of each compound was integrated. <sup>b</sup> Analyzed as disulfide dimer.

Figure 2.21. Effect of pH on S→N acyl migration in S-acyl tetrapeptide 2.2.9a.
Conclusion

In summary, this work demonstrates an efficient and convenient synthetic pathway for the preparation of several S-acyl isopeptides containing internal cysteine residues. The chemical ligation studies of these S-acyl peptides via 5-, 8-, 11- and 14-membered cyclic transition states show that the 8-membered transition state is clearly disfavored, whereas the 11- and 14-membered transition states are relatively favored for long-range ligation approach. These results indicate that the transition states studied in this present paper are decreasingly favored in the order 5>>14>11>>8.

Furthermore, a series of novel S-acyl peptides containing β- and/or γ-amino acid residues, which are useful intermediates in various synthetic and biological applications, were synthesized according to original protocols. The ligation step was investigated under MW heating and was found to be sensitive to concentration of the ligating fragments. The observed variation in ligation yield allows for reactivity scaling. The native peptides obtained after chemical ligation of tetrapeptides via 15- and 16-membered cyclic transition states were isolated in modest to good yields. The experimental results show that the extent of chemical ligation via 14-TS, 15-TS and 16-TS are similar.

Experimental

General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded in CDCl₃, DMSO-<sub>d₆</sub> or CD₃OD-<sub>d₄</sub> on Gemini or Varian NMR operating at 300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C with TMS as an internal standard. Elemental analyses were performed on a Carlo Erba-1106 instrument. All microwave assisted reactions were carried out with a single
mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). L-Cystine, Boc-Gly-OH and L-Leucine were purchased from Sigma-Aldrich. Cbz-L-Ala-OH and Fmoc-Gly-OH were purchased from Chem-Impex International. L-Phenylalanine and glycine methyl ester hydrochloride were purchased from TCI-US. All commercially available starting materials were used without further purification. Fmoc-Gly-Bt (Bt = benzotriazol-1-yl), Cbz-L-Ala-Bt, Boc-Gly-Bt and N,N-di-Boc-cystine were prepared according to known procedures. The phosphate buffer (NaH$_2$PO$_4$/Na$_2$HPO$_4$) (0.4 M, pH 7.8) was degassed by bubbling argon through the buffer. HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (2.1 x 150 mm; 5 µm) + guard column (2 x 4 mm) or Thermoscientific Hypurity C8 (5µm; 2.1 x 100 mm + guard column) using 0.2% acetic acid in H$_2$O/methanol as mobile phases; wavelength = 254 nm; and mass spectrometry was done with electro spray ionization (ESI). Product ratios were obtained from HPLC-MS semiquantitation. The area of ion-peak resulting from the sum of the intensities of the [M+H]$^+$ and [M+Na]$^+$ ions of each compound was integrated. Semi-preparative and analytical HPLC were carried out on Phenomenex Luna 10 µm C18(2) columns. Methanol:water (70:30) was used as eluent for the isolation of compounds.

**Experimental Details for Compounds 2.1.2, 2.1.3, 2.1.4, 2.1.8, 2.1.9, 2.1.15, 2.1.16, 2.1.21 and 2.1.22**

(2.1.2). A solution of N,N'-di-Boc-cystine (1.32 g, 3 mmol) in dry THF (10 mL) under argon was cooled to -15 °C in an ice bath with stirring. N-Methylmorpholine (0.67 g, 6.6 mmol), followed by isobutylchloroformate (0.91 g, 6.6 mmol) were added. After 5 min, a solution of glycine methyl ester hydrochloride (0.75 g, 6 mmol) and N-methylmorpholine (0.67 g, 6.6 mmol) in DMF (15 mL) was added. The ice bath was removed after 5 min and the solution was allowed to stir for 24 h at room temperature. The solution was concentrated under vacuum and the residue was dissolved in a mixture of ethyl acetate (20 mL) and water (5 mL). After extraction, the aqueous phase was discarded and the organic phase washed successively with saturated Na₂CO₃ (2 x 10 mL), water (10 mL) and 2N HCl (10 mL). The solution was dried over dry MgSO₄, filtered and concentrated under vacuum. The peptide was recrystallized from ethyl acetate:hexanes to give desired product. White microcrystals, 79% yield, mp 140-143 °C; Anal. Calcd for C₂₂H₃₈N₄O₁₀S₂: C 45.35; H 6.57; N 9.62. Found: C 45.68; H 6.62; N 9.18; ¹H NMR (300 MHz, CDCl₃) δ 1.41 (s, 18H), 2.89 (dd, J = 14.3, 10.2 Hz, 2H), 3.04 (dd, J = 14.7, 3.9 Hz, 2H), 3.70 (s, 6H), 3.87 (dd, J = 17.6, 5.3 Hz, 2H), 4.11 (dd, J = 18.0, 6.3 Hz, 2H), 4.90 (br s, 2H), 5.56 (d, J = 9.6 Hz, 2H), 8.13 (s, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 28.5, 41.1, 46.9, 52.4, 54.7, 80.5, 156.1, 169.9, 171.0.

(R)-Methyl 2-((tert-butoxycarbonyl)amino)-3-mercaptopropanamido)-acetate (2.1.3). (6R,11R)-methyl 11-((tert-butoxycarbonyl)amino)-6-((2-methoxy-2-oxoethyl)carbamoyl)-2,2-dimethyl-4,12-dioxo-3-oxa-8,9-dithia-5,13-diazapentadecan-15-oate (400 mg, 0.69 mmol) was treated with PBu₃ (277 mg, 0.34 mL, 1.37 mmol) in 12 mL of MeOH:water (9:1) for 2h at room temperature. The reaction mixture was concentrated under vacuum and the residue was dissolved in diethylether (15 mL). The
solution was dried over magnesium sulfate, and then concentrated under vacuum. The peptide was purified by column chromatography on silica gel (Eluent hexane:AcOEt, 2:1) and recrystallized from Et<sub>2</sub>O:hexane to give 3. White microcrystals, 55% yield, mp 55-57 °C; Anal. Calcd for C<sub>11</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>S: C 45.19; H 6.90; N 9.58. Found: C 45.48; H 7.16; N 9.45; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.44 (s, 9H), 1.70 (dd, <i>J</i> = 10.4, 7.6 Hz, 1H), 2.70–2.80 (m, 1H), 3.09-3.18 (m, 1H), 3.76 (s, 3H), 3.98-4.15 (m, 2H), 4.45 (br s, 1H), 5.58 (d, <i>J</i> = 8.1 Hz, 1H), 7.0 (br s, 1H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 27.3, 28.5, 41.4, 52.6, 55.7, 80.8, 155.6, 170.2, 170.8.

(R)-Methyl 2-((tert-butoxycarbonyl)amino)-3-mercapto-propanamido)acetate (2.1.4). Dipeptide Boc-L-Cys-Gly-OMe (0.29 g, 1 mmol) together with an equimolar amount of Fmoc-Gly-Bt (0.40 g, 1 mmol) was suspended in acetonitrile (20 mL) at 25 °C. Then, 12 mL of 0.1 N KHCO<sub>3</sub> in water was added dropwise. The solution was stirred at the same temperature and monitored by TLC for starting material consumption. After completion of the reaction, the solution was acidified with 2 N HCl (15 mL), acetonitrile was removed under reduced pressure. The residue formed was dissolved in ethyl acetate (40 mL), extracted with 2 N HCl (3 × 15 mL), sat. NaCl solution (20 mL) and dried over MgSO<sub>4</sub>. Ethyl acetate was removed under reduced pressure and the residue was dissolved in dichloromethane (15 mL), hexanes were added until the solution is turbid and the solution was left to crystallize in the freezer. The solid obtained was filtered, dried to give the corresponding target. White microcrystals, 88% yield, mp 108-112 °C; Anal. Calcd for C<sub>28</sub>H<sub>33</sub>N<sub>3</sub>O<sub>8</sub>S: C 58.83; H 5.82; N 7.35. Found: C 58.93; H 5.87; N 7.38; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.44 (s, 9H), 3.25 (dd, <i>J</i> = 14.1, 7.6 Hz, 1H), 3.40 (dd, <i>J</i> = 14.0, 5.0 Hz, 1H), 3.71 (s, 3H), 4.01 (d, <i>J</i> = 5.4 Hz, 2H), 4.14 (d, <i>J</i> = 5.5 Hz,
2H), 4.23 (t, J = 6.9 Hz, 2H), 4.42 (s, 1H), 4.44 (s, 1H), 5.42 (d, J = 7.9 Hz, 1H), 5.63 (br s, 1H), 6.94 (br s, 1H), 7.30 (t, J = 7.2 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.59 (d, J = 7.1 Hz, 2H), 7.76 (d, J = 7.4 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 28.5, 30.9, 41.4, 47.3, 50.9, 52.6, 54.2, 67.5, 80.9, 120.2, 125.2, 127.3, 128.0, 141.5, 143.9, 155.8, 156.5, 170.1, 170.5, 198.5.

(7R,12R)-3,6,13,16-Tetraoxo-2,17-dioxa-9,10-dithia-5,14-diazaoctadecane-7,12-diaminium chloride (2.1.8). HCl gas was passed through a solution of peptide 2.1.2 (4 mmol) in methanol (15 mL) for 30 minutes. The methanol solution was concentrated under vacuum and diethyl ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry ethyl acetate (10 mL) and then with diethyl ether (10 mL) dried to give the corresponding deprotected peptide 7. White solid, 85% yield, mp 112-116 °C; Anal. Calcd for C$_{12}$H$_{24}$N$_4$O$_6$S$_2$: C 31.65; H 5.75; N 12.30. Found: C 31.25; H 5.63; N 10.94; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 3.18-3.37 (m, 4H), 3.64 (s, 6H), 3.93 (d, J = 5.4 Hz, 4H), 4.21-4.22 (m, 2H), 8.63 (br s, 6H), 9.38 (d, J = 5.4 Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 38.5, 40.8, 51.1, 52.0, 167.5, 169.7.

(Gly-L-Cys-Gly-OCH$_3$)$_2$ hydrochloride (2.1.21). HCl gas was passed through a solution of peptide 2.1.9 (4 mmol) in methanol (15 mL) for 30 minutes. The methanol solution was concentrated under vacuum and diethyl ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry ethyl acetate (10 mL) and then with diethyl ether (10 mL) dried to give the corresponding deprotected peptide 21. White microcrystals, 92% yield, mp 209-212 °C; Anal. Calcd for C$_{16}$H$_{30}$Cl$_2$N$_6$O$_8$S$_2$.H$_2$O: C 32.71; H 5.49; N 14.30. Found: C 32.50; H
5.27; N 13.13; \(^1^\)H NMR (300 MHz, CD\(_3\)OD) \(\delta\) 2.98 (dd, \(J = 13.7, 6.5\) Hz, 1H), 3.23-3.28 (m, 1H), 3.69 (s, 3H), 3.87 (s, 2H), 3.96 (s, 2H); \(^1^\)\(^3\)C NMR (75 MHz, CD\(_3\)OD) \(\delta\) 41.8, 42.2, 53.0, 54.1, 168.1, 171.8, 172.6.

\((\text{Boc-Gly-L-Phe-Gly-L-Cys-Gly-OCH}_3)_2\) (2.1.2). A solution of 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-phenylpropanoic acid 2.1.21 (0.97 g, 3 mmol) in dry THF (10 mL) under argon was cooled to -15 °C in an ice bath with stirring. N-Methylmorpholine (0.33g, 3.2 mmol), followed by isobutylchloroformate (0.45 g, 3.2 mmol) were added. After 3 min, a solution of 2.1.21 (0.85 g, 1.5 mmol) and N-methylmorpholine (0.7 g, 1.6 mmol) in DMF (10 mL) was added. The ice bath was removed after 5 minutes and the solution was allowed to stir for 12 h at room temperature. The solution was concentrated under vacuum and the residue was dissolved in ethyl acetate (30 mL) and water (5 mL). After extraction, the aqueous phase was discarded and the organic phase washed successively with saturated Na\(_2\)CO\(_3\) (2 x 15 mL), water (10 mL), 2N HCl (15 mL) and water (10 mL). The solution was dried over dry MgSO\(_4\), filtered and then concentrated under vacuum. The peptide was recrystallized from ethyl acetate/hexane to give desired (Boc-Gly-L-Phe-Gly-L-Cys-Gly-OCH\(_3\))\(_2\). White microcrystals, 74% yield, mp 180-185 °C; Anal. Calcd for C\(_{48}\)H\(_{68}\)N\(_{10}\)O\(_{16}\)S\(_2\): C 52.16; H 6.20; N 12.67. Found: C 52.44; H 6.60; N 11.83; \(^1^\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) 1.35 (br s, 18H), 2.75-2.89 (m, 4H), 2.99-3.15 (m, 4H), 3.44-3.54 (m, 4H), 3.61 (br s, 6H), 3.67-3.74 (m, 2H), 3.84-3.91 (m, 6H), 4.52 (br s, 2H), 4.62 (br s, 2H), 6.88 (t, \(J = 5.5\) Hz, 2H), 7.22 (br s, 10H), 8.03 (d, \(J = 7.8\) Hz, 2H), 8.30 (d, \(J = 8.1\) Hz, 2H), 8.38 (br s, 2H), 8.52 (br s, 2H). \(^1^\)\(^3\)C NMR (75 MHz, DMSO-d\(_6\)) \(\delta\) 28.2, 37.6,
General Procedure for Boc Deprotection of Peptides 2.1.4, 2.1.11, 2.1.18 and 2.1.24 to Give the Corresponding Unprotected Peptides 2.1.5, 2.1.12, 2.1.19 and 2.1.25

HCl gas was passed through a solution of peptide 2.1.4, 2.1.11, 2.1.18 or 2.1.25 in methanol (15 mL) for 30 minutes. The methanol solution was concentrated under vacuum and diethyl ether (20 mL) was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry ethyl acetate (10 mL) and diethyl ether (10 mL) dried to give the corresponding deprotected peptide 2.1.5, 2.1.12, 2.1.19 and 2.1.25

(R)-1-(9H-Fluoren-9-yl)-3,6,10,13-tetraoxo-2,14-dioxo-7-thia-4,11-diazapentadecan-9-aminium chloride (2.1.5). White solid, 85% yield, mp 203-206 °C; Anal. Calcd for C_{23}H_{25}N_{3}O_{6}S·HCl·2H_{2}O: C 50.78; H 5.56; N 7.72. Found: C 50.81; H 5.07; N 7.47; \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6) \delta 3.29-3.42 (m, 2H), 3.61 (s, 3H), 3.90 (d, J = 5.4 Hz, 2H), 3.96 (d, J = 5.9 Hz, 2H), 4.08 (br s, 1H), 4.24 (t, J = 6.8 Hz, 1H), 4.34 (d, J = 6.8 Hz, 2H), 7.33 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.71 (d, J = 7.4 Hz, 2H), 7.88 (d, J = 7.4 Hz, 2H), 8.13 (t, J = 5.9 Hz, 1H), 8.54 (br s, 3H), 9.12 (t, J = 5.4 Hz, 1H); \textsuperscript{13}C NMR (75 MHz, DMSO-\textit{d}_6) \delta 28.6, 40.9, 46.7, 50.5, 51.3, 52.0, 66.1, 120.3, 125.3, 127.2, 127.8, 140.8, 143.8, 156.6, 167.3, 169.6, 197.9.

(5S,9R)-methyl 9-(2-aminoacetamido)-5-methyl-3,6,10-trioxo-1-phenyl-2-oxa-7-thia-4,11-diazatridecan-13-oate hydrochloride (2.1.12). White microcrystals, 85% yield, mp 130-135 °C; Anal. Calcd for C_{19}H_{27}ClN_{4}O_{8}S·1.5H_{2}O: C 44.06; H 5.84; N 10.82. Found: C 43.95; H 6.05; N 10.78; \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6) \delta 1.26 (d, J = 7.0 Hz,
3H), 2.73-2.77 (m, 1H), 3.00-3.06 (m, 1H), 3.62 (br s, 5H), 3.85 (d, J = 5.2 Hz, 2H), 4.20 (t, J = 7.1 Hz, 1H), 4.54-4.56 (m, 1H), 5.02-5.11 (m, 2H), 7.37 (br s, 5H), 8.08-8.10 (m, 1H), 8.15 (br s, 3H), 8.70-8.72 (m, 1H), 8.82 (d, J = 8.5 Hz, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 17.4, 28.2, 30.3, 40.8, 51.8, 56.7, 65.8, 127.8, 128.0, 128.4, 136.8, 155.9, 166.1, 169.7, 169.9, 201.7.

H-Gly-L-Phe-L-Cys(S-L-Cbz-Ala)-Gly-OCH$_3$ hydrochloride (2.1.19). White microcrystals, 86% yield, mp 172-175 °C; Anal. Calcd for C$_{28}$H$_{36}$ClN$_5$O$_8$S$\cdot$2H$_2$O: C 49.88; H 5.38; N 10.39. Found: C 50.06; H 5.70; N 10.76; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 1.27 (d, J = 7.0 Hz, 3H), 2.72-2.80 (m, 1H), 3.07-3.11 (m, 2H), 3.23 (dd, J = 13.0, 5.7 Hz, 1H), 3.62 (br s, 5H), 3.86 (d, J = 5.1 Hz, 2H), 4.20 (t, J = 7.2 Hz, 1H), 4.40-4.44 (m, 1H), 4.61-4.64 (m, 1H), 5.05 (dd, J = 17.1, 12.4 Hz, 2H), 7.14-7.44 (m, 10 H), 8.11 (d, J = 7.3 Hz, 1H), 8.18 (br s, 3H), 8.49 (br s, 1H), 8.71 (d, J = 7.9 Hz, 1H), 8.79 (d, J = 8.0 Hz, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 17.4, 30.1, 37.8, 40.8, 51.8, 54.3, 56.7, 65.8, 126.4, 127.8, 127.9, 128.1, 128.4, 129.3, 136.7, 137.5, 155.8, 165.7, 169.9, 170.7, 201.9.

H-Gly-L-Ph-L-Gly-Cys(S-L-Cbz-Ala)-Gly-OCH$_3$ hydrochloride (2.1.25). White microcrystals, 85% yield, mp 131-134 °C; Anal. Calcd for C$_{30}$H$_{39}$ClN$_6$O$_9$S: C 51.83; H 5.65; N 12.09. Found: C 51.78; H 5.76; N 12.26; $^1$H NMR (300 MHz, CD$_3$OD) δ 1.40 (d, J = 7.1 Hz, 3H), 2.98-3.06 (m, 1H), 3.24-3.28 (m, 2H), 3.43-3.50 (m, 1H), 3.66-3.91 (m, 5H), 3.98-4.06 (m, 4H), 4.35 (t, J = 6.9 Hz, 1H), 4.60-4.67 (m, 2H), 5.14 (s, 2H), 7.29-7.39 (m, 10H); $^{13}$C NMR (75 MHz, CD$_3$OD) δ 18.0, 31.0, 38.3, 41.8, 42.1, 43.7, 52.9, 53.9, 57.0, 58.4, 68.0, 128.0, 128.9, 129.2, 12.6, 129.7, 130.4, 138.2, 138.4, 158.5, 167.9, 171.6, 171.7, 172.5, 173.8, 203.7.
Chemical Ligation of Cys-(S-(Fmoc-L-Ala))-Gly-O-Me Hydrochloride (2.1.5) to Form Native Tripeptide (2.1.6)

(R)-1-(9H-Fluoren-9-yl)-3,6,10,13-tetraoxo-2,14-dioxo-7-thia-4,11-diaza-pentadecan-9-aminium chloride 2.1.5 (0.58 g, 1 mmol) was dissolved in a mixture of water (8 mL) and acetonitrile (24 mL). Triethylamine (0.168 mL, 1.2 mmol) was added and the mixture was stirred at room temperature for 1 h under argon. The reaction was acidified to pH 1 using 2N HCl and extracted with ethyl acetate (2 × 10 mL). The ethyl acetate layer was washed with 2N HCl (3 × 15 mL), sat. NaCl solution (20 mL) and dried over MgSO₄. The ethyl acetate solution was concentrated under vacuum and hexane was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and dried to give the corresponding native tripeptide 2.1.6. White microcrystals, 72% yield, mp 88-92 °C; Anal. Calcd for C₂₃H₂₅N₃O₆S: C 58.59; H 5.34; N 8.91. Found: C 58.72; H 5.17; N 8.62; ¹H NMR (300 MHz, CDCl₃) δ 2.89-2.97 (m, 1H), 3.02-3.07 (m, 1H), 3.64 (s, 3H), 3.70 (t, J = 7.3 Hz, 1H), 3.93-9.99 (m, 2H), 4.05-4.09 (m, 2H), 4.21-4.24 (m, 1H), 4.39 (d, J = 6.9 Hz, 2H), 5.54-5.59 (m, 1H), 6.17 (br s, 1H), 7.13-7.19 (m, 1H), 7.28 (t, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.75 (d, J = 7.4 Hz, 2H), 8.47 (br s, 1H); ¹³C NMR (75 MHz, DMSO-d₆) δ 40.8, 43.4, 46.7, 51.8, 62.2, 65.8, 74.9, 78.6, 120.1, 125.3, 127.1, 127.7, 140.7, 143.9, 156.6, 169.4, 170.0, 170.5.

General Procedure for the Synthesis of Peptides 2.1.10, 2.1.17 and 2.1.23

A mixture of tributylphosphine (0.607 g, 3 mmol) and dimer peptide 2.1.9, 2.1.16 or 2.1.22 (1.5 mmol) in MeOH:water (9:1, 20 mL) was stirred at rt for 2 h under argon. The solvent was evaporated and the residue was dissolved in diethyl ether (15 mL). The solution was dried over magnesium sulfate and concentrated under reduced pressure.
The crude peptides were purified according to the following procedures. Peptide 2.1.10 was recrystallized from diethyl ether:hexanes. Compounds 2.1.17 and 2.1.23 were recrystallized from MeOH:diethyl ether. The precipitates were washed with cold hexanes (5 mL) and CH$_2$Cl$_2$ (5 mL) and dried under reduced pressure.

(R)-Methyl 9-(mercaptomethyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (2.1.10). White microcrystals, 67% yield, mp 77-79 °C; Anal. Calcd for C$_{13}$H$_{23}$N$_3$O$_6$S: C 44.69; H 6.63; N 12.03. Found: C 44.29; H 6.77; N 11.67; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.45 (s, 9H), 1.85 (dd, $J = 11.3$, 6.5 Hz, 1H), 2.64-2.72 (m, 1H), 3.3 (br s, 1H), 3.74 (s, 3H), 3.82 (t, $J = 5.2$ Hz, 2H), 3.87-3.95 (m, 1H), 4.10-4.18 (m, 1H), 4.75-4.79 (m, 1H), 5.28 (br s, 1H), 7.19 (br s, 1H), 7.30 (br s, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 27.1, 28.5, 41.4, 44.6, 52.5, 54.3, 80.6, 156.8, 170.3, 170.7.

Boc-Gly-L-Phe-L-Cys-Gly-OCH$_3$ (2.1.17). White microcrystals, 62% yield, mp 158-162 °C; Anal. Calcd for C$_{22}$H$_{32}$N$_4$O$_7$S: C 53.21; H 6.50; N 11.28. Found: C 52.83; H 6.43; N 10.91; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 1.35 (br s, 9H), 2.31 (t, $J = 8.2$ Hz, 1H), 2.72-2.84 (m, 3H), 3.01-3.05 (m, 1H), 3.45-3.60 (m, 2H), 3.63 (br s, 3H), 3.87 (br s, 2H), 4.42-4.44 (m, 1H), 4.58 (br s, 1H), 6.90 (br s, 1H), 7.22 (br s, 5H), 7.97 (d, $J = 7.6$ Hz, 1H), 8.30 (d, $J = 7.6$ Hz, 1H), 8.37 (br s, 1H); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 26.2, 28.2, 37.4, 40.7, 43.1, 51.8, 53.8, 54.9, 78.1, 126.3, 128.0, 129.3, 137.6, 155.7, 169.3, 170.1, 171.1.

Boc-Gly-L-Phe-Gly-L-Cys-Gly-OCH$_3$ (2.1.23). White microcrystals, 67% yield, mp 188-193 °C; Anal. Calcd for C$_{24}$H$_{38}$N$_5$O$_8$S$_1$: C 52.07; H 6.37; N 12.65. Found: C 52.00; H 6.62; N 11.79; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 1.35 (br s, 9H), 2.34 (br s, 1H), 2.68-2.83 (m, 3H), 3.00-3.05 (m, 1H), 3.62 (br s, 5H), 3.72-4.00 (m, 4H), 4.46-4.50 (m, 2H), 4.70-4.75 (m, 1H), 6.92 (br s, 1H), 7.19 (br s, 1H), 7.30 (br s, 1H), 7.35 (br s, 1H); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 26.2, 28.2, 37.4, 40.7, 43.1, 51.8, 53.8, 54.9, 78.1, 126.3, 128.0, 129.3, 137.6, 155.7, 169.3, 170.1, 171.1.
6.88 (br s, H), 7.23 (br s, 5H), 8.07 (d, J = 5.6 Hz, 2H), 8.41 (br s, 1H), 8.52 (br s, 1H); 
$^{13}$C NMR (75 MHz, CD$_3$OD) δ 27.1, 28.9, 38.2, 42.1, 43.9, 44.7, 52.8, 56.8, 57.2, 81.0, 
128.0, 129.7, 130.4, 138.4, 158.7, 171.8, 172.8, 174.5.

**General Procedure for the Preparation of Dimer Peptides 2.1.9 and 2.1.16**

Isobutyl chloroformate (0.6 g, 4.4 mmol) was added to a solution of N-Boc-Gly-OH or the respective Boc-protected dipeptide 2.1.15 (4 mmol) and N-methylmorpholine (0.45 g, 4.4 mmol) in dry THF (30 mL) at -10 °C. After 5 min a mixture of {2-amino-3-[2-amino-2-(methoxycarbonylmethyl-carbamoyl)-ethylisulfanyl]-propionylamino}-acetic acid methyl ester dihydrochloride 2.1.8 (0.91 g, 2 mmol) and N-methylmorpholine (0.45 g, 4.4 mmol) in dry DMF (10 mL) was added at -10 °C. The reaction mixture was stirred at rt for 12 h under argon. The THF was removed under reduced pressure, water (30 mL) was added and the resulting solution was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were subsequently washed with 2N HCl (2 x 50 mL), water (30 mL), 5% Na$_2$CO$_3$ (2 x 50 mL), brine (30 mL) and dried over MgSO$_4$. Evaporation of the solvent gave the desired products, which were purified by recrystallization from CH$_2$Cl$_2$:hexanes.

(9R,9'R)-Dimethyl 9,9'-(disulfanediylbis(methylene))bis(2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate) (2.1.9). White microcrystals, 62% yield, mp 88-92 °C; Anal. Calcd for C$_{26}$H$_{44}$N$_6$O$_{12}$S$_2$H$_2$O: C 43.69; H 6.49; N 11.76. Found: C 43.81; H 6.58; N 11.49; $^1$H NMR (300 MHz, CDCl$_3$) δ 1.44 (s, 18H), 2.89-3.08 (m, 4H), 3.76 (s, 6H), 3.82-3.96 (m, 6H), 4.01 (d, J = 4.8 Hz, 2H), 4.09-4.17 (m, 2H), 5.43 (br s, 2H), 5.64 (br s, 2H), 7.26 (s, 2H), 8.41 (br s, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 28.5, 41.6, 44.4, 45.2, 52.6, 53.2, 80.4, 156.3, 170.2, 170.5, 170.6.
(Boc-Gly-L-Phe-L-Cys-Gly-OCH₃)₂ (2.1.16). White microcrystals, 72% yield, mp 155-159 °C; Anal. Calcd for C₄₄H₆₂N₈O₁₄S₂: C 53.32; H 6.71; N 11.31. Found: C 52.93; H 6.49; N 10.85; ¹H NMR (300 MHz, DMSO-d₆) δ 1.35 (br s, 18H), 2.73-2.95 (m, 4H), 3.03-3.16 (m, 4H), 3.45-3.56 (m, 4H), 3.62 (br s, 6H), 3.86 (br s, 4H), 4.58-4.63 (m, 4H), 6.90 (br s, 2H), 7.22 (br s, 10H), 7.95-7.97 (m, 2H), 8.38 (br s, 2H), 8.48 (d, J = 7.3 Hz, 2H); ¹³C NMR (75 MHz, DMSO-d₆) δ 28.2, 37.6, 40.8, 43.1, 51.8, 51.9, 53.8, 78.1, 126.2, 128.0, 129.3, 137.5, 155.7, 169.3, 169.9, 170.2, 171.1.

**General Procedure for the Preparation of Boc-Protected Dipeptides 2.1.15**

Boc-Gly-Bt (10 mmol) was added at 25 °C to a solution of the respective amino acid 2.1.14 (10 mmol) in MeCN:H₂O (30 mL : 10 mL) in the presence of Et₃N (10 mmol). The reaction mixture was stirred at 25 °C for 2 h. Aq. 4N HCl solution (5 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (50 mL), and the organic extract was washed with 4N HCl (3 × 30 mL), brine (30 mL) and dried over MgSO₄. Evaporation of the solvent gave the desired product, which was purified by recrystallization from ethyl acetate:hexanes to yield the desired Boc-protected dipeptides as solid compounds.

(S)-2-(2-(((tert-Butoxycarbonyl)amino)acetamido)-3-phenylpropanoic acid 2.1.15.

White microcrystals, 69% yield, mp 147-148 °C; Anal. Calcd for C₁₃H₂₄N₂O₅: C 59.62; H 6.88; N 8.69. Found: C 59.58; H 7.02; N 8.64; ¹H NMR (300 MHz, DMSO-d₆) δ 1.36 (br s, 9H), 2.84-2.95 (m, 1H), 3.00-3.06 (m, 1H), 3.35 (br s, 3H), 3.48-3.60 (m, 2H), 4.41-4.46 (m, 1H), 6.93 (t, J = 5.9 Hz, 1H), 7.19-7.26 (m, 5H), 8.02 (d, J = 8.0 Hz, 1H); ¹³C NMR (75 MHz, DMSO-d₆) δ 28.2, 36.8, 43.0, 53.4, 78.0, 78.0, 126.5, 128.2, 129.1, 137.4, 155.7, 169.2, 172.8.
General Procedure for the Preparation of S-Acyl Peptides 2.1.11, 2.1.18 and 2.1.24

Cbz-L-Ala-Bt (0.325 g, 1 mmol) was added to a mixture of 2.1.10, 2.1.17 or 2.1.23 (1 mmol) and triethylamine (0.1 g, 1 mmol) in acetonitrile (20 mL). The mixture was stirred for 3 h at rt and the solvent was removed under reduced pressure. The crude compounds were purified according to the following procedure. The residue was dissolved in ethyl acetate (20 mL), extracted with 2N HCl (2 x 20 mL), water (15 mL), and brine (10 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. Compound 2.1.11 was recrystallized from CH₂Cl₂:hexanes. The S-acyl peptides 2.1.18 and 2.1.24 were recrystallized from ethyl acetate:hexanes. The solids obtained were filtered, washed with ether (5 mL) and dried.

\[ S\)-S-\((R)\)-2-\(2-\)\(2-\)\((\text{tert-butoxycarbonyl})\)amino)acetamido)-4-\((\text{methoxycarbonyl})\)amino)-3-oxobutyl 2-\((\text{benzyloxy})\)-carbonyl)amino)propanethioate (10).

White microcrystals, 82% yield, mp 62-67 °C; Anal. Calcd for C₂₄H₃₄N₄O₉S: C 51.98; H 6.18; N 10.10. Found: C 51.70; H 5.87; N 9.75; \(^1\)H NMR (300 MHz, CDCl₃) δ 1.44 (s, 12H), 3.37-3.41 (m, 2H), 3.72 (br s, 4H), 3.81-3.88 (m, 1H), 3.95-4.08 (m, 2H), 4.38 (t, J = 7.2 Hz, 1H), 4.66 (br s, 1H), 5.06-5.20 (m, 2H), 5.43 (d, J = 6.6 Hz, 1H), 5.55 (br s, 1H), 6.89 (d, J = 7.4 Hz, 1H), 7.18 (br s, 1H), 7.36 (br s, 5H); \(^1^3\)C NMR (75 MHz, CDCl₃) δ 18.2, 28.5, 29.7, 41.4, 44.5, 52.5, 53.2, 57.2, 67.6, 80.6, 128.3, 128.5, 128.8, 136.1, 156.3, 156.6, 170.0, 170.1, 170.8, 202.5.

Boc-Gly-L-Phe-L-Cys(S-L-Cbz-Ala)-Gly-OCH₃ (2.1.18). White microcrystals, 92% yield, mp 164-167 °C; Anal. Calcd for C₃₃H₄₅N₅O₁₀S: C 56.48; H 6.18; N 9.98. Found: C 56.17; H 6.20; N 9.97; \(^1\)H NMR (300 MHz, DMSO-d₆) δ 1.26 (d, J = 6.9 Hz, 3H), 1.35 (s, 9H), 2.77 (dd, J = 13.7, 9.3 Hz, 1H), 3.00-3.06 (m, 2H), 3.21 (dd, J = 13.2, 5.4 Hz, 1H), 3.63 (br s, 5H), 3.87 (br s, 2H), 4.19 (t, J = 7.3 Hz, 1H), 4.38-4.47 (m, 1H), 4.54 (br s,
H), 5.01-5.10 (m, 2H), 6.90 (t, \( J = 5.4 \) Hz, 1H), 7.17-7.28 (m, 5H), 7.35 (br s, 5H), 7.92 (d, \( J = 7.7 \) Hz, 1H), 8.09 (d, \( J = 7.3 \) Hz, 1H), 8.38 (t, \( J = 5.1 \) Hz, 1H), 8.45 (d, \( J = 7.8 \) Hz, 1H); \(^{13}\)C NMR (75 MHz, DMSO-\( \delta_6 \)) δ 18.6, 28.9, 30.9, 38.8, 42.1, 44.7, 52.8, 54.0, 56.2, 58.4, 68.0, 80.9, 128.0, 128.9, 129.2, 129.6, 129.7, 130.5, 138.1, 138.3, 158.4, 171.5, 172.2, 172.6, 173.4, 203.6.

Boc-Gly-L-Phe-Gly-L-Cys(S-L-Cbz-Ala)-Gly-OCH\(_3\) (2.1.24). White microcrystals, 86% yield, mp 126-128 °C; Anal. Calcd for C\(_{35}\)H\(_{46}\)N\(_6\)O\(_{11}\)S: C 55.40; H 6.11; N 11.07. Found: C 55.02; H 6.05; N 11.00; \(^1\)H NMR (300 MHz, CD\(_3\)OD) δ ppm 1.36 (d, \( J = 7.3 \) Hz, 3H), 1.43 (s, 9H), 2.96 (dd, \( J = 13.9, 8.7 \) Hz, 1H), 3.17-3.24 (m, 2H), 3.43 (dd, \( J = 13.9, 5.1 \) Hz, 1H), 3.60-3.69 (m, 1H), 3.70 (br s, 5H), 3.97 (br s, 3H), 4.29 (q, \( J = 7.3 \) Hz, 1H), 4.56-4.66 (m, 2H), 5.11 (s, 2H), 7.19-7.36 (m, 10H); \(^{13}\)C NMR (75 MHz, CD\(_3\)OD) δ ppm 18.0, 28.9, 31.0, 38.4, 42.2, 43.8, 44.7, 52.8, 54.0, 56.5, 58.4, 68.0, 81.0, 127.9, 128.9, 129.2, 129.7, 130.4, 138.2, 138.5, 158.5, 171.6, 172.5, 172.8, 174.2, 203.4

**General Procedure for Chemical Ligation of S-Acyl Peptides 2.1.12, 2.1.19 and 2.1.25**

The respective S-acyl peptide hydrochloride 2.1.12, 2.1.19 or 2.1.25 (0.05 mmol) was suspended in degassed phosphate buffer (NaH\(_2\)PO\(_4\)/Na\(_2\)HPO\(_4\)) (0.4 M, pH 7.8, 7 mL) and acetonitrile (~1 mL) was added dropwise until the starting material was dissolved. The mixture was subjected to microwave irradiation (for compounds 2.1.19 and 2.1.25: 50 °C, 50 W, 1 h; for compound 2.1.12: 70 °C, 50 W, 3 h) under argon. The reaction was allowed to cool to room temperature, acetonitrile was removed under reduced pressure and the residue was acidified with 2N HCl to pH = 1. The mixture was extracted with ethyl acetate (3 x 20 mL), the combined organic extracts were dried over MgSO\(_4\) and the solvent was removed under reduced pressure. The ligation mixture was
weighed and then a solution in methanol (1 mg/mL) was analyzed by HPLC-MS.

Compounds 2.1.20a, 2.1.20b and 2.1.26 were subsequently isolated by semi-preparative HPLC and characterized by analytical HPLC and HRMS analysis

**Preparations of Compounds 2.2.2a, 2.2.3a, 2.2.4a, 2.2.5a, 2.2.6a, 2.2.7a, 2.2.8a and 2.2.9a**

Boc-β-Ala-Bt (2.2.4b). A solution of N-Boc-β-Ala-OH (3.00 g, 15.9 mmol) in CH₂Cl₂ (30 mL) was added to a solution of dicyclohexylcarbodiimide (3.27 g, 15.9 mmol) and 1H-benzotriazole (1.89 g, 15.9 mmol) in CH₂Cl₂ (10 mL). The reaction mixture was stirred at room temperature for 1.5 h. The precipitate was filtered and the solution was passed through 6.00 g of celite. The solution was evaporated under reduced pressure and the crude mixture obtained was dissolved in EtOAc (50 mL). The organic layer was washed with saturated Na₂CO₃ (3 x 30 mL), brine (20 mL) and dried over MgSO₄. Concentration under reduced pressure produced the final product.

Recrystallization from CH₂Cl₂:hexanes gave Boc-β-Ala-Bt. White microcrystals, 3.46g, 63% yield; mp 112-115 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.43 (s, 9H), 3.66-3.70 (m, 4H), 5.11 (br s, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 8.14 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 8.2 Hz, 1H); ¹³C NMR (CDCl₃, 75 MHz): δ 28.5, 35.8, 36.6, 79.8, 114.4, 120.4, 126.4, 130.7, 131.1, 146.3, 155.9, 171.5; Anal. Calcd for C₁₄H₁₈N₄O₃: C 57.92, H 6.25, N 19.30. Found: C 58.00, H 6.33, N 19.31.

Boc-β-Ala-L-Leu-OH (2.2.5a). A solution of L-Leucine (0.81 g, 6.20 mmol) and triethylamine (0.63 g, 6.20 mmol) in acetonitrile (10 mL) and water (5 mL) was added to the suspension of Boc-β-Ala-Bt (1.50 g, 5.17 mmol) in acetonitrile (30 mL). The solution was stirred for 15 h and then the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate. The organic phase was washed with 2.0 N
HCl (3 x 10 mL) and brine (10 mL). The solution was dried over MgSO₄, filtered and then concentrated under vacuum. The solid was recrystallized from ethyl acetate:hexanes to yield Boc-β-Ala-L-Leu-OH. White microcrystals, 1.13 g, 72% yield; mp 124-127 °C; ¹H NMR (DMSO-d₆, 300 MHz): δ 0.84 (d, J = 6.4 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H), 1.37 (s, 9H), 1.45-1.53 (m, 2H), 1.54-1.68 (m, 1H), 2.21-2.32 (m, 2H), 3.10 (dd, J = 13.3, 7.2 Hz, 2H), 4.15-4.23 (m, 1H), 6.68 (t, J = 5.2 Hz, 1H), 8.11 (d, J = 7.9, Hz 1H), 12.50 (br s, 1H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 21.3, 22.9, 24.3, 28.2, 35.5, 36.7, 39.9, 50.1, 77.6, 155.4, 170.4, 174.2; Anal. Calcd for C₁₄H₂₆N₂O₅: C 55.61, H 8.67, N 9.26. Found: C 55.98, H 8.91, N 9.22.

(Boc-Gly-L-Cys-OCH₃)₂ (2.2.2a). A solution of Boc-Gly-OH (1.13 g, 6.4 mmol) in dry THF (10 mL) under argon was cooled to -15 °C in an ice bath with stirring. N-methylmorpholine (0.65 g, 6.4 mmol), followed by isobutylchloroformate (0.84 g, 6.4 mmol) were added. After 5 minutes, a solution of L-Cystine dimethyl ester dihydrochloride (1.00 g, 2.9 mmol) and N-methylmorpholine (0.65 g, 6.4 mmol) in DMF (5 mL) were added. The ice bath was removed after 5 minutes and the solution was allowed to stir for 24 h at room temperature. The solution was concentrated under vacuum; the residue was taken up in ethyl acetate (20 mL) and 2N HCl (5 mL). The organic phase was washed successively with saturated Na₂CO₃ (3 x 10 mL), and 2N HCl (3 x 10 mL). The solution was dried over dry MgSO₄, filtered and then concentrated under vacuum. The peptide was recrystallized from diethyl ether:hexanes to give (Boc-Gly-L-Cys-OCH₃)₂. White microcrystals, 1.13 g, 66% yield; mp 51-54 °C; ¹H NMR (DMSO-d₆, 300 MHz): δ 1.38 (br s, 18H), 2.96 (dd, J = 13.9, 8.3 Hz, 2H), 3.12 (dd, J = 13.4, 4.8 Hz, 2H), 3.58 (d, J = 5.8 Hz, 4H), 3.65 (s, 6H), 4.55-4.65 (m, 2H), 6.94 (t, J
= 5.8 Hz, 2H), 8.35 (d, J = 7.9 Hz, 2H); \(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz): \(\delta\) 28.2, 39.1, 42.9, 51.2, 52.2, 78.1, 155.7, 169.6, 170.8; Anal. Calcd for C\(_{22}\)H\(_{38}\)N\(_4\)O\(_{10}\)S\(_2\): C 45.35, H 6.57, N 9.62. Found: C 45.64, H 6.76, N 9.45.

(Gly-L-Cys-OCH\(_3\))\(_2\) hydrochloride (2.2.3a). HCl gas was passed through a solution of (Boc-Gly-L-Cys-OCH\(_3\))\(_2\) (0.58 g, 1.0 mmol) in methanol (15 mL) for 30 minutes. The methanol solution was concentrated under vacuum and ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry diethyl ether (10 mL) dried to give the corresponding (Gly-L-Cys-OCH\(_3\))\(_2\) hydrochloride. White microcrystals, 0.38 g, 83% yield; mp 185-189 °C; \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): \(\delta\) 2.98 (dd, J = 13.9, 8.5 Hz, 2H), 3.15 (dd, J = 13.7, 5.0 Hz, 2H), 3.60 (s, 4H), 3.66 (s, 6H), 4.57-4.68 (m, 2H), 8.36 (s, 6H), 9.31 (d, J = 7.4 Hz, 2H); \(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz): \(\delta\) 39.9, 51.5, 52.4, 166.3, 170.4; Anal. Calcd for C\(_{12}\)H\(_{24}\)Cl\(_2\)N\(_4\)O\(_6\)S\(_2\).H\(_2\)O: C 30.45, H 5.54, N 11.84. Found: C 30.33, H 5.53, N 11.98.

(Boc-\(\beta\)-Ala-L-Leu-Gly-L-Cys-OCH\(_3\))\(_2\) (2.2.6a). A solution of Boc-\(\beta\)-Ala-L-Leu-OH (0.91 g, 3 mmol) in dry THF (10 mL) under argon was cooled to -15 °C in an ice bath with stirring. N-methylmorpholine (0.33g, 3.2 mmol), followed by isobutylchloroformate (0.45 g, 3.2 mmol) were added. After 4 min, a solution of (Gly-L-Cys-OCH\(_3\))\(_2\) hydrochloride (0.68 g, 1.5 mmol) and N-methyl-morpholine (0.7 g, 1.6 mmol) in DMF (5 mL) was added. The ice bath was removed after 5 min and the solution was allowed to stir for 12 h at room temperature. The solution was concentrated under vacuum; the residue was taken up in ethyl acetate (30 mL) and water (5 mL). The organic phase was washed successively with saturated Na\(_2\)CO\(_3\) (2 x 15 mL), water (10 mL), 2N HCl (15 mL) and water (10 mL). The solution was dried over MgSO\(_4\), filtered and then
concentrated under vacuum. The peptide was recrystallized from ethyl acetate:hexanes to give (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH₃)₂. White microcrystals, 0.93 g, 65% yield; mp 105-111 °C; ¹H NMR (DMSO-d₆, 300 MHz): δ 0.83 (d, J = 6.4 Hz, 6H), 0.88 (d, J = 6.6 Hz, 6H), 1.36 (s, 18H), 1.44 (t, J = 7.1, 4H), 1.53-1.63 (m, 2H), 2.25-2.31 (m, 4H), 2.95 (dd, J = 13.8, 8.5 Hz, 2H), 3.07-3.16 (m, 6H), 3.65 (s, 6H), 3.70-3.75 (m, 4H), 4.19-4.26 (m, 2H), 4.58 (dd, J = 13.3, 8.2 Hz, 2H), 6.70 (t, J = 5.5 Hz, 2H), 8.07 (d, J = 7.3 Hz, 2H), 8.22 (t, J = 5.6 Hz, 2H), 8.30 (d, J = 7.9 Hz, 2H); ¹³C NMR (Acetone-d₆, 75 MHz): δ 19.4, 22.2, 23.4, 25.4, 28.7, 36.9, 37.8, 40.6, 41.3, 43.5, 52.8, 53.4, 78.8, 156.7, 170.2, 171.5, 173.2, 174.1; Anal. Calcd for C₄₀H₇₀N₈O₁₄S₂: C 50.51, H 7.42, N 11.78. Found: C 50.35, H 7.56, N 11.39.

Boc-β-Ala-L-Leu-Gly-L-Cys-OCH₃ (2.2.7a). (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH₃)₂ (1.36 g, 1.43 mmol) was treated with P(Bu)₃ (0.58 g, 0.71 mL, 2.86 mmol) in 20 mL of 9:1 MeOH:water for 2 h at rt under argon gas. The reaction mixture was concentrated under vacuum and the residue was taken up in ethyl acetate (15 mL). The solution was dried over MgSO₄, and then concentrated under vacuum. The peptide was recrystallized from ethyl acetate:hexane to give Boc-β-Ala-L-Leu-Gly-L-Cys-OCH₃.

White microcrystals, 1.02 g, 75% yield; mp 140-142 °C; ¹H NMR (DMSO-d₆, 300 MHz): δ 0.83 (d, J = 6.4 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H), 1.25 (br s, 1H), 1.37 (s, 9H), 1.44 (t, J = 7.3 Hz, 2H), 1.53-1.65 (m, 1H), 2.23-2.34 (m, 2H), 2.74-2.90 (m, 2H), 3.11 (dd, J = 12.9, 7.0 Hz, 2H), 3.65 (s, 3H), 3.73 (d, J = 4.6 Hz, 2H), 4.16-4.24 (m, 1H), 4.46-4.53 (m, 1H), 6.72 (t, J = 6.1 Hz, 1H), 8.08-8.13 (m, 2H), 8.32 (t, J = 6.1 Hz, 1H); ¹³C NMR (Acetone-d₆, 75 MHz): δ 22.2, 23.4, 25.4, 26.7, 28.7, 36.9, 37.8, 41.1, 43.5, 52.6, 53.6,
Boc-β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH₃ (2.2.8a). (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH₃ (500 mg, 1.25 mmol) were suspended in acetonitrile (15 mL) and a solution of (10S,16R)-methyl 10-isobutyl-16-(mercaptomethyl)-2,2-dimethyl-4,8,11,14-tetraoxo-3-oxa-5,9,12,15-tetraazaheptadecan-17-oate (596 mg, 1.25 mmol) in acetonitrile:water (10 mL 4:1) containing an equivalent amount of triethylamine (127 mg, 1.26 mmol) was added. The mixture was stirred at 25 °C for 3 h until completion. Acetonitrile was removed under reduced pressure and the residue was taken in ethyl acetate (30 mL), extracted with 2N HCl (2 x 20 mL), water (15 mL) and brine (10 mL). Ethyl acetate was concentrated under reduced pressure and hexane was added; the turbid solution was left to crystallize overnight at -20 °C. The solid obtained was filtered, washed with diethyl ether (3 mL), CH₂Cl₂ (3 mL) and dried to give the corresponding Boc-β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH₃. White microcrystals, 739 mg, 87% yield; mp 100-105 °C; ¹H NMR (DMSO-δ₆, 300 MHz): δ 0.83 (d, J = 6.4 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H), 1.25 (d, J = 7.3 Hz, 3H), 1.36 (s, 9H), 1.44 (t, J = 7.2 Hz, 2H), 1.52-1.64 (m, 1H), 2.28 (t, J = 6.5 Hz, 2H), 3.06-3.15 (m, 3H), 3.24 (dd, J = 14.1, 6.4 Hz, 1H), 3.62 (s, 3H), 3.70 (t, J = 6.1 Hz, 2H), 4.14-4.26 (m, 2H), 4.36-4.43 (m, 1H), 5.06 (s, 2H), 6.70 (t, J = 5.0 Hz, 1H), 7.30-7.42 (m, 5H), 8.07 (t, J = 6.8 Hz, 1H), 8.18 (t, J = 5.6 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H); ¹³C NMR (Acetone-δ₆, 75 MHz): δ 18.0, 22.2, 23.4, 25.4, 28.7, 37.8, 41.2, 43.2, 52.7, 53.3, 57.9, 67.1, 78.8, 128.7, 129.3, 138.0, 156.7, 156.9, 169.9, 171.2, 173.0, 173.4, 202.0; Anal. Calcd for C₃₁H₄₇N₅O₁₀S: C 54.61, H 6.95, N 10.27. Found: C 54.58, H 6.80, N 10.08.
β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH₃ hydrochloride (2.2.9a). HCl gas was passed through a solution of Boc-β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH₃ (600 mg, 0.88 mmol) dissolved in methanol for 1.5 h at rt. The solvent was then evaporated under reduced pressure and the solid was recrystallized from methanol:ether to yield (5S,9R,15S)-15-isobutyl-9-(methoxycarbonyl)-5-methyl-3,6,11,14,17-pentaoxo-1-phenyl-2-oxa-7-thia-4,10,13,16-tetraazanonadecan-19-aminium chloride. White microcrystals, 0.45 g, 82% yield; mp 174-185 °C; ¹H NMR (DMSO-ｄ₆, 300 MHz): δ 0.86 (dd, J = 12.9, 6.4 Hz, 6H), 1.25 (d, J = 7.2 Hz, 3H), 1.42-1.50 (m, 2H), 1.54-1.64 (m, 1H), 2.92-3.00 (m, 2H), 3.09 (dd, J = 13.4, 7.4 Hz, 1H), 3.23 (dd, J = 14.6, 6.7 Hz, 1H), 3.62 (br s, 3H), 3.65 (br s, 2H), 3.70-3.76 (m, 2H), 3.80 (dd, J = 14.6, 6.7 Hz, 1H), 4.14-4.22 (m, 1H), 4.29 (dd, J = 15.0, 7.5 Hz, 1H), 4.32 (q, J = 6.8 Hz, 1H), 5.06 (s, 2H), 7.30-7.42 (m, 5H), 7.90 (br s, 3H), 8.10 (d, J = 7.6 Hz, 1H), 8.26 (t, J = 5.4 Hz, 1H), 8.33-8.37 (m, 1H), 8.40 (d, J = 7.3 Hz, 1H); ¹³C NMR (CD₃OD, 75 MHz): δ 18.0, 22.2, 23.5, 26.0, 30.7, 32.8, 37.2, 41.4, 41.9, 43.3, 53.3, 54.0, 58.4, 67.9, 128.8, 129.2, 129.6, 138.2, 158.4, 171.5, 172.0, 172.9, 175.4, 203.2; Anal. Calcd for C₁₂₆H₄₀Cl₅N₅O₈S₃H₂O: C 46.46, H 6.90, N 10.42. Found: C 46.53, H 6.72, N 10.54.

Preparations of Compounds 2.2.2b, 2.2.3b, 2.2.4a, 2.2.5b, 2.2.6b, 2.2.7b, 2.2.8b and 2.2.9b

Boc-β-Ala-L-Phe-OH (2.2.5b). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-OH (2.2.5a). White microcrystals, 1.02 g, 65% yield; mp 154 -157 °C; ¹H NMR (DMSO-ｄ₆, 300 MHz): δ 1.37 (br s, 9H), 2.19-2.25 (m, 2H), 2.84 (dd, J = 13.7, 9.5, Hz, 1H), 3.00-3.11 (m, 3H), 4.37-4.44 (m, 1H), 6.63 (br s, 1H), 7.17-7.30 (m, 5H), 8.23 (d, J = 8.0 Hz, 1H); ¹³C NMR (DMSO-ｄ₆, 75 MHz): δ
28.3, 35.5, 36.6, 36.7, 53.4, 77.6, 126.4, 128.1, 129.1, 137.7, 155.4, 170.3, 173.0; Anal.
Calcd for C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>: C 60.70, H 7.19, N 8.33. Found: C 60.29, H 7.22, N 8.19.

(Boc-β-Ala-L-Cys-OCH<sub>3</sub>)<sub>2</sub> (2.2.2b). The compound was prepared according to the method for preparation of (Boc-Gly-L-Cys-OCH<sub>3</sub>)<sub>2</sub> (2.2.2a). White microcrystals, 1.27 g, 72% yield; mp 117-119 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz): δ 1.37 (s, 18H), 2.29 (t, J = 7.3 Hz, 4H), 2.92 (dd, J = 13.8, 8.8 Hz 2H), 3.06-3.13 (m, 6H), 3.64 (s, 6H), 4.53 (dd, J = 13.2, 8.1 Hz, 2H), 6.72 (br s, 2H), 8.46 (d, J = 7.4 Hz, 2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 75 MHz): δ 28.2, 35.4, 36.5, 51.1, 52.2, 77.6, 155.4, 170.6, 170.9; Anal. Calcd for C<sub>24</sub>H<sub>42</sub>N<sub>4</sub>O<sub>10</sub>S<sub>2</sub>: C 47.20, H 6.93, N 9.17. Found: C 47.33, H 7.12, N 9.01.

(β-Ala-L-Cys-OCH<sub>3</sub>)<sub>2</sub> hydrochloride (2.2.2b). The compound was prepared according to the method for preparation of (Gly-L-Cys-OCH<sub>3</sub>)<sub>2</sub> hydrochloride (2.2.2a). White microcrystals, 0.20 g, 82% yield; mp 87-92 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz): δ 2.58 (t, J = 7.4 Hz, 4H), 2.92-3.00 (m, 6H), 3.12 (dd, J = 14.1, 4.9 Hz, 2H), 3.66 (br s, 6H), 4.53-4.60 (m, 2H), 8.04 (br s, 6H), 8.81 (d, J = 7.7 Hz, 2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 75 MHz): δ 32.6, 32.7, 51.9, 52.9, 170.3, 171.4; Anal. Calcd for C<sub>14</sub>H<sub>28</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>6</sub>S<sub>2</sub>·0.75H<sub>2</sub>O: C 33.84, H 5.98, N 11.27. Found: C 34.06, H 6.01, N 10.88.

(Boc-β-Ala-L-Phe-β-Ala-L-Cys-OCH<sub>3</sub>)<sub>2</sub> (2.2.6b). The compound was prepared according to the method for preparation of (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH<sub>3</sub>)<sub>2</sub> (2.2.6a). White microcrystals, 0.45 g, 52% yield; mp 187-197 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MH): δ 1.35 (s, 18H), 2.13-2.23 (m, 4H), 2.28 (t, J = 7.5 Hz, 4H), 2.73 (dd, J = 13.6, 9.6 Hz, 2H), 2.91-3.03 (m, 8H), 3.10 (dd, J = 13.7, 5.3 Hz, 2H), 3.16-3.27 (m, 4H), 3.63 (s, 6H), 4.38-4.45 (m, 2H), 4.52-4.59 (m, 2H), 6.61 (t, J = 5.3 Hz, 2H), 7.12-7.27 (m, 10H), 8.09 (bs, 2H), 8.15 (d, J = 8.5 Hz, 2H), 8.57 (d, J = 7.6 Hz, 2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 75 MHz): δ 28.3, 35.5, 36.6, 36.7, 53.4, 77.6, 126.4, 128.1, 129.1, 137.7, 155.4, 170.3, 173.0; Anal.
MHz): $\delta$ 28.2, 34.8, 35.2, 35.5, 36.6, 37.8, 51.2, 52.2, 54.0, 77.6, 126.2, 127.9, 129.1, 138.0, 155.3, 170.1, 170.6, 170.9, 171.1; Anal. Calcd for C$_{48}$H$_{70}$N$_8$O$_{14}$S$_2$: C 55.05, H 6.74, N 10.70. Found: C 55.00, H 7.15, N 10.72.

Boc-$\beta$-Ala-L-Phe-$\beta$-Ala-L-Cys-OCH$_3$ (2.2.7b). The compound was prepared according to the method for preparation of Boc-$\beta$-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (2.2.7a). White microcrystals, 0.21 g, 68% yield; mp 137-142 °C; $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 0.84-0.94 (m, 1H), 1.36 (br s, 9H), 2.10-2.25 (m, 2H), 2.31 (t, $J$ = 7.2 Hz, 2H), 2.68-2.86 (m, 3H), 2.93-3.04 (m, 3H), 3.15-3.30 (m, 2H), 3.46 (s, 3H), 4.38-4.49 (m, 2H), 6.62 (bs, 1H), 7.17-7.27 (m, 5H), 8.04 (bs, 1H), 8.12 (d, $J$ = 8.6 Hz, 1H), 8.43 (d, $J$ = 7.6 Hz, 1H); $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 26.7, 28.9, 36.3, 37.1, 37.3, 38.0, 39.1, 53.2, 56.3, 56.4, 80.3, 127.9, 129.6, 130.4, 138.7, 158.3, 172.3, 173.7, 173.9; Anal. Calcd for C$_{24}$H$_{36}$N$_4$O$_7$: C 54.95, H 6.92, N 10.68. Found: C 55.02, H 7.01, N 11.00.

Boc-$\beta$-Ala-L-Phe-$\beta$-Ala-L-Cys(S-L-Cbz-Ala)-OCH$_3$ (2.2.8b). The compound was prepared according to the method for preparation of Boc-$\beta$-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH$_3$ (2.2.8a). White microcrystals, 0.25 g, 81% yield; mp 158-162 °C; $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.24 (d, $J$ = 7.2 Hz, 3H), 1.35 (br s, 9H), 2.12-2.30 (m, 4H), 2.71 (dd, $J$ = 13.6, 9.2 Hz, 1H), 2.91-3.10 (m, 4H), 3.16-3.26 (m, 3H), 3.61 (s, 3H), 4.14-4.22 (m, 1H), 4.34-4.48 (m, 2H), 5.05 (s, 2H), 6.61 (br s, 1H), 7.16-7.30 (m, 5H), 7.30-7.42 (m, 5H), 7.98-8.02 (m, 1H), 8.07-8.12 (m, 2H), 8.47 (d, $J$ = 7.6 Hz, 1H); $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 18.0, 28.9, 30.7, 36.2, 37.1, 37.3, 38.0, 39.1, 53.3, 53.4, 56.4, 58.4, 67.9, 80.3, 127.9, 128.9, 129.2, 129.6, 130.4, 138.2, 138.7, 158.4, 172.2, 173.8, 203.3; Anal. Calcd for C$_{38}$H$_{47}$N$_5$O$_{10}$S: C 57.60, H 6.49, N 9.60. Found: C 57.42, H 6.70, N 9.44.
\[ \beta-\text{Ala-L-Phe-\beta-\text{Ala-L-Cys(S-L-Cbz-Ala)-OCH}_3 \text{ hydrochloride}} \] (2.2.9b). The compound was prepared according to the method for preparation of \( \beta-\text{Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH}_3 \) hydrochloride (2.2.9a). White microcrystals, 0.16 g, 81\% yield; mp 158-162 °C; \(^1\)H NMR (DMSO-\( d_6 \), 300 MHz): \( \delta \) 1.24 (d, \( J = 7.2 \) Hz, 3H), 2.24-2.30 (m, 2H), 2.40 (dd, \( J = 15.4,7.7 \) Hz, 2H), 2.74 (dd, \( J = 13.5, 9.6 \) Hz, 1H), 2.82-2.88 (m, 2H), 2.97 (dd, \( J = 13.7, 4.7 \) Hz, 1H), 3.08 (dd, \( J = 13.6, 8.0 \) Hz, 1H), 3.18-3.29 (m, 4H), 3.62 (br s, 3H), 4.13-4.23 (m, 1H), 4.34-4.48 (m, 2H), 5.06 (s, 2H), 7.16-7.28 (m, 5H), 7.28-7.37 (m, 5H), 7.91 (br s, 3H), 8.10 (d, \( J = 7.3 \) Hz, 1H), 8.17 (t, \( J = 5.4 \) Hz, 1H), 8.43 (d, \( J = 8.5 \) Hz, 1H), 8.53 (d, \( J = 7.7 \) Hz, 1H); \(^{13}\)C NMR (CD\( _3 \)OD, 75 MHz): \( \delta \) 17.9, 30.6, 32.8, 36.1, 36.9, 37.1, 38.9, 53.2, 56.5, 58.4, 67.8, 127.9, 128.8, 129.1, 129.5, 130.3, 138.1, 138.5, 158.4, 172.1, 173.8, 203.5; Anal. Calcd for C\(_{30}\)H\(_{40}\)ClN\(_5\)O\(_8\)S.H\(_2\)O: C 52.66, H 6.19, N 10.24. Found: C 52.64, H 6.62, N 10.13.

**Preparations of Compounds 2.2.2b, 2.2.3b, 2.2.4b, 2.2.5b, 2.2.6b, 2.2.7b, 2.2.8b and 2.2.9b**

Boc-GABA-Bt (2.2.4b). A solution of \( N \)-Boc-GABA-OH (3.23 g, 15.9 mmol) in CH\(_2\)Cl\(_2\) (30 mL) was added to a solution of dicyclohexylcarbodiimide (3.27 g, 15.9 mmol) and 1H-benzotriazole (1.89 g, 15.9 mmol) in CH\(_2\)Cl\(_2\) (10 mL). The reaction mixture was stirred at room temperature for 1.5 hours. The precipitate was filtered and the solution was passed through 6.00 g of celite. The solvent was evaporated under reduced pressure and the crude mixture obtained was dissolved in EtOAc (50 mL). The organic layer was washed with saturated Na\(_2\)CO\(_3\) (3 x 30 mL), brine (20 mL) and dried over MgSO\(_4\). Recrystallization from CH\(_2\)Cl\(_2\):hexanes gave Boc-GABA-Bt. White microcrystals, 4.68 g, 97\% yield; mp 108-110 °C; \(^1\)H NMR (CDCl\(_3\), 300 MHz): \( \delta \) 1.41 (br s, 9H), 2.12 (p, \( J = 7.0 \) Hz, 2H), 3.29-3.36 (m, 2H), 3.49 (t, \( J = 7.2 \) Hz, 2H), 4.72 (br s,
1H), 7.52 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H), 7.67 (ddd, J = 8.2, 7.2, 1.0 Hz, 1H), 8.13 (ddd, J = 8.2, 0.8, 0.8 Hz, 1H), 8.29 (ddd, J = 8.6, 1.1, 0.8 Hz, 1H); 13C NMR (CDCl3, 75 MHz): δ 25.0, 28.5, 32.9, 39.9, 79.5, 114.6, 120.3, 126.3, 130.6, 131.3, 146.3, 156.2, 172.2; Anal. Calcd for C15H20N4O3: C 59.20, H 6.62, N 18.41. Found: C 59.34, H 6.71, N 18.39.

Boc-GABA-L-Phe-OH (2.2.5c). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-OH (2.2.5a). White microcrystals, 1.82 g, 68% yield; mp 78-81 °C; 1H NMR (DMSO-d6, 300 MHz): δ 1.37 (br s, 9H), 1.49 (p, J = 7.2 Hz, 2H), 1.98-2.05 (m, 2H), 2.79-2.87 (m, 3H), 3.04 (dd, J = 13.9, 5.0 Hz, 1H), 4.36-4.44 (m, 1H), 6.76 (t, J = 5.5 Hz, 1H), 7.16-7.30 (m, 5H), 8.15 (d, J = 8.2 Hz, 1H); 13C NMR (DMSO-d6, 75 MHz): δ 26.0, 28.4, 32.7, 36.9, 53.5, 77.7, 126.5, 128.3, 129.2, 137.8, 155.7, 172.1, 173.3; Anal. Calcd for C18H26N2O5: C 61.70, H 7.48, N 7.99. Found: C 61.32, H 7.55, N 7.59.

(Boc-GABA-L-Phe-Gly-L-Cys-OCH3)2 (2.2.6c). The compound was prepared according to the method for preparation of (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH3)2 (2.2.6c). White microcrystals, 0.33 g, 52% yield; mp 110-115 °C; 1H NMR (DMSO-d6, 300 MHz): δ 1.37 (br s, 18H), 1.41-1.52 (m, 4H), 2.02 (t, J = 7.2 Hz, 4H), 2.70-2.82 (m, 6H), 2.93-3.06 (m, 4H), 3.15 (dd, J = 13.7, 4.3 Hz, 2H), 3.65 (br s, 6H), 3.72 (dd, J = 16.5, 5.0 Hz, 2H), 3.82 (dd, J = 17.0, 5.8 Hz, 2H), 4.43-4.54 (m, 2H), 4.55-4.64 (m, 2H), 6.74 (br s, 2H), 7.14-7.32 (m, 10H), 8.12 (d, J = 8.2 Hz, 2H), 8.33 (br s, 2H), 8.40 (d, J = 7.7 Hz, 2H); 13C NMR (DMSO-d6, 75 MHz): δ 25.7, 28.3, 32.6, 37.4, 41.6, 51.3, 52.3, 54.1, 77.4, 126.2, 128.0, 129.1, 138.0, 155.5, 169.0, 170.7, 171.7, 172.0; Anal. Calcd for C48H70N8O14S2: C 55.05, H 6.74, N 10.70. Found: C 55.12, H 6.46, N 10.56.
Boc-GABA-L-Phe-Gly-L-Cys-OCH$_3$ (2.2.7c). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (2.2.7a). White microcrystals, 0.27 g, 66% yield; mp 84-90 °C; $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 0.88 (dd, $J = 13.5$, 7.1 Hz, 1H), 1.37 (br s, 9H), 1.42-1.51 (m, 2H), 2.02 (t, $J = 7.4$ Hz, 2H), 2.71-2.88 (m, 5H), 3.02 (dd, $J = 13.5$, 4.2 Hz, 1H), 3.65 (br s, 3H), 3.69-3.83 (m, 2H), 4.46-4.53 (m, 2H), 6.75 (br s, 1H), 7.15-7.26 (m, 5H), 8.15 (d, $J = 8.0$ Hz, 1H), 8.19 (d, $J = 7.9$ Hz, 1H), 8.38 (d, $J = 5.4$ Hz, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 25.4, 25.7, 28.3, 32.6, 37.3, 41.8, 51.2, 54.2, 54.5, 77.4, 126.2, 128.0, 129.1, 138.0, 155.6, 16.9, 170.5, 171.9, 172.2; Anal. Calcd for C$_{24}$H$_{36}$N$_4$O$_7$S: C 54.95, H 6.92, N 10.68. Found: C 54.84, H 6.59, N 10.67.

Boc-GABA-L-Phe-Gly-L-Cys(S-L-Cbz-Ala)-OCH$_3$ (2.2.8c). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH$_3$ (22a). White microcrystals, 0.27 g, 87% yield; mp 91-96 °C; $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.26 (d, $J = 7.2$ Hz, 3H), 1.37 (br s, 9H), 1.42-1.50 (m, 2H), 2.01 (t, $J = 7.4$ Hz, 2H), 2.70-2.82 (m, 3H), 3.02 (dd, $J = 14.0$, 3.8 Hz, 1H), 3.10 (dd, $J = 13.7$, 7.7 Hz, 1H), 3.25 (dd, $J = 12.9$, 5.0 Hz, 1H), 3.63 (br s, 3H), 3.65-3.82 (m, 2H), 4.15-4.25 (m, 1H), 4.38-4.54 (m, 2H), 5.06 (br s, 2H), 6.74 (br s, 1H), 7.14-7.40 (m, 10H), 8.08-8.11 (m, 2H), 8.29 (t, $J = 5.4$ Hz, 1H), 8.40 (d, $J = 7.5$ Hz, 1H); $^{13}$C NMR (Acetone-$d_6$, 75 MHz): $\delta$ 18.1, 26.9, 28.8, 33.4, 37.9, 40.3, 52.8, 56.3, 57.9, 67.1, 78.7, 127.3, 128.7, 129.2, 129.3, 130.1, 138.0, 138.8, 156.9, 157.2, 169.9, 171.3, 172.6, 174.0, 202.1; Anal. Calcd for C$_{35}$H$_{47}$N$_5$O$_{10}$S: C 57.60, H 6.49, N 9.60. Found: C 57.49, H 6.66, N 9.46.
GABA-L-Phe-Gly-L-Cys(S-L-Cbz-Ala)-OCH$_3$ hydrochloride (2.2.9c). The compound was prepared according to the method for preparation of β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH$_3$ hydrochloride (23a). White microcrystals, 0.17 g, 87% yield; mp 125-131 °C; $^1$H NMR (CD$_3$OD, 300 MHz): δ 1.36 (d, $J$ = 7.2 Hz, 3H), 1.79-1.88 (m, 2H), 2.24-2.41 (m, 2H), 2.76-2.85 (m, 2H), 2.91 (dd, $J$ = 13.7, 9.4 Hz, 1H), 3.16-3.27 (m, 2H), 3.43 (dd, $J$ = 13.8, 5.3 Hz, 1H), 3.67-3.74 (m, 5H), 4.29 (q, $J$ = 7.3 Hz, 1H), 4.56-4.72 (m, 2H), 5.06-5.16 (m, 2H), 7.18-7.36 (m, 10H); $^{13}$C NMR (CD$_3$OD, 75 MHz): δ 18.0, 24.3, 30.7, 33.5, 38.6, 40.3, 43.3, 53.4, 56.7, 58.4, 68.0, 128.0, 128.8, 129.2, 129.6, 130.4, 138.2, 138.6, 158.5, 171.4, 171.9, 17.3, 174.8, 203.3; Anal. Calcd for C$_{30}$H$_{40}$ClN$_5$O$_8$S.3H$_2$O: C 50.03, H 6.44, N 9.72. Found: C 50.06, H 6.24, N 9.55.

**General Procedure for Long-Range Acyl Migration of S-(Pg-α-aminoacyl)tetrapeptide 2.2.9a,b,c to Form Native Peptides 2.2.10, and 2.2.12a,b**

The N-terminus unprotected S-(Pg-α-aminoacyl)peptide 2.2.9a,b,c (0.02 mmol) was suspended in degassed phosphate buffer (NaH$_2$PO$_4$/Na$_2$HPO$_4$) (1 M, pH = 6.2-8.2 for 2.2.9a and 7.6 for 2.2.9b and c 9.6 mL) and acetonitrile (0.4 mL) was added dropwise until the starting material was dissolved. The mixture was subjected to microwave irradiation 50 °C, 50 W, 1 h. The reaction was allowed to cool to rt and acetonitrile was removed under reduced pressure and the residue was acidified with 2N HCl to pH = 1. The mixture was extracted with ethyl acetate (3 x 10 mL), the combined organic extracts were dried over MgSO$_4$ and the solvent was removed under reduced pressure. Native peptides 2.2.10, and 2.2.12a,b were subsequently isolated as disulfide dimer by semi-preparative HPLC on Phenomenex Luna C18(2) columns.

Cbz-L-Ala-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (2.2.10). The compound was prepared from β-Ala-L-Leu-Gly-L-Cys(S-L-Cbz-Ala)-OCH$_3$ hydrochloride according to general
procedure for long-range acyl migration. Cbz-L-Ala-β-Ala-L-Leu-Gly-L-Cys-OCH₃ was subsequently isolated as disulfide dimer by semi-preparative HPLC. Colorless oil, 3.1 mg, 31% yield; ¹H NMR (DMSO-d₆, 300 MHz): δ 0.83-0.88 (m, 12H), 1.17 (d, J = 7.2 Hz, 6H), 1.45 (t, J = 7.2 Hz, 4H), 1.56-1.64 (m, 2H), 2.26-3.36 (m, 4H), 2.97 (dd, J = 13.8, 8.4 Hz, 2H), 3.13 (dd, J = 13.9, 5.1 Hz, 2H), 3.21-3.28 (m, 4H), 3.65 (br s, 6H), 3.69-3.80 (m, 4H), 3.95-4.01 (m, 2H), 4.25 (q, J = 7.4 Hz, 2H), 4.57-4.61 (m, 2H), 4.97-5.05 (m, 4H), 7.28-7.32 (m, 2H), 7.32-7.38 (m, 10H), 7.87 (t, J = 5.8 Hz, 2H), 8.09 (d, J = 7.4 Hz, 2H), 8.21 (t, J = 5.8 Hz, 2H), 8.32 (d, J = 7.7 Hz, 2H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 18.2, 21.5, 23.0, 24.2, 35.0, 35.3, 40.5, 41.5, 50.0, 51.2, 51.3, 52.2, 65.4, 127.7, 127.8, 128.3, 137.0, 155.6, 169.1, 170.7, 170.8, 172.4, 172.5; ESI-MS m/z: 1183 (M+Na). HRMS (ESI) calcd for C₅₂H₇₆N₁₀O₁₆S₂Na [M+Na]⁺ 1183.4774, found 1183.4733.

Cbz-L-Ala-β-Ala-L-Phe-β-Ala-L-Cys-OCH₃ (2.2.12a). The compound was prepared from β-Ala-L-Phe-β-Ala-L-Cys(S-L-Cbz-Ala)-OCH₃ hydrochloride according to general procedure for long-range acyl migration. Cbz-L-Ala-β-Ala-L-Phe-β-Ala-L-Cys-OCH₃ was subsequently isolated as disulfide dimer by semi-preparative HPLC. Colorless oil, 2.2 mg, 42% yield; ESI-MS m/z: 1256 (M+H). HRMS (ESI) calcd for C₆₀H₇₆N₁₀O₁₆S₂Na [M+Na]⁺ 1279.4774, found 1279.4718.

Cbz-L-Ala-GABA-L-Phe-Gly-L-Cys-OCH₃ (2.2.12b). The compound was prepared from GABA-L-Phe-Gly-L-Cys(S-L-Cbz-Ala)-OCH₃ hydrochloride according to general procedure for long-range acyl migration. Cbz-L-Ala-GABA-L-Phe-Gly-L-Cys-OCH₃ was subsequently isolated as disulfide dimer by semi-preparative HPLC.
Colorless oil, 1.9 mg, 42% yield; ESI-MS $m/z$: 1256 (M+H). HRMS (ESI) calcd for $C_{60}H_{76}N_{16}O_{16}S_2Na [M+Na]^+$ 1279.4774, found 1279.4775.
CHAPTER 3
SCOPE AND MECHANISTIC ASPECTS OF S TO N LONG-RANGE ACYL MIGRATION

Introduction

S-Acyl isopeptide methods have the major advantages of utilizing conventional amino acids and providing an isomer of the native peptide that can be isomerized under a variety of controlled conditions. In contrast to conventional NCL, in which two unprotected peptides are condensed by thioester-mediated amide bond formation, S-isopeptide-based strategies (Figure 3-1) require conventional chemical synthesis of an amide or (thio)ester linked peptide which subsequently rearranges to a native peptide bond.

Figure 3-1. Long range S→N acyl migration to form native peptide analogs.

The results for the long-range acyl migration via 5-, 8-, 11-, 14-, 15- and 16-membered cyclic transitions states were analyzed in conjunction with our group’s

previous studies on the long-range ligation through cyclic intermediates.\textsuperscript{57,58} HPLC-MS (ESI) analysis of each crude product’s desired peptide/transacylated product ratio was used to provide preliminary analysis of the formation rate of each desired product. The result indicates that the rate of long-range \textit{S} to \textit{N} acyl migration is strongly dependent on the size of the cyclic transition state involved in precursor peptide.

Studies on the rates of lactone formation have shown a similar dependence on ring size.\textsuperscript{59} The formation of five and six membered lactones is favorable but in contrast, seven and eight membered lactones are difficult to form. However, cyclization becomes more favorable as the size of the lactone ring increases (Figure 3-2, 3-3).\textsuperscript{59}

\begin{figure}[ht]
\centering
\includegraphics[width=0.5\textwidth]{cyclization.png}
\caption{Cyclization reaction to form lactone}
\end{figure}

Figure 3-2. Cyclization reaction to form lactone

Our long-range ligation experiments show a similar trend. This chapter discusses the long-range acyl migration \textit{via} different transition state sizes by replacing one or more \textalpha- amino acid units by \textbeta- or \textgamma-aminooacyl units. The purpose of this study is to identify sequence and geometry requirements that enable long-range acyl migration by a study of “chemical ligations” of isotri- and isotetrapeptides containing \textalpha- or \textgamma- amino acid units \textit{via} 9- and 13-membered cyclic TS to further understand scope and mechanistic aspect of the \textit{S} to \textit{N} long-range acyl migration.\textsuperscript{60}
Results and Discussion

The Feasibility of S-Acyl Monoisotripeptide to Undergo S→N Acyl Migration via a 9-Membered Cyclic Transition State

Monoisotripeptide 3.7 was prepared for the study of S→N acyl migration via a 9-membered cyclic transition state as illustrated in Scheme 3-2. Boc-protected β-alanine 3.1 was converted into the corresponding benzotriazolide 3.2 by the standard method. L-Cysteine was reacted with the Boc-β-alanine benzotriazolide 3.2 in aqueous acetonitrile (MeCN/H₂O, 7/3) containing 1 equiv of Et₃N for 1 h at 20 °C to give the Boc-β-alanyl-cysteine dipeptide 3.4 (68%). Subsequent S-acylation of 3.4 with Z-L-Ala-Bt 3.5 at room temperature in the presence of KHCO₃ furnished the Boc-protected S-acyl monoisotripeptide 6 (70%), and Boc-group deprotection of 3.6 with dioxane saturated with hydrochloric acid gas gave S-acyl monoisotripeptide 3.7 as its hydrochloride salt (Figure 3-4).
Chemical ligation via the 9-membered cyclic transition state was attempted by microwave irradiation of a solution of 3.7 at a 2 mM concentration in 0.4 M NaH$_2$PO$_4$/Na$_2$HPO$_4$ buffer (pH = 8.2) and acetonitrile (24:1) for 4 h at 50 °C. However, HPLC-MS (ESI) analysis of the reaction mixture (Figure 3-4, Table 3-1) revealed that the major product 3.9 arose from intermolecular disproportionation 3.7→3.9, indicating that intermolecular aminolysis of one molecule thioester 3.7 by another (3.7+7→3.9) is favored over intramolecular attack through a 9-membered ring. Ligated product 8 was also present (Table 3-1) but was formed in only 4% yield.

Figure 3-4. Chemical ligation of S-acyl monoisotripeptide 3.7.
Table 3-1. Attempted chemical ligation of S-acyl isopeptides 3.7 and 3.12

<table>
<thead>
<tr>
<th>Product R and relative amounts of each product (%)</th>
<th>Product characterization by HPLC-MS transacylation product (TA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[M+H](^+) found</td>
</tr>
<tr>
<td></td>
<td>ligated peptide monomer (LM)</td>
</tr>
<tr>
<td>R</td>
<td>LM</td>
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<tr>
<td>(5)</td>
<td>(4)</td>
</tr>
<tr>
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<td>3.13</td>
</tr>
<tr>
<td>(1)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

*a* the combined crude yield was calculated according to the following equation: combined crude yield = ([ligated peptide] + 2 x [transacylation product])/[starting material]

*b* Determined by HPLC-MS semiquantitative. The area of ion-peak resulting from the sum of the intensities of the [M+H]\(^+\) and [M+Na]\(^+\) ions of each compound was integrated

*c* R = recovered reactant, LM = ligation monomer, LD = ligation dimer, TA = transacylation

*d* amounts are corrected for LD = 2 mmol, LM = 1 mmol

Similar observation was also reported in the study of molecular machines for peptide synthesis, in which the amino acids are *preorganized* in a supramolecular architecture for the synthesis of small peptides (Figure 3-5).\(^{63,64}\) Leigh and co-workers reported a conceptually new approach to peptide synthesis, in which the amino acids are preorganized in a supramolecular architecture for the synthesis of small peptides.\(^{64}\) For reactions in this molecular machine, close analogies can be drawn to natural ribosomal and nonribosomal peptide biosynthesis. Ribosomal peptides are synthesized by translation of the mRNA, whereas nonribosomal machinery for peptide synthesis uses multi-enzyme complexes as an assembly line to catalyze the peptide condensation in a stepwise manner. This molecular machine design can be considered a major breakthrough in the area of supramolecular chemistry that will open up new methods for peptide synthesis using artificial nanomachines. The design was based on the S-to-N long-range acyl transfer via 11-, 14- and 17-membered cyclic TS, and in order for the machine operate successfully, a Gly-Gly dipeptide spacer between the
cysteine residue and the amine of the peptide-elongation site was required to avoid the slow ligation reaction via small cyclic TS.59

Figure 3-5. A rotaxane-based molecular machine for the synthesis of small peptides

**Demonstration of S→N acyl Migration in S-acyl Monoisotetrapeptide via a 13-Membered Cyclic Transition State**

Coupling of N-terminal amino-unprotected S-acyl monoisotripeptide 3.7 with Boc-β-Ala-Bt 3.2 gave 3.11. Deprotection of the Boc group in 3.11 produced S-acyl monoisotetrapeptide 3.12 (Figure 3-6), which was subjected to microwave irradiation at 50 °C for 4 h in NaH2PO4/Na2HPO4 buffer at pH 8.2. HPLC-MS (ESI) analysis of the crude ligation mixture revealed the successful ligation of 3.15 via a 13-membered cyclic transition state. Ligation product 3.15 was the major component (82%) (molecular ion of the disulfide dimer [M+H]+ m/z: 934, vs 468 for the [M+H]+ molecular ion of the starting S-(Z-Ala)tripeptide 3.12). The HPLC-MS (ESI) also confirmed that a substantial amount of 3.14 was formed by intermolecular trans-acylation (Table 3-1, Figure 3-6). Thus the feasibility of long-range acyl migration via a 13-membered cyclic transition state is
confirmed favorable in complete agreement with a previous study. The findings of this investigation hence offer prospects for a convergent assembly of peptides and proteins with $\beta$-amino acid architecture.

Figure 3-6. Chemical ligation of S-acyl monoisotetrapeptide 3.12.

**Conclusion**

In summary, stable, amino-unprotected S-acyl-monoisotri- and S-acyl-monoisotetra-cysteine-peptides containing $\alpha$-, and/or $\beta$-amino acid residues undergo chemical ligations in which the cysteine S-acyl groups migrate to the N-terminal amino acids via 9- and 13-membered cyclic transition states to form the corresponding native tri- and tetrapeptide analogs. The work describes a range of novel long range S- to N-acyl migrations and offers evidence that relates to the challenging problem of coupling large peptides and peptide analog fragments.
Experimental

General Methods

Melting points were determined on a capillary point apparatus equipped with a
digital thermometer. NMR spectra were recorded in CDCl₃, DMSO-d₆ or CD₃OD-d₄
operating at 300 MHz for ¹H and 75 MHz for ¹³C with TMS as an internal standard. All
microwave assisted reactions were carried out with a single mode cavity Discover
Microwave Synthesizer. The reaction mixtures were transferred into a 10 mL glass
pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed
with a silicon septum and the reaction mixture was subjected to microwave irradiation
(Discover mode; run time: 60 sec.; PowerMax-cooling mode). The starting Boc-β-Ala-Bt
3.2, and Z-L-Ala-Bt 3.5, and Boc-Gly-Bt were prepared in 90-95% yields from
Corresponding N-protected amino acids following previously published one-step
procedures.

Procedures for Preparations of Compounds 3.4, 3.6 and 3.7

Synthesis of peptide 3.4. N-protected Bt-derivative 3.2 (2 mmol) was dissolved in
acetonitrile (20 mL) was added to a solution of L-cysteine (2 mmol) and Et₃N (2 mmol)
in water (10 mL) at rt and stirred for 3 hours. Mixture was then evaporated, acidified with
1N citric acid solution to pH = 2 and extracted with ethyl acetate (2x30 mL). The organic
layer was dried over magnesium sulfate and concentrated under reduced pressure.
Compounds 3.4 was recrystallized from CH₂Cl₂:hexanes.

Boc-β-Ala-L-Cys-OH (3.4). White microcrystals, 68% yield, mp 94-96 oC; ¹H
NMR (300 MHz, CDCl₃) δ 9.30 (br s, 1H), 5.34 (br s, 1H), 4.90-4.85 (m, 1H), 3.45-3.43
(m, 2H), 3.10-3.03 (m, 2H), 2.58-2.50 (m, 2H), 1.42 (s, 9H); ¹³C NMR (75 MHz, CDCl₃)
δ 172.3, 173.1, 156.8, 80.4, 54.0, 37.2, 36.6, 28.4, 26.6. Anal. Calcd. For C_{11}H_{20}N_{2}O_{5}S:
C 45.19; H 6.90; N 9.58. Found: C 45.50; H 7.11; N 9.40.

Preparation of S-acyl peptides 3.6. (0.33 g, 1 mmol) was added to a mixture of
3.4 (1 mmol) and KHCO$_3$ (0.1 g, 1 mmol) in acetonitrile (20 mL). The mixture was stirred
for 3 h at rt and solvent was then removed under reduced pressure. The residue was
dissolved in ethyl acetate (20 mL), extracted with 2N HCl (2 x 20 mL), water (15 mL),
and brine (10 mL). The organic layer was dried over magnesium sulfate and
concentrated under reduced pressure. Compound 3.6 was recrystallized from
CH$_2$Cl$_2$:hexanes.

Boc-β-Ala-L-Cys(S-Z-L-Ala)-OH (3.6). White microcrystals, 70% yield, mp 65-69
°C; $^1$H NMR (300 MHz, CD$_3$OD) δ 7.26-7.15 (m, 5H), 4.99 (s, 2H), 4.51-4.48 (m, 1H),
4.19-4.15 (m, 1H), 3.41-3.34 (m, 1H), 3.18-3.15 (m, 2H), 3.10-3.00 (m, 1H), 2.27 (t, $J =$
6.9 Hz, 2H), 1.29 (s, 9H), 1.22 (d, $J = 7.2$ Hz, 3H); $^{13}$C NMR (75 MHz, CD$_3$OD) δ 203.4,
174.0, 173.2, 158.4, 138.2, 139.6, 129.2, 128.9, 80.3, 68.0, 58.5, 53.3, 50.0, 38.0, 37.1,
32.9, 30.9, 28.9; Anal. Calcd. For C$_{22}$H$_{31}$N$_{3}$O$_{8}$S: C, 53.11; H, 6.28; N, 8.45. Found: C
53.44; H 6.46; N 8.40.

Procedure for Boc deprotection of peptides 3.6 to give the corresponding
unprotected peptides 3.7. HCl gas was passed through a solution of peptide 3.6 in
dioxane (15 mL) for 30 minutes. The dioxane solution was concentrated under vacuum
and diethyl ether (20 mL) was added. The turbid solution was left to crystallize in the
freezer overnight. The solid formed was filtered and washed with dry ethyl acetate (10
mL) and diethyl ether (10 mL) to give corresponding deprotected peptide 3.7.
H-β-Ala-L-Cys(S-Z-L-Ala)-OH hydrochloride (3.7). White microcrystals, 100% yield, mp 120-123 °C; 1H NMR (300 MHz, CD3OD) δ 7.23-7.14 (m, 5H), 4.98 (s, 2H), 4.60-4.55 (m, 1H), 4.15-4.12 (m, 1H), 3.42-3.35 (m, 1H), 3.17-3.14 (m, 1H), 3.05-2.98 (m, 3H), 2.48 (t, J = 6.6 Hz, 2H), 1.20 (d, J = 7.5 Hz, 3H); 13C NMR (75 MHz, CD3OD) δ 203.7, 173.0, 172.3, 158.6, 138.2, 129.6, 129.2, 128.8, 68.0, 58.5, 53.1, 37.1, 32.7, 30.9, 17.9; Anal. Calcd. For C17H24ClN3O6S: C 47.06; H 5.58; N, 9.68. Found: C 46.69; H 5.48; N 9.69.

Procedures for Preparations of Compounds 3.11 and 3.12

Synthesis of S-Acyl isotetrapeptides 3.11. 1 mmol of 3.2 was added to solution of 3.7 (1 mmol) and DIPEA (2 mmol) in acetonitrile (20 mL). Mixture was stirred for 4 h at rt. Solvent was then evaporated and residue diluted with ethyl acetate (50 mL) followed by washing with 1N HCl. Organic layer was dried over anhydrous sodium sulfate and evaporated. Residue was separated by column chromatography using ethyl acetate to give S-acyl isotetrapeptide 3.11.

Boc-β-Ala-β-Ala-L-Cys(S-Z-L-Ala)-OH (3.11). White microcrystals, 62% yield, mp 108-112 °C; 1H NMR (300 MHz, CD3OD) δ 7.11-7.05 (m, 5H), 4.89-4.82 (m, 2H), 4.42-4.38 (m, 1H), 4.08-4.05 (m, 1H), 3.50-3.37 (m, 3H), 3.31-3.18 (m, 3H), 2.99-2.91 (m, 1H), 2.23-2.09 (m, 4H), 1.18 (s, 9H), 1.12 (d, J = 7.2 Hz, 3H); 13C NMR (75 MHz, CD3OD) δ 203.4, 174.0, 173.8, 173.1, 158.3, 138.1, 129.6, 129.1, 129.0, 128.9, 80.2, 67.9, 58.4, 53.3, 38.1, 37.4, 37.0, 36.4, 30.9, 28.9, 18.0.

H-β-Ala-β-Ala-L-Cys(S-Z-L-Ala)-OH hydrochloride (3.12). The compound was prepared according to the method for preparation of H-β-Ala-L-Cys(S-Z-L-Ala)-OH hydrochloride (3.7). White microcrystals, 97% yield, mp 95-98°C; 1H NMR (300 MHz, CD3OD) δ 7.22-7.11 (m, 5H), 5.00-4.84 (m, 2H), 4.53-4.46 (m, 1H), 4.20-4.08 (m, 1H),
3.40-3.20 (m, 4H), 3.16-2.92 (m, 6H), 2.51-2.42 (m, 2H), 2.35-2.23 (m, 2H), 1.19 (t, J = 7.4 Hz, 3H); $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 203.5, 173.8, 173.1, 172.3, 158.4, 138.1, 129.6, 129.1, 129.0, 128.8, 67.9, 58.4, 53.2, 48.0, 37.4, 37.0, 36.3, 33.1, 30.9; HRMS (ESI) calcd. for $[C_{20}H_{29}N_4O_7S]^{+}$ m/z 469.1751, found 459.1774.

**General Procedure for Chemical Ligation of S-Acyl Peptides 3.7 and 3.12**

The respective S-acyl peptide hydrochloride 3.7 or 3.12 (0.05 mmol) was suspended in degassed phosphate buffer ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) (0.4 M, pH 7.8, 7 mL) and acetonitrile (~1 mL) was added dropwise until the starting material was dissolved. The mixture was subjected to microwave irradiation (50 °C, 50 W, 1 h) under argon. The reaction was allowed to cool to room temperature, acetonitrile was removed under reduced pressure and the residue was acidified with 2N HCl to pH = 1. The mixture was extracted with ethyl acetate (3 x 20 mL), the combined organic extracts were dried over MgSO$_4$ and the solvent was removed under reduced pressure. The ligation mixture was weighed and then a solution in methanol (1 mg/mL) was analyzed by HPLC-MS. Ligation products were subsequently purified by semi-preparative HPLC and characterized by analytical HPLC and HRMS analysis. Products were characterized as dimeric sulfides.
CHAPTER 4
LIGATION AT HYDROXYPROLINE SITE VIA SALICYLALDEHYDE CAPTURE AND IMINIUM INDUCED REARRANGEMENT

Introduction

NCL involves the reaction between an unprotected peptidyl C-terminal thioester with another peptide bearing an N-terminal cysteine. Following rate-determining trans-thioacylation, a fast S→N acyl transfer passing through a five-membered tetrahedral intermediate leads to product.65

Currently, there is much interest in the development of chemoselective ligations at a hydroxyproline site to access larger peptides for pharmacological targets. Proline site ligation in general is among the most challenging, as evident by its possessing the longest reaction times as well as the paucity of current synthetic protocols. The unique cyclic structure of a prolyl residue orientates the αN carbonyl on oxygen in close proximity to the thioester carbonyl C atom,66,67 and consequent n →π* increases electron density and reduces electrophilicity of thioester carbonyl (Figure 4-1 and 4-2).68,69

Figure 4-1. The unique challenges of proline ligation arise from an n →π* increase electron density.
Incidentally, this same effect has been postulated to confer the unique structural rigidity that is implicated in the polyproline triple helices of collagen and the PrAMPs. A way to realize derivatized proline ligations was recently advanced by Danishefsky et al. using thio- and selenoprolines. As mentioned previously, NCL is predicated on the bifunctional 1,2-mercaptoamine group of cysteine, which is present in the thioproline. This worked showed that ligation at a derivatized-proline site was achievable. However, this advancement in proline-site ligation has limited applications: thio-proline is not a naturally occurring amino acid; it requires a thiol installation; and later, a dethylation involving metals catalysis, which has drawbacks. We were attracted by the imine-induced proximity acyl-transfer approach. Such a strategy was originally introduced by Kemp and fully developed by Tam to ligate a C-terminal glycolaldehyde peptide with another peptide containing a Cys, Thr, or Ser residue at the N-terminus to furnish a coupled product with a pseudoproline structure (thiazolidine or oxazolidine) at the ligation site. Taken together, we proposed an imine-induced ligation using hydroxyproline by adopting Tam’s reaction conditions (Figure 4-3).
A) Known literature ligation methods
1. Advanced proline ligation

![Diagram of ligation reaction]

2. Chemoselective Ligation Serine/Threonine Sites

![Diagram of chemoselective ligation]

B) This study: hydroxy proline ligation:

![Diagram of hydroxy proline ligation]

Figure 4-3. (A) Schemes of the previous advanced proline ligation strategy. Our reaction represented in (B) exhibiting the similarities in strategy.

In this study (Figure 4-2A), salicaldehyde was installed at the phenolic position of a mono- or dipeptide using conventional EDCI/DMAP coupling techniques, which furnished an electrophilic aldehyde group that is receptive to the α-amine capture of 4-hydroxyproline. An iminium species was thereby formed through a Schiff’s base-type mechanism. Owing to the second functional group of 4-hydroxyproline, the compound rearranges chemoselectively, because the iminium formed induces the nucleophilic attack of the 4-hydroxy group forming a stable bridged bicyclic intermediate. This
removed charges from nitrogen and restores its nucleophilicity. The N-terminal carboxyl group is brought into proximity by the phenolic attachment of salicylaldehyde, which undergoes an O→N trans-esterification with the now-nucleophilic α-amine of 4-hydroxyproline connected to SAE as an aminal. The aldehyde can then reform at the 4-hydroxy position, and this can then be acidolyzed, yielding the native peptide bond at the hydroxy position (Figure 4-4).

Figure 4-4. Scheme for hydroxyproline-site ligation. After installation of O-salicylaldehyde, the proline is captured forming an iminium, which then rearranges ultimately allowing for the native peptide bond to be formed.
Results and Discussion

Primary Study

In order to develop a practical chemoselective Hyp peptide ligation based on the imine induced strategy, two questions must be addressed: (i) whether the imine capture step or the acyl transfer step can be accelerated (it is not known which step is the rate-determining step) and (ii) whether the formed pseudo bridged proline moiety can be readily transformed into natural peptide linkages. The second question is more challenging and critical because the removal conditions should ideally be compatible with other functionalities in peptides.

Among many candidates, we tentatively identified the salicylaldehyde ester as a potential functional donor at C terminal. We hypothesized that, when the benzaldehyde ester is used, either the hydroxy attack to the imine or O to N acyl transfer would proceed faster. However, as the penalty, the acyl transfer has to progress through a disfavored long-range acyl transfer via a 6-membered TS. On the other hand, the formed N,O-benzyldiene acetal group is expected to be readily removed under weak acidic conditions.

The hypothesis was first tested with the commercially available salicylaldehyde. As a model study, we prepared Fmoc-Ala salicylaldehyde ester 4.3a by coupling Fmoc-protected alanine 4.1a with a salicylaldehyde 4.2 at the C-terminus using coupling reagent EDCI (Figure 4-5).

Installing salicylaldehyde (SAE) phenolically on the commercially procured Fmoc protected amino acid was carried out in anhydrous DCM (dried refluxed under calcium hydride) under N₂ gas pressure. The reaction mixture was left overnight to ensure reaction completion, with yields >77%.
Compound 4.3a was purified by recrystallization from DCM/hexanes and fully characterized by $^1$H and $^{13}$C NMR spectroscopy. $^1$H NMR spectra indicated the formation of the desired ligation product salicylaldehyde ester with appearance of newly formed aldehyde proton signals.

Having Fmoc-Ala-SAE in hand we next studied the ligation of 4.3a with Hyp-OMe (4.4a). The ligation step was achieved by introducing the hydroxyproline to a solution of the O-salicylaldehyde ester and pyridine/acetic acid (Figure 4-6). After 5 hours, the solvent was removed, and in the second step, the aminal is treated with a mixture of TFA/H$_2$O/i-Pr$_3$SiH yielding the native peptide bond at the hydroxyproline ligation site. The products were purified by recrystallization and preparative HPLC.

Proton spectra of the ligation product 4.5a revealed the major component to be the expected ligation product 3a with the absence of the aldehyde peak around 10 ppm. Purification of 4.5a by semipreparative HPLC allowed isolation of 91% of pure proline containing dipeptide 4.5a (Table 4.1, Entry 1).
Table 4-1. Scope of the reaction between Hyp peptides and salicylaldehyde esters

<table>
<thead>
<tr>
<th>Entry</th>
<th>Peptide at C-side Ligation</th>
<th>Product (Yield [%])</th>
<th>HRMS [M+Na]^+ found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ala</td>
<td>Fmoc-Ala-Hyp-OMe (4.5a)</td>
<td>91 461.1677</td>
</tr>
<tr>
<td>2</td>
<td>Val</td>
<td>Fmoc-Val-Hyp-OMe (4.5b)</td>
<td>85 481.2623</td>
</tr>
<tr>
<td>3</td>
<td>Leu</td>
<td>Fmoc-Leu-Hyp-OMe (4.5c)</td>
<td>88 503.2146</td>
</tr>
<tr>
<td>4</td>
<td>Val</td>
<td>Fmoc-Val-Phe-Hyp-OMe (4.5d)</td>
<td>86 636.2685</td>
</tr>
<tr>
<td>5</td>
<td>Phe</td>
<td>Fmoc-Ala-Phe-Hyp-OMe (4.5e)</td>
<td>91 608.2372</td>
</tr>
<tr>
<td>6</td>
<td>Leu</td>
<td>Fmoc-Leu-Phe-Hyp-OMe (4.5f)</td>
<td>85 650.2837</td>
</tr>
<tr>
<td>7</td>
<td>Ala-</td>
<td>Fmoc-Leu-Ala-Hyp-OMe (4.5g)</td>
<td>92 574.2524</td>
</tr>
<tr>
<td>8</td>
<td>Phe</td>
<td>Fmoc-Gly-Phe-Hyp-OMe (4.5h)</td>
<td>78 594.2211</td>
</tr>
<tr>
<td>9</td>
<td>Phe</td>
<td>Fmoc-Val-Phe-Hyp-Leu-OMe (4.5i)</td>
<td>81 739.4247</td>
</tr>
<tr>
<td>10</td>
<td>Phe</td>
<td>Fmoc-Val-Phe-Hyp-B-Ala-Phe-Leu-OMe (4.5j)</td>
<td>88 967.4611</td>
</tr>
</tbody>
</table>

Mechanistic Study of Formation of the Aminal Intermediate

The key steps in formation of the native peptide bond in the Hyp ligation are the formation of the bridged bicyclic aminal and the phenolic ester has been known to directly condense with amines to afford amides. To rule out this direct condensation possibility and evaluate the chemoselectivity, two experiments were carried out to confirm the formation of the aminal intermediate. According to our hypothesized mechanism, a stable bridged bicyclic aminal should be formed (Figure 4-7). There general scheme for these studies is shown below.
Figure 4-7. Formation of the aminal intermediate.

We attempted to isolate this intermediate via a reaction of reactants in dry DCM for 3 hours before adding a catalytic amount of p-toluenesulfonic acid, and then heated under reflux for at 45 °C additional 60 h. But formation of the bicyclic aminal intermediate was not observed. Next a study was attempted which produced the aminal. A solution of salicylaldehyde Fmoc-Ala ester (4.3a), Hyp-OMe (4.4a), and a catalytic amount of p-toluenesulfonic acid combined in dry toluene (70-80 mL) was heated. The formation of the aminal was observed by HPLC-MS analysis. HPLC-MS (ESI) analysis of the crude reaction mixture showed the presence of the MW expected with the aminal compound to provide evidence the presence of the intermediate. The aminal 4.6 was latter treated with a mixture of TFA/H2O/i-Pr3SiH leading to formation of the native peptide 4.5a. The results of these studies suggested that the native amide bond proceeds though an aminal intermediate following the capture/rearrangement process.

**Salicylaldehyde Iminium-Induced Ligation of Dipeptide via Hydroxyproline Capture-Rearrangement**

We next explored the scope of the coupling reaction between N-terminal Hyp containing peptides and the C-terminal salicylaldehyde ester of hindered branched amino acid sites (Val, Leu, and Phe). These β-branched amino acids when used at the C-terminus generally reduce the rate of peptide coupling step dramatically; thus, most known ligation methods require a prolonged time for completion at these amino acid sites or are limited to less hindered amino acids at the C-terminal site.\(^{39}\) However, these
amino acids possessing a salicylaldehyde ester at the C-terminus react with Hyp derived peptides, resulted in >70% conversion after 30 min and completion within 5 to 8 h (Table 4.1, Figure 4-8). Treatment with (TFA/H2O/i-Pr3SiH) gave rise to peptides with natural peptide bonds at the ligation site (Table 4.1). The products were isolated in diastereomERICally pure form, and the epimerized diastereomer were not detected by LCMS and NMR.

Figure 4-8. Hyp Ligation for synthesis of native peptides

To demonstrate the feasibility of this ligation strategy in peptide segment ligation, a two- step sequential ligation was carried out between the O- salicylaldehyde ester dipeptide 4.3d and N-terminal Hyp derived peptide 4.4c possessing (Table 4-1, entry 10). With only one purification, the desired hexapeptide 4.5j with a natural peptide bond (Phe-Hyp) at the ligation site was obtained in 88% yield. It is noteworthy that peptide 4.5j contains Hyp which possesses a hydroxy group at the fourth position. Hyp can be modified to access functionalized proline residues that have diverse applications (Figure 4-9).74
Conclusion

Salicylaldehyde capture iminium-induced rearrangement, chemoselective ligation at the hydroxyproline site was demonstrated by forming a series oligopeptides. In this study, salicaldehyde was installed at the phenolic position of a mono- or dipeptide using conventional coupling techniques, which furnishes an electrophilic aldehyde group that is receptive to the α-amine capture of 4-hydroxyproline. An iminium species is thereby formed. Owing to the second functional group of 4-hydroxyproline, the compound rearranges chemoselectively, because iminium intermediate induces the nucleophilic attack of the 4-hydroxy group forming a stable bridged bicyclic intermediate. This removed the charge on the nitrogen and restores its nucleophilicity. The N-terminal carboxyl group is brought into proximity by the phenolic attachment of salicylaldehyde, which undergoes an O to N trans-esterification with the now-nucleophilic α-amine of 4-hydroxyproline connected to SAE as an aminal. The aldehyde can then reform at the 4-hydroxyl position, and this can then be acidolyzed, yielding the native peptide bond at the N terminal position.
This study represents the first reported ligation at the hydroxyproline site by employing the above-described chemoselective capture/rearrangement methodology. Furthermore, the relatively high yields suggest this methodology is worthy of further study. Our novel methodologies in combination with convergent synthesis schemes should provide the key elements for the total chemical synthesis of natural or fully unnatural linear and cyclic peptides containing proline derivatives with yet to be discovered properties.

**Experimental**

**General Methods**

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$, DMSO-$_d_6$, acetone-$_d_6$, or CD$_3$OD using a 300 or 500 MHz spectrometer (with TMS as an internal standard). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublets, ddd = doublet of doublets of doublets, and dt = doublet of triplets. HPLC–MS analyses were performed on a reverse phase gradient using 0.2% acetic acid in H$_2$O/methanol as mobile phases; wavelength = 254 nm; mass spectrometry was done with electrospray ionization (ESI), matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or atmospheric-pressure chemical ionization (APCI). Ether refers to diethyl ether.

**General Preparations for Salicylaldehyde Ester**

A stirred suspension of protected amino acid 1 (1 equiv.) and 4-dimethylaminopyridine (DMAP) (15% mole) in dry Dichloromethane (DCM) (100 ml/0.4 g of the protected peptide substrate was treated with 1-Ethyl-3-(3-
dimethylaminopropyl)carbodiimide (EDCI) (1.3 equiv.). After cooling at 0 °C for 10 minutes, salicylaldehyde (SAE) (1.1 equiv.) was added to the reaction. After 24 h stirring at room temperature, the reaction mixture was diluted with DCM (150 ml). The organic layer was then washed successively with brine (3 x 250ml) and HCl (2N) (3 x 250ml). The organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure resulting in the SAE-coupled, protected amino acid 3a.

Fmoc-L-Ala-SAE (4.3a) 1.03 g, 2.47 mmol, 77% yield, sticky orange oil. ¹H NMR (300 MHz, Chloroform-d) δ 10.08 (br s, 1H), 7.89 (d, J = 7.7, 1H), 7.75 (d, J = 7.4, 2H), 7.69 – 7.53 (m, 3H), 7.48 – 7.35 (m, 3H), 7.34 – 7.24 (m, 2H), 7.20 (d, J = 7.9 Hz, 1H), 5.48 (d, J = 7.7 Hz, 1H), 4.72 (p, J = 7.4 Hz, 1H), 4.50 – 4.33 (m, 2H), 4.23 (q, J = 7.1 Hz, 1H), 1.57 (d, J = 7.2 Hz, 3H). ¹³C NMR (75 MHz, Chloroform-d) δ 188.9, 176.4, 155.9, 143.7, 135.4, 131.8, 127.7, 127.1, 126.8, 125.1, 123.3, 120.0, 67.2, 50.0, 47.1, 18.5, 18.1.

Fmoc-L-Val-SAE (4.3b), 0.35 g, 0.57 mmol; 77% yield, White microcrystals., mp 74.4 – 78.9 °C. ¹H NMR (300 MHz, Chloroform-d) δ 10.38 – 9.99 (br s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 7.1 Hz, 2H), 7.36 (p, J = 7.5 Hz, 3H), 7.43 – 7.29 (m, 3H), 7.31 – 7.22 (m, 2H), 7.22 – 7.12 (m, 1H), 5.62 (d, J = 8.3 Hz, 1H), 4.69 – 4.56 (m, 1H), 4.44 (d, J = 7.1 Hz, 2H), 4.23 (t, J = 4.7 Hz, 1H), 2.51 – 2.33 (m, 1H), 1.11 (d, J = 6.1 Hz, 3H), 1.06 (d, J = 6.5 Hz, 2H). ¹³C NMR (75 MHz, Chloroform-d) δ 172.7, 171.4, 156.8, 155.0, 144.0, 141.4, 127.8, 127.2, 127.2, 125.3, 125.3, 120.1, 73.6, 70.3, 67.3, 58.0, 57.8, 55.8, 55.5, 52.4, 47.2, 43.2, 37.7, 31.3, 19.7, 19.3, 17.7, 14.7.

Fmoc-L-Leu-SAE (4.3c), 0.28 g, 0.62 mmol; Sticky oil. 81% yield. ¹H NMR (300 MHz, Chloroform-d) δ 10.15 (s, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H),
7.61 (d, J = 7.5 Hz, 3H), 7.40 (q, J = 7.2 Hz, 3H), 7.29 (t, J = 7.5 Hz, 2H), 7.20 (d, J = 8.2 Hz, 1H), 5.41 (d, J = 8.9 Hz, 1H), 4.66 (m, 1H), 4.46 (d, J = 7.0 Hz, 2H), 4.25 (t, J = 7.0 Hz, 1H), 2.22 – 2.06 (m, 1H), 1.71 – 1.51 (m, 1H), 1.42 – 1.21 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H), 1.01 (t, J = 8.9 Hz, 3H). $^{13}C$ NMR (75 MHz, Chloroform-$d$) δ 188.9, 176.7, 171.7, 156.4, 151.5, 143.8, 141.5, 135.5, 131.4, 127.9, 127.2, 126.9, 125.2, 123.5, 120.1, 67.3, 53.0, 47.3, 41.1, 25.1, 23.2, 21.8.

Fmoc-Val-Phe-SAE (4.5d) 0.48g, 0.81 mmol; 82% yield. White microcrystals, mp = 155.5 – 160.0 °C. $^1H$ NMR (300 MHz, Chloroform-$d$) δ 9.90 (s, 1H), 7.81 – 7.72 (m, 2H), 7.65 – 7.49 (m, 4H), 7.39 (dd, J = 8.0, 4.7 Hz, 2H), 7.34 – 7.10 (m, 6H), 7.09 – 6.90 (m, 3H), 5.66 – 5.32 (m, 1H), 4.82 (dq, J = 12.4, 6.4 Hz, 1H), 4.37 (ddd, J = 39.7, 16.7, 7.3 Hz, 2H), 4.26 – 3.97 (m, 1H), 3.42 – 3.13 (m, 1H), 3.12 – 2.85 (m, 1H), 2.13 – 1.92 (m, 1H), 1.17 – 0.55 (m, 6H). $^{13}C$ NMR (75 MHz, Chloroform-$d$) δ 189.21, 173.57, 171.51, 156.77, 143.92, 141.49, 136.12, 135.82, 135.57, 131.95, 129.51, 129.03, 128.69, 128.00, 127.32, 126.95, 125.23, 123.35, 120.23, 67.59, 60.53, 53.44, 47.31, 37.44, 31.25, 19.30, 18.18.

Fmoc-Ala-Phe-SAE (4.5e) 0.52g, 0.92 mmol; 91% yield. White microcrystals, mp = 95.7 – 100.2 °C. $^1H$ NMR (300 MHz, Chloroform-$d$) δ 10.30 – 9.52 (m, 1H), 7.89 – 7.62 (m, 3H), 7.60 – 7.49 (m, 4H), 7.44 – 7.33 (m, 3H), 7.33 – 7.16 (m, 4H), 7.09 – 6.96 (m, 3H), 5.85 – 5.47 (m, 1H), 4.55 – 4.25 (m, 3H), 4.20 – 3.98 (m, 1H), 3.41 – 2.99 (m, 2H), 1.40 – 1.26 (m, 3H). $^{13}C$ NMR (126 MHz, cd$_3$od) δ 173.94, 171.90, 158.32, 145.49, 145.32, 142.69, 138.22, 137.89, 130.89, 130.71, 130.50, 129.54, 128.91, 128.30, 127.92, 126.35, 121.04, 70.84, 59.63, 52.86, 32.88, 23.83, 14.57.
**Fmoc-Leu-Phe-SAE (4.5f)** 2.18 g, 3.60 mmol, 81% yield; Sticky Oil. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 9.89 (s, 1H), 7.78 – 7.70 (m, 2H), 7.62 – 7.48 (m, 5H), 7.37 (dd, $J$ = 7.8, 5.2 Hz, 2H), 7.33 – 7.16 (m, 4H), 7.08 – 6.93 (m, 4H), 5.32 – 5.13 (m, 1H), 5.18 – 5.02 (m, 1H), 4.45 – 4.38 (m, 1H), 4.27 – 4.16 (m, 3H), 4.24 – 4.14 (m, 1H), 3.40 – 3.29 (m, 1H), 3.29 – 3.18 (m, 1H), 2.04 (s, 1H), 1.76 – 1.30 (m, 2H), 1.19 – 1.07 (m, 1H), 1.02 – 0.89 (m, 3H), 0.89 – 0.64 (m, 3H). $^{13}$C NMR (75 MHz, Chloroform-$d$) $\delta$ 196.8, 174.2, 169.9, 143.9, 143.8, 137.2, 135.4, 133.9, 130.4, 129.9, 128.9, 128.0, 127.5, 126.8, 125.3, 123.3, 120.1, 117.8, 70.2, 60.6, 53.8, 47.3, 41.5, 37.7, 29.9, 24.7.

**Fmoc-Leu-Ala-SAE (4.5g)** 0.45 g, 0.85 mmol; 78% yield. White microcrystals, mp = 119.8 – 121.7 °C. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 9.91 (s, 0H), 7.75 (dd, $J$ = 7.4, 4.1 Hz, 2H), 7.61 – 7.47 (m, 3H), 7.47 – 7.34 (m, 2H), 7.34 – 7.21 (m, 5H), 5.37 – 5.08 (m, 1H), 4.59 – 4.31 (m, 3H), 4.30 – 4.04 (m, 4H), 1.81 – 1.45 (m, 9H), 1.16 – 0.58 (m, 6H). $^{13}$C NMR (75 MHz, Chloroform-$d$) $\delta$ 189.22, 172.57, 171.04, 156.51, 143.93, 141.45, 135.62, 132.03, 127.92, 127.25, 126.96, 125.21, 123.50, 120.16, 67.32, 53.57, 48.64, 47.28, 41.35, 24.80, 23.09, 22.19, 17.68.

**Fmoc-Gly-Phe-SAE (4.5h)** 0.27 g, 0.49 mmol; 85% yield. White microcrystals, mp = 90.7 – 95.2 °C. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 9.88 (d, $J$ = 10.8 Hz, 1H), 7.76 (d, $J$ = 7.6 Hz, 2H), 7.67 – 7.51 (m, 4H), 7.46 – 7.14 (m, 8H), 7.15 – 6.84 (m, 3H), 4.55 – 4.30 (m, 2H), 4.22 (t, $J$ = 7.0 Hz, 2H), 4.03 – 3.57 (m, 3H), 3.31 (dd, $J$ = 13.0, 6.7 Hz, 1H). $^{13}$C NMR (75 MHz, Chloroform-$d$) $\delta$ 189.27, 171.10, 169.42, 156.80, 143.88, 141.43, 137.18, 135.51, 133.92, 130.21, 129.48, 128.98, 128.71, 127.94, 127.28, 125.24, 123.35, 120.17, 117.78, 115.81, 69.68, 67.51, 54.00, 47.18, 34.19.
Synthesis of Aminal, Mechanistic Study

A solution of salicylaldehyde Fmoc-Ala ester 415.14 mg (1 m Mol), 145.07 mg Hyp-OMe (1 m Mol) and a catalytic amount of p-toluenesulfonic acid (14 µl, 0.1 m Mol, 10 mol%) where combined in dry toluene (60 mL) was heated under reflux at a temperature of 100-125 °C for 3 hours. The solution mixture was evaporated to 5 mL, replaced with 100 mL of DCM, and washed successively with NaHCO₃, water, and brine solution, then dried over MgSO₄ and evaporated to yield final crude product. The crude product was then directly subjected to HPLC-MS analysis.

Mass Spectrometry: ThermoFinnigan (San Jose, CA) LCQ Classic with electrospray ionization (ESI). ESI: sheath gas(N₂) = 65; aux gas(N₂) = 5; heated capillary temperature = 250 °C. (+)ESI: spray voltage = 3.3 kV; heated capillary voltage = 12.5 V; tube lens offset = 0 V. HPLC: Agilent (Palo Alto, CA) 1100 series system consisting of G1322A degasser and G1312A binary pump. Column: Hypurity C8 (5um; 2.1 x 100 mm + guard col). Mobile Phases #1: A = Water + 0.2% acetic acid + 2.0 mM NH₄OAc. B = methanol + 0.2% acetic acid + 2.0 mM NH₄OAc. Compound 4.6, 2.5 uL of a 1/10th dilution via C8 HPLC/254 nm UV/(+)ESI-MSn.

[M+H]⁺ MW 542 isomers were detected: There were two m/z 543 ion-peaks MW 542, RT 34.44 min. This isomer produced m/z 543 (top) which was dissociated to m/z 265, 220, and 178.

General Procedure Hydroxyproline Ligation 4.5a-j

The hydroxyproline segment 4.4 (1.1 equiv.) and the O-salicylaldehyde ester segment 4.3 (1.0 equiv.) were dissolved in pyridine/acetic acid (1:1 mole/mole) at a concentration of 0.05 M. The reaction was stirred at room temperature. The reaction was monitored using TCL. After the completion of the reaction (within 5 hours), the
solvent was removed by reduced pressure the residue obtained was treated with TFA/H₂O/i-Pr₃SiH (94/5/1, v/v/v) for 5 to 10 min to give the coupled product with a natural peptide bond at the ligation site. The solvent could be blown off by a stream of air for purification. The products were purified by HPLC or recrystallization from DCM/hexanes.

**Fmoc-Ala-Hyp-OMe (4.5a)** 1.05 g, 2.25 mmol; 91% yield, sticky oil. ¹H NMR (500 MHz, DMSO-δ₆) δ 7.89 (d, J = 7.5 Hz, 2H), 7.72 (t, J = 6.2 Hz, 2H), 7.62 (t, J = 7.7 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 4.40 – 4.17 (m, 4H), 4.35 (d, J = 8.0 Hz, 1H), 4.33 – 4.27 (m, 2H), 4.26 – 4.17 (m, 2H), 4.00 (m, 1H), 3.67 (br s, 3H), 3.33 (br s, 2H), 2.10 (dd, J = 12.7, 8.8 Hz, 1H), 1.89 (ddd, J = 13.0, 8.4, 4.7 Hz, 1H), 1.24 (m, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 176.0, 172.6, 172.2, 156.3, 143.9, 141.4, 127.9, 127.3, 125.3, 120.1, 70.5, 67.4, 58.0, 55.4, 52.6, 49.7, 48.5, 47.3, 37.6, 29.9, 18.6, 18.0.

**Fmoc-Val-Hyp-OMe (4.5b)**, 0.33 g, 0.67 mmol. White microcrystals mp 107.2 – 115.6 °C. 85% yield. ¹H NMR (500 MHz, Chloroform-d) δ 7.81 – 7.68 (m, 2H), 7.63 – 7.50 (m, 2H), 7.44 – 7.35 (m, 2H), 7.35 – 7.27 (m, 2H), 5.54 (d, J = 8.9 Hz, 0H), 5.45 – 5.25 (m, 1H), 4.75 – 4.59 (m, 1H), 4.58 – 4.48 (m, 1H), 4.46 – 4.28 (m, 2H), 4.26 – 4.12 (m, 1H), 3.75 (br s, 3H), 3.33 (p, J = 6.9 Hz, 1H), 2.75 (d, J = 13.9 Hz, 1H), 2.47 – 2.13 (m, 1H), 2.11 – 1.98 (m, 1H), 1.70 (br s, 1H), 1.37 – 1.15 (m, 1H), 1.12 – 1.01 (m, 2H), 1.01 – 0.86 (m, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 188.6, 170.8, 156.6, 151.6, 144.0, 143.9, 141.5, 135.5, 131.0, 128.2, 127.9, 127.3, 126.9, 125.2, 123.4, 67.4, 59.6, 47.4, 31.1, 19.6, 17.8.
Fmoc-Leu-HYPOMe (4.5c) 0.36 g, 0.71 mmol. Sticky oil; 88% yield. $^1$H NMR (300 MHz, Methanol-$d_4$) $\delta$ 7.82 – 7.69 (m, 2H), 7.68 – 7.50 (m, 2H), 7.43 – 7.14 (m, 4H), 4.59 – 4.42 (m, 1H), 4.41 – 4.24 (m, 2H), 4.23 – 4.04 (m, 2H), 3.89 – 3.76 (m, 1H), 3.75 – 3.63 (m, 2H), 2.44 – 2.19 (m, 1H), 2.19 – 1.91 (m, 1H), 1.84 – 1.67 (m, 1H), 1.63 – 1.44 (m, 2H), 1.00 – 0.91 (m, 3H), 0.90 – 0.63 (m, 3H). $^{13}$C NMR (75 MHz, Methanol-$d_4$) $\delta$ 174.3, 174.2, 173.9, 173.6, 158.7, 145.6, 145.5, 145.3, 145.2, 142.7, 128.9, 128.3, 128.2, 126.4, 121.2, 121.0, 75.4, 71.2, 68.3, 68.1, 59.5, 59.4, 56.4, 54.2, 53.9, 53.0, 52.6, 49.1, 41.6, 41.4, 38.4, 35.6, 26.0, 25.8, 23.7, 23.6, 22.4, 21.9.

Fmoc-Val-Phe-Hyp-OMe (4.5d) 0.120 mg, 0.19 mmol; 88% yield. White microcrystals, mp = 181.1 – 181.5 °C. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.79 – 7.73 (m, 2H), 7.65 – 7.52 (m, 2H), 7.40 (t, $J$ = 7.5 Hz, 2H), 7.31 (t, $J$ = 7.5 Hz, 2H), 7.28 – 7.08 (m, 5H), 5.54 – 5.29 (m, 1H), 5.00 – 4.80 (m, 1H), 4.69 – 4.26 (m, 4H), 4.26 – 3.91 (m, 2H), 3.86 – 3.60 (m, 3H), 3.33 – 2.79 (m, 3H), 2.33 – 1.81 (m, 3H), 1.16 – 0.47 (m, 6H). $^{13}$C NMR (75 MHz, Chloroform-$d$) $\delta$ 171.69, 171.50, 171.08, 156.62, 143.98, 141.47, 136.25, 129.82, 129.53, 128.82, 128.70, 127.91, 127.28, 125.30, 120.16, 70.30, 67.32, 58.00, 55.52, 52.56, 47.34, 38.46, 31.78, 22.85, 19.34, 17.60, 14.32.

Fmoc-Ala-Phe-Hyp-OMe (4.5e) 0.62 mg, 0.1 mmol; 85% yield. Sticky oil. $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.77 (d, $J$ = 7.3 Hz, 2H), 7.64 – 7.51 (m, 2H), 7.40 (t, $J$ = 7.4 Hz, 2H), 7.36 – 7.29 (m, 2H), 7.29 – 7.07 (m, 4H), 5.45 (t, $J$ = 8.4 Hz, 1H), 5.06 – 4.62 (m, 1H), 4.47 – 4.26 (m, 2H), 4.26 – 4.13 (m, 2H), 3.82 – 3.53 (m, 3H), 3.35 – 2.82 (m, 4H), 1.54 – 1.23 (m, 3H). $^{13}$C NMR (75 MHz, Methanol-$d_4$) $\delta$ 173.10, 172.62, 170.59, 156.85, 144.01, 141.37, 136.57, 129.18, 128.22, 127.59, 126.99, 126.61, 125.04,
110.73, 69.66, 66.85, 58.35, 55.00, 51.55, 37.10, 31.56, 28.97, 22.51, 17.00, 13.27. MS (ESI) (m/z): [M+Na]^+ calcd for C_{43}H_{53}N_{3}O_{7}Na, 608.24, found 608.2.

Fmoc-Leu-Phe-Hyp(OMe) (4.5f) 0.55 mg, 0.09 mmol; 85% yield. Sticky oil. ^1H NMR (300 MHz, Methanol-d$_4$) δ 7.78 (d, J = 7.4 Hz, 2H), 7.70 – 7.59 (m, 2H), 7.40 – 7.35 (m, 4H), 7.20 (m, 5H), 4.51 – 4.40 (d, J = 6.4 Hz, 3H), 4.17 – 4.29 (m, 1H), 4.18 (dd, J = 6.9, 6.9 Hz, 1H), 4.13 – 4.03 (m, 1H), 3.67 (br s, 3H), 3.33 – 3.29 (m, 1H), 3.08 (m, 1H), 2.96 – 2.75 (m, 1H), 2.25 – 2.06 (m, 1H), 2.03 – 1.88 (m, 1H), 1.74 – 1.52 (m, 1H), 1.51 – 1.39 (m, 1H), 1.37 – 1.17 (m, 2H), 1.00 – 0.88 (m, 3H), 0.88 – 0.75 (m, 3H).

^13C NMR (75 MHz, Methanol-d$_4$) δ 174.0, 172.3, 145.3, 142.8, 137.9, 130.9, 129.6, 128.9, 128.3, 128.0, 126.4, 121.1, 71.0, 68.0, 59.7, 56.3, 55.05, 53.9, 52.9, 49.2, 42.2, 38.9, 38.5, 25.9, 23.6, 22.0.

Fmoc-Leu-Ala-Hyp-OMe (4.5g) 0.28 mg, 0.05 mmol; 77% yield. White microcrystals, mp = 106.3 – 126.5 °C. ^1H NMR (300 MHz, Chloroform-d) δ 7.81 – 7.68 (m, 2H), 7.57 (dd, J = 7.4, 4.5 Hz, 2H), 7.43 – 7.33 (m, 2H), 7.32 – 7.23 (m, 2H), 5.77 – 5.44 (m, 1H), 4.83 – 4.57 (m, 1H), 4.55 – 4.09 (m, 5H), 3.94 – 3.48 (m, 4H), 2.39 – 2.18 (m, 1H), 2.07 – 1.84 (m, 1H), 1.73 – 1.41 (m, 4H), 1.30 (t, J = 12.8, 6.9 Hz, 3H), 1.05 – 0.71 (m, 6H). ^13C NMR (75 MHz, Methanol-d$_4$) δ 175.10, 174.18, 172.97, 158.50, 145.28, 142.73, 128.92, 128.29, 126.33, 121.05, 71.09, 68.02, 59.46, 56.19, 54.78, 52.94, 48.12, 42.16, 38.41, 25.98, 23.67, 21.97, 17.62, 17.19. HRMS (ESI) (m/z): [M+Na]^+ calcd for C$_{30}$H$_{37}$N$_{3}$O$_{7}$Na, 574.2534, found 574.2524.

Fmoc-Gly-Phe-Hyp-OMe (4.5h) 0.75 m, 0.92 mmol; 78% yield. White microcrystals, mp = 105.7 – 108.5 °C. ^1H NMR (300 MHz, Methanol-d$_4$) δ 7.82 (d, J = 7.8 Hz, 2H), 7.68 (d, J = 6.6 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.34 (dd, J = 7.4, 1.6 Hz, 2H), 7.23 (m, 2H), 7.15 (d, J = 8.6 Hz, 2H), 7.04 – 6.93 (m, 2H), 6.89 (d, J = 7.9 Hz, 2H), 6.77 (d, J = 7.4 Hz, 2H), 6.61 (d, J = 7.4 Hz, 2H), 6.54 (d, J = 7.4 Hz, 2H), 6.40 (d, J = 7.4 Hz, 2H), 6.27 (d, J = 7.4 Hz, 2H), 6.15 (d, J = 7.4 Hz, 2H), 6.03 (d, J = 7.4 Hz, 2H), 5.90 (d, J = 7.4 Hz, 2H), 5.77 (d, J = 7.4 Hz, 2H), 5.64 (d, J = 7.4 Hz, 2H), 5.51 (d, J = 7.4 Hz, 2H), 5.38 (d, J = 7.4 Hz, 2H), 5.25 (d, J = 7.4 Hz, 2H), 5.12 (d, J = 7.4 Hz, 2H), 5.00 (d, J = 7.4 Hz, 2H), 4.87 (d, J = 7.4 Hz, 2H), 4.74 (d, J = 7.4 Hz, 2H), 4.61 (d, J = 7.4 Hz, 2H), 4.48 (d, J = 7.4 Hz, 2H), 4.35 (d, J = 7.4 Hz, 2H), 4.22 (d, J = 7.4 Hz, 2H), 4.10 (d, J = 7.4 Hz, 2H), 3.97 (d, J = 7.4 Hz, 2H), 3.84 (d, J = 7.4 Hz, 2H), 3.71 (d, J = 7.4 Hz, 2H), 3.58 (d, J = 7.4 Hz, 2H), 3.45 (d, J = 7.4 Hz, 2H), 3.32 (d, J = 7.4 Hz, 2H), 3.19 (d, J = 7.4 Hz, 2H), 3.06 (d, J = 7.4 Hz, 2H), 2.93 (d, J = 7.4 Hz, 2H), 2.80 (d, J = 7.4 Hz, 2H), 2.67 (d, J = 7.4 Hz, 2H), 2.54 (d, J = 7.4 Hz, 2H), 2.41 (d, J = 7.4 Hz, 2H), 2.28 (d, J = 7.4 Hz, 2H), 2.15 (d, J = 7.4 Hz, 2H), 2.02 (d, J = 7.4 Hz, 2H), 1.90 (d, J = 7.4 Hz, 2H), 1.77 (d, J = 7.4 Hz, 2H), 1.64 (d, J = 7.4 Hz, 2H), 1.51 (d, J = 7.4 Hz, 2H), 1.38 (d, J = 7.4 Hz, 2H), 1.25 (d, J = 7.4 Hz, 2H), 1.12 (d, J = 7.4 Hz, 2H), 0.99 (d, J = 7.4 Hz, 2H), 0.86 (d, J = 7.4 Hz, 2H), 0.73 (d, J = 7.4 Hz, 2H), 0.59 (d, J = 7.4 Hz, 2H), 0.46 (d, J = 7.4 Hz, 2H), 0.32 (d, J = 7.4 Hz, 2H).
2H), 7.29 – 7.15 (m, 5H), 4.71 (dd, J = 7.9, 5.3 Hz, 1H), 4.55 – 4.32 (m, 3H), 4.25 (d, J = 7.0 Hz, 1H), 3.87 – 3.63 (m, 5H), 3.26 – 2.84 (m, 3H), 2.35 – 1.72 (m, 2H). $^{13}$C NMR (75 MHz, Methanol-$d_4$) δ 173.92, 171.98, 171.53, 159.05, 145.34, 142.66, 138.06, 130.46, 129.58, 128.91, 128.30, 127.99, 126.35, 121.04, 70.69, 68.34, 59.67, 56.11, 55.00, 53.71, 52.88, 44.85, 43.66, 38.47. HRMS (ESI) (m/z): [M+Na]$^+$ calcd for C$_{32}$H$_{33}$N$_3$O$_7$Na, 594.2211, found 594.2233.

Fmoc-Val-Phe-Hyp-Leu-OMe (4.5i) 4.5e) 0.52g, 71.5 µmol; 81% yield. Sticky oil.

$^1$H NMR (300 MHz, Methanol-$d_4$) δ 7.85 – 7.73 (m, 2H), 7.65 (t, J = 6.8 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.34 – 7.24 (m, 4H), 7.16 – 7.07 (m, 3H), 4.53 – 4.25 (m, 3H), 4.25 – 4.16 (m, 1H), 3.87 – 3.75 (m, 2H), 3.75 – 3.51 (m, 3H), 3.25 – 3.11 (m, 1H), 3.05 – 2.76 (m, 1H), 2.36 – 2.10 (m, 1H), 2.00 – 1.84 (m, 7H), 1.79 – 1.66 (m, 1H), 1.60 (dd, J = 14.1, 7.1 Hz, 1H), 1.19 – 0.54 (m, 12H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 172.93, 172.47, 170.33, 170.26, 156.03, 154.23, 143.92, 143.76, 140.70, 129.43, 129.32, 127.62, 127.04, 125.35, 120.06, 69.91, 67.74, 65.69, 57.91, 51.78, 50.28, 46.74, 30.15, 27.67, 27.56, 24.14, 22.78, 21.34, 21.19, 19.34, 18.85, 18.16. HRMS (ESI) (m/z): [M+H]$^+$ calcd for C$_{41}$H$_{51}$N$_4$O$_8$, 727.3701, found 727.3877

Fmoc-Val-Phe-Hyp-B-Ala-Phe-Leu-OMe (4.5j) 0.12 mg, 12.4 µmol; 88% yield. Sticky oil.

$^1$H NMR (300 MHz, Methanol-$d_4$) δ 7.78 (d, J = 7.5 Hz, 2H), 7.65 (t, J = 6.9 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.33 – 7.23 (m, 6H), 7.23 – 6.97 (m, 6H), 4.71 – 4.47 (m, 2H), 4.45 – 4.25 (m, 3H), 4.25 – 4.07 (m, 3H), 3.95 – 3.74 (m, 3H), 3.74 – 3.57 (m, 4H), 3.15 (dd, J = 13.9, 5.2 Hz, 1H), 3.02 – 2.77 (m, 2H), 2.55 – 2.12 (m, 3H), 2.08 – 1.79 (m, 2H), 1.76 – 1.53 (m, 4H), 1.21 – 1.12 (m, 2H), 0.99 – 0.88 (m, 12H). $^{13}$C NMR (75 MHz, Methanol-$d_4$) δ 175.46, 175.33, 175.10, 174.48, 174.01, 173.52, 158.70,
145.57, 145.35, 142.82, 139.41, 138.68, 130.60, 130.48, 129.70, 129.42, 129.06,
128.46, 128.06, 127.69, 126.47, 73.27, 68.27, 62.20, 61.00, 57.02, 55.97, 55.09, 52.99,
52.47, 41.75, 39.38, 37.00, 32.25, 29.48, 28.87, 26.14, 23.63, 22.11, 20.07, 19.67,
18.32. HRMS (MALDI) (m/z): [M+Na]$^+$ calcd for C$_{53}$H$_{64}$N$_6$O$_{10}$Na, 967.4576, found 967.4611
CHAPTER 5
A CONVENIENT SYNTHESIS OF MEDIUM_SIDED CYCLIC PEPTIDES BY STAUDINGER MEDIATED RING-CLOSURE

Introduction

Small cyclic peptides have attracted attention for decades. Due to their metabolic stability, defined conformations, and importance as a source of new drug candidates, Natural cyclic dipeptides possess diverse biological activities including antibiotic/antifungal, plant growth retardation and cytotoxicity. Small cyclic peptides show potent cytotoxic effects by a variety of mechanisms and display neuroprotective properties.

Ring size is a significant factor in the success of macrolactamisation in the synthesis of a cyclic peptide. Six-membered dipeptides (2,5-diketopiperazines) are readily accessible and often observed as undesired side-products in the synthesis of peptide constructs. In sharp contrast, synthesis of their 7- and 8-membered analogs is much more challenging.

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Ring-closure to give small cyclic peptide by direct head-to-tail lactamization is difficult and no cyclisation could be accomplished using the most powerful coupling reagents such as EDC–HOBt, BOP, TBTU and PfPyU at high dilution (Figure 5-1).\textsuperscript{88,89,90}

To overcome problems in synthesis of small and medium cyclo-peptides, an auxiliary-based method for the synthesis of \textit{bis}-lactams was developed (Figure 5-2).\textsuperscript{89} The novel auxiliary was designed to be inserted into the backbone of a linear peptide facilitating the mutually reactive terminal groups to approach one another for a cyclization reaction. A subsequent ring contraction mechanism leads to formation of the desired cyclic products with the remainings of the auxiliary still attached. Functionalized seven- and eight-membered cyclic dipeptides have been synthesized.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5-2}
\caption{A pincer auxiliary to force difficult lactamization.}
\end{figure}

However, removal of the auxiliary from the final cyclic products can be difficult; moreover, possible side reactions that can occur.

Other strategies are based on cyclizations on solid phase,\textsuperscript{91} chemoenzymatic\textsuperscript{92} and dendrimeric nanoreactors (Figure 5-3).\textsuperscript{93} But, these methods are still limited to specific sequences.
Figure 5-3. Wedge-shaped carbosilane dendrimeric carbodiimides to cyclize homodiketopiperazines through a site-isolation mechanism

The cyclization of linear peptides containing more than seven amino acids usually proceeds well. As a rule of thumb, di- and tripeptides possessing 7–15-membered rings are less accessible and can frequently only be synthesized with difficulty. The main hurdle to the cyclization of peptides to afford 7- to 15-membered rings is the preferential *transoid* alignment of amide bonds in their acyclic precursors which leads to a preferred extended structure, placing the termini which need to react far apart.94,95

Among other strategies, traceless Staudinger ligation has been the focus of numerous attempts to overcome problems in the coupling of large peptide fragments.53 Intramolecular traceless Staudinger ligation has been applied to access cyclic polypeptides and medium-sized (7–10-membered) lactams, but this methodology required protection of the phosphine-containing auxiliary by a borane complex to avoid undesired premature Staudinger reaction.96 The cleavage of the borane protecting group may require harsh conditions and, the phosphinothioester peptide can undergo oxidation during the borane deprotection process to produce undesired by-products.

This chapter focuses on the development of innovative and efficient cyclization procedures to prepare 7- and 8-membered cyclic di- and 10-membered cyclic
tripeptides containing α-, β- or γ-amino acid residues via a solution phase Staudinger ligation-like reaction (Figure 5-4).

![Reaction Mechanism](image)

Figure 5-4. Ring construction to form cyclic oligopeptides by Staudinger ring closure.

**Results and Discussion**

**Synthesis of 7-Membered and 8-Membered Cyclic Dipeptide Using Staudinger Assisted Ring Closure**

In the first phase of the project, the synthesis of 7- and 8-membered cyclic dipeptides was investigated from azidopeptidoyl thioesters. The general procedure for the synthesis of the starting azidopeptidoyl thioesters 5.5a-f is illustrated in Figure 5-5. First, chloroalkylcarbonyl chlorides 5.1a-d were reacted with amino acids 5.2a-e to form dipeptide derivatives 5.3a-f which, with an excess of sodium azide in DMF at 80 °C or MeOH at reflux, afforded the corresponding azidoprotected dipeptides 5.4a-f. Mixed anhydride coupling of 5.4a-f with 1.2 equiv. of thiophenol furnished the azidopeptidoyl thioesters 5.5a-f (45% to 72%). Preparative conditions for the Staudinger-mediated ring-closure were first optimized on compound 5.5a: treatment with 1.5 equiv. of PBu₃ in dry THF under microwave heating at 50 °C and 50 W for 5 min. The desired cyclic peptide 5.6a was precipitated from the crude mixture on addition of hexanes and obtained in high purity.
Figure 5-5. Synthesis of starting azido dipeptide thioesters (Table 5-1).

Next synthesis of (S)-3-benzyl-[1,4]diazepane-2,5-dione (5.6b), a challenging target difficult to obtain by direct macrolactamization was studied.\textsuperscript{6d,6f} Reaction of 5.5b with tributylphosphine using the optimized conditions described above gave product 5.6b, isolated in 63% yield after purification. This protocol was then applied to the synthesis of 3,3-dimethyl-1,4-diazepane-2,5-dione (5.6c) by cyclization of precursor 5.5c. Novel cyclic dipeptide 5.6c was obtained in 72% yield after purification. These three examples show the advantages of our microwave-assisted Staudinger-mediated cyclization for synthesis of difficult diketopiperazines. The higher yield in the reaction of 5.5a in comparison to 5.5b suggests that the cyclization is sensitive to steric congestion at the C-terminal fragment.\textsuperscript{10} The unexpected high yield obtained for compound 5.5c is attributed to a Thorpe-Ingold effect ($C^\alpha$-dimethyl substitution).

The few literature examples of 8-membered cyclic dipeptide are endowed with promising biological activity.\textsuperscript{12} To broaden the scope of our methodology, 1,4-diazocane-2,5-dione 5.6d and 1,5-diazocane-2,6-dione 5.6e were considered as potential targets. The macrolactamizations of 5.5d-f were each initiated by treatment with 1.5 equiv. of PBu$_3$ in dry THF at room temperature. The unfunctionalized 8-membered bislactams 6d and 6e were isolated after purification in 55% and 57% yield, respectively.
Table 5-1. En route to 7- and 8-membered cyclic dipeptides.

<table>
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<th>X</th>
<th>n</th>
<th>m</th>
<th>R¹</th>
<th>R²</th>
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<th>Yield (%)</th>
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<td>H</td>
<td>H</td>
<td><img src="image" alt="Structure 5.6f" /></td>
<td>51</td>
</tr>
</tbody>
</table>

a The yield of 5.6d in italics was calculated from ^1^H NMR spectrum (500 MHz) of the crude mixture.

The impact of the ring-size and the sequence on the activation barrier were studied. Transition states for the cyclization of model aza-ylide thioester intermediates (PH₃ as model phosphine) were calculated at the B3LYP/6-31+G** level of theory and
the nature of the stationary points was determined by analytical calculation of the Hessian. A model sequence N$_3$-Gly-Gly-SPh was selected as a reference. Its cyclization through a six-membered TS required an activation barrier of 16.3 kcal·mol$^{-1}$, while the cyclization of the sequence N$_3$-Gly-$\beta$-Ala-SPh through a 7-membered TS required 25.1 kcal·mol$^{-1}$. Interestingly, the cyclization of the reversed sequence (N$_3$-$\beta$-Ala-Gly-SPh, leading to 5.6a) proceeded with a lower activation barrier ($\Delta G^\ddagger = 14.7$ kcal·mol$^{-1}$). The conformational flexibility given to the aza-ylide nucleophile by the $\beta$-Ala residue at the N-terminus of the azido dipeptide thioester seemed to have great impact on the activation barrier. Incorporation of C$^\alpha$-thioester substituent led a slight increase of the activation barrier: 15.1 and 20.2 kcal·mol$^{-1}$ for the cyclization of N$_3$-$\beta$-Ala-Phe-SPh (leading to 5.6b) and for the N$_3$-$\beta$-Ala-Aib-Sph (leading to 5.6c), respectively. The cyclization of the eight-membered cyclic peptide 5.6d required 22.0 kcal·mol$^{-1}$.

**Synthesis of a 10-Membered Cyclic Tripeptide Using Staudinger Assisted Ring Closure**

The procedure was applied to the cyclization of azidotripeptidoyl thiester 5.11 for synthesis of the cyclic 10-membered tripeptide 5.12. Cyclotripeptides constitute a very important class of compounds with high potential in drug discovery but their synthesis using traditional activated carboxylic acids led to low yield and a number of side-products.$^{10}$ Azido tripeptide thioester 5.11 was prepared according to the strategy used for compounds 5.5a-f. The conditions for the cyclization of 5.11 were similar to those used for the cyclization of 5.5d-f and gave cyclo(Gly-Phe-$\beta$-Ala) 5.12 in 48% yield (Figure 5-6).
Figure 5-6. Solution phase synthesis of a 10-membered cyclic tripeptide.

**Conformational Studies of 7-Membered Cyclic Dipeptides**

Monocrystals were grown from acetone/methanol mixtures for compounds 5.6a and 5.6c, the structures of which were unambiguously assigned by X-ray diffraction.

The conformational behavior of medium-sized cyclic peptides has been rarely studied in contrast to the decades of research dedicated to biological activity of cyclic peptides.\(^{6f-6k,14}\) We now find that, in cyclic dipeptides 5.6a and 5.6c, the seven-membered ring is formed by two puckered planes (envelope-like), in agreement with reported data for similar compounds (Figure 5-7).\(^{97}\)

Empirical force-field calculations were performed to predict the conformational preferences of 1,5-diazocane-2,6-dione 5.6a and 3,3-dimethyl-1,4-diazepane-2,5-dione 5.6c. A high overlay similarity (93.7 and 98.5%, respectively) between the X-ray structure and the predicted conformers was found and encouraged us to study the conformational preferences of the eight-membered cyclodipeptides 5.6d and 5.6e, for which no X-ray data were available. These cyclic dipeptides could adopt two possible conformers: a C\(_2\)-symmetric twisted-boat or a centro-symmetric chair. Analysis of the empirical force-field calculations showed an exclusive chair-like conformation for both compounds 5.6d and 5.6e (Figure 5-8).
Figure 5-7. X-ray structures, traditional representations and computed structures of A) 1,5-diazocane-2,6-dione (5.6a) and B) 3,3-dimethyl-1,4-diazepane-2,5-dione (5.6c).

Figure 5-8. Predicted conformers for compounds A) 1,5-diazocane-2,6-dione (5.6d) and B) 1,4-diazocane-2,5-dione (5.6e).

**Conclusion**

In summary, we have developed a new, straightforward and powerful strategy towards small cyclic di- and tripeptides that relies on a Staudinger assisted ring closure. This was demonstrated by the successful ring-closure of a series of azido peptide thioesters yielding a small library of 7-, 8- and 10-membered cyclopeptides, which could not be prepared efficiently using the previously reported methods. A model of reactivity
based on ring size and sequence was developed using computational chemistry. The conformational behavior of cyclodipeptides was studied by X-ray on the homodiketopiperazine series. The conformers obtained by empirical force-field calculations showed high levels of similarity with the X-ray results. Empirical force-field calculations were also used to predict the conformational behavior of diazocanes. Homodiketopiperazines have showed envelope-like conformational preferences, while diazocanes prefer chair-like conformations.

**Experimental**

**General Methods**

All commercial materials (Aldrich, Fluka) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher Solvents were dried using standard protocols kept under a dry atmosphere of nitrogen. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$, DMSO-$_d_6$, Acetone-$d_6$ or CD$_3$OD-$d_4$ using a 300 or 500 MHz spectrometer (with TMS as an internal standard) at ambient temperature unless otherwise stated. Chemical shifts are reported in parts per million relative to residual solvent CDCl$_3$ ($^1$H, 7.26 ppm; $^{13}$C, 77.23 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublets, quint = pentet. All $^{13}$C NMR spectra were recorded with complete proton decoupling. The data have been reported in order to provide the maximum amount of information regarding coupling constants, which has necessarily led to integrals reported following a group of peaks in some instances. High-resolution and high-performance liquid chromatography mass spectral analyses were performed by the University of Florida chemistry department.
facility staff. Reactions were carried out in oven-dried glassware under an argon or nitrogen atmosphere unless otherwise noted. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). Analytical TLC was performed on E. Merck silica gel 60 F254 plates and visualized by UV and potassium permanganate staining. Flash column chromatography was performed on E. Merck silica gel 60 (40–63 mm). Yields refer to chromatographically and spectroscopically pure compound-

**General Procedure I for the Preparation of Compounds 5.3a-f**

To a suspension of amino acid (5.2a-e) in dry THF (20 mL/1 mmol) at 0 °C was added halogen acyl chloride (5.1a-d) (1.5 eq.) and the resulting mixture was refluxed for 2.5 h. The reaction mixture was filtered hot and the solvent was evaporated under reduced pressure. Then diethyl ether (30 mL/1 mmol 2a-e) was added to the residue and left in the fridge to recrystallize. The solid obtained was washed with hexanes (5 mL/1 mmol), cold diethyl ether (5 mL/1 mmol) and dried to give desired products (5.3a-f).

3-(2-Chloroacetamido)propanoic acid (5.3a). The compound was synthesized following the general procedure I from chloroacetyl chloride (4.77 mL, 60 mmol) and β-alanine (3.56 g, 40 mmol) in 75% yield (5.06 g, 28 mmol). White microcrystals, mp 99-100 °C. $^1$H NMR (DMSO- $d_6$, 300 MHz): $\delta$ 2.40 (t, $J = 6.8$ Hz, 2H), 3.24-3.34 (m, 2H), 4.04 (s, 2H), 8.26 (br s, 1H), 12.27 (br s, 1H); $^{13}$C NMR (DMSO- $d_6$, 75 MHz): $\delta$ 33.5,
Anal. Calcd for C₅H₈ClNO₃: C, 36.27; H, 4.87; N, 8.46. Found: C, 36.33; H, 4.95; N, 8.33.

(S)-2-(3-Bromopropanamido)-3-phenylpropanoic acid (5.3b). The compound was synthesized following the general procedure I from 3-bromopropyl chloride (3.63 mL, 36 mmol) and L-phenylalanine (4.00 g, 24 mmol) in 88% yield (6.30 g, 21 mmol). White microcrystals, mp 94-98 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 2.57 (dt, J = 6.5, 1.9 Hz, 2H), 2.86 (dd, J = 14.0, 9.2 Hz, 1H), 3.05 (dd, J = 13.8, 5.1 Hz, 1H), 3.69 (t, J = 6.3 Hz, 2H), 4.41-4.50 (m, 1H), 7.16-7.30 (m, 5H), 8.37 (d, J = 8.0 Hz, 1H), 12.75 (br s, 1H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 36.9, 38.0, 40.7, 53.5, 126.5, 128.2, 129.2, 137.5, 168.9, 172.9; Anal. Calcd for C₁₂H₁₄BrNO₃: C, 48.02; H, 4.70; N, 4.67. Found: C, 47.94; H, 4.50; N, 4.91.

2-(3-Chloropropanamido)-2-methylpropanoic acid (5.3c). The compound was synthesized following the general procedure I from 3-chloropropyl chloride (5.6 g, 44 mmol) and 2-methylalanine (3.00 g, 29 mmol) in 73% yield (4.10 g, 21 mmol). White microcrystals, mp 167-169 ºC. ¹H NMR (DMSO-d₆, 300 MHz): δ 1.33 (br s, 6H), 2.56 (t, J = 6.3 Hz, 2H), 3.74 (t, J = 6.5 Hz, 2H), 8.18 (br s, 1H), 12.16 (br s, 1H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 25.0, 38.0, 40.9, 54.9, 168.3, 175.4. HRMS (m/z): [M-H]⁻ calcd for C₇H₁₁BrNO₃ 192.0433, found 192.0442.

3-(3-Chloropropanamido)propanoic acid (5.3d). The compound was synthesized following the general procedure I from 3-chloropropyl chloride (7.48 g, 59 mmol) and 3-aminopropanoic acid (3.50 g, 39 mmol) in 56% yield (3.95 g, 22 mmol). White microcrystals, mp 116-120 ºC. ¹H NMR (DMSO-d₆, 300 MHz): δ 2.37 (t, J = 6.9 Hz, 2H),
2.54 (t, $J = 6.3$ Hz, 2H), 3.25 (q, $J = 6.4$ Hz, 2H), 3.76 (t, $J = 6.5$ Hz, 2H), 8.09 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 33.9, 34.9, 38.2, 41.1, 169.0, 172.9.

4-(2-Chloroacetamido)butanoic acid (5.3e). The compound was synthesized following the general procedure I from chlororacetyl chloride (2.31 mL, 29 mmol) and $\gamma$-aminobutyric acid (2.00 g, 19 mmol) in 72% yield (2.51 g, 14 mmol). White microcrystals, mp 75-79 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.64 (quint, $J = 7.2$ Hz, 2H), 2.22 (t, $J = 7.4$ Hz, 2H), 3.06-3.13 (m, 2H), 4.03 (s, 2H), 8.23 (br s, 1H), 12.08 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): 24.3, 30.9, 38.3, 42.7, 165.9, 174.1; Anal. Calcd for C$_6$H$_{10}$ClNO$_3$: C, 40.13; H, 5.61; N, 7.80. Found: C, 39.78; H, 5.73; N, 7.45.

2-(4-Chlorobutanamido)acetic acid (5.3f). The compound was synthesized following the general procedure I from 4-chlorobutanoyl chloride (4.79 mL, 50 mmol) and glycine (2.50 g, 33 mmol) in 38% yield (2.27 g, 13 mmol). Sticky solid. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.93 (q, $J = 6.9$ Hz, 2H), 2.28 (t, $J = 7.2$ Hz, 2H), 3.64 (t, $J = 6.5$ Hz, 2H), 3.72 (d, $J = 6.0$ Hz, 2H), 8.24 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): 28.3, 32.1, 40.6, 45.0, 171.4, 171.6.

**General Procedure II for the Preparation of Compounds 5.4a-f**

Dipeptide derivatives 5.3a-f was dissolved in a minimum amount of methanol (5.4a) or DMF (4b-f) and then sodium azide (5 eq.) was added to the solution. The suspension was heated at 80 °C for 72 h (methanol) or 24 h (DMF). The solvent was removed under reduced pressure and brine was added to the residue until extra sodium azide was dissolved. The resulting mixture was acidified slowly with HCl to pH 5 and extracted with ethyl acetate (3 x 10 mL/1 mmol 5.3a-f). The organic layers were combined, then dried over anhydrous MgSO$_4$ and filtered. The solvent was evaporated.
under reduced pressure to give the desired products 5.4a-f. Compounds 5.4b and 5.4c were recrystallized from a CH$_2$Cl$_2$:hexanes mixture

3-(2-Azidoacetamido)propanoic acid (5.4a). The compound was synthesized following the general procedure II from 3-(2-chloroacetamido)propanoic acid (5.3a) (2.00 g, 12.1 mmol) in 63% yield (1.31 g, 7.6 mmol). Colorless oil. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.62 (t, $J = 6.0$ Hz, 2H), 3.53-3.60 (m, 2H), 3.99 (s, 2H), 6.87 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 33.7, 34.9, 52.8, 167.4, 176.5. HRMS ($m/z$): [M-H]$^-$ calcd for C$_5$H$_7$N$_4$O$_3$, 171.0524, found 171.0531.

(S)-2-(3-Azidopropanamido)-3-phenylpropanoic acid (5.4b). The compound was synthesized following the general procedure II from (S)-2-(3-bromoropropanamido)-3-phenylpropanoic acid (5.3b) (3.51 g, 11.7 mmol) in 54% yield (1.66 g, 6.3 mmol). White microcrystals, mp 128-129 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.37 (t, $J = 6.2$ Hz, 2H), 2.86 (dd, $J = 13.8$, 9.3 Hz, 1H), 3.06 (dd, $J = 13.7$, 5.0 Hz, 1H), 3.42 (t, $J = 6.3$ Hz, 2H), 4.41-4.49 (m, 1H), 7.17-7.31 (m, 5H), 8.35 (d, $J = 8.1$ Hz, 1H), 12.72 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 34.3, 36.8, 46.8, 53.5, 126.4, 128.1, 129.1, 137.5, 169.5, 172.9.

(3-Azidopropanamido)-2-methylpropanoic acid (5.4c). The compound was synthesized following the general procedure II from (2-(3-chloropropanamido)-2-methylpropanoic acid (5.3c) (3.00 g, 15.5 mmol) in 82% yield (2.54 g, 12.7 mmol). Yellow microcrystals, mp 145-152 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.33 (s, 6H), 2.36 (t, $J = 6.5$ Hz, 2H), 3.46 (t, $J = 6.5$ Hz, 2H), 8.17 (br s, 1H), 12.30 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 24.9, 34.4, 46.8, 54.8, 168.9, 175.4. HRMS ($m/z$): [M-H]$^-$ calcd for C$_7$H$_{11}$N$_4$O$_3$ 199.08371, found 199.0843.
3-(3-Azidopropanamido)propanoic acid (5.4d). The compound was synthesized following the general procedure II from 3-(3-chloropropanamido)propanoic acid (5.3d) (2.00 g, 11.1 mmol) in 63% yield (1.31 g, 7.0 mmol). Yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 2.41 (t, $J = 6.3$ Hz, 2H), 2.60 (t, $J = 5.8$ Hz, 2H), 3.54 (q, $J = 6.0$ Hz, 2H), 3.60 (t, $J = 6.5$ Hz, 4H), 6.38 (br s, 1H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 33.9, 35.2, 36.0, 47.5, 170.9, 176.3. HRMS (m/z): [M-H]$^-$ calcd for C$_6$H$_9$N$_4$O$_3$, 185.0680, found 185.0686.

4-(2-Azidoacetamido)butanoic acid (5.4e). The compound was synthesized following the general procedure II from 4-(2-chloroacetamido)butanoic acid (5.3e) (1.50 g, 8.35 mmol) in 68% yield (1.06 g, 5.68 mmol). Yellow sticky solid. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.89 (quint, $J = 6.8$ Hz, 2H), 2.51 (t, $J = 6.9$ Hz, 2H), 3.36 (q, $J = 6.6$ Hz, 2H), 4.00 (s, 2H), 6.59 (br s 1H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 24.2, 32.7, 38.5, 52.8, 167.3, 169.1.

2-(4-Azidobutanamido)acetic acid (5.4f). The compound was synthesized following the general procedure II from 2-(4-chlorobutanamido)acetic acid (5.3f) (2.00 g, 11.2 mmol) in 63% yield (1.31 g, 7.0 mmol). Yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.75 (q, $J = 7.1$ Hz, 2H), 2.20 (t, $J = 7.4$ Hz, 2H), 3.33 (t, $J = 6.9$ Hz, 2H), 3.73 (d, $J = 6.0$ Hz, 2H), 8.22 (br s, 1H), 12.52 (br s, 1H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 24.5, 31.9, 40.6, 50.1, 171.4, 171.7.

**General Procedure III for the Preparation of Compounds 5.5a-f**

A solution of azidoprotected dipeptides 5.4a-e and N-methylmorpholine (1 eq.) in dry THF (10 mL/1 mmol) was cooled to 0 °C under argon atmosphere. To the resulting solution, isobutyl chloroformate was added (1 eq.). After 5 min, thiophenol (1.1 eq.) was added and the mixture was stirred for 24 h at room temperature. The solvent was
evaporated under reduced pressure and the residue was taken up with ethyl acetate (30 mL/ 1 eq.). The organic layer was washed with Na₂CO₃ (3 x 15 mL/ 1 eq.), dried over anhydrous MgSO₄, filtered and concentrated under reduced pressure. The crude product was recrystallized from a CH₂Cl₂/hexanes mixture to give 5.5a-f.

S-Phenyl 3-(2-azidoacetamido)propanethioate (5.5a). The compound was synthesized following the general procedure III from 3-(2-azidoacetamido)propanoic acid (5.4a) (0.93 g, 5.38 mmol) in 57% yield (0.81 g, 3.07 mmol). White microcrystal, mp 63-64 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.93 (t, J = 6.2 Hz, 2H), 3.55-3.65 (m, 2H), 3.96 (s, 2H), 6.79 (br s, 1H), 7.40-7.46 (m, 5H); ¹³C NMR (CDCl₃, 75 MHz): δ 35.3, 42.8, 52.7, 127.1, 129.5, 129.9, 134.7, 166.9, 197.3; Anal. Calcd for C₁₁H₁₂N₄O₂S: C, 49.99; H, 4.58; N, 21.20. Found: C, 50.11; H, 4.57; N, 21.24.

(S)-S-Phenyl 2-(3-azidopropanamido)-3-phenylpropanethioate (5.5b) The compound was synthesized following the general procedure III from (S)-2-(3-azidopropanamido)-3-phenylpropanoic acid (5.4b) (1.41 g, 5.4 mmol) in 66% yield (1.26 g, 3.6 mmol). White microcrystals, mp 86-87 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 2.54-2.73 (m, 2H), 2.92 (dd, J = 13.8, 10.2 Hz, 1H), 3.15 (dd, J = 14.0, 5.0 Hz, 1H), 3.71-3.78 (m, 2H), 4.67-4.76 (m, 1H), 7.18-7.31 (m, 5H), 7.34-7.39 (m, 2H), 7.45-7.48 (m, 3H), 8.90 (d, J = 8.1 Hz, 1H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 34.4, 36.6, 46.7, 60.6, 126.6, 127.2, 128.2, 129.1, 129.3, 129.5, 134.5, 136.9, 170.2, 198.3. HRMS (m/z): [M+Na]⁺ calcd for C₁₈H₁₆N₄O₂SNa, 370.1043, found 370.0656.

S-Phenyl 2-(3-azidopropanamido)-2-methylpropanethioate (5.5c) The compound was synthesized following the general procedure III from 2-(3-azidopropanamido)-2-methylpropanoic acid (5.4c) (1.75 g, 5.99 mmol) in 64% yield (1.12 g, 3.83 mmol).
Yellow microcrystals, mp 55-57 °C. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.58 (br s, 6H), 2.43 (t, $J = 6.5$ Hz, 2H), 3.61 (t, $J = 6.3$ Hz, 2H), 6.41 (br s, 1H), 7.34-7.39 (m, 5H);
$^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 25.6, 36.3, 47.3, 63.1, 127.6, 129.3, 129.5, 135.2, 169.7, 201.3. HRMS (m/z): [M+Na]$^+$ calcd for C$_{13}$H$_{16}$N$_4$O$_2$SNa, 315.0886, found 315.0894.

S-Phenyl 3-(3-azidopropanamido)propanethioate (5.5d). The compound was synthesized following the general procedure III from 3-(3-azidopropanamido)propanoic acid (5.4d) (0.20 g, 1.07 mmol) in 65% yield (0.19 g, 0.70 mmol). Pale yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 2.34 (t, $J = 6.5$ Hz, 2H), 2.89 (t, $J = 5.9$ Hz, 2H), 3.55 (q, $J = 5.9$ Hz, 4H), 6.21 (br s, 1H), 7.39-7.43 (m, 5H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 35.4, 36.0, 42.9, 47.5, 127.2, 129.5, 129.9, 134.7, 170.1, 197.9. HRMS (m/z): [M+Na]$^+$ calcd for C$_{12}$H$_{14}$N$_4$O$_3$Na, 301.0730, found 301.0739

S-Phenyl 4-(2-azidoacetamido)butanethioate (5.5e). The compound was synthesized following the general procedure III from 4-(2-azidoacetamido)butanoic acid (5.4e) (0.20 g, 1.07 mmol) in 55% yield (0.16 g, 0.59 mmol). Pale yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.89 (quint, $J = 7.1$ Hz, 2H), 2.67 (t, $J = 7.2$ Hz, 2H), 3.27-3.33 (m, 2H), 3.89 (s, 2H), 6.58 (br s, 1H), 7.37 (s, 5H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 25.7, 34.0, 47.5, 51.1, 127.3, 129.5, 129.9, 134.7, 170.2, 197.9. HRMS (m/z): [M+Na]$^+$ calcd for C$_{12}$H$_{14}$N$_4$O$_3$Na, 301.0730, found 301.0736.

S-Phenyl 2-(4-azidobutanamido)ethanethioate (5.5f). The compound was synthesized following the general procedure III from 2-(4-azidobutanamido)acetic acid (5.4f) (1.50 g, 8.06 mmol) in 65% yield (1.46 g, 5.25 mmol). Pale yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.92 (q, $J = 6.8$ Hz, 2H), 2.33 (t, $J = 7.2$ Hz, 2H), 3.35 (t, $J = 6.5$ Hz, 2H), 4.24-4.27 (m, 2H), 6.43 (br s, 1H), 7.35-7.43 (m, 5H); $^{13}$C NMR (CDCl$_3$, 75
MHz): δ 24.8, 33.0, 49.1, 50.8, 127.3, 129.5, 130.0, 134.9, 172.4, 195.7. HRMS (m/z): [M+Na]^+ calcld for C_{12}H_{14}N_{4}O_{3}Na, 301.0730, found 301.0738.

**General Procedure for the Cyclization of Azido Dipeptidoyl Thioesters (5.5e-f) to Form Compounds 5.6a-f**

**General Procedure IVA for the Preparation of Compounds 5.6a-c.**

To a solution of azido dipeptidoyl thioesters 5.5a-c in dry THF (10 mL/1 mmol), PBu₃ (1.5 eq.) was added under argon atmosphere. The solution was subjected to microwave irradiation (50 °C, 50 W, 5 min). The reaction was allowed to cool to room temperature, and then diluted with CH₂Cl₂ (10 mL/1 mmol). Hexanes was added until the solution turned turbid and was then left to crystallize in the freezer. The solid obtained was filtered off, washed with diethyl ether (2 mL/1 mmol) and dried under high vacuum yielding pure products 5.6a-c.

1,4-Diazepane-2,5-dione (5.6a). The compound was synthesized following the general procedure IVA from (S)-phenyl 3-(2-azidoacetamido)propanethioate (5.5a) (200.00 mg, 0.76 mmol) in 73% yield (70.43 mg, 0.55 mmol). White microcrystals, mp 247-248 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 2.52-2.59 (m, 2H), 3.26-3.30 (m, 2H), 3.70 (d, J = 2.7 Hz, 2H), 7.73 (br s, 1H), 7.81 (br s, 1H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 35.5, 37.3, 45.5, 170.6, 172.4; HRMS (m/z): [M+Na]^+ calcld for C₅H₆N₂O₂Na, 151.0479, found 151.0482.

(S)-3-Benzyl-1,4-diazepane-2,5-dione (5.6b). The compound was synthesized following the general procedure IVA from (S)-phenyl 2-(3-azidopropanamido)-3-phenylpropanethioate (5.5b) (200.00 mg, 0.56 mmol) in 66% yield (80.75 mg, 0.37 mmol). White microcrystals, mp 222-223 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 2.31-2.39 (m, 1H), 2.58-2.66 (m, 1H), 2.77 (dd, J = 8.6, 4.7 Hz, 1H), 3.03-3.12 (m, 2H), 3.61-
3.67 (m, 1H), 4.52-4.58 (m, 1H), 7.19 (t, \( J = 4.2 \) Hz, 1H), 7.28 (t, \( J = 4.4 \) Hz, 2H), 7.35 (d, \( J = 4.5 \) Hz, 2H), 7.45 (br s, 1H), 7.84 (br s, 1H); \(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz): \( \delta \) 34.9, 35.8, 36.1, 52.9, 126.2, 128.1, 129.4, 138.3, 171.5, 172.0; Anal. Calcd for \( \text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2 \): C 66.04, H 6.47, N 12.83. Found: C 65.74, H 6.61, N 12.60. HRMS (\textit{m}/\textit{z}): [M+Na]\(^+\) calcd for \( \text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2\text{Na} \), 241.0947, found 241.0955.

3,3-Dimethyl-1,4-diazepane-2,5-dione (5.6c). The compound was synthesized following the general procedure IVA from (S)-phenyl 2-(3-azidopropanamido)-3-phenylpropanethioate (5.5c) (0.19 g, 0.68 mmol) in 75% yield (79.61 mg, 0.51 mmol). White microcrystals, mp 253-256 °C. \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): \( \delta \) 1.34 (s, 6H), 2.44-2.51 (m, 2H), 3.15-3.21 (m, 2H), 7.34 (s, 1H), 7.86 (br s, 1H); \(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz): \( \delta \) 29.3, 37.0, 37.4, 57.4, 173.7, 173.9; HRMS (\textit{m}/\textit{z}): [M+Na]\(^+\) calcd for \( \text{C}_7\text{H}_{12}\text{N}_2\text{O}_2\text{Na} \), 179.0791, found 179.0792.

**General Procedure IVB for the Preparation of Compounds 5.6d,e.**

To a solution of thioesters 5.5d-f in dry THF (100 mL/1 mmol), PBu\(_3\) (1.5 eq.) was added dropwise under argon atmosphere. The mixture was stirred for 24 h at room temperature. The solvent was evaporated under reduced pressure and the residue was taken up with ethyl acetate and washed with an aqueous solution of \( \text{Na}_2\text{CO}_3 \). The organic layer was dried with anhydrous MgSO\(_4\), filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate:hexanes (gradient) as eluent to yield pure 5.6d,e.

1,5-Diazocane-2,6-dione (5.6d). The compound was synthesized following the general procedure IVB from (S)-phenyl 4-(2-azidoacetamido)butanethioate (5.5e) (200.00 mg, 0.72 mmol) in 55% yield (56.83 mg, 0.40 mmol). White microcrystals, mp
295-300 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.57 (t, $J = 7$ Hz, 4H), 3.38 (q, $J = 4.3$ Hz, 4H), 7.54 (t, $J = 4.2$ Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 36.2, 37.4, 172.4; HRMS ($m/z$): [M+Na]$^+$ calcd for C$_6$H$_{10}$N$_2$O$_2$Na 165.0634, found 165.0641

1,4-Diazocane-2,5-dione (5.6e) The compound was synthesized following the general procedure IVB from (S)-phenyl 4-(2-azidoacetamido)butanethioate (5.5e) (200.00 mg, 0.72 mmol) in 57% yield (58.23 mg, 0.41 mmol) or S-phenyl 2-(4-azidobutanamido)ethanethioate (5.5f) (0.20 g, 0.72 mmol) in 51% yield (52.57 mg, 0.37 mmol). Colorless oil. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.97-2.04 (m, 2H), 2.58 (t, $J = 4.8$ Hz, 2H), 3.70 (t, $J = 4.4$ Hz, 2H), 4.11 (br s, 2H), 8.43 (br s, 2H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 17.1, 32.5, 42.9, 44.8, 167.1, 176.4; MS ($m/z$): [M+H]$^+$ calcd for C$_6$H$_{11}$N$_2$O$_2$ 143.16, found 143.0.

**En Route for the Preparation of Cyclic Tripeptide 5.1.2**

(S)-2-(3-Aminopropanamido)-3-phenylpropanoic acid hydrochloride (5.8) HCl gas was passed through a solution of Boc-$\beta$-Ala-L-Phe (5.7) (336.17 mg, 1.00 mmol) in dioxane (25 mL) for 1 h. The dioxane solution was concentrated under vacuum and ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry diethyl ether (5 mL), dried under high vacuum to give the corresponding $\beta$-Ala-L-Phe hydrochloride 5.8 in 88% yield (239.44 mg, 0.88 mmol). White sticky solid. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.41-2.60 (m, 2H), 2.81-3.00 (m, 3H), 3.06 (dd, $J = 8.6$, 5.3 Hz, 1H), 4.38-4.46 (m, 1H), 7.12-7.30 (m, 5H), 8.09 (br s, 3H), 8.57 (d, $J = 8.1$ Hz, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 32.5, 35.8, 37.3, 54.3, 127.1, 128.8, 129.7, 138.2, 170.0, 173.5.
(S)-2-(3-(2-Chloroacetamido)propanamido)-3-phenylpropanoic acid (5.9). The compound was prepared according to the general method I for the preparation of compounds 5.3a-f from chloroacetyl chloride (0.12 mL, 1.5 mmol) and (S)-2-(3-aminopropanamido)-3-phenylpropanoic acid hydrochloride (5.8) (272.09 mg, 1.00 mmol) in 81% yield (5.06 g, 0.81 mmol). White microcrystals, mp 188-189 °C. 

\[ \text{NMR (DMSO-}d_6, 300 \text{ MHz): } \delta 2.18-2.36 (m, 2H), 2.85 (dd, J = 13.7, 9.5 \text{ Hz, 1H}), 3.05 (dd, J = 14.0, 5.0 \text{ Hz, 1H}), 3.17-3.24 (m, 2H), 4.01 (s, 2H), 4.39-4.47 (m, 1H), 7.16-7.30 (m, 5H), 8.17 (t, J = 5.6 \text{ Hz, 1H}), 8.28 (d, J = 8.2 \text{ Hz, 1H}), 12.72 (br s, 1H); \]

\[ \text{C NMR (DMSO-}d_6, 75 \text{ MHz): } \delta 34.6, 35.6, 36.8, 42.6, 53.4, 126.4, 128.2, 129.1, 137.6, 165.8, 170.3, 173.1. \]

HRMS (m/z): [M-H]^- calcd for C_{14}H_{16}ClN_{2}O_{4}, 311.0804, found 311.0813.

(S)-2-(3-(2-Azidoacetamido)propanamido)-3-phenylpropanoic acid (5.10). The compound was prepared according to the general method II for the preparation of compounds 5.4a-f from (S)-2-(3-(2-chloroacetamido)propanamido)-3-phenylpropanoic acid (5.9) (624.18 mg, 2.00 mmol) in 61% yield (389.34 mg, 1.22 mmol). White microcrystals, mp 160-161 °C. 

\[ \text{NMR (DMSO-}d_6, 300 \text{ MHz): } \delta 2.18-2.34 (m, 2H), 2.84 (dd, J = 13.8, 9.6 \text{ Hz, 1H}), 3.04 (dd, J = 13.7, 5.0 \text{ Hz, 1H}), 3.16-3.23 (m, 2H), 3.75 (s, 2H), 4.37-4.46 (m, 1H), 7.17-7.30 (m, 5H), 8.07 (t, J = 5.6 \text{ Hz, 1H}), 8.26 (d, J = 8.2 \text{ Hz, 1H}), 12.7 (br s, 1H); \]

\[ \text{C NMR (DMSO-}d_6, 75 \text{ MHz): } \delta 34.8, 35.2, 36.7, 50.7, 53.4, 126.4, 128.1, 129.0, 137.7, 167.2, 170.2, 173.1. \]

HRMS (m/z): [M-H]^- calcd for C_{14}H_{16}N_{5}O_{4}, 318.1208, found 318.1223.

(S)-S-Phenyl 2-(3-(2-azidoacetamido)propanamido)-3-phenylpropanethioate (3.11). The compound was prepared according to the general method III for the preparation of compounds 5.5a-f from (S)-2-(3-(2-azidoacetamido)propanamido)-3-
phenylpropanoic acid (5.10) (500 mg, 1.57 mmol) in 51% yield (328.91 mg, 0.80 mmol). White microcrystals, mp 94-97 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.26-2.46 (m, 2H), 2.93 (dd, $J = 13.7, 10.4$ Hz, 1H), 3.15 (dd, $J = 13.8, 4.8$ Hz, 1H), 3.23-3.31 (m, 2H), 3.76 (s, 2H), 4.66-4.74 (m, 1H), 5.19-5.32 (m, 5H), 7.35-7.39 (m, 2H), 7.45-7.48 (m, 1H), 7.81 (t, $J = 5.6$ Hz, 1H), 8.84 (d, $J = 8.0$ Hz, 1H); $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 34.8, 35.1, 36.6, 50.7, 60.5, 126.6, 127.3, 128.3, 129.1, 129.3, 129.4, 134.5, 137.0, 167.3, 170.9, 198.5; Anal. Calcd for C$_{20}$H$_{21}$N$_5$O$_3$S: C, 58.38; H, 5.14; N, 17.02. Found: C, 58.50; H, 5.26; N, 16.94.

(S)-3-Benzyl-1,4,7-triazecane-2,5,8-trione (5.12) The compound was prepared according to the general method IVB for the preparation of compounds 5.6e-d from (S)-phenyl 2-(3-(2-azidoacetamido)propanamido)-3-phenylpropanethioate (5.11) (205.57 mg, 0.50 mmol) in 48% yield (66.03 mg, 0.24 mmol). Colorless oil. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.92 (dd, $J = 8.3, 6.2$ Hz, 1H), 3.13 (dd, $J = 8.4, 3.0$ Hz, 1H), 3.41 (q, $J = 7.4$ Hz, 2H), 4.15 (t, $J = 3.9$ Hz, 2H), 4.66-4.71 (m, 1H), 5.37 (dd, $J = 3.6, 1.2$ Hz, 2H), 7.34-7.37 (m, 2H), 7.42-7.48 (m, 2H), 7.49-7.52 (m, 1H), 8.13 (t, $J = 3.0$ Hz, 1H), 8.18 (d, $J = 2.1$ Hz, 1H), 8.82 (d, $J = 4.5$ Hz, 1H); ESI-MS $m/z$: 298 (M+Na)$^+$

Crystal Data for Compounds 5.6a and 5.6c†

Compounds 5.6a and 5.6c were recrystallized from DMSO/Acetone.

Compound 5.6a: Colorless (block), dimensions 0.55 × 0.45 × 0.41 mm, crystal system monoclinic, space group P2(1)/c, $Z = 4$, $a = 9.243(3)$, $b = 7.153(2)$, $c = 8.902(3)$ Å, $\beta = 91.489(4)^\circ$, $V = 588.3(3)$ Å$^3$, $\rho = 1.447$ g cm$^{-3}$, $T = 100(2)$ K, $\Theta_{\text{max}} = 31.42^\circ$.

† Crystal data were obtained by Professor Matthias Zeller at Department of Chemistry of Youngstown State University, Youngstown, USA
radiation Mo-Kα, λ = 0.71073 Å, 0.3 ω-scans with CCD area detector, covering a whole sphere in reciprocal space, 6608 reflections measured, 1871 unique (Rint = 0.0245), 1728 observed (I > 2σ(I)), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, μ = 0.113 mm⁻¹, Tmin = 0.6701, Tmax = 0.7462, structure solved by direct methods and refined against F with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 164 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 1.053 for observed reflections, final residual values R1(F) = 0.0346, wR2(F) = 0.0943 for observed reflections. CCDC 881662.

Compound 5.6c: colorless (block), dimensions 0.55 × 0.36 × 0.32 mm, crystal system triclinic, space group P1̅, Z = 2, a = 5.6500(16), b = 7.884(2), c = 9.777(3) Å, β = 90.330(4)°, V = 381.49(19) Å³, ρ = 1.360 g cm⁻³, T = 100(2) K, Θmax = 30.80°, radiation Mo-Kα, λ = 0.71073 Å, 0.3 ω-scans with CCD area detector, covering a whole sphere in reciprocal space, 7746 reflections measured, 2304 unique (Rint = 0.0160), 2184 observed (I > 2σ(I)), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, μ = 0.101 mm⁻¹, Tmin = 0.6852, Tmax = 0.7462, structure solved by direct methods and refined against F with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 164 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 1.039 for observed reflections, final residual values R1(F) = 0.0353, wR2(F) = 0.0954 for observed reflections. CCDC 881663.
Computational Details‡

Computational details for the cyclization of aza-ylides and cartesian coordinates of stationary points

All calculations were performed with the density functional theory (DFT) using the B3LYP functional with the GAUSSIAN 03 series of programs,\textsuperscript{98} using the Pople 6-31+G** polarized basis set. The analytical second derivatives are used to determine the nature of the stationary points and a full thermochemical analysis was performed (Table S1). Computations were performed on model compounds (PH3 as model phosphine)

Table 5-2. Activation parameters

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<th>Sequences</th>
<th>DH‡ (kcal)</th>
<th>DG‡ (kcal)</th>
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<th>DH‡ (kcal)</th>
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‡ Computational studies were performed Dr. Girinath G. Pillai at the Department of Chemistry of University of Tartu, Tartu, Estonia and Dr. Jean-Christophe M. Monbaliu at the Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
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Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-Gly-Gly-SPh (6-membered ring)

Figure 5-9. TS in the cyclization of the aza-ylide thioester corresponding to azido-Gly-Gly-SPh
Table 5-4. Cartesian coordinates for the TS in the cyclization of the aza-ylide thioester derived from azido-Gly-Gly-SPh

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E(B3LYP/6-31+G**)= -1388.34371254 Hartree

Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-Gly-bAla-SPh (7-membered ring)
Table 5-5. Cartesian coordinates for the TS in the cyclization of the aza-ylide thioester derived from azido-Gly-β-Ala-SPh 5.5.a

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\[ E(\text{B3LYP/6-31+G**}) = -1427.65765139 \text{ Hartree} \]
Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-β-Ala-Gly-SPh (7-membered ring)

Figure 5-11. TS in the cyclization of the aza-ylide thioester corresponding to azido-β-Ala-Gly-SPh
Table 5-6. Cartesian coordinates for the TS in the cyclization of the aza-ylide thioester derived from azido-Gly-β-Ala-SPh 5.5C (7-membered ring)

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E(B3LYP/6-31+G**) = -1427.65788122 Hartree

Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-β-Ala-Aib-SPh 5.5C (7-membered ring)
Table 5-7. Cartesian coordinates for the TS in the cyclization of the aza-ylide thioester derived from azido-β-Ala-Aib-SPh 5.5c

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E(B3LYP/6-31+G**) = -1506.28401397 Hartree
Figure 5-12. TS in the cyclization of the aza-ylide thioester corresponding to azido-β-Ala-Alb-SPh 5.5c

Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-β-Ala-Phe-SPh 5.5b (7-membered ring)

Figure 5-13. TS in the cyclization of the aza-ylide thioester corresponding to azido-β-Ala-Phe-SPh 5.5b
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\[ E(\text{B3LYP/6-31+G**}) = -1698.03267142 \text{ Hartree} \]
Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-β-Ala-β-Ala-SPh 5.5d (8-membered ring)

Figure 5-14. TS in the cyclization of the aza-ylide thioester corresponding to azido-β-Ala-β-Ala-SPh 5.5d

Table 5-9. Cartesian coordinates for the TS in the cyclization of the aza-ylide thioester derived from azido-β-Ala-β-Ala-SPh 5.5d

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\[ E(\text{B3LYP/6-31+G**}) = -1466.96681296 \text{ Hartree} \]

Cartesian coordinates for the TS associated with the cyclization of the aza-ylide thioester derived from azido-Gly-GABA-SPh 5.5e (8-membered ring)

Figure 5-15. TS in the cyclization of the aza-ylide thioester corresponding to azido-Gly-GABA-SPh 5.5e
Table 5-10. Cartesian coordinates for the TS in the cyclization of the aza-ylide thioester derived from azido-Gly-GABA-SPh 5.5e

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<tr>
<td>H</td>
<td>0.751145</td>
<td>3.410023</td>
</tr>
<tr>
<td>H</td>
<td>2.374274</td>
<td>2.167468</td>
</tr>
<tr>
<td>H</td>
<td>1.912785</td>
<td>-0.192633</td>
</tr>
<tr>
<td>H</td>
<td>-0.193068</td>
<td>-1.286117</td>
</tr>
<tr>
<td>H</td>
<td>-1.819160</td>
<td>-0.038767</td>
</tr>
</tbody>
</table>

E(B3LYP/6-31+G**) = -1466.96642431 Hartree
Conformational analysis of compounds 5.6a, 5.6c, 5.6d and 5.6e

A series of conformers were generated using Marvin Suite99 (ChemAxon Kft, Hungary) and each conformer was optimized using HyperChem.100 For both 5.6a and 5.6c, the most stable conformer was envelop-like. A similarity analysis between the predicted conformers for 5.6a and 5.6c and the Xray results show in each case high levels of overlay similarity (Table 5-11).

Table 5-11. Similarity analysis between ball and stick model of both Xray crystal structure (yellow) and theoretical conformer (green)

<table>
<thead>
<tr>
<th>Entry</th>
<th>3D Superimpose</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6a</td>
<td>93.7</td>
<td></td>
</tr>
<tr>
<td>5.6c</td>
<td>98.5</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 6
SYNTHESIS OF CYCLIC PEPTIDES BY CYCLO-OLIGOMERIZATION OF DIPEPTIDOYL BENZOTRIAZOLIDES *

Introduction

Among the various methods for the synthesis of cyclic peptides, a common approach is the head-to-tail ring-closing of the linear precursor.5 Macrocyclizations are best performed on an insoluble polymer using peptide coupling reagents.101 A protecting-group strategy of at least three dimensions of orthogonality is required to construct the linear peptide, deprotect the N- and C-termini, cyclize in a head-to-tail fashion, and finally cleave the product from the solid support.5,102 However, the reaction set-ups are often rather sophisticated, insertion of the solid-support into the peptide backbone requires more extended synthetic procedure and low yields and partial epimerizations are observed in many cases. In addition, the direct head-to-tail macro-lactamization for small and medium ring-sized cyclic peptides failed even when using the most powerful coupling reagents at high dilution.26,103,20 Among contemporary strategies for construction of peptide macro-cycles, cyclooligomerization has proved to be a powerful method to get fast access to different peptide macro-cycles.104,105,106

Cyclooligomerization is a valuable entry to large macrocycles and has been used to provide libraries of metal-binding macrocyclic ligands that are likely to reveal novel ion-binding and transport interactions and can serve as scaffolds for supramolecular and combinatorial chemistry.107,108,109,110,111 In most cases, cyclic dimers, trimers and tetramers are the major products and the ratio of dimer-, trimer-, tetramer-, and larger

macrocycles obtained in cyclooligomerization process is kinetically controlled and dependent on the relative stereochemistry of the backbone α-carbons (Fig.1).\textsuperscript{3,4,113,105,107} Peptides containing β-turn inducing constraints like oxazolines, oxazoles, thiazolines, thiazoles and imidazoles, are ideal precursors for cyclooligomerization reaction\textsuperscript{109,114} for formation of peptide macrocycles because the imidate linkage fused into their five membered rings has a rigid \textit{transoid} conformation leading to an extended structure with the mutually reactive terminal groups far apart (Figure 6-1).\textsuperscript{105,109}

![Diagram](image)

**Figure 6-1.** Cyclooligomerization method to give fast access to different peptide macrocycles.

![Diagram](image)

**Figure 6-2.** Cyclooligomerization method to give fast access to different peptide macrocycles.
This chapter describes the development of innovative and efficient cyclization procedures for construction of peptide macrocycles.\textsuperscript{115,116,117} A versatile strategy for the synthesis of different small and medium ring-sized cyclo-peptide from dipeptidoyl benzotriazolides by a palladium promoted tandem deprotection/cyclization method is reported. Open chain \textit{N}-Cbz-dipeptidoyl benzotriazole sequences containing a $\beta$-turn-inducing constraint (Figure 6-2) were converted into either the corresponding homodiketopiperrazines in a intramolecular cyclization pathway or a library of macrocyclic scaffolds \textit{via} cyclooligomerization.

\textbf{Results and Discussion}

\textbf{Synthesis of Benzotriazolide Cyclization Substrate}

The first objective was to obtain the Cbz-N-dipeptidoyl benzotriazolide precursors for the cyclization study. As outlined in Scheme 1, Cbz-N-dipeptidoyl benzotriazolides 6.5a-g were synthesized in a 3-step procedure starting from commercially available $\beta$-amino acids 6.1a-c. Cbz-N-protected $\beta$-amino acid 6.1a-c was first converted into the benzotriazolides 6.2a-c; reaction with turn-introducers 6.3a-c gave dipeptides 6.4a-g, which were converted into Cbz-dipeptidoyl benzotriazolides 6.5a-g (Figure 6-3).\textsuperscript{118}

\begin{center}
\includegraphics[width=\textwidth]{figure6_3.png}
\end{center}

Figure 6-3. Synthesis of N-protected dipeptidoyl benzotriazolides.
Cyclization of Dipeptidoyl Benzotriazolides

Preparative conditions for intramolecular cyclization were first investigated using compound 6.5a Cbz-β-Ala-D-Pro-Bt. To avoid intermolecular oligomerization, a dilute solution of 6.5a was added dropwise to a suspension of Pd/C in dry methanol. On stirring at a concentration of 0.1 mM under hydrogen in the presence of Pd/C (10 wt%) at 20 °C, Cbz-β-Ala-D-Pro-Bt 6.5a was cyclized into homo-diketopiperazine 6.6a (Figure 6-4, Table 6-1). HPLC–MS analysis confirmed the major product is the 7-membered cyclic dipeptide cyclo-(β-Ala-D-Pro-) 6.6a. Similarly, cyclo-(β-Homo-Ala-L-Pro-) 6.6b was synthesized in 68% yield by deprotection/cyclization of Cbz-β-Homo-Ala-L-Pro Bt 6.5b (Figure 6-4A, Table 6-1).

Figure 6-4. Intramolecular cyclization of N-protected dipeptidoyl benzotriazolides: a) using Pro as turn-introducer; b) using Hyp as turn introducer.

Table 6-1. Benzotriazole auxiliary route to homo-diketopiperazines

<table>
<thead>
<tr>
<th>Entry</th>
<th>n</th>
<th>R¹</th>
<th>R²</th>
<th>Producta</th>
<th>HMRS [M+H]+</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>calc.</td>
<td>found</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>cyclo[β-Ala-D-Pro-], 6.6a</td>
<td>169.0972</td>
<td>169.0985</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Me</td>
<td>H</td>
<td>cyclo[β-Homo-Ala-L-Pro-], 6.6b</td>
<td>183.1128</td>
<td>183.114</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>H</td>
<td>OH</td>
<td>nd</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

[a] The purity was determined by HPLC-MS of the crude product.
Next, we studied *cis*-L-4-hydroxyproline as a turn-inducing unit in cyclization of Cbz-β-Ala-L-Hyp(Bn)-Bt 6.5d; however, ring-closure to give 7-membered ring cyclic dipeptide *cyclo*(β-Ala-L-Hyp-) 6d by head-to-tail lactamization of 6.5d (Figure 6-4B) is difficult and no cyclization was accomplished. The main reason for the cyclization failure may the predominant *trans* arrangement of the amide bond in the linear hydroxyproline precursor, preventing a correct spatial positioning of the terminal amine and the activated carboxylic group. Due to a strong noncovalent interaction between adjacent amide bonds through n→π* interaction in the C4-endopuckered of C4-hydroxyproline, C4-OH substituted proline preferes endo-ring pucker of with *trans* amide bonds which prevent the cyclization ring contraction event to form the 7-membered ring cyclic dipeptide *cyclo*(β-Ala-L-Hyp-) 6.6d (Figure 6-5). In addition, an endo-ring pucker comformation of *cis* L-Hyp is more favored by an attractive H-bonding between the hydroxyl substituent at C4 and the carbonyl group of the benzotriazole moiety (Figure 6-5B).

![Figure 6-5. A) cis/trans conformers of proline containing amide bonds; B) C4-endo conformations of L-Hyp-Bt with H-bond donor stabilization at C4.](image)

Unexpectedly, treatment of Cbz-β-Ala-3-Aze-Bt 6.5e under similar reaction conditions to formation of cyclo-dimerization product *cyclo*(β-Ala-3-Aze)₂ 6.7g as a
result of LCMS and MS-MS analysis (Figure 6-6). Probably, this is due to the unfavored eight-membered ring as a result of intramolecular reaction pathway since formation of the 16-membered cyclic tetrapeptide dimer would avoid the ring-strain of the eight-membered cyclo-dipeptide.\textsuperscript{89,122}

![Chemical structure](image)

**Figure 6-6. Intermolecular cyclodimerization of Cbz-β-Ala-3-Aze-Bt 6.5e.**

Next we studied the cyclo-oligomerization of N-Cbz protected dipeptidoyl benzotriazolides 6.5a-c,h to access different peptide macrocycles. Since the cyclooligomerization cascade is kinetically controlled,\textsuperscript{105,109} we postulate that at higher concentration of the substrate, the preferred ring size of the macrocycles is determined by the relative configurations of the cyclization precursors 6.5a-c,h and the β-turn introducer. Increased substrate concentration\textsuperscript{113} with higher Pd/C load should allow for the spontaneous formation of larger macrocycles. During formation of intramolecular 7-membered rings, the ground-state E geometry of the peptide bond prevents the peptides from attaining the ring-like conformation conducive to cyclization. However, larger ring sized products of cyclo-oligomerization do not pose this problem because they accommodate E peptide bonds more easily. To test the above hypothesis, we prepared a 15 mM solution of Cbz-β-Ala-D-Pro-Bt 6.5a and examined its reaction with Pd/C in the present of hydrogen gas. The reaction was monitored by TLC and analyzed
and analyzed by HPLC and MS. After stirring at rt for 36 h, the reaction products were isolated and characterized. HPLC revealed that the one-pot cyclooligomerization of the benzotriazolide Cbz-β-Ala-D-Pro-Bt 6.5a produced a novel series of constrained macrocycles cyclo-[β-Ala-D-Pro-]$_n$ (n=2-6) containing up to 12 amino acids with 42 atoms in the cycle. Molecular weights were confirmed by the precise match of peak shapes to calculated isotopic distribution patterns and by their HR-ESI mass spectra. Thus, incremental differences in mass units of cyclo-[β-Ala-D-Pro-] and unique isotopic patterns characterized each macrocycle (Figure 6-7).

![Figure 6-7. Structures of compounds cyclooligomerization products of 6.5a](image)

We identified 3 major products from this reaction, including the cyclic dimer 6.7a (72%), cyclic trimer 6.8a (15%), and cyclic tetramer 6.9a (10%) with formula cyclo-[β-Ala-D-Pro-]$_n$ in which n=2-4 (Table 6-2, Scheme 6-4). No direct intra-molecular cyclization product of 6.5a cyclo-[β-Ala-D-Pro] was observed. All of the products were confirmed by LC-MS and HMRS analysis. Cyclic dimer 6.7a was purified by gradient
chromatography (MeOH/ether) and obtained in 68% isolated yield and characterized characterized fully by $^1$H and $^{13}$C NMR spectroscopy and HRMS (ESI).

![Chemical structure](image)

**Figure 6-8.** Intermolecular cyclo-oligomerization of N-protected dipeptidoyl benzotriazolides

**Dependence of Cyclo-Oligomerization on Substrate Concentration**

We also probed the influence of substrate concentration on the Pd promoted macrocyclization reaction of Cbz-β-Ala-D-Pro-Bt 6.5a and the product distribution. It was expected that substrate concentration would have a major influence on the ratios of intermolecular and intramolecular reactions. For these studies, we used higher load of Pd/C in order to shorten the reaction time and facilitate the monitoring. As shown in Table 6-2, with higher substrate concentration, the yields of cyclic dimer 6.7a increased. The formation of cyclic oligomeric peptides 6.7a, 6.8a and 6.9a in the product distribution suggests again that the relative ring size of the peptidoyl macrocycles is determined by kinetic control effected by the relative concentration of Cbz-β-Ala-D-Pro-Bt 6.5a. Another notable trend revealed in Table 6-2 is that the reaction gave a higher percentage of dimeric cyclo-tetrapeptide 6.7a (β-Ala-L-Pro)$_2$ to total cyclo-oligomeric products with the increased concentration of 6.5a. This trend was expected because increased substrate concentrations should favor intermolecular reactions significantly.
The main factor contributing to the formation of cyclo-tetrapeptide 6.7a may be the relative energies of transition state during the ring construction event of cyclic dimer 6.7a. Figure 6-7 implies that Cbz-deprotection of Cbz-1-N-benzotriazolyl dipeptide 6.5a forms N-unprotected intermediate β-Ala-D-Pro-Bt which could coordinate to the Pd could form a pre-organized tetrapeptide complex. The formation of the Pd complex in turn could lower the activation energy in the formation of the intermolecular dimerization/cyclization product 6.7a.\textsuperscript{18,123}

Table 6-2. Product distributions of Pd-promoted tandem deprotection/cyclization reactions of various concentrations of Cbz-β-Ala-L-Pro-Bt 6.5a

<table>
<thead>
<tr>
<th>Concentration of 6.5a (mM)</th>
<th>Intramolecular cyclization product 6.6a\textsuperscript{b} (%)</th>
<th>Cyclic-dimer 6.7a (%)</th>
<th>Cyclic-trimer 6.8a (%)</th>
<th>Cyclic-trimer 6.9a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1\textsuperscript{a}</td>
<td>99.9</td>
<td>0.1</td>
<td>ND\textsuperscript{c}</td>
<td>ND</td>
</tr>
<tr>
<td>0.2\textsuperscript{a}</td>
<td>91\textsuperscript{d}</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>ND</td>
<td>72</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>82</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>ND</td>
<td>55</td>
<td>5</td>
<td>ND\textsuperscript{e}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction were performed under drop-wised condition over 24 h with 16-36 h additional stirring \textsuperscript{b} Yields derived from HPLC analysis of crude products; \textsuperscript{c} Not detected; \textsuperscript{d} 6% is hydrolyzed side product; \textsuperscript{e} many linear oligomers was detected

To prove the generality of our approach, we studied the cyclo-oligomerization of 6.5b-d, 6.5g,h which consist of different β-amino acids and turn-inducing constraint units (Table 3). Our one-pot tandem oligomerization/cyclization procedure was employed to perform cyclization of N-Cbz protected dipeptidoyl benzotriazolide 6.5b (Figure 6-7, Table 6-3). The reaction was carried out under optimized conditions using 0.15 mM of Cbz-β-Homo-D,L-Ala-L-Pro-Bt. After reaction at room temperature for 204 h in the presence of Pd/C and H\textsubscript{2} gas, the anticipated dimeric cyclic tetrapeptide 6.7b was detected in 78% crude yield, along with a small amount of the cyclic trimer 6.8b and
tetramer 6.9b, in 8% and 2% yields, respectively (Table 3, entry 1). Cyclo-tetrapeptide 6.7b was later obtained in 69% yield after purification. The cyclo-oligomerization of 6.5c Cbz-β-D,L-Phe-L-Pro-Bt offered the cyclo-dimerization product as the major product (88%), and the trimeric cyclic product was also observed in a small quantity (<3%) (Table 6-3, entry 2). However, the reaction of peptide Cbz-β-Ala-L-Hyp(Bzl)-Bt gave only methyl ester and hydrolyzed side products with little dimerization or trimerization (Table 6-3, entry 3).

**The Turn-Introducer Effect on Cyclooligomerization of Dipeptidoyl Benzotriazols**

To determine the turn-introducer effect required for a dipeptidoyl benzotriazole to prefer cyclooligomerization vs direct head-to-tail cyclization, we then prepared benzotriazolides 6.5f,g containing a 4 membered ring L-2-Aze and 6 membered ring L-Homo-Pro. Subsequently, 0.12-0.15 mM peptide solutions were treated with catalytic amounts of Pd/C under hydrogen gas, and the reactions were monitored by HPLC and HR-ESI MS.

Interestingly, HPLC-MS analysis showed that Cbz-β-Ala-L-Aze-Bt 6f formed only trimeric cyclic product as the major product (90%) (Table 6-3, entry 4) and no dimerization, tetramerization or higher oligomerization products were detected. The change of ring size from 5-membered ring in proline to 4-membered ring in azetidine results in remarkable changes in ring structures. The bond angle of C’-N-Cα for the Aze peptide increased by 2-9° from those of the Pro containing peptide,124 which could result in more favorable formation of lager ring sized trimeric cyclic product vs the dimer product in cyclooligomerization reaction of Cbz-β-Ala-L-Aze-Bt 6.5f. No cyclooligomerization products were observered, when 0.12-0.15 mM peptide solutions of 6.5g were treated with similar conditions.
Table 6-3. Product distributions of Pd-promoted tandem deprotection/cyclization reactions of Cbz-protected dipeptidoyl benzotriazolides 6.5b-d, 6.5g,h

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cyclization substrates</th>
<th>Cyclic-dimer 6.7 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cyclic-trimer 6.8 (%)</th>
<th>Cyclic-tetramer 6.9 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cbz&lt;sub&gt;6.5b&lt;/sub&gt;</td>
<td>78</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Cbz&lt;sub&gt;6.5c&lt;/sub&gt;</td>
<td>88</td>
<td>trace</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Cbz&lt;sub&gt;6.5d&lt;/sub&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>Cbz&lt;sub&gt;6.5f&lt;/sub&gt;</td>
<td>ND</td>
<td>90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Cbz&lt;sub&gt;6.5g&lt;/sub&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratios derived from HPLC analysis of crude products; <sup>b</sup> Only trace amount was detected, mainly methyl ester and hydrolyzed side product were detected; <sup>c</sup> Only cyclic dimer was found

**Conclusion**

We have demonstrated that open chain N-Cbz-dipeptidoyl benzotriazole sequences containing a β-amino acid and a β-turn-inducing constraint were selectively converted into either the corresponding homo-diketopiperazines in an intramolecular cyclization pathway or macrocyclic scaffolds via cyclooligomerization. The relative ring size of the macrocycles is under kinetic control determined by the concentration of the substrate and the relative configurations of turn-introducers. At lower concentrations, β-amino acid containing N-Cbz-dipeptidoyl benzotriazoles formed 7-membered ring under
treatment with Pd/C in the present of hydrogen gas. As concentration of the substrate increases, kinetic preference shifts to the formation of larger 14-, 21-, and 28-membered ring systems to avoid the ring-strain of small peptide cycles. Dipeptidoyl benzotriazolides containing proline as turn-introducer favor formation of cyclic dimer. In contrast, the use of azetidine building block allows the formation of even larger trimeric peptide macrocycles. The approach described here provides a convenient access to generate combinatorial petide macrocycle libraries with conceivable uses as new antibiotics, new metal sequestrants, or as scaffolds for developing macromolecular devices and protein mimetics.

**Experimental**

**General Methods**

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$, DMSO-$d_6$, acetone-$d_6$, or CD$_3$OD using a 300 or 500 MHz spectrometer (with TMS as an internal standard). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublets, ddd = doublet of doublets of doublets, and dt = doublet of triplets. HPLC–MS analyses were performed on a reverse phase gradient using 0.2% acetic acid in H$_2$O/methanol as mobile phases; wavelength = 254 nm; mass spectrometry was done with electrospray ionization (ESI), matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) or atmospheric-pressure chemical ionization (APCI). Ether refers to diethyl ether.
General Procedure for the Preparation of Benzotriazolides 6.2a-c

A stirred solution of 1H-benzotriazole (BtH) (4 equiv.) in dry tetrahydrofuran (THF) (10 mL/1 g) was treated at 20 °C with thionyl chloride (SOCl$_2$) (1 equiv.). After 20 minutes, a solution of (Cbz-protected amino acid-OH) 1 equiv.) in dry THF (10 mL/1 g) was added drop–wise and the resulting solution was then stirred for 2 h at 20 °C. Upon completion, the mixture was filtered, and THF was removed under reduced pressure. The residue was dissolved by dichloromethane (CH$_2$Cl$_2$ 50 mL/1 g of 1a-b) and washed successively with HCl (4N, 2 × 1 mL/1 mL CH$_2$Cl$_2$), aq. Na$_2$CO$_3$ (10%, 2 × 1 mL/1 mL CH$_2$Cl$_2$) and brine (1 mL/1 mL). The organic layer was dried over magnesium sulfate (MgSO$_4$), filtered and evaporated to give the crude product. The solid was recrystallized from CH$_2$Cl$_2$/hexanes to yield benzotriazolides 6.2a-c.

(Cbz-β-Ala-Bt) 6.2a. White solid, 4.61 g, 5.10 mmol, 82% yield; mp 111.0–112.0 °C. $^1$H NMR (CDCl$_3$, 300 MHz): δ 3.60–3.70 (m, 2H), 3.75 (t, $J$ = 6.0 Hz, 2H), 5.07 (br s, 2H), 5.36 (br s, 1H), 7.24–7.32 (m, 5H), 7.50 (dt, $J$ = 7.2, 1.0 Hz, 1H), 7.64 (dt, $J$ = 7.2, 1.0 Hz, 1H), 8.10 (dd, $J$ = 8.2, 0.9 Hz, 1H), 8.23 (dd, $J$ = 8.2, 0.9 Hz, 1H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 36.2, 36.4, 67.1, 114.5, 120.5, 126.5, 128.4, 128.7, 130.8, 131.1, 136.5, 146.3, 156.4, 171.5. Anal. Calcd for C$_{17}$H$_{16}$N$_4$O$_3$: C 62.95, H 4.97, N 17.27. Found: C 63.01, H 4.94, N 17.61.

(Cbz-D,L-β-Phe-Bt) 6.2b. White solid, 1.10 g, 2.75 mmol, 82% yield; mp 166.8–167.5 °C. $^1$H NMR (CDCl$_3$, 300 MHz): δ 3.92 (dd, $J$ = 16.7, 5.6 Hz, 1H), 4.02–4.18 (m, 1H), 5.02–5.51 (m, 2H), 5.59 (dd, $J$ = 13.5, 6.6 Hz, 1H), 7.21–7.45 (m, 10H), 7.46–7.55 (m, 1H), 7.59–7.67 (m, 1H), 8.08–8.14 (m, 1H), 8.18–8.25 (m, 1H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 41.7, 51.8, 67.0, 114.4, 120.2, 126.4, 128.0, 128.1, 128.5, 128.9, 130.6, 146.2, 155.6, 169.6.
(Cbz-H-β-Ala-Bt) 6.2c. White Solid, 1.25 g, 3.70 mmol, 88% yield; mp 99.8–100.9 °C. \(^1\)H NMR (CDCl\(_3\), 300 MHz): δ 1.41 (d, \(J = 6.4\) Hz, 3H), 3.58 (dd, \(J = 16.4, 5.9\) Hz, 1H), 3.70 (dd, \(J = 12.8, 6.0\) Hz, 1H), 4.40–4.55 (m, 1H), 4.97 (br s, 2H), 5.05 (br s, 2H), 5.20–5.42 (m, 1H), 7.20–7.42 (m, 5H), 7.47–7.54 (m, 1H), 7.62–7.68 (m, 1H), 8.09–8.13 (m, 1H), 8.24–8.28 (m, 1H). \(^13\)C NMR (CDCl\(_3\), 75 MHz): δ 21.0, 42.0, 44.4, 66.9, 114.6, 120.4, 126.4, 128.3, 128.5, 128.6, 128.8, 130.7, 136.3, 146.3, 155.7, 170.4. Anal. Calcd for C\(_{18}\)H\(_{18}\)N\(_4\)O\(_3\): C 63.89, H 5.36, N 16.56. Found: C 64.11, H 5.49, N 16.70.

**General Procedure for the Preparation of Dipeptides 6.4a-g**

Benzotriazolides 6.2a-c (1 equiv.) were each suspended in acetonitrile/water (3:1) (25 mL/1 g) and a solution of amino acid (1 equiv.) in water (5 mL/1 g of amino acid) containing triethylamine (1.0–1.1 equiv.) was added slowly. The mixtures were stirred at 20 °C for 16–24 h until TLC revealed consumption of the starting materials. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed with 4N HCl (3 × 1.5 mL/1 mL of ethyl acetate) and brine (1 mL/1 mL of ethyl acetate). Recrystallization from ethyl acetate/hexanes yielded dipeptides 6.4a-g.

Cbz-β-Ala-L-Pro-OH (6.4a). \(^1\)H NMR (CDCl\(_3\), 300 MHz, Methanol-\(d_4\)) δ 7.30 (dd, \(J = 11.1, 3.6\) Hz, 5H), 5.30–4.92 (m, 2H), 4.44–4.36 (m, 1H), 3.63–3.45 (m, 2H), 3.40 (t, \(J = 6.8\) Hz, 2H), 2.56 (t, \(J = 6.7\) Hz, 2H), 2.17 (qd, \(J = 9.7, 8.1, 3.6\) Hz, 1H), 2.03–1.80 (m, 3H). \(^13\)C NMR (CDCl\(_3\), 75 MHz): δ 172.8, 172.7, 171.9, 171.4, 60.8, 60.7, 58.6, 47.6, 45.1, 44.7, 42.8, 29.4, 29.2, 24.7, 24.4, 23.6, 21.0.

Cbz-β-Homo-Ala-D-Pro -OH (6.4b). \(^1\)H NMR (300 MHz, Methanol-\(d_4\)) δ 7.50–7.19 (m, 5H), 5.04 (d, \(J = 8.0\) Hz, 2H), 4.49–4.22 (m, 1H), 4.05 (dt, \(J = 13.6, 5.8\) Hz, 1H), 3.80–3.65 (m, 2H), 2.66 (d, \(J = 17.1\) Hz, 1H), 2.43 (p, \(J = 7.8\) Hz, 1H), 2.21 (dt, \(J = 7.0, 3.6\) Hz, 2H).
= 15.8, 7.4 Hz, 1H), 2.05 – 1.91 (m, 3H), 1.25 – 1.12 (m, 3H). $^{13}$C NMR (75 MHz, cd3od) δ 175.7, 172.0, 157.9, 138.5, 129.6, 68.9, 67.4, 60.2, 47.6, 47.5, 45.9, 45.5, 42.2, 41.9, 32.2, 32.2, 30.4, 30.4, 26.6, 25.8, 25.7, 23.6, 20.9.

Cbz-β-Phe-L-Pro-OH (6.4c). $^1$H NMR (500 MHz, Methanol-d$_4$) δ 7.40 – 7.17 (m, 10H), 5.22 – 5.08 (m, 1H), 5.08 – 4.97 (m, 2H), 4.34 (ddd, $J$ = 26.8, 8.7, 3.4 Hz, 1H), 3.60 – 3.40 (m, 2H), 2.99 – 2.52 (m, 2H), 2.19 – 2.03 (m, 1H), 1.97 – 1.69 (m, 3H). $^{13}$C NMR (75 MHz, DMSO) δ 172.8, 167.5, 154.7, 142.8, 136.6, 127.8, 127.3, 126.3, 126.0, 125.8, 64.9, 57.9, 50.8, 46.1, 28.4, 26.2, 23.9.

Cbz-β-Ala-L-Hyp(O-Bn)-OH (6.4d). $^1$H NMR (299 MHz, Methanol-d$_4$) δ 7.40 – 7.23 (m, 10H), 5.06 (d, $J$ = 3.1 Hz, 2H), 4.61 – 4.49 (m, 2H), 4.45 (t, $J$ = 8.0 Hz, 1H), 4.26 (d, $J$ = 9.4 Hz, 1H), 3.77 – 3.62 (m, 1H), 3.42 – 3.34 (m, 3H), 2.52 (t, $J$ = 6.7 Hz, 2H), 2.52 – 2.32 (m, 1H), 2.07 (ddd, $J$ = 13.0, 8.0, 4.9 Hz, 1H). $^{13}$C NMR (75 MHz, Methanol-d$_4$) δ 175.7, 172.7, 158.7, 139.5, 138.4, 129.6, 129.1, 129.0, 127.3, 78.5, 72.2, 67.5, 59.1, 53.8, 37.9, 36.2, 35.5.

Cbz-β-Ala-3-Aze-OH (6.4e). $^1$H NMR (300 MHz, Methanol-d$_4$) δ 7.41 – 7.26 (m, 5H), 5.07 (s, 2H), 4.35 – 4.24 (m, 1H), 4.19 – 4.02 (m, 1H), 3.36 (td, $J$ = 6.8, 4.3 Hz, 2H), 3.07 (dt, $J$ = 3.1, 1.5 Hz, 1H), 2.50 (t, $J$ = 6.8 Hz, 2H), 2.34 (t, $J$ = 6.7 Hz, 2H). $^{13}$C NMR (75 MHz, Methanol-d$_4$) δ 175.57, 173.56, 158.75, 138.44, 129.59, 129.12, 67.55, 53.95, 51.82, 37.83, 32.95, 32.38.

Cbz-β-Ala-L-2-Aze-OH (6.4f). $^1$H NMR (300 MHz, Methanol-d$_4$) δ 7.45 – 7.23 (m, 5H), 5.07 (s, 2H), 4.69 (dd, $J$ = 9.6, 5.4 Hz, 1H), 4.24 – 4.02 (m, 1H), 3.93 (q, $J$ = 6.9, 6.3 Hz, 1H), 3.38 (t, $J$ = 5.0 Hz, 2H), 2.76 – 2.53 (m, 1H), 2.45 – 2.26 (m, 2H), 2.28 –
2.12 (m, 1H). $^{13}$C NMR (75 MHz, Methanol-$d_4$) δ 174.1, 173.7, 158.8, 138.5, 129.6, 129.0, 127.2, 67.5, 62.8, 60.8, 37.8, 32.4, 21.1.

Cbz-β-Ala-D,L-Homo-Pro-OH (6.4g). $^1$H NMR (300 MHz, Methanol-$d_4$) δ 7.34 (d, $J = 4.5$ Hz, 5H), 5.07 (s, 2H), 4.12 – 3.76 (m, 1H), 3.46 – 3.34 (m, 2H), 2.76 – 2.57 (m, 1H), 2.52 (t, $J = 6.7$ Hz, 1H), 2.25 (d, $J = 13.4$ Hz, 1H), 1.53 – 1.22 (m, 4H), 0.94 (dd, $J = 9.7$, 6.5 Hz, 3H). $^{13}$C NMR (75 MHz, CD$_3$OD) δ 174.4, 173.6, 158.8, 138.4, 129.6, 129.1, 128.9, 67.5, 53.4, 44.8, 34.5, 27.9, 26.4, 22.0, 19.5.

**General Procedure for the Preparation of Dipeptidoyl Bentrotriazolides 6.5a-g**

A stirred solution of BtH (4 equiv.) in dry tetrahydrofuran (THF) (15 mL/1 g) was treated at -20 °C with SOCl$_2$ (1 equiv.). After 20 minutes, the solution of BtH and SOCl$_2$ was cooled down on ice and salt (NaCl) for 5mins and a solution of 6.4a-g (1 equiv.) in dry THF (20 mL/1 g) was added drop-wise and the resulting solutions were then stirred for 4 h at -20 °C. THF was removed under reduced pressure and the residue was dissolved ethyl acetate (85 mL/1 g) and washed successively with Na$_2$CO$_3$ 10 wt.-% in water ($3 \times 1$ mL/1 mL of ethyl acetate), HCl (4N, $1 \times 1$ mL/1 mL of CH$_2$Cl$_2$), and brine ($1 \times 30$ mL). The organic layer was dried over magnesium sulfate, filtered and evaporated. The crude product was then recrystallized from CH$_2$Cl$_2$/hexanes to yield dipeptidoyl bentrotriazolides 6.5a-g.

Cbz-β-Ala-L-Pro-Bt (6.5a). $^1$H NMR (300 MHz, Chloroform-$d$) δ 8.25 (d, $J = 8.2$ Hz, 1H), 8.12 (d, $J = 8.3$ Hz, 1H), 7.65 (t, $J = 7.6$ Hz, 1H), 7.51 (t, $J = 7.6$ Hz, 1H), 7.42 – 7.22 (m, 5H), 5.93 (dd, $J = 9.0$, 3.9 Hz, 1H), 5.56 (t, $J = 6.3$ Hz, 1H), 5.09 (s, 2H), 3.83 – 3.58 (m, 2H), 3.58 – 3.43 (m, 2H), 2.71 – 2.58 (m, 2H), 2.58 – 2.47 (m, 1H), 2.26 – 2.06 (m, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 170.4, 170.3, 156.4, 145.7, 136.5, 130.9, 130.3,
Cbz-β-Homo-Ala-D-Pro-Bt (6.5b). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.29 (d, $J = 8.3$ Hz, 1H), 8.21 (d, $J = 8.3$ Hz, 1H), 7.80 (t, $J = 7.7$ Hz, 1H), 7.64 (t, $J = 7.8$ Hz, 1H), 7.42 – 7.18 (m, 6H), 5.72 (dt, $J = 8.6$, 4.1 Hz, 1H), 5.01 (d, $J = 4.2$ Hz, 2H), 3.98 – 3.82 (m, 1H), 3.79 – 3.62 (m, 2H), 2.65 (dt, $J = 15.3$, 5.5 Hz, 1H), 2.42 (dt, 2H), 2.26 – 2.11 (m, 1H), 2.11 – 1.97 (m, 2H), 1.11 (dd, $J = 6.6$, 2.1 Hz, 3H). $^{13}$C NMR (75 MHz, DMSO) δ 170.7, 169.0, 145.4, 137.2, 131.0, 130.6, 128.3, 127.7, 126.7, 120.2, 114.0, 65.1, 58.8, 58.7, 47.1, 29.1, 29.0, 24.8, 24.7.

Cbz-β-Phe-L-Pro-Bt (6.5c). $^1$H NMR (300 MHz, Chloroform-$d$) δ 8.34 – 8.19 (m, 1H), 8.17 – 7.98 (m, 1H), 7.71 – 7.59 (m, 1H), 7.58 – 7.46 (m, 1H), 7.46 – 7.14 (m, 10H), 5.90 – 5.74 (m, 1H), 5.23 – 5.10 (m, 1H), 5.10 – 4.98 (m, 3H), 3.78 – 3.32 (m, 3H), 2.95 – 2.80 (m, 1H), 2.81 – 2.32 (m, 2H), 2.23 – 1.96 (m, 2H), 1.96 – 1.65 (m, 2H), 1.18 – 0.78 (m, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 170.4, 170.3, 169.7, 156.0, 146.1, 136.7, 131.4, 131.3, 130.7, 130.6, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.0, 127.8, 127.7, 127.6, 127.5, 126.6, 126.5, 126.3, 126.2, 125.8, 120.3, 120.1, 115.0, 114.6, 114.5, 109.9, 73.0, 66.8, 59.6, 59.5, 52.4, 47.8, 45.1, 29.8, 29.6, 27.2, 25.1, 25.0, 23.3.

Cbz-β-Ala-L-Hyp(O-Bn)-Bt (6.5d). $^1$H NMR (299 MHz, Chloroform-$d$) δ 8.27 (d, $J = 8.2$ Hz, 1H), 8.13 (d, $J = 8.2$ Hz, 1H), 7.66 (t, $J = 7.6$ Hz, 1H), 7.52 (t, $J = 7.1$ Hz, 1H), 7.46 – 7.24 (m, 10H), 5.66 – 5.42 (m, 1H), 5.08 (s, 2H), 4.71 – 4.41 (m, 2H), 3.90 – 3.61 (m, 1H), 3.61 – 3.24 (m, 4H), 2.68 – 2.16 (m, 3H), 2.12 – 2.01 (m, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ 172.91, 170.89, 156.67, 146.26, 137.66, 136.83, 131.38, 130.77,
Cbz-β-Ala-3-Aze-Bt (6.5e). $^1$H NMR (299 MHz, Chloroform-d) δ 8.33 – 8.22 (m, 1H), 8.17 – 8.10 (m, 1H), 7.76 – 7.65 (m, 1H), 7.60 – 7.50 (m, 1H), 7.39 – 7.27 (m, 5H), 5.58 – 5.45 (m, 1H), 5.09 (br s, 2H), 4.79 – 4.64 (m, 1H), 4.59 – 4.36 (m, 2H), 3.78 (q, J = 5.7 Hz, 1H), 3.69 (t, J = 5.7 Hz, 1H), 3.49 (q, J = 5.9 Hz, 2H), 2.37 (t, J = 5.4 Hz, 2H).

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 171.39, 170.1, 156.3, 146.0, 136.5, 130.7, 130.3, 128.3, 127.8, 126.5, 126.1, 120.2, 114.0, 66.5, 51.5, 49.9, 36.3, 33.2, 31.1.

Cbz-β-Ala-L-2-Aze-Bt (6.5f). $^1$H NMR (299 MHz, Chloroform-d) δ 8.28 – 8.18 (m, 1H), 8.16 – 8.05 (m, 1H), 7.73 – 7.59 (m, 1H), 7.57 – 7.42 (m, 1H), 7.38 – 7.22 (m, 5H), 6.09 (dd, J = 9.8, 5.5 Hz, 1H), 5.72 – 5.53 (m, 1H), 5.15 – 5.00 (m, 2H), 4.31 – 4.07 (m, 1H), 3.80 – 3.60 (m, 1H), 3.57 – 3.40 (m, 2H), 3.09 – 2.81 (m, 1H), 2.55 – 2.28 (m, 3H).

$^{13}$C NMR (75 MHz, Chloroform-d) δ 171.7, 168.7, 156.4, 145.8, 136.6, 130.8, 130.6, 128.3, 127.8, 126.7, 126.4, 120.0, 114.1, 66.3, 59.6, 48.2, 36.4, 31.0, 21.0.

Cbz-β-Ala-D,L-Homo-Pro-Bt (6.5g). $^1$H NMR (500 MHz, Chloroform-d) δ 8.07 (dt, J = 8.4, 0.9 Hz, 1H), 7.78 – 7.66 (m, 1H), 7.50 (ddd, J = 8.1, 7.0, 1.0 Hz, 1H), 7.43 – 7.29 (m, 6H), 6.52 (dd, J = 6.8, 2.4 Hz, 1H), 5.36 5.30 (m, 1H), 5.11 (br s, 2H), 4.48 – 4.25 (m, 1H), 4.08 (dtd, J = 31.2, 8.2, 6.2 Hz, 3H), 3.49 (q, J = 6.1 Hz, 1H), 3.16 (ddd, J = 13.0, 8.2, 4.5, 2.4 Hz, 1H), 2.64 – 2.46 (m, 2H), 2.45 – 2.31 (m, 1H), 2.28 – 2.11 (m, 2H), 1.01 – 0.74 (m, 1H).

**General Procedure for the Cyclizations of Dipeptides 6.6a-g**

The dipeptidoyl benzotriazolides 6-g (100 mg) were dissolved in methanol with the desired concentration (0.1-50 mM). To the solutions, 150 mg of 10 wt % Pd/C was added slowly. The mixture was stirred for 24-72h at room temperature, under 40 psi
pressure of hydrogen gas. After the completion of reaction, the mixture was filtered through a celite pad and washed thoroughly with methanol to separate Pd/C. The solvent was evaporated under reduced pressure to yield the crude mixture of products. For 6.5a,b,c the mixture was separated to its components by gradient column chromatography. For 6.5d,e,f,g the crude product mixture was analyzed by HPLC-MS to determine the percentage of each component. The samples were analyzed via reverse phase gradient C18 HPLC/UV(254 nm)/(+)ESI-MSn. The details of mass spectrometry and HPLC. Mass Spectrometry: ThermoFinnigan (San Jose, CA) LCQ with electrospray ionization (ESI  ESI: sheath gas(N2) = 65; aux gas(N2) = 3; heated capillary temperature = 250 C (+)ESI: spray voltage = 3.3 kV; heated capillary voltage = 12.5 V; tube lens offset = 0 V. HPLC: Agilent (Palo Alto, CA) 1100 series binary pump. Column: thermoScientific Hypurity C8 (2.1 x 100 mm; 5 um) + Phenomenex (Torrace, CA) C18 guard column (2mm x 4 mm) .Mobile Phases: A = 0.2% Acetic acid in H2O (HPLC-grade, B&J-HPLC, Honeywell Burdick & Jackson) B = 0.2% acetic aicd in acetonitrile (LC-MS grade, Honeywell Burdick & Jackson). Acetic Acid (glacial, ACS Certified PLUS, Thermo Scientific). Gradient: @ 0.25 mL/min: A:B(min) = 100:0(0) => 5:95(45-60). Injector: Rheodyne 7125 manual injector, 25 mL injection loop; 25 mL Hamilton 1702 gastight syringe. UV: Agilent 1100 G1314A UV/Vis detector; wavelength = 254 nm.

For Cbz-β-Ala-L-Hyp(O-Bn)-Bt (6.5d) and Cbz-β-Ala-D,L-Homo-Pro-Bt (6.5g), no significant cyclization was observed at low concentrations of 0.1 mM or higher concentrations of 50 mM.
**Cyclizations of dipeptide Cbz-β-Ala-Pro-Bt (6.5a)**

[Cbz-β-Ala-D-Pro]ₙ. Cyclization of Cbz-β-Ala-D-Pro 6.5a. 0.1, 0.2, 13, 15 and 50 mM of 6.5a was treated with conditions stated in general procedure. The sample was analyzed via reverse phase gradient C18 HPLC/UV/(+)ESI-TOF-HRMS or via reverse phase gradient C8 HPLC/254 nm UV/(+)ESI-MSn. All of the products 6.6a, 6.7a, 6.8a and 6.9a of interest were detected with 13 and 15 mM concentrations. Intramolecular cyclization product and 6.6a were detected with 0.1 and 0.2 mM. Products 6.7a, 6.8a and 6.9a were detected with 50 mM of 6.5a.

Intramolecular cyclization product MW 168 eluted at RT 14.98 min. Intramolecular cyclization product MW 168 (RT 14.98 min) produced an abundant m/z 169 [M+H]+ ion. Dimeric products 6.7a MW 336 eluted at RT 43.65 min. 6.7a MW 336 produced an m/z 337 [M+H]+ ion and m/z 359 [M+Na]+ ions (top) along with several fragment ions. Trimeric 6.8a MW 504, eluted at RT 49.60 min. MW 504, RT 49.60 min. 6.8a the MW 504 produced m/z 505 [M+H]+ and m/z 527 [M+Na]+ ions. Tetrameric 6.9a MW 672 eluted at RT 55.59 min. The MW 672 produced m/z 673 [M+H]+ and m/z 695 [M+Na]+ ions. Products distributions were calculated based on mass area.

Cyclo-(β-Ala-D-Pro)₃.(6.8a) was purified by gradient chromatography DCM:Ether 1:9 to 1:1. Colorless oil, 139 mg, 0.41 mmol, 35 % yield. ¹H NMR (500 MHz, Methanol-d₄) δ 5.56 – 5.42 (m, 3H), 4.43 (dddd, J = 15.1, 12.0, 5.3, 3.4 Hz, 3H), 4.28 – 4.20 (m, 3H), 4.16 (t, J = 6.8 Hz, 3H), 3.86 (dq, J = 15.4, 5.3 Hz, 3H), 3.47 (t, J = 3.8 Hz, 3H), 3.44 (dd, J = 4.3, 3.3 Hz, 3H), 3.24 – 3.12 (m, 6H), 2.72 (dq, J = 13.6, 7.0 Hz, 3H), 2.50 (p, J = 6.9 Hz, 6H). ¹³C NMR (75 MHz, Methanol-d₄) δ 173.9, 171.9, 58.0, 37.5, 29.1, 23.6. Anal Calcd. for HRMS (MALDI-TOF-MS) C₂₄H₃₇N₆O₆: 505.2769. Found: 505.2771.
Cyclo-(β-Ala-D-Pro)$_5$ Cyclic pentamer was detected using MALDI-TOF-MS. HRMS MALDI-TOF-MS calcd. for C$_{40}$H$_{61}$N$_{10}$O$_{10}$ [M + H]$^+$ : 841.4572. Found: 841.4521.

Cyclo-(β-Ala-D-Pro)$_6$ Cyclic hexamer was detected using MALDI-TOF-MS. HRMS MALDI-TOF-MS calcd. for C$_{48}$H$_{73}$N$_{12}$O$_{12}$ [M + H]$^+$ : 1009.5470. Found: 109.5428.

**Cyclizations of dipeptide Cbz-D,L-Homo-β-Ala-D-Pro-Bt (6.5b)**

Cyclo-oligomerization of Cbz-D,L-β-Homo-Ala-D-Pro 6.5b. 0.1 mM or 15 mM of 6.5a was treated with conditions stated in general procedure. The sample was analyzed via reverse phase gradient C18 HPLC/UV/(+)ESI-TOF-HRMS. All of the products 6.6b, 6.7b, 6.8a and 6.9b of interest were detected. HPLC: Agilent (Palo Alto, CA) 1100 series binary pump. Mobile Phases: MP A: 0.2% HOAc in H$_2$O MP B: 0.2% HOAc in Methanol. Gradient: @ 0.17 mL/min: A:B(min) = 95:5(0) => 5:95(65-80). Product distributions were determined based on weight area.

At 0.1 mM concentration only intramolecular cyclization product Cyclo-(D,L-β-Homo-Ala-D-Pro) MW 182 detected at RT 13.6 min. HRMS (ESI) calcd for C$_9$H$_{15}$N$_2$O$_2$. [M + H]$^+$ 183.1128, found 183.114.

At higher concentration of 15 mM higher oligomers were detected. Cyclo-(D,L-β-Homo-Ala-D-Pro)$_2$ 6.7b MW 364: With a 20 ppm window about the m/z 365.2183 and m/z 387.2003 ions expected of MW 364, there were a number of ion-peaks due to combination of diastereomers detected. RT 15.9 min. HRMS ESI-MS calcd. for C$_{18}$H$_{29}$N$_4$O$_4$ [M + H]$^+$ 365.2183. Found: 365.2204. RT 21.8 min. HRMS ESI-MS calcd. for C$_{18}$H$_{29}$N$_4$O$_4$ [M + H]$^+$ 365.2183. Found: 365.2198. RT 21.8 min. HRMS ESI-MS calcd. for C$_{18}$H$_{29}$N$_4$O$_4$ [M + H]$^+$ 365.2193, found 365.2183. Cyclo-(β-Ala-D-Pro)$_3$. (6.8a) was purified by gradient chromatography DCM:Ether 1:9 to 1:1.
Cyclo-(D,L-Homo-β-Ala-D-Pro-)_2 (6.7b) was purified by gradient chromatography DCM:Ether 1:9 to 1:1. Colorless oil, 146 mg, 0.40 mmol, 68% yield. ¹H NMR (CDCl₃, 300 MHz): δ 1.23 (d, J = 6.4 Hz, 3H), 1.28 (d, J = 6.4 Hz, 3H), 1.76–1.88 (m, 4H), 2.04–2.15 (m, 2H), 2.37–2.45 (m, 2H), 2.48–2.61 (m, 2H), 2.89 (dd, J = 17.3, 2.9 Hz, 1H), 3.12 (t, J = 13.4 Hz, 1H), 3.43–3.53 (m, 3H), 3.54–3.60 (m, 1H), 3.69–3.76 (m, 1H), 4.03–4.09 (m, 1H), 4.77 (t, J = 6.9 Hz, 1H). ¹³C NMR (CDCl₃, 75 MHz): 21.0, 23.6, 24.4, 24.7, 29.2, 29.4, 42.8, 44.7, 45.1, 47.7, 58.6, 60.7, 171.4, 172.0, 172.7, 172.9. HRMS (ESI) calcd for C₁₈H₂₉N₄O₄ [M + H]⁺ 365.2193, found 365.2183.


Cyclo-(D,L-Homo-β-Ala-D-Pro)_4 6.9b MW 728, RT 28.9 min: There were several m/z 751 ion-peaks (bottom) but only the RT 28.8 ion-peak correlated with the m/z 729 ion-peak. HRMS ESI-MS calcd. for C₃₆H₅₆N₆O₈ [M + H]⁺: 729.4294. Found: 729.429

**Cyclizations of dipeptide Cbz-D,L-Homo-β-Ala-D-Pro-Bt (6.5c)**

Cyclo-oligomerization of Cbz-D,L-β-Phe-D-Pro 6.5c. 15 mM of 6.5a was treated with conditions stated in general procedure. The samples were analyzed via reverse phase gradient C18 HPLC/UV/(+)ESI-MSn. Mobile Phases: A = 0.2% HOAc in H₂O (B&J) B = 0.2% HOAc in Methanol (B&J). Acetic Acid (glacial, ACS Cerified Plus; Fisher Scientific) Gradient: @ 0.15 mL/min: A:B(min) = 100:0(0) => 5:95(45-60). Cyclo-(D,L-β-Phe-D-Pro)_2 6.7c MW 488: A number of m/z 489 and m/z 511 ion-peaks were detected and examined. MW 488 isomers: There appeared to be at least two isomers present due to combinations of diastereomers with RT 43.96 and RT 67.37. MW 488:
There was a co-eluting compound which produced an m/z 488.2 ion-peak which did not correlate to the m/z 489 ion-peak of MW 488. [M+Na]+, m/z 511 RT 49.42 min.

Cyclo-(D,L-β-Phe-D-Pro-)$_2$ (6.7c) was purified by gradient chromatography DCM:Ether 1:9 to 1:1. Colorless oil, 75 mg, 0.15 mmol, 61% yield. $^1$H NMR (CDCl$_3$, 300 MHz): δ 1.76–1.96 (m, 6H), 2.02–2.18 (m, 2H), 2.62–2.76 (m, 4H), 3.45–3.58 (m, 4H), 4.53 (dd, $J = 8.1, 4.0$ Hz, 2H), 4.77 (dd, $J = 13.2, 2.7$ Hz, 2H), 5.89 (br s, 2H), 7.05–7.38 (m, 10H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 22.9, 23.6, 28.8, 29.9, 43.9, 46.9, 56.9, 59.8, 126.0, 126.4, 128.6, 128.8, 129.0, 129.5, 142.0, 169.1, 170.2. HRMS (ESI) calcd for C$_{29}$H$_{32}$N$_4$O$_4$Na [M + Na]$^+$ 511.2316, found 511.2303.

Cyclo-(D,L-β-Phe-D-Pro)$_4$ 6.9c MW 998. The m/z 977 ion-peaks “co-eluted” with m/z 500 ion-peaks. In addition, MS/MS of the m/z 997 ion-peaks produced m/z 500 via loss of 477 u. These are MW 477 compounds which produced m/z 500 [M+Na]$^+$ and m/z 997 [M+Na+M]$^+$ ions

**Cyclizations of dipeptide Cbz-β-Ala-3-Aze-Bt (6.5e)**

Cyclo-oligomerization of Cbz-β-Ala-3-Aze-Bt 6.5e. 0.1 mM of 6.5e was treated with conditions stated in general procedure.

HPLC: Agilent (Palo Alto, CA) 1100 series binary pump. Column: waters XTerra MS C18 3.5 um (2.1 x 150 mm; S/N=T03401K); with Phenomenex C18 Security Guard Column (2 x 4 mm). Mobile Phases: MP A: 0.2% HOAc in H$_2$O ; MP B: 0.2% HOAc in Methanol H$_2$O (Optima; LCMS-grade; Fisher Scientific). Methanol (MeOH; Optima; LCMS-grade; Fisher Scientific). Acetic acid, glacial: Certified ACS Plus (Fisher Scientific). Gradient: @ 0.17 mL/min: A:B(min) = 95:5(0)=>65:35(10)> 5:95(65-80)
Only cyclo[-β-Ala-3-Aze]$_2$ was detected with MW 308 RT 38.7. The (+)ESI-MS was dominated by the m/z 331 ion, a possible [M+Na]$^+$ ion. The m/z 331 was dissociated to m/z 275, 208, 180 and several other product ions.

**Cyclizations of dipeptide Cbz-β-Ala-L-2-Aze-Bt (6.5f)**

Cyclo-oligomerization of Cbz-β-Ala-L-2-Aze-Bt (6.5f). 15 mM of 6.5f was treated with conditions stated in general procedure. Only cyclic trimer [β-Ala-L-2-Aze]$_3$ was detected. HPLC: Agilent (Palo Alto, CA) 1100 series binary pump.

Column: Waters XTerra MS C18 3.5 um(2.1 x 150 mm; S/N=T03401K); with Phenomenex C18 Security Guard Column (2 x 4 mm). Mobile Phases: MP A: 0.2% HOAc in H$_2$O; MP B: 0.2% HOAc in Methanol H$_2$O (Optima; LCMS-grade; Fisher Scientific). Methanol (MeOH; Optima; LCMS-grade; Fisher Scientific). Acetic acid, glacial: Certified ACS Plus (Fisher Scientific). Gradient: @ 0.17 mL/min: A:B(min) = 95:5(0)=>65:35(10)> 5:95(65-80).

Cyclo[-β-Ala-L-2-Aze]$_3$. MW 462, RT 67.75: At RT 67.7 were matching m/z 485 and 463 ion-peaks. MW 462 RT 67.7 min: While the (+)ESI-MS (top) contains the expected m/z 463 and m/z 485 ions (along with m/z 507) indicative of a MW 462, the m/z 463 ion was relatively stable towards CID as m/z 463 was still the most abundant ion.
CHAPTER 7
CONFORMATIONALLY ASSISTED LACTAMIZATIONS FOR THE SYNTHESIS OF BIS-2,5-DIKETOPIPERAZINES

Introduction

2,5-Diketopiperazines (2,5-DKPs) occur in numerous natural products, often as such, but also embedded in larger, more complex molecular architectures in a variety of natural products from fungi, bacteria, the plant kingdom, and mammals (Figure 7-1). 2,5-DPKs have the ability to bind to a wide range of receptors, together with several characteristics, attractive in scaffolds for drug discovery. DKPs are small, conformationally constrained heterocyclic molecules stable to proteolysis. Diversity can be introduced at up to six positions and stereochemistry controlled at up to four positions. Recent advances in solid-phase methodology have increased their availability for combinatorial drug discovery.

In sharp contrast to numerous studies dedicated to the synthesis and biological properties of DKPs, relatively few bis-DKPs have been reported. They have, however, shown considerable biological activity: (i) (+)-WIN 64821, from Aspergillus flavus cultures is a potent competitive P antagonist with submicromolar potency for both the human neurokinin 1 and the cholecystokinin B receptors; (ii) dimeric diketopiperazine (−)-ditryptophenaline alkaloids and (−)-N1-(2-phenylethylene)ditryptophenaline inhibit the former receptor; (iii) (+)-11,11'-dideoxyverticillin A is a tyrosine kinase inhibitor with potent antitumor activity; (iv), (v)

naturally occurring *bis*-DPKs chaetocin and chetomin are inhibitors of HIF-1α-p300/CBP interaction, although the inhibition mechanism remains unclear.\textsuperscript{133} Comparative analysis has shown that *bis*-DKPs impact highly on the expression level of hypoxia-inducible genes and have more genome-wide effects than DPKs;\textsuperscript{134} (vi) X-ray crystallographic studies show that xyylene-linked *bis*-2,5-DKPs obtained by direct C3 alkylation of the \( N \)-substituted 2,5-DKP core via carbanion chemistry, can adopt open- and closed conformations, which enable them to serve as building blocks for metallo-supramolecular assemblies, metal-organic polygons and other metal-organic materials.\textsuperscript{135}

Figure 7-1. Representative of bioactive natural products containing DKP

Head-to-tail condensation between the \( N \)- and \( C \)- termini of the corresponding linear peptides represents the most straightforward synthesis for *bis*-2,5-DKPs (Figure 7-2).\textsuperscript{136,137} However, head-to-tail condensation may require harsh conditions which causes partial epimerization, other side reactions, and low yields. Dimeric DKPs can
also be obtained by radical dimerization (Figure 7-3)\textsuperscript{129,138,139} or direct modification of the $N$-alkylated DKP core via carbanion chemistry (Figure 7-4)\textsuperscript{134,135} but these procedures are challenging because multiple protection and deprotection steps limit the methodology to specific peptide sequences.

Figure 7-2. Synthesis of bis-DKP via head-to-tail condensation between the N- and C-termini

Figure 7-3. Radical dimerization in synthesis of bis-DKP

Figure 7-4. Synthesis of bis-DKP using carbanion chemistry
This chapter describes a method for the synthesis of both symmetrical and unsymmetrical bis-2,5-DKPs by triethylamine-promoted macrolactamization from peptidoyl benzotriazolides containing proline as a turn introducer (Figure 7-5). Proline has high tendency to induce reverse turns in polypeptides, because it can accommodate both the cis and the trans conformers of a tertiary Xaa–Pro amide bond (where Xaa represents any L-α-amino-acid).\textsuperscript{5} It has therefore been utilized to introduce reverse turns to achieve short end-to-end distance in peptide chains.\textsuperscript{140}

\[ \text{Peptide} \xrightarrow{\text{base promoted macrolactamization}} \text{Peptide} \]

\( \text{BTH: benzotriazole; Turn-introducer: proline; Pg: protecting group} \)

Figure 7-5. Ring construction strategy to form DKPs

**Results and Discussion**

**Synthesis of Symmetrical Bis-DPKs**

Dimeric DKP 7.5a was synthesized in 4 steps (Figure 7-6): (i) \( N^a-N^a\)-bis-Cbz-L-cystine (7.1a) was converted to benzotriazolide 7.2a in 86\% yield; (ii) reaction of L-Cbz-L-cystinyl benzotriazole 7.2a with D-proline according to our previously reported procedure,\textsuperscript{141,142} was complete within 3 h at 20 °C and produced dipeptide dimer bis-Cbz-L-Cys-D-Pro-OH 7.3a; (iii) the reaction of 7.3a with BtS(O)Bt, generated \textit{in situ}, at -20 °C in dry THF, led without epimerization to the Cbz \( N \)-protected dipeptidoyl benzotriazolides 7.4a (82\%); (iv) macrocyclization of 7.4a formed \textit{bis-}(Cbz-L-Cys-D-Pro) 7.5a (Figure 7-6, Table 7-1).

Optimum preparative conditions, co-reagents and solvents for cyclization of 7.4a were examined: little reaction was observed when compound 7.4a was treated under
microwave in acetonitrile without additive for 3 h, and conversion reached just 10% after 18 h under reflux in acetonitrile. Addition of triethylamine (2.2 equiv.), however gave the desired novel cyclic bis-(Cbz-L-Cys-D-Pro) 7.5a in 76% yield (Figure 7-6, Table 1). The presence of water (MeCN/H₂O, 9 : 1) in the reaction mixture caused minimal (<5%) hydrolysis of 7.4a.

![Chemical structures](image)

Figure 7-6. Cyclization of bis-Cbz-dipeptidoyl benzotriazoles 7.4a-e

No epimerization of 7.5a was detected by HPLC. Thus, HPLC analysis [chirobiotic T column (250 mm x 4.6 mm), detection at 254 nm, flow rate 5 mL min⁻¹, MeOH] showed a single peak, retention time 12.0 min on 7.5a, which confirmed the absence of diastereomers in the lactamization product bis-2,5-DKP 7.5a. To provide further racemization-free evidence during Bt-mediated lactamization, dimeric 2,5-DKP bis-(Cbz-L-Cys-D,L-Pro) 7.5b was synthesized (Figure 7-6, Table 7-1). HPLC analysis [chirobiotic T column (250 mm – 4.6 mm), detection at 230 nm, flow rate 0.25 mL/min,
MeOH] on 7.5a (single peak, retention time 19.0 min) and 7.5b (two equal peaks, retention times 18.9 and 20.6 min) confirmed that product 7.5a is enantiomerically pure. Compounds 7.5a,b were characterized by $^1$H and $^{13}$C NMR and HRMS (Table 7-1).

Table 7-1. *En route* to symmetrical bis-DKPs

<table>
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<tr>
<th>Entry</th>
<th>Time (h)</th>
<th>HMRS (M+Na$^+$)</th>
<th>Product</th>
<th>Yield (%)</th>
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A similar protocol was used to synthesize *bis*-2,5-DPK 7.5c *bis*-((Cbz-Homo-D,L-Cys-D-Pro) (83%) by cyclization of its precursor 7.4c (Figure 7-6, Table 7-1). Compound 7.5c was purified by semi-preparative HPLC and fully characterized by $^1$H and $^{13}$C NMR spectroscopy, HRESI-MS, and analytical HPLC. $^{13}$C NMR spectra indicated formation of
desired lactamization product 7.5c (appearance of two more up-field amide carbon signals), and was corroborated by $^1$H NMR data. Similarly, bis-2,5-DPK bis-(Cbz-L-Cys-L-Hyp(OtBu)) 7.5d and bis-2,5-DPK bis-(Cbz-Homo-D,L-Cys-L-Hyp(OtBu)) 7.5e were synthesized in 84% and 88% respectively (Figure 7-6, Table 7-1).

**Further Development of the Method for Synthesis of Symmetrical Bis-2,5-DPKs**

To broaden the utility of our method, we applied the procedure to the cyclization of open chain peptidoyl benzotriazolides 7.10a-c for the synthesis of symmetrical dimeric DPKs 7.11a-c (Table 7-2) with aliphatic “mimetic” linkers (Figure 7-7 and 7-8, Table 7-2).

![Figure 7-7. Synthesis of bis-benzotriazoles 7.10a-c](image)

For R, X and yields refer to table 7-2

![Figure 7-8. Synthesis of aliphatic “mimetic” linked bis-2,5-DKPs 7.11a-c](image)

*Bis*-DKP derivative ICRF-159 with an aliphatic linker between the DPK units showed unique preclinical properties: chelation with divalent cations, potential amelioration of anthracycline cardiac toxicity and possible antimetastatic effects.$^{143,144}$

Dicarboxylic acids: 3,3-dimethylglutaric and trans-1,4-cyclohexanedicarboxylic acids were chosen as linker-precursors for the DPKs units. To study the macrolactamization
reaction to form symmetrical bis-DKPs 7.11a-c, the starting materials 7.10a-c were obtained in a six-step procedure starting from commercially available bis-benzotriazolides 7.6a,b.

Table 7-2. En route to symmetrical bis-DKPs 7.11a-c

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<th>HMRS (M+Na⁺)</th>
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<th>Yield (%)</th>
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The symmetrical N-Cbz-protected tripeptide derivatives 7.7a-b were prepared in yields of 72–78% by coupling bis-benzotriazolides 7.6a-b with N⁵-Cbz-L-lysine, or N-Cbz-L-cysteine. Compounds 7.7a-b were then converted into N-(Cbz-α-aminoacyl)-benzotriazoles 7.8a-b (78%-83%) which were subsequently coupled with D-proline or L-proline to afford the side chain linked tetrapeptides 7.9a-c. Treatment of N-Cbz-tetrapeptides 7.9a-c with eight equivalents of BtH and two equivalents of thionyl chloride in tetrahydrofuran at −45 °C for 6 h, gave N-protected α-tetrapeptidoyl benzotriazolides 7.10a–c in yields 68–88% (Figure 7-7 and 7-8, Table 7-2).
Cyclization of *bis*-benzotriazolides 7.10a-c was carried out in dry acetonitrile in the presence of 3.5 equivalents of triethylamine. The macro lactamization mixture was stirred at room temperature until the TLC revealed complete reaction. Open chain *N*-Cbz-protected-peptidoyl benzotriazolides 7.10a-c were converted into symmetrical *bis*-2,5-diketopiperazines 7.11a-c in yields 91%, 85% and 81% correspondingly by our cyclization strategy (Figure 7-8, Table 7-2).

**Synthesis of Unsymmetrical *Bis*-2,5-DKPs**

Literature examples of unsymmetrical *bis*-DPKs include compounds endowed with antagonist and anticancer activity.\(^{77,145,146}\) We therefore targeted *bis*-DKPs with unsymmetrical linkers to broaden the scope of our methodology. Our strategy included three key transformations: (i) preparation of unsymmetrical linkers (7.15a-e) derived from amino dicarboxylic acids (7.12a,b) to provide functional groups in one of the amino acid constituents of the DKPs (Figure 7-9); (ii) coupling of linkers (7.15a-e) at both C-termini with a turn-introducer proline unit as a second amino acid that forms a DPK unit forming intermediates (7.17a-e) (Figure 7-10); (iii) lactamization of (7.17a-e) to form the designed *bis*-2,5-DKPs (7.19a-e). Cbz *N*-protected L-aspartic and L-glutamic acids were chosen as linker-precursors. Figure 7-9 shows coupling reactions with side chain functional groups in the linkers. Cbz-L-Asp-OH (7.12a) and Cbz-L-Glu-OH (7.12b) were each converted into *N*-(Cbz-\(\alpha\)-aminoacyl)-benzotriazolides 7.13a,b (86% and 92% respectively). Coupling of these benzotriazole derivatives 7.13a,b with functional groups of the side chains in Cbz-*N*-protected-\(\alpha\)-aminoacids 7.14a-c in the presence of diisopropylethylamine (for 7.15a,b) or triethylamine (for 7.15c-e) gave 7.15a-e (88–93%) (Figure 7-9).
Figure 7-9. Preparation of 7.15a-e

Figure 7-10. Synthesis of bis-DKPs 7.19a-e with unsymmetrical linkers
<table>
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<tr>
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<th>HMRS (M+Na+)</th>
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</table>

Peptides 7.15a-e were converted into the corresponding benzotriazolides 7.16a-e (68–78%, Figure 7-10). Coupling 7.16a-e with Cbz-protected L-proline or D-proline in acetonitrile-water (3:1) in the presence of triethylamine provided peptides 7.17a-e, which were subsequently treated with \textit{in-situ} generated BtS(O)Bt to afford the peptide benzotriazolides 7.18a-e in 62-72% yields. Cyclization of 7.18a-e occurred under
conditions similar to those used for the macrocyclization of 7.4a-c and gave the desired bis-2,5-DPKs 7.19a-e in yields of 72-88% (Figure 7-10, Table 7-3). Samples 7.19a-e (Table 7-3) showed the advantage of our base-assisted lactamization of open chain \(N\)-Cbz-protected peptidoyl benzotriazolides for synthesis of DKPs derivatives since direct macrolactamization of \(N\)-protected dipetides to form DKPs using peptide coupling reagents often requires harsh conditions,\(^{77,147,148}\) and the deprotection/cyclization strategy can lead to extended procedures and lower yields.\(^{77,149}\)

**Conclusion**

In summary, we have developed novel, straightforward and versatile Bt-mediated macrolactamizations for the synthesis of bis-2,5-DKPs with both symmetrical and unsymmetrical linkers. The methodology was utilized to ring-close a series of peptidoyl benzotriazolides yielding a small library of bis-DKPs with novel features, which could not be prepared efficiently using previously reported methods. The approach described herein should provide a convenient entry to the design and synthesis of a variety of bis-DKPs with potential utility in drug discovery, biological catalysis and materials chemistry. Our Bt-assisted macrocyclization offers the following advantages: (i) macrolactamization at room temperature; (ii) formation of bis-2,5-DKPs in good yields with no detectable racemization; (iii) the use of commercially available and inexpensive reagents, and (iv) easy purification. Given that there are an increasing number of studies involving the synthesis of symmetrical and unsymmetrical DKPs to evaluate their biological activities, we believe this new approach represents a significant development in the field.
Experimental Section

General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$, DMSO-$d_6$, acetone-$d_6$, or CD$_3$OD using a 300 or 500 MHz spectrometer (with TMS as an internal standard). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublets, ddd = doublet of doublets of doublets, and dt = doublet of triplets. 3,3-DMG refers to 3,3 dimethyl glutarate, and trans-1,4-CHD refers to trans-cyclohexane-1,4-dicarboxylate. HPLC–MS analyses were performed on a reverse phase gradient using 0.2% acetic acid in H$_2$O/methanol as mobile phases; wavelength = 254 nm; mass spectrometry was done with electrospray ionization (ESI), matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) or atmospheric-pressure chemical ionization (APCI). Ether refers to diethyl ether.

General Procedure I for the Preparation of Bis-Benzotriazolides 7.2a-b and 7.13a-b

A stirred solution of 1H-benzotriazole (BtH) (8 equiv.) in dry tetrahydrofuran (THF) (10 mL/1 g) was treated at 20 °C with thionyl chloride (SOCl$_2$) (2 equiv.). After 20 minutes, a solution of 7.1a-b or 7.12a-b (1 equiv.) in dry THF (10 mL/1 g) was added drop–wise and the resulting solution was then stirred for 2 h at 20 °C. Upon completion, the mixture was filtered, and THF was removed under reduced pressure. The residue was dissolved by dichloromethane (CH$_2$Cl$_2$ 50 mL/1 g of 7.2a-b or 7.13a-b) and washed successively with HCl (4N, 2 × 1 mL/1 mL CH$_2$Cl$_2$), aq. Na$_2$CO$_3$ (10%, 2 × 1 mL/1 mL CH$_2$Cl$_2$) and brine (1 mL/1 mL). The organic layer was dried over magnesium sulfate.
(MgSO₄), filtered and evaporated to give the crude product. The solid was recrystallized from CH₂Cl₂/hexanes to yield bis-benzotriazolides 7.2a-b and 7.13a-b.

(Cbz-L-Cys-Bt)₂ (7.2a). The compound was prepared according to general procedure I for preparation of bis-bentrotiazolides from (Cbz-Cys-OH)₂ (3.17 g, 6.25 mmol), BtH (5.96 g, 50.0 mmol) and SOCl₂ (0.91 mL, 12.5 mmol). White microcrystals, 3.82 g, 5.38 mmol, 86% yield; mp 152–156 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.20–3.37 (m, 2H), 3.38–3.50 (m, 2H), 5.11 (br s, 4H), 5.91 (d, J = 7.2 Hz, 2H), 5.97–6.07 (m, 2H), 7.26–7.38 (m, 10H), 7.51 (t, J = 7.3 Hz, 2H), 7.65 (t, J = 7.2 Hz, 2H), 8.05 (d, J = 8.2 Hz, 2H), 8.19 (d, J = 8.2 Hz, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 41.0, 54.3, 67.7, 114.5, 120.6, 126.9, 128.4, 128.7, 131.2, 136.1, 146.2, 155.9, 169.4. Anal. Calcd for C₃₄H₃₀N₈O₆S₂: C 57.45, H 4.25, N 15.76. Found: C 57.66, H 4.09, N 15.40.

(Cbz-Homo-D,L-Cys-Bt)₂ (7.2b). The compound was prepared according to general procedure I for preparation of symmetrical bis-benzotriazolides from BtH (3.56 g, 29.8 mmol), SOCl₂ (0.54 mL, 7.5 mmol) and (Cbz-D,L-Homo-Cys-OH)₂ (2.0 g, 3.7 mmol). White microcrystals, 1.86 g, 2.5 mmol, 68% yield; mp 98–104 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.21–2.32 (m, 2H), 2.48–2.62 (m, 2H), 2.85–2.96 (m, 4H), 5.15 (br s, 4H), 5.87–5.95 (m, 2H), 6.00–6.13 (m, 2H), 7.16 (br s, 2H), 7.28–7.42 (m, 8H), 7.50–7.57 (m, 2H), 7.66–7.70 (m, 2H), 8.09–8.15 (m, 2H), 8.26 (t, J = 7.6 Hz, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 33.1, 34.7, 54.2, 67.6, 114.5, 120.6, 126.8, 128.5, 128.7, 131.1, 131.3, 136.1, 146.2, 156.3, 171.3. HRMS (ESI) calcd for C₃₆H₃₄N₈O₆S₂Na [M + Na]+ 761.1935, found 761.1949.

Cbz-L-Asp-(Bt)-Bt (7.13a). The compound was prepared according to general procedure I for preparation of symmetrical bis-benzotriazolides BtH (3.39 g, 28.5 mmol),
SOCl₂ (0.52 mL, 7.1 mmol) and Cbz-L-Asp-OH 7.12a (4.00 g, 3.74 mmol). White microcrystals, 1.51 g, 3.22 mmol, 86% yield; mp 132–134 °C. \(^1\)H NMR (CDCl₃, 300 MHz): δ 4.35–4.54 (m, 2H), 5.13 (br s, 2H), 6.08–6.13 (m, 1H), 6.19–6.26 (m, 1H), 7.26–7.36 (m, 5H), 7.46–7.71 (m, 4H), 8.07–8.19 (m, 3H), 8.26 (d, \(J = 8.5\) Hz, 1H).

Cbz-L-Glu-(Bt)-Bt (7.13b). The compound was prepared according to general procedure I for preparation of symmetrical bis-benzotriazolides from BtH (13.56 g, 113.84 mmol), SOCl₂ (2.07 mL, 26.46 mmol) and Cbz-L-Glu-OH 7.12b (4.00 g, 14.23 mmol). White microcrystals, 6.33 g, 13.09 mmol, 92% yield; mp 156–157 °C. \(^1\)H NMR (CDCl₃, 300 MHz): δ 2.47–2.61 (m, 1H), 2.73–2.87 (m, 1H), 3.72 (t, \(J = 7.1\) Hz, 2H), 5.10 (br s, 2H), 5.80–6.04 (m, 2H), 7.20–7.38 (m, 5H), 7.45–7.71 (m, 4H), 8.07–8.26 (m, 4H). \(^13\)C NMR (CDCl₃, 75 MHz): δ 27.5, 31.9, 54.3, 67.7, 114.5, 120.4, 120.7, 126.5, 126.9, 128.4, 128.7, 130.7, 131.1, 131.2, 136.0, 146.3, 156.1, 171.1, 171.3. Anal. Calcd for C\(_{25}\)H\(_{21}\)N\(_7\)O\(_4\): C 62.11, H 4.38, N 20.28. Found: C 62.15, H 4.26, N 20.56.

**General Procedure II for the Preparation of Di-sulfide Dipeptides 7.3a-e**

Di-sulfide dipeptide benzotriazolides 7.2a-b (1 equiv.) were each suspended in acetonitrile/water (3:1) (25 mL/1 g) and a solution of D-proline, D,L-proline or Hyp-(OtBu) (2 equiv.) in water (10 mL/1 g of proline) containing triethylamine (2–2.2 equiv.) was added slowly. The mixtures were stirred at 20 °C for 15 h until TLC revealed consumption of the starting materials. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed with 4N HCl (3 x 1.5 mL/1 mL of ethyl acetate) and brine (1 mL/1 mL of ethyl acetate). Recrystallization from ethyl acetate/hexanes yielded di-sulfide dipeptides 7.3a-e.

(Cbz-L-Cys-D-Pro-OH)\(_2\) (7.3a). The compound was prepared according to general procedure II for preparation of di-sulfide dipeptides from benzotriazolide 7.2a.
(Cbz-L-Cys-Bt)$_2$ (3.00 g, 4.22 mmol) and D-proline (0.97 g, 8.44 mmol). White microcrystals, 2.22 g, 3.17 mmol, 75% yield; mp 78–80 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.78–1.96 (m, 6H), 2.08–2.18 (m, 2H), 2.85 (dd, $J = 13.9, 9.9$ Hz, 2H), 3.02 (dd, $J = 13.7, 3.9$ Hz, 2H), 3.56–3.68 (m, 4H), 4.24 (dd, $J = 8.8, 3.8$ Hz, 2H), 4.48–4.62 (m, 2H), 5.03 (br s, 4H), 7.28–7.51 (m, 10H), 7.78 (d, $J = 8.2$ Hz, 2H), 12.37 (br s, 2H). $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 26.0, 30.2, 40.7, 53.6, 60.7, 68.0, 129.0, 129.1, 129.6, 138.2, 158.6, 171.5, 175.3. HRMS (ESI) calcd for C$_{32}$H$_{37}$N$_4$O$_{10}$S$_2$ [M – H]$^-$ 701.1945, found 701.1959.

(Cbz-L-Cys-D,L-Pro-OH)$_2$ (7.3b). The compound was prepared according to general procedure II for preparation of di-sulfide dipeptide from benzotriazolide 7.2a (Cbz-L-Cys-Bt)$_2$ (2.00 g, 2.82 mmol) and D,L-proline (0.65 g, 5.63 mmol). White microcrystals, 1.54 g, 2.20 mmol, 78% yield; mp 68–72 °C. $^1$H and $^{13}$C NMR were identical to 7.3a.

(Cbz-D,L-Homo-Cys-D-Pro-OH)$_2$ (7.3c). The compound was prepared according to general procedure II for preparation of di-sulfide dipeptides from (Cbz-D,L-Homo-Cys-Bt)$_2$ 7.2c (1.0 g, 1.35 mmol) and D-proline (0.31 g, 2.70 mmol). Sticky gel, 0.77 g, 1.05 mmol, 78% yield. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.80–2.00 (m, 8H), 2.02–2.24 (m, 4H), 2.62–2.82 (m, 4H), 3.64 (br s, 4H), 4.144.27 (m, 2H), 5.01 (t, $J = 3.4$ Hz, 2H), 5.04 (br s, 4H), 7.24–7.40 (m, 10H), 7.81 (d, $J = 8.3$ Hz, 2H). $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 26.0, 30.2, 32.2, 35.6, 53.0, 54.1, 67.8, 127.2, 128.9, 129.1, 129.5, 138.1, 158.7, 174.2. HRMS (ESI) calcd for C$_{34}$H$_{41}$N$_4$O$_{10}$S$_2$ [M – H]$^-$ 729.2259, found 729.2268.

Bis-Cbz-L-Cys-L-Hyp(O-tBu)-OH (7.3d). The compound was prepared according to general procedure II for preparation of di-sulfide dipeptides from (Cbz-L-Cys-Bt)$_2$ 7.2c.
and Hyp(O-tBu). Sticky oil, 1.47 g, 1.73 mmol, 82% yield. \( ^1\text{H} \) NMR (CD\(_3\)OD, 300 MHz): \( \delta \) 7.42 – 7.21 (m, 10H), 5.09 (br s, 4H), 5.15 – 5.03 (m, 4H), 4.80 (dd, 2H), 4.54 – 4.32 (m, 4H), 3.92 (dd, \( J = 10.7 \), 5.5 Hz, 2H), 3.66 (dd, 2H), 3.17 (dd, \( J = 14.1 \), 4.8 Hz, 2H), 2.89 (dd, \( J = 14.3 \), 9.2 Hz, 2H), 2.25 – 1.98 (m, 4H), 1.19 (br s, 18H).

\( ^{13}\text{C} \) NMR (CD\(_3\)OD, 75 MHz): \( \delta \) 171.5, 158.5, 138.1, 130.0, 129.6, 129.4, 129.1, 129.0, 128.9, 75.6, 71.1, 68.4, 68.0, 59.6, 55.5, 53.5, 41.0, 38.2, 29.1, 28.7. HRMS (ESI): calcd for \( \text{C}_{40}\text{H}_{53}\text{N}_{4}\text{O}_{12}\text{S}_{2}[\text{M} - \text{H}]^{-} \) 845.3095, found 845.3091.

Bis-Cbz-Homo-D,L-Cys-L-Hyp(O-tBu)-OH (7.3e). The compound was prepared according to general procedure II for preparation of di-sulfide dipeptides from (Cbz-Homo-D,L-Cys\(_2\)) 7.2b and Hyp(O-tBu). White solid, 0.24 g, 0.65 mmol, 77% yield; mp 79.0 – 83.0 °C. \( ^1\text{H} \) NMR (CD\(_3\)OD, 300 MHz): \( \delta \) 7.38 – 7.26 (m, 10H), 5.18 – 5.04 (m, 4H), 4.66 – 4.58 (m, 2H), 4.49 (t, \( J = 6.7 \) Hz, 2H), 4.46 – 4.29 (m, 2H), 3.92 – 3.82 (m, 2H), 3.74 – 3.62 (m, 2H), 2.87 – 2.63 (m, 4H), 2.23 – 2.13 (m, 4H), 2.13 – 2.05 (m, 2H), 2.05 – 1.91 (m, 2H), 1.33 – 1.11 (m, 18H). \( ^{13}\text{C} \) NMR (CD\(_3\)OD, 75 MHz): \( \delta \) 175.5, 172.6, 158.5, 138.3, 129.6, 129.2, 129.1, 129.0, 110.2, 75.7, 71.2, 69.3, 67.9, 67.8, 59.5, 59.3, 55.7, 54.2, 52.6, 38.4, 38.3, 35.6, 35.0, 32.5, 28.74, 28.71. HRMS (ESI): calcd for \( \text{C}_{42}\text{H}_{57}\text{N}_{4}\text{O}_{12}\text{S}_{2}[\text{M} - \text{H}]^{-} \) 873.3408, found 873.3401.

**General Procedure III for the Preparation of Di-Sulfide Dipeptidoyl Bentrotriazolides 7.4a-e**

A stirred solution of BtH (8 equiv.) in dry tetrahydrofuran (THF) (15 mL/1 g) was treated at -20 °C with SOCl\(_2\) (1 equiv.). After 20 minutes, a solution of 7.3a-e (1 equiv.) in dry THF (15 mL/1 g 7.3a-e) was added drop-wise and the resulting solutions were then stirred for 1.5 h at -20 °C. The ice bath was then removed and the reaction mixture was stirred for an additional 1.5 h at room temperature. The mixture was filtered and
THF was removed under reduced pressure. The residue was dissolved CH\textsubscript{2}Cl\textsubscript{2} (100 mL/1 g 7.3a-c) and washed successively with HCl (4N, 2 × 0.7 mL/1 mL of CH\textsubscript{2}Cl\textsubscript{2}), Na\textsubscript{2}CO\textsubscript{3} 10 wt.-% in water (2 × 1 mL/1 mL of CH\textsubscript{2}Cl\textsubscript{2}) and brine (1 × 30 mL). The organic layer was dried over magnesium sulfate, filtered and evaporated. The crude product was then recrystallized from CH\textsubscript{2}Cl\textsubscript{2}/hexanes to yield di-sulfide dipeptidoyl bentrotiazolides 7.4a-e.

(Cbz-L-Cys-D-Pro-Bt)\textsubscript{2} (7.4a). The compound was prepared according to general procedure III for preparation of di-sulfide benzotriazolide from 7.3a (Cbz-L-Cys-D-Pro-OH)\textsubscript{2} (1.50 g, 2.13 mmol), BtH (2.03 g, 17.07 mmol) in and SOCl\textsubscript{2} (0.31 mL, 4.27 mmol). White microcrystals, 1.58 g, 1.75 mmol, 82% yield; mp 119–124 °C. \(^1\)H NMR (DMSO-\textsubscript{d}\textsubscript{6}, 300 MHz): \(\delta 2.02–2.26 (m, 6H), 2.38–2.47 (m, 2H), 2.89 (dd, \(J = 13.7, 8.8 \) Hz, 2H), 3.14 (dd, \(J = 13.7, 5.1 \) Hz, 2H), 3.74–3.95 (m, 4H), 4.72–4.82 (m, 2H), 5.09 (br s, 4H), 5.68–5.78 (m, 2H), 7.28–7.42 (m, 10H), 7.64 (dt, \(J = 8.3, 1.2 \) Hz, 2H), 7.81 (dt, \(J = 8.3, 1.1 \) Hz, 2H), 7.84 (d, \(J = 8.6 \) Hz, 2H), 8.22 (d, \(J = 8.3 \) Hz, 2H), 8.29 (d, \(J = 8.3 \) Hz, 2H). \(^{13}\)C NMR (CDCl\textsubscript{3}, 75 MHz): \(\delta 25.2, 29.9, 41.2, 48.0, 52.1, 60.0, 67.3, 114.7, 120.4, 126.6, 128.2, 128.3, 128.7, 130.7, 131.3, 136.3, 146.2, 155.9, 169.0, 170.3\). HRMS (ESI) calcd for C\textsubscript{44}H\textsubscript{44}N\textsubscript{10}O\textsubscript{8}S\textsubscript{2}Na [M + Na]\textsuperscript{+} 927.2677, found 927.2681.

(Cbz-L-Cys-D,L-Pro-Bt)\textsubscript{2} (7.4b). The compound was prepared according to general procedure III for the preparation of di-sulfide benzotriazolide from 7.3b (Cbz-L-Cys-D,L-Pro-OH)\textsubscript{2} (1.00 g, 1.42 mmol), BtH (1.36 g, 11.39 mmol) and SOCl\textsubscript{2} (0.21 mL, 2.84 mmol). White microcrystals, 1.09 g, 1.21 mmol, 85% yield; mp 111–114 °C. \(^1\)H and \(^{13}\)C NMR were identical to 7.4a.
(Cbz-D,L-Homo-Cys-D-Pro-Bt)$_2$ (7.4c). The compound was prepared according to general procedure III for preparation of di-sulfide dipeptidoyl benzotriazolides from BtH (5.95 g, 50.0 mmol), SOCl$_2$ (0.90 mL, 12.5 mmol) and (Cbz-D,L-Homo-Cys-D-Pro-OH)$_2$ 7.3c (4.57 g, 6.25 mmol). White microcrystals, 5.32 g, 5.7 mmol 91% yield; mp 83–84 °C. $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 1.78–2.00 (m, 4H), 2.01–2.26 (m, 8H), 2.66–2.86 (m, 4H), 3.49–4.29 (m, 4H), 4.34–4.74 (m, 2H), 5.03 (br s, 4H), 5.21–5.32 (m, 1H), 5.60–5.80 (m, 1H), 7.24–7.46 (m, 12H), 7.56–7.84 (m, 4H), 8.14–8.24 (m, 2H), 8.26–8.34 (m, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 25.2, 25.5, 29.9, 32.4, 34.1, 47.9, 51.5, 59.9, 67.2, 114.6, 120.4, 126.4, 126.6, 128.3, 128.7, 128.9, 130.8, 136.3, 146.1, 156.3, 170.2. HRMS (ESI) calcd for C$_{46}$H$_{48}$N$_{10}$O$_{8}$S$_2$Na [M + Na]$^+$ 955.2990, found 955.2996.

(Cbz-L-Cys-L-Hyp(O-tBu)-Bt)$_2$ (7.4d). The compound was prepared according to general procedure III for preparation of di-sulfide dipeptidoyl benzotriazolides from BtH, SOCl$_2$ and (Cbz-L-Cys-L-Hyp(O-tBu)-OH)$_2$ 7.3d). White solid, 0.84 g, 0.80 mmol, 68% yield; mp 74.0–80.0 °C. $^1$H NMR (CDCl$_3$, 300 MHz): δ 8.25 (d, $J = 8.0$ Hz, 2H), 8.12 (d, $J = 8.4$ Hz, 2H), 7.71–7.57 (m, 2H), 7.56–7.46 (m, 2H), 7.45–7.21 (m, 10H), 6.12–5.92 (m, 2H), 5.87 (d, $J = 8.9$ Hz, 2H), 5.20–5.03 (m, 4H), 5.03–4.82 (m, 2H), 4.73–4.19 (m, 2H), 4.18–3.86 (m, 2H), 3.75 (t, $J = 12.9$ Hz, 2H), 3.41–2.71 (m, 4H), 2.60–2.40 (m, 2H), 2.37–2.19 (m, 2H), 1.19 (s, 18H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 170.4, 169.4, 166.7, 156.1, 152.5, 146.2, 136.3, 131.3, 130.7, 128.8, 128.7, 128.3, 128.2, 128.1, 127.7, 126.6, 120.4, 114.7, 74.8, 74.6, 69.9, 69.4, 67.6, 67.3, 67.1, 58.9, 58.8, 55.0, 54.0, 52.2, 42.1, 41.4, 39.3, 38.5, 37.8, 29.9, 28.4. HRMS (ESI): calcd for C$_{52}$H$_{60}$N$_{10}$O$_{10}$S$_2$Na [M + Na]$^+$ 1071.3827, found 1071.3825.
(Cbz-D,L-Homo-Cys-L-Hyp(O-tBu)-Bt)2 (7.4e). The compound was prepared according to general procedure III for preparation of di-sulfide dipeptidoyl benzotriazolides from BtH, SOCl₂ and (Cbz-D,L-Homo-Cys-L-Hyp(O-tBu)-OH)₂ 7.3e. White solid, 0.94 g, 0.87 mmol, 76% yield; mp 77.0–82.0 °C. ¹H NMR (CDCl₃, 300 MHz): δ 8.31 – 8.19 (m, 2H), 8.18 – 8.05 (m, 2H), 7.74 – 7.57 (m, 2H), 7.57 – 7.44 (m, 2H), 7.40 – 7.27 (m, 10H), 6.13 – 5.95 (m, 2H), 5.93 – 5.58 (m, 2H), 5.19 – 5.00 (m, 4H), 4.86 – 4.71 (m, 2H), 4.55 – 4.40 (m, 2H), 4.15 – 3.89 (m, 2H), 3.79 – 3.56 (m, 2H), 2.88 – 2.65 (m, 4H), 2.62 – 2.43 (m, 2H), 2.34 – 2.21 (m, 6H), 1.25 – 1.12 (m, 18H). ¹³C NMR (CDCl₃, 75 MHz): δ 170.5, 170.4, 156.3, 146.3, 136.4, 131.3, 130.8, 128.9, 128.7, 128.4, 128.3, 128.2, 126.6, 126.3, 120.5, 114.7, 74.9, 70.0, 69.7, 67.2, 58.6, 54.7, 51.5, 37.6, 32.5, 29.9, 28.4, 28.3. HRMS (ESI): calcd for C₅₄H₆₄N₁₀O₁₀S₂Na [M + Na]⁺ 1099.4140, found 1099.4135.

General Procedure IV for the Cyclization of Di-Sulfide Dipeptidoyl Bentrotiazolides 7.4a-c to Form Symmetrical Bis-DKPs 7.5a-e

A solution of di-sulfide dipeptidoyl bentrotiazolides 7.4a-e (1 equiv.) and triethylamine (2.2 equiv.) in dry acetonitrile (15 mL/1 g) was stirred at room temperature until the TLC revealed completion of the reaction. The mixture was then concentrated under vacuum and the residue was dissolved in ethyl acetate. The organic layer was washed with 4N HCl (3 × 1 mL/1 mL of ethyl acetate) and Na₂CO₃ 10 wt.-% in water (3 × 1 mL/1 mL of ethyl acetate), and purified by column chromatography (hexanes/ethyl acetate gradient) to give the corresponding symmetrical bis-DKPs 7.5a-e.

Bis-[cyclo-(Cbz-L-Cys-D-Pro)] (7.5a). The compound was prepared according to general procedure IV for preparation of symmetrical bis-DKPs from (Cbz-L-Cys-D-Pro-Bt)₂ 7.4a (0.90 g, 1.00 mmol). White microcrystals, 0.51 g, 0.76 mmol, 76% yield; mp
116–120 °C. \(^1^H\) NMR (CDCl\(_3\), 300 MHz): δ 1.86–1.94 (m, 2H), 1.97–2.11 (m, 4H), 2.38–2.46 (m, 2H), 3.23 (d, \(J = 6.1\) Hz, 2H), 3.42–3.63 (m, 6H), 4.39 (dd, \(J = 9.1, 6.9\) Hz, 2H), 5.04 (dd, \(J = 5.8, 5.8\) Hz, 2H), 5.27 (d, \(J = 7.5\) Hz, 2H), 5.31 (d, \(J = 7.5\) Hz, 2H), 7.31–7.41 (m, 10H). \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): δ 22.5, 29.7, 40.6, 45.8, 60.0, 60.7, 69.7, 128.6, 128.9, 134.7, 152.1, 163.1, 167.3. HRMS (ESI) calcd for C\(_{32}\)H\(_{34}\)N\(_4\)O\(_8\)S\(_2\)Na [M + Na]\(^+\) 689.1710, found 689.1731.

Bis-[cyclo-(Cbz-L-Cys-DL-Pro)] (7.5b). The compound was prepared according to general procedure IV for the preparation of symmetrical bis-DKPs from (Cbz-L-Cys-DL-Pro-Bt)\(_2\) 7.4b (0.75 g, 0.83 mmol). Isolated as mixture of diastereomers. White microcrystals, 0.43 g, 0.65 mmol, 78% yield; mp 96–101 °C. \(^1^H\) and \(^{13}\)C NMR were identical to 7.5a. HRMS (ESI) calcd for C\(_{32}\)H\(_{34}\)N\(_4\)O\(_8\)S\(_2\)Na [M + Na]\(^+\) 689.1710, found 689.1724.

Bis-[cyclo-(Cbz-Homo-DL-Cys-D-Pro)] (7.5c). The compound was prepared according to general procedure IV for preparation of symmetrical bis-DKPs from 7.4c (Cbz-DL-Homo-Cys-D-Pro-Bt)\(_2\) (0.90 g, 0.50 mmol). White microcrystals, 0.29 g, 0.42 mmol 83% yield; mp 85–86 °C. \(^1^H\) NMR (CDCl\(_3\), 300 MHz): δ 1.86–2.24 (m, 10H), 2.35–2.48 (m, 2H), 2.64–2.78 (m, 4H), 3.48–3.59 (m, 4H), 4.26 (t, \(J = 7.6\) Hz, 2H), 4.85 (t, \(J = 7.7\) Hz, 2H), 5.29 (br s, 4H), 7.26–7.44 (m, 10H). \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): δ 23.1, 29.6, 32.0, 34.4, 46.0, 59.9, 60.7, 69.8, 128.8, 129.0, 129.1, 135.0, 152.5, 164.9, 167.7. HRMS (ESI) calcd for C\(_{34}\)H\(_{38}\)N\(_4\)O\(_8\)S\(_2\)Na [M + Na]\(^+\) 717.2023, found 717.2034.

Cyclo-[Bis-Cbz-L-Cys-L-Hyp(O-tBu)] (7.5d). The compound was prepared according to general procedure IV for preparation of symmetrical bis-DKPs from 7.5d (Cbz-L-Cys-L-Hyp(O-tBu)-Bt)\(_2\). Sticky oil, 227 mg, 0.28 mmol, 84% yield. \(^1^H\) NMR
(CD$_3$OD, 300 MHz): δ 7.51 – 7.22 (m, 10H), 5.48 (dd, $J$ = 4.0, 2.0 Hz, 2H), 5.34 (s, 4H), 4.59 (dd, $J$ = 5.5, 4.1 Hz, 2H), 3.78 (dd, $J$ = 11.6, 5.6 Hz, 2H), 3.59 (dd, $J$ = 11.8, 4.1 Hz, 2H), 3.47 (dd, $J$ = 14.3, 4.5 Hz, 2H), 3.35 (dd, $J$ = 4.2, 0.9 Hz, 2H), 2.88 (dd, $J$ = 14.2, 5.2, 1.0 Hz, 2H), 2.33 (dd, $J$ = 14.2, 5.8 Hz, 2H), 2.23 – 2.20 (m, 2H), 2.17 – 1.95 (m, 2H), 1.25 – 1.16 (m, 18H).

$^{13}$C NMR (CD$_3$OD, 75 MHz): δ 168.6, 167.3, 166.3, 164.7, 152.5, 136.7, 136.6, 131.6, 130.1, 130.0, 129.7, 129.6, 129.4, 127.2, 75.9, 71.6, 70.6, 69.2, 60.1, 59.2, 57.0, 55.5, 52.7, 39.8, 38.6, 34.0, 32.4, 28.7. HRMS (ESI): calcd for C$_{40}$H$_{50}$N$_{4}$O$_{10}$S$_{2}$Na [M + Na]$^+$ 833.2860, found 833.2862.

Cyclo-[Bis-Cbz-Homo-$\Delta$-L-Cys-L-Hyp(O-tBu)] (7.5e). The compound was prepared according to general procedure IV for preparation of symmetrical bis-DKPs from 7.5e (Cbz- Cbz-Homo-$\Delta$-L-Cys-L-Hyp(O-tBu)-Bt)$_2$. Sticky oil, 240 mg, 0.29 mmol, 88% yield. $^1$H NMR (CD$_3$OD, 500 MHz): δ 7.49 – 7.25 (m, 10H), 5.35 – 5.24 (m, 4H), 4.98 – 4.87 (m, 2H), 4.72 – 4.50 (m, 2H), 4.48 – 4.33 (m, 2H), 3.80 – 3.69 (m, 2H), 3.60 – 3.38 (m, 2H), 2.83 – 2.65 (m, 4H), 2.53 – 2.43 (m, 2H), 2.34 – 2.18 (m, 4H), 2.17 – 1.95 (m, 2H), 1.25 – 1.16 (m, 18H). $^{13}$C NMR (CD$_3$OD, 75 MHz): δ 172.1, 169.6, 169.0, 166.7, 153.5, 136.7, 136.6, 130.0, 129.8, 129.7, 129.6, 129.4, 129.2, 129.0, 121.2, 76.0, 75.6, 71.2, 70.4, 69.4, 68.9, 68.7, 67.7, 61.4, 60.2, 59.6, 59.0, 55.5, 53.9, 53.4, 44.8, 39.0, 37.8, 35.4, 35.3, 32.3, 30.9, 28.7, 28.6. HRMS (ESI): calcd for C$_{42}$H$_{54}$N$_{4}$O$_{10}$S$_{2}$Na [M + Na]$^+$ 861.3173, found 861.3175.

General Procedure V for the O-, S- and N-Acylation of Bis-Benzotriazolides 7.6a-b and 7.13a-b for Preparation of Compounds 7.7a-b and 7.15a-e

Bis-benzotriazolides 7.6a-b or 7.13a-b (1 equiv.) were each suspended in acetonitrile/water (3:1) (20 mL/1 g) (for S- and N-acylations) or acetonitrile (for O-acylation) and a solution of Cbz-L-Cys-OH (2-2.1 equiv.) or Cbz-L-Lys-OH (2-2.1 equiv.)
in water (10 mL/1 g) containing triethylamine (2.2 equiv.) or Cbz-L-Ser-OH (2-2.1 equiv.) in acetonitrile (10 mL/1 g) containing diisopropylamine (DIPEA) (6.5 equiv.) was added slowly. The mixtures were stirred at 20 °C for 16-72 h until the TLC revealed consumption of the starting materials. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (30 mL/1 g of 7.6a-b, 7.13a-b). The organic layer was washed with 4N HCl (3 × 1 mL/1 mL of ethyl acetate) evaporated and the products were recrystallized from ethyl acetate/hexanes to yield 7.7a-b or 7.15a-e.

3,3-DMG-(Cbz-L-Lys-OH)$_2$ (7.7a). The compound was prepared by N-acylation of 3,3-DMG-(Bt)$_2$ 7.6a (3.94 g, 10.87 mmol) by Cbz-L-Lys-OH (6.09 g, 21.73 mmol) according to general procedure V. Sticky gel, 5.36 g, 7.83 mmol, 72% yield. $^1$H NMR (CD$_3$OD, 300 MHz): δ 1.08 (s, 6H), 1.36–1.61 (m, 8H), 1.64–1.75 (m, 2H), 1.80–1.92 (m, 2H), 2.25 (s, 2H), 2.35 (s, 2H), 3.18 (t, $J = 6.8$ Hz, 2H), 3.26 (t, $J = 6.8$ Hz, 2H), 4.09–4.17 (m, 2H), 5.09 (s, 4H), 7.27–7.38 (m, 10H). $^{13}$C NMR (CD$_3$OD, 75 MHz): δ 24.4, 28.7, 30.0, 32.5, 34.1, 40.2, 47.0, 55.3, 67.7, 128.9, 129.1, 129.6, 138.2, 158.7, 174.4, 176.0. HRMS (ESI) calcd for C$_{35}$H$_{47}$N$_4$O$_{10}$ [M – H]$^-$ 683.3287, found 683.3293.

Trans-1,4-CHD-(Cbz-L-Cys-OH)$_2$ (7.7b). The compound was prepared by S-acylation of trans-1,4-CHD-(Bt)$_2$ 7.6b (1.12 g, 2.99 mmol) according to general procedure V. Sticky gel, 1.51 g, 2.33 mmol, 78% yield. $^1$H NMR (CD$_3$OD, 300 MHz): δ 1.52–1.75 (m, 4H), 1.78–2.06 (m, 4H), 2.40–2.68 (m, 2H), 3.13 (dd, $J = 13.7, 8.9$ Hz, 2H), 3.50 (dd, $J = 13.8, 4.5$ Hz, 2H), 4.36 (dd, $J = 8.6$ Hz, 4.4 Hz, 2H), 5.18–5.01 (m, 4H), 7.21–7.41 (m, 10H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 26.2, 30.6, 49.3, 53.7, 67.4,
Compound 7.7b was characterized by $^1$H and $^{13}$C NMR.

Cbz-L-Asp-(Cbz-L-Ser-OH)-Cbz-L-Ser-OH (7.15a). The compound was prepared by $O$-acylation of Cbz-L-Asp-(Bt)-Bt 7.13a (4.00 g, 8.52 mmol) according to general procedure V. Sticky gel, 5.32 g, 7.50 mmol, 88% yield. $^1$H NMR (CD$_3$OD, 300 MHz): $\delta$ 2.79 (dd, $J = 16.9$, 7.0 Hz, 1H), 2.87 (dd, $J = 16.9$, 7.0 Hz, 1H), 3.79 (ddd, $J = 18.0$, 11.4, 4.5 Hz, 1H), 4.26–4.42 (m, 2H), 4.45–4.63 (m, 4H), 5.07 (br s, 6H), 7.26–7.40 (m, 15H). $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 37.2, 51.9, 54.6, 57.8, 63.2, 65.6, 66.0, 68.0, 68.1, 127.2, 128.9, 129.1, 129.3, 129.5, 137.9, 138.0, 158.4, 158.5, 171.7, 172.0, 172.4, 172.6. Anal. Calcd for C$_{34}$H$_{35}$N$_3$O$_{14}$: C 57.54, H 4.97, N 5.92. Found: C 57.37, H 5.02, N 5.96.

Cbz-L-Glu-(Cbz-L-Ser-OH)-Cbz-L-Ser-OH (7.15b). The compound was prepared by $O$-acylation of Cbz-L-Glu-(Bt)-Bt 7.13b (4.0 g, 8.28 mmol) according to general procedure V. Sticky gel, 5.51 g, 7.62 mmol, 92% yield. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.93–2.04 (m, 1H), 2.21–2.40 (m, 2H), 2.42–2.47 (m, 1H), 3.66 (d, $J = 4.8$ Hz, 1H), 4.00–4.12 (m, 1H), 4.20 (dd, $J = 10.5$, 6.9 Hz, 1H), 4.31–4.42 (m, 2H), 4.48 (dd, $J = 10.8$, 4.5 Hz, 1H), 4.70 (dd, $J = 9.8$, 2.6 Hz, 1H), 4.99–5.07 (m, 4H), 5.15–5.26 (m, 2H), 7.24–7.46 (m, 15H), 7.62–7.80 (m, 2H), 7.91 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 20.9, 30.6, 52.7, 56.6, 58.3, 61.3, 64.0, 65.4, 65.7, 67.3, 127.5, 127.7, 128.1, 128.3, 135.4, 136.8, 150.5, 156.0, 170.5, 170.9, 172.0, 172.8. HRMS (ESI) calcd for C$_{35}$H$_{36}$N$_3$O$_{14}$ [M – H]$^-$ 722.2192, found 722.2181.

Cbz-L-Asp-(Cbz-L-Cys-OH)-Cbz-L-Cys-OH (7.15c). The compound was prepared by $S$-acylation of Cbz-L-Asp-(Bt)-Bt 7.13a (0.97 g, 2.07 mmol) according to
general procedure V. Sticky gel, 1.38 g, 1.86 mmol, 90% yield. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 2.84–3.19 (m, 6H), 4.02–4.19 (m, 2H), 4.42–4.68 (m, 1H), 5.03 (br s, 4H), 5.07 (br s, 2H), 7.22–7.41 (m, 15H), 7.75 (d, $J = 9.0$ Hz, 2H), 8.18 (t, $J = 9.0$ Hz, 1H). $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 27.1, 31.8, 45.9, 54.9, 59.2, 67.9, 128.9, 129.1, 129.6, 138.2, 158.5, 165.0, 173.4, 196.7. HRMS (ESI) calcd for C$_{34}$H$_{34}$N$_3$O$_{12}$S$_2$ [M – H]$^-$ 740.1578, found 740.1594.

Cbz-L-Glu-(Cbz-L-Cys-OH)-Cbz-L-Cys-OH (7.15d). The compound was prepared by S-acylation of Cbz-Glu-(Bt)-Bt 7.13b (4.00 g, 14.2 mmol) according to general procedure V. Sticky gel, 9.65 g, 12.78 mmol, 90% yield. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.94–2.08 (m, 1H), 2.14–2.49 (m, 2H), 2.50–2.71 (m, 1H), 3.05–3.38 (m, 3H), 3.43–3.58 (m, 1H), 4.47–4.80 (m, 3H), 4.99–5.26 (m, 6H), 5.56 (d, $J = 9.0$ Hz, 1H), 5.87 (d, $J = 9.0$ Hz, 1H), 7.23–7.40 (m, 14H), 7.53 (br s, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 22.5, 30.8, 53.3, 65.3, 67.5, 68.8, 128.3, 128.4, 128.7, 134.8, 136.0, 150.8, 156.2, 173.1, 173.7, 198.3. HRMS (ESI) calcd for C$_{35}$H$_{36}$N$_3$O$_{12}$S$_2$ [M – H]$^-$ 754.1735, found 754.1716.

Cbz-L-Asp-(Cbz-L-Lys-OH)-Cbz-L-Lys-OH (7.15e). The compound was prepared by N-acylation of Cbz-L-Asp-(Bt)-Bt 7.7b (4.0 g, 8.52 mmol) by Cbz-L-Lys-OH (4.78 g, 17.05 mmol) according to general procedure V. Sticky gel, 6.27g, 7.92 mmol, 93% yield. $^1$H NMR (CD$_3$OD, 300 MHz): $\delta$ 1.37–1.45 (m, 5H), 1.57–1.72 (m, 5H), 1.80–1.90 (m, 2H), 2.64 (dd, $J = 5.7, 5.7$ Hz, 1H), 2.67 (dd, $J = 5.6, 5.6$ Hz, 1H), 3.00 (dd, $J = 10.7, 5.6$ Hz, 1H), 3.52 (t, $J = 3.9$ Hz, 3H), 4.111–4.15 (m, 2H), 4.32–4.39 (m, 1H), 5.06 (s, 2H), 5.08 (s, 4H), 7.25–7.31 (m, 3H), 7.32–7.38 (m, 12H). $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 23.8, 27.9, 29.7, 32.1, 36.1, 39.4, 51.1, 51.2, 55.1, 67.6, 67.9, 68.0, 128.7, 128.9, 129.1, 129.6, 134.8, 136.0, 150.8, 156.2.
129.4, 137.8, 138.1, 158.1, 158.6, 175.8, 176.9, 178.3. Anal. Calcd for C_{40}H_{49}N_{5}O_{12}: C 60.67, H 6.24, N 8.84. Found: C 60.61, H 6.27, N 9.91.

**General Procedure VI for the Preparation of Bis-Benzo triazolides 7.8a-b and 7.16a-e**

A stirred solution of BtH (8 equiv.) in dry THF (10 mL/1 g) was treated at 20 °C by SOCl2 (2 equiv.). After 20 minutes, a solution of 7.8a-b or 7.15a-e (1 equiv.) in dry THF (15 mL/1 g) was added drop-wise at 20 °C and each resulting solution was stirred for 2 h at -20 °C. The ice bath was removed and each reaction mixture was stirred for additional 2 h at room temperature. The mixtures were filtered, and THF was removed under reduced pressure. Each residue was dissolved in ethyl acetate (50 mL/1 g of 7.7a-b or 7.15a-e) and washed successively with HCl (4N, 2 × 1.5 mL/1 mL of ethyl acetate), Na2CO3 10 wt.-% in water (2 × 1.5 mL/1 mL of ethyl acetate) and brine (1 × 1 mL/1 mL of ethyl acetate). The organic layers were dried over sodium sulfate, filtered, evaporated, and recrystallized from CH2Cl2/hexane to give the corresponding bis-benzotriazolides 7.8a-b or 7.16a-e.

3,3-DMG-(Cbz-L-Lys-Bt)2 (7.8a). The compound was prepared according to general procedure VI for preparation of bis-benzo triazolide from BtH. Sticky gel, 1.01 g, 1.14 mmol, 78% yield. 

\[ ^1H \text{NMR (DMSO-}d_6, 300 MHz): \delta 1.12 (s, 6H), 1.34–1.50 (m, 8H), 1.79–1.96 (m, 4H), 2.25 (s, 4H), 2.92–3.06 (m, 4H), 5.03 (br s, 4H), 5.28–5.52 (m, 2H), 7.28–7.38 (m, 10H), 7.53–7.66 (m, 2H), 7.74–7.81 (m, 2H), 7.85–7.92 (m, 2H), 8.16–8.29 (m, 4H). \]

\[ ^{13}C \text{NMR (CDCl}_3, 75 MHz): \delta 24.5, 28.5, 29.1, 29.8, 30.9, 32.6, 34.1, 44.4, 69.4, 114.6, 120.1, 125.9, 126.3, 127.7, 128.5, 130.5, 131.1, 138.9, 146.1, 156.5, 170.9, 172.4. \]

HRMS (ESI) calcd for C_{47}H_{54}N_{10}O_8Na [M + Na]^+ 909.4018, found 909.4038.
Trans-1,4-CHD-(Cbz-L-Cys-Bt)2 (7.8b). The compound was prepared according to general procedure VI for preparation of bis-benzotriazolides from BtH. Sticky gel, 0.70 g, 0.83 mmol, 83% yield. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.38–1.83 (m, 8H), 2.42–2.58 (m, 2H), 3.51 (dd, $J = 14.6, 4.7$ Hz, 2H), 3.76 (dd, $J = 14.6, 5.3$ Hz, 2H), 5.08–5.17 (m, 4H), 5.87–6.20 (m, 4H), 7.20–7.42 (m, 10H), 7.51 (t, $J = 7.8$ Hz, 2H), 7.64 (t, $J = 7.8$ Hz, 2H), 8.11–8.24 (m, 4H).

$^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 25.7, 26.2, 31.3, 49.1, 54.2, 67.4, 114.3, 120.4, 126.6, 128.2, 128.5, 130.9, 146.1, 155.6, 168.8, 200.9.

HRMS (ESI) calcd for C$_{42}$H$_{40}$N$_8$O$_8$S$_2$Na [$M + Na]^+$ 871.2303, found 871.2322.

Cbz-L-Asp-(Cbz-L-Ser-Bt)-Cbz-L-Ser-Bt (7.16a). The compound was prepared according to general procedure VI from BtH (3.5 g, 29.6 mmol), SOCl$_2$ (0.54 mL, 7.5 mmol) and Cbz-L-Asp-(Cbz-L-Ser-OH)-Cbz-L-Ser-OH 7.15a (2.00 g, 3.7 mmol). White microcrystals, 1.86 g, 2.5 mmol, 68%; mp 80–83 °C. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 2.76 (dd, $J = 17.0, 4.4$ Hz, 1H), 2.87 (dd, $J = 17.4, 4.5$ Hz, 1H), 4.50–4.59 (m, 2H), 4.65–4.73 (m, 1H), 4.77–4.96 (m, 2H), 5.02–5.17 (m, 6H), 5.79–5.90 (m, 1H), 5.94–6.60 (m, 2H), 6.24 (t, $J = 10.2$ Hz, 2H), 7.20–7.37 (m, 15H), 7.43–7.59 (m, 4H), 8.05–8.14 (m, 4H).

$^{13}$C NMR (DMSO–d$_6$, 75 MHz): $\delta$ 35.5, 50.1, 53.6, 63.1, 63.6, 65.7, 66.11, 113.9, 120.22, 126.8, 127.7, 127.9, 128.4, 130.6, 130.9, 131.2, 136.5, 136.6, 136.8, 145.3, 155.7, 156.1, 168.4, 168.5, 169.5, 170.3. HRMS (ESI) calcd for C$_{46}$H$_{41}$N$_9$O$_{12}$Na [$M + Na]^+$ 934.2767, found 934.2756.

Cbz-L-Glu-(Cbz-L-Ser-Bt)-Cbz-L-Ser-Bt (7.16b). The compound was prepared according to general procedure VI for preparation of bis-benzotriazolides from BtH (3.56 g, 29.8 mmol), SOCl$_2$ (2.17 mL, 7.5 mmol) and Cbz-L-Glu-(Cbz-L-Ser-OH)-Cbz-L-Ser-OH 7.15b (2.00 g, 3.7 mmol). White microcrystals, 1.86 g, 2.5 mmol, 68% yield; mp 71–
75 °C. ¹H NMR (CDCl₃, 300 MHz): δ 1.97–2.13 (m, 1H), 2.22–2.37 (m, 1H), 2.39–2.66 (m, 2H), 4.44 (dd, J = 12.3, 5.1 Hz, 1H), 4.66 (dd, J = 9.3, 2.9 Hz, 1H), 4.68 (dd, J = 9.3, 2.3 Hz, 1H), 4.78–4.86 (m, 2H), 5.04–5.20 (m, 4H), 5.21–5.34 (m, 2H), 5.81 (d, J = 7.5 Hz, 1H), 5.94–6.08 (m, 2H), 6.09–6.19 (m, 1H), 6.39–6.52 (m, 1H), 7.28–7.42 (m, 15H), 7.48–7.60 (m, 2H), 7.61–7.70 (m, 2H), 8.09–8.32 (m, 4H). ¹³C NMR (CDCl₃, 75 MHz): δ 21.8, 21.9, 31.2, 54.9, 58.8, 59.0, 65.3, 67.8, 69.0, 114.4, 115.1, 120.7, 126.3, 127.1, 128.4, 128.5, 128.7, 131.1, 131.3, 135.0, 136.0, 138.7, 146.2, 151.5, 156.1, 168.0, 170.8, 172.7, 173.6. HRMS (ESI) calcd for C₄₇H₄₃N₉O₁₂Na [M + Na]⁺ 948.2923, found 948.2938.

Cbz-L-Asp-(Cbz-L-Cys-Bt)-Cbz-L-Cys-Bt (7.16c). The compound was prepared according to general procedure VI for the preparation of bis-benzotriazolides. Sticky gel, 0.99 g, 1.05 mmol, 78% yield. ¹H NMR (CDCl₃, 300 MHz): δ 3.25–3.71 (m, 4H), 3.76–4.00 (m, 2H), 4.50–4.96 (m, 1H), 5.15 (br s, 6H), 5.40–5.80 (m, 2H), 5.80–6.20 (m, 3H), 7.24–7.40 (m, 15H), 7.42–7.59 (m, 3H), 7.60–7.78 (m, 1H), 7.85–8.28 (m, 4H). ¹³C NMR (CDCl₃, 75 MHz): δ 31.6, 36.7, 45.2, 53.4, 54.2, 57.4, 67.9, 115.1, 120.6, 126.3, 126.9, 128.3, 128.8, 131.2, 136.0, 138.8, 146.2, 155.9, 168.9, 196.6. HRMS (ESI) calcd for C₄₆H₄₁N₉O₁₂S₂Na [M + Na]⁺ 966.2310, found 966.2304.

Cbz-L-Glu-(Cbz-L-Cys-Bt)-Cbz-L-Cys-Bt (7.16d). The compound was prepared by general procedure VI for preparation of bis-benzotriazolides. Sticky gel, 0.93 g, 0.97 mmol, 68% yield. ¹H NMR (CD₃OD, 300 MHz): δ 1.92–2.04 (m, 1H), 2.28–2.50 (m, 1H), 2.51–2.81 (m, 2H), 3.47–3.70 (m, 4H), 4.18–4.46 (m, 1H), 5.075.17 (m, 5H), 5.195.29 (m, 1H), 5.88–5.99 (m, 2H), 7.27–7.40 (m, 15H), 7.57–7.69 (m, 2H), 7.71–7.85 (m, 2H), 8.15–8.22 (m, 2H), 8.23–8.28 (m, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 22.6, 27.5, 30.7,
Cbz-L-Asp-(Cbz-L-Lys-Bt)-Cbz-L-Lys-Bt (7.16e). The compound was prepared according to general procedure VI for preparation of bis-benzotriazolide from BtH (2.41 g, 20.22 mmol), SOCl₂ (0.37 mL, 5.05 mmol) and Cbz-L-Asp-(Cbz-L-Lys-OH)-Cbz-L-Lys-OH 7.15e (2.00 g, 2.53 mmol). Sticky gel, 1.86 g, 1.87 mmol 74% yield. ¹H NMR (CDCl₃, 300 MHz): δ 1.44–1.71 (m, 7H), 1.72–1.83 (m, 1H), 1.85–1.98 (m, 2H), 2.05–2.16 (m, 2H), 2.70–2.88 (m, 1H), 2.99–3.06 (m, 2H), 3.51–3.62 (m, 3H), 4.21–4.32 (m, 1H), 5.04–5.13 (m, 7H), 5.64–5.77 (m, 3H), 5.79–5.85 (m, 1H), 6.00 (br s, 1H), 7.10 (br s, 1H), 7.26–7.38 (m, 15H), 7.52 (t, J = 4.7 Hz, 2H), 7.66 (t, J = 4.4 Hz, 2H), 8.12 (d, J = 5.1 Hz, 2H), 8.25 (d, J = 4.8 Hz, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 22.3, 26.6, 32.1, 35.6, 38.3, 50.1, 54.5, 67.2, 114.4, 120.3, 126.5, 128.1, 128.2, 128.3, 128.5, 130.8, 131.1, 135.8, 136.1, 145.9, 156.0, 156.3, 171.7, 174.6, 176.1. HRMS (ESI) calcd for C₅₂H₅₅N₁₁O₁₀Na [M + Na]⁺ 1016.4026, found 1016.4033.

**General Procedure VII for the Coupling of Bis-Benzotriazolides 8a-b and 16a-e with Proline to Prepare Compounds 7.9a-c and 7.17a-e**

Bis-benzotriazolides 7.8a-b or 7.17a-e (1 equiv.) were each suspended in acetonitrile/water (3:1) (15 mL/1 g) and a solution of D- or L-proline (2-2.1 equiv.) in water (10 mL/1 g) containing triethylamine (2-2.2 equiv.) was added slowly. The mixtures were stirred at 20 °C for up to 16 h until the TLC revealed consumption of the starting materials. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (30 mL/1 g of 8a-b or 16a-e). The organic layer was washed with 4N HCl (3 x 1 mL/1 mL of ethyl acetate ) and Na₂CO₃ 10 wt.-% in water (3 x 1 mL/1
mL of ethyl acetate), and purified by column chromatography (ethyl acetate/hexanes
gradient) to yield compounds 7.9a-c or 7.17a-e.

3,3-DMG-(Cbz-L-Lys-D-Pro-OH)2 (7.9a). The compound was prepared by
coupling of bis-benzotriazolide 3,3-DMG-(Cbz-L-Lys-Bt)2 7.8a (2.00 g, 2.30 mmol) with
D-proline (0.52 g, 4.6 mmol) according to general procedure VII. Sticky gel, 1.67 g, 1.89
mmol, 84% yield. 1H NMR (CD3OD, 300 MHz): δ 1.03 (s, 6H), 1.28–1.40 (m, 4H), 1.45–
1.59 (m, 4H), 1.60–1.77 (m, 4H), 1.78–2.12 (m, 5H), 2.13–2.31 (m, 3H), 2.52 (s, 4H),
3.14–3.25 (m, 1H), 3.41–3.55 (m, 1H), 3.58–3.69 (m, 1H), 3.68–3.77 (m, 4H), 3.80–3.87
(m, 1H), 4.00–4.22 (m, 2H), 4.31–4.53 (m, 2H), 5.03–5.15 (m, 4H), 7.28–7.37 (m, 10H).
13C NMR (CD3OD, 75 MHz): δ 24.0, 24.4, 25.8, 27.8, 28.5, 30.1, 32.2, 40.0, 47.0, 54.0,
55.3, 60.8, 67.8, 127.2, 128.9, 129.5, 140.1, 158.2, 174.2, 175.4, 176.0. HRMS (ESI)

Trans-1,4-CHD-(Cbz-L-Cys-D-Pro-OH)2 (7.9b). The compound was prepared by
coupling of bis-benzotriazolide trans-1,4-CHD-(Cbz-L-Cys-Bt)2 7.8b (1.25 g, 1.47 mmol)
with D-proline (0.34 g, 2.95 mmol) according to general procedure VII. Sticky gel, 0.99
g, 1.18 mmol, 80% yield. 1H NMR (DMSO-d6, 300 MHz): δ 1.30–1.99 (m, 14H), 2.01–
2.23 (m, 2H), 2.58–2.74 (m, 2H), 2.91 (dd, J = 13.3, 9.3 Hz, 2H) 3.18 (dd, J = 13.7, 5.0
Hz, 2H), 3.51–3.72 (m, 4H), 4.19 (dd, J = 9.1, 3.7 Hz, 2H), 4.41–4.54 (m, 1H), 4.95–
5.12 (m, 5H), 7.23–7.42 (m, 10H), 7.60–7.76 (m, 2H), 12.45 (br s, 2H). 13C NMR
(DMSO-d6, 75 MHz): δ 24.0, 25.4, 28.4, 29.8, 46.2, 48.2, 51.2, 58.4, 65.2, 127.2, 127.4,
127.9, 136.6, 155.4, 156.6, 167.7, 171.4, 172.6, 200.6, 201.2. Compound 7.9b was
characterized by 1H and 13C NMR.
Trans-1,4-CHD-(Cbz-L-Cys-L-Pro-OH)2 (7.9c). The compound was prepared by coupling of bis-benzotriazolide trans-1,4-CHD-(Cbz-L-Cys-Bt)2 7.8b (1.25 g, 1.47 mmol) with L-proline (0.34 g, 2.95 mmol) according to general procedure VII. Sticky gel, 0.93 g, 1.11 mmol, 75% yield. 1H and 13C NMR were identical to 7.9b.

Cbz-L-Asp-(Cbz-L-Ser-L-Pro-OH)-Cbz-L-Ser-L-Pro-OH (7.17a). The compound was prepared by coupling of bis-benzotriazolide Cbz-L-Asp-(Cbz-L-Ser-Bt)-Cbz-L-Ser-Bt 7.16a (4.0 g, 4.44 mmol) with L-proline (1.40 g, 8.77 mmol) according to general procedure VII. White microcrystals, 3.69 g, 4.08 mmol, 92% yield; Sticky gel. 1H NMR (DMSO-d6, 300 MHz): δ 1.62–1.94 (m, 6H), 1.98–2.20 (m, 2H), 2.72 (dd, J = 16.5, 8.0 Hz, 1H), 2.84 (dd, J = 16.8, 4.7 Hz, 1H), 3.56–3.66 (m, 3H), 3.93–4.05 (m, 2H), 4.22–4.27 (m, 2H), 4.30–4.37 (m, 2H), 4.44–4.53 (m, 2H), 4.57–4.65 (m, 2H), 4.93–5.06 (m, 6H), 7.24–7.37 (m, 15H), 7.71 (d, J = 8.3 Hz, 1H), 7.80 (t, J = 7.6 Hz, 2H). 13C NMR (CD3OD, 75 MHz): δ 25.8, 30.1, 37.1, 51.8, 53.1, 60.6, 64.9, 65.2, 68.0, 115.7, 127.2, 128.7, 128.9, 129.1, 129.5, 138.0, 140.0, 158.3, 169.8, 171.8, 172.2, 175.2. HRMS (ESI) calcd for C44H48N5O16 [M – H]– 902.3091, found 902.3099.

Cbz-L-Glu-(Cbz-L-Ser-D-Pro-OH)-Cbz-L-Ser-D-Pro-OH (7.17b). The compound was prepared by coupling Cbz-L-Glu-(Cbz-L-Ser-Bt)-Cbz-L-Ser-Bt 7.16b (1.0 g, 1.08 mmol) and D-proline (0.25 g, 2.16 mmol) according to general procedure VII. Sticky gel, 0.81 g, 0.89 mmol, 82% yield. 1H NMR (CD3OD, 300 MHz): δ 1.78–2.10 (m, 6H), 2.10–2.32 (m, 3H), 2.34–2.46 (m, 2H), 2.48–2.62 (m, 1H), 3.41–3.78 (m, 1H), 3.57–3.75 (m, 2H), 4.03–4.18 (m, 1H), 4.18–4.46 (m, 3H), 4.48–4.60 (m, 1H), 4.62–4.73 (m, 2H), 4.76–4.89 (m, 1H), 5.04–5.11 (m, 4H), 5.12–5.30 (m, 2H), 7.23–7.38 (m, 15H). 13C NMR (CD3OD, 75 MHz): δ 22.8, 26.2, 30.7, 32.3, 53.1, 60.7, 61.1, 66.0, 68.4, 69.8,
Cbz-L-Asp-(Cbz-L-Cys-L-Pro-OH)-Cbz-L-Cys-L-Pro-OH (7.17c). The compound was prepared by coupling of bis-benzotriazolide Cbz-L-Asp-(Cbz-L-Cys-Bt)-Cbz-L-Cys-Bt 7.16c (1.04 g, 1.10 mmol) with d-Pro-OH (0.25 g, 2.20 mmol) according to general procedure VII. Sticky gel, 0.89 g, 0.95 mmol, 86% yield. **1H NMR** (DMSO-d6, 300 MHz): δ 1.50–2.02 (m, 5H), 2.02–2.35 (m, 2H), 2.62–3.20 (m, 7H), 3.48–3.73 (m, 3H), 3.95–4.14 (m, 1H), 4.14–4.29 (m, 2H), 4.39–4.74 (m, 2H), 4.93–5.18 (m, 7H), 7.25–7.42 (m, 15H), 7.73 (d, J = 8.1 Hz, 2H), 7.80–8.21 (m, 1H). **13C NMR** (CD3OD, 300 MHz): δ 25.8, 30.4, 31.9, 45.9, 53.2, 54.9, 59.2, 60.8, 68.0, 128.9, 129.1, 129.6, 138.2, 158.2, 158.5, 170.6, 173.5, 175.4, 196.7. HRMS (ESI) calcd for C44H48N5O14S2 [M – H]– 934.2634, found 934.2649.

Cbz-L-Glu-(Cbz-L-Cys-D-Pro-OH)-Cbz-L-Cys-D-Pro-OH (7.17d). The compound was prepared by coupling of bis-benzotriazolide Cbz-L-Glu-(Cbz-L-Cys-Bt)-Cbz-L-Cys-Bt 7.16d (1.0 g, 1.04 mmol) with d-Pro-OH (0.24 g, 2.09 mmol) according to general procedure VII. Sticky gel, 0.81 g, 0.89 mmol, 82% yield. **1H NMR** (CD3OD, 300 MHz): δ 1.75–2.06 (m, 8H), 2.12–2.31 (m, 3H), 2.32–2.74 (m, 3H), 2.99–3.18 (m, 2H), 3.31–3.62 (m, 2H), 3.63–3.78 (m, 2H), 4.24–4.48 (m, 3H), 4.54–4.74 (m, 1H), 5.02–5.12 (m, 5H), 5.13–5.26 (m, 2H), 7.23–7.48 (m, 15H). **13C NMR** (CD3OD, 75 MHz): δ 23.4, 25.8, 30.4, 31.7, 32.1, 40.7, 48.0, 52.8, 53.4, 60.8, 61.7 66.8, 68.0, 69.5, 128.9, 129.2, 129.3 129.4, 129.6, 136.6, 138.2, 152.1, 158.3, 158.4, 170.4, 170.7, 175.4, 176.1, 200.4. HRMS (ESI) calcd for C45H50N5O14S2 [M – H]– 948.2790, found 948.2772.
Cbz-L-Asp-(Cbz-L-Lys-D-Pro-OH)-Cbz-L-Lys-D-Pro-OH (7.17e). The compound was prepared by coupling of *bis*-benzotriazolide Cbz-L-Asp-(Cbz-L-Lys-Bt)-Cbz-L-Lys-Bt 7.16e (2.0 g, 2.01 mmol) with D-proline (0.46 g, 4.03 mmol) according to general procedure VII. Sticky gel, 0.77 g, 1.53 mmol, 76% yield. ¹H NMR (CD₃OD, 300 MHz): δ 1.22–1.52 (m, 6H), 1.53–1.80 (m, 7H), 1.82–1.94 (m, 2H), 1.95–2.08 (m, 3H), 2.16–2.32 (m, 2H), 2.63 (dd, J = 5.9, 5.9 Hz, 1H), 2.66 (dd, J = 5.7, 5.7 Hz, 1H), 3.00 (dd, J = 10.8, 5.4 Hz, 1H), 3.08–3.19 (m, 1H), 3.46–3.55 (m, 4H), 3.56–3.67 (m, 1H), 3.78–3.85 (m, 1H), 4.32–4.42 (m, 3H), 4.43–4.48 (m, 1H), 5.05–5.10 (m, 7H), 7.27–7.40 (m, 15H). ¹³C NMR (CDCl₃, 75 MHz): δ 21.8, 22.8, 24.7, 26.9, 28.8, 31.6, 35.6, 28.4, 47.3, 50.0, 52.2, 53.6, 59.5, 66.9, 67.2, 76.8, 77.3, 77.7, 136.0, 136.3, 156.2, 156.5, 171.5, 174.0, 175.0, 176.5. HRMS (ESI) calcd for C₅₀H₆₂N₇O₁₄ [M – H]⁻ 984.4349, found 984.4362.

**General Procedure VIII for the Preparation of Cyclization Precursors 7.10a-c and 7.18a-e**

A stirred solution of BtH (8 equiv.) in dry THF (10 mL/1 g) was treated at 0 °C with SOCl₂ (2 equiv.). After 20 minutes, the reaction mixture was cooled to -45 °C in acetone/dry ice bath and a solution of 7.9a-c or 7.17a-e (1 equiv.) in dry THF (15 mL/1 g) was added drop-wise. The resulting solution was stirred for 6 h at -45.0 °C, after which THF was removed under reduced pressure. The residue was dissolved by ethyl acetate (20 mL/1 g of 9a-c or 18a-e) and washed with brine (2 × 1.5 mL/1 mL), HCl (4N, 2 × 1 mL/1 mL ethyl acetate), Na₂CO₃ 10 wt.-% in water (2 × 1 mL/1 mL ethyl acetate). The organic layer was dried over sodium sulfate, filtered and evaporated. The residue was recrystallized with CH₂Cl₂/hexane to yield compounds 7.10a-c or 7.18a-e.

3,3-DMG-(Cbz-L-Lys-D-Pro-Bt)₂ (7.10a). The compound was prepared by general procedure VIII for preparation of *bis*-benzotriazolide cyclization precursor from BtH (2.17
g, 18.2 mmol), SOCl$_2$ (0.33 mL, 4.6 mmol) and 3,3-DMG-(Cbz-L-Lys-D-Pro-OH)$_2$ 7.9a (2.0 g, 2.30 mmol). Sticky gel, 1.67 g, 1.55 mmol, 68% yield. $^1$H NMR (CDCl$_3$, 300 MHz): δ 1.07 (br s, 6H), 1.14–1.31 (m, 6H), 1.33–1.87 (m, 10H), 2.10–2.31 (m, 4H), 2.50 (br s, 4H), 3.26 (br s, 2H), 3.52–3.58 (m, 1H), 3.68–3.85 (m, 4H), 3.90–4.08 (m, 1H), 4.56–4.70 (m, 2H), 5.03–5.18 (m, 4H), 5.51–5.67 (m, 2H), 5.68–5.82 (m, 2H), 5.88–5.98 (m, 2H), 7.27–7.42 (m, 10H), 7.52 (t, $J$ = 7.2 Hz, 2H), 7.66 (t, $J$ = 7.7 Hz, 2H), 8.13 (d, $J$ = 8.1 Hz, 2H), 8.27 (d, $J$ = 8.4 Hz, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 22.3, 25.2, 27.9, 28.9, 29.3, 29.8, 32.3, 39.0, 46.6, 47.7, 52.5, 59.8, 67.1, 114.7, 120.4, 126.5, 128.1, 128.7, 130.7, 131.4, 136.6, 146.2, 156.0, 170.3, 170.7, 172.2. HRMS (ESI) calcd for C$_{57}$H$_{68}$N$_{12}$O$_{10}$Na [M + Na]$^+$ 1103.5074, found 1103.5096.

$^*$Trans-1,4-CHD-(Cbz-L-Cys-D-Pro-Bt)$_2$ (7.10b). The compound was prepared by general procedure VIII from BtH (0.95 g, 8.00 mmol), SOCl$_2$ (0.15 mL, 2.00 mmol) and trans-1,4-CHD-(Cbz-L-Cys-D-Pro-OH)$_2$ 7.9b (0.84 g, 1.00 mmol). Sticky gel, 0.72 g, 0.85 mmol, 85% yield. $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 1.79–1.52 (m, 5H), 2.05–2.78 (m, 5H), 2.06–2.31 (m, 6H), 2.47–2.71 (m, 2H), 3.30 (dd, $J$ = 14.1, 4.2 Hz, 2H), 3.38 (dd, $J$ = 13.5, 6.6Hz, 2H), 3.64–4.09 (m, 4H), 4.84–4.80 (m, 2H), 5.03–5.18 (m, 4H), 5.70 (dd, $J$ = 14.2 Hz, 8.0 Hz, 2H), 5.84 (dd, $J$ = 8.4 Hz, 3.9 Hz, 2H), 7.26–7.42 (m, 10H), 7.46–7.56 (m, 2H), 7.59–7.69 (m, 2H), 8.12 (d, $J$ = 8.4 Hz, 2H), 8.25 (d, $J$ = 8.4 Hz, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 25.1, 26.1, 29.7, 31.2, 47.6, 49.3, 51.8, 59.8, 66.9, 114.5, 120.2, 126.4, 127.9, 128.0, 128.5, 130.5, 131.2, 136.4, 146.0, 155.8, 168.4, 170.0, 201.6. HRMS (ESI) calcd for C$_{52}$H$_{54}$N$_{10}$O$_{10}$S$_2$Na [M + Na]$^+$ 1065.3358, found 1065.3372.

$^*$Trans-1,4-CHD-(Cbz-L-Cys-L-Pro-Bt)$_2$ (7.10c). The compound was prepared by general procedure VIII from BtH (1.00 g, 8.42 mmol), SOCl$_2$ (0.16 mL, 2.11 mmol) and
trans-1,4-CHD-(Cbz-L-Cys-L-Pro-OH)\(_2\) 7.9c (0.88 g, 1.05 mmol). Sticky gel, 0.78 g, 0.92 mmol, 88% yield. \(^1\)H and \(^{13}\)C NMR were identical to 7.10b.

Cbz-L-Asp-(Cbz-L-Ser-L-Pro-Bt)-Cbz-L-Ser-L-Pro-Bt (7.18a). The compound was prepared according to general procedure VIII from BtH (0.528 g, 4.43 mmol), SOCl\(_2\) (0.16 mL, 2.21 mmol) and Cbz-L-Asp-(Cbz-L-Ser-L-Pro-OH)-Cbz-L-Ser-L-Pro-OH 7.17a (0.5 g, 0.553 mmol). Sticky gel, 0.44 g, 0.40 mmol, 72% yield; \(^1\)H NMR (DMSO-\(d_6\), 300 MHz): δ 1.64–1.98 (m, 2H), 1.98–2.30 (m, 6H), 2.68–2.96 (m, 2H), 3.72–3.90 (m, 3H), 3.95–4.17 (m, 2H), 4.28–4.58, (m, 4H), 4.64–4.80 (m, 2H), 5.02 (br ss, 6H), 5.68–5.84 (m, 2H), 7.24–7.42 (m, 15H), 7.56–7.68 (m, 3H), 7.68–7.85 (m, 4H), 8.08–8.30 (m, 4H). \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): δ 25.3, 29.8, 36.7, 47.9, 50.9, 51.8, 60.1, 67.3, 114.5, 120.3, 126.5, 127.7, 128.2, 128.6, 130.8, 131.2, 136.3, 146.0, 156.2, 167.6, 169.9, 170.4. HRMS (ESI) calcd for C\(_{56}\)H\(_{55}\)N\(_{11}\)O\(_{14}\)Na [M + Na]\(^+\) 1128.3828, found 1128.3845.

Cbz-L-Glu-(Cbz-L-Ser-D-Pro-Bt)-Cbz-L-Ser-D-Pro-Bt (7.18b). The compound was prepared according to general procedure VIII from BtH (1.56 g, 13.07 mmol), SOCl\(_2\) (0.24 mL, 3.27 mmol) and Cbz-L-Glu-(Cbz-L-Ser-D-Pro-OH)-Cbz-L-Ser-D-Pro-OH 7.17b (1.50 g, 1.63 mmol). White microcrystals, 1.24 g, 1.11 mmol 68% yield; mp 85–89 °C. \(^1\)H NMR (CDCl\(_3\), 300 MHz): δ 1.88–2.10 (m, 6H), 2.14–2.23 (m, 2H), 2.28–2.48 (m, 2H), 2.48–2.76 (m, 2H), 3.45–3.68 (m, 3H), 3.82–3.94 (m, 1H), 4.19–4.42 (m, 3H), 4.48–4.66 (m, 3H), 4.92–5.08 (m, 2H), 5.12–5.17 (m, 1H), 5.20–5.35 (m, 4H), 5.74 (d, J = 8.2 Hz, 2H), 5.86–5.93 (m, 3H), 7.28–7.40 (m, 15H), 7.50–7.56 (m, 2H), 7.64–7.70 (m, 2H), 8.13–8.16 (m, 2H), 8.2–8.27 (m, 2H). \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): δ 21.9, 22.3, 25.2, 29.6, 29.9, 31.2, 45.8, 52.1, 58.8, 59.8, 60.1, 64.8, 67.4, 68.7, 67.0, 69.9, 114.6,

Cbz-L-Asp-(Cbz-L-Cys-L-Pro-Bt)-Cbz-L-Cys-L-Pro-Bt (7.18c). The compound was prepared according to general procedure VIII from BtH (2.00 g, 16.8 mmol), SOCl₂ (0.30 mL, 4.2 mmol) and Cbz-L-Asp-(Cbz-L-Cys-D-Pro-OH)-Cbz-L-Cys-D-Pro-OH (2.00 g, 2.1 mmol). Sticky gel, 1.60 g, 1.40 mmol, 67% yield. ¹H NMR (CDCl₃, 300 MHz): δ 1.89–2.32 (m, 8H), 2.40–2.72 (m, 2H), 2.78–3.45 (m, 4H), 3.48–3.82 (m, 3H), 3.82–4.08 (m, 2H), 4.34–4.94 (m, 2H), 4.95–5.28 (m, 6H), 5.46–5.78 (m, 1H), 5.78–5.98 (m, 2H), 5.98–6.12 (m, 1H), 6.18–6.50 (m, 1H), 7.15–7.44 (m, 15H), 7.50–7.62 (m, 2H), 7.62–7.74 (m, 2H), 8.08–8.20 (m, 2H), 8.20–8.32 (m, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 24.9, 28.6, 29.6, 31.3, 40.6, 44.7, 47.6, 51.5, 51.9, 57.4, 59.5, 59.8, 67.1, 67.3, 114.3, 114.8, 120.1, 120.2, 125.7, 126.3, 126.5, 127.9, 130.5, 130.9, 131.0, 135.9, 136.1, 138.5, 145.8, 155.9, 168.7, 169.7, 195.9, 199.5. HRMS (ESI) calcd for C₅₆H₅₅N₁₁O₁₂S₂Na [M + Na]+ 1160.3365, found 1160.3355.

Cbz-L-Glu-(Cbz-L-Cys-D-Pro-Bt)-Cbz-L-Cys-D-Pro-Bt (7.18d). The compound was prepared by general procedure VIII from BtH (2.00 g, 16.8 mmol), SOCl₂ (0.30 mL, 4.2 mmol) and Cbz-L-Glu-(Cbz-L-Cys-D-Pro-OH)-Cbz-L-Cys-D-Pro-OH (2.0 g, 2.1 mmol). White microcrystals, 1.50 g, 1.3 mmol 62% yield; mp 93–98 °C. ¹H NMR (CDCl₃, 300 MHz): δ 1.80–2.09 (m, 1H), 2.10–2.34 (m, 6H), 2.362.78 (m, 5H), 3.10–3.40 (m, 3H), 3.40–3.65 (m, 2H), 3.88–4.44 (m, 3H), 4.77 (dd, J = 9.5, 2.3 Hz, 1H), 4.78–4.94 (m, 1H), 4.98–5.16 (m, 5H), 5.20–5.38 (m, 2H), 5.73 (d, J = 9 Hz, 2H), 5.76–5.88 (m, 3H), 7.33–7.45 (m, 15H), 7.46–7.55 (m, 2H), 7.60–7.68 (m, 2H), 8.00–8.15 (m, 2H), 8.20–
8.26 (m, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 22.6, 25.2, 29.9, 31.0, 31.7, 47.7, 51.3, 60.0, 60.3, 65.3, 67.2, 68.7, 114.6, 120.4, 125.8, 126.3, 128.3, 128.7, 130.8, 131.3, 135.1, 136.3, 146.1, 151.1, 155.9, 168.0, 170.1, 173.2, 199.0. HRMS (ESI) calcd for C$_{57}$H$_{57}$N$_{11}$O$_{12}$S$_2$Na $[\text{M + Na}]^+$ 1174.3522, found 1174.3541.

Cbz-L-Asp-(Cbz-L-Lys-D-Pro-Bt)-Cbz-L-Lys-D-Pro-Bt (7.18e). The compound was prepared by general procedure VIII for preparation of bis-benzotriazolide cyclization precursor from BtH (0.97 g, 8.12 mmol), SOCl$_2$ (0.15 mL, 2.03 mmol) and Cbz-L-Asp-(Cbz-L-Lys-D-Pro-OH)-Cbz-L-Lys-D-Pro-OH 7.17e (1.00 g, 1.01 mmol). Sticky gel, 0.83 g, 0.70 mmol, 69% yield. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.28–1.42 (m, 5H), 1.56–1.64 (m, 5H), 1.64–1.84 (m, 5H), 2.12–2.26 (m, 5H), 2.50–2.66 (m, 2H), 3.03 (dd, $J = 10.8$, 5.4 Hz, 2H), 3.50–3.66 (m, 3H), 3.68–3.74 (m, 2H), 3.92–3.98 (m, 1H), 4.36–4.48 (m, 2H), 4.55–4.61 (m, 1H), 5.03–5.13 (m, 7H), 5.60–5.68 (m, 2H), 5.92 (dd, $J = 5.4$, 2.1 Hz, 2H), 5.98 (d, $J = 3.0$ Hz, 1H), 7.27–7.36 (m, 17H), 7.48–7.54 (m, 2H), 7.65 (t, $J = 4.4$ Hz, 2H), 8.11–8.14 (m, 2H), 8.22–8.27 (m, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 21.8, 21.9, 25.1, 26.9, 29.7, 32.4, 35.9, 38.5, 47.6, 50.1, 52.1, 59.7, 67.0, 67.4, 114.7, 120.4, 126.6, 127.9, 128.1, 128.2, 128.3, 128.4, 128.7, 130.7, 131.3, 136.4, 146.1, 156.2, 156.3, 170.3, 170.7, 174.7, 176.1. HRMS (ESI) calcd for C$_{62}$H$_{69}$N$_{13}$O$_{12}$Na $[\text{M + Na}]^+$ 1210.5081, found 1210.99.

**General Procedure IX for the Cyclization of Precursor 7.10a-c to Form Symmetrical Bis-DKPs 7.11a-c and 7.18a-e to Form Unsymmetrical Bis-DKPs 7.19a-e**

A solution of 7.10a-c or 7.18a-e (1 equiv.) and triethylamine (2–2.2 equiv.) in dry acetonitrile (100 mL) was stirred for 48–84 h until TLC revealed cyclization was complete. Each mixture was concentrated under vacuum and the residue was dissolved in ethyl acetate (20 mL/1 g of 7.11a-c or 7.19a-e). The organic layer was washed with
4N HCl (3 x 1 mL/1 mL ethyl acetate) and Na₂CO₃ 10 wt.-% in water (3 x 1 mL/1 mL ethyl acetate), and purified by column chromatography (CH₂Cl₂/hexanes gradient) to give the corresponding symmetrical bis-DKPs 7.11a-c or unsymmetrical bis-DKPs 7.19a-e.

3,3-DMG-cyclo-(Cbz-L-Lys-D-Pro)]₂ (7.11a). The unsymmetrical bis-DKP was prepared by macro-cyclization of 3,3-DMG-(Cbz-L-Lys-D-Pro-Bt)₂ 7.10a (0.50 g, 0.46 mmol) according to general procedure IX. Sticky gel, 0.35 g, 0.42 mmol, 91% yield. ¹H NMR (CD₃OD, 300 MHz): δ 0.98 (br s, 6H), 1.22–1.34 (m, 8H), 1.42–1.63 (m, 6H), 1.78–2.00 (m, 4H), 2.13–2.24 (m, 1H), 2.28–2.37 (m, 1H), 2.47 (br s, 4H), 3.07–3.19 (m, 1H), 3.37–3.52 (m, 2H), 3.54–3.61 (m, 1H), 3.62–3.73 (m, 3H), 3.73–3.83 (m, 1H), 4.02–4.38 (m, 2H), 4.40–4.82 (m, 2H), 4.99–5.27 (m, 4H), 7.21–7.36 (m, 8H), 7.37–7.44 (m, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 22.8, 24.8, 27.8, 29.4, 29.9, 31.4, 38.6, 46.5, 52.5, 59.6, 61.5, 67.1, 69.9, 128.1, 128.7, 129.0, 134.9, 156.1, 165.3, 167.9, 172.3. HRMS (ESI) calcd C₄₅H₅₈N₆O₁₀Na [M + Na]⁺ 865.4107, found 865.4125.

Trans-1,4-CHD-[cyclo-(Cbz-L-Cys-D-Pro)]₂ (7.11b). The compound was prepared by macro-cyclization of bis-benzotriazolide precursor trans-1,4-CHD-(Cbz-L-Cys-D-Pro-Bt)₂ 7.10b (1.05 g, 1.24 mmol) according to general procedure IX. Sticky gel, 0.85 g, 1.05 mmol, 85% yield. ¹H NMR (CD₃OD, 300 MHz): δ 1.49–1.67 (m, 3H), 1.74–2.12 (m, 12H), 2.34–2.46 (m, 2H), 2.50–2.58 (m, 1H), 3.34–3.59 (m, 8H), 4.66 (dd, J = 8.0, 8.0 Hz, 2H), 4.95 (dd, J = 7.1, 7.1 Hz, 2H), 5.22–5.36 (m, 4H), 7.22–7.45 (m, 10H). ¹³C NMR (CD₃OD, 75 MHz): δ 23.5, 27.2, 27.4, 30.3, 46.9, 50.0, 61.3, 61.7, 70.4, 129.6, 129.7, 129.8, 136.7, 153.6, 165.4, 169.0, 202.3. HRMS (ESI) calcd for C₄₀H₄₄N₄O₁₀Na [M + Na]⁺ 827.2391, found 827.2371.
**Trans-1,4-CHD-[cyclo-(Cbz-L-Cys-L-Pro)]₂ (7.11c).** The compound was prepared by macro-cyclization of bis-benzotriazolide precursor trans-1,4-CHD-(Cbz-L-Cys-L-Pro-Bt)₂ 7.10c (0.78 g, 0.93 mmol) according to general procedure IX. Sticky gel, 0.61 g, 0.75 mmol, 81% yield. ¹H and ¹³C NMR were identical to 7.11b. HRMS (ESI) calcd for C₄₀H₄₄N₄O₁₀Na [M + Na]⁺ 827.2391, found 827.2398.

Cbz-L-Asp-[cyclo-(Cbz-L-Ser-L-Pro)]-cyclo-(Cbz-L-Ser-L-Pro) (7.19a). The compound was prepared by macro-cyclization of bis-benzotriazolide precursor Cbz-L-Asp-(Cbz-L-Ser-L-Pro-Bt)-Cbz-L-Ser-L-Pro-Bt 7.18a (1.0 g, 0.9 mmol) according to general procedure IX. Sticky gel, 0.56 g, 0.65 mmol, 72% yield. ¹H NMR (DMSO-ᴅ₆, 300 MHz): δ 1.69–1.98 (m, 6H), 2.02–2.24 (m, 2H), 2.54–2.78 (m, 2H), 3.54–3.62 (m, 4H), 3.68–4.06 (m, 2H), 4.23–4.80 (m, 6H), 4.82–4.92 (m, 1H), 4.94–5.06 (m, 4H), 5.20–5.29 (m, 2H), 7.24–7.43 (m, 15H), 7.68–7.70 (m, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ 22.4, 22.8, 29.6, 31.8, 36.1, 45.8, 50.3, 60.0, 64.0, 65.2, 66.0, 67.3, 67.5, 69.8, 128.3, 128.5, 128.7, 128.8, 134.6, 136.0, 152.2, 156.0, 162.5, 167.3, 170.3. HRMS (ESI) calcd for C₄₄H₄₅N₅O₁₄Na [M + Na]⁺ 890.2855, found 890.2828.

Cbz-L-Glu-[cyclo-(Cbz-L-Ser-D-Pro)]-cyclo-(Cbz-L-Ser-D-Pro) (7.19b). The bis-DKP was prepared by macro-cyclization of Cbz-L-Glu-(Cbz-L-Ser-D-Pro-Bt)-Cbz-L-Ser-D-Pro-Bt 7.18b (0.25 g, 0.23 mmol) according to general procedure IX. White microcrystals, 178.4 mg, 0.20 mmol, 88% yield; mp 70–72 °C. ¹H NMR (CD₂OD, 300 MHz): δ 1.91–2.10 (m, 6H), 2.26–2.45 (m, 4H), 2.46–2.60 (m, 2H), 3.41–3.62 (m, 4H), 4.364.44 (m, 2H), 4.49 (dd,   𝐽 = 12.0, 4.5 Hz, 2H), 4.57 (dd,  𝐽 = 11.7, 4.2 Hz, 2H), 4.73 (dd,  𝐽 = 9.5, 2.9 Hz, 1H), 5.01 (t,  𝐽 = 4.2 Hz, 2H), 5.13–5.30 (m, 2H), 5.31 (br s, 4H), 7.28–7.39 (m, 12H), 7.43–7.47 (m, 3H). ¹³C NMR (CD₂OD, 75 MHz): δ 22.6, 23.2, 26.7,
Cbz-L-Asp-[cyclo-(Cbz-L-Ser-L-Pro)]-cyclo-(Cbz-L-Ser-L-Pro) (7.19c). The compound was prepared by macro-cyclization of bis-benzotriazolide precursor Cbz-L-Asp-(Cbz-L-Cys-L-Pro-Bt)-Cbz-L-Cys-L-Pro-Bt 7.18c (1.14 g, 1.00 mmol) according to general procedure IX. Sticky gel, 0.69 g, 0.76 mmol, 76% yield. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 1.72–2.04 (m, 8H), 2.18–2.24 (m, 2H), 3.21–3.30 (m, 2H), 3.38–3.42 (m, 6H), 4.60 (dd, $J = 9.8$, 5.6 Hz, 2H), 4.76–4.83 (m, 1H), 4.91 (t, $J = 4.4$ Hz, 1H), 4.98–5.12 (m, 3H), 5.20–5.34 (m, 4H), 7.22–7.51 (m, 16H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 22.2, 28.6, 29.4, 37.9, 45.3, 59.0, 59.5, 65.6, 66.0, 68.4, 127.7, 127.9, 128.2, 135.2, 136.6, 136.9, 151.7, 151.9, 155.8, 162.3, 162.4, 166.9, 167.3, 199.6, 199.9. HRMS (ESI) calcd for C$_{44}$H$_{45}$N$_5$O$_{14}$S$_2$Na $[M + Na]^+$ 922.2398, found 922.2382.

Cbz-L-Glu-[cyclo-(Cbz-L-Cys-D-Pro)]-cyclo-(Cbz-L-Cys-D-Pro) (7.19d). The compound was prepared by macro-cyclization of bis-benzotriazolide precursor Glu-(Cbz-L-Cys-D-Pro-Bt)-Cbz-L-Cys-D-Pro-Bt 7.18d (0.25 g, 0.22 mmol) according to general procedure IX. Sticky gel, 0.17 g, 0.19 mmol, 86% yield. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 1.88–2.37 (m, 9H), 2.38–2.52 (m, 3H), 2.53–2.67 (m, 1H), 3.29 (dd, $J = 14.1$, 5.8 Hz, 2H), 3.24–3.43 (m, 3H), 3.46–3.66 (m, 4H), 4.50 (dd, $J = 9.3$, 7.2 Hz, 2H), 4.78 (dd, $J = 9.0$, 2.4 Hz, 1H), 4.93 (dd, $J = 8.7$, 5.7 Hz, 1H), 5.04–5.16 (m, 1H), 5.18–5.37 (m, 6H), 7.25–7.45 (m, 16H). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 22.5, 22.6, 28.9, 29.4, 30.9, 45.7, 59.7, 60.3, 65.1, 68.7, 69.8, 128.3, 128.4, 128.5, 128.7, 128.9, 134.6, 135.0,
Cbz-L-Asp-[cyclo-(Cbz-L-Lys-D-Pro)]-cyclo-(Cbz-L-Lys-D-Pro) (7.19e). The unsymmetrical bis-DKP was prepared by macro-cyclization of Cbz-L-Asp-(Cbz-L-Lys-D-Pro-Bt)-Cbz-L-Lys-D-Pro-Bt 7.18e (0.35 g, 0.29 mmol) according to general procedure IX. Sticky gel, 0.24 g, 0.26 mmol, 88% yield. \(^1\)H NMR (CD\(_3\)OD, 300 MHz): \(\delta\)

- 1.34–1.44 (m, 4H), 1.49–1.68 (m, 3H), 1.77–1.89 (m, 2H), 1.90–2.01 (m, 5H), 2.03–2.12 (m, 2H), 2.33–3.39 (m, 2H), 2.62 (dd, \(J = 5.0\), 5.0 Hz, 1H), 2.66 (dd, \(J = 5.0\), 5.0 Hz, 1H), 2.95–3.03 (m, 2H), 3.43–3.53 (m, 6H), 4.33–4.40 (m, 2H), 4.47–4.54 (m, 1H), 4.68–4.75 (m, 2H), 5.08 (br s, 4H), 5.26–5.32 (m, 2H), 7.25–7.38 (m, 13H), 7.42–7.46 (m, 2H). \(^1\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\)

- 21.9, 22.6, 22.7, 26.3, 29.2, 30.4, 36.1, 38.1, 45.8, 50.0, 59.4, 60.8, 67.4, 69.4, 128.3, 128.5, 128.7, 128.8, 134.8, 136.1, 152.1, 156.3, 165.1, 167.6, 174.9, 176.5. HRMS (MALDI-TOF) calcd for C\(_{50}\)H\(_{59}\)N\(_7\)O\(_{12}\)Na [M + Na]\(^+\) 972.4114, found 972.4115.
CHAPTER 8
SYNTHESIS OF POLYKETIDE FRAGMENT OF APRATOXINS

Introduction

Apratoxins\textsuperscript{150,151,152} (Figure 8-1) are marine natural products of mixed biogenetic origin. Apratoxins were isolated from cyanobacterial \textit{Lyngbya} spp. (now known as \textit{Moorea bouillonii}) collected in Guam and Palau.\textsuperscript{32,153,154} They are cyclodepsipeptides that embody both polypeptide and polyketide domains. In contrast to most known potent anticancer natural products, the cellular and molecular basis of apratoxin action is the inhibition of cotranslational translocation.\textsuperscript{155}

![Chemical structures of apratoxins]

Figure 8-1. Structures of natural apratoxins

The first member, apratoxin A, was isolated in 2001 by Moore, Paul and co-workers\textsuperscript{31} from the marine cyanobacterium \textit{Lyngbya majuscula} (Finger's Reef, Apra Harbor, Guam). Apratoxin A exhibited potent \textit{in vitro} cytotoxicity against KB and LoVo cancer cell lines, with IC\textsubscript{50} values of 0.52 nM and 0.38 nM, respectively. Apratoxin A is composed of discrete polyketide and polypeptide domains, joined via amide and ester
linkages (Figure 8-1). The polypeptide domain contains three methylated L-amino acid residues (O-methyltyrosine, N-methyl alanine, N-methylisoleucine), one L-proline residue, a modified D-cysteine residue; and the polyketide domain contains four stereogenic centers. The slight structural differences in the apratoxin family showed mixed biogenetic origins. This variation is common among cyanobacteria, which often produce more than one member of a certain class of compounds due to relaxed specificity of biosynthetic enzymes during biosynthesis of precursor.

Apratoxin A was rigorously profiled and shown to possess broad-spectrum but differential in vitro activity. Because of their biological activity and intriguing structures, they have been subject to several total syntheses and SAR studies. Recently apratoxins were found to prevent cotranslational translocation and thereby downregulate various receptors, including receptor tyrosine kinases (RTKs). They inhibit trafficking of other secretory molecules, including growth factors that act on RTKs.

<table>
<thead>
<tr>
<th>Apratoxin</th>
<th>IC_{50} (nM) cell viability</th>
<th>IC_{50} (nM) VEGF-A secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>S4 (30S,34-Me)</td>
<td>1.43</td>
<td>0.32</td>
</tr>
<tr>
<td>S7 (30S,34-H_2)</td>
<td>1.25</td>
<td>0.30</td>
</tr>
<tr>
<td>S8 (30S,34-Me_2)</td>
<td>1.99</td>
<td>0.47</td>
</tr>
<tr>
<td>S9 (30R,34-Me)</td>
<td>0.69</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Figure 8-2. Activities of synthetic apratoxins

A novel more potent apratoxin S4 was synthesized, and showed 2–3-fold improved in vitro activity, even though the new analog still had the potential to be deactivated by dehydration. However, the new apratoxin was found to be stable during repurifications and stability tests. It is important to develop synthetic approaches to the novel apratoxin derivatives to further study their biological applications.
The synthesis of apratoxin A was first described by Forsyth. Their strategy for constructing the sensitive thiazoline moiety was a one-pot Staudinger reduction/intramolecular aza-Wittig reaction. Other routes toward apratoxin A have also been reported by Takahashi, Doi and Ma. As shown in Figure 8-3, apratoxin A could be disconnected to yield tetrapeptide and the polyketide fragments containing a thiazoline ring (Figure 8-3, Strategy I).

Figure 8-3. Retrosynthetic analysis of apratoxin A.

The most common and effective strategy toward total synthesis of apratoxin A is based on condensation of the peptide and polyketide domains with the proline residue (Figure 8-3, Strategy II). This strategy was used with a higher yielding amide formation between the isoleucine carboxylate of tripeptide and the proline amine moiety. A useful strategy in the syntheses of apratoxin A is also the late stage assembly of the thiazoline moiety, which is oxidatively sensitive and potentially prone to unwanted side reactions. On the other hand, tripeptide fragments can theoretically be accessed in a relatively
straightforward way using standard peptide synthesis procedures. The polyketide and thiazoline portions of the structure present a significant synthetic challenge, which necessitated the development of new synthetic strategies.

Figure 8-4 shows detailed retro synthetic analysis of synthetic apratoxin S4. The key step for the synthesis of apratoxin S4 is the formation of the thiazoline ring in the intermediate from the open-chain cyclization precursor (Figure 8-4). Experiments showed that different degrees of methylation at C34 or different configuration at C30 affected the ring formation. Compound 8.3 (Figure 8-4) can be disconnected into a modified cysteine and
proline ester-based carboxylic acid 8.4 (Figure 8-4), which in turn can be synthesized from aldehyde 8.5.

This chapter describes the large-scale synthesis of aldehyde 8.5 and its asymmetric crotylation. Aldehyde 8.5 can be used for synthesis of modified apratoxins for further biological studies.

**Results and Discussion**

**Multigram Synthesis of the Key Aldehyde**

The synthesis of aldehyde 8.5 has been reported using different strategies, but, the scale of preparation was typically only a few grams. Aldehyde 8.5 can be prepared from alcohol 8.6 (Figure 8-5) via a 6 step procedure. However, its preparation on a large scale is a challenging undertaking because the alcohol substrate 8.2 and diene ester 8.8 have low molecular weights and boiling points. The preparations involve highly reactive bases which can ignite spontaneously if exposed to air. Moreover, the ring closing metathesis of diene 8.8 leading to lactone 8.9 requires high dilution ring-closing conditions (Figure 8-5).

![Chemical Structure](image)

Figure 8-5. Synthesis of aldehyde 8.5

The synthesis of compound 8.5 can be achieved from alcohol 8.6 which can be synthesized from pivalaldehyde 8.13 (Figure 8-6). To install the C-40 stereocenter, β-
hydroxy ketone 8.14 was prepared via a D-proline catalyzed aldol reaction of pivalaldehyde 8.13 with acetone. Ketone 8.14 then underwent protection with TBS followed by reduction with NaBH₄, and elimination through mesylate to form 155 g of TBS protected alkenyl alcohol 8.17. TBS deprotection of 8.17 afforded allyl alcohol 8.6, which is the key step in the preparation of 8.6 due to its high volatile property. The solvent (Et₂O and THF) can be removed by distillation using a Vigreux fractionation column in small-scale reactions. However, for large-scale reactions, this was not feasible and we used a combination of a cooling method and Vigreux fraction, which gave 78 g of 8.6 in 96% yield.

Alcohol 8.6 was then treated with acryloyl chloride 8.7 in the presence of TEA to form diene ester 8.8 (Figure 8-5). Ring closing metathesis (RCM) of diene 8.8 to form lactone 8.9 can be carried out in the presence of first generation or second generation of Grubbs’ catalysts (Figure 8-7). In addition, titanium tetra-isopropoxide can be added in catalytic amount to increase the yield of the RCM product. The RCM reaction of diene ester 8.8 also require highly diluted conditions to favor the intramolecular cyclization product. High dilution conditions can be challenging to carry out on a large scale reaction.
The RCM of the diene ester 8.8 was carried out under several conditions that are presented in Table 8-1. Under similar conditions, second generation of Grubbs' catalyst proved to be more efficient (Table 8-1 entries 1, 2). Higher substrate concentration resulted in an unsatisfactory yield of the desired compound (Table 8-1, entry 3).

Table 8-1. Conditions for ring closing metathesis of diene 8.8

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst, mol%</th>
<th>Amount of 8.8 (g)</th>
<th>Ti(OiPr)$_4$ (mol%)</th>
<th>Concentration of substrate 8.8 (mM)</th>
<th>Reaction time (h)</th>
<th>Yield, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2$^{nd}$ gr, 10%</td>
<td>0.523</td>
<td>NA</td>
<td>2</td>
<td>6</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>1$^{st}$ gr, 15%</td>
<td>0.523</td>
<td>NA</td>
<td>2</td>
<td>6</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>1$^{st}$ gr, 15%</td>
<td>0.523</td>
<td>NA</td>
<td>3</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>2$^{nd}$ gr, 15%</td>
<td>0.523</td>
<td>20</td>
<td>2</td>
<td>6</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>2$^{nd}$ gr, 15%</td>
<td>6</td>
<td>20</td>
<td>2</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>2$^{nd}$ gr, 15%</td>
<td>6</td>
<td>NA</td>
<td>2</td>
<td>4</td>
<td>69</td>
</tr>
</tbody>
</table>

When the diene ester 8.8 was converted to the corresponding lactone 8.9 in the presence of 20 mol% Ti(OiPr)$_4$ and 15 mol% of second generation of Grubbs' catalyst, the RCM reaction gave lactone 8.9 in higher yields (Table 8-1, entry 4). Using second generation of Grubbs' catalyst with Ti(OiPr)$_4$ additive, however, the reaction gave the product 8.9 in similar yields (Table 8-1, entries 4 and 5). Considering the price of
reagents for a scale-up preparation and the product yield, we chose the coupling conditions of entry 4 for the improved RCM process of ester 8.8 (Table 8-2).

Table 8-2: Ring closing metathesis of compound 8.5

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amount of 8.8 (g)</th>
<th>Ti(OiPr)$_4$ (mL)</th>
<th>Solvent (DCM, L)</th>
<th>Reaction times (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>6.2</td>
<td>4.5</td>
<td>5.5</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>6.4</td>
<td>4.8</td>
<td>5</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>6.3</td>
<td>4.7</td>
<td>4.8</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>6.4</td>
<td>4.5</td>
<td>5.6</td>
<td></td>
</tr>
</tbody>
</table>

Lactone 8.9 was then subjected to a conjugate addition with methyllithium and copper (I) cyanide to provide the methyl-substituted lactone 8.10 as the only detectable stereoisomer (Figure 8-8, Table 8-3).

Figure 8-8. 1,4-Addition to lactone 8.8 in the presence of Cu (I) salt

The stereochemical outcome resulted from the mixed higher order cyanocuprate-mediated 1,4-conjugated addition. The kinetically controlled conjugate addition to a conjugated enone takes place preferentially by way of the chair-like enolate, which resulted from the more stable half-chair conformer. As shown in Figure 8-9, attack on the top face of the more stable conformation gives the chairlike enolate ion, which then in turn produced 8.10. The sterically hindered t-butyl group enhanced the selectivity via formation of the most stable conformer.
Figure 8-9. Stereoselectivity in 1,4–addition of lactone 8.9

Table 8-3. Copper (I) mediated 1,4–addition of α,β-unsaturated lactone 8.9

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amount of lactone 8.9 (g)</th>
<th>Amount of CuCN (g), 1.6 M in diethyl ether</th>
<th>Reaction time (h)</th>
<th>Yield (g,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>2.1, 30</td>
<td>10</td>
<td>2.81, 86%</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>4.3, 62</td>
<td>11</td>
<td>5.83, 88%</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>5.6, 85</td>
<td>14</td>
<td>7.24, 82%</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>5.6, 88</td>
<td>12</td>
<td>17, 87%</td>
</tr>
<tr>
<td>5</td>
<td>7.3</td>
<td>5.6, 88</td>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

Ring opening of the lactone 8.10 with N,O-dimethyl hydroxylamine gave the corresponding alcohol 8.11 (Figure 8-1). p-Methoxybenzyl bromide (PMB) protection of
alcohol 8.11 was achieved using PMBBr\textsuperscript{162,163} or PMBOC(NH)CCl\textsubscript{3}\textsuperscript{164} (Figure 8-10, Table 8-4).

**Figure 8-10. PMB protection of alcohol 8.11**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Protecting reagent</th>
<th>Base, equiv.</th>
<th>Catalyst, mol%</th>
<th>Conversion (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PMB-Br</td>
<td>NaH, 1</td>
<td>TBAI, 15</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>PMB-Br</td>
<td>NaH, 1.5</td>
<td>TBAI, 15</td>
<td>&lt;20%</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>PMB-Br</td>
<td>NaH, 2</td>
<td>TBAI, 15</td>
<td>&lt;30%</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>PMBOC(NH)CCl\textsubscript{3}</td>
<td>None</td>
<td>TfOH, 1</td>
<td>75%</td>
<td>92%</td>
</tr>
<tr>
<td>5</td>
<td>PMBOC(NH)CCl\textsubscript{3}</td>
<td>None</td>
<td>TfOH, 1.5</td>
<td>80%</td>
<td>91%</td>
</tr>
<tr>
<td>6</td>
<td>PMBOC(NH)CCl\textsubscript{3}</td>
<td>None</td>
<td>Sc(TfO)\textsubscript{3}, 10</td>
<td>50%</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>PMBOC(NH)CCl\textsubscript{3}</td>
<td>None</td>
<td>La(TfO)\textsubscript{3}, 10</td>
<td>56%</td>
<td>ND</td>
</tr>
</tbody>
</table>

The conversion was low (<30\%) when PMBBr was used to protect the hydroxy group of 8.11 in the presence of NaH/tetra-n-butyl-ammonium iodide (TBAI) in THF. PMBOC(NH)CCl\textsubscript{3}/TfOH was effective to convert alcohol 8.11 into corresponding PMB ether in 80\% conversion and 91\% yield. Weinreb amide 8.12 was obtained in 32 g using
optimized conditions. Amide 8.12 was readily converted into the corresponding aldehyde 8.12 by treatment with DIBAL-H (Figure 8-5).

**Anti-Crotylation of the C35-44 Aldehyde Fragment**

Asymmetric aldehyde crotylation reactions are widely used for the synthesis of natural product containing a polyketide fragments. Because of their important applications, many crotylating reagents have been developed (Figure 8-11).

![Contemporary crotylating reagents for anti-addition of aldehyde](image)

The most widely used reagents are crotylboranes. Despite several important conceptual and/or practical advances, since the development of Brown’s and Roush’s crotylboronates, the development of an asymmetric aldehyde crotylation method that is characterized by (i) wide scope and generality with respect to the aldehyde substrate, (ii) access to either diastereomer and (3) sustainable, safe, inexpensive, and scalable procedures remains a major problem.

To construct the C34(Me)–C35(OH) chiral unit of the key polyketide fragment (Figures 8-1 and 8-4), there are only two *anti*-selective allylation methods that have...
shown to be effective, the Brown protocol\textsuperscript{179,180} and the recently reported EZ-CrotylMix silicon based methodology (Figure 8-12 and 8-13).\textsuperscript{32}

The Brown protocol requires the metellation of butene under cryogenic conditions, and quite often suffers from difficulties in product isolation. By contrast, the EZ-CrotylMixes is a highly attractive option for large scale reactions and in particular for complex aldehydes.\textsuperscript{181} Therefore, Leighton reagent CrotylMixes were chosen.

Next, we set our goal to synthesize the crotylating reagent. The preparation of the EZ-CrotylMixes 8.18 involves the reaction of the requisite enantiomer of the diamine ligand 8.22 with \textit{trans}-crotyltrichlorosilane and DBU, followed by
concentration, trituration, and filtration of the DBU·HCl salts with pentane, recrystallization of the crotylsilane reagent, and mixing with the Sc(OTf)$_3$ catalyst, all under strictly anhydrous conditions (Figure 8-14).$^{182,183,184}$

![Chemical structures](image)

**Figure 8-14. Synthesis of Leighton crotylating reagent**

Although, reagent 8.18 (Figure 8-13) was obtained in 80% crude yield and $^1$H spectra analysis clearly showed the formation of the compound, we were unable to purify the product. Indeed, all attempts led to decomposition due to extended exposure to air and moisture.

Other asymmetric methods for crotylation reported in the literature suffer from one or more drawbacks such as air or moisture sensitivity, the need for a multistep preparation of chiral reagents, unreproducibility or incompatibility with aldehydes. This prompted us to develop an efficient alternative to replace the capricious crotylation reaction for the preparation of the polyketide fragment. For this purpose, we
investigated a simple proline-mediated aldolization/homologation synthetic sequence, which does not require the handling of sensitive reactants or the use of complex catalyst (Figure 8-15).

Figure 8-15. Synthesis of polyketide fragment of apratoxins

In order to evaluate this strategy, the synthesis of the alcohol 8.6 was studied in the course of a two-step procedure. Using the L-proline catalyzed cross aldol asymmetric reaction\(^\text{185}\) between aldehyde 8.5 and propionaldehyde afforded the intermediate 8.23. Aldehyde 8.23 was then used as a crude substrate for Tebbe's homologation conditions\(^\text{186}\) to furnish the expected homoallylic alcohol 8.19 without loss of stereoselectivity and excellent control of the stereoselectivity. No other diastereomer was detected by NMR and alcohol 8.19 was obtained in 52% yield from crude intermediate 8.23.

**Conclusion**

A large-scale preparation route to syntheses of the key aldehyde leading to polyketide fragments of modified cyclic peptide apratoxins has been developed. Advancing apratoxins or analogs thereof into further applications will require more efficient synthetic routes that are sustainable, safe, inexpensive, technically simple to perform, and readily scalable. As a result of optimizing the reaction conditions and improving efficiency, this route is suitable for a large-scale preparation. Key steps involve asymmetric allylation, RCM, and asymmetric conjugated additions.
Enantioselective aldehyde crotylation of the key aldehyde is the major step in the total synthesis of apratoxins, but despite many conceptual advances over the years, most of the current crotylating methods are elusive. We developed a novel enantioselective aldol/olefin homologation reaction, which may be a valuable alternative to classical stereoselective crotylation methods.

**Experimental**

**General Methods**

All commercial reagents were used without further purification unless otherwise noted. Tetrahydrofuran (THF) and diethyl ether (Et₂O) were distilled from sodium chips in the presence of a small amount of benzophenone; CH₂Cl₂ and toluene were distilled from CaH₂; MeCN, N,N-dimethylformamide (DMF) were dried with 4 Å molecular sieves (MS) and MeOH dried with 3 Å MS; 4 M hydrochloric acid (HCl) solution in ethyl acetate was prepared by dissolving HCl gas (yielding by dropping aqueous hydrochloric acid (34%) to concentrated sulfuric acid (98%)) to ethyl acetate. All reactions were performed in heat-gun dried flasks (400 °C under reduced pressure) under an inert atmosphere of anhydrous Ar unless otherwise noted. Thin layer chromatography was performed on EMD silica gel 60 Å F254 glass plates and preparative thin layer chromatography was performed on Whatman silica gel 60 Å F254 glass plates (layer thick 1000 μm). Flash column chromatography was performed with Fisher 170–400 mesh silica gel. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 MHz. Chemical shifts for proton nuclear magnetic resonance (¹H NMR) spectra are reported in parts per million relative to the signal residual CDCl₃ at 7.26 ppm. Chemicals shifts for carbon nuclear magnetic resonance (¹³C NMR) spectra are reported in parts per million relative to the center line of the CDCl₃ triplet at 77.16 ppm.
The abbreviations s, d, dd, ddd, dddd, t, q, br, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublets, triplet, quartet, broad and multiplet, respectively. LR-MS data was obtained using a 3200 QTrap triple quadrupole mass spectrometer and detection by electrospray ionization-MS in the positive ion mode.

**Preparation of Compounds 8.6, 8.14-17**

(S)-5,5-dimethyl-4-hydroxyhexan-2-one (8.14).\(^{156}\) D-proline (18.94, 164.53 mmol) was added to the mixture of acetone (0.8 L) and DMSO (3.2 L) at room temperature and the mixture was stirred at the same temperature for 1h before pivalaldehyde (61 mL, 0.548 mol) was added. After stirring at room temperature for 4 days, the mixture was cooled to 0 °C with an ice-water bath and saturated aqueous NH\(_4\)Cl solution (2 L) was added to quench this reaction. The mixture was extracted with ethyl acetate (1.5 L ×3) and the extract was concentrated under reduced pressure to remove most of the ethyl acetate and acetone. Then the concentrated mixture was diluted with another 800 mL of ethyl acetate and washed with small portions of water (200 mL × 5) to remove most of the DMSO. The organic layer was dried with anhydrous MgSO\(_4\) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8-20% ethyl acetate in hexane) to give product 8.14 (65 g, 82%) as a colorless liquid. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 3.72 (ddd, \(J = 10.8, 3.6, 2.0\) Hz, 1H), 2.85 (m, 1H), 2.63 (dd, \(J = 17.6, 2.4\) Hz, 1H), 2.48 (dd, \(J = 17.2, 10.8\) Hz, 1H), 2.20 (s, 3H), 0.90 (s, 9H).

(S)-5,5-Dimethyl-4-(tert-butyldimethylsilyloxy)hexan-2-one (8.15).\(^{32}\) 3,4 tert-Butyldimethylsilyl chloride (TBS-Cl) (102 g, 0.68 mol) and imidazole (89.65 g, 1.32 mol) were added to the solution of compound 8.14 (62.66 g, 0.434 mol) in DMF (100 mL). After stirring at room temperature for 24 h under Ar, the reaction was quenched by...
addition of 150 mL methanol and 1.5 L water. The mixture was extracted with ethyl acetate (1.5 L × 3), the combined organic layers were concentrated in vacuo and purified by column chromatography on silica gel (5% ethyl acetate in hexane) to give product 8.15 (95.5, 85%) as a colorless liquid. $^1$H NMR (400 MHz, CDCl$_3$): δ 3.95 (dd, $J = 6.0$, 4.0 Hz, 1H), 2.61 (dd, $J = 17.2$ Hz, 1H), 2.49 (dd, $J = 17.6$, 6.4 Hz, 1H), 2.15 (s, 3H), 0.86 (s, 9H), 0.84 (s, 9H), 0.06 (s, 3H), 0.06 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 207.9, 75.1, 48.2, 35.7, 31.43, 26.2, 26.0, 18.4, -4.0, -4.8.

(S)-tert-Butyl (1-tert-butylbut-3-enyloxy)dimethylsilane (8.16).$^{32}$ NaBH$_4$ (95 g, 2.5 mol) was added to the solution of compound 8.15 (324 g, 1.25 mol) in MeOH (3.5 L) at 0 °C. After stirred at the 0 °C for 40 min, the reaction was concentrated first and then 300 mL water was added at 0 °C. The mixture was extracted with ethyl acetate (3 L × 3), washed sequentially with brine (3L × 2) and water (2 L × 2), dried with anhydrous MgSO$_4$, evaporated in vacuo to give the crude alcohol 10 which was used in the next step without further purification.

(S)-tert-butyl((2,2-dimethylhex-5-en-3-yl)oxy)dimethylsilane (8.17).$^{179}$ Et$_3$N (99 ml, 715 mmol) and MsCl (55.4 ml, 715 mmol) were added to the solution of the crude 8.16 in dry CH$_2$Cl$_2$ (1 L) at 0 °C under Ar atmosphere. After stirring at the same temperature for 2.5 h, this reaction was quenched by brine (0.5 L). The organic layer was separated and water layer was extracted with CH$_2$Cl$_2$ (300 mL × 2). The combined CH$_2$Cl$_2$ fractions were washed with water (300 mL × 2), dried with anhydrous MgSO$_4$ and evaporated in vacuo to give the crude mesylate.

$t$-BuOK (132.3 g, 1.18 mol) was added to the solution of the above crude mesylate in dried toluene (1.2 L). The suspended mixture was heated to reflux for 1 h,
cooled to room temperature and 0.6 L water was added. The organic layer was separated and the water layer was extracted with heptane (0.9 L \times 3). The combined toluene and heptane layer was washed with brine (0.6 L \times 3) and water (0.6 L \times 2), dried with anhydrous MgSO$_4$, evaporated in vacuo and purified by column chromatography (100% hexane) to give product (80 g, 90 % 3 steps) as a colorless liquid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.88 (dddd, $J$ = 16.8, 9.6, 7.2, 7.2 Hz, 1H), 5.00 (m, 1H), 3.32 (dd, $J$ = 8.0, 4.0 Hz, 1H), 2.35 (m,1H), 2.13 (m, 1H), 0.90 (s, 9H), 0.87 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 137.9, 115.7, 80.2, 38.5, 36.3, 26.7, 26.3, 18.5, -3.1, -4.1.

(S)-2,2-Dimethyl-5-hexen-3-ol (8.6). The solution of tetra-n-butylammonium floride trihydrate (TBAF) (82.07 g, 260.116 mmol) in THF (220 mL) was added to the mixture of compound 8.11 (160.3 g, 21.0 g, 0.662 mol) and 4 Å molecular sieves (pre dried at 450 °C under reduced pressure 1,5 h) in anhydrous THF (2.3 L) at 0 °C. Then the reaction mixture was stirred at room temperature overnight and filtered through a small pad of Celite (washed with diethyl ether). The filtrate was quenched with 1.5 L of water, extracted with diethyl ether (2.2 L \times 3), washed with brine (2.2 L \times 2), dried over anhydrous MgSO$_4$, concentrated with cooling/condensing fraction distillation system under moderate vacuum, and further concentrated by Vigreux fraction distillation column. The concentrated mixture was purified by column chromatography eluted by 3–5% diethyl ether in pentane. The eluted product fractions were also concentrated by cooling/condensing fraction distillation system under moderate vacuum to give product 8.6 and further distilled by Vigreux fraction distillation column to provide product 8.12 (78 g g, 92%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.86 (dddd, $J$ = 14.4, 10.4, 8.8, 6.0 Hz,
1H), 5.14 (m, 2H), 3.25 (dd, \( J = 10.4, 2.0 \) Hz, 1H), 2.39–2.33 (m, 1H), 1.98 (ddd, \( J = 13.6, 9.6, 9.6 \) Hz, 1H), 0.91 (s, 9H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 136.7, 117.9, 78.2, 36.7, 34.7, 25.9.

**Synthesis of Key Aldehyde 8.5**

(S)-2,2-dimethylhex-5-en-3-yl acrylate (8.8).\(^{159,180}\) Acryloyl chloride (62 mL, 0.76 mol) and triethylamine (242 mL, 1.756 mol) were added sequentially to the solution of compound 8.6 (75, 0.585 mol) in anhydrous diethyl ether (2.5 L) at 0 °C. The reaction was warmed to room temperature and stirred at room temperature for 4 h, then poured into cold water (2 L) and extracted with diethyl ether (2 L × 4). The organic layer of extractions was washed with saturated NaHCO\(_3\) (2 L × 2), saturated NH\(_4\)Cl (2 L), brine (2 L), dried with anhydrous MgSO\(_4\), concentrated (first by cooling/condensing fraction distillation system under moderate vacuum and further by Vigreux fraction distillation column) and purified by column chromatography to give product 8.8 (101 g, 95%).

Because the compound can easily evaporates along with solvent, the product fraction of column was also concentrated first by cooling/condensing fraction distillation system\(^{187,188}\) under moderate vacuum and further by Vigreux fraction distillation column.

**General procedure for RCM**

Procedure A:\(^{32}\) Ti(OiPr)\(_4\) (20 mol\% to substrate 8.8) was added to the solution of diene 8 in CH\(_2\)Cl\(_2\) (resulted in 2 or 3 mM of substrate 8.8) under Ar. The resulting solution was refluxing for 1 h, then Grubb’s second generation catalyst (15 mol \%) in CH\(_2\)Cl\(_2\) (degassed) was added under refluxing. The reaction mixture continued to reflux for another 4-6 h, was then cooled to room temperature, evaporated in vacuo and purified by column chromatography (eluted by diethyl ether/pentane 2:7) to give product as a colorless oil.
Procedure B: Ar gas was passed through to the solution of diene 8.8 in CH$_2$Cl$_2$ (resulted in 2 or 3 mM of substrate 8.8) for 1-1.5 h. Then Grubb’s second or first generation catalyst (10 or 15 mol%) in CH$_2$Cl$_2$ (degassed) was added under refluxing. The reaction mixture continued to reflux for another 4-6 h, was then cooled to room temperature, evaporated in vacuo and purified by column chromatography (eluted by diethyl ether/pentane 2:7) to give product as a colorless oil.

(6S)-6-tert-Butyl-5,6-dihydro-pyran-2-one (8.9). Colorless oil (65 g, 75%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 6.91 (ddd, $J$ = 11.0, 6.4, 2.8 Hz, 1H), 6.01 (m, 1H), 4.06 (dd, $J$ = 12.0, 4.4 Hz, 1H), 2.39-2.24 (m, 1H), 1.00 (s, 9H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.1, 145.7, 121.3, 85.4, 34.0, 25.6, 24.7.

Procedure for conjugated methyl addition

Methyllithium (1.6 M in diethyl ether, 10-120 mL, 2.6 equiv) was added slowly (over 1 h) to the suspension of CuCN (1.2 equiv) in diethyl ether at -78 °C. After the mixture was stirred at the same temperature for 40 min, it was moved to an ice-bath for another 40 min, then re-cooled to -78 °C before compound lactone 8.9 (0.5-8g mmol) was added slowly (over 1 h) in dried diethyl ether (15 mL/ 1g). The reaction mixture was kept at -78 °C 40 min, warmed to -50 – -40 ° C 40 min, -20 ° C 1 h, was then quenched with 5% FeCl$_3$, extracted with diethyl ether, washed with brine, dried with anhydrous MgSO$_4$, concentrated in vacuo, purified by column chromatography (eluted by diethyl ether/pentane 1:4) to give product 8.10 as a colorless oil.$^{160}$

(4R,6S)-6-tert-Butyl-4-methyl-tetrahydro-pyran-2-one (8.10). Colorless oil (67 g, 85%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.97 (dd, $J$ = 11.8, 3.6 Hz, 1H), 2.53-2.46 (m, 1H), 2.21-2.16 (m, 1H), 1.80 (dddd, $J$ = 14.0, 11.6, 7.2 Hz, 1H),1.49 (ddd, $J$ = 14.4, 3.2, 3.2 Hz, 1H), 1.09 (d, $J$ = 6.4, 3H), 0.95 (s, 9H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 173.4,
83.8, 37.1, 34.1, 29.9, 25.6, 24.1. MS (m/z): [M+H]⁺ calcd for C₁₀H₁₉O₂ 171.1, found 171.1.

**Procedure for ring opening of lactone 8.10**

Trimethylaluminum ((CH₃)₃Al) (2M in hexane, 3 equiv) was added to the solution of N,O-dimethylhydroxylamine hydrochloride (3 equiv.) in CH₂Cl₂ (1.5 mL/1g) at -78 °C, then warmed to room temperature and kept at room temperature overnight. The solution of compound 10 (Table 8-3, 1-8g) in CH₂Cl₂ (2.5 mL/1 g of 8.10) was added slowly to the above solution over 1 h at 0 °C. The reaction mixture was stirred at 0 °C for 40 min and room temperature for another 5 h, was then concentrated to about 100 mL, quenched with sodium potassium tartrate (Rochelle's salt) solution, extracted with ethyl acetate, washed with brine, dried with anhydrous MgSO₄ and purified by column chromatography (eluted by ethyl acetate/hexane 1:2) to give product 8.11.¹⁵⁹

(3R,5S)-5-Hydroxy-3,6,6-trimethyl-heptanoic acid methoxy-methyl-amide (8.11). Thick colorless oil (72 g, 90%). ¹H NMR (400 MHz, CDCl₃): δ 3.68 (s, 3H), 3.18 (s, 3H), 3.13 (dd, J = 10.4, 2.4 Hz, 1H), 2.71 (br, 1H), 2.47-2.41 (m, 1H), 2.31-2.28 (m, 2H), 1.43-1.29 (m, 2H), 1.02 (d, J = 6.4 Hz, 3H), 0.87 (s, 9H). ¹³C NMR (100 MHz, CDCl₃): δ 175.0, 76.5, 61.3, 39.6, 38.3, 34.8, 32.4, 26.4, 26.0, 22.5.

**General procedure for PMB protection**

Procedure A¹⁶⁴,¹⁶³ using PMBOC(NH)CCl₃. 4-Methoxybenzyl-2,2,2-trichloroacetimidate (2 equiv) and trifluoromethane sulfonic acid (TfOH) (1 or 1.5 mol%) or Sc(TfO)₃ (10 mol%) or La(TfO)₃ (10 mol%) was added sequentially to the solution of 8.11 (025-8g) in THF (1.5 mL/1g) at 0 °C. The resulting mixture was stirred at room temperature overnight and was then diluted with ethyl acetate, quenched with saturated NaHCO₃, extracted with ethyl acetate, dried with anhydrous MgSO₄, and evaporated in
Hexane was added to the residue, which resulted in the precipitation of a white solid (2,2,2-trichloroacetimidate). The solid was filtered off, and the filtrate was concentrated and purified by column chromatography (eluted by 20–50% ethyl acetate in hexane) to give product 12 (50-80% conversion) (recovered starting material is used in next batch).

Procedure B using PMBBr. To a suspension of NaH (1-2 equiv) in THF at 0 °C were added solutions of amide 8.11, TBAI (15 mol%) dissolving in THF PMB-Br (1-1.1 equiv) in THF via a cannula. The reaction mixture was stirred at room temperature for 16 h and then quenched with cold water. Volatiles were removed under reduced pressure and the residue was extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous solution of NH₄Cl, brine, and dried over MgSO₄. Conversion was determined by proton NMR analysis.

(3R,5S)-5-(4-Methoxy-benzyloxy)-3,6,6-trimethylheptanoic acid methoxy-methylamide (8.12). Colorless oil (32 g, 85%). ¹H NMR (400 MHz, CDCl₃): δ 7.31 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 4.60 (d, J = 10.4 Hz, 1H), 4.49 (d, J = 10.4, 1H), 3.78 (s, 3H), 3.67 (s, 3H), 3.19 (s, 3H), 3.07 (dd, J = 8.4, 2.8 Hz, 1H), 2.52-2.50 (m, 1H), 2.32-2.22 (m, 2H), 1.54-1.40 (m, 2H), 1.01 (d, J = 6.4 Hz, 3H), 0.93 (s, 9H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 174.2, 159.0, 131.5, 129.5, 129.3, 113.8, 113.6, 85.7, 74.2, 61.3, 55.3, 39.1, 38.7, 36.3, 32.2, 27.8, 26.6, 21.7. MS (m/z): [M+H]+ calcd for C₂₀H₃₄NO₄ 352.2, found 352.1.
Procedure for reduction of Weinreb amide

Diisobutylaluminum hydride (1M in toluene, 8.27, 28.4 mmol) was added dropwise to the solution of 8.12 (4g, 11.34 mmol) in THF (240 mL) at -78 °C. The reaction mixture was quenched with 10% Rochelle’s salt solution (400 mL) and diethyl ether (400 mL) when stirred at -78 °C for 30 min. After the two phase mixture had been stirred vigorously at room temperature for 2 h, it was extracted with ethyl acetate (400 mL ×3), washed with brine (180 mL ×3), dried with anhydrous MgSO\(_4\), concentrated in vacuo and purified by column chromatography (eluted by 8% ethyl acetate in hexane) to give product 2.8 g (85%) as a colorless oil.\(^{32}\)

(3R,5S)-5-(4-Methoxy-benzyloxy)-3,6,6-trimethylheptanal (8.5). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 9.68 (s, 1H), 7.28 (d, \(J = 8.4\) Hz, 2H), 6.87 (d, \(J = 8.4\) Hz, 2H), 4.57 (d, \(J = 10.8\) Hz, 1H), 4.48 (d, \(J = 10.8\), 1H), 3.79 (s, 3H), 2.45-2.49 (m, 1H), 2.21-2.10 (m, 2H), 1.50-1.38 (m, 2H), 1.00 (d, \(J = 6.4\) Hz, 3H), 0.94 (s, 9H) ppm. \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 202.9, 159.1, 131.2, 129.3, 113.8, 85.3, 74.5, 55.3, 50.2, 38.8, 36.3, 26.6, 25.7, 21.7.

Synthesis of Leighton Anti-Crotylating Reagent

(1E,1′E)-N,N′-((1S,2S)-cyclohexane-1,2-diyl)bis(1-(4-bromophenyl)methanimine) (8.21).\(^{184}\) To (1S,2S)-(+)1,2-diaminocyclohexane L-tartrate (4.8 g, 18.2 mmol, 1.0 equiv.) in water (85 mL) and EtOH (42.5 mL) was added K\(_2\)CO\(_3\) (5.02 g, 36.3 mmol, 2.0 equiv.) followed by a solution of 4-bromobenzaldehyde (6.72 g, 36.3 mmol, 2.0 equiv.) and methanesulfonic acid (141 \(\mu\)L, 2.18 mmol, 0.12 equiv.) in DCM (85 mL) via dropping funnel over the course of 1 hour. The mixture was stirred at room temperature for 15 hours and then at reflux for 1 hour. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to a volume of ca. 50 mL.
Then water (50 mL) was added and the mixture was filtered. The collected solid was dried under vacuum to give the title diimin. White solid (6.92 g, 85%). ¹H NMR (400 MHz, CDCl₃, ppm): δ 8.11 (s, 2H), 7.45 (s, 8H), 3.41-3.34 (m, 2H), 1.91-1.74 (m, 6H), 1.54-1.44 (m, 2H). ¹³C NMR (300 MHz, CDCl₃, ppm): δ 159.7, 135.1, 131.6, 129.3, 124.6, 73.7, 32.8, 24.4. 8, 1400, 1376, 1341, 1295, 1068, 1010.

(1S,2S)-N₁,N₂-bis(4-bromobenzyl)cyclohexane-1,2-diamine (8.22). The diimin solid 21 (6.9 g, 15.39 mmol) was suspended in MeOH (30 mL) and cooled to 0 °C before NaBH₄ (1.71 g, 45.4 mmol, 2.95 equiv.) was added in portions. After bubbling had ceased (ca. 2 hours), the reaction was heated at reflux temperature for 90 minutes. It was then concentrated under reduced pressure, 1M NaOH (50 mL) and hexane/EtOAc 1:1 (50 mL) were added, the phases were separated, and the aqueous layer was extracted with hexane/EtOAc 1:1 (twice 50 mL). The combined organic layers were washed with brine (15 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. Diamine 8.22 (6.5 g, 14.36 mmol, 92%) was obtained as a yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 7.44-7.38 (m, 4H), 7.20-7.14 (m, 4H), 3.83 (d, J = 13.4, 2H), 3.59 (d, J = 13.4 Hz, 2H), 2.25-2.16 (m, 2H), 2.16-2.07 (m, 2H), 1.82 (br s, 2H), 1.77-1.65 (m, 2H), 1.31-1.12 (m, 2H), 1.09-0.92 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 140.2, 131.5, 129.9, 120.6, 61.0, 50.4, 31.7, 25.1.

(3aS,7aS)-1,3-Bis(4-bromobenzyl)-2-((E)-but-2-enyl)-2-chloro-octahydro-1H-benzo[d]-[1,3,2]-diazasilole (8.23). To a solution of ((E)-but-2-enyl)-trichlorosilane (2 mL, 13.2 mmol, 1.1 equiv) in dry CH₂Cl₂ (24 mL) at 0 °C was added DBU (3.9 mL, 26.4 mmol, 2.2 equiv). A solution of (1S,2S)-N,N₀-bis-(4-bromobenzyl)-cyclohexane-1,2-diamine (5.43 g, 12 mmol, 1 equiv) in CH₂Cl₂ (12 mL) was then added
slowly. The reaction mixture was allowed to warm to room temperature. After 12 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was diluted in pentane (36 mL) and vigorously stirred overnight to ensure complete precipitation of DBU salts. The solution of pentane with desired reagent was then transferred to another flash using a cannula. The solution was concentrated and washed with pentane to furnish the expected crude product as a yellow powder (2.34 g, 35%). 1H NMR (300 MHz, CDCl3) δ 7.50–7.23 (m, 8H), 5.32–5.17 (m, 2H), 4.15–3.96 (m, 2H), 3.86–3.70 (m, 2H), 2.80–2.66 (m, 2H), 1.71–1.50 (m, 9H), 1.34–0.85 (m, 4H)

**Synthesis of Compound 8.19**

(3R,4S,6S,8S)-8-((4-methoxybenzyl)oxy)-3,6,9,9-tetramethyldec-1-en-4-ol (8.19).

To a solution of aldehyde 8.5 (0.3 g, 10.25 mmol, 1 equiv) and (L)-proline (16 mg, 0.14 mmol, 0.2 equiv) in DMF (0.7 mL) at 0 °C was added slowly a solution of propionaldehyde (0.1 mL, 1.5 mmol, 2 equiv) in DMF (0.5 mL). After 72 h, the solvent was partially removed under reduced pressure the reaction was quenched with water and extracted with EtOAc/toluene 2:1. The organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated. The crude intermediate 8.23 was used without purification. Yellow oil (216 mg, 0.616 mmol, 60%)185

The crude product 8.23 was dissolved in THF (12 mL). The Tebbe’s reagent186 (0.5 M in toluene, 0.9 mL, 1.5 equiv) was then added at 0 °C. After 1 h, the reaction mixture was quenched with an aqueous solution of 10% NaOH, diluted in diethyl ether, and filtered through Celite. After removal of the solvents, the residue was purified by flash column chromatography (EtOAc/Hexanes 10:90) to furnish the expected product as a colorless oil (111.6 mg, 52%). 1H NMR(400 MHz, CDCl3): δ 7.30 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 5.75 (m, 1H), 5.10–5.14 (m, 2H), 4.63 (d, J = 10.8 Hz, 1H),
4.51 (d, $J = 10.8$ Hz, 1H), 3.79 (s, 3 H), 3.50 (m, 1H), 3.11 (dd, $J = 9.3$, 2.9 Hz, 1H), 2.17 (m, 1H), 1.96 (m, 1H), 1.57 (ddd, $J = 13.7$, 10.7, 2.9 Hz, 1H), 1.47 (dd, $J = 14.2$, 8.8, 3.9 Hz, 1H), 1.35 (dd, $J = 14.2$, 9.3, 2.4 Hz, 1H), 1.12 (ddd, $J = 13.7$, 9.3, 2.4 Hz, 1H), 1.03 (d, $J = 7.3$ Hz, 3H), 0.96 (d, $J = 6.8$ Hz, 3H), 0.93 (s, 9H).
Figure A-1. $^1$H NMR spectrum of compound 8.14 in CDCl$_3$ (400 MHz)
Figure A-2. $^{13}$C NMR spectrum of compound 8.14 in CDCl$_3$ (100 MHz)
Figure A-3. $^1$H NMR spectrum of compound 8.6 in CDCl$_3$ (400 MHz)
Figure A-4. $^1$H NMR spectrum of compound 8.8 in CDCl$_3$ (400 MHz)
Figure A-5. $^1$H NMR spectrum of compound 8.9 in CDCl$_3$ (400 MHz)
Figure A-6. $^{13}$C NMR spectrum of compound 8.9 in CDCl$_3$ (100 MHz)
Figure A-7. $^1$H NMR spectrum of compound 8.10 in CDCl$_3$ (400 MHz)
Figure A-8. $^{13}$C NMR spectrum of compound 8.10 in CDCl3 (100 MHz)
Figure A-9. $^1$H NMR spectrum of compound 8.11 in CDCl3 (400 MHz)
Figure A-10. $^{13}$C NMR spectrum of compound 8.11 in CDCl$_3$ (100 MHz)
Figure A-11. $^1$H NMR spectrum of compound 8.12 in CDCl$_3$ (400 MHz)
Figure A-12. $^{13}$C NMR spectrum of compound 8.12 in CDCl$_3$ (100 MHz)
Figure A-13. $^1$H NMR spectrum of compound 8.5 in CDCl$_3$ (400 MHz)
Figure A-14. $^{13}$C NMR spectrum of compound 8.5 in CDCl$_3$ (100 MHz)
Figure A-15. $^1$H NMR spectrum of PMB-Br in CDCl$_3$ (400 MHz)
Figure A-16. $^{13}$C NMR spectrum of PMB-Br in CDCl$_3$ (100 MHz)
Figure A-17. $^1$H NMR spectrum of PMB-trichloroacetamide in CDCl$_3$ (400 MHz)
Figure A-18. $^{13}$C NMR spectrum of PMB-trichloroacetamide in CDCl$_3$ (100 MHz)
Figure A-19. $^1$H NMR spectrum of compound 8.21 in CDCl$_3$ (400 MHz)
Figure A-20. $^{13}$C NMR spectrum of compound 8.21 in CDCl$_3$ (100 MHz)
Figure A-21. $^1$H NMR spectrum of compound 8.22 in CDCl$_3$ (400 MHz)
Figure A-22. $^{13}$C NMR spectrum of compound 8.22 in CDCl$_3$ (100 MHz)
Figure A-23. $^1$H NMR spectrum of trichloro($E$-crotyl)silane in CDCl$_3$ (400 MHz)
Figure A-24. $^1$H NMR spectrum of compound 8.18 in CDCl$_3$ (400 MHz)
Figure A-25. $^1$H NMR spectrum of compound 8.18 in CDCl$_3$ (100 MHz)
Figure A-26. $^1$H NMR spectrum of compound 8.19 in CDCl$_3$ (400 MHz)
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BIOGRAPHICAL SKETCH

Khanh Ha was born in Thai Nguyen, Vietnam. In 2002, after graduating from Thai Nguyen High School for Gifted Students, he was awarded Full Academic Scholarship by the Ministry of Training and Education of Vietnam to study Chemical Engineering at Irkutsk State Technical University in Russia. In 2006, he started his undergraduate research at the Favorsky Irkutsk Institute of Chemistry, a Siberian Branch of the Russian Academy of Sciences under supervision of Professor Galina Levkovskaya as a Favorsky Scholar. His diploma thesis was appreciated by the State Board of Examiners, and he was awarded the Young Scientist certificate and the Bachelor Degree with Highest Honor in 2009. In 2010, he began his graduate studies at Chemistry Department under the supervision of Professor Alan Katritzky. In 2014, he moved to Dr. Luesch’s lab at the Center for Natural Products, Drug Discovery and Development to finish up his thesis. Khanh’s research focuses on methodologies for cyclization and coupling of peptides and synthesis of apratoxin polyketide fragments, which remains one of the main problems of contemporary organic chemistry. Dr. Ha has published seven papers during his stay at the University of Florida. In addition, he has participated in several internationally renowned conferences and delivered either a poster or an oral presentation. Khanh was recognized with the Certificate of Outstanding Achievement, the Proctor & Gamble Award for Research Excellence, the Graduate Student Mentoring Award, the Alec Courtelis Award for excellent academic performance, the Science for Life Graduate Student Award, Presidential Service Award, Synfacts Prize and recently the Leadership Development Award from The Younger Chemists Committee of the American Chemical Society. Dr. Ha has been serving in reviewer panel for journals of the Royal Society of Chemistry since 2012.