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</tr>
<tr>
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<tr>
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<tr>
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<td>Diphenyl phosphoryl azide</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>Ethyl acetate</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide snythase</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Fmoc</td>
<td>Fluorenlymethyloxycarbonyl</td>
</tr>
<tr>
<td>GBM</td>
<td>Glioblastoma multiforme</td>
</tr>
<tr>
<td>Glu</td>
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</tr>
<tr>
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<td>Glycine</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
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<td>J</td>
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<tr>
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<tr>
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<td>N-Methylmorpholine</td>
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<tr>
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<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>nNOS</td>
<td>Neuronal nitric oxide synthase</td>
</tr>
<tr>
<td>NOC</td>
<td>N-Nitroso compounds</td>
</tr>
<tr>
<td>Pd</td>
<td>Palladium</td>
</tr>
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<td>Pentafluorophenyl</td>
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<tr>
<td>Pg</td>
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<tr>
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<td>Phenylalanine</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>RGD</td>
<td>Arginine-glycine-aspartic</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
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<td>Thionyl chloride</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine</td>
</tr>
<tr>
<td>tᵣ</td>
<td>Retention time</td>
</tr>
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<td>Tryptophan</td>
</tr>
<tr>
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<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFFH</td>
<td>N, N’-Tetramethylfluoroformamidinium hexafluorophosphate</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
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</table>
The thesis is concerned with synthesis of lysine and arginine containing peptides, 5-(substituted amino)-1,2,3,4-thiatriazoles and 8-azaquinazolinone analogue, NBI-74330. Lysine and arginine are essential alpha-amino acids and are constituent of various biologically active peptides. 5-(Substituted amino)-1,2,3,4-thiatriazoles show interesting biological properties. NBI-74330 is the CXCR3 receptor antagonist.

Benzotriazole methodology enables convenient syntheses of natural and unnatural di- and tripeptides derived from L-lysine by extension at N(alpha)-, N(epsilon)-, and/or C- termini, under mild reaction conditions. Extensions at N-termini have been carried out by acylation with N-(Cbz-or Fmoc-alpha-aminoacyl)benzotriazoles or N-protected dipeptidoylbenzotriazoles. Extensions at C-terminii were performed similarly by coupling the N-protected acylbenzotriazole derivative of lysine and its dipeptides with unprotected alpha-amino acids. These reactions are performed in aqueous acetonitrile solution, require short reaction times; involve simple work up procedures and form enantiopure products in high yields.

Arginine containing di- and tripeptides are synthesized by chain elongation at either the N- or C-terminus of N(omega)-L-nitroarginine or L-arginine. Coupling of N(omega)-L-nitroarginine with N-(Cbz-or Fmoc-alpha-aminoacyl)benzotriazoles or N-protected dipeptidoylbenzotriazoles
form di- or tripeptides in good yields. The acylbenzotriazole derivative of Cbz(alpha)-N(omega)-
L-nitroarginine is coupled with free amino acids to obtain the corresponding dipeptides. All
isolated peptides were enantiopure and obtained in good yields. The protected RGD peptide
sequence was synthesized using benzotriazole methodology.

A convenient one-pot protocol for the synthesis of 5-(substituted amino)-1,2,3,4-
thiatriazoles from bis(1H-benzotriazol-1-yl)methanethione is described. Reactions of amines or
amino acid esters with bis(1H-benzotriazol-1-yl)methanethione followed by addition of aqueous
sodium azide at room temperature resulted with formation of corresponding 5-(monosubstituted
amino)-1,2,3,4-thiatriazoles in 73-97% yields, in short reaction times, without the use of column
chromatographic purification.

The CXCR3 receptor antagonist NBI-74330 was synthesized and tested for its biological
activity by Dr. Jeffrey K. Harrison group for treatment of malignant gliomas.
1.1 Benzotriazole and its Properties

\( \text{\textit{H}} \)-Benzotriazole 1.1 is a stable, inexpensive, white solid, and a readily available compound. It is almost insoluble in water, but soluble in aqueous sodium carbonate, aqueous hydrochloric acid, ethanol, benzene, toluene, chloroform, tetrahydrofuran and dimethylformamide. Benzotriazole is a good synthetic auxiliary which offers many advantages. As an aspect of a synthetic auxiliary, a group must be able to be introduced readily at the beginning of the sequence and it must be easy to remove at the end of the synthetic sequence. It should be stable during various synthetic operations, and if possible, exert an activating influence on the other parts of molecule. Benzotriazole (pK\( \text{a} \) 8.2) is readily removed from a reaction mixture by simply washing with aqueous base. Because of its chemical properties, it is an excellent synthetic auxiliary.\(^{1-3}\) Facile chemical reactivity of benzotriazole auxiliaries arise due to activation of the carbon atom attached to the benzotriazolyl group.

The benzotriazolyl group can activate substrates in a variety of ways: as a leaving group 1.2a and 1.2b, as an electron withdrawing group assisting deprotonation 1.3 and as an electron donating group assisting ionization 1.4 (Figure 1-1). The benzotriazolyl group is also an ambident anion directing group and it can act as a radical or carbanion precursor.\(^1\)

![Figure 1-1. Benzotriazole and its properties](image-url)
N-Substituted benzotriazoles in which the N-substituent contains an α-heteroatom (N, S, O or halogen) can ionize in two ways, either (i) yielding a benzotriazole anion and a cation derived from the heteroatom 1.5 or (ii) ionizing off the α-substituent 1.6 (Scheme 1-1).

Scheme 1-1. Typical reactions of N-substituted benzotriazoles

N-Substituted benzotriazoles undergo several types of reactions including: N-, C-, O-, and S-acylation; imidoylation; thioacylation; amino-, amido-, alkoxy-, and thio-alkylation; sulfonylation; carbon insertion; benzotriazole ring cleavage reactions and radical reactions. Relevant to our present work, acylation, thioacylation and thiocarbamoylation using benzotriazole reagents will be discussed in this general introduction.

1.2 Synthetic Utility of Benzotriazole

1.2.1 N-Acylbenzotriazoles and its application in Acylation and Peptide Synthesis

N-Acylbenzotriazoles have been employed for:

(i) N-acylation of primary, secondary and tertiary amines, amino acids, sulfonamides, hydroxylamines, hydrazines, and aminosugars;

(ii) C-acylation of ketones, cyanides, sulfones, heterocycles, esters, thioesters, nitroalkanes, organometallics;

(iii) O-acylation in addition to aldehydes, O-acylation of sugars and steroids (Scheme 1-2)
Scheme 1-2. N-, C- and O-Acylation with N-acylbenzotriazoles

N-acylation of amines results in the formation of an amide bond and this methodology has been successfully extended for the synthesis of peptides. Thus, the carboxyl group of the $\alpha$-amino acid 1.7 is activated by converting it to the corresponding $N$-acylbenzotriazole derivative 1.8 and reacted with another amino acid 1.9 to obtain a dipeptide 1.10. The resulting dipeptide is further activated to the corresponding benzotriazole derivative 1.11 and reacted with another $\alpha$-amino acid 1.12 to form a tripeptide 1.13 (Scheme 1-3).

Scheme 1-3. General scheme for peptide synthesis

The first step in the peptide synthesis, conversion of $N$-protected $\alpha$-amino acids to benzotriazole derivatives is carried out by one of the following methods: (i) by treatment with
sulfonylbenzotriazole in the presence of triethyl amine or (ii) by reacting with excess of benzotriazole in the presence of thionyl chloride (Scheme 1-4). The thionyl chloride method has been extensively used for N-(Cbz-or –Fmoc)α-amino acids. It is carried out at room temperature in high yields and is more convenient than the former one which requires heating. Thus, a wide range of N-(protected α-aminoacyl)benzotriazoles 1.8 have been prepared from the corresponding N-protected α-amino acids 1.7 with retention of chirality. Moreover, this methodology can be applied to N-protected α-amino acids with unprotected side chain functionality such as in Tyr, Trp, Cys, Met and Gln (containing phenolic OH, NH of indole, SH, SMe and amide groups); and in Ser, Asn, Glu, Asp, Cys (containing aliphatic OH, primary amide and CO₂H groups and the S–S linkage). These acyl derivatives which are stable and crystalline solids, couple with free α-amino acids 1.9 with unprotected side chain functionality in aqueous CH₃CN to give the corresponding dipeptides 1.10 with no detectable epimerization.

![Scheme 1-4. Synthesis of N-protected dipeptides](image)

Similarly, dipeptides 1.16 and 1.17 have been synthesized by acylation of the amino groups of free aspartic and glutamic acids with various N-(protected α-aminoacyl)benzotriazoles 1.8 with complete retention of chirality (Scheme 1-5). Also, aspartic and glutamic acids were selectively extended at each of the alternative C-terminals to afford diverse natural and unnatural N-protected dipeptides 1.20, 1.21, 1.24, 1.25 by reactions between N-(protected α-
aminoacyl)benzotriazoles 1.18, 1.19, 1.22, 1.23 and free amino acids 1.7, again with complete retention of chirality (Scheme 1-6).24

Scheme 1-5. Dipeptides by extension at N-terminus of aspartic and glutamic acids

![Scheme 1-5](image)

Scheme 1-6. Dipeptides by extension at C-terminus of aspartic and glutamic acids

However, benzotriazole derivative of N-protected glutamic ester, 1.23 cyclizes to form 1.26 in aqueous CH$_3$CN in the presence of Et$_3$N. Similarly, coupling of N-protected bis-benzotriazole derivative of glutamic acid 1.27 with α-amino acids (Ala, Phe, Val) 1.7 in aqueous CH$_3$CN in the presence of Et$_3$N produced N-protected pyroglutamyl pseudopeptides 1.28 in 55-88% yields (Scheme 1-7).25
Scheme 1-7. Preparation of N-protected pyroglutamyl-amino acids

Tripeptides 1.13 have been synthesized by stepwise coupling utilizing N-protected dipeptidoylbenzotriazoles 1.11 with unprotected amino acids 1.12. Similarly, tripeptides 1.13 have also been synthesized from N-(protected-α-aminoacyl)benzotriazoles 1.8 and free dipeptides by fragment condensation in aqueous CH₃CN solution.

Scheme 1-8. Stepwise and fragment coupling for synthesis of peptides

N-(Fmoc-α-aminoacyl)benzotriazoles have been employed in solid-phase peptide synthesis under microwave irradiation to synthesize tri-, tetra-, penta-, hexa-, and heptapeptides (20-68%).

Chapter 2 describes the preparation of peptides containing lysine by extension at both C- and N-terminus of lysine by utilizing N-(Cbz-or Fmoc-α-aminoacyl)benzotriazoles as efficient coupling reagents.
In chapter 3 the preparation of peptides containing arginine by utilizing \( N-(Cbz-\alpha\text{-aminoacyl}) \)benzotriazoles as efficient coupling reagents will be discussed. Synthesis of protected integrin recognition sequence Arg-Gly-Asp will be described utilizing benzotriazole methodology.

1.2.2 Bis(1\( H \)-Benzotriazol-1-yl)methanethione and its Application in Thiocarbamoylation and Related Reactions

Classical thioacylating agents such as thiophosgene\(^{27,28}\) and carbon disulfide\(^{29}\) have been converted to the corresponding thiocarbamoyl derivatives\(^{30-32}\) for further reactions with nucleophiles. Alternatively, the thiocarbonyl group can be introduced by using thionating agents such as sulfur dihydride,\(^{33}\) phosphorus pentasulfide\(^{34,35}\) and Lawesson’s reagent.\(^{33}\)

A valuable alternative reagent to the classical thioacylating reagents is bis(1\( H \)-benzotriazol-1-yl)methanethione 1.30 (Scheme 1-9), which is more stable and less hygroscopic.\(^{36}\) This reagent has been utilized in the synthesis of thioureas,\(^{6,37}\) \( N \)-hydroxythioureas,\(^{38}\) thioamides,\(^{6}\) thiocarbamates,\(^{6}\) thionoesters,\(^{6}\) dithiocarbamates,\(^{6,39}\) thiocarbonates,\(^{6}\) dithiocarbonates,\(^{6}\) thiosemicarbazides,\(^{38}\) guanidines\(^{40}\) and \( \beta \)-enamino thioic acid derivatives.\(^{41}\)

Thus, the reagent 1.30 undergoes substitution reactions with \( N \)-, \( O \)- and \( S \)-nucleophiles with selective substitution of only one benzotriazoly group at ambient temperature affording the corresponding benzotriazole derivatives 1.31-1.33. However, reactions with Grignard reagents and organolithium failed to afford thiocarbonylbenzotriazole.\(^{6}\) Imination with triphenylphosphine imides\(^{40}\) and hetero Diels–Alder cycloaddition with dienes\(^{42,43}\) affords 1.34-1.36 respectively (Scheme 1-9).
Enamino thiocarbonylbenzotriazoles 1.37 were synthesized by the reaction of 1.30 with ketimines (Scheme 1-9). The second benzotriazolyl group in the intermediates obtained may be further substituted with different N-, O-, S- and C-nucleophiles under different reaction conditions depending on the type of substituent present in the starting compounds.

The benzotriazole intermediate 1.31 can be used as thiocarbamoylating agent. This class of compounds is prepared by the reaction of 1.30 and a variety of primary and secondary amines (Scheme 1-9); however the reaction with primary aryl amines is accompanied by elimination of the benzotriazole molecule and results in formation of aryl isothiocyanates (Scheme 1-10).

Scheme 1-9. Reactions of bis(1H-benzotriazol-1-yl)methanethione

Scheme 1-10. Formation of isothiocyanates
The synthetic applications of thiocarbamoyl benzotriazoles 1.31 include: nucleophilic substitutions with C-, N-, O- and S-nucleophiles to obtain thiocarbamides 1.38, thioureas 1.39, thiocarbamates 1.40 and dithiocarbamates 1.41 (Scheme 1-11).

Scheme 1-11. Synthetic applications of thiocarbamoyl benzotriazoles

In chapter 4, easy and convenient one-pot synthesis of 5-(substituted amino)-1,2,3,4-thiatriazoles will be described from bis(1H-benzotriazol-1-yl)methanethione 1.30.

In chapter 5, detailed synthesis of CXCR3 antagonist 8-azaquinazolinone analogue, NBI-74330 is described. It was tested by Harrison et. al. for the treatment of malignant gliomas.

Results of the biological testing are summarized.
CHAPTER 2
SYNTHESIS OF PEPTIDES BY EXTENSIONS AT THE N- OR C-TERMINII OF LYSINE

2.1 Introduction

Lysine is an essential α-amino acid and is basic in nature. It is important for proper growth and it plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acid into energy and helping low cholesterol. Lysine plays major role in calcium absorption and formation of collagens.\textsuperscript{44} The most promising role of lysine is its use in preventing painful herpes sores caused by herpes simplex viruses (HSV).\textsuperscript{45}

α-Lysine (Figure 2-1) residue occurs in various biologically active peptides, for example, in the active portions of adrenocorticotropic hormones,\textsuperscript{46} melanotropic hormones,\textsuperscript{47} thrombolytically active therapeutic agents such as P6A (obtained from fibrinogen degradation),\textsuperscript{48-50} adhesive proteins of marine mussel,\textsuperscript{51,52} chemotactic peptides,\textsuperscript{53} biocompatible telomers,\textsuperscript{54} hybrid peptides\textsuperscript{55} and analogues like somatostatin.\textsuperscript{56} Basic amino acid L-lysine is also a constituent of sweet tasting peptide. N-Acetyl-L-phenlyalanyl-L-lysine possessed a potential sweetness 20 times stronger than sucrose.\textsuperscript{57}

![Figure 2-1. Chemical structure and coupling sites of L-lysine](image)

Lysine has an ε-amino group at the end of the side chain. So, in addition to α-peptides, it is also capable of forming alternative ε-peptides or unnatural peptides or isopeptides.
Consequently, considerable efforts have been made to incorporate Lys residues in the peptide chain extension. Previous preparations of peptides containing $N^\epsilon$-Cbz-lysine utilized coupling reagents like carbodiimides DCC,\textsuperscript{48,52,55,58-64} and BOP.\textsuperscript{54} (Figure 2-2, Scheme 2-1). Peptide coupling has also been achieved using the mixed anhydride method,\textsuperscript{53,56,65-67} the azide method,\textsuperscript{68,69} the active ester method (formyl-substituted nitrophenylthio esters) in Scheme 2-2.\textsuperscript{70}

![DCC](image1)

![BOP](image2)

Figure 2-2. Peptide coupling reagents

\[
\text{Boc-Ala-OH} + \text{DCC} + \text{HOBT} \xrightarrow{(i) \text{Anhd THF, 0 °C, RT, 24 h}} \text{Boc-Ala-Lys(Cbz)-OBz}
\]

\[
\text{(ii) Lys(Cbz)-OBz, HCl, NMM, Anhd THF, RT, 24 h}}
\]

Scheme 2-1. Literature procedures for lysine peptide using DCC/HOBt

\[
\text{Cl-NO}_2 \xrightarrow{\text{Na}_2\text{S}, 9\text{H}_2\text{O}} \text{NaS-NO}_2 \xrightarrow{\text{Fmoc-AA-Cl, THF-DMF, -78-50 °C, 3 h}} \text{Fmoc-AA-S-NO}_2
\]

\[
\text{Fmoc-AA-S-NO}_2 + \text{HCl-Lys-(Cbz)-OBu} \xrightarrow{\text{DIPEA, NMM, DMF-H}_2\text{O (9:1)}} \text{Fmoc-AA-Lys-(Cbz)-OBu}
\]

Scheme 2-2. Formyl-substituted nitrophenylthioester method

However, all these methodologies require long reaction times (18-24 h). For example, the coupling of N-protected amino acid with $N^\epsilon$-Cbz-L-Lys or its corresponding ester in the presence of DCC/HOBt requires 18-24 h to achieve completion.\textsuperscript{55,56,59,63-65,70} In some cases, the final product is an ester and thus requires hydrolysis or hydrogenolysis as an additional step to obtain the desired free peptide.\textsuperscript{48,49,55,65,67,69,70}
\( \varepsilon \)-Lysine peptide is the fundamental structural unit of clavicepamines. \(^{71}\) The \( \varepsilon \)-amino group of lysine is also involved in the amide bond formation in collagen, bovine growth hormone and some natural products such as biocytin and bacitracin. \(^{67,72}\) \( \varepsilon \)-Lysine peptides were previously prepared utilizing various procedures involving carbodiimides, mixed carboxylic-carbonic anhydrides (MCA) (Scheme 2-3), \(^{67,73}\) or using lysine Cu complex. \(^{71,73}\) (Scheme 2-4). According to Theodoropoulos, in the case of lysine, the Cu complex formation gives the best protection for isopeptide coupling of lysine at C-terminal. \(^{73}\) Both the carbodiimide and MCA method reportedly require reaction times of 5-12 h, and the \(-\text{COOH}\) group of \( N^\alpha \)-Cbz-\( L \)-Lys needs to be protected and thus there is an additional hydrolysis step to obtain the free peptide.

Scheme 2-3. Literature procedure for \( \varepsilon \)-lysine peptides using mixed anhydride method

Scheme 2-4. Literature procedure for \( \varepsilon \)-lysine peptides using Cu-complex.
In continuation of our research on peptide chain extensions, an easy and convenient preparation of diverse di- and tripeptides containing $N^\varepsilon$-Cbz-lysine and $N^\alpha$-Cbz-lysine units by extension at both N- and C-terminus of protected lysine is described using benzotriazole methodology.

2.2 Results and Discussion

2.2.1 Preparation of Dipeptides 2.3a-g, and the Diastereomeric Mixtures (2.3a+2.3a'), (2.3d+2.3d') Using the $N^\alpha$-Terminus of $N^\varepsilon$-Cbz-L-Lys 2.2a

Peptide coupling reactions were successfully carried out between $N$-(Cbz- or Fmoc-$\alpha$-aminoacyl)benzotriazoles 2.1a-g derived from $L$-Phe, $L$-Ala, $L$-Trp, $L$-Met and $N^\varepsilon$-Cbz-$L$-Lys 2.2a in partially aqueous CH$_3$CN solution in the presence of Et$_3$N over 30-45 min. (Scheme 2-5, Table 2-1). By comparison, literature methods require 18-24 h for completion.

$N$-(Cbz- or Fmoc-$\alpha$-aminoacyl)benzotriazoles 2.1a-g were prepared following literature procedure. $^1$H NMR analysis for each compound 2.3 revealed two sets of doublets for the two –NH protons ranging from 7.40–8.30 ppm (Figure 2-3). The methyl protons from the $L$-Ala fragment in 2.3a showed a clear doublet and two sets of doublet for the –NH protons, supporting the enantiopurity of the $LL$-dipeptide. However in the case of the diastereomeric mixture (2.3a+2.3a'), although the methyl protons from $L$-Ala fragment also showed a clear doublet but one of the –NH protons showed a multiplet in the range of 8.00-8.15 ppm instead of a clear doublet (Figure 2-4).

![Scheme 2-5. Preparation of dipeptides 2.3a-g and the diastereomeric mixtures (2.3a+2.3a'), (2.3d+2.3d')](attachment:image.png)
Figure 2-3. $^1$H NMR spectrum for $2.3a$

Figure 2-4. $^1$H NMR spectrum for $(2.3a+2.3a')$
The 13C NMR for (2.3a+2.3a') showed two singlets for each aliphatic and carbonyl carbons. In the case of (2.3d+2.3d'), one of the –NH protons showed an apparent triplet at 7.47 ppm. 13C NMR of (2.3d+2.3d') also gave two singlets for each aliphatic and carbonyl carbons. The enantiopurity of the dipeptides 2.3a-g was further confirmed by HPLC analyses using Chirobiotic T column (detection at 254 nm, flow rate 1.0 mL/min, and MeOH as solvent). For each of the LL-dipeptide 2.3a and 2.3d, the HPLC results showed a single peak. By contrast two peaks were observed for the corresponding diastereomeric mixtures (2.3a+2.3a') and (2.3d+2.3d') confirming the enantiopurity of the LL-dipeptide 2.3a and 2.3d. These results also indicated shorter retention times for the LL-configuration as compared to the LD-configuration as summarized in Table 2-1.

Table 2-1. Preparation of dipeptides 2.3a-g and the diastereomeric mixtures (2.3a+2.3a'), (2.3d+2.3d')

<table>
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<th>Entry</th>
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<th>Product</th>
<th>Yielda (%)</th>
<th>mp (°C)</th>
<th>[α]23D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cbz-L-Ala-Bt 2.1a</td>
<td>Cbz-L-Ala-Nε-Cbz-L-Lys-OH 2.3a</td>
<td>85</td>
<td>92–94</td>
<td>+4.04</td>
</tr>
<tr>
<td>2</td>
<td>Cbz-DL-Ala-Bt (2.1a+2.1a')</td>
<td>Cbz-DL-Ala-Nε-Cbz-L-Lys-OH (2.3a+2.3a')d</td>
<td>88</td>
<td>105–108</td>
<td>−1.86</td>
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<tr>
<td>3</td>
<td>Cbz-L-Phe-Bt 2.1b</td>
<td>Cbz-L-Phe-Nε-Cbz-L-Lys-OH 2.3b</td>
<td>95</td>
<td>117–119</td>
<td>−4.53</td>
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<tr>
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<td>Cbz-L-Trp-Nε-Cbz-L-Lys-OH 2.3c</td>
<td>85</td>
<td>114–116</td>
<td>−15.44</td>
</tr>
<tr>
<td>5</td>
<td>Cbz-L-Met-Bt 2.1d</td>
<td>Cbz-L-Met-Nε-Cbz-L-Lys-OH 2.3d</td>
<td>83</td>
<td>122–123</td>
<td>−1.94</td>
</tr>
<tr>
<td>6</td>
<td>Cbz-DL-Met-Bt (2.1d+2.1d')</td>
<td>Cbz-DL-Met-Nε-Cbz-L-Lys-OH (2.3d+2.3d')g</td>
<td>91</td>
<td>45–46</td>
<td>−0.40</td>
</tr>
<tr>
<td>7</td>
<td>Fmoc-L-Trp-Bt 2.1e</td>
<td>Fmoc-L-Trp-Nε-Cbz-L-Lys-OH 2.3e</td>
<td>80</td>
<td>91–93</td>
<td>−14.71</td>
</tr>
<tr>
<td>8</td>
<td>Fmoc-L-Met-Bt 2.1f</td>
<td>Fmoc-L-Met-Nε-Cbz-L-Lys-OH 2.3f</td>
<td>80</td>
<td>122–124</td>
<td>−7.06</td>
</tr>
<tr>
<td>9</td>
<td>Fmoc-L-Phe-Bt 2.1g</td>
<td>Fmoc-L-Phe-Nε-Cbz-L-Lys-OH 2.3g</td>
<td>92</td>
<td>128–132</td>
<td>−13.03</td>
</tr>
</tbody>
</table>

aIsolated yields; b Lit. data not available in 74-76; c Retention time = 3.07 min; d Retention time = 3.13, 3.62 min, for conditions, see experimental section; e Lit. Ref 77 mp 117-121 °C; [α]25D = −6.90 (c, 1.0, DMF); f Retention time = 2.99 min; g Retention time = 2.99, 3.58 min.
2.2.2 Preparation of Unnatural Dipeptides 2.3h–j Using $N^\alpha$-Terminus of $N^\alpha$-Cbz-$L$-Lys 2.2b

Peptide coupling reactions were carried out successfully at the side chain $\varepsilon$-amino functionality of lysine using $N^\alpha$-Cbz-$L$-Lys 2.2b and $N$-(Cbz-or Fmoc-$\alpha$-aminoacyl)benzotriazoles 2.1b,c,f and (2.1h+2.1h')\(^1\) derived from $L$-Phe, $L$-Trp, $L$-Met, $DL$-Phe (Scheme 2-6, Table 2-2) in a similar procedure as adopted for natural dipeptides. Interestingly, a shorter reaction time (<15min) sufficed compared to the natural peptides. \(^1\)H NMR and HPLC analysis showed no detectable epimerization (<1%) for the enantiopure $LL$-dipeptides 2.3h–j. For compounds 2.3h–j, \(^1\)H NMR analysis for each compound revealed three sets of doublets for the three –NH protons ranging from 7.45–8.10 ppm. The observed \(^1\)H NMR pattern was different for these unnatural dipeptides as compared to the natural dipeptides. For example, in the case of 2.3h and 2.3i, the –CH protons arising from the $L$-Phe and $L$-Trp fragment were shifted upfield (2.3h: 3.89 ppm and 2.3i: 3.91 ppm) as compared to those in the corresponding fragment in the natural dipeptides 2.3b (4.80 ppm) and 2.3c (4.37 ppm). The HPLC results showed a single peak for compounds 2.3h–j. By contrast two peaks were observed for the corresponding diastereomeric mixture (2.3h+2.3h') confirming the enantiopurity of the $LL$-dipeptide 2.3h.

\[ \text{Scheme 2-6. Preparation of unnatural dipeptides 2.3h–j and the diastereomeric mixture (2.3h+2.3h') } \]

\(^1\) Compound numbers written in the bracket represent racemic compound or the diastereomeric mixture.
Table 2-2. Preparation of unnatural dipeptides \(2.3h\text{--}j\) and the diastereomeric mixture \((2.3h+2.3h')\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield(^a) (%)</th>
<th>mp (°C)</th>
<th>([\alpha]^{23}_{D})</th>
<th>(t_R) (min)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cbz-L-Phe-Bt (2.1b)</td>
<td>(N^\alpha\text{-Cbz}-N^\varepsilon\text{-}(Cbz-L-Phe)-L-Lys-OH (2.3h^c))</td>
<td>79</td>
<td>162-164</td>
<td>-12.88</td>
<td>3.60</td>
</tr>
<tr>
<td>2</td>
<td>Cbz-DL-Phe-Bt ((2.1b+2.1b'))</td>
<td>(N^\alpha\text{-Cbz}-N^\varepsilon\text{-}(Cbz-DL-Phe)-L-Lys-OH ((2.3h+2.3h')))</td>
<td>95</td>
<td>93-95</td>
<td>-5.00</td>
<td>3.16, 3.60</td>
</tr>
<tr>
<td>3</td>
<td>Cbz-L-Trp-Bt (2.1c)</td>
<td>(N^\alpha\text{-Cbz}-N^\varepsilon\text{-}(Cbz-L-Trp)-L-Lys-OH (2.3i))</td>
<td>93</td>
<td>87-89</td>
<td>-17.94</td>
<td>3.34</td>
</tr>
<tr>
<td>4</td>
<td>Fmoc-L-Met-Bt (2.1f)</td>
<td>(N^\alpha\text{-Cbz}-N^\varepsilon\text{-}(Fmoc-L-Met)-L-Lys-OH (2.3j))</td>
<td>94</td>
<td>83-85</td>
<td>-9.70</td>
<td>2.55</td>
</tr>
</tbody>
</table>

\(^a\)Isolated yields; \(^b\)t\(_R\): Retention time; for conditions, see the experimental section; \(^\circ\)Physical characterization data is not available in the literature.

2.2.3. Preparation of Dipeptides \(2.3k\text{--}n\), and the Diastereomeric mixture \((2.3l+2.3l')\) by Chain Elongation at the C-Terminus of Lysine Using \(N^\alpha\text{-Fmoc}-N^\varepsilon\text{-Cbz}-L\text{-Lys-Bt} \(2.1h\)\)

Preparation of \(N^\alpha\text{-Fmoc}-N^\varepsilon\text{-Cbz}-L\text{-Lys-Bt} \(2.1h\)\) was carried out using reported procedure\(^{21}\) from \(N^\alpha\text{-Fmoc}-N^\varepsilon\text{-Cbz}-L\text{-Lys-OH} \(2.2c\)\) using \(1H\)-benzotriazole in the presence of SOCl\(_2\). Peptide coupling between \(N^\alpha\text{-Fmoc}-N^\varepsilon\text{-Cbz}-L\text{-Lys-Bt} \(2.1h\)\) and \(L\)-amino acids \(2.2a,c\text{-}e\) and \(DL\)-Met \((2.2c+2.2c')\) proceeded in CH\(_3\)CN/H\(_2\)O in the presence of Et\(_3\)N for 30 min (Scheme 2-7, Table 2-3). The crude products were washed with 4N HCl to remove the by-product. NMR analysis showed no detectable epimerization (<1%) for the enantiopure \(LL\)-dipeptides \(2.3k\text{-}n\).

![Scheme 2-7](image-url)

Scheme 2-7. Preparation of dipeptides \(2.3k\text{-}n\) and the diastereomeric mixture \((2.3l+2.3l')\) from \(N^\alpha\text{-Fmoc}-N^\varepsilon\text{-Cbz}-L\text{-Lys-Bt} \(2.1h\)\)
Table 2-3. Preparation of dipeptides 2.3k-n and the diastereomeric mixture (2.3l+2.3l') from N-Nα-Fmoc-Nε-Cbz-L-Lys-Bt 2.1h

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield</th>
<th>mp (°C)</th>
<th>[α]D</th>
<th>tR (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>L-Met</td>
<td>Nα-Fmoc-Nε-Cbz-L-Lys-L-Met-OH 2.3l</td>
<td>95</td>
<td>138–140</td>
<td>+2.09</td>
<td>2.57</td>
</tr>
<tr>
<td>3</td>
<td>DL-Met (2.2c+2.2c')</td>
<td>Nα-Fmoc-Nε-Cbz-L-Lys-DL-Met-OH (2.3l+2.3l')</td>
<td>85</td>
<td>80–81</td>
<td>−3.94</td>
<td>2.57, 3.59</td>
</tr>
<tr>
<td>4</td>
<td>L-Trp</td>
<td>Nα-Fmoc-Nε-Cbz-L-Lys-L-Trp-OH 2.3m</td>
<td>88</td>
<td>67–69</td>
<td>+2.40</td>
<td>2.98</td>
</tr>
<tr>
<td>5</td>
<td>L-Ser</td>
<td>Nα-Fmoc-Nε-Cbz-L-Lys-L-Ser-OH 2.3n</td>
<td>80</td>
<td>85–89</td>
<td>−1.47</td>
<td>3.10</td>
</tr>
</tbody>
</table>

aIsolated Yields; b tR: Retention time; for conditions, see the experimental section.

For compounds 2.3k-n, 1H NMR of each compound revealed two sets of doublets for the two –NH protons ranging from 7.53–8.25 ppm. The HPLC results showed a single peak for 2.3k-n. By contrast two peaks were observed for the corresponding diastereomeric mixture (2.3l+2.3l') confirming the enantiopurity of the LL-dipeptide 2.3l.

2.2.4. Preparation of N-Protected Dipeptidoylbenzotriazoles 2.5a-c, and the Diastereomeric Mixture (2.5b+2.5b') from N-Protected Dipeptides 2.4a,b, 2.3b and (2.4b+2.4b')

N-Cbz-Dipeptides 2.4a,b, 2.3b and the diastereomeric mixture (2.4b+2.4b') were successfully converted into their corresponding benzotriazole derivatives 2.5a-c and the diastereomeric mixture (2.5b+2.5b') (Scheme 2-8, Table 2-4). The reaction was carried out at –15 °C for the formation of N-protected dipeptidoylbenzotriazoles following literature procedure. Progress of the reaction was monitored by 1H NMR. In the case of 2.5a, after the removal of THF, the residue was treated with EtOAc, and the crystalline material formed was filtered to give the desired product in pure form. In the case of 2.5c, 1.5 equivalent of SOCl₂ was used instead of 1 equivalent to achieve completion. In addition, 2.5c could not be isolated by the
usual acid (4N HCl) or base (dilute Na₂CO₃) work up and the crude product was purified by reprecipitation using EtOAc/hexanes, in order to remove excess 1H-benzotriazole.

Scheme 2-8. Preparation of N-Cbz-dipeptidoylbenzotriazoles 2.5a-c and the diastereomeric mixture (2.5b+2.5b')

Table 2-4. Preparation of N-Cbz-dipeptidoylbenzotriazoles 2.5a-c and the diastereomeric mixture (2.5b+2.5b')

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield(^a) (%)</th>
<th>mp (°C)</th>
<th>([\alpha]_{23}^D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cbz-L-Ala-L-Trp-OH 2.4(^a) Cbz-L-Phe-L-Met-OH 2.4b</td>
<td>Cbz-L-Ala-L-Trp-Bt 2.5a Cbz-L-Phe-L-Met-Bt 2.5b</td>
<td>70</td>
<td>162–164</td>
<td>-36.99</td>
</tr>
<tr>
<td>2</td>
<td>Cbz-L-Phe-DL-Met-OH (2.4b+2.4b') Cbz-L-Phe-Cbz(^z)-L-Lys-OH 2.3b</td>
<td>Cbz-L-Phe-DL-Met-Bt (2.5b+2.5b') Cbz-L-Phe-Cbz(^z)-L-Lys-Bt 2.5c</td>
<td>65</td>
<td>140–141</td>
<td>-13.86</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>139–141</td>
<td>-17.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Isolated yields

2.2.5. Preparation of Tripeptides 2.6a-e, and the Diastereomeric Mixtures (2.6b+2.6b'), (2.6e+2.6e')

Tripeptides 2.6a-e and the diastereomeric mixtures (2.6b+2.6b'), (2.6e+2.6e') were prepared by two different approaches:

(i) Tripeptides 2.6a,b and the diastereomeric mixture (2.6b+2.6b'), by extension from the N\(^\alpha\)-terminus of N\(^\varepsilon\)-Cbz-L-Lys 2.2a were obtained by coupling reactions between N-Cbz-dipeptidoylbenzotriazoles 2.5a,b and the diastereomeric mixture (2.5b+2.5b') with N\(^\varepsilon\)-Cbz-Lys 2.2a (Scheme 2-9, Table 2-5)

(ii) Tripeptides 2.6c-e and the diastereomeric mixture (2.6e+2.6e'), by extension at the C-terminus were obtained by coupling reaction between Cbz-L-Phe-N\(^\varepsilon\)-Cbz-L-Lys-Bt 2.5c and free amino acid 2.2c,d,f and (2.2f+2.2f') (Scheme 2-10, Table 2-6)
Scheme 2-9. Preparation of tripeptides 2.6a,b and the diastereomeric mixture (2.6b+2.6b')

Table 2-5. Preparation of tripeptides 2.6a,b and the diastereomeric mixture (2.6b+2.6b')

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactant</th>
<th>Product</th>
<th>Yield (a) (%)</th>
<th>mp (°C)</th>
<th>[α]23D</th>
<th>tR (min) (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cbz-L-Ala-L-Trp-Bt 2.5a</td>
<td>Cbz-L-Ala-L-Trp-Ne-Cbz-L-Lys-OH 2.6a</td>
<td>75</td>
<td>137–139</td>
<td>-8.94</td>
<td>3.16</td>
</tr>
<tr>
<td>2</td>
<td>Cbz-L-Phe-L-Met-Bt 2.5b</td>
<td>Cbz-L-Phe-L-Met-Ne-Cbz-L-Lys-OH 2.6b</td>
<td>72</td>
<td>135–140</td>
<td>-7.76</td>
<td>3.09</td>
</tr>
<tr>
<td>3</td>
<td>Cbz-L-Phe-DL-Met-Bt (2.5b+2.5b')</td>
<td>Cbz-L-Phe-DL-Met-Ne-Cbz-L-Lys-OH (2.6b+2.6b')</td>
<td>75</td>
<td>126–127</td>
<td>-6.98</td>
<td>3.09, 3.60</td>
</tr>
</tbody>
</table>

*Isolated yields; *t*R: Retention time; for conditions, see the experimental section.

Scheme 2-10. Preparation of tripeptides 2.6c-e and the diastereomeric mixture (2.6e+2.6e')

1H NMR showed three separate sets of doublets for the tripeptides 2.6c-e ranging from 7.54-9.38 ppm. In case of 2.6e, the L-Ala fragment showed a clear doublet at 1.24 ppm, but gave a multiplet in the range of 1.20-1.30 ppm for the corresponding diastereomeric mixture (2.6e+2.6e'). One of the –NH protons in the diastereomeric mixture (2.6e+2.6e') showed a multiplet in the range of 8.18-8.32 ppm, instead of a clear doublet as observed in the enantiopure 2.6e.
Table 2-6. Preparation of tripeptides 2.6c-e and the diastereomeric mixture (2.6e+2.6e’)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reactant</th>
<th>Product</th>
<th>Yielda (%)</th>
<th>mp (°C)</th>
<th>[α]23bD</th>
<th>tR (min)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-Met 2.2c</td>
<td>Cbz-L-Phe-ε-Cbz-L-Lys-L-Met-OH 2.6c</td>
<td>73</td>
<td>137–139</td>
<td>−10.99</td>
<td>2.73, 3.14</td>
</tr>
<tr>
<td>2</td>
<td>L-Trp 2.2d</td>
<td>Cbz-L-Phe-ε-Cbz-L-Lys-L-Trp-OH 2.6d</td>
<td>88</td>
<td>71–73</td>
<td>−2.09</td>
<td>3.08, 3.61</td>
</tr>
<tr>
<td>3</td>
<td>L-Ala 2.2f</td>
<td>Cbz-L-Phe-ε-Cbz-L-Lys-L-Ala-OH 2.6e</td>
<td>74</td>
<td>129–131</td>
<td>−9.60</td>
<td>3.37</td>
</tr>
<tr>
<td>4</td>
<td>DL-Ala (2.2f+2.2f’)</td>
<td>Cbz-L-Phe-ε-Cbz-L-Lys-DL-Ala-OH (2.6e+2.6e’)</td>
<td>90</td>
<td>99–101</td>
<td>−7.35</td>
<td>3.37, 3.58</td>
</tr>
</tbody>
</table>

aIsolated Yields; b tR: Retention time; for conditions, see the experimental section; 
Diastereomeric ratio = 9:1

The HPLC results showed a single peak for 2.6a,b,e. By contrast two peaks were observed for the corresponding diastereomeric mixture (2.6b+2.6b’) and (2.6e+2.6e’) confirming the enantiopurity of the LL-dipeptides 2.6b and 2.6e. However, two peaks were observed for 2.6c,d in the ratio of 9:1 suggesting either epimerization or the existence of rotamers; however, no precise reason can yet be attributed to this observation.

2.2.6. Preparation of Unnatural Tripeptide 2.6f by Extension from the Nε-Terminus of Nα-Cbz-L-Lys 2.2b

Peptide coupling reaction was carried out successfully at the side chain amino functionality of lysine using Nα-Cbz-L-Lys 2.2b and N-Cbz-L-Ala-L-Trp-Bt (Scheme 2-11) in a similar procedure to that adopted for the natural tripeptides. Unnatural tripeptide 2.6f was obtained in 82% yield and enantiopurity.

Scheme 2-11. Preparation of unnatural tripeptide 2.6f
2.3 Conclusion

In summary, convenient preparations of natural and unnatural di- and tripeptides derived from lysine by extension at the \(N^\alpha\)-, \(N^\varepsilon\)-, and \(C\)- termini has been demonstrated under mild reaction conditions. The reactions gave di- and tripeptides in high yields with easy isolation and purification procedures.

2.4 Experimental Section

2.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in CDCl\(_3\) or DMSO-\(d_6\) with TMS as an internal standard for \(^1\text{H}\) (300 MHz) or solvent as an internal standard for \(^{13}\text{C}\) (75 MHz). Elemental analyses were performed on a CarloErba-1106 instrument. Optical rotation values were measured with the use of sodium D line. \(N\)-Cbz- and Fmoc-\(\alpha\)-amino acids were purchased from Fluka and Acros, were used without further purification. HPLC analyses were performed on Beckman system gold programmable solvent module 126 using Chirobiotic T column (4.6 x 250 mm), detection at 254 nm, flow rate of 1.0 mL/min and MeOH as an eluting solvent.

2.4.2 General Procedure for the Preparation of Cbz-\(\text{DL}\)-Met-Bt (2.1d+2.1d') and \(N^\alpha\)-Fmoc-\(N^\varepsilon\)-Cbz-\(L\)-Lys-Bt 2.1h

Cbz-\(\text{DL}\)-Met-Bt (2.1d+2.1d') and \(N^\alpha\)-Fmoc-\(N^\varepsilon\)-Cbz-\(L\)-Lys-Bt 2.1h were prepared using the reported procedure.\(^{21}\) To a solution of 1\(H\)-benzotriazole (8 mmol) in dry THF (10 mL), SOCl\(_2\) (3 mmol) was added and the reaction mixture was stirred for 20 min at room temperature. To this solution, N-protected amino acids Cbz-\(\text{DL}\)-Met-OH or \(N^\alpha\)-Fmoc-\(N^\varepsilon\)-Cbz-\(L\)-Lys-OH 2.2c (2 mmol) was directly added and reaction mixture was stirred for 2 h at room tempreature. Progress of the reaction was monitored by \(^1\text{H}\) NMR. The white precipitate obtained was filtered off and the filtrate was concentrated under vacuum. To the residue obtained, ethyl acetate (100 mL) was
added and the solution was washed with 4N HCl (3 x 50 mL) solution followed by brine (25 mL). The organic layer was dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. Compounds were recrystallized from CHCl₃/hexanes for elemental analysis.

**Benzyl-N-[1-(1H-1,2,3-benzotriazol-1-ylcarbonyl)-3-(methylsulfanyl)propanoyl]carbamate, Cbz-DL-Met-Bt, (2.1d+2.1d')**: Colorless microcrystals (93%); mp 93–94 °C; ¹H NMR (DMSO-d₆) δ 2.05 (s, 3H), 2.20–2.34 (m, 2H), 2.58–2.78 (m, 2H), 5.06 (s, 2H), 5.60–5.72 (m, 1H), 7.30–7.42 (m, 5H), 7.66 (t, J = 7.6 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 8.20–8.34 (m, 3H). ¹³C NMR (DMSO-d₆) δ 14.2, 29.6, 29.7, 53.4, 65.9, 114.0, 120.2, 126.8, 127.9, 128.0, 128.4, 130.7, 131.2, 136.7, 145.4, 156.4, 171.9. Anal. Calcd for C₁₉H₂₀N₄O₃S: C, 59.36; H, 5.24; N, 14.57. Found: C, 59.22; H, 5.20; N, 14.66.

**9H-Fluoren-9-ylmethyl-N-[(1S)-5-benzyloxycarbonylamino-1-(1H-1,2,3-benzotriazol-1-yl-carbonyl)pentyl]carbamate, Nα-Fmoc-Nε-Cbz-L-Lys-Bt, 2.1h**: Colorless microcrystals (70%); mp 148–149 °C; [α]23 D = −35.94 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.25–1.60 (m, 4H), 1.71–2.03 (m, 2H), 2.90–3.07 (m, 2H), 4.20–4.29 (m, 1H), 4.30–4.40 (m, 2H), 4.97 (s, 2H), 5.48–5.50 (m, 1H), 7.20–7.38 (m, 9H), 7.42 (t, J = 7.4 Hz, 2H), 7.65 (t, J = 7.7 Hz, 1H), 7.72 (d, J = 7.3 Hz, 2H), 7.82 (t, J = 7.7 Hz, 1H), 7.90 (d, J = 7.4 Hz, 2H), 8.24 (d, J = 8.5 Hz, 1H), 8.30 (d, J = 8.5 Hz, 1H). ¹³C NMR (CDCl₃) δ 22.4, 29.2, 32.2, 40.1, 47.1, 54.4, 66.6, 67.1, 114.3, 119.9, 120.3, 125.0, 126.5, 127.0, 127.7, 128.0, 128.4, 130.7, 131.0, 136.4, 141.2, 143.6, 143.7, 145.9, 156.2, 156.6, 171.7. Anal. Calcd for C₃₅H₃₃N₅O₅: C, 69.64; H, 5.51; N, 11.60. Found: C, 69.39; H, 5.64; N, 11.30.

### 2.4.3 General Procedure for the Preparation of LL-Dipeptides 2.3a-g and the Diastereomeric Mixture (2.3b+2.3b') and (2.3d+2.3d')

N-(Protected α-aminoacyl)benzotriazoles 2.1a-g (0.5 mmol) were added to a solution of Nε-Cbz-L-Lys 2.2a (0.5 mmol) in CH₃CN/H₂O (5 mL/10 mL) in the presence of Et₃N (0.6
mmol) at room temperature. The reaction mixture was then stirred at room temperature until the starting material was completely consumed as observed on TLC using hexanes/EtOAc (2:1) as the eluent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH₃CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), then dried over anhydrous MgSO₄. Evaporation of the solvent gave the desired product in pure form, which was further recrystallized from CHCl₃/hexanes unless specified, otherwise.

(S)-6-Benzyloxy carbonylamino-2-[(S)-2-
benzyloxy carbonylaminopropanoylaminol]hexanoic acid, N-Cbz-L-Ala-Nε-Cbz-L-Lys-OH,

2.3a: Colorless microcrystals (89%); mp 92–94 °C; [α]²³_D = +4.04 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.20 (d, J = 7.1 Hz, 3H), 1.23–1.48 (m, 4H), 1.49–1.78 (m, 2H), 2.97 (apparent d, J = 5.8 Hz, 2H), 4.03–4.20 (m, 2H), 5.00 (s, 4H), 7.18–7.40 (m, 11H), 7.41 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 7.7 Hz, 1H), 12.55 (s, 1H). ¹³C NMR (DMSO-d₆) δ 18.2, 22.7, 29.0, 30.8, 40.1, 49.8, 51.8, 65.2, 65.4, 127.8, 128.4, 137.1, 137.3, 155.6, 156.1, 172.7, 173.6. Anal. Calcd for C₂₅H₃₁N₃O₇: C, 61.84; H, 6.44; N, 8.65. Found: C, 62.10; H, 6.62; N, 8.35.

(S)-6-Benzyloxy carbonylamino-2-(2-
benzyloxy carbonylaminopropanoylaminol)hexanoic acid, N-Cbz-DL-Ala-Nε-Cbz-L-Lys-OH, (2.3a+2.3a'): Colorless microcrystals (88%); mp 105–108 °C; [α]²³_D = −1.86 (c 1.0, DMF);
¹H NMR (DMSO-d₆) δ 1.20 (d, J = 7.1 Hz, 3H), 1.20–1.46 (m, 4H), 1.50–1.78 (m, 2H), 2.88–3.04 (m, 2H), 4.03–4.22 (m, 2H), 4.99 (s, 4H), 7.16–7.50 (m, 12H), 8.00–8.15 (m, 1H), 12.58 (br s, 1H). ¹³C NMR (DMSO-d₆) δ 18.2, 18.7, 22.6, 22.7, 29.0, 29.1, 30.8, 41.0, 49.8, 50.0, 51.6, 51.8, 65.2, 65.4, 127.8, 128.4, 137.1, 137.3, 155.6, 155.7, 156.1, 172.5, 172.7, 173.6. Anal. Calcd for C₂₅H₃₁N₃O₇: C, 61.84; H, 6.44; N, 8.65. Found: C, 61.92; H, 6.50; N, 8.82.
(S)-6-Benzylxycarbonylamino-2-[(S)-2-benzyloxycarbonylamino-3-phenylpropanoylamino]hexanoic acid, N-Cbz-L-Phe-Nε-Cbz-L-Lys-OH, 2.3b: White microcrystals (95%); mp 117–119 °C; [α]$_{23}^{23}$ = –4.53 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ
1.20–1.50 (m, 4H), 1.50–1.82 (m, 2H), 2.61–2.80 (m, 1H), 2.88–3.15 (m, 3H), 4.11–4.23 (m, 1H), 4.23–4.36 (m, 1H), 4.92 (s, 2H), 5.00 (s, 2H), 7.08–7.44 (m, 16H), 7.48 (d, $J = 8.8$ Hz, 1H), 8.27 (d, $J = 7.1$ Hz, 1H), 12.63 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 22.9, 29.2, 30.9, 37.6, 40.2, 52.1, 56.1, 65.3, 65.4, 126.4, 127.0, 127.6, 127.8, 127.9, 128.2, 128.4, 128.5, 129.4, 137.1, 137.4, 138.2, 156.0, 156.3, 172.0, 173.7. Anal. Calcd for C$_{31}$H$_{35}$N$_3$O$_7$: C, 66.30; H, 6.28; N, 7.48. Found: C, 66.47; H, 6.47; N, 7.21.

(S)-6-Benzylxycarbonylamino-2-[(S)-2-benzyloxycarbonylamino-3-(1H-indol-3-yl)propanoylamino]hexanoic acid, N-Cbz-L-Trp-Nε-Cbz-L-Lys-OH, 2.3c: White microcrystals (85%); mp 114–116 °C; [α]$_{23}^{23}$ = –15.44 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ
1.20–1.50 (m, 4H), 1.53–1.83 (m, 2H), 2.83–2.99 (m, 3H), 3.04–3.18 (m, 1H), 4.13–4.28 (m, 1H), 4.28–4.41 (m, 1H), 4.92 (s, 2H), 4.99 (s, 2H), 6.97 (t, $J = 7.1$ Hz, 1H), 7.06 (t, $J = 7.7$ Hz, 1H), 7.16 (s, 1H), 7.18–7.42 (m, 13H), 7.66 (d, $J = 7.4$ Hz, 1H), 8.25 (d, $J = 7.4$ Hz, 1H), 10.81 (s, 1H), 12.61 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 22.8, 27.9, 29.1, 30.8, 40.2, 52.0, 55.3, 65.2, 65.3, 110.2, 111.3, 118.2, 118.7, 120.9, 124.0, 127.3, 127.5, 127.7, 127.8, 128.4, 136.1, 137.0, 137.3, 155.9, 156.2, 172.1, 173.7. Anal. Calcd for C$_{33}$H$_{35}$N$_4$O$_7$: C, 65.99; H, 6.04; N, 9.33. Found: C, 65.84; H, 6.10; N, 9.20.

(S)-6-Benzylxycarbonylamino-2-[(S)-2-benzyloxycarbonylamino-4-methylsulfanylbutanoylamino]hexanoic acid, N-Cbz-L-Met-Nε-Cbz-L-Lys-OH, 2.3d: White microcrystals (83%); mp 122–123 °C; [α]$_{23}^{23}$ = –1.94 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ
1.24–1.48 (m, 4H), 1.48–1.64 (m, 1H), 1.64–1.74 (m, 1H), 1.76–1.94 (m, 2H), 2.02 (s, 3H),
2.42–2.54 (m, 2H), 2.92–3.04 (m, 2H), 4.05–4.20 (m, 2H), 5.00 (s, 2H), 5.01 (s, 2H), 7.18–7.45 (m, 11H), 7.48 (d, J = 8.2 Hz, 1H), 8.12 (d, J = 7.7 Hz, 1H), 12.56 (s, 1H). 13C NMR (DMSO-d\text{6}) δ 14.6, 22.7, 29.5 (2C), 30.6, 31.9, 51.3, 53.7, 65.5, 127.7 (2C), 128.3 (2C), 137.0, 137.3, 155.9, 156.1, 171.6, 173.6. Anal. Calcd for C27H35N3O7S: C, 59.43; H, 6.47; N, 7.70. Found: C, 59.66; H, 6.69; N, 7.43.

(S)-6-Benzoxycarbonylamino-2-(2-benzyloxycarbonylamino-4-methylsulfanylbutanoylamino)hexanoic acid, N-Cbz-DL-Met-N\text{ε}-Cbz-L-Lys-OH,

(2.3d+2.3d'): Colorless microcrystals (91%); mp 45–46 °C; [\alpha]^23\text{D} = −0.40 (c 1.0, DMF); 1H NMR (DMSO-d\text{6}) δ 1.20–1.47 (m, 4H), 1.50–1.75 (m, 2H), 1.75–1.90 (m, 2H), 2.01 (s, 1.5H), 2.02 (s, 1.5H), 2.49–2.55 (m, 2H), 2.90–3.02 (m, 2H), 4.07–4.22 (m, 2H), 5.00 (s, 2H), 5.01 (s, 2H), 7.20–7.40 (m, 11H), 7.47 (t, J = 9.1 Hz, 1H), 8.14 (d, J = 7.1 Hz, 1H), 12.60 (s, 1H). 13C NMR (DMSO-d\text{6}) δ 14.6, 14.7, 22.6, 22.8, 29.0, 29.1, 29.6, 29.7, 30.6, 30.8, 31.9, 32.0, 51.7, 51.9, 53.7, 53.8, 65.2, 65.4, 65.5, 127.7, 127.8, 127.8, 128.4, 137.0, 137.3, 156.0, 156.1, 171.5, 171.6, 173.5, 173.6. Anal. Calcd for C27H35N3O7S: C, 59.43; H, 6.47; N, 7.70. Found: C, 59.38; H, 6.48; N, 7.74.

(S)-6-Benzoxycarbonylamino-2-[(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-methylsulfanylbutanoylamino]hexanoic acid, N-Fmoc-L-Trp-N\text{ε}-Cbz-L-Lys-OH, 2.3e:

White microcrystals (88%); mp 91–93 °C; [\alpha]^23\text{D} = −14.71 (c 1.0, DMF); 1H NMR (DMSO-d\text{6}) δ 1.21–1.49 (m, 4H), 1.52–1.68 (m, 1H), 1.68–1.82 (m, 1H), 2.87–3.04 (m, 3H), 3.06–3.18 (m, 1H), 4.08–4.18 (m, 3H), 4.18–4.27 (m, 1H), 4.30–4.45 (m, 1H), 4.98 (s, 2H), 6.97 (t, J = 7.1 Hz, 1H), 7.06 (t, J = 7.7 Hz, 1H), 7.17–7.46 (m, 12H), 7.52 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 7.7 Hz, 1H), 7.86 (d, J = 7.4 Hz, 2H), 8.27 (d, J = 7.4 Hz, 1H), 10.82 (s, 1H), 12.62 (br s, 1H). 13C NMR (DMSO-d\text{6}) δ 22.8, 27.8, 29.11, 30.8, 46.6, 52.0, 55.3, 65.2, 65.7,
(S)-6-Benzylxycarbonylamino-2-[(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-methylsulfanylbutanoylamino]hexanoic acid, N-Fmoc-L-Met-N\textsuperscript{ε}-Cbz-L-Lys-OH, 2.3f:

White microcrystals (85%); mp 122–124 °C; [\alpha]_{D}^{23} = −7.06 (c 1.0, DMF); \(1^H\) NMR (DMSO-d\textsubscript{6}) \(\delta\) 1.20–1.48 (m, 4H), 1.50–1.63 (m, 1H), 1.63–1.76 (m, 1H), 1.76–1.97 (m, 2H), 2.03 (s, 3H), 2.43–2.54 (m, 2H), 2.90–3.02 (m, 2H), 4.08–4.19 (m, 2H), 4.19–4.31 (m, 3H), 4.98 (s, 2H), 7.20–7.36 (m, 8H), 7.41 (t, \(J = 7.4\) Hz, 2H), 7.57 (d, \(J = 8.2\) Hz, 1H), 7.72 (t, \(J = 6.3\) Hz, 2H), 7.88 (d, \(J = 7.4\) Hz, 2H), 8.13 (d, \(J = 8.4\) Hz, 1H), 12.56 (s, 1H). \(13^C\) NMR (DMSO-d\textsubscript{6}) \(\delta\) 14.7, 22.7, 29.0, 29.6, 30.6, 31.9, 46.7, 51.9, 53.6, 65.1, 65.6, 120.1, 125.3, 127.0, 127.6, 127.7, 128.3, 137.2, 140.7, 143.7, 143.9, 156.9, 156.0, 171.6, 173.5. Anal. Calcd for C\textsubscript{34}H\textsubscript{39}N\textsubscript{3}O\textsubscript{7}: C, 64.44; H, 6.20; N, 6.63. Found: C, 64.23; H, 6.26; N, 6.61.

(\(S\))-6-Benzylxycarbonylamino-2-[(\(S\))-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoylamino]hexanoic acid, N-Fmoc-L-Phe-N\textsuperscript{ε}-Cbz-L-Lys-OH, 2.3g: White microcrystals (92%); mp 128–130 °C; [\alpha]_{D}^{23} = −13.03 (c 1.0, DMF); \(1^H\) NMR (DMSO-d\textsubscript{6}) \(\delta\) 1.24–1.50 (m, 4H), 1.55–1.69 (m, 1H), 1.69–1.82 (m, 1H), 2.70–2.85 (m, 1H), 2.90–3.10 (m, 3H), 4.05–4.25 (m, 4H), 4.25–4.40 (m, 1H), 4.99 (s, 2H), 7.10–7.45 (m, 15H), 7.57–7.68 (m, 3H), 7.87 (d, \(J = 7.4\) Hz, 2H), 8.28 (d, \(J = 7.4\) Hz, 1H), 12.65 (s, 1H). \(13^C\) NMR (DMSO-d\textsubscript{6}) \(\delta\) 22.8, 29.1, 30.8, 37.5, 40.2, 46.6, 52.0, 56.0, 65.2, 65.7, 120.1, 125.3, 125.4, 126.3, 127.1, 127.7, 127.8, 128.1, 128.4, 129.2, 129.3, 137.3, 138.3, 140.7, 143.7, 143.8, 155.9, 156.2, 171.9, 173.7. Anal. Calcd for C\textsubscript{38}H\textsubscript{39}N\textsubscript{3}O\textsubscript{7}: C, 70.25; H, 6.05; N, 6.47. Found: C, 70.60; H, 6.21; N, 6.44.
2.4.4 General Procedure for the Preparation of unnatural LL-Dipeptides 2.3h-j and the Diastereomeric Mixture (2.3h+2.3h')

N-(Protected α-aminoacetyl)benzotriazoles 2.1h-j and (2.3h+2.3h') (0.5 mmol) were added to a solution of Nα-Cbz-L-Lys 2.2b (0.5 mmol) in CH3CN/H2O (5 mL/5 mL) in the presence of Et3N (0.6 mmol) at room temperature. The reaction mixture was then stirred at room temperature until the starting material was completely consumed as observed on TLC using hexane/EtOAc (2:1) as the eluent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH3CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), then dried over anhydrous MgSO4. Evaporation of the solvent gave the desired product in pure form, which was further recrystallized from EtOAc/hexanes unless specified, otherwise.

(S)-2-Benzylxocarbonylamino-6-[2-benzyloxycarbonylamino-3-phenylpropanoylamino]hexanoic acid, Nα-Cbz-Nε-(Cbz-L-Phe)-L-Lys-OH, 2.3h: White microcrystals (79%); mp 162-164 °C; [α]23 D = −12.88 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.19–1.42 (m, 4H), 1.45–1.75 (m, 2H), 2.68–2.81 (m, 1H), 2.85–3.15 (m, 3H), 3.85–3.95 (m, 1H), 4.10–4.22 (m, 1H), 4.85–5.07 (m, 4H), 7.10–7.40 (m, 15H), 7.49 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.99 (br s, 1H), 12.58 (s, 1H). 13C NMR (DMSO-d6) δ 22.9, 28.6, 30.4, 37.8, 38.3, 53.8, 56.3, 65.2, 65.4, 126.2, 127.5, 127.7, 127.8, 127.8, 128.0, 128.3, 128.4, 129.2, 137.0, 137.1, 138.1, 155.8, 156.2, 171.2, 174.1. Anal. Calcd for C31H35N3O7: C, 66.30; H, 6.28; N, 7.48. Found: C, 66.17; H, 6.34; N, 7.59.

(S)-2-Benzylxocarbonylamino-6-[2-benzyloxycarbonylamino-3-phenylpropanoylamino]hexanoic acid, Nα-Cbz-Nε-(Cbz-DL-Phe)-L-Lys-OH, (2.3h+2.3h'): White microcrystals (95%); mp 93–95 °C; [α]23 D = −5.00 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.20–1.45 (m, 4H), 1.48–1.75 (m, 2H), 2.69–2.81 (m, 1H), 2.85–3.15 (m, 3H), 3.85–3.95 (m,
(S)-2-Benzoyloxy carbamolino-6-[(S)-2-benzoyloxy carbamolino-3-(1H-indol-3-yl)propanoylamino]hexanoic acid, Nα-Cbz-Nε-([Cbz-L-Trp]-L-Lys-OH, 2.3i: White microcrystals (93%); mp 87–89 °C; [α]d23 = −17.94 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.20–1.45 (m, 4H), 1.48–1.75 (m, 2H), 2.83–2.97 (m, 1H), 2.97–3.11 (m, 3H), 3.85–3.97 (m, 1H), 4.15–4.27 (m, 1H), 4.94 (s, 2H), 4.98 (d, J = 12.9 Hz, 1H, A part of AB system), 5.04 (d, J = 12.6 Hz, 1H, B part of AB system), 6.96 (t, J = 7.3 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 7.13 (s, 1H), 7.20–7.40 (m, 12H), 7.56 (d, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.95–8.05 (m, 1H), 10.79 (s, 1H), 12.52 (br s, 1H). 13C NMR (DMSO-d6) δ 23.0, 28.1, 28.6, 29.8, 30.5, 38.3, 46.7, 53.3, 53.7, 65.3, 65.5, 110.3, 111.3, 118.2, 118.6, 120.9, 123.9, 127.3, 12.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 136.1, 137.1, 155.9, 156.3, 171.7, 174.1. Anal. Calcd for C33H36N4O7: C, 65.99; H, 6.04; N, 9.33. Found: C, 65.94; H, 6.10; N, 9.20.

(S)-2-Benzoyloxy carbamolino-6-[(S)-2-(9H-fluoren-9-ylmethoxy carbamolino)-4-methylsulfanylbutanoylamino]hexanoic acid, Nα-Cbz-Nε-([Fmoc-L-Met]-L-Lys-OH, 2.3j: White microcrystals (94%); mp 83–85 °C; [α]d23 = −9.70 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.20–1.47 (m, 4H), 1.50–1.75 (m, 2H), 1.75–1.95 (m, 2H), 2.03 (s, 3H), 2.36–2.50 (m, 2H) 2.95–3.14 (m, 2H), 3.87–3.98 (m, 1H), 3.98–4.09 (m, 1H), 4.18–4.33 (m, 3H), 5.02 (s, 2H), 7.25–7.38 (m, 6H), 7.41 (t, J = 7.4 Hz, 3H), 7.52–7.56 (m, 2H), 7.68–7.77 (m, 2H), 7.89 (d, J = 8.4 Hz, 3H), 12.56 (s, 1H). 13C NMR (DMSO-d6) δ 14.6, 23.0, 28.6, 29.8, 30.4, 31.0, 31.7, 38.3, 46.7,
2.4.5 General Procedure for the Preparation of \textit{LL}-Dipeptides 2.3k-n and the Diastereomeric Mixture (2.3l+2.3l') from Extension at the C-Terminus of Benzotriazole derivative, $N^\alpha$-Fmoc-$N^\varepsilon$-Cbz-$L$-Lys-Bt 2.1h

$N^\alpha$-Fmoc-$N^\varepsilon$-Cbz-$L$-Lys-Bt 2.1h (0.4 mmol) was added to a solution of $N^\varepsilon$-Cbz-$L$-Lys 2.2a or unprotected amino acids ($L$-Met, \textit{DL}-Met, $L$-Trp, $L$-Ser), 2.2a,c-e and (2.2c+2.2c') (0.4 mmol) in CH$_3$CN (5 mL)/H$_2$O (10 mL) in the presence of Et$_3$N (0.5 mmol) at room temperature. The reaction mixture was then stirred at room temperature until the starting material was completely consumed as observed on TLC using EtOAc/hexanes (1:2) as the solvent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH$_3$CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), and then dried over anhydrous MgSO$_4$. Evaporation of the solvent gave the desired product in pure forms, which were further recrystallized from CHCl$_3$/hexanes.

\textit{(S)}-6-Benzylxycarbonylamino-2-\{\textit{(S)}-6-benzyloxy carbonylamino-2-(9H-fluoren-9-ylmethoxy carbonylamino)hexanoylamino\}hexanoic acid, $N^\alpha$-Fmoc-$N^\varepsilon$-Cbz-$L$-Lys-$N^\varepsilon$-Cbz-$L$-Lys-OH, 2.3k: White microcrystals (74%); mp 103–104 °C; $[\alpha]^{23}_D = -4.52$ (c 1.0; DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.20–1.48 (m, 8H), 1.48–1.75 (m, 4H), 2.88–3.05 (m, 4H), 3.90–4.15 (m, 2H), 4.18–4.32 (m, 3H), 4.90–5.10 (m, 4H), 7.20–7.44 (m, 16H), 7.48 (d, $J = 8.0$ Hz, 1H), 7.63–7.76 (m, 2H), 7.88 (d, $J = 7.1$ Hz, 2H), 8.08 (d, $J = 7.9$ Hz, 1H), 12.47 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 22.7, 22.8, 22.9, 29.1, 29.2, 30.7, 31.7, 40.2, 46.7, 51.9, 53.9, 54.4, 65.2, 65.6, 120.1, 125.4, 127.1, 127.4, 127.7, 127.8, 128.4, 137.3, 140.7, 143.8, 144.0, 156.0, 156.1, 156.2,
172.1, 173.6. Anal. Calcd for C_{43}H_{48}N_{4}O_{9}: C, 67.52; H, 6.33; N, 7.32. Found: C, 67.77; H, 6.54; N, 6.93.

(S)-2-[(S)-6-Benzoxycarbonylamino-2-(9H-fluoren-9-ylmethoxycarbonylamino)hexanoylamino]-4-(methylsulfanyl)butanoic acid, N^α-Fmoc-N^ε-Cbz-L-Lys-L-Met-OH, 2.3l: White microcrystals (95%); mp 138–140 °C; [α]^{23}\text{D} = -2.09 (c 1.0, DMF); \textsuperscript{1}H NMR (DMSO-d_6) δ 1.20–1.51 (m, 4H), 1.51–1.75 (m, 2H), 1.75–1.98 (m, 2H), 2.01 (s, 3H), 2.37–2.61 (m, 2H), 2.90–3.10 (m, 2H), 3.93–4.11 (m, 1H), 4.15–4.45 (m, 4H), 5.00 (s, 2H), 7.20–7.46 (m, 10H), 7.49 (d, J = 8.2 Hz, 1H), 7.68–7.80 (m, 2H), 7.88 (d, J = 7.4 Hz, 2H), 8.16 (d, J = 7.7 Hz, 1H), 12.70 (br s, 1H). \textsuperscript{13}C NMR (DMSO-d_6) δ 14.6, 22.8, 29.2, 29.6, 30.8, 31.5, 40.2, 46.7, 50.9, 54.4, 65.1, 65.6, 120.1, 125.4, 127.1, 127.7, 127.8, 128.4, 137.3, 140.7, 143.8, 143.9, 156.0, 156.1, 172.3, 173.3. Anal. Calcd for C_{34}H_{39}N_{3}O_{7}S: C, 64.44; H, 6.20; N, 6.63. Found: C, 64.25; H, 6.23; N, 6.50.

2-[(S)-6-Benzoxycarbonylamino-2-(9H-fluoren-9-ylmethoxycarbonylamino)hexanoyl-amino]-4-(methylsulfanyl)butanoic acid, N^α-Fmoc-N^ε-Cbz-L-Lys-DL-Met-OH, (2.3l+2.3l'): White microcrystals (90%); mp 80–81 °C; [α]^{23}\text{D} = -3.94 (c 1.0, DMF); \textsuperscript{1}H NMR (DMSO-d_6) δ 1.22–1.47 (m, 4H), 1.50–1.74 (m, 2H), 1.75–2.05 (m, 2H), 2.00 (s, 1.5H), 2.01 (s, 1.5H), 2.40–2.55 (m, 2H), 2.92–3.04 (m, 2H), 3.95–4.08 (m, 1H), 4.15–4.38 (m, 4H), 4.99 (s, 2H), 7.20–7.36 (m, 8H), 7.41 (t, J = 7.4 Hz, 2H), 7.49 (d, J = 7.5 Hz, 1H), 7.68–7.76 (m, 2H), 7.89 (d, J = 7.7 Hz, 2H), 8.15 (d, J = 7.7 Hz, 0.5 H), 8.21 (d, J = 8.0 Hz, 0.5 H), 12.67 (s, 1H). \textsuperscript{13}C NMR (DMSO-d_6) δ 14.5, 14.6, 22.8, 29.1, 29.2, 29.6, 30.8, 31.5, 32.0, 40.1, 46.7, 50.8, 50.8, 54.4, 65.1, 65.6, 65.7, 120.1, 125.4, 127.1, 127.7, 127.8, 128.4, 137.3, 140.7, 143.8, 143.9, 155.9, 155.9, 156.1, 172.1, 172.2, 173.2, 173.2. Anal. Calcd for C_{34}H_{39}N_{3}O_{7}S: C, 64.44; H, 6.20; N, 6.63. Found: C, 64.43; H, 6.17; N, 6.43.
(S)-2-[(S)-6-Benzoxycarbonylamino-2-(9H-fluoren-9-ylmethoxycarbonylamino)hexanoyl-amino]-3-(1H-indol-3-yl)propanoic acid, Nα-Fmoc-Nε-Cbz-L-Lys-L-Trp-OH, 2.3m: White microcrystals (88%); mp 67–69 °C; [α]$_D^{23}$ = +2.40 (c 1.0, DMF); $^{1}$H NMR (DMSO-$d_6$) δ 1.20–1.75 (m, 6H), 2.90–3.04 (m, 2H), 3.00–3.25 (m, 2H), 3.85–4.08 (m, 1H), 4.15–4.36 (m, 3H), 4.40–4.55 (m, 1H), 5.00 (s, 2H), 6.96 (t, $J = 7.2$ Hz, 1H), 7.05 (t, $J = 7.2$ Hz, 1H), 7.15 (s, 1H), 7.70–7.59 (m, 12H), 7.64 (d, $J = 8.0$ Hz, 1H), 7.73 (d, $J = 7.1$ Hz, 2H), 7.88 (d, $J = 8.11$, 2H), 8.11 (d, $J = 7.4$ Hz, 1H), 10.86 (s, 1H), 12.65 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 22.9, 27.1, 29.2, 31.7, 40.2, 46.7, 52.9, 54.5, 65.2, 65.7, 109.6, 111.4, 118.2, 118.4, 120.2, 120.9, 123.7, 125.4, 127.1, 127.3, 127.7, 127.8, 128.4, 136.1, 137.3, 140.8, 143.8, 144.0, 156.0, 156.1, 172.2, 173.3. Anal. Calcd for C$_{40}$H$_{40}$N$_4$O$_7$: C, 69.75; H, 5.85; N, 8.13. Found: C, 69.81; H, 6.14; N, 7.92.

(S)-2-[(S)-6-Benzoxycarbonylamino-2-(9H-fluoren-9-ylmethoxycarbonylamino)hexanoylamino]-3-hydroxypropanoic acid, Nα-Fmoc-Nε-Cbz-L-Lys-L-Ser-OH, 2.3n: White microcrystals (80%); mp 85–89 °C; [α]$_D^{23}$ = −1.47 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 1.20–1.50 (m, 4H), 1.50–1.73 (m, 2H), 2.90–3.05 (m, 2H), 3.55–3.65 (m, 1H), 3.65–3.78 (m, 1H), 4.00–4.15 (m, 1H), 4.15–4.35 (m, 4H), 4.99 (s, 2H), 7.20–7.36 (m, 8H), 7.41 (t, $J = 7.4$ Hz, 2H), 7.50 (d, $J = 8.5$ Hz, 1H), 7.68–7.76 (m, 2H), 7.88 (d, $J = 7.4$ Hz, 2H), 8.04 (d, $J = 7.0$ Hz, 1H), $-\text{OH and } -\text{COOH from } -\text{Ser missing is not observed in } ^1\text{H NMR.}$ $^{13}$C NMR (DMSO-$d_6$) δ 22.8, 29.2, 31.7, 40.2, 46.7, 54.4, 54.6, 61.4, 65.2, 65.7, 120.1, 125.4, 127.1, 127.7, 127.8, 128.4, 137.3, 140.7, 143.8, 144.0, 156.0, 156.1, 172.0, 172.2. Anal. Calcd for C$_{32}$H$_{35}$N$_3$O$_8$: C, 65.18; H, 5.98; N, 7.13. Found: C, 65.25; H, 6.07; N, 7.02.
2.4.6 General Procedure for Preparation of Dipeptidoylbenzotriazoles 2.5a-c and the Diastereomeric Mixture (2.5b+2.5b’)

To a solution of 1H-benzotriazole (8 mmol) in dry THF (10 mL), SOCl₂ (2.2 mmol) was added and the reaction mixture was stirred for 20 min at room temperature. It was then cooled down to –15 °C in ice salt bath. A cold solution of dipeptides 2.4 a,b, 2.3b and the diastereomeric mixture (2.4b+2.4b’)(2 mmol) dissolved in dry THF (5 mL) was added drop wise, and the reaction mixture was stirred for 6 h at –15 °C. Progress of reaction was monitored by ¹H NMR. The white precipitate obtained was filtered off and the filtrate was concentrated under vacuum. To the residue obtained, ethyl acetate was added and the solution was washed with dilute Na₂CO₃ and brine. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. Compounds were recrystallized from CHCl₃/hexanes for elemental analysis.

(S)-2-Benzylxycarbonylamino-N-[(1S)-2-(1H-1,2,3-benzotriazol-1-yl)-1-(1H-indol-3-ylmethyl)-2-oxoethyl]propanamide, N-Cbz-L-Ala-L-Trp-Bt, 2.5a: White microcrystals (70%); mp 162–164 °C; [α]²³ D = −36.99 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.24 (d, J = 7.0 Hz, 3H), 3.20–3.55 (m, 2H), 4.15–4.25 (m, 1H), 4.97 (d, J = 12.5 Hz, 1H, A part of AB system), 5.00 (d, J = 12.6 Hz, 1H, B part of AB system), 5.83–5.95 (m, 1H), 6.91 (t, J = 7.2 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 7.20–7.40 (m, 7H), 7.46 (d, J = 7.7 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.77 (t, J = 7.7 Hz, 1H), 8.18 (d, J = 8.2 Hz, 1H), 8.23 (d, J = 8.2 Hz, 1H), 8.79 (d, J = 6.0 Hz, 1H), 10.89 (s, 1H). ¹³C NMR (DMSO-d₆) δ 18.1, 27.0, 49.6, 53.8, 65.4, 108.8, 111.5, 114.0, 118.1, 118.5, 120.2, 121.1, 124.3, 126.7, 126.9, 127.9, 128.4, 130.6, 131.1, 136.1, 137.0, 145.3, 155.7, 171.5, 173.3 Anal. Calcd for C₂₈H₂₆N₆O: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.67; H, 5.19; N, 16.36.
(S)-2-Benzylloxycarbonylamino-N-[(1S)-1-(1H-1,2,3-benzotriazol-1-ylcarbonyl)-3-(methylsulfanyl)propyl]-3-phenylpropanamide, N-Cbz-L-Phe-L-Met-Bt, 2.5b: Colorless microcrystals (76%); mp 110-112 °C; [α]$_D^{23}$ = −36.88 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 2.07 (s, 3H), 2.10–2.23 (m, 1H), 2.23–2.40 (m, 1H), 2.55–2.85 (m, 3H), 2.93–3.10 (m, 1H), 4.30–4.48 (m, 1H), 4.94 (s, 2H), 5.70–5.85 (m, 1H), 7.12–7.40 (m, 10H), 7.59 (d, $J$ = 7.5 Hz, 1H), 7.65 (t, $J$ = 7.6 Hz, 1H), 7.82 (t, $J$ = 7.8 Hz, 1H), 8.24 (d, $J$ = 8.2 Hz, 1H), 8.31 (d, $J$ = 8.2 Hz, 1H), 8.96 (d, $J$ = 6.5 Hz, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 14.4, 29.6, 30.1, 37.3, 51.9, 55.8, 65.3, 114.1, 120.2, 126.3, 126.7, 127.5, 127.7, 128.1, 128.3, 129.2, 130.7, 131.1, 136.9, 138.0, 145.4, 155.9, 171.1, 172.5. Anal. Calcd for C$_{28}$H$_{29}$N$_5$O$_4$S: C, 63.26; H, 5.50; N, 13.17. Found: C, 63.36; H, 5.45; N, 13.15.

(S)-2-Benzylloxycarbonylamino-N-[1-(1H-1,2,3-benzotriazol-1-ylcarbonyl)-3-phenylpropanamide, N-Cbz-L-Phe-DL-Met-Bt, (2.5b+2.5b'): Colorless (65%); mp 140-141 °C; [α]$_D^{23}$ = −13.86 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 2.03 (s, 1H), 2.06 (s, 1H), 2.10–2.25 (m, 1H), 2.25–2.50 (m, 1H), 2.55–2.85 (m, 2H), 2.90–3.10 (m, 1H), 4.30–4.50 (m, 1H), 4.93 (s, 2H), 5.69–5.85 (m, 1H), 7.15–7.52 (m, 11H), 7.55 (t, $J$ = 7.7 Hz, 1H), 7.65 (t, $J$ = 7.8 Hz, 1H), 7.81 (t, $J$ = 7.7 Hz, 1H), 8.24 (d, $J$ = 8.4 Hz, 1H), 8.30 (d, $J$ = 8.4 Hz, 1H), 8.85–9.05 (m, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 14.3, 14.4, 29.5, 29.6, 29.9, 30.1, 37.3, 38.1, 51.7, 52.0, 55.8, 65.3, 114.1, 120.3, 126.3, 126.4, 126.8, 127.0, 127.5, 127.6, 127.8, 128.1, 128.2, 128.3, 129.3, 130.7, 131.1, 137.0, 137.8, 138.0, 145.4, 155.8, 155.9, 171.1, 171.2, 172.2, 172.5. Anal. Calcd for C$_{28}$H$_{29}$N$_5$O$_4$S: C, 63.26; H, 5.50; N, 13.17. Found: C, 62.97; H, 5.46; N, 12.85.

Benzyl-[(S)-6-benzotriazol-1-yl-5-((S)-2-benzylloxycarbonylamino-3-phenylpropanoyl-amino)-6-oxohexyl]pentanoate, Cbz-L-Phe-$N^6$-Cbz-L-Lys-Bt, 2.5c: Colorless microcrystals
(75%); mp 139–141 °C; $[\alpha]_{D}^{23} = -17.19$ (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.20–1.60 (m, 4H), 1.80–2.15 (m, 2H), 2.66–2.82 (m, 1H), 2.92–3.10 (m, 3H), 4.33–4.59 (m, 1H), 4.93 (s, 2H), 4.97 (s, 2H), 5.57–5.68 (m, 1H), 7.10–7.42 (m, 16H), 7.55 (d, $J = 8.7$ Hz, 1H), 7.65 (t, $J = 7.7$ Hz, 1H), 7.81 (t, $J = 7.7$ Hz, 1H), 8.24 (d, $J = 8.2$ Hz, 1H), 8.31 (d, $J = 8.4$ Hz, 1H), 8.91 (d, $J = 6.2$ Hz, 1H). $^{13}$C NMR (DMSO-$d_6$) 22.8, 29.1, 30.4, 37.4, 52.9, 55.7, 65.2, 65.3, 114.0, 120.3, 126.3, 126.8, 127.5, 127.8, 128.1, 128.3, 128.4, 129.2, 130.6, 131.1, 137.0, 137.3, 138.0 145.4, 155.9, 156.1, 171.44, 172.5. Anal. Calcd for C$_{37}$H$_{38}$N$_{6}$O$_{6}$: C, 67.05; H, 5.78; N, 12.68. Found: C, 66.89; H, 5.85; N, 12.32.

2.4.7 General Procedure for Preparation of **LLL-Triptides 2.6a,b and the Diastereomeric Mixture (2.6b+2.6b')**

$N$-Cbz-dipeptidoylbenzotriazoles 2.5a,b and (2.5b+2.5b') (0.6 mmol) were added at –15 °C to a solution of $N^\varepsilon$-Cbz-L-Lys 2.2a (0.6 mmol) in CH$_3$CN (15 mL)/H$_2$O (5 mL) in the presence of Et$_3$N (0.7 mmol). The reaction mixtures were then stirred at –15 °C until the starting material was completely consumed as observed on TLC using EtOAc/hexanes (1:2) as the eluent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH$_3$CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), and then dried (anhydrous MgSO$_4$). Evaporation of the solvent gave the desired products, which were purified by dissolving in ethyl acetate and precipitating with hexanes. Compounds were recrystallized from EtOAc/hexanes for elemental analysis.

(S)-6-Benzoxycarbonylamino-2-[(S)-2-((S)-2-benzoxycarbonylaminopropanoylamino)-3-(1H-indol-3-yl)propanoylamino]hexanoic acid, $N$-Cbz-$L$-Ala-$L$-Trp-$N^\varepsilon$-Cbz-$L$-Lys-OH, **2.6a**: White microcrystals (75%); mp 137–139 °C; $[\alpha]_{D}^{23} = -8.94$ (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.13 (d, $J = 7.1$ Hz, 3H), 1.20–1.49 (m,
4H), 1.50–1.80 (m, 2H), 2.89–3.05 (m, 3H), 3.09–3.22 (m, 1H), 4.02 (quintet, J = 7.1 Hz, 1H),
4.11–4.23 (m, 1H), 4.50–4.67 (m, 1H), 4.92–5.10 (m, 4H), 6.96 (t, J = 7.2 Hz, 1H), 7.05 (t, J =
7.4 Hz, 1H), 7.15 (s, 1H), 7.20–7.40 (m, 12H), 7.43 (d, J = 7.4 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H),
7.93 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 7.4 Hz, 1H), 10.82 (s, 1H), 12.63 (br s, 1H). 13C NMR
(DMSO-d6) δ 18.2, 22.7, 27.9, 29.1, 30.9, 40.2, 50.2, 52.0, 53.1, 65.2, 65.5, 109.9, 111.3, 118.2,
118.5, 120.9, 123.7, 127.5, 127.8, 128.4, 136.1, 137.0, 137.3, 155.7, 156.2, 171.5, 172.3, 173.6.
Anal. Calcd for C36H41N5O8: C, 64.37; H, 6.15; N, 10.43. Found: C, 64.47; H, 6.16; N, 10.38.

(S)-6-Benzyloxy carbonylamino-2-[((S)-2-(((S)-2-benzyloxy carbonylamino)-4-(methylsulfanyl)butanoylamino)hexanoic acid, N-Cbz-L-Phe-L-
Met-N°-Cbz-L-Lys-OH, 2.6b: White microcrystals (72%); mp 135–140 °C; [α]23
D = −7.76 (c
1.0, DMF); 1H NMR (DMSO-d6) δ 1.20–1.50 (m, 4H), 1.50–1.78 (m, 2H), 1.78–2.00 (m, 2H),
2.04 (s, 3H), 2.40–2.55 (m, 2H), 2.65–2.85 (m, 1H), 2.85–3.07 (m, 3H), 4.07–4.48 (m, 3H), 4.97
(s, 2H), 4.99 (s, 2H), 7.00–7.21 (m, 16H), 7.53 (d, J = 8.5 Hz, 1H), 8.14–8.19 (m, 2H),
12.61(brs, 1H). 13C NMR (DMSO-d6) δ 14.7, 22.8, 29.1, 29.3, 30.6, 32.4, 37.4, 51.7, 52.0, 56.1,
65.2, 65.3, 126.3, 127.5, 127.6, 127.7, 127.8, 128.1, 128.3, 128.4, 129.3, 137.0, 137.3,
138.1,155.9, 156.1, 171.0, 171.5, 173.6. Anal. Calcd for C36H44N4O8S: C, 62.41; H, 6.40; N,
8.09. Found: C, 62.07; H, 6.42; N, 8.08.

(S)-6-Benzyloxy carbonylamino-2-[(S)-2-[(S)-2-benzyloxy carbonylamino-3-
phenylpropanoylamino)-4-(methylsulfanyl)butanoylamino]hexanoic acid, N-Cbz-L-Phe-
DL-Met-N°-Cbz-L-Lys-OH, (2.6b+2.6b'): White microcrystals (78%); mp126–127 °C; [α]23
D = −6.98 (c 1.0, DMF); 1H NMR (DMSO-d6) 1.20–1.51 (m, 4H), 1.51–1.78 (m, 2H), 1.78–1.98 (m, 
2H), 1.97 (s, 1.5H), 2.04 (s, 1.5H), 2.45–2.55 (m, 2H), 2.67–2.95 (m, 1H), 2.90–2.99 (m, 3H),
4.08–4.23 (m, 1H), 4.23–4.48 (m, 2H), 4.93 (s, 2H), 4.99 (s, 2H), 7.05–7.20 (m, 16H), 7.53 (d, J
= 8.5 Hz, 0.6H), 7.65 (d, J = 7.7 Hz, 0.3H), 8.01 (d, J = 7.4 Hz, 0.3H), 8.05–8.29 (m, 1H), 8.31 (d, J = 8.0 Hz, 0.40H), 12.56 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 14.5, 14.7, 22.5, 22.8, 28.9, 29.1, 29.2, 29.3, 30.5, 30.7, 31.9, 32.3, 37.3, 37.5, 51.7, 56.1, 56.3, 65.1, 65.2, 65.3, 126.3, 127.4, 127.6, 127.7, 127.8, 128.1, 128.3, 128.4, 129.2, 136.9, 137.0, 137.3, 137.7, 138.1, 155.9, 156.1, 170.9, 171.0, 171.5, 173.2, 173.4, 173.5. Anal. Calcd for $C_{36}H_{44}N_4O_8S$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.12, H, 6.53; N, 7.92.

### 2.4.8 General Procedure for Preparation of LLL-Tripeptides 2.6c-e and the Diastereomeric Mixture (2.6e+2.6e')

$N$-Cbz-$L$-Phe-$N^\varepsilon$-Cbz-$L$-Lys-Bt 2.5c (0.6 mmol) was added at −15 °C to a solution of free amino acid ($L$-Met, $L$-Trp, $L$-Ala, $DL$-Ala) 2.2c,d.f and (2.2f+2.2f') (0.6 mmol) in CH$_3$CN (15 mL)/H$_2$O (5 mL) in the presence of Et$_3$N (0.7 mmol). The reaction mixtures were then stirred at −15 °C until the starting material was completely consumed as observed on TLC using EtOAc/hexanes (1:2) as the eluent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH$_3$CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), and then dried (anhydrous MgSO$_4$). Evaporation of the solvent gave the desired products, which were purified by dissolving in ethyl acetate and precipitating with hexanes. Compounds were recrystallized from EtOAc/hexanes for elemental analysis.

$\text{(S)}$-$2\text{-}[(\text{S})$-6$\text{-Benzyloxy carbonylamino-2-}$-(\text{S})$-2$-benzyloxy carbonylamino$-3$-phenyl propanoylamino$]$hexanoylamino$]-4$-methylsulfanybutanoic acid, $N$-Cbz-$L$-Phe-$N^\varepsilon$-Cbz-$L$-Lys-$L$-Met-OH, 2.6c: White microcrystals (73%); mp 137–139 °C; $[\alpha]^{23}_D = -10.99$ (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 1.20–1.49 (m, 4H), 1.50–1.78 (m, 2H), 1.78–2.05 (m, 2H), 2.03 (s, 3H), 2.35–2.60 (m, 2H), 2.65–2.81 (m, 1H), 2.87–3.09 (m, 3H), 4.13–4.40 (m, 3H), 4.94 (s, 2H), 5.00 (s, 2H), 7.00–7.40 (m, 16H), 7.50 (d, $J = 8.5$ Hz, 1H), 8.10 (d, $J = 7.7$ Hz, 1H), 8.26
(d, $J = 7.4$ Hz, 1H), 12.66 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 14.6, 22.5, 29.3, 29.6, 30.7, 31.9, 37.4, 50.9, 52.0, 52.4, 56.1, 65.2, 65.2, 126.3, 127.5, 127.7, 127.8, 128.1, 128.3, 128.4, 129.2, 137.0, 137.3, 138.2, 155.9, 156.1, 171.5, 171.7, 173.2. Anal. Calcd for C$_{36}$H$_{44}$N$_4$O$_8$S: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.44; H, 6.53; N, 7.90.

(S)-2-[(S)-6-Benzoxycarbonylamino-2-[(S)-2-benzyloxycarbonylamino-3-phenylpropanoylamino]hexanoylamino]-3-(1H-indol-3-yl)propanoic acid, N-Cbz-L-Phe-$^{N^6}$-Cbz-L-Lys-L-Trp-OH, 2.6d: White microcrystals (88%); mp 71–73 °C; [$\alpha$]$^{23}$~D = −2.09 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 1.10–1.80 (m, 6H), 2.60–2.80 (m, 1H), 2.82–3.25 (m, 5H), 4.20–4.38 (m, 2H), 4.40–4.55 (m, 1H), 4.93 (s, 2H), 5.00 (s, 2H), 6.97 (t, $J = 7.2$ Hz, 1H), 7.05 (t, $J = 7.3$ Hz, 1H), 7.05–7.20 (m, 18H), 7.48 (d, $J = 8.2$ Hz, 1H), 7.53 (d, $J = 7.7$ Hz, 1H), 8.04 (d, $J = 7.7$ Hz, 1H), 8.19 (d, $J = 7.4$ Hz, 1H), 10.85 (s, 1H), 12.65 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 22.5, 27.0, 29.1, 29.3, 32.1, 37.4, 52.4, 53.9, 56.0, 65.2, 66.2, 109.6, 111.4, 118.2, 118.4, 120.9, 123.7, 126.2, 127.3, 127.5, 127.7, 127.8, 128.0, 128.3, 128.4, 129.2, 136.1, 137.0, 137.3, 138.2, 155.9, 156.1, 171.4, 171.6, 173.2. Anal. Calcd for C$_{42}$H$_{45}$N$_5$O$_8$: C, 67.45; H, 6.06; N, 9.36. Found: C, 67.20; H, 6.28; N, 9.22.

(S)-2-[(S)-6-Benzoxycarbonylamino-2-[(S)-2-benzyloxycarbonylamino-3-phenylpropanoylamino]hexanoylamino]propanoic acid, N-Cbz-L-Phe-$^{N^6}$-Cbz-L-Lys-L-Ala-OH, 2.6e: White microcrystals (74%); mp 129–131 °C; [$\alpha$]$^{23}$~D = −9.60 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 1.20–1.80 (m, 6H), 1.27 (d, $J = 7.4$ Hz, 3H), 2.65–2.78 (m, 1H), 2.87–3.04 (m, 3H), 4.12–4.34 (m, 3H), 4.93 (s, 2H), 5.00 (s, 2H), 7.10–7.40 (m, 16H), 7.50 (d, $J = 8.8$ Hz, 1H), 8.04 (d, $J = 8.2$ Hz, 1H), 8.22 (d, $J = 7.1$ Hz, 1H), 12.55 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) δ 17.1, 22.5, 29.3, 32.1, 37.5, 47.5, 52.0, 52.2, 56.1, 65.2, 65.2, 126.3, 127.4, 127.6, 127.7, 127.8, 128.0,
128.3, 128.4, 129.2, 137.0, 137.3, 138.2, 155.9, 156.1, 171.3, 171.4, 174.0. Anal. Calcd for C_{34}H_{40}N_{4}O_{8}: C, 64.54; H, 6.37; N, 8.85. Found: C, 64.42; H, 6.63; N, 8.68.

2-[(S)-6-Benzoylcarbonylamino-2-[(S)-2-benzoylcarbonylamino-3-phenylpropanoylamino]hexanoylamino]propanoic acid, N-Cbz-L-Phe-N^ε-Cbz-L-Lys-DL-Ala-OH, (2.6e+2.6e'): White microcrystals (90%); mp 99-101 °C; [α]^23_D = –7.35 (c 1.0, DMF); 1H NMR (DMSO-\textit{d}_6) δ 1.20–1.80 (m, 9H), 2.65–2.80 (m, 1H), 2.88–3.08 (m, 3H), 4.12–4.40 (m, 3H), 4.93 (s, 2H), 4.99 (s, 2H), 7.10–7.42 (m, 16H), 7.50 (d, \textit{J} = 8.6 Hz, 1H), 8.03 (d, \textit{J} = 8.3 Hz, 1H), 8.19–8.34 (m, 1H), 12.57 (s, 1H). 13C NMR (DMSO-\textit{d}_6) δ 17.1, 17.4, 22.5, 22.8, 29.1, 29.2, 29.3, 30.8, 30.9, 32.1, 32.3, 37.4, 47.4, 47.5, 52.0, 52.2, 56.0, 56.1, 65.1, 65.2, 126.3, 127.4, 127.5, 127.6, 127.7, 127.8, 128.1, 128.3, 128.4, 129.3, 130.9, 136.9, 137.0, 137.3, 138.2, 155.9, 156.1, 171.2, 171.3, 171.4, 171.8, 173.6, 174.0, 174.1. Anal. Calcd for C_{34}H_{40}N_{4}O_{8}: C, 64.54; H, 6.37; N, 8.85. Found: C, 64.25; H, 6.46; N, 8.46.

2.4.9 Procedure for Preparation of unnatural LLL-Tripeptide 2.6f

\textit{N}-Cbz-dipeptidoylbenzotriazole 2.5a (0.6 mmol) was added at –15 °C to a solution of Cbz^α-L-Lys (0.6 mmol) in CH₃CN (15 mL)/H₂O (5 mL) in the presence of Et₃N (0.7 mmol). The reaction mixtures were then stirred at –15 °C until the starting material was completely consumed as observed on TLC using EtOAc/hexanes (1:2) as the eluent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH₃CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), and then dried over anhydrous MgSO₄. Evaporation of the solvent gave the desired products, which was purified by dissolving in ethyl acetate and precipitating with hexanes. Compound was recrystallized from EtOAc/hexanes for elemental analysis.
(S)-2-Benzoxycarbonylamino-6-[(S)-2-[(S)-2-
benzyloxycarbonylaminopropanoylamino]-3-(1H-indol-3-yl)propanoylamino]hexanoic
acid, Nα-Cbz-Nε-(Cbz-L-Ala-L-Trp)-L-Lys-OH, 2.6f: White microcrystals (82%); mp 62–65
°C; [α]$_{23}^D$ = −10.90 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.14 (d, $J = 6.9$ Hz, 3H), 1.19–1.40
(m, 4H), 1.45–1.70 (m, 2H), 2.88–3.14 (m, 4H), 3.84–3.94 (m, 1H), 3.97–4.10 (m, 1H), 5.01 (s,
4H), 6.95 (t, $J = 7.3$ Hz, 1H), 7.03 (t, $J = 7.4$ Hz, 1H), 7.10 (s, 1H), 7.20–7.43 (m, 11H), 7.47 (d,
$J = 7.9$ Hz, 1H), 7.55 (d, $J = 7.7$ Hz, 2H), 7.81–7.95 (m, 2H), 10.80 (s, 1H), 12.57 (br s, 1H). $^{13}$C
NMR (DMSO-$d_6$) $\delta$ 18.0, 22.9, 27.8, 28.4, 30.4, 38.3, 50.2, 53.4, 53.7, 65.3, 65.4, 110.0, 111.1,
118.1, 118.4, 120.7, 123.4, 127.3, 127.7, 127.7, 128.3, 136.0, 136.9, 137.0, 155.7, 156.1, 170.8,
172.1, 173.9. Anal. Calcd for C$_{36}$H$_{41}$N$_5$O$_8$: C, 64.37; H, 6.15; N, 10.43. Found: C, 64.03; H, 6.36;
N, 10.67.
CHAPTER 3
SYNTHESIS OF PEPTIDES BY EXTENSION AT N- OR C-TERMINUS OF ARGinine

3.1 Introduction

The essential amino acid L-Arginine with its guanidine group is involved in numerous diverse biological processes connected with cell division, healing wounds, removal of ammonia from immune functions, and hormone release.78

L-Arginine is an immediate precursor of nitric oxide (NO) in a reaction catalyzed by isoforms of NO synthase (NOS).79 Nitric oxide is a potent biological signal for diverse physiological processes within the cardiovascular, immune, and nervous systems.80 Overproduction of NO can lead to chronic neurodegenerative diseases including Alzheimer’s, Parkinson81-85 and inflammatory diseases such as arthritis86 and colitis.87 On the other hand, impaired NO production is responsible for hypertension88 and atherosclerosis.89 Therefore, many studies have been conducted on novel substrates and isoform-selective NOC inhibitors in attempt to find treatment for pathological NO production in biological systems. N$^{\omega}$-Methyl-L-arginine and N$^{\omega}$-ethyl-L-arginine show limited selective inhibition of the NOC isoforms90; however, high selectivity was estimated for N$^{\omega}$-nitroarginine and phenylalanine containing dipeptides and dipeptides esters.91 Important selective inhibitors of nNOS over eNOS include nonbiological dipeptides amides and peptidomimetics, built on an N$^{\omega}$-NO$_2$-L-arginine scaffold 3.1a-c (Figure 3-1).85

Figure 3-1. N$^{\omega}$-NO$_2$-L-arginine scaffold
Arginine is the preferred residue at the P1 position of substrates for serine proteases\textsuperscript{92} such as trypsin,\textsuperscript{93} factor Xa\textsuperscript{94} and others of the coagulation cascade, and an essential residue in the integrin recognition sequence Arg-Gly-Asp.\textsuperscript{95} Arg-Gly-Asp (RGD), is a well known characteristic and conservative sequence that acts as cell recognition site for many adhesive proteins present in extracellular matrices (ECM) and in blood including fibrinogen. RGD and some synthetic RGD containing peptides acting as competitive, reversible inhibitors for adhesive protein binding have been used to study adhesive interaction between cells and suppress tumor metastasis and platelet aggregation.\textsuperscript{95,96}

The geometry, charge distribution and ability to form multiple H-bonds make arginine ideal for binding negatively charged groups and preferably located on the outside of the proteins it can interact with the polar environment. Certain peptides containing high percentage of cationic amino acids are known to efficiently translocate through the cell membrane. Short oligomers of arginine can migrate across the plasma membrane of a cell.\textsuperscript{97,98} Synthetic arginine-rich peptides are efficient carriers for transporting various types of biomolecules into cytoplasmic and nuclear compartments of living cells,\textsuperscript{99} including nucleic acids,\textsuperscript{100-101} peptides, proteins.\textsuperscript{102} As a result of extensive physiological function arginine containing peptides and other conjugates are therapeutic agents of diverse activity\textsuperscript{103} and drugs in anti-cancer therapy.\textsuperscript{104-107} Considerable effort has been devoted to the synthesis of arginine peptides and peptidomimetics\textsuperscript{108,109} utilizing solution and solid phase methodologies.\textsuperscript{110-114} The highly basic nature and nucleophilic character of the guanidine moiety in arginine requires appropriate protection before chemical manipulations. Various C-terminal arginine peptides have been prepared from $N^α$-Cbz-$N^ω$-NO$_2$-$L$-arginine by the mixed anhydride method.\textsuperscript{115-118} Similar
methods gave \(N\)-acetylated peptides from \(N^\omega\)-\(\text{NO}_2\)-L-arginine ester\(^{119}\) selective anticoagulant tripeptide \(D\text{-Phe-Pro-Arg}\)^\(^{120}\) and peptides from tribenzoxycarbonyl-L-arginine.\(^{121,122}\)

Other approaches include Arg(Cbz)\(_2\)-OH with TFFH/collidine in CH\(_2\)Cl\(_2\)^\(^{123}\) coupling agents: DCC,\(^{124}\) DCC/DNP, DCC/HOBt, DPPA\(^{125}\) and \(N\)-carboxyanhydride\(^{126}\) and pyrophosphate.\(^{127}\) Protected Leu-Arg-Pro tripeptides are prepared using NMM/pivaloyl chloride/HOBt in DMF or CH\(_2\)Cl\(_2\).\(^{128}\) Unprotected arginine couples with activated amino acid pentafluorophenyl ester (Pfp) in DMF and utilization of orthogonal protection affords free tripeptide 3.4 (Scheme 3-1).\(^{129,130}\)

\[
\begin{align*}
3.2 & \quad \text{a. L-Arg-OH/DMF} \quad \text{85\%} \\
3.3 & \quad \text{b. H}_2, \text{Pt/C, MeOH} \\
3.4 & \quad \text{c. Cbz-Asp(Cbz)-OPfp/DMF} \\
& \quad \text{d. H}_2, \text{Pt/C, MeOH} \\
& \quad \text{b. 96\%} \\
& \quad \text{c. 81\%} \\
& \quad \text{d. 98\%}
\end{align*}
\]

Scheme 3-1. Literature method for preparation of arginine peptides

After carboxyl activation of protected arginines, intramolecular \(\delta\)-lactam formation competes with coupling\(^{108,125,131,132}\) to an extent depending on the carboxyl activation and also on the amino acid component (Scheme 3-2). This side reaction is a serious problem, especially when the reaction involves a weak nucleophile. Mixed anhydride coupling favors \(\delta\)-lactam formation\(^{125,126}\) as also does EDC/HOBt/NMM in CH\(_3\)CN (Scheme 3-3).\(^{132}\) Deprotection of the guanidino function favours lactam formation,\(^{123}\) the side products also were minimized by the DPPA method.\(^{125}\)

\[
\begin{align*}
\text{PG} & \quad \text{R = H or NO}_2 \\
\text{PG} & \quad \text{R} = \text{Protecting group} \\
\text{X} & \quad \text{X} = \text{Activated group}
\end{align*}
\]

Scheme 3-2. Intramolecular \(\delta\)-lactam formation during carboxyl activation of arginine
Scheme 3-3. Intramolecular δ-lactam formation during carboxyl activation with EDC/HOBt

Because of the wide range of application for RGD and related peptides, several approaches such as mixed anhydride and DCC have been used for their synthesis. Very recently, protected RGD tripeptide Bz-RGD-OEt was synthesized by chemo-enzymatic synthesis. First Gly-Asp was synthesized by chloroacetylation of L-aspartic acid and ammonolysis of chloroacetyl L-aspartic acid. In this linkage of third amino acid Bz-Arg-OEt to Gly-Asp-(OEt)₂ was completed by enzymatic method in organic solvent (Scheme 3-4).⁹⁶

\[
\begin{align*}
\text{L-Asp} & \quad + \quad \text{ClCH₂COCl} & \quad \rightarrow \quad \text{ClCH₂COAsp} \\
\text{ClCH₂COAsp} & \quad + \quad \text{NH₃} & \quad \rightarrow \quad \text{NH₂CH₂COAsp} \\
\text{NH₂CH₂COAsp} & \quad + \quad \text{HCl (gas)} & \quad \rightarrow \quad \text{NH₂CH₂COAsp-(COOEt)₂} \\
\text{Bz-Arg-OEt} & \quad + \quad \text{NH₂CH₂COAsp-(COOEt)₂} & \quad \rightarrow \quad \text{Bz-Arg-Gly-Asp-(OEt)₂}
\end{align*}
\]

Scheme 3-4. Chemo-enzymatic synthesis of the protected RGD peptide

Free RGD peptide has also been synthesized by N-carboxyanhydride method. In this case first Gly-Asp was synthesized as previously. Second N⁶-Cbz-Arg was reacted with Gly-Asp to yield RGD tripeptide by N-carboxyanhydride method (Scheme 3-5).¹²⁶

\[
\begin{align*}
\text{ClCH₂COAsp} & \quad + \quad \text{NH₃} & \quad \rightarrow \quad \text{Gly-Asp} \\
\text{Cbz-Arg} & \quad + \quad \text{PBTr} & \quad \rightarrow \quad \text{NCA-Arg} \\
\text{Arg-Gly-Asp} & \quad \quad & \quad \quad
\end{align*}
\]

Scheme 3-5. Synthesis step of the free RGD peptide
In continuation of our extensive research on use of \( N \)-acybenzotriazoles in peptide synthesis herein, convenient procedures for preparation of arginine peptides by extension at \( C \)- and \( N \)-terminus of arginine is described. In addition a novel synthesis of the protected RGD tripeptide by utilizing benzotriazole methodology is also described.

### 3.2 Results and Discussion

#### 3.2.1 Preparation of \( LL \)-Dipeptides 3.9a-e and the Diastereomeric mixtures (3.9b+3.9b'), (3.9c+3.9c') by Extension at the \( N^\omega \)-Terminus of \( N^\omega \)-NO\(_2\)-L-Arg-OH 3.8

\( N^\omega \)-NO\(_2\)-L-Arg-OH 3.8 couples with \( N \)-(Cbz-\( \alpha \)-aminoacyl)benzotriazoles 3.7a-e derived from chiral \( L \)-Phe, \( L \)-Met, \( L \)-Ala, \( L \)-Trp and di-Cbz-\( L \)-cystine-di-Bt, and corresponding racemic mixtures (3.7b+3.7b') and (3.7c+3.7c'), in aqueous acetonitrile (CH\(_3\)CN/H\(_2\)O, 1:2) containing Et\(_3\)N in 30 min at room temperature to give dipeptides 3.9a-e, and diastereomeric mixtures (3.9b+3.9b') and (3.9c+3.9c') (75–95%), all isolated without column chromatography (Scheme 3-6, Table 3-1). Diastereomeric mixtures were prepared to compare that the original chirality of amino acids and peptides used is maintained during coupling reactions by means of HPLC and NMR analysis.

![Scheme 3-6. Preparation of \( N^\omega \)-NO\(_2\)-L-Arginine dipeptides 3.9a-e and diastereomeric mixtures (3.9b+3.9b') and (3.9c+3.9c')](image)

\(^1\)H NMR analysis of dipeptides 3.9a-e revealed that each \( LL \)-dipeptide displayed two sets of doublets for two amide NH protons ranging from 7.30 to 8.40 ppm, supporting their enantiopurity. However, for each of the diastereomeric mixtures (3.9b+3.9b') and (3.9c+3.9c'),
one of the amide NH protons showed as a multiplet. The $^{13}$C NMR of (3.9b+3.9b′) and (3.9c+3.9c′) each showed doubled signals for each aliphatic and carbonyl carbon. The guanidine NH$_2$ protons all appeared as broad signals ranging from 7.30 to 8.30 ppm. The NH protons of the guanidine group appear as a broad singlet at ~ 8.50 ppm.

Table 3-1. Preparation of arginine dipeptides 3.9a-e, (3.9b+3.9b′) and (3.9c+3.9c′) from $\text{NO}_2$-L-Arg-OH 3.8

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>$[\alpha]^{23}_D$</th>
<th>$t_R$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cbz-L-Phe-Bt</td>
<td>Cbz-L-Phe-$\text{NO}_2$-L-Arg-OH</td>
<td>90</td>
<td>173–175</td>
<td>−6.20</td>
<td>11.16</td>
</tr>
<tr>
<td>3.7a</td>
<td>3.9a$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-L-Met-Bt</td>
<td>Cbz-L-Met-$\text{NO}_2$-L-Arg-OH</td>
<td>80</td>
<td>141–143</td>
<td>−4.15</td>
<td>10.13</td>
</tr>
<tr>
<td>3.7b</td>
<td>3.9b$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-DL-Met-Bt</td>
<td>Cbz-DL-Met-$\text{NO}_2$-L-Arg-OH (3.9b+3.9b′)</td>
<td>95</td>
<td>61–64</td>
<td>−2.67</td>
<td>9.42, 10.03</td>
</tr>
<tr>
<td>(3.7b+3.7b′)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-L-Ala-Bt</td>
<td>Cbz-L-Ala-$\text{NO}_2$-L-Arg-OH</td>
<td>93</td>
<td>168–169</td>
<td>+3.84</td>
<td>10.79</td>
</tr>
<tr>
<td>3.7c</td>
<td>3.9c$^d$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-DL-Ala-Bt</td>
<td>Cbz-DL-Ala-$\text{NO}_2$-L-Arg-OH (3.9c+3.9c′)</td>
<td>92</td>
<td>133–134</td>
<td>−2.33</td>
<td>8.64, 10.45</td>
</tr>
<tr>
<td>(3.7c+3.7c′)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-L-Trp-Bt</td>
<td>Cbz-L-Trp-$\text{NO}_2$-L-Arg-OH</td>
<td>80</td>
<td>65–68</td>
<td>−19.60</td>
<td>11.12</td>
</tr>
<tr>
<td>3.7d</td>
<td>3.9d$^e$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di-Cbz-L-Cystine-di-Bt 3.7e</td>
<td>Di-Cbz-L-Cystine-di-$\text{NO}_2$-L-Arg-OH 3.9e</td>
<td>75</td>
<td>105–108</td>
<td>−84.3</td>
<td>3.04</td>
</tr>
</tbody>
</table>

$^a$Isolated yields, $^b,c,e$ Lit.$^{118}$ mp $^b$174–176 °C, $^c140–143$ °C, $^e68–75$ °C. $^d$Lit.$^{115}$ mp 171-172 °C. $^f_{t_R}$ Retention time for HPLC

The enantiopurity of each of the LL-dipeptides 3.9a-e was further supported by HPLC analyses by using a Chirobiotic T column [detection at 220 nm, flow rate 0.4-1.0 mL/min; eluting with MeOH/H$_2$O (1:1) for 3.9a,c,d and (3.9c+3.9c′), MeOH/H$_2$O (9:1) for 3.9b and (3.9b+3.9b′); MeOH for 3.9e]. Enantiopure compound 3.9b showed a single retention time at 10.13 min whereas the corresponding diastereomeric mixture (3.9b+3.9b′) showed two retention times with equal intensity at 9.42 and 10.03 min. Similarly, in the case of compound 3.9c one single retention time at 10.79 min was observed, whereas the diastereomeric mixture
(3.9c+3.9c') showed two retention times at 8.64 and 10.45 min. Enantiopure compounds 3.9a, 3.9d and 3.9e showed single retention times at 11.16, 11.12, and 3.04 min respectively.

3.2.2 Preparation of LL-Dipeptides 3.11a-d and the Diastereomeric mixture (3.11b+3.11b') by Chain Elongation at the N°-Terminus of L-Arg-OH 3.10

L-Arginine containing dipeptides 3.11a-d and the diastereomeric mixture (3.11b+3.11b') were prepared by extension at the N°-terminus of L-arginine 3.10 by coupling with N-(Cbz-α-aminoacyl)benzotriazoles 3.7a-d, and the diasteromeric mixture (3.7b+3.7b') in aqueous CH$_3$CN without Et$_3$N at room temperature for 6 h (free arginine affords required base medium). After evaporation of solvent the residue was purified by reprecipitation from MeOH/Et$_2$O. Repetition of this procedure three times afforded complete removal of byproduct 1H-Benzotriazole and gave pure dipeptides 3.11a-d and the diastereomeric mixture (3.11b+3.11b') in 75–83% yields (Scheme 3-7, Table 3-2). NMR analysis of the compounds revealed no detectable epimerization (<1 %). For each of the enantiopure compounds 3.11a-d, two sets of doublets were observed for the amide NH protons. For the diastereomeric mixture (3.11b+3.11b'), one of the amide NH protons appeared as a multiplet and the $^{13}$C NMR spectrum also showed doubling of the signals for the aliphatic and carbonyl carbons.

Scheme 3-7. Preparation of arginine dipeptides 3.11a-d and (3.11b+3.11b') from L-Arg-OH 3.10.

HPLC analyses (flow rate 1.0 mL/min with MeOH as eluent) of LL-dipeptides 3.11a, 3.11b, 3.11c and 3.11d each showed single retention times at 1.91, 1.84, 1.92 and 2.88 min
respectively. However, in case of the diatseromeric mixture (3.11b+3.11b'), two retention time at 1.85 and 1.98 min was obtained.

### Table 3-2. Preparation of arginine dipeptides 3.11a-d and (3.11b+3.11b') from L-Arg-OH 3.10.

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield (a) (%)</th>
<th>Mp (°C)</th>
<th>[α]D23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cbz-L-Phe-Bt</td>
<td>Cbz-L-Phe-L-Arg-OH</td>
<td>83</td>
<td>130–132</td>
<td>−10.37</td>
</tr>
<tr>
<td>3.7a</td>
<td>3.11a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-L-Met-Bt</td>
<td>Cbz-L-Met-L-Arg-OH</td>
<td>81</td>
<td>143–144</td>
<td>−7.20</td>
</tr>
<tr>
<td>3.7b</td>
<td>3.11b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-DL-Met-Bt</td>
<td>Cbz-DL-Met-L-Arg-OH</td>
<td>76</td>
<td>121–124</td>
<td>+5.86</td>
</tr>
<tr>
<td>(3.7b+7b')</td>
<td>(3.11b+3.11b')</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-L-Trp-Bt</td>
<td>Cbz-L-Trp-L-Arg-OH</td>
<td>83</td>
<td>135–137</td>
<td>−18.29</td>
</tr>
<tr>
<td>3.7c</td>
<td>3.11c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cbz-L-Val-Bt</td>
<td>Cbz-L-Val-L-Arg-OHd</td>
<td>75</td>
<td>121–123</td>
<td>−0.99</td>
</tr>
<tr>
<td>3.7d</td>
<td>3.11d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Isolated yields, bLit mp 131-133 °C, cReference133, dReference134*

### 3.2.3 Preparation from Nα-Cbz-Nω-NO2-L-Arg-Bt 3.13 of Arginine LL-dipeptides 3.15a-c and the Diastereomeric mixture (3.15a+3.15a') by Extension at the C-Terminus of Nα-Cbz-Nω-NO2-L-Arg-OH 3.14

To extend at the arginine C-terminus, carboxyl group need to be activated. First, I tried to synthesize benzotriazole derivative 3.13 of Nα-Cbz-L-Arg-OH 3.12. However, the desired product 3.13 could not be isolated due to competing intramolecular cyclization product of 3.13 in the presence of free guanidine moiety (Scheme 3.8). Next, Nα-Cbz-Nω-NO2-L-Arg-OH 3.14 was used instead of Nα-Cbz-L-Arg-OH 3.12 for formation of activated benzotriazole derivative 3.19 (Scheme 3-9).

![Scheme 3-8. Attempted synthesis of benzotriazole derivative of Nα-Cbz-L-Arg-OH 3.12](image-url)
Nα-Cbz-Nω-NO2-L-Arg-OH 3.14 was treated with 1H-benzotriazole and SOCl2 in THF at 20 °C to give benzotriazole derivative Nα-Cbz-Nω-NO2-L-Arg-Bt 3.15 after acidic work up in 97% yield; this reaction was completed in 45 min without formation of side products. Coupling Nα-Cbz-Nω-NO2-L-Arg-Bt 3.15 with free amino acids 3.16a-d, (3.16a+3.16a') gave chiral N-terminal arginine dipeptides 3.17a-d and the diastereomeric mixture (3.17a+3.17a') in yields of 65-80% (Scheme 3-9, Table 3-3).

Scheme 3-9. Preparation of Nα-Cbz-Nω-NO2-L-Arg-Bt 3.15 and arginine dipeptides 3.17a-d, (3.17a+3.17a')

Table 3-3. Preparation of Nα-Cbz-Nω-NO2-L-Arg-Bt 3.15 and arginine dipeptides 3.17a-d, (3.17a+3.17a')

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yielda (%)</th>
<th>mp (°C)</th>
<th>[α]23D</th>
<th>tRc (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Phe-OH</td>
<td>3.16a</td>
<td>68</td>
<td>217-219</td>
<td>+5.32</td>
<td>3.01d</td>
</tr>
<tr>
<td>DL-Phe-OH (3.16a+3.16a')</td>
<td>3.16a</td>
<td>65</td>
<td>205-207</td>
<td>+8.14</td>
<td>3.16, 3.94d</td>
</tr>
<tr>
<td>L-Met-OH</td>
<td>3.16b</td>
<td>66</td>
<td>144-146</td>
<td>-4.60</td>
<td>3.34d</td>
</tr>
<tr>
<td>L-Ser-OH</td>
<td>3.16c</td>
<td>65</td>
<td>83-85</td>
<td>-5.58</td>
<td>3.57d</td>
</tr>
<tr>
<td>L-Gly-OH</td>
<td>3.16d</td>
<td>80</td>
<td>115-117</td>
<td>-1.54</td>
<td>4.15d</td>
</tr>
</tbody>
</table>

a Isolated Yields; b Lit.135 mp 114–117; c tR = Retention Time; d Flow rate: 1.0 mL/min, eluent MeOH.
The procedure was similar to that utilized above for the preparation of 3.9a-c (Scheme 3-5). Purification of crude product by recrystallization in MeOH/Et2O gave pure dipeptides 3.17a-c and the diastereomeric mixture (3.17a+3.17a’), while 3.17d was isolated by acidifying at -15 °C. 1H NMR analysis showed no detectable racemization (<5 %) for the LL-dipeptides 3.17a-d and the diastereomeric mixture (3.17a+3.17a’). However monitoring by TLC, disclosed that a side product 3.18 (10-30%) was formed in all these reactions, and could be isolated from the filtrate. The structure of 3.18 was revealed (by 1H and 13C NMR) to be the intramolecular cyclization product (δ-lactam); 3.18 formed competitively with the expected dipeptide during coupling of benzotriazole activated nitroarginine 3.15 with free amino acids. In case of coupling with Gly-OH 31.6d, extent of intramolecular cyclization product was less as compared to other amino acid L-Phe, L-Met and L-Ser 3.16a-c.

HPLC analyses for the enantiopure LL-peptides 3.17a-d showed single retention times, while the diastereomeric mixture (3.17a+3.17a’) showed two retention times (Table 3-3).

3.2.4 Preparation of C-Terminal Arginine Tripeptides 3.22a-c and (3.22a+3.22a’) by Extension at the Nα-Terminus of Nω-NO2-L-Arg-OH 3.8

N-Cbz-Dipeptidoylbenzotriazoles 3.22b,c were obtained as reported previously.136 Analogs 3.22a and (3.22a+3.22a’) were prepared from Cbz-L-Asp(OBz)-OH 3.19 (Scheme 3-10) similarly to a described procedure.23 Cbz-L-Asp(OBz)-Bt 3.20 was prepared from Cbz-L-Asp(OBz)-OH 3.19 by reaction with 1H-benzotriazole in CH2Cl2 in the presence of SOCl2. Further coupling of 3.20 with L-Phe and DL-Phe yielded Cbz- protected dipeptide 3.21a and the diastereomeric mixture (3.21a+3.21a’) which were then converted to their dipetidoylbenzotriazole derivative 3.22a and (3.22a+3.22a’) (Scheme 3-10).
Scheme 3-10. Preparation of Cbz-L-Asp(OBz)-Bt 3.20

Scheme 3-11. Synthesis of N-Cbz-dipetidoylbenotriazole 3.22 and (3.22a+3.22a')

N\\(^{\omega}\)-NO\\(_{2}\)-L-Arg-OH 3.8 was coupled with 3.22a-c, (3.22a+3.22a') in aqueous CH\\(_{3}\)CN in the presence of Et\\(_{3}\)N for 2 h at -15 °C to give tripeptides 3.23a-c and diastereomeric mixture (3.23a+3.23a') in 66-85% yields (Scheme 3-12, Table 3-4).

Scheme 3-12. Synthesis of N\\(^{\omega}\)-NO\\(_{2}\)-L-arginine tripeptides 3.23a-c and (3.23a+3.23a')
The $^1$H and $^{13}$C NMR spectra of the optically pure $LLL$-tripeptides 3.23a-c showed the absence of epimerization (<1%). $^1$H NMR showed three sets of doublets for the amide NH protons for each of the enantiopure compounds. In the case of diastereomeric mixture (3.23a+3.23a'), each set of amide NH protons appeared as split doublets and $^{13}$C NMR showed doubling of the signals for the aliphatic and carbonyl carbons. The room temperature $^1$H NMR for 3.22c showed the existence of two rotameric forms, which underwent coalescence in a high temperature $^1$H NMR experiment.

Table 3-4. Preparation of $L$-arginine tripeptides 3.23a-c and the diastereomeric mixture (3.23a+3.23a')

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>$[\alpha]^{23}_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cbz-$L$-Asp(OBz)-$L$-Phe-Bt 3.22a</td>
<td>Cbz-$L$-Asp(OBz)-$L$-Phe-$N^\omega$-NO$_2$-$L$-Arg-OH 3.23a</td>
<td>84</td>
<td>128–129</td>
<td>−11.71</td>
</tr>
<tr>
<td>Cbz-$L$-Asp(OBz)-$DL$-Phe-Bt (3.22a+3.22a')</td>
<td>Cbz-$L$-Asp(OBz)-$DL$-Phe-$N^\omega$-NO$_2$-$L$-Arg-OH (3.23a+3.23a')</td>
<td>82</td>
<td>60.4–65.0</td>
<td>−6.31</td>
</tr>
<tr>
<td>Cbz-$L$-Ala-$L$-Trp-Bt 3.22b</td>
<td>Cbz-$L$-Ala-$L$-Trp-$N^\omega$-NO$_2$-$L$-Arg-OH 3.23b</td>
<td>66</td>
<td>150–151</td>
<td>−10.24</td>
</tr>
<tr>
<td>Cbz-$L$-Phe-$L$-Met-Bt 3.22c</td>
<td>Cbz-$L$-Phe-$L$-Met-$N^\omega$-NO$_2$-$L$-Arg-OH 3.23c</td>
<td>75</td>
<td>77–79</td>
<td>−7.98</td>
</tr>
</tbody>
</table>

*Isolated Yields

HPLC analysis of tripeptide 3.23b shows one retention time at 3.17 min supporting its enantiopurity. Tripeptide 3.23a is a protected analogue of H-Asp-Phe-Arg-OH 3.4, recently adopted as a catalyst for asymmetric Michael addition reactions. The classical method for the preparation of 3.4, (Scheme 3-1) utilizing the pentafluorophenyl ester of amino acid in DMF, requires prolonged reaction times and complicated isolation procedures; our methodology advantageously includes coupling of benzotriazole activated amino acid in aqueous media, short reaction times and simple work up procedures affording final chiral tripeptide 3.23a, and the diastereomeric mixture (3.23a+3.23a') in 82-84% yields.
3.2.5 Application of Benzotriazole Methodology in the Preparation of the Protected RGD Peptide \(N^\alpha\)-Cbz-N\(^{\omega}\)-NO\(_2\)-L-Arg-Gly-L-Asp-(OH)\(_2\) 3.26

We used benzotriazole methodology to synthesize protected arginyl-glycyl-\(\alpha\)-aspartyl "RGD" tripeptide Cbz-Arg(NO\(_2\))-Gly-\(\alpha\)-Asp(OH)\(_2\) 3.26 (Scheme 3-13). A recent literature\(^{135}\) preparation of di-benzyl ester derivative Cbz-Arg(NO\(_2\))-Gly-\(\alpha\)-Asp(\(\beta\)-OBz)OBz utilizes amino acid esters, requires low temperatures of −5 to −8 °C, prolonged reaction times (14–16 h) and coupling reagents (DCC/ HOBt). Di-benzyl ester protected tripeptide was finally deprotected by conventional method to give Arg-Gly-Asp-(OH)\(_2\).\(^{135}\) Our methodology utilized benzotriazole activated amino acid 3.15 and finally synthesized protected RGD peptide 3.26 from Cbz\(^{\alpha}\)-L-\(^{\omega}\)NO\(_2\)-Arg-Gly-Bt 3.24 and free aspartic acid 3.25, by modification of the coupling procedure adopted for the preparation of other tripeptides. First \(N^\alpha\)-Cbz-N\(^{\omega}\)-NO\(_2\)-L-Arg-Gly-OH 3.17d was synthesized as described in Scheme 3-9 and then converted to its dipeptidoylbenzotriazole derivative \(N^\alpha\)-Cbz-N\(^{\omega}\)-NO\(_2\)-L-Arg-Gly-Bt 3.24. Further coupling of 3.24 with free aspartic acid 3.25 was achieved in CH\(_3\)CN/H\(_2\)O/THF in presence of Et\(_3\)N at -15 °C for 3.5 h (Scheme 3-13). THF was added to reaction mixture because of very low solubility of 3.24.

Scheme 3-13. Preparation of protected RGD peptide 3.26
In comparison, our methodology offers simple preparative and workup procedures, takes less time to complete, uses inexpensive reagents, gives high yields, and allows the use of free amino acids as coupling components affording 3.26 in good yield.

### 3.3 Conclusion

In conclusion, convenient and efficient preparation of arginine di- and tripeptides in short reaction times utilizing simple workup procedures, inexpensive reagents and free amino acids as coupling components are described. The peptides can be prepared by chain elongation at either the N- or C-terminus of L-arginine. We have successfully synthesized protected RGD peptide sequence with our benzotriazole methodology, using free aspartic acid as coupling component.

### 3.4 Experimental Section

#### 3.4.1 General Methods

Melting points were determined on a capillary point apparatus and are uncorrected. NMR spectra were recorded in CDCl$_3$ or DMSO-$d_6$ with TMS as an internal standard for $^1$H (300 MHz) and solvent as an internal standard for $^{13}$C (75 MHz). N-Cbz- and Fmoc-amino acids and free amino acids were purchased from Fluka and Acros, were used without further purification. Optical rotation values were measured with the use of sodium D line. HPLC analyses were performed using Chirobiotic T column (4.6 x 250 mm), detection at 220 nm, flow rate of 0.4-1.0 mL/min and MeOH or MeOH/H$_2$O as an eluting solvent.

#### 3.4.2 General Procedure for the Preparation of LL-Dipeptides 3.9a-e and the Diastereomeric mixture (3.9b+3.9b'), (3.9c+3.9c')

$N$-(Cbz-$\alpha$-aminoacyl)benzotriazoles 3.7a-d, (3.7b+3.7b'), (3.7c+3.7c') (0.5 mmol) were added at 20 °C to a solution of $N^\omega$-NO$_2$-L-Arg-OH 3.2 (0.5 mmol) in CH$_3$CN/H$_2$O (5 mL/10 mL) in the presence of Et$_3$N (0.6 mmol). The reaction mixture was then stirred at 20 °C until the starting material was completely consumed as observed by TLC using EtOAc/hexanes (1:2) as
the eluent. After addition of 4N HCl (1 mL), the solution was concentrated under reduced pressure to remove CH₃CN. Residue was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL), and saturated NaCl (10 mL) then dried over anhydrous MgSO₄. Evaporation of the solvent gave the desired product in pure form, which was further recrystallized from MeOH/Et₂O unless specified, otherwise.

(5S)-2-(5S)-2-Benzoylcarbonylamino-3-phenylpropanoylamino)-5-nitroguanidinopentanoic acid (Cbz-L-Phe-N⁰(NO₂)-L-Arg-OH, 3.9a): White microcrystals (90%); mp 173–175 °C (Lit¹¹⁸ 174–176 °C); [α]²⁳ D = −6.2 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.40–1.70 (m, 3H), 1.70–1.87 (m, 1H), 2.60–2.80 (m, 1H), 2.90–3.05 (m, 1H), 3.05–3.25 (m, 2H), 4.10–4.35 (m, 2H), 4.92 (s, 2H), 7.00–7.40 (m, 10H), 7.51 (d, J = 8.4 Hz, 1H), 7.60–8.25 (m, 2H), 8.32 (d, J = 7.4 Hz, 1H), 8.56 (br s, 1H), 12.65 (br s, 1H). ¹³C NMR (DMSO-d₆) δ 24.9, 28.3, 37.4, 40.2, 51.7, 56.0, 65.2, 126.3, 127.5, 127.7, 128.1, 128.3, 129.2, 137.0, 138.1, 155.9, 159.3, 171.8, 173.4. Anal. Calcd for C₂₃H₂₈N₆O₇: C, 55.19; H, 5.64; N, 16.79. Found: C, 54.88; H, 5.76; N, 16.63.

(5S)-2-(5S)-2-Benzoylcarbonylamino-4-methylsulfanylbutyrylamino)-5-nitroguanidinopentanoic acid, (Cbz-L-Met-N⁰(NO₂)-L-Arg-OH, 3.9b): White microcrystals (80%); mp 141–143 °C (Lit¹¹⁸ 140–143 °C); [α]²³ D = −4.15 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.40–1.70 (m, 3H), 1.70–1.95 (m, 3H), 2.03 (s, 3H), 2.40–2.60 (m, 2H), 3.00–3.20 (m, 2H), 4.05–4.25 (m, 2H), 4.93-5.10 (m, 2H), 7.24–7.42 (m, 5H), 7.50 (d, J = 8.2 Hz, 1H), 7.60–8.16 (m, 2H), 8.21 (d, J = 7.1 Hz, 1H), 8.54 (br s, 1H). ¹³C NMR (DMSO-d₆) δ 14.7, 25.0, 28.2, 29.6, 31.9, 40.3, 51.7, 53.8, 65.6, 127.8, 127.9, 128.5, 137.1, 156.0, 159.4, 171.8, 173.5. Anal. Calcd for C₁₉H₂₈N₆O₇S: C, 47.10; H, 5.82; N, 17.34. Found: C, 47.23; H, 5.86; N, 17.07.
(S)-2-(2-Benzylxocarbonylamino-4-methylsulfanylbutanoylamido)-5-nitroguanidinopentanoic acid (Cbz-DL-Met-N°-NO₂-L-Arg-OH, (3.9b+3.9b')): White microcrystals (95%); mp 61–64 °C; [α]²³D = –2.67 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.40–1.95 (m, 6H), 2.02 (s, 3H), 2.35–2.50 (m, 2H), 3.05–3.25 (m, 2H), 4.05–4.25 (m, 2H), 5.02 (s, 2H), 7.25–7.40 (m, 5H), 7.40–7.53 (m, 1H), 7.55–8.20 (m, 2H), 8.21 (d, J = 6.9 Hz, 1H), 8.50 (br s, 1H), 12.67 (br s, 1H). ¹³C NMR (DMSO-d₆) δ 14.6, 24.9, 28.1, 28.4, 29.6, 29.7, 31.8, 32.0, 51.6, 51.7, 53.7, 53.9, 65.0, 65.5, 127.8, 127.8, 127.9, 128.4, 137.0, 156.0, 156.0, 159.3, 171.6, 171.7, 173.3, 173.4. Anal. Calcd for C₁₉H₂₈N₆O₇S: C, 47.10; H, 5.82; N, 17.34. Found: C, 46.92; H, 5.87; N, 17.06.

(S)-2-((S)-2-Benzylxocarbonylaminopropanoylamino)-5-nitroguanidinopentanoic acid (Cbz-L-Ala-N°-NO₂-L-Arg-OH, 3.9c): White microcrystals (93%); mp 168–169 °C (lit,¹¹⁶ 171–172 °C); [α]²³D = +3.84 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.10–1.32 (d, J = 6.9 Hz, 3H), 1.35–1.85 (m, 4H), 3.00–3.25 (m, 2H), 4.00–4.38 (m, 2H), 4.98 (d, J = 12.2 Hz, 1H, A part of AB system), 5.37 (d, J = 12.6 Hz, 1H, B part of AB system), 7.20–7.40 (m, 5H), 7.44 (d, J = 7.2 Hz, 1H), 8.11 (d, J = 7.4 Hz, 1H), 7.50–8.20 (m, 2H), 8.52 (bs, 1H), 12.56 (s, 1H). ¹³C NMR (DMSO-d₆) δ 18.2, 24.8, 28.3, 49.8, 51.5, 65.4, 127.8, 128.4, 137.1, 155.6, 159.3, 172.7, 173.4. Anal. Calcd for C₁₇H₂₄N₆O₇: C, 48.11; H, 5.70; N, 19.80. found: C, 48.19; H; 5.73; N, 19.74.

(S)-2-(2-Benzylxocarbonylaminopropanoylamino)-5-nitroguanidinopentanoic acid (Cbz-DL-Ala-N°-NO₂-L-Arg-OH, (3.9c+3.9c')): White microcrystals (92%); mp 133–134 °C; [α]²³D = –2.33 (c 1.0, DMF); ¹H NMR (DMSO-d₆) δ 1.21 (d, J = 6.6 Hz, 3H), 1.39–1.85 (m, 4H), 3.05–3.22 (m, 2H), 4.02–4.27 (m, 2H), 4.98 (d, J =12.6 Hz, 1H, A part of AB system), 5.03 (d, J = 12.6 Hz, 1H, B part of AB system), 7.25–7.40 (m, 5H), 7.44 (d, J = 8.0 Hz, 1H), 7.50–8.20 (m, 2H), 8.05–8.20 (m, 1H), 8.53 (br s, 1H), 12.67 (s, 1H). ¹³C NMR (DMSO-d₆) δ 18.2, 18.7, 24.8,
(S)-2-[(S)-2-Benzoylcarbonylamino-3-(1H-indol-3-yl)-propanoylamino]-5-nitroguanidino pentanoic acid (Cbz-L-Trp-\textsuperscript{\textomega}NO\textsubscript{2}-L-Arg-OH, 3.9d): White microcrystals (80%); mp 71–73 °C (lit\textsuperscript{118} mp 68–75 °C); [\alpha]\textsuperscript{23}D = -19.55 (c 1.0, DMF); \textsuperscript{1}H NMR (DMSO-\textit{d}\textsubscript{6}) \delta 1.45–1.85 (m, 4H), 2.84–2.97 (m, 1H), 3.05–3.33 (m, 3H), 4.18–4.40 (m, 2H), 4.92 (s, 2H), 6.94–7.00 (m, 1H), 7.04 (t, J = 7.4 Hz, 1H), 7.16 (s, 1H), 7.18–7.22 (m, 7H), 7.66 (d, J = 8.0 Hz, 1H), 7.60–8.40 (m, 2H), 8.32 (d, J = 7.7 Hz, 1H), 8.55 (br s, 1H), 10.80 (s, 1H), 12.68 (s, 1H). 

\textsuperscript{13}C NMR (DMSO-\textit{d}\textsubscript{6}) \delta 26.9, 27.8, 28.3, 40.2, 51.7 55.3, 65.3, 110.1, 11.3, 118.2, 118.6, 120.9, 124.0, 127.3, 127.5, 127.7, 128.3, 136.1, 137.0, 155.8, 159.3, 172.2, 173.5.

(6S,9S,14S,17S)-1-Amino-9,14-bis(benzoylcarbonylamino)-6-carboxy-17-(3-(nitroguani-dino) propyl)-1-(nitroimino)-8,15-dioxo-11,12-dithia-2,7,16-triazaoctadecan-18-oic acid (di-Cbz-L-Cystine-di-\textsuperscript{\textomega}NO\textsubscript{2}-L-Arg-OH, 3.9e): White microcrystals (75%); mp 102–108 °C; [\alpha]\textsuperscript{23}D = -84.22 (c 1.0, DMF); \textsuperscript{1}H NMR (DMSO-\textit{d}\textsubscript{6}) \delta 1.32–1.82 (m, 8H), 2.75–2.93 (m, 2H), 3.00–3.22 (m, 6H), 4.12–4.42 (m, 4H), 5.03 (s, 4H), 7.21–7.40 (m, 10H), 7.62 (d, J = 8.5 Hz, 2H), 7.65–8.20 (m, 4H), 8.28 (d, J = 7.4 Hz, 2H), 8.53 (br s, 2H). –COOH protons are missing. \textsuperscript{13}C NMR (DMSO-\textit{d}\textsubscript{6}) \delta 24.6, 28.1, 40.2, 40.5, 51.8, 53.8, 65.6, 127.8, 127.9, 128.4, 136.9, 156.0, 159.3, 170.3, 173.2. HRMS Calcd for \([\text{C}_{34}\text{H}_{46}\text{N}_{12}\text{O}_{14}\text{S}_{2}+\text{H}]^{+}\) : 911.2771. Found, 911.2721.

3.4.3 General Procedure for the Preparation of \textit{LL}-Dipeptides 3.11a-d and the Diastereomeric Mixture (3.11b+3.11b')

\textit{N}(Cbz-\textalpha-Aminoacyl)benzotriazoles 3.7a-d and (3.7b+3.7b') (0.5 mmol) were added at 20 °C to a solution of \textit{L}-Arg-OH 3.10 (0.5 mmol) in CH\textsubscript{3}CN/H\textsubscript{2}O (5 mL/3 mL) The reaction
mixture was then stirred at room temperature until the starting material was completely consumed as observed by TLC using EtOAc/hexanes (1:2) as the eluent. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in a minimum amount of MeOH and then product was reprecipitated with Et₂O. This was repeated three times to remove all the 1H-Benzotriazole from the reaction mixture.

(S)-2-((S)-2-Benzylxycarbonylamino-3-phenyl-propionylamino)-5-guanidinopentanoic acid, \((\text{Cbz-L-Phe-L-Arg-OH, 3.11a})\): White microcrystals (83%); mp 131–132°C (lit\(^{130}\), 131-133 °C); \([\alpha]^{23}_D = -10.37 \, (c \, 1.0, \text{DMF}); \) \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 1.35–1.75 (m, 4H), 1.65–1.80 (m, 1H), 2.95–3.18 (m, 3H), 3.85–4.00 (m, 1H), 4.12–4.25 (m, 1H), 4.93 (s, 2H), 7.05–7.35 (m, 10H), 7.35–7.55 (m, 3H), 7.55–7.70 (m, 2H), 9.41 (br s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 25.3, 29.8, 37.4, 40.4, 53.7, 56.7, 65.2, 126.2, 127.3, 127.7, 128.1, 128.3, 129.2, 137.1, 138.4, 155.9, 157.4, 170.4, 175.5.

(S)-2-((S)-2-Benzylxycarbonylamino-4-methylsulfanylbutanoylamino)-5-guanidinopentanoic acid (Cbz-L-Met-L-Arg-OH, 3.11b): White microcrystals (81%); mp 143–144 °C; \([\alpha]^{23}_D = -7.20 \, (c \, 1.0, \text{DMF}); \) \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 1.30–1.70 (m, 4H), 1.70–1.95 (m, 2H), 2.01 (s, 3H), 2.35–2.55 (m, 2H), 2.95–3.10 (m, 2H), 3.80–3.90 (m, 1H), 5.03 (s, 2H), 7.02–7.40 (m, 6H), 7.47 (d, \(J = 6.9 \text{ Hz}, 1H\)), 7.67 (d, \(J = 7.2 \text{ Hz}, 1H\)), 7.40–8.00 (m, 2H), 9.46 (br s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 14.6, 25.3, 29.8, 30.0, 31.8, 53.6, 54.2, 65.5, 127.0, 127.6, 127.8, 128.4, 137.0, 156.0, 157.4, 170.3, 175.3. HRMS Calcd for \([\text{C}_{19}\text{H}_{29}\text{N}_5\text{O}_5\text{S}+\text{H}]^+\): 440.1962. Found, 440.1966.

(S)-2-(2-Benzylxycarbonylamino-4-methylsulfanyl-butanoylamino)-5-guanidinopentanoic acid (Cbz-DL-Met-L-Arg-OH, (3.11b+3.11b')): White microcrystals (76%); mp 121–124 °C; \([\alpha]^{23}_D = +5.86 \, (c \, 1.0, \text{DMF}); \) \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 1.30–1.70 (m, 4H), 1.70–1.95
(m, 2H), 2.01 (s, 3H), 2.40–2.60 (m, 2H), 2.90–3.12 (m, 2H), 3.80–3.95 (m, 1H), 4.00–4.15 (m, 1H), 5.03 (s, 2H), 7.20–7.40 (m, 6H), 7.40–7.90 (m, 2H), 7.49 (d, \( J = 5.2 \) Hz, 1H), 7.60–7.70 (m, 1H), 9.40 (br s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \( \delta \) 14.5, 14.6, 25.1, 25.2, 29.8, 29.8, 30.0, 31.6, 31.7, 53.5, 54.1, 65.4, 127.6, 127.8, 128.4, 137.0, 155.9, 156.0, 157.3, 170.2, 170.3, 175.1, 175.2.


**(S)-2-[(S)-2-Benzzyloxy carbonylamino-3-(1H-indol-3-yl)-propanoyl amino]-5-guanidinopentanoic acid, (Cbz-L-Trp-L-Arg-OH, 3.11c):** White microcrystals (83%), mp 135–137 °C (lit\(^{133}\)); \([\alpha]^{23}_D = -18.29 \) (c 1.0, DMF); \(^1\)H NMR (DMSO-\(d_6\)) \( \delta \) 1.35–1.80 (m, 4H), 2.83–2.97 (m, 1H), 2.97–3.12 (m, 2H), 3.12–3.23 (m, 1H), 3.87–4.00 (m, 1H), 4.15–4.31 (m, 1H), 4.94 (s, 2H), 6.95 (t, \( J = 7.4 \) Hz, 1H), 7.00–7.10 (m, 1H), 7.14 (s, 1H), 7.10–7.36 (m, 7H), 7.40–8.20 (m, 5H), 9.42 (br s, 1H), 10.84 (s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \( \delta \) 25.4, 27.8, 29.9, 40.5, 53.7, 56.2, 65.3, 110.5, 111.4, 118.3, 118.4, 120.9, 123.8, 127.3, 127.4, 127.5, 127.7, 128.4, 136.2, 137.1, 155.9, 157.5, 170.7, 175.5. HRMS Calcd for \([C_{23}H_{29}N_{5}O_{5}+H]^+\): 495.2350. Found: 495.2341.

**(S)-2-((S)-2-Benzzyloxy carbonylamino-3-methyl-butanoylamino)-5-guanidinopentanoic acid, (Cbz-L-Val-L-Arg-OH, 3.11d):** White microcrystals (75%), mp 121–123 °C (lit,\(^{134}\)); \([\alpha]^{23}_D = -0.99 \) (c 1.0, DMF); \(^1\)H NMR (DMSO-\(d_6\)) \( \delta \) 0.70–0.95 (m, 6H), 1.30–1.80 (m, 4H), 1.90–2.15 (m, 1H), 2.90–3.15 (m, 2H), 3.76–3.95 (m, 2H), 5.04 (s, 2H), 7.20–7.40 (m, 5H), 7.44 (d, \( J = 6.9 \) Hz, 1H), 7.53 (d, \( J = 8.9 \) Hz, 1H), 7.40–8.15 (m, 3H), 9.37 (br s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \( \delta \) 18.0, 19.5, 25.5, 30.0, 30.3, 53.7, 60.7, 65.6, 127.7, 127.9, 128.5, 137.2, 156.4, 157.6, 170.2, 175.5. HRMS. Calcd for \(C_{19}H_{29}N_{5}O_{5}\): 408.2241 Found: 408.2204, \([M+H]^+\)
3.4.4 Preparation of \(N^\alpha\)-Cbz-\(N^\omega\)-NO\(_2\)-L-Arg-Bt, 3.15

\(N^\alpha\)-Cbz-\(N^\omega\)-NO\(_2\)-L-Arg-Bt 3.15 was prepared from \(N^\alpha\)-Cbz-\(N^\omega\)-NO\(_2\)-L-Arg-OH 3.14 using a reported procedure.\(^{135}\) \(N^\alpha\)-Cbz-\(N^\omega\)-NO\(_2\)-L-Arg-OH (0.5 mmol) was added to a solution of 1\(H\)-benzotriazole (2.0 mmol) in anhydrous THF (15 mL), at room temperature in the presence of SOCl\(_2\) (0.6 mmol). Recation mixture was stirred for 45 min at room temperature. The white precipitate formed during the reaction was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (75 mL) and the solution was washed with 4N HCl (3 × 20), brine (20 mL), and dried over MgSO\(_4\). Removal of solvent under reduced pressure gave 3.15 which was further purified by reprecipitation from MeOH/Et\(_2\)O mixture for the elemental analysis.

**Benzyl N-[(1S)-4-{{amino(nitroimino)methyl}amino}-1-(1\(H\)-1,2,3-benzotriazol-1-ylcarbon-yl)-butyl]carbamate (\(N^\alpha\)-Cbz-\(N^\omega\)-NO\(_2\)-L-Arg-Bt, 3.15):** White microcrystals (97%); mp 146–148°C; \([\alpha]^{23}_D = -16.21\) (c 1.0, DMF); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 1.58–2.10 (m, 4H), 3.08–4.23 (m, 2H), 5.05 (s, 2H), 5.43–5.55 (m, 1H), 7.28–7.44 (m, 5H), 7.66 (t, \(J = 7.7\) Hz, 1H), 7.83 (t, \(J = 7.7\) Hz, 1H), 7.74–8.16 (m, 2H), 8.20–8.36 (m, 3H), 8.45 (br s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 24.8, 28.1, 40.2, 54.1, 65.9, 114.0, 120.3, 126.8, 127.9, 128.0, 128.4, 130.6, 131.2, 136.7, 145.4, 156.4, 159.3, 171.8. Anal. Calcd for C\(_{20}\)H\(_{22}\)N\(_8\)O\(_5\): C, 52.86; H, 4.88; N, 24.66. Found: C, 52.96; H, 4.89; N, 24.50.

3.4.5 General Procedure for the Preparation of LL-Dipeptides 3.17a-d and the Diastereomeric mixture (3.17a+3.17a')

\(N^\alpha\)-Cbz-\(N^\omega\)-NO\(_2\)-L-Arg-Bt 3.15 (0.5 mmol) was added at 20 °C to a solution of free amino acid 3.16a-d and the racemic mixture (3.16a+3.16a') (0.5 mmol) in CH\(_3\)CN/H\(_2\)O (10 mL/10 mL) in the presence of Et\(_3\)N (0.6 mmol). The reaction mixture was then stirred at 20 °C until the starting material was completely consumed as observed by TLC using EtOAc/hexanes (1:2) as
the eluent. After addition of 4N HCl (1 mL), the solution was concentrated under reduced pressure to remove CH3CN. Residue was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL), and saturated NaCl (10 mL) then dried over anhydrous MgSO4. Evaporation of the solvent gave the crude mixture of the desired product and the intramolecular cyclization product 3.18. Crude mixture was purified by reprecipitation from MeOH/Et2O. Intramolecular cyclization product was found to be soluble in Et2O. Insoluble product was filtered off and washed further with Et2O to give desired product in pure form. In the case of 3.17d, after completion, reaction mixture was acidified with 4N HCl at -15 to -10 °C. Solid precipitated out was filtered off and washed with excess of Et2O to remove intramolecular cyclization product 3.18. Product 3.17d was isolated in pure form.

(S)-2-((S)-2-Benzylxocarbonylamino-5-(nitroguanidino)pentanoylamino)-3-phenylproanoic acid, (Nα-Cbz-Nω-NO2-L-Arg-L-Phe-OH, 3.17a): White microcrystals (68%); mp 220–222 °C (lit,118 mp 225-226 °C); [α]23D = +5.32 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.30–1.80 (m, 4H), 2.80–2.98 (m, 1H), 2.98–3.22 (m, 3H), 3.90–4.15 (m, 1H), 4.37–4.54 (m, 1H), 5.02 (s, 2H), 7.07–7.50 (m, 11H), 7.60–8.25 (m, 2H), 8.12 (d, J = 7.4 Hz, 1H), 8.48 (br s, 1H), 12.78 (br s, 1H). 13C NMR (DMSO-d6) δ 24.7, 29.2, 36.7, 53.3, 54.2, 65.5, 126.5, 127.7, 128.2, 128.4, 129.2, 137.0, 137.4, 155.9, 159.3, 171.7, 172.8. Anal. Calcd for C23H28N6O7: C, 55.19; H, 5.64; N, 16.79 Found: C, 54.84; H, 5.76; N, 16.63.

2-((S)-2-(Benzyloxycarbonylamino)-5-(nitroguanidino)pentanamido)-3-phenylproanoic acid (Nα-Cbz-Nω-NO2-L-Arg-DL-Phe-OH, (3.17a+3.17a')): White microcrystals (65%); mp 205–207 °C; [α]23D = +8.14 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.28–1.73 (m, 4H), 2.82–3.01 (m, 1H), 3.02–3.23 (m, 3H), 3.96–4.13 (m, 1H), 4.40–4.55 (m, 1H), 5.06 (s, 2H), 7.10–7.50 (m, 11H), 7.50–8.30 (m, 2H), 8.08–8.30 (m, 1H), 8.52 (br s, 1H),
12.82 (br, s). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.7, 29.2, 36.7, 37.0, 53.2, 53.3, 54.2, 65.4, 126.4, 127.0, 127.7, 127.8, 128.1, 128.4, 129.2, 137.0, 137.3, 155.8, 159.3, 171.6, 172.7. Anal. Calcd for C$_{23}$H$_{28}$N$_6$O$_7$: C, 55.19; H, 5.64; N, 16.79. Found: C, 55.49; H; 5.57; N; 16.47.

(S)-2-((S)-2-Benzoylcarbonylamino-5-nitroguanidino-pentanoylamino)-4-methylsulfanyl butanoic acid, ($N^\alpha$-Cbz-$N^\omega$-NO$_2$-L-Arg-L-Met-OH, 3.17b): White microcrystals (66%); mp 144−146 °C; $\left[\alpha\right]_{\text{D}}^{23} = -4.60$ (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.45−1.65 (m, 4H), 1.65−2.00 (m, 2H), 2.03 (s, 3H), 2.40−2.60 (m, 2H), 3.05−3.20 (m, 2H), 3.95−4.05 (m, 1H), 4.25−4.37 (m, 1H), 5.01 (s, 2H), 7.20−7.40 (m, 5H), 7.46 (d, $J = 8$ Hz, 1H), 7.46−8.20 (m, 2H), 8.19 (d, $J = 7.7$ Hz, 1H), 8.50 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 14.6, 24.7, 29.1, 29.6, 30.8, 50.8, 54.1, 65.4, 127.8, 127.8, 137.0, 137.0, 155.9, 159.3, 171.9, 173.2. Anal. Calcd for C$_{19}$H$_{28}$N$_6$O$_7$: C, 47.10; H, 5.82; N, 17.34. Found: C, 47.46; H, 5.74; N, 17.64.

(S)-2-((S)-2-Benzoylcarbonylamino-5-nitroguanidinopentanylarnino)-3-hydroxypropanoic acid ($N^\alpha$-Cbz-$N^\omega$-NO$_2$-L-Arg-L-Ser-OH, 3.17c): White microcrystals (65%); mp 83−85 °C; $[\alpha]_{\text{D}}^{23} = -5.58$ (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40−1.85 (m, 4H), 3.03−3.24 (m, 2H), 3.54−3.80 (m, 2H), 4.03−4.18 (m, 1H) 4.21−4.31 (m, 1H), 5.02 (s, 1H), 7.23−7.40 (m, 5H), 7.44 (d, $J = 8.2$ Hz, 1H), 7.50−8.20 (m, 2H), 8.05 (d, $J = 7.3$ Hz, 1H), 8.48 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.6, 29.3, 40.2, 54.0, 54.6, 61.3, 65.4, 127.7, 127.8, 128.4, 137.0, 155.9, 159.3, 171.9. Anal. Calcd for C$_{17}$H$_{24}$N$_6$O$_8$: C, 46.36; H, 5.49; N, 19.08. Found: C; 46.72; H, 5.63; N, 18.77.

((S)-2-Benzoylcarbonylamino-5-nitroguanidinopentanamido)ethanoic acid ($N^\alpha$-Cbz-$N^\omega$-NO$_2$-L-Arg-Gly-OH, 3.17d): White microcrystals (80%); mp 116−117 °C (lit, 136 114−117 °C); $[\alpha]_{\text{D}}^{23} = -1.54$ (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.43−1.80 (m, 4H), 3.04−3.21 (m, 2H), 3.68−3.84 (m, 2H), 3.98−4.10 (m, 1H), 5.03 (s, 2H), 7.26−7.42 (m, 5H), 7.46
(d, J = 8.0 Hz, 1H), 7.60-8.16 (m, 2H), 8.23 (t, J = 5.6 Hz, 1H) 8.50 (br s, 1H). $^{13}$C NMR
(DMSO-$_d_6$) δ 24.7, 29.2, 40.7, 45.7, 54.6, 65.5, 127.8, 127.9, 128.4, 137.0, 156.0, 159.3, 171.2, 172.2.

3.4.6 Procedure for the Preparation of N-Cbz-Dipeptidoylbenzotriazole Derivatives 3.22a and (3.22a+3.22a') from Cbz-L-Asp(OBz)-OH 3.19

Compound Cbz-L-Asp(OBz)-Bt, 3.20 was prepared from Cbz-L-Asp(OBz)-OH 3.19 following the literature procedure.$^{23}$ Cbz-L-Asp(OBz)-Bt 3.20 was coupled with L-Phe and DL-Phe to afford corresponding protected dipeptides 3.21a and the diastereomeric mixture (3.21a+3.21a'), which were finally converted to their benzotriazole derivatives 3.22a and (3.22a+3.22a').$^{23}$

3.4.6.1 Procedure for the preparation of Cbz-L-Asp(OBz)-Bt, 3.20

To a solution of $1H$-benzotriazole (8 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL), SOCl$_2$ (3 mmol) was added and the reaction mixture was stirred for 20 min at room temperature. To this solution, N-protected amino acid Cbz-L-Asp(OBz)-OH 3.19 (2 mmol) was directly added and reaction mixture was stirred for 2 h at room temperature. Progress of the reaction was monitored by $^1$H NMR. The white precipitate obtained was filtered off and the filtrate was concentrated under vacuum. To the residue obtained, ethyl acetate (100 mL) was added and the solution was washed with aqueous Na$_2$CO$_3$ (3 x 50 mL) solution followed by brine (25 mL). The organic layer was dried over anhydrous MgSO$_4$ and the solvent was removed under reduced pressure. Compounds were recrystallized from CHCl$_3$/hexanes for elemental analysis

(S)-Benzyl 4-$(1H$-benzotriazol-1-yl)-3-(benzyloxy carbonylamino)-4-oxobutanoate
(Cbz-L-Asp(OBz)-Bt, 3.21) White microcrystals (99%); mp 119 °C; $[\alpha]^{20}_D = –9.23$ (c 1.0, DMF); $^1$H NMR (CDCl$_3$) δ 3.28 (dd, J = 16.5, 4.8 Hz, 1H), 3.43 (dd, J = 16.8, 4.8 Hz, 1H), 5.07 (s, 2H), 5.13 (s, 2H), 5.88–6.00 (m, 1H), 6.06 (d, J = 8.1 Hz, 1H), 7.14–7.48 (m, 10H), 7.54 (t, J
= 7.6 Hz, 1H), 7.68 (t, \( J = 8.2 \) Hz, 1H), 8.14 (d, \( J = 8.2 \) Hz, 1H), 8.23 (d, \( J = 8.2 \) Hz, 1H). \(^{13}\)C
NMR (CDCl\(_3\)) \( \delta \) 37.3, 51.7, 67.0, 67.3, 114.3, 120.2, 126.5, 128.0, 128.2, 128.2, 128.3, 128.4,
130.8, 131.0, 134.9, 135.8, 145.8, 155.6, 169.2, 169.8. Anal. Calcd for C\(_{25}\)H\(_{22}\)N\(_4\)O\(_5\): C; 65.75; H,
4.84; N, 12.22; Found: C, 65.75; H, 4.90; N, 12.00.

3.4.6.2 Procedure for preparation of Cbz-\(L\)-Asp(OBz)-\(L\)-Phe-OH 3.21a and the
diastereomeric mixture Cbz-\(L\)-Asp(OBz)-\(DL\)-Phe-OH (3.21a+3.21a’)

Cbz-\(L\)-Asp(OBz)-Bt 3.20 (0.5 mmol) was added at 20 °C to a solution of \(L\)-Phe 3.16a or
\(DL\)-Phe (3.16a+3.16a’) (0.5 mmol) in CH\(_3\)CN/H\(_2\)O (10 mL/10 mL) in the presence of Et\(_3\)N (0.6
mmol). The reaction mixture was then stirred at room temperature until the starting material was
completely consumed as observed by TLC using EtOAc/hexanes (1:2) as the eluent. After
addition of 4N HCl (1 mL), the solution was concentrated under reduced pressure to remove
CH\(_3\)CN. Residue was extracted with EtOAc (20 mL), and the organic extract was washed with
4N HCl (5 mL), and saturated NaCl (10 mL) then dried over anhydrous MgSO\(_4\). Evaporation of
the solvent gave the desired product in pure form, which was further recrystallized from
CHCl\(_3\)/hexanes unless specified, otherwise.

(S)-2-((S)-4-(benzyloxy)-2-(benzyloxycarbonylamino)-4-oxobutanamido)-3-
phenylpropanoic acid (Cbz-\(L\)-Asp(OBz)-\(L\)-Phe-OH, 3.21a): White microcrystals (97%); mp
154–156 °C; \([\alpha]\)\(^{23}\)\(_D\) = –5.22 (c 1.0, DMF); \(^1\)H NMR (DMSO-d\(_6\)) \( \delta \) 2.53–2.63 (m, 1H), 2.70–2.82
(m, 1H), 2.85–2.98 (m, 1H), 2.99–3.10 (m, 1H), 4.35–4.52 (m, 2H), 5.01 (s, 2H), 5.08 (s, 2H),
7.10–7.45 (m, 15H), 7.62 (d, \( J = 8.2 \) Hz, 1H), 8.10 (d, \( J = 8.0 \) Hz, 1H), 12.81 (s, 1H). \(^{13}\)C NMR
(CDCl\(_3\)) \( \delta \) 36.1, 37.3, 50.9, 53.4, 67.0, 67.4, 115.0, 126.2, 127.1, 128.1, 128.2, 128.3, 128.4,
128.5, 129.3, 135.2, 135.6, 135.8, 138.5, 156.2, 170.4, 171.5, 174.1. Anal. Calcd for
2-((S)-4-(Benzyloxy)-2-(benzyloxycarbonylamino)-4-oxobutanamido)-3-phenylpropanoic acid (Cbz-L-Asp(OBz)-DL-Phe-OH, (3.21a+3.21a')): White microcrystals (98%); mp 94–96 °C; [α]_{D}^{23} = −7.67 (c 1.0, DMF); 1H NMR (DMSO-d$_6$) δ 2.35–2.63 (m, 1H), 2.63–2.70 (m, 1H), 2.70–2.97 (m, 1H), 2.98–3.11 (m, 1H), 4.34–4.52 (m, 2H), 4.93–5.17 (m, 4H), 7.15–7.50 (m, 15H), 7.58 (d, J = 8.8 Hz, 0.5H), 7.61 (d, J = 7.7 Hz, 0.5H), 8.09 (d, J = 6.9 Hz, 0.5H), 8.18 (d, J = 7.7 Hz, 0.5 H), 12.80 (br s, 1H). 13C NMR (DMSO-d$_6$): δ 24.8, 29.2, 42.6, 54.2, 65.6, 113.8, 120.2, 126.7, 127.8, 127.9, 128.4, 130.6, 131.0, 137.0, 145.3, 156.1, 159.4, 168.6, 172.9. Anal. Calcd for C$_{28}$H$_{28}$N$_2$O$_7$: C, 66.66; H, 5.59; N, 5.62. Found: C, 66.81; H, 5.72; N, 5.56.

3.4.6.3 General procedure for preparation of Cbz-L-Asp(OBz)-L-Phe-Bt, 3.22a and the diastereomeric mixture (3.22a+3.22a')

To a solution of 1H-benzotriazole (2 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL), at room temperature SOCl$_2$ (0.8 mmol) was added and stirred for 20 min. The reaction mixture was cooled to -15 °C and Cbz-L-Asp(OBz)-L-Phe-OH 3.21a (0.6 mmol) or Cbz-L-Asp(OBz)-DL-Phe-OH (3.21a+3.21a') (0.6 mmol) was added as solid and stirred for 4 h at -15 °C. The white precipitate formed during the reaction was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (75 mL) and the solution was washed with aqueous sodium carbonate (3 × 20), brine (20 mL), and dried over MgSO$_4$. Removal of solvent under reduced pressure gave corresponding N-(Cbz-protected)dipeptidoylbenzotriazole derivatives which were further recrystallized by reprecipitation from CHCl$_3$/hexanes for the elemental analysis.

(S)-Benzyl-4-(((S)-1-(1H-benzotriazol-1-yl)-1-oxo-3-phenylpropan-2-ylamino)-3-(benzyloxycarbonylamino)-4-oxobutanoate (Cbz-L-Asp(OBz)-L-Phe-Bt, 3.22a): White microcrystals (75%); mp 140–142 °C; [α]_{D}^{23} = +11.23 (c 1.0, DMF); 1H NMR (CDCl$_3$) δ 2.73
(dd, J = 17.3, 6.5 Hz, 1H), 2.96–3.15 (m, 1H), 3.20 (dd, J = 14.0, 7.8 Hz, 1H), 3.45 (dd, J = 14.1, 5.0 Hz, 1H), 4.58–4.72 (m, 1H), 5.00–5.20 (m, 4H), 5.90 (d, J = 8.5 Hz, 1H), 6.10–6.22 (m, 1H), 7.02–7.16 (m, 2H), 7.16–7.44 (m, 14H), 7.55 (t, J = 7.2 Hz, 1H), 7.68 (t, J = 7.2 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 8.22 (d, J = 8.2 Hz, 1H). \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 36.0, 38.2, 50.7, 54.3, 66.9, 67.3, 114.2, 120.3, 126.5, 127.3, 128.1, 128.2, 128.3, 128.5, 128.5, 128.7, 129.2, 130.7, 130.9, 134.9, 135.2, 135.9, 145.9, 156.0, 170.0, 170.3, 171.6. Anal. Calcd for C\(_{34}\)H\(_{31}\)N\(_5\)O\(_6\): C, 67.43; H, 5.16; N, 11.56. Found: C, 67.46; H, 5.22; N, 11.45.

(3S)-Benzy 4-(1-(1H-benzotriazol-1-yl)-1-oxo-3-phenylpropan-2-ylamino)-3-
(benzyloxy carbonylamino)-4-oxobutanoate (Cbz-L-Asp(OBz)-DL-Phe-Bt, (3.22a+3.22a')):
White microcrystals (80%); mp 129 °C; \([\alpha]^{23}_D = -10.52\) (c 1.0, DMF); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 2.58–2.80 (m, 1H), 2.96–3.10 (m, 1H), 3.13–3.27 (m, 1H), 3.40–3.52 (m, 1H), 4.59–4.21 (m, 1H), 5.01–5.20 (m, 4H), 5.83–5.99 (m, 1H), 6.10–6.25 (m, 1H), 7.06–7.16 (m, 2H), 7.18–7.44 (m, 14H), 7.55 (t, J = 7.7 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 8.22 (d, J = 8.2 Hz, 0.5 H), 8.23 (d, J = 8.2 Hz, 0.5H). \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 36.0, 38.3, 38.5, 50.7, 50.8, 54.2, 54.4, 67.0, 67.3, 114.3, 120.4, 126.5, 127.4, 128.1, 128.1, 128.3, 128.3, 128.4, 128.6, 128.7, 128.7, 129.2, 130.8, 131.0, 134.9, 134.9, 135.2, 135.2, 135.8, 146.0, 156.0, 170.0, 170.2, 170.3, 171.5, 171.7. Anal. Calcd for C\(_{34}\)H\(_{31}\)N\(_5\)O\(_6\): C, 67.43; H, 5.16; N, 11.56. Found: C, 67.11; H, 5.01; N, 11.38.

3.4.7 General Procedure for Preparation of Arginine LLL-Tripeptides 3.23a-c and the Diastereomeric Mixture (3.23a+3.23a')

\(\text{N-Cbz-dipeptidoylbenzotriazoles 3.22a-c and (3.22a+3.22a')} (0.5 \text{ mmol}) \) were added at \(-15 \degree\text{C}\) to a solution of \(\text{N}^\omega-\text{NO}_2-\text{L-Arg-OH 3.2} (0.5 \text{ mmol}) \) in CH\(_3\)CN (15 mL)/H\(_2\)O (5 mL) in the presence of Et\(_3\)N (0.6 mmol). The reaction mixtures were then stirred at \(-15 \degree\text{C}\) until the starting material was completely consumed as observed on TLC using EtOAc/hexanes (1:2) as the
eluent. After 1 mL of 4N HCl was added, the solution was concentrated under reduced pressure to remove CH₃CN. The solution was extracted with EtOAc (20 mL), and the organic extract was washed with 4N HCl (5 mL) and saturated NaCl (10 mL), and then dried (anhydrous MgSO₄). Evaporation of the solvent gave the desired products, which were purified by recprecipitaion from MeOH/Et₂O.

(5S,8S,11S)-8-Benzyl-5-(2-(benzyloxy)-2-oxoethyl)-11-(3-(nitroguanidino)propyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic acid (N-Cbz-L-Asp(OBz)-L-Phe-N°-NO₂-L-Arg-OH, 3.23a): White microcrystals (84%); mp 128–129 °C; [α]²³_D = –11.71 (c 1.0, DMF); ¹H NMR (DMSO-δ̄₀) δ 1.35–1.85 (m, 4H), 2.50–2.62 (m, 1H), 2.65–2.90 (m, 2H), 2.90–3.21 (m, 3H), 4.10–4.20 (m, 1H), 4.22–4.70 (m, 2H), 5.00 (s, 2H), 5.07 (s, 2H), 7.00–7.50 (m, 15H), 7.50–8.20 (m, 5H), 8.54 (br s, 1H), 12.73 (br s, 1H). ¹³C NMR (DMSO-δ̄₀) δ 24.8, 28.3, 36.2, 37.4, 40.2, 51.2, 51.7, 53.6, 65.6, 126.3, 127.8, 127.9, 128.0, 128.4, 129.3, 136.1, 136.8, 137.5, 155.8, 159.3, 170.1, 170.3, 170.8, 171.3. Anal. Calcd for C₃₄H₃₉N₇O₁₀: C, 57.87; H, 5.57; N; 13.89. Found C, 57.47; H; 5.51; N; 13.91.

(5S,11S)-8-Benzyl-5-(2-(benzyloxy)-2-oxoethyl)-11-(3-(2-nitroguanidino)propyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic acid (N-Cbz-L-Asp(OBz)-DL-Phe-N°-NO₂-L-Arg-OH, (3.23a+3.23a')): White microcrystals (82%); mp 60-65 °C; [α]²³_D = –6.31 (c 1.0, DMF); ¹H NMR (DMSO-δ̄₀) δ 1.35–1.85 (m, 4H), 2.35–2.60 (m, 1H), 2.65–2.87 (m, 2H), 2.90–3.22 (m, 3H), 4.12–4.28 (m, 1H), 4.34–4.70 (m, 2H), 4.92–5.13 (m, 4H), 7.00–7.50 (m, 15H), 7.50–8.40 (m, 5H), 8.53 (br s, 1H), 12.69 (br s, 1H). ¹³C NMR (DMSO-δ̄₀) δ 24.8, 28.1, 28.3, 28.6, 36.2, 36.3, 37.4, 38.3, 40.2, 51.2, 51.5, 51.7, 53.7, 65.6, 65.7, 126.3, 127.7, 127.9, 128.0, 128.4, 136.0, 136.1, 136.8, 136.9, 137.5, 155.8, 159.3, 169.9, 170.1, 170.2, 170.4, 170.7, 170.8, 171.3.
170.9, 173.3. Anal. Calcd for C\textsubscript{34}H\textsubscript{39}N\textsubscript{7}O\textsubscript{10}: C, 57.87; H, 5.57; N, 13.89. Found C, 57.47; H, 5.63, N, 13.65. HRMS Calcd for C\textsubscript{34}H\textsubscript{39}N\textsubscript{7}O\textsubscript{10}+\textsubscript{H}\textsuperscript{+}: 706.2831. Found: 706.2822.

(S)-2-[(S)-2-((S)-2-Benzoyloxycarbonylaminopropanoylamino)-3-(1H-indol-3-yl)propanoylamino]-5-nitroguanidinopentanoic acid (Cbz-L-Ala-L-Trp-\textsuperscript{N\textdegree}-NO\textsubscript{2}-L-Arg-OH, 3.23b): White microcrystals (66%); mp 150−151 °C; [\alpha]\textsubscript{23}D = −10.24 (c 1.0, DMF); \textsuperscript{1}H NMR (DMSO-\textsubscript{d\textsubscript{6}}) δ 1.13 (d, J = 7.1 Hz, 3H), 1.43−1.85 (m, 4H), 2.90−3.15 (m, 1H), 3.15−3.25 (m, 3H), 3.95−4.10 (m, 1H), 4.15−4.20 (m, 1H), 4.50−4.65 (m, 1H), 4.98 (d, J = 12.4 Hz, 1H, A part of AB system.), 5.03 (d, J = 12.6 Hz, 1H, B part of AB system), 6.96 (t, J = 7.4 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 7.15 (s, 1H), 7.20−7.40 (m, 6H), 7.41 (d, J = 7.4 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.65−8.40 (m, 2H), 7.96 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.54 (br s, 1H), 10.82 (s, 1H), 12.71 (br s, 1H). \textsuperscript{13}C NMR (DMSO-\textsubscript{d\textsubscript{6}}) δ 18.1, 24.8, 27.5, 28.4, 50.1, 51.6, 53.1, 65.4, 109.8, 111.2, 118.2, 118.4, 120.8, 123.6, 127.4, 127.8, 128.4, 136.0, 137.0, 155.7, 159.3, 171.5, 172.3, 173.3. Anal. Calcd for C\textsubscript{28}H\textsubscript{34}N\textsubscript{8}O\textsubscript{8}·H\textsubscript{2}O: C, 53.50; H, 5.77; N; 17.82. Found C, 53.18; H, 5.70; N, 17.37. HRMS calcd for [C\textsubscript{28}H\textsubscript{34}N\textsubscript{8}O\textsubscript{8}+Na\textsuperscript{+}]: 633.2391. Found 633.2371.

(5\textsubscript{S},8\textsubscript{S},11\textsubscript{S})-5-Benzyl-8-(2-(methylthio)ethyl)-11-(3-(nitroguanidino)propyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic acid (N-Cbz-L-Phe-L-Met-\textsuperscript{N\textdegree}-NO\textsubscript{2}-L-Arg-OH, 3.23c): White microcrystals (75%); mp 77−79 °C; [\alpha]\textsubscript{23}D = −7.98 (c 1.0, DMF); \textsuperscript{1}H NMR (DMSO-\textsubscript{d\textsubscript{6}}) δ 1.40−1.97 (m, 6H), 1.94−2.10 (m, 3H), 2.10−2.55 (m, 2H), 2.60−2.84 (m, 1H), 2.85−3.05 (m, 1H), 3.05−3.23 (m, 2H), 4.11−4.45 (m, 3H), 4.93 (s, 2H), 7.00−7.40 (m, 10H), 7.52 (d, J = 8.8 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 7.4 Hz, 1H), 7.60−8.40 (m, 2H), 8.53 (br s, 1H), 12.69 (br s, 1H). \textsuperscript{13}C NMR (DMSO-\textsubscript{d\textsubscript{6}}) δ 14.5, 14.7, 24.8, 28.1, 29.2, 29.3, 32.3, 37.3, 51.5, 51.7, 56.0, 65.2, 65.4, 126.3, 127.4, 127.6, 127.7, 128.1, 128.3, 129.2, 136.9,
3.4.8 Preparation of $N^a$-Cbz-$N^\omega$-NO$_2$-L-Arg-Gly-Bt, 3.24

To a solution of $1H$-benzotriazole (2.1 mmol) in anhydrous THF (5 mL), at 20 °C, SOCl$_2$ (0.8 mmol) was added and stirred for 20 min. The reaction mixture was cooled to -15 °C and then added $N^a$-Cbz-$N^\omega$-NO$_2$-L-Arg-Gly-OH 3.16d (0.54 mmol) as solid and stirred for 5.5 h at -15 °C. The white precipitate formed during the reaction was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (75 mL) and the solution was washed with 4N HCl (3 × 20), brine (20 mL), and dried over MgSO$_4$. Removal of solvent under reduced pressure gave 3.24 which was further purified by reprecipitation from MeOH/Et$_2$O mixture for the elemental analysis.

(S)-Benzyl 1-(2-(1$H$-benzotriazol-1-yl)-2-oxoethylamino)-5-(nitroguanidino)-1-oxopentan-2-ylcarbamate ($N^a$-Cbz-$N^\omega$-NO$_2$-L-Arg-Gly-Bt, 3.24): White microcrystals (80%); mp 119–122 °C; $[\alpha]^{23}_D$ = −6.00 (c 1.0, DMF); $^1$H NMR (DMSO-$d_6$) δ 1.47–1.90 (m, 4H), 3.08–3.27 (m, 2H), 4.11–4.25 (m, 1H), 4.92–5.05 (m, 2H), 5.06 (s, 2H), 5.11 (m, 5H), 7.58 (d, $J = 8.0$ Hz, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.81 (t, $J = 7.6$ Hz, 1H), 7.70–8.40 (m, 2H), 8.23 (d, $J = 8.2$ Hz, 1H), 8.29 (d, $J = 8.2$ Hz, 1H), 8.55 (br s, 1H), 8.70 (t, $J = 5.2$ Hz, 1H). $^{13}$C NMR (DMSO-$d_6$): δ 24.8, 29.2, 42.6, 54.2, 65.6, 113.8, 120.2, 126.7, 127.8, 127.9, 128.4, 130.6, 131.0, 137.0, 145.3, 156.1, 159.4, 168.6, 172.9. Anal. Calcd for C$_{22}$H$_{25}$N$_9$O$_6$: C, 51.66; H, 4.99; N, 24.65. Found: C, 52.01; H, 4.99; N, 24.94.

3.4.9 Preparation of $N^a$-Cbz-$N^\omega$-NO$_2$-L-Arg-Gly-L-Asp-(OH)$_2$, 3.26

$N^a$-Cbz-$N^\omega$-NO$_2$-L-Arg-Gly-Bt, 3.24 (0.5 mmol) was dissolved in a minimum amount of THF and added dropwise at -15 °C to a solution of L-Asp-OH 3.25 (0.55 mmol) in CH$_3$CN (5
mL) / H₂O (3 mL) in the presence of Et₃N (2.1 mmol). The reaction mixture was stirred at -15 °C and progress was monitored using TLC by disappearance of the Cbz⁶⁻NO₂⁻L-Arg-Gly-Bt, 3.24. After 3.5 h reaction mixture was concentrated under reduced pressure to remove CH₃CN. The reaction mixture was acidified with 4N HCl (2 mL) under cold condition, and the solution was extracted with EtOAc (100 mL) after adding solid NaCl to the acidified solution. The organic extract was washed with 4N HCl and saturated NaCl (10 mL) and then dried over anhydrous MgSO₄. Evaporation of solvent under reduced pressure, gave the product which was purified by reprecipitation from MeOH/Et₂O.

(S)-2-(2-((S)-2-(Benzyloxycarbonylamino) -5-(2-nitroguanidino)pentanamido)acetamido)succinic acid (N²-Cbz-N¹⁴-NO₂⁻L-Arg-Gly-L-Asp-(OH)₂), 3.26: White microcrystals (60%); mp 75−79 ºC; [α]²³₃ = -13.7 (c 1.0, DMF); 1H NMR (DMSO-d6) δ 1.40−1.80 (m, 4H), 2.59 (dd, J = 16.8, 6.6 Hz, 1H), 2.70 (dd, J = 16.5, 5.5 Hz, 1H), 3.03−3.22 (m, 2H), 3.65−3.83 (m, 2H), 3.95−4.08 (m, 1H), 4.50−4.62 (m, 1H), 5.00 (d, J = 12.6 Hz, A part of AB system, 1H), 5.05 (d, J = 12.6 Hz, B part of AB system, 1H), 7.25−7.42 (m, 5H), 7.52 (d, J = 7.4 Hz, 1H), 7.55−8.10 (m, 2H), 8.07−8.30 (m, 2H), 8.48 (br s, 1H) 12.60 (br s, 2H). 13C NMR (DMSO-d6): δ 24.7, 29.0, 36.1, 41.6, 45.6, 48.5, 54.4, 65.0, 127.8, 128.4, 136.9, 156.1, 159.3, 168.6, 171.6, 172.0, 172.3. HRMS Calcd for [C₂₁H₃₁N₇O₁₀⁺²Na]⁺: 570.1530. Found: 570.1531.
CHAPTER 4
CONVENIENT ONE-POT SYNTHESIS OF 5-(SUBSTITUTED AMINO)-1,2,3,4-
THIATRIAZOLES

4.1 Introduction

Azoles are one important family of heterocycles; important as biologically active compounds and synthetic intermediates. My research interest focused on thiaatriazoles, particularly monosubstituted aminothiaatriazoles, i.e. 5-substituted amino-1,2,3,4-thiaatriazole because of their interesting biological properties including antihypertensive, antibacterial, antitubercular, antiviral, fungicidal, anticancer, and central nervous system stimulant and muscle relaxant activities. Monosubstituted aminothiaatriazoles are also synthetic intermediates for the preparation of 3-oxo-$\Delta 4$-1,2,4-thiadiazolin-5-yl ureas, fused thiazolidine, fused thiazoline and fused 1,2,4-thiadiazoles (Figure 4-1).

Reported synthesis of 5-(monosubstituted amino)-1,2,3,4-thiaatriazoles include: (i) reaction of thiosemicarbazides with nitrous acid, which is the most widely used method, (ii) reaction of isothiocyanates with hydrazoic acid, trimethylsilyl azide or sodium azide that proceed through 1,3-dipolar cycloadditions and electrocyclizations,
(iii) aza transfer procedure with diazonium salts (Scheme 4-1).\textsuperscript{166} The disadvantages associated with the thiosemicarbazide method are: use of strong acid which may affect the acid sensitive functional group, if present in the molecule, and use of hazardous reagent (hydrazine) during the preparation of thiosemicarbazide.\textsuperscript{150,151} Similarly, the hydrazoic acid method accomplish with moderate yields and formation of side product. Though, the reaction conditions using trimethylsilyl azide and sodium azide methods are milder, there is no detail synthetic study to show the generality of the method.

Scheme 4-1. Literature methods for synthesis of monosubstituted aminothiatriazoles 4.1

Recently Batey et al. reported synthesis of substituted aminothiatriazoles 4.12 from corresponding thiocarbamoylimidazolium salts 4.11 by treatment with sodium azide followed by electrocyclization in 50-96% yield (Scheme 4-2).\textsuperscript{167} Thiocarbamoylimidazolium salts 4.11 were synthesized from corresponding amines 4.8 with thiocarboxyldiimidazole 4.9 followed by methylation with iodomethane.

Scheme 4-2. Literature procedure for synthesis of substituted aminothiatriazoles 4.12
Though this procedure is milder as compared to previously described classical methods, it suffers from disadvantages such as: utilization of excess (10 equivalents) of methyl iodide and long reaction time (27-43 h).

From the foregoing account, it is obvious that, mild, efficient and convenient methods are limited in number, and there is a great demand for such a reaction. Therefore, in continuation of our group extensive research on the utility of benzotriazole functionalized reagents, I was interested to find the possibility of such a reagent that could fulfill the demand. In earlier work from our group, 1,1'-carbonyl-bisbenzotriazole 4.13,\(^{168}\) di(1H-benzotriazol-1-yl)methanimine 4.14,\(^{169}\) and bis(1H-benzotriazole-1-yl)methanethione 4.15\(^{41}\), (Figure 4-2) have provided convenient synthesis of substituted ureas from 4.13,\(^{168}\) of di- and trisubstituted thioureas from 4.15,\(^{37}\) of tri- and tetrasubstituted guanidines from 4.14,\(^{169}\) and of 1,2,3- trisubstituted guanidines from 4.15.\(^{40}\) Analogous to this, Batey and coworkers have utilized the imidazolyl reagents 4.16 and 4.17 for the synthesis of ureas\(^{170}\) and thioureas\(^{171}\) in comparable yields. However, the use of imidazole methodology needed an extra step of conversion \textit{in situ} into quaternary derivatives 4.11, 4.18 as compared to the benzotriazole methodology for the synthesis of ureas and thioureas. The synthesis of substituted guanidines from both the reagents 4.14\(^{169}\) and 4.9\(^{172}\) are comparable in yields and number of steps.

![Figure 4-2. Structure of bis(1H-benzotriazol-1-yl) and bisimidazol-1-yl reagents](image-url)
Herein, I describe a convenient method for synthesis of 5-(substituted amino)-1,2,3,4-thiatriazoles 4.1 from bis(1H-benzotriazol-1-yl)methanethione 4.15, which is superior to Batey’s thiocarbonyldiimidazole method.167

4.2 Results and Discussion

4.2.1 Two-Step Synthesis of 5-(Substituted amino)-1,2,3,4-thiatriazoles 4.1a-i

Bis(1H-benzotriazol-1-yl)methanethione 4.15 was prepared in 99% yield from thiophosgene by modification of the literature procedure.41 The reaction was carried out at room temperature for 3 h instead at 0 °C. Yield was considerably improved from 80 to 99%.

Thiocarbamoylbenzotriazoles 4.20a-g,j,k were easily prepared from 4.15 and amines 4.19a-g,j,k in anhydrous CH$_2$Cl$_2$ at 20 °C in shorter duration of time, 1-4 h (Scheme 4-3, Table 4-1) as compared to literature procedure.37,40 According to the literature reaction of aromatic amine, p-anisidine 4.19g with 4.15 did not yield the corresponding thiocarbamoyl derivative 4.20g, only corresponding isothiocyanate was obtained (Scheme 4-4). However, in my case, following same literature procedure,37 corresponding thiocarbamoyl derivative 4.20g was obtained in 40% yield. Optimizing the reaction condition, by decreasing reaction time from 18 h to 45 min yielded desired product 4.20g in 80% yield. Structure of the product was established from $^1$H NMR, $^{13}$C NMR and elemental analysis.

Thiocarbamoylbenzotriazoles 4.20i,j were prepared from bis(1H-benzotriazol-1-yl)methanethione 4.15 and amino acid ester hydrochlorides 4.19h,i in aqueous CH$_3$CN in the presence of Et$_3$N (Scheme 4-3, Table 4-1). Subsequent treatment of 4.20a-i with NaN$_3$ in aqueous CH$_3$CN at room temperature for 1-4 h yielded 5-(substituted amino)-1,2,3,4-thiatriazoles 4.1a-i after 1,3 dipolar cycloaddition followed by electrocyclization in 65-96% yields and overall yields of 50-94% starting from bis(1H-benzotriazol-1-yl)methanethione 4.15 (Scheme 4-3, Table 4-2). Monosubstituted aminothiatriazoles 4.1a-i were isolated without any
chromatographic purification. It was observed that these compounds 4.1a-i were decomposed slowly at room temperature.

Scheme 4-3. Two step synthesis of 5-(substituted amino)-1,2,3,4-thiatriazoles 4.1a-i from bis(1H-benzotriazol-1-yl)methanethione 4.15

Scheme 4-4. Literature report for reaction of bis(1H-benzotriazol-1-yl)methanethione 4.15 and p-anisidine 4.19g

Table 4-1. Preparation of thiocarbamoylbenzotriazoles 4.20a-k

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amines 4.19 (R)</th>
<th>Product 4.20</th>
<th>Yield (%)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-n-Propyl</td>
<td>4.20a</td>
<td>89</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>-Benzyl</td>
<td>4.20b</td>
<td>95</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>-Methylbenzyl</td>
<td>4.20c</td>
<td>93</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td>-(Furan-2-yl)methyl</td>
<td>4.20d</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>5.</td>
<td>-Cyclohexyl</td>
<td>4.20e</td>
<td>95</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>-Allyl</td>
<td>4.20f</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>7.</td>
<td>-4-Methoxyphenyl</td>
<td>4.20g</td>
<td>90</td>
<td>0.8</td>
</tr>
<tr>
<td>8.</td>
<td>-CH₂COOCH₃</td>
<td>4.20h</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>9.</td>
<td>-(R)CH(CH₃)COOCH₃</td>
<td>4.20i</td>
<td>68</td>
<td>3.5</td>
</tr>
<tr>
<td>10.</td>
<td>-CH₂CH₂CH₂CH₂-</td>
<td>4.20j</td>
<td>97</td>
<td>3.5</td>
</tr>
<tr>
<td>11.</td>
<td>-(CH₃)₂</td>
<td>4.20k</td>
<td>68</td>
<td>2</td>
</tr>
</tbody>
</table>

"For structure of products 4.20 see Table 4-2. "Isolated Yields."
Table 4-2. Preparation of 5-(substituted amino)-1,2,3,4-thiatriazoles 4.1a-i from thiocarbamoylbenzotrizoles 4.20a-i

<table>
<thead>
<tr>
<th>Entry</th>
<th>Thiocarbamoylbenzotriazoles 4.20a-i</th>
<th>Product 4.1a-i</th>
<th>Time (h)</th>
<th>Yield&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Overall yield&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
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</table>

<sup>a</sup>Isolated yields.  <sup>b</sup>Overall yield after two steps in scheme 4-3.  <sup>c</sup>Lit. references for known compounds are indicated in experimental section.

4.2.2 One-Pot Synthesis of 5-(Substituted amino)-1,2,3,4-thiatriazoles 4.1a-g,i,l

Success of this two step reaction scheme, and generation of a stable byproduct (1H-benzotriazole) during the first step led me to attempt one-pot synthesis of aminothiatriazoles
4.1a-g,i,l. In the one-pot reactions, in case of amines 4.19a-g,l, after completion of the first step, CH₂Cl₂ was removed under reduced pressure and the residue (a crude mixture of 4.20a-g,l and by-product, 1H-benzotriazole), was treated with NaN₃ in aqueous CH₃CN solution for 1-4 h to obtain 5-(substituted amino)-1,2,3,4-thiatriazoles 4.1a-g,i,j in 73-97% yields (Scheme 4-5, Table 4-3). 1H-Benzotriazole, the only by-product of the conversion was removed by simply washing the organic layer with saturated aqueous Na₂CO₃. All products 4.1a-g,l were isolated after one-pot reaction without chromatographic purification. In the case of 4.1i, the first step of coupling 4.15 with D-Ala-OMe ester hydrochloride 4.19i was carried in CH₃CN/H₂O in presence of Et₃N, instead of CH₂Cl₂ (Scheme 4-6, Table 4-3). After completion of first step, an aqueous solution of NaN₃ was directly added to the reaction mixture, without evaporation of CH₃CN. In the case of 4.1l, after evaporating CH₃CN from the reaction mixture, EtOAc was added for work up. Since the product was insoluble, it was directly filtered off to give 4.1l. Since trace of 1H-benzotriazole was evident from ¹H NMR, the crude product was further stirred in MeOH for 1 h and filtered off to give 4.1l in pure form.

![Scheme 4-5. One-pot synthesis of 5-(substituted amino)-1,2,3,4-thiazirole from bis(1H-benzotriazol-1-yl)methanethione 4.15 and primary amines 4.19a-g,l](image)

![Scheme 4-6. One-pot synthesis of 5-(substituted amino)-1,2,3,4-thiazirole 4.1i from bis(1H-benzotriazol-1-yl)methanethione 4.15 and amino acid ester hydrochloride 4.19i](image)

96
### Table 4-3. One-pot synthesis of 5-(substituted amino)-1,2,3,4-thiatriazoles 4.1a-g,i,l from 4.15

<table>
<thead>
<tr>
<th>Entry</th>
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<th>Product 4.1</th>
<th>Time (h)</th>
<th>Yield(^a) (%)</th>
<th>Lit Yield(^b) (%)</th>
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<td>4.1g</td>
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<tr>
<td>8</td>
<td>4.19i</td>
<td>4.1i</td>
<td>7</td>
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</tr>
<tr>
<td>9</td>
<td>4.19l</td>
<td>4.1l</td>
<td>8</td>
<td>73</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)Isolated yields after one pot reaction. \(^b\)Lit. references for known compounds are indicated in experimental section.

This represents a general method for the synthesis of 5-(monosubstituted amino)-1,2,3,4-thiatriazoles since a variety of substrates such as aliphatic, aromatic and allylic amines and α-
amino acid esters have been utilized (Table 4-3). Amines 4.19a-e undergo one-pot reaction to the corresponding aminothiatriazoles 4.1a-e in excellent yields (92-97%). Similarly, the allylamine 4.19f has been used to prepare 4.1f in 85% yield. Interestingly, aromatic amine 4.19g can also be converted to the corresponding aminothiatriazole 4.1g in 81% yield. In the case of amino acid, D-Ala-OMe ester hydrochloride 4.19i, the product 4.1i was obtained in 74% yield. The substituted bis-aminothiatriazoles 4.1l was also prepared in 73% yield from 4.19l. Yields obtained from one-pot procedure were higher than the two step reaction scheme.

**4.2.3. Attempted Synthesis of 5-(Disubstituted amino)-1,2,3,4-thia triazoles**

In attempts to synthesize 5-(disubstituted amino)-1,2,3,4-thiatriazole, thiocarbamoylbenzotriazole 4.20j was treated with NaN₃ in aqueous CH₃CN at room temperature under the same reaction condition (Scheme 4-7). No product was obtained. Continuing the reaction for 48 h yielded no desired product. All the starting material was recovered from reaction mixture. Refluxing the reaction mixture for 12 h did not indicate any product formation by TLC. Changing the solvent from CH₃CN/H₂O to THF/H₂O did not show any product formation at room temperature for 28 h and refluxing for 24 h. Also changing the substrate from 4.20j to 4.20k did not yield the desired product under room temperature for 48 h or refluxing for 24 h (Scheme 4-8).

![Scheme 4-7. Attempted synthesis of 5-(disubstituted amino)-1,2,3,4-thiatriazole 4.1j](image)

![Scheme 4-8. Attempted synthesis of 5-(disubstituted amino)-1,2,3,4-thiatriazole 4.1k](image)
Failure of the formation of disubstituted aminothiatriazole may be explained by the lack of formation of isothiocyanate intermediate. However, in the cases of imidazole derivatives, disubstituted aminothiatriazoles have been reported to form in about 50% yields. Thus, the formation of isothiocyanate intermediate may not be the correct explanation. When thiocarbamoylimidazole 4.10 (Scheme 4-2) including disubstituted amine derivatives were converted to its imidazolium salt 4.11, those underwent facile reactions with sodium azide to form the corresponding thiatriazole compounds. This may be explained by the gain of electrophilicity of the thiocarbonyl carbon atom due to the positive charge on the nitrogen atom of the imidazole ring. This may also be true for benzotriazole derivatives. Thus, in the cases of 4.20j and 4.20k the thiocarbonyl carbon atoms are not sufficiently electrophilic for the attack of the azide nucleophile to displace the benzotriazole group.

4.3 Conclusion

In conclusion, a simple, mild and convenient novel one-pot synthetic route for preparation of 5-(monosubstituted amino)-1,2,3,4-thiatriazoles has been developed. This method is versatile, general and involves easily accessible starting materials, and can be carried out at room temperature to obtain products in 73-97% yields without the necessity of column purification. Moreover, these yields are better than the reported yields. Thus, it represents a superior method than the reported methods.

4.4 Experimental

4.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. $^1$H (300 MHz) and $^{13}$C (75 MHz) NMR spectra were recorded in CDCl$_3$ or DMSO-$d_6$. $^1$H chemical shifts in CDCl$_3$ were reported in ppm ($\delta$ units) downfield from tetramethylsilane.
Solvent peaks were used as internal references for all $^{13}$C NMR. Solvent peaks were used as internal references for all $^{13}$C NMR.

All the amines were purchased from Fluka or Aldrich, and were used without further purification. Elemental analyses were performed on a Carlo Erba-1106 instrument.

### 4.4.2 Synthesis for the Preparation of Bis(1H-benzotrizol-1-yl)methanethione 4.15

Thiophosgene (1 mmol) was dropwise added to a solution of 1H-benzotriazole (6.3 mmol) dissolved in CH$_2$Cl$_2$ (100 mL) at room temperature. The reaction mixture was stirred for 3 h. After completion, the reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL) and washed with saturated sodium carbonate solution (3 x 100 mL) in order to remove excess of 1H-benzotriazole. The organic layer was dried over anhydrous Na$_2$SO$_4$, and evaporated under vacuum to give bis(1H-benzotrizol-1-yl)methanethione in pure form.

**Bis(1H-benzotrizol-1-yl)methanethione 4.15:** Yellow microcrystals (99%); mp. 172-173°C (Lit. mp 169-170°C); $^1$H NMR (CDCl$_3$) $\delta$ 7.61 (t, $J = 7.4$ Hz, 2H), 7.75 (t, $J = 7.5$ Hz, 2H), 8.23 (d, $J = 8.2$ Hz, 2H), 8.28 (d, $J = 8.4$ Hz, 2H). $^{13}$C NMR (CDCl$_3$) $\delta$ 113.9, 121.0, 126.9, 130.6, 133.1, 146.8, 169.6.

### 4.4.3 General Procedure for the Preparation of Thiocarbamoylbenzotriazoles 4.20a-g,k

Thiocarbamylbenzotriazoles 4.20a-g,j,k were synthesized by adding bis(1H-benzotriazol-1-yl)methanethione 4.15 (1 mmol) to appropriate amine (1 mmol) 4.19a-g,j,k dissolved in CH$_2$Cl$_2$ (10 mL) at room temperature for 1-5 h according to reported procedure. Reaction mixture was diluted with CH$_2$Cl$_2$, washed with saturated Na$_2$CO$_3$ (3 x 25 mL) followed by brine (25 mL) and dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure to give desired thiocarbamoylbenzotriazoles in pure form. It was further recrystallized from CHCl$_3$/hexanes for elemental analysis. Melting point and spectral data was used to characterize
known 4.20b,d-f,j and were found to be identical to reported values. 4.20b: mp 108-109 °C (Lit.172 mp 108-109 °C); 4.20d: mp 118 °C (Lit.40 mp 117 °C); 4.20e: mp 72-73 °C (Lit.40 mp 72-73 °C); 4.20f: mp 57-58 °C (Lit.37 mp 56-57 °C); 4.20j mp 144-145 °C (Lit.40 mp 144-145 °C);

Lit data not available for 4.20k.

Compounds 4.20a,c,g are novel and has been fully characterized by 

\[ ^1H \text{ and } ^{13}C \text{ NMR spectroscopy and elemental analysis. Compound } \text{4.20k was also fully characterized.} \]

**N-Propyl-1H-benzotriazole-1-carbothioamide 4.20a:** White microcrystals (89%); mp 89−90 °C; \(^1H\) NMR (CDCl\(_3\)) \(\delta\) 1.09 (t, \(J = 7.4\) Hz, 3H), 1.85 (sextet, \(J = 7.4\) Hz, 2H), 3.83 (q, \(J = 6.7\) Hz, 2H), 7.49 (t, \(J = 7.6\) Hz, 1H), 7.65 (t, \(J = 7.4\) Hz, 1H), 8.11 (d, \(J = 8.4\) Hz, 1H), 8.94 (d, \(J = 8.5\) Hz, 1H), 9.11 (br s, 1H). \(^{13}C\) NMR (CDCl\(_3\)) \(\delta\) 11.5, 21.4, 46.7, 116.0, 120.1, 125.6, 130.2, 132.3, 147.0, 174.3. Anal. Calcd for C\(_{10}\)H\(_{12}\)N\(_4\)S: C, 54.52; H, 5.49; N, 25.43. Found: C, 54.77; H, 5.50; N, 25.36.

**N-(1-Phenylethyl)-1H-benzotriazole-1-carbothioamide 4.20c:** White Microcrystals (98%); mp 95 °C; \(^1H\) NMR (CDCl\(_3\)) \(\delta\) 1.77 (d, \(J = 6.9\) Hz, 1H), 5.79 (quintet, \(J = 7.1\) Hz, 1H), 7.28-7.52 (m, 6H), 7.64 (t, \(J = 7.8\) Hz, 1H), 8.10 (d, \(J = 8.2\) Hz, 1H), 8.91 (d, \(J = 7.5\) Hz, 1H), 9.32 (d, \(J = 6.6\) Hz, 1H). \(^{13}C\) NMR (CDCl\(_3\)) \(\delta\) 21.0, 54.0, 116.0, 120.2, 125.6, 126.4, 127.9, 128.8, 130.2, 132.3, 141.0, 147.0, 173.3. Anal. Calcd for C\(_{15}\)H\(_{14}\)N\(_4\)S: C, 63.80; H, 5.00; N, 19.84. Found: C, 63.45; H, 4.59; N, 19.63.

**N-(4-Methoxyphenyl)-1H-benzotriazole-1-carbothioamide 4.20g:** White Microcrystals (80%); mp 101 °C; \(^1H\) NMR (CDCl\(_3\)) \(\delta\) 3.87 (s, 3H), 7.00 (d, \(J = 8.8\) Hz, 2H), 7.52 (t, \(J = 7.7\) Hz, 1H), 7.63 (d, \(J = 9.1\) Hz, 2H), 7.68 (t, \(J = 7.4\) Hz, 1H), 8.15 (d, \(J = 8.2\) Hz, 1H), 8.96 (d, \(J = 8.4\) Hz, 1H), 10.60 (br s, 1H). \(^{13}C\) NMR (CDCl\(_3\)) \(\delta\) 112.5, 114.6, 119.6, 126.2, 127.5, 128.8, 129.4,

**N,N-Dimethyl-1H-benzotriazole-1-carbothioamide 4.20k:** Yellow Microcrystals (68%); mp 41-43 °C; {^1}H NMR (CDCl\textsubscript{3}) \( \delta \) 1.32 (t, \( J = 6.7 \) Hz, 3H), 1.48 (t, \( J = 6.7 \) Hz, 3H), 3.51 (q, \( J = 6.9 \) Hz, 2H), 4.12 (q, \( J = 6.7 \) Hz, 2H), 7.42 (t, \( J = 7.6 \) Hz, 1H), 7.57 (t, \( J = 7.7 \) Hz, 1H), 7.94-8.12 (m, 2H). \(^{13}\)C NMR (CDCl\textsubscript{3}) \( \delta \) 10.6, 13.7, 47.9, 48.0, 113.1, 119.4, 124.7, 128.5, 132.9, 174.4.

Anal. Calcd for C\textsubscript{9}H\textsubscript{10}N\textsubscript{4}S: C, 52.41; H, 4.89; N, 27.16. Found: C, 52.11; H, 4.80, N, 27.30.

**4.4.4 General Procedure for the Preparation of Thiocarbamoylbzotriazoles 4.20h,i:**

Thiocarbamylbenzotriazoles 4.20h,i were synthesized by adding bis(1H-benzotriazol-1-yl)methanethione 4.15 (1 mmol) to appropriate amino acid ester hydrocholate (1 mmol) 4.19h,i dissolved in CH\textsubscript{3}CN/H\textsubscript{2}O (4 mL/1 mL) in the presence of Et\textsubscript{3}N (1.1 mmol) at room temperature for 1-5 h. After completion of reaction, CH\textsubscript{3}CN was evaporated from the reaction mixture, EtOAc (75 mL) was added and the resulting solution was washed with saturated Na\textsubscript{2}CO\textsubscript{3} (3 x 25 mL) followed by brine (25 mL) and dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and evaporated under reduced pressure to give desired thiocarbamoylbzotriazoles 4.20h,i in pure form. Compounds were further recrystallized from CHCl\textsubscript{3}/hexanes for elemental analysis.

**Methyl 2-[(1H-1,2,3-benzotriazol-1-ylcarbothioyl)amino]acetate 4.20h:** White microcrystals (70%); mp 120 °C; \(^{1}\)H NMR (CDCl\textsubscript{3}) \( \delta \) 3.87 (s, 3H), 4.62 (d, \( J = 4.8 \) Hz, 2H), 7.67 (t, \( J = 7.8 \) Hz, 1H), 7.53 (t, \( J = 7.8 \) Hz, 1H), 8.12 (d, \( J = 8.3 \) Hz, 1H), 8.87 (d, \( J = 8.5 \) Hz, 1H), 9.50 (br s, 1H). \(^{13}\)C NMR (CDCl\textsubscript{3}) \( \delta \) 46.1, 52.8, 115.7, 120.3, 125.8, 132.3, 146.9, 168.5, 174.7.

Anal. Calcd for C\textsubscript{10}H\textsubscript{10}N\textsubscript{4}O\textsubscript{2}S: C, 47.99; H, 4.03; N, 22.39. Found: C, 47.63; H, 4.01; N, 22.25.

**Methyl (2R)-2-[(1H-1,2,3-benzotriazol-1-ylcarbothioyl)amino]propanoate 4.20i:**

White microcrystals (68%); mp 103-105 °C; \(^{1}\)H NMR (CDCl\textsubscript{3}) \( \delta \) 1.71 (d, \( J = 7.1 \) Hz, 3H), 3.85
(s, 3H), 5.12-5.30 (m, 1H), 7.50 (t, J = 7.2 Hz, 1H), 7.66 (dt, J = 8.2, 0.8 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 8.88 (d, J = 8.5 Hz, 1H), 9.45 (br s, 1H). 13C NMR (CDCl3) 17.3, 52.9, 53.0, 115.9, 120.3, 125.8, 130.4, 132.4, 147.1, 171.9, 174.0. Anal. Calcd for C11H12N4O2S: C, 49.99; H, 4.58; N, 21.20. Found: C, 49.76; H, 4.49; N, 21.05.

4.4.5 General Procedure for the Preparation of 5- (Substituted amino)-1,2,3,4-thiatriazoles 4.1a-i from Thiocarbamoylbenzotriazoles 4.20a-i

Thiocarbamoylbenzotriazoles (1 mmol) 4.20a-i were added to a solution of NaN3 (2.5 mmol) dissolved in CH3CN/H2O (12 mL/2 mL) at room temperature. Reaction mixture was stirred at room temperature. When TLC (15% EtOAc/hexanes) indicated completion of reaction, CH3CN was evaporated under vacuum and EtOAc (100 mL) was added. The solution was washed with saturated Na2CO3 (3 x 50 mL) followed by brine (50 mL) and dried over anhydrous Na2SO4. Evaporation under vacuum yielded the corresponding monosubstituted aminothiatriazoles 4.1a-i which were recrystallized from CH2Cl2/hexanes or CHCl3/hexanes.

N-Propyl-1,2,3,4-thiatriazol-5-amine 4.1a: Colorless microcrystals (84%); mp 59–60 °C, (Lit.157 mp 58–59 °C); 1H NMR (CDCl3), δ 1.05 (t, J = 7.4 Hz, 3 H); 1.80 (sextet, J = 7.3 Hz, 2H); 3.27–3.42 (m, 2 H); 7.18 (brs, 1 H). 13C NMR (CDCl3) δ 11.3, 22.0, 52.0, 179.6.

N-Benzyl-1,2,3,4-thiatriazol-5-amine 4.1b: Colorless microcrystals (96%); mp 78–80 °C, (Lit.166 mp 78–80 °C); 1H NMR (CDCl3) δ 4.60 (d, J = 5.4 Hz, 2 H), 7.30–7.45 (m, 6 H). 13C NMR (CDCl3) δ 52.5, 127.9, 128.5, 129.0, 134.7, 179.1. Anal. Calcd for C8H8N4S: C, 49.98; H, 4.19; N, 29.14. Found: C, 50.18; H, 4.13; N, 29.00.

N-(1-Phenylethyl)-1,2,3,4-thiatriazol-5-amine 4.1c: Colorless microcrystals (94%); mp 120–121 °C; 1H NMR (CDCl3) δ 1.79 (d, J = 6.9 Hz, 3H); 4.45 (quintet, J = 6.5 Hz, 1 H), 7.28–7.46 (m, 5 H), 8.32–8.74 (m, 1 H). 13C NMR (CDCl3) δ: 23.8, 59.7, 126.7, 128.5, 129.1, 140.0,
N-(Furan-2-ylmethyl)-1,2,3,4-thiatriazol-5-amine 4.1d: Colorless microcrystals (91%); mp 68–69 °C; $^1$H NMR (CDCl$_3$) $\delta$ 4.59 (d, $J$ = 4.4 Hz, 2H), 6.25–6.40 (m, 1 H), 6.42 (d, $J$ = 2.6 Hz, 1 H), 7.25–7.45 (m, 1 H), 7.80–8.00 (m, 1 H). $^{13}$C NMR (CDCl$_3$) $\delta$: 44.8, 109.9, 110.5, 143.3, 148.0, 178.5. Anal. Calcd for C$_6$H$_6$N$_4$OS: C, 39.55; H, 3.32; N, 30.75. Found: C, 39.69; H, 3.12; N, 30.48.

N-Cyclohexyl-1,2,3,4-thiatriazol-5-amine 4.1e: Colorless microcrystals (87%); mp 118 °C (Lit.$^{166}$ mp 113–115 °C); $^1$H NMR (CDCl$_3$) $\delta$ 1.20–1.72 (m, 6 H); 1.74–1.92 (m, 2 H); 2.04–2.20 (m, 2 H), 3.12–3.30 (m, 1 H), 6.88–7.30 (m, 1 H). $^{13}$C NMR (CDCl$_3$) $\delta$ 24.4, 25.1, 31.9, 59.8, 178.9. Anal. Calcd for C$_7$H$_{12}$N$_4$S: C, 45.63; H, 6.56; N, 30.41. Found: C, 45.99; H, 6.4; N, 30.54.

N-Allyl-1,2,3,4-thiatriazol-5-amine 4.1f: Colorless microcrystals (78%); mp 62–64 °C (Lit.$^{155}$ mp 53–53.5 °C). $^1$H NMR (CDCl$_3$) $\delta$ 4.03 (t, $J$ = 5.7 Hz, 2H); 5.31–5.45 (m, 2 H), 5.82–5.98 (m, 1 H), 7.10–7.40 (m, 1H). $^{13}$C NMR (CDCl$_3$) $\delta$ 51.3, 119.5, 130.8, 179.2.

N-(4-Methoxyphenyl)-1,2,3,4-thiatriazol-5-amine 4.1g: Colorless microcrystals (65%); mp 142 °C (Lit.$^{142}$ mp 140 °C); $^1$H NMR (CDCl$_3$) $\delta$ 3.85 (s, 3 H), 7.01 (dd, $J$ = 8.9, 2.1 Hz, 2 H), 7.23–7.40 (m, 2 H), 10.31 (br s, 1 H). $^{13}$C NMR (CDCl$_3$) $\delta$ 55.6, 115.3, 120.7, 132.8, 157.5, 176.5.

Methyl 2-(1,2,3,4-thiatriazol-5-ylamino)acetate 4.1h: Colorless microcrystals (86%), mp 142 °C; $^1$H NMR (CDCl$_3$) $\delta$ 3.86 (s, 3H), 4.33 (d, $J$ = 4.3 Hz, 2H), 6.90 (br s, 1H). $^{13}$C NMR (CDCl$_3$) $\delta$ 47.8, 53.0, 169.3, 177.1. Anal. Calcd for C$_4$H$_6$N$_4$O$_2$S: C, 27.58; H, 3.47; N, 32.17. Found: C, 27.45; H, 3.40; N, 32.01.
(R)-Methyl 2-((1,2,3,4-thiatriazol-5-ylamino)propanoate 4.1i: Colorless microcrystals (74%); mp 88–91 °C; [α]$_D^{23}$ = -3.31 (c, 1.0, CHCl$_3$); $^1$H NMR (CDCl$_3$) δ 1.65 (d, $J$ = 7.1 Hz, 3H), 3.83 (s, 3 H), 4.22–4.62 (m, 1 H), 7.32 (s, 1 H). $^{13}$C NMR (CDCl$_3$) δ: 17.8, 53.0, 55.3, 172.6, 176.7. Anal. Calcd for C$_5$H$_8$N$_4$O$_2$S: C, 31.91; H, 4.28; N, 29.77. Found: C, 32.32; H, 4.14; N, 29.39.

### 4.4.6 General One-Pot Procedure for the Preparation of 5-(Substituted amino)-1,2,3,4-thiatriazoles 4.1a-g

To a solution of corresponding amine 4.19a-g (1 mmol) in CH$_2$Cl$_2$ (10 mL) was added bis(1H-benzotriazol-1-yl)methanethione 4.15 (1 mmol) and the reaction mixture was stirred at room temperature for 1-3 h. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was evaporated to remove CH$_2$Cl$_2$. NaN$_3$ (2.5 mmol) dissolved in CH$_3$CN/H$_2$O (12 mL/2 mL) was directly added to the residue obtained. The reaction mixture was stirred for 1-4 h at room temperature. When TLC (15% EtOAc/hexane) indicated complete reaction, CH$_3$CN was evaporated under vacuum and EtOAc (100 mL) was added. The solution was washed with saturated Na$_2$CO$_3$ (3 x 50 mL) followed by brine (50 mL) and dried over anhydrous Na$_2$SO$_4$. Evaporation under vacuum yielded the corresponding monosubstituted aminothiatriazoles which were recrystallized from CH$_2$Cl$_2$/hexanes or CHCl$_3$/hexanes. Yields and melting point are indicated. Spectral characterization is same as for product obtained from two step synthesis.

**N-Propyl-1,2,3,4-thiatriazol-5-amine 4.1a:** Colorless microcrystals (92%); mp 59–60 °C (Lit.$^{157}$ mp 58–59 °C).

**N-Benzyl-1,2,3,4-thiatriazol-5-amine 4.1b:** Colorless microcrystals (96%); mp 78–80 °C (Lit.$^{166}$ mp 78–80 °C).

**N-(1-Phenylethyl)-1,2,3,4-thiatriazol-5-amine 4.1c:** Colorless microcrystals (97%); mp 120–121 °C.
N-(Furan-2-ylmethyl)-1,2,3,4-thiatriazol-5-amine 4.1d: Colorless microcrystals (93%); mp 68–69 °C.

N-Cyclohexyl-1,2,3,4-thiatriazol-5-amine 4.1e: Colorless microcrystals (93%); mp 118 °C (Lit.\(^{166}\) mp 113–115 °C).

N-Allyl-1,2,3,4-thiatriazol-5-amine 4.1f: Colorless microcrystals (85%); mp 62–64 °C (Lit.\(^{155}\) mp 53–53.5 °C).

N-(4-Methoxyphenyl)-1,2,3,4-thiatriazol-5-amine 4.1g: Colorless microcrystals (81%); mp 142 °C (Lit.\(^{142}\) mp 140 °C).

4.4.7 One-Pot Procedure for the Preparation of 5-(Substituted amino)-1,2,3,4-thiatriazoles 4.1i

To a solution of D-alanine methyl ester hydrochloride 4.19i (1 mmol) in CH\(_3\)CN/H\(_2\)O (6 mL/1 mL) and Et\(_3\)N (1.1 mmol) was added bis(1H-benzotriazol-1-yl)methanethione 4.15 (1 mmol) and the reaction mixture was stirred at room temperature for 3 h. Progress of the reaction was monitored by TLC. After completion of reaction, a solution of NaN\(_3\) (2.5 mmol) in H\(_2\)O (2 mL) was added directly to reaction mixture. The reaction mixture was stirred for 3.5 h at room temperature. When TLC (15% EtOAc/hexanes) indicated complete reaction, CH\(_3\)CN was evaporated under vacuum and EtOAc (100 mL) was added. The solution was washed with saturated Na\(_2\)CO\(_3\) (3 x 50 mL) followed by brine (50 mL) and dried over anhydrous Na\(_2\)SO\(_4\). Evaporation under vacuum yielded the corresponding 5-(monosubstituted amino)-1,2,3,4-thiatriazole 4.1i which was recrystallized from CHCl\(_3\)/hexanes.

(R)-Methyl 2-(1,2,3,4-thiatriazol-5-ylamino)propanoate 4.1i. Colorless microcrystals (74%); mp 88–91 °C.

4.4.8. One-Pot Procedure for the Preparation of 5-(Substituted amino)bis(1,2,3,4-thiatriazol) 4.1l

To a solution of amine 4.19l (1 mmol) in CH\(_2\)Cl\(_2\) (35 mL) was added bis(1H-benzotriazol-1-yl)methanethione 4.15 (2 mmol) and the reaction mixture was stirred at room temperature for 3 h. Progress of the reaction was monitored by TLC (20% EtOAc/hexanes. After completion of
reaction, the reaction mixture was evaporated to remove CH$_2$Cl$_2$ and to the residue was added a solution NaN$_3$ (5 mmol) in CH$_3$CN/H$_2$O (60 mL/10 mL). The reaction mixture was stirred for 5 h at room temperature. When TLC (40% EtOAc/hexanes) indicated complete reaction, CH$_3$CN was evaporated under vacuum and EtOAc (100 mL) was added. The product was insoluble in EtOAc. It was directly filtered off and dried to give 5-(substituted amino) bis(1,2,3,4-aminothiatriazole) 4.11. The product was insoluble in MeOH, so it was further stirred in MeOH for 1 h and filtered off to get pure 4.11 without trace of 1H-benzotriazole.

$N,N'$(1,4-Phenylenemethylene)bis(1,2,3,4-thiatriazol-5-amine) 4.11. Colorless microcrystals (75%); mp 141 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 4.59 (s, 4H), 7.36 (s, 4H), 9.38 (br s, 2H) $^{13}$C NMR (DMSO-$d_6$) $\delta$ 49.3, 127.9, 136.6, 177.2. HRMS Calcd for [C$_{10}$H$_{10}$N$_8$S$_2$+Na]$^+$: 329.0362. Found: 329.0355.
CHAPTER 5
SYNTHESIS OF 8-AZAQUINAZOLINONE ANALOGUE, NBI-74330 AS CXCR3 RECEPTOR ANTAGONIST AND ITS APPLICATION IN TREATMENT OF MALIGNANT GLIOMAS

5.1 Introduction

Chemokines are a family of small proteins secreted by leukocytes or tissue cells that induce chemotaxis of responsive cells and are attractive molecules to mediate the migration of immune cells into tumor. In addition to these chemoattractive functions, chemokines also exert direct effects on tumor growth, angiogenesis, and metastasis.\textsuperscript{174,175} Depending on the number and spacing of conserved cysteine residues, chemokines are classified into four major groups CC, CXC, CX3C and XC.\textsuperscript{176,177} Like all the chemokine receptors, CXC chemokine receptor-3 (CXCR3) belongs to super family of G-protein coupled receptor predominantly expressed on activated inflammatory T lymphocytes that promote Th1 response.\textsuperscript{178} More recently, the related 3H-pyrido[2,3-d] pyrimidin-4-one compounds AMG 487, 5.1 and NBI-74330, 5.2 (Figure 5-1) have been reported as nanomolar CXCR3 antagonists and these ligands are currently under clinical investigation.\textsuperscript{179} These ligands are the most potent CXCR3 ligands reported to date, showing affinity values for the human CXCR3 receptor in nanomolar range.

\textbf{Figure 5-1.} Structure of chemokines receptors AMG-487 5.1 and NBI-74330 5.2

Malignant gliomas are the most frequent and lethal type of brain cancer originating in the central nervous system (CNS). Human glioblastoma multiforme (GBM) [World Health
Organization (WHO) grade IV astrocytoma, the primary brain tumor associated with dismal prognosis, is the most biologically aggressive subtype of malignant gliomas.\textsuperscript{180} The current standard for the treatment of GBM patients is surgical resection of the tumor mass, followed by adjuvant radiation therapy and chemotherapy with oral alkylating agent temozolomide.\textsuperscript{180} Due to relative ineffectiveness of these traditional treatments, immunotherapy is currently being evaluated as better alternative treatment for GBM.\textsuperscript{181}

Microglia are macrophage-like cells present within the central nervous system (CNS).\textsuperscript{182} Macrophage/microglial cells play a critical role in mediating potent immune response to infectious challenges in the human brain and are the largest immune cell population infiltrating human glioma. The marked presence of glioma infiltrating microglia and lymphocytes suggests that targeting immune system could be one way to treat GBM.\textsuperscript{175} A number of recent studies have demonstrated that multiple toll like receptors expressed in murine microglial cells are very crucial in detecting and generating innate immune response in CNS.\textsuperscript{182} Murine microglia/macrophages are also able to activate CD4\textsuperscript{+} receptor and helper T cells.\textsuperscript{183} The mechanism by which the various immune cells grow into tumor is not yet very clear.

Very recently, Liu et. al. studied effect of chemokine receptor CX3CR1 and its ligands CX3CL1 in gliomagenesis, using GL261 murine model of glioma.\textsuperscript{174} Based on the techniques of \textit{in situ} hybridization analysis,\textsuperscript{184} CX3CR1 and CX3CL1 expression in GL261 glioma was established \textit{in vivo} from wild type C57BL/6 mice. Tumor growth in CX3CR1 deficient mice indicated larger tumor size in homozygous animal (-/-) as compared to heterozygous animal (+/−).\textsuperscript{175} These results indicated that CX3CR1 has little or no impact on the glioma formation or migration of microglia and lymphocytes into gliomas.
The present study was undertaken to evaluate the role of various chemokine systems in gliomagenesis utilizing CXCR3 gene disruption and with CXCR3 antagonism. Herein, synthesis of 8-azaquinazolinone analogue NBI-74330 5.2 is described by literature method\textsuperscript{185} with some modifications, and its biological application as CXCR3 receptor antagonist in treatment of human gliomas is briefly described.

5.2 Results and Discussion

5.2.1 Procedure for the Preparation of 8-Azaquinazolinone Analogue, NBI-74330 5.2

Synthesis of desired compound NBI-74330 5.2 was done following literature method\textsuperscript{185} (Scheme 5-1). Boc-D-Ala 5.3 was activated with \textit{iso}-butylchloroformate 5.4 under basic conditions in anhydrous CH\textsubscript{2}Cl\textsubscript{2} under nitrogen atmosphere at -20 °C, and then allowed to react with 2-aminonicotinic acid 5.6. Intermediate 5.7 was isolated after acidic work up with saturated citric acid. Crude intermediate was immediately reacted with \textit{p}-phenitidine in anhydrous CH\textsubscript{2}Cl\textsubscript{2} under nitrogen atmosphere at -20 °C for 1.5 h. Intermediate 5.9 was isolated after acidic work with saturated citric acid and used in next step without any further purification. Subsequent ring closure of intermediate 5.9 was achieved in presence of \textit{N}-methylmorpholine (NMM) and \textit{iso}-butylchloroformate in anhydrous CH\textsubscript{2}Cl\textsubscript{2} under nitrogen atmosphere, at -20 °C for 4 h. Crude product 5.10 was purified by reprecipitation in \textit{tert}-butyl methyl ether/heptane solvent mixture. Compound 5.10 was isolated in overall yield of 22% after three steps. Deprotection of –Boc group with trifluoroacetic acid (TFA) 5.11 afforded intermediate compound 5.12 in 94% yields. Reductive amination of intermediate 5.12 with pyridine-3-carboxaldehyde and sodium triacetoxyborohydride at room temperature for 18 h afforded crude product 5.15 in 97% yield which was further used in the final step without any purification.
Scheme 5-1. Synthesis of CXCR3 receptor NBI-74330 \textbf{5.2}

In the final step coupling of crude product \textbf{5.15} with 4-fluoro-3-trifluoroacetic acid \textbf{5.16} with 1-ethyl-3-(3’-dimethylaminopropyl)carbodiimide hydrochloride (EDC), hydroxybenzotriazole (HOBt), and NMM in anhydrous DMF at room temperature for 22 h afforded the final crude product \textbf{5.2} as sticky solid. Crude product could not be purified by trituration with \textit{tert}-butyl methyl ether as reported.\textsuperscript{181} So, it was purified by flash column chromatography (10\% MeOH/EtOAc) on silica gel. Pure product after column was dissolved in
minimum amount of tert-butyl methyl ether, and precipitated by adding hexane to the solution. White solid precipitated was filtered off from solution to give final pure compound $5.2$ in 43% yield. Procedure described in the patent$^{185}$ did not indicate the yield for final step. However, according to literature,$^{186}$ final step was obtained in only 19% yield. Overall yield for product $5.2$ was 8.6%. Room temperature $^1$H NMR for final compound was very complicated due to presence of rotamers. Peak splitting due to presence of rotamers were confirmed by high temperature $^1$H NMR measurement (Figure 5-2) in DMSO-$d_6$ at 120 °C. Compound $5.6$ under reverse phase HPLC shows 100% purity (Figure 5-3).

![Figure 5-2. High temperature $^1$H NMR for the compound $5.2$ at increasing temperature up to 120 °C](image)
5.2.2 Role of NBI-74330 as CXCR3 Receptor Antagonist for Treatment of Malignant Gliomas

This work was done by Harrison et al.¹ The role of the CXCR3 chemokine receptor system in gliomagenesis and associated immune response was studied using murine GL261 model of malignant gliomas. Gliomas were established from GL261 cells grown as adherent (GL261-AD) monolayers. A prominent microglia infiltration and various types of tumor infiltrated lymphocytes (CD4+, CD8+, Foxp3+ and Ly49G2+) were present in both GL261-AD and GL261-NS derived tumors. *In situ* hybridization analysis determined that chemokines, CXCL9/MIG and CXCL10/IP-10 (chemokines ligands of CXCR3 receptor), are expressed by the tumors *in vivo*. The role of CXCR3 in GL261 gliomagenesis was therefore evaluated in

¹ Biological studies were carried out by Dr. Jeffrey K. Harrison, Defang Luo and Che Liu in the Department of Pharmacology and Therapeutics, College of Medicine, University of Florida.
CXCR3-deficient mice as well as glioma-bearing mice treated with CXCR3 receptor antagonist, NBI-74330. A brief summary of results are as follows

- CXCL9 and CXCL10 are expressed in murine GL261 gliomas established in C57BL/6 wild type CXCR3-deficient, and NBI-74330 treated mice.
- CXCR3 deficient mice displayed reduced tumor growth and animal survival.
- Reduced levels of NKT cells were found within GL261 tumors established in CXCR3 deficient mice.
- CXCR3 antagonist NBI-74330 reduced tumor growth and prolonged survival in tumor bearing mice in a manner independent of host CXCR3.

5.3 Conclusion

In conclusion CXCR3 antagonism is a potential therapeutics approach for treatment of malignant gliomas.

5.4 Experimental Section

5.2.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in CDCl$_3$ or DMSO-$d_6$ with TMS as an internal standard for $^1$H (300 MHz) and solvent as an internal standard for $^{13}$C (75 MHz). Boc-$D$-Ala was purchased from Fluka. All other chemicals were purchased from Fluka or Aldrich and were used without further purification. Elemental analyses were performed on a Carlo Erba-1106 instrument.

5.2.2 Procedure for Preparation of Compound 5.10

Synthesis of compound 5.10 was performed in three steps, starting from Boc-$D$-Ala-OH 5.3.

**Step 1.** $N$-Methylmorpholine (NMM) 5.5 (25 mmol) was added to a solution of Boc-$D$-Ala-OH 5.3 (10 mmol) in CH$_2$Cl$_2$ (40 mL) under nitrogen atmosphere and the reaction mixture
was stirred for 15 min. The solution was cooled to -20 °C and iso-butylchloroformate 5.4 (22 mmol) dissolved in CH₂Cl₂ (10 mL) was added dropwise. After stirring the reaction mixture for 45 min at -20 °C, 2-aminonicotinic acid 5.6 (10 mmol) was added and the reaction mixture was allowed to warm to room temperature for 18 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and washed with saturated citric acid (3 x 50 mL), and brine (75 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum to give 88% of yellow orange oil 5.7. The crude product from step 1 was used in step 2 without any further purification.

Step 2. Crude product 5.7 (8.72 mmol) was dissolved in CH₂Cl₂ (60 mL) and cooled to -20 °C under a nitrogen atmosphere and p-phenetidine 5.8 (8.72 mmol) was added dropwise. The reaction mixture was stirred for 1.5 h at -20 °C to 0 °C. After completion, the reaction mixture was diluted with CH₂Cl₂ (75 mL) and washed with saturated citric acid solution (3 x 75 mL) and saturated sodium bicarbonate solution (3 x 50 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum to give 65% of crude bis-amide product 5.9. The crude product from step 2 was used in step 3 without any further purification.

Step 3. N-Methylmorpholine (NMM) (0.58 mL) and iso-butylchloroformate were added to the crude bis-amide product 5.9 (5.3 mmol) from step 2 dissolved in anhydrous CH₂Cl₂ (60 mL) under nitrogen atmosphere at -20 °C. The reaction mixture was stirred at -20 °C to -15 °C for 4 h. The reaction mixture was diluted with of CH₂Cl₂ (250 mL) and washed successively with saturated citric acid (3 x 75 mL), saturated sodium bicarbonate solution (3 x 75 mL) and brine (50 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum to give the crude product as brown viscous oil. The crude product was dissolved in tert-butyl methyl ether (50 mL) and stirred at room temperature. The product started precipitating out
of the solution. Heptane (50 mL) was then added and stirred at 0 °C. The resulting precipitate was collected by vacuum filtration, washed with heptane and dried to afford 5.10 as off white solid in overall yield of 22% after three steps.

(R)-tert-Butyl 1-(3-(4-ethoxyphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)ethylcarbamate, 5.10: Colorless microcrystals (22%); mp 152 °C; [α]_{D}^{23} = +3.90 (c, 1.0, CHCl₃); ¹H NMR (CDCl₃) δ 1.31 (d, J = 6.7 Hz, 3H), 1.40 (s, 9H), 1.46 (t, J = 7.0 Hz, 3H), 4.10 (q, J = 6.9 Hz, 2H), 4.58–4.73 (m, 1H), 5.80 (d, J = 9.2 Hz, 1H), 7.04 (dd, J = 8.7, 2.7 Hz, 1H), 7.10 (dd, J = 8.8, 2.8 Hz, 1H), 7.16 (dd, J = 8.8, 2.7 Hz, 1H), 7.30–7.38 (m, 1H), 7.45 (dd, J = 8.0, 4.6 Hz, 1H), 8.61 (dd, J = 7.9, 2.0 Hz, 1H), 8.99 (dd, J = 4.4, 2.0 Hz, 1H). ¹³C NMR (CDCl₃) δ 14.7, 20.7, 28.2, 47.7, 63.7, 79.5, 115.5, 115.8, 116.2, 122.4, 127.4, 129.3, 136.8, 154.9, 156.0, 157.2, 159.7, 162.6, 162.8. Anal Calcd for C₂₂H₂₆N₄O₄: C, 64.37; H, 6.38; N, 13.65. Found: C, 64.64; H, 6.60; N, 13.58.

5.2.3 Procedure for the Preparation of Compound 5.12

(R)-2-(1-Aminoethyl)-3-(4-ethoxyphenyl)pyrido[2,3-d]pyrimidin-4(3H)-one, 5.12: Colorless microcrystals (94%); mp 185-190 °C; [α]_{D}^{23} = −14.15 (c, 1.0, CHCl₃); ¹H NMR (CDCl₃) δ 1.18 (d, J = 6.5 Hz, 3H), 1.38 (t, J = 6.9 Hz, 3H), 1.94 (br s, 2H), 3.53 (q, J = 6.4 Hz, 1H), 4.11 (q, J = 6.7 Hz, 2H), 6.97–7.15 (m, 2H), 7.30–7.45 (m, 2H), 7.55 (dd, J = 7.8, 4.5 Hz, 1H), 8.49 (dd, J = 8.0, 2.0 Hz, 1H), 8.99 (dd, J = 4.5, 1.9 Hz, 1H). ¹³C NMR (CDCl₃) δ 14.6, 23.1, 48.8, 63.8, 115.6, 115.7, 115.9, 122.2, 127.9, 128.9, 129.3, 136.7, 156.1, 157.4, 159.6, 162.8, 165.8.

5.2.4 Procedure for the Preparation of Compound 5.15

To a solution of 5.12 (5.17 mmol) in dichloroethane (100 mL), was added pyridine-3-carboxaldehyde, 5.13 (5.43 mmol) followed by sodium triacetoxy borohydride (7.24 mmol). The
Reaction mixture was allowed to stir at room temperature for 18 h. The reaction mixture was diluted with CH₂Cl₂ (250 mL) and washed with 1M ammonium hydroxide (2 x 250 mL). The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum to afford 97% of (R)-3-(4-Ethoxyphenyl)-2-(1-(pyridin-3-ylmethylamino)ethyl)pyrido[2,3-d]pyrimidin-4(3H)-one, 5.15 as white microcrystals.

(R)-3-(4-Ethoxyphenyl)-2-(1-(pyridin-3-ylmethylamino)ethyl)pyrido[2,3-d]pyrimidin-4(3H)-one, 5.15: White microcrystals (97%); mp 61-63 °C; [α]₂³⁰D = +9.96 (c, 1.0, CHCl₃); ¹H NMR (DMSO-d₆) δ 1.20 (d, J = 6.5 Hz, 3H), 1.35 (t, J = 6.9 Hz, 3H), 3.23-3.40 (m, 1H), 3.54 (d, J = 14.0 Hz, 1H, A part of AB system), 3.75 (d, J = 14.0 Hz, 1H, B part of AB system), 3.97-4.12 (m, 2H), 6.86 (dd, J = 8.8, 2.7 Hz, 1H), 7.02 (dd, J = 8.7, 2.6 Hz, 1H), 7.17 (dd, J = 8.7, 2.3 Hz, 1H), 7.29 (dd, J = 7.7, 4.8 Hz, 1H), 7.36 (dd, J = 8.8, 2.3 Hz, 1H), 7.57 (dd, J = 7.8, 4.3 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 8.41 (d, J = 3.5 Hz, 1H), 8.46 (s, 1H), 8.51 (dd, J = 7.8, 1.7 Hz, 1H), 9.00 (dd, J = 6.7, 1.7 Hz, 1H). ¹³C NMR (CDCl₃) δ 14.6, 21.4, 48.8, 54.2, 63.7, 115.5, 115.6, 116.0, 122.3, 123.3, 127.5, 128.7, 128.9, 135.0, 135.8, 136.8, 148.2, 149.3, 156.1, 157.5, 159.6, 162.7, 164.7. HRMS Calcd for [C₂₃H₂₃N₅O₂+H]⁺: 402.1925, found: 402.1939.

5.2.5 Procedure for the Preparation of 8-Azaquinazolinone Analogue NBI 74330 5.2

To a solution of 4-fluoro-3-trifluoroacetic, 5.16 (16.4 mmol) in anhydrous DMF (45 mL) was added 1-ethyl-3-(3’-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (21.3 mmol), hydroxybezotriazole (HOBt) (16.4 mmol), N-methylmorpholine (NMM) (24.6 mmol). After stirring for 30 min, (8.2 mmol) of (R)-3-(4-ethoxyphenyl)-2-(1-(pyridin-3-ylmethylamino)ethyl)pyrido[2,3-d]pyrimidin-4(3H)-one 5.15 was added. The reaction mixture was allowed to stir at room temperature for 22 h. The reaction mixture was diluted with CH₂Cl₂ (500 mL), and washed with water (3 x 500 mL), saturated sodium bicarbonate solution (3 x 300 mL) and brine (300 mL). The organic extract was dried over anhydrous Na₂SO₄, filtered and
concentrated under vacuum. Crude product obtained was purified by flash column chromatography (10% MeOH/EtOAc) on silica gel. Pure product after column was dissolved in minimum amount of tert-butyl methyl ether, and precipitated by adding hexane to the solution. White solid precipitated was filtered off from solution to give 43% of (R)-N-(1-(3-(4-ethoxyphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)ethyl)-2-(4-fluoro-3-(trifluoromethyl)phenyl)-N-(pyridin-3-ylmethyl)acetamide, 5.2. The product obtained was further recrystallized from EtOAc/hexanes for elemental analysis.

(R)-N-(1-(3-(4-Ethoxyphenyl)-4-oxo-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)ethyl)-2-(4-fluoro-3-(trifluoromethyl)phenyl)-N-(pyridin-3-ylmethyl)acetamide, 5.2: White microcrystal (43%); mp 186 °C; [α]$_D^{23}$ = -42.22 (c, 1.0, CHCl$_3$); $^1$H NMR (DMSO-d$_6$) [High temperature NMR at 120 °C, Figure 5-2] δ 1.33 (d, $J$ = 6.9 Hz, 3H), 2.89 (d, $J$ = 6.7 Hz, 3H), 2.85 (s, 1H), 3.60 (d, $J$ = 16.4 Hz, 1H), 4.09 (q, $J$ = 6.9 Hz, 2H), 4.75 (br s, 2H), 5.20–5.35 (m, 1H), 7.00–7.22 (m, 4H), 7.23–7.34 (m, 1H), 7.34–7.48 (m, 3H), 7.49–7.60 (m, 2H), 8.29–8.40 (m, 2H), 8.41–8.40 (m, 1H), 8.96–9.04 (m, 1H). Anal. Calcd for C$_{32}$H$_{27}$F$_4$N$_5$O$_3$: C, 63.47; H, 4.49; N, 11.41. Found: C, 63.23; H, 4.40; N, 11.41. HRMS Calcd for [C$_{32}$H$_{27}$F$_4$N$_5$O$_3$+H]$^+$: 606.2123, found: 606.2129.
LIST OF REFERENCES

The references citation system employed throughout this dissertation is from Journal of Organic Chemistry, from American Chemical Society (ACS) publication.

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LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Geeta Meher (Geeta) daughter of Uma Shanker Ram Prajapati and Lalita Prajapati was born in 1978 in Poonapar, Uttar Pradesh, India. She received her Bachelor of Science degree in 1998 from Udai Pratap College, Varanasi, and obtained her Master of Science degree in Chemistry from Banaras Hindu University, Varanasi, India in 2000. After completing her Master in Technology degree in “Modern Methods of Chemical Analysis” from Indian Institute of Technology, Delhi, India in 2001, she joined in a polymer laboratory at Indian Institute of Technology, Bombay, and worked there from 2002 to 2004. Later, she joined Dr. Alan R. Katritzky Research group in March 2005 and there on worked with him towards her Doctor of Philosophy degree in the field of peptides and heterocyclic chemistry.