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LIST OF ABBREVIATIONS

α  Alpha locant
[α]D  Specific rotation
Å  Angstrom(s)
Ac  Acetyl
Ala  Alanine
Ar  Aryl
AZT  3′-Azidothymidine
β  Beta locant
Bn  Benzyl
Boc  t-Butoxycarbonyl
br  Broad
Bt  Benzotriazol-1-yl
C  Carbon
°C  Degree Celcius
Calcd  Calculated
Cbz  Carbobenzyloxy
CDCl3  Deuterated chloroform
CTH  Catalytic hydrogen transfer
CuSO4·H2O  Copper(II) sulfate pentahydrate
Cys  Cysteine
δ  Chemical shift in parts per million downfield from tetramethysilane
d  Days; Douplet (spectral)
D  Dextrorotatory (right)
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC \( N,N'-\text{Dicyclohexylcarbodiimide} \)
DCM \( \text{Dichloromethane} \)
DIPEA \( \text{Diisopropylethylamine} \)
DMF \( \text{Dimethylformamide} \)
DMSO \( \text{Dimethylsulfoxide} \)
\( \text{D}_2\text{O} \) \( \text{Deuterium oxide} \)
EDC \( 1\text{-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide} \) (stands as an abbreviation for EDAC and EDCI as well)
Et \( \text{Ethyl} \)
et al. \( \text{And others} \)
ESI \( \text{Electrospray ionization} \)
\( \text{Et}_3\text{N} \) \( \text{Triethylamine} \)
EtOAc \( \text{Ethyl acetate} \)
EtOH \( \text{Ethanol} \)
Equiv \( \text{Equivalent(s)} \)
g \( \text{Gram(s)} \)
Gly \( \text{Glycine} \)
h \( \text{Hour} \)
H \( \text{Hydrogen} \)
HCl \( \text{Hydrochloric acid} \)
HPLC \( \text{High performance liquid chromatography} \)
HRMS \( \text{High resolution mass spectrometry} \)
Hz \( \text{Hertz} \)
IR \( \text{Infrared} \)
\( J \) \( \text{Coupling constant} \)
L \( \text{Levorotatory (left)} \)
<table>
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<th>Abbreviation</th>
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<tr>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>Lit</td>
<td>Literature</td>
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<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
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<tr>
<td>MeOH</td>
<td>Methanol</td>
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<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Magnesium sulfate</td>
</tr>
<tr>
<td>mol</td>
<td>Mole(s)</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry/Mass spectra</td>
</tr>
<tr>
<td>MW</td>
<td>Microwave</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>Sodium carbonate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>Sodium Sulfate</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>o</td>
<td>Ortho locant</td>
</tr>
<tr>
<td>O</td>
<td>Oxygen</td>
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<tr>
<td>OEt</td>
<td>Ethoxy</td>
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<td>OH</td>
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OMe  methoxy

p  Para locant

Pd  Palladium

Pd(OAc)$_2$  Palladium(II)acetate

Pd(Ph$_3$)$_4$  Tetrakis(triphenylphosphine)palladium(0)

Ph  Phenyl

Phe  Phenylalanine

PPh$_3$  Triphenylphosphine

ppm  Part per million

Pro  Proline

Py  Pyridine

q  Quartet

R  Rectus (right)

ref.  Reference

rt  Room temperature

s  Singlet

S  Sinister (left)

S  Sulphur

Ser  Serine

SOCl$_2$  Thionyl chloride

SO$_2$Cl$_2$  Sulfuryl chloride

t  Time; Triplet (spectral)

T  Tertiary

TLC  Thin-layer chromatography

TMS  Trimethylsilane
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<td>Trp</td>
<td>Tryptophan</td>
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<tr>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per unit volume (volume-to-volume ratio)</td>
</tr>
<tr>
<td>W</td>
<td>Watt(s)</td>
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The theme of this work is the development of novel methodologies for the efficient preparation of a variety of organic compounds. Chapter 1 presents a general overview of the work presented in subsequent chapters together with a brief discussion of the importance of benzotriazole methodology in organic synthesis.

Chapter 2 describes the role of benzotriazole for the synthesis of a novel diazotransfer reagent and examines the utility of the reagent in the preparation of organic azide targets. This methodology is also employed in Chapter 3, where the tolerance of azide as a protecting group is examined in various acylation reactions.

Chapter 4 presents the use of the microwave in organic synthesis. This chapter reports on the palladium-catalyzed reactions of N-acylbenzotriazoles with epoxides affording pseudohalohydrin ester surrogates as single regioisomers.

Chapter 5 focuses on the development of a mild protocol towards the synthesis of O-acylisodipeptides from serine and threonine amino acid residues using benzotriazole methodology. This mild protocol is visited again in Chapter 6, where it was employed in peptide ligations via large transition states. Cysteine-free microwave-assisted chemical ligations are examined in this chapter using O-acylpeptides from serine residues.
Chapter 7 summarizes the achievements together with the conclusions.
CHAPTER 1
GENERAL INTRODUCTION

1H-Benzotriazole\textsuperscript{1,9} is an inexpensive, stable synthetic auxiliary that is soluble in many organic solvents, sparingly soluble in water and is highly soluble in basic solutions. Since it is an acid of appreciable strength (pKa = 8.2), and a base (pKa = 1.6) benzotriazole can be readily removed from the reaction mixture by simply washing with a base or an acid.\textsuperscript{1-3}

Benzotriazole displays the characteristics of an ideal synthetic auxiliary and possesses both electron-donor and electron-acceptor properties.\textsuperscript{1-3} The application of benzotriazole in organic chemistry will be highlighted in many aspects in this thesis (Scheme 1-1).

Scheme 1-1. Diverse application of benzotriazole highlighted in this thesis.
The chemistry of azides started with the preparation of the first organic azide, phenyl azide, the discovery of hydrazoic acid and Curtius rearrangement reported in 1890.\textsuperscript{10-15} Since then, several syntheses and applications of organic azides have been developed. These valuable intermediates have been used in the synthesis of various nitrogen-containing heterocycles, in peptide chemistry, in azidonucleosides for the treatment of AIDS and for the preparation of bioconjugates via Staudinger ligation.\textsuperscript{16-18}

In the course of investigations on the use of benzotriazole, Chapter 2 demonstrates the use of benzotriazole in the synthesis of a novel diazotransfer reagent, benzotriazol-1-yl-sulfonyl azide, and the reagent’s utility in diazotransfer reactions. Chapter 3 examines the azido protecting group tolerance in various acylations using azidoacylbenzotriazoles.

The use of heterocyclic acylazoles as acylating agents offers many advantages.\textsuperscript{3} In particular, N-acylbenzotriazoles are useful synthetic auxiliaries since they can be installed and removed readily. Benzotriazole is comparable in many ways to a halogen substituent because of its leaving group ability, but regarded as a tame halogen substituent in view of its stability. Benzotriazole can be acylated at N-1 or N-2 position. In solution, it is substituted at the N-1 position.

\textit{N-}Acylbenzotriazoles are: (i) solids (highly crystalline compounds), (ii) soluble in organic solvents and can be used in aqueous media (relatively stable to hydrolysis), (iii) non-hygroscopic making them easy to handle and store, (iv) compatible with a wide range of functionality, (v) chirally stable for long periods and (vi) efficient neutral acylating agents, where benzotriazole can be easily recovered and recycled.\textsuperscript{3,5,19} More importantly, they can be prepared directly from RCO\textsubscript{2}H in near quantitative yields.
(Scheme 1-2). Chapters 3-6 describe the synthesis and reactions of N-acylbenzotriazoles demonstrating their application in microwave synthesis, azide and peptide chemistry.

![Scheme 1-2. Synthesis of N-acylbenzotriazole.](image)

The use of microwave (MW) activation is of great interest as a non-conventional energy source accelerating a wide range of organic reactions.\textsuperscript{20,21-23} It is described as a green eco-friendly approach since many reactions are run under solvent-free conditions, with reduced reaction times, enhanced conversions and sometimes selectivity. The number of publications using microwave irradiation in organic synthesis is growing exponentially (Figure 1-1).\textsuperscript{20,21-23}
Most organic reactions have been performed using conventional heat transfer equipment such as oil baths, sand baths and heating jackets. However, these methods are rather slow and a temperature gradient can develop within the sample. In addition, local overheating may cause decomposition of the product, substrate or reagent. In contrast, in microwave dielectric heating, the microwave energy is introduced into the chemical reactor remotely. The microwave radiation passes through the walls of the vessel and heats only the reactants and solvent, not the reaction vessel itself. Chapters 4 and 6 demonstrate the application of microwave in the synthesis of pseudohalohydrin surrogates and in chemical ligations of peptides.

Scientists have long sought to understand how the structure of a protein molecule gives rise to its functional properties. Thus, the chemical synthesis of peptides and proteins is of great importance. A mild protocol towards the synthesis of chirally pure O-acyldipeptides is described in Chapter 5. This mild protocol is utilized in Chapter 6 for the ‘traceless’ chemical ligation of O-acylpeptides using serine.

Figure 1-1. Number of publication with microwave irradiation.\textsuperscript{24,25}
Chapter 7 presents a summary of achievements together with conclusions.
CHAPTER 2
BENZOTRIAZOL-1-YL-SULFONYL AZIDE

2.1 Introduction

Organic azides,\textsuperscript{11,12,17,26} discovered by Peter Grieß more than 140 years ago, play an important role at the interface between chemistry, biology, medicine, and material science. These nitrogen-rich molecules have been utilized: (i) as building blocks,\textsuperscript{12,27,28} exemplified by the synthesis of natural products,\textsuperscript{29-33} (ii) in photoaffinity labeling,\textsuperscript{34-41} (iii) as drugs, such as anti-HIV medication (3'-azidothymidine or zidovudine, AZT),\textsuperscript{42} and (iv) as masked amines as in the synthesis of oseltamivir phosphate Tamiflu.\textsuperscript{12,43}

Aliphatic azides\textsuperscript{12,43} can be prepared by classical nucleophilic displacement (S\textsubscript{N}2 type) with a highly nucleophilic azide anion.\textsuperscript{44,45} This is commonly performed with sodium azide (or other alkali azides, tetraalkylammonium azides, polymer-bound azides or highly explosive silver azides) that displaces a good leaving group (e.g. a halide, carboxylate, sulfonate, mesylate, nosylate or triflates) in a polar aprotic solvent. However such reactions at sp\textsuperscript{3} carbon may cause inversion, epimerization,\textsuperscript{46} or concurrent elimination and solvents such as DMF and DMSO can hinder isolation of the azide product.

Organic azides can also be prepared by: (i) reactions of aryldiazonium salts with inorganic azides;\textsuperscript{47} (ii) catalyzed cross-coupling of between sodium azide and aryl and vinyl boronic acids,\textsuperscript{48} (iii) catalyzed coupling reaction of aryl halides and vinyl halides with sodium azide,\textsuperscript{49} or (iv) diazo transfer of primary amines.

Preparation of azides from amines by diazo transfer\textsuperscript{50} avoids epimerization, inversion, and elimination. The conversion occurs efficiently in the presence of a catalytic amount of divalent metal ion that is believed to complex with the amine before attacking the electrophilic azide.\textsuperscript{50-53}

An ideal diazotransfer reagent should be crystalline (for ease of purification, handling\textsuperscript{54} and stability), nonexplosive, easily prepared, and of general applicability for diazotransfer. \textit{p}-Tosyl azide (2.1), the classical diazotransfer reagent, melts at 21-22 °C,\textsuperscript{55} and requires relatively harsh conditions that limit its use.\textsuperscript{56} Suggested replacements reported in the literature include: (i) mesyl azide (2.2),\textsuperscript{57} an oil needing distillation at 56 °C (0.5 mm. Hg); (ii) polystyrene-supported benzenesulfonyl azide (2.3),\textsuperscript{58} a safe-to-handle but insoluble resin; (iii) oligomer-bound benzenesulfonyl azide (2.4),\textsuperscript{59} which is insoluble in most organic solvents, lacks long-term stability, and needs to be utilized within 1-2 weeks; (iv) imidazole-1-sulfonyl azide (2.5),\textsuperscript{50,54} a colorless oil used as crystalline hydrochloride salt; and (v) the most commonly used “diazo-transfer reagent”, trifluoromethanesulfonyl azide (TfN\textsubscript{3}, 2.6),\textsuperscript{43,50,53,54,56} prepared from sodium azide and trifluoromethanesulfonic anhydride, which has a poor shelf life and must be used in situ as a solution because of its explosive nature (Scheme 2-1).\textsuperscript{48} Thus, the synthesis of an improved diazotransfer reagent is of considerable interest. The preparation of benzotriazol-1-sulfonyl azide,\textit{2.7}, as an improved, novel diazo donor is described (Scheme 2-2).\textsuperscript{51,52}
Scheme 2-1. Diazotransfer reagents.

2.2 Results and Discussion

2.2.1 Preparation and Characterization of Benzotriazol-1-yl-sulfonyl Azide 2.7

The reaction of chlorosulfonyl azide, prepared in situ from sodium azide and sulfuryl chloride, with benzotriazole (2 equiv) and pyridine (1 equiv) in MeCN gave benzotriazol-1-yl-sulfonyl azide 2.7 (70%, obtained after aqueous workup) as a white crystalline solid (mp 85.3-88.3 °C) requiring no further purification (Scheme 2-2). These conditions proved to be optimal. Initial investigation of the effect of various parameters toward reaction optimization has shown that in the absence of pyridine, the reaction needed 2.5 days and gave 45% of 2.7. The use of other bases and solvent systems resulted in either partial or complete decomposition products (Table 2-1).

Scheme 2-2. Synthesis of benzotriazol-1-yl-sulfonyl azide 2.7.
Table 2-1. Optimization of reaction conditions for 2.7.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>Yield of 2.7 (%)</th>
<th>Decomposition&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetonitrile, BtH (2 equiv), 2.5 d</td>
<td>45</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Acetone, BtH (2 equiv), 12h</td>
<td>-</td>
<td>Decomposed</td>
</tr>
<tr>
<td>3</td>
<td>Acetonitrile, BtH (2 equiv), Et₃N (2 equiv), 4h</td>
<td>-</td>
<td>Decomposed</td>
</tr>
<tr>
<td>4</td>
<td>Acetonitrile, BtH (2 equiv), Et₃N (1 equiv), 4h</td>
<td>-</td>
<td>Decomposed</td>
</tr>
<tr>
<td>5</td>
<td>Acetonitrile, BtH (2 equiv), py (1 equiv), 12h</td>
<td>70</td>
<td>None</td>
</tr>
</tbody>
</table>

<sup>a</sup>Determined by TLC and <sup>1</sup>H NMR.

Reagent 2.7 as a dry solid has a long shelf life at room temperature and can be utilized several months after its preparation. <sup>1</sup>H NMR on 2.7 after storage in a closed clear glass vial at room temperature for a period of 6 weeks and even for several months, revealed no decomposition thus demonstrating its longevity. A moderate exothermic decomposition was noted when the hammer test was performed. Although no trouble was experienced when utilizing the reagent, appropriate safety measures must be taken at all times with high-energy azides.¹²,⁴³,⁵¹,⁵²,⁶⁰ [An acidic workup should be avoided because of the possible formation of explosive hydrazoic acid resulting from trace amounts of residual sodium azide. Procedure misuse has resulted in an explosion by a visiting research scholar (in our laboratories)].

Reagent 2.7 is soluble in many organic solvents as well as in partially aqueous media (e.g., MeCN, CH₂Cl₂, MeOH, EtOAc, MeCN/H₂O (1:1)).

The detailed molecular structure of benzotriazol-1-yl-sulfonyl azide 2.7 was established by X-ray diffraction analysis (Figure 2-1). Thermal properties of 2.7 were studied by TGA and DSC. Thermogravimetric analysis (TGA) shows that 64 wt% of 2.7 is lost around 112 °C (Figure 2-2). Differential scanning calorimetry (DSC) shows that
2.7 is thermally stable below 95 °C, melting and resolidifying (see Figure 2-3, which shows 2 cycles of heating to 95 °C and cooling to -100 °C). The heats of fusion (166.7 J/g for cycle 1 and 163.8 J/g for cycle 2) and heats of freezing (114.1 J/g for cycle 1 and 101.4 J/g for cycle 2) show that there is negligible material loss.

![Figure 2-1. X-ray of benzotriazol-1-sulfonyl azide 2.7.](image1)

![Figure 2-2. Thermogravimetric analysis of benzotriazol-1-sulfonyl azide 2.7.](image2)
Figure 2-3. Differential scanning calorimetry of benzotriazol-1-sulfonyl azide 2.7: (a) cycle 1 and (b) cycle 2.
2.2.2 Preparation of Azides from Primary Amines

Benzotriazol-1-yl-sulfonyl azide 2.7 was found to be an efficient diazo donor reagent with various aliphatic and aromatic amines. BtSO$_2$N$_3$ 2.7 converted amine compounds 2.8a-f into the corresponding azides 2.9a-f (in 47-85% yields), without requiring a base. In a typical reaction, benzotriazol-1-yl-sulfonyl azide 2.7 reacted with an amine in methanol at room temperature in the presence of copper(II) sulfate (Table 2-2). It is noteworthy that the reaction also works in absence of catalyst; for example, the reaction of 2.7 with $p$-methoxyphenylamine 2.8a without catalyst gave $p$-methoxyphenyl azide 2.9a (57%) after 24 h.
Table 2-2. Synthesis of Azides from Primary Amines Utilizing 2.7.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate 2.8</th>
<th>Product 2.9</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeO-(\text{NH}_2)</td>
<td>2.8a</td>
<td>2.9a</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Br-(\text{NH}_2)</td>
<td>2.8b</td>
<td>2.9b</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>(\text{NO}_2)-(\text{NH}_2)</td>
<td>2.8c</td>
<td>2.9c</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>(\text{NH}_2)</td>
<td>2.8d</td>
<td>2.9d</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>(\text{NH}_2)</td>
<td>2.8e</td>
<td>2.9e</td>
<td>12</td>
</tr>
<tr>
<td>6(^a)</td>
<td>(\text{HCl})</td>
<td>2.8f</td>
<td>2.9f</td>
<td>7</td>
</tr>
</tbody>
</table>

\(^a\) Et\(_3\)N (1 equiv) was required.

2.2.3 Preparation of \(\alpha\)-Azido Acids

Reagent 2.7 converted \(\alpha\)-amino acids 2.10 into their corresponding \(\alpha\)-azido acids 2.11 without any observable racemization. This mild transformation was achieved when 2.7 was reacted with free amino acids 2.10a-e, (2.10a + 2.10a') at 20 °C in aqueous CH\(_3\)CN in the presence of Et\(_3\)N and catalytic amount of copper(II) sulfate to give corresponding \(\alpha\)-azido acids 2.11a-e, (2.11a + 2.11a') in good yield (60-87%)(Table 2-3).
Table 2-3. Synthesis of α-Azido Acids 2.11a-e,(2.11a + 2.11a').

\[
\begin{array}{ccc}
\text{entry} & \text{substrate, 2.10} & \text{Product, 2.11} & \text{yield (\%)} \\
1 & L-Phe, 2.10a & 2.11a & 65 \\
2 & L-Leu, 2.10b & 2.11b & 65 \\
3 & L-Ala, 2.10c & 2.11c & 60 \\
4 & DL-Phe, (2.10a+2.10a') & (2.11a+2.11a') & 65 \\
5 & L-Val, 2.10d & 2.11d & 87 \\
6 & (L-Cys), 2.10e & 2.11e & 77 \\
\end{array}
\]

HPLC analysis [chirobiotic T column (250 mm × 4.6 mm), detection at 254 nm, flow rate 0.5 mL/min, MeOH] on 2.11a (single peak, retention time 7.2 min) and (2.11a + 2.11a') (two equal peaks, retention times 6.7 and 7.2 min) confirmed that product 2.11a is enantiomerically pure.

The mechanistic details of this interconversion are not well established. A proposed mechanism for diazotransfer involves a tetrazole intermediate and incorporates a divalent metal ion that complexes with the amine. The amine in this complex is then thought to attack the electrophilic azide.\(^{50}\)

2.2.4 Preparation of Diazo Compounds

Diazo compounds are versatile synthetic building blocks\(^{61}\) with rich transition-metal-catalyzed chemistry.\(^{62-64}\) Thus, carbene insertion into C-H bonds has increased in
importance since its discovery by Meerwein and Werner.\textsuperscript{65} We utilized benzotriazol-1-yl-sulfonyl azide 2.7 in the preparation of diazo compounds 2.13a,b containing activated methylene groups, and the results are summarized in Table 2-4. In general, yields and reaction times compare favorably with those reported in the literature using the most recent diazotransfer reagent, imidazole-1-sulfonyle azide hydrochloride (2.5.HCl).\textsuperscript{54}

Table 2-4. Synthesis of diazo compounds 2.13 Utilizing 2.7.

<table>
<thead>
<tr>
<th>Product</th>
<th>R</th>
<th>R’</th>
<th>time [lit]\textsuperscript{a} (h)</th>
<th>yield [lit]\textsuperscript{a} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.13a</td>
<td>CN</td>
<td>CO\textsubscript{2}Et</td>
<td>12[9]</td>
<td>65[61]</td>
</tr>
<tr>
<td>2.13b</td>
<td>SO\textsubscript{2}Ph</td>
<td>CO\textsubscript{2}Et</td>
<td>14[48]</td>
<td>56[-]\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Reaction with imidazole-1-sulfonyle azide (2.5). \textsuperscript{b}No reaction was observed using imidazole-1-sulfonyle azide (2.5.HCl).\textsuperscript{16}

I believe that the formation of diazo compounds from activated CH\textsubscript{2} groups via diazotransfer occurs through nucleophilic attack of an intermediate enolate 2.14 onto benzotriazol-1-ylsulfonyl azide 2.7, followed by protonation to give intermediate 2.15. In the presence of base, intermediate 2.16 is formed, which fragments to the desired diazo product 2.13 (Scheme 2-3).

Scheme 2-3. Proposed mechanism for the formation of diazo compounds 2.13.
2.3 Conclusions

Benzotriazol-1-yl-sulfonyl azide 2.7 is a new, thermally stable, and safe to handle crystalline diazotransfer reagent with a long shelf life and high solubility in organic and aqueous solvents, which allows convenient and efficient synthesis of a wide range of azides and diazo compounds.

2.4 Experimental

2.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. The NMR spectra were recorded in CDCl₃, DMSO-d₆ with TMS for ¹H (300 MHz) and ¹³C (75 MHz) as an internal reference. Silica was used as the stationary phase for column chromatography. Phosphomolybdic acid was used to detect compounds that were not UV active.

2.4.2 Preparation of Benzotriazol-1-yl-Sulfonyl Azide 2.7

Sulfuryl chloride (6.23 mL, 0.08 mol) was added portion-wise to a suspension of NaN₃ (5 g, 0.08 mol) in MeCN (25 mL) at 0 °C, and the mixture was stirred overnight at room temperature. Benzotriazole (18.35 g, 0.15 mol) was dissolved in pyridine (6.46 mL, 0.08 mol) and acetonitrile (10 mL), and the solution was added to the suspension at 0 °C. The resulting suspension was stirred for 10 h at room temperature. The unreacted solid was filtered, and the yellow-orange filtrate evaporated and diluted with ethyl acetate (~30 mL). The organic layer was washed with a saturated solution of sodium carbonate to remove excess benzotriazole, dried over anhydrous MgSO₄, and filtered. The filtrate was concentrated under reduced pressure to give a light brown solid that was later used directly. Recrystallization using hexane/EtOAc 7:3 gave a white crystalline solid 2.7 (12.5 g, 70%); mp 85.3 - 88.3 °C; ¹H NMR (300 MHz, CDCl₃): δ 8.17
(d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.72 (t, J = 7.2 Hz, 1H), 7.57 (t, J = 7.2 Hz, 1H); \(^{13}\text{C} \text{NMR} \ (75 \text{ MHz, } \text{CDCl}_3): \delta 145.3, 131.6, 126.9, 121.3, 112.1. \text{ Anal. Calcd for } \text{C}_6\text{H}_4\text{N}_6\text{O}_2\text{S: C, 32.14; H, 1.80; N, 37.48. Found: C, 32.18; H, 1.74; N, 37.27. NOTE: } ^1\text{H} \text{ NMR on reagent 2.7 after storage in a closed clear glass vial at room temperature for a period of 6 weeks, showing no decomposition. }

A moderate exothermic decomposition was noted when the hammer test was performed on 2.7. [Caution: Although no trouble was experienced when utilizing sodium azide, the in situ generated chlorosulfonyl azide, or any of the synthesized organic azides, appropriate safety measures must always be taken at all times because azides are often found to be high energy compounds.\(^{10,12,51,52,60}\)]

2.4.3 General Procedure for the Preparation of Azides 2.9a-f

Benzotriazol-1-yl-sulfonyl azide 2.7 (0.50 g, 2.23 mmol) was added to the amine (2.23 mmol) in MeOH (20 mL). CuSO\(_4\).5H\(_2\)O (2.5 mg, 10 \(\mu\)mol) was then added, and the mixture was stirred at room temperature for the specified time (Table 2-1). The mixture was concentrated, diluted with water (20 mL), and extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO\(_4\), filtered, and concentrated. Purification was performed via flash column chromatography to give the corresponding azide 2.9.

1-Azido-4-methoxybenzene (2.9a). 4-Methoxyaniline (275 mg, 2.23 mmol) was treated according to the above procedure [flash chromatography (hexane/EtOAc, 9:1)] to give 1-azido-4-methoxybenzene 2.9a as a pale yellow oil (233 mg, 70%); \(^1\text{H} \text{ NMR} \ (300 \text{ MHz, } \text{CDCl}_3): \delta 6.98 - 6.87 \text{ (m, 4H), 3.80 \text{ (s, 3H); } ^{13}\text{C} \text{NMR} \ (75 \text{ MHz, } \text{CDCl}_3): \delta 156.9, 132.3, 120.0, 115.1, 55.6.}
1-Azido-4-bromobenzene (2.9b). 4-Bromoaniline (384 mg, 2.23 mmol) was treated according to the above procedure [flash chromatography (hexane)] to give 1-azido-4-bromobenzene 2.9b as a pale yellow oil (332 mg, 75%); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.47 - 7.44 (m, 2H), 6.92 - 6.88 (m, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 139.2, 132.7, 120.6, 117.7.

1-Azido-3-nitrobenzene (2.9c). 3-Nitroaniline (308 mg, 2.23 mmol) was treated according to the above procedure [flash chromatography (hexane/EtOAc, 9.8:0.2)] to give 1-azido-3-nitrobenzene 2.9c as a yellow solid (209 mg, 57%); mp 53.1 - 54.5 °C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.01 - 7.98 (m, 1H), 7.89 (t, $J = 2.1$ Hz, 1H), 7.54 (t, $J = 8.1$ Hz, 1H), 7.35 (dd, $J = 1.8$, 7.5 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 141.9, 130.6, 124.9, 119.7, 114.1.

3-Azidopyridine (2.9d). Pyridin-3-amine (210 mg, 2.23 mmol) was treated according to the above procedure [flash chromatography (hexane)] to give 3-azidopyridine 2.9d as a pale yellow oil (126 mg, 47%); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.41 - 8.35 (m, 2H), 7.38 - 7.27 (m, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 145.8, 141.1, 137.0, 125.8, 124.0.

(2-Azidoethyl)benzene (2.9e). 2-Phenylethanamine (270 mg, 2.23 mmol) was treated according to the above procedure [chromatography(hexane)] to give (2-azidoethyl)benzene 2.9e as colorless oil (279 mg, 85%); $^1$H NMR (300 MHz,CDCl$_3$): $\delta$ 7.38 - 7.23(m, 5H), 3.52 (t, $J = 7.4$ Hz, 2H), 2.92 (t, $J = 7.2$ Hz, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 137.9, 128.6, 128.5, 126.7, 52.4, 35.3.

(1S,2S,3S,5R)-3-(Azidomethyl)-2,6,6-trimethylbicyclo[3.1.1]-heptanes (2.9f). (+)-3-Pinanemethylamine hydrochloride (454 mg, 2.23 mmol) and triethyl amine (1 equiv)
were treated according to the above procedure [flash chromatography (hexane)] to give 
\((1S,2S,3S,5R)-3-(azidomethyl)-2,6,6\text{-trimethylbicyclo}[3.1.1]\text{heptanes 2.9f} as a yellow 
oil (220 mg, 51%); \([\alpha]_D^{20} +6.9 (c 1.5, \text{CHCl}_3); ^1\text{H NMR} (300 MHz, \text{CDCl}_3): \delta 3.32 (dd, J = 5.7, 11.7 Hz, 1H), 3.18 (dd, J = 7.5, 11.7 Hz, 1H), 2.35 - 2.26 (m, 1H), 2.22 - 2.12 (m, 1H), 2.04 - 1.89 (m, 2H), 1.81 - 1.68 (m, 2H), 1.59 - 1.51 (m, 1H), 1.20 (s, 3H), 1.07 (dd, J = 1.5, 7.2 Hz, 3H), 1.01 (s, 3H); ^13\text{C NMR}(75 MHz, \text{CDCl}_3): \delta 59.6, 47.7, 41.3, 40.5, 38.8, 36.5, 33.5, 32.2, 27.9, 22.9, 21.6; \text{HRMS m/z for } C_{11}H_{20}N [M-N_2+H]^+ \text{ calcd} 166.1590, \text{found} 166.1593.

2.2.4 General Procedure for the Preparation of \(\alpha\)-Azido Acids 2.11a-e

(2.11a+2.11a’)

Each amino acid (4.46 mmol, 2 equiv) was dissolved in MeCN/H2O (1:1, 20 mL) 
and triethyl amine (0.78 mL, 5.58 mmol). Benzotriazol-1-yl-sulfonyl azide 2.7 (0.50 g, 
2.23 mmol) was added to the solution followed by CuSO\(_4\).5H\(_2\)O(2.5 mg, 10 \(\mu\)mol), and 
the mixture stirred at room temperature for 12 h. The mixture was acidified with 6N HCl, 
concentrated, diluted with ethyl acetate and washed with 6N HCl to remove 
benzotriazole. The organic layer was collected, dried over anhydrous MgSO\(_4\), filtered, 
and concentrated to give the corresponding \(\alpha\)-azido acid 2.11.

(2S)-2-Azido-3-phenylproanoic Acid (2.11a). L-Phenylalanine (736 mg, 4.46 
mmol) was treated according to the above procedure to give (2S)-2-azido-3-
phenylproanoic acid 2.11a as a pale yellow oil (278 mg, 65%); ^1\text{H NMR} (300 MHz, 
\text{CDCl}_3): \delta 9.90 (s, 1H), 7.38-7.27 (m, 5H), 4.15 (dd, J = 4.8, 9.0 Hz, 1H), 3.22 (d, J = 5.1 
Hz, 1H), 3.08 - 3.03 (m, 1H); ^13\text{C NMR} (75 MHz, \text{CDCl}_3): \delta 175.7, 135.6, 129.2, 128.8, 
127.4, 63.1, 37.5.
(S)-2-Azido-4-methylpentanoic Acid (2.11b). L-Leucine (586 mg, 4.46 mmol) was treated according to the above procedure to give (S)-2-azido-4-methylpentanoic acid 2.11b as a pale yellow oil (228 mg, 65%); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 8.95 (s, 1H), 3.88 (dd, \(J = 5.7, 9.0\) Hz, 1H), 1.89 - 1.77 (m, 1H), 1.76 - 1.64 (m, 2H), 1.00 - 0.96 (m, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 177.0, 60.0, 39.8, 25.0, 22.7, 21.4.

(S)-2-Azidopropanoic Acid (2.11c). L-Alanine (396 mg, 4.46 mmol) was treated according to the above procedure to give (S)-2-azidopropanoic acid 2.11c as a yellow oil (154 mg, 60%); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 4.56 (s, 1H), 4.03 (q, \(J = 7.1\) Hz, 1H), 1.54 (d, \(J = 7.2\) Hz, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 177.0, 57.0, 16.6.

2-Azido-3-phenylpropanoic Acid (2.11a+2.11a'). D,L-Phenylalanine (736 mg, 4.46 mmol) was treated according to the above procedure to give 2-azido-3-phenylpropanoic acid (2.11a+2.11a') as a pale yellow oil (278 mg, 65%); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.38 - 7.25 (m, 5H), 4.17 (dd, \(J = 5.0, 8.9\) Hz, 1H), 3.25 (dd, \(J = 5.0, 14.0\) Hz, 1H), 3.05 (dd, \(J = 8.9, 14.0\) Hz, 1H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 175.6, 135.5, 129.2, 128.8, 127.4, 63.0, 37.5.

(S)-2-Azido-3-methylbutanoic Acid (2.11d). L-Valine (523 mg, 4.46 mmol) was treated according to the above procedure to give (S)-2-azido-3-methylbutanoic acid 2.11d as a yellow oil (277 mg, 87%); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 11.8 (s, 1H), 3.76 (d, \(J = 5.7\) Hz, 1H), 2.27 - 2.17 (m, 1H), 0.99 (d, \(J = 6.9\) Hz, 3H), 1.04 (d, \(J = 6.9\) Hz, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 176.5, 67.8, 30.8, 19.3, 17.6.

2-Azido-3-(((\(\alpha\))-2-azido-2-carboxyethyl)disulfanyl)propanoic Acid (2.11e). L-Cystine (268 mg, 1.12 mmol) was treated according to the above procedure to give 2-azido-3-(((\(\alpha\))-2-azido-2-carboxyethyl)disulfanyl)propanoic acid 2.11e as an orange-
brown oil (252 mg, 77%); $[\alpha]_D^{20} \cdot 37.7$ (c 1.3, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.59 (bs, 2H), 4.43 (dd, $J = 5.0$, 7.1 Hz, 2H), 3.32 (dd, $J = 5.0$, 14.0 Hz, 2H), 3.03 (dd, $J = 7.2$, 13.8 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 174.4, 60.8, 39.9; HRMS m/z for C$_6$H$_8$O$_4$N$_6$S$_2$Na [M+Na]$^+$ calcd 314.9941, found 314.9940.

2.2.5 General Procedure for Preparation of Diazo Compounds 2.13a-b

Pyridine (3.5 equiv) was added to the substrate 2.12 (1 equiv) in MeCN (15 mL) and was stirred for 30 minutes before adding benzotriazol-1-yl-sulfonyl azide 2.7 (0.50 g, 2.23 mmol). The mixture was stirred for the specified time (Table 2-3), concentrated under reduced pressure, acidified with 4N hydrochloric acid and extracted with ethyl acetate (50 mL). The extracts were dried over anhyd MgSO$_4$, filtered and concentrated under reduced pressure. Purification was performed via flash column chromatography to give the corresponding diazo compound 2.13.

Ethyl Cyanodiazooacetate (2.13a). Ethyl cyanoacetate 2.12a (0.2 mL, 2.23 mmol) was treated according to the above procedure, using pyridine (0.62 mL, 7.81 mmol) [flash chromatography (hexane:EtOAc, 9.8:0.2)], to give ethyl cyanodiazooacetate 2.13a as a yellow oil (0.20 g, 65%); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 4.35 (q, $J = 7.2$ Hz, 2H), 1.34 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 107.3, 63.5, 29.7, 14.3.

Ethyl 2-diazo-2-(phenylsulfonyl)acetate (2.13b). Ethyl 2-(phenylsulfonyl)acetate 2.12b (0.51 mg, 2.23 mmol) was treated according to the above procedure, using pyridine (0.62 mL, 7.81 mmol) [flash chromatography (hexane:EtOAc, gradient)], to give ethyl 2-diazo-2-(phenylsulfonyl)acetate 2.13b as an orange solid (0.32 g, 56%); mp 52.0-54.0 °C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.04 - 8.00 (m, 2H), 7.69 - 7.63 (m, 1H), 7.58 - 7.53 (m, 2H), 4.21 (q, $J = 6.9$ Hz, 2 H), 1.24 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 159.5, 141.6, 134.0, 129.1, 127.8, 62.3, 14.1.
CHAPTER 3
BENZOTRIAZOL-1-YL-SULFONYL AZIDE FOR PREPARATION OF
AZIDOACYLBENZOTRIAZOLES

3.1 Introduction

Chemical synthesis is a powerful method for creating complex molecules with tailored biological and physical properties for drug discovery, engineering, nanotechnology, and the study of biological processes and is based on the concourse of reagents and catalysts to attain the clean formation of new bonds. Suitable protecting groups are also often required to avoid the formation of undesired bonds and side reactions.

Although, the necessity to temporarily mask a functional group was first recognized by Emil Fischer, it was not until 1932 that Bergmann and Zervas reported the first “modern” protecting group, the benzyloxy carbonyl (Z).

The use of protecting groups influences the length, efficiency and complexity of the synthesis and are often responsible for its success or failure. In choosing a protecting group, the following characteristics should be kept in mind: (i) should be easily introduced into the functional group under mild conditions, in a selective manner and in high yield (giving no additional stereocenters); (ii) should be stable to a broad range of reaction conditions, with a stabilizing effect on the molecule and should suppress racemization or epimerization; and (iii) should be easy to remove at the end of the synthetic process or when the functional group requires manipulation. Additionally, other protecting groups present in the molecule and unprotected functionalities should not be affected by the cleavage conditions.

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Orthogonality, the concept of having two or more protecting groups that belong to independent classes and removed by distinct mechanisms, is of consideration in designing the retrosynthesis of a main target. The differential protection of functional groups of comparable reactivity is a major challenge with conventional protecting-group strategies, namely orthogonal protection and modulated lability. In particular, the development of effective protective schemes for polyfunctional molecules is not trivial.

Chiral, nonracemic natural products, including amino acids, have long been utilized as building blocks for organic synthesis. Amino groups in peptide syntheses need protection, which is required to prevent polymerization of the amino acid once it is activated.

Azides have found application as “protected” amines for sensitive substrates such as oligosaccharides, aminoglycoside antibiotics, glycosoaminoglycans, and peptidonucleic acids (PNA) and in solid-phase peptide synthesis.

In α-azido acyl groups the azide both masks the amine functionality and strongly activates the carboxyl moiety, thus facilitating the formation of peptide bonds. The small size of the azide unit in comparison to, e.g., Boc or Fmoc may be advantageous in the coupling of hindered compounds. The azide group is stable under both acidic and basic conditions and toward osmium and ruthenium-catalyzed dihydroxylation or alkylation.

Choice of activation for an α-azido acid is important: (i) acyl halides tend to be over-activated and require base for neutralizing the hydrogen halide formed; (ii)
acid anhydrides easily form imides with ammonia and primary amines; (iii) esters are frequently under-activated and require basic catalysts and/or high pressure.\textsuperscript{1,87}

The use of acylazoles as acylating agents, reported by H. A. Staab in the 1960’s, offer several advantages.\textsuperscript{3} In particular, benzotriazole is an inexpensive, stable synthetic auxiliary that is soluble in many organic solvents (e.g. ethanol, benzene, toluene, chloroform, and DMF). It is sparingly soluble in water but highly soluble in basic solutions.\textsuperscript{3}

It is noteworthy that for a synthetic auxiliary group to be useful, it should demonstrate the following main characteristics: (i) be easy to remove at the end of the synthetic sequence (it is an added advantage if it can be recovered and used again); (ii) be able to be introduced readily at the beginning of the sequence; and (iii) should be stable during various synthetic operations, and, if possible, exert an activating influence on other parts of the molecule. Benzotriazole displays all of these characteristics to a high degree and possesses both electron-donor and electron-acceptor properties.\textsuperscript{1-3}

\textit{N}-Acylbenzotriazoles are efficient neutral acylating agents and form amide bonds at ambient temperatures with unprotected amino acids in aqueous/organic solvents resisting side reactions in the preparation of \textit{N}-terminal protected peptides.\textsuperscript{3,5,19} Thus, \textit{N}-(protected-\textalpha-\text{aminoacyl})benzotriazoles have enabled fast preparations of biologically relevant peptides and peptide conjugates in high yields and purity, under mild reaction conditions, with full retention of the original chirality.\textsuperscript{89}

Herein, the functional group tolerance of azido, as a protecting group, in \textit{N}-, \textit{O}-, \textit{S}- and \textit{C}- acylations is examined. The synthesis and the reliability of \textit{N}-(\textalpha-\text{azidoacyl})benzotriazoles as acylating agents is reported.
3.2 Results and Discussion

3.2.1 α-Azido Acids

Reagent 2.7 converted α-amino acids 2.10 into their corresponding α-azido acids 2.11 without any racemization at 20 °C in aqueous CH$_3$CN in the presence of Et$_3$N and copper(II) sulfate in good yields (60-87%)(chapter 2, Table 2-3).$^{51,52}$

3.2.2 Preparation of N-(α-Azidoacyl)benzotriazoles

N-(α-Azidoacyl)benzotriazoles 3.1a-d, (3.1a+3.1') were prepared in good yields (65-98%) by the treatment of the corresponding α-azido acids 2.11 with 1.2 equiv of thionyl chloride and 2 equiv of benzotriazole in methylene chloride (Table 3-1).

Table 3-1. Synthesis of N-(α-Azidoacyl)benzotriazoles 3.1 from α-Azido Acids 2.11.

<table>
<thead>
<tr>
<th>α-azido acids, 2.11</th>
<th>product 3.1</th>
<th>yield (%)</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_3$-L-Phe, 2.11a</td>
<td>3.1a</td>
<td>98</td>
<td>66.1-68.3</td>
</tr>
<tr>
<td>N$_3$-L-Leu, 2.11b</td>
<td>3.1b</td>
<td>96</td>
<td>47.3-49.0</td>
</tr>
<tr>
<td>N$_3$-L-Ala, 2.11c</td>
<td>3.1c</td>
<td>65</td>
<td>77.0-77.9</td>
</tr>
<tr>
<td>N$_3$-DL-Phe, (2.11a+2.11a')</td>
<td>(3.1a+3.1a')</td>
<td>98</td>
<td>Oil</td>
</tr>
<tr>
<td>N$_3$-L-Val, 2.11d</td>
<td>3.1d</td>
<td>72</td>
<td>Oil</td>
</tr>
</tbody>
</table>

3.2.3 N-Acylation

The reliability of N-(α-azidoacyl)benzotriazoles as acylating agents was tested on a variety of N-nucleophiles 3.2 to provide amides 3.3a-k in 62-87% yields (Table 3-2). The azide demonstrated its functional group tolerance as a protecting group in N-
acylation of aromatic, aliphatic amines and free amino acids (including free cysteine), nucleobases, nucleosides, and sulfonamides. HPLC analysis [chiracel OD-H column (250 mm × 4.6 mm), detection at 254 nm, flow rate 0.5 mL/min, hexane/isopropyl alcohol (90:10)] on 3.3a (single peak, retention time 50.8 min) and 3.3g (two equal peaks, retention times 47.6 and 51.4 min) confirmed that product 3.3a is enantiomerically pure (Figure 3-1); this result was further confirmed by a co-injection. Entries 9 and 10 (Table 3-2) were performed without added base with the objective of forming S-acylated products according to a reported literature method by our group where S-acylation was performed on aryl N-acylbenzotriazoles.90 As expected S-acylation occurs first giving the thioester product, followed by S- to N-shift to provide the amide linkage. Interestingly, in this case ligation occurs spontaneously providing the N-acylated dipeptides containing free thiol in the absence of base.
Table 3-2. Synthesis of Amides 3.3 from N-(α-Azidoacyl)benzotriazoles 3.1.

\[
\begin{array}{cccccc}
\text{entry} & N-(\alpha\text{-azidoacyl})- & N-Nu, & \text{optimized reaction} & \text{product, 3.3} & \text{yield (\%)} \\
& \text{benzotriazole, 3.1} & 3.2 & \text{conditions} & & [\text{m.p. (\degree C)}] \\
\hline
1 & N_3\text{-}L\text{-PheBt, 3.1a} & p\text{-Anisidine, 3.2a} & \text{MeCN, 12 h} & \text{3.3a} & 79 [79-81] \\
2 & N_3\text{-}L\text{-LeuBt, 3.1b} & p\text{-Anisidine, 3.2a} & \text{MeCN, 12 h} & \text{3.3b} & 84 [43-45] \\
3 & N_3\text{-}L\text{-LeuBt, 3.1b} & \text{Adenine, 3.2b} & \text{DMSO, 8 h} & \text{3.3c} & 68 [187-188] \\
4 & N_3\text{-}L\text{-PheBt, 3.1a} & p\text{-Toluene-sulfonamide, 3.2c} & \text{Et}_3\text{N (1.1 equiv), MeCN, 12 h} & \text{3.3d} & 79 [120-122] \\
\end{array}
\]
Table 3-2. Synthesis of Amides 3.3 from N-(α-Azidoacyl)benzotriazoles 3.1. (Continued)

<table>
<thead>
<tr>
<th>Step</th>
<th>Initial Amide</th>
<th>Pivotal Residue</th>
<th>Conditions</th>
<th>Yield</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-LeuBt, 3.1b</td>
<td>p-Toluene-sulfonamide, 3.2c</td>
<td>Et&lt;sub&gt;3&lt;/sub&gt;N (1.1 equiv), MeCN, 8 h</td>
<td>77%</td>
<td>[55-56]</td>
</tr>
<tr>
<td>6</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- DL-PheBt, (3.1a+3.1a')</td>
<td>Cytidine, 3.2d</td>
<td>DMF, 12 h</td>
<td>62%</td>
<td>[glassy solid]</td>
</tr>
<tr>
<td>7</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- DL-PheBt, (3.1a+3.1a')</td>
<td>p-Anisidine, 3.2a</td>
<td>MeCN, 12 h</td>
<td>79%</td>
<td>[oil]</td>
</tr>
<tr>
<td>8</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-PheBt, 3.1a</td>
<td>L-Leu, 3.2e</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O:MeCN (1:1), Et&lt;sub&gt;3&lt;/sub&gt;N (2.5 equiv)</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-PheL-Leu 3.3h</td>
<td>87%</td>
</tr>
<tr>
<td>9</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-PheBt, 3.1a</td>
<td>L-Cys, 3.2f</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O:MeCN (1:1)</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-PheL-Cys 3.3i</td>
<td>80%</td>
</tr>
<tr>
<td>10</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-LeuBt, 3.1b</td>
<td>L-Cys, 3.2f</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O:MeCN (1:1)</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-LeuL-Cys 3.3j</td>
<td>83%</td>
</tr>
<tr>
<td>11</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-PheBt, 3.1a</td>
<td>L-Ala, 3.2g</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O:MeCN (1:1), Et&lt;sub&gt;3&lt;/sub&gt;N (2.5 equiv)</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;- L-PheL-Ala 3.3k</td>
<td>80%</td>
</tr>
</tbody>
</table>
Figure 3-1. HPLC analysis for compounds 3.3a and 3.3g.

3.2.4 O-, S-, and C- Acylation

Similar to N-acylation, the reactivity of N-(α-azidoacyl)benzotriazoles 3.1a-c, (3.1a + 3.1a') was tested against a variety of O-, S-, and C- nucleophiles (Table 3-3). As expected, azide as a protecting group is well tolerated, and N-(α-azidoacyl)benzotriazoles 3.1 could be used in the acylation of phenols, alcohols (including sterols), thiols, and stabilized enolates (Table 3-3).
Table 3.3. O-, S-, and C-Acylations Utilizing N-\((\alpha\)-Azidoacyl\)benzotriazoles 3.1.

<table>
<thead>
<tr>
<th>entry</th>
<th>N-((\alpha)-azidoacyl)-benzotriazole, 3.1</th>
<th>Nu, 3.4</th>
<th>optimized reaction conditions</th>
<th>product, 3.5</th>
<th>yield (%)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N\textsubscript{3}α L-PheBt, 3.1a</td>
<td>Phenol, 3.4a</td>
<td>K\textsubscript{2}CO\textsubscript{3} (2 equiv), MeCN, 12 h</td>
<td>3.5a</td>
<td>84</td>
<td>[oil]</td>
</tr>
<tr>
<td>2</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Phenol, 3.4a</td>
<td>K\textsubscript{2}CO\textsubscript{3} (2 equiv), MeCN, 24 h</td>
<td>3.5b</td>
<td>63</td>
<td>[oil]</td>
</tr>
<tr>
<td>3</td>
<td>N\textsubscript{3}α DL-PheBt, (3.1a+3.1a\textsuperscript{'})</td>
<td>Cholesterol, 3.4b</td>
<td>THF, DMAP (cat.), 3 h</td>
<td>3.5c</td>
<td>78</td>
<td>[56-58]</td>
</tr>
<tr>
<td>4</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Cholesterol, 3.4b</td>
<td>CHCl\textsubscript{3}, Et\textsubscript{3}N (1 equiv), 72 h</td>
<td>3.5d</td>
<td>61</td>
<td>[81-83]</td>
</tr>
<tr>
<td>5</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>β-Sitosterol, 3.4c</td>
<td>CHCl\textsubscript{3}, Et\textsubscript{3}N (1 equiv), 12 h</td>
<td>3.5e</td>
<td>70</td>
<td>[57-60]</td>
</tr>
<tr>
<td>6</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Thiophenol, 3.4d</td>
<td>CH\textsubscript{3}Cl\textsubscript{2}, py (1 equiv), 18 h</td>
<td>3.5f</td>
<td>72</td>
<td>[oil]</td>
</tr>
<tr>
<td>7</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>2-Mercaptoacetic acid, 3.4e</td>
<td>EtOAc, Et\textsubscript{3}N (2 equiv), 18 h</td>
<td>3.5g</td>
<td>82</td>
<td>[oil]</td>
</tr>
<tr>
<td>8</td>
<td>N\textsubscript{3}α L-PheBt, 3.1a</td>
<td>Methyl 2-mercaptoacetate, 3.4f</td>
<td>EtOAc, Et\textsubscript{3}N (1 equiv), 18 h</td>
<td>3.5h</td>
<td>57</td>
<td>[oil]</td>
</tr>
<tr>
<td>9</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Meldrum’s acid, 3.4g</td>
<td>CH\textsubscript{3}CN, Et\textsubscript{3}N (1 equiv), 14 h</td>
<td>3.5i</td>
<td>72</td>
<td>[oil]</td>
</tr>
<tr>
<td>10</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Cyclohexane-1,3-dione, 3.4h</td>
<td>MeCN, Et\textsubscript{3}N (1 equiv), 14 h</td>
<td>3.5j</td>
<td>95</td>
<td>[oil]</td>
</tr>
<tr>
<td>11</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Ethyl 2-cyanoacetate, 3.4i</td>
<td>CH\textsubscript{3}Cl\textsubscript{3}, Et\textsubscript{3}N (1 equiv), 18 h</td>
<td>3.5k</td>
<td>69</td>
<td>[oil]</td>
</tr>
<tr>
<td>12</td>
<td>N\textsubscript{3}α L-LeuBt, 3.1b</td>
<td>Ethanol, 3.4j</td>
<td>EtOH, Et\textsubscript{3}N (1 equiv), 12 h</td>
<td>3.5l</td>
<td>95</td>
<td>[oil]</td>
</tr>
</tbody>
</table>
3.3 Conclusions

Benzotriazol-1-yl-sulfonyl azide 2.7 allowed convenient and efficient synthesis of a wide range of azides, including \(N\)-(\(\alpha\)-azidoacyl)-benzotriazoles 3.1, which are efficient \(N\)-, \(S\)-, \(C\)-, and \(O\)-acylating agents and enable facile preparation of azido-peptides. In addition, the azido, as a protecting group, was shown to be well-tolerated in these acylations.

3.4 Experimental

3.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. The NMR spectra were recorded in \(\text{CDCl}_3\), DMSO-\(d_6\) with TMS for \(^1\text{H}\) (300 MHz) and \(^{13}\text{C}\) (75 MHz) as an internal reference. Silica was used as the stationary phase for column chromatography. Phosphomolybdic acid was used to detect compounds that were not UV active. [Caution: Although no trouble was experienced when utilizing sodium azide, the \textit{in situ} generated chlorosulfonyl azide, or any of the synthesized organic azides, appropriate safety measures must be taken at all times because azides are often found to be high energy compounds.\(^{10,12,51,52,60}\)]

3.4.2 General Procedure for the Preparation of \(N\)-(\(\alpha\)-Azidoacyl)benzotriazoles, 3.1a-c, (3.1a+3.1a′)

Thionyl chloride (0.01 mol, 1.2 equiv) was added to a solution of \(1H\)-benzotriazole (2.38 g, 0.02 mol, 2 equiv) in methylene chloride (10 mL) to give a clear yellow solution and was stirred for 15 min. at room temperature. The \(\alpha\)-azido acid 2.11 (0.01 mol, 1 equiv) was then added to give a suspension which was stirred for 2.5 h at room temperature. The precipitate was filtered, the filtrate evaporated, the residue dissolved in ethyl acetate and the solution washed with a saturated solution of sodium carbonate.
The organic portions were combined and dried over anhyd MgSO$_4$, filtered and dried to give the corresponding N-(α-azidoacyl)benzotriazoles 3.1.

(S)-2-Azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-phenylpropan-1-one (3.1a). (2S)-2-Azido-3-phenylpropanoic acid 2.11a (1.91 g, 0.01 mol) was treated according to the above procedure to give (S)-2-azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-phenylpropan-1-one 3.1a as a brown yellow solid (2.86 g, 98%); mp 66.1 - 68.3 °C; [α]$_D^{20}$ +32.5 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 8.20 (d, $J = 8.1$ Hz, 1H), 8.06 (d, $J = 8.1$ Hz, 1H), 7.62 (t, $J = 7.5$ Hz, 1H), 7.47 (t, $J = 7.7$ Hz, 1H), 7.42 - 7.18 (m, 5H), 5.48 (dd, $J = 5.3$, 8.9 Hz, 1H), 3.42 (dd, $J = 5.1$, 13.8 Hz, 1H), 3.30 - 3.15 (m, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 168.8, 146.0, 135.2, 130.9, 130.8, 129.2, 128.7, 127.4, 126.7, 120.4, 114.2, 62.4, 37.6; Anal. Calcd for C$_{15}$H$_{12}$N$_6$O: C, 61.64; H, 4.14; N, 28.75. Found: C, 61.90; H, 4.04; N, 28.57.

(S)-2-Azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-4-methylpentan-1-one (3.1b). (S)-2-Azido-4-methylpentanoic acid 2.11b (1.57 g, 0.01 mol) was treated according to the above procedure to give (S)-2-azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-4-methylpentan-1-one 3.1b as a yellow solid (2.48 g, 96%); mp 47.3 - 49.0 °C; [α]$_D^{20}$ +42.4 (c 0.6, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 8.30 (d, $J = 8.1$ Hz, 1H), 8.15 (d, $J = 8.4$ Hz, 1H), 7.71 (t, $J = 7.5$ Hz, 1H), 7.55 (t, $J = 7.5$ Hz, 1H) 5.28 (d, $J = 9.3$ Hz, 1H), 2.04-1.89 (m,3H), 1.08 (d, $J = 5.4$, Hz, 3H), 1.03 (d, $J = 5.4$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 170.2, 146.1, 130.9, 126.7, 120.4, 114.3, 59.5, 39.9, 25.4, 22.9, 21.3; Anal. Calcd for C$_{12}$H$_{14}$N$_6$O: C, 55.80; H, 5.46; N, 32.54. Found: C, 55.78; H, 5.76; N, 32.19.

(S)-2-Azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)propan-1-one (3.1c). (S)-2-Azidopropanoic acid 2.11c (1.15 g, 0.01 mol) was treated according to the above
procedure to give (S)-2-azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)propan-1-one **3.1c** as a brown solid (1.41 g, 65%); mp 77.0 - 77.9 °C; [α]D 20 +32.4 (c 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 8.28 (d, J = 8.1 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.73 - 7.67 (m, 1H), 7.57 - 7.51 (m, 1H), 5.35 (q, J = 7.0 Hz, 1H), 1.80 (d, J = 7.2 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 170.0, 146.1, 130.9, 126.7, 120.4, 114.3, 56.8, 17.0; HRMS m/z for C₉H₉NO₆ [M + H]+ calcd 217.0838, found 217.0826.

2-Azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-phenylpropan-1-one **(3.1a+3.1a')**. 2-Azido-3-phenylpropanoic acid **(2.11a+2.11a')** (1.91 g, 0.01 mol) was treated according to the above procedure to give 2-azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-phenylpropan-1-one **(3.1a+3.1a')** as a brown oil (2.86 g, 98%); ¹H NMR (300 MHz, CDCl₃): δ 8.20 (d, J = 8.1 Hz, 1H), 8.06 (d, J = 8.1 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.47 - 7.18 (m, 5H), 5.48 (d, J = 5.1, 8.7 Hz, 1H), 3.42 (dd, J = 5.1, 13.8 Hz, 1H), 3.20 (dd, J = 9, 13.5 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃): δ 168.9, 146.2, 135.2, 131.0, 131.0, 129.2, 128.8, 127.5, 126.8, 120.5, 114.3, 62.5, 37.7; Anal. Calcd for C₁₅H₁₂N₆O: C, 61.64; H, 4.14; N, 28.75. Found: C, 61.61; H, 4.13; N, 28.94.

(S)-2-Azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-methylbutan-1-one **(3.1d)**. (S)-2-Azido-3-methylbutanoic acid **(2.11d)** (1.43 g, 0.01 mol) was treated according to the above procedure to give (S)-2-azido-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-methylbutan-1-one **3.1d** as brown oil (1.76 g, 72%); [α]D 20 + 0.6 (c 6.5, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.85 (td, J = 0.9, 8.1 Hz, 1H), 7.69 - 7.66 (m, 1H), 7.28 - 7.22 (m, 1H), 7.13 - 7.07 (m, 1H), 4.76 (d, J = 6.9 Hz, 1H), 2.13 (octet, J = 6.6 Hz, 1H), 0.71 (dd, J = 6.9, 11.4 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 168.9, 145.7, 130.5, 126.3, 120.0, 114.0,
3.4.3 General Procedure for 3.3a-k

The appropriate N-nucleophile 3.2 was reacted under the optimized conditions described in Table 3-2. N-(α-Azidoacyl)benzotriazoles 3.1 (1 equiv) was then added to the reaction mixture. This was stirred for the specified time (Table 3-2) at room temperature before isolation of products 3.3a-k in yields of 62-87%.

(S)-2-Azido-N-(4-methoxyphenyl)-3-phenylpropanamide (3.3a). p-Anisidine 3.2a (260 mg, 2.11 mmol, 1.5 equiv) was treated with 3.1a (412 mg, 1.41 mmol) according to the above procedure. The solvent was then evaporated, the residue diluted with EtOAc and washed with 6N HCl. The organic portion was collected, dried over anhyd MgSO₄, filtered, and the filtrate concentrated under reduced pressure to give (S)-2-azido-N-(4-methoxyphenyl)-3-phenylpropanamide 3.3a (328 mg, 1.11 mmol) as a beige solid: 79%; mp 79.4 - 81.0 °C; [α]D²⁰ -14.6 (c 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 8.00 (bs, 1H), 7.40 - 7.26 (m, 7H), 6.82 (d, J = 8.4 Hz, 2H), 4.27 (dd, J = 4.5, 7.8 Hz, 1H), 3.75 (s, 3H), 3.37 (dd, J = 4.1, 13.7 Hz, 1H), 3.08 (dd, J = 8.1, 14.1 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃): δ 166.6, 156.7, 135.9, 129.6, 129.3, 128.6, 127.1, 122.1, 114.0, 65.4, 55.3, 38.5; Anal. Calcd for C₁₆H₁₆N₄O₂: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.53; H, 5.59; N, 18.66.

(S)-2-Azido-N-(4-methoxyphenyl)-4-methylpentanamide (3.3b). p-Anisidine 3.2a (251 mg, 2.04 mmol, 1.5 equiv) was treated with 3.1b (351 mg, 1.36 mmol) according to the above procedure. The solvent was then evaporated and the residue was diluted with EtOAc, washed with 6N HCl. The organic portion was then collected, dried over anhyd MgSO₄, filtered, and the filtrate concentrated under reduced pressure to give (S)-2-
azido-N-(4-methoxyphenyl)-4-methylpentanamide \(3.3b\) (300 mg, 1.14 mmol) as a white solid: 84%; mp 42.5 - 45.0 °C; \([\alpha]_D^{20} +55.43\) (c 0.49, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): \(\delta\) 8.10 (s, 1H), 7.45 - 7.41 (m, 2H), 6.88 - 6.83 (m, 2H), 4.08 - 4.03 (m, 1H), 3.78 (s, 3H), 1.88 - 1.74 (m, 3H), 1.00 (d, \(J = 2.4\) Hz, 3H), 0.98 (d, \(J = 2.4\) Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): \(\delta\) 167.8, 156.7, 130.0, 121.8, 114.1, 63.1, 55.4, 41.3, 25.0, 23.0, 21.5; Anal. Calcd for C$_{13}$H$_{18}$N$_4$O$_2$: C, 59.53; H, 6.92; N, 21.36. Found: C, 59.86; H, 7.18; N, 20.99.

(S)-2-Azido-4-methyl-N-(9H-purin-6-yl)pentanamide \(3.3c\). Adenine \(3.2b\) (268 mg, 1.99 mmol, 1 equiv) was treated with \(3.1b\) (514 mg, 1.99 mmol) according to the above procedure. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with water (2 x 50 mL). The organic layer was dried over anhyd MgSO$_4$, filtered and the filtrate evaporated. The residue was separated by column chromatography (acetonitrile) to give (S)-2-azido-4-methyl-N-(9H-purin-6-yl)pentanamide \(3.3c\) (370 mg, 1.35 mmol) as a white solid: 68%; mp 187.0 - 188.0 °C; \([\alpha]_D^{20} +47.5\) (c 0.3, CHCl$_3$); $^1$H NMR (300 MHz, DMSO-$d_6$): \(\delta\) 12.26 (bs, 1H), 11.60 (bs, 1H), 8.67 (s, 1H), 8.48 (s, 1H), 4.30 - 4.19 (m, 1H), 1.90 - 1.60 (m, 3H), 1.10 - 0.90 (m, 6H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): \(\delta\) 170.7, 151.2, 146.4, 143.1, 59.7, 39.3, 24.9, 22.7, 21.5; Anal. Calcd for C$_{11}$H$_{14}$N$_8$O: C, 48.17; H, 5.14; N, 40.85. Found: C, 48.09; H, 4.96; N, 40.61.

(S)-2-Azido-3-phenyl-N-tosylpropanamide \(3.3d\). p-Toluenesulfonamide \(3.2c\) (140 mg, 0.82 mmol, 1.2 equiv) was treated with \(3.1a\) (200 mg, 0.68 mmol) according to the above procedure. The solvent was evaporated, the residue diluted with EtOAc and washed with 6N HCl. The organic portion was then collected, dried over anhyd MgSO$_4$, filtered and the filtrate concentrated under reduced pressure to give (S)-2-azido-3-phenyl-N-tosylpropanamide \(3.3d\) (185 mg, 0.54 mmol) as a beige solid: 79%; mp 119.5
- 121.7 °C; \([\alpha]_D^{20} +4.9\) (c 1.0, CHCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 8.76 (bs, 1H), 7.91 (d, \(J = 8.1\) Hz, 2H), 7.37 - 7.05 (m, 7H), 4.23 - 4.19 (m, 1H), 3.21 (dd, \(J = 4.2, 14.1\) Hz, 1H), 2.99 (dd, \(J = 7.5, 14.1\) Hz, 1H), 2.47 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 166.7, 145.4, 134.8, 134.7, 129.6, 129.3, 128.7, 128.6, 127.4, 64.8, 38.0, 21.7; Anal. Calcd for C\(_{16}\)H\(_{16}\)N\(_4\)O\(_3\)S: C, 55.80; H, 4.68; N, 16.27. Found: C, 55.91; H, 4.74; N, 16.24.

(S)-2-Azido-4-methyl-N-tosylpentanamide (3.3e). \(p\)-Toluensulfonamide 3.2c (119 mg, 0.70 mmol, 1.2 equiv) was treated with 3.1b (151 mg, 0.58 mmol) according to the above procedure. The solvent was evaporated, the residue diluted with EtOAc and washed with 6N HCl. The organic portion was then collected, dried over anhyd MgSO\(_4\), filtered and the filtrate concentrated under reduced pressure to give (S)-2-azido-4-methyl-N-tosylpentanamide 3.3e (140 mg, 0.45 mmol) as white microcrystals: 77%; mp 55.3 - 56.0 °C; \([\alpha]_D^{20} +69.1\) (c 0.3, CH\(_3\)OH); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 9.29 (bs, 1H), 7.96 (dd, \(J = 1.8, 6.6\) Hz, 2H), 7.39 - 7.33 (m, 2H), 3.96 - 3.38 (m, 1H), 2.44 (s, 3H), 1.86 - 1.60 (m, 3H), 1.00 - 0.87 (m, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 168.0, 145.5, 134.9, 129.6, 128.5, 62.3, 40.4, 24.7, 22.7, 21.7, 21.4; Anal. Calcd for C\(_{13}\)H\(_{18}\)N\(_4\)O\(_3\)S: C, 50.31; H, 5.85; N, 18.05. Found: C, 50.12; H, 6.02; N, 17.78.

2-Azido-N-(1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-3-phenylpropanamide (3.3f): Cytidine 3.2d (404 mg, 1.66 mmol, 1 equiv) was treated with (3.1a+3.1a') (486 mg, 1.66 mmol) according to the above procedure. DMF was evaporated and the residue chromatographed using MeOH:CH\(_2\)Cl\(_2\) (gradient) to give 2-azido-N-(1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)-3-phenylpropanamide 3.3f (428 mg, 1.03 mmol) as a glassy white solid: 62%; mp (glassy
solid); [α]_D$^20$ +57.3 (c 1.0, CH$_3$OH); ¹H NMR (300 MHz, DMSO-$d_6$): δ 8.51 (d, J = 7.2 Hz, 1H), 7.36 - 7.31 (m, 5H), 7.17 (d, J = 7.5 Hz, 1H), 5.78 (d, J = 2.1 Hz, 1H), 5.53 (d, J = 4.5 Hz, 1H), 5.21 (t, J = 5.0 Hz, 1H), 5.08 (d, J = 4.8 Hz, 1H), 4.32 (dd, J = 5.1, 9.3 Hz, 1H), 3.99 - 3.87 (m, 3H), 3.77 - 3.71 (m, 1H), 3.62 - 3.57 (m, 1H), 3.21 (dd, J = 5.0, 13.7 Hz, 1H), 2.96 (dd, J = 9.8, 13.7 Hz, 1H); ¹³C NMR (75 MHz, DMSO-$d_6$): δ 170.6, 161.9, 154.4, 146.0, 136.4, 129.1, 128.5, 126.9, 95.3, 90.3, 84.3, 74.6, 68.6, 63.1, 62.8, 59.9, 36.6; Anal. Calcd for C$_{18}$H$_{20}$N$_6$O$_6$: C, 51.92; H, 4.84; N, 20.18. Found: C, 52.27; H, 4.35; N, 20.32.

2-Azido-N-(4-methoxyphenyl)-3-phenylpropanamide (3.3g). p-Anisidine 3.2a (261 mg, 2.12 mmol, 1.5 equiv) was treated with (3.1a+3.1a) (411 mg, 1.41 mmol) according to the above procedure. The solvent was evaporated, the residue diluted with EtOAc and washed with 6 N HCl. The organic portion was then collected, dried over anhyd MgSO$_4$, filtered, and the filtrate concentrated under reduced pressure to give 2-azido-N-(4-methoxyphenyl)-3-phenylpropanamide 3.3g (328 mg, 1.11 mmol) as a brown oil: 79%; ¹H NMR (300 MHz, CDCl$_3$): δ 7.79 (br s, 1H), 7.33 - 7.21 (m, 7H), 6.84 - 6.80 (m, 2H), 4.30 (dd, J = 4.4, 8.0 Hz, 1H), 3.75 (s, 3H), 3.38 (dd, J = 4.2, 14.1 Hz, 1H), 3.08 (dd, J = 8.3, 14.0 Hz, 1H); ¹³C NMR (75 MHz, CDCl$_3$): δ 166.6, 156.8, 135.9, 129.6, 129.4, 128.6, 127.2, 122.1, 114.1, 65.6, 55.4, 38.7; Anal. Calcd for C$_{16}$H$_{16}$N$_4$O$_2$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.81; H, 5.51; N, 18.97.

(S)-2-((S)-2-Azido-3-phenylpropanamido)-4-methylpentanoic acid (3.3h). L-Leu 3.2e (1.02 g, 7.74 mmol, 2 equiv) was treated with 3.1a (1.13 g, 3.87 mmol) according to the above procedure. The MeCN was evaporated, the residue diluted with EtOAc and washed with 6N HCl. The organic layer was then collected, dried over anhyd MgSO$_4$, etc.
filtered and the filtrate concentrated under reduced pressure to give (S)-2-((S)-2-azido-3-phenylpropanamido)-4-methylpentanoic acid 3.3h (1.02 g, 3.37 mmol) as a bright yellow oil: 87%; [α]$_D^{20}$ +27.8 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 11.20 (bs, 1H), 7.28 - 7.25 (m, 5H), 6.72 (d, $J$ = 8.1 Hz, 1H), 4.58 - 4.54 (m, 1H), 4.37 - 4.31 (m, 1H), 4.12 (d, $J$ = 7.2 Hz, 1H), 3.31 (dd, $J$ = 3.8, 14.3 Hz, 1H), 3.06 (dd, $J$ = 7.5, 14.1 Hz, 1H), 1.68 - 1.58 (m, 1H), 1.54 - 1.40 (m, 1H), 0.88 - 0.86 (m, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 176.5, 169.0, 135.7, 129.5, 129.2, 128.6, 127.2, 65.0, 50.6, 40.9, 38.2, 24.6, 22.7, 21.7; Anal. Calcd for C$_{15}$H$_{20}$N$_4$O$_3$: C, 59.20; H, 6.62; N, 18.41. Found: C, 59.19; H, 6.55; N, 18.60.

(R)-2-((S)-2-Azido-3-phenylpropanamido)-3-mercaptopropanoic acid (3.3i). L-Cys 3.2f (165 mg, 1.36 mmol, 2 equiv) was treated with 3.1a (197 mg, 0.68 mmol) according to the above procedure. The MeCN was evaporated, the residue diluted with EtOAc and the solution washed with 6N HCl. The organic layer was then collected, dried over anhyd MgSO$_4$, filtered and the filtrate concentrated under reduced pressure to give (R)-2-((S)-2-azido-3-phenylpropanamido)-3-mercaptopropanoic acid 3.3i (160 mg, 0.54 mmol) as a beige solid: 80%; mp 159.0 - 161.0 °C; [α]$_D^{20}$ +2.5 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 8.51 (bs, 1H), 7.31 - 7.28 (m, 5H), 7.17 (d, $J$ = 7.5 Hz, 1H), 4.85 (t, $J$ = 3.6 Hz, 1H), 4.43 - 4.39 (m, 1H), 3.29 (dd, $J$ = 3.8, 14.1 Hz, 1H), 3.15 (dd, $J$ = 6.6, 14.1 Hz, 1H), 3.00 - 2.92 (m, 1H), 2.84 - 2.70 (m, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 172.9, 169.0, 135.2, 129.6, 128.7, 127.5, 64.7, 53.3, 38.1, 26.4; Anal. Calcd for C$_{12}$H$_{14}$N$_4$O$_3$S: C, 48.97; H, 4.79; N, 19.04. Found: C, 49.13; H, 4.47; N, 19.03.

(R)-2-((S)-2-Azido-4-methylpentanamido)-3-mercaptopropanoic acid (3.3j). L-Cys 3.2f (242 mg, 2.00 mmol, 2 equiv) was treated with 3.1b (258 mg, 1.00 mmol) according
to the above procedure. The MeCN was evaporated, the residue diluted with EtOAc and the solution washed with 6N HCl. The organic layer was then collected, dried over anhyd MgSO₄, filtered and the filtrate concentrated under reduced pressure to give (R)-2-((S)-2-azido-4-methylpentanamido)-3-mercaptopropanoic acid 3.3j (216 mg, 0.83 mmol) as a colorless oil: 83%; [α]D²⁰ -15.2 (c 0.3, CH₂OH); ¹H NMR (300 MHz, CDCl₃): δ 10.22 (s, 2H), 8.68 (s, 1H), 4.54 - 4.40 (m, 1H), 3.87 - 3.78 (m, 1H), 3.00 - 2.80 (m, 2H), 1.80 - 1.50 (m, 3H), 0.98 - 0.85 (m, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 172.0, 170.5, 60.0, 52.9, 31.8, 31.0, 25.2, 23.2, 22.3; HRMS m/z for C₉H₁₇N₄O₃S [M + H]+ calcd 261.1016, found 261.1007.

2-((S)-2-Azido-3-phenylpropanamido)propanoic acid (3.3k). L-Ala 3.2g (611 mg, 6.86 mmol, 2 equiv) was treated with 3.1a (1.00 g, 3.43 mmol) according to the above procedure. The MeCN was evaporated, the residue diluted with EtOAc and washed with 6N HCl. The organic layer was then collected, dried over anhyd MgSO₄, filtered and the filtrate concentrated under reduced pressure to give 2-((S)-2-azido-3-phenylpropanamido)propanoic acid 3.3k (0.72 g, 2.74 mmol) as white microcrystals: 80%; mp 113.0 - 114.0 °C; [α]D²⁰ -10.7 (c 1.0, CH₃OH); ¹H NMR (300 MHz, CDCl₃): δ 7.58 (bs, 1H), 7.34 - 7.23 (m, 5H), 6.81 (d, J = 7.2 Hz, 1H), 4.61 - 4.50 (m, 1H), 4.32 - 4.27 (m, 1H), 3.32 (dd, J = 3.9, 14.1 Hz, 1H), 3.05 (dd, J = 7.8, 14.1 Hz, 1H), 1.37 (s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ 176.3, 168.7, 135.6, 129.5, 128.6, 127.3, 65.0, 48.0, 38.4, 17.9; Anal. Calcd for C₁₂H₁₄N₄O₃: C, 54.96; H, 5.38; N, 21.36. Found: C, 55.25; H, 5.21; N, 21.13.

3.4.4 General Procedure for 3.5a-l

The appropriate nucleophile 3.4 was reacted under the optimized conditions described in Table 3-3. N-(α-Azidoacyl)benzotriazoles 3.1 (1 equiv) was added to the
reaction mixture. This was allowed to stir for the specified time (Table 3-3) at room temperature before isolation of products 3.5a-l in yields of 57-95%.

(S)-Phenyl 2-azido-3-phenylpropanoate (3.5a). Phenol 3.4a (70 mg, 0.75 mmol, 1.1 equiv) was treated with 3.1a (198 mg, 0.68 mmol) according to the above procedure. The solvent was then evaporated, diluted with ether and washed with 1 M NaOH. The organic layer was then collected, dried over anhyd MgSO₄, filtered and the filtrate concentrated under reduced pressure to give (S)-phenyl 2-azido-3-phenylpropanoate 3.5a (153 mg, 0.57 mmol) as a brown oil: 84%; [α]D²⁰⁻3.1 (c 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.41 - 7.23 (m, 10H), 7.03 - 6.98 (m, 1H), 4.36 - 4.28 (m, 1H), 3.32 (dd, J = 6.0, 13.8 Hz, 1H), 3.19 (dd, J = 8.1, 13.8 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃): δ 168.4, 150.0, 135.4, 129.5, 129.3, 128.7, 127.4, 126.3, 121.5, 121.1, 63.1, 37.7; Anal. Calcd for C₁₅H₁₃N₃O₂: C, 67.40; H, 4.90; N, 15.72. Found: C, 67.86; H, 5.09; N, 15.28.

(S)-Phenyl 2-azido-4-methylpentanoate (3.5b). Phenol 3.4a (104 mg, 1.10 mmol, 1.1 equiv) was treated with 3.1b (258 mg, 1.00 mmol) according to the above procedure. The solvent was then evaporated, diluted with ether and washed with 1 M NaOH. The organic layer was then collected and chromatographed using EtOAc:Hexanes (1:5) to give (S)-phenyl 2-azido-4-methylpentanoate 3.5b (146 mg, 0.63 mmol) as a colorless oil: 63%; [α]D²⁰⁻59.5 (c 0.5, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.38 (d, J = 8.1 Hz, 2H), 7.25 (t, J = 7.5 Hz, 1H), 7.12 (d, J = 8.7 Hz, 2H), 4.05 (t, J = 6.3 Hz, 1H), 1.99 - 1.80 (m, 3H), 1.05 - 1.00 (m, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 169.5, 150.2, 129.5, 126.3, 121.1, 60.3, 39.8, 25.1, 22.8, 21.6; Anal. Calcd for C₁₂H₁₅N₃O₂: C, 61.79; H, 6.48; N, 18.01. Found: C, 61.71; H, 6.75; N, 17.61.
(8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-azido-3-phenylpropanoate (3.5c). Cholesterol 3.4b (178 mmol, 0.46 mmol, 1 equiv) was treated with (3.1a+3.1a') (135 mg, 0.46 mmol) according to the above procedure. The solvent was evaporated, the oil obtained chromotographed using hexane to give (8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-azido-3-phenylpropanoate 3.5c (200 mg, 0.36 mmol) as a white powder: 78%; mp 56.0 - 58.0 °C; [α]D 20 -10.5 (c 1.0, CHCl3); 1H NMR (300 MHz, CDCl3): δ 7.15 - 7.03 (m, 5H), 5.19 (bs, 1H), 4.49 - 4.44 (m, 1H), 3.82 (t, J = 6.3 Hz, 1H), 3.00 - 2.78 (m, 2H), 2.15 - 2.05 (m, 2H), 1.84 - 1.48 (m, 2H), 1.34 - 0.65 (m, 42 H), 0.49 (s, 3H); 13C NMR (75 MHz, CDCl3): δ 169.3, 139.1, 135.9, 129.2, 128.6, 127.2, 123.1, 75.8, 63.2, 56.6, 56.1, 49.9, 42.3, 40.3, 39.7, 39.5, 37.6, 36.9, 36.5, 36.1, 35.8, 31.8, 28.2, 28.0, 27.6, 24.3, 23.8, 22.8, 22.5, 21.0, 19.3, 18.7, 11.8; Anal. Calcd for C36H53N3O2: C, 77.24; H, 9.54; N, 7.51. Found: C, 77.29; H, 9.37; N, 7.52.

(8S,9S,10R,13R,14S,17R)-10,13-Dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-azido-4-methylpentanoate (3.5d). Cholesterol 3.4b (387 mg, 1.00 mmol, 1 equiv) was treated with 3.1b (259 mg, 1.00 mmol) according to the above procedure. The solvent was evaporated, the oil obtained chromotographed using hexane:EtOAc (1:40) to give (8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-azido-4-methylpentanoate 3.5d (320 mg, 0.61 mmol) as a white powder: 61%; mp
81.0 - 83.0 °C; [α]_D^{20} -29.2 (c 0.6, CHCl₃); \(^1\)H NMR (300 MHz, CDCl₃): δ 5.50 - 5.35 (m, 1H), 4.83 - 4.62 (m, 1H), 3.90 - 3.70 (m, 1H), 2.50 - 2.29 (m, 2H), 2.15 - 0.68 (m, 44H), 0.68 - 0.66 (s, 6H); \(^1\)C NMR (75 MHz, CDCl₃): δ 170.4, 139.2, 123.0, 75.6, 60.4, 56.7, 56.1, 50.0, 42.3, 39.9, 39.7, 39.5, 38.0, 36.9, 36.6, 36.2, 35.8, 31.8, 28.2, 28.0, 27.7, 25.0, 24.3, 23.8, 22.8, 22.6, 21.6, 21.0, 19.3, 18.7, 11.9.; HRMS m/z for C₃₃H₅₅N₃NaO₂ [M + Na]+ calcd 548.4186, found 548.4194.

(S)-(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-azido-4-methylpentanoate (3.5e). \(\beta\)-Sitosterol 3.4c (211 mg, 0.51 mmol, 1 equiv) was treated with 3.1b (133 mg, 0.51 mmol) according to the above procedure. Chloroform was evaporated, residue diluted with EtOAc and flash chromatographed with hexanes:EtOAc (45:5). The organic fractions were evaporated to give (S)-(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 2-azido-4-methylpentanoate (3.5e) (200 mg, 0.36 mmol) as a white solid: 70%; mp 57.1 - 60.0 °C; [α]_D^{20} +43.7 (c 1.0, CHCl₃); \(^1\)H NMR (300 MHz, CDCl₃): δ 5.40 (s, 1H), 2.36 (d, J = 6.9Hz, 2H), 1.99 - 0.68 (m, 54H); \(^1\)C NMR (75 MHz, CDCl₃): δ 170.5, 139.2, 123.1, 75.6, 61.7, 60.3, 56.7, 56.0, 50.0, 45.8, 42.6, 42.3, 39.9, 38.0, 36.9, 36.6, 33.7, 31.8, 28.2, 27.7, 26.1, 25.0, 24.3, 22.8, 21.6, 21.0, 19.3, 18.8, 14.2, 11.9; Anal. Calcd for C₃₅H₅₉N₃O₂: C, 75.90; H, 10.74; N, 7.59. Found: C, 75.51; H, 10.60; N, 7.44.

(S)-S-Phenyl 2-azido-4-methylpentanethioate (3.5f). Thiophenol 3.4d (165 mg, 1.50 mmol, 1.5 equiv) was treated with 3.1b (258 mg, 1.00 mmol) according to the
above procedure (pyridine (0.08 mL, 1 equiv) was added to a mixture of 3.4d and 3.1b at 0 °C and then left to stir at room temperature). The reaction mixture was filtered and filtrate evaporated. The residue was diluted Et₂O (30 mL) and washed with 5% solution of sodium hydroxide (2 x 50 mL) and water (2 x 30 mL). The organic layer was dried over anhyd MgSO₄, filtered and the filtrate evaporated. The residue was separated by column chromatography using EtOAc:Hexanes (1:5) to give (S)-S-phenyl 2-azido-4-methylpentanethioate 3.5f (179 mg, 0.72 mmol) as a colorless oil: 72%; [α]D²⁰ +106.9 (c 0.3, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.48 - 7.35 (m, 5H), 4.08 - 4.00 (m, 1H), 1.92 - 1.71 (m, 3H), 1.08 - 0.96 (m, 6H); ¹³C NMR (75 MHz,CDCl₃): δ 197.1, 134.5, 129.7, 129.3, 126.6, 67.5, 40.7, 25.0, 22.9, 21.5; Anal. Calcd for C₁₂H₁₅N₃O₃S:C, 57.81; H, 6.06; N, 16.85. Found: C, 58.16; H, 6.18; N, 17.11.

(S)-2-((2-Azido-4-methylpentanoyl)thio)acetic acid (3.5g). 2-Mercaptoacetic acid 3.4e (183 mg, 1.99 mmol, 1 equiv) was treated with 3.1b (513 mg, 1.99 mmol) according to the above procedure. The solvent was evaporated, the residue diluted with EtOAc and washed with 6 N HCl. The organic portions were dried over anhyd MgSO₄, filtered, and the filtrate evaporated to give (S)-2-((2-azido-4-methylpentanoyl)thio)acetic acid 3.5g (376 mg, 1.63 mmol) as a yellow oil: 82%;[α]D²⁰ -10.6 (c 0.7, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 10.8 (bs, 1H), 4.04 (t, J = 7.2 Hz, 1H), 3.84 - 2.74 (m, 2H), 1.85 - 1.75 (m, 1H), 1.74 - 1.64 (m, 2H), 0.99 (d, J = 4.8 Hz, 3H), 0.97 (d, J = 4.8 Hz, 3H); ¹³C NMR (75 MHz,CDCl₃): δ 190.8, 167.4, 60.5, 33.6, 24.1, 17.8, 15.9, 14.3; HRMS m/z for C₁₈H₁₄N₃O₃S [M+H]+ calcd 323.0750, found 323.0742.

(S)-Methyl 2-((2-azido-3-phenylpropanoyl)thio)acetate (3.5h). Methyl 2-Mercaptoacetate 3.4f (106 mg, 1.00 mmol, 1 equiv) was treated with 3.1a (292 mg, 1.00
mmol) according to the above procedure. The solvent was evaporated, the residue chromatographed using hexane: EtOAc (1:5) to give (S)-methyl 2- ((2-azido-3-phenylpropanoyl)thio)acetate \(3.5h\) (160 mg, 0.57 mmol) as a yellow oil: 57%; \([\alpha]_{D}^{20} +19.2\) (c 0.8, CHCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.36 - 7.18 (m, 5H), 4.25 - 4.20 (m, 1H), 3.75 - 3.70 (m, 5H), 3.25 - 3.21 (m, 1H), 3.05 - 2.87 (m, 1H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 196.7, 168.4, 135.4, 129.2, 128.6, 127.2, 70.3, 52.8, 38.3, 31.1; Anal. Calcd. for C\(_{12}\)H\(_{13}\)N\(_3\)O\(_3\): C, 51.60; H, 4.69; N, 15.04. Found: C,51.99; H, 4.55; N,14.93.

(S)-5-(2-Azido-1-hydroxy-4-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione \(3.5i\). Meldrum’s acid \(3.4g\) (176 mg, 1.22 mmol, 1 equiv) was treated with \(3.1b\) (316 mg, 1.22 mmol) according to the procedure above. The solvent was evaporated, the residue was separated by column chromatography [EtOAc] to give (S)-5-(2-azido-1-hydroxy-4-methylpentylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione \(3.5i\) (250 mg, 0.88 mmol) as a yellow oil; 72%; \([\alpha]_{D}^{20} +113.0\) (c 0.5, CH\(_3\)OH); \(^1\)H NMR (300 MHz, Acetone-\(d_6\)): \(\delta\) 5.08 - 5.02 (m, 1H), 1.77 (dd, \(J = 6.6, 13.2\) Hz, 1H), 1.65 - 1.53 (m, 8H), 0.98 - 0.94 (m, 6H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 193.4, 170.4, 159.4, 105.6, 90.7, 58.6, 39.7, 26.9, 26.7, 25.3, 23.0, 21.1; Anal. Calcd for C\(_{12}\)H\(_{17}\)N\(_3\)O\(_5\): C, 50.88; H, 6.05; N, 14.83. Found: C, 51.01; H, 6.08; N,14.31.

(S)-2-(2-Azido-4-methylpentanoyl)cyclohexane-1,3-dione \(3.5j\). Cyclohexane-1,3-dione \(3.4h\) (449 mg,4.00 mmol, 2 equiv) was treated with \(3.1b\) (517 mg, 2.00 mmol) according to the procedure above. The solvent was then evaporated, the residue diluted with EtOAc (40 mL) and washed with 6 N HCl (3 x 50 mL). The organic layer was dried with over anhyd MgSO\(_4\), filtered and the filtrate evaporated. This residue was separated by column chromatography [EtOAc:Hexanes (1:1)] to give (S)-2-(2-azido-4-
methylpentanoyl)cyclohexane-1,3-dione 3.5j (480 mg, 1.90 mmol) as a colorless oil; 95%; [α]$_D^{20}$ +70.3 (c 0.3, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 17.34 (s, 1H), 5.07 (dd, $J$ = 4.1, 9.8 Hz, 1H), 2.73 (t, $J$ = 6.5 Hz, 2H), 2.51 (dd, $J$ = 6.0, 7.2 Hz, 2H), 2.11 - 1.90 (m, 3H), 1.65 - 1.55 (m, 2H), 1.08 (d, $J$ = 6.6 Hz, 3H), 1.00 (dd, $J$ = 2.4, 6.6 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 203.7, 198.0, 194.9, 111.5, 62.7, 39.5, 38.4, 32.5, 25.6, 23.3, 20.9, 18.9; Anal. Calcd for C$_{12}$H$_{17}$N$_3$O$_3$: C, 57.36; H, 6.82; N, 16.72. Found: C, 57.56; H, 7.10; N, 16.98.

(4S)-Ethyl 4-azido-2-cyano-6-methyl-3-oxoheptanoate (3.10k). Ethyl cyanoacetate 3.4i (452 mg, 4.00 mmol, 2 equiv) was treated with 3.1b (517 mg, 2.00 mmol) according to the procedure above. The solvent was evaporated, the residue diluted with EtOAc (40 mL) and washed with 6 N HCl (3 x 50 mL). The organic layer was dried with anhyd MgSO$_4$, filtered and the filtrate evaporated. This residue was separated by column chromatography [EtOAc:Hexanes (1:1)] to give (4S)-ethyl 4-azido-2-cyano-6-methyl-3-oxoheptanoate 3.5k (350 mg, 1.38 mmol) as a colorless oil; 69%; [α]$_D^{20}$ +125.9 (c 0.4, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 13.84 (s, 1H), 4.42 - 4.35 (m, 2H), 4.30 - 4.26 (m, 1H), 1.89 - 1.67 (m, 3H), 1.42 - 1.37 (m, 3H), 1.04 - 0.98 (m, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ 186.2, 169.7, 113.1, 81.3, 63.2, 59.3, 39.7, 25.0, 22.6, 21.8, 14.0; Anal. Calcd for C$_{11}$H$_{16}$N$_4$O$_3$: C, 52.37; H, 6.39; N, 22.21. Found: C, 52.67; H, 6.60; N, 22.14.

(S)-Ethyl 2-azido-4-methylpentanoate (3.5l). Ethanol 3.4j (solvent, 10 mL) was treated with 3.1b (39 mg, 0.85 mmol) according to the procedure above. The solvent was evaporated, the residue diluted with EtOAc (25 mL) and washed with saturated sodium carbonate solution to remove benzotriazole. The organic layer was dried with
over anhyd MgSO$_4$, filtered and the filtrate evaporated to give (S)-ethyl 2-azido-4-methylpentanoate 3.5I (150 mg, 0.81 mmol) as a yellow oil; 95%; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 4.24 (q, $J$ = 7.2 Hz, 2H), 3.81 - 3.75 (m, 1H), 1.81 - 1.58 (m, 3H), 1.33 - 1.24 (m, 3H), 0.98 - 0.93 (m, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 171.0, 61.7, 60.3, 39.9, 25.0, 22.8, 21.5, 14.2.
CHAPTER 4
MICROWAVE ASSISTED REGIOSPECIFIC SYNTHESIS OF PSEUDOHALOHYDRIN ESTERS

4.1 Introduction

Halohydrin esters are important intermediates in the asymmetric synthesis of a wide range of biologically active natural and synthetic products\textsuperscript{91-96} including drugs,\textsuperscript{97} $\beta$-aminoalcohols,\textsuperscript{98} pyrrolidines\textsuperscript{99} and functionalized cyclopropanes.\textsuperscript{100}

Halohydrin esters\textsuperscript{101-103} are commonly prepared by (i) direct reaction of an epoxide with an acyl halide,\textsuperscript{91,94,95,102,103} (ii) the ring-opening of epoxides by halogen nucleophiles followed by the $O$-acylation of the resulting halohydrin derivatives\textsuperscript{104-109} and (iii) from 1,2-diols.\textsuperscript{96,110} In general, these strategies produce mixtures of regioisomers and side-products. No previous general method has achieved the high regioselectivity which I now report.

Microwave heating is a powerful tool in promoting a variety of reactions in organic synthesis and functional group transformations without solvents. The use of a single-mode cavity microwave synthesizer helps achieve reproducibility, safety, reduced pollution, and simplicity in processing and handling.\textsuperscript{20,21,23-25,111,112}

$N$-Acylbenzotriazoles, are more stable than acid chlorides towards hydrolysis and have replaced them advantageously in many acylations, often reducing side reactions.\textsuperscript{3,51,113,114} $N$-Acylbenzotriazoles have thus enabled both Friedel-Crafts and Vilsmeier-Haack acylations.\textsuperscript{3,115} Obase et al.\textsuperscript{116} previously synthesized a pseudohalohydrin ester (Scheme 4-1) from an $N$-acylbenzotriazole. However, the single example reported gave a mixture of three products, the parent benzotriazole, and two

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isomeric pseudohalohydrin esters A and B. The structures of A (yield 13%) and B (yield 19%) were assigned only on the basis of their UV spectra as the “1-” and “2-” substituted benzotriazoles. In addition to A and B, there is the third possible isomeric benzotriazole product C which wasn’t reported or identified. The only evidence advanced for the structures of A and B was derived from ultraviolet spectroscopy. This work^116 is limited to a single example and neither substrate scope nor optimization of reaction conditions were investigated.

Scheme 4-1. Reported^116 synthesis of a pseudohalohydrin ester from an N-acylbenzotriazole and an epoxide.

To the best of my knowledge, the palladium catalyzed synthesis of pseudohalohydrin esters from N-acylbenzotriazoles and epoxides has not been previously attempted. An improved protocol for the regiospecific synthesis of β-(benzotriazol-1-yl)ethyl pseudohalohydrin esters 4.4, with palladium catalysis under microwave-assisted, solvent free conditions is described.

Microwave heating is a powerful tool in promoting a variety of reactions in organic synthesis and functional group transformations without solvents. The use of a single-
mode cavity microwave synthesizer helps achieve reproducibility, safety, reduced pollution, and simplicity in processing and handling.\textsuperscript{20,21,23-25,111,112}

4.2 Results and Discussion

4.2.1 Optimization of reaction conditions for 4.4a.

I found that the palladium catalyzed thermal reaction of N-acylbenzotriazole 1a\textsuperscript{117-119} with epoxide 4.3a gave single regioisomer \(\beta\)-(benzotriazol-1-yl)ethyl ester 4.4a. Although thermal isomerization of 4.1a to 4.1a', followed by oxidative addition of 4.1a' to palladium (0) was reported\textsuperscript{33} recently to give 4.1a'', I detected no 1,4-benzoxazine 4.2a (Scheme 4-2).\textsuperscript{120}

Scheme 4-2. Palladium catalyzed thermal reaction of N-acylbenzotriazole 4.1a with epoxide 4.3a.

Reaction of (1H-benzotriazol-1-yl)(4-ethylphenyl)methanone (4.1a) with styrene oxide 4.3a in the presence of 10 mol% of Pd(PPh\textsubscript{3})\textsubscript{4} under microwave irradiation (130 \(^\circ\)C, 50 W) for 30 minutes, gave 2-(1H-benzotriazol-1-yl)-1-phenylethyl 4-ethylbenzoate (4.4a) as a single regioisomer in 87% yield (Scheme 4-2). These conditions proved to
be generally successful but in the absence of palladium catalyst no reaction occurred during 30 min and prolonged reaction times resulted in decomposition. Other catalyst systems including CuSO$_4$ anhydrous, CuSO$_4$.5H$_2$O, Pd(OAc)$_2$ were less effective as were lower temperatures, power and catalyst loadings (100 °C, 20 W and 100 °C, 50 W, 130 °C, 50 W, 5 mol% Pd(PPh$_3$)$_4$) (Table 4-1).
Table 4-1. Optimization of reaction conditions for the synthesis of 4.4a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Reaction conditions</th>
<th>4.4a Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>130 °C, 50 W, 30-90 min</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10 mol%Pd(PPh₃)₄</td>
<td>130 °C, 50 W, 30 min</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>10 mol% Pd(Ph₃)₄</td>
<td>rt, CHCl₃, 12h</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>10 mol% Pd(Ph₃)₄</td>
<td>reflux, CHCl₃, 12h</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>10 mol% Pd(Ph₃)₄</td>
<td>130 °C, 12h</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>5 mol% Pd(Ph₃)₄</td>
<td>130 °C, 50 W, 30 min</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>10 mol% CuSO₄ anhyd.</td>
<td>130 °C, 50 W, 30 min</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>10 mol% CuSO₄•5H₂O</td>
<td>130 °C, 50 W, 30 min</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>10 mol% Pd(Oac)₂</td>
<td>130 °C, 50 W, 90 min</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>10 mol% PPh₃</td>
<td>130 °C, 50 W, 40 min</td>
<td>35</td>
</tr>
</tbody>
</table>

4.2.2 Synthesis of β-(Benzotriazol-1-yl)ethyl esters 4.4

I examined the scope of the palladium-catalyzed reaction of \(N\)-acylbenzotriazoles 4.1a-f with epoxides 4.3a-c, using the optimized conditions as detailed in Table 4-2. β-
(Benzotriazol-1-yl)ethyl esters 4.4 were obtained as single isomers in 52-87% yields. The reaction proceeded faster with N-aroylbenzotriazoles (reaction time, 30 min.) than with N-alkylbenzotriazoles (reaction time, 60 min) and aromatic as well as alkyl substituted epoxides were tolerated (Table 4-2).

Table 4-2. Synthesis of β-(Benzotriazol-1-yl)ethyl esters 4.4.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate 4.1</th>
<th>Substrate 4.3</th>
<th>Product 4.4, yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-CH₃CH₂-C₆H₄, 4.1a</td>
<td>R'&quot;=H, R'(^{\prime})=C₆H₅, 4.3a</td>
<td>4.4a, 87</td>
</tr>
<tr>
<td>2</td>
<td>p-CH₃CH₂-C₆H₄, 4.1a</td>
<td>R'&quot;=C₆H₅, R'(^{\prime})=C₆H₅, 4.3b</td>
<td>4.4b, 62</td>
</tr>
<tr>
<td>3</td>
<td>C₆H₅-CH₂CH₂, 4.1b</td>
<td>R'&quot;=H, R'(^{\prime})=CH₃(CH₂)₃, 4.3c</td>
<td>4.4c, 72</td>
</tr>
<tr>
<td>4</td>
<td>1-Naphthyl-, 4.1c</td>
<td>R'&quot;=H, R'(^{\prime})=C₆H₅, 4.3a</td>
<td>4.4d, 62</td>
</tr>
<tr>
<td>5</td>
<td>1-Naphthyl-, 4.1c</td>
<td>R'&quot;=H, R'(^{\prime})=CH₃(CH₂)₃, 4.3c</td>
<td>4.4e, 73</td>
</tr>
<tr>
<td>6</td>
<td>p-NO₂-C₆H₄, 4.1d</td>
<td>R'&quot;=H, R'(^{\prime})=C₆H₅, 4.3a</td>
<td>4.4f, 70</td>
</tr>
<tr>
<td>7</td>
<td>C₆H₅, 4.1e</td>
<td>R'&quot;=H, R'(^{\prime})=C₆H₅, 4.3a</td>
<td>4.4g, 75</td>
</tr>
<tr>
<td>8</td>
<td>C₆H₅, 4.1e</td>
<td>R'&quot;=H, R'(^{\prime})=CH₃(CH₂)₃, 4.3c</td>
<td>4.4h, 76</td>
</tr>
<tr>
<td>9</td>
<td>1-Adamantane-CH₂-, 4.1f</td>
<td>R'&quot;=H, R'(^{\prime})=CH₃(CH₂)₃, 4.3c</td>
<td>4.4i, 52</td>
</tr>
</tbody>
</table>
I propose initial oxidative addition of Pd(0) forming $4.6$. The interaction of $4.6$ with epoxide $4.3$ forms $4.6'$, which is then attacked by benzotriazole giving $4.4$ as a single regioisomer (Scheme 4-3).

Scheme 4-3. Possible mechanism.

The mechanism appears to be similar to the recent report of coupling reaction of acyl halides and epoxides affording halohydrin esters as mixtures of regioisomers (Scheme 4-4). However, the palladium-catalyzed pathway proved to be regioselective providing a single regioisomer in each case.

Scheme 4-4. Acid halides and epoxides in halohydrin-ester synthesis.
Reaction of Z-L-Phe-Bt 4.1g with 4.3c however, gave 4.5, the alcohol derived from ring-opening of the epoxide by the benzotriazole anion (Scheme 4-5). In this case the epoxide oxygen is protonated rather than acylated.

\[
\text{4.1g} \quad \text{4.3c} \quad \text{Pd(PPh\textsubscript{3})\textsubscript{4}} \quad \text{MW, 130 °C, 50 W (70%)} \quad \text{4.5}
\]

Scheme 4-5. Synthesis of pseudohalohydrin 4.5.

### 4.3 Conclusions

In conclusion, an efficient Pd-catalyzed one-step, regioselective pathway towards pseudohalohydrin esters is reported.

### 4.4 Experimental

#### 4.4.1 General Methods

All reagents were available commercially. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. The NMR spectra were recorded in CDCl\textsubscript{3} with TMS for \textsuperscript{1}H (300 MHz) and \textsuperscript{13}C (75 MHz) as an internal reference. Silica gel was utilized for column chromatography. Aliphatic and aromatic epoxides and carboxylic acid groups were purchased from Aldrich and TCI America and used without further purification.

#### 4.4.2 General Procedure for the Preparation of N-Acylbenzotriazoles 4.1

\[
\text{R COOH} \quad \xrightarrow{\text{SOCl\textsubscript{2}, BtH}} \quad \text{R CO} \quad \text{N=N} \quad \text{4.1}
\]

\[
\text{CH\textsubscript{2}Cl\textsubscript{2}, rt}
\]
Thionyl chloride (0.6 mL, 8.00 mmol, 1.2 equiv) was added to a solution of 1\(^H\)-benzotriazole (3.17 g, 26.67 mmol, 4 equiv) in methylene chloride to give a clear yellow solution that was stirred for 15 min at room temperature. The carboxylic acid (6.67 mmol, 1 equiv) was then added to give a suspension which was stirred for 2.5 h at room temperature. The suspension was filtered, the filtrate evaporated, the residue dissolved in EtOAc and the solution washed with a saturated solution of sodium carbonate. The organic portion was dried over anhydrous MgSO\(_4\), filtered, and dried to give the corresponding \(N\)-acylbenzotriazoles 4.1.

\((1H\text{-}Benzotriazol\text{-}1-yl)(4\text{-}ethylphenyl)methanone (4.1a)\). White microcrystals (91%); mp 112.0 - 113.0 °C; \(^1\)H NMR (CDCl\(_3\)): \(\delta 7.96 (d, J = 8.1\) Hz, 1H), 7.88 - 7.64 (m, 3H), 7.26 (t, \(J = 7.1\) Hz, 1H), 7.11 (t, \(J = 7.2\) Hz, 1H), 7.01 - 6.98 (m, 2H), 2.36 (q, \(J = 7.3\) Hz, 2H), 0.90 (t, \(J = 7.7\) Hz, 3H); \(^{13}\)C NMR (CDCl\(_3\)): \(\delta 166.3, 150.7, 145.5, 132.2, 131.9, 130.1, 128.6, 127.9, 126.0, 119.9, 114.6, 28.9, 15.0\); Anal. Calcd for C\(_{15}\)H\(_{13}\)N\(_3\)O: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.96; H, 5.56; N, 16.97.

\(1\text{-}(1H\text{-}Benzotriazol\text{-}1-yl)\text{-}3\text{-}phenylpropan\text{-}1\text{-}one (4.1b)\). White microcrystals (90%); mp 71.0 - 72.0 °C (lit.\(^{23}\) mp 62.0 - 64.0 °C); \(^1\)H NMR (CDCl\(_3\)): \(\delta 8.26 - 8.22\) (m, 1H), 8.07
- 8.04 (m, 1H), 7.59 (tt, J = 8.1, 1.5 Hz, 1H), 7.45 (tt, J = 7.2, 1.5 Hz, 1H), 7.28 - 7.24 (m, 4H), 7.21 - 7.15 (m, 1H), 3.72 (td, J = 7.8, 1.1 Hz, 2H), 3.19 (t, J = 7.7 Hz, 2H); 13C NMR (CDCl3): δ 171.6, 146.1, 139.8, 131.0, 130.4, 128.6, 128.4, 126.5, 126.1, 120.1, 114.4, 37.1, 30.1; Anal. Calcd for C15H13N3O: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.91; H, 5.59; N, 16.99.

(1H-Benzotriazol-1-yl)(naphthalen-2-yl)methanone (4.1c). White microcrystals (76%); mp 140.0 - 142.0 °C (lit.122 mp 136.0 - 137.0 °C); 1H NMR (CDCl3): δ 8.50 - 8.47 (m, 1H), 8.19 - 8.09 (m, 3H), 7.98 - 7.91 (m, 2H), 7.77 - 7.71 (m, 1H), 7.63 - 7.53 (m, 4H); 13C NMR (CDCl3): δ 167.8, 146.3, 133.7, 133.2, 132.2, 131.2, 130.7, 130.4, 129.5, 128.9, 128.1, 126.9, 126.7, 124.9, 124.5, 120.5, 114.9.

(1H-Benzo[d][1,2,3]triazol-1-yl)(4-nitrophenyl)methanone (4.1d). White microcrystals (75%); mp 199.0 - 200.0 °C (lit.123 mp 194.0 - 196.0 °C); 1H NMR (CDCl3): δ 8.42 - 8.34 (m, 5H), 8.18 (d, J = 8.1 Hz, 1H), 7.75 (t, J = 7.7 Hz, 1H), 7.59 (t, J =7.7 Hz, 1H); 13C NMR (CDCl3): δ 165.2, 150.6, 146.0, 137.1, 132.8, 131.1, 131.2, 127.2, 123.7, 120.7, 114.9.
(1H-Benzotriazol-1-yl)(phenyl)methanone (4.1e). White microcrystals (90%); mp 119.0 - 120.0 °C (lit.\textsuperscript{124} mp 112.0 - 113.0 °C); \(^1\text{H} \text{NMR (CDCl}_3\text{)}: \delta 8.36 \text{ (d, } J = 8.4 \text{ Hz, 1H), 8.23 - 8.15 \text{ (m, 3H), 7.70 - 7.49 \text{ (m, 5H); } ^{13}\text{C} \text{NMR (CDCl}_3\text{)}: \delta 166.8, 145.9, 133.8, 132.5, 131.9, 131.6, 130.5, 128.6, 126.5, 120.3, 114.9.}

2-((3r,5r,7r)-Adamantan-1-yl)-1-(1H-benzo[d][1,2,3]triazol-1-yl)ethanone (4.1f). White microcrystals (74%); mp 92.0 - 93.0 °C (lit.\textsuperscript{125} mp 84.0 - 85.0 °C); \(^1\text{H} \text{NMR (CDCl}_3\text{)}: \delta 8.33 - 8.29 \text{ (m, 1H), 8.11 - 8.07 \text{ (m, 1H), 7.65 - 7.59 \text{ (m, 1H), 7.51 - 7.45 \text{ (m, 1H), 3.19 \text{ (s, 2H), 1.96 \text{ (br s, 3H), 1.73 - 1.74 \text{ (m, 12H); } ^{13}\text{C} \text{NMR (CDCl}_3\text{)}: \delta 171.1, 146.5, 131.2, 130.4, 126.2, 120.3, 114.9, 48.4, 42.7, 36.8, 34.7, 28.8.}

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (4.1g). White microcrystals (90%); mp 150.0 - 152.0 °C (lit.\textsuperscript{5} mp 152.0 - 153.0 °C); \(^1\text{H} \text{NMR (CDCl}_3\text{)}: \delta 8.23 \text{ (d, } J = 7.8 \text{ Hz, 1H), 8.15 \text{ (d, } J = 7.8 \text{ Hz, 1H), 7.68 \text{ (t,}
\[ J = 7.4 \text{ Hz}, 1H \), 7.54 (t, \( J = 7.5 \text{ Hz}, 1H \)), 7.32 - 7.23 (m, 7H), 7.14 (br s, 3H), 6.09 (d, \( J = 4.2 \text{ Hz}, 1H \)), 5.57 (d, \( J = 6.6 \text{ Hz}, 1H \)), 5.08 (s, 2H), 3.48 (d, \( J = 9.6 \text{ Hz}, 1H \)), 3.24 (d, \( J = 7.8 \text{ Hz}, 1H \)); \] 
\[ ^{13}C \text{ NMR (CDCl}_3\text{): } \delta 170.8, 155.7, 146.0, 135.9, 134.9, 131.0, 130.8, 129.2, 128.7, 128.5, 128.1, 127.4, 126.5, 120.4, 114.3, 67.2, 55.6, 38.8. \]

**4.4.3 General Procedure for the Preparation of β-(Benzotriazol-1-yl)ethyl Esters 4.4a-l and 4.5**

![Chemical Reaction](attachment:reaction.png)

To a mixture of \( N \)-acylbenzotriazoles 4.1 (0.20 mmol) and \( \text{Pd(PPh}_3\text{)}_4 \) (23.11 mg, 10 mol%) in a microwave tube was added epoxide 4.3 (1.5 equiv). The mixture was stirred at 130 \( ^{\circ} \text{C} \) and 50 W for 30 min. (\( N \)-arylbenezotriazoles) or 60 min. (\( N \)-alkylbenzotriazoles). The residue was dissolved in MeOH and purified by silica gel column chromatography to obtain the corresponding hydrin esters 4.4. Table 4-1 summarizes optimization of the reaction conditions.

![Chemical Structure](attachment:structure.png)

2-(1H-Benzotriazol-1-yl)-1-phenylethyl 4-ethylbenzoate (4.4a). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 7:3) to obtain a yellow oil, (87%); \( ^1H \text{ NMR (CDCl}_3\text{): } \delta 7.83 (d, J = 8.1 \text{ Hz}, 1H), 7.69 (d, J = 8.1 \text{ Hz}, 1H), 7.27 - \]
7.14 (m, 10H), 7.06 - 7.01 (m, 1H), 6.29 - 6.25 (m, 1H), 5.00 - 4.86 (m, 2H), 2.48 (q, $J = 7.4$ Hz, 2H), 1.03 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 165.3, 150.3, 136.7, 133.4, 129.9, 128.9, 128.0, 127.4, 126.3, 125.7, 123.9, 120.0, 109.3, 74.3, 52.8, 29.0, 15.2; HRMS m/z for C$_{23}$H$_{22}$N$_3$O$_2$ [M+H]$^+$ calcd. 372.1707, found 372.1703.

2-(1H-Benzotriazol-1-yl)-1,2-diphenylethyl 4-ethylbenzoate (4.4b). Purified by gradient silica gel column chromatography (hexanes to hexanes:CH$_2$Cl$_2$, 3:2, then hexanes:CH$_2$Cl$_2$, 1:1) to obtain beige microcrystals, (62%), mp 109.0 - 110.0 °C; $^1$H NMR (CDCl$_3$): $\delta$ 8.09 (dd, $J = 8.3$, 1.4 Hz, 1H), 7.68 - 7.60 (m, 2H), 7.54 - 7.47 (m, 1H), 7.45 - 7.30 (m, 6H), 7.27 - 7.20 (m, 6H), 7.14 - 7.11 (m, 2H), 6.34 (dd, $J = 9.2$, 1.4 Hz, 1H), 2.82 - 2.73 (m, 1 H), 2.65 (q, $J = 7.5$ Hz, 2H), 1.35 - 1.28 (m, 2H), 1.22 (td, $J = 7.8$, 2.1 Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 165.0, 149.9, 136.8, 134.6, 133.3, 130.2, 129.6, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.7, 127.3, 127.2, 126.8, 125.9, 123.9, 120.1, 109.6, 76.8, 67.4, 28.8, 15.1; HRMS m/z for C$_{29}$H$_{26}$N$_3$O$_2$ [M+H]$^+$ calcd.448.2020, found 448.2022.
1-(1H-Benzo[d][1,2,3]triazol-1-yl)hexan-2-yl 3-phenylpropanoate (4.4c). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 4:1) to obtain yellow oil, (72%); \(^1\)H NMR (CDCl\(_3\)): δ 8.05 - 8.01 (m, 1H), 7.49 - 7.42 (m, 2H), 7.36 - 7.31 (m, 1H), 7.29 - 7.07 (m, 5H), 5.28 - 5.21 (m, 1H), 4.74 (dd, \(J = 14.6, 4.8\) Hz, 1H), 4.69 (dd, \(J = 14.5, 6.1\) Hz, 1H), 2.97 - 2.40 (m, 4H), 1.59 - 1.52 (m, 2H), 1.30 - 1.19 (m, 4H), 0.83 (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C NMR (CDCl\(_3\)): δ172.4, 145.9, 140.27, 133.7, 128.6, 128.4, 127.7, 126.5, 124.2, 120.2, 109.7, 72.5, 50.9, 35.9, 31.5, 30.9, 27.3, 22.6, 14.1.

2-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-phenylethyl 1-naphthoate (4.4d). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 9.3:0.7) to obtain white solid, (62%); mp 62.0 - 63.0°C \(^1\)H NMR (CDCl\(_3\)): δ 8.60 - 8.56 (m, 1H), 8.09 - 7.97 (m, 3H), 7.85 - 7.79 (m, 1H), 7.50 - 7.26 (m, 11H), 6.58 (dd, \(J = 7.5, 4.7\) Hz, 1H), 5.19 (dd, \(J = 14.6, 7.5\) Hz, 1H), 5.11 (dd, \(J = 14.6, 4.7\) Hz, 1H); \(^{13}\)C NMR (CDCl\(_3\)): δ 166.1, 146.0, 136.9, 134.0, 133.9, 133.6, 131.4, 130.6, 129.2, 128.7, 128.1, 127.7, 126.6, 126.5, 125.7, 124.6, 124.1, 120.3, 109.5, 74.7, 53.1; Anal. Calcd for C\(_{25}\)H\(_{19}\)N\(_3\)O\(_2\): C, 76.32; H, 4.87; N, 10.68. Found: C, 75.97; H, 5.31; N, 10.45.
1-(1H-Benzo[d][1,2,3]triazol-1-yl)hexan-2-yl 1-naphthoate (4.4e). Purified by gradient silica gel column chromatography (hexanes to EtOAc:hexanes, 9.3:0.7) to obtain a yellow oil (73%); $^1$H NMR (CDCl$_3$): $\delta$ 8.64 - 8.59 (m, 1H), 8.02 - 7.89 (m, 3H), 7.79 - 7.75 (m, 1H), 7.51 - 7.17 (m, 6H), 5.61 - 5.53 (m, 1H), 4.88 (d, $J$ = 5.3 Hz, 2H), 1.78 - 1.70 (m, 2H), 1.51 - 1.22 (m, 4H), 0.81 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 166.8, 146.1, 133.9, 133.7, 131.5, 130.5, 128.7, 128.0, 127.6, 126.5, 126.4, 125.7, 124.6, 124.1, 120.2, 109.9, 72.8, 51.0, 31.7, 27.6, 22.6, 14.1; Anal. Calcd for C$_{69}$H$_{73}$N$_9$O$_8$: C, 71.67; H, 6.36; N, 10.90. Found: C, 71.53; H, 6.38; N, 10.93.

2-(1H-Benzo[d][1,2,3]triazol-1-yl)-1-phenylethyl 4-nitrobenzoate (4.4f). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 9:1) to obtain a yellow oil (70%); $^1$H NMR (CDCl$_3$): $\delta$ 8.31 - 8.11 (m, 7H), 7.52 - 7.34 (m, 5H), 6.44 (dd, $J$ = 8.4, 3.6 Hz, 1H), 4.82 (dd, $J$ = 12.0, 8.4 Hz, 1H), 4.71(dd, $J$ =12.2, 3.8 Hz, 1H); $^{13}$C NMR (CDCl$_3$): $\delta$ 164.5, 164.0, 151.0, 135.6, 135.2, 135.1, 131.1, 131.0, 129.5, 129.3, 126.9, 123.9, 75.1, 67.3.
2-(1H-Benztetrazol-1-yl)-1-phenylethyl benzoate (4.4g). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 9:1) to obtain a yellow oil (75%); $^1$H NMR (CDCl$_3$): $\delta$ 8.02 - 7.93 (m, 3H), 7.55 - 7.27 (m, 11H), 6.46 (dd, $J = 7.5$, 4.8 Hz, 1H), 5.15 (dd, $J = 14.5$, 7.3 Hz, 1H), 5.07 (dd, $J = 14.5$, 4.8, 1H); $^{13}$C NMR (CDCl$_3$): $\delta$ 165.4, 145.9, 136.8, 133.6, 129.9, 129.5, 129.2, 129.1, 128.6, 127.6, 126.5, 124.1, 120.2, 109.4, 74.7, 52.9; HRMS m/z for C$_{21}$H$_{18}$N$_3$O$_2$ [M+H]$^+$ calcd. 344.1394, found 344.1384.

1-(1H-Benzo[d][1,2,3]triazol-1-yl)hexan-2-yl benzoate (4.4h). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 9:1) to obtain a yellow oil (76%); $^1$H NMR (CDCl$_3$): $\delta$ 8.03 - 8.00 (m, 1H), 7.91 - 7.88 (m, 2H), 7.55 - 7.49 (m, 2H), 7.40 - 7.28 (m, 4H), 5.55 - 5.48 (m, 1H), 4.90 (d, $J = 5.2$ Hz, 2H), 1.76 - 1.67 (m, 2H), 1.47 - 1.26 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 166.1, 146.1, 133.7, 133.5, 129.9, 129.7, 128.6, 127.6, 124.1, 120.2, 109.9, 72.9, 50.9, 31.5, 27.5, 22.6, 14.0; HRMS m/z for C$_{19}$H$_{22}$N$_3$O$_2$ [M+H]$^+$ calcd. 324.1707, found 324.1719.
(4.4i). Purified by gradient silica gel column chromatography (hexanes to hexanes:EtOAc, 9.3:0.7) to obtain a yellow oil (52%); $^1$H NMR (CDCl$_3$): $\delta$ 8.00 - 7.97 (m, 1H), 7.56 (d, $J$ = 8.4 Hz, 1H), 7.47 - 7.41 (m, 1H), 7.33 - 7.28 (m, 1H), 5.27 - 5.19 (m, 1H), 4.72 - 4.69 (m, 2H), 2.05 - 1.88 (m, 3H), 1.84 - 1.80 (m, 2H), 1.63 - 1.55 (m, 8H), 1.49 - 1.46 (m, 2H), 1.37 - 1.18 (m, 8H), 0.82 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 171.3, 146.0, 133.8, 127.7, 124.1, 120.2, 109.9, 71.9, 50.9, 49.0, 42.5, 42.4, 36.9, 36.8, 32.9, 31.7, 28.8, 28.7, 27.5, 22.6, 14.1; HRMS m/z for C$_{24}$H$_{33}$N$_3$O$_2$Na [M+Na]$^+$ calcd. 418.2465, found 418.2480.

(4.5). Purified by gradient silica gel column chromatography (hexanes to EtOAc:hexanes, 4:1) to obtain a yellow oil (70%); $^1$H NMR (CDCl$_3$): $\delta$ 7.96 (dd, $J$ = 8.4, 0.9 Hz, 1H), 7.60 (dd, $J$ = 8.7, 0.9 Hz, 1H), 7.50 - 7.45 (m, 1H), 7.36 - 7.30 (m, 1H), 4.71 - 4.64 (m, 2H), 4.56 - 4.49 (m, 1H), 4.25 (br s, 1H), 1.65 - 1.34 (m, 6H), 0.92 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 145.5, 133.8, 128.5, 127.4, 124.0, 119.7, 109.9, 71.0, 54.0, 34.3, 27.6, 22.6, 14.0; HRMS m/z for C$_{12}$H$_{17}$N$_3$ONa [M+Na]$^+$ calcd. 242.1264, found 242.1266.
CHAPTER 5
SOLUTION-PHASE SYNTHESIS OF CHIRAL O-ACYL ISODIPEPTIDES

5.1 Introduction

The synthesis of peptides and proteins is of great importance to the understanding of biological function. Automated solid-phase peptide synthesis has empowered rapid and convenient preparation of target peptides. However, the phenomena of aggregation in the synthesis of long peptides, proteins and peptides with “difficult sequence” remains problematic, resulting in low yields and purity. The aggregation is attributed to intermolecular hydrophobic interactions in “difficult sequences” in solution. Hydrogen bond networks in resin-bound peptides can form extended structures such as β-sheets (Figure 5-1). This phenomenon depends on the nature of the peptide and side chain protecting groups, commonly occurring in peptides with sequences containing numerous Ala, Val, Ile, Asn and Gln residues.

Figure 5-1. β-Sheets

Existing strategies to overcome such associations comprise “external factors”

like solvent composition, elevated temperature, and use of chaotropic salts or solubilizing protecting groups but have been reported to have variable efficiencies.\textsuperscript{136}

Sheppard and Johnson et al. developed an N-alkylation system, the 2-hydroxy-4-methoxybenzyl (Hmb) building block, introduced as an amide protecting group, thus preventing hydrogen-bonding.\textsuperscript{140} Mutter introduced pseudo-proline units which are essentially Ser/Thr-derived oxazolidine and Cys-derived thiazolodine derivatives. These have been reported to induce a “kink” conformation in the peptide backbone, originating in the preference for cis amide bond formation, thus, preventing peptide aggregation, self-association. However, this methodology requires 2-6 step modification of Fmoc amino acids to synthesize the building blocks in solution. In addition, the removal of such building blocks proved to be difficult, requiring strong acid treatments (Figure 5-2).\textsuperscript{139,141,142}

Figure 5-2. Pseudo-prolines.\textsuperscript{128,130,131}

Kiso et al.\textsuperscript{130} demonstrated that the introduction of an O-acyl in place of an N-acyl residue within a peptide backbone significantly altered the secondary structure of native
Furthermore, these “O-acyl isopeptides” or “click peptides” (Kiso denotes O-acyl peptides as “click peptides” because of their easy conversion to target native peptides under physiological conditions) are more hydrophilic, and easier to purify by HPLC. The α-hydroxy-β-amino acids have higher water solubility because of the newly formed and ionized amino group. He found that a subsequent O-N intramolecular acyl migration, triggered by change in pH, could rapidly generate a target natural peptide under physiological conditions (pH 7.4) (Figure 5-3). This “O-acyl isopeptide method” has been used to develop new water-soluble taxoid prodrugs, HIV-1 protease inhibitors, the anti-tumor agent, paclitaxel, difficult sequence-containing peptides including Ac-Val-Val-Ser-Val-Val-NH₂, Alzeheimer's disease-related amyloid β peptide (Aβ) 1–42, and cyclic peptides.

![Figure 5-3. O-Acyl isopeptide methodology.](image)

However, epimerization during the esterification step in the solid-phase synthesis of O-acyl isopeptides remained a major problem. Based on the hypothesis that epimerization during esterification should be suppressed in solution by a faster coupling rate compared to that on a solid support, Kiso synthesized O-acyl isodipeptides in three steps (Scheme 5-1): (i) protection of the carboxylic acid group in serine or threonine by benzyl esterification, (ii) O-acylation and (iii) deprotection using
Pd/C. Treatment of Cbz-protected isodipeptides containing Cys and Met with H₂ over Pd/C failed, although catalytic hydrogen transfer (CTH) to Cys- and Met-containing protected isodipeptides gave 45% of the desired product.¹²⁹

Scheme 5-1. Literature reported synthetic scheme of “O-acyl isodipeptide unit”.¹²⁹

The chapter reports an efficient single-step preparation of chiral O-acyl isodipeptides from serine and threonine. Advantageous N-acylbenzotriazole methodology was used for such transformations.¹⁹,⁵¹,⁸⁹ N-(Protected-α-aminoacyl)benzotriazoles have enabled fast preparations of biologically relevant peptides and peptide conjugates in high yields and purity, under mild reaction conditions, with full retention of the original chirality.⁸⁹

5.2 Results and Discussion

5.2.1 Synthesis of Serine-based O-Acylisodipeptides

O-Acyl isoserinedipeptides 5.3a–h were prepared by O-acylation of Boc-protected serine 5.1a with various N-Pg-(α-aminoacyl)benzotriazoles 5.2 in the
presence of diisopropylethylamine in CH$_3$CN at 23 °C for 12 h in yields of 74–90%.

These proved to be the optimum conditions under which neither epimerization of 5.3 nor hydrolysis of 5.2 occurred. The presence of water (MeCN/H$_2$O, 9 : 1) in the reaction caused minimal hydrolysis of 5.2 (4%) but no epimerization was detected by HPLC-MS analysis on 5.3b and (5.3b + 5.3b'). The O-acylated serine targets were characterized by NMR and elemental analysis (Table 5-1).
Table 5-1. The preparation of serine-based O-acyl-isodipeptides 5.3a-h.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>O-acyl isodipeptides 5.3a-g</th>
<th>Yield %</th>
<th>mp/° C</th>
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</thead>
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<td>Boc-L-Ser(Cbz-L-Ala)OH 5.3a</td>
<td>84</td>
<td>oil</td>
</tr>
<tr>
<td>2</td>
<td>Boc-L-Ser(Cbz-L-Phe)OH 5.3b</td>
<td>81</td>
<td>oil</td>
</tr>
<tr>
<td>3</td>
<td>Boc-L-Ser(Cbz-DL-Phe)OH(5.3b+5.3b')</td>
<td>81</td>
<td>60-62</td>
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<td>Boc-L-Ser(Cbz-L-Trp)OH 5.3c</td>
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<td>67-69</td>
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<tr>
<td>5</td>
<td>Boc-L-Ser(Cbz-L-Met)OH 5.3d</td>
<td>74</td>
<td>oil</td>
</tr>
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<td>Boc-L-Ser(Cbz-L-Val)OH 5.3e</td>
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<tr>
<td>7</td>
<td>Boc-L-Ser(Cbz-Gly)OH 5.3f</td>
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<td>57-58</td>
</tr>
<tr>
<td>8</td>
<td>Boc-L-Ser(Cbz-L-Cys(Bzl)OH 5.3g</td>
<td>85</td>
<td>oil</td>
</tr>
<tr>
<td>9</td>
<td>Boc-L-Ser(Boc-Gly)OH 5.3h</td>
<td>82</td>
<td>58-59</td>
</tr>
</tbody>
</table>

HPLC analysis [chirobiotic T column (250 mm × 4.6 mm), detection at 254 nm, flow rate 2.5 mL min⁻¹, MeOH] on 5.3b (single peak, retention time 1.3 min) and (5.3b + 5.3b') (two peaks, retention times, 1.3 min and 1.5 min) as well as HPLC-MS and (-) ESI-MS
on 5.3b (Figure 5-4) confirmed the absence of racemization in the targeted isodipeptides.

Figure 5-4. HPLC-MS and (−)ESI-MS analysis of 5.3b.

5.2.2 Synthesis of Threonine-based O-Acylisodipeptides

O-Acylated threonine-based isodipeptides 5.4a-h were also prepared by O-acylation of Boc-protected threonine 5.1b with various N-Pg-(α-aminoacyl)benzotriazoles 5.2 in the presence of diisopropylethylamine in CH₃CN at room temperature in yields of 86–91% (Table 5-2). The synthesized compounds were characterized by NMR and elemental analysis.
Table 5-2. The preparation of threonine-based O-acyl-isodipeptides 5.4a-h.

![Chemical formula and reaction scheme]

<table>
<thead>
<tr>
<th>Entry</th>
<th>O-acyl isodipeptides 5.4a-f</th>
<th>Yield (%)</th>
<th>mp/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boc-L-Thr(Cbz-L-Ala)-OH 5.4a</td>
<td>91</td>
<td>oil</td>
</tr>
<tr>
<td>2</td>
<td>Boc-L-Thr(Cbz-L-Phe)-OH 5.4b</td>
<td>87</td>
<td>oil</td>
</tr>
<tr>
<td>3</td>
<td>Boc-L-Thr(Cbz-D,L-Phe)-OH (5.4b+5.4b')</td>
<td>87</td>
<td>60-62</td>
</tr>
<tr>
<td>4</td>
<td>Boc-L-Thr(Cbz-L-Trp)-OH 5.4c</td>
<td>91</td>
<td>89-90</td>
</tr>
<tr>
<td>5</td>
<td>Boc-L-Thr(Cbz-L-Met)-OH 5.4d</td>
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<td>6</td>
<td>Boc-L-Thr(Cbz-Gly)-OH 5.4e</td>
<td>86</td>
<td>54-58</td>
</tr>
<tr>
<td>7</td>
<td>Boc-L-Thr(Cbz-L-Cys(Bzl))-OH 5.4f</td>
<td>86</td>
<td>oil</td>
</tr>
<tr>
<td>8</td>
<td>Boc-L-Thr(Boc-Gly)-OH 5.4g</td>
<td>85</td>
<td>64-65</td>
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<td>9</td>
<td>Boc-L-Thr(Boc-L-Phe)-OH 5.4h</td>
<td>90</td>
<td>47-50</td>
</tr>
</tbody>
</table>

HPLC analysis [chirobiotic T column (250 mm × 4.6 mm), detection at 254 nm, flow rate 0.5 mL min⁻¹, MeOH : H₂O, 4 : 1] on 5.4b (single peak, retention time 7.23 min) and (5.4b + 5.4b') (two peaks, retention times, 6.54 min and 7.20 min) confirmed the retention of chirality and lack of racemization in the desired isodipeptides.
5.3 Conclusions

In conclusion, the mild protocol reported herein enables the efficient preparation of optically pure O-acyl isopeptides from serine and threonine without protection of their carboxyl groups.

5.4 Experimental

5.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. The NMR spectra were recorded in CDCl₃ with TMS for ¹H (300 MHz) and ¹³C (75 MHz) as an internal reference.

5.4.2 General Procedure for the Preparation of N-(Pg-α-Aminoacyl) Benzotriazoles 5.2

*N-(Z-α-Aminoacyl)benzotriazoles 5.2.* Thionyl chloride (0.6 mL, 8.00 mmol, 1.2 equiv) was added to a solution of ¹H-benzotriazole (3.17 g, 26.67 mmol, 4 equiv) in methylene chloride to give a clear yellow solution that was stirred for 15 min at room temperature. The amino acid (6.67 mmol, 1 equiv) was then added to give a suspension which was stirred for 2.5 h at room temperature. The suspension was filtered, the filtrate evaporated, the residue dissolved in EtOAc and the solution was washed with a saturated solution of sodium carbonate. The organic portion was dried over anhyd MgSO₄, filtered, and evaporated to give the corresponding N-(Z-α-aminoacyl)benzotriazole 5.2.

*N-(Boc-α-Aminoacyl)benzotriazoles 5.2.* Boc-protected amino acid (0.03 mol) was added to a solution of DCC (1 equiv) in methylene chloride under an atmosphere of nitrogen. After 30 min., BtH (1 equiv) was added and the mixture was stirred for 12 h. The suspension was filtered through a bed of silica and celite, the filtrate evaporated,
and the residue dissolved in EtOAc, then filtered through a bed of silica and celite and washed with a solution of saturated sodium carbonate, then with water and brine. The organic portion was dried over anhyd MgSO$_4$, filtered on a bed of silica, and evaporated to give the corresponding N-(Boc-α-aminoacyl)benzotriazole 5.2. $^1$H NMR and mp of Boc-Gly-Bt 5.2h and Boc-L-Phe-Bt 5.2i were found in agreement with that reported in the literature.$^{157,158}$

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxopropan-2-yl)carbamate (5.2a).
White solid (90%); mp 115 °C (lit.$^{159}$ mp 113 - 115 °C); $^1$H NMR (CDCl$_3$): $\delta$ 8.16 (d, $J$ = 8.1 Hz, 1H), 8.04 (d, $J$ = 8.4 Hz, 1H), 7.57 (t, $J$ = 7.8 Hz, 1H), 7.43 (t, $J$ = 7.7 Hz, 1H), 7.40 - 7.03 (m, 6H), 5.80 - 5.60 (m, 2H), 5.10 - 4.99 (m, 1H), 1.59 (d, $J$ = 6.3 Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 172.2, 155.6, 145.9, 136.0, 131.0, 130.6, 128.4, 128.1, 126.4, 120.2, 114.3, 67.1, 50.5, 19.0.

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (5.2b). White solid (90%); mp 150 - 152 °C (lit.$^{160}$ mp 149.0 - 150.0 °C); $^1$H NMR (CDCl$_3$): $\delta$ 8.23 (d, $J$ = 7.8 Hz, 1H), 8.15 (d, $J$ = 7.8 Hz, 1H), 7.68 (t, $J$ = 7.4 Hz, 1H), 7.54 (t, $J$ = 7.5 Hz, 1H), 7.32 - 7.23 (m, 7H), 7.14 (br s, 3H), 6.09 (d, $J$ = 4.2 Hz, 1H), 5.57 (d, $J$ = 6.6 Hz, 1H), 5.08 (s, 2H), 3.48 (d, $J$ = 9.6 Hz, 1H), 3.24 (d, $J$ = 7.8 Hz, 1H); $^{13}$C NMR (CDCl$_3$): $\delta$ 170.8, 155.7, 146.0, 135.9, 134.9, 131.0, 130.8, 129.2, 128.7, 128.5, 128.1, 127.4, 126.5, 120.4, 114.3, 67.2, 55.6, 38.8.

Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (5.2b'). White solid (90%); mp 141 - 142 °C (lit.$^{22}$ mp 141 - 142 °C); $^1$H NMR (CDCl$_3$): $\delta$ 8.23 (d, $J$ = 7.8 Hz, 1H), 8.15 (d, $J$ = 7.8 Hz, 1H), 7.68 (t, $J$ = 7.4 Hz, 1H), 7.54 (t, $J$ = 7.5 Hz, 1H), 7.32 - 7.23 (m, 7H), 7.14 (br s, 3H), 6.09 (d, $J$ = 4.2 Hz, 1H),
5.57 (d, J = 6.6 Hz, 1H), 5.08 (s, 2H), 3.48 (d, J = 9.6 Hz, 1H), 3.24 (d, J = 7.8 Hz, 1H);

$^{13}$C NMR (CDCl$_3$): $\delta$ 170.8, 155.7, 146.0, 135.9, 134.9, 131.0, 130.8, 129.2, 128.7, 128.5, 128.1, 127.4, 126.5, 120.4, 114.3, 67.2, 55.6, 38.8.

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate (5.2c). Yellow solid (80%); mp 101 °C (lit.$^{157}$ mp 98 - 100 °C); $^1$H NMR (CDCl$_3$): $\delta$ 8.23 (br s, 1H), 8.14 - 8.06 (m, 2H), 7.57 (t, J = 7.5 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.31 - 7.24 (m, 4H), 7.20 (s, 1H), 7.10 - 7.04 (m, 1H), 6.95 - 6.90 (m, 2H), 6.87 - 6.85 (m, 1H), 6.14 - 6.10 (m, 1H), 5.70 (d, J = 7.5 Hz, 1H), 5.03 (s, 2H), 3.58 (dd, J = 15.0, 4.5 Hz, 1H), 3.40 (dd, J = 14.9, 7.7 Hz, 1H); $^{13}$C NMR (CDCl$_3$): $\delta$ 171.1, 155.9, 145.8, 136.1, 131.0, 130.6, 128.4, 128.1, 127.0, 126.4, 123.2, 122.2, 120.2, 119.7, 118.3, 114.3, 111.2, 108.9, 67.2, 55.1, 28.7.

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-4-(methylthio)-1-oxobutan-2-yl)carbamate (5.2d). Beige solid (85%); mp 108 - 109 °C (lit.$^{157}$ mp 108 - 109 °C); $^1$H NMR (CDCl$_3$): $\delta$ 8.15 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.44 (t, J = 7.7 Hz, 1H), 7.30 - 7.24 (m, 5H), 5.83 - 5.79 (m, 1H), 5.67 - 5.65 (m, 1H), 5.03 (s, 2H), 2.59 (t, J = 7.1 Hz, 2H), 2.35 (br s, 1H), 2.10 - 2.03 (m, 1H), 1.97 (s, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$ 171.2, 155.7, 130.9, 146.0, 130.8, 128.4, 128.5, 128.3, 126.6, 120.4, 114.3, 67.4, 54.2, 32.4, 30.0, 15.4; Anal. Calcd for C$_{19}$H$_{20}$N$_{4}$O$_{3}$S: C, 59.36; H, 5.23; N, 14.57. Found: C, 59.69; H, 5.23; N, 14.54.

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (5.2e). White powder (87%); mp 107 °C (lit.$^{160}$ mp 73 - 74 °C); $^1$HNMR (CDCl$_3$): $\delta$ 8.28 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 7.68 (dd, J = 8.0, 7.4 Hz, 1H), 7.54 (dd, J =
8.1, 7.3 Hz, 1H), 7.37 (br s, 5H), 5.78 - 5.74 (m, 1H), 5.57 (d, J = 9.2 Hz, 1H), 5.14 (s, 2H), 2.61 - 2.43 (m, 1H), 1.13 (d, J = 6.6 Hz, 3H), 0.97 (d, J = 6.6 Hz, 3H); $^{13}$C NMR (CDCl$_3$): δ 171.5, 156.2, 146.0, 136.0, 131.0, 130.7, 128.5, 128.2, 126.5, 120.3, 114.3, 67.3, 59.4, 31.6, 19.7, 17.0.

(S)-Benzyl (2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-oxoethyl) carbamate ($5.2f$). White solid (90%); mp 110 - 111 °C (lit.$^{161}$ mp 106 - 108 °C); $^1$H NMR (CDCl$_3$): δ 8.24 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.39 - 7.34 (m, 5H), 6.03 (br s, 1H), 5.21 (s, 2H), 5.09 (d, J = 5.7 Hz, 2H); $^{13}$C NMR (CDCl$_3$): δ 168.3, 156.4, 145.9, 136.0, 130.8, 128.5, 128.2, 128.1, 126.5, 120.3, 114.0, 67.4, 44.8.

(R)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-(benzylthio)-1-oxopropan-2-yl)carbamate ($5.2g$). Yellow oil (87%); $^1$H NMR (CDCl$_3$): δ 8.31 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.1 Hz, 1H), 7.74 (t, J = 8.1 Hz, 1H), 7.60 (t, J = 8.1 Hz, 1H), 7.47 - 7.17 (m, 10H), 6.03 (br s, 2H), 5.22 (br s, 2H), 3.79 (br s, 2H), 3.28 - 2.89 (m, 2H); $^{13}$C NMR (CDCl$_3$): δ 169.8, 155.7, 145.9, 136.0, 135.9, 130.8, 128.8, 128.4, 128.2, 128.1, 127.1, 126.6, 125.8, 120.3, 114.3, 67.4, 53.7, 36.1, 33.6; Anal. Calcd for C$_{24}$H$_{22}$N$_4$O$_3$S: C, 64.56; H, 4.51; N 12.55. Found: C, 64.26; H, 4.93; N 12.90.

5.4.3 General Procedure for the Preparation of O-Acyl Isodipeptides 5.3 and 5.4

DIPEA (0.44 mL, 3 equiv) was added to a solution of Boc-LSerOH or Boc-L-ThrOH (0.49 mmol, 1 equiv) in MeCN (15 mL). The appropriate N-(Pg-a-aminoacyl)benzotriazole $5.2$ (0.49 mmol, 1 equiv) dissolved in MeCN (5 mL) was added to the clear solution and the mixture was stirred for 12 h at room temperature.

Complete reaction was judged by the disappearance of starting material. The solution was acidified with 1N HCl and evaporated; the residue was dissolved in EtOAc
and washed with 1N HCl. The organic portion was dried over anhyd Na₂SO₄, filtered and evaporated to give the corresponding O-acyl isodi peptide. All samples were then freeze-dried and fully characterized.

(R)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)propanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3a). Prepared from 5.2a. Colorless oil (84%); ¹H NMR (CDCl₃): δ 10.2 (br s, 1H), 7.27 - 7.15 (m, 5H), 5.62 - 5.49 (m, 1H), 5.25 - 4.96 (m, 2H), 4.63 - 4.29 (m, 4H), 4.10 - 3.98 (m, 1H), 1.36 (s, 9H); ¹³C NMR (CDCl₃): δ 172.8, 172.4, 156.1, 155.6, 136.0, 128.4, 128.1, 80.6, 67.1, 64.9, 52.7, 49.7, 28.2, 18.1; Anal. Calcd for C₁₉H₂₆N₂O₈: C, 55.60; H, 6.39; N, 6.83. Found: C, 55.31; H, 6.41; N, 6.70.

(R)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3b). Prepared from 5.2b. Yellow transparent oil (81%); ¹H NMR (CDCl₃): δ 10.8 (bs, 1H), 7.31 - 7.18 (m, 10H), 7.11 - 7.05 (m, 2H), 5.53 - 5.44 (m, 1H), 5.10 - 4.97 (m, 2H), 4.64 - 4.55 (m, 2H), 4.41 - 4.32 (m, 2H), 3.04 - 2.98 (m, 1H), 1.42 (br s, 9H); ¹³C NMR (CDCl₃): δ 172.6, 171.2, 156.0, 135.5, 129.3, 129.1, 128.6, 128.5, 128.4, 128.1, 128.0, 127.2, 80.6, 67.2, 65.2, 54.9, 52.5, 38.0, 28.2; Anal. Calcd for C₂₅H₃₀N₂O₈: C, 61.72; H, 6.22; N, 5.76. Found: C, 61.79; H, 6.34; N, 5.42.

3-(((S)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3b + 5.3b'). Prepared from (5.2b + 5.2b'). Colorless solid (81%); mp 60 - 62 °C; ¹H NMR (CDCl₃): δ 8.85 (br s, 1H), 7.33 - 7.10 (m, 10H), 7.05 - 6.95 (m, 2H), 5.59 - 5.41 (m, 1H), 5.03 (bs, 2H), 4.64 - 4.17 (m, 3H), 3.03 (bs, 2H), 1.41 (bs, 9H), 1.23 - 1.18 (m, 3H); ¹³C NMR (CDCl₃): δ 175.2, 172.5, 171.1, 156.0, 155.5, 135.9, 135.5, 129.1, 128.5, 128.4, 128.0, 127.1, 80.5, 67.1, 65.2, 60.5,
54.9, 53.6, 52.5, 38.8, 37.9, 37.7, 29.6, 28.2, Anal. Calcd for C\textsubscript{25}H\textsubscript{30}N\textsubscript{2}O\textsubscript{8}: C, 61.72; H, 6.22; N, 5.76. Found: C, 61.79; H, 6.35; N, 5.42.

\((S)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-3-(1H-indol-3-yl)propanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3c)\). Prepared from 5.2c. Yellow gel (85%); \(^1\)H NMR (CDCl\textsubscript{3}): δ 8.37 - 8.31 (m, 1H), 7.92 (br s, 1H), 7.55 - 7.42 (m, 1H), 7.25 - 7.04 (m, 7H), 6.90 - 6.81 (m, 1H), 5.50 - 5.44 (m, 1H), 5.12 - 4.98 (m, 2H), 4.70 - 4.65 (m, 1H), 4.54 (br s, 1H), 4.38 - 4.28 (m, 1H), 3.31 - 3.21 (m, 2H), 1.44 (br s, 9H); \(^{13}\)C NMR (CDCl\textsubscript{3}): δ 175.9, 172.8, 171.6, 156.1, 155.7, 136.1, 128.5, 128.1, 127.3, 123.0, 122.0, 120.0, 118.3, 111.4, 109.3, 80.7, 67.2, 65.0, 54.7, 52.7, 28.3; Anal. Calcd for C\textsubscript{27}H\textsubscript{31}N\textsubscript{3}O\textsubscript{8}: C, 61.71; H, 5.95; N, 8.00. Found: C, 61.96; H, 6.00; N, 7.61.

\((R)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-4-(methylthio)butanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3d)\). Prepared from 5.2d. Yellow oil (74%); \(^1\)H NMR(CDCl\textsubscript{3}): δ 9.40 (bs,1H), 7.27 - 7.12 (m, 5H), 5.72 - 5.62 (m, 1H), 5.08 - 4.97 (m, 2H), 4.57 - 4.42 (m, 3H), 2.49 - 2.41 (m, 2H), 2.22 - 1.84 (m, 5H), 1.36 (br s, 9H); \(^{13}\)C NMR (CDCl\textsubscript{3}): δ 175.7, 172.7, 171.4, 156.2, 155.5, 135.8, 128.4, 128.1, 80.6, 67.2, 65.1, 53.2, 52.7, 31.5, 29.7, 28.2,15.2; Anal. Calcd for C\textsubscript{21}H\textsubscript{30}N\textsubscript{2}O\textsubscript{8}S: C, 53.60; H, 6.43; N, 5.95. Found: C, 53.94; H, 6.56; N, 5.52.

\((S)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-3-methylbutanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3e)\). Prepared from 5.2e. Yellow oil (90%); \(^1\)H NMR (CDCl\textsubscript{3}): δ 9.54 (br s, 1 H), 7.34 - 7.22 (m, 5H), 5.80 - 5.51 (m, 1H), 5.12 - 5.00 (m, 2H), 4.62 - 4.02 (m, 3H), 2.13 - 2.00 (m, 1H), 1.39 (br s, 9H), 0.96 - 0.83 (m, 6H); \(^{13}\)C NMR (CDCl\textsubscript{3}): δ 172.5, 171.4, 156.5, 155.5, 135.9,128.4, 128.1, 80.5, 67.2, 64.7, 59.1,
52.7, 31.0, 28.2, 18.9, 17.4; Anal. Calcd for C$_{21}$H$_{30}$N$_2$O$_8$: C 57.52; H 6.90; N 6.39.

Found: C:57.79, H 6.69, N 6.45.

(S)-3-(2-(((Benzyloxy)carbonyl)amino)acetoxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3f). Prepared from 5.2f. Colorless solid (87%); mp 57 - 58 ºC; $^1$H NMR (CDCl$_3$): δ 10.18 (br s, 1H), 7.13 - 7.11 (m, 5H), 5.62 - 5.47 (m, 1H), 4.90 (br s, 2H), 4.41 - 4.17 (m, 2H), 3.73 (br s, 2H), 1.23 (br s, 9H); $^{13}$C NMR (CDCl$_3$): δ 172.5, 169.7, 156.7, 155.5, 135.9, 128.4, 128.0, 80.5, 67.2, 65.0, 52.5, 42.5, 28.2; Anal. Calcd for C$_{18}$H$_{24}$N$_2$O$_8$: C, 54.54; H, 6.10; N, 7.07. Found: C, 54.72; H, 6.04; N, 6.73.

(S)-3-(((R)-2-(((Benzyloxy)carbonyl)amino)-3-(benzylthio)propanoyl)oxy)-2-((tert-butoxycarbonyl)amino)propanoic acid (5.3g). Prepared from 5.2g. Yellow gel (85%); $^1$H NMR (CDCl$_3$): δ 10.9 (br s, 1H), 7.49 - 7.16 (m, 11H), 5.81 - 5.71 (m, 1H), 5.09 - 4.98 (m, 2H), 4.61 - 4.32 (m, 3H), 3.66 (s, 2H), 2.79 - 2.73 (m, 2H), 1.36 (br s, 9H); $^{13}$C NMR (CDCl$_3$): δ 172.3, 170.1, 156.0, 155.4, 137.2, 135.7, 128.8, 128.4, 128.0, 127.1, 80.4, 67.2, 65.2, 53.3, 52.5, 36.1, 33.1, 28.1; Anal. Calcd for C$_{26}$H$_{32}$N$_2$O$_8$S: C, 58.63; H, 6.06; N, 5.26. Found: C, 58.95; H, 6.08; N, 5.12.

(S)-2-(((Tert-butoxycarbonyl)amino)-3-((tert-butoxycarbonyl)amino)acetoxy)propanoic acid (5.3h). Prepared from 5.2h. White microcrystals (82%); mp 58 - 59 ºC; $^1$H NMR (CDCl$_3$): δ 7.38 - 7.28 (s, 2H), 5.59 - 5.20 (m, 1H), 4.58 - 4.45 (m, 3H), 3.86 (d, J = 4.2 Hz, 2H), 1.38 (s,18H); $^{13}$C NMR (CDCl$_3$): δ 172.3, 170.1,156.1, 155.6, 126.1, 114.9, 82.1, 80.5, 65.1, 52.7, 43.9, 42.3, 28.3; Anal. Calcd for C$_{15}$H$_{26}$N$_2$O$_8$: C, 49.72; H, 7.23; N, 7.73. Found:C, 50.41; H, 7.29; N, 8.16.

(2S)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)propanoyl)oxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4a). Prepared from 5.2a. Colorless oil (91%); $^1$H
NMR (CDCl₃): δ 9.73 (bs, 1H), 7.13 - 7.10 (m, 6H), 5.56 - 5.53 (m, 1H), 5.28 (br s, 1H), 4.89 (br s, 2H), 4.31 - 3.82 (m, 2H); 13C NMR (CDCl₃): δ 173.8, 173.2, 171.8, 156.1, 136.0, 128.4, 128.1, 80.3, 71.9, 67.0, 58.2, 57.0, 49.6, 28.2, 18.0, 16.6; Anal. Calcd for C₂₀H₂₈N₂O₈: C, 56.60; H, 6.65; N, 6.60. Found: C, 56.21; H, 6.55; N, 6.40.

(2S)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoyl)oxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4b). Prepared from 5.2b. Colorless oil (87%); 1H NMR (CDCl₃): δ 9.86 (bs, 1H), 7.31 - 7.13 (m, 10 H), 7.13 (br s, 2H), 5.42 (br s, 1H), 5.10 - 4.99 (m, 2H), 4.79 - 4.40 (m, 2H), 3.03 (d, J = 6.0 Hz, 2H), 1.46 (s, 9H), 1.28 - 1.18 (m, 3H); 13C NMR (CDCl₃): δ 173.4, 170.6, 156.1, 135.7, 129.2, 128.5, 128.0, 127.0, 80.3, 72.2, 67.1, 56.8, 54.8, 38.1, 28.2, 16.6; Anal. Calcd for C₂₆H₃₂N₂O₈: C, 62.39; H, 6.44; N, 5.60. Found: C, 62.46; H, 6.50; N, 5.53.

(2S)-3-((2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoyl)oxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4b + 5.4b'). Prepared from (5.2b + 5.2b*).
Yellow solid (87%); mp 60 - 62 °C; 1H NMR (CDCl₃): δ 9.56 (br s, 1H), 7.38 - 7.19 (m, 10H), 7.08 (br s, 2H), 5.36 - 5.30 (m, 2H), 5.01 (br s, 2H), 4.64 - 4.44 (m, 2H), 3.13 - 2.99 (m, 3H), 1.42 (br s, 9H), 1.19 (d, J = 22.8 Hz, 3H); 13C NMR (CDCl₃): δ 175.7, 173.5, 170.8, 156.0, 136.0, 135.6, 129.3, 128.5, 128.4, 128.1, 127.0, 80.4, 72.3, 67.0, 56.8, 54.6, 38.1, 37.7, 28.2, 16.7; Anal. Calcd for C₂₆H₃₂N₂O₈: C, 62.39; H, 6.44; N, 5.60. Found: C, 62.46; H, 6.50; N, 5.59.

(2S)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-3-(1H-indol-3-yl)propanoyl)oxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4c). Prepared from 5.2c. Yellow oil (90%); 1H NMR (CDCl₃): δ 8.38 - 8.15 (m, 1H), 7.54 (d, J = 5.4 Hz, 1H), 7.42 - 6.90 (m, 12H),
5.52 - 5.39 (m, 2H), 5.10 - 4.96 (m, 3H), 3.22 (s, 2H), 1.45 (s, 9H), 1.25 - 1.14 (m, 3H); 13C NMR (CDCl₃): δ 172.7, 170.9, 156.1, 136.0, 128.5, 128.1, 127.5, 122.9, 122.3, 119.7, 118.6, 111.2, 109.6, 80.5, 72.0, 67.2, 56.8, 54.6, 28.3, 27.7, 16.5; Anal. Calcd for C₇₈H₉₃N₃O₈: C, 62.33; H, 6.16; N, 7.79. Found: C, 62.34; H, 6.30; N, 7.60.

(2R)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)-4-(methylthio)butanoyl)oxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4d). Prepared from 5.2d. Yellow oil (90%); 1H NMR (CDCl₃): δ 8.84 (br s, 1H), 8.58 (br s, 1H), 7.34 - 7.27 (m, 5H), 5.86 - 5.83 (m, 1H), 5.71 (t, J = 8.9 Hz, 1H), 5.52 (br s, 1H), 5.10 (br s 2H), 4.63 - 4.38 (m, 1H), 2.54 - 2.47 (m, 2H), 2.05 - 1.91 (m, 4H), 1.38 (s, 9H), 1.28 - 1.27 (m, 3H); 13C NMR (CDCl₃): δ 173.6, 170.7, 156.3, 156.1, 135.9, 128.4, 128.1, 80.4, 72.1, 67.2, 56.8, 53.2, 31.5, 29.8, 28.2, 16.7, 15.4; Anal. Calcd for C₂₂H₂₆N₂O₈S: C, 54.53; H, 6.66; N, 5.78. Found: C, 54.87; H, 6.77; N, 5.76.

(2S)-3-2-(((Benzyloxy)carbonyl)amino)acetoxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4e). Prepared from 5.2f. Colorless solid (86%); mp 54 - 57 °C; 1H NMR (CDCl₃): δ 7.33 - 7.31 (m, 5H), 7.01 (bs, 1H), 5.62 - 5.40 (m, 2H), 5.10 (s, 2H), 3.90 (d, J = 5.7 Hz, 2H), 1.45 (br s, 9H), 1.29 (d, J = 6.9 Hz,3H); 13C NMR (CDCl₃): δ 173.3, 169.2, 156.5, 156.0, 136.0, 128.5, 128.1, 80.5, 72.0, 67.2, 56.8, 42.6, 28.2, 16.8; HRMS m/z for C₂₂H₂₆N₂O₈Na [M + Na]+ calcd 433.1581, found 433.1597.

(2S)-3-(((R)-2-(((Benzyloxy)carbonyl)amino)-3-(benzylthio)propanoyl)oxy)-2-((tert-butoxycarbonyl)amino)butanoic acid (5.4f). Prepared from 5.2g. Yellow gel (86%); 1H NMR (CDCl₃): δ 9.05 (br s, 1H), 7.50 - 7.09 (m, 11H), 5.83 - 5.78 (m, 1H), 5.44 - 5.39 (m, 1H), 5.08 - 4.96 (m, 2H), 4.56 - 4.44 (m, 1H), 3.62 (s, 2H), 2.82 - 2.50 (m, 2H), 1.39
(br s, 9H), 1.20 (d, J = 6.3 Hz, 3H); $^{13}$C NMR (CDCl$_3$): δ 173.2, 169.5, 156.0, 137.4, 135.8, 128.8, 128.4, 128.0, 127.1, 80.3, 72.4, 67.1, 56.7, 53.3, 36.2, 33.0, 28.2, 16.6; HRMS m/z for C$_{27}$H$_{34}$N$_2$O$_8$SNa [M + Na]$^+$ calcd. 569.1928, found 569.1939.

(2S,3S)-2-((Tert-butoxycarbonyl)amino)-3-(2-((tert-butoxycarbonyl)amino)acetoxy)butanoic acid (5.4g). Prepared from 5.2h. White microcrystals (85%); mp 64 - 65 °C; $^1$H NMR (CDCl$_3$): δ 7.89 (s, 2H), 5.58 - 5.38 (m, 3H), 4.47 - 4.45 (m, 1H), 3.84 (s, 2H), 1.43 and 1.42 (overlapped s, 18H), 1.31 (d, J = 5.7 Hz, 3H); $^{13}$C NMR(CDCl$_3$): δ 173.2, 169.7, 156.6, 156.1, 81.8, 80.4, 72.0, 56.9, 43.6, 28.3, 16.8; Anal. Calcd for C$_{16}$H$_{28}$N$_2$O$_8$: C, 51.05; H, 7.50; N, 7.44. Found: C, 50.72; H, 7.64; N, 7.41.

(2S,3S)-2-((Tert-butoxycarbonyl)amino)-3-(((S)2-((tert-butoxycarbonyl)amino)-3-phenylpropanoyl)oxy)butanoic acid (5.4h). Prepared from 5.2i. White microcrystals (90%); mp 47 - 50 °C; $^1$H NMR (CDCl$_3$): δ 7.07 - 7.01 (m, 5H), 6.97 - 6.95 (m, 2H), 5.21 (s, 1H), 4.92 - 4.80 (m, 3H), 4.31 - 4.25 (m, 2H), 2.83 (s, 1H), 1.28 (s, 9H), 1.21 (s, 9H), 1.06 - 1.05 (m, 3H); $^{13}$C NMR (CDCl$_3$): δ 171.0, 156.1, 155.5, 129.2, 128.6, 127.1, 80.4, 72.1, 56.8, 54.4, 38.1, 28.3, 16.6; Anal. Calcd for C$_{23}$H$_{34}$N$_2$O$_8$: C, 59.21; H, 7.35; N, 6.00. Found: C, 59.62; H, 7.92; N, 6.54.
CHAPTER 6
‘TRACELESS’ CYSTEINE-FREE CHEMICAL LIGATIONS FROM O-ACYL SERINE SITES

6.1 Introduction

Proteins are biological macromolecules that are involved in most biochemical functions of the cell. The total chemical synthesis of proteins has already contributed to knowledge of the relationship of protein structure to their function in important biological processes. Once synthetic access to a protein has been established, chemical synthesis allows the researcher to: (i) effect, at-will, any required change in the covalent structure of a protein molecule and (ii) label a protein without limitation as to the number and kind of labels introduced.

Merrifield’s linear solid-phase peptide synthesis (SPPS) is a common tool used in the synthesis of polypeptides. However, the linear SPPS of a very large polypeptide can be expensive. The development of a technique to achieve a convergent synthesis using smaller polypeptide fragments then becomes critical in terms of both reducing the cost of production of peptide therapeutics and realizing chemical synthesis of proteins.

The development of chemical ligation has facilitated the synthesis of large peptides by linking the C-terminus of one unprotected peptide with the N-terminus of another (Figure 6-1).

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Native chemical ligation (NCL), first reported by Wieland\textsuperscript{175} and later developed by Kent,\textsuperscript{162,165} is a chemoselective and regioselective reaction of the thiolate of an N-terminal Cys-peptide with the carbon of a C-terminal thioester in another peptide that results in a native amide bond at the ligation site through a rapid NCL S- to N- acyl transfer via a cyclic transition state.\textsuperscript{162-167} The bifunctional nature of the N-terminal cysteine 1,2-mercaptoamine moiety is responsible for the observed chemoselectivity in NCL (Figure 6-2).\textsuperscript{169,176}

While of great importance, NCL has limitations that include the requirement of a N-terminal cysteine residue at the ligation site to afford a peptide containing an internal
cysteine. The low abundance of cysteine in human proteins (1.7% of the residues) also presents another limitation.\textsuperscript{174,176-180}

Considerable effort has been devoted to developing thiol auxiliary groups in attempts to overcome the problem of low abundance of cysteine, (Figure 6-3), but, such ligations were found: (i) difficult to complete due to steric hindrance\textsuperscript{174,178-186} and (ii) problematic since extraneous groups in the ligated product can be troublesome to remove.\textsuperscript{174,178-186} Another approach to overcome this limitation involves the conversion of a cysteine residue into a serine residue after NCL,\textsuperscript{174,179} however, this requires post-NCL modifications after NCL peptide synthesis.\textsuperscript{179}

![Diagram](Figure 6-3. Auxiliary method.)

To address these limitations, our group recently reported\textsuperscript{159,187,188} ligations of S-acylated cysteine peptides to form native peptides through expanded transition states with 11- and 14-membered rings. The developed methodology required no auxiliary groups and enabled the selective S-acylation of cysteine peptides by N-acylbenzotriazoles in good yields and under mild conditions followed by microwave-assisted chemical ligations of S-acyl isopeptides. However, the challenge of ligation through an 8-membered transition state and the low abundance of cysteine remained
an obstacle. Our current approach is therefore focused on serine which possesses the 1,2-hydroxylamine bifunctionality\textsuperscript{169} (mimicking the SH/NH\textsubscript{2} bifunctionality of cysteine) and thus offers the possibility of chemoselective ligation by O- to N- acyl transfer without the need of cysteine residues.

Initially, two problems existed for the acylation of the hydroxyl group of serine: (i) difficulty in achieving O-acylation without epimerization (especially in solid-phase synthesis)\textsuperscript{127,130,168} and (ii) the facile hydrolysis of O-acyl serine ester linkages\textsuperscript{114} under the aqueous conditions of classical NCL. These problems were successfully overcome by our recently reported methodology for the preparation of chirally pure O-acyl isopeptides in a single step (74-91\%) and under anhydrous conditions.\textsuperscript{114}

Kiso et al.\textsuperscript{130,168} demonstrated that O-acyl residues within a backbone significantly altered the secondary native peptide structure. “O-Acyl isopeptides” are more hydrophilic and easier to purify by HPLC\textsuperscript{119,155} than their corresponding native peptides. N-Terminal serine isopeptides rapidly generate, by O→N intramolecular acyl migration, the corresponding native peptide \textit{via} a 5-membered transition state (Figure 6-4).\textsuperscript{128}

![Figure 6-4. O-Acyl isopeptide methodology.\textsuperscript{128}]

That this classic O- to N- acyl shift \textit{via} a 5-membered transition state can be extended to eight-membered and eleven-membered transition states is now described. Thus, “traceless” chemical ligation involving O- to N- acyl shift (at a Ser site) involving
neither cysteine nor an auxiliary group at the ligation site can be utilized for the synthesis of longer peptides.

6.2 Results and Discussion

6.2.1 ‘Traceless’ Chemical Ligation by O- to N- Acyl Shift via an eight-membered-TS

Traceless chemical ligation by O- to N- acyl shift via an eight-membered-TS at Ser site was demonstrated in 6.4a,c. Protected N-(Pg-α-aminoacyl)benzotriazoles 6.1a-c were coupled with L-Ser-OH using benzotriazole methodology\textsuperscript{51,113} giving intermediates 6.2a-c, which on O-acylation provided 6.3a-c. Deprotection of the Cbz/Boc group of 6.3 by hydrogenation with Pd/C or by HCl-dioxane afforded O-acyl isopeptides 6.4a-c (Scheme 6-1).

Scheme 6-1. Preparation of O-acyl isopeptides 6.4a-c.
Intermediates \textbf{6.4a,c} underwent ligation under microwave irradiation in piperidine-DMF 20 v/v\%, 50 \( ^\circ \)C, 50 W, 1 h (Scheme 6-2). Anhydrous conditions were chosen to avoid ester hydrolysis.

Indeed, HPLC-MS indicated the formation of the desired intra-molecular ligated products \textbf{6.5a} (57\%, retention time 23.07 min.) and \textbf{6.5c} (22\%, retention time 39.50 min.) and the presence of starting materials \textbf{6.4a} (43\%, retention time, 19.61 min.) and \textbf{6.4c} (78\%, retention time, 38.65 min.). The retention times and fragmentation patterns of \textbf{6.4a} and \textbf{6.4c} were also studied by control experiments (HPLC-MS of pure \textbf{6.4a} or \textbf{6.4c}). HPLC-MS, via (-)ESI-MS/MS confirmed that compounds \textbf{6.4a} and \textbf{6.5a} with MW 409 have different fragmentation patterns. This data indeed proved the formation of intramolecular ligated products \textbf{6.5a} and \textbf{6.5c}. Moreover, product \textbf{6.5a} was isolated and the structure confirmed by HRMS.

![Scheme 6-2](image)

\textit{Scheme 6-2. Chemical ligation of O-acyl isopeptides 6.4a,c.}

\textbf{6.2.2 ‘Traceless’ Chemical Ligation by O- to N- Acyl Shift via an eleven-membered-TS}

Traceless chemical ligation by O- to N- acyl shift from a Ser site via an 11-membered TS was achieved in isopeptides \textbf{6.7a-b}. Amino-unprotected O-acyl isopeptides \textbf{6.4a-b} (Scheme 6-1) were coupled with Pg"-Gly-Bt to give \textbf{6.6a-b} which
after deprotection of the protecting group Pg" provided O-acyl isopeptides 6.7a-b (Scheme 6-3).

Intermediates 6.7a-b underwent ligation (Scheme 6-3) under anhydrous conditions (piperidine 20 v/v% in DMF, MW 50 °C, 50 W, 1 h (for 6.7a) and 3h (for 6.7b). HPLC-MS showed formation of the expected intramolecular ligated product 6.8a (99%, retention time 21.67 min.), hydrolysed form 6.9a (1%) and none of the intermolecular by-product 6.10a. As for ligation on 7b, HPLC-MS indicated the formation of the desired 6.8b (18%, retention time 17.86 min.), hydrolysed form 6.9b (8%) and intermolecular by-product 6.10b (31%). The retention times and fragmentation patterns of 6.7a and 6.7b were also studied by control experiments (HPLC-MS of pure 6.7a and 6.7b).

HPLC-MS, via (-)ESI-MS/MS demonstrated that products 6.7a and 6.8a, each of MW
466, produced different fragmentation patterns. In addition, product 6.10a was isolated and structure confirmed by HRMS.

The ligation of 6.7a was also examined under aqueous conditions, (pH 7.6, 1 M buffer strength, MW 50 °C, 50 W, 1 h). HPLC-MS of the aqueous product, disclosed a small amount of the ligated product 6.8a and a major signal of MW 366 which corresponds to removal of the Boc-group either from 6.7a or from the ligated product 6.8a.

6.2.3 Computational Studies for ‘Traceless’ Chemical Ligation by O- to N- Acyl Shift

The eight-membered ring transition state in S-acyl tripeptides is sterically hindered and poorly organized for binding, but the structurally similar O-acyl tripeptide 6.4a demonstrated a preferential internal O- to N- acyl shift. This counter-intuitive reactivity of O-acyl tripeptides is however rationalized by the same computational protocol of virtual screening and quantum chemical calculations performed by Dr. Alexander Oliferenko. The effectiveness of conformational preorganization was defined in terms of the $b$(N-C) scoring function, i.e. the geometrical distance between the nucleophilic amine nitrogen and the electrophilic ester carbon atom. A full conformational search was performed using the MMX force field (as implemented in PCModel v.9.3 software), resulting in 572 conformations which were subsequently ranked in descending order of the $b$(N-C) scoring function. The best preorganised conformer shown in Figure 6-2 had a value for $b$(N-C) of 3.242 Å significantly smaller than the value of 3.591 Å found in the similar S-acyl structure (The GMMX routine of PCModel and MMX force field were used for scanning all rotatable bonds).

The quantum chemical reaction energy $E_{\text{react}}$ is defined as $E_{\text{react}} = E_2 - E_1$, where $E_1$ and $E_2$ are the energies of the starting and final, geometry-optimised structures, respectively. Geometry optimization resulted in $E_{\text{react}} = -62$ kcal/mol, which is about 33 kcal/mol more favorable than the previously studied S-acyl structure.\textsuperscript{188} The cyclic transition state looks rather more organized than in the corresponding S-acyl structure (previously reported in literature in Figure 2b in ref.\textsuperscript{188}).

6.3 Conclusions

In conclusion, chemical ligation via O- to N-acyl transfer with 8- and 11-transition states occurs successfully without the use of either cysteine or an auxiliary group. The reactivity of O-acyl peptides in traceless chemical ligation reactions is supported by theoretical and computational studies.
6.4 Experimental

6.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. The NMR spectra were recorded with TMS for $^1$H (300 MHz) and $^{13}$C (75 MHz) as an internal reference. Starting materials were available commercially. HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (C18): (2 x 150 mm; 4 um) + C18 guard column (2 x 4 mm) using 0.2% acetic acid in H$_2$O/acetonitrile as mobile phases or 0.4 mM ammonium formate in H$_2$O/methanol; wavelength = 254 nm; flow rate 0.2 mL/min; and mass spectrometry was done with electro spray ionization (ESI).

6.4.2 General Procedure for the Preparation of $N$-(Z-$\alpha$-Aminoacyl)benzotriazoles

![Chemical structure](image)

$6.1$’

Thionyl chloride (0.6 mL, 8.00 mmol, 1.2 equiv) was added to a solution of $1H$-benzotriazole (3.17 g, 26.67 mmol, 4 equiv) in methylene chloride to give a clear yellow solution that was stirred for 15 min at room temperature. The amino acid $6.1$ (6.67 mmol, 1 equiv) was then added to give a suspension which was stirred for 2.5 h at room temperature. The suspension was filtered, the filtrate evaporated, the residue dissolved in EtOAc and the solution was washed with a saturated solution of sodium carbonate.
The organic portion was dried over anhyd MgSO₄, filtered, and dried to give the corresponding N-(Z-a-aminoacyl)benzotriazole 6.1'.

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (6.1a'). White solid (90%); mp 150 - 152 °C (lit. mp 149.0 - 150.0 °C); ¹H NMR (CDCl₃): δ 8.23 (d, J = 7.8 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 7.4 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.32 - 7.23 (m, 7H), 7.14 (br s, 3H), 6.09 (d, J = 4.2 Hz, 1H), 5.57 (d, J = 6.6 Hz, 1H), 5.08 (s, 2H), 3.48 (d, J = 9.6 Hz, 1H), 3.24 (d, J = 7.8 Hz, 1H); ¹³C NMR (CDCl₃): δ 170.8, 155.7, 146.0, 135.9, 134.9, 131.0, 130.8, 129.2, 128.7, 128.5, 128.1, 127.4, 126.5, 120.4, 114.3, 67.2, 55.6, 38.8.

(S)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxopropan-2-yl)carbamate (6.1c'). White solid (90%); mp 115 °C (lit. mp 113 - 115 °C); ¹H NMR (CDCl₃): δ 8.16 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.40 - 7.03 (m, 6H), 5.80 - 5.60 (m, 2H), 5.10 - 4.99 (m, 1H), 1.59 (d, J = 6.3 Hz, 3H); ¹³C NMR (CDCl₃): δ 172.2, 155.6, 145.9, 136.0, 131.0, 130.6, 128.4, 128.1, 126.4, 120.2, 114.3, 67.1, 50.5, 19.0.

6.4.3 General procedure for the preparation of N-(Boc-α-aminoacyl)benzotriazoles 6.1'

Boc-protected amino acid 6.1 (0.03 mol) was added to a solution of DCC (1 equiv) in methylene chloride under an atmosphere of nitrogen. After 30 min., BtH (1 equiv) was
added and the mixture stirred for 12 h. The suspension was filtered on a bed of silica and celite, the filtrate evaporated, the residue dissolved in EtOAc, filtered on a bed of silica and celite and washed with a solution of saturated sodium carbonate, then with water and brine. The organic portion was dried over anhyd MgSO$_4$, filtered on a bed of silica, and dried to give the corresponding N-(Boc-α-aminoacyl)benzotriazole. $^1$H NMR and mp of Boc-L-Phe-Bt and Boc-Gly-Bt matched that reported in the literature.$^{157,158}$

6.4.4 General Procedure for the Preparation of Serine-containing dipeptides 6.2a-c

$N$-(Pg-α-Aminoacyl)benzotriazoles 6.1$'$ (1.0 mmol) in MeCN (5 mL) was added dropwise to a solution of L-Ser (1.5 mmol) and Et$_3$N (3.0 mmol) in MeCN/H$_2$O (9:1, 15 mL) at room temperature and stirred for 4 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 3N HCl (5 x 50 mL). The organic portion was dried over anhyd. NaSO$_4$, filtered and concentrated to give 6.2a-c.

(S)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-hydroxypropanoic acid (6.2a). White solid (85%); mp 156 - 157 °C; $^1$H NMR (CD$_3$OD): $\delta$ 8.16 (d, $J = 7.8$ Hz, 1H), 7.38 - 7.20 (m, 10 H), 5.05 - 4.80 (m, 2H), 4.52 - 4.42 (m, 2H), 3.95 - 3.80 (m, 2H), 3.23 - 3.16 (m, 1H), 2.90 - 2.81 (m, 1H); $^{13}$C NMR (CD$_3$OD): $\delta$ 174.3, 173.2, 158.4, 138.7, 138.2, 130.5, 129.6, 129.0, 128.8, 127.8, 67.7, 63.0, 57.9, 56.2, 39.3; Anal. Calcd for C$_{20}$H$_{22}$N$_2$O$_6$: C, 62.17; H, 5.74; N, 7.25; Found: C, 62.47; H, 5.82; N, 7.21.
(S)-2-((S)-2-(((Tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-hydroxypropanoic acid (6.2b). White solid (81%); 63.0 - 65.0 °C; \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.52 (br s, 1H), 7.26 - 7.17 (m, 5H), 6.98 (br s, 1H), 4.62 - 4.57 (m, 2H), 4.05 - 3.87 (m, 2H), 3.16 - 2.88 (m, 2H), 1.34 (s, 9H); \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 172.7, 172.1, 156.1, 136.3, 129.4, 128.5, 126.9, 80.8, 62.6, 55.5, 54.7, 38.7, 28.2, 28.0; Anal. Calcd for C\(_{17}\)H\(_{24}\)N\(_2\)O\(_6\): C, 57.94; H, 6.86; N, 7.95; Found: C, 57.83; H, 7.34; N, 7.47.

(S)-2-((S)-2-(((Benzzyloxy)carbonyl)amino)propanamido)-3-hydroxypropanoic acid (6.2c). White solid (73%); 195.0 - 197.0 °C; (lit. \(^19\) mp 192.0 - 194.0 °C); \(^1\)H NMR (DMSO-\(d_6\)): \(\delta\) 7.99 (d, \(J = 7.8\) Hz, 1H), 7.46 (d, \(J = 7.8\) Hz, 1H), 7.36 - 7.29 (m, 5H), 5.02 (s, 2H), 4.29 - 4.23 (m, 1H), 4.17 - 4.10 (m, 1H), 3.72 (dd, \(J = 11, 5\) Hz, 1H), 3.62 (dd, \(J = 11, 4\) Hz, 1H), 1.21 (d, \(J = 7.1\) Hz, 3H); \(^{13}\)C NMR (DMSO-\(d_6\)): \(\delta\) 172.5, 171.9, 155.6, 137.0, 128.3, 127.8, 127.7, 65.4, 61.3, 54.6, 49.8, 18.3.

6.4.5 General Procedure for the Preparation of O-Acyl Isopeptides 6.3a-c

![Diagram of the reaction](image-url)

Compound 6.2 (1.0 mmol) was added to a solution of Pg'-AA-Bt (1.0 mmol) and DIPEA (3.0 mmol) in MeCN (20 mL) at room temperature and stirred for 12 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 2N HCl.
(3 x 50 mL). The organic portion was dried over anhyd. NaSO₄, filtered and concentrated to give 6.3.

(S)-2-((S)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-(2-((tert-butoxycarbonyl)amino)acetoxyl)propanoic acid (6.3a). White solid. (86%); mp 86 - 90 °C; 

1H NMR (CD₃OD): δ 8.22 (d, J = 8.1 Hz, 1H), 7.20 - 7.04 (m, 10 H), 4.88 - 4.85 (m, 2H), 4.64 - 4.60 (m, 1H), 4.44 (dd, J = 11.4, 3.6 Hz, 1H), 4.35 - 4.23 (m, 2H), 3.65 (s, 2H), 3.05 (dd, J = 13.8, 4.5 Hz, 1H), 2.76 - 2.67 (m, 1H), 1.29 (s, 9H); 

13C NMR (CD₃OD): δ 174.3, 171.8, 158.6, 158.3, 138.6, 138.2, 130.5, 129.5, 129.0, 128.8, 127.8, 80.9, 67.7, 65.0, 57.8, 53.0, 43.0, 39.2, 28.9; 


(S)-3-(((S)-2-(((Benzyloxy)carbonyl)amino)propanoyl)oxy)-2-((S)-2-(((tert-butoxycarbonyl)amino)-3-phenylpropanamido)propanoic acid (6.3b). White solid. (73%); mp 72.0 - 73.0 °C; 

1H NMR (CDCl₃): δ 7.85 (br s, 2H), 7.36 - 7.16 (m, 10 H), 5.65 (br s, 1H), 5.21 - 4.98 (m, 2H), 4.80 - 4.69 (m, 2H), 4.53 - 4.23 (m, 3H), 3.24 - 2.88 (m, 2H), 1.39 - 1.28 (m, 12H); 

13C NMR (CDCl₃): δ 172.4, 172.1, 171.3, 156.4, 155.7, 136.7, 135.8, 129.3, 128.5, 128.2, 128.1, 126.7, 80.3, 67.3, 63.7, 55.8, 51.8, 49.9, 38.3, 28.2, 17.5; 

Anal. Calcd for C₂₈H₃₅N₃O₉: C, 60.31; H, 6.33; N, 7.54; Found C, 60.05; H, 6.77; N, 7.39.

(S)-2-((S)-2-(((Benzyloxy)carbonyl)amino)propanamido)-3-(((S)-2-(((tert-butoxycarbonyl)amino)-3-phenylpropanoyl)oxy)propanoic acid (6.3c). White solid. (70%); mp 66.0 - 68.0 °C; 

1H NMR (CDCl₃) δ 7.03 - 7.09 (m, 10 H), 5.17 - 4.83 (m, 3H), 4.53 - 4.36 (m, 4H), 3.07 - 2.78 (m, 2H), 1.38 (d, J = 2.5 Hz, 3H), 1.34 (s, 9H); 

13C NMR (CDCl₃) δ 173.3, 171.9, 171.6, 156.4, 155.9, 136.3, 136.0, 129.4, 128.9, 128.7, 128.4,
128.3, 127.3, 80.8, 67.3, 64.0, 54.7, 51.8, 50.7, 38.0, 28.4, 18.8; Anal. Calcd for C_{28}H_{35}N_{3}O_{9}: C, 60.31; H, 6.33; N, 7.54; Found C, 60.34; H, 6.74; N, 7.37.

6.4.6 General Procedure for the Preparation of O-Acyl Isopeptides 6.4a-c and 6.7a-b

6.4.6.1 For deprotection of the Cbz-protecting group

Compound 6.3 (6.6) (1.0 mmol) was dissolved in anhydrous MeOH (30 mL) and stirred under an atmosphere of hydrogen in the presence of a catalytic amount of Pd/C for 4 h. Filtration through a bed of celite and evaporation afforded 6.4 (6.7).

6.4.6.2 For deprotection of the Boc-protecting group

Compound 6.3 (6.6) (1.0 mmol) was dissolved in either HCl-dioxane (4.0 M in dioxane) or freshly prepared HCl-MeOH (prepared by bubbling HCl in MeOH) (30 mL) and stirred for 1 h. Solvent was evaporated, and ether was added to the residue and
stirred for 2h. Filtration gave a white solid 6.4 (6.7) (when sticky solid resulted, decantation of ether several times was performed instead).

(S)-2-((S)-2-Amino-3-phenylpropanamido)-3-(2-((tert-butoxycarbonyl)amino)acetoxy)propanoic acid (6.4a). White solid (80%); mp 170 °C; ¹H NMR (CD₃OD): δ 7.32 - 7.30 (m, 5H), 4.60 - 4.50 (m, 1H), 4.39 (s, 2H), 4.25 - 4.18 (m, 1H), 3.77 (s, 2H), 3.30 - 3.00 (m, 2H), 1.38 (s, 9H); ¹³C NMR (CD₃OD): δ 174.2, 172.8, 169.5, 158.8, 135.5, 130.7, 130.3, 129.0, 81.5, 66.5, 55.9, 55.6, 43.1, 38.2, 28.8; HRMS m/z for C₁₉H₂₈N₃O₇ [M+H]⁺ calcd. 410.1922, found 410.1909.

(S)-2-((S)-2-Amino-3-phenylpropanamido)-3-(((S)-2-(((benzyloxy)carbonyl)amino)propanoyl)oxy)propanoic acid (6.4b). White microcrystals (79%); mp 103.0 - 104.0 °C; ¹H NMR (DMSO-d₆): δ 9.15 (d, J = 8.1 Hz, 1H), 8.37 (br s, 3H), 7.82 (d, J = 7.2 Hz, 1H), 7.39 - 7.23 (m, 10H), 4.99 (dd, J = 15.7, 12.6 Hz, 2H), 4.65 - 4.59 (m, 1H), 4.36 (dd, J = 11.3, 4.7 Hz, 1H), 4.27 (dd, J = 11.3, 5.9 Hz, 1H), 4.16 - 4.05 (m, 2H), 3.20 (dd, J = 14.3, 5.7 Hz, 1H), 3.03 (dd, J = 14.3, 7.5 Hz), 1.29 (d, J = 7.4 Hz, 3H); ¹³C NMR (DMSO-d₆): δ 172.6, 170.0, 168.2, 155.9, 136.9, 134.8, 129.7, 128.5, 128.4, 127.8, 127.1, 66.4, 65.6, 53.2, 51.2, 49.3, 36.7, 16.9; Anal. Calcd for C₄₆H₅₈N₆O₁₅: C, 54.93; H, 5.81; N, 8.35; Found C, 54.63; H, 6.27; N, 8.02.

(S)-2-((S)-2-Aminopropanamido)-3-(((S)-2-((tert-butoxycarbonyl)amino)phenylpropanoyl)oxy)propanoic acid (6.4c). White solid (80%); mp 150.0 - 152.0 °C; ¹H NMR (CD₃OD): δ 7.26 - 7.19 (m, 5H), 4.53 - 4.33 (m, 4H), 3.18 (dd, J = 13.9, 4.7 Hz, 1H), 2.87 (dd, J = 13.9, 9.5 Hz, 1H), 1.53 (d, J = 6.7 Hz, 3H), 1.36 (s, 9H); ¹³C NMR (CD₃OD): δ 174.2, 173.4, 170.8, 157.8, 138.5, 130.5, 130.3, 129.4, 127.7, 80.6, 66.5,
56.5, 55.6, 50.4, 38.4, 28.7, 17.5; Anal. Calcd for C$_{20}$H$_{29}$N$_{3}$O$_{7}$: C, 56.73; H, 6.90; N, 9.92; Found C, 56.61; H, 7.33; N, 9.18.

(S)-2-((S)-2-(2-Aminoacetamido)-3-phenylpropanamido)-3-(2-((tert-butoxycarbonyl)amino)acetoxy)propanoic acid (6.7a). White solid (85%) yield; mp 168 - 173 °C; $^1$H NMR (CD$_3$OD): δ 7.18 - 6.88 (m, 5H), 4.55 - 4.40 (m, 1H), 4.39 - 4.26 (m, 2H), 4.20 - 4.10 (m, 1H), 3.70 - 3.40 (m, 3H), 3.04 - 2.95 (m, 1H), 2.78 - 2.40 (m, 4H), 1.20 (s, 9H); $^{13}$C NMR (CD$_3$OD): δ 174.9, 173.2, 172.2, 167.8, 158.7, 138.5, 130.5, 129.7, 128.0, 80.9, 66.0, 56.6, 54.7, 44.8, 43.1, 38.8, 28.9; HRMS m/z for C$_{21}$H$_{30}$N$_{4}$O$_{8}$Na [M+Na]$^+$ calcd. 489.1956, found 489.1965.

(5S,9S,12S)-12-Benzyl-9-carboxy-5-methyl-3,6,11,14-tetraoxo-1-phenyl-2,7-dioxao,4,10,13-triazapentadecan-15-aminium chloride (6.7b). White solid (78%) yield; mp 93.0 - 94.0°C; $^1$H NMR (CD$_3$OD) δ 7.35 - 7.18 (m, 10H), 5.14 - 5.02 (m, 3H), 4.61 (dd, $J$ = 10.6, 4 Hz, 1H), 4.39 (dd, $J$ = 10.6, 5 Hz, 1H), 4.27 - 4.22 (m, 1H), 3.75 - 3.67 (m, 2H), 3.59 - 3.55 (m, 1H), 3.24 (dd, $J$ =13.7, 5 Hz, 1H), 2.92 (dd, $J$ =13.7, 9.2Hz, 1H), 1.38 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (CD$_3$OD) δ 174.5, 173.4, 172.0, 167.4, 158.8, 138.2, 138.0, 130.4, 129.6, 129.6, 129.2, 128.9, 128.0, 68.2, 68.0, 65.1, 56.2, 53.2, 41.6, 38.9, 17.6; HRMS m/z for C$_{25}$H$_{30}$N$_{4}$O$_{8}$ [M+H]$^+$ calcd. 515.2136, found 515.2137.

6.4.7 General Procedure for the Preparation of O-Acyl Isopeptides 6.6a-b
Pg"-Gly-Bt (1.0 mmol) was added to a solution of 6.4 (1.0 mmol) and DIPEA (3.0 mmol) in MeCN:H₂O (9.5:0.5, 20 mL) at room temperature and stirred for 12 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 2N HCl (3 x 50 mL). The organic portion was dried over anhyd. NaSO₄, filtered and concentrated to give 6.6.

(8S,11S)-8-Benzyl-11-((2-((tert-butoxycarbonyl)amino)acetoxy)methyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic acid (6.6a). White solid (89%), converted to compound 6.7a after checking NMR 6.6a: mp 180 °C (decomposed). ¹H NMR (CD₃OD): δ 7.20 - 7.03 (m, 10H), 4.89 (s, 2H), 4.56 - 4.44 (m, 2H), 4.38 - 4.30 (m, 1H), 4.24 - 4.18 (m, 1H), 3.60 - 3.47 (m, 4H), 3.11 - 2.87 (m, 1H), 2.78 - 2.58 (m, 4H), 1.23 (s, 9H); ¹³C NMR (CD₃OD) δ 177.0, 173.6, 173.4, 172.1, 171.9, 171.8, 159.1, 138.3, 130.5, 129.6, 129.1, 129.0, 127.9, 127.2, 80.9, 68.0, 65.0, 55.7, 53.0, 44.0, 43.0, 38.7, 28.6.

(9S,12S)-9-Benzyl-12-(((S)-2-(((benzyloxy)carbonyl)amino)propanoyl)oxy)methyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oic acid (6.6b). Colorless oil (89%); converted to compound 6.7 after checking NMR of 6.6b; ¹H NMR (CDCl₃) δ 9.70 (br s, 2H), 7.34 (d, J = 7.4 Hz, 1H), 7.25 - 7.07 (m, 10H), 6.03 (d, J = 7.4 Hz, 1H), 5.07 - 4.57 (m, 4H), 4.29 - 4.14 (m, 2H), 3.84 - 3.55 (m, 3H), 3.12 - 2.89 (m, 2H), 1.36 (d, J = 2.8 Hz, 3H), 1.32 (s, 9H); ¹³C NMR (CDCl₃): δ 155.5, 155.1, 135.1, 134.9, 128.2, 127.5, 127.4, 127.1, 127.0, 125.9, 124.4, 66.1, 62.6, 53.0, 50.9, 48.8, 42.8, 36.5, 29.3, 27.2, 16.4.

6.4.8 ‘Traceless’ Chemical Ligation

(9S,12S)-9-Benzyl-12-(hydroxymethyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oic acid (6.5). Compound 6.4 (20 mg, 0.05 mmol) was dissolved in
piperidine 20 v/v% in DMF (1 mL) and stirred at 50 °C and 50 W for 1h. The mixture was then evaporated and purified by HPLC to give ligated product 6.5 (for example 6.5a, 57%); The sample was analyzed via reverse phase gradient C18 HPLC/UV/(-)ESI-MSn to give a retention time of 23.07 min (for 6.5a). Structure 6.5a was confirmed by HRMS, m/z for C_{19}H_{26}N_{3}O_{7} [M-H]^{+} calcd. 408.1776, found 408.1794.

(12S,15S)-12-benzyl-15-(hydroxymethyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oic acid (6.8). Compound 6.7 (20 mg, 0.04 mmol) was dissolved in piperidine 20 v/v% in DMF (1 mL) and stirred at 50 °C and 50 W for 1h (3h for 6.8b). The mixture was then evaporated and purified by HPLC to give ligated product 8 (for example 6.8a, 99.39%); The sample was analyzed via reverse phase gradient C18 HPLC/UV (254 nm/ESI-MSn to give a retention time of 21.67 min (for 6.8a). To confirm structure, HRMS for 6.8a m/z for C_{21}H_{29}N_{4}O_{8} [M-H]^{+} calcd. 465.2064, found 465.1992.
CHAPTER 7
CONCLUSIONS AND SUMMARY OF ACHIEVEMENTS

Chapter 1 provides a general introduction to the main themes presented throughout this thesis. These include the chemistry of N-substituted benzotriazole, azides, peptides and the role of the microwave in organic synthesis.

Chapter 2 presents the synthesis of a novel diazotransfer reagent, benzotriazol-1-yl-sulfonyl azide 2.7, which was characterized by $^1$H, $^{13}$C NMR, elemental analysis and X-ray diffraction. Thermogravimetric analysis, differential scanning calorimetry and the hammer test were performed on the reagent. The reagent (2.7) proved to have a long shelf-life and to be highly soluble in many organic and partially aqueous solvents. The synthesis of a wide range of azides and diazo compounds starting from aliphatic and aromatic amines, including α-amino acids, and activated methylenes were examined to demonstrate the utility of the reagent.

In Chapter 3, azide chemistry is again examined but in a protecting group context. Reagent 2.7 was used to prepare N-(α-azidoacyl)-benzotriazoles 3.1; these proved to be efficient N-, S-, C-, and O-acylating agents and enabled the facile preparation of azido-peptides. The utility of benzotriazole: (i) to activate an α-azido acid carboxylic acid group and (ii) as a good leaving group is presented for the first time. In addition, masking of the primary amine in α-amino acids by an azide proved to be an excellent choice when performing acylation reactions.

The palladium-catalyzed reaction of N-acylbenzotriazoles with epoxide gave unexpected pseudohalohydrin ester surrogates 4.4. Chapter 4 describes the reaction conditions and the scope of this efficient, one-step microwave-assisted, solvent-free
reaction towards the synthesis β-(benzotriazol-1-yl)ethyl esters 4.4, as single regioisomers.

Peptide chemistry is introduced in Chapters 5 and 6. A mild protocol towards the synthesis of enantiomerically pure O-acylisodipeptides from serine and threonine using benzotriazole methodology is described in Chapter 5. This mild protocol was used to study eight and eleven membered transition states in cysteine-free ‘traceless’ chemical ligations using serine residues as described in Chapter 6.
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BIOGRAPHICAL SKETCH

Mirna was born in Beirut, Lebanon of Jordanian parents, but grew up in Cyprus. She graduated from the Grammar School in Nicosia, Cyprus in June 2003 with honors and also received the “Ioannis & Gregoriou Memorial Award” as the top student in both chemistry and in pure mathematics as well as the London Examinations General Certificate (advanced level in biology, chemistry and pure mathematics).

She received her Bachelor of Science degree in chemistry with a minor in biology in June 2006 and her Masters of Science in chemistry in June 2008 from the American University of Beirut, in Lebanon. During her Masters, Mirna worked under the supervision of Professor Makhluf J. Haddadin on the “Synthesis of some Quinoxalines, Quinoxaline 1,4-Dioxides, and Quinoxalinocinnoline N-Oxides”. In August 2008, Mirna joined the graduate program in the Department of Chemistry at the University of Florida and pursued her Ph.D. in organic chemistry under the guidance of Professor Alan R. Katritzky at the Center of Heterocyclic Compounds where she worked on the novel synthetic utility of benzotriazole as a synthetic auxiliary chiefly in azide and peptide chemistry.

During her course of study, Mirna has participated in several internationally renowned conferences and delivered either a poster or an oral presentation. In addition, Mirna was recognized with the Certificate of Outstanding Achievement three times (2010-2012) for maintaining a GPA of 4.0, the Proctor & Gamble Award for Research Excellence (2011), the GSC Travel Grant (2011) and the best FloHet-13 poster award (2012).