INNOVATIVE STRATEGIES FOR THE SYNTHESIS OF HETEROCYCLES OF POTENTIAL INTEREST IN MEDICINAL CHEMISTRY

By

LUCAS K. BEAGLE

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UNIVERSITY OF FLORIDA

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To my wife, without whom this would not have been possible
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<td>Å</td>
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</tr>
<tr>
<td>AcOH</td>
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</tr>
<tr>
<td>Ac₂O</td>
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</tr>
<tr>
<td>OAc</td>
<td>Acetoxy</td>
</tr>
<tr>
<td>pKₐ</td>
<td>Acid dissociation constant</td>
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<td>Anal.</td>
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</tr>
<tr>
<td>aq.</td>
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<tr>
<td>Bn</td>
<td>Benzyl</td>
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<tr>
<td>br s</td>
<td>Broad singlet</td>
</tr>
<tr>
<td>BtH</td>
<td>Benzotriazole</td>
</tr>
<tr>
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<tr>
<td>Cbz</td>
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</tr>
<tr>
<td>°C</td>
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<tr>
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<tr>
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<td>CHCl₃</td>
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<td>Cu</td>
<td>Copper</td>
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<td>CRF</td>
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</tr>
<tr>
<td>hDA</td>
<td>Hetero Diels-Alder</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
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<td>Hydrochloric acid</td>
</tr>
<tr>
<td>N₂H₄</td>
<td>Hydrazine</td>
</tr>
<tr>
<td>H₂</td>
<td>Hydrogen (gas)</td>
</tr>
<tr>
<td>NH₂OH</td>
<td>Hydroxylamine</td>
</tr>
<tr>
<td>H₂SO₄</td>
<td>Sulfuric acid</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>Half-life</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>IRC</td>
<td>Intrinsic reaction coordinate</td>
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<td>Iron</td>
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<tr>
<td>i-Bu</td>
<td>iso-butyl</td>
</tr>
<tr>
<td>ΔHᵣₒ</td>
<td>Isodesmic heat</td>
</tr>
<tr>
<td>JAKs 1-3</td>
<td>Janus-associated kinases 1-3</td>
</tr>
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<td>Literature</td>
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<tr>
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</tr>
<tr>
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</tr>
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<td>Melting point</td>
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<td>Meta</td>
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<td>Methyl</td>
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<td>Methanol</td>
</tr>
<tr>
<td>μM</td>
<td>Micromole</td>
</tr>
</tbody>
</table>
mL  Milliliter
mm  Millimeter
mmol Millimole
min Minute
MW Molecular weight
m Multiplet
NO Nitric oxide
NMOR Nitrosomorpholine
HNO Nitroxyl
N Normal
NMR Nuclear magnetic resonance
ox oxidize
p Para
ppm Parts per million
% Percent
% w/w Percent weight by weight
Ph Phenyl
K₂CO₃ Potassium carbonate
PG Protecting group
¹H NMR Proton nuclear magnetic resonance
q Quartet
RSE Radical stabilization energy
rt Room temperature
sec Second
s Singlet
<table>
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</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>SPPS</td>
<td>Solid-phase peptide synthesis</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butyl carboxylate</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>SOCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Thionyl chloride</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>Ts</td>
<td>Tosyl</td>
</tr>
<tr>
<td>TS</td>
<td>Transition State</td>
</tr>
<tr>
<td>Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>Tf</td>
<td>Triflate</td>
</tr>
<tr>
<td>TFA&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Trifluoroacetate anion</td>
</tr>
<tr>
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<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethyl silyl</td>
</tr>
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<tr>
<td>TYK2</td>
<td>Tyrosine-associated kinase 2</td>
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<td>uv</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>vs.</td>
<td>Versus</td>
</tr>
<tr>
<td>W</td>
<td>Weight</td>
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</table>
INNOVATIVE STRATEGIES FOR THE SYNTHESIS OF HETEROCYCLES OF POTENTIAL INTEREST IN MEDICINAL CHEMISTRY

By
Lucas K. Beagle
August 2012

Chair: Alan R. Katritzky
Major: Chemistry

This thesis focuses on expanding the role of benzotriazole-mediated reactions leading to compounds of potential interest in medicinal chemistry. Chapter 1 reviews the common themes covered in this research which includes: the role in synthesis of 1$H$-benzotriazole, a brief overview of peptides and microwave-assisted synthesis.

In Chapter 2, 3,5-diamino-1,2,4-triazoles were synthesized under optimized microwave irradiation conditions. Exocyclic and ring acylation of 3,5-diamino-1,2,4-triazoles were studied using $N$-(protected α-aminoacyl)benzotriazoles and $N$-(protected dipeptidoyl)benzotriazoles.

Chapters 3 and 4 describe the formation of cyclic 2,5-diketopiperazines using novel cyclization methodology. In Chapter 3, the turn-inducer proline was used to facilitate head-to-tail cyclization of $N$-(Cbz-protected dipeptidoyl)benzotriazoles to selectively form cis- or trans-2,5-diketopiperazines through a tandem deprotection/cyclization or cyclization/epimerization strategy. Chapter 4 discusses the use of Staudinger protocols in forming 2,5-diketopiperazines from a head-to-tail cyclization of azido-protected dipeptidoyl thioesters.
In Chapter 5, the synthesis of a novel class of α-leaving group nitroso compounds is discussed. These compounds, which utilize benzotriazole as the leaving group, were investigated for reactivity as dienophiles in hetero Diels-Alder reactions and as potential NO donors.
CHAPTER 1
GENERAL INTRODUCTION

The three themes that pervade this thesis are heterocycles, cyclic peptides and microwave-assisted synthesis that are mediated by the use of benzotriazole as a synthetic auxiliary.

1.1 Heterocycles in Synthesis

Heterocycles are cyclic compounds composed of more than one type of atom in the ring (Figure 1-1). The natural antithesis of a heterocycle is the homocycle, in which cyclic compounds have a single type of atom in the ring.[86JCE860] Heterocycles include multiple heteroatoms, varying degrees of unsaturation, single to multiple rings, and a range of ring sizes.

Figure 1-1. Selected examples of common heterocycles

Heterocycles are extremely important in organic chemistry and many research programs have been dedicated to their synthesis, structure and reactivity.[03HC1, 08CHC1, 11PHC1] In particular 1H-benzotriazole is of great importance to this research, and some of its properties and reactivity are described below.

Figure 1-2. Tautomerization of 1H- and 2H- benzotriazole
Benzotriazole is an interesting molecule in which there are two fused rings, a six-membered benzenoid ring and a five-membered 1,2,3-triazole ring. It naturally exists in two tautomeric forms (Figure 1-2), and has the ability to both accept (pK$_{aH} < 0$) and donate a proton (pK$_a = 8.2$). Benzotriazole has the ability to act as a proton activator 1.10, an ambient ion director 1.12, a radical activator 1.13, and an anion precursor 1.14. Often behaving like a mild halogen in most reactions, the synthetic utility of benzotriazole coupled with its stabilizing ability through electron donation offers the organic chemist a valuable tool.

The use of benzotriazole as a leaving group is of greatest importance to this research. Comparable to cyano and sulfonyl groups in its pure leaving group ability, it forms a stable anion in solution.
benzotriazole offers a modulated reactivity due to its ability to stabilize the carbonyl, which offers an advantage over the more reactive acyl halides and acyl tosylates. [91T2683, 98CR409, 03CEJ4586] Easy displacement by nucleophilic reagents offers a wide substrate scope for activation by benzotriazole methodologies. [94CSR363]

Synthesis of acyl benzotriazolide 1.17 occurs under mild conditions starting from acyl chlorides or carboxylic acids (Figure 1-4). Formation from the carboxylic acid 1.15 involves in situ generation of thionyl benzotriazole (from an excess of 1H-benzotriazole and thionyl chloride) or benzotriazole mesylate and triethylamine. [00JOC8069, 03S2795, 92T7817]

![Figure 1-4. Synthesis of acyl benzotriazolides](image)

Benzotriazole-mediated acylation has been shown to be effective for C-, O-, N- and S- nucleophiles (Figure 1-5). [03JOC4932, 03JOC5720, 00S2029] The unique reactivity and stabilization effect of benzotriazole allow these transformations under mild
conditions and often in high yields compared to literature methods. [96JOC1624, 00JOC8240, 06S411]

Figure 1-5. Acylation reactions involving C-, O-, N- and S- nucleophiles

Benzotriazole-based reagents have become an important part of substitution chemistry, especially involving acylation of peptides with other compounds. Benzotriazole-mediated reactions are an important part of the research included in this thesis. The use of benzotriazole-based acylating reagents are presented in Chapters 2-4, and the ability of benzotriazole as a stabilizing agent is described in Chapter 5.

In Chapter 2, N-(protected α-aminoacyl)benzotriazoles and N-(protected dipeptidoyl)benzotriazoles are used to acylate 3,5-diamine-1,2,4-triazole compounds giving novel compounds as potential peptidomimetics. Proline containing N-(protected dipeptidoyl)benzotriazoles are used in Chapter 3 to selectively form cis- and trans-2,5-diketopiperazines under mild conditions. Aminoacyl-benzotriazoles are used in the synthesis of starting materials which form 2,5-diketopiperazines under Staudinger ligation conditions in Chapter 4.
In Chapter 5, benzotriazole plays a different role, being used a stabilizing agent in the generation of NO from novel α-benzotriazoyl nitroso compounds.

1.2 Peptides in Synthesis

Peptides are oligomeric sequences made from natural and non-natural amino acids, connected through amide (peptide) bonds.[02PBC5] Peptides are generically differentiated from proteins by the length of their sequences; peptides are generally under 50 residues whereas proteins are longer. They are named and characterized directionally going from an N- to a C- terminus on the amino acid sequence. Some small peptides have shown biological activity but suffer from hydrolysis especially by native peptidases \textit{in vivo.}[99QJM1]

While peptides are biosynthesized from N- to C- terminus, chemical synthesis usually occurs from C- to N- terminus (Figure 1-6).[02PBC5] Synthetic preparation occurs by attack of the amine moiety on an activated carboxyl group in a sequential manner.[12CSR1826] While many peptides are available from natural sources, synthesis has become a more reliable method, often at lower cost and in higher purity.

\begin{center}
\textbf{Laboratory synthesis C- to N- terminus}
\end{center}

\begin{center}
\textbf{Biosynthesis N- to C- terminus}
\end{center}

Figure 1-6. Synthesis of peptides
Two main methods are available for the preparation of peptides in the laboratory. The classical method relies on solution-phase coupling by which chemists have successfully prepared many peptides. The methods are often tedious, require complicated purification and long reaction times.[12CSR1826] Solid-phase peptide synthesis (SPPS) is a paradigm shift that allowed significantly simpler purification, shorter reaction times and the ability to solubilize larger peptides.[88ARB957] SPPS relies on attachment of peptides to polymer resins in which they are immobilized, while the reactants and byproducts are easily washed away. Peptide synthesis using SPPS methods is a four part cycle (couple-wash-deprotection-wash) that is continued until synthesis is complete and ready for cleavage from the resin. For the most part, SPPS has replaced solution-phase synthesis due to the above advantages, but solution-phase synthesis remains more effective for industrial preparation of peptides.[07EJB279]

Chapter 3 uses the classical solution phase approach in the selective synthesis of cis- and trans-2,5-diketopiperazines. Whereas both methods are used in Chapter 4 for the synthesis of 2,5-diketopiperazines using Staudinger ligation protocols. An overview comparing both solution- and solid-phase peptide syntheses is included in Chapter 4.

Peptide conjugates are an important class of compounds involved in peptide chemistry, since they merge the properties of two compounds often leading to enhanced activity.[08PBDD1] Common peptide conjugates include phosphopeptides,[90TL2497] glycopeptides,[06G113R] lipopeptides,[09CEJB258] and pharmaceutical peptide conjugates.[01JMC1341, 08PBDD1] These compounds are valuable targets for a library of synthetic materials as one target molecule can simultaneously be reacted with many different peptides.
1.3 Microwaves in Synthesis

Microwave-assisted synthesis, once one of the hottest topics in synthesis, is an area that continues to receive intense investigation. The use of microwave reactors has grown considerably since their introduction in 1986.[86TL279] Almost any reaction can be performed under microwave irradiation, and since its inception, reactions involving microwave assisted chemistry include, but are not limited to, reductions, catalytic reactions, and substitutions.[04AA66] Many reviews have been written on the subject and the methodology has been applied to virtually every kind of substrate. Microwave-assisted chemistry is characterized by its efficient heating and drastic decreases in reaction times while often providing superior yields.[03N571, 04ACIE6250] These advantages are seen in Chapters 2-4 where decreases in reaction times and improved yields using microwave-assisted synthesis are integral.

Microwave-assisted heating has been an area of debate as to what causes the acceleration of reactions. Although pronounced effects are seen, the energy of microwave photons in the frequency of microwave reactors (2.45 GHz) are too low to break chemical bonds and therefore cannot induce chemical reactions which are rationalized by simple thermal/kinetics concerns.[91CSR1, 98CSR213] Heating from microwaves occurs in one of two ways: i) dipolar polarization and ii) ionic conduction.[05CSR164]

Dipolar polarization is the most common mode of heating in microwave chemistry, as it is dependent on the solvent. Solvents which are dipolar and therefore have high dielectric constants interact with the microwave fields. These molecules attempt to align with the field causing motion and therefore heat transfer to the system.[04AA66] A measure of the ability of solvents to interact with microwave irradiation is known as loss
factor and is represented as $\tan\delta$ (Figure 1-7).[04ACIE6250] Solvents with extremely low loss factors are known as microwave transparent (i.e. benzene, hexanes) and show little to no affect from microwave heating. Heating of transparent solvents can still be accomplished by the addition of electrical conductive materials such as catalysts or ionic liquids which are intense microwave absorbers.[05CSR164]

Table 1-1. Values of $\tan\delta$ for common solvents[04ACIE6250]

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\tan\delta$</th>
<th>Solvent</th>
<th>$\tan\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylene glycol</td>
<td>1.350</td>
<td>chloroform</td>
<td>0.091</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.941</td>
<td>acetonitrile</td>
<td>0.062</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.825</td>
<td>ethyl acetate</td>
<td>0.059</td>
</tr>
<tr>
<td>methanol</td>
<td>0.659</td>
<td>acetone</td>
<td>0.054</td>
</tr>
<tr>
<td>acetic acid</td>
<td>0.174</td>
<td>THF</td>
<td>0.047</td>
</tr>
<tr>
<td>DMF</td>
<td>0.161</td>
<td>DCM</td>
<td>0.042</td>
</tr>
<tr>
<td>water</td>
<td>0.123</td>
<td>hexane</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Ionic conduction is an important characteristic in microwave heating. The phenomenon is due to microwave electric fields causing oscillations of ions, which in turn is transferred to the system as heat. Ions dissolved in solution can cause “hot spots” which often are much higher temperature than the surrounding medium.[04AA66]

Advantages of microwave heating versus conventional heating have been extensively discussed.[04ACIE6250, 02MOS253] In contrast, conventional heating relies simply on convection currents, thus is limited by the ability of the reactor to transfer heat from the source to the reaction medium. Conventional heating depends on the thermal conductivity of the solvent, whereas microwave irradiation relies on the dielectric properties of the solvent to increase heating.[04AA66] Superheating of a solvent is an important consideration; reactions carried out in sealed tubes under microwave reactions employ this technique efficiently.[92JCSCC674]
Libraries of compounds may be synthesized in short time periods using microwave-assisted heating and are utilized in prospective drug discovery. With sensitive catalytic systems, efficient heating and reduction of reaction times often enables the catalyst to last longer, often resulting in higher yields.[04AA66, 06NR51]
CHAPTER 2
EFFICIENT MICROWAVE-ASSISTED SYNTHESIS OF 1,2,4-TRIAZOLE-BASED PEPTIDOMIMETICS USING BENZOTRIAZOLE METHODOLOGY¹

2.1 Literature Overview

1,2,4-Triazole derivatives have attracted interest among medicinal chemists because of their versatile biological properties and the bioisosteric replacement of cis-amide bonds. By coupling microwave-assisted reactions with traditional organic synthesis, a large range of compounds can be synthesized using shorter reaction times and with increased yields.

2.1.1 Biological Properties of Selected 3,5-Disubstituted-1,2,4-Triazoles

Compounds bearing the 1,2,4-triazole moiety show interesting biological activities that including: potent inhibitors of CRF1 antagonist, regulators of long term stress responses in the pituitary gland [01BMCL3165], and binding with muscarinic receptor ligands as potential therapeutics in the treatment of Alzheimer's disease. [92JMC1280], [92JMC2392] Several examples of 1,2,4-triazoles have undergone biological testing showing activity in vivo (Figure 2-1). Ribavirin (2.1) is effective against a number of viral types, including HIV, and has been used to treat leukemic cell proliferation. [73JMC935, 09AR1971] JNJ-07706621 (2.2) inhibits Janus associated kinases (TYK2 and JAKs 1-3), and cyclin-dependent kinases (CDKs) by affecting the early G1 phase of the cell cycle. [10BMCL7454, 06BMCL3639] AH22216 (2.3) is a H2-receptor antagonist, and in vivo testing shows it to be 20-30 times more effective than cimetidine in oral potency. [83BR871]

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2.1.2 Previous Methods for the Synthesis of 1,2,4-Triazole-3,5-diamines

Efficient methods for the preparation of 1,2,4-triazole-3,5-diamine derivatives include: \( N \)-cyanoguanidines,[58JACS3929, 77JHC443] \( S,S \)-dimethyl-\( N \)-cyanodithioimidocarbonate,[86JHC401] and diphenyl cyanocarbonimidate.[82JHC1205, 87JHC275, 93T165, 98TL7983] Due to the limited scope of \( N \)-cyanoguanidines and the relatively expensive starting materials of diphenyl cyanocarbonimidate, \( S,S' \)-dimethyl-\( N \)-cyanodithioimidocarbonate 2.4 is the most widely used reagent (Figure 2-2).

\[
\begin{align*}
\text{2.4} & \quad \xrightarrow{\text{NHR}_2} \quad \text{2.5} & \quad \text{2.6} & \quad \xrightarrow{\text{H}_2\text{NNH}_2} \quad \text{2.7}
\end{align*}
\]

Figure 2-2. Synthesis of 1,2,4-triazoles-3,5-diamine 2.7 from \( S,S' \)-dimethyl-\( N \)-cyanodithioimidocarbonate 2.4

2.1.3 Peptidomimetics

Bioactive peptides function as hormones, enzyme inhibitors and neurotransmitters, but their clinical application is limited due to their rapid hydrolysis by peptidase enzymes.[00CMC945, 02DD847] One approach to overcome this is the use of peptidomimetics. These are small protein- or peptide-like molecules designed to mimic natural peptides.[09EJOC5099] The bioisosteric replacement of the amide bond is
important in the design of peptidomimetics (Figure 2-3).[96CR3147] In particular, 1,2,4-triazole derivatives are utilized as amide bond mimetics with increased hydrolytic stability.[98BMCL775, 09EJOC5099]

![1,2,4-triazole and cis-Amine Bond](image)

Figure 2-3. 1,2,4-triazoledes as surrogates of cis-amide bonds

Peptidomimetic drugs, such as Remikiren 2.10, resist degradation by peptidases (Figure 2-4), and compound 2.10 has shown great efficacy in vivo towards hypertension as an inhibitor of the liver enzyme renin. Unfortunately, it also has a short half-life on oral delivery and was recently replaced by Aliskiren 2.11.[08NRDD399]

![Remikiren and Aliskiren](image)

Figure 2-4. Peptidomimetic drugs which showed anti-hypertensive properties

### 2.2 Results and Discussion

*N*-Acylbenzotriazoles are stable solids, easy to handle and useful for *N*, *O*, *C*- and *S*-acylation.[09S2392] A novel microwave-assisted approach for the synthesis of 1,2,4-triazole-based peptidomimetics using benzotriazole methodology and starting from inexpensive and versatile starting materials is described.
2.2.1 Starting Material Synthesis

Table 2-1. Synthesis of Isothioureas intermediates 2.12-14

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Product</th>
<th>Conv. Method</th>
<th>Microwave</th>
<th>Mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Time (min)</td>
<td>Yield (%)</td>
<td>YIELD (%)</td>
</tr>
<tr>
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<td>-(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;O(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>2.12</td>
<td>240</td>
<td>5</td>
<td>76&lt;sup&gt;d&lt;/sup&gt;</td>
<td>131-132</td>
</tr>
<tr>
<td>2</td>
<td>PhCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
<td>2.13</td>
<td>300</td>
<td>89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Ph</td>
<td>H</td>
<td>2.14</td>
<td>240</td>
<td>63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>CHCl<sub>3</sub>, reflux, <sup>b</sup>Diethyl ether, RT, <sup>c</sup>EtOH, reflux, <sup>d</sup>Diethyl ether, MW, 45 °C, 50 W, <sup>e</sup>EtOH, MW, 90 °C, 100 W

Isothiourea intermediates 2.12-14 were prepared following literature procedures by reacting commercially available S,<s>\text{S'}</s>-dimethyl-N-cyanodithioimidocarbonate 2.4 with primary (aniline and benzyl amine) and secondary amines (morpholine) (Table 2-1).[86JHC401, 87AP608, 70JOC2067] The conventional heating method gave isothioureas 2.12-14 (63-89% yield), but long reaction times were necessary (4-5 hours). Carrying out the reaction under microwave irradiation led to significantly reduced reaction times (5-30 minutes) and afforded 2.12-14 in comparable yields (61-86%).

Isothiourea intermediates 2.12-14 were reacted with hydrazine hydrate in refluxing ethanol to give 3,5-diamino-1,2,4-triazoles 2.15-17 (75-90% yield). Significant reduction in reaction times was again seen under microwave irradiation giving 2.15-17 in 85-95% yield (4-5 h to 5-10 min). The optimized microwave-assisted protocol was carried out at 80 °C at 100 W under a continuous flow of nitrogen.
Table 2-2. Synthesis of 3,5-diamino-1,2,4-triazoles 2.15-17 from isothiourea intermediates 2.12-14

<table>
<thead>
<tr>
<th>Entry</th>
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<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Product</th>
<th>Conv. heating&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Microwave&lt;sup&gt;b&lt;/sup&gt;</th>
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<td></td>
<td></td>
<td></td>
<td>Time (min)</td>
<td>Yield (%)</td>
<td>Time (min)</td>
</tr>
<tr>
<td>1</td>
<td>-(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;O(CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>2.15</td>
<td>240</td>
<td>83</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>PhCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
<td>2.16</td>
<td>300</td>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Ph</td>
<td>H</td>
<td>2.17</td>
<td>240</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup>EtOH, reflux, <sup>b</sup>EtOH, MW, 80 °C, 100 W

Isothiourea intermediate 2.13 was used in the synthesis of N<sup>1</sup>-methyl-protected 3,5-diamino-1,2,4-triazole 2.18 (Scheme 2-1). Methyl hydrazine (2 equivalents) was reacted with isothiourea 2.13 in refluxing ethanol to give 2.18 (51% yield).[86JHC401] Under microwave heating a 50/50 mixture of regioisomers was obtained and could not be separated.

Scheme 2-1. Formation of ring protected 3,5-diamino-1,2,4-triazole 2.18

2.2.2 Ring Acylation of 2.15 and 2.17.

Initial experiments showed reaction of 2.15 with Cbz-Ala-Bt under reflux conditions was incomplete after 12 hours. However, when heating under microwave irradiation was employed, 2.23 was obtained in 95% yield (Entry 1, Table 2-3). Compound 2.15 (Entries 2-4) was therefore reacted with N-(protected α-aminoacyl)benzotriazoles 2.20-22 to afford the corresponding N-aminoacyl triazoles 2.24-26 in good to excellent yields (70-
95%). Reaction of 2.17 (Table 2-2) with Boc-protected glycyl benzotriazole gave 2.27 in 65% yield (Entry 5). This methodology showed compatibility with a wide range of amino acid protecting groups.

Table 2-3. Synthesis of ring acylated 3,5-diamino-1,2,4-triazoles 2.23-27

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>PG</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.23</td>
<td>-(CH₂)₂O(CH₂)₂⁻</td>
<td>CH₃</td>
<td>Cbz</td>
<td>95</td>
<td>205-207</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.24</td>
<td>-(CH₂)₂O(CH₂)₂⁻</td>
<td>H</td>
<td>Fmoc</td>
<td>70</td>
<td>211-214</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.25</td>
<td>-(CH₂)₂O(CH₂)₂⁻</td>
<td>PhCH₂</td>
<td>Cbz</td>
<td>95</td>
<td>217-218</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.26</td>
<td>-(CH₂)₂O(CH₂)₂⁻</td>
<td>H</td>
<td>Boc</td>
<td>73</td>
<td>212-215</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.27</td>
<td>Ph</td>
<td>H</td>
<td>H</td>
<td>Boc</td>
<td>65</td>
<td>230-233</td>
</tr>
</tbody>
</table>

Table 2-4. Ring acylation using Cbz-protected dipeptidoyl benzotriazoles on 2.15

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R¹</th>
<th>R²</th>
<th>R⁴</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.31</td>
<td>CH₃</td>
<td>PhCH₂</td>
<td>95</td>
<td>188-190</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.32</td>
<td>PhCH₂</td>
<td>CH₃</td>
<td>86</td>
<td>194-195</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.33</td>
<td>i-Bu</td>
<td>H</td>
<td>76</td>
<td>102-106</td>
<td></td>
</tr>
</tbody>
</table>
Ring acylation was observed in good to excellent yields (65-95%) with \(N\)-(protected \(\alpha\)-aminoacyl)benzotriazoles 2.19-22 and \(N\)-(protected dipeptidoyl)benzotriazoles 2.28-30, showing tolerance for an array of standard amino acid and peptide protecting groups.

2.2.3 Exocyclic Acylation of 2.18

Ring acylation was seen exclusively with 2.23-27 and 2.31-33, but exocyclic acylation was also required. Reaction of 2.18 with \(N\)-Cbz-Alaninyl-benzotriazole 2.19, gave exocyclic product 2.34 in 57% yield (Scheme 2-2), and reaction with dipeptidoyl 2.28 gave exocyclic dipeptidoyl acylation of 2.35 in 71% yield.

Scheme 2-2. Exocyclic acylation of 2.18 under microwave irradiation

2.2.4 Determination of Acylation Site by \(^1\)H NMR Experiments

Acylation of the ring nitrogen was confirmed by observation of an amino signal (2H) in the \(^1\)H NMR spectrum. The downfield shift of the amino group signal (> 7 ppm) indicates that ring acylation has taken place at the \(N^1\) position proximal to the amino group. This is consistent with the findings of Reiter et al. who showed that the exocyclic
amino group proximal to the methyl substituted ring nitrogen in 1-methyl-1H-1,2,4-triazole-3,5-diamine induced a downfield shift whereas the distal exocyclic amino group was shifted upfield by approximately 2 ppm.[86JHC401] Studies by D’Andrea et al. found a similar trend with an analog of compound JNJ-7706621 2.2, for which the chemical shift of the amino group upon acylation at N\(^2\) was upfield (6.25 ppm) whereas acylation at N\(^1\) showed a considerable downfield shift of 7.95 ppm.[10BMCL7454] Compounds (2.23-27 and 2.31-33) share this chemical shift pattern where the downfield signal of the amino group indicates acylation of the ring nitrogen (N\(^1\)) proximal to the primary amino group (Figure 2-5).

![Figure 2-5. \(^1\)H NMR shifts of ring acylated compounds](image)

2.3 Summary

An efficient, fast and convenient method for microwave-assisted preparation of 1,2,4-triazole substituted amino acids and dipeptides was developed. This method offers short reaction times, good to excellent yields (57-95%) and is compatible with a variety of standard protecting groups.

2.4 Experimental

2.4.1 General Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in CDCl\(_3\) or DMSO-\(d_6\) on Gemini or
Varian NMR operating at 300 MHz for $^1$H and 75 MHz for $^{13}$C with TMS as an internal standard. Elemental analyses were performed on a Carlo Erba-1106 instrument. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). All $N$-(protected $\alpha$-aminoacyl)benzotriazoles and $N$-(protected dipeptidoyl)benzotriazoles used have been prepared according to our previously published methods.$^{25}$

**2.4.2 Synthesis 2.12-14 and 2.15-17**

2.4.2.1 General procedures for the microwave-assisted preparation of isothioureas 2.12-14

A mixture of $S,S'$-dimethyl-$N$-cyanodithioimidocarbonate 2.4 (0.44 g, 3.0 mmol) and the respective primary or secondary amine (3.0 mmol) in diethyl ether (5 mL) or ethanol (5 mL) was subjected to microwave irradiation (Table 1). Compounds 2.12-14 were collected, washed with diethyl ether (2 x 5 mL) and dried under vacuum. Compound 2.14 was crystallized from ethanol:hexanes, filtered, washed with hexanes (2 x 5 mL) and dried under vacuum.

Methyl $N$-cyanomorpholine-4-carbimidothioate 2.12. White microcrystals, 76% yield, mp 131-132 °C (lit. mp 125-126 °C);[81TL2285] $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 3.82 (t, $J$ = 4.7 Hz, 4H), 3.70 (t, $J$ = 4.7 Hz, 4H), 2.76 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 169.4, 114.9, 66.4, 48.7, 16.3. Anal. Calcd for C$_7$H$_{11}$N$_3$OS: C 45.39; H 5.99; N 22.68. Found: C 45.24; H 5.96; N 22.64.
Methyl N-benzyl-N'-cyanocarbamimidothioate 2.13. White microcrystals, 86% yield, mp 158-161 °C (lit. mp 156-157 °C);[05BCSJ873] $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.91 (s, 1H), 7.38-7.25 (m, 5H), 4.50 (s, 2H), 2.63 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 170.3, 137.4, 128.4, 127.3, 127.2, 115.8, 46.2, 14.1. Anal. Calcd for C$_{10}$H$_{11}$N$_3$S: C 58.51; H 5.40; N 20.47. Found: C 58.13; H 5.29; N 20.68.

Methyl N'-cyano-N-phenylcarbamimidothioate 2.14. White microcrystals, 61% yield, mp 195-198 °C (lit. mp 194-196 °C);[70JOC2067] $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.16 (s, 1H), 7.52-7.18 (m, 5H), 2.70 (s, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 170.2, 137.2, 128.8, 126.4, 124.2, 114.8, 14.9. Anal. Calcd for C$_9$H$_9$N$_3$S: C 56.52; H 4.74; N 21.97. Found: C 56.25; H 4.60; N 21.95.

2.4.2.2 General procedures for microwave-assisted synthesis of 1,2,4-triazoles 2.15-17

A reaction mixture of the appropriate isothiourea 2.12-14 (2.0 mmol) and 72% hydrazine hydrate (0.2 g, 4.0 mmol) in ethanol (5 mL) was subjected to microwave irradiation (80 °C, 100 W, 5-10 min). On completion of the reaction (TLC), the solvent was removed under reduced pressure and the residue was crystallized from CHCl$_3$:hexanes.

3-Morpholino-1H-1,2,4-triazol-5-amine 2.15. White microcrystals, 89% yield, mp 165-166 °C (lit. mp 167-168 °C);[86JHC401] $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.90 (br s, 1H), 5.99 (br s, 2H), 3.66 (t, $J = 4.4$ Hz, 4H), 3.15 (t, $J = 4.4$ Hz, 4H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 163.1, 156.9, 66.3, 47.4.

N$^2$-Benzyl-1H-1,2,4-triazole-3,5-diamine 2.16. White microcrystals, 90% yield, mp 147-148 °C (lit. mp 151-153 °C);[86JHC401] $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.47-7.10 (m, 5H), 6.20 (s, 1H), 5.42 (s, 2H), 4.23 (d, $J = 5.7$ Hz, 2H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 160.1, 157.8, 141.1, 128.0, 127.2, 126.4, 46.2.
**N^2-Phenyl-1-1,2,4-triazole-3,5-diamine 2.17.** White microcrystals, 95% yield, mp 166-169 °C (lit. mp 161-162 °C); [86JHC401] \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 11.20 (br s, 1H), 8.62 (s, 1H), 7.49 (d, \(J = 7.9\) Hz, 2H), 7.15 (t, \(J = 7.8\) Hz, 2H), 6.71 (t, \(J = 7.3\) Hz, 1H), 5.87 (br s, 2H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 157.6, 155.6, 142.6, 128.4, 118.3, 115.5. Anal. Calcd for C\(_8\)H\(_9\)N\(_5\): C 54.85; H 5.18; N 39.98. Found: C 54.48; H 5.08; N 39.81.

**2.4.2.3 Synthesis of ring-protected 1,2,4-triazeole 2.18**

**N^6-Benzyl-1-methyl-1-1,2,4-triazole-3,5-diamine 2.18.** Methyl \(N\)-benzyl-\(N\)-cyanocarbamimidothioate (2.0 g, 10.0 mmol) and methylhydrazine (1.1 mL, 20.0 mmol) were heated under reflux in ethanol (50 mL) for 4 hours. The solvent was removed under reduced pressure and the crude residue was recrystallized from acetonitrile/hexanes. White microcrystals, 51% yield, mp 159-162 °C (lit. mp 159-161 °C); [86JHC401] \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 7.40-7.16 (m, 5H), 6.74 (t, \(J = 6.0\) Hz, 1H), 4.90 (br s, 2H), 4.39 (d, \(J = 6.0\) Hz, 2H), 3.34 (s, 3H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 160.2, 154.8, 140.4, 128.2, 127.1, 126.7, 46.6, 32.5.

**2.4.3 Ring Acylation of 1,2,4-Triazoles 2.23-27 and 2.31-33**

**2.4.3.1 General procedures for microwave-assisted synthesis of compounds 2.23-27**

A mixture of the respective \(N\)-(protected \(\alpha\)-aminoacyl)benzotriazole (1.0 mmol) and 1,2,4-triazole 2.15 and 2.17 (1.0 mmol) in dry THF (3 mL) was subjected to microwave irradiation (70 °C, 100 W, 30 min). The products were isolated and purified according to the following procedures. The reaction mixtures of compounds 2.23 and 2.25 were quenched with water (2 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with Na\(_2\)CO\(_3\) solution (10% w/w, 3 x 20 mL), water (3 x 20 mL), dried over MgSO\(_4\) and the solvent was removed under reduced
The residues were recrystallized from CH$_2$Cl$_2$:hexanes to give the desired products 2.23 and 2.25. In case of compounds 2.24 and 2.26 the reaction mixtures were evaporated under reduced pressure and the crude products were recrystallized from methanol. The reaction mixture of compound 2.27 was allowed to cool to room temperature and crystallized from a mixture of THF, CH$_2$Cl$_2$ and hexanes. The precipitate was collected, washed with CH$_2$Cl$_2$ (2 x 10 mL) and dried under vacuum.

(S)-Benzyl (1-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-1-oxopropan-2-yl)carbamate 2.23. White microcrystals, 95% yield, mp 205-207 °C; $^1$H NMR (300 MHz, DMSO- $d_6$) $\delta$ 7.85 (d, $J$ = 7.2 Hz, 1H), 7.61 (br s, 2H), 7.39-7.31 (m, 5H), 5.04-5.01 (m, 2H), 4.91-4.81 (m, 1H), 3.67-3.62 (m, 4H), 3.31-3.24 (m, 4H), 1.35 (d, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, DMSO- $d_6$) $\delta$ 172.5, 163.0, 157.2, 155.9, 136.9, 128.4, 127.9, 65.6, 49.4, 45.6, 34.4, 16.2. HRMS calcd. for C$_{17}$H$_{22}$N$_{6}$O$_4$ [M+H]$^+$: 375.1775. Found [M+H]$^+$: 375.1786.

(9H-Fluoren-9-yl)methyl (2-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-2-oxoethyl)carbamate 2.24. White microcrystals, 70% yield, mp 211-214 °C; $^1$H NMR (300 MHz, DMSO- $d_6$) $\delta$ 7.90 (d, $J$ = 7.2 Hz, 2H), 7.84-7.67 (m, 3H), 7.58 (br s, 2H), 7.48-7.30 (m, 4H), 4.44-4.09 (m, 5H), 3.63 (br s, 4H), 3.28 (br s, 4H); $^{13}$C NMR (75 MHz, DMSO- $d_6$) $\delta$ 169.3, 163.7, 157.5, 157.3, 144.4, 141.4, 128.3, 127.7, 125.9, 120.8, 66.5, 66.2, 47.3, 46.2, 44.0. Anal. Calcd for C$_{23}$H$_{24}$N$_{6}$O$_4$: C 61.60; H 5.39; N 18.74. Found: C 61.91; H 5.53; N 18.53.

(S)-Benzyl (1-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate 2.25. White microcrystals, 95% yield, mp 217-218 °C; $^1$H NMR (300 MHz, DMSO- $d_6$) $\delta$ 7.88 (d, $J$ = 7.2 Hz, 2H), 7.76-7.75 (m, 4H) 7.57 (br s, 2H), 7.45-7.40
(m 4 H), 4.34-4.30 (m, 2H), 4.27-4.21 (m, 2H), 3.63 (d, J = 4.8 Hz, 4H), 3.28 (d, J = 4.8 Hz, 4H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 166.7, 163.0, 156.8, 156.6, 143.8, 140.7, 127.6, 127.0, 125.2, 120.1, 65.8, 65.5, 46.6, 45.5, 43.4. Anal. Calcd for C$_{23}$H$_{26}$N$_6$O$_4$: C 61.32; H 5.82; N 18.65. Found: C 61.44; H 5.45; N 18.28.

**tert-Butyl (2-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-2-oxoethyl)-carbamate** 2.26. White microcrystals, 73% yield, mp 212-215 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.55 (br s, 2H), 7.14 (t, J = 6.1 Hz, 1H), 4.14 (d, J = 6.1 Hz, 2H), 3.64 (t, J = 4.7 Hz, 4H), 3.27 (t, J = 4.7 Hz, 4H), 1.39 (s, 9H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 169.0, 163.0, 156.8, 155.9, 78.2, 65.6, 45.5, 43.0, 28.2. Anal. Calcd for C$_{13}$H$_{22}$N$_6$O$_4$: C 47.84; H 6.79; N 25.75. Found: C 48.17; H 6.97; N 25.95.

**tert-Butyl (2-(5-amino-3-(phenylamino)-1H-1,2,4-triazol-1-yl)-2-oxoethyl)-carbamate** 2.27. White microcrystals, 65% yield, mp 230-233 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 9.29 (s, 1H), 7.65 - 7.52 (m, 4H), 7.29 - 7.17 (m, 3H), 6.85 (t, J = 7.2 Hz, 1H), 4.27 (d, J = 6.0 Hz, 2H), 1.41 (s, 9H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 168.9, 158.4, 155.8, 140.9, 128.6, 120.0, 116.7, 78.3, 43.2, 28.2. Anal. Calcd for C$_{15}$H$_{20}$N$_6$O$_3$: C 54.21; H 6.07; N 25.29. Found: C 54.43; H 6.23; N 24.57.

2.4.3.2 General procedures for microwave-assisted synthesis of compounds 2.31-33.

A mixture of the appropriate N-(protected dipeptidoyl)benzotriazole (1.0 mmol) and 3-morpholino-1H-1,2,4-triazol-5-amine (0.169 g, 1.0 mmol) in dry THF (3 mL) was subjected to microwave irradiation (70 °C, 100 W, 30 min). The reaction mixtures were allowed to cool to room temperature and evaporated to give crude products. Compound 2.31 was dissolved in ethyl acetate (30 mL), washed with Na$_2$CO$_3$ solution (10% w/w, 3 x 20 mL), water (3 x 20 mL), dried over MgSO$_4$ and the solvent was removed under reduced pressure to give the desired product. Compound 2.32 was recrystallized from
methanol. Compound 2.33 was recrystallized from diethyl ether:hexanes. The precipitates were collected, washed with hexanes (2 x 5 mL) and dried under vacuum.

Benzyl ((S)-1-(((S)-1-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate 2.31. White microcrystals, 95% yield, mp 188-190 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.36 (d, $J = 6.5$ Hz, 1H), 7.89 (d, $J = 7.7$ Hz, 2H), 7.57 (m, 3H), 7.46-7.28 (m, 6H), 5.10-5.00 (m, 1H), 4.30-4.17 (m, 3H), 3.68-3.62 (m, 6H), 3.32-3.24 (m, 4H), 1.36 (d, $J = 7.0$ Hz, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 171.9, 169.0, 162.9, 157.1, 156.4, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 65.7, 65.6, 47.6, 46.6, 45.5, 42.9, 16.3. Anal. Calcd for C$_{26}$H$_{31}$N$_7$O$_5$: C 59.87; H 5.99; N 18.80. Found: C 60.21; H 5.60; N 18.42.

Benzyl ((S)-1-(((S)-1-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)-amino)-1-oxopropan-2-yl)carbamate 2.32. White microcrystals, 86% yield, mp 194-195 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.26 (d, $J = 7.7$ Hz, 1H), 7.56 (br s, 2H), 7.40-7.20 (m, 10H), 7.19-7.11 (m, 1H), 5.28-5.05 (m, 1H), 4.93 (d, $J = 2.6$ Hz, 2H), 4.12-3.96 (m, 1H), 3.67-3.54 (m, 4H), 3.37-3.22 (m, 4H), 3.14 (dd, $J = 13.6$, 3.3 Hz, 1H), 2.81 (dd, $J = 13.8$, 9.8 Hz, 1H), 1.14 (d, $J = 7.1$ Hz, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 172.8, 170.6, 163.0, 157.1, 155.5, 137.6, 137.0, 129.0, 128.3, 128.2, 127.8, 126.5, 65.6, 65.3, 53.8, 49.7, 45.5, 35.7, 18.2. Anal. Calcd for C$_{26}$H$_{31}$N$_7$O$_5$: C 59.87; H 5.99; N 18.80. Found: C 59.72; H 6.04; N 18.92.

(S)-Benzyl (2-((1-(5-amino-3-morpholino-1H-1,2,4-triazol-1-yl)-4-methyl-1-oxopentan-2-yl)amino)-2-oxoethyl)carbamate 2.33. White microcrystals, 76% yield, mp 102-106 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.26 (d, $J = 8.0$ Hz, 1H), 7.57 (br s, 2H), 7.43 (t, $J = 6.2$ Hz, 1H), 7.39-7.29 (m, 5H), 5.18-5.07 (m, 1H), 5.02 (s, 2H), 3.74-3.58
(m, 6H), 3.31-3.22 (m, 4H), 1.79-1.36 (m, 3H), 0.97-0.78 (m, 6H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 171.9, 169.4, 162.8, 157.1, 156.4, 137.0, 128.3, 127.7, 65.5, 65.4, 50.3, 45.5, 43.1, 24.6, 23.2, 20.9. Anal. Calcd for C$_{22}$H$_{31}$N$_7$O$_3$: C 55.80; H 6.60; N 20.71. Found: C 55.86; H 6.55; N 20.33.

2.4.4 Exocyclic Acylation of 1,2,4-Triazoles 2.34-35

(S)-Benzyl (1-((1-methyl-5-(phenylamino)-1H-1,2,4-triazol-3-yl)amino)-1-oxopropan-2-yl)-carbamate 2.34. A mixture of Cbz-L-Ala-Bt 2.19 (0.32 g, 1.0 mmol) and N$_5$-benzyl-1-methyl-1H-1,2,4-triazole-3,5-diamine 2.18 (0.20 g, 1.0 mmol) in dry THF (3 mL) was subjected to microwave irradiation (70 °C, 100 W, 30 min). The reaction was quenched with water (2 mL), and extracted with ethyl acetate (3 x 10 mL). The combined organics were washed with Na$_2$CO$_3$ solution (10% w/w, 3 x 20 mL), water (3 x 20 mL) and dried over MgSO$_4$. The solvent was then removed under reduced pressure and the residue was recrystallized from CH$_2$Cl$_2$/hexanes. White microcrystals, 57% yield, mp 184-185 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.47 (d, $J = 7.6$ Hz, 1H), 7.38-7.29 (m, 11H), 7.28-7.18 (br s, 1H), 5.00 (s, 2H), 4.41 (d, $J = 5.9$ Hz, 2H), 4.14 (d, $J = 1.3$ Hz, 1H), 3.48 (s, 3H), 1.21 (d, $J = 7.1$ Hz, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 155.6, 154.7, 140.0, 128.3, 128.2, 127.7, 127.0, 126.7, 65.3, 46.4, 33.0, 18.0. Anal. Calcd for C$_{21}$H$_{24}$N$_6$O$_3$: C 61.75; H 5.92; N 20.57. Found: C 61.37; H 5.92; N 20.63.

Benzyl ((S)-1-(((S)-1-((5-(benzylamino)-1-methyl-1H-1,2,4-triazol-3-yl)amino)-1-oxo-3-phenyl-propan-2-yl)amino)-1-oxopropan-2-yl)carbamate 2.35. A mixture of Cbz-L-Ala-L-Phe-Bt 2.28 (0.48 g, 1.0 mmol) and N$_5$-benzyl-1-methyl-1H-1,2,4-triazole-3,5-diamine 2.18 (0.20 g, 1.0 mmol) in dry THF (3 mL) was subjected to microwave irradiation (70 °C, 100 W, 30 min). The reaction was quenched with water (2 mL), and extracted with ethyl acetate (3 x 10 mL). The combined organics were washed with
Na$_2$CO$_3$ solution (10% w/w, 3 x 20 mL), water (3 x 20 mL) and dried over MgSO$_4$. The solvent was then removed under reduced pressure and the residue was recrystallized from CH$_2$Cl$_2$/hexanes. White microcrystals, 71% yield, mp 107-109 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.27 (br s, 1H), 7.95 (d, $J$ = 7.9 Hz, 1H), 7.45-7.14 (m, 16H), 7.07 (br s, 1H), 5.06-4.91 (m, 2H), 4.72-4.51 (m, 1H), 4.42 (d, $J$ = 6.0 Hz, 2H), 4.10-3.94 (m, 1H), 3.50 (s, 3H), 3.09-2.93 (m, 1H), 2.89-2.68 (m, 1H), 1.12 (d, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 172.9, 169.5, 156.2, 155.3, 152.4, 140.6, 138.1, 137.6, 130.0, 129.0, 128.9, 128.6, 128.4, 127.7, 127.4, 126.9, 66.1, 54.7, 50.7, 47.1, 38.2, 33.7, 18.9. Anal. Calcd for C$_{30}$H$_{33}$N$_7$O$_4$: C 64.85; H 5.99; N 17.65. Found: C 64.55; H 6.04; N 17.55.
CHAPTER 3
A NEW BENZOTRIAZOLE-MEDIATED, STEREOFLEXIBLE GATEWAY TO HETERO-2,5-DIKETOPIPERAZINES

3.1 Literature Overview

Small cyclic peptides are an important subgroup of peptidic structures. A large library of cyclic structures can be generated from available natural or synthetic amino acids. The 2,5-diketopiperazine (DKP) backbone, the smallest cyclic peptidoyl sequence, appears in many naturally occurring molecules.[11CEJ1388, 11OL2770, 11TL2262, 09P833] Proline plays a prominent role in protein folding conferring on DKPs the ability to mimic protein domains such as β-turns.[11EJOC217] The biological activity associated with DKPs includes antibiotic [08JACS6281, 03JNP1299], insecticidal [03TL6003], antimitotic [08BMC4626], chemosensitizing [02MCT417, 86TL6361], and antiviral [10OBC5179] properties.

3.1.1 Properties of Proline-Containing Cyclic Peptides

The proline-containing 2,5-diketopiperazine (DKP) scaffold 3.1 has three positions available for functionalization, two of which are stereocenters (Figure 3-1). [10BC210, 09JPS474, 08OBC3989, 08TL906, 07ACIE7488, 07JOC195] Cyclo(L-Leu-d-Pro) (3.2) exhibits antibiotic properties against Vibrio anguillarum at 0.13 μg/mL concentrations.[03JNP1299] Spirostyrptostatin A 3.3 [99JACS2417, 96T12651] and tryptostatin A and B 3.5 [98TL7009] show ability to inhibit the mammalian cell cycle at the G2/M phase during cell division, making them potential therapeutics for cancer treatment. Fumitremorgin B 3.4 [86TL6217], isolated from toxigenic food-borne fungi Aspergillus fumigates, exhibits strong tremorgenic actions in mice. Protubonines A and

B 3.6 [11JNP1284], isolated from marine fungus, are effective against human cancer cells lines.

![Figure 3-1. Selected examples of proline-containing 2,5-diketopiperazines](image)

Cyclic 2,5-diketopiperazines show improved bioavailability and increased resistance to enzymatic degradation relative to linear analogs. The lipophilicity of the lateral chains can be tuned by structural changes [07JACS11802], thus making DKPs important building blocks for the generation of new therapeutic agents.

Most naturally occurring DKPs possess a cis-configuration since they originate from (L)-α-amino acids and considerable attention has been given to their synthesis.[11EJOC217, 07T9923, 02T3297] cis-Cyclic dipeptide synthesis usually starts from protected amino acids or peptide subunits in solution or on a solid phase.[07T9923, 02T3297, 07CCHTS857] Literature cyclizations fall into three groups:
head-to-tail condensation, [10ACIE9262, 10TL1303, 09ACB1051, 09T5343, 08ACIE1485, 06JCC915, 06T4784] dimerization of two peptidic subunits, [10TL4558, 08EJOC5418] and non-peptidic coupling methods.[10JACS2889, 09JOC4267]

Several recent reports include trans-DKPs bearing non proteinogenic (d)-α-amino acids.[09ACB1051] trans-DKPs have also been used as building blocks for foldamers.[06JOC8691] (d)-Proline-containing DKP backbones show a number of properties including retardation of metabolism pharmaceuticals [02JMC1559], and increased mimicry of substrates.[04JMC5713, 12BJ23] trans-DKPs have been synthesized from the more expensive (d)-α-amino acids [10OBC5179, 02JMC1559, 10OL2162, 10BMCL7327] or by epimerization of cis-DKPs.[03JNP1299, 74JACS3985] Epimerization, often reported as a side reaction upon cyclization [10OL2418, 05S3412] or further functionalization, [03JNP1299, 08T3713, 00BMC2407] leads to mixtures of cis-/trans-DKPs.

3.1.2 Turn-inducers in Peptide Synthesis

Turn-inducing moieties are common features in proteins and other molecules. Their incorporation in small peptide sequences mimics protein domains (α-helices and β-turns) is used to study complex interactions in the secondary structure.[11EJOC217] Turn-inducers allow favorable geometric conformations and facilitate cyclizations. Common turn-inducers include proline,[88JMB221] pseudoprolines,[10OL3136] unnatural amino acids [06JMC616, 08CBDD125] and non proteinogenic residues.[09T240]
3.2 Results and Discussion

Despite considerable research, there is a need for further development of alternative, flexible and cost-effective strategies for the synthesis of cyclic peptides. Our longstanding involvement in benzotriazole-mediated oligopeptide chemistry prompted the design of a new, versatile and flexible strategy to provide cis- or trans-configured DKPs starting from identical (L,L)-dipeptidoyl benzotriazolides. trans-DKPs were synthesized by a tandem triethylamine-catalyzed cyclization/epimerization, whereas a tandem deprotection/cyclization strategy led to cis-DKPs.

3.2.1 Starting Material Synthesis

Dipeptides 3.17-23 were prepared from N-(Cbz-α-aminoacyl)benzotriazoles 3.9-15 and L-proline 3.16. Coupling was performed without racemization using procedures optimized by Katritzky et al.[09S2392] The reaction was complete in acetonitrile/water (3:1) in the presence of triethylamine (Et$_3$N) at room temperature in less than 3 hours (Table 3-1 Condition A). Significant improvements in yield and reaction times were seen under microwave heating giving dipeptides 3.17-23 in 15 min and in 85-95% yield (Table 3-1 Condition B).
Table 3-1. Synthesis of Cbz-protected dipeptides 3.17-23 through benzotriazole-mediated coupling

![Chemical structure of Cbz-protected dipeptides 3.9-15, 3.16, and 3.17-23](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>Time (min)</th>
<th>Yield (%)</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.17</td>
<td>H</td>
<td>120</td>
<td>76</td>
<td>5</td>
<td>87</td>
</tr>
<tr>
<td>3.18</td>
<td>Me</td>
<td>120</td>
<td>92</td>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td>3.19</td>
<td>PhSCH₂</td>
<td>180</td>
<td>89</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td>3.20</td>
<td>i-Pr</td>
<td>300</td>
<td>77</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>3.21</td>
<td>i-Bu</td>
<td>180</td>
<td>91</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td>3.22</td>
<td>Bn</td>
<td>180</td>
<td>89</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>3.23</td>
<td>INDM[c]</td>
<td>180</td>
<td>91</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

[a] Condition A: MeCN/water 3:1, 1 eq. Et₃N, rt.  
[b] Condition B: MeCN/water 3:1, 1 eq. Et₃N, MW, 50W, 50 °C.  
[c] 1H-Indol-3-ylmethyl.

Benzotriazole-activated dipeptides 3.24-30 (Scheme 3-1) were synthesized from dipeptides 3.17-23. The process was carried out at -10 °C with thionyl benzotriazole generated in situ to yield 3.24-30 (61-87%). [09S2392]

Scheme 3-1. Formation of dipeptidoyl benzotriazole compounds 3.24-30

3.2.2 Synthesis of trans-2,5-Diketopiperazines.

Conditions for cyclization were optimized using 3.24 (Table 3-2). Different solvents, co-reagents, and reaction conditions including the effects of microwave irradiation, were studied. Under reflux conditions for 18 hours in acetonitrile and in the absence of co-reagent, 14% of 3.31 was isolated, while no product was detected under
microwave irradiation (Entry 1). Attempts using pyridine and sodium carbonate also led to no product formation (Entries 2 and 3).

Table 3-2. Optimization of cyclization conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Co-reagent (eq.)</th>
<th>t (min)[a]</th>
<th>Isolated 3.31 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeCN</td>
<td>None</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>MeCN</td>
<td>Pyridine (1)</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>MeCN</td>
<td>NaHCO₃ (5)</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>MeCN</td>
<td>Et₃N (1)</td>
<td>20</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>THF</td>
<td>Et₃N (1)</td>
<td>40</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>MeCN</td>
<td>Et₃N (0.1)</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>MeCN</td>
<td>Et₃N (0.01)</td>
<td>40</td>
<td>72</td>
</tr>
<tr>
<td>8</td>
<td>MeCN</td>
<td>DBU (1)</td>
<td>20</td>
<td>0[b]</td>
</tr>
</tbody>
</table>

[a] 80 °C, 70 W. [b] Complete degradation was observed.

Reaction in the presence of 1 equivalent of triethylamine (Et₃N), however gave 3.31 in 83% yield (Entry 4), with THF as an effective solvent (Entry 5). Catalysis by sub-stoichiometric amounts of Et₃N, 0.1 equivalents (Entry 6) led to a 75% yield of 3.31 and 0.01 equivalents of Et₃N gave 3.31 in 72% yield (Entry 7). Chiral HPLC on 3.31 revealed complete racemization.

Scheme 3-2. Formation of racemic 3.31 from chiral 3.32

Cyclization with enantiomer 3.32 again showed racemization and which led to an investigation of the reaction mechanism.
The unexpected racemization, was probably due to base-catalyzed enolization of the 2,5-diketopiperazine. Lacking a stereo center at position 3, there was no chiral memory to induce stereoselectivity during reprotonation (Figure 3-3). Literature sources suggest that proline-containing DKPs are prone to acid- or base-induced prototropy, although racemization was also observed in the cyclization of Cbz-N-glycyl-L-leucyl benzotriazole 3.36 (Scheme 3-3).[74JACS3985, 01JOC6333] According to recent literature however, cyclization under harsh conditions utilizing a methoxybenzyl-protection scheme proceeded without racemization.[08T3713]

![Figure 3-3. Cyclization and racemization of substrates 3.24 and 3.32](image)

Scheme 3-3. Cyclization/racemization of 3.36

On the basis of our preliminary observations, we postulated that inclusion of a stereocenter at position 3 of the DKP would influence the stereochemistry of
reprotonation. Authentic stereoisomeric samples of 3.25 and 3.27 were synthesized using previously described procedures. Cyclization of 3.25 and 3.27 using the optimized reaction conditions was then examined by chiral HPLC (Table 3-3).

Table 3-3. Cyclization of 3.25 and 3.27 to form trans- 3.38 and 3.39[^a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>(X,Y)</th>
<th>Yield (%)</th>
<th>R_t (min)</th>
<th>Stereochem.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(DL,L)-3.25</td>
<td>70</td>
<td>42.0, 43.3</td>
<td>(L,D)+(D,L)-3.38</td>
</tr>
<tr>
<td>2</td>
<td>(L,L)-3.25</td>
<td>69</td>
<td>43.3</td>
<td>(L,D)-3.38</td>
</tr>
<tr>
<td>3</td>
<td>(L,D)-3.25</td>
<td>72</td>
<td>43.3</td>
<td>(L,D)-3.38</td>
</tr>
<tr>
<td>4</td>
<td>(D,L)-3.25</td>
<td>71</td>
<td>41.9</td>
<td>(D,L)-3.38</td>
</tr>
<tr>
<td>5</td>
<td>(D,D)-3.25</td>
<td>63</td>
<td>42.0</td>
<td>(D,L)-3.38</td>
</tr>
<tr>
<td>6</td>
<td>(L,L)-3.27</td>
<td>75</td>
<td>35.2</td>
<td>(L,D)-3.39</td>
</tr>
<tr>
<td>7</td>
<td>(L,D)-3.27</td>
<td>77</td>
<td>35.1</td>
<td>(L,D)-3.39</td>
</tr>
<tr>
<td>8</td>
<td>(D,L)-3.27</td>
<td>79</td>
<td>37.9</td>
<td>(D,L)-3.39</td>
</tr>
<tr>
<td>9</td>
<td>(D,D)-3.27</td>
<td>69</td>
<td>37.9</td>
<td>(D,L)-3.39</td>
</tr>
</tbody>
</table>

[^a] Chiral HPLC was performed using a Chirobiotic T column. Flow rate = 0.1 mL/min; eluent = methanol.

The initial condition (Entry 1), starting from a mixture of DL-alanine and L-proline yielded a racemic mixture of thermodynamically favored trans-DKPs (L,D)-3.38 and (D,L)-3.38. The enantiomers were co-crystallized and the relative stereochemistry was assigned unambiguously by single crystal X-ray diffraction (Figure 3-4). Compound (L,D)-3.38, obtained from entries 2 and 3, resulted in identical HPLC profiles with a retention time of 43.3 minutes. The diastereomer (D,L)-3.38 (Entries 4 and 5) showed similar results with a unique retention time of 42.0 minutes. The results were supported
by optical rotation measurements showing \((\text{L,D})-3.38\) having \([\alpha]_D^{21} = 101.0\) and \((\text{D,L})-3.38\) having \([\alpha]_D^{21} = -129.7\) in agreement with literature values. This data allowed the absolute stereochemical assignments of the trans-DKPs regardless of the stereochemistry of the starting material.

Figure 3-4. Single crystal X-ray diffraction structure of \((\text{L,D})\) 3.38 showing the trans-configuration

Results obtained for the 3.39 series (Entries 6-9) were also consistent with formation of trans-DKPs. In entry 6, the absolute stereochemistry was assigned unambiguously by X-ray diffraction (Figure 3-5).

Figure 3-5. Single Crystal X-ray diffraction structure of 3.39 showing the absolute stereochemistry of the L,D configuration
Computations performed by Dr. Jean-Christophe Monbaliu, utilizing the B3LYP/6-31+G(d) level of theory (Figure 3-6) to compare the thermodynamic stability of the enols and the parent DKPs. The presence of a Cbz-protecting group (modeled as a methyl carbamate) considerably increased the thermodynamic stability of the corresponding enol (3.40-41).[10EJOC1711]

![Diagram showing thermodynamic stabilities of enols and mother DKPs](image)

**Figure 3-6.** Thermodynamic stabilities of enols and mother DKPs showing the effect of the protecting group

Computational conformational analysis, by Dr. Jean-Christophe Monbaliu, showed the most stable conformer of the proline-containing dipeptide is the twisted, ready-to-cyclize conformation. Nonproline-containing dipeptides, however, were in a linear transoid configuration in their lowest energy conformation. Investigation revealed two possible mechanisms: a unimolecular transition state with benzotriazole as a base and a leaving group, and a bimolecular transition state with Et₃N as a catalyst and benzotriazole solely as a leaving group. The latter was energetically favored over the
unimolecular mechanism, a result in agreement with the experimental observations (Figure 3-7).[12CEJ2632]

![Figure 3-7. Pictures of the transition states associated with the unimolecular unassisted mechanism (TS\textsuperscript{uni}, left) and with the bimolecular assisted mechanism (TS\textsuperscript{bi}, right). Cbz and benzotriazole groups are modeled by a methyl carbamate and a triazole group, respectively][12CEJ2632]

Table 3-4. Tandem cyclization/epimerization for the formation of 3.28-29 and 3.44-47

<table>
<thead>
<tr>
<th>Compound</th>
<th>R\textsuperscript{1}</th>
<th>Isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.38</td>
<td>Me</td>
<td>69</td>
</tr>
<tr>
<td>3.44</td>
<td>BnSCH\textsubscript{2}</td>
<td>72</td>
</tr>
<tr>
<td>3.45</td>
<td>i-Pr</td>
<td>70</td>
</tr>
<tr>
<td>3.39</td>
<td>i-Bu</td>
<td>75</td>
</tr>
<tr>
<td>3.46</td>
<td>Bn</td>
<td>71</td>
</tr>
<tr>
<td>3.47</td>
<td>INDM\textsuperscript{[a]}</td>
<td>73</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} \textit{1H}-Indol-3-ylmethyl.
A small library of proline-containing DKPs 3.38-39, 3.44-47 were synthesized in 69-75% yield (Table 3-4), to give exclusively the trans-DKPs 3.38-39, 3.44-47 by the tandem cyclization/epimerization reaction. All reactions were carried out with 1 equivalent of Et₃N to ensure short reaction times. Reaction products were purified through flash column chromatography and fully characterized.

Compound 3.39 was deprotected by hydrogenation in the presence of Pd/C for 24 hours at room temperature (Scheme 3-4), affording 3.48 in 87% yield as the free trans-2,5-diketopiperazine after recrystallization.

![Scheme 3-4](image)

Scheme 3-4. Formation of 3.48 as the free 2,5-diketopiperazine form Cbz-protected 3.39

### 3.2.3 Synthesis of cis-2,5-Diketopiperazines

Complimentary to the trans-2,5-diketopiperazines, formation of the cis-configured DKPs was accomplished by a tandem deprotection/cyclization strategy. Compound 3.24 was stirred under hydrogen with Pd/C (10% wt) for 18 hours at room temperature. Filtration through celite removed palladium yielding compound 3.49 (Scheme 3-5) in 72% as a single enantiomer by recrystallization. The optical rotation value of 3.49 coincided with the known literature value, thus confirming the tandem deprotection/cyclization without epimerization. Compounds 3.25 and 3.27 were cyclized producing 3.50-51 (65-69% yield) as cis-2,5-diketopiperazines. Compounds 3.51 and
3.48 had $[\alpha]_D^{20}$ values that coincided with the literature cis- and trans-DKP values.[09ACB1051]

![Chemical structure](image)

Scheme 3-5. Tandem deprotection/cyclization for the formation of cis-DKPs 3.49-51

### 3.2.4 Reaction Kinetics for the Formation of 3.31

The kinetics of the cyclization were studied in CD$_3$CN (0.34 M) over a temperature range of 25 °C – 65 °C (Table 3-2). Pseudo-first-order kinetics were observed and an Arrhenius plot ($\ln(k)$ vs $1/T$) (Figure 3-8) and an Eyring plot ($\ln(k/T)$ vs $1/T$) were constructed (Figure 3-9). The Arrhenius activation energy was 40 kJ/mol with $\Delta H^\neq$=37 kJ/mol and $\Delta S^\neq$=-146 J/K mol. This large negative value of $\Delta S^\neq$ coincides with the proposed highly ordered 6-membered cyclic transition state.

![Arrhenius plot](image)

Figure 3-8. Arrhenius plot for the cyclization of 3.24 to 3.31 under conventional heating
Figure 3-9. Eyring plot for the cyclization of 3.24 to 3.31 under conventional heating

Significant effects of microwave irradiation on cyclization were shown by pseudo-first-order rate constants of $k_{\text{obs}}^{\text{MW}} = 0.59 \text{ s}^{-1}$ at 65 °C under microwave heating and $k_{\text{obs}}^{\text{CONV}} = 0.31 \text{ s}^{-1}$ under conventional heating.

3.3 Summary

Proline-containing 2,5-diketopiperazines were selectively synthesized in cis- and trans-configurations from their corresponding aminoacyl benzotriazolides. The D-amino acids were accessed from inexpensive L-amino acid precursors under mild conditions showing compatibility with a wide range of amino acids. Mechanism and stereoselectivity were rationalized by chiral HPLC, kinetics, and computational methods. Compared to literature precedent, the methodology illustrates stereoflexibility and atom-efficiency as well as shorter reaction times.
3.4 Experimental

3.4.1 General Methods.

$^1$H NMR spectra were recorded at 300 MHz and $^{13}$C NMR spectra were recorded at 75 MHz on Gemini or Varian spectrometers at room temperature. The chemical shifts are reported in ppm relative to TMS as internal standard ($^1$H NMR) or to solvent residual peak ($^{13}$C NMR). The NMR experiments at variable temperatures (35, 45, 55 and 65 °C) were recorded on a Varian Inova NMR spectrometer operating at 500 MHz. Chiral HPLC experiments were performed on a Chirobiotic-T column using methanol as mobile phase. Compounds were analyzed at a flow rate of 0.1 mL/min (detection wavelength = 230 nm, solvent = methanol). HRMS spectra were recorded on a LC TOF (ES) apparatus. Elemental analysis was performed on a Carlo Erba-1106 instrument. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. $[\alpha]_D^{20}$ values were recorded on a Perkin-Elmer polarimeter. $[\alpha]_D^{20}$ values are given in deg·cm$^3$·cm$^{-1}$·g$^{-1}$ and concentration are given in mg/100cm$^3$. Flash chromatography was performed on silica gel 60 (230-400 mesh). All solvents were dried according to standard procedures. Triethylamine was distilled prior use. All commercially available substrates were used as received without further purification. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-
cooling mode). N-Cbz protected amino acids were purchased from Chem-Impex International.

3.4.2 General Procedures for the Tandem Cyclization/Epimerization Sequence and Characterization of the Corresponding rac-Diketopiperazines 3.31 and 3.37 and trans-Diketopiperazines 3.38-39 and 3.44-47

A solution of Cbz N-protected dipeptidoyl benzotriazole 3.24-30 (1 mmol) and triethylamine (1 mmol) in dry acetonitrile (4 mL) was subjected to microwave irradiation (20 min, 70W, 80 °C). Upon completion, the reaction mixture was concentrated under vacuum and the crude mixture was purified by column chromatography (hexanes/ethyl acetate gradient) to give the corresponding rac-diketopiperazines 3.31, 3.37 or trans-diketopiperazines 3.38-39 and 3.43-46.

cyclo(Z-Gly-D-Pro) 3.31. White microcrystals, yield: 83% (0.23 g), mp 110-111 °C. \([\alpha]_D^{20} = 0 \) (c = 0.2 in CHCl₃). \(^1\)H NMR (300 MHz, CDCl₃): \(\delta = 7.43-7.27 \) (m, 5H), \(5.29 \) (d, \(J = 12.3 \) Hz, 1H), \(5.26 \) (d, \(J = 12.3 \) Hz, 1H), \(4.72 \) (d, \(J = 16.5 \) Hz, 1H), \(4.29-4.08 \) (m, 2H), \(3.54 \) (dd, \(J = 8.2, 5.8 \) Hz, 2H), \(2.50-2.20 \) (m, 2H), \(2.08-1.82 \) (m, 2H) ppm. \(^{13}\)C NMR (75 MHz, CDCl₃): \(\delta = 167.4, 163.1, 151.9, 134.7, 128.8, 128.4, 69.5, 60.4, 50.0, 45.4, 28.2, 23.2 \) ppm. Anal. calcd. for C₁₅H₁₆N₂O₄: C 62.49; H 5.59; N 9.72. Found: C 62.09; H 5.61; N 9.64.

cyclo(Z-L-Ala-D-Pro) (L,D)-3.38. White microcrystals, yield: 69% (0.20 g), mp 149-150 °C. \([\alpha]_D^{20} = 101.0 \) (c = 0.2 in CHCl₃). \(^1\)H NMR (300 MHz, CDCl₃): \(\delta = 7.50-7.31 \) (m, 5H), \(5.30 \) (s, 2H), \(4.86 \) (q, \(J = 7.2 \) Hz, 1H), \(4.20 \) (dd, \(J = 9.3, 7.2 \) Hz, 1H), \(3.62-3.52 \) (m, 2H), \(2.49-2.39 \) (m, 1H), \(2.25-1.85 \) (m, 3H) \(1.53 \) (d, \(J = 7.2 \) Hz, 3H) ppm. \(^{13}\)C NMR (75 MHz, CDCl₃): \(\delta = 167.4, 165.9, 151.9, 134.8, 128.8, 128.7, 128.3, 69.3, 59.4, 57.6, 45.6,
29.2, 22.8, 17.4 ppm. Anal. calcd. for C\textsubscript{16}H\textsubscript{18}N\textsubscript{2}O\textsubscript{4}: C 63.56; H 6.00; N 9.27. Found: C 63.92; H 6.08; N 9.29.

cyclo(Z-D-Ala-L-Pro) (D,L)-3.38. White solid, yield: 71% (0.21 g), mp 153-155 °C. \([\alpha]_D^{20} = -129.7 \ (c = 0.2 \text{ in CH}_2\text{Cl}_2)\). \(^1\text{H}\) and \(^{13}\text{C}\) NMR were identical to its enantiomer (L,D)-4b. Anal. calcd. for C\textsubscript{16}H\textsubscript{18}N\textsubscript{2}O\textsubscript{4}: C 63.56; H 6.00; N 9.27; Found: C 63.62; H 6.09; N 9.21.

cyclo(Z-L-(BnS)-Cys-D-Pro) 3.44. White solid, yield: 72% (0.31 g), mp 115-116 °C. \([\alpha]_D^{20} = 79.8 \ (c = 0.2 \text{ in CH}_2\text{Cl}_2)\). \(^1\text{H}\) NMR (300 MHz, CDCl\textsubscript{3}): \(\delta = 7.44-7.16 \ (m, 10H), 5.29 (d, J = 12.0, 1H), 5.26 (d, J = 12.0, 1H), 5.01 (t, J = 6.0 Hz, 1H), 4.41 (dd, J = 10.5, 7.5 Hz, 1H), 3.71-3.43 (m, 4H), 2.96 (t, J = 6.0 Hz, 2H), 2.46-2.29 (m, 1H), 2.14-1.74 (m., 3H) ppm. \(^{13}\text{C}\) NMR (75 MHz, CDCl\textsubscript{3}): \(\delta = 167.4, 164.0, 152.3, 137.1, 134.7, 129.2, 128.8, 128.5, 127.5, 69.6, 61.0, 60.2, 45.7, 37.0, 33.6, 29.7, 22.5 ppm. HRMS (ESI): m/z calcd for C\textsubscript{23}H\textsubscript{24}N\textsubscript{2}O\textsubscript{4}S+Na\textsuperscript{+}: 447.1354 [M+Na\textsuperscript{+}]; found 447.1356.

cyclo(Z-L-Val-D-Pro) 3.45. White microcrystals, yield: 70% (0.23 g), mp. 117-119 °C. \([\alpha]_D^{20} = 108.9 \ (c = 0.2 \text{ in CH}_2\text{Cl}_2)\). \(^1\text{H}\) NMR (300 MHz, CDCl\textsubscript{3}): \(\delta = 7.50-7.16 \ (m, 5H), 5.29 (s, 2H), 4.62 (d, J = 9.6 Hz, 1H), 4.26 (t, J = 8.1 Hz, 1H), 3.68-3.45 (m, 2H), 2.51-2.36 (m, 1H), 2.22-1.81 (m, 4H), 1.08 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H) ppm. \(^{13}\text{C}\) NMR (75 MHz, CDCl\textsubscript{3}): \(\delta = 168.1, 164.9, 152.4, 134.7, 128.6, 128.5, 128.3, 69.2, 66.6, 59.8, 45.6, 31.4, 29.6, 22.7, 19.5, 19.4 ppm. Anal. calcd. for C\textsubscript{18}H\textsubscript{22}N\textsubscript{2}O\textsubscript{4}: C 65.44; H 6.71; N 8.48; Found: C 65.15; H 6.77; N 8.18.

cyclo(Z-L-Leu-D-Pro) (L,D)-3.39. White microcrystals, yield: 75% (0.26 g), mp 114-115 °C. \([\alpha]_D^{20} = 109.0 \ (c = 0.2 \text{ in CH}_2\text{Cl}_2)\). \(^1\text{H}\) NMR (300 MHz, CDCl\textsubscript{3}): \(\delta 7.44-7.28 \ (m, 5H), 5.28 (s, 2H), 4.85 (dd, J = 8.9, 6.5 Hz, 1H), 4.22 (dd, J = 9.0, 7.2 Hz, 1H), 3.64-
3.45 (m, 2H), 2.50-2.30 (m, 1H), 2.26-2.09 (m, 1H), 2.06-1.81 (m, 2H), 1.77-1.54 (m, 3H), 0.95 (d, J = 6.1 Hz, 3H), 0.90 (d, J = 6.1 Hz, 3H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 167.9, 165.4, 152.1, 134.7, 128.7, 128.5, 69.3, 60.6, 45.7, 41.1, 29.4, 24.9, 23.0, 22.8, 22.0$ ppm. Anal. Calcd. for $C_{19}H_{24}N_2O_4$: C 66.26; H 7.02; N 8.13; found: C 66.18; H 7.25; N 8.07.

Cyclo(Z-D-Leu-L-Pro) (D,L)-3.39. Yield: 79% (0.27 g), white solid. m.p. 118-119 °C. $[\alpha]_D^{20} = -102.2$ (c = 0.2 in CH$_2$Cl$_2$). $^1$H and $^{13}$C NMR were identical to its enantiomer (L,D)-4e. Anal. Calcd. for $C_{19}H_{24}N_2O_4$: C 66.26; H 7.02; N 8.13; found: C 66.11; H 7.31; N 8.01.

Cyclo(Z-L-Phe-D-Pro) 3.46. Colorless gel, yield: 71% (0.27 g). $[\alpha]_D^{20} = 79.89$ (c = 0.2 in CH$_2$Cl$_2$). $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 7.43-7.34$ (m, 5H), 7.28-7.23 (m, 3H), 7.13-7.09 (m, 2H), 5.29 (d, J = 12.3 Hz, 1H), 5.23 (d, J = 12.3 Hz, 1H) 5.09 (t, J = 5.0 Hz, 1H), 3.58-3.48 (m, 1H), 3.44-3.36 (m, 1H), 3.31-3.18 (m, 2H), 2.60 (dd, J = 9.8, 6.8 Hz, 1H), 2.18-2.04 (m, 1H), 1.92-1.77 (m, 2H), 1.70-1.56 (m, 1H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 167.4, 164.3, 152.1, 135.1, 134.8, 130.1, 128.8, 128.4, 127.8, 69.3, 62.6, 58.9, 45.1, 38.5, 29.4, 22.1$ ppm. Anal. Calcd. for $C_{22}H_{22}N_2O_4$/$\text{H}_2$O: C 68.20; H 5.98; N 7.23; found: C 68.30; H 6.01; N 6.92.

Cyclo(Z-L-Trp-D-Pro) 3.47. White solid, yield: 73% (0.27 g), mp 77-79 °C. $[\alpha]_D^{20} = 135.5$ (c = 0.2 in CH$_2$Cl$_2$). $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 8.70$ (br s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.43-7.28 (m, 6H), 7.20-7.04 (m, 2H), 6.86 (d, J = 2.4 Hz, 1H), 5.31 (d, J = 12.0, 1H), 5.20 (d, J = 12.3 Hz, 1H), 5.11 (dd, J = 5.1, 3.6 Hz, 1H), 3.58 (dd, J = 15.0, 3.6 Hz, 1H), 3.48-3.28 (m, 2H), 3.16-3.03 (m, 1H), 2.31-2.27 (m, 1H), 1.99-1.84 (m, 1H), 1.79-1.57 (m, 2H), 1.30-1.07 (m, 1H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 168.1,$
165.1, 152.1, 136.3, 134.8, 128.8, 128.5, 127.2, 124.8, 122.6, 119.9, 118.9, 111.5, 109.0, 69.3, 62.2, 59.1, 45.1, 29.5, 28.6, 21.8 ppm. Anal. Calcd. for C_{24}H_{23}N_{3}O_{4}:
C 69.05; H 5.55; N 10.07, Found: C 69.04; H 5.56; N 10.07.

**cyclo(Z-Gly-Leu) 3.37.** White Solid, Yield: 42% (0.13 g), mp 96-98 °C. [α]_D^{20} = 0 (c = 0.2 in CH_2Cl_2). ^1H NMR (300 MHz, CDCl_3): δ = 7.72 (br s, 1H), 7.44-7.25 (m, 5H), 5.31 (s, 2H), 4.42 (d, J = 17.4 Hz, 1H), 4.32 (d, J = 17.4 Hz, 1H), 4.08-3.93 (m, 1H), 1.51-1.88 (m, 3H), 0.97 (d, J = 6.0 Hz, 3H), 0.94 (d, J = 5.7 Hz, 3H) ppm. ^13C NMR (75 MHz, CDCl_3): δ = 167.2, 168.9, 152.2, 134.7, 128.8, 128.4, 69.5, 55.2, 48.0, 41.8, 24.4, 23.1, 21.4 ppm. Anal. Calcd. for C_{16}H_{20}N_{2}O_{4}: C 63.14; H 6.65; N 9.20; Found: C 62.81; H 6.65; N 9.25.

### 3.4.3 Deprotection of Compound 3.39 and Characterization of Compound 3.48

A solution of (3S,8aS)-benzyl-3-isobutyl-1,4-dioxohexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate (3.39) (5.8 mmol) in dry ethanol (40 mL) in the presence of Pd/C (10 wt.-%) was stirred for 24 hours at room temperature under an atmosphere of hydrogen. Upon completion, the crude mixture was filtered on Celite and concentrated under reduced pressure. (3S,8aR)-3-isobutyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (3.48) was recrystallized from an ethyl acetate/hexanes mixture.

**cyclo(L-Leu-D-Pro) 3.48.** White Solid, yield: 87% (1.06 g), mp 142-145 °C (lit. 146-149 °C). [α]_D^{21} = 88.6 (c = 0.2 in EtOH) (lit. [α]_D^{21} = 98.9, c = 0.9 in EtOH). ^1H NMR (300 MHz, DMSO-d_6): δ = 8.36 (br d, J = 4.1 Hz, 1H), 4.17 (dd, J = 8.8, 6.8 Hz, 1H), 3.65-3.57 (m, 1H), 3.48-3.24 (m, 2H), 2.19-2.06 (m, 1H), 1.90-1.62 (m, 4H), 1.60-1.49 (m, 1H), 1.47-1.35 (m, 1H), 0.91 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.3 Hz, 3H) ppm. ^13C NMR (75 MHz, DMSO-d_6): δ = 168.7, 166.0, 57.3, 55.2, 45.0, 42.1, 28.4, 23.8, 22.8,
21.8, 21.5 ppm. Anal. Calcd. for C_{11}H_{18}N_{2}O_{2}: C 62.83; H 8.63; N 13.32; Found: C 62.63, H 8.96; N 13.09.

3.4.4 General Procedures for the Tandem Deprotection/Cyclization Sequence and Characterization of Compound 3.49-51

A solution of N-Cbz protected (L,L)-dipeptidoyl benzotriazole 3.24-25 or 3.27 (5 mmol) in dry ethanol (50 mL) was stirred for 18 h at room temperature in the presence of Pd/C (10 wt.-%) under an atmosphere of hydrogen. Upon completion, the crude mixture was filtered on Celite and concentrated under reduced pressure. (S)-Hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (3.49), (3S,8aS)-3-methyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (3.50) and (3S,8aS)-3-isobutyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (3.51) were recrystallized from ethanol/hexanes mixtures.

cyclo(Gly-L-Pro) 3.49. White microcrystals yield: 72% (0.55 g), mp 218-221 °C (lit. mp 220-223 °C). [α]_{D}^{20} = -194.2 (c = 0.2 in EtOH) (lit. [α]_{D}^{20} = -179.6, c = 0.8 in EtOH).

1H NMR (300 MHz, CDCl3) δ = 7.25 (s, 1H), 4.15-4.04 (m, 2H), 3.90 (dd, J = 16.6, 4.4 Hz, 1H), 3.69-3.51 (m, 2H), 2.43-2.33 (m, 1H), 2.15-1.83 (m, 3H) ppm; 13C NMR (75 MHz, CDCl3) δ = 170.3, 163.7, 58.7, 46.8, 45.5, 28.6, 22.6. Anal. Calcd. for C_{7}H_{10}N_{2}O_{2}: C 54.54; H 6.54; N 18.17; Found: C 54.35; H 6.55; N 18.10.

cyclo(L-Leu-L-Pro) 3.51. White microcrystals, yield: 69% (0.73 g), mp 160-163 °C (lit. mp 162-168 °C). [α]_{D}^{20} = -135.19 (c = 0.2 in EtOH) (lit. [α]_{D}^{20} = -134.3, c = 1.1 in EtOH). 1H NMR (300 MHz, DMSO-d6) δ = 8.02 (br s, 1H), 4.19 (t, J = 7.9 Hz, 1H), 4.00 (t, J = 6.3 Hz, 1H), 3.42-3.26 (m, 2H), 2.17-2.05 (m, 1H), 1.99-1.68 (m, 5H), 1.35 (ddd, J = 13.6, 7.5, 5.7 Hz, 1H), 0.87 (d, J = 2.4 Hz, 3H), 0.85 (d, J = 2.7 Hz, 3H) ppm; 13C NMR (75 MHz, DMSO-d6) δ = 170.2, 166.4, 58.4, 52.5, 44.8, 37.7, 27.4, 24.0, 22.8,
22.4, 21.8 ppm. Anal. Calcd. for C_{11}H_{18}N_{2}O_{2}: C 62.83; H 8.63; N 13.32; Found C 62.63; H 8.96; N 13.09.

3.4.5 X-Ray Data for 3.38-39

Crystal data for compound 3.38: colorless crystal (block), dimensions 0.38 x 0.25 x 0.23 mm, crystal system monoclinic, space group P2_1/n, Z = 4, a = 10.0745(15), b = 11.0092(19), c = 12.972(2) Å, β = 94.768(12)°, V = 1433.8(4) Å³, ρ = 1.401 g cm⁻³, T = 113(2) K, Θmax = 20.32°, radiation Mo-Kα, λ = 0.71073 Å, 0.3 ω-scans with CCD area detector, covering a whole sphere in reciprocal space, 9260 reflections measured, 1681 unique (R_{int} = 0.0554), 2770 observed (I > 2σ(I)), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, m = 0.102 mm⁻¹, Tmin = 0.795, Tmax = 1.00, structure solved by direct methods and refined against F² with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 199 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 0.930 for observed reflections, final residual values R₁(F) = 0.0473, wR(F²) = 0.1129 for observed reflections. CCDC 844865, 844866.

Crystal data for compound 3.39: colorless crystal (block), dimensions 0.12 x 0.10 x 0.09 mm, crystal system monoclinic, space group P2(1), Z = 2, a = 9.8547(5), b = 8.7811(4), c = 10.6717(6) Å, β = 106.843(2)°, V = 883.86(8) Å³, ρ = 1.294 g cm⁻³, T = 100(2) K, Θmax = 66.59°, radiation Cu-Kα, λ = 1.54178 Å, 0.3 ω-scans with CCD area detector, covering a whole sphere in reciprocal space, 9033 reflections measured, 2926 unique (R_{int} = 0.0191), 2907 observed (I > 2σ(I)), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, m = 0.744 mm⁻¹,
Tmin = 0.7086, Tmax = 0.7528, structure solved by direct methods and refined against F2 with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 228 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 1.055 for observed reflections, final residual values R1(F) = 0.0233, wR(F2) = 0.0611 for observed reflections. CCDC 846343.

### 3.4.6 Kinetic Data for the Formation of 3.31

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Reaction kinetics were studied using a 0.34 M solution of 3.24 in CD$_3$CN, monitoring the disappearance of 3.24 and formation of 3.31 using the benzotriazole aromatic protons in $^1$H NMR. One milliter of the solution of 3.31 in CD$_3$CN (0.34 mmol)
was injected via syringe into a standard 5 mm NMR sample tube. The lock and shim was set with the initial sample in order to maximize the efficiency of the experiment. At t=0, the sample was removed from the machine and 1 equivalent of Et3N (0.05 mL, 0.34 mmol) was injected into the sample. The tube was shaken to ensure proper mixing and injected into the NMR probe. Proper adjustments to the lock and shim were done prior to each scan. Scans were taken every 2 minutes for 30 minutes. Integration of the appropriate signals allowed for determination of concentration and the percentage of product formed. (Table 3-5) As the reaction is in a 1:1 ratio with respect to the starting material and product, a simple determination of concentration is possible.

![Kinetics 45 °C 1 eq Et₃N 0.34M](image)

Figure 3-10. Plot showing the percent completion over time

The data obtained from the kinetics experiment was then plotted to show percent completion versus time which shows the useful data to be between 0-12 minutes (Figure 3-10). A plot of the natural log of the concentration versus time (0-12 minutes) shows the reaction to be pseudo first order (Figure 3-11). By linear regression, the rate constant, \( k_{obs} \), is obtained as the slope of the determined linear regression line equation as 0.1465 s⁻¹.
The procedure was repeated at various temperature (25, 35, 45, 55, 65 °C) as well as the analysis to determine the value of $k_{obs}$. After analysis and acquisition of the rate constant data, an Arrhenius plot was constructed (Figure 3-12). The equation obtained from linear regression analysis allows for the determination of the Arrhenius activation parameters ($k=Ae^{-Ea/RT}$) as the slope of the regression is the $E_a$ parameter. From the Arrhenius activation parameters, an Erying plot was constructed using the natural log of the concentration over time.
rate constant divided by the temperature versus the inverse of the temperature (Figure 3-13). The equation obtained by linear regression analysis shows the slope to be the negative value of the enthalpy of the reaction divided by the universal gas constant (-\(\Delta H/R\)).

![Eyring Plot](image)

Figure 3-13 Eyring plot for the cyclization of 3.24

Z-Gly-D-Pro-Bt 3.24
Conv

<table>
<thead>
<tr>
<th>Conv</th>
<th>k</th>
<th>(\ln (k))</th>
<th>C°</th>
<th>K</th>
<th>1/K</th>
<th>1/T</th>
<th>k/T</th>
<th>(\ln (k/T))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0748</td>
<td>-2.59294</td>
<td>35</td>
<td>308.15</td>
<td>0.00325</td>
<td>0.00325</td>
<td>0.00024</td>
<td>-8.32352</td>
<td></td>
</tr>
<tr>
<td>0.1379</td>
<td>-1.98123</td>
<td>45</td>
<td>318.15</td>
<td>0.00314</td>
<td>0.00314</td>
<td>0.00043</td>
<td>-7.74375</td>
<td></td>
</tr>
<tr>
<td>0.1811</td>
<td>-1.70871</td>
<td>55</td>
<td>328.15</td>
<td>0.00305</td>
<td>0.00305</td>
<td>0.00055</td>
<td>-7.50218</td>
<td></td>
</tr>
<tr>
<td>0.3146</td>
<td>-1.15645</td>
<td>65</td>
<td>338.15</td>
<td>0.00296</td>
<td>0.00296</td>
<td>0.00093</td>
<td>-6.97994</td>
<td></td>
</tr>
</tbody>
</table>

\[
\ln [A] = 12.937 - \Delta H/R = -4453.3 \quad \ln(k_0/h) + \Delta S/R = 6.160
\]

\[
-Ea/R = -4775.9 - \Delta H = 37006.9 \quad \Delta S = -146.2
\]

\[
E_a = 39687.73 \quad \ln(k_0/h) = 23.7595
\]

Figure 3-14 Data extrapolated from the Arrhenius and Eyring plots

The same general procedure was followed for determination of the kinetics for formation of 3.31 were used with one exception, the samples were heated under microwave irradiation then checked immediately after every minute by \(^1\)H NMR.
CHAPTER 4
STAUDINGER LIGATION IN THE FORMATION OF 2,5-DIKETOPIPERAZINES

4.1 Literature Overview

There is great interest in the study of cyclic peptides, the smallest of which are the 2,5-diketopiperazines (DKPs). A large number of cyclic peptides are available from natural sources, but their synthesis is often considered difficult and a considerable challenge. Solid-phase peptide synthesis (SPPS) is a convenient method for the formation of peptides, having many advantages over conventional solution-phase procedures. Staudinger ligation is also a powerful method for the formation of amide bonds and has been used in the synthesis of large cyclic peptides.

4.1.1 Biological Properties of 2,5-Diketopiperazines

Small peptides and peptide-like structures show an interesting array of biological properties. These are limited in vivo due to enzymatic degradation and hydrolysis. Cyclic peptides, with their constrained structure, are more resilient to peptidases and hydrolysis, thereby making them valuable targets for medicinal and synthetic chemists.

The 2,5-diketopiperazine scaffold (DKP) 4.1 appears in many natural products (Figure 1-1). Cyclic glycine-leucine 4.2 is a natural antibiotic that is effective against Bacillus subtilis interacting with the cytochrome P450 complex.[10B7282] Cyclic tyrosine-tyrosine 4.3 and cyclic tyrosine-phenylalanine 4.4 are efficient in binding with μ-opoid receptor [05JP305], and with L-type calcium channels.[04BMCL1717] Cyclic glycine dimers 4.5 and 4.6 are effective against many types of virus in μg/mL concentrations [04NNNA1815] Cyclic glycine dimer 4.7 is an effective inhibitor of glycogen phosphorylase (while not affecting the glucosidases) and an interesting target for type 2 diabetes.[03G93R]
Figure 4-1. Selected examples of biologically active 2,5-diketopiperazines

4.1.2 Synthesis of 2,5-Diketopiperazines

Figure 4-2. Common synthetic methods for cyclic peptides
The synthesis of small cyclic peptides can be divided into three groups: i) head-to-tail condensation of linear peptides, ii) dimerization of two amino acid subunits and iii) transition metal mediated peptide coupling reactions (Figure 4-2).[97CR2243, 04T2447] These methods often afford low yields, require protection-deprotection schemes, long reaction times and harsh conditions.[68JOC864, 83BCSJ568, 02BJ23, 06CC2884, 06T7484, 09JACS3033]

A recent example (Scheme 4-1) demonstrates the use of Brønsted acid to activate the C-terminus for attack followed by dehydration.[09T3688] Inherent stereochemistry is retained under acidic conditions, but when the cyclization or the dehydration step is carried out under basic conditions racemization is observed.

Scheme 4-1. Head to tail condensation of \(N\)-\(\alpha\)-ketoacyl amino acid amides

Dimerization of two subunits (4.12), using phosphorus-promoted cyclization under continuous microwave irradiation formed symmetrical and unsymmetrical DKPs (Scheme 4-2).[08EJOC5418] Unsymmetrical cases require a highly hindered substrate coupled with a minimally hindered substrate. The less hindered amino acid was in excess and was added after activation of the hindered substrate to minimize homodimer formation.
Organometallic coupling methods have been reported, ranging from oxidative phenolic coupling to olefin metathesis using Grubbs second generation catalyst.\cite{01T353, 02JOC8247} Jackson et al. featured an intramolecular Negishi cross-coupling reaction to form cyclic peptides (Scheme 4-3).\cite{09JOC8280}

Recent developments relied on the use of turn-inducers requiring specific residues to be included in the amino acid sequence, but these methodologies limit the scope of the reaction and leave turn-inducer residues in the cyclic peptide.\cite{12CEJ2632, 10OL3136}

Head-to-tail condensation of $N$- to $C$- terminus of linear dipeptides is of most relevance to this research. The method often leads to formation of the cyclic homodimer of the linear dipeptide and not the cyclic heterodimer. Formation of the homodimer was
minimized by high dilution conditions.[09BMCL3928] Investigations into efficient and atom-economical syntheses are important in the cyclic peptide field.[97CR2243, 04T2447]

### 4.1.3 Solid-Phase Supported Synthesis

Table 4-1. Advantages and disadvantage of SPPS methodology

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy separation of byproducts or excess</td>
<td>Incompatibility of resin with growing</td>
</tr>
<tr>
<td>starting material</td>
<td>peptide chain.</td>
</tr>
<tr>
<td>No purification between steps</td>
<td>Lack of stability of peptide resin linkers.</td>
</tr>
<tr>
<td>No mechanical loss in purification or</td>
<td>Interaction with functional groups on supports.</td>
</tr>
<tr>
<td>mechanical transfer.</td>
<td></td>
</tr>
<tr>
<td>Solubility of growing peptide independent</td>
<td>Formation of erroneous peptides due to truncation or failed coupling</td>
</tr>
<tr>
<td>of its solubility.</td>
<td>sequences.</td>
</tr>
<tr>
<td>Automation (e.g. automated peptide synthesizers).</td>
<td>Peptide conformational changes in macroscopic environments.</td>
</tr>
<tr>
<td>Single cleavage step for final product</td>
<td></td>
</tr>
<tr>
<td>Shorter reaction times.</td>
<td></td>
</tr>
<tr>
<td>Recyclability of spent resin.</td>
<td></td>
</tr>
</tbody>
</table>

Bruce Merrifield pioneered SPPS in 1963, and optimized a C- to N- terminus synthesis under mild conditions.[63JACS2149] Many advantages over solution-phase synthesis exist in SPPS methodology (Table 4-1) since column chromatography or recrystallization are avoided thereby reducing mechanical loss of the product during purification. Some SPPS methods enable the coupling of peptides in as little as 30 minutes, and coupling can be accomplished on the solid support independent of the solubility of growing residues. A more complete review of the advantages and disadvantages of SPPS is shown in Table 4-1.[88ARB957, 12CSR1826]

The solid-phase in SPPS is a polymer support having a functional group attached (Figure 4-3). Resins 4.16-18 are used for Boc-protected amino acid SPPS, while resins 4.19-20 are used for Fmoc-protected peptides. A synthetic linker is used to bind the
resin and substrate, acting as a spacer varying in length from four to six atoms.\cite{00LPS17,98JPR303}

Figure 4-3. Selected resin solid-phase supports

DMF is used to swell the resin and effectively solvate the attached peptide. Use of Boc-protecting groups allows for simple deprotection and neutralization performed \textit{in situ}.\cite{88ARB957,97ME14,92IJPPR180} Elongation may involve many coupling reagents, but commonly DCC is used due to easy removal of the urea salt.\cite{04T2447,12CSR1826} Monitoring reactions in SPPS is accomplished by ninhydrin tests, and much care must be taken to characterize the product upon removal from the resin since the intermediate stages are not characterized.\cite{88ARB957}

\textbf{4.1.4 Staudinger Ligation}

Staudinger ligation is a powerful tool for the creation of amide bonds,\cite{09JOC2203,10BMC3679} and has been used for protein engineering,\cite{00S2007,02PNAS19} labeling of specific nucleic acids,\cite{03BC697} studies in
An azide reacts with triphenylphosphine to form an iminophosphorane 4.24, with enhanced nucleophilic activity.

Figure 4-4. Mechanism of the formation of the activated iminophosphorane [05JACS2686]

Raines et al. used a phosphinthioester (4.25) in a Staudinger ligation to form a linear dipeptide 4.27 (Scheme 4-4). While effective, this methodology cannot be used to form cyclic peptides since the azide is reduced before the desired reaction.

Scheme 4-4. Raines and co-workers’ Staudinger ligation methodology using phosphinothioester and azide
4.2 Results and Discussion

In pursuit of efficient methods for the preparation of small cyclic peptides a novel, mild and atom economic tandem deprotection-cyclization strategy is demonstrated. Utilizing a solution- and solid-phase Staudinger ligation allowed rapid, convenient and cost effective cyclization.

4.2.1 Synthesis of Starting Materials for Solution-Phase Staudinger Ligation Reactions of 4.38-40 and 4.43

4.2.1.1 Synthesis of chloro-dipeptides 4.32-34

Commercially available (L)-amino acids 4.28-30 were reacted with chloroacetyl chloride 4.31 under reflux conditions in THF yielding chloro-dipeptides 4.32-34 (Scheme 4-5). Purification by crystallization from ether gave 4.32-34 in 38-78% yields.

[03RCB2197, 12ACIE548]

Scheme 4-5. Synthesis of chloro-dipeptides 4.32-34 from L-amino acid and chloroacetyl chloride

4.2.1.2 Synthesis of azido dipeptides 4.35-37

Chloro-dipeptides 4.32-34 were reacted with sodium azide in DMF/water, since water increased the solubility of the azide ion. Azido dipeptides 4.35-37 were obtained in 61-78% yields after recrystallization from ethyl acetate and hexanes (Scheme 4-6).
4.2.1.3 Synthesis of azido thioester dipeptides 4.38-40

Azido-protected dipeptide thioesters 4.38-40 were synthesized by mixed anhydride coupling of 4.35-37 with thiophenol, utilizing an in situ activation of the dipeptide. Compounds 4.38-40 were purified by a recrystallization from ethyl acetate and hexanes (Scheme 4-7) in 42-75% yield.

Scheme 4-7. Synthesis of azido thioester dipeptides 4.38-40 from azido dipeptides 4.35-37

Azido methyl ester dipeptide 4.43 was synthesized (Scheme 4-8) to examine the effect of the leaving group on cyclization. The L-leucine methyl ester was acylated with chloroacetyl chloride 4.31 yielding chloro methyl ester dipeptide 4.42 in 65% yield. Compound 4.42 was treated with sodium azide to give azido methyl ester dipeptide 4.43 in 35% yield.
4.2.2 Synthesis of Starting Materials for Solid-Phase Staudinger Ligation of 4.59-61 and 4.68-69

4.2.2.1 Synthesis of boc-protected amino acid linkers 4.48-50

Compounds 4.48-50 were prepared by reaction of commercially available 3-mercaptopropionic acid 4.47 with Boc-protected aminoacyl-benzotriazolides 4.44-46. Acylation of the thiol afforded the Boc-protected amino acid linkers in good yields (57-85%) (Scheme 4-9). [92IJPXR180]

Scheme 4-9. Synthesis of Boc-protected amino acid linkers 4.48-50

4.2.2.2 Synthesis of azido-protected solid-phase supported dipeptides 4.59-61.

Boc-protected amino acid linkers 4.48-50, were attached to the aminomethyl (AM) resin (4.51), chosen due to its high loading capacity (1.66 mmol/g), using DCC (0.5 M in DCM) coupling overnight in a mechanical shaker. Boc-deprotection gave the TFA salts 4.55-57. Azido-glycyl benzotriazole 4.58 was then reacted with 4.55-57 under the basic conditions to yield the azido-protected solid-phase supported dipeptides 4.59-61 (Scheme 4-10).
Scheme 4-10. Synthesis of solid-phase supported azido-protected dipeptides 4.59-61

The spent resin 4.63 was recycled for a second cycle (Scheme 4-11). Boc-protected amino acid 4.62 was reacted with 4.63 to yield 4.64. The solid-phase supported amino acid was then carried out through the synthesis to give azido-protected solid supported dipeptide 4.60 (Scheme 4-11).

Scheme 4-11. Recyclability of resin 4.63 to give intermediate 4.53

4.2.2.3 Synthesis of azido-protected solid-phase supported tripeptides 4.68-69

Due to the modular design of the SPPS, intermediates from the dipeptide synthesis were used as starting materials in the tripeptide synthesis (Scheme 4-12). TFA salts 4.55 and 4.56 were reacted with Boc-protected alaninyl benzotriazolide 4.46 under basic conditions to give Boc-protected solid supported dipeptides 4.64 and 4.65.
respectively. Deprotection with TFA, gave salts 4.66 and 4.67 which were reacted with azido glycyI benzotriazole 4.58 to give the solid supported azido-protected tripeptides 4.68-69.

Scheme 4-12. Synthesis of azido-protected solid supported tripeptides 4.68-69

4.2.3 Staudinger Ligation of 4.38-40, 4.43, 4.59-61, and 4.68-69 for the Formation of 2,5-Diketopiperazines 4.63-4.65

A screening of the reaction was undertaken to indentify optimal conditions for the proposed Staudinger ligation. Initial investigation with 4.38 utilizing 1.5 equivalents of tributylphosphine in dry DCM under standard reflux conditions (Entry 1) served as the starting point (Table 4-2). Solvent, addition or exclusion of water, and different phosphines were examined for optimization. Microwave irradiation gave a higher yield.
with shorter reaction time (Table 4-2, Entry 2). An increase in yield was seen upon addition of 5 equivalents of water, facilitating hydrolysis of the phosphonium salt (Table 4-2, Entry 3). The lower polarity of DCM aids in filtration of insoluble 4.70, indentifying it as the best solvent (Table 4-2, Entries 4-6). Reaction with triphenylphosphine resulted in lower yields and difficulty was seen in separation of triphenylphosphine oxide from 4.70 (Table 4-2, Entry 7). Thus using tributylphosphine, in DCM, under continuous microwave irradiation (50 W, 50 °C, 5 min), with 5 equivalents of water (Table 4-2, Entry 3) gave the best yield of 4.70.

Table 4-2. Initial conditions for optimization of preperation of 4.70

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBu₃, CH₂Cl₂ (dry)ᵃ, rt, 12 h</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>PBu₃, CH₂Cl₂ (dry)ᵃ, MWᵇ, 30 min</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>PBu₃, CH₂Cl₂, H₂O (5 eq.), MWᵇ, 5 min</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>PBu₃, THF, H₂O (5 eq.), MWᵇ, 5 min</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>PBu₃, CH₃CN, H₂O (5 eq.), MWᵇ, 5 min</td>
<td>65</td>
</tr>
<tr>
<td>6</td>
<td>PBu₃, toluene, H₂O (5 eq.), MWᵇ, 5 min</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>PPh₃, CH₂Cl₂, H₂O (5 eq.), MWᵇ, 5 min</td>
<td>58</td>
</tr>
</tbody>
</table>

ᵃ Reaction was quenched with H₂O;ᵇ 50 °C, 50 W

Methyl ester 4.43 was treated under the optimum conditions (60% yield of 4.70) but was less reactive then the thioester 4.38 (Scheme 4-13). A computational investigation, at the B3LYP/6-31G(d) level of theory, supported the experimental evidence by showing a lower activation barrier for thiophenol (21.6 kcal/mol) versus the methyl ester (30.0 kcal/mol). [Unpublished work by Dr. Jean Christophe Monbaliu]
Scheme 4-13. Reaction of methyl ester derivative 4.43 in the Staudinger ligation under optimized conditions

Compounds 4.39 and 4.40 gave 4.71 and 4.72 (74% and 78% yields) using method A for solution phase synthesis (Table 4-3).

Table 4-3. Reaction of 4.38-40 and 4.59-61 to form 4.70-72

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i-Bu (4.38)</td>
<td>78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Bn (4.39)</td>
<td>74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Me (4.40)</td>
<td>78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>i-Bu (4.59)</td>
<td>79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Bn (4.60)</td>
<td>72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Me (4.61)</td>
<td>81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Bn (4.60)</td>
<td>82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Method A (solution phase); <sup>b</sup>Method B (solid phase); <sup>c</sup>Linker-aminomethyl resin 4.63 was recovered and reused
Reactions of the solid-phase substrates 4.59-4.61 were carried out using the optimized solution-phase conditions (Table 4-3), and gave similar yields to those found in the solution-phase method.

Recycled resin 4.60 (Entry 7) was reacted under the optimized Staudinger protocols yielding 4.71 in 83% yield thus demonstrated recyclability.

Formation of cyclic tripeptides was attempted (Scheme 4-14) by solid-phase methodology. Unfortunately, no evidence was obtained for cyclic tripeptide formation under Staudinger ligation protocols.

Scheme 4-14. Attempted solid-phase cyclic tripeptide formation of 4.73 and 4.74 under Staudinger ligation protocols

However, formation of cyclic dipeptide 4.72 was observed from 4.68 and 4.69 due to an unprecedented cyclization/cleavage of the amide bond (Scheme 4-15).

Scheme 4-15. Formation of the unexpected cyclic peptide 4.72 from the linear tripeptides 4.68 and 4.69

Computational investigation, performed by Dr. Jean-Christophe Monbaliu, utilizing the B3LYP/6-31G(d) level of theory on model compounds, including PH₃ as a model
phosphine, showed the energy difference between the competitive cyclization pathways. The 9-membered cyclic transition state (TS$_9$ 4.78) was favored by only 2.4 kcal/mol over the 6-membered cyclic transition state (TS$_6$ 4.77). Increase in steric congestion around the thioester and the higher phosphine may lead to a reversal of the tendency to cyclize to the tripeptide.

![Figure 4-5](image)

**Figure 4-5. Competitive cyclization through TS$_6$ and TS$_9$ for the formation of 4.72**

### 4.3 Summary

2,5-Diketopiperazines (4.70-72) were synthesized from the corresponding azido thioester dipeptides in excellent yields, using a straightforward Staudinger ligation protocol. The starting materials were synthesized from commercially available $\alpha$-amino acids by known procedures, and syntheses were accomplished with short reaction times and simple purification procedures. The modified aminomethyl resin 4.63 was recycled and reused without loss in yield providing a cost-efficient and eco-friendly method compared to conventional SPPS procedures. Further work should include revisiting the tripeptide sequence design, but taking steric factors into consideration.
4.4 Experimental

4.4.1 General Methods

$^1$H NMR spectra were recorded at 300 MHz and $^{13}$C NMR spectra were recorded at 75 MHz on Gemini or Varian spectrometers at room temperature. The chemical shifts are reported in ppm relative to TMS as internal standard ($^1$H NMR) or to solvent residual peak ($^{13}$C NMR). HRMS spectra were recorded on a LC TOF (ES) apparatus. Elemental analysis was performed on a Carlo Erba-1106 instrument. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. Flash chromatography was performed on silica gel 60 (230-400 mesh). All solvents were dried according to standard procedures. Triethylamine was distilled prior use. All commercially available substrates were used as received without further purification. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stir bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 120 sec.; PowerMax-cooling mode). Amino acids were purchased from Chem-Impex International, and the aminomethyl (AM) resin was purchased from Chempep. Quantum chemical calculations were done using Gaussian 03W version 6.1.

4.4.2 General Methods for the Synthesis of 4.70-72

To a stirred solution of 4.38-40 (1 mmol) in CH$_2$Cl$_2$ (4 mL), tributylphosphine (0.37 mL, 1.5 mmol) was added and stirring was continued for 5 min at room temperature. Water (0.1 mL, 5 mmol) was added and stirring was continued for 5 min. The reaction mixture was then subjected to microwave irradiation (50 W, 50 °C, 5 min). Hexane (4
ml) was then added to the reaction to induce crystallization and the mixture was placed in the freezer. The reaction was filtered and washed with DCM (5 mL) and hexanes (15 mL) and dried under vacuum to yield pure 4.70-72.

To a stirred suspension of 4.59-61 (1 mmol) in CH₂Cl₂ (4 mL), tributylphosphine (0.37 mL, 1.5 mmol) was added and stirring was continued for 5 min at room temperature. Water (0.1 mL, 5 mmol) was added and stirring was continued for 5 min. The reaction mixture was then subjected to microwave irradiation (50 W, 50 °C, 5 min). The solids were filtered and washed with CH₂Cl₂, the remaining solid was treated with hot methanol and the mother liquor was collected, cooled in the freezer and the precipitate collected to yield pure 4.70-72.

To a stirred suspension of 4.68-69 (1 mmol) in CH₂Cl₂ (4 mL), tributylphosphine (0.37 mL, 1.5 mmol) was added and stirring was continued for 5 min at room temperature. Water (0.1 mL, 5 mmol) was added and stirring was continued for 5 min. The reaction mixture was subjected to microwave irradiation (50 W, 50 °C, 5 min), the solids filtered and washed with CH₂Cl₂. The remaining solid was treated with hot methanol and the resulting mother liquor was collected, cooled in the freezer and the precipitate collected and dried under vacuum to yield pure 4.72.

(S)-3-Isobutylpiperazine-2,5-dione 4.70. White microcrystals, yield: 78% (0.13 g), mp 248.0-250.0 °C. ¹H NMR (300 MHz, DMSO-d₆): δ = 8.26 (br s, 1H), 8.26 (br s, 1H), 7.99 (br s, 1H), 3.89 - 3.79 (m, 1H), 3.70 - 3.56 (m, 2H), 1.84 - 1.69 (m, 1H), 1.56 - 1.49 (m, 2H), 0.89 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H) ppm. ¹³C NMR (75 MHz, DMSO-d₆): δ = 168.8, 166.3, 52.9, 44.2, 42.1, 23.6, 22.9, 21.8 ppm. Anal. Calcd. for C₈H₁₄N₂O₂: C 56.45; H 8.29; N 16.46; Found: C 56.63; H 8.41; N 16.28.
(S)-3-Benzylpiperazine-2,5-dione 4.71. White microcrystals, yield: 74% (0.15 g), mp 251.0-252.0 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 8.18$-8.11 (m, 1H), 7.90-7.84 (m, 1H), 7.32-7.21 (m, 3H), 7.18-7.10 (m, 2H), 4.09-4.02 (m, 1H), 3.42-3.26 (m, 2H), 3.08 (dd, $J = 13.5$, 4.4 Hz, 1H), 2.87 (dd, $J = 13.5$, 4.9 Hz, 1H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 167.4$, 166.0, 136.1, 130.2, 128.3, 127.0, 55.7, 43.8 ppm. Anal. Calcd. for C$_{11}$H$_{12}$N$_2$O$_2$: C 64.49; H 5.92; N 13.72; Found: C 64.43; H 6.06; N 13.65.

(S)-3-Methylpiperazine-2,5-dione 4.72. White microcrystals, yield: 78% (0.10 g), mp 236-238 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 8.17$ (br s, 1H), 7.99 (br s, 1H), 3.88 (dq, $J = 0.6$, 6.9 Hz, 1H), 3.76 (s, 2H), 1.30 (d, $J = 6.9$ Hz, 3H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 168.8$, 166.2, 49.7, 44.5, 18.6. ppm.

4.4.3 General Methods for the Synthesis of Compounds 4.32-40

4.4.3.1 General procedures for the synthesis of compounds 4.32-34

To a suspension of the appropriate amino acid 4.28-30 (20 mmol) in THF (50 mL), chloroacetyl chloride 4.31 (2.4 mL, 30 mmol) was added and the mixture was heated under reflux for 2 h. After cooling, water (20 mL) and brine (30 mL) were added and the mixture was extracted with ethyl acetate (3 x 75 mL). The combined organics were dried over MgSO$_4$ and the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether and stirred at 0 °C for 30 min. The mixture was filtered and the white solid was collected to yield pure 4.32-34.

(S)-2-(2-Chloroacetamido)-4-methylpentanoic acid 4.32. White microcrystals, yield: 77% (3.19 g), mp 139.0-141.0 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 8.46$ (d, $J = 7.9$ Hz, 1H), 4.28 - 4.17 (m, 1H), 4.13 - 4.02 (m, 2H), 1.70 - 1.58 (m, 1H), 1.58 - 1.48 (m, 2H), 0.88 (d, $J = 6.3$ Hz, 3H), 0.88 (d, $J = 6.3$ Hz, 3H), 0.84 (d, $J = 6.3$ Hz, 3H) ppm. $^{13}$C
NMR (75 MHz, DMSO-$d_6$): $\delta$ = 21.9, 23.4, 25.0, 40.6, 43.0, 51.2, 166.6, 174.2 ppm.

Anal. Calcd. for C$_8$H$_{14}$ClNO$_3$: C 46.27; H 6.80; N 6.75; found: C 46.64; H 6.61; N 6.70.

(S)-2-(2-Chloroacetamido)-3-phenylpropanoic acid 4.33. White microcrystals, yield: 61% (2.97 g), mp 125.0-127.0 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 8.53 (d, $J$ = 7.9 Hz, 1H), 7.37-7.21 (m, 5H), 4.58-4.46 (m, 1H), 4.10 (s, 1H), 4.09 (s, 1H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 172.5, 170.0, 137.3, 129.2, 128.3, 126.6, 53.9, 42.4, 36.7 ppm. Anal. Calcd. for C$_{11}$H$_{12}$ClNO$_3$: C 54.67; H 5.00; N 5.80; found: C 54.63; H 5.04; N 5.71.

(S)-2-(2-Chloroacetamido)propanoic acid 4.34. White microcrystals, yield: 38% (1.29 g), mp 84.0-87.0 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 12.79-12.58 (m, 1H), 8.51 (d, $J$ = 7.2 Hz, 1H), 4.41-4.14 (m, 1H), 4.12 (s, 1H), 1.32 (d, $J$ = 7.3 Hz, 3H) ppm. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 173.7, 165.7, 47.9, 42.4, 17.1 ppm. Anal. Calcd. for C$_5$H$_8$ClNO$_3$: C 36.27; H 4.87; N 8.46; found: C 36.63; H 4.96; N 8.29.

4.4.3.2 General procedures for the synthesis of compounds 4.35-37

4.32-34 (10 mmol) was dissolved in a DMF/water 2:1 mixture (45 mL), sodium azide (2.92 g, 45 mmol) was added and the suspension was stirred for 12 h at room temperature. Water (30 mL) was added and the mixture stirred for 30 min, and extracted with ethyl acetate (3 x 50 mL). The combined organics were washed with water (3 x 50 mL) and brine (3 x 50 mL), dried over MgSO$_4$ and solvent was removed under reduced pressure. The residue was recrystallized from ethyl acetate/hexanes to afford pure 4.35-37.

(S)-2-(2-Azidoacetamido)-4-methylpentanoic acid 4.35. White microcrystals, yield: 61% (1.31 g), mp 105.1-107.0 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 12.75 (s, 1H), 8.47 (dd, $J$ = 28.6, 7.9 Hz, 1H), 4.35-4.20 (m, 1H), 4.13 (d, $J$ = 2.0 Hz, 1H), 3.89 (d, $J$ = 0.8 Hz, 1H) ppm. Anal. Calcd. for C$_{10}$H$_{14}$N$_3$: C 46.27; H 6.48; N 14.48; found: C 45.91; H 6.51; N 14.58.
Hz, 1H), 1.75-1.49 (m, 3H), 0.91 (dd, \( J = 12.7, 6.3 \) Hz, 6H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 173.7, 167.5, 50.6, 50.4, 50.3, 42.4, 24.3, 22.9, 21.3 \) ppm. HRMS calcd for 

\[ \text{C}_8\text{H}_{14}\text{N}_4\text{O}_3 \] [M-H]: 213.1005. Found 213.1002

(S)-2-(2-Azidoacetamido)-3-phenylpropanoic acid 4.36. White microcrystals, yield: 78% (1.94 g), mp 99.4-103.0 °C. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta = 12.86 \) (br s, 1H), 8.42 (d, \( J = 8.0 \) Hz, 1H), 7.31-7.15 (m, 5H), 4.47 (dt, \( J = 8.7, 5.0 \) Hz, 1H), 3.79 (d, \( J = 5.3 \) Hz, 2H), 3.08 (dd, \( J = 13.8, 5.0 \) Hz, 1H), 2.89 (dd, \( J = 13.8, 9.2 \) Hz, 1H) ppm. \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 172.6, 167.4, 137.4, 129.1, 128.3, 126.6, 53.6, 50.5, 36.7 \) ppm. Anal. Calcd for \( \text{C}_{11}\text{H}_{12}\text{N}_4\text{O}_3 \): C 53.22; H 4.87; N 22.57; found: C 53.46; H 4.92; N 22.45.

(S)-2-(2-Azidoacetamido)propanoic acid 4.37. Yield: 65% (1.12 g), white microcrystals. m.p. 100.1-101.0 °C. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta = 12.68 \) (br s, 1H), 8.45 (d, \( J = 7.3 \) Hz, 1H), 4.33-4.22 (m, 1H), 3.88 (br s, 2H), 1.32 (d, \( J = 7.3 \) Hz, 3H) ppm. \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 173.7, 167.1, 50.4, 47.6, 17.2 \) ppm. Elemental analysis calcd (% for \( \text{C}_5\text{H}_8\text{ClNO}_3 \): C, 36.27; H, 4.87; N, 8.46; found: C, 36.63; H, 4.96; N, 8.29.

4.4.3.3 General methods for the synthesis of 4.38-40

Isobutyl chloroformate (0.72 mL, 5.5 mmol) and N-methyl morpholine (0.60 mL, 5.5 mmol) were mixed and reacted for 5 minutes with a stirred solution of 4.35-37 (5 mmol) in THF (30 mL). Thiophenol (0.56 mL, 5.5 mmol) was added and the reaction was warmed to room temperature and allowed to stir for 18 h. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (50 mL), washed with 10% NaOH (3 x 25 mL), and brine (25 mL) then dried over MgSO\(_4\). The solvent was removed under reduced pressure, and the residue was recrystallized from a mixture of DCM/hexanes to yield pure 4.38-40.
(S)-S-Phenyl 2-(2-azidoacetamido)-4-methylpentanethioate 4.38. Yield: 75% (1.15 g), white microcrystals. m.p. 138.0-140.0 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 7.50 - 7.35 (m, 5H), 7.34 - 7.27 (m, 1H), 7.09 (d, $J$ = 8.3 Hz, 1H), 4.91 - 4.74 (m, 1H), 4.13 - 4.06 (m, 2H), 4.01 (s, 2H), 1.84 - 1.53 (m, 3H), 0.96 (d, $J$ = 6.3 Hz, 3H), 0.93 (d, $J$ = 6.0 Hz, 3H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 198.9, 168.6, 168.6, 167.8, 134.8, 129.8, 129.5, 126.9, 58.0, 52.6, 43.2, 41.5, 25.1, 23.2, 21.7 ppm. Elemental analysis calcd (%) for C$_{16}$H$_{21}$N$_4$O$_3$S: C, 52.88; H, 5.82; N, 19.27; found: C, 52.94; H, 6.01; N, 19.14.

(S)-S-Phenyl 2-(2-azidoacetamido)-3-phenylpropanethioate 4.39. Yield: 57% (0.97 g), white microcrystals. m.p. 135.0 - 137.0 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 12.68 (br s, 1H), 8.45 (d, $J$ = 7.3 Hz, 1H), 4.33-4.22 (m, 1H), 3.88 (br s, 2H), 1.32 (d, $J$ = 7.3 Hz, 3H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 173.7, 167.1, 50.4, 47.6, 17.2 ppm. Elemental analysis calcd (%) for C$_{17}$H$_{16}$N$_4$O$_3$S: C, 59.98; H, 4.74; N, 16.46; found: C, 59.74; H, 4.68; N, 16.22.

(S)-S-Phenyl 2-(2-azidoacetamido)propanethioate 4.40. Yield: 42% (0.56 g), white microcrystals. m.p. 124.5-126.0 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 7.29-7.22 (m, 5H), 6.65 (d, $J$ = 7.7 Hz, 1H), 4.71 (qd, $J$ = 14.4, 7.2 Hz, 1H), 3.90 (d, $J$ = 1.3 Hz, 2H), 1.43-1.39 (m, 2H), 1.36 (d, $J$ = 7.2 Hz, 3H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 173.0, 166.6, 134.9, 130.0, 129.6, 54.9, 52.8, 19.1 ppm. Elemental analysis calcd (%) for C$_{11}$H$_{12}$N$_4$O$_3$S: C, 49.99; H, 4.58; N, 21.20; found: C, 50.12; H, 4.43; N, 21.57.
CHAPTER 5
A-BENZOTRIAZOYL NITROSO DERIVATIVES: POTENTIAL NOVEL NO DONORS

5.1 Literature Overview

Nitroso compounds are of great interest due to their reactivity and transient nature. While many of these compounds have shown both therapeutic and mutagenic activity, attention has also been focused on their use in synthetic processes. Nitrogen oxides and their derivatives form a family of compounds involved in many physical, chemical and biological phenomena, ranging from atmospheric pollution to immune response.

5.1.1 Biological Properties of Selected Nitroso Compounds

$N$-Nitroso amines are a class of nitrosation agents that act as potent mutagens to mammalian cells which may lead to heptacarcinoma (liver cancer).[81CR5039] Nitrosomorpholine (NMOR) 5.1, is a potent mutagen forming liver cancer in rats \textit{in vivo}. [79JOC1563] Although nitroso proline 5.2 and nitroso hydroxyproline 5.3 are non-carcinogenic compounds found in nature, consumption may lead to trans-nitrosation with morpholine to form NMOR.[81CR5039] Cyclic $N$-nitroso amines have been shown to be more carcinogenic than their acyclic counter parts.[81CR5039]

\begin{center}
\begin{tikzpicture}
\node at (0,0) {5.1};
\node at (1,0) {5.2};
\node at (2,0) {5.2};
\end{tikzpicture}
\end{center}

Figure 5-1. N-Nitroso compounds showing interesting biological activity

However, NO plays an important role in neurotransmission,[91TN60] immune regulation,[88B8706, 89JEM1011, 91RI565] vascular smooth muscle relaxation,[86PNAS9164, 89PR651] and inhibition of platelet aggregation.[86JP411, 87JP687, 87BPRC1482] Naturally occurring $S$-nitrosothiols, $S$-nitroso-$L$-cysteine (5.4)
and S-nitroso-L-glutathione (5.5), are important NO releasers in vivo.[00CS507] An important class of enzymes known as NO synthase enzymes (NOS), catalyze the sequential oxidation of L-arginine to release NO and L-citrulline.[89PNAS444] The mechanism involves a Cu$^+$ species for the in vivo release of NO from thiols and thiol-based NO releasing drugs.[00CS507]

![Figure 5-2. S-Nitroso compounds of biological relevance](image)

**5.1.2 Different Forms of Nitroso Compounds**

Many compounds with the nitroso moiety have been studied (Figure 5-3).[11ACIE5630, 05CC3514] Our research will pay a particular interest to compounds 5.8-9 involving an α-leaving group.

![Figure 5-3. Examples of various nitroso compounds](image)

Recent literature has shown that reactivity is significantly affected by the structure of the nitroso compound.[10JSST49] The electrophilicity scale (Figure 5-4) shows that acyl nitrosos are the most reactive, thus they must be generated in situ.[11ACIE5630]
While the alkyl nitrosos are the least electrophilic, making them more stable and sluggish to react.

![Electrophilicity Scale](image)

Figure 5-4. Global electrophilicity scale of common nitroso compounds[10JST49]

Nitroxyl 5.6 is the simplest form of a nitroso compound and is highly reactive. It is not usually used in synthesis due to its fleeting nature and is a decomposition product of nitroso compounds.[05CCR433] Dimerization of 5.6 is a major issue, leading to loss of \( \text{H}_2\text{O} \) and formation of \( \text{N}_2\text{O} \).[96JACS3550, 06JACS9687]

\( \alpha \)-Chloronitroso compounds 5.8 are subject to rapid decomposition, due to their highly polarized nature.[98T1317] With chloro as a weak electron withdrawing leaving group, the nitroso is slow to react as an electrocyclization reagent, but has been well studied in that capacity.[84TL5377, 98T1317, 00JCS(P1)329] \( \alpha \)-Chloronitroso compounds are readily synthesized from oximes and electrophilic chlorine reagents (e.g. t-BuOCl).[84TL5377, 00JCS(P1)329]

\( \alpha \)-Acetoxy nitroso compounds 5.9 are more stable than the \( \alpha \)-chloro nitroso analogs, compound 5.9 can be synthesized from oxime precursors by oxidation with lead (IV) tetraacetate or IBX.[06JACS9687, 05CTMC665] \( \alpha \)-Acetoxy nitroso compounds are also important in studying the release of NO and HNO.[05CCR433, 11ARS1637]

\( \alpha \)-Acyl nitroso species 5.15 are extremely unstable, but can be reacted \textit{in situ} as transient species.[81T4007] They are commonly synthesized by oxidation of oxime or isocyanate precursors and either reacted or trapped as cycloadducts with 9,10-dimethylandthracene.[79CJC1712, 81T4007] They have found considerable use in
hetero Diels-Alder reactions because of their high reactivity. Their existence was first proved by reaction with various nucleophiles (Figure 5-5), although the first direct proof was provided by Schwarz and co-workers in 1991 through neutralization-reionization mass spectrometry when liberated from 9,10-dimethylanthracene adducts.

Figure 5-5. Transient acyl nitroso compound 5.15 and their derivatives 5.17-19 on reaction with nucleophiles

α-Aryl nitroso compounds 5.13-14 are stable compounds whose aryl group acts to modulate the reactivity of the nitroso moiety. This dampening of reactivity can be regulated by introducing electron withdrawing groups into the aryl ring which enhances reactivity. Normally these compounds are synthesized by oxidation of the corresponding oximes, but may also be generated by oxidation in situ of the corresponding aromatic amines.

Cyano based nitroso compounds fall into two main groups: nitrosocyanamide 5.7 (direct attachment of the nitroso to the cyano group) and α-cyano nitroso compounds 5.10 (the cyano is an α-leaving group). The use of nitrosocyanamide 5.7 is limited due to its high reactivity and is usually stored as a...
cycloadduct with 9,10-dimethyl anthracene.[80JCS(P1)1587, 81JCS(P1)1802] The α-cyano nitroso 5.10 is a useful reagent as a surrogate of α-chloro nitroso compounds where excessive reactivity is a concern.[96T7585, 09JOC1450]

5.1.3 Reactions of Nitroso Compounds

Three common reactions of nitroso compounds described in the literature are: i) nitroso Diels-Alder reactions, ii) nitroso-ene reactions and iii) nitroso aldol reactions. Most other “reactions” involve decompositions of nitroso compounds where either NO or HNO is released.

5.1.3.1 Diels-Alder reactions of nitroso compounds

The Diels-Alder reaction is a pericyclic reaction discovered in 1928 by Otto Paul Hermann Diels and Kurt Alder.[28JLAC98] Nitroso compounds act as activated dienophiles, the exception being vinyl nitrosos that can also act as a diene.[11ACIE5630, 98T1317] The nitroso Diels-Alder reaction results in useful building blocks such as 1,2-oxazine rings.[94S1107] Thus with the α-leaving group nitroso, the nitroso Diels-Alder reaction is a three step process (Figure 5-6). Initially the 1,2-oxazine ring 5.21 is formed by pericyclic reaction of 5.8 and 5.20. The acetoxy or chloro group leaves forming iminium salt 5.22 which is then hydrolyzed yielding the free 1,2-oxazine 5.23. If the reaction takes place in a polar protic solvent such as methanol, solvolysis occurs immediately on formation of the iminium salt 5.22.[10JST49]

\[
\begin{array}{c}
\text{5.8} \\
\text{5.20} \\
\text{5.21} \\
\text{5.22} \\
\text{5.23}
\end{array}
\]

Figure 5-6. Three step process for the formation of 1,2-oxazines in nitroso hetero Diels-Alder reactions using α-leaving group nitroso 5.8
Much attention has been given to enantio- and diastereoselective Diels-Alder reactions, and chiral nitroso compounds are used to induce stereoselectivity.[05CC3514, 98T1317, 07BCJ595] Yamamoto et al. have recently written a comprehensive review on stereoselective nitroso hetero Diels-Alder reactions.[06EJOC2031] For example, chiral nitroso reagent 5.25 was employed as a key step in Kibayashi’s asymmetric hDA synthesis of (-)-epibatidine (Figure 5-7).[98JOC8397]

![Chemical结构式](image)

**Figure 5-7.** Asymmetric hDA utilized in the synthesis of (-)-epibatidine from chiral nitroso reagent 5.25

### 5.1.3.2 Nitroso-ene reactions

The nitroso-ene reaction is another reaction associated with nitroso compounds (Figure 5-8).[05CC3514, 07BCJ595] The electron-deficient nitroso 5.28 acts as an enophile that reacts with the allylic system 5.27 to give the pericyclic product 5.29.[01JCSPT(1)1908] Asymmetric versions have also been developed using chiral nitroso compounds.[81JACS3173, 82JOC1302, 00JACS9846]

![Chemical结构式](image)

**Figure 5-8.** The nitroso-ene reaction involving an allylic system and a nitroso compound
5.1.3.3 Nitroso aldol reactions

In the nitroso-aldol reaction the nitroso acts as a surrogate carbonyl compound. Mukaiyama and co-workers report the reaction in 1963, isolating azomethine 5.32 from reaction of nitrosobenzene 5.31 with diethyl malonate 5.30.[63BCJ970]

\[
\begin{align*}
\text{EtO} & \quad \text{Cat. NaOH} \quad \text{EtOH} \\
\text{O} & \quad \text{O} \\
\text{N}_{\text{Ph}} & \quad \text{EtO} \\
5.30 & \quad \xrightarrow{5.31} \quad 5.32 \\
\text{O} & \quad \text{EtO}
\end{align*}
\]

Figure 5-9. The nitroso aldol reaction of nitrosobenzene and diethyl malonate

Later, however, it was found that both the nitrogen and oxygen could be involved in the nitroso aldol reaction (Figure 5-10).[07BCJ595] Yamamoto and co-workers also reported the catalytic enantioselective addition of enolates to nitroso. By varying the equivalents of silver in the catalyst, they were able to induce selective addition to either the O- or N-moiety of the nitroso group.[04JACS5360, 04JACS5962]

\[
\begin{align*}
\text{O} & \quad \text{N}_{\text{Ph}} \\
\text{O} & \quad \text{O} \\
\text{N}_{\text{Ph}} & \quad \text{O} \\
5.34 & \quad \xrightarrow{2 \text{ eq AgOTf}, (R)-BINAP} \quad 5.33 \quad + \quad 5.31 \quad \xrightarrow{1 \text{ eq AgOTf}, (R)-BINAP} \quad 5.35 \\
\text{O} & \quad \text{OH}
\end{align*}
\]

Figure 5-10. Selective addition to the O- or N-moiety of nitroso compounds

5.1.3.4 Release of NO and HNO

The release of NO or HNO by decomposition of nitroso compounds is important in medicinal and pharmaceutical chemistry,[11JMC1059, 11ARS1637] since NO and HNO have therapeutic value as vasodilators (Chapter 5.1.1).[00PR471] King and co-workers investigated the release of HNO from -acetoxy nitroso compound 5.36 and found
addition of base gave 5.37, which decomposed to release HNO 5.6 and cyclohexanone 5.38 (Figure 5-17).[06JACS9687]

Figure 5-11. Release of HNO from α-acetoxy nitroso compounds

The bond dissociation energy of C-Nitroso compounds is approximately 36-40 kcal/mol which is similar to the O-NO BDE of organic nitrate esters (a common NO donor).[95JPC10815, 78JCS(P2)1110, 02JPCA12386, 05CTMC687] Thus NO release occurs by homolytic bond cleavage of the nitroso moiety from the parent C-nitroso compound.[01JACS8868]

Nitrate esters are effective NO donors, but unfortunately require co-catalysts (protons, base, enzymes) and discharge NO in various oxidation states (·NO vs +NO).[05CCR433, 92S1898, 99BPA1411, 05CRT790, 10CC3788] However, Toone et al. used α-cyano nitroso compound 5.10 to selectively release NO without a co-catalyst (Figure 5-18).[09JOC1450]

Figure 5-12. Release of NO radical from α-cyano nitroso compound 5.10
5.2 Results and Discussion

5.2.1 Synthesis of α-Leaving Group Benzotriazole Nitroso Compounds

The starting oximes 5.40-45 were synthesized in 67-91% yields by literature methods. Lead (IV) acetate/benzotriazole reagent, generated \textit{in situ} from lead (IV) tetracetate with 10 equivalents of 1H-benzotriazole in DCM or THF on addition of 5.40-45, the reaction mixture turned blue-green signaling formation of 5.46-51 (Table 5-1).

Table 5-1. Synthesis of α-benzotriazoyl nitroso compounds 5.46-51

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R^1)</th>
<th>(R^2)</th>
<th>Solvent</th>
<th>Yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.46</td>
<td>Cyclohexyl</td>
<td>DCM</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>5.46</td>
<td>Cyclohexyl</td>
<td>THF</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>5.47</td>
<td>Cyclopentyl</td>
<td>THF</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>5.48</td>
<td>Cycloheptyl</td>
<td>THF</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>5.49</td>
<td>Cyclooctyl</td>
<td>THF</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>5.50</td>
<td>Me</td>
<td>Me</td>
<td>THF</td>
<td>76</td>
</tr>
<tr>
<td>5.51</td>
<td>Me</td>
<td>Et</td>
<td>THF</td>
<td>72</td>
</tr>
</tbody>
</table>

It should be noted that α-aromatic oxime 5.52, derived from acetophenone, failed to produce nitroso compound 5.53.

Figure 5-13. α-Aromatic substrate 5.52 which failed to react to produce α-benzotriazoyl nitroso 5.53

β-Aromatic nitroso 5.54 (Figure 5-14), derived from the methyl ibuprofen ketoxime, decomposed rapidly (within minutes), and 5.55 could not be isolated.
Figure 5.14. $\alpha$-Benzotriazoyl nitroso derived from methyl ketoxime 5.54

5.2.2 Hetero Diels-Alder Reaction of 5.46

No reaction was observed between 5.46 and either 2,3-methyl butadiene 5.56 or cyclopentadiene 5.57 (Table 5-2), and it was concluded that 5.46 was not a suitable substrate for nitroso Diels-Alder reactions.

In an effort to understand the lack of reactivity, a computational investigation, performed by Dr. Jean-Christophe Monbaliu, on the cycloaddition of $\alpha X$-NO derivatives with dienes 5.56-57. The computations were carried out using 2-(2-nitrosopropan-2-yl)-2-$H$-benzo[d][1,2,3]triazole (5.61, $X=\text{Bt}$), 2-chloro-2-nitrosopropane (5.62, $X=\text{Cl}$) and 2-nitrosopropan-2-yl acetate (5.63, $X=\text{AcO}$) as model dienophiles. 2-Nitrosopropane (5.64, $X=\text{H}$) was selected as a reference for studying the nature of the leaving group on the process. The global electrophilicity was obtained by the method of Domingo.

[07CPL341] [06EJOC2570] Full geometry optimization and verification of the Hessian were performed by the G03 program package (revision E.01). [09GE01] Transition states were optimized and localized at the B3LYP/6-31+G* level with zero point energy correction and were verified by frequency and IRC calculations. All reactants were optimized in gas phase at the B3LYP/6-31+G* level.
Table 5.2. Reaction of 5.46 in the nitroso Diels-Alder reaction

<table>
<thead>
<tr>
<th>αBtNO</th>
<th>Diene</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>neat</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>toluene</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>DCM</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>diethyl ether</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>THF</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>DCM/EtOH</td>
<td>rt</td>
<td>48</td>
<td>Degradation</td>
</tr>
<tr>
<td>5.46</td>
<td>5.56</td>
<td>MeOH</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.57</td>
<td>MeOH</td>
<td>rt</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>5.46</td>
<td>5.57</td>
<td>MeOH</td>
<td>MW</td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

The computed global electrophilicity (ω) allowed for a classification of the selected nitroso compounds according to their reactivity. The following electrophilicity scale was found: 5.64 ~ 5.61 (ω=2.6 eV) < 5.62 (ω=2.7 eV) < 5.63 (ω=2.9 eV), showing that these compounds are moderate electrophiles within the electrophilicity scale, and that the nature of leaving group X has minimal effect on global reactivity.

Figure 5-15. Picture of the TSs associated with the cycloaddition of the selected nitroso compounds with butadiene. For each situation, 4 isomeric TSs have been isolated from endo/exo approach of the dienophile and the syn/anti orientation of the X group vs N=O. The results shown are the most stable TSs (endo/anti) isolated.
Activation barriers for the cycloaddition step were calculated, and showed relative insensitivity towards the nature of X (Figure 5-15). The transition state associated with the most electrophilic species (5.63) is also the lowest in energy.

5.2.3 Computational Investigation into the Release of NO

The possibility of 5.46 as a NO releasing agent was investigated, since 5.46 did not participate in a hDA. Attempts were made to show NO release in our facilities, and an outside laboratory was also utilized. In an effort to provide evidence for the formation of NO, a computational investigation was undertaken. The computational work was carried out by Dr. Jean-Christophe Monbaliu.

Isodesmic heat ($\Delta H^{iso}$) for radical exchange and radical stabilization energies (RSE) [10PCCP9597] were computed for a variety of substituted cyclohexyl substrates, showing that the Bt$^2$ substituent stabilizes a radical better than phenyl does (Table 5-3).

Table 5-3. Isodesmic heat ($\Delta H^{iso}$) for radical exchange on different cyclohexyl substrates and corresponding radical stabilization energy (RSE)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^1=$</th>
<th>$\Delta H^{iso}$ (kcal/mol)</th>
<th>RSE (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.65</td>
<td>Me</td>
<td>-10.8</td>
<td>10.8</td>
</tr>
<tr>
<td>5.66</td>
<td>Cl</td>
<td>-10.2</td>
<td>10.2</td>
</tr>
<tr>
<td>5.67</td>
<td>Ph</td>
<td>-19.3</td>
<td>19.3</td>
</tr>
<tr>
<td>5.68</td>
<td>$^2$Bt</td>
<td>-30.9</td>
<td>30.9</td>
</tr>
</tbody>
</table>

Homolytic bond dissociation energies (BDE) for the release of nitric oxide were computed for compounds 5.65-68, and revealed that the homolytic bond rupture for 5.46 is similar to that of reference compound 5.10 (Table 5-4), [09JOC1450]
emphasizing that compound 5.46 could release nitric oxide forming a stable tertiary radical.

Table 5-4. Homolytic bond dissociation energies (BDE) for compounds 5.8, 5.10 and 5.46

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>BDE (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.46</td>
<td>^2Bt</td>
<td>22.4</td>
</tr>
<tr>
<td>5.10</td>
<td>CN</td>
<td>19.8</td>
</tr>
<tr>
<td>5.8</td>
<td>Cl</td>
<td>30.6</td>
</tr>
</tbody>
</table>

In contrast, the heterolytic bond dissociation leading to nitroxyl was found to be extremely disfavored for 5.46 compared to 5.8 [06JACS9687], the reference for HNO donors (Table 5-5).

Table 5-5. Heterolytic bond dissociation energies (BDE) for compounds 5.8, 5.36, and 5.46

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>BDE (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.46</td>
<td>^2Bt</td>
<td>189.4</td>
</tr>
<tr>
<td>5.36</td>
<td>O-</td>
<td>37.1</td>
</tr>
<tr>
<td>5.8</td>
<td>Cl</td>
<td>211.1</td>
</tr>
</tbody>
</table>

Indirect proof for the release of NO in situ was found when 5.46 was dissolved in methanol and allowed to stir for 4 hours open to air (Figure 5-16). The characteristic blue color of 5.46 disappeared and on removal of the solvent a white solid was obtained. The structure was determined unambiguously by X-ray diffraction of a single crystal as 5.69 (Figure 5-17).
Figure 5-16. Indirect proof for the release of NO through radical intermediate 5.70

![Chemical structure](image)

Figure 5-17. X-ray structure of α-benzotriazole nitro 5.69

The formation of nitro compound 5.69 from the nitroso has literature precedent and involves oxidation by molecular oxygen.[08EJOC3279] Oxidized N₂O reacts with the stabilized radical 5.70 to yield 5.69. The formation of NO₂ from NO is a well known and has been investigated by the oxidation of nitroso compounds by N₂O₄.[08EJOC3279]

**5.2.4 Properties of α-Benzotriazole Nitroso Compounds 5.46-51**

C-Nitroso compounds have a characteristic blue-green to deep blue color associated with their monomeric form.[96JOC1047, 09JOC1450] This blue coloration is due an \( n \rightarrow \pi^* \) transition of the nitroso group.[58QRCS321, 76T467] However nitroso compounds have a tendency to dimerize since the dimer is a lower energy state (6-10 kcal/mol for the dimerization and 20-30 kcal/mol for dissociation).[70JACS1460,
The dimers are unreactive, and must dissociate for a reaction to occur.\[96\text{JOC1047}\]

Compound 5.46 is deep blue in color in both solution and solid state indicating the monomer. In contrast, compounds 5.50-51 dimerize in the solid state forming a white powder. Compound 5.46 is both air and moisture stable for at least six months, and a X-ray structure showed a 95:5 ratio of nitroso to nitro compound, probably due to a small amount of NO release and oxidation to NO\(_2\) in solution during recrystallization.

Figure 5-18. X-ray structure of 5.46 with minor impurities from the nitro compound 5.69

Kinetic information was obtained by Ms. Judit Kovacs for the release of nitric oxide from compound 5.46 and 5.50-51 using UV-spectrophotometry at 20 °C. Effects of different solvents were seen by following the disappearance of the nitroso signal (\(\lambda_{\text{max}} = 655.7\) nm, 10 mg mL\(^{-1}\)). The observed dependency of the reaction rates on the solvent polarity supports a homolytic mechanism for release of NO since the half-life of 5.46 in MeOH, CH\(_3\)CN, and CH\(_2\)Cl\(_2\) was 2.47, 345.6, and 495.1 min, respectively. Inclusion of water in acetonitrile (7:3 acetonitrile/water mixture) increased the half life slightly (\(t_{1/2} = 385.1\) min). First order rate constants \(k_{\text{obs}} = 3.1, 2.0, 1.8\) and \(1.4 \times 10^{-3}\) s\(^{-1}\) were determined in methanol, acetonitrile/water, acetonitrile and dichloromethane (Figure 5-19).
Figure 5-19. Half-lives of 5.46 as measured in various solvents

Additional experiments using different concentrations (5 and 20 mg·mL⁻¹) of compound 5.46 in acetonitrile gave half-lives of $t_{1/2} = 266.6$ and 495.1 min. This is consistent with the formation of an “inactive reservoir” of nitroso compound 5.46 as its azodioxy dimer at higher concentration (Figure 5-20). As per Figure 5-21, the steric hindrance of the backbone on α-carbon has an impact on the release of NO ($t_{1/2} = 223.6$ (5.50), 247.6 (5.51) and 495.1(5.46) min).

Figure 5-20. Kinetics for dissociation at various concentrations in methanol of 5.46
5.3 Summary

A novel class of α-benzotriazoyl nitroso reagents 5.26-51 were synthesized in good yields utilizing a lead (IV) benzotriazole/acetate reagent. This method provided 5.46 as a stable reagent that is air and moisture insensitive for at least six months. The α-benzotriazoyl nitrosos did not react as dienophiles in hetero Diels-Alder reactions, but their potential as NO donors was investigated. A computation investigation supported NO release, and indirect evidence from the isolation of α-benzotriazoyl nitro 5.69 was obtained. Further laboratory testing is needed to learn more about the nature of 5.46 as a candidate for NO release.

5.4 Experimental

5.4.1 General Methods

$^1$H NMR spectra were recorded at 300 MHz and $^{13}$C NMR spectra were recorded at 75 MHz on Gemini or Varian spectrometers at room temperature. The chemical shifts are reported in ppm relative to TMS as internal standard ($^1$H NMR) or to solvent residual peak ($^{13}$C NMR). The NMR experiments at variable temperatures (35, 45, 55 and 65 °C)
were recorded on a Varian Inova NMR spectrometer operating at 500 MHz. Chiral HPLC experiments were performed on a Chirobiotic-T column using methanol as mobile phase. Compounds were analyzed at a flow rate of 0.1 mL/min (detection wavelength = 230 nm, solvent = methanol). HRMS spectra were recorded on a LC TOF (ES) apparatus. Elemental analysis was performed on a Carlo Erba-1106 instrument. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. Flash chromatography was performed on silica gel 60 (230-400 mesh). All commercially available substrates were used as received without further purification. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). Quantum chemical calculations were done using Gaussian 03W version 6.1.

5.4.2 Synthesis of Nitroso Compounds 5.46-51

A solution of lead (IV) tetraacetate (4.43 g, 10.0 mmol) and 1H-benzotriazole (11.9 g, 100.0 mmol) in THF (100 mL) was stirred for 15 min at 0 °C. The resulting homogeneous solution was treated dropwise by a solution of oxime 5.40-45 (10.0 mmol) in THF (25 mL) over 15 min at 0°C. After 2 h, the solvent was removed under reduced pressure and the brown residue was washed several times with hexanes (5 x 50 mL). The combined organic fractions were evaporated under reduced pressure and the greenish oily residue was purified over silica gel (hexanes /ethyl acetate 10/1) to
yield pure $\alpha$-benzotriazole nitrosos 5.46-51. Slow crystallization from a hexanes/diethyl ether mixture gave 5.46 as blue crystals.

2-(1-Nitrosocyclohexyl)-2H-benzo[d][1,2,3]triazole 5.46. Yield: 48% (1.95 g), blue microcrystals. m.p. 125.0-127.0 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.34-1.51 (m, 2H), 1.55-1.67 (m, 1H), 1.67-1.80 (m, 1H), 1.94-2.06 (m, 2H), 2.65-2.86 (m, 4H), 7.36-7.45 (m, 2H), 7.84-7.93 (m, 2H) ppm. $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 21.7, 24.6, 29.2, 118.7, 127.0, 128.1, 144.9 ppm. Elemental analysis calcd (%) for C$_{12}$H$_{14}$N$_4$O$_1$: C, 62.59; H, 6.13; N, 24.33; found: C, 62.20; H, 5.90; N, 24.41.

**5.4.3 X-Ray Data for 5.46 and 5.69**

Crystal data for compound 5.46: blue crystal (plates), dimensions 0.4 x 0.1 x 0.04 mm, crystal system monoclinic, space group P2(1)/c, Z = 4, $a = 11.7352(5)$, $b = 8.5659(3)$, $c = 12.1021(4)$ Å, $\beta = 110.616(2)^\circ$, $V = 1138.63(7)$ Å$^3$, $\rho = 1.349$ g cm$^{-3}$, $T = 100(2)$ K, $\Theta_{\text{max}} = 30.60^\circ$, radiation Mo-K$\alpha$, $\lambda = 0.71073$ Å, 0.3 $\omega$-scans with CCD area detector, covering a whole sphere in reciprocal space, 14435 reflections measured, 3407 unique ($R_{\text{int}} = 0.0218$), 2873 observed ($I > 2\sigma(I)$), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, $m = 0.091$ mm$^{-1}$, $T_{\text{min}} = 0.6840$, $T_{\text{max}} = 0.7461$, structure solved by directmethods and refined against $F^2$ with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 164 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 1.069 for observed reflections, final residual values $R1(F) = 0.0376$, $wR(F^2) = 0.0976$ for observed reflections.CCDC 883509.

Crystal data for compound 5.69: white crystal (rods), dimensions 0.55 x 0.29 x 0.26 mm, crystal system orthorhombic, space group P2(1)2(1)2(1), Z = 4, $a = 5.9246(9)$, $b = 5.3906(9)$, $c = 18.6750(18)$ Å, $\beta = 90.00^\circ$, $V = 520.50(2)$ Å$^3$, $\rho = 1.349$ g cm$^{-3}$, $T = 296(2)$ K, $\Theta_{\text{max}} = 30.40^\circ$, radiation Mo-K$\alpha$, $\lambda = 0.71073$ Å, 0.3 $\omega$-scans with CCD area detector, covering a whole sphere in reciprocal space, 14675 reflections measured, 3926 unique ($R_{\text{int}} = 0.0238$), 3303 observed ($I > 2\sigma(I)$), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, $m = 0.091$ mm$^{-1}$, $T_{\text{min}} = 0.6855$, $T_{\text{max}} = 0.7457$, structure solved by directmethods and refined against $F^2$ with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 167 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 1.059 for observed reflections, final residual values $R1(F) = 0.0323$, $wR(F^2) = 0.0907$ for observed reflections.CCDC 883510.

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b = 11.7114(19), c = 16.898(3) Å, β = 90.00 °, V = 1172.5(3) Å³, ρ = 1.395 g cm⁻³, T = 100(2) K, Θmax = 31.73°, radiation Mo-Kα, λ = 0.71073 Å, 0.3 ω-scans with CCD area detector, covering a whole sphere in reciprocal space, 8626 reflections measured, 2124 unique (Rint = 0.0554), 2045 observed (I > 2σ(I)), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS22 based on the Laue symmetry of the reciprocal space, m = 0.099 mm⁻¹, Tmin = 0.6747, Tmax = 0.7463, structure solved by direct methods and refined against F2 with a Full-matrix least-squares algorithm using the SHELXL-97 software package, 163 parameters refined, hydrogen atoms were treated using appropriate riding models, goodness of fit = 1.071 for observed reflections, final residual values R1(F) = 0.0346, wR(F2) = 0.0888 for observed reflections.CCDC 883510.
CHAPTER 6
SUMMARY OF ACHIEVEMENTS

Synthetic organic chemistry continues to play an important role in materials science, medicinal chemistry, and biochemistry. Heterocyclic compounds are found in a significant segment of biologically active compounds, and the work in this thesis extends the use of mild and efficient methods for the synthesis of heterocyclic compounds.

Chapter 1 includes the common research themes: heterocyclic compounds, peptides and microwave-assisted synthesis. In addition, the properties and reactivity of benzotriazole are reviewed, as developed in the Katritzky laboratories. Properties and general information on the synthesis of peptides, as well as general information regarding microwave heating are outlined.

Chapter 2 discusses microwave-assisted synthesis of 3,5-diamino-1,2,4-triazole compounds. Novel compounds were obtained by ring acylation using N-(protected α-aminoacyl)benzotriazoles and N-(protected dipeptidoyl)benzotriazoles, however with the ring protected, exocyclic acylation was exclusively observed.

Chapter 3 discusses the formation of proline-containing 2,5-diketopiperazines; stereoflexible strategies lead selectively to cis- or trans- configured DKPs. Starting from Cbz-protected dipeptidoyl benzotriazoles cis-configured DKPs were synthesized from a tandem deprotection/cyclization step, whereas trans- DKPs were formed utilizing a tandem cyclization/epimerization strategy. Through a series of computational and experimental investigations, a full rationalization of the phenomenon was accomplished.

Chapter 4 discusses the formation of cyclic peptides without the use of a turn-inducer. The use of Staudinger protocols in the formation of a phospho-aza-ylide
allowed for the convenient synthesis of 2,5-diketopiperazines from easily prepared starting materials. The methodology was extended to a novel solid-phase protocol involving an aminomethyl (AM) resin solid support.

Finally, Chapter 5 describes the synthesis and reactivity of a novel class of α-benzotriazoyl nitroso compounds. Reaction with dienes showed α-benzotriazoyl nitroso compounds to be poor dienophiles for nitroso Diels-Alder reactions. Computational investigation of NO release by α-benzotriazoyl compounds gave promising results, including indirect experimental evidence. Further experimental testing is needed to assess the full potential of α-benzotriazoyl nitroso compounds as NO release candidates.
LIST OF REFERENCES


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N. Asano, *Glycobiology*, 2003, **13**, 93R.


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BIOGRAPHICAL SKETCH

Lucas K. Beagle, born in 1982 in Beaver County, Pennsylvania, was the first of two children of Timothy C. and Ellen K. Beagle. He graduated with a Bachelor of Science in biological sciences from Wright State University in Dayton, Ohio in 2005. In 2007, he enrolled at Youngstown State University studying under Dr. Peter Norris and Dr. Allen Hunter earning a Master of Science in chemistry in 2008. Immediately following he enrolled at the University of Florida, joining the Florida Center for Heterocyclic Compounds in 2010 under the direction of Prof. Alan R. Katritzky. Lucas received his Ph. D. from the University of Florida in the summer of 2012, and accepted an assistant professor position in the Department of Chemistry at the University of Georgia in August 2012. Lucas’s research interest includes benzotriazole-activation methodology, cyclic peptide synthesis, and novel stabilized nitroso compounds.