EXPANDING BENZOTRIA-ZOLE METHODOLOGY: DIFFICULT PEPTIDES AND OTHER MOLECULES OF BIOLOGICAL INTEREST

By

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To my parents, Kingsley and Beverley Haase
   To my brother, Kevin Haase
To my family and dear friends, for their continued love and support
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>alpha locant</td>
</tr>
<tr>
<td>[α]</td>
<td>specific rotation [expressed without units; the units, (deg mL)/(g dm) are understood]</td>
</tr>
<tr>
<td>Ala</td>
<td>alanine</td>
</tr>
<tr>
<td>Alk</td>
<td>alkyl</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>Asp</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>β</td>
<td>beta locant</td>
</tr>
<tr>
<td>br</td>
<td>broad (spectral)</td>
</tr>
<tr>
<td>Bt</td>
<td>benzotriazol-1-yl</td>
</tr>
<tr>
<td>BtH</td>
<td>1H-benzotriaizole</td>
</tr>
<tr>
<td>C</td>
<td>carbon</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celcius</td>
</tr>
<tr>
<td>calcd</td>
<td>calculated</td>
</tr>
<tr>
<td>Cbz</td>
<td>benzyloxycarbonyl</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>Cu</td>
<td>copper</td>
</tr>
<tr>
<td>Cys</td>
<td>cysteine</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift in parts per million downfield from tetramethylsilane</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>D (10 point)</td>
<td>dextrorotary (right)</td>
</tr>
<tr>
<td>DCC</td>
<td>(N,N)^'-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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</table>
DTT 1,4-Dithiothreitol
EDT 1,2-Ethanedithiol
equiv equivalent(s)
Et ethyl
et al. and others
Et$_3$N triethylamine
Fe iron
Fmoc 9-fluorenlymethoxycarbonyl
g gram(s)
Gly glycine
Glu glutamic acid
Gln glutamine
h hour
H hydrogen
HRMS high resolution mass spectrometry
Hz hertz
i iso (as in i-Pr; never i-propyl)
Ile isoleucine
i-Pr isopropyl
IR infrared
$J$ coupling constant (in NMR spectroscopy)
L (10 point) levorotary (left)
Leu leucine
lit literature
Lys lysine
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>m</td>
<td>multiplet (spectral); metre(s); milli</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>Met</td>
<td>methionine</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>mol</td>
<td>mole(s)</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>o</td>
<td>ortho locant</td>
</tr>
<tr>
<td>O</td>
<td>oxygen</td>
</tr>
<tr>
<td>OEt</td>
<td>ethoxy</td>
</tr>
<tr>
<td>OMe</td>
<td>methoxy</td>
</tr>
<tr>
<td>Oxone®</td>
<td>potassium peroxymonosulfate</td>
</tr>
<tr>
<td>p</td>
<td>para locant</td>
</tr>
<tr>
<td>Pd</td>
<td>palladium</td>
</tr>
<tr>
<td>Pg</td>
<td>protecting group</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>Phe</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>Pro</td>
<td>proline</td>
</tr>
<tr>
<td>Pz</td>
<td>pyrazine</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>R</td>
<td>rectus (right) (naming groups around a central carbon) (opposite of S)</td>
</tr>
<tr>
<td>ref.</td>
<td>reference</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet (spectral)</td>
</tr>
<tr>
<td>S</td>
<td>sinister (left) (naming groups around a central carbon) (opposite of R)</td>
</tr>
<tr>
<td>Ser</td>
<td>serine</td>
</tr>
<tr>
<td>SOCl₂</td>
<td>thionyl chloride</td>
</tr>
<tr>
<td>t</td>
<td>triplet (spectral)</td>
</tr>
<tr>
<td>t</td>
<td>tertiary</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>Thr</td>
<td>threonine</td>
</tr>
<tr>
<td>TIPS</td>
<td>triisopropylsilane</td>
</tr>
<tr>
<td>TLC</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilane</td>
</tr>
<tr>
<td>Tr</td>
<td>(triphenylmethane) trityl</td>
</tr>
<tr>
<td>Trp</td>
<td>tryptophan</td>
</tr>
<tr>
<td>Tyr</td>
<td>tyrosine</td>
</tr>
<tr>
<td>Val</td>
<td>valine</td>
</tr>
<tr>
<td>W</td>
<td>watt(s)</td>
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</tbody>
</table>
1H-Benzotriazole has found wide applicability as a highly effective synthetic auxiliary in solution and solid phase reactions. The aim of this study was to develop practical synthetic routes of potential importance for the construction of biologically active structural motifs and compounds of biological interest. This thesis is divided into eight parts. Chapter 1 gives an overview of 1H-benzotriazole methodology, highlighting recent applications in synthetic organic chemistry. Chapter 2 describes the C-thiocarbamoxylation and C-aminoimidoylation of ester enolates and other nucleophiles. Chapter 3 extends this work to the syntheses of novel O-aryl-benzotriazole-1-carbothioates, S-aryl-benzotriazole carbodithioates, benzotriazole-1-carboximidates and explores their potential to C-alkoxylate- and C-arylthioimidoylate nucleophiles.

Chapter 4 describes the preparation of N-Fmoc-(α-aminoacyl)benzotriazoles and Chapter 5 their application in the microwave-assisted solid phase peptide synthesis (SPPS) of oligopeptides. Chapter 6 extends the utility of benzotriazole-mediated syntheses to the assembly of “difficult” peptides. In Chapter 7, potential applications of benzotriazole methodology in the syntheses of azole-based peptides and water-soluble coupling reagents for peptide synthesis are
presented. Finally, Chapter 8 provides conclusions, a summary of achievements and future directions.
Benzazoles, in particular, 1H-benzotriazole (1.1) play a unique role in heterocyclic chemistry (Figure 1-1). [09MRC142] 1H-Benzotriazole is an intriguing molecule that possesses interesting chemical and biological activities. [54SCI989, 95EJMC77, 91T2683] The chemistry of 1H-benzotriazole has been extensively studied by Katritzky and coworkers over the last two decades and is a mature field, however, new discoveries are constantly being made. Currently there is a resurgence of interest in 1H-benzotriazole and its derivatives due to wide applicability in fields such as medicine, environmental science, technology, polymer science and synthetic organic chemistry. [09MRC142]

![Tautomeric equilibrium of benzotriazole](image)

**Figure 1-1.** Tautomeric equilibrium of benzotriazole

1.2 1H-Benzotriazole as a Synthetic Auxiliary

1H-Benzotriazole, a benzofused heterocycle possessing an azole ring, has three contiguous nitrogen atoms (Figure 1-1). The presence of concatenated nitrogen atoms in 1H-benzotriazole is responsible for its innate properties such as a $pK_a$ of 8.2, indicating a high N-H acidity and a $pK_{\text{ah}} < 0$ for proton addition. In other words, the 1H-benzotriazole ring is able to accept or donate electrons. [91T2683]
A synthetic auxiliary is a compound that is temporarily incorporated during the synthesis of a chemical entity. Numerous neutral and charged heterocyclic species, for example 1H-benzotriazole, can act as efficient synthetic auxiliaries (Figure 1-2).

As an ideal synthetic auxiliary, 1H-benzotriazole is:

- Economically favored, that is, it is inexpensive and readily available
- Readily attached to the substrate by substitution, addition or three component condensation reactions
- A good leaving group and thus is easily displaced, for example, by hydrolysis, reduction, or Pd-catalyzed substitution
- Readily separated from the product, usually with minimal effort
- Easily recovered and reused
- Exhibits interesting and diverse reactivity patterns

Figure 1-2. Cycle of 1H-benzotriazole-assisted syntheses

As an ideal synthetic auxiliary, 1H-benzotriazole is:
The use of 1H-benzotriazole as a versatile synthetic auxiliary, for both solution and solid phase syntheses, was previously established by Katritzky and coworkers. [91T2683, 98CR409, 03CEJ4586] The benzotriazolyl group is an excellent leaving group and can replace halogens in many reactions. [98CR409] Additionally, the benzotriazolyl moiety is known to activate carbon atoms to which it is attached, thereby promoting common synthetic transformations. [91T2683, 98CR409, 03CEJ4586] The benzotriazolyl moiety conveys its activation of carbon atoms in several ways, for example as (i) a proton activator, (ii) a cation stabilizer, (iii) an ambident anion directing group, (iv) a radical precursor and (v) an anion precursor (Figure 1-3).

The leaving group ability, CH activation towards proton loss and electron donor properties of 1H-benzotriazole have been compared with other activating groups, such as the cyano and phenyl groups. [91T2683, 98CR409, 03CEJ4586] It was determined that 1H-benzotriazole is comparable to cyano and phenylsulfonyl with respect to leaving group ability and in its ability to enable deprotonation (Figure 1-4). [98CR409] However, when compared with the electron donor properties of the phenyl and vinyl groups, 1H-benzotriazole exhibits advantages. Examples of activating groups that possess all three aforementioned attributes, that is, they are good leaving groups, electron donors and activators of CH proton loss, are rare. 1H-benzotriazole and the phenylthio group are examples of activating groups possessing these three properties, however when compared, the former has considerable advantages (Figure 1-4). [91T2683, 98CR409, 03CEJ4586]

In general, benzotriazole chemistry is straightforward and easy to comprehend. The benzotriazole derivatives, many of which are odorless and stable solids, are usually prepared in high yields and are capable of a plethora of reactions. Now, an overview of the numerous
synthetic possibilities of benzotriazole derivatives and recent developments in the syntheses of these derivatives are provided in Section 1.2.

Figure 1-3. Multiple activating influences of the benzotriazolyl group
1.3 Recent Developments in Benzotriazole Methodology

1.3.1 Synthesis of α-Benzotriazolylamides

α-Benzotriazolylamides 1.2 are biologically active and are synthetic precursors to pyrid-2-ones. [88FA29, 97JOC6210, 09OL995] In the literature, the synthesis of α-benzotriazolylamides has been achieved by (i) the reaction of sodium benzotriazolate with halides in average yields of 74% [97JOC6210] and (ii) an Ugi-Smiles/deallylation/diazotization sequence in average yields of 65% [09OL995] (Figure 1-5).

Figure 1-5. Literature methods for the preparation of α-benzotriazolylamides 1.2
In addition to being a powerful addition to the available synthesis of α-benzotriazolylamides 1.2, the Ugi-Smiles/deallylation sequence is useful for the syntheses of other privileged structures, for example, benzimidazoles 1.3. [09OL995]

1.3.2 1H-Benzotriazole and its Derivatives in Cross Coupling Reactions

Over the last twenty years, cross coupling reactions have emerged as an integral tool for the syntheses of natural products and polymers. [06EJO3283] The Cu-mediated C-N, C-S and C-O bond forming reactions have attracted much attention as a result of its economy and efficiency when compared to Pd-catalyzed cross coupling reactions. [07TL4207] However, the benefits of Cu-catalyzed bond formation reactions are often masked by the lack of highly efficient ligands and alternate copper sources. [03AGE5400]

The use of 1H-benzotriazole and its derivatives as ligands in transition metal mediated cross coupling reactions is relatively unexplored. A search of the literature revealed few examples in which 1H-benzotriazole and its derivatives were used as ligands. Recently, Verma and coworkers assessed the potential of 1H-benzotriazole as a ligand for Cu-catalyzed C-N and C-S bond formation. [07TL4207, 07TL7199] 1H-benzotriazole is moisture insensitive, inert to air and has excellent coordinating capabilities, so Verma and coworkers considered it a potential ligand in Cu-mediated bond formations. [07TL4207] As a result of its coordinating abilities, it was postulated that 1H-benzotriazole would be useful in stabilizing catalytic species and in that way promote catalytic cycles under mild conditions. In a study on the preparation of N-arylimidazoles 1.4 [07TL4207] (Figure 1-6) and aryl sulfides 1.5 [07TL7199] (Figure 1-7) via Cu-mediation, 1H-benzotriazole was found to be an exceptional ligand and afforded 1.4 and 1.5 in good to excellent yields.
A possible mechanism for the formation of N-arylimidazoles 1.4 and aryl sulfides 1.5 is shown in Scheme 1-1. [07TL4207, 07TL7199] It is postulated that chelation of Cu(I) with 1H-benzotriazole results in the formation of species L1. Reactive species L1 readily participates in the oxidative addition reaction with aryl halides (ArX) to form species L2. Intermediate L3 is then generated by reaction of L2 with thiols or imidazoles in the presence of base. Subsequent reductive elimination releases the product 1.4 or 1.5 and regenerates the active Cu(I) species. The described activation mode affords compounds 1.4 and 1.5 which are significant components of numerous bioactive compounds. [07TL4207, 07TL7199]

Verma and coworkers also utilized benzotriazol-1-ylmethanol 1.7 as a ligand in the Cu-catalyzed tandem synthesis of multiring heterocycles. [09AGE1138] Multiring heterocyclic indolo- and pyrrolo[2,1-a]isoquinolines 1.8 were synthesized in good yields with excellent regioselectivity from indoles 1.9 and ortho haloarylalkynes 1.10 in the presence of catalytic CuI, base and hydroxymethyl benzotriazole 1.7 (Figure 1-8). [09AGE1138] The proposed mechanistic pathways are illustrated in Scheme 1-2. [09AGE1138]
Scheme 1-1. A plausible mechanism for the Cu-catalyzed N-arylation of imidazoles and S-arylation of thiols to produce 1.4 and 1.5 respectively

In one pathway, copper complex 1.11 is generated from CuI and 1.7. Oxidative addition and ensuing π complexation with the ortho haloarylalkyne produces intermediate 1.12. Intermolecular and an intramolecular nucleophilic attack on 1.12, followed by elimination of HBr results in the formation of Cu complex 1.15 via intermediates 1.13 and 1.14 respectively. Product 1.8 is then generated from the reductive elimination of 1.15.

Figure 1-8. Cu(I) mediated tandem synthesis of indolo- and pyrrolo[2,1-a]isoquinolines 1.8

Contrary to its use as a ligand in Cu-mediated cross coupling reactions, substituted benzotriazoles have also been the synthetic targets. [08SL3068] The Cu-catalyzed N-arylation of 1H-benzotriazole 1.1 with aromatic and heteroaromatic chlorides 1.17 provided the corresponding aryl-functionalized benzotriazole derivatives 1.18 in good to excellent yields (Figure 1-9). [08SL3068]
More recently, Nakamura and coworkers [09OL1055] demonstrated that benzotriazole derivatives are synthetic equivalents of 2-haloanilides in Pd-mediated reactions. Nakamura and coworkers reported the construction of polysubstituted indole derivatives $1.19$ via the Pd-catalyzed denitrogenative indolization of $N$-arylbenezotriazoles $1.20$ and disubstituted alkynes $1.21$ (Figure 1-10). [09OL1055]

The mechanism proposed by the authors [09OL1055] (Scheme 1-3) involves insertion of Pd(0) into the C-N bond of the diazonium species $1.22$ to produce intermediate $1.23$. Subsequent insertion of the alkyne $1.21$ into the C-Pd bond of $1.23$ generates the palladacycle species $1.24$. Reductive elimination of Pd(0) from $1.24$ provides the polysubstituted indole derivatives $1.19$. [09OL1055]
1.3.3 Uses of 1H-Benzotriazole and its Derivatives in Materials Chemistry

1.3.3.1 Benzotriazole derivatives as corrosion inhibitors

Triazole containing compounds, such as 1H-benzotriazole, are known to be efficient corrosion inhibitors. [09JAE269, 09JMPT1729, 09ME367] 1H-benzotriazole reduces the rate of decay of metals or alloys such as Cu and Fe. [09JAE269] Recently, benzotriazole derivatives, 1-(2-thienylcarbonyl)benzotriazole 1.25 and 1-(2-pyrolecarbonyl)benzotriazole 1.26 have been examined for their potential efficacy as corrosion inhibitors (Figure 1-11). [09JAE269]
1.3.3.2 Benzotriazole as a component of conjugated polymers

Conjugated polymers have diverse applications, especially in sensors, LEDs, electrochromic devices and solar cells. [08CM7510] The efficacy of electrochromic materials depends on its ability to reversibly change colors on modification of the redox state of the material. [08CM7510] Despite major advances in materials chemistry, there still exists a demand for optically responsive materials with electrochromic properties for use in data storage and display devices. [08CM7510] Accordingly, Toppare and coworkers synthesized a novel donor-acceptor type polymer poly(4,7-bis(2,3-dihydrothieno[3,4-b][1,4]dioxin-5-yl)-2-dodecyl-2H-benzo[1,2,3]triazole) (PBEBT) 1.27 and investigated its electrochromic properties. Their results indicated that the spectroelectrochemistry of PBEBT was nearly identical to the well known poly(ethylenedioxythiophene) (PEDOT) 1.28 with a maximum absorption of 618 nm (Figure 1-12). Also, 1.27 displayed advantages over 1.28 with respect to optical contrast, switching time and coloration efficiency. Moreover 1.27 is capable of p- and n-type doping, thus it is superior to 1.28 and a better option for use in electrochromic display devices.
1.3.4 Aspects of Benzotriazole-mediated Syntheses

Benzotriazole-mediated acylation and imidoylation are examples of synthetically useful reactions that have been extensively studied. [03CEJ4586] In many instances the benzotriazole-assisted strategy represents the most efficient routes to synthetic intermediates and target molecules. [91T2683, 98CR409, 03CEJ4586]

1.3.4.1 Benzotriazole-assisted imidoylations

Imidoylation reactions have received much attention by numerous researchers. Recently, Katritzky and coworkers developed guanylating reagents di(benzotriazolyl)methanimine 1.29 and benzotriazolylcarboximidoyl chlorides 1.30. As illustrated in Scheme 1-4, the reaction of 1H-benzotriazole and cyanogen bromide affords 1.29, while 1.30 is easily prepared from the reaction of isonitriles and 1-chlorobenzotriazole. Guanylating reagents 1.29 and 1.30 were then utilized in the preparation of tri- and tetra-substituted guanidines (Schemes 1-4). [03CEJ4586]

Scheme 1-4. Preparation of guanidines from guanylating reagents 1.29 and 1.30

Chapter 1 of this study introduces novel C-thiocarbamoylating reagents and describes their application in the preparation of C-aminoimidoylating reagents. Subsequently, reactions of the C-aminoimidoylating and C-thiocarbamoylating reagents with nucleophiles were explored. Chapter 2, an extension of Chapter 1, describes the preparation of C-alkoxyimidoylating reagents.
1.3.4.2 Benzotriazole-assisted N-acylations

N-Acylbenzotriazoles can be prepared from (i) acyl halides and benzotriazole in the presence of a base, (ii) carboxylic acids and sulfonylbenzotriazole in the presence of Et₃N [00JOC8210] and (iii) carboxylic acids treated with thionyl chloride and excess benzotriazole [03S2795] (Scheme 1-5).

Scheme 1-5. Preparation of N-acylbenzotriazoles 1.31

It is of particular importance that N-acylbenzotriazoles can be prepared from N-protected amino acids and that these N-protected-(α-aminoacyl)benzotriazoles are chemically stable, crystalline and chirally pure. Chapters 4-7 will discuss aspects of the preparation of N-Fmoc-(α-aminoacyl)benzotriazoles and their use in microwave-mediated peptide chain extension.

1.4 Concluding Remarks

The exciting discoveries within the last few years demonstrate the incredible potential of 1H-benzotriazole methodology. This study contributes to the major advances in 1H-benzotriazole chemistry by expanding the scope of 1H-benzotriazole methodology to the syntheses of biologically important structural motifs and other molecules of interest. The aim of this study was to (i) discover and/or optimize synthetic routes to interesting compound classes, and (ii) assess the potential limitations of this methodology.
Herein the application of $1H$-benzotriazole methodology in the syntheses of precursors to heterocycles and small biomolecules is reported.
CHAPTER 2
C-AMINOIMIDOYLATION AND C-THIOCARBAMOYLATION OF ESTERS

2.1 Background

Recently there has been widespread focus on isothiocyanates (Figure 2-1) as a result of their usefulness in pharmacology, medicine and industry. [06JPCA13195] Isothiocyanates, phytochemicals that frequently occur in cruciferous plants and vegetables, form one of several classes of organic compounds possessing cumulative double bonds. [06JPCA13195, 91CR1, 05EJO1184] Additional sources of isothiocyanates are blue-green algae, fungi and marine organisms. [05EJO1184]

\[
\begin{align*}
R & \quad N=C=S \\
\text{Isothiocyanate} & \quad \text{N=C=N} \\
R^1 & \\
\text{Carbodiimide} &
\end{align*}
\]

Figure 2-1. General structure of isothiocyanates and carbodiimides

Isothiocyanates possess a range of activity, but are widely exploited for their \textit{in vitro} and \textit{in vivo} anticancer activity. [06JPCA13195] In addition to their biological activity, they act as valuable synthetic intermediates for the preparation of biologically active heterocycles, for example, thiohydantoins (2.1), thiopyrimidone derivatives (2.2) and pyridopyrimidinethiones (2.3) (Figure 2-2). [91CR1]

Figure 2-2. Formation of heterocycles from isothiocyanates
Similarly, carbodiimides are heterocumelenes that are useful for the construction of biologically heterocycles such as imidazolidinones (2.4) and quinazolines (2.5) (Figure 2-3). [81CR589] In syntheses, carbodiimides are frequently employed as dehydrating agents in the preparation of peptides and nucleotides. [81CR589] In contrast to isothiocyanates, carbodiimides are not naturally occurring and multiple methods have been devised for the syntheses of carbodiimides, many of which utilize thioureas. [81CR589]

![Figure 2-3. Biologically active heterocycles synthesized from carbodiimides](image)

While isothiocyanates and carbodiimides are valuable reagents, their utility has been somewhat limited by their great reactivity, moisture sensitivity, and the need for careful handling and storage. Therefore reactions involving the use of these compound classes can be quite tedious.

In preceding work, Katritzky and coworkers synthesized 1-(alkyl/arylthiocarbamoyl)-benzotriazoles (2.6) [04JOC2976] and benzotriazole-1-carboxamidines (2.7) [05HCA1664], which are synthetic equivalents of isothiocyanates and carbodiimides respectively. 1-(Alkyl/arylthiocarbamoyl)benzotriazoles (2.6) and benzotriazole-1-carboxamidines (2.7) are mainly stable solids that are moisture insensitive. [04JOC2976, 05HCA1664] It is postulated that reactions involving 2.6 and 2.7 occur via i) an E1cB mechanism with a concomitant in situ generation of the corresponding isothiocyanates and carbodiimides or ii) an addition-elimination mechanism (Scheme 2-1).
Scheme 2-1. Representative reactions of 2.6 and 2.7 with nucleophiles

Recently, the utility of 1-(alkyl/arylthiocarbamoyl)benzotriazole thiocarbamoylating reagents (2.6) was reported in the syntheses of di- and trisubstituted thioureas (2.8 and 2.9) [04JOC2976], N-hydroxythioureas (2.10) and thiosemicarbazides (2.11) [06ARK226] (Scheme 2-2). Benzotriazole-1-carboxamidine N-aminoimidoylating reagents (2.7) were also reported for the synthesis of 1,2,3-trisubstituted guanidines (2.12) [05HCA1664], N-hydroxy- and N-amino-guanidines (2.13 and 2.14) [06JOC6753] (Scheme 2-3).

To expand the efficacy of 2.6 and 2.7 the reaction of 1-(alkyl/arylthiocarbamoyl)-benzotriazole thiocarbamoylating reagents (2.6) and benzotriazole-1-carboxamidine N-aminoimidoylating reagents (2.7) with carbon nucleophiles was examined.
Scheme 2-2. Reactions of 1-(alkyl/arylthiocarbamoyl)benzotriazoles 2.6

Scheme 2-3. N-Aminoimidoylation with benzotriazole-1-carboxamidines 2.7

2.2 Introduction

The wide availability of acylating and imidoylating reagents reflects the wide attention given to C-acylation [47JACS119, 03JOC1443, 59JACS4882] and C-imidoylation [97TL6771, 99OL977, 02JOC4667]. By contrast, C-aminoimidoylation and C-thiocarbamoylation are both
relatively unexplored synthetically. In the literature, examples of compounds that could conceptually have been made by C-aminoimidoylation and C-thiocarbamoylation have generally been obtained via multiple steps. [04JOC188, 00JOC1583, 79S343, 83LA290]

A search of the literature disclosed no examples of the direct C-aminoimidoylation or C-thiocarbamoylation of esters. Examples of the C-aminoimidoylation products 2.16 were obtained by the nucleophilic attack of amines on isoxazolones 2.15 leading to ring opening and concomitant loss of CO₂ (Scheme 2-4).[81JOC4068]

![Scheme 2-4. Preparation of C-aminoimidoylation product 2.16](image)

Similarly potential C-thiocarbamoylation products 2.17 were prepared from isothiocyanates, alcohols and carboxylic acids (Scheme 2-5). [04JOC188, 00JOC1583, 79S343, 83LA290]

![Scheme 2-5. Preparation of C-thiocarbamoylation product 2.17](image)

Therefore a general straightforward approach to the synthesis of C-aminoimidoylation and C-thiocarbamoylation products would be useful. Now the C-aminoimidoylation and C-
thiocarbamoylation of esters *via* 1-(alkyl/arylthiocarbamoyl)benzotriazoles (2.6a-c) and benzotriazole-1-carboxamidines (2.7a-b) is reported.

### 2.3 Results and Discussion

#### 2.3.1 Preparation of 1-(Alkyl/aryl-thiocarbamoyl)benzotriazoles 2.6a–d and Benzotriazole-1-carboxamidines 2.7a–c.

Bis(benzotriazolyl)methanethione [78JOC337] 2.18 and amines afforded 1-(alkylthiocarbamoyl)benzotriazoles 2.6a–f,h–k or 1-(arylthiocarbamoyl)benzotriazoles 2.6g, which were converted in turn by iminophosphoranes into the benzotriazole-1-carboxamidines 2.7a–i (Scheme 2-6).

![Scheme 2-6. Preparation of reagents 2.6 and 2.7](image)

#### 2.3.2 C-Aminoimidoylation and C-Thiocarbamoylation of Doubly Activated Esters

Reactions of 2.0 equiv. of ester enolates (from ester 2.19a-c with 2.5 equiv potassium tert-butoxide) with 1.0 equiv of benzotriazole-1-carboxamidines 2.7a–c or 1-(alkylthiocarbamoyl)benzotriazoles 2.6b,d afforded 2.20a–c, 2.21a,b respectively; after limited optimization, yields of 20-51% were obtained (Scheme 2-7, Table 2-1). Elemental analysis, HRMS and NMR spectral data support the structural assignments (See 2.5 Experimental Section).
Scheme 2-7. Preparation from esters 2.19a-c of the C-aminoimidoylation products 2.20a-c and the C-thiocarbamoylation products 2.21a,b

Table 2-1. C-Aminoimidoylation and C-thiocarbamoylation of esters 2.19a–c to give 2.20a-c, 2.21a,b

<table>
<thead>
<tr>
<th>Ester</th>
<th>R¹</th>
<th>R²</th>
<th>Reagent</th>
<th>R³</th>
<th>R</th>
<th>Product</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.19a</td>
<td>CO₂Et</td>
<td>Et</td>
<td>2.7a</td>
<td>CH₃(CH)Ph</td>
<td>m-CNC₆H₄</td>
<td>2.20a</td>
<td>24</td>
</tr>
<tr>
<td>2.19b</td>
<td>CO₂Me</td>
<td>Me</td>
<td>2.7b</td>
<td>CH₂(CH(CH₃)CH₂CH₃</td>
<td>p-Tol</td>
<td>2.20b</td>
<td>51</td>
</tr>
<tr>
<td>2.19c</td>
<td>CN</td>
<td>Et</td>
<td>2.7c</td>
<td>Allyl</td>
<td>m-CNC₆H₄</td>
<td>2.20c</td>
<td>20</td>
</tr>
<tr>
<td>2.19b</td>
<td>CO₂Me</td>
<td>Me</td>
<td>2.6b</td>
<td>CH₃(CH)Ph</td>
<td>-</td>
<td>2.21a</td>
<td>27</td>
</tr>
<tr>
<td>2.19a</td>
<td>CO₂Et</td>
<td>Et</td>
<td>2.6d</td>
<td>(CH₂)₂Ph</td>
<td>-</td>
<td>2.21b</td>
<td>49</td>
</tr>
</tbody>
</table>

Different tautomeric structures are possible for the ester products. The determination of the precise tautomeric structure of the compounds was of interest due to the influence of tautomeric structures on the physical properties [for example, boiling point, solubility] and chemical properties [for example: acidity/basicity, reactivity] of compounds.

X-ray data suggests that the compounds 2.21a,b would exist as the keto (CH) rather than enol (OH) tautomer in the solid state (Figure 2-4).[07JOC6742] Conceptually, in solution three tautomeric forms of 2.21 can be present, that is, the keto (2.21), enol (2.21') and the enethiol (2.21'') forms may coexist in a dynamic equilibrium (Figure 2-4). [05TEC284] Also, it is known that the extent of the keto-enol-enethiol tautomerism of compounds is dependent on the compound structure and the nature of the solvent. [05TEC284] Compounds 2.21a,b were examined in chloroform-d at 25 ºC and 2D-NMR verified the existence of a single form, the keto (CH) tautomer, under the conditions examined (Figure 2-5).
Compounds 2.20a-c were examined in chloroform-\(d\) at 25 °C to determine whether they exist in the enol or keto forms. On examination in chloroform-\(d\) at 25 °C, the NMR spectra indicated the absence of keto tautomers. Coupling of one of the NH protons with the alpha protons on \(R^3\) allowed assignment of the NH protons, and supported the conclusion that 2.20a-c are present in solution as the keto-enamine tautomers (2.20) and not the corresponding amidine-enol isomers (2.20′), as depicted in Figure 2-6. For 2.20c, two conformers are possible in solution, however, the NMR spectra displayed signals from either a single stereoisomer (\(E\)
isomer) or an average structure as the rotation about the C=C is quite rapid when compared with the NMR timescale. [04JOC188]

Additionally, there are four probable C-N rotameric conformations for 2.20a-c. These are the syn syn (ss), syn anti (sa), anti syn (as) and anti anti (aa) conformations (Scheme 2-8).

[04JOC188] A survey of the literature disclosed that 2.20a,b should adopt the unusual sterically demanding aa conformation as the two stabilizing intramolecular hydrogen bonds compensate for the steric hindrance between R and R³. On the other hand, 2.20c should preferentially adopt the more favored sa or as conformation, but may assume the aa conformation although it is capable of a single stabilizing hydrogen bond. [4JOC188]

Figure 2-6. Relevant ¹H and ¹³C chemical shifts in CDCl₃ of keto-enamine tautomeric structures 2.20a-c

¹H chemical shifts- blue
¹³C chemical shifts- black
Scheme 2-8. Four possible rotameric conformations of **2.20a-c**

For **2.20a-c**, the formation of the keto-enamine tautomer was further corroborated by IR spectroscopy. The IR spectra of **2.20c** indicated the presence of a hydrogen bonded NH at 3246 cm\(^{-1}\), while the carbonyl and alkene stretching frequencies were observed at 1655 and 1605 cm\(^{-1}\) respectively. The carbonyl frequency was characteristic for a conjugation effect of the aminal group and intramolecular hydrogen bonding of the aminal NH with the carbonyl group.

[04JOC188]

In the literature compounds **2.20a-c** are known as \(N,N'\)-disubstituted ketene aminals, important compounds for the construction of heterocyclic systems.[04JOC188] Also, the \(N,N'\)-disubstituted ketene aminals, referred to as 1,1-enediamines and \(\alpha,\alpha\)-dioxoketene aminals, are considered bioisosteres of urea and are an important class of compounds in the fields of agriculture and medicine. [04JOC188] The heterocyclic analogues to \(N,N'\)-disubstituted ketene aminals, heterocyclic ketene aminals (HKAs) have also garnered much attention in recent decades. [07SL761]

Literature methods for the preparation of \(N,N'\)-disubstituted ketene aminals include the reaction of (i) activated methylene compounds and isothiocyanates, [04JOC188] ii) oxoketene \(N,S\)-acetals and lithiated secondary amines or aniline [00JOC1583], or (iii) tris(dimethylamino)ethoxymethane and simple ketones [79S343, 83LA290]. Methods i-iii (Scheme 2-9) each consist of multiple steps with average overall yields of \textit{ca.} 30\%, whereas the
current C-aminoimidoylation method provides a direct access to these compound classes in comparable yields.

![Chemical Structure](image1)

Scheme 2-9. Literature methods for the preparation of ketene aminals 2.22

2.3.3 C-Aminoimidoylation and C-Thiocarbamoylation of Unactivated Esters and Other Activated Compounds

2.3.3.1 Reactions with unactivated esters

Reactions of 2.0 equiv of ester enolates (from ester 2.19d-f with 2.5 equiv potassium tert-butoxide) with 1.0 equiv of benzotriazole-1-carboxamidines 2.7d,e or 1-(alkylthiocarbamoyl)-benzotriazoles 2.6b,e,f afforded 2.23a,b, 2.24a-d respectively; after limited optimization, yields of 28-49% were obtained (Scheme 2-10, Table 2-2). Elemental analysis, HRMS and NMR spectral data support the structural assignments (See 2.5 Experimental Section).

![Chemical Structures](image2)

Scheme 2-10. Preparation from esters 2.19d-f of the C-aminoimidoylation products 2.23a,b and the C-thiocarbamoylation products 2.24a-c
<table>
<thead>
<tr>
<th>Ester</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Reagent</th>
<th>R&lt;sup&gt;3&lt;/sup&gt;</th>
<th>R</th>
<th>Product</th>
<th>Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.19d</td>
<td>H</td>
<td>i-Pr</td>
<td>2.7d</td>
<td>i-Pr</td>
<td>p-Tol</td>
<td>2.23a</td>
<td>33</td>
</tr>
<tr>
<td>2.19d</td>
<td>H</td>
<td>i-Pr</td>
<td>2.7e</td>
<td>i-Pr</td>
<td>p-CO&lt;sub&gt;2&lt;/sub&gt;iPrC₆H₄</td>
<td>2.23b</td>
<td>49</td>
</tr>
<tr>
<td>2.19d</td>
<td>H</td>
<td>i-Pr</td>
<td>2.6f</td>
<td>Bn</td>
<td>-</td>
<td>2.24a</td>
<td>28</td>
</tr>
<tr>
<td>2.19e</td>
<td>H</td>
<td>Me</td>
<td>2.6b</td>
<td>CH(CH₃)Ph</td>
<td>-</td>
<td>2.24b</td>
<td>40</td>
</tr>
<tr>
<td>2.19e</td>
<td>H</td>
<td>Me</td>
<td>2.6e</td>
<td>i-Pr</td>
<td>-</td>
<td>2.24c</td>
<td>71</td>
</tr>
<tr>
<td>2.19f</td>
<td>Cyclopropyl</td>
<td>Et</td>
<td>2.6e</td>
<td>i-Pr</td>
<td>-</td>
<td>2.24d</td>
<td>45</td>
</tr>
</tbody>
</table>

Examination of the C-aminoimidoylation and C-thiocarbamoylation of unactivated esters yielded O-alkyl isoureas 2.23<sub>a,b</sub> and O-alkyl thiocarbamates 2.24<sub>a-d</sub> and not the expected C-aminoimidoylated and C-thiocarbamoylated ester products. Although potassium t-butoxide is a bulky base, the bulkiness of the t-butyl group did not hinder the nucleophilic addition of the potassium t-butoxide to the carbonyl group of the ester 2.19<sub>d-f</sub>. [06JACS14268] The subsequent reaction of the alkoxide of the ester 2.19<sub>d-f</sub> and benzotriazole-1-carboxamidines 2.7<sub>d,e</sub> or 1-(alkylthiocarbamoyl)benzotriazoles 2.6<sub>b,c,f</sub> afforded the products 2.23<sub>a,b</sub> and 2.24<sub>a-d</sub>. In the case of benzotriazole-1-carboxamidine 2.7<sub>e</sub> transesterification of the aromatic ester substituent also occurred.

As expected the <sup>1</sup>H NMR spectra of O-alkyl thiocarbamates 2.24<sub>a-d</sub> and O-alkyl isoureas 2.23<sub>a,b</sub> indicated the presence of rotamers in a ratio of approximately 1:1. In the solid state it is well established that thioamides, for example 2.24<sub>a-d</sub>, are stable and exist in a single form. However, in solution thioamides exist as a dynamic equilibrium of the cis and trans amide forms due to the relatively low energy barrier to rotation of the amino group in thioamides (Figure 2-7). [88BKCS236] While the energy barrier to rotation around the C-N bond of a thioamide is relatively low, it is somewhat larger than the barrier to rotation about the C-N bond of an amide (usually 16-20 kcal mol<sup>-1</sup> in magnitude). Thus the electron delocalization in amides and
thioamides probably results in a higher rotational barrier around the C-N bond when compared to a normal C-N bond. The presence of conformational isomers in the O-alkyl isoureas 2.23a,b can also be rationalized by moderately low rotational barriers.

![Thioamides diagram](image)

Figure 2-7. Trans and cis forms of thioamides

The formation of O-alkyl isoureas was further corroborated by IR spectroscopy. Compound 2.23b displayed strong IR absorption bands for a non-hydrogen bonded NH, a carbonyl group and amidine C=N at 3373, 1701 and 1641 cm\(^{-1}\) respectively.

Mostly, the C-aminoimidoylation and C-thiocarbamoylation of unactivated esters proceeded by reaction of the alkoxides of the unactivated esters, however reactions of benzyl acetate with 2.6j,k and 2.7e with methyl 2-(napthalen-1-yl)acetate did not yield products.

In addition to O-alkyl isoureas 2.23, the hydrolysis of C-aminoimidoylating reagents 2.7f,h yielded ureas 2.25 (Scheme 2-11).

![Scheme 2-11](image)

Scheme 2-11. Preparation of ureas 2.25a,b from benzotriazole-1-carboxamidines 2.7f,h

The functions of O-alkyl isoureas 2.23, O-alkyl thiocarbamates 2.24 and ureas 2.25 are diverse. O-Alkyl thiocarbamates and O-alkyl isoureas find potential use as anti-HIV drugs [93PR1076] and synthetic intermediates in the preparation of primary alkyl bromides [08S3565].
respectively. Versatile ureas are often used as neutral and extremely directional binding units in anion receptors. [06CCR3200]

2.3.3.2 Reactions with sulfones and cyano compounds

C-Aminoimidoylation of carbon nucleophiles derived from sulfones was examined. The reaction of C-aminoimidoylating reagent 2.7f with ethyl phenyl sulfone (2.0 equiv.) in the presence of potassium t-butoxide (2.5 equiv.) failed to generate the desired product. Therefore, the reaction was repeated using 2.7a and 2.7g and benzyl phenyl sulfone (2.0 equiv.) in the presence of n-BuLi (2.0 equiv.). The product of the reaction of n-BuLi with 2.7g was the replacement of the benzotriazoyl group with the butyl group. Next, the more sterically demanding LDA was reacted with 2.7a, but again no product was formed.

Likewise, the reaction of (i) 2.6b,e with malononitrile in the presence of potassium t-butoxide and (ii) 2.6b and malononitrile in the presence of NaH did not afford the corresponding C-thiocarbamoylation products. C-thiocarbamoylating reagents 2.6c,i,f were reacted with ethyl cyanoacetate in the presence of potassium t-butoxide, but again, no products were formed.

2.3.3.3 Reactions with nitro compounds

C-Aminoimidoylation and C-thiocarbamoylation of nitro compounds were examined in the presence of 2.0 equiv. of the nitronate anion (made from the nitro compound and 2.5 equiv. of potassium t-butoxide). In most cases, with the exception of 2.6f, the desired C-thiocarbamoylation and C-aminoimidoylation products were not obtained. Changing the solvent to DMSO and the base to NaH did not alter the outcome of the reaction.

The reaction of 2.6f with ethyl nitroacetate and nitromethane produced the corresponding C-thiocarbamoylation products, however, these compounds were unstable and decomposed subsequent to the acquisition of the 1H NMR (For example of product 1H NMR, see Figure 2-8).
2.4 Conclusions and Outlook

Nontraditional approaches to the preparation of C-thiocarbamoylated ester products 2.21, N,N’-disubstituted ketene aminals 2.20, O-alkyl isoureas 2.23 and O-alkyl thiocarbamates 2.24 were outlined. Compounds 2.20 and 2.21 were prepared via the successful C-aminoimidoylation and C-thiocarbamoylation of esters, in 34% average yield under mild reaction conditions. While optimization of the reaction conditions is required, this method still provides an easy access to interesting classes of compounds that may be used for further synthetic transformations.

C-Aminoimidoylating reagents 2.7 possess the amidine functional group. Amidines are important in organic chemistry and are often employed as bases. [08ARK153] The presence of the electron donor benzotriazolyl group may introduce interesting reactivity to 2.7 and may warrant investigation.
To summarize, C-thiocarbamoylating reagents 2.6 and C-aminoimidoylating reagents 2.7 hold great promise in synthetic organic chemistry. The chemical reactivity of 2.6 and 2.7 require greater examination in order for the full potential of these reagents to be evaluated.

2.5 Experimental Section

2.5.1 General

Column chromatography was conducted on flash silica gel (200-425 mesh). Visualization of TLC plates was via UV and phosphomolybdic acid staining. Melting points were determined on a hot-stage apparatus and are uncorrected. $^{1}$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were determined in CDCl$_3$ with TMS as the internal standard.

2.5.2 General Procedure for the Preparation of 2.20a-c, 2.21a,b, 2.23a,b, 2.24a-d, 2.25a,b

To a solution of the desired ester 2.19 (2.0 mmol) in THF (15 mL), was added potassium t-butoxide (2.5 mmol). After stirring the mixture for 30 min, 1.0 mmol of the desired reagent 2.6 or 2.7 were added to the reaction mixture. The progress of the reaction was monitored by TLC. Upon completion, water (20 mL) was added to quench the reaction followed by extraction with dichloromethane (3 x 30 mL). The combined extracts were dried over magnesium sulfate and the solvent removed under vacuum. The crude mixture was purified by gradient column chromatography over silica gel (EtOAc-hexanes) to give the desired products in moderate yields.

**Diethyl-2-(3-cyanoanilino)[1-phenethyl]amino]methylene]malonate (2.20a).**

Recrystallized from EtOAc-hexanes to give white microcrystals (24%); mp 99.6-100.6 °C; $^{1}$H NMR (CDCl$_3$) δ 10.72 (br s, 1H), 10.03 (d, $J$ = 6.0 Hz, 1H), 7.36-7.34 (m, 2H), 7.24-7.23 (m, 5H), 6.87-6.84 (m, 2H), 4.26-4.12 (m, 5H), 1.40 (d, $J$ = 6.0 Hz, 3H), 1.32 (t, $J$ = 15.0 Hz, 6H); $^{13}$C NMR (CDCl$_3$) δ 171.1, 162.0, 142.6, 140.8, 130.0, 128.7, 127.8, 127.5, 126.4, 125.3, 118.1, 113.2, 103.4, 81.2, 60.2, 54.7, 24.5, 14.3. HRMS calcd for C$_{23}$H$_{25}$N$_3$O$_4$ [M +H]$^+$ 408.1918, found 408.1917.
Dimethyl-2-[(2-methylbutyl)amino](4-methyltoluidino)methylene]malonate (2.20b). Yellow oil (51%); $^1$H NMR (CDCl$_3$) $\delta$ 10.93 (s, 1H), 9.82 (s, 1H), 7.11 (d, $J = 9.0$ Hz, 2H), 6.97 (d, $J = 6.0$ Hz, 2H), 3.74 (s, 6H), 2.97-2.58 (m, 2H), 2.31 (s, 3H), 1.56-1.44 (m, 1H), 1.31-1.21 (m, 1H), 1.06-1.04 (m, 1H), 0.84 (d, $J = 9.0$ Hz, 3H), 0.77 (t, $J = 9.0$ Hz, 6.0 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$171.6, 163.5, 137.2, 134.1, 129.6, 122.5, 77.8, 51.1, 35.1, 26.6, 20.8, 17.1, 11.1. Anal. Calcd for C$_{18}$H$_{26}$N$_2$O$_4$: C, 64.65; H, 7.84; N, 8.38. Found: C, 65.03; H, 8.17; N, 8.50.

Ethyl-3-(allylamino)-2-cyano-3-[(3-cyanophenyl)imino]propionate (2.20c). Yellow oil (20%); $^1$H NMR (CDCl$_3$) $\delta$ 10.76 (br s, 1H), 9.36 (br s, 1H), 7.50-7.33 (m, 4H), 5.76-5.70 (m, 1H), 5.24 (m, 2H), 4.24 (quartet, $J = 9.0$ Hz, 2H), 3.57 (t, $J = 6.0$ Hz, 2H), 1.34 (t, $J = 9.0$ Hz, 6.0 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 169.9, 162.5, 140.4, 132.3, 130.9, 128.4, 125.6, 124.4, 119.4, 118.6, 118.4, 114.1, 61.0, 47.8, 30.1, 14.9. HRMS calcd for C$_{16}$H$_{16}$N$_4$O$_2$ [M+H]$^+$ 297.1346, found 297.1341.

Dimethyl 2-[(1-phenylethyl)amino]carbothioyl]malonate (2.21a). Yellow oil (27%); $^1$H NMR (CDCl$_3$) $\delta$ 9.43 (br s, 1H), 7.39-7.26 (m, 5H), 5.66 (quintet, $J = 9.0$ Hz, 1H), 5.03 (s, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 1.63 (d, $J = 9.0$ Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 187.3, 165.6, 165.4, 140.7, 128.3, 127.2, 125.8, 65.1, 54.7,53.2, 53.1, 20.1. Anal. Calcd for C$_{14}$H$_{17}$NO$_4$S: C, 56.93; H, 5.80; N, 4. 74. Found: C, 57.30; H, 6.02; N, 4.65.

Diethyl 2-[(1-phenethyl)amino]carbothioyl]malonate (2.21b). Yellow oil (49%); $^1$H NMR (CDCl$_3$) $\delta$ 9.23 (br s, 1H), 7.33-7.23 (m, 5H), 4.96 (s, 1H), 4.20 (quartet, $J = 6.0$ Hz, 4H), 3.95 (quartet, $J = 6.0$ Hz, 2H), 2.99 (t, $J = 9.0$ Hz, 6.0 Hz, 2H), 1.25 (t, $J = 9.0$ Hz, 6.0 Hz, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 189.2, 165.2, 137.9, 128.5, 128.4, 126.5, 65.6, 62.6, 47.3, 33.3, 13.6. Anal. Calcd for C$_{16}$H$_{21}$NO$_4$S: C, 59.42; H, 6.54; N, 4. 33. Found: C, 59.36; H, 6.67; N, 4.36.
1-\{[(Z)-Isopropoxy(isopropylamino)methylidene|amino]-4-methylbenzene (2.23a).

Yellow oil (33%); $^1$H NMR (CDCl$_3$) $\delta$ 7.08 (d, $J = 7.8$ Hz, 2H), 6.76 (d, $J = 8.1$ Hz, 2H), 5.19 (quintet, $J = 14.7$, 6.0 Hz, 1H), 3.79-3.69 (overlapped m, 1H), 3.68 (overlapped br s, 1H), 2.30 (s, 3H), 1.33 (d, $J = 6.3$ Hz, 6H), 1.04 (d, $J = 6.3$, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 152.9, 146.8, 131.6, 130.5, 123.1, 68.6, 43.6, 23.9, 22.4, 21.2. Anal. Calcd for C$_{14}$H$_{22}$N$_2$O: C, 71.76; H, 9.46; N, 11.95. Found: C, 71.50; H, 9.93; N, 11.59.

Isopropyl 4-\{[(Z)-isopropoxy(phenethylamino)methylidene|amino]benzoate (2.23b).

Colorless oil (49%); $^1$H NMR (CDCl$_3$) $\delta$ 7.90 (d, $J = 8.7$ Hz, 2H), 7.31-7.18 (m, 3H) 7.10 (d, $J = 6.9$ Hz, 2H), 6.80 (d, $J = 8.7$ Hz, 2H), 5.27-5.10 (m, 2H), 3.92 (br s, 1H), 3.31 (quartet, $J = 5.9$ Hz, 2H), 2.72 (t, $J = 6.9$, 2H), 1.37-1.31 (m, 12H); $^{13}$C NMR (CDCl$_3$) $\delta$ 166.2, 153.8, 152.1, 138.8, 131.1, 128.8, 128.6, 126.5, 124.5, 122.6, 69.1, 67.8, 42.8, 36.7, 22.0. HRMS calcd for C$_{22}$H$_{28}$N$_2$O$_3$ [M+H]$^+$ 369.2173, found 369.2180.

O-Isopropyl N-benzylcarbamothioate (2.24a). Colorless oil (28%); $^1$H NMR (CDCl$_3$) (ca. 1:1 mixture of rotamers) 7.40-7.19 (m, 5H), 7.09 (br s, 1H), 6.42 (br s, 1H), 5.62-5.48 (m, 1H), 4.74 (d, $J = 5.4$ Hz, 1H), 4.40 (d, $J = 5.7$ Hz, 1H), 1.32 (d, $J = 6.3$ Hz, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 189.8, 189.0, 136.8, 136.7, 128.7, 128.6, 127.9, 127.8, 127.7, 127.6, 76.0, 74.0, 49.0, 47.0, 21.8, 21.7. Anal. Calcd for C$_6$H$_{15}$NOS $\cdot$ 0.2H$_2$O: C, 62.05; H, 7.29; N, 6.58. Found: C, 62.41; H, 7.55; N, 6.78.

O-Methyl N-(1-phenylethyl)carbamothioate (2.24b). Yellow oil (40%); $^1$H NMR (CDCl$_3$) (ca. 1:1 mixture of rotamers) $\delta$ 7.38-7.23 (m, 10H), 7.01 (br s, 1H), 6.49 (br s, 1H), 5.45 (quintet, $J = 7.5$ Hz, 1H), 4.97 (quintet, $J = 7.1$ Hz, 1H), 4.00 (s, 3H), 3.97 (s, 3H), 1.57 (d, $J = 6.9$ Hz, 3H), 1.50 (t, $J = 6.8$ Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 190.3, 190.1, 142.2, 142.0, 128.6,
O-Methyl N-isopropylcarbamothioate (2.24c). Colorless oil (71%); $^1$H NMR (CDCl$_3$) (ca. 1:1 mixture of rotamers) δ 7.03 (br s, 1H), 6.30 (br s, 1H), 4.46-4.28 (m, 1H), 4.07 (overlapped s, 3H), 4.06-3.96 (overlapped m, 1H), 3.96 (overlapped s, 3H), 1.24 (d, $J = 6.6$ Hz, 6H), 1.18 (d, $J = 6.5$ Hz, 6H); $^{13}$C NMR (CDCl$_3$) δ 189.5, 189.4, 57.9, 56.4, 47.0, 45.3, 22.1, 21.7. Anal. Calcd for C$_5$H$_{11}$NOS: C, 45.08; H, 8.32; N, 10.51. Found: C, 45.06; H, 8.55; N, 10.34.

O-Ethyl N-isopropylcarbamothioate (2.24d). Colorless oil (45%); $^1$H NMR (CDCl$_3$) (ca. 1:1 mixture of rotamers) δ 6.71 (br s, 1H), 6.11 (br s, 1H), 4.56 (q, $J = 7.1$ Hz, 2H), 4.45 (overlapped q, $J = 7.0$ Hz, 2H), 4.41-4.31 (overlapped m, 1H), 4.09-3.94 (overlapped m, 1H), 1.37 (t, $J = 7.1$, 3H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.24 (d, $J = 6.6$ Hz, 6H), 1.18 (d, $J = 6.6$ Hz, 6H); $^{13}$C NMR (CDCl$_3$) δ 189.4, 68.1, 66.3, 47.3, 45.6, 22.7, 22.3, 14.6. Anal. Calcd for C$_6$H$_{13}$NOS: C, 48.94; H, 8.90; N, 9.51. Found: C, 49.72; H, 9.37; N, 9.29.

1-Butyl-3-(3-cyanophenyl)urea (2.25a). Colorless oil (40%); $^1$H NMR (CDCl$_3$) δ 7.78 (s, 1H), 7.62 (s, 1H), 7.53 (d, $J = 8.7$ Hz, 1H), 7.30 (overlapped t, $J = 8.0$ Hz, 1H), 7.24 (overlapped t, $J = 7.4$ Hz, 1H), 5.64 (t, $J = 5.4$ Hz, 1H), 3.23 (q, $J = 6.6$ Hz, 2H), 1.53-1.41 (m, 2H), 1.40-1.24 (m, 2H), 0.89 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (CDCl$_3$) δ 155.9, 140.1, 129.8, 125.9, 123.4, 122.1, 118.8, 112.5, 40.0, 32.0, 20.0, 13.7. Anal. Calcd for C$_{12}$H$_{15}$N$_3$O: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.58; H, 6.37; N, 18.86.

1-Phenyl-3-p-tolyurea (2.25b). White solid (65%); $^1$H NMR (CDCl$_3$) δ 7.44-7.29 (m, 5H), 7.26 (d, $J = 4.5$ Hz, 2H), 7.07 (d, $J = 8.1$ Hz, 2H), 3.72 (s, 2H), 2.28 (s, 3H); $^{13}$C NMR
(CDCl$_3$) $\delta$ 169.4, 135.4, 134.9, 134.5, 130.0, 129.8, 129.6, 128.0, 120.3, 21.2. HRMS calcd for C$_{14}$H$_{14}$N$_2$O [M+H]$^+$ 226.1242, found 226.1106.
Figure 2-9. IR spectrum of 2.20c

Figure 2-10. IR spectrum of 2.23b
Figure 2-11. High Resolution Mass Spectrum of **2.23b**
Figure 2-12. $^1$H NMR spectrum of 2.24d

Figure 2-13. $^{13}$C NMR spectrum of 2.24d
Figure 2-14. \textsuperscript{1}H NMR spectrum of 2.25a

Figure 2-15. \textsuperscript{13}C NMR spectrum of 2.25a
Figure 2-16. $^1$H NMR spectrum of 2.25b

Figure 2-17. $^{13}$C NMR spectrum of 2.25b
CHAPTER 3
C-ALKOXYIMIDOYLATION AND C-ARYLTHIOIMIDOYLATION

3.1 Introduction

Isothioureas [96TL2619, 08JOC2003, 05TL7597], isoureas [95RCR929, 04T61], ketene $O,N$- and $S,N$-acetals [90S195] are all useful building blocks in the construction of many heterocycles [96TL2619, 08JOC2003, 04T61, 95RCR929, 90S195] and frequently form part of the structure of biologically active compounds (For examples, see Figures 3-2, 3-3 and 3-4).

Figure 3-1. General Structure of isothioureas, isoureas, ketene $O,N$- and $S,N$-acetals

The applications of isothioureas are diverse, and they are widely employed, especially in the fields of medicine and agriculture. Examples include 3.1 [08JOC2003], an isothiourea that possesses potent herbicidal activity, and 3.2 [05TL7597], a commercial drug known as Clobenpropit$^\text{®}$ that is an anticonvulsant (Figure 3-2).

Figure 3-2. Specific examples of isothioureas applied in medicine and agriculture

Isothioureas are widely used, for example, (i) as solubility modulators [04JOC1571], (ii) potent inhibitors of nitric oxide synthases (NOSs) [96TL2619, 96EJP341, 96PBB179], (iii) to treat acute kidney failure, septic shock, and to prevent organ rejection after transplant surgery [96TL2619, 96EJP341, 96PBB179]. Also, isothioureas are postulated to be potent agonists of
neurodegenerative diseases, such as Alzheimer’s disease (AD). [96TL2619]. In synthesis, isothioureas are valuable intermediates in the construction of heterocycles as well as guanidines. [05TL7597, 07JOM545]

Isoureas, frequently used as synthetic intermediates in carbodiimide-based peptide coupling, are known appetite suppressants, agricultural agents, inhibitors of pheromone synthesis and antihypertensive agents [04T61, 02JCA293, 08BMC8210]. Trehazolin (3.3), the first natural cyclic isourea derivative of carbohydrates, exhibits potent activity as a trehalase inhibitor (Figure 3-3). [04T61] Trehalase is a glycoside hydrolase enzyme found in most animals and is responsible for the conversion of trehalose to glucose.

Figure 3-3. Trehazolin (3.3), a natural cyclic isourea

Ketene $O,N$- and $S,N$-acetals serve as versatile building blocks in the preparation of functionalized heterocycles. Ketene $O,N$- and $S,N$-acetals, for example trifluoroacetylketene $O,N$- and $S,N$-acetals (3.4) and (3.5) respectively, are easily accessed and potentially useful medicinal and agricultural agents (Figure 3-4). [90S195]

Figure 3-4. Examples of ketene $O,N$ and $S,N$-acetals
Multiple methods are available for the synthesis of these four compound classes; however, their use as potential drugs is limited due to the lack of general synthetic routes. Previously, Katritzky and coworkers synthesized (i) 1,2,3-trisubstituted guanidines [05HCA1664], N-hydroxy- and N-amino-guanidines [04JOC2976] (ii) di- and trisubstituted thioureas [06JOC6753], N-hydroxythioureas and thiosemicarbazides [06ARK226] using novel 1-(alkyl/arylthiocarbamoyl)benzotriazole thiocarbamoylating reagents.

Now, the syntheses of novel benzotriazole-1-carboximidates 3.12 and benzotriazole-1-carboximidothioates 3.13 are described. It will be demonstrated that esters, ketones and amines can be C-alkoxyimidoylated by benzotriazole-1-carboximidates 3.12 and C-arylthioimidoylated by benzotriazole-1-carboximidothioates 3.13 to provide isothioureas, isoureas, ketene O,N and S,N-acetals. This simple procedure comprises nucleophilic substitution of the benzotriazolyl group in benzotriazole-1-carboximidates 3.12 and benzotriazole-1-carboximidothioates 3.13 by the appropriate carbon and nitrogen nucleophiles.

3.2 Results and Discussion

Bis(benzotriazolyl)methanethione 3.6 [78JOC337] reacted with phenols (3.7) to afford O-aryl-benzotriazole carbothioates 3.9. Similarly, the reaction of 3.6 with p-toluenethiol 3.8 afforded S-aryl-benzotriazole carbodithioate 3.10. The synthesis of benzotriazole-1-carboximidates 3.12 was achieved by reacting 3.9 with iminophosphoranes 3.11 (Scheme 3-1).

Reaction of 2.0 equiv of ester enolates (from ester 3.14 with 2.5 equiv potassium tert-butoxide) with 1.0 equiv of benzotriazole-1-carboximidates 3.12a, afforded 3.15; after limited optimization, a yield of 54% was obtained (Scheme 3-2).
Scheme 3-1. Preparation of $O$-aryl-benzotriazole carbothioate (3.9), $S$-aryl-benzotriazole carbodithioate (3.10) and benzotriazole-1-carboximidates (3.12)

Scheme 3-2. Synthesis of product 3.14

3.3 Conclusion

A facile route to the synthesis of C-alkoxyimidoylated ester products has been developed. Optimization of the procedure is still required and further experimental work is necessary to determine the scope of these reactions.

3.4 Experimental Section

Melting points were determined on a hot-stage apparatus and are uncorrected. $^1$H (300 MHz, with TMS as the internal standard) and $^{13}$C NMR (75 MHz) NMR spectra were recorded in CDCl$_3$. Elemental analysis was carried out in an Eager 200 CHN analyzer.

3.4.1 General Procedure for the Preparation of $O$-Arylbenzotriazole Carbothioate 3.9 or $S$-Arylbenzotriazole Carbodithioate 3.10a

Bis-benzotriazol-1-yl-methanethione 3.6 (1 mmol) was dissolved in methylene chloride (10 mL). In another flask, the appropriate phenol 3.7 or thiol 3.8 (1 mmol) and sodium hydride
(5 mmol) were combined in methylene chloride (10 mL) and stirred for 15 min. The sodium salt of the former was added to the bis-bezotriazol-1-yl-methanethione solution and the resulting mixture stirred at room temperature. The progress of the reaction was monitored by TLC. On completion of the reaction, water (20 mL) was added to the reaction mixture, which was then extracted with methylene chloride (3 x 30 mL). The combined organic extracts was washed with 10% sodium carbonate solution (3 x 30 mL), dried over magnesium sulfate and the solvent removed in vacuo. The crude mixture was purified by gradient column chromatography over silica gel (Ethyl acetate/hexanes) to give O-aryl-benzotriazole-1-carbothioates 3.9a-c or S-aryl-benzotriazole-1-carbodithioates 3.10a in 34-61% yield.

**O-(4-Ethylphenyl) 1H-1,2,3-benzotriazole-1-carbothioate (3.9a).** Recrystallized from EtOAc-hexanes to give pale yellow crystals (34%); mp 91.7-94.1 °C; 1H NMR δ 8.49 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.55 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H), 2.74 (q, J = 7.6 Hz, 2H), 1.30 (t, J = 7.5 Hz); 13C NMR δ 182.4, 150.6, 146.6, 143.3, 131.6, 130.7, 129.2, 126.3, 121.7, 120.8, 115.1, 28.3, 15.3. Anal. Calcd for C15H13N3O: C, 63.58; H, 4.62; N, 14.83. Found: C, 63.77; H, 4.54; N, 14.88.

**O-(2,6-Dimethylphenyl) 1H-1,2,3-benzotriazole-1-carbothioate (3.9b).** Yellow oil (35%) 1H NMR δ 8.53 (d, J = 8.4 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 7.56 (t, J = 7.7 Hz, 1H), 7.22-7.16 (m, 3H), 2.27 (s, 6H); 13C NMR δ 180.1, 150.0, 146.7, 131.7, 130.8, 130.4, 129.1, 127.1, 126.3, 120.9, 115.1, 16.3. HRMS calcd for C15H13N3OS [M+H]^+ 284.0852, found 284.0837.

**O-(3,5-Dimethylphenyl) 1H-1,2,3-benzotriazole-1-carbothioate (3.9c).** Recrystallized from EtOAc-hexanes to give yellow crystals (37%); mp 127.7-129.8 °C; 1H NMR δ 8.48 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.67 (t, J = 7.7 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.03 (s,
1H), 6.90 (s, 2H), 2.40 (s, 6H); $^{13}$C NMR δ 182.3, 152.6, 146.6, 139.9, 131.6, 130.7, 128.9,
126.2, 120.8, 119.4, 115.1, 21.3. Anal. Calcd for C$_{15}$H$_{13}$N$_{3}$O: C, 63.58; H, 4.62; N, 14.83. Found:
C, 63.70; H, 4.53; N, 14.78.

4-Methylphenyl 1H-1,2,3-benzotriazole-carbodithioate (3.10a). Recrystallized from
EtOAc-hexanes to give yellow crystals (61%); mp 86.6-88.4 °C; $^1$H NMR δ 8.71 (d, J = 8.4 Hz, 
1H), 8.17 (d, J = 8.1 Hz, 1H), 7.66 (t, J = 7.8 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.47 (d, J = 8.1
Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 2.47 (s, 3H); $^{13}$C NMR δ 198.4, 146.9, 141.0, 135.8, 132.0,
130.7, 130.2, 126.2, 125.4, 120.3, 115.3, 21.2. Anal. Calcd for C$_{14}$H$_{11}$N$_{3}$S$_{2}$: C, 58.92; H, 3.88; N,
14.72. Found: C, 58.98; H, 3.75; N, 14.69.

3.4.2 General Procedure for the Preparation of Benzotriazole-1-carboximidates (3.12)

To a solution of O-aryl-benzotriazole-1-carbothioates 3.9 (1 mmol) in toluene (12 mL),
was added the iminophosphorane 3.11 (1 mmol). The mixture was stirred for 5 min then refluxed
for 12 h. The solvent was removed in vacuo and the crude product was chromatographed over
silica gel using a gradient of ethyl acetate/hexanes. Further purification by crystallization from
ethyl acetate/hexanes yielded pure benzotriazole-1-carboximidates 3.12a and 3.12b in 13 and
58% respectively.

3,5-Dimethylphenyl N-(4-methylphenyl)-1H-1,2,3-benzotriazole-1-carboximidoate
(3.12a). Recrystallized from EtOAc-hexanes to give white crystals (13%); mp 139.6-142.1 °C; $^1$H
NMR δ 8.36 (br s, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.65 (t, J = 7.7 Hz, 1H), 7.49 (t, J = 7.7, 6.0 Hz,
1H), 7.22-7.03 (m, 4H), 6.74-6.56 (m, 3H), 2.31 (s, 3H), 2.20 (s, 6H); $^{13}$C NMR δ 170.0, 154.1,
146.2,140.5, 139.7, 135.1, 131.9, 129.5, 129.4, 126.2, 125.4, 122.9, 120.2, 115.0, 114.1, 21.2,
20.9. Anal. Calcd for C$_{22}$H$_{20}$N$_{4}$O: C, 74.14; H, 5.66; N, 15.72. Found: C, 74.40; H, 5.62; N,
15.58. Anal. Calcd for C$_{22}$H$_{20}$N$_{4}$O: C, 74.14; H, 5.66; N, 15.58. Found: C, 74.40; H, 5.62; N,
15.58.
4-Ethylphenyl N-(4-chlorophenyl)-1H-1,2,3-benzotriazole-1-carboximidoate (3.12b).
Recrystallized from EtOAc-hexanes to give white crystals (58%); $^1$H NMR $\delta$ 8.25 (s, 1H), 8.11 (d, $J = 8.1$ Hz, 1H), 7.64 (t, $J = 7.5$ Hz, 1H), 7.48 (t, $J = 7.7$ Hz, 1H), 7.21 (d, $J = 8.4$ Hz, 2H), 7.06 (d, $J = 7.2$ Hz, 3H), 6.97 (s, 2H), 2.56 (quartet, $J = 7.2$ Hz, 2H), 1.17 (t, $J = 7.5$ Hz); $^{13}$C NMR $\delta$ 152.2, 146.5, 142.3, 141.4, 141.3, 132.2, 130.9, 130.1, 130.0, 129.6, 129.5, 129.4, 126.1, 126.0, 124.4, 120.8, 118.2, 114.2, 28.5, 15.9. Anal. Calcd for C$_{21}$H$_{17}$ClN$_4$O: C, 66.93; H, 4.55; N, 14.87. Found: C, 66.90; H, 4.54; N, 14.67.

3.4.3 General Procedure for the Preparation of 3.14
To a solution of potassium tert-butoxide (2.5 equiv) in THF (15 mL) was added dimethylmalonate (3.13) (2.0 equiv). After the solution was stirred for 30 min, 3,5-dimethylphenyl N-(4-methylphenyl)-1H-1,2,3-benzotriazole-1-carboximidoate (3.12a) (1.0 equiv) was added and the resulting mixture stirred at room temperature. The progress of the reaction was monitored by TLC. On completion of the reaction, water (15 mL) was added to the reaction mixture, which was then extracted with dichloromethane (3 x 30 mL). The combined extracts were dried over magnesium sulfate and the solvent removed in vacuo. The crude mixture was purified by gradient column chromatography over silica gel (ethyl acetate/hexanes) to give a yellow oil, tetramethyl 2-($p$-tolylamino)prop-1-ene-1,1,3,3-tetracarboxylate (3.14) (0.03 g, 54%).

Tetramethyl 2-($p$-tolylamino)prop-1-ene-1,1,3,3-tetracarboxylate (3.14). Yellow oil (54%); $^1$H NMR $\delta$ 11.72 (br s, 1H), 7.17 (d, $J = 8.1$ Hz, 2H), 7.08 (d, $J = 8.1$ Hz, 2H), 4.76 (s, 1H), 3.76 (s, 3H), 3.71 (s, 6H), 3.68 (s, 3H), 2.36 (s, 3H); $^{13}$C NMR $\delta$ 170.2, 167.8, 165.2, 158.5, 138.0, 134.3, 130.3, 126.8, 103.5, 93.9, 53.1, 52.3, 51.7, 21.1. HRMS calcd for C$_{18}$H$_{21}$NO$_8$ [M+H]$^+$ 380.1340, found 380.1341.
CHAPTER 4
N-(FMOC-α-AMINOACYL)BENZOTRIAZOLES: VERSATILE SYNTHETIC REAGENTS
FROM PROTEINOGENIC AMINO ACIDS

4.1 Introduction

N-Protected α-amino acids need activation of the carboxylic acid function to facilitate peptide bond formation. Usually, activation of the carboxylic acid moiety is achieved by reaction with an appropriate peptide coupling reagent. [04T2447] Subsequent reaction of the activated amino acid with the amino group of a second amino acid generates the amide bond.

\[
\text{amino acid 1} + \text{amino acid 2} \xrightarrow{\text{peptide coupling reagent, base}} \text{dipeptide}
\]

Scheme 4-1. Amide bond formation

Activation methods can be in situ with no isolable intermediates as exemplified by the use of carbodiimides, EDC, DCC and DIC, in combination with additives such as HOBt and HOAt. [04T2447]. Alternatively, isolated activated intermediates include N-protected-α-amino acid halides and azides.

\(N\)-Acylbenzotriazoles have been utilized in (i) N-acylation for the preparation of primary, secondary and tertiary amides [00JOC2810, 06S3231, 08OBC2400] and \(N\)-acylsulfonamides, [04ARK14] (ii) S-acylation for the synthesis of thiol esters [04S1806] and (iii) C-acylation for the preparation of ketones, diketones, \(β\)-ketosulfones, [03JOC1443] enaminones [00S2029] and C-acylated pyrroles, indoles, [04CCA175] 2-methylfurans and thiophenes [04CCA175]. Previously the \(N\)-(Boc-, Fmoc- and Cbz-α-aminoacetyl)benzotriazoles of some amino acids were prepared by Katritzky and coworkers. [05S397, 02ARK134,
Now stable, crystalline $N$-Fmoc-$\alpha$-aminoacyl)benzotriazoles derivatives of 18 proteinogenic amino acids and their utilization in the synthesis of chiral $\alpha$-($N$-Fmoc-amino)acid amides are reported.

### 4.2 Results and Discussion

#### 4.2.1 Preparation of $N$-(Fmoc-$\alpha$-aminoacyl)benzotriazoles 4.1a-r

Eighteen of the twenty natural, $N$-Fmoc-$\alpha$-amino acids 4.1a-r (purchased from Peptides International, Louisville, KY, USA and used without further purification) when treated with 1$H$-benzotriazole and thionyl chloride in THF at 20 °C for 2 hours [05S397] (Scheme 4-2, Table 4-1), afforded crystalline $N$-(Fmoc-$\alpha$-aminoacyl)benzotriazoles 4.2a-r in 69-90% yields. Novel 4.2b-l, q-r were characterized by $^1$H and $^{13}$C NMR spectroscopy, elemental analysis and high resolution mass spectrometry (HRMS); known 4.2a and m-p were verified by comparison of the melting points and spectroscopic data with that of the literature. [07CBDD45, 06S4135, 06S411]

The spectra of 4.2b-l, q-r displayed the expected $^{13}$C NMR chemical shifts at ca. $\delta$ 131, 127, 120, and 114 ppm, and that of the amide and carbamate carbonyl carbons at ca. $\delta$ 170 and 155 ppm, respectively.[02ARK134]

![Scheme 4-2. Preparation of $N$-Fmoc-(\(\alpha\)-aminoacyl)benzotriazoles 4.2a-r from the corresponding \(N\)-protected \(\alpha\)-amino acids 4.1a-r](image)

For structural designation of R in 4.1a-r and 4.2a-r, see Table 4-1

The $N$-Fmoc-(\(\alpha\)-aminoacyl)benzotriazoles of arginine and asparagine were not obtained under these conditions; they appeared to be formed but were rapidly hydrolyzed before they could be isolated.
### Table 4-1. Conversions of the 18 N-Fmoc-α-amino acids 4.1a-r into N-(Fmoc-α-aminoacetyl) benzotriazoles 4.2a-r

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield (^{a})</th>
<th>mp (°C) (^{c})</th>
<th>[(\alpha)](\text{D})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc-L-Ile-OH (4.1a)</td>
<td>Fmoc-L-Ile-Bt (4.2a)</td>
<td>78</td>
<td>165.4-167.25 (^{c}) (168.8-170.0)</td>
<td>-32.8</td>
</tr>
<tr>
<td>Fmoc-L-Val-OH (4.1b)</td>
<td>Fmoc-L-Val-Bt (4.2b)</td>
<td>84</td>
<td>148.3-149.8</td>
<td>-40.4</td>
</tr>
<tr>
<td>Fmoc-L-Thr(tBu)-OH (4.1c)</td>
<td>Fmoc-L-Thr(tBu)-Bt (4.2c)</td>
<td>80</td>
<td>62.2-65.0</td>
<td>-30.0</td>
</tr>
<tr>
<td>Fmoc-L-Lys(Boc)-OH (4.1d)</td>
<td>Fmoc-L-Lys(Boc)-Bt (4.2d)</td>
<td>75</td>
<td>138.4-140.6</td>
<td>-33.3</td>
</tr>
<tr>
<td>Fmoc-L-Glu(OrBu)-OH (4.1e)</td>
<td>Fmoc-L-Glu(OrBu)-Bt (4.2e)</td>
<td>81</td>
<td>65.5-67.6</td>
<td>-21.2</td>
</tr>
<tr>
<td>Fmoc-L-Ser(tBu)-OH (4.1f)</td>
<td>Fmoc-L-Ser(tBu)-Bt (4.2f)</td>
<td>70</td>
<td>91.7-92.4</td>
<td>-14.8</td>
</tr>
<tr>
<td>Fmoc-L-Tyr(tBu)-OH (4.1g)</td>
<td>Fmoc-L-Tyr(tBu)-Bt (4.2g)</td>
<td>83</td>
<td>138.4-139.3</td>
<td>+15.0</td>
</tr>
<tr>
<td>Fmoc-L-Gln(Trt)-OH (4.1h)</td>
<td>Fmoc-L-Gln(Trt)-Bt (4.2h)</td>
<td>69</td>
<td>167.0-168.0</td>
<td>-16.3</td>
</tr>
<tr>
<td>Fmoc-L-Asp(OrBu)-OH (4.1i)</td>
<td>Fmoc-L-Asp(OrBu)-Bt (4.2i)</td>
<td>73</td>
<td>102.0-104.0</td>
<td>-11.1</td>
</tr>
<tr>
<td>Fmoc-L-Cys(Trt)-OH (4.1j)</td>
<td>Fmoc-L-Cys(Trt)-Bt (4.2j)</td>
<td>88</td>
<td>96.0-98.0</td>
<td>-11.0</td>
</tr>
<tr>
<td>Fmoc-L-His(Trt)-OH (4.1k)</td>
<td>Fmoc-L-His(Trt)-Bt (4.2k)</td>
<td>73</td>
<td>137.4-139.5</td>
<td>+13.0</td>
</tr>
<tr>
<td>Fmoc-L-Leu-OH (4.1l)</td>
<td>Fmoc-L-Leu-Bt (4.2l)</td>
<td>80</td>
<td>121.3-123.2</td>
<td>+53.1</td>
</tr>
<tr>
<td>Fmoc-L-Trp-OH (4.1m)</td>
<td>Fmoc-L-Trp-Bt (4.2m)</td>
<td>90</td>
<td>92.5-93.6(^{c}); 192.4-195.2(^{b}) (88.0-90.0)(^{d})</td>
<td>+9.0</td>
</tr>
<tr>
<td>Fmoc-L-Phe-OH (4.1n)</td>
<td>Fmoc-L-Phe-Bt (4.2n)</td>
<td>85</td>
<td>159.1-160.2(^{c}) (136.5-137.4)(^{d})</td>
<td>+3.4</td>
</tr>
<tr>
<td>Fmoc-L-Met-OH (4.1o)</td>
<td>Fmoc-L-Met-Bt (4.2o)</td>
<td>82</td>
<td>122.7-123.35(^{c}) (98.0-100.0)(^{e})</td>
<td>-44.7</td>
</tr>
<tr>
<td>Fmoc-L-Ala-OH (4.1p)</td>
<td>Fmoc-L-Ala-Bt (4.2p)</td>
<td>72</td>
<td>160.0-160.3(^{c}) (160.7-161.3)(^{d})</td>
<td>-60.8</td>
</tr>
<tr>
<td>Fmoc-L-Pro-OH (4.1q)</td>
<td>Fmoc-L-Pro-Bt (4.2q)</td>
<td>89</td>
<td>163.5-165.4(^{c})</td>
<td>-60.5</td>
</tr>
<tr>
<td>Fmoc-Gly-OH (4.1r)</td>
<td>Fmoc-Gly-Bt (4.2r)</td>
<td>88</td>
<td>161.5-161.9(^{c})</td>
<td>Non-chiral</td>
</tr>
</tbody>
</table>

\(^{a}\) Isolated yield, \(^{b}\) mp of polymorph, \(^{c}\) 07CBDD465, \(^{d}\) 06S4135, \(^{e}\) 06S411

### 4.2.1 Preparation of α-(N-Fmoc-amino)acid Amides 4.3a,b and 4.4a,b

α-Methylbenzylamides of \(N\)-protected amino acids provide criteria for optical purity and stability towards racemization. \(N\)-Fmoc-(α-aminoacetyl)benzotriazoles 4.2b,g were separately reacted with L-α-methylbenzylamine 4.5 and D-α-methylbenzylamine 4.6 in THF at 20 °C to
afford amides \(4.3a, b\) and \(4.4a, b\) in 66-74% yields (average 72%, Scheme 4-3, 4.4 Experimental Section).

The diastereomeric excess (de) for the \(\alpha\)-(N-Fmoc-amino)acid amides \(4.3a, b\) and \(4.4a, b\) were determined by \(^1\)H NMR and HPLC. A comparison of the \(^1\)H NMR spectra obtained from the derivatization of \(4.2b\) with D/L- and L-\(\alpha\)-methylbenzylamine respectively demonstrated the chirality of \(4.3a\) and thus the conversion of the \(4.1b\) to \(4.2b\) also occurred with retention of chirality. HPLC analyses of \(4.3a, 4.4a\) and corresponding diastereomeric mixtures provided further evidence for the smooth conversion of chiral \(4.1b, g\) to chiral \(4.2b, g\) (Table 4-2).

![Chemical structures](Image)

For structural designation of \(R\) in \(4.2b, g, 4.3a, b\) and \(4.4a, b\) see Table 4-2

Scheme 4-3. Preparation of \(N\)-(acylamino)amides \(4.3a, b\) and \(4.4a, b\) from \(N\)-(Fmoc-\(\alpha\)-aminoacyl) benzotriazoles \(4.2b, g\) and L- or D-PhCH(Me)NH\(_2\) (\(4.5\) or \(4.6\))

Table 4-2. Preparation of \(N\)-(acylamino)amides \(4.3a, b\) and \(4.4a, b\) from \(N\)-(Fmoc-\(\alpha\)-aminoacyl) benzotriazoles \(4.2b, g\) and L- or D-PhCH(Me)NH\(_2\) (\(4.5\) or \(4.6\))

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield(^a) (%)</th>
<th>Retention time, (t_R) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc-L-Val-Bt ((4.2b))</td>
<td>Fmoc-L-Val-L-NHCH(Me)Ph ((4.3a))</td>
<td>73</td>
<td>5.42</td>
</tr>
<tr>
<td>Fmoc-L-Val-Bt ((4.2b))</td>
<td>Fmoc-L-Val-D/L-NHCH(Me)Ph ((4.3a+4.4a))</td>
<td>74</td>
<td>5.41, 8.00</td>
</tr>
<tr>
<td>Fmoc-L-Tyr(tBu)-Bt ((4.2g))</td>
<td>Fmoc-L-Tyr(tBu)-D/L-NHCH(Me)Ph ((4.3b))</td>
<td>74</td>
<td>1.91</td>
</tr>
<tr>
<td>Fmoc-L-Tyr(tBu)-Bt ((4.2g))</td>
<td>Fmoc-L-Tyr(tBu)-D/L-NHCH(Me)Ph ((4.3b+4.4b))</td>
<td>66</td>
<td>1.73, 1.91</td>
</tr>
</tbody>
</table>

\(^a\) Isolated yield. \(^b\) For conditions, see 4.4 Experimental Section

HOBt-based aminium- and phosphonium derivatives (PyBOP, HBTU, HATU, etc), \(1\) \(N\)-hydroxysuccinimide esters, [64JACS1839] and \(p\)-nitrophenyl esters [02TL7717] are widely used
in peptide synthesis but the preparative routes require multiple steps. \(N\)-Fmoc-(\(\alpha\)-aminoacyl)benzotriazoles are easily prepared peptide coupling reagents whose generality has been demonstrated in the solution phase syntheses of sterically hindered peptides [07JOC5794] or peptide conjugates [08JOC511] and the solid phase preparation of simple oligopeptides [07CBDD465]. Additionally, \(N\)-Fmoc-(\(\alpha\)-aminoacyl)benzotriazoles are fully amenable to microwave-assisted syntheses. [07CBDD465, 07JOC5794]

4.3 Conclusion

In summary, the convenient, cost effective preparation of \(N\)-(Fmoc-\(\alpha\)-aminoacyl)benzotriazoles 4.2a-r (69-90%) was described. \(N\)-(Fmoc-\(\alpha\)-aminoacyl)benzotriazoles 4.2a-r are storable at 20 °C for months without special handling. \(^1\)H NMR and HPLC analyses of \(N\)-(Fmoc-\(\alpha\)-aminoacyl)amides 4.3a,b and 4.4a,b, easily prepared in high yields, demonstrated that the chirality is maintained during amide bond formation.

4.4 Experimental Section

Reagents were obtained as follows: \(N\)-Fmoc-L-amino acids from Peptides International, Louisville, KY, USA; \(1H\)-benzotriazole, dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate (EtOAc), hexanes, magnesium sulfate (MgSO\(_4\)) and sodium carbonate (Na\(_2\)CO\(_3\)) from Fischer Scientific, Fair Lawn, NJ, USA. Melting points were determined on a hot-stage apparatus and are uncorrected. \(^1\)H (300 MHz, with TMS as the internal standard) and \(^13\)C NMR (75 MHz) NMR spectra were recorded in CDCl\(_3\). Optical rotations were recorded on Perkin Elmer 241 polarimeter. HPLC analyses were performed on a Shimadzu instrument using a Zorbax Rx-C18 reverse phase column (4.6 x 150 mm) with UV detection at 210 nm, a flow rate of 1.0 mL/min and MeOH:H\(_2\)O as the eluting solvent. High resolution mass spectrometry was performed in the ESI (electrospray ionization) mode on an Agilent 6210 LC-TOF (liquid
chromatography-time of flight) instrument. Elemental analysis was carried out in an Eager 200 CHN analyzer.

4.4.1 General Procedure for the Preparation of 4.2b-l, q, r

Thionyl chloride (5 mmol) was added dropwise to a solution of 1H-benzotriazole (20 mmol) in THF (50 mL). After stirring at room temperature for 30 min, N-Fmoc-amino acid (5 mmol) was added in one portion. After stirring for 2 h at room temperature, the solvent was evaporated in vacuo. The crude mixture obtained was dissolved in EtOAc (30 mL) and the organic layer washed with saturated Na₂CO₃ solution (30 mL x 3) and dried over MgSO₄. Concentration under reduced pressure gave the desired product, which was precipitated from dichloromethane-hexanes.

S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (Fmoc-L-Val-Bt, 4.2b). White microcrystals (84%), mp 148.3-149.8 °C: [α]²⁴D = -40.4 (c = 1.5, CHCl₃); ¹H NMR δ 8.28 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.69 (t, J = 7.8 Hz, 1H) 7.61 (d, J = 7.2 Hz, 2H), 7.54 (t, J = 7.5 Hz, 1H), 7.39 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.2 Hz, 2H), 5.6 (dd, J = 9.5, 5.0 Hz, 1H), 5.63 (d, J = 9.3 Hz, 1H), 4.44 (d, J = 6.3 Hz, 2H), 4.24 (t, J = 7.2, 6.3 Hz, 1H), 2.58-2.42 (m, 1H), 1.13 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H); ¹³C NMR δ 172.0, 156.7, 146.4, 144.2, 144.1, 141.7, 141.1, 128.1, 127.5, 127.0, 125.4, 120.8, 120.4, 114.8, 67.6, 59.8, 47.6, 32.1, 20.1, 17.5. Anal. Calcd for C₂₆H₂₄N₄O₃: C, 70.89; H, 5.49; N, 12.72; Found: C, 71.25; H, 5.57; N, 12.82.

S-(9H-Fluoren-9-yl)methyl(2S,3R) 1-(1H-benzotriazol-1-yl)-3-tert-butoxy-1-oxobutan-2-ylcarbamate (Fmoc-L-Thr(tBu)-Bt, 4.2c). White microcrystals (80%), mp 62.2-65.0 °C: [α]²⁴D = -30.0 (c = 1.5, CHCl₃); ¹H NMR δ 8.28 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 7.5 Hz, 2H) 7.72-7.64 (m, 3H), 7.54 (t, J = 7.8 Hz, 1H), 7.45-7.31 (m, 4H), 5.94 (d, J = 9.6 Hz, 1H), 5.67 (dd, J = 9.6, 1.5 Hz, 1H), 4.62-4.51 (m, 1H), 4.43 (t, J = 6.6 Hz, 2H),
4.30 (t, J = 7.2 Hz, 1H), 1.43 (d, J = 6.0 Hz, 3H), 0.92 (s, 9H); ^{13}C NMR δ169.9, 156.8, 145.8, 143.9, 143.7, 141.3, 131.1, 130.8, 127.7, 127.1, 126.5, 125.2, 125.2, 120.3, 120.0, 114.2, 74.3, 68.0, 67.4, 60.6, 47.1, 28.0, 27.8, 21.1. Anal. Calcd for C_{29}H_{30}N_{4}O_{4}: C, 69.86; H, 6.06; N, 11.24; Found: C, 70.04; H, 6.23; N, 11.14.

S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-6-(tert-butoxycarbonylamino)-1-oxohexan-2-ylcarbamate (Fmoc-L-Lys(Boc)-Bt, 4.2d). White microcrystals (75%), mp 138.4 - 140.6 °C; [α]^{24}_D = -30.0 (c = 1.5, CHCl₃); ^{1}H NMR δ 8.27 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 7.5 Hz, 2H), 7.68 (overlapped t, J = 7.5 Hz, 1H), 7.65-7.60 (m, 2H) 7.54 (t, J = 7.8 Hz, 1H), 7.40 (t, J = 7.1 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 5.87 (d, J = 7.8 Hz, 1H), 5.82-5.72 (m, 1H), 4.62 (br s, 1H), 4.49-4.37 (m, 2H), 4.24 (t, J = 6.9 Hz, 1H), 3.20-3.00 (m, 2H), 2.20-1.90 (m, 2H), 1.60-1.50 (m, 4H), 1.43 (s, 9H); ^{13}C NMR δ 171.7, 156.2, 146.0, 143.8, 143.6, 141.2, 131.1, 130.7, 127.7, 127.1, 126.5, 125.1, 120.3, 120.0, 114.4, 79.3, 67.2, 54.5, 47.1, 39.6, 32.2, 29.6, 28.4, 22.5. Anal. Calcd for C_{32}H_{35}N_{5}O_{5}: C, 67.47; H, 6.19; N, 12.29; Found: C, 67.38; H, 6.22; N, 11.90.

S-tert-butyl 4-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-5-(1H-benzotriazol-1-yl)-5-oxopentanoate (Fmoc-L-Glu(OtBu)-Bt, 4.2e). White microcrystals (81%), mp 65.5-67.6 °C, [α]^{20}_D = -21.2° (c = 2.4, CHCl₃); ^{1}H NMR δ 8.19 (d, J = 8.1 Hz, 1H), 8.08 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 7.5 Hz, 2H), 7.65-7.39 (m, 4H), 7.33 (t, J = 6.9 Hz, 2H), 7.24 (t, J = 7.2 Hz, 2H), 7.00 (br s, 1H), 5.91 (d, J = 8.1 Hz, 1H), 5.78-5.68 (m, 1H), 4.42-4.27 (m, 2H), 4.16 (t, J = 6.9 Hz, 1H), 2.41-2.28 (m, 2H), 2.25-2.10 (m, 2H), 1.36 (s, 9H); ^{13}C NMR δ 172.0, 171.2, 156.1, 146.0, 143.8, 143.6, 141.2, 131.1, 130.8, 127.7, 127.0, 126.6, 125.1, 120.3, 119.9, 114.4, 81.2, 67.2, 54.5, 47.1, 31.6, 28.0, 27.5. HRMS calcd for C_{30}H_{30}N_{4}O_{5} [M+Na]^{+} 549.2108, found 549.2071.
**S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-3-tert-butoxy-1-oxopropan-2-ylcarbamate (Fmoc-L-Ser(tBu)-Bt, 4.2f)**. White microcrystals (70%), mp 91.7-92.4 °C, $[\alpha]_{D}^{20} = -14.8^\circ$ (c = 2.4, CHCl$_3$); $^1$H NMR $\delta$ 8.30 (d, $J = 8.4$ Hz, 1H), 8.15 (d, $J = 8.1$ Hz, 1H), 7.78 (d, $J = 7.5$ Hz, 2H), 7.72-7.63 (m, 2H), 7.58-7.52 (m, 2H), 7.44-7.26 (m, 4H), 6.02 (d, $J = 9.0$ Hz, 1H), 5.88-5.86 (m, 1H), 4.47-4.35 (m, 2H), 4.31-4.22 (m, 2H), 3.92 (dd, $J = 9.0$, 3.2 Hz, 1H), 1.03 (s, 9H); $^{13}$C NMR $\delta$ 169.5, 156.2, 143.9, 143.7, 141.3, 131.2, 131.0, 127.8, 127.1, 126.5, 126.1, 125.2, 120.3, 120.0, 114.4, 74.0, 67.5, 62.9, 55.9, 47.1, 27.1. HRMS calcd for C$_{28}$H$_{28}$N$_4$O$_4$ [M+Na]$^+$ 507.2003, found 507.1986.

**S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-3-(4-tert-butoxyphenyl)-1-oxopropan-2-ylcarbamate (Fmoc-L-Tyr(tBu)-Bt, 4.2g)**. White microcrystals (83%), mp 138.4-139.3 °C, $[\alpha]_{D}^{20} = +15.0^\circ$ (c = 1.9, CHCl$_3$); $^1$H NMR $\delta$ 8.19 (d, $J = 8.4$ Hz, 1H), 8.09 (d, $J = 8.4$ Hz, 1H), 7.72 (d, $J = 7.2$ Hz, 2H), 7.62 (t, $J = 7.5$ Hz, 1H), 7.55 (t, $J = 4.5$ Hz, 2H), 7.48 (t, $J = 7.5$ Hz, 1H), 7.38-7.23 (m, 4H), 7.03 (d, $J = 8.1$ Hz, 2H), 6.83 (d, $J = 7.8$ Hz, 2H), 6.13-6.06 (m, 1H), 5.83 (d, $J = 8.4$ Hz, 1H), 4.38 (t, $J = 6.6$ Hz, 2H), 4.20-4.16 (m, 1H), 3.39 (dd, $J = 13.5$, 5.4 Hz, 1H), 3.21 (dd, $J = 13.5$, 8.0 Hz, 1H), 1.24 (s, 9H); $^{13}$C NMR $\delta$ 170.9, 155.7, 154.4, 145.8, 143.6, 143.5, 141.1, 130.8, 130.6, 129.7, 129.6, 127.6, 126.9, 126.4, 124.9, 124.2, 120.2, 119.8, 114.1, 78.4, 67.1, 55.6, 46.9, 38.2, 28.6. Anal. Calcd for C$_{34}$H$_{32}$N$_4$O$_4$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.84; H, 6.00; N, 9.70.

**S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-1,5-dioxo-5-(tritylamino)pentan-2-ylcarbamate (Fmoc-L-Gln(Trt)-Bt, 4.2h)**. White microcrystals (69%), mp 167.0-168.0 °C, $[\alpha]_{D}^{20} = -16.3$ (c = 1.4, CHCl$_3$); $^1$H NMR $\delta$ 8.26 (d, $J = 8.4$ Hz, 1H), 8.15 (d, $J = 8.4$ Hz, 1H), 7.75 (d, $J = 6.9$ Hz, 2H), 7.68 (t, $J = 7.2$ Hz, 1H), 7.59-7.51 (m, 2H), 7.44-7.38 (m, 2H), 7.32-7.26 (m, 12H), 7.23-7.20 (m, 6H), 6.81 (s, 1H), 6.14 (d, $J = 7.2$ Hz, 1H), 5.83-5.22 (m, 1H), 5.52-5.42 (m, 1H), 4.92-4.82 (t, $J = 4.5$ Hz, 2H), 4.32-4.22 (m, 1H), 3.92 (dd, $J = 9.0$, 3.2 Hz, 1H), 1.03 (s, 9H); $^{13}$C NMR $\delta$ 169.5, 156.2, 143.9, 143.7, 141.3, 131.2, 131.0, 127.8, 127.1, 126.5, 126.1, 125.2, 120.3, 120.0, 114.4, 74.0, 67.5, 62.9, 55.9, 47.1, 27.1. HRMS calcd for C$_{28}$H$_{28}$N$_4$O$_4$ [M+Na]$^+$ 507.2003, found 507.1986.
4.52-4.45 (m, 1H), 4.36 (t, J = 6.6 Hz, 1H), 4.23 (t, J = 6.3 Hz, 1H), 2.57 (t, J = 6.6 Hz, 2H), 2.44 (br s 1H), 2.27 (br s 1H); 13C NMR δ 171.1, 170.8, 156.5, 146.1, 144.5, 143.9, 143.7, 141.3, 131.1, 130.8, 128.7, 128.1, 127.8, 127.2, 126.6, 125.2, 120.4, 120.0, 114.5, 70.9, 67.3, 54.7, 47.2, 35.6, 27.7. Anal. Calcd for C45H37N5O4: C, 75.93; H, 5.24; N, 9.84. Found: C, 75.82; H, 5.46; N, 9.89.

*S*-tert-butyl 3-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-4-(1H-benzotriazol-1-yl)-4-oxobutanoate (Fmoc-L-Asp(OtBu)-Bt, 4.2i). White microcrystals (73%), mp 102.0-104.0 °C, [α]20D = -11.1 (c = 2.5, CHCl3); 1H NMR δ 8.29 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 7.2 Hz, 2H), 7.68 (t, J = 7.5 Hz, 1H), 7.60-7.51 (m, 3H), 7.42-7.28 (m, 4H), 6.15 (d, J = 6.9 Hz, 1H), 6.00-5.86 (m 1H), 4.44-4.40 (m, 2H), 4.27-4.25 (m, 1H), 3.26 (dd, J = 15.5, 5.6 Hz, 1H), 3.14 (dd, J = 18.6, 5.4 Hz, 1H), 1.38 (s, 9H); 13C NMR δ 169.5, 169.1, 155.7, 145.9, 143.7, 143.5, 141.2, 131.1, 131.8, 127.7, 127.0, 126.6, 125.1, 120.3, 120.0, 114.3, 82.3, 67.3, 51.9, 47.0, 38.5, 27.9. Anal. Calcd for C29H28N4O5: C, 67.96; H, 5.51; N, 10.93. Found: C, 67.98; H, 5.81; N, 10.96.

*R*-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-1-oxo-3-(tritylthio)-propan-2-ylcarbamate (Fmoc-L-Cys(Trt)-Bt, 4.2j). White microcrystals (88%), mp 96.0-98.0 °C, [α]20D = -11.0 (c = 2.0, CHCl3); 1H NMR δ 8.24 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 7.78-7.76 (m, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.64-7.56 (m, 3H), 7.41-7.38 (m, 2H), 7.33-7.30 (m, 7H), 7.19-7.11 (m, 11H), 5.78-5.70 (m, 1H), 5.52 (d, J = 7.2 Hz, 1H), 4.41 (d, J = 6.9 Hz, 2H), 4.24 (t, J = 6.9 Hz, 1H), 3.13. (dd, J = 14.1, 5.7 Hz, 1H), 2.92 (dd, J = 12.6, 6.2 Hz, 1H); 13C NMR δ 169.1, 155.6, 146.0, 144.0, 143.5, 141.3, 131.1, 130.8, 129.4, 128.0, 127.8, 127.1, 127.0, 126.7, 125.2, 120.4, 120.0, 114.5, 67.5, 67.3, 53.9, 47.1, 34.1. Anal. Calcd for C43H34N4O3S: C, 75.20; H, 4.99; N, 8.16. Found: C, 75.09; H, 5.28; N, 7.82.
S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-1-oxo-3-(1-trityl-1H-imidazol-4-yl)propan-2-ylcarbamate (Fmoc-L-His(Trt)-Bt, 4.2k). Yellow microcrystals (73%), mp 137.4-139.5 °C, [α]$_{D}^{24}$ = -60.5 (c = 1.5, CHCl$_3$); $^1$H NMR (300 Hz, CDCl$_3$) δ 8.19 (d, $J = 8.1$ Hz, 1H), 8.07 (d, $J = 8.1$ Hz, 1H), 7.72 (d, $J = 7.5$ Hz, 2H), 7.66-7.57 (m, 3H), 7.42-7.44 (m, 3H), 7.38-7.31 (m, 4H), 7.26-7.24 (m, 15H), 6.45 (s, 1H), 6.08-5.80 (m, 1H), 4.40-4.20 (m, 3H), 3.50-3.38 (m, 2H); $^{13}$C NMR (75Hz, CDCl$_3$) δ 170.5, 156.3, 145.8, 143.8, 142.0, 141.1, 139.0, 135.5, 130.5, 129.6, 129.6, 128.0, 127.6, 127.0, 126.3, 125.2, 120.2, 119.8, 114.3, 103.3, 77.2, 70.0, 60.4, 55.4, 30.3. HRMS calcd for C$_{46}$H$_{36}$N$_6$O$_3$ [M+H]$^+$ 721.2922, found 721.2919.

S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-4-methyl-1-oxopentan-2-ylcarbamate (Fmoc-L-Leu-Bt, 4.2l). White microcrystals (80%), mp 121.3-123.2 °C: [α]$_{D}^{24}$ = +53.1 (c = 1.5, DMF); $^1$H NMR δ 8.27 (d, $J = 8.2$ Hz, 1H), 8.16 (d, $J = 8.1$ Hz, 1H), 7.77 (d, $J = 7.3$ Hz, 2H), 7.69 (overlapped t, $J = 7.1$ Hz, 1H), 7.62-7.40 (m, 2H), 7.54 (overlapped t, $J = 7.8$ Hz, 1H), 7.41 (t, $J = 7.0$ Hz, 2H), 7.32 (t, $J = 7.1$ Hz, 2H), 5.85 (t, $J = 7.8$ Hz, 1H), 5.54-5.44 (m, 1H), 4.45 (d, $J = 7.0$ Hz, 2H), 4.25 (t, $J = 6.6$ Hz, 1H), 1.88 (m, 2H), 1.82-1.71 (m, 1H), 1.11 (d, $J = 4.9$ Hz, 3H), 0.99 (d, $J = 5.4$ Hz, 3H); $^{13}$C NMR δ 172.4, 156.1, 146.0, 143.8, 143.6, 141.3, 131.1, 130.7, 127.7, 127.0, 126.5, 125.0, 120.3, 120.0, 114.4, 67.1, 53.0, 47.1, 41.9, 25.2, 23.2, 21.3. Anal. Calcd for C$_{27}$H$_{26}$N$_4$O$_3$: C, 71.35; H, 5.77; N, 12.33; Found: C, 71.19; H, 6.06; N, 12.21.

S-(9H-Fluoren-9-yl)methyl 2-(1H-benzotriazole-1-carbonyl)pyrrolidine-1-carboxylate (Fmoc-L-Pro-Bt, ca. 1:1 mixture of rotamers, 4.2q). White microcrystals (89%), mp 163.5-165.4 °C, [α]$_{D}^{24}$ = -60.5 (c = 1.5, DMF); $^1$H NMR δ 8.29 (d, $J = 8.2$ Hz, 0.5H), 8.20 (d, $J = 8.2$ Hz, 0.5H), 8.14 (d, $J = 8.1$ Hz, 1H), 7.78 (d, $J = 7.5$ Hz, 2H), 7.74-7.28 (m, 6H), 7.21 (t, $J = 6.0$ Hz, 1.5H), 7.09 (t, $J = 6.7$ Hz, 0.5H), 6.89-6.78 (m, 1H), 5.89 (d, $J = 4.2$ Hz, 0.5H), 5.86 (d, $J =$
4.2 Hz, 0.5H), 5.44 (d, J = 3.3 Hz, 0.5H), 5.41 (d, J = 3.9 Hz, 0.5H), 4.61-4.53 (m, 1H), 4.52-4.43 (m, 0.5H), 4.40-4.26 (m, 0.5H), 4.02 (t, J = 5.0 Hz, 0.5H), 3.90-3.81 (m, 0.5H), 3.77-3.57 (m, 1.5H), 2.71-2.57 (m, 0.5H), 2.56-2.42 (m, 0.5H), 2.31-2.19 (m, 1.5H), 2.18-2.00 (m, 1H), 1.99-1.88 (m, 1.5H); 13C NMR δ 171.0, 170.6, 154.9, 154.1, 146.0, 144.0, 143.8, 143.5, 141.3, 141.0, 140.8, 131.2, 131.2, 130.5, 130.5, 127.7, 127.4, 127.1, 126.9, 126.8, 126.5, 126.4, 126.4, 125.2, 125.1, 124.1, 124.0, 120.2, 120.2, 120.0, 119.7, 119.4, 114.6, 114.5, 67.7, 66.5, 60.0, 59.2, 47.2, 47.0, 46.9, 31.6, 30.7, 24.5, 23.2. Anal. Calcd for C26H22N4O3: C, 71.22; H, 5.06; N, 12.78; Found: C, 71.16; H, 5.03; N, 13.12.

(9H-Fluoren-9-yl)methyl 2-(1H-benzotriazol-1-yl)-2-oxoethylcarbamate (Fmoc-Gly-Bt, 4.2r). White microcrystals (88%), mp 161.5-161.9 °C: 1H NMR δ 8.25 (d, J = 8.2 Hz, 1H), 8.15 (d, J = 8.4Hz, 1H), 7.77 (d, J = 7.4Hz, 2H), 7.71-7.68 (overlapped t, J = 7.8 Hz, 1H), 7.64 (d, J = 7.5 Hz, 1H), 7.54 (t, J = 7.7 Hz, 2H), 7.41 (t, J = 7.2 Hz, 1H), 7.33 (t, J = 7.2 Hz, 2H), 7.14 (br s, 1H), 5.59 (t, J = 5.4 Hz, 1H), 5.10 (d, J = 5.7 Hz, 2H), 4.49 (d, J = 6.9 Hz, 2H), 4.28 (t, J = 6.9 Hz, 1H); 13C NMR δ 168.4, 156.5, 146.0, 143.7, 141.3, 130.9, 130.8, 127.7, 127.1, 126.6, 125.1, 120.4, 120.0, 114.0, 67.4, 47.0, 44.8. Anal. Calcd for C23H18N4O3: C, 69.34; H, 4.55; N, 14.03; Found: C, 69.40; H, 4.36; N, 14.08.

4.4.2 General Procedure for the Preparation of 4.3a,b, 4.4a,b, (4.3a+4.4a) and (4.3b+4.4b)

N-(Fmoc-α-aminoacyl)benzotriazoles 4.2b,g (1 mmol) was dissolved in THF and L-α-methylbenzylamine 4.5, D-α-methylbenzylamine 4.6 or α-methylbenzylamine (4.5+4.6) (1 mmol) was added to the solution. The mixture was stirred at room temperature and monitored by TLC. On completion of the reaction the solvent was evaporated in vacuo. The resulting solid was dissolved in EtOAc (30 mL) and washed with saturated Na2CO3 (30 mL x 3) and dried with MgSO4. The solution was reduced to dryness in vacuo to yield 4.3a,b, 4.4a,b, (4.3a+4.4a) and (4.3b+4.4b).
(9H-Fluoren-9-yl)methyl S-3-methyl-1-oxo-1-((R)-1-phenylethylamino)butan-2-ylcarbamate (4.3a). White powder (73%), mp 205.7-206.2 °C, [α]D = -32.6 (c = 2.4, CHCl3); 1H NMR δ 7.82 (d, J = 7.8 Hz, 2H), 7.63 (d, J = 7.2 Hz, 2H), 7.45 (t, J = 7.2 Hz, 2H), 7.38-7.32 (m, 7H), 6.34 (d, J = 6.3 Hz, 1H), 5.57 (d, J = 8.1 Hz, 1H), 5.17 (quintet, J = 7.2 Hz, 15.6 Hz, 1H), 4.50-4.35 (m, 2H), 4.28-4.20 (m, 1H), 4.01 (t, J = 5.6 Hz, 1H), 2.18-2.06 (m, 1H), 1.75 (s, 1H), 1.52 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.0 Hz, 6H); 13C NMR 170.3, 156.5, 143.7, 142.8, 141.3, 128.6, 127.7, 127.4, 127.1, 126.1, 125.0, 120.0, 67.0, 60.5, 48.9, 47.1, 31.3, 21.7, 19.2, 17.9. Anal. Calcd for C28H30N2O3: C, 75.99; H, 6.83; N, 6.33; Found: C, 75.90; H, 6.99; N, 6.09.

(9H-Fluoren-9-yl)methyl S-3-(4-tert-butoxyphenyl)-1-oxo-1-((R)-1-phenylethylamino)propan-2-ylcarbamate (4.3b). White microcrystals (74%), mp 180.1-181.1, [α]D = +8.6 (c = 1.0, CHCl3); 1H NMR δ 7.81 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.45 (t, J = 6.9 Hz, 2H), 7.38-7.26 (m, 5H), 7.21-7.15 (m, 4H), 6.98 (d, J = 7.8 Hz, 2H), 5.76 (d, J = 5.1 Hz, 1H), 5.53 (d, J = 5.5 Hz, 1H), 5.04 (quintet, J = 6.9 Hz, 1H), 4.45-4.41 (m, 3H), 4.24 (t, J = 6.6 Hz, 1H), 3.18 (br s, 1H), 2.99-2.96 (m, 1H), 1.42-1.31 (m, 12H); 13C NMR 169.6, 154.5, 143.8, 143.7, 142.8, 141.3, 129.9, 128.7, 127.8, 127.5, 127.1, 126.0, 125.1, 124.4, 120.0, 78.5, 77.3, 67.0, 56.6, 49.0, 47.1, 38.5, 28.8, 21.6. Anal. Calcd for C36H40N2O5 .H2O: C, 74.46; H, 6.94; N, 4.82; Found: C, 74.49; H, 7.07; N, 4.58.

(9H-Fluoren-9-yl)methyl S-3-methyl-1-oxo-1-((S)-1-phenylethylamino)butan-2-ylcarbamate (4.4a). White powder (77%), mp 166.3-168.8 °C, [α]D = +22.1 (c = 2.2, CHCl3); 1H NMR δ 7.75 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.34-7.27 (m, 7H), 6.24 (d, J = 7.8 Hz, 1H), 5.44 (d, J = 8.7 Hz, 1H), 5.18-5.06 (m, 1H), 4.43-4.23 (m, 2H), 4.22-4.13 (m, 1H), 4.00-3.90 (m, 1H), 2.21-2.06 (m, 1H), 1.48 (d, J = 6.6 Hz, 3H), 0.97 (2 overlapped d, J = 7.7 Hz, 6H); 13C NMR 170.1, 169.9, 143.8, 142.6, 141.3, 128.7, 127.7, 127.4,
127.1, 126.1, 125.0, 119.9, 67.0, 60.5, 48.9, 47.1, 31.1, 21.7, 19.2, 18.0. Anal. Calcd for C_{28}H_{30}N_{2}O_{3}: C, 75.99; H, 6.83; N, 6.33; Found: C, 76.10; H, 7.01; N, 6.51.

(9H-fluoren-9-yl)methyl S-3-(4-tert-butoxyphenyl)-1-oxo-1-((S)-1-phenylethylamino)propan-2-ylcarbamate (4.4b). White powder (79%), mp 196.3-197.7 °C, [α]_{D}^{24} = -6.2 (c = 1.0, CHCl_{3}); ^{1}H NMR δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.33-7.22 (m, 5H) 7.12 (d, J = 7.2 Hz, 2H), 7.00-6.97 (m, 2H), 6.83 (d, J = 8.1 Hz, 2H), 5.88 (br s, 1H), 5.42 (br s, 1H), 5.06-5.01 (m, 1H), 4.45-4.35 (m, 2H), 4.23-4.13 (m, 1H), 3.11-3.00 (m, 1H), 2.98-2.86 (m, 1H), 1.91-1.82 (m, 1H), 1.39 (d, J = 6.9 Hz, 3H), 1.31 (s, 9H); ^{13}C NMR 169.9, 154.6, 143.9, 142.6, 141.5, 130.0, 128.8, 127.9, 127.6, 127.3, 126.3, 125.2, 124.5, 120.2, 78.6, 77.4, 67.2, 56.6, 49.2, 47.3, 38.3, 29.0, 21.7. Anal. Calcd for 2(C_{72}H_{78}N_{4}O_{9})•H_{2}O: C, 75.63; H, 6.88; N, 4.90; Found: C, 75.59; H, 6.82; N, 4.77.

(9H-fluoren-9-yl)methyl S-3-methyl-1-oxo-1-(1-phenylethylamino)butan-2-ylcarbamate (4.3a+4.4a). Yellow oil (74%); ^{1}H NMR δ 7.69 (d, J = 7.5 Hz, 2H), 7.52 (d, J = 7.2 Hz, 2H), 7.43 (d, J = 7.2 Hz, 1H), 7.34-7.12 (m, 21H), 6.01 (s, 0.46H), 5.15-5.05 (m, 1.58H), 3.94 (t, J = 6.5 Hz, 1H), 3.74 (q, J = 6.0 Hz, 1H), 3.16 (t, J = 3.0 Hz, 2H), 2.96 (dd, J = 12.0, 6.2 Hz, 1H), 2.82 (dd, J = 12.2, 6.9 Hz, 1H), 2.34-2.16 (m, 2H), 1.42 (d, J = 6.9 Hz, 6H), 1.24 (d, J = 6.6 Hz, 3H), 0.91 (t, J = 7.5 Hz, 6H), 0.78 (d, J = 6.9 Hz, 6H), 0.68 (d, J = 6.6 Hz, 3H); ^{13}C NMR 173.8, 173.7, 146.4, 146.4, 146.0, 144.0, 143.8, 141.6, 141.6, 129.1, 129.0, 129.0, 128.8, 127.6, 127.6, 127.5, 127.4, 127.2, 127.1, 126.6, 126.5, 125.0, 125.0, 121.4, 120.3, 120.2, 120.1, 60.4, 60.4, 58.6, 51.1, 48.5, 48.4. HRMS calcd for C_{28}H_{30}N_{2}O_{3} [M+H-CO_{2}]^{+} 399.2436, found 399.2393.

(9H-fluoren-9-yl)methyl S-3-(4-tert-butoxyphenyl)-1-oxo-1-(1-phenylethylamino)propan-2-ylcarbamate (4.3b+4.4b). Off-white powder (66%), mp 170.8-
176.8 °C; $^1$H NMR $\delta$ 7.76 (d, $J = 6.9$ Hz, 4H), 7.55 (d, $J = 6.9$ Hz, 4H), 7.40 (t, $J = 7.0$ Hz, 4H), 7.34-7.20 (m, 12H), 7.18-7.06 (m, 6H), 6.92 (d, $J = 7.8$ Hz, 2H), 6.83 (d, $J = 8.1$, 2H), 5.94 (br s, 1H), 5.74 (br s, 1H), 5.50 (br s, 2H), 5.30-4.92 (m, 2H), 4.46-4.26 (m, 6H), 4.22-4.12 (m, 2H), 3.22-2.78 (m, 4H), 1.33 (s, 18H), 1.32 (overlapped d, $J = 6.3$ Hz, 6H); $^{13}$C NMR 169.8, 169.6, 154.4, 154.4, 143.7, 143.7, 142.4, 142.4, 141.3, 129.9, 129.8, 128.6, 128.6, 127.7, 127.4, 127.4, 127.1, 126.1, 126.0, 125.0, 124.4, 124.3, 120.0, 78.5, 78.4, 77.2, 67.0, 56.6, 48.9, 47.1, 38.5, 38.1, 28.8, 21.5. HRMS calcd for $C_{36}H_{38}N_2O_4$ [M+H]$^+$ 563.2910, found 563.2904.
CHAPTER 5
MICROWAVE-ASSISTED SOLID PHASE PEPTIDE SYNTHESIS UTILIZING N-FMOC-PROTECTED(α-AMINOACYL)BENZOTRIAZOLES

5.1 Introduction

Peptides and proteins are ubiquitous and essential to all cellular processes (for example, cell division, biochemical control, storage and transport, etc.). [97MI1, 02MI2] To solve biological problems, it is of interest to understand the function of peptides and proteins in such processes. Moreover, peptides and peptide-related drugs are widely used in the medicine, hence the constant demand for new and improved approaches directed toward peptide and protein syntheses. [97MI1, 02MI2]

Peptides and proteins are constructed via the sequential coupling of amino acid residues, with peptides containing fewer amino acid residues when compared to proteins. The groundbreaking synthesis of the first peptides was achieved by Fischer and Curtius over a century ago. However in the last thirty years, Bruce Merrifield further revolutionized peptide synthesis by the invention of the solid phase peptide synthesis (SPPS) technique. [97MI1, 02MI2] Despite major advances in peptide synthesis, the simple and efficient preparation of many peptides, especially the so called “difficult” sequences remains a challenge. [97MI1, 02MI2]

Compared with classical solution synthesis, SPPS, a simple and rapid technique, offers greater ease of separation of the products from the reagents, with the elimination of inherent product losses associated with conventional chemistries (filtration, recrystallization, etc.); SPPS also utilizes an excess of reagents that promotes the rapid completion of the coupling reaction. The increased efficiency of SPPS when compared to conventional solution phase methodologies has resulted in its near-exclusive use for the preparation of peptides and in turn led to better coupling reagents, shorter coupling times, and better yields over the last three decades. [95JACS5401, 06CEJC285, 04JMC5662, 05JMC3060]
Microwave heating can enhance the rate of a variety of reactions including the solid phase assembly of peptides. Although this area is still relatively unexplored, a search of the current literature disclosed faster coupling times and improved peptide yields with the incorporation of microwave heating. [02S1592, 05OL1521, 92JOC4781, 06JOC3051]

Acylbenzotriazoles are easily prepared, chirally stable, nonhydroscopic synthetic equivalents of acid halides. [02ARK134, 05ARK116, 07JOC407, 06S411] Recently, Katritzky and coworkers reported that the solution phase peptide coupling reactions of N-protected-(α-aminoacyl)benzotriazoles with unprotected amino acids proceed with minimal epimerization in partially aqueous media under mild conditions. [05S397] Additionally, the preparation of di-, tri- and tetrapeptides, [06S411, 05S397, 04S2645] the C-acylation of activated heterocycles, [05JOC4993] as well as the O-aminoacylation of hydroxysteroids [06ST660] and terpenes [06S4135] can be achieved using N-protected-(α-aminoacyl)benzotriazoles.

Now, the convenient microwave-enhanced solid phase syntheses of simple tetra-, penta-, and hexapeptides using N-Fmoc-(α-aminoacyl)benzotriazoles [07CBDD465] is reported.

5.2 Results and Discussion

5.2.1 Preparation of N-Fmoc-(α-aminoacyl)benzotriazoles (5.2a-g)

N-Fmoc-(α-aminoacyl)benzotriazoles 5.2a-g were prepared in yields of 60-90% from the reaction of Nα-Fmoc-protected amino acids 5.1a-g (purchased from Peptides International and used without further purification) with precomplexed 1H-benzotriazole (4.0 equiv) and SOCl₂ (1.0 equiv) in THF at 20°C for 2 h following the published procedure (Scheme 5-1, Table 5-1). The original chirality was preserved in all cases (>95% as evidenced by NMR comparison of the diastereomers and the corresponding diastereomeric mixture). [05S397, 05JOC4993, 09ARK47]
Scheme 5-1. Preparation of N-Fmoc-protected (α-aminoacyl)benzotriazoles 5.2a–g

Table 5-1. N-Fmoc-protected (α-aminoacyl)benzotriazoles 5.2a–g utilized for peptide synthesis

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound 2</th>
<th>mp (°C)</th>
<th>Lit. mp (°C) [ref.]</th>
<th>[α]_D^23</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fmoc-L-Ala-Bt (5.2a)</td>
<td>160.0-160.3</td>
<td>160-161 [06S4135]</td>
<td>-60.8^b</td>
</tr>
<tr>
<td>2</td>
<td>Fmoc-L-Trp-Bt (5.2b)</td>
<td>92.5-93.6</td>
<td>88-90 [06S411]</td>
<td>+9.0^b</td>
</tr>
<tr>
<td>3</td>
<td>Fmoc-L-Met-Bt (5.2c)</td>
<td>122.7-123.3</td>
<td>98-100 [06S411]</td>
<td>-44.7^b</td>
</tr>
<tr>
<td>4</td>
<td>Fmoc-L-Pro-Bt (5.2d)</td>
<td>163.0-165.0</td>
<td>163.5-165.4 [09ARK47]</td>
<td>-60.0^b</td>
</tr>
<tr>
<td>5</td>
<td>Fmoc-L-Phe-Bt (5.2e)</td>
<td>159.1-160.2</td>
<td>136-137 [06S4135]</td>
<td>+3.4^b</td>
</tr>
<tr>
<td>6</td>
<td>Fmoc-Gly-Bt (5.2f)</td>
<td>160.9-161.5</td>
<td>161.5-161.9 [09ARK47]</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Fmoc-L-Leu-Bt (5.2g)</td>
<td>121.0-122.8</td>
<td>121.3-123.2 [09ARK47]</td>
<td>+50.2</td>
</tr>
</tbody>
</table>

^a Isolated yields; ^b Lit. Optical rotation 5.2a [06S4135]: [α]_D^23 = -96.8 (c 1.6, DMF), 5.2b [06S411]: [α]_D^25 = +12.7 (c 1.5, DMF), 5.2c [06S411]: [α]_D^25 = -75.1 (c 1.5, DMF), 5.2d [06S4135]: [α]_D^24 = -60.5 (c 1.5, DMF), 5.2e [06S4135]: [α]_D^24 = +35.6 (c 1.6, DMF), 5.2g [06S4135]: [α]_D^24 = +53.1 (c 1.5, DMF)

5.2.2 Peptide Syntheses

Standard Fmoc SPPS was performed manually in a 25 mL Discover SPS (solid phase synthesis) reaction vessel. The peptides 5.1-5.3, were prepared on Rink amide 4-methylbenzhydrylamine (MBHA) resin (0.1 mmol). Following the swelling of the resin in DCM (4 mL, 0.5 h) and treatment with 20% piperidine-DMF for 20 min. to afford the free-base amide resin, a DMF-DCM (5:1 ca 3 mL) solution of the appropriate N-Fmoc-(α-aminoacyl)-benzotriazole (0.5 mmol) and the resin were combined and coupled using microwave irradiation (75 °C, 80 W, 15 min). The completion of the coupling reaction was monitored by the ninhydrin (Kaiser) test. Successive N-Fmoc-(α-aminoacyl)benzotriazoles were coupled to the growing peptide in this manner. Deprotection of the N-Fmoc-(α-aminoacyl)benzotriazole was achieved with 20% piperidine-DMF. Finally, the resulting peptidyl resin was cleaved with cleavage cocktail B (88% TFA/ 5% phenol/ 5% water/ 2% TIPS), K (82.5% TFA/ 5% phenol/ 5% water/
5% thioanisole/5% EDT) or L (88% TFA/5% DTT/5% water/2% TIPS) for 1.5-2.0 h to afford the crude peptides 5.3-5.5.

Table 5-2. Synthesis of peptides 5.3-5.4

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure (C-N terminus)</th>
<th>Crude Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Crude Yield (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>After HPLC separation Purity (%)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Purity (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Retention time, t&lt;sub&gt;R&lt;/sub&gt;(min)</th>
<th>HRMS [M+H]&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-Trp-Met-Trp-Pro (5.3)</td>
<td>73</td>
<td>39</td>
<td>&gt;95</td>
<td>82</td>
<td>14.79</td>
<td>618.2502</td>
</tr>
<tr>
<td>2</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-Ala-Phe-Gly-Met-Leu (5.4)</td>
<td>68</td>
<td>24</td>
<td>&gt;99</td>
<td>92</td>
<td>11.67</td>
<td>537.2626</td>
</tr>
<tr>
<td>3</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-Ala-Phe-Gly-Met-Leu-Pro (5.5)</td>
<td>73</td>
<td>31</td>
<td>&gt;99</td>
<td>54</td>
<td>12.42</td>
<td>634.3678</td>
</tr>
</tbody>
</table>

<sup>a</sup> Samples were weighed after precipitation of the cleaving mixture, <sup>b</sup> Estimated from the crude peptide HPLC trace, <sup>c</sup> Samples were weighed after HPLC purification, <sup>d</sup> Purity after HPLC purification.

The preparation of peptides 5.3-5.5 was straightforward. In the literature the thioether side-chain of Met has reportedly been accompanied by alkylation and oxidation side reactions, either during the synthetic process or during subsequent handling of the Met-containing peptides.[00MI3] However, no oxidation product was observed during the preparation of Met-containing peptides 5.3-5.5, although other works reported relatively easy partial oxidation of Met to its sulphone upon prolonged exposure to air.[87JACS620] Characterization of the purified compounds 5.3-5.5 by HPLC revealed complete retention of configuration (see 5.4 Experimental Section).

5.3 Conclusion

A plethora of coupling reagents is widely available for SPPS. Often, when the more conventional carbodiimide-based methods are employed long coupling times are required. Onium- and aminium-based coupling reagents react rapidly, but are costly. N-Fmoc-(α-aminoacyl)benzotriazoles are easily accessed, cheap, chirally pure reagents and were demonstrated to be useful alternatives for both solid and solution phase peptide syntheses. As
summarized in Table 5-2, application of benzotriazole methodology in SPPS afforded peptides 5.3-5.5 in crude yields of 68-73%.

5.4 Experimental Section

Analytical reversed-phase HPLC was performed on a Rainin HPXL system with a Vydac C-18 (5 μm. 2.1 x 250 mm) silica column at a 1mL/min flow rate. Peptides were eluted using a 10-80% gradient of solvent B (0.1% TFA in acetonitrile) vs. solvent A (0.1% TFA in water) and the peaks were detected at λ 214 nm. The identification of the products was achieved by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF, ABI 4700 Proteomics Analyzer) with a α-cyano-4-hydroxy cinnamic acid matrix.

MS/MS peptide fragmentation was obtained on the crude peptides by way of low resolution MS and tandem mass spectrometry (MSn) data obtained using an Agilent (Palo Alto, CA) 1100 series HPLC equipped with a Phenomenex Synergi 4u Hydro-RP 80A (2 x 150 mm, 4 μm) column plus a C18 guard column (2 mm x 4 mm). Mass Analysis was performed using a ThermoFinnigan-LCQ ion trap mass spectrometer (San Jose, CA) in electrospray ionization (ESI) mode.

The peptides yielded abundant [M+H]+ and [M+Na]+ ions under the (+)ESI-MS conditions used here. With (+)ESI-MS/MS and MSn of each peptide’s [M+H]+ ion, dissociation of the peptide proceeds, in general, due to fragmentation at the amide bonds as indicated below to yield a series of y and b ions which then form z and a ions, respectively.
Figure 5-1. General Peptide Fragmentation
Figure 5-2. The HPLC profile of peptide 5.3 (Pro-Trp-Met-Trp-NH₂): a) after purification and b) crude

Figure 5-3. Expected product ions from the (+)ESI-MSn of the m/z 618 [M+H]^+ ion. The shaded ions were observed in the spectra.
Figure 5-4. The HPLC profile of peptide 5.4 (Leu-Met-Gly-Phe-Ala-NH₂): a) after purification and b) crude.

<table>
<thead>
<tr>
<th>L-M-G-F-A(NH₂)</th>
<th>MW = 536.3</th>
<th>[M+H]⁺ = 537.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1-ions (loss of CO)</td>
<td>86.1</td>
<td>217.1</td>
</tr>
<tr>
<td>b-ions</td>
<td>N-term</td>
<td>114.1</td>
</tr>
<tr>
<td>Residue</td>
<td>H</td>
<td>L</td>
</tr>
<tr>
<td>Residue mass</td>
<td>1.0</td>
<td>113.1</td>
</tr>
<tr>
<td>y-ions</td>
<td>537.3</td>
<td>424.2</td>
</tr>
<tr>
<td>Loss of NH₃</td>
<td>520.3</td>
<td>407.2</td>
</tr>
</tbody>
</table>

Figure 5-5. Expected product ions from the (+)ESI-MSn of the m/z 537 [M+H]⁺ ion. The shaded ions were observed in the spectra.
Figure 5-6. The profile of peptide 5.5 (Pro-Leu-Met-Gly-Phe-Ala-NH₂): a) after purification b) crude

<table>
<thead>
<tr>
<th>Peptide</th>
<th>MW</th>
<th>[M+H]^+</th>
<th>Residue mass</th>
<th>Residue</th>
<th>a1-Ions (loss of CO)</th>
<th>b-Ions</th>
<th>y-Ions</th>
<th>Loss of NH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-L-M-G-F-A(NH₂)</td>
<td>633.3</td>
<td>634.4</td>
<td>1.0</td>
<td>H</td>
<td>70.1</td>
<td>589.3</td>
<td>634.4</td>
<td>617.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>183.1</td>
<td>556.3</td>
<td>537.3</td>
<td>520.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L</td>
<td>314.2</td>
<td>518.3</td>
<td>424.2</td>
<td>407.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>371.2</td>
<td>399.2</td>
<td>293.2</td>
<td>276.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G</td>
<td>518.3</td>
<td>546.3</td>
<td>236.2</td>
<td>219.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>634.4</td>
<td>617.3</td>
<td>89.1</td>
<td>72.1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>556.3</td>
<td>520.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-NH₂</td>
<td>518.3</td>
<td>546.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5-7. Expected product ions from the (+)ESI-MSn of the m/z 634 [M+H]^+ ion. The shaded ions were observed in the spectra.
CHAPTER 6
BENZOTRIAZOLE-ASSISTED SOLID-PHASE ASSEMBLY OF LEU-ENKEPHALIN, AMYLOID-\(\beta\) SEGMENT 34-42, AND OTHER "DIFFICULT" PEPTIDE SEQUENCES

6.1 Introduction

Solution phase and especially the solid phase peptide synthesis (SPPS) of potentially bioactive peptides is of great interest. [00MI3, 97MI1] While SPPS has enabled major advances in scope, yields, purities, and reaction times, [00MI3, 88JCS2895] SPPS [63JACS2149, 86S341] has also encountered “difficult” peptide sequences when incomplete aminoacylation and/or deprotection reactions at various stages in the synthetic scheme [88ARB957, 90JACS6039, 94IJPPR431, 95JACS12058] have resulted in low yields and purities [00MI3]. Such difficulties can arise in attempting to form a peptide link due (i) to steric effects when both amino acid units possess \(\beta\)-branched side chains (e.g. valine, isoleucine and threonine) [90JACS6039] and (ii) as a result of the formation of secondary structures by intra- and interchain hydrogen-bonded associations [88ARB957, 90JACS6039, 94IJPPR431, 95JACS12058]. In such cases, the synthesized peptides can be partly racemized and/or adulterated with deletion sequences or form aspartimides and related side products [00MI3, 03JPS518]

Some of the problems associated with these difficult sequences in Fmoc-based SPPS have been alleviated by the use of (i) bases such as DBU and piperazine (less nucleophilic than the conventional piperidine) to suppress racemization and to reduce aspartimide formation [03JPS518, 07JPS143]; (ii) chemical ligation techniques, for example the ‘\(O\)-Acyl isopeptide method’ which can significantly reduce isomerization of the peptide backbone [04CC124, 06TL3013]; (iii) microwave acceleration of the deprotection and coupling steps [07JPS143, 07CBDD465], which decreases racemization as the growing peptide has less time available for \(\alpha\)-carbon epimerization.
The formation of aspartimides and related side products remains a problem in SPPS. Backbone protection using the 2,4-dimethoxybenzyl (Dmb), 2-hydroxy-4-methoxybenzyl (Hmb), 2,4,6-trimethoxybenzyl (Tmb) or 2-nitrobenzyl (Nbzl) groups [95TL7523, 95JACS11656, 00JOC5460] can help, but requires additional steps.

Recently, N-Fmoc-(α-aminoacyl)benzotriazoles of proteinogenic amino acids have been utilized in the syntheses of tri- to heptapeptides in crude yields of 65-77% on the Rink amide MBHA solid support. [07CBDD465] The microwave-assisted syntheses of six short “difficult” α-peptide sequences is now described in attempts to examine the extent of racemization, incomplete aminoacylation/ deprotection reactions and the formation of aspartimides when N-Fmoc-(α-aminoacyl)benzotriazoles are used as activating reagents for SPPS.

6.2 Results and Discussion

N-Fmoc-(α-aminoacyl)benzotriazoles 6.2a-l (76-91%) were prepared as previously described [09ARK47] by treatment of purchased Fmoc-L-protected amino acids 6.1a-l with 4 equivalents of benzotriazole and 1 equivalent of SOCl₂ in THF at room temperature for 2 hours (Scheme 6-1, Table 6-1).

![Scheme 6-1. Preparation of N-Fmoc-(α-aminoacyl)benzotriazoles](image)

A standard SPPS approach was employed in the syntheses of the “difficult” peptides (6.3-6.10), in which the appropriate N-Fmoc-(α-aminoacyl)benzotriazole (2) was coupled in turn to the growing peptide (Scheme 6-2). Subsequent cleavage [TBAB] from the Rink amide MBHA resin and purification of the crude peptide provided the desired product.
Table 6-1. Preparation of N-Fmoc-(α-aminoacyl)benzotriazoles (6.2) from the corresponding Fmoc-protected-α-amino acids (6.1)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Yield (a) (%)</th>
<th>mp (°C)</th>
<th>Lit mp (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fmoc-L-Val-Bt (6.2a)</td>
<td>84</td>
<td>151.9-152.6</td>
<td>148.3-149.8</td>
<td>09ARK47</td>
</tr>
<tr>
<td>2</td>
<td>Fmoc-L-Thr(tBu)-Bt (6.2b)</td>
<td>80</td>
<td>64.6-66.8</td>
<td>62.2-65.0</td>
<td>09ARK47</td>
</tr>
<tr>
<td>3</td>
<td>Fmoc-L-Ser(tBu)-Bt (6.2c)</td>
<td>78</td>
<td>63.8-65.4</td>
<td>91.7-92.4</td>
<td>b</td>
</tr>
<tr>
<td>4</td>
<td>Fmoc-L-Tyr(tBu)-Bt (6.2d)</td>
<td>84</td>
<td>99.0-100.5</td>
<td>138.4-139.3</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>Fmoc-L-Ile-Bt (2e)</td>
<td>78</td>
<td>165.4-167.2</td>
<td>168.8-170.0</td>
<td>09ARK47</td>
</tr>
<tr>
<td>6</td>
<td>Fmoc-L-Lys(Boc)-Bt (6.2f)</td>
<td>78</td>
<td>138.2-141.7</td>
<td>138.4-140.6</td>
<td>09ARK47</td>
</tr>
<tr>
<td>7</td>
<td>Fmoc-Gly-Bt (6.2g)</td>
<td>76</td>
<td>161.8-163.3</td>
<td>161.5-161.8</td>
<td>09ARK47</td>
</tr>
<tr>
<td>8</td>
<td>Fmoc-L-Asp(OtBu)-Bt (6.2h)</td>
<td>81</td>
<td>102.1-104.3</td>
<td>102.0-104.0</td>
<td>09ARK47</td>
</tr>
<tr>
<td>9</td>
<td>Fmoc-L-Phe-Bt (6.2i)</td>
<td>78</td>
<td>157.0-158.3</td>
<td>159.1-160.2</td>
<td>09ARK47</td>
</tr>
<tr>
<td>10</td>
<td>Fmoc-L-Leu-Bt (6.2j)</td>
<td>87</td>
<td>75.3-78.6</td>
<td>121.3-123.2</td>
<td>b</td>
</tr>
<tr>
<td>11</td>
<td>Fmoc-L-Met-Bt (6.2k)</td>
<td>91</td>
<td>129.1-131.8</td>
<td>122.7-123.3</td>
<td>09ARK47</td>
</tr>
<tr>
<td>12</td>
<td>Fmoc-L-Ala-Bt (6.2l)</td>
<td>88</td>
<td>160.5-161.8</td>
<td>160.0-160.3</td>
<td>09ARK47</td>
</tr>
</tbody>
</table>

(a) Isolated. (b) See 6.5 Experimental Section for characterization of the polymorphs 6.2c,d,j

Scheme 6-2. SPPS approach using the Rink amide MBHA resin and N-Fmoc-(α-aminoacyl)benzotriazoles

β-Hydroxy-α-amino acids, such as serine, 3-hydroxyproline, threonine, and certain analogues (for example, β-hydroxyphenylalanine and β-hydroxytyrosine) are widely distributed as components of biologically active natural products. [06JOC7106, 07TA1667, 00JOC7663, 97JACS11734] The synthesis of peptides containing β-hydroxy-α-amino acids can be challenging due i) to the risk of racemization of such residues during both the stepwise and convergent approaches to Fmoc SPPS [06TL3013, 98TL8529] and ii) because of aggregation,
which may commence as early as the addition of the fifth amino acid residue in certain β-
hydroxy-α-amino acid containing sequences [06TL3013, 98TL8529]. Hydrophobic and branched
chain amino acids (BCAAs), such as valine, isoleucine and leucine, promote aggregation during
peptide synthesis and purification, particularly when a large percentage of such hydrophobic
residues is present.

In the literature the effect of the amino acid hydrophobicity is evident in the DIPCDI-
HOBt (1,3-diisopropylcarbodiimide-hydroxybenzotriazole) stepwise SPPS of H-Val-Val-Ser-
Val-Val-NH₂ (3) [04CC124, 06TL3013] where the undesired N-protected peptide amide, Fmoc-
Val-Val-Ser-Val-Val-NH₂ was produced as the major compound (1.1 fold higher than the
desired peptide 6.3 as evidenced by HPLC [04CC124]). In the presently reported work, using N-
Fmoc-(α-aminoacyl)benzotriazoles, peptide 6.3 was obtained as the major product (Table 6-2,
Figure 6-1; Figure 6-13, 6.5 Experimental Section) with no evidence of the undesired Fmoc-Val-
Val-Ser-Val-Val-NH₂ peptide or any racemized product. A possible explanation for the absence
of micro-aggregates in the benzotriazole-assisted synthesis of 6.3 when compared to the
DIPCDI-HOBt route [04CC124, 06TL3013] could be the impact of microwave irradiation on the
environment of the growing peptide. In the present work, the alteration of the microenvironment
by microwave irradiation may hinder the formation of insoluble micro-aggregates and facilitate
the removal of the Fmoc groups from the resin bound peptide 3. [07JPS143] Additionally, our
microwave-assisted stepwise protocol reduced the total coupling time for the synthesis of 6.3
from 10 hours [04CC124] to 2.5 hours (open vessel; 1.5 hours when closed vessel conditions are
applied) and improved the overall yield from 1.4% [04CC124] to 7%. While using the O-acyl
isopeptide chemical ligation technique to synthesize 6.3 may provide a peak overall yield of 28%
(extrapolated from the yield of the $O$-acyl isopeptide of 6.3), this requires a coupling time of 40 h. [06TL3013]
Next synthesized peptide 6.5 (Table 6-2; Figures 6-19 and 6-20, 6.5 Experimental Section) possessing 80% BCAAs and with a hydrophobicity similar to 6.3, was isolated in 28% yield, with no evidence of interference by aggregation (Figures 6-19 and 6-22, 6.5 Experimental Section; during purification the peptide amide was hydrolyzed to the corresponding acid).

Previously, the Boc-based SPPS strategy along with the DCC/HOBt active ester coupling method was applied in the synthesis of 6.5, while our present work utilized the milder Fmoc-based strategy along with N-(Fmoc-protected-α-aminoacyl)benzotriazoles. A comparison of the yield of 6.5 via the literature Boc-based SPPS with our yield by the Fmoc-based benzotriazole-assisted strategy could not be calculated from the information provided by the previous authors.

[01JPS641]
Hexapeptide 6.6 contains only $\beta$-branched side chains and is less hydrophobic than 6.3 and 6.4. Peptide 6.6 (Table 6-2; Figures 6-23, 6-24 and 6-25, 6.5 Experimental Section) was prepared by six successive coupling with (i) Fmoc-Val-Bt, (ii) Fmoc-Thr(tBu)-Bt, (iii) Fmoc-Val-Bt, (iv) Fmoc-Thr(tBu)-Bt, (v) Fmoc-Val-Bt (vi) Fmoc-Thr(tBu)-Bt. The linear geometry of 6.6 can be attributed to destabilizing steric effects and the restricted rotational freedom of peptides containing only $\beta$-branched amino acids. [94B12022] Peptides, such as 6.6 could be useful building blocks in tests for the stabilizing or destabilizing effect of BCAAs in peptide and protein $\alpha$-helix formation. [94B12022, 04JOC8804] The nearest literature comparison appears to be the synthesis by Jolliffe et al. [04JOC8804] of the TBS-protected linear peptide amide (Val-Thr)$_3$ as an intermediate in the formation of cyclo (Val-Thr)$_3$; again no direct comparison of yields is possible because of lack of data in the literature reference. [04JOC8804]

In the SPPS of peptides containing asparagine or aspartic acid, aspartimides are frequently formed. [00MI3, 03JPS518, 00LIPS107] Aspartimide formation can occur under both acidic and basic conditions. [00MI1] In base-catalyzed aspartimide formation the proportion of aspartimide side products attained depends on the base used for removal of the Fmoc group [03JPS518], the nature of the preceding amino acid residue [03JPS518, 00LIPS107] and to a lesser extent, the protecting group on the aspartyl residue [03JPS518, 00LIPS107]. Next hexapeptide 6.7 [03JPS518, 06TL4121] was examined; structure 6.7 is the peptide fragment 1-6 of toxin II from the scorpion *Androctonus australis* Hector [87T5961]) and contains the Asp-Gly fragment. Syntheses of compound 6.7 (Table 6-2; Figures 6-26 and 6-27, 6.5 Experimental Section) and analogue 6.8 [03JPS518] (Table 6-2; Figures 6-29 and 6-30, 6.5 Experimental Section) containing Asp-Val serve to test the tendency for aspartimide formation using $N$-Fmoc-(\(\alpha\)-aminoacyl)benzotriazoles. As anticipated, this synthesis of peptide amide 6.7 (\(m/z\) 735.4419, $t_R$...
8.31 min; Figures 6-26 and 6-28, 6.5 Experimental Section) did produce the corresponding aspartimide \((m/z 675.3808, t_R 8.11 \text{ min})\); Figures 6-26 and 6-28, 6.5 Experimental Section) as a by product. However, the presently described synthesis of peptide amide 6.8 (Table 6-2; Figures 6-29 and 6-31, 6.5 Experimental Section) proceeded without any of the analogous aspartimide by product, the formation of which was evidently suppressed by replacement of the glycine residue with valine. For both 6.7 and 6.8 5% piperazine-DMF (and not 20% piperidine-DMF) was used for the removal of the Fmoc group and this eliminated ring opening of the aspartimides to the corresponding piperidides.

Although 7 was used as a test peptide by four sets of authors [03JPS518, 00LIPS107, 06TL4121, 87T5961], no comparison of the yield from this benzotriazole-assisted synthesis with the literature can be made because each literature case reported only product purity and no yield was provided. [03JPS518, 00LIPS107, 06TL4121, 87T5961] Again, for 6.8, only the product purity was provided in the literature [03JPS518], making a yield comparison impossible.

6.3 Preparation of Leu-Enkephalin (9) and Amyloid-β Segment (34-42) (10)

Two biologically important “difficult” peptides 6.9 [00OL1815] and 6.10 [04PPL377] were prepared by the currently described microwave-enhanced and benzotriazole-mediated methodology. Leu-enkephalin (6.9) (Table 6-2, Figure 6-2) is a natural peptide neurotransmitter and a powerful painkiller. For 6.9 no direct yield comparison with the literature [00OL1815] can be made; as no literature yield is provided.

Alzheimer’s disease (AD) is slowly progressive and is characterized by dementia. Intracellular β-amyloid aggregates are regularly associated with Alzheimer’s disease (AD). Formation of these aggregates heralds the formation of insoluble plaque, the principal biological marker indicating the development and progression of AD. The formation of the aggregates observed in an AD afflicted individual is due to the hydrophobic interaction between the core
hydrophobic amino acid residues in the polypeptide. The preparation of hydrophobic segment (34-42) \(6.10\) of \(\beta\)-amyloid (Table 6-2) was previously described by Halverson [90B2639] and coworkers who used Kaiser oxime resin with the alanine residue already attached; they then coupled \(N\)-Boc-protected amino acids eight times to assemble \(6.10\), stating that the solubilization was “extremely difficult” and recovery subsequent to HPLC analysis was “extremely sensitive” but claiming a 40% yield of material that was “quite susceptible” to oxidation.

Figure 6-2. HPLC profiles of a) crude and b) pure peptide \(6.9\) obtained after SPPS using 20% piperidine-DMF for Fmoc cleavage.

### 6.4 Conclusions and Directions

#### 6.4.1 Conclusions

As summarized in Table 6-2, benzotriazole-assisted solid phase assembly affords difficult peptides in crude yields of 34-86%. These benzotriazole-assisted syntheses, in tandem with microwave acceleration have the following advantages over the previously used carbodiimide based coupling methods: (i) no base is required for the coupling reactions, (ii) the conditions are comparatively mild, (iii) the reactions are more rapid and (iv) importantly the products are chirally homogeneous.

1-Hydroxybenzotriazole hydrate (HOBr) is a widely used coupling additive in peptide synthesis that suppresses racemization when used in combination with carbodiimides such as
DCC. Other 1-hydroxybenzotriazole derivatives, for example, 1-hydroxy-7-azabenzotriazole (HOAt), 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt), phosphonium and aminium salts of hydroxybenzotriazoles are also used as additives. Recently, the availability of HOAt has decreased due to the propensity for an explosion during transportation. [05JHM1] Thus, this shows the efficacy of N-Fmoc-(α-aminoacyl)benzotriazoles in peptide synthesis.

There is a present and growing need for efficient synthetic methods for the assembly of proteins and peptides needed to study diverse systems in the body and/or develop a cure for neurodegenerative diseases such as AD. N-Fmoc-(α-aminoacyl)benzotriazoles were demonstrated to be useful for the solid phase assembly of “difficult” peptides, and represent viable alternatives as SPPS reagents.

6.4.2 Directions

In order to access longer chain peptides with the benzotriazole-mediated strategy, aggregation of the growing peptide must be controlled. One approach is to apply backbone amide protecting groups, such as 2-hydroxy-4-methoxybenzyl (Hmb), to disrupt hydrogen-bonded associations. This would permit better solvation of the peptide chain and lead to more efficient coupling and deprotection steps. Investigations into the preparation of N-Hmb,N-Fmoc-dipeptidoylbenzotriazole derivatives for use in SPPS are underway.

6.5 Experimental Section

Reagents were obtained as follows: N-α-fluorenylmethoxycarbonyl amino acids, and the Rink amide MBHA resin (substitution 0.43 meq/g) from Peptides International, Louisville, KY, USA; the Ninhydrin test kit from AnaSpec, San Jose, CA, USA; N,N-dimethylformamide (DMF), dichloromethane (DCM) and trifluoroacetic acid (TFA) from Fischer Scientific, Fair Lawn, NJ, USA; triisopropylsilane (TIS) and piperidine from Sigma Aldrich, St. Louis, MO; piperazine from Acros Organics, NJ, USA. A Discover® Benchmate, upgraded for peptide
synthesis, 10 mL vials and 25 mL Discover solid phase synthesis (SPS) reaction vessels from CEM Corporation, Matthews, NC, USA were used for manual SPS. For SPS, the set temperature was monitored using an internal fiber optic probe.

Melting points were determined on a hot-stage apparatus and are uncorrected. NMR spectra were recorded in CDCl₃ using TMS as the internal standard for ¹H NMR (300 MHz) and the solvent, CDCl₃, as the internal standard for ¹³C NMR (75 MHz).

Analytical reversed-phase HPLC was performed on a Rainin HPXL system, equipped with a Vydac C-18 (5 μm, 2.1 x 250 mm) silica column, and using a flow rate of 1.0 mL/min. Peptides were eluted using a 10-80% gradient of solvent B (0.1% TFA in acetonitrile) versus solvent A (0.1% TFA in water) and peaks were detected at 214 nm. The identification of the products was achieved by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF, ABI 4700 Proteomics Analyzer) with α-cyano-4-hydroxy cinnamic acid as the matrix.

MS/MS peptide fragmentation was obtained on the peptides. Tandem mass spectrometry (MSn) via HPLC-UV/(+)ESI-MS and -MSn was acquired using a ThermoFinnigan (San Jose, CA) LCQ ion trap mass spectrometer in electrospray ionization (ESI) mode at a wavelength of 220 nm. High resolution mass spectrometry (HRMS) via flow-injection positive [(+)ESI]-time of flight (TOF) was obtained on an Agilent 1200 series spectrometer.

6.5.1 Solid Phase Protocol for the Preparation of Peptides 3-10

Standard stepwise solid phase synthesis was performed manually in a 25 mL Discover SPS reaction vessel. Peptides 6.3-6.10, were each prepared on Rink amide MBHA resin. After swelling the resin (0.1 mM) in DCM (4 mL, 0.5 h) and treatment with 20% piperidine-DMF (ca. 3 mL) for 20 min, the free-base amide resin was filtered off, washed with DMF (5 mL x 3) and DCM (5 mL x 3), dried and a DMF-DCM (5:1 ca. 3 mL) solution of the appropriate N-Fmoc-(α-
aminoacyl)benzotriazole (0.5 mM) was added. The coupling was induced using microwave irradiation (70-75 °C, 70-80 W, 10-30 min) and the completion of the coupling reaction was assessed by a negative ninhydrin (Kaiser) test. Successive N-Fmoc-(α-aminoacyl)benzotriazole were similarly coupled to the growing peptide. Deprotection of the N-Fmoc-(α-aminoacyl)benzotriazole was achieved at each stage with 20% piperidine-DMF or 5% piperazine-DMF. Finally, the resulting peptidyl resin was cleaved with cleavage cocktail B [TBAB] (88% TFA/ 5 % phenol/ 5% water/2% TIPS), K [TBAB] (82.5% TFA/ 5% phenol/ 5% water/ 5% thioanisole/ 5% EDT) or L [TBAB] (88% TFA/ 5% DTT/ 5% water/ 2% TIPS) for 1.5-2.0 h. Following cleavage, the peptide was precipitated with cold diethyl ether, the ether-peptide mixture incubated for 24 h at 4 °C and lyophilized to afford the crude peptides 6.3-6.10.

6.5.2 Parameters for Microwave Reactions

Microwave experiments were performed using single mode irradiation in pulsed temperature control mode (SPS mode). Open vessel reactions were performed in 25 mL Discover SPS reaction vessels as previously described (See Experimental). In addition to open vessel conditions, closed vessel syntheses were performed in a capped 10 mL vial for 3-9. Similar results were obtained under both conditions, however, closed vessel reaction were marginally faster than the corresponding open vessel reaction. Average coupling times per N-Fmoc-(α-aminoacyl)benzotriazole under open vessel conditions were 12-15 min, when compared to closed vessel reactions which were complete in ~ 10 min. Most N-Fmoc-α-aminoacylbenzotriazole produced a negative ninhydrin (Kaiser) test after the 10-15 min coupling time; however, double coupling was frequently required for Fmoc-L-Val-Bt (2a).

The characterization data presented for peptides 3-10 are for open vessel SPS, unless indicated.
6.5.3 Peptide Analysis

Analyses of the peptides 6.3-6.10 (Table 2) were carried out on an Agilent (Palo Alto, CA) 1100 series HPLC equipped with a Phenomenex Synergi 4u Hydro-RP 80A (2 x 150mm, 4 μm) column plus a C18 guard column (2 mm x 4 mm). Mass Analysis was performed using a ThermoFinnigan-LCQ ion trap mass spectrometer (San Jose, CA) in electrospray ionization (ESI) mode.

6.5.4 Characterization of Polymorphic Compounds 2c,d,j and 3-10

Full characterization data for N-Fmoc-(α-aminoacyl)benzotriazoles 2a,b,e-i,k,l are reported in the literature. [09ARK47]

*S-(9H-Fluoren-9-yl)methyl1-(1H-benzotriazol-1-yl)-3-tert-butoxy-1-oxopropan-2-ylcarbamate (Fmoc-L-Ser(tBu)-Bt, 6.2c). Recrystallized from EtOAc-hexanes to give white crystals (78%); mp 63.8-65.4 °C; ¹H NMR δ 8.31 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 7.8 Hz, 2H), 7.72-7.64 (m, 3H), 7.55 (t, J = 7.7 Hz, 1H), 7.42 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H) 5.99 (d, J = 9.0 Hz, 1H), 5.90-5.84 (m, 1H), 4.50-4.48 (m, 2H), 4.31-4.26 (overlapped m, 1H), 4.24 (overlapped dd, J = 10.0 Hz, 3.0 Hz, 1H), 3.91 (dd, J = 9.8 Hz, 3.2 Hz, 1H), 1.03 (s, 9H); ¹³C NMR δ 169.7, 156.6, 146.2, 144.3, 144.1, 141.7, 131.6, 131.2, 128.1, 127.5, 126.9, 125.6, 125.5, 120.7, 120.4, 114.8, 74.4, 67.8, 63.2, 56.2, 47.5, 37.8, 27.7, 27.5. Anal. Calcd. for C₂₈H₂₈N₄O₄: C, 69.40; H, 5.82; N, 11.56. Found: C, 69.00; H, 5.86; N, 11.33.

*S-(9H-Fluoren-9-yl)methyl1-(1H-benzotriazol-1-yl)-3-(4-tert-butoxyphenyl)-1-oxopropan-2-ylcarbamate (Fmoc-L-Tyr(tBu)-Bt, 6.2d). Recrystallized from EtOAc-hexanes to give white crystals (84%); mp 99.0-100.5 °C; ¹H NMR δ 8.19 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.62 (overlapped t, J = 7.7 Hz, 1H), 7.58-7.54 (m, 2H), 7.48 (overlapped t, J = 7.7 Hz, 1H), 7.360(t, J = 7.4 Hz, 2H), 7.27 (t, J = 7.2 Hz, 2H) 7.02 (d, J = 7.8 Hz, 2H), 6.83 (2H, J = 7.8 Hz, 2H), 6.10-6.08 (m, 1H), 5.79 (d, J = 7.5 Hz, 1H), 4.43-4.35 (m, 2H), 4.18 (t, J = 6.8 Hz, 1H),
3.38 (dd, J = 10.4 Hz, 5.6 Hz, 1H), 3.21 (dd, J = 13.8 Hz, 7.5 Hz, 1H), 1.24 (s, 9H); 13C NMR δ
170.9, 155.6, 154.5, 145.8, 143.6, 143.5, 141.1, 130.8, 130.6, 129.7, 129.6, 127.6, 126.9, 126.4,
124.9, 124.2, 120.2, 119.8, 114.1, 78.4, 67.0, 55.6, 46.9, 38.3, 28.6. Anal. Calcd. for
C₃₄H₃₂N₄O₄: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.84; H, 6.00; N, 9.70.

S-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-4-methyl-1-oxopentan-2-ylcarbamate (Fmoc-L-
Leu-Bt, 6.2j). Recrystallized from EtOAc-hexanes to give white crystals (87%); mp 75.3-78.6 °C;
1H NMR δ 8.20 (d, J = 8.1 Hz, 1H), 8.08 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 7.2 Hz, 2H), 7.61 (t, J
= 7.5 Hz, 1H), 7.58-7.50 (m, 2H), 7.46 (t, J = 7.7 Hz, 1H), 7.33 (t, J = 7.1 Hz, 2H), 7.25 (t, J =
7.0 Hz, 2H), 5.82-5.72 (m, 1H), 5.43 (d, J = 8.7 Hz, 1H), 4.37 (d, J = 6.9 Hz, 2H), 4.17 (t, J = 6.5
Hz, 1H), 1.88-1.64 (m, 3H), 1.04 (d, J = 4.8 Hz, 3H), 0.91 (d, J = 5.1 Hz, 3H); 13C NMR δ 172.4,
156.1, 146.0, 143.8, 143.6, 141.3, 131.1, 130.7, 127.7, 127.0, 126.5, 125.1, 120.3, 120.0, 114.4,
67.1, 53.4, 47.1, 41.9, 25.2, 23.2, 21.3. HRMS calcd for C₂₇H₂₆N₄O₃ [M+Na]⁺ 477.1897, found
477.1898.
Figure 6-3. $^1$H NMR spectrum of 6.2c in CDCl$_3$

Figure 6-4. $^{13}$C NMR spectrum of 6.2c in CDCl$_3$
Figure 6-5. $^1\text{H}$ NMR spectrum ($\delta$ 8.4-6.7) of 6.2d in CDCl$_3$

Figure 6-6. $^1\text{H}$ NMR spectrum ($\delta$ 6.2-1.1) of 6.2d in CDCl$_3$
Figure 6-7. $^{13}$C NMR spectrum of 6.2d in CDCl$_3$
Figure 6-8. $^1\text{H}$ NMR spectrum of 6.2j in CDCl$_3$

Figure 6-9. $^{13}\text{C}$ NMR spectrum of 6.2j in CDCl$_3$
Figure 6-10. High Resolution Mass Spectrum of 6.2j
<table>
<thead>
<tr>
<th>H-V-V-S-V-V-NH₂</th>
<th>MW = 500.4</th>
<th>[M+H]⁺ = 501.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-ions-H₂O</td>
<td></td>
<td>268.2 367.2 466.3</td>
</tr>
<tr>
<td>a-ions (loss of CO)</td>
<td>72.1 171.1 258.2 357.2 456.3</td>
<td></td>
</tr>
<tr>
<td>b-ions N-term</td>
<td>100.1 199.1 286.2 385.2 484.3</td>
<td>C-term</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residue</th>
<th>H</th>
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<th>S</th>
<th>V</th>
<th>V</th>
<th>-NH₂</th>
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<td>99.07</td>
<td>87.03</td>
<td>99.07</td>
<td>99.07</td>
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<tr>
<td>y-ions</td>
<td>501.4</td>
<td>402.3</td>
<td>303.2</td>
<td>216.2</td>
<td>117.1</td>
<td></td>
</tr>
<tr>
<td>Loss of NH₃</td>
<td>484.4</td>
<td>385.3</td>
<td>286.2</td>
<td>199.2</td>
<td>100.1</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6-11. Expected product ions from the (+)ESI-MSn of crude 6.3 (m/z 501 [M+H]⁺ ions). The shaded ions were observed.

Figure 6-12. Fragmentation of crude 6.3
Figure 6-13. High Resolution Mass Spectrum of crude 6.3
Figure 6-14. HPLC profiles of a) crude and b) pure 6.3 obtained after SPPS using 20% piperidine-DMF for Fmoc cleavage. In this instance 6.3 was synthesized using closed vessel conditions.

<table>
<thead>
<tr>
<th>H-V-V-S-V-V-NH₂</th>
<th>MW = 500.4</th>
<th>[M+H]⁺ = 501.4</th>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>b-ions-H₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-ions (loss of CO)</td>
<td>72.1</td>
<td>171.1</td>
</tr>
<tr>
<td>b-ions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-term</td>
<td>100.1</td>
<td>199.1</td>
</tr>
<tr>
<td>C-term</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Residue mass</th>
<th>H</th>
<th>V</th>
<th>V</th>
<th>S</th>
<th>V</th>
<th>V</th>
<th>-NH₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>99.07</td>
<td>99.07</td>
<td>87.03</td>
<td>99.07</td>
<td>99.07</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>y-ions</td>
<td>501.4</td>
<td>402.3</td>
<td>303.2</td>
<td>216.2</td>
<td>117.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of NH₃</td>
<td>484.4</td>
<td>385.3</td>
<td>286.2</td>
<td>199.2</td>
<td>100.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of H₂O</td>
<td>466.4</td>
<td>367.2</td>
<td>268.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6-15. Expected product ions from the (+)ESI-MSn of crude 6.3 (m/z 501 [M+H]⁺ ions). There was a series of ions resulting from the loss of the serine (Ser) hydroxyl group as loss of H₂O from the b-ions and other product ions. The shaded ions were observed. In this instance 6.3 was synthesized using closed vessel conditions.
Figure 6-16. HPLC profile of crude 6.4

<table>
<thead>
<tr>
<th>H-V-V-Y-S-V-V-NH₂</th>
<th>MW = 599.4</th>
<th>[M+H]^+ = 600.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-ions-H₂O</td>
<td></td>
<td>367.2 488.3 585.4</td>
</tr>
<tr>
<td>a-ions (loss of CO)</td>
<td>72.1 171.1 270.2 367.2 468.3 565.4</td>
<td></td>
</tr>
<tr>
<td>b-ions</td>
<td>N-term</td>
<td>100.1 199.1 290.2 365.2 464.3 583.4  C-term</td>
</tr>
</tbody>
</table>

Figure 6-17. Expected product ions from the (+)ESI-MSn dissociation of crude 6.4 (m/z 600 [M+H]^+ ion). The shaded ions were observed.
Figure 6-18. High Resolution Mass Spectrum of crude 6.4 (a) zoomed-in view, (b) full)
Figure 6-19. HPLC profiles of a) crude and b) pure 6.5 obtained after SPPS using 20% piperidine-DMF for Fmoc cleavage.

<table>
<thead>
<tr>
<th>H-V-I-V-I-G-NH₂</th>
<th>MW = 498.4</th>
<th>[M+H]+ = 499.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-ions (loss of CO)</td>
<td>72.1 185.2 284.2 397.3 454.3</td>
<td></td>
</tr>
<tr>
<td>b-ions</td>
<td>N-term 100.1 213.2 312.2 425.3 482.3 C-term</td>
<td></td>
</tr>
<tr>
<td>Residue mass</td>
<td>H 99.07 113.08 99.07 113.08 57.02 16.0</td>
<td></td>
</tr>
<tr>
<td>y-ions</td>
<td>499.4 400.3 287.2 188.2 75.1</td>
<td></td>
</tr>
<tr>
<td>Loss of NH₃</td>
<td>482.4 383.3 270.2 171.2 58.1</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6-20. Expected product ions from the (+)ESI-MSn collision induced dissociation (CID) of crude 6.5 (m/z 499 [M+H]+ ion) with the product ions resulting from traditional cleavage along the peptide backbone. The shaded product ions were observed.
<table>
<thead>
<tr>
<th>Residue</th>
<th>H</th>
<th>V</th>
<th>I</th>
<th>V</th>
<th>I</th>
<th>G</th>
<th>-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue mass</td>
<td>1.0</td>
<td>99.1</td>
<td>113.1</td>
<td>99.1</td>
<td>113.1</td>
<td>57.0</td>
<td>17.0</td>
</tr>
<tr>
<td>y-ions</td>
<td>500.3</td>
<td>401.3</td>
<td>288.2</td>
<td>189.1</td>
<td>76.0</td>
<td>C-Term</td>
<td></td>
</tr>
<tr>
<td>z-ions (y-NH₃)</td>
<td>483.3</td>
<td>384.3</td>
<td>271.2</td>
<td>172.1</td>
<td>59.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-CO</td>
<td>455.3</td>
<td>356.3</td>
<td>243.2</td>
<td>144.1</td>
<td>31.0</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 6-21. Expected product ions from the (+)ESI-MSn CID of pure 6.5 (m/z 500 [M+H]+ ion) with the product ions resulting from traditional cleavage along the peptide backbone. The shaded product ions were observed.

Figure 6-22. High Resolution Mass Spectrum of pure 6.5
Figure 6-23. HPLC profiles of a) crude and b) pure 6.6 obtained after SPPS using 20% piperidine-DMF for Fmoc cleavage.

**H-T-V-T-V-T-V-NH₂**  
MW = 617.4  
[M+H]^+ = 618.4

<table>
<thead>
<tr>
<th>a₁-Ions (loss of CO)</th>
<th>74.1</th>
<th>173.1</th>
<th>274.2</th>
<th>373.2</th>
<th>474.3</th>
<th>573.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-Ions N-term</td>
<td>102.1</td>
<td>201.1</td>
<td>302.2</td>
<td>401.2</td>
<td>502.3</td>
<td><strong>601.4</strong></td>
</tr>
<tr>
<td>Residue</td>
<td>H</td>
<td>T</td>
<td>V</td>
<td>T</td>
<td>V</td>
<td>T</td>
</tr>
<tr>
<td>Residue mass</td>
<td>1.0</td>
<td>101.1</td>
<td>99.1</td>
<td>101.1</td>
<td>99.1</td>
<td>101.1</td>
</tr>
<tr>
<td>y-Ions</td>
<td>618.4</td>
<td>517.4</td>
<td>418.3</td>
<td>317.2</td>
<td>218.2</td>
<td>117.1</td>
</tr>
<tr>
<td>Loss of NH₃</td>
<td>601.4</td>
<td>500.4</td>
<td>401.3</td>
<td>300.2</td>
<td>201.2</td>
<td>100.1</td>
</tr>
</tbody>
</table>

| b-Ions | 302 | 401 | 502 | 601 |
| -H₂O   | 284 | 383 | 484 | 583 |
| -H₂O   | 266 | 365 | 466 | 565 |

**Figure 6-24.** Product ions from the (+)ESI-MSn dissociation of crude 6.6 (m/z 618 [M+H]^+ ion). In addition to the traditional b and y ions, the b-Ions yielded a number of ions due to successive losses of H₂O (from Thr, threonine).
Figure 6-25. High Resolution Mass Spectrum of pure 6.6
Figure 6-26. HPLC profiles of a) crude and b) pure 6.7 obtained after SPPS using 5% piperazine-DMF for Fmoc cleavage.

<table>
<thead>
<tr>
<th>Residue</th>
<th>H</th>
<th>V</th>
<th>K</th>
<th>D</th>
<th>G</th>
<th>Y</th>
<th>I</th>
<th>-NH₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue mass</td>
<td>1.0</td>
<td>99.07</td>
<td>128.0</td>
<td>115.0</td>
<td>3</td>
<td>57.02</td>
<td>163.06</td>
<td>113.08</td>
</tr>
<tr>
<td>y-ions</td>
<td>693.4</td>
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<td>466.2</td>
<td>351.2</td>
<td>294.2</td>
<td>131.1</td>
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<td></td>
</tr>
<tr>
<td>Loss of NH₃</td>
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<td>449.2</td>
<td>334.2</td>
<td>277.2</td>
<td>114.1</td>
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</table>

Figure 6-27. Expected product ions from the (+)ESI-MSn of crude 6.7 (m/z 693 [M+H]⁺ ion). The shaded ions were observed.
Scheme 6-3. Possible (+)ESI-MSn dissociation pathway for the formation of m/z 449 and m/z 336 product ions from the m/z 693 [M+H]^+ ion of 6.7.
Figure 6-28. High Resolution Mass Spectrum of crude 6.7
Figure 6-29. HPLC profiles of a) crude and b) pure 6.8 obtained after SPPS using 5% piperazine-DMF for Fmoc cleavage.

<table>
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<tr>
<th>H-V-K-D-V-Y-I-NH₂</th>
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<td>C-term</td>
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<td>V</td>
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<td>D</td>
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<td>491.3</td>
<td>376.3</td>
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</table>

Figure 6-30. Expected product ions from the (+)ESI-CID-MS/MS of crude 6.8 (m/z 735 [M+H]⁺ ion). The shaded product ions were observed.
Figure 6-31. High Resolution Mass Spectrum of crude 6.8
<table>
<thead>
<tr>
<th>Residue</th>
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<th>Y</th>
<th>G</th>
<th>G</th>
<th>F</th>
<th>L</th>
<th>-NH₂</th>
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<td>57.0</td>
<td>57.0</td>
<td>147.1</td>
<td>113.1</td>
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<td>N-Term</td>
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<td>392.2</td>
<td>335.2</td>
<td>278.2</td>
<td>131.1</td>
<td>C-Term</td>
</tr>
<tr>
<td>z-ions (y-NH₃)</td>
<td>538.3</td>
<td>375.2</td>
<td>318.2</td>
<td>261.2</td>
<td>114.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-CO</td>
<td>510.3</td>
<td>347.2</td>
<td>290.2</td>
<td>233.2</td>
<td>86.1</td>
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</tbody>
</table>

Table 6-32. Expected product ions according to classical cleavage along the amide backbone to create b-, a-, y-, and z- ions. The shaded ions were observed for pure 6.9.

Figure 6-32. High Resolution Mass Spectrum of pure 6.9
Figure 6-34. Gradient analysis: The major compound was the expected peptide 6.9 (MW 554, shaded peaks). There was also a minor compound with a MW 555 eluting shortly after 6.9.
Figure 6-35. The (+)ESI-MS spectrum of peptide 6.9 produced m/z 555 [M+H]+, m/z 577 [M+Na]+ and m/z 1109 [M+H+M]+ ions. Collision-induced dissociation in the source region of the mass spectrometer (SCID) was used to produce some characteristic fragment ions. Some of these fragment ions were then chosen for MSn scans.
Figure 6-36. The (+)ESI-MS/MS of 6.9 (m/z 555 [M+H]⁺ ion) (top) and (+)ESI-MS/MS/MS of m/z 538 (middle) and m/z 510 (bottom) primary product ions of 6.9
Figure 6-37. HPLC profiles of a) crude and b) pure 6.10 obtained after SPPS using 20% piperidine-DMF for Fmoc cleavage.

Figure 6-38. High Resolution Mass Spectrum of pure 6.10.
Figure 6-39. The (+)ESI-MS of peptide 6.10 predominantly produced its m/z 857 [M+H]+ ion (top). The m/z 857 [M+H]+ ion then underwent collision-induced dissociation (CID) to form m/z 840 and numerous other primary product ions (middle). The m/z 840 product ion was further dissociated to yield m/z 741 and other secondary product ions (bottom).
Figure 6-40. The traditional amide cleavage of 6.10 during (+)ESI-MS and –MSn produced the expected a, b, y, and y-NH\textsubscript{3} ions. In addition, there were abundant ions due to loss of NH\textsubscript{3} from the a-ions. The shaded ions were detected.
CHAPTER 7
SYNTHESES OF AZOLE-BASED AMINO ACIDS AND PEPTIDES, AND WATER-SOLUBLE COUPLING REAGENTS

7.1 Azole-based Amino Acids and Peptides

7.1.1 Introduction

Recently there has been considerable interest in the syntheses of modified peptides and novel β-amino acids possessing a heterocyclic moiety due to their promising biological activity. Amino acid and peptide conjugates with 1,3,4-oxadiazole moieties display various properties including but not limited to antidepressant, antimicrobial, hypoglycemic and analgesic effects. Furthermore, 1,3,4-oxadiazoles are known to be bioisosteres of amides and esters. Compounds with 1,2,4-triazole and 1,3,4-thiadiazole components also display a considerable range of biological activity including antimiycotic, antibacterial, antidepressant, HIV inhibitory and antimycobacterial effects.

In nature, a vast array of biologically active peptides is present. Despite the abundance of bioactive peptides and proteins, peptide drugs are rare due to the prevalence of human peptidases. α- and γ-Amino acids and peptides containing such amino acid residues occur less frequently in nature and are more stable to human peptidase, thus may be useful in peptidomimetics.

The attention of many research groups has focused on the syntheses of hybrid amino acids and peptides for use in peptidomimetics. Hamzé et al. reported the synthesis of various 3-substituted 1,2,4-oxadiazole-containing chiral β3- and α-amino acids from Fmoc-protected aspartic acid. Recently, Katritzky and coworkers described the synthesis of chiral 1,2,4-oxadiazoles using N-protected(α-aminoacyl)benzotriazoles of some amino acids in 70-94% yield. A search of the literature disclosed very few
reported syntheses of 1,2,4-triazolo- or 1,3,4-thiadiazolo-substituted amino acids or peptides. [07MOL103, 07HAC316] Prompted by the potential biological activities of these compounds and in continuation of the work on the versatility of \(N\)-protected(\(\alpha\)-aminoacyl)benzotriazoles, the proposed synthetic route to 1,2,4-oxadiazolo-substituted dipeptides, 1,2,4-triazolo-substituted amino acids and peptides and 1,3,4-thiadiazolo-substituted amino acids and peptides is now described.

### 7.1.2 Proposed Synthetic Route to Azolo-based Amino Acids and Dipeptides

A practical method for the synthesis of 1,2,4-triazolo-, 1,2,4-oxadiazolo- 1,3,4-thiadiazolo amino acid derivatives involves the reaction of \(N\)-protected-(\(\alpha\)-aminoacyl)benzotriazoles 7.1 with amidrazones 7.2, amidoximes 7.3 and thiohydrazides/thiosemicarbazides 7.4/7.5 respectively (Figure 7-1).

**Figure 7-1. Synthesis of 1,2,4-triazolo-, 1,2,4-oxadiazolo- 1,3,4-thiadiazolo amino acid derivatives 7.6-7.7**
Similarly azolo-containing dipeptides can be made from dipeptidoylbenzotriazoles 7.10 and amidrazones 7.2, amidoximes 7.3 and thiohydrazides/thiosemicarbazides 7.4/7.5 (Figure 7-2).

Figure 7-2. Synthesis of 1,2,4-triazolo-, 1,2,4-oxadiazolo- 1,3,4-thiadiazolo dipeptide derivatives 7.11-7.14

7.1.3 Preparation of N-Protected(α-aminoacyl)benzotriazoles 7.1

N-Protected-(α-aminoacyl)benzotriazoles 7.1 were prepared from commercial N-protected-L-amino acids according to the literature (Scheme 7-1). [09ARK47]

Scheme 7-1. Preparation of N-protected-(α-aminoacyl)benzotriazoles 7.1
7.1.4 Preparation of Amidrazones 7.2

Amidrazones 7.2, also referred to as hydrozonamides, can be obtained by several routes. This includes the reaction of hydrazines with (i) nitriles [70CR151], (ii) imidates and their salts [92JCS(PK2)671, 68JOC1679], (iii) imidoyl halides [70CR151], (iv) amides or thioamides in the presence of POCl3 [50JACS2783, 58JOC1931], (v) ketenimines [65JOC3718], and (vi) imidoyl benzotriazoles [06JOC9051].

Although the reaction of nitriles with hydrazines appears to be simple, it is known that the reaction of nitriles with hydrazine can lead to the formation of dihydrotetrazines and subsequently tetrazines by oxidation [70CR151], however the reaction outcome is largely controlled by the nature of the nitrile. [06JOC9051] Initially, the reaction of nitriles 7.15 with hydrazine 7.16 yielded the corresponding dihydrotetrazines 7.17 rather than the amidrazone as the major product (Scheme 7-2). For nitrile 7.15a a trace amount of the amidrazone was present as evidenced in the high resolution mass spectrum (See 7.3 Experimental Section). Subsequently, the reaction of amidine 7.18 with hydrazine 7.16 afforded the smooth conversion to amidrazone 7.2b.

![Scheme 7-2. Preparation of amidrazones 7.2 and dihydrotetrazines 7.17](image-url)
7.1.5 Preparative routes to Amidoximes 7.3, Thiohydrazides 7.4 and Semicarbazides 7.5

Amidoximes 7.3 can be readily prepared following the protocol of Hamzé and coworkers [03JOC7316]. Using this procedure amidoximes 7.3 will be synthesized by reacting nitriles 7.15 and hydroxylamines 7.19 (Scheme 7-3).

![Scheme 7-3. Preparation of amidoximes 7.3](image)

The key intermediate in the preparation of thiohydrazides 7.4 and semicarbazides 7.5 is hydrazine 7.16. Thiohydrazides 7.4 will be readily available via the reaction of various thiocarbonylbenzotriazoles 7.20 with hydrazine 7.16 (Scheme 7-4). [05JOC7866] Similarly, semicarbazides 7.5 can be obtained by reacting thiocarbamoylbenzotriazoles 7.21 with hydrazine 7.16 (Scheme 7-4). [07JOC6742]

![Scheme 7-4. Preparation of thiohydrazides 7.4 and semicarbazides 7.5](image)

7.1.6 Summary and Future Prospect

Precursors toazole-based amino acids and peptides such as novel \(N\)-Cbz-(\(\alpha\)-aminoacyl)benzotriazoles 7.1a and amidrazone 7.2b were synthesized and will be utilized in subsequent coupling reactions. Previously, \(N\)-protected(\(\alpha\)-aminoacyl)benzotriazoles 7.1 and amidoximes were coupled by refluxing in ethanol in the presence of triethylamine. [05ARK36]

Following the syntheses of a vast array of intermediates 7.2-7.5, investigations into the optimum
conditions for the microwave assisted couplings of \(N\)-protected(\(\alpha\)-aminoacyl)benzotriazoles 7.1 with amidrazones 7.2, amidoximes 7.3 and thiohydrazides/thiosemicarbazides 7.4/7.5 will be explored.

### 7.1.7 Experimental Section

Melting points were determined on a hot-stage apparatus and are uncorrected. \(^1\)H (300 MHz, with TMS as the internal standard) and \(^{13}\)C NMR (75 MHz) NMR spectra were recorded in CDCl\(_3\) or DMSO-\(d_6\). Elemental analysis was carried out in an Eager 200 CHN analyzer.

#### 7.1.7.1 General procedure for the preparation of \(N\)-protected(\(\alpha\)-aminoacyl)benzotriazoles 7.1

\(N\)-protected(\(\alpha\)-aminoacyl)benzotriazoles 7.1 were prepared using established protocol. [09ARK47]. Preparative details and characterization data for 7.1b,c were described in 4.4 Experimental Section.

**Benzyl \(N\)-(1S)-1-(1\(H\)-1,2,3-benzotriazol-1-ylcarbonyl)-3-methylbutyl|carbamate (7.1a).** Recrystallized from EtOAc-hexanes to give white crystals (86%); mp 61.7-63.9 °C; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.23 (d, \(J = 8.4\) Hz , 1H), 8.11 (d, \(J = 8.4\) Hz, 1H), 7.65 (t, \(J = 7.7\) Hz, 1H), 7.50 (t, \(J = 7.7\) Hz, 1H), 7.39-7.26 (m, 5H), 7.09 (br s, 1H), 5.88-5.78 (m, 1H), 5.51 (d, \(J = 8.7\) Hz, 1H), 5.11 (s, 2H), 1.90-1.80 (m, 2H), 1.80-1.64 (m, 1H), 1.08 (d, \(J = 5.1\) Hz, 3H), 0.95 (d, \(J = 6.0\) Hz, 3H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 172.4, 146.0, 130.7, 128.5, 128.2, 128.1, 126.4, 120.3, 114.4, 67.3, 53.4, 42.0, 25.2, 23.2, 21.3. Anal. Calcd for C\(_{20}\)H\(_{22}\)N\(_4\)O\(_3\): C, 65.56; H, 6.05; N, 15.29. Found: C, 65.66; H, 6.43; N, 14.96.

#### 7.1.7.2 General procedure for the preparation of amidrazone 7.2b and dihydrotetrazine 7.17a,b

To an ethanolic solution of benzamidine hydrochloride (8 mmol) was added hydrazine (8 mmol). The mixture was refluxed for 12 h, and then allowed to cool to room temperature. On
cooling, an off-white solid precipitated. This solid was filtered and washed with cold ethanol to afford (Z)-benzohydrazonamide 7.2b as an off-white powder that turned pink on standing.

Similarly, dihydrotetrazines 7.17 were prepared by adding hydrazine (8 mmol) to an ethanolic solution of 4-methoxyphenylacetonitrile 7.15a (8 mmol) or p-tolunitrile 7.15b (1 mol). The mixture was refluxed for 12 h, and then allowed to cool to room temperature. On cooling, an off-white solid precipitated. This solid was filtered and washed with cold ethanol to afford dihydrotetrazines 7.17a,b.

(Z)-Benzohydrazonamide (7.2b). Recrystallized from isopropanol to give an off-white powder (65%); mp 183.6-184.6 °C; 1H NMR (DMSO-\textit{d}_6) \(\delta\) 7.87-7.81 (m, 1H), 7.50-7.40 (overlapped m, 4H), 7.35 (br s, 4H); 13C NMR (DMSO-\textit{d}_6) \(\delta\) 148.0, 130.3, 128.6, 126.0. HRMS calcd for C\(_7\)H\(_9\)N\(_3\) [M+H]\(^+\) 136.0865, found 136.0869.

3,6-Di-p-tolyl-1,2-dihydro-1,2,4,5-tetrazine (7.17a). Recrystallized from ethanol to give pink crystals (71%); mp 218.4-219.6 °C; 1H NMR (CDCl\(_3\)) \(\delta\) 7.55 (d, \(J = 8.1\) Hz, 4H), 7.28 (d, \(J = 6.9\) Hz, 4H), 2.42 (s, 6H); 13C NMR (CDCl\(_3\)) \(\delta\) 143.8, 132.2, 130.0, 129.7, 126.0, 22.0. HRMS calcd for C\(_{16}\)H\(_{16}\)N\(_4\) [M+H]\(^+\) 265.1403, found 265.1453.

3,6-Bis(4-methoxybenzyl)-1,2-dihydro-1,2,4,5-tetrazine (7.17b). Recrystallized from ethanol to give pink crystals (60%); mp 192.2-196.9 °C; 1H NMR (CDCl\(_3\)) \(\delta\) 7.16 (d, \(J = 8.7\) Hz, 4H), 6.82 (d, \(J = 8.7\) Hz, 4H), 4.09 (s, 6H), 3.77 (s, 4H); 13C NMR (CDCl\(_3\)) \(\delta\) 158.7, 154.3, 129.7, 127.5, 114.4, 55.4, 30.2. Anal. Calcd for C\(_{18}\)H\(_{20}\)N\(_4\)O\(_2\): C, 66.56; H, 6.21; N, 17.27. Found: C, 66.33; H, 6.19; N, 17.32.

7.2 Synthesis of Water-soluble Coupling Reagents

7.2.1 Introduction

Solid phase peptide synthesis (SPPS) is a simple and rapid technique that offers ease of separation of products from reagents, while eliminating inherent product losses associated with
conventional solution phase methodologies (filtration, recrystallization, etc.). [97MI1] When compared with conventional solution phase methodologies, the increased efficiency of SPPS has resulted in its near-exclusive use for the preparation of peptides over the last thirty years. [73JACS4501, 06CEJC285] However, there is concern regarding the environmentally friendly yet safe disposal of large amounts of organic solvents required for SPPS. [01JPS615, 04TL9293, 06PPL189] Reactions performed in aqueous media could reduce the copious amount of solvents required for SPPS.

Published SPPS in aqueous media water has used water soluble N-protected amino acids. In standard N-C SPPS water soluble coupling reagents can be made by (i) protecting the N-terminus with a polar group and (ii) activating the C-terminus with a polar group. To date (i) aqueous SPPS with water soluble N-protecting groups [75IJPPR295, 76CB3693, 78JOC4808, 06PPL189, 04CPB422], has been emphasized with little attention to (ii) water soluble N-protected amino acids via the activation of the C-terminus [04TL9293, 06PPL189, 79JACS3394]. Water soluble N-protecting groups utilized include the methylsulfonylethoxycarbonyl [75IJPPR295], 2-(triphenylphosphino)ethoxycarbonyl [76CB3693], 9-(2-sulfo)fluorenylmethoxycarbonyl [78JOC4808], 2-[phenyl(methyl)sulfonio]ethoxycarbonyl (Pms) [06PPL189], 2-(4-sulfophenyl)ethoxycarbonyl (Sps) [06PPL189] and N-ethanesulfonylethoxycarbonyl (Esc) [04CPB422] groups (Figure 7-3).

![Figure 7-3. Structures of some of water soluble N-protecting groups](image-url)
Conversely, a search of the literature revealed a few methods involving SPPS using water soluble carbodiimides (WSCD) and water soluble active esters (Scheme 7-4). [06PPL189, 04TL9293, 79JACS3394]

Figure 7-4. Structures of water soluble activating groups

Recently the preparation of \(N\)-(Fmoc-\(\alpha\)-aminoacyl)benzotriazoles of 18 proteinogenic amino acids, their utility in the microwave accelerated synthesis of tri- to heptapeptides in crude yields of 65-77% [09ARK47, 07CBDD465] and short “difficult” peptides in crude yields of 34-86% [09JOC2028] on the Rink amide MBHA solid support was reported. \(N\)-(Fmoc-\(\alpha\)-aminoacyl)benzotriazoles are sparingly soluble in water, therefore, novel water-soluble coupling reagents 7.22 and 7.23 were designed with the aim of performing SPPS in aqueous media (Figure 7-5). Herein preparative routes to benzotriazole-6-sulfonic acid derivatives 7.22 and \(N\)-(2-(2-pyridyl)ethoxycarbonyl)-\(\alpha\)-aminoacyl)benzotriazoles 7.23 as well as their potential for application in SPPS is described.

Figure 7-5. Potential water-soluble coupling reagents 7.22 and 7.23
7.2.2.1 Retrosynthetic analysis for 7.22

The retrosynthetic pathway leading to title compound 7.22 is shown in Figure 7-6. The water-soluble C-activating group, 1H-benzotriazole-6-sulfonic acid 7.29 was readily obtained via nitro group reduction and subsequent cyclization of commercially available sodium 4-amino-3-nitrosulfonate 7.30 (Scheme 7-5).

![Figure 7-6. Retrosynthetic analysis of 7.22](image)

Scheme 7-5. Preparation of 1H-benzotriazole-6-sulfonic acid 7.29
The preparation of the $N$-protecting group is more involved and is now described. To commence, the reaction of 4-mercaptophenol with bromoethanol in the presence of NaOH will yield 4(2-hydroxyethylsulfanyl)phenol $\mathbf{7.25}$. [03OPRD418] Oxidation of $\mathbf{7.25}$ with oxone $\mathbf{7.33}$ and subsequent reaction with bis(benzotriazo-1-yl)methanone $\mathbf{7.26}$ in the presence of Et$_3$N should afford $\mathbf{7.32}$ (Scheme 7-6). [62USP3068278] Reaction of $\mathbf{7.32}$ with sultone and further coupling with free L-amino acids $\mathbf{7.28}$ should furnish $\mathbf{7.24}$ (Scheme 7-6).

Once $\mathbf{7.24}$ is obtained the coupling reaction with 1H-benzotriazole-6-sulfonic acid $\mathbf{7.24}$ can be performed (Scheme 7-7).

Scheme 7-6. Preparation of $\mathbf{7.24}$ from $\mathbf{7.25}$

Scheme 7-7. Proposed route to $\mathbf{7.22}$
7.2.2.3 Retrosynthetic analysis for 7.23

The retrosynthesis for possible water-soluble \(N-(2-(4-pyridyl)ethoxycarbonyl)-\alpha\)-aminoacyl)benzotriazoles 7.23 is shown in Figure 7-7. The reaction of 2-(pyridin-4-yl)ethanol with bis(benzotriazo-1-yl)methanone 7.26 in the presence of Et\(_3\)N should afford 7.36 (Scheme 7-8). Further reaction of 7.36 with amino acids should yield 7.34, which will be reacted with 1\(H\)-benzotriazole or 7-azabenzotriazole to afford 7.23 (Scheme 7-8).

Figure 7-7. Proposed synthetic route to 7.23

Scheme 7-8. Proposed preparation of 7.23 from 7.35
7.2.2.4 General procedure for the preparation of $^{1}H$-benzotriazole-6-sulfonic acid 7.29

Sodium 4-amino-3-nitrosulfonate 7.30 (1.50 g, 6.25 mmol) was dissolved in water (25 mL) and palladium/carbon (50 % wet, 0.66 g, 0.312 mmol, 20 mol %) was slowly added to the solution. The mixture was stirred under hydrogen for 3 h. After 3 h the mixture was filtered through a celite bed and the red-brown filtrate lyophilized for 24 hours to afford a red brown liquid, sodium 3,4-diaminobenzenesulfonate 7.31 (1.19 g, 5.66 mmol, 90%).

To an acidic solution of the sodium 3,4-diaminobenzenesulfonate 7.31 (0.50 g, 2.379 mmol) was slowly added NaNO$_2$ solution (0.16 g, 2.379 mmol) at 0 °C. The solution was stirred at room temperature for 4 h then neutralized with 2N NaOH. The water was then evaporated to yield $^{1}H$-benzotriazole-6-sulfonic acid 7.29 (0.32 g, 1.447 mmol, 60%). The dissociation of MW 199 compound 7.29 is shown in Figure 7-10. In Figure 7-10 compound 7.29 formed a m/z 200 $[M+H]^+$ ion (top spectrum) that dissociated to yield a number of product ions (middle spectrum). Further dissociation of the m/z 136 product ion of 7.29 is also illustrated in Figure 7-10 (bottom spectrum). Additionally, the m/z 198 $[M-H]^-$ ion of 7.29, a number of self adduct ions (for example, m/z 397 [(M-H)+M]) and the product ions of dissociation are shown in Figure 7-11.

**Sodium 3,4-diaminobenzenesulfonate (7.31).** Red-brown liquid (90%); $^1$H NMR (D$_2$O) δ 7.08 (d, $J = 2.1$ Hz, 1H), 7.04 (dd, $J = 8.4$ Hz, 2.1 Hz, 1H), 6.74 (d, $J = 8.1$ Hz, 1H); $^{13}$C NMR (D$_2$O) δ 137.2, 133.1, 133.0, 117.6, 115.7, 113.6. HRMS calcd for C$_6$H$_7$N$_2$O$_3$S $[M-Na]^-$ 187.0181, found 187.0183.

**$^{1}H$-Benzotriazole-6-sulfonic acid (7.29).** Brown wax (60%); $^1$H NMR (D$_2$O) δ 8.38 (s, 1H), 8.00 (d, $J = 8.7$ Hz, 1H), 7.83 (dd, $J = 8.7$, 1.6 Hz, 1H); $^{13}$C NMR (D$_2$O) 138.9, 122.2, 115.3, 113.6. HRMS calcd for C$_6$H$_4$N$_3$O$_3$S [M] $^+$ 197.9973, found 197.8068.
Figure 7-8. $^1$H NMR spectrum of 7.29 in D$_2$O

Figure 7-9. $^{13}$C NMR spectrum of 7.29 in D$_2$O
Figure 7-10. The m/z 200 [M+H]$^+$ ion of 7.29 (top) plus the primary and secondary product ions of dissociation (middle and bottom)
Figure 7-11. The m/z 198 [M-H]⁺ ion of 7.29, self-adduct ions (top) plus primary and secondary product ions of dissociation (bottom)
Scheme 7-9. Probable (-)ESI-MSn dissociation of the m/z 198 [M-H]- ion of 7.29
7.2.2.5. Future work

Subsequent to the synthesis of the 1\textit{H}-benzotriazole-6-sulfonic acid amino acid derivatives 7.22 and \(N\)-(2-(2-pyridyl)ethoxycarbonyl)-\(\alpha\)-aminoacyl)benzotriazoles 7.23, water solubility studies will be conducted on 7.22 and 7.23. In anticipation of a favorable outcome, the syntheses of Leu- and Met-enkephalin will be attempted with water-soluble coupling reagents 7.22 and 7.23 (Scheme 7-10) and a comparison of the results made with the previous synthesis of Leu-enkephalin (See Section 6.2.1).

Scheme 7-10. Proposed SPPS using potential water-soluble reagents 7.22 or 7.23
CHAPTER 8
CONCLUSIONS, SUMMARY OF ACHIEVEMENTS AND FUTURE OUTLOOK

Synthetic organic chemistry had its genesis in the study of natural products. In the broadest sense, the development of new methodologies still finds its inspiration from nature which is a constant source of intellectual challenge. Unlocking the mysteries surrounding the biogenesis of heterocycles and other pertinent biological molecules, as well as understanding their properties and functions in nature requires a focused, multidisciplinary approach and synthetic organic chemistry has been a valuable tool in this process.

Chapter 1 presented an overview $1H$-benzotriazole methodology and current applications of $1H$-benzotriazole and its derivatives in varying fields of chemistry. This chapter illustrated the renewed interest of research groups in $1H$-benzotriazole chemistry. From Chapter 1, it is apparent that there has been an explosion in research into some of the less studied aspects of $1H$-benzotriazole and its derivatives such as its use in cross-coupling reactions.

Chapter 2 of this study predominantly focused on C-aminoimidoylation and C-thiocarbamoylation of ester enolates. Chapter 3, an extension of the methodology applied in Chapter 2, covered the preparation of C-alkoxyimidoylating reagents. C-Amino and C-alkoxyimidoylation, as well as C-thiocarbamoylation are extremely useful reactions for the formation of precursors to heterocycles. These precursors to heterocycles were obtained in one step from the C-aminoimidoylating and C-thiocarbamoylating reagents, whereas previous methodologies required multiple steps. Furthermore, when compared with the previous methodologies, the present C-aminoimidoylation and C-thiocarbamoylation methodologies provided comparable product yields. Although the discussions on C-amino, C-alkoxy and C-arylthioimidoylation, as well as C-thiocarbamoylation are by no means comprehensive, the present methodologies provide viable access to a range of important compound classes. It is
likely that further optimization of the C-amino, C-alkoxy and C-arylthioimidoylation, as well as C-thiocarbamoylation reactions will provide even more method generality. Thus these reactions warrant further research.

Over the last four decades there has been an escalation in the demand for synthetic peptides and proteins. The search for more promising bioactive peptides, vaccine development and elucidating the factors influencing the three dimensional structure of proteins are a few reasons for the increased demand of synthetic peptides. Traditional solution phase methodologies involve the elaboration of the peptide or protein one amino acid at a time. This process is slow and can be a limiting factor in some studies. Thus, SPPS evolved in response to the need for a rapid, reliable and inexpensive method for peptide syntheses. Today, both the solution and solid phase techniques for peptide synthesis have their niche in synthetic organic chemistry.

In response to the increasing need for methods to access synthetic peptides, Chapters 4-7 of this study has expanded well-developed and reproducible benzotriazole-based methods to the novel syntheses of structural motifs frequently found in biologically relevant compounds, including peptides and proteins. N-Protected-(α-aminoacyl)benzotriazoles of 18 proteinogenic amino acids were prepared from precomplexed 1H-benzotriazole and thionyl chloride. In this study N-protected-(α-aminoacyl)benzotriazoles were demonstrated to be highly efficient tools for the syntheses of peptides and peptide conjugates. The scope of this aminoacylation reaction is quite general and affords a rapid and easy access to peptides and peptide conjugates. Evidently, the ability to synthesize peptides and proteins (Chapters 4-6), for example, β-amyloid can greatly expand our comprehension of the significant role that these biomolecules play in the progression of neurodegenerative diseases such as Parkinson’s and Alzheimer’s. Thus an
understanding of the degeneration process in AD and Parkinson’s disease will assist with finding cures.

Extensions of the benzotriazole-based strategy of peptide synthesis via N-protected-(α-aminoacyl)benzotriazoles was discussed in Chapter 7. In Chapter 7 the syntheses of azole-based peptides can provide access to compound classes that may serve as valuable tools for peptidomimetics. Although work on the water-based synthesis of peptides (Chapter 7) using 1H-benzotriazole methodology is still in its infancy, the development of this aqueous-based methodology is expected to have a profound impact on SPPS and by extension solution syntheses.

To summarize, in this study synthetically useful benzotriazole-assisted methods were applied to the syntheses of a variety of significant compounds (N,N′-disubstituted ketene aminals, peptides etc.). Among the challenges for the future is the development of 1H-benzotriazole-mediated aqueous synthetic methods. While the Katritzky group has explored many aspects of 1H-benzotriazole chemistry over the last three decades, the maximum potential of 1H-benzotriazole as a synthetic auxiliary has not been attained and many novel and interesting chemical transformations and applications await discovery. Undoubtedly the renewed interest in the chemistry of 1H-benzotriazole and its derivatives will lead to insights at the interface of a range of chemistry related disciplines.
LIST OF REFERENCES

The reference citation system employed throughout this dissertation is that from “Advances in Heterocyclic Chemistry” (vol. 96) Academic Press, 2008 (Ed. A. R. Katritzky).

Each time a reference is cited a number-letter code is designated to the corresponding reference with the first two, or four if before 1910, numbers indicating the year followed by the letter code of the journal and the page number in the end.

Additional notes to this reference system are as follows:

(i) Each reference code is followed by the conventional literature citation as depicted in the Advances in Heterocyclic Chemistry instruction for authors

(ii) Less commonly used books and journals are coded as “MI” for miscellaneous

(iii) The list of references are arranged according to the designated code in the order of (a) year, (b) journal in alphabetical order, (c) page number


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A. A. Aly and A. M. Nour-El-Din, Arkivoc, (i), 153 (2008).


TBAB        Technical Bulletin, Applied Biosystems
BIOGRAPHICAL SKETCH

Danniebelle N. Haase was born in 1979, in St. Andrew, Jamaica, West Indies. She was the first of two children. She spent her formative years at the Herrick Basic and Dunrobin Primary schools and later attended the Immaculate Conception High School. Subsequently, she attended the University of the West Indies where she read for a Bachelor of Science in Chemistry and Management. Upon graduation in the summer of 2000, she taught science at the Merl Grove High School for girls. Once again, in fall 2002 she matriculated as a student in the Master of Philosophy program in the Department of Chemistry at the University of the West Indies. She was awarded the Master of Philosophy in the summer of 2005, after which she traveled to the USA to pursue doctoral studies, specializing in organic chemistry at the University of Florida. In the fall of 2005 she joined Professor Alan R. Katritzky’s research group at the Florida Center for Heterocyclic Chemistry.