Chapter 3

An efficient synthesis of N\textsuperscript{α}-protected amino acid azides employing carbonyldiimidazole: Application to the one-pot preparation of ureidopeptides

In this chapter, the synthesis of N\textsuperscript{α}-protected amino acyl azides starting from corresponding acids employing carbonyldiimidazole (CDI) is described. The protocol is extended for one-pot preparation of ureidopeptides which is devoid of the isolation of acyl azide and isocyanates. The reaction was accomplished without using any other additives and base. The protocol is simple, clean, high yielding and free from racemization.

3.1. INTRODUCTION

3.1.1. N-Protected amino acid azides

N-Protected amino acid azides are well known and efficient acylating agents in peptide chemistry. They were first reported by Curtius during his efforts to develop mild, racemization free route for the peptide synthesis.\textsuperscript{1,2} Acid azide method, frequently used in the segment condensation of peptide fragments by the divergent approach, is still practiced by the peptide chemists especially for coupling bifunctional amino acids \textit{viz.} His, Thr, Trp and Ser.\textsuperscript{3} Bodanszky \textit{et al.}, explored the utility of acyl azide method for the synthesis of secretin.\textsuperscript{4} The total synthesis of the 124 amino acid sequence of bovine pancreatic ribonuclease (RNase) A, reported by Haruaki Yajima is an outstanding application of acyl azides.\textsuperscript{5-10} Merrifield employed amino acid azides in the stepwise synthesis of octapeptide-oxytocin on SPPS.\textsuperscript{11} Schwyzer \textit{et al.}, used acyl azides during cyclodimerization of cyclohexapeptides.\textsuperscript{12} Amino acid azides have also been used for polymerization reactions as reported by Hofmann \textit{et al.}\textsuperscript{13} Additionally, they are useful as building blocks for partially modified retro-inverso peptides,\textsuperscript{14-16} gem-diaminoalkylamines,\textsuperscript{17-19} $\beta$-amino acids\textsuperscript{20} and $\beta$-amino alcohols.\textsuperscript{21} In general, acyl azides are extensively used in organic synthesis for the preparation of amides, nitriles and certain class of heterocycles and also in cycloaddition reactions.\textsuperscript{22,23}

The protocols generally employed to access acyl azides involve reaction of an azide ion with carboxy acids activated in the form of acid chlorides,\textsuperscript{24-29} mixed anhydrides\textsuperscript{30-33} or acyl benzotriazoles.\textsuperscript{34} The acid chloride method is less advantageous because of the danger of hydrolysis, racemization, cleavage of acid labile protecting groups and other side reactions.
such as degradation of N-Boc-amino acid chlorides to oxazolidin-2,5-diones (Leuch’s anhydride). Though, the mixed anhydride method is beneficial compared to acid chlorides, use of toxic chloroformates limits the wider application of this approach. Katritzky’s group reported the synthesis of acyl azides by a reaction of NaN₃ with acyl benzotriazoles, in a two-step protocol, which requires 16 h for completion. On the other hand, Boc as well as Z protected α-amino acid azides have been obtained from their acyl hydrazides via diazotization. This route requires the use of NO⁺ equivalents and is not so compatible with Fmoc urethane. Other reported protocols involve treatment of carboxy acids with diphenylphosphoryl azide (DPPA) or Deoxo-fluor/trimethylsilyl azide (TMSN₃) and that of aldehydes with Dess-Martin reagent/NaN₃ (Scheme 3.1). Although some of these methods are beneficial for the preparation of acyl azides, there are drawbacks such as longer reaction time, toxic and expensive reagents, byproduct formation and tedious reaction/work-up conditions. In view of the vast utility of acyl azides, development of an expedient, mild method is warranted.

Scheme 3.1
Recently, interest has turned towards the synthesis of acyl azides employing peptide-coupling agents. The efficacy of these coupling reagents for carboxy group activation has been fully exploited in peptide chemistry. Some of the important advantages of using coupling agents are commercial availability, solubility in a wide range of solvents, easy removal of byproducts by extraction, low epimerization and high yield.\(^{43-45}\) In this regard, our group had reported the synthesis of acyl azides employing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 2-(1\(H\)-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (Scheme 3.2).\(^{46}\)

\[
\begin{align*}
\text{R-CHO} & \xrightarrow{\text{EDC, 0 °C, CH}_2\text{Cl}_2} \xrightarrow{\text{NaN_3/DMSO, 0 °C, 20 min}} \text{R-CN} \\
& \text{SCHEME 3.2}
\end{align*}
\]

With the continued interest in this area and our knowledge over various classes of carboxy activating reagents, we become interested towards a mild reagent of this class—carbonyldiimidazole (CDI, Figure 3.1) which is also known for its ability to transfer carbonyl group.\(^{47-49}\) CDI is commonly used on a large scale in peptide chemistry due to the innocuous nature of the byproducts. It possesses several advantages over the traditional coupling agents such as inexpensiveness, ability to offer high yield and purity of the products and racemization free coupling. Keeping these advantages in mind, it was reasoned that it could be a useful reagent for generation of acyl azides from corresponding carboxy acids.

![FIGURE 3.1. Carbonyldiimidazole](image)
First application of CDI in the synthesis of peptides was demonstrated by Wieland and Schneider via acylation of imidazole ring of methyl N-benzoyl-L-histidine.\textsuperscript{50} Later, Anderson’s group developed a simple and general procedure for the CDI mediated peptide synthesis (Scheme 3.3).\textsuperscript{51}

\textbf{SCHEME 3.3}

CDI was also employed for the aqueous phase synthesis of peptides and peptide thioesters from free amino acids and thiols via one pot two step protocol.\textsuperscript{52-54} In the first step CDI reacts with amino acid in water to form N-carboxyanhydride (NCA) which is then subsequently condensed with amino acid or thiol to obtain peptide or thioester (Scheme 3.4).

\textbf{SCHEME 3.4}

Zhang and coworkers employed CDI as a carbonyl transfer reagent in the synthesis of protease inhibitors GE 20372 and MAPI containing urea linkage (Scheme 3.5).\textsuperscript{55}

\textbf{SCHEME 3.5}
CDI has been widely used as a carbonyl transfer reagent in the synthesis of tetramic acid derivative (Scheme 3.6).\textsuperscript{56}

\[
\begin{align*}
\text{R} &= \text{c-Hex, s-Bu} \\
\end{align*}
\]

**SCHEME 3.6**

Vaidyanathan \textit{et al.}, described the CDI mediated amidation of carboxylic acid catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).\textsuperscript{57} Recently Dube’s group employed CDI as a promoter for the Lossen rearrangement of various hydroxamic acids to isocyanates which were then trapped with amine or alcohol to obtain urea or carbamate (Scheme 3.7).\textsuperscript{58}

\[
\begin{align*}
\text{R}^{1}\text{NH}_{2} &\xrightarrow{\text{CDI}} [\text{O} \quad \text{O}] \\
\text{R}^{1}-\text{N}=\text{C}=\text{O} &\xrightarrow{-\text{CO}_{2}} \text{R}^{1}\text{N}\text{H}
\end{align*}
\]

**SCHEME 3.7**

Despite such widespread application, there are no reports on the utility of CDI for the synthesis of acyl azides. With an aim to demonstrate a new application of this reagent, we prepared acyl azides from carboxy acids using CDI/Na\textsubscript{2}N\textsubscript{3}, as an efficient reagent combination. Further, a one-pot preparation of ureidopeptides through CDI mediated carboxy activation has been demonstrated owing to the importance of urea derivatives.
3.1.2. Ureidopeptides

Insertion of urea moiety in place of amide bond has led to ureidopeptidomimetics some of which are screened as HIV-1 protease inhibitors, [Leu] 5 enkephalin analogs, angiotensins and γ-secretaries that have shown increased metabolic stability. 59-65 Several families of peptidomimetics with a urea backbone including \( N,N' \)-linked oligoureas \([N(\text{CONIIR})-(\text{CII})_m^-]_n\) , \( N,N' \)-linked oligoureas \([\text{NIH-CHR-CII}_2-\text{NIH-CO}]_n\) and ureidopeptides \([\text{NR-CH}_2-\text{CH}_2-\text{NH-CO}]_n\) have been found to display antiparallel β-sheet conformations. 66-68 These molecules are relatively rigid compared to native peptides due to strong hydrogen bonding properties and possess metabolic stability, solubility, improved pharmacokinetic properties such as absorption, transport characteristics and low toxicity. Natural products containing urea linkages are also known in the literature. 69 In addition, urea linkage is used to build special class of molecules such as dendrimers and self-assembling organic nanotubes. 70

One of the most followed and straightforward method for preparation of ureido conjugates is reaction of an amine with isocyanate, the latter can be prepared either by treatment of substituted amine with phosgene and related reagents or through Curtius rearrangement of acyl azides. Among the procedures reported for the generation of amino acid derived isocyanates, some of the frequently discussed and noteworthy methods are presented below.

A very early report from Goldschmidt et al., described conversion of amino acid ester hydrochlorides into corresponding isocyanates by treating with phosgene in refluxing toluene. 71 Later, Nowick et al., improved this method by using a solution of phosgene in
toluene instead of gaseous phosgene and importantly, the reaction was carried out at 0 °C for 2 h in presence of pyridine (Scheme 3.8).

![Chemical structure](image)

**SCHEME 3.8**

The same group further improved the synthesis of isocyanates by employing modified Schotten-Baumann conditions. This involved the addition of a solution of phosgene or triphosgene in toluene to an ice-cold suspension of amino acid ester or peptide ester hydrochloride salt in biphasic mixture of CH₂Cl₂ and saturated aqueous NaHCO₃ solution (Scheme 3.9). Though the handling of solid triphosgene is safe, easier and yields are comparable with that of phosgene, the difficulty in the removal of excess triphosgene at the end of reaction makes it less preferable.

![Chemical structure](image)

**SCHEME 3.9**

In peptide chemistry, apart from conversion of amine group of the amino acid into isocyanates to synthesize ureidopeptides, much research has also been devoted to convert the carboxy terminus into isocyanates and use them to assemble ureidopeptidomimetics. Curtius rearrangement of acyl azides has been a major choice followed by Lossen rearrangement of hydroxamic acids and Hofmann rearrangement of amides. The protocols are executed either via isolation of isocyanates or in situ trapping with desired amines. In some cases such
isocyanates are also converted into stable carbamates and later used in solution as well as solid phase synthesis of urea conjugates.

Guichard et al., reported the solid phase synthesis of oligoureas using O-succinimidyl-(9H-fluoren-9-ylmethoxycarbonylamino)ethylcarbamate derivatives as active monomers.\(^{74}\) The required building blocks were prepared starting from Fmoc-\(\beta\)-amino acids \(via\) the Curtius rearrangement of the corresponding acyl azides followed by the treatment of resulting isocyanates with \(N\)-hydroxysuccinimide (Scheme 3.10).

\[
\begin{align*}
\text{FmocHN} & \quad \text{COOH} \quad \text{NMM, ECF} \quad \text{NaN}_3 \quad \text{R} \\
\text{NMM, ECF} & \quad \text{NaN}_3 \quad \text{R} \\
\text{toluene} & \quad 65^\circ \text{C} \quad \text{FmocHN} \quad \text{CON}_3 \\
\text{FmocHN} & \quad \text{NCO} \\
\text{N-hydroxysuccinimide} & \quad \text{pyridine} \\
\text{FmocHN} & \quad \text{NCO} \\
\end{align*}
\]

**SCHEME 3.10**

Sureshbabu et al., were the first to isolate both the \(N^\alpha\)-protected amino acid azides as well as isocyanates as solids and characterized.\(^{75-77}\) Thus isolated isocyanates were efficiently employed in solution phase synthesis of \(\alpha\)-ureidopeptides. They also explored the utility of \(N\)-protected amino acid derived isocyanates for building oligoureas, glycopeptidomimetics and other synthetically useful active carbamates (Scheme 3.11).

\[
\begin{align*}
\text{FmocHN} & \quad \text{R}^1 \quad \text{OH} \quad \text{NMM, ECF} \quad \text{NaN}_3 \quad \text{R}^1 \\
\text{NMM, ECF} & \quad \text{NaN}_3 \quad \text{R}^1 \\
\text{MW or } \Delta & \quad \text{or i)} \quad \text{FmocHN} \quad \text{N=C=O} \\
\text{NH}_2-\text{CH(R}^2\text{)-COOX} & \quad \text{FmocHN} \quad \text{R}^1 \quad \text{OX} \\
\text{FmocHN} & \quad \text{R}^1 \\
\text{X = Me or Bn} & \quad \text{R}^2 \\
\end{align*}
\]

**SCHEME 3.11**
Application of Hofmann rearrangement for accessing ureidopeptides was reported by Lipton and co-workers. In one of the reports, [bis(trifluoroacetoxy)iodo]benzene (PIFA) was employed for converting $N$-protected amino acid amide into isocyanate which was coupled, without isolation, with an amine to obtain ureidopeptides (Scheme 3.12).$^{78}$

\[
\text{PgHN} \quad \text{NH}_2 \quad \text{PIFA} \quad \text{pyridine} \quad \left[ \begin{array}{c}
\text{PgHN} \\
\text{NCO}
\end{array} \right] \quad \text{NH}_2\text{CH(R^2)COOX} \quad \text{PgHN} \\
\text{O} \quad \text{R^1} \quad \text{R^2} \quad \text{O} \quad \text{X}
\]

$\text{Pg} = \text{Boc, Z, Fmoc or acyl groups}$  
$\text{X} = \text{Me, Bn}$

**SCHEME 3.12**

Another important route to access ureas *via* isocyanate intermediate is the Lossen rearrangement. This protocol involves transformation of hydroxamic acids into isocyanates for which a variety of reagents such as $p$-toluenesulfonyl chloride ($p$-TsCl),$^{79}$ cyanuric chloride,$^{80}$ 1,1'-carbonyldiimidazole (CDI),$^{58}$ bromodimethyl sulfide (BDMS),$^{81}$ $N$-benzyl-$N$-(3-dimethylaminopropyl)carbodiimide (BDC)$^{82}$ and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)$^{83}$ have been employed (Scheme 3.13).

\[
\begin{array}{c}
\text{R} \\
\text{O} \\
\text{N} \quad \text{OH} \\
\end{array} \quad \text{BDC or EDC} \quad \text{$p$-TsCl} \quad \text{BDMS} \quad \text{CDI} \quad \text{cyanuric chloride}
\]

**SCHEME 3.13**

Apart from the isocyanate intermediacy, several custom made reagents (such as carbonylating reagents and alkyl/aryl chloroformates) are available that link two amine
functionalities via a carbonyl group, resulting in symmetrical or unsymmetrical ureas. Pena and co-workers used $N,N'$-carbonyldiimidazole (CDI)\textsuperscript{55} in the presence of an organic base where the reaction proceeds via the imidazole intermediate. Ogura et al., described the synthesis of ureas through succinimidyl carbonate derivative using $N,N'$-disuccinimidyl carbonate (DSC).\textsuperscript{64} Katritzky et al., synthesized unsymmetrical tetra substituted ureas employing 1,1'-carbonyl-bis-benzotriazole (CBT,\textsuperscript{55} Scheme 3.14).

Schultz et al., described the solid phase synthesis of oligoureas via azido 4-nitrophenyl carbamate monomers.\textsuperscript{86} In their synthesis, $N$-Boc/2-(trimethylsilyl)ethoxycarbonyl (Teoc)-protected amino alcohol was converted to the corresponding azide via the mesylation using methanesulfonyl chloride (MsCl) followed by the removal of Boc and Teoc groups by aqueous HCl or tetrabutylammonium fluoride (TBAF). The resulting free amine was converted to the active monomer by treatment with 4-nitrophenyl chloroformate (NPbOCOCI). The solid phase synthesis of oligoureas was initiated by coupling a monomer to the free amine of resin followed by the reduction of azide group (Scheme 3.15).
SCHEME 3.15

Liskamp and co-workers employed Fmoc and Boc protection strategy in solid phase to synthesize oligourepeptidomimetics. In their protocol, they synthesized the oligoureapeptidomimetics of [Leu\(^1\)]enkephalin, employing \(p\)-nitrophenyl car bamate (R-NH-\(\text{CO-ONp}\)) as monomer (Scheme 3.16).

SCHEME 3.16

Classical Curtius rearrangement employed to access ureidopeptides is a multistep protocol, which involves preparation of an acid azide which in turn was obtained by the reaction of sodium azide with acid chloride or mixed anhydride, its isolation, transformation into isocyanate followed by coupling with an amine. Instability of acid chlorides, explosive nature of acyl azide, toxic reagents makes this process less attractive. On the other hand, the Hofmann protocol is useful only in Boc and Z chemistry. Its utility in base labile Fmoc chemistry is not satisfactory due to the necessity of higher equivalents of a base. In Lossen transformation, the common drawback is the accumulation of isocyanate before the complete
activation of hydroxamic acid leading to self-condensed byproducts. Therefore a simple, improved, and scalable protocol which produces higher yields of the urea and also tolerates different urethane protecting groups is desirable. In view of this, we have conducted experiments on the applicability of CDI as a mild reagent to promote acid azide preparation and also for one-pot synthesis of ureidopeptides. The results are described in the following section of this chapter.
3.2. PRESENT WORK

3.2.1. Acyl azides

In the first part of our work, N-protected amino acyl azides were synthesized from corresponding acids. In a typical reaction, a chilled solution of Fmoc-Ala-OH 3.1a in THF was treated with an equimolar quantity of CDI. After 15 min, NaN₃ (dissolved using a few drops of DMSO) was added and the reaction was continued for another 20 min. The resulting acyl azide 3.3a was isolated after a simple workup as a solid in 89% yield. Imidazole byproduct was removed completely in aqueous workup. Initial characterization by IR revealed the presence of a strong peak at around 2135 cm⁻¹ corresponding to the acyl azide. Further analyses through mass and NMR studies confirmed the desired product formation (Scheme 3.17). The acyl azide formation is a two-step process involving first, the activation of carboxy group as acyl imidazole (imidazolide) 3.2 followed by its azidolysis. The IR analysis of the isolated imidazolide had a signal at around 1650 cm⁻¹, which underwent rapid azidolysis on addition of NaN₃.

\[
\begin{align*}
\text{PgHN}^\bigvee \text{COOH} & \quad \text{CDI, THF} \quad 0 \degree C, 15 \text{ min} \quad \text{PgHN}^\bigvee \text{CON}_3 \\
\text{3.1} & \quad \text{3.2} & \quad \text{3.3} \\
\text{NaN}_3/\text{DMSO} & \quad 0 \degree C, 20 \text{ min} \\
\end{align*}
\]

\[\text{Pg} = \text{Fmoc, Z or Boc group}\]

\[\text{R}^1 = \text{Ph, COO}^\bigvee\text{Bu, OBn}\]

**SCHEME 3.17.** Synthesis of urethane protected amino acyl azides

The importance of the protocol lies in the fact that the method doesn’t require the addition of an organic base. Since the carboxy activation as imidazolide leads to expulsion of
one free imidazole into solution, this itself can act as a base. This would contribute to
circumvent the base catalyzed side reactions including racemization. The efficacy of the
protocol was demonstrated by preparing few other Fmoc-protected amino acid azides 3.3a-d
as well. Encouraged by this result, the protocol was extended to synthesize some Z-protected
amino acid azides 3.3e-h including one bifunctional amino acid derived acyl azide 3.3f. In
addition, two dialkyl amino acid azides Boc-Aib-N₃ 3.3i and Boc-Gpn-N₃ 3.3j were also
prepared (Table 3.1).

**Table 3.1. List of Urethane Protected Acyl Azides 3.3**

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Acyl azide</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
<th>HRMS [M+Na]+ Obsd (calcd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3a</td>
<td>Fmoc-Ala-N₃</td>
<td>89</td>
<td>162 (163)⁷⁵</td>
<td>359.1123 (359.1120)</td>
</tr>
<tr>
<td>3.3b</td>
<td>Fmoc-Val-N₃</td>
<td>87</td>
<td>168 (169)⁷⁵</td>
<td>387.1425 (387.1433)</td>
</tr>
<tr>
<td>3.3c</td>
<td>Fmoc-Glu(O'Bu)-N₃</td>
<td>78</td>
<td>169 (168-170)⁷⁵</td>
<td>473.17 (473.18)³⁴</td>
</tr>
<tr>
<td>3.3d</td>
<td>Fmoc-Ser(Bn)-N₃</td>
<td>75</td>
<td>146</td>
<td>481.1285 (481.1278)⁶⁴</td>
</tr>
<tr>
<td>3.3e</td>
<td>Z-Phe-N₃</td>
<td>88</td>
<td>145 (146)³⁴</td>
<td>347.1093 (347.1120)</td>
</tr>
<tr>
<td>3.3f</td>
<td><img src="image" alt="Structure" /></td>
<td>76</td>
<td>Gum</td>
<td>327.0712 (327.0705)</td>
</tr>
<tr>
<td>3.3g</td>
<td>Z-Val-N₃</td>
<td>70</td>
<td>Gum</td>
<td>299.13 (299.11)³⁴</td>
</tr>
<tr>
<td>3.3h</td>
<td>Z-Gpn-N₃</td>
<td>74</td>
<td>Gum</td>
<td>353.1595 (353.1590)</td>
</tr>
</tbody>
</table>
3.3i  Boc-Aib-N₃  78  Gum  251.1125  
(251.1120)

3.3j  Boc-Gpn-N₃  77  Gum  335.1489  
(335.1485)ᵇ

ᵃESI-MS, ᵇ[M+K]^+

3.2.2. Curtius rearrangement of acyl azides

As a second objective, we undertook the one-pot synthesis of ureidopeptides. N-Protected amino acid azides especially those derived from N-Boc and Z-protected α-amino acids are not stable to the extent of isolation. Thus, it is desirable to develop one-pot protocols which involve tandem transformation of acyl azides into isocyanates and the target molecules thereafter. To execute this, a solution of Fmoc- Ala-N₃ 3.3a prepared as described previously, was subjected to ultrasonication for 10 min. Our group had previously reported that N-protected amino acid azides can be converted to isocyanates using ultrasonic energy in a short time, almost quantitatively. The reaction can also be followed by simple IR measurements of the reaction mixture. After the completion of reaction, the in situ generated isocyanate was treated with leucinyl methyl ester. This part of the reaction was also continued under ultrasonication with an anticipation of faster generation of the urea product. As expected, the reaction was complete in 15 min. This was evident by TLC analysis and precipitation of insoluble urea adduct 3.4a from the reaction mixture. An excess of hexane was added to complete the precipitation and the product was collected by filtration. A single recrystallization afforded 3.4a in good yield with excellent purity as stable solid (Scheme 3.18).

It is to note that in conventional protocols for urea synthesis by coupling isocyanates to amines, either a base or an additive such as DMAP is added to promote the reaction.
However, in the present case, the reaction mixture already consists of two equivalents of free imidazole which can act as catalyst to conduct this reaction smoothly without the aid of any other external promoters.

\[
\begin{align*}
\text{PgHN} & \quad \text{COOH} \\
\rightarrow & \quad \text{1) CDI, NaN₃, 0 °C} \\
\text{PgHN} & \quad \text{N} \\
\text{N} & \quad \text{O} \\
\text{COOMe} & \quad \text{2) H₂N-CH(R²)-COOMe} \\
\text{Pg = Fmoc, Boc or Z group}
\end{align*}
\]

**SCHEME 3.18.** One-pot synthesis of ureidopeptides

The sequence of Curtius rearrangement was confirmed by carrying out a separate experiment without adding amine component to the reaction mixture. The acyl azide generated by the reaction of NaN₃/DMSO with N-protected amino acid derived imidazolide, underwent Curtius transformation on subjecting to ultrasonication to afford the corresponding isocyanate. This was confirmed through IR spectroscopy (2245 cm⁻¹) and complete disappearance of acyl azide peak was observed after 10 min. The protocol was extended for the synthesis of few other Fmoc-protected ureas 3.4a-c, as well as Boc/Z-protected ureidopeptides 3.4d-i. All the products were obtained in excellent yield and purity (Table 3.2).

### 3.2.3. Test for racemization

To examine whether the course of the reaction involving formation of acyl azide as well as its conversion into ureidopeptide *in situ* suffers racemization, two diastereomeric ureidopeptides were prepared by treating Z-Val-CON₃ with (R) and (S)-1-phenylethylamine. Both the products were found to be enantiomerically pure as determined by NMR analysis carried out on the crude samples of the two. The methyl group resonances
TABLE 3.2. List of Urethane Protected Ureidoacetamides

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Urea</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
<th>HRMS [M+Na]^+ Obsd (calcd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4a</td>
<td><img src="image" alt="Structure" /></td>
<td>94</td>
<td>200 (199)^a</td>
<td>476.2153 (473.2161)</td>
</tr>
<tr>
<td>3.4b</td>
<td><img src="image" alt="Structure" /></td>
<td>89</td>
<td>167-168</td>
<td>568.2432 (568.2424)</td>
</tr>
<tr>
<td>3.4c</td>
<td><img src="image" alt="Structure" /></td>
<td>88</td>
<td>171</td>
<td>640.2432 (640.2425)^b</td>
</tr>
<tr>
<td>3.4d</td>
<td><img src="image" alt="Structure" /></td>
<td>89</td>
<td>168 (169)^a</td>
<td>422.1681 (422.1692)</td>
</tr>
<tr>
<td>3.4e</td>
<td><img src="image" alt="Structure" /></td>
<td>91</td>
<td>188</td>
<td>450.23 (420.20)^a</td>
</tr>
<tr>
<td>3.4f</td>
<td><img src="image" alt="Structure" /></td>
<td>92</td>
<td>172 (170)^a</td>
<td>340.1845 (340.1848)</td>
</tr>
<tr>
<td>3.4g</td>
<td><img src="image" alt="Structure" /></td>
<td>79</td>
<td>174-175 (175)^a</td>
<td>398.2052 (398.2057)^b</td>
</tr>
<tr>
<td>3.4h</td>
<td><img src="image" alt="Structure" /></td>
<td>85</td>
<td>155-157</td>
<td>392.1944 (392.1950)</td>
</tr>
<tr>
<td>3.4i</td>
<td><img src="image" alt="Structure" /></td>
<td>83</td>
<td>155-157</td>
<td>392.1945 (392.1950)</td>
</tr>
</tbody>
</table>

^aESI-MS, ^b[M+K]^+.
of the phenylethylamine residue in Z-Val-$\psi$(NHCONH)-R-(+)-1-phenylethylamine 3.4h and Z-Val-$\psi$(NHCONH)-S-(−)-1-phenylethylamine 3.4i were observed as distinct doublets at $\delta$ 1.30, 1.32 and $\delta$ 1.29, 1.31, respectively. This clearly proves that there was no formation of an epimeric mixture (absence of two –CH$_3$ doublets when optically pure phenylethylamines were coupled) during the reaction. This in turn confirmed that the entire protocol is free from racemization.

In summary, a simple route for the preparation of N-Fmoc/Boc/Z-protected $\alpha$-amino acyl azides is reported employing CDI as carboxy activating agent. The reaction is mild and high yielding. The protocol has also been designed to carry out a one-pot synthesis of ureidopeptides starting from $N$-protected amino acids. The protocol obviates the isolation of acyl azide and isocyanate intermediates en route to urea conjugates, thus promises to be a better option and safer to operate. In addition the chirality of the starting amino acid is retained during the whole process due to the mild reaction condition. All the products were isolated and characterized by NMR and mass spectrometry.
3.3. EXPERIMENTAL

3.3.1. General procedure for the preparation of N-protected acid azides

\[ \text{PgHN} - \text{COOH} \xrightarrow{\text{CDI, NaN}_3/\text{DMSO}} \text{PgHN} - \text{CON}_3 \]

To a solution of N-protected amino acid (1.0 mmol) in THF (10.0 mL), was added CDI (162 mg, 1.0 mmol) at 0 °C and the reaction mixture was stirred for 15 min. To this, NaN\(_3\) (1.1 mmol, 72 mg) in DMSO (1-2 drops) was added and the stirring was continued for another 20 min. The solvent was removed under vacuum and diluted with CH\(_2\)Cl\(_2\) (20 mL). The organic layer was washed with 5% Na\(_2\)CO\(_3\) solution (2 x 10 mL), water (2 x 10 mL), brine (10 mL) and dried over anhydrous Na\(_2\)SO\(_4\). Solvent was evaporated in vacuo to afford the product in 88-90% yield.

3.3.1.1. (S)-(9H-Fluoren-9-yl)methyl 1-azido-1-oxopropan-2-ylcarbamate (Fmoc-Ala-N\(_3\)) (3.3a)

Yield: 299 mg (89%); white solid; mp 162 °C; \( R_f = 0.30 \) (n-hexane and EtOAc, 9:1); IR (KBr): 1698, 2135 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 1.42 \) (d, \( J = 7.2 \) Hz, 3H), 3.88 (m, 1H), 4.35 (t, \( J = 3.8 \) Hz, 1H), 4.48 (d, \( J = 6.8 \) Hz, 2H), 6.89 (br, 1H), 7.31-7.92 (m, 8H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 18.6, 47.5, 51.9, 67.5, 120.5, 125.4, 127.6, 128.3, 141.8, 144.1, 150.6, 180.9.\)

HR-MS: \( m/z \) calcd for C\(_{16}\)H\(_{14}\)N\(_4\)O\(_3\): 359.1120 [M+Na]\(^+\); found: 359.1123.
3.3.1.2. (S)-(9H-Fluoren-9-yl)methyl 1-azido-3-methyl-1-oxobutan-2-ylcarbamate \{Fmoc-Val-N\textsubscript{3}\} (3.3b)

Yield: 317 mg (87%); white solid; mp 168 °C; \(R_f = 0.30\) (n-hexane and EtOAc, 9:1); IR (KBr): 1716, 2138 cm\textsuperscript{-1}.

\(^1\text{H} \text{NMR (400 MHz, CDCl}_3\):} \(\delta = 0.93\) (d, \(J = 6.2\) Hz, 6H), 2.15 (m, 1H), 3.99 (br, 1H), 4.12 (t, \(J = 3.8\) Hz, 1H), 4.25 (d, \(J = 6.4\) Hz, 2H), 5.89 (br, 1H), 7.22-7.75 (m, 8H).

\(^1\text{C} \text{NMR (100 MHz, CDCl}_3\):} \(\delta = 17.7, 31.4, 47.7, 61.2, 67.6, 125.4, 125.5, 127.6, 128.23, 141.8, 144.2, 156.8, 180.1\).

HR-MS: \(m/z\) calcd for C\textsubscript{29}H\textsubscript{22}N\textsubscript{4}O\textsubscript{3}: 387.1433 [M+Na]\textsuperscript{+}; found: 387.1425.

3.3.1.3. (S)-tert-Butyl-4(9H-fluoren-9-yl)methoxy)carbonyl)-5-azido-5-oxopentanoate \{Fmoc-Glu(O\textsubscript{t}Bu)-N\textsubscript{3}\} (3.3c)

Yield: 351 mg (78%); white solid; mp 169 °C; \(R_f = 0.35\) (n-hexane and EtOAc, 9:1); IR (KBr): 1695, 1735, 2136 cm\textsuperscript{-1}.

\(^1\text{H} \text{NMR (400 MHz, CDCl}_3\):} \(\delta = 1.38\) (s, 9H), 1.79-1.82 (m, 2H), 2.10 (br, 1H), 2.18-2.25 (m, 2H), 3.87 (m, 1H), 4.12-4.27 (m, 3H), 7.33-7.89 (m, 8H).

\(^1\text{C} \text{NMR (100 MHz, CDCl}_3\):} \(\delta = 27.7, 31.3, 38.5, 46.5, 51.5, 65.5, 79.6, 127.2, 127.4, 127.6, 129.0, 140.6, 142.5, 155.8, 168.0, 171.5\).

ESI-MS: \(m/z\) calcd for C\textsubscript{2}\textsubscript{3}H\textsubscript{28}N\textsubscript{4}O\textsubscript{5}: 473.18 [M+Na]\textsuperscript{+}; found: 473.17.
3.3.1.4. (S)-(9H-Fluoren-9-yl)methyl 1-azido-3-(benzyleoxy)-1-oxopropan-2-ylcarbamate \{Fmoc-Ser(Bn)-N_3\} (3.3d)

Yield: 332 mg (75%); white solid; mp 146 °C; \( R_f = 0.32 \) (n-hexane and EtOAc, 9:1); IR (KBr): 1697, 2142 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 3.28 \) (s, 2H), 3.65 (d, \( J = 4.6 \) Hz, 2H), 4.15 (t, \( J = 3.8 \) Hz, 1H), 4.27 (d, \( J = 6.4 \) Hz, 2H), 4.65 (m, 1H), 6.95 (br, 1H), 7.22-7.81 (m, 13H).

\(^1^3\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 28.5, 47.6, 52.5, 68.5, 69.8, 127.0, 127.6, 128.1, 128.4, 128.7, 128.9, 129.2, 137.5, 141.1, 143.8, 156.1, 171.9.

HR-MS: \( m/z \) calcd for C\(_{28}\)H\(_{22}\)N\(_4\)O\(_4\): 481.1278 [M+K]\(^{+}\); found: 481.1285.

3.3.1.5. (S)-Benzy1 1-azido-1-oxo-3-phenylpropan-2-ylcarbamate \{Z-Phe-N\(_3\}\} (3.3e)

Yield: 285 mg (88%); white solid; mp 145 °C; \( R_f = 0.32 \) (n-hexane and EtOAc, 9:1); IR (KBr): 1695, 2132 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 2.51 \) (d, \( J = 5.6 \) Hz, 2H), 3.67 (m, 1H), 5.09 (s, 2H), 6.98 (br, 1H), 7.13-7.35 (m, 10H).

\(^1^3\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 38.1, 57.2, 67.6, 128.1, 128.6, 128.9, 129.1, 129.3, 129.7, 135.9, 136.7, 156.4, 179.8.

HR-MS: \( m/z \) calcd for C\(_{19}\)H\(_{16}\)N\(_4\)O\(_3\): 347.1120 [M+Na]\(^{+}\); found: 347.1093.

3.3.1.6. (S)-Benzy1 4-(2-azido-2-oxoethyl)-5-oxooxazolidine-3-carboxylate \{Z-Asp(oxa)-N\(_3\}\} (3.3f)

Yield: 231 mg (76%); gum; \( R_f = 0.35 \) (n-hexane and EtOAc, 9:1); IR (neat): 1712, 2146 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 2.89 \) (d, \( J = 6.4 \) Hz, 2H), 4.01 (t, \( J = 4.2 \) Hz, 2H).
Hz. 1H), 5.16 (s, 2H), 5.65 (s, 2H), 7.19-7.21 (m, 5H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 21.4, 55.6, 67.7, 79.0, 128.5, 128.9, 129.2, 136.9, 153.3, 172.6, 180.3.

HR-MS: m/z calcd for C$_{13}$H$_{12}$N$_4$O$_3$: 327.0705 [M+Na]$^+$; found: 327.0712.

3.3.1.7. (S)-Benzyl 1-azido-3-methyl-1-oxobutan-2-ylcarbamate {Z-Val-N$_3$} (3.3g)

Yield: 189 mg (70%); gum; $R_f$ = 0.32 (n-hexane and EtOAc, 9:1); IR (neat): 1702, 2142 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 0.92 (d, $J$ = 6.4 Hz, 6H), 2.18 (m, 1H), 3.95 (br, 1H), 5.36 (s, 2H), 6.23 (br, 1H), 7.22-7.70 (m, 5H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 17.5, 30.4, 53.5, 67.8, 127.2, 127.8, 128.9, 139.9, 155.8, 179.5.

ESI-MS: m/z calcd for C$_{13}$H$_{16}$N$_4$O$_3$: 299.11 [M+Na]$^+$; found: 299.13.

3.3.1.8. Benzyl 1-(azidocarbonyl)cyclohexylcarbamate {Z-Gpn-N$_3$} (3.3h)

Yield: 244 mg (74%); gum; $R_f$ = 0.35 (n-hexane and EtOAc, 9:1); IR (KBr): 1699, 2141 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 1.18-1.25 (m, 10H), 2.35 (s, 2H), 2.89 (br, 2H), 5.25 (s, 2H), 6.99 (br, 1H), 7.19-7.21 (m, 5H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 21.8, 24.7, 28.8, 37.5, 39.3, 41.8, 65.6, 126.9, 127.5, 128.9, 139.8, 155.6, 171.3.

HR-MS: m/z calcd for C$_{17}$H$_{22}$N$_4$O$_3$: 353.1590 [M+Na]$^+$; found: 353.1595.

3.3.1.9. tert-Butyl 1-azido-2-methyl-1-oxopropan-2-ylcarbamate {Boc-Aib-N$_3$} (3.3i)

Yield: 178 mg (78%); gum; $R_f$ = 0.35 (n-hexane and EtOAc, 9:1); IR (neat): 1694, 2138 cm$^{-1}$.

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\[^1\text{H} \text{NMR} \ (400 \text{ MHz, CDCl}_3): \delta = 1.39 \text{ (s, 9H), 1.47 (s, 6H), 5.02 (br, 1H)}.\]

\[^{13}\text{C} \text{NMR} \ (100 \text{ MHz, CDCl}_3): \delta = 24.5, 28.9, 52.3, 79.3, 155.6, 180.1.\]

HR-MS: \(m/z\) calcd for C\(_9\)H\(_{16}\)N\(_3\)O\(_3\): 251.1120 [M+Na]\(^+\); found: 251.1125

3.3.1.10. tert-Butyl 1-(azidocarbonyl)cyclohexylcarbamate (Boc-Gpn-N\(_3\)) (3.3j)

Yield: 228 mg (77%); gum; \(R_f = 0.35\) (n-hexane and EtOAc, 9:1); IR (neat): 1702, 2138 cm\(^{-1}\).

\[^1\text{H} \text{NMR} \ (400 \text{ MHz, CDCl}_3): \delta = 1.36 \text{ (s, 9H), 1.19-1.43 (m, 10H), 2.33 (s, 2H), 2.92 (br, 2H), 6.95 (br, 1H)}.\]

\[^{13}\text{C} \text{NMR} \ (100MHz, \text{CDCl}_3): \delta = 21.5, 24.3, 27.5, 28.3, 36.4, 39.2, 42.5, 78.6, 156.5, 171.9.\]

HR-MS: \(m/z\) calcd for C\(_{14}\)H\(_{24}\)N\(_4\)O\(_3\): 335.1485 [M+K]\(^+\); found: 335.1489.

3.3.2. General procedure for the one pot preparation of ureidopeptides

\[
\begin{align*}
\text{PgHN} &\quad \text{R}^1^\text{I} \\
\text{COOH} &\quad 1) \text{CDI, NaN}_3 \\
\text{2) H}_2\text{N-CH(R}^2\text{-COOMe} &\quad \text{PgHN} \\
\text{3.1} &\quad \text{R}^1\text{O} \\
\text{3.4} &\quad \text{R}^2\text{COOMe}
\end{align*}
\]

To a chilled solution of \(N\)-protected amino acid (1.0 mmol) in THF (10 mL), CDI (162 mg, 1.0 mmol) was added and the reaction mixture was stirred for 15 min. NaN\(_3\) (72 mg, 1.1 mmol in few drops of DMSO) was added and the stirring was continued for 20 min. Then the reaction mixture was ultrasonicated for 10 min, amino acid ester (1.1 mmol) was added and the ultrasonication was continued for another 15 min. Urea product, which precipitated out from the reaction mixture, was filtered and recrystallized using DMSO-water system. Otherwise, the product was isolated through aqueous work up.
3.3.2.1. (S)-Methyl 2-(3-((S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)ethyl)ureido)-4-methylpentanoate \{Fmoc-Ala-\psi[NHCONH]-Leu-OMe\} (3.4a)  

Yield: 426 mg (94%); white solid; mp 200 °C; \( R_f = 0.40 \) (CHCl\(_3\) and MeOH, 9:1); IR (KBr): 1649, 1698, 1739 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 0.92 \) (d, \( J = 6.4 \) Hz, 6H), 1.17 (d, \( J = 6.4 \) Hz, 3H), 1.32 (m, 2H), 1.52 (m, 1H), 3.61 (s, 3H), 3.67-3.82 (m, 2H), 4.12 (d, \( J = 7.2 \) Hz, 2H), 4.21 (t, \( J = 4.4 \) Hz, 1H), 5.01 (d, \( J = 7.2 \) Hz, 1H), 5.35 (br, 1H), 6.98 (br, 1H), 7.25-7.81 (m, 8H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.2, 22.3, 23.2, 40.1, 47.2, 48.8, 50.4, 61.6, 66.5, 120.1, 124.6, 127.2, 127.6, 141.5, 143.8, 155.5, 156.6, 171.5.

HR-MS: \( m/z \) calc for C\(_{25}\)H\(_{31}\)N\(_3\)O\(_5\): 476.2161 [M+Na]\(^+\); found: 476.2153.

3.3.2.2. (S)-Methyl 2-(3-((S)-1-(((9H-fluoren-9-yl)methoxy)carbonyl)-2-(benzzyloxy)ethyl)ureido)-3-methylbutanoate \{Fmoc-Ser(Bn)-\psi[NHCONH]-Val-OMe\} (3.4b)  

Yield: 486 mg (89%); white solid; mp 167-168 °C; \( R_f = 0.42 \) (CHCl\(_3\) and MeOH, 9:1); IR (KBr): 1648, 1701, 1738 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 0.94 \) (d, \( J = 6.4 \) Hz, 6H), 2.18 (m, 1H), 3.28 (s, 2H), 3.62 (d, \( J = 4.6 \) Hz, 2H), 3.68 (s, 3H), 3.98-4.02 (m, 2H), 4.12 (d, \( J = 7.4 \) Hz, 2H), 4.27 (t, \( J = 4.2 \) Hz, 1H), 5.82 (br, 1H), 6.51-6.72 (m, 2H), 7.33-7.75 (m, 13H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.5, 31.4, 47.3, 50.5, 52.5, 61.9, 65.9, 68.3, 69.8, 120.5, 125.6, 127.1, 127.4, 127.9, 128.8, 129.1, 136.9, 141.5, 144.7, 155.5, 156.9, 171.1.

HR-MS: \( m/z \) calc for C\(_{31}\)H\(_{35}\)N\(_3\)O\(_{6}\): 568.2424 [M+Na]\(^+\); found: 568.2432.

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3.3.2.3. (S)-tert-Butyl-4(((9H-fluoren-9-yl)methoxy)carbonyl)-4-((3-((S)-1-methoxy-1-oxo-3-phenylpropan-2-yl)ureido)butanolate \{Fmoc-Glu(O'Bu)-ψ[NHCONH]-Phe-OMe\} (3.4c)

Yield: 529 mg (88%); white solid; mp 171 °C; \( R_f = 0.45 \) (CHCl₃ and MeOH, 9:1); IR (KBr): 1644, 1692, 1731 cm⁻¹.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 1.42 \) (s, 9H), 2.11 (t, \( J = 7.2 \) Hz, 2H), 2.52 (m, 2H), 2.87 (d, \( J = 6.8 \) Hz, 2H), 3.65 (s, 3H), 4.15 (t, \( J = 4.4 \) Hz, 1H), 4.29 (d, \( J = 7.2 \) Hz, 2H), 4.40-4.47 (m, 2H), 5.65 (d, \( J = 6.0 \) Hz, 1H), 6.75-6.91 (m, 2H), 7.54-7.85 (m, 13H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 27.8, 35.3, 37.7, 46.5, 51.8, 54.3, 61.5, 62.4, 66.6, 73.5, 119.8, 124.6, 126.1, 126.8, 127.2, 128.6, 129.2, 137.6, 141.1, 143.6, 155.3, 156.6, 170.8, 171.4.

HR-MS: \( m/z \) calcd for C₄₂H₃₉N₅O₇: 640.2425 [M+K]⁺; found: 640.2432.

3.3.2.4. (S)-Methyl 2-((3-((S)-1-(benzyloxy)carbonyl)-2-phenylethyl)ureido)propanoate \{Z-Phe-ψ[NHCONH]-Ala-OMe\} (3.4d)

Yield: 355 mg (89%); white solid; mp 168 °C; \( R_f = 0.45 \) (CHCl₃ and MeOH, 9:1); IR (KBr): 1647, 1699, 1734 cm⁻¹.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 1.42 \) (d, \( J = 7.2 \) Hz, 3H), 2.72 (d, \( J = 5.2 \) Hz, 2H), 3.56 (s, 3H), 3.89 (m, 2H), 5.25 (s, 2H), 6.76-6.98 (m, 3H), 7.12-7.19 (m, 10H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.9, 36.5, 42.8, 45.7, 52.5, 62.3, 125.6, 126.3, 127.1, 127.8, 128.3, 128.9, 138.5, 139.9, 155.5, 156.5, 171.1.

HR-MS: \( m/z \) calcd for C₂₃H₂₃N₃O₅: 422.1692 [M+Na]⁺; found: 422.1681.
3.3.2.5. (S)-Methyl 2-(3-((S)-1-(benzoylcarbonyl)-2-methylpropyl)ureido)-3-phenyl propanoate \{Z-Val-$\psi$[NHCONH]-Phe-OMe\} (3.4e)

Yield: 389 mg (91%); white solid; mp 188 °C; \( R_f = 0.45 \) (CHCl\(_3\) and MeOH, 9:1); IR (KBr): 1652, 1701, 1738 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 0.78 \) (d, \( J = 5.6 \) Hz, 6H), 1.75 (m, 1H), 2.89 (d, \( J = 7.4 \) Hz, 2H), 3.56 (s, 3H), 4.51-4.82 (m, 2H), 5.25 (s, 2H), 5.69 (br, 1H), 6.96 (m, 2H), 7.14-7.21 (m, 10H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.8, 25.6, 36.8, 41.2, 48.5, 52.5, 65.9, 125.6, 126.9, 127.1, 127.9, 128.2, 128.8, 139.1, 141.6, 155.6, 156.9, 169.9.

ESI-MS: \( m/z \) calcd for C\(_{23}\)H\(_{29}\)N\(_3\)O\(_5\): 450.20 [M+Na]\(^+\); found: 450.23.

3.3.2.6. (S)-Methyl 2-(3-((S)-1-(tert-butoxycarbonyl)ethyl)ureido)-3-methylbutanoate \{Boc-Ala-$\psi$[NHCONH]-Val-OMe\} (3.4f)

Yield: 292 mg (92%); white solid; mp 172 °C; \( R_f = 0.48 \) (CHCl\(_3\) and MeOH, 9:1); IR (KBr): 1649, 1705, 1739 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 0.84 \) (d, \( J = 6.0 \) Hz, 6H), 1.25 (d, \( J = 7.2 \) Hz, 3H), 1.36 (s, 9H), 2.12 (m, 1H), 3.64 (s, 3H), 4.25-4.37 (m, 2H), 4.76 (br, 1H), 6.22 (br, 1H), 6.41 (br, 1H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.6, 17.7, 27.6, 31.9, 47.5, 51.2, 62.5, 75.4, 154.6, 156.2, 173.6.

HR-MS: \( m/z \) calcd for C\(_{14}\)H\(_{27}\)N\(_3\)O\(_5\): 340.1848 [M+Na]\(^+\); found: 340.1845.
3.3.2.7. (S)-Methyl 2-(3-((S)-1-(tert-butoxycarbonyl)-3-methylbutyl)ureido)-3-methylbutanoate \{Boc-Leu-$\psi$[NHCONH]-Val-OMe\} (3.4g)

Yield: 284 mg (79%); white solid; mp 174-175 °C; \( R_f = 0.50 \) (CHCl₃ and MeOH, 9:1); IR (KBr): 1645, 1702, 1738 cm⁻¹.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 0.93 \) (m, 12H), 1.21 (m, 2H), 1.38 (s, 9H), 1.72 (m, 1H), 2.12 (m, 1H), 3.63 (s, 3H), 4.21 (m, 1H), 4.32 (m, 1H), 5.03 (br, 1H), 6.41-6.53 (m, 2H).

\(^1^3\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.8, 18.5, 23.7, 31.0, 46.9, 52.9, 55.1, 58.3, 63.1, 76.3, 155.8, 156.3, 172.6. \)

HR-MS: \( m/z \) calcd for C₁₇H₃₃N₅O₅: 398.2057 [M+K]⁺; found: 398.2052.

3.3.2.8. (R)-Benzyl 4-methyl-3-(3-((R)-1-phenylethyl)ureido)pentanoate \{Z-Val-$\psi$[NHCONH]-(R)-1-phenylethylamine\} (3.4h)

Yield: 314 mg (85%); white solid; mp 155-157 °C; \( R_f = 0.55 \) (CHCl₃ and MeOH, 9:1); IR (KBr): 1648, 1699 cm⁻¹.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 0.98 \) (d, \( J = 5.8 \) Hz, 6H), 1.30 (d, \( J = 8.0 \) Hz, 3H), 3.28-3.41 (m, 1H), 5.10-20 (m, 2H), 5.35 (s, 2H), 6.53-6.70 (m, 3H), 7.25-7.43 (m, 10H).

\(^1^3\)C NMR (100 MHz, DMSO-\(d_6\)): \( \delta = 17.8, 18.5, 23.7, 31.0, 46.9, 52.9, 55.1, 58.3, 63.1, 76.3, 155.8, 156.3, 172.6. \)

HR-MS: \( m/z \) calcd for C₂₃H₂₇N₅NaO₃: 392.1950 [M+Na]⁺; found: 392.1944.
3.3.2.9. (R)-Benzy1 4-methyl-3-(3-((S)-1-phenylethyl)ureido)pentanoate \{Z-Val-$\psi$[NHCONH]-(S)-1-phenylethylamine\} (3.4i)

Yield: 306 mg (83%); white solid; mp 155-157 °C; $R_f = 0.55$
(CHCl$_3$ and MeOH, 9:1); IR (KBr): 1645, 1702 cm$^{-1}$.

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 0.93$ (d, $J = 6.0$ Hz, 6H), 1.29 (d, $J = 8.0$ Hz, 3H), 3.21-3.29 (m, 1H), 5.12-5.19 (m, 2H), 5.32 (s, 2H), 6.75-6.84 (m, 3H), 7.29-7.52 (m, 10H).

$^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta = 17.1, 18.3, 23.0, 30.8, 46.5, 52.3, 55.8, 58.5, 63.7, 76.1, 155.3, 156.2, 171.9$.

HR-MS: $m/z$ calcd for C$_{23}$H$_{27}$N$_3$NaO$_3$: 392.1950 [M+Na]$^+$; found: 392.1945.
3.4. SPECTRA

\( ^1H \) NMR spectrum of Fmoc-Ala-N\(_3\) 3.3a

\( ^{13}C \) NMR spectrum of Fmoc-Ala-N\(_3\) 3.3a
\(^1\)H NMR spectrum of Fmoc-Val-N\(_3\) 3.3b

\[ \text{Calcd Mass} = 387.1433 \text{ (M-Na)} \]

\[ 1 \text{ TOF MS ES}^+ \]

150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000

IIR-MS spectrum of Fmoc-Val-N\(_3\) 3.3b
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IR spectrum of Fmoc-Val-N₃ 3.3b

HR-MS spectrum of Fmoc-Ser(Bn)-N₃ 3.3d
$^1$H NMR spectrum of Z-Phe-N$_3$ 3.3e

HR-MS spectrum of Z-Gpn-N$_3$ 3.3h
$^1$H NMR spectrum of Boc-Aib-N$_3$ 3.3i

HR-MS spectrum of Boc-Aib-N$_3$ 3.3i

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HR-MS spectrum of Fmoc-Ala-ψ[NHCONH]-Leu-OMe 3.4a

$^1$H NMR spectrum of Boc-Ala-ψ[NHCONH]-Val-OMe 3.4f
$^1$H NMR spectrum of Z-Val-ψ[HNCONH]-(R)-1-phenylethylamine 3.4h

$^1$H NMR spectrum of Z-Val-ψ[HNCONH]-(S)-1-phenylethylamine 3.4i
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RP-HPLC trace of Fmoc-Ala-\(\psi\)[NCONH]-Leu-OMe 3.4a \((\lambda = 254\ nm, \text{flow: } 0.5\ \text{mL/min};\text{ column: Agilent eclipse XDB-C-18, pore size - 5}\mu\text{m, diameter } \times \text{ length } = 4.6 \times 150\ \text{mm};\text{ method: gradient } 0.1\%\ TFA \text{ water-CH}_3\text{CN; CH}_3\text{CN }30-100\%\ \text{in } 30\ \text{min})\)

RP-HPLC trace of Boc-Ala-\(\psi\)[NCONH]-Val-OMe 3.4f \((\lambda = 254\ nm, \text{flow: } 0.5\ \text{mL/min};\text{ column: Agilent eclipse XDB-C-18, pore size - 5}\mu\text{m, diameter } \times \text{ length } = 4.6 \times 150\ \text{mm};\text{ method: gradient } 0.1\%\ TFA \text{ water-CH}_3\text{CN; CH}_3\text{CN }30-100\%\ \text{in } 30\ \text{min})\)

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3.5. REFERENCES


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