Application of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) for the synthesis of acid azides

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Conversion of acids to acyl azides using peptide coupling agent TBTU has been demonstrated. The procedure is simple and applicable to a range of carboxylic acids under mild reaction conditions. The protocol is extended to the synthesis of urethane protected amino acid azides as well.

Keywords: Acid chlorides, acyl azides, peptide coupling agent, TBTU

Azides present themselves as energy-rich and flexible intermediates among many synthetic precursors and have continued to attract immense interest. An azide can be considered as a masked amino group and is used for amine protection adding an extra dimension to protection group orthogonality. Azides as dipoles undergo cycloaddition reactions with olefinic species popularly known as Huisgen reaction. They are also important intermediates in Ugi-multicomponent reactions. A member of this family, acyl azides introduced by Theodor Curtius, is one of the widely used and highly reactive species. The discovery of the rearrangement of acyl azides to the corresponding isocyanates was appropriately termed as ‘Curtius rearrangement’, which are useful precursors in the synthesis of biologically important compounds containing formamides and unnatural amino acids. Acyl azides are used for the preparation of amides and a variety of heterocycles. On the other hand, the art and practice of peptide synthesis has come a long way dating back to the beginning of the last century. Over the years, it has allowed chemists to unravel many synthetic methodologies which paved the way for the advent of new routes that rendered the syntheses more efficient and less cumbersome.

Curtius carried out the acylation of amino acids under Schotten-Baumann conditions using N-acylamino acid azides. These acylating agents have been employed in the synthesis of peptides, peptidomimetics, partially modified retro-inverso peptides and ureidopeptidomimetics. Using azide procedure, total synthesis of bovine pancreatic ribonuclease with 124 amino acids was accomplished by successive assembly of 30 peptide fragments by Yajima group. Peptidyl ureas prepared from acyl azide with high biological activity are the HIV-1 proteases, TAR-binding fragment of the Tat protein, γ-secretase and microbial alkaline proteinase inhibitors. The ureido analogs of [Leu5]enkephalin, angiotensins, gastrin antagonists, and protease inhibitors have been prepared.

Acyl azides are usually prepared from acid chlorides or mixed anhydrides, with azide ions or by the reaction of acyl hydrazines with nitrosyl precursors (Scheme I). Emil Fischer proposed the use of acid chlorides for peptide bond formation. Acid chlorides are in turn obtained from carboxylic acids by treating with SOCl2. But the longer reaction duration and the handling problems and storage along with their inconvenience in tolerating acid sensitive groups are some of the drawbacks of this method. Use of an inorganic azide, NaN3 which has poor solubility in organic solvents further compounded the problems with the use of acid chlorides. Phase transfer catalyst was also used as an additive to overcome the solubility problems. Thereafter, acid chlorides were generated in situ using cyanuric chloride/N-methylmorpholine, N,N-chloromethylenedimethylammonium chloride and subsequent coupling with an azide. Since N-Boc/Z-amino acid chlorides are unstable, these methods were not applicable and required alternate protocols. Acyl azide procedure has gained popularity, finding widespread application in coupling of amino acid residues for synthesis of peptides in solution. The main advantage of the azide procedure over acid chloride method, which frequently leads to side reactions and racemization, is the low tendency for oxazol-5(4H)-one formation due to their moderate activation and thus low affinity of the α-carbonyls towards nucleophilic oxygens. Direct conversion of carboxylic acids to acyl azides

Note
can also be achieved via mixed anhydrides by the use of alkyl chloroformate. Recently, Katritzky et al., demonstrated the synthesis of acyl azides from corresponding N-acylbenzotriazoles.

Acyl azides are also prepared by the reaction of acids with azido transfer reagent such as diphenylphosphoryl azide (DPPA) in a single step. Sureshbabu et al., reported the synthesis of Fmoc-amino acid azides and their successful application as peptide coupling agents. Recently, the applicability of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was demonstrated in the synthesis of acid azides, ureas and carbamates from carboxylic acids.

Alternatively, acid fluorides were generated in situ in the synthesis of ureidopeptides and urea linked glycosylated amino acids using Deoxo-Fluor/TMS-N₃ (Ref 33). Tetrabutylammonium nitrite was used as auxiliary reagent for the preparation of peptide azides. Another reported protocol involves the treatment of acids with triphosgene/triethylamine but is not applicable to N-Boc-amino acids because of the formation of oxazolidin-2,5-diones (Leuch’s anhydrides). Dess-Martin periodinane/NaN₃ has been used in the direct conversion of aldehydes to acyl azides. These reported protocols involve the use of expensive reagents, longer reaction duration and controlled reaction conditions which warrant an alternate protocol for the efficient and practical synthesis of acyl azides.

Peptide coupling agents including phosphonium, uronium, carbodiimides, immonium and imidazolium and organophosphorous reagents are known. These reagents enable the activation of carboxy group and its coupling with amine. Use of peptide coupling agents has numerous advantages providing scalable procedures, minimizing side reactions and easy applicability to a wide range of substrates in diverse reaction conditions and minimal synthetic steps. Carbodiimide and active ester techniques have been replaced with onium salts based upon 1-hydroxybenzotriazole (HOBT) and 7-aza-1-hydroxybenzotriazole (HOAt). Several uronium reagents such as O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), O-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and 2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TDBTU) have found widespread applications as coupling agents in solid phase peptide synthesis (SPPS), for fragment condensations and other purposes of peptide and protein chemistry.

In continuation of the interest in the development of methodologies which contribute to the synthesis of peptides and peptidomimetics, the application of peptide coupling agent, TBTU is envisaged for the synthesis of acyl azides including urethane protected amino acid azides starting from corresponding carboxylic acids.
Results and Discussion

*N*-Protected α-amino acid azides are useful precursors for peptide as well as peptidomimetic syntheses. This group has reported the synthesis of Fmoc-α-amino acid azides using acid chlorides and mixed anhydrides. These azides were isolated as shelf stable compounds. In continuation of this pursuit, for the conversion of an acid into acid azide using peptide coupling agent, TBTU, a preliminary experiment was undertaken. It was reasoned that the use of TBTU would readily facilitate the rapid conversion of an acid into acid azide due to the ready activation of the carboxy group. In a typical reaction, to a solution of 1d in THF at 0°C was added an equivalent each of TBTU and diisopropylethylamine (DIEA) followed by the addition of NaN₃ in DMSO. Formation of acyl

\[
\begin{align*}
\text{PgH}_2\text{N} & \text{COOH} \quad \text{TBTU, DIPEA} \\
& \text{NaN}_3 \text{in DMSO} \\
0^\circ \text{C} \\
\text{PgH}_2\text{N} & \text{CON}_2\text{Y} \\
\text{Pg} & = \text{Fmoc: 2a-e}; \text{Pg} = \text{Z: 2f-2g}
\end{align*}
\]

**Scheme II** — Synthesis of *N*-protected α-amino acid azides

<table>
<thead>
<tr>
<th>Compd</th>
<th>Pg-Xaa-CON₃</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
<th>HRMS [M+Na]^+ (m/z) Found (Calcd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>FmocHN</td>
<td>84</td>
<td>165(^a)</td>
<td>359.1123 (359.1120)</td>
</tr>
<tr>
<td>2b</td>
<td>FmocHNPh</td>
<td>83</td>
<td>173(^a)</td>
<td>435.1434 (435.1433)</td>
</tr>
<tr>
<td>2c</td>
<td>FmocHN</td>
<td>82</td>
<td>166(^a)</td>
<td>387.1424 (387.1433)</td>
</tr>
<tr>
<td>2d</td>
<td></td>
<td>76</td>
<td>74(^a)</td>
<td>385.1274 (385.1277)</td>
</tr>
<tr>
<td>2e</td>
<td>FmocHN</td>
<td>85</td>
<td>124(^a)</td>
<td>401.1592 (401.1590)</td>
</tr>
<tr>
<td>2f</td>
<td>ZHN</td>
<td>79</td>
<td>124</td>
<td>271.0816 (271.0807)</td>
</tr>
<tr>
<td>2g</td>
<td>ZN(^b)</td>
<td>78</td>
<td>Gum</td>
<td>327.0714 (327.0705)</td>
</tr>
</tbody>
</table>

\(^{a}\text{Ref 32}\)

**Table I** — List of *N*-urethane protected amino acid azides
azide was complete in about 15 min. A simple work-up led to the isolation of 2d in 76% yield (Scheme II, Table I), which was initially confirmed by IR (strong peak at 2135 cm\(^{-1}\) characteristic of azido group) and then confirmed through mass and NMR analysis.

The reaction was repeated to prepare N-protected amino acid azides 2a-f in good yield and purity. Acid azide 2g which is the product of the conversion of \(\mu\)-COOH of Z-Asp-OH was similarly prepared from Z-Asp-5-oxazolidinone. Except 2g, all the azides synthesized were isolated as stable solids. It is noteworthy that the present protocol is efficient in terms of execution and also facilitates the easy isolation of products.

It was then sought to extend the current protocol to the synthesis of organic azides from the corresponding acids. For this purpose, to furan-2-carboxylic acid 3a in THF at 0°C was added TBTU and DIEA followed by the addition of sodium azide in DMSO. The reaction was found to be complete in 10 min (as monitored by IR analysis). A simple workup enabled the isolation of the corresponding acid azide 4a in 82% yield (Scheme III). Similarly, TBTU was further used to prepare a series of diversely functionalized acyl azides 4a-f in excellent yield and purity (Table II).

**Experimental Section**

All solvents were freshly distilled prior to use. Melting points were determined using capillary method and are uncorrected. IR spectra were recorded on a Nicolet model impact 400 D FT-IR spectrometer.
(KBr pellets, 3 cm⁻¹ resolution). ¹H NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer. Mass spectra were recorded using high resolution mass spectrometer. The TLC analysis was carried out on pre-coated silica gel glass plates using the solvent systems ethyl acetate:hexane (10:90).

General procedure for the synthesis of Fmoc/Z-amino acid azides 2
To a solution of Fmoc/Z-amino acid (1.0 mmol) in dry THF (10.0 mL) were added DIEA (1.3 mmol) and TBTU (1.1 mmol) at 0°C followed by NaN₃ (1.5 mmol) in DMSO (1 mL). The reaction mixture was stirred for 15 min. After the completion of the reaction, the solvent was removed under reduced pressure and the reaction mixture was diluted with CH₂Cl₂. The organic layer was successively washed with 5% sodium carbonate solution, water and brine and dried over anhydrous Na₂SO₄. Solvent was removed in vacuo to obtain the desired products.

Characterization data
(R)-9H-Fluoren-9-yl)methyl 1-azido-1-oxopropan-2-ylcarbamate {Fmoc-Ala-N₃} 2a: ¹H NMR (CDCl₃, 400 MHz): δ 1.61 (s, 3H), 2.83 (br, 2H), 4.47-4.61 (m, 4H), 7.28-7.34 (m, 8H); ¹³C NMR (CDCl₃, 100 MHz): δ 17.4, 51.8, 67.5, 125.9, 126.7, 127.3, 128.3, 129.1, 129.5, 136.7, 141.2, 144.1, 157.9, 179.1; HRMS: Calcd for C₁₅H₂₀N₅O₅; m/z 359.1120 (M⁺+Na). Found: 359.1123.

(9H-Fluoren-9-yl)methyl 1-azido-1-oxopropan-2-ylcarbamate {Fmoc-Leu-N₃} 2b: ¹H NMR (CDCl₃, 400 MHz): δ 1.09 (s, 3H), 1.33 (m, 3H), 1.39-1.42 (m, 1H), 2.06 (s, 3H), 2.47 (s, 3H), 4.46-4.50 (m, 2H), 5.11 (s, 2H), 7.28-7.34 (m, 6H); ¹³C NMR (CDCl₃, 100 MHz): δ 21.6, 24.5, 26.1, 41.9, 43.6, 52.7, 66.2, 126.6, 127.8, 128.1, 141.1, 144.5, 156.2, 178.5; HRMS: Calcd for C₂₀H₁₈N₄O₅; m/z 385.1277 (M⁺+Na). Found: 385.1274.

(9H-Fluoren-9-yl)methyl 1-azido-4-methyl-1-oxo-2-descarbonylpyrrolidine-1-carboxylate {Fmoc-Pro-N₃} 2d: ¹H NMR (CDCl₃, 400 MHz): δ 1.45-1.95 (m, 4H), 3.21 (m, 2H), 4.25 (m, 2H), 4.52 (d, 2H, J = 5.9 Hz), 7.22-7.65 (m, 8H); ¹³C NMR (CDCl₃, 100 MHz): δ 24.5, 25.1, 41.9, 43.6, 52.7, 66.2, 126.6, 127.8, 128.1, 141.1, 144.5, 156.2, 178.5; HRMS: Calcd for C₂₀H₁₈N₄O₅; m/z 385.1277 (M⁺+Na). Found: 385.1274.

(9H-Fluoren-9-yl)methyl 1-azido-4-methyl-1-oxo-pentan-2-ylcarbamate {Fmoc-Leu-N₃} 2e: ¹H NMR (CDCl₃, 400 MHz): δ 1.38 (s, 3H), 5.11 (m, 1H), 5.12 (s, 2H), 7.23-7.45 (m, 5H); ¹³C NMR (CDCl₃, 100 MHz): δ 18.7, 51.8, 67.5, 125.7, 127.3, 127.5, 128.3, 129.0, 141.2, 156.1, 180.2; HRMS: Calcd for C₁₃H₂₂N₄O₅; m/z 371.0807 (M⁺+Na). Found: 371.0816.

Benzyl-1-azido-1-oxopropan-2-ylcarbamate Ze-Ala-N₃ 2f: ¹H NMR (CDCl₃, 400 MHz): δ 1.09 (s, 3H), 1.33 (m, 3H), 1.39-1.42 (m, 1H), 2.06 (s, 3H), 2.47 (s, 3H), 4.46-4.50 (m, 2H), 5.11 (s, 2H), 7.28-7.34 (m, 6H); ¹³C NMR (CDCl₃, 100 MHz): δ 21.6, 55.0, 67.8, 79.2, 128.7, 128.3, 129.6, 136.2, 153.3, 172.3; ESMS: Calcd for C₁₀H₁₄N₅O₅; m/z 327.0705 (M⁺+Na). Found: 327.0714.

Furan-2-carboxylic acid azide 4a: ¹H NMR (CDCl₃, 400 MHz): δ 6.80 (m, 1H), 7.33 (d, 1H, J = 7.0 Hz), 7.13 (d, 1H, J = 6.9 Hz); ¹³C NMR (CDCl₃, 100 MHz): δ 112.4, 120.0, 144.3, 146.5, 178.5; HRMS: Calcd for C₃H₅N₅O₂; m/z 160.0123 (M⁺+Na). Found: 160.0126.

Thiophene-2-carboxylic acid azide 4b: ¹H NMR (CDCl₃, 400 MHz): δ 6.92 (d, 1H, J = 6.8 Hz), 7.31 (d, 1H, J = 6.1 Hz), 7.32 (m, 1H); ¹³C NMR (CDCl₃, 100 MHz): δ 129.0, 130.5, 132.6, 142.5, 174.4; HRMS: Calcd for C₃H₅N₅O₂; m/z 175.9870 (M⁺+Na). Found: 175.9880.
2-(1H-Indol-3-yl) acetyl azide 4c: Rf 0.4 (n-hexane/EtOAc 8:2); IR (KBr): 2131, 1748 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 3.63 (s, 2H), 7.12 (d, 1H, J = 2.0 Hz), 7.05-7.53 (m, 4H), 8.82 (s, 1H); ¹³C NMR (CDCl₃, 100 MHz): δ 31.4, 111.1, 111.3, 120.3, 122.0, 123.4, 123.9, 127.9, 136.6, 172.3; HRMS: Calcd for C₁₆H₁₄N₂O: m/z 201.0776 (M⁺+H). Found: 201.0780.

Phenyl acetyl azide 4d: Rf 0.6 (n-hexane/EtOAc 9:1); IR (KBr): 2133, 1747 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 3.88 (s, 2H), 7.13 (m, 1H), 7.18 (d, 2H, J = 6.8 Hz), 7.38 (m, 2H); ¹³C NMR (CDCl₃, 100 MHz): δ 38.8, 127.8, 129.1, 129.9, 141.3, 181.5; HRMS: Calcd for C₉H₇N₂O: m/z 184.0487 (M⁺+Na). Found: 184.0481.

Pyridine-3-carboxylic acid azide 4e: Rf 0.3 (n-hexane/EtOAc 8:2); IR (KBr): 2133, 1744 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 7.58-7.69 (m, 2H), 8.01 (d, 1H, J = 7.6 Hz), 8.31 (s, 1H); ¹³C NMR (CDCl₃, 100 MHz): δ 126.4, 129.1, 135.3, 151.1, 152.4, 172.5; HRMS: Calcd for C₇H₈N₃O: m/z 171.0283 (M⁺+Na). Found: 171.0285.

p-Toluoyl azide 4f: Rf 0.4 (n-hexane/EtOAc 8:2); IR (KBr): 2138, 1737 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz): δ 2.76 (s, 3H), 7.62 (d, J = 8.6 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H); ¹³C NMR (CDCl₃, 100 MHz): δ 23.3, 128.4, 128.6, 129.7, 129.8, 132.5, 145.2, 180.1; HRMS: Calcd for C₁₀H₇N₃O: m/z 184.0487 [M⁺+Na]. Found: 184.0486.

Conclusion
In conclusion, the application of the peptide coupling agent, TBTU for the direct conversion of acids into acid azides is demonstrated. The use of TBTU as carboxy activator has resulted in a mild and facile synthesis of N-Fmoc/Z-protected α-amino acid azides including a wide range of organic azides. All the azides have been obtained in good yields.

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References
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