N-(Fmoc-α-aminoacyl)benzotriazoles: versatile synthetic reagents from proteinogenic amino acids

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Dedicated to Prof. Nicolo Vivona on the occasion of his 70th anniversary

Abstract
N-Fmoc-α-amino acids were smoothly converted into stable, crystalline N-(Fmoc-α-aminoacyl)benzotriazoles 2a-r (69-90%). Compounds 2b,g reacted with the chiral derivatizing reagent, (+/-)α-methylbenzylamine (5 or 6), to afford α-(N-Fmoc-amino)acid amides 3a,b and 4a,b (average yield 72%) with no detectable racemization.

Keywords: N-(Fmoc-α-aminoacyl)benzotriazoles, N-Fmoc-α-amino acids, α-aminoacylation, α-(N-Fmoc-amino)acid amides

Introduction
N-Protected α-amino acids need activation of the carboxylic acid function to allow peptide 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.1

N-Acylbenzotriazoles have been utilized in (i) N-acylation for the preparation of primary, secondary and tertiary amides2a-c and N-acylsulfonamides,2d (ii) S-acylation for the synthesis of thiol esters3 and (iii) C-acylation for the preparation of ketones,4a diketones,4b β-ketosulfones,4c enaminones4d and C-acylated pyroles, indoles,4e 2-methylfurans and thiophenes4f. We have previously prepared the N-(Boc-, Fmoc- and Cbz-α-aminoacyl)benzotriazoles of some amino acids.5a-c We now report stable, crystalline N-(Fmoc-α-aminoacyl)benzotriazoles derivatives of 18 proteinogenic amino acids and their utilization in the synthesis of chiral α-(N-Fmoc-amino)acid amides.
Results and Discussion

Preparation of N-(Fmoc-α-aminoacyl)benzotriazoles 2a-r
Eighteen of the twenty natural, N-Fmoc-α-amino acids 1a-r (purchased from Peptides International, Louisville, KY, USA and used without further purification) when treated with 1H-benzotriazole and thionyl chloride in THF at 20 °C for 2 hours\(^5a\) (Scheme 1, Table 1), afforded crystalline N-(Fmoc-α-aminoacyl)benzotriazoles 2a-r in 69-90% yields. Novel 2b-l, q-r were characterized by \(^1\)H and \(^{13}\)C NMR spectroscopy, elemental analysis and high resolution mass spectrometry; known 2a and m-p were verified by comparison of the melting points and spectroscopic data with that of the literature. The spectra of 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.\(^5b\)

\[
\text{Scheme 1. Preparation of } N-(\text{Fmoc-α-aminoacyl})\text{benzotriazoles } 2a-r \text{ from the corresponding } N-\text{protected α-amino acids } 1a-r.
\]

The N-(Fmoc-α-aminoacyl)benzotriazoles of arginine and aspargine were not obtained under these conditions; they appeared to be formed but were rapidly hydrolyzed before they could be isolated.

Preparation of α-(N-Fmoc-amino)acid amides 3a,b and 4a,b
\(\alpha\)-Methylbenzylamides of N-protected amino acids provide criteria for optical purity and stability towards racemization. N-(Fmoc-α-aminoacyl)benzotriazoles 2b,g were separately reacted with L-\(\alpha\)-methylbenzylamine 5 and D-\(\alpha\)-methylbenzylamine 6 in THF at 20 °C to afford amides 3a,b and 4a,b in 66-74 % yields (average 76 %, Scheme 2, Experimental Section).

The diastereomeric excess (\(de\)) for the α-(N-Fmoc-amino)acid amides 3a,b and 4a,b were determined by \(^1\)H NMR and HPLC. A comparison of the \(^1\)H NMR spectra obtained from the derivatization of 2b with D/L- and L-\(\alpha\)-methylbenzylamine respectively demonstrated the chirality of 3a and thus the conversion of the 1b to 2b also occurred with retention of chirality. HPLC analyses of 3a, 4a and corresponding diastereomeric mixtures provided further evidence for the smooth conversion of chiral 1b,g to chiral 2b,g (Table 2).
<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>mp (°C)</th>
<th>[α]&lt;sub&gt;D&lt;/sub&gt;</th>
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<tr>
<td>Fmoc-L-Ile-OH&lt;sup&gt;(1a)&lt;/sup&gt;</td>
<td>Fmoc-L-Ile-Bt&lt;sup&gt;(2a)&lt;/sup&gt;</td>
<td>78</td>
<td>165.4-167.2&lt;sup&gt;5c&lt;/sup&gt; (168.8-170.0)</td>
<td>-32.8</td>
</tr>
<tr>
<td>Fmoc-L-Val-OH&lt;sup&gt;(1b)&lt;/sup&gt;</td>
<td>Fmoc-L-Val-Bt&lt;sup&gt;(2b)&lt;/sup&gt;</td>
<td>84</td>
<td>148.3-149.8</td>
<td>-40.4</td>
</tr>
<tr>
<td>Fmoc-L-Thr(tBu)-OH&lt;sup&gt;(1c)&lt;/sup&gt;</td>
<td>Fmoc-L-Thr(tBu)-Bt&lt;sup&gt;(2c)&lt;/sup&gt;</td>
<td>80</td>
<td>62.2-65.0</td>
<td>-30.0</td>
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<tr>
<td>Fmoc-L-Lys(Boc)-OH&lt;sup&gt;(1d)&lt;/sup&gt;</td>
<td>Fmoc-L-Lys(Boc)-Bt&lt;sup&gt;(2d)&lt;/sup&gt;</td>
<td>75</td>
<td>138.4-140.6</td>
<td>-33.3</td>
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<tr>
<td>Fmoc-L-Glu(OtBu)-OH&lt;sup&gt;(1e)&lt;/sup&gt;</td>
<td>Fmoc-L-Glu(OtBu)-Bt&lt;sup&gt;(2e)&lt;/sup&gt;</td>
<td>81</td>
<td>65.5-67.6</td>
<td>-21.2</td>
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<tr>
<td>Fmoc-L-Ser(tBu)-OH&lt;sup&gt;(1f)&lt;/sup&gt;</td>
<td>Fmoc-L-Ser(tBu)-Bt&lt;sup&gt;(2f)&lt;/sup&gt;</td>
<td>70</td>
<td>91.7-92.4</td>
<td>-14.8</td>
</tr>
<tr>
<td>Fmoc-L-Tyr(tBu)-OH&lt;sup&gt;(1g)&lt;/sup&gt;</td>
<td>Fmoc-L-Tyr(tBu)-Bt&lt;sup&gt;(2g)&lt;/sup&gt;</td>
<td>83</td>
<td>138.4-139.3</td>
<td>+15.0</td>
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<tr>
<td>Fmoc-L-Gln(Trt)-OH&lt;sup&gt;(1h)&lt;/sup&gt;</td>
<td>Fmoc-L-Gln(Trt)-Bt&lt;sup&gt;(2h)&lt;/sup&gt;</td>
<td>69</td>
<td>167.0-168.0</td>
<td>-16.3</td>
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<tr>
<td>Fmoc-L-Asp(OtBu)-OH&lt;sup&gt;(1i)&lt;/sup&gt;</td>
<td>Fmoc-L-Asp(OtBu)-Bt&lt;sup&gt;(2i)&lt;/sup&gt;</td>
<td>73</td>
<td>102.0-104.0</td>
<td>-11.1</td>
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<tr>
<td>Fmoc-L-Cys(Trt)-OH&lt;sup&gt;(1j)&lt;/sup&gt;</td>
<td>Fmoc-L-Cys(Trt)-Bt&lt;sup&gt;(2j)&lt;/sup&gt;</td>
<td>88</td>
<td>96.0-98.0</td>
<td>-11.0</td>
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<tr>
<td>Fmoc-L-His(Trt)-OH&lt;sup&gt;(1k)&lt;/sup&gt;</td>
<td>Fmoc-L-His(Trt)-Bt&lt;sup&gt;(2k)&lt;/sup&gt;</td>
<td>73</td>
<td>137.4-139.5</td>
<td>+13.0</td>
</tr>
<tr>
<td>Fmoc-L-Leu-OH&lt;sup&gt;(1l)&lt;/sup&gt;</td>
<td>Fmoc-L-Leu-Bt&lt;sup&gt;(2l)&lt;/sup&gt;</td>
<td>80</td>
<td>121.3-123.2</td>
<td>+53.1</td>
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<tr>
<td>Fmoc-L-Trp-OH&lt;sup&gt;(1m)&lt;/sup&gt;</td>
<td>Fmoc-L-Trp-Bt&lt;sup&gt;(2m)&lt;/sup&gt;</td>
<td>90</td>
<td>92.5-93.6&lt;sup&gt;5c&lt;/sup&gt;; 192.4-195.2&lt;sup&gt;b&lt;/sup&gt; (88.0-90.0)&lt;sup&gt;5e&lt;/sup&gt;</td>
<td>+9.0</td>
</tr>
<tr>
<td>Fmoc-L-Phe-OH&lt;sup&gt;(1n)&lt;/sup&gt;</td>
<td>Fmoc-L-Phe-Bt&lt;sup&gt;(2n)&lt;/sup&gt;</td>
<td>85</td>
<td>159.1-160.2&lt;sup&gt;5c&lt;/sup&gt; (136.5-137.4)&lt;sup&gt;5d&lt;/sup&gt;</td>
<td>+3.4</td>
</tr>
<tr>
<td>Fmoc-L-Met-OH&lt;sup&gt;(1o)&lt;/sup&gt;</td>
<td>Fmoc-L-Met-Bt&lt;sup&gt;(2o)&lt;/sup&gt;</td>
<td>82</td>
<td>122.7-123.3&lt;sup&gt;5c&lt;/sup&gt; (98.0-100.0)&lt;sup&gt;5e&lt;/sup&gt;</td>
<td>-44.7</td>
</tr>
<tr>
<td>Fmoc-L-Ala-OH&lt;sup&gt;(1p)&lt;/sup&gt;</td>
<td>Fmoc-L-Ala-Bt&lt;sup&gt;(2p)&lt;/sup&gt;</td>
<td>72</td>
<td>160.0-160.3&lt;sup&gt;5c&lt;/sup&gt; (160.7-161.3)&lt;sup&gt;5d&lt;/sup&gt;</td>
<td>-60.8</td>
</tr>
<tr>
<td>Fmoc-L-Pro-OH&lt;sup&gt;(1q)&lt;/sup&gt;</td>
<td>Fmoc-L-Pro-Bt&lt;sup&gt;(2q)&lt;/sup&gt;</td>
<td>89</td>
<td>163.5-165.4&lt;sup&gt;5c&lt;/sup&gt;</td>
<td>-60.5</td>
</tr>
<tr>
<td>Fmoc-Gly-OH&lt;sup&gt;(1r)&lt;/sup&gt;</td>
<td>Fmoc-Gly-Bt&lt;sup&gt;(2r)&lt;/sup&gt;</td>
<td>88</td>
<td>161.5-161.9&lt;sup&gt;5c&lt;/sup&gt;</td>
<td>Non-chiral</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolated yield. <sup>b</sup> mp of polymorph.
Scheme 2. Preparation of N-(acylamino)amides 3a,b and 4a,b from N-(Fmoc-α-aminoacyl) benzotriazoles 2b,g and L- or D-PhCH(Me)NH2 (5 or 6).

HOBt-based aminium- and phosphonium derivatives (PyBOP, HBTU, HATU, etc), N-hydroxysuccinimide esters, and p-nitrophenyl esters are widely used in peptide synthesis but the preparative routes require multiple steps. N-(Fmoc-α-aminoacyl)benzotriazoles are easily prepared peptide coupling reagents whose generality has been demonstrated in the solution phase syntheses of sterically hindered peptides or peptoids and the solid phase preparation of simple oligopeptides. Additionally, N-(Fmoc-α-aminoacyl)benzotriazoles are fully amenable to microwave assisted syntheses.

Table 2. Preparation of N-(acylamino)amides 3a,b and 4a,b from N-(Fmoc-α-aminoacyl) benzotriazoles 2b,g and L- or D-PhCH(Me)NH2 (5 or 6)

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fmoc-L-Val-Bt (2b)</td>
<td>Fmoc-L-Val-L-NHCH(Me)Ph (3a)</td>
<td>73</td>
<td>5.42</td>
</tr>
<tr>
<td>Fmoc-L-Val-Bt (2b)</td>
<td>Fmoc-L-Val-D/L-NHCH(Me)Ph (3a+4a)</td>
<td>74</td>
<td>5.41, 8.00</td>
</tr>
<tr>
<td>Fmoc-L-Tyr(tBu)-Bt (2g)</td>
<td>Fmoc-L-Tyr(tBu)-D-NHCH(Me)Ph (3b)</td>
<td>74</td>
<td>1.91</td>
</tr>
<tr>
<td>Fmoc-L-Tyr(tBu)-Bt (2g)</td>
<td>Fmoc-L-Tyr(tBu)-D/L-NHCH(Me)Ph (3b+4b)</td>
<td>66</td>
<td>1.73, 1.91</td>
</tr>
</tbody>
</table>

*Isolated yield. *b For conditions, see the experimental section.

In summary, we describe the convenient, cost effective preparation of N-(Fmoc-α-aminoacyl)benzotriazoles 2a-r (69-90%) storable at 20 °C for months without special handling. 1H NMR and HPLC analyses of N-(Fmoc-α-aminoacyl)amides 3a,b and 4a,b, easily prepared in high yields, demonstrated that the chirality is maintained during amide bond formation.
Experimental Section

General Procedures. 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 (MgSO4) and sodium carbonate (Na2CO3) from Fischer Scientific, Fair Lawn, NJ, USA. Melting points were determined on a hot-stage apparatus and are uncorrected. 1H (300 MHz, with TMS as the internal standard) and 13C (75 MHz) NMR spectra were recorded in CDCl3. 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:H2O 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.

General procedure for the preparation of 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 was 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, 2b). White microcrystals (84%); mp 148.3-149.8 °C; [α]24 D = -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, 131.4, 131.1, 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, 2c). White microcrystals (80%); mp 62.2-65.0 °C; [α]24 D = -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); 13C 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, 2d). White microcrystals (75%); mp 138.4-140.6 °C; [α]_{D}^{24} = -30.0° (c = 1.5, CHCl_{3}); \textsuperscript{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); 13C 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, 2e). White microcrystals (81%); mp 65.5-67.6 °C; [α]_{D}^{20} = -21.2° (c = 2.4, CHCl_{3}); \textsuperscript{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); 13C 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, 2f). White microcrystals (70%); mp 91.7-92.4°C; [α]_{D}^{20} = -14.8° (c = 2.4, CHCl_{3}); \textsuperscript{1}H NMR δ 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); 13C NMR δ 169.5, 156.2, 154.4, 145.8, 143.6, 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-tert-butoxy-1-oxopropan-2-ylcarbamate (Fmoc-L-Tyr(tBu)-Bt, 2g). White microcrystals (83%); mp 138.4-139.3°C; [α]_{D}^{20} = 15.0° (c = 1.9, CHCl_{3}); \textsuperscript{1}H NMR δ 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); 13C NMR δ 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, 2h). White microcrystals (69%); mp 167.0-168.0 °C; [α]20D -16.3° (c = 1.4, CHCl3); 1H NMR δ 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), 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. Caled 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(ObBu)-Bt, 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. Caled for C29H28N4O5: C, 67.98; H, 5.81; N, 9.84. Found: C, 75.82; H, 5.46; N, 9.89.

R-(9H-Fluoren-9-yl)methyl 1-(1H-benzotriazol-1-yl)-1-oxo-3-(tritylthio)-propan-2-ylcarbamate (Fmoc-L-Cys(Trt)-Bt, 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. Caled for C43H34N4O5S: C, 72.90; 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, 2k). Yellow microcrystals (73%); mp 137.4-139.5°C; [α]24°D = -60.5° (c = 1.5, CHCl3); 1H NMR (300 Hz, CDCl3) δ 8.19 (d, J = 8.1 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.72 (d, J = 7.5 Hz, 7H), 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); 13C NMR (75Hz, CDCl3) δ 170.5, 156.3, 145.8, 143.8, 142.0, 141.1, 139.0, 135.5, 130.5, 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 calced for C43H36N6O3 [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, 2l). White microcrystals (80%); mp 121.3-123.2 °C; [α]24°D = + 53.1° (c = 1.5, DMF); 1H 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 = 7.8 Hz, 1H).
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); 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, 125.0, 120.3, 120.0, 114.4, 67.1, 53.0, 47.1, 41.9, 25.2, 23.2, 21.3. Anal. Calcd for C27H26N4O3: 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, 2q).** White microcrystals (89%); mp 163.5-165.4 °C; [α]24D = -60.5° (c = 1.5, DMF); 1H NMR δ 8.29 (d, J = 8.2 Hz, 0.5H), 8.20 (d, J = 8.4 Hz, 0.5H), 8.14 (d, J = 7.8 Hz, 1.0H), 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, 120.0, 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.

**General procedure for the preparation of 3a,b, 4a,b, (3a+4a) and (3b+4b)**

N-(Fmoc-α-aminoacyl)benzotriazoles 2b,g (1 mmol) was dissolved in THF and L-α-methylbenzylamine 5, D-α-methylbenzylamine 6 or α-methylbenzylamine (5+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 3a,b, 4a,b, (3a+4a) and (3b+4b).

**S-(9H-Fluoren-9-yl)methyl 2-(1H-benzotriazol-1-yl)-2-oxoethylicarbamate (Fmoc-Gly-Bt, 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 = 8.4 Hz, 1H), 7.77 (d, J = 7.4 Hz, 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, 126.7, 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 C23H18N4O3: C, 69.34; H, 4.55; N, 14.03; Found: C, 69.40; H, 4.36; N, 14.08.

**(9H-Fluoren-9-yl)methyl S-3-methyl-1-oxo-1-((R)-1-phenylethylamino)butan-2-ylcarbamate (3a).** White powder (73%); mp 205.7-206.2 °C; [α]25D = -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, 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 C_{28}H_{30}N_{2}O_{5}: 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 (3b). White microcrystals (74%); mp 180.1-181.1; [α]_{D}^{25} = +8.6° (c = 1.0, CHCl_{3}); 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 C_{36}H_{40}N_{2}O_{5}: 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 (4a). White powder (77%); mp 166.3-168.8°C; [α]_{D}^{25} = +22.1° (c = 2.2, CHCl_{3}); 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 (two overlapped doublets, J = 7.7 Hz, 6H); 13C NMR 170.1, 169.9, 143.8, 142.6, 141.3, 128.7, 127.9, 127.8, 127.5, 127.1, 125.0, 124.7, 124.6, 124.3, 121.8, 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_{5}: 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 (4b). White powder (79%); mp 196.3-197.7°C; [α]_{D}^{25} = -6.2° (c = 1.0, CHCl_{3}); 1H 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); 13C 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 C_{27}H_{28}N_{2}O_{5}•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 (3a+4a). Yellow oil (74%); 1H 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, 2H), 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); 13C NMR 173.8, 173.7, 146.4, 146.0, 144.0, 143.8, 141.6, 141.6, 129.1, 129.0, 129.0, 128.8, 127.8, 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\cdot COO]^− 399.2436, found 399.2393

(9H-Fluoren-9-yl)methyl S-3-(4-tert-butoxyphenyl)-1-oxo-1-(1-phenylethylamino)propan-2-ylcarbamate (3b+4b). Off-white powder (66%); mp 170.8-176.8°C; 1H NMR δ 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, 4H).
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), 4.22-4.12 (m, 2H), 3.22-2.78 (m, 4H), 1.33 (s, 18H), 1.32 (overlapped d, J = 6.3 Hz, 6H); 13C 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_{2}O_{4} [M+H]^+ 563.2910, found 563.2904.

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References