Supporting Information for

Title: A Facile Synthesis and Crystallographic Analysis of N-Protected β-Amino Alcohols and Short Peptaibols

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General Experimental Details

All amino acids, N-hydroxysuccinimide, Isobutyl Chloroformate, DiPEA, NaBH₄, TFA, were purchased from Aldrich. THF, EtOAc, were purchased from Merck. Di-tert-butyl dicarbonate obtained from spectrochem and used without further purification. THF and DiPEA were distilled over sodium prior to use. Column chromatography was performed on Merck silica gel (120-200 mesh). Reactions were monitored by thin-layer chromatography (TLC) using 60 F₂₅₄ Merck precoated silica gel plates. Visualization was accomplished with UV light and phosphomolybdic acid (PMA) or ninhydrin stain followed by charring on hot-plate. Yields refer to chromatographically pure compounds unless otherwise stated. The ¹H spectra were recorded over Jeol 400 MHz (or 100 MHz for ¹³C ) spectrometer using residual solvents signals as an internal reference (CDCl₃ δ_H, 7.24 ppm, δ_c 77.0 ppm), unless otherwise stated. The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Specific rotations were recorded using MeOH as a solvent (Rudolph Analytical Research) at ambient temperature. Melting points were determined over Veego VMP-DS hot stage apparatus and were not corrected. Chiral HPLC was performed on Waters 600 equipped with empower software using CHIRALPAK® AD Analytical Column, which was purchased from Daicel Chemical Industries. Mass spectra obtained from MALDI TOF/TOF (Applied Biosciences) and LCMS/MS (waters). Data for X-ray structure determination were obtained with Bruker APEXII DUO diffractometer using Mo-Kα (λ = 0.71073 Å) graphite monochromated radiation.
General procedure for the synthesis of Fmoc- or Boc- protected β-amino alcohol from N-hydroxysuccinimide esters:

\[
\text{PG} \quad \text{OH} \quad \text{N} \quad \text{R} \quad \rightarrow \quad \text{PG} \quad \text{OH} \\
\text{DCC, NaBH}_4 \quad \text{THF, } 0 \degree \text{C} \\
\text{70} - \text{90%}
\]

PG = Fmoc- or Boc-

Scheme 1
To a solution of Fmoc- or Boc-protected amino acid (2 mmol) and N-hydroxysuccinimide (0.345 g, 3 mmol) at 0 °C in THF (5 mL), was added DCC (0.413 g, 2 mmol), and reaction was stirred at this temperature for 1 h. precipitated DCU was filtered and washed with THF (3 X 2 mL), combined organic layer was cooled to ice temperature and the solution of sodium borohydride (0.152 g, 4 mmol) in water (1 mL) was added in one portion which leads to the vigorous evolution of gas. After 5 min, 5 mL of 0.5 N HCl was added to quench unreacted NaBH₄. Reaction mixture was extracted in EtOAc (3 X 10 mL), and combined organic layer was washed with 5% Na₂CO₃ (3 X 10 mL), brine (3 X 10 mL), dried over Na₂SO₄, and concentrated in vacuo. After work up procedure, Boc protected beta amino alcohols were obtained as pure product over 80-90% yield. Fmoc protected Products were purified over silica gel column chromatography to get pure beta amino alcohol over 70-90% yield.

General procedure for the synthesis of N-protected β-amino alcohol using mixed anhydride method:¹⁻³

\[
\text{PG} \quad \text{HN} \quad \text{R} \quad \text{O} \quad \text{OH} \\
\text{Cl} \quad \text{O} \\
\text{DiPEA} \\
\text{THF, -15 to -20 °C} \\
\text{PG} \quad \text{HN} \quad \text{R} \quad \text{OH} + \text{PG} \quad \text{N} \quad \text{R} \quad \text{O} \\
\text{NaBH}_4 \\
\text{0 - 15%}
\]

PG= Fmoc-, Boc-, etc.,

Scheme 2
Isobutyl chloroformate (0.26 mL, 2 mmol) was added dropwise to the solution of N-protected amino acid (2 mmol) and DiPEA (0.35 mL, 2 mmol) at -15 to -20 °C in THF (5 mL). After 5 min, precipitate of DiPEA hydrochloride salt was filtered and washed with THF (2 X 3 mL). Combined organic layer was cooled in salt-ice temperature and the solution of sodium borohydride (0.152 g, 4 mmol) in water (1 mL) was added in one portion which leads to strong evolution of gas. After 5 min, 5 mL of 0.5 N HCl was added to quench unreacted NaBH₄. The reaction mixture was extracted in EtOAc (3 X 10 mL), washed with 5% Na₂CO₃ (3 X 10 mL), brine (3 X 10 mL), dried over Na₂SO₄ and concentrated in vacuo.

**Peptide synthesis:** Dipeptide and tripeptide were synthesized by conventional solution-phase methods by using a fragment-condensation strategy. The tert-butyloxy carbonyl group was used for N-terminus protection, and the C-terminus was protected as a methyl ester. Deprotections were performed with trifluoroacetic acid and saponification for the N- and C-terminals, respectively. Couplings were mediated by dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt). The tripeptide Boc-Aib-Ala-Leu-OMe was prepared by [2+1] condensation involving an N-terminus dipeptide acid Boc-Aib-Ala-OH and H-Leu-OMe using DCC/N-Hydroxysuccinimide (HOSu). Another Tripeptide Boc-Ala-Leu-Val-OMe was prepared by [2+1] condensation involving N-terminus dipeptide acid Boc-Ala-Leu-OH and H-Val-OMe, by using DCC/N-Hydroxysuccinimide (HOSu).

**General procedure for the synthesis of Boc protected dipeptide alcohol and tripeptide alcohol (Peptaibol)**

\[
\begin{align*}
\text{Boc-AA-AA-OH} & \quad \xrightarrow{\text{DCC, NaBH}_4} \quad \text{Boc-AA-AA-OH} \\
\text{or} & \\
\text{Boc-AA-AA-OH} & \quad \xrightarrow{\text{THF, O}^\circ\text{C}} \quad \text{Boc-AA-AA-OH} \\
& \quad \xrightarrow{70\%-85\%} \\
\end{align*}
\]

**Scheme 3**

The previous procedure was utilized to synthesize Boc protected dipeptides and tripeptides and pentapeptide alcohols. Products were obtained in pure form after work up procedure, and were obtained over 70-90% yield.
Crystal structure information Fmoc-Valol and Boc-Ala-Valol:

Crystal structure of Fmoc-Valol (3):\(^{28}\) C\(_{20}\)H\(_{23}\)N\(_{1}\)O\(_{3}\); A colourless crystal with approximate dimensions 0.85 x 0.50 x 0.25 mm gave a Monoclinic with space group P2(1); a = 4.942(4), b =11.622(11), c = 15.783(14) Å, \(\alpha = 90^\circ\) \(\beta = 93.573(14)^\circ\) \(\gamma = 90^\circ\); \(V = 904.7(14)\) Å\(^3\); \(T = 296\) (2) K; \(Z = 2\); \(\rho_{calc} =1.195\) Mgm\(^{-3}\); 2\(\theta_{max} = 56.56^\circ\); MoK\(\alpha\lambda = 0.71073\) Å. Fine-focus sealed tube source with graphite monochromator. \(R = 0.410\) (for 2760 reflections with \(I>2\sigma(I)\)); \(wR = 0.0508\) which was refined against \(|F|^2\) and \(S = 1.456\), for 220 parameters and 4239 unique reflections. The structure was obtained by direct methods using SHELXS-97.\(^{29}\) All non-hydrogen atoms were refined isotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. \(\mu = 0.08\) mm\(^{-1}\); Minimum/maximum residual electron density –0.161/0.107 eÅ\(^{-3}\).

Crystal structure of Boc-Ala-Valol (16):\(^{28}\) C\(_{13}\)H\(_{26}\)N\(_{2}\)O\(_{4}\); A colourless crystal with approximate dimensions 0.45 x 0.35 x 0.30 mm gave orthorhombic space group P 21 21 21; a = 9.6178(4), b =13.321(2), c = 25.115(4) Å, \(\alpha = \beta = \gamma = 90^\circ\); \(V = 3217.8(8)\) Å\(^3\); \(T = 100\) (2) K; \(Z = 8\); \(\rho_{calc} =1.133\) Mgm\(^{-3}\); 2\(\theta_{max} = 56.76^\circ\); MoK\(\alpha\lambda = 0.71073\) Å. Fine-focus sealed tube source with graphite monochromator. \(R = 0.0580\) (for 5149 reflections with \(I>2\sigma(I)\)); \(wR = 0.1345\) which was refined against \(|F|^2\) and \(S = 0.851\), for 357 parameters and 7971 unique reflections. The structure was obtained by direct methods using SHELXS-97.\(^{29}\) All non-hydrogen atoms were refined isotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded, \(\mu = 0.083\) mm\(^{-1}\); Minimum/maximum residual electron density –0.233/0.224 eÅ\(^{-3}\).

**TABLE 1**

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<th>Residue</th>
<th>(\phi)</th>
<th>(\psi)</th>
<th>(\omega)</th>
<th>(\chi_1)</th>
<th>(\chi_2)</th>
<th>(\chi_3)</th>
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<td>Val-ol</td>
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<td>-68</td>
<td>---</td>
<td>-62/175</td>
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<td>---</td>
</tr>
<tr>
<td>Ala1</td>
<td>-82</td>
<td>153</td>
<td>178</td>
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<tr>
<td>(-75)</td>
<td>(153)</td>
<td>(168)</td>
<td>---</td>
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<tr>
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<td>47</td>
<td>--</td>
<td>-48/-171</td>
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</tr>
<tr>
<td>(-127)</td>
<td>(139)</td>
<td>---</td>
<td>(67/-161)</td>
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</table>
The conformation of Fmoc-Valol was studied in the crystals. The crystal structure of Fmoc-Valol is shown in Fig. 2A. The crystals were obtained after slow evaporation of ethyl acetate. The crystal structure showed that, the OH group of the alcohol is in gauche conformation with Val side chain (g+g- conformation) and the NH group. The torsional angles $\phi$ and $\psi$ were found to be $-96^\circ$ and $-70^\circ$, respectively. This energetically unfavorable conformation is stabilized by the intermolecular H-bonding of NH and OH groups with the urethane carbonyl of neighboring molecule. In the case of dipeptide alcohol 16, single crystals were obtained after slow evaporation of methanol solution. The crystal structure is shown in Fig. 1B. Two molecules are appeared in the asymmetric unit with relevant variation in the torsional values. The torsional variables are given in the Table 1. The dipeptide alcohol adapted irregular structure in the crystal packing. Surprisingly, energetically unfavorable cis Boc-urethane bond is observed in the molecule B (Fig.1B). The reason for adapting trans and cis conformations of Boc-urethane in the same molecule is unclear.
Spectroscopic Data for N-protected β-amino alcohols obtained from the N-hydroxysuccinimide method

(R)-(9H-fluoren-9-yl)methyl 1-hydroxypropan-2-ylcarbamate (1) : white solid (0.505 g, 85%), mp 148-151 °C; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.77 (d, $J = 7.36$ Hz, 2H), 7.58 (d, $J = 7.32$ Hz, 2H), 7.40 (t, $J = 7.50$ Hz, 2H), 7.31 (td, $J = 0.85$ Hz, $J = 7.60$ Hz, 2H), 4.94 (bs, 1H), 4.42 (d, $J = 6.44$ Hz, 2H), 4.22 (t, $J = 6.44$ Hz, 1H), 2.40 (bs, 1H), 1.18 (d, $J = 6.43$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 156.56, 143.85, 141.29, 127.67, 127.02, 124.99, 119.94, 66.78, 48.91, 47.21, 33.87, 25.72, 24.92, 17.23; MALDI TOF/TOF - m/z calcd. for C$_{18}$H$_{19}$NO$_3$ [M+K]$^+$ 336.1002, obsrvd. 335.9430. $[\alpha]_D^{25} = +2$ (c = 1, MeOH).

(S)-(9H-fluoren-9-yl)methyl 1-hydroxypropan-2-ylcarbamate (2) : white solid (0.534 g, 90%); mp 150-153°C; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.77 (d, $J = 7.36$ Hz, 2H), 7.60 (d, $J = 7.32$ Hz, 2H), 7.47 (t, $J = 7.52$ Hz, 2H), 7.32 (td, $J = 0.9$ Hz, $J = 7.62$ Hz, 2H), 4.86 (bs, 1H), 4.43 (d, $J = 6.44$ Hz, 2H), 4.22 (t, $J = 6.44$ Hz, 1H), 3.83 (bs, 1H), 1.18 (d, $J = 6.44$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 156.60, 143.86, 141.32, 127.69, 127.04, 125.01, 119.97, 66.92, 66.62, 48.94, 47.23, 17.34; MALDI TOF/TOF - m/z calcd. for C$_{18}$H$_{19}$NO$_3$ [M+K]$^+$ 336.1002, obsrvd. 335.9779, $[\alpha]_D^{25} = -2.0$ (c = 1, MeOH).
(S)-(9H-fluoren-9-yl)methyl 1-hydroxy-3-methylbutan-2-ylcarbamate (3): white solid (0.520 g, 80%), mp 121-122 °C, $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.75 (d, $J = 7.33$ Hz, 2H), 7.59 (d, $J = 7.30$ Hz, 2H), 7.45 (t, $J = 7.51$ Hz, 2H), 7.32 (td, $J = 0.9$ Hz, $J = 7.62$ Hz, 2H), 4.90 (bs, 1H), 4.45 (d, $J = 6.44$ Hz, 2H), 4.21 (t, $J = 6.44$ Hz, 1H), 3.66 (m, 2H), 3.46 (m, 1H), 2.04 (bs, 1H), 1.83 (m, 1H), 0.94 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 157.05, 143.86, 141.32, 127.65, 127.02, 124.97, 119.94, 66.54, 63.72, 58.53, 47.29, 29.15, 19.47, 18.61; MALDI TOF/TOF - m/z calcd. for C$_{20}$H$_{23}$NO$_3$ [M+Na]$^+$ 348.1576, obsrvd. 348.0117, $[\alpha]_D^{25}$ = -13.0 (c = 1, MeOH).

(S)-(9H-fluoren-9-yl)methyl 1-hydroxy-4-methylpentan-2-ylcarbamate (4): white solid (0.549 g, 81%), mp 133-135°C, $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.77 (d, $J = 7.76$ Hz, 2H), 7.59 (d, $J = 7.32$ Hz, 2H), 7.40 (t, $J = 7.32$ Hz, 2H), 7.32 (t, $J = 7.32$ Hz, 2H), 4.78 (bs, 1H), 4.45 (d, $J = 6.88$ Hz, 2H), 4.21 (t, $J = 6.88$ Hz, 1H), 3.85 (m, 1H), 3.58 (m, 1H), 1.63 (m, 3H), 1.32 (m, 1H), 0.92 (dd, $J = 2.28$ Hz, $J = 6.2$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.76, 143.85, 141.33, 127.67, 127.03, 125.0, 119.95, 66.47, 66.05, 51.30, 47.30, 40.34, 24.72, 23.04, 22.10; MALDI TOF/TOF - m/z calcd. for C$_{21}$H$_{25}$NO$_3$ [M+K]$^+$ 378.1472, obsrvd. 377.9655, $[\alpha]_D^{25}$ = -20.1 (c = 1, MeOH).
(9H-fluoren-9-yl)methyl (2S,3R)-1-hydroxy-3-methylpentan-2-ylcarbamate (5): white solid (0.423 g, 65%), mp 114-116 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.77 (d, \(J = 7.8 \text{ Hz}, 2\)H), 7.60 (d, \(J = 7.32 \text{ Hz}, 2\)H), 7.41 (t, \(J = 7.36 \text{ Hz}, 2\)H), 7.32 (dt, \(J = 1.36 \text{ Hz}, J = 7.32 \text{ Hz}, 2\)H), 4.88 (bd, \(J = 8.24 \text{ Hz}, 1\)H), 4.44 (d, \(J = 6.4 \text{ Hz}, 2\)H), 4.22 (t, \(J = 6.4 \text{ Hz}, 1\)H), 3.54 (m, 2H), 5.28 (bd, \(J = 7.76 \text{ Hz}, 1\)H), 4.41 (m, 2H), 4.23 (t, \(J = 6.88 \text{ Hz}, 1\)H), 3.96 (m, 1H), 3.66 (m, 3H), 2.86 (bs, 1H), 1.21 (s, 9H), 1.16 (d, \(J = 5.96 \text{ Hz}, 3\)H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 157.00, 143.85, 141.32, 127.65, 127.02, 124.98, 119.94, 66.51, 63.49, 57.36, 47.31, 35.83, 25.40, 15.48, 11.35; MALDI TOF/TOF - m/z calcd. for C\(_{21}\)H\(_{25}\)NO\(_3\) [M+K]+ 378.1472, obsrvd. 377.9658, \([\alpha]_D^{25} = -11.7 \) (c = 1, MeOH).

(9H-fluoren-9-yl) methyl (2R, 3S)-3-tert-butoxy-1-hydroxybutan-2-ylcarbamate (6): colorless oil (0.651 g, 85%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.76 (d, \(J = 7.76 \text{ Hz}, 2\)H), 7.61 (d, \(J = 7.6 \text{ Hz}, 2\)H), 7.40 (t, \(J = 7.32 \text{ Hz}, 2\)H), 7.32 (t, \(J = 7.32 \text{ Hz}, 2\)H), 5.28 (bd, \(J = 7.76 \text{ Hz}, 1\)H), 4.41 (m, 2H), 4.23 (t, \(J = 6.88 \text{ Hz}, 1\)H), 3.96 (m, 1H), 3.66 (m, 3H), 2.86 (bs, 1H), 1.21 (s, 9H), 1.16 (d, \(J = 5.96 \text{ Hz}, 3\)H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 157.28, 144.16, 141.56, 127.94, 127.30, 125.34, 120.23, 74.59, 67.45, 67.06, 63.96, 57.39, 47.53, 28.91, 20.36; MALDI TOF/TOF - m/z calcd. for C\(_{23}\)H\(_{29}\)NO\(_4\) [M+Na]+ 406.1994, obsrvd. 406.0422; \([\alpha]_D^{25} = +6.1 \) (c = 1, MeOH).
(S)-tert-butyl 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-hydroxybutanoate (7): white solid (0.730 g, 92%), mp 93-96 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.77 (d, \(J = 7.8\) Hz, 2H), 7.59 (d, \(J = 7.32\) Hz, 2H), 7.40 (t, \(J = 7.32\) Hz, 2H), 7.31 (t, \(J = 7.32\) Hz, 2H), 5.54 (bs, 1H), 4.40 (d, \(J = 6.92\) Hz, 2H), 4.22 (t, \(J = 6.92\) Hz, 1H), 4.03 (m, 1H), 3.74 (m, 2H), 2.57-2.52 (m, 2H), 1.46 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 171.10, 156.30, 143.79, 141.28, 127.69, 127.03, 125.02, 119.96, 82.00, 67.95, 66.84, 64.53, 49.92, 47.15, 37.26, 28.00; MALDI TOF/TOF- m/z calcd. for C\(_{23}\)H\(_{27}\)NO\(_5\) [M+K]+ 436.1526, obsrvd. 435.9662; \([\alpha]_D^{25}\) = -7.4 (c = 1, MeOH).

(S)-(9H-fluoren-9-yl)methyl6-((tert-butoxycarbonyl)amino)-1-hydroxyhexan-2-ylcarbamate (8): white solid (0.681 g, 75%), mp 136-138 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.75 (d, \(J = 7.77\) Hz, 2H), 7.60 (d, \(J = 7.31\) Hz, 2H), 7.41 (t, \(J = 7.32\) Hz, 2H), 7.32 (t, \(J = 7.32\) Hz, 2H), 5.10 (bs, 1H), 4.60 (bs, 1H), 4.40 (d, \(J = 6.90\) Hz, 2H), 4.21 (t, \(J = 6.90\) Hz, 1H), 3.62 (bs, 3H), 3.18-3.07 (m, 2H), 2.56 (bs, 1H), 1.67 (m, 2H), 1.47 (m, 2H), 1.43 (s, 9H), 1.36 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.78, 156.53, 144.00, 141.42, 127.77, 127.15, 125.16, 120.05, 80.00, 66.69, 64.78, 53.03, 47.38, 39.61, 30.43, 30.09, 28.50, 22.60; MALDI TOF/TOF- m/z calcd. for C\(_{26}\)H\(_{34}\)N\(_2\)O\(_5\) [M+Na]+ 477.2399, obsrvd. 477.2375 and m/z calcd. for C\(_{21}\)H\(_{26}\)N\(_2\)O\(_3\) [M-Boc+H]+ 355.2022, obsrvd. 355.1945; \([\alpha]_D^{25}\) = -6.9 (c = 1, MeOH).
(S)-(9H-fluoren-9-yl)methyl 1-hydroxy-4-oxo-4-(tritylamino)butan-2-ylcarbamate (9): white solid (1.048 g, 90%)\(^{\text{a}}\), mp 155-159 °C; \(^1\text{H NMR} (400 \text{ MHz, CDCl}_3) \delta 7.74-7.17 (\text{ m, 23H}), 7.03 (\text{ bs, 1H}), 5.77 (\text{ d, } J = 7.28 \text{ Hz, 1H}), 4.32 (\text{ m, 2H}), 4.15 (\text{ t, } J = 6.88 \text{ Hz, 1H}), 3.91 (\text{ m, 1H}), 3.64 (\text{ m, 2H}), 3.43 (\text{ bs, 1H}), 2.71 (\text{ m, 2H}); \(^{13}\text{C NMR} (100 \text{ MHz, CDCl}_3) \delta 171.00, 144.07, 141.25, 128.53, 128.02, 127.70, 125.05, 119.96, 80.7, 70.95, 66.88, 49.97, 47.10, 39.50; \text{MALDI TOF/TOF- } m/z \text{ calcd. for C}_{38}\text{H}_{34}\text{N}_2\text{O}_4 [\text{M+Na}]^+ 605.2416, \text{ obsrvd. 605.2652}; [\alpha]_D^{25} = -12.3 (c = 1, \text{ MeOH}).

(S)-(9H-fluoren-9-yl)methyl 1-hydroxy-5-oxo-5-(tritylamino)pentan-2-ylcarbamate (10): white solid (1.013 g, 85%), mp 74-77 °C; \(^1\text{H NMR} (400 \text{ MHz, CDCl}_3) \delta 7.72 (\text{ d, } J = 7.36 \text{ Hz, 2H}), 7.56 (\text{ d, } J = 7.36 \text{ Hz, 2H}), 7.39-7.16 (\text{ m, 19H}), 6.93 (\text{ s, 1H}), 5.36 (\text{ d, } J = 8.72 \text{ Hz, 1H}), 4.50 (\text{ bs, 1H}), 4.37 (\text{ m, 2H}), 4.17 (\text{ t, } J = 6.88 \text{ Hz, 1H}), 5.53 (\text{ m, 1H}), 3.39 (\text{ m, 2H}), 2.28 (\text{ m, 2H}), 1.79 (\text{ m, 2H}); \(^{13}\text{C NMR} (100 \text{ MHz, CDCl}_3) \delta 172.40, 156.83, 144.54, 143.94, 141.41, 128.73, 128.06, 127.80, 127.18, 125.19, 120.08, 70.75, 66.59, 64.09, 52.76, 47.36, 33.57, 26.40; \text{MALDI TOF/TOF- } m/z \text{ calcd. for C}_{39}\text{H}_{36}\text{N}_2\text{O}_4 [\text{M+Na}]^+ 619.2573, \text{ obsrvd. 619.2513}; [\alpha]_D^{25} = -5.8 (c = 1, \text{ MeOH}).
(S)-tert-butyl 1-hydroxypropan-2-ylcarbamate (11): colorless oil (0.280 g, 80%); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.74 (bs, 1H), 3.77 (m, 1H), 3.57 (m, 2H), 1.44 (s, 9H), 1.15 (d, $J$ = 6.88 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.45, 79.72, 67.15, 48.57, 28.44, 17.39; LCMS/MS - m/z calcd. for C$_8$H$_{17}$NO$_3$ [M+Na]$^+$ 198.1106, obsrvd. 198.1172; $[\alpha]_{D}^{25} = -8.5$ (c = 1, MeOH).

(S)-tert-butyl 1-hydroxy-3-methylbutan-2-ylcarbamate (12): colorless oil (0.312 g, 77%); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.83 (bs, 1H), 3.52 (m, 2H), 3.43 (m, 1H), 3.10 (bs, 1H), 1.82 (m, 1H), 0.94 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.97, 79.57, 64.05, 58.04, 29.33, 28.34, 19.58, 18.53; LCMS/MS - m/z calcd. for C$_{10}$H$_{21}$NO$_3$ [M+Na]$^+$ 226.1419, obsrvd. 226.1481; $[\alpha]_{D}^{25} = -17.6$ (c = 1, MeOH).

(S)-tert-butyl 1-hydroxy-4-methylpentan-2-ylcarbamate (13): colorless oil (0.369 g, 85%); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.75 (bs, 1H), 3.71 (m, 1H), 3.55 (m, 2H), 2.98 (bs, 1H), 1.67 (m, 1H), 1.45 (s, 9H), 0.93 (dd, $J$ = 1.4 Hz, $J$ = 6.42 Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 156.64, 79.60, 66.35, 50.97, 40.58, 28.64, 25.00, 24.86, 23.12, 22.27; LCMS/MS - m/z calcd. for C$_{11}$H$_{23}$NO$_3$ [M+Na]$^+$ 240.1576, obsrvd. 240.1544; $[\alpha]_{D}^{25} = -23.2$ (c = 1, MeOH).
(S)-tert-butyl 2-(hydroxymethyl)pyrrolidine-1-carboxylate (14): colorless oil (0.313 g, 78%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 3.98 (m, 1H), 3.59 (m, 2H), 3.92 (m, 2H), 2.04 (m, 2H), 1.82 (m, 2H), 1.47 (s, 9H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 157.27, 80.35, 67.69, 60.21, 47.63, 30.09, 24.38, 22.03; LCMS/MS - m/z calcd. for C\(_{10}\)H\(_{19}\)NO\(_3\) [M+Na]\(^+\) 224.1263, obsrvd. 224.1223; \([\alpha]\) \(D\)\(_{25}\) = -45.7 (c = 1, MeOH).

(S)-tert-butyl 1-hydroxy-3-(1H-indol-3-yl)propan-2-ylcarbamate (15): white solid (0.414 g, 75%), mp 99-103 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.30 (bs, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.19 (t, J = 7.32 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 7.00 (d, J = 6.88 Hz, 1H), 4.92 (bs, 1H), 3.97 (m, 1H), 3.60 (m, 2H), 2.97 (d, J = 5.96 Hz, 2H), 2.78 (bs, 1H), 1.42 (s, 9H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 156.60, 136.35, 127.73, 122.90, 122.15, 119.46, 118.93, 111.71, 111.31, 79.83, 64.85, 53.22, 28.46, 27.01; LCMS/MS - m/z calcd. for C\(_{16}\)H\(_{22}\)N\(_2\)O\(_3\) [M+K]\(^+\) 329.1267, obsrvd. 328.9698; \([\alpha]\) \(D\)\(_{25}\) = -22.2 (c = 1, MeOH).

Spectroscopic Data for the Boc-protected peptaibols

tert-butyl (S)-1-((S)-1-hydroxy-3-methylbutan-2-ylamino)-1-oxopropan-2-ylcarbamate (16): off-white solid (0.471 g, 86%); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.57 (bs, 1H), 5.14 (bs, 1H), 5.14 (bs, 1H),
4.13 (m, 1H), 3.70 (m, 2H), 3.50 (m, 1H), 1.89 (m, 1H), 1.44 (s, 9H), 1.37 (d, J = 6.88 Hz, 3H), 0.93 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.59, 155.99, 80.58, 63.76, 57.34, 50.57, 33.98, 28.36, 25.01, 19.61, 18.67; MALDI TOF/TOF- m/z calcd. for C$_{13}$H$_{26}$N$_2$O$_4$ [M+Na]$^+$ 297.1790, obsrvd. 297.0301; [$\alpha$]$_D^{25}$ = -22.2 (c = 1, MeOH).

$tet$-butyl(S)-1-((S)-1-hydroxy-3-methylbutan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate (17) : off-white solid (0.495 g, 82%); $^1$H NMR (400 MHz, CDCl$_3$) δ 6.36 (bs, 1H), 5.12 (bs, 1H), 3.84 (m, 1H), 3.67 (m, 3H), 1.89 (m, 2H), 1.44 (s, 9H), 0.96 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.59, 155.99, 80.58, 63.76, 57.34, 50.57, 33.98, 29.01, 28.36, 25.67, 25.01, 19.60, 18.67; MALDI TOF/TOF- m/z calcd. for C$_{15}$H$_{30}$N$_2$O$_4$ [M+K]$^+$ 341.1843, obsrvd. 341.0229; [$\alpha$]$_D^{25}$ = -34.7 (c = 1, MeOH).

$tet$-butyl(S)-1-((S)-1-hydroxy-4-methylpentan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate (18) : off-white solid (0.518 g, 82%); $^1$H NMR (400 MHz, CDCl$_3$) δ 6.11 (bs, 1H), 5.02 (bs, 1H), 4.08 (m, 1H), 3.84-3.66 (m, 2H), 3.53 (m, 1H), 2.16 (m, 1H), 1.62 (m, 2H), 1.45 (s, 9H), 1.35 (m, 1H), 0.93 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.27, 156.23, 80.31, 65.93, 60.71, 50.14, 39.99, 30.42, 28.34, 24.87, 23.15, 22.11, 19.43; MALDI TOF/TOF- m/z calcd. for C$_{16}$H$_{32}$N$_2$O$_4$ [M+K]$^+$ 355.1999, obsrvd. 355.0220; [$\alpha$]$_D^{25}$ = -35.9 (c = 1, MeOH).
tert-butyl  (S)-1-((S)-1-((S)-1-hydroxy-3-methylbutan-2-ylamino)-4-methyl-1-oxopentan-2-ylamino)-1-oxopropan-2-ylcarbamate (19) : white solid (0.612 g, 79%); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.69 (m, 1H), 5.10 (d, $J = 5.36$ Hz, 1H), 4.36 (m, 1H), 4.22 (d, $J = 7.36$ Hz, 1H), 4.11 (m, 1H), 3.66 (m, 2H), 3.47 (m, 1H), 3.03 (bs, 1H), 1.71 (m, 4H), 1.45 (s, 9H), 1.36 (d, $J = 7.08$ Hz, 3H), 1.16-0.89 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 173.35, 172.43, 156.92, 80.96, 63.86, 57.66, 49.22, 40.51, 34.01, 30.39, 28.93, 25.68, 25.02, 23.12, 19.61, 19.06, 17.85; MALDI TOF/TOF - m/z calcd. for C$_{19}$H$_{37}$N$_3$O$_5$ [M+K]$^+$ 426.2370, obsrvd. 426.0512; $[\alpha]_D^{25} = -34.59$ (c = 1, MeOH).

tert-butyl  1-((S)-1-((S)-1-hydroxy-4-methylpentan-2-ylamino)-1-oxopropan-2-ylamino)-2-methyl-1-oxopropan-2-ylcarbamate (20) : white solid (0.567 g, 76%); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.07 (bs, 1H), 6.57 (bd, $J = 5.04$ Hz, 1H), 5.10 (bs, 1H), 4.22 (m, 1H), 3.70 (m, 2H), 3.47 (m, 1H), 3.09 (bs, 1H), 1.94-1.68 (m, 12H), 1.46 (s, 9H), 0.90 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 175.10, 172.58, 81.37, 65.67, 60.51, 57.01, 50.26, 39.55, 34.01, 30.39, 28.31, 25.68, 25.02, 22.27, 17.80, 14.27; MALDI TOF/TOF - m/z calcd. for C$_{18}$H$_{35}$N$_3$O$_5$ [M+K]$^+$ 412.2214, obsrvd. 412.0356; $[\alpha]_D^{25} = -13.6$ (c = 1, MeOH).
**tert-butyl (3S,6S,9S,12S,15S)-15-(hydroxymethyl)-6-isobutyl-12-isopropyl-2,9,17-trimethyl-4,7,10,13-tetraoxo-5,8,11,14-tetraazaotdecacan-3-yl carbamate:** white solid (0.032 g, 65%);

$^1$H NMR (400 MHz, DMSO $d_6$) $\delta$ 7.97 (d, $J = 8$ Hz, 1H, -NH of Ala$_3$), 7.79 (d, $J = 8.24$ Hz, 1H, -NH of Leu$_2$), 7.57 (d, $J = 8.72$ Hz, 1H, -NH of Val$_4$), 7.46 (d, $J = 8.60$ Hz, 1H, -NH of Leu$_5$), 6.71 (d, $J = 8.72$ Hz, 1H, -NH of Val$_1$), 4.60 (t, $J = 5.96$ Hz, 1H, -OH), 4.25 (m, 2H, $\alpha$ CH of Leu$_2$ and Ala$_3$), 3.97 (m, 1H, $\alpha$ CH of Val$_4$), 3.68 (m, $\alpha$ CH of Val$_1$ and $\beta$ CH of Leu$_5$), 3.22-3.09 (m, 2H, $\alpha$ CH$_2$ of Leu$_5$), 1.85 (m, 2H, $\beta$ CH of Val$_1$ and Val$_4$), 1.52 (m, $\gamma$ CH of Leu$_2$ and $\delta$ CH of Leu$_5$), 1.36 (m, 2H, $\beta$ CH$_2$ of Leu$_2$), 1.31 (s, 9H, $t$ Boc of Val$_1$), 1.20 (m, 2H, $\gamma$ CH$_2$ of Leu$_5$), 1.10 (d, $J = 6.88$ Hz, 3H, $\beta$ CH$_3$ of Ala$_3$), 0.69-0.81 (m, 24H, $\gamma$ CH$_3$ of Val$_1$ and Val$_4$, $\delta$ CH$_3$ of Leu$_2$, $\omega$ CH$_3$ of Leu$_5$);

MALDI TOF/TOF - m/z calcd. for C$_{30}$H$_{57}$N$_5$O$_7$ [M+K]$^+$ 638.3895, obsrvd. 638.1087 and m/z calcd. for C$_{30}$H$_{57}$N$_5$O$_7$ [M- $t$ Boc + Na]$^+$ 522.3631, obsrvd. 522.1472.

**Chiral HPLC trace**

![Chiral HPLC trace](image)

**Fmoc-Ile-ol (5):** The HPLC was performed on chiral pack AD column using 90% isopropanol in $n$-hexane as a solvent system at isocratic mode with the flow rate of 1 mL/min.
HPLC traces for short peptaibols

**Boc-Ala-Valol (16):** Used a HPLC gradient system equipped with a detector (220 nm) and a reversed-phase C18 column (4.6 mm × 250 mm; 5 µm). A linear gradient of 60–95% (vol/vol) solvent B in solvent A for 50 min was used. (flow rate 1 mL min − 1; solvent A: 0.1% (vol/vol) TFA in H₂O, solvent B: 0.1% (vol/vol) TFA in MeOH)
**Boc-Aib-Ala-Leuol(20):** Used a HPLC gradient system equipped with a detector (220 nm) and a reversed-phase C18 column (4.6 mm × 250 mm; 5 µm). A linear gradient of 60–95% (vol/vol) solvent B in solvent A for 50 min was used. (flow rate 1 mL min − 1; solvent A: 0.1% (vol/vol) TFA in H$_2$O, solvent B: 0.1% (vol/vol) TFA in MeOH)

**Boc-Val-Leu-Ala-Val-Leuol(21):** Reverse HPLC gradient system equipped with a detector (220 nm) and a reversed-phase C18 column (4.6 mm × 250 mm; 5 µm). A linear gradient of 60–95% (vol/vol) solvent B in solvent A for 50 min was used. (flow rate 1 mL min − 1; solvent A: 0.1% (vol/vol) TFA in H$_2$O, solvent B: 0.1% (vol/vol) TFA in MeOH)
Copies of $^1$H NMR, $^{13}$C NMR and Mass Spectra for Products
Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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![Chemical Structure Image]

54
Final - Shots 750 - HNG GROUP; Label G8

328.9988 (R3928, S2123)

calcd. [M+K]+ 329.1267

Mass (m/z)
69
Final - Shots 750 - HNG GROUP; Label G10

355.0220(R8209,S9599)


Mass (m/z)
Final - Shots 750 - HNG GROUP; Label F10

Calcd. [M+K]^+ 426.2370

Mass (m/z)
Final - Shots 750 - HNG GROUP; Label G13

412.0356 (R596, S1617)

calc. IM+k^+ 412.2214

Mass (m/z)


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Final - Shots 750 - IISER; Label A1

638.1087 (R8644, S3129)

\[ [M + K]^{+} \]

Calcd. \[ [M + K]^{+} = 638.3895 \]

\[ [M - \text{Boc + Na}]^{+} = 522.3631 \]

522.1472 (R8194, S1018)

\[ [M - \text{Boc + Na}]^{+} \]

538.1145 (R7648, R998)

622.1596 (R7895, S285)

\[ [M_{2}\text{Boc + K}]^{+} \]

Mass (m/z)

99.0 279.8 460.6 641.4 822.2 1003.0 3875.0
References


2. G. Kokotos, Synthesis, 1990, 299-301,