Supporting Information for

Azide and Alkyne-Functionalised α- and β^3^-Amino Acids

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1. General experimental

For reactions conducted under anhydrous conditions glassware was dried overnight in an oven at 150 °C and was allowed to cool in a desiccator over anhydrous KOH. Anhydrous reactions were carried out under nitrogen. Reagents/solvents for anhydrous reactions were obtained from a Pure Process Technology Glass Contour solvent purification system (SPS) (CH₂Cl₂, THF, DMF) or were dried over 3Å molecular sieves (MeCN, MeOH, PhMe, NMP, pyridine). Commercially acquired chemicals were used without further purification unless otherwise stated. Sulfate buffer was prepared by dissolving 1.5 mol of Na₂SO₄ in 0.5 mol H₂SO₄ and adding water to give a total volume of 2000 ml. A diazomethane distillation kit from Sigma Aldrich was used for the distillation of diazomethane and applied according to the manufacturer’s instructions using Diazald® as the diazomethane precursor. Thin layer chromatography was carried out on commercially available pre-coated aluminium sheets (Merck 60F254) and visualised using UV light (254 nm) or with I₂/SiO₂ or Ninhydrin stain. The quoted Rₜ values are rounded to the nearest 0.05. Flash column chromatography was carried out according to the published procedure using silica gel 60 (40-63 μm mesh).¹ Dry column vacuum chromatography was carried out according to the published procedure using Merck silica gel 60 (15-40 μm mesh).²

NMR was run on a Bruker Avance 400 or 500 NMR spectrometer (400 and 500 MHz, respectively). Chemical shifts are reported in parts per million (ppm) with reference to tetramethylsilane (TMS) or the appropriate NMR solvent as internal standard. Coupling constants (J) are reported in Hertz (Hz), rounded to the nearest 0.5 Hz. Signal assignment was made from unambiguous chemical shifts and COSY, HMQC, HMBC, APT, DEPTQ, and/or NOESY experiments.

HPLC was recorded on an Agilent 1200 series system at 254 nm on a C₁₈ analytical Xbridge RP column (5 cm × 2.1 mm, 2.7 μm) using the following gradient: 0-1 min 95% solvent A, 1-10 min 95% solvent A to 100% solvent B, 10-11 min 100% solvent B, 11-12 min 100% solvent B to 95% solvent A, 12-14 min 95% solvent A, [Solvent A: 0.2% formic acid, 99.8% water (v/v); Solvent B: 0.2% formic acid, 99.8% acetonitrile (v/v)] with a flow rate of 1 ml/min.

HPLC-MS was recorded on an Agilent 1100 LC-MS system on a C₁₈ analytical Xbridge RP column (5 cm × 2.1 mm, 2.7 μm) using the following gradient: 0-1 min 100% solvent A, 1-6 min 100% solvent A to 100% solvent B, 6-10 min 100% solvent B [solvent A: 0.1% formic acid, 5% acetonitrile, 94.9% water (v/v/v); Solvent B: 0.05% formic acid, 5% water, 94.95% acetonitrile (v/v/v)] with a flow rate of 1 ml/min.
High resolution mass spectra (HRMS) were recorded on a time of flight (TOF) MS system, coupled to an analytical HPLC and electrospray ionisation (ESI) mass detector. HRMS HPLC was performed on a C\textsubscript{18} RP column (25 cm × 4.6 mm, 5 μm) with a linear gradient (10 % to 100 % MeOH in H\textsubscript{2}O, containing 0.1 % TFA, in 20 min, v/v) at a flow rate of 1 ml/min and UV detection at 215 nm.

Infrared spectra were recorded on a Perkin-Elmer Spectrum One FT-IR apparatus with a universal ATR accessory and are reported in wavenumbers (cm\textsuperscript{-1}).

Optical rotation was recorded on a Perkin Elmer 241 polarimeter at 23 °C using the sodium D line (589 nm) and are reported in units of 10\textsuperscript{-1} deg dm\textsuperscript{2} g\textsuperscript{-1}.

2. Synthetic procedures

(S)-2-\{[(9H-Fluoren-9-yl)methoxy]carbonyl]amino\}-6-azidohexanoic acid, (S)-1

\textit{Fmoc-L-Lys(N$_3$)-OH}

Fmoc-L-Lys-OH (S)-7 (10.0 g, 27 mmol), NaHCO$_3$ (8.0 g, 95 mmol) and copper (II) sulfate pentahydrate (1.07 g, 4.30 mmol) was suspended in MeOH/H$_2$O [8:2 (v/v), 100 ml]. \textit{IH}-Imidazole-1-sulfonyl azide×HCl 8 (6.81 g, 32.50 mmol) was added and the pH was adjusted and maintained at 8–9 by addition of sat. aq. NaHCO$_3$ during the course of the reaction. The blue reaction mixture was stirred for 5 hr at room temperature, was diluted with aqueous sulfate buffer (300 ml) and extracted with EtOAc (3 × 150 ml). The organic layer was washed with saturated aqueous brine (2 × 150 ml), dried (Na$_2$SO$_4$), filtered and concentrated \textit{in vacuo} to give Fmoc-L-Lys(N$_3$)-OH, (S)-1 (8.88 g, 84%) as a yellow oil that required no further purification.

[α]$_D^{23}$ −1.8 (c 2.5, MeOH).

\textbf{IR} \textit{v}_{\text{max}} \textit{(neat) 2948, 2099, 1714, 1241 cm}^{-1}.

\textbf{1H NMR} (500 MHz; CDCl$_3$) δ = 7.77 (2H, d, \textit{J} = 8, Ar), 7.60 (2H, d, \textit{J} = 8, Ar), 7.42 (2H, t, \textit{J} = 7.5, Ar), 7.32 (2H, t, \textit{J} = 7.5, Ar), 5.27 (1H, br d, \textit{J} = 8, NH), 4.60-4.22 (3H, m, Fmoc-CH$_2$ and α-CH), 4.24 (1H, t, \textit{J} = 7, Fmoc-CH), 3.31 (2H, t, \textit{J} = 6.5, CH$_2$N$_3$), 2.01-1.43 (6H, m, 3 × CH$_2$).

\textbf{13C NMR} (125 MHz; CDCl$_3$) δ = 175.8 (CO$_2$H), 156.1 (Carbamate C=O), 143.6, 141.3, 127.8, 127.1, 125.0, 120.0 (6 × Ar), 67.1 (Fmoc-CH$_2$), 53.4 (α-CH), 51.1 (CH$_2$), 47.2 (Fmoc-CH), 31.8, 28.4, 22.4 (3 × CH$_2$).

3
HPLC R_T (min) = 8.6 min.
HRMS (+ESI) m/z found: MNa⁺, 417.1533 (C_{21}H_{22}N_{4}O_{4}Na requires M, 417.1539).

(R)-2-\{[(9H-Fluoren-9-yl)methoxy]carbonyl]amino\}-6-azidohexanoic acid, (R)-1

Fmoc-d-Lys(N_{3})-OH

Fmoc-d-Lys(N_{3})-OH was synthesised by the same method described for the enantiomer to give azide (R)-1 (3.63 g, 94%) as a yellow oil that required no further purification. 

\([\alpha]_{D}^{23} +1.7 \text{ (c 2.5, MeOH).}\\

HPLC R_T (min) = 8.7 min.
HRMS (+ESI) m/z found: MNa⁺, 417.1578 (C_{21}H_{22}N_{4}O_{4}Na requires M, 417.1539).
All analytical data was in agreement with that reported above for Fmoc-L-Lys(N_{3})-OH, (S)-1.

(S)-2-\{[(9H-Fluoren-9-yl)methoxy]carbonyl]amino\}pent-4-ynoic acid, 2

Fmoc-L-Pra-OH

Fmoc-L-Pra-OH (2.0 g, 17.7 mmol) was dissolved in a mixture of 10% K_{2}CO_{3} in water (w/v) (10 ml) and dioxane (15 ml) and cooled to 0 °C. Fmoc-succinimide (6.56 g, 19.5 mmol) dissolved in dioxane (15 ml) was added dropwise over 30 minutes. After stirring for 4.5 hr H_{2}O (100 ml) was added and the mixture was washed with EtOAc (100 ml). The aqueous layer was acidified with sulfate buffer (100 ml) and extracted with EtOAc (3 × 100 ml). The combined organic phases were washed with H_{2}O (3 × 50 ml), saturated aqueous brine (3 × 50 ml), dried (Na_{2}SO_{4}), filtered and concentrated in vacuo. The crude product was recrystallised from EtOAc to give Fmoc-L-Pra-OH 2 (3.72 g, 63%) as a white amorphous solid. 

\([\alpha]_{D}^{23} +9.0 \text{ (c 1.0, MeOH).}\\

IR \nu_{\text{max}} \text{ (neat) 3408, 3294, 1721, 1697, 1523, 1233, 1191 cm}^{-1}.

^{1}H \text{ NMR (400 MHz; DMSO)} \delta = 12.97 \text{ (1H, s, CO}_{2}\text{H), 7.90 (2H, d, } J = 7.5, \text{ Ar), 7.74 (2H, d, } J = 7.5, \text{ Ar), 7.67 (1H, br d, } J = 5.5, \text{ NH), 7.43 (2H, t, } J = 7.5, \text{ Ar), 7.34 (2H, t, } J = 7.5, \text{ Ar), 4.32-4.23 and 4.16-4.10 (2 \times m, 3H + 1H, Fmoc-CH}_{2}\text{CH and } \alpha-\text{CH), 2.88 (1H, t, } J = 2.5, \equiv \text{CH), 2.67 (1H, ddd, } J = 16.5, 5, 2.5, \text{ CH}_{\alpha}\text{H}_{\beta}\text{C}\equiv), 2.57 (1H, ddd, } J = 16.5, 5, 2.5, \text{ CH}_{\alpha}\text{H}_{\beta}\text{C}\equiv).
$^{13}$C NMR (100 MHz; DMSO) $\delta = 171.9$ (CO$_2$H), $155.8$ (Carbamate C=O), 143.7, 140.7, 127.6, 127.1, 125.3, 120.1 (6 × Ar), 80.7 (C≡CH), 72.9 (C≡CH), 65.8 (Fmoc-CH$_2$), 52.9 ($\alpha$-CH), 46.6 (Fmoc-CH$_2$), 21.1 ($\beta$-CH$_2$).

HPLC $R_T$ (min) = 7.9 min.

HRMS (+ESI) $m/z$ found: MH$^+$, 336.1256 (C$_{20}$H$_{18}$NO$_4$ requires M, 336.1230).

$(S)$-2-[(((9H-Fluoren-9-yl)methoxy)carbonyl]amino]-3-(prop-2-yn-1-yloxy)propanoic acid, 3

$^{1}$H NMR (400 MHz; MeOD) $\delta = 7.79$ (2H, d, $J = 7.5$, Ar), 7.69-7.66 (2H, m, Ar), 7.38 (2H, t, $J = 7.5$, Ar), 7.31 (2H, t, $J = 7.5$, Ar), 4.42-4.40 (1H, m, Fmoc-CH), 4.39-4.31 (2H, m, Fmoc-CH$_2$), 4.24 (1H, t, $J = 7$, C-H), 4.19 (2H, d, $J = 2.5$, CH$_2$C≡), 3.92 (1H, dd, $J = 9.5$, 5, $\beta$-CH$_2$H$_B$), 3.82 (1H, dd, $J = 9.5$, 3.5, $\beta$-CH$_AH_B$), 2.86 (1H, t, $J = 2.5$, C=CH).  

$^{13}$C NMR (100 MHz; DMSO) $\delta = 171.4$ (CO$_2$H), 156.0 (Carbamate C=O), 143.8, 140.7, 127.6, 127.0, 125.3, 120.0 (Ar), 79.8 (C≡CH), 77.4 (C≡CH), 68.7 and 65.8 ($\beta$-CH$_2$ and Fmoc-CH$_2$), 57.7 (CH$_2$C≡), 53.9 ($\alpha$-CH), 46.6 (Fmoc-CH$_2$).

HPLC $R_T$ (min) = 8.0 min.

LRMS (+ESI) $m/z$ found: MIfmoc$^+$, 388.1 (C$_{21}$H$_{19}$NO$_5$Na requires M, 388.1).

HRMS (+ESI) $m/z$ found: MIfmoc$^+$, 388.1171 (C$_{21}$H$_{19}$NO$_5$Na requires M, 388.1161).
(S)-3-{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino]-7-azidoheptanoic acid, (S)-4

**Fmoc-L-β<sup>3</sup>-Lys(N<sub>3</sub>)-OH**

Ketene (S)-9 (1.25 g, 2.98 mmol) was dissolved in THF/H<sub>2</sub>O [8:2 (v/v), 10 ml] and wrapped in aluminium foil to exclude light. Silver trifluoroacetate (72 mg, 0.33 mmol) was added and the reaction flask was sonicated in the dark for 3 hr at room temperature. An empty balloon was fitted on the reaction flask to prevent pressure build up. The reaction mixture was filtered through a plug of cotton wool and concentrated in vacuo. The residue was purified by flash column chromatography [50% EtOAc in n-heptane containing 0.5% AcOH (v/v)] to give β<sup>3</sup>-Lys(N<sub>3</sub>)-OH (S)-4 (820 mg, 66%) as a brown oil. 

[α]<sub>D</sub><sup>23</sup> = +2.3 (c 2.5, MeOH).

**TLC** R<sub>f</sub> = 0.60 (50% EtOAc in heptane containing 0.5% AcOH, v/v).

**IR** v<sub>max</sub> (neat) 3328, 2944, 2095, 1690, 1534, 1254 cm<sup>-1</sup>.

**1H NMR** (500 MHz; CDCl<sub>3</sub>) δ = 7.75 (2H, d, J = 7.5, Ar), 7.57 (2H, d, J = 7, Ar), 7.39 (2H, t, J = 7.5, Ar), 7.31 (2H, t, J = 8, Ar), 5.14 (1H, d, J = 9.5, NH), 4.42 (2H, d, J = 6.5, Fmoc-CH<sub>2</sub>), 4.21 (1H, t, J = 7, Fmoc-CH), 4.03-3.91 (1H, m, β-CH), 3.27 (2H, t, J = 6.5, CH<sub>2</sub>N<sub>3</sub>), 2.73-2.52 (2H, m, α-CH<sub>2</sub>), 1.75-1.32 (6H, m, 3 × CH<sub>2</sub>).

**13C NMR** (125 MHz; CDCl<sub>3</sub>) δ = 175.7 (CO<sub>2</sub>H), 155.9 (Carbamate C=O), 143.8, 141.3, 127.7, 127.0, 125.0, 120.0 (6 × Ar), 66.6 (Fmoc-CH<sub>2</sub>), 51.2 (CH<sub>2</sub>), 47.8 (β-CH), 47.3 (Fmoc-CH), 38.7, 33.8, 28.5, 23.3 (4 × CH<sub>2</sub>).

**HPLC** R<sub>T</sub> (min) = 8.6 min; HRMS (+ESI) m/z found: MNa<sup>+</sup>, 431.1724 (C<sub>22</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>Na requires M, 431.1690).

(R)-3-{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino]-7-azidoheptanoic acid, (R)-4

**Fmoc-D-β<sup>3</sup>-Lys(N<sub>3</sub>)-OH**

Fmoc-D-β<sup>3</sup>-Lys(N<sub>3</sub>)-OH was synthesised as described for the enantiomer to give azide (R)-4 (1.11 g, 59%) as a yellow oil. [α]<sub>D</sub><sup>23</sup> = +2.2 (c 2.5, MeOH); HPLC R<sub>T</sub> (min) = 8.6 min; HRMS (+ESI) m/z found: MNa<sup>+</sup>, 431.1680 (C<sub>22</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>Na requires M, 431.1695); All analytical data was in agreement with that reported for Fmoc-L-Lys(N<sub>3</sub>)-OH, (S)-4.
(S)-3-([((9H-Fluoren-9-yl)methoxy)carbonyl]amino)hex-5-ynoic acid, 5

**Fmoc-L-β3-Pra-OH**

Fmoc-L-Pra-ketene 12 (0.27 g, 0.74 mmol) was dissolved in a mixture of THF/H2O [8:2 (v/v), 10 ml] and wrapped in aluminium foil to exclude light. Silver trifluoroacetate (16 mg, 0.07 mmol) was added and the reaction flask was sonicated in the dark for 8 hr at ambient temperature. An empty balloon was fitted on the reaction flask to prevent pressure build up. Additional silver trifluoroacetate (11 mg, 0.11 mmol) was added after 4 hr. The reaction mixture was filtered through a cotton plug, concentrated *in vacuo* and purified by flash column chromatography [90% EtOAc in *n*-heptane containing 0.5% AcOH (v/v)] to give Fmoc-L-β3-Pra-OH 5 (159 mg, 60%) as a yellow solid.

$\left[\alpha\right]_D^{23} = +3.9 \ (c \ 1.0, \text{MeOH}).$

**TLC** $R_f = 0.75$ (90% EtOAc in *n*-heptane containing 0.5% AcOH, v/v).

**IR** $\nu_{\text{max}}$ (neat) 3347, 3294, 1699, 1691, 1531, 1270 cm$^{-1}$.

**$^1$H NMR** (400 MHz; CD$_3$OD) $\delta = 7.79$ (2H, d, $J = 7.5$, Ar), 7.65 (2H, d, $J = 7.5$, Ar), 7.38 (2H, t, $J = 7.5$, Ar), 7.30 (2H, t, $J = 7.5$, Ar), 4.38-4.28 (2H, m, Fmoc-CH$_2$), 4.21 (1H, t, $J = 7$, Fmoc-CH), 4.09 (1H, p, $J = 6.5$, β-CH), 2.67 (1H, dd, $J = 16$ and 6, α- or γ-CH$_2$H$_6$), 2.56 (1H, dd, $J = 16$ and 8, α- or γ-CH$_2$H$_6$), 2.47 (2H, m, α- or γ-CH$_2$), 2.33 (1H, $J = 2.5$, ≡CH); $^{13}$C NMR (100 MHz; CD$_3$OD) $\delta = 174.8$ (CO$_2$H), 158.1 (Carbamate C=O), 145.4, 142.6, 128.8, 128.2, 126.3, 120.9 (6 × Ar), 81.2 (C≡CH), 72.0 (≡CH), 67.8 (Fmoc-CH$_2$), 48.5 (β-CH or Fmoc-CH, one signal not observed due to overlap with CD$_3$OD solvent signal), 39.0 (α-CH$_2$), 24.9 (γ-CH$_2$).

**HPLC** $R_T$ (min) = 7.9 min.

**LRMS** (+ESI) $m/z$ found: MNa$, 372.1 \ (C$_{21}$H$_{19}$NO$_4$Na requires M, 372.1).

**HRMS** (+ESI) $m/z$ found: MNa$, 372.1206 \ (C$_{21}$H$_{19}$NO$_4$Na requires M, 372.1212).
(R)-3-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino}-4-(prop-2-yn-1-yloxy)butanoic acid, 6

**Fmoc- L-β3-Ser(Prp)-OH**

![Chemical Structure](image)

Fmoc-L-Ser(Prp)-OH 3 (0.67 g, 1.84 mmol) was dissolved in anhydrous THF (3 ml) and cooled to –20 °C. N-Methylmorpholine (212 μl, 1.93 mmol) and iso-butylchloroformate (251 μl, 1.93 mmol) were added and the reaction mixture was stirred for 30 minutes at –20 °C. The temperature was raised to 0 °C and diazomethane (approx. 386 mg, 9.20 mmol) was distilled directly into the reaction flask over a period of 1.5 hour and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with Et₂O (20 ml), washed with saturated aqueous NaHCO₃ (2 × 20 ml), saturated aqueous NH₄Cl (2 × 20 ml), saturated aqueous brine (2 × 20 ml), dried (Na₂SO₄), filtered and concentrated *in vacuo* to give Fmoc-L-Ser(Prp)-ketene (0.64 g) as a yellow oil that was used with no further purification. LRMS (+ESI) *m/z* found: MNa⁺, 412.1 (C₂₂H₂₂N₆O₃Na requires M, 412.13).

Crude Fmoc-L-Ser(Prp)-ketene (0.64 g) was dissolved in THF/H₂O [8:2 (v/v), 10 ml] and wrapped in aluminium foil to exclude light. Silver trifluoroacetate (41 mg, 0.18 mmol) was added and the reaction flask was sonicated in the dark for 6.5 hr at ambient temperature. An empty balloon was fitted on the reaction flask to prevent pressure build up. Additional silver trifluoroacetate (61 mg, 0.28 mmol) was added to the reaction after 3.5 hr. The reaction mixture was filtered through a cotton plug, concentrated *in vacuo* and purified by flash column chromatography [90% EtOAc in n-heptane containing 0.5% AcOH (v/v)] to give Fmoc-L-β3-Ser(Prp)-OH 6 (0.26 g, 36%, 3 steps) as a tan oil.

**TLC** *Rf* = 0.40 [50% EtOAc in heptane (v/v)].

[α] <sup>23</sup> +7.7 (c 1.0, MeOH).

**IR** ν<sub>max</sub> (neat) 3289, 2961, 1707, 1239 cm<sup>−1</sup>.

**¹H NMR** (300 MHz; CDCl₃) δ = 7.75 (2H, d, *J* = 7.5, Ar), 7.58 (2H, d, *J* = 7, Ar), 7.42-7.37 (2H, m, Ar), 7.33-7.28 (2H, m, Ar), 5.39 (1H, d, *J* = 8.5, NH), 4.42-4.40 (2H, m, Fmoc-CH₂), 4.25-4.16 (5H, m, CH₂C≡, β-CH and Fmoc-CH), 3.70-3.61 (2H, m, β-CHCH₂O), 2.71 (2H, d, *J* = 5.5, α-CH₂), 2.44 (1H, t, *J* = 2.5, ≡CH).

**¹³C NMR** (75 MHz; CDCl₃) δ = 175.8 (CO₂H), 156.1 (Carbamate C=O), 144.0, 141.5, 127.9, 127.2, 125.2, 120.1 (6 × Ar), 79.3 (C≡CH), 77.4 (≡CH), 75.2, 70.5, 67.1 (Fmoc-CH₂), 58.7, 47.4 (Fmoc-CH), 35.9 (α-CH₂).
HPLC $R_T$ (min) = 7.9 min.
HRMS (+ESI) \( m/z \) found: MH\(^+\), 380.1506 (C\(_{22}\)H\(_{22}\)NO\(_5\) requires M, 380.1493).

**IH-Imidazole-1-sulfonyl azide×HCl, 8**

According to the method of Goddard-Borger and Stick\(^3\) sodium azide (13.1 g, 200 mmol) was suspended in anhydrous MeCN and cooled in an ice-water bath. Freshly distilled sulfuryl chloride (16.1 ml, 200 mmol) was added drop-wise and the reaction mixture was stirred overnight at room temperature. The reaction mixture was cooled in an ice-water bath and imidazole (25.9 g, 380 mmol) was added portion-wise and the resulting slurry was stirred for 3 hours at room temperature. EtOAc (400 ml) was added and the organic layer was extracted with water (2 × 400 ml), sat. aq. NaHCO\(_3\) (2 × 200 ml), dried (Na\(_2\)SO\(_4\)) and filtered to give a clear colourless solution. A solution of HCl in EtOAc [obtained by the drop-wise addition of acetyl chloride (21.3 ml, 300 mmol) to ice-cooled absolute ethanol (75 ml)] was added drop-wise to the stirred filtrate. The product precipitated as the hydrochloric salt, was collected by filtration and washed with EtOAc (3 × 100 ml) to give sulfonyl azide 8 (27.5 g, 66%) as a white powder. The product was dried rigorously in a vacuum desiccator over KOH and stored in a freezer at –20 \(^\circ\)C (Stable >10 months).

**IR** $\nu_{\text{max}}$ (neat) 2496, 2167, 1425, 1160 cm\(^{-1}\).

**\(^1\)H NMR** (300 MHz; D$_2$O) $\delta$ = 9.20-8.84 (1H, m, C-1), 7.95-7.79 (1H, m), 7.54-7.36 (1H, m) (C-3 and C-4).

**\(^{13}\)C NMR** (75 MHz; D$_2$O) $\delta$ = 137.7 (C-1), 123.3, 120.1 (C-3 and C-4).

**LRMS** (+ESI) \( m/z \) found: MH\(^+\), 174.2. (C\(_3\)H\(_4\)N\(_5\)O\(_2\)S requires M, 174.2).

All analytical data was in agreement with that previously published.\(^3\)

\(^{(S)}\)-(9H-Fluoren-9-yl)methyl (7-azido-1-diazo-2-oxoheptan-3-yl)carbamate, \(^{(S)}\)-9

**Fmoc-L-Lys(N\(_3\))-ketene**

Fmoc-L-Lys(N\(_3\))-OH (461.10 mg, 1.16 mmol) was dissolved in anhydrous THF (5 ml) and cooled to –20 \(^\circ\)C. N-Methylmorpholine (134.60 \(\mu\)l, 1.22 mmol) and isoo-butylchloroformate (159 \(\mu\)l, 1.22 mmol) were added and the reaction mixture was stirred for 30 minutes at –20
°C. The temperature was raised to 0 °C and diazomethane (approx. 420 mg, 14 mmol) was distilled directly into the reaction flask over a period of 1 hour. The reaction mixture was stirred at room temperature overnight, diluted with Et₂O (20 ml), washed with saturated aqueous NaHCO₃ (2 × 20 ml), saturated aqueous NH₄Cl (2 × 20 ml), saturated aqueous brine (2 × 20 ml), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash column chromatography [50% EtOAc in n-heptane (v/v)] to give ketene (S)-9 (0.47 g, 98%) as a yellow oil.

TLC Rₜ = 0.60 (50% EtOAc in heptane, v/v).

LRMS (+ESI) m/z found: MNa⁺, 441.2 (C₂₂H₂₂N₆O₃Na requires M, 441.2).

(R)-(9H-fluoren-9-yl)methyl (7-azido-1-diazo-2-oxoheptan-3-yl)carbamate, (R)-9

Fmoc-d-Lys(N₃)-ketene

Fmoc-d-Lys(N₃)-ketene was synthesised as described for the enantiomer to give ketene (R)-9 (1.94 g, 85%) as a yellow oil. The crude product was used with no further purification. TLC and LRMS data was in agreement with that reported above for Fmoc-L-Lys(N₃)-ketene (S)-9.

(R)-Methyl 2-[(S)-2-[((9H-fluoren-9-yl)methoxy)carbonyl]amino]-6-azidohexanamido]-3-methylbutanoate, 10

Fmoc-L-Lys(N₃)-Val-OMe

Fmoc-Lys(N₃)-OH (S)-1 (100 mg, 0.25 mmol), L-valine-methylester×HCl (47 mg, 0.28 mmol), HOBr (41 mg, 0.30 mmol), EDCI×HCl (53 mg, 0.28 mmol) and (iPr)₂NEt (176 μl, 1.01 mmol) were dissolved in CH₂Cl₂ (3 ml) and stirred for 72 hr. The reaction mixture was diluted with EtOAc (15 ml) and extracted with sulfate buffer (3 × 5 ml), saturated aqueous NaHCO₃ (3 × 5 ml), saturated aqueous brine (3 × 5 ml), dried (Na₂SO₄), filtered and concentrated in vacuo. The crude residue was analysed by NMR followed by purification by flash column chromatography [33% EtOAc in n-heptane (v/v)] to give dipeptide 10 (67 mg, 52%) as a white amorphous solid.

TLC Rₜ = 0.70 (33% EtOAc in heptane, v/v).
IR ν<sub>max</sub> (neat) 3294, 2948, 2097, 1735, 1692, 1650, 1535, 1253 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ = 7.77 (2H, d, J = 7.5, Ar), 7.58 (2H, d, J = 7.5, Ar), 7.41 (2H, t, J = 7.5, Ar), 7.32 (2H, t, J = 7.5, Ar), 6.34 (1H, br d, J = 9, NH), 5.34 (1H, br d, J = 8, NH), 4.55 (1H, dd, J = 9 and 5, α-CH), 4.47-4.37 (2H, m, Fmoc-CH<sub>2</sub>), 4.23 (1H, t, J = 7, Fmoc-CH), 4.23-4.18 (1H, m, α-CH), 3.75 (3H, s, OMe), 3.30 (1H, t, J = 6.5, CH<sub>2</sub>), 2.28-2.11 (1H, m, α-CHCH), 1.97-1.25 (6H, m, 3 × CH<sub>2</sub>), 0.94 (3H, d, J = 8, CH<sub>3</sub>), 0.91 (3H, d, J = 8, CH<sub>3</sub>).

<sup>13</sup>C NMR (125 MHz; CDCl<sub>3</sub>) δ = 172.1 (CO<sub>2</sub>H), 171.3 (CO<sub>2</sub>Me), 156.1 (Carbamate C=O) 143.8, 141.3, 127.8, 127.1, 125.0, 120.0 (6 × Ar), 67.2 (Fmoc-CH<sub>2</sub>), 57.2 (OMe), 54.7 (α-CHCH), 52.3 (α-CHCH<sub>2</sub>), 51.1 (CH<sub>2</sub>), 47.1 (Fmoc-CH), 32.1 (CH<sub>2</sub>), 31.2 (CH), 28.5, 22.6 (2 × CH<sub>2</sub>), 18.9, 17.7 (2 × CH<sub>3</sub>).

HRMS (+ESI) m/z found: MNa<sup>+</sup>, 530.2404 (C<sub>27</sub>H<sub>33</sub>N<sub>5</sub>O<sub>5</sub>Na requires M, 530.2374).

(S)-Methyl 2-{((S)-3-[[[(9H-fluoren-9-yl)methoxy]carbonyl]amino]-7-azidoheptanamido}-3-methylbutanoate, 11

<sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ = 7.69 (2H, d, J = 7.5, Ar), 7.53 (2H, m, Ar), 7.33 (2H, t, J = 7.5, Ar), 7.24 (2H, t, J = 7.5, Ar), 6.02 (1H, br d, J = 7.5, NH), 5.52 (1H, br d, J = 8, NH), 4.51-4.41 (1H, m, α-CH), 4.35-4.25 (2H, m, Fmoc-CH<sub>2</sub>), 4.14 (1H, t, J = 7, Fmoc-CH), 3.86 (1H, m, β-CH), 3.66 (3H, s, OCH<sub>3</sub>), 3.20 (2H, br t, J = 6, CH<sub>2</sub>), 2.59-2.33 (2H, m, α-CH<sub>2</sub>), 2.09 (1H, dq, J = 12 and 6, α-CHCH), 1.69-1.06 (6H, m, 3 × CH<sub>2</sub>), 0.86 (3H, d, J = 7, CH<sub>3</sub>), 0.83 (3H, d, J = 7, CH<sub>3</sub>).
$^{13}$C NMR (125 MHz; CDCl$_3$) $\delta$ = 172.3 (CO$_2$H), 168.8 (CO$_2$Me), 155.3 (Carbamate C=O), 144.0, 141.3, 127.7, 127.0, 125.1, 120.0 (6 × Ar), 66.7 (Fmoc-CH$_2$), 57.0, 52.2, 51.2 (CH$_2$), 48.6, 47.3, 40.4 (CH$_2$), 33.8 (CH$_2$), 31.1 ($\alpha$-CHCH), 28.5 (CH$_2$), 23.5 (CH$_2$), 19.0, 17.8 (2 × CH$_3$).

LRMS (+ESI) m/z found: MH$^+$, 522.3 (C$_{28}$H$_{36}$N$_5$O$_5$ requires M, 522.3).

HRMS (+ESI) m/z found: MNa$^+$, 544.2518 (C$_{28}$H$_{35}$N$_5$O$_5$Na requires M, 544.2536).

(S)-(9H-Fluoren-9-yl)methyl (1-diazo-2-oxohex-5-yn-3-yl)carbamate, 12

*Fmoc-L-Pra-ketene*

\[
\text{Fmoc}-\text{L-Pra-OH} \rightarrow \text{Fmoc}-\text{L-Pra-Ketene}
\]

Fmoc-L-Pra-OH 2 (1.2 g, 3.6 mmol) was dissolved in anhydrous THF (6 ml) and cooled to –20 °C. N-Methylmorpholine (418 $\mu$l, 3.8 mmol) and iso-butylchloroformate (496 $\mu$l, 3.8 mmol) were added and the reaction mixture was stirred for 30 minutes at –20 °C. The temperature was raised to 0 °C and diazomethane in ether (approx. 760 mg, 18.1 mmol) was distilled directly into the reaction flask over 1.5 hr. The reaction mixture was stirred at room temperature overnight, diluted with ether (10 ml), washed with saturated aqueous NaHCO$_3$ (50 ml), saturated aqueous NH$_4$Cl (50 ml), saturated aqueous brine (50 ml), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography [50% EtOAc in heptane (v/v)] to give ketene 12 (0.50 g, 39%) as a white amorphous solid.

TLC R$_f$ = 0.60 (50% EtOAc in heptane, v/v).

LRMS (+ESI) m/z found: MNa$^+$, 382.1 (C$_{21}$H$_{17}$N$_3$O$_3$Na requires M, 382.1).

(S)-Methyl 2-{{(S)-2-[((9H-fluoren-9-yl)methoxy)carbonyl]amino}pent-4-ynamido}-3-methylbutanoate, 13

*Fmoc-Pra-Val-OMe*

\[
\text{Fmoc}-\text{L-Pra-OH} \rightarrow \text{Fmoc}-\text{Pra-Val-OMe}
\]

Fmoc-L-Pra-OH 2 (76 mg, 0.23 mmol), L-valine methyl ester×HCl (42 mg, 0.25 mmol), EDCI×HCl (34 mg, 0.25 mmol), HOBt (52 mg, 0.27 mmol) and (Pr)$_2$NEt (0.16 ml, 0.74 mmol) were dissolved in anhydrous CH$_2$Cl$_2$ (5 ml) and stirred overnight. The reaction
mixture was diluted with EtOAc (3 ml) and washed with sulfate buffer (3 × 5 ml), saturated aqueous NaHCO₃ (3 × 5 ml), saturated aqueous brine (3 × 5 ml), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was analysed by NMR followed by purification by flash column chromatography [50% EtOAc in n-heptane (v/v)] to give dipeptide 13 (36 mg, 47%) as a white amorphous powder.

**TLC** R_f = 0.65 (50% EtOAc in n-heptane, v/v).

**IR** ν_max (neat) 3286, 2966, 1733, 1650, 1549 cm⁻¹.

**¹H NMR** (400 MHz; CDCl₃) δ = 7.70 (2H, d, J = 7.5, Ar), 7.52 (2H, d, J = 7.5, Ar), 7.34 (2H, t, J = 7.5, Ar), 7.25 (2H, t, J = 7.5, Ar), 6.65 (1H, br d, J = 8, NH), 5.52 (1H, br s, NH), 4.48 (1H, dd, J = 5 and 8.5, α-CH), 4.34-3.33 (3H, m, Fmoc-CH₂ and CH), 4.17 (1H, t, J = 7, CH), 3.66 (3H, br s, OCH₃), 2.77 (1H, m, CH₃H₂B≡C), 2.74 (1H, m, CH₃H₃B≡C), 2.18-2.06 (1H, m, α-CH(CH)), 2.06 (1H, t, J = 2.5, =CH), 0.87 (3H, d, J = 7, CHCH₃), 0.84 (3H, d, J = 7, CHCH₃).

**¹³C NMR** (100 MHz; CDCl₃) δ = 171.8 (CO₂H), 169.6 (CO₂Me), 155.9 (Carbamate C=O), 143.6, 141.3, 127.8, 127.1, 125.0, 120.0 (Ar), 77.2 (C≡CH), 72.1 (C≡CH), 67.4 (Fmoc-CH₂), 57.5 (OMe), 53.4 (α-CHCH), 52.2 (α-CHCH₂), 47.1 (Fmoc-CH), 31.3 (α-CHCH₂), 22.5 (α-CHCH), 18.9, 17.8 (2 × CH₃).

**LRMS** m/z (+ESI) found: MH⁺, 449.2 (C_{26}H_{29}N_{2}O_{5} requires M, 449.2).

**HRMS** (+ESI) m/z found: MH⁺, 449.2054 (C_{26}H_{29}N_{2}O_{5} requires M, 449.2071).

(S)-Methyl 2-[(S)-3-[[((9H-fluoren-9-yl)methoxy)carbonyl]amino]hex-5-ynamido]-3-methylbutanoate, 14

**Fmoc-β¹-L-Pra-Val-OMe**

Fmoc-β¹-L-Pra-OH 5 (20 mg, 0.057 mmol), L-valine methyl ester×HCl (11 mg, 0.063 mmol), EDCI×HCl (9 mg, 0.063 mmol), HOBt (13 mg, 0.069 mmol) and (Pr)₂NEt (40 μl, 0.22 mmol) were dissolved in anhydrous CH₂Cl₂ (3 ml) and stirred overnight. The reaction mixture was diluted with EtOAc (3 ml) and washed with sulfate buffer (3 × 2 ml), saturated aqueous NaHCO₃ (3 × 3 ml), saturated aqueous brine (3 × 3 ml), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was analysed by NMR followed by purification by flash column chromatography [50% EtOAc in heptane (v/v)] to give dipeptide 14 (12 mg, 45%) as a white amorphous powder.
TLC $R_f = 0.30$ (50% EtOAc in heptane, v/v).

IR $\nu_{\text{max}}$ (neat) 3314, 3278, 2966, 1731, 1686, 1640, 1537, 1268 cm$^{-1}$.

$^1$H NMR (400 MHz; CDCl$_3$) $\delta = 7.69$ (2H, d, $J = 7.5$, Ar), 7.52 (2H, d, $J = 7.5$, Ar), 7.33 (2H, t, $J = 7.5$, Ar), 7.24 (2H, t, $J = 7.5$, Ar), 6.07 (1H, br s, NH), 5.65 (1H, br s, NH), 4.48 (1H, dd, $J = 9$ and 5, $\alpha$-CH), 4.35-4.24 (2H, m, Fmoc-CH$_2$), 4.18-4.12 (2H, m, Fmoc-CH and $\beta$-CH), 3.67 (3H, s, OCH$_3$), 2.70-2.63 and 2.60-2.44 (1H and 3H, 2 × m, CH$_2$C≡ and CH$_2$C=O), 2.13-2.07 (1H, m, $\alpha$-CHCH), 2.02-2.01 (1H, m, ≡CH), 0.87 (3H, d, $J = 7$, CHCH$_3$), 0.84 (3H, d, $J = 7$, CHCH$_3$).

$^{13}$C NMR (100 MHz; CDCl$_3$) $\delta =$172.2 (CO$_2$H), 170.3 (CO$_2$Me), 155.7 (Carbamate C=O), 143.9, 141.3, 127.7, 127.1, 125.1, 120.0 (Ar), 77.2 (C≡CH), 71.3 (C≡CH), 67.0 (Fmoc-CH$_2$), 57.1 (OMe), 52.2 ($\alpha$-CCHCH), 47.2 (Fmoc-CH), 38.8 ($\alpha$-CH$_2$), 31.3 (CH$_3$C≡), 23.9 ($\alpha$-CHCH), 19.0, 17.8 (2 × CH$_3$).

LRMS $m/z$ (+ESI) found: MH$^+$, 463.2 (C$_{27}$H$_{31}$N$_2$O$_5$ requires M, 463.2).

HRMS (+ESI) $m/z$ found: MH$^+$, 463.2220 (C$_{27}$H$_{31}$N$_2$O$_5$ requires M, 463.2227).

(S)-2-[(tert-Butoxycarbonyl)amino]-3-(prop-2-yn-1-loyloxy)propanoic acid, 16

*Boc-L-Ser(Prp)-OH*

Boc-L-serine 15 (5.0 g, 24.4 mmol) was dissolved in DMF (50 ml), cooled to 0 °C and NaH (60% dispersion in mineral oil, 2.14 g, 53.60 mmol) was added. After stirring for 30 min at 0 °C 3-bromopropyne (80% solution in toluene, 2.85 ml, 26.80 mmol) was added dropwise. After stirring at 0 °C for 30 minutes the ice bath was removed and stirring continued overnight at ambient temperature. Aqueous sulfate buffer (150 ml) and saturated aqueous brine (150 ml) was added and the mixture was extracted with EtOAc (3 × 100 ml), washed with saturated aqueous brine (3 × 100 ml), dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was purified by dry column vacuum chromatography [10% MeCN in t-BuOMe, (v/v)] to give alkyne 16 (5.6 g, 94%) as a yellow gum.

$\left[\alpha\right]_{D}^{23} +0.10$ (c 1.0, MeOH).

TLC $R_f = 0.70$ (75% MeCN in t-BuOMe, v/v).

IR $\nu_{\text{max}}$ (neat) 3293, 2978, 1707, 1157 cm$^{-1}$.

$^1$H NMR (300 MHz; CDCl$_3$) $\delta = 5.39$ (1H, br d, $J = 8.5$, NH), 4.50 (1H, dt, $J = 9$ and 3.5, $\alpha$-CH), 4.19 (2H, t, $J =2$, CH$_2$C≡), 4.01 (1H, dd, $J = 9.5$ and 3, $\beta$-CH$_4$H$_B$), 3.82 (1H, dd, $J = 9.5$ and 3.5, $\beta$-CH$_4$H$_B$), 2.49 (1H, t, $J = 2.5$, ≡CH), 1.47 (9H, s, 3 × CH$_3$).
$^{13}$C NMR (125 MHz; CDCl$_3$) $\delta = 175.0$ (CO$_2$H), 155.7 (Carbamate C=O), 80.4 (CMe$_3$), 78.7 (C≡CH), 75.3 (C≡CH), 69.4 ($\beta$-CH$_2$), 58.7 (OCH$_2$C≡), 53.6 ($\alpha$-CH), 28.3 (CMe$_3$).

HRMS (+ESI) $m/z$ found: MH$^+$, 244.1183 (C$_{11}$H$_{18}$NO$_5$ requires M, 244.1179).

(S)-Methyl 2-{$(R)$-3-[[((9H-fluoren-9-yl)methoxy)carbonyl]amino]-4-(prop-2-yn-1-yloxy)butanamido}-3-methylbutanoate, 17

$^{13}$C NMR (125 MHz; CDCl$_3$)$\delta = 172.3$ (C=O), 143.9, 141.3, 127.7, 127.1, 125.2, 120.0 (6 $\times$ Ar), 79.3 (C≡CH), 77.6 (C≡CH), 74.9, 70.6, 66.9, (Fmoc-CH$_2$), 58.5, 57.1, 52.2, 47.2 (Fmoc-CH), 37.6, 29.7 ($\alpha$-CHCH$_3$ and $\alpha$-CH$_2$), 19.0, 17.8 (2 $\times$ CH$_3$).

LRMS (+ESI) $m/z$ found: MH$^+$, 493.3 (C$_{28}$H$_{33}$N$_2$O$_6$ requires M, 493.2).

HRMS (+ESI) $m/z$ found: MH$^+$, 493.2325 (C$_{28}$H$_{33}$N$_2$O$_6$ requires M, 493.2333).
(S)-Methyl 2-\{((S)-2-\{((9H-fluoren-9-yl)methoxy)carbonyl)amino\}-3-(prop-2-yn-1-yloxy)propanamido\}-3-methylbutanoate, 18

**Fmoc-Ser(Prp)-Val-OMe**

\[\text{Fmoc-Ser(Prp)-Val-OMe} \]

Fmoc-\(\beta\)-L-Ser(Prp)-OH 3 (87 mg, 0.24 mmol), L-valine methyl ester×HCl (44 mg, 0.26 mmol), HOBt (54 mg, 0.28 mmol), EDCI×HCl (35 mg, 0.26 mmol), and \((\text{Pr})_2\text{NEt}\) (163 \(\mu\)l, 0.94 mmol) were dissolved in anhydrous CH\(_2\)Cl\(_2\) (5 ml) and stirred overnight. The reaction mixture was diluted with EtOAc (5 ml), washed with sulfate buffer (3 × 5 ml), saturated aqueous NaHCO\(_3\) (3 × 5 ml), saturated aqueous brine (3 × 5 ml), dried (Na\(_2\)SO\(_4\)), filtered and concentrated \textit{in vacuo}. The residue was analysed by NMR followed by purification by flash column chromatography [50% EtOAc in heptane (v/v)] to give dipeptide 18 (42 mg, 49%) as a yellow oil.

**TLC** \(R_f = 0.80\) (90% EtOAc in heptane, v/v).

**IR** \(v_{\text{max}}\) (neat) 3290, 2967, 2163, 1733, 1692, 1651, 1531, 1212 cm\(^{-1}\).

**\(^1\)H NMR** (400 MHz; CDCl\(_3\)) \(\delta = 7.70\) (2H, d, \(J = 7.5, \text{ Ar}\)), 7.53 (2H, d, \(J = 7, \text{ Ar}\)), 7.33 (2H, t, \(J = 7.5, \text{ Ar}\)), 7.25 (2H, t, \(J = 7.5, \text{ Ar}\)), 6.87 (1H, br d, \(J = 6.5, \text{ NH}\)), 5.63 (1H, br s, \text{ NH}), 4.46 (1H, dd, \(J = 8.5, 5, \alpha\)-CH), 4.36-4.34 (3H, m, \text{ CH\(_2\)-Fmoc and CH}), 4.18-4.15 (2H, m), 3.91-3.89 (1H, m), 3.67-3.66 (4H, br s, OCH\(_3\) and \(\alpha\)-CHCH\(_2\)H\(_3\)O), 3.59-3.55 (1H, m, \(\alpha\)-CHCH\(_3\)CH\(_3\)O), 2.39 (1H, t, \(J = 2.5, \equiv\text{CH}\)), 2.20-2.05 (1H, m, \(\alpha\)-CHCH), 0.88 (3H, d, \(J = 7, \text{ CH}_3\)), 0.84 (3H, d, \(J = 7, \text{ CH}_3\)).

**\(^{13}\)C NMR** (100 MHz; CDCl\(_3\)) \(\delta = 171.9\) (CO\(_2\)H), 169.7 (CO\(_2\)Me), 156.1 (Carbamate C=O), 143.7, 141.3, 127.7, 127.1, 125.1, 120.0 (Ar), 78.7 (C≡CH), 77.2 (C≡CH), 75.4, 69.4, 67.3, 58.7, 57.5, 52.2, 47.1 (Fmoc-CH), 31.2 (C-CHCH), 19.0, 17.7 (2 × CH\(_3\)).

**LRMS** (+ESI) \(m/z\) found: MH\(^+\) , 479.2 (C\(_{27}\)H\(_{31}\)N\(_2\)O\(_6\) requires M, 479.2).
3. NMR spectra
4. HPLC chromatograms compounds 1 to 6

- **(S)-1** FmocN₁⁰CO₂H (8.6 min)
- **(S)-4** FmocN₁⁰CO₂H (8.6 min)
- **(R)-1** FmocN₁⁰CO₂H (8.7 min)
- **(R)-4** FmocN₁⁰CO₂H (8.6 min)
TS1-50-D: UV Chromatogram, 252-256 nm

TS1-45.D: UV Chromatogram, 252-256 nm

TS1-38A.D: UV Chromatogram, 252-256 nm

TS1-54C.D: UV Chromatogram, 252-256 nm

Compounds:
1. Fmoc-HN-CO2H
2. 7.9 min
3. 8.0 min
4. 7.9 min
5. 7.9 min
6. 7.9 min
5. Reference List