Enzymatically-stable Oxetane-based Dipeptide Hydrogels

Laura McDougall,[a] Emily R. Draper,[a] Jonathan D. Beadle,[b] Michael Shipman,[b] Piotr Raubo,[c] Andrew G. Jamieson[a,*] and Dave J. Adams[a,*]

a. School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, U.K.
b. Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, U.K.
c. Medicinal Chemistry, Oncology, IMED Biotech Unit, AstraZeneca, Cambridge, U.K.

Electronic Supplementary Information

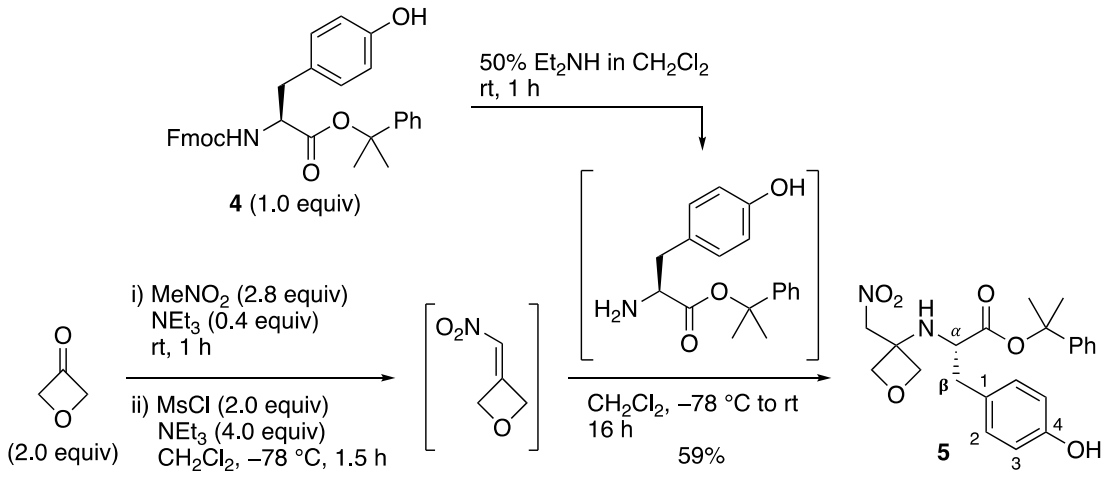
1. Synthetic methods and characterisation data for 1, 2, 3, 5 and 6

General. Anhydrous solvents were purchased from Sigma-Aldrich or Acros Organics in Sure-Seal™ bottles for use as reaction solvents. All other solvents were reagent grade and used as received. Petroleum ether refers to the fraction that boils in the range 40-60 °C. Commercially available starting materials were used without purification unless otherwise stated. All amino acids are of *L*-configuration unless otherwise stated. Fmoc-Tyr-OCumyl **4**,[1] Fmoc-GOx-Phe-OCumyl **7**[2] and Fmoc-GOx-Val-OCumyl **8**[2] were prepared following previously described literature procedures.

Thin layer chromatography was performed on pre-coated aluminium-backed plates (Merck Silicagel 60 F254), visualised by UV 254 nm and then stained with phosphomolybdic acid (PMA) dip. Flash column chromatography was performed using Aldrich 40-63 μm silica gel. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker DPX (300 or 400 MHz), or AV (500 MHz) spectrometers at the University of Warwick. The ^{1}H and ^{13}C NMR spectra for **1** post-gelation were collected using a Bruker Avance III HD 400 MHz spectrometer at the University of Glasgow in deuterated DMSO. Chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent residual peaks ($CDCl_3$ δ_H: 7.26 ppm, δ_C: 77.16 ppm; DMSO-d_6 δ_H: 2.50 ppm, δ_C 39.52 ppm; MeOD δ_H: 3.31 ppm, δ_C: 49.00 ppm). Coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (b), or combination of these. NMR assignments were deduced using 2D experiments (COSY, HSQC and HMBC).

Low-resolution mass spectra were recorded on an Agilent Technologies 6130 Quadrupole LC-MS instrument. High-resolution mass spectra were recorded using a Bruker MaXis Impact. Infrared spectra were recorded with a Bruker ALPHA Platinum ATR apparatus and are reported as observed. Optical rotations $[\alpha]_D^T$ were measured using an AA-1000 polarimeter and reported as observed.

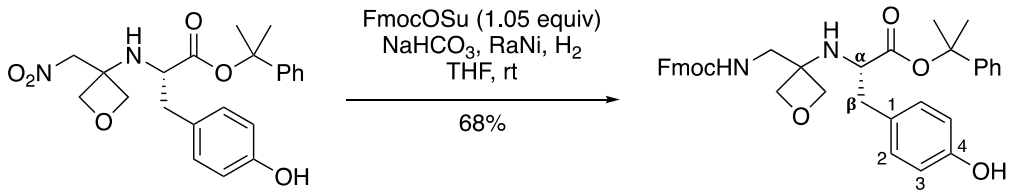
2-Phenylpropan-2-yl [3-(nitromethyl)oxetan-3-yl]-*L*-tyrosinate 5



Fmoc-Tyr-OCumyl **4**[1] (825 mg, 1.58 mmol) in 50% diethylamine in CH_2Cl_2 (4 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH_2Cl_2 (3 x 15 mL/mmol) and concentrated under reduced pressure to give the crude amine. Meanwhile, oxetan-3-one (203 µL, 3.16 mmol), nitromethane (237 µL, 4.42 mmol) and triethylamine (88 µL, 0.63 mmol) were stirred at room temperature for 1 h. CH_2Cl_2 (13 mL) was added and the reaction mixture cooled to –78 °C. Triethylamine (881 µL, 6.32 mmol) was added followed by the dropwise addition of a solution of methanesulfonyl chloride (245 µL, 3.16 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was stirred at –78 °C for 1.5 h. The crude amine in CH_2Cl_2 (7 mL) was added to the oxetane mixture *via* syringe at –78 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h. A saturated solution of NH_4Cl (20 mL) was added and stirred for 10 min. The layers were separated and the aqueous extracted with CH_2Cl_2 (2 x 30 mL) and EtOAc (3 x 30 mL). The combined organics were washed with saturated $NaHCO_3$ (2 x 20 mL), then brine (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by column chromatography (30% EtOAc in petroleum ether) gave **5** (389 mg, 0.94 mmol, 59%) as a yellow solid. $\mathbf{R_f}$ (30% EtOAc in petroleum ether) 0.26; $[\alpha]_D^{24}$ +6.5 (*c* 0.16, $CHCl_3$); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.36-7.22 (5H, m, ArH), 7.05 (2H, d, *J* = 8.3 Hz, ArH-2), 6.74 (2H, d, *J* = 8.4 Hz, ArH-3), 4.84 (1H, bs, OH), 4.74 (1H, d, *J* = 12.7 Hz, NO_2CHH), 4.66 (1H, d, *J* = 12.6 Hz, NO_2CHH), 4.45 (1H, d, *J* = 7.1 Hz, OC*H*H-Ox), 4.36-4.26 (3H, m, OCH*H*-Ox and OCH_2-Ox), 3.71-3.61 (1H, m, CHα-Tyr), 2.93 (1H, dd, *J* = 13.5, 5.9 Hz, C*H*Hβ-Tyr), 2.76 (1H, dd, *J* = 13.5, 7.5 Hz, CH*H*β-Tyr), 2.29 (1H, d, *J* = 8.7 Hz, NH), 1.77 (3H, s, CH_3-cumyl), 1.73 (3H, s, CH_3-cumyl); **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 173.5 (C=O), 154.6 (C, Ar-4), 144.8 (C, Ar-cumyl), 130.9 (2 x CH, Ar-

2), 129.1 (C, Ar-1), 128.5 (2 x CH, Ar), 127.6 (CH, Ar), 124.5 (2 x CH, Ar), 115.3 (2 x CH, Ar-3), 83.3 (C, cumyl), 79.0 (NO_2CH_2), 78.8 (OCH_2, Ox), 78.7 (OCH_2, Ox), 59.5 (C, Ox), 58.0 (CH, α-Tyr), 39.9 (CH_2, β-Tyr), 28.7 (CH_3, cumyl), 27.6 (CH_3, cumyl); **IR** (film): 3765 (OH), 3194 (NH), 2973, 1732 (C=O), 1552, 1514, 1380, 1242, 1076 cm^{-1}; **MS** (ESI^+) *m/z* 437 $[M+Na]^+$, 453 $[M+K]^+$; **HRMS** (ESI^+) Calcd for $C_{22}H_{26}N_2O_6Na$ $[M+Na]^+$: 437.1683, found 437.1684.

Fmoc-GOx-Tyr(OH)-OCumyl 6

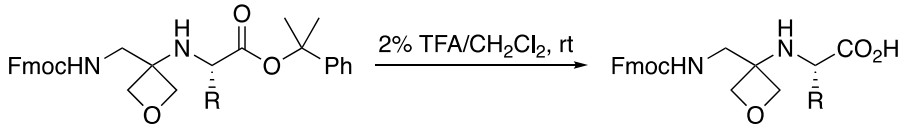


5 **6**

To **5** (356 mg, 0.86 mmol) in THF (9 mL) was added Fmoc *N*-hydroxysuccinimide ester (305 mg, 0.90 mmol), $NaHCO_3$ (152 mg, 1.81 mmol) and Raney Ni (1 mL, slurring in H_2O). The reaction mixture was stirred at room temperature under an atmosphere of H_2 (balloon) until consumption of starting material (MS monitoring). The reaction mixture was filtered through a plug of Celite® eluting with EtOAc. The eluent was washed with a saturated solution of Na_2CO_3 (3 x 10 mL) and brine (5 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by column chromatography (5-20% EtOAc in CH_2Cl_2) gave **6** (357 mg, 0.59 mmol, 68%) as a white solid. $\mathbf{R}_f$ (20% EtOAc in CH_2Cl_2) 0.22; **mp** 82-85 °C; $[\alpha]_D^{24}$ –5.0 (*c* 0.27, $CHCl_3$); **^{1}H-NMR** (500 MHz, $CDCl_3$) δ 7.88-7.70 (2H, m, Ar-Fmoc), 7.65-7.51 (2H, m, ArH-Fmoc), 7.46-7.37 (2H, m, ArH-Fmoc), 7.37-7.19 (7H, m, ArH), 7.04 (2H, d, *J* = 8.1 Hz, ArH-2), 6.85-6.73 (0.25H, m, minor rotamer ArH-3), 6.63 (1.75H, d, *J* = 8.1 Hz, major rotamer ArH-3), 4.76-4.54 (2H, m, OH and NH-GOx), 4.53-4.29 (2H, m, CH_2-Fmoc), 4.27-4.02 (5H, m, CH-Fmoc and 2 x OCH_2-Ox), 3.52-3.17 (3H, m, CH_2-GOx and CHα-Tyr), 3.00 (1H, dd, *J* = 13.4, 4.5 Hz, C*H*Hβ-Tyr), 2.56 (1H, dd, *J* = 13.3, 9.7 Hz, CH*H*β-Tyr), 1.98 (1H, bs, NH-Tyr), 1.79 (3H, s, CH_3-cumyl), 1.77 (3H, s, CH_3-cumyl); **^{13}C-NMR** (125 MHz, $CDCl_3$) δ 174.2 (C=O, Tyr), 156.7 (C, Ar-4), 154.6 (C=O, Fmoc), 144.8 (C, Ar-cumyl), 144.2 (C, Ar-Fmoc), 144.0 (C, Ar-Fmoc), 141.5 (C, Ar-Fmoc), 141.3 (C, Ar-Fmoc), 130.7 (2 x CH, Ar-2), 129.5 (C, Ar-1), 128.5 (2 x CH, Ar), 127.9 (CH, Ar), 127.8 (CH, Ar), 127.6 (CH, Ar), 127.23 (CH, Ar), 127.18 (CH, Ar), 125.2 (2 x CH-Ar), 124.5 (2 x CH, Ar), 120.2 (CH, Ar), 120.1 (CH, Ar), 115.6 (2 x CH, Ar-3), 83.2 (C, cumyl), 80.1 (CH_2-Ox), 79.4 (CH_2, Ox), 66.5 (CH_2-Fmoc), 59.3 (C, Ox), 58.0 (CH, α-Tyr), 47.5 (CH, Fmoc),

45.0 (CH_2, GOx), 39.9 (CH_2, β-Tyr), 28.7 (CH_3, cumyl), 28.0 (CH_3, cumyl); **IR** (film): 3675 (OH), 3320 (NH), 2987, 2901, 1718 (C=O), 1695 (C=O), 1514, 1227, 1100 cm^{-1}; **MS** (ESI+) *m/z* 607 $[M+H]^+$, 629 $[M+Na]^+$, 645 $[M+K]^+$; **HRMS** (ESI+) Calcd for $C_{37}H_{38}N_2O_6Na$ $[M+Na]^+$: 629.2622, found 629.2622.

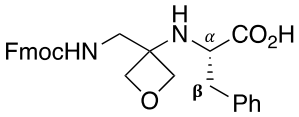
General Procedure for Synthesis of 1 and 3



7, **6** **1**, **3**

Cumyl esters [**7**, **6**] in 2% TFA/CH_2Cl_2 (0.05 M) were stirred at room temperature until consumption of starting material (TLC monitoring). The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH_2Cl_2 (5 x 20 mL/mmol) and concentrated under reduced pressure. To this crude acid was added Et_2O (60 mL/mmol) and the suspension vigorously stirred at 0 °C for 15 min. The precipitate was collected by vacuum filtration and washed with cold Et_2O to give the free carboxylic acid.

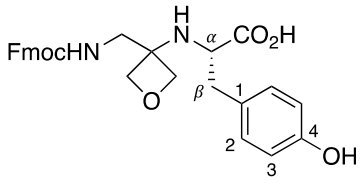
Fmoc-GOx-Phe-OH 1



Following the general procedure, Fmoc-GOx-Phe-OCumyl **7**[2] (376 mg, 0.62 mmol) was treated with 2% TFA/CH_2Cl_2 (13 mL) to give **1** (178 mg, 0.38 mmol, 61%) as a white solid. **mp** 183-186 °C; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ 7.90 (2H, d, *J* = 7.6 Hz, ArH-Fmoc), 7.70 (2H, d, *J* = 7.4 Hz, ArH-Fmoc), 7.42 (2H, t, *J* = 7.4 Hz, ArH-Fmoc), 7.34 (2H, t, *J* = 7.4 Hz, ArH-Fmoc), 7.30-7.10 (6H, m, ArH-Phe and NH-GOx), 4.40-4.31 (2H, m, CH_2-Fmoc), 4.31-4.18 (4H, m, CH-Fmoc, OCH_2-Ox and OC*H*H-Ox), 4.12 (1H, d, *J* = 6.2 Hz, OCH*H*-Ox), 3.82-3.61 (1H, m, CHα-Phe), 3.53-3.14 (2H, m, CH_2-GOx, overlapping with H_2O signal), 2.87 (1H, dd, *J* = 13.3, 6.8 Hz, C*H*Hβ-Phe), 2.77 (1H, dd, *J* = 13.0, 7.0 Hz, CH*H*β-Phe) [Note: NH-Phe and CO_2H not assigned]; **^{13}C-NMR** (125 MHz, DMSO-d_6) δ 176.0 (C=O, Phe), 156.9 (C=O, Fmoc), 143.9 (C, Ar-Fmoc), 143.8 (C, Ar-Fmoc), 140.7 (2 x C, Ar-Fmoc), 137.8 (C, Ar-Phe), 129.3 (2 x CH, Ar), 128.0 (2 x CH, Ar), 127.6 (2 x CH, Ar), 127.1 (2 x CH, Ar), 126.3 (CH, Ar), 125.1 (2 x CH, Ar), 120.1 (2 x CH, Ar), 78.08 (OCH_2, Ox), 77.98 (OCH_2, Ox), 65.5 (CH_2, Fmoc), 59.8 (C, Ox), 56.9 (CH, α-Phe), 46.7 (CH, Fmoc), 44.4 (CH_2, GOx), 39.5 (CH_2, β-Phe) [Note: C=O-Phe assigned using HMBC and CH_2β-Phe assigned using HSQC]; **MS** (ESI^+) *m/z* 473 $[M+H]^+$, 495 $[M+Na]^+$, 511 $[M+K]^+$, 967 $[2M+Na]^+$; **IR** (film): 3410 (NH),

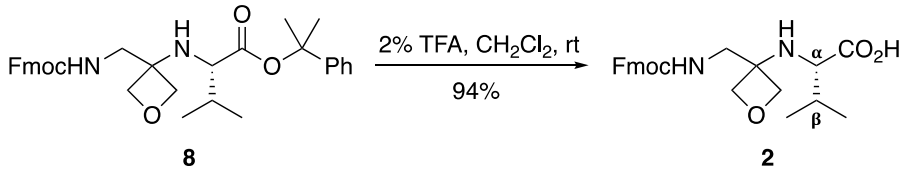
3063, 2884, 1702 (C=O), 1625 (C=O), 1524, 1225, 993 cm^{-1}; **MS** (ESI^-) 471 $[M-H]^-$, 585 $[M+TFA-H]^-$, 943 $[2M-H]^-$; **HRMS** (ESI^+) Calcd for $C_{28}H_{28}N_2O_5Na$ $[M+Na]^+$: 495.1890, found 495.1891; **HRMS** (ESI^-) Calcd for $C_{28}H_{27}N_2O_5$ $[M-H]^-$: 471.1925, found 471.1925. NOTE: No optical rotation as compound insoluble in suitable solvents.

Fmoc-GOx-Tyr-OH 3



Following the general procedure, **6** (152 mg, 0.25 mmol) was treated with 2% TFA/CH_2Cl_2 (5 mL) to give **3** (100 mg, 0.20 mmol, 82%) as a white solid. **mp** 232-235 °C (decomposition); **^{1}H-NMR** (500 MHz, DMSO-d_6) δ 9.17 (1H, s, ArOH), 7.90 (2H, d, *J* = 7.5 Hz, ArH-Fmoc), 7.70 (2H, d, *J* = 7.3 Hz, ArH-Fmoc), 7.45-7.39 (2H, m, ArH-Fmoc), 7.37-7.26 (3H, m, ArH-Fmoc and NH-GOx), 7.02 (2H, d, *J* = 8.3 Hz, ArH-2), 6.64 (2H, d, *J* = 8.2 Hz, ArH-3), 4.39-4.29 (2H, m, CH_2-Fmoc), 4.29-4.17 (4H, m, OCH_2-Ox, OC*H*H-Ox and CH-Fmoc), 4.12 (1H, d, *J* = 6.2 Hz, OCH*H*-Ox), 3.67-3.50 (1H, m, CHα-Tyr), 3.47-3.25 (2H, m, CH_2-GOx, overlapping with H_2O signal), 2.75 (1H, dd, *J* = 13.3, 6.7 Hz, C*H*Hβ-Tyr), 2.64 (1H, dd, *J* = 13.2, 7.0 Hz, CH*H*β-Tyr) [Note: NH-Tyr and CO_2H not assigned]; **^{13}C-NMR** (125 MHz, DMSO-d_6) δ 176.9 (C=O, Tyr), 157.3 (C=O, Fmoc), 156.3 (C, Ar-4), 144.4 (C, Ar-Fmoc), 144.3 (C, Ar-Fmoc), 141.2 (2 x C, Ar-Fmoc), 130.7 (2 x CH, Ar-2), 128.4 (C, Ar-1), 128.1 (2 x CH, Ar-Fmoc), 127.6 (2 x CH, Ar-Fmoc), 125.6 (2 x CH, Ar-Fmoc), 120.6 (2 x CH, Ar-Fmoc), 115.3 (2 x CH, Ar-3), 78.7 (OCH_2, Ox), 78.6 (OCH_2, Ox), 66.0 (CH_2, Fmoc), 60.3 (C, Ox), 57.7 (CH, α-Tyr), 47.2 (CH, Fmoc), 45.1 (CH_2, GOx), 39.4 (CH_2, β-Tyr) [Note: CH_2, β-Tyr assigned using HSQC]; **IR** (film): 3676 (OH), 3350 (NH), 3101, 2987, 2900, 1712 (C=O), 1589, 1267, 1243 cm^{-1}; **MS** (ESI^-) 487 $[M-H]^-$, 975 $[2M-H]^-$; **HRMS** (ESI^+) Calcd for $C_{28}H_{28}N_2O_6Na$ $[M+Na]^+$: 511.1840, found 511.1842. NOTE: No optical rotation as compound insoluble in suitable solvents.

Fmoc-GOx-Val-OH 2



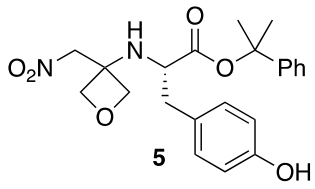
8 **2**

Fmoc-GOx-Val-OCumyl **8**[2] (446 mg, 0.82 mmol) in 2% TFA/CH_2Cl_2 (16 mL) was stirred at room temperature until consumption of starting material (TLC monitoring, 2 h). The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly

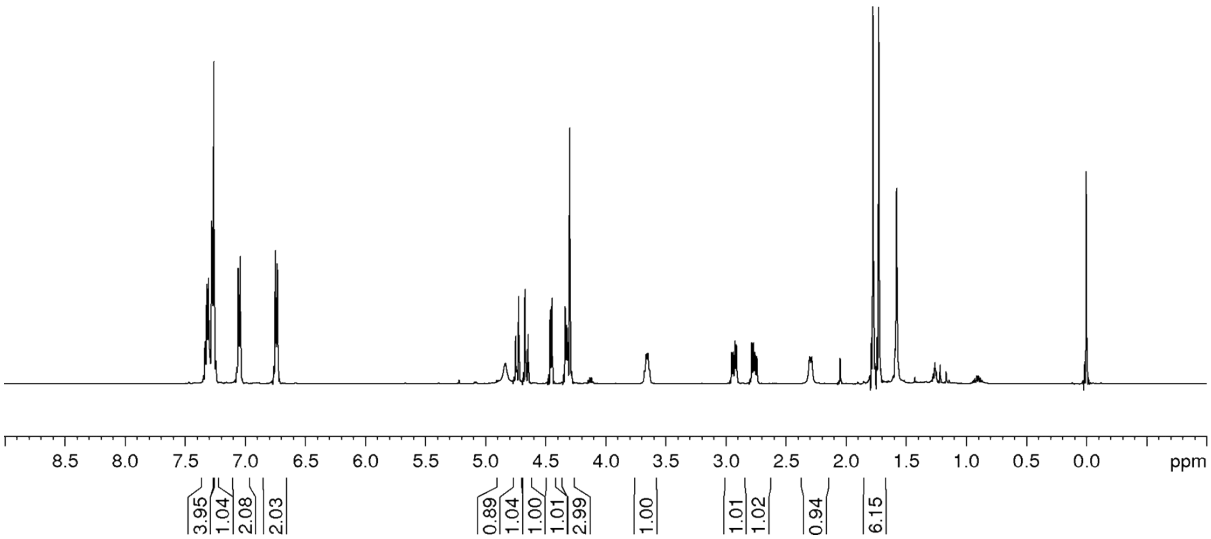
dissolved in CH_2Cl_2 (5 x 15 mL) and concentrated under reduced pressure. Purification by column chromatography (0-10% MeOH in CH_2Cl_2) gave **2** (329 mg, 0.77 mmol, 94%) as a white solid. $\mathbf{R_f}$ (10% MeOH in CH_2Cl_2) 0.32; **mp** 103-107 °C; $[\alpha]_D^{24}$ +2.0 (*c* 0.60, $CHCl_3$); **^{1}H-NMR** (500 MHz, MeOD) δ 7.80 (2H, d, *J* = 7.5 Hz, ArH-Fmoc), 7.65 (2H, d, *J* = 7.4 Hz, ArH-Fmoc), 7.39 (2H, t, *J* = 7.4 Hz, ArH-Fmoc), 7.31 (2H, t, *J* = 7.4 Hz, ArH-Fmoc), 4.69 (1H, d, *J* = 7.2 Hz, OC*H*H-Ox), 4.59 (1H, d, *J* = 7.3 Hz, OC*H*H-Ox), 4.48 (1H, d, *J* = 7.3, OCH*H*-Ox), 4.46-4.35 (3H, m, OCH*H*-Ox and CH_2-Fmoc), 4.23 (1H, t, *J* = 6.6 Hz, CH-Fmoc), 3.74 (1H, d, *J* = 4.0 Hz, CHα-Val), 3.70 (1H, d, *J* = 14.9 Hz, C*H*H-GOx), 3.60 (1H, d, *J* = 14.8 Hz, CH*H*-GOx), 2.19-2.04 (1H, m, CHβ-Val), 1.06 (3H, d, *J* = 6.9 Hz, CH_3-Val), 1.02 (3H, d, *J* = 6.8 Hz, CH_3-Val); **^{13}C-NMR** (125 MHz, MeOD) δ 174.9 (C=O, Val), 159.7 (C=O, Fmoc), 145.3 (C, Ar-Fmoc), 145.2 (C, Ar-Fmoc), 142.6 (2 x C, Ar-Fmoc), 128.8 (2 x CH, Ar-Fmoc), 128.2 (2 x CH, Ar-Fmoc), 126.13 (CH, Ar-Fmoc), 126.11 (CH, Ar-Fmoc), 121.0 (2 x CH, Ar-Fmoc), 78.03 (OCH_2-Ox), 78.00 (OCH_2-Ox), 68.1 (CH_2-Fmoc), 63.0 (CH, α-Val), 62.7 (C, Ox), 48.4 (CH, Fmoc), 45.1 (CH_2, GOx), 32.1 (CH, β-Val), 18.9 (CH_3, Val), 18.5 (CH_3, Val); **IR** (film): 3304 (NH), 3040, 2966, 2882, 2882, 1671 (C=O), 1250, 1181, 1134 cm^{-1}; **MS** (ESI^+) *m/z* 447 $[M+Na]^+$, 871 $[2M+Na]^+$; **MS** (ESI^-) *m/z* 423$[M-H]^-$, 847 $[2M-H]^-$; **HRMS** (ESI^+) Calcd for $C_{24}H_{28}N_2O_5Na$ $[M+Na]^+$: 447.1890, found 447.1879. Compound previously prepared by Carreira *et al.*[3] *via* an alternative route.

2. ^{1}H and ^{13}C NMR spectra of 1, 2, 3, 5 and 6

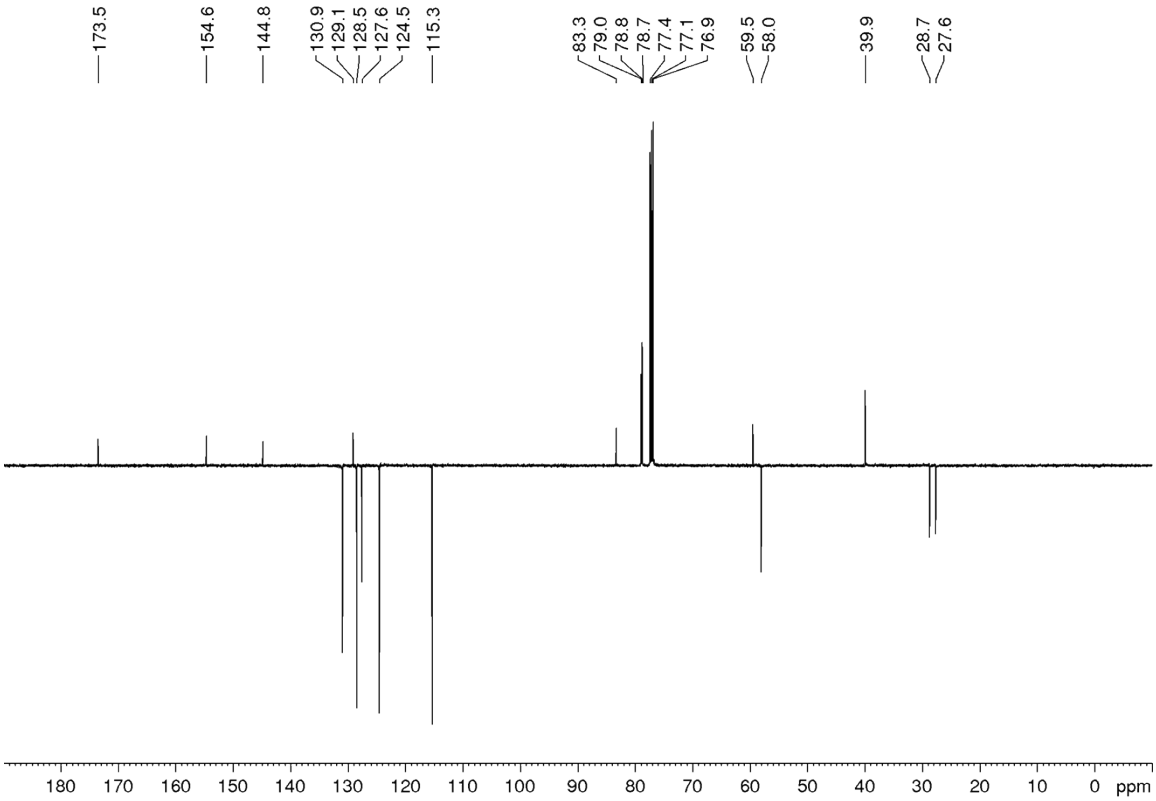
5 **^{1}H-NMR** (500 MHz, $CDCl_3$)



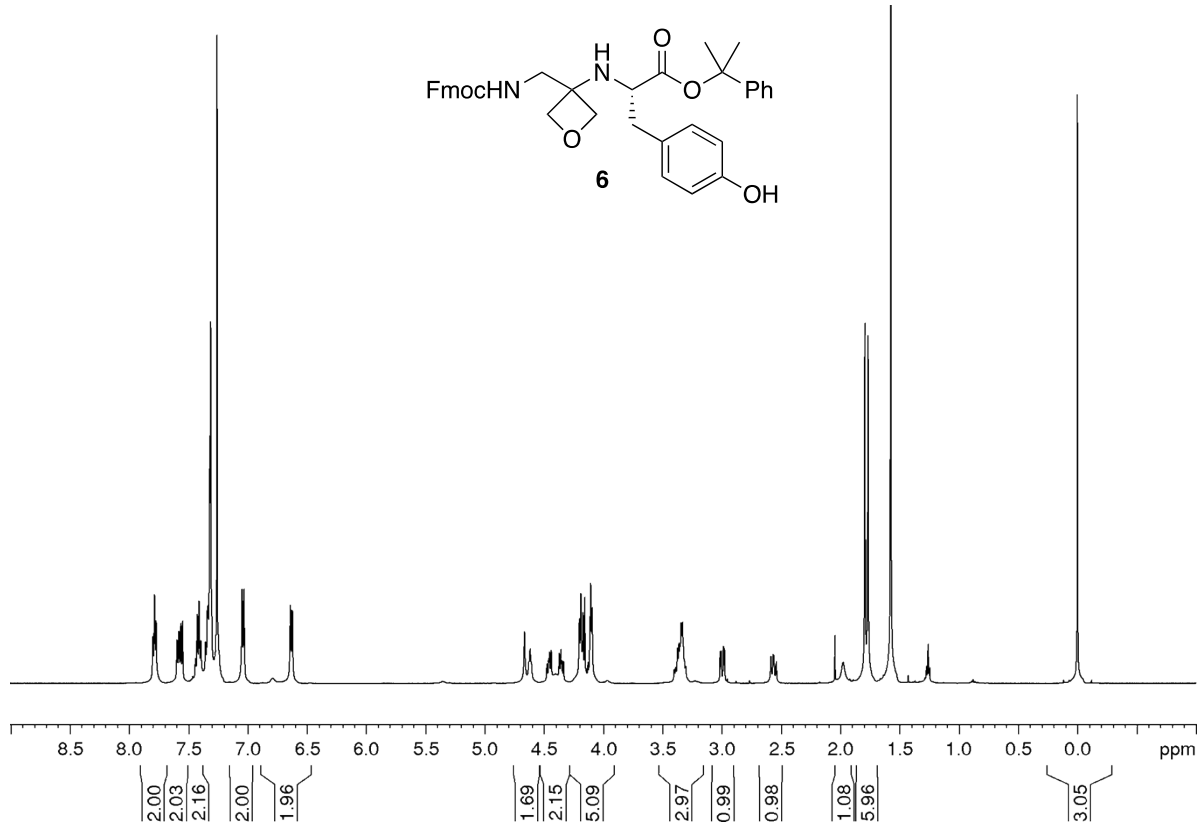
5



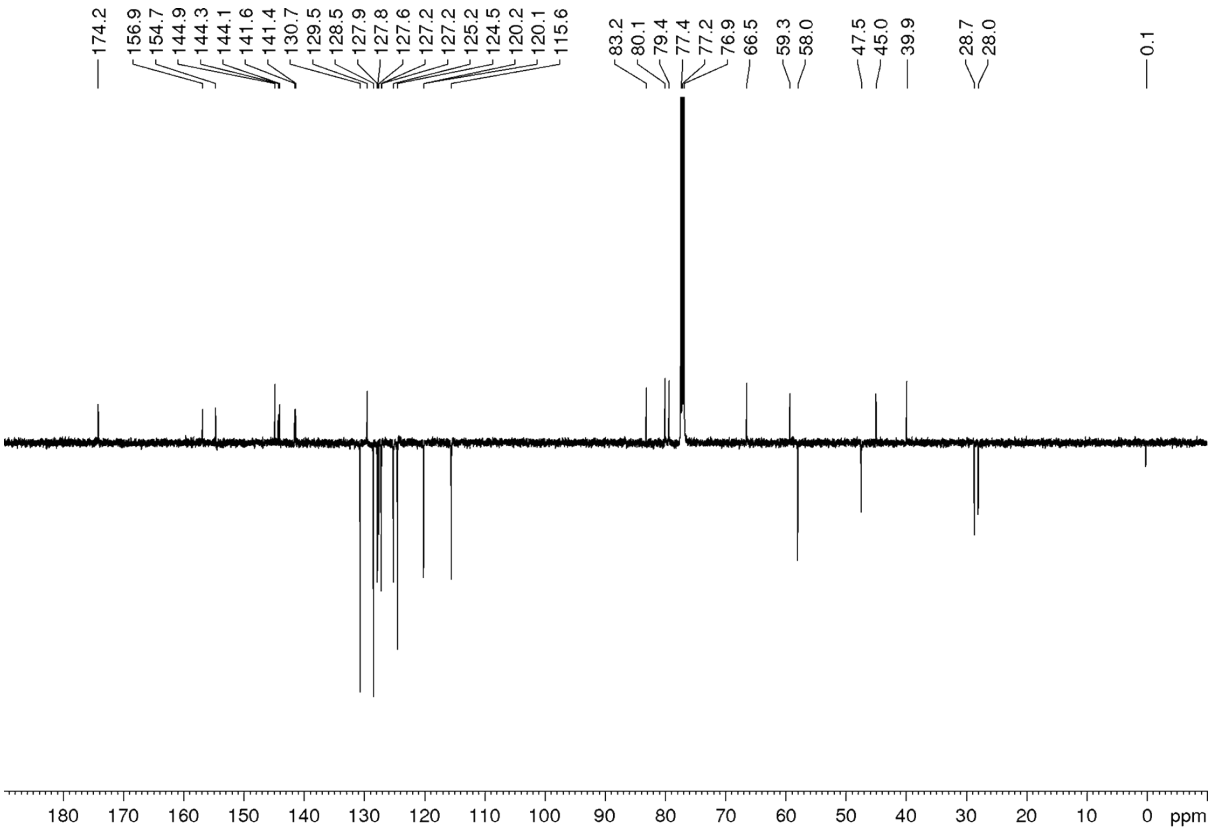
^{13}C-NMR (125 MHz, $CDCl_3$)



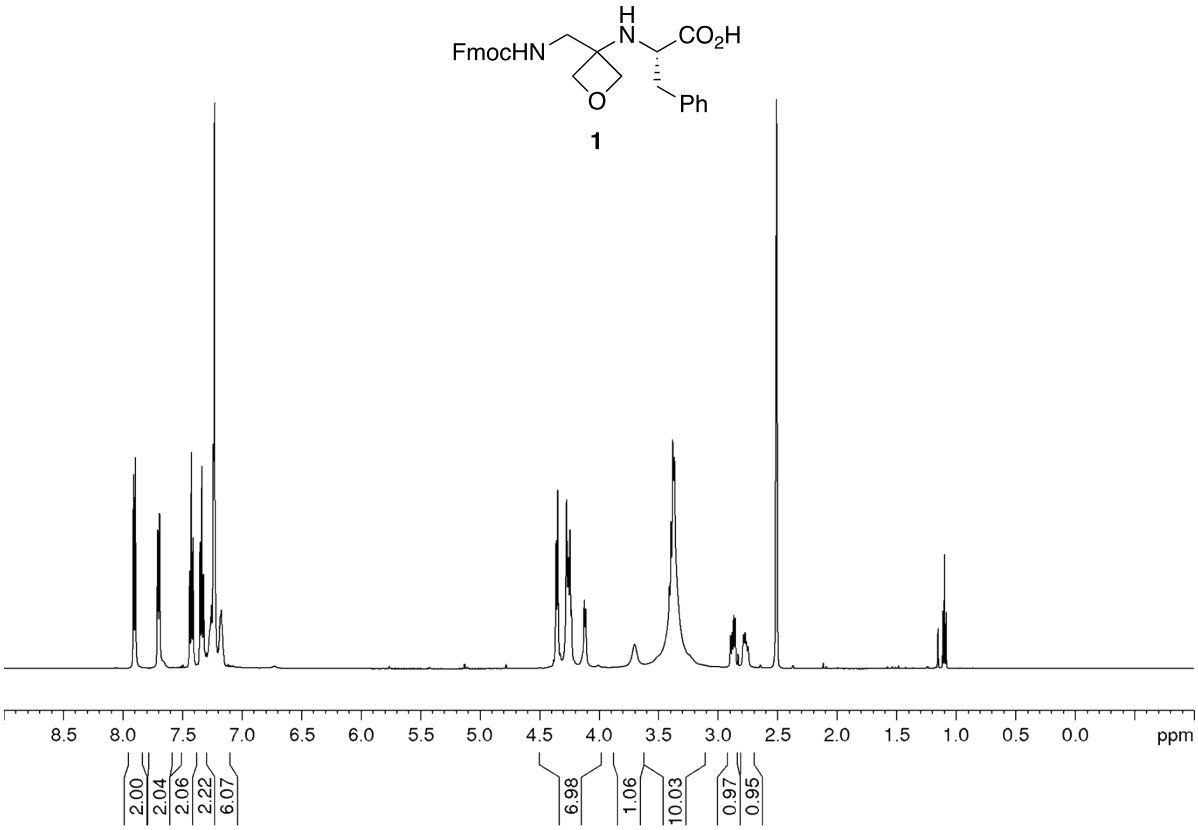
6 **^{1}H-NMR** (500 MHz, $CDCl_3$)



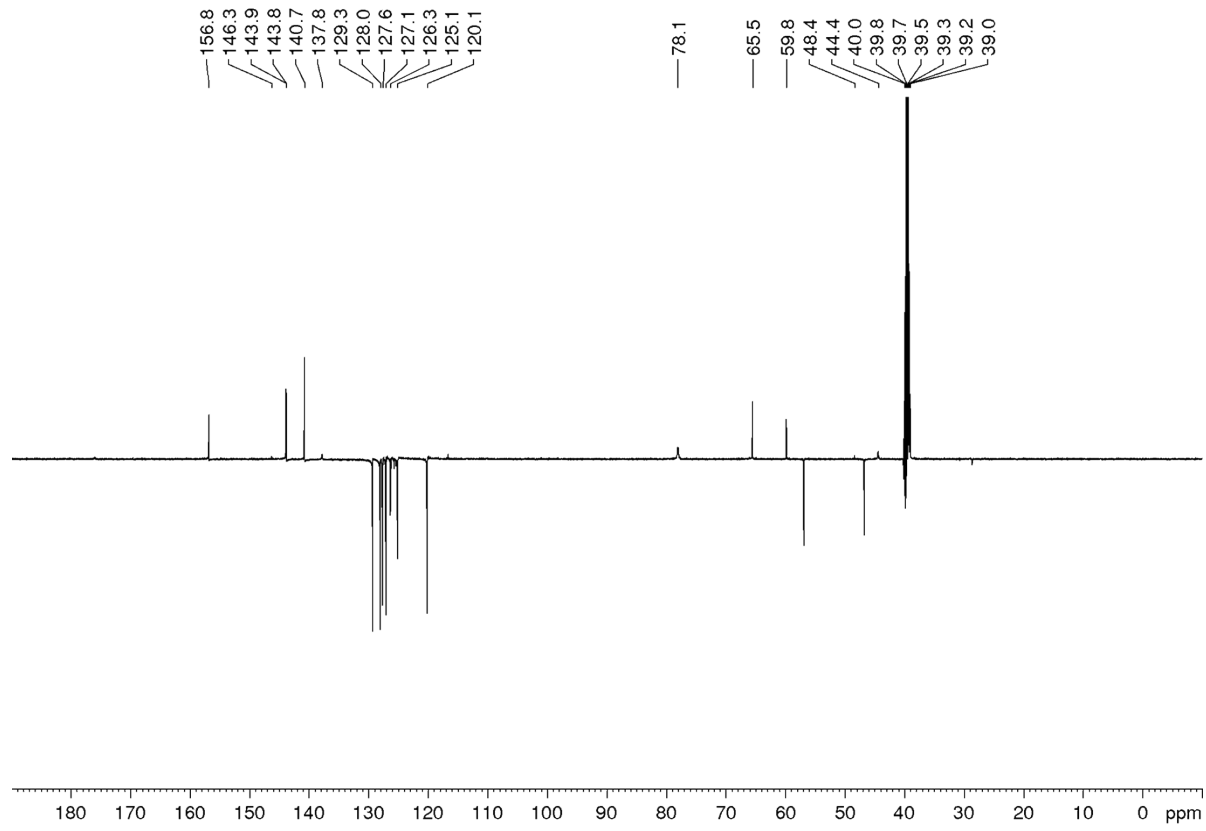
^{13}C-NMR (125 MHz, $CDCl_3$)



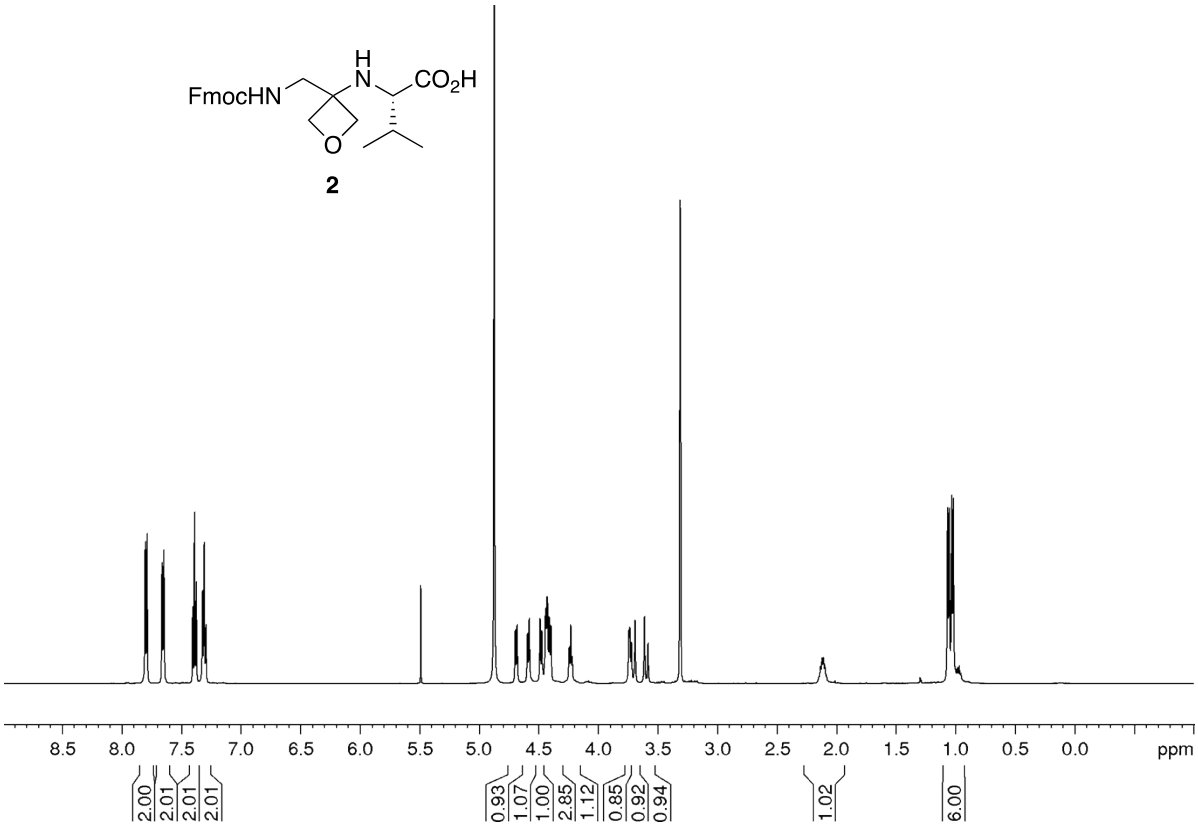
1 **^{1}H-NMR** (500 MHz, DMSO-d_6)



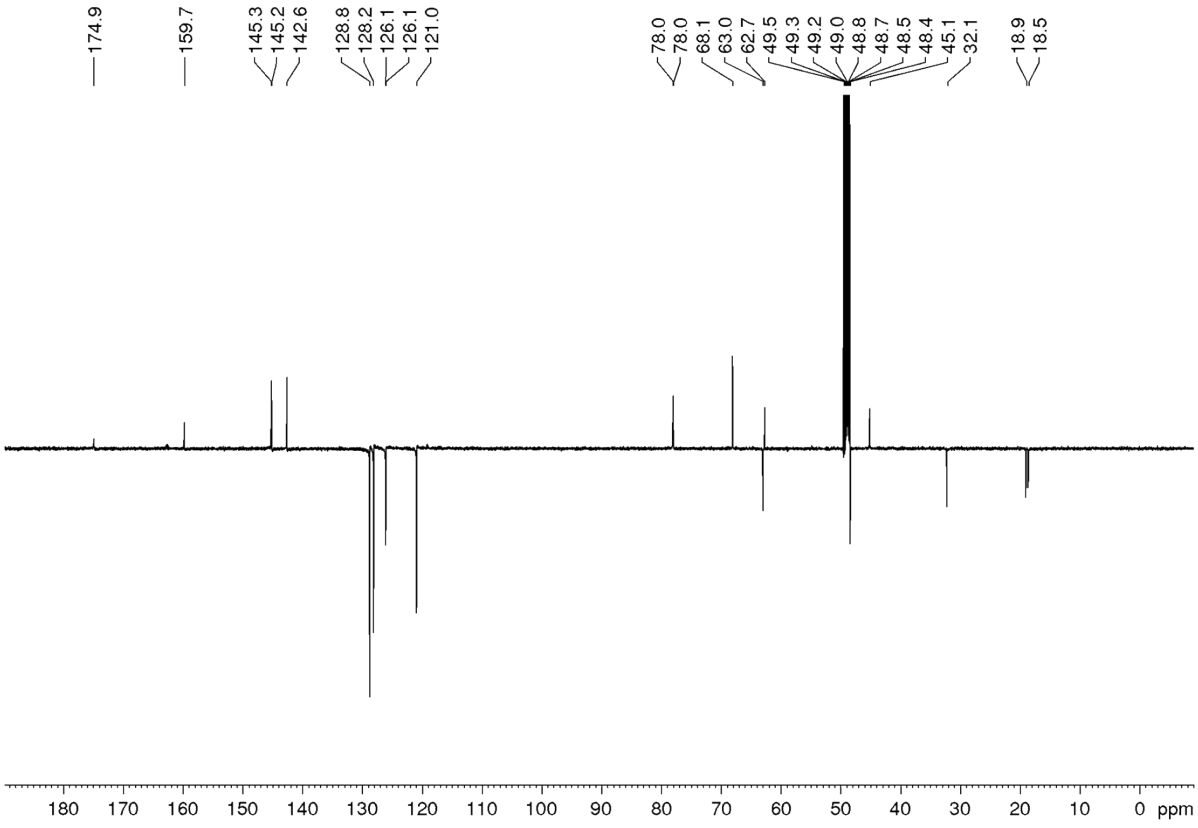
^{13}C-NMR (125 MHz, DMSO-d_6)



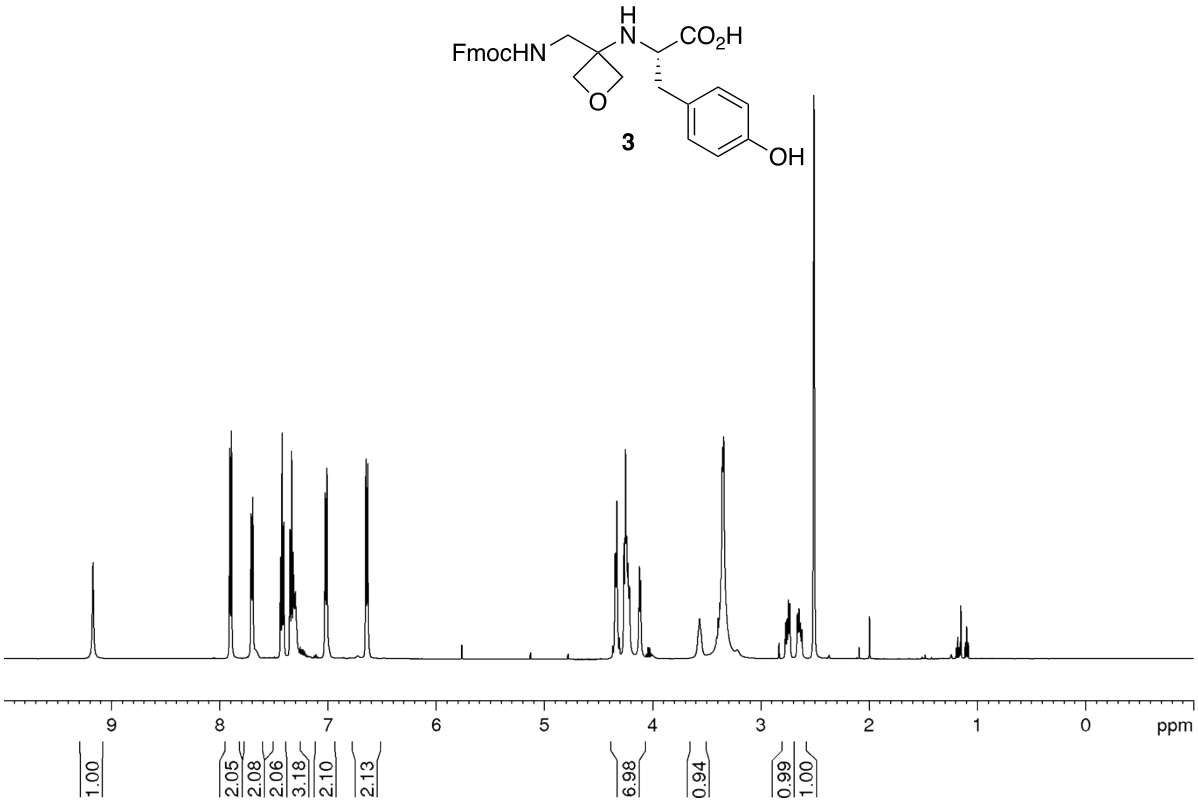
2 ^{1}H-NMR (500 MHz, MeOD)



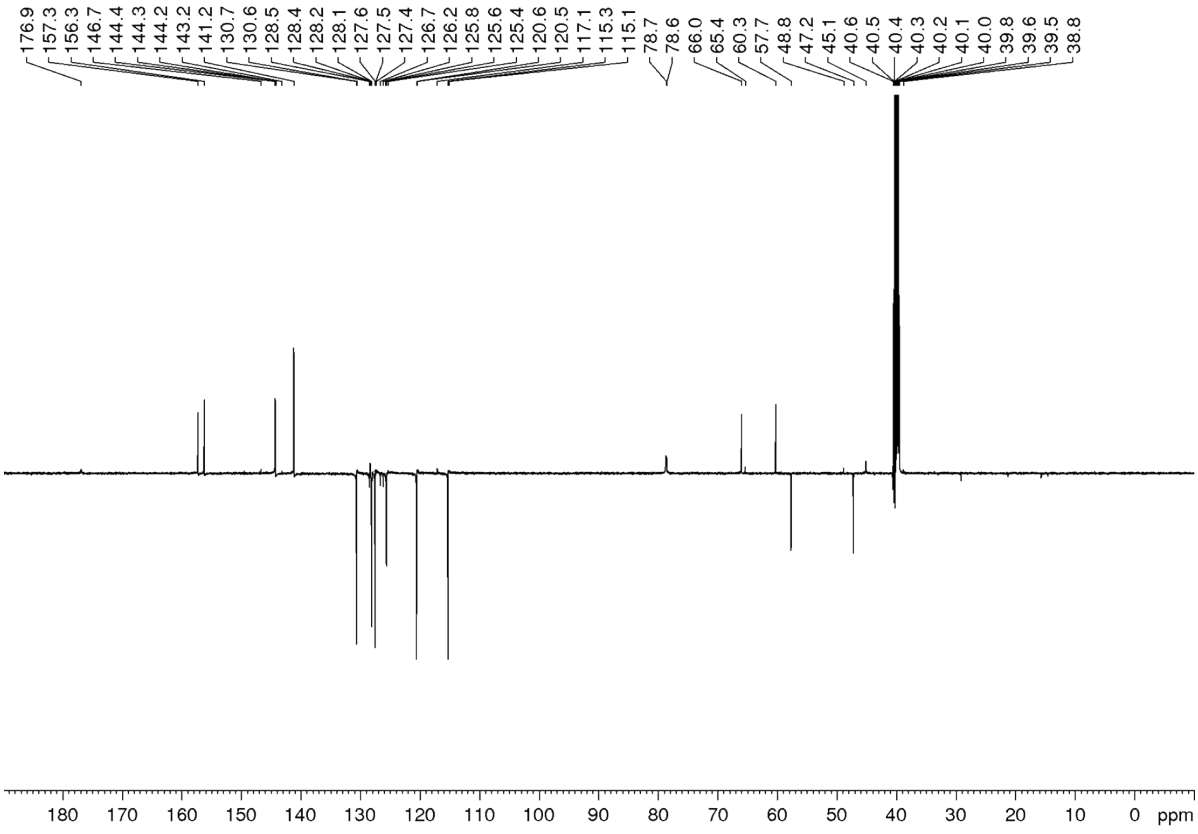
^{13}C-NMR (125 MHz, MeOD)



3 **^{1}H-NMR** (500 MHz, DMSO-d_6)



^{13}C-NMR (125 MHz, DMSO-d_6)



3. Gelation Studies

Preparation of Gels. 1 (6 mg) was suspended in water (1.87 mL). Sodium carbonate (0.13 mL of a 10 mg/mL aqueous solution) was added. The sample was placed in an ultrasonic bath for 10 minutes, after which time a clear solution was formed. To form a gel, this solution was added to a pre-weighed aliquot of glucono-δ-lactone (GdL) (16 mg). After swirling to dissolve the GdL, the sample was allowed to stand for 18 hours to form a gel.

Photographs. Photographs of the solutions and gels were taken on an iPhone7.

Rheology. All rheological measurements were carried out on an Anton Paar Physica MCR 301 or 101. Strain and frequency sweeps were performed using a vane and cup geometry. For this 2 mL of gel was made as described as above in 7 mL Sterlin vials in which they could be measured directly rather than having to transfer onto the rheometer. This ensured samples were not damaged and for reproducibility. All gels were measured in triplicate at 25 °C and were left for 16 hours before measuring. Time sweeps were conducted using a parallel plate geometry, using a 25 mm plate. Gels were made directly on the plate and the plate lowered on top of the solution. This was achieved by pre-weighing an amount of GdL into a vial and adding the gelator solution and mixing quickly and transferring straight on to the plate. Once the plate had been lowered on to gelling solution, the outside of the plate was flooded with oil to prevent evaporation.

Strain sweep: Strain sweep were performed between 0.1 – 1000 % strain at a constant frequency of 10 rad/s, measuring 10 points per decade. The yield point is determined at which strain G′ deviated from linearality and the flow point as were G′ and G″ crossed over (if at all). The linear viscoelastic region (LVR) can be defined as where strain has no impact upon G′ and G″. All measurements were carried out in triplicate at 25 °C.

Frequency sweep: Frequency sweeps were performed between 0.1 – 100 rad/s at a constant strain of 0.5 %, measuring 10 points per decade. It is crucial to perform the frequency sweeps within the LVR as determined by the strain sweep. G′ and G″ were quoted at 10 rad/s. Again, all measurements were carried out in triplicate at 25 °C.

Time sweep: Monitoring gelation over time was done with a constant strain of 0.5 % and a frequency of 10 rad/s. A measurement was recorded every minute until a plateau in G′ had been achieved, this was roughly after 16 hours. The gap distance was 1 mm and a normal of 0 N was maintained throughout.

Fluorescence Spectroscopy. Fluorescence spectra were collected on an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer in a 1 cm pathlength PMMA fluorescence cuvette. Spectra were collected using an excitation of 265 nm from 280-400 nm with a slit width of 1.5 nm. Samples were prepared as stated previously but in the cuvettes.

Fourier-Transform Infra-red Spectroscopy. FTIR spectra were collected on an Agilent Technologies Cary 630 FTIR using to the ATR attachment. Samples were prepared as described above, a small amount of either solution or gel was removed by a pipette (for solution) or carefully by spatula and measured on the FTIR.

Scanning Electron Microscopy (SEM). Images were collected using a Hitachi S-4800 FE SEM in decceleration mode at 2 kV at a height of 3 mm.. The FE-SEM measurement scale bar was calibrated using certified SIRA calibration standards. The samples were prepared in a vial as described above and a small amount was transferred onto a glass cover slip. This was allowed to dry overnight in air before being attached to a 15 mm Hitachi M4 aluminium stub with a sticky carbon tab. Samples were not coated before measuring. Areas of the gel were chosen at random to image from as to make sure the area being imaged was representative of the whole sample. Fibre width measurements from the SEM images were carried out using ImageJ. This was calibrated using the scale bars on the images. 70 measurements were done using a few of the images and a histogram of these widths was plotted. An average fibre could then be calculated and a standard deviation from this average.

Circular and linear dichroism. Circular dichroism spectra were recorded using a Jasco J-810 spectropolarimeter. Samples were analysed at 20 °C in a quartz cuvette (0.01cm pathlength). Interval scans were recorded at 100nm/min at 300 sec intervals using a bandwidth of 1 nm. Linear dichroism spectra were collected on the same spectropolarimeter, as were the HT data and the UV-vis spectra.

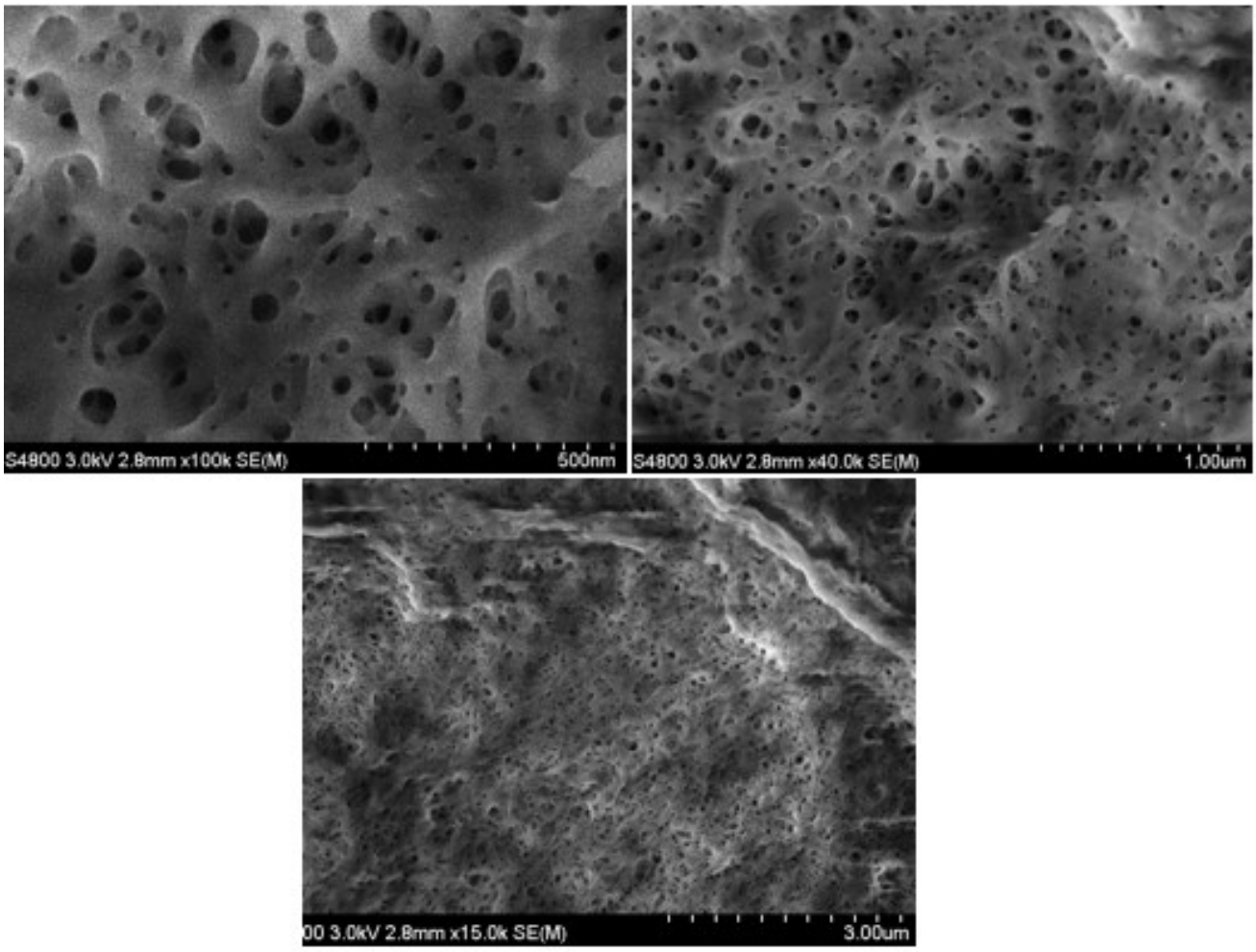


Figure S1. Additional SEM images for the xerogel of **1**.

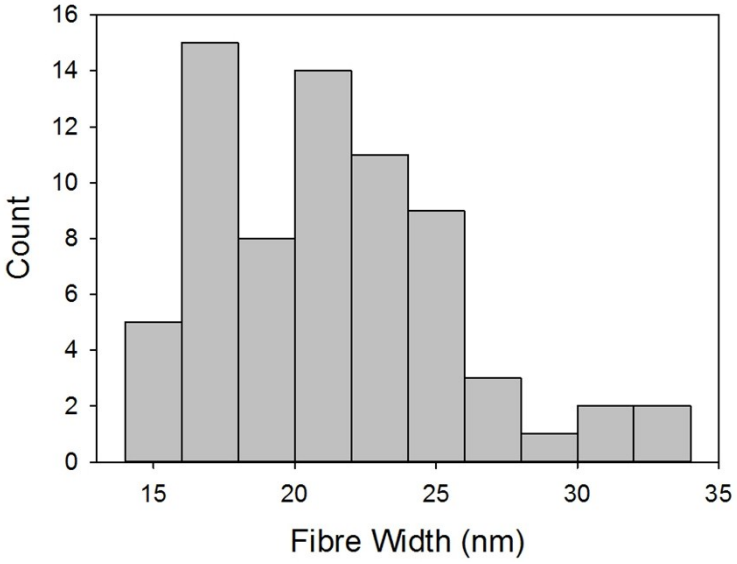


Figure S2. Histogram of fibre widths measured from the SEM images in Fig. 3d (main paper) and Figure S1.

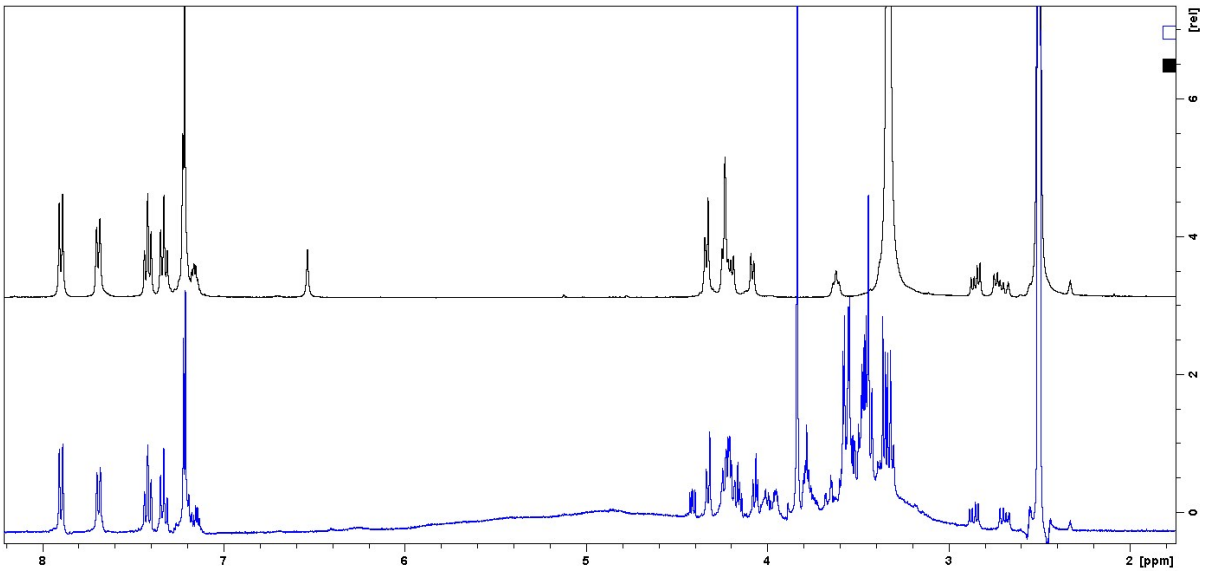


Figure S3. Overlay of the ^{1}H NMR (in d_6-DMSO) of **1** (as prepared, in black) with **1** recovered from the gel by freeze-drying (blue). In addition to the peaks from **1**, there are also the peaks from the hydrolysis products of GdL, as well as a broad baseline due to the presence of the sodium carbonate.

Assignment	Peak position – as prepared / ppm	Peak position – after gelation / ppm	Difference
C=O, Fmoc	156.9	157.2	-0.3
C, Ar-Fmoc	143.9	144.34	-0.44
C, Ar-Fmoc	143.8	144.31	-0.51
2 x C, Ar-Fmoc	140.7	141.2	-0.5
C, Ar-Phe	137.8	139	-1.2
2 X CH, Ar	129.3	129.8	-0.5
2 x CH, Ar	128	128.4	-0.4
2 X CH, Ar	127.6	128.1	-0.5
2 x CH, Ar	127.1	127.6	-0.5
CH, Ar	126.3	126.6	-0.3
2 x CH, Ar	125.1	125.6	-0.5
2 x CH, Ar	120.1	120.6	-0.5
OCH_2, Ox	78.08	78.78	-0.7
OCH_2, Ox	77.98	78.66	-0.68
CH_2, Fmoc	65.5	66	-0.5
C, Ox	59.8	60.2	-0.4
CH, a-Phe	56.9	57.1	-0.2
CH, Fmoc	46.7	47.2	-0.5
CH2, Gox	44.4	45.1	-0.7
CH_2, b-Phe	39.5	Under DMSO	N/A
DMSO	39.5	40.0	-0.5

Table S1. Comparison of the peaks in the ^{13}C NMR for **1** as freshly prepared and after recovery from the gel by freeze-drying. The peaks for the GdL hydrolysis products have not been assigned or included here. There is a slight difference in the absolute peak positions arising from the calibration of the DMSO peak between two different NMR spectrometers. There is also no doubling up of the signals in the aromatic region, oxetane region and other regions suggesting that there isn't any other dipeptide-related material present indicating that no chemical reaction to the oxetane-dipeptide buidling block has occured.

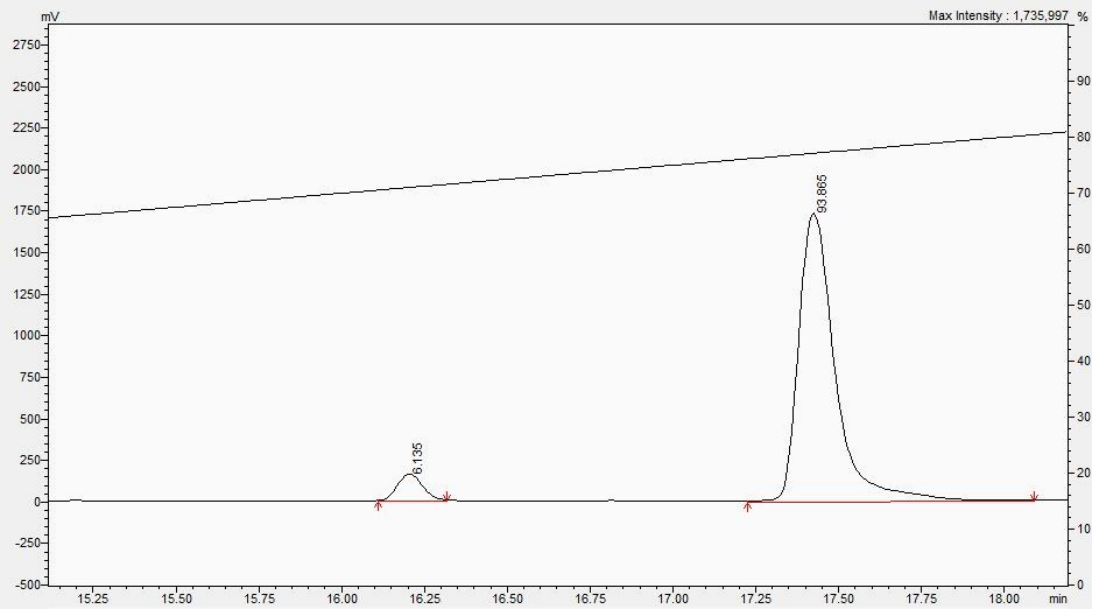


Figure S4. HPLC of **1** recovered by freeze-drying the gel. This indicates presence of an impurity (6%) assigned as product of ring opening of oxetane by water on the basis of the mass spectroscopy data.

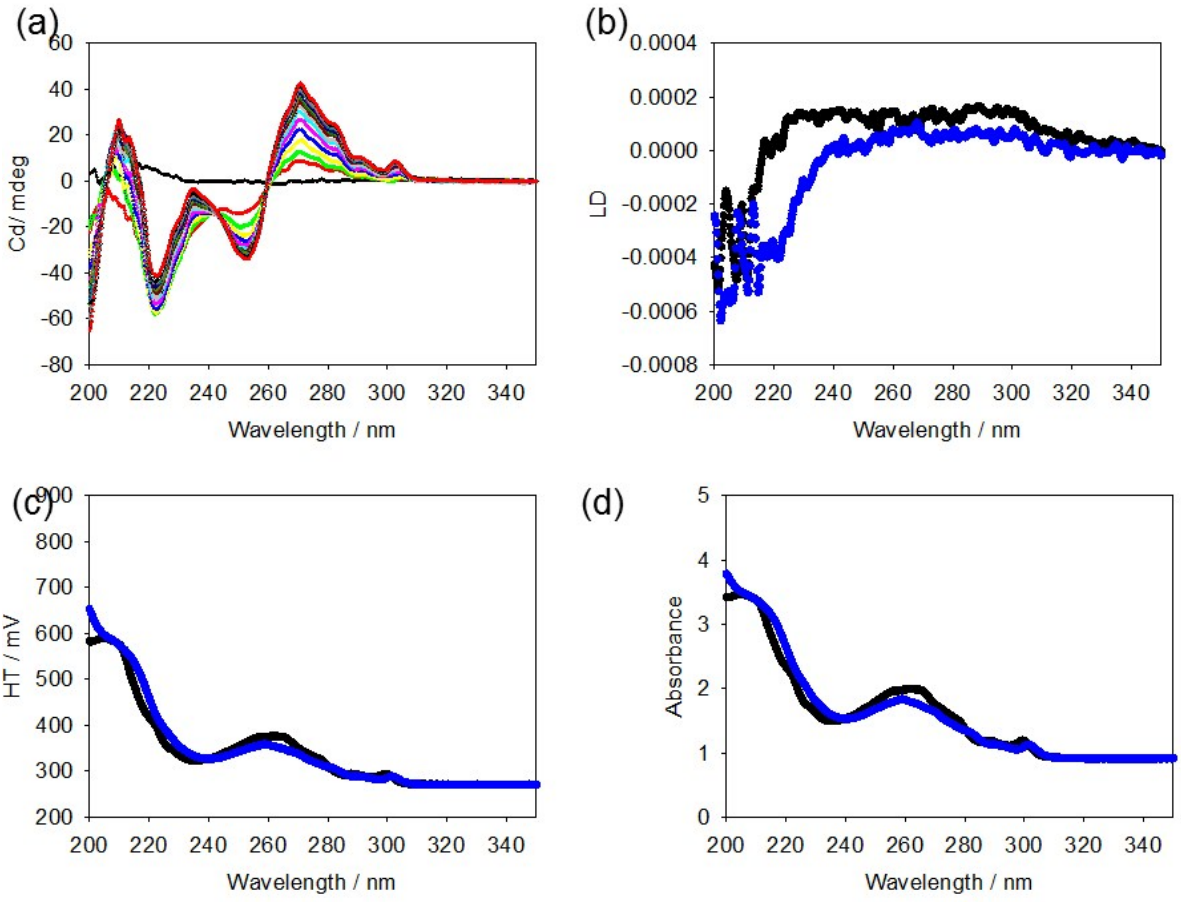


Figure S5. (a) Evolution of CD data over time. The black line is for the solution prior to the addition of GdL and the coloured lines show the peaks increasing in magnitude over two hours. (b) LD data collected prior to the additon of GdL (black data) and after the sample had gelled overnight after adding GdL (blue data). (c) HT data collected prior to the additon of GdL (black data) and after the sample had gelled overnight after adding GdL (blue data). (d) UV-Vis spectra collected prior to the additon of GdL (black data) and after the sample had gelled overnight after adding GdL (blue data).

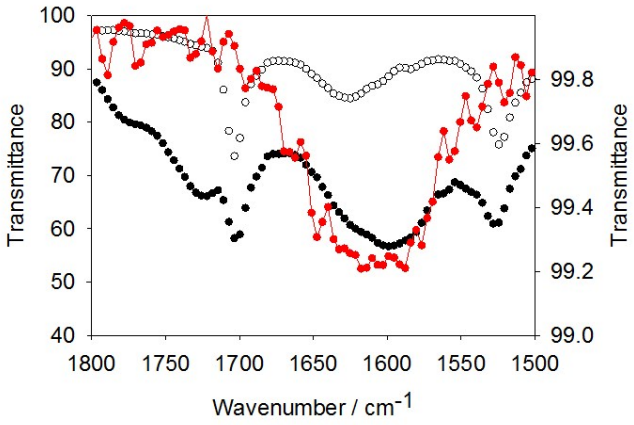


Figure S6. Overlay of the IR data for **1** (as prepared, open circles), the gel formed from **1** (filled circles) with a solution of sodium carbonate and GdL after hydrolysis for 24 hours (red circles. The intensity for the solution of sodium carbonate and GdL is at a much lower intensity and so is plotted on the right hand axis.

4. Enzymatic digestion of Fmoc-Dipeptides (FmocGlyPhe and 1)

Solutions of peptides (FmocGlyPhe or **1**) were prepared by dissolving in Tris buffer (0.05M, pH 7.9) to a concentration of 1 mg/mL. A stock solution containing 26 mg/mL Carboxypeptidase A was diluted ten times to a final concentration of 2.6 mg/mL (5.7 μM) and 7.7 μL was added to the peptide solutions (1:50 ratio of enzyme to peptide by mass). The enzyme containing solutions were incubated at 25°C and 100 μL aliquots taken at t=0, 1 min, 5 min, 1 h, 4 h and 24 h. The enzyme was quenched with 200 μL MeCN and centrifuged at 12,000 rpm for 5 min. The supernatant was taken and analysed by analytical HPLC using a Shimadzu HPLC system and Aeris C18 column (150 x 4.6 mm, particle size: 5 μm, pore size: 100 Å) at a flow rate of 1 mL/min. Samples were run with a linear gradient of 0-100% buffer B over 15 mins (buffer A: 95:5 v/v H_2O/MeCN + 0.1% TFA and buffer B: 95:5 v/v MeCN/H_2O + 0.1% TFA).

References

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2. Beadle, J. D.; Knuhtsen, A.; Hoose, A.; Raubo, P.; Jamieson, A. J.; Shipman, M. *Org. Lett.*, **2017**, *19*, 3303-3306.
3. McLaughlin, M.; Yazaki, R.; Fessard, T. C.; Carreira, E. M. *Org. Lett.*, **2014**, *16*, 4070-4073.