Development of Oxetane Modified Building Blocks for Peptide Synthesis

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Table of Contents

1. General experimental information

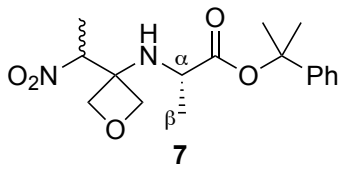
Reaction mixtures were stirred magnetically. All chemicals were purchased from Acros Organics, Alfa Aesar, Fluorochem or Sigma-Aldrich and used as received unless otherwise mentioned. Anhydrous solvents were purchased from Sigma-Aldrich or Acros Organics in Sure-Seal™ bottles. All other solvents were reagent grade and used as received. Petroleum ether (PE) refers to the fraction that boils in the range of 40–60 °C. All amino acids are of *L*-configuration unless otherwise stated. Fmoc-Ala-OCumyl,[1] NO_2-GOx-Thr(*t*Bu)-OCumyl[1] and 3-(1-nitroethylidene)oxetane (**5**)[2] were prepared following known literature procedures.

^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded in $CDCl_3$ on a Bruker HD300 (300 MHz), HD400 (400 MHz), AV500 (500 MHz) or AV600 (600 MHz) Fourier transform spectrometer. Chemical shifts (δ_H) are quoted in parts per million (ppm) and referred to the residual protic solvent signal of $CDCl_3$ (7.26 ppm). ^{1}H NMR coupling constants (*J*) are reported in hertz (Hz) and refer to apparent multiplicities. Data are reported as follows: chemical shift, multiplicity (s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublet, etc.), coupling constant, integration, and assignment. ^{13}C NMR spectra were recorded at 101, 126 or 151 MHz. Chemical shifts (δ_C) are quoted in ppm referenced to $CDCl_3$ (77.16 ppm). NMR assignments were deduced using 2D experiments (COSY, HSQC and HMBC).

Low-resolution mass spectra were recorded on an Agilent 6130B single Quad (ESI) instrument. High-resolution mass spectra were recorded using a Bruker MaXis Impact. All infrared spectra were recorded on the neat compounds using a Bruker ALPHA-Platinum FTIR spectrometer, irradiating between 4000 cm^{-1} and 600 cm^{-1}. Only strong and selected absorbances (ν_{max}) are reported. Analytical TLC was performed on aluminium backed silica plates (Merck, Silica Gel 60 F_{254}, 0.25 mm). Compounds were visualised by fluorescence quenching or by staining the plates with 5% solution of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) in EtOH or 1% solution of potassium permanganate ($KMnO_4$) in water followed by heating. Flash column chromatography was performed on silica gel (Aldrich, Silica Gel 60, 40–63 μm). All mixed solvent eluents are reported as v/v solutions. Optical rotations were obtained using an AA-1000 polarimeter at 589 nm (Na D-line) in a cell with a path length of 2 dm. Specific rotation values are given in (deg mL)/(g dm). Melting points were measured with a Gallenkamp melting point apparatus.

2. Synthesis and characterisation of oxetane modified alanine building blocks

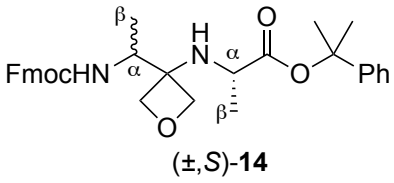
2-Phenylpropan-2-yl [3-((±)-1-nitroethyl)oxetan-3-yl]-L-alaninate (7)



7

Fmoc-Ala-OCumyl[1] (976 mg, 2.27 mmol, 1.5 equiv) in 50% diethylamine in CH_2Cl_2 (5.0 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 × 20 mL) and concentrated under reduced pressure to give crude amine **6**. To **6** in CH_2Cl_2 (7.5 mL) was added 3-(1-nitroethylidene)oxetane (**5**)[2] (196 mg, 1.51 mmol, 1.0 equiv) in CH_2Cl_2 (7.5 mL). The reaction mixture was stirred at room temperature until consumption of the nitroalkene (^{1}H NMR monitoring, 8 h) and then concentrated under reduced pressure to give a crude product which ^{1}H-NMR showed to consist of a 50:50 mixture of diastereoisomers. Purification by column chromatography (SiO_2, PE/EtOAc 9:1→4:1) gave **7** (436 mg, 1.30 mmol, 86%) as a colourless oil as a 55:45 mixture of diastereoisomers. **R_f** (PE/EtOAc 4:1) 0.29; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.40–7.30 (m, 4H, ArH), 7.30–7.22 (m, 1H, ArH), 4.97–4.86 (m, 1H, NO_2CH), 4.61–4.57 (m, 1.55H, 2 × major diastereoisomer OC*H*H-Ox and minor diastereoisomer OC*H*H-Ox), 4.55 (d, *J* = 7.5 Hz, 1H, OCH*H*-Ox), 4.48 (d, *J* = 7.6 Hz, 0.45H, minor diastereoisomer OC*H*H-Ox), 4.45 (d, *J* = 7.6 Hz, 0.45H, minor diastereoisomer OCH*H*-Ox), 4.41 (d, *J* = 7.8 Hz, 0.55 H, major diastereoisomer OCH*H*-Ox), 3.79–3.69 (m, 0.45H, minor diastereoisomer CHα-Ala), 3.68–3.57 (m, 0.55H, major diastereoisomer CHα-Ala), 2.36 (br. s, 0.45H, minor diastereoisomer NH), 2.19 (br. s, 0.55H, major diastereoisomer NH), 1.81–1.77 (m, 6H, 2 × CH_3, cumyl), 1.68 (d, *J* = 7.1 Hz, 1.35H, minor diastereoisomer $NO_2CHC\mathit{H}_3$), 1.67 (d, *J* = 7.0 Hz, 1.65H, major diastereoisomer $NO_2CHC\mathit{H}_3$), 1.34 (d, *J* = 7.1 Hz, 1.35H, minor diastereoisomer CH_3β-Ala), 1.32 (d, *J* = 7.1 Hz, 1.65H, major diastereoisomer CH_3β-Ala); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 174.9 (C=O), 174.7 (C=O), 145.1 (C), 128.52 (2 × CH), 128.5 (2 × CH), 127.53 (CH), 127.48 (CH), 124.39 (2 × CH), 124.37 (2 × CH), 86.9 (NO_2CH), 85.3 (NO_2CH), 82.83 (C, cumyl), 82.78 (C, cumyl), 78.2 (OCH_2), 77.9 (OCH_2), 77.8 (OCH_2), 76.9 (OCH_2), 62.6 (C, Ox), 62.2 (C, Ox), 51.9 (CH, α-Ala), 51.7 (CH, α-Ala), 28.52 (CH_3, cumyl), 28.47 (CH_3, cumyl), 28.45 (CH_3, cumyl), 28.3 (CH_3, cumyl), 21.3 (CH_3, $NO_2CHC\mathit{H}_3$), 21.0 (CH_3, $NO_2CHC\mathit{H}_3$), 14.0 (CH_3, β-Ala), 13.5 (CH_3, β-Ala); **ν_{max}** (neat) = 3342 (NH), 2981, 2937, 2883, 1730 (C=O), 1549, 1448, 1366, 1271, 1132, 1101 cm^{-1}; **MS** (ESI$^+$) *m/z* 359 [M+Na]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{17}H_{24}N_2O_5Na$ [M+Na]$^+$ 359.1577, found 359.1571.

Fmoc-L-AOx-Ala-OCumyl and Fmoc-D-AOx-Ala-OCumyl ((±,*S*)-14)

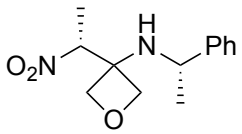


(±,*S*)-**14**

Following general procedure 4, **7** (377 mg, 1.12 mmol, 1.0 equiv, dr 55:45) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 19:1→4:1), (±,*S*)-**14** (467 mg, 0.88 mmol, 79%) as a white solid. HPLC analysis showed a 58:42 mixture of diastereoisomers. **R_f** (CH_2Cl_2/EtOAc 85:5) 0.47; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.6 Hz, 2H, ArH), 7.63–7.54 (m, 2H, ArH), 7.45–7.20 (m, 9H, ArH), 5.69–4.91 (m, 1H, NH), 4.78–4.02 (m, 8H, $CHCH_2$-Fmoc, CHα-AOx, 2 × OCH_2-Ox), 4.01–3.63 (m, 1H, CHα-Ala), 1.82 (s, 3H, CH_3, cumyl), 1.79 (s, 1.8H, major diastereoisomer CH_3, cumyl), 1.78 (s, 1.2H, minor diastereoisomer CH_3, cumyl), 1.38

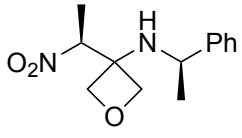
(d, J = 6.9 Hz, 3H, CH_3β-Ala), 1.23–0.90 (m, 3H, CH_3β-AOx); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 175.6 (2 × C=O), 156.5 (C=O, Fmoc), 156.3 (C=O, Fmoc), 145.17 (C), 145.15 (C), 144.12 (C), 144.09 (C), 144.06 (C), 141.5 (C), 128.53 (CH), 128.48 (CH), 127.82 (CH), 127.79 (CH), 127.49 (CH), 127.47 (CH), 127.2 (CH), 125.36 (CH), 125.27 (CH), 125.19 (CH), 124.4 (CH), 124.3 (CH), 120.12 (CH), 120.08 (CH), 82.80 (C, cumyl), 82.78 (C, cumyl), 79.1 (OCH_2), 79.0 (OCH_2), 78.9 (OCH_2), 77.9 (OCH_2), 66.8 (CH_2, Fmoc), 66.7 (CH_2, Fmoc), 62.5 (C, Ox), 62.4 (C, Ox), 52.1 (CH, α-Ala), 52.0 (CH, Fmoc or α-AOx), 51.2 (CH, α-Ala), 47.5 (CH, Fmoc or α-AOx), 47.4 (CH, Fmoc), 28.8 (CH_3, cumyl), 28.2 (CH_3, cumyl), 28.1 (CH_3, cumyl), 21.7 (CH_3, β-Ala), 20.8 (CH_3, β-Ala), 14.9 (CH_3, β-AOx), 14.6 (CH_3, β-AOx); **ν_{max}** (neat) = 3330 (NH), 2976, 2940, 2875, 1718 (C=O), 1496, 1448, 1241, 1132, 1101 cm^{-1}; **MS** (ESI$^+$) *m/z* 529 $[M+H]^+$, 551 $[M+Na]^+$; **HRMS** (ESI$^+$) calcd. for $C_{32}H_{36}N_2O_5Na$ $[M+Na]^+$ 551.2516, found 551.2515; **HPLC** (Chiralcel OD-H (0.46 cm × 25 cm, 5 μm), 20% isopropanol in hexane, 25 °C, 1.0 mL/min, λ = 254 nm) t_R = 54 min (major), 93 min (minor).

3-((*R*)-1-Nitroethyl)-*N*-((*S*)-1-phenylethyl)oxetan-3-amine ((*R*,*S*)-9)


(*R*,*S*)-**9**

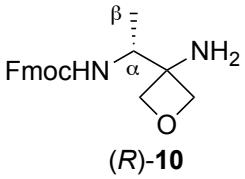
To 3-(1-nitroethylidene)oxetane (**5**)[2] (100 mg, 0.77 mmol, 1.0 equiv) in CH_2Cl_2 (8.0 mL) was added (*S*)-(−)-α-methylbenzylamine ((*S*)-**8**) (120 μL, 0.93 mmol, 1.2 equiv) and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a crude product which ^{1}H NMR showed to consist of a 60:40 mixture of diastereoisomers. Purification by column chromatography (SiO_2, PE/EtOAc 7:1) gave the major diastereoisomer (*R*,*S*)-**9** (108 mg, 0.43 mmol, 56%, dr >95:5) as a white solid. **R_f** (PE/EtOAc 4:1) 0.29; **mp** 76–79 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.39–7.29 (m, 4H, ArH), 7.28–7.21 (m, 1H, ArH), 5.05 (q, J = 6.9 Hz, 1H, NO_2CH), 4.53 (d, J = 7.2 Hz, 1H, OC*H*H-Ox), 4.43 (d, J = 7.6 Hz, 1H, OC*H*H-Ox), 4.41 (d, J = 7.6 Hz, 1H, OCH*H*-Ox), 4.30–4.22 (m, 2H, OCH*H*-Ox, NHC*H*), 1.84 (br. s, 1H, NH), 1.73 (d, J = 6.9 Hz, 3H, NO_2CHCH_3), 1.37 (d, J = 6.7 Hz, 3H, $NHCHCH_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 146.7 (C), 128.9 (CH), 127.4 (CH), 126.3 (CH), 86.2 (NO_2CH), 77.8 (OCH_2), 76.9 (OCH_2), 62.4 (C, Ox), 52.7 (NHCH), 26.3 (NO_2CHCH_3), 14.4 ($NHCHCH_3$); **ν_{max}** (neat) = 3330 (NH), 2977, 2968, 2925, 2881, 1546, 1389, 1289, 974 cm^{-1}; **MS** (ESI$^+$) *m/z* 251 [M+H], 273 $[M+Na]^+$, 289 $[M+K]^+$; **HRMS** (ESI$^+$) calcd. for $C_{13}H_{19}N_2O_3$ $[M+H]^+$ 251.1390, found 251.1397; $[\alpha]_D^{18}$ −10.3 (*c* 0.60, $CHCl_3$).

3-((*S*)-1-Nitroethyl)-*N*-((*R*)-1-phenylethyl)oxetan-3-amine ((*S*,*R*)-9)


(*S*,*R*)-**9**

To 3-(1-nitroethylidene)oxetane (**5**)[2] (1.06 g, 8.17 mmol, 1.0 equiv) in CH_2Cl_2 (82 mL) was added (*R*)-(+)-α-methylbenzylamine ((*R*)-**8**) (1.25 mL, 9.81 mmol, 1.2 equiv) and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to give a crude product which ^{1}H NMR showed to consist of 60:40 mixture of diastereoisomers. Purification by column chromatography (SiO_2, PE/EtOAc 9:1→85:15) gave the major diastereoisomer (*S*,*R*)-**9** (1.03 g, 4.12 mmol, 50%, dr >95:5) as a white solid. **mp** 71–74 °C; $[\alpha]_D^{19}$ +7.9 (*c* 0.19, $CHCl_3$). Other analytical data as described for (*R*,*S*)-**9**.

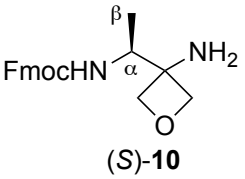
Fmoc-D-AOx-NH$_2$ ((*R*)-10)



(*R*)-**10**

To (*R,S*)-**9** (531 mg, 2.12 mmol, 1.0 equiv) in MeOH (21 mL) was added 20% $Pd(OH)_2/C$ (212 mg). The reaction mixture was stirred under an atmosphere of H_2 (balloon) at room temperature for 4 d. The mixture was filtered through a plug of Celite eluting with MeOH and the filtrate was concentrated under reduced pressure to give the crude diamine. To the crude diamine were added CH_2Cl_2 (10 mL), $NaHCO_3$ (356 mg, 4.24 mmol, 2.0 equiv) and Fmoc *N*-hydroxysuccinimide ester (715 mg, 2.12 mmol, 1.0 equiv) and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc and washed with saturated Na_2CO_3 (2 × 20 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, EtOAc → CH_2Cl_2 → CH_2Cl_2/MeOH 19:1) gave (*R*)-**10** (415 mg, 1.23 mmol, 58%) as a sticky white gum. **R_f** (CH_2Cl_2/MeOH 9:1) 0.44; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.77 (d, *J* = 7.4 Hz, 2H, ArH), 7.59 (d, *J* = 7.4 Hz, 2H, ArH), 7.40 (t, *J* = 7.4 Hz, 2H, ArH), 7.32 (t, *J* = 7.4 Hz, 2H, ArH), 5.13 (d, *J* = 8.2 Hz, 1H, NH), 4.57 (d, *J* = 6.6 Hz, 1H, OC*H*H-Ox), 4.54 (d, *J* = 6.3 Hz, 1H, OC*H*H-Ox), 4.49–4.37 (m, 2H, CH_2-Fmoc), 4.36–4.26 (m, 2H, 2 × OCH*H*-Ox), 4.26–4.15 (m, 2H, CH-Fmoc, CHα-AOx), 1.57 (s, 2H, NH_2), 1.11 (d, *J* = 6.5 Hz, 3H, CH_3β-AOx). *N.B.* NH_2 overlapping with H_2O signal; **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 156.3 (C=O, Fmoc), 144.1 (C), 144.0 (C), 141.5 (C), 127.8 (CH), 127.2 (CH), 125.2 (CH), 125.1 (CH), 120.14 (CH), 120.10 (CH), 83.0 (OCH_2), 82.4 (OCH_2), 66.7 (CH_2, Fmoc), 58.9 (C, Ox), 51.5 (CH, α-AOx), 47.5 (CH, Fmoc), 14.8 (CH_3, β-AOx). *N.B.* One aromatic C signal not observed; **ν_{max}** (neat) = 3298 (NH), 2946, 2868, 1700 (C=O), 1529, 1448, 1242, 1050, 970 cm^{-1}; **MS** (ESI^+) *m/z* 339 $[M+H]^+$, 361 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{18}H_{20}N_5NaO_2$ $[M+Na]^+$ 361.1509, found 361.1511; $[\alpha]_D^{18}$ –9.6 (*c* 0.45, $CHCl_3$).

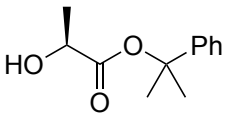
Fmoc-L-AOx-NH$_2$ ((*S*)-10)



(*S*)-**10**

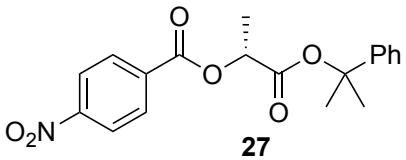
To (*S,R*)-**9** (605 mg, 2.42 mmol, 1.0 equiv) in MeOH (24 mL) was added 20% $Pd(OH)_2/C$ (242 mg). The reaction mixture was stirred under an atmosphere of H_2 (balloon) at room temperature for 4 d. The mixture was filtered through a plug of Celite eluting with MeOH and the filtrate was concentrated under reduced pressure to give the crude diamine. To the crude diamine were added CH_2Cl_2 (10 mL), $NaHCO_3$ (407 mg, 4.84 mmol, 2.0 equiv) and Fmoc *N*-hydroxysuccinimide (816 mg, 2.42 mmol, 1.0 equiv) and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc and washed with saturated Na_2CO_3 (2 × 20 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, EtOAc → CH_2Cl_2 → CH_2Cl_2/MeOH 19:1) gave (*S*)-**10** (407 mg, 1.20 mmol, 50%) as a sticky white gum. $[\alpha]_D^{22}$ +7.1 (*c* 0.47, $CHCl_3$). Other analytical data as described for (*R*)-**10**.

2-Phenylpropan-2-yl (*S*)-2-hydroxypropanoate ((*S*)-12)


(*S*)-**12**

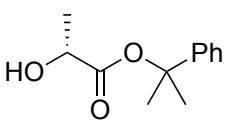
Following general procedure 1, L-lactic acid (1.05 g, 11.6 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 9:1→85:15), (*S*)-**12** (1.47 g, 7.07 mmol, 61%) contaminated with traces of 2-phenyl-2-propanol (~19:1 by ^{1}H NMR) as a colourless oil. $\mathbf{R_f}$ (PE/EtOAc 85:15) 0.30; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.39–7.32 (m, 4H, ArH), 7.31–7.24 (m, 1H, ArH), 4.24 (q, *J* = 6.9 Hz, 1H, CH), 2.76 (br. s, 1H, OH), 1.82 (s, 3H, CH_3, cumyl), 1.80 (s, 3H, CH_3, cumyl), 1.45 (d, *J* = 6.9 Hz, 3H, CH*C*H_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 174.6 (C=O), 145.1 (C), 128.5 (CH), 127.5 (CH), 124.3 (CH), 83.5 (C, cumyl), 67.0 (CH), 28.8 (CH_3, cumyl), 28.4 (CH_3, cumyl), 20.6 (CH*C*H_3); $\boldsymbol{\nu_{max}}$ (neat) = 3358 (OH), 3058, 2977, 2937, 1721 (C=O), 1446, 1368, 1219, 1123, 1042 cm^{-1}; **MS** (ESI$^+$) *m/z* 231 [M+Na]$^+$, 439 [2M+Na]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{12}H_{16}NaO_3$ [M+Na]$^+$ 231.0992, found 231.0994; $[\boldsymbol{\alpha}]_D^{22}$ −3.5 (*c* 0.50, $CHCl_3$).

(*R*)-1-Oxo-1-[(2-phenylpropan-2-yl)oxy]propan-2-yl 4-nitrobenzoate (27)


27

To (*S*)-**12** (1.27 g, 6.08 mmol, 1.0 equiv) in CH_2Cl_2 (61 mL) was added triphenylphosphine (2.39 g, 9.12 mmol, 1.5 equiv) and 4-nitrobenzoic acid (1.52 g, 9.12 mmol, 1.5 equiv) and the reaction mixture cooled to 0 °C. Diethyl azodicarboxylate (1.44 mL, 9.12 mmol, 1.5 equiv) was added dropwise and the mixture stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the crude mixture dissolved in EtOAc (150 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ (3 × 50 mL) and brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, PE/EtOAc 9:1) gave **27** (2.06 g, 5.77 mmol, 95%) as a yellow oil. $\mathbf{R_f}$ (PE/EtOAc 9:1) 0.30; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.30–8.26 (m, 2H, ArH), 8.26–8.21 (m, 2H, ArH), 7.39–7.29 (m, 4H, ArH), 7.28–7.22 (m, 1H, ArH), 5.35 (q, *J* = 7.1 Hz, 1H, CH), 1.82 (s, 3H, CH_3, cumyl), 1.80 (s, 3H, CH_3, cumyl), 1.67 (d, *J* = 7.1 Hz, 3H, CH*C*H_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 168.7 (C=O), 164.2 (C=O), 150.8 (C), 145.0 (C), 135.2 (C), 131.1 (CH), 128.5 (CH), 127.5 (CH), 124.3 (CH), 123.6 (CH), 83.7 (C, cumyl), 70.3 (CH), 28.8 (CH_3, cumyl), 28.3 (CH_3, cumyl), 17.0 (CH*C*H_3); $\boldsymbol{\nu_{max}}$ (neat) = 3112, 3058, 2985, 2940, 2877, 1753 (C=O), 1726 (C=O), 1525, 1346, 1268, 1213, 1098 cm^{-1}; **MS** (ESI$^+$) *m/z* 380 [M+Na]$^+$, 396 [M+K]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{19}H_{19}NNaO_6$ [M+Na]$^+$ 380.1105, found 380.1110; $[\boldsymbol{\alpha}]_D^{22}$ +6.9 (*c* 1.60, $CHCl_3$).

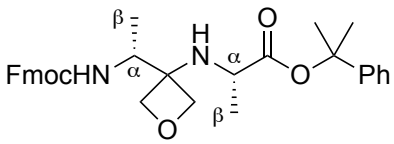
2-Phenylpropan-2-yl (*R*)-2-hydroxypropanoate ((*R*)-12)


(*R*)-**12**

To **27** (3.12 g, 8.74 mmol, 1.0 equiv) in MeOH (87 mL) was added K_2CO_3 (1.27 g, 9.17 mmol, 1.05 equiv) and the reaction mixture stirred at room temperature for 5 min before it was quenched with a saturated solution of aqueous NH_4Cl (150 mL). The resulting mixture was extracted with EtOAc (3 × 150 mL) and the combined organic layers were washed with brine (50 mL), dried

over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, PE/EtOAc 9:1→85:15) to give (*R*)-**12** (1.52 g, 7.32 mmol, 84%) as a colourless oil that crystallised to a white solid upon storage at −20 °C. **mp** 58–60 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.38–7.32 (m, 4H, ArH), 7.31–7.24 (m, 1H ArH), 4.23 (qd, *J* = 6.8, 5.4 Hz, 1H, CH), 2.76 (d, *J* = 5.4 Hz, 1H, OH), 1.82 (s, 3H, CH_3, cumyl), 1.80 (s, 3H, CH_3, cumyl), 1.45 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$); $[\alpha]_D^{21}$ +1.0 (*c* 0.70, $CHCl_3$). Other analytical data as described for (*S*)-**12**.

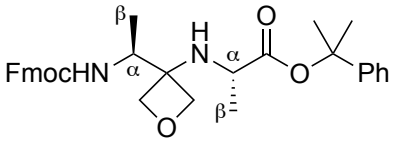
Fmoc-D-AOx-Ala-OCumyl ((*R*,*S*)-14)



(*R*,*S*)-**14**

The synthesis of (*R*,*S*)-**14** was adapted from a procedure originally published by Carreira and co-workers.[3] To (*R*)-**12** (459 mg, 2.20 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) at −20 °C was added DIPEA (825 µL, 4.74 mmol, 2.15 equiv) followed by the dropwise addition of a solution of triflic anhydride (408 µL, 2.42 mmol, 1.1 equiv) in CH_2Cl_2 (2.5 mL). The reaction mixture was allowed to reach room temperature and stirred for 1 h. Petroleum ether (12.5 mL) was added and the reaction mixture was filtered through a plug of silica gel eluting with 50% CH_2Cl_2 in petroleum ether. The eluent was concentrated under reduced pressure to give triflate **13** (629 mg, 1.85 mmol, 84%), which was used immediately without further purification. To triflate **13** in MeCN (4.0 mL) was added a solution of (*R*)-**10** (377 mg, 1.12 mmol, 1.0 equiv) and DIPEA (322 µL, 1.85 mmol, 1.65 equiv) in MeCN (4.0 mL). The reaction mixture was stirred at 30 °C for 20 h and then concentrated under reduced pressure. The crude material was purified by repeat column chromatography (SiO_2, PE/EtOAc 7:3→3:2; CH_2Cl_2/EtOAc 9:1→4:1) to give (*R*,*S*)-**14** (340 mg, 0.64 mmol, 57%, dr 98:2) as a white solid. **R_f** (PE/EtOAc 7:3) 0.21; **mp** 63–65 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.4 Hz, 2H, ArH), 7.58 (d, *J* = 7.4 Hz, 2H, ArH), 7.43–7.23 (m, 9H, ArH), 5.20 (d, *J* = 8.3 Hz, 1H, NH), 4.61–4.40 (m, 8H, $CHCH_2$-Fmoc, CHα-AOx, 2 × OCH_2-Ox), 3.88–3.76 (m, 1H, CHα-Ala), 2.07 (br. s, 1H, NH), 1.82 (s, 3H, CH_3, cumyl), 1.79 (s, 3H, CH_3, cumyl), 1.38 (d, *J* = 6.9 Hz, 3H, CH_3β-Ala), 1.12 (d, *J* = 6.4 Hz, 3H, CH_3β-AOx); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 175.6 (C=O), 156.3 (C=O, Fmoc), 145.2 (C), 144.1 (C), 141.5 (C), 128.5 (CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 125.20 (CH),125.18 (CH), 124.3 (CH), 120.1 (CH), 82.8 (C, Ox), 79.0 (OCH_2), 77.9 (OCH_2), 66.7 (CH_2, Fmoc), 62.5 (C, Ox), 52.0 (CH, α-AOx), 51.2 (CH, α-Ala), 47.5 (CH, Fmoc), 28.8 (CH_3, cumyl), 28.2 (CH_3, cumyl), 21.7 (CH_3, β-Ala), 14.6 (CH_3, β-AOx). *N.B.* Two aromatic C signals not observed; **ν_{max}** (neat) = 3332 (NH), 2977, 2942, 2875, 1719 (C=O), 1496, 1447, 1270, 1131 cm^{-1}; **MS** (ESI^+) *m/z* 529 $[M+H]^+$, 551 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{32}H_{36}N_2NaO_5$ $[M+Na]^+$ 551.2516, found 551.2520; $[\alpha]_D^{22}$ −18.4 (*c* 0.38, $CHCl_3$); **HPLC** (Chiralcel OD-H (0.46 cm × 25 cm, 5 µm), 20% isopropanol in hexane, 25 °C, 1.0 mL/min, λ = 254 nm) t_R = 55 min (major), 97 min (minor).

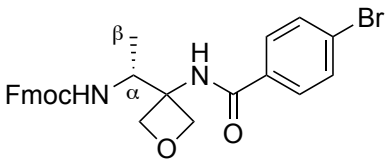
Fmoc-L-AOx-Ala-OCumyl ((*S*,*S*)-14)



(*S*,*S*)-**14**

The synthesis of (*S*,*S*)-**14** was adapted from a procedure originally published by Carreira and co-workers.[3] To (*R*)-**12** (458 mg, 2.20 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) at −20 °C was added DIPEA (824 µL, 4.73 mmol, 2.15 equiv) followed by the dropwise addition of a solution of triflic anhydride (407 µL, 2.42 mmol, 1.1 equiv) in CH_2Cl_2 (2.5 mL). The reaction mixture was allowed to reach room temperature and stirred for 1 h. Petroleum ether (12.5 mL) was added and the reaction mixture filtered through a plug of silica gel eluting with 50% CH_2Cl_2 in petroleum ether. The eluent was concentrated under reduced pressure to give triflate **13** (734 mg, 2.16 mmol, 98%) which was used immediately without further purification. To triflate **13** in MeCN (4.0 mL) was added a solution of (*S*)-**10** (338 mg, 1.00 mmol, 1.0 equiv) and DIPEA (376 µL, 2.16 mmol, 2.16 equiv) in MeCN (4.0 mL). The reaction mixture was stirred at 30 °C for 20 h and then concentrated under reduced pressure. The crude material was purified by repeat column chromatography (SiO_2, PE/EtOAc 7:3→3:2; CH_2Cl_2/EtOAc 9:1→4:1) to give (*S*,*S*)-**14** (264 mg, 0.50 mmol, 50%, dr >98:2) as a white solid. $\mathbf{R_f}$ (PE/EtOAc 7:3) 0.20; **mp** 73–76 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.5 Hz, 2H, ArH), 7.59 (d, *J* = 7.5 Hz, 2H, ArH), 7.44–7.21 (m, 9H, ArH), 5.57 (d, *J* = 7.1 Hz, 1H, NH), 4.67–3.81 (m, 8H, 2 × OCH_2-Ox, CH_2CH-Fmoc, CHα-AOx), 3.79–3.64 (m, 1H, CHα-Ala), 1.82 (s, 3H, CH_3, cumyl), 1.80–1.70 (m, 4H, CH_3, cumyl, NH), 1.38 (d, *J* = 6.8 Hz, 3H, CH_3β-Ala), 1.16 (d, *J* = 5.9 Hz, 3H, CH_3β-AOx); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 175.5 (C=O), 156.5 (C=O, Fmoc), 145.2 (C), 144.12 (C), 144.10 (C), 141.5 (C), 128.5 (CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 125.4 (CH), 125.3 (CH), 124.4 (CH), 120.1 (CH), 82.8 (C, cumyl), 79.1 (OCH_2), 78.9 (OCH_2), 66.8 (CH_2, Fmoc), 62.4 (C, Ox), 52.1 (CH, α-Ala), 52.0 (CH, α-AOx), 47.4 (CH, Fmoc), 28.9 (CH_3, cumyl), 28.1 (CH_3, cumyl), 20.8 (CH_3, β-Ala), 14.9 (CH_3, β-AOx). *N.B.* One aromatic C signal not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3329 (NH), 2976, 2937, 1718 (C=O), 1497, 1448, 1242, 1132, 1077 cm^{-1}; **MS** (ESI$^+$) *m/z* 529 $[M+H]^+$, 551 $[M+Na]^+$, 567 $[M+K]^+$; **HRMS** (ESI$^+$) calcd. for $C_{32}H_{36}N_2NaO_5$ $[M+Na]^+$ 551.2516, found 551.2512; $[\boldsymbol{\alpha}]_D^{18}$ −13.4 (*c* 0.25, $CHCl_3$); **HPLC** (Chiralcel OD-H (0.46 cm × 25 cm, 5 µm), 20% isopropanol in hexane, 25 °C, 1.0 mL/min, λ = 254 nm) t_R = 56 min (minor), 87 min (major).

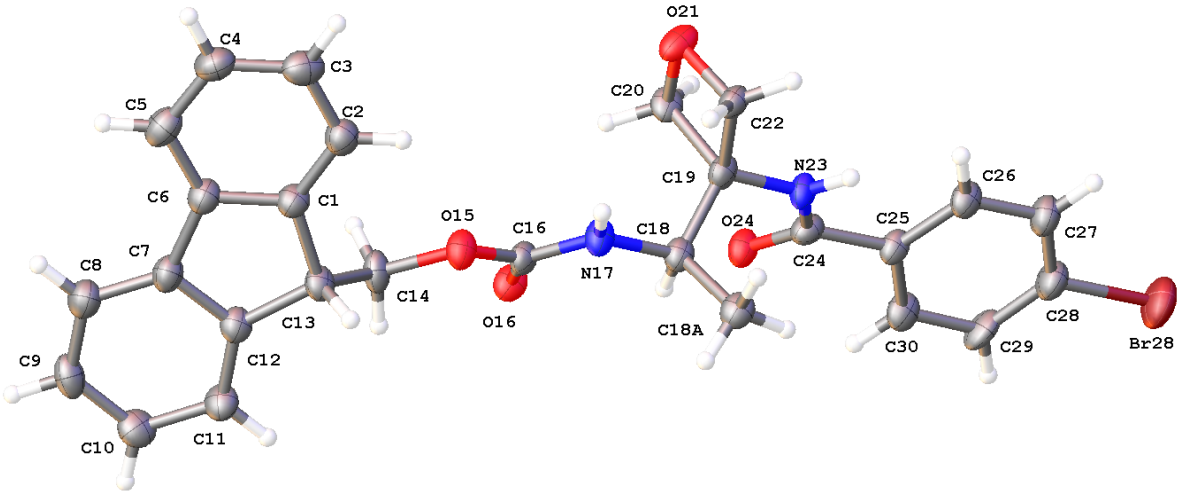
(9*H*-Fluoren-9-yl)methyl (*R*)-(1-(3-(4-bromobenzamido)oxetan-3-yl)ethyl)carbamate (11)



11

To (*R*)-**10** (289 mg, 0.85 mmol, 1.0 equiv) in CH_2Cl_2 (9.0 mL) was added DIPEA (297 µL, 1.71 mmol, 2.0 equiv) and 4-bromobenzoyl chloride (375 mg, 1.71 mmol, 2.0 equiv). The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with a saturated solution of $NaHCO_3$ (20 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (SiO_2, PE/EtOAc 2:3) gave **11** (384 mg, 0.74 mmol, 87%) as a white solid. A crystal suitable for X-ray analysis was grown from toluene. $\mathbf{R_f}$ (PE/EtOAc 2:3) 0.43; **mp** 164–167 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.6 Hz, 2H, ArH), 7.67 (d, *J* = 8.0 Hz, 2H, ArH),

7.61–7.53 (m, 4H, ArH), 7.44–7.36 (m, 2H, ArH), 7.33–7.25 (m, 2H, ArH), 6.91 (s, 1H, NH), 5.93 (d, J = 7.1 Hz, 1H, NH), 4.98 (d, J = 6.6 Hz, 1H, OC*H*H-Ox), 4.92 (d, J = 6.6 Hz, 1H, OC*H*H-Ox), 4.67 (d, J = 6.6 Hz, 1H, OCH*H*-Ox), 4.57 (d, J = 6.6 Hz, 1H, OCH*H*-Ox), 4.46–4.27 (m, 3H, CH_2-Fmoc, CHα-AOx), 4.23–4.16 (m, 1H, CH-Fmoc), 1.36 (d, J = 6.4 Hz, 3H, CH_3β-AOx); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 167.1 (C=O), 157.0 (C=O, Fmoc), 143.94 (C), 143.89 (C), 141.5 (C), 141.4 (C), 132.6 (C), 132.1 (CH), 128.8 (CH), 127.9 (CH), 127.2 (CH), 127.1 (C), 125.2 (CH), 120.1 (CH), 78.0 (OCH_2), 77.5 (OCH_2), 67.1 (CH_2, Fmoc), 60.7 (C, Ox), 52.6 (CH, α-AOx), 47.3 (CH, Fmoc), 15.5 (CH_3, β-AOx); **ν_{max}** (neat) = 3371 (NH), 3352 (NH), 2967, 2893, 1693 (C=O), 1643 (C=O), 1516, 1248, 989 cm^{-1}; **MS** (ESI$^+$) *m/z* 543 [M(^{79}Br)+Na]$^+$, 545 [M(^{81}Br)+Na]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{27}H_{25}BrN_2NaO_4$ [M+Na]$^+$ 543.0890 (^{79}Br) and 545.0874 (^{81}Br), found 543.0890 (^{79}Br) and 545.0873 (^{81}Br); $[\alpha]_D^{22}$ +23.5 (*c* 0.50, $CHCl_3$).

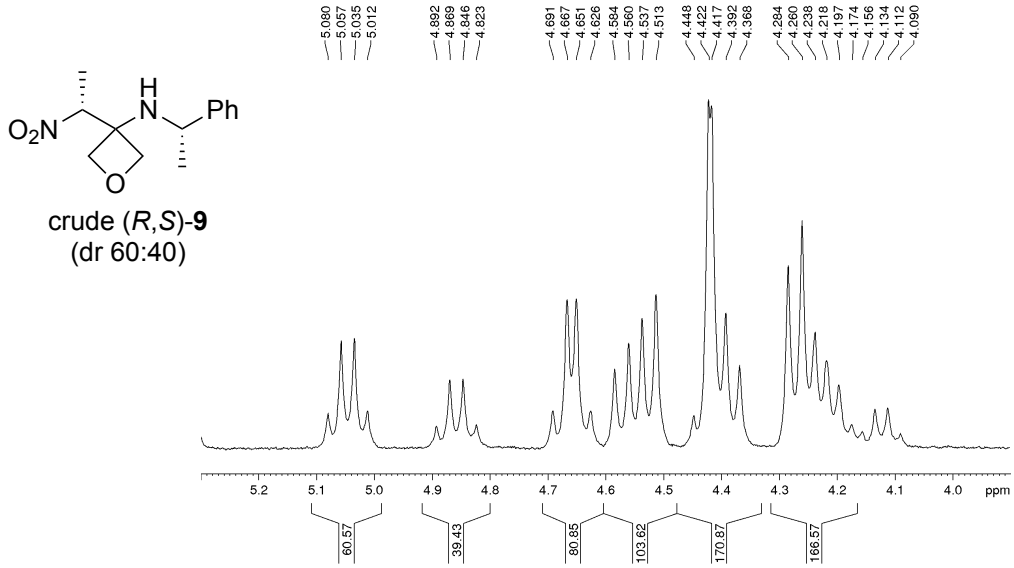


Crystal Data for $C_{28.75}H_{27}BrN_2O_4$ (CCDC 2005273, M = 544.43 g/mol): monoclinic, space group C2 (no. 5), a = 25.7843(4) Å, b = 5.30700(10) Å, c = 18.5521(3) Å, β = 95.2280(10)°, V = 2528.06(7) Å^3, Z = 4, T = 100(2) K, μ(CuKα) = 2.526 mm^{-1}, *Dcalc* = 1.430 g/cm^3, 22540 reflections measured (6.884° ≤ 2Θ ≤ 136.32°), 4460 unique (R_{int} = 0.0686, R_{sigma} = 0.0454) which were used in all calculations. The final R_1 was 0.0722 (I > 2σ(I)) and wR_2 was 0.2005 (all data).

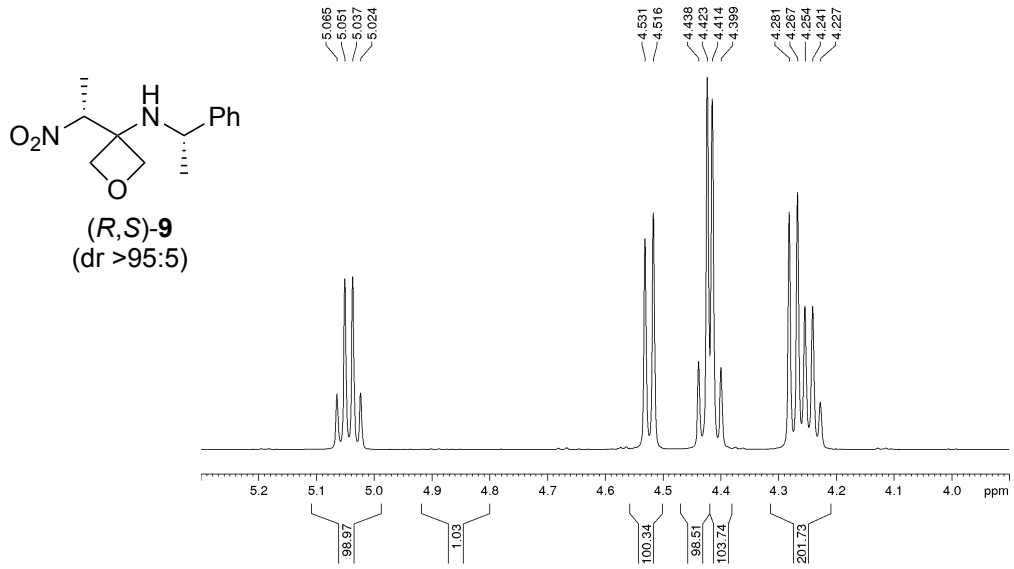
Diastereomeric purity of (*R,S*)-9 and (*S,R*)-9

Confirmation that (*R,S*)-**9** was isolated as a single diastereoisomer (dr >95:5) was demonstrated by comparison of the NO_2CH signals in the crude ^1H NMR (300 MHz, $CDCl_3$) spectrum with the same region in the ^1H NMR (500 MHz, $CDCl_3$) spectrum after column chromatography. Integration of the signals at 5.05 (q, J = 6.8 Hz) ppm and 4.86 (q, J = 6.9 Hz) ppm in spectrum **A** relative to that in spectrum **B** confirmed dr >95:5. ^1H NMR analysis of (*S,R*)-**4** was conducted in the same fashion and also confirmed dr >95:5.

A. Expanded ^1H NMR (300 MHz, $CDCl_3$) of crude (*R,S*)-**9**



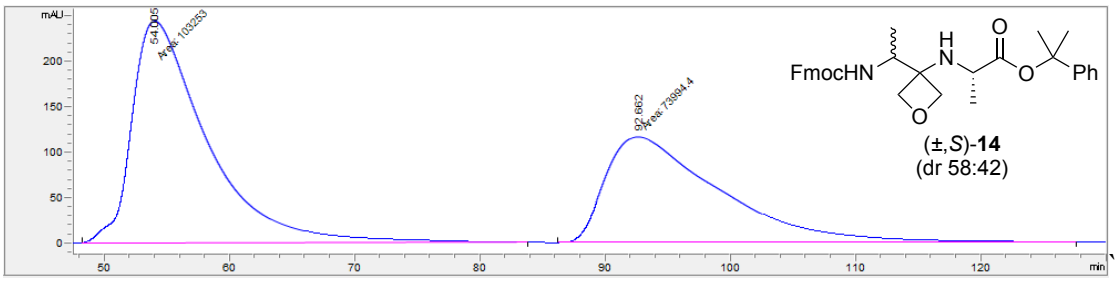
B. Expanded ^1H NMR (500 MHz, $CDCl_3$) of (*R,S*)-**9** after column chromatography



HPLC Analysis of (±,*S*)-14, (*R*,*S*)-14 and (*S*,*S*)-14

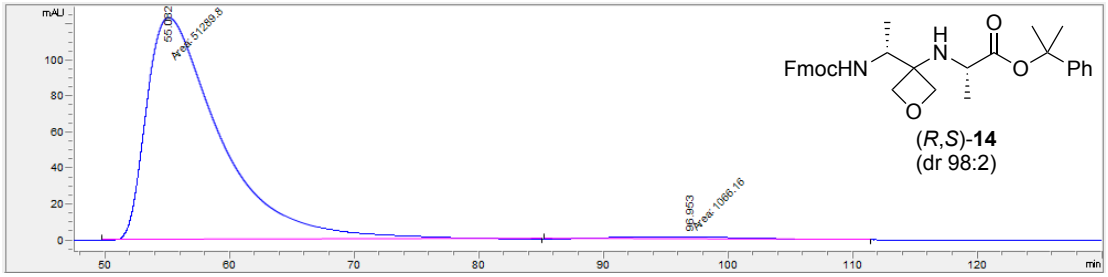
HPLC analysis was conducted on an Agilent Technologies 1200 Series HPLC, using Chiralcel OD-H column (0.46 cm × 25 cm, 5 µm) at 25 °C, with detection by UV at 254 nm. Samples were eluted at a flow rate of 1.0 mL/min with a mobile phase system composed of 20% isopropanol in hexane. Retention times (t_R) are reported in minutes.

(±,*S*)-**14**



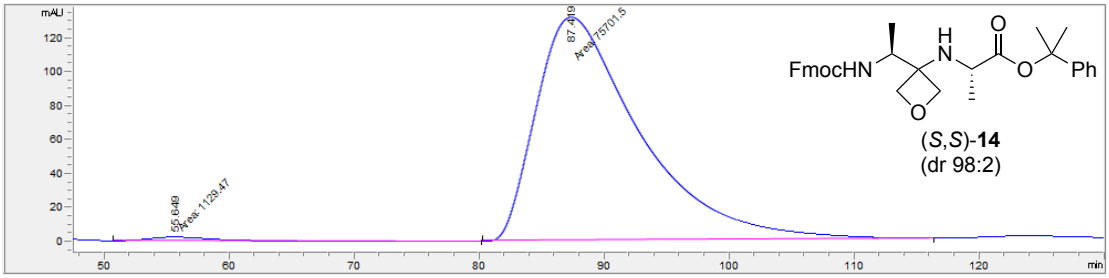
#	Time	Area	Height	Width	Area%	Symmetry
1	54.005	103252.9	245.4	7.0114	58.254	0.423
2	92.662	73994.4	116.8	10.5588	41.746	0.391

(*R*,*S*)-**14**



#	Time	Area	Height	Width	Area%	Symmetry
1	55.082	51289.8	123.4	6.929	97.964	0.41
2	96.953	1066.2	1.3	13.2996	2.036	1.216

(*S*,*S*)-**14**



#	Time	Area	Height	Width	Area%	Symmetry
1	55.649	1129.5	2.3	8.1607	1.470	0.399
2	87.419	75701.5	131.1	9.6274	98.530	0.498

3. Solid phase peptide synthesis

General information

All peptides were synthesised on a NovaSyn® TGR R (0.18 mmol/g, 0.025 mmol) resin (Novabiochem) on a Prelude peptide synthesizer (ProteinTechnologies Inc.) using an Fmoc/*t*Bu protecting group strategy. Couplings were performed under standard coupling conditions using HATU/DIPEA or HCTU/NMM. Oxetane building blocks were manually incorporated by double coupling using 2 equiv of building block, 1.9 equiv HATU and 6 equiv DIPEA. Fmoc-deprotection was achieved with 20% piperidine in DMF and capping after every coupling step with Ac_2O (5%) and NMM (5%) in DMF. Crude peptides were purified by preparative HPLC (Agilent Infinity 1260) on a Agilent PLRP-S column (100 Å, 8 µm, 150×25 mm).

Loading the resin with Fmoc-Tyr(tBu)-OH

NovaSyn® TGR R (0.18 mmol/g, 139 mg, 0.025 mmol, 1.0 equiv) resin was pre-swollen in DMF for 1 h and washed with DMF. Fmoc-Tyr(*t*Bu)-OH (0.25 mmol, 115 mg, 10 equiv), PyBOP (0.25 mmol, 130 mg, 10 equiv) and DIPEA (0.5 mmol, 87 µL, 20 equiv) in DMF (1.5 mL) were added to the resin and the mixture was agitated for 1 h at room temperature. The solvent was removed and the resin washed several times with DMF. Loading was repeated in case of a positive TNBS-test.

Peptide synthesis

Peptides were synthesized on the peptide synthesizer using N-α-Fmoc protected amino acids (10 equiv), HCTU (10 equiv) and NMM (20 equiv). The first twelve amino acids were attached by double coupling (45 min each), whereas for the last five amino acids triple couplings were performed. After each amino acid, the peptide was capped with Ac_2O:NMM:DMF (5:5:90; v/v/v) at room temperature for 2 × 15 min. After the last amino acid, the Fmoc group was cleaved manually with 20% piperidine in DMF at room temperature for 20 min and the N-terminus was capped with Ac_2O:NMM:DMF (5:5:90; v/v/v) at room temperature for 2 × 20 min.

In case of the oxetane-modified peptides, the oxetane-containing dipeptide building blocks were first deprotected as follows; (*S*,*S*)-**14** or **15** (2.0 equiv) in 2% TFA/CH_2Cl_2 (0.05 M) was stirred at room temperature until consumption of starting material. The reaction mixture was concentrated under reduced pressure and the resulting residue dissolved in CH_2Cl_2 and concentrated under reduced pressure (3×) to give the crude acid **16** or **17**. The crude acid was manually double coupled using HATU (1.9 equiv) and DIPEA (6.0 equiv) at room temperature for 2-12 h until a negative TNBS test was observed. In case of the N-terminal modified peptides the last two coupling steps with alanine and lysine were also done manually under standard conditions.

In all cases, cleavage from the resin was achieved with 95% TFA, 2.5% TIS, 2.5% water. The cleavage solution was concentrated under a stream of nitrogen and the peptide was precipitated in ice-cold ether. The crude peptide was re-dissolved in water/acetonitrile and purified by preparative HPLC (A: H_2O (0.1 % TFA), B: MeCN (0.1 % TFA)).

Parent peptide **18**
Gradient: 0–5 min, 5% B; 5–30 min, 5–50% B, 30–35 min, 50–100% B; t_R: 19.3 min.

Central AlaOX **19**
Gradient: 0–5 min, 5% B; 5–35 min, 5–35% B, 35–40 min, 50–100% B; t_R: 22.7 min.

Terminal AlaOX **20**
Gradient: 0–3 min, 5% B; 3–22 min, 5-30% B, 22–27 min, 30–100% B; t_R: 17.2 min.

Treminal GlyOX **21**
Gradient: 0–3 min, 5% B; 3–22 min, 5–30% B, 22–27 min, 30–100% B; t_R: 16.2 min.

Analytical data

Pure peptides were analysed on an Agilent Technologies 1200 Series RP-HPLC, using an Agilent Eclipse Plus C18 (5 µm, 4.6 × 150 mm) at 25 °C, with detection by UV at 214 and 280 nm. Gradients were run using a solvent system consisting of solution A (5% MeCN in H_2O + 0.1% TFA) and B (5% H_2O in MeCN + 0.1% TFA). Two gradients were used to characterise each peptide; a gradient from 5-95% solution B over 20 min and a gradient from 5-50% solution B over 20 min. Analytical RP-HPLC data is recorded as column retention time (t_R) in min. High-resolution mass spectra were recorded using a Bruker MaXis Impact.

Table S1. Peptide characterisation data

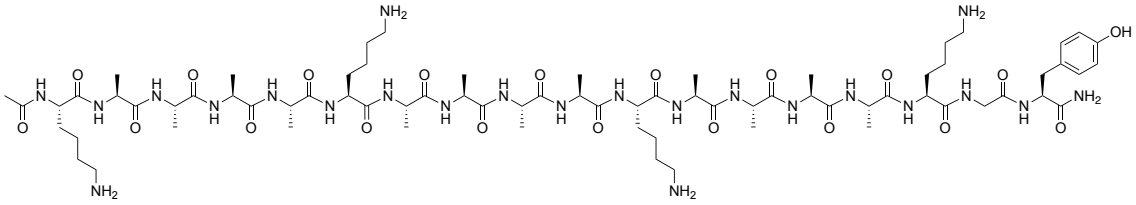
Entry	Peptide Sequence	t_R (min)[a]	Purity (%)[b,c]
1	Ac-KAAAA-KAAAA-KAAAA-KGY-NH_2, **18** parent	8.75 + 10.46	92
2	Ac-KAAAA-KA**AOx**AA-KAAAA-KGY-NH_2, **19** central AOx	7.83 + 8.61	90
3	Ac-KA**AOx**AA-KAAAA-KAAAA-KGY-NH_2, **20** *N*-terminal AOx	9.39 + 11.47	76
4	Ac-KA**GOx**AA-KAAAA-KAAAA-KGY-NH_2, **21** *N*-terminal GOx	9.35 + 11.38	90

[a] First t_R is the gradient from 5–95% solution B over 20 min; second number is the gradient from 5–50% over 20 min. [b] Measured at 214 nm. [c] Lowest purity of the two gradients runs.

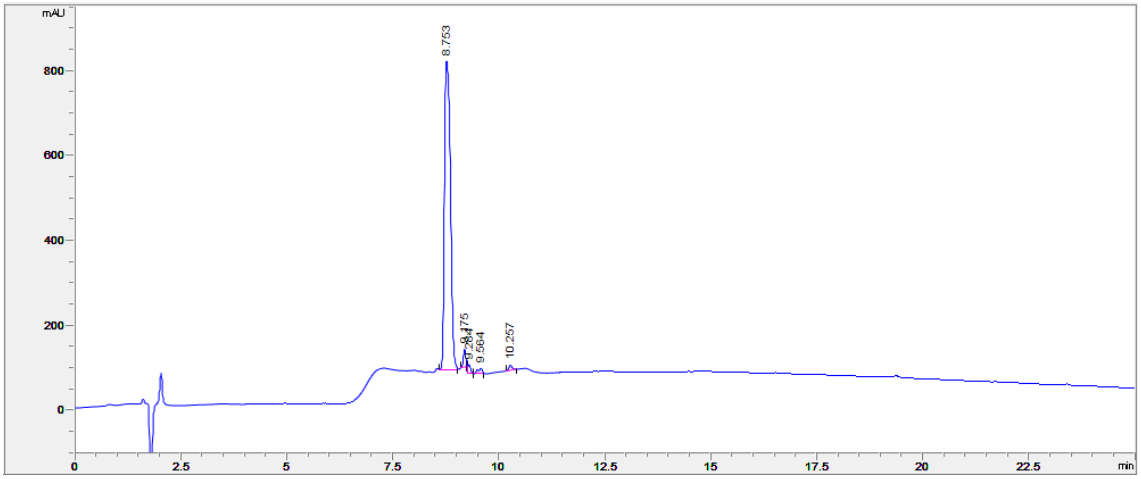
Table S2. Peptide characterisation data

Entry	Peptide Sequence	HRMS		ΔMW (ppm)
		Calculated	Observed	
1	Ac-KAAAA-KAAAA-KAAAA-KGY-NH_2, **18** parent	822.9808 $[M+2H]^{2+}$	822.9808 $[M+2H]^{2+}$	0.0
2	Ac-KAAAA-KA**AOx**AA-KAAAA-KGY-NH_2, **19** central AOx	858.9784 $[M+2Na]^{2+}$	858.9766 $[M+2Na]^{2+}$	2.1
3	Ac-KA**AOx**AA-KAAAA-KAAAA-KGY-NH_2, **20** *N*-terminal AOx	558.3334 $[M+3H]^{3+}$	558.3331 $[M+3H]^{3+}$	–0.5
4	Ac-KA**GOx**AA-KAAAA-KAAAA-KGY-NH_2, **21** *N*-terminal GOx	851.9706 $[M+2Na]^{2+}$	851.9701 $[M+2Na]^{2+}$	–0.6

Ac-KAAAA-KAAAA-KAAAA-KGY-NH_2 (18)

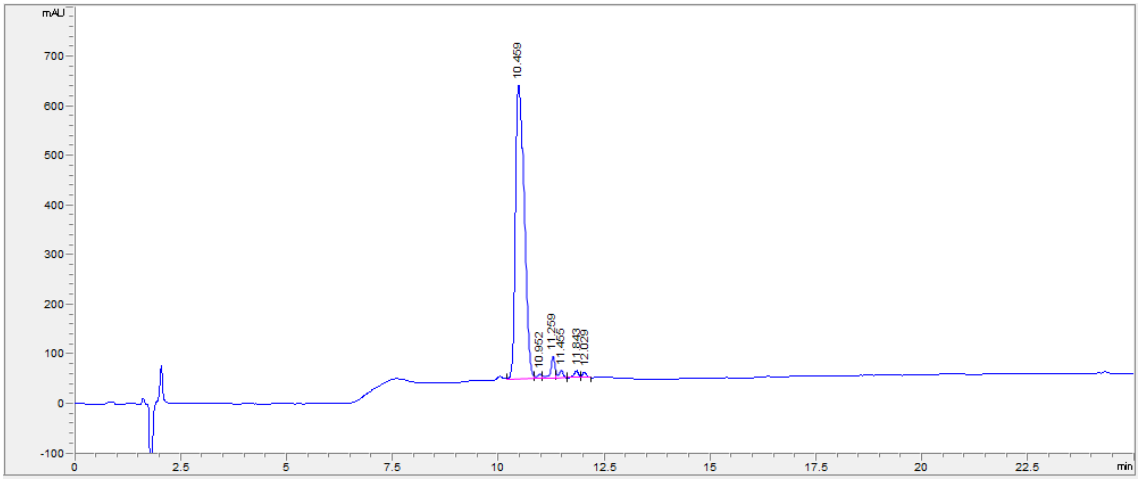


Analytical HPLC (5-95% gradient)



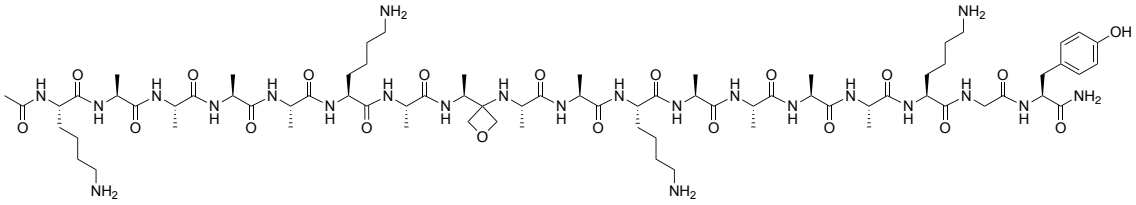
#	Time	Area	Height	Width	Area%	Symmetry
1	8.753	7078.9	726.6	0.1624	94.512	0.562
2	9.175	163.4	42.7	0.0637	2.182	0.965
3	9.284	70.2	20.2	0.0578	0.937	0.332
4	9.564	107.8	13.9	0.1293	1.440	2.122
5	10.257	69.7	12.5	0.093	0.930	0.803

Analytical HPLC (5-50% gradient)

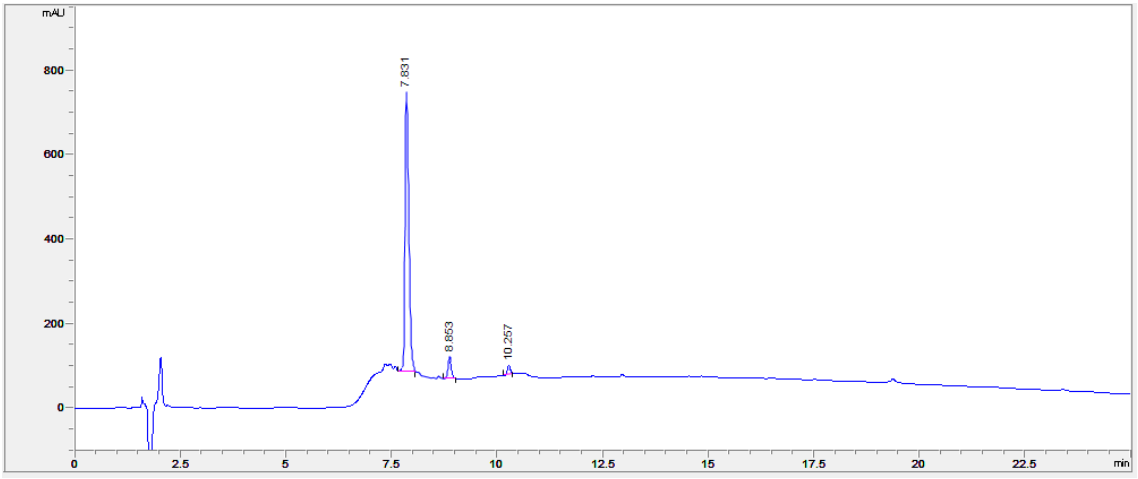


#	Time	Area	Height	Width	Area%	Symmetry
1	10.459	8226.4	593.6	0.2237	92.041	0.583
2	10.952	85.2	9.8	0.1231	0.954	1.169
3	11.259	321.6	44.8	0.1057	3.598	1.171
4	11.455	109.8	16	0.1038	1.228	0.985
5	11.843	118.9	14.9	0.1173	1.330	1.272
6	12.029	75.9	11.2	0.103	0.849	0.892

Ac-KAAAA-KAAOxAA-KAAAA-KGY-NH_2 (19)

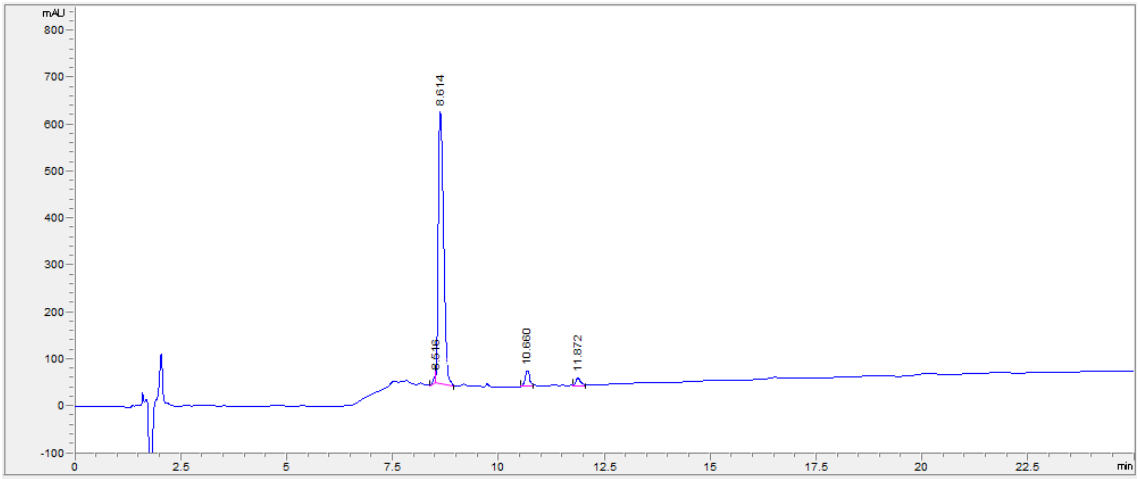


Analytical HPLC (5-95% gradient)



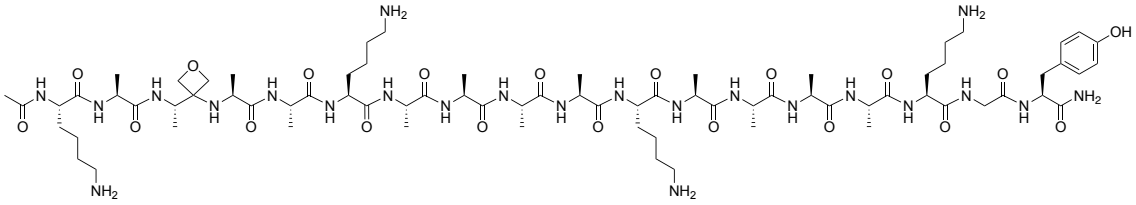
#	Time	Area	Height	Width	Area%	Symmetry
1	7.831	4160.5	662.8	0.1046	90.857	0.581
2	8.853	308.9	52.3	0.0984	6.746	0.801
3	10.257	109.8	21.6	0.0848	2.398	0.896

Analytical HPLC (5-50% gradient)

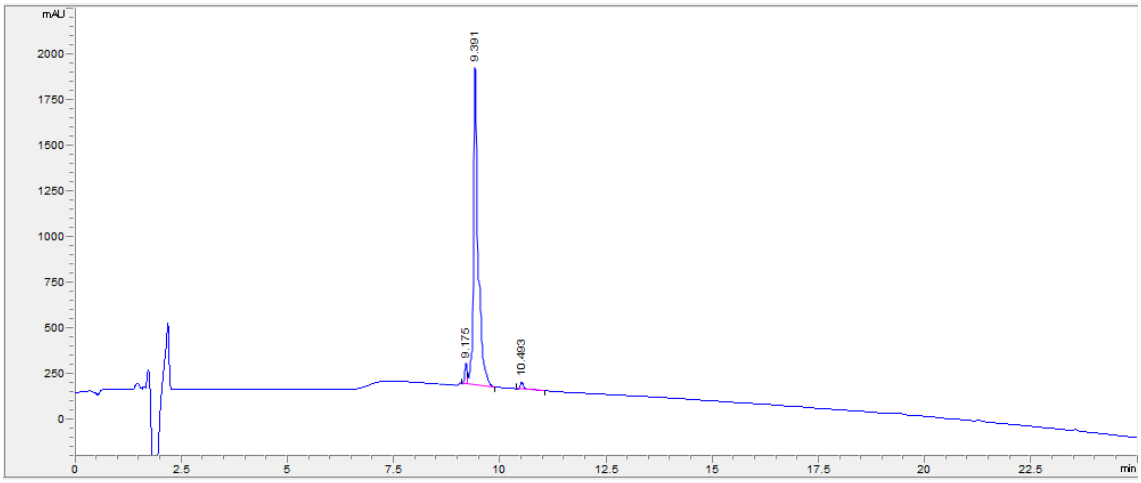


#	Time	Area	Height	Width	Area%	Symmetry
1	8.516	92.3	17.9	0.0858	1.654	1.228
2	8.614	5027.7	581	0.1442	90.059	0.58
3	10.66	292.8	34	0.1434	5.244	0.759
4	11.872	169.9	18.7	0.1514	3.043	0.617

Ac-KAAOxAA-KAAAA-KAAAA-KGY-NH_2 (20)

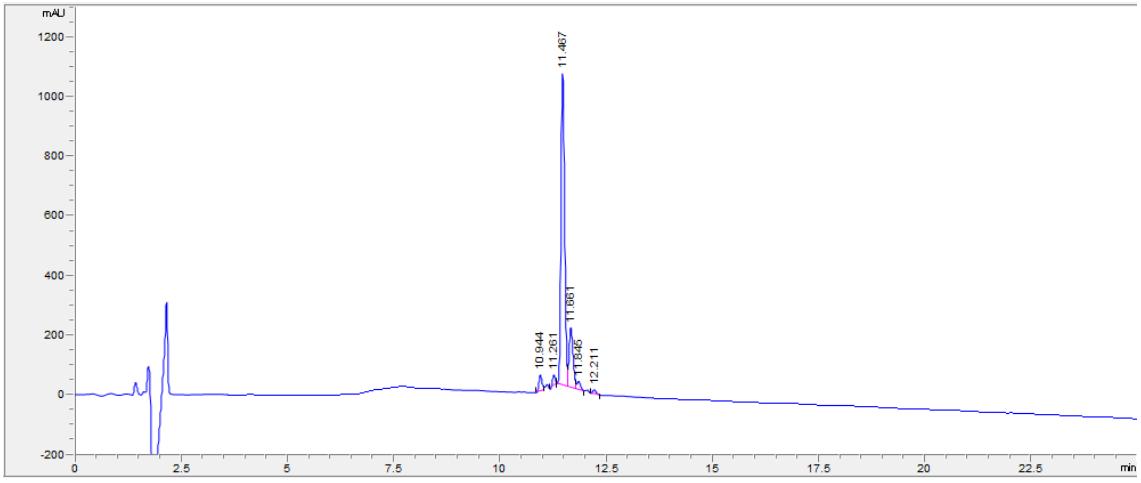


Analytical HPLC (5-95% gradient)



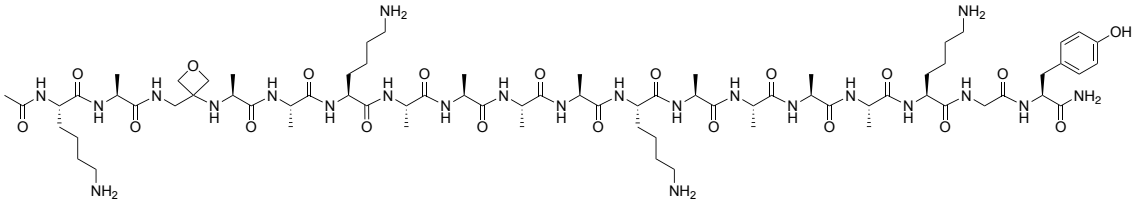
#	Time	Area	Height	Width	Area%	Symmetry
1	9.175	479.8	114.9	0.0647	3.423	0.845
2	9.391	13290.4	1741.4	0.109	94.829	0.508
3	10.493	245	38.4	0.0945	1.748	0.536

Analytical HPLC (5-50% gradient)

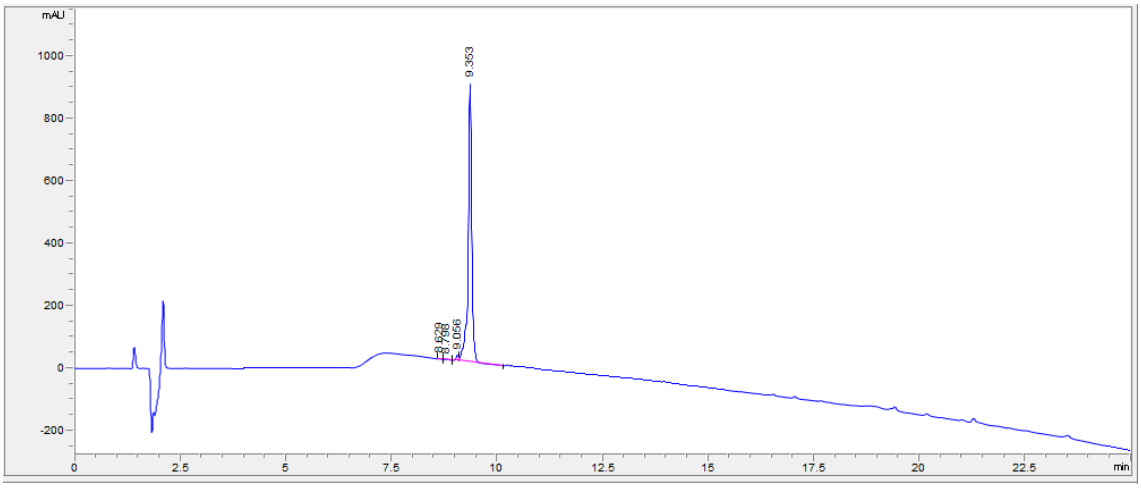


#	Time	Area	Height	Width	Area%	Symmetry
1	10.944	300.1	55.7	0.0809	3.705	0.728
2	11.261	172.1	36.6	0.0769	2.125	1.063
3	11.467	6127.7	1043.2	0.0925	75.667	0.749
4	11.661	1284.4	197.6	0.0978	15.861	0.723
5	11.845	135.2	25.5	0.0798	1.670	0.739
6	12.211	78.7	14.5	0.0875	0.972	0.757

Ac-KAGOxAA-KAAAA-KAAAA-KGY-NH_2 (21)

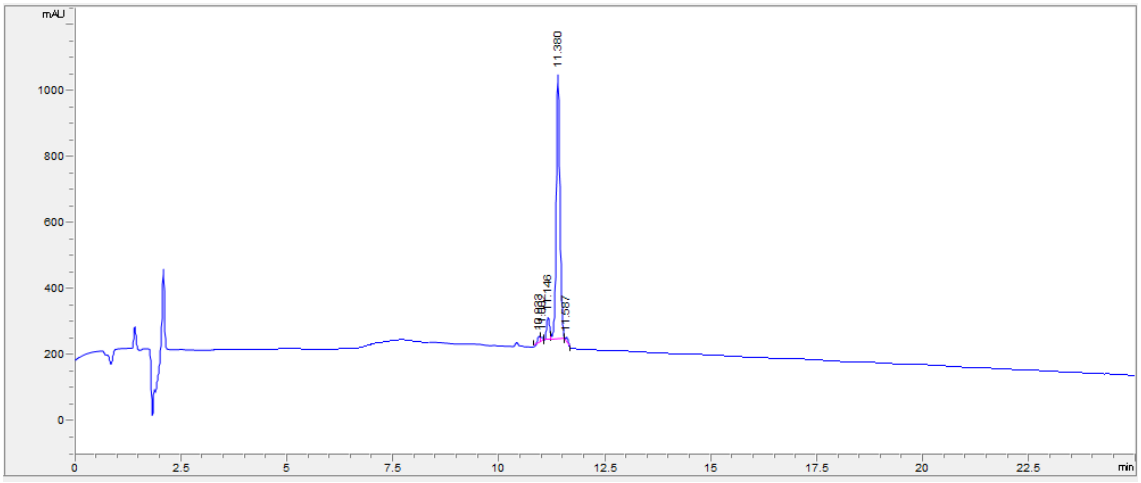


Analytical HPLC (5-95% gradient)



#	Time	Area	Height	Width	Area%	Symmetry
1	8.629	16.3	3.2	0.075	0.300	0.668
2	8.798	11.7	2.1	0.0815	0.216	0.427
3	9.056	86.1	19	0.0669	1.586	1.094
4	9.353	5313.7	895.1	0.0873	97.898	1.12

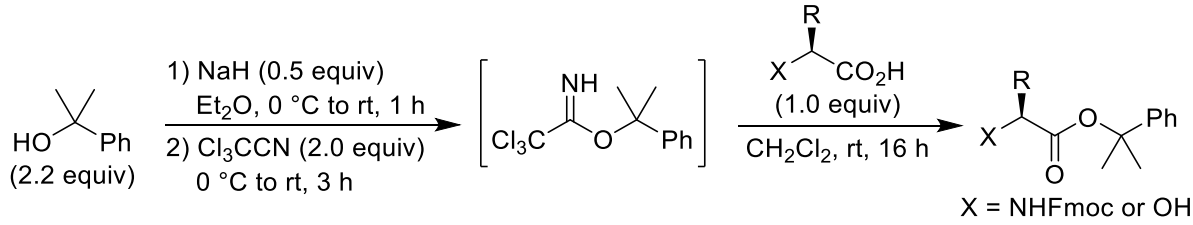
Analytical HPLC (5-50% gradient)



#	Time	Area	Height	Width	Area%	Symmetry
1	10.933	102.9	22.4	0.0696	1.857	1.332
2	11.002	51.6	14.6	0.0552	0.931	0.998
3	11.146	367.4	69.3	0.0838	6.630	0.796
4	11.38	4968.7	803.4	0.0942	89.666	0.833
5	11.587	50.7	13.1	0.0631	0.915	0.419

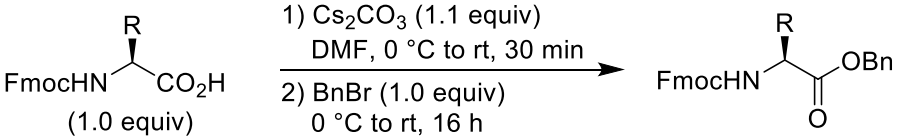
4. General procedures

General procedure 1: *Synthesis of cumyl esters with 2-phenylisopropyltrichloroacetimidate*



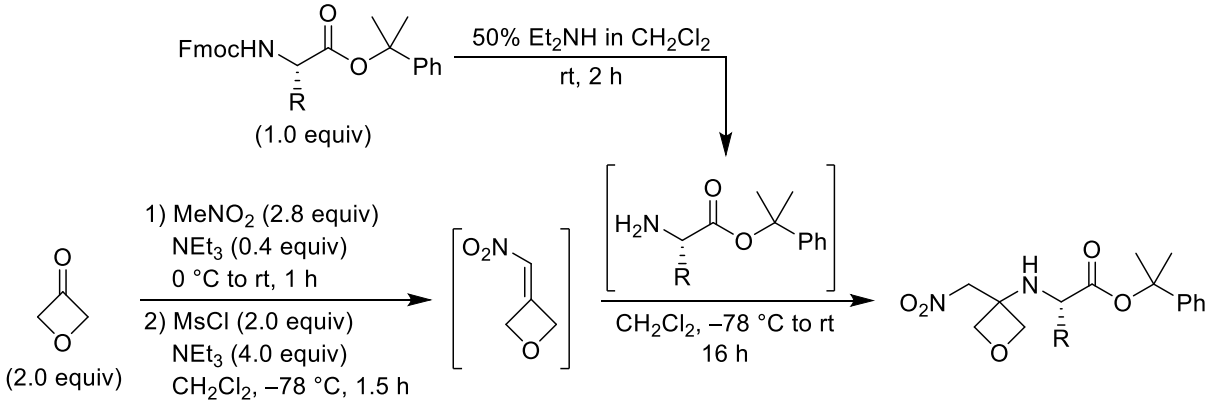
Cumyl esters were prepared following a modified procedure by Potier and co-workers.[4] To sodium hydride (60% dispersion in mineral oil, 0.5 equiv) in Et_2O (0.5 M) at 0 °C was added 2-phenyl-2-propanol (2.2 equiv) in Et_2O (2.0 M) and the mixture was stirred at room temperature for 1 h. The reaction mixture was cooled to 0 °C, trichloroacetonitrile (2.0 equiv) was added slowly and stirring was continued for 3 h at room temperature. The solvent was removed under reduced pressure and the residue re-dissolved in petroleum ether (4.0 M), MeOH (0.5 equiv) was added dropwise and this mixture was stirred at room temperature for 10 min. The reaction mixture was filtered through a plug of Celite eluting with petroleum ether. The filtrate was concentrated under reduced pressure to give the crude imidate. To the crude imidate in CH_2Cl_2 (0.15 M) was added a carboxylic acid (1.0 equiv) and the reaction mixture was stirred at room temperature for 16 h. The mixture was filtered through a plug of Celite eluting with CH_2Cl_2 to remove precipitated trichloroacetamide. The crude product was concentrated *in vacuo* and purified by column chromatography (SiO_2, PE/EtOAc or toluene/EtOAc) to give the pure cumyl ester.

General procedure 2: *Synthesis of benzyl esters*



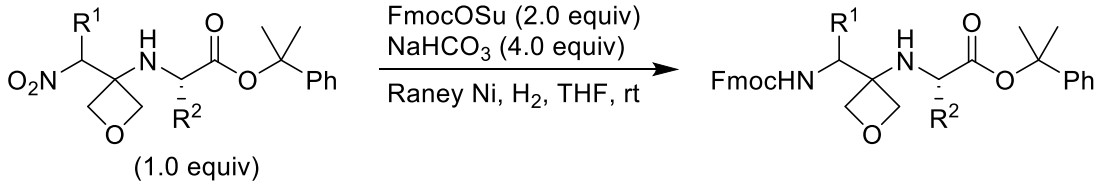
To a solution of Fmoc-protected amino acid (1.0 equiv) in DMF (0.25 M) was added Cs_2CO_3 (1.1 equiv) at 0 °C, the mixture was allowed to warm to room temperature and stirred for 30 min. Benzyl bromide (1.0 equiv) was added slowly at 0 °C, the mixture was allowed to warm to room temperature and stirred for 16 h. The mixture was filtered through a plug of Celite eluting with diethyl ether. The filtrate was washed with water, the aqueous phase was extracted with diethyl ether (2 × 5.0 mL/mmol), the combined organic layers were washed with water (2 × 5.0 mL/mmol) and brine (5.0 mL/mmol), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was used without further purification.

General procedure 3: *Synthesis of oxetane containing nitro amino cumyl esters*



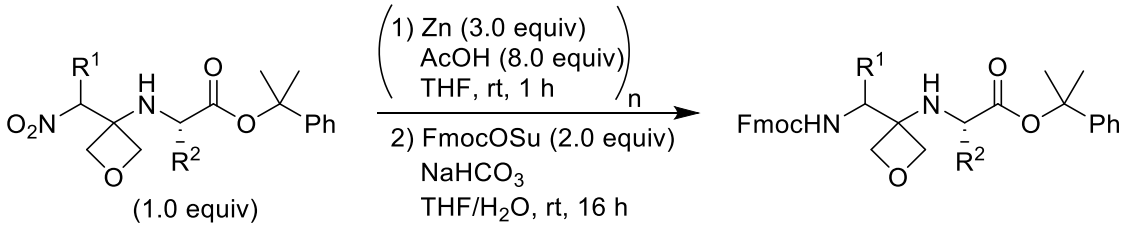
An *N*-Fmoc amino cumyl ester (1.0 equiv) in 50% diethylamine/CH_2Cl_2 (0.5 M) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH_2Cl_2 (3 × 5.0 mL/mmol) and concentrated under reduced pressure to give the crude amine. Meanwhile in a second reaction vessel, oxetan-3-one (2.0 equiv), nitromethane (2.8 equiv) and triethylamine (0.4 equiv) were combined at 0 °C and stirred for 1 h at room temperature. CH_2Cl_2 (0.2 M) was added and the reaction mixture cooled to −78 °C. Triethylamine (4.0 equiv) was added followed by the dropwise addition of a solution of methanesulfonyl chloride (2.0 equiv) in CH_2Cl_2 (1.0 M). The reaction mixture was stirred at −78 °C for 1.5 h. The crude amine in CH_2Cl_2 (0.25 M) was added slowly 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 (8 mL/mmol) was added and stirred for 10 min. The layers were separated and the aqueous extracted with CH_2Cl_2 (2 × 5.0 mL/mmol) and EtOAc (2 × 5.0 mL/mmol). The combined organic layers were washed with saturated $NaHCO_3$ (2 × 8.0 mL/mmol), then brine (5.0 mL/mmol), dried over $MgSO_4$, filtered and concentrated *in vacuo*, and the crude product purified by column chromatography (SiO_2, PE/EtOAc).

General procedure 4: *Nitro reduction and Fmoc protection using H_2/Raney Ni*



To a nitro alkane (1.0 equiv) in THF (0.1 M) was added Fmoc *N*-hydroxysuccinimide ester (2.0 equiv), $NaHCO_3$ (4.0 equiv) and Raney Ni (1.0 mL/mmol, slurry in H_2O). The reaction mixture was placed under an atmosphere of nitrogen, evacuated, filled with hydrogen (balloon) and stirred at room temperature until full conversion of the starting material (MS monitoring). The reaction mixture was filtered through a plug of Celite eluting with EtOAc. The filtrate was washed with saturated Na_2CO_3 (3 × 8.0 mL/mmol) and brine (5.0 mL/mmol), dried over $MgSO_4$, filtered, concentrated under reduced pressure, and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc).

General procedure 5: *Nitro reduction and Fmoc protection using Zn/AcOH*

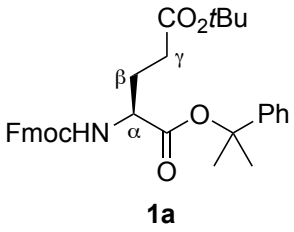


To a solution of nitro alkane (1.0 equiv) in THF (0.05 M) was added zinc powder (3.0 equiv) and acetic acid (8.0 equiv) and the reaction mixture was vigorously stirred with a glass-coated magnetic stir bar at room temperature for 1h. Additional zinc powder (3.0 equiv) and acetic acid (8.0 equiv) were added and the mixture was stirred at ambient temperature for 1 h (repeat until all starting material has been consumed, MS monitoring). The mixture was cooled to 0 °C and saturated aqueous $NaHCO_3$ solution (20 mL/mmol) was added followed by Fmoc *N*-hydroxysuccinimide ester (2.0 equiv) and the solution was stirred for 16 h at room temperature. Brine (15 mL/mmol) was added and the mixture as extracted with EtOAc (3 × 10 mL/mmol). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (20 mL/mmol) and brine (20 mL/mmol), dried over $MgSO_4$, filtered, concentrated *in vacuo*, and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc).

5. Synthesis and characterisation of oxetane modified glycine building blocks

5.1 Synthesis of Fmoc-GOx-Glu(tBu)-OCumyl

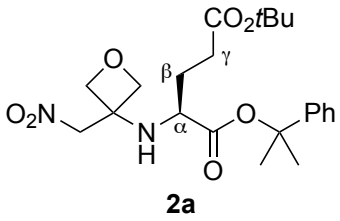
Fmoc-Glu(*t*Bu)-OCumyl (1a)



1a

Following general procedure 1, Fmoc-Glu(*t*Bu)-OH (6.38 g, 15.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1), **1a** (5.95 g, 10.9 mmol, 73%) contaminated with traces of 2-phenyl-2-propanol as a pale yellow viscous oil. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.49; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.4 Hz, 2H, ArH), 7.58 (d, *J* = 7.4 Hz, 2H, ArH), 7.41–7.28 (m, 9H, ArH), 5.39 (d, *J* = 7.9 Hz, 1H, NH), 4.56–4.23 (m, 3H, CH_2-Fmoc, CHα-Glu), 4.20 (t, *J* = 6.9 Hz, 1H, CH-Fmoc), 2.48–2.09 (m, 3H, CH_2γ-Glu, C*H*Hβ-Glu), 2.03–1.90 (m, 1H, CH*H*β-Glu), 1.82 (s, 3H, CH_3, cumyl), 1.80 (s, 3H, CH_3, cumyl), 1.47 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.3 (C=O), 170.7 (C=O), 156.1 (C=O, Fmoc), 145.0 (C), 144.0 (C), 143.9 (C), 141.4 (C), 128.5 (CH), 128.4 (CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 126.8 (CH), 125.3 (CH), 124.5 (CH), 120.1 (CH), 83.6 (C, cumyl), 80.9 (C, *t*Bu), 67.2 (CH_2, Fmoc), 54.0 (CH, α-Glu), 47.3 (CH, Fmoc), 31.6 (CH_2, γ-Glu), 28.8 (CH_3, cumyl), 28.3 (CH_3, cumyl), 28.2 (CH_3, *t*Bu), 27.9 (CH_2, β-Glu). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3340 (NH), 2977, 1720 (C=O), 1514, 1448, 1366, 1249, 1136, 957, 841, 739, 698 cm^{-1}; **MS** (ESI$^+$) *m/z* 494 $[M+Na]^+$, 510 $[M+K]^+$; **HRMS** (ESI$^+$) calcd. for $C_{30}H_{33}NNaO_4$ $[M+Na]^+$ 494.2302, found 494.2299; $[\boldsymbol{\alpha}]_D^{26}$ –0.1 (*c* 1.03, $CHCl_3$).

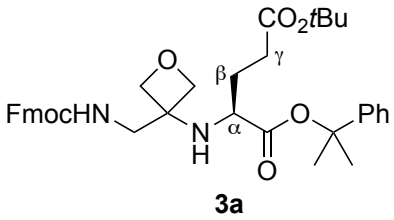
NO_2-GOx-Glu(*t*Bu)-OCumyl (2a)



2a

Following general procedure 3, **1a** (5.97 g, 10.9 mmol, 1.0 equiv), oxetan-3-one (1.28 mL, 20.0 mmol, 1.8 equiv) and nitromethane (1.52 mL, 28.0 mmol, 2.5 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **2a** (2.36 g, 5.41 mmol, 49%) as an orange oil. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.27; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.39–7.31 (m, 4H, ArH), 7.29–7.24 (m, 1H, ArH), 4.78 (d, *J* = 12.8 Hz, 1H, NO_2C*H*H), 4.71 (d, *J* = 12.8 Hz, 1H, NO_2CH*H*), 4.56 (d, *J* = 7.1 Hz, 1H, OC*H*H-Ox), 4.52 (d, *J* = 7.1 Hz, 1H, OC*H*H-Ox), 4.43 (d, *J* = 7.1 Hz, 1H, OCH*H*-Ox), 4.39 (d, *J* = 7.1 Hz, 1H, OCH*H*-Ox), 3.55 (t, *J* = 10.1 Hz, 1H, CHα-Glu), 2.38–2.28 (m, 2H, CH_2γ-Glu), 2.25 (d, *J* = 10.5 Hz, 1H, NH), 2.04 (dd, *J* = 13.5, 6.3 Hz, 1H, C*H*Hβ-Glu), 1.80 (s, 6H, 2 × CH_3, cumyl), 1.70 (dd, *J* = 13.5, 7.4 Hz, 1H, CH*H*β-Glu), 1.46 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.0 (C=O), 172.5 (C=O), 144.8 (C), 128.5 (CH), 127.6 (CH), 124.5 (CH), 83.2 (C, cumyl), 80.7 (C, *t*Bu), 78.9 (NO_2CH_2, OCH_2), 78.6 (OCH_2), 59.5 (C, Ox), 55.4 (CH, α-Glu), 31.5 (CH_2, γ-Glu), 29.4 (CH_2, β-Glu), 28.4 (CH_3, cumyl), 28.2 (CH_3, *t*Bu); $\boldsymbol{\nu_{max}}$ (neat) = 3331 (NH), 2978, 1723 (C=O), 1555, 1367, 1272, 1137, 979, 836, 763, 700 cm^{-1}; **MS** (ESI$^+$) *m/z* 437 $[M+H]^+$, 459 $[M+Na]^+$, 475 $[M+K]^+$; **HRMS** (ESI$^+$) calcd. for $C_{22}H_{32}N_2NaO_7$ $[M+Na]^+$ 459.2102, found 459.2105; $[\boldsymbol{\alpha}]_D^{28}$ –0.8 (*c* 0.22, $CHCl_3$).

Fmoc-GOx-Glu(*t*Bu)-OCumyl (3a)

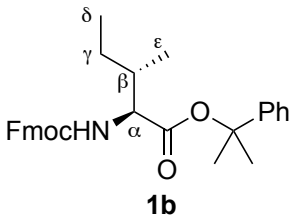


3a

Following general procedure 4, **2a** (2.22 g, 5.09 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1→4:1), **3a** (1.38 g, 2.19 mmol, 43%) as a colourless oil. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.32; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.77 (d, *J* = 7.4 Hz, 2H, ArH), 7.61 (d, *J* = 7.4 Hz, 2H, ArH), 7.43–7.26 (m, 9H, ArH), 5.44 (t, *J* = 7.0 Hz, 1H, NH), 4.46–4.35 (m, 3H, CH_2-Fmoc, C*H*H-Ox), 4.31 (s, 2H, CH_2-Ox), 4.27–4.16 (m, 2H, CH*H*-Ox, CH-Fmoc), 3.66 (dd, *J* = 13.6, 5.1 Hz, 1H, C*H*HGOx), 3.45 (dd, *J* = 13.6, 4.1 Hz, 1H, CH*H*GOx), 3.38 (dd, *J* = 8.0, 3.3 Hz, 1H, CHα-Glu), 2.48–2.31 (m, 2H, CH_2γ-Glu), 2.14–1.92 (m, 2H, C*H*Hβ-Glu, NH), 1.80 (s, 6H, 2 × CH_3, cumyl), 1.79–1.72 (m, 1H, CH*H*β-Glu), 1.47 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.7 (C=O), 172.7 (C=O), 157.1 (C=O, Fmoc), 144.8 (C), 144.09 (C), 144.05 (C), 141.4 (C), 128.5 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.4 (CH), 120.1 (CH), 83.1 (C, cumyl), 80.8 (C, *t*Bu), 79.8 (OCH_2), 79.6 (OCH_2), 67.0 (CH_2, Fmoc), 59.6 (C, Ox), 55.4 (CH, α-Glu), 47.3 (CH, Fmoc), 45.5 (CH_2, GOx), 32.1 (CH_2, γ-Glu), 29.6 (CH_2, β-Glu), 28.4 (CH_3, cumyl), 28.3 (CH_3, cumyl), 28.2 (CH_3, *t*Bu). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3332 (NH), 2977, 1720 (C=O), 1518, 1449, 1366, 1240, 1133, 972, 838, 740, 699 cm^{-1}; **MS** (ESI$^+$) *m/z* 629 $[M+H]^+$, 651 $[M+Na]^+$, 667 $[M+K]^+$; **HRMS** (ESI$^+$) calcd. for $C_{37}H_{44}N_2NaO_7$ $[M+Na]^+$ 651.3041, found 651.3039; $[\boldsymbol{\alpha}]_D^{26}$ +0.7 (*c* 1.00, $CHCl_3$).

5.2 Synthesis of Fmoc-GOx-Ile-OCumyl

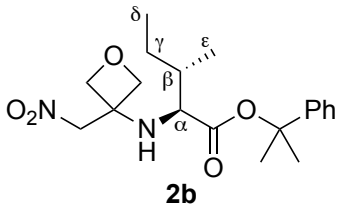
Fmoc-Ile-OCumyl (1b)



1b

Following general procedure 1, Fmoc-Ile-OH (5.30 g, 15.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1), **1b** (6.26 g, 13.3 mmol, 89%) as a white solid. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.37; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.4 Hz, 2H, ArH), 7.58 (d, *J* = 7.4 Hz, 2H, ArH), 7.43–7.27 (m, 9H, ArH), 5.26 (d, *J* = 8.7 Hz, 1H, NH), 4.60–4.27 (m, 3H, CHα-Ile, CH_2-Fmoc), 4.22 (t, *J* = 7.0 Hz, 1H, CH-Fmoc), 1.96 (br. m, 1H, CHβ-Ile), 1.82 (s, 3H, CH_3, cumyl), 1.81 (s, 3H, CH_3, cumyl), 1.46–1.34 (m, 1H, C*H*Hγ-Ile), 1.23–1.13 (m, 1H, CH*H*γ-Ile), 1.01–0.86 (m, 6H, CH_3δ-Ile, CH_3ε-Ile); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.8 (C=O), 156.3 (C=O, Fmoc), 145.2 (C), 144.1 (C), 144.0 (C), 141.4 (C), 128.4 (CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 125.3 (CH), 124.6 (CH), 120.1 (CH), 83.3 (C, cumyl), 67.1 (CH_2, Fmoc), 58.9 (CH, α-Ile), 47.4 (CH, Fmoc), 38.4 (CH, β-Ile), 28.6 (CH_3, cumyl), 28.4 (CH_3, cumyl), 24.9 (CH_2, γ-Ile), 15.7 (CH_3, ε-Ile), 11.9 (CH_3, δ-Ile). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3346 (NH), 2963, 1706 (C=O), 1536, 1448, 1342, 1245, 1202, 1134, 1086, 1042, 825, 757, 737, 697 cm^{-1}; **MS** (ESI$^+$) *m/z* 566 $[M+Na]^+$, 582 $[M+K]^+$; **HRMS** (ESI$^+$) calcd. for $C_{33}H_{37}NNaO_6$ $[M+Na]^+$ 566.2513, found 566.2507; $[\boldsymbol{\alpha}]_D^{28}$ –2.5 (*c* 0.63, $CHCl_3$).

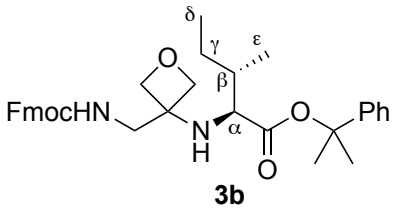
NO_2-GOx-Ile-OCumyl (2b)



2b

Following general procedure 3, **1b** (5.75 g, 12.2 mmol, 1.0 equiv), oxetan-3-one (1.54 mL, 24.0 mmol, 2.0 equiv) and nitromethane (1.82 mL, 33.6 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1→2:1), **2b** (3.26 g, 8.95 mmol, 73%) as an orange oil. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.14; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.42–7.30 (m, 4H, ArH), 7.29–7.26 (m, 1H, ArH), 4.77 (d, *J* = 12.7 Hz, 1H, NO_2CHH), 4.66 (d, *J* = 12.7 Hz, 1H, NO_2CHH), 4.56 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.48 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.43 (d, *J* = 7.0 Hz, 2H, 2 × OCH*H*-Ox), 3.36 (d, *J* = 2.9 Hz, 1H, CHα-Ile), 2.24 (br. s, 1H, NH), 1.82 (s, 3H, CH_3, cumyl), 1.80 (s, 3H, CH_3, cumyl), 1.78–1.72 (m, 1H, CHβ-Ile), 1.42–1.31 (m, 1H, C*H*Hγ-Ile), 1.07 (dq, *J* = 10.8, 7.4 Hz, 1H, CH*H*γ-Ile), 0.93 (d, *J* = 7.2 Hz, 3H, CH_3ε-Ile), 0.90 (t, *J* = 7.4 Hz, 3H, CH_3δ-Ile); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.1 (C=O), 144.9 (C), 128.4 (CH), 127.6 (CH), 124.7 (CH), 82.9 (C, cumyl), 79.2 (NO_2CH_2), 78.8 (OCH_2), 78.5 (OCH_2), 60.7 (CH, α-Ile), 59.7 (C, Ox), 39.2 (CH, β-Ile), 28.5 (CH_3, cumyl), 28.0 (CH_3, cumyl), 24.5 (CH_2, γ-Ile), 16.0 (CH_3, ε-Ile), 11.9 (CH_3, δ-Ile); $\boldsymbol{\nu_{max}}$ (neat) = 3346 (NH), 2963, 1707 (C=O), 1536, 1449, 1342, 1246, 1202, 1133, 1086, 828, 757, 697 cm^{-1}; **MS** (ESI$^+$) *m/z* 387 [M+Na]$^+$, 403 [M+K]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{19}H_{28}N_2NaO_5$ [M+Na]$^+$ 387.1890, found 387.1894; $[\boldsymbol{\alpha}]_D^{28}$ –3.6 (*c* 0.69, $CHCl_3$).

Fmoc-GOx-Ile-OCumyl (3b)

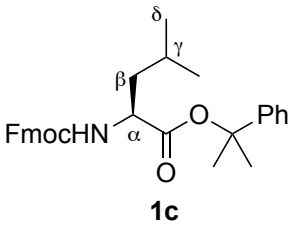


3b

Following general procedure 4, **2b** (3.15 g, 8.64 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 19:1→9:1→4:1), **3b** (1.50 g, 2.69 mmol, 31%) as a sticky colourless oil. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.23; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.77 (d, *J* = 7.4 Hz, 2H, ArH), 7.59 (d, *J* = 6.8 Hz, 2H, ArH), 7.43–7.27 (m, 9H, ArH), 5.24 (t, *J* = 5.3 Hz, 1H, NH), 4.47–4.18 (m, 7H, 2 × OCH_2-Ox, CH_2-Fmoc, CH-Fmoc), 3.70 (dd, *J* = 13.4, 5.7 Hz, 1H, C*H*HGOx), 3.36 (dd, *J* = 13.4, 3.7 Hz, 1H, CH*H*GOx), 3.22 (d, *J* = 3.5 Hz, 1H, CHα-Ile), 1.96 (br. s, 1H, NH), 1.83 (s, 3H, CH_3, cumyl), 1.80 (s, 3H, CH_3, cumyl), 1.80–1.74 (m, 1H, CHβ-Ile), 1.46–1.37 (m, 1H, C*H*Hγ-Ile), 1.19–1.09 (m, 1H, CH*H*γ-Ile), 1.00 (d, *J* = 6.3 Hz, 3H, CH_3ε-Ile), 0.94 (t, *J* = 7.0 Hz, 3H, CH_3δ-Ile); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.8 (C=O), 156.9 (C=O, Fmoc), 144.9 (C), 144.1 (C), 144.0 (C), 141.4 (C), 128.4 (CH), 127.8 (CH), 127.6 (CH), 127.1 (CH), 125.2 (CH), 124.6 (CH), 120.1 (CH), 82.9 (C, cumyl), 79.7 (OCH_2), 79.5 (OCH_2), 67.0 (CH_2, Fmoc), 60.6 (CH, α-Ile), 59.5 (C, Ox), 47.3 (CH, Fmoc), 45.8 (CH_2, GOx), 39.2 (CH, β-Ile), 28.6 (CH_3, cumyl), 28.0 (CH_3, cumyl), 24.6 (CH_2, γ-Ile), 16.2 (CH_3, ε-Ile), 11.9 (CH_3, δ-Ile). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3334 (NH), 2961, 1720 (C=O), 1515, 1448, 1237, 1130, 972, 739, 698 cm^{-1}; **MS** (ESI$^+$) *m/z* 557 [M+H]$^+$, 579 [M+Na]$^+$, 595 [M+K]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{34}H_{40}N_2NaO_5$ [M+Na]$^+$ 579.2829, found 579.2826; $[\boldsymbol{\alpha}]_D^{29}$ +10.9 (*c* 0.58, $CHCl_3$).

5.3 Synthesis of Fmoc-GOx-Leu-OCumyl

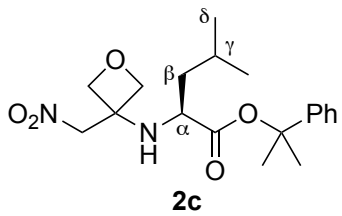
Fmoc-Leu-OCumyl (1c)



1c

Following general procedure 1, Fmoc-Leu-OH (7.07 g, 20.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1), **1c** (8.61 g, 18.3 mmol, 91%) as a viscous colourless oil. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.29; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.75 (d, *J* = 7.4 Hz, 2H, ArH), 7.57 (d, *J* = 7.4 Hz, 2H, ArH), 7.41–7.23 (m, 9H, ArH), 5.13 (d, *J* = 8.6 Hz, 1H, NH), 4.42–4.35 (m, 3H, CH_2-Fmoc, CHα-Leu), 4.20 (t, *J* = 6.8 Hz, 1H, CH-Fmoc), 1.79 (s, 6H, 2 × CH_3, cumyl), 1.73–1.61 (m, 2H, CHγ-Leu, C*H*Hβ-Leu), 1.56–1.46 (m, 1H, CH*H*β-Leu), 1.04–0.87 (m, 6H, 2 × CH_3δ-Leu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 171.8 (C=O), 156.1 (C=O, Fmoc), 145.3 (C), 144.1 (C), 143.9 (C), 141.4 (C), 128.5 (CH), 127.8 (CH), 127.4 (CH), 127.2 (CH), 125.2 (CH), 124.4 (CH), 120.1 (CH), 83.2 (C, cumyl), 67.0 (CH_2, Fmoc), 53.0 (CH, α-Leu), 47.3 (CH, Fmoc), 42.0 (CH_2, β-Leu), 28.59 (CH_3, cumyl), 28.55 (CH_3, cumyl), 24.9 (CH, γ-Leu), 23.1 (CH_3, δ-Leu), 22.0 (CH_3, δ-Leu). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3343 (NH), 2958, 1707 (C=O), 1518, 1448, 1248, 1133, 1045, 739, 698 cm^{-1}; **MS** (ESI^+) *m/z* 494 $[M+Na]^+$, 510 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{30}H_{33}NNaO_4$ $[M+Na]^+$ 494.2302, found 480.2294; $[\boldsymbol{\alpha}]_D^{29}$ –16.3 (*c* 0.61, $CHCl_3$).

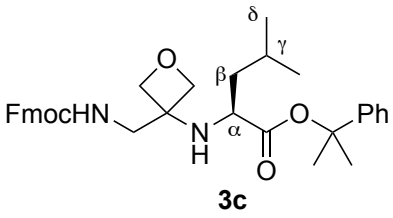
NO_2-GOx-Leu-OCumyl (2c)



2c

Following general procedure 3, **1c** (8.49 g, 18.0 mmol, 1.0 equiv), oxetan-3-one (2.31 mL, 36.0 mmol, 2.0 equiv) and nitromethane (2.73 mL, 50.4 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **2c** (4.19 g, 11.5 mmol, 64%) as an orange oil. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.44; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.38–7.32 (m, 4H, ArH), 7.30–7.22 (m, 1H, ArH), 4.80 (d, *J* = 12.6 Hz, 1H, NO_2C*H*H), 4.72 (d, *J* = 12.6 Hz, 1H, NO_2CH*H*), 4.57 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.52 (d, *J* = 7.1 Hz, 1H, OC*H*H-Ox), 4.46 (d, *J* = 7.1 Hz, 1H, OCH*H*-Ox), 4.36 (d, *J* = 7.0 Hz, 1H, OCH*H*-Ox), 3.47 (dd, *J* = 12.8, 8.4 Hz, 1H, CHα-Leu), 2.17 (d, *J* = 10.0 Hz, 1H, NH), 1.81 (s, 3H, CH_3, cumyl), 1.78 (s, 3H, CH_3, cumyl), 1.77–1.70 (m, 1H, CHγ-Leu), 1.54–1.46 (m, 1H, C*H*Hβ-Leu), 1.39–1.31 (m, 1H, CH*H*β-Leu), 0.94 (d, *J* = 6.9 Hz, 3H, CH_3δ-Leu), 0.92 (d, *J* = 6.9 Hz, 3H, CH_3δ-Leu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.8 (C=O), 145.0 (C), 128.5 (CH), 127.6 (CH), 124.5 (CH), 82.9 (C, cumyl), 79.0 (NO_2CH_2 or OCH_2), 78.9 (NO_2CH_2 or OCH_2), 78.8 (OCH_2), 59.7 (C, Ox), 54.9 (CH, α-Leu), 43.7 (CH_2, β-Leu), 28.6 (CH_3, cumyl), 27.9 (CH_3, cumyl), 24.7 (CH, γ-Leu), 23.1 (CH_3, δ-Leu), 22.2 (CH_3, δ-Leu); **IR** (neat) = 3332 (NH), 2957, 1729 (C=O), 1555, 1381, 1271, 1193, 1132, 978, 763 cm^{-1}; **MS** (ESI^+) *m/z* 387 $[M+Na]^+$, 403 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{19}H_{28}N_2NaO_5$ $[M+Na]^+$ 387.1890, found 387.1884; $[\boldsymbol{\alpha}]_D^{28}$ –5.3 (*c* 0.10, $CHCl_3$).

Fmoc-GOx-Leu-OCumyl (3c)

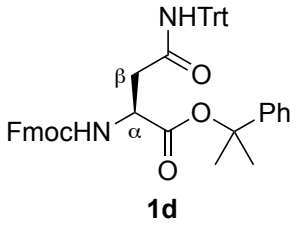


3c

Following general procedure 4, **2c** (1.40 g, 3.84 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 19:1→9:1), **3c** (1.09 g, 1.96 mmol, 51%) as a sticky colourless oil. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.40; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.77 (d, *J* = 7.4 Hz, 2H, ArH), 7.58 (d, *J* = 7.4 Hz, 2H, ArH), 7.44–7.26 (m, 9H, ArH), 5.24 (t, *J* = 5.6 Hz, 1H, NH), 4.54–4.17 (m, 7H, 2 × OCH_2-Ox, CH_2CH-Fmoc), 3.71 (dd, *J* = 13.6, 5.7 Hz, 1H, C*H*HGOx), 3.41 (dd, *J* = 13.6, 4.1 Hz, 1H, CH*H*GOx), 3.33 (dd, *J* = 8.0, 5.0 Hz, 1H, CHα-Leu), 1.93–1.83 (m, 2H, CHγ-Leu, NH), 1.81 (s, 3H, CH_3, cumyl), 1.78 (s, 3H, CH_3, cumyl), 1.56–1.47 (m, 1H, C*H*Hβ-Leu), 1.43–1.34 (m, 1H, CH*H*β-Leu), 0.98 (d, *J* = 7.8 Hz, 3H, CH_3δ-Leu), 0.96 (d, *J* = 8.5 Hz, 3H, CH_3δ-Leu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 175.6 (C=O), 156.9 (C=O, Fmoc), 145.0 (C), 144.1 (C), 144.0 (C), 141.4 (C), 128.5 (CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 125.2 (CH), 124.5 (CH), 120.1 (CH), 82.8 (C, cumyl), 80.0 (OCH_2), 79.7 (OCH_2), 67.0 (CH_2, Fmoc), 59.5 (C, Ox), 54.6 (CH, α-Leu), 47.4 (CH, Fmoc), 45.6 (CH_2, GOx), 43.8 (CH_2, β-Leu), 28.6 (CH_3, cumyl), 28.0 (CH_3, cumyl), 24.9 (CH, γ-Leu), 23.3 (CH_3, δ-Leu), 22.0 (CH_3, δ-Leu). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3331 (NH), 2954, 1720 (C=O), 1515, 1449, 1227, 1131, 971, 739, 699 cm^{-1}; **MS** (ESI^+) *m/z* 557 $[M+H]^+$, 579 $[M+Na]^+$, 595 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{34}H_{40}N_2NaO_5$ $[M+Na]^+$ 579.2829, found 579.2824; $[\boldsymbol{\alpha}]_D^{30}$ +0.2 (*c* 0.52, $CHCl_3$).

5.4 Synthesis of Fmoc-GOx-Asn(Trt)-OCumyl

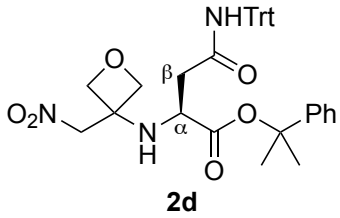
Fmoc-Asn(Trt)-OCumyl (1d)



1d

Following general procedure 1, Fmoc-Asn(Trt)-OH (5.97 g, 10.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **1d** (6.79 g, 9.50 mmol, 95%) as a white foam. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.34; **mp** 101–103 °C; **^{1}H NMR** (300 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.5 Hz, 2H, ArH), 7.59 (d, *J* = 7.5 Hz, 2H, ArH), 7.40 (t, *J* = 7.5 Hz, 2H, ArH), 7.33–7.20 (m, 16H, ArH), 7.19–7.12 (m, 6H, ArH), 6.68 (s, 1H, NH), 6.14 (d, *J* = 8.4 Hz, 1H, NH), 4.57 (dt, *J* = 8.8, 3.9 Hz, 1H, CHα-Asn), 4.42 (dd, *J* = 9.9, 7.4 Hz, 1H, C*H*H-Fmoc), 4.30 (dd, *J* = 9.9, 7.4 Hz, 1H, CH*H*-Fmoc), 4.20 (t, *J* = 7.4 Hz, 1H, CH-Fmoc), 3.10 (dd, *J* = 16.1, 3.9 Hz, 1H, C*H*Hβ-Asn), 2.85 (dd, *J* = 16.1, 3.9 Hz, 1H, CH*H*β-Asn), 1.72 (s, 3H, CH_3, cumyl), 1.67 (s, 3H, CH_3, cumyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 169.7 (C=O), 169.4 (C=O), 156.5 (C=O, Fmoc), 145.5 (C), 144.5 (C), 144.1 (C), 143.9 (C), 141.42 (C), 141.39 (C), 128.8 (CH), 128.4 (CH), 128.2 (CH), 127.8 (CH), 127.3 (CH), 127.2 (CH), 125.4 (CH), 125.3 (CH), 124.4 (CH), 120.1 (CH), 83.7 (C, cumyl), 71.1 (C, Trt), 67.4 (CH_2, Fmoc), 51.6 (CH, α-Asn), 47.3 (CH, Fmoc), 38.7 (CH_2, β-Asn), 28.7 (CH_3, cumyl), 27.9 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3311 (NH), 2932, 1721 (C=O), 1491, 1447, 1216, 1137, 1033, 740, 697 cm^{-1}; **MS** (ESI^+) *m/z* 737 $[M+Na]^+$, 753 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{47}H_{42}N_2NaO_5$ $[M+Na]^+$ 737.2986, found 737.2980; $[\boldsymbol{\alpha}]_D^{27}$ +3.3 (*c* 0.20, $CHCl_3$).

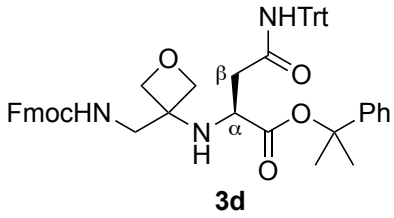
NO_2-GOx-Asn(Trt)-OCumyl (2d)



2d

Following general procedure 3, **1d** (8.22 g, 11.5 mmol, 1.0 equiv), oxetan-3-one (1.47 mL, 23.0 mmol, 2.0 equiv) and nitromethane (1.74 mL, 32.2 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 2:1→1:1), **2d** (3.58 g, 5.89 mmol, 51%) as an orange foam. $\mathbf{R_f}$ (PE/EtOAc 1:1) 0.57; **mp** 68–70 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.32–7.25 (m, 14H, ArH), 7.20 (d, *J* = 7.3 Hz, 6H, ArH), 7.16 (s, 1H, NH), 4.69 (d, *J* = 13.8 Hz, 1H, NO_2CHH), 4.65 (d, *J* = 13.8 Hz, 1H, NO_2CHH), 4.60 (d, *J* = 7.2 Hz, 1H, OC*H*H-Ox), 4.46 (d, *J* = 7.3 Hz, 1H, OC*H*H-Ox), 4.33 (d, *J* = 7.3 Hz, 1H, OCH*H*-Ox), 4.29 (d, *J* = 7.2 Hz, 1H, OCH*H*-Ox), 3.94 (br. s, 1H, CHα-Asn), 2.80–2.67 (m, 2H, C*H*Hβ-Asn, NH), 2.49 (dd, *J* = 14.8, 9.3 Hz, 1H, CH*H*β-Asn), 1.74 (s, 6H, 2 × CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.1 (C=O), 168.6 (C=O), 144.7 (C), 144.5 (C), 128.8 (CH), 128.5 (CH), 128.1 (CH), 127.6 (CH), 127.3 (CH), 124.4 (CH), 83.8 (C, cumyl), 79.3 (NO_2CH_2), 78.5 (OCH_2), 77.7 (OCH_2), 70.9 (C, Trt), 59.3 (C, Ox), 53.6 (CH, α-Asn), 41.8 (CH_2, β-Asn), 28.3 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3367 (NH), 2980, 1724 (C=O), 1683, 1552, 1491, 1447, 1271, 1134, 1032, 971, 824, 751, 697 cm^{-1}; **MS** (ESI^+) *m/z* 630 $[M+Na]^+$, 646 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{36}H_{37}N_3NaO_6$ $[M+Na]^+$ 630.2575, found 630.2571; $[\boldsymbol{\alpha}]_D^{26}$ –8.4 (*c* 0.44, $CHCl_3$).

Fmoc-GOx-Asn(Trt)-OCumyl (3d)

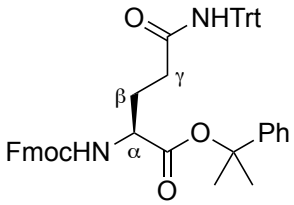


3d

Following general procedure 4, **2d** (1.22 g, 2.00 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 19:1→9:1→4:1), **3d** (335 mg, 0.42 mmol, 21%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.22; **mp** 107–110 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.68 (d, *J* = 7.3 Hz, 2H, ArH), 7.46–7.38 (m, 2H, ArH), 7.31 (dd, *J* = 12.2, 6.5 Hz, 2H, ArH), 7.26–7.15 (m, 23H, ArH, NH), 5.54 (t, *J* = 5.7 Hz, 1H, NH), 4.35–4.27 (m, 3H, OCH_2-Ox, OC*H*H-Ox), 4.16 (t, *J* = 7.7 Hz, 2H, CH_2-Fmoc), 3.99–3.86 (m, 2H, OCH*H*-Ox, CH-Fmoc), 3.85 (d, *J* = 9.9 Hz, 1H, CHα-Asn), 3.58 (dd, *J* = 14.4, 6.0 Hz, 1H, C*H*HGOx), 3.50 (dd, *J* = 14.4, 4.8 Hz, 1H, CH*H*GOx), 2.70 (d, *J* = 14.2 Hz, 1H, C*H*Hβ-Asn,), 2.34 (dd, *J* = 14.2, 11.1 Hz, 2H, CH*H*β-Asn, NH), 1.70 (s, 3H, CH_3, cumyl), 1.68 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.6 (C=O), 169.0 (C=O), 157.4 (C=O, Fmoc), 144.6 (C), 144.5 (C), 144.3 (C), 143.9 (C), 141.4 (C), 141.2 (C), 128.9 (CH), 128.5 (CH), 128.1 (CH), 127.72 (CH), 127.66 (CH), 127.6 (CH), 127.2 (CH), 127.1 (CH), 125.7 (CH), 125.2 (CH), 124.4 (CH), 120.0 (CH), 119.9 (CH), 83.4 (C, cumyl), 79.8 (OCH_2), 79.5 (OCH_2), 71.0 (C, Trt), 67.2 (CH_2, Fmoc), 59.8 (C, Ox), 53.4 (CH, α-Asn), 47.1 (CH, Fmoc), 45.4 (CH_2, GOx), 41.3 (CH_2, β-Asn), 28.5 (CH_3, cumyl), 28.2 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3316 (NH), 2934, 1722 (C=O), 1672, 1492, 1447, 1244, 1134, 1031, 967, 827, 742, 697 cm^{-1}; **MS** (ESI^+) *m/z* 800 $[M+H]^+$, 822 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{51}H_{49}N_3NaO_6$ $[M+Na]^+$ 822.3514, found 822.3517; $[\boldsymbol{\alpha}]_D^{27}$ +16.9 (*c* 0.20, $CHCl_3$).

5.5 Synthesis of Fmoc-GOx-Gln(Trt)-OCumyl

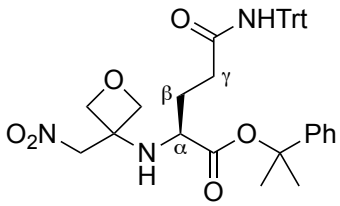
Fmoc-Gln(Trt)-OCumyl (1e)



1e

Following general procedure 1, Fmoc-Gln(Trt)-OH (12.2 g, 20.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **1e** (13.2 g, 18.1 mmol, 91%) as a white foam. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.26; **mp** 62–66 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.67 (d, *J* = 7.4 Hz, 2H, ArH), 7.49 (d, *J* = 7.4 Hz, 2H, ArH), 7.33–7.13 (m, 24H, ArH), 6.94 (s, 1H, NH), 5.47 (d, *J* = 7.8 Hz, 1H, NH), 4.40–4.21 (m, 3H, CH_2-Fmoc, CHα-Gln), 4.12 (t, *J* = 6.8 Hz, 1H, CH-Fmoc), 2.38–2.16 (m, 3H, CH_2γ-Gln, C*H*Hβ-Gln), 1.90–1.79 (m, 1H, CH*H*β-Gln), 1.73 (s, 3H, CH_3, cumyl), 1.68 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 171.2 (C=O), 170.7 (C=O), 156.6 (C=O, Fmoc), 144.9 (C), 144.7 (C), 144.0 (C), 143.7 (C), 141.42 (C), 141.39 (C), 128.8 (CH), 128.5 (CH), 128.1 (CH), 127.8 (CH), 127.5 (CH), 127.19 (CH), 127.17 (CH), 125.2 (CH), 124.4 (CH), 120.1 (CH), 83.6 (C, cumyl), 70.8 (C, Trt), 67.1 (CH_2, Fmoc), 54.0 (CH, α-Gln), 47.3 (CH, Fmoc), 33.6 (CH_2, γ-Gln), 29.0 (CH_2, β-Gln), 28.9 (CH_3, cumyl), 28.2 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3331 (NH), 2953, 1716 (C=O), 1493, 1447, 1220, 1134, 824, 740, 697 cm^{-1}; **MS** (ESI^+) *m/z* 751 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{48}H_{44}N_2NaO_5$ $[M+Na]^+$ 751.3142, found 751.3129; $[\boldsymbol{\alpha}]_D^{29}$ +5.5 (*c* 0.20, $CHCl_3$).

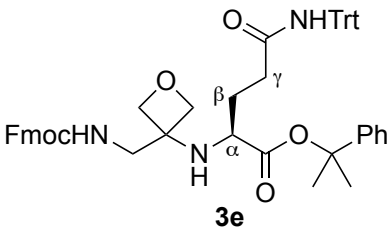
NO_2-GOx-Gln(Trt)-OCumyl (2e)



2e

Following general procedure 3, **1e** (12.8 g, 17.5 mmol, 1.0 equiv), oxetan-3-one (2.31 mL, 36.0 mmol, 2.0 equiv) and nitromethane (2.73 mL, 50.4 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 2:1→1:1) and recrystallisation from EtOAc, **2e** (5.29 g, 8.51 mmol, 49%) as an off-white solid. $\mathbf{R_f}$ (PE/EtOAc 1:1) 0.30; **mp** 162–164 °C (decomposition); **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.30–7.15 (m, 20H, ArH), 6.59 (s, 1H, NH), 4.63 (d, *J* = 12.0 Hz, 1H, NO_2C*H*H), 4.63 (d, *J* = 12.0 Hz, 1H, NO_2CH*H*), 4.48 (d, *J* = 7.1 Hz, 1H, OC*H*H-Ox), 4.43 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.35 (d, *J* = 7.1 Hz, 1H, OCH*H*-Ox), 4.25 (d, *J* = 7.0 Hz, 1H, OCH*H*-Ox), 3.43 (br. m, 1H, CHα-Gln), 2.42–2.23 (m, 2H, CH_2γ-Gln), 2.20 (d, *J* = 5.8 Hz, 1H, NH), 2.04 (td, *J* = 13.6, 7.1 Hz, 1H, C*H*Hβ-Gln), 1.73 (s, 6H, 2 × CH_3, cumyl), 1.67 (dd, *J* = 15.2, 7.6 Hz, 1H, CH*H*β-Gln); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.9 (C=O), 171.2 (C=O), 144.8 (C), 128.8 (CH), 128.5 (CH), 128.1 (CH), 127.6 (CH), 127.2 (CH), 124.5 (CH), 83.2 (C, cumyl), 78.9 (OCH_2), 78.7 (OCH_2), 78.6 (NO_2CH_2), 70.6 (C, Trt), 59.6 (C, Ox), 55.7 (CH, α-Gln), 33.1 (CH_2, γ-Gln), 29.3 (CH_2, β-Gln), 28.4 (CH_3, cumyl), 28.3 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3351 (NH), 2980, 1682 (C=O), 1551, 1489, 1269, 1138, 971, 841, 753, 696 cm^{-1}; **MS** (ESI^+) *m/z* 623 $[M+H]^+$, 645 $[M+Na]^+$, 661 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{37}H_{39}N_3NaO_6$ $[M+Na]^+$ 645.2731, found 645.2726; $[\boldsymbol{\alpha}]_D^{29}$ +12.5 (*c* 0.20, $CHCl_3$).

Fmoc-GOx-Gln(Trt)-OCumyl (3e)

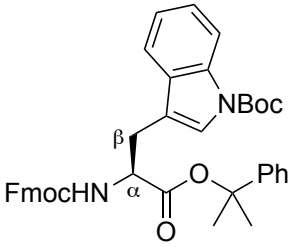


3e

Following general procedure 4, **2e** (2.82 g, 4.54 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1→4:1), **3e** (2.30 g, 2.83 mmol, 62%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 4:1) 0.33; **mp** 85–87 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.82 (d, *J* = 7.1 Hz, 2H, ArH), 7.61 (t, *J* = 6.8 Hz, 2H, ArH), 7.46–7.26 (m, 24H, ArH), 6.70 (s, 1H, NH), 5.51 (t, *J* = 5.2 Hz, 1H, NH), 4.57–4.22 (m, 6H, CH_2-Fmoc, 2 × OCH_2-Ox), 4.21 (t, *J* = 6.5 Hz, 1H, CH-Fmoc), 3.64 (dd, *J* = 13.6, 5.8 Hz, 1H, C*H*HGOx), 3.52–3.30 (m, 2H, CH*H*GOx, CHα-Gln), 2.64–2.31 (m, 2H, CH_2γ-Gln), 2.20 (dt, *J* = 12.8, 6.9 Hz, 1H, C*H*Hβ-Gln), 2.12 (br. s, 1H, NH), 1.92–1.86 (m, 1H, CH*H*β-Gln), 1.85 (s, 3H, CH_3, cumyl), 1.84 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.7 (C=O), 171.2 (C=O), 157.1 (C=O, Fmoc), 144.8 (C), 144.7 (C), 144.1 (C), 141.40 (C), 141.37 (C), 128.8 (CH), 128.5 (CH), 128.1 (CH), 127.8 (CH), 127.6 (CH), 127.21 (CH), 127.17 (CH), 127.15 (CH), 125.3 (CH), 124.5 (CH), 120.0 (CH), 83.1 (C, cumyl), 79.9 (OCH_2), 79.6 (OCH_2), 70.7 (C, Trt), 67.0 (CH_2, Fmoc), 59.8 (C, Ox), 55.5 (CH, α-Gln), 47.3 (CH, Fmoc), 45.4 (CH_2, GOx), 33.4 (CH_2, γ-Gln), 29.7 (CH_2, β-Gln), 28.5 (CH_3, cumyl), 28.3 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3302 (NH), 2943, 1716 (C=O), 1490, 1447, 1243, 1132, 969, 741 cm^{-1}; **MS** (ESI^+) *m/z* 814 $[M+H]^+$, 836 $[M+Na]^+$, 852 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{52}H_{51}N_3NaO_6$ $[M+Na]^+$ 836.3670, found 836.3661; $[\boldsymbol{\alpha}]_D^{29}$ +11.8 (*c* 0.20, $CHCl_3$).

5.6 Synthesis of Fmoc-GOx-Trp(Boc)-OCumyl

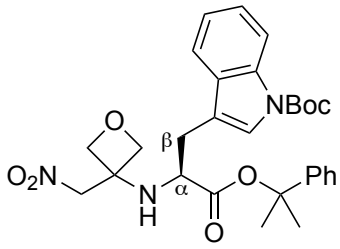
Fmoc-Trp(Boc)-OCumyl (1f)



1f

Following general procedure 1, Fmoc-Trp(Boc)-OH (5.27 g, 10.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 4:1), **1f** (6.06 g, 9.40 mmol, 94%) contaminated with traces of 2-phenyl-2-propanol as a white solid. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.61; **mp** 56–60 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.18 (d, *J* = 6.3 Hz, 1H, ArH), 7.76 (d, *J* = 7.4 Hz, 2H, ArH), 7.63 (d, *J* = 7.5 Hz, 1H, ArH), 7.57–7.27 (m, 11H, ArH), 7.26–7.21 (m, 3H, ArH), 5.42 (br. s, 1H, NH), 4.78 (d, *J* = 6.0 Hz, 1H, CHα-Trp), 4.44–4.38 (m, 2H, CH_2-Fmoc), 4.21 (t, *J* = 6.9 Hz, 1H, CH-Fmoc), 3.35–3.24 (m, 2H, CH_2β-Trp), 1.77 (s, 3H, CH_3, cumyl), 1.75 (s, 3H, CH_3, cumyl), 1.65 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.4 (C=O), 155.9 (C=O, Fmoc), 149.6 (C=O, Boc), 145.0 (C), 144.0 (C), 143.9 (C), 141.4 (C), 135.5 (C), 130.8 (C), 128.4 (CH), 127.8 (CH), 127.4 (CH), 127.2 (CH), 125.3 (CH), 125.2 (CH), 124.7 (CH), 124.5 (CH), 124.3 (CH), 122.9 (CH), 120.1 (CH), 119.2 (CH), 115.4 (CH), 115.3 (C), 83.9 (C, cumyl), 83.7 (C, *t*Bu), 67.3 (CH_2, Fmoc), 54.6 (CH, α-Trp), 47.2 (CH, Fmoc), 28.8 (CH_3, cumyl), 28.3 (CH_3, *t*Bu), 28.1 (CH_2, β-Trp), 28.0 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 2979, 1722 (C=O), 1450, 1368, 1309, 1152, 1085, 758, 739, 698 cm^{-1}; **MS** (ESI^+) *m/z* 667 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{40}H_{40}N_2NaO_6$ $[M+Na]^+$ 667.2779, found 667.2780; $[\boldsymbol{\alpha}]_D^{29}$ +10.5 (*c* 1.02, $CHCl_3$).

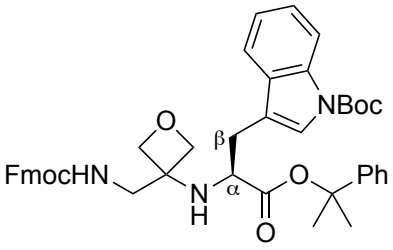
NO_2-GOx-Trp(Boc)-OCumyl (2f)



2f

Following general procedure 3, **1f** (3.22 g, 5.00 mmol, 1.0 equiv), oxetan-3-one (640 µL, 10.0 mmol, 2.0 equiv) and nitromethane (760 µL, 14.0 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1), **2f** (1.35 g, 2.51 mmol, 50%) as a yellow sticky solid. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.37; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.17 (d, J = 7.4 Hz, 1H, ArH), 7.60 (d, J = 7.6 Hz, 1H, ArH), 7.49–7.26 (m, 4H, ArH), 7.25–7.09 (m, 4H, ArH), 4.77 (d, J = 12.8 Hz, 1H, NO_2CHH), 4.70 (d, J = 12.8 Hz, 1H, NO_2CHH), 4.51 (d, J = 7.2 Hz, 1H, OC*H*H-Ox), 4.39 (d, J = 4.6 Hz, 1H, OC*H*H-Ox), 4.38 (d, J = 4.6 Hz, 1H, OCH*H*-Ox), 4.29 (d, J = 7.2 Hz, 1H, OCH*H*-Ox), 3.84 (br. m, 1H, CHα-Trp), 3.10 (dd, J = 14.3, 6.3 Hz, 1H, C*H*Hβ-Trp), 2.97 (dd, J = 14.3, 6.9 Hz, 1H, CH*H*β-Trp), 2.97 (br. s, 1H, NH), 1.75 (s, 3H, CH_3, cumyl), 1.69 (s, 3H, CH_3, cumyl), 1.65 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.5 (C=O), 149.7 (C=O, Boc), 144.8 (C), 135.6 (C), 130.4 (C), 128.4 (CH), 127.5 (CH), 124.7 (CH), 124.6 (CH), 124.4 (CH), 122.8 (CH), 119.1 (CH), 115.6 (C), 115.4 (CH), 83.7 (C, cumyl), 83.5 (C, *t*Bu), 78.9 (NO_2CH_2 or OCH_2), 78.8 (NO_2CH_2 or OCH_2), 78.7 (OCH_2), 59.6 (C, Ox), 56.6 (CH, α-Trp), 30.4 (CH_2, β-Trp), 28.7 (CH_3, cumyl), 28.3 (CH_3, *t*Bu), 27.5 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 2979, 1721 (C=O), 1451, 1368, 1220, 1152, 1085, 1055, 758, 739 cm^{-1}; **MS** (ESI^+) *m/z* 560 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{29}H_{35}N_3NaO_7$ $[M+Na]^+$ 560.2367, found 560.2372; $[\boldsymbol{\alpha}]_D^{29}$ +6.2 (*c* 1.09, $CHCl_3$).

Fmoc-GOx-Trp(Boc)-OCumyl (3f)

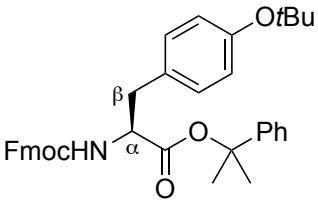


3f

Following general procedure 4, **2f** (2.28 g, 4.23 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 19:1→9:1→4:1), **3f** (1.35 g, 1.85 mmol, 44%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 4:1) 0.44; **mp** 85–90 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 8.16 (d, J = 7.0 Hz, 1H, ArH), 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.60 (d, J = 7.6 Hz, 1H, ArH), 7.54 (t, J = 6.7 Hz, 2H, ArH), 7.49 (s, 1H, ArH), 7.44–7.26 (m, 8H, ArH), 7.24 (d, J = 7.2 Hz, 3H, ArH), 5.07 (br. s, 1H, NH), 4.50–4.18 (m, 6H, 2 × OCH_2-Ox, CH_2-Fmoc), 4.16 (s, 1H, CH-Fmoc), 3.70 (t, J = 6.4 Hz, 1H, CHα-Trp), 3.63 (dd, J = 13.8, 6.3 Hz, 1H, C*H*HGOx), 3.30 (dd, J = 13.8, 4.7 Hz, 1H, CH*H*GOx), 3.16 (dd, J = 14.3, 5.3 Hz, 1H, C*H*Hβ-Trp), 2.94 (dd, J = 14.3, 8.1 Hz, 1H, CH*H*β-Trp), 1.78 (s, 3H, CH_3, cumyl), 1.73 (s, 3H, CH_3, cumyl), 1.64 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.2 (C=O), 156.9 (C=O, Fmoc), 149.7 (C=O, Boc), 144.8 (C), 144.1 (C), 141.4 (C), 135.7 (C), 130.4 (C), 128.4 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.9 (CH), 124.4 (CH), 122.9 (CH), 120.1 (CH), 119.1 (CH), 116.0 (C), 115.7 (CH), 83.9 (C, cumyl), 83.4 (C, *t*Bu), 79.8 (OCH_2), 79.7 (OCH_2), 67.0 (CH_2, Fmoc), 59.6 (C, Ox), 59.5 (CH, α-Trp), 47.3 (CH, Fmoc), 45.4 (CH_2, GOx), 30.5 (CH_2, β-Trp), 28.7 (CH_3, cumyl), 28.3 (CH_3, *t*Bu), 27.6 (CH_3, cumyl). *N.B.* Two aromatic C and one aromatic CH signal not observed; $\boldsymbol{\nu_{max}}$ (neat) = 2979, 2933, 1722 (C=O), 1450, 1368, 1254, 1152, 1055, 758, 739, 698 cm^{-1}; **MS** (ESI^+) *m/z* 730 $[M+H]^+$, 752 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{44}H_{47}N_3NaO_7$ $[M+Na]^+$ 752.3306, found 752.3300; $[\boldsymbol{\alpha}]_D^{29}$ –1.8 (*c* 1.00, $CHCl_3$).

5.7 Synthesis of Fmoc-GOx-Tyr(tBu)-OCumyl

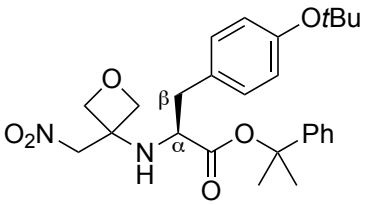
Fmoc-Tyr(*t*Bu)-OCumyl (1g)



1g

Following general procedure 1, Fmoc-Tyr(*t*Bu)-OH (4.59 g, 10.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1), **1g** (5.49 g, 9.50 mmol, 95%) as a colourless gum. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.36; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.4 Hz, 2H, ArH), 7.56 (d, *J* = 7.4 Hz, 2H, ArH), 7.40 (t, *J* = 7.4 Hz, 2H, ArH), 7.34–7.25 (m, 7H, ArH), 7.08 (d, *J* = 7.8 Hz, 2H, ArH), 6.92 (d, *J* = 7.4 Hz, 2H, ArH), 5.27 (d, *J* = 7.8 Hz, 1H, NH), 4.63 (dd, *J* = 13.1, 6.3 Hz, 1H, CHα-Tyr), 4.40 (t, *J* = 7.9 Hz, 1H, C*H*H-Fmoc), 4.32 (dd, *J* = 9.8, 7.5 Hz, 1H, C*H*H-Fmoc), 4.20 (t, *J* = 6.9 Hz, 1H, CH-Fmoc), 3.12 (dd, *J* = 14.6, 6.8 Hz, 1H, C*H*Hβ-Tyr), 3.06 (dd, *J* = 14.6, 7.2 Hz, 1H, CH*H*β-Tyr), 1.75 (s, 3H, CH_3, cumyl), 1.72 (s, 3H, CH_3, cumyl), 1.32 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.4 (C=O), 155.7 (C=O, Fmoc), 154.5 (C), 145.0 (C), 143.9 (C), 141.4 (C), 130.9 (C), 130.1 (CH), 128.5 (CH), 127.8 (CH), 127.4 (CH), 127.2 (CH), 125.3 (CH), 125.2 (CH), 124.5 (CH), 124.3 (CH), 120.1 (CH), 83.6 (C, cumyl), 78.5 (C, *t*Bu), 67.1 (CH_2, Fmoc), 55.3 (CH, α-Tyr), 47.3 (CH, Fmoc), 37.9 (CH_2, β-Tyr), 29.0 (CH_3, *t*Bu), 28.9 (CH_3, cumyl), 28.0 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3328 (NH), 2977, 1717 (C=O), 1505, 1448, 1365, 1236, 1159, 1136, 1102, 1031, 896, 826, 739 cm^{-1}; **MS** (ESI^+) *m/z* 600 $[M+Na]^+$, 616 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{37}H_{39}NNaO_5$ $[M+Na]^+$ 600.2720, found 600.2725; $[\boldsymbol{\alpha}]_D^{28}$ +16.0 (*c* 0.80, $CHCl_3$).

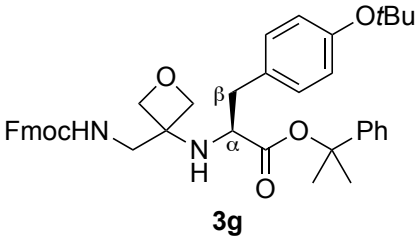
NO_2-GOx-Tyr(*t*Bu)-OCumyl (2g)



2g

Following general procedure 3, **1g** (5.78 g, 10.0 mmol, 1.0 equiv), oxetan-3-one (1.28 mL, 20.0 mmol, 2.0 equiv) and nitromethane (1.52 mL, 28.0 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **2g** (2.91 g, 6.18 mmol, 62%) as an orange oil. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.46; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.38–7.22 (m, 5H, ArH), 7.10 (d, *J* = 7.9 Hz, 2H, ArH), 6.93 (d, *J* = 7.9 Hz, 2H, ArH), 4.72 (d, *J* = 12.7 Hz, 1H, NO_2C*H*H), 4.65 (d, *J* = 12.7 Hz, 1H, NO_2CH*H*), 4.44 (d, *J* = 7.1 Hz, 1H, OC*H*H-Ox), 4.32 (d, *J* = 7.1 Hz, 1H, OCH*H*-Ox), 4.28–4.23 (m, 2H, OCH_2-Ox), 3.67 (q, *J* = 7.9 Hz, 1H, CHα-Tyr), 2.96 (dd, *J* = 13.4, 5.7 Hz, 1H, C*H*Hβ-Tyr), 2.77 (dd, *J* = 13.4, 7.9 Hz, 1H, CH*H*β-Tyr), 2.32 (d, *J* = 7.9 Hz, 1H, NH), 1.76 (s, 3H, CH_3, cumyl), 1.70 (s, 3H, CH_3, cumyl), 1.32 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.5 (C=O), 154.4 (C), 144.9 (C), 132.0 (C), 130.2 (CH), 128.5 (CH), 127.6 (CH), 124.5 (CH), 124.3 (CH), 83.3 (C, cumyl), 79.0 (NO_2CH_2), 78.8 (OCH_2), 78.7 (OCH_2), 78.6 (C, *t*Bu), 59.5 (C, Ox), 58.1 (C, α-Tyr), 40.3 (CH_2, β-Tyr), 29.0 (CH_3, *t*Bu), 28.8 (CH_3, cumyl), 27.6 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3336 (NH), 2976, 1727 (C=O), 1555, 1505, 1366, 1236, 1160, 1135, 981 cm^{-1}; **MS** (ESI^+) *m/z* 493 $[M+Na]^+$, 509 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{26}H_{34}N_2NaO_6$ $[M+Na]^+$ 493.2309, found 493.2310; $[\boldsymbol{\alpha}]_D^{28}$ +5.3 (*c* 1.47, $CHCl_3$).

Fmoc-GOx-Tyr(*t*Bu)-OCumyl (3g)

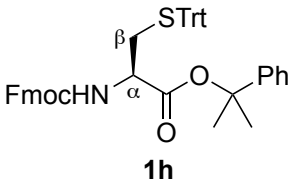


3g

Following general procedure 4, **2g** (2.76 g, 5.87 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1→4:1), **3g** (2.26 g, 3.41 mmol, 58%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.37; **mp** 60–62 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.77 (d, *J* = 7.5 Hz, 2H, ArH), 7.58 (t, *J* = 7.2 Hz, 2H, ArH), 7.40 (t, *J* = 7.4 Hz, 2H, ArH), 7.35–7.29 (m, 6H, ArH), 7.26–7.21 (m, 1H, ArH), 7.14 (d, *J* = 7.6 Hz, 2H, ArH), 6.94 (d, *J* = 7.6 Hz, 2H, ArH), 4.95 (t, *J* = 5.5 Hz, 1H, NH), 4.38 (dd, *J* = 9.8, 8.1 Hz, 1H, C*H*H-Fmoc), 4.31 (t, *J* = 8.5 Hz, 1H, CH*H*-Fmoc), 4.25–4.18 (m, 2H, CH-Fmoc, OC*H*H-Ox), 4.17–4.09 (m, 3H, OCH_2-Ox, OCH*H*-Ox), 3.58–3.40 (m, 2H, CHα-Tyr, C*H*HGOx), 3.33 (dd, *J* = 13.7, 4.6 Hz, 1H, CH*H*GOx), 3.03 (dd, *J* = 12.9, 3.6 Hz, 1H, C*H*Hβ-Tyr), 2.69 (dd, *J* = 12.9, 9.3 Hz, 1H, CH*H*β-Tyr), 2.03 (br. s, 1H, NH), 1.77 (s, 3H, CH_3, cumyl), 1.73 (s, 3H, CH_3, cumyl), 1.29 (s, 9H, 3 × CH_3, *t*Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.2 (C=O), 156.9 (C=O, Fmoc), 154.5 (C), 144.9 (C), 144.2 (C), 144.1 (C), 141.4 (C), 132.3 (C), 130.0 (CH), 128.5 (CH), 127.8 (CH), 127.5 (CH), 127.2 (CH), 125.3 (CH), 124.5 (CH), 120.1 (CH), 83.2 (C, cumyl), 79.8 (OCH_2), 79.5 (OCH_2), 78.5 (C, *t*Bu), 66.9 (CH_2, Fmoc), 59.5 (C, Ox), 58.1 (CH, α-Tyr), 47.3 (CH, Fmoc), 45.3 (CH_2, GOx), 40.3 (CH_2, β-Tyr), 28.9 (CH_3, *t*Bu), 28.7 (CH_3, cumyl), 27.8 (CH_3, cumyl). *N.B.* One aromatic C and one aromatic CH signal not observed; $\mathbf{\nu_{max}}$ (neat) = 3329 (NH), 2976, 1720 (C=O), 1505, 1448, 1365, 1235, 1159, 1133, 1100, 974, 895, 831, 740 cm^{-1}; **MS** (ESI^+) *m/z* 663 $[M+H]^+$, 685 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{41}H_{46}N_2NaO_6$ $[M+Na]^+$ 685.3248, found 685.3254; $[\boldsymbol{\alpha}]_D^{28}$ –0.1 (*c* 0.40, $CHCl_3$).

5.8 Synthesis of Fmoc-GOx-Cys(Trt)-OCumyl

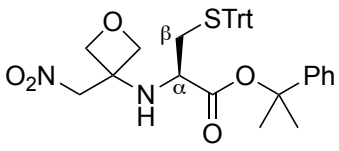
Fmoc-Cys(Trt)-OCumyl (1h)



1h

Following general procedure 1, Fmoc-Cys(Trt)-OH (4.15 g, 7.10 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1), **1h** (3.88 g, 5.51 mmol, 78%) as a white foam. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.22; **mp** 60–62 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.81 (d, *J* = 7.4 Hz, 2H, ArH), 7.64 (d, *J* = 7.4 Hz, 2H, ArH), 7.51–7.39 (m, 8H, ArH), 7.36–7.26 (m, 16H, ArH), 5.33 (d, *J* = 8.1 Hz, 1H, NH), 4.47–4.37 (m, 3H, CH_2-Fmoc, CHα-Cys), 4.27 (t, *J* = 6.8 Hz, 1H, CH-Fmoc), 2.73 (dd, *J* = 11.4, 6.6 Hz, 1H, C*H*Hβ-Cys), 2.65 (dd, *J* = 11.4, 2.8 Hz, 1H, CH*H*β-Cys), 1.81 (s, 6H, 2 × CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 169.2 (C=O), 155.7 (C=O, Fmoc), 145.0 (C), 144.5 (C), 144.0 (C), 143.9 (C), 141.4 (C), 129.6 (CH), 128.4 (CH), 128.2 (CH), 127.8 (CH), 127.4 (CH), 127.2 (CH), 127.0 (CH), 125.3 (CH), 124.5 (CH), 120.1 (CH), 83.9 (C, cumyl), 67.3 (CH_2, Fmoc), 67.0 (C, Trt), 53.5 (CH, α-Cys), 47.2 (CH, Fmoc), 34.5 (CH_2, β-Cys), 28.43 (CH_3, cumyl), 28.41 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\mathbf{\nu_{max}}$ (neat) = 3317 (NH), 2979, 1719 (C=O), 1493, 1446, 1201, 1134, 1031, 831, 734 cm^{-1}; **MS** (ESI^+) *m/z* 726 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{46}H_{41}NNaO_4S$ $[M+Na]^+$ 726.2649, found 726.2642; $[\boldsymbol{\alpha}]_D^{29}$ +20.1 (*c* 0.40, $CHCl_3$).

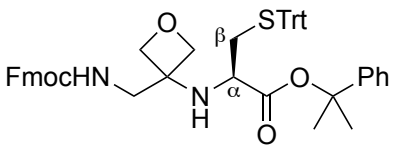
NO_2-GOx-Cys(Trt)-OCumyl (2h)



2h

Following general procedure 3, **1h** (3.85 g, 5.47 mmol, 1.0 equiv), oxetan-3-one (699 μL, 10.9 mmol, 2.0 equiv) and nitromethane (829 μL, 15.3 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **2h** (1.48 g, 2.45 mmol, 45%) as an orange gum. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.36; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.45 (d, *J* = 7.5 Hz, 6H, ArH), 7.35–7.25 (m, 14H), 4.70 (d, *J* = 13.4 Hz, 1H, NO_2CHH), 4.66 (d, *J* = 13.4 Hz, 1H, NO_2CHH), 4.55 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.46–4.36 (m, 3H, OCH*H*-Ox, OCH_2-Ox), 3.23 (br. s, 1H, CHα-Cys), 2.50 (dd, *J* = 12.7, 5.4 Hz, 1H, C*H*Hβ-Cys), 2.44 (dd, *J* = 12.7, 7.8 Hz, 1H, CH*H*β-Cys), 2.40 (br. s, 1H, NH), 1.75 (s, 3H, CH_3, cumyl), 1.73 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.1 (C=O), 144.69 (C), 144.67 (C), 129.7 (CH), 128.4 (CH), 128.2 (CH), 127.6 (CH), 126.9 (CH), 124.5 (CH), 83.6 (C, cumyl), 78.91 (NO_2CH_2 or OCH_2), 78.87 (NO_2CH_2 or OCH_2), 78.4 (OCH_2), 67.2 (C, Trt), 59.2 (C, Ox), 56.4 (CH, α-Cys), 36.3 (CH_2, β-Cys), 28.4 (CH_3, cumyl), 28.1 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3367 (NH), 2979, 1721 (C=O), 1492, 1446, 1200, 1334, 1031, 831, 740 cm^{-1}; **MS** (ESI^+) *m/z* 619 $[M+Na]^+$, 635 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{35}H_{36}N_2NaO_5S$ $[M+Na]^+$ 619.2237, found 619.2243; $[\boldsymbol{\alpha}]_D^{28}$ +20.0 (*c* 0.14, $CHCl_3$).

Fmoc-GOx-Cys(Trt)-OCumyl (3h)

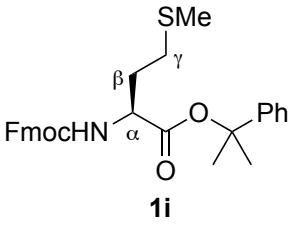


3h

Following general procedure 5, **2h** (878 mg, 1.47 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 19:1→9:1), **3h** (427 mg, 0.54 mmol, 37%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.48; **mp** 69–71 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.79 (d, *J* = 7.4 Hz, 2H, ArH), 7.65–7.55 (m, 2H, ArH), 7.48 (d, *J* = 7.4 Hz, 6H, ArH), 7.42 (t, *J* = 7.4 Hz, 2H, ArH), 7.34–7.21 (m, 16H, ArH), 5.54 (t, *J* = 5.3 Hz, 1H, NH), 4.48–4.24 (m, 5H, CH_2-Fmoc, OCH_2-Ox, OC*H*H-Ox), 4.19 (d, *J* = 6.0 Hz, 1H, OCH*H*-Ox), 4.13 (t, *J* = 7.3 Hz, 1H, CH-Fmoc), 3.57 (dd, *J* = 13.6, 5.7 Hz, 1H, C*H*HGOx), 3.47 (dd, *J* = 13.6, 2.7 Hz, 1H, CH*H*GOx), 2.97 (br. s, 1H, CHα-Cys), 2.60–2.40 (m, 2H, CH_2β-Cys), 2.04 (br. s, 1H, NH), 1.77 (s, 3H, CH_3, cumyl), 1.72 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 172.8 (C=O), 157.0 (C=O, Fmoc), 144.6 (C), 144.2 (C), 144.0 (C), 141.4 (C), 141.3 (C), 129.7 (CH), 128.4 (CH), 128.2 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 127.0 (CH), 125.4 (CH), 125.3 (CH), 124.4 (CH), 120.0 (CH), 83.4 (C, cumyl), 80.2 (OCH_2), 79.6 (OCH_2), 67.5 (C, Trt), 67.1 (CH_2, Fmoc), 59.2 (C, Ox), 56.0 (CH, α-Cys), 47.3 (CH, Fmoc), 44.7 (CH_2, GOx), 36.5 (CH_2, β-Cys), 28.6 (CH_3, cumyl), 28.0 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3338 (NH), 2978, 1722 (C=O), 1492, 1446, 1201, 1134, 1031, 831, 740 cm^{-1}; **MS** (ESI^+) *m/z* 789 $[M+H]^+$, 811 $[M+Na]^+$, 827 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{50}H_{48}N_2NaO_5S$ $[M+Na]^+$ 811.3176, found 811.3176; $[\boldsymbol{\alpha}]_D^{29}$ +41.3 (*c* 0.20, $CHCl_3$).

5.9 Synthesis of Fmoc-GOx-Met-OCumyl

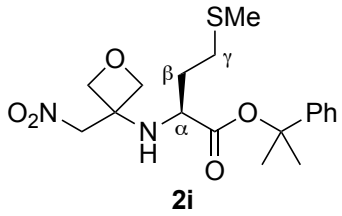
Fmoc-Met-OCumyl (1i)



1i

Following general procedure 1, Fmoc-Met-OH (3.71 g, 10.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 4:1), **1i** (4.65 g, 9.50 mmol, 95%) contaminated with traces of 2-phenyl-2-propanol (~ 3:1 by ^{1}H NMR) as a colourless oil. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.60; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.77 (d, *J* = 7.4 Hz, 2H, ArH), 7.59 (d, *J* = 7.4 Hz, 2H, ArH), 7.43–7.28 (m, 9H, ArH), 5.40 (br. s, 1H, NH), 4.50 (s, 1H, CHα-Met), 4.44–4.36 (m, 2H, CH_2-Fmoc), 4.22 (t, *J* = 6.7 Hz, 1H, CH-Fmoc), 2.66–2.39 (m, 2H, CH_2γ-Met), 2.34–2.17 (m, 1H, C*H*Hβ-Met), 2.12 (s, 3H, CH_3, Met), 2.00 (dd, *J* = 13.4, 7.4 Hz, 1H, CH*H*β-Met), 1.82 (s, 3H, CH_3, cumyl), 1.75 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.6 (C=O), 156.0 (C=O, Fmoc), 144.9 (C), 144.0 (C), 143.9 (C), 141.4 (C), 128.5 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.2 (CH), 124.4 (CH), 120.1 (CH), 83.6 (C, cumyl), 67.1 (CH_2, Fmoc), 53.7 (CH, α-Met), 47.3 (CH, Fmoc), 32.4 (CH_2, β-Met), 30.0 (CH_2, γ-Met), 28.7 (CH_3, cumyl), 28.4 (CH_3, cumyl), 15.7 (CH_3, Met). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3329 (NH), 3062, 2977, 2917, 1705 (C=O), 1447, 1218, 1135, 759, 740 cm^{-1}; **MS** (ESI$^+$) *m/z* 512 [M+Na]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{29}H_{31}NNaO_4S$ [M+Na]$^+$ 512.1866, found 512.1869; $[\boldsymbol{\alpha}]_D^{29}$ –0.9 (*c* 1.10, $CHCl_3$).

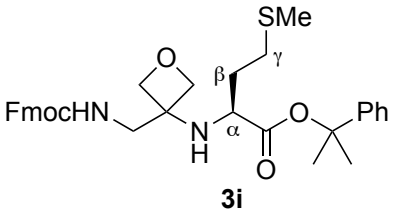
NO_2-GOx-Met-OCumyl (2i)



2i

Following general procedure 3, **1i** (4.50 g, 9.19 mmol, 1.0 equiv), oxetan-3-one (1.18 mL, 18.4 mmol, 2.0 equiv) and nitromethane (1.40 mL, 25.7 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1), **2i** (1.96 g, 5.12 mmol, 56%) contaminated with traces of 2-phenyl-2-propanol (~ 3:1 by ^{1}H NMR) as a sticky yellow gum. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.49; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.38–7.27 (m, 5H, ArH), 4.80 (d, *J* = 12.8 Hz, 1H, NO_2C*H*H), 4.70 (d, *J* = 12.8 Hz, 1H, NO_2CH*H*), 4.63 (d, *J* = 7.2 Hz, 1H, OC*H*H-Ox), 4.51 (d, *J* = 7.2 Hz, 1H, OC*H*H-Ox), 4.45 (d, *J* = 7.2 Hz, 2H, 2 × OCH_2-Ox), 3.67 (d, *J* = 4.6 Hz, 1H, CHα-Met), 2.62–2.56 (m, 2H, CH_2γ-Met), 2.30 (br. s, 1H, NH), 2.11 (s, 3H, CH_3, Met), 2.11–1.96 (m, 1H, C*H*Hβ-Met), 1.80 (s, 6H, 2 × CH_3, cumyl), 1.75–1.69 (m, 1H, CH*H*β-Met); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.3 (C=O), 144.8 (C), 128.6 (CH), 127.7 (CH), 124.5 (CH), 83.3 (C, cumyl), 79.2 (NO_2CH_2 or OCH_2), 78.7 (NO_2CH_2 or OCH_2), 78.5 (OCH_2), 59.6 (C, Ox), 54.8 (CH, α-Met), 33.3 (CH_2, β-Met), 30.4 (CH_2, γ-Met), 28.5 (CH_3, cumyl), 28.2 (CH_3, cumyl), 15.3 (CH_3, Met); $\boldsymbol{\nu_{max}}$ (neat) = 3366 (NH), 2979, 2919, 2880, 1726 (C=O), 1553, 1380, 1272, 1199, 1133, 1101, 824 cm^{-1}; **MS** (ESI$^+$) *m/z* 405 [M+Na]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{18}H_{26}N_2NaO_5S$ [M+Na]$^+$ 405.1455, found 405.1460; $[\boldsymbol{\alpha}]_D^{29}$ –18.9 (*c* 1.04, $CHCl_3$).

Fmoc-GOx-Met-OCumyl (3i)

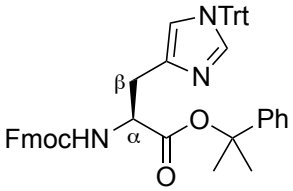


3i

Following general procedure 4, **2i** (710 mg, 1.86 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1), **3i** (280 mg, 0.49 mmol, 26%) as a sticky colourless gum. Alternatively following general procedure 5, **2i** (312 mg, 0.82 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1), **3i** (173 mg, 0.30 mmol, 37%) as a sticky colourless gum. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.54; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.76 (d, *J* = 7.4 Hz, 2H, ArH), 7.58 (d, *J* = 7.4 Hz, 2H, ArH), 7.44–7.27 (m, 9H, ArH), 5.36 (br. s, 1H, NH), 4.43 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.39 (d, *J* = 7.0 Hz, 2H, CH_2-Fmoc), 4.34 (d, *J* = 7.0 Hz, 1H, OCH*H*-Ox), 4.30 (d, *J* = 6.5 Hz, 1H, OC*H*H-Ox), 4.25 (d, *J* = 6.5 Hz, 1H, OCH*H*-Ox), 4.20 (t, *J* = 7.0 Hz, 1H, CH-Fmoc), 3.68 (dd, *J* = 13.9, 5.0 Hz, 1H, C*H*HGOx), 3.54 (dd, *J* = 9.1, 3.6 Hz, 1H, CHα-Met), 3.45 (dd, *J* = 13.9, 5.0 Hz, 1H, CH*H*GOx), 2.72–2.60 (br. m, 2H, CH_2γ-Met), 2.11 (s, 3H, CH_3, Met), 2.05–1.99 (m, 1H, C*H*Hβ-Met), 1.80 (s, 6H, 2 × CH_3, cumyl), 1.78–1.72 (m, 1H, CH*H*β-Met); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 174.9 (C=O), 157.0 (C=O, Fmoc), 144.8 (C), 144.0 (C), 141.4 (C), 128.5 (CH), 127.84 (CH), 127.80 (CH), 127.6 (CH), 127.2 (CH), 125.2 (CH), 125.1 (CH), 124.4 (CH), 120.1 (CH), 83.1 (C, cumyl), 79.9 (OCH_2), 79.6 (OCH_2), 66.9 (CH_2, Fmoc), 59.6 (C, Ox), 54.5 (CH, α-Met), 47.4 (CH, Fmoc), 45.6 (CH_2, GOx), 33.1 (CH_2, β-Met), 30.6 (CH_2, γ-Met), 28.4 (CH_3, cumyl), 28.3 (CH_3, cumyl), 15.3 (CH_3, Met). *N.B.* Two aromatic C signals not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3329 (NH), 3063, 2929, 2873, 1718 (C=O), 1511, 1448, 1240, 1132, 1100, 970, 759, 740, 698 cm^{-1}; **MS** (ESI$^+$) *m/z* 575 $[M+H]^+$, 597 $[M+Na]^+$; **HRMS** (ESI$^+$) calcd. for $C_{33}H_{38}N_2NaO_5S$ $[M+Na]^+$ 597.2394, found 597.2397; $[\boldsymbol{\alpha}]_D^{30}$ –8.9 (*c* 1.06, $CHCl_3$).

5.10 Synthesis of Fmoc-GOx-His(Trt)-OCumyl

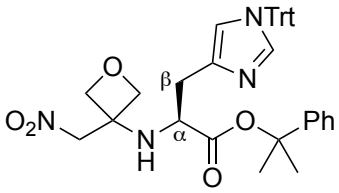
Fmoc-His(Trt)-OCumyl (1j)



1j

Following general procedure 1, Fmoc-His(Trt)-OH (6.20 g, 10.0 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, PE/EtOAc 1:1), **1j** (4.90 g, 6.64 mmol, 66%) contaminated with traces of 2-phenyl-2-propanol (~33:1 by ^{1}H NMR) as a white solid. $\mathbf{R_f}$ (PE/EtOAc 1:1) 0.50; **mp** 73–76 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.75 (d, *J* = 7.4 Hz, 2H, ArH), 7.60 (d, *J* = 7.4 Hz, 2H, ArH), 7.41–7.27 (m, 15H, ArH), 7.25–7.14 (m, 4H, ArH), 7.12–7.05 (m, 6H, ArH), 6.62 (s, 1H, ArH), 6.48 (d, *J* = 8.1 Hz, 1H, NH), 4.62 (dd, *J* = 13.0, 5.2 Hz, 1H, CHα-His), 4.38–4.24 (m, 2H, CH_2-Fmoc), 4.21 (t, *J* = 7.5 Hz, 1H, CH-Fmoc), 3.23–2.99 (m, 2H, CH_2β-His), 1.72 (s, 3H, CH_3, cumyl), 1.71 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 170.4 (C=O), 156.3 (C=O, Fmoc), 145.6 (C), 144.2 (C), 144.1 (C), 142.5 (C), 141.4 (C), 138.9 (CH), 136.5 (C), 129.9 (CH), 128.4 (CH), 128.1 (CH), 127.7 (CH), 127.20 (CH), 127.17 (CH), 125.52 (CH), 125.47 (CH), 124.4 (CH), 120.0 (CH), 119.6 (CH), 83.1 (C, cumyl), 75.4 (C, Trt), 67.3 (CH_2, Fmoc), 54.6 (CH, α-His), 47.3 (CH, Fmoc), 30.3 (CH_2, β-His), 28.7 (CH_3, cumyl), 28.4 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3293 (NH), 3060, 2979, 1718 (C=O), 1493, 1446, 1135, 741, 699 cm^{-1}; **MS** (ESI$^+$) *m/z* 738 $[M+H]^+$, 760 $[M+Na]^+$; **HRMS** (ESI$^+$) calcd. for $C_{49}H_{44}N_3O_4$ $[M+H]^+$ 738.3326, found 738.3329; $[\boldsymbol{\alpha}]_D^{29}$ –0.6 (*c* 1.02, $CHCl_3$).

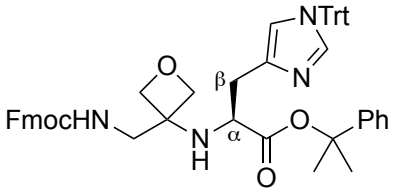
NO_2-GOx-His(Trt)-OCumyl (2j)



2j

Following general procedure 3, **1j** (3.69 g, 5.00 mmol, 1.0 equiv), oxetan-3-one (640 µL, 10.0 mmol, 2.0 equiv) and nitromethane (760 µL, 14.0 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 1:1→1:3), **2j** (2.14 g, 3.39 mmol, 68%) as an orange foam; $\mathbf{R_f}$ (EtOAc) 0.77; **mp** 65–69 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.39 (s, 1H, ArH), 7.35–7.27 (m, 14H, ArH), 7.22 (t, *J* = 7.1 Hz, 1H, ArH), 7.16–7.10 (m, 5H, ArH), 6.61 (s, 1H, ArH), 4.74 (d, *J* = 13.2 Hz, 1H, NO_2CHH), 4.64 (d, *J* = 13.2 Hz, 1H, NO_2CHH), 4.46 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.40 (d, *J* = 7.2 Hz, 1H, OC*H*H-Ox), 4.37 (d, *J* = 7.2 Hz, 1H, OCH*H*-Ox), 4.31 (d, *J* = 7.0 Hz, 1H, OCH*H*-Ox), 3.83 (dd, *J* = 8.6, 4.0 Hz, 1H, CHα-His), 3.05 (dd, *J* = 14.3, 4.0 Hz, 1H, C*H*Hβ-His), 2.97 (dd, *J* = 14.3, 8.6 Hz, 1H, CH*H*β-His), 2.52 (br. s, 1H, NH), 1.76 (s, 3H, CH_3, cumyl), 1.74 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.5 (C=O), 145.2 (C), 142.5 (C), 138.7 (CH), 136.8 (C), 129.9 (CH), 128.4 (CH), 128.2 (CH), 128.1 (CH), 127.4 (CH), 124.5 (CH), 120.0 (CH), 82.9 (C, cumyl), 79.0 (NO_2CH_2 or OCH_2), 78.8 (NO_2CH_2 or OCH_2), 78.5 (OCH_2), 75.4 (C, Trt), 59.3 (C, Ox), 56.5 (CH, α-His), 33.1 (CH_2, β-His), 28.6 (CH_3, cumyl), 28.1 (CH_3, cumyl); $\boldsymbol{\nu_{max}}$ (neat) = 3326 (NH), 3060, 2927, 2878, 1729 (C=O), 1552, 1131, 979, 747, 698 cm^{-1}; **MS** (ESI^+) *m/z* 631 $[M+H]^+$, 653 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{38}H_{39}N_4O_5$ $[M+H]^+$ 631.2915, found 631.2916; $[\boldsymbol{\alpha}]_D^{30}$ –11.4 (*c* 1.02, $CHCl_3$).

Fmoc-GOx-His(Trt)-OCumyl (3j)

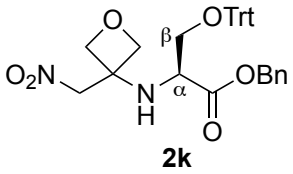


3j

Following general procedure 4, **2j** (1.26 g, 2.00 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 1:1), **3j** (1.01 g, 1.23 mmol, 61%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 1:1) 0.52; **mp** 76–80 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.75 (d, *J* = 7.4 Hz, 2H, ArH), 7.53 (d, *J* = 7.4 Hz, 2H, ArH), 7.49 (s, 1H, ArH), 7.34–7.20 (m, 19H, ArH), 7.16–7.00 (m, 5H, ArH), 6.63 (s, 1H, ArH), 6.60 (s, 1H, NH), 4.34 (d, *J* = 6.2 Hz, 1H, OC*H*H-Ox), 4.30 (d, *J* = 6.2 Hz, 1H, OCH*H*-Ox), 4.21 (d, *J* = 6.6 Hz, 4H, OCH_2-Ox, CH_2-Fmoc), 3.97 (t, *J* = 7.2 Hz, 1H, CH-Fmoc), 3.70 (t, *J* = 8.6 Hz, 1H, CHα-His), 3.66–3.58 (m, 2H, CH_2GOx), 3.07 (d, *J* = 14.2 Hz, 1H, C*H*Hβ-His), 2.71 (dd, *J* = 14.2, 10.2 Hz, 1H, CH*H*β-His), 1.78 (s, 3H, CH_3, cumyl), 1.73 (s, 3H, CH_3, cumyl); **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C 173.9 (C=O), 157.2 (C=O, Fmoc), 145.1 (C), 144.20 (C), 144.17 (C), 142.4 (C), 141.3 (C), 139.0 (CH), 137.1 (C), 129.8 (CH), 128.5 (CH), 128.19 (CH), 128.16 (CH), 127.7 (CH), 127.5 (CH), 127.2 (CH), 125.4 (CH), 125.3 (CH), 124.4 (CH), 120.1 (CH), 120.0 (CH), 82.8 (C, cumyl), 80.7 (OCH_2), 80.3 (OCH_2), 66.9 (CH_2, Fmoc), 59.6 (C, Ox), 56.0 (CH, α-His), 47.3 (CH, Fmoc), 44.6 (CH_2, GOx), 32.2 (CH_2, β-His), 28.7 (CH_3, cumyl), 28.2 (CH_3, cumyl). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3324 (NH), 3060, 2928, 2869, 1716 (C=O), 1446, 1237, 1131, 974, 741 cm^{-1}; **MS** (ESI^+) *m/z* 823 $[M+H]^+$, 845 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{53}H_{51}N_4O_5$ $[M+H]^+$ 823.3854, found 823.3850; $[\boldsymbol{\alpha}]_D^{30}$ +3.9 (*c* 1.03, $CHCl_3$).

5.11 Synthesis of Fmoc-GOx-Ser(Trt)-OBn

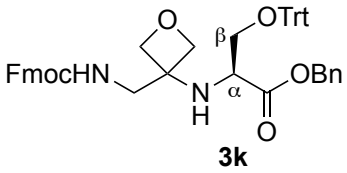
NO_2-GOx-Ser(Trt)-OBn (2k)



2k

Following general procedure 2, Fmoc-Ser(Trt)-OH (5.70 g, 10.0 mmol, 1.0 equiv), benzyl bromide (1.19 mL, 10.0 mmol, 1.0 equiv) and Cs_2CO_3 (3.58 g, 11.0 mmol, 1.1 equiv) in DMF (40 mL) gave Fmoc-Ser(Trt)-OBn, which was used without further purification. Following general procedure 3, Fmoc-Ser(Trt)-OBn, oxetan-3-one (1.28 mL, 20.0 mmol, 2.0 equiv) and nitromethane (1.52 mL, 28.0 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 4:1→2:1), **2k** (1.72 g, 3.12 mmol, 31% over two steps) as a pale yellow foam. $\mathbf{R_f}$ (PE/EtOAc 2:1) 0.32; **mp** 42–43 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.40–7.36 (m, 6H, ArH), 7.35–7.26 (m, 14H, ArH), 5.18 (s, 2H, OCH_2Ph), 4.80 (s, 2H, NO_2CH_2), 4.58 (d, *J* = 6.9 Hz, 1H, OC*H*H-Ox), 4.55–4.43 (m, 3H, OCH_2-Ox, OCH*H*-Ox), 3.69 (dd, *J* = 8.5, 4.5 Hz, 1H, CHα-Ser), 3.44–3.33 (m, 2H, CH_2β-Ser), 2.76 (d, *J* = 9.7 Hz, 1H, NH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 173.6 (C=O), 143.6 (C), 135.2 (C), 128.74 (CH), 128.70 (CH), 128.68 (CH), 128.0 (CH), 127.3 (CH), 86.9 (C, Trt), 79.0 (NO_2CH_2 or OCH_2), 78.8 (NO_2CH_2 or OCH_2), 78.7 (NO_2CH_2 or OCH_2), 67.6 (CH_2, Bn), 66.0 (CH_2, β-Ser), 59.6 (C, Ox), 56.3 (CH, α-Ser). *N.B.* One aromatic CH not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3342 (NH), 2954, 2878, 1733 (C=O), 1553, 1491, 1448, 1377, 1183, 1080, 979, 746, 696 cm^{-1}; **MS** (ESI^+) *m/z* 475 $[M+Na]^+$, 491 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{33}H_{32}N_2NaO_6$ $[M+Na]^+$ 475.2153, found 475.2147; $[\boldsymbol{\alpha}]_D^{28}$ +1.2 (*c* 0.22, $CHCl_3$).

Fmoc-GOx-Ser(Trt)-OBn (3k)

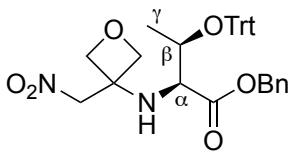


3k

Following general procedure 4, **2k** (1.64 g, 2.97 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1→4:1), **3k** (1.48 g, 1.99 mmol, 67%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2/EtOAc 9:1) 0.28; **mp** 73–74 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.75 (d, *J* = 7.5 Hz, 2H, ArH), 7.56 (d, *J* = 6.9 Hz, 2H, ArH), 7.39 (d, *J* = 7.3 Hz, 8H, ArH), 7.32–7.19 (m, 16H, ArH), 5.24 (br. s, 1H, NH), 5.17 (s, 2H, OCH_2Ph), 4.45–4.33 (m, 3H, CH_2-Fmoc, OC*H*H-Ox), 4.31 (d, *J* = 6.4 Hz, 1H, OC*H*H-Ox), 4.27 (d, *J* = 6.4 Hz, 1H, OCH*H*-Ox), 4.24 (d, *J* = 6.4 Hz, 1H, OCH*H*-Ox), 4.19 (t, *J* = 6.7 Hz, 1H, CH-Fmoc), 3.63 (dd, *J* = 13.7, 5.7 Hz, 1H, C*H*HGOx), 3.53–3.50 (m, 1H, CHα-Ser), 3.43 (dd, *J* = 13.7, 4.5 Hz, 1H, CH*H*GOx), 3.40 (dd, *J* = 8.4, 4.8 Hz, 1H, C*H*Hβ-Ser), 3.35 (dd, *J* = 8.4, 4.4 Hz, 1H, CH*H*β-Ser), 2.39 (br. s, 1H, NH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 174.1 (C=O), 157.0 (C=O, Fmoc), 144.1 (C), 144.0 (C), 143.6 (C), 141.4 (C), 135.2 (C), 128.8 (CH), 128.74 (CH), 128.70 (CH), 128.6 (CH), 128.1 (CH), 127.8 (CH), 127.4 (CH), 127.2 (CH), 125.2 (CH), 120.1 (CH), 87.1 (C, Trt), 79.8 (OCH_2), 79.7 (OCH_2), 67.6 (CH_2, Bn), 67.0 (CH_2, Fmoc), 66.0 (CH_2, β-Ser), 59.5 (C, Ox), 56.2 (CH, α-Ser), 47.4 (CH, Fmoc), 45.4 (CH_2, GOx). *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3330 (NH), 2947, 2875, 1719 (C=O), 1515, 1491, 1448, 1221, 1152, 1080, 741, 697 cm^{-1}; **MS** (ESI^+) *m/z* 767 $[M+Na]^+$, 783 $[M+K]^+$; **HRMS** (ESI^+) calcd. for $C_{48}H_{44}N_2NaO_6$ $[M+Na]^+$ 767.3092, found 767.3098; $[\boldsymbol{\alpha}]_D^{29}$ +3.4 (*c* 0.50, $CHCl_3$).

5.12 Synthesis of Fmoc-GOx-Thr(Trt)-OBn

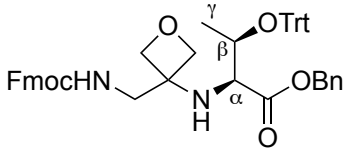
NO_2-GOx-Thr(Trt)-OBn (2l)



2l

Following general procedure 2, Fmoc-Thr(Trt)-OH (5.84 g, 10.0 mmol, 1.0 equiv), benzyl bromide (1.19 mL, 10.0 mmol, 1.0 equiv) and Cs_2CO_3 (3.58 g, 11.0 mmol, 1.1 equiv) in DMF (40 mL) gave Fmoc-Thr(Trt)-OBn, which was used without further purification. Following general procedure 3, Fmoc-Thr(Trt)-OBn, oxetan-3-one (1.28 mL, 20.0 mmol, 2.0 equiv) and nitromethane (1.52 mL, 28.0 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 9:1→4:1→2:1), **2l** (1.58 g, 2.79 mmol, 28% over two steps) as a pale yellow foam. $\mathbf{R_f}$ (PE/EtOAc 4:1) 0.09; **mp** 46–47 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H ppm 7.45 (d, J = 6.8 Hz, 6H, ArH), 7.37–7.32 (m, 3H, ArH), 7.31–7.25 (m, 11H, ArH), 5.19 (d, J = 12.0 Hz, 1H, OC*H*HPh), 4.99 (d, J = 12.0 Hz, 1H, OCH*H*Ph), 4.64 (s, 2H, NO_2CH_2), 4.42 (d, J = 6.8 Hz, 2H, 2 × OC*H*H-Ox), 4.38 (d, J = 6.8 Hz, 1H, OCH*H*-Ox), 4.34 (d, J = 6.8 Hz, 1H, OCH*H*-Ox), 3.87–3.80 (m, 1H, CHβ-Thr), 3.12 (dd, J = 10.0, 2.5 Hz, 1H, CHα-Thr), 2.67 (d, J = 10.0 Hz, 1H, NH), 0.98 (d, J = 5.9 Hz, 3H, CH_3γ-Thr); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 174.0 (C=O), 144.8 (C), 135.2 (C), 129.1 (CH), 128.9 (CH), 128.7 (CH), 128.6 (CH), 127.8 (CH), 127.2 (CH), 87.0 (C, Trt), 78.9 (NO_2CH_2), 78.7 (OCH_2), 78.4 (OCH_2), 72.2 (CH, β-Thr), 67.6 (CH_2, Bn), 60.0 (CH, α-Thr), 59.4 (C, Ox), 17.3 (CH_3, γ-Thr); $\boldsymbol{\nu_{max}}$ (neat) = 3333 (NH), 2947, 2873, 1728 (C=O), 1553, 1490, 1447, 1377, 1174, 1082, 980, 746, 696 cm^{-1}; **MS** (ESI$^+$) *m/z* 589 [M+Na]$^+$, 605 [M+K]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{34}H_{34}N_2NaO_6$ [M+Na]$^+$ 589.2309, found 589.2306; $[\boldsymbol{\alpha}]_D^{27}$ +11.7 (*c* 0.29, $CHCl_3$).

Fmoc-GOx-Thr(Trt)-OBn (3l)

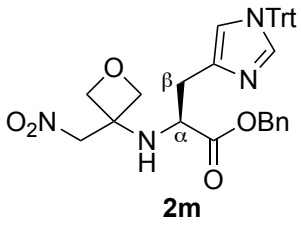


3l

Following general procedure 4, **2l** (1.45 g, 2.56 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1→4:1), **3l** (1.16 g, 1.53 mmol, 60%) as a white foam. $\mathbf{R_f}$ (CH_2Cl_2:EtOAc 9:1) 0.37; **mp** 79–81 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.80 (d, J = 7.6 Hz, 2H, ArH), 7.63 (d, J = 7.3 Hz, 2H, ArH), 7.47 (d, J = 6.7 Hz, 6H, ArH), 7.37–7.24 (m, 18H, ArH), 5.20 (d, J = 12.2 Hz, 1H, OC*H*HPh), 5.17 (br. s, 1H, NH), 4.98 (d, J = 12.2 Hz, 1H, OCH*H*Ph), 4.45 (d, J = 6.6 Hz, 2H, CH_2-Fmoc), 4.34–4.15 (m, 5H, 2 × OCH_2-Ox, CH-Fmoc), 3.87–3.80 (m, 1H, CHβ-Thr), 3.49 (dd, J = 13.4, 5.2 Hz, 1H, C*H*H-GOx), 3.34 (dd, J = 13.4, 3.9 Hz, 1H, CH*H*-GOx), 2.99 (br. s, 1H, CHα-Thr), 2.37 (br. s, 1H, NH), 1.05 (d, J = 5.5 Hz, 3H, CH_3γ-Thr); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C 174.7 (C=O), 156.9 (C=O, Fmoc), 144.7 (C), 144.1 (C), 144.0 (C), 141.5 (C), 135.2 (C), 129.1 (CH), 128.8 (CH), 128.72 (CH), 128.66 (CH), 127.8 (CH), 127.3 (CH), 127.2 (CH), 125.2 (CH), 120.1 (CH), 87.1 (C, Trt), 79.6 (OCH_2), 79.4 (OCH_2), 72.2 (CH, β-Thr), 67.5 (CH_2, Bn), 67.0 (CH_2, Fmoc), 59.9 (CH, α-Thr), 59.4 (C, Ox), 47.4 (CH, Fmoc), 45.5 (CH_2, GOx), 17.5 (CH_3, γ-Thr). *N.B.* One aromatic C and one aromatic CH signal not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3345 (NH), 2942, 2873, 1720 (C=O), 1515, 1490, 1218, 1184, 1081, 972, 741, 697 cm^{-1}; **MS** (ESI$^+$) *m/z* 781 [M+Na]$^+$; **HRMS** (ESI$^+$) calcd. for $C_{49}H_{46}N_2NaO_6$ [M+Na]$^+$ 781.3248, found 781.3251; $[\boldsymbol{\alpha}]_D^{27}$ +29.5 (*c* 0.52, $CHCl_3$).

5.13 Synthesis of Fmoc-GOx-His(Trt)-OBn

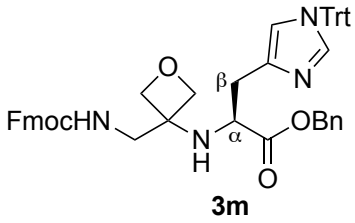
NO_2-GOx-His(Trt)-OBn (2m)



2m

Following general procedure 2, Fmoc-His(Trt)-OH (6.20 g, 10.0 mmol, 1.0 equiv), benzyl bromide (1.19 mL, 10.0 mmol, 1.0 equiv) and Cs_2CO_3 (3.58 g, 11.0 mmol, 1.1 equiv) in DMF (40 mL) gave Fmoc-His(Trt)-OBn, which was used without further purification. Following general procedure 3, Fmoc-His(Trt)-OBn, oxetan-3-one (1.28 mL, 20.0 mmol, 2.0 equiv) and nitromethane (1.52 mL, 28.0 mmol, 2.8 equiv) were combined to give, after column chromatography (SiO_2, PE/EtOAc 3:1→2:1→1:1→EtOAc), **2m** (3.84 g, 6.37 mmol, 64% over two steps) as an orange foam. $\mathbf{R_f}$ (EtOAc) 0.41; **mp** 62–64 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H ppm 7.38 (s, 1H, ArH), 7.36–7.28 (m, 14H, ArH), 7.15–7.08 (m, 6H, ArH), 6.57 (s, 1H, ArH), 5.11 (d, *J* = 12.1 Hz, 1H, OC*H*HPh), 5.03 (d, *J* = 12.1 Hz, 1H, OCH*H*Ph), 4.78 (d, *J* = 13.2 Hz, 1H, NO_2C*H*H), 4.71 (d, *J* = 13.2 Hz, 1H, NO_2CH*H*), 4.48 (d, *J* = 6.9 Hz, 1H, OC*H*H-Ox), 4.43 (d, *J* = 7.0 Hz, 1H, OC*H*H-Ox), 4.38 (d, *J* = 7.0 Hz, 1H, OCH*H*-Ox), 4.32 (d, *J* = 6.9 Hz, 1H, OCH*H*-Ox), 3.83 (br. s, 1H, CHα-His), 2.95 (dd, *J* = 14.2, 4.1 Hz, 1H, C*H*Hβ-His), 2.80 (dd, *J* = 14.1, 8.1 Hz, 1H, CH*H*β-His), 2.64 (br. s, 1H, NH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C ppm 174.6 (C=O), 142.4 (C), 136.4 (C), 135.4 (C), 129.9 (CH), 128.7 (CH), 128.6 (CH), 128.21 (CH), 128.20 (CH), 120.1 (CH), 79.2 (OCH_2), 78.6 (OCH_2), 78.3 (NO_2CH_2), 75.4 (C, Trt), 67.2 (CH_2, Bn), 59.4 (C, Ox), 56.3 (CH, α-His), 32.8 (CH_2, β-His). *N.B.* Two aromatic CH signals not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3332 (NH), 2946, 2872, 1724 (C=O), 1550, 1445, 1155, 981, 744, 697 cm^{-1}; **MS** (ESI^+) *m/z* 603 $[M+H]^+$, 625 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{36}H_{35}N_4O_5$ $[M+H]^+$ 603.2602, found 603.2601; $[\boldsymbol{\alpha}]_D^{28}$ –5.1 (*c* 0.22, $CHCl_3$).

Fmoc-GOx-His(Trt)-OBn (3m)

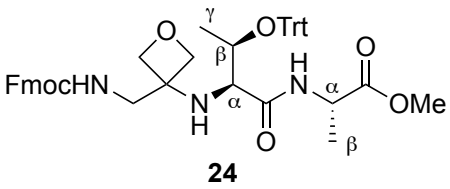


3m

Following general procedure 4, **2m** (3.71 g, 6.16 mmol, 1.0 equiv) gave, after column chromatography (SiO_2, CH_2Cl_2/EtOAc 1:1→EtOAc), **3m** (3.40 g, 4.28 mmol, 69%) as an off-white foam. $\mathbf{R_f}$ (EtOAc) 0.39; **mp** 73–74 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H ppm 7.75 (d, *J* = 7.4 Hz, 2H, ArH), 7.55 (d, *J* = 7.2 Hz, 2H, ArH), 7.47 (s, 1H, ArH), 7.41–7.27 (m, 9H, ArH), 7.26–7.20 (m, 9H, ArH), 7.12–7.04 (m, 6H, ArH), 6.78 (s, 1H, NH), 6.57 (s, 1H, ArH), 5.14 (d, *J* = 12.0 Hz, 1H, OC*H*HPh), 5.08 (d, *J* = 12.0 Hz, 1H, OCH*H*Ph), 4.46–4.15 (m, 6H, 2 × OCH_2-Ox, CH_2-Fmoc), 3.98 (t, *J* = 6.9 Hz, 1H, CH-Fmoc), 3.79 (d, *J* = 8.7 Hz, 1H, CHα-His), 3.68 (dd, *J* = 13.8, 6.1 Hz, 1H, C*H*HGOx), 3.61 (dd, *J* = 13.8, 3.0 Hz, 1H, CH*H*GOx), 2.99 (dd, *J* = 14.6, 2.2 Hz, 1H, C*H*Hβ-His), 2.80 (dd, *J* = 14.6, 9.2 Hz, 1H, CH*H*β-His), 1.85 (br. s, 1H, NH); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C ppm 175.0 (C=O), 157.3 (C=O, Fmoc), 144.21 (C), 144.16 (C), 142.3 (C), 141.4 (C), 139.0 (CH), 137.0 (C), 135.4 (C), 129.9 (CH), 129.81 (CH), 128.80 (CH), 128.7 (CH), 128.6 (CH), 128.19 (CH), 128.15 (CH), 127.7 (CH), 127.17 (CH), 127.15 (CH), 125.4 (CH), 125.3 (CH), 120.03 (CH), 119.99 (CH), 80.8 (OCH_2), 80.2 (OCH_2), 75.5 (C, Trt), 67.2 (CH_2, Bn), 66.9 (CH_2, Fmoc), 59.6 (C, Ox), 55.5 (CH, α-His), 47.3 (CH, Fmoc), 44.4 (CH_2, GOx), 32.0 (CH_2, β-His); *N.B.* One aromatic C not observed; $\boldsymbol{\nu_{max}}$ (neat) = 3331 (NH), 2947, 2870, 1716 (C=O), 1493, 1446, 1238, 1155, 974, 742, 699 cm^{-1}; **MS** (ESI^+) *m/z* 795 $[M+H]^+$, 817 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{51}H_{46}N_4NaO_5$ $[M+Na]^+$ 817.3360, found 817.3358; $[\boldsymbol{\alpha}]_D^{28}$ +7.1 (*c* 0.54, $CHCl_3$).

6. Peptide couplings of selected oxetane modified glycine building blocks

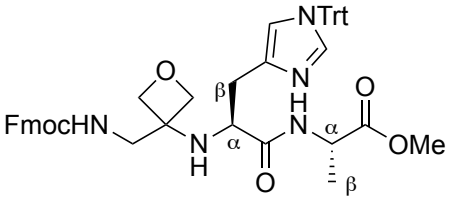
Fmoc-GOx-Thr(Trt)-Ala-OMe (24)



24

To a solution of Fmoc-GOx-Thr(Trt)-OBn (**3l**) (379 mg, 0.50 mmol, 1.0 equiv) in DMF (5.0 mL) was added 10 wt% Pd/C (38 mg, 10 wt%) and the reaction flask was evacuated, filled with nitrogen, evacuated, and placed under an atmosphere of hydrogen (balloon). The reaction mixture was stirred at room temperature for 2 h, placed under nitrogen and filtered through a plug of Celite, which was washed with DMF (15 mL). H-Ala-OMe·HCl (140 mg, 1.00 mmol, 2.0 equiv), HATU (190 mg, 0.50 mmol, 1.0 equiv) and DIPEA (523 µL, 3.00 mmol, 5.0 equiv) were added subsequently, and the reaction mixture was stirred at room temperature for 20 h. The mixture was concentrated under reduced pressure, CH_2Cl_2 (20 mL) was added and washed with 10% citric acid (2 × 20 mL) and saturated $NaHCO_3$ solution (2 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, PE:EtOAc 1:1→EtOAc) to give tripeptide **24** (244 mg, 0.32 mmol, 65%) as a white foam. **R_f** (PE:EtOAc 1:1) 0.16; **mp** 81–83 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H ppm 7.76 (d, *J* = 7.5 Hz, 2H, ArH), 7.59 (dd, *J* = 7.1, 4.3 Hz, 2H, ArH), 7.53 (d, *J* = 7.7 Hz, 6H, ArH), 7.39 (t, *J* = 7.4 Hz, 2H, ArH), 7.35–7.26 (m, 12H, ArH, NH), 5.72 (t, *J* = 4.7 Hz, 1H, NH), 4.52 (quint, *J* = 7.2 Hz, 1H, CHβ-Thr), 4.38–4.30 (m, 2H, CH_2-Fmoc), 4.20 (t, *J* = 7.0 Hz, 2H, CH-Fmoc, OC*H*H-Ox), 4.11 (d, *J* = 5.9 Hz, 1H, OCH*H*-Ox), 4.07 (d, *J* = 6.4 Hz, 1H, OC*H*H-Ox), 4.03 (d, *J* = 6.4 Hz, 1H, OC*H*H-Ox), 3.85 (quint, *J* = 4.9 Hz, 1H, CHα-Ala), 3.79 (s, 3H, OCH_3), 3.47 (dd, *J* = 13.6, 7.0 Hz, 1H, C*H*H-GOx), 3.03 (dd, *J* = 13.6, 3.4 Hz, 1H, CH*H*-GOx), 2.53 (d, *J* = 3.2 Hz, 1H, CHα-Thr), 2.39 (br. s, 1H, NH), 1.36 (d, *J* = 7.2 Hz, 3H, CH_3γ-Thr), 1.19 (d, *J* = 6.0 Hz, 3H, CH_3β-Ala); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C ppm 173.5 (C=O), 173.1 (C=O), 157.1 (C=O, Fmoc), 144.3 (C), 144.1 (C), 141.4 (C), 128.8 (CH), 128.3 (CH), 127.8 (CH), 127.7 (CH), 127.2 (CH), 125.4 (CH), 125.3 (CH), 120.1 (CH), 88.4 (C, Trt), 79.6 (OCH_2), 79.2 (OCH_2), 73.3 (CH, α-Ala), 67.0 (CH_2, Fmoc), 59.6 (C, Ox), 58.6 (CH, α-Thr), 52.6 (OCH_3), 48.6 (CH, β-Thr), 47.3 (CH, Fmoc), 45.5 (CH_2, GOx), 18.0 (CH_3, γ-Thr), 16.4 (CH_3, β-Ala). *N.B*. Two aromatic C signals not observed. **ν_{max}** (neat) = 3332 (NH), 2948, 2874, 1718 (C=O), 1669 (C=O), 1514, 1448, 1219, 1152, 1012, 741, 706 cm^{-1}; **MS** (ESI^+) *m/z* 776 $[M+Na]^+$; **HRMS** (ESI^+) calcd. for $C_{46}H_{47}N_3NaO_7$ $[M+Na]^+$ 776.3306, found 776.3297; **$[\alpha]_D^{28}$** +13.2 (*c* 0.50, $CHCl_3$).

Fmoc-GOx-His(Trt)-Ala-OMe (25)

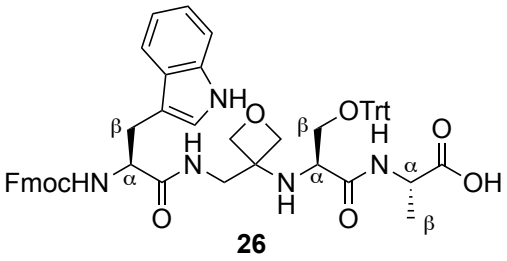


25

To a solution of Fmoc-GOx-His(Trt)-OBn (**3m**) (397 mg, 0.50 mmol, 1.0 equiv) in DMF (5.0 mL) was added 10 wt% Pd/C (40 mg, 10 wt%) and the reaction flask was evacuated, filled with nitrogen, evacuated, and placed under an atmosphere of hydrogen (balloon). The reaction mixture was stirred at room temperature for 2 h, placed under nitrogen and filtered through a plug of Celite, which was washed with DMF (15 mL). H-Ala-OMe·HCl (140 mg, 1.00 mmol, 2.0 equiv), HATU (190 mg, 0.50 mmol,

1.0 equiv) and DIPEA (523 µL, 3.00 mmol, 5.0 equiv) were added subsequently, and the reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure, CH_2Cl_2 (20 mL) was added and washed with 10% citric acid (2 × 20 mL) and saturated $NaHCO_3$ solution (2 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 97.5:2.5→95:5) to give tripeptide **25** (221 mg, 0.28 mmol, 56%) as a pale yellow oil. $\mathbf{R_f}$ (CH_2Cl_2/MeOH 95:5) 0.16; **MS** (ESI$^+$) *m/z* 790 $[M+H]^+$, 812 $[M+Na]^+$; **HRMS** (ESI$^+$) calcd. for $C_{48}H_{48}N_5O_6$ $[M+Na]^+$ 790.3599, found 790.3605.

Fmoc-Trp-GOx-Ser(Trt)-Ala-OH (26)



26

To a solution of Fmoc-GOx-Ser(Trt)-OBn (**3k**) (372 mg, 0.50 mmol, 5.0 equiv) in DMF (2.0 mL) was added 10 wt% Pd/C (37 mg, 10 wt%) and the reaction flask was evacuated, filled with nitrogen, evacuated, and placed under an atmosphere of hydrogen (balloon). The reaction mixture was stirred at room temperature for 1.5 h, placed under nitrogen and filtered through a plug of Celite, which was washed with DMF (8.0 mL). The filtrate of Fmoc-GOx-Ser(Trt)-OH (**4k**) was used for SPPS without further purification. Fmoc-Ala-2-chlorotrityl resin (192 mg, 0.10 mmol, 1.0 equiv) was placed in a 10 mL reaction vessel and the resin was pre-swollen in DMF (2.0 mL) for 30 min. The Fmoc group was deprotected with 20% piperidine in DMF (2.0 mL) for 20 min at room temperature and the resin was washed with DMF (5 × 2.0 mL). HATU (190 mg, 0.50 mmol, 5.0 equiv) and DIPEA (174 µL, 1.00 mmol, 10 equiv) were added to the filtrate of **4k** and the coupling solution was added to the resin. The coupling reaction was allowed to proceed for 2 h at room temperature under slight agitation. The resin was filtered, washed with DMF (5 × 2.0 mL) before the Fmoc group was removed with 20% piperidine in DMF (2.0 mL) for 20 min at room temperature. After washing the resin with DMF (5 × 2.0 mL), Fmoc-Trp-OH (213 mg, 0.50 mmol, 5.0 equiv) was coupled with HATU (190 mg, 0.50 mmol, 5.0 equiv), DIPEA (174 µL, 1.00 mmol, 10 equiv) in DMF (2.0 mL) for 1 h at room temperature. The resin was washed with DMF (5 × 2.0 mL) before the Fmoc-group was removed as described before. The tetrapeptide was then cleaved from the resin with TFE in CH_2Cl_2 (1:4, 2.5 mL) for 1 h at room temperature. This was repeated twice and the combined cleavage solutions were evaporated to dryness under reduced pressure. Tetrapeptide **26** was obtained as an off-white solid (84 mg, 92 µmol) in 92% crude yield over all steps. **MS** (ESI$^-$) *m/z* 910 $[M-H]^-$; **HRMS** (ESI$^-$) calcd. for $C_{55}H_{52}N_5O_8$ $[M-H]^-$ 910.3821, found 910.3820.

7. References

[1] S. Roesner, G. J. Saunders, I. Wilkening, E. Jayawant, J. V. Geden, P. Kerby, A. M. Dixon, R. Notman and M. Shipman, *Chem. Sci.*, 2019, **10**, 2465–2472.

[2] J. P. Phelan, E. J. Patel and J. A. Ellman, *Angew. Chem. Int. Ed.*, 2014, **53**, 11329–11332.

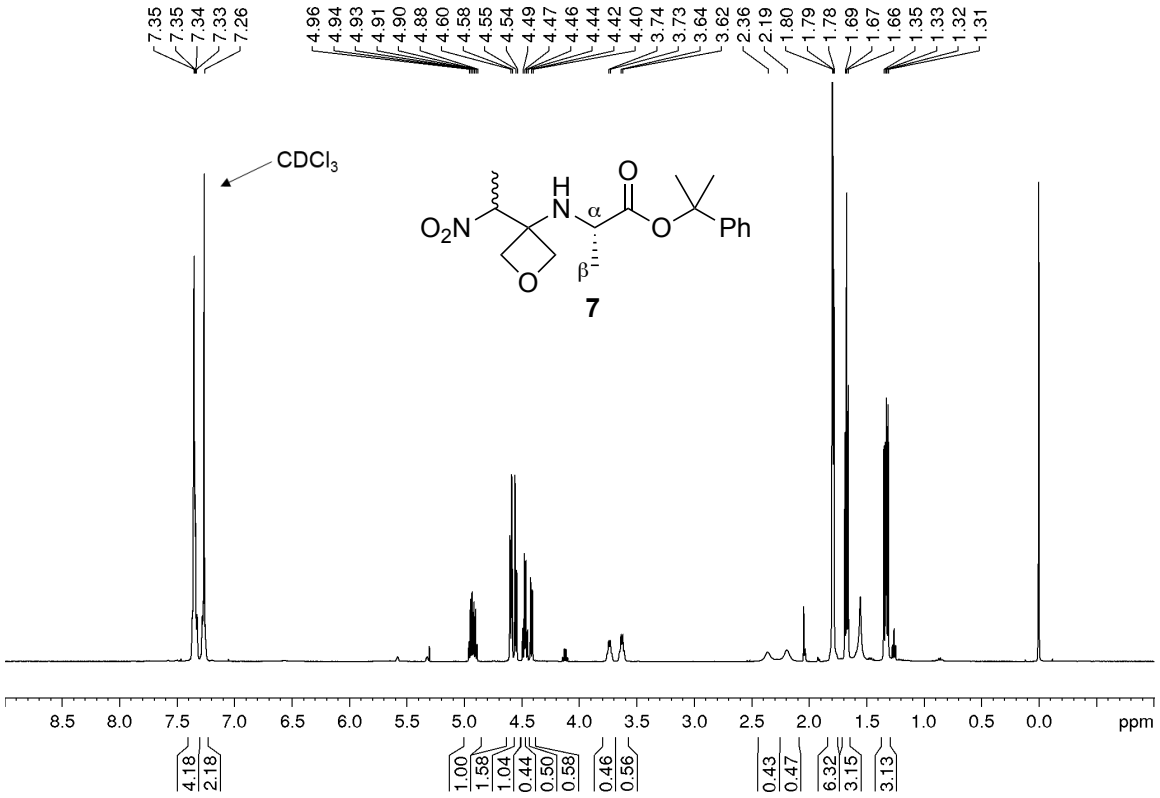
[3] G. P. Möller, S. Müller, B. T. Wolfstädter, S. Wolfrum, D. Schepmann, B. Wünsch and E. M. Carreira, *Org. Lett.*, 2017, **19**, 2510–2513.

[4] C. Yue, J. Thierry and P. Potier, *Tetrahedron Lett.*, 1993, **34**, 323–326.

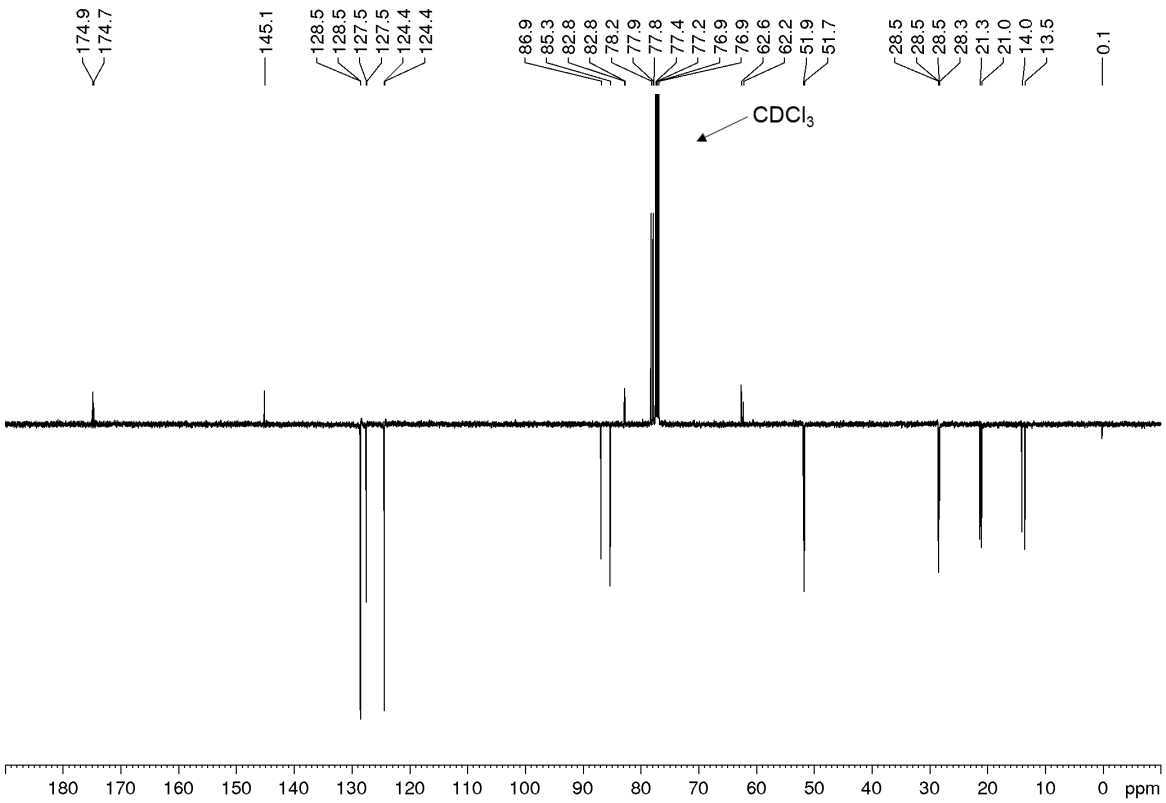
8. ^{1}H NMR and ^{13}C NMR spectra

2-Phenylpropan-2-yl [3-((±)-1-nitroethyl)oxetan-3-yl]-L-alaninate (7)

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

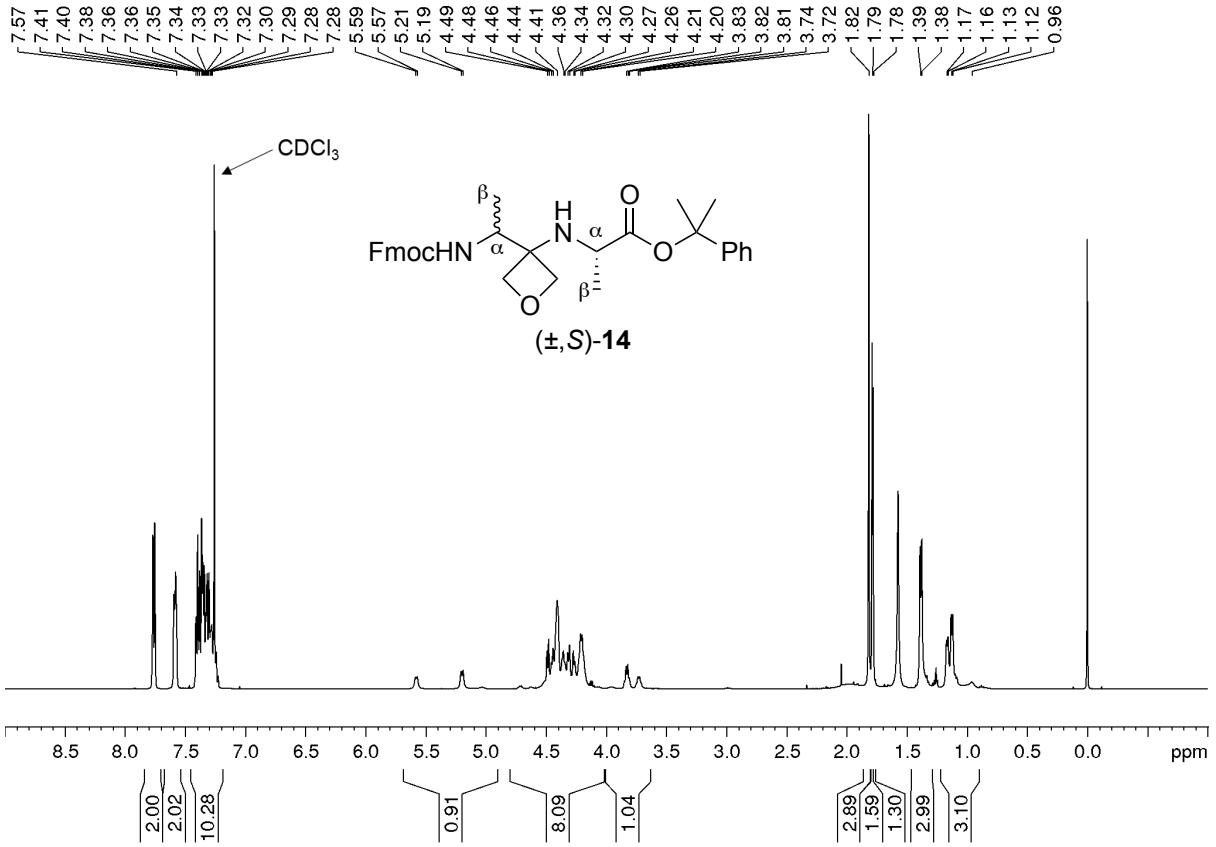


^{13}C NMR (126 MHz, $CDCl_3$)

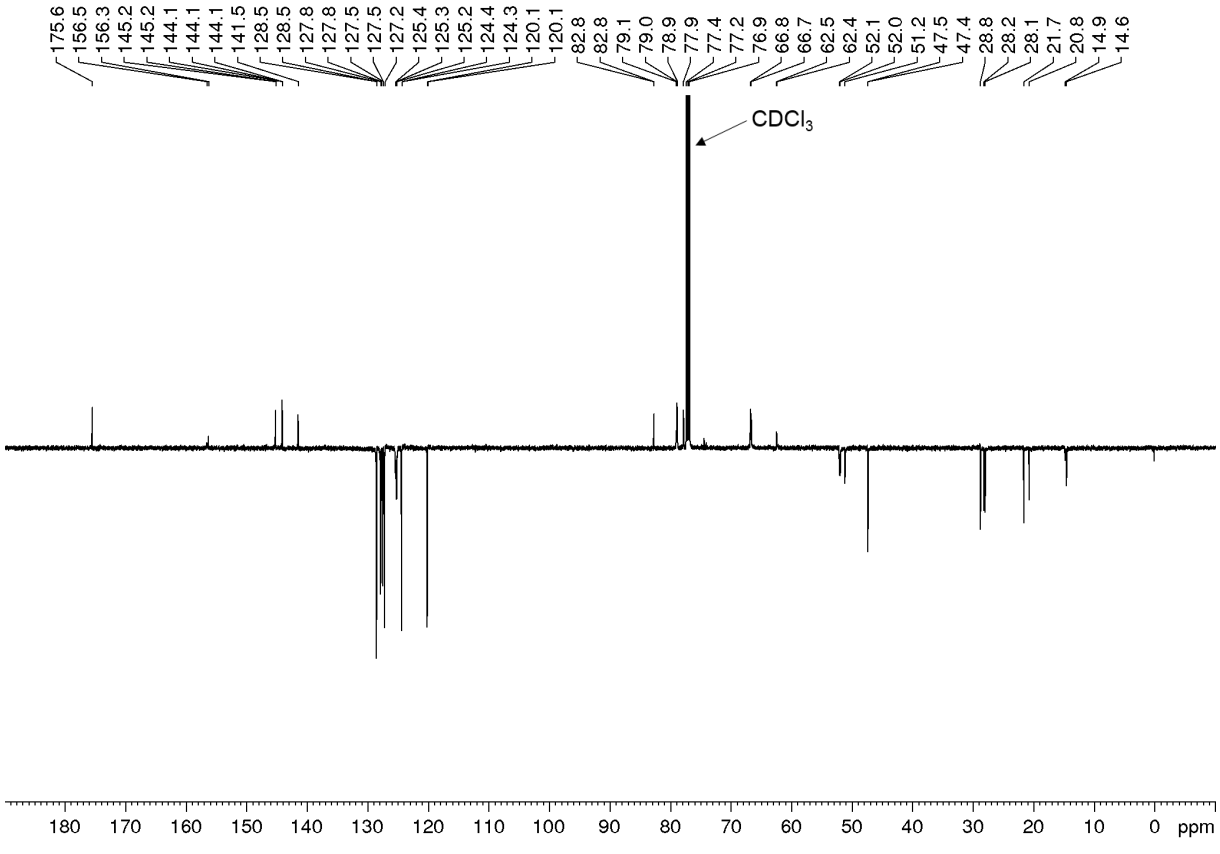


Fmoc-L-AOx-Ala-OCumyl and Fmoc-D-AOx-Ala-OCumyl ((±,*S*)-14)

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

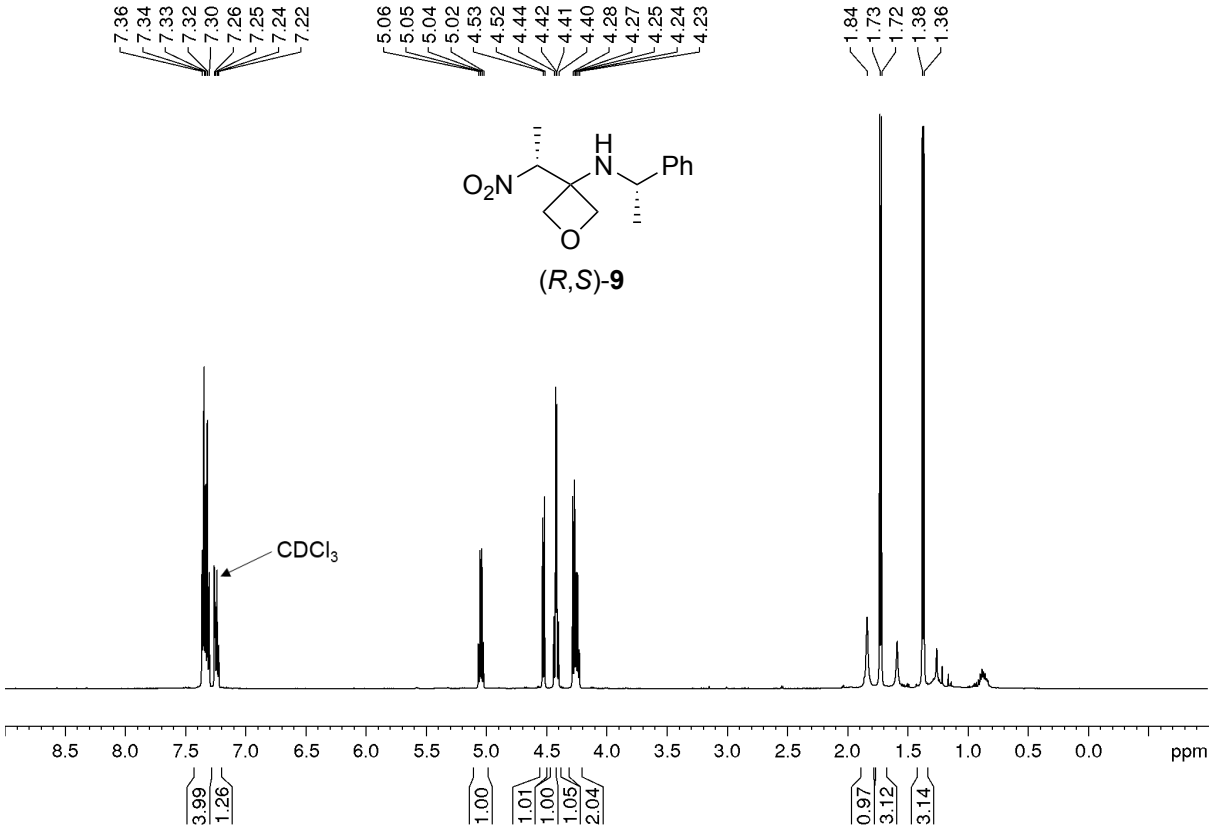


^{13}C NMR (126 MHz, $CDCl_3$)

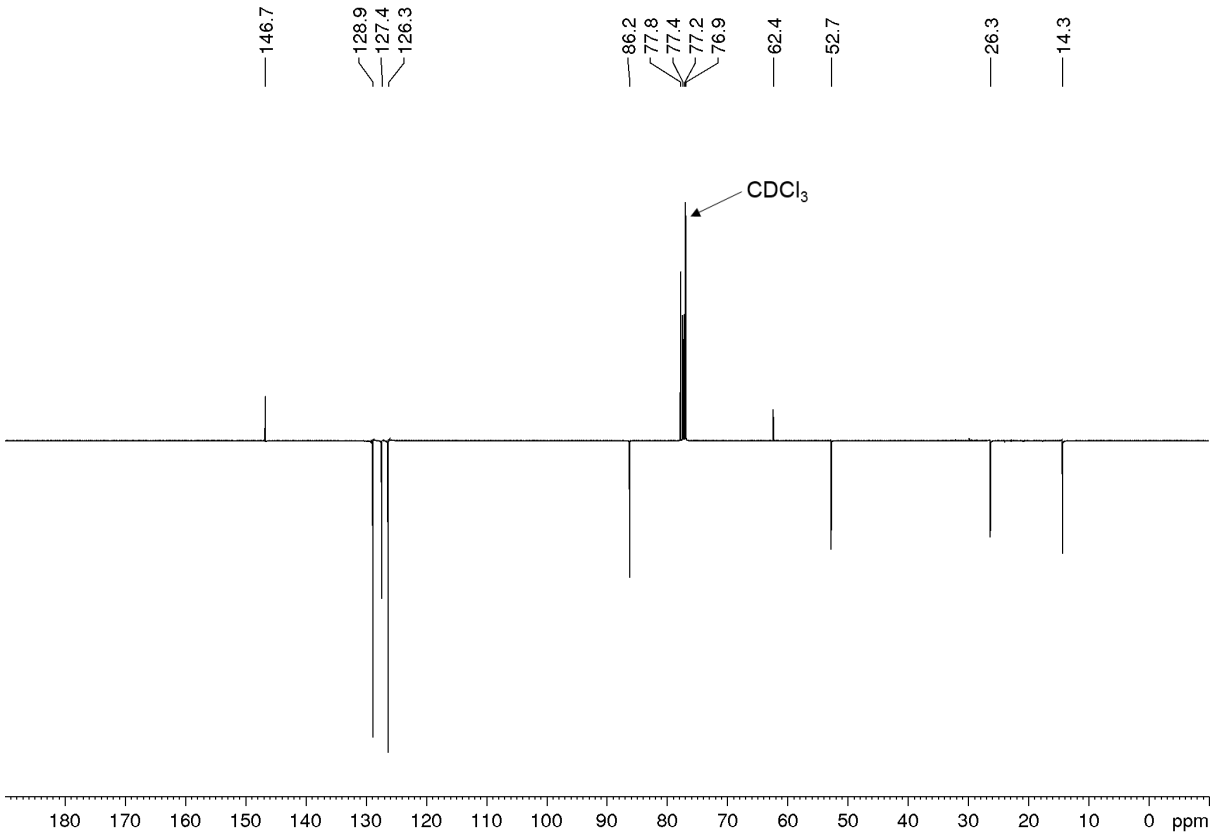


3-((*R*)-1-Nitroethyl)-*N*-((*S*)-1-phenylethyl)oxetan-3-amine ((*R*,*S*)-9)

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

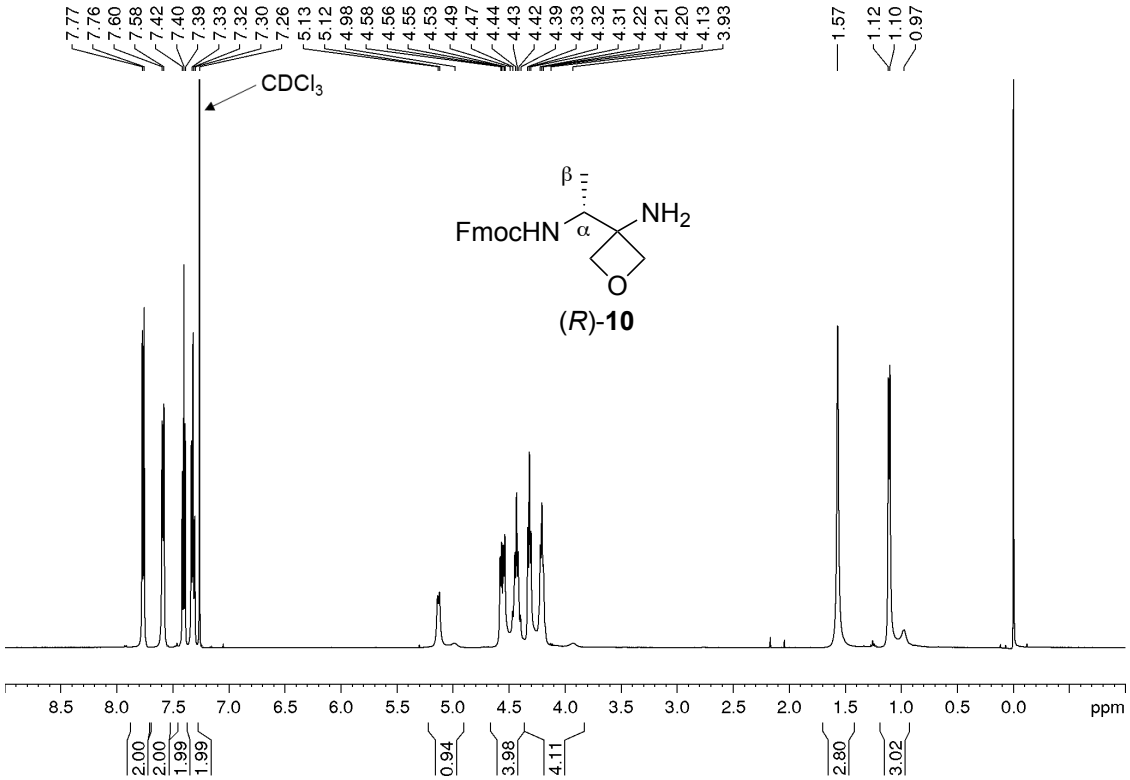


^{13}C NMR (126 MHz, $CDCl_3$)

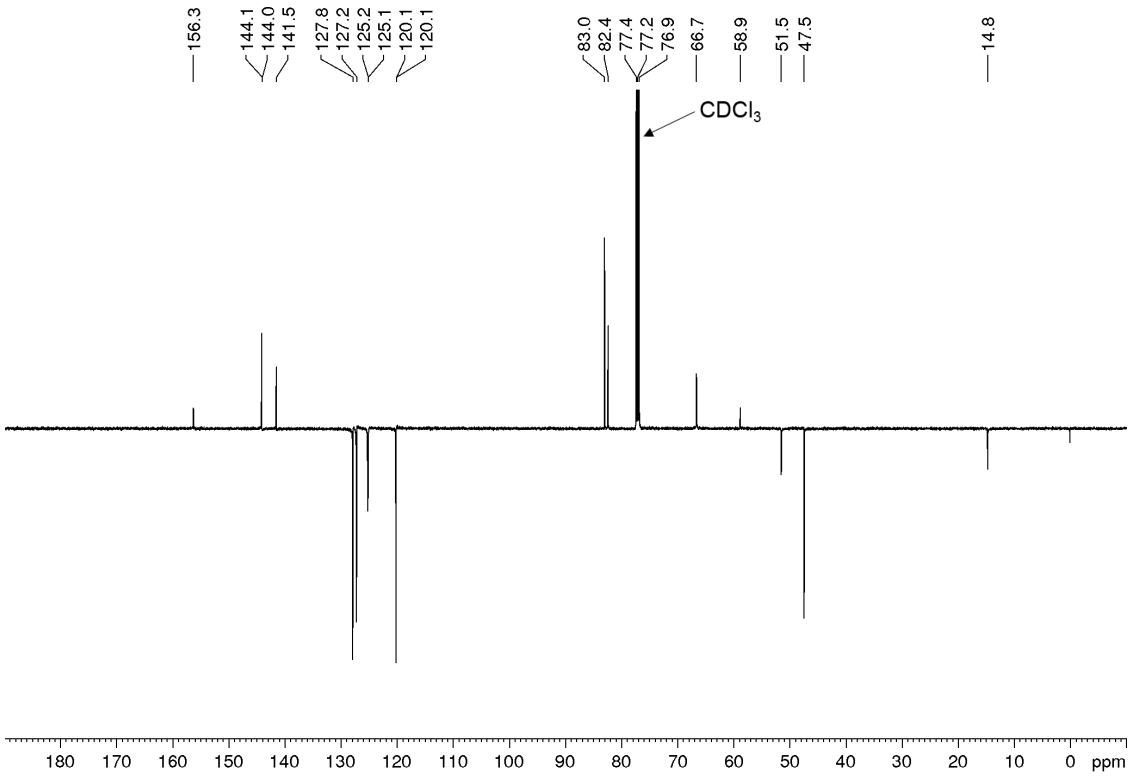


Fmoc-D-AOx-NH_2 ((*R*)-10)

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

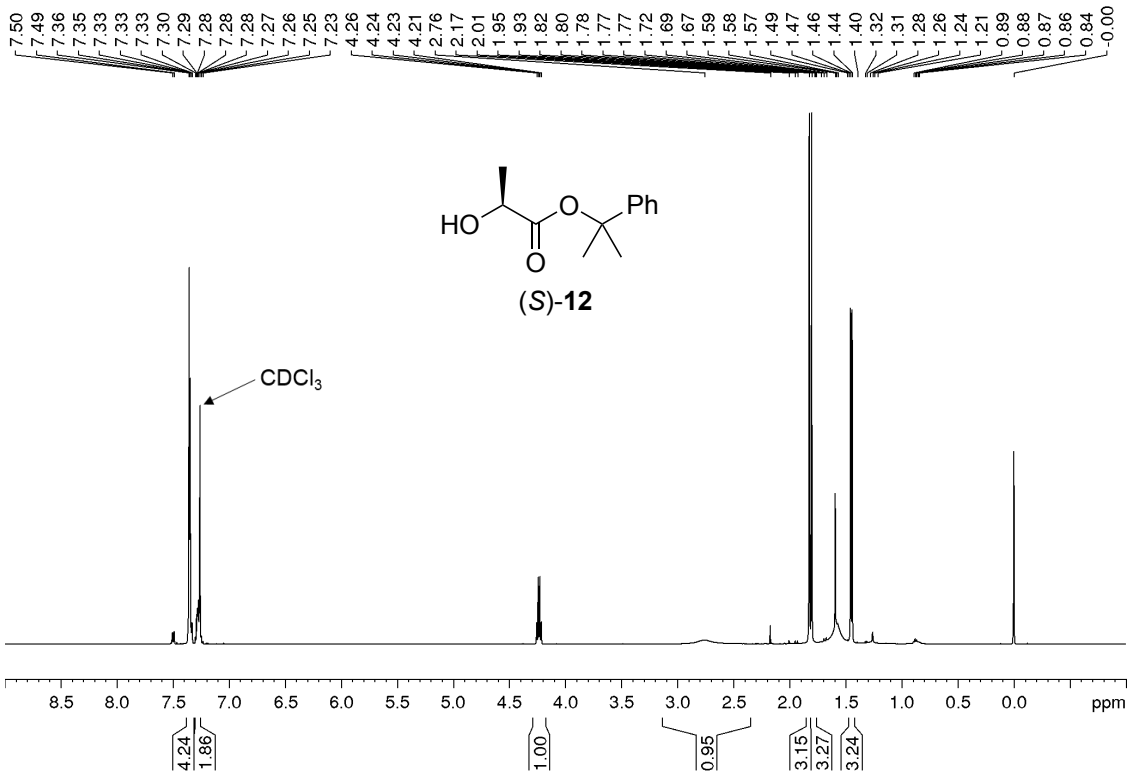


^{13}C NMR (126 MHz, $CDCl_3$)

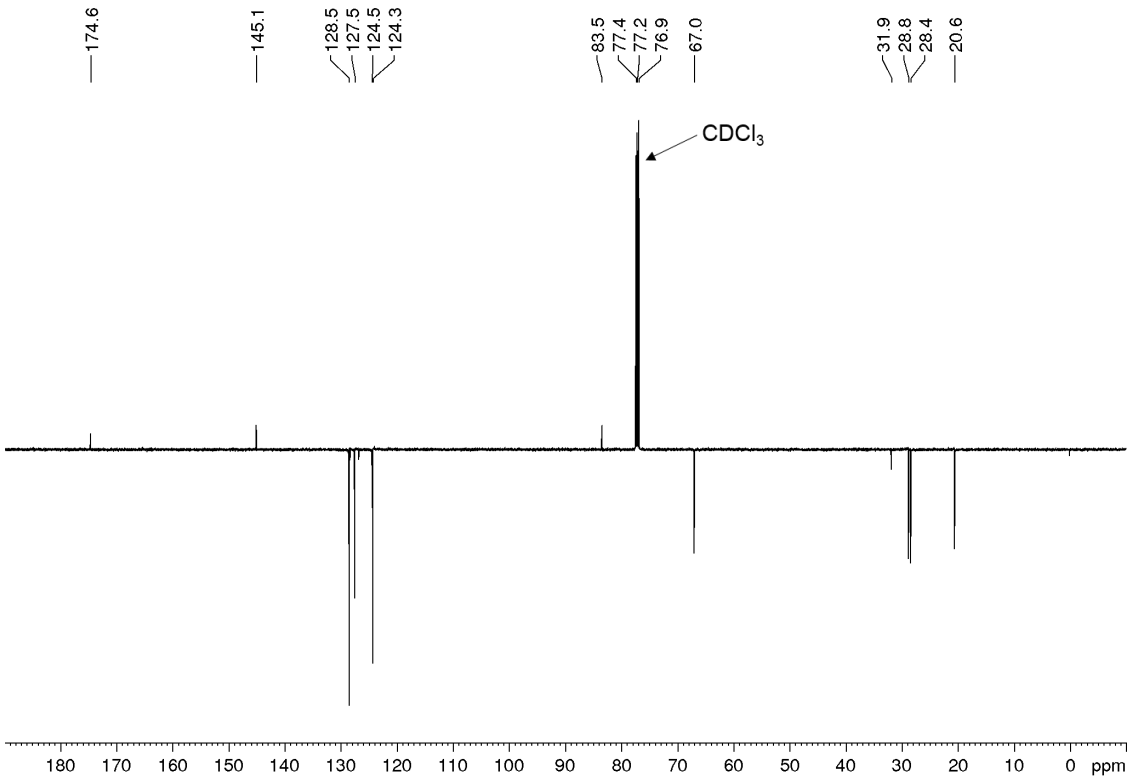


2-Phenylpropan-2-yl (*S*)-2-hydroxypropanoate ((*S*)-12)

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

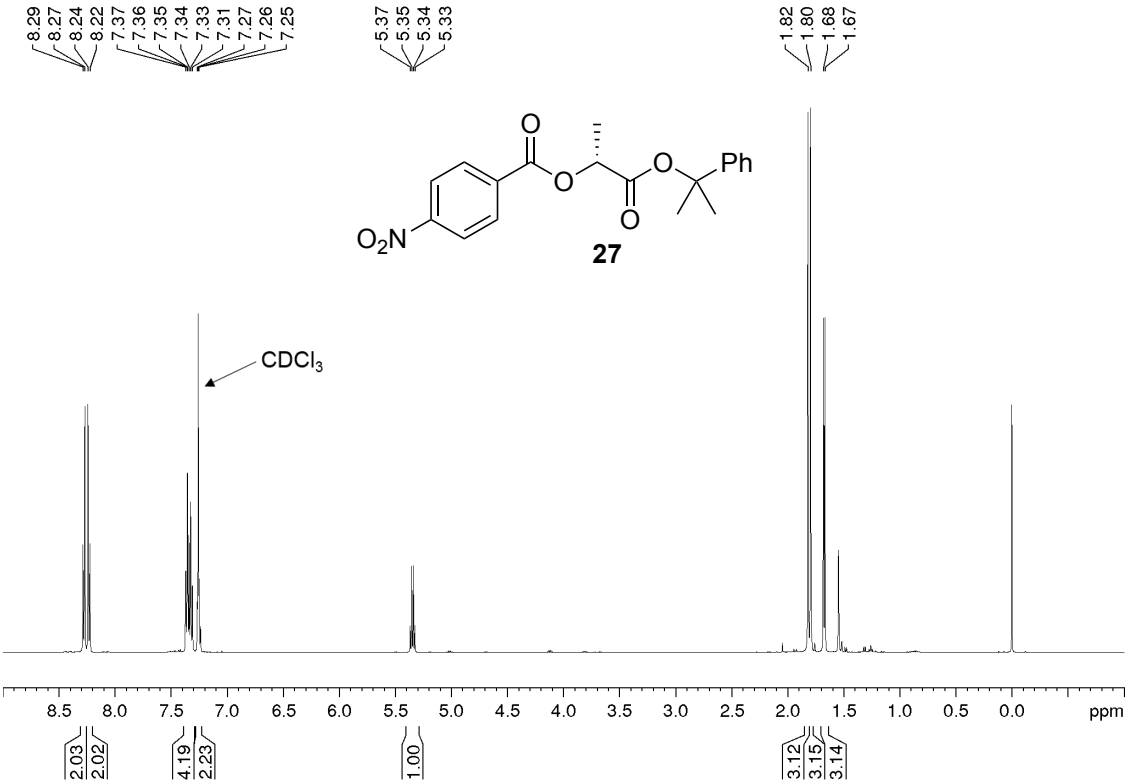


^{13}C NMR (126 MHz, $CDCl_3$)

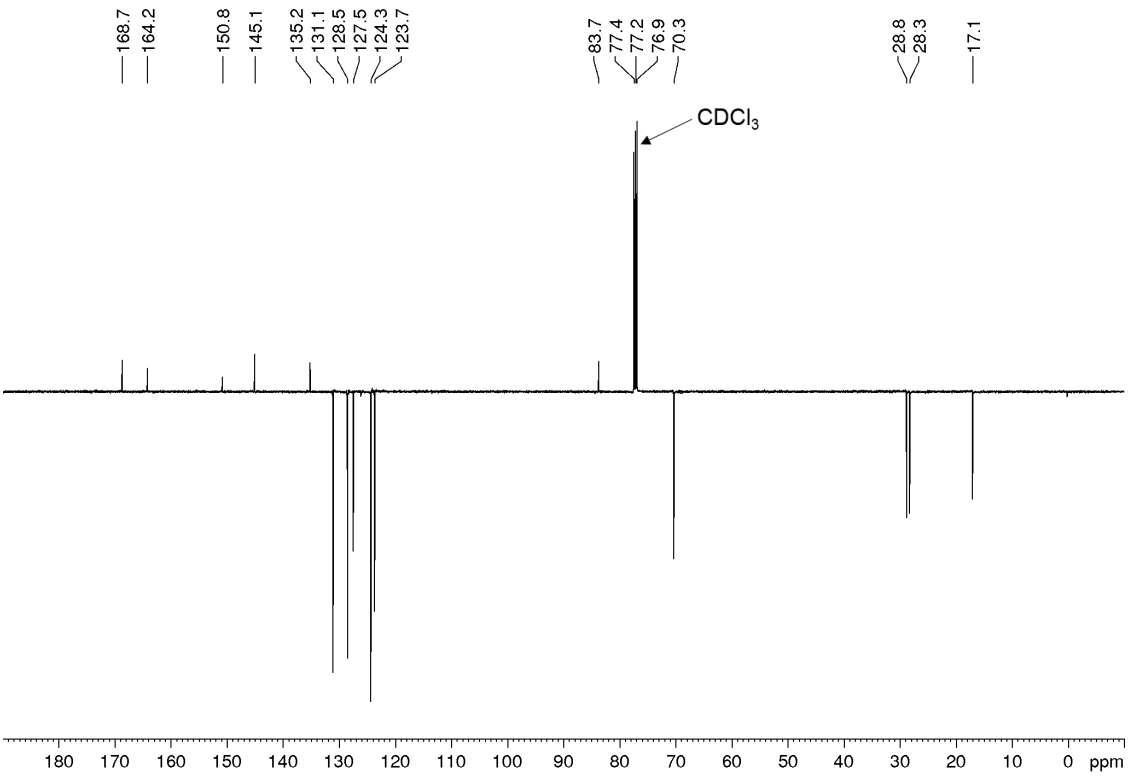


(*R*)-1-Oxo-1-[(2-phenylpropan-2-yl)oxy]propan-2-yl 4-nitrobenzoate (27)

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

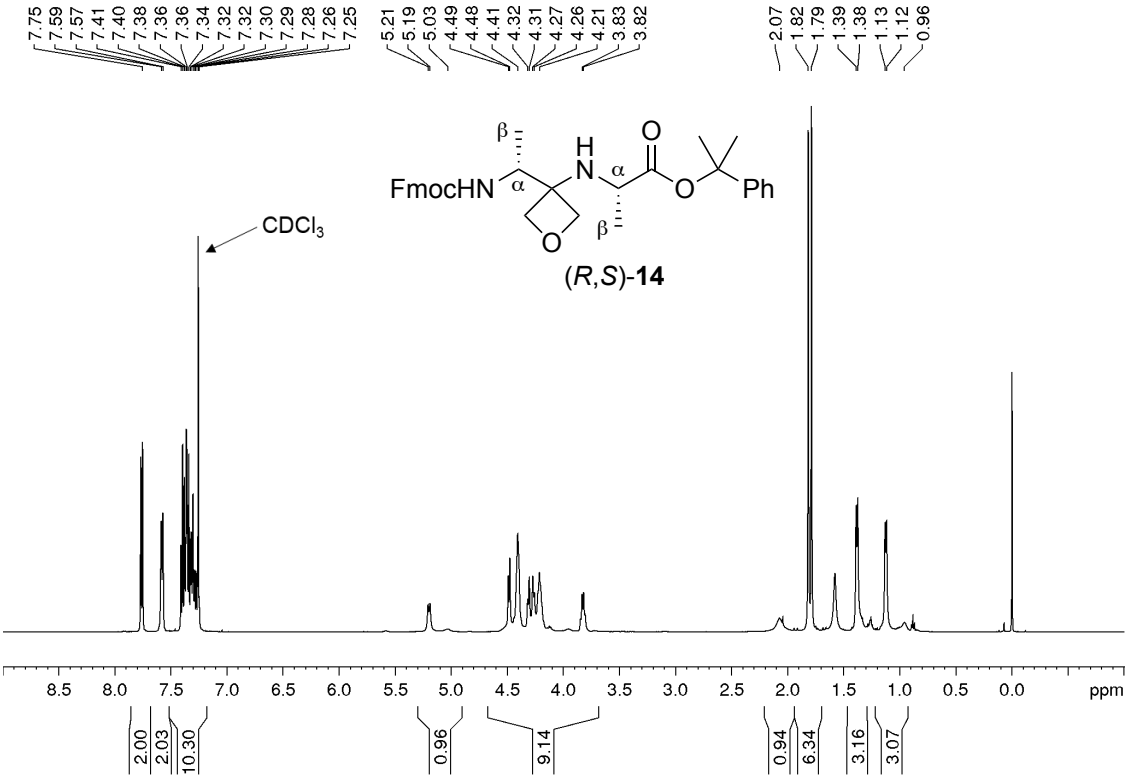


^{13}C NMR (126 MHz, $CDCl_3$)

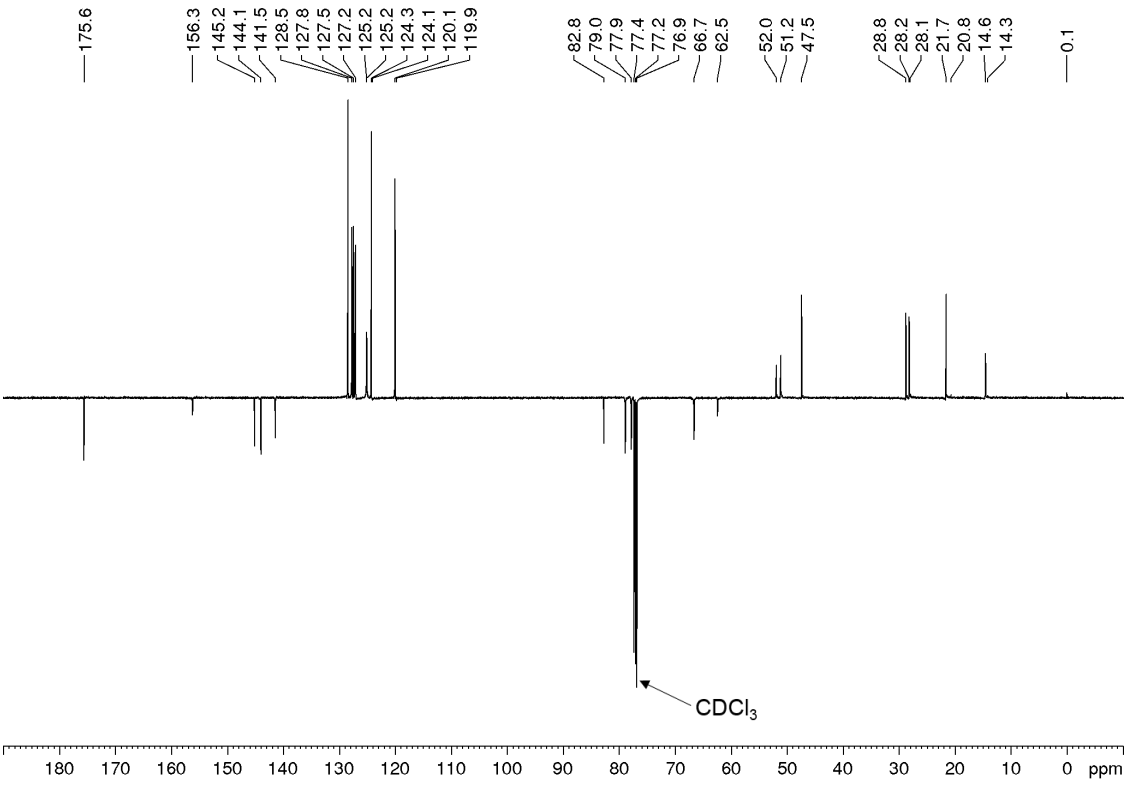


Fmoc-D-AOx-Ala-OCumyl ((*R*,*S*)-14)

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

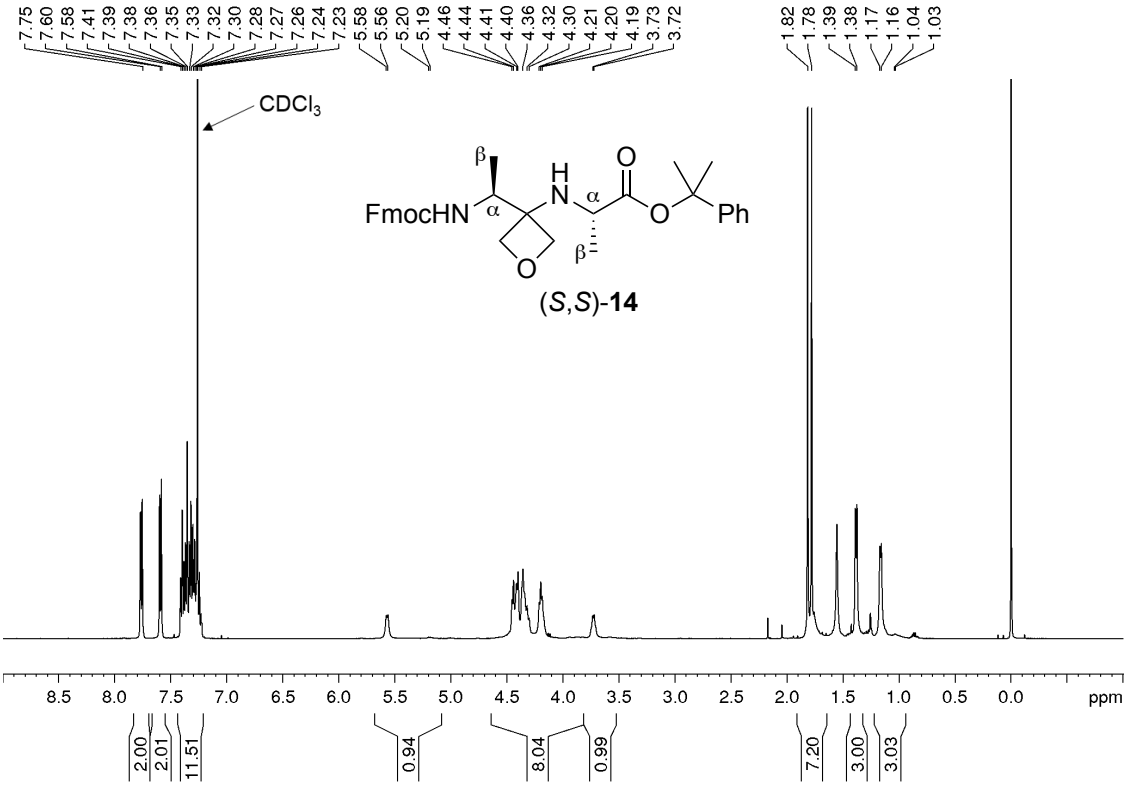


^{13}C NMR (126 MHz, $CDCl_3$)

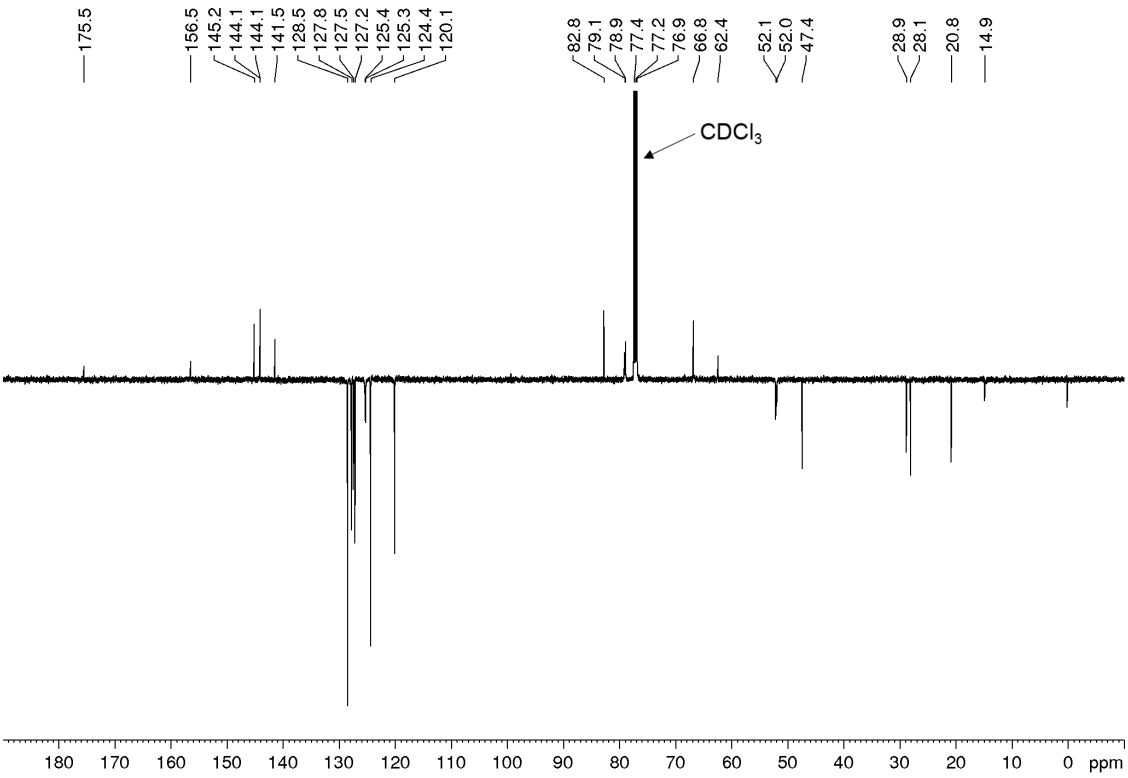


Fmoc-L-AOx-Ala-OCumyl ((*S*,*S*)-14)

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

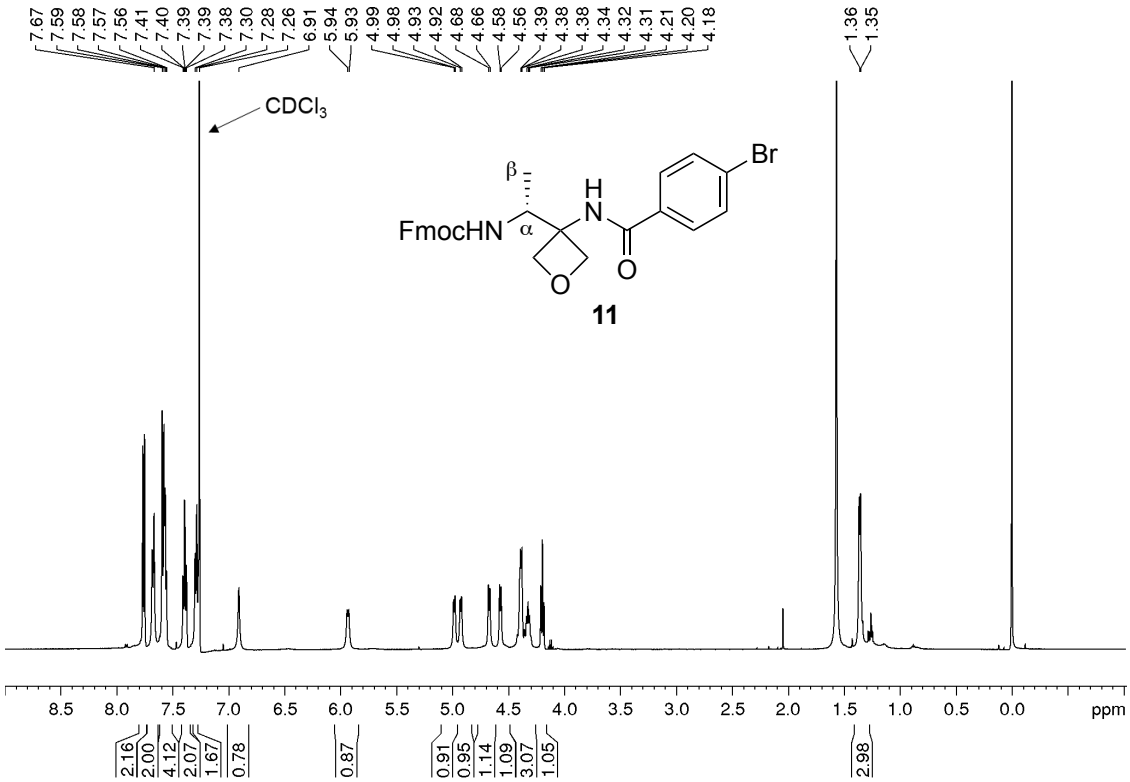


^{13}C NMR (126 MHz, $CDCl_3$)

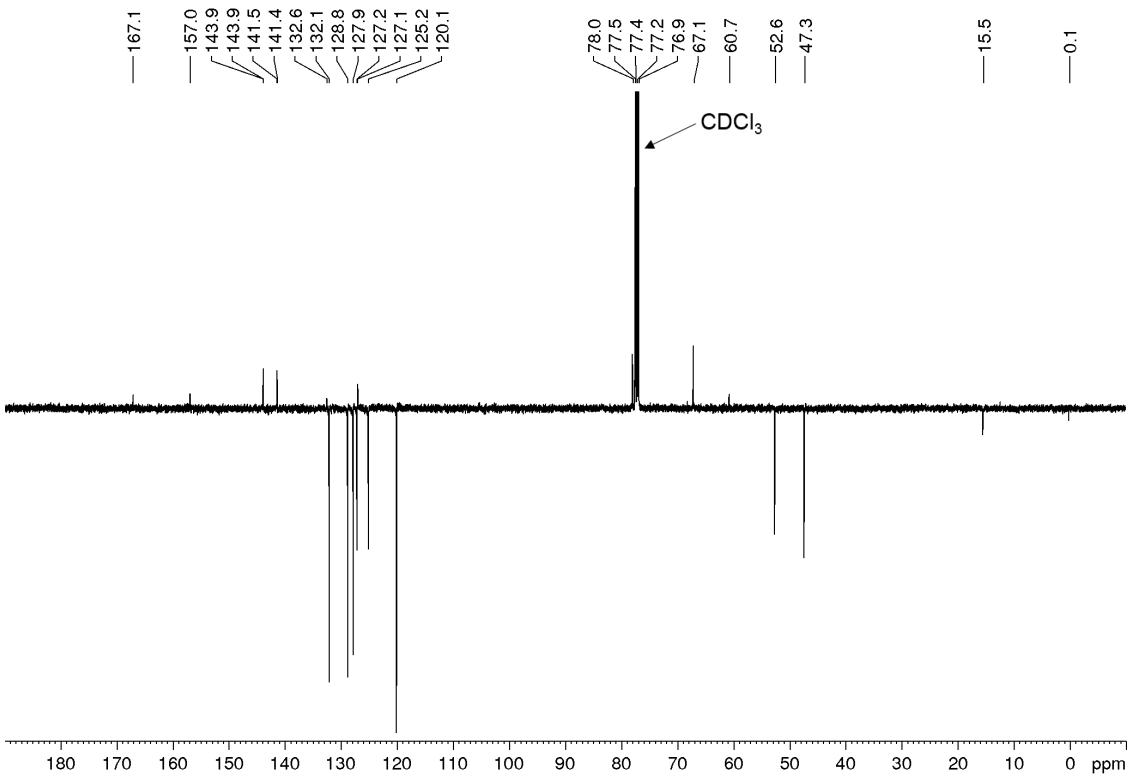


(9*H*-Fluoren-9-yl)methyl (*R*)-(1-(3-(4-bromobenzamido)oxetan-3-yl)ethyl)carbamate (11)

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

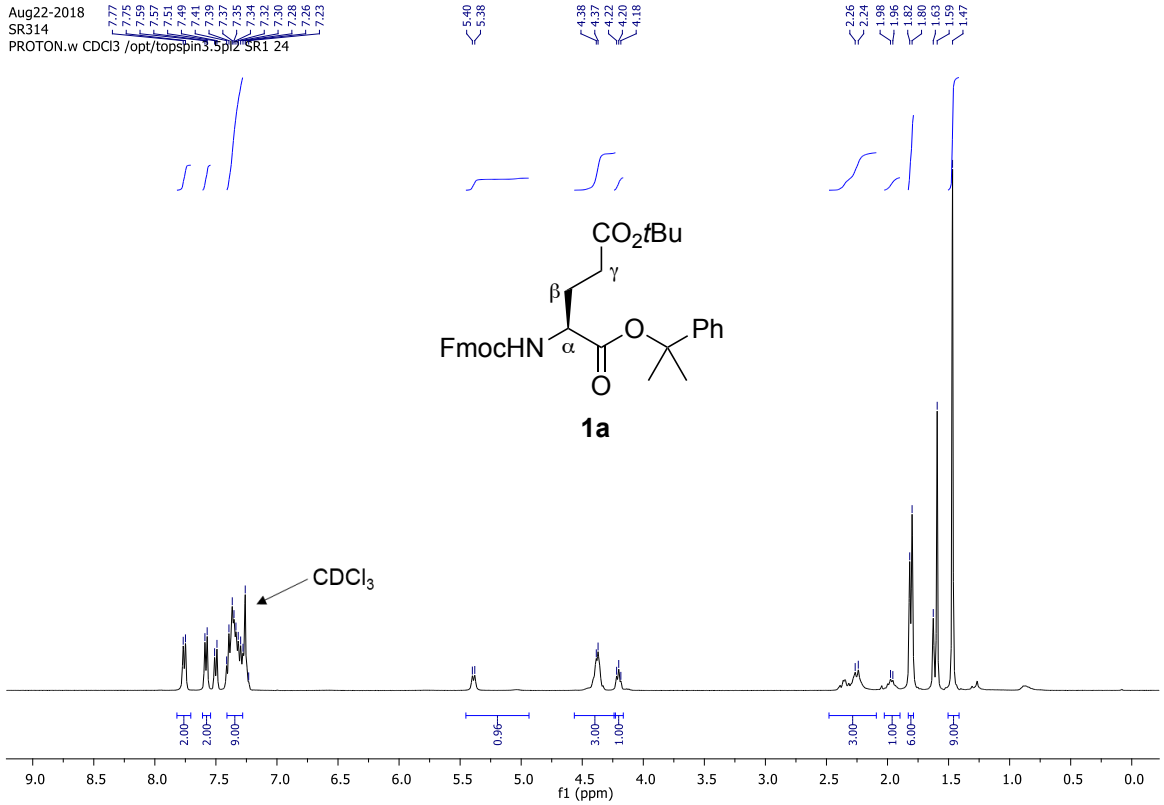


^{13}C NMR (126 MHz, $CDCl_3$)

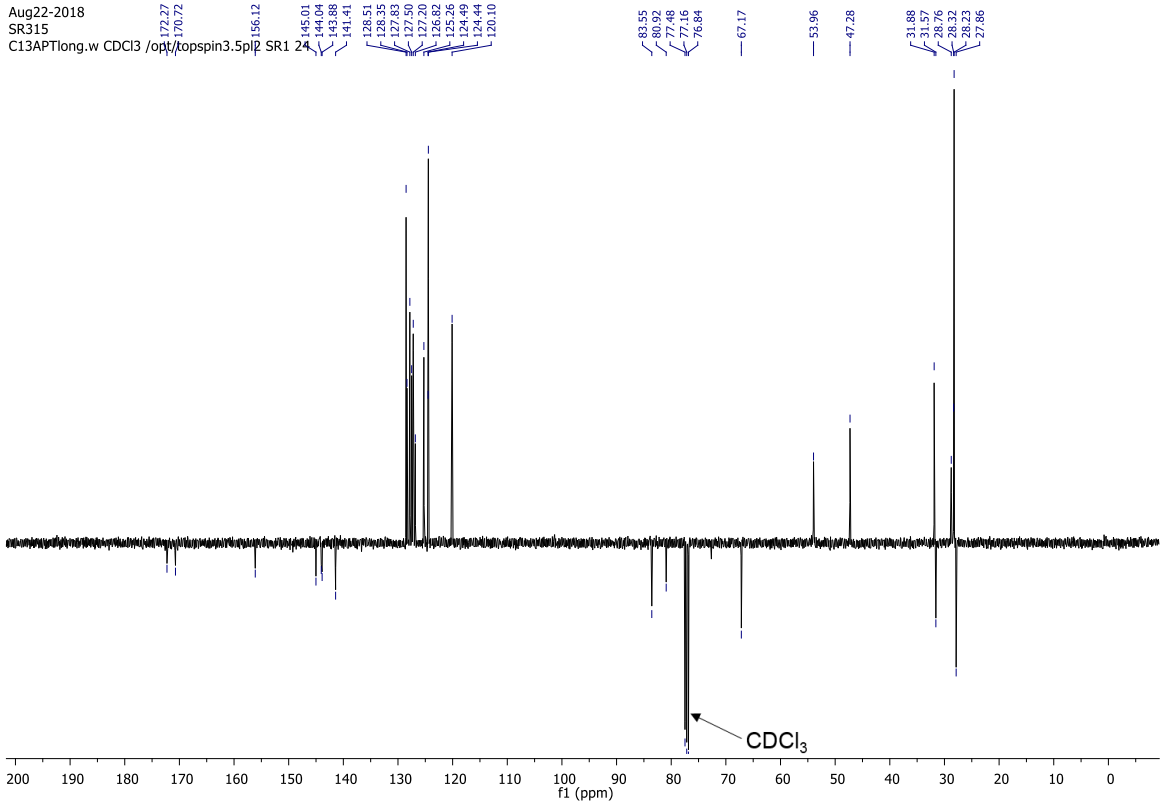


Fmoc-Glu(*t*Bu)-OCumyl (1a)

^{1}H NMR (400 MHz, $CDCl_3$)

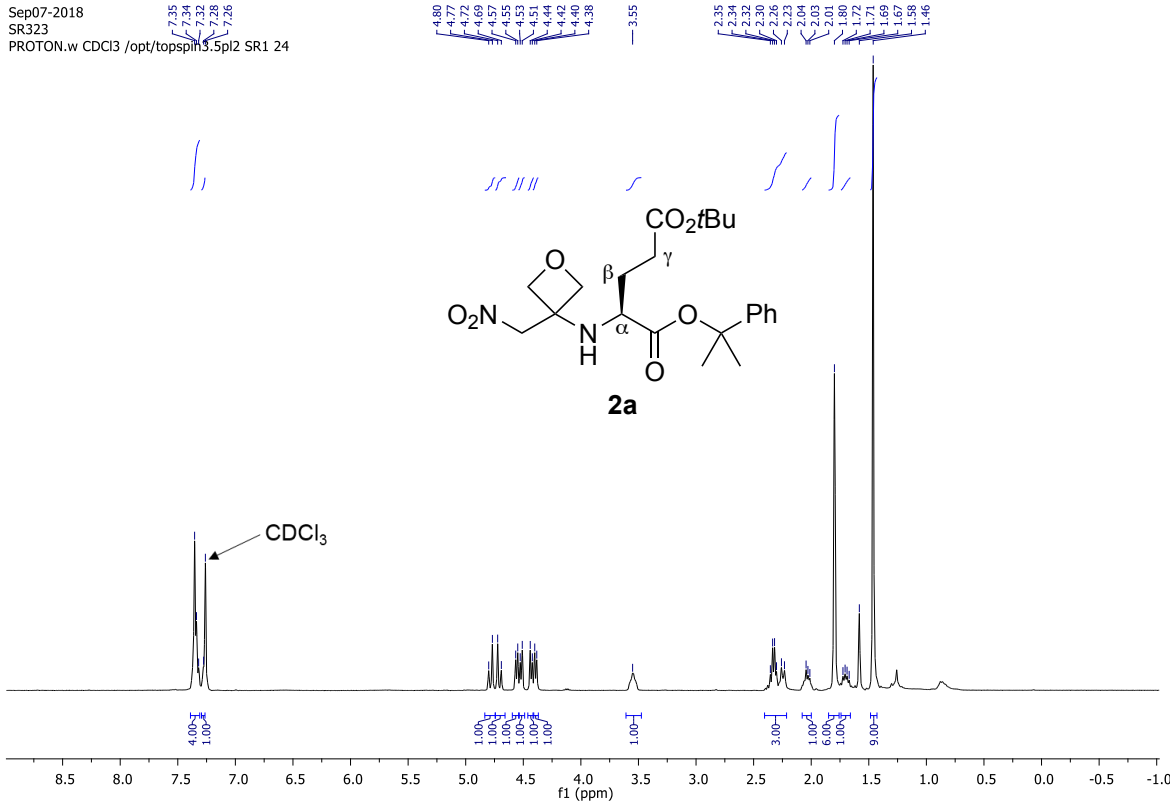


^{13}C NMR (101 MHz, $CDCl_3$)

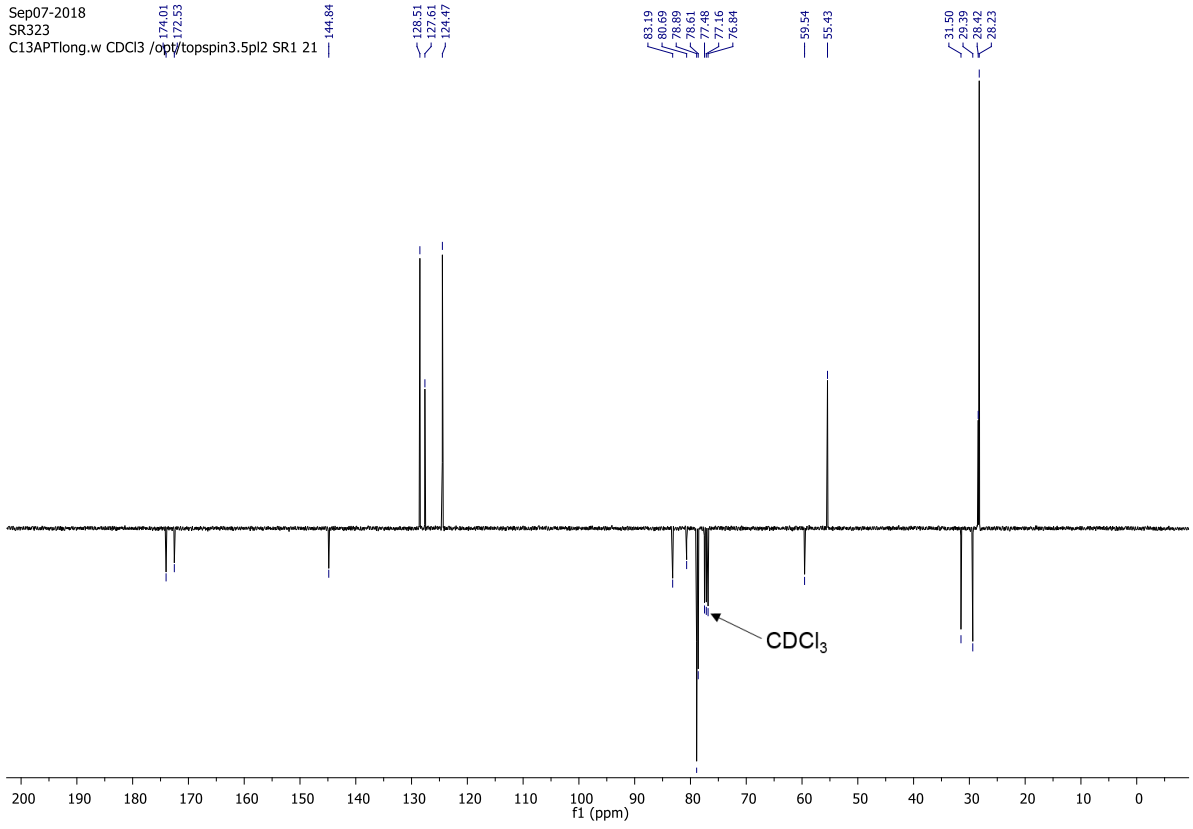


O_2N-GOx-Glu(*t*Bu)-OCumyl (2a)

^{1}H NMR (400 MHz, $CDCl_3$)

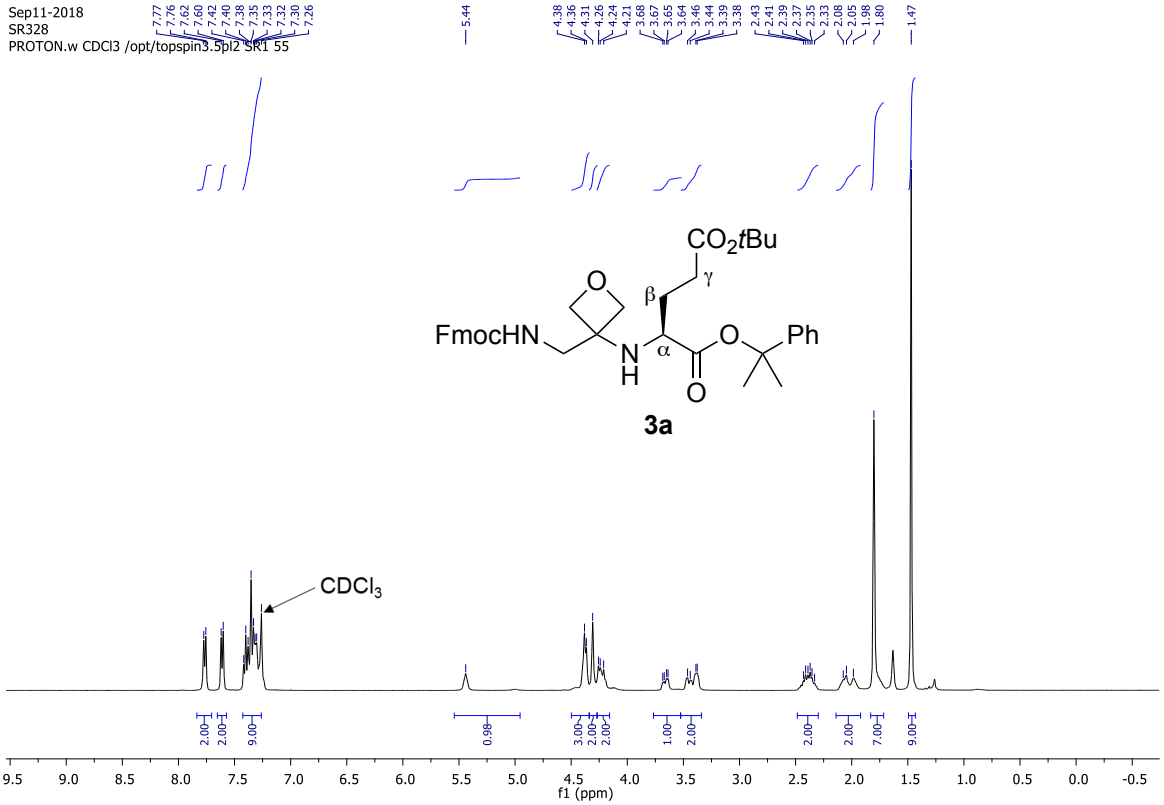


^{13}C NMR (101 MHz, $CDCl_3$)

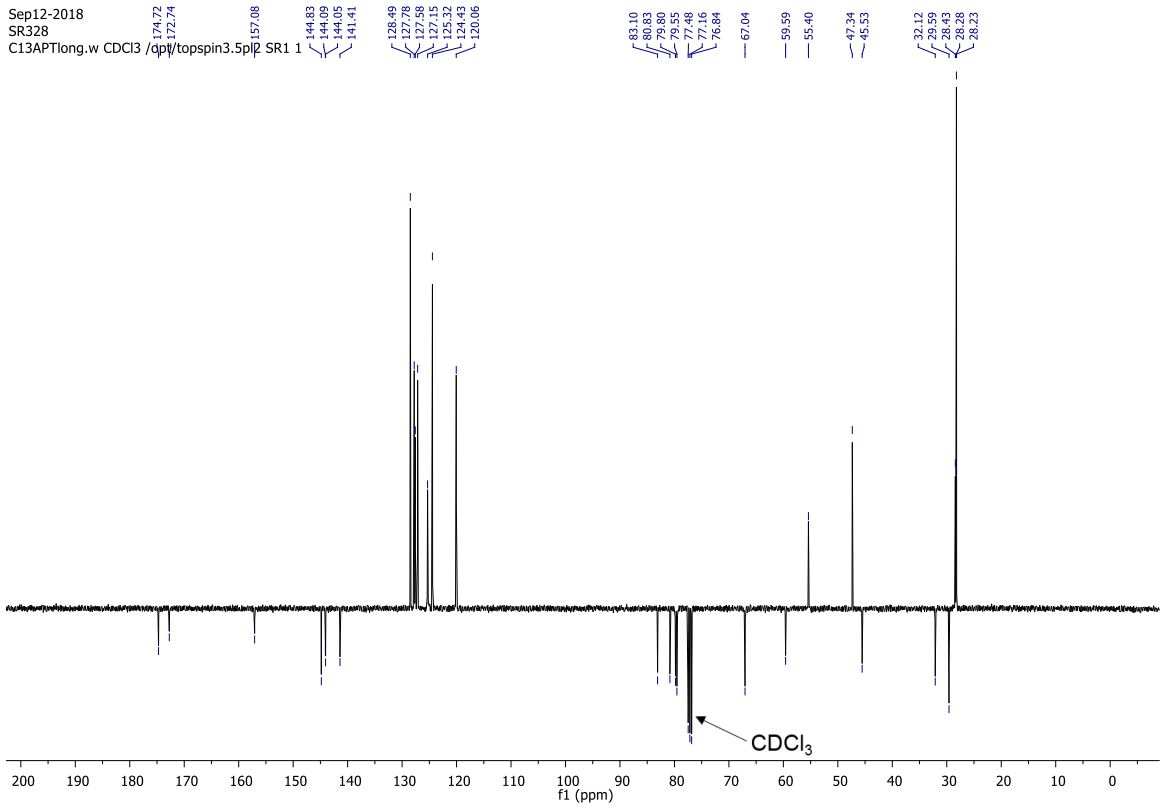


Fmoc-GOx-Glu(*t*Bu)-OCumyl (3a)

^{1}H NMR (400 MHz, $CDCl_3$)

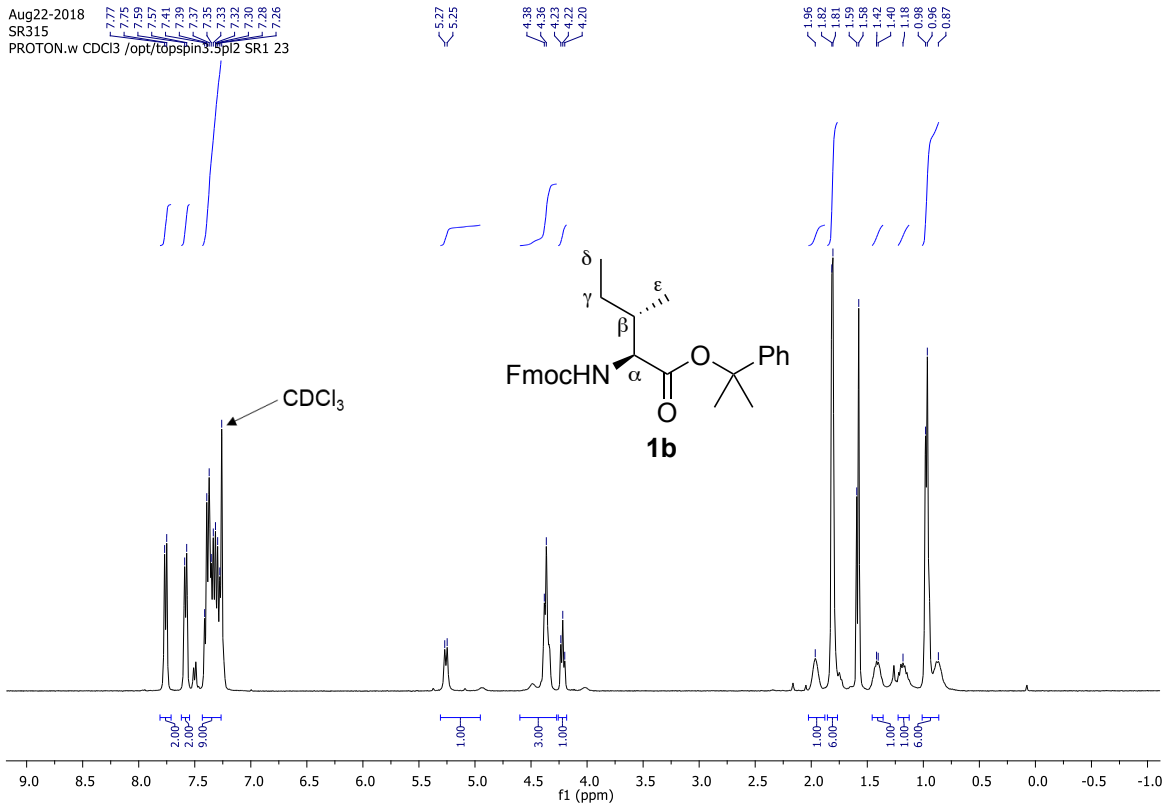


^{13}C NMR (101 MHz, $CDCl_3$)

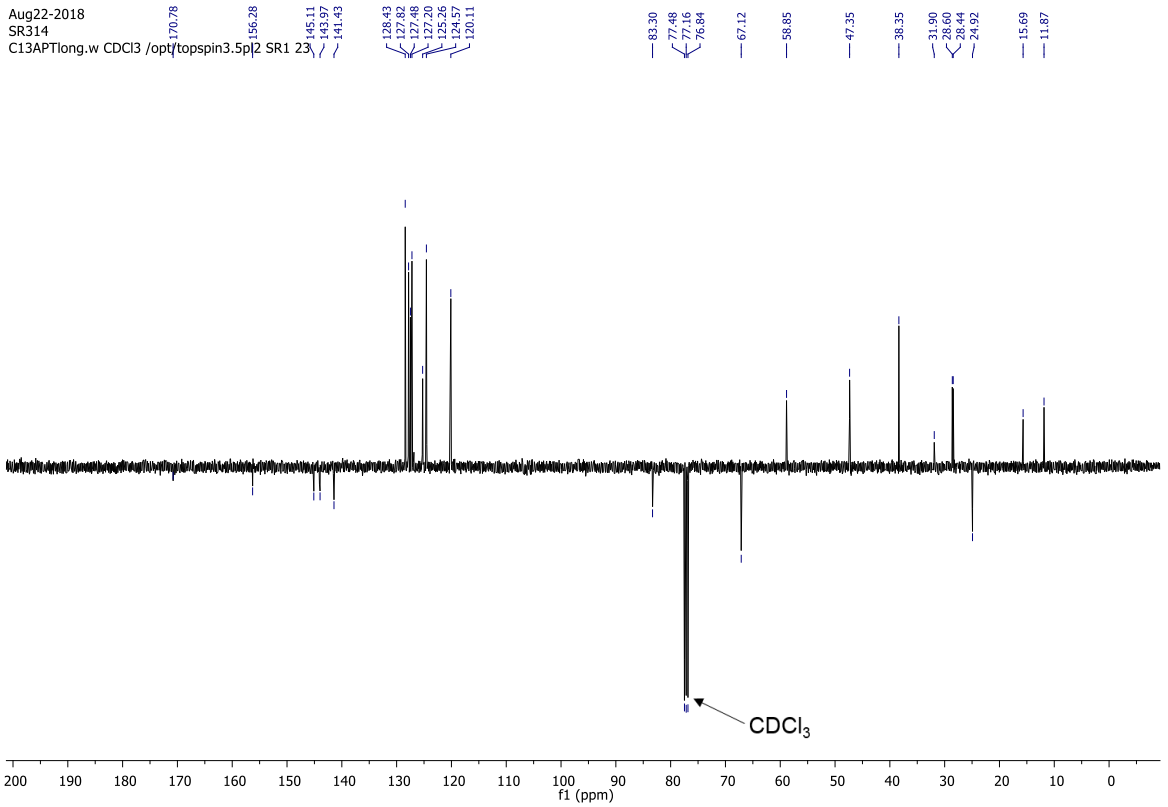


Fmoc-Ile-OCumyl (1b)

^{1}H NMR (400 MHz, $CDCl_3$)

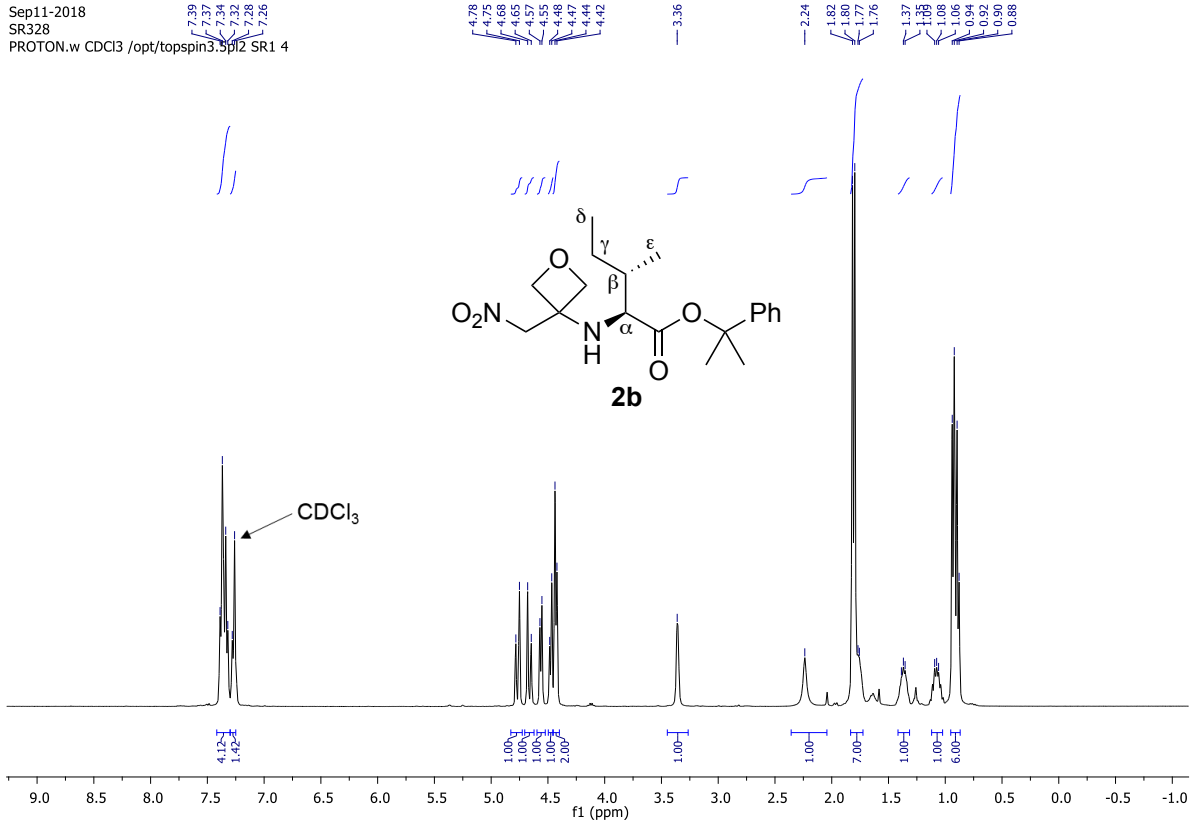


^{13}C NMR (101 MHz, $CDCl_3$)

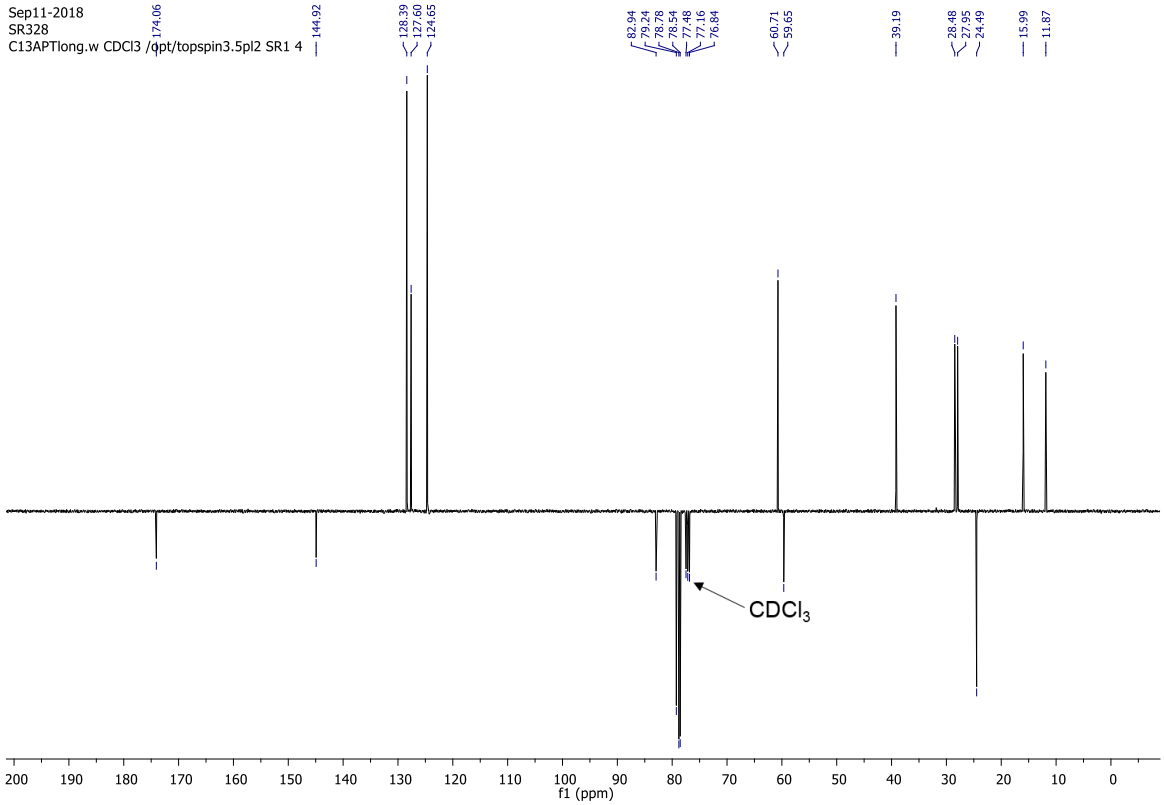


NO_2-GOx-Ile-OCumyl (2b)

^{1}H NMR (400 MHz, $CDCl_3$)

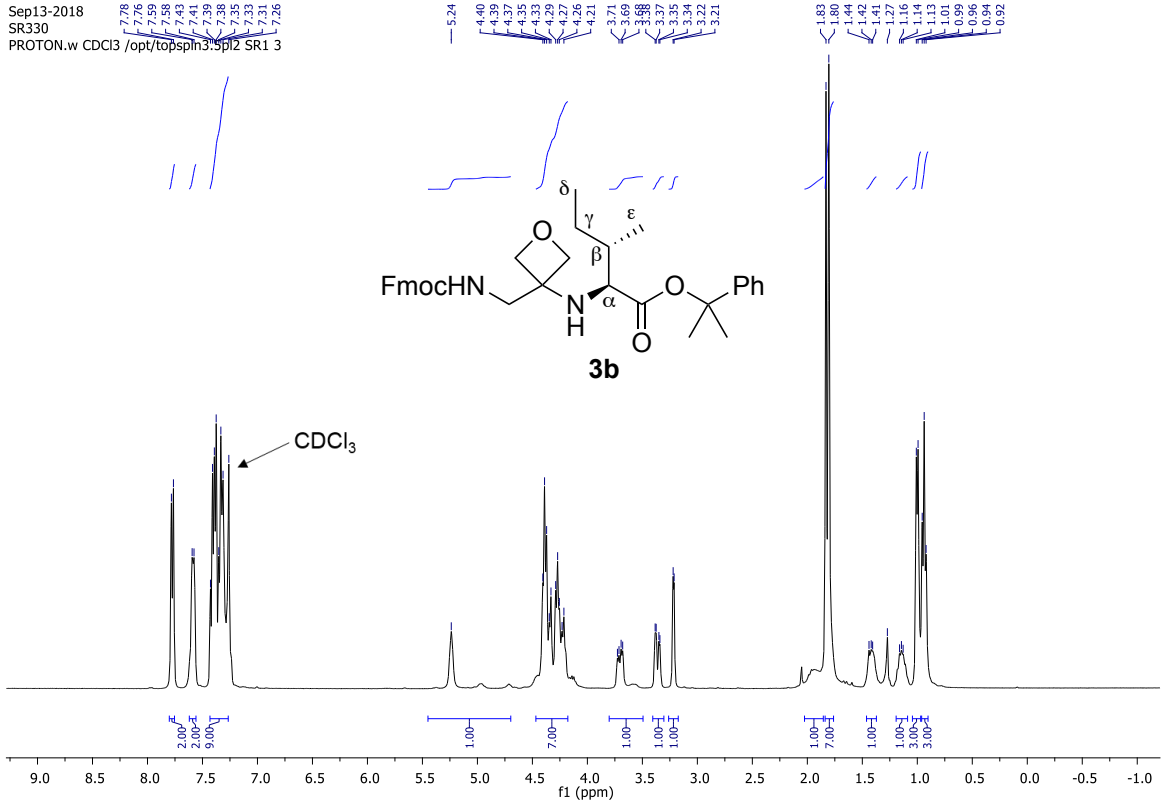


^{13}C NMR (101 MHz, $CDCl_3$)

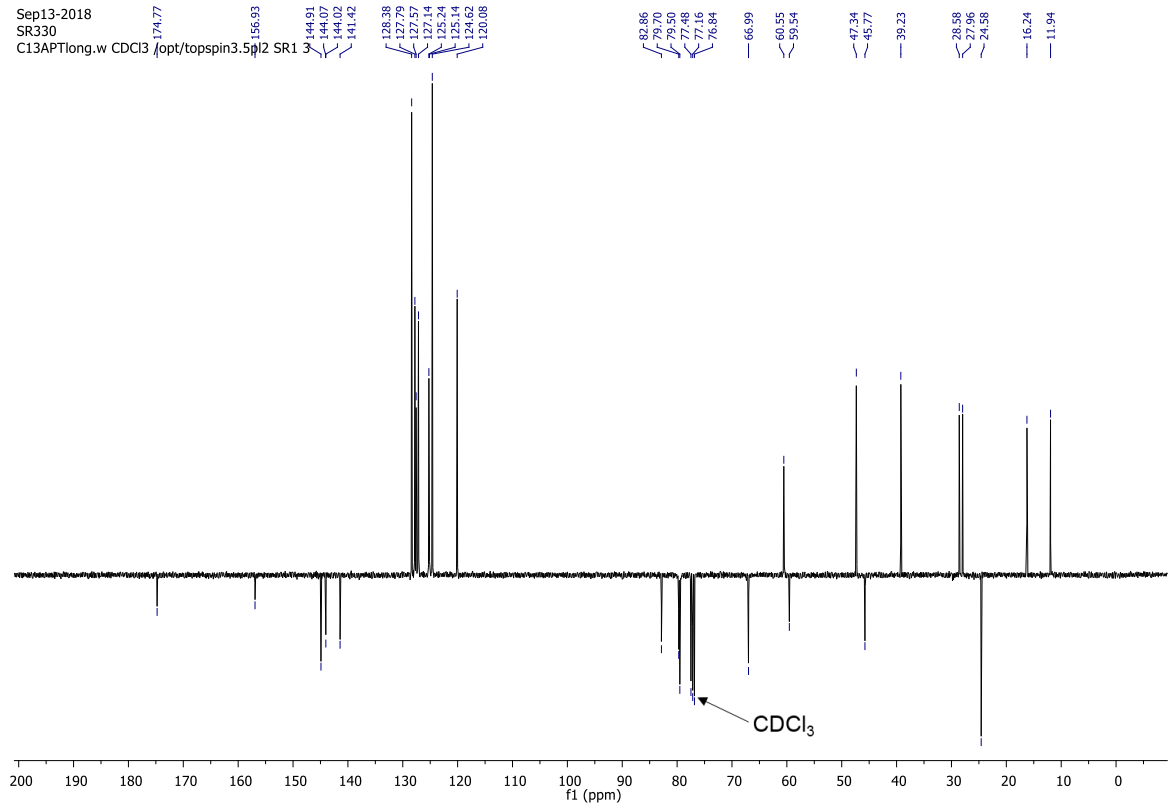


Fmoc-GOx-Ile-OCumyl (3b)

^{1}H NMR (400 MHz, $CDCl_3$)

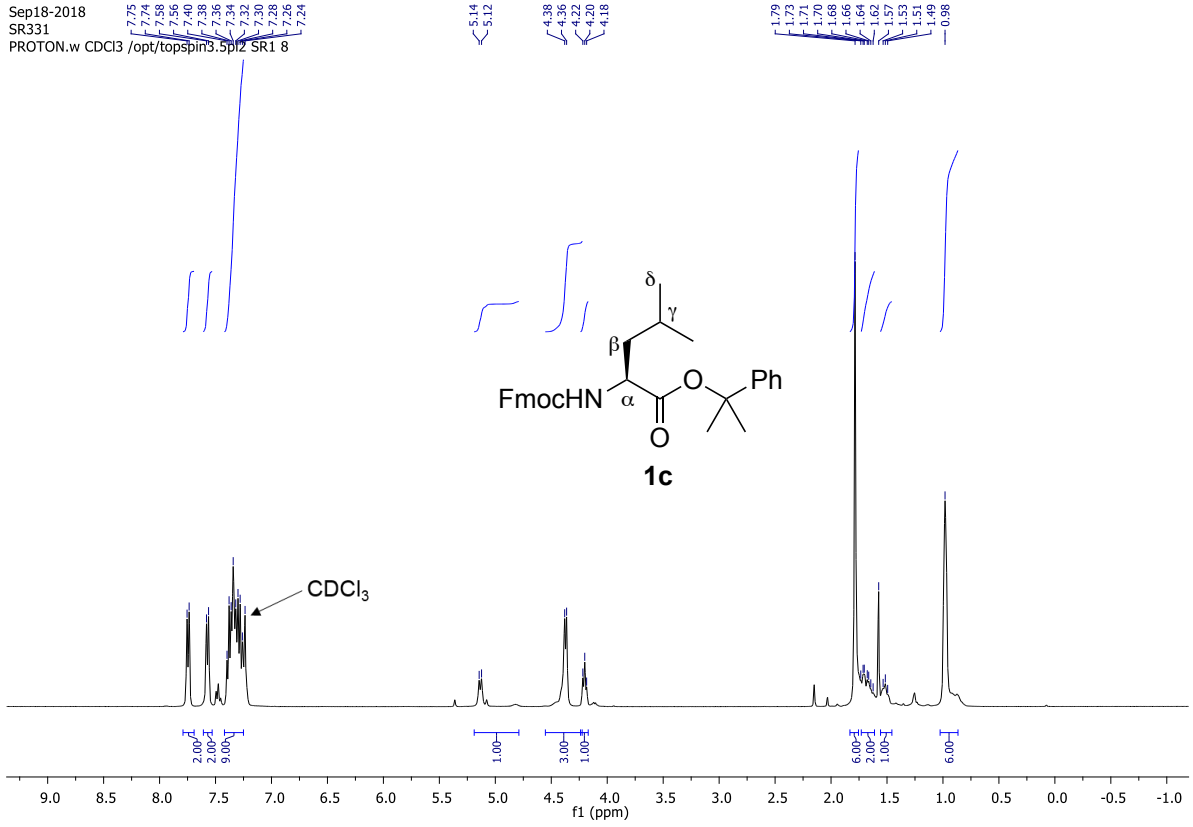


^{13}C NMR (101 MHz, $CDCl_3$)

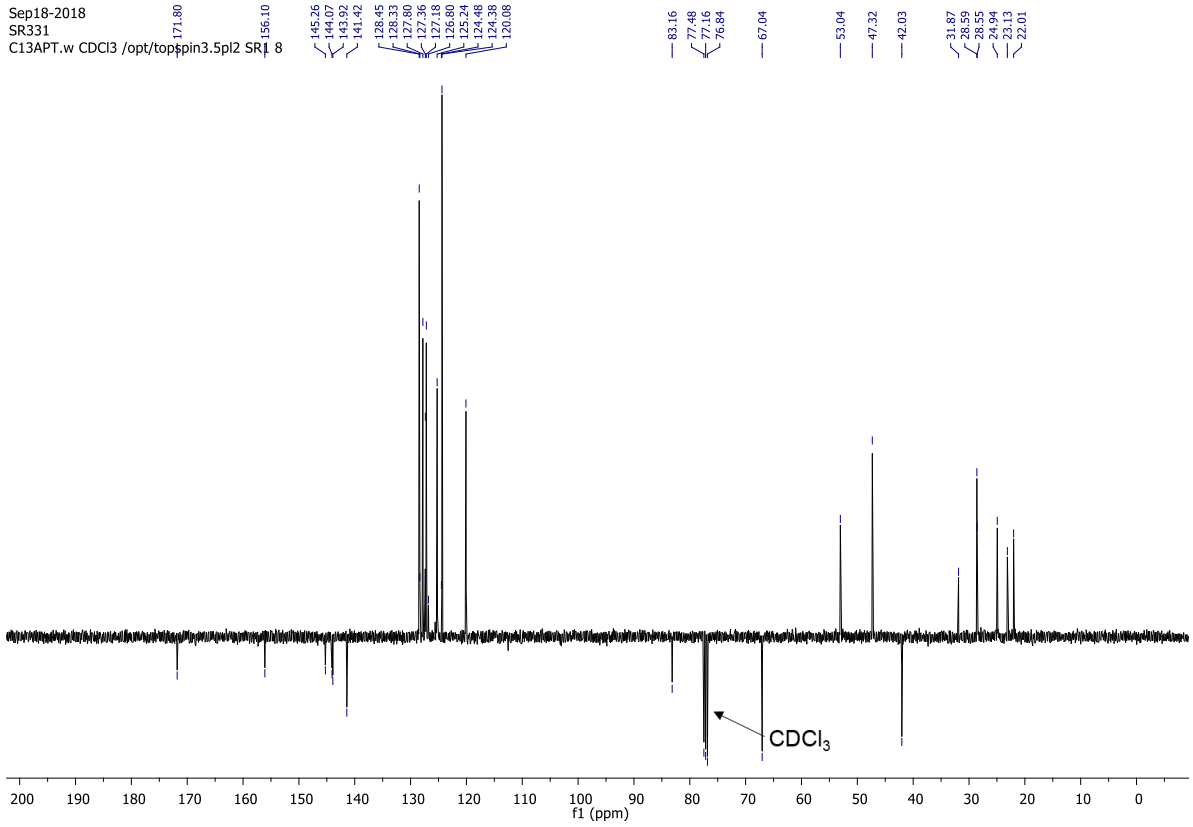


Fmoc-Leu-OCumyl (1c)

^{1}H NMR (400 MHz, $CDCl_3$)

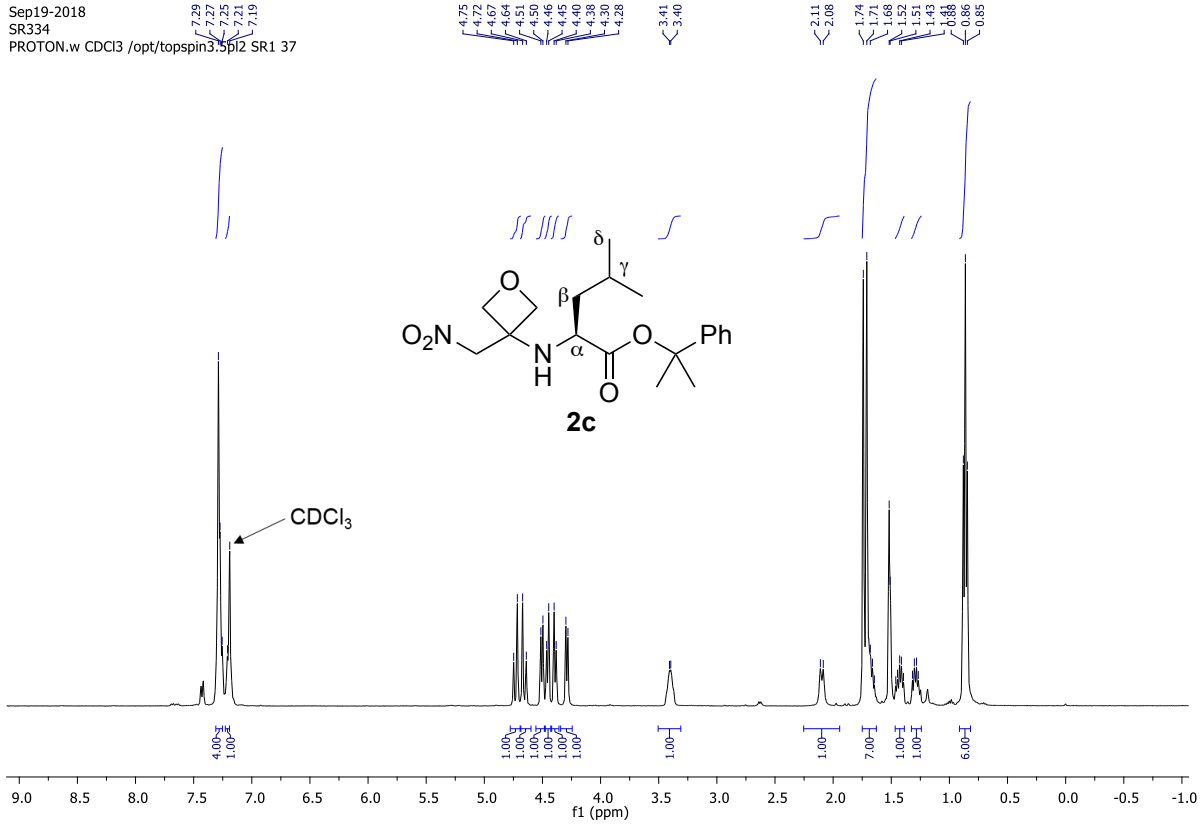


^{13}C NMR (101 MHz, $CDCl_3$)

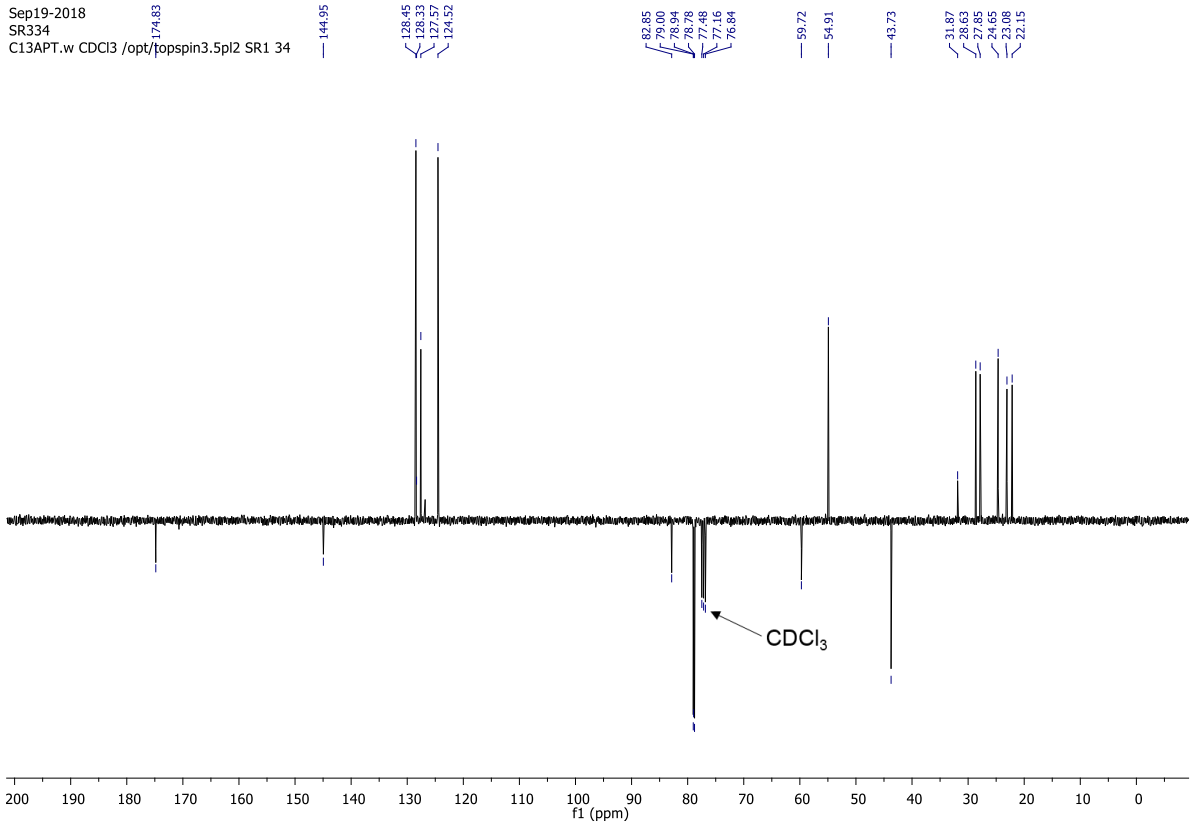


NO_2-GOx-Leu-OCumyl (2c)

^{1}H NMR (400 MHz, $CDCl_3$)

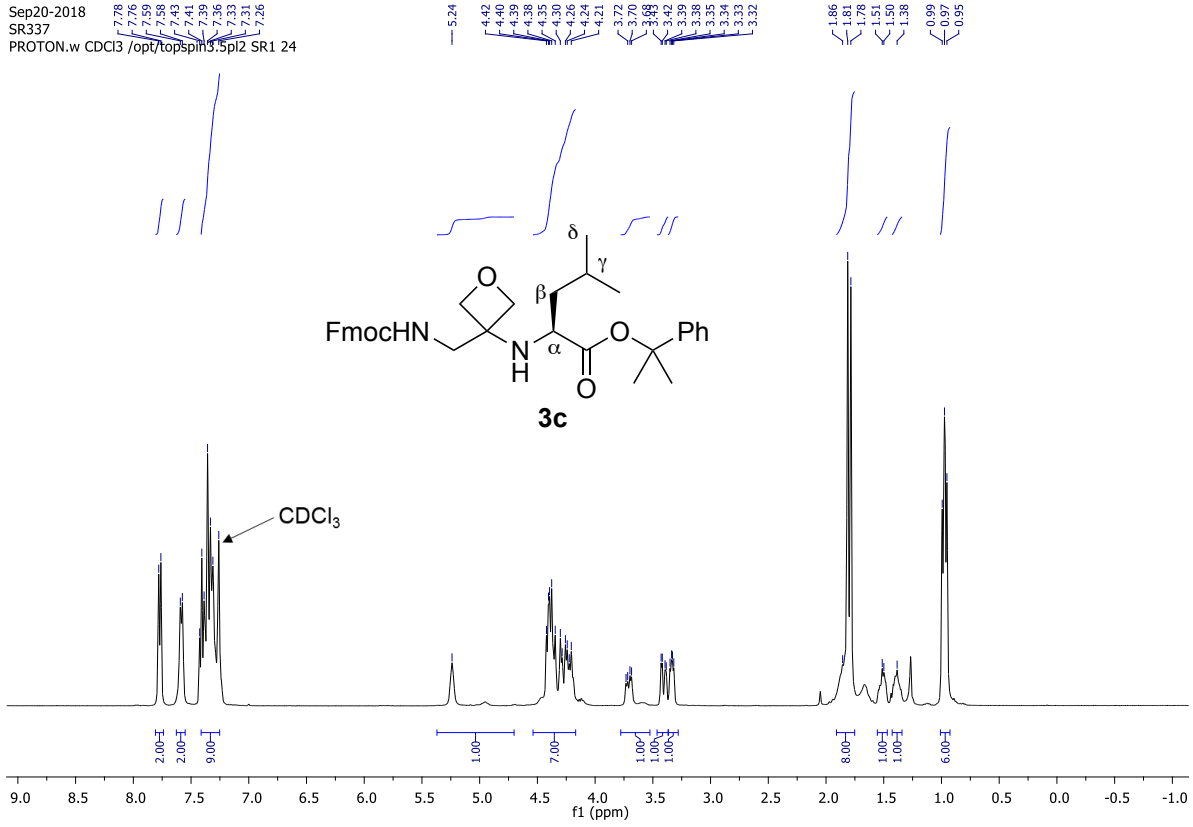


^{13}C NMR ($CDCl_3$, 101 MHz)

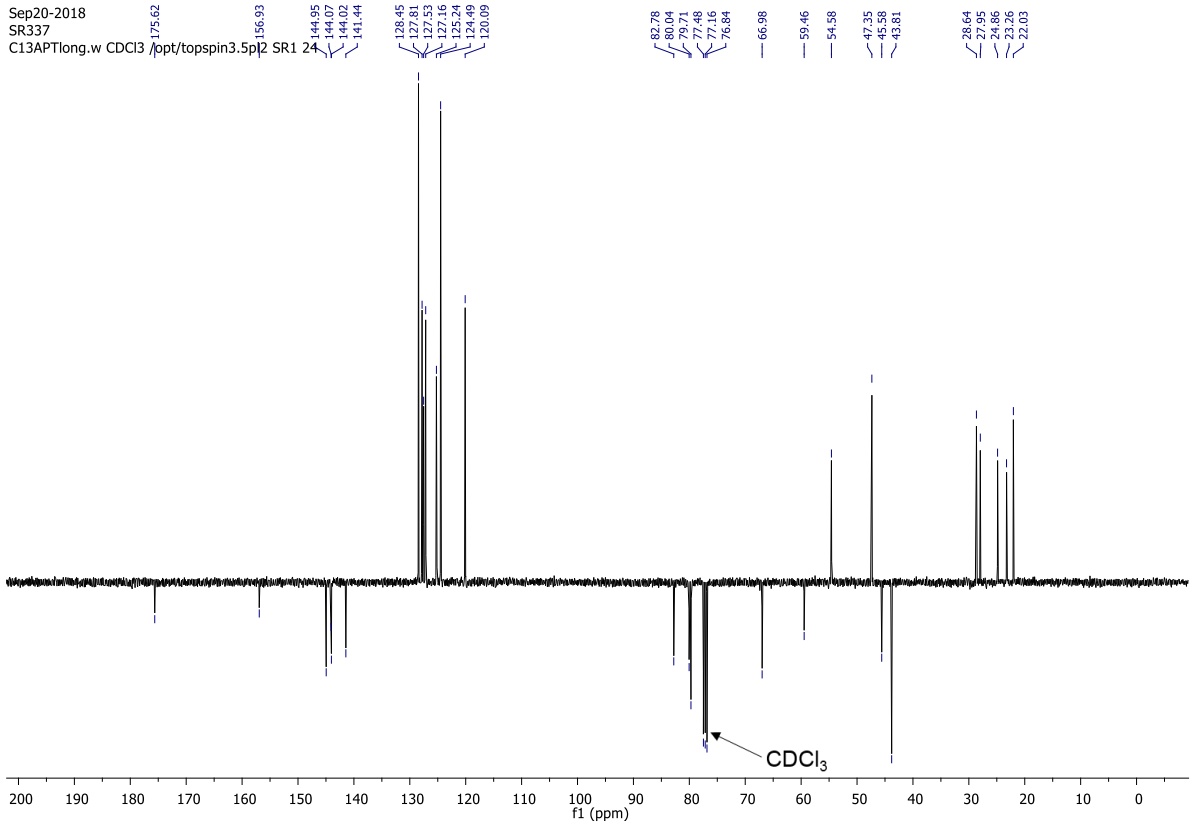


Fmoc-GOx-Leu-OCumyl (3c)

^{1}H NMR (400 MHz, $CDCl_3$)

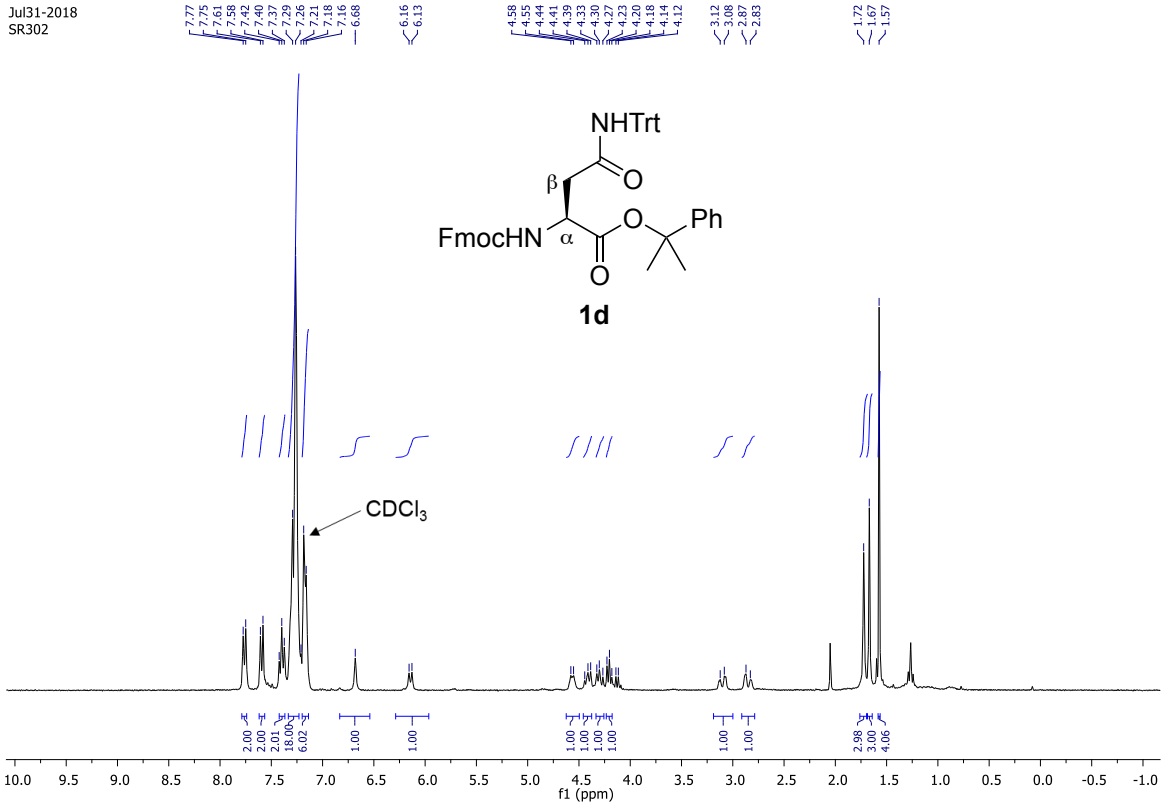


^{13}C NMR (101 MHz, $CDCl_3$)

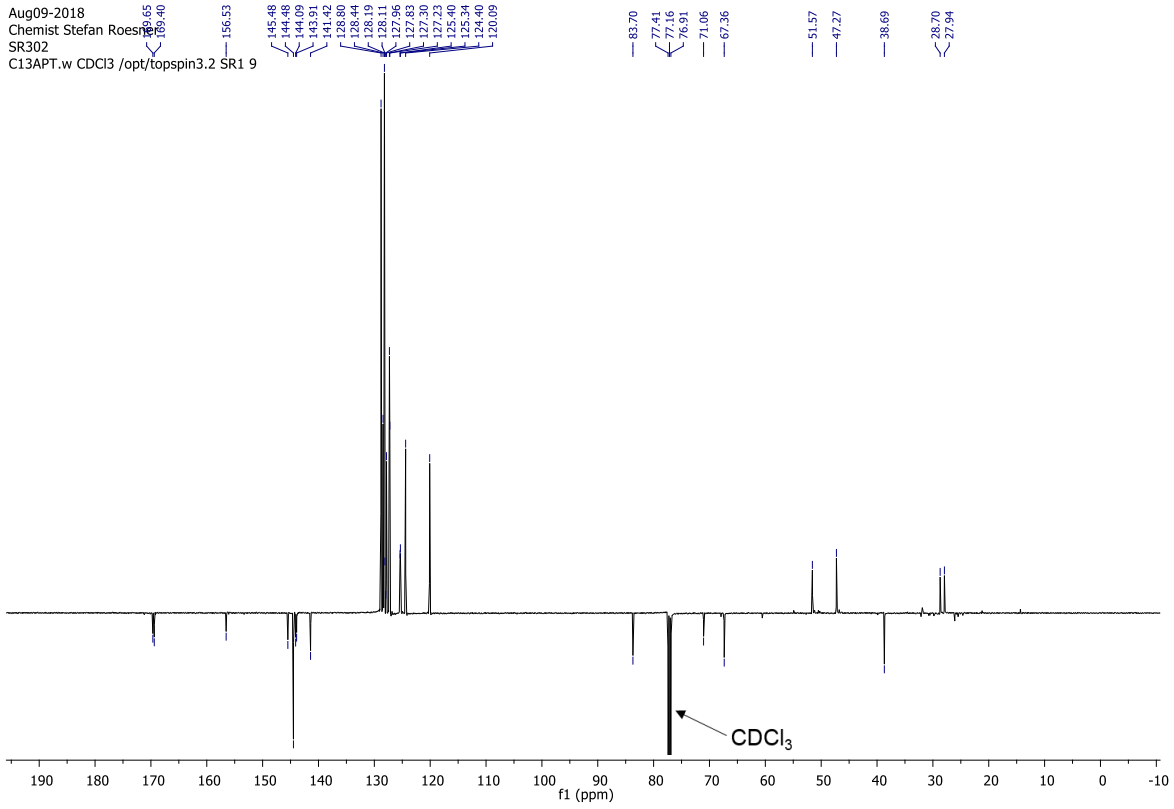


Fmoc-Asn(Trt)-OCumyl (1d)

^{1}H NMR (300 MHz, $CDCl_3$)

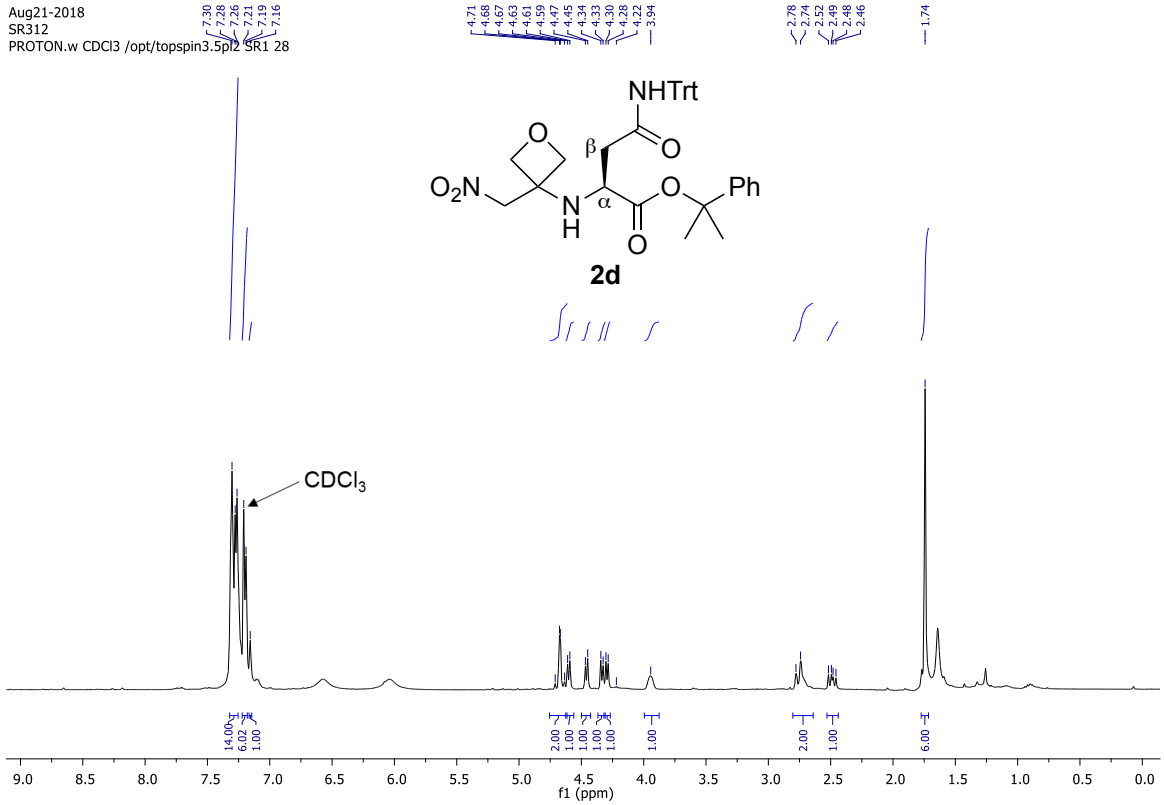


^{13}C NMR (126 MHz, $CDCl_3$)

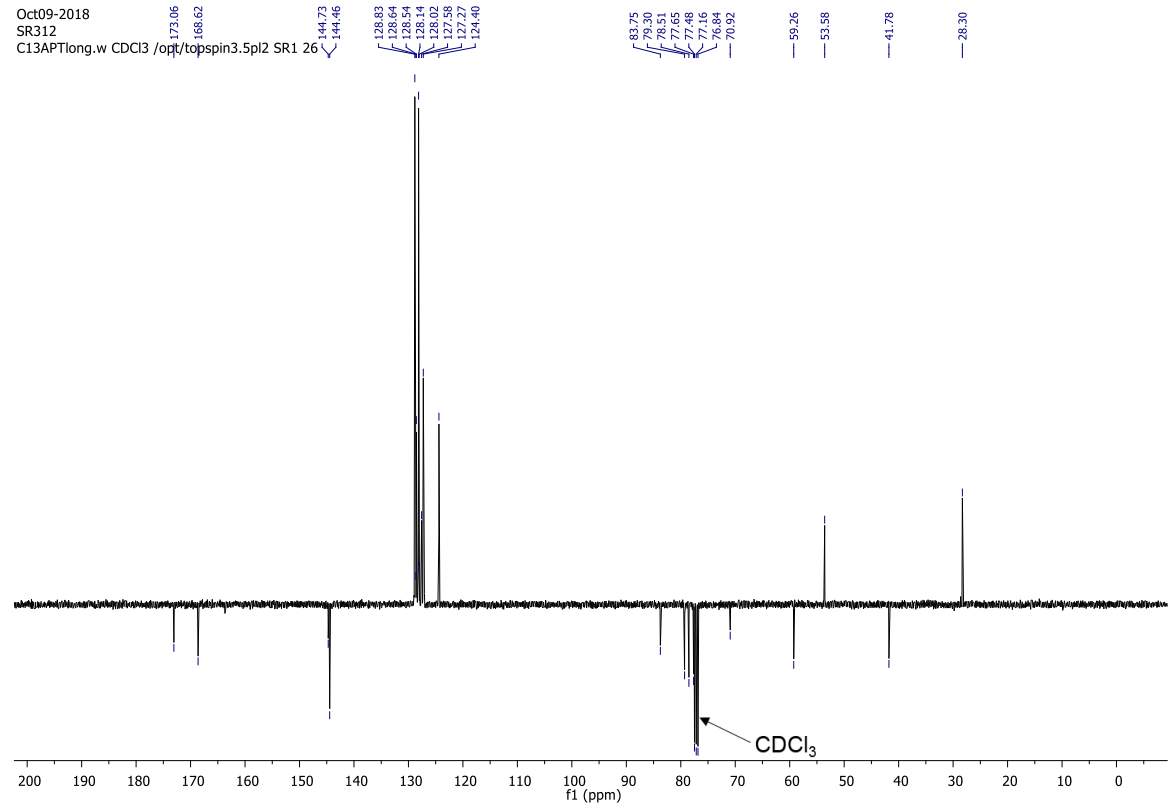


O_2N-GOx-Asn(Trt)-OCumyl (2d)

^{1}H NMR (400 MHz, $CDCl_3$)

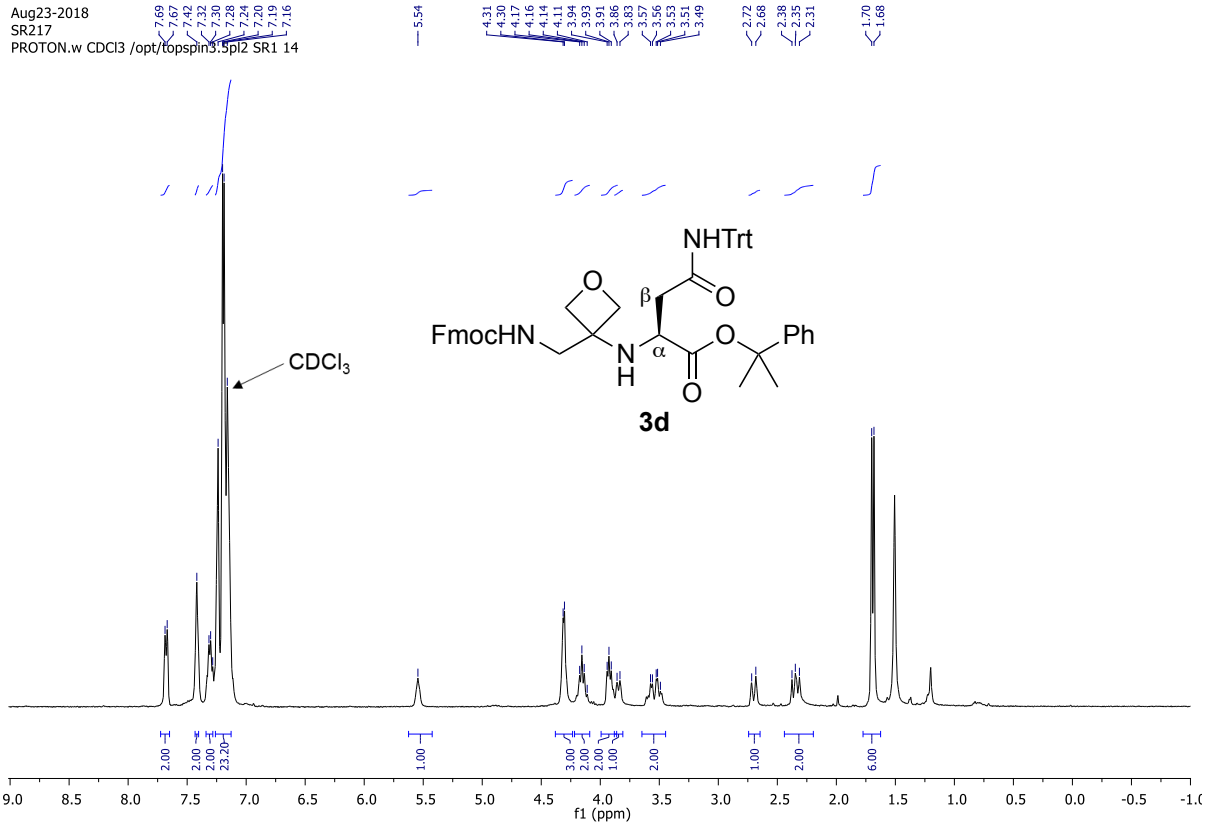


^{13}C NMR (101 MHz, $CDCl_3$)

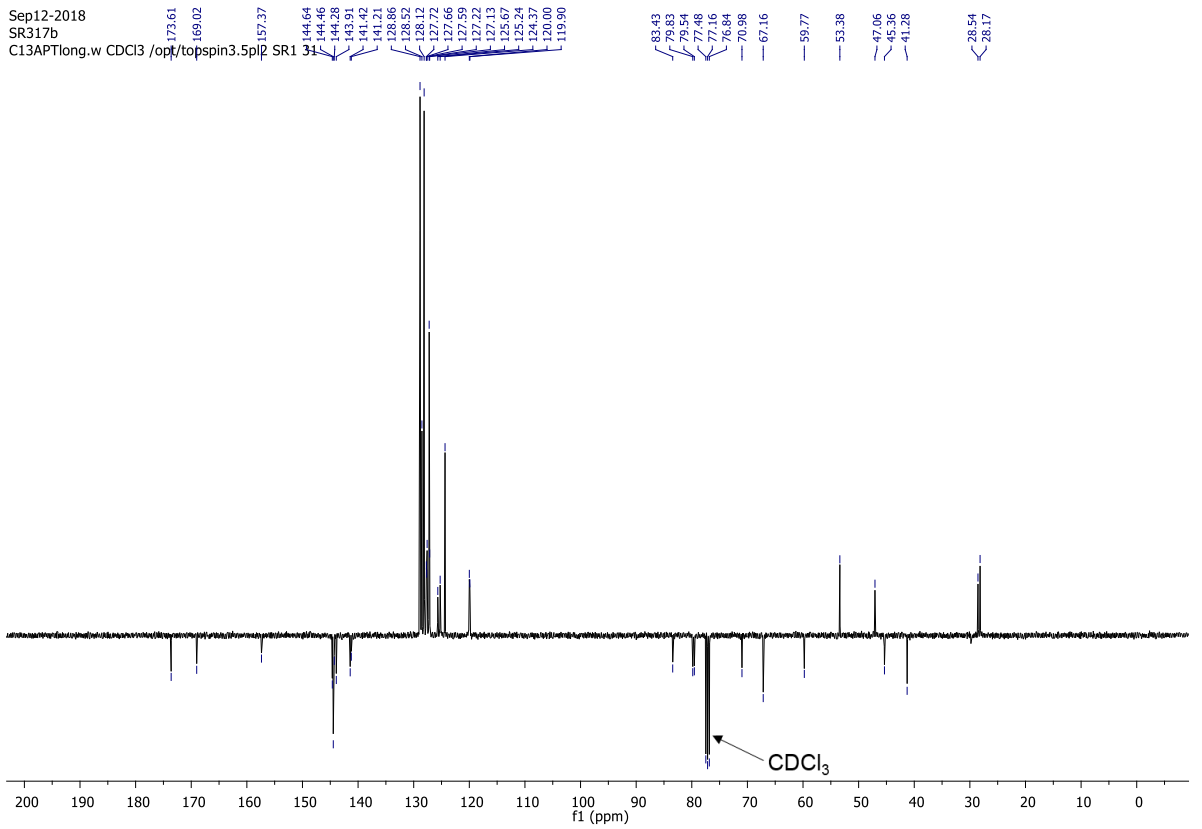


Fmoc-GOx-Asn(Trt)-OCumyl (3d)

^{1}H NMR (400 MHz, $CDCl_3$)

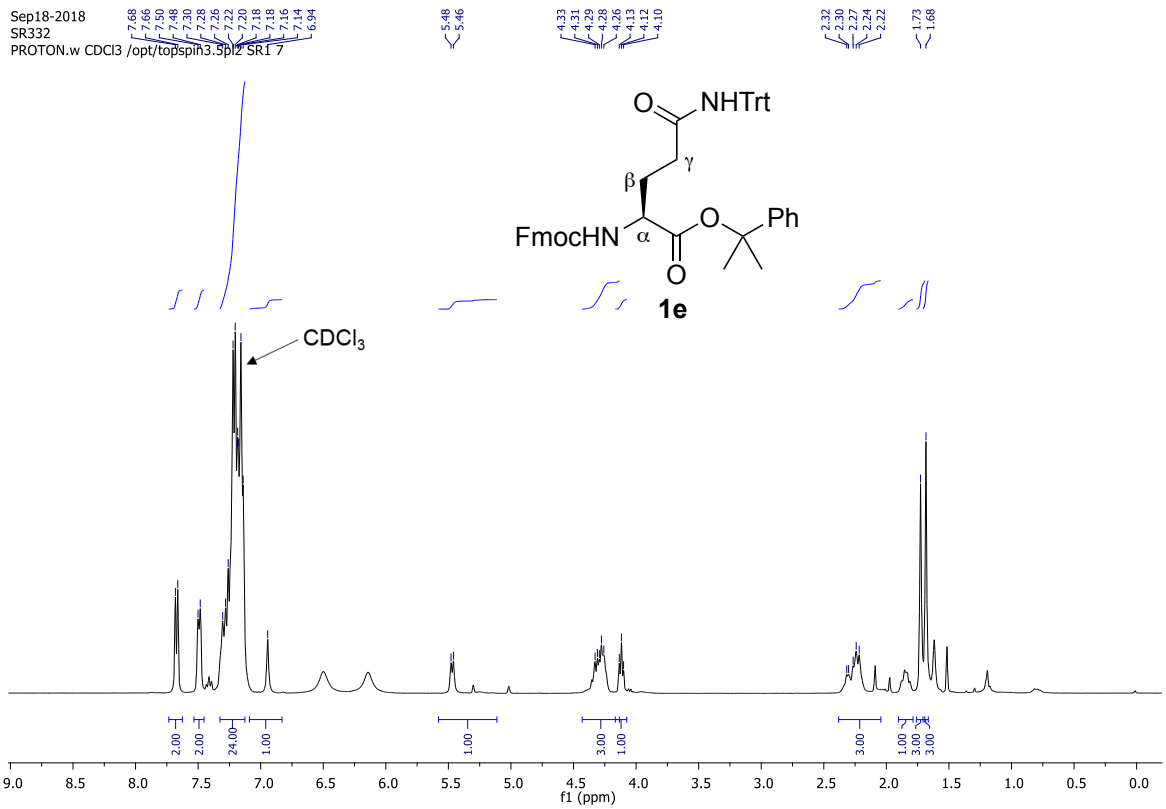


^{13}C NMR (101 MHz, $CDCl_3$)

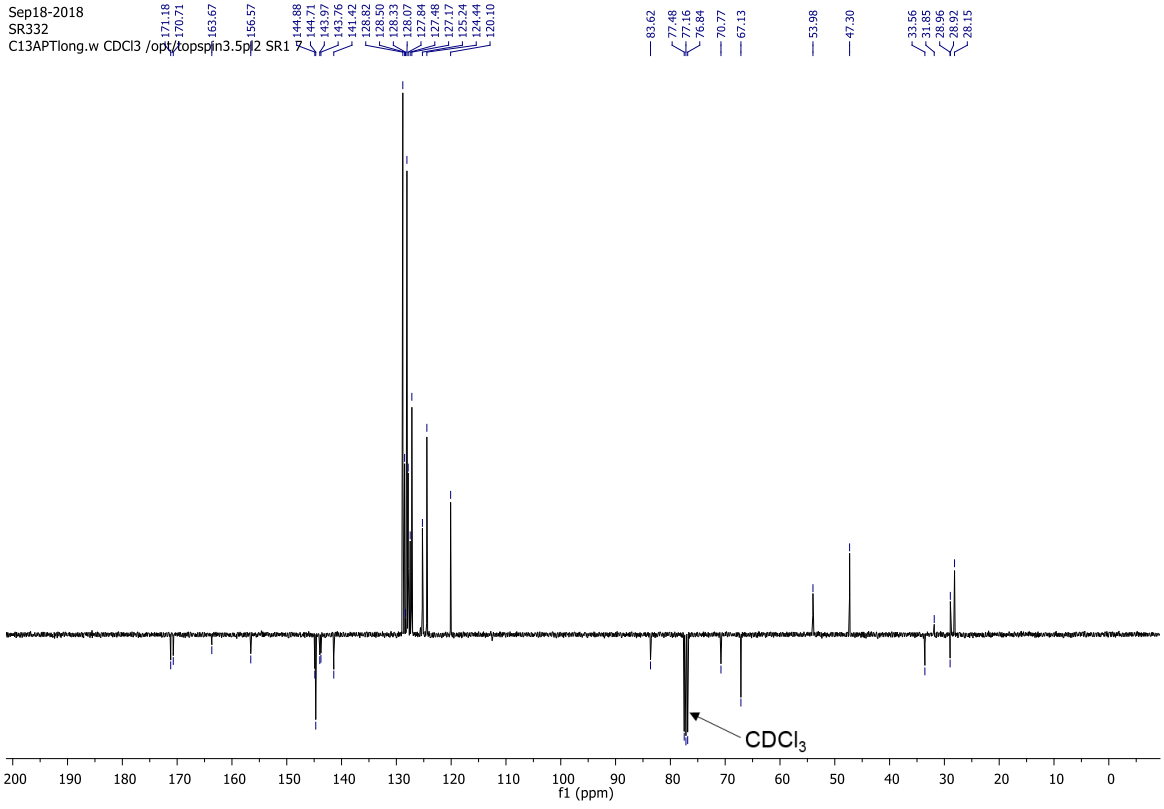


Fmoc-Gln(Trt)-OCumyl (1e)

^{1}H NMR (400 MHz, $CDCl_3$)

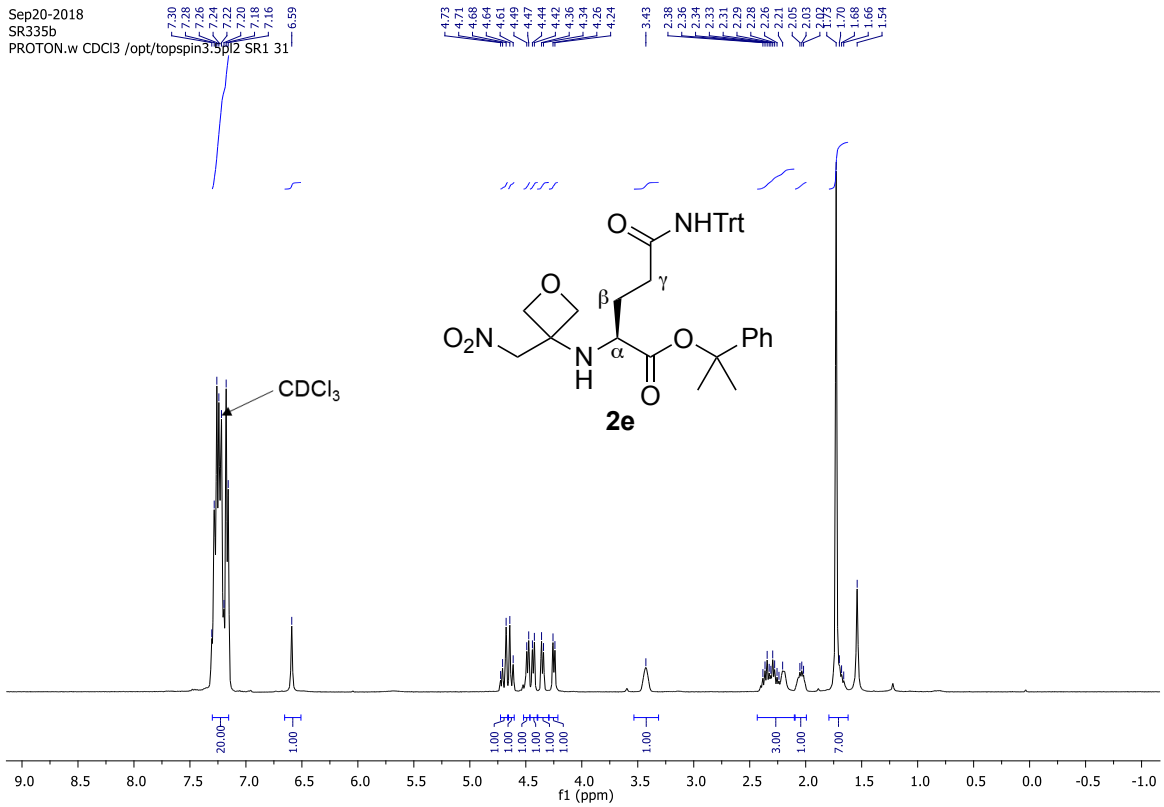


^{13}H NMR (101 MHz, $CDCl_3$)

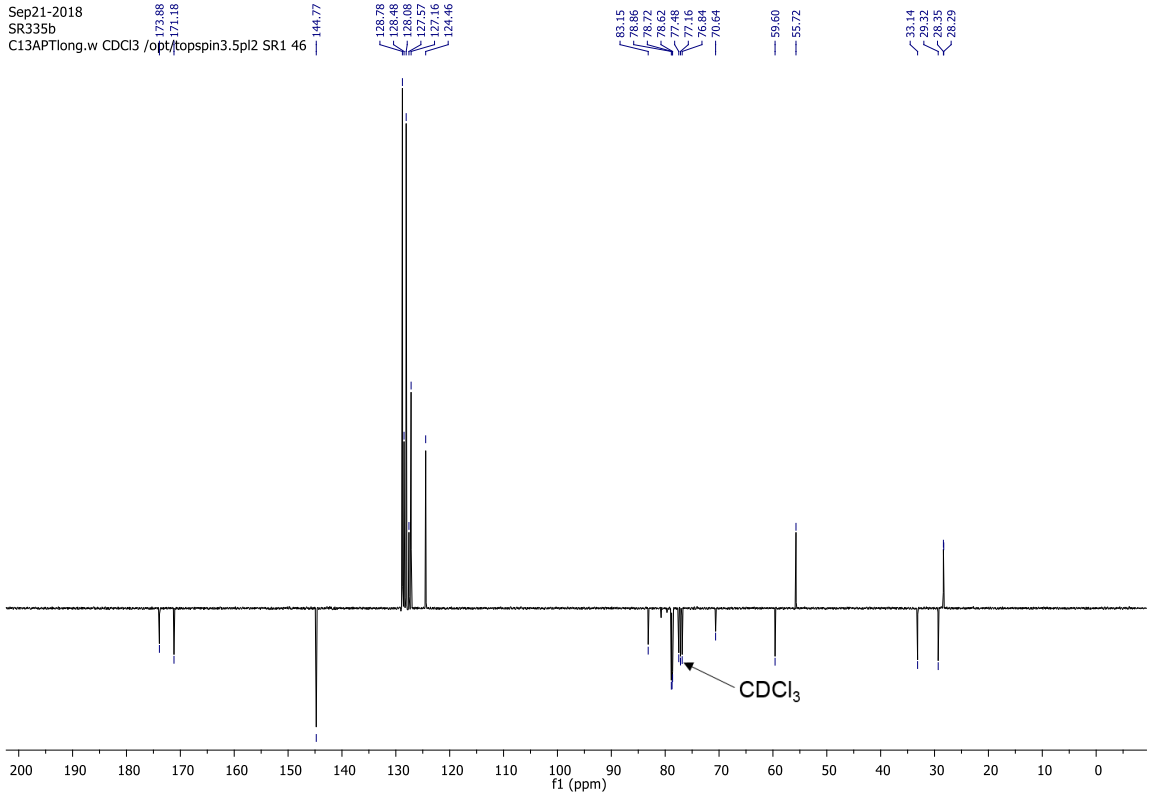


O_2N-GOx-Gln(Trt)-OCumyl (2e)

^{1}H NMR (400 MHz, $CDCl_3$)

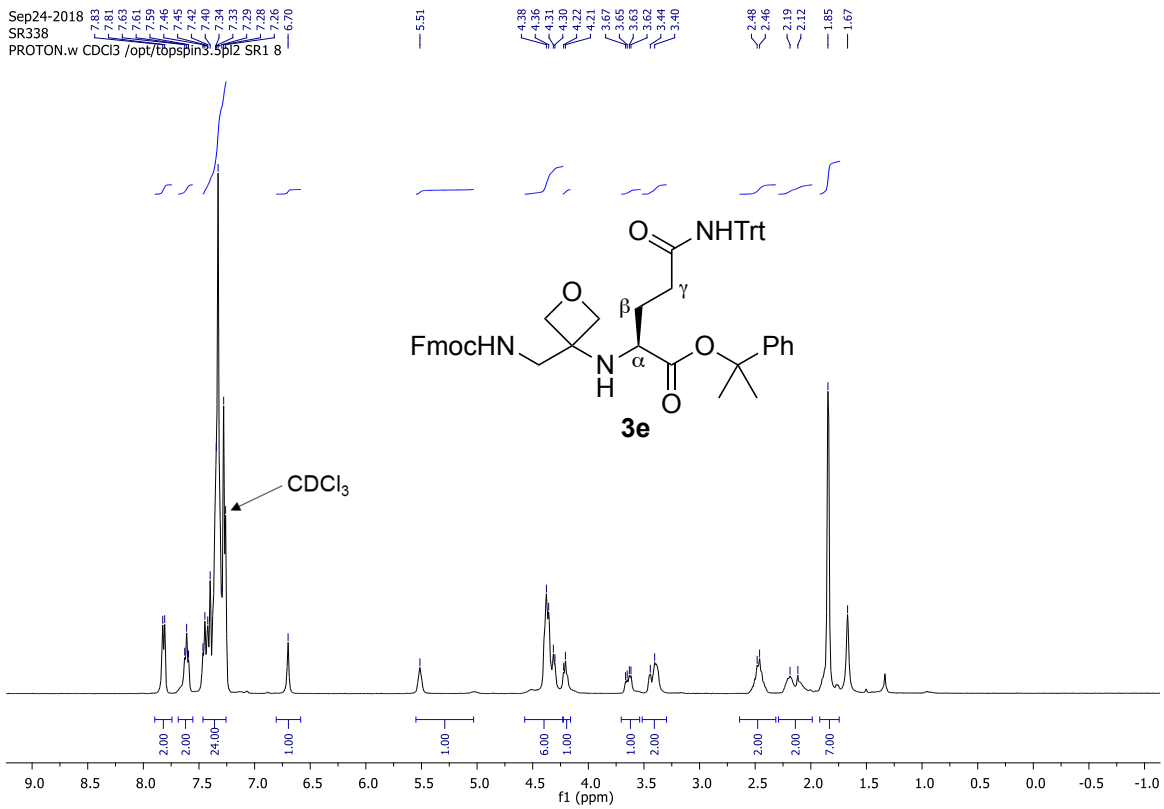


^{13}C NMR (101 MHz, $CDCl_3$)

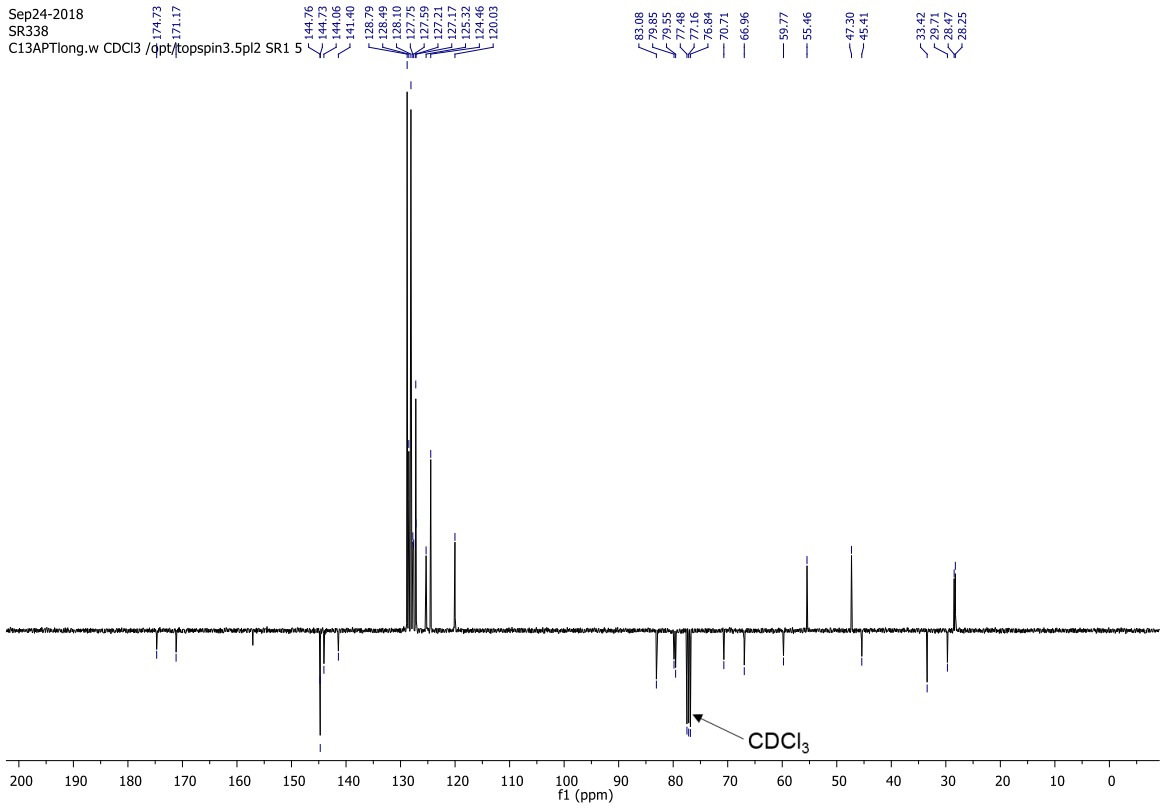


Fmoc-GOx-Gln(Trt)-OCumyl (3e)

^{1}H NMR (400 MHz, $CDCl_3$)

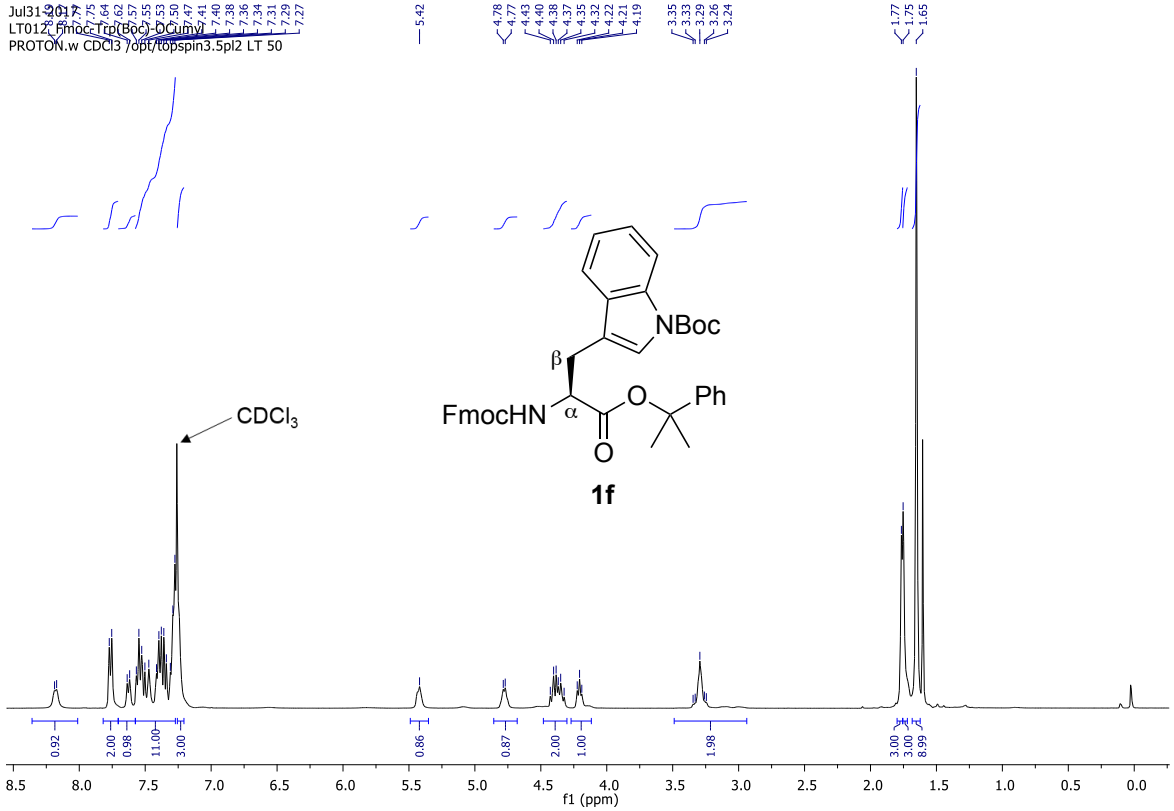


^{13}C NMR (101 MHz, $CDCl_3$)

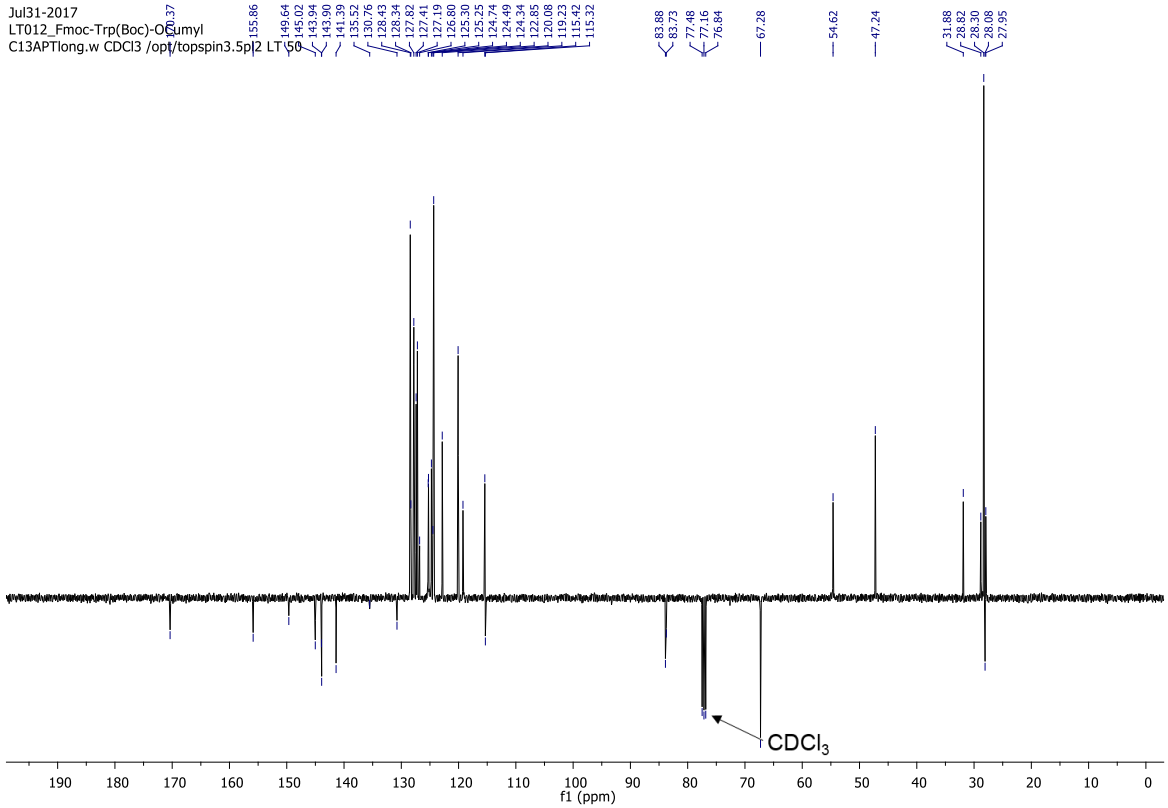


Fmoc-Trp(Boc)-OCumyl (1f)

^{1}H NMR (400 MHz, $CDCl_3$)

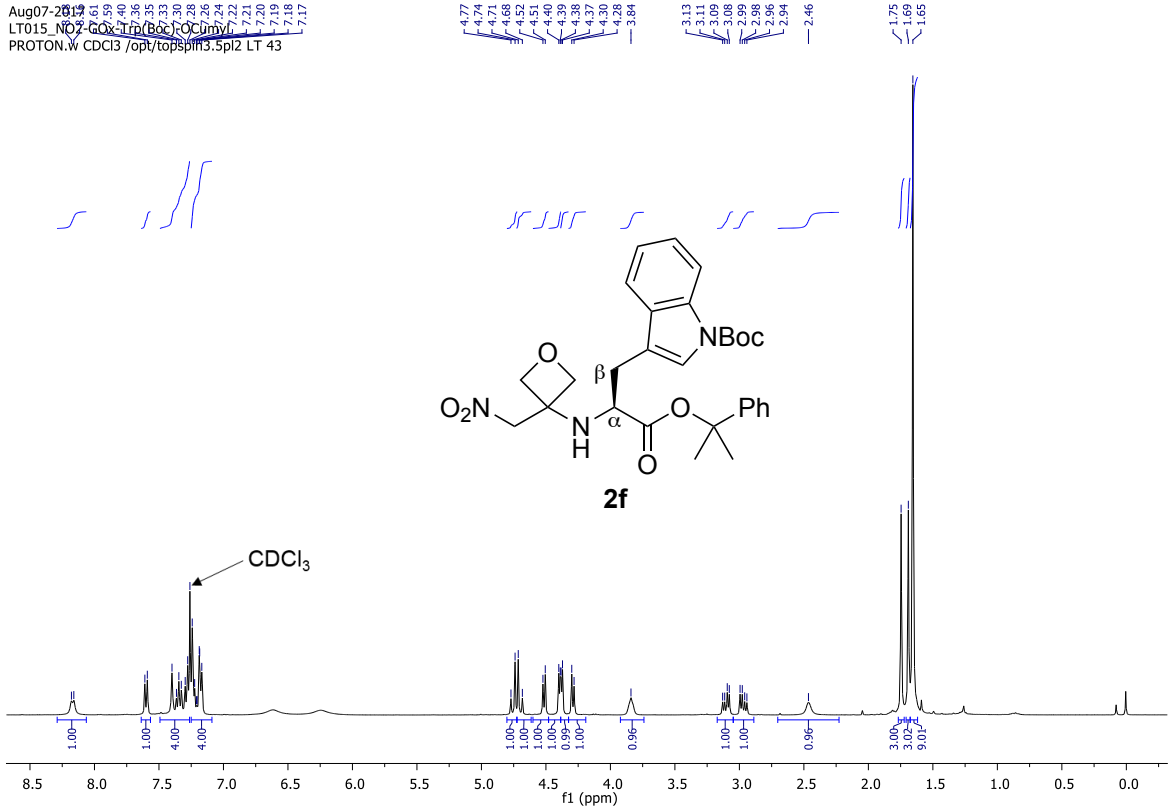


^{13}C NMR (101 MHz, $CDCl_3$)

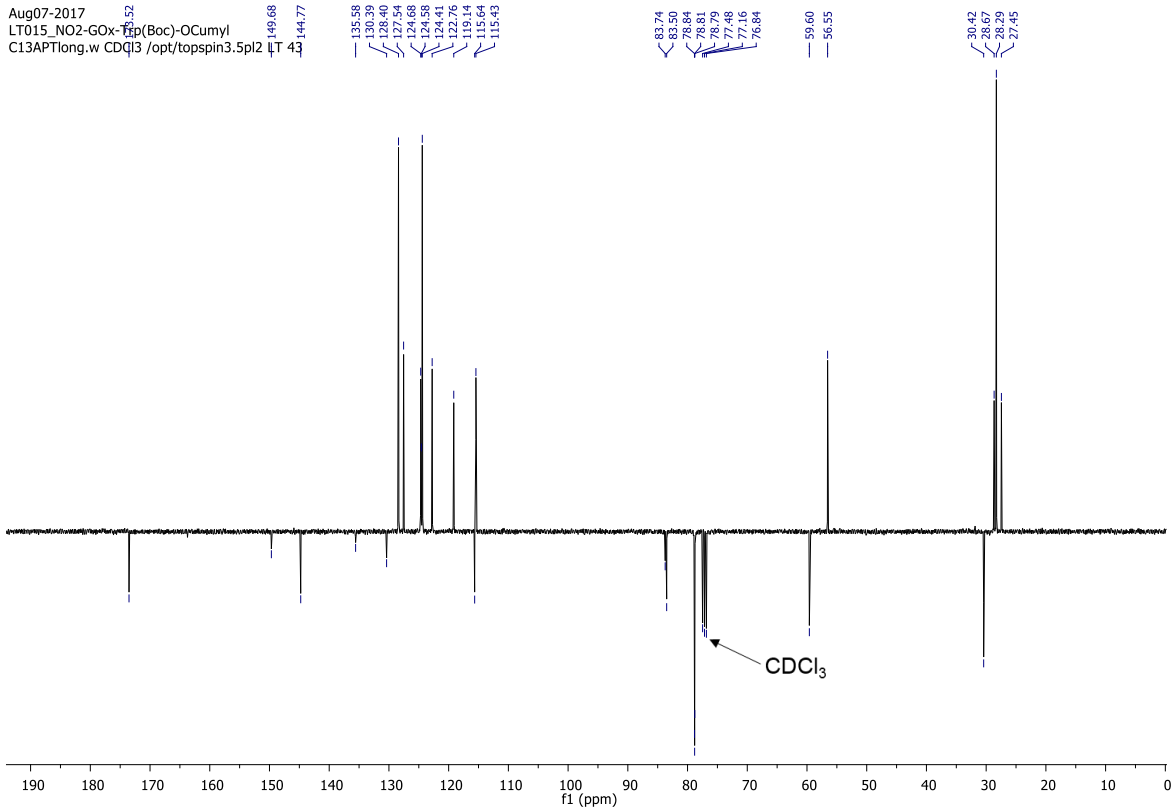


NO_2-GOx-Trp(Boc)-OCumyl (2f)

^{1}H NMR (400 MHz, $CDCl_3$)

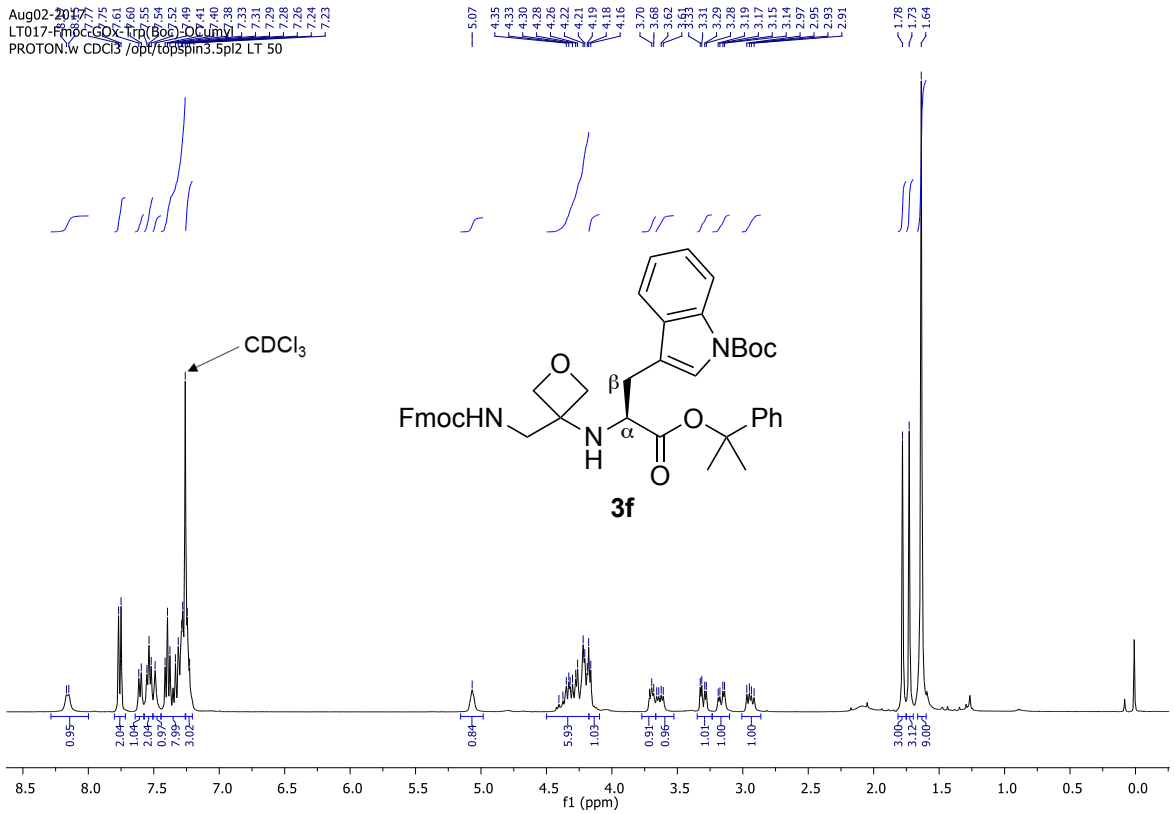


^{13}C NMR (101 MHz, $CDCl_3$)

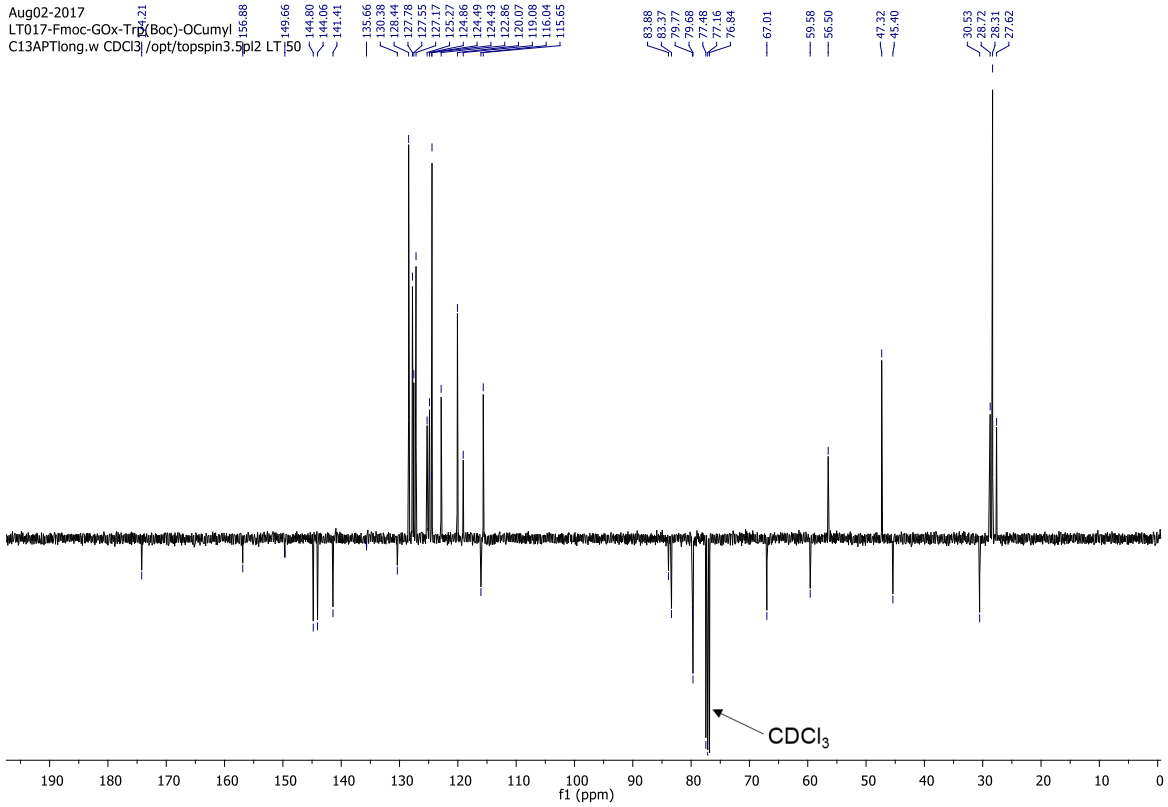


Fmoc-GOx-Trp(Boc)-OCumyl (3f)

^{1}H NMR (400 MHz, $CDCl_3$)

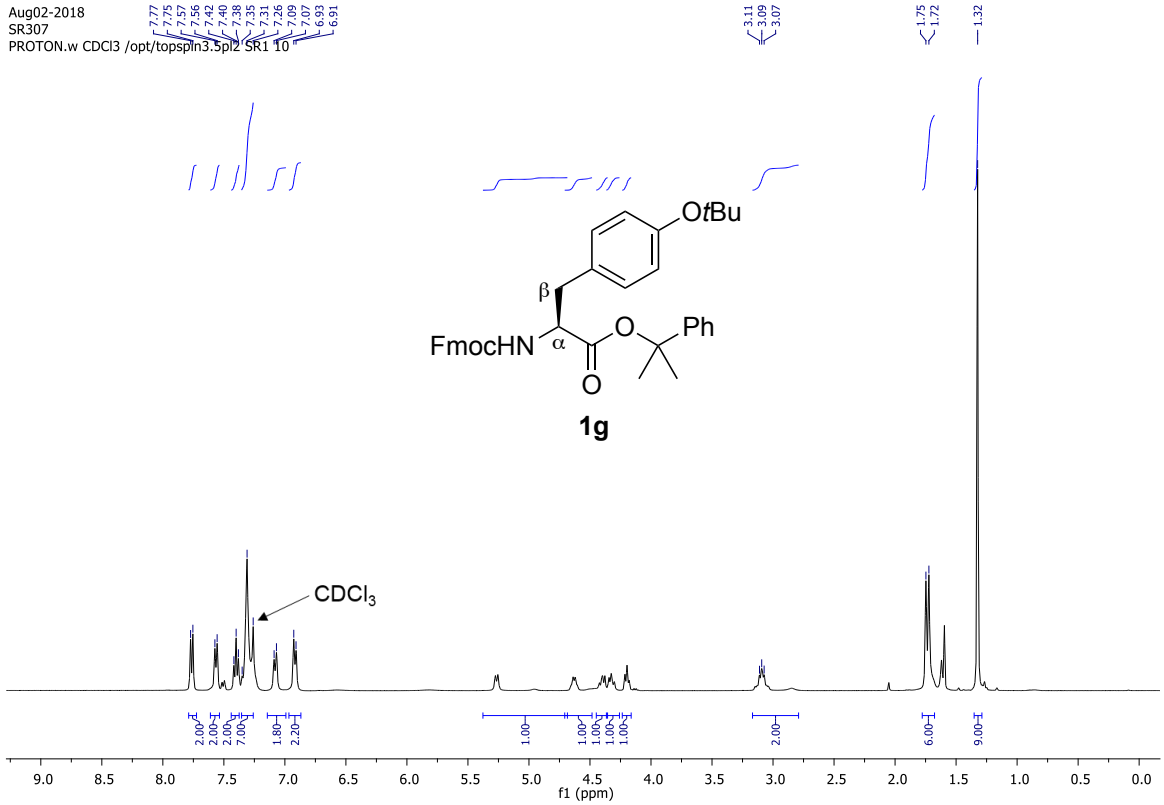


^{13}C NMR (101 MHz, $CDCl_3$)

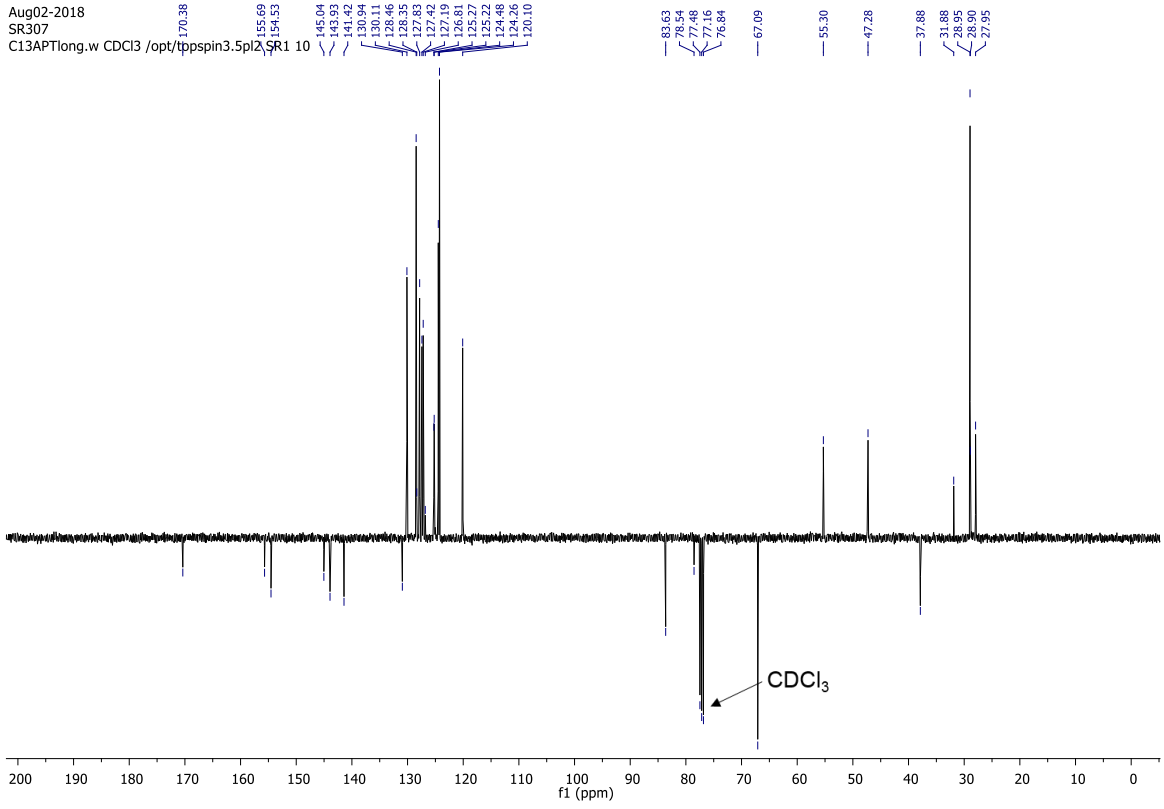


Fmoc-Tyr(*t*Bu)-OCumyl (1g)

^{1}H NMR (400 MHz, $CDCl_3$)

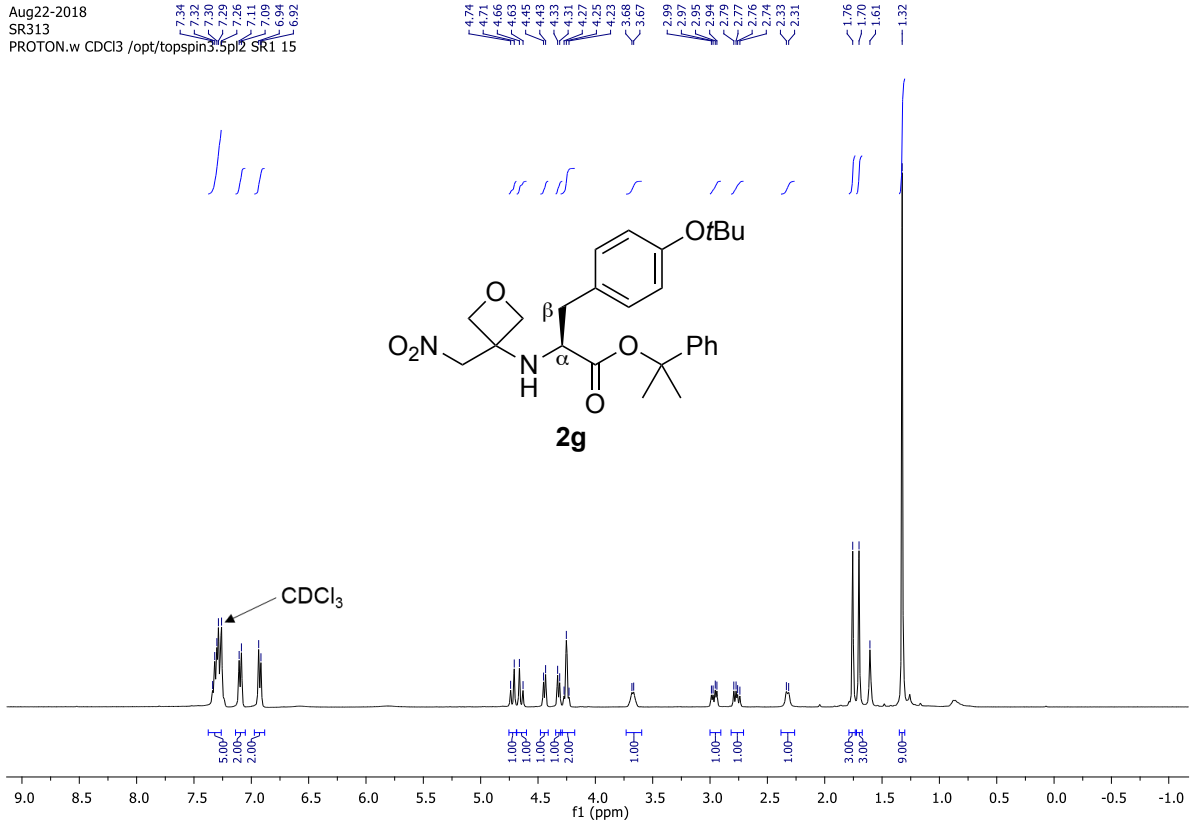


^{13}C NMR (101 MHz, $CDCl_3$)

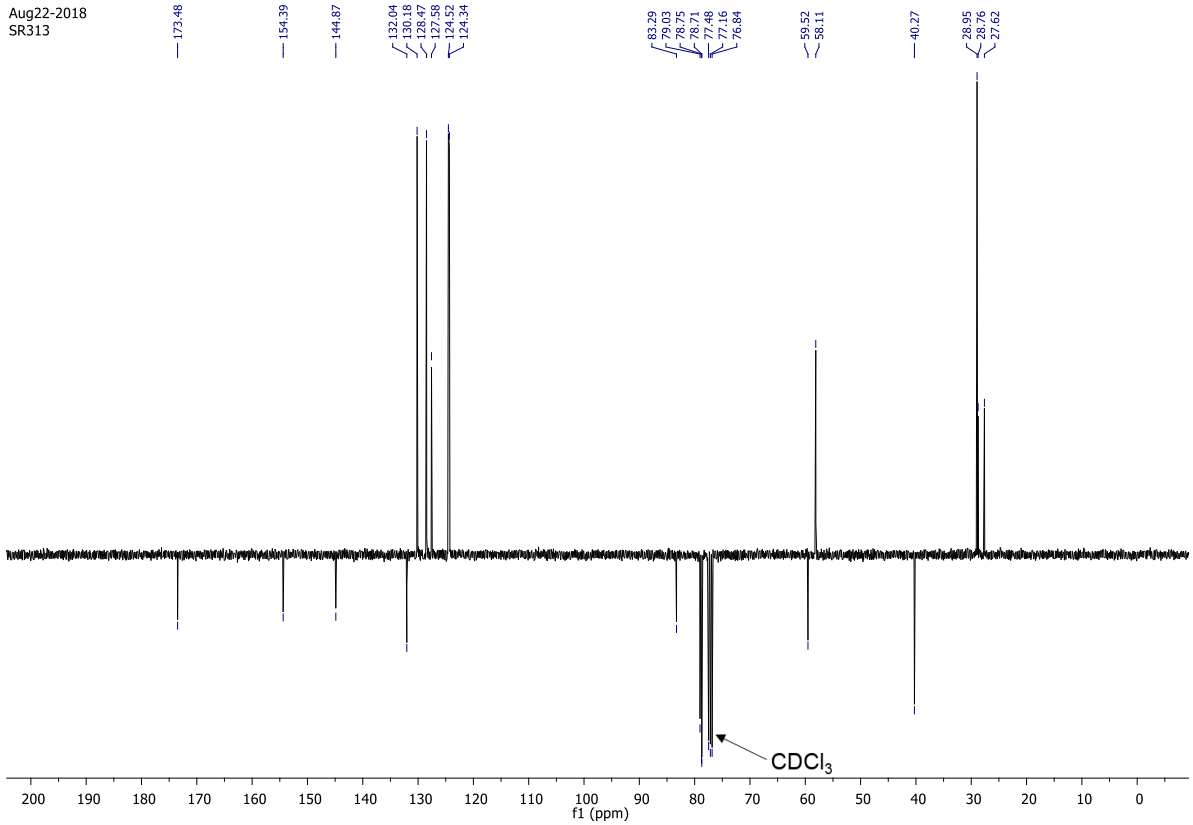


O_2N-GOx-Tyr(*t*Bu)-OCumyl (2g)

^{1}H NMR (400 MHz, $CDCl_3$)

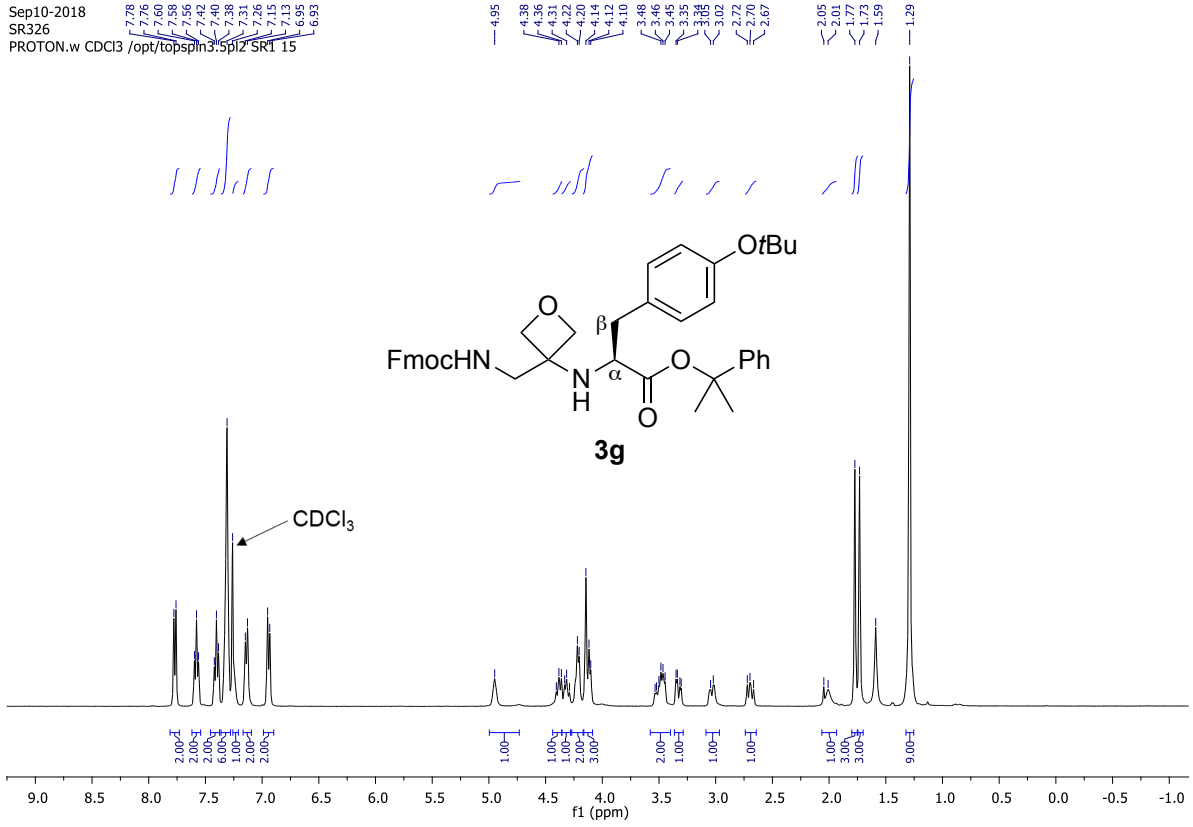


^{13}C NMR (101 MHz, $CDCl_3$)

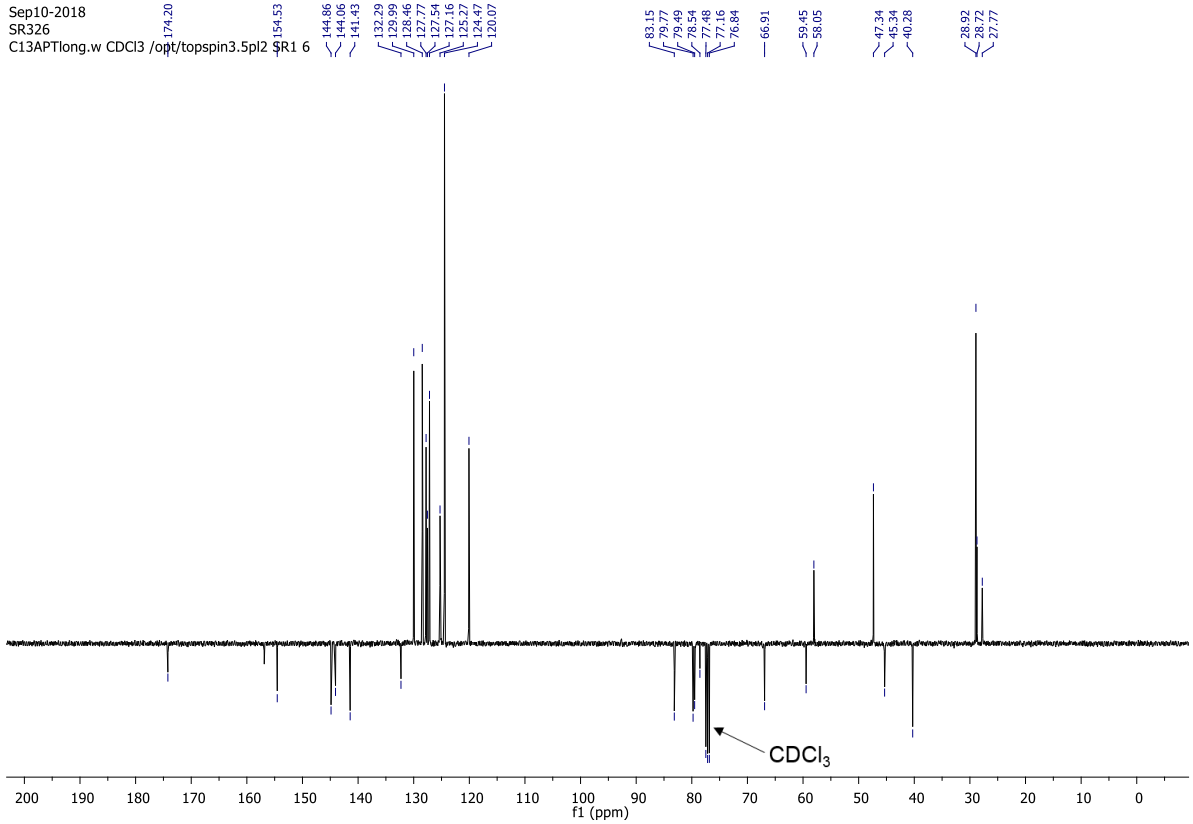


Fmoc-GOx-Tyr(*t*Bu)-OCumyl (3g)

^{1}H NMR (400 MHz, $CDCl_3$)

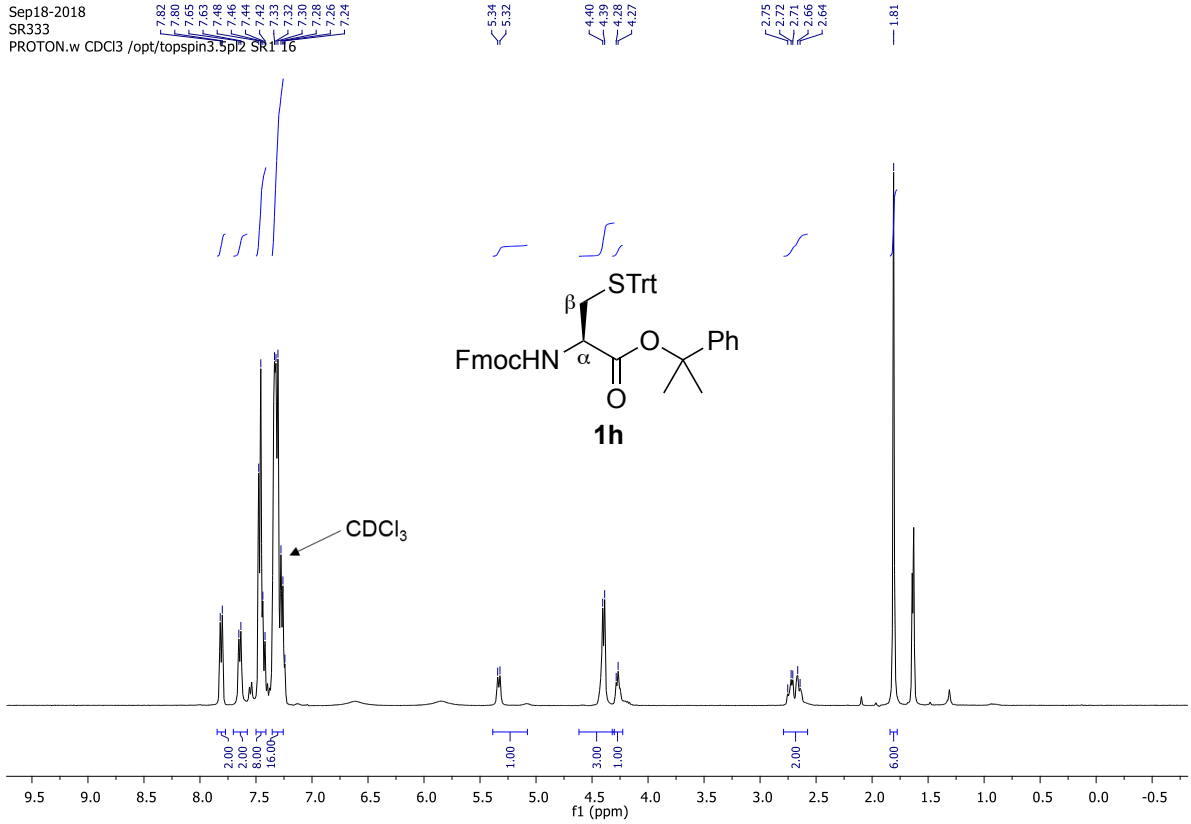


^{13}C NMR (101 MHz, $CDCl_3$)

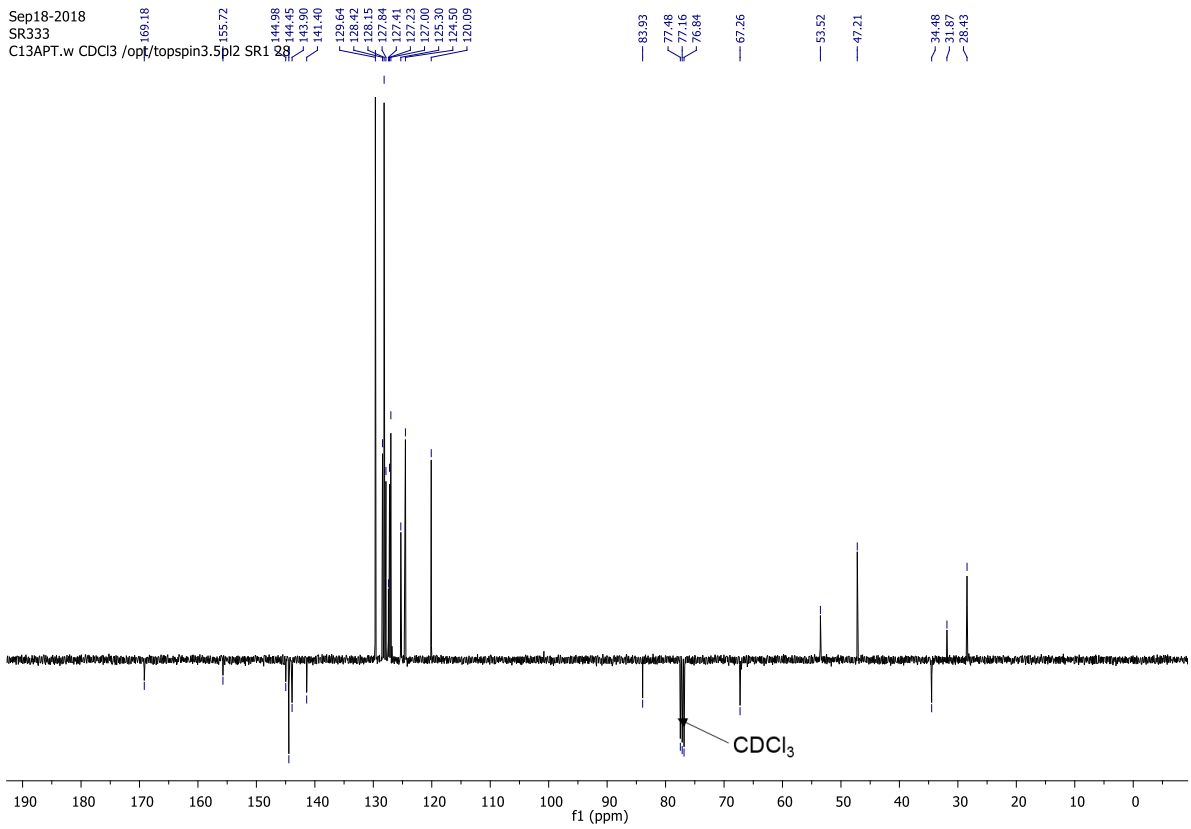


Fmoc-Cys(Trt)-OCumyl (1h)

^{1}H NMR (400 MHz, $CDCl_3$)

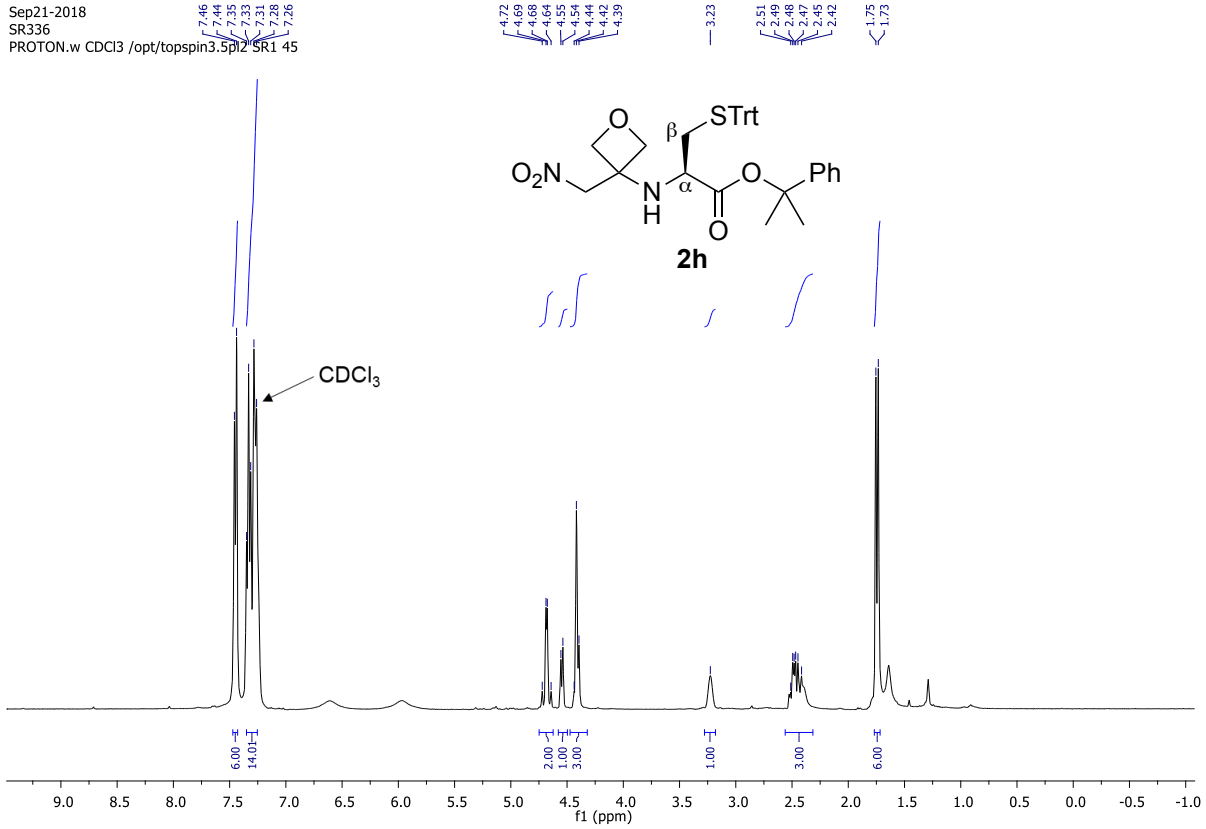


^{13}C NMR (101 MHz, $CDCl_3$)

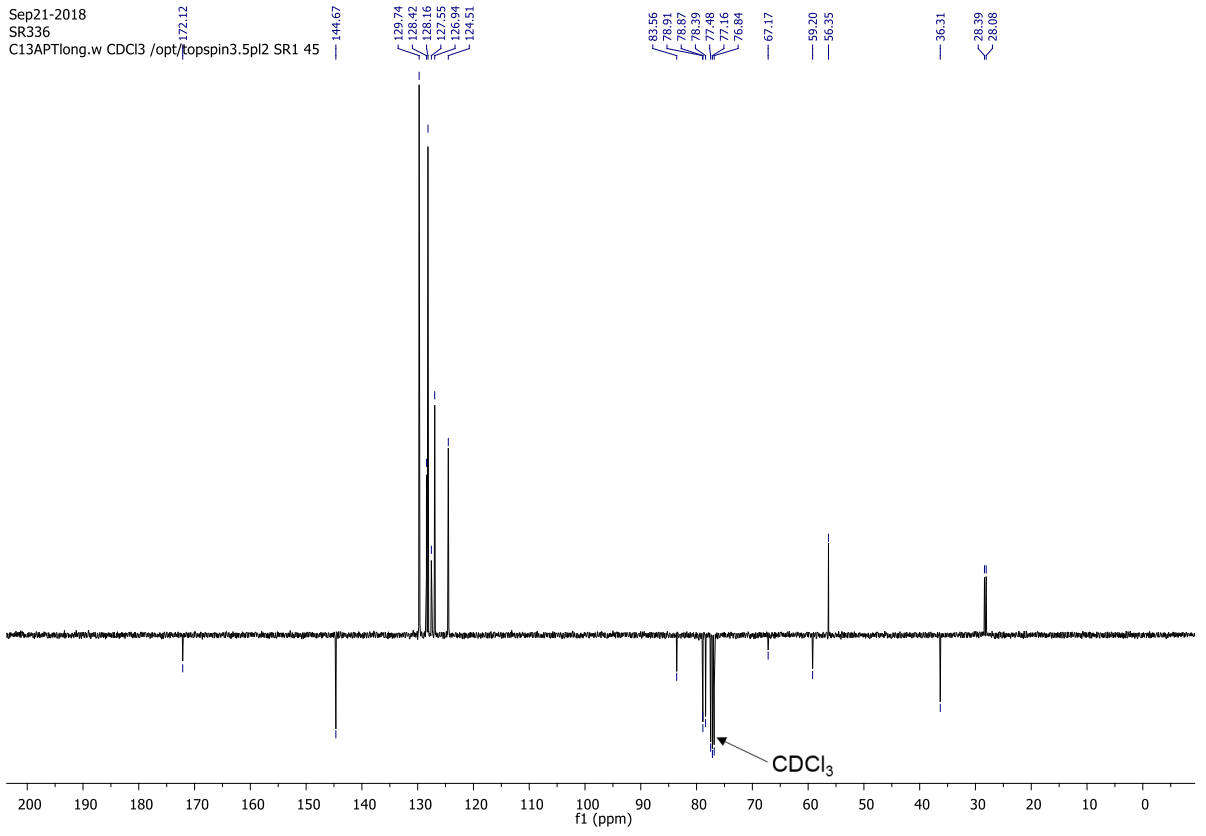


NO_2-GOx-Cys(Trt)-OCumyl (2h)

^{1}H NMR (400 MHz, $CDCl_3$)

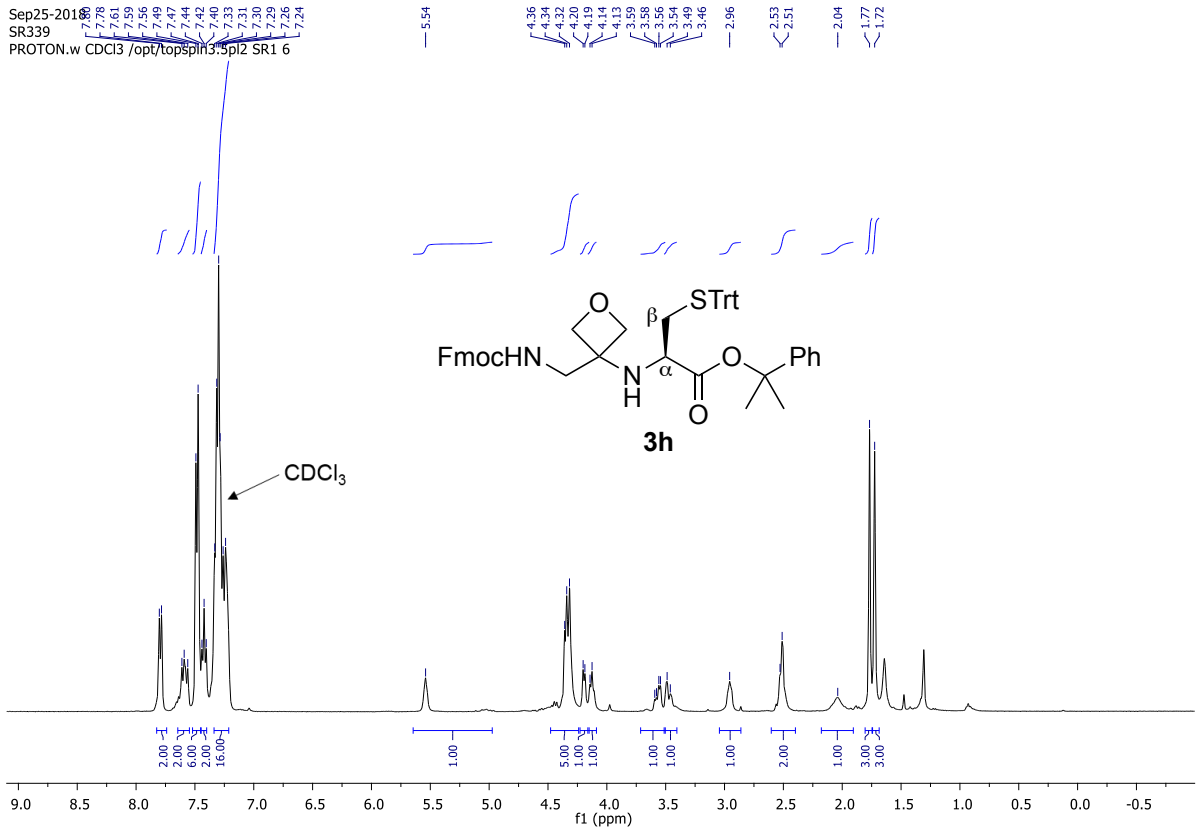


^{13}C NMR (101 MHz, $CDCl_3$)

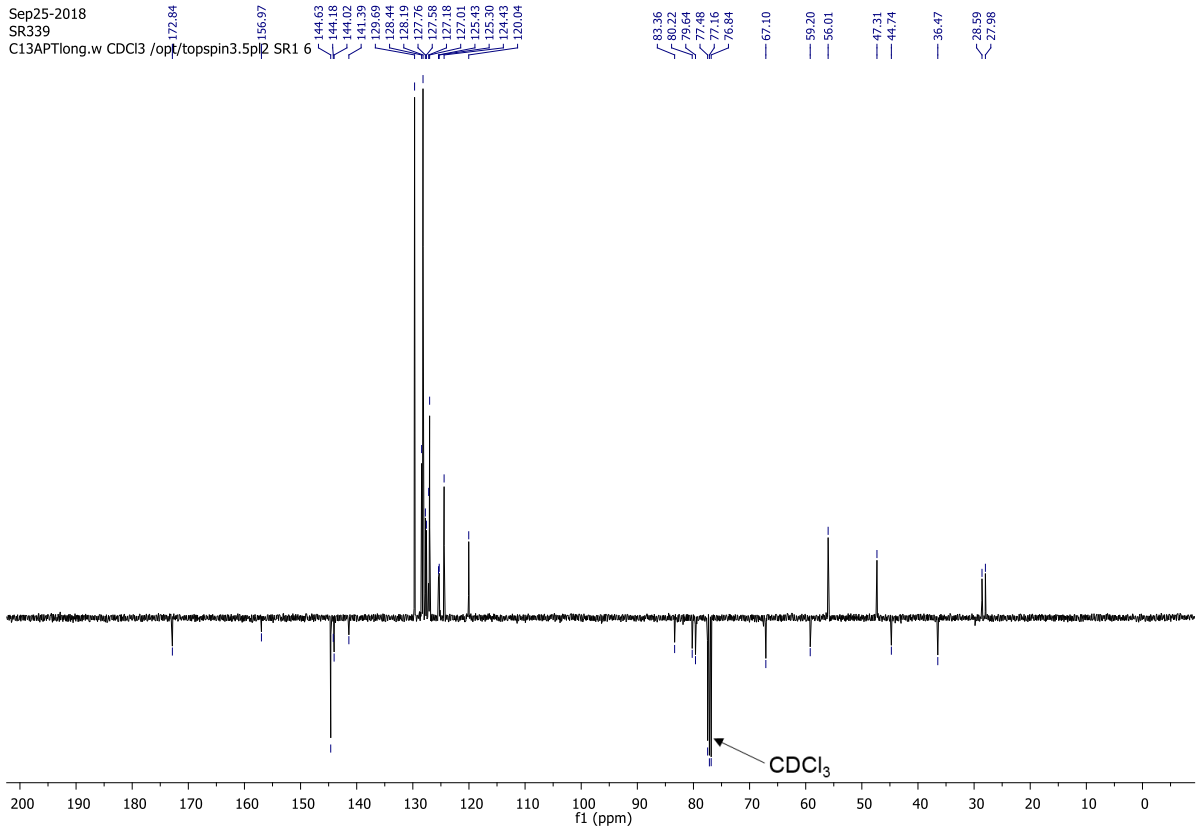


Fmoc-GOx-Cys(Trt)-OCumyl (3h)

^{1}H NMR (400 MHz, $CDCl_3$)

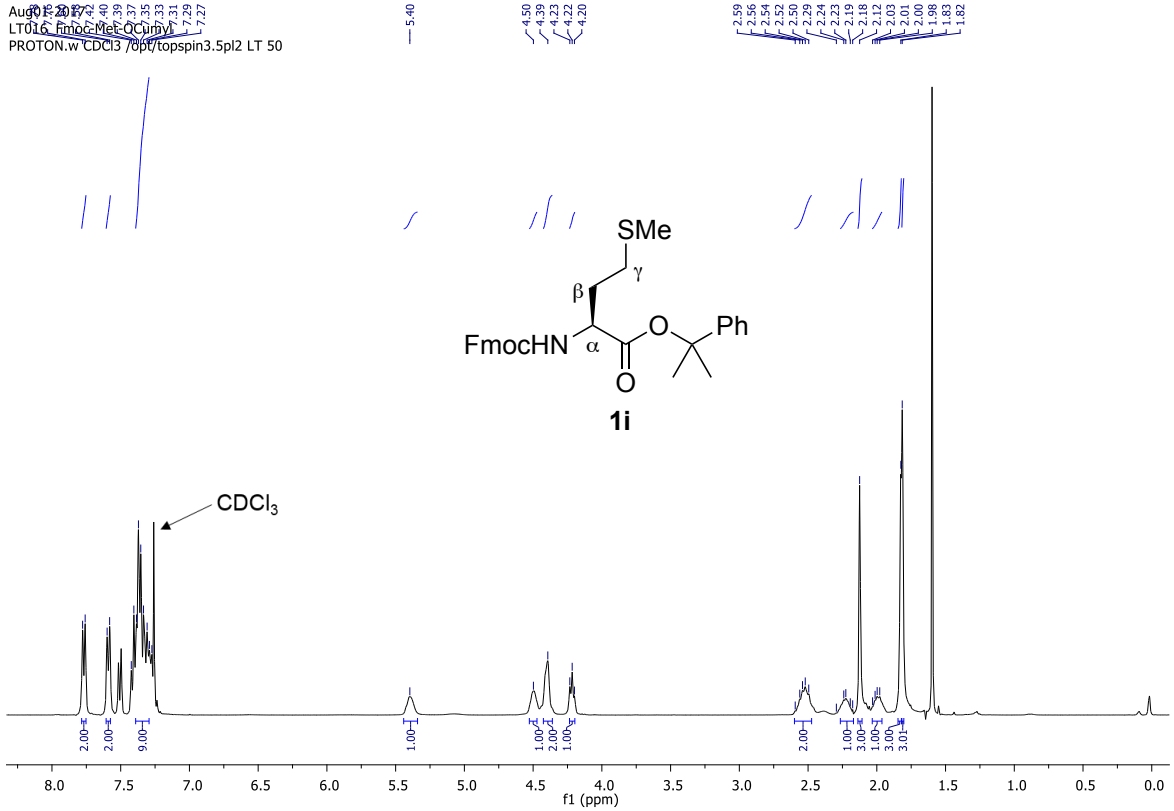


^{13}C NMR (101 MHz, $CDCl_3$)

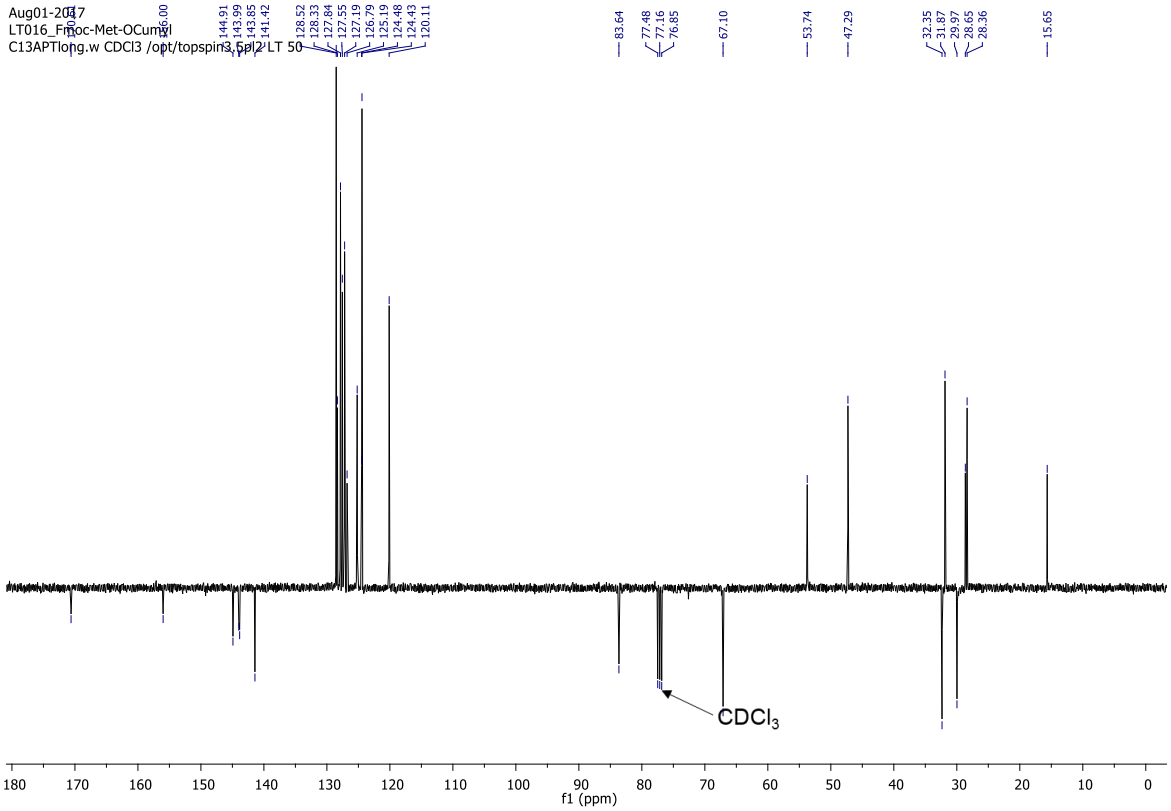


Fmoc-Met-OCumyl (1i)

^{1}H NMR (400 MHz, $CDCl_3$)

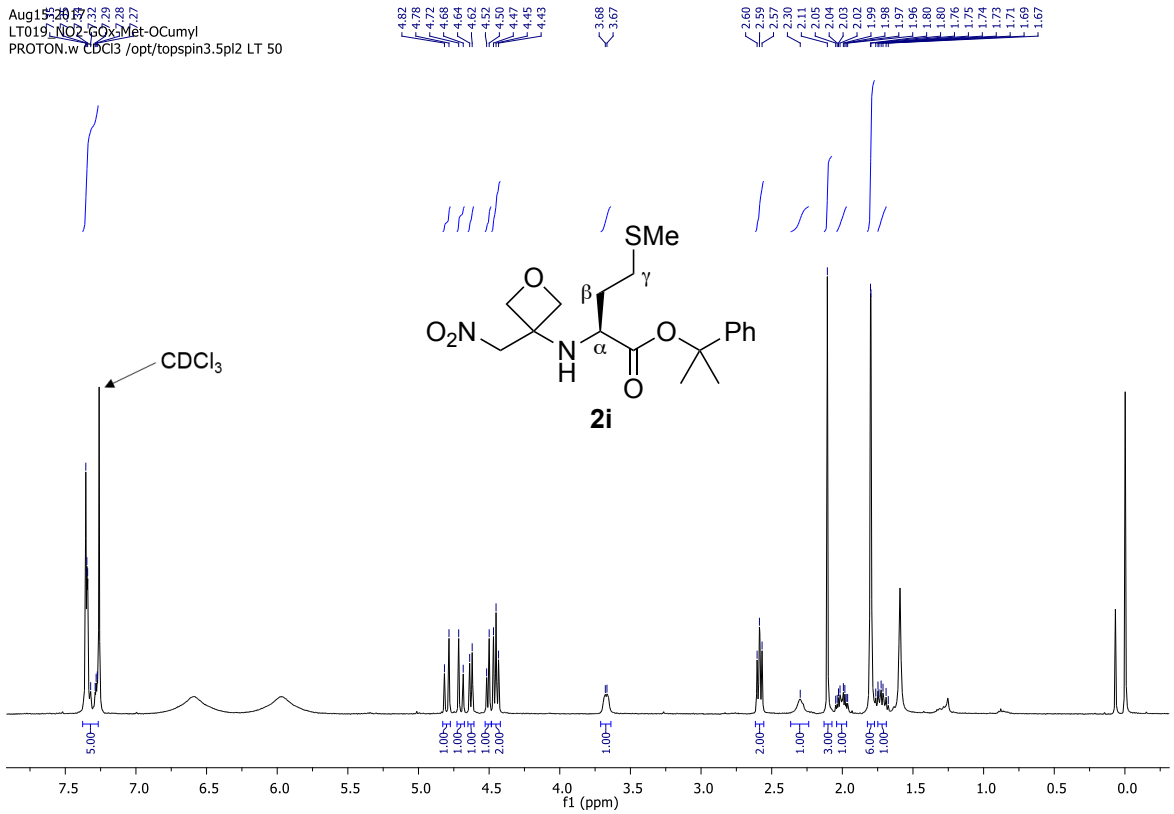


^{13}C NMR (101 MHz, $CDCl_3$)

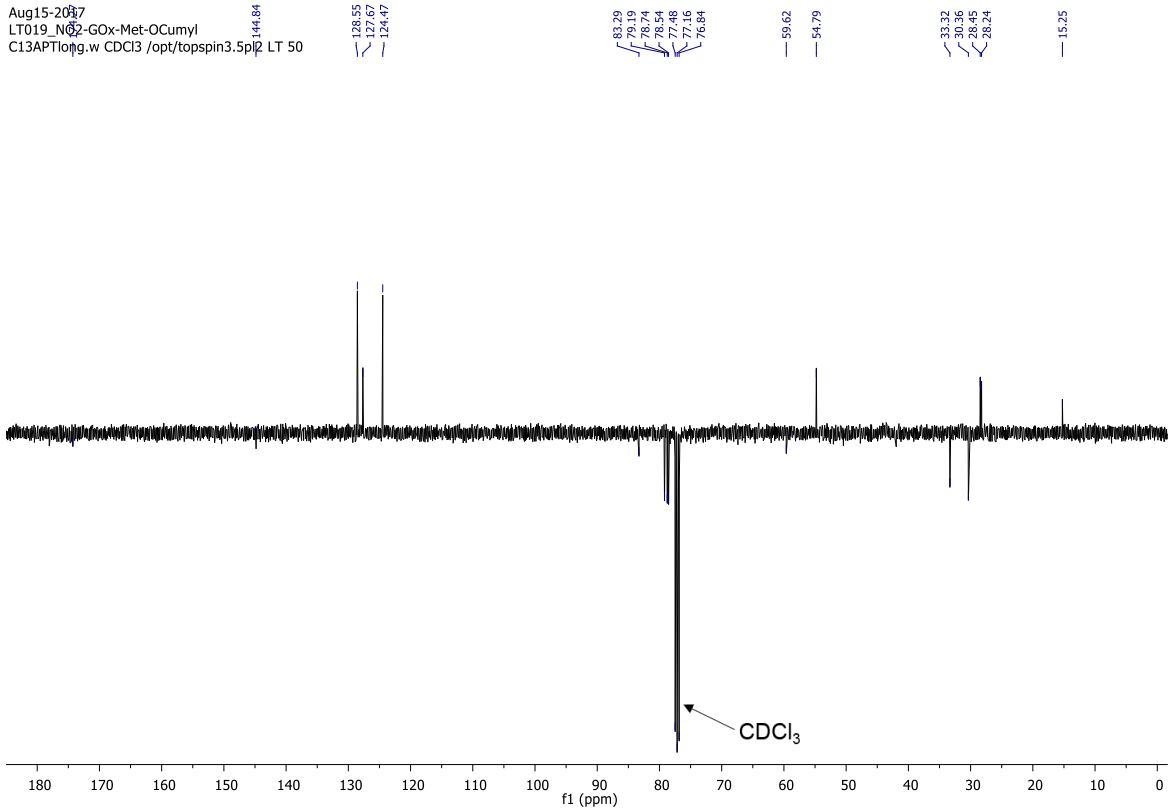


NO_2-GOx-Met-OCumyl (2i)

^{1}H NMR (400 MHz, $CDCl_3$)

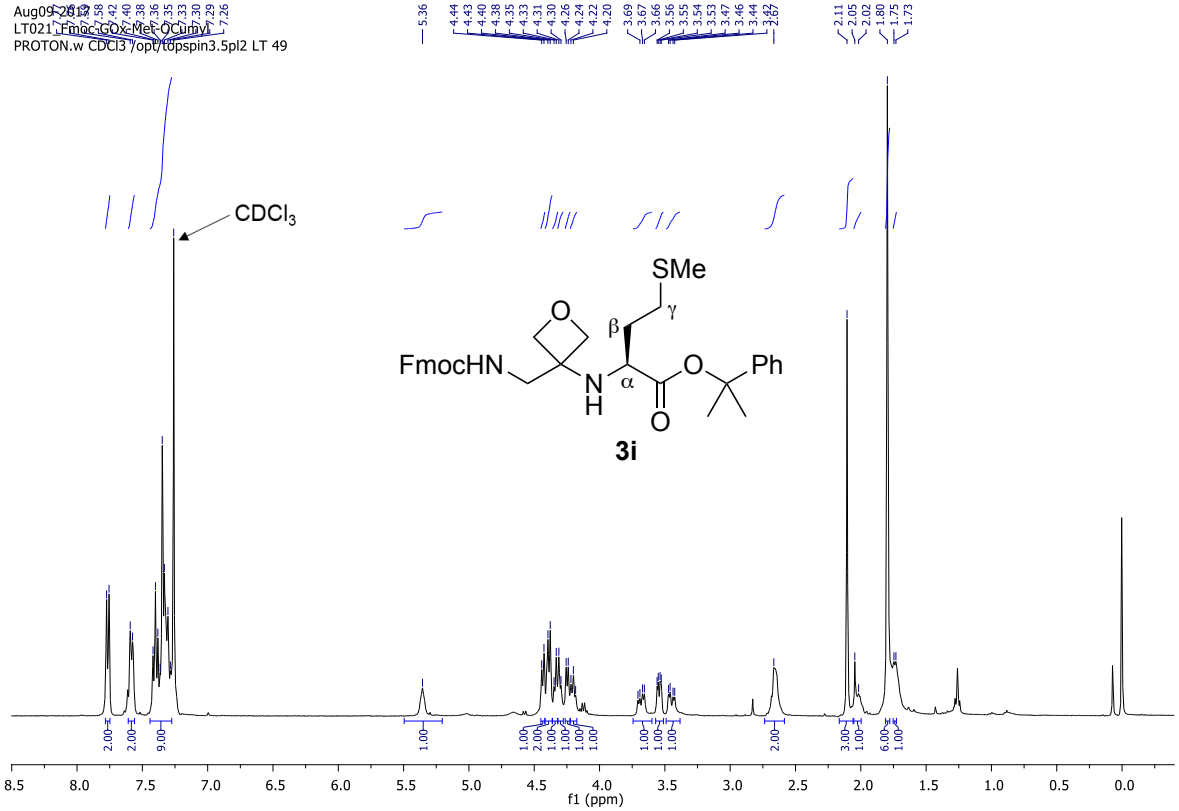


^{13}C NMR (101 MHz, $CDCl_3$)

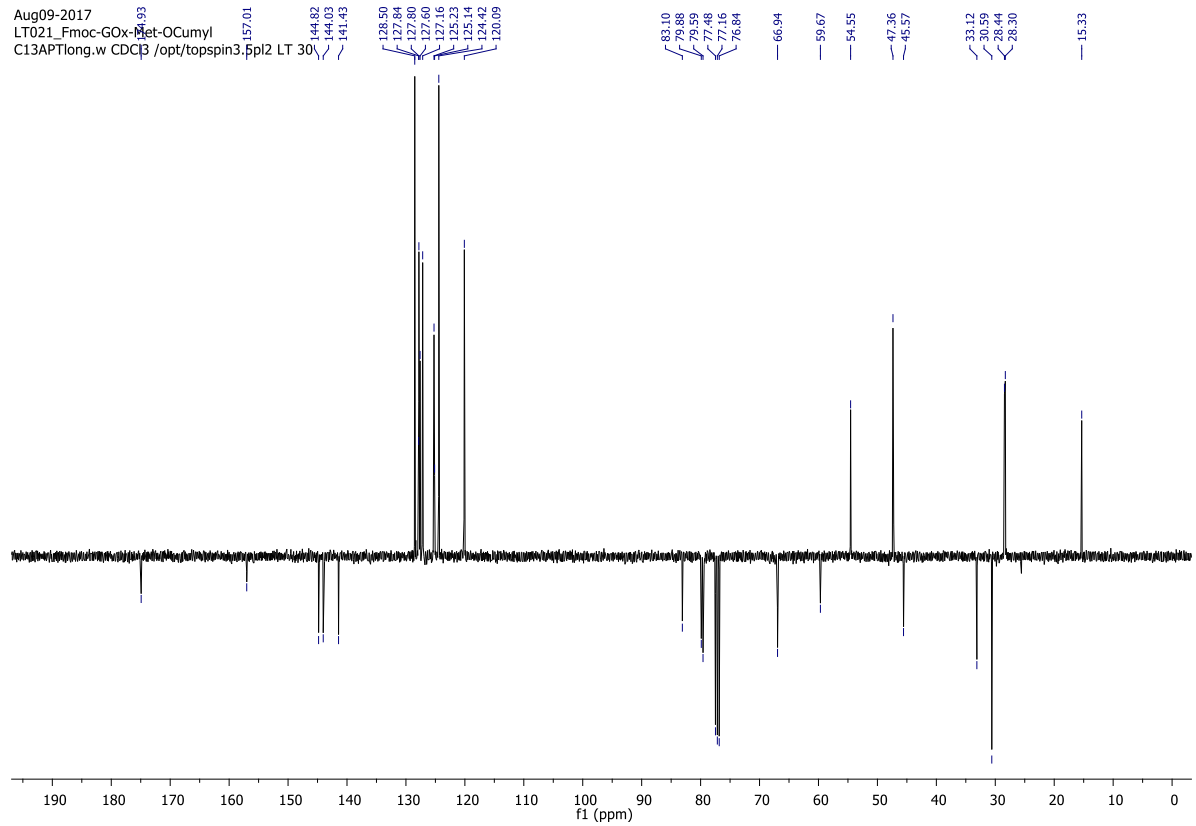


Fmoc-GOx-Met-OCumyl (3i)

^{1}H NMR (400 MHz, $CDCl_3$)

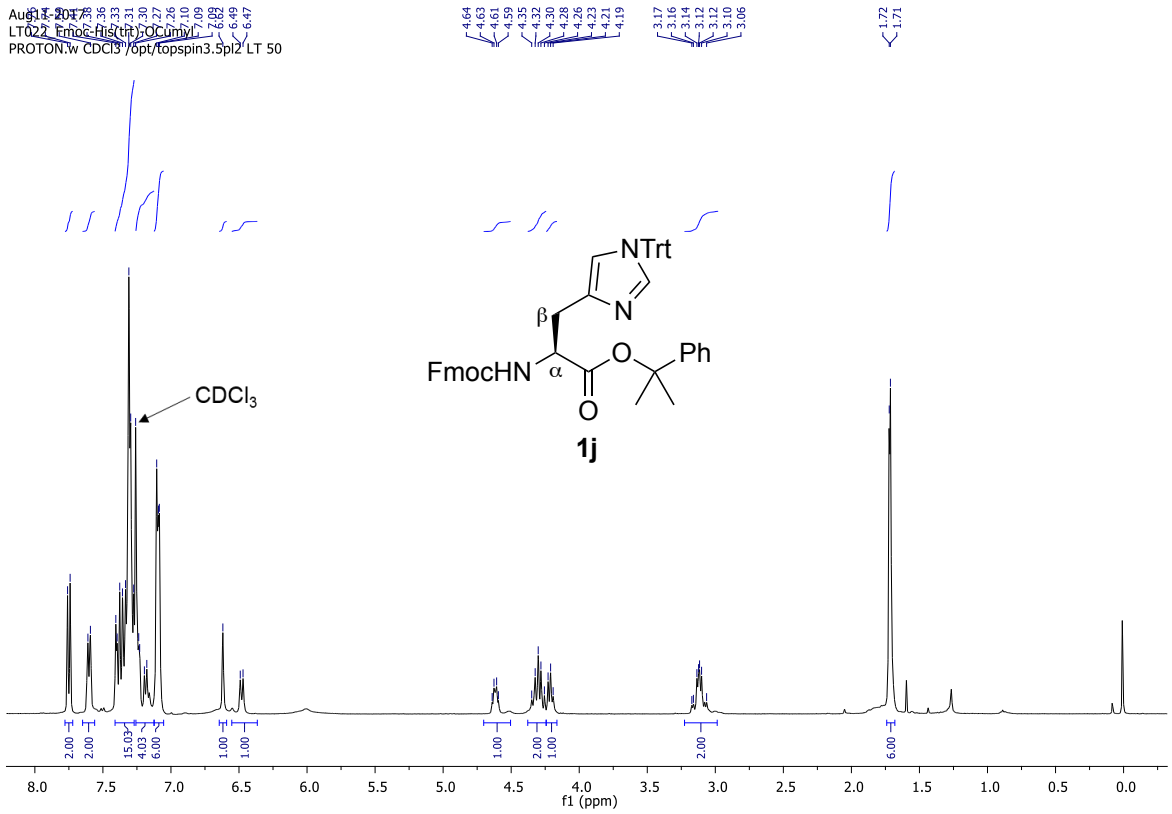


^{13}C NMR (101 MHz, $CDCl_3$)

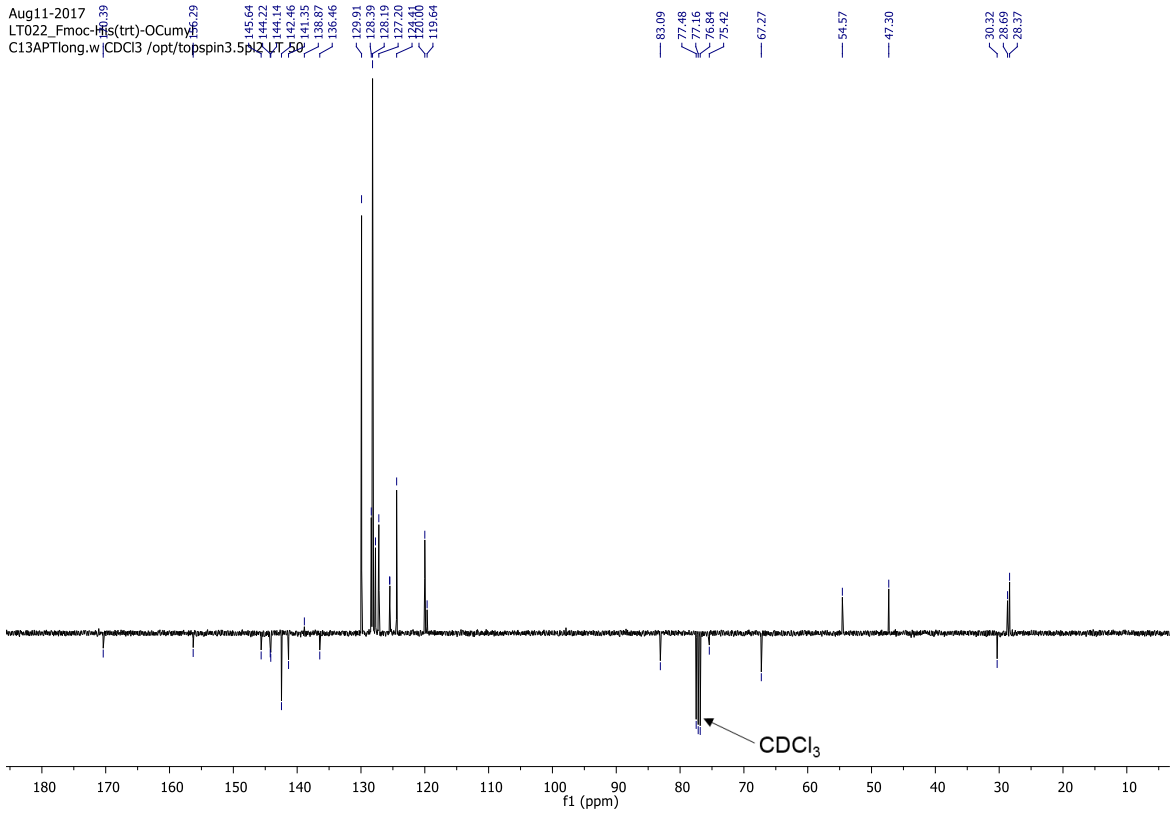


Fmoc-His(Trt)-OCumyl (1j)

^{1}H NMR (400 MHz, $CDCl_3$)

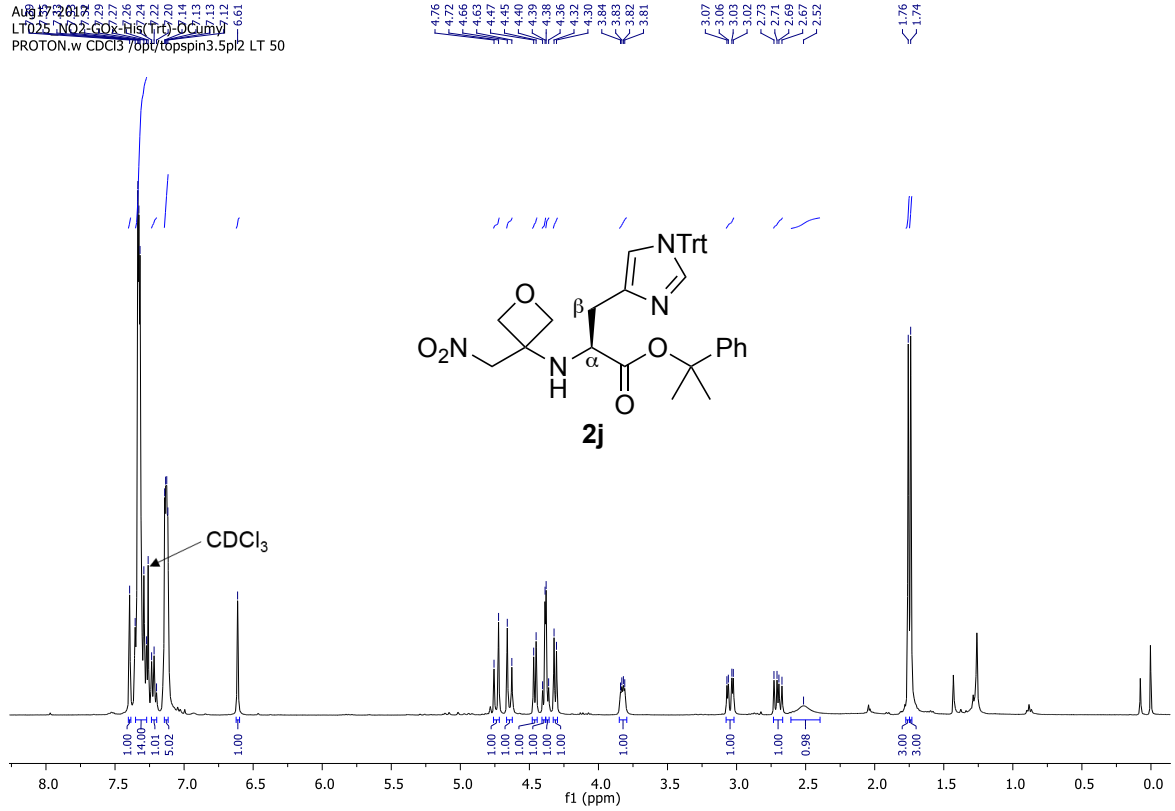


^{13}C NMR (101 MHz, $CDCl_3$)

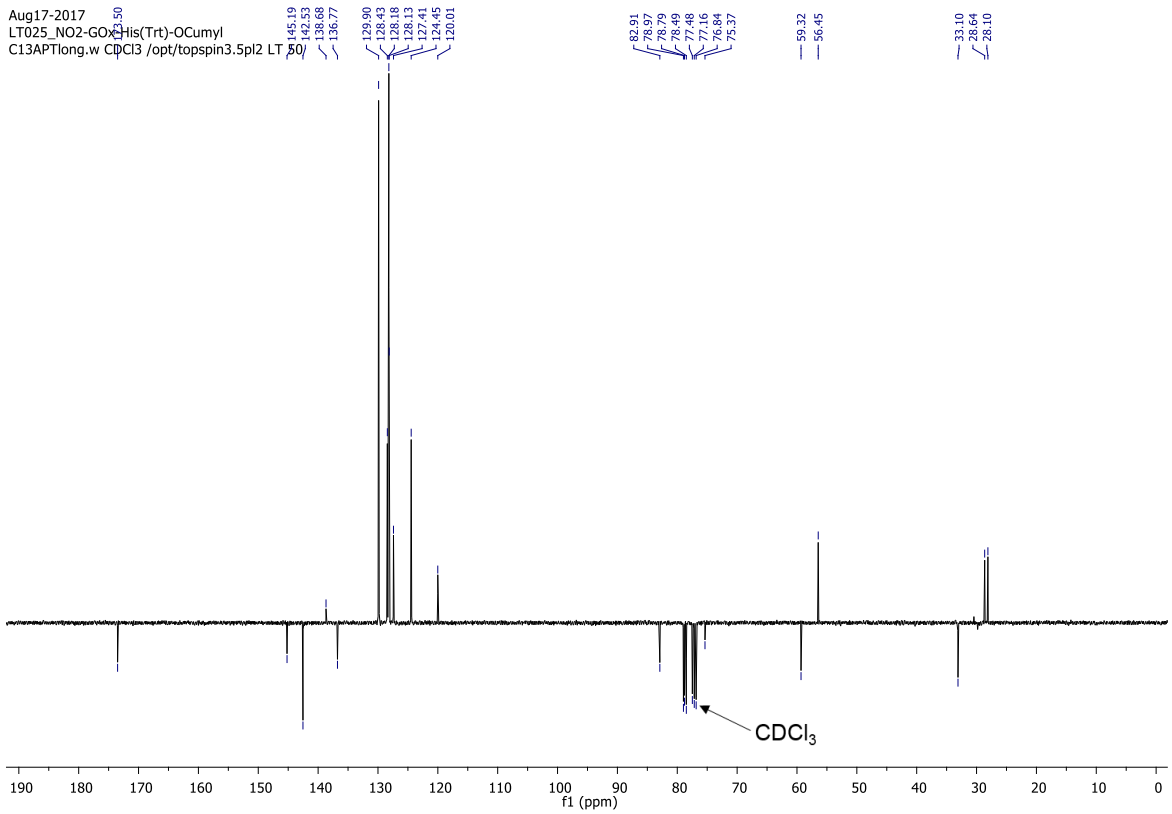


NO_2-GOx-His(Trt)-OCumyl (2j)

^{1}H NMR (400 MHz, $CDCl_3$)

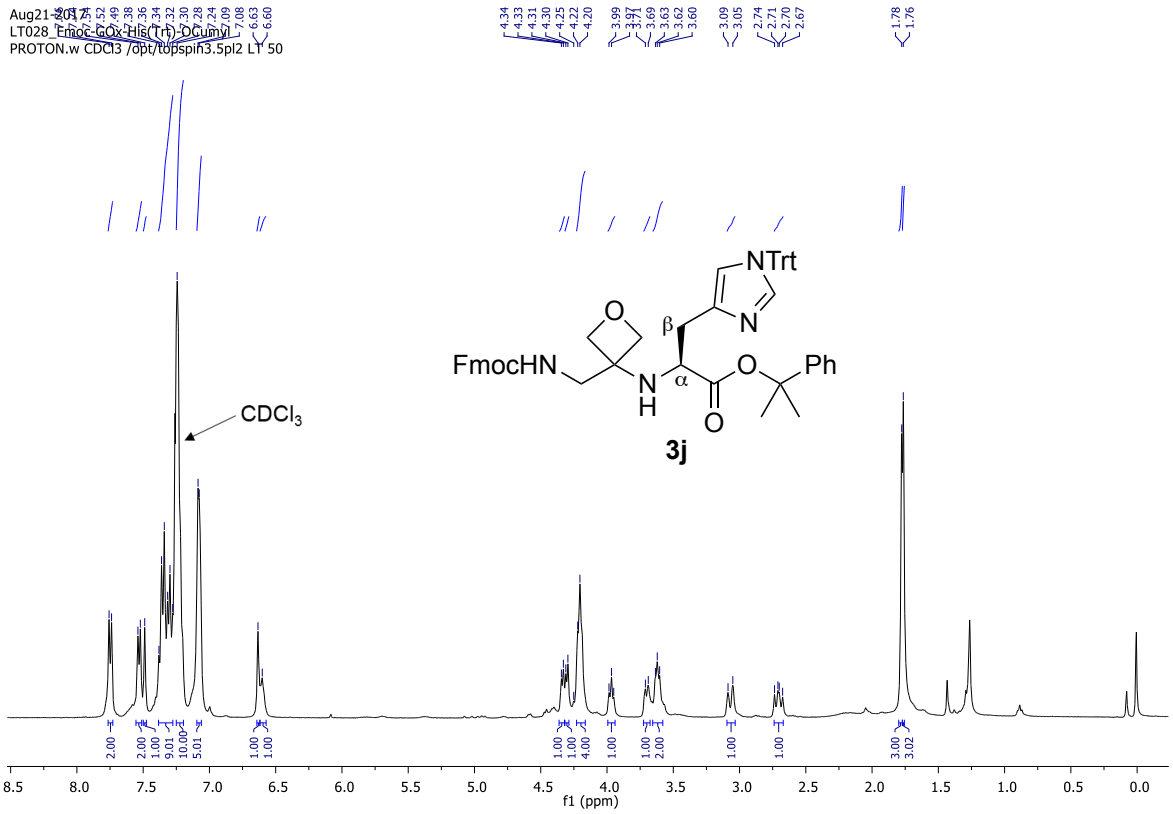


^{13}C NMR (101 MHz, $CDCl_3$)

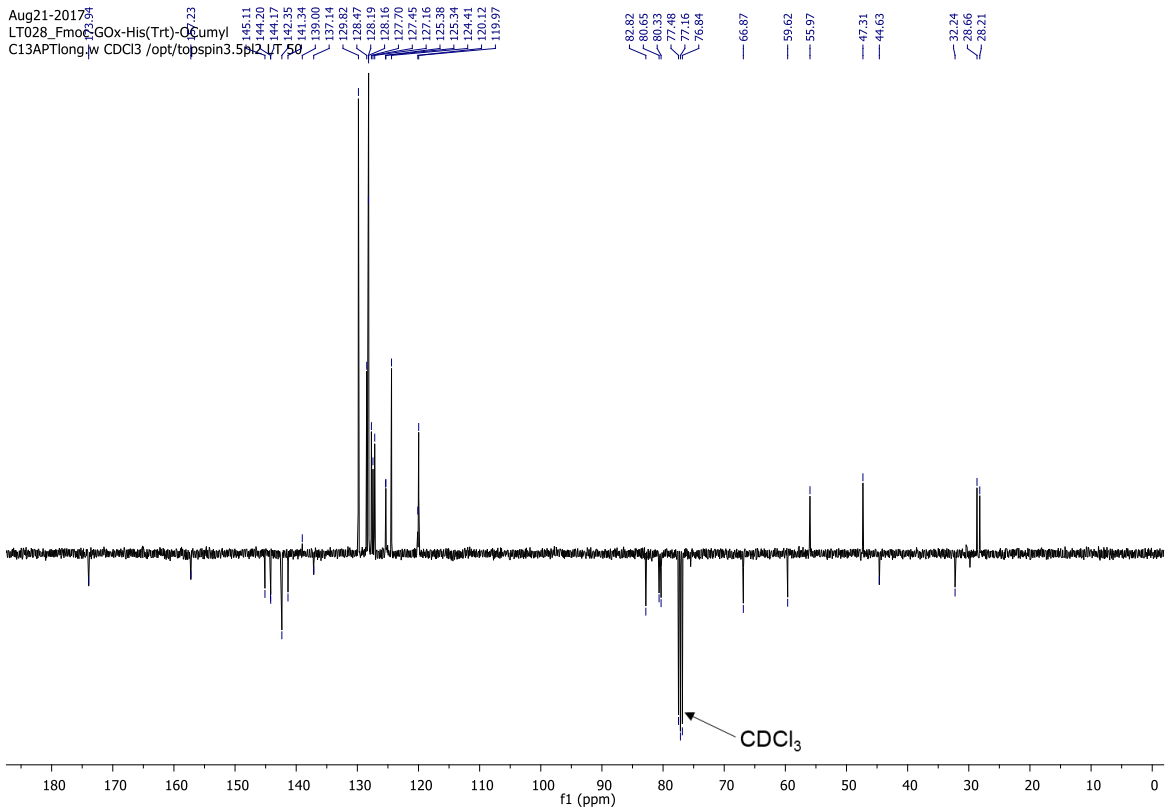


Fmoc-GOx-His(Trt)-OCumyl (3j)

^{1}H NMR (400 MHz, $CDCl_3$)

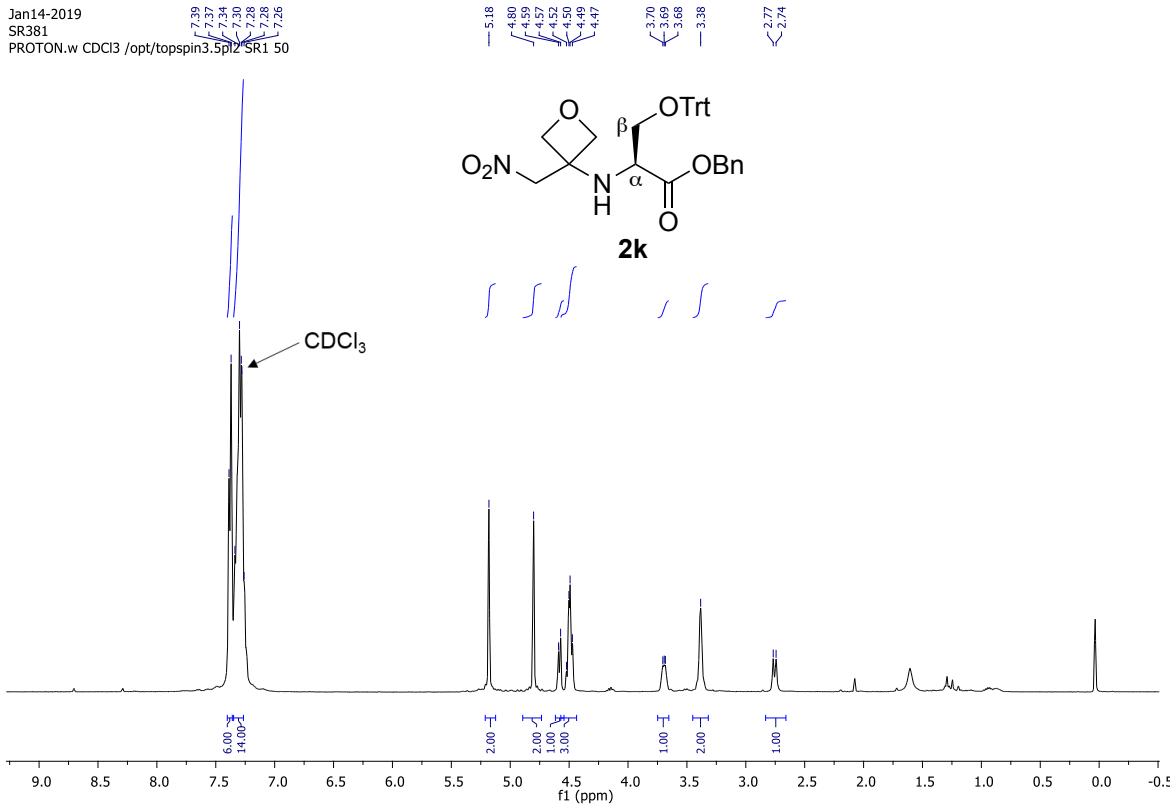


^{13}C NMR (101 MHz, $CDCl_3$)

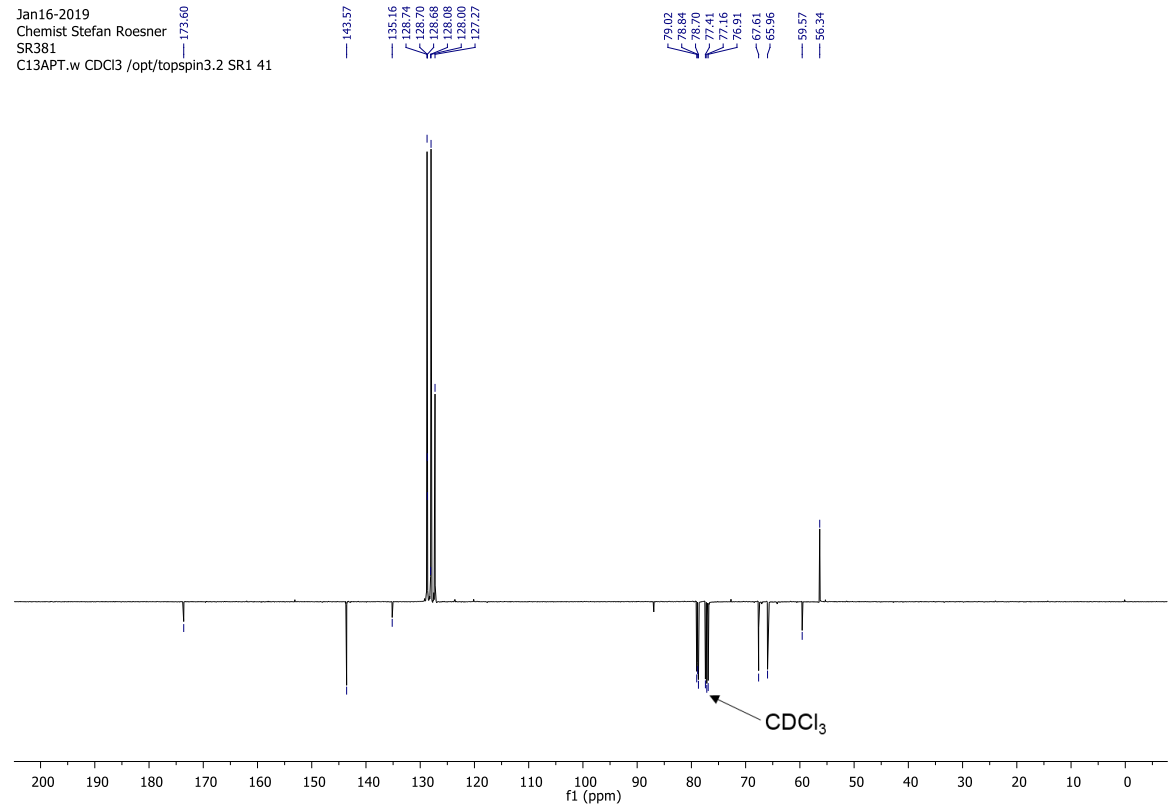


NO_2-GOx-Ser(Trt)-OBn (2k)

^{1}H-NMR (400 MHz, $CDCl_3$)

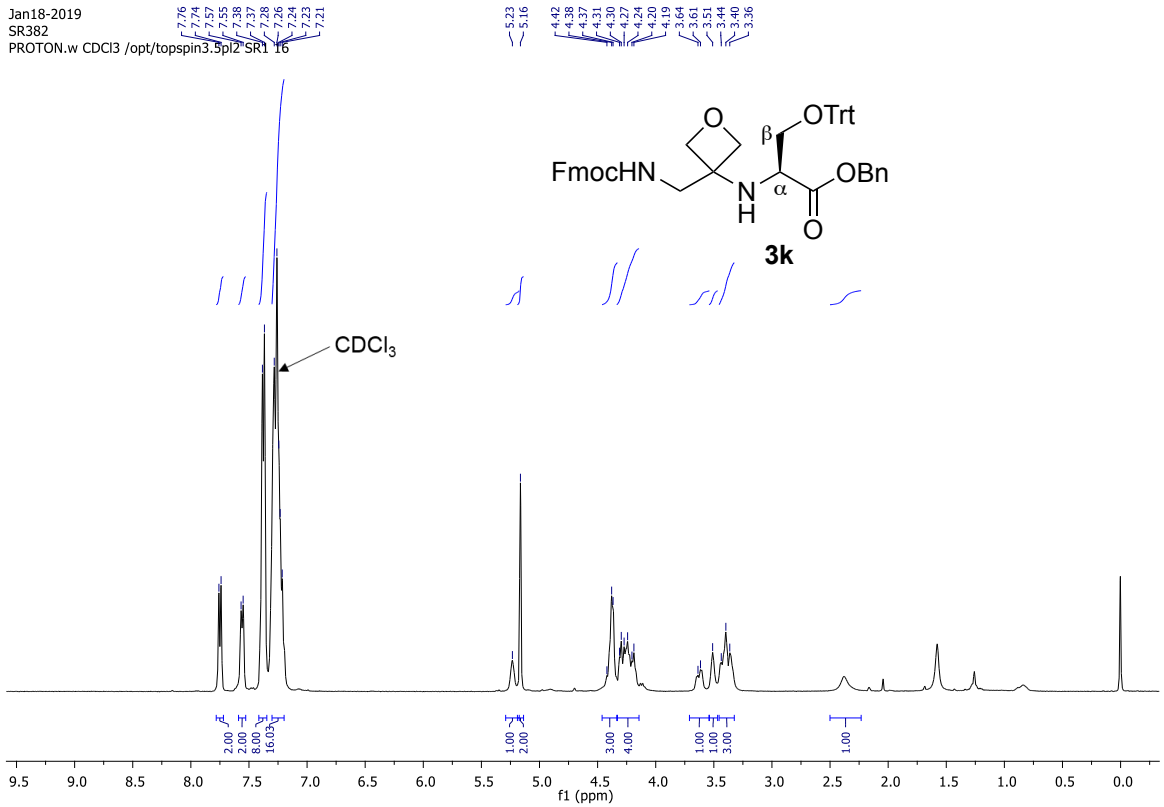


^{13}C-NMR (126 MHz, $CDCl_3$)

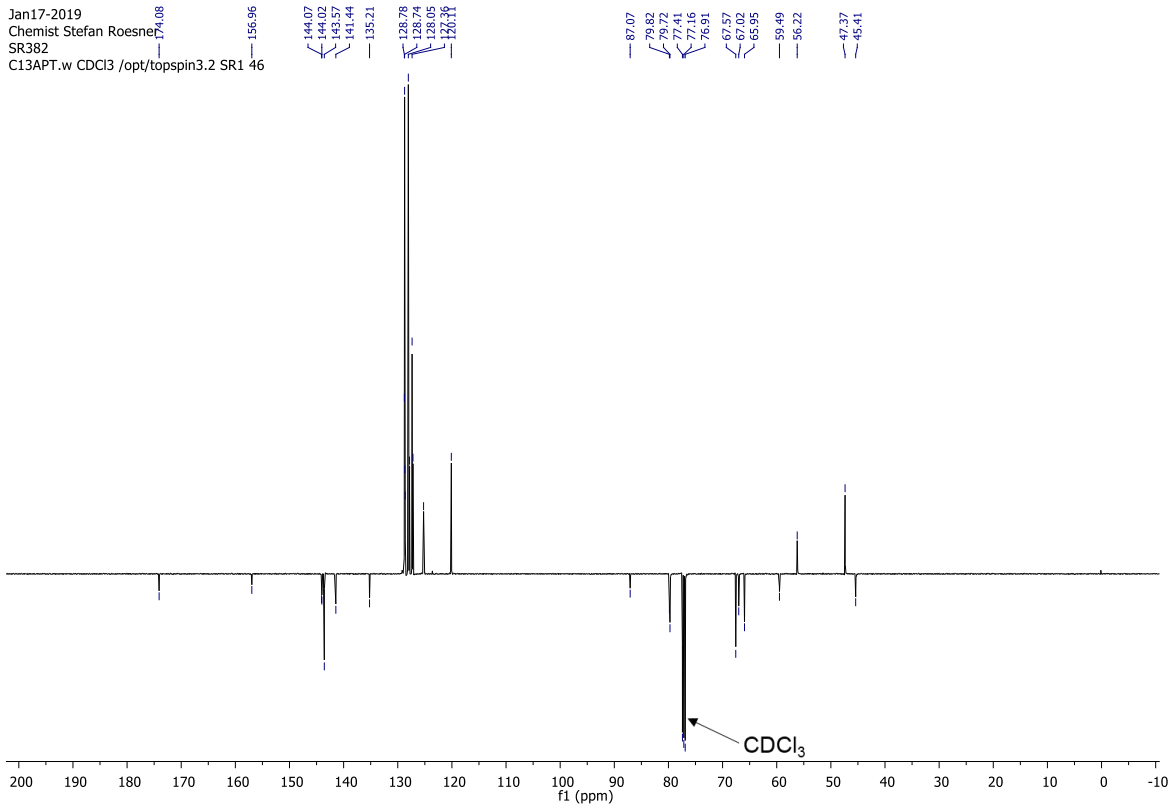


Fmoc-GOx-Ser(Trt)-OBn (3k)

^{1}H-NMR (400 MHz, $CDCl_3$)

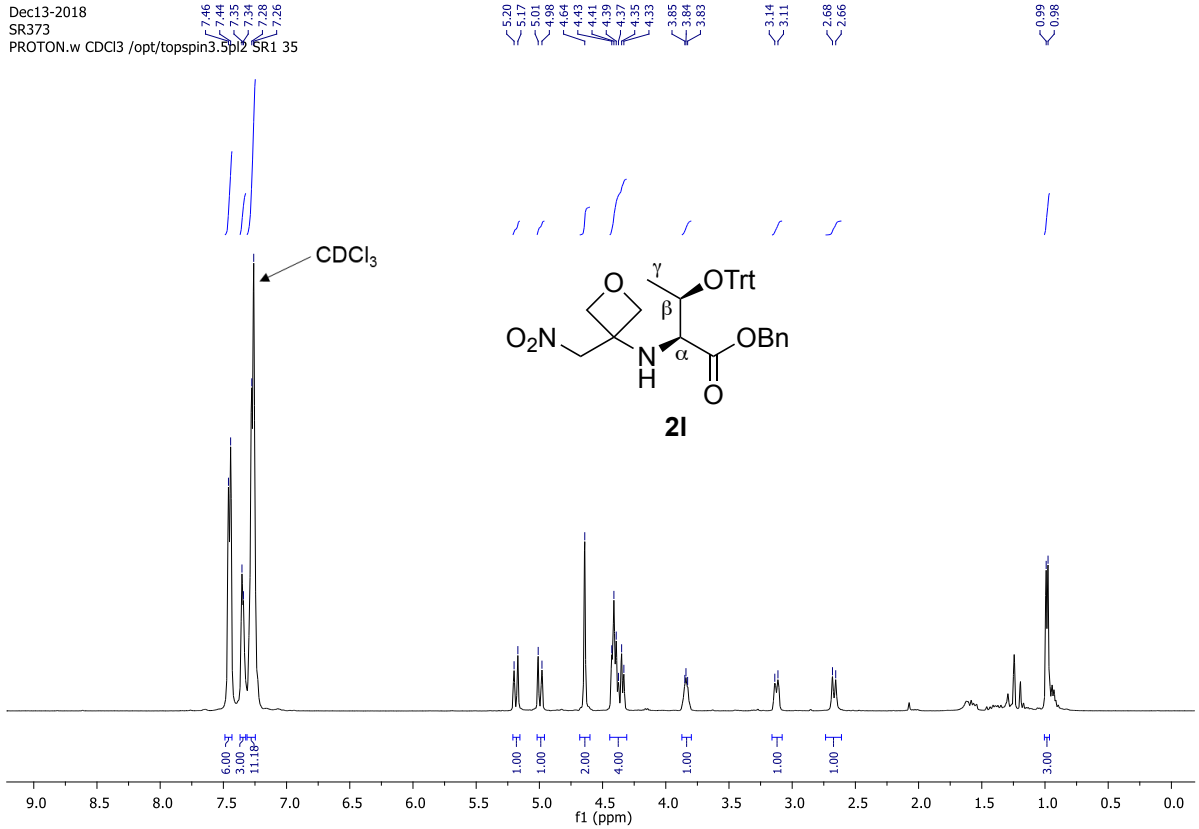


^{13}C-NMR (126 MHz, $CDCl_3$)

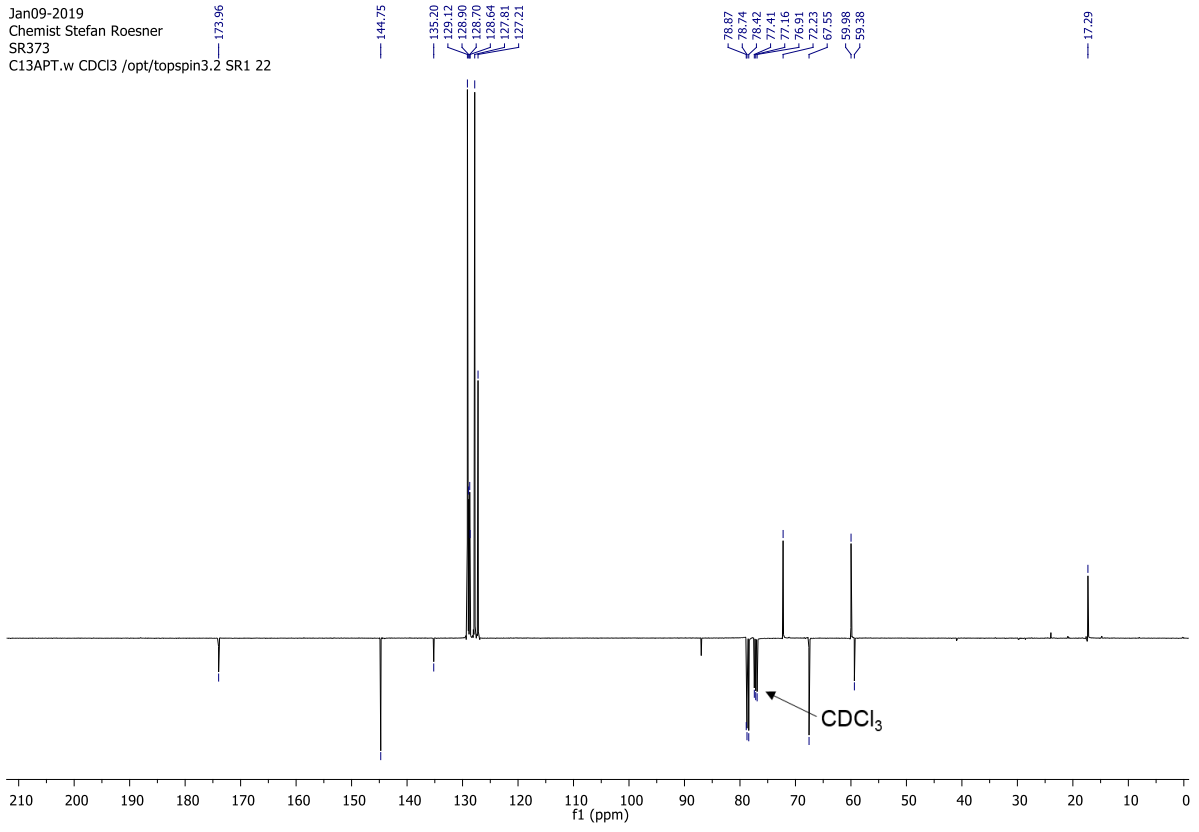


NO_2-GOx-Thr(Trt)-OBn (2l)

^{1}H NMR (400 MHz, $CDCl_3$)

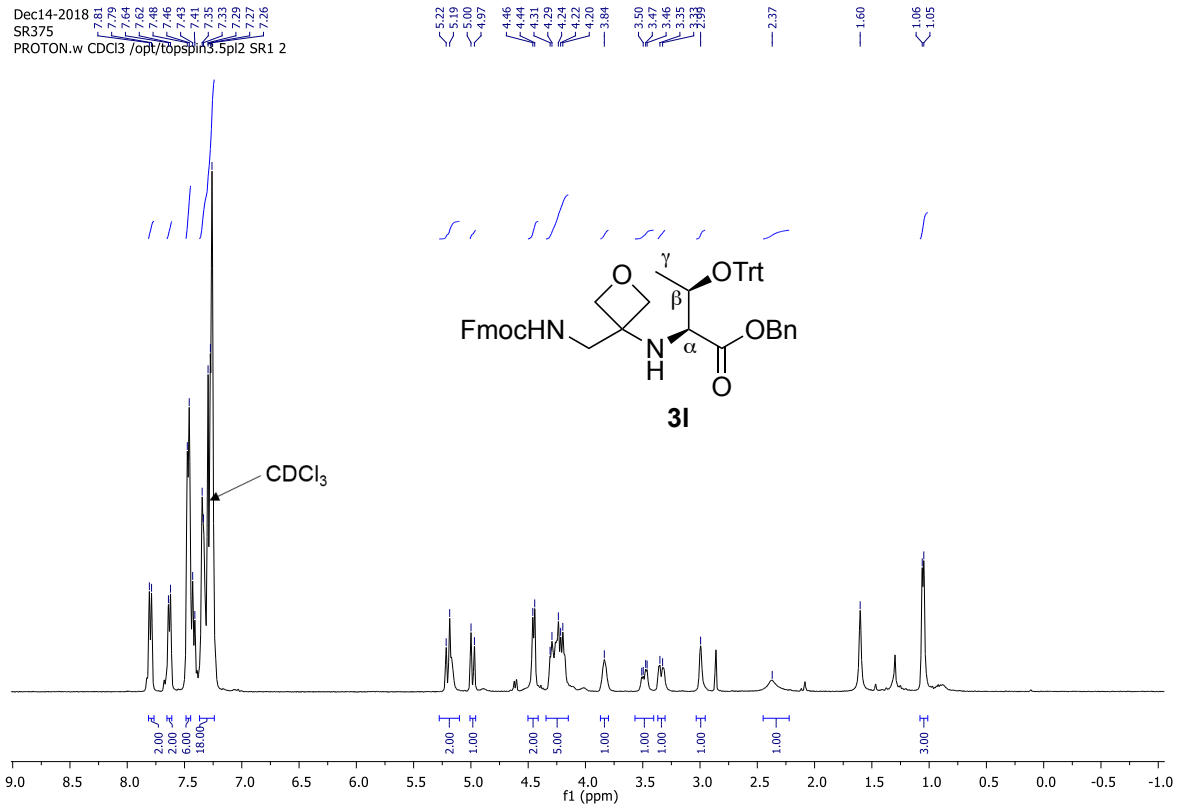


^{13}C NMR (126 MHz, $CDCl_3$)

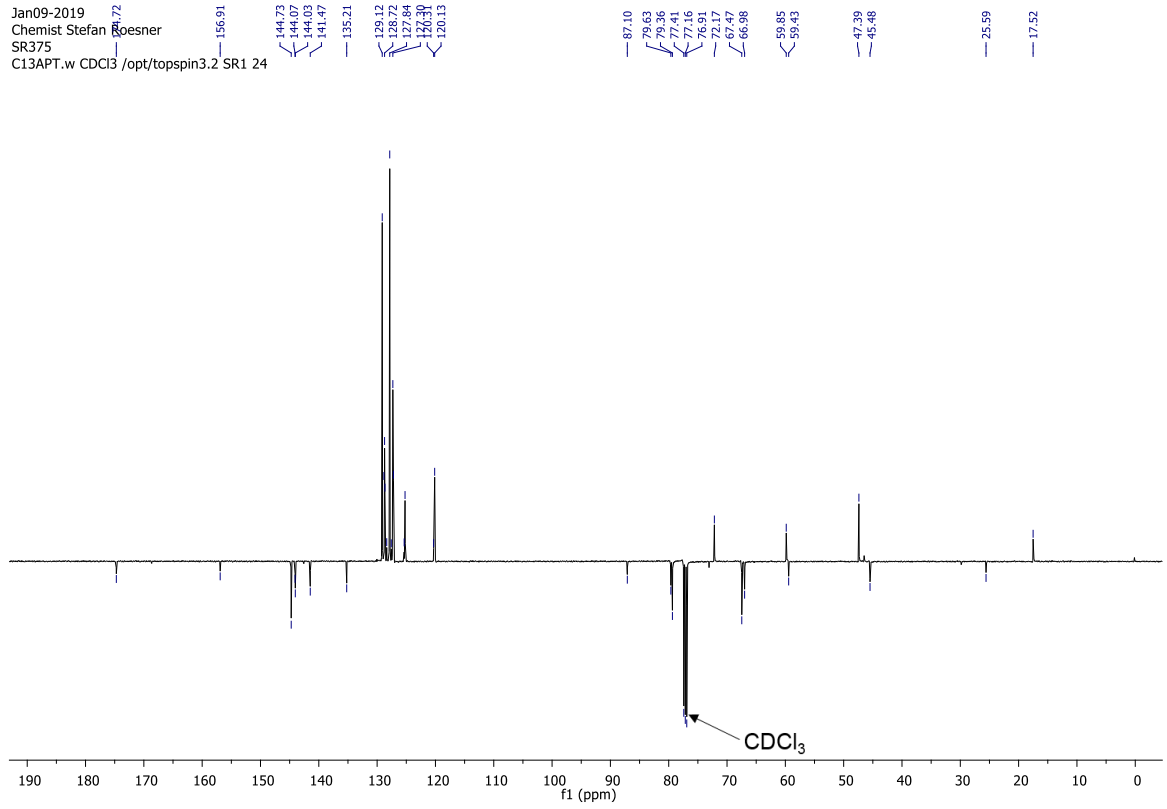


Fmoc-GOx-Thr(Trt)-OBn (3l)

^{1}H NMR (400 MHz, $CDCl_3$)

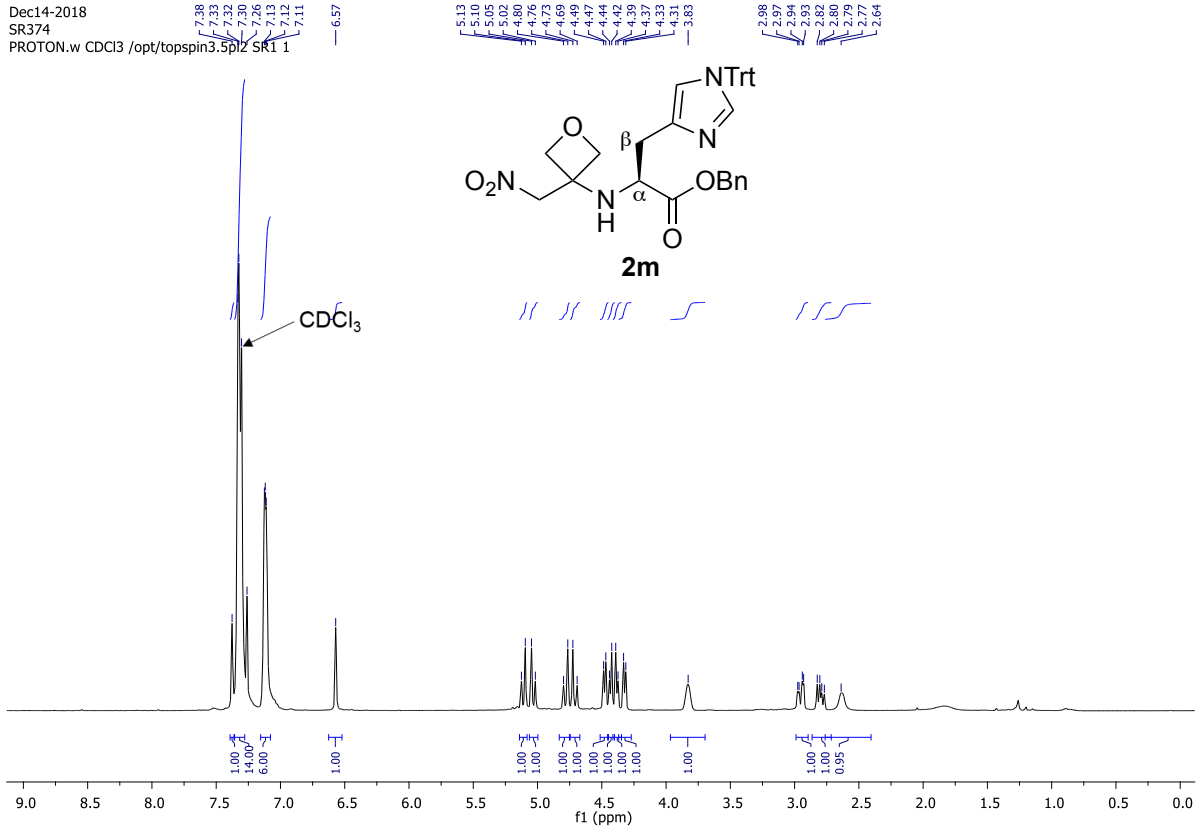


^{13}C NMR (126 MHz, $CDCl_3$)

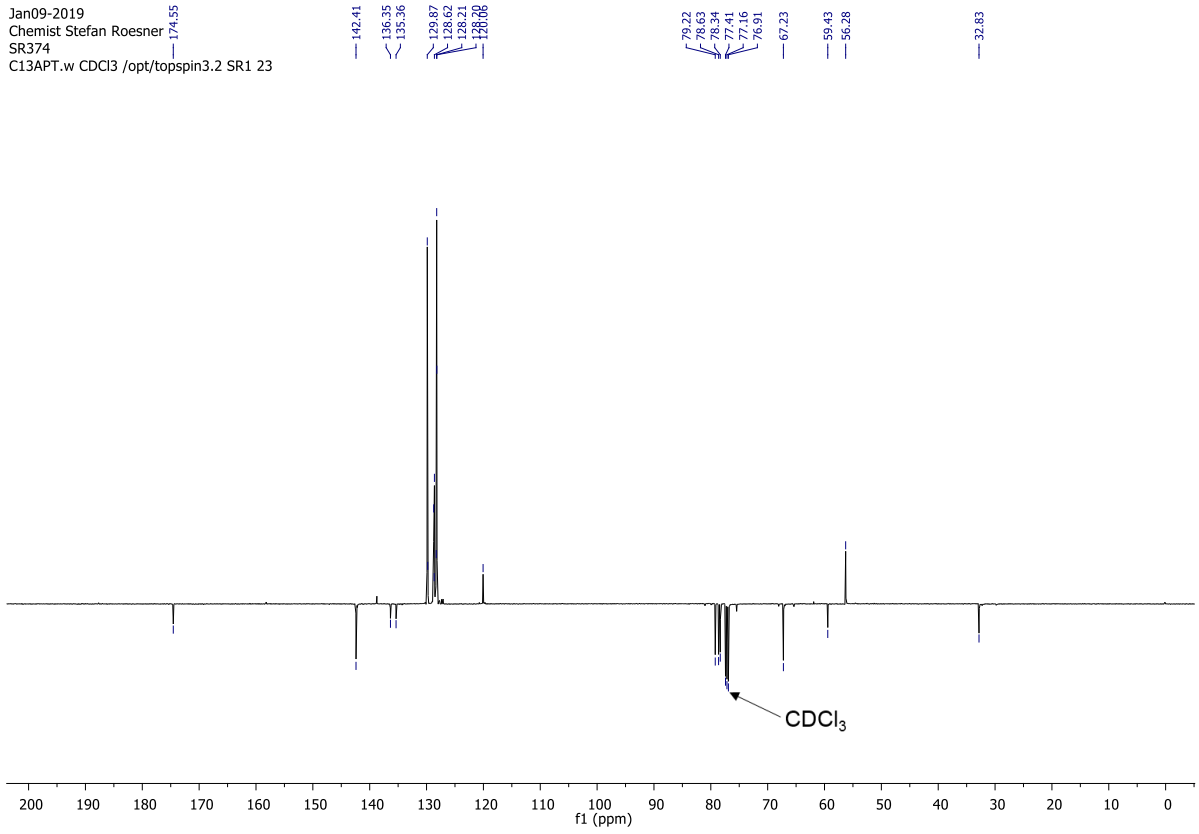


NO_2-GOx-His(Trt)-OBn (2m)

^{1}H NMR (400 MHz, $CDCl_3$)

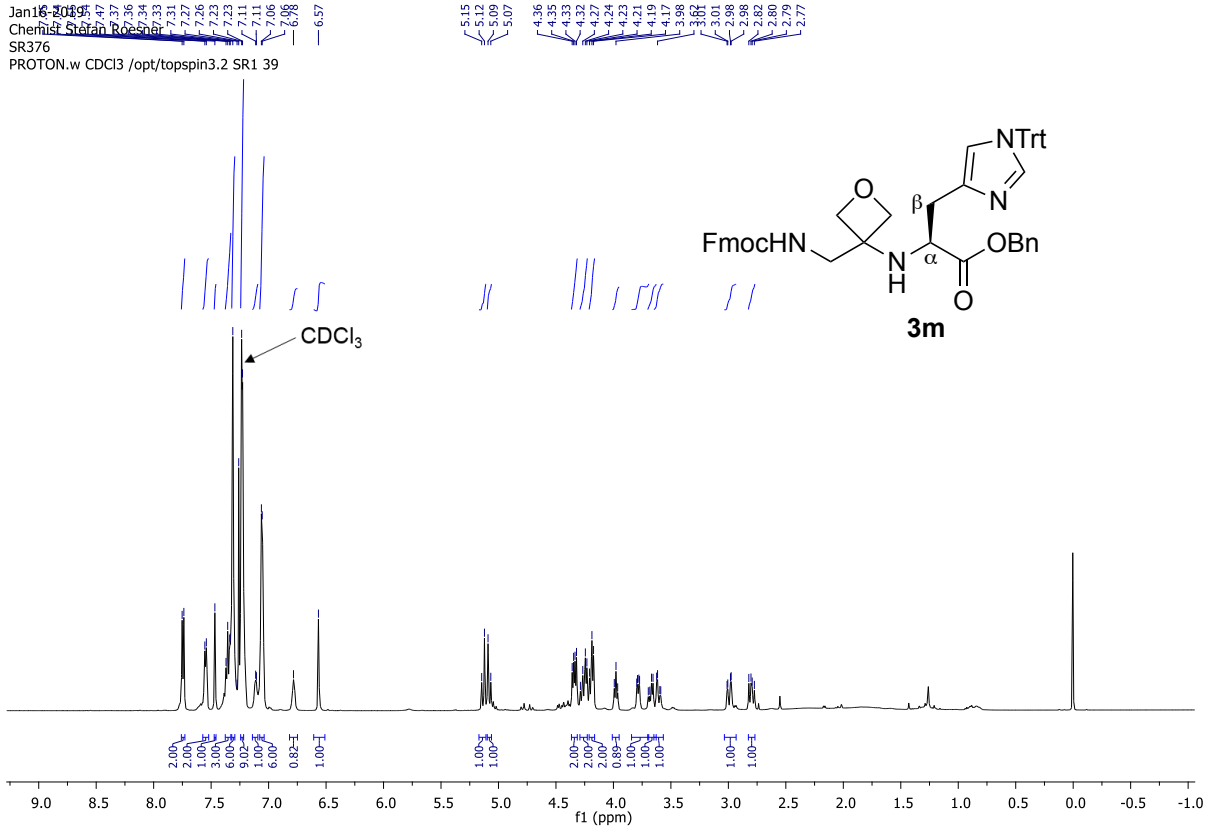


^{13}C NMR (126 MHz, $CDCl_3$)

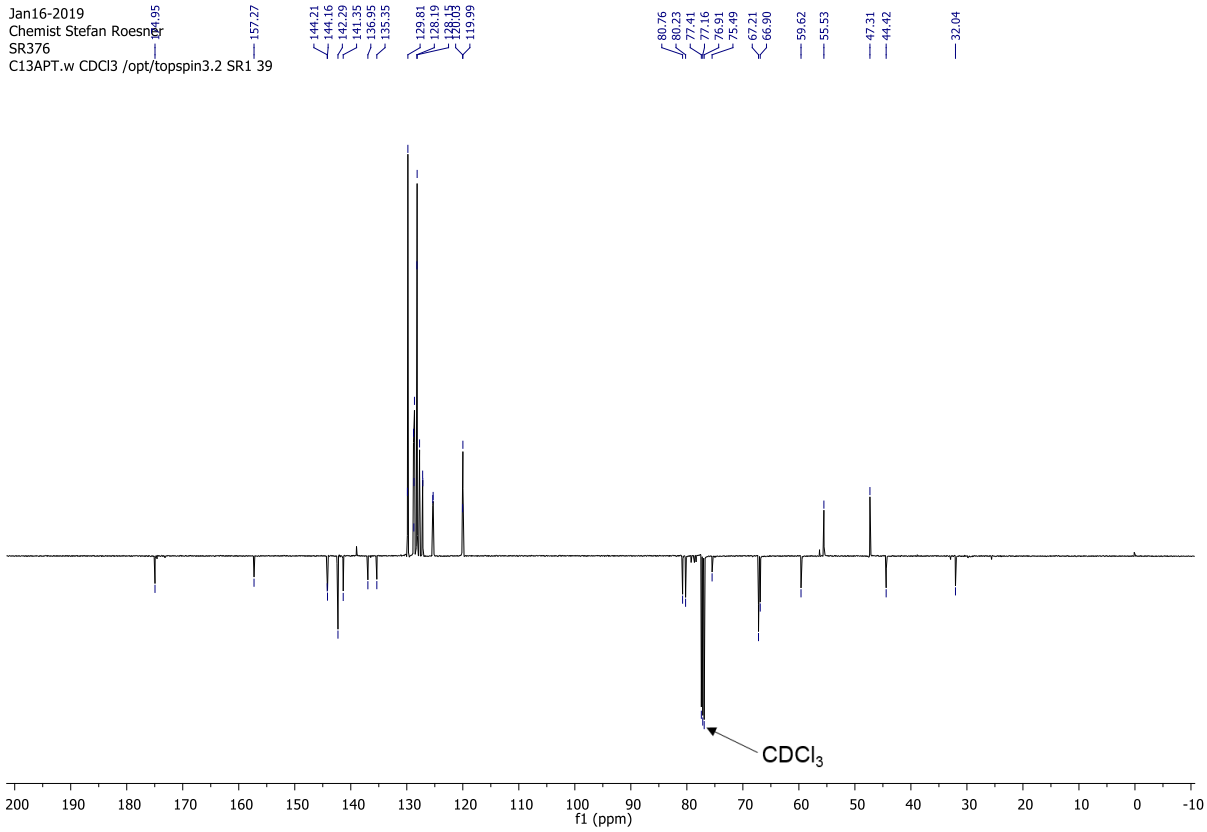


Fmoc-GOx-His(Trt)-OBn (3m)

^{1}H NMR (400 MHz, $CDCl_3$)

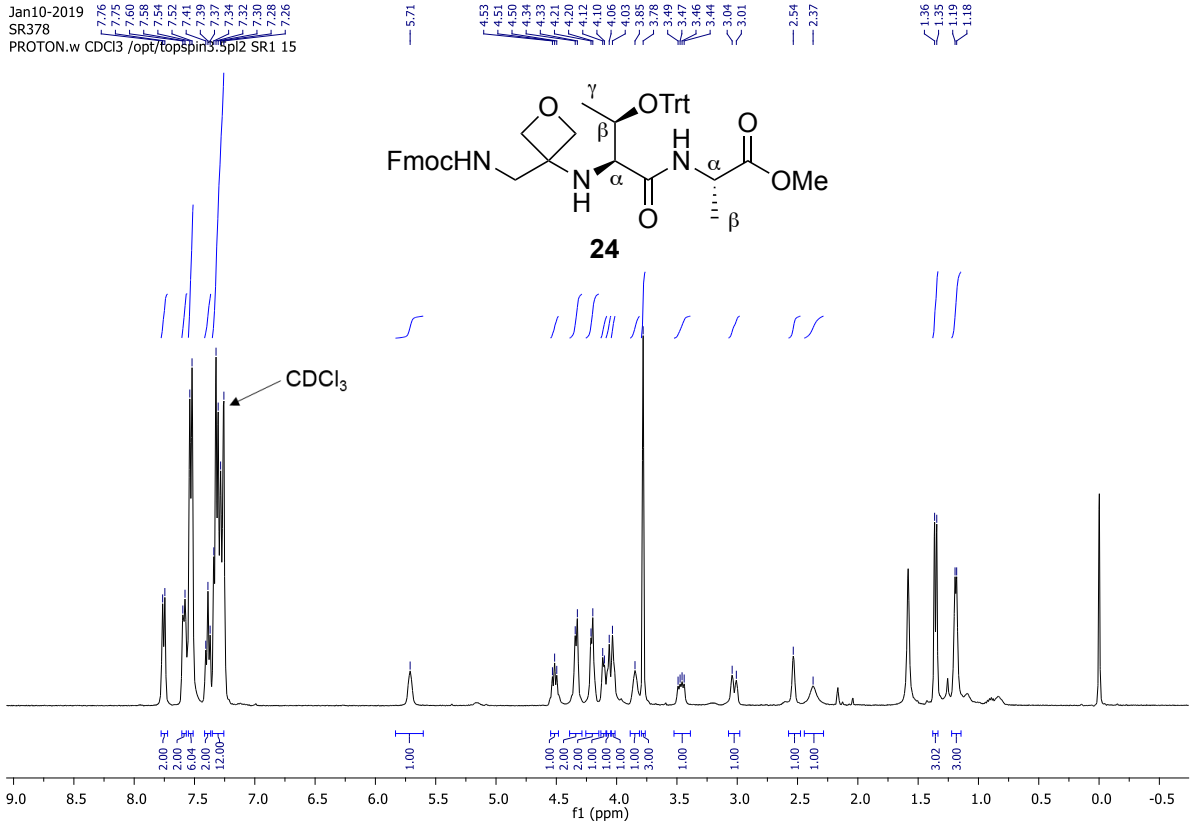


^{13}C NMR (126 MHz, $CDCl_3$)



Fmoc-GOx-Thr(Trt)-OBn (24)

^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)