Supporting information

Synthesis of Strained Cyclic Peptides via an Aza-Michael/Acyl-Transfer Reaction Cascade

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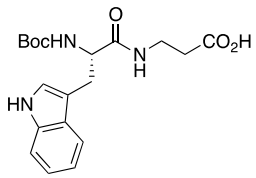
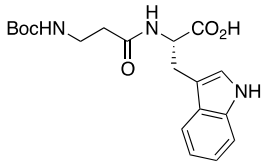
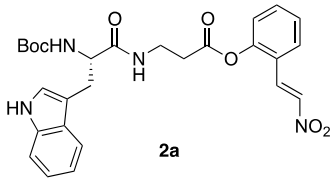
General:
All reactions were monitored by TLC using a Merck TLC plastic roll 500 x 20 cm silica gel 60 F254. Flash column chromatography was performed on Biosolve 60 Å (0.032-0.063 mm) silica gel using the indicated solvent mixtures (PE = Petroleum Ether 40-60). All solvents were bought from Biosolve (AR grade) and used without further purification unless stated otherwise. Starting materials were purchased from Sigma-Aldrich, Fluka or Acros and used without further purification unless stated otherwise. The NMR spectra were recorded in $CDCl_3$ or CD_3OD solutions using a Bruker ARX 400 and a Varian Inova 500 spectrometer. Spectra are reported in δ units (ppm) an J values (Hz) using the solvent as internal standard. HRMS (FAB^+) were recorded with a JEOL JMS SX/SX 102A four sector mass spectrometer. HRMS (ESI^+) were recorded with ApexUltra Fourier transform ion cyclotron resonance mass spectrometer. The LC-MS experiments were performed using a Finnigan LXQ Ion Trap apparatus. LC was carried out with an XTerra C18 3.5 µm column using gradients between H_2O/0,1% HCO_2H (Solvent A) and CH_3CN/0,1% HCO_2H (Solvent B). Electrospray ionisation mass spectra (positive ions) were recorded in full scan mode (m/z = 100 – 2000). Infrared (IR) spectra were obtained with a Bruker Alpha-P diamant-point spectrometer and reported in wave numbers (cm^{-1}). Melting points were determined with a Büchi melting point apparatus B-545 and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Chiral HPLC was performed using a Shimadzu LC-20AD with an SPD-M20A diode array detector (254 nm) and DGU-20A5 degasser with a Chiralpak® AD (Chiral Technologies Europe, 0.46 cm x 25 cm) Column.



Boc-Trp-ßAla-OH. Boc-Trp-OSu (10.0 g, 24.9 mmol, 1 equiv) was dissolved in EtOH:acetone = 1:4 (100 mL). A solution of H-ßAla-OH (2.24 g, 25.1 mmol, 1.01 equiv) and $NaHCO_3$ (5.23 g, 62.2 mmol, 2.5 equiv), dissolved in water (100 mL) was added and the resulting mixture was stirred for 18 h at rt. The reaction mixture was neutralised using a 10% HCl solution and extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the volatiles, the resulting oil was purified using flash chromatography (3% MeOH in dichloromethane→10% MeOH in dichloromethane) to yield Boc-Trp-ßAla-OH as a white foam (7.68 g, 20.5 mmol, 82%). mp 84.9 – 87.8 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.60 (d, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.10 (t, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 4.29 (t, *J* = 6.8 Hz, 1H), 3.50 – 2.99 (m, 4H), 2.44 – 2.30 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 173.83, 173.38, 136.62, 127.41, 123.10, 120.99, 118.35, 117.97, 110.84, 110.79, 109.60, 79.25, 55.72, 48.23, 48.02, 47.80, 47.59, 47.38, 47.16, 46.95, 34.83, 32.94, 27.88, 27.21. IR ν 3325, 2978, 2931, 1698, 1655, 1508, 1637, 1165, 743. HRMS (FAB+) *m/z* found for $C_{19}H_{26}N_3O_5$ $[M+H]^+$ 376.1872, calcd 376.1872. $[\alpha]^{20}_{589nm}$ = -13,8 (c=0,0083 g/mL, MeOH).

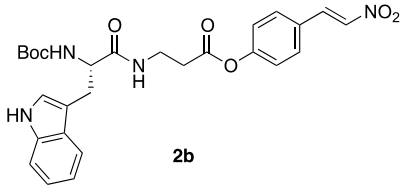


Boc-ßAla-Trp-OH. Boc-ßAla-OSu (14.3 g, 50.0 mmol) was dissolved in EtOH:acetone = 1:4 (100 mL). A solution of H-Trp-OH (10.3 g, 50.5 mmol, 1.01 equiv) and $NaHCO_3$ (10.5 g, 125 mmol, 2.5 equiv) in water was added and the mixture was stirred for 18 h at rt. The reaction mixture was neutralised using a 10% HCl solution and extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the volatiles Boc-ßAla-Trp-OH was obtained as a white solid (18.6 g, 49.6 mmol, 99%). mp 89.9 - 95.1 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.58 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.15 – 7.06 (m, 2H), 7.03 (td, *J* = 7.6, 1.0 Hz, 1H), 4.74 (q, *J* = 12.0, 6.0 Hz, 1H), 3.44 -3.12 (m, 4H), 2.36 (td, *J* = 6.9, 1.7 Hz, 2H), 1.43 (s, 8H). ^{13}C NMR (101 MHz, MeOD) δ 173.89, 172.34, 156.85, 136.61, 127.44, 122.94, 120.98, 118.39, 117.83, 110.86, 109.65, 78.75, 53.30, 48.24, 48.03, 47.82, 47.60, 47.39, 47.18, 46.96, 36.47, 35.62, 27.32, 27.10, 24.86. IR ν 3392, 3290, 2974, 2926, 1692, 1652, 1518, 1339, 1273, 743 cm^{-1}. HRMS (FAB+) *m/z* found for $C_{19}H_{26}N_3O_5$ $[M+H]^+$ 376.1872, calcd 376.1872. $[\alpha]^{20}_{589nm}$ = 101 (c=0,0141 g/mL, MeOH).



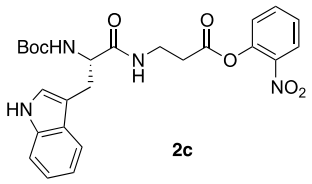
2a

2a. To a solution of Boc-Trp-ßAla-OH (0.50 g, 1.30 mmol, 1 equiv) in THF (75mL), HATU (0.76 g, 2.00 mmol, 1.5 equiv), DiPEA (0.52 g, 0.66 mL, 4.00 mmol, 3 equiv) and (*E*)-2-(2-nitrovinyl)phenol 0.33 g, 2.0 mmol, 1.5 equiv) were added and the resulting reaction mixture was stirred for 18 h at rt. The reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography (EtOAc:PE = 1:1→1:0) to yield **2a** (0.33 g, 0.63 mmol, 49%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.93 (d, *J* = 13.7 Hz, 1H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.60 – 7.44 (m, 3H), 7.30 (t, *J* = 8.2 Hz, 2H), 7.23 – 7.09 (m, 3H), 7.06 (d, *J* = 1.7 Hz, 1H), 6.27 (t, *J* = 4.7 Hz, 1H), 5.19 (d, *J* = 2.1 Hz, 1H), 4.41 (d, *J* = 4.9 Hz, 1H), 3.67 – 3.40 (m, 2H), 3.32 (dd, *J* = 14.2, 4.2 Hz, 1H), 3.16 (dd, *J* = 14.3, 7.6 Hz, 1H), 2.76 – 2.47 (m, 2H), 1.40 (s, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.29, 170.14, 149.91, 138.60, 136.34, 133.18, 132.97, 128.89, 127.55, 126.83, 123.68, 123.33, 122.79, 122.46, 119.92, 119.01, 111.39, 110.72, 55.51, 34.93, 34.04, 29.83, 28.55, 28.40. IR ν 3325, 2979, 2928, 1703, 1661, 1519, 1340, 1241, 1132, 742. HRMS (FAB+) *m/z* found for $C_{27}H_{30}N_4O_7$ 523.2202 [M+H], calcd 523.2193. $[\alpha]^{20}_{589nm}$ = -2,14 (c=0,0422 g/mL, MeOH).



2b

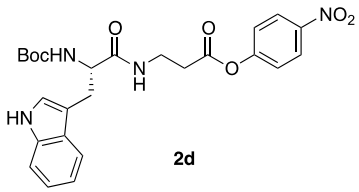
2b. To a solution of Boc-Trp-ßAla-OH (50 mg, 0.13 mmol, 1 equiv), HATU (51 mg, 0.13 mmol, 1 equiv), DiPEA (35 mg, 44 µL, 0.27 mmol, 2 equiv) and (*E*)-4-(2-nitrovinyl)phenol (44 mg, 0.27 mmol, 2 equiv) in THF (10 mL) were added and the reaction mixture was stirred for 16 h. After evaporation of the volatiles, the residue was purified using flash chromatography (EtOAc:PE = 1:1→1:0) to yield **2b** (59 mg, 0.11 mmol, 87%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 21.6 Hz, 1H), 8.05 – 7.92 (m, 1H), 7.65 (t, *J* = 9.4 Hz, 1H), 7.55 (ddd, *J* = 6.6, 6.1, 4.4 Hz, 3H), 7.38 – 7.30 (m, 1H), 7.25 – 7.03 (m, 5H), 6.32 (t, *J* = 6.1 Hz, 1H), 5.17 (s, 1H), 4.42 (d, *J* = 5.5 Hz, 1H), 3.67 – 3.39 (m, 2H), 3.34 (dd, *J* = 14.3, 4.9 Hz, 1H), 3.19 (dd, *J* = 14.5, 7.5 Hz, 1H), 2.73 – 2.44 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.02, 170.14, 155.47, 153.13, 137.91, 137.19, 136.20, 130.35, 127.75, 127.41, 123.12, 122.63, 122.37, 119.81, 118.90, 111.25, 110.61, 80.22, 55.36, 34.70, 34.02, 28.29. IR ν 3407, 2967, 2930, 1758, 1657, 1498, 1384, 1337, 1167, 737. HRMS (FAB+) *m/z* found for $C_{27}H_{30}N_4O_7$ 523.2182 [M+H], calcd 523.2193. $[\alpha]^{20}_{589nm}$ = 5,93 (c=0,0961 g/mL, MeOH).



2c

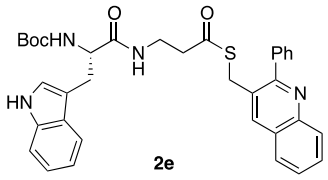
2c. A solution was made of Boc-Trp-ßAla-OH (50 mg, 0.13 mmol, 1 equiv), HATU (51 mg, 0.13 mmol, 1 equiv), DiPEA (35 mg, 44 µL, 0.27 mmol, 2 equiv) and 2-nitrophenol (37 mg, 0.27 mmol, 2 equiv) in THF (10 mL) and was stirred for 16 h at rt. The reaction mixture was concentrated *in vacuo* and the residue was purified using flash chromatography (EtOAc: PE = 1:1→1:0) to yield **2c** (55 mg, 0.11 mmol, 88%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s,

1H), 8.10 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.71 – 7.61 (m, 2H), 7.46 – 7.38 (m, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.23 – 7.09 (m, 3H), 7.07 (d, *J* = 1.8 Hz, 1H), 6.40 (s, 1H), 5.23 (d, *J* = 6.7 Hz, 1H), 4.46 (d, *J* = 4.5 Hz, 1H), 3.53 (d, *J* = 5.9 Hz, 2H), 3.40 – 3.14 (m, 2H), 2.78 – 2.56 (m, 2H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.43, 172.09, 169.90, 155.48, 143.75, 141.47, 136.22, 134.96, 127.48, 126.81, 125.83, 125.21, 123.15, 122.20, 119.65, 119.50, 118.87, 111.39, 111.24, 110.55, 80.08, 77.37, 77.05, 76.73, 55.34, 34.69, 33.92, 28.42, 28.26. IR ν 3309, 2978, 2931, 1698, 1661, 1528, 1348, 1161, 744. HRMS (FAB+) *m/z* found for $C_{25}H_{28}N_4O_7$ 497.2038 [M+H], calcd 497.2036. $[\alpha]^{20}_{589nm}$ = -4,01 (c=0,0174 g/mL, MeOH).



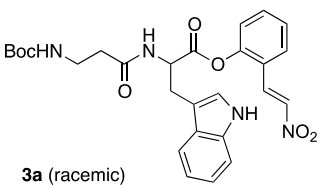
2d

2d. A solution of Boc-Trp-ßAla-OH (100 mg, 0.27 mmol, 1 equiv), HATU (15 mg, 0.40 mmol, 1.5 equiv), DiPEA (100 mg, 130 µL, 0.80 mmol, 3 equiv) and 4-nitrophenol (56 mg, 0.40 mmol, 1.5 equiv) in THF (20 mL) was made and stirred for 16 h at rt. The reaction mixture was concentrated *in vacuo* and the residue purified by flash chromatography (EtOAc:PE = 1:1→1:0) to yield **2d** (130mg, 0.26 mmol, 97%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (m, 3H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.22 – 7.10 (m, 4H), 7.06 (s, 1H), 6.31 (s, 1H), 5.14 (s, 1H), 4.40 (d, *J* = 4.6 Hz, 1H), 3.59 – 3.37 (m, 2H), 3.32 (dd, *J* = 13.9, 4.4 Hz, 1H), 3.17 (dd, *J* = 14.4, 7.4 Hz, 1H), 2.71 – 2.48 (m, 1H), 1.40 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.56, 169.83, 155.70, 155.11, 145.51, 136.35, 127.46, 125.28, 123.29, 122.58, 122.49, 120.01, 118.97, 111.39, 34.85, 34.07, 28.38. IR ν 3406, 2931, 1785, 1635, 1509, 1210, 1141, 834. HRMS (FAB+) *m/z* found for $C_{25}H_{28}N_4O_7$ 497.2038 [M+H], calcd 497.2036. $[\alpha]^{20}_{589nm}$ = 1,79 (c=0,0393 g/mL, MeOH).



2e

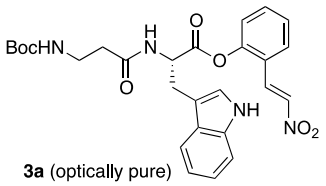
2e. A solution of (2-phenylquinolin-3-yl)methanethiol[1] (141 mg, 0.56 mmol, 1 equiv), dicyclohexylcarbodiimide (230 mg, 1.12 mmol, 2 equiv), hydroxybenzotriazole (152 mg, 1.12 mmol, 2 equiv) and Boc-Trp-ßAla-OH (420 mg, 1.12 mmol, 2 equiv) in DMF (5 mL) was made and stirred for 16 h at rt. The reaction mixture was filtered and diluted with dichloromethane (10 mL). The organic solution was washed three times with water, once with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc:hexanes = 1:4→3:7→1:1) to yield **2e** as a clear oil (212 mg, 0.35 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.19 (s, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.68 (t, *J* = 7.2 Hz, 1H), 7.61 – 7.36 (m, 8H), 7.21 (d, *J* = 7.9 Hz, 1H), 7.08 (dt, *J* = 14.6, 7.0 Hz, 2H), 6.88 (s, 1H), 6.33 (s, 1H), 5.27 (s, 1H), 4.37 (s, 1H), 4.23 – 4.09 (m, 2H), 3.56 – 3.02 (m, 5H), 2.46 (dd, *J* = 25.3, 19.7 Hz, 2H), 1.93 – 1.59 (m, 1H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.75, 171.95, 159.80, 157.27, 155.45, 146.74, 139.59, 137.66, 136.24, 130.00, 128.98, 128.95, 128.84, 128.66, 128.56, 127.37, 127.31, 126.95, 123.21, 122.05, 119.47, 118.71, 111.34, 110.16, 80.06, 55.35, 49.02, 42.62, 35.08, 33.85, 30.90, 28.51, 28.28, 25.57, 24.91. IR ν 3309, 2978, 2930, 1686, 1488, 1367, 1242, 1165, 1045, 742, 702. HRMS (FAB+) *m/z* found for $C_{35}H_{36}N_4O_4S$ 609.2536 [M+H], found 609.2536. $[\alpha]^{20}_{589nm}$ = -6,05 (c=0,0385 g/mL, MeOH).



3a (racemic)

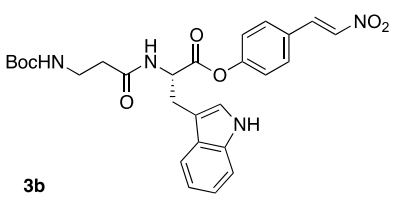
3a (racemate) A solution of Boc-ßAla-Trp-OH (0.50 g, 1.30 mmol, 1 equiv), HATU (0.76 g, 2.00 mmol, 1.5 equiv), DiPEA (0.52g, 0.66 mL, 4.00 mmol, 3 equiv) and (*E*)-2-(2-nitrovinyl)phenol (0.33 g, 2.00 mmol, 1.5 equiv) in THF (75 mL) was stirred 18 h at rt. The reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography (EtOAc:hexanes = 1:1→1:0) to yield **3a** (0.31 g, 0.59 mmol, 46%) as a yellow solid. Optical rotation measurement and further analysis by chiral HPLC revealed that full racemisation had occurred. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.96 (d, *J* = 13.7 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.57 – 7.47 (m, 3H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.22 – 7.16 (m, 1H), 7.15 – 7.12 (m, 1H), 7.11 – 7.06 (m, 2H), 7.04 (d, *J* = 1.9 Hz, 1H), 6.31 (t, *J* = 6.0 Hz, 1H), 5.16 (s, 1H), 4.40 (d, *J* = 5.5 Hz, 1H), 3.61 – 3.37 (m, 2H), 3.30 (dd, *J* = 14.3, 4.6 Hz, 1H), 3.16 (dd, *J* = 14.5, 7.4 Hz, 1H), 2.69 – 2.49 (m, 2H), 1.40 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.25, 170.24, 155.61, 153.24, 138.03, 137.28, 136.34, 131.57, 130.47, 127.85, 127.52,

123.30, 122.72, 122.41, 121.76, 119.85, 118.95, 116.77, 111.40, 110.58, 80.33, 55.49, 34.84, 34.09, 28.38. IR ν 3404, 2925, 1766, 1692, 1519, 1342, 1163,745. HRMS (FAB+) *m/z* found for $C_{27}H_{30}N_4O_7$ 523.2202 [M+H], calcd 523.2193.



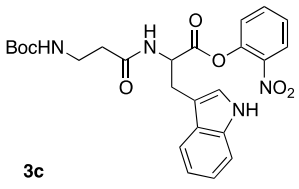
3a (optically pure)

3a. Boc-Trp-OH (1.00 g, 3.30 mmol, 1 equiv), HATU (2.50 g, 6.50 mmol, 2 equiv), DiPEA (1.30 g, 1.60 mL, 9.90 mmol, 3 equiv) and (*E*)-2-(2-nitrovinyl)phenol (1.10 g, 6.50 mmol, 2 equiv) were dissolved in THF (70 mL) and stirred for 20 h at rt. The reaction mixture was concentrated, diluted with EtOAc and washed with 1M $KHSO_4$ (three times), sat. $NaHCO_3$ (three times) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (EtOAc:PE = 1:2→1:0) to yield the anticipated ester as a yellow solid (1.40 g, 3.00 mmol, 91%). mp 168.5-170.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.94 (d, *J* = 13.7 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.56 – 7.48 (m, 2H), 7.47 – 7.36 (m, 2H), 7.33 – 7.25 (m, 2H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.17 (d, *J* = 2.1 Hz, 1H), 7.12 (t, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 5.14 (d, *J* = 7.0 Hz, 1H), 4.90 (dd, *J* = 13.1, 6.3 Hz, 1H), 3.45 (d, *J* = 6.2 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.28, 155.55, 150.21, 138.76, 136.45, 133.04, 132.92, 128.66, 127.47, 126.80, 123.50, 123.18, 122.70, 120.12, 118.94, 111.53, 109.81, 100.14, 80.69, 54.99, 28.41, 27.98. IR ν 3411, 2978, 2932, 1765, 1700, 1517, 1341, 1160, 738. HRMS (FAB+) *m/z* found for $C_{24}H_{25}N_3O_6$ 452.1828 [M+H], calcd 452.1822. $[\alpha]^{20}_{589nm}$ = -5,75 (c=0,040 g/mL, MeOH)
Subsequent removal of the Boc protective group was accomplished by dissolving of the above ester (0.25 g, 0.55 mmol, 1 equiv) in TFA:dichloromethane = 95:5 (10 mL) followed by stirring for 5 min at rt, and concentration *in vacuo*. To the residue was added THF (25 mL), DiPEA (0.36 g, 0.46 mL, 2.80 mmol, 5 equiv), HBTU (0.53 g, 1.40 mmol, 2.5 equiv) and Boc-ßAla-OH (0.26 g, 1.40 mmol, 2.5 equiv). The resulting mixture was stirred for 2 h at rt and concentrated *in vacuo*. EtOAc (50 mL) was added and the organic solution was washed with 1M $KHSO_4$ (three times), sat. $NaHCO_3$ (three times) and brine. The solution was dried over Na_2SO_4, filtered and concentrated. Flash chromatography of the residue (EtOAc:PE = 1:1→1:0) yielded optically pure **3a** (0.13 g, 0.25 mmol, 69%) as a yellow solid. mp 145.5-149.9 °C. $[\alpha]^{20}_{589nm}$ = -17,64 (c=0,0171 g/mL, MeOH). Further spectral data were analogues to racemic **3a**.



3b

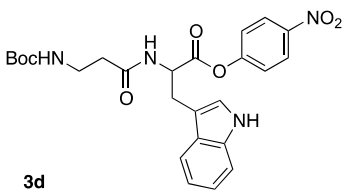
3b. A solution of Boc-ßAla-Trp-OH (50 mg, 0.13 mmol, 1 equiv), HATU (51 mg, 0.13 mmol, 1 equiv), DiPEA (35 mg, 44 µL, 0.27 mmol, 2 equiv) and (*E*)-4-(2-nitrovinyl)phenol (44 mg, 0.27 mmol, 2 equiv) in THF (10 mL) was stirred for 16 h at rt. The reaction mixture was concentrated and purified using flash chromatography (EtOAc:PE = 1:1→1:0) to yield **3b** (30 mg, 0.057 mmol, 44%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.94 (d, *J* = 13.7 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.50 (m, 3H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.16 – 7.08 (m, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.36 (s, 1H), 5.10 (q, *J* = 13.1, 6.2 Hz, 2H), 3.50 – 3.33 (m, 4H), 2.40 (t, *J* = 5.8 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.36, 156.23, 153.25, 137.97, 137.40, 136.38, 130.50, 128.09, 127.55, 123.11, 122.77, 122.66, 120.20, 118.69, 111.65, 109.70, 53.49, 36.32, 28.54, 27.75. IR ν 3400, 2976, 2931, 1761, 1682, 1635, 1519, 1506, 1340, 1167, 837. HRMS (FAB+) *m/z* found for $C_{27}H_{30}N_4O_7$ 523.2187 [M+H], calcd 523.2193.



3c

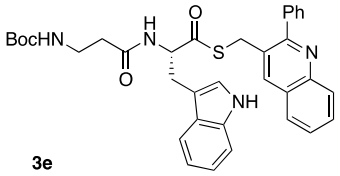
3c. A solution of Boc-ßAla-Trp-OH (50 mg, 0.13 mmol, 1 equiv), HATU (51 mg, 0.13 mmol, 1 equiv), DiPEA (35 mg, 44 µL, 0.27 mmol, 2 equiv) and 2-nitrophenol (37 mg, 0.27 mmol, 2 equiv) in THF (10 mL) was stirred for 16 h at rt. The reaction mixture was concentrated and the residue was purified by flash chromatography (EtOAc:PE = 1:1→1:0) to yield **3c** (17 mg, 0.034 mmol, 26%) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 7.63 (t, *J* = 8.6 Hz, 2H), 7.41 (dd, *J* = 11.2, 8.2 Hz, 2H), 7.25 – 7.20 (m, 1H), 7.19 – 7.12 (m, 3H), 6.16 (s, 1H), 5.23 – 5.03 (m, 2H), 3.59 (dd, *J* = 14.9, 5.4 Hz, 1H), 3.42 (dd, *J* = 15.0, 7.3 Hz, 1H), 3.36 (s, 2H), 2.35 (d, *J* = 5.6 Hz, 2H), 1.41 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.16, 169.71, 136.41, 134.95, 127.70, 127.06, 125.94, 125.40, 123.37, 122.68, 120.16, 118.67, 111.60, 109.96, 76.91, 53.31, 53.29, 36.29, 29.85,

28.54, 27.11. IR ν 3289, 2979, 2918, 1776, 1682, 1649, 1532, 1357, 1146, 753. HRMS (FAB+) *m/z* found for $C_{25}H_{28}N_4O_7$ 497.2038 [M+H], calcd 497.2036.



3d

3d. A solution of Boc-ßAla-Trp-OH (0.10 g, 0.27 mmol, 1 equiv), HATU (0.15 g, 0.4 mmol, 1.5 equiv), DiPEA (0.10 g, 130 µL, 0.80 mmol, 3 equiv) and 4-nitrophenol (0.056 g, 0.40 mmol, 1.5 equiv) in THF (20 mL) was stirred for 16 h at rt. The reaction mixture was concentrated en purified using flash chromatography (EtOAc:PE = 1:1→1:0) to yield **3d** (0.10 g, 0.20 mmol, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 – 8.16 (m, 3H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.17 – 7.09 (m, 2H), 7.04 (d, *J* = 9.1 Hz, 2H), 6.34 (s, 1H), 5.10 (q, *J* = 13.2, 6.3 Hz, 1H), 3.49 – 3.37 (m, 4H), 2.43 (t, *J* = 6.0 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.69, 170.02, 155.11, 145.67, 136.39, 127.46, 125.32, 123.09, 122.91, 122.44, 120.32, 118.67, 111.68, 109.65, 53.62, 36.80, 36.34, 28.54, 27.75. IR ν 3417, 2979, 2930, 1765, 1692, 1664, 1524, 1347, 1162, 845. HRMS (FAB+) *m/z* found for $C_{25}H_{28}N_4O_7$ 497.2038 [M+H], calcd 497.2036.



3e

3e. A solution of (2-phenylquinolin-3-yl)methanethiol[1] (82 mg, 0.33 mmol, 1 equiv), dicyclohexylcarbodiimide (134 mg, 0.65 mmol, 2 equiv), hydroyxbenzotriazole (88 mg, 0.65 mmol, 2 equiv) and Boc-ßAla-Trp-OH (245 mg, 0.65 mmol, 2 equiv) in DMF (5 mL) was stirred for 16 h at rt. The reaction mixture was filtered and diluted with dichloromethane. The organic solution was washed three times with water, once with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography using silica (EtOAc:hexanes = 1:4→3:7→1:1) to yield **3e** as a clear oil (181 mg, 0.30 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 – 8.08 (m, 3H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.53 – 7.41 (m, 6H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.68 (s, 1H), 6.11 (s, 1H), 5.15 – 4.92 (m, 2H), 4.27 – 4.12 (m, 2H), 3.41 – 3.14 (m, 4H), 2.30 (s, 2H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.66, 174.22, 171.56, 156.12, 138.31, 136.22, 130.34, 129.02, 128.91, 128.73, 127.55, 127.53, 127.42, 127.18, 123.16, 122.54, 120.05, 118.46, 111.58, 109.25, 100.12, 59.29, 36.50, 36.26, 31.26, 28.50, 28.18. IR ν 3308, 2978, 2930, 1684, 1489, 1367, 1239, 1165, 1043, 742, 701. HRMS (FAB+) *m/z* found for $C_{35}H_{36}N_4O_4S$ 609.2536 [M+H], calcd 609.2536. $[\propto]^{20}_{589nm}$ = -29,26 (c=0,0682 g/mL, MeOH).

Solid phase synthesis of Boc-Phe-Tyr(t-Bu)-Ala-Gly-OH

Solid phase peptide synthesis (SPPS) chemistry was performed in a round bottom flask where agitation was achieved via rotation. After each step the organic solution was removed by filtration. Peptide synthesis consisted of the following steps.

Resin activation:
The resin (2-chlorotrityl) was washed once with DMF and three times with DCM. Subsequently, a DCM:$SOCl_2$=10:1 mixture (10mL per gram of resin) was added and the resin was agitated for 30 minutes. After filtration the resin was washed three times with DCM.

Coupling of first peptide:
A solution of Fmoc-AA-OH (4.0 equiv) and D*i*PEA (4.0 equiv) in NMP was added to the resin which was agitated for 1 hour followed by washing three times with MeOH. Capping of the remaining free 2-chlorotrityl groups was performed by addition of D*i*PEA (4.0 equiv) in MeOH. The resin was agitated in this solution for 30 minutes after which the resin was washed three times with MeOH and three times with NMP.

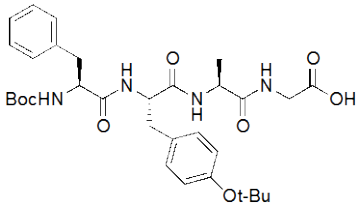
Fmoc removal:
The resin was agitated 3 times in a NMP:piperidine=4:1 mixture (10 mL per gram of resin) for 5 minutes. Removal of the fluorenemethyl derivative and piperidine traces was assured by washing the resin with NMP three times.

PG-AA-OH coupling:
A solution of the appropriate amino acid (4.0 equiv), HBTU (4.0 equiv), HOBt (4.0 equiv) and D*i*PEA (8.0 equiv) in NMP was added to the resin, which was agitated for at least 2 hours. After peptide

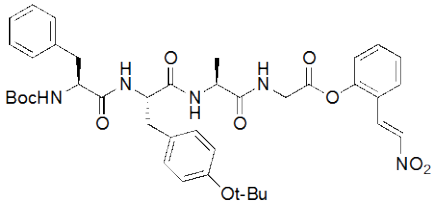
coupling, three washing steps with NMP were performed in order to remove side products and residual coupling reagents.

Peptide cleavage:

The resin was suspended in a 1%TFA in DCM solution (10 mL per gram of resin) and agitated 5 minutes. The filtrate was collected and the above-mentioned step was repeated until the resin had colored an intense dark red. The resin was then washed with DCM three times. The combined organic solutions were diluted with toluene (to approximately 1:1) and concentrated *in vacuo*.

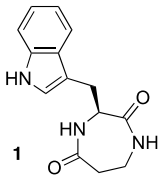


Boc-Phe-Tyr(tBu)-Ala-Gly-OH: The synthesis was performed by SPPS using 2-chloro trityl resin (loading 1.27 mmol/g, 4.0 g, 5.1 mmol) to yield **Boc-Phe-Tyr(tBu)-Ala-Gly-OH** (2.4 g, 3.9 mmol, 77%) as a white solid. mp 113.8-115.1 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.36 – 7.12 (m, 8H), 6.90 (dd, *J* = 8.4, 4.4 Hz, 2H), 4.63 (dt, *J* = 13.7, 5.2 Hz, 1H), 4.45 – 4.36 (m, 1H), 4.29 (dd, *J* = 9.2, 4.9 Hz, 1H), 3.98 – 3.82 (m, 2H), 3.14 – 2.88 (m, 4H), 2.72 (dd, *J* = 13.7, 9.7 Hz, 1H), 1.35 (br. s, 12H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 174.85, 174.27, 172.91, 172.66, 155.41, 138.53, 133.19, 131.05, 130.33, 129.38, 127.67, 125.18, 116.34, 80.75, 79.47, 57.46, 55.90, 50.16, 41.77, 39.12, 38.17, 29.21, 28.66, 18.07. IR ν 3291, 2977, 2931, 1723, 1634, 1507, 1366, 1159, 697. HRMS (FAB+) *m/z* found for $C_{32}H_{44}N_4O_8$ 613.3230 [M+H], calcd 613.3237. $[\propto]^{20}_{589nm}$ = -20.06 (c=0,0176 g/mL, MeOH)



Boc-Phe-Tyr(tBu)-Ala-Gly-O-(E)-2-(2-nitrovinyl)phenyl: A solution of **Boc-Phe-Tyr(tBu)-Ala-Gly-OH** (2.06 g, 3.37 mmol, 1.0 equiv), HATU (1.28 g, 3.37 mmol, 1.0 equiv), D*i*PEA (1.11 mL, 0.87 g, 6.73 mmol, 2.0 equiv) and (*E*)-2-(2-nitrovinyl)phenol (1.11 g, 6.73 mmol 2.0 equiv) in THF (50 mL) was stirred 4 hours. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in EtOAC. The organic solution was washed three times with 1M $KHSO_4$ (aq), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc:MeOH=1:0→95:5) yielding **Boc-Phe-Tyr(tBu)-Ala-Gly-O-(E)-2-(2-nitrovinyl)phenyl** (1.96 g, 2.58 mmol, 77%) as a yellow solid. mp 122.3-125.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 13.8 Hz, 1H), 7.50 – 7.43 (m, 2H), 7.40 – 7.35 (m, 1H), 7.27 – 7.17 (m, 6H), 7.02 (dd, *J* = 7.7, 1.6 Hz, 2H), 6.85 – 6.74 (m, 5H), 4.72 (s, 1H), 6.36 (s, 1H), 4.50 – 4.37 (m, 2H), 4.20 (qd, *J* = 17.9, 5.8 Hz, 2H), 4.10 – 4.07 (m, 1H), 3.04 – 2.91 (m, 2H), 2.81 (dd, *J* = 14.2, 5.9 Hz, 1H), 2.72 (dd, *J* = 14.1, 8.2 Hz, 1H), 1.25 (d, *J* = 7.3 Hz, 3H), 1.21 (s, 9H), 1.18 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.21, 172.73, 170.75, 167.91, 154.90, 149.92, 138.76, 135.45, 133.04, 132.96, 130.02, 129.49, 129.05, 129.00, 128.92, 127.52, 126.66, 124.34, 123.63, 122.94, 81.49, 78.49, 60.42, 56.93, 54.62, 49.16, 41.71, 37.36, 35.96, 28.85, 28.03, 17.18, 14.20. IR ν 3272, 2977, 2933, 1772, 1630, 1506, 1340, 1159, 844, 699. HRMS (FAB+) *m/z* found for $C_{40}H_{49}N_5O_{10}$ 760.3558 [M+H], calcd 760.3558. $[\propto]^{20}_{589nm}$ = -5.28 (c=0,0123 g/mL, MeOH)

1. General procedures.

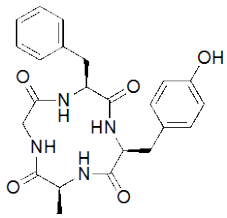


A: The linear precursor (0.06 mmol) was dissolved in TFA:dichloromethane = 95:5 (5 mL) and stirred for 10 min at rt. The reaction mixture was concentrated *in vacuo* and the residual oil was taken up in EtOAc (10 mL). The solution was added over 52 h to a heated (50 °C) suspension of $NaHCO_3$ (2.0 g) in EtOAc (50 mL). The reaction mixture was stirred for two additional hours, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc:MeOH = 1:0→24:1) to yield **1** as a clear oil. ^{1}H NMR (400 MHz, MeOD) δ 7.57 (t, *J* = 8.9 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.16 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 1H), 4.66 (dd, *J* = 8.3, 5.9 Hz, 1H), 3.66 (ddd, *J* = 15.4, 11.7, 3.7 Hz, 1H), 3.18 (dt, *J* = 15.4, 4.7 Hz, 1H), 3.05 (dd, *J* = 14.9, 8.4 Hz, 1H), 2.61 (dt, *J* = 17.8, 3.8 Hz, 1H), 2.55 – 2.44 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 175.87, 175.35, 164.22, 163.88, 163.54, 163.20, 138.75, 129.06, 125.62, 123.20, 120.55, 119.63, 113.00, 111.03, 54.39, 50.42, 37.76, 37.50, 27.63. IR ν 3413, 2926, 1679, 1444, 1208, 1138, 845, 803, 725. HRMS (FAB+) *m/z* found for $C_{14}H_{15}N_3O_2$ 258.1241 [M+H], calcd 258.1241 [M+H]. $[\propto]^{20}_{589nm}$ = 19,9 (c=0,02325 g/mL, MeOH).

B: The linear precursor was dissolved in freshly distilled dichloromethane and methyl trifluoromethanesulfonate (1 equiv) was added. The mixture was stirred for 2 h and the reaction was monitored by LCMS. In case of an incomplete reaction another equivalent of methyl trifluoromethanesulfonate was added and the reaction mixture was stirred for another 2 h. The reaction mixture was concentrated *in vacuo* and subsequently dissolved in TFA:dichloromethane = 95:5, stirred for 10 min and concentrated. The resulting salt was diluted with acetonitrile to a concentration of 1 mM. To this solution triethylamine (50 equiv) was added. The reaction mixture was heated at 50 ºC and stirred for 24 h. The reaction mixture was concentrated and the residue was purified by flash chromatography (EtOAc:MeOH = 1:0→24:1) to yield **1** as a clear oil.
C: The linear precursor was dissolved in TFA:dichloromethane = 95:5 (10 mM), stirred for 10 min and concentrated. The resulting salt was dissolved in acetonitrile to obtain a final concentration of 1 mM. To this solution triethylamine (50 equiv) was added and the reaction mixture was heated at 50 ºC and stirred for 24 h. The reaction mixture was concentrated and the residue was purified by flash chromatography (EtOAc:MeOH = 1:0→24:1) to yield **1** as a clear oil.

The obtained yields of **1** are collected in the Table below.

Cyclisation precursor	Method	Yield (%)	Optical purity
2a	**A**	62	>99%
2b	**A**	Trace	n.d.
2c	**A**	13	n.d.
2d	**A**	12	n.d.
2e	**B**	22	n.d.
2e	**C**	44	n.d.
3a	**A**	67	>99%
3b	**A**	15	n.d.
3c	**A**	26	n.d.
3d	**A**	23	n.d.
3e	**B**	64	82%
3e	**C**	34	n.d.

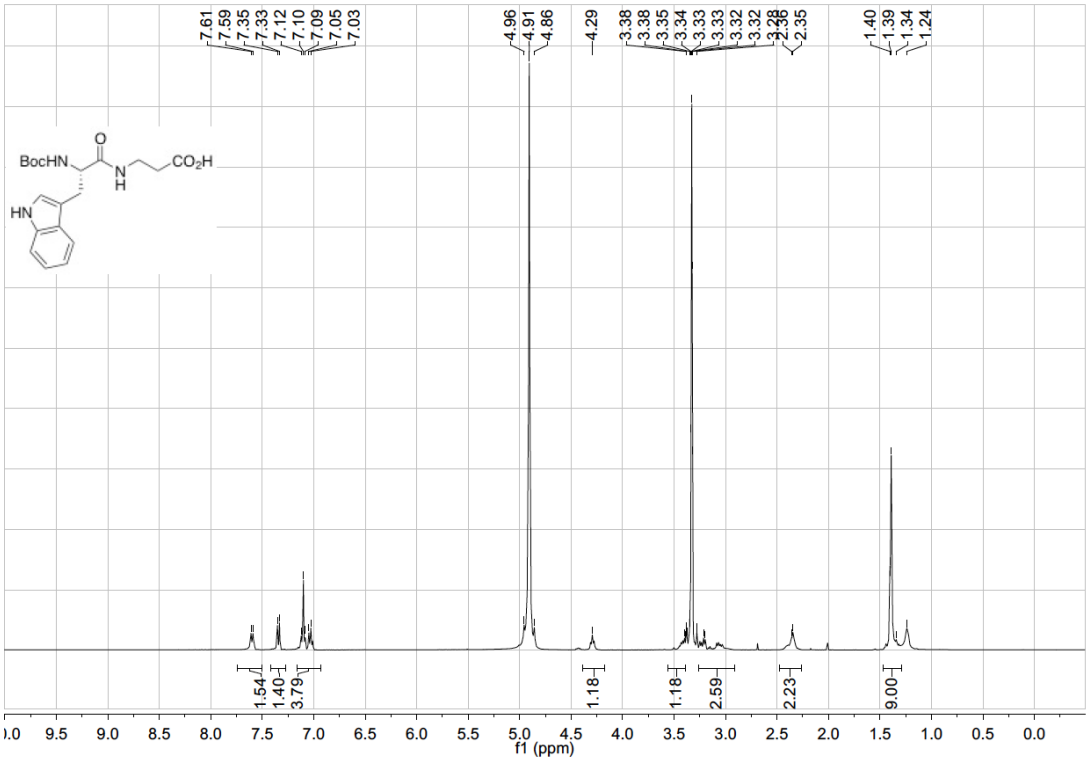


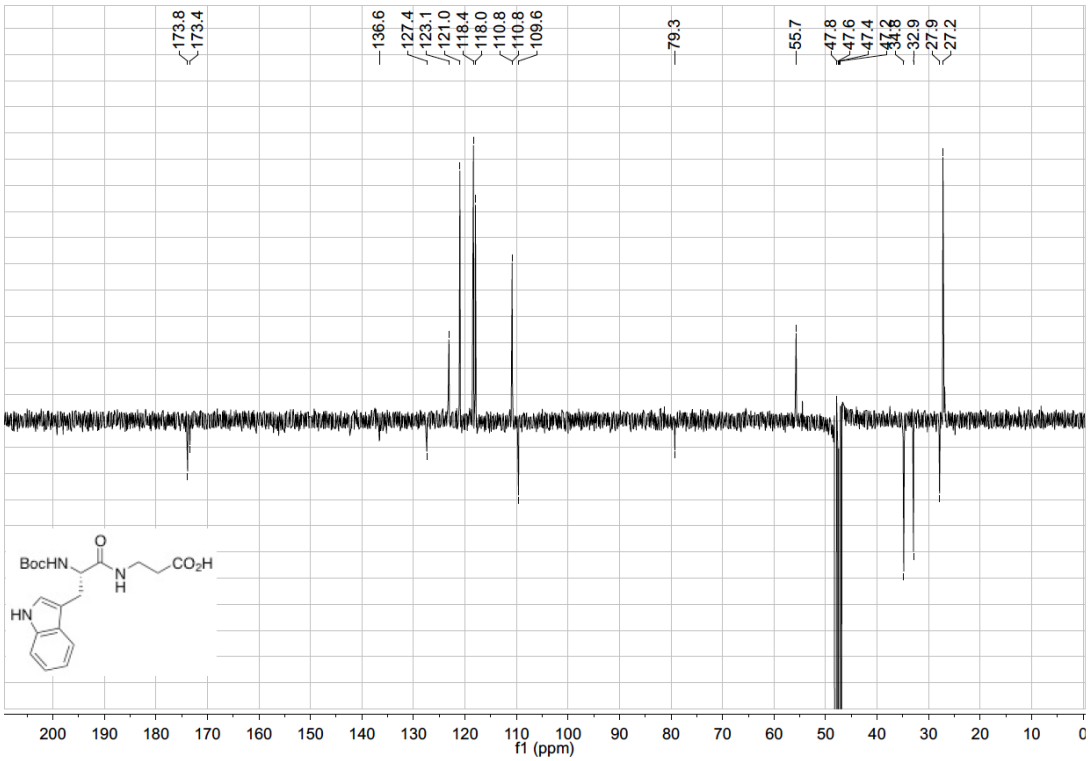
cyclo[Phe-Tyr-Ala-Gly]: A solution of **Boc-Phe-Tyr(tBu)-Ala-Gly-O-(E)-2-(2-nitrovinyl)phenyl** (1.24 g, 1.63 mmol, 1.0 equiv) in TFA:DCM=95:5 (10 mL) was stirred 30 minutes before being concentrated *in vacuo*. The residue was dissolved in EtOAc (10mL) and the organic solution was added to a heated (65 ºC) suspension of $NaHCO_3$ (2.0 g) in EtOAc (2.0 L). The reaction mixture was stirred 48 hours. The suspension was filtered and the filtrate was concentrated *in vacuo* and the residue was purified via flash column chromatography (EtOAC:MeOH=1:0→99:1→98:2→96:4) yielding **cyclo[Phe-Tyr-Ala-Gly]** (0.203 g, 0.463 mmol, 28%) as a yellow solid. mp 182.5-192.1 °C. ^{1}H NMR (500 MHz, DMSO 75 °C) δ 8.96 (br. s, 1H), 7.97 (br. s, *J* = 7.1 Hz, 1H), 7.51 (br d*J* = 57.9 Hz, 2H), 7.22 (br. s, 2H), 7.09 (s, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 7.9 Hz, 2H), 4.39 (br. s, 2H), 4.06 (br. s, 1H), 2.85 (br. s, 2H), 1.28 (br. s, 3H). ^{13}C NMR (126 MHz, DMSO 75 °C) δ 129.67, 129.33, 128.85, 128.42, 127.72, 125.85, 114.63, 109.14, 67.29, 59.25, 47.82, 28.54, 23.07, 20.24, 17.89, 13.65. IR ν 3306, 1653, 1540, 1517, 1455, 1192, 1135, 800, 723. HRMS (ESI) *m/z* found for $C_{23}H_{27}N_4O_5$ 439.1968 [M+H], calcd 439.1981. $[\alpha]^{20}_{589nm}$ = -6.68 (c=0,0283 g/mL, MeOH)

[1] Leleu, S.; Penhoat, M.; Bouet, A.; Dupas, G.; Papamicael, C.; Marsais, F.; Levacher, V., *J. Am. Chem. Soc.* **2005,** 127, 15668-15669.

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

Boc-Trp-ßAla-OH





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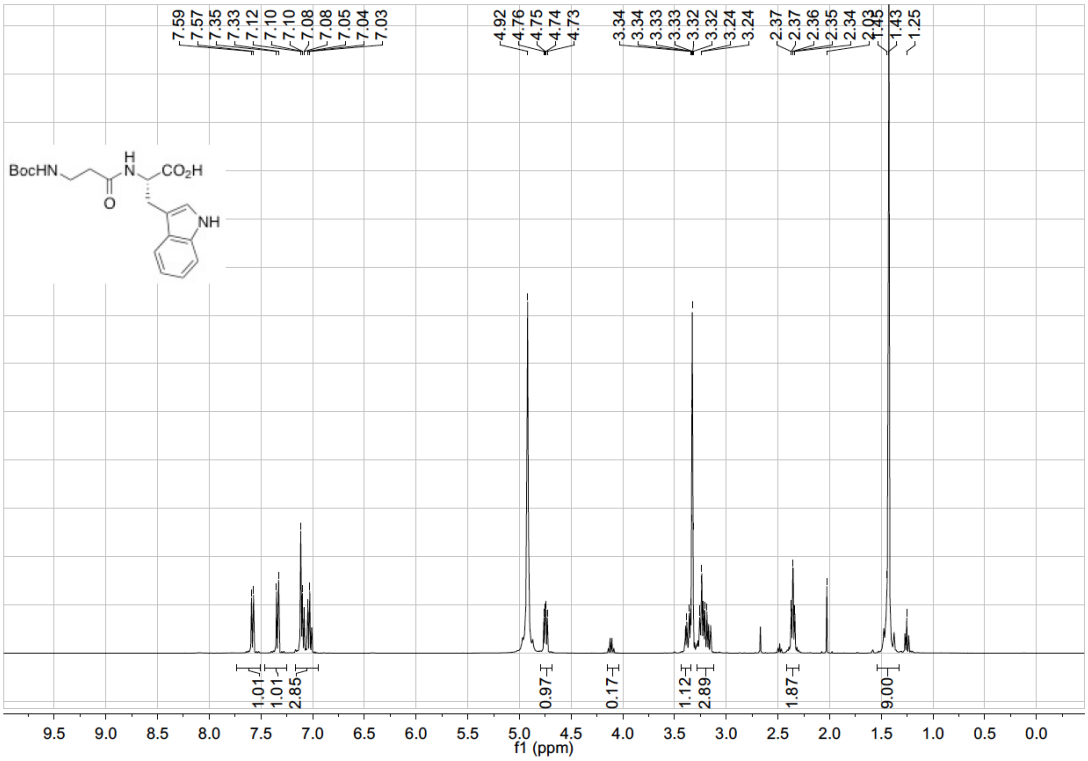
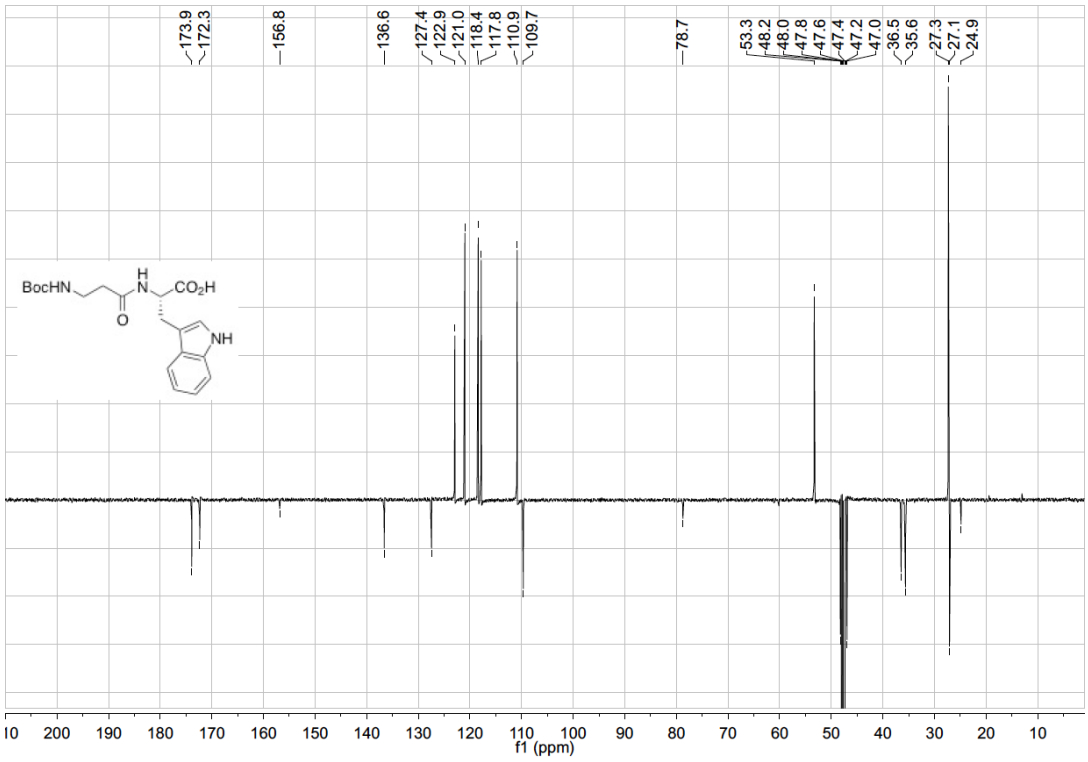
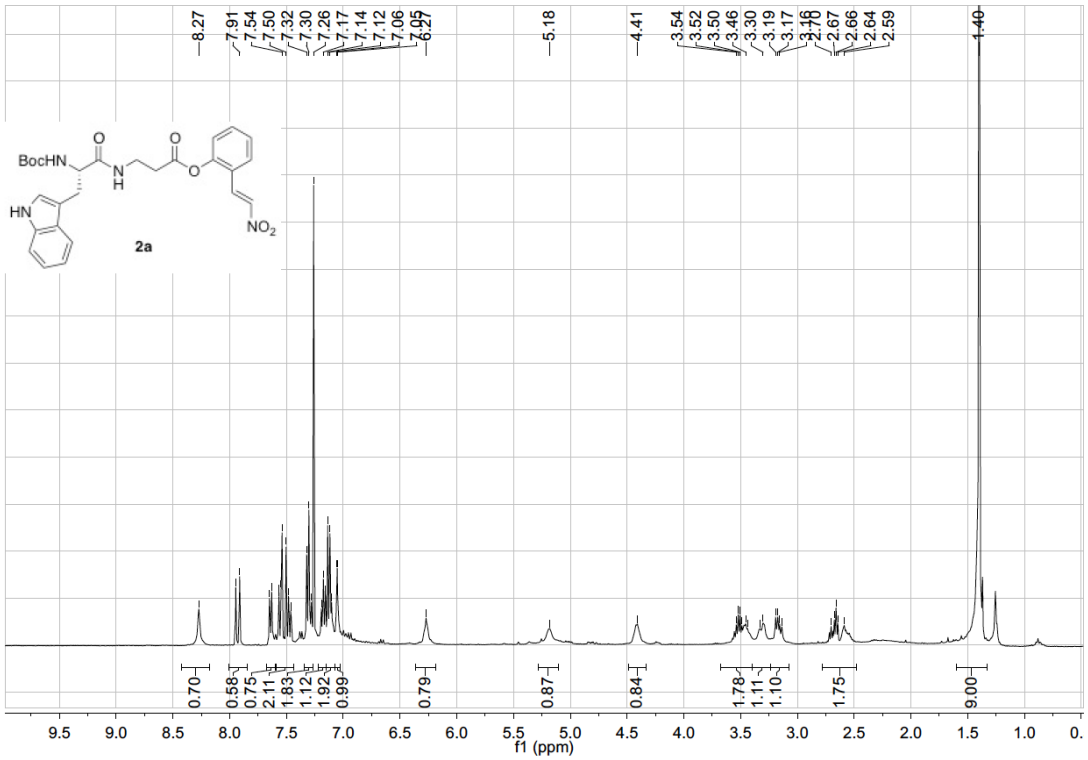




2a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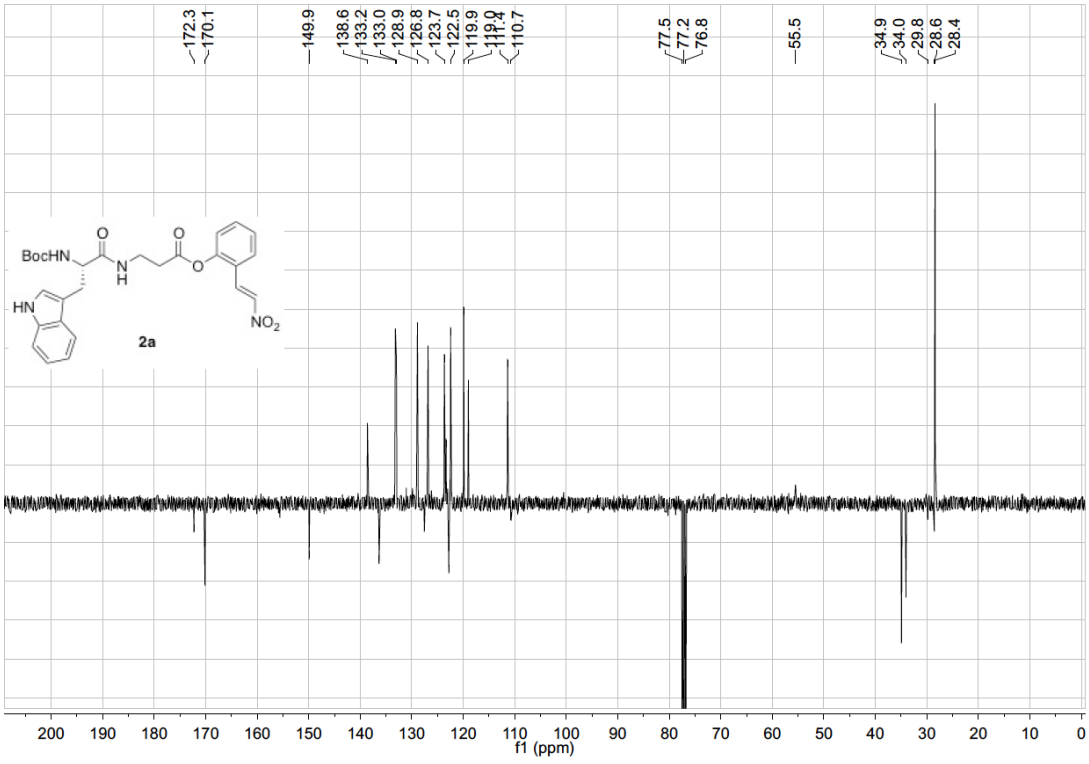
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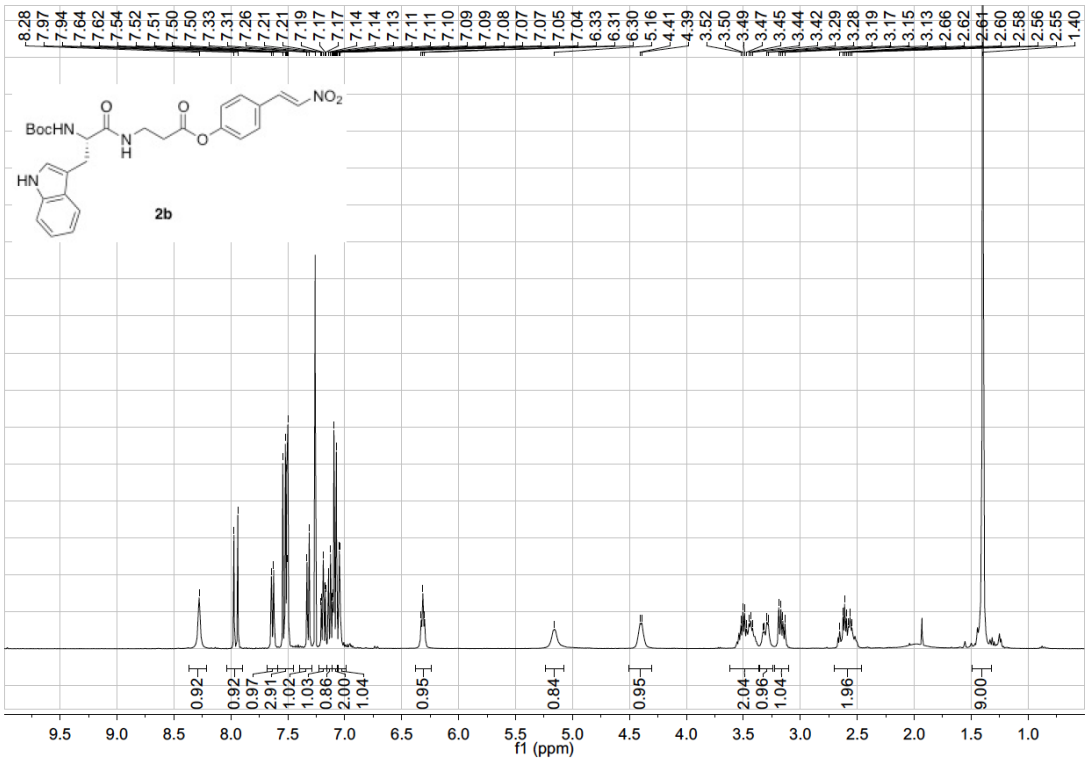
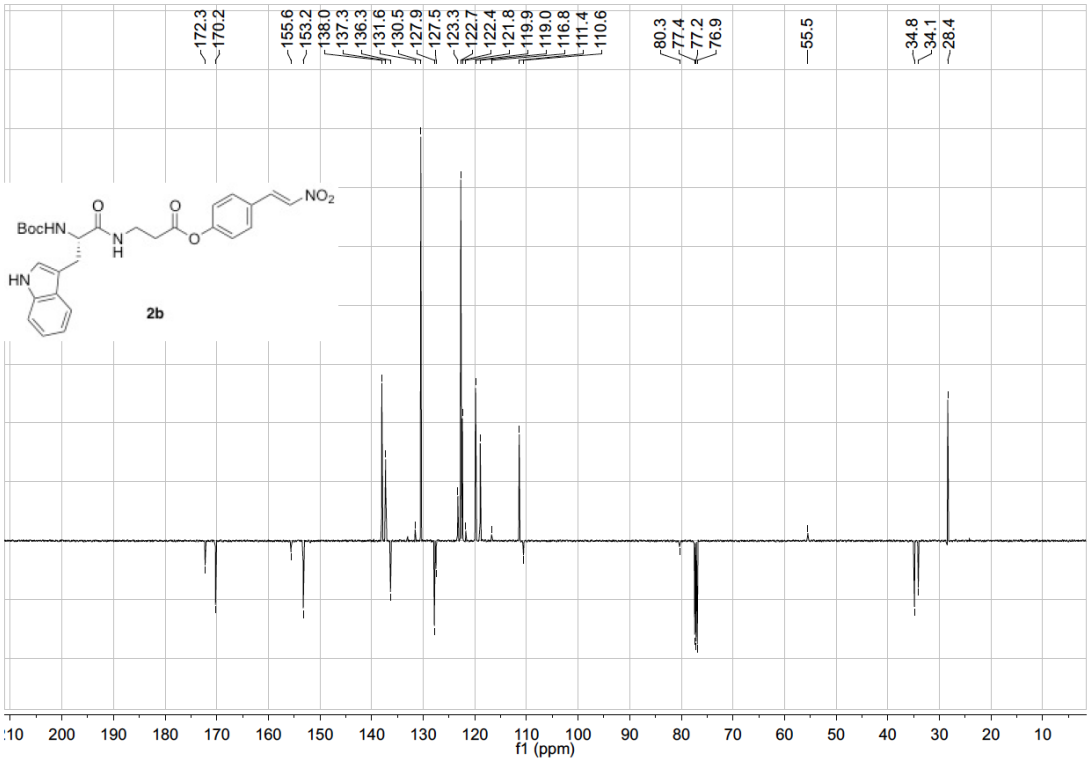
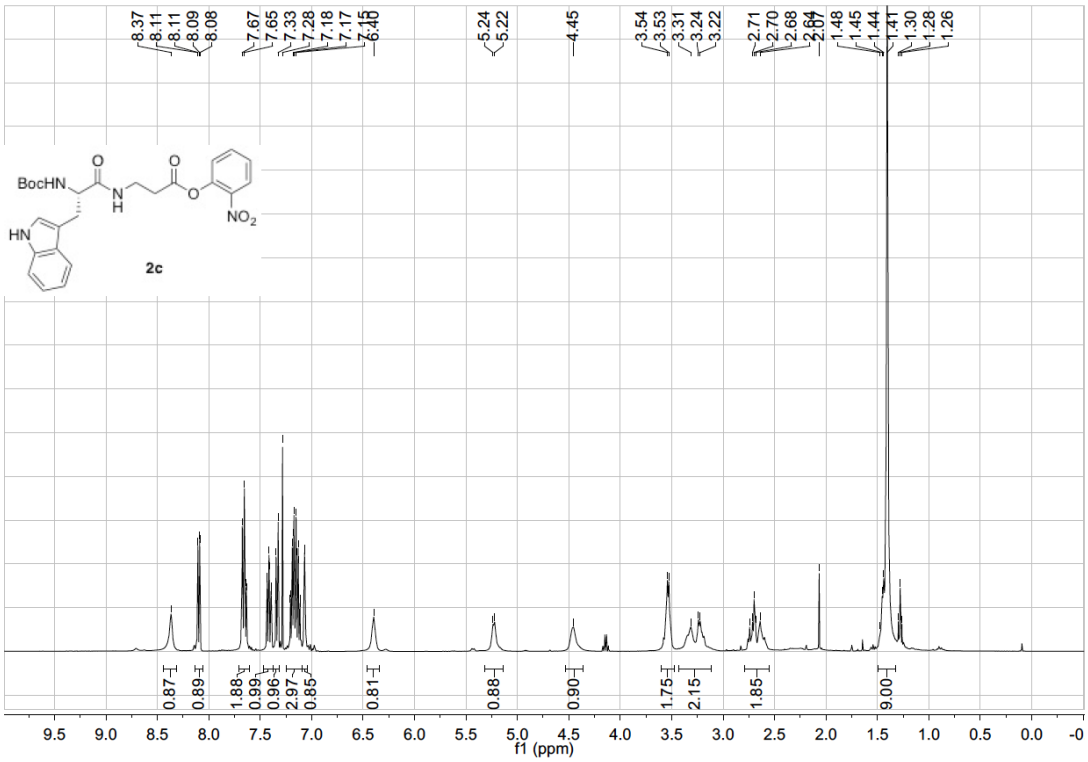
f1 (ppm)

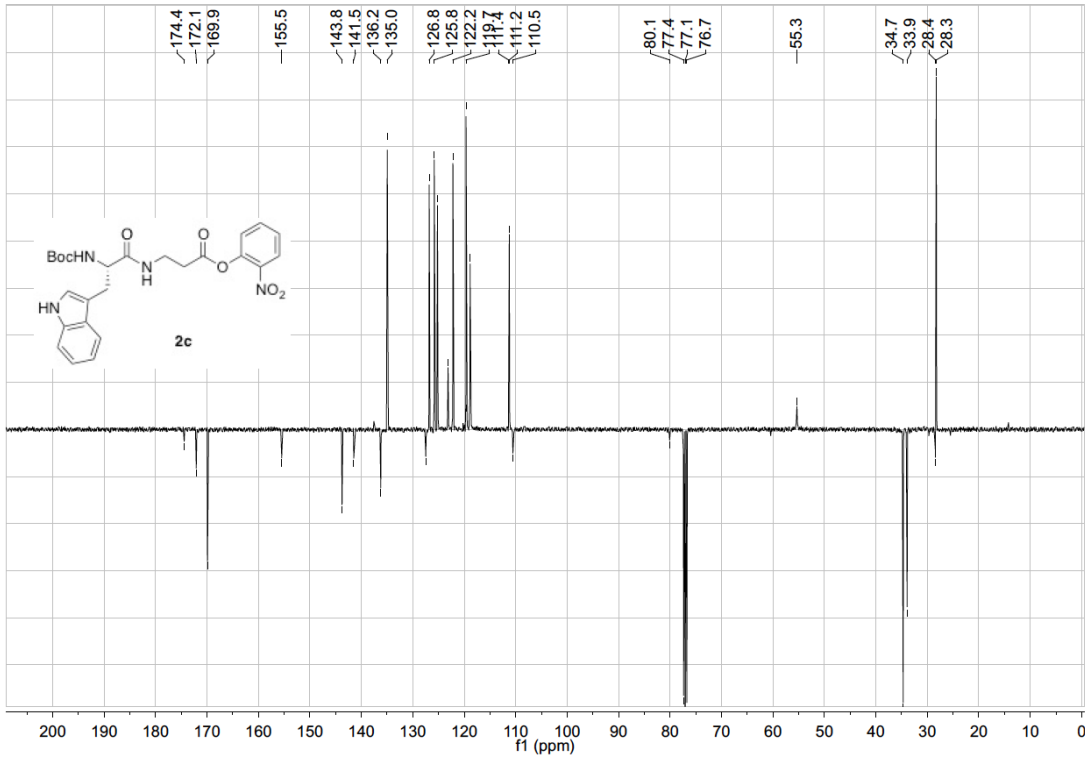
2b



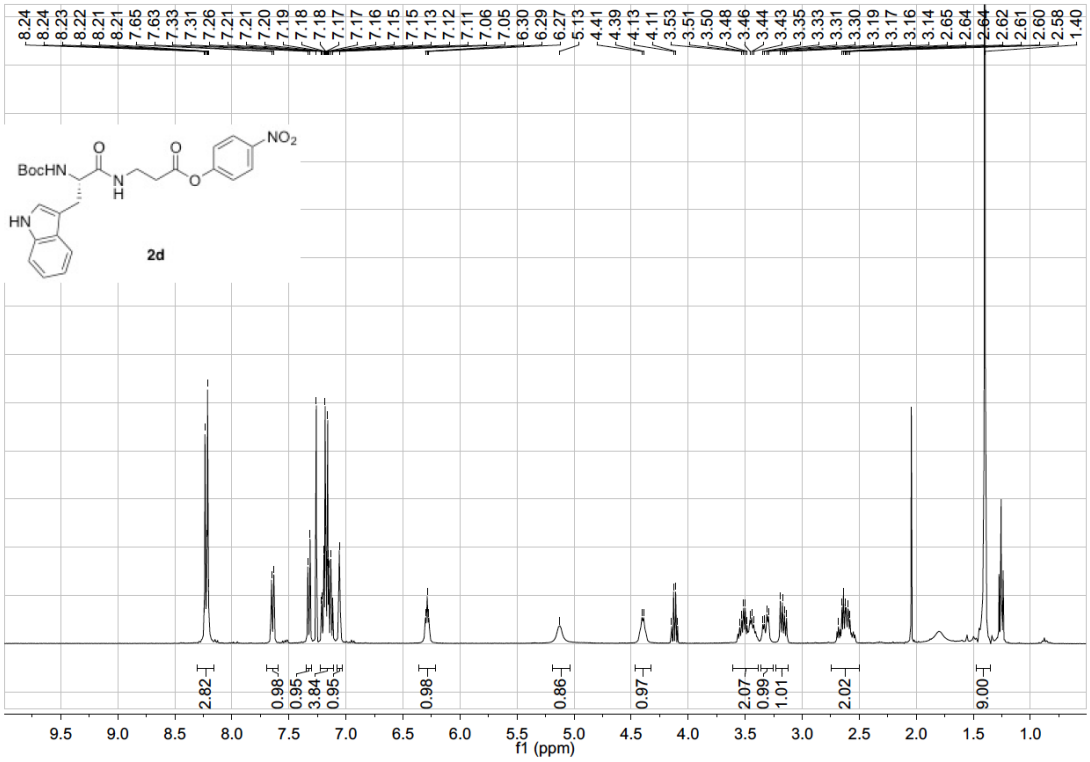


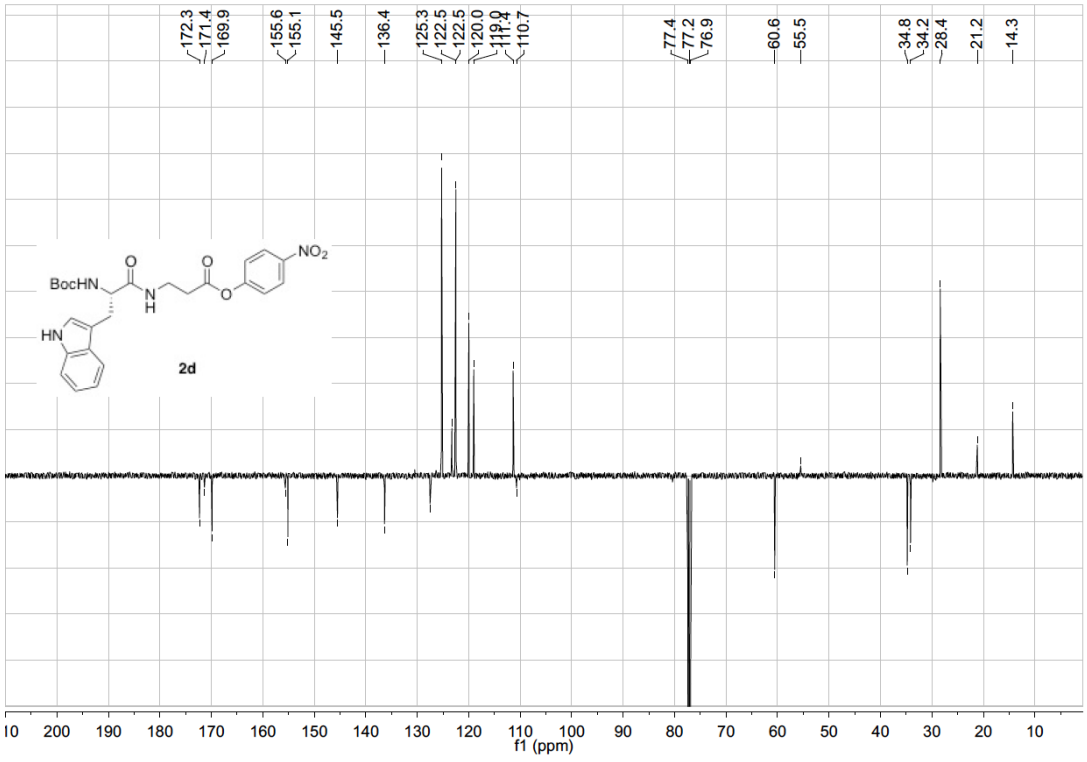
2c


8.37
8.11
8.09
8.08
7.67
7.65
7.33
7.28
7.18
7.17
7.15
6.40
5.24
5.22
4.45
3.54
3.53
3.31
3.24
3.22
2.71
2.70
2.68
2.64
2.07
1.48
1.45
1.44
1.41
1.30
1.28
1.26
BocHN
HN
NO2
2c
0.87
0.89
1.88
0.99
0.96
2.97
0.85
0.81
0.88
0.90
1.75
2.15
1.85
9.00
f1 (ppm)

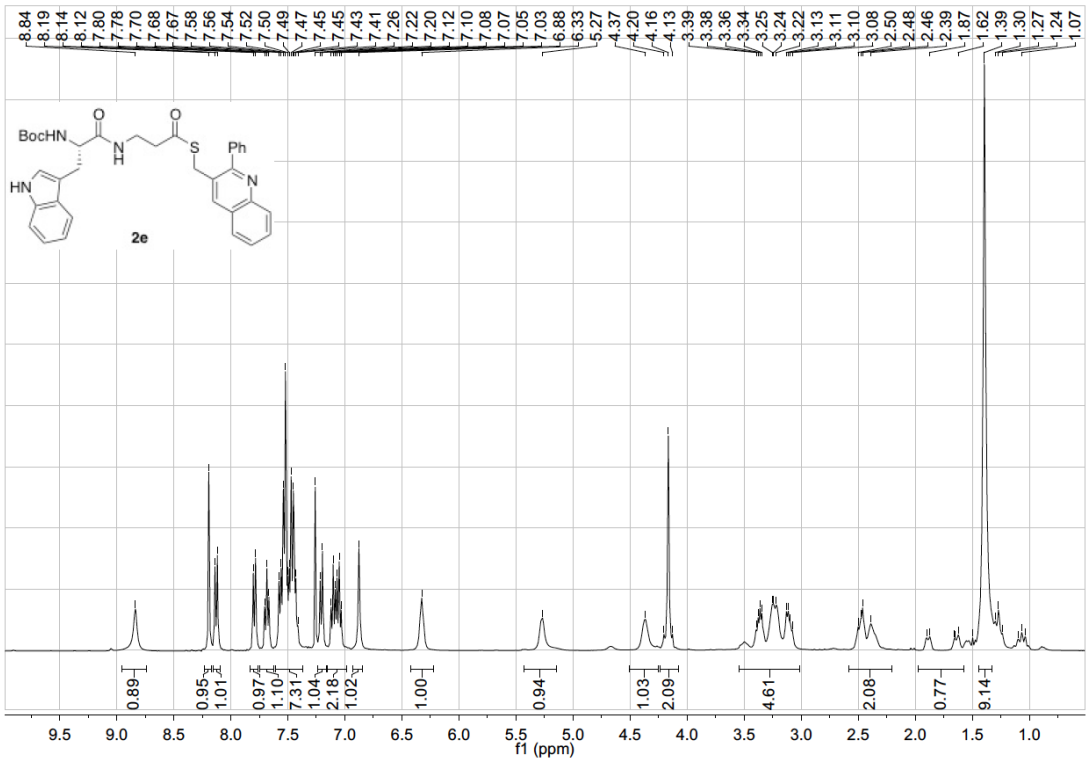

174.4
172.1
169.9
155.5
143.8
141.5
136.2
135.0
126.8
125.8
122.2
119.7
111.2
110.5
80.1
77.4
77.1
76.7
55.3
34.7
33.9
28.4
28.3
BocHN
HN
NO2
2c
f1 (ppm)

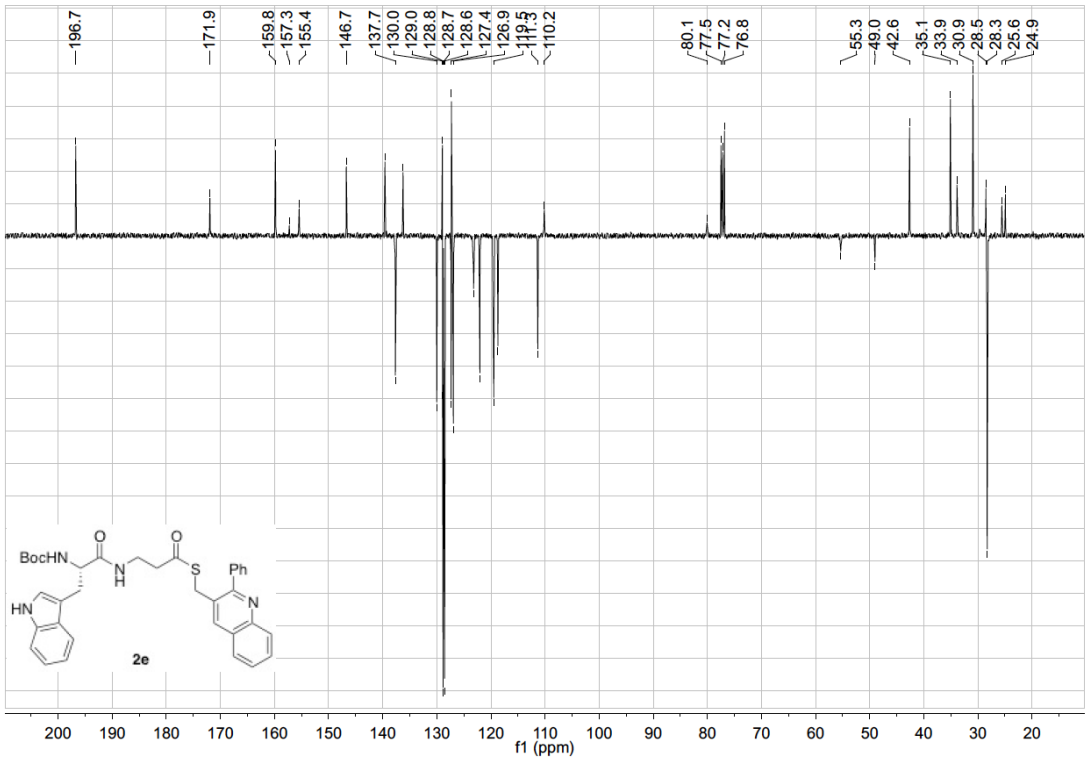
2d


8.24
8.24
8.23
8.22
8.21
8.21
7.65
7.63
7.33
7.31
7.26
7.21
7.21
7.20
7.19
7.18
7.18
7.17
7.17
7.16
7.15
7.15
7.13
7.12
7.11
7.06
7.05
6.30
6.29
6.27
5.13
4.41
4.39
4.13
4.11
3.53
3.51
3.50
3.48
3.46
3.44
3.43
3.35
3.33
3.31
3.30
3.19
3.17
3.16
3.14
2.65
2.64
2.64
2.62
2.61
2.60
2.58
1.40
BocHN
O
N
H
O
O
NO2
HN
2d
2.82
0.98
0.95
3.84
0.95
0.98
0.86
0.97
2.07
0.99
1.01
2.02
9.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

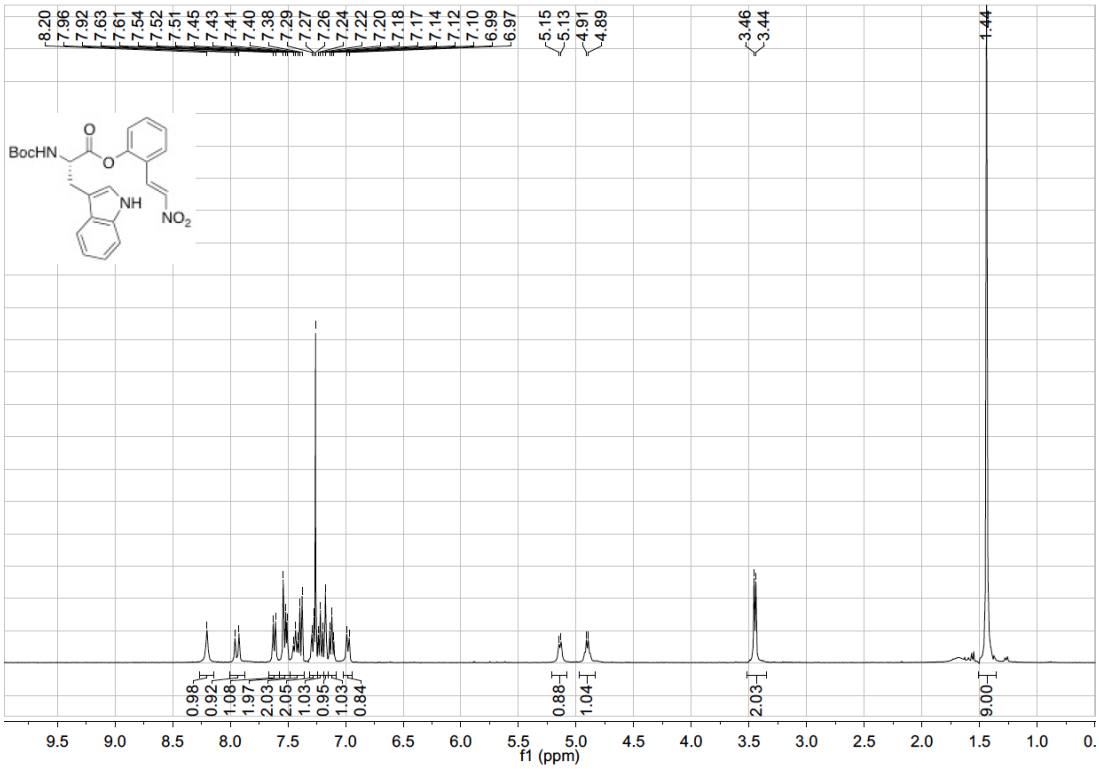

172.3
171.4
169.9
155.6
155.1
145.5
136.4
125.3
122.5
122.5
120.0
111.9
110.7
77.4
77.2
76.9
60.6
55.5
34.8
34.2
28.4
21.2
14.3
BocHN
O
N
H
O
O
NO2
HN
2d
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

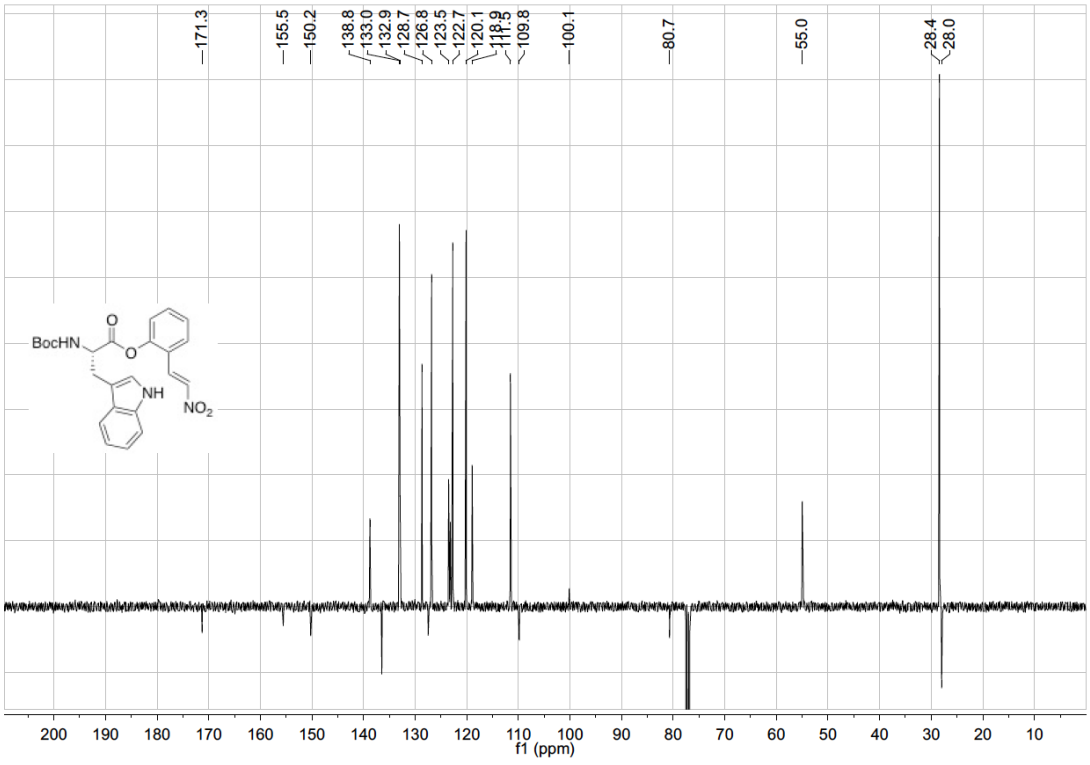
2e


8.84 8.19 8.14 8.12 7.80 7.78 7.70 7.68 7.67 7.58 7.56 7.54 7.52 7.50 7.49 7.47 7.45 7.43 7.41 7.26 7.22 7.20 7.12 7.10 7.08 7.07 7.05 7.03 6.88 6.33 5.27 4.37 4.20 4.16 4.13 3.39 3.38 3.36 3.34 3.25 3.24 3.22 3.13 3.11 3.10 3.08 2.50 2.48 2.46 2.39 1.87 1.62 1.39 1.30 1.27 1.24 1.07
BocHN
HN
O
N
H
O
S
Ph
N
2e
0.89 0.95 1.01 0.97 1.10 7.31 1.04 2.18 1.02 1.00 0.94 1.03 2.09 4.61 2.08 0.77 9.14
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

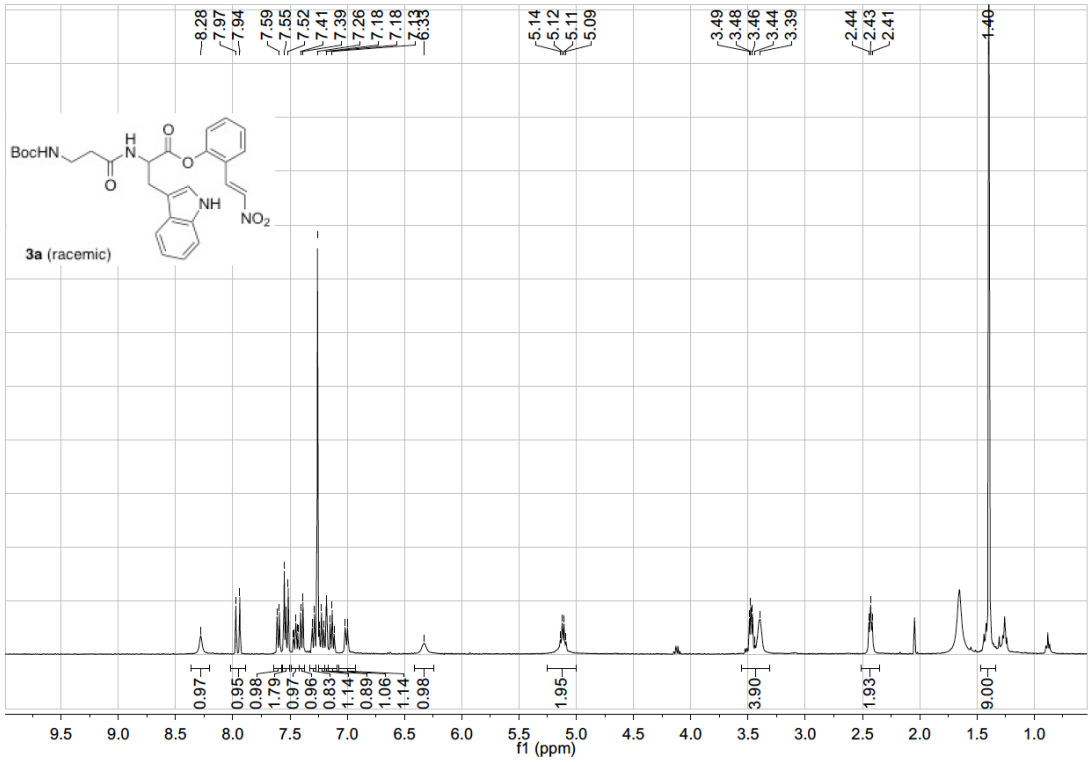

196.7 171.9 159.8 157.3 155.4 146.7 137.7 130.0 129.0 128.8 128.7 128.6 127.4 126.9 119.5 119.3 110.2
80.1 77.5 77.2 76.8
55.3 49.0 42.6 35.1 33.9 30.9 28.5 28.3 25.6 24.9
BocHN
HN
O
N
H
O
S
Ph
N
2e
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

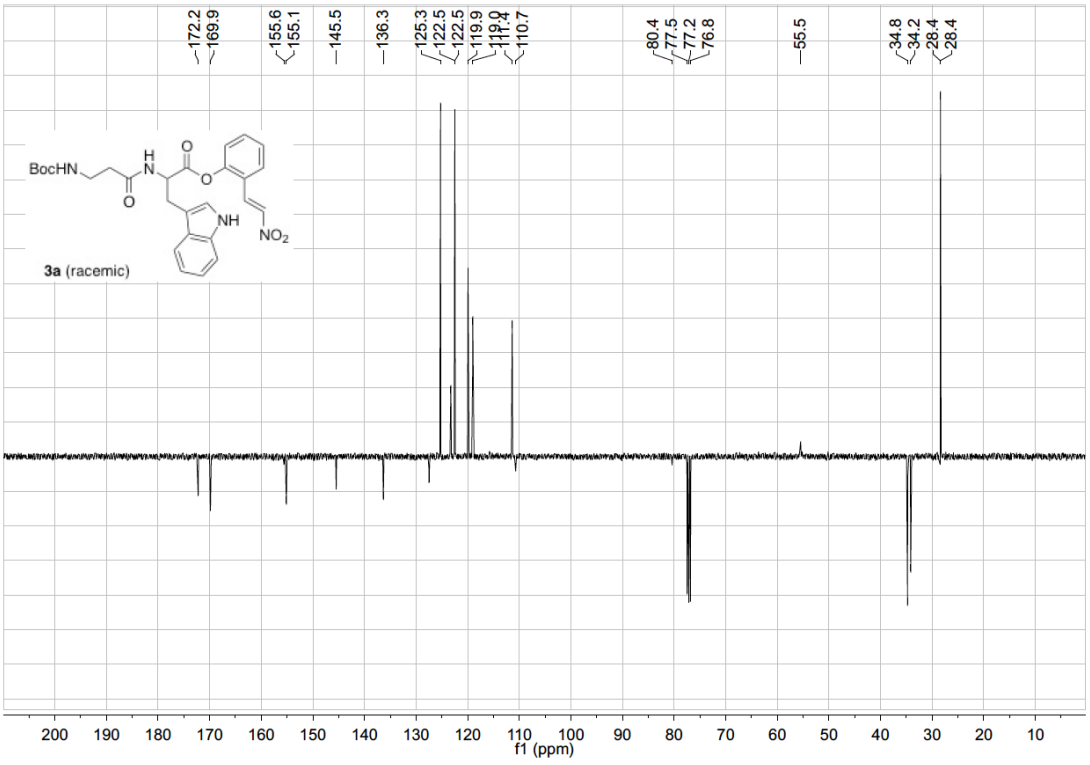
Boc-Trp-OAr Building block for optically pure synthesis 3a



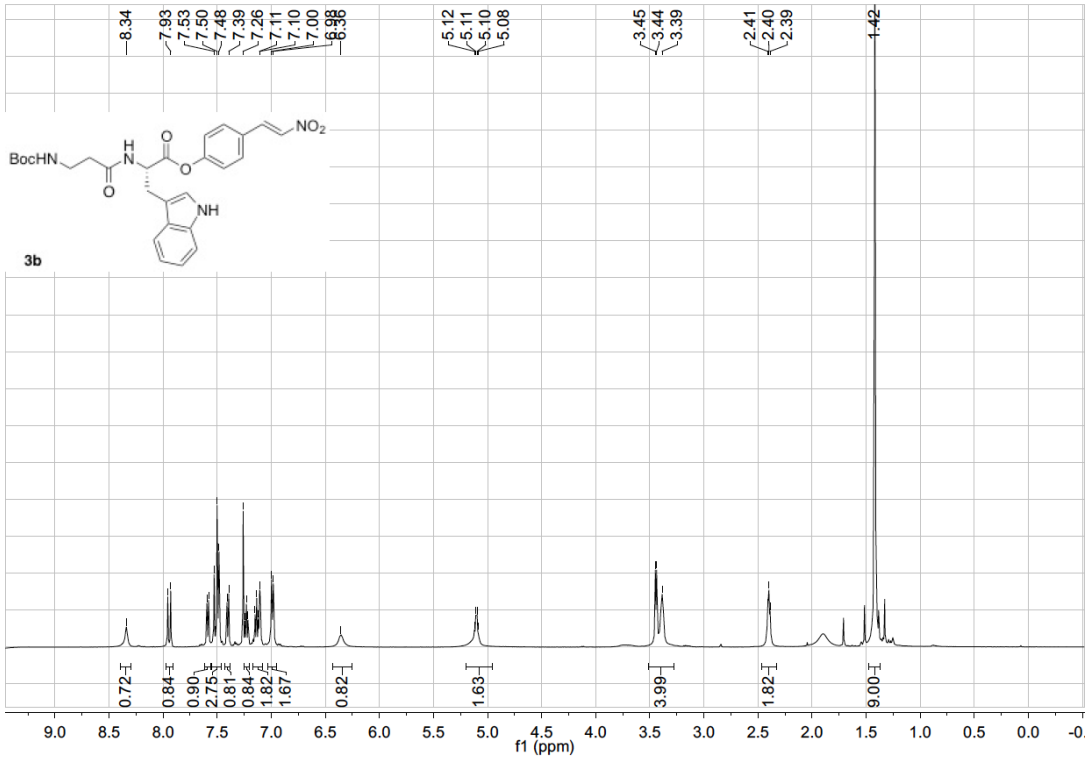


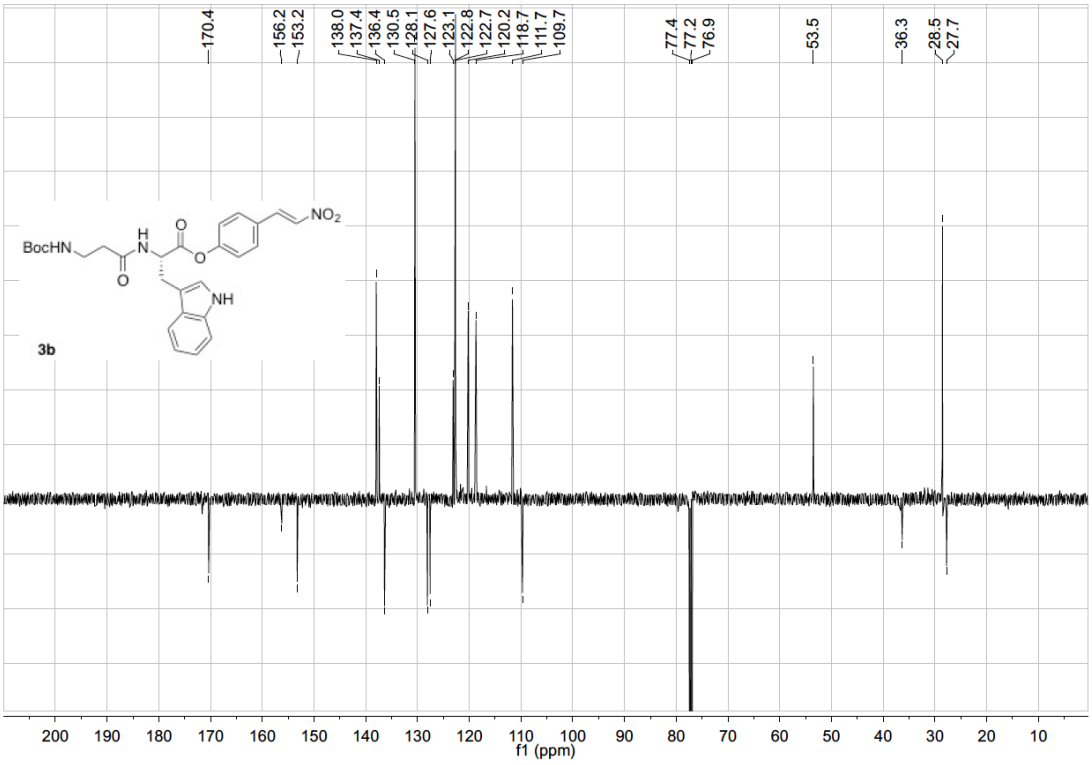
3a


8.28
7.97
7.94
7.59
7.55
7.52
7.41
7.39
7.26
7.18
6.33
5.14
5.12
5.11
5.09
3.49
3.48
3.46
3.44
3.39
2.44
2.43
2.41
1.40
BocHN
NH
NO2
3a (racemic)
0.97
0.95
0.98
1.79
0.97
0.96
0.83
1.14
0.89
1.06
1.14
0.98
1.95
3.90
1.93
9.00
f1 (ppm)

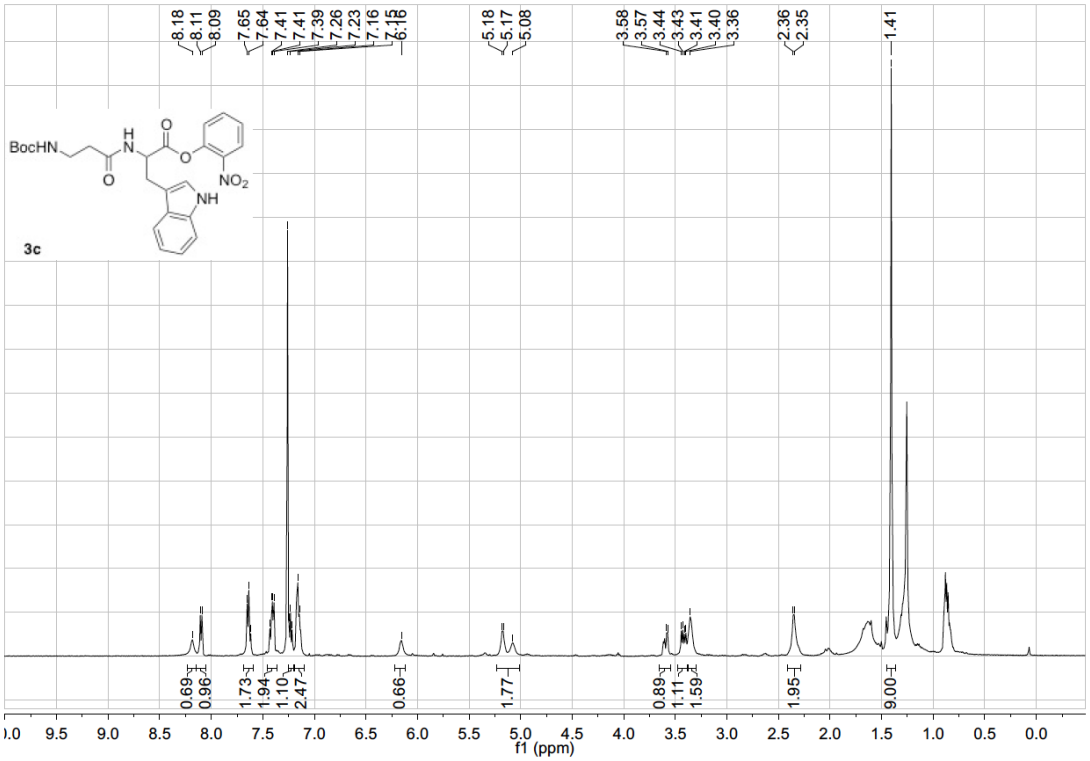

172.2
169.9
155.6
155.1
145.5
136.3
125.3
122.5
122.5
119.9
111.4
110.7
80.4
77.5
77.2
76.8
55.5
34.8
34.2
28.4
28.4
BocHN
NH
NO2
3a (racemic)
f1 (ppm)

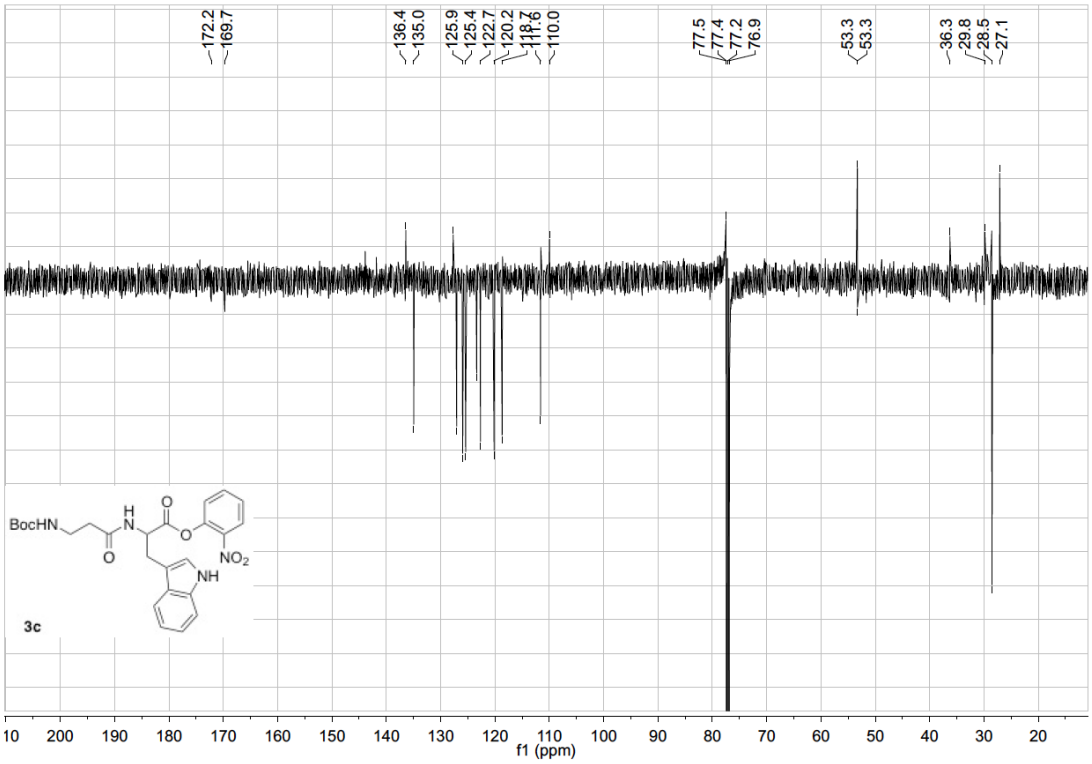
3b


8.34
7.93
7.53
7.50
7.48
7.39
7.26
7.11
7.10
7.00
6.98
6.96
5.12
5.11
5.10
5.08
3.45
3.44
3.39
2.41
2.40
2.39
1.42
BocHN
NH
NO2
3b
0.72
0.84
0.90
2.75
0.81
0.84
1.82
1.67
0.82
1.63
3.99
1.82
9.00
f1 (ppm)

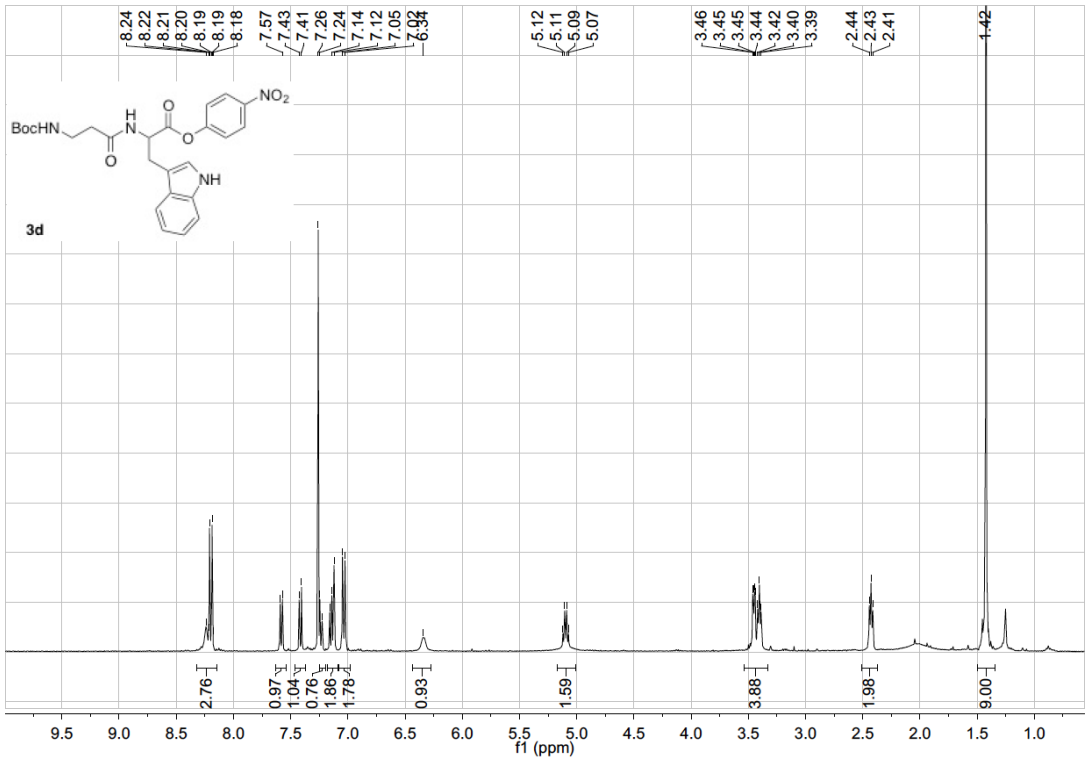

170.4
156.2
153.2
138.0
137.4
136.4
130.5
128.1
127.6
123.1
122.8
122.7
120.2
118.7
111.7
109.7
77.4
77.2
76.9
53.5
36.3
28.5
27.7
BocHN
NH
NO2
3b
f1 (ppm)

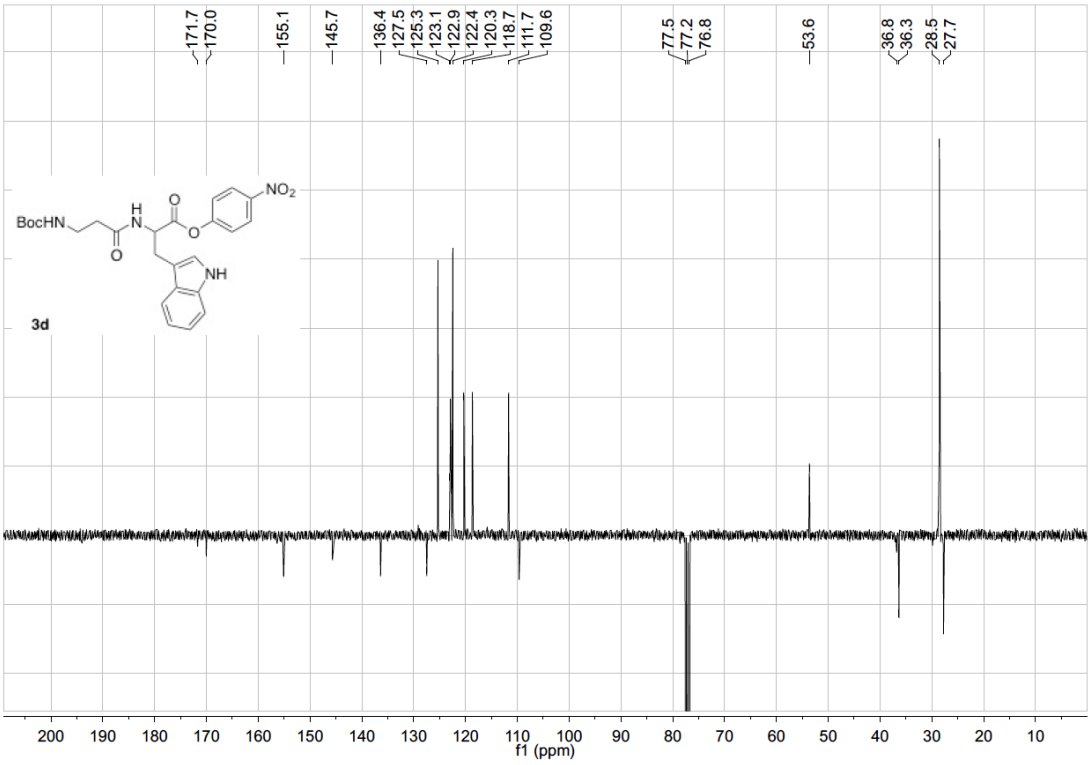
3c


8.18
8.11
8.09
7.65
7.64
7.41
7.41
7.39
7.26
7.23
7.16
7.15
6.16
5.18
5.17
5.08
3.58
3.57
3.44
3.43
3.41
3.40
3.36
2.36
2.35
1.41
BocHN
NO2
NH
3c
0.69
0.96
1.73
1.94
1.10
2.47
0.66
1.77
0.89
1.11
1.59
1.95
9.00
f1 (ppm)

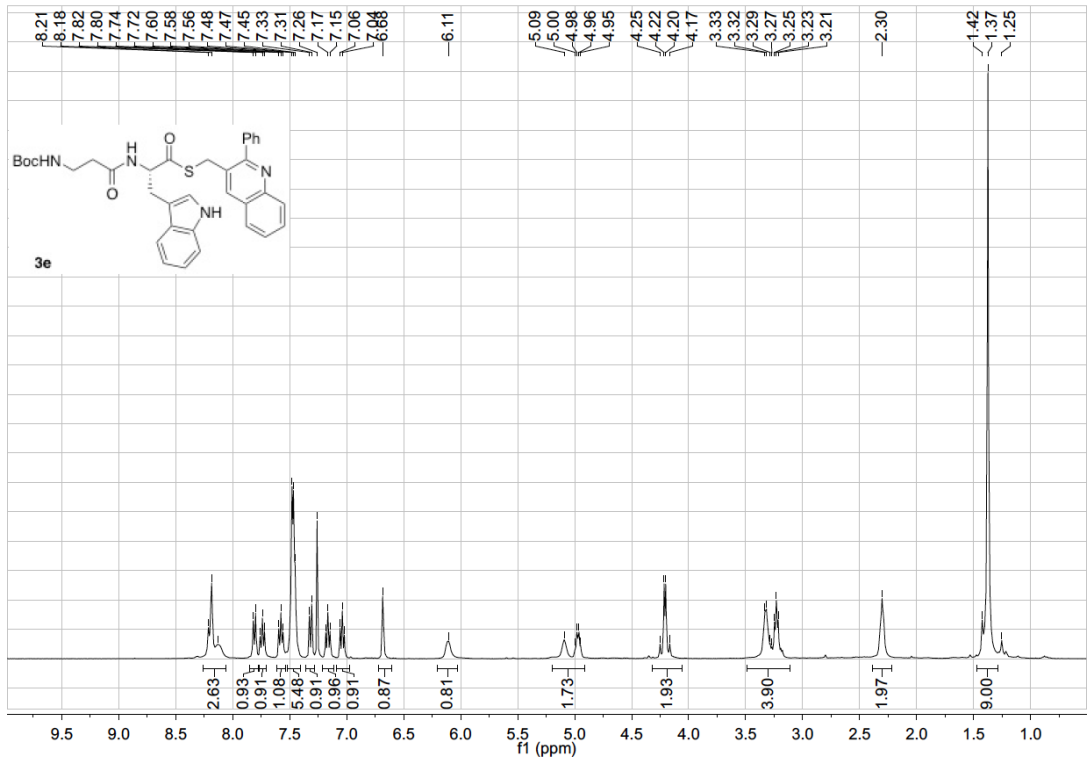

172.2
169.7
136.4
135.0
125.9
125.4
122.7
120.2
111.6
110.0
77.5
77.4
77.2
76.9
53.3
53.3
36.3
29.8
28.5
27.1
BocHN
NO2
NH
3c
f1 (ppm)

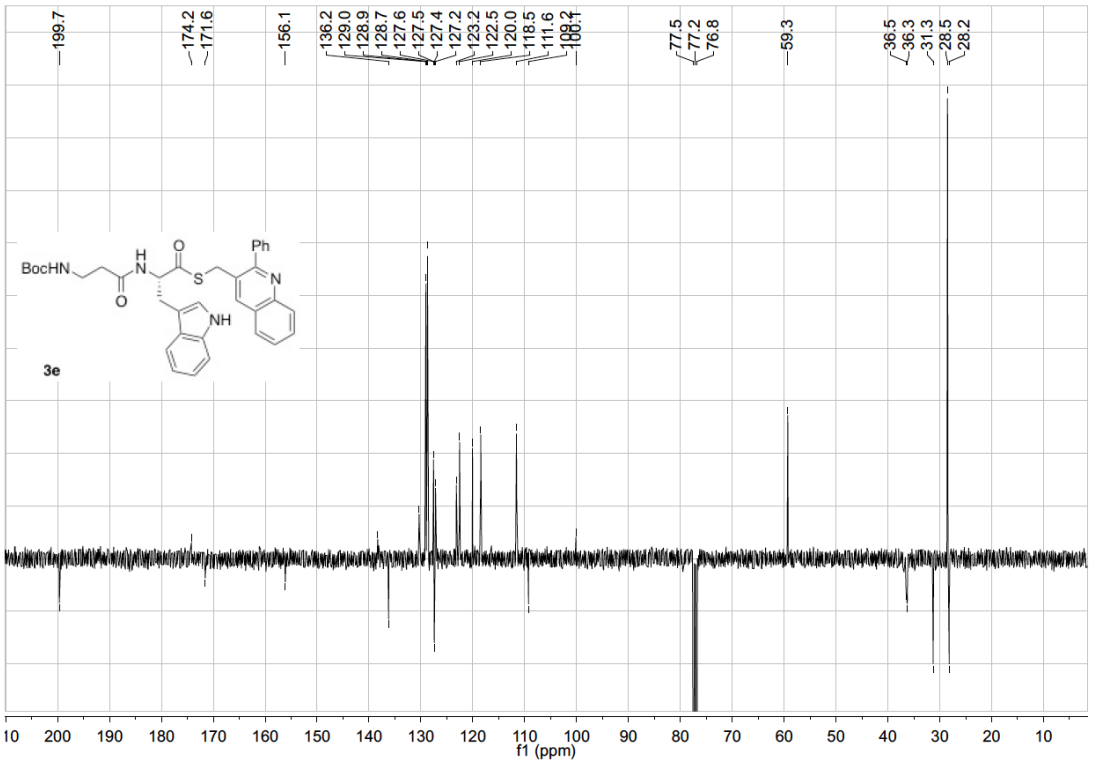
3d


8.24
8.22
8.21
8.20
8.19
8.19
8.18
7.57
7.43
7.41
7.26
7.24
7.14
7.12
7.05
6.94
6.94
5.12
5.11
5.09
5.07
3.46
3.45
3.45
3.44
3.42
3.40
3.39
2.44
2.43
2.41
1.42
BocHN
NH
NO2
3d
2.76
0.97
1.04
0.76
1.86
1.78
0.93
1.59
3.88
1.98
9.00
f1 (ppm)

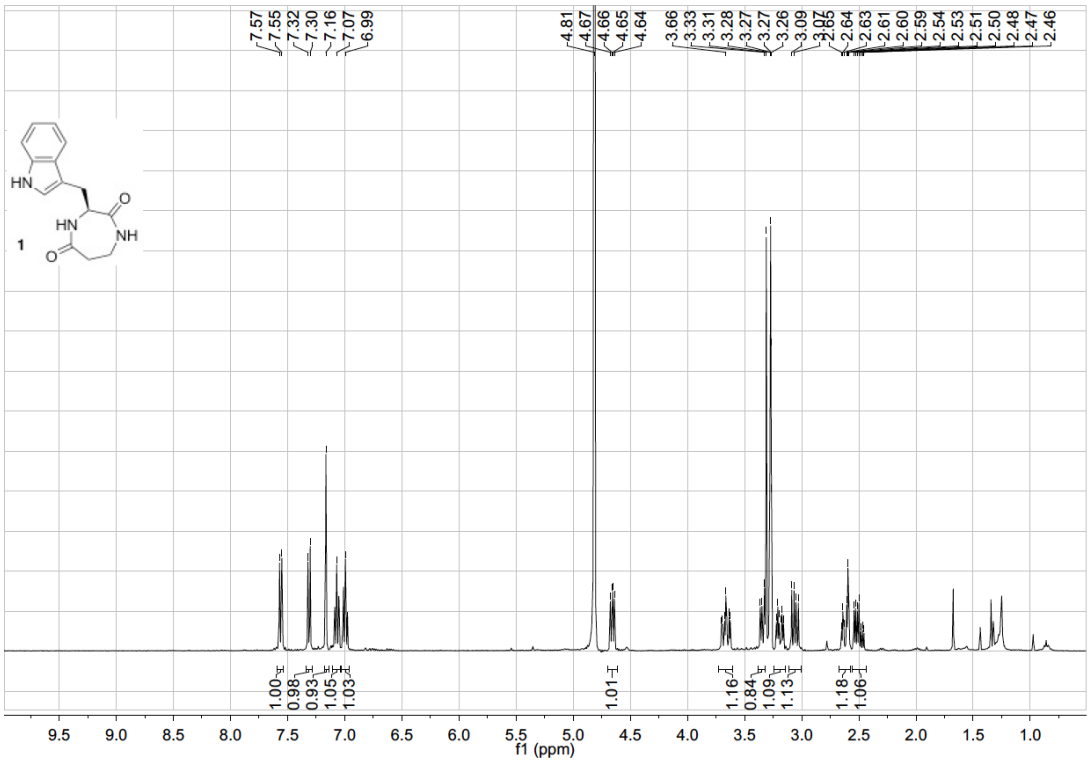

171.7
170.0
155.1
145.7
136.4
127.5
125.3
123.1
122.9
122.4
120.3
118.7
111.7
109.6
77.5
77.2
76.8
53.6
36.8
36.3
28.5
27.7
BocHN
NH
NO2
3d
f1 (ppm)

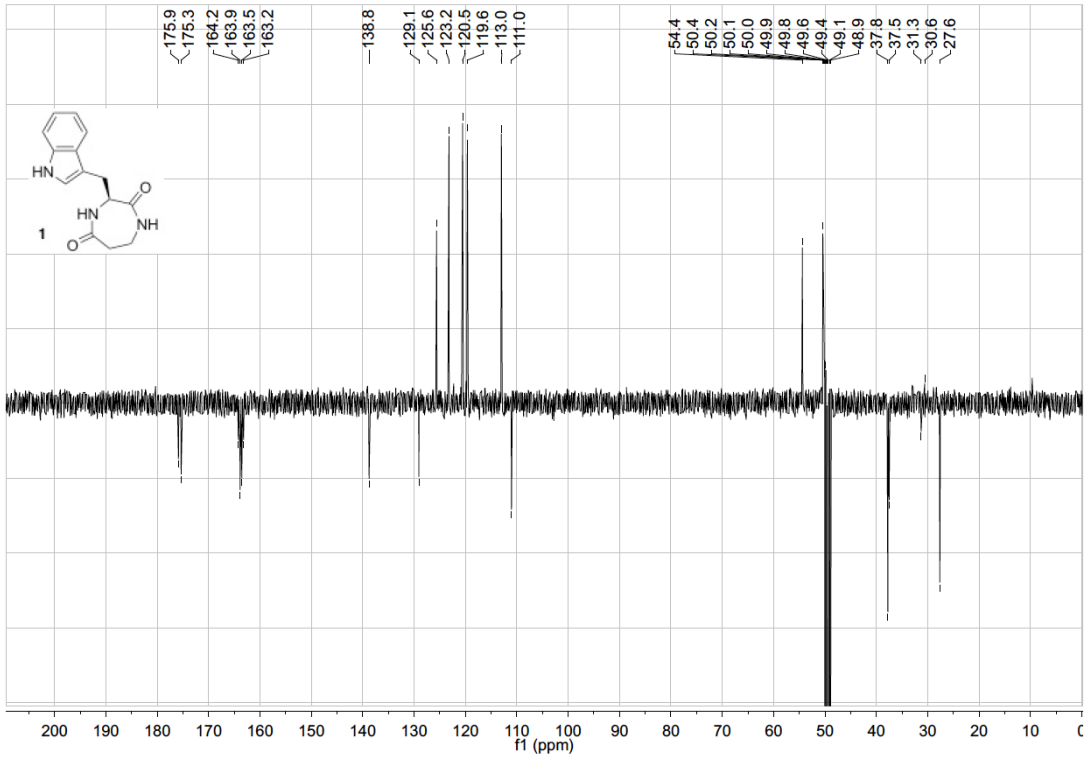
3e



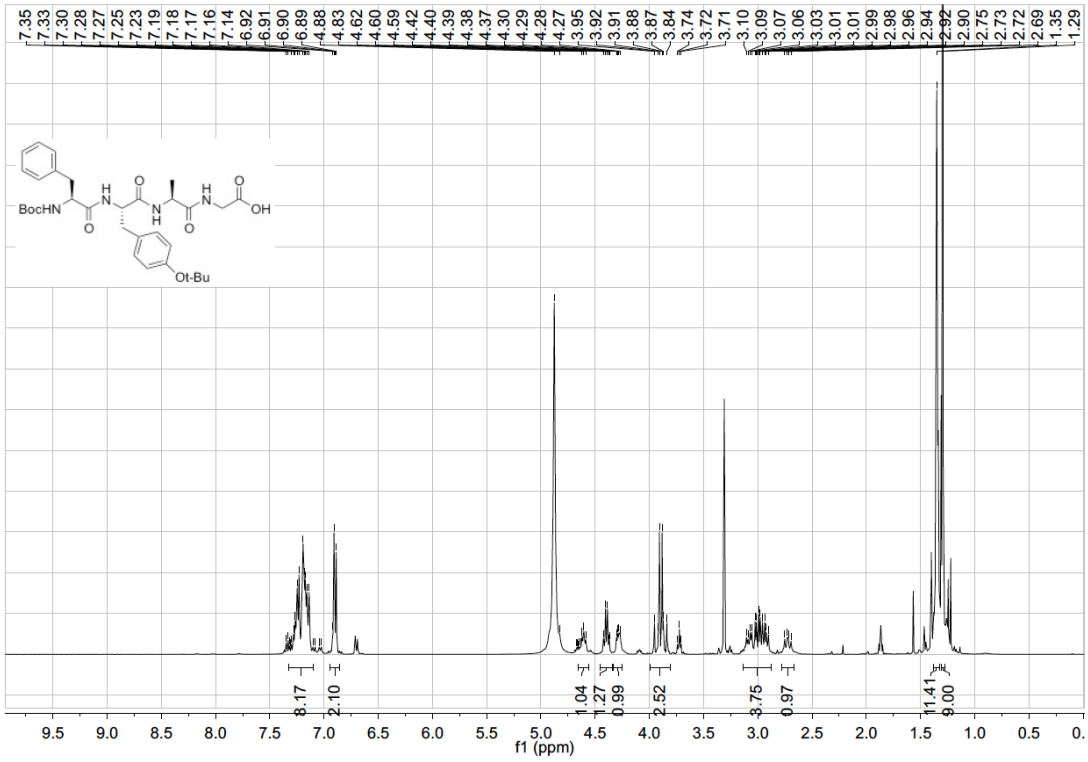


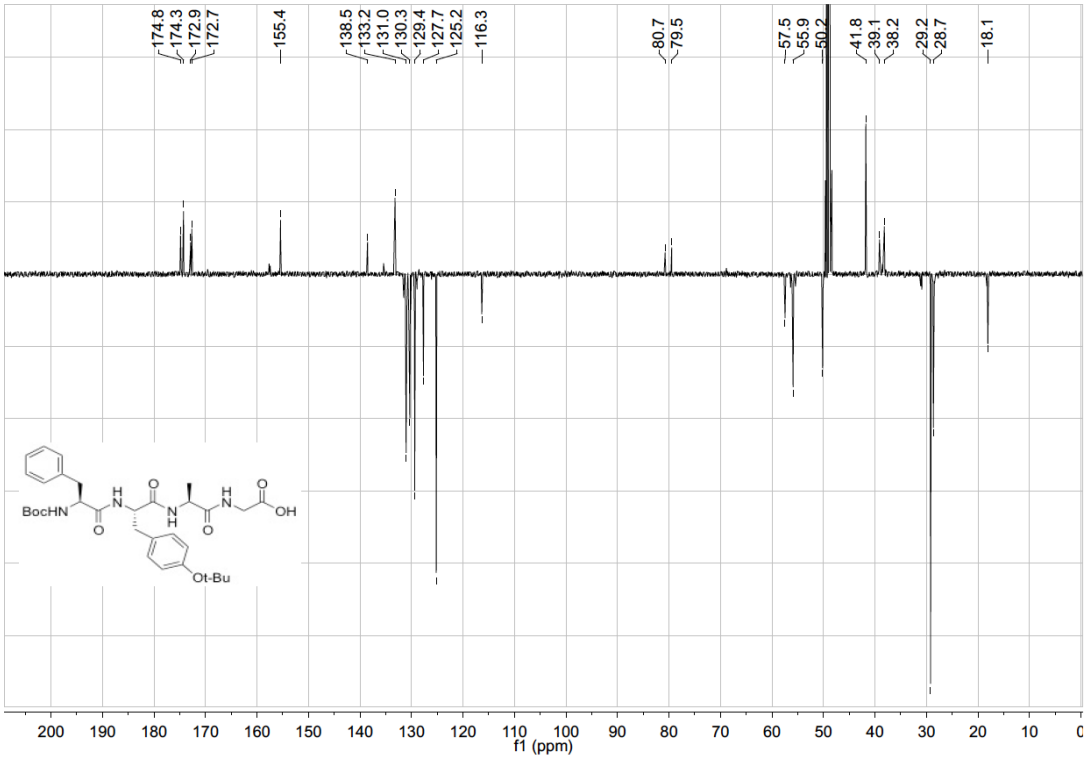
1


7.57
7.55
7.32
7.30
7.16
7.07
6.99
4.81
4.67
4.66
4.65
4.64
3.66
3.33
3.31
3.28
3.27
3.27
3.26
3.09
2.64
2.63
2.61
2.60
2.59
2.54
2.53
2.51
2.50
2.48
2.47
2.46
HN
HN
O
NH
O
1
1.00
0.98
0.93
1.05
1.03
1.01
1.16
0.84
1.09
1.13
1.18
1.06
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)

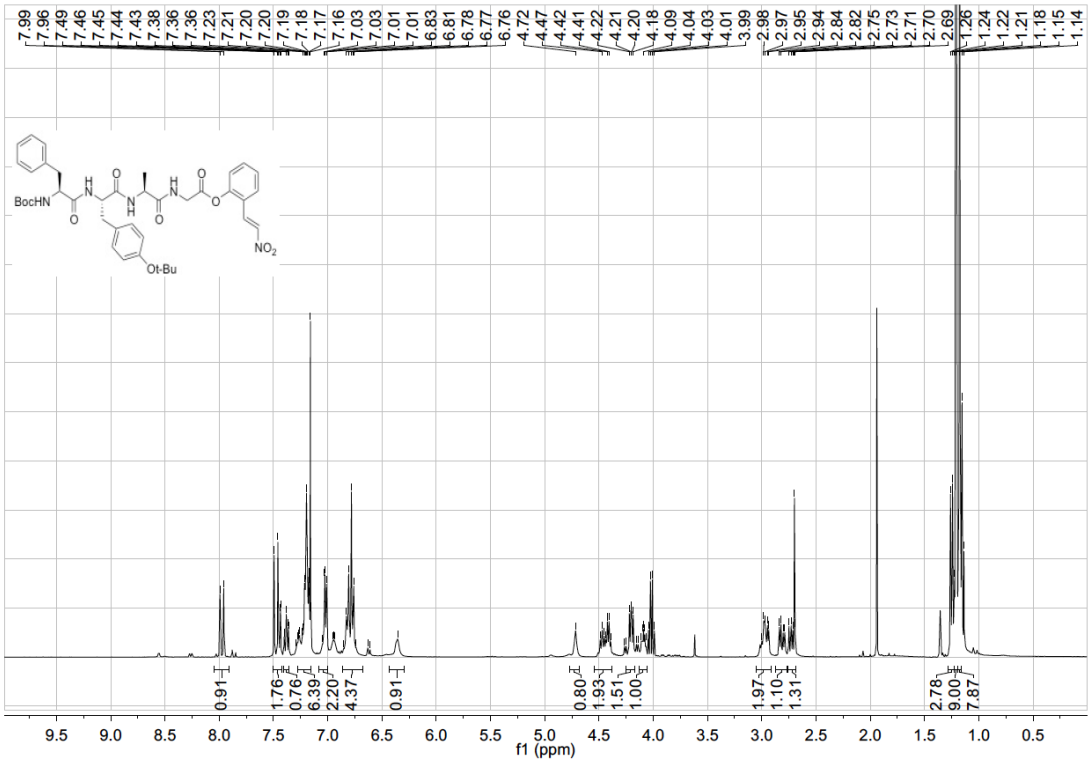

175.9
175.3
164.2
163.9
163.5
163.2
138.8
129.1
125.6
123.2
120.5
119.6
113.0
111.0
54.4
50.4
50.2
50.1
50.0
49.9
49.8
49.6
49.4
49.1
48.9
37.8
37.5
31.3
30.6
27.6
HN
HN
O
NH
O
1
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

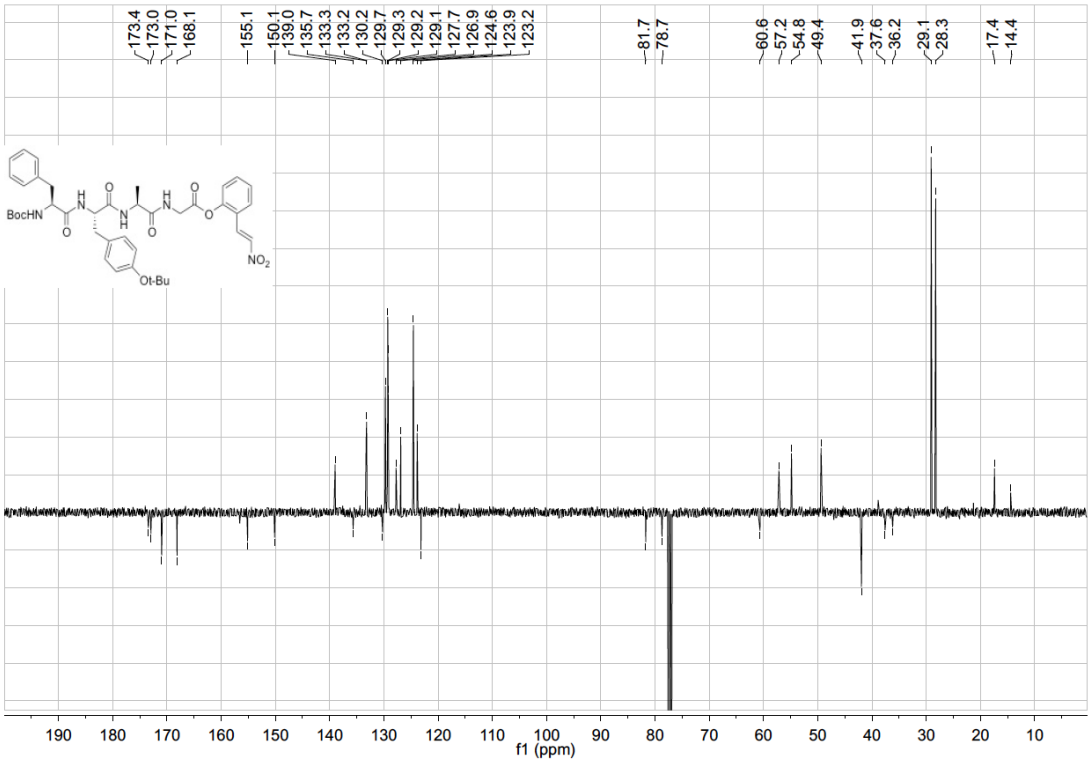
Boc-Phe-Tyr(tBu)-Ala-Gly-OH



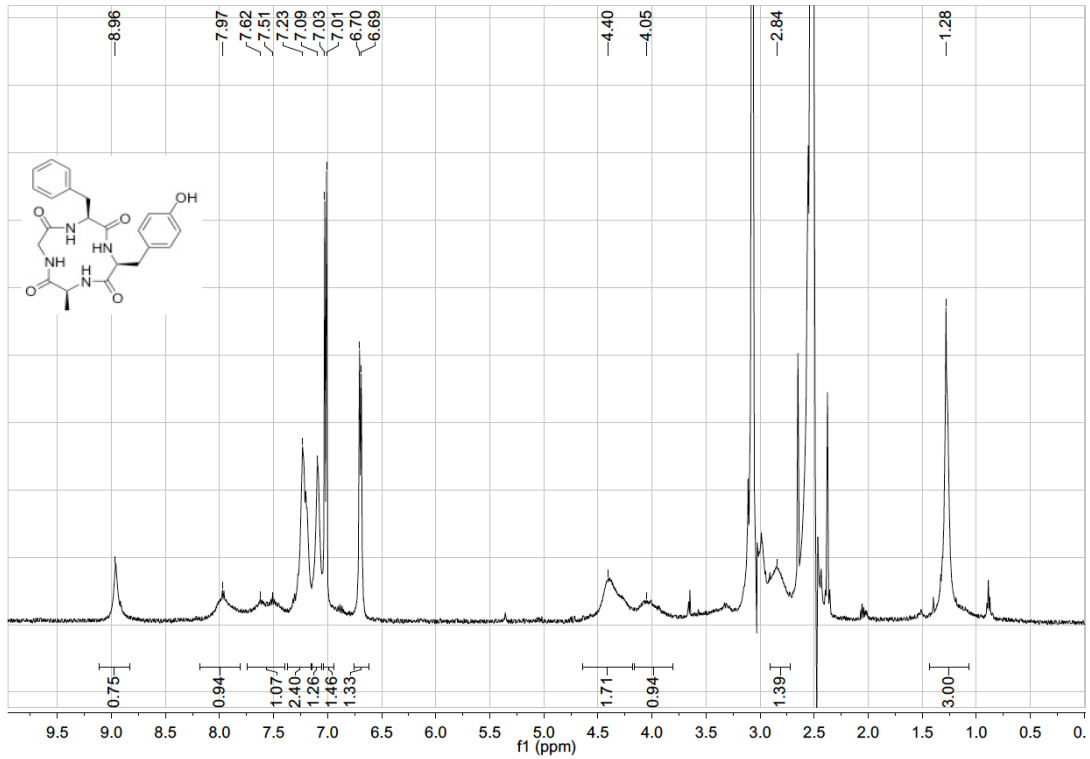


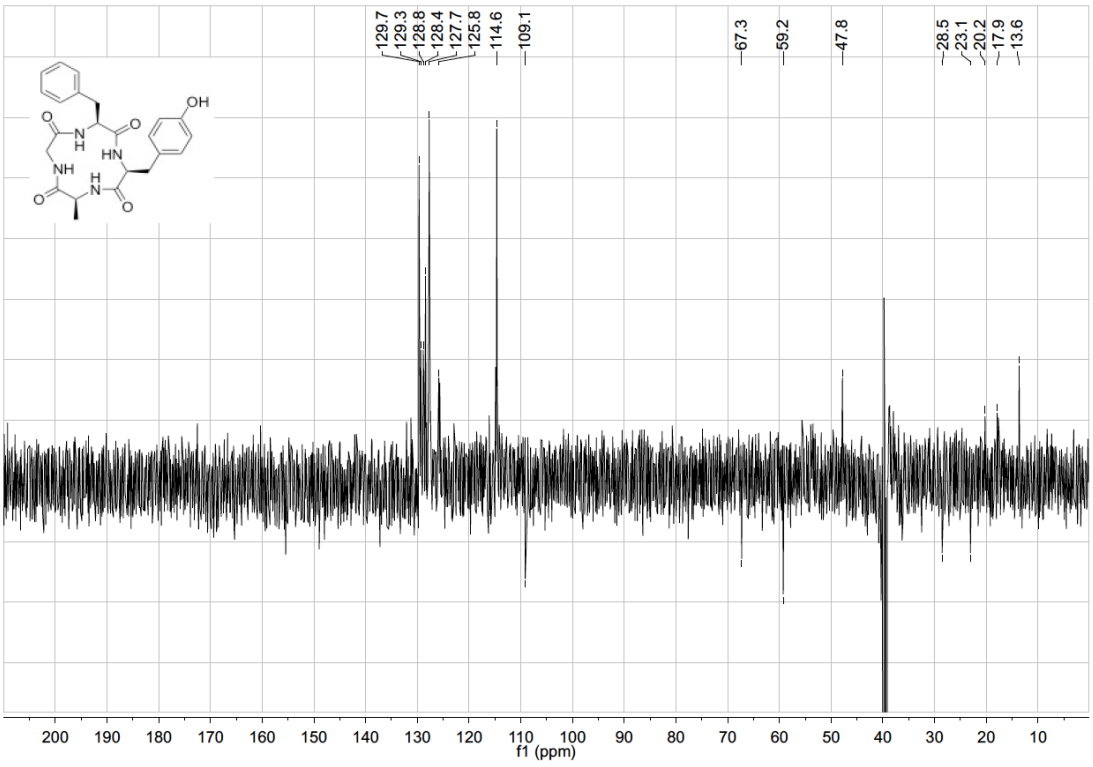
Boc-Phe-Tyr(tBu)-Ala-Gly-O-(E)-2-(2-nitrovinyl)phenyl





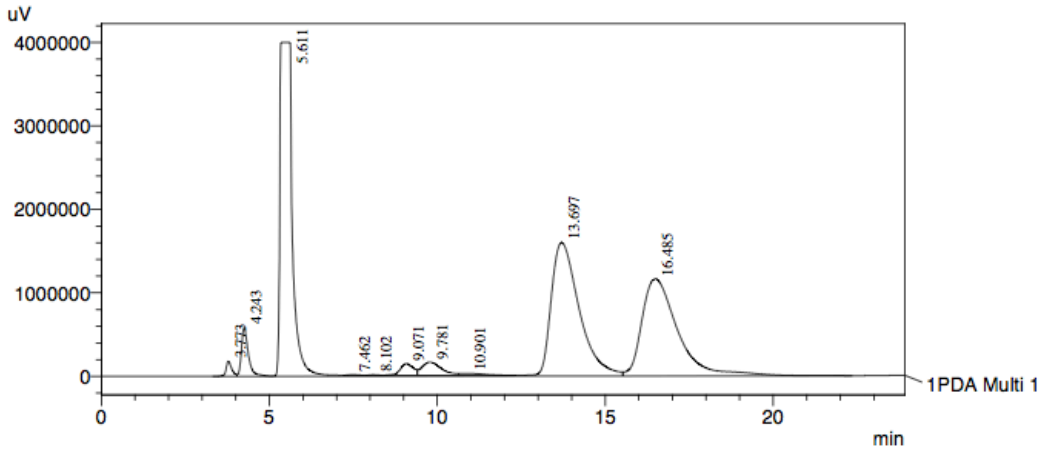
Cyclo[Phe-Tyr-Ala-Gly]





Chiral HPLC traces

3a (racemic)



1 PDA Multi 1 / 220nm 4nm

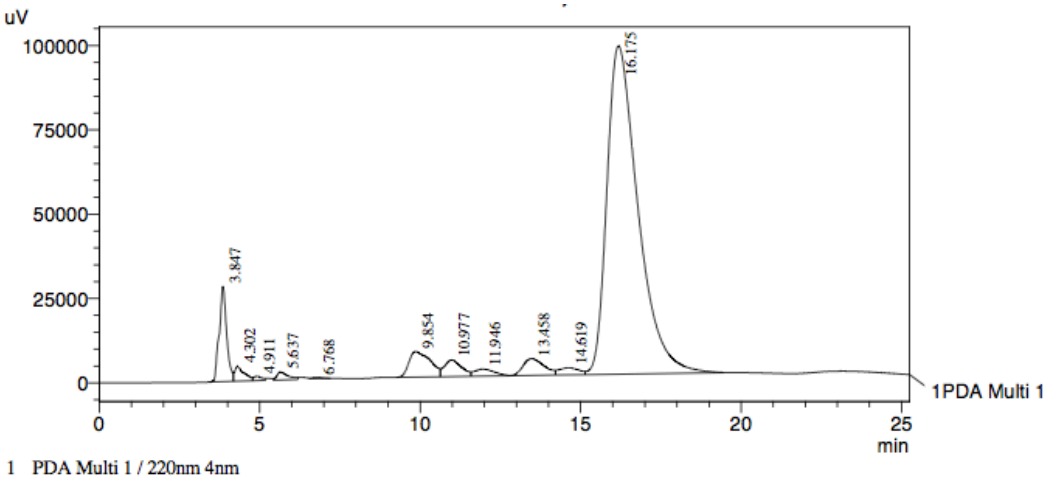
Results

PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.773	2140621	177417	0.700	2.259
2	4.243	8350044	584273	2.732	7.440
3	5.611	104895260	3999701	34.324	50.929
4	7.462	115792	6403	0.038	0.082
5	8.102	85893	4972	0.028	0.063
6	9.071	3765544	138741	1.232	1.767
7	9.781	6385707	156836	2.090	1.997
8	10.901	1171676	23790	0.383	0.303
9	13.697	92440506	1598482	30.249	20.354
10	16.485	86250924	1162857	28.223	14.807
Total		305601967	7853472	100.000	100.000

3a (optically pure)



1 PDA Multi 1 / 220nm 4nm

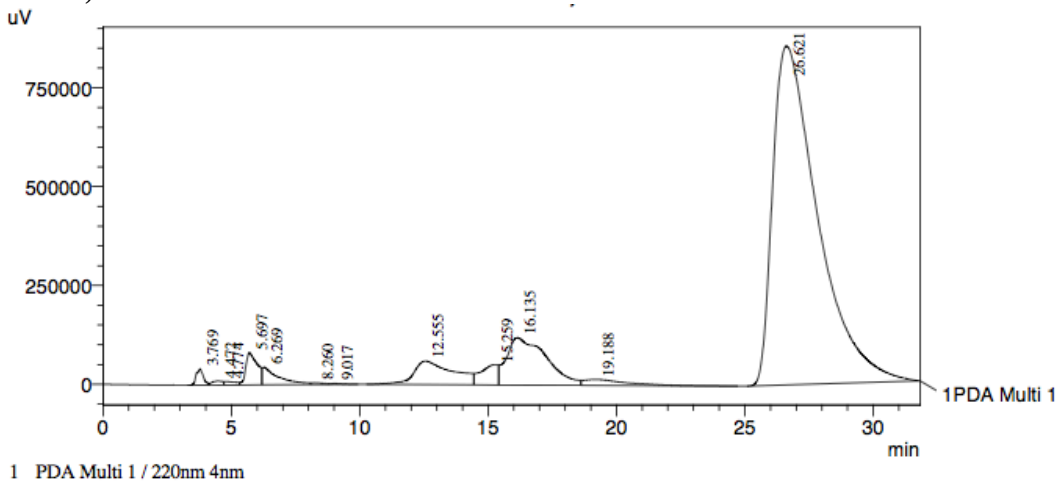
Results

PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.847	457306	28306	5.618	18.184
2	4.302	90848	4413	1.116	2.835
3	4.911	22599	1252	0.278	0.805
4	5.637	56179	2322	0.690	1.492
5	6.768	10279	445	0.126	0.286
6	9.854	350436	7595	4.305	4.879
7	10.977	184044	4894	2.261	3.144
8	11.946	89549	2076	1.100	1.334
9	13.458	224990	4957	2.764	3.185
10	14.619	91867	2067	1.129	1.328
11	16.175	6561995	97337	80.613	62.530
Total		8140092	155664	100.000	100.000

1 (from **2a**)



1 PDA Multi 1 / 220nm 4nm

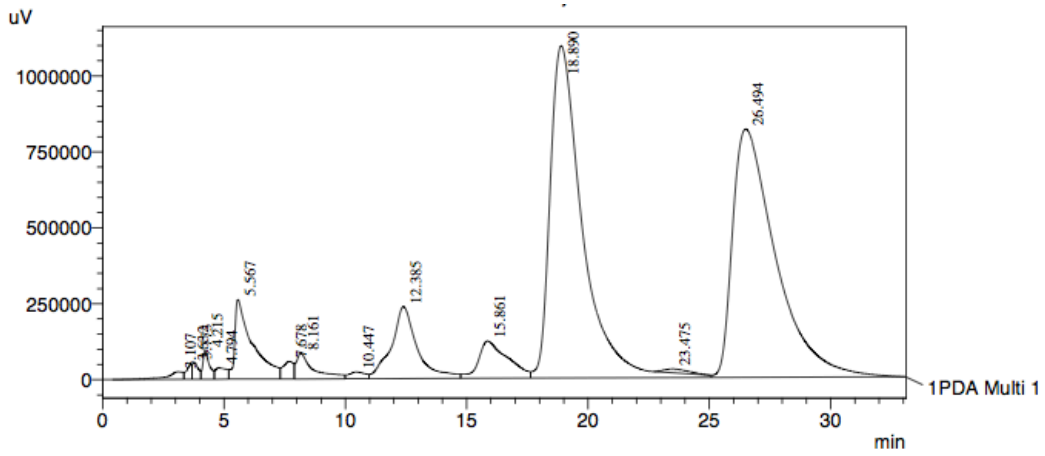
Results

PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.769	802232	40938	0.599	3.162
2	4.472	306022	10171	0.229	0.786
3	4.774	279705	9082	0.209	0.701
4	5.697	2576246	81618	1.925	6.304
5	6.269	1957164	44181	1.462	3.413
6	8.260	152770	4527	0.114	0.350
7	9.017	82519	2299	0.062	0.178
8	12.555	6413378	59303	4.792	4.581
9	15.259	2388479	50888	1.785	3.931
10	16.135	12892463	119838	9.633	9.256
11	19.188	1813022	15217	1.355	1.175
12	26.621	104178980	856617	77.837	66.164
Total		133842981	1294679	100.000	100.000

1 (from **3a** (racemic))



1 PDA Multi 1 / 220nm 4nm

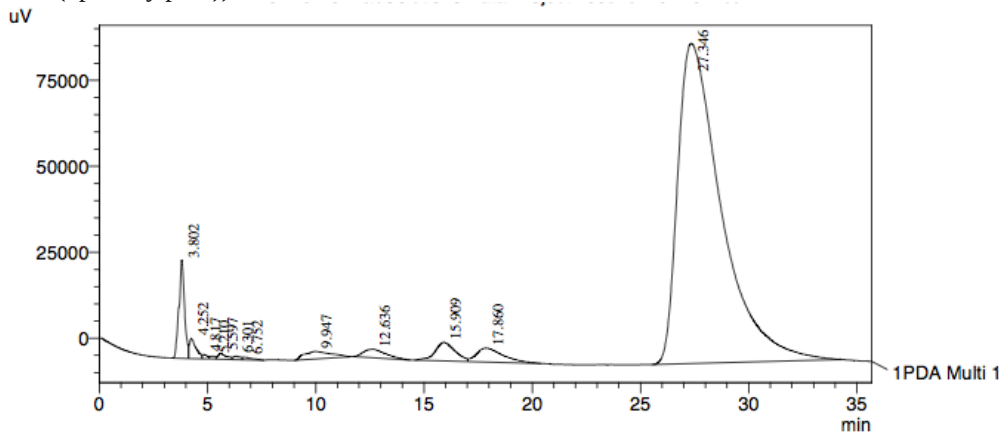
Results

PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.107	1190532	25135	0.462	0.846
2	3.632	723300	49248	0.281	1.657
3	3.773	940401	55440	0.365	1.865
4	4.215	1779181	93326	0.690	3.139
5	4.794	1223163	37257	0.475	1.253
6	5.567	13346439	263183	5.178	8.853
7	7.678	1749435	57455	0.679	1.933
8	8.161	4159369	85565	1.614	2.878
9	10.447	1045275	22248	0.406	0.748
10	12.385	17525390	236604	6.799	7.959
11	15.861	10251090	122548	3.977	4.122
12	18.890	101903135	1094800	39.534	36.829
13	23.475	889655	12309	0.345	0.414
14	26.494	101033599	817577	39.197	27.503
Total		257759964	2972693	100.000	100.000

1 (from **3a** (optically pure))



1 PDA Multi 1 / 220nm 4nm

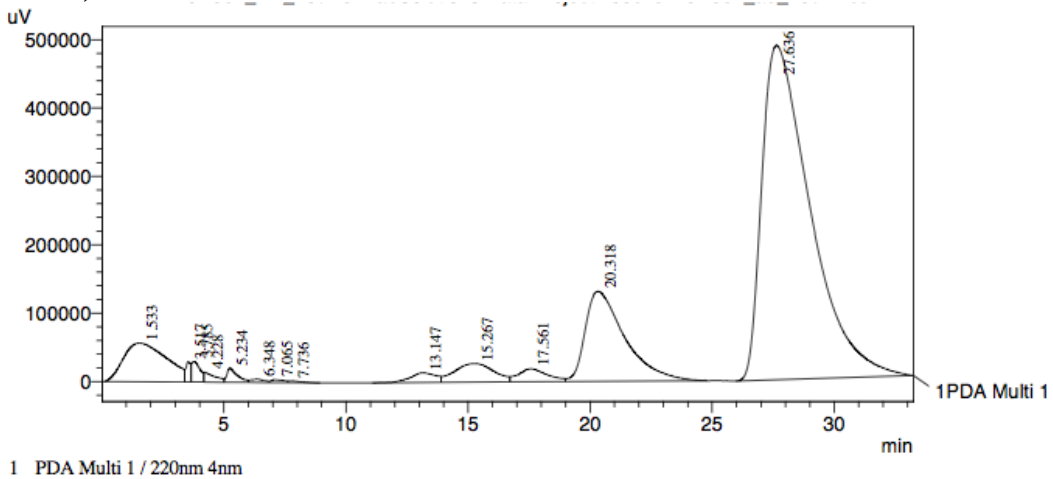
Results

PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.802	513040	28650	3.458	19.502
2	4.252	124523	5750	0.839	3.914
3	4.817	19748	1223	0.133	0.832
4	5.210	14311	797	0.096	0.542
5	5.597	43825	1697	0.295	1.155
6	6.301	22982	945	0.155	0.643
7	6.752	22450	685	0.151	0.467
8	9.947	186606	2197	1.258	1.496
9	12.636	174962	2433	1.179	1.656
10	15.909	344653	5392	2.323	3.670
11	17.860	346736	4038	2.337	2.748
12	27.346	13022283	93105	87.774	63.376
Total		14836120	146910	100.000	100.000

1 (from **3e**)



1 PDA Multi 1 / 220nm 4nm

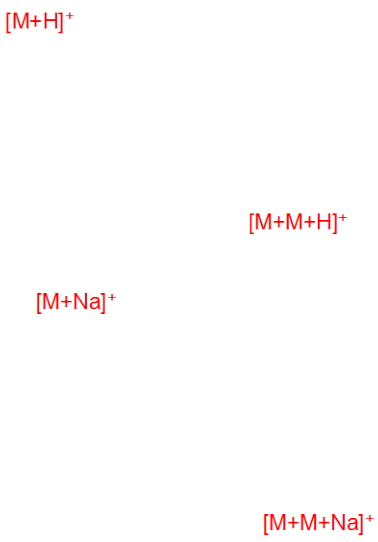
Results

PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.533	6893057	56492	7.091	6.702
2	3.517	413004	29365	0.425	3.484
3	3.785	722484	30201	0.743	3.583
4	4.228	445824	13907	0.459	1.650
5	5.234	606080	20390	0.623	2.419
6	6.348	184237	4957	0.190	0.588
7	7.065	150511	4349	0.155	0.516
8	7.736	87491	2430	0.090	0.288
9	13.147	1039704	14167	1.070	1.681
10	15.267	3036331	27260	3.123	3.234
11	17.561	1567486	19052	1.612	2.260
12	20.318	14823689	131522	15.249	15.603
13	27.636	67243648	488847	69.171	57.993
Total		97213545	842938	100.000	100.000

LCMS trace **cyclo[Phe-Tyr-Ala-Gly]**



MS-MS

cyclo[Phe-Tyr-Ala-Gly]

Mass spectrometric analysis

Apparatus Mass spectral data were acquired using an ApexUltra Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a 7 T magnet, an Apollo II-Dual source and an infrared multiphoton dissociation (IRMPD) laser system.

MS/MSMS data were acquired by direct nano-spray infusion at a flow rate of 300 nl/min. Instrument mass calibration was better then 2 ppm. Resolution of the mass signal of the cyclic peptide ion with m/z 439.19675 was better then 250000. MSMS data were acquired by mass selection of the cyclic peptide ion in the Q-sector, followed by 14 eV collision-induced dissociation (CID) in the hexapole at an argon pressure of about $6*10^{-6}$ mbar (measured at the pressure gauge) and detection of the fragment ions in the ICR cell.