Electronic Supplementary Material (ESI) for ChemComm.

Rapid access to Asp/Glu sidechain hydrazides as thioester precursors for peptide cyclization and glycosylation.

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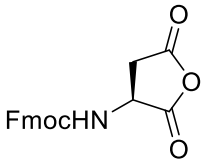
Supporting information

General experimental details.

Chemicals were purchased from Sigma-Aldrich, Across, Fischer Scientific, Merck Biosciences, and Cambridge Reagents, and were used without further purification. Dry solvents were purchased from Fischer Scientific and used as supplied. Flash chromatography was carried out with silica gel 60 A (35-70 microns) purchased from Merck. Thin layer chromatography was carried out on aluminium sheets coated with Merck silica gel 60 A F254. Staining was performed with *p*-anisaldehyde dip. NMR experiments were recorded at room temperature on Bruker AMX 300, 500 and 600 MHz instruments. Chemical shifts (δ H and δ C) are recorded in ppm with the stated solvent resonance as the internal reference. Signals are denoted as s= singlet, d=doublet, t=triplet, q=quartet, dd=doublet and apt=apparent triplet.

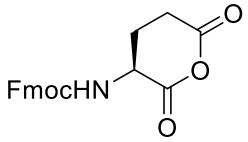
Preparative reversed-phase high performance liquid chromatography (RP-HPLC) was performed using a Dionex Ultimate 3000 system equipped with a Phenomenex Jupiter 10µ Proteo 90A, C_{12}, 250 x 21.2 mm column. Separations involved a mobile phase of 0.1% TFA (v/v) in water (solvent A)/acetonitrile (solvent B) over a 5-60% acetonitrile gradient over 45 min, and were monitored at wavelengths 230 nm, 254 nm, and 280 nm. Analytical reversed-phase high performance liquid chromatography (RP-HPLC) was performed using a Dionex Ultimate 3000 equipped with a Phenomenex SphereClone 5µ ODS, C_{18}, 250 x 4.6 mm column. Separations involved a mobile phase of 0.1% TFA (v/v) in water (solvent A)/acetonitrile (solvent B) over a 5-95% acetonitrile gradient, and were monitored at wavelengths 230 nm, 254 nm, and 280 nm. Analytical LC-MS was performed using Waters Acquity UPLC SQD and Thermo Q-Exactive plus instruments. Separations involved a mobile phase of 0.1% formic acid (v/v) in water (solvent A)/acetonitrile (solvent B) over a 5-95% acetonitrile gradient, and were monitored at 254 nm.

***N*-Fmoc-L-aspartic anhydride**[1]



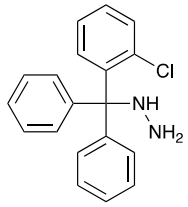
Fmoc-L-aspartic acid (5 g, 14 mmol) was added to acetic anhydride (15 mL) and heated to 110 °C until dissolved. Once fully dissolved, the reaction mixture was put on ice and quickly cooled to room temperature. The precipitate was filtered, washed with ice cold diethyl ether (2 x 10 mL) and dried under vacuum. The overall yield of fmoc-L-aspartic anhydride was 82% (3.89 g, 12 mmol). **^{1}H NMR** (600 MHz, DMSO) δ 8.21 (d, *J* = 7.7 Hz, 1H, NH), 7.90 (d, *J* = 7.7 Hz, 2H, fmoc), 7.67 (d, *J* = 7.4, 0.9 Hz, 2H, fmoc), 7.42 (t, *J* = 7.7 Hz, 2H, fmoc), 7.34 (dt, *J* = 7.4, 1.1 Hz, 2H, fmoc), 4.65 (ddd, *J* = 10.1, 7.7, 6.2 Hz, 1H, CH_{α}), 4.36-4.44 (m, 2H, fmoc CH_2), 4.25 (t, *J* = 6.6 Hz, 1H, fmoc), 3.24 (dd, *J* = 18.5, 7.7 Hz, 1H, β-CH_{2A}), 2.85 (dd, *J* = 18.5, 6.2 Hz, 1H, β-CH_{2B}). **^{13}C NMR** (151 MHz, DMSO) δ 172.1, 169.9, 167.1, 155.9, 143.7, 140.8, 127.7, 127.2, 125.1, 120.2, 66.1, 50.4, 46.6, 34.7, 22.1. **ESI-MS** Calc m/z for $C_{19}H_{14}NO_5$ $[M-H]^-$ = 336.1, observed m/z = 336.9.

***N*-Fmoc-L-Glutamic anhydride**



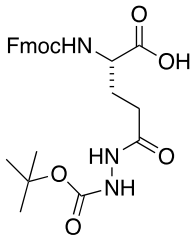
Fmoc-L-Glutamic acid (5.0 g, 13.6 mmol) was added to acetic anhydride (15 mL) and heated to 110 °C until dissolved. Once fully dissolved, the reaction mixture was cooled to room temperature and then concentrated in vacuo. The residue was washed with ice-cold isopropyl ether (2 x 10 ml) and dried under vacuum to yield Fmoc-L-Glutamic anhydride (4.7 g, 98%) as a white solid. **^{1}H NMR** (600 MHz, DMSO) δ_H/ppm: 7.96 (d, *J* = 8.4 Hz, 1H, NH), 7.89 (d, *J* = 7.6 Hz, 2H, Fmoc), 7.71 (dd, *J* = 7.6, 3.3 Hz, 2H, Fmoc), 7.42 (t, *J* = 7.4 Hz, 2H, Fmoc), 7.34 (t, *J* = 7.4 Hz, 2H, Fmoc), 4.61-4.55 (m, 1H, CH_{α}), 4.40-4.36 (m, 2H, Fmoc-CH_2), 4.25 (t, *J* = 6.7 Hz, 1H, Fmoc), 3.03 – 2.94 (m, 1H, γ-CH_{2A}), 2.87-2.78 (m, 1H, γ-CH_{2B}), 2.14 – 2.04 (m, 1H, β-CH_{2A}), 1.96 – 1.87 (m, 1H, β-CH_{2B}). **^{13}C NMR** (151 MHz, DMSO) δ_C/ppm: 167.9, 167.1, 156.0 (C=O), 143.7, 140.7 (Fmoc-**C**), 127.7, 127.1, 125.2, 120.2 (Fmoc-**C**H), 67.2 (**C**H_{α}), 65.9 (Fmoc-**C**H_2), 49.9 (Fmoc-**C**H), 29.5 (**C**H_2), 22.0 (**C**H_2).). **ESI-MS**: m/z calculated for $C_{20}H_{18}NO_5$ $[M+H]^+$ 352.1185, major peak observed corresponded to the hydrolysed Fmoc-glutamic acid and a small peak for the anhydride, observed $[M+H]^+$ 352.1177

((2-Chlorophenyl)diphenylmethyl)hydrazine



A solution of 2-chlorotrityl chloride (1.5 g, 4.8 mmol) in THF (5 mL) was added dropwise to a solution of hydrazine hydrate (0.77 g, 24 mmol) in THF (10 mL) and stirred at room temperature overnight. After this time the solvent was removed under vacuum. The resulting oil was crystallised from ethanol to afford ((2-Chlorophenyl)diphenylmethyl)hydrazine as a white solid (71%, 0.8 g, 2.6 mmol). **^{1}H NMR (600 MHz, $CDCl_3$) δ** 7.63 (d, *J* = 7.3 Hz, 1H, ArH), 7.49 – 7.45 (m, 4H, ArH), 7.42 – 7.36 (m, 1H, ArH), 7.33 – 7.24 (m, 6H, ArH), 7.25-7.20 (m, 2H, ArH), 4.35 (s, 1H, NH), 2.85 (s, 2H, NH_2). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 145.6, 133.2, 131.2, 128.6, 128.4, 128.1, 126.7, 126.3, 73.1. **ESI-MS:** m/z calculated for $C_{19}H_{18}ClN_2$ $[M + H]^+$, 309.1158, observed m/z = 309.1152.

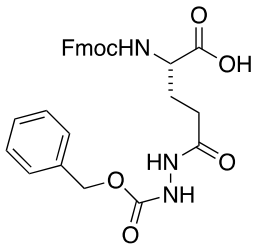
***N*^{2}-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*N*^{5}-((*tert*-butoxycarbonyl)amino)-*L*-glutamine (1)**



Fmoc-L-glutamic anhydride (1.0 g, 2.84 mmol) and *tert*-butyl carbazate (0.57 g, 4.26 mmol) were dissolved in DMSO (1.5 ml) and then stirred at room temperature for 2 h. Water (5 ml) was then added to the reaction mixture and stirred for further 10 mins. A white gum formed and to it was added DCM (10 ml) and the organic layer extracted. The organic layer was then washed with 1N HCl (1 x 5 ml) and sat NaCl (2 x 5 ml), dried over $MgSO_4$ and concentrated on vacuo to afford the crude product. The crude product was then flash chromatographed on silica gel with EtOAc/MeOH 20:1 and the product was isolated a white solid (0.85 g, 62%). **^{1}H NMR** (600 MHz, DMSO) δ_H/ppm: 9.56 (s, 1H, NH), 8.67 (s, 1H, NH), 7.89 (d, *J* = 7.6 Hz, 2H, Fmoc), 7.70 (d, *J* = 7.4 Hz, 2H, Fmoc), 7.41 (t, *J* = 7.6 Hz, 2H, Fmoc), 7.33 (t, *J* = 7.5 Hz, 2H, Fmoc), 4.25-4.21 (m, 3H, Fmoc-CH_2, Fmoc-H), 4.00-3.97 (m, 1H, CH_α), 2.14-2.09 (m, 2H, γ-CH_2), 1.98 – 1.85 (m, 1H, β-CH_{2A}), 1.83 – 1.75 (m, 1H, β-CH_{2B}), 1.39 (s, 9H, *t*-butyl). **^{13}C NMR** (151 MHz, DMSO) δ_C/ppm: 171.6, 170.4, 155.7, 155.3 (**C**=O), 143.9, 140.7, 127.7, 127.1, 125.4, 120.1 (Fmoc), 79.1 (**C**- *t*-butyl), 65.8 (Fmoc-**C**H_2), 59.8 (**C**H_2), 54.9 (**C**H_α), 46.7 (Fmoc-**C**H), 29.8

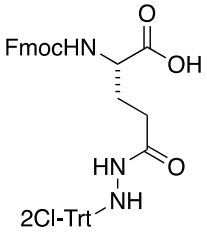
(CH_2), 28.1 (**C**H_3- *t*-butyl). **ESI-MS**: m/z calculated for $C_{25}H_{30}N_3O_7$[M + H] $^+$, 484.2084, observed [M+H]$^+$ 484.2080

N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^5-(((benzyloxy)carbonyl)amino)-*L*-glutamine (2)



Fmoc-L-glutamic anhydride (0.5g, 1.42 mmol) and benzyl carbazate (0.36 g, 2.13 mmol) were dissolved in DMSO (1.0 ml) and then stirred at room temperature for 2 h. To the reaction mixture was added DCM (10 ml) then washed with 1N HCl (2 x 10 ml) and sat. aq. NaCl (2 x 10 ml), dried over $MgSO_4$ and concentrated on vacuo. A white solid was obtained which was then washed with diethyl ether (2 x 10 ml) and dried under vacuum. Isolated yield was (0. 65 g, 88%). **^{1}H NMR** (600 MHz, DMSO) δ_H/ppm: 9.71 (s, 1H, NH), 9.15 (s, 1H, NH), 7.88 (d, *J* = 7.5 Hz, 2H, Fmoc), 7.73 (t, *J*=8.2 Hz, 2H, Fmoc), 7.48 – 7.15 (m, 9H, 4xFmocH, 5xArH), 5.06 (s, 2H, PhC$\underline{H}_2$), 4.32-4.16 (m, 3H, Fmoc-CH_2, Fmoc-H), 4.05-3.95 (m, 1H, CH_α), 2.32 – 1.99 (m, 2H, γ-CH_2), 1.95 – 1.72 (m, 2H, β-CH_2). **^{13}C NMR** (151 MHz, DMSO) δ_C/ppm: 171.7, 169.1, 156.2, 155.0 (**C**=O), 143.9, 140.7 (Fmoc), 136.6 (ArC), 128.5 (ArC), 128.1 (ArC), 127.9 (ArC), 127.7 , 127.1, 125.4, 120.2 (Fmoc), 65.9 (Fmoc-**C**H_2), 64.9 (Ph**C**H_2), 54.9 (**C**H_2), 52.7 (**C**H_α), 46.5 (Fmoc-**C**H), 29.8 (**C**H_2). **ESI-MS**: m/z calculated for $C_{28}H_{28}N_3O_7$[M+H] $^+$, 518.1927 observed [M+H]$^+$ 518.1921

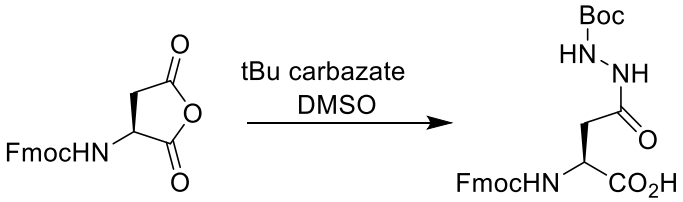
N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^5-(((2-chlorophenyl)diphenylmethyl)amino)-*L*-glutamine (3)



Fmoc-L-glutamic anhydride (0.5 g, 1.42 mmol) and 2-chlorotrityl hydrazine (0.66 g, 2.14 mmol) were dissolved in DMSO (1.0 ml) and stirred at room temperature for overnight. To the reaction mixture was added DCM (10 ml) and then washed with 1N HCl (2 x 10 ml) and sat. aq. NaCl (2 x 10 ml), dried over $MgSO_4$ and concentrated on vacuo. A viscous oil was obtained which was recrystalised with Pet/EtOAc to yield a white solid product (0.68 g, 73%). **^{1}H NMR** (600 MHz, DMSO) δ_H/ppm: 9.14 (d, *J*

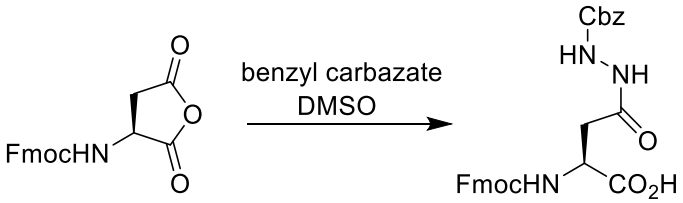
= 8.1 Hz, 1H, NH), 7.89 (d, *J*= 7.7 Hz, 2H, FmocH), 7.79 (d, *J*= 7.7 Hz, 1H, Trt), 7.68 (d, *J*= 7.8 Hz, 2H, Fmoc), 7.45 – 7.14 (m, 17H, 4 x FmocH, 13 x Trt-H), 6.10 (d, *J* = 8.1 Hz, 1H, NH), 4.27 – 4.15 (m, 3H, Fmoc-CH_2, Fmoc-H), 3.66-3.59 (m, 1H, CH_α), 1.87-1.73 (m, 2H, γ-CH_2), 1.54–1.36 (m, 2H, β-CH_2). **^{13}C NMR** (151 MHz, DMSO) δ_C/ppm: 176.1, 170.2, 155.4 (**C**=O), 143.9 (Fmoc), 143.8 (Trt), 142.4 (Trt), 140.7 (Fmoc), 133.7 (Trt), 131.6, 131.4, 128.9, 128.3, 127.6, 127.1, 126.5, 126.2, 125.2, 120.2, 73.0, 65.4 (Fmoc-**C**H_2), 54.9 (**C**H_α), 46.8 (Fmoc-**C**H), 30.7, 27.3. **ESI-MS**: m/z calculated for $C_{39}H_{35}ClN_3O_5$ $[M+H]^+$ 660.2265, observed $[M+H]^+$ 660.2252

N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4-((*tert*-butoxycarbonyl)amino)asparagine (4)



Fmoc-L-aspartic anhydride (1 g, 3 mmol) and tert-butyl carbazate (0.588g, 4.5 mmol, 1.5 eq) were dissolved in DMSO (1 mL) and stirred at room temperature for 1 h. After this time, water (5 mL) was added and the reaction mixture was extracted into DCM (10 mL). The organic layer was washed with HCl (1M, 10 mL) then brine (10 mL), dried ($MgSO_4$) and solvent evaporated to afford **4** as a white solid (1.08 g, 77%). **^{1}H NMR (600 MHz, DMSO) δ** 12.72 (s, 1H, OH), 9.61 (s, 1H, NH), 8.80 (d, *J* = 7.0 Hz, 1H, NH), 7.89 (d, *J* = 7.4 Hz, 2H, fmoc), 7.77-7.70 (m, 2H, fmoc), 7.58 (d, *J* = 8.4 Hz, 1H, NH), 7.42 (t, *J* = 7.4 Hz, 2H, Fmoc), 7.33 (dt, *J* = 7.4, 3.2 Hz, 2H, Fmoc), 4.45-4.35 (m, 1H, CH_α), 4.29 – 4.19 (m, 3H, Fmoc-CH_2, Fmoc-CH), 2.69-2.54 (m, 2H, β-CH_2), 1.39 (s, 9H, tbutyl). **^{13}C NMR (151 MHz, DMSO) δ** 173.0, 169.0, 155.9, 155.3, 143.8, 143.7, 127.7, 127.2, 125.3, 120.2, 79.3, 65.8, 54.9, 46.6, 40.2, 34.8, 28.1. **ESI-MS:** Calc m/z for $C_{24}H_{28}N_3O_7$ $[M+H]^+$ = 470.1927, observed m/z = 470.1921.

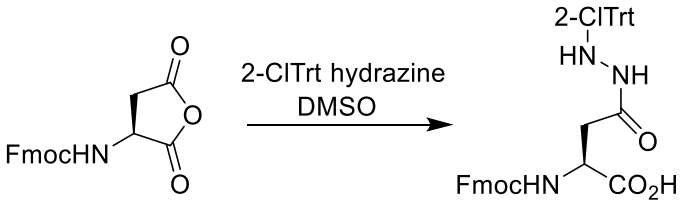
N^2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-N^4-(((benzyloxy)carbonyl)amino)asparagine (5) [2]



Fmoc-L-aspartic anhydride (0.5 g, 1.5 mmol, 1 eq) and benzyl carbazate (0.369 g, 2.2 mmol, 1.5 eq) were dissolved in DMSO (0.5 mL) and stirred at room temperature for 1 h. After this time, water (5 mL) was added and the reaction mixture was extracted into DCM (10 mL). The organic layer was washed with HCl (1M, 10 mL) then brine (10 mL), dried ($MgSO_4$) and solvent evaporated to afford **5** as

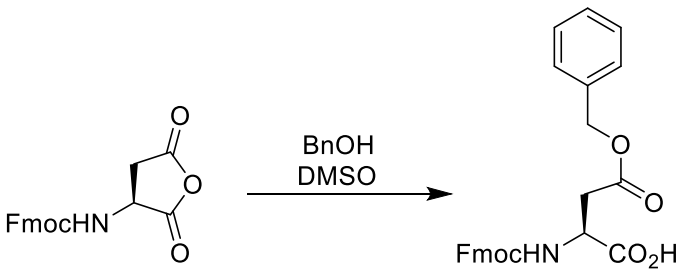
a white foam (0.470 g, 63%). **^{1}H NMR (600 MHz, DMSO) δ** 9.26 (s, 1H, NH), 7.88 (d, *J* = 7.4 Hz, 2H, Fmoc), 7.71 (d, *J* = 7.4 Hz, 2H, Fmoc), 7.57 – 7.47 (m, 1H, NH), 7.41 (t, *J* = 1.4 Hz, 2H, Fmoc), 7.39 – 7.34 (m, 5H, ArH), 7.33 (d, *J* = 7.4 Hz, 2H, fmoc), 5.07 (s, 2H, CH_2Ph), 4.45 – 4.35 (m, 1H, CH_α), 4.28 – 4.11 (m, 3H Fmoc-CH_2, Fmoc-H), 2.69-2.53 (m, 2H, β-CH_2). **^{13}C NMR (151 MHz, DMSO) δ** 173.0, 169.1, 156.2, 155.9, 143.8, 140.7, 136.6, 128.5, 128.1, 127.9, 127.7, 127.2, 125.4, 120.2, 65.9, 65.7, 54.9, 46.6, 34.9. **ESI-MS:** Calc m/z for $C_{27}H_{26}N_3O_7$ $[M+H]^+$ = 504.1765, observed m/z = 504.1776.

N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4-(((2-chlorophenyl)diphenylmethyl)amino) asparagine (6)



Fmoc-L-aspartic anhydride (1 g, 3 mmol, 1 eq) and 2-chlorotrityl hydrazine (1.386g, 4.5 mmol, 1.5 eq) were dissolved in DMSO (1 mL) and stirred at room temperature overnight. After this time, water (5 mL) was added and the reaction mixture was extracted into DCM (10 mL). The organic layer was washed with HCl (1M, 10 mL) then brine (10 mL), dried ($MgSO_4$) and solvent evaporated. The crude oil was purified by flash column chromatography (1 % to 5% methanol in chloroform) to afford **6** as a white solid (66%, 1.27 g, 2 mmol). **^{1}H NMR (600 MHz, DMSO) δ** 7.90 (d, *J* = 7.4 Hz, 2H, Fmoc), 7.73 (d, *J* = 7.9 Hz, 1H, Trt), 7.70 – 7.66 (m, 2H, Fmoc), 7.47 – 7.23 (m, 15H, 4 Fmoc, 11 Trt), 7.22-7.14 (m, 2H, Trt), 6.14 (d, *J* = 8.3 Hz, 1H, NH), 4.34-4.04 (m, 4H, Fmoc-CH_2, Fmoc-H, CH_α), 2.18-2.00 (m, 2H, β-CH_2). **^{13}C NMR (151 MHz, DMSO) δ** 172.8, 166.9, 155.7, 145.0, 143.8, 140.7, 133.7, 129.7, 129.2, 128.7, 128.5, 128.3, 128.2, 128.1, 127.8, 127.7, 127.1, 126.6, 126.4, 125.3, 120.2, 73.2, 65.6, 46.6, 40.4, 34.8. **ESI-MS** Calc m/z $C_{38}H_{33}ClN_3O_5$ $[M+H]^+$ = 646.21, observed m/z = 646.7

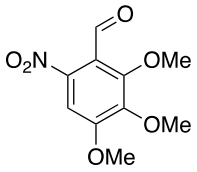
Fmoc-Asp(OBzl)-OH



Fmoc-aspartic anhydride (0.5 g, 1.5 mmol) and benzyl alcohol (0.15 ml, 1.5 mmol) were dissolved in DMSO (1 mL) and stirred at room temperature. After 2 hours, another equivalent of benzyl alcohol (0.15 ml, 1.5 mmol) was added and the reaction mixture stirred at room temperature overnight. After this time, water (5 mL) was added and the reaction mixture was extracted into DCM (10 mL), dried ($MgSO_4$) and solvent evaporated. The crude oil was purified by flash column chromatography (0 % to 2% methanol in chloroform) to afford the product as an off-white solid (36%, 0.24 g, 0.5 mmol). **^{1}H NMR (600 MHz, DMSO)** δ 12.94 (br s, 1H, OH), 7.90 (d, *J* = 7.6 Hz, 2H, fmoc), 7.79 (d, *J* = 8.6 Hz, 1H, NH), 7.71 (d, *J* = 7.5 Hz, 2H, fmoc), 7.42 (t, *J* = 7.5 Hz, 2H, fmoc), 7.39 – 7.30 (m, 7H, 2 fmoc, 5xArH), 5.11 (s, 2H, PhCH_2), 4.44-4.38 (m, 1H, CH_α), 4.34 – 4.26 (m, 2H, fmoc CH_2), 4.24-4.22 (m, 1H, fmoc CH), 2.88 (dd, *J* = 16.2, 5.5 Hz, 1H, β-CH_{2A}), 2.73 (dd, *J* = 16.2, 8.3 Hz, 1H, β-CH_{2B}). **^{13}C NMR (151 MHz, DMSO)** δ 172.5, 170.1, 155.9, 143.8, 140.8, 136.0, 128.4, 128.0, 127.9, 127.7, 127.1, 125.3, 120.2, 65.8, 65.7, 50.4, 46.6, 35.9.

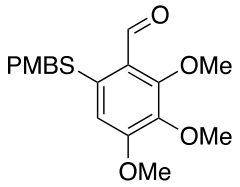
Synthesis of sugar-linked auxiliary **11** [3]

2,3,4-Trimethoxy-6-nitrobenzaldehyde



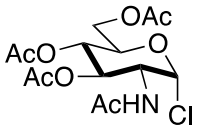
A mixture of 2,3,4-Trimethoxybenzaldehyde (20.0 g, 0.10 mol) and glacial acetic acid (50 ml) were cooled to -15 °C and 70% concentrated HNO_3 (50 ml) was added slowly. The temperature was increased to 0 °C and the reaction mixture was stirred for 1 h. After an hour, the red solution formed was poured into ice-water (350 ml) and a yellow precipitate formed which was filtered under vacuum. The solid was washed with Et_2O to afford the product (15.5 g, 62%) as pale-yellow crystals. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 10.25 (s, 1H, CHO), 7.25 (s, 1H, ArH), 3.98 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3) . **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C (ppm): 189.1 (C=O), 159.3 (C_{Ar}-OCH_3), 157.4 (C_{Ar}-OCH_3), 141.8 (C_{Ar}-OCH_3), 124.3 (C_{Ar}-NO_2), 123.2 (C_{Ar}-COH), 107 (CH_{Ar}), 62.1, 60.8, 56.0 (-OCH_3). **ESI-MS**: m/z calculated for $C_{10}H_{11}NO_6[M]^+$ 241.06, found [M+H] 242.06. Data obtained matched that reported in the literature.[3]

2,3,4-Trimethoxy-6-((4-methoxybenzyl) thiol)benzaldehyde



2,3,4-Trimethoxy-6-nitrobenzaldehyde (1.5 g, 6.21 mmol) and 4-methoxybenzylmercaptan (0.96 g, 6.21 mmol) were dissolved in DMF (13 ml) and to the mixture a solution of KOH (0.63 g, 11.2 mmol) in water (2 ml) was added dropwise. The mixture was heated at 80 °C for 6 h and the resulting dark red solution was poured into ice-water (75 ml). The resulting brown precipitate was filtered, washed with cold water and dried under vacuum. The solid was then washed with Et_2O to afford the product (0.69 g, 48%) as light brown crystals. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H/ppm: 10.33 (s, 1H, CHO), 7.35 (d, *J*= 8.7Hz, 2H, ArH), 6.85 (d, *J*= 8.7Hz, 2H, ArH), 6.58 (s, 1H, ArH), 4.10 (s, 2H, Ar$\underline{CH_2}$S), 3.98 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C (ppm): 189.1 (C=O), 159.1 (C_{PMB}-OCH_3), 158.3 (C_{Ar}-OCH_3), 158.1 (C_{Ar}-OCH_3), 139.8 (C_{Ar}-OCH_3), 138.5 (C_{Ar}-S), 130.0 (CH_{PMB}-H), 128.0 (C_{PMB}-CH_2), 120.2 (C_{Ar}), 114.2 (CH_{PMB}), 104.8 (CH_{Ar}), 62.5, 61.1, 56.2, 53.4 (-OCH_3), 36.7 (CH_2). **ESI-MS**: m/z calculated for $C_{18}H_{20}O_5S$ $[M]^+$ 348.10, found [M+H] 349.07. Data obtained matched that reported in the literature.[3]

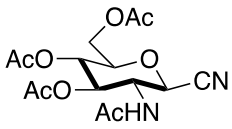
2-Acetamido-2-deoxy-3, 4, 6-tri-O-acetyl-α-D-glucopyranosyl chloride



N-acetyl glucosamine (10.17 g, 46 mmol) was added to stirring acetyl chloride (20 ml) in a flask equipped with drying tube. The suspension was stirred for 18 h and formed an amber coloured syrup, to which chloroform (80 ml) was added. The resulting solution was poured into ice (80 g) and water (20 ml) with stirring. The organic layer was added to saturated $NaHCO_3$ (80 ml) with neutralisation being completed in the separating funnel. The organic layer was then dried over anhydrous $MgSO_4$ for 15 min, filtered and evaporated to dryness in vacuo to afford crude product as a light brown solid. The crude solid was purified by flash column chromatography with EtOAc/Pet (85:15) to give product as a pale yellow solid (3.05 g, 18 %). mpt. 119- 121.6°C (lit. 127 -128 °C). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H/ppm: 6.18 (1H, d, *J*= 3.8 Hz, H-1), 5.81 (1H, d, *J*=8.7 Hz, NH), 5.32 (1H, dd, *J*= 9.5 Hz, H-3), 5.21 (1H, apt, *J*= 9.6 Hz, H-4), 4.56 – 4.50 (1H, m, H-2), 4.30 – 4.24 (2H, m, H-6_b, H-6_a), 4.15 – 4.10 (1H, m, H-5), 2.10 (3H, s, CH_3), 2.05 (6H, s, 2x CH_3), 1.98 (3H, s, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ_C/ppm: 171.6, 170.3,

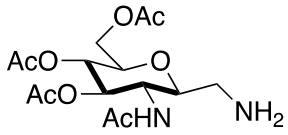
170.1, 169.2 (C=O), 93.7 (C_1), 71.0 (C_5), 70.3 (C_3), 67.0 (C_4), 61.1 (C_6), 53.6 (C_2), 23.2 (NAc), 20.8, 20.7, 20.6 (OAc). **ESI-MS**: m/z calculated for $C_{14}H_{20}NO_8Cl[M]^+$ 365.09, found $[M+H]^+$ 366.09.

2-Acetamido-2-deoxy-3, 4, 6-tri-O-acetyl-α-D-glucopyranosyl cyanide [4]



Glycosyl chloride (2.50 g, 6.90 mmol), tetrabutylammonium hydrogen sulphate (2.34 g, 6.90 mmol) and potassium cyanide (2.24 g, 34.5 mmol) were stirred vigorously at room temperature in a mixture of CH_2Cl_2 (40 ml) and 1M sodium carbonate (40 ml). The reaction was monitored by TLC and after 6 h, complete consumption of the chloride was observed. To the biphasic mixture was added CH_2Cl_2 (200 ml) and washed with H_2O (2 x 150 ml). The organic layer was then washed with sat. aq NaCl (150 ml), dried over $MgSO_4$, filtered and evaporated to dryness in vacuo to afford crude product as a brown oil. The product was purified by flash chromatography over silica gel (EtOAc/hexanes 4:1) to afford the cyanide (0.5 g, 21%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H/ppm: 5.82 (d, *J*=8.4Hz, 1H, NH), 5.37 (apt, 1H, *J* = 9.7 Hz, H_3), 5.07 (apt, 1H, *J* = 9.7 Hz, H_4), 4.71 (d, 1H, *J* = 10.7 Hz, H_1), 4.23 (dd, *J* = 12.7, 4.9 Hz, 1H, H_{6a}), 4.16 – 4.04 (m, 2H, H_{6b}, H_2), 3.77-3.70 (m, 1H, H_5), 2.10 (s, 3H, CH_3), 2.06 (3H, s, CH_3), 2.03 (3H, s, CH_3) 2.00 (3H, s, CH_3). **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C/ppm: 170.9, 170.8, 170.7, 169.3 (C=O), 115.0 (CN), 76.5 (C_5), 71.8 (C_4), 67.9 (C_3), 66.9 (C_1), 61.7 (C_6), 53.4 (C_2), 23.3 (NAc), 20.8, 20.7, 20.6 (OAc). **ESI-MS**: m/z calculated for $C_{15}H_{20}N_2O_8$ $[M]^+$, 356.1298 found [M+H], 357.1289. Data obtained matched that previously reported in the literature.[4]

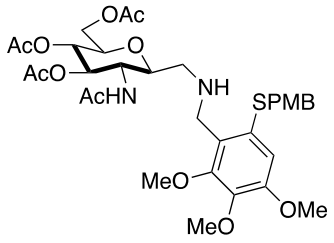
2-Acetamido-2-deoxy-3, 4, 6-tri-O-acetyl-α-D-glucopyranosyl methylamine



Glycosyl cyanide (0.4 g, 0.9 mmol) and 10% Pd/C (0.14g) were added to a 2-necked flask which was then evacuated and purged with nitrogen 3 times. To the solid was added dry THF-MeOH (9:1) ratio (20 ml) and the flask evacuated and purged with nitrogen 3 times again. The reaction mixture was stirred under hydrogen at room temperature overnight. TLC confirmed complete consumption of starting material and the reaction mixture was filtered through a celite plug and evaporated to dryness in vacuo to yield the methylamine (0.39 g) as a white solid. The product was used in the next steps

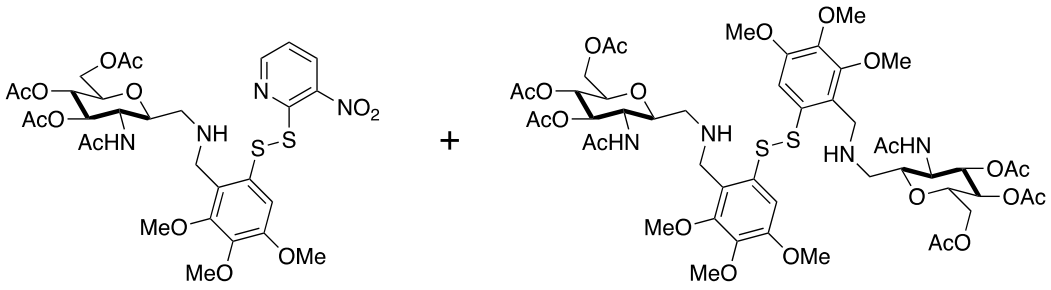
without further purification. **^{1}H NMR** (600MHz, $CDCl_3$) δ_H/ppm: 5.81 (d, *J* = 9.2 Hz, 1H, NH), 5.11 – 4.99 (m, 2H, H-3, H-4), 4.24 (dd, *J* = 12.3, 4.7 Hz, 1H, H-6_a), 4.11 – 4.03 (m, 2H, H-6_b, H-2), 3.67 – 3.60 (m, 1H, H-5), 3.59-3.51 (m, 1H, H-1), 2.78 (d, *J* = 4.8 Hz, 2H, CH_2N), 2.08 (s, 3H, CH_3), 2.02 (s, 6H, 2 x CH_3), 1.94 (s, 3H, CH_3). **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C/ppm: 171.6, 170.9, 170.5, 169.6 (C=O), 78.6 (C_5), 75.9 (C_4), 74.2 (C_3), 68.5 (C_1), 62.4 (C_6), 51.9 (C_2), 50.3 (CH_2), 23.4 (NAc), 21.0, 20.9, 20.8 (OAc). **ESI-MS**: m/z calculated for $C_{15}H_{25}N_2O_8$ [M + H], 361.1611 found [M+H], 361.1602. Data obtained matched that previously reported in the literture.[5]

(2*R*,3*S*,4*R*,5*S*,6*S*)-5-acetamido-2-(acetoxymethyl)-6-(((2,3,4-trimethoxy-6-((4-methoxybenzyl)thio)benzyl)amino)methyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate.[5]



Benzaldehyde auxiliary (0.42 g, 1.2 mmol) was dissolved in a solution of 2% AcOH in CH_2Cl_2 (6 ml). Glycosyl methylamine (0.36 g, 1.0 mmol) was then added, followed by sodium triacetoxyborohydride (0.233, 1.1 mmol). The reaction was stirred at room temperature and monitored by TLC. After 2 h, complete consumption of starting material was observed, and the mixture was then diluted with dichloromethane (10 ml) and neutralised with sat $NaHCO_3$ solution (10 ml). Extraction was completed in a separating funnel and the organic layer was washed with brine (2 x 5 ml), dried over $MgSO_4$, filtered and evaporated to dryness in vacuo. The crude product was purified by flash chromatography over silica gel (EtOAc/MeOH 9:1) to afford the amine (0.36 g, 52%) as a yellow oil. **^{1}H NMR** (600MHz, $CDCl_3$) δ_H/ppm: %): 7.10 (d, *J*= 8.6 Hz, 2H, 2xArH), 6.80 (d, *J*= 8.6 Hz, 2H, 2xArH), 6.60 (s, 1H, Ar), 5.70 (s, 1H, NH), 5.04 (m, 2H, H-3, H-4), 4.20 - 4.08 (m, 2H, H-1, H-6_a), 4.06 – 3.93 (m, 4H, H-2, H-6_b, CH_2), 3.86 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 3.72 (s, 2H, CH_2), 3.58 – 3.55 (m, 2H, H-5, CH_{2a}), 2.76 (m, 3H, CH_{2b}, CH_2), 2.04 – 1.98 (s, 12H, 4 x CH_3). **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C/ppm: 171.6, 170.9,170.5, 169.6 (C=O), 159.0 (C_{PMB}), 152.9, 152.5, 141.7 (C_{Ar}), 130.4 (C_{PMB}), 130.2 (CH_{PMB}), 129.4 (C_{Ar}), 114.2 (CH_{PMB}), 112.4 (CH_{Ar}), 77.3 (C_1), 75.7 (C_5), 74.3 (C_3), 68.5 (C_4), 62.5 (C_6), 61.4 (C_2), 60.9, 56.0, 55.4, 52.2 (OCH_3), 49.7 (CH_2), 45.6 (CH_2), 40.4 (CH_2), 23.4 (NAc), 20.9, 2 x 20.8 (OAc). **ESI-MS**: m/z calculated for $C_{33}H_{45}N_2O_{12}S$[M+H] 693.2693, found [M+H] 693.2692.

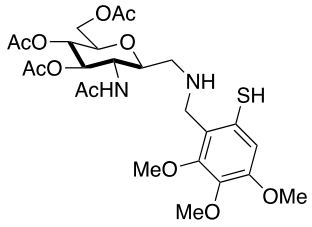
(2*R*,3*S*,4*R*,5*S*,6*S*)-5-acetamido-2-(acetoxymethyl)-6-(((2,3,4-trimethoxy-6-((3-nitropyridin-2-yl)disulfaneyl)benzyl)amino)methyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate.[6]



2 : 1

To a solution of the reductive amination product (68 mg , 0.10 mmol) in 2,2,2-trifluroethanol (8 ml) was added a solution of 3-nitro-2-pyridinesufenyl chloride (20 mg, 0.11 mmol) in CH_2Cl_2 (2 ml). The reaction was stirred at room temperature for 2 h and more 3-nitro-2-pyridinesufenyl chloride (10 mg) was added. The mixture was stirred for another 30 min and concentrated in vacuo. The crude product was flash chromatographed on silica gel with EtOAc/MeOH 9:1 solvent system. Two disulfide product were obtained as a mixture from the column and were used in the next step.

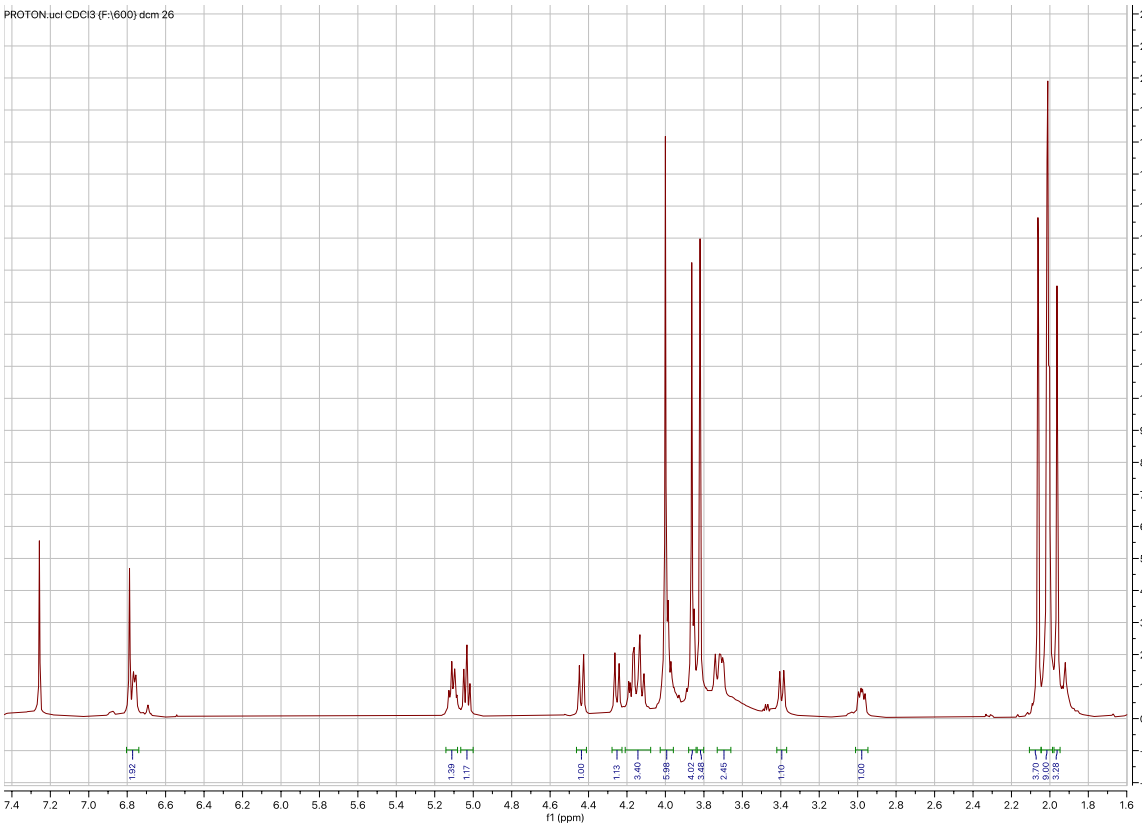
(2*R*,3*S*,4*R*,5*S*,6*S*)-5-acetamido-2-(acetoxymethyl)-6-(((6-mercapto-2,3,4 trimethoxybenzyl)amino)methyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate

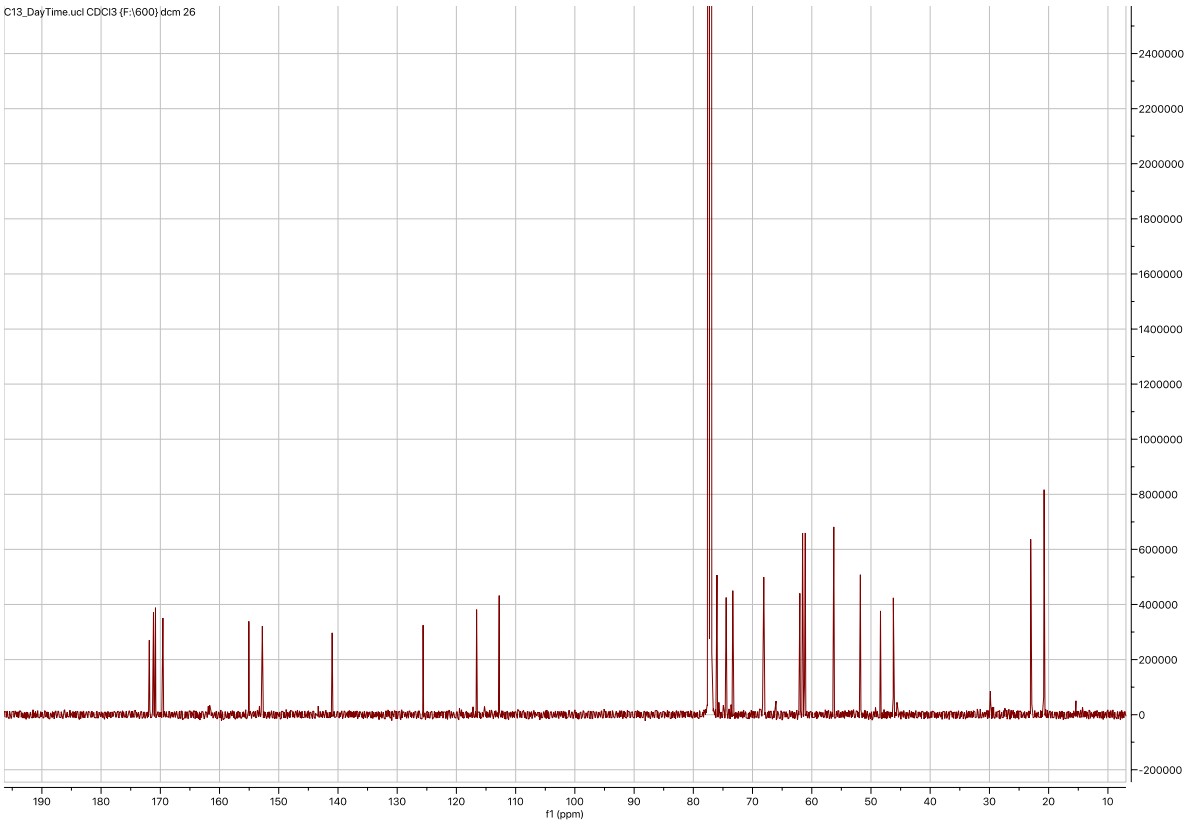


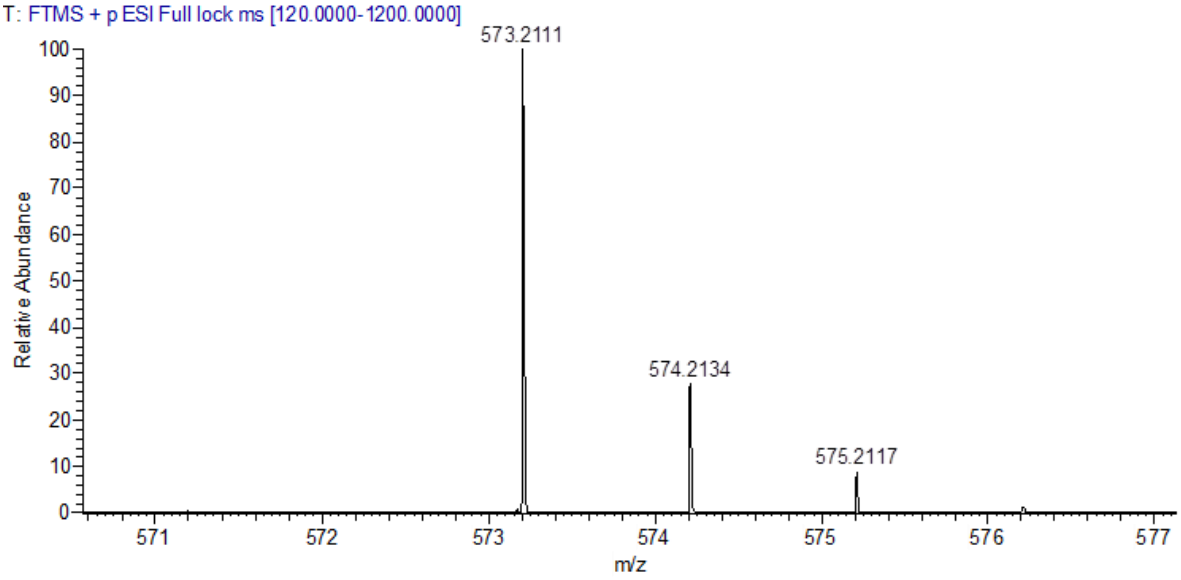
11

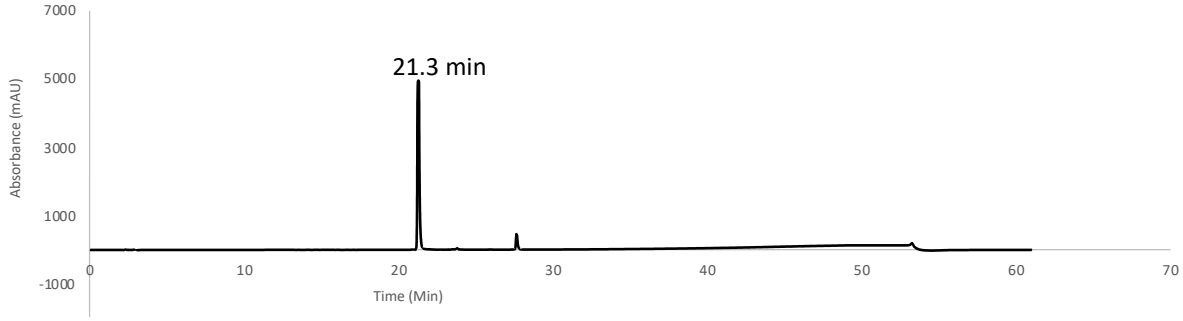
The disulfdes (100 mg) were dissolved in ligation buffer (6M Gn·HCl in 0.1 M sodium phosphate buffer pH 7.0) (10 ml), and TCEP was added to a final concentration of 30 mM. The thiol was purified on preparative HPLC (t_R= 33 min) and lyophilised to yield the thiol (35 mg). ^{1}H NMR (600MHz, $CDCl_3$) δ_H/ppm: 6.78 (s, 1H, ArH), 6.76 (d, *J*= 7.8 Hz, 1H, NH), 5.15-5.07 (m, 1H, H-3), 5.05-4.99 (m, 1H, H-4), 4.43 (d, *J*=13.4Hz, 1H, CH_{AB}), 4.25 (d, *J*=13.4, 1H, CH_{AB}), 4.20 - 4.10 (m, 2H, H-6_a, H-6_b), 4.03 – 3.95 (m, 5H, $ArCH_{2AB}$,H-2, OCH_3, CH_2), 3.89-3.84 (m, 4H, $ArCH_{2AB}$, OCH_3), 3.81 (s, 3H, OCH_3), 3.58 – 3.55 (m, 2H, H-5, H-1), 2.98 (1H, s(br), SH), 2.06 (3H, s, CH_3), 2.01 (6H, s, 2 x CH_3), 1.96 (3H, s, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ_C/ppm: 172.0, 171.1,170.8, 169.5 (C=O), 155.0, 152.7, 140.9, 125.6, 116.4 (C_{Ar}), 112.7 (CH_{Ar}), 76.0 (C_1), 74.5 (C_5), 73.3 (C_3), 68.1 (C_4), 62.0 (C_6), 61.5 (C_2), 61.0, 56.3, 51.8 (OCH_3), 48.4 (CH_2), 46.2 (CH_2), 23.1 (NAc), 3 x 20.8 (OAc). ESI-MS: m/z calculated for $C_{25}H_{37}N_2O_{11}S$[M+H]

573.2118, found [M+H] 573.2111.









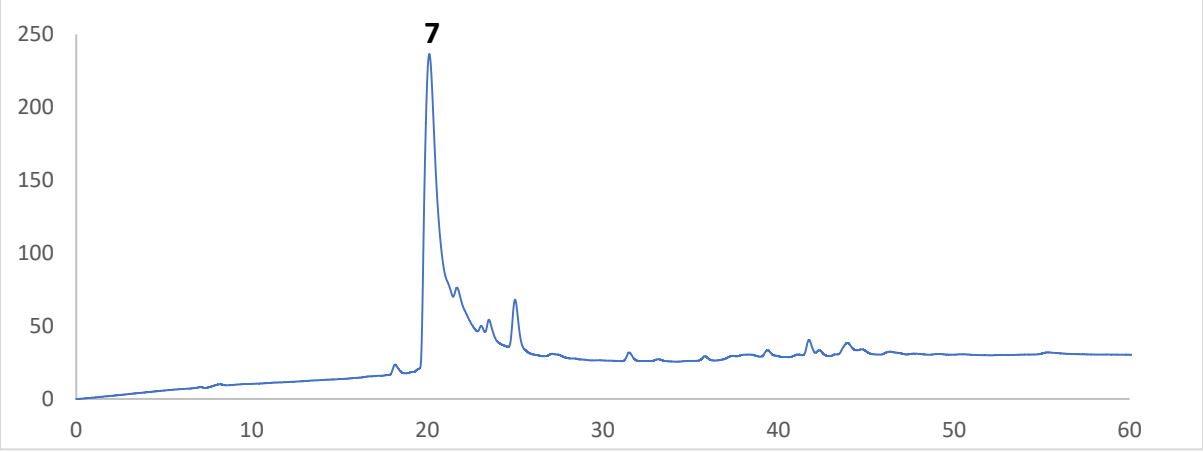
Analytical HPLC trace of **11.**

General Solid Phase Peptide Synthesis. Peptide synthesis was carried out on an ABI 433A automated synthesiser using standard Fmoc amino acids on a 0.05 mmol scale, and employing either Rink Amide MBHA resin (for peptides **7** and **8**) or pre-loaded FmocGly-NovaSyn®TGT resin (loading = 0.19 mmolg^{-1}) for peptides **12** and **13**. Cleavage of the assembled peptide chain was carried out in TFA (95% v/v)/1,2-ethanedithiol (2.5%)/H_2O(2.5%) for 4.5 h before filtration and 2 cycles of precipitation into cold ether followed by centrifugation (3000 rpm, 4°C, 15 min). Peptides were dissolved in the minimum volume of water and purified via preparative RP-HPLC as described above, and fractions containing the desired peptide were identified by LC-MS and lyophilized to afford the products as fluffy white solids.

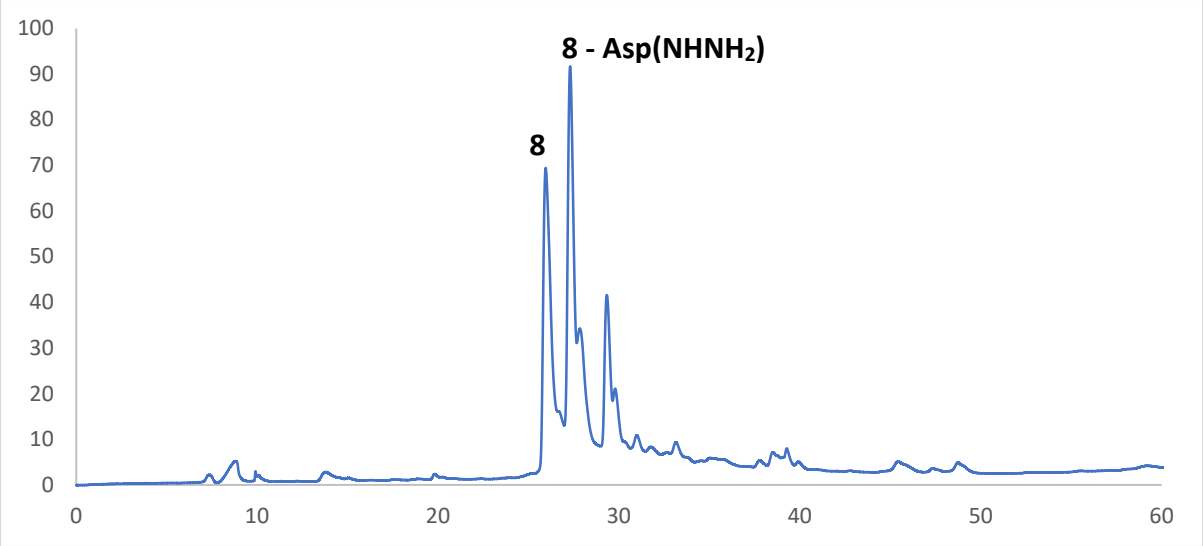
Symmetrical Anhydride method for introduction of building blocks **4** or **6** into **8**. [7]

Building blocks (**4** or **6**, 0.5 mmol) were dissolved in dry DCM 1.0 ml and cooled to 0°C. DIC (0.25mmol) was dissolved in dry DCM (1.0 mL) and added dropwise. The reaction was stirred at 0°C for 20 min then evaporated to dryness. The residue was dissolved in dry DMF (2.0 mL) and added to resin bound peptide. **8** was isolated as a white solid (8 mg, 14 %)

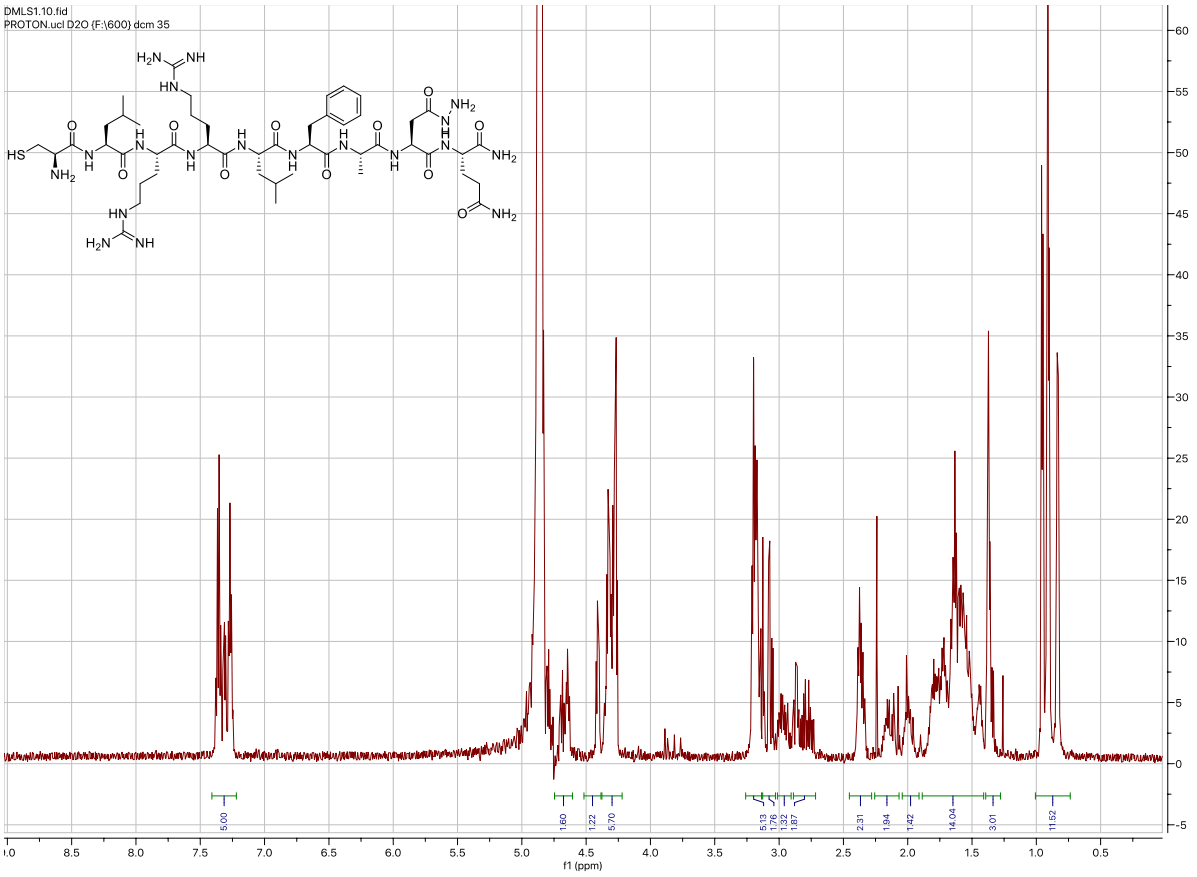
Microcin J25 derived peptide **7:** isolated yield 12 mg, 25%.



Preparative HPLC trace for sidechain Glu-Hydrazide peptide **7**.

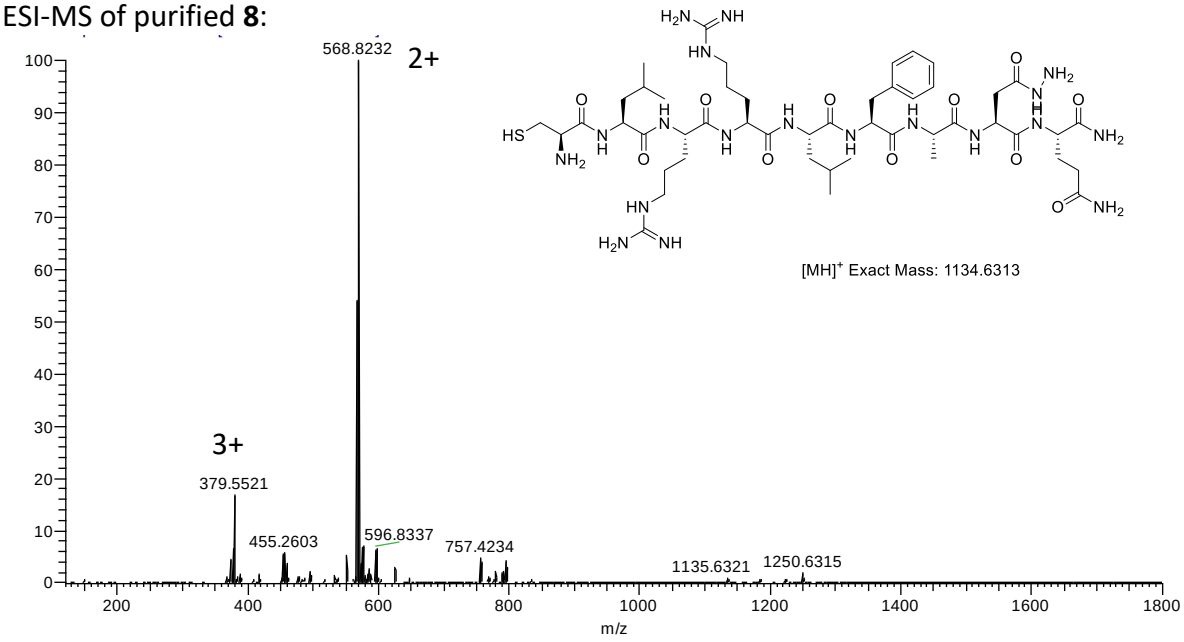


Preparative HPLC trace for sidechain Glu-Hydrazide peptide **8** employing symmetrical anhydride coupling with **4**.



^{1}H NMR Spectrum of isolated **8**.

ESI-MS of purified **8**:



Analytical cyclisation reactions of model peptide 7

A stock solution of **7** was prepared by dissolving in water to a final concentration of approx. 2.0 mg/ml (approx. 2 mM).

Acetyl acetone activation/cyclisation.
Stock solution (0.5 mL, 1.06 µmol) was diluted to 1.0 mL with sodium phosphate buffer (0.5 M, pH 3.0) to give a final concentration of approx. 1 mM peptide in 250 mM sodium phosphate buffer pH 3.0. Acetyl acetone (acac,0.1 M, 20 µL, 2 µmol) was added from a freshly prepared 0.1 M solution in ddH_2O, along with MESNa (50 mg, 300 µmol). The pH of the reaction mixture was confirmed (approx. pH 3) and then the acidic solution was shaken at room temperature for 2 h in an Eppendorf thermomixer (700rpm). The pH of the reaction mixture was then adjusted to pH7 by careful addition of 0.1 M NaOH and shaking was continued for 1h (until LC-MS indicated that the reaction was complete). 20 µL aliquots of reaction mixture were also analysed by analytical HPLC after 2h at pH 3 and 1h at pH7.

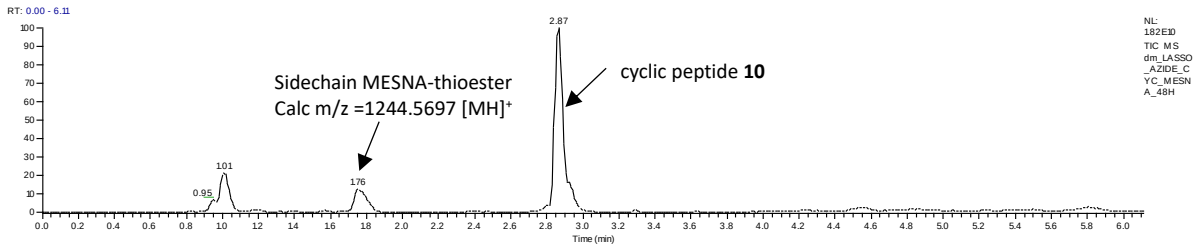
$NaNO_2$ activation/cyclisation.
Peptide stock solution (0.5 mL, 1.06 µmol) was diluted to 1.0 mL with sodium phosphate buffer (0.5 M, pH 3.0) to give a final concentration of approx. 1 mM and cooled to 0 ^oC. 1.2 equiv. of $NaNO_2$ was added (12 µL of a 0.1 M stock solution prepared in water) and the reaction was allowed to stand on ice, with occasional agitation, for 20 min.

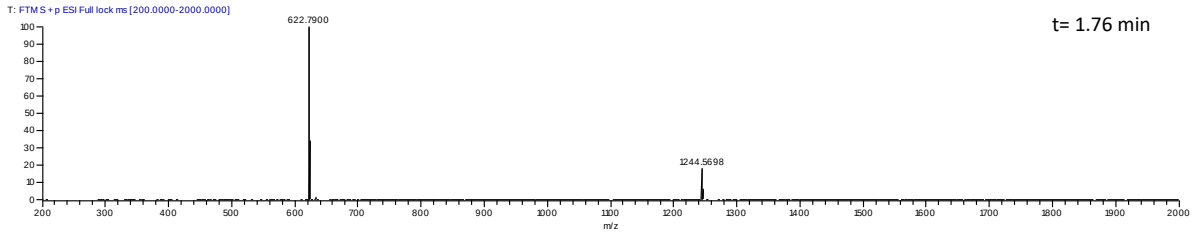
MesNa (50 mg, 300 µmol) was added and the pH was adjusted to pH 7 by careful addition of 0.1 M NaOH and the reaction was continued at room temperature in in Eppendorf thermomixer for 2h (when LC-MS indicated that the reaction was complete). 20 µL aliquots of reaction mixture were also analysed by analytical HPLC after 1h at pH7.

Preparative cyclisation reactions of model peptide 8

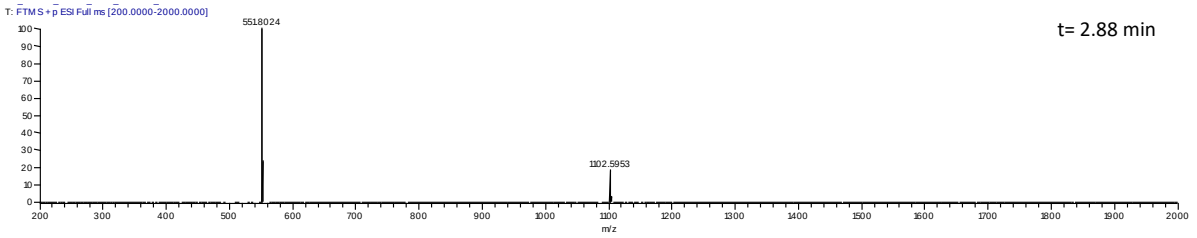
8 (8.0 mg, 7.06 µmol) was dissolved in a 1:1 mixture of 6M guanidine.HCl and 0.5 M Na Phosphate buffer to a final concentration of approx. 2.0 mg/ml (approx. 2 mM). The pH was adjusted to pH 3 with 2 M HCl and cooled on ice for 0.5 h. Freshly prepared sodium nitrite solution (0.1 M, 96µL) was added and the reaction was continued at 0 ^oC with occasional agitation for 30 min. MESNa (200 mg, 1.22 mmol) was then added and the reaction pH was adjusted to pH 7 by careful addition of 0.1 M NaOH. The reaction was then placed on an orbital shaker (300 rpm) for 48h at room temperature and monitored by LC-MS. After 48 h the reaction mixture was purified by preparative HPLC to afford the product (5.0 mg, 65%) as a fluffy white solid.

LC-MS analysis of cyclisation reaction after 48h at 25°C:



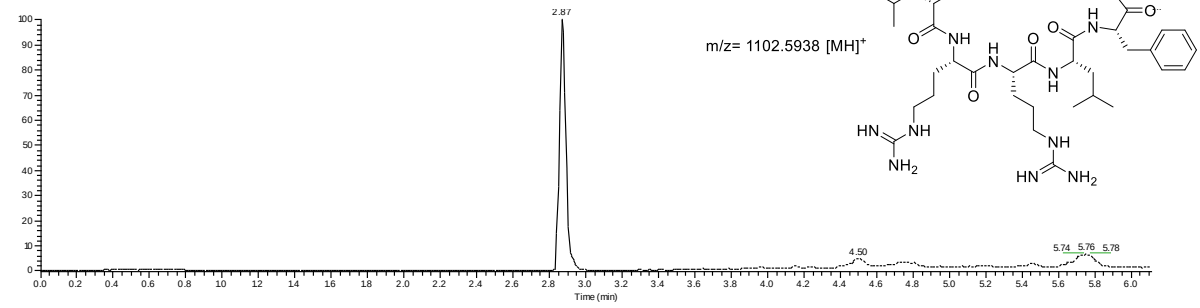


t= 1.76 min

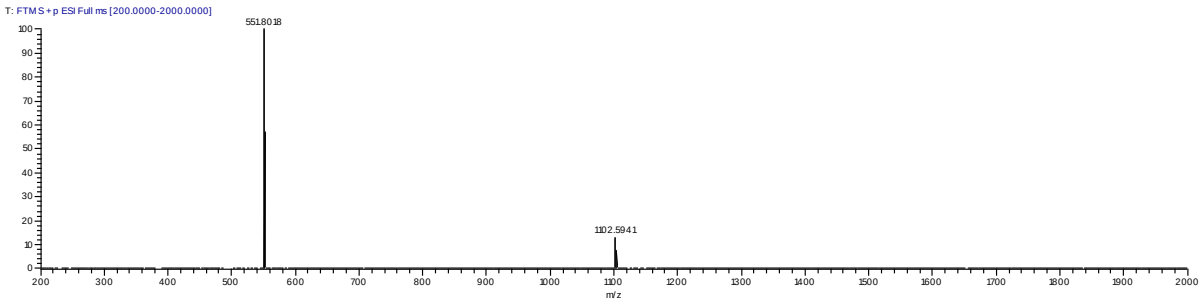


t= 2.88 min

LC-MS analysis of HPLC purified **10**



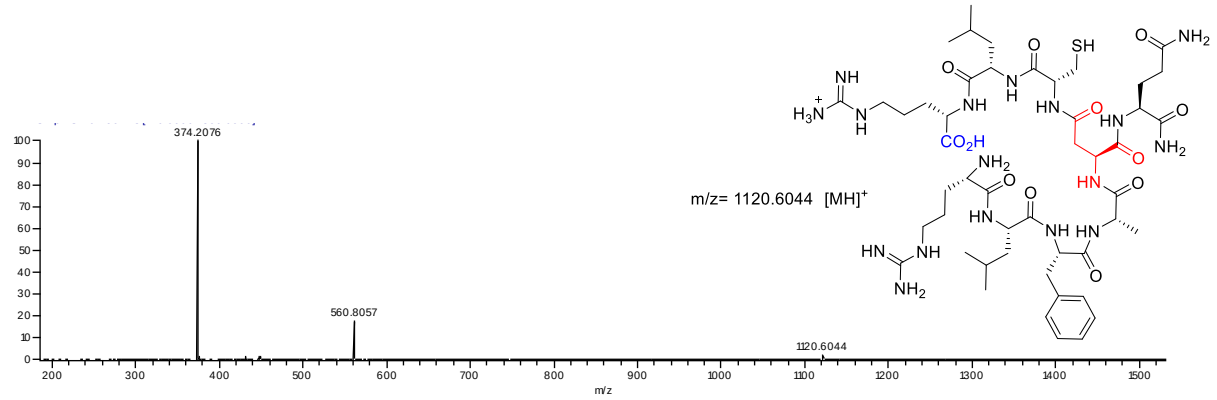
m/z= 1102.5938 [MH]+

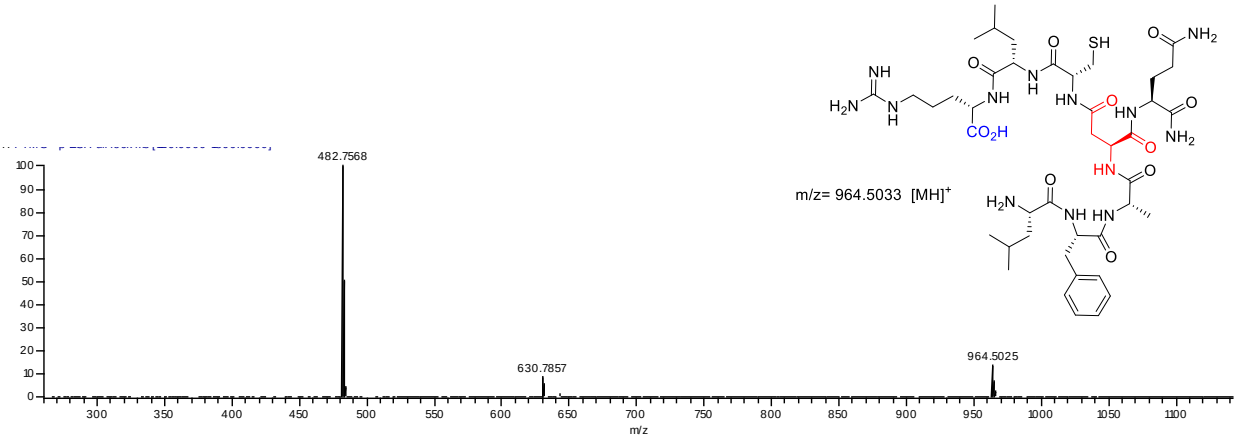


Tryptic Digest of cyclic peptide **10**

10, 0.5 mg was dissolved in 50 mM $(NH_4)HCO_3$ and incubated at 30 ºC for 1.5 h. A sample (2µL) was analysed by LC-MS.

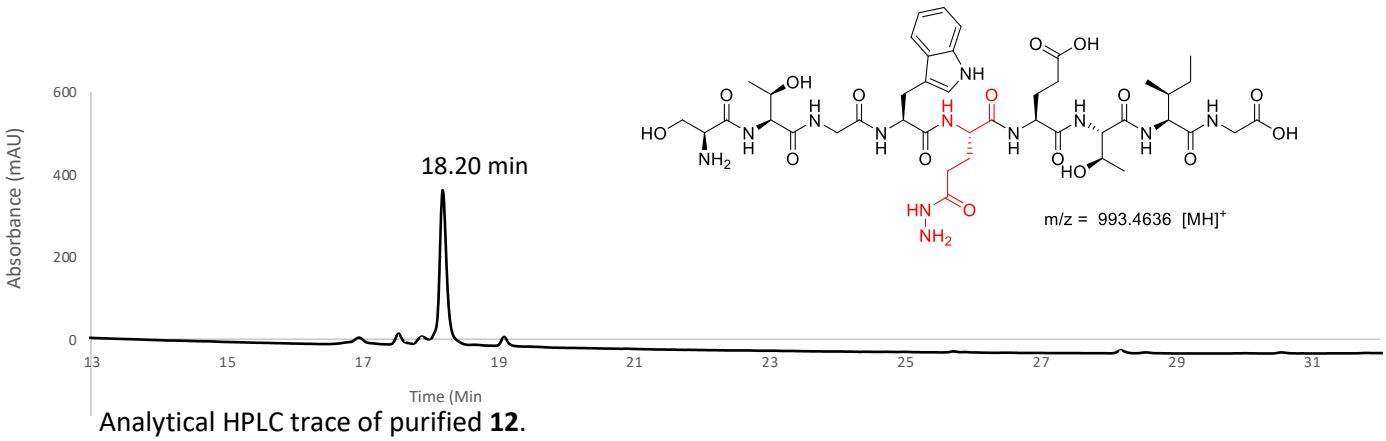
2 major species were observed were observed: m/z = 1120 ($+H_2O$) and 964 (-Arg), consistent with the cyclic product:



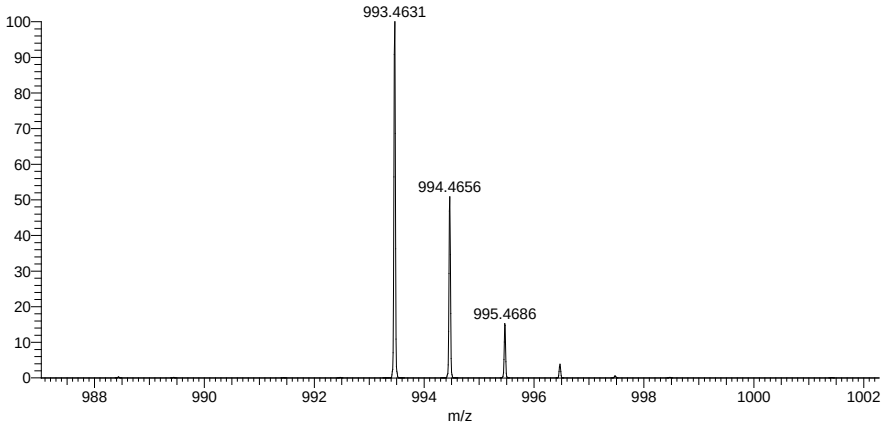


Synthesis of model Interferon derived peptide **12**

A model peptide with glutamic acid sidechain hydrazide **(**at natural glycosylation site 80 of β-interferon) was constructed using the general method above at 0.05 mmol scale, on Fmoc-Gly-NovaSyn-TGT resin. Glutamic acid hydrazide was introduced as the 2-Chlorotrityl protected analogue (**3**). After lyophilisation of the semi-preparative reverse phase (RP) HPLC fractions (t_R = 25.5 min), peptide hydrazide was isolated as a white fluffy solid (10 mg, 20%). The peptide was characterised by HPLC and mass spectrometry. **ESI-MS**: m/z calculated for $C_{42}H_{65}N_{12}O_{16}[MH]^+$ 993.4636, found [M+H] 993.4631.

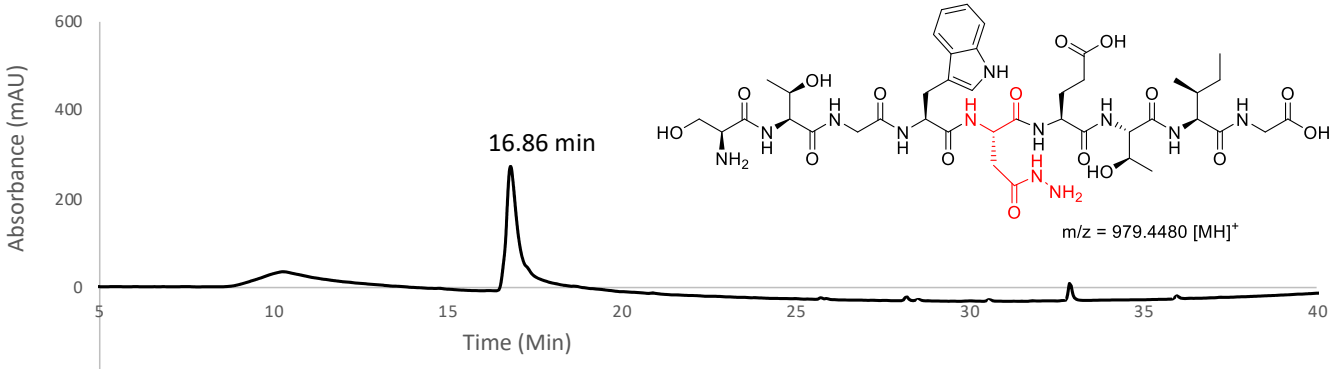


Analytical HPLC trace of purified **12**.

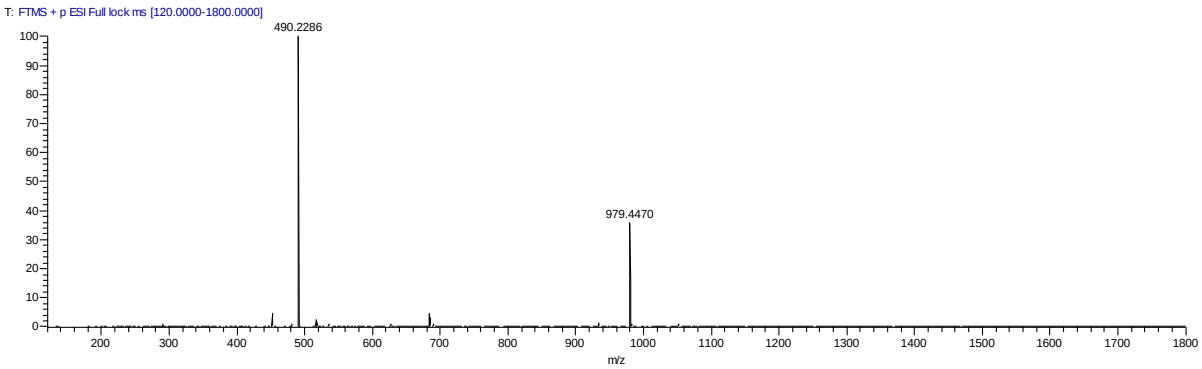


ESI mass spectrum of **12**

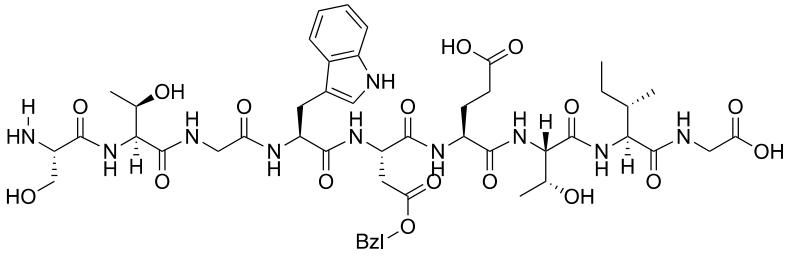
<u>Preparation of Asp hydrazide **13**</u>



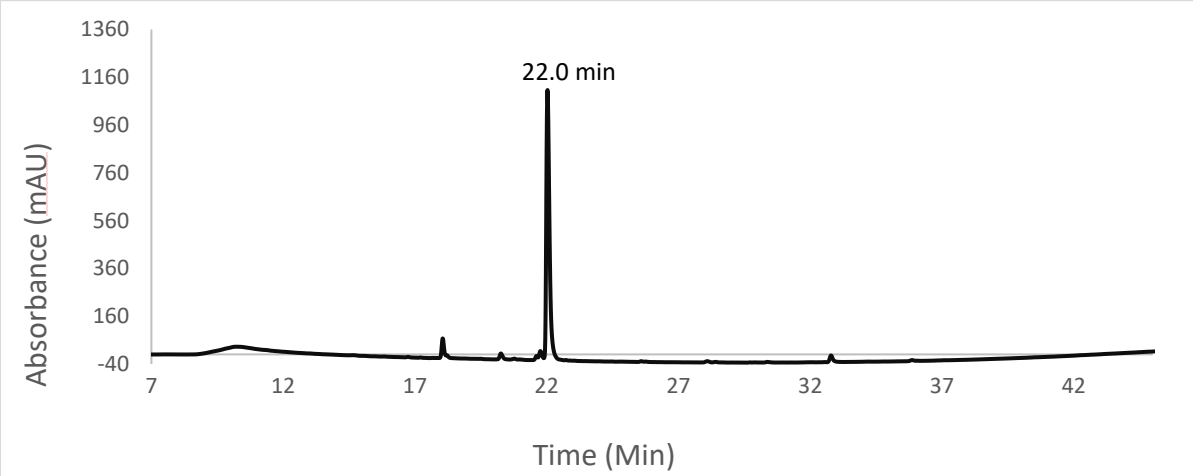
Analytical HPLC trace of IFN (77 - 83) model peptide **13**.



Alternative procedure for preparation **13,** *via* sidechain benzyl ester.

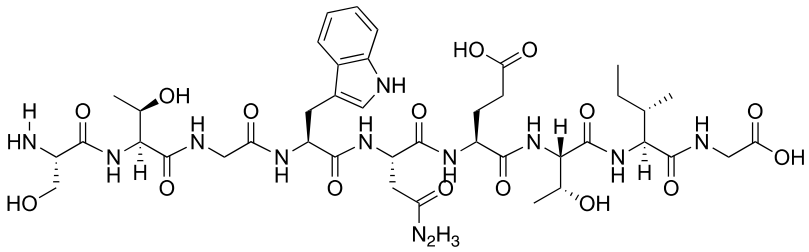


β-Interferon derived model peptide with and Asp sidechain benzyl ester at the natural glycosylation site was constructed using the general method described above on pre-loaded Fmoc-Gly-NovaSyn-TGT resin. After lyophilisation of the semi-preparative reverse phase (RP) HPLC fractions (t_R = 35.5 min), peptide ester was isolated as a white fluffy solid in 19% yield. The peptide was characterised by mass spectrometry. **ESI-MS**: m/z calculated for $C_{48}H_{67}N_{10}O_{17}[M]^+$ 1055.47, found [M+H] 1055.77.



Analytical RP-HPLC trace of interferon model peptide with aspartic acid sidechain benzyl ester.

Hydrazinolysis of benzyl ester to afford **13**

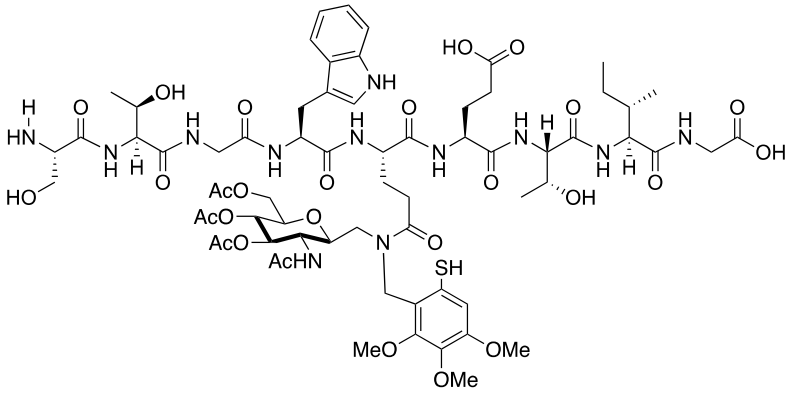


Hydrazine hydrate (5% hydrazine in H_2O, 200 μL) was added to 8 mg of peptide benzyl ester in DMF. The mixture was stirred at room temperature for 1 h and hydrazinolysis was monitored by LC-MS. The crude mixture was purified on semi-preparative reverse phase (RP) HPLC (t_R = 26 min) and lyophilised to yield the hydrazide (6.8 mg, 92%). **ESI-MS**: m/z calculated for $C_{41}H_{63}N_{12}O_{16}[M]^+$ 979.4485, found [M+H] 979.4484

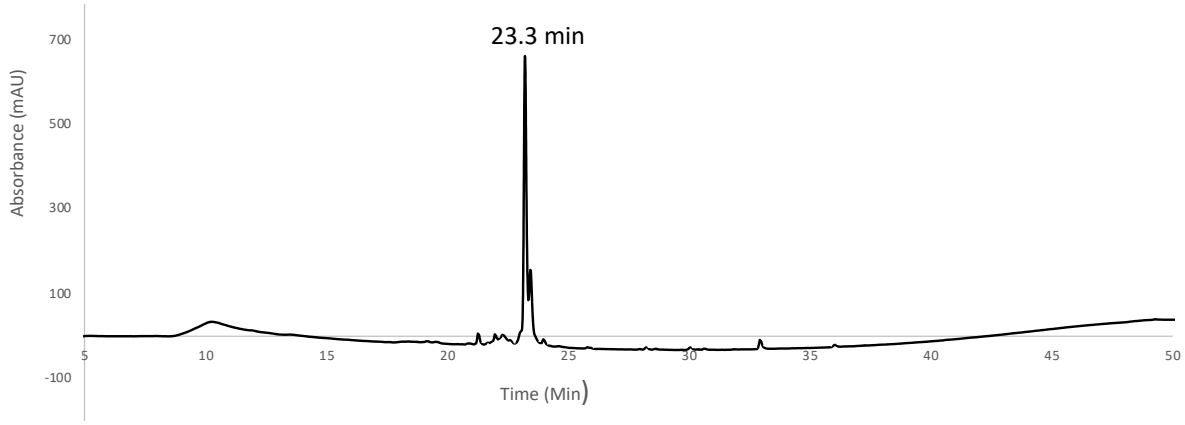
General method for auxiliary mediated ligations using the acac activation method:

Peptide hydrazides were dissolved in ligation buffer comprised of 0.1 M sodium phosphate buffer pH 3.0-4.0 in 6.0 M guanidinium hydrochloride to a concentration of 2 mg/ml. Peptide solution (0.5 ml) was added to an Eppendorf tube followed by 0.25 M acetylacetone (10 µl) and 0.2 M MPAA (0.5 ml) and the mixture shaken at 700rpm on an Eppendorf thermomixer for 2 h at room temperature. **11** (1 – 2 eq) and TCEP (final concentration 5 mM) were added and the pH was adjusted within a range of 5.5 – 7.0 using 1 M NaOH. The reactions were monitored by LC-MS.

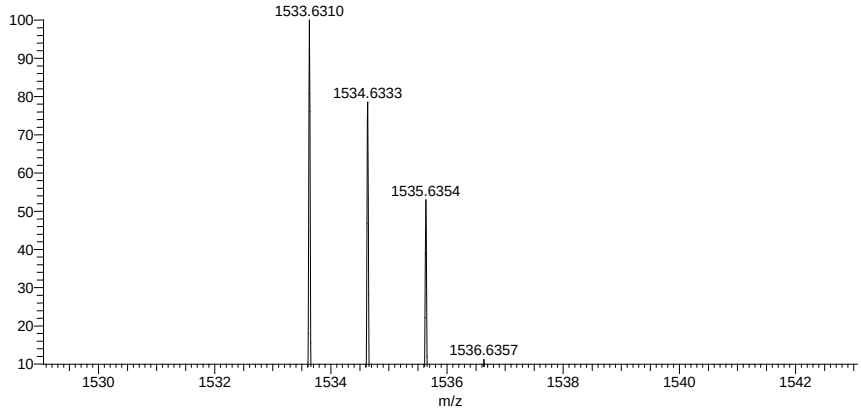
Synthesis of a Glu sidechain glycopeptide **14**



Acac activation of the peptide hydrazide **12** (6.9 mg, 6.9 µmol) was conducted as above. 1.2 eq of **11** was employed and the pH was adjusted to pH 7. After 24 h the crude reaction mixture was purified by semi-preparative reverse phase (RP) HPLC (t_R = 41.2 min) and lyophilised to yield the hydrazide (6.1 mg, 58 %). $C_{67}H_{97}N_{12}O_{27}S[M]^+$ 1533.6307, found $[M+H]\ ]^+$ 1533.6310

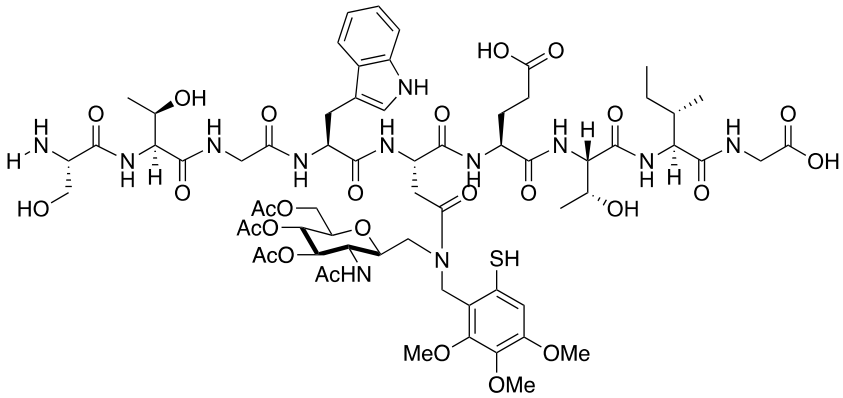


Analytical RP-HPLC trace of isolated glycopeptide **14**.

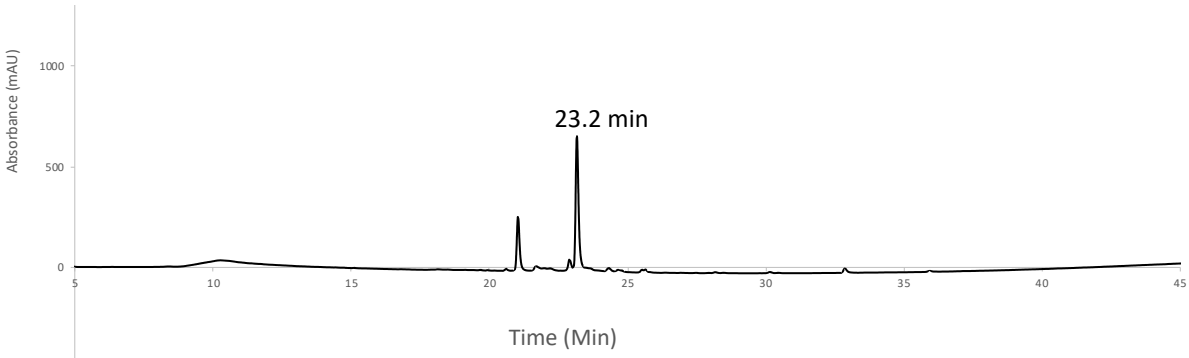


High Resolution ESI-MS spectrum of isolated glycopeptide **14**.

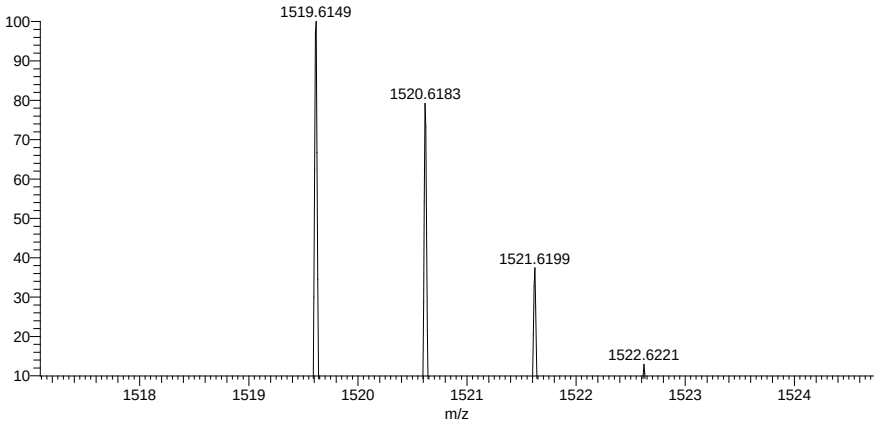
Synthesis of Asp sidechain glycopeptide **15**



Glycopeptide **15** was synthesised following the general ligation method above using acac. 1.2 eq of **11** was employed and the final pH was adjusted to pH 6. The reaction was completed after 48 h and the crude mixture was purified by semi-preparative reverse phase (RP) HPLC (t_R = 41.0 min). Fractions containing the desired product were collected and lyophilised to afford **15** (36 %). $C_{66}H_{95}N_{12}O_{27}S[M]^+$ 1519.6150, found $[M+H]^+$ 1519.6149.

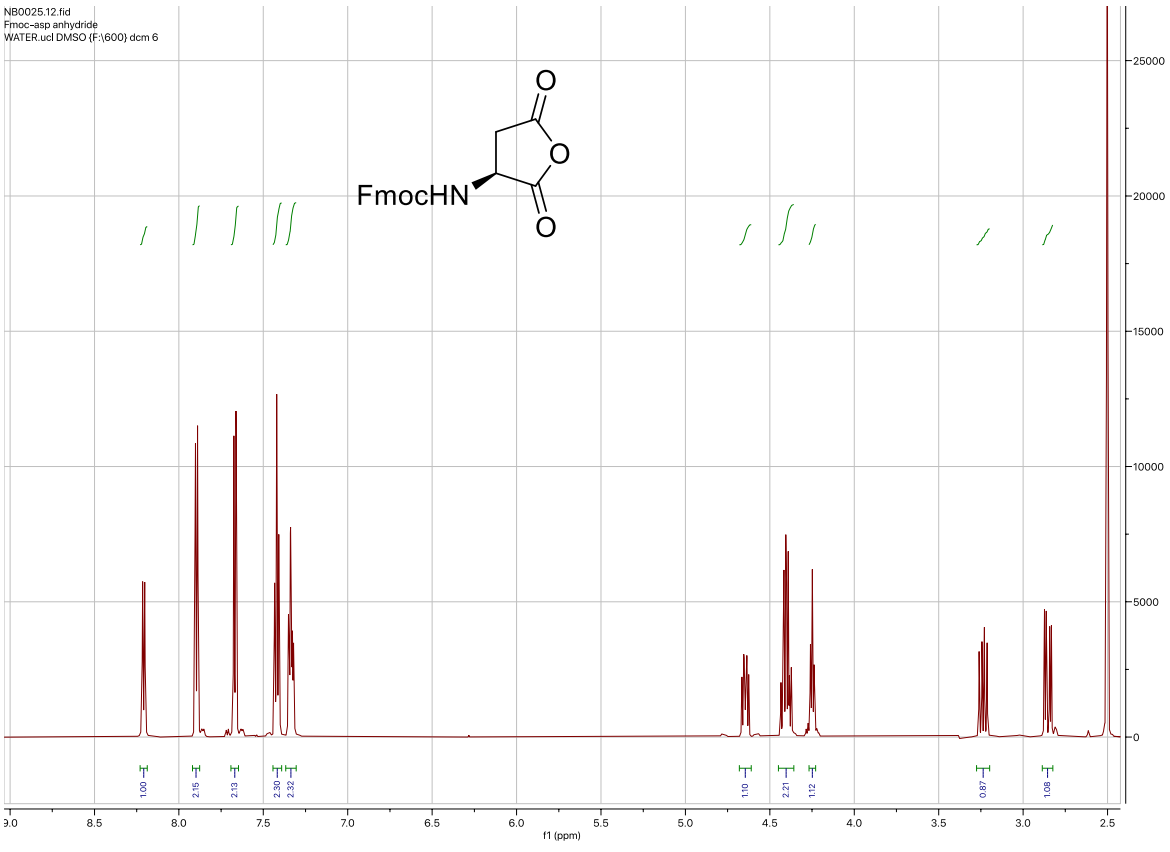


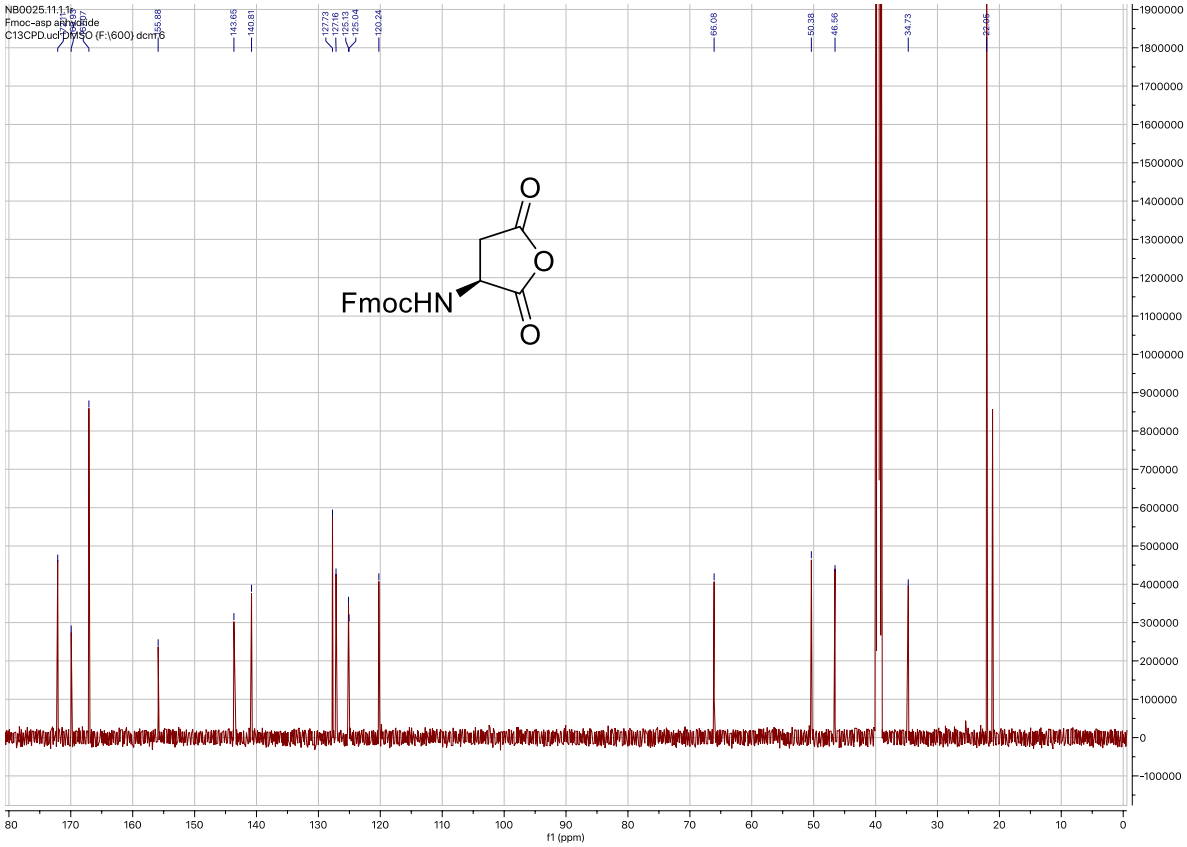
Analytical RP-HPLC trace of isolated glycopeptide **15**.



High Resolution ESI-MS spectrum of glycopeptide **15**.

Selected analytical data:

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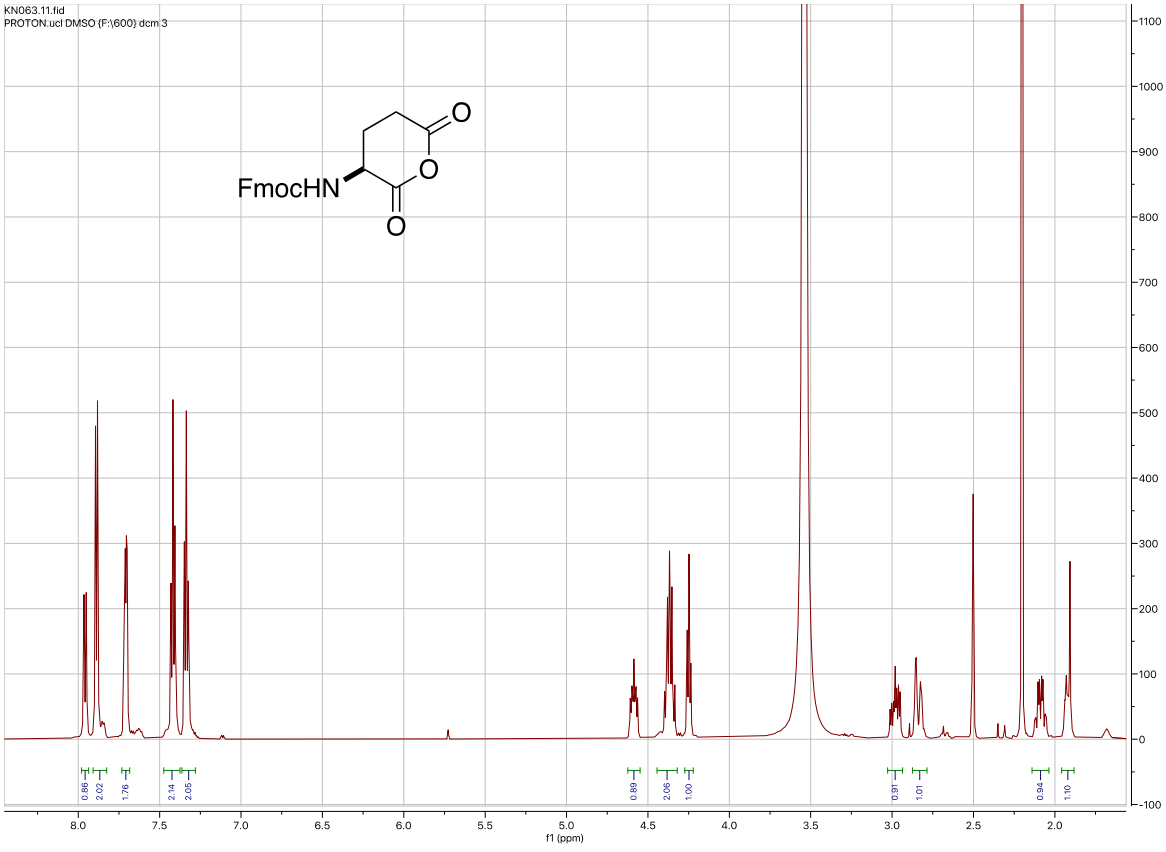
143.66
140.81
127.73
127.16
125.93
125.04
120.24
66.08
50.38
46.56
34.73
22.66
FmocHN
O
O
O
f1 (ppm)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2.05
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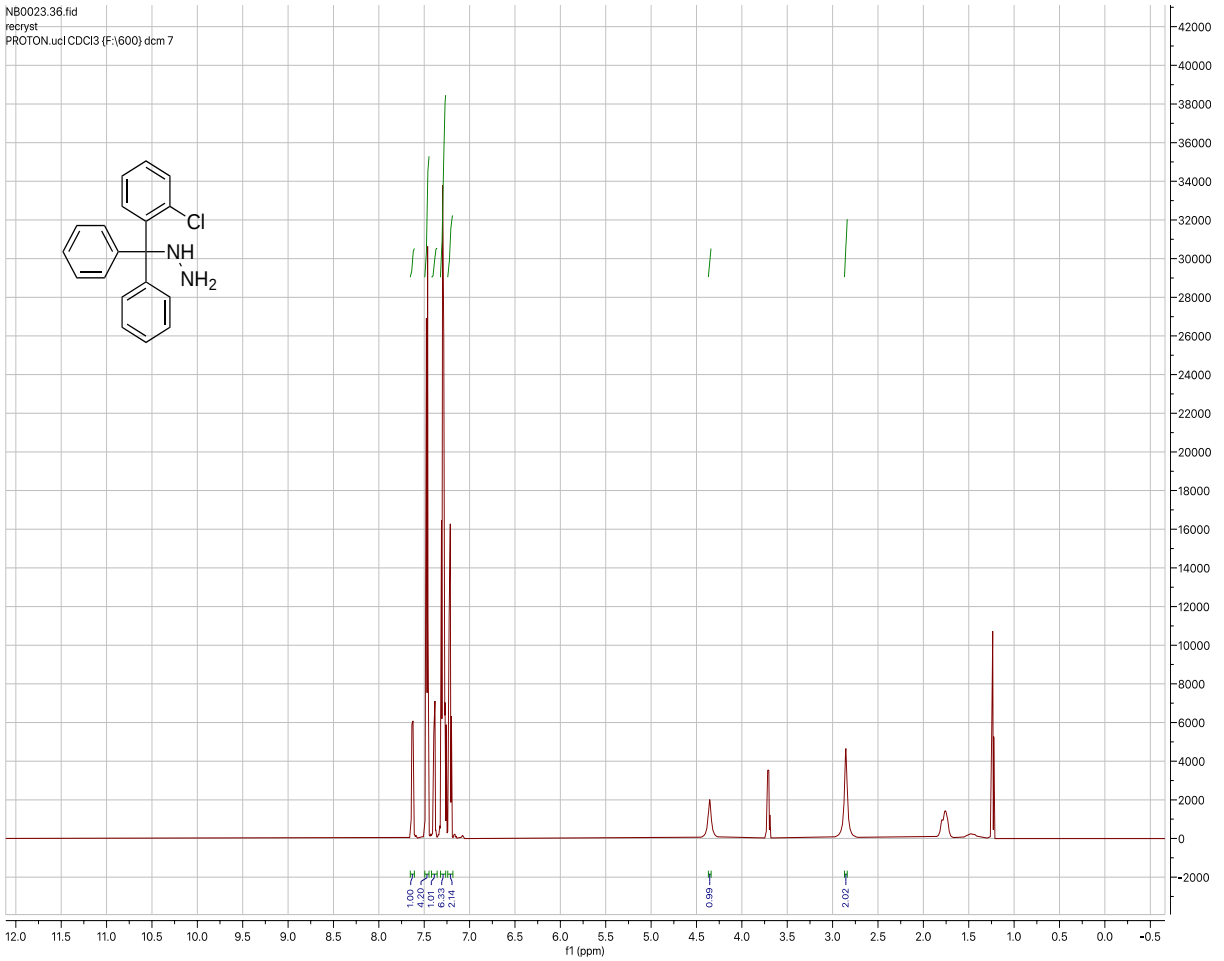
2.06
1.00
0.91
1.01
0.94
1.10
f1 (ppm)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recryst
PROTON.ucl CDCl3 (F:\600) dcm 7
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NH2
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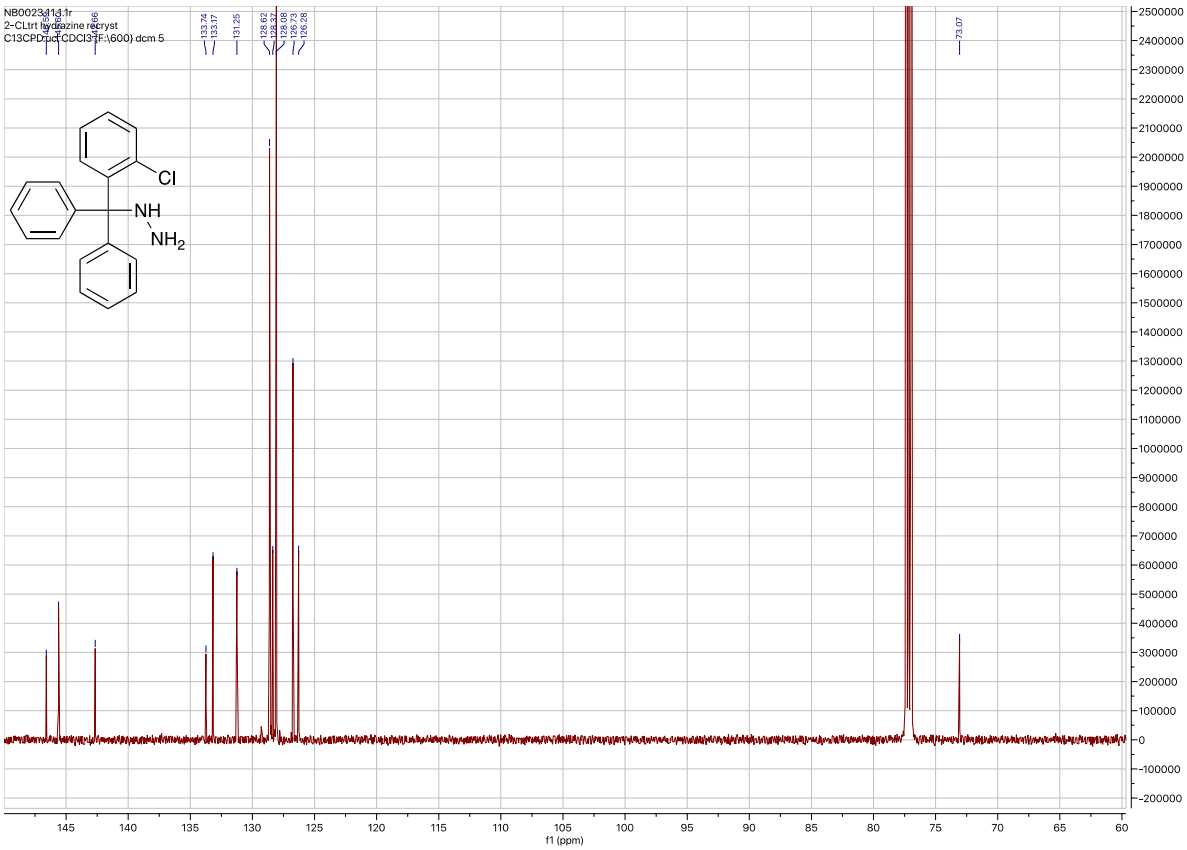
4.20
1.01
6.33
2.14
0.99
2.02
f1 (ppm)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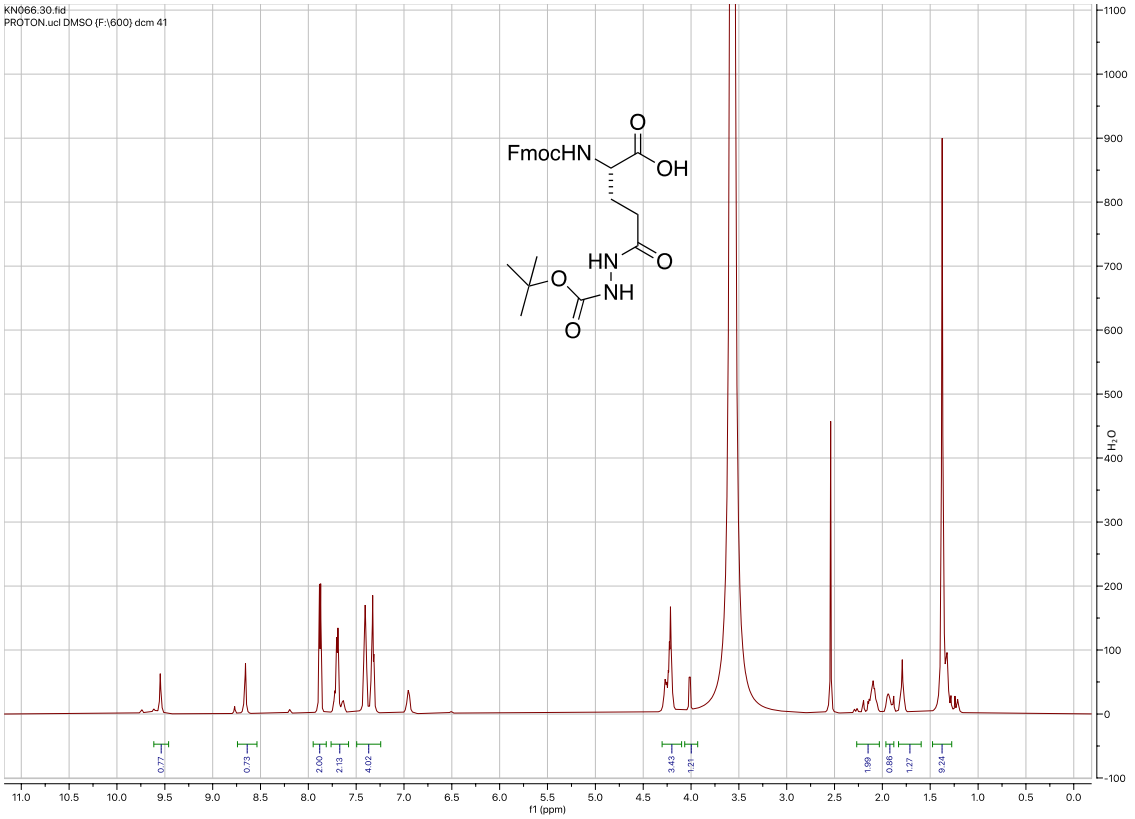
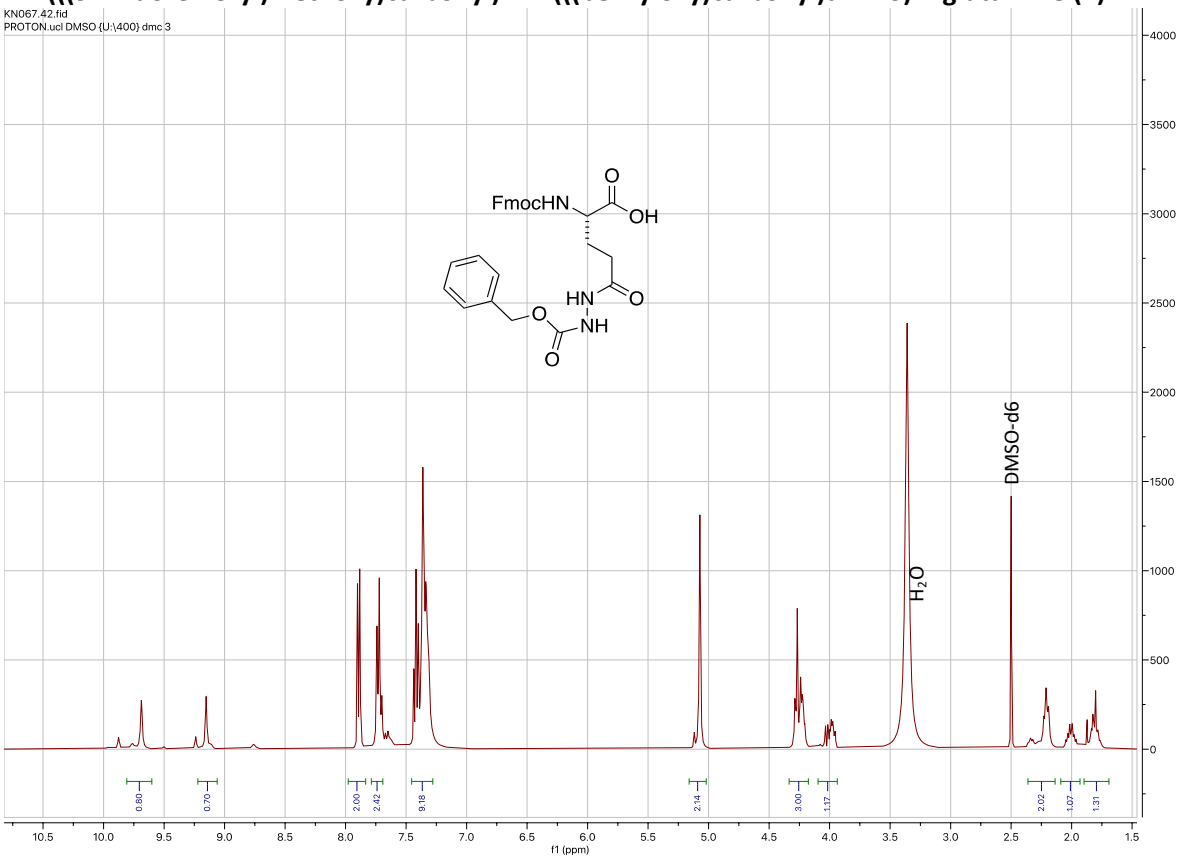
133.17
131.25
128.62
128.37
128.08
126.73
125.28
73.07
Cl
NH
NH2
f1 (ppm)

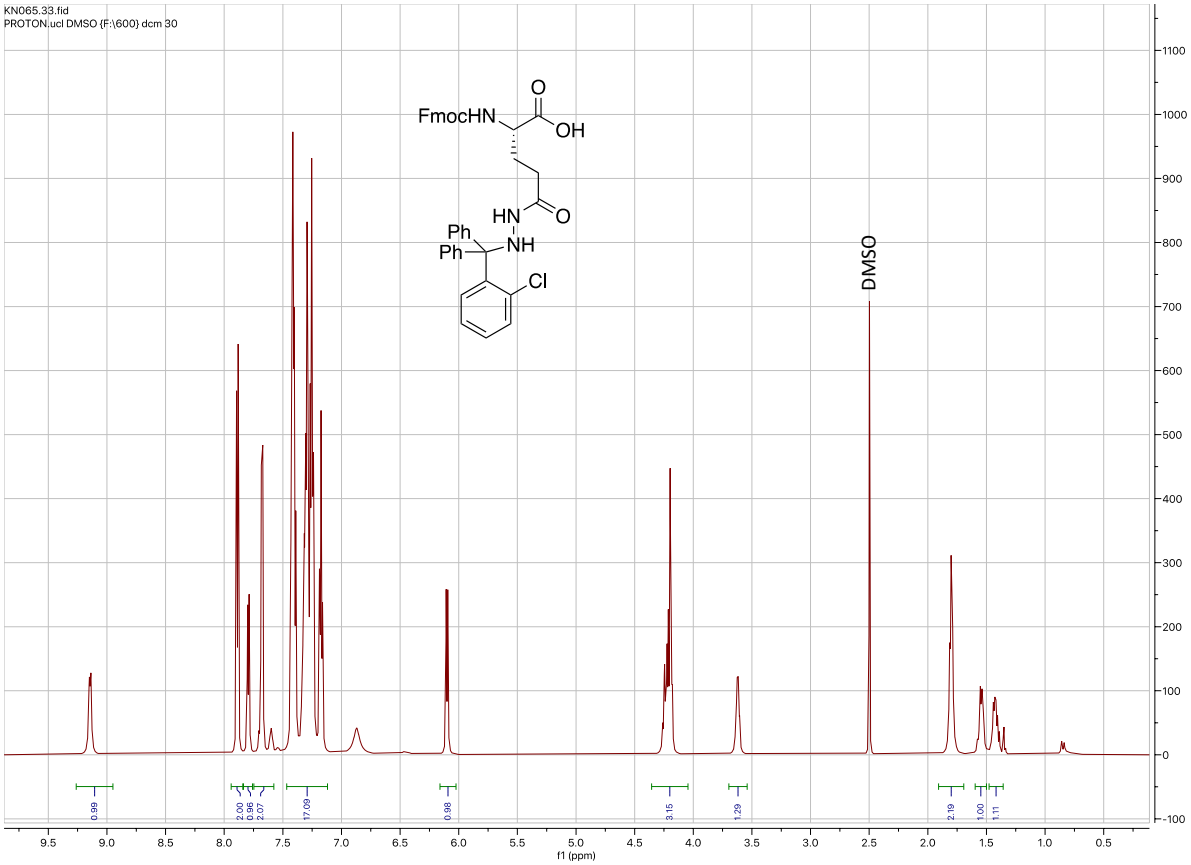
N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^5-((*tert*-butoxycarbonyl)amino)-*L*-glutamine (1)



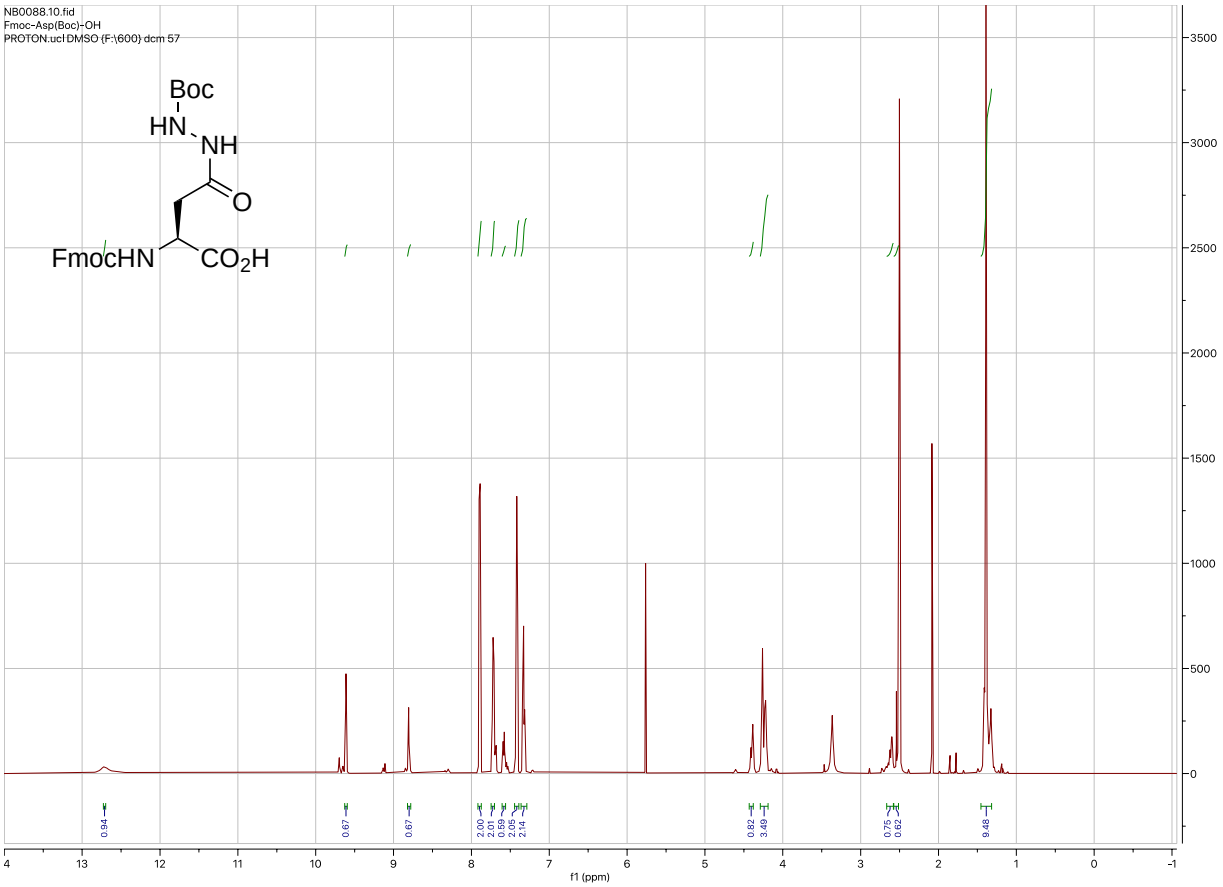
N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^5-(((benzyloxy)carbonyl)amino)-*L*-glutamine (2)



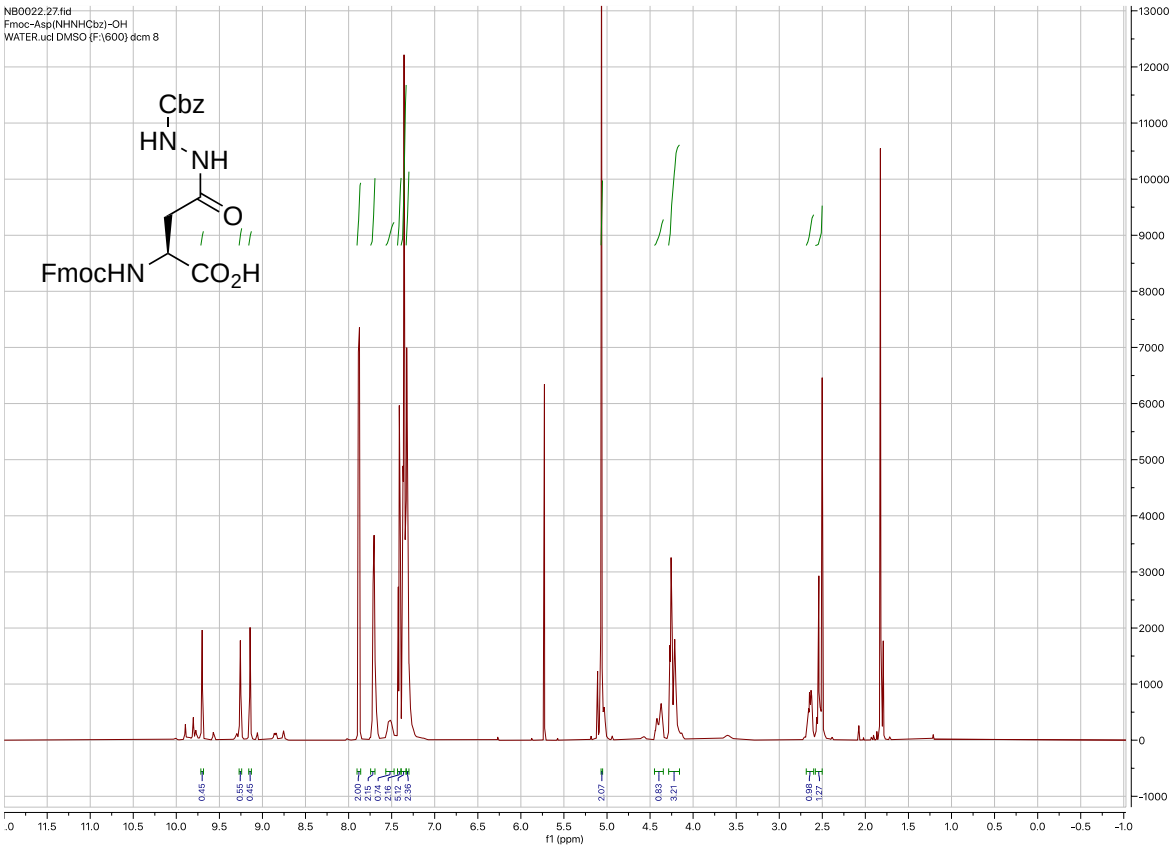
N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^5-(((2-chlorophenyl)diphenylmethyl)amino)-*L*-glutamine (3)



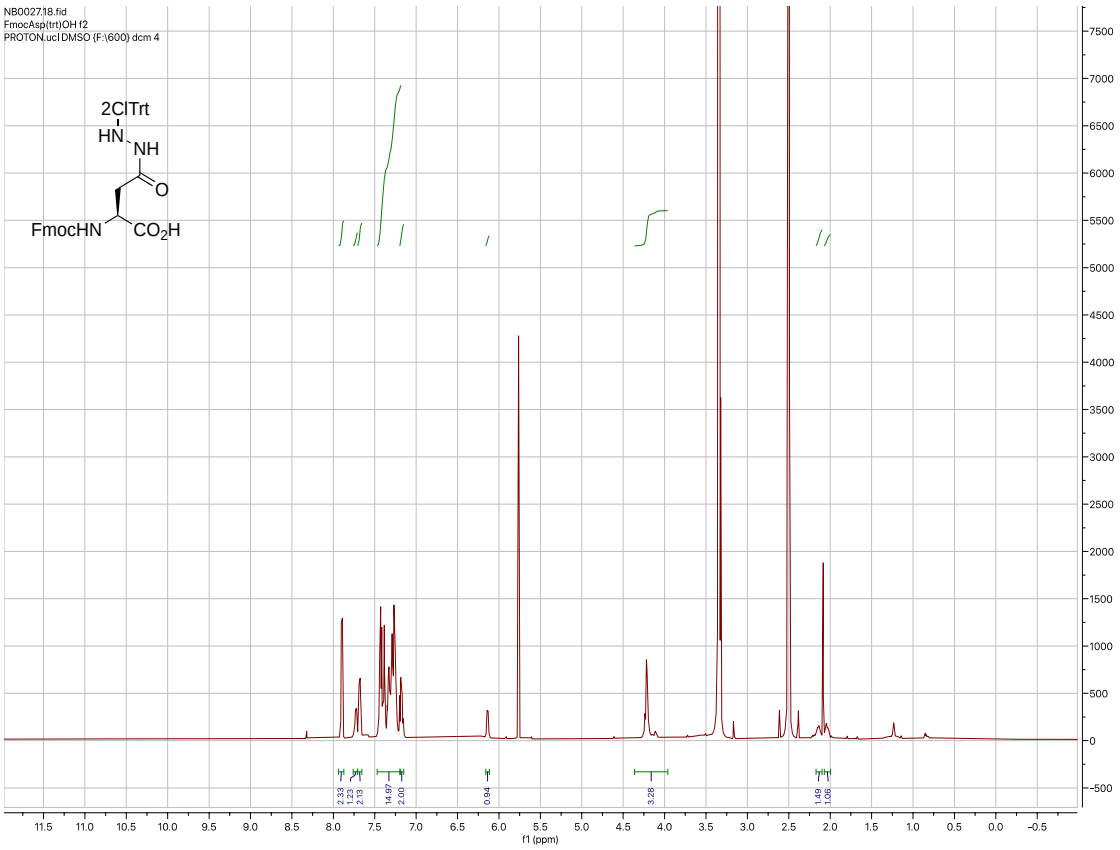
N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4-((*tert*-butoxycarbonyl)amino)asparagine (4)



N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4-((*tert*-butoxycarbonyl)amino)asparagine (5)



N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4-(((2-chlorophenyl)diphenylmethyl)amino) asparagine (6)



References

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(2) J. Lu, X.B. Tian, and W Huang, *Chinese Chem. Lett.* 2015, ***26*** (8), 946–950.

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