SUPPORTING INFORMATION FOR "SOLID PHASE SYNTHESIS OF THIOETHER-LINKED GLYCOPEPTIDE MIMICS FOR APPLICATION TO GLYCOPROTEIN SEMI-SYNTHESIS" by D. Macmillan et al.

General

Peptide synthesis was carried out using pre-loaded NovaSyn-TGT resin, 4-sulfamylbutylryl-AM resin and Fmoc amino acids from Novabiochem. Mass spectra were obtained on a Micromass Platform II electrospray LC-MS. LC-MS was performed using a Phenomenex Luna C18 LC-MS column (2×50 mm) and a gradient of 10-90% acetonitrile containing 0.1% TFA over 20 minutes (flow rate of 0.2 mL/min). Semi-preparative HPLC was performed using a Phenomenex Sphereclone C18 column (10mm×250mm) and a gradient of 10-90% acetonitrile containing 0.1% TFA over 50 minutes (flow rate of 3.0 mL/min). All other chemical reagents were obtained form Aldrich. ^{1}H-NMR spectra were acquired on Brucker WP 250 SY and WP 360 SY spectrometers. ^{13}C NMR spectra were obtained at 62.9 MHz (250 MH spectrometer) and 90.6 MHz (360 MHz spectrometer).

General Solid-phase peptide synthesis

Solid-phase peptide synthesis was carried out on a 0.1 mmol scale. Manual solid-phase peptide synthesis was conducted using 0.5mmol of each Fmoc amino acid and HBTU/HOBt as coupling reagents. On average the coupling time was 4 h and the reaction progress was monitored using LC-MS and the Kaiser ninhydrin test.

General StBu deprotection.

DTT (100 mg) was dissolved in dry DMF (0.9 ml) and solid ammonium carbonate was added. After stirring for 5 minutes the supernatant was decanted and transferred to a peptide synthesis vessel containing resin-bound StBu protected peptide. (NOTE. The ammonium carbonate could be replaced by 2.5 % v/v diisopropylethylamine). After 16 h the resin was filtered and washed exhaustively with DMF then DCM. The deprotection can be repeated if necessary.

General iodoacetamide couplings.

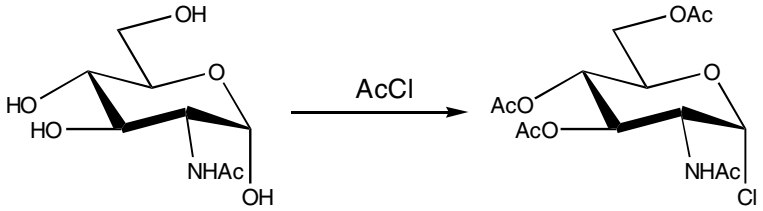
Iodoacetamides (3. equivalents) were dissolved in 2.5 % v/v pyridine in DMF (1.0 ml) and transferred to a peptide synthesis vessel containing resin-bound StBu deprotected peptide (40-50 mg). The reaction was allowed to proceed from 1-3 h in the absence of light. After this time, the resin was filtered and washed exhaustively with DMF then DCM.

General TFA cleavage and purification.

The "thio" CD52 glycopeptide mimics prepared on pre-loaded NOVAsyn TGT resin were cleaved from the resin using 90 % TFA, 5 % ethanedithiol, 5 % water for 3 h. After this time, the resin was filtered off and the filtrate was poured into ether (10 volumes). The precipitated peptide was then collected by centrifugation (3000 rpm, 15 mins). The precipitate was re-suspended in ether (5 volumes) and collected by centrifugation once again (3000 rpm, 15 mins). The crude glycopeptide mimics were dissolved in 30 % MeCN/water and loaded directly onto a semi preparative HPLC column (250 mm × 10 mm) using a gradient of 5 % to 95 % acetonitrile (containing 0.1 % TFA) over 50 minutes. Fractions containing the glycopeptide products were identified by mass spectrometry and lyophilised to obtain the purified products as fluffy white solids.

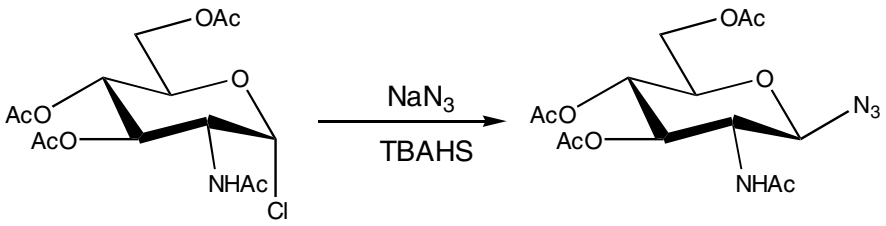
Synthesis of saccharide iodoacetamides : Iodoacetamides **1** and **3**

2-Acetamido-2-Deoxy-3, 4, 6-Tri-*O*-Acetyl-α-D-Glucopyranosyl Chloride.



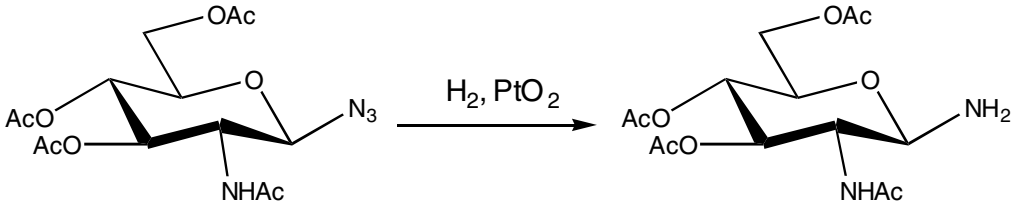
N-Acetyl glucosamine (5.00g; 22.6mmol) was added to stirring acetyl chloride (10.0cm^3) and the resulting suspension was stirred magnetically for 16h. Chloroform (40.0cm^3) was then added to the amber solution and the resulting solution was poured into ice (40.0g) and water (10.0cm^3) with stirring. The organic layer was immediately separated and run into saturated aqueous $NaHCO_3$ (40.0cm^3) and ice with stirring, the neutralisation being completed in a separating funnel. The organic layer was then separated and dried for 10mins. The organic layer was then filtered with suction and the residue washed thoroughly with dichloromethane. The resulting orange solution was concentrated to approximately 10cm^3 under vacuum. Anhydrous ether (50.0cm^3) was then added and the product crystallised out immediately. The product was stoppered and left to stand at room temperature for 16h. The pale yellow solid was then filtered with suction, washed with anhydrous ether (2× 15.0cm^3) and allowed to dry for 5 minutes to afford the crude product which was subsequently purified by flash chromatography over silica (100% ethylacetate) to afford the product (4.17g; 50%) as a colourless crystalline solid, mpt. 124-125°C (lit. 127-128°C), υ_{max} 3239(NH), 1742 and 1642(CO) cm^{-1}, δ_H (250MHz; $CDCl_3$) 6.17(1H, d, J=3.8Hz, H-1), 5.86(1H, d, J=8.7Hz, NH), 5.31(1H, dd, J=9.8Hz, H-3), 5.19(1H, dd, J=9.6Hz, H-4), 4.57-4.46(1H, m, H-2), 4.31-4.06(3H, m, H-6_b, H-6_a and H-5), 2.09(3H, s, $COCH_3$), 2.04(6H, s, 2×$COCH_3$) and 1.97(3H, s, $COCH_3$), Found: m/z (FAB-MS), 366(M^+, 90%), 330(M^+-Cl, 100%), $C_{14}H_{20}NO_8Cl$ requires m/z, 366 (M^+).

2-Acetamido-2-Deoxy-3, 4, 6-Tri-*O*-Acetyl-β-D-Glucopyranosyl Azide.



To a solution of the glucosyl chloride (1.00g; 2.7mmol), TBAHS (0.92g; 2.7mmol) and sodium azide (0.53g; 8.1 mmol) in dichloromethane (10.0cm^3) was added saturated aqueous $NaHCO_3$ (10.0cm^3). The resulting biphasic solution was stirred vigorously at room temperature for 1h. Ethyl acetate (100.0cm^3) was then added and the organic layer was separated and washed with saturated aqueous $NaHCO_3$ (1×20.0cm^3), water (2×20.0cm^3) and saturated aqueous NaCl (1×10.0cm^3). The organic phase was then dried over Na_2SO_4 and the solvent removed under vacuum to afford the pure (by TLC) azide (0.91g ; 91%) as a white solid mpt. 158-161°C (lit. 166-167°C), υ_{max} 3622 (NH), 2118 (N_3), 1747 and 1642 (CO) cm^{-1}, δ_H (250MHz; $CDCl_3$) 5.84(1H, d, J=8.9Hz , NH), 5.24(1H, dd, J=9.7Hz, H-3), 5.08(1H, dd, J=9.7Hz, H-4), 4.76(1H, d, J=9.3Hz, H-1), 4.26(1H, dd,$J_{H6a-H6b}$ =12.5Hz J_{H6b-H5} =4.8Hz, H-6_b), 4.14(1H, dd, J_{H6a-H5} =2.4Hz, H-6_a), 3.90(1H, m, H-2), 3.82-3.75(1H, m, H-5) and 2.09, 2.02, 2.01, and 1.96(12H, 4s, $COCH_3$)ppm, Found: m/z (FAB-MS), 373 (MH^+), $C_{14}H_{20}N_4O_8$ requires m/z, 372.3 (M^+).

2-Acetamido-2-Deoxy-3,4,6-Tri-*O*-Acetyl-β-D-Glucopyranosyl Amine.

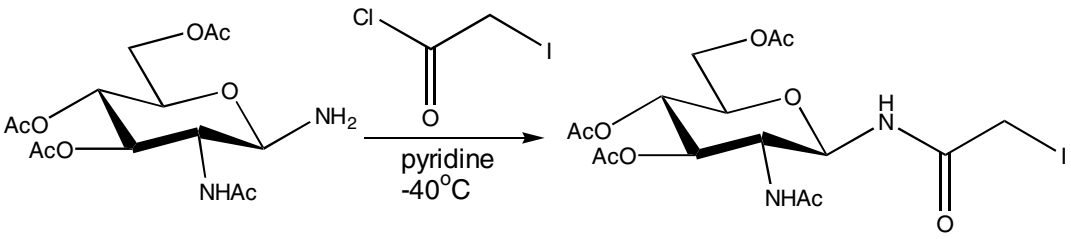


A solution of the glycosyl azide (1.50g; 4.0mmol) in anhydrous THF (27.0cm^3) was catalytically hydrogenated at atmospheric pressure with PtO_2 for 1h. The catalyst was then removed by filtration through Celite and the filtrate was evaporated to dryness under vacuum to afford the pure (by TLC) amine (1.03g ; 74%) as a pale grey solid mpt.=159°C , υ_{max} 1744 and 1682 (CO) cm^{-1} ,δ_H (250MHz; $CDCl_3$) 5.72(1H, d, J=8.9Hz ,NH), 5.10-5.00(2H, m ,H-3 and H-4), 4.20(1H, dd, $J_{H6a-H6b}$ =12.3Hz J_{H6b-H5} = 4.8Hz, H-6_b), 4.13-3.97(2H, m, H-6_a and H-2), 3.99(1H, d, J=9.5, H-1) 3.66-3.60(1H, m, H-5) and 2.08, 2.02, 2.01, and 1.96(12H, 4s, 4×$COCH_3$), Found: m/z (FAB-MS), 347(MH^+), $C_{14}H_{22}N_2O_8$ requires m/z, 346.3.

2-Iodo-Acetyl Chloride.

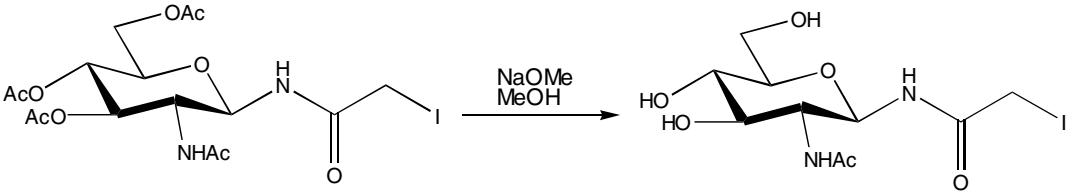
Thionyl chloride (0.1cm^3; 1.30mmol) was added dropwise over 5 mins. with stirring to iodoacetic acid (200mg; 1.08mmol) and the resulting pink suspension was stirred at room temperature with the exclusion of atmospheric moisture and light. After 24h the excess thionyl chloride was removed under vacuum to afford the acid chloride (214 mg, 97.2%) as a pink oil. δ_H (250MHz; $CDCl_3$) 4.23(s, CH_2I), δ_C ($CDCl_3$) 167.12(COCl) and 4.12(CH_2I), Found: m/z (EI-MS), 203.9 (M^+), C_2H_2OClI requires m/z, 203.8 (M^+).

2-Acetamido-2-Deoxy-3,4,6-Tri-*O*-Acetyl-1-*N*-[1-(2-Iodo)Acetyl]-β-D-Glucopyranose (3).



A solution of the protected glucopyranosylamine (187mg; 0.54mmol) in anhydrous DCM (4.0cm^3) was treated with pyridine (0.044cm^3; 0.54mmol) and cooled to -40°C with the exclusion of light under Nitrogen. This solution was then treated dropwise with stirring with 2-iodo-acetyl-chloride (110mg; 0.54mmol) and the reaction mixture was stirred at -40°C. After 0.5h the reaction was quenched with chloroform (40.0cm^3) and the resulting yellow solution was washed with saturated aqueous $NaHCO_3$ (2×15.0cm^3) water (1×15.0cm^3). The organic phase was then dried, filtered, concentrated under vacuum to approx. 2cm^3 and then co-evaporated with toluene (2×5.0cm^3) to afford the iodoacetamide (264mg; 95%) as a yellow solid mpt.=200-202°C(decomp.), υ_{max} 1745 and 1684(CO) cm^{-1} , δ_H (250MHz; $CDCl_3$) 7.65(1H , d, J=8.1Hz, NH), 6.57(1H, d, J=8.4Hz, NH), 5.15-4.98(3H, m, H-1, H-3 and H-4), 4.29(1H, dd, $J_{H6b-H6a}$=12.5Hz, J_{H6b-H5}=4.3Hz, H-6_b), 4.22-4.12(1H, m, H-2), 4.08(1H, dd, J_{6b-5} =2.1Hz, H-6_a), 3.79-3.73(1H, m, H-5), 3.62(2H, s, CH_2I) and 2.07, 2.06, 2.03 and 1.99(12H, 4×s, 4×$COCH_3$), Found: 36.5%C;4.3%H;5.2%N, m/z (FAB-MS), 515(MH^+, 63%), 389(M-I, 41%) $C_{16}H_{23}N_2O_9I$ requires 37.4%C;4.5%H;5.5%N, m/z, 515 (MH^+).

2-Acetamido-2-Deoxy-1-*N*-[1-(2-Iodo)Acetyl]-β-D-Glucopyranose (1).



The iodoacatamide (65.0mg; 0.13mmol) as a solution in anhydrous methanol (10.0cm^3) was treated with a solution of sodium methoxide in methanol (0.5M; 0.078cm^3) under nitrogen with the exclusion of light and the resulting solution was stirred at room temperature for 3h. The solution was then treated

with a solution of NH_4Cl (0.5M; 0.078cm^3) and the solvent was removed under vacuum. The residue was dissolved in water (14.0cm^3), extracted with ethyl acetate (2×3.5cm^3) and the aqueous layer was evaporated to dryness to afford the pure (by TLC) deprotected product (56.4mg; 100%) as an orange solid. R_f=0.68 ($CHCl_3$: MeOH:H_2O, 12:12:3), δ_H (250MHz; D_2O) 5.33(1H , d, J=9.7Hz ,H-1), 3.82(1H, dd, $J_{H6a-H6b}$=12.3Hz J_{H6b-H5}=2.1Hz, H-6_b), 3.76(1H, dd, $J_{2,3}$ =9.8Hz, H-2), 3.69(1H, dd, J_{6b-5} =4.8Hz, H-6_a), 3.67(2H, q, J_{AB} =10.0Hz, CH_2I), 3.55(1H, dd, $J_{3,4}$ =9.5Hz, H-3), 3.45(1H, m, H-5), 3.42(1H, dd, $J_{4,5}$ =10.1Hz, H-4) and 1.98(3H, s, $COCH_3$), δ_C (D_2O) 174.7 and 173.1 (CO), 78.6(C(1)H), 77.7 (C(5)H), 74.1(C(3)H), 69.4(C(4)H), 60.4 (C(6)H_2), 54.3 (C(2)H), 22.4($CO\underline{C}H_3$) and -3.6(CH_2I), Found: m/z (FAB-MS), 389.019 (MH^+), $C_{10}H_{17}N_2O_6I$ requires m/z, 389.020 (MH^+).

General synthesis Of Glycosyl (glucosyl and lactosyl starting from glucose and lactose) Bromoacetamides (Prepared as for iodoacetamide above but using the commercially available 2-bromoacetyl bromide).

A solution of the protected glycopyranosylamines in anhydrous DCM were treated with pyridine and cooled to -40°C with the exclusion of light under nitrogen. This solution was then treated dropwise with stirring with 2-bromoacetyl bromide and the reaction mixture was stirred at -40°C. After 0.5h the reaction was quenched with chloroform and the resulting yellow solution was washed with 2M HCl, saturated aqueous $NaHCO_3$, and water. The organic phase was then dried, filtered, concentrated under to afford the bromoacetamides as pale yellow solids.

Selected NMR data for synthetic bromoacetamides 2,4 and 5.

glucosyl bormoacetamide (2)
^{1}H NMR (D_2O) δ 4.96 (d, *J*=9.0 Hz, 1H, H_1), 3.96 (s, 2H, CH_2Br), 3.86 (dd, *J*=2.1 and 12.4 Hz, 1H, H_{6a}), 3.71 (dd, *J*=5.2 and 12.4 Hz, 1H, H_{6b}), 3.57-3.49 (m, 2H), 3.44-3.40 (m, 2H). ^{13}C NMR (D_2O) δ 171.5, 80.0, 78.0, 76.7, 72.1, 69.5, 60.8, 28.1 ppm.
$C_8H_{14}BrNO_6$ requires m/z = 300.10, found m/z (ESI-MS) = 321.8, 323.8 (MNa^+)

Peracetylated lactosyl bromoacetamide (per-acetylated 5)
^{1}H NMR ($CDCl_3$) δ 7.10 (d, *J*=9.0 Hz, 1H, NH), 5.37-5.30 (m, 2H, H_3 H'$_3$), 5.1-5.09 (m, 2H, H_1 H'$_1$), 4.98-4.90 (m, 2H, H_2 H'$_2$), 4.49 (d, *J*=7.9 Hz, 1H, H_{6a}), 4.44-4.43 (d,), 4.18-4.06 (m, 3H), 3.89-3.77 (m, 3H), 3.83 (, 2H, CH_2Br), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.98 (s, 3H, OAc). ^{13}C NMR ($CDCl_3$) δ 170.9, 170.2, 170.0, 169.2, 168.8, 166.2, 100.7, 78.4, 75.7, 74.5, 72.0, 70.8, 70.6, 70.3, 68.8, 66.4, 61.7, 60.7, 28.1, 20.7, 20.6, 20.5, 20.4 ppm.
$C_{28}H_{38}BrNO_{18}$ requires m/z = 756.50, found m/z (ESI-MS) = 777.7, 779.8 (MNa^+)

Lactosyl bromoacetamide (5)
^{1}H NMR (D_2O) δ 4.99 (d, *J*=9.2 Hz, 1H), 4.45 (d, *J*=7.6 Hz, 1H), 3.97 (s, 2H, CH_2Br), 3.90-3.85 (m, 2H), 3.82-3.6 (m, 7H), 3.59-3.42 (m, 1 or 2H). ^{13}C NMR (D_2O) δ 171.5, 103.2, 79.9, 78.0, 76.9, 75.7, 75.4, 72.8, 71.8, 71.3, 68.9, 61.4, 60.2, 28.2 ppm.
$C_{14}H_{24}BrNO_{11}$ requires m/z = 462.24, found m/z (ESI-MS) = 485.6, 487.6 (MNa^+).

GlcNAc bromoacetamide (1-bromoacetamide)
^{1}H NMR (D_2O) δ 4.97 (d, *J*=9.6 Hz, 1H), 3.85-3.72 (m, 2H), 3.76 (s, 2H, CH_2Br), 3.70-3.68 (m, 1H), 3.67-3.60 (m, 1H), 3.57-3.47 (m, 1H), 3.43-3.36 (m, 1H), 1.90 (s, 3H, OAc). ^{13}C NMR (D_2O) δ 175.2, 171.1, 79.1, 78.1, 74.3, 69.8, 60.8, 54.7, 27.8, 22.4 ppm.
$C_{10}H_{17}BrN_2O_6$ requires m/z = 341.16, found m/z (ESI-MS) = 365.2, 367.2 (MNa^+)

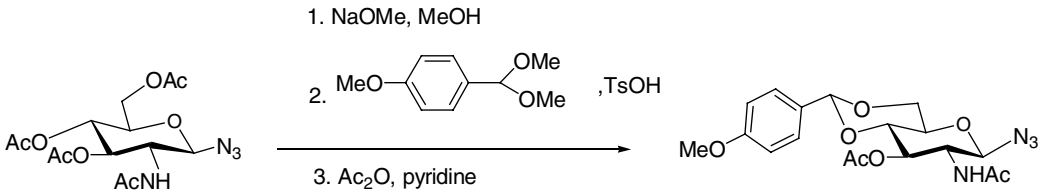
Chitobiose Bomoacetamide (4)

^{1}H NMR (D_2O) δ 7.43 (1H, NH), 7.08(1H, NH), 5.08(1H, d, J=9.7 Hz, H-1), 4.61((1H, d, J=8.4 Hz, H-1'), 3.94-3.83 (5H, m, 3H and C<u>H</u>$_2$Br), 3.78-3.75 (3H, m), 3.70-3.63 (2H, m), 3.61-3.55 (2H, m), 3.51-3.47 (3H, m), 2.06(3H, s, NHAc), 2.01(3H, s, NHAc) ppm.
$C_{18}H_{30}BrN_3O_{11}$ requires m/z = 544.35, found m/z (ESI-MS) = 566.2, 568.2 (MNa^+)

<u>Synthesis of chitobiosyl β-azide required for the synthesis of chitobiosyl bromoacetamide (4).</u>

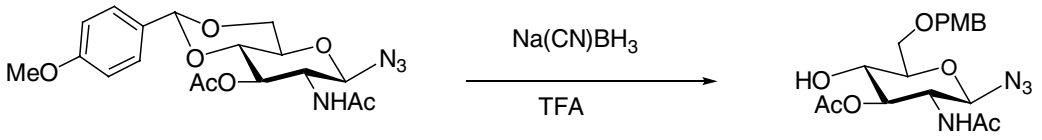
(After the β-azide was synthesized, the bromoacetamide was prepared as for all other compounds described above)

2-Acetamido-2-Deoxy-4,6-O-*p*-Methoxybenzylidine-β-D-Glucopyranosyl Azide.



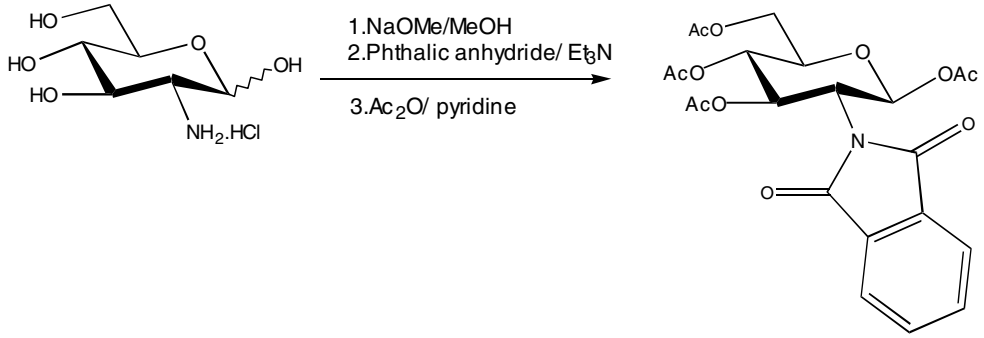
The azide (3.00g, 8.07mmol) was dissolved in dry methanol (15.0 ml) and sodium methoxide (200 µl of a 0.5 M solution in methanol) was added and the reaction mixture was stirred for 2 h at room temp. The reaction mixture was then neutralized by the addition of acetic acid (10 µl) and concentrated under reduced pressure to afford the crude deacetylated product as a pale yellow foam. The crude azide was dissolved in anhydrous DMF (10.0 ml) and *p*-anisaldehyde dimethylacetal (3.37 g, 18.54 mmol) and *p*-Tosic acid (0.28 g, 1.61 mmol) were added. After stirring for 1.5 h at 50 °C, the reaction mixture was concentrated under vacuum. The residue was poured into a cold mixture of sat. aq. sodium bicarbonate (30.0 ml) and dichloromethane (30.0 ml) and cooled for 10 min at 4°C. The precipitate was filtered off and washed with water and dichloromethane. The remaining precipitate was crystallised from ethyl acetate (30.0 ml). The product was filtered, dried under vacuum and isolated as a white solid. The resulting acetal (2.95 g, 8.07 mmol) was dissolved in pyridine (20.0 ml) and acetic anhydride (10.0 ml) was added. The mixture was stirred for 24 h at room temp. then concentrated under vacuum. Dichloromethane (150 ml) was then added and the organic phase was washed with water (1×20.0 ml), sat. aq. sodium hydrogencarbonate (1×20.0 ml), and again with water(1×20.0 ml). The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum to afford the crude product as a white solid which was crystallised from ethyl acetate (1.19 g, 70 % over three steps). R_f=0.3 (100% EtOAc), δ_H (250MHz; $CDCl_3$/ CD_3OD) 7.24(2H, d, J=8.9Hz, 2×ArH), 6.77(2H, d, J=8.9Hz, 2×ArH), 5.37(1H, s, ArC<u>H</u>), 5.08(1H, dd, J_{34}=9.3Hz, H3), 4.55(1H, d, J_{12}=9.3Hz, H1), 4.21(1H, dd, $J_{H6a\text{-}H6b}$=10.4, $J_{H6b\text{-}H5}$=4.8Hz, $H6_b$), 3.92(1H, dd, J_{23}=10.2Hz, H2), 3.69(1H, dd, H6a), 3.68(3H, s, OCH_3), 3.60(1H, dd, J_{45}=9.3Hz, H4), 3.50(1H, m, H5) and 1.95 and 1.85(6H, 2×$COCH_3$) ppm. δ_C, 171.4, 170.9, 159.8 and 128.8(qC), 127.1(2×ArCH), 113.2(2×ArCH), 101.2(CH), 88.8(CH), 78.1(CH), 71.4(CH), 68.1(CH), 67.9(CH_2), 54.9(O<u>C</u>H_3), 53.3(CH) and 22.2 and 20.3(CO<u>C</u>H_3)ppm. $C_{18}H_{22}N_4O_7$ requires m/z = 407.4, found m/z (ESI-MS) = 407(MH^+).

2-Acetamido-2-Deoxy-3-O-Acetyl-6-*p*-Methoxybenzyl-β-D-Glucopyranosyl Azide.



Trifluoroacetic acid (4.19 g, 36.8 mmol) in dry DMF (22.1 ml) cooled to 0°C, was added dropwise to a mixture of the *p*-methoxybenzylidene acetal (1.5 g, 3.68 mmol), sodium cyanoborohydide (1.16 g, 18.40 mmol) and 3Å molecular sieves in dry DMF (29.4 ml) at 0°C in an ice bath. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at room temperature for 16 h. Next, the reaction was filtered with suction. The filtrate was poured into ice-cold sat. aq. sodium hydrogencarbonate and the aqueous phase was extracted dichloromethane (5×60.0 ml). The combined organic extracts were washed with sat. aq. sodium hydrogencarbonate (80.0 ml) and dried over sodium sulfate. The drying agent was then filtered of and the solvent removed under reduced pressure. The crude product was purified by flash chromatography over silica (100% ethylacetate) and was isolated as a white solid (1.0 g, 66.00 % yield). R_f [100% EtOAc]: 0.2; δ_H (250MHz; $CDCl_3$) 7.28(2H, d, J=8.7Hz, 2×ArH), 6.91(2H, d, J=8.7Hz, 2×ArH), 6.02(1H, d, J=9.2Hz, NH), 5.08(1H, dd, H-3), 4.59(1H, d, J=9.3Hz, H-1), 4.54(2H, q, O$\underline{C}H_2$Ar), 4.09-3.95(1H, m, H-2), 3.83(3H, s, OCH_3), 3.83-3.74(3H, m, H-4 and 2×H6), 3.83-3.74(1H, m, H5), 3.31(1H, d, J=4.1 Hz, OH) 2.12 and 1.99(6H, 2×s, 2×$COCH_3$), δ_C 171.9, 170.9, 159.2 (3×qC), 129.3(2×ArCH), 113.7(2×ArCH), 88.3 (CH), 76.8(CH), 75.1(CH), 73.3($\underline{C}H_2$-Ar), 69.0(CH), 69.0($\underline{C}H_2$), 55.1(OCH_3), 53.1(CH), 22.7 and 20.7 (2×C, 2×OC-$\underline{C}H_3$); Found: m/z(ESI): 431 $[M+Na]^+$, $C_{18}H_{24}N_4O_7$ requires m/z, 408.4(M^+).

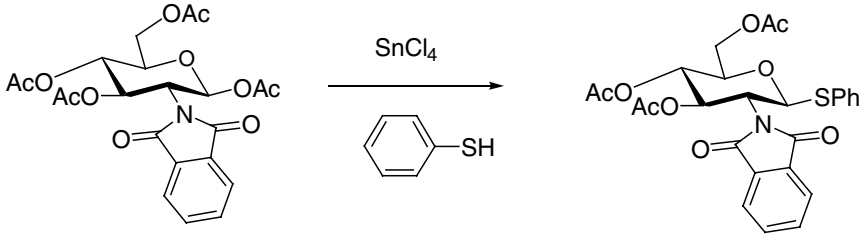
2-Phthalamido-2-Deoxy-1,3,4,6-Tetra-*O*-Acetyl-β-D-Glucopyranose.



To a stirred solution of sodium methoxide (3.0g; 46.3mmol) in anyhdrous methanol (75.0cm^3) was added glucosamine hydrochloride (10.0g; 46.3mmol). The reaction mixture was stirred for 10min at room temperature and then filtered with suction. Phthalic anhydride (3.5g; 23mmol) was then added to the filtrate and stirring was continued for a further 20mins. A further portion of phthalic anhydride (3.5g; 23mmol) was then added followed by triethylamine (7.6ml; 55.6mmol). The reaction mixture was stirred at room temperature for 10 mins and then at 50°C for 0.5h. The resulting mixture

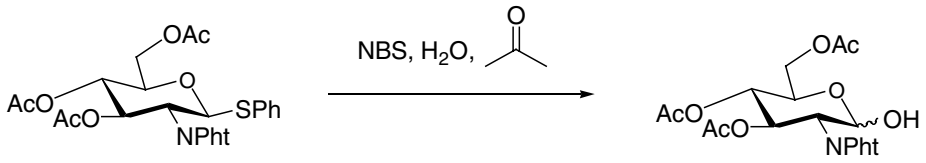
(containing a thick white precipitate) was cooled for 1h in an ice bath and then filtered with suction. The precipitate was washed with cold methanol (2×20.0cm^3) and dried under high vacuum. The dry white solid was then suspended in acetic anhydride (44.5cm^3), cooled to ice bath temperature and then pyridine (22.7cm^3) was added carefully with stirring. The reaction was then stirred at room temperature for 16h. After 16h the reaction mixture was poured into ice/water (200cm^3) and extracted with chloroform (3×200cm^3). The combined organic extracts were washed with 5% HCl (1×120cm^3), saturated $NaHCO_3$ (1×120cm^3), water (1×120cm^3) and brine (1×100cm^3). The organic phase was then dried (Na_2SO_4), filtered and the solvent was removed under vacuum to afford the crude product as an orange oil which was purified by flash column chromatography over silica (hexane/Ethyl acetate 1:1) to afford the product (8.65g, 40%) as a white foam. δ_H (250MHz; $CDCl_3$) 7.86-7.75(4H, m, 4×ArH), 6.50(1H, d, $J_{1,2}$=10.0Hz, H-1), 5.86(1H, dd, $J_{3,4}$=9.5Hz, H-3), 5.20(1H, dd, $J_{4,5}$=9.9Hz, H-4), 4.45(1H, dd, $J_{2,3}$=10Hz, H-2), 4.37(1H, dd, $J_{H6b\text{-}H6a}$=11.4Hz, $J_{H6b\text{-}H5}$=4.1Hz, H-6_b), 4.12(1H, dd, $J_{H6a\text{-}H5}$=2.9Hz, H-6_a), 4.03(1H, ddd, H-5), 2.11, 2.05, 2.00 and 1.87(12H, 4×s, 4×COCH$_3$), δ_C($CDCl_3$), 170.5, 170.0, 169.3, 168.5 and 167.2 (qC), 134.4(2×ArCH), 131.1 (qC), 123.7(2×ArCH), 89.6(CH), 72.5(CH), 70.4(CH), 68.1 (CH), 61.4 (CH_2), 53.3(CH), 20.6(2×OCH$_3$), 20.4(OCH$_3$) and 20.3(COCH$_3$)ppm.$C_{22}H_{23}NO_{11}$ requires (m/z) 477.42, found m/z (ESI) = 500.3(MNa^+).

2-Phthalamido-2-Deoxy-3,4,6-Tri-*O*-Acetyl-1-Thiophenyl-β-D-Glucopyranoside.



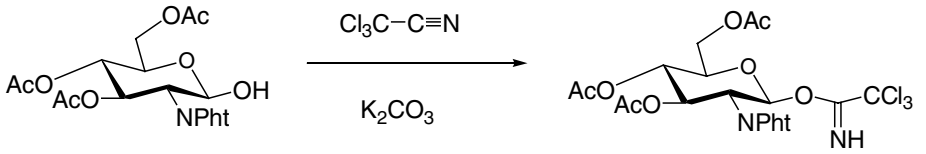
A solution of the acetylated N-phthalamido sugar (5.0 g, 11.84 mmol) and tin tetrachloride (4.26 ml of a 1M solution in DCM, 4.26 mmol) in dry DCM (75.0 ml) under nitrogen were treated with thiophenol (3.06 g, 27.82 mmol). The reaction mixture was stirred at room temp. for 16 h and then diluted with DCM (100 ml). This solution was washed with water (1×100 ml), sat. aq. sodium bicarbonate (1×100 ml) and again with water(1×100 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed under vacuum to afford the crude product, which was purified by flash chromatography over silica (ethylacetate/ pet. ether, 1:1) and was isolated as a white solid (78.9% yield). ^{1}H NMR ($CDCl_3$, 250 MHz), δ = 7.87-7.72(4H, m, ArH), 7.42-7.21(5H, m, ArH), 5.78(1H, dd, J_{34} 9.1 Hz, H-3), 5.69(1H, d, J_{12}=10.6 Hz, H-1), 5.13(1H, dd, J_{45}=10.2 Hz, H-4), 4.34(1H, dd, J_{23}=10.5, H-2), 4.26-4.15(2H, m, H-6_B, H-6_A), 3.93-3.85(1H, m, H-5), 2.09, 2.01 and 1.82(9H, 3×s, 3×COCH$_3$); δc(62 MHz) 170.5, 170.0, 169.3, 167.7, 166.8(5×qC), 134.3(2×ArCH), 123.6(2×ArCH), 134.3(ArCH), 133.1(2×ArCH), 130.8(qC), 128.7(2×ArCH), 128.3(ArCH), 123.6(2×ArCH)(5C, ArH), 82.9(CH), 75.8(CH), 71.5(CH), 68.6(CH), 62.1(CH$_2$), 53.4(CH), 20.6, 20.5 and 20.3 (3×CH$_3$) ppm; $C_{26}H_{25}NO_9S$ requires 527.54, found m/z (ESI): 551 [MNa^+].

2-Phthalamido-2-Deoxy-3,4,6-Tri-*O*-Acetyl-β-D-Glucopyranose.



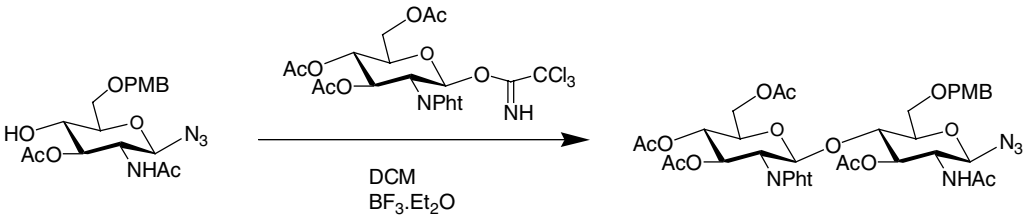
The thiophenyl glycoside (4.5 g, 8.54 mmol) and N-bromosuccinamide (4.5 g, 25.62 mmol) were dissolved in acetone (40.5ml) and water (4.5 ml) was added. The mixture was stirred for 0.5 h at room temp. after which time further N-bromosuccinamide (4.5 g, 25.62 mmol) was added. After a further 0.5 h, the reaction mixture was diluted with dichloromethane (180 ml) and washed with water (100 ml), sat. aq. sodium thiosulfate (100 ml) and again with water (100 ml). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure to afford the crude product as a white solid, which was recrystallized from diethylether to afford the hemi-acetal as a white solid (90.3 % yield). $C_{20}H_{21}NO_{10}$ requires m/z =435.38, found m/z (ESI) = 458 $[M+Na]^+$

O-(2-Phthalamido-2-Deoxy-3,4,6-Tri-O-Acetyl-β-D-Glucopyranosyl)- Trichloroacetimidate.



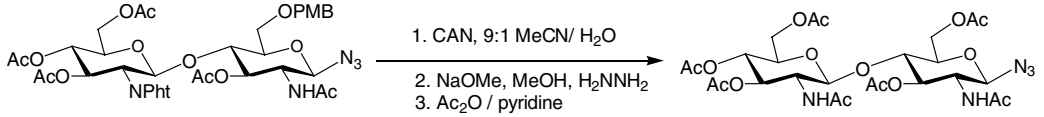
To a solution of the hemi-acetal (667 mg, 1.26 mmol) and trichloroacetonitrile (1.2 ml, 12.39 mmol) in dry dichloromethane containing molecular sieves (3 Å) was added anhydrous potassium carbonate (0.49 g, 3.99 mmol). The reaction mixture was then stirred under nitrogen at room temp. After 18 h, the reaction was filtered with suction and the solvent was removed under reduced pressure. The product was purified by flash chromatography over silica (ethylacetate/ pet. ether, 1:1) and was isolated as a white solid (57% yield). R_f [EtOAc/pet. Ether (1:1)]=0.37; δ_H (250MHz; $CDCl_3$) 7.84-7.69(4H, m, 4×Ar<u>H</u>), 6.60(1H, d, $J_{1,2}$=8.9Hz, H-1), 5.90(1H, dd, $J_{3,4}$=9.1Hz, H-3), 5.27(1H, dd, $J_{4,5}$=10.2Hz, H-4), 4.62(1H, dd, $J_{2,3}$=10.7Hz, H-2), 4.38(1H, dd, $J_{H6b\text{-}H6a}$=12.5Hz, $J_{H6b\text{-}H5}$=4.3Hz, H-6_b), 4.18(1H, dd, $J_{H6a\text{-}H5}$=2.2Hz, H-6_a), 4.05(1H, ddd, H-5), 2.11, 2.03 and 1.87(9H, 3×s, 3×COC<u>H</u>$_3$). δ_C, 170.6, 170.0, 169.3, 167.3 and 160.4 (qC), 134.3(2×ArCH), 131.1 (qC), 123.6(2×ArCH), 93.4(CH), 72.7(CH), 70.3(CH), 68.3 (CH), 61.4 (CH_2), 53.4(CH), 20.6(OC<u>C</u>H$_3$), 20.5(OC<u>C</u>H$_3$) and 20.3(OC<u>C</u>H$_3$)ppm. $C_{22}H_{21}Cl_3N_2O_{10}$ requires m/z = 579.77, found m/z (ESI)= 601.4 $[MNa]^+$

2-*N*-phthalamido-2-deoxy-3,4,6-tri-*O*-Acetyl-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3-*O*-acetyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl azide.



A solution of the azide (159.00 mg, 0.392 mmol), the trichloroacetimidate (250.00 mg, 0.431 mmol) and 3Å molecular sieves in dry dichloromethane (10.0 ml) was cooled down to 0°C in an ice bath, under nitrogen. Borontrifluoride diethyletherate (12 µl, 0.098 mmol) was then added. The ice bath was removed and the reaction was allowed to warm to 20 °C. The reaction mixture was stirred for 4 h. After this time further borontrifluoride diethyl etherate (6 µl, 0.049 mmol) was added. The mixture was stirred for further 16 h. The reaction mixture was then diluted with dichloromethane (50.0 ml) and filtered with suction. The filtrate was then washed with sat. aq. sodium bicarbonate (1× 25.0 ml) and water (1× 25.0 ml). The organic phase was dried over sodium sulfate, the drying agent was filtered off and the solvent was removed under reduced pressure. The resulting white solid was purified by flash chromatography over silica (100% ethyl acetate) and the purified product was isolated as a white solid (110 mg, 34 %); δ_H(360MHz; $CDCl_3$) 7.78-7.64(4H, m, 4×ArH), 7.18(2H, d, J=8.8Hz, 2×ArH), 6.84(2H, d, J=8.8Hz, 2×ArH), 6.16(1H, d, J=9.5 Hz, NH), 5.68(1H, dd, H-3'), 5.37(1H, d, $J_{1,2}$=8.4Hz, H-1'), 5.06(1H, dd, H-4'), 4.96(1H, dd, H-3), 4.36-4.26(3H, m, H-1, CH_2OPMB), 4.15-3.89(3H, m, 3×CH), 3.77(3H, s, OCH_3), 3.61-3.24(4H, m, 4×CH), 2.06, 2.02, 1.97, 1.92 and 1.77(15H, 5×s, 5×$COCH_3$). δ_C, 171.0(qC), 170.3(qC), 169.9(qC), 169.3(qC), 158.9(qC), 134.3(ArCH), 130.9(qC), 129.8(qC), 129.0(2×ArCH(PMB)), 123.4(ArCH), 113.5(2×ArCH(PMB)), 96.9(CH), 88.3(CH), 76.4(CH), 73.0(CH), 72.7(CH), 72.2(OCH_2Ar), 71.5(CH), 70.3(CH), 68.1(CH), 66.6(CH_2OPMB), 61.2 (CH_2), 55.0(OCH_3), 54.7(CH), 53.0(CH), 23.0, 20.6, 20.4, 20.4 and 20.1(5×CH_3) ppm. $C_{38}H_{43}N_5O_{16}$ requires m/z = 825.77, found m/z (ESI): 848.4 $[MNa]^+$

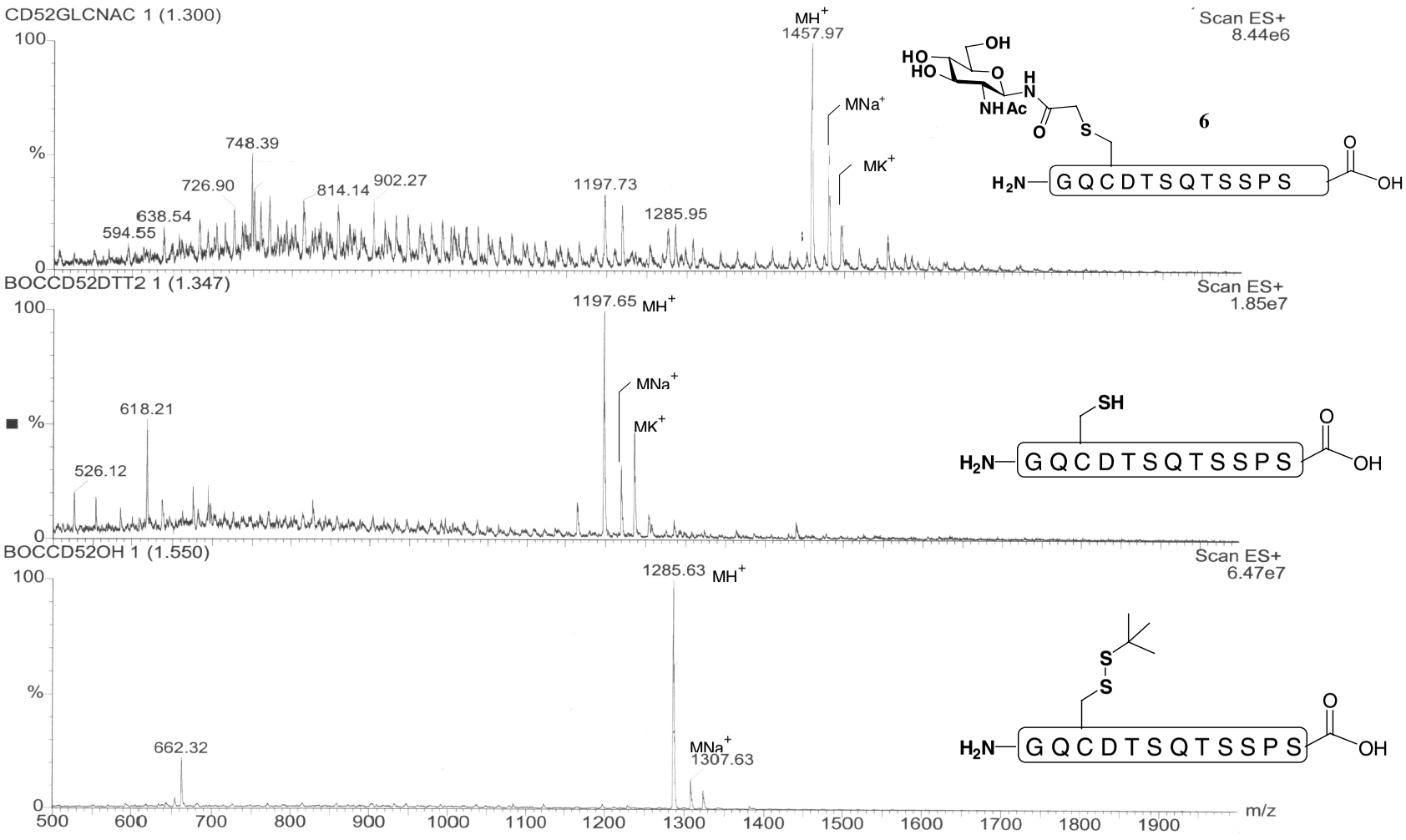
2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-3,6-di-*O*-acetyl-β-D-glucopyranosylazide.



The fully protected disaccharide (170mg, 0.207 mmol) was dissolved in 9:1 MeCN/ water (1.0 mL) and cerium ammonium nitrate (226 mg, 0.414 mmol) was added. After 0.5 h TLC indicated that the reaction was complete and the reaction was diluted with dichloromethane (25.0 ml) and washed with Sat. aq. $NaHCO_3$ (10.0 ml). The aqueous phase was extracted with further DCM (25.0 ml). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed under vacuum to afford the crude product as a pale yellow gum. The crude product was purified by flash chromatography over silica (short column, R_f =0.28, 100% ethylacetate) and the product was isolated as a white solid (129

mg, 88 %). This partially deprotected disaccharide (122 mg, 0.173 mmol) was then dissolved in anhydrous methanol (5.0 ml) and sodium methoxide (20 µl of a 0.5 M solution in methanol) was added. The reaction mixture was stirred for 1 h and after this time the reaction was neutralised with acetic acid (2 µL) and the solvent removed under reduced pressure. The residue was dissolved in ethanol (10.0 ml) and hydrazine hydrate (200 µl) was added. This suspension (containing a white precipitate) was heated under reflux for 16 h after which time the solid had dissolved. Next, the solvent was removed under reduced pressure and the residue was suspended in pyridine (5.0 ml) and acetic anhydride (2.5 ml) was added. The reaction mixture was then stirred for 16 h at room temp. The solvent was then removed under reduced pressure. The residue was dissolved in DCM (50 ml) and washed with 2 M HCl (1× 25 ml), sat. aq. sodium hydrogencarbonate (1× 25 ml) and water (1× 25 ml). Finally the solvent was removed under removed pressure and the product was purified by flash chromatography over silica (ethyl acetate/methanol 95:5) and was isolated as a white solid (107 mg, 93.8%), δ_H(360MHz; $CDCl_3$) 6.60(1H, d,(br), NH), 6.49(1H, d(br), NH), 5.28-5.03(3H, m, 3×CH), 4.66-4.59(2H, m, 2×CH), 4.49-4.38(2H, m, 2×CH), 4.35-4.29(1H, m, CH), 4.20-4.01(2H, m, 2×CH), 3.95-3.85(1H, m, CH), 3.70-3.62(3H, m, 3×CH), 2.20-1.91(14H, m, 7×CH_3)ppm. $C_{26}H_{37}N_5O_{15}$ requires m/z = 659.60, found m/z (ESI) = 660.1 (MH^+).

Crude TFA cleavage products for the chemistry depicted in manuscript figure 1 (synthesis of glycopeptide **6** using "thio CD52 and GlcNAc iodoacetamide **1**)

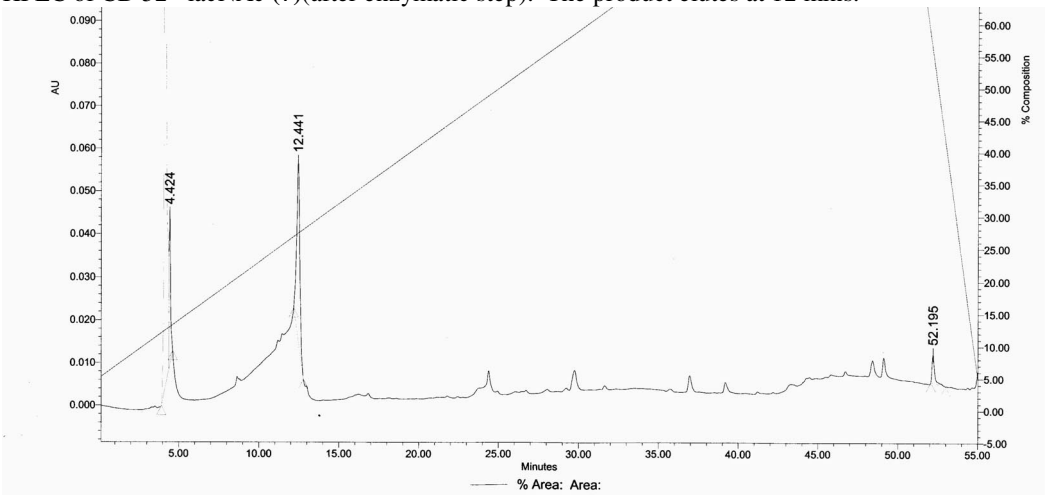


Synthesis of LacNAc (glycopeptide 7) using β1-4 Galactosyltransferase

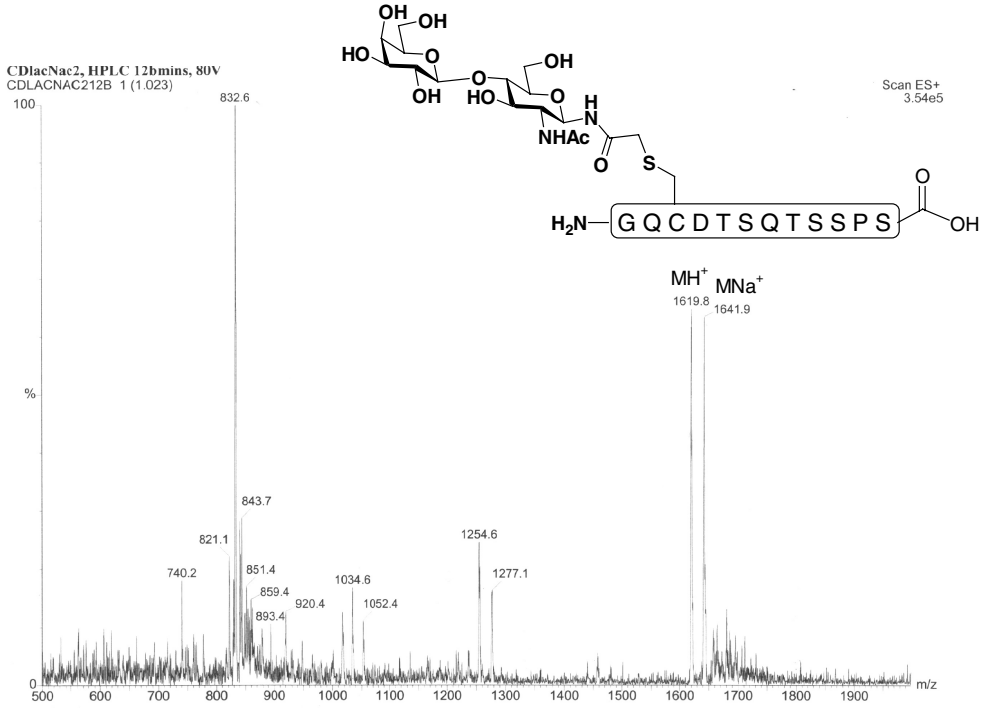
To an eppendorf tube containing UDP-Gal (0.5 mg) was added GlcNAc peptide **6** (250 µL of 2.5 mg/mL solution in 100 mM sodium cacodylate buffer pH 7.54), galactosyl transferase (19 µL, 0.038 units), $MnCl_2$ (to give final concentration of 5 mM), PMSF (7 µL of 40mM solution) and shrimp alkaline phosphatase (0.1 units). This was then incubated at 37 ˚C for 24 hours. Products were purified by HPLC with fractions collected at 11.5 and 12.0 minutes containing the desired product when analysed by mass spectroscopy.

HPLC purified glycopeptides:

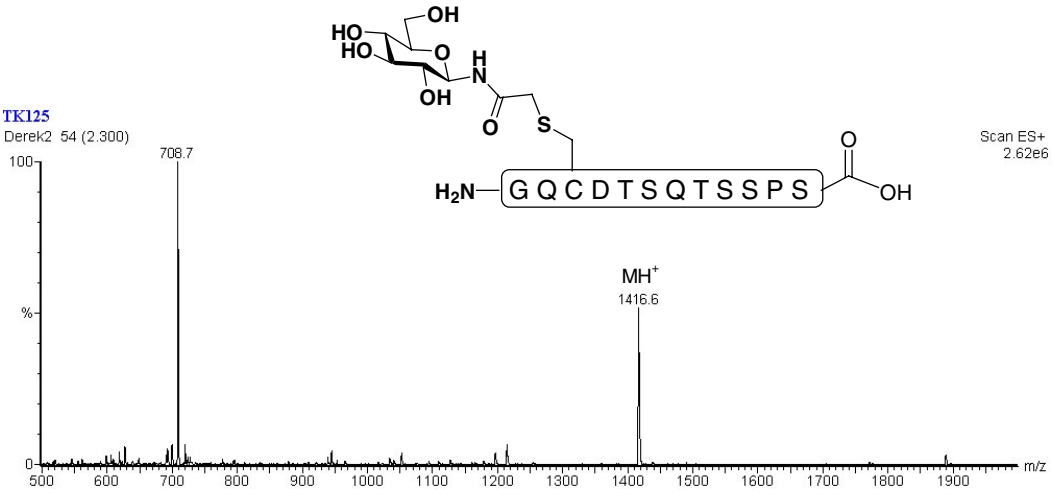
HPLC of CD 52 –lacNAc **(7)**(after enzymatic step). The product elutes at 12 mins.



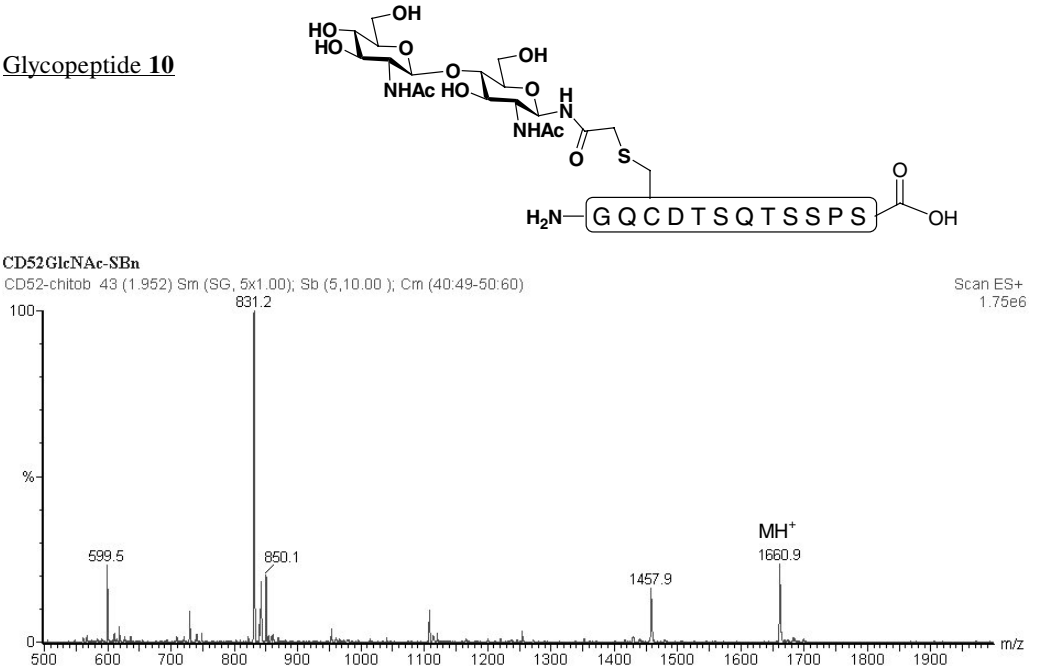
MS of Glycopeptide **7** (12 min fraction)



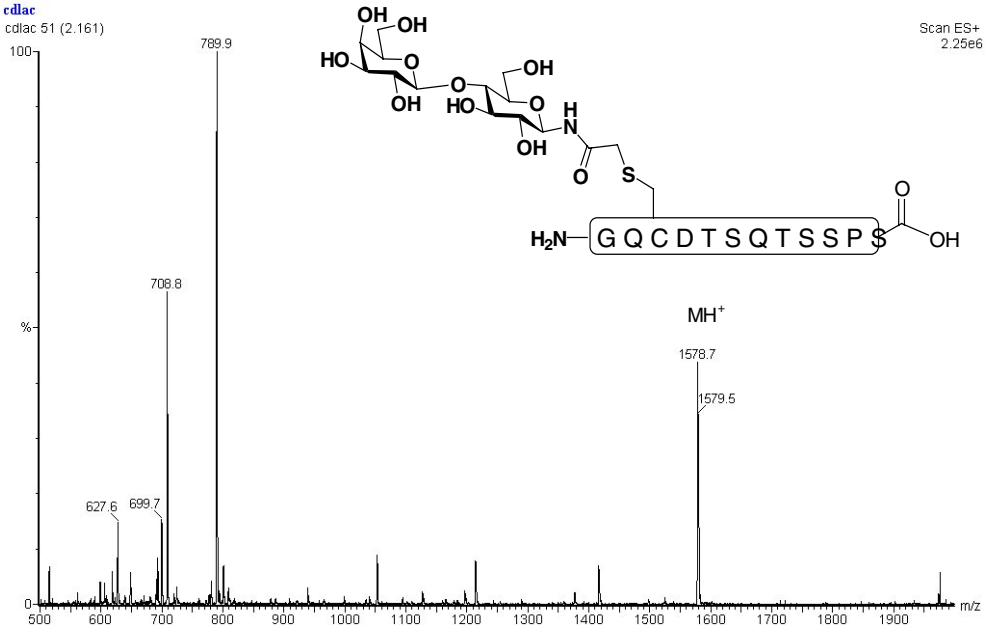
Glycopeptide **8**



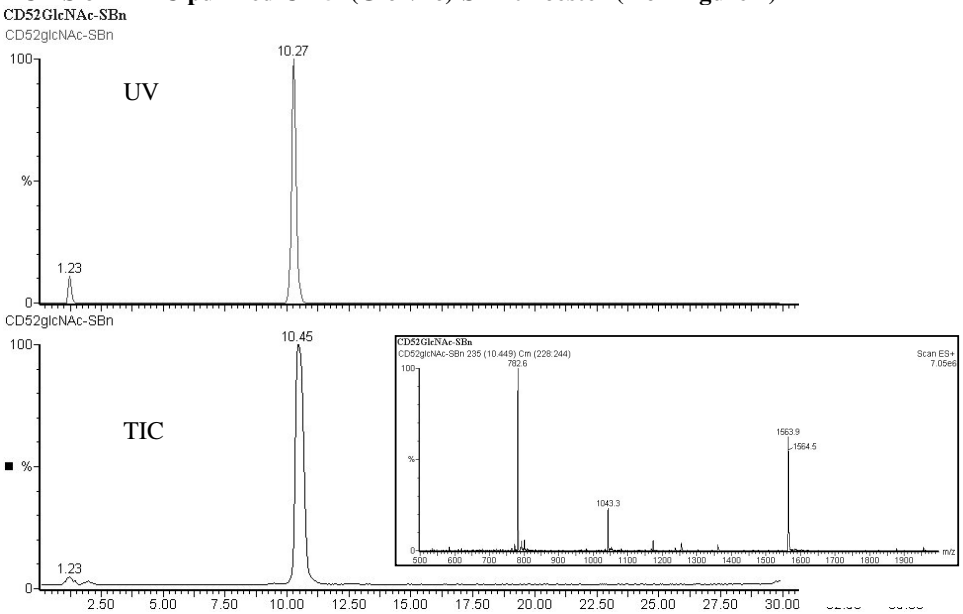
Glycopeptide **10**



Glycopeptide **11**



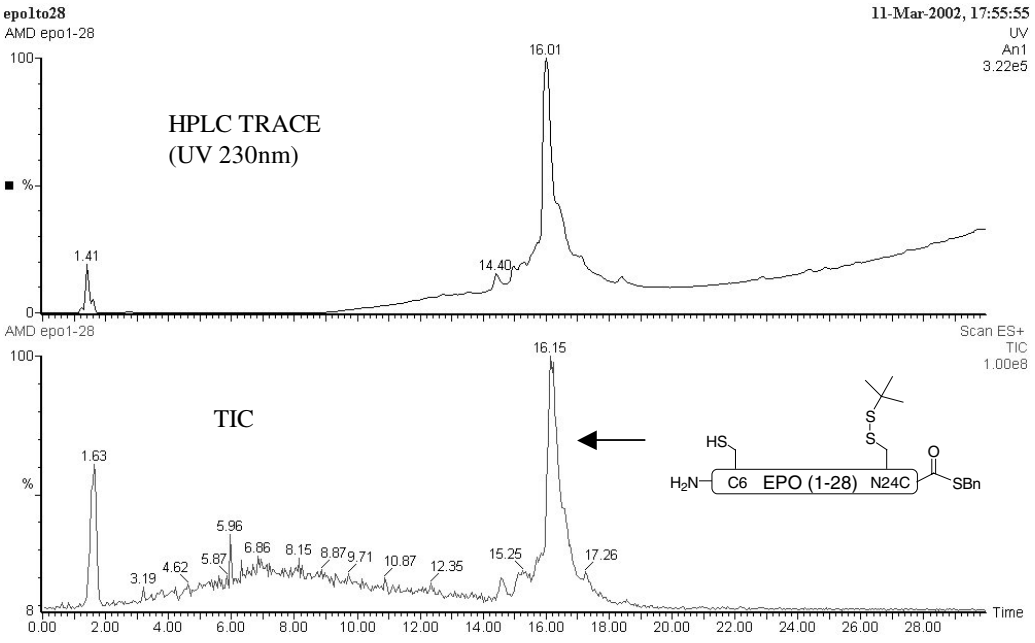
LCMS of HPLC purified CD 52(GlcNAc)-SBn thioester (from figure 2)



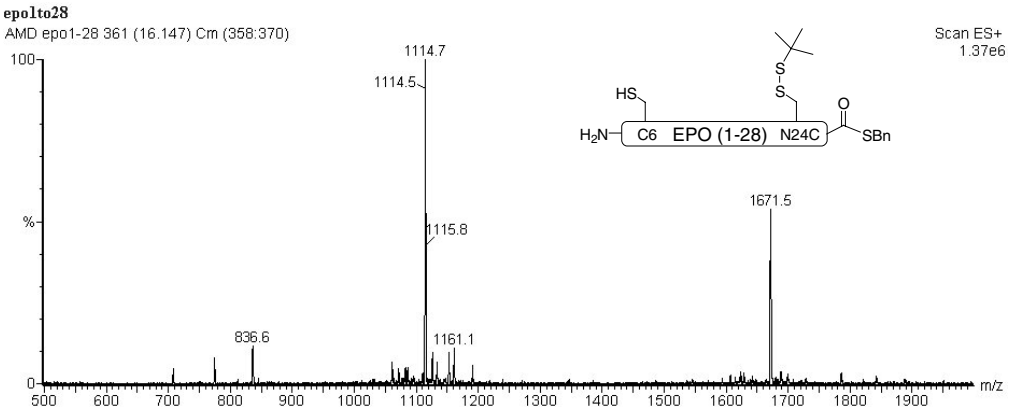
Erythropoietin (EPO) residues 1 to 28

To demonstrate the success of our peptide synthesis we first cleaved an aliquot of unglycosylated peptide (EPO residues 1 to 28) from a 20mg sample of 4-sulfamylbutyryl resin:

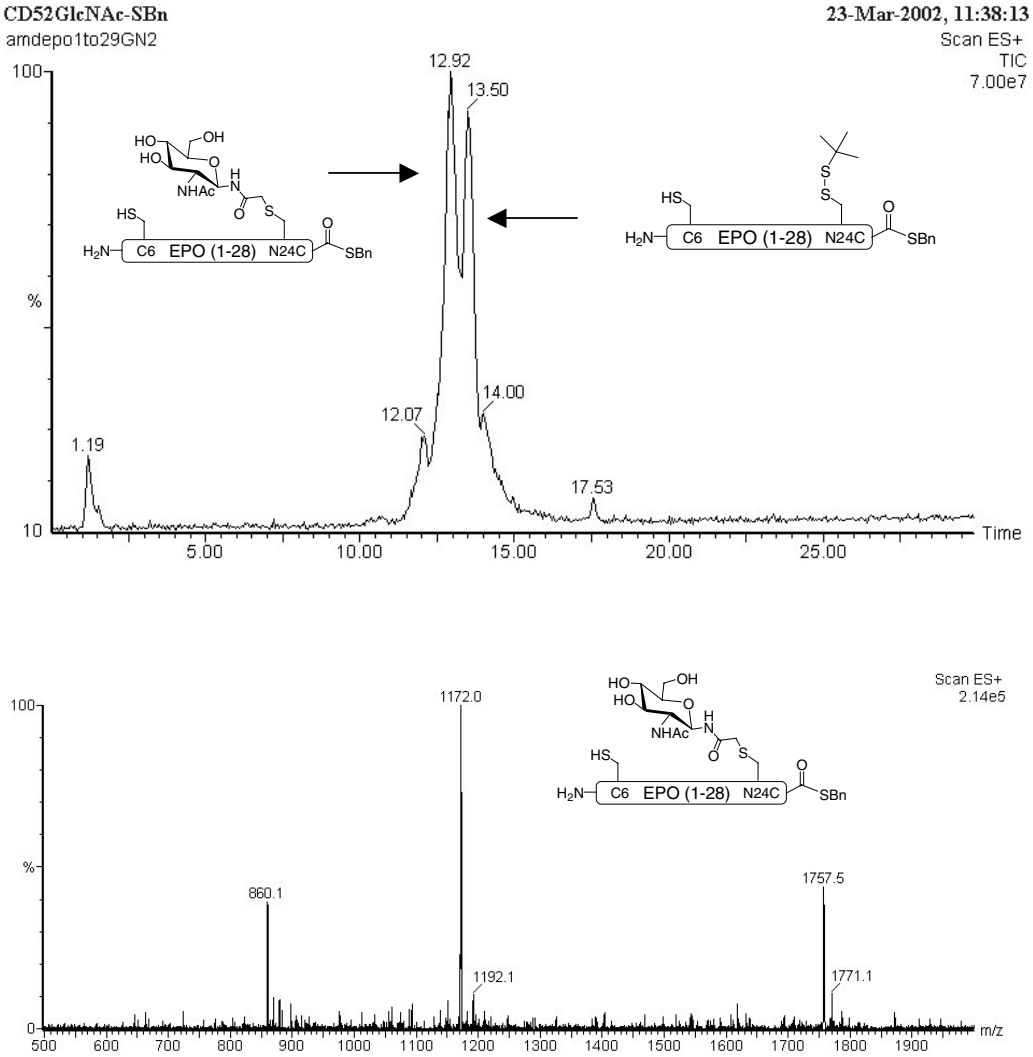
LC-MS of <u>crude</u> EPO fragment (residues 1 to 28). Interestingly, cleavage of the peptide backbone protecting groups with 95 % TFA, 2.5 % H_2O, 2.5 % EDT leaves the S-tBu disulfide protecting group intact. Cleavage with 85 % TFA, 5 % H_2O, 5 % EDT, 5 % thioanisole does not.



Mass spectrum of 16min peak (corresponds to H_2N-EPO residues 1 to 28(+ StBu)-SBn thioester as expected)



We then coupling and deprotection conditions of figure 3 were applied to EPO residues 1 to 28 applied the same analysis to the glycosylated fragment:



In this case the peptide is modified greater than 50 % (not quantitative however) as a result of incomplete StBu deprotection using the deprotection conditions in figure 3. Quantitative S-tBu removal requires longer incubation times and slightly elevated temperatures (40-50°C). More

generally and like standard peptide synthesis complete sulfhydryl modification may require “double coupling”.