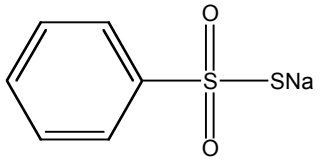
Glycosyl phenylthiosulfonates (Glyco-PTS): Novel Protein Glycosylating Reagents

David P. Gamblin, Philippe Garnier, Sarah J. Ward, Neil J. Oldham, Antony .J. Fairbanks,* and Benjamin G. Davis*

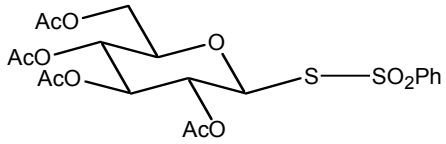
Supporting Information

Sodium phenylthiosulfonate (NaPTS)



Sodium benzenesulfinate (10 g, 61 mmol) and sulfur (1.95 g, 61 mmol) were dissolved in anhydrous pyridine (60 mL) to give a yellow solution. The reaction was stirred under argon and after 1 h gave a white suspension. The reaction was filtered and washed with anhydrous diethyl ether. Recrystalisation from anhydrous ethanol afforded sodium phenylthiosulfonate (10.5 g, 88%) as a white crystalline solid m.p. 305-306 ˚C [Lit. 287 ˚C][1]; δ_H (200 MHz, DMSO-d_6) 7.28-7.76 (5H, m, Ar-H); m/z (ES) 219 (MNa^+, 100%).

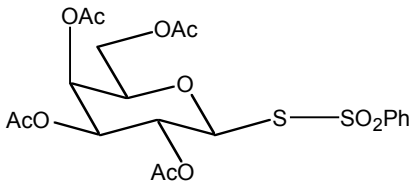
2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl phenylthiosulfonate 2a



2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **4a** (207 mg, 0.5 mmol) was dissolved in anhydrous acetonitrile (5 mL). To this sodium phenylthiosulfonate (201 mg, 1 mmol) and tetrabutylammonium bromide (16 mg, 0.05 mmol) were added. The resulting mixture was stirred under argon at 70 ºC. After a 4.5 h period, t.l.c.

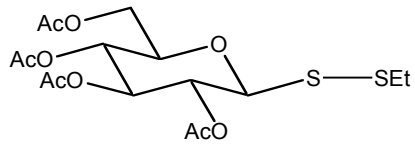
(petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.5) with complete consumption of the starting material (R_f 0.3). The solution was concentrated *in vacuo*. The crude solid was partitioned between DCM (20 mL) and water (20 mL), and the aqueous layer re-extracted with DCM (2 x 20 mL). The combined organics were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:1) to afford 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate **2a** (225 mg, 88%) as a white crystalline solid mp 129-130˚C;$[\alpha]_D^{25}$ +51.2 (c, 1.0 in $CHCl_3$); υ_{max} (KBr) 1754 (s, C=O), 1376 (s, C=C) cm^{-1}; δ_H (400 MHz, C_6D_6) 1.68, 1.72, 1.73, 1.75 (4 x 3H, 4 x s, 4 x OAc), 3.09 (1H, ddd, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 2.4 Hz, $J_{5,6'}$ 4.2 Hz, H-5), 3.83 (1H, dd, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.7 Hz, H-6), 4.08 (1H, dd, $J_{5,6'}$ 4.2 Hz, $J_{6,6'}$ 12.6 Hz, H-6'), 5.17-5.23 (2H, m, H-2, H-4), 5.40 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 5.44 (1H, at, *J* 9.4 Hz, H-3), 6.98-7.03 (3H, m, Ar-H), 7.90-7.92 (2H, m, Ar-H); δ_C (100 MHz, C_6D_6) 20.1, 20.1, 20.3 (3 x s, 4 x CH_3) 61.4 (t, C-6), 68.0, 69.4 (2 x d, C-2, C-4), 74.0 (d, C-3), 76.6 (d, C-5), 87.4 (d, C-1), 127.4, 128.0, 128.2, 128.4, 128.6, 129.3 (6 x d, 5 x Ar-C), 146.9 (s, SO_2-C), 169.1, 169.3, 169.8, 170.0 (4 x s, 4 x CO); m/z (HRMS TOF ES^+) Calcd. for $C_{20}H_{28}NO_{11}S_2$ (MNH_4^+) 522.1104. Found 522.1110).

2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl phenylthiosulfonate 2c



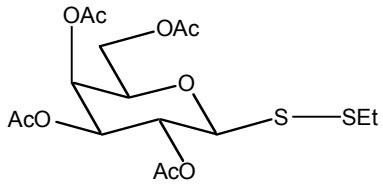
2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **4c** (2.0 g, 5 mmol) was dissolved in anhydrous acetonitrile (80 mL). To this sodium phenylthiosulfonate (2.02 g, 10.3 mmol) and tetrabutylammonium bromide (160 mg, 0.5 mmol) were added. The resulting mixture was stirred under argon at 70 ºC. After a 5 h period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.4) with complete consumption of the starting material (R_f 0.6). The solution was concentrated *in vacuo*. The crude oil was partitioned between DCM (50 mL) and water (50 mL), and the aqueous layer re-extracted with DCM (2 x 50 mL). The combined organics were washed brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 2:1) to afford 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate **2c** (1.7 g, 65%, 2 steps) as a white crystalline solid mp 53-54˚C;$[\alpha]_D^{27}$ +24.2 (c, 1.0 in $CHCl_3$); λ_{max} (KBr) 1756 (s, C=O), 1366 (s, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.98, 2.03, 2.06, 2.11 (4 x 3H, 4 x s, 4 x OAc), 3.85 (1H, dd, $J_{5,6}$ 8.8 Hz, $J_{6,6'}$ 14.0 Hz, H-6), 3.95-4.00 (2H, m, H-5, H-6), 5.11 (1H, dd, $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.23 (1H, at, J 10.3 Hz, H-2), 5.25 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 5.43 (1H, dd, $J_{3,4}$ 3.6 Hz, $J_{4,5}$ 1.0 Hz, H-4), 7.54-7.68 (3H, m, Ar-H), 7.93-7.97 (2H, m, Ar-H); δ_C (100 MHz, C_6D_6) 20.4, 20.5, 20.6 (3 x d, 4 x CH_3), 60.7 (t, C-6), 65.9 (d, C-2), 66.8 (d, C-4), 71.5 (d, C-3), 75.0 (d, C-5), 87.3 (d, C-1), 127.0, 128.0, 129.2, 129.3, 134.0 (5 x d, 5 x Ar-C), 146.0 (s, SO_2-C), 169.6, 167.0 (2 x s, 4 x CO); m/z LRMS (ES^+) 525 MNa^+ 100%;m/z HRMS (ES^+) Calcd. for $C_{20}H_{28}NO_{11}S_2$ (MNH_4^+) 522.1104 Found 522.1109.

Ethyl 2,3,4,6-tetra-O-acetyl-1-dithio-β-D-glucopyranosyl disulfide 5a



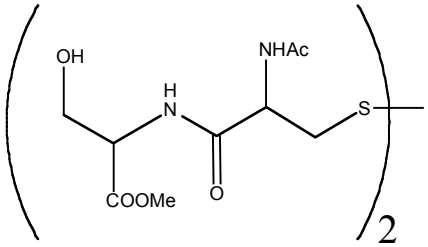
2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl phenylthiosulfonate **2 a** (100 mg, 0.2 mmol) and triethylamine (0.03 mL, 0.2 mmol) were dissolved in anhydrous DCM (10 mL) and stirred at RT under an atmosphere of argon. A solution of ethane thiol (0.016 mL, 0.2 mmol) in anhydrous DCM (10 mL) was slowly added dropwise *via* a syringe pump over a 30 min period. After a 40 min period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.3). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:1) to afford ethyl 2,3,4,6-tetra-O-acetyl-1-dithio-β-D-glucopyranosyl disulfide **5a** (70 mg, 82%**)** as a white crystalline solid mp 95-96 ˚C [Lit. 100-102˚C][2]; $[\alpha]_D^{22}$ -164.9 (c, 0.2 in $CHCl_3$) [Lit. $[\alpha]_D^{24}$ -178.0 (c, 1.0 in MeOH)][2]; υ_{max} (KBr) 1749 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.30 (1H, t, *J* 7.4 Hz, CH_3), 2.00, 2.02, 2.03, 2.06 (4 x 3H, 4 x s, 4 x CH_3), 2.79 (2H, dq, $J_{CH3\text{-}H}$ 7.5 Hz, J_{HH} 2.7 Hz), 3.73 (1H, ddd, $J_{4,5}$ 10.2 Hz, $J_{5,6}$ 2.5 Hz, $J_{5,6'}$ 4.8 Hz, H-5), 4.14 (1H, dd, $J_{5,6}$ 2.4 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.22 (1H, dd, $J_{5,6'}$ 4.7 Hz, $J_{6,6'}$ 12.4 Hz, H-6'), 4.52 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 5.10 (1H, at, *J* 9.8 Hz, H-4), 5.21-5.26 (2H, m, H-2, H-3); δ_C (100 MHz, $CDCl_3$) 14.2 (q, $CH_2\underline{C}H_3$), 20.5, 20.6 (2 x q, 4 x CH_3), 34.0 (t, $\underline{C}H_2CH_3$), 62.0 (t, C-6), 68.0,(d, C-4), 69.1, 73.8 (2 x d, C-2, C-3), 76.0 (d, C-5), 88.0 (d, C-1), 169.1, 169.4, 170.2, 170.5, (4 x s, 4 x C=O); m/z (ES+) 447.074 (MNa^+, 100%); m/z HRMS (ES^+) Calcd. For $C_{16}H_{24}O_9NaS_2$ (MNa^+) 447.0759. Found 447.0764.

Ethyl 2,3,4,6-tetra-O-acetyl-1-dithio-β-D-galactopyranosyl disulfide 5c



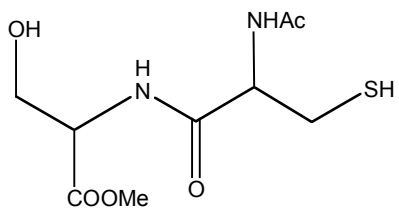
2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl phenylthiosulfonate **2 c** (100 mg, 0.2 mmol) and triethylamine (0.03 mL, 0.2 mmol) were dissolved in anhydrous DCM (10 mL) and stirred at RT under an atmosphere of argon. A solution of ethane thiol (0.016 mL, 0.2 mmol) in anhydrous DCM (10 mL) was slowly added dropwise *via* a syringe pump over a 30 min period. After a 40 min period, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.4) along with complete consumption of the starting material (R_f 0.3). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:1) to afford ethyl 2,3,4,6-tetra-O-acetyl-1-dithio-β-D-galactopyranosyl disulfide **5c** (78 mg, 91%**)** as a white crystalline solid mp 65-66 ˚C; $[\alpha]_D^{25}$ -52.1 (c, 1.4 in $CHCl_3$); υ_{max} (KBr) 1746 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.30 (1H, t, *J* 7.4 Hz, CH_3), 1.95, 2.01, 2.02, 2.13 (4 x 3H, 4 x s, 4 x CH_3), 2.79 (2H, dq, $J_{CH3\text{-}H}$ 7.2 Hz, J_{HH} 1.7 Hz), 3.94 (1H, td, $J_{4,5}$ 0.9 Hz, $J_{5,6}$ 6.3 Hz, $J_{5,6'}$ 7.0 Hz, H-5), 4.06 (1H, dd, $J_{5,6}$ 6.3 Hz, $J_{6,6'}$ 11.3 Hz, H-6), 4.12 (1H, dd, $J_{5,6'}$ 7.0 Hz, $J_{6,6'}$ 11.2 Hz, H-6'), 4.51 (1H, d, $J_{1,2}$ 9.9 Hz, H-1), 5.05 (1H, dd, $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.6 Hz, H-3), 5.35-5.40 (2H, m, H-2, H-4); δ_C (100 MHz, $CDCl_3$) 14.1 (q, $CH_2\underline{C}H_3$), 20.5, 20.6, 20.7, 21.0 (4 x q, 4 x CH_3), 34.1 (t, $\underline{C}H_2CH_3$), 61.5 (t, C-6), 66.7, 67.2 (2 x d, C-2, C-4), 71.8 (d, C-3), 74.7 (d, C-5), 89.8 (d, C-1), 169.3, 170.0, 170.1, 170.3, (4 x s, 4 x C=O); m/z (ES+) 447 (MNa^+, 100%), 442 (MNH_4^+, 90%); m/z HRMS (ES^+) Calcd. For $C_{16}H_{24}O_9S_2Na$ (MNa^+) 447.0759. Found 447.0768.

***bis*-*N*-Acetyl-L-cysteinyl-L-serine methylester**



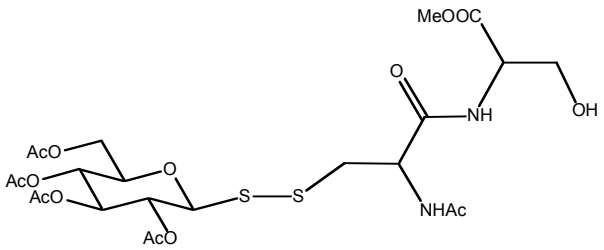
bis-L-Cysteinyl-L-serine methylester (100 mg, 0.23 mmol) was dissolved in methanol (5 mL). To this solution acetic anhydride (0.09 mL, 0.92 mmol) and pyridine (0.075 mL, 0.92 mmol) were added. After a 15 min period, t.l.c. (ethyl acetate:methanol 5:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.1). The reaction was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol 5:1) to afford *bis*-*N*-acetyl-L-cysteinyl-L-serine methylester (60 mg, 50%) as a white crystalline solid mp 145-147 ˚C; $[\alpha]_D^{25}$ -33.4 (c, 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.04 (3H, s, $COCH_3$), 2.96 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\_H}$ 4.7 Hz, CysC<u>H</u>H), 3.23 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\alpha H}$ 4.7 Hz, CysCH<u>H</u>), 3.76 (3H, s, OMe), 3.83 (1H, dd, $J_{CH,H}$ 11.4 Hz, $J_{CH,\alpha H}$ 4.1 Hz, SerC<u>H</u>H), 3.93 (1H, dd, $J_{CH,H}$ 11.3 Hz, $J_{CH,\alpha H}$ 4.9 Hz, SerC<u>H</u>H), 4.55 (1H, t, *J* 4.3 Hz, αHSer), 4.87 (1H, t, *J* 4.8, αHCys).

***N*-Acetyl-L-cysteinyl-L-serine methylester**



bis-*N*-Acetyl-L-cysteinyl-L-serine methylester (1.92 g, 3.96 mmol) was dissolved in wet chloroform (100 mL) and methanol (10 mL) and stirred. To this stirred solution tributylphosphine (1.1 mL, 4.36 mmol) was added. After a 2 h period, t.l.c. (ethyl acetate:methanol 10:1) indicated the formation of a product (R_f 0.6) along with complete consumption of the starting material (R_f 0.3). The reaction was concentrated *in vacuo*. Recrystallization from ethyl acetate/methanol afforded *N*-acetyl-L-cysteinyl-L-serine methylester (1.77 g, 93%) as a white crystalline solid mp 127-128 °C; $[\alpha]_D^{25}$ -32.0 (c, 1.0 in MeOH); δ_H (400 MHz, $CDCl_3$) 1.89 (1H, at, *J* 8.9 Hz, SH), 2.06 (3H, s, $COCH_3$), 2.84-2.93 (1H, m, CysC<u>H</u>H), 2.97-3.04 (1H, m, CysCH<u>H</u>), 3.79 (3H, s, OMe), 3.91 (1H, dd, $J_{CH,H}$ 11.4 Hz, $J_{CH,\alpha H}$ 3.1 Hz, SerC<u>H</u>H), 4.03 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 4.2 Hz, SerC<u>H</u>H), 4.61-4.65 (1H, m, αHSer), 4.71-4.76 (1H, m, αHCys), 6.93 (1H, d, $J_{\alpha H,NH}$ 7.8 Hz, NHCys), 7.73 (1H, d, $J_{\alpha H,NH}$ 7.4 Hz, NHSer).

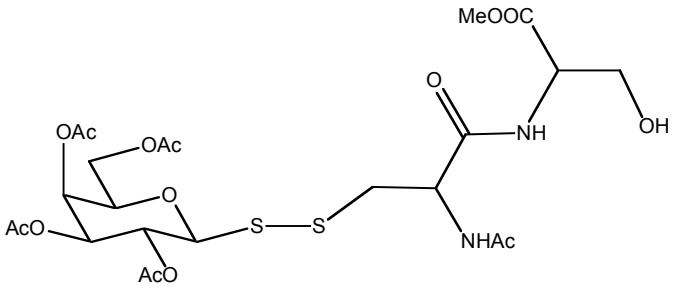
_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-acetyl-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester 6a



2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl phenylthiosulfonate (61 mg, 0.12 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of argon. To this *N*-acetyl-L-cysteine-L-serine methylester (32 mg, 0.12 mmol) and triethylamine (0.015 mL, 0.11mmol) in anhydrous DCM (10 mL) and anhydrous methanol (0.5 mL) were slowly added dropwise *via* a syringe pump over a 4 h period.

After a 5 h period, t.l.c. (ethyl acetate:methanol, 10:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.3, (t.l.c system (petrol:ethyl acetate, 1:1)). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol, 10:1) to afford *N*-acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester (75 mg, 99%**)** as a white crystalline solid mp 126-128 ˚C [Lit. 125-128˚C][2]; $[\alpha]_D^{25}$ -47.9 (c, 0.7 in $CHCl_3$) [Lit. $[\alpha]_D^{24}$ -178.0 (c, 1.0 in MeOH)][2]; υ_{max} (KBr) 3306 (bs, OH, NH), 1746 (s, C=O), 1639, 1543 (s, NH) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.03, 2.06, 2.07, 2.11 (5 x 3H, 4 x s, 5 x CH_3), 3.05 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\alpha H}$ 8.8 Hz, CysC<u>H</u>H), 3.28 (1H, dd, $J_{CH,H}$ 13.9 Hz, $J_{CH,\alpha H}$ 4.8 Hz, CysCH<u>H</u>), 3.80 (3H, s, OMe), 3.89 (1H, ddd, $J_{4,5}$ 10.0 Hz, $J_{5,6}$ 2.2 Hz, $J_{5,6'}$ 4.1 Hz, H-5), 3.94 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.0 Hz, SerC<u>H</u>H), 4.00 (1H, dd, $J_{CH,H}$ 13.8 Hz, $J_{CH,\alpha H}$ 3.7 Hz, SerCH<u>H</u>), 4.23 (1H, dd, $J_{5,6}$ 4.2 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.38 (1H, dd, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.5 Hz, H-6'), 4.62-4.65 (1H, m, αHSer), 4.64 (1H, d, $J_{1,2}$ 9.5 Hz, H-1), 4.90-4.94 (1H, m, αHCys), 5.18 (1H, at, *J* 10.1 Hz, H-4), 5.24-5.29 (2H, m, H-2, H-3), 6.94 (1H, d, $J_{NH,H}$ 7.9 Hz, NHAc), 7.52 (1H, d, $J_{NH,H}$ 7.6 Hz, NHSer); δ_C (100 MHz, $CDCl_3$) 20.5, 20.7 (2 x q, 5 x CH_3), 42.2 (t, CysCH_2), 52.5 (q, OCH_3), 54.8, 54.9 (d, αHSer, αHCys), 61.6 (t, SerCH_2), 62.3 (t, C-6), 67.7,(d, C-4), 65.9 (d, C-3), 73.6 (d, C-2), 76.0 (d, C-5), 88.4 (d, C-1), 169.2, 169.3, 170.0, 170.3, 170.4, 170.5, 170.7, 171.2 (8 x s, 7 x <u>C</u>O); m/z (ES+) 627 (MH^+, 60%), 649 (MNa^+, 100%); m/z HRMS (ES^+) Calcd. For $C_{23}H_{34}O_{14}N_2Na^{23}S_2$ (MNa^+) 649.1349. Found 649.1352.

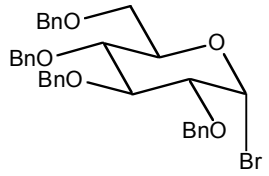
***N*-Acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-_-D-galactopyranosyl disulfide)-L-serine methylester 6c**



2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate **2c** (50 mg, 0.1 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of argon. A solution of *N*-acetyl-L-cysteine-L-serine methylester (31 mg, 0.12 mmol) and triethylamine (0.015 mL, 0.11mmol) in anhydrous DCM (10 mL) and anhydrous methanol (0.5 mL) was slowly added dropwise *via* a syringe pump over a 2 h period. After a 2 h period, t.l.c. (ethyl acetate:methanol, 10:1) indicated the formation of a major product (R_f 0.5) along with complete consumption of the starting material (R_f 0.5, (t.l.c system (petrol:ethyl acetate, 1:1))). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate:methanol, 10:1) to afford *N*-acetyl-L-cysteine (2,3,4,6-tetra-*O*-acetyl-1-dithio-β-D-galactopyranosyl disulfide)-L-serine methylester **6c** (59 mg, 95%**)** as a white amorphous solid; $[\alpha]_D^{25}$ -48.8 (c, 0.25 in $CHCl_3$); υ_{max} (KBr) 3306 (bs, OH, NH), 1746 (s, C=O), 1639, 1543 (s, NH) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.99, 2.04, 2.05, 2.08, 2.18 (5 x 3H, 4 x s, 5 x CH_3), 2.80 (1H, bs, OH), 2.99 (1H, dd, $J_{CH,H}$ 14.1 Hz, $J_{CH,\alpha H}$ 9.2 Hz, CysC<u>H</u>H), 3.32, 3.77 (3H, s, OMe), 3.92 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.0 Hz, SerC<u>H</u>H), 4.01 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.7 Hz, SerC<u>H</u>H), 4.06-4.14 (2H, m, H-5, H-6), 4.20-4.26 (1H, m, H-6'), 4.61-4.63 (1H, m, αHSer), 4.65 (1H, d, $J_{1,2}$ 9.8 Hz, H-1), 4.88-4.93 (1H, m, αHCys), 5.11 (1H, dd, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.3 Hz,

H-3), 5.42-5.47 (2H, m, H-2, H-4), 6.68 (1H, d, $J_{NH,H}$ 7.8 Hz, NHAc), 7.28 (1H, d, $J_{NH,H}$ 8.1 Hz, NHSer); δ_C (100 MHz, $CDCl_3$) 20.6, 20.7, 20.8, 21.0, 23.1 (5 x q, 5 x CH_3), 42.0 (t, CysCH_2), 52.7 (q, d, OCH_3, αHCys), 54.8 (d, αHSer), 61.7 (t, C-6), 62.4 (t, SerCH_2), 66.5, 67.2 (2 x d, C-2, C-4), 71.7 (d, C-3), 74.9 (d, C-5), 89.7 (d, C-1), 169.5, 170.0, 170.2, 170.3, 170.4, 170.9, 171.0 (7 x s, 7 x CO); m/z (ES+) 627 (MH^+, 100%), 649 (MNa^+, 20%). m/z HRMS (ES^+) Calcd. For $C_{23}H_{35}O_{14}N_2S_2$ (MH^+) 627.1530. Found 627.1528).

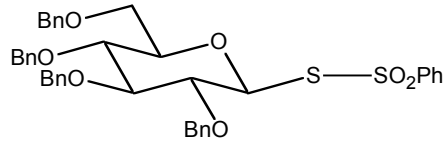
2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose bromide 4b



2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (1.0 g, 1.9 mmol) was dissolved in anhydrous DCM (6 mL) and anhydrous DMF (0.4 mL) under argon. The resulting solution was stirred at 0 ˚C. Oxalyl bromide (4 mL, 2M in DCM, 24 mmol) was added dropwise over a 5 min period . The reaction was stirred at RT. After a 40 min period, t.l.c. (petrol:ethyl acetate, 2:1) indicated the formation of a major product (R_f 0.7). The reaction was cooled to 0˚C and quenched with ice cold water (30 mL) added over a 5 min period. The reaction was partitioned between DCM (20 mL) and water. The aqueous layer was re-extracted with DCM (3 x 20 mL), the combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 2,3,4,6-tetra-*O*-benzyl-D-α-glucopyranosyl bromide (1.10 g, 95%) as a crude yellow oil; δ_H (400 MHz, $CDCl_3$), 3.57 (1H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.1 Hz, H-2), 3.68 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 11.0 Hz, H-6), 3.79-3.84 (2H, m, H-4, H-6'), 4.07 (1H, at, J 9.1 Hz, H-3), 4.07-4.11 (1H, m, H-5), 4.47-4.62 (3H, m, PhCH_2), 4.74 (s, 2H,

PhC<u>H</u>$_2$), 4.84-4.89 (2H, m, PhC<u>H</u>$_2$), 5.10 (1H, d, *J* 11.1 Hz, PhC<u>H</u>$_2$), 6.46 (1H, d, H-1), 7.15-7.41 (20H, m, Ar-H).

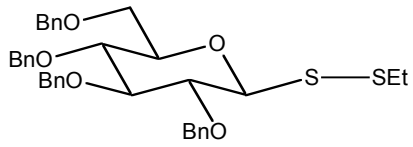
2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate 2b



2,3,4,6-Tetra-*O*-benzyl-D-α-glucopyranosoyl bromide **4b** (3.55 g, 5.88 mmol) and sodium phenylthiosulfonate (4.76 g, 24.3 mmol) were dissolved in anhydrous 1,4 dioxane (90 mL). The reaction was heated to 70 ˚C under argon. After 20 h, t.l.c. (petrol:ethyl acetate, 2:1) indicated the formation of a major product (R_f 0.6) with complete consumption of the starting material (R_f 0.7). The reaction was cooled to RT and filtered, the precipitate was washed with petrol/ethyl acetate and the filtrate concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 4:1) to afford 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl phenylthiosulfonate (3.18 g, 78%) as a white viscous gum as a mixture of α,β compounds both in an β:α ratio of 3:1. Selective re-crystallisation from ethyl acetate/petrol afforded pure 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate **2b** as a white crystalline solid m.p. 106-108 ºC; $[\alpha]_D^{22}$ +21.4 (c, 0.35 in $CHCl_3$); υ_{max} (Thin film) 1328, 1362 (s, C=C) cm^{-1}; δ_H (500 MHz, C_6D_6) 3.21 (1H, ddd, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 1.4 Hz, $J_{5,6'}$ 3.8 Hz, H-5), 3.29 (1H, dd, $J_{5,6}$ 1.4 Hz, $J_{6,6'}$ 11.1 Hz, H-6), 3.34 (1H, dd, $J_{1,2}$ 9.9 Hz, $J_{2,3}$ 8.7 Hz, H-2), 3.49 (1H, dd, $J_{5,6}$ 3.8 Hz, $J_{6,6'}$ 11.1 Hz, H-6'), 3.51 (1H, at, *J* 9.4 Hz, H-3), 3.60 (1H, at, *J* 9.4 Hz, H-4), 4.15, 4.25 (2H, ABq, *J* 12.1 Hz, $PhCH_2$), 4.52, 4.58 (2H, ABq, *J* 11.0 Hz, $PhCH_2$), 4.72,

4.76 (2H, ABq, *J* 11.3 Hz, $PhCH_2$), 4.78, 4.52 (2H, ABq, *J* 11.3 Hz, $PhCH_2$), 5.25 (1H, d, $J_{1,2}$ 10.2 Hz, H-1), 6.82-6.88 (3H, m, Ar-H), 7.05-7.26 (20H, m, Ar-H), 7.96-7.98 (2H, m, Ar-H); δ_C (125 MHz, C_6D_6) 69.1 (t, C-6), 73.8, 75.1, 75.7, 75.9 (4 x d, 4 x Ph<u>C</u>H_2), 77.7 (d, C-4), 80.0 (d, C-2), 86.9 (d, C-3), 89.2 (d, C-1), 127.8, 128.1, 128.3, 128.3, 128.5, 128.6, 128.7, 128.8, 128.9, 129.2 (9 x d, 25 x Ar-C), 138.4, 139.0, 139.3, 139.4 (4 x s, 4 x Ar-C), 147.7 (s, SO_2-C); m/z (ES) 714 (MNH_4^+, 48%), 719 (MNa^+, 100%). m/z HRMS (ES^+) Calcd. for $C_{40}H_{40}O_7S_2Na^{23}$ (MNa^+) 719.2115. Found 719.2115. (Found: C, 68.80%; H, 5.80%. $C_{40}H_{40}O_7S_2$ requires: C, 68.94%; H, 5.79%).

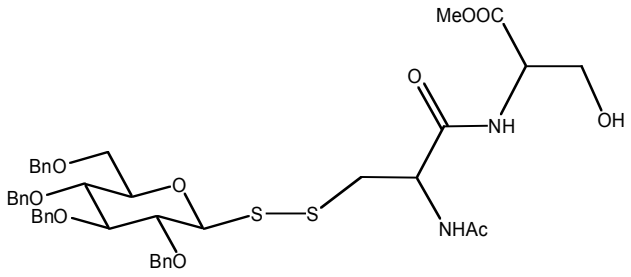
Ethyl 2,3,4,6-tetra-O-benzyl-1-dithio-β-D-glucopyranosyl disulfide 5b



2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate **2b** (100 mg, 0.14 mmol) and triethylamine (0.02 mL, 0.14 mmol) were dissolved in anhydrous DCM (10 mL) and stirred at RT under an atmosphere of Ar. To this ethane thiol (11 μL, 0.14 mmol) in anhydrous DCM (10 mL) was slowly added dropwise *via* a syringe pump over a 90 min period. After a 90 min period, t.l.c. (petrol:ethyl acetate, 6:1) indicated the formation of a major product (R_f 0.4) along with complete consumption of the starting material (R_f 0.2). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 7:1) to afford ethyl 2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyranosyl disulfide **5b** (83 mg, 95%**)** as a clear oil; $[\alpha]_D^{22}$ -164.9 (c, 0.2 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ -

80.0 (c, 3.0 in MeOH)][2]; υ_{max} (KBr) 3019 (s, C-H), 1495, 1526 (m, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.22 (1H, t, *J* 7.3 Hz, CH_3), 2.68-2.86 (2H, m, CH_2), 3.24 (1H, ddd, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 3.3 Hz, $J_{5,6'}$ 2.1 Hz, H-5), 3.56-3.60 (2H, m, H-6, H-6'), 3.61 (1H, at, *J* 9.1 Hz, H-3), 3.72 (1H, at, *J* 9.4 Hz, H-4), 3.89 (1H, at, *J* 9.1 Hz, H-2), 4.34 (1H, d, $J_{1,2}$ 9.7 Hz, H-1), 4.37, 4.31 (2H, Abq, *J* 12.2 Hz, PhC$\underline{H}_2$), 4.56, 4.83 (2H, Abq, *J* 11.3 Hz, PhC$\underline{H}_2$), 4.77-4.83 (2H, m, PhC$\underline{H}_2$), 4.90 (1H, d, *J* 11.1 Hz, PhC$\underline{H}$H), 4.97 (1H, d, *J* 10.7 Hz, PhC$\underline{H}$H), 7.07-7.21 (14H, m, Ar-H), 7.25-7.27 (2H, m, Ar-H), 7.29-7.31 (2H, m, Ar-H), 7.36-7.38 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 14.8 (q, $CH_2\underline{C}H_3$), 34.6 (t, $\underline{C}H_2CH_3$), 69.6 (t, C-6), 73.8, 75.2, 75.7, 75.8 (4 x t, 4 x Ph$\underline{C}H_2$), 78.2 (d, C-4), 79.8 (d, C-5), 80.2 (d, C-2), 87.2 (d, C-3), 90.5 (d, C-1), 127.9, 128.0, 128.1, 128.2, 128.6, 128.8, 128.9 (7 x d, 16 x Ar-C), 139.2, 139.4, 139.7 (3 x s, 4 x Ar-C); m/z (ES+) 634 (MNH_4^+, 100%), 639 (MNa^+, 90%); m/z HRMS (ES^+) Calcd. For $C_{36}H_{44}O_5NS_2$ (MNH_4^+) 634.2661. Found 634.2656.

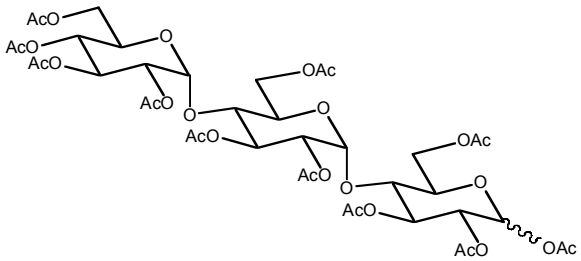
_N_-Acetyl-L-cysteine (2,3,4,6-tetra-_O_-benzyl-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester 6b



2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl phenylthiosulfonate **2b** (50 mg, 0.07 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of Ar. To this *N*-acetyl-L-cysteine-L-serine methylester (19 mg, 0.07 mmol) and triethylamine (11 μL, 0.08 mmol) in anhydrous DCM (5 mL) and

anhydrous methanol (0.5 mL) was slowly added dropwise *via* a syringe pump over a 5 h period. After a 5 h period, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.6) along with complete consumption of the starting material (R_f 0.9). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford *N*-acetyl-L-cysteine (2,3,4,6-tetra-*O*-benzyl-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester **6b** (48 mg, 82%**)** as a white crystalline solid mp 96-97 ˚C; $[\alpha]_D^{22}$ +56.2 (c, 1 in $CHCl_3$); υ_{max} (KBr) 3274 (bs, OH, NH), 1743 (s, C=O), 1640, 1543 (s, NH), 1372 (s, C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.03 (3H, s, $COCH_3$), 3.19 (1H, dd, $J_{CH,H}$ 14.0 Hz, $J_{CH,\alpha H}$ 8.3 Hz, CysC<u>H</u>H), 3.37 (1H, dd, $J_{CH,H}$ 14.3 Hz, $J_{CH,\alpha H}$ 6.0 Hz, CysCH<u>H</u>), 3.64 (1H, ddd, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 1.8 Hz, $J_{5,6'}$ 3.9 Hz, H-5), 3.72 (1H, at, *J* 9.2 Hz, H-4), 3.77 (1H, at, *J* 8.8 Hz, H-3), 3.82 (3H, s, OMe), 3.84-3.90 (4H, m, SerCH<u>H</u>, H-2, H-6, H-6'), 3.96 (1H, dd, $J_{CH,H}$ 11.7 Hz, $J_{CH,\alpha H}$ 3.3 Hz, SerC<u>H</u>H), 4.50 (1H, d, $J_{1,2}$ 9.6 Hz, H-1), 4.51, 4.70 (2H, ABq, *J* 11.6 Hz, Ph<u>C</u>H_2), 4.55, 4.85 (2H, ABq, *J* 10.4 Hz, Ph<u>C</u>H_2), 4.59-4.62 (1H, m, αHSer), 4.81, 4.87 (2H, ABq, *J* 10.6 Hz, Ph<u>C</u>H_2), 4.91, 4.97 (2H, ABq, *J* 11.0 Hz, Ph<u>C</u>H_2), 4.93-4.98 (1H, m, αHCys), 6.88 (1H, bd, $J_{NH,H}$ 7.9 Hz, NHAc), 7.13-7.39 (20H, m, 20 x Ar-C), 7.48 (1H, d, $J_{NH,H}$ 7.6 Hz, NHSer); δ_C (125 MHz, $CDCl_3$) 20.3 (q, $COCH_3$), 41.6 (t, Cys<u>C</u>H_2), 52.6 (q, OCH_3), 53.1 (d, αCSer), 54.9 (d, αCCys), 62.3 (t, SerCH_2), 68.7 (t, C-6), 73.3, 74.9,75.3, 75.5 (4 x t, 4 x PhCH_2), 77.2,(d, C-4), 78.6 (d, C-5), 79.0 (d, C-2), 86.3 (d, C-3), 88.9 (d, C-1), 127.5, 127.6, 127.8, 128.1, 128.3, 128.5 (6 x d, 24 x Ar-C), 136.137.6, 137.7, 138.3 (4 x s, 4 x Ar-C), 170.3, 170.4, 170.6 (3 x s, 3 x <u>C</u>O); m/z (ES+) 819 (MH^+, 95%), 841 (MNa^+, 100%); m/z HRMS (ES^+) Calcd. For $C_{43}H_{51}O_{10}N_2S_2$ (MH^+) 819.2985. Found 819.3011.

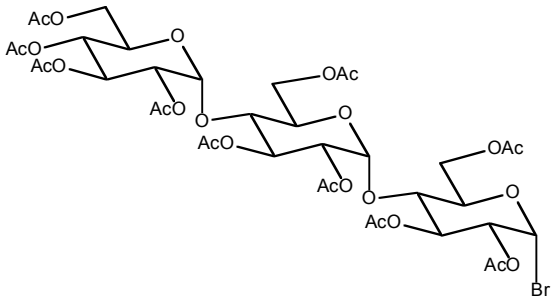
1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-D-glucopyranose



Sodium acetate (700 mg, 8.3 mmol) was added to acetic anhydride (50 mL) and heated to reflux, at which point maltotriose (3.00 g, 6.0 mmol) was added and stirred vigorously. After 90 min, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0.0). The reaction was allowed to cool to RT and diluted with DCM (50 mL) and partitioned with water (100 mL). The phases were separated and the aqueous layer was re-extracted with DCM (2 x 50 mL). The combined organic layers were washed with sodium hydrogen carbonate (400 mL of a saturated aqueous solution) until pH 8 was obtained, brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-D-glucopyranose as a mixture of anomers (α/β, 2/11) as an amorphous white solid; υ_{max} (KBr) 1758 (s, C=O); For β compound δ_H (500 MHz, $CDCl_3$) 2.05, 2.07, 2.10, 2.14, 2.15, 2.19, 2.21, 2.27 (30H, 8 x s, 10 x OAc), 3.92 (1H, ddd, $J_{4,5}$ 9.5 Hz, $J_{5,6}$ 2.9 Hz, $J_{6,6}$ 4.1 Hz, H-5a), 3.95-4.01 (3H, m, H-4b, H-5b, H-5c), 4.05 (1H, at, *J* 9.1 Hz, H-4a), 4.09 (1H, dd, $J_{5,6}$ 2.5 Hz, $J_{6,6'}$ 12.7 Hz, H-6c), 4.21 (1H, dd, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 12.6 Hz, H-6b), 4.29 (1H, dd, $J_{5,6}$ 3.4 Hz, $J_{6,6'}$ 12.4 Hz, H-6'c), 4.35 (1H, dd, $J_{5,6}$ 4.3 Hz, $J_{6,6'}$ 12.3 Hz, H-6a), 4.48-4.52 (2H, m, H-6'a, H-6'b), 4.78 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.3 Hz, H-2b), 4.90 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2c), 5.01 (1H, dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2a), 5.11 (1H, at,

J 10.1 Hz, H-4c), 5.31 (1H, d, $J_{1,2}$ 3.9 Hz, H-1b), 5.32-5.44 (3H, m, H-3a, H-3b, H-3c), 5.45 (1H, d, $J_{1,2}$ 4.1 Hz, H-1c), 5.79 (1H, d, $J_{1,2}$ 8.2 Hz, H-1a); δ_C (125 MHz, $CDCl_3$) 20.3, 20.5, 20.7, 20.8, 20.9, 22.0 (6 x q, 10 x Ac), 61.2 (t, C-6c), 62.1 (t, C-6b), 62.5 (t, C-6c), 67.7 (d, C-4c), 68.4, 68.9, 72.3 (3 x d, C-4b, C-5b, C-5c), 69.2, 71.5 (2 x d, C-3b, C-3c), 69.9 (d, C-2c), 70.3 (d, C-2b), 70.8 (d, C-2a), 72.8 (d, C-5a, 73.3 (d, C-4a), 75.0 (d, C-3a), 91.1 (d, C-1a), 95.5, 95.8 (2 x d, C1b, C-1c), 168.7, 169.3, 169.5, 169.7, 169.8, 170.2, 170.3, 170.4, 170.5 (9 x s, 10 x CO); For α compound selected data only, δ_H (500 MHz, $CDCl_3$) 2.08, 2.09, 2.12, 2.18, 2.21, 2.23, 2.26 (30H, 8 x s, 10 x OAc), 5.07 (1H, at, *J* 9.9 Hz), 6.28 (1H, d, $J_{1,2}$ 3.8 Hz, H-1a). Remaining signals lie in the following multiplet regions, 3.85-3.89, 3.90-3.98, 3.99-4.07, 4.15-4.18, 4.23-4.27, 4.29-4.32, 4.43-4.49, 4.74-4.76, 4.84-4.87, 4.98-4.94, 5.25-5.54; m/z (ES+) 984 (MNH_4^+, 30%), 989 (MNa^+, 100%); m/z HRMS (ES^+) Calcd. For $C_{40}H_{58}O_{27}N$ (MNH_4^+) 984.3196 Found 984.3199.

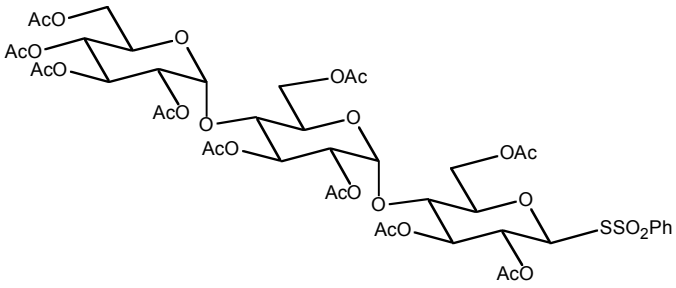
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl bromide 4d



1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-D-glucopyranose (200 mg, 0.21 mmol) was dissolved in anhydrous DCM (5 mL). To this hydrogen bromide (33% in acetic acid, 2 mL) was added. The mixture was left under argon at RT. After a 30 min period,

t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a product (R_f 0.6) with complete consumption of the starting material (R_f 0.3). The reaction mixture was partitioned between DCM (10 mL) and water (10 mL), and the aqueous layer re-extracted with DCM (3 x 10 mL). The combined organic layers were washed with sodium hydrogen carbonate (20 mL of a saturated aqueous solution) until pH 8 was obtained, brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl bromide **4d** (203 mg, 98%) as a white foam; $[\alpha]_D^{22}$ +152.2 (c, 1.0 in $CHCl_3$); υ_{max} (KBr) 1758 (s, C=O); δ_H (400 MHz, $CDCl_3$) 2.03, 2.05, 2.06, 2.08, 2.10, 2.13, 2.18, 2.21 (30H, 10 x $COCH_3$), 3.93-3.99 (3H, m, H-4b, H-5a, H-5b), 4.05-4.10 (2H, m, H-4c, H-6a), 4.20 (1H, dd, $J_{5,6}$ 1.8 Hz, $J_{6,6'}$ 12.2 Hz, H-6b), 4.26-4.34 (2H, m, H-5c, H-6a'), 4.35 (1H, dd, $J_{5,6}$ 3.5 Hz, $J_{6,6'}$ 12.7 Hz, H-6c), 4.52 (1H, dd, $J_{5,6}$ 0.6 Hz, $J_{6,6'}$ 12.2 Hz, H-6b'), 4.57 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6c''), 4.74 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 9.9 Hz, H-2c), 4.78 (1H, dd, $J_{1,2}$ 4.2 Hz, $J_{2,3}$ 10.2 Hz, H-2b), 4.88 (1H, dd, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 10.5 Hz, H-2a), 5.10 (1H, at, *J* 9.7 Hz, H-4a), 5.32 (1H, d, $J_{1,2}$ 4.0 Hz, H-1b), 5.39 (1H, at, *J* 9.9 Hz, H-3q), 5.43-5.46 (1H, m, H-3b), 5,45 (1H, d, $J_{1,2}$ 3.8 Hz, H-1a), 5.64 (1H, at, *J* 9.5 Hz, H-3c), 6.53 (1H, d, $J_{1,2}$ 3.9 Hz, H-1c); δ_C (100 MHz, MeOD) 20.5, 20.6, 20.8, (3 x q, 10 x CO<u>C</u>H_3), 61.3 (t, C-6a), 61.9 (t, C-6b), 62.1 (t, C-6c), 67.9 (d, C-4a), 68.4 (d, C-5a), 69.1, 72.2 (2 x d, C-4b, C-5b), 69.3 (d, C-3a), 70.0 (d, C-2a), 70.4 (d, C-2b), 71.0 (d, C-2c), 71.6 (d, C-3b), 72.2 (d, C-3c), 72.5 (2 x d, C-4c, C-5c), 85.9 (d, C-1c), 95.6 (d, C-1b), 95.9 (d, C-1a), 169.4, 169.6, 169.8, 170.3, 170.5, 170.6, 170.8 (7 x s, 10 x <u>C</u>OCH_3); m/z (ES+) 1004, 1006 (MNH_4^+, 100%), 1009, 1011 (MNa^+, 70%).

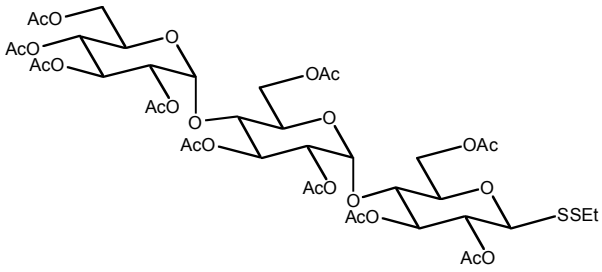
2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate 2d



2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranosyl bromide **4d** (200 mg, .21 mmol) was dissolved in anhydrous acetonitrile (10 mL). To this sodium benzenethiosulfonate (80 mg, 0.41 mmol) and tetrabutylammonium iodide (10 mg, 0.02 mmol) were added. The resulting mixture was stirred under argon at 70 ºC. After a 2 h period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a UV active product (R_f 0.5) with complete consumption of the starting material (R_f 0.5). At which point the solution was allowed to cool to RT and filtered, the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:2) to afford 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate **2d** (140 mg, 62%) as a white amorphous solid; $[\alpha]_D^{22}$ +69.9 (c, 0.75 in $CHCl_3$); υ_{max} (KBr) 2963 (m, C-H), 1748 (s, C=O), 1372 (s, C=C) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.03, 2.04, 2.06, 2.08, 2.11, 2.15, 2.19, (30H, 10 x $COCH_3$), 3.77-3.79 (1H, m, H-5a), 3.94-4.00 (4H, m, H-4a, H-4c, H-5b, H-5c), 4.10 (1H, dd, $J_{5,6}$ 2.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6b), 4.17-4.22 (3H, m, H-6a, H-6c, H-6a'), 4.29 (1H, dd, $J_{5,6}$ 3.3 Hz, $J_{6,6'}$ 12.6 Hz, H-6b'), 4.46 (1H, dd, $J_{5,6}$ 1.9 Hz, $J_{6,6'}$ 12.4 Hz, H-6c'), 4.76 (1H, dd, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 10.4 Hz, H-2a), 4.89-4.94 (2H, m, H-2b, H-2c), 5.12 (1H, at, *J* 9.9 Hz, H-4b), 5.28 (1H, d, $J_{1,2}$ 3.8 Hz, H-1a), 5.34 (1H, d, $J_{1,2}$ 9.7 Hz,

H-1c), 5.37 (1H, at, *J* 9.1 Hz, H-3c), 5.41 (1H, at, *J* 10.1 Hz, H-3b), 5.41-5.45 (2H, m, H-1b, H-3a), 7.62-7.65 (2H, m, Ar-H), 7.71 (1H, m, Ar-H), 8.00-8.02 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 20.3, 20.4, 20.5, 20.6, 20.7 (5 x q, 10 x CO<u>C</u>H_3), 61.3 (t, C-6b), 62.2 (t, C-6a), 62.5 (t, C-6c), 67.8 (d, C-4b), 68.4, 68.9, 72.4, 73.2 (4 x d, C-4a, C-4c, C-5a, C-5b), 69.2 (d, C-3a), 69.3, 70.0 (2 x d, C-2b, C-2c), 70.3 (d, C-2), 71.4 (d, C--3a), 75.5 (d, C-3c), 76.4 (d, C-5c), 86.2 (d, C-1c), 95.6, 95.7 (2 x d, C-1a, C-1b),126.9, 129.2 (2 x d, 5 x Ar-C), 133.9 (s, Ar-C), 169.5, 170.4 (2 x s, 10 x <u>C</u>OCH_3); m/z (ES+) 1103 (MNa^+, 100%). (Found: C, 48.62%; H, 5.01%. $C_{44}H_{39}O_{27}S_2$ requires: C, 48.89%; H, 5.22%).

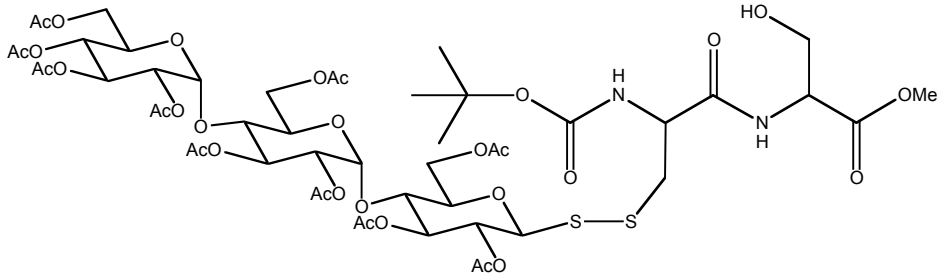
Ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)- 1-dithio-β-D-glucopyranosyl disulfide 5d



2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate **2d** (50 mg, 0.05 mmol) was dissolved in anhydrous DCM (10 mL) and stirred at RT under an atmosphere of argon. A solution of triethylamine (7 μL, 0.05 mmol) and ethane thiol (3 μL, 0.05 mmol) and anhydrous DCM (10 mL) was slowly added dropwise *via* a syringe pump over a 1 h period. After a 1 h period, t.l.c. (petrol:ethyl acetate, 1:2) indicated the formation of a major product (R_f 0.6) along with complete

consumption of the starting material (R_f 0.4). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 1:2) to afford ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-1-dithio-β-D-glucopyranosyl disulfide **5d** (43 mg, 93 %**)** as a clear oil; $[\alpha]_D^{24}$ +26.4 (c, 1.5 in $CHCl_3$); υ_{max} (KBr) 1752 (s, C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.30 (1H, t, *J* 7.2 Hz, CH_3), 2.04, 2.05, 2.06, 2.07, 2.10, 2.14, 2.19, 2.20 (30H, 8 x s, 10 x $COCH_3$), 2.75-2.87 (2H, m, $C\underline{H}_2CH_3$), 3.77-3.81 (1H, m, H-5a), 3.96-4.00 (3H, m, H-4b, H-5c, H-5b), 4.03 (1H, at, *J* 9.3 Hz, H-4a), 4.10 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.6 Hz, H-6c), 4.22 (1H, dd, $J_{5,6}$ 2.9 Hz, $J_{6,6'}$ 12.4 Hz, H-6b), 4.29 (1H, dd, $J_{5,6}$ 3.7 Hz, $J_{6,6'}$ 12.4 Hz, H-6'c), 4.33 (1H, dd, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$12.4 Hz, H-6a), 4.51 (1H, dd, $J_{5,6'}$ 1.8 Hz, $J_{6,6'}$ 12.4 Hz, H-6b', 4.57 (1H, dd, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 12.4 Hz, H-6a'), 4.58 (1H, d, $J_{1,2}$ 9.9 Hz, H-1a), 4.79 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2b), 4.90 (1H, dd, $J_{1,2}$ 4.3 Hz, $J_{2,3}$ 10.4 Hz, H-2c), 5.11 (1H, at, *J* 9.9 Hz, H-4c), 5.16 (1H, at, *J* 9.5 Hz, H-2a), 5.33 (1H, d, $J_{1,2}$ 4.1 Hz, H-1b), 5.37 (1H, at, *J* 8.9 Hz, H-3a), 5.38-5.44 (2H, m, H-3b, H-3c), 5.45 (1H, d, $J_{1,2}$ 4.1 Hz, H-1c); δ_C (125 MHz, $CDCl_3$) 14.6 (q, $CH_2\underline{C}H_3$), 20.4, 20.5, 20.6, 20.8, 20.9 (5 x q, 10 x $CO\underline{C}H_3$), 34.4 (t, $\underline{C}H_2CH_3$), 61.8 (t, C-6c), 62.7 (t, C-6b), 63.3 (t, C-6), 68.4 (d, C-4c), 68.9, 69.4, 72.9 (3 x d, C-4b, C-5b, C-5c), 69.8 (d, C-3c), 70.3 (d, C-2), 70.5 (d, C-2c), 70.9 (d, C-2b), 72.2 (d, C-3b), 73.9 (d, C-4), 76.8 (2 x d, C-5, C-3), 87.7 (d, C-1), 96.1, 96.3 (2 x d, C-1b, C-1c), 169.3, 169.5, 169.7, 170.2, 170.4, 170.5, 171.0 (7 x s, 10 x C=O); m/z (ES+) 1017 (MNH_4^+, 95%), 1022 (MNa^+, 100%). (Found: C, 48.03%; H, 5.61%. $C_{40}H_{56}O_{25}S_2$ requires: C, 48.00%; H, 5.64%).

***N*-Butoxycarbonyl-L-cysteine (2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester 6d**



2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate **2d** (89 mg, 0.08 mmol) was dissolved in anhydrous DCM (5 mL) and stirred at RT under an atmosphere of argon. A solution of triethylamine (0.014 mL, 0.2 mmol) and *N*-butoxycarbonyl-L-cysteinyl-L-serine methylester (30 mg, 0.09 mmol) in anhydrous DCM (10 mL) and anhydrous methanol (1 mL) was slowly added dropwise *via* a syringe pump over a 3 h period. After a 3 h period, t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.6) along with complete consumption of the starting material (R_f 0.7). The solution was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford *N*-Butoxycarbonyl-L-cysteine (2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-1-dithio-β-D-glucopyranosyl disulfide)-L-serine methylester **6d** (66 mg, 74%) as an amorphous white solid; $[\alpha]_D^{24}$ +25.1 (c, 1.25 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 1.47 (9H, s, $C(CH_3)_3$), 2.00, 2.01, 2.02, 2.03, 2.06, 2.09, 2.15, 2.18 (30H, 8 x s, 10 x $COCH_3$), 2.75-2.87 (1H, m, C<u>H</u>HCys), 3.16-3.19 (1H, m, CH<u>H</u>Cys), 3.27 (1H, t, *J* 6.2 Hz, OH), 3.81 (3H, s, OMe), 3.83-3.85 (1H, m, H-5a), 3.92-4.01 (6H, m, H-4b, H-5b, H-5c, H6a, H-6a', CH<u>H</u>Ser), 4.06 (1H,

dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 12.2 Hz, H-6c), 4.09-4.16 (2H, m, H-4a, H-6b), 4.25 (1H, dd, $J_{5,6}$ 3.2 Hz, $J_{6,6'}$ 12.3 Hz, H-6c'), 4.39-4.41 (1H, m, CH<u>H</u>Ser), 4.52-4.67 (4H, m, αHSer, αHCys, H-1a, H-6'b), 4.74 (1H, dd, $J_{1,2}$ 4.1 Hz, $J_{2,3}$ 10.3 Hz, H-2b), 4.85 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.5 Hz, H-2c), 5.07 (1H, at, *J* 9.9 HZ, H-4c), 5.11-5.13 (1H, m, H-2a), 5.28 (1H, d, $J_{1,2}$ 4.1 Hz, H-1b), 5.32-5.41 (4H, m, H-3a, H-3b, H-3c, NHCys), 5.42 (1H, d, $J_{1,2}$ 3.9 Hz, H-1c), 7.25 (1H, bd, $J_{NH,\alpha H}$ 6.7 Hz, NHSer); δ_C (125 MHz, $CDCl_3$) 20.4, 20.5, 20.7, 20.8 (4 x q, 10 x CO<u>C</u>H_3), 28.6 (3 x q, C(<u>C</u>$H_3)_3$), 33.8 (s, <u>C</u>$(CH_3)_3$)), 42.7 (t, CH_2Cys), 53.1 (q, OMe), 54.4 (d, αCCys), 55.4 (d, αCSer), 61.7 (t, C-6c), 62.4 (t, C-6b), 62.7 (t, CH_2Ser), 63.0 (t, C-6a), 68.4 (d, C-4c), 69.7, 72.1, 76.7 (3 x d. C-3a, C-3b, C-3c), 70.0 (2 x d, C-4b, C-5c), 70.3 (d, C-2a), 70.5 (d, C-2b), 70.7 (d, C-2c), 72.6 (d, C-5b), 73.7 (d, C-4a), 77.3 (d, C-5a), 87.3 (d, C-1a), 95.9, 96.3 (2 x d, C-1b, C-1c), 169.3, 169.6, 169.7, 170.0, 170.2, 170.4, 170.5 (7 x s, 13 x C=O); m/z (ES+) 1260 (MH^+, 20%), 1277 (MNH_4^+, 35%), 1282 (MNa^+, 100%).

Protein glycosylation procedure for 7a

SBLS156C mutant (24 mg, 0.89 μmol) was dissolved in aqueous buffer solution (2.4 mL, 70 mM HEPES, 2 mM $CaCl_2$, pH 6.9). 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenylthiosulfonate **2a** (50mg, 0.1 mmol) was dissolved in water/acetonitrile (1.6 mL, 9/7 v/v). A portion of the sugar solution (50 μL) was added to the protein solution and placed on an end-over-end rotator. After 25 min, the absence of free thiol was shown by Ellman's analysis,[3] at which point another portion of sugar solution (50 μL) was added. The reaction was placed on an end-over-end rotator for a further 5 min, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with (70 mM HEPES, 2 mM $CaCl_2$ pH 7.0). The protein fraction was collected and dialysed (MWCO 12-14 KDa) against 10 mM

MES, 1 mM $CaCl_2$, pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford **7a** m/z (ES) found 27072 calcd. 27078.

Protein glycosylation procedure for 7c

SBLS156C mutant (24 mg, 0.89 μmol) was dissolved in aqueous buffer solution (2.4 mL, 70 mM HEPES, 2 mM $CaCl_2$, pH 6.9). 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate **2c** (50mg, 0.1 mmol) was dissolved in water/acetonitrile (1.0 mL, 1/1 ratio). The sugar solution (50 μL) was added to the protein solution and placed on an end-over-end rotator. After 25 min, the absence of free thiol was shown by Ellman's analysis,[3] at which point another portion of sugar solution (50 μl) was added. The reaction was placed on an end-over-end rotator for a further 5 min, at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with (70 mM HEPES, 2 mM $CaCl_2$ pH 7.0). The protein fraction was collected and dialysed (MWCO 12-14 KDa) against 10 mM MES, 1 mM $CaCl_2$, pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford **7c** m/z (ES) found 27072 calcd. 27078.

Protein glycosylation procedure for 7d

SBLS156C mutant (10 mg, 0.37 μmol) was dissolved in degassed aqueous buffer solution (1 mL, 70 mM CHES, 5mM MES, 2 mM $CaCl_2$, pH 9.5). 2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*O*-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranosyl phenylthiosulfonate **2d** (30mg, 0.03 mmol) was dissolved in acetonitrile (150 μL). The sugar solution (75 μL) was added to the protein solution and placed on an end-over-end rotator. After 30 min, the absence of free thiol was shown by Ellman's analysis,[3] at which point the reaction mixture was

loaded onto a PD10 Sephadex® G25 column and eluted with (70 mM HEPES, 2 mM $CaCl_2$ pH 7.0). The protein fraction was collected and dialysed (MWCO 12-14 KDa) against 10 mM MES, 1 mM $CaCl_2$, pH 5.8, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford **7d** m/z (ES) found 27654 calcd. 27653.

Protein glycosylation procedures for 8a

BSA (10 mg, 0.14 μmol) was dissolved in aqueous buffer solution (1 mL, 50 mM Tris, pH 7.7). 2,3,4,6-Tetra-*O*-acetyl- β-D-glucopyranosyl phenylthiosulfonate **2a** (10mg, 0.02 mmol) was dissolved in water/acetonitrile (1.0 mL, 8/2 ratio). The sugar solution (150 μl) was added to the protein solution and placed on an end-over-end rotator. After 30 min, the absence of free thiol was shown by Ellman's analysis,[3] at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with (70 mM HEPES, 2 mM $CaCl_2$ pH 7.0). The protein fraction was collected and dialysed (MWCO 12-14 KDa) against pure water, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford **8a** m/z (ES) found 66798 calcd. 66794.

Protein glycosylation procedure for 8c

BSA (10 mg, 0.14 μmol) was dissolved in aqueous buffer solution (1 mL, 50 mM Tris, pH 7.7). 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl phenylthiosulfonate **2c** (25mg, 0.05 mmol) was dissolved in acetonitrile (0.5 mL). The sugar solution (75 μL) was added to the protein solution and placed on an end-over-end rotator. After 30 min, the absence of free thiol was shown by Ellman's analysis,[3] at which point the reaction mixture was loaded onto a PD10 Sephadex® G25 column and eluted with (70 mM HEPES, 2 mM $CaCl_2$ pH 7.0). The protein fraction was collected and

dialysed (MWCO 12-14 KDa) against pure water, (1 x 4L for 1 h, 2 x 2L for 30 min), to afford **8c** m/z (ES) found 66792 calcd. 66794.

Protein ESI-MS summary

Protein system	Mass found	Theoretical mass
SBL156C-β-$GlcAc_4$	27072	27078
SBL156C-β-$GalAc_4$	27072	27078
SBL156C- β-$GlcAc_3$(α1,4)$GlcAc_3$(α1,4)$GlcAc_4$	27654	27653
BSA-β-$GlcAc_4$	66798	66794
BSA-β-$GalAc_4$	66792	66794

References for Experimental

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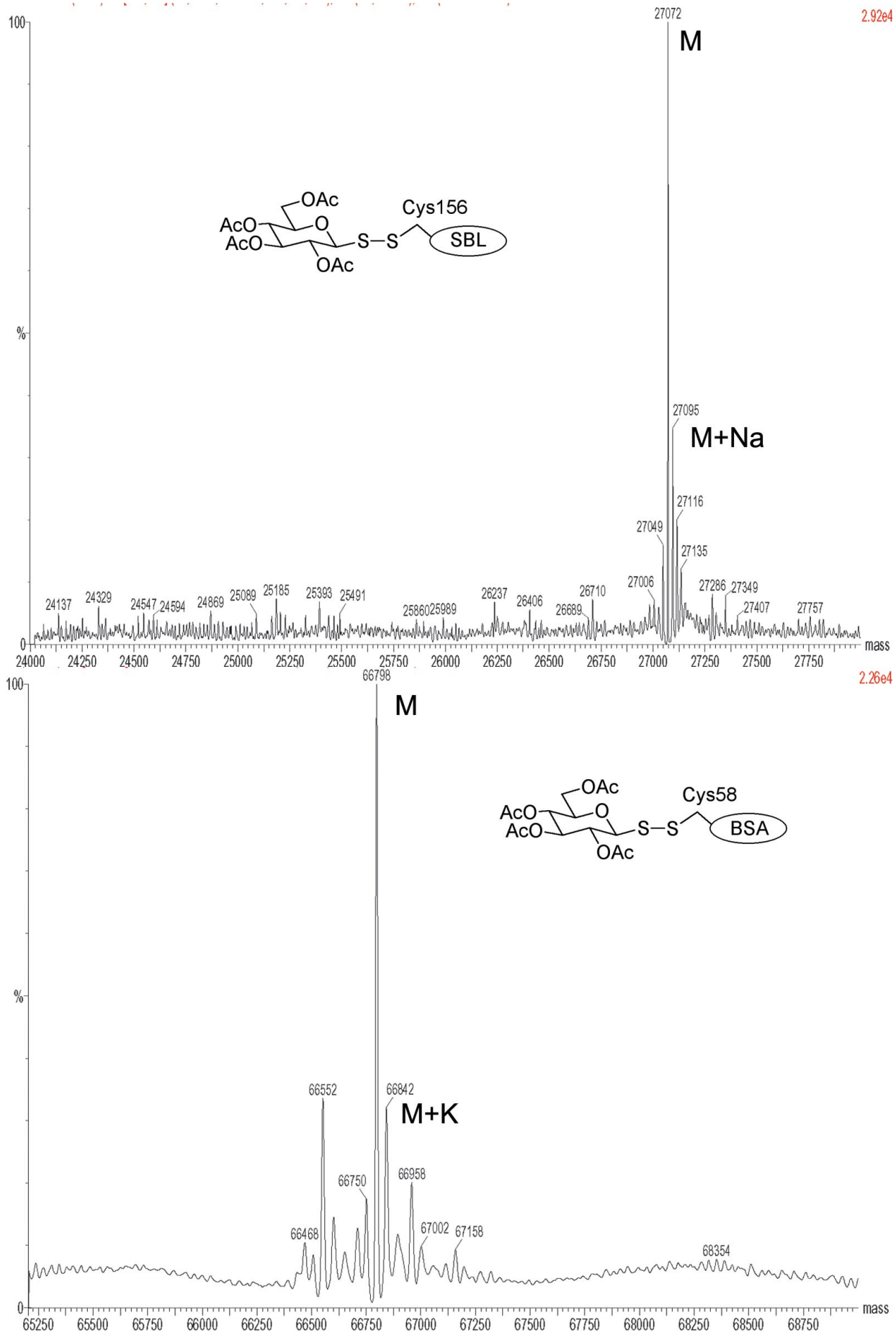
2. Davis, B. G.; Ward, S. J.; Rendle, P. M. *Chem. Commun.* **2001**, 189
3. Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70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24137
24329
24547
24594
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25185
25393
25491
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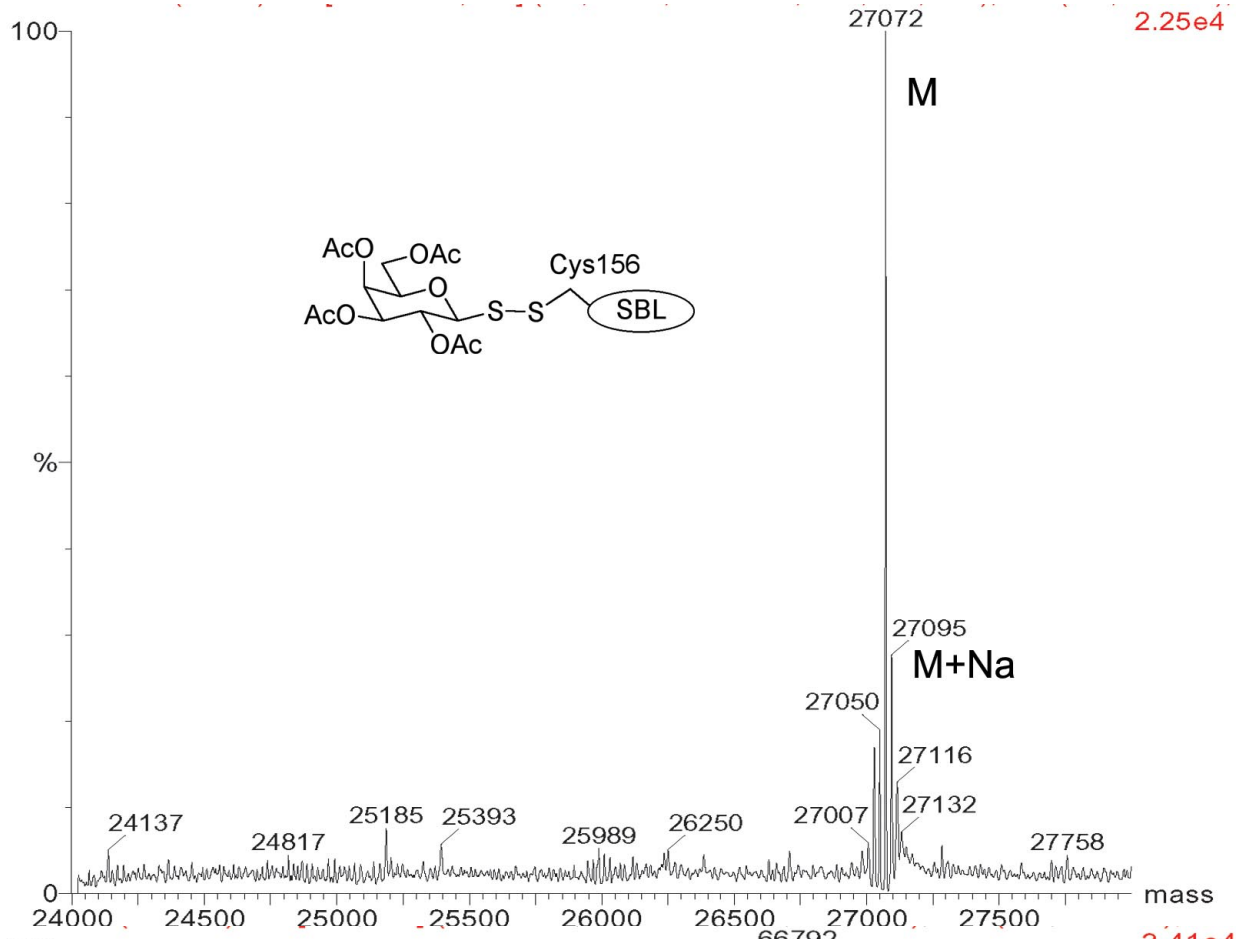
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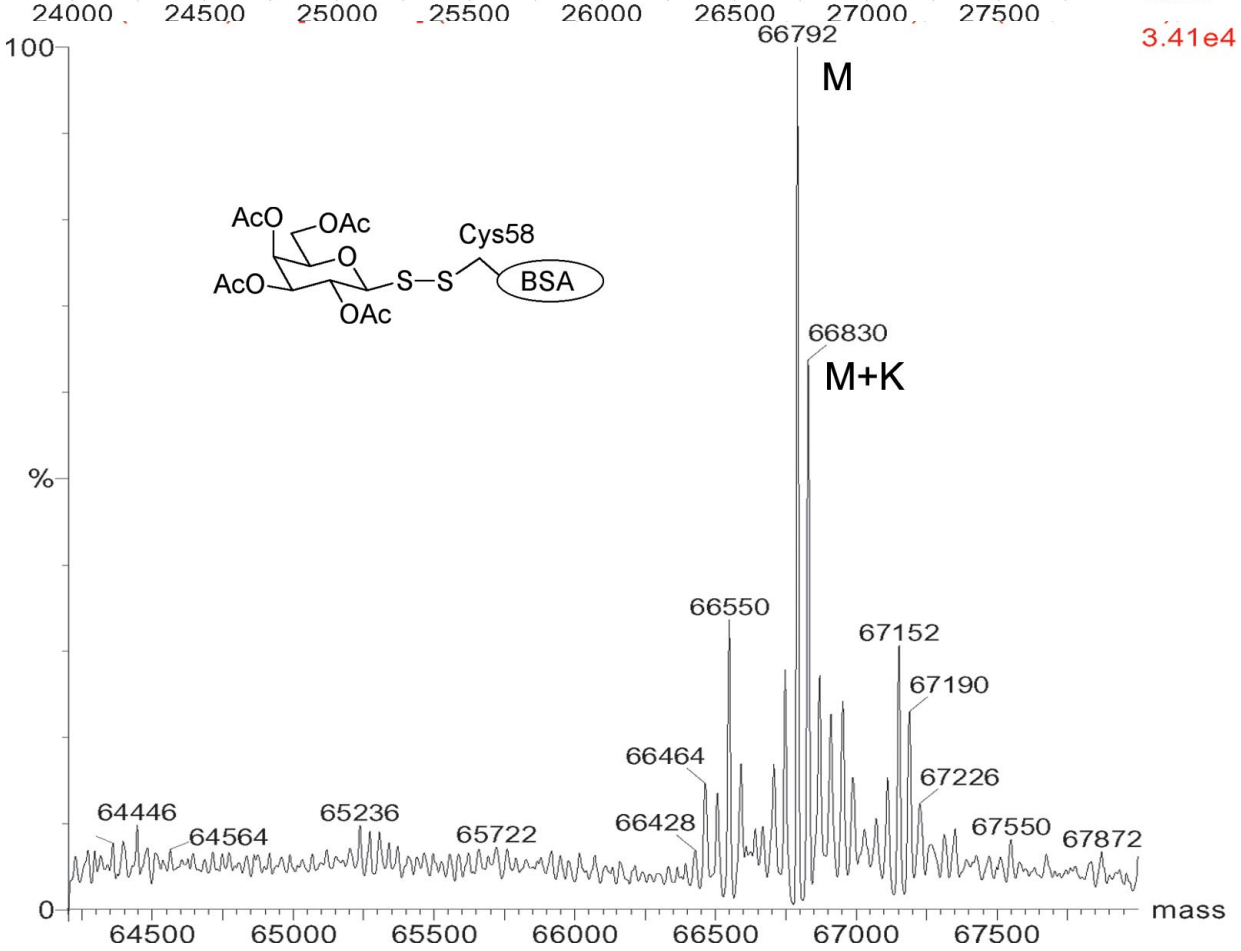
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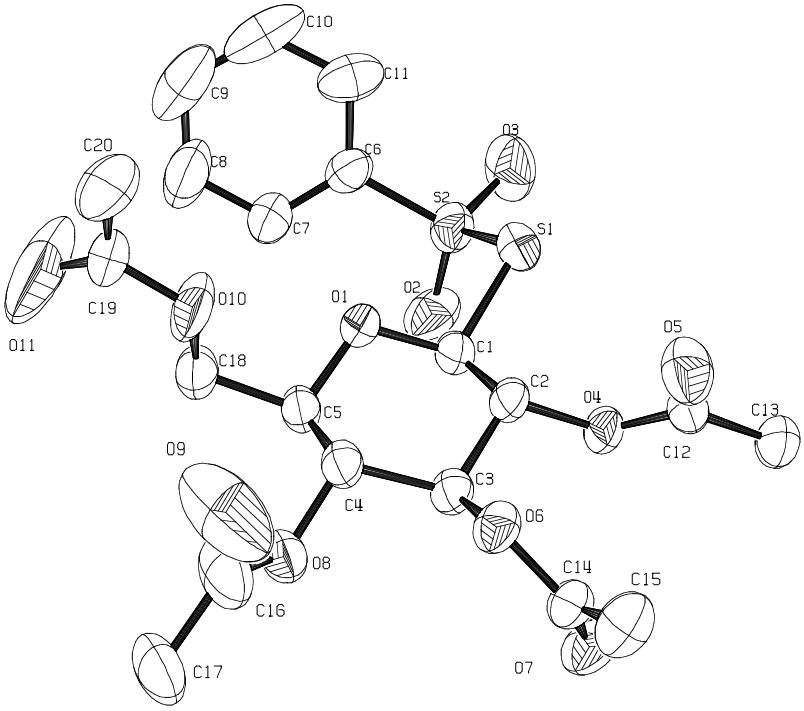
Chemistry Crystallography Service

Single-crystal X-ray diffraction report for

Andrew R. Cowley
E-mail: a_r_cowley@hotmail.com
Tel. (2)70827



H atoms not shown

Crystals of **ARC395** were grown by *. A large single crystal was cut to give a fragment having dimensions approximately 0.28 x 0.32 x 0.40 mm. This was mounted on a glass fibre using perfluoropolyether oil and cooled rapidly to 200K in a stream of cold N_2 using an Oxford Cryosystems CRYOSTREAM unit. Diffraction data were measured using an Enraf-Nonius KappaCCD diffractometer (graphite-monochromated MoK_α radiation, λ = 0.71073 Å). Intensity data were processed using the DENZO-SMN package[1].

Examination of the systematic absences of the intensity data showed the space group to be either *P* 2_1 or *P* $2_1/m$, the latter being precluded by the chiral nature of the compound. The structure was solved in the space group *P* 2_1 using the direct-methods program SIR92[2], which located all non-hydrogen atoms. Subsequent full-matrix least-squares refinement was carried out using the CRYSTALS program suite[3]. Coordinates and anisotropic thermal parameters of all non-hydrogen atoms were refined. Hydrogen atoms were positioned geometrically after each cycle of refinement. A 4-term Chebychev polynomial weighting scheme was applied. Refinement converged satisfactorily to give R = 0.0417, wR = 0.0465.

Attached is a thermal ellipsoid plot (ORTEP-3[4]) at 40% probability. A summary of crystallographic data is given below, as are full lists of atomic coordinates, anisotropic thermal parameters and those bond lengths and angles not concerning H atoms.

Comment:

On cooling the crystal below 180K, additional peaks become visible in the diffraction pattern. This indicates that a phase change takes place. However, it has not proved to be possible to index these peaks and thus to determine the structure of the low-temperature phase. It should be noted that at 200K the carbonyl O atoms of two of the acetyl substituents (O(9) and O(11)) and to a lesser extent the other atoms of these groups and the phenyl ring have large and anisotropic thermal ellipsoids, suggesting that there may be unresolved disorder. The phase change is likely to be associated with ordering of some or all of these groups.

References for Crystallography:

1 Z. Otwinowski and W. Minor, *Processing of X-ray Diffraction Data Collected in Oscillation Mode, Methods Enzymol.*, 1997, **276**, Eds C. W. Carter and R. M. Sweet, Academic Press.

2 A. Altomare, G. Cascarano, G. Giacovazzo, A. Guagliardi ,M. C. Burla, G. Polidori and M. Camalli, *J. Appl. Cryst*. 1994, **27**, 435.

3 D. J. Watkin, C. K. Prout, J. R. Carruthers, P. W. Betteridge and R. I. Cooper, CRYSTALS issue 11, Chemical Crystallography Laboratory, Oxford, UK, 2001.

4 ORTEP-3 v. 1.0.2, C. K. Johnson and M. K. Burnett, 1998.

Table 1: Crystal data and refinement details

Crystal identification	ARC395
Chemical formula	$C_{20}H_{24}O_{11}S_2$
Formula weight	504.52
Temperature (K)	150
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P\,2_1$
a (Å)	10.7470(3)
b (Å)	8.3113(2)
c (Å)	13.5613(4)
α (°)	90
β (°)	100.4321(12)
γ (°)	90
Cell volume (Å^3)	1191.3
Z	2
Calculated density (Mg/m^3)	1.406
Absorption coefficient (mm^{-1})	0.280
F_{000}	528.648
Crystal size (mm)	0.28 x 0.32 x 0.40
Description of crystal	Colourless fragment
Absorption correction	Semi-empirical from equivalent reflections
Transmission coefficients (min,max)	0.89, 0.92
θ range for data collection (°)	$5.0 \leq \theta \leq 27.5$
Index ranges	$-13 \leq h \leq 13$, $-9 \leq k \leq 10$, $0 \leq l \leq 17$
Reflections measured	10203
Unique reflections	4759
R_{int}	0.024
Observed reflections ($I > 3\sigma(I)$)	3881
Refinement method	Full-matrix least-squares on *F*
Parameters refined	298
Weighting scheme	Chebychev 4-term polynomial
Goodness of fit	1.0631
R	0.0417
wR	0.0465
Residual electron density (min,max) (eÅ^{-3})	-0.40, 0.36

Table 2: Atomic coordinates and equivalent isotropic thermal parameters ($Å^2$) of non-hydrogen atoms

Atom	x	y	z	U_{equiv}
O(1)	0.22336(17)	0.1065(2)	0.21698(13)	0.0467
C(1)	0.3348(2)	0.2003(4)	0.2232(2)	0.0442
C(2)	0.3256(2)	0.3445(3)	0.2911(2)	0.0402
C(3)	0.3080(2)	0.2892(3)	0.39476(19)	0.0395
C(4)	0.1986(2)	0.1711(3)	0.3861(2)	0.0424
C(5)	0.2133(2)	0.0366(4)	0.3121(2)	0.0450
S(1)	0.34526(8)	0.27211(12)	0.09878(6)	0.0558
S(2)	0.39905(7)	0.05903(12)	0.03654(6)	0.0543
O(2)	0.4797(2)	-0.0269(3)	0.11365(19)	0.0710
O(3)	0.4435(3)	0.1116(4)	-0.0514(2)	0.0835
C(6)	0.2598(3)	-0.0526(4)	-0.0006(2)	0.0493
C(7)	0.2400(4)	-0.1853(5)	0.0535(3)	0.0814
C(8)	0.1295(5)	-0.2721(7)	0.0255(4)	0.1145
C(9)	0.0427(4)	-0.2275(9)	-0.0554(4)	0.1037
C(10)	0.0640(4)	-0.0956(7)	-0.1100(4)	0.0911
C(11)	0.1740(3)	-0.0031(5)	-0.0837(2)	0.0679
O(4)	0.44155(15)	0.4336(2)	0.29907(14)	0.0435
C(12)	0.4338(3)	0.5963(3)	0.28954(19)	0.0437
O(5)	0.3351(2)	0.6669(3)	0.2788(2)	0.0657
C(13)	0.5613(3)	0.6698(4)	0.2952(2)	0.0595
O(6)	0.27377(15)	0.4272(2)	0.44855(14)	0.0436
C(14)	0.3662(2)	0.5080(4)	0.5112(2)	0.0449
O(7)	0.47348(18)	0.4613(3)	0.53123(17)	0.0585
C(15)	0.3169(3)	0.6575(4)	0.5477(3)	0.0616
O(8)	0.19953(17)	0.0973(3)	0.48230(14)	0.0476
C(16)	0.0938(3)	0.1131(5)	0.5247(3)	0.0737
O(9)	0.0049(3)	0.1897(6)	0.4872(4)	0.1434
C(17)	0.1038(4)	0.0176(5)	0.6183(3)	0.0805
C(18)	0.1025(3)	-0.0780(4)	0.2928(2)	0.0534
O(10)	-0.00972(18)	0.0183(3)	0.2596(2)	0.0743
C(19)	-0.1157(3)	-0.0546(5)	0.2315(3)	0.0686
O(11)	-0.1203(4)	-0.1949(5)	0.2331(5)	0.1885
C(20)	-0.2249(3)	0.0525(7)	0.2000(3)	0.0911

Table 3: Atomic coordinates and isotropic thermal parameters ($Å^2$) of hydrogen atoms

Atom	x	y	z	U_{iso}
H(11)	0.4111	0.1345	0.2508	0.0519
H(21)	0.2512	0.4127	0.2623	0.0467
H(31)	0.3881	0.2375	0.4295	0.0464
H(41)	0.1181	0.2312	0.3625	0.0498
H(51)	0.2904	-0.0251	0.3431	0.0528
H(71)	0.3044	-0.2193	0.1126	0.0953
H(81)	0.1134	-0.3688	0.0652	0.1378
H(91)	-0.0368	-0.2913	-0.0750	0.1266
H(101)	-0.0002	-0.0644	-0.1700	0.1051
H(111)	0.1897	0.0941	-0.1231	0.0793
H(131)	0.5526	0.7892	0.2880	0.0713
H(132)	0.6150	0.6435	0.3614	0.0713
H(133)	0.6018	0.6260	0.2400	0.0713
H(151)	0.3865	0.7143	0.5935	0.0738
H(152)	0.2474	0.6311	0.5850	0.0738
H(153)	0.2834	0.7285	0.4895	0.0738
H(171)	0.0251	0.0321	0.6470	0.1002
H(172)	0.1786	0.0552	0.6679	0.1002
H(173)	0.1144	-0.0987	0.6030	0.1002
H(181)	0.0950	-0.1369	0.3558	0.0629
H(182)	0.1140	-0.1572	0.2397	0.0629
H(201)	-0.3031	-0.0137	0.1795	0.1065
H(202)	-0.2358	0.1241	0.2571	0.1065
H(203)	-0.2101	0.1197	0.1421	0.1065

Table 4: Anisotropic thermal parameters (Å^2)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	0.0440(9)	0.0447(11)	0.046(1)	0.0066(9)	-0.0050(8)	-0.0142(8)
C(1)	0.0377(12)	0.0458(16)	0.0462(14)	0.0103(12)	0.000(1)	-0.0088(11)
C(2)	0.0292(11)	0.0402(15)	0.0474(14)	0.0063(11)	-0.003(1)	-0.007(1)
C(3)	0.0282(11)	0.0405(14)	0.0473(13)	0.0058(12)	0.0004(9)	-0.002(1)
C(4)	0.0304(11)	0.0418(15)	0.0524(14)	0.0136(12)	0.000(1)	-0.003(1)
C(5)	0.0378(12)	0.0415(16)	0.0528(14)	0.0144(12)	0.000(1)	-0.0073(11)
S(1)	0.0702(5)	0.0478(4)	0.0481(4)	0.0082(3)	0.0071(3)	-0.0134(4)
S(2)	0.0462(3)	0.0610(5)	0.0574(4)	-0.0009(4)	0.0137(3)	-0.0120(3)
O(2)	0.0482(11)	0.0721(17)	0.0843(16)	-0.0057(13)	-0.0100(11)	0.0039(11)
O(3)	0.0901(18)	0.091(2)	0.0816(17)	-0.0023(15)	0.0470(14)	-0.0252(16)
C(6)	0.0455(14)	0.0590(19)	0.0426(13)	-0.0083(13)	0.0059(11)	-0.0061(13)
C(7)	0.095(3)	0.086(3)	0.0580(19)	0.0037(19)	-0.0022(18)	-0.048(2)
C(8)	0.121(4)	0.145(5)	0.078(3)	-0.025(3)	0.021(3)	-0.091(4)
C(9)	0.065(2)	0.151(5)	0.101(3)	-0.063(4)	0.030(2)	-0.046(3)
C(10)	0.0507(19)	0.123(4)	0.089(3)	-0.056(3)	-0.0154(19)	0.019(2)
C(11)	0.0655(19)	0.075(3)	0.0572(18)	-0.0176(17)	-0.0037(15)	0.0179(17)
O(4)	0.0306(8)	0.0440(11)	0.053(1)	0.0066(9)	-0.0003(7)	-0.0099(7)
C(12)	0.0548(15)	0.0383(16)	0.0381(12)	0.0029(11)	0.0084(11)	-0.0103(12)
O(5)	0.0635(14)	0.0457(13)	0.0880(17)	0.0102(12)	0.0141(12)	0.0000(11)
C(13)	0.0647(19)	0.063(2)	0.0503(16)	0.0006(15)	0.0099(14)	-0.0293(16)
O(6)	0.0311(8)	0.0460(11)	0.052(1)	-0.0011(9)	0.0033(7)	-0.0034(8)
C(14)	0.0337(12)	0.0537(17)	0.0453(13)	0.0031(12)	0.002(1)	-0.0113(11)
O(7)	0.038(1)	0.0632(15)	0.0672(13)	-0.0030(11)	-0.0089(9)	-0.003(1)
C(15)	0.0500(16)	0.064(2)	0.070(2)	-0.0129(17)	0.0103(14)	-0.0089(15)
O(8)	0.0406(9)	0.0487(12)	0.054(1)	0.0116(9)	0.0095(8)	-0.0009(8)
C(16)	0.0509(17)	0.085(3)	0.091(3)	0.026(2)	0.0271(17)	-0.0070(18)
O(9)	0.0580(15)	0.218(5)	0.168(3)	0.109(3)	0.0563(19)	0.043(2)
C(17)	0.097(3)	0.079(3)	0.075(2)	0.015(2)	0.039(2)	-0.025(2)
C(18)	0.0478(15)	0.0428(17)	0.0666(18)	0.0102(14)	0.0021(13)	-0.0096(13)
O(10)	0.038(1)	0.0559(15)	0.119(2)	0.0138(14)	-0.0115(11)	-0.019(1)
C(19)	0.0589(19)	0.073(3)	0.068(2)	0.0110(18)	-0.0029(15)	-0.0366(18)
O(11)	0.109(3)	0.083(3)	0.329(7)	0.025(4)	-0.078(4)	-0.051(2)
C(20)	0.0402(15)	0.129(4)	0.097(3)	0.013(3)	-0.0059(16)	-0.023(2)

Table 5: Bond lengths (Å)

O(1) - C(1)	1.419(3)
O(1) - C(5)	1.437(3)
C(1) - C(2)	1.525(4)
C(1) - S(1)	1.812(3)
C(2) - C(3)	1.523(4)
C(2) - O(4)	1.437(3)
C(3) - C(4)	1.520(3)
C(3) - O(6)	1.442(3)
C(4) - C(5)	1.529(4)
C(4) - O(8)	1.440(3)
C(5) - C(18)	1.510(4)
S(1) - S(2)	2.0880(12)
S(2) - O(2)	1.423(3)
S(2) - O(3)	1.431(3)
S(2) - C(6)	1.755(3)
C(6) - C(7)	1.362(5)
C(6) - C(11)	1.383(4)
C(7) - C(8)	1.383(5)
C(8) - C(9)	1.356(8)
C(9) - C(10)	1.366(8)
C(10) - C(11)	1.401(6)
O(4) - C(12)	1.359(4)
C(12) - O(5)	1.197(4)
C(12) - C(13)	1.489(4)
O(6) - C(14)	1.362(3)
C(14) - O(7)	1.200(3)
C(14) - C(15)	1.471(5)
O(8) - C(16)	1.370(4)
C(16) - O(9)	1.183(5)
C(16) - C(17)	1.484(5)
C(18) - O(10)	1.450(4)
O(10) - C(19)	1.285(4)
C(19) - O(11)	1.168(5)
C(19) - C(20)	1.473(6)

Note – H atoms have been excluded

Table 6: Bond angles (°)

C(1) - O(1) - C(5)	111.65(19)
O(1) - C(1) - C(2)	108.7(2)
O(1) - C(1) - S(1)	108.62(17)
C(2) - C(1) - S(1)	108.97(19)
C(1) - C(2) - C(3)	110.6(2)
C(1) - C(2) - O(4)	107.5(2)
C(3) - C(2) - O(4)	109.71(19)
C(2) - C(3) - C(4)	110.3(2)
C(2) - C(3) - O(6)	108.2(2)
C(4) - C(3) - O(6)	106.8(2)
C(3) - C(4) - C(5)	110.6(2)
C(3) - C(4) - O(8)	109.1(2)
C(5) - C(4) - O(8)	107.4(2)
O(1) - C(5) - C(4)	109.1(2)
O(1) - C(5) - C(18)	106.6(2)
C(4) - C(5) - C(18)	113.8(2)
C(1) - S(1) - S(2)	99.53(11)
S(1) - S(2) - O(2)	107.75(12)
S(1) - S(2) - O(3)	103.69(14)
O(2) - S(2) - O(3)	120.98(17)
S(1) - S(2) - C(6)	106.29(11)
O(2) - S(2) - C(6)	108.63(15)
O(3) - S(2) - C(6)	108.55(16)
S(2) - C(6) - C(7)	118.8(2)
S(2) - C(6) - C(11)	119.0(3)
C(7) - C(6) - C(11)	122.3(3)
C(6) - C(7) - C(8)	119.0(4)
C(7) - C(8) - C(9)	120.7(5)
C(8) - C(9) - C(10)	120.0(4)
C(9) - C(10) - C(11)	121.2(4)
C(6) - C(11) - C(10)	116.9(4)
C(2) - O(4) - C(12)	117.9(2)
O(4) - C(12) - O(5)	122.5(3)
O(4) - C(12) - C(13)	111.3(3)
O(5) - C(12) - C(13)	126.2(3)
C(3) - O(6) - C(14)	118.93(19)
O(6) - C(14) - O(7)	123.0(3)
O(6) - C(14) - C(15)	111.1(2)
O(7) - C(14) - C(15)	125.9(3)
C(4) - O(8) - C(16)	118.0(2)
O(8) - C(16) - O(9)	122.5(3)
O(8) - C(16) - C(17)	111.7(3)
O(9) - C(16) - C(17)	125.7(3)
C(5) - C(18) - O(10)	106.9(2)
C(18) - O(10) - C(19)	118.3(3)
O(10) - C(19) - O(11)	120.2(4)
O(10) - C(19) - C(20)	114.7(4)
O(11) - C(19) - C(20)	125.0(4)

Note – H atoms have been excluded