A Trisulfide-linked glycoprotein

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General procedure

Melting points were recorded on a Kofler hot block and are uncorrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Bruker AV400 (400 MHz), a Bruker DPX400 (400 MHz) spectrometer, or by Dr. B. Odell or Dr. T. Claridge on a Bruker AVC500 (500 MHz) spectrometer. Proton spectra were assigned using COSY. Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Bruker AV400 (100.7 MHz) spectrometer or by Dr. B. Odell or Dr. T. Claridge on a Bruker AVC 500 (125.8 MHz) spectrometer. Spectra were assigned using HMQC; multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ-scale in ppm using residual solvent as the internal standard.

Infrared spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer using thin films on NaCl plates, or KBr discs, absorption maxima being recorded in wavenumbers (cm^{-1}) and classified as s (strong) or br (broad). Only signals representing functional groups are reported; C-H absorptions as well as the fingerprint region are not listed.

Low resolution mass spectra were recorded on a Micromass Platform 1 spectrometer using electrospray ionization (ESI) or by Mr. Robin Proctor using a Walters 2790-Micromass LCT electrospray ionization mass spectrometer using chemical ionization (NH_3, Cl) techniques as stated. High resolution mass spectra were recorded by Mr. Robin Proctor on a Walters 2790-Micromass LCT electrospray ionization mass

spectrometer using chemical ionization (NH_3, Cl) techniques as stated. *m/z* values are reported in Daltons and are followed by their percentage abundance in parentheses.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml.

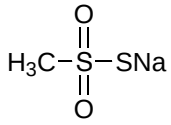
Thin layer chromatography (t.l.c.) was carried out using Merck Kieselgel $60F_{254}$ silica gel pre-coated glass-backed plates or Merck aluminium backed sheets coated with $60F_{254}$ silica gel. Visualisation of the silica plates/sheets was achieved using a u.v. lamp (λ_{max} = 254 or 365 nm), and/or ammonium molybdate (5% in 2M H_2SO_4), or potassium permanganate (5% in 1M NaOH). Flash column chromatography was carried out using Sorbsil C60 40/60 silica.

Anhydrous solvents including were purchased from Fluka. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reagents were purchased from Aldrich, and were used as supplied. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40-60ºC. All reactions using anhydrous conditions were performed using flame dried apparatus under an atmosphere of argon.

Protein Mass Spectrometry: Liquid chromatography/mass spectrometry was performed on a Micromass LCT (ESI-TOF-MS) coupled to a Waters Alliance 2790 HPLC using a Phenomenex Jupiter C4 column (250 x 4.6 mm x 5µm). Water:acetonitrile, 95:5 (solvent A) and acetonitrile (solvent B), each containing 0.1% of formic acid, were used as the mobile phase at a flow rate of 0.2 mL min^{-1}. The gradient was programmed as

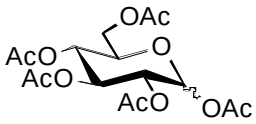
follows: 95% A (3 min isocratic) to 100% B after 16 min then isocratic for 2 min. The electrospray source of LCT was operated with a capillary voltage of 3.2 kV and a cone voltage of 25 V. Nitrogen was used as the nebuliser and desolvation gas at a total flow of 400 l hr^{-1}. Myoglobin (horse heart) was used as a calibration standard and to test the sensitivity of the system.

Sodium methanethiosulfonate (Na-MTS)[1] (1)



Sodium sulfide monohydrate (72.1 g, 0.3 mol) was dissolved in water (80 mL) with gentle heating to 60 °C. The solution was cooled to 0 °C in an ice bath with stirring. Freshly distilled methanesulfonyl chloride (23.3 mL, 0.3 mol) was added dropwise over a 1 h period. The mixture turned yellow, then orange, and finally colourless. The mixture was heated under reflux for 18 h and then cooled to room temperature. The solution was concentrated *in vacuo* to give a white solid and dried for a further 24 h period in a dessicator under vacuum. The white solid was extracted with dry ethanol (10 x 100 mL) and the slurry filtered after each extraction. The filtrate was evaporated to give sodium methanethiosulfonate **1** (32.5 g, 87%) as a white crystalline solid; m.p. 270-272 ºC [Lit. 272-273 ºC][1]; δ_H (400 MHz, D_2O) 3.26 (3H, s, CH_3); δ_C (100.7 MHz, D_2O) 54.9 (q, CH_3); *m/z* (ES^+) 156 (MNa^+, 100%).

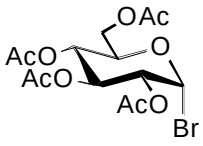
1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranoside[2, 3]



D-Glucose (50.0 g, 278 mmol) was dissolved in pyridine (200 mL) under an atmosphere of argon. Acetic anhydride (250 mL) was added portionwise over 30 min and the mixture was left to stir at RT. After 22 h, t.l.c. (petrol:ethyl acetate, 1:1) indicated the formation of a product (R_f 0.6) with complete consumption of starting material (R_f 0.0). The reaction mixture was co-evaporated with ethanol until no pyridine or acetic anhydride remained. The residue was recrystallised (ethanol) to afford 1,2,3,4,6-penta-

O-acetyl-D-glucopyranoside (90.5 g, 84%) as a white crystalline solid being a mixture of anomers (α:β, 1:1.2); m.p. 98-100 ºC (ethanol) [Lit. 100-102 ºC][3]; $[\alpha]_D^{21}$ +51.3 (c, 1.01 in $CHCl_3$) [Lit. $[\alpha]_D$ +54.5 (c, 3.8 in $CHCl_3$)][2]; δ_H (400 MHz, $CDCl_3$) 2.02, 2.03, 2.09, 2.11, 2.18 (15H, 5 x s, 5 x C(O)CH_3α), 2.02, 2.03, 2.09, 2.12, 2.18 (15H, 5 x s, 5 x C(O)CH_3β), 3.82-3.86 (1H, m, H-5β), 4.08-4.14 (3H, m, H-5α, H-6'α, H-6'β), 4.25-4.31 (2H, m, H-6α, H-6β), 5.08-5.17 (4H, m, H-2α, H-4α, H-2β, H-4β), 5.25 (1H, at, *J* 9.4 Hz, H-3β), 5.47 (1H, at, *J* 9.8 Hz, H-3α), 5.72 (1H, d, $J_{1,2}$ 8.3 Hz, H-1β), 6.33 (1H, d, $J_{1,2}$ 3.6 Hz, H-1α).

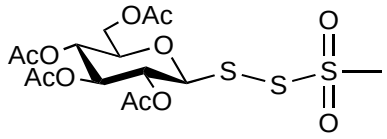
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide[4] (2)



1,2,3,4,6-Penta-*O*-acetyl-D-glucopyranoside (20 g, 51.2 mmol) was dissolved in anhydrous DCM (200 mL) and to this hydrogen bromide (33% w/w in acetic acid, 150 mL) was added. The mixture was stirred under argon at RT. After a 2 h period, t.l.c. (petrol:ethyl acetate, 3:1) indicated the formation of a product (R_f 0.4) with complete consumption of starting material (R_f 0.1). Ice water (250 mL) was added and the mixture stirred for 10 min. The two phases were separated and the aqueous layer re-extracted with DCM (3 x 50 mL). The combined organic layers were washed with sodium hydrogen carbonate (saturated aqueous solution) until pH 8 was obtained. The combined organics were washed with brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation (ethyl acetate/petrol) afforded 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **2** (17.5 g, 83%) as a white crystalline solid; m.p. 84-86 ºC (ethyl acetate/petrol) [Lit. 89.5-90.5 ºC][4]; $[\alpha]_D^{22}$ +182.1 (c, 1.01 in

$CHCl_3$) [Lit. $[\alpha]_D$ +186 (c, 6 in CH_2Cl_2)][4]; δ_H (400 MHz, $CDCl_3$) 2.04, 2.06, 2.10, 2.11 (12H, 4 x s, 4 x C(O)CH_3), 4.14 (1H, dd, $J_{5,6}$ 1.9 Hz, $J_{6,6'}$ 12.6 Hz, H-6), 4.28-4.36 (2H, m, H-5, H-6'), 4.85 (1H, dd, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 10.0 Hz, H-2), 5.17 (1H, at, *J* 9.8 Hz, H-4), 5.56 (1H, at, *J* 9.7 Hz, H-3), 6.25 (1H, d, $J_{1,2}$ 4.0 Hz, H-1).

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl methanedithiosulfonate (4)



2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide **2** (200 mg, 0.486 mmol) was dissolved in anhydrous dioxane (3 mL), and the mixture heated to 70 ºC under an atmosphere of argon. Sodium methanethiosulfonate **1** (392 mg, 2.918 mmol) was added to the above solution and the resulting mixture stirred for 70 h. The reaction was concentrated *in vacuo*. The white residue was washed with ethyl acetate (3 x 15 mL), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 6:5) and recrystallization from ether to afford 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl methanedithiosulfonate **4** (27 mg, 13%) as a white crystalline solid: R_f 0.4 (ethyl acetate:petrol, 7:3); m.p. 147-148 ºC (ether); $[\alpha]_D^{25}$ -238 (c, 0.5 in $CHCl_3$); υ_{max} (KBr disc) 1753 (s, C=O) 1326 (s, SO_2) 1258, 1226 (2 x s, S-S) 1141 (s, SO_2) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.02, 2.04, 2.08, 2.09 (12H, 4 x s, 4 x C(O)CH_3), 3.39 (3H, s, CH_3SO_2), 3.83 (1H, ddd, $J_{4,5}$ 10.4 Hz, $J_{5,6}$ 4.6 Hz, $J_{5,6'}$ 2.1 Hz, H-5), 4.12 (1H, dd, $J_{5,6}$ 4.6 Hz, $J_{6,6'}$ 12.8 Hz, H-6), 4.34 (1H, dd, $J_{5.6'}$ 2.1 Hz, $J_{6,6'}$ 12.8 Hz, H-6'), 4.76 (1H, d, $J_{1,2}$ 10.0 Hz, H-1), 5.11 (1H, at, *J* 9.8 Hz, H-4), 5.16 (1H, at, *J* 9.6 Hz, H-2), 5.28 (1H, at, *J* 9.3 Hz, H-3); δ_C (128.8 MHz, $CDCl_3$) 20.5, 20.6, 20.7 (3 x q, 4 x C(O)$\underline{C}H_3$), 47.3 (q, CH_3SO_2), 61.4 (t, C-6), 67.5 (d, C-4),

69.6 (d, C-2), 73.3 (d, C-3), 76.6 (d, C-5), 87.2 (d, C-1), 169.3, 169.4, 169.9, 170.3 (4 x s, 4 x $\underline{C}(O)CH_3$); *m/z* (ES^+) 497 (MNa^+, 100%) 533 ($MMeCNNH_4^+$, 30%). (HRMS (ES^+) Calcd. for $C_{15}H_{22}NaO_{11}S_3$ (MNa^+) 497.0216. Found: 497.0215); (Found: C, 37.73%; H, 4.68%, S, 20.02%. $C_{15}H_{22}O_{11}S_3$ requires: C, 37.97%; H, 4.67%, S, 20.27%);

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl methanethiosulfonate[5] **3** (70 mg, 33%) was also obtained as a white crystalline solid**:** R_f 0.5 (ethyl acetate:petrol, 7:3); m.p. 149-151 ºC (ether) [Lit. 151-152 ºC (ether)][5]; $[\alpha]_D^{23}$ -24.5 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{27}$ -19.0 (c, 1.24 in $CHCl_3$)][5]; δ_H (400 MHz, $CDCl_3$) 2.02, 2.06, 2.07, 2.09 (12H, 4 x s, 4 x $C(O)CH_3$), 3.45 (3H, s, CH_3SO_2), 3.82-3.87 (1H, m, H-5), 4.11 (1H, dd, $J_{5,6}$ 6.1 Hz, $J_{6,6'}$ 12.8 Hz, H-6), 4.33 (1H, dd, $J_{5.6'}$ 1.5 Hz, $J_{6,6'}$ 12.8 Hz, H-6'), 5.07 (1H, at, *J* 9.6 Hz, H-4), 5.08 (1H, dd, $J_{1,2}$ 10.5 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.27 (1H, d, $J_{1,2}$ 10.5 Hz, H-1), 5.31 (1H, at, *J* 9.5 Hz, H-3); δ_C (100.7 MHz, $CDCl_3$) 20.5, 20.6, 20.7 (3 x q, 4 x $C(O)\underline{C}H_3$), 52.8 (q, CH_3SO_2), 61.8 (t, C-6), 67.8 (d, C-4), 68.6 (d, C-2), 73.2 (d, C-3), 76.5 (d, C-5), 86.4 (d, C-1), 169.4, 169.5, 169.9, 170.3 (4 x s, 4 x $\underline{C}(O)CH_3$); *m/z* (ES^+) 465 (MNa^+, 100%). (HRMS (ES^+) Calcd. for $C_{15}H_{22}NaO_{11}S_2$ (MNa^+) 465.0501. Found: 465.0502); (Found: C, 40.26%; H, 5.03%. $C_{15}H_{22}O_{11}S_2$ requires: C, 40.72%; H, 5.01%);

Protein Glycosylation Procedure

SBLS156C (2.5 mg, 0.94 μmol) was dissolved in buffer solution (0.5 mL, 70 mM CHES, 5mM MES, 2mM $CaCl_2$, pH 9.5). 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl methanedithiosulfonate (Glc-MDTS) **4** (6 mg, 0.013 mmol) was dissolved in acetonitrile (200 μL). The sugar solution (100 μL, 50 equivalents) was added to the protein solution and placed on an end-over-end rotator. After 10 min, the reaction was purified by size exclusion chromatography using a PD-10 column and analysed by LC-mass spectrometry (observed mass, 27104; calculated mass, 27110).

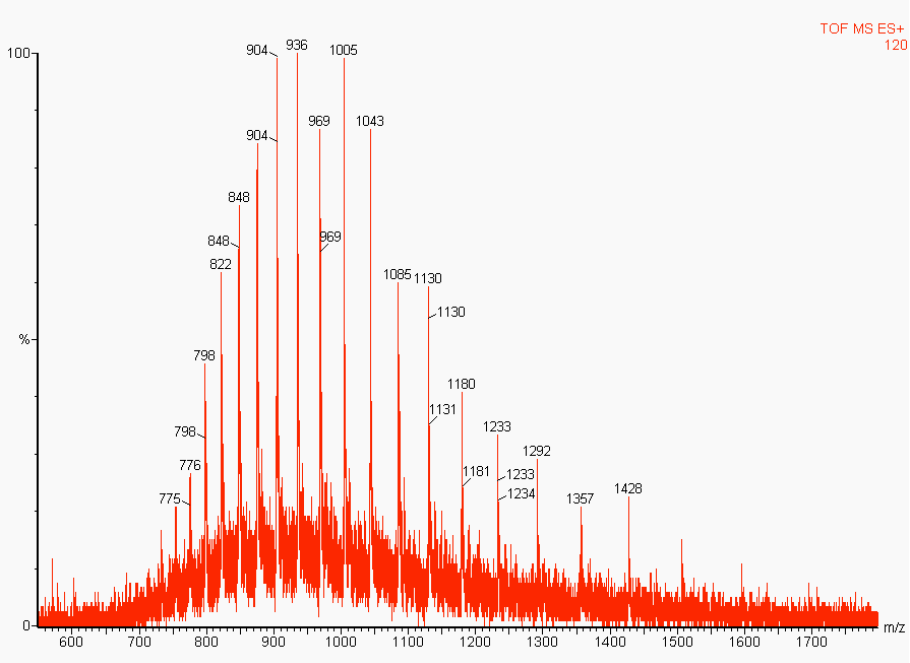


Figure 1. ESI mass spectrum of SBL-SSS-**4**.

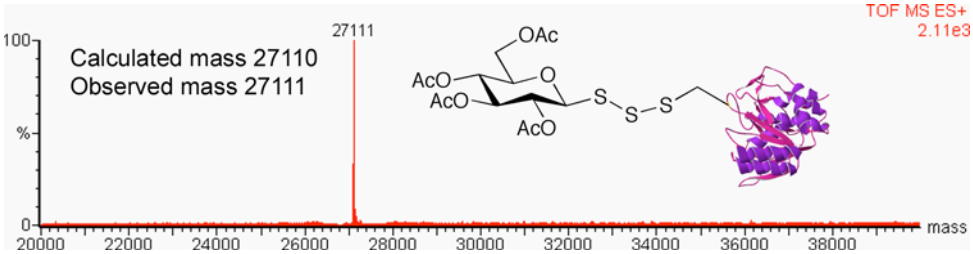


Figure 2. Deconvoluted ESI mass spectrum of SBL-SSS-**4**.

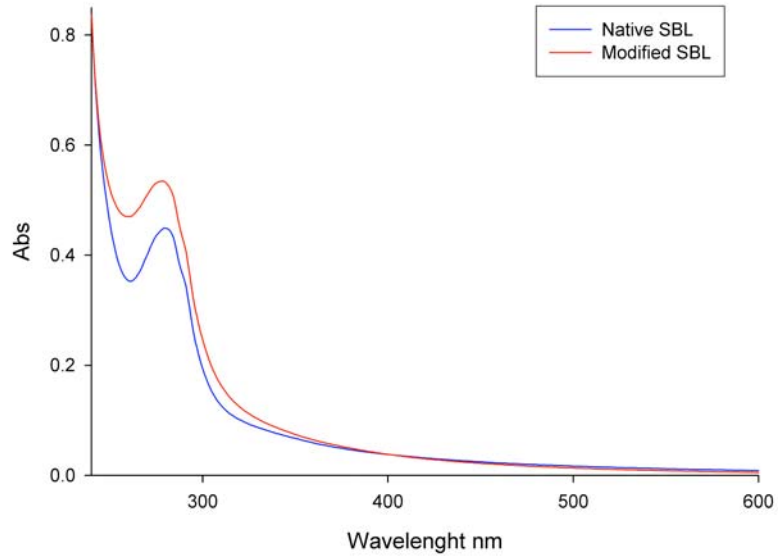


Figure 3. UV spectrum of SBL-SSS-**4**.

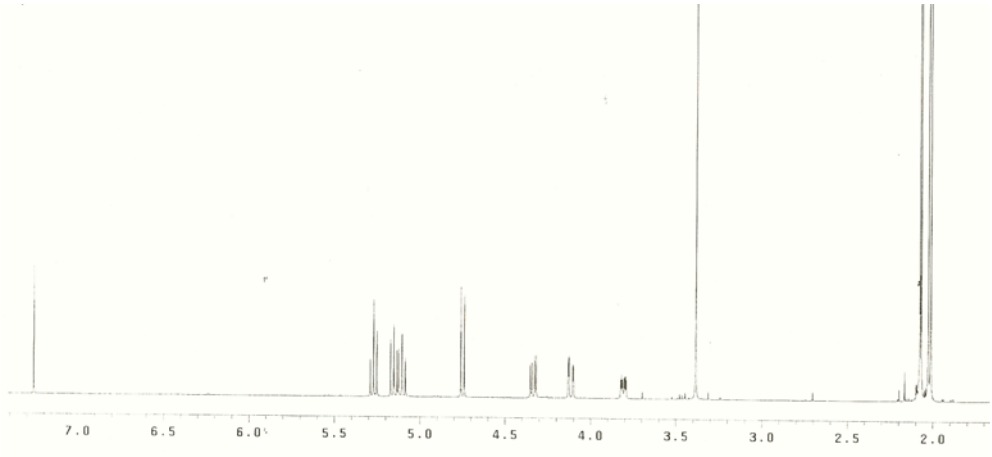


Figure 4. 1H-NMR of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl methanedithiosulfonate (Glc-DMTS) **4**.

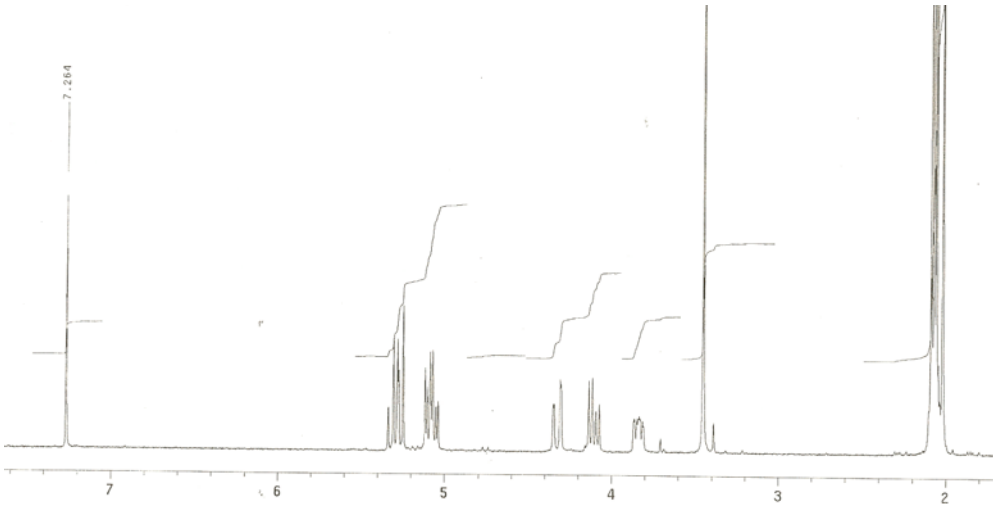


Figure 5. 1H-NMR of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl methanethiosulfonate (Glc-MTS) **3**.

References

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