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Carbasugar–thioether pseudodisaccharides related to N-glycan biosynthesis

Ian Cumpstey [a,*], Dominic S. Alonzi [b], Terry D. Butters [b]

[a] Department of Organic Chemistry, Stockholm University, Arrhenius Laboratory, 106 91 Stockholm, Sweden
[b] Department of Biochemistry, Oxford Glycobiology Institute, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

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ABSTRACT

Analogues of the α-Glc*p*-(1→3)-α-Glc*p* and α-Glc*p*-(1→3)-α-Man*p* disaccharides (representing the two α-*gluco* linkages cleaved by α-Glucosidase II in N-glycan biosynthesis) in which the non-reducing-end sugar is replaced by a carbasugar and the inter-glycosidic oxygen by a sulfur were synthesised. The key coupling step was an S_N2 displacement of an equatorial triflate at C-1 of the carbasugar by C-3 *gluco* or *manno* thiolates with inversion of configuration to give thioether pseudodisaccharides with axial substitution at C-1 of the carbasugar. The deprotected pseudodisaccharides failed to inhibit the action of α-Glucosidase II as measured both by an in vitro assay and by free oligosaccharide (FOS) analysis from cell studies.

1. Introduction

During N-glycan biosynthesis, a $Glc_3Man_9GlcNAc_2$ tetradecasaccharide is assembled with a dolichol phosphate aglycon and transferred to newly synthesised polypeptides in the endoplasmic reticulum before completion of folding to give the finished glycoproteins.[1] After glycosyl transfer to the protein, the oligosaccharide is remodelled by glycosidases and glycosyltransferases to give high mannose, complex and hybrid structures, this process begins with sequential cleavage of the terminal glucose residues by the two α-Glucosidases I and II (Fig. 1a). The transient presence of the three α-glucosides has been proposed to be important for glycosyl transfer by oligosaccharyltransferase (OST) complex,[2] and also the monoglucosylated oligosaccharide is bound by the lectin chaperones Calnexin and Calreticulin in a quality control process to ensure correct protein folding.[3] A recently described lectin with high selectivity for the diglucosylated oligosaccharide may also be relevant in N-glycan biosynthesis.[4]

It has been proposed that α-Glucosidase II has two different active sites, each responsible for the cleavage of one of the α-Glc*p*-(1→3)-α-Glc*p* or α-Glc*p*-(1→3)-α-Man*p* linkages.[5] A difference in the rate of cleavage of these two glucose residues suggests that the enzyme recognises a larger part of the structure than just the terminal monosaccharide. The two disaccharides are substrates for the enzyme,[6] and in designing inhibitors for α-Glucosidase II, disaccharide-mimicking structures may give more enzyme-specific inhibition than a monosaccharide-mimicking inhibitor, such as *N*-butyl deoxynojirimycin (NB-DNJ).[7] Formal replacement of the glucose ring-oxygen with a methylene group to give a carbasugar[8,9] renders the pseudodisaccharide stable to the hydrolytic action of the enzyme. In this paper, we report our results on the synthesis of thioether-linked carbasugar pseudodisaccharides **1** and **2** mimicking the two linkages cleaved by α-Glucosidase II (Fig. 1b and c).

1-Thio-carbasugars have been shown to inhibit enzymes before.[10–13] Only one example of a thioether-linked saturated carbasugar pseudodisaccharide appears to be known, mimicking α-Glc*p*-(1→4)-Glc*p*.[14] It acts as a glycosidase inhibitor, albeit a weak one. It has been shown that the 1-amino-carbasugars valienamine (C-5=C-5a unsaturated) and validamine (saturated) inhibit α-Glucosidases I and II.[15] A valienamine-based mimic of α-Glc*p*-(1→2)-Glc*p* failed to inhibit α-Glucosidase I.[16] A mimic of the α-Glc*p*-

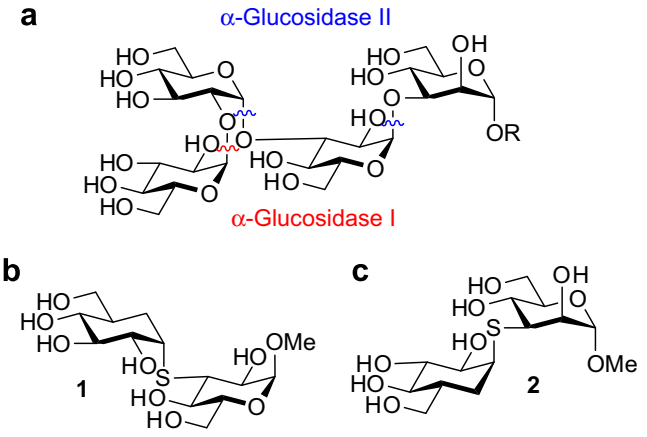

Figure 1.

* Corresponding author. Tel.: +46 8 16 2481; fax: +46 8 15 4908.
E-mail address: cumpstey@organ.su.se (I. Cumpstey).

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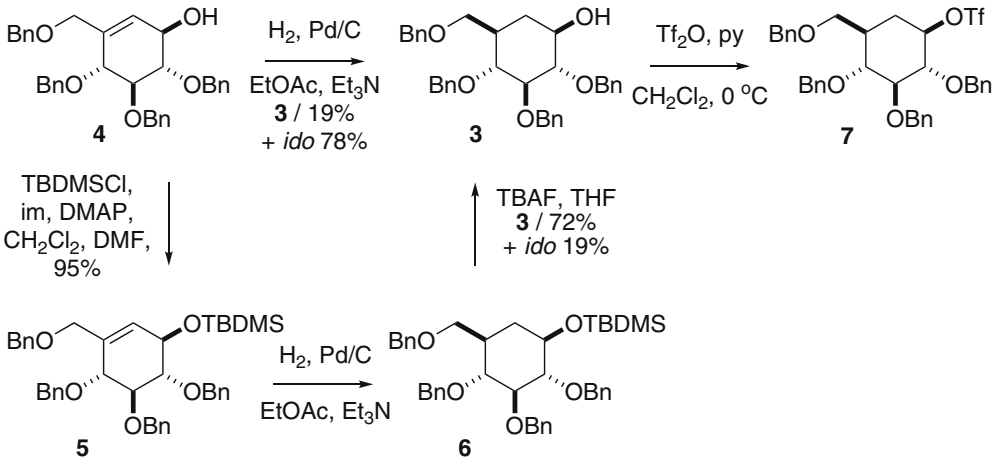


Scheme 1.

(1→3)-Glc*p* disaccharide in which the non-reducing-end glucose is replaced by an inositol and with an inter-residue amine bridge has been synthesised, but this compound failed to inhibit α-Glucosidase II.[17,18]

2. Results and discussion

Our synthetic strategy for pseudodisaccharide formation was based on thioetherification under conditions favouring S_N2 displacement of an equatorial triflate at C-1 of a carbasugar by *gluco* and *manno* C-3 thiolates to ensure formation of the axial thioether linkages.[19] Similar S_N2 displacement of carbohydrate triflates by anomeric thiolates is a well-known method for the synthesis of thiooligosaccharides.[20–22] The synthesis of the carbaglucose alcohol **3** has been described,[11] but here we detail an alternative route from the unsaturated carbocycle **4**, which is accessible from L-sorbose by a ring-closing metathesis approach.[23] Hydrogenation of the carbon–carbon double bond in **4** gave mainly the *ido* diastereomer (*ido*/*gluco*, 4:1), but protection with a bulky TBDMS ether blocked the top face, and hydrogenation of fully protected species **5** reversed the diastereoselectivity, and the required *gluco* diastereomer was the major product (*ido*/*gluco*, 1:3). Silyl ether removal and triflation gave the S_N2 electrophile **7** (Scheme 1).

The required S-3 *gluco* **8** and *manno* **9**[24] compounds were obtained by thioacetate displacement of triflates of the OH-3 *allo* **10**[25] and *altro* **11**[26] alcohols. The moderate yields are explained by competing elimination reactions (Scheme 2). For the formation of the *gluco* compound **8**, best results were found using THF as solvent (**8**, 51%; elimination, 13%), while DMF (**8**, 30%; elimination, 42%) gave rather worse selectivity.[†]

In contrast, in the formation of the *manno* compound **9**, better selectivity for substitution was found with DMF as solvent (**9**, 38%; elimination, 53%)[24] than THF (**5**, 26%; elimination, 60%). The origin of this difference in selectivity is not clear.

The *manno* thiol was formed by methanolysis of the thioester protection in **9** using excess sodium methoxide, and then coupled with the carbasugar triflate **7** to give the pseudodisaccharide **13**. For the *gluco* nucleophile, both the 3-thioacetate and the 2-benzoate were removed from **8** with excess methoxide, and after coupling with the carbasugar triflate **7**, it was necessary to acetylate the free OH-2 in the pseudodisaccharide product **12** to allow purification. Both pseudodisaccharides **12** and **13** were formed as single diastereomers with inversion of configuration at C-1 of the carbasugar in the thioether-forming reaction.

Deprotection of the pseudodisaccharides was carried out under Birch conditions: the presence of the sulfur ruled out catalytic hydrogenolysis for benzyl ether deprotection, but the Birch reduction worked well, and peracetylation facilitated purification of the pseudodisaccharides **14** and **15**. Deacetylation with sodium methoxide then gave the free pseudodisaccharides **1** and **2** (Scheme 3).

Both pseudodisaccharides were non-inhibitory to α-Glucosidase II activity at concentrations of 100 μM when measured using both $Glc_1Man_7GlcNAc_2$ and $Glc_2Man_7GlcNAc_2$ oligosaccharides labelled with anthranilic acid (2AA) in vitro. In contrast, NB-DNJ had an IC_{50} of 9.7 ± 0.7 μM and an IC_{90} of 110 ± 0.9 μM for $Glc_1Man_7GlcNAc_2$, and was totally inhibitory at 100 μM for α-Glucosidase-mediated hydrolysis of $Glc_2Man_7GlcNAc_2$–2AA. Using $Glc_2Man_5GlcNAc_1$–2AA as a substrate, NB-DNJ had an IC_{50} value of 10.8 ± 1.13 μM.[27]

The pseudodisaccharides were evaluated for α-Glucosidase II inhibition in HL60 cells for 24 h at a non-cytotoxic concentration of 100 μM, and no inhibition was observed using the free oligosaccharide (FOS) assay.[27] NP-HPLC profiles of fluorescently labelled oligosaccharides were identical to non-inhibitor treated FOS profiles (results not shown). The combination of a cell-free and a cellular-based assay provides convincing evidence for the lack of inhibition of α-Glucosidase II by the pseudodisaccharides synthesised in this paper.

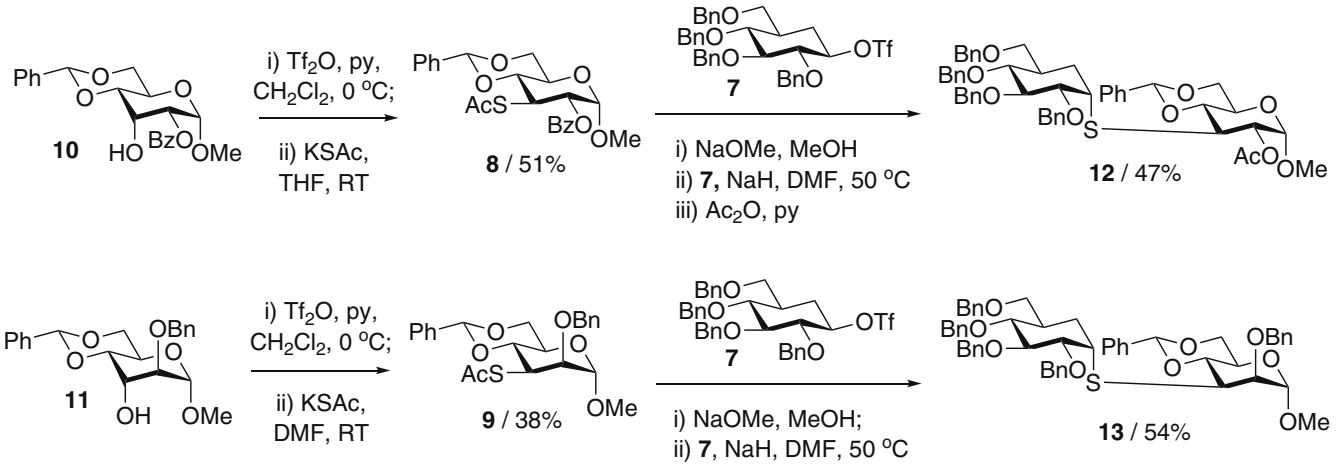
To conclude, thioether α-(1–3)-linked carbasugar pseudodisaccharides are synthesised by an S_N2 route by displacement of an equatorial carbasugar C-1 triflate by carbohydrate thiolates. The pseudodisaccharides failed to inhibit α-Glucosidase II. It is possible that better inhibition would be obtained with C-5=C-5a unsaturated carbasugars or analogues where the inter-glycosidic heteroatom is nitrogen rather than sulfur.[14] Nitrogen may be protonated when bound to the enzyme, and may bind more tightly than a neutral ligand either by mimicking the charge build-up at the transition state of the cleavage reaction or because of favourable electrostatic interactions with carboxylate side-chains in the active site.[28] We hope to report further results of our investigations in this area shortly.

3. Experimental

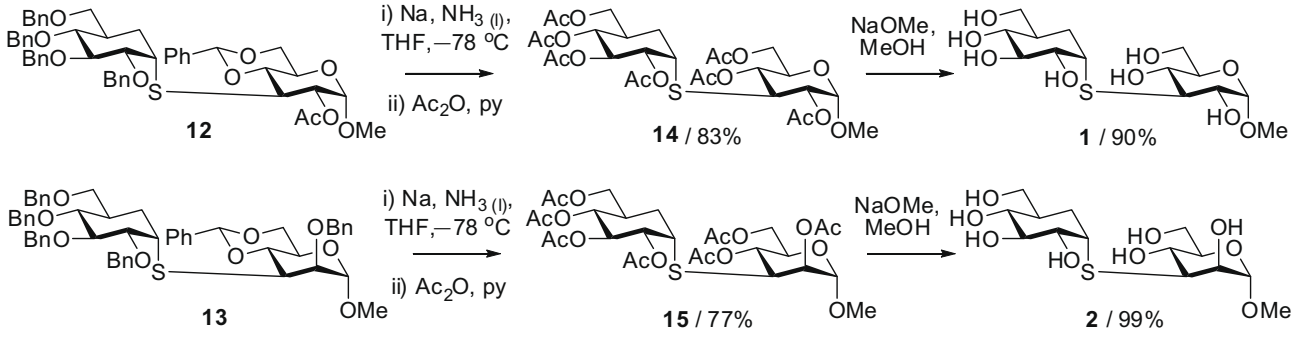
3.1. General methods

Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^1H) spectra were recorded on Bruker Avance II 400

[†] Acetonitrile (**8**, 42%; elimination, 32%) and butanone 80 °C (**8**, 42%; elimination, 48%) were also tested.



Scheme 2.



Scheme 3.

(400 MHz) or 500 (500 MHz) or Varian Mercury 400 (400 MHz) spectrometers; multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), apparent triplet (at), apparent triplet of doublets (atd), doublet of apparent triplets (dat), quartet (q), apparent quartet (aq), AB quartet (ABq) or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded on Bruker Avance II 400 (100 MHz) or 500 (125 MHz) or Varian Mercury 400 (100 MHz) spectrometers, and multiplicities assigned by DEPT. Spectra were assigned using COSY, HSQC and DEPT experiments. All chemical shifts are quoted on the δ scale in parts per million (ppm). Residual solvent signals were used as an internal reference ($CDCl_3$: 7.26 and 77.16 ppm); spectra in D_2O were calibrated with either MeOH (3.34 and 49.50 ppm) or AcOH (2.08 and 21.03 ppm). Low- and high-resolution (HRMS) electrospray (ESI) mass spectra were recorded using a Bruker Microtof instrument. Infra-red spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer using the thin film method on a NaCl plate. 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 (TLC) was carried out on Merck Kieselgel sheets, pre-coated with $60F_{254}$ silica. Plates were visualised with UV light and developed using 10% sulfuric acid, or an ammonium molybdate (10% w/v) and cerium (IV) sulfate (2% w/v) solution in 10% sulfuric acid. Flash column chromatography was carried out on silica gel (35–70 μm, Grace). Reactions that were performed under an atmosphere of hydrogen or nitrogen were maintained by an inflated balloon.

3.2. 2,3,4,6-Tetra-*O*-benzyl-1-*O*-(*tert*-butyldimethylsilyl)-5a-carba-β-D-xylo-hex-5(5a)-enopyranose (5)

Alcohol **4** (278 mg, 0.52 mmol) was dissolved in a mixture of CH_2Cl_2 (5 mL) and DMF (5 mL). Imidazole (106 mg, 1.56 mmol) and TBDMSCl (233 mg, 1.55 mmol) were added, and the mixture stirred at rt. After 20 h, TLC (pentane–EtOAc 3:1) showed formation of a single product (R_f = 0.9) and no remaining starting material (R_f = 0.3). The mixture was added to brine (50 mL) and extracted with Et_2O (2 × 50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2) to give the silyl ether **5** (320 mg, 95%) as a colourless oil; $[\alpha]_D^{21}$ −47.4 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.12, 0.14 (6H, 2 × s, 2 × CH_3), 0.95 (9H, s, $C(CH_3)_3$), 3.62 (1H, dd, $J_{2,3}$ = 10.5 Hz, J = 7.8 Hz, H-2 or H-3), 3.81 (1H, dd, J = 7.8 Hz, H-2 or H-3), 3.93 (1H, d, $J_{6,6'}$ = 12.3 Hz, H-6), 4.24 (1H, dd, H-6′), 4.35 (1H, d, J = 7.8 Hz, H-1 or H-4), 4.41 (1H, d, J = 7.8 Hz, H-1 or H-4), 4.50, 4.55 (2H, ABq, J_{AB} = 11.9 Hz, PhCH_2), 4.73, 4.85 (2H, ABq, J_{AB} = 10.9 Hz, PhCH_2), 4.80, 4.98 (2H, ABq, J_{AB} = 11.0 Hz, PhCH_2), 4.89, 4.92 (2H, ABq, J_{AB} = 11.3 Hz, PhCH_2), 5.63 (1H, s, H-5a), 7.26–7.38 (20H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.4, −4.4 (2 × q, $Si(CH_3)_2$), 18.2 (s, Si*C*$(CH_3)_3$), 26.0 (q, SiC(CH_3)$_3$), 70.0 (t, C-6), 72.3, 74.9, 75.6, 75.6 (4 × t, 4 × PhCH_2), 73.2, 80.5 (2 × d, C-1, C-4), 84.7, 85.1 (2 × d, C-2, C-3), 127.5, 127.6, 127.7, 127.9, 128.0, 128.4, 128.5, 128.5, 128.5, 129.2 (10 × d, Ar-CH, C-5a), 135.3 (s, C-5), 138.4, 138.8, 138.8, 139.0 (4 × s, 4 × Ar-C); *m/z* (ESI^+) 673 ([M + Na]$^+$, 100%); HRMS (ESI^+) calcd for $C_{41}H_{50}O_5SiNa$ [M+Na]$^+$: 673.3320, found 673.3320.

3.3. 2,3,4,6-Tetra-*O*-benzyl-5a-carba-β-D-glucopyranose (3)

Silyl ether **5** (314 mg, 0.49 mmol) was dissolved in EtOAc (10 mL), and Et_3N (0.1 mL, 0.74 mmol) was added. Palladium (10% on carbon, 30 mg) was added, and the mixture was degassed and stirred under H_2. After 18 h, the mixture was filtered through Celite and concentrated in vacuo.

The residue was dissolved in THF (3 mL), TBAF (1 M in THF, 1.5 mL, 1.5 mmol) was added, and the mixture was stirred at 50 °C. After 2 h 30 min, TLC (pentane–EtOAc, 9:1) showed formation of product ($R_f = 0$) and no remaining starting material ($R_f = 0.8$). The mixture was concentrated in vacuo, and the residue was purified by flash column chromatography (CH_2Cl_2–Et_2O 30:1) to give the *ido* alcohol (49 mg, 19%) and the *gluco* alcohol **3** (186 mg, 72%)[11] as white crystals, mp 97–99 °C (EtOAc–pentane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (1H, aq, $J = 12.6$ Hz, H-5a), 1.76 (1H, m, H-5), 2.04 (1H, dat $J_{5a,5a'} = 13.2$ Hz, $J_{at} = 4.2$ Hz, H-5a′), 3.33 (1H, ddd, $J = 2.2$ Hz, $J = 6.9$ Hz, $J = 9.1$ Hz), 3.51 (1H, dd, $J_{5,6} = 2.6$ Hz, $J_{6,6'} = 8.9$ Hz, H-6), 3.55–3.60 (3H, m), 3.63 (1H, dd, $J_{5,6'} = 5.0$ Hz, H-6′), 4.47 (2H, s, PhCH_2), 4.56 (1H, d, $J = 10.8$ Hz, PhC*H*H′), 4.71, 5.01 (2H, ABq, $J_{AB} = 11.3$ Hz, PhCH_2), 4.88–4.96 (3H, m, PhCH_2, PhCH*H*′), 7.22–7.37 (20H, m, Ar-H).

3.4. Methyl 3-*S*-acetyl-2-*O*-benzoyl-4,6-*O*-benzylidene-3-deoxy-3-thio-α-D-glucopyranoside (8)

Alcohol **10** (1.00 g, 2.59 mmol) was dissolved in CH_2Cl_2 and cooled to 0 °C under N_2. Pyridine (0.46 mL, 5.72 mmol) and Tf_2O (0.47 mL, 2.78 mmol) were added. After 5 min, the ice-bath was removed. After 30 min, TLC (pentane–EtOAc 3:1) showed the presence of a single reaction product ($R_f = 0.3$). The mixture was poured into ice-water (100 mL) and extracted with CH_2Cl_2 (100 + 30 mL). The organic phases were dried (Na_2SO_4), filtered and concentrated in vacuo to give crude triflate (1.34 g), which was used without further purification.

Triflate (497 mg, 0.96 mmol) was dissolved in THF. KSAc (2.19 mg, 1.92 mmol) was added, and the mixture stirred at rt under N_2. After 72 h, brine (50 mL) was added, and the mixture was extracted with Et_2O (2 × 50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane–EtOAc 5:1) to give the thioacetate **8** (219 mg, 51%) as an oil, which was recrystallised to give white crystals, mp 129–132 °C (EtOAc–pentane); $[\alpha]_D^{22}$ +107 (*c* 1.0, $CHCl_3$); ν 1703, 1722 (2 × C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (3H, s, C(O)CH_3), 3.43 (3H, s, OCH_3), 3.66 (1H, dd, $J_{3,4} = 11.3$ Hz, $J_{4,5} = 9.2$ Hz, H-4), 3.77 (1H, at $J = 10.4$ Hz, H-6), 4.02 (1H, atd, $J = 9.7$ Hz, $J_{5,6'} = 4.8$ Hz, H-5), 4.32 (1H, dd, $J_{6,6'} = 10.4$ Hz, H-6′), 4.40 (1H, at $J = 11.3$ Hz, H-3), 5.04 (1H, d, $J_{1,2} = 3.6$ Hz, H-1), 5.14 (1H, dd, $J_{2,3} = 11.4$ Hz, H-2), 5.54 (1H, s, PhC*H*), 7.34–7.49 (7H, m, Ar-H), 7.57 (1H, m, A-H), 8.03–8.06 (2H, m, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.0 (q, C(O)*C*H$_3$), 44.3 (d, C-3), 55.5 (q, OCH_3), 64.6 (d, C-5), 69.1 (t, C-6), 71.8 (d, C-2), 78.2 (d, C-4), 97.5 (d, C-1), 102.0 (d, Ph*C*H), 126.4, 128.4, 128.6, 129.2, 130.2, 133.6 (6 × d, Ar-CH), 129.3, 137.2 (2 × s, 2 × Ar-C), 166.0 (s, O*C*=O), 193.8 (s, S*C*=O); *m/z* (ESI$^+$) 911 ([2M+Na]$^+$, 20), 467 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{23}H_{24}O_7SNa$ [M+Na]$^+$: 467.1135, found 467.1128. Anal. Calcd for $C_{23}H_{24}O_7S$: C, 62.15; H, 5.44; S, 7.21. Found: C, 62.17; H, 5.60; S, 7.07.

Elimination products (46 mg, 13%), two isomers that were inseparable were also produced.

3.5. Methyl 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-thio-α-D-glucopyranoside (12)

Carbasugar alcohol **3** (100 mg, 0.19 mmol) was dissolved in CH_2Cl_2 (2 mL) under N_2 and cooled to 0 °C. Pyridine (39 μL, 0.48 mmol) and Tf_2O (39 μL, 0.23 mmol) were added, and the mixture was stirred at 0 °C. After 30 min, TLC (pentane–EtOAc 4:1) showed product formation ($R_f = 0.8$) and remaining starting material ($R_f = 0.1$). Further pyridine (39 μL, 0.48 mmol) and Tf_2O (39 μL, 0.23 mmol) were added. After a further 30 min, TLC showed that only traces of starting material remained. The reaction mixture was poured into ice-water (25 mL) and extracted with CH_2Cl_2 (25 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give the triflate **7** that was used without further purification.

Sodium (9 mg, 0.39 mmol) was dissolved in MeOH (2 mL) and added to the thioacetate **8** (55 mg, 0.12 mmol). The solution was degassed and stirred at 50 °C under N_2. After 25 min, TLC (pentane–EtOAc 4:1) showed the complete consumption of starting material ($R_f = 0.7$) and the formation of two products ($R_f = 0.8$ and 0.3). After a further 35 min, the less polar component had been completely converted into the more polar component. The reaction mixture was added to NH_4Cl (satd aq, 30 mL) and extracted with CH_2Cl_2 (25 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give the thiol, which was used without further purification.

Crude triflate **7** was dissolved in DMF (2 × 1.5 mL) and added to the thiol. The mixture was degassed and left under N_2. Sodium hydride (60% in oil, 20 mg, 0.50 mmol) was added, and the mixture was stirred at 50 °C. After 30 min, TLC (pentane–EtOAc 2:1) showed the presence of a major component ($R_f = 0.3$). The mixture was added to NH_4Cl (satd aq, 30 mL) and extracted with Et_2O (2 × 30 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane–EtOAc 2:1). Product-containing fractions were concentrated in vacuo, and the residue was dissolved in pyridine (1.5 mL) and Ac_2O (1.5 mL) and stirred at rt. After 18 h, the mixture was diluted with EtOAc (30 mL) and washed with H_2SO_4 (1 M, 30 mL) and $NaHCO_3$ (satd aq, 30 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane–EtOAc 3:1) to give the thioether pseudodisaccharide **12** (50 mg, 47%) as a colourless oil; $[\alpha]_D^{20}$ +67.5 (*c* 1.0, $CHCl_3$); ν 1746 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71 (1H, m, H-5a^{II}), 2.03 (1H, dat, $J_{5a,5a'} = 14.5$ Hz, $J = 3.3$ Hz, H-5a′^{II}), 2.11 (3H, s, C(O)CH_3), 2.35 (1H, m, H-5^{II}), 3.36 (1H, at, $J = 11.1$ Hz, H-3^{I}), 3.40–3.53 (3H, m, H-2^{II}, H-4^{II}, H-6^{II}), 3.43 (3H, s, OCH_3), 3.62 (1H, dd, $J_{3,4} = 10.5$ Hz, $J_{4,5} = 9.3$ Hz, H-4^{I}), 3.45–3.53 (3H, m, H-6^{I}, H-3^{II}, H-6′^{II}), 3.84 (1H, aq, $J = 4.2$ Hz, H-1^{II}), 3.89 (1H, atd, $J_{5,6'} = 4.8$ Hz, $J = 9.8$ Hz, H-5^{I}), 4.20, 4.57 (2H, ABq, $J_{AB} = 12.2$ Hz, PhCH_2), 4.27 (1H, dd, $J_{6,6'} = 10.2$ Hz, H-6′^{I}), 4.42, 4.47 (2H, ABq, $J_{AB} = 12.0$ Hz, PhCH_2), 4.48, 4.86 (2H, ABq, $J_{AB} = 10.7$ Hz, PhCH_2), 4.67, 4.92 (2H, ABq, $J_{AB} = 10.7$ Hz, PhCH_2), 4.78 (1H, dd, $J_{1,2} = 3.6$ Hz, $J_{2,3} = 11.5$ Hz, H-2^{I}), 4.89 (1H, d, H-1^{I}), 5.56 (1H, s, PhC*H*), 7.02–7.46 (25H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.0 (q, C(O)*C*H$_3$), 29.6 (t, C-5a^{II}), 37.8 (d, C-5^{II}), 44.8 (d, C-3^{I}), 45.2 (d, C-1^{II}), 55.3 (q, OCH_3), 64.2 (d, C-5^{I}), 69.2 (t, C-6^{I}), 70.3 (t, C-6^{II}, PhCH_2), 71.4 (d, C-2^{I}), 73.2, 75.4, 75.7 (3 × t, 3 × PhCH_2), 80.8 (d, C-4^{II}), 81.3 (d, C-2^{II}), 83.1 (d, C-4^{I}), 83.8 (d, C-3^{II}), 97.3 (d, C-1^{I}), 102.8 (d, Ph*C*H), 126.6, 127.3, 127.5, 127.6, 127.6, 127.8, 128.0, 128.2, 128.4, 128.5, 128.5, 129.5 (12 × d, Ar-CH), 137.4, 138.2, 138.7, 139.0, 139.2 (5 × s, 5 × Ar-C), 170.3 (s, C=O); *m/z* (ESI$^+$) 1744 ([2M+Na]$^+$, 5), 899 ([M+K]$^+$, 10), 883 ([M+Na]$^+$, 100), 878 ([M+NH$_4$]$^+$, 45), 861 ([M+H]$^+$, 25%). HRMS (ESI$^+$) calcd for $C_{51}H_{57}O_{10}S$ [M+H]$^+$: 861.3667, found 861.3691.

3.6. Methyl 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-3-thio-α-D-mannopyranoside (13)

As described above, Triflate **7** was prepared from alcohol **3** (83 mg, 0.15 mmol), pyridine (54 μL, 66 mmol), and Tf_2O (54 μL, 32 mmol) in CH_2Cl_2 (1 mL), and was used crude.

Sodium (10 mg, 0.43 mmol) was dissolved in MeOH (1.5 mL) and added to a solution of thioacetate **9** (108 mg, 0.25 mmol) in MeOH (1.5 mL). The solution was degassed and stirred at rt under N_2. After 1 h, TLC (pentane–EtOAc 3:1) showed the complete consumption of starting material (R_f = 0.5) and the formation of a major product (R_f = 0.6) along with traces of a by-product (R_f = 0.4). The reaction mixture was added to NH_4Cl (satd aq, 50 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography to give the thiol (83 mg, 85%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.04 (1H, d, $J_{SH,3}$ = 8.3 Hz, SH-3), 3.38 (3H, s, OCH_3), 3.42 (1H, m, H-3), 3.72 (1H, dd, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 3.78–3.82 (3H, m, H-4, H-5, H-6), 4.24 (1H, m, H-6′), 4.68, 4.73 (2H, ABq, J_{AB} = 11.5 Hz, PhC*H*$_2$), 4.71 (1H, d, H-1), 5.60 (1H, s, PhC*H*), 7.32–7.53 (10H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 40.7 (d, C-3), 55.0 (q, OCH_3), 65.7, 79.6 (2 × d, C-4, C-5), 68.9 (t, C-6), 74.0 (t, PhCH_2), 79.8 (d, C-2), 98.3 (d, C-1), 102.1 (d, PhC*H*), 126.2, 128.2, 128.4, 128.4, 128.6, 129.1 (6 × d, Ar-CH), 137.5, 137.6 (2 × s, Ar-C).

Thiol (83 mg, 0.19 mmol) was dissolved in DMF (2 × 1.5 mL) and added to the crude triflate **7**. The mixture was degassed and left under N_2. Sodium hydride (60% in oil, 20 mg, 0.50 mmol) was added, and the mixture was stirred at 50 °C. After 40 min, TLC (pentane–EtOAc, 3:1) showed the presence of a major component (R_f = 0.4), no remaining triflate (R_f = 0.6), some thiol remaining (R_f 0.5) and some more polar by-products. The mixture was added to NH_4Cl (satd aq, 30 mL) and extracted with Et_2O (2 × 30 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane–EtOAc 4:1) to give the thioether pseudodisaccharide **13** (72 mg, 54%) as a colourless oil; $[\alpha]_D^{22}$ +20.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65 (1H, ddd, $J_{1,5a}$ = 3.0 Hz, $J_{5,5a}$ = 12.2 Hz, $J_{5a,5a'}$ = 14.9 Hz, H-5a^{II}), 2.01 (1H, dat, *J* = 3.3 Hz, H-5a′^{II}), 2.43 (1H, m, H-5^{II}), 3.23 (1H, dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 11.0 Hz, H-3^{I}), 3.35 (3H, s, OCH_3), 3.39 (1H, dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.7 Hz, H-4^{II}), 3.49–3.53 (2H, m, H-2^{II}, H-6^{II}), 3.69 (1H, dd, $J_{5,6'}$ = 5.0 Hz, $J_{6,6'}$ = 9.1 Hz, H-6′^{II}), 3.71 (1H, dd, $J_{1,2}$ = 2.0 Hz, H-2^{I}), 3.78 (1H, at, *J* = 9.4 Hz, H-3^{II}), 3.82–3.85 (3H, m, H-5^{I}, H-6^{I}, H-1^{II}), 4.07–4.12 (2H, m, H-4^{I}, PhC*H*H′), 4.22 (1H, m, H-6′^{I}), 4.42, 4.46 (2H, ABq, J_{AB} = 11.9 Hz, PhCH_2), 4.47, 4.87 (2H, ABq, J_{AB} = 10.8 Hz, PhCH_2), 4.55–4.58 (2H, m, PhCH*H*′, H-1^{I}), 4.64, 4.75 (2H, ABq, J_{AB} = 11.7 Hz, PhCH_2), 4.71, 4.97 (2H, ABq, J_{AB} = 10.8 Hz, PhCH_2), 5.60 (1H, s, PhC*H*), 6.98–7.45 (30H, m, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.3 (t, C-5a^{II}), 37.3 (d, C-5^{II}), 44.7 (d, C-1^{II}), 45.4 (d, C-3^{I}), 55.0 (q, OCH_3), 65.7 (d, C-5^{I}), 69.2 (t, C-6^{I}), 69.7, 73.1, 74.3, 75.5, 75.7 (5 × t, 5 × PhCH_2), 70.4 (t, C-6^{II}), 80.6, 80.7, 81.0, 81.1 (4 × d, C-2^{I}, C-4^{I}, C-2^{II}, C-4^{II}), 83.6 (d, C-3^{II}), 98.8 (d, C-1^{I}), 102.9 (d, PhC*H*), 126.5, 127.2, 127.4, 127.6, 127.7, 127.9, 128.0, 128.1, 128.1, 128.2, 128.3, 128.3, 128.4, 128.5, 129.3 (15 × d, Ar-CH), 137.8, 137.8, 138.3, 138.7, 139.0, 139.3 (6 × s, 6 × Ar-C); *m/z* (ESI$^+$) 947 ([M+K]$^+$, 40), 931 ([M+Na]$^+$, 50), 926 ([M+NH$_4$]$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{56}H_{60}O_9SNa$ [M+Na]$^+$: 931.3850, found 931.3843.

3.7. Methyl 2,3,4,6-tetra-*O*-acetyl-5a-carba-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3-deoxy-3-thio-α-D-glucopyranoside (14)

Ammonia (ca. 25 mL) was condensed into a flask at −78 °C. Sodium (ca. 200 mg, 8 mmol) was added, and the mixture turned deep blue. The pseudodisaccharide **12** (25 mg, 0.029 mmol) was dissolved in THF (1.5 mL) and transferred into the reaction vessel by pipette. Then, MeOH (2 × 50 μL, 2.5 mmol) was added, and the reaction mixture was stirred for a further 5 min. The blue colour persisted throughout this sequence. After this time, NH_4Cl was added until the blue colour disappeared, and the mixture was allowed to warm to rt. The resulting solid was extracted with MeOH/$CHCl_3$, 1:1, and filtered, and the filtrate was concentrated in vacuo.

The residue was suspended in pyridine (2 mL) and Ac_2O (2 mL), and was stirred at rt. After 18 h, the mixture was diluted with EtOAc (30 mL) and washed with H_2SO_4 (1 M, 30 mL) and $NaHCO_3$ (satd aq, 30 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane–EtOAc 1:2; 1% Et_3N) to give the heptaacetate **14** (18 mg, 93%) as a colourless oil; $[\alpha]_D^{21}$ +61.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.88 (1H, atd, $J_{1,5a}$ = 3.2 Hz, *J* = 13.7 Hz, H-5a^{II}), 1.98 (1H, m (obs), H-5a′^{II}), 1.99, 2.01, 2.05, 2.09, 2.11, 2.20 (21H, 6 × s, 7 × C(O)CH_3), 2.25 (1H, m, H-5^{II}), 3.38 (3H, s, OCH_3), 3.40 (1H, m (obs), H-3^{I}), 3.72 (1H, m, H-1^{II}), 3.86 (1H, m, H-5^{I}), 3.88 (1H, dd, $J_{5,6}$ = 2.8 Hz, $J_{6,6'}$ = 12.6 Hz, H-6^{II}), 4.07–4.10 (2H, m, H-6^{I}, H-6′^{II}), 4.19 (1H, dd, $J_{5,6'}$ = 4.8 Hz, $J_{6,6'}$ = 12.2 Hz, H-6′^{I}), 4.74 (1H, dd, $J_{1,2}$ = 2.9 Hz, $J_{2,3}$ = 11.3 Hz, H-2^{I}), 4.89–4.93 (2H, m, H-1^{I}, H-4^{I}), 4.95 (1H, at *J* = 10.0 Hz, H-4^{II}), 5.03 (1H, dd, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.2 Hz, H-2^{II}), 5.45 (1H, at, *J* = 9.6 Hz, H-3^{II}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 20.8, 20.9, 20.9, 21.0 (5 × q, 7 × C(O)*C*H$_3$), 30.6 (t, C-5a^{II}), 35.9 (d, C-5^{II}), 44.8 (d, C-1^{II}), 46.9 (d, C-3^{I}), 55.5 (q, OCH_3), 62.5 (t, C-6^{I}), 63.1 (t, C-6^{II}), 67.8 (d, C-4^{I}), 68.7 (d, C-5^{I}), 71.6 (d, C-4^{II}), 71.8 (d, C-3^{II}), 74.0 (d, C-2^{II}), 74.6 (d, C-2^{I}), 96.3 (d, C-1^{I}), 169.8, 169.9, 170.1, 170.2, 170.9 (5 × s, 7 × C=O); *m/z* (ESI$^+$) 687 (M+Na$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{28}H_{40}O_{16}SNa$ [M+Na]$^+$: 687.1929, found 687.1918.

3.8. Methyl 2,3,4,6-tetra-*O*-acetyl-5a-carba-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3-deoxy-3-thio-α-D-mannopyranoside (15)

Ammonia (ca. 10 mL) was condensed into a flask at −78 °C. Sodium (ca. 70 mg, 3 mmol) was added, and the mixture turned deep blue. The pseudodisaccharide **13** (35 mg, 0.039 mmol) was dissolved in THF (2 mL) and transferred into the reaction vessel by pipette. After 5 min, MeOH (20 μL, 0.49 mmol) was added, and the reaction mixture was stirred for a further 20 min. The blue colour persisted throughout this sequence. After this time, NH_4Cl was added until the blue colour disappeared, and the mixture was allowed to warm to rt. The resulting solid was extracted with MeOH–$CHCl_3$, 1:1, and filtered, and the filtrate was concentrated in vacuo.

The residue was suspended in pyridine (3 mL) and Ac_2O (3 mL), and was stirred at rt. After 3 h, the mixture was diluted with EtOAc (30 mL) and washed with HCl (1 M, 30 mL) and $NaHCO_3$ (satd aq, 30 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane–EtOAc 1:1; 1% Et_3N) to give the heptaacetate **15** (19 mg, 77%) as a colourless oil; $[\alpha]_D^{21}$ +47.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.83 (1H, ddd, $J_{1,5a}$ = 3.6 Hz, $J_{5,5a}$ = 12.8 Hz, $J_{5a,5a'}$ = 14.7 Hz, H-5a^{II}), 1.94 (1H, dat (obs), *J* = 3.3 Hz, H-5a′^{II}), 1.98, 2.01, 2.04, 2.07, 2.09, 2.14, 2.17 (21H, 7 × s, 7 × C(O)CH_3), 2.25 (1H, m, H-5^{II}), 3.35 (1H, dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 11.2 Hz, H-3^{I}), 3.38 (3H, s, OCH_3), 3.57 (1H, aq, *J* = 3.5 Hz, H-1^{II}), 3.85–3.91 (2H, m, H-5^{I}, H-6^{II}), 4.04 (1H, dd, $J_{5,6'}$ = 5.1 Hz, $J_{6,6'}$ = 11.3 Hz, H-6′^{II}), 4.08 (1H, dd, $J_{5,6}$ = 2.4 Hz, $J_{6,6'}$ = 12.3 Hz, H-6^{I}), 4.21 (1H, dd, $J_{5,6'}$ = 5.6 Hz, H-6′^{I}), 4.65 (1H, d, $J_{1,2}$ = 1.3 Hz, H-1^{I}), 4.92 (1H, dd, $J_{4,5}$ = 9.5 Hz, H-4^{II}), 4.97–5.06 (3H, m, H-2^{I}, H-4^{I}, H-2^{II}), 5.42 (1H, at, *J* = 9.8 Hz, H-3^{II}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 20.8, 20.8, 20.9, 21.0 (5 × q, C(O)*C*H$_3$), 30.2 (t, C-5a^{II}), 35.6 (d, C-5^{II}), 43.5 (d, C-1^{II}), 46.5 (d, C-3^{I}), 55.3 (q, OCH_3), 63.0 (t, C-6^{I}), 63.2 (t, C-6^{II}), 66.6 (d, C-4^{I}), 69.5 (d, C-5^{I}), 70.5 (d, C-2^{I}), 71.6 (d, C-4^{II}), 71.9 (d, C-3^{II}), 73.9 (d, C-2^{II}), 97.8 (d, C-1^{I}), 169.9, 170.1, 170.1, 170.2, 170.3, 170.8, 170.9 (7 × s, 7 × C=O); *m/z* (ESI$^+$) 687 ([M+Na]$^+$, 100), 682 ([M+NH$_4$]$^+$, 45%); HRMS (ESI$^+$) calcd for $C_{28}H_{40}O_{16}SNa$ [M+Na]$^+$: 687.1929, found 687.1911.

3.9. Methyl 5a-carba-α-D-glucopyranosyl-(1→3)-3-deoxy-3-thio-α-D-glucopyranoside (1)

Sodium (6 mg, 0.26 mmol) was dissolved in MeOH (1.5 mL) and added to the pseudodisaccharide heptaacetate **14** (16 mg, 0.024 mmol). After 4 h, TLC (CMAW) showed the presence of a single component (R_f = 0.2). Dowex 50X8 resin (H^+) was added, and the mixture was filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc–MeOH–water 7:2:1) to give the deprotected pseudodisaccharide **2** (8 mg, 90%) as a colourless oil; $[\alpha]_D^{22}$ +101 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.67 (1H, ddd, $J_{5a,5a'}$ = 14.3 Hz, $J_{5,5a}$ = 13.1 Hz, $J_{1,5a}$ = 3.5 Hz, H-5a^{II}), 1.88 (1H, m, H-5^{II}), 2.08 (1H, dat, J = 3.3 Hz, H-5a′^{II}), 2.93 (1H, at, J = 10.9 Hz, H-3^{I}), 3.24 (1H, dd, J = 9.1 Hz, J = 10.7 Hz, H-4^{II}), 3.43 (3H, s, OCH_3), 3.45–3.48 (2H, m, H-4^{I}, H-1^{II}), 3.51 (1H, at, J 9.5 Hz, H-3^{II}), 3.55 (1H, dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 11.2 Hz, H-2^{I}), 3.62–3.68 (3H, m, H-5^{I}, H-2^{II}, H-6^{II}), 3.72–3.76 (2H, m, H-6^{I}, H-6′^{II}), 3.85 (1H, dd, $J_{5,6'}$ = 2.2 Hz, $J_{6,6'}$ = 12.2 Hz, H-6′^{I}), 4.8 (1H, obs, H-1^{I}); ^{13}C NMR (125 MHz, D_2O): δ 31.2 (t, C-5a^{II}), 40.3 (d, C-5^{II}), 49.2 (c, C-1^{II}), 54.5 (d, C-3^{I}), 55.5 (q, OCH_3), 61.4 (t, C-6^{I}), 62.9 (t, C-6^{II}), 69.5 (d, C-4^{I}), 70.1 (d, C-2^{I}), 73.2, 74.2 (2 × d, C-5^{I}, C-2^{II}), 73.6 (d, C-4^{II}), 76.3 (d, C-3^{II}), 99.3 (d, C-1^{I}); *m/z* (ESI$^+$) 763 ([2M+Na]$^+$, 10), 393 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{14}H_{26}O_9SNa$ [M+Na]$^+$: 393.1190, found 393.1207.

3.10. Methyl 5a-carba-α-D-glucopyranosyl-(1→3)-3-deoxy-3-thio-α-D-mannopyranoside (2)

Sodium (6 mg, 0.26 mmol) was dissolved in MeOH (2 mL) and added to the pseudodisaccharide heptaacetate **15** (19 mg, 0.029 mmol). After 4 h, TLC (CMAW) showed the presence of a single component (R_f = 0.2). Dowex 50X8 resin (H^+) was added, and the mixture was filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc–MeOH–water, 7:2:1) and freeze dried to give the deprotected pseudodisaccharide **2** (11 mg, 99%) as a white solid; $[\alpha]_D^{22}$ +45 (*c*, 0.25, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.85 (1H, ddd, $J_{5a,5a'}$ = 14.3 Hz, $J_{5,5a}$ = 12.9 Hz, $J_{1,5a}$ = 3.2 Hz, H-5a^{II}), 2.11 (1H, m, H-5^{II}), 2.19 (1H, dat, J = 3.4 Hz, H-5a′^{II}), 3.29 (1H, dd, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 10.6 Hz, H-3^{I}), 3.40 (1H, dd, J = 9.0 Hz, J = 10.7 Hz, H-4^{II}), 3.59 (1H, s, OCH_3), 3.68–3.73 (2H, m, H-1^{II}, H-3^{II}), 3.79–3.94 (6H, m, H-4^{I}, H-5^{I}, H-6^{I}, H-2^{II}, H-6^{II}, H-6′^{II}), 4.05 (1H, dd, $J_{5,6'}$ = 1.8 Hz, $J_{6,6'}$ = 12.2 Hz, H-6′^{I}), 4.12 (1H, dd, $J_{1,2}$ = 1.7 Hz, H-2^{I}), 4.88 (1H, d, H-1^{I}); ^{13}C NMR (125 MHz, D_2O): δ 29.1 (t, C-5a^{II}), 38.0 (d, C-5^{II}), 47.6 (d, C-1^{II}), 49.9 (d, C-3^{I}), 53.2 (q, OCH_3), 59.7 (t, C-6^{I}), 0.8 (t, C-6^{II}), 65.3 (d, C-4^{I}), 69.5 (d, C-2^{I}), 71.7, 72.2, 72.2 (3 × d, C-2^{II}, C-4^{II}, C-5^{I}), 74.0 (d, C-3^{II}), 98.5 (d, C-1^{I}); *m/z* (ESI$^+$) 393 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) calcd for $C_{14}H_{26}O_9SNa$ [M+Na]$^+$: 393.1190, found 393.1198.

3.11. In vitro glucosidase inhibition

Fluorescently labelled substrates $Glc_1Man_7GlcNAc_2$ and $Glc_2Man_7GlcNAc_2$ were prepared using anthranilic acid (2AA) and added to separate 1.5 mL centrifuge tubes with varying concentrations of the compounds (0–100 μM). Sufficient purified rat liver α-glucosidase II was added to generate 25% hydrolysis of $Glc_1Man_7GlcNAc_2$–2AA in a 90 min reaction time or 2 h for $Glc_2Man_7GlcNAc_2$–2AA. Linear degradation of substrate occurred over the time of incubation. The reactions were stopped by the addition of 3 μL glacial acetic acid and 30 μL acetonitrile. Following enzyme digestion, samples were centrifuged through a 10,000 molecular weight cut-off filter at 7000 rpm for 45 min (which had been pre-washed with 150 μL of water) to remove protein before NP-HPLC analysis as described previously.[27]

3.12. Free oligosaccharides (FOS) analysis in cells following compound treatment

HL60 Cells were cultured in RPMI media containing 10% foetal calf serum, 2 mM L-glutamine and 1% penicillin-streptomycin (Invitrogen), before the medium was replaced with fresh medium containing compounds at 100 μM. After 24 h incubation, the medium was removed, and the cells were washed three times with PBS by centrifugation. Washed cells were stored at −20 °C for a short time before thawing and dounce homogenisation in water. The FOS were labelled with 2-anthranilic acid, purified using Concanavalin A (Con A)-Sepharose 4B beads (100 μL packed resin) and analysed by NP-HPLC using a 4.6 × 250 mM TSK gel Amide-80 column (Anachem, Luton, UK) as described previously.[27]

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Supplementary data

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References

1. Kornfield, R.; Kornfield, S. *Annu. Rev. Biochem.* **1985**, *54*, 631–664.
2. Castro, O.; Movsichoff, F.; Parodi, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 14756–14760.
3. Helenius, A.; Trombetta, E. S.; Hebert, D. N.; Simons, J. F. *Trends Cell Biol.* **1997**, *7*, 193–200.
4. Schallus, T.; Jaeckh, C.; Feher, K.; Palma, A. S.; Liu, Y.; Simpson, J. C.; Mackeen, M.; Stier, G.; Gibson, T. J.; Feizi, T.; Pieler, T.; Muhle-Goll, C. *Mol. Biol. Cell* **2008**, *19*, 3404–3414.
5. Alonso, J. M.; Santa-Cecilia, A.; Calvo, P. *Eur. J. Biochem.* **1993**, *215*, 37–42.
6. Cumpstey, I.; Butters, T. D.; Tennant-Eyles, R. J.; Fairbanks, A. J.; France, R. R.; Wormald, M. R. *Carbohydr. Res.* **2003**, *338*, 1937–1949.
7. Mellor, H. R.; Neville, D. C. A.; Harvey, D. J.; Platt, F. M.; Dwek, R. A.; Butters, T. D. *Biochem. J.* **2004**, *381*, 867–875.
8. McCasland, G. E.; Furuta, S.; Durham, L. J. *J. Org. Chem.* **1966**, *31*, 1516–1521.
9. Ogawa, S. *Trends Glycosci. Glyc.* **2004**, *16*, 33–53.
10. Zanardi, F.; Battistini, L.; Marzocchi, L.; Acquotti, D.; Rassu, G.; Pinna, L.; Auzzas, L.; Zambrano, V.; Casiraghi, G. *Eur. J. Org. Chem.* **2002**, *12*, 1956–1965.
11. Tsunoda, H.; Ogawa, S. *Liebigs Ann.* **1995**, 267–277.
12. Bourderioux, A.; Lefoix, M.; Gueyrard, D.; Tatibouet, A.; Cottaz, S.; Arzt, S.; Burmeister, W. P.; Rollin, P. *Org. Biomol. Chem.* **2005**, *3*, 1872–1879.
13. Whalen, L. J.; Halcomb, R. L. *Org. Lett.* **2004**, *19*, 3221–3224.
14. Tsunoda, H.; Sasaki, S.-I.; Furuya, T.; Ogawa, S. *Liebigs Ann.* **1996**, 159–166.
15. Takeuchi, M.; Kamata, K.; Yoshida, M.; Kameda, Y.; Matsui, K. *J. Biochem.* **1990**, *108*, 42–46.
16. Ogawa, S.; Ashiura, M.; Uchida, C. *Carbohydr. Res.* **1998**, *370*, 83–95.
17. Carvalho, I.; Haines, A. H. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1795–1800.
18. Haines, A. H.; Carvalho, I. *Chem. Commun.* **1998**, 817–818.
19. Cumpstey, I. *Synlett* **2006**, 1711–1714.
20. Driguez, H. *ChemBioChem* **2001**, *2*, 311–318.
21. Pachamuthu, K.; Schmidt, R. R. *Chem. Rev.* **2006**, *106*, 160–187.
22. Szilagyi, L.; Varela, O. *Curr. Org. Chem.* **2006**, *10*, 1745–1770.
23. Cumpstey, I.; Gehrke, S.; Erfan, S.; Cribiu, R. *Carbohydr. Res.* **2008**, *343*, 1676–1693.
24. Izquierdo, I.; Plaza, M. T.; Ramirez, A.; Aragon, F. *J. Heterocycl. Chem.* **1996**, *33*, 1239–1242.
25. Manthorpe, J. M.; Szpilman, A. M.; Carreira, E. M. *Synthesis* **2005**, 3380–3388.
26. Akhtar, T.; Eriksson, L.; Cumpstey, I. *Carbohydr. Res.* **2008**, *343*, 2094–2100.
27. Alonzi, D. S.; Neville, D. C. A.; Lachmann, R. H.; Dwek, R. A.; Butters, T. D. *Biochem. J.* **2008**, *409*, 571–580.
28. Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515–553.