



Synthesis of potential metal-binding group compounds to examine the zinc dependency of the GPI de-*N*-acetylase metalloenzyme in *Trypanosoma brucei*

Nuha Z. Abdelwahab, Michael D. Urbaniak, Michael A. J. Ferguson*, Arthur T. Crossman

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, The University of Dundee, DD1 5EH Dundee, Scotland, United Kingdom

ARTICLE INFO

Article history:

Received 15 December 2010

Received in revised form 1 February 2011

Accepted 2 February 2011

Available online 24 February 2011

Keywords:

Glycosylphosphatidylinositol (GPI)
biosynthesis

Zinc metalloenzyme inhibitor

Zinc-binding group

Branched monosaccharides,

Phosphatidylinositol de-*N*-acetylase

ABSTRACT

A small zinc-binding group (ZBG) library of deoxy-2-C-branched-monosaccharides, for example, 1,5-anhydroglucitols, consisting of either monodentate ligand binding carboxylic acids or bidentate ligand binding hydroxamic acids, were prepared to assess the zinc affinity of the putative metalloenzyme 2-acetamido-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 6)-phosphatidylinositol de-*N*-acetylase (EC 3.5.1.89) of glycosylphosphatidylinositol biosynthesis. The *N*-ureido thioglucoside was also synthesised and added to the ZBG library because a previous *N*-ureido analogue, synthesised by us, had inhibitory activity against the aforementioned de-*N*-acetylase, presumably via the *N*-ureido motif.

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1. Introduction

Glycosylphosphatidylinositol (GPI) acts as a membrane anchor for a small but significant proportion of higher eukaryote cell-surface glycoproteins that are particularly abundant in protozoan parasites such as *Trypanosoma brucei*, the causative agent of African sleeping sickness in humans and the related disease Nagana in cattle.¹ The structure, biosynthesis, and function of GPI anchors and related molecules have been extensively reviewed.^{1–4} Disruption of GPI biosynthesis in the clinically relevant bloodstream form of *T. brucei* has been genetically^{5–8} and chemically⁹ validated as a drug target.

A key early step in the biosynthesis of the GPI anchors is the de-*N*-acetylation of 2-acetamido-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 6)-phosphatidylinositol¹⁰ [α -D-GlcpNAc-PI (**1**, Fig. 1)] to form α -D-GlcpNH₂-PI (**2**, Fig. 1). De-*N*-acetylation is a prerequisite for subsequent processing of **2** that leads to mature GPI anchor precursors.¹¹ In *T. brucei*, de-*N*-acetylation is followed by mannosylation and subsequent inositol-acylation of **2**, whereas in mammalian cells the order of these reactions is reversed.^{12,13}

Previously, we have shown¹⁴ that mammalian and trypanosomal α -D-GlcpNAc-PI de-*N*-acetylases are zinc metalloenzymes, proposed a mechanism of action similar to that of zinc peptidases and postulated that known zinc binding motifs^{15,16} such as the *N*-hydroxyurea analogue **3** (Fig. 1),¹⁷ could act as inhibitors. Here,

we have designed and synthesised a small library of deoxymonosaccharides [**5–12** (Fig. 2)] containing recognisable zinc binding groups (ZBGs), that is, carboxylic acids and hydroxamic acids, as well as a potentially new ZBG, the ureido derivative, that should continue to probe the trypanosomal α -D-GlcpNAc-PI de-*N*-acetylase.

A good starting point for our compound library was the earlier work by Hindsgaul and co-workers^{18,19} which demonstrated the effectiveness of 1,5-anhydro-2-deoxy-D-glucitol hydroxamic acids, for example **7**,¹⁹ as ZBG probes. The hydroxamic acid **7** was resynthesised and included in the compound library because **7** was shown to be a potent inhibitor of LpxC,¹⁹ presumably via zinc chelation, and could serve as the standard by which to compare the potency of the other analogues in the library. Therefore, compounds **5**, **6** and **8**[†] resemble those of Hindsgaul et al. whereby the 2-C appendage is either a hydroxamic acid or a carboxylic acid ZBG moiety. Compounds **9–11** were synthesised to supply potential glycosyl donors for another project but might also exhibit some degree of inhibition towards the trypanosome de-*N*-acetylase enzyme. Lastly, the *N*-ureido thioglycoside **12** was fashioned because of previous inhibitory data of the *N*-ureido-GlcpNAc-PI derivative **4**²⁰ (Fig. 1) against the trypanosome de-*N*-acetylase enzyme. Analogue **12** is a truncated version of **4** which focuses on, what we believe to be the most potent inhibitory component of **4**, the *N*-ureido motif.

* Corresponding author. Tel.: +44 1382 384219.

E-mail address: m.a.j.ferguson@dundee.ac.uk (M.A.J. Ferguson).

[†] Compound **8** is in the literature, Jackman, J. E. et al. *J. Biol. Chem.* **2000**, 275, 11002–11009, however, no preparative details are given therein.

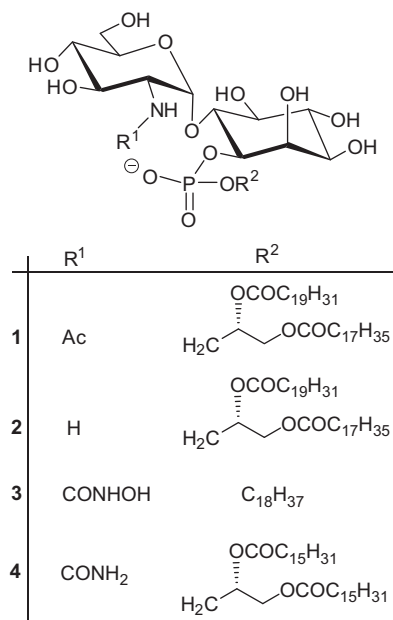


Figure 1.

2. Results and discussion

The synthesis, of the analogues **5–8**, is based on a successful approach^{18,19} used previously (Scheme 1).

The first three steps, benzylation→ozonolysis→Pinnick²¹ oxidation, from the known¹⁸ 2-C-allyl derivative **13** was accomplished straightforwardly to furnish the pivotal carboxylic acid **14**.¹⁹ The carboxylic acid analogue **14**¹⁹ and the corresponding intermediates from **13**¹⁸ were not fully characterised in the literature. Consequently, we have included the analytical data for those intermediates, and that of compound **14**,¹⁹ in this paper as [Supplementary data](#). Hydrogenolysis of the benzylidene protecting group of compound **14** furnished the target analogue **5** in 59% yield; alternatively, the yield could be improved to 70% by using aqueous TFA.

The synthesis of carboxylic acid **6** emerged from the de-O-benzylation of **14**,¹⁹ under Zemplén conditions, followed by hydrogenolysis over 10% palladium on carbon to give the crude derivative **6** (Scheme 1). The analogue **6** was then purified by reversed phase chromatography (RPC) to afford the final target glucitol **6** in 80% yield.

The carboxylic acid derivative **14**¹⁹ was coupled with *O*-benzylhydroxylamine hydrochloride (BnONH₂·HCl) using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDAC) to give the known¹⁹ hydroxyamide **16** (see the [Supplementary data](#) for the

analytical data of **16**). The benzyloxyamide **16** was hydrogenated, as described in the literature, to give the hydroxamic acid **7**; ¹H NMR assignments for **7** were identical to those reported in the literature¹⁹ and see the [Supplementary data](#) for the ¹³C NMR assignments of **7**. The ZBG analogue **8** was synthesised following the sequence **16**→**17**¹⁸→**8**, as previously described for **6**. An alternative synthesis of the derivative **17**¹⁸ is described in the [Supplementary data](#).

The synthesis of the targeted carboxylic acid **9** (Scheme 2) began from the acetolysis of the 1,6-anhydro derivative **18**²² to give, exclusively, the α-2-C-allyl derivative **19** [*J*_{1,2} = 3.1 Hz]. The tetraacetate derivative **19** proved to be a very useful intermediate because **19** could be altered to supply analogues **10** and **11**, as well. Thus, a portion of the 2-C-allyl intermediate **19** was ozonised to give the aldehyde **20**, which was oxidised, following Pinnicks' protocols,²¹ to furnish the carboxylic acid **21** in 94% yield. Lastly, the tetraacetate **21** was de-O-acetylated with 0.03 M methanolic sodium methoxide to produce the fully deprotected carboxylic acid analogue **9** in 51% yield, as a mixture of α/β anomers.

Another portion of the 2-C-allyl derivative **19** was transformed into the corresponding α- and β-phenylthioglucoisides **22** and **23**, respectively, via Lewis acid (BF₃·Et₂O) catalysed substitution of the anomeric acetate with thiophenol in refluxing dichloromethane.²³ These two anomers were separated by radial band chromatography to furnish the α-anomer **22** (*J*_{1,2} = 4.9 Hz) and the β-anomer **23** (*J*_{1,2} = 10.9 Hz) in 48% and 13% yields, respectively. The closing sequences **22**→**24**→**26**→**10** and **23**→**25**→**27**→**11** were then conducted without incident, essentially as those described for **9**; the exception being **26**→**10** which was achieved via acid hydrolysis²⁴ (Scheme 2).

A synthesis of 1-thiophenyl-2-deoxy-2-ureido-β-D-glucopyranoside **12** was obtained on treatment of the known amine²⁵ **28** with potassium cyanate (KOCN) and water at room temperature in total darkness^{26,27} (Scheme 3). After evaporation to dryness, the crude ureido compound was purified by reversed phase chromatography to give crystalline **12** (65% yield; characteristic ¹³C carbonyl carbon at δ 158.47 ppm).

Details of the results of enzymatic studies with the above ZBG analogues will be reported elsewhere in due course.

3. Experimental

3.1. General methods

¹H, ¹³C, ³¹P NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer using deuteriochloroform as a solvent and tetramethylsilane as the internal standard, unless otherwise indicated. All coupling constants (*J*) are given in Hertz. High resolution electrospray ionisation mass spectra (HRESIMS) and liquid chromatography mass spectra (LCMS) were recorded with a Bruker

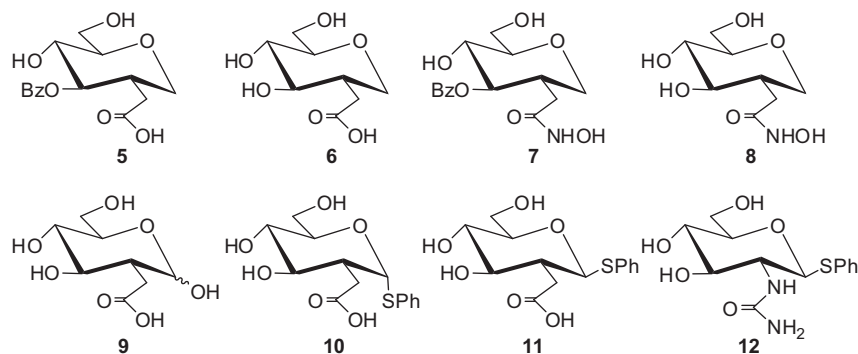
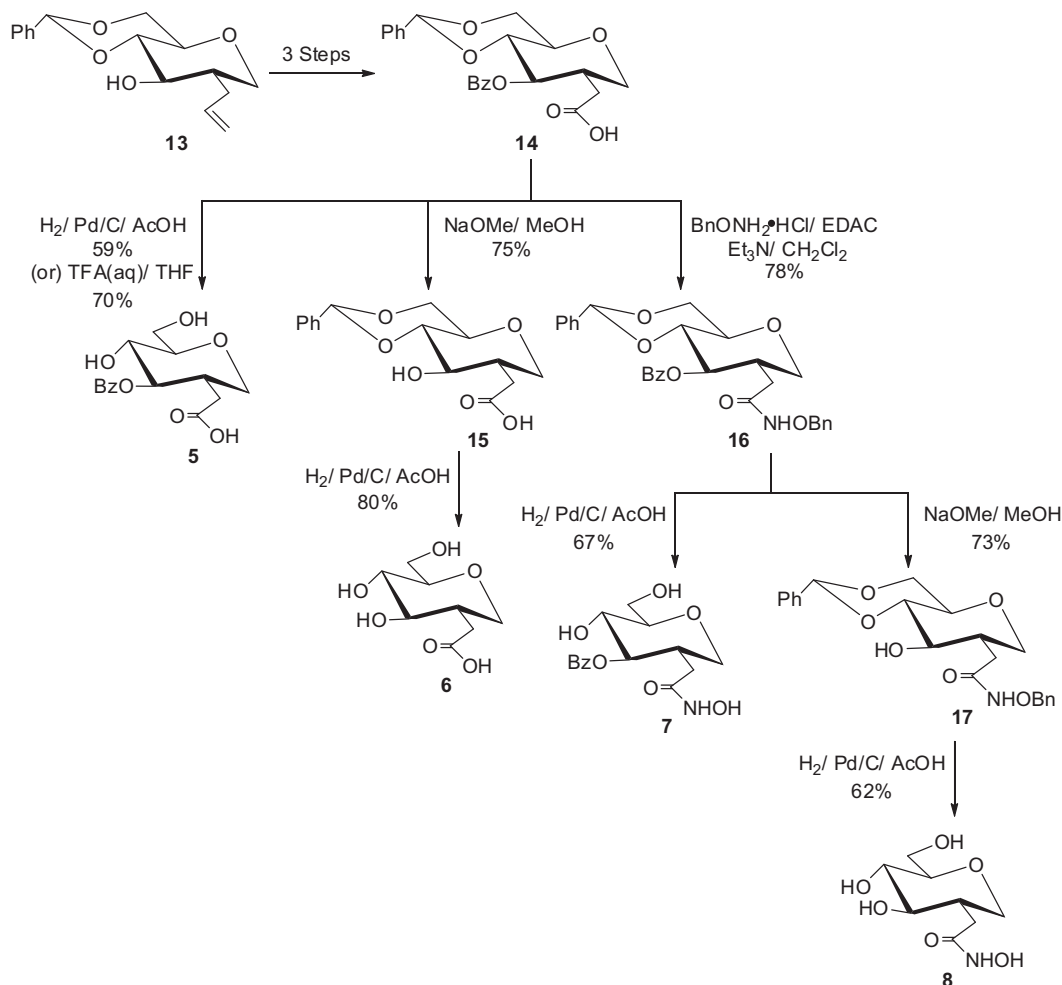


Figure 2. A small library of zinc chelator probes.



Scheme 1.

microTof spectrometer. Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 343 polarimeter. Thin layer chromatography (TLC) was performed on Kieselgel 60 F₂₅₄ (Merck) or RP-18 F_{254s} (Merck) plates with various solvent systems as developers, followed by detection under UV light or by charring using either sulfuric acid–water–ethanol (15:85:5), phosphomolybdic acid, orcinol or ninhydrin spray reagents. Flash column chromatography (FCC) was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). Reversed phase chromatography was performed on a C18 cartridge supplied by Sigma–Aldrich. Radial-band chromatography (RBC) was performed using a Chromatotron (model 7924T, TC Research UK) with silica gel F₂₅₄ TLC standard grade as the adsorbent. All reactions were carried out in commercially available dry solvents, unless otherwise stated. Light petroleum refers to the fraction having a boiling range 60–80 °C, unless indicated otherwise.

3.2. Synthesis of the ZBG library

3.2.1. 1,5-Anhydro-3-O-benzoyl-2-C-carboxymethyl-2-deoxy-D-glucitol (**5**)

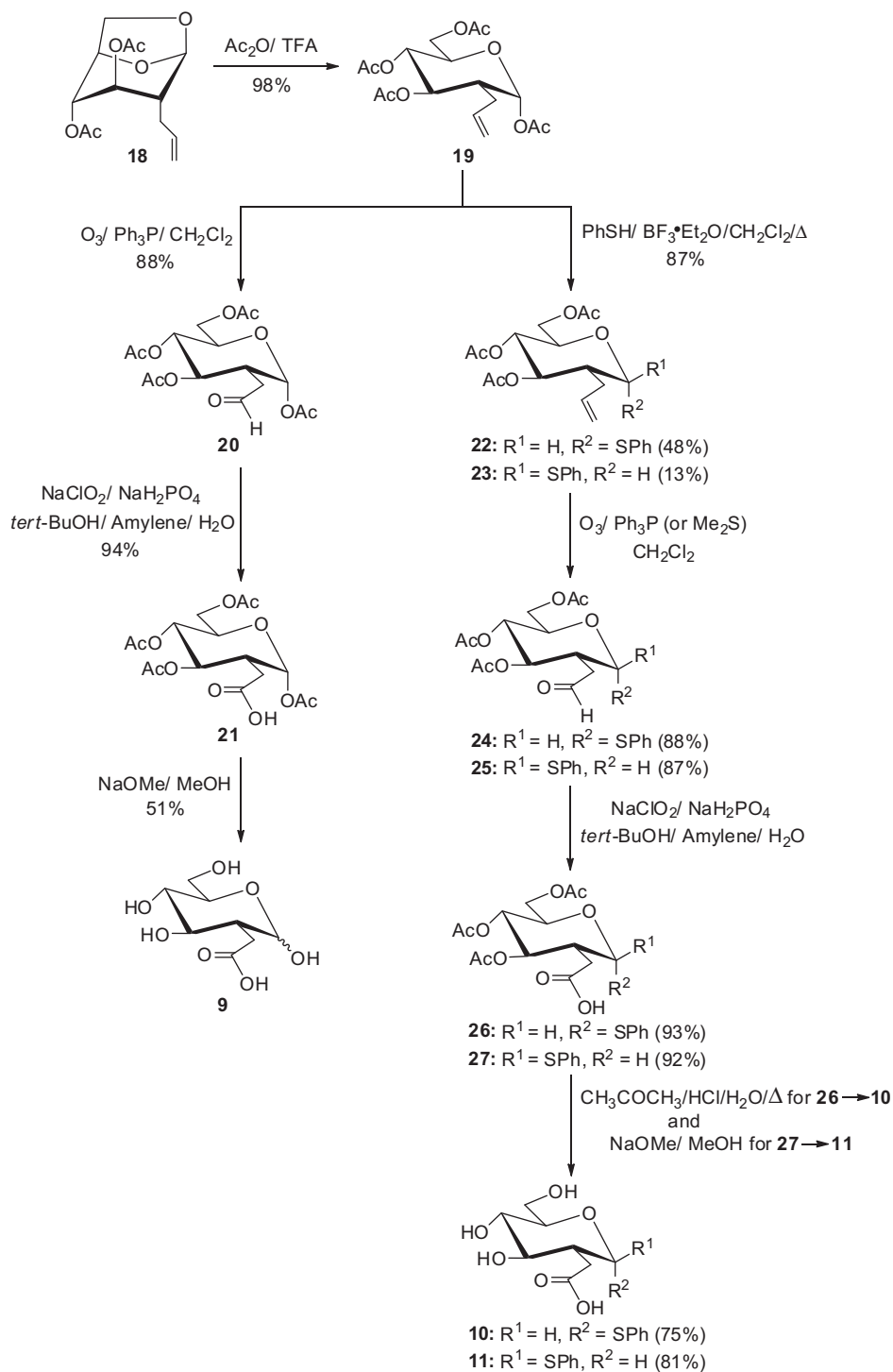
3.2.1.1. Method A. A solution of the benzylidene compound **14**¹⁹ (20 mg, 0.05 mmol) in AcOH (2 mL) containing 10% palladium on carbon (10 mg) was stirred under a slight overpressure of hydrogen at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced

pressure. The residue was purified by FCC (10:1:0.02 CHCl₃–MeOH–AcOH) to furnish a brown paste **5** (9 mg, 59%), which was indistinguishable from that obtained by the following procedure.

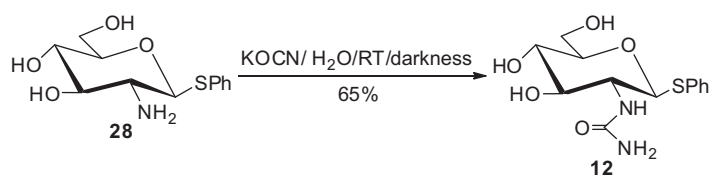
3.2.1.2. Method B. A solution of the benzylidene compound **14**¹⁹ (40 mg, 0.10 mmol) in THF (2 mL) and 96% (aq) TFA (0.5 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (2 × 5 mL). The residue was purified with the same solvent system as in method A to give the acid **5** (21 mg, 70%); *R*_f 0.20 (10:1:0.02 CHCl₃–MeOH–AcOH); $[\alpha]_{\text{D}}^{25} +8.9$ (c 1.0, MeOH); ¹H NMR (CD₃OD, 500 MHz): δ 8.10–7.49 (m, 5H, Ph), 5.10 (dd, 1H, *J*_{2,3} 10.7, *J*_{3,4} 9.3 Hz, H-3), 4.10 (dd, 1H, *J*_{1a,2} 4.7, *J*_{1a,1b} 11.5 Hz, H-1a), 3.88 (dd, 1H, *J*_{5,6a} 2.1, *J*_{6a,6b} 11.8 Hz, H-6a), 3.71 (dd, 1H, H-6b), 3.60 (t, 1H, *J*_{4,5} 9.3 Hz, H-4), 3.40 (t, 1H, *J*_{1a,1b} 11.5 Hz, H-1b), 3.37–3.34 (m, 1H, H-5), 2.50–2.41 (m, 1H, H-2), 2.35 (dd, 1H, *J*_{2,7a} 4.8, *J*_{7a,7b} 16.0 Hz, H-7a), 2.17 (dd, 1H, H-7b); ¹³C NMR (CD₃OD, 125 MHz): δ 175.49 (C=O), 168.15 (PhCO), 134.29–129.56 (C-Ph), 82.75 (C-5), 79.92 (C-3), 70.79 (C-4), 70.56 (C-1), 62.94 (C-6), 40.03 (C-2), 34.10 (C-7). HRESIMS: Calcd for [C₁₅H₁₈O₇–H][–]: 309.0980. Found *m/z*: 309.0967.

3.2.2. 1,5-Anhydro-4,6-O-benzylidene-2-C-carboxymethyl-2-deoxy-D-glucitol (**15**)

A methanolic 0.03 M NaOMe (0.6 mL, 0.018 mmol) solution was added to the benzoate derivative **14**¹⁹ (60 mg, 0.15 mmol) in THF–MeOH (1:4 5 mL) and the reaction mixture was stirred over-



Scheme 2.



Scheme 3.

night at room temperature. Afterwards, the reaction mixture was neutralised with Amberlite IR-120 (H⁺) ion-exchange resin, filtered and the filtrate concentrated under reduced pressure and co-evaporated with water (5 × 5 mL). The residue was purified by FCC (20:1:0.02 CH₂Cl₂–MeOH–AcOH) to give the crystalline acid **15** (33 mg, 75%); mp 183–185 °C; *R*_f 0.24 (20:1:0.02 CH₂Cl₂–MeOH–AcOH); [α]_D²⁵ –209.0 (c 1.0, MeOH); ¹H NMR (CD₃OD, 500 MHz): δ 7.50–7.33 (m, 5H, Ph), 5.58 (s, 1H, PhCH), 4.20 (dd, 1H, *J*_{5,6a} 5.0, *J*_{6a,6b} 10.3 Hz, H-6a), 4.02 (dd, 1H, *J*_{1a,2} 4.7, *J*_{1a,1b} 11.4 Hz, H-1a), 3.70 (t, 1H, *J*_{6a,6b} 10.3 Hz, H-6b), 3.50–3.45 (m, 2H, H-3, H-4), 3.37–3.28 (m, 2H, H-1b, H-5), 2.77 (dd, 1H, *J*_{2,7a} 3.0, *J*_{7a,7b} 15.8 Hz, H-7a), 2.21–2.14 (m, 1H, H-2), 2.11 (dd, 1H, H-7b); ¹³C NMR (CD₃OD, 125 MHz): δ 176.06 (C=O), 139.32–127.56 (C-Ph), 103.06 (PhCH), 84.55 (C-4), 73.57 (C-3), 73.17 (C-5), 71.46 (C-1), 69.84 (C-6), 41.88 (C-2), 33.58 (C-7). HRESIMS: Calcd for [C₁₅H₁₈O₆–H][–]: 293.1031. Found *m/z*: 293.1030.

3.2.3. 1,5-Anhydro-2-C-carboxymethyl-2-deoxy- β -D-glucitol (6)

A solution of the benzylidene derivative **15** (58 mg, 0.20 mmol) in AcOH (10 mL) containing 10% palladium on carbon (29 mg) was stirred under a slight overpressure of hydrogen at room temperature for 3 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The resulting residue was purified by an RPC C18 column (10% MeOH) to furnish the carboxylic acid **6** (32 mg, 80%); *R*_f 0.40 (10% MeOH); [α]_D²⁵ +379.6 (c 0.5, MeOH); ¹H NMR (CD₃OD, 500 MHz): δ 3.99 (dd, 1H, *J*_{1a,2} 3.9, *J*_{1a,1b} 11.5 Hz, H-1a), 3.83 (dd, 1H, *J*_{5,6a} 2.1, *J*_{6a,6b} 11.8 Hz, H-6a), 3.62 (dd, 1H, H-6b), 3.23 (t, 1H, *J*_{3,4} = *J*_{4,5} = 8.6 Hz, H-4), 3.19–3.15 (m, 3H, H-1b, H-3, H-5), 2.77–2.71 (m, 1H, H-7a), 2.08–2.00 (m, 2H, H-2, H-7b); ¹³C NMR (CD₃OD, 125 MHz): δ 176.44 (C=O), 82.71 (C-5), 77.59 (C-3), 73.12 (C-4), 70.81 (C-1), 63.26 (C-6), 41.34 (C-2), 33.83 (C-7). HRESIMS: Calcd for [C₈H₁₄O₆–H][–]: 205.0718. Found *m/z*: 205.0724.

3.2.4. 1,5-Anhydro-2-C-(carboxymethyl *N*-hydroxyamide)-2-deoxy- β -D-glucitol (8)

10% Palladium on carbon (40 mg) was added to a solution of the benzyloxyamide **17**¹⁸ (40 mg, 0.10 mmol) in AcOH (10 mL). The reaction mixture was stirred under a slight over pressure of hydrogen at room temperature for 4 h. After filtration through a pad of Celite the solvent was concentrated under reduced pressure. The resulting residue was purified by an RPC C18 column (10% MeOH) to furnish the hydroxamic acid **8** (13.6 mg, 62%); *R*_f 0.38 (10% MeOH); [α]_D²⁵ +38.9 (c 1.3, MeOH); ¹H NMR (CD₃OD, 500 MHz): δ 3.92 (dd, 1H, *J*_{1a,2} 4.6, *J*_{1a,1b} 11.5 Hz, H-1a), 3.83 (dd, 1H, *J*_{5,6a} 1.9, *J*_{6a,6b} 11.8 Hz, H-6a), 3.61 (dd, 1H, H-6b), 3.24–3.13 (m, 4H, H-1b, H-3, H-4, H-5), 2.54 (dd, 1H, *J*_{2,7a} 3.9, *J*_{7a,7b} 14.3 Hz, H-7a), 2.06–1.95 (m, 1H, H-2), 1.84 (dd, 1H, H-7b); ¹³C NMR (CD₃OD, 125 MHz): δ 171.44 (C=O), 82.72, 77.99, 73.08, 70.66 (C-1), 63.26 (C-6), 41.64 (C-2), 32.66 (C-7). HRESIMS: Calcd for [C₈H₁₅NO₆+Na]⁺: 244.0792. Found *m/z*: 244.0795.

3.2.5. 1,3,4,6-Tetra-O-acetyl-2-C-allyl-2-deoxy- α -D-glucopyranose (19)

A solution of the known²² 1,6-anhydro derivative **18** (0.865 g, 3.2 mmol) in Ac₂O–trifluoroacetic acid (9:1, 20 mL) was stirred at room temperature overnight, whereafter it was neutralised with a solution of satd NaHCO₃. The aqueous solution was extracted with CH₂Cl₂ (2 × 200 mL) and the organic extracts were combined, washed with H₂O (200 mL), brine (200 mL), dried with MgSO₄ and then concentrated under reduced pressure. The residue was purified by FCC (5:1→2:1 light petroleum–EtOAc) to give the tetraacetate **19** as white needles (1.17 g, 98%); mp 99–101 °C (from 10:1 light petroleum–EtOH); *R*_f 0.20 (1:1 light petroleum–EtOAc); [α]_D²⁵ +123.0 (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 500 MHz): δ 6.06 (d, 1H, *J*_{1,2} 3.1 Hz, H-1), 5.65–5.57 (m, 1H, H-8), 5.20 (t, 1H,

*J*_{2,3} = *J*_{3,4} = 10.8 Hz, H-3), 5.01–4.91 (m, 3H, H-4, H-9a,b), 4.23 (dd, 1H, *J*_{5,6a} 4.0, *J*_{6a,6b} 12.4 Hz, H-6a), 3.97–3.93 (m, 2H, H-5, H-6b), 2.16–2.12 (m, 2H, H-2, H-7a), 2.09, 2.00, 1.97, 1.96 (4 × s, 12H, 4 × CH₃CO), 1.97–1.93 (m, 1H, H-7b); ¹³C NMR (CDCl₃, 125 MHz): δ 169.78, 169.60, 168.83, 167.96 (4 × C=O), 133.06 (C-8), 116.54 (C-9), 90.68 (C-1), 70.81 (C-3), 68.73 (C-5), 68.09 (C-4), 60.90 (C-6), 41.89 (C-2), 30.71 (C-7), 19.82, 19.76, 19.70, 19.53, (4 × CH₃CO). HRESIMS: Calcd for [C₁₇H₂₄O₉+Na]⁺: 395.1313. Found *m/z*: 395.1298.

3.2.6. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-C-formylmethyl- α -D-glucopyranose (20)

Ozone was passed through a solution of the allyl compound **19** (150 mg, 0.403 mmol) in CH₂Cl₂ (20 mL) at –78 °C until the solution turned blue. The excess ozone was removed by a stream of argon until the solution was clear and then followed by the addition of triphenylphosphine (264.3 mg, 1.01 mmol). The mixture was allowed to warm to room temperature for 2 h, concentrated under reduced pressure and purified by RBC (6:1→2:1 light petroleum–EtOAc) to give the aldehyde **20** (84 mg, 88%); *R*_f 0.28 (1:1 light petroleum–EtOAc); [α]_D²⁵ +172.7 (c 0.6, CHCl₃); ¹H NMR (CDCl₃, 500 MHz): δ 9.61 (t, 1H, *J* 1.2 Hz, HC=O), 6.20 (d, 1H, *J*_{1,2} 3.4 Hz, H-1), 5.18 (dd, 1H, *J*_{2,3} 11.4, *J*_{3,4} 9.5 Hz, H-3), 5.03 (t, 1H, *J*_{4,5} 9.5 Hz, H-4), 4.23 (dd, 1H, *J*_{5,6a} 4.6, *J*_{6a,6b} 13.0 Hz, H-6a), 4.01–3.97 (m, 2H, H-5, H-6b), 2.76–2.70 (m, 1H, H-2), 2.38 (m, 2H, H-7a, H-7b), 2.09, 2.02, 19.96, 19.94 (4 × s, 12H, 4 × CH₃CO); ¹³C NMR (CDCl₃, 125 MHz): δ 197.80 (HC=O), 169.70, 169.52, 168.66, 167.88 (4 × C=O), 90.86 (C-1), 70.18 (C-3), 68.84 (C-5), 67.80 (C-4), 60.73 (C-6), 41.42 (C-7), 36.92 (C-2), 19.85, 19.80, 19.70, 19.62, (4 × CH₃CO). HRESIMS: Calcd for [C₁₆H₂₂O₁₀+Na]⁺: 397.1313. Found *m/z*: 397.1298.

3.2.7. 1,3,4,6-Tetra-O-acetyl-2-C-carboxymethyl-2-deoxy- α -D-glucopyranose (21)

A solution of sodium chlorite (2.58 g, 28.56 mmol) and sodium dihydrogen phosphate (3.92 g, 32.63 mmol) in water (20 mL) was added dropwise to a solution of the aldehyde **20** (724 mg, 1.931 mmol) in *tert*-BuOH (56.7 mL, 604 mmol) and amylene (17 mL, 203 mmol). The reaction mixture was stirred for 1 h then diluted with ice water and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (30 mL), dried (Na₂SO₄), and concentrated under reduced pressure. The residue was purified by FCC (1:1:0.02 light petroleum–EtOAc–AcOH) to furnish the acid **21** (709 mg, 94%); *R*_f 0.27 (1:1:0.02 light petroleum–EtOAc–AcOH); [α]_D²⁵ +88.3 (c 1.3, CHCl₃); ¹H NMR (CDCl₃, 500 MHz): δ 6.25 (d, 1H, *J*_{1,2} 3.4 Hz, H-1), 5.22 (dd, 1H, *J*_{2,3} 11.4, *J*_{3,4} 9.5 Hz, H-3), 5.03 (t, 1H, *J*_{4,5} 9.5 Hz, H-4), 4.28 (dd, 1H, *J*_{5,6a} 4.1, *J*_{6a,6b} 12.4 Hz, H-6a), 4.08–4.02 (m, 2H, H-5, H-6b), 2.61–2.56 (m, 1H, H-2), 2.34 (dd, 1H, *J*_{2,7a} 5.8, *J*_{7a,7b} 16.3 Hz, H-7a), 2.27 (dd, 1H, H-7b), 2.04, 2.01, 2.00, 19.99 (4 × s, 12H, 4 × CH₃CO); ¹³C NMR (CDCl₃, 125 MHz): δ 174.59, 172.40, 172.14, 171.44, 170.75 (5 × C=O), 93.16 (C-1), 72.67 (C-3), 71.13 (C-5), 70.45 (C-4), 63.16 (C-6), 41.79 (C-2), 32.98 (C-7), 20.79, 20.75, 20.70, 20.65 (4 × CH₃CO). HRESIMS: Calcd for [C₁₆H₂₂O₁₁–H][–]: 389.1089. Found *m/z*: 389.1085.

3.2.8. 2-C-Carboxymethyl-2-deoxy- β -D-glucopyranose (9)

To a solution of benzoylated compound **21** (93 mg, 0.238 mmol) in MeOH (2 mL) was added 0.03 M sodium methoxide in MeOH (6.2 mL, 0.186 mmol) at room temperature. After 48 h, the reaction mixture was neutralised with Amberlite IR-120 (H⁺) ion-exchange resin, filtered and the filtrate was concentrated under reduced pressure; followed by co-evaporation with water (5 × 5 mL). The residue was purified by FCC (3:1:0.02 CH₂Cl₂–MeOH–AcOH) to give the carboxylic acid **9** as an α : β (1.5:1) mixture (27 mg, 51%); *R*_f 0.25 (3:1:0.02 CH₂Cl₂–MeOH–AcOH); ¹H NMR (CD₃OD,

500 MHz): δ 5.23 (d, 1H, $J_{1,2}$ 3.1 Hz, H-1 α), 4.63 (d, $J_{1,2}$ 8.6 Hz, H-1 β), 3.85 (dd, $J_{5,6a}$ 1.9, $J_{6a,6b}$ 11.7 Hz, H-6a β), 3.80–3.76 (m, 2H, H-5, H-6a α), 3.70 (dd, $J_{6a,6b}$ 11.4 Hz, H-6b α), 3.66 (dd, H-6b β), 3.51 (dd, $J_{2,3}$ 10.9, $J_{3,4}$ 8.9 Hz, H-3 α), 3.36 (dd, $J_{2,3}$ 10.7, $J_{3,4}$ 8.1 Hz, H-3 β), 3.32–3.21 (m, 3H, H-4 α , H-4 β , H-5 β), 2.71 (dd, $J_{2,7a}$ 3.3, $J_{7a,7b}$ 16.6 Hz, H-7a α), 2.60 (dd, $J_{2,7a}$ 4.1, $J_{7a,7b}$ 16.1 Hz, H-7a β), 2.46 (dd, H-7b β), 2.39 (dd, H-7b α), 2.08–2.03 (m, 1H, H-2 α), 1.91–1.85 (m, 1H, H-2 β); ^{13}C NMR (CD_3OD , 125 MHz): δ 177.02, 175.55 (2 $\times$ C=O), 98.23 (C-1 β), 93.79 (C-1 α), 77.99 (C-5 β), 76.06 (C-3 β), 73.25 (C-5 α), 73.17 (C-3 α), 72.90 (C-4), 63.06 (C-6 β), 63.02 (C-6 α) 47.62 (C-2 β), 44.83 (C-2 α), 33.62 (C-7 α), 33.51 (C-7 β). HRESIMS: Calcd for $[\text{C}_8\text{H}_{14}\text{O}_7\text{H}]^-$: 221.0667. Found m/z : 221.0659.

3.2.9. Phenyl 3,4,6-tri-O-acetyl-2-C-allyl-2-deoxy-1-thio- α - and β -D-glucopyranoside (22) and (23)

To a stirred solution of the tetraacetate **19** (200 mg, 0.537 mmol) in freshly distilled CH_2Cl_2 (10 mL) at room temperature under argon was added thiophenol (110 μL , 1.074 mmol) and boron trifluoride diethyl etherate (270 μL , 2.148 mmol). The resulting mixture was heated to reflux for 3 h, cooled to room temperature, and then diluted with CH_2Cl_2 (10 mL), washed with satd NaHCO_3 (10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. RBC (10:1 $\rightarrow$ 4:1 light petroleum–EtOAc) of the residue provided the α -anomer **22** (95 mg, 48%), the β -anomer **23** (25.7 mg, 13%), as well as, an α/β mixture (51.5 mg, 26%).

α -Anomer **22**: R_f 0.28 (4:1 light petroleum–EtOAc); $[\alpha]_D^{25} +281.0$ (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 7.47–7.27 (m, 5H, Ph) 5.76–5.68 (m, 1H, H-8), 5.46 (d, 1H, $J_{1,2}$ 4.9 Hz, H-1), 5.23–5.18 (m, 2H, H-3, H-9a), 5.08 (dd, 1H, J 9.8 Hz, H-9b), 5.00 (t, 1H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 4.63–4.60 (m, 1H, H-5), 4.31 (dd, 1H, $J_{5,6a}$ 5.2, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.01 (dd, 1H, H-6b), 2.44–2.38 (m, 1H, H-2), 2.32–2.26 (m, 1H, H-7a), 2.25–2.16 (m, 1H, H-7b) 2.05, 2.04, 2.03 (3 $\times$ s, 12H, 3 $\times$ CH_3CO); ^{13}C NMR (CDCl_3 , 125 MHz): δ 170.63, 170.31, 169.97 (3 $\times$ C=O), 134.06 (C-8), 133.57–127.64 (C-Ph), 117.94 (C-9), 88.09 (C-1), 72.46 (C-3), 69.98 (C-4), 68.80 (C-5), 62.41 (C-6), 45.04 (C-2), 33.03 (C-7), 20.73, 20.72, 20.69 (3 $\times$ CH_3CO). HRESIMS: Calcd for $[\text{C}_{21}\text{H}_{26}\text{O}_7\text{S}+\text{Na}]^+$: 445.1291. Found m/z : 445.1294.

β -Anomer **23**: R_f 0.24 (4:1 light petroleum–EtOAc); $[\alpha]_D^{25} +60.0$ (c 0.2, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 7.56–7.31 (m, 5H, Ph) 5.80–5.71 (m, 1H, H-8), 5.14–5.08 (m, 3H, H-3, H-9a, H-9b), 4.94 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 4.55 (d, 1H, $J_{1,2}$ 10.9 Hz, H-1), 4.24 (dd, 1H, $J_{5,6a}$ 5.6, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.13 (dd, 1H, H-6b), 3.65–3.60 (m, 1H, H-5), 2.45–2.40 (m, 1H, H-7a), 2.34–2.29 (m, 1H, H-7b), 2.07 (s, 3H, CH_3CO), 2.06–2.02 (m, 1H, H-2), 2.01, 2.00 (2 $\times$ s, 6H, 2 $\times$ CH_3CO); ^{13}C NMR (CDCl_3 , 125 MHz): δ 170.71, 170.29, 169.94 (3 $\times$ C=O), 132.47 (C-8), 132.79–128.11 (C-Ph), 118.93 (C-9), 86.45 (C-1), 75.32 (C-5), 73.16 (C-3), 69.86 (C-4), 62.70 (C-6), 43.81 (C-2), 32.05 (C-7), 20.79, 20.75, 20.70 (3 $\times$ CH_3CO).

3.2.10. Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-C-formylmethyl-1-thio- α -D-glucopyranoside (24)

This compound was prepared from the allyl derivative **22** (95 mg, 0.225 mmol) and then quenched with triphenylphosphine (147 mg, 0.562 mmol) essentially as described for **20**. RBC (6:1 $\rightarrow$ 2:1 light petroleum–EtOAc) of the residue yielded the aldehyde **24** (84 mg, 88%); R_f 0.21 (2:1 light petroleum–EtOAc); $[\alpha]_D^{25} +268.27$ (c 0.9, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 9.74 (s, 1H, HC=O), 7.44–7.28 (m, 5H, Ph), 5.75 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.16 (dd, 1H, $J_{2,3}$ 11.5, $J_{3,4}$ 9.5 Hz, H-3), 5.04 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.61–4.56 (m, 1H, H-5), 4.32 (dd, 1H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.05 (dd, 1H, H-6b), 3.03–2.95 (m, 1H, H-2), 2.76 (dd, 1H, $J_{2,7a}$ 8.1, $J_{7a,7b}$ 18.3 Hz, H-7a), 2.61 (dd, 1H, H-7b), 2.07, 2.04, 2.02 (3 $\times$ s, 9H, 3 $\times$ CH_3CO); ^{13}C NMR (CDCl_3 , 125 MHz): δ 198.86

(HC=O), 170.63, 170.31, 169.85 (3 $\times$ C=O), 132.96–127.85 (C-Ph), 87.52 (C-1), 71.92 (C-3), 69.54 (C-4), 68.62 (C-5), 62.24 (C-6), 43.37 (C-7), 39.73 (C-2), 20.72, 20.69, 20.67 (3 $\times$ CH_3CO). HRESIMS: Calcd for $[\text{C}_{20}\text{H}_{24}\text{O}_8\text{S}+\text{Na}]^+$: 447.1084. Found m/z : 447.1096.

3.2.11. Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-C-formylmethyl-1-thio- β -D-glucopyranoside (25)

This compound was prepared from the allyl derivative **23** (25 mg, 0.059 mmol) essentially as described for the previous α -derivative **24**. However, dimethyl sulfide (130 μL , 0.177 mmol) was used in place of triphenylphosphine. The residue was purified by RBC (6:1 $\rightarrow$ 2:1 light petroleum–EtOAc) to afford the aldehyde **25** (21.8 mg, 87%); R_f 0.21 (2:1 light petroleum–EtOAc); $[\alpha]_D^{25} +11.0$ (c 1.5, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 9.59 (s, 1H, HC=O), 7.53–7.31 (m, 5H, Ph), 5.15 (dd, 1H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.5 Hz, H-3), 4.96 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.84 (d, 1H, $J_{1,2}$ 10.7 Hz, H-1), 4.27 (dd, 1H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.17 (dd, 1H, H-6b), 3.76–3.71 (m, 1H, H-5), 2.84 (dd, 1H, $J_{2,7a}$ 3.8, $J_{7a,7b}$ 16.4 Hz, H-7a), 2.57 (dd, 1H, H-7b), 2.45–2.38 (m, 1H, H-2), 2.10, 2.01, 1.97 (3 $\times$ s, 9H, 3 $\times$ CH_3CO); ^{13}C NMR (CDCl_3 , 125 MHz): δ 198.94 (HC=O), 170.69, 170.31, 169.82 (3 $\times$ C=O), 132.87–128.45 (C-Ph), 86.51 (C-1), 75.67 (C-5), 74.49 (C-3), 69.26 (C-4), 62.47 (C-6), 43.09 (C-7), 40.51 (C-2), 20.81, 20.67, 20.61 (3 $\times$ CH_3CO). HRESIMS: Calcd for $[\text{C}_{20}\text{H}_{24}\text{O}_8\text{S}+\text{Na}]^+$: 447.1084. Found m/z : 447.1096.

3.2.12. Phenyl 3,4,6-tri-O-acetyl-2-C-carboxymethyl-2-deoxy-1-thio- α -D-glucopyranoside (26)

Pinnick²¹ oxidation of the aldehyde **24** (0.383 g, 0.902 mmol) in the presence of sodium chlorite (1.20 g, 13.04 mmol), sodium dihydrogen phosphate (1.83 g, 15.24 mmol), *tert*-BuOH (26.5 mL, 282 mmol), amylene (7.96 mL, 94.71 mmol) and water (10 mL), essentially as described for compound **21**, furnished a crude residue of **26**. This residue was purified by FCC (1:1:0.02 hexane–Et₂O–AcOH) to give the white crystalline carboxylic acid **26** (0.369 g, 93%); mp 95–98 °C; R_f 0.25 (1:1:0.02 hexane–Et₂O–AcOH); $[\alpha]_D^{25} +195.0$ (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 7.46–7.27 (m, 5H, Ph), 5.77 (d, 1H, $J_{1,2}$ 5.1 Hz, H-1), 5.18 (dd, 1H, $J_{2,3}$ 11.7, $J_{3,4}$ 9.1 Hz, H-3), 5.03 (dd, 1H, $J_{4,5}$ 10.1 Hz, H-4), 4.61–4.56 (m, 1H, H-5), 4.32 (dd, 1H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.05 (dd, 1H, H-6b), 2.90–2.85 (m, 1H, H-2), 2.61 (dd, 1H, $J_{2,7a}$ 8.4, $J_{7a,7b}$ 17.0 Hz, H-7a), 2.50 (dd, 1H, H-7b), 2.05, 2.04, 2.02 (3 $\times$ s, 9H, 3 $\times$ CH_3CO); ^{13}C NMR (CDCl_3 , 125 MHz): δ 176.64, 170.86, 170.43, 170.03 (4 $\times$ C=O), 133.10–127.79 (C-Ph), 87.65 (C-1), 71.94 (C-3), 69.75 (C-4), 68.65 (C-5), 62.30 (C-6), 41.70 (C-2), 33.78 (C-7), 20.84, 20.70, 20.61 (3 $\times$ CH_3CO). HRESIMS: Calcd for $[\text{C}_{20}\text{H}_{24}\text{O}_9\text{S}-\text{H}]^-$: 439.1068. Found m/z : 439.1085.

3.2.13. Phenyl 3,4,6-tri-O-acetyl-2-C-carboxymethyl-2-deoxy-1-thio- β -D-glucopyranoside (27)

Pinnick²¹ oxidation of the aldehyde **25** (20 mg, 0.047 mmol) in the presence of sodium chlorite (63 mg, 0.695 mmol), sodium dihydrogen phosphate (95 mg, 0.794 mmol), *tert*-BuOH (1.40 mL, 14.71 mmol), amylene (413.5 μL , 4.94 mmol) and water (10 mL), essentially as described for compound **21**, gave the crude compound **27**. This material was purified by FCC (1:1:0.02 hexane–Et₂O–AcOH) and gave the acid **27** as white crystals (19.4 mg, 92%); mp 95–98 °C; R_f 0.25 (1:1:0.02 hexane–Et₂O–AcOH); $[\alpha]_D^{25} +10$ (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 7.50–7.31 (m, 5H, Ph), 5.26 (dd, 1H, $J_{2,3}$ 10.6 Hz, $J_{3,4}$ 9.3, H-3), 4.95 (t, 1H, $J_{4,5}$ 9.3 Hz, H-4), 4.92 (d, 1H, $J_{1,2}$ 10.9 Hz, H-1), 4.25 (dd, 1H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.17 (dd, 1H, H-6b), 3.75–3.71 (m, 1H, H-5), 2.69 (dd, 1H, $J_{2,7a}$ 4.0, $J_{7a,7b}$ 17.1 Hz, H-7a), 2.61 (dd, 1H, H-7b), 2.35–2.28 (m, 1H, H-2), 2.09, 2.01, 1.99 (3 $\times$ s, 9H, 3 $\times$ CH_3CO); ^{13}C NMR (CDCl_3 , 125 MHz): δ 176.26, 170.75, 170.49, 169.85 (4 $\times$ C=O), 132.96–128.37 (C-Ph), 86.24 (C-1), 75.59 (C-5), 73.97 (C-3), 69.43 (C-4), 62.53 (C-6), 41.93 (C-2), 32.94 (C-7), 20.81,

20.70, 20.61 ($3 \times \text{CH}_3\text{CO}$). HRESIMS: Calcd for $[\text{C}_{20}\text{H}_{24}\text{O}_9\text{S}-\text{H}]^-$: 439.1068. Found m/z : 439.1085.

3.2.14. Phenyl 2-C-carboxymethyl-2-deoxy-1-thio- α -D-glucopyranoside (10)

To a stirred mixture of the triacetate **26** (75 mg, 0.170 mmol) in acetone (10 mL) at 56 °C was added dropwise a solution of concentrated hydrochloric acid (1 mL) in water (1.8 mL). Stirring was continued overnight at 56 °C, whereafter the mixture was neutralised with TEA, concentrated under reduced pressure and co-evaporated with toluene (2×5 mL). The residue was purified by an RPC C18 column (55% MeOH) to furnish the carboxylic acid as white needles **10** (40 mg, 75%): mp 144–146 °C; R_f 0.38 (55% MeOH); $[\alpha]_D^{25} +247$ (c 1.0, MeOH); ^1H NMR (CD_3OD , 500 MHz): δ 7.49–7.26 (m, 5H, Ph), 5.61 (d, 1H, $J_{1,2}$ 4.5 Hz, H-1), 4.12–4.09 (m, 1H, H-5), 3.80 (dd, 1H, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.76 (dd, 1H, $J_{5,6b}$ 4.9 Hz, H-6b), 3.42–3.34 (m, 2H, H-3, H-4), 2.88 (dd, 1H, $J_{2,7a}$ 3.7, $J_{7a,7b}$ 16.0 Hz, H-7a), 2.56–2.46 (m, 2H, H-2, H-7b); ^{13}C NMR (CD_3OD , 125 MHz): δ 174.48 (COOH), 136.11–128.54, (C-Ph), 90.54 (C-1), 75.13 (C-5), 74.09, 72.93, 62.56 (C-6), 45.29 (C-2), 34.85 (C-7). HRESIMS: Calcd for $[\text{C}_{14}\text{H}_{18}\text{O}_6\text{S}-\text{H}]^-$: 313.0751. Found m/z : 313.0766.

3.2.15. Phenyl 2-C-carboxymethyl-2-deoxy-1-thio- β -D-glucopyranoside (11)

A methanolic 0.03 M NaOMe (0.43 mL, 0.013 mmol) solution was added to the triacetate **27** (19 mg, 0.043 mmol) in MeOH (1 mL) and the reaction mixture was stirred at room temperature overnight. Afterwards, it was neutralised with Amberlite IR-120 (H^+) ion-exchange resin, filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by an RPC C18 column (55% MeOH) to give the triol as white needles **11** (11 mg, 81%): mp 205–208 °C; R_f 0.42 (55% MeOH); $[\alpha]_D^{25} -50.0$ (c 0.6, MeOH); ^1H NMR (CD_3OD , 500 MHz): δ 7.54–7.27 (m, 5H, Ph), 4.85 (d, 1H, $J_{1,2}$ 10.7 Hz, H-1), 3.86 (dd, 1H, $J_{6a,6b}$ 11.9 Hz, H-6a), 3.68 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6b), 3.51 (t, 1H, $J_{2,3} = J_{3,4} = 8.9$ Hz, H-3), 3.34–3.29 (m, 1H, H-5), 3.26 (t, 1H, $J_{4,5}$ 8.9 Hz, H-4), 2.73 (dd, 1H, $J_{2,7a}$ 3.2, $J_{7a,7b}$ 16.6 Hz, H-7a), 2.60 (dd, 1H, H-7b), 2.04–1.96 (m, 1H, H-2); ^{13}C NMR (CD_3OD , 125 MHz): δ 174.59 (COOH), 135.78–128.22 (C-Ph), 88.85 (C-1), 81.82 (C-5), 77.85 (C-3), 72.83 (C-4), 63.10 (C-6), 45.59 (C-2), 36.85 (C-7). HRESIMS: Calcd for $[\text{C}_{14}\text{H}_{18}\text{O}_6\text{S}-\text{H}]^-$: 313.0751. Found m/z : 313.0766.

3.2.16. Phenyl 2-(N-aminocarbonyl)amino-2-deoxy-1-thio- β -D-glucopyranoside (12)

Potassium cyanate (278 mg, 3.42 mmol) was added to a suspension of the known²⁵ amino-glucopyranoside **28** (607 mg, 2.24 mmol) in water (15 mL). The mixture was stirred in total darkness at room temperature for 4 days. Whereafter, the water was evaporated to dryness under reduced pressure and the residue was co-evaporated with toluene (3×20 mL). RPC (25% CH_3CN) of the residue yielded the ureido compound **12** (458 mg, 65%): mp 220–222 °C (MeOH); R_f 0.40 (25% CH_3CN); $[\alpha]_D^{25} -32.0$ (c 1.5, DMSO); ^1H NMR (DMSO, 500 MHz): δ 7.41–7.17 (m, 5H, Ph), 6.02 (d, 1H, J 8.7 Hz, NH), 5.52 (s, 2H, NH_2), 5.07 (d, 2H, J 5.2 Hz, OH-3 and OH-4), 4.81 (d, 1H, $J_{1,2}$ 10.3 Hz, H-1), 4.62 (dd, 1H, J 5.8, J 11.5 Hz, 6-OH), 3.70 (ddd, 1H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.45 (d, 1H, H-6b), 3.40 (ddd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 3.30 (dt, 1H, $J_{3,4}$ 9.0 Hz, H-3), 3.24–3.21 (m, 1H, H-5), 3.13 (dt, 1H, $J_{4,5}$ 9.0 Hz, H-4); ^{13}C NMR (DMSO, 125 MHz): δ 158.47 (C=O), 136.17–

125.85 (C-Ph), 86.56 (C-1), 80.87 (C-5), 76.04 (C-3), 70.54 (C-4), 60.97 (C-6), 54.94 (C-2). HRESIMS: Calcd for $[\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_5\text{S}+\text{H}]^+$: 315.1009. Found m/z : 315.1012.

Acknowledgements

This work was supported by a programme grant from The Wellcome Trust (085622). One of the authors (N. Z. Abdelwahab) is indebted to the BBSRC for a Ph.D. studentship. We also thank Dr. A. V. Nikolaev for his interest and advice.

Supplementary data

Supplementary data (additional experimental procedures and partial characterisation data for those intermediates obtained from the sequence **13**→**14**, hydroxamates **16** and **17**, plus the hydroxamic acid **7**) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.02.004.

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