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


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A small zinc-binding group (ZBG) library of deoxy-2-C-branched-monosaccharides, for example, 1,5-anhydroglucitol, 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→6)-phosphatidylinositol de-N-acetylase (EC 3.5.1.89) of glycosylphosphatidylinositol biosynthesis. The N-ureido thioglycoside 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 cells. The structure, biosynthesis, and function of GPI anchors have been extensively reviewed.1–4 Disruption of GPI biosynthesis in the clinically relevant bloodstream form of T. brucei has been genetically5–8 and chemically9 validated as a drug target. Lastly, the N-ureido thioglycoside 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.

We have designed and synthesised a small library of deoxymonoaccharides [5–12 (Fig. 1)] 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-GlcNAc-PI de-N-acetylase.

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→6)-phosphatidylinositol10 [α-D-GlcNAc-PI (1, Fig. 1)] to form α-D-GlcNAc-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-GlcNAc-PI de-N-acetylases are zinc metalloenzymes, proposed a mechanism of action similar to that of zinc peptidases and postulated that known zinc binding motifs15,16 such as the N-hydroxyurea analogue 3 (Fig. 1),17 could act as inhibitors. Here, we have designed and synthesised a small library of deoxymonoaccharides [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-GlcNAc-PI de-N-acetylase.

A compound library was the earlier work by Hindsgaul and co-workers18,19 which demonstrated the effectiveness of 1,5-anhydro-2-deoxy-2-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 trypanosomal de-N-acetylase enzyme. Lastly, the N-ureido thioglucoside 12 was fashioned because of previous inhibitory data of the N-ureido-GlcNAc-PI derivative 410 (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.

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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, benzoylation→ozonolysis→Pinnick\(^{21}\) oxidation, from the known \(^{18}\) 2-C-allyl derivative 13 was accomplished straightforwardly to furnish the pivotal carboxylic acid 14.\(^{18}\) 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-benzoylation 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 (BnONH\(_2\)·HCl) using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) to give the known\(^{18}\) hydroxamic acid 16 (see the Supplementary data for the analytical data of 16). The benzoxoyamide 16 was hydrogenated, as described in the literature, to give the hydroxamic acid 7.\(^{18,19}\) \(^{1}H\) NMR assignments for 7 were identical to those reported in the literature\(^{19}\) and see the Supplementary data for the \(^{13}C\) NMR assignments of 7. The ZBG analogue 8 was synthesised following the sequence 16→17→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 acetylolation of the 1,6-anhydro derivative 18\(^{22}\) to give, exclusively, the α-2-C-allyl derivative 19 \((J_{1,2} = 3.1 \text{ Hz})\). 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 Pinnick’s 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-phenylthioglucosides 22 and 23, respectively, via Lewis acid (BF\(_3\)-Et\(_2\)O) catalysed substitution of the anomeric acetate with thiophenol in refluxing dichloromethane.\(^{24}\) These two anomers were separated by radial band chromatography to furnish the α-anomer 22 \((J_{1,2} = 4.9 \text{ Hz})\) and the β-anomer 23 \((J_{1,2} = 10.9 \text{ Hz})\) 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 hydrolysis\(^{24}\) (Scheme 2).

A synthesis of 1-thiophenyl-2-deoxy-2-ureido-β-D-glucopyranoside 12 was obtained on treatment of the known amine\(^{25}\) 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 \(^{13}C\) carbonyl carbon at \(\delta\) 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

\(^{1}H, \, ^{13}C, \, ^{31}P\) 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
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 F254s (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 (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 (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%): Rf 0.20 (10:1:0.02 CHCl3–MeOH–AcOH); ½ a138C25 D +8.9 (c 1.0, MeOH); 1H NMR (CD3OD, 500 MHz): δ 8.10–7.49 (m, 5H, Ph), 5.10 (dd, 1H, J2,3 10.7, J3,4 9.3 Hz, H-3), 4.10 (dd, 1H, J1a,2 4.7, J1a,1b 11.5 Hz, H-1a), 3.88 (dd, 1H, J5,6a 2.1, J6a,6b 11.8 Hz, H-6a), 3.71 (dd, 1H, J5,6b 2.1, J6b,6c 11.8 Hz, H-6b), 3.60 (t, 1H, J4,5 9.3 Hz, H-4), 3.40 (t, 1H, J1a,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, J2,7a 4.8, J7a,7b 16.0 Hz, H-7a), 2.17 (dd, 1H, H-7b); 13C NMR (CD3OD, 125 MHz): δ 175.49 (C=O), 168.15 (PhC=O), 134.29–129.56 (C-Ph), 82.75 (C-5), 79.92 (C-3), 70.56 (C-1), 62.94 (C-6), 40.03 (C-2), 34.10 (C-7). HRESIMS: Calcd for [C15H18O7Na]+: 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 MaOMe (0.6 mL, 0.018 mmol) solution was added to the benzate derivative 14 (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; Rf 0.24 (20:1:0.02 CH₂Cl₂−MeOH−AcOH); [α]D 20 = −209.0 (c 1.0, MeOH); ¹H NMR (CDCl₃, 500 MHz): δ 7.50−7.33 (m, 5H, PhH), 5.58 (s, 1H, PhCH₂), 4.20 (dd, 1H, J6,6a 5.0, J6,8a 10.3 Hz, H-6a), 4.02 (dd, 1H, J1a,2 4.7, J8a,10 11.4 Hz, H-1a), 3.70 (t, 1H, J1a,6b 10.3 Hz, H-6b), 3.50−3.45 (m, 2H, H-3), 3.37−3.32 (m, 2H, H-1b, H-5), 2.77 (dd, 1H, J1a,2a 3.0, J3,7b 15.8 Hz, H-7a), 2.21−2.14 (m, 1H, H-1b), 2.11 (dd, 1H, H-7b): ¹³C NMR (CDCl₃, 125 MHz): δ 176.06 (C-1), 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 [C₈H₁₆NO₆+Na⁺]: 395.1313. Found m/z: 395.1298.

3.2.6. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-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%): Rf 0.28 (1:2 light petroleum−EtOAc); [α]D 20 = +172.7 (c 0.8, CHCl₃); ¹H NMR (CDCl₃, 500 MHz): δ 9.61 (t, 1H, J1,2 1.2 Hz, H-2), 6.10 (2H, 2H, H-1a, H-1b), 5.18 (dd, 1H, J3,4 9.5 Hz, H-3), 5.03 (t, 1H, J4,5 9.5 Hz, H-4), 4.23 (dd, 1H, J5,6a 6.0, J6a,8b 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-1a, H-7b), 2.09, 2.02, 19.96, 19.94 (4 × s, 12H, 4 × CH₂CO); ¹³C NMR (CDCl₃, 125 MHz): δ 189.70 (H-1c), 169.70, 169.52, 168.66, 167.88 (4 × C-0), 90.86 (C-1), 70.18 (C-3), 68.84 (C-6), 67.80 (C-4), 60.73 (C-6), 49.76 (C-7), 41.65 (C-4), 21.85, 19.80, 19.70, 16.70, 4 (× CH₂CO). HRESIMS: Calcd for [C₁₆H₂₂O₁₀Na⁺]: 397.1313. Found m/z: 397.1298.

3.2.7. 1,3,4,6-Tetra-O-acetyl-2-carboxymethyl-2-carboxymethyl-2-deoxy-α-D-glucopyranose (21)
A solution of sodium chloride (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, 1.91 mmol) in tert-ButOH (56.7 mL, 604 mmol) and anisylene (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%): Rf 0.27 (1:1:0.02 light petroleum−EtOAc−AcOH); [α]D 20 = +88.3 (c 1.3, CHCl₃); ¹H NMR (CDCl₃, 500 MHz): δ 6.25 (d, 1H, J1,2 3.4 Hz, H-1), 5.22 (dd, 1H, J3,4 11.4 Hz, H-3), 5.03 (t, 1H, J4,5 9.5 Hz, H-4), 4.28 (dd, 1H, J5,6a 4.1, J6a,8a 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, J2a,7a 5.8, J7a,8a 13.6 Hz, H-7a), 2.27 (dd, 1H, H-7b, 2.04, 2.01, 19.99 (4 × s, 12H, 4 × CH₂CO); ¹³C NMR (CDCl₃, 125 MHz): δ 174.59, 172.40, 172.14, 171.44, 170.75 (5 × C-0), 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, 20.65 (4 × CH₂CO). HRESIMS: Calcd for [C₇₁H₃₂O₃₅Na⁺]: 389.1089. Found m/z: 389.1085.

3.2.8. 2-Carboxymethyl-2-deoxy-α-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 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 (1:3.1) mixture (27 mg, 5%; Rf 0.25 (3:1:0.02 CH₂Cl₂−MeOH−AcOH); ¹H NMR (CDCl₃,
500 MHz): δ 5.23 (d, 1H, J1,2 3.1 Hz, H-1a), 4.63 (d, J3,4 8.6 Hz, H-1p), 3.85 (dd, J5a,b 11.7 Hz, H-6aβ), 3.80–3.76 (m, 2H, H-5, H-6α), 3.70 (dd, J6a,b 11.4 Hz, H-6b), 3.66 (dd, H-6b), 3.51 (dd, J3,4 8.9 Hz, H-3x), 3.36 (dd, J5,6a 10.7 Hz, J3,4 8.1 Hz, H-3β), 3.32–3.21 (m, 3H, H-4x, H-4β, H-5β), 2.71 (dd, J2,3 3.3, J2,7b 16.6 Hz, H-7α), 2.60 (dd, J2,3 4.1, J2,7b 16.1 Hz, H-7α), 2.46 (dd, H-7β), 2.39 (dd, H-7bx), 2.08–2.03 (m, 1H, H-2x), 1.91–1.85 (m, 1H, H-2β), 13C NMR (CDCl3, 125 MHz): δ 177.22, 175.55 (2 × C=O), 98.23 (C-1), 93.79 (C-1α), 77.99 (C-5β), 76.06 (C-3β), 73.25 (C-5α), 71.37 (C-19), 72.90 (C-4α), 63.06 (C-6β), 63.02 (C-6α) 47.26 (C-2β), 44.83 (C-2α), 33.62 (C-7α), 33.51 (C-7β). HRE-SIMS: Calcd for [C22H24O6S2Na]+: 421.1067. Found m/z: 421.1059.

3.2.9. Phenyl 3,4,6-tri-0-acetyl-2-callyl-2-deoxy-1-thio-β- and β-0-glucopyranoside (22) and (23)

To a stirred solution of the tetraacetate 19 (200 mg, 0.537 mmol) in freshly distilled CH2Cl2 (10 mL) at room temperature under argon was added triphenylphosphine (110 μL, 1.074 mmol) and boron trifluoride diethyl etherate (270 μL, 2.14 mmol). The resulting mixture was heated to reflux for 3 h, cooled to room temperature, and then diluted with CH2Cl2 (10 mL), washed with satd NaHCO3 (10 mL), brine (10 mL), dried (Na2SO4), and concentrated under reduced pressure. RBC (1:0.1:4.1 light petroleum–EtOAc) was used in place of triphenylphosphine. The residue was purified by RBC (6:1 2:1 light petroleum–EtOAc) to afford the aldehyde 23 (25 mg, 0.562 mmol) essentially as described for the previous α-derivative 24. However, dimethyl sulfide (130 μL, 1.77 mmol) was used in place of triphenylphosphine. The residue was purified by RBC (6:1 2:1 light petroleum–EtOAc) to afford the aldehyde 25 (21.8 mg, 87%): Rf 0.21 (2:1 light petroleum–EtOAc); δ 10.7 (c 1.5, CHCl3); 1H NMR (CDCl3, 500 MHz): δ 9.59 (s, 1H, H-CO), 7.53–7.31 (m, 5H, Ph), 5.15 (dd, 1H, J2,3 10.7, J3,4 9.5 Hz, H-3), 4.96 (t, 1H, J4,5 9.5 Hz, H-4), 4.84 (d, 1H, J1,2 10.7 Hz, H-1), 4.27 (dd, 1H, J5a,b 5.3, J6a,b 12.2 Hz, H-6a), 4.17 (dd, 1H, H-6b), 3.76–3.71 (m, 1H, H-5), 2.84 (dd, 1H, J7a,b 3.8, J7a,b 16.4 Hz, H-7a), 2.57 (dd, 1H, H-7b), 2.45–2.38 (m, 3H, H-2, H-1), 2.10, 2.01, 1.97 (3 × 9H, 3 × CH3CO); 13C NMR (CDCl3, 125 MHz): δ 198.94 (HC=O), 170.69, 170.31, 168.92 (3 × C=O), 132.87–128.45 (C-Ph), 86.51 (C-1), 76.57 (C-5), 74.49 (C-3), 69.26 (C-24), 64.27 (C-63), 40.93 (C-7), 40.51 (C-22), 20.81, 20.67, 20.61 (3 × CH3CO). HRE-SIMS: Calcd for [C22H20O8S2Na]+: 447.1064. Found m/z: 447.1056.

3.2.10. Phenyl 3,4,6-tri-0-acetyl-2-callyl-2-deoxy-1-thio-β and β-0-glucopyranoside (24)

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, 1.77 mmol) was used in place of triphenylphosphine. The residue was purified by RBC (6:1 2:1 light petroleum–EtOAc) to afford the aldehyde 25 (21.8 mg, 87%): Rf 0.21 (2:1 light petroleum–EtOAc); δ 10.7 (c 1.5, CHCl3); 1H NMR (CDCl3, 500 MHz): δ 9.59 (s, 1H, H-CO), 7.53–7.31 (m, 5H, Ph), 5.15 (dd, 1H, J2,3 10.7, J3,4 9.5 Hz, H-3), 4.96 (t, 1H, J4,5 9.5 Hz, H-4), 4.84 (d, 1H, J1,2 10.7 Hz, H-1), 4.27 (dd, 1H, J5a,b 5.3, J6a,b 12.2 Hz, H-6a), 4.17 (dd, 1H, H-6b), 3.76–3.71 (m, 1H, H-5), 2.84 (dd, 1H, J7a,b 3.8, J7a,b 16.4 Hz, H-7a), 2.57 (dd, 1H, H-7b), 2.45–2.38 (m, 3H, H-2, H-1), 2.10, 2.01, 1.97 (3 × 9H, 3 × CH3CO); 13C NMR (CDCl3, 125 MHz): δ 198.94 (HC=O), 170.69, 170.31, 168.92 (3 × C=O), 132.87–128.45 (C-Ph), 86.51 (C-1), 76.57 (C-5), 74.49 (C-3), 69.26 (C-24), 64.27 (C-63), 40.93 (C-7), 40.51 (C-22), 20.81, 20.67, 20.61 (3 × CH3CO). HRE-SIMS: Calcd for [C22H20O8S2Na]+: 447.1064. Found m/z: 447.1056.
To a stirred mixture of the triacetate, filtered, and the filtrate was concentrated overnight. Afterwards, it was neutralised with Amberlite IR-120 (1 mL) and the reaction mixture was stirred at room temperature. The residue was purified by an RPC C18 column (55% MeOH) to give the triol as white needles (11 mg, 75%): mp 144–146°C; Rf 0.42 (55% MeOH); 1H NMR (CD3OD, 500 MHz): δ 5.74–7.26 (m, 5H, Ph), 3.68 (dd, 1H, J3,4 = 8.9 Hz, H-3), 3.34–3.29 (m, 1H, H-5), 3.26 (dd, 1H, J5,6a = 8.9 Hz, H-4), 2.73 (dd, 1H, J7a,7b = 16.6 Hz, H-7a), 2.60 (dd, 1H, J7b,7a = 16.6 Hz, H-7b). This mixture was co-evaporated with toluene (20 mL). RPC (25% CH3CN) of the residue yielded the ureido compound (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 x 20 mL). RPC (25% CH3CN) of the residue yielded the ureido compound (12) (458 mg, 65%): mp 220–222°C (MeOH); Rf 0.40 (25% CH3CN); 1H NMR (DMSO-D6, 500 MHz): δ 2.21–2.15 (m, 2H, H-5), 1.68–1.56 (m, 4H, H-7), 1.43–1.35 (m, 4H, H-5), 0.93–0.86 (m, 2H, H-4). 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, and 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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