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Note

Synthesis of 1-D-6-*O*-[2-(*N*-hydroxyaminocarbonyl)amino-2-deoxy-α-D-glucopyranosyl]-*myo*-inositol 1-(*n*-octadecyl phosphate): a potential metalloenzyme inhibitor of glycosylphosphatidylinositol biosynthesis

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Abstract—1-D-6-*O*-[2-(*N*-Hydroxyaminocarbonyl)amino-2-deoxy-α-D-glucopyranosyl]-*myo*-inositol 1-(*n*-octadecyl phosphate) was prepared to probe the reaction mechanism of the putative zinc-dependent metalloenzyme 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→6)-phosphatidylinositol de-*N*-acetylase of glycosylphosphatidylinositol biosynthesis.

Keywords: Glycosylphosphatidylinositol (GPI) membrane anchors; *N*-Hydroxyurea; Zinc metalloenzyme inhibitor; *N*-Acetyl-D-glucosaminylphosphatidylinositol de-*N*-acetylase

Glycoconjugates on the cell surface of parasitic protozoa of the trypanosomatidae (including African trypanosomes, American trypanosomes and *Leishmania* spp. that are human and animal pathogens) have a crucial role in determining parasite survival and infectivity.[1–3] Many glycoconjugates are attached to the plasma membrane by means of glycosylphosphatidylinositol (GPI) anchors,[1,4] whose principal function is to provide stable association of protein or oligosaccharide with the lipid bilayer. Although this type of anchor is not confined to the protozoa, it does appear to be used with a much greater frequency in these organisms than in higher eukaryotes.[1] A key, early step in the biosynthesis of the GPI anchors involves the de-N-acetylation of 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→6)-phosphatidylinositol,[5] [α-D-Glc*p*NAc-PI (**1**, Fig. 1)], to form α-D-Glc*p*NH_2-PI (**2**, Fig. 1).

De-N-acetylation is a prerequisite for subsequent processing of **2** that leads to mature GPI anchor precursors.[6] In *Trypanosoma brucei*, the causative agent of African sleeping sickness, de-N-acetylation, is followed by mannosylation and subsequent inositol-acylation of

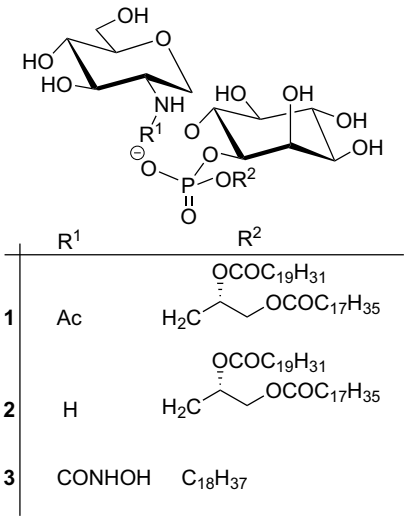

Figure 1.

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2,[7] whereas in mammalian cells the order of these reactions is reversed.[8] Disruption of GPI biosynthesis is fatal to the bloodstream form parasite in culture,[9–11] and a conditional null mutant of the gene encoding the α-D-Glc*p*NAc-PI de-*N*-acetylase in *T. brucei* (TbGPI12) has genetically validated this enzyme as a drug target.[10]

Recently, we have shown[12] that mammalian and trypanosomal α-D-Glc*p*NAc-PI de-*N*-acetylases are zinc metalloenzymes, proposed a mechanism of action similar to that of zinc peptidases and postulated that known zinc binding moieties[13] such as *N*-hydroxyurea could act as inhibitors. As previous studies have demonstrated that the diacylglycerol portion of **1** is not specifically recognised by the enzyme, and may be efficiently replaced by an octadecyl chain,[14] we synthesised, therefore, the *N*-hydroxyurea analogue 1-D-6-*O*-[2-(*N*-hydroxyaminocarbonyl)amino-2-deoxy-α-D-glucopyranosyl]-*myo*-inositol 1-(*n*-octadecyl phosphate) (**3**, Fig. 1).

The synthesis of the *N*-hydroxyurea analogue **3** began by reducing the azide to the amine of the known pseudodisaccharide[15] **4** by a Staudinger reaction,[16] followed by immediate treatment with 1-(4-nitrophenol)-*N*-(*O*-benzylhydroxy) carbamate[13] in the presence of triethylamine to give the crude *O*-benzyl protected *N*-hydroxyurea **5** (Scheme 1).

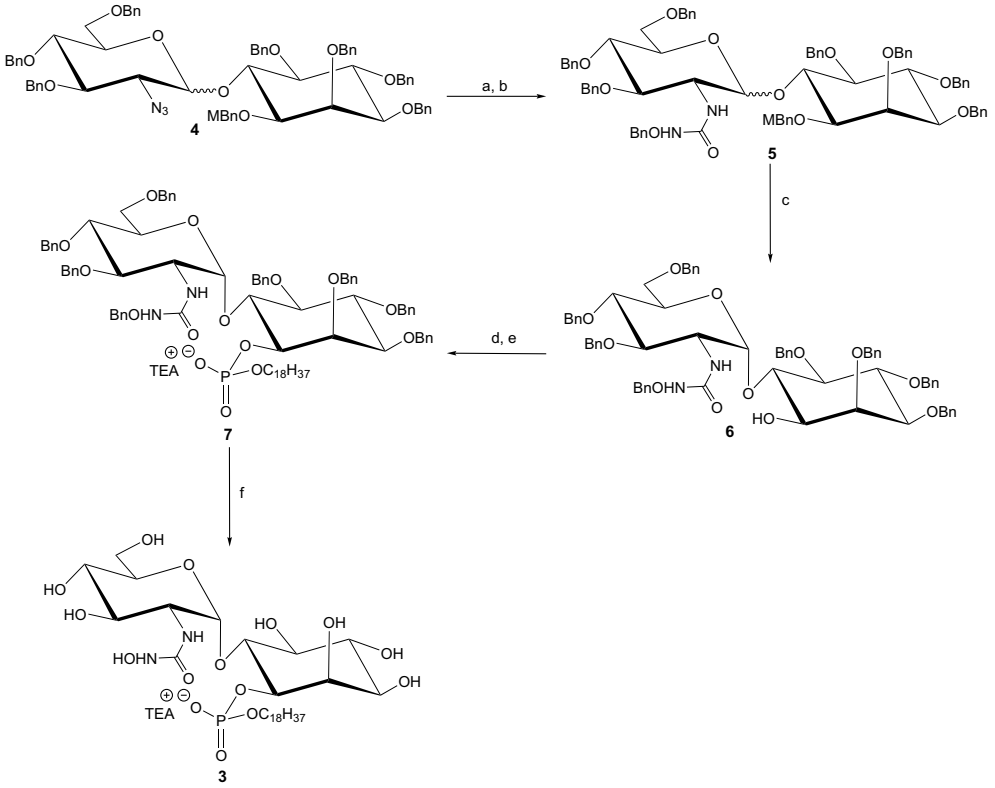
Trifluoroacetic acid removal of the 4-methoxybenzyl group followed by radial-band chromatography afforded the α-coupled compound **6** in 82% yield over three steps.

The phospholipid moiety was introduced by the reaction of 1-OH of the benzylated pseudodisaccharide **6** with triethylammonium *n*-octadecyl hydrogenphosphonate[17] in the presence of pivaloyl chloride in pyridine to give a mixture of diastereoisomeric phosphonic diesters that was converted into the phosphoric diester **7** on oxidation in situ with iodine in wet pyridine.[18] The final transformation (**7**→**3**) was accomplished by hydrogenolysis over 20% $Pd(OH)_2$ on carbon. Details of the results of enzymic studies with the *N*-hydroxyurea analogue **3** will be reported elsewhere.

1. Experimental

1.1. General methods

Chemicals were purchased from commercial sources and were used without further purification. ^1H and ^{13}C NMR plus COSY, HSQC and DEPT45 spectra were recorded on a Bruker Avance 500 MHz spectrometer using tetramethylsilane as the internal standard. ^{31}P NMR spectra used 85% phosphoric acid in D_2O as the external standard. Optical rotations were measured using a Perkin Elmer 343 polarimeter. High resolution



Scheme 1. Reagents and conditions: (a) Ph_3P, THF/H_2O {10:1}, 60 °C, 3 h; (b) 1-(4-nitrophenol)-*N*-(*O*-benzylhydroxy) carbamate,[13] Et_3N, rt, overnight; (c) TFA, CH_2Cl_2, rt, 2 h, 82% over 3 steps; (d) triethylammonium *n*-octadecyl hydrogenphosphonate,[17] PivCl, pyridine, rt, 2 h; (e) I_2, pyridine/H_2O {19:1}, rt, 45 min, 58%; (f) H_2, 20% $Pd(OH)_2$/C, *n*-propanol/THF {1:1}, 3 atm, rt, 3 h, 94%.

electrospray ionisation mass spectra (HRESIMS) were recorded with a Bruker microTOF spectrometer. HPLC was performed using a Dionex P680 HPLC pump, an Alltech ELSD 800 detector and a Vydac protein C4 column (250 mm × 46 mm) employing a gradient of 10% *n*-propanol + 0.05% TFA in water → 95% *n*-propanol + 0.05% TFA in water. TLC was performed on Kieselgel 60 F_{254} (Merck) with detection under UV light or by charring with sulfuric acid–water–ethanol (15:85:5). Radial-band chromatography (RBC) was performed using a Chromatotron (model 7924T, TC Research, UK) with TLC standard grade silica gel (2–25 μm) (Aldrich) as the adsorbent.

1.2. 1-D-6-*O*-[2-(*N*-Benzyloxyaminocarbonyl)amino-2-deoxy-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl]-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol (6)

To a stirred α,β mixture of **4**[15] (50 mg, 0.04 mmol) in 10:1 THF–water (3 mL) at 60 °C was added Ph_3P (31 mg, 0.12 mmol). After 3 h, the reaction mixture was cooled to rt and then Et_3N (78 μL, 0.56 mmol) and 1-(4-nitrophenol)-*N*-(*O*-benzylhydroxy) carbamate[13] (29 mg, 0.10 mmol) were added. Stirring of the mixture was continued overnight, whereafter it was evaporated and co-evaporated with toluene (3 × 10 mL) under reduced pressure. A solution of the residue in EtOAc was percolated through a short column of silica gel (further elution with EtOAc) and the eluent was concentrated under reduced pressure to give the crude derivative **5**. A solution of this methoxybenzyl compound **5** in CH_2Cl_2 (2 mL) containing TFA (27 μL, 0.36 mmol) was set aside at rt for 2 h, whereafter it was neutralised with Et_3N, washed successively with water and brine, dried ($MgSO_4$) and concentrated under reduced pressure. RBC of the residue (elution first with hexane and then with 1:1 hexane–EtOAc) afforded the α-linked *O*-benzyl protected *N*-hydroxyurea pseudodisaccharide **6** (37 mg, 82% over 3 steps); $[\alpha]_D^{25}$ +63 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–6.90 (40H, 8 × Ph), 6.25 (d, 1H, $J_{2',NH}$ 9.3 Hz, N*H*C(O)NHOBn), 5.39 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 5.05–4.16 (16H, 8 × CH_2Ph), 4.12 (m, 1H, H-2′), 4.03 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 3.94 (br d, 1H, $J_{4',5'}$ 9.9 Hz, H-5′), 3.85 (t, 1H, $J_{1,6} = J_{5,6}$ 9.4 Hz, H-6), 3.81 (t, 1H, $J_{1,2} = J_{2,3}$ 2.2 Hz, H-2), 3.76 (t, 1H, $J_{3',4'}$ 9.7 Hz, H-4′), 3.69 (t, 1H, $J_{2',3'}$ 9.2 Hz, H-3′), 3.31 (dd, 1H, H-3), 3.25 (dd, 1H, $J_{5',6'a}$ 2.8, $J_{6'a,6'b}$ 11.3 Hz, H-6′a), 3.19 (m, 2H, H-5, 6′b), 3.09 (dt, 1H, H-1), 2.33 (d, 1H, $J_{1,OH}$ 9.6 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.6 (C═O), 138.0–126.0 (C–Ph), 97.4 (C-1′), 80.6 (C-4), 80.1 (C-5), 80.0 (C-3, 3′), 77.7 (C-6), 77.4, 76.8 (C-4′), 76.6, (C-2), 76.3, 76.0, 75.8, 74.8, 74.4, 73.9, 73.8, 73.6, 72.2, 72.0 (C-1), 71.9, 69.9 (C-5′), 66.9 (C-6′), 52.4 (C-2′); HRESIMS: calcd for $[C_{69}H_{72}N_2O_{12}+H]^+$: 1121.5158. Found *m*/*z*: 1121.5158.

1.3. Triethylammonium 1-D-6-*O*-[2-(*N*-benzyloxyaminocarbonyl)amino-2-deoxy-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl]-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 1-(*n*-octadecyl phosphate) (7)

Each of compounds **6** (60 mg, 0.05 mmol) and triethylammonium *n*-octadecyl hydrogenphosphonate[17] (47 mg, 0.11 mmol) was dried overnight over P_2O_5 in a vacuum desiccator, whereafter anhyd pyridine (5 mL) was evaporated therefrom. They were then dissolved in dry pyridine (5 mL), pivaloyl chloride (42 μL, 0.34 mmol) was added and the resulting solution was stirred under argon at rt for 2 h. A freshly prepared solution of iodine (53 mg. 0.21 mmol) in 19:1 pyridine–water (10 mL) was then added and stirring of the reaction was continued for 45 min. After the addition of CH_2Cl_2 (25 mL), the organic solution was washed successively with 5% $NaHSO_3$ (25 mL), water (25 mL) and 1 M TEAB buffer solution (3 × 15 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (19:1 $CHCl_3$–MeOH) of the residue afforded the TEA phosphate derivative **7** (45 mg, 58%); $[\alpha]_D^{25}$ +40 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.60 (s, 1H, N*H*(CH_2CH_3)$_3$), 9.65 (s, 1H, N*H*OBn), 7.40–6.95 (40H, 8 × Ph), 6.35 (d, 1H, $J_{2',NH}$ 8.8 Hz, N*H*C(O)NHOBn), 5.16 (s, 1H, H-1′), 5.03–4.34 (14H, 7 × CH_2Ph), 4.30 (s, 1H, H-2), 4.23 (d, 1H, $J_{1,6}$ 11.0 Hz, H-1), 4.12–3.99 (m, 5H, H-2′, 4, 6, CH_2Ph), 3.91 (d, 1H, $J_{4',5'}$ 9.3 Hz, H-5′), 3.77–3.60 (m, 4H, H-3′, 4′, OCH_2), 3.42 (d, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.30 (t, 1H, $J_{4,5} = J_{5,6}$ 8.2 Hz, H-5), 2.95 (dd, 2H, $J_{6'a,6'b}$ 11.0 Hz, H-6′a,b), 2.77 (m, 6H, 3 × C$H_2$$CH_3$), 1.50 (m, 2H, OCH_2CH_2), ~1.15 (30H, $[CH_2]_{15}$), 1.00 (t, 9H, *J* 7.2 Hz, 3 × CH_2CH_3), 0.81 (t, 3H, *J* 7.0 Hz, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.1 (C═O), 138.4–126.2 (C–Ph), 98.9 (C-1′), 81.2, 81.1, 80.0 (C-5), 79.8 (C-3), 77.3, 76.6, 76.3, 76.0, 75.8, 75.5 (C-2), 74.8, 74.2, 74.1, 73.5, 72.1, 71.4, 69.8 (C-5′), 66.8 (C-6′), 64.6 (OCH_2), 52.3, 44.1 (3 × C$H_2$$CH_3$), 30.9, 30.2, 30.1, 29.3, 28.7, 28.5, 28.4, 25.5, 25.0, 21.7, 13.1 (CH_2CH_3), 7.3 (3 × CH_2CH_3); ^{31}P NMR (202 MHz, $CDCl_3$): δ_P −1.68 (with ^{1}H heteronuclear decoupling); HRESIMS: calcd for $[C_{93}H_{124}N_3O_{15}P-NEt_3-H]^-$: 1451.7493. Found *m*/*z*: 1451.7459.

1.4. Triethylammonium 1-D-6-*O*-[2-(*N*-hydroxyaminocarbonyl)amino-2-deoxy-α-D-glucopyranosyl]-*myo*-inositol 1-(*n*-octadecyl phosphate) (3)

A solution of the TEA salt **7** (30 mg, 0.02 mmol) in 1:1 *n*-propanol–THF (10 mL) containing 20% $Pd(OH)_2$ on carbon (20 mg) was stirred under 3 atm of hydrogen for 3 h before it was percolated through a short column of Chelex 100 on a bed of Celite (further elution with 1:1 *n*-propanol–THF). The eluent was concentrated under reduced pressure and then purified via HPLC to give

the deprotected product **3** (15 mg, 94%); $[\alpha]_D^{25}$ +21 (*c* 1.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.10 (d, 1H, $J_{1',2'}$ 3.2 Hz, H-1′), 3.84 (m, 1H, H-4′), 3.65 (m, 2H, H-2, 6′a), 3.57 (br t, 3H, *J* 6.5 Hz, H-6, OCH_2), 3.40 (m, 2H, H-1, 6′b), 3.20 (q, 6H, *J* 7.3 Hz, 3 × CH_2CH_3), 2.35 (m, 1H, H-4), 2.00–1.78 (m, 5H, H-2′, 3, 3′, 5, 5′), 1.60 (m, 2H, OCH_2CH_2), ~1.30 (39H, 3 × CH_2CH_3, $[CH_2]_{15}$), 0.90 (t, 3H, *J* 7.5 Hz, CH_2CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 177.3 (C=O), 105.1 (C-1′), 68.0 (C-4′), 67.2, 62.8, 62.1, 47.9 (3 × CH_2CH_3), 33.2, 33.1, 31.8, 31.4, 30.8, 30.5, 28.9, 26.3, 24.4, 23.4, 14.5 (CH_2CH_3), 9.25; ^{31}P NMR (202 MHz, $CDCl_3$): δ_P 0.12 (with ^{1}H heteronuclear decoupling); HRESIMS: calcd for $[C_{37}H_{76}N_3O_{15}P-NEt_3-H]^-$: 731.3737. Found *m*/*z*: 731.3749.

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