Parasite glycoconjugates. Part 3.[1] Synthesis of substrate analogues of early intermediates in the biosynthetic pathway of glycosylphosphatidylinositol membrane anchors

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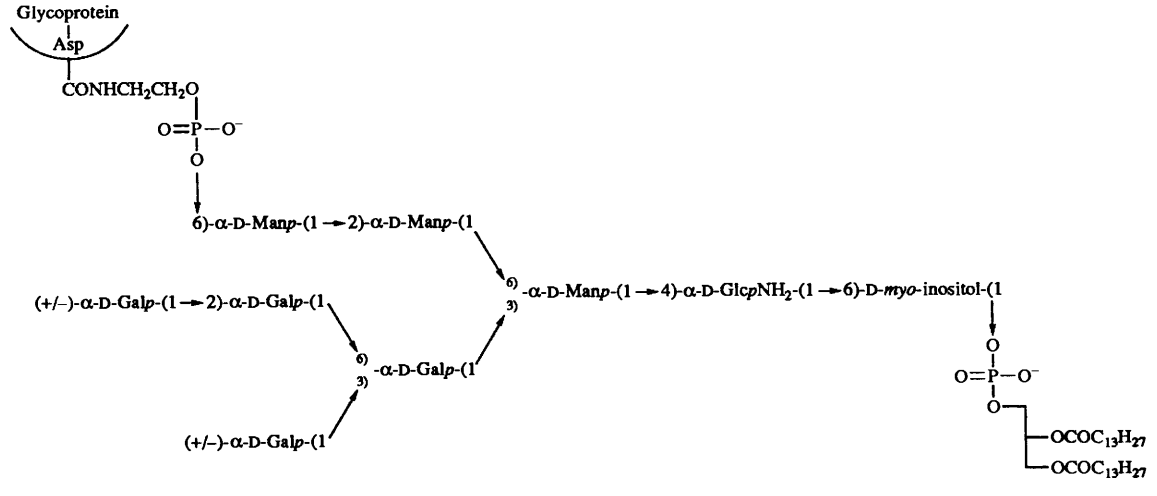
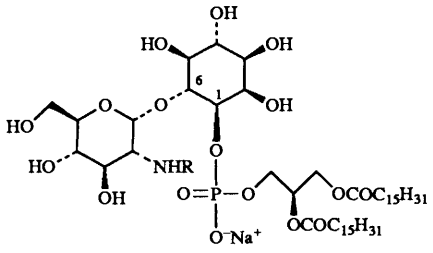
Substrate analogues of sodium 1D-6-*O*-(2-[$^3H_3$]acetamido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-[*sn*-2,3-bis(palmitoyloxy)propyl phosphate] **3**, including the lipid-depleted compounds **9**, **11** and **15**, have been prepared for biological evaluation with a partially purified de-N-acetylase from the bloodstream form of the parasitic protozoan *Trypanosoma brucei*. Such analogues as 2-deoxy-α-D-Glc*p*-PI **25** and α-D-Glc*p*-PI **33** have also been prepared and tested as potential inhibitors of this de-N-acetylase.

Introduction

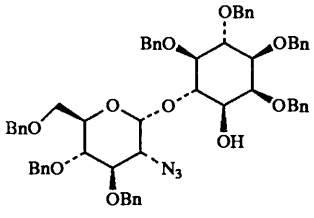
Glycosyl phosphatidylinositol (GPI) membrane anchors are distributed widely among the eukaryotes.[2] Their principal function is to anchor proteins or oligosaccharides to the outer leaflet of the plasma membrane.[3,4] Protein-linked GPI anchors and GPI-related glycolipids, such as the lipophosphoglycans and glycoinositol phospholipids of the *Leishmania*, are particularly abundant in the protozoa.[5] The tsetse fly-transmitted African trypanosomes, which cause human sleeping sickness and a variety of livestock diseases, are able to survive in the host's bloodstream by virtue of a dense cell-surface coat consisting of 10 million copies of a GPI-anchored glycoprotein called the variant surface glycoprotein (VSG).[6] A schematic representation of the GPI-membrane anchor of the VSG of *Trypanosoma brucei*, an African protozoan parasite that causes a disease related to sleeping sickness in domestic cattle, is shown in formula **1**.[4]

The complete GPI biosynthetic pathway in trypanosomes has been elucidated,[7-14] while that in other protozoa[15] and in mammalian cells[16] appears to be broadly similar. The first step in the biosynthetic pathway involves the transfer of α-D-Glc*p*NAc from UDP-D-Glc*p*NAc (where UDP = uridine 5'-diphospho) to form α-D-Glc*p*NAc-(1→6)-PI, which is rapidly de-N-acetylated to form α-D-Glc*p*NH_2-(1→6)-PI.[7] Each of the three α-D-Man*p* residues is then transferred in turn from dolichol phosphate D-mannose to form α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→4)-α-D-Glc*p*NH_2-(1→6)-PI,[8,9] to which is added ethanolamine phosphate (from phosphatidylethanolamine) at the terminal α-D-Man*p* residue.[10] The resulting structure undergoes a complex series of fatty-acid-remodelling reactions[11] (to yield an *sn*-dimyristoyl-PI moiety) before the preassembled GPI precursor (known as glycolipid A) is transferred *en bloc* to newly synthesized protein.[12] Some α-D-galactosylation of the GPI anchor occurs in the endoplasmic reticulum,[13] the principal site of biosynthesis, but mainly in the Golgi apparatus during transport to the surface membrane,[14] when as many as five α-D-Gal*p* residues may be added to the GPI anchors of *T. brucei* VSG.[2] The extraordinary dependence of parasitic protozoa on GPI-anchored proteins and/or GPI-related molecules for survival and infectivity makes the GPI biosynthetic pathway an attractive target for the development of chemotherapeutic agents. This approach is predicated on the belief that disruption of GPI biosynthesis would seriously impair the parasite's ability to survive in the host's bloodstream.





2 R = H
3 R = COC^3H_3



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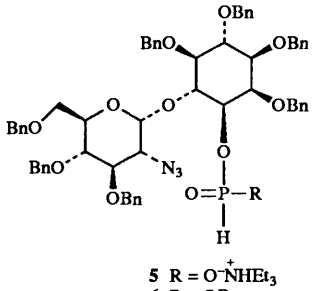
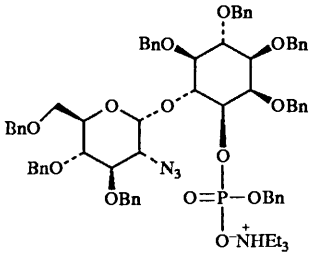


5 R = $O^-\overset{+}{N}HEt_3$
6 R = OBn



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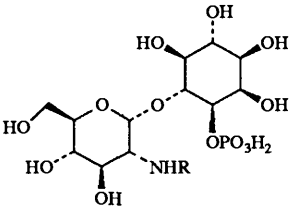
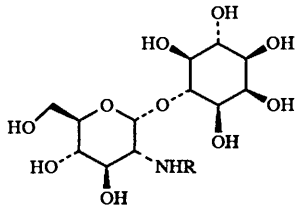
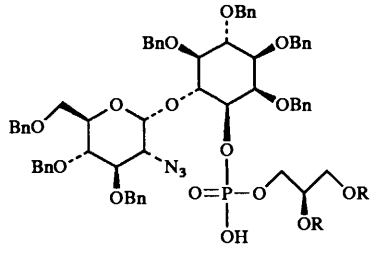


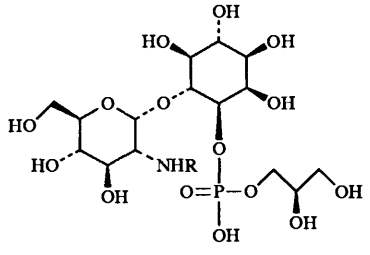
8 R = H
9 R = COC^3H_3



10 R = H
11 R = COC^3H_3



12 R = $COC_{15}H_{31}$
13 R = H



14 R = H
15 R = COC^3H_3

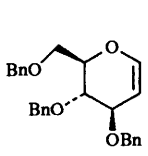
Results and discussion

In our initial investigation [17] along these lines, the glycosylphosphatidylinositol **2** was synthesized and, after N-[^{3}H]acetylation (⟶ **3**), was used [18] as a substrate in the partial purification of the α-D-Glc*p*NAc-PI de-N-acetylase of the bloodstream form of *T. brucei*. Having achieved a partial purification of the *T. brucei* de-N-acetylase, it should now be possible to probe its substrate specificity by using synthetic substrate analogues, and also to examine potential inhibitors of the enzymic de-N-acetylation of the close substrate analogue **3**, which differs from the enzyme's natural substrate only in that the lipid component is esterified with palmitic acid rather than other long-chain fatty acids. Routes to the substrate analogues **9**, **11** and **15** and the potential inhibitors **25** and **33** are described herein. The substrate analogues **9**, **11** and **15** were chosen with a view to assessing the roles of the lipid, phospholipid and fatty acid moieties, respectively, in substrate recognition, whereas 2-deoxy-α-D-Glc*p*-PI **25** and α-D-Glc*p*-PI **33** retain all the components of the substrate analogue **3** except that the vital NHAc group at C-2 of the sugar moiety is replaced by either H or OH.

Although several routes have now been devised [19,20] for the synthesis of 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-phosphate **8**, which is also of interest for its possible insulin-like activity,[20] we chose to use an adaptation of the hydrogen phosphonate procedure.[21] Thus, the glycosyl inositol derivative **4** [17] reacted with phosphorous acid in pyridine to give, after conversion into the triethylammonium (TEA) salt, the hydrogen phosphonate **5**. Conventional treatment of the latter compound with benzyl alcohol in the presence of pivaloyl chloride in pyridine [22,23] furnished the phosphonic diester **6**, as a mixture of diastereoisomers. Oxidation of the phosphonic diester **6** *in situ* with iodine in aq. pyridine [21] gave the phosphoric diester, which was isolated and characterised as the TEA salt **7**. Hydrogenolysis of the free-acid form of the phosphoric diester **7** in the presence of 20% palladium hydroxide on carbon yielded the deprotected compound **8**, whose conversion into the *N*-tritritioacetyl derivative **9** for enzymic studies has been described elsewhere.[18] Also prepared from the glycosyl-inositol derivative **4** [17] by similar hydrogenolysis was the dephosphorylated material **10**. The preparation of the *N*-tritritioacetyl derivative **11** from compound **10** and enzymic studies thereon have been described elsewhere.[18]

Deacylation of the previously described [17] di-*O*-palmitoyl compound **12** with sodium methoxide gave the glycerophosphate derivative **13**, which on catalytic hydrogenolysis produced the phosphoric diester **14** required for conversion [18] into the *N*-tritritioacetyl derivative **15**. Biological assay of this and the other tritiated compounds **3**, **9** and **11** with the *T. brucei* de-N-acetylase was based on the initial rate of release of [^{3}H]acetic acid.[18]

The first step in the synthesis of the 2'-deoxy analogue **25** of the proven [18] substrate **3** for the *T. brucei* de-N-acetylase involved the coupling of 3,4,6-tri-*O*-benzyl-D-glucal **16** with the 1D-*myo*-inositol derivative **17** [17] in the presence of triphenylphosphine–hydrogen bromide.[24] The α-coupled product **18** ($J_{1',2'ax}$ 3.5, $J_{1',2'eq}$ ~ 1 Hz), obtained in 56% yield, was then treated with ammonium cerium(IV) nitrate [25] (CAN) in aq. acetonitrile to remove the 4-methoxybenzyl group at O-1 of the D-*myo*-inositol residue (⟶**19**). Thereafter the hydrogen phosphonate approach [21] was used to introduce the phospholipid component at O-1. This involved the reaction of compound **19** with the hydrogen phosphonate **20** [22] in the presence of pivaloyl chloride in pyridine [22,23] to give the



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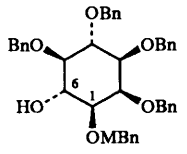
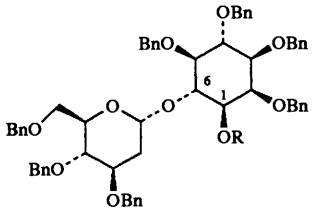
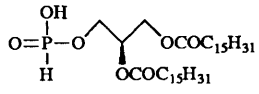


17

MBn = *p*-methoxybenzyl



18 R = MBn
19 R = H



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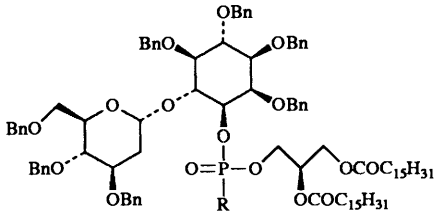
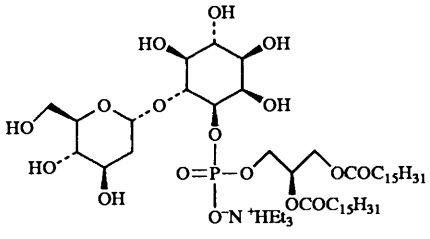


21 R = H
22 R = OH
23 R = O⁻NHEt₃
24 R = O⁻Na⁺



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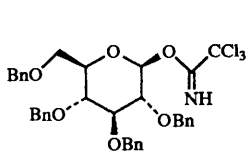

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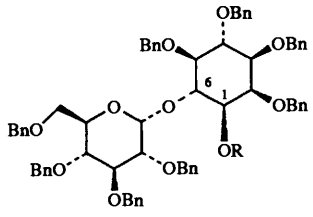
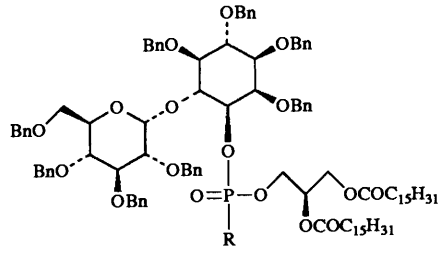
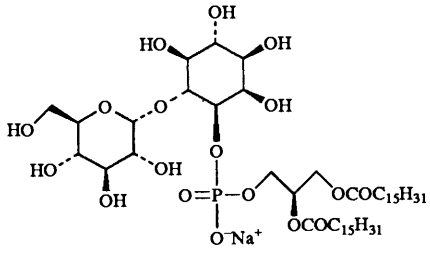


27 R = MBn
28 R = H



29 R = H
30 R = OH
31 R = O⁻NHEt₃
32 R = O⁻Na⁺



33

phosphonic diester **21** as a mixture of diastereoisomers. Oxidation of this diester with iodine in aq. pyridine[21] afforded the phosphoric diester **22**, which was isolated and characterised at the TEA salt **23**. Hydrogenolysis of the corresponding sodium salt **24** in the presence of palladium hydroxide on carbon provided the fully deprotected 2-deoxy-α-D-Glc*p*-PI **25** (isolated as the TEA salt).

For the synthesis of α-D-Glc*p*-PI **33**, the L-*myo*-inositol derivative **17**[17] was coupled in diethyl ether with the trichloroacetimidate **26**[26] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give the fully protected α-D-glucopyranosylinositol **27** ($J_{1',2'}$ 3.4 Hz) in 76% yield. Thereafter, the compound **27** was taken through an identical sequence of reactions, *viz.* **27** ⟶ **28** ⟶ **29** ⟶ **30** ⟶ **31** ⟶ **32** ⟶ **33**, to that used in preparing 2-deoxy-α-Glc*p*-PI **25**.

The results of biological evaluation of α-D-GlcNAc*p*-PI **3** and the substrate analogues **9**, **11** and **15** have been reported elsewhere.[18] Briefly, α-D-Glc*p*NAc-PI **3** proved to be a good exogenous substrate for a partially purified *T. brucei* de-N-acetylase. The analogue **15**, lacking the fatty acid components, was a relatively poor substrate for the enzyme, indicating that the lipid component plays an important role in substrate recognition and/or presentation to the enzyme in detergent micelles. This view is reinforced by the fact that neither of the substrate analogues **9** or **11**, which lack the lipid and phospholipid components, respectively, was recognised by the enzyme. α-D-Glc*p*NH_2-PI **2**,[17] a product of enzymic action, showed weak inhibition, implying that product inhibition is unlikely to play a regulatory role in the GPI biosynthetic pathway. The substrate/product analogues 2-deoxy-α-D-Glc*p*-PI **25** and α-D-Glc*p*-PI **33** were, respectively, much better and much poorer inhibitors of the hydrolysis of α-D-Glc*p*NAc-PI **3** by the *T. brucei* de-N-acetylase than is α-D-Glc*p*NH_2-PI **2**.

Recent studies[27] on the first α-D-mannosyl transferase of the GPI biosynthetic pathway have demonstrated that, in comparison with the close substrate analogue **2**, the delipidated compounds **8** and **10** were poor substrates. The compound **14**, which differs from the natural substrate in having the fatty acid residues removed, was a slightly better substrate than was either of the compounds **8** or **10**, whereas neither 2-deoxy-α-D-Glc*p*-PI **25** nor α-D-Glc*p*-PI **33** was recognised by the enzyme. Details of these studies, which highlight the importance of the NH_2 group in substrate recognition by this α-D-mannosyl transferase, will be published elsewhere in due course.

Experimental

Mps were determined on a Reichert hot-plate apparatus and are uncorrected. TLC was performed on silica gel 60 GF_{254}

(Merck) and spots were detected with UV light or by charring with dil. sulfuric acid as appropriate. Flash-column chromatography was performed on silica gel 60 (230–400 mesh, Merck). ^{1}H NMR spectra were recorded on Bruker AM 200 MHz or AC 500 MHz spectrometers with deuteriochloroform as solvent and tetramethylsilane as internal reference, unless otherwise indicated; J values are given in Hz. Mass spectra were measured with VG 250/70SE or VG Fisons Quattro instruments. Optical rotations were obtained using a Perkin-Elmer 141 polarimeter at ambient temperature, and are given in units of 10^{-1} deg cm^2 g^{-1}.

Triethylammonium 1D-6-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 1-(hydrogenphosphonate) 5

A solution of compound **4**[17] (85 mg, 85 μmol; previously dried by co-evaporation with pyridine) and pivaloyl chloride (0.06 cm^3, 0.49 mmol) in 2 mol dm^{-3} phosphorous acid in pyridine (0.45 cm^3, 0.9 mmol) was stirred at room temp. for 3 h before the reaction was stopped by the addition of 1 mol dm^{-3} aq. TEA hydrogen carbonate. The resulting solution was diluted with diethyl ether, and the ethereal solution was washed twice with 1 mol dm^{-3} aq. TEA hydrogen carbonate, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [chloroform–methanol (7:3)] of the residue gave the TEA hydrogen phosphonate derivative **5** (75 mg, 76%), $[\alpha]_D$ +63 (*c* 1.4, $CHCl_3$); δ_H 1.22 (9 H, t, 3 × CH_2Me), 2.93 (6 H, q, 3 × CH_2Me), 3.15 (1 H, dd, $J_{5',6'a}$ 1.9, $J_{6'a,6'b}$ 11.7, 6'-H^a), 3.26 (1 H, dd, $J_{5',6'b}$ 3.9, 6'-H^b), 3.27 (1 H, dd, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.2, 2'-H), 3.45 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.3, 5-H), 3.57 (1 H, dd, $J_{2,3}$ 2.5, $J_{3,4}$ 10.0, 3-H), 3.70 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.3, 4'-H), 3.98 (1 H, t, 3'-H), 4.06 (1 H, m, 5'-H), 4.10 (1 H, t, 4-H), 4.20 (1 H, dd, $J_{1,6}$ 9.2, 1-H), 4.29 (1 H, t, 6-H), 4.63 (1 H, t, 2-H), 5.74 (1 H, d, 1'-H), 7.13 (1 H, d, $J_{H,P}$ 622, HP) and 7.00–7.49 (35 H, m, 7 × Ph).

Triethylammonium 1D-6-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol 1-(benzyl phosphate) 7

A mixture of the hydrogenphosphonate **5** (13 mg, 11 μmol), benzyl alcohol (5 mm^3, 48 μmol) and pivaloyl chloride (8 mm^3, 65 μmol) in dry pyridine (0.25 cm^3) was stirred at room temp. for 2 h to give the diastereoisomeric hydrogenphosphonates **6**. Oxidation of the hydrogenphosphonates **6** was accomplished by the addition of 0.1 mol dm^{-3} iodine in 2% aq. pyridine (0.2 cm^3) to the mixture, which was then stirred at room temp. for 1 h and diluted with diethyl ether. The resulting solution was washed with 5% aq. sodium hydrogen sulfite, dried ($MgSO_4$), and concentrated under reduced pressure. After flash-column chromatography [first with chloroform and then with chloroform–methanol (9:1)], an ethereal solution of the residue was washed twice with 1 mol dm^{-3} aq. TEA hydrogen carbonate, dried ($MgSO_4$), and concentrated under reduced pressure to give the TEA phosphate derivative **7** (12 mg, 85%), $[\alpha]_D$ +45 (*c* 1.2, $CHCl_3$); δ_H 1.17 (9 H, t, 3 × CH_2Me), 2.87 (6 H, q, 3 × CH_2Me), 3.19 (1 H, dd $J_{1',2'}$ 3.4, $J_{2',3'}$ 10.2, 2'-H), 3.39 (2 H, m, 6'-H_2), 3.46 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.5, 5-H), 3.52 (1 H, dd, $J_{3,4}$ 9.5, 3-H), 3.71 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.5, 4'-H), 4.02 (1 H, t, 3'-H), 4.08 (1 H, t, 4-H), 4.14 (1 H, m, 5'-H), 4.35 (2 H, m, 1-, 6-H), 4.74 (1 H, t, 2-H), 5.03 (2 H, m, $POCH_2Ph$), 5.92 (1 H, d, 1'-H) and 6.97–7.40 (40 H, m, 8 × Ph); δ_P($CDCl_3$; ext. ref. 85% H_3PO_4) −0.39 (with ^{1}H heteronuclear decoupling).

1D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-dihydrogen phosphate) 8

A solution of the free-acid form of the phosphoric diester **7** (prepared from the TEA salt, 12 mg, 9.4 μmol) in chloroform–methanol (2 cm^3; 1:3) containing 20% palladium hydroxide on carbon (10 mg) was shaken under a slight overpressure of hydrogen at room temp. for 20 h and was then percolated through a short column packed with a layer of Celite on top of silica gel, with washing with methanol. The filtrate and the washings were combined, and concentrated under reduced pressure, and the residue was subjected to flash-column chromatography [acetonitrile–water (7:3) and then (6.5:3.5)] to give the phosphate **8** (3 mg, 75%), $[\alpha]_D$ +75 (*c* 0.2, H_2O), (lit.,[19] +73 (*c* 0.6, H_2O); ES$^-$ MS *m*/*z* 420 (M$^-$ − H).

1D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 10

A solution of compound **4**[17] (15 mg, 15 μmol) in chloroform–methanol (3 cm^3; 1:2) containing 20% palladium hydroxide on carbon (15 mg) was shaken under a slight overpressure of hydrogen at room temperature for 12 h and was then filtered through a Celite pad and concentrated under reduced pressure. The residue was dissolved in water, and the aqueous solution was filtered and freeze-dried to give the glycosyl inositol **10** (5 mg, 97.5%), $[\alpha]_D$ +82 (*c* 0.5, MeOH); δ_H(D_2O; int. ref. Me_2CO at δ_H 2.07) 3.19 (1 H, dd, $J_{1',2'}$ 3.4, $J_{2',3'}$ 10.7, 2'-H), 3.23 (1 H, m, 5-H), 3.37 (1 H, dd, $J_{2,3}$ 3.0, $J_{3,4}$ 9.8, 3-H), 3.40 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.4, 4'-H), 3.49 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.8, 4-H), 3.59 (2 H, m, 1-, 6-H), 3.67 (2 H, m, 6'-H_2), 3.76 (1 H, dd, 3'-H), 3.87 (1 H, dd, $J_{1,2}$ 2.1, 2-H), 3.92 (1 H, m, 5'-H) and 5.29 (1 H, d, 1'-H). FAB$^+$ MS *m*/*z* 342 (MH$^+$).

1D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(*sn*-2,3-dihydroxypropyl hydrogen phosphate) 14

A solution of compound **12** (as the sodium salt,[17] 45 mg, 27.3 μmol) and 1 mol dm^{-3} sodium methoxide in methanol (30 mm^3) in diethyl ether–methanol (3 cm^3; 1:2) was stirred at room temp. for 30 min and was then deionised with Amberlite IR 120 (H$^+$) resin. Removal of the solvents under reduced pressure and flash chromatography [first with chloroform and then with chloroform–methanol (9:1)] of the residue gave the deacylated compound **13** (30 mg, 95.5%); FAB$^+$ MS *m*/*z* 1151 (M$^+$ − H).

A solution of compound **13** (30 mg, 26 μmol) in methanol (2 cm^3) containing 20% palladium hydroxide on carbon (30 mg) was shaken under a slight overpressure of hydrogen at room temp. for 12 h and was then processed as described in the previous experiment. An aqueous solution of the residue was passed down a short column of Sephadex LH20–100 and the eluent was freeze-dried to give the phosphoric diester **14** (9 mg, 67%), $[\alpha]_D$ +54 (*c* 0.9, H_2O); δ_H(D_2O; int. ref. Me_2CO at δ 2.07) 3.20 (1 H, dd, $J_{1',2'}$ 3.8, $J_{2',3'}$ 10.4, 2'-H), 3.27 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.3, 5-H), 3.39 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.9, 4'-H), 3.40 (1 H, dd, $J_{2,3}$ 2.3, $J_{3,4}$ 10.4, 3-H), 3.45 and 3.52 (2 H, m, 3-H_2 propyl), 3.54 (1 H, dd, 4-H), 3.67 (2 H, m, 6'-H_2), 3.75 (1 H, dd, 3'-H), 3.77 (1 H, m, 2-H propyl), 3.79 (1 H, t, $J_{1,6} = J_{5,6}$ = 9.9, 6-H), 3.84 (2 H, m, 1-H_2 propyl), 3.96 (1 H, m, 5'-H), 4.04 (1 H, t, $J_{1,2} = J_{2,3}$ = 2.3, 2-H), 4.08 (1 H, m, 1-H) and 5.40 (1 H, d, 1'-H); δ_P(D_2O; ext. ref. 85% H_3PO_4) 4.25 (with ^{1}H heteronuclear decoupling). ES$^-$ MS *m*/*z* 494 (M$^-$ − H).

2,3,4,5-Tetra-*O*-benzyl-1D-6-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-α-D-*arabino*-hexopyranosyl)-*myo*-inositol 19

A mixture of 3,4,6-tri-*O*-benzyl-D-glucal **16** (Fluka, 40 mg, 96 μmol), the D-*myo*-inositol derivative **17**[17] (60 mg, 91 μmol), and triphenylphosphine–hydrogen bromide (1 mg, 2.9 μmol) in dry dichloromethane (0.35 cm^3) was stirred at room temp. for 24 h. Flash-column chromatography [cyclohexane–diethyl ether (3:1)] of the reaction mixture gave the α-coupled derivative **18** (55 mg, 56%), $[\alpha]_D$ +38 (*c* 5.5, $CHCl_3$); δ_H(*inter alia*) 1.63 (1 H, ddd, $J_{1',2'ax}$ 3.5, $J_{2'ax,3'}$ 11.9, $J_{2'ax,2'eq}$ 12.6, 2'-H^{ax}), 2.19 (1 H ddd, $J_{1',2'eq}$ ~ 1, $J_{2'eq,3'}$ 4.9, 2'-H^{eq}), 3.78 (3 H, s, OMe), 5.59 (1 H, dd, 1'-H) and 6.83–7.40 (39 H, C_6H_4 and 7 × Ph).

A solution of compound **18** (30 mg, 28 μmol) in acetonitrile–

water (1 cm^3; 9:1) containing CAN (80 mg, 144 μmol) was stirred at 0 °C for 1 h and was then diluted with diethyl ether. The ethereal solution was washed twice with aq. sodium chloride, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [toluene–butanone (9:1)] of the residue gave the 2-deoxyglycosylinositol **19** (18 mg, 67.5%), mp 108–110 °C (from diethyl ether–hexane); $[\alpha]_D$ +71 (*c* 1.8, $CHCl_3$) (Found: C, 76.6; H, 6.85. $C_{61}H_{64}O_{10}$ requires C, 76.5; H, 6.7%); δ_H(*inter alia*) 1.69 (1 H, ddd, $J_{1'.2'ax}$ 3.5, $J_{2'ax.3'}$ 11.5, $J_{2'ax.3'eq}$ 12.7, 2'-H^{ax}), 2.33 (1 H, ddd, $J_{1'.2'eq}$ ~ 1, $J_{2'eq.3'}$ 4.9, 2'-H^{eq}), 5.64 (1 H, dd, 1'-H) and 6.99–7.36 (35 H, m, 7 × Ph).

Triethylammonium 1D-2,3,4,5-tetra-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-α-D-*arabino*-hexopyranosyl)-*myo*-inositol 1-[*sn*-2,3-bis(palmitoyloxy)propyl phosphate] 23

A mixture of compound **19** (66 mg, 69 μmol), *sn*-2,3-bis(palmitoyloxy)propyl hydrogen hydrogenphosphonate[22] **20** (50 mg, 79 μmol) and pivaloyl chloride (40 mm^3, 0.32 mmol) in anhydrous pyridine (3 cm^3) was stirred at room temp. for 10 min to form a mixture of the diastereoisomeric hydrogenphosphonates **21**. Oxidation of the hydrogenphosphonates was accomplished by the addition of 0.1 mol dm^{-3} iodine in 2% aq. pyridine (1.6 cm^3) to the mixture, which was then stirred at room temp. for 10 min and diluted with diethyl ether. The ethereal solution was washed with 5% aq. sodium hydrogen sulfite, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [first with chloroform and then with chloroform–methanol (19:1)] of the residue gave the phosphoric diester **22**, which was taken up in diethyl ether, and the ethereal solution was washed with 1 mol dm^{-3} aq. TEA hydrogen carbonate, and concentrated under reduced pressure to give the TEA phosphate derivative **23** (94 mg, 81%), $[\alpha]_D$ +40 (*c* 1.3, $CHCl_3$); δ_H 0.88 (6 H, t, *J* 7.3, 2 × CH_2Me), 1.19 (9 H, t, 3 × CH_2Me), 1.24 (48 H, m, 2 × $[CH_2]_{12}$), 1.56 (4 H, m, 2 × $COCH_2CH_2$), 1.63 (1 H, ddd, 2'-H^{ax}), 2.23 and 2.25 (4 H, 2 t, 2 × $COCH_2$), 2.53 (1 H, ddd, $J_{1'.2'eq}$ ~ 1, $J_{2'eq.3'}$ 4.8, $J_{2'ax.2'eq}$ 13.7, 2'-H^{eq}), 2.96 (6 H, q, 3 × CH_2Me), 3.26 (2 H, m, 6'-H_2), 3.36 (1 H, t, $J_{4.5} = J_{5.6}$ = 8.8, 5-H), 3.54 (1 H, dd, $J_{2.3}$ 2.4, $J_{3.4}$ 10.0, 3-H), 3.62 (1 H, t, $J_{3'.4'} = J_{4'.5'}$ = 9.0, 4'-H), 3.97 (1 H, dt, 5'-H), 4.02 (1 H, m, 3'-H), 4.07 (1 H, t, 4-H), 4.10 and 4.37 (4 H, 2 m, 2 × CH_2 propyl), 4.19 (1 H, m, 1-H), 4.26 (1 H, dd, $J_{1.6}$ 7.8, 6-H), 4.58 (1 H, br t, 2-H), 5.24 (1 H, m, 2-H propyl), 5.64 (1 H, dd, $J_{1'.2'ax}$ 2.5, 1'-H) and 6.99–7.45 (35 H, m, 7 × Ph); δ_P($CDCl_3$; ext. ref. 85% H_3PO_4) 0.08 (with ^1H heteronuclear decoupling).

Triethylammonium 1D-6-*O*-(2-deoxy-α-D-*arabino*-hexopyranosyl)-*myo*-inositol 1-[*sn*-2,3-bis(palmitoyloxy)propyl phosphate] 25

The sodium salt **24** was obtained quantitatively upon stirring of a solution of the TEA phosphate **23** in methanol with Amberlite DPI (Na^+) resin for 3 h, filtration, and concentration of the filtrate under reduced pressure. A solution of the sodium salt **24** (47 mg, 29 μmol) in methanol–tetrahydrofuran (THF) (4 cm^3; 1:1) containing 20% palladium on carbon (50 mg) was shaken under a slight overpressure of hydrogen at room temp. for 20 h and was then percolated through a short column packed with a layer of Celite on top of silica gel, with washing with methanol–THF (1:1). The eluent and washings were combined, and concentrated under reduced pressure. Flash-column chromatography [first with chloroform and then with chloroform–methanol (1:1)] gave the debenzylated compound, which was taken up in butan-1-ol and shaken with 1 mol dm^{-3} aq. TEA hydrogen carbonate. Concentration of the organic phase under reduced pressure gave the TEA phosphate derivative **25** (17 mg, 55%), $[\alpha]_D$ +28 [*c* 0.8, $CHCl_3$–MeOH (9:1)]; δ_H(*inter alia*) ($CDCl_3$-CD_3OD, 9:1), 0.88 (6 H, t, *J* 7.3, 2 × CH_2Me), 1.28 (48 H, m, 2 × $[CH_2]_{12}$), 1.32 (9 H, t, 3 × CH_2Me), 1.58 (1 H, ddd, 2'-H^{ax}), 1.60 (4 H, m, $COCH_2CH_2$), 2.27 (1 H, ddd, $J_{1'.2'eq}$ ~ 1, $J_{2'eq.3'}$ 4.5, 2'-H^{eq}), 2.29 and 2.33 (4 H, 2 t, 2 × $COCH_2$), 4.15 (1 H, m, 1-H^a propyl), 4.39 (1 H, dd, $J_{1b.2}$ 3.2, $J_{1a.1b}$ 12.2, 1-H^b propyl), 4.23 (1 H, br t, 2-H), 5.21 (1 H, dd, 1'-H) and 5.23 (1 H, m, 2-H propyl); δ_P($CDCl_3$–CD_3OD, 9:1) −0.15 (with ^1H heteronuclear decoupling). ES^- MS *m/z* 955.5 (M^- − TEA).

1D-2,3,4,5-Tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*myo*-inositol 27

To a cooled (−60 °C) mixture of the trichloroacetimidate **26**[26] (200 mg, 290 μmol), the D-*myo*-inositol derivative **17**[17] (150 mg, 230 μmol) and powdered 3 Å molecular sieves (0.5 g) in anhydrous diethyl ether (5 cm^3) under nitrogen was added dropwise 0.02 mol dm^{-3} TMSOTf in anhydrous diethyl ether (0.25 cm^3). The reaction mixture was stirred at −60 °C for 0.5 h, neutralised with triethylamine (0.5 cm^3), and then percolated through a short column of silica gel, with washing with diethyl ether. The combined eluent was concentrated under reduced pressure and the residue was subjected to flash-column chromatography [toluene–butanone (50:1)] to give the D-glucopyranosylinositol **27** (196 mg, 76%), $[\alpha]_D$ +38 (*c* 1.5, $CHCl_3$); δ_H(*inter alia*) 3.75 (3 H, s, OMe) and 5.85 (1 H, d, $J_{1'.2'}$ 3.4, 1'-H); δ_C($CDCl_3$) 96.36 (C-1'). ES^+ MS *m/z* 1205.6 (M^+ + Na).

1D-2,3,4,5-Tetra-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*myo*-inositol 28

A solution of compound **27** (73 mg, 62 μmol) in 1% trifluoroacetic acid in anhydrous dichloromethane (8 cm^3) was stirred at room temp. for 1.5 h, and was then neutralised with triethylamine (0.5 cm^3) and concentrated under reduced pressure. Flash-column chromatography [cyclohexane–diethyl ether (3:1)] of the residue gave the alcohol **28** (53 mg, 81%), $[\alpha]_D$ +45 (*c* 2, $CHCl_3$); δ_H 3.09 (1 H, dd, $J_{5'.6'a}$ 1.7, $J_{6'a.6'b}$ 11.0, 6'-H^a), 3.38 (1 H, t, $J_{4.5} = J_{5.6}$ = 9.0, 5-H), 3.40 (1 H, dd, $J_{5'.6'b}$ 2.6, 6'-H^b), 3.45 (1 H, dd, $J_{2.3}$ 2.5, $J_{3.4}$ 10.2, 3-H), 3.59 (1 H, dd, $J_{1'.2'}$ 3.5, $J_{2'.3'}$ 9.6, 2'-H), 3.60 (1 H, dd, $J_{1.2}$ 2.5, 1-H), 3.73 (1 H, t, $J_{3'.4'} = J_{4'.5'}$ = 9.3, 4'-H), 3.90 (1 H, dt, 5'-H), 3.97 (1 H, t, 6-H), 4.01 (1 H, t, 3'-H), 4.05 (1 H, t, 2-H), 4.10 (1 H, t, 4-H), 4.05–4.96 (12 H, m, 8 × CH_2Ph), 5.17 (1 H, d, 1'-H) and 6.99–7.45 (40 H, m, 8 × Ph). ES^+ MS *m/z* 1085.5 (M^+ + Na).

Triethylammonium 1D-2,3,4,5-tetra-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*myo*-inositol 1-[*sn*-2,3-bis(palmitoyloxy)propyl phosphate] 31

A mixture of compound **28** (42 mg, 39.5 μmol), the hydrogenphosphonate **20**[22] (28 mg, 44 μmol) and pivaloyl chloride (25 mm^3, 0.2 mmol) in anhydrous pyridine (2 cm^3) was stirred at room temp. for 20 min to form the diastereoisomeric hydrogenphosphonates **29**, which were converted into the phosphoric diester **30** and thereafter into the TEA phosphate derivative **31** (43 mg, 61%), $[\alpha]_D$ +36 (*c* 1.45, $CHCl_3$), as described for the corresponding 2'-deoxy derivative **21**; δ_H(*inter alia*) 0.88 (6 H, t, *J* 7.3, 2 × CH_2Me), 1.11 (9 H, t, 3 × CH_2Me), 1.20 (48 H, m, 2 × $[CH_2]_{12}$), 1.52 (4 H, m, 2 × $COCH_2CH_2$), 2.17 and 2.20 (4 H, 2 t, 2 × $COCH_2$), 2.79 (6 H, q, 3 × CH_2Me), 3.44 (2 H, m, 6'-H_2), 3.56 (1 H, dd, 2'-H), 3.61 (1 H, t, 4'-H), 4.01 (1 H, t, 3'-H), 4.35 (1 H, m, 5'-H), 5.20 (1 H, m, 2-H propyl), 6.01 (1 H, d, $J_{1'.2'}$ 3.4, 1'-H) and 6.89–7.52 (40 H, m, 8 × Ph). ES^- MS *m/z* 1692.1 (M^- − TEA).

Sodium 1D-6-*O*-α-D-glucopyranosyl-*myo*-inositol 1-[*sn*-2,3-bis(palmitoyloxy)propyl phosphate] 33

A solution of the sodium salt **32** (30 mg, 17.7 μmol; prepared from the TEA derivative **31** as described for compound **24**) in methanol–dichloromethane (3 cm^3; 1:1) containing 20%

palladium hydroxide on carbon (10 mg) was shaken under a slight overpressure of hydrogen at room temp. for 12 h, and was then filtered with the aid of Celite and concentrated under reduced pressure to give the α-D-Glc*p*-PI **33** (10 mg, 96%), $[\alpha]_D$ +12.5 (*c* 1, Me_2SO), as a solid, which TLC [chloroform–methanol (3:2)] indicated to have a purity >90%; $\delta_H[(CD_3)_2SO]$ 0.84 (6 H, t, *J* 7.3, 2 × CH_2Me), 1.23 (48 H, m, 2 × $[CH_2]_{12}$), 1.49 (4 H, m, 2 × $COCH_2CH_2$), 2.27 (4 H, m, 2 × $COCH_2$), 4.10 (1 H, dd, $J_{1a.2}$ 7.5, $J_{1a.1b}$ 11.2, 1-H^a propyl), 4.29 (1 H, dd, $J_{1b.2}$ 2.5, 1-H^b propyl), 4.98 (1 H, d, $J_{1'.2'}$ 3.5, 1'-H) and 5.12 (1 H, m, 2-H propyl). ES$^-$ MS *m*/*z* 971.5 (M^- − Na). This material was further purified by semi-preparative HPLC, essentially as described[18] for α-D-Glc*p*NAc-PI **3**, before being used in inhibition studies[18] with the *T. brucei* de-N-acetylase.

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