Parasite glycoconjugates. Part 7.[1] Synthesis of further substrate analogues of early intermediates in the biosynthetic pathway of glycosylphosphatidylinositol membrane anchors

Arthur Crossman, Jr.,[a] John S. Brimacombe *,[a] and Michael A. J. Ferguson[b]

[a] *Department of Chemistry, University of Dundee, Dundee, UK DD1 4HN*
[b] *Department of Biochemistry, University of Dundee, Dundee, UK DD1 4HN*

Substrate analogues of 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-[*sn*-2,3-bis(myristoyloxy)propyl phosphate], an early intermediate in the biosynthesis of glycosylphosphatidylinositol (GPI) membrane anchors, have been prepared for biological evaluation with the α-(1→4)-D-mannosyltransferase of the protozoan parasite *Trypanosoma brucei*. The analogue α-D-Glc*p*NH$_2$-(1→6)-2-OMe-PI 2 is a substrate for the protozoan α-(1→4)-D-mannosyltransferase but not for the corresponding mammalian enzyme, whereas the analogues 3 and 4, in which the fatty-acid groups of the natural substrate are replaced by alkyl groups, are acceptable substrates for both the protozoan and mammalian enzymes.

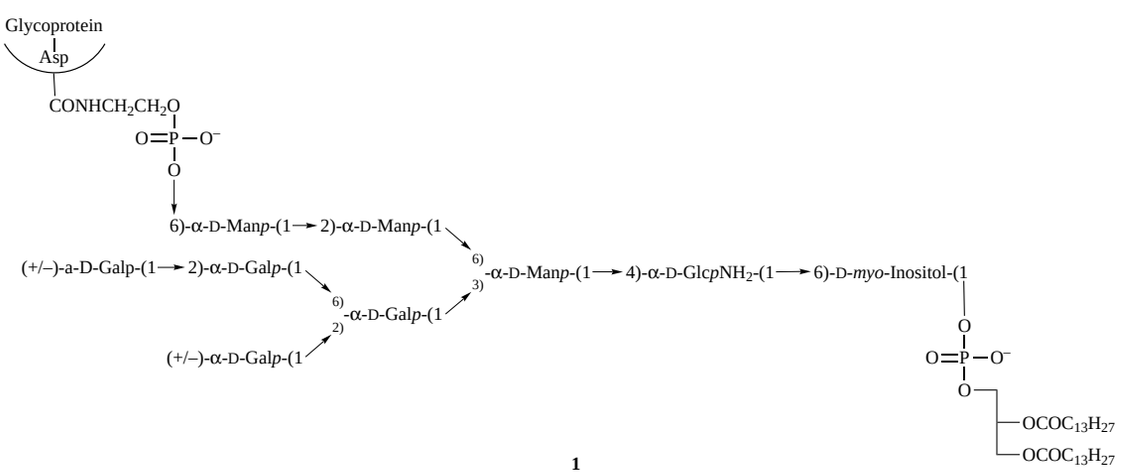
Introduction

Each year millions of people and domestic animals in the tropics are afflicted by parasitic diseases, which are often seriously debilitating and sometimes fatal. Throughout their life cycle parasites live in a hostile environment, whether in the host or in the insect vector. Consequently, parasites have evolved intricate defence mechanisms in order to survive and reproduce. The goal of chemotherapy is to kill the parasite by somehow breaching its defences. Drug treatment of most parasitic diseases is fairly rudimentary and often produces unpleasant side-effects without effecting a long-term cure. A better understanding, at a molecular level, of how parasites evade the host's immune system is clearly of fundamental importance and is increasingly being realised.[2]

Glycoconjugates on the cell surface of parasitic protozoa of the Trypanosomatidae (including, for example, African and American trypanosomes and *Leishmania spp.*) frequently have a crucial role in determining parasite survival and infectivity.[2] Many glycoconjugates are attached to the plasma membrane by means of glycosylphosphatidylinositol (GPI) anchors, whose principal function is to provide a stable association of protein or oligosaccharide with the lipid bilayer.[3] Studies in our laboratories have largely focused on GPI membrane anchors in the belief that disruption of GPI biosynthesis would seriously impair the parasite's ability to survive in the host.[4,5]

A schematic representation of the GPI membrane anchor of the variant surface glycoprotein (VSG) of *Trypanosoma brucei*, an African protozoan parasite that causes a disease related to sleeping sickness in domestic cattle, is shown in formula **1**.[6] All GPI anchors that have been characterised to date (from protozoan, yeast, slime mould, fish and mammalian sources) contain an identical ethanolamine phosphate-6-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)-D-*myo*-inositol backbone, suggesting that this sequence is likely to be conserved in all GPI anchors.[2] This conserved region may be furbished, as in *T. brucei*, with other sugars in a species- and stage-specific manner. The lipid moieties of GPI anchors can also vary in a species- and stage-specific manner.

The biosynthesis of GPI membrane anchors in bloodstream forms of *T. brucei*[7,8] occurs in the endoplasmic reticulum and involves the sequential glycosylation of phosphatidylinositol (PI) as follows: α-D-Glc*p*NAc is transferred from UDP-D-Glc*p*NAc to PI to form α-Glc*p*NAc-(1→6)-PI, which is then de-N-acetylated to form α-D-Glc*p*NH$_2$-(1→6)-PI. An α-D-Man*p* residue is then transferred from dolichol phosphate D-mannose to form α-D-Man*p*-(1→4)-α-D-Glc*p*NH$_2$-(1→6)-PI, to which is added a fatty acyl group (for example, palmitoyl) at 2-OH of the D-*myo*-inositol residue. Two further α-D-Man*p* residues are 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)-2-*O*-acyl-PI. Ethanolamine phosphate (from phosphatidyl ethanolamine) is subsequently added at the terminal α-D-Man*p* residue and the fatty acyl group is then



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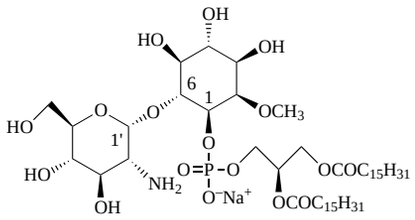
removed from the D-*myo*-inositol residue. The resulting structure undergoes a complex series of fatty acid remodelling reactions before the preassembled GPI precursor (known as glycolipid A) is transferred *en bloc* to newly synthesised protein. Some α-D-galactosylation of the GPI anchor occurs in the endoplasmic reticulum but mainly in the Golgi apparatus during transport to the outer surface of the cell membrane, when as many as five α-D-Gal*p* residues may be added to the GPI membrane anchors of *T. brucei* VSG. The GPI biosynthetic pathway in mammalian cells appears to be broadly similar to that outlined for the bloodstream form of *T. brucei*. One notable difference is that acylation of 2-OH of the D-*myo*-inositol residue occurs before the first α-D-Man*p* residue is attached.[7,8]

We have used chemically synthesised analogues[9,10] of some early intermediates in the GPI biosynthetic pathway of bloodstream forms of *T. brucei* to assist in the purification and/or characterisation of both the de-N-acetylase,[4] which is responsible for converting α-D-Glc*p*NAc-(1→6)-PI into α-D-Glc*p*NH_2-(1→6)-PI, and the first α-D-mannosyltransferase,[5] which converts the latter compound into α-D-Man*p*-(1→4)-α-D-Glc*p*NH_2-(1→6)-PI. Mannosylation *in vivo* requires prior de-N-acetylation of α-D-Glc*p*NAc-(1→6)-PI.[2] Such studies have revealed that an analogue lacking the fatty acid residues is a relatively poor substrate for the de-N-acetylase, which also fails to recognise substrate analogues lacking the glycerophosphate component.[4] Similar probing of the acceptor specificity of the α-(1→4)-D-mannosyltransferase showed that it requires the *myo*-inositol residue of α-D-Glc*p*NH_2-(1→6)-PI to have the 1D-configuration and the presence of the sugar NH_2 group, since neither α-D-Glc*p*-PI nor 2-deoxy-α-D-Glc*p*-PI acted as substrates.[5] Moreover, neither of the latter compounds inhibited the D-mannosylation of α-D-Glc*p*NH_2-PI. Once again, the fatty acid and phosphoglycerol components of α-D-Glc*p*NH_2-PI are important in enhancing substrate presentation and substrate recognition, respectively, by the enzyme; α-D-Glc*p*NH_2-(1→6)-D-*myo*-inositol appears to be the minimum structure that can support detectable acceptor activity.

Bearing in mind these key structural features, we have synthesised another group of substrate analogues related to α-D-Glc*p*NH_2-PI with either 2-OH of the D-*myo*-inositol residue methylated (*i.e.*, compound **2**) or the fatty acids replaced by O-alkyl groups (*i.e.*, compounds **3** and **4**). In yet another analogue, compound **5**, the phosphate group is esterified with an octyl group rather than with a lipid moiety. The first modification **2** is of interest since some mammalian and protozoan GPI membrane anchors and/or biosynthetic intermediates have an additional fatty acid attached to O-2 of the D-*myo*-inositol ring.[8] The addition of a fatty acid to the inositol ring would be obstructed with the methylated analogue **2**, thereby allowing the role of inositol acylation in GPI biosynthesis to be assessed. Replacement of the fatty acid residues in the lipid moiety was occasioned by the need to prepare substrates which did not degrade through loss of fatty acid residues on storage and which would allow greater flexibility in subsequent synthetic manipulations. The introduction of ether-linked substituents at O-2 and O-3 of the glyceryl residue is, therefore, advantageous providing the analogues are acceptable substrates for the enzymes concerned. Expectations for this modification, as in compounds **3** and **4**, were high, since the lipid moiety of several GPI anchors contains both ether- and ester-linked alkyl chains.[2] The synthesis of the octylated analogue **5** and its N-acetyl derivative was undertaken to define further the substrate specificities of the *T. brucei* enzymes referred to earlier, particularly with regard to the role of the phosphodiester linkage.



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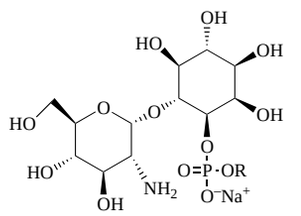
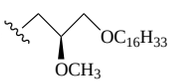
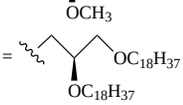


3 R = 

4 R = 

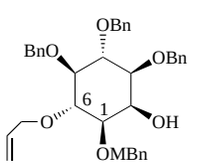
5 R = C_8H_{17}

Results and discussion

The synthesis of the glycosylphosphatidylinositol analogues **2**–**5** is based on a successful approach[9,10] used previously. In the first step of this approach, the glycosyl fluoride **12** is coupled with 6-OH of an appropriately protected D-*myo*-inositol derivative, whereafter 1-OH of the coupled product is exposed (as in compound **27**) for further coupling with a hydrogenphosphonate derivative (*e.g.*, compound **16**).[11,12] The mixture of diastereoisomeric phosphonic diesters so obtained is oxidised with iodine in pyridine–water[13] to give the corresponding phosphoric diester, which is transformed into a fully deprotected α-D-Glc*p*NH_2-PI on hydrogenolysis.

Synthesis of α-D-Glc*p*NH_2-(1→6)-2-OMe-PI **2** required the D-*myo*-inositol derivative **8** as the glycosyl acceptor. This compound was readily obtained by methylation of the known[9] alcohol **6**, followed by deallylation of the methylated product **7**.

An alternative route to the one reported previously[9] was used to prepare the glycosyl donor **12** from the benzylated D-glucal **9**. Azido-phenylselenylation of the glycal **9**, as described by Czernecki and Ayadi,[14] furnished the phenyl 2-azido-2-deoxy-1-seleno-α-D-glucopyranoside **10** as the principal product. In order to avoid a difficult work-up procedure with mercury trifluoroacetate,[14] we chose to hydrolyse the selenoglucoside **10**



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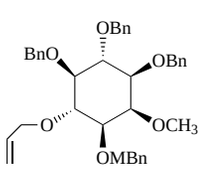
MBn = *p*-methoxybenzyl



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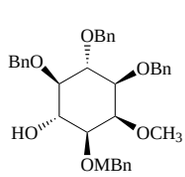

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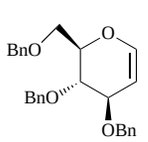

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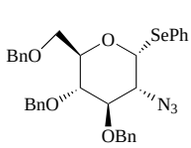

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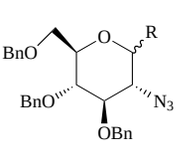
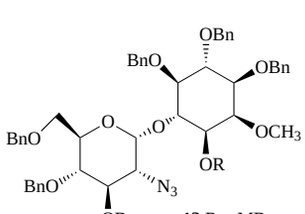
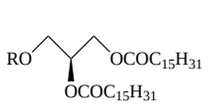


11 R = OH
12 R = F



13 R = MBn
14 R = H

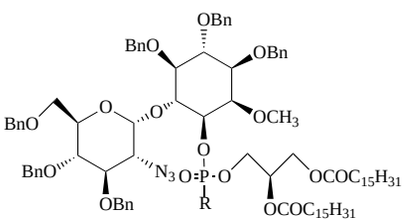


15 R = H
16 R = $HPO_2^-NHEt_3^+$

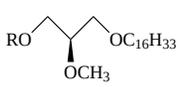
(⟶ **11**) by using silver triflate[15] in wet tetrahydrofuran (THF). The resulting 2-azido-2-deoxy-D-glucopyranose derivative **11** was then transformed into the glycosyl fluoride **12** as previously described.[9]

The D-*myo*-inositol derivative **8** reacted with the glycosyl fluoride **12**[9] in dry diethyl ether in the presence of zirconocene dichloride and silver perchlorate[16] to produce, *inter alia*, the α-(1→6)-linked compound **13**, which was converted into the alcohol **14** on treatment with trifluoroacetic acid (TFA) in CH_2Cl_2. Following preparative thin-layer chromatography (PLC), the pure α-coupled product **14** ($J_{1',2'}$ 3.5 Hz) was obtained in 55% yield over the two steps. It now remained to couple the pseudo-disaccharide derivative **14** to the hydrogenphosphonate **16**.

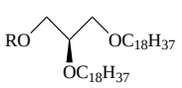
A synthesis of the hydrogenphosphonate **16** has already been described.[11] However, we preferred an alternative route using salicyl chlorophosphite as the phosphitylating reagent.[17] The *sn*-2,3-dipalmitoyloxy compound **15** reacted with salicyl chlorophosphite in pyridine to give, after quenching with 1 M triethylammonium hydrogen carbonate (TEAB) buffer solution, the TEA salt **16**. This salt condensed with the alcohol **14** in the presence of pivaloyl chloride[12,13] to give the phosphonic diester **17** as a mixture of diastereoisomers. The phosphonic diester **17** was oxidised with iodine in pyridine–water[13] to the phosphoric diester **18**, which was isolated and characterised as the TEA salt **19**. Finally, hydrogenolysis of the corresponding sodium salt **20** over 20% $Pd(OH)_2$/C furnished α-D-Glc*p*NH_2-(1→6)-2-OMe-PI **2**.



17 R = H
18 R = OH
19 R = $\bar{O}\,\overset{+}{N}HEt_3$
20 R = $\bar{O}\,\overset{+}{N}a$



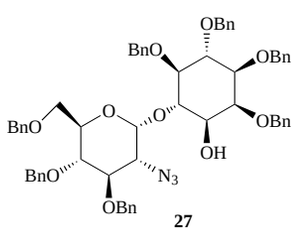
21 R = H
22 R = $HPO_2^-\,\overset{+}{N}HEt_3$



23 R = H
24 R = $HPO_2^-\,\overset{+}{N}HEt_3$

$C_8H_{17}OR$

25 R = H
26 R = $HPO_2^-\,\overset{+}{N}HEt_3$



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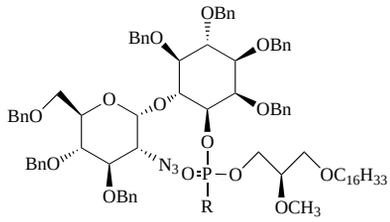
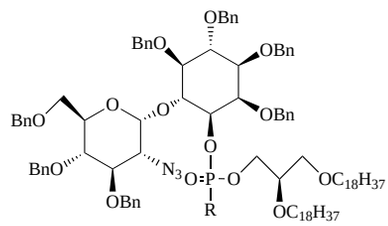
An identical approach was used to prepare the other hydrogenphosphonates from their respective alcohols, namely **21**† ⟶ **22**, **23**[18] ⟶ **24** and **25** ⟶ **26**. Each of the hydrogenphosphonates **22**, **24** and **26** was coupled as already described to 1-OH of the known[9] D-*myo*-inositol derivative **27**. Thereafter the coupled products were transformed into the phosphoric diesters **28**, **30** and **32**. Hydrogenolysis of the corresponding sodium salts **29**, **31** and **33** over $Pd(OH)_2$/C furnished the glycosylphosphatidylinositol analogues **3**, **4** and **5**, respectively.

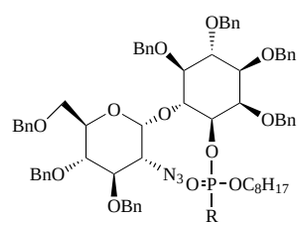
† Purchased from Sigma.



28 R = $\bar{O}\,\overset{+}{N}HEt_3$
29 R = $\bar{O}\,\overset{+}{N}a$



30 R = $\bar{O}\,\overset{+}{N}HEt_3$
31 R = $\bar{O}\,\overset{+}{N}a$



32 R = $\bar{O}\,\overset{+}{N}HEt_3$
33 R = $\bar{O}\,\overset{+}{N}a$

Details of the results of enzymic studies with the above analogues will be reported elsewhere in due course. In brief, α-D-Glc*p*NH_2-(1→6)-2-OMe-PI **2** is a substrate for the α-(1→4)-D-mannosyltranferase of the protozoan parasite *T. brucei*, but not for the corresponding mammalian enzyme. This is the first direct evidence that the trypanosomal and mammalian α-(1→4)-D-mannosyltransferases have different substrate specificities. The alkylated analogues **3** and **4** served as acceptable substrates for both trypanosomal and mammalian mannosyltransferases.

Experimental

Mps were determined on a Reichert hot-plate and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter at ambient temperature and are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR and COSY spectra were recorded on Bruker AM 200 MHz or AC 500 MHz spectrometers using deuteriochloroform as the solvent and tetramethylsilane as the internal reference, and ^{31}P spectra were performed at 81 MHz using aq. 85% H_3PO_4 as the external reference, unless otherwise indicated. Chemical shifts (δ) and *J*-values are given in ppm and Hz, respectively. ES mass spectra were recorded with a VG Quattro instrument. TLC was performed on silica gel $60F_{254}$ (Merck) with *A*, diethyl ether–hexane (1:1); *B*, chloroform–methanol (19:1); *C*, chloroform–methanol–water. (10:10:3); and *D*, butanol–ethanol–water (4:2:2) as developers and detection by UV light or by charring with sulfuric acid–water–ethanol (15:85:5) as appropriate. PLC was performed using a Chromatotron (Model 7924T, TC Research, UK) with Adsorbosil Plus-P (6–15 μm) (Alltech) as the adsorbent.

1D-6-*O*-Allyl-3,4,5-tri-*O*-benzyl-2-*O*-methyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 7

To a stirred and cooled (0 °C) mixture of the alcohol **6**[9] (0.10 g, 0.16 mmol) and sodium hydride (15 mg, 0.64 mmol) in *N*,*N*-dimethylformamide (10 cm^3) was added methyl iodide (15 mm^3, 0.24 mmol), whereafter the mixture was stirred for 1 h at 0 °C before methanol was added to decompose the excess of sodium hydride. The resulting solution was extracted with diethyl ether (25 cm^3) and the organic extract was washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure to give an oil which solidified on storage to give the *methyl derivative* **7** (90 mg, 90%), mp 88–90 °C (from hexane); $[\alpha]_D$ −11 (*c* 1, $CHCl_3$) (Found: C, 74.7; H, 6.9. $C_{39}H_{44}O_7$ requires C, 75.0; H, 6.9%); δ_H 3.22 (1 H, dd, *J* 2.4 and 10, 1- or 3-H), 3.25 (1 H, dd, *J* 2.5 and 10, 3- or 1-H), 3.36 (1 H, t, $J_{4,5} = J_{5,6} = 9.0$, 5-H), 3.62 (3 H, s, OCH_3), 3.65 (1 H, t, 2-H), 3.81 (1 H, t, 4- or 6-H), 3.82 (3 H, s, $ArOCH_3$), 3.92 (1 H, t, 6- or 4-H), ~4.35 (2 H, m, $CH_2CH{=}CH_2$), 4.52–4.95 (8 H, m, 4 × CH_2Ph), 5.10–5.21 (2 H, m, $CH_2CH{=}CH_2$), 5.89–6.08 (1 H, m, $CH_2CH{=}CH_2$) and 6.80–7.50 (19 H, m, C_6H_4 and 3 × Ph).

1D-3,4,5-Tri-*O*-benzyl-2-*O*-methyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 8

A solution of the allyl derivative **7** (80 mg, 0.13 mmol) in anhydrous dimethyl sulfoxide (10 cm^3) containing potassium *tert*-butoxide (127 mg, 1.13 mmol) was heated and stirred at 60 °C for 1 h, cooled, and poured into ice–water (75 cm^3). The aqueous solution was extracted with ethyl acetate (3 × 25 cm^3), and the organic extract was washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting propenyl derivative was heated under reflux for 10 min in 1M hydrochloric acid–acetone (20 cm^3; 1:9) and the solvents were removed under reduced pressure. Crystallisation of the residue from hexane–diethyl ether (5:1) gave the *deallylated compound* **8** (65 mg, 87%), mp 43–45 °C; $[\alpha]_D$ −24 (*c* 1, $CHCl_3$) (Found: C, 74.2; H, 6.9. $C_{36}H_{40}O_7$ requires C, 73.95; H, 6.9%); δ_H 3.15 (1 H, dd, *J* 2.0 and 9.8, 3-H), 3.35 (2 H, m, 1- and 5-H), 3.60 (3 H, s, OCH_3), 3.72 (1 H, t, 2-H), 3.82 (3 H, s, $ArOCH_3$), 4.00 (2 H, m, 4- and 6-H), 4.50–5.00 (8 H, m, 4 × CH_2Ph) and 6.80–7.50 (19 H, m, C_6H_4 and 3 × Ph).

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose 11

To a solution of the phenyl selenoglucopyranoside **10**[14] (0.20 g, 0.32 mmol) in THF (10 cm^3) were added silver triflate (0.49 g, 1.92 mmol) and water (17.3 mm^3, 0.96 mmol). After stirring of the reaction mixture at room temperature for 30 min, potassium carbonate (1.3 g, 9.6 mmol) was added and stirring was continued for a further 30 min. The reaction mixture was then percolated through a short column of silica gel with diethyl ether as the eluent. The eluate was washed successively with saturated aq. potassium carbonate and brine, dried (Na_2SO_4), and concentrated under reduced pressure. Crystallisation of the residue from diethyl ether–hexane gave the hemiacetal **11** (0.10 g, 66%), mp 97–98 °C; $[\alpha]_D$ +18 (*c* 1, $CHCl_3$) {lit.,[19] mp 96–100 °C; $[\alpha]_D$ +18.1 (*c* 1, $CHCl_3$)}.

1D-6-*O*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-*O*-benzyl-2-*O*-methyl-*myo*-inositol 14

To a stirred solution of the acceptor **8** (41 mg, 70 μmol) and the glycosyl fluoride **12**[9] (46.5 mg, 98 μmol) in anhydrous diethyl ether (10 cm^3) were added zirconocene dichloride (112 mg, 385 μmol) and powdered 4 Å molecular sieves (0.2 g). After 15 min at room temperature, the mixture was cooled to 0 °C and predried silver perchlorate (79 mg, 350 μmol) suspended in anhydrous diethyl ether (10 cm^3) was added dropwise, followed by 1,1,3,3-tetramethylurea (10 mm^3, 84 μmol). The mixture was stirred vigorously at 0 °C under argon overnight. It was then percolated through a short column of silica gel (elution with diethyl ether) and the eluate was concentrated under reduced pressure to give the crude methoxybenzyl derivative **13**, which was dissolved in CH_2Cl_2 (5 cm^3) and treated with TFA (110 mm^3, 1.4 mmol) at room temperature for 1 h. The reaction mixture was neutralised with triethylamine before concentration under reduced pressure. PLC (1:2 diethyl ether–hexane) of the residue gave the α-linked compound **14** (35 mg, 55%) as an opaque oil; R_f 0.19 (solvent *A*); $[\alpha]_D$ +31 (*c* 1, $CHCl_3$); δ_H 3.05 (1 H, dd, $J_{5',6'a}$ 2.4, $J_{6'a,6'b}$ 10.8, 6′-H[a]), 3.22 (1 H, dd, $J_{5',6'b}$ 2.3, $J_{6'b,6'a}$ 10.8, 6′-H[b]), 3.35 (1 H, t, $J_{4,5} = J_{5,6} = 9.5$, 5-H), 3.44 (1 H, dd, $J_{2,3}$ 2.2, $J_{3,4}$ 9.9, 3-H), 3.50 (1 H, dd, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10, 2′-H), 3.58 (1 H, dd, $J_{1,2}$ 2.2, $J_{1,6}$ 9.5, 1-H), 3.67 (3 H, s, OCH_3), 3.70 (1 H, m, $J_{3',4'} = J_{4',5'} = 9.5$, 4′-H), 3.73 (1 H, m, 2-H), 3.89–3.95 (3 H, m, 3′-, 5′- and 6-H), 4.00 (1 H, t, 4-H), 4.40–5.02 (12 H, 6 × CH_2Ph), 5.41 (1 H, d, $J_{1',2'}$ 3.5, 1′-H) and 7.05–7.35 (30 H, m, 6 × Ph); ESMS(−): *m/z* 956 $[M + Cl]^-$.

General procedure for the synthesis of hydrogenphosphonate triethylammonium (TEA) salts

The corresponding alcohol (0.2–0.8 mmol) was dried by co-evaporation with pyridine and was then dissolved in dry THF–pyridine (10:1; 5–11 cm^3). This solution was added dropwise over a period of 30 min to a stirred solution of salicyl chlorophosphite (1.22 mol equiv.) in dry THF (5 cm^3) under argon at room temperature. When TLC analysis (100:15:1 $CHCl_3$–methanol–water) showed complete conversion of the starting material into a product of lower mobility (R_f ~0.3), the reaction mixture was quenched with 1 M aq. TEAB buffer (15 cm^3) and was stirred for 15 min. $CHCl_3$ (25 cm^3) was added and the organic layer was washed with 1 M aq. TEAB (3 × 10 cm^3), dried ($MgSO_4$), and was then concentrated under reduced pressure to give the crude hydrogenphosphonate TEA salts which were used without further purification.

Triethylammonium *sn*-2,3-bis(palmitoyloxy)propyl hydrogenphosphonate 16. 0.13 g, 99%; $[\alpha]_D$ +16 (*c* 3, $CHCl_3$); δ_P 4.50 (with heteronuclear decoupling), J_{PH} 624 {lit.,[11] $[\alpha]_D$ +16.8 (*c* 3.0, $CHCl_3$); δ_P 4.60, J_{PH} 626}; ESMS(−): *m/z* 631 $[M - NEt_3 - H]^-$.

Triethylammonium *sn*-3-hexadecyloxy-2-methoxypropyl hydrogenphosphonate 22. 0.14 g, 94%; δ_P 4.50 (with heteronuclear decoupling), J_{PH} 627; ESMS(−): *m/z* 393 $[M - NEt_3 - H]^-$.

Triethylammonium *sn*-2,3-bis(octadecyloxy)propyl hydrogenphosphonate 24. 0.35 g, 92%; δ_P 4.80 (with heteronuclear decoupling), J_{PH} 629; ESMS(−); *m/z* 660 $[M - NEt_3 - H]^-$.

Triethylammonium octyl hydrogenphosphonate 26. 0.20 g, 87%; δ_P 4.10 (with heteronuclear decoupling), J_{PH} 631; ESMS(−): *m/z* 193 $[M - NEt_3 - H]^-$.

General procedure for the synthesis of the fully protected triethylammonium 2-azido-2-deoxy-α-D-glucopyranosyl-*myo*-inositol phosphoric diesters

A mixture of either the compound **14** (30 mg, 0.033 mmol) or **27**[9] (0.060–0.070 mmol) and the hydrogenphosphonate TEA salt (2 mol equiv.) was dried by evaporation of anhydrous pyridine (3 × 2 cm^3) therefrom. The residue was dissolved in dry pyridine (5–10 cm^3), pivaloyl chloride (6.2 mol equiv., with respect to the hydrogenphosphonate) was added, and the solution was stirred under argon for 1–2 h at room temperature; TLC then indicated a mixture of diastereoisomeric hydrogenphosphonates had formed. A freshly prepared solution of iodine (2 mol equiv., with respect to the hydrogenphosphonate) in 95:5 pyridine–water (10 cm^3) was added followed, after 30–45 min, by $CHCl_3$ (20 cm^3). The organic layer was washed successively with 5% aq. $NaHSO_3$ (20 cm^3) and 1 M aq. TEAB buffer (3 × 15 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to give the TEA phosphate derivative. These derivatives were purified by PLC (Chromatotron), with elution first with $CHCl_3$ and then with 19:1 $CHCl_3$–methanol.

Triethylammonium 1D-6-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-*O*-benzyl-2-*O*-methyl-*myo*-inositol 1-[*sn*-2,3-bis(palmitoyloxy)propyl phosphate] 19. Con-

centration of the appropriate fractions from the Chromatotron gave the TEA phosphate derivative **19** (36 mg, 67%); $[a]_D$ +28 (*c* 0.5, $CHCl_3$); R_f 0.11 (solvent *B*); δ_H 0.88 (6 H, t, *J* 7.0, 2 × CH_2*Me*), ~1.25 (57 H, m, 3 × CH_2*Me* and 2 × $[CH_2]_{12}$), 1.55 (4 H, br t, 2 × $COCH_2CH_2$), 2.30 (4 H, m, 2 × $COCH_2$), 3.00 (1 H, dd, $J_{5',6'}$ 2.5, $J_{6'a,6'b}$ 9.3, 6′-H[a]), 3.10 and 3.15 (7 H, m, 6′-H[b] and 3 × CH_2Me), 3.35 (1 H, t, $J_{4,5}$ 9.1, 5-H), 3.49 (2 H, m, 3- and 2′-H), 3.64 (3 H, s, OCH_3), 3.70 (1 H, t, $J_{3',4'}$ 9.7, 4′-H), 3.93 (1 H, t, $J_{3',4'}$ 9.7, 3′-H), 4.00 (2 H, m, 5′- and 4-H), 4.08 (1 H, m, 2-H), 4.17 (2 H, m, 6- and 1-H), 4.23 and 4.35 (4 H, 2 m, 2 × CH_2 propyl), 4.45–5.10 (12 H, m, 6 × CH_2Ph), 5.26 (1 H, m, 2-H propyl), 5.75 (1 H, d, $J_{1',2'}$ 4.0, 1′-H) and 7.00–7.40 (30 H, m, 6 × Ph); δ_P −1.00 (with heteronuclear decoupling); ESMS(−): *m/z* 1550 $[M - NEt_3 - H]^-$.

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-[*sn*-3-hexadecyloxy-2-methoxypropyl phosphate] 28. Concentration of the appropriate fractions from the Chromatotron gave the TEA phosphate derivative **28** (54 mg, 60%); $[a]_D$ +15 (*c* 1.3, $CHCl_3$); R_f 0.20 (solvent *B*); δ_H 0.88 (3 H, t, *J* 7.0, CH_2*Me*), ~1.25 (35 H, m, 3 × CH_2*Me* and $[CH_2]_{13}$), 1.55 (2 H, m, OCH_2CH_2), 2.92 (6 H, q, 3 × CH_2Me), 3.19 (1 H, dd, $J_{1',2'}$ 3.0, $J_{2',3'}$ 10.0, 2′-H), 3.29 (2 H, m, 6′-H_2), 3.35–3.45 (7 H, m, OCH_3 and 2 × CH_2 propyl), 3.50 (1 H, m, 5-H), 3.54 (1 H, m, 3-H), 3.74 (1 H, t, $J_{3',4'} = J_{4',5'} = 10$, 4′-H), 4.02 (2 H, m, 3′- and 2-H propyl), 4.08 (1 H, m, 4-H), 4.13 (1 H, m, $J_{4',5'}$ 10, 5′-H), 4.32 (1 H, m, 1-H), 4.34 (1 H, m, 6-H), 4.5–5.0 (14 H, m, 7 × CH_2Ph), 4.75 (1 H, m, 2-H), 5.88 (1 H, d, $J_{1',2'}$ 3.0, 1′-H) and 7.00–7.50 (35 H, m, 7 × Ph); δ_P −1.40 (with heteronuclear decoupling); ESMS(−): *m/z* 1389 $[M - NEt_3 - H]^-$.

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-[*sn*-2,3-bis(octadecyloxy)propyl phosphate] 30. Concentration of the appropriate fractions from the Chromatotron gave the TEA phosphate derivative **30** (67 mg, 60%); $[a]_D$ +10 (*c* 1.4, $CHCl_3$); R_f 0.28 (solvent *B*); δ_H 0.85 (6 H, t, *J* 7.0, 2 × CH_2*Me*), 1.10–1.35 (69 H, m, 2 × $[CH_2]_{15}$ and 3 × CH_2*Me*), 1.45 (4 H, m, 2 × OCH_2CH_2), 2.85 (6 H, q, 3 × CH_2Me), 3.10 (1 H, dd, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10, 2′-H), 3.31 (2 H, m, 6′-H_2), 3.41 (1 H, t, $J_{4,5} = J_{5,6} = 9.3$, 5-H), 3.50 (4 H, m, 2 × OCH_2), 3.52 (1 H, dd, $J_{2,3}$ 3.0, $J_{3,4}$ 10.0, 3-H), 3.60 (4 H, m, 2 × CH_2 propyl), 3.64 (1 H, m, $J_{3',4'}$ 6, 4′-H), 3.80 (1 H, t, $J_{3',4'}$ 6, 3′-H), 3.95 (1 H, m, 2-H propyl), 4.05 (1 H, t, $J_{4,5}$ 9.3, 4-H), 4.10 (1 H, dd, $J_{4',5'}$ 11, $J_{5',6'}$ 2.1, 5′-H), 4.28 (1 H, m, 1-H), 4.31 (1 H, m, $J_{5,6}$ 9.3, 6-H), 4.40–5.00 (14 H, m, 7 × CH_2Ph), 4.69 (1 H, t, 2-H), 5.85 (1 H, d, $J_{1',2'}$ 3.7, 1′-H) and 6.90–7.50 (35 H, m, 7 × Ph); δ_P −0.80 (with heteronuclear decoupling); ESMS(−): *m/z* 1655 $[M - NEt_3 - H]^-$.

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-[octyl phosphate] 32. Concentration of the appropriate fractions from the Chromatotron gave the TEA phosphate derivative **32** (71 mg, 79%); $[a]_D$ +20 (*c* 1.8, $CHCl_3$); R_f 0.10 (solvent *B*); δ_H 0.80 (3 H, t, *J* 7.3, CH_2*Me*), 1.15 (9 H, t, 3 × CH_2*Me*), ~1.18 (10 H, m, $[CH_2]_5$), 1.54 (2 H, m, OCH_2CH_2), 2.90 (6 H, q, 3 × CH_2Me), 3.15 (1 H, dd, $J_{1',2'}$ 2.8, $J_{2',3'}$ 10.1, 2′-H), 3.35 (2 H, m, 6′-H_2), 3.47 (1 H, t, $J_{4,5}$ 9.5, 5-H), 3.50 (1 H, br d, $J_{3,4}$ 9.5, 3-H), 3.65 (1 H, t, $J_{4',5'}$ 9.6, 4′-H), 3.87 (2 H, m, OCH_2), 3.95 (1 H, t, $J_{3',4'}$ 9.6, 3′-H), 4.01 (1 H, t, $J_{3,4}$ 9.5, 4-H), 4.09 (1 H, dt, $J_{4',5'}$ 9.6, 5′-H), 4.25 (1 H, m, 1-H), 4.30 (1 H, m, 6-H), 4.50 (1 H, t, $J_{1,2}$ 3.0, 2-H), 4.55–5.00 (14 H, m, 7 × CH_2Ph), 5.85 (1 H, d, $J_{1',2'}$ 2.8, 1′-H) and 6.90–7.50 (35 H, m, 7 × Ph); δ_P −1.80 (with heteronuclear decoupling); ESMS(−): *m/z* 1188 $[M - NEt_3 - H]^-$.

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

The sodium salt **20** was obtained quantitatively upon stirring of the TEA salt **19** in 1:1 diethyl ether–methanol with Amberlite DP-1 (Na^+) resin for 3 h. A solution of the sodium salt **20** (28 mg, 18 μmol) in 1:1 THF–methanol (5 cm^3) containing 20% $Pd(OH)_2$/C was shaken under a slight overpressure of hydrogen at room temperature for 24 h, whereafter the mixture was centrifuged and the supernatant was removed. The catalyst was further washed with 1:1 THF–methanol (2 × 5 cm^3), and the supernatant and washings were combined, and concentrated under reduced pressure to give the inositol derivative **2** (18 mg, 95%), $[a]_D$ +3 [*c* 0.45, $CHCl_3$–MeOH–water (10:10:3)]; R_f 0.76 (solvent *D*); δ_H 0.70 (6 H, m, 2 × CH_2*Me*), ~1.25 (48 H, m, 2 × $[CH_2]_{12}$), 1.55 (4 H, m, 2 × $COCH_2CH_2$), 2.25 (4 H, m, 2 × $COCH_2$), 3.21 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.5$, 4′-H), 3.50 (2 H, m, 6′-H_2), 3.70 (3 H, s, OCH_3) and 5.30 (1 H, d, $J_{1',2'}$ 3.9, 1′-H); δ_P 5.10 (with heteronuclear decoupling); ESMS(−): *m/z* 984 $[M - Na]^-$. This material was further purified for use in biological experiments by octyl-Sepharose chromatography (elution with propan-1-ol–water 5% ⟶ 60%); pure title compound **2** eluted at ~30% propan-1-ol–water.

Sodium 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-[*sn*-3-hexadecyloxy-2-methoxypropyl phosphate] 3

A solution of the sodium salt **29** (65 mg, 50 μmol; prepared from the TEA salt **28**) in 1:1 THF–methanol (10 cm^3) containing 20% $Pd(OH)_2$/C was shaken under a slight overpressure of hydrogen at room temperature for 24 h. Processing as described for the bis(palmitoyloxy) derivative **2** gave the diether **3** (38 mg, 97%), $[a]_D$ −8 [*c* 0.45, $CHCl_3$–MeOH–water (10:10:3)]; R_f 0.57 (solvent *D*) and 0.82 (solvent *C*); δ_H 0.80 (3 H, t, *J* 7.0, CH_2*Me*), ~1.20 (26 H, m, $[CH_2]_{13}$), 1.50 (2 H, m, OCH_2CH_2), 3.10 (1 H, d, $J_{1',2'}$ 4.0, 2′-H), 3.35 (3 H, s, OCH_3), 3.72 (1 H, t, $J_{1,6} = J_{5,6} = 9.5$, 6-H), 3.80 (1 H, t, $J_{1,2} = J_{2,3} = 2.5$, 2-H) and 5.35 (1 H, d, $J_{1',2'}$ 4.0, 1′-H); δ_P 4.8 (with heteronuclear decoupling); ESMS(−): *m/z* 732 $[M - Na]^-$. A portion of the sodium salt **3** was further purified for use in biological experiments by PLC (Chromatotron), and elution first with 9:1 $CHCl_3$–methanol and then with 10:10:3 $CHCl_3$–methanol–1 M ammonium hydrogen carbonate to give the corresponding ammonium salt.

Sodium 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-[*sn*-2,3-bis(octadecyloxy)propyl phosphate] 4

A solution of the sodium salt **31** (10 mg, 6 μmol; prepared from the TEA salt **30**) in 1:1:1 THF–propan-1-ol–water (5 cm^3) containing 20% $Pd(OH)_2$/C was shaken under a slight overpressure of hydrogen at room temperature for 24 h. Processing essentially as described for the bis(palmitoyloxy) derivative **2**, but with 1:1 propan-1-ol–water (2 × 5 cm^3) for washing of the spent catalyst, gave the bis(octadecyloxy) derivative **4** (6 mg, 98%), $[a]_D$ −9 [*c* 0.5, $CHCl_3$–MeOH–water (10:10:3)]; R_f 0.70 (solvent *D*) and 0.65 (solvent *C*); δ_H 0.80 (6 H, t, *J* 7.0, 2 × CH_2*Me*), ~1.20 (60 H, m, 2 × $[CH_2]_{15}$), 1.50 (4 H, m, 2 × OCH_2CH_2), 3.18 (1 H, dd, $J_{1',2'}$ 3.8, $J_{2',3'}$ 8.0, 2′-H), 3.33 (1 H, m, 4′-H), 3.52–3.60 (8 H, m, 2 × OCH_2 and 2 × CH_2 propyl), 3.62 (2 H, dd, $J_{5',6'}$ 2.5, $J_{6'a,6'b}$ 12, 6′-H_2), 3.75 (1 H, br t, $J_{2',3'}$ 8.0, 3′-H), 3.90–4.11 (3 H, m, 2-H propyl, and 1- and 5′-H), 4.13 (1 H, t, $J_{1,2} = J_{2,3} = 3.0$, 2-H) and 5.45 (1 H, d, $J_{1',2'}$ 3.8, 1′-H); δ_P 5.50 (with heteronuclear decoupling); ESMS(−): *m/z* 998 $[M - Na]^-$. This material was further purified in a similar manner to compound **2** before being used in biological experiments.

Sodium 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-[octyl phosphate] 5

A solution of the sodium salt **33** (19 mg, 15.5 μmol; prepared from the TEA salt **32**) in 1:1 THF–methanol (5 cm^3) containing 20% $Pd(OH)_2$/C was shaken under a slight overpressure of hydrogen at room temperature for 24 h. Processing as described for the bis(palmitoyloxy) derivative **2** gave the phosphoric diester **5** (8 mg, 93%), $[a]_D$ +5 [*c* 0.5, $CHCl_3$–MeOH–water (10:10:3)]; R_f 0.63 (solvent *D*); δ_H 0.88 (3 H, t, *J* 7.0, CH_2*Me*), ~1.25 (10 H, m, $[CH_2]_5$), 1.54 (2 H, m, OCH_2CH_2), 3.30 (1 H,

dd, $J_{1',2'}$ 2.0, 2′-H), 3.45 (1 H, m, 4′-H), 3.58 (1 H, m, 3-H), 3.65 (2 H, ddd, $J_{5',6'b}$ 2.0, $J_{5',6'a}$ 3.5, $J_{6'a,6'b}$ 12, 6′-H_2), 3.72 (1 H, m, $J_{5,6}$ 9.2, 6-H), 3.75 (1 H, m, 3′-H), 3.85 (1 H, m, 2-H), 3.87 (2 H, m, OCH_2), 4.09 (1 H, m, 1-H), 4.11 (1 H, m, 5′-H) and 5.27 (1 H, d, $J_{1',2'}$ 2.0, 1′-H); δ_P 2.10 (with heteronuclear decoupling); ESMS(−): *m/z* 532 $[M - Na]^-$. This material was further purified in a similar manner to compound **2** before being used in biological experiments.

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