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Synthesis of some second-generation substrate analogues of early intermediates in the biosynthetic pathway of glycosylphosphatidylinositol membrane anchors☆

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Abstract

1-D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-2-*O*-octyl-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate) (**23**) and the corresponding 2-*O*-hexadecyl-D-*myo*-inositol compound **24** have been prepared as substrate analogues of an early intermediate in the biosynthetic pathway of glycosylphosphatidylinositol (GPI) membrane anchors. 1-D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-octyl-*sn*-glycerol 3-phosphate) has also been prepared as a substrate analogue. Biological evaluation of the analogues **23** and **24** revealed that they are neither substrates nor inhibitors of GPI biosynthetic enzymes in the human (HeLa) cell-free system but are potent inhibitors at different stages of GPI biosynthesis in the *Trypanosoma brucei* cell-free system.

Keywords: Glycosylphosphatidylinositol (GPI) membrane anchors; 1-D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-2-*O*-octyl- and -2-*O*-hexadecyl-*myo*-inositol 1-(1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate); 1-D-6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-(1,2-di-*O*-octyl-*sn*-glycerol 3-phosphate); Inhibition of GPI biosynthesis

1. Introduction

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 infectivity [2]. Many glycoconjugates are attached to the plasma membrane by means of glycosylphosphatidylinositol (GPI) anchors, the principal function of which is to provide a stable association of protein or oligosaccharide with the lipid bilayer [3]. Studies in our laboratories have focused largely on those enzymes involved in the early stages of GPI biosynthesis in the belief that disruption of GPI-anchor biosynthesis would seriously impair the parasite's ability to survive in the host [4,5].

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*-

☆ Parasite glycoconjugates, Part 10. For Part 9, see Ref. [1].

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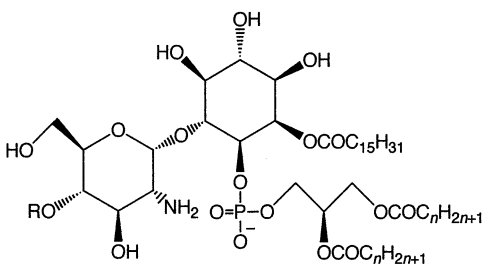
(1→4)-α-D-Glc*p*NH_2-(1→6)-D-*myo*-inositol backbone, suggesting that this sequence is likely to be conserved in all GPI anchors [2]. To this conserved region may be added other sugars in a species- and stage-specific manner.

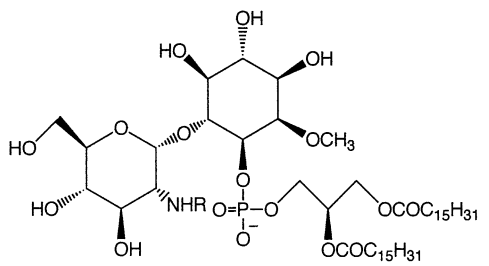
The biosynthesis of GPI membrane anchors in bloodstream forms of *Trypanosoma brucei* [6,7] occurs in the endoplasmic reticulum and involves the sequential glycosylation of phosphatidylinositol (PI) as follows: α-D-Glc*p*NAc is transferred from UDP-D-Glc*p*NAc to PI to form α-D-Glc*p*NAc-(1→6)-PI, which is then N-deacetylated 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 [e.g. hexadecanoyl(palmitoyl)] at 2-OH of the D-*myo*-inositol residue to give the 2-*O*-acyl compound **1**. 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 removed from the D-*myo*-inositol residue. The resulting structure undergoes a complex series of fatty acid remodelling reactions before the pre-assembled GPI precursor (known as glycolipid A) is transferred en bloc to newly synthesised protein. Some α-D-galactosylation of glycolipid A takes place in the endoplasmic reticulum but mainly in the Golgi apparatus during transport to the outer surface of the cell membrane.

The GPI biosynthetic pathway in mammalian cells [8] 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 to form, for example, α-D-Glc*p*NH_2-(1→6)-2-*O*-acyl-PI **2**.



1 R = α-D-Man*p*
2 R = H

Methylation of 2-OH of the D-*myo*-inositol residue, as in the synthetic analogue **3** [9], prevents inositol acylation and allows the role of inositol acylation in GPI biosynthesis to be assessed. Studies with the methylated analogue **3** showed [10] that it is recognised and D-mannosylated by the GPI biosynthetic enzymes of *T. brucei* but not by those of human (HeLa) cells, thereby providing the first direct evidence that trypanosomal and mammalian α-(1→4)-D-mannosyltransferases have different substrate specificities and that inositol 2-*O*-acylation of α-D-Glc*p*NH_2-(1→6)-PI (→**2**) is a prerequisite for D-mannosylation (→**1**) in the HeLa cell-free system. Furthermore, they confirmed that inositol 2-*O*-acylation is required in trypanosomes for the efficient addition of ethanolamine phosphate [7], since 2-*O*-methylation prevented the addition of ethanolamine phosphate to a later biosynthetic intermediate, namely 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*-methyl-PI. It is also notable that α-D-Glc*p*NAc-2-*O*-methyl-PI **4** is a substrate for both trypanosomal and human N-deacetylases, exhibiting turnover rates of roughly 60% of that of α-D-Glc*p*NAc-PI [10,11].



3 R = H
4 R = Ac

The finding [10] that the synthetic analogue **3** is recognised and D-mannosylated by the GPI biosynthetic pathway of *T. brucei*, but not by that of human (HeLa) cells, suggests that the discovery and development of inhibitors that are selective for parasite GPI pathways are attainable goals. With this in mind, we have prepared for biological testing a number of analogues in which the inositol 2-*O*-methyl group of the compound **3** is re-

placed by a medium or long alkyl chain, as in the 2-*O*-octyl and 2-*O*-hexadecyl analogues **23** and **24**, respectively. Following on from earlier work [9], we also wished to test as a substrate for the various D-mannosyltransferases of GPI anchor biosynthesis, the ether-substituted analogue **31** in which octyl groups are introduced at O-1 and O-2 of the glycerol residue. The corresponding octadecyl compound **32** [9] proved to be an excellent substrate for the GPI D-mannosyltransferases and it was of interest to discover whether shorter alkyl chains would suffice. Our reasons for wanting ether-linked substituents on the glycerol residue have been recorded earlier [9].

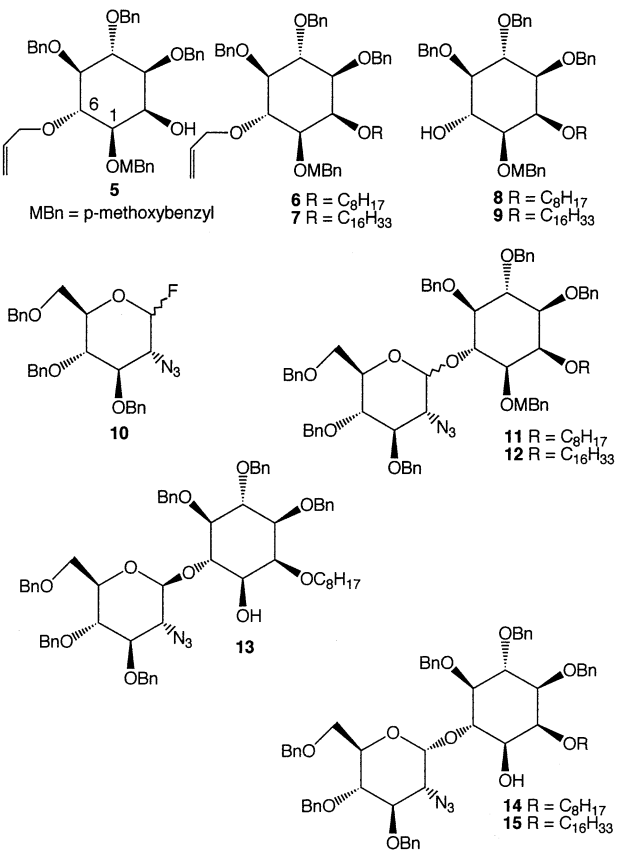
2. Results and discussion

Alkylation of 2-OH of the otherwise protected D-*myo*-inositol **5** [12] with octyl or hexadecyl bromide in *N*,*N*-dimethylformamide in the presence of sodium hydride produced the 2-*O*-octyl and 2-*O*-hexadecyl derivatives **6** and **7**, respectively. These derivatives gave the deallylated compounds **8** and **9** following isomerisation of the *O*-allyl group with potassium *tert*-butoxide in dimethyl sulfoxide and mild acid hydrolysis of the resulting propenyl derivatives (Scheme 1).

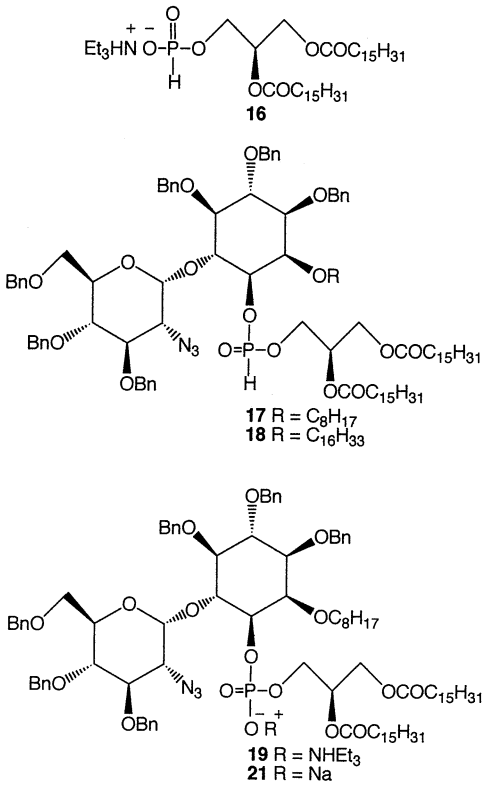
The 2-*O*-octyl derivative **8** gave a ~2:1 mixture of the α- and β-linked pseudodisaccharides **11** on coupling with the glycosyl fluoride **10** [9,12] in dry diethyl ether in the presence of zirconocene dichloride and silver perchlorate [13]. Treatment of the coupled products with trifluoroacetic acid (TFA) in CH_2Cl_2 then afforded a mixture of the compounds **13** ($J_{1',2'}$ 7.9 Hz) and **14** ($J_{1',2'}$ 3.6 Hz), which was resolved by a combination of crystallisation (of **13**) and radial-band chromatography. Similar coupling of the glycosyl fluoride **10** [9,12] and the 2-*O*-hexadecyl derivative **9** gave a ~3:1 mixture of the α- and β-linked pseudodisaccharides **12**, which, following hydrolysis with TFA and radial-band chromatography, furnished the required α-linked compound **15** ($J_{1',2'}$ 3.6 Hz).

In following the serviceable hydrogenphosphonate route to phosphonic diesters [9,12], the hydrogenphosphonate **16** [9,14] reacted with each of the alcohols **14** and **15** in the presence of pivaloyl chloride [15,16] to give the phosphonic diesters **17** and **18**, respectively, as mixtures of diastereoisomers (Scheme 2). These were oxidised with iodine in pyridine–water [16] to the phosphoric diesters, which were isolated and characterised as the triethylammonium (TEA) salts **19** and **20**. Finally, hydrogenolysis of the corresponding sodium salts **21** and **22** over 20% $Pd(OH)_2$/C gave α-D-Glc*p*NH_2-(1→6)-2-*O*-octyl-PI **23** and the 2-*O*-hexadecyl derivative **24**, respectively (Scheme 3).

In pursuing a similar approach to the 1,2-di-*O*-octyl-*sn*-glycerol-PI analogue **31**, the pseudodisaccharide **25** [12] is required to react with the hydrogenphosphonate **27**, which is readily prepared by a standard procedure [17] from the enantiopure alcohol **26** [18]. The reaction of compounds **25** and **27** in the presence of pivaloyl chloride [15,16] gave a mixture of diastereoisomeric phosphonic esters **28**, which, as before, was transformed first into the TEA phosphate derivative **29** for

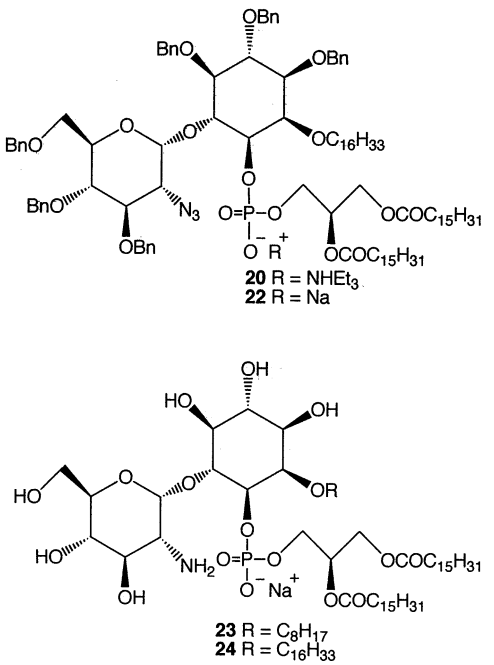


Scheme 1.

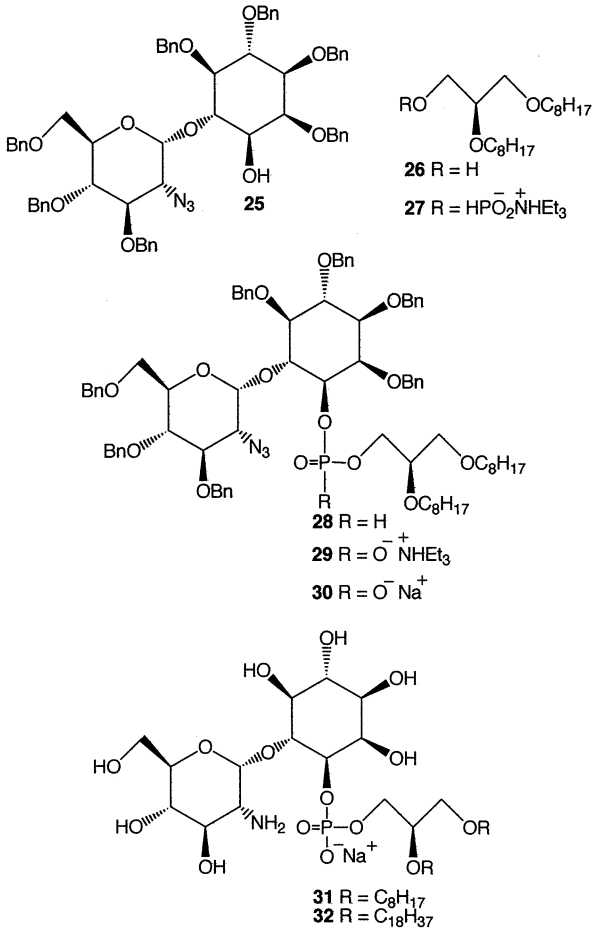


Scheme 2.

characterisation purposes and thereafter into the sodium salt **30**. Hydrogenolysis of the sodium salt **30** over 20% $Pd(OH)_2/C$ furnished the 1,2-di-*O*-octyl-*sn*-glycerol-PI **31** (Scheme 4).



Scheme 3.



Scheme 4.

Details of the results of enzymic studies with the above analogues are reported elsewhere [11,19]. In brief, neither trypanosomal nor HeLa α-(1→4)-D-mannosyltransferases can act on α-D-Glc*p*NH_2-2-*O*-hexadecyl-PI **24**, which turns out to be an inhibitor of trypanosomal α-(1→4)-D-mannosyltransferase but not of the corresponding HeLa enzyme. α-D-Glc*p*NH_2-2-*O*-octyl-PI **23** is neither a substrate for, nor an inhibitor of, any of the early GPI biosynthetic enzymes in the HeLa cell-free system but is a potent inhibitor of inositol 2-*O*-acylation of D-mannosylated species in the *T. brucei* cell-free system. The importance of inositol 2-*O*-acylation in the trypanosomal GPI biosynthetic pathway [7], coupled with the selective inhibition of this activity with the 2-*O*-octyl analogue **23**, suggest trypanosomal inositol acyltransferase as a potential target for the development of trypanosome-specific therapeutic agents.

The 1,2-di-*O*-octyl-*sn*-glycerol-PI analogue **31** resembled the corresponding octadecyl analogue **32** [9] in being an excellent substrate for both mammalian and trypanosomal D-mannosyltransferases.

3. Experimental

Melting points were determined on a Reichert hot-plate and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter at ambient temperature. ^{1}H NMR and COSY spectra were recorded on a Bruker AM 200 MHz or AC 500 MHz spectrometer 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 $60F_{254}$ (E. Merck) with A, a 1:1 diethyl ether–hexane; B, 9:1 $CHCl_3$–MeOH; C, 19:1 $CHCl_3$–MeOH; D, 10:10:3 $CHCl_3$–MeOH–water; and E, 2:1:1 butanol–EtOH–water as developers and detection by UV light or by charring with 3:17:1 H_2SO_4–water–EtOH as appropriate. Radial-band chromatography (RBC) was performed using a Chromatotron (model 7924T, TC Research, UK) with Adsorbosil Plus-P (6–15 mm) (Alltech) as the adsorbent.

1-D-*6*-O-*Allyl*-*3,4,5*-*tri*-O-*benzyl*-*1*-O-*(4-methoxybenzyl)*-*2*-O-*octyl*-myo-*inositol* (**6**).—To a stirred solution of the alcohol **5** [12] (514 mg, 0.84 mmol) in DMF (15 mL) at 0 °C were added sodium hydride (78 mg, 3.25 mmol) and octyl bromide (212 μL, 1.23 mmol). The cooling bath was removed and the solution was stirred for 24 h, whereafter MeOH was added to decompose any excess of sodium hydride. The resulting solution was extracted with diethyl ether (25 mL) and the organic extract was washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure to an oil which solidified on standing. Recrystallisation from hexane gave the octyl derivative **6** (0.6 g, 98%), mp 32–33 °C; $[\alpha]_D$ −7° (*c* 1.2, $CHCl_3$); δ_H 0.80 (t, 3 H, *J* 7.1, CH_2Me), ~1.20 (10 H, $[CH_2]_5$), 1.47 (m, 2 H, OCH_2CH_2), 3.13 (dd, 1 H, $J_{2,3}$ 2.3, $J_{3,4}$ 9.8, H-3), 3.20 (dd, 1 H, $J_{1,2}$ 2.3, $J_{1,6}$ 9.8, H-1), 3.25 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.8, H-5), 3.64 (t, 2 H, *J* 6.5, OCH_2), 3.70 (4 H, $ArOCH_3$ and H-2), 3.75 (t, 1 H, H-4), 3.88 (t, 1 H, H-6), 4.20–4.35 (m, 2 H, $CH_2CH{=}CH_2$), 4.45–4.85 (8 H, 4 × CH_2Ar), 5.00–5.20 (m, 2 H, $CH_2CH{=}CH_2$), 5.85–5.94 (m, 1 H, $CH_2CH{=}CH_2$), 6.75–7.40 (19 H, C_6H_4 and 3 × Ph); Anal. Calcd for $C_{46}H_{58}O_7$: C, 76.4; H, 8.1. Found: C, 76.1; H, 8.2.

1-D-*6*-O-*Allyl*-*3,4,5*-*tri*-O-*benzyl*-*2*-O-*hexadecyl*-*1*-O-*(4-methoxybenzyl)*-myo-*inositol* (**7**).—To a stirred solution of the alcohol **5** [12] (1.0 g, 1.6 mmol) in DMF (30 mL) at 0 °C were added sodium hydride (0.16 g, 6.7 mmol) and hexadecyl bromide (734 μL, 2.4 mmol). The reaction mixture was stirred for 2 h at room temperature (rt) before MeOH was added to quench any excess of sodium hydride. The resulting solution was extracted with diethyl ether (3 × 25 mL) and the combined extracts were washed successively with water (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated under reduced pressure. RBC (1:2 diethyl ether–cyclohexane) of the residue gave the hexadecyl derivative **7** (1.32 g, 96.5%), mp 42–43 °C (from hexane); $[\alpha]_D$ −7° (*c* 1.0, $CHCl_3$); δ_H 0.80 (t, 3 H, *J* 7.0, CH_2Me), ~1.20 (26 H, $[CH_2]_{13}$), 1.48 (m, 2 H, OCH_2CH_2), 3.13 (dd, 1 H, $J_{2,3}$ 2.2, $J_{3,4}$ 9.8, H-3), 3.18 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,6}$ 9.8, H-1), 3.28 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.8, H-5), 3.62 (m, 2 H, OCH_2), 3.69 (4 H, $ArOCH_3$ and H-2), 3.75 (t, 1 H, H-4), 3.86 (t, 1 H, H-6), 4.15–4.35 (m, 2 H, $CH_2CH{=}CH_2$), 4.45–4.85 (8 H, 4 × CH_2Ar), 5.00–5.25 (m, 2 H, $CH_2CH{=}CH_2$), 5.85–5.94 (m, 1 H, $CH_2CH{=}CH_2$), 6.75–7.30 (19 H, C_6H_4 and 3 × Ph). Anal. Calcd for $C_{54}H_{74}O_7$: C, 77.7; H, 8.9. Found: C, 77.4; H, 8.9.

1-D-*3,4,5*-*Tri*-O-*benzyl*-*1*-O-*(4-methoxybenzyl)*-*2*-O-*octyl*-myo-*inositol* (**8**).—A solution of the allyl derivative **6** (491 mg, 0.68 mmol) in anhyd Me_2SO (15 mL) containing potassium *tert*-butoxide (662 mg, 5.9 mmol) was heated and stirred at 60 °C for 1 h, cooled and then poured into ice-water (75 mL). The resulting aqueous solution was extracted with EtOAc (3 × 25 mL) and the combined organic

extracts were washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting propenyl derivative in 1 M hydrochloric acid–acetone (20 mL, 1:9) was boiled under reflux for 10 min, whereafter the solvents were removed under reduced pressure. A solution of the residue in diethyl ether was percolated through a short column of silica gel and the eluent was concentrated under reduced pressure to give the deallylated compound **8** (0.37 g, 80%), mp 62–63 °C (from hexane), $[\alpha]_D$ −14° (*c* 1.1, $CHCl_3$); δ_H 0.85 (t, 3 H, *J* 7.1, CH_2Me), ~1.25 (10 H, $[CH_2]_5$), 1.55 (m, 2 H, OCH_2CH_2), 2.45 (s, 1 H, OH), 3.10 (d, 1 H, $J_{2,3}$ 2.2, $J_{3,4}$ 9.8, H-3), 3.31 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,6}$ 9.8, H-1), 3.34 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.8, H-5), 3.70 (m, 2 H, OCH_2), 3.80 (s, 3 H, $ArOCH_3$), 3.84 (t, 1 H, H-2), 3.95 (t, 1 H, H-6), 4.05 (t, 1 H, H-4), 4.45–4.90 (8 H, 4 × CH_2Ar), 6.80–7.40 (19 H, C_6H_4 and 3 × Ph). Anal. Calcd for $C_{43}H_{54}O_7$: C, 75.6; H, 8.0. Found: C, 75.6; H, 8.3.

1-D-*3,4,5-Tri*-O-*benzyl-2*-O-*hexadecyl-1*-O-*(4-methoxybenzyl)*-myo-*inositol* (**9**).—A solution of the allyl derivative **7** (800 mg, 0.96 mmol) in anhyd Me_2SO (10 mL) containing potassium *tert*-butoxide (931 mg, 8.3 mmol) was heated and stirred at 60 °C for 1 h, cooled, and poured into ice-water. The resulting solution was extracted with EtOAc (3 × 25 mL) and the combined organic extracts were washed successively with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting propenyl derivative was boiled under reflux in 1 M hydrochloric acid–acetone (20 mL; 1:9) for 10 min, after which the solvents were removed under reduced pressure. Purification of the residue by RBC (elution with 1:3 diethyl ether–cyclohexane) gave the deallylated compound **9** (0.72 g, 94%), mp 44–46 °C (from hexane); $[\alpha]_D$ −11° (*c* 1.4, $CHCl_3$); δ_H 0.80 (t, 3 H, *J* 7.1, CH_2Me), ~1.20 (26 H, $[CH_2]_{13}$), 1.50 (m, 2 H, OCH_2CH_2), 2.50 (s, 1 H, OH), 3.03 (dd, 1 H, $J_{2,3}$ 2.0, $J_{3,4}$ 9.8, H-3), 3.24 (dd, 1 H, $J_{1,2}$ 2.1, $J_{1,6}$ 9.8, H-1), 3.27 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.8, H-5), 3.60 and 3.65 (m, 2 H, OCH_2), 3.68 (s, 3 H, $ArOCH_3$), 3.78 (t, 1 H, H-2), 3.90 (t, 1 H, H-6), 4.00 (t, 1 H, H-4), 4.40–4.80 (8 H, 4 × CH_2Ar), 6.75–7.30 (19 H, C_6H_4 and 3 × Ph). Anal. Calcd for $C_{51}H_{70}O_7$: C, 77.0; H, 8.9. Found: C, 76.9; H, 9.0.

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy-α,β*-D-*glucopyranosyl)-3,4,5-tri*-O-*benzyl-1*-O-*(4-methoxybenzyl)-2*-O-*octyl*-myo-*inositol* (**11**).—The glycosyl fluoride **10** [12] (140 mg, 0.29 mmol) and the acceptor **8** (143 mg, 0.21 mmol) were dried overnight under vacuum in a desiccator containing P_2O_5. They were then dissolved in anhyd diethyl ether (10 mL) and zirconocene dichloride (338 mg, 1.16 mmol) and 4 Å molecular sieves (1.0 g) were added to the solution. After 15 min at rt, the mixture was cooled to 0 °C and pre-dried silver perchlorate (236 mg, 1.05 mmol) suspended in anhyd diethyl ether (10 mL) was added dropwise, followed by 1,1,3,3-tetramethylurea (30 μL, 251 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 eluent was concentrated under reduced pressure. Purification of the residue by RBC (1:6 diethyl ether–hexane) gave the pseudodisaccharide **11** (119 mg, 51%) as a mixture of the α, β anomers in the ratio of ~2:1 (determined by ^1H NMR spectroscopy); $[\alpha]_D$ +17.5° (*c* 1.2, $CHCl_3$); δ_H 0.80 (t, 3 H, *J* 7.1, CH_2Me), ~1.20 (10 H, $[CH_2]_5$), 1.50 (m, 2 H, OCH_2CH_2), 3.09 (dd, 1 H, $J_{5',6'a}$ 2.2, $J_{6'a,6'b}$ 11.0, H-6′a), 3.14 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.5, H-2′ α anomer), 3.16 (dd, 1 H, $J_{5',6'b}$ 2.0, H-6′b), 3.27 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,6}$ 9.8, H-1), 3.31 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.5, H-5), 3.34 (dd, 1 H, $J_{2,3}$ 2.2, $J_{3,4}$ 9.5, H-3), 3.60 (3 H, OCH_2 and H-4′), 3.74 (s, 3 H, $ArOCH_3$), 3.78 (t, 1 H, H-2), 3.88 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 10.5, H-3′), 3.96 (2 H, H-5′, 6), 4.09 (t, 1 H, H-4), 4.32 (d, 1 H, $J_{1',2'}$ 8.5, H-1′ β anomer), 4.40–4.95 (14 H, 7 × CH_2Ar), 5.65 (d, 1 H, 1′-H α anomer), 6.80–7.40 (34 H, C_6H_4 and 6 × Ph); ESMS (+): *m/z* 1157 $[M + NH_4]^+$.

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy-α,β*-D-*glucopyranosyl)-3,4,5-tri*-O-*benzyl-2*-O-*hexadecyl-1*-O-*(4-methoxybenzyl)*-myo-*inositol* (**12**).—After drying overnight over P_2O_5 in a vacuum desiccator, the glycosyl donor **10** [12] (280 mg, 0.59 mmol) and the acceptor **9** (340 mg, 0.43 mmol) were dissolved in anhyd diethyl ether (15 mL) containing zirconocene dichloride (691 mg, 2.36 mmol)

and 4 Å molecular sieves (1 g). After stirring of the mixture at rt for 15 min under argon, it was cooled to 0 °C and pre-dried silver perchlorate (484 mg, 2.15 mmol) and 1,1,3,3-tetramethylurea (62 μL, 520 μmol) were added. Stirring of the mixture under argon at 0 °C was continued overnight, whereafter it was percolated through a short column of silica gel (elution with diethyl ether) and the eluent was concentrated under reduced pressure. RBC (1:4 diethyl ether–cyclohexane) of the residue gave a mixture of the α- and β-linked compounds **12** (377 mg, 70%) in the ratio of ~3:1 (determined by ^{1}H NMR spectroscopy); $[\alpha]_D$ +18.4° (*c* 1.3, $CHCl_3$); δ_H 0.80 (t, 3 H, *J* 7.1, CH_2*Me*), ~1.20 (26 H, $[CH_2]_{13}$), 1.47 (m, 2 H, OCH_2CH_2), 3.07 (dd, 1 H, $J_{5',6'a}$ 2.1, $J_{6'a,6'b}$ 11.0, H-6′a), 3.10–3.15 (2 H, H-2′, 6′b), 3.25 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,6}$ 9.8, H-1), 3.29–3.35 (2 H, H-3, 5), 3.55–3.61 (3 H, OCH_2, H-4′), 3.69 (s, 3 H, $ArOCH_3$), 3.76 (t, 1 H, $J_{2,3}$ 2.2, H-2), 3.87 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 10.2, H-3′), 3.94 (2 H, H-5′, 6), 4.07 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5, H-4), 4.32 (d, 1 H, $J_{1',2'}$ 8.2, H-1′ β anomer), 4.30–5.00 (m, 14 H, 7 × CH_2Ar), 5.64 (d, 1 H, $J_{1',2'}$ 3.7, H-1′ α anomer), 6.70–7.30 (34 H, C_6H_4 and 6 × Ph); ESMS(+): *m*/*z* 1274 $[M + Na]^+$.

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-3,4,5-tri*-O-*benzyl-2*-O-*octyl*-myo-*inositol* (**14**).—A solution of the methoxybenzyl derivative **11** (110 mg, 96.5 μmol) in CH_2Cl_2 (10 mL) containing TFA (104 μL, 1.35 mmol) was set aside at rt for 1 h, whereafter it was neutralised with Et_3N, washed successively with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. Trituration of the residue with hexane deposited the crystalline β anomer **13** (20 mg, 20%), mp 137–139 °C; $[\alpha]_D$ −34° (*c* 0.3, $CHCl_3$); δ_H 4.57 (d, 1 H, $J_{1',2'}$ 7.9, H-1′); ESMS(+): *m*/*z* 1037 $[M + NH_4]^+$. Anal. Calcd for $C_{62}H_{73}O_{10}N_3$: C, 73.0; H, 7.2; N, 4.1. Found: C, 73.0; H, 7.6; N, 4.1. RBC (1:2 diethyl ether–hexane) of the mother liquor furnished the α-linked compound **14** (45 mg, 46%) as an opaque oil; R_f 0.40 (solvent A); $[\alpha]_D$ +18° (*c* 1.1, $CHCl_3$); δ_H 0.81 (t, 3 H, *J* 7.1, CH_2*Me*), ~1.25 (10 H, $[CH_2]_5$), 1.52 (m, 2 H, OCH_2CH_2), 3.02 (dd, 1 H, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 11, H-6′a), 3.08 (d, 1 H, $J_{1,OH}$ 6.9, HO-1), 3.17 (dd, 1 H, $J_{5',6'b}$ 2.3, H-6′b), 3.27 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.4, H-5), 3.34 (dd, 1 H, $J_{2,3}$ 2.3, $J_{3,4}$ 9.8, H-3), 3.40 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10, H-2′), 3.51 (m, 1 H, H-1), 3.56 and 3.86 (2 × m, 2 H, OCH_2), 3.65 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.3, H-4′), 3.75 (t, 1 H, $J_{1,2} = J_{2,3}$ 2.3, H-2), 3.80–3.88 (3 H, H-3′, 5′, 6), 3.93 (t, 1 H, H-4), 4.05–4.95 (12 H, 6 × CH_2Ph), 5.45 (d, 1 H, H-1′), 6.90–7.50 (30 H, 6 × Ph); ESMS(+): *m*/*z* 1037 $[M + NH_4]^+$.

1-D-*6*-O-*(2-Azido-3,4,6-tri*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-3,4,5-tri*-O-*benzyl-2*-O-*hexadecyl*-myo-*inositol* (**15**).—To a stirred solution of the methoxybenzyl compound **12** (280 mg, 0.22 mmol) in CH_2Cl_2 (10 mL) at rt was added TFA (241 μL, 3.1 mmol). After 1 h, the solution was neutralised with Et_3N and washed successively with water and brine, dried $MgSO_4$, and evaporated under reduced pressure. RBC (1:1 diethyl ether–hexane) of the residue gave the α-coupled compound **15** (169 mg, 67%) as an oil; R_f 0.48 (solvent A); $[\alpha]_D$ +21° (*c* 1.2, $CHCl_3$); δ_H 0.80 (t, 3 H, *J* 7.1, CH_2*Me*), ~1.20 (26 H, $[CH_2]_{13}$), 1.52 (m, 2 H, OCH_2CH_2), 3.02 (dd, 1 H, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 11.0, H-6′a), 3.07 (d, 1 H, $J_{1,OH}$ 6.9, HO-1), 3.17 (dd, 1 H, $J_{5',6'b}$ 2.1, H-6′b), 3.27 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.5, H-5), 3.33 (dd, 1 H, $J_{2,3}$ 2.2, $J_{3,4}$ 9.5, H-3), 3.40 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10.3, H-2′), 3.50 (m, 1 H, H-1), 3.55 and 3.82 (2 × m, 2 H, OCH_2), 3.65 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.4, H-4′), 3.75 (t, 1 H, $J_{1,2} = J_{2,3}$ 2.2, H-2), 3.85 (3 H, H-3′, 5′, 6), 3.93 (t, 1 H, H-4), 4.05–5.00 (12 H, 6 × CH_2Ph), 5.43 (d, 1 H, 1′-H), 6.90–7.40 (30 H, 6 × Ph); ESMS(−): *m*/*z* 1130 $[M–H]^-$.

Triethylammonium 1-D-*6*-O-*(2-azido-3,4,6-tri*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-3,4,5-tri*-O-*benzyl-2*-O-*octyl*-myo-*inositol 1-(1,2-di*-O-*hexadecanoyl*-sn-*glycerol 3-phosphate)* (**19**).—Compound **14** (45 mg, 0.044 mmol) and 1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-hydrogenphosphonate TEA salt **16** [9] (65 mg, 0.089 mmol) were each dried overnight over P_2O_5 and dried further by evaporation of anhyd pyridine (3 × 2 mL) therefrom. To a solution of the mixture in dry pyridine (10 mL) was added pivaloyl chloride (68 μL, 0.55 mmol), and the resulting solution was stirred under argon for 1 h; TLC (9:1

toluene–EtOAc) then indicated that a mixture of diastereoisomeric hydrogenphosphonates **17** had formed. Oxidation of the hydrogenphosphonates was accomplished by the addition of a freshly prepared solution of iodine (46 mg, 0.18 mmol) in 19:1 pyridine–water (10 mL). After 45 min, $CHCl_3$ (25 mL) was added and the resulting solution was washed successively with 5% aq $NaHSO_3$ (20 mL) and 1 M aq triethylammonium hydrogen carbonate (TEAB) buffer (3 × 15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. RBC (elution first with $CHCl_3$ and then with 19:1 $CHCl_3$–MeOH) of the residue gave the TEA salt **19** (54 mg, 70%); $[\alpha]_D$ +42° (*c* 1.3, $CHCl_3$); R_f 0.45 (solvent B); δ_H 0.81 (t, 9 H, *J* 6.9, 3 × CH_2Me), ~1.23 (67 H, 3 × CH_2Me, $[CH_2]_5$, 2 × $[CH_2]_{12}$), 1.55 (6 H, OCH_2CH_2, 2 × $COCH_2CH_2$), 2.22 (m, 4 H, 2 × $COCH_2$), 3.00 (m, 6 H, 3 × CH_2Me), 3.12 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10.2, H-2′), 3.25–3.45 (4 H, H-3, 5, 6′a,b), 3.64 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.7, H-4′), 3.75 (t, 2 H, *J* 6.5, OCH_2), 3.95–4.00 (2 H, H-3′, 4), 4.05–4.25 (5 H, H-1, 5′, 6, CH_2 -1 or -3 glycerol), 4.34 (3 H, H-2, CH_2 -1 or -3 glycerol), 4.45–4.90 (12 H, 6 × CH_2Ph), 5.20 (m, 1 H, 2-H glycerol), 5.70 (d, 1 H, 1′-H), 6.90–7.40 (30 H, 6 × Ph); δ_P −0.98 (with heteronuclear decoupling); ESMS(−): *m*/*z* 1649 $[M-NEt_3-H]^-$.

*Triethylammonium 1-*D*-6-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-3,4,5-tri-*O*-benzyl-2-*O*-hexadecyl*-myo*-inositol 1-(1,2-di-*O*-hexadecanoyl*-sn*-glycerol 3-phosphate)* (**20**).—After drying overnight over P_2O_5, the TEA salt **16** [9] (129 mg, 0.176 mmol) and the α-compound **15** (100 mg, 0.088 mmol) were dissolved in anhyd pyridine (10 mL) under argon and pivaloyl chloride (135 μL, 1.1 mmol) was added to the solution. After 1 h at rt, TLC (9:1 toluene–EtOAc) indicated the presence of two diastereoisomeric hydrogenphosphonates **18**, which were then oxidised using a freshly prepared solution of iodine (89 mg, 0.35 mmol) in 19:1 pyridine–water (10 mL). $CHCl_3$ (20 mL) was added after 45 min and the organic solution was washed successively with 5% $NaHSO_3$ (20 mL) and 1 M aq TEAB buffer (3 × 15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. RBC (elution first with $CHCl_3$ and then with 19:1 $CHCl_3$–MeOH) of the residue furnished the TEA phosphate salt **20** (150 mg, 91%); $[\alpha]_D$ +30° (*c* 1.3, $CHCl_3$); R_f 0.48 (solvent C); δ_H 0.80 (t, 9 H, J 7.1, 3 × CH_2Me), ~1.20 (87 H, 3 × CH_2Me, $[CH_2]_{13}$, 2 × $[CH_2]_{13}$), 1.50 (m, 6 H, OCH_2CH_2, 2 × $COCH_2CH_2$), 2.20 (m, 4 H, 2 × $COCH_2$), 2.95 (m, 6 H, 3 × CH_2Me), 3.10 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10.3, H-2′), 3.30 (dd, 1 H, $J_{5',6'a}$ 2.2, $J_{6'a,6'b}$ 11, H-6′a), 3.32–3.42 (3 H, H-3, 5, 6′b), 3.64 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.7, H-4′), 3.74 (t, 2 H, *J* 6.6, OCH_2), 3.90–4.00 (2 H, H-3′, 4), 4.04–4.25 (5 H, H-1, 5′, 6, CH_2 -1 or -3 glycerol), 4.36 (3 H, H-2, CH_2 -1 or -3 glycerol), 4.45–4.90 (12 H, 6 × CH_2Ph), 5.20 (m, 1 H, H-2 glycerol), 5.72 (d, 1 H, H-1′), 6.90–7.30 (30 H, 6 × Ph); δ_P −1.6 (with heteronuclear decoupling); ESMS(−): *m*/*z* 1761.3 $[M-NEt_3-H]^-$.

*Sodium 1-*D*-6-*O*-(2-amino-2-deoxy-α-*D*-glucopyranosyl)-2-*O*-octyl*-myo*-inositol 1-(1,2-di-*O*-hexadecanoyl*-sn*-glycerol 3-phosphate)* (**23**).—A solution of the sodium salt **21** (33 mg, 0.02 mmol; prepared from the corresponding TEA salt **19** as described below) in 4:4:1 THF–propan-1-ol–water (9 mL) containing 20% $Pd(OH)_2/C$ was shaken under a slight overpressure of hydrogen at rt for 24 h. It was then percolated through a short column containing Chelex 100 on a bed of Celite (elution with 1:1 propan-1-ol–water) and the eluent was concentrated under reduced pressure to give the inositol derivative **23** (20 mg, 92%); $[\alpha]_D$ +24° (*c* 1, 10:10:3 $CHCl_3$–MeOH–H_2O); R_f 0.68 (solvent D) and 0.61 (solvent E); δ_H (10:10:3 $CDCl_3$–CD_3OD–D_2O) 0.60 (m, 9 H, 3 × CH_2Me), ~1.00 (58 H, $[CH_2]_5$, 2 × $[CH_2]_{12}$), 1.25 (6 H, OCH_2CH_2, 2 × $COCH_2CH_2$), 2.05 (m, 4 H, 2 × $COCH_2$), 2.85 (dd, 1 H, $J_{1',2'}$ 3.4, $J_{2',3'}$ 9.0, H-2′), 3.00 (m, 1 H, H-5), 3.15 (2 H, H-3, 4′), 3.35 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.0, H-4), 3.42 (m, 2 H, OCH_2), 3.50 (4 H, H-2, 3′, 6′a,b), 3.60 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.5, H-6), 3.70 (m, 2 H, CH_2 -1 or -3 glycerol), 3.79 (m, 1 H, H-5′), 3.85 (3 H, H-1, CH_2 -1 or -3 glycerol), 4.90 (m, 1 H, H-2 glycerol), 5.20 (d, 1 H, H-1′); δ_P 4.70 (with heteronuclear decoupling); ESMS(−): *m*/*z* 1082.2 $[M-Na]^-$.

*Sodium 1-*D*-6-*O*-(2-amino-2-deoxy-α-*D*-glucopyranosyl)-2-*O*-hexadecyl*-myo*-inositol 1-(1,2-di-*O*-hexadecanoyl*-sn*-glycerol 3-phosphate)* (**24**).—A solution of the TEA salt **20**

(54 mg, 0.029 mmol) in 1:1 THF–MeOH (10 mL) was stirred in the presence of Amberlite DP-1 (Na^+) resin for 3 h, filtered, and concentrated under reduced pressure to afford quantitatively the sodium salt **22**. A solution of the sodium salt **22** (40 mg, 0.022 mmol) in 4:4:1 THF–propan-1-ol–water (9 mL) containing 20% $Pd(OH)_2/C$ (50 mg), was shaken under a slight overpressure of hydrogen at rt, for 24 h. It was then percolated through a short column containing Chelex 100 on a bed of Celite (elution with 1:1 propan-1-ol–water) and the eluent was concentrated under reduced pressure to give the inositol derivative **24** (20 mg, 74%); $[\alpha]_D$ +6° (*c* 1, 10:10:3 $CHCl_3$–MeOH–H_2O); R_f 0.76 (solvent D) and 0.58 (solvent E); δ_H (10:10:3 $CDCl_3$–CD_3OD–D_2O) 0.60 (t, 9 H, *J* 7.0, 3 × CH_2*Me*), ~0.90 (78 H, 3 × $[CH_2]_{13}$), 1.30 (6 H, OCH_2CH_2, 2 × $COCH_2CH_2$), 2.03 (m, 4 H, 2 × $COCH_2$), 2.87 (dd, 1 H, $J_{1',2'}$ 3.6, $J_{2',3'}$ 10, H-2′), 3.00 (m, 1 H, H-5), 3.15 (m, 1 H, H-4′), 3.34 (2 H, H-3, 4), 3.43 (3 H, OCH_2, H-6′a), 3.53 (3 H, H-2, 3′, 6′b), 3.61 (t, 1 H, $J_{1,6} = J_{5,6}$ 9.3, H-6), 3.69 (m, 2 H, CH_2 -1 or -3 glycerol), 3.80 (m, 1 H, H-5′), 3.90 (3 H, H-1, CH_2 -1 or -3 glycerol), 4.95 (m, 1 H, H-2 glycerol), 5.23 (d, 1 H, H-1′); δ_P 0.95 (with heteronuclear decoupling): ESMS(−): *m*/*z* 1194.6 $[M-Na]^-$.

Triethylammonium 1,2-di-O-*octyl*-sn-*glycerol 3-hydrogenphosphonate* (**27**).—The alcohol **26** [18] (150 mg, 0.475 mmol) was dried by evaporation of pyridine therefrom and was afterwards dissolved in dry 10:1 THF–pyridine (11 mL). This solution was added dropwise over 30 min to a stirred solution of salicyl chlorophosphite (134 mg, 0.66 mmol) in dry THF (5 mL) under argon at rt. After 1 h, TLC (19:1 $CHCl_3$–MeOH) indicated complete conversion of the starting material into a product of lower mobility (R_f 0.26). The reaction mixture was quenched with 1 M TEAB buffer solution (15 mL) and the resulting aqueous solution was stirred for 30 min. $CHCl_3$ (25 mL) was then added and the organic layer was separated and washed with 1 M TEAB solution (3 × 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude hydrogenphosphonate TEA salt **27** (215 mg, 94%) so obtained was used in the next experiment without further purification; δ_P 5.33 (with heteronuclear decoupling), J_{PH} 640; ESMS(-): *m*/*z* 379 $[M-NEt_3-H]^-$.

Triethylammonium 1-D-*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-(1,2-di*-O-*octyl*-sn-*glycerol 3-phosphate)* (**29**).—The compounds **25** [12] (127 mg, 0.13 mmol) and **27** (170 mg, 0.35 mmol) were dried over P_2O_5 overnight, whereafter anhydrous pyridine (5 mL) was evaporated from them. They were then dissolved in dry pyridine (10 mL), pivaloyl chloride (269 μL, 2.19 mmol) was added and the resulting solution was stirred under argon at rt for 1 h. A freshly prepared solution of iodine (177 mg, 0.7 mmol) in 19:1 pyridine–water (10 mL) was then added and stirring of the reaction mixture was continued for 45 min. After the addition of $CHCl_3$ (25 mL), the organic solution was washed successively with 5% aq $NaHSO_3$ (25 mL), water (25 mL), and 1 M aq TEAB buffer (3 × 15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. RBC of the residue (elution first with $CHCl_3$ and then with 25:1 $CHCl_3$–MeOH) afforded the TEA phosphate derivative **29** (150 mg, 80%); $[\alpha]_D$ +51° (*c* 1.1, $CHCl_3$); R_f 0.45 (solvent C); δ_H 0.86 (t, 6 H, *J* 7.0, 2 × CH_2*Me*), ~1.24 (29 H, 3 × CH_2*Me*, 2 × $[CH_2]_5$), 1.50 (m, 4 H, 2 × OCH_2CH_2), 2.92 (m, 6 H, 3 × CH_2Me), 3.18 (dd, 1 H, $J_{1',2'}$ 2.2 and $J_{2',3'}$ 10.2, H-2′), 3.35 (m, 2 H, OCH_2CH_2), 3.40 (m, 2 H, H-6′a,b), 3.46 (t, 1 H, $J_{4,5} = J_{5,6}$ 9.0, H-5), 3.52 (2 H, m, OCH_2CH_2), 3.54 (m, 4 H, 2 × CH_2 glycerol), 3.62 (1 H, H-3), 3.71 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.3, H-4′), 3.96–4.14 (4 H, H-3′, 4, 5′, H-2 glycerol), 4.32 (1 H, H-6), 4.52 (1 H, H-1), 4.72 (1 H, H-2), 5.86 (d, 1 H, H-1′), 4.20–5.00 (14 H, 7 × CH_2Ph), 6.80–7.40 (35 H, 7 × Ph); δ_P −0.96 (with heteronuclear decoupling); ESMS(−): *m*/*z* 1374.1 $[M-NEt_3-H]^-$.

Sodium 1-D-*6*-O-*(2-amino-2-deoxy-α*-D-*glucopyranosyl)*-myo-*inositol 1-(1,2-di*-O-*octyl*-sn-*glycerol 3-phosphate)* (**31**).—The sodium salt **30** was obtained quantitatively by stirring a solution of the TEA salt **29** (73 mg, 0.049 mmol) in 1:1 THF–MeOH with Amberlite DP-1 (Na^+) resin for 3 h, followed by filtration and concentration of the filtrate under reduced pressure. A solution of the sodium salt **30** (68.5 mg, 0.049 mmol) in 4:4:1

THF–propan-1-ol–water (9 mL) containing 20% $Pd(OH)_2/C$ was shaken under a slight overpressure of hydrogen at rt for 24 h. It was then percolated through a short column containing Chelex 100 on a bed of Celite (elution with 10:10:3 $CHCl_3$–MeOH–water) and the eluent was concentrated under reduced pressure to give the di-O-octyl derivative **31** (35 mg, 96%); $[\alpha]_D$ +25° (*c* 1.1, 10:10:3 $CHCl_3$–MeOH–H_2O); R_f 0.72 (solvent D) and 0.60 (solvent E); δ_H (10:10:3 $CDCl_3$–CD_3OD–D_2O) 0.55 (t, 6 H, *J* 7.0, $2 \times CH_2Me$), ~0.94 (20 H, $2 \times [CH_2]_5$), 1.24 (m, 4 H, $2 \times OCH_2CH_2$), 2.85 (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.6, H-2′), 3.02 (dd, 1 H, $J_{2,3}$ 2.0, $J_{3,4}$ 9.8, H-3), 3.12 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.7, H-4′), 3.16 and 3.26 (m, 4 H, $2 \times OCH_2$), 3.32 (5 H, H-4, $2 \times CH_2$ glycerol), 3.40 (dd, 1 H, $J_{5',6'a}$ 4.9, $J_{6'a,6'b}$ 12, H-6′a), 3.50 (dd, 1 H, $J_{5',6'b}$ 2.1, H-6′b), 3.54 (dd, 1 H, $J_{3',4'}$ 9.7, H-3′), 3.60 (2 H, H-2, H-2 glycerol), 3.77 (2 H, H-5′, 6), 3.84 (dd, 1 H, $J_{1,2}$ 2.0, $J_{1,6}$ 9.5, H-1), 5.21 (d, 1 H, H-1′); δ_P 1.02 (with heteronuclear decoupling); ESMS(−): *m*/*z* 718.4 $[M-Na]^-$.

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