Parasite Glycoconjugates. Part 1. The Synthesis of Some Early and Related Intermediates in the Biosynthetic Pathway of Glycosyl-phosphatidylinositol Membrane Anchors

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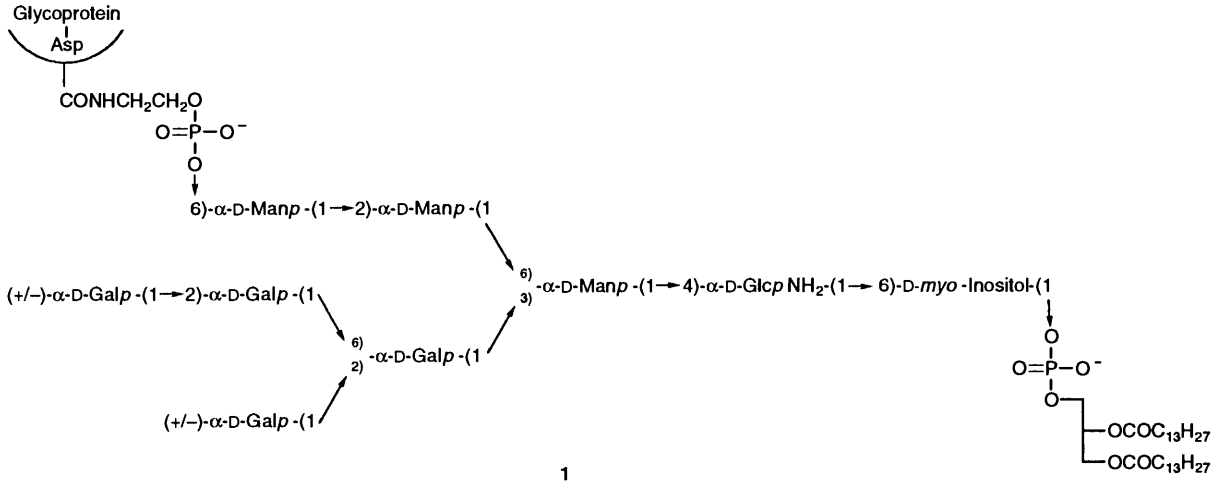
The enantio-pure 1D- and 1L-*myo*-inositol derivatives **3D** and **3L** have been used to prepare sodium 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol *sn*-2,3-dipalmitoyloxypropyl phosphate **21** and a related 1,6-disubstituted 1L-*myo*-inositol **28**, respectively. The hydrogenphosphonate approach was effective in coupling together the phosphonolipid moiety **16** and the protected 6-*O*-(2-azido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositols **15** *and* **24**, respectively.

Glycoconjugates on the cell surface of parasitic protozoa of the Trypanosomatidae (including, for example, African trypanosomes and *Leishmania spp.*) frequently have a crucial role in determining parasite survival and infectivity. It has become clear over the past five years that many glycoconjugates are attached to the plasma membrane by means of glycosylphosphatidylinositol (GPI) anchors.[1] Although this type of anchor is not confined to the protozoa, it does appear to be used with a much greater frequency in these organisms than in higher eukaryotes. The most fundamental function of GPI-membrane anchors is to provide a stable association of protein or oligosaccharide with the lipid bilayer.[1,2]

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**.[2] All the 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)-1D-*myo*-inositol backbone, suggesting that this sequence is likely to be conserved in all GPI anchors.[3] The tetrasaccharide backbone may be adorned, as in the *T. brucei* GPI anchor **1**, 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, and some mammalian and protozoan anchors have an additional fatty acid attached to the inositol ring.[4]

The biosynthesis of GPI-membrane anchors in bloodstream forms of *T. brucei* 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 de-*N*-acetylated to form α-D-Glc*p*NH_2-(1→6)-PI.[5] 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,[6] to which is added ethanolamine phosphate (from phosphatidylethanolamine) at the terminal α-D-Man*p* residue.[7] The resulting structure undergoes a complex series of fatty acid remodelling reactions[8] (to yield an *sn*-2,3-dimyristoyl-PI moiety) before the preassembled GPI precursor (known as glycolipid A) is transferred *en bloc* to newly synthesised protein.[9] Some α-D-galactosylation of the GPI anchor occurs in the endoplasmic reticulum[10] but mainly in the Golgi apparatus during transport to the surface membrane,[11] when as many as five α-D-Gal*p* residues may be added to the GPI anchors of *T. brucei* VSG.[3]

Although none of the enzymes involved in the biosynthesis of GPI-membrane anchors has been purified, their activities can be demonstrated using cell-free membrane preparations.[3] Such assays depend on the presence of endogenous GPI intermediates in the membrane. Since the GPI biosynthetic enzymes are themselves integral membrane proteins, their purification requires access to pure biosynthetic intermediates which can be added as exogenous acceptors to detergent-solubilised preparations. In this paper, we describe the synthesis of sodium 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol *sn*-2,3-dipalmitoyloxypropyl phosphate **21**, which will be used to develop *in vitro* assays for the α-D-Man*p*-(1→4)-α-D-



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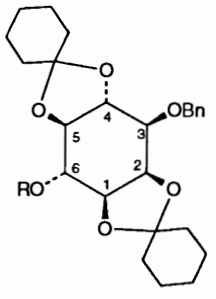
Glc*p*NH_2-(1→6)-PI α-D-mannosyltransferase and, after *N*-tritritioacetylation (→ **22**), for the D-Glc*p*NAc-PI de-*N*-acetylase. A positional isomer **28** of the glycosyl-phosphatidylinositol **21** was also synthesized for testing with these enzyme systems. Although with different perspectives in mind, the synthesis of similar and/or larger fragments of the GPI-membrane anchors of trypanosomes have been reported by other groups.[12,13]

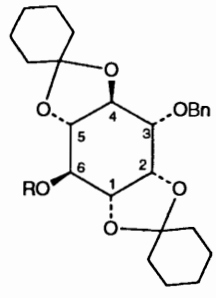
Results and Discussion

Our approach to the glycosyl-phosphatidylinositol **21** entailed coupling of the protected 1D-*myo*-inositol **10D** at 6-OH with a similarly protected 2-azidoglucosyl fluoride **13**, and, after demethoxybenzylation (→ **15**), at 1-OH with the phosphonolipid moiety **16** using the hydrogenphosphonate procedure.[14] After generation of the phosphoric diester **18**, complete deprotection and conversion of the 2′-azido group into a 2′-amino group could be accomplished in a single operation.

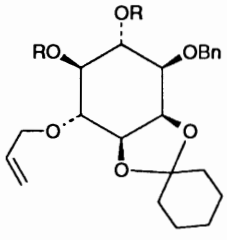
A synthesis of the differentially protected 1D-*myo*-inositol **10D** was pursued from enantio-pure 1D-3-*O*-benzyl-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol **3D**, which was derived from the (*S*)-camphanate **2D** using the literature procedure.[15,16] It is incumbent on us to point out that the structures originally assigned[15,16] to the diastereoisomeric camphamates **2D** and **2L** have been revised in a recent corrigendum.[17] The more polar diastereoisomer obtained directly by crystallisation is reassigned[17] the structure **2D**, whereas the less polar diastereoisomer recovered from the mother liquor has the structure **2L**.* An independent proof of the structure of the *myo*-inositol derivative **3D** is given below.



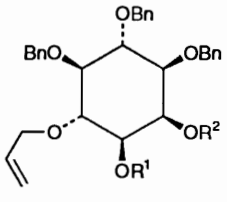
2D R = (*S*)-(−)-camphanoyl
3D R = H
4D R = $CH_2CH{=}CH_2$



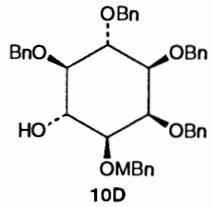
2L R = (*S*)-(−)-camphanoyl
3L R = H
4L R = $CH_2CH{=}CH_2$



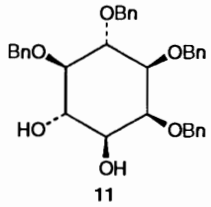
5D R = H
6D R = Bn



7D $R^1 = R^2 = H$
8D R^1 = MBn, R^2 = H
9D R^1 = MBn. R^2 = Bn
MBn = *p*-methoxybenzyl



10D



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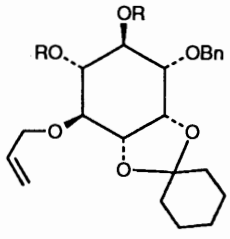
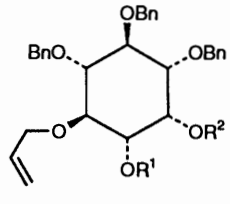
In proceeding with the synthesis, the *myo*-inositol derivative **3D** was transformed into the allylated compound **4D**, from which the less stable *trans*-fused 4,5-*O*-cyclohexylidene group was removed selectively by means of acetal exchange. Benzylation of the resulting diol **5D** gave the fully protected derivative **6D**, which was transformed into the *cis*-diol **7D** on acid-catalysed methanolysis. Regioselective 4-methoxybenzylation of the equatorial 1-OH group of the *cis*-diol **7D** was accomplished by way of the 1,2-*O*-dibutylstannylene derivative to give the axial alcohol **8D**, and, after benzylation, the differentially protected *myo*-inositol **9D**. Deallylation of the latter compound was achieved conventionally[19] in two steps by way of the corresponding propenyl derivative to furnish 1D-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol **10D**.

The absolute configuration of the *myo*-inositol derivative **10D** and its antecedents was readily confirmed by removal of the 4-methoxybenzyl group with ammonium cerium(IV) nitrate in aq. acetonitrile[20] to yield 1D-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol **11**, having physical constants indistinguishable from those of the authentic D-enantiomer prepared by an alternative route.[21]

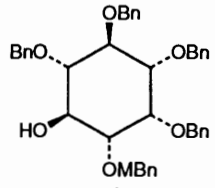
The 1L-*myo*-inositol derivative **3L**[16,17] was also taken through an identical sequence of reactions, *viz.* **3L** → **4L** → **5L** → **6L** → **7L** → **8L** → **9L** → 1L-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol **10L**.



5D R = H
6D R = Bn



7L $R^1 = R^2 = H$
8L R^1 = MBn, R^2 = H
9L R^1 = MBn. R^2 = Bn



10L

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose[22] **12** reacted with diethylaminosulfur trifluoride (DAST)[23] in ethylene dichloride at −30 °C to give the glycosyl fluoride **13** (α:β ratio 2:1), which was coupled to the *myo*-inositol derivative **10D** in the presence of zirconocene dichloride and silver perchlorate in anhydrous diethyl ether[24] to give, after oxidative removal[20] of the 4-methoxybenzyl group from the intermediate product **14**, the α-coupled compound **15** in 34% yield over the two steps. The α-configuration of the anomeric linkage of the pseudo-disaccharide **15** was assigned from the ^{1}H NMR spectrum, which contained a signal for 1′-H as a doublet at δ_H 5.44 with $J_{1',2'}$ 3.5 Hz.

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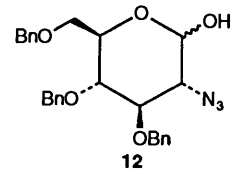
* We found the D and L convention adopted for the *myo*-inositol derivatives in Scheme 2 of ref. 16 to be misleading and chose to ignore it, relying on the information given in the corrigendum[17] to fix structures.

An error has been noted[18] in the sign of the specific rotation of 1D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol reported in ref. 15. Regrettably, this error seems to have escaped notice in a recent synthesis[12] of a part structure of the GPI anchor of *T. brucei*.

The phospholipid moiety was introduced by reaction of 1-OH of the benzylated pseudo-disaccharide **15** with *sn*-2,3-dipalmitoyloxypropyl hydrogen hydrogenphosphonate[25] **16** in the presence of pivaloyl chloride in pyridine[25,26] to give the phosphonic diester **17** as a mixture of diastereoisomers. Oxidation of the phosphonic diester **17** with iodine in pyridine–water[14] afforded the phosphoric diester **18**, which was transformed into the triethylammonium (TEA) phosphate derivative **19** for the purpose of the characterisation and, thereafter, into the sodium phosphate derivative **20**. The structure of the latter compound was supported by the presence of a peak at *m/z* 1652 ($M^+ + Na + H$) in the positive-ion FAB mass spectrum. Hydrogenolysis of the phosphate **20** in the presence of 20% palladium hydroxide on charcoal provided the fully deprotected 2-amino-2-deoxy-D-glucosyl-phosphatidyl-inositol **21**, whose structure was confirmed by its ^1H NMR and FAB mass spectra. The *N*-tritritioacetyl derivative **22** proved to be a good substrate for a partially purified D-Glc*p*NAc-PI de-*N*-acetylase from *T. brucei*,[27] details of which will be published elsewhere in due course.

An analogous sequence of reactions was performed on the L-*myo*-inositol derivative **10L**, which, on coupling with the glycosyl fluoride **13**, furnished the pseudo-disaccharide derivative **23** and, thereafter, the crystalline 1-OH compound **24**, whose ^1H NMR spectrum revealed 1′-H as a doublet at δ_H 5.38 with $J_{1',2'}$ 3.7 Hz. The hydrogenphosphonate approach[14] was then used to obtain the phosphonic diester **25**, oxidation of which afforded the phosphoric diester **26** (characterised as the TEA phosphate derivative **27**). Finally, hydrogenolysis of the phosphoric diester **26** gave the 1,6-disubstituted L-*myo*-inositol derivarive **28** (characterised by its ^1H NMR and FAB mass spectra). The *N*-tritritioacetyl derivative **29** is currently undergoing biological evaluation with a view to exploring the substrate specificity of the enzyme system referred to above. In this context, compound **29** might be regarded as a 3,4-positional isomer of the 1,6-disubstituted D-*myo*-inositol **22** since the 1- and 6-position of *myo*-inositol itself (plane of symmetry between C-2 and C-5) are enantiotopic with the 3- and 4-position, respectively.



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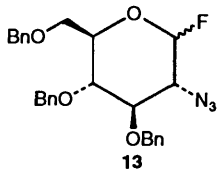
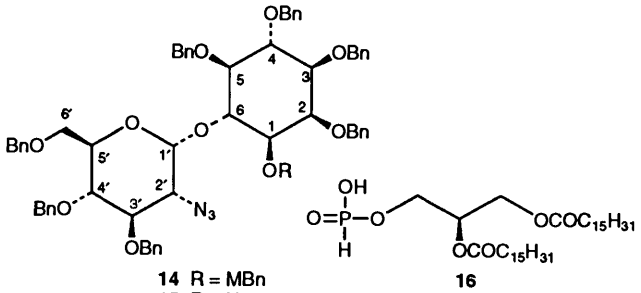
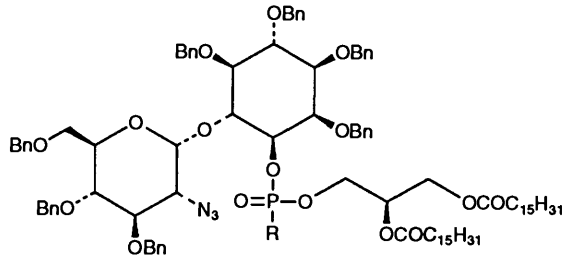


13

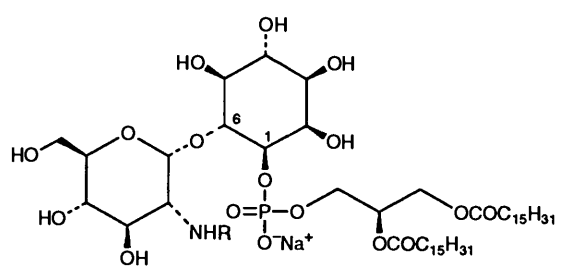


14 R = MBn
15 R = H

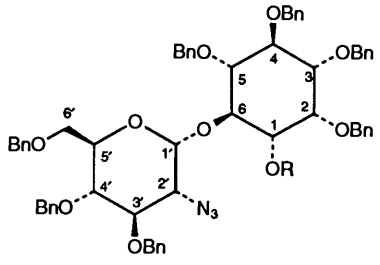
16



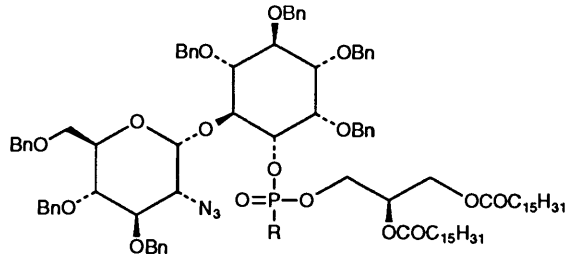
17 R = H
18 R = OH_4^+
19 R = O^-NHEt_3
20 R = O^-Na^+



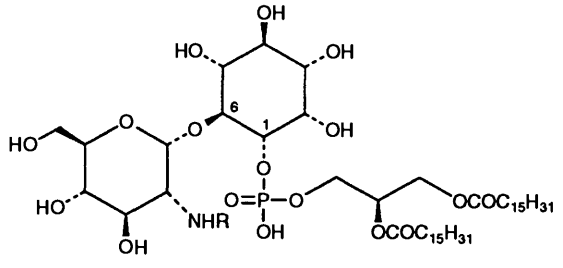
21 R = H
22 R = COC^3H_3



23 R = MBn
24 R = H



25 R = H
26 R = OH_4^+
27 R = O^-NHEt_3



28 R = H
29 R = COC^3H_3

Experimental

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). ^1H NMR spectra were recorded on Bruker AM 200 MHz or AC 500 MHz spectrometers usually using deuteriochloroform as the solvent and

tetramethylsilane as internal reference. *J* Values are given in Hz. M.p.s were determined on a Reichert hot-plate apparatus and are uncorrected. FAB mass spectra were measured in the positive ionisation mode with a VG 250/70 SE instrument, unless otherwise indicated; thioglycerol–glycerol was used as the liquid matrix. 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}. Light petroleum refers to the fraction having the boiling range 60–80 °C, unless indicated otherwise.

1D-6-O-*Allyl*-3-O-*benzyl*-1,2:4,5-*di*-O-*cyclohexylidene*-myo-*inositol* **4D**.—To a stirred solution of the enantio-pure compound **3D**[16,17] (3.02 g, 7.02 mmol) in *N*,*N*-dimethylformamide (DMF) (60 cm^3) at 0 °C were added sodium hydride (0.75 g, 31.25 mmol) and allyl bromide (0.75 cm^3, 8.7 mmol). The reaction mixture was stirred at room temperature for 1.5 h before methanol was added to decompose the excess of sodium hydride. The resulting solution was extracted with diethyl ether and the extract was washed successively with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. Crystallisation of the residue from ethanol gave the *allyl ether* **4D** (3.23 g, 99%), m.p. 95–96 °C; $[\alpha]_D$ −45 (*c* 1.1, $CHCl_3$) (Found: C, 71.2; H, 7.9. $C_{28}H_{38}O_6$ requires C, 71.5; H, 8.1%); δ_H 1.30–1.80 (20 H, m, 10 × CH_2), 3.27 (1 H, dd, $J_{4,5}$ 9.8, $J_{5,6}$ 10.9, 5-H), 3.63 (1 H, dd, $J_{1,6}$ 6.1, 6-H), 3.73 (1 H, dd, $J_{3,4}$ 10.9, 3-H), 3.98–4.05 (2 H, m, 1-, 4-H), ~4.30 (2 H, m, CH_2CH=CH$_2$), 4.33 (1 H, dd, $J_{1,2}$ 4.9, $J_{2,3}$ 3.6, 2-H), 4.85 (2 H, ABq, J_{AB} 11.0, PhCH_2), 5.18–5.33 (2 H, m, CH$_2$CH=CH_2), 5.90–5.98 (1 H, m, CH$_2$CH=CH$_2$) and 7.27–7.43 (5 H, m, Ph).

An identical procedure starting from the enantiomeric compound **3L**[16,17] gave 1L-6-O-*allyl*-3-O-*benzyl*-1,2:4,5-*di*-O-*cyclohexylidene*-myo-*inositol* **4L** (98%), m.p. 93–94 °C; $[\alpha]_D$ +45 (*c* 1.15, $CHCl_3$) (Found: C, 71.2; H, 8.4%).

1D-6-O-*Allyl*-3-O-*benzyl*-1,2-O-*cyclohexylidene*-myo-*inositol* **5D**.—A solution of the diketal **4D** (2.96 g, 6.3 mmol) in acetonitrile–chloroform (60 cm^3; 1:1) containing ethylene glycol (3.9 cm^3, 70 mmol) and toluene-*p*-sulfonic acid monohydrate (0.1 g) was stirred at room temperature for 30 min and then neutralised with triethylamine. The mixture was diluted with chloroform and the resulting solution was washed successively with aq. sodium hydrogen carbonate and water, dried ($MgSO_4$), and concentrated under reduced pressure. Crystallisation of the residue from acetone–hexane gave the trans-*diol* **5D** (1.83 g, 74%), m.p. 136–137 °C; $[\alpha]_D$ +20 (*c* 1.7, $CHCl_3$) (Found C, 67.8; H, 7.65. $C_{22}H_{30}O_6$ requires C, 67.7; H, 7.7%); δ_H 1.34–1.80 (10 H, m, 5 × CH_2), ~2.65 (2 H, m, 4-, 5-OH), 3.33 (1 H, ddd, $J_{5,6}$ 9.8, 5-H), 3.46 (1 H, dd, $J_{1,6}$ 7.3, 6-H), 3.51 (1 H, dd, $J_{3,4}$ 9.8, 3-H), 3.94 (1 H, ddd, $J_{4,5}$ 9.8, 4-H), 4.01 (1 H, dd, $J_{1,2}$ 4.9, 1-H), ~4.17 and 4.41 (2 H, m, CH_2CH=CH$_2$), 4.31 (1 H, dd, $J_{2,3}$ 4.9, 2-H), 4.76 (2 H, ABq, J_{AB} 11.0, PhCH_2), 5.17–5.30 (2 H, m, CH$_2$CH=CH_2), 5.90–5.98 (1 H, m, CH$_2$CH=CH$_2$) and 7.29–7.40 (5 H, m, Ph).

Flash-column chromatography [ethyl acetate–light petroleum (1:1)] afforded a further quantity (0.1 g, total yield 78%) of the *trans*-diol **5D**.

An identical procedure starting from the diketal **4L** gave 1L-6-O-*allyl*-3-O-*benzyl*-1,2-O-*cyclohexylidene*-myo-*inositol* **5L** (83%), m.p. 134–135 °C; $[\alpha]_D$ −19 (*c* 1.1, $CHCl_3$) (Found: C, 68.1; H, 7.5%).

1D-6-O-*Allyl*-3,4,5-*tri*-O-*benzyl*-myo-*inositol* **7D**.—To a stirred and cooled (0 °C) mixture of the diol **5D** (0.2 g, 0.51 mmol) and sodium hydride (0.1 g, 4.2 mmol) in DMF (5 cm^3) was gradually added benzyl bromide (170 mm^3, 1.43 mmol). The mixture was stirred at room temperature overnight, methanol was then added to decompose the excess of sodium hydride, and the resulting solution was extracted with diethyl ether. The extract was washed successively with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [cyclohexane–diethyl ether (1:1)] of the residue gave the benzylated compound **6D** (0.27 g), which was dissolved in anhydrous methanol (9 cm^3), and to the cooled (0 °C) methanolic solution was gradually added acetyl chloride (130 mm^3). The reaction mixture was set aside at room temperature overnight and then concentrated under reduced pressure. Flash-column chromatography [ethyl acetate–light petroleum(1:2)] of the residue gave the cis-*diol* **7D** (0.215 g, 86%), m.p. 119–120 °C (from acetone–hexane); $[\alpha]_D$ −35 (*c* 1.5, $CHCl_3$) (Found: C, 73.25; H, 6.7. $C_{30}H_{34}O_6$ requires C, 73.4; H, 7.0%); δ_H 2.47 (2 H, br s, 1-, 2-OH), 3.39–3.46 (3 H, m, 1-, 3-, 5-H), 3.70 (1 H, t, $J_{1,6} = J_{5,6}$ = 9.8, 6-H), 3.92 (1 H, t, $J_{3,4} = J_{4,5}$ = 8.5, 4-H), 4.22 (1 H, t, $J_{1,2} = J_{2,3}$ = 2.5, 2-H), ~4.25 and 4.41 (2 H, m, CH_2CH=CH$_2$), 4.68–4.90 (6 H, m, 3 × CH_2Ph), 5.16–5.28 (2 H, m, CH$_2$CH=CH_2), 5.91–5.99 (1 H, m, CH$_2$CH=CH$_2$) and 7.27–7.36 (15 H, m, 3 × Ph).

An identical procedure starting from the compound **5L** gave 1L-6-O-*allyl*-3,4,5-*tri*-O-*benzyl*-myo-*inositol* **7L** (76%), m.p. 119–120 °C; $[\alpha]_D$ +37 (*c* 1.1, $CHCl_3$) (Found: C, 73.4; H, 7.15%).

1D-6-O-*Allyl*-3,4,5-*tri*-O-*benzyl*-1-O-(4-*methoxybenzyl*)-myo-*inositol* **8D**.—A mixture of the *cis*-diol **7D** (2.6 g, 5.3 mmol) and dibutyltin(IV) oxide (1.87 g, 7.5 mmol) in anhydrous methanol (140 cm^3) was boiled under reflux for 2 h, cooled, and concentrated under reduced pressure. Toluene was added to, and distilled from, the residue twice. A mixture of the residue, caesium fluoride (1.12 g, 7.7 mmol), potassium iodide (1.17 g, 7.05 mmol) and 4-methoxybenzyl chloride (1 cm^3, 7.4 mmol) in DMF (150 cm^3) was stirred under nitrogen at room temperature overnight and then concentrated under reduced pressure. Flash-column chromatography [cyclohexane–diethyl ether (1:1)] of the residue gave the *methoxybenzyl derivative* **8D** (2.19 g, 72%), m.p. 105–106 °C (from diethyl ether–light petroleum); $[\alpha]_D$ −10 (*c* 1.2, $CHCl_3$) (Found: C, 74.9; H, 6.8. $C_{38}H_{42}O_7$ requires C, 74.7; H, 6.9%); δ_H 2.40 (1 H, s, 2-OH), 3.30 (1 H, dd, *J* 2.4 and 9.5, 1- or 3-H), 3.35 (1 H, dd, *J* 2.4 and 9.5, 3- or 1-H), 3.38 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.5, 5-H), 3.81 (3 H, s, OMe), 3.81 (1 H, dd, 4- or 6-H), 3.94 (1 H, dd, 6- or 4-H), 4.15 (1 H, t, 2-H), ~4.35 (2 H, m, CH_2CH=CH$_2$), 4.56–4.89 (8 H, m, 4 × CH_2Ph), 5.14–5.29 (2 H, m, CH$_2$CH=CH_2), 5.94–6.01 (1 H, m, CH$_2$CH=CH$_2$), and 6.86–6.89 and 7.26–7.35 (2 H and 17 H, 2 m, C_6H_4 and 3 × Ph).

An identical procedure starting from the *cis*-diol **7L** afforded 1L-6-O-*allyl*-3,4,5-*tri*-O-*benzyl*-1-O-(4-*methoxybenzyl*)-myo-*inositol* **8L** (77%), m.p. 104–105 °C; $[\alpha]_D$ +10 (*c* 1.3, $CHCl_3$) (Found: C, 75.3; H, 7.05%).

1D-2,3,4,5-*Tetra*-O-*benzyl*-1-O-(4-*methoxybenzyl*)-myo-*inositol* **10D**.—Benzyl bromide (0.65 cm^3, 5.46 mmol) was added dropwise to a stirred and cooled (0 °C) mixture of compound **8D** (2.1 g, 3.44 mmol) and sodium hydride (0.35 g, 14.6 mmol) in DMF (40 cm^3), after which the mixture was stirred at room temperature for 2 h. Methanol was added to decompose the excess of sodium hydride and the resulting solution was diluted with diethyl ether, washed successively with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The crude tetrabenzyl derivative **9D** (2.79 g) was dissolved in anhydrous dimethyl sulfoxide (60 cm^3) containing potassium *tert*-butoxide (3.9 g, 34.75 mmol) and the mixture was heated at 60 °C and stirred for 1.5 h, cooled, and poured into ice–water. The aqueous phase was extracted with ethyl acetate, and the extract was washed successively with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure.

A solution of the resulting propenyl derivative in 1 mol dm^{-3} hydrochloric acid–acetone (90 cm^3; 1:9) was boiled under reflux

for 10 min and then the solvents were removed. Flash-column chromatography [cyclohexane–diethyl ether (3:1)] of the residue furnished the *alcohol* **10D** (1.63 g, 72%), m.p. 77–78 °C [from diethyl ether–light petroleum (boiling range 40–60 °C)]; $[\alpha]_D$ −9 (*c* 1, $CHCl_3$) {lit.,[12] (L-enantiomer) m.p. 77–77.5 °C; $[\alpha]_D$ +8.8 (*c* 2.59, $CHCl_3$) see below and footnote}.*

An identical procedure starting from the compound **8L** gave 1L-2,3,4,5-*tetra*-*O*-*benzyl*-1-*O*-(4-*methoxybenzyl*)-myo-*inositol* **10L** (91%), m.p. 76–77 °C; $[\alpha]_D$ +8 (*c* 1.5, $CHCl_3$) {lit.,[12] m.p. 77–77.5 °C; $[\alpha]_D$ +8.8 (*c* 2.59, $CHCl_3$)}.*

1D-2,3,4,5-*Tetra*-O-*benzyl*-myo-*inositol* **11**.—A solution of the methoxybenzyl derivative **10D** (0.03 g, 0.045 mmol) in acetonitrile–water (4.4 cm^3; 9:1) containing ammonium cerium(IV) nitrate (0.1 g, 0.18 mmol) was kept at 0 °C for 5 min and at room temperature for 45 min and was then diluted with chloroform. The resulting solution was washed with aq. sodium hydrogen carbonate, dried ($MgSO_4$), and concentrated under reduced pressure. Crystallisation of the residue from acetone–hexane gave the *tetrabenzylated derivative* **11** (0.015 g, 61%), m.p. 150–152 °C; $[\alpha]_D$ +14 (*c* 1.5, $CHCl_3$) {lit.,[21] m.p. 153–154 °C; $[\alpha]_D$ +14 (*c* 1, $CHCl_3$)}.

2-*Azido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-D-*glucopyranosyl Fluoride* **13**.—DAST (350 mm^3, 2.63 mmol) was added dropwise to a stirred solution of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose[22] **12** (0.3 g, 0.63 mmol) in ethylene dichloride (5 cm^3) at −30 °C, and the reaction mixture was allowed to attain room temperature over 30 min. Ice–water was then added carefully, followed by chloroform, and the organic layer was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [cyclohexane–diethyl ether (4:1)] of the residue gave the *glycosyl fluoride* **13** (0.3 g, 99%) as a syrup containing the α and β anomers in the ratio 1:2 (Found: C, 67.8; H, 5.95; N, 8.95. $C_{27}H_{28}FN_3O_4$ requires C, 67.9; H, 5.9; N, 8.8%); δ_H 5.04 (dd, $J_{1,2}$ 6.1, $J_{1,F}$ 52.5, 1-H β-anomer) and 5.66 (dd, $J_{1,2}$ 2.5, $J_{1,F}$ 53.7, 1-H α-anomer).

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* **15**.—To a stirred solution of the *myo*-inositol derivative **10D** (0.28 g, 0.42 mmol) and the glycosyl fluoride **13** (0.28 g, 0.59 mmol) in anhydrous diethyl ether (10 cm^3) at 0 °C were added powdered 3 Å molecular sieves (0.98 g) and zirconocene dichloride (0.67 g, 2.3 mmol), followed by predried silver perchlorate (0.47 g) suspended in anhydrous diethyl ether (12 cm^3), and then tetramethylurea (60 mm^3, 0.52 mmol). The mixture was allowed to attain room temperature and was stirred vigorously under nitrogen overnight. It was then percolated through a short column of silica gel with diethyl ether as the eluent and concentrated under reduced pressure.

A solution of the residue (containing compound **14** and the corresponding β-anomer) in acetonitrile–water (20 cm^3; 9:1) containing ammonium cerium(IV) nitrate (0.5 g, 0.9 mmol) was stirred at 0 °C for 1 h and was then diluted with diethyl ether. The resulting solution was washed successively with saturated aq. sodium hydrogen carbonate and brine, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [toluene–butanone (50:1)] gave the *α-coupled compound* **15** (0.145 g, 34%), $[\alpha]_D$ +44 (*c* 1.1, $CHCl_3$) (Found: C, 73.3; H, 6.3; N, 4.1. $C_{61}H_{63}N_3O_{10}$ requires C, 73.4; H, 6.3; N, 4.2%); δ_H 3.03 (1 H, dd, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ 11.1, 6'-H[a]), 3.22 (1 H, dd, $J_{5',6'b}$ 2.5, 6'-H[b]), 3.25 (1 H, d, 1-OH), 3.37 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.5, 5-H), 3.44 (1 H, dd, $J_{2,3}$ 2.5, $J_{3,4}$ 4.4, 3-H), 3.50 (1 H, dd, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10.2, 2'-H), 3.61 (1 H, m, 1-H), 3.72 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.6, 4'-H), 3.90 (1 H, ddd, 5'-H), 3.96 (1 H, t, 3'-H), 3.98 (1 H, t, $J_{1,6}$ 9.5, 6-H), 4.00 (1 H, t, 2-H), 4.10 (1 H, t, 4-H), 4.11–5.10 (14 H, 7 × ABq, 7 × CH_2Ph), 5.44 (1 H, d, $J_{1',2'}$ 3.5, 1'-H) and 7.01–7.40 (35 H, m, 7 × Ph). The ^{1}H NMR spectrum of compound **15** was indistinguishable from that of the same compound prepared by another route.[28]

Sodium 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* sn-2,3-*Dipalmitoyloxypropyl Phosphate* **20**.—A mixture of compound **15** (80 mg, 80 μmol), *sn*-2,3-dipalmitoyloxypropyl hydrogen hydrogenphosphonate[25] **16** (80 mg, 126 μmol), and pivaloyl chloride (60 mm^3, 0.5 mmol) in anhydrous pyridine (2 cm^3) was stirred at room temperature for 15 min to form a mixture of the diastereoisomeric hydrogenphosphonates **17**. Oxidation of the hydrogenphosphonates **17** was accomplished by the addition of 0.1 mol dm^{-3} iodine in 2% aq. pyridine (2.6 cm^3) to the mixture, which was then stirred at room temperature for 20 min and diluted with diethyl ether. The resulting 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 **18**, which was taken up in diethyl ether and the ethereal solution was washed with 1 mol dm^{-3} aq. triethylammonium hydrogen carbonate and concentrated under reduced pressure to give the TEA phosphate derivative **19** (0.132 g, 95%); δ_H 0.87 (6 H, t, *J* 7.3, 2 × CH_2*Me*), 1.21 (9 H, t, 3 × CH_2*Me*), 1.25 (48 H, m, 2 × $[CH_2]_{12}$), 1.55 (4 H, br t, 2 × $COCH_2CH_2$), 2.24 and 2.25 (4 H, 2 t, 2 × $COCH_2$), 2.92 (6 H, q, 3 × CH_2Me), 3.18 (1 H, dd, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.3, 2'-H), 3.39 (2 H, m, 6'-H_2), 3.47 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.3, 5-H), 3.56 (1 H, dd, $J_{2,3}$ 2.0, $J_{3,4}$ 9.8, 3-H), 3.71 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.5, 4'-H), 4.02 (1 H, t, 3'-H), 4.10 and 4.37 (4 H, 2 m, 2 × CH_2 propyl), 4.12 (1 H, ddd, 5'-H), 4.28 (1 H, m, $J_{1,2}$ 2.1, $J_{1,6}$ 9.3, 1-H), 4.35 (1 H, t, 6-H), 4.77 (1 H, t, 2-H), 5.26 (1 H, m, 2-H propyl), 5.88 (1 H, d, 1'-H) and 6.98–7.42 (35 H, m, 7 × Ph); $\delta_P(CDCl_3)$ −0.57 (with ^{1}H heteronuclear decoupling).

The sodium phosphate derivative **20**, $[\alpha]_D$ +50 (*c* 0.9, $CHCl_3$), was obtained quantitatively on stirring of the TEA phosphate derivative **19** in diethyl ether–methanol (1:1) with Amberlite DP1 (Na^+) resin for 3 h, filtration, and concentration of the filtrate under reduced pressure; FAB mass spectrum *m/z* 1652 (M^+ + Na + H).

Sodium 1D-6-O-(2-*Amino*-2-*deoxy*-α-D-*glucopyranosyl*)-myo-*inositol* sn-2,3-*Dipalmitoyloxypropyl Phosphate* **21**.—A solution of the sodium phosphate derivative **20** (54 mg, 33 μmol) in chloroform–methanol–water (2.8 cm^3; 10:7:2) containing 20% palladium hydroxide on carbon (110 mg) was shaken under a slight overpressure of hydrogen at room temperature for 3 h and was then percolated through a short column packed with a layer of Celite on top of silica gel, with washing with methanol–tetrahydrofuran (THF) (1:2). The filtrate and washings were combined, and concentrated under reduced pressure. The residue was subjected to semi-preparative HPLC on Kromasil 100 [250 × 10 mm, 5 μm, using chloroform–methanol–water (10:10:3)] to give the *glycosyl-phosphatidylinositol* **21** (16 mg, 49%), $[\alpha]_D$ +43 [*c* 0.75, MeOH–THF (1:1)]; δ_H[$(CD_3)_2$SO] 0.83 (6 H, t, *J* 7.3, 2 × CH_2*Me*), 1.23 (48 H, m, 2 × $[CH_2]_{12}$), 1.49 (4 H, m, 2 × $COCH_2CH_2$), 2.25 and 2.27 (4 H, 2 t, 2 × $COCH_2$), 2.82 (1 H, dd, 2'-H), 3.10 (2 H, m, 3-, 5-H), 3.22 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.7, 4'-H), 3.43 (1 H, t, 4-H), 3.51 (1 H, dd, $J_{5',6'a}$ 3.9, $J_{6'a,6'b}$ 11.7, 6'-H[a]), 3.58 (1 H, dd, $J_{5',6'b}$ 1.5, 6'-H[b]), 3.63 (1 H, t, $J_{2',3'}$ 9.7, 3'-H), 3.75 (1 H, t, $J_{1,6} = J_{5,6}$ =

* In the light of the next experiment and other evidence,[18] this compound should be assigned the 1L configuration rather than the 1D configuration assigned originally.[12]

9.3, 6-H), 3.80 (1 H, t, 2-H), 3.81 (1 H, m, 3-H^a propyl*), 3.84 (1 H, m, 3-H^b propyl†), 3.98 (1 H, m, 1-H), 3.99 (1 H, ddd, 5'-H), 4.11 (1 H, m, 1-H^a propyl*), 4.28 (1 H, dd, $J_{2,1b}$ 2.0, $J_{1a,1b}$ 12.2, 1-H^b propyl†), 5.10 (1 H, m, 2-H propyl) and 5.40 (1 H, d, $J_{1',2'}$ 3.4, 1'-H); δ_P[$(CD_3)_2SO$] 5.46 (with ^{1}H heteronuclear decoupling). The FAB mass spectrum of compound **21** exhibited peaks, *inter alia*, at *m/z* 970.5873 corresponding to $C_{47}H_{89}NO_{17}P$ ($M^+ - H$) (requires *m/z* 970.5868) and *m/z* 972.6048 corresponding to $C_{47}H_{91}NO_{17}P$ ($M^+ + H$) (requires *m/z* 972.6024).

1L-6-O-(2-*Azido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-α-D-*glucopyranosyl*)-2,3,4,5-*tetra*-O-*benzyl*-myo-*inositol* **24**.—To a stirred solution of the *myo*-inositol derivative **10L** (0.54 g, 0.82 mmol) and the glycosyl fluoride **13** (0.63 g, 1.32 mmol) in anhydrous diethyl ether (40 cm^3) at 0 °C were added powdered 3Å molecular sieves (2 g), zirconocene dichloride (1.56 g, 5.34 mmol), and silver perchlorate hydrate (1.1 g). The mixture was allowed to attain room temperature and was then stirred overnight. After neutralisation with triethylamine, solids were removed by filtration through Celite and the filtrate was washed successively with aq. sodium hydrogen carbonate and brine, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography [cyclohexane–diethyl ether (4:1)] furnished the coupled compounds (0.64 g, 70%) as an unresolved mixture of the α-anomer **23** and the corresponding β-anomer in the ratio ~3:1 (determined by ^{1}H NMR spectroscopy).

A solution of these compounds (0.575 g, 0.51 mmol) and ammonium cerium(IV) nitrate (1.5 g, 2.7 mmol) in acetonitrile–water (66 cm^3; 10:1) was stirred at 0 °C for 1 h and then processed as described for the 1D-*myo*-inositol derivative **15**. Crystallisation of the residue from diethyl ether gave the α-*compound* **24** (0.21 g, 41%), m.p. 150–151 °C; $[\alpha]_D$ +37 (*c* 1.1, $CHCl_3$) (Found: C, 73.6; H, 6.2; N, 4.5. $C_{61}H_{63}N_3O_{10}$ requires C, 73.8; H, 6.3; N, 4.2%); δ_H(*inter alia*) 2.79 (1 H, br d, 1-OH), 3.34 (1 H, dd, $J_{2',3'}$ 9.8, 2'-H), 5.38 (1 H, d, $J_{1',2'}$ 3.7, 1'-H) and 7.12–7.35 (35 H, m, 7 × Ph).

1L-6-O-(2-*Azido*-3,4,6-*tri*-O-*benzyl*-2-*deoxy*-α-D-*glucopyranosyl*)-2,3,4,5-*tetra*-O-*benzyl*-myo-*inositol* sn-2,3-*Dipalmitoyloxypropyl Hydrogen Phosphate* **26**.—A mixture of compound **24** (0.1 g, 0.1 mmol), *sn*-2,3-dipalmitoyloxypropyl hydrogen hydrogenphosphonate[25] **16** (0.126 g, 0.2 mmol), and pivaloyl chloride (75 mm^3, 0.61 mmol) in anhydrous pyridine (2.5 cm^3) was stirred at room temperature overnight; TLC [toluene–ethyl acetate (9:1)] then revealed complete conversion into the phosphonic diester **25**, which was obtained as a mixture of diastereoisomers (R_f 0.35 and 0.4).

To the foregoing solution was added a solution of 0.1 mol dm^{-3} iodine in 2% aq. pyridine (2 cm^3), and the mixture was stirred at room temperature for 20 min before being processed as described for the 1D isomer **18**. Flash-column chromatography [first with chloroform and then with chloroform–ethanol (19:1)] gave the *phosphoric diester* **26** (0.152 g, 93%), $[\alpha]_D$ +18.5 (*c* 4, $CHCl_3$) as an oil (Found: C, 70.35; H, 8.3; N, 2.5. $C_{96}H_{130}N_3O_{17}P$ requires C, 70.8; H, 8.1; N, 2.6%); δ_P($CDCl_3$) −0.85 (with ^{1}H heteronuclear decoupling).

For the purpose of characterisation, a portion of the phosphoric diester **26** was converted quantitatively into the TEA phosphate derivative **27** by washing of an ethereal solution with 1 mol dm^{-3} aq. triethylammonium hydrogen carbonate and concentration of the ethereal solution under reduced pressure; δ_H 0.88 (6 H, t, *J* 7.3, 2 × CH_2Me), 1.07 (9 H, t, 3 × CH_2Me), ~1.25 (48 H, m, 2 × $[CH_2]_{12}$), 1.52 (4 H, m, 2 × $COCH_2CH_2$), 2.16 and 2.21 (4 H, 2 t, 2 × $COCH_2$), 2.76 (6 H, q, 3 × CH_2Me), 3.29 (1 H, dd, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.5, 2'-H), 3.57 (1 H, dd, 3-H), 3.61 (1 H, t, $J_{4,5} = J_{5,6}$ = 9.5, 5-H), 3.68 (1 H, dd, $J_{5',6'a}$ 10.2, $J_{6'a,6'b}$ ~12, 6'-H^a), 3.82 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.8, 4'-H), 3.91 (1 H, dd, $J_{5',6'b}$ 13.5, 6'-H^b), 3.98 (1 H, t, 3'-H), ~4.03 and 4.28 (4 H, 2 m, 1-, 3-H$_2$ propyl), 4.08 (1 H, m, 1-H), 4.10 (1 H, t, 4-H), 4.35 (1 H, t, $J_{1,6}$ 9.8, 6-H), 4.59 (1 H, m, 5'-H), 4.76 (1 H, t, 2-H), 5.21 (1 H, m, 2-H propyl), 5.65 (1 H, d, 1'-H) and 7.15–7.48 (35 H, m, 7 × Ph).

1L-6-O-(2-*Amino*-2-*deoxy*-α-D-*glucopyranosyl*)-myo-*inositol* sn-2,3-*Dipalmitoyloxypropyl Hydrogen Phosphate* **28**.—A solution of the phosphoric diester **26** (52 mg, 32 μmol) in chloroform–methanol–water (2 cm^3; 10:7.5:2) containing 20% palladium hydroxide on carbon (100 mg) was shaken under a slight overpressure of hydrogen at room temperature for 4 h, and then filtered through Celite, and concentrated under reduced pressure. A solution of the residue in chloroform–methanol–water (10:10:3) was subjected to semi-preparative HPLC on Kromasil-100 (using conditions identical with those employed for the 1D isomer **21**) to give the *glycosylphosphatidylinositol* **28** (12 mg, 39%), $[\alpha]_D$ +21 (*c* 0.47, Me_2SO); FAB mass spectrum (negative ionisation mode): *m/z* 971.5930, corresponding to $C_{47}H_{90}NO_{17}P$ (M^-) (requires *m/z* 971.5946) and *m/z* 970.5903, corresponding to $C_{47}H_{89}NO_{17}P$ ($M^- - H$) (requires *m/z* 970.5868); δ_H[$(CD_3)_2SO$–D_2O (49:1)] (*inter alia*) 0.83 (6 H, t, *J* 6.8, 2 × CH_2Me), ~1.22 (48 H, m, 2 × $[CH_2]_{12}$), 1.49 (4 H, m, 2 × $COCH_2CH_2$), 2.23 (4 H, m, 2 × $COCH_2$), 2.88 (1 H, dd, $J_{1',2'}$ 3.3, $J_{2',3'}$ 9.8, 2'-H), 4.30 (1 H, dd, $J_{1a,2}$ 2.0, $J_{1a,1b}$ 11.7, 1-H^a propyl), 5.09 (1 H, m, 2-H propyl) and 5.39 (1 H, d, $J_{1',2'}$ 3.0, 1'-H); δ_P[$(CD_3)_2SO$–D_2O (49:1)] 3.40 (with ^{1}H heteronuclear decoupling).

*,† These assignments may be interchanged.

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