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SYNTHESIS OF 1-*O*-(1,2-DI-*O*-PALMITOYL-*SN*-GLYCERO-3-PHOSPHORYL)-2-*O*-α-*D*-MANNOPYRANOSYL-*D*-*MYO*-INOSITOL: A FRAGMENT OF MYCOBACTERIAL PHOSPHOLIPIDS

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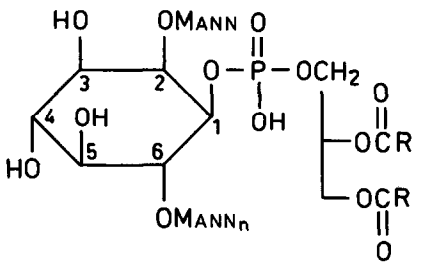
Abstract. Optically active and partially benzylated 1-*O*-(2-*O*-α-*D*-mannopyranosyl)-*D*-*myo*-inositol was coupled, *via* a trivalent phosphorus method, with 1,2-di-*O*-palmitoyl-*sn*-glycerol. Oxidation of the intermediate phosphite-triester, and subsequent removal of the *P*(V)- and *O*-benzyl protecting groups, afforded the chiral title compound.

Some years ago Anderson *et al.*[1] demonstrated for the first time that *myo*-inositol and *myo*-inositol dimannosides are part of Mycobacterium phospholipids. Later on, structure elucidation by Ballou *et al.*[2] showed that this mycobacterial phospholipid consisted of a trisubstituted *D*-*myo*-inositol (see structure I) of which OH-1 is esterified to phosphatidic acid, OH-2 α-linked to one *D*-mannopyranose unit and OH-6 which is similarly bound to a linear oligo-*D*-mannan (n=2-5) having solely α(1-2) or α(1-6) interglycosidic linkages.

As part of a programme[3] to prepare chiral pure and biologically active *myo*-inositol derivatives, we report the synthesis of 1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero-3-*O*-phosphoryl)-2-*O*-α-*D*-mannopyranosyl-*D*-*myo*-inositol (6c).

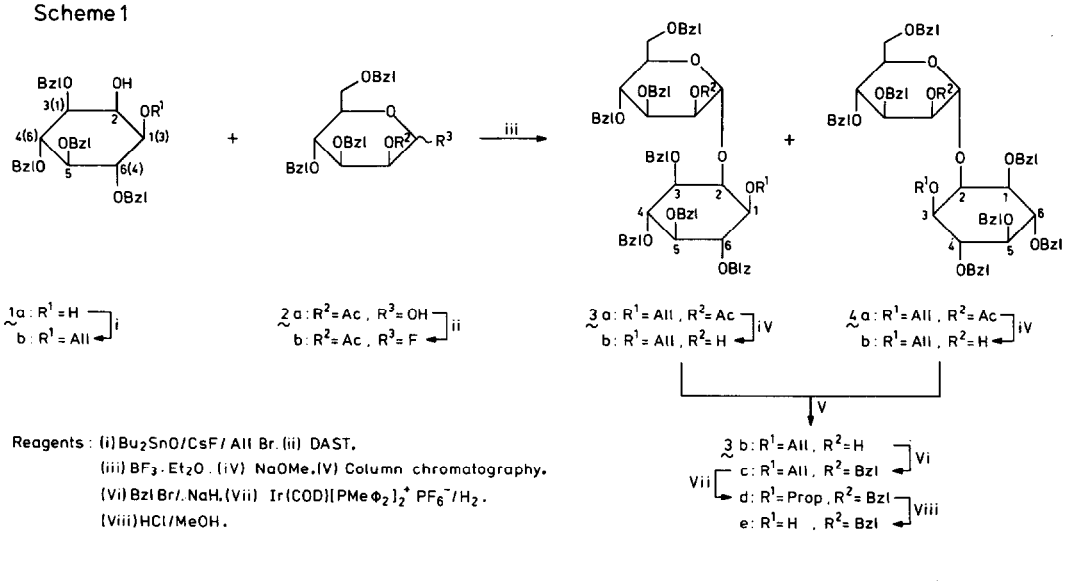


I MANN = α-D-mannopyranosyl; n=0-5

The strategy we followed for the assemblage of the target molecule 6c consisted of the following three stages. Synthesis (Scheme 1) of the chiral pure and benzyl-protected mani-nositose 3e having a free hydroxyl at C-1 of the *D*-*myo*-inositol moiety. Phosphorylation (Scheme 2) of 3e with the phosphitylated diglyceride 5 followed by oxidation of the intermediate phosphite-triester and, finally, a two-step deprotection of the thus obtained fully benzylated product.

In an earlier study Shvets *et al.*[4] showed that α-glycosylation of 1-*O*-propenyl-3,4,5,6-tetra-*O*-benzyl-*D*-*myo*-inositol (**1b**, R^1= -CH=CHCH$_3$) could be performed using 3,4,6-tri-*O*-acetyl-1,2-*O*-*t*-butylorthoacetyl-β-*D*-mannopyranose as the glycosyl donor. The recovery of the fully protected 2-*O*-mannosyl-*myo*-inositol was however not completely satisfactory. We found that effective and stereoselective glycosylation of the axially orientated OH-2 of racemic 1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**1b**) could be realized using 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α/β-*D*-mannopyranosyl fluoride (**2b**) as the glycosyl donor. The donor compound was easily accessible by fluorination[5] of the anomeric hydroxyl in **2a**[6] with (diethylamino)sulfur trifluoride. The pentasubstituted inositol acceptor **1b** was prepared by stannylidene-mediated[7] regioselective allylation of racemic **1a**[8] with allyl bromide in the presence of cesium fluoride[9]. Condensation of **1b** with **2b** using boron trifluoride etherate as the catalyst[10] afforded a mixture of the two diastereoisomers **3a** and **4a** in a total yield of 95%. Separation of **3a** and **4a** at this stage of the synthesis by column chromatography proved to be difficult due to the small difference in R_F-values. Zemplèn deacetylation of **3a** and **4a** gave the corresponding derivatives **3b** and **4b**, which could be separated conveniently by short column chromatography, to afford **3b** and its enantiomorph **4b** in a yield of 40% and 43%, respectively.

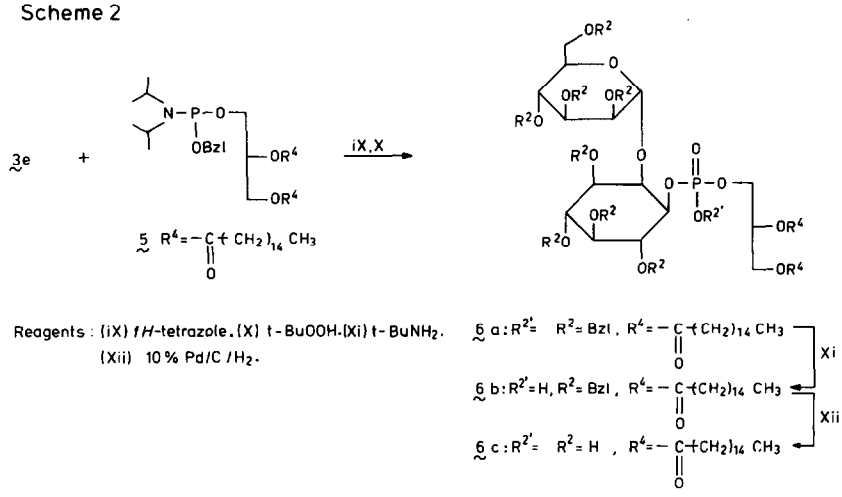
Scheme 1



Determination of the *D*- or *L*-configuration of the *myo*-inositol constituents in the individual stereoisomers **3b** and **4b** was corroborated by acidic hydrolysis[11] of the α-linkage in diastereoisomer **3b** and by measuring the specific optical rotation of the thus isolated *myo*-inositol (*i.e.* *D*- or *L*-**1b**). The outcome of this degradation purification procedure indicated that the pentasubstituted *myo*-inositol **1b** had the same $[\alpha]_D^{20}$-value as earlier recorded[4] for 1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*D*-*myo*-inositol. However, the relatively small negative

$[\alpha]_D^{20}$-value of *D*-1b urged us to subject also 3d to the same degradation procedure. In this particular case, acidic hydrolysis of the glycosidic linkage will be accompanied by concomitant removal of the *trans*-prop-1-enyl group to afford the expected tetrasubstituted *D-myo*-inositol 1a the absolute configuration of which has been firmly established[12]. Indeed the $[\alpha]_D^{20}$-value of 1a was in excellent agreement with that reported for 3,4,5,6-tetra-*O*-benzyl-*D-myo*-inositol: hence the maninositose 3b contains the required *D-myo*-inositol moiety. The conversion of 3b into 3d could be accomplished by a well-established two-step procedure. Thus benzylation of 3b gave 3c in an excellent yield. Isomerization of the allyl group in 3c by the H_2-activated catalyst 1,5-cyclooctadiene-*bis*[methyldiphenylphosphine]-iridium hexafluorophosphate[13] resulted in a nearly quantitative isolation of the *trans*-prop-1-enyl derivative 3d. Complete removal of the prop-1-enyl group could be effected by treatment with hydrogen chloride in methanol to give, after work-up, key-intermediate 3e in an excellent yield. The latter result is in sharp contrast with the acidic hydrolysis ($HCl-H_2O$-MeOH) conditions used before by Shvets *et al.*[4]. They observed that the acidic removal of the prop-1-enyl group (R^1) in 3a, in which the benzyl groups of the *D*-mannopyranosyl unit were replaced by acetyl groups, was not compatible with the α-glycosidic linkage: thus a considerable loss of the maninositol took place. The efficacy of the HCl-MeOH reagent was also recently illustrated[12c] in the removal of a cyclohexylidene group in the presence of *p*-methoxybenzyl ether functions.

Scheme 2



Reagents: (iX) 1*H*-tetrazole, (X) t-BuOOH, (Xi) t-$BuNH_2$, (Xii) 10% Pd/C / H_2.

6 a: $R^{2'}$= R^2= Bzl, R^4= -C(=O)-$(CH_2)_{14}$ CH_3 — Xi

6 b: $R^{2'}$=H, R^2= Bzl, R^4= -C(=O)-$(CH_2)_{14}$ CH_3 — Xii

6 c: $R^{2'}$= R^2= H, R^4= -C(=O)-$(CH_2)_{14}$ CH_3

The introduction of a phosphodiester bond between the secundary OH-1 of a properly protected myo-inositol unit in 3e was realized for the first time by Shvets *et al.*[15] *via* a phosphodiester approach: *i.e.* arenesulfonyl chloride mediated phosphorylation of 3e, in which the *D*-mannose moiety was protected by acetyl groups, with 1,2-di-*O*-palmitoyl-*sn*-glycer-3-yl-phosphate. On the other hand, Ward *et al.*[16] showed that the same goal could be

achieved by a phosphotriester methodology: *i.e.* arenesulfonyl chloride assisted coupling of a properly protected *D*-myo-inositol with 1,2-di-*O*-palmitoyl-*sn*-glycer-3-yl-phenylphosphate and subsequent hydrogenolysis of the *P*(V)-phenyl protecting group. In our case, we adopted a recently by us explored[3d] phosphite-triester method (see Scheme 2). Thus 1*H*-tetrazole assisted phosphitylation of 3e with 5, which was obtained in a high yield by reaction of 1,2-di-*O*-palmitoyl-*sn*-glycerol with benzyloxy-*bis*(*N*,*N*-diisopropylamino)phosphine, afforded a phosphite-triester intermediate. *In situ* oxidation of the latter with *t*-butyl hydroperoxide[17] gave, after purification by silica gel chromatography, homogeneous phosphotriester 6a in a yield of 80%. The removal of all benzyl-protecting groups from 6a was performed in two steps. First, the *P*(V)-benzyl ($R^{2'}$) group was removed by treating 6a with *t*-butylamine[18] to give phosphodiester 6b. The *O*-benzyl groups were then deblocked by hydrogenation over palladium on charcoal of 6b (sodium-salt) in *t*-butanol-water, to afford homogeneous mycobacterial phospholipid 6c as a solid. In this respect it is interesting to note that several attempts to remove the *P*(V)- and *O*-benzyl groups from 6a in a one-step procedure by catalytic transfer or palladium on charcoal hydrogenation did not result in the isolation of homogeneous 6c. The latter may be ascribed to an increase in rate[19] of the acidic hydrolysis of the α-mannosidic linkage in 6b(c) by the neighbouring phosphodiester function. The identity and homogeneity of 6c thus obtained was unambiguously ascertained by ^{31}P-, ^{13}C- and ^{1}H NMR spectroscopy.

In conclusion, we believe that the results described in this paper may be of great value for the future synthesis of other naturally occurring mycobacterial phospholipids (*e.g.* I, n = 1-5).

EXPERIMENTAL

Tetrahydrofuran, triethylamine, dichloromethane, *N*,*N*-dimethylformamide and 1,2-dichloroethane were dried by refluxing with calcium hydride (5 g per litre) for 16 h and distilled. Tetrahydrofuran was stored over molecular sieves 5Å and the other liquids over molecular sieves 4Å. Tetrahydrofuran and 1,2-dichloro-ethane were redistilled from lithium aluminium hydride (2 g per litre) before use. Methanol was dried by refluxing with magnesium methoxide, distilled and stored over molecular sieves 4Å. Toluene and ether were distilled from phosphorus pentoxide and stored over sodium wire. Benzyl alcohol was distilled under reduced pressure. Triethyl ammonium bicarbonate (TEAB) was prepared by passing a stream of carbon dioxide gas through a cooled (ice-water bath) solution of triethylamine in de-ionized water (2 molar) until a neutral solution was obtained. 1*H*-Tetrazole was purchased from Janssen Chimica. Schleicher and Schüll DC Fertigfolien F1500 LS254 were used for TLC analysis. The following eluents were used: System I (dichloromethane/ acetone, 97/3, v/v), System II (*n*-hexane/ether, 1/1, v/v), System III (*n*-hexane/ether, 1/2, v/v), System IV (dichloromethane/methanol, 9/1, v/v). Compounds were detected by charring with 20% sulfuric acid in methanol, or by spraying with 1% potassium permangenate in 5% aqueous potassium carbonate for compounds containing an double bond, or by spraying with a solution of ammonium molybdate (25 g) and ammonium cerium sulphate (10 g) in 10% aqueous sulphuric acid. Short column chromatography was performed on Kieselgel 60 (230-400 mesh ASTM, Merck), or on Sephadex LH-20 suspended in dichloromethane/methanol (2/1, v/v). ^{1}H NMR spectra were measured at 300 MHz, using a Bruker WM-300 spectrometer interfaced with an ASPECT-2000 computer, operating in the Fourier transform mode. ^{13}C NMR spectra were measured at 50.1 MHz, using a JEOL JNM-FX 200 spectrometer, equipped with an ASPECT-2000 computer, operating in the Fourier transform mode. Tetramethylsilane (TMS) was used as internal standard for samples in $CDCl_3$. Chemical shifts are given in ppm (δ) relative to TMS. ^{31}P NMR spectra were

measured at 80.7 MHz, proton-noise decoupled, using a JEOL JNM-FX 200 spectrometer, operating in the Fourier transform mode. Chemical shifts are given in ppm (δ), relative to 85% H_3PO_4 as external standard. Optical rotations were measured at 20°C using a Perkin Elmer 241 Polarimeter.

1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (1b)

A solution of **1a** (5.3 g , 9.8 mmol) and dibutyltin oxide (2.5 g , 10.0 mmol) in dry methanol (75 ml) was refluxed for 2.5 h and subsequently concentrated. The residue was coevaporated with toluene (3 x 50 ml). The resulting oil was dissolved in *N,N*-dimethylformamide (60 ml) to which was added cesium fluoride (2.0 g , 13.2 mmol) and allyl bromide (1.25 ml, 14.45 mmol). The reaction mixture was stirred for 16 h at 20°C, when TLC analysis (system I) indicated the formation of **1b** to be complete. The solution was concentrated *in vacuo* and the oily residue was taken up in diethyl ether (200 ml), washed with water (100 ml), aqueous sodium bicarbonate (100 ml, 10%, w/v), water (100 ml), dried ($MgSO_4$) and evaporated to dryness to give crude **1b** as an oil. Crystallization from diisopropyl ether/*n*-hexane afforded **1b** (4.7 g , 8.1 mmol); m.p. 68-70 °C; Rf 0.55 (system I); ^{1}H NMR ($CDCl_3$) δ 3.28 and 3.31 (dd 1 H, H-1, $J_{1,2}$ 2.70 Hz, $J_{1,6}$ 9.60 Hz), 3.39 and 3.42 (dd, 1 H, H-3, $J_{2,3}$ 2.73 Hz), 3.44 (t, 1 H, H-5, $J_{4,5}$ 9.47 Hz), 3.95 (t, 1 H, H-4, $J_{3,4}$ 9.55 Hz), 3.99 (t, 1 H, H-6, $J_{5,6}$ 9.50 Hz), 4.16 and 4.19 (dt(b), 2 H, OCH_2, allyl), 4.23 (t, 1 H, H-2), 4.73 and 4.91 (m, 8 H, 4 x CH_2, benzyl), 5.16-5.32 (m, 2 H, $=CH_2$, allyl), 5.88-5.98 (m, 1 H, -CH=, allyl), 7.04-7.42 (m, 20 H, $H_{arom.}$ 4 x benzyl); ^{13}C NMR ($CDCl_3$) δ 67.3, 79.4, 79.7, 80.9, 82.9, (C-1, C-2, C-3, C-4, C-5, C-6), 71.6 (CH_2, benzyl), 72.4 (OCH_2, allyl), 75.9 (3 x CH_2, benzyl), 117.2 ($=CH_2$, allyl), 127.3-128.3 (20 x $CH_{arom.}$ benzyl), 134.5 (-CH=, allyl), 137.8 ($C_{arom.}$ benzyl), 138.5 (3 x $C_{arom.}$ benzyl).

Anal. Calc. for $C_{37}H_{40}O_6$: C, 76.53; H, 6.94. Found : C, 76.52; H, 7.05.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranose (2a)

The title compound was obtained[6] by acidic hydrolysis of 3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-1,2-(2-methyl-ortho-acetate).

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranosyl fluoride (2b)

To a solution of **2a** (4.4 g, 8.94 mmol) in tetrahydrofuran (50 ml), under a blanket of nitrogen, was added at -30°C (diethylamino)sulfur trifluoride (1.31 ml, 10.73 mmol). After stirring for 1 h at 20°C, TLC analysis (system II) showed conversion of the starting material into **2b**. The mixture was cooled (-30°C) and excess DAST was quenched with methanol (0.6 ml) and concentrated *in vacuo*. The resulting oil was taken up in dichloromethane (150 ml), washed with potassium fluoride (1 M, 75 ml), water (75 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give an oil. Column chromatography (100 g silica gel, *n*-hexane/diethyl ether, 2:1, v/v, followed by *n*-hexane/diethyl ether, 1:1, v/v) of the crude product gave α-fluoride **2b** as an oil (3.05 g, 6.17 mmol) and the crystalline β-fluoride **2b** (0.5 g, 1.01 mmol).

α-Fluoride: Rf 0.62 (system II); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3 H, acetyl), 3.69-3.73 (m, 1 H), 3.80-3.83 (m, 1 H), 3.96 (s, 3 H), 4.47-4.87 (m, 8 H, 4 x CH_2, benzyl), 5.47 (t, 1 H, H-2, $J_{2,3}$ 2.14 Hz, $J_{2,F}$ 4.0 Hz), 5.33-5.70 (dd, 1 H, H-1, $J_{1,2}$ 2.02 Hz, $J_{1,F}$ 49.1 Hz), 7.10-7.45 (m, 15 H, $H_{arom.}$ 3 x benzyl); ^{13}C NMR ($CDCl_3$) δ 20.6 (CH_3, acetyl), 66.9 (d, C-2, $J_{2,F}$ 19.6 Hz), 73.1, 73.6, 77.0 (C-3, C-4, C-5), 68.0 (C-6), 71.9, 73.2 and 75.0 (3 x CH_2, benzyl), 105.3 (d, C-1, $J_{C-1,F}$ 219.8 Hz, $J_{H-1,C-1}$ 184.64 Hz), 127.5-128.2 (15 x $CH_{arom.}$ benzyl), 137.4, 137.8 and 138.0 (3 x $C_{arom.}$ benzyl), 169.7 ($C_{carb.}$ acetyl).

β-Fluoride: m.p. 75°C (diisopropyl ether/*n*-hexane); Rf 0.47 (system II); ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3 H, acetyl), 3.60-3.87 (m, 5 H, H-3, H-4, H-5, $H\text{-}6_{endo}$ and $H\text{-}6_{exo}$), 4.49-4.82 (m, 6 H, 3 x CH_2, benzyl), 5.31-5.47 (dd, 1 H, H-1, $J_{1,2}$ 1.54 Hz, $J_{1,F}$ 49.47 Hz), 7.16-7.34 (m, 15 H, $H_{arom.}$ 3 x benzyl); ^{13}C NMR ($CDCl_3$) δ 20.8 (CH_3, acetyl), 66.9 (d, C-2, $J_{2,F}$ 19.1 Hz), 73.4 (C-5), 75.0 (d, C-4, $J_{4,F}$ 4.4 Hz), 68.8 (C-6), 71.8, 73.4 and 74.5 (3 x CH_2, benzyl), 77.9 (d, C-3, $J_{3,F}$ 7.3 Hz), 104.7 (d, C-1, $J_{1,F}$ 218.3 Hz), 127.6-128.9 (15 x $CH_{arom.}$ benzyl), 137.2, 137.8 and 138.0 (3 x $C_{arom.}$ benzyl), 170.1 ($C_{carb.}$ acetyl).

Anal. Calc. for $C_{29}H_{31}O_6F$: C, 70.43; H, 6.32; F, 3.84. Found : C, 70.19; H, 6.54; F, 3.35.

1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-2-*O*-(2'-*O*-acetyl-3',4',6'-tri-*O*-benzyl-α-D-mannopyranosyl)-DL-*myo*-inositol (3a and 4a)

Compound **1b** (2.85 g, 5.77 mmol) and **2b** (2.23 g, 3.85 mmol) were dried by coevaporation with toluene (3 x 25 ml). Boron trifluoride etherate (1 M, 0.58 ml) was now added to a stirred

solution of 1b, 2b and activated molecular sieves 4Å (2.0 g) in dichloromethane (25 ml) under a blanket of nitrogen. TLC analysis (system II), after 1 h at 20°C, revealed the formation of a diastereomeric mixture (3a and 4a). The reaction was stopped with triethylamine (1 ml) and the mixture was diluted with dichloromethane (100 ml). The solids were removed by filtration and the clear filtrate was evaporated to dryness. The residual oil was applied to a column of Sephadex LH-20 (120 x 3 cm) which was eluted with dichloromethane/methanol (v/v, 2:1) to give a mixture of 3a and 4a as a colourless oil. Yield 3.9 g (3.67 mmol); Rf 0.36 (3a) and 0.32 (4a) (system II); ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3 H, CH_3, acetyl), 3.18-4.98 (m, 28 H, 7 x CH_2 benzyl, OCH_2 allyl, H-2', H-3', H-4', H-5', $H\text{-}6_{endo}$, $H\text{-}6_{exo}$, H-1, H-2, H-3, H-4, H-5 and H-6), 5.07-5.24 (m, 2 H, $=CH_2$, allyl), 5.50 (d, 1 H, H-1', $J_{1',2'}$ 10.9 Hz), 5.71-5.93 (m, 1 H, -CH=, allyl), 7.10-7.48 (m, 35 H, $H_{arom.}$ 7 x benzyl); ^{13}C NMR ($CDCl_3$) δ 20.7 (CH_3, acetyl), 68.4, 71.0, 71.9, 73.9, 77.2, 78.1, 80.3, 80.8 and 82.9 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5 and C-6), 68.1, 71.2, 71.4, 73.0, 73.2, 74.6, 75.4 and 75.7 (7 x CH_2 benzyl, C-6' and 2 x OCH_2 allyl), 98.4 (C-1', $J_{1',H}$ 177.3 Hz), 116.2 and 116.3 (2 x $=CH_2$, allyl), 126.8-128.0 (35 x $CH_{arom.}$ benzyl), 134.1 and 134.4 (2 x -CH=, allyl), 137.5, 137.6, 137.8 and 138.3 (14 x $C_{arom.}$ benzyl), 169.5 and 169.6 (2 x $C_{carb.}$ acetyl).

Anal. Calc. for $C_{66}H_{70}O_{12}$: C, 75.12; H, 6.69. Found : C, 75.68; H, 6.78.

1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-2-*O*-(3',4',6'-tri-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (3b) and 1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-2-*O*-(3',4',6'-tri-*O*-benzyl-α-D-mannopyranosyl)-L-*myo*-inositol (4b)

To a solution of 3a and 4a (3.9 g, 3.67 mmol) in dry methanol (100 ml) methanolic sodium methoxide (1 M, 5.50 ml) was added, and the mixture was stirred for 2 h at 20°C. The solution was neutralized with Dowex 50 XW4 (H^+-form) resin (100-200 mesh), filtered and concentrated. The two diastereoisomers 3b and 4b were separated by column chromatography (50 g silica gel, eluted with *n*-hexane/diethyl ether, 1:1, v/v, 200 ml, followed by *n*-hexane/diethyl ether, 1:3, v/v, 250 ml) to give 3b (1.5 g, 1.47 mmol, 40%), $[\alpha]_D^{20}$ +35° (c 1 $CHCl_3$) and 4b (1.6 g, 1.57 mmol, 43%), $[\alpha]_D^{20}$ +26° (c 1 $CHCl_3$) as colourless oils. Rf 0.16 (3b, system III) and 0.42 (4b, system III); ^{1}H NMR ($CDCl_3$) 3b: δ 3.23-3.33 (m, 3 H), 3.39-3.46 (m, 2 H), 3.77-3.97 (m, 4 H), 4.07 (bs, 1 H, H-2'), 4.12-4.18 (m, 3 H), 4.28-4.45(m, 3H), 4.53-4.64 (m, 2 H), 4.71-4.94 (m, 1 H, 2'-OH), 5.14-5.30 (m, 2 H, OCH_2, allyl), 5,35 (d, 1 H, H-1', $J_{1',2'}$ 1.71 Hz), 5.83-5.96 (m, 1 H, -CH=, allyl), 7.05-7.42 (m, 35 H, $H_{arom.}$ 7 x benzyl); ^{13}C NMR ($CDCl_3$) 3b: δ 68.3, 70.4, 72.0, 73.7, 78.7, 78.9, 80.2, 80.8, 81.1 and 83.0 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5 and C-6), 68.2, 71.5, 71.6, 72.1, 73.2, 74.7, 75.4, 75.5 and 75.8 (7 x CH_2 benzyl, C-6' and OCH_2 allyl), 100.3 (C-1'), 116.8 ($=CH_2$, allyl), 126.9 (35 x $CH_{arom.}$ benzyl), 134.2 (-CH=, allyl), 137.2, 137.8, 137.9 and 138.3, (7 x $C_{arom.}$ benzyl).

Anal. Calc. for $C_{64}H_{68}O_{11}$: C, 75.87; H, 6.76. Found : C, 75.15; H, 6.62.

^{13}C NMR ($CDCl_3$) 4b: δ 68.1, 70.5, 71.5, 73.7, 78.3, 78.7, 80.3, 80.5, 80.9 and 82.8 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5 and C-6), 68.7, 70.9, 71.4, 72.5, 73.2, 74.5, 75.2, 75.3 and 75.6 (7 x CH_2 benzyl, C-6' and OCH_2 allyl), 100.1 (C-1'), 116.1 ($=CH_2$, allyl), 127.2-130.3 (7 x $CH_{arom.}$ benzyl), 134.3 (-CH=, allyl), 137.3, 137.4, 137.6, 138.1 and 138.2 (7 x $C_{arom.}$ benzyl).

1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-2-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (3c)

To a cooled (0°C) and stirred suspension of 3b (1.5 g, 1.47 mmol) and sodium hydride (0.1 g, 4.15 mmmol) in dry *N*,*N*-dimethylformamide (50 ml) was added benzyl bromide (0.3 ml, 2.52 ml). The mixture was stirred for 24 h at 20°C and excess sodium hydride was quenched with dry methanol (5 mmol). The solution was concentrated *in vacuo*, diluted with ether (150 ml), and successively washed with water (75 ml), aqueous sodium bicarbonate (75 ml, 10%, w/v), water (75 ml), dried ($MgSO_4$) and concentrated. Crude 3c was purified on a column of silica gel (40 g) which was eluted with *n*-hexane/diethyl ether (2:1, v/v, 150 ml) to give 3c as an oil (1.5 g, 1.35 mmol); $[\alpha]_D^{20}$ +19° (c 1 $CHCl_3$); Rf 0.43 (system II); ^{1}H NMR ($CDCl_3$) δ 3.21-3.52 (m, 5 H), 3.58-3.62 (m, 1 H), 3.65-3.73 (m, 1 H), 3.76-3.77 (t, 1 H, H-2', $J_{2',3'}$ 2.06 Hz), 3.80-3.86 (m, 2 H), 4.04-4.15 (m, 4 H, OCH_2 allyl and CH_2 benzyl), 5.14-5.31 (m, 2 H, $=CH_2$, allyl), 5.42-5.43 (d, 1 H, H-1', $J_{1',2'}$ 1.61 Hz), 5.84-5.97 (m, 1 H, -CH=, allyl), 7.04-7.35 (m, 40 H, $H_{arom.}$ 8 x benzyl); ^{13}C NMR ($CDCl_3$) δ 71.7, 71.9, 74.4, 74.7, 78.8, 79.0, 80.9, 81.0, 81.3 and 83.3 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5 and C-6), 68.8, 71.8, 72.0, 72.1, 73.3, 74.9, 75.5, 75.7 and 76.0 (8 x CH_2 benzyl, C-6'

and OCH_2 allyl), 98.3 (C-1'), 117.2 (=CH_2, allyl), 125.2-128.9 (35 x $CH_{arom.}$ benzyl), 134.5 (-CH=, allyl), 137.7, 138.1, 138.2, 138.4, 138.7 and 138.8 (8 x $C_{arom.}$ benzyl).

3,4,5,6-Tetra-*O*-benzyl-1-*O*-prop-1-enyl-2-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (3d)

Compound 3c (1.5 g, 1.35 mmol) was dissolved in 1,2-dichloroethane (10 ml). The solution was alternatingly degassed and placed under helium (3x). 1,5-Cyclooctadiene-*bis*[methyldiphenylphosphine]iridium hexafluorophosphate (20 mg) was added and again the solution was degassed and placed under helium (3x). The catalyst was activated by passing over a stream of hydrogen for 2 min. Once again the reaction mixture was degassed and, thereafter, left under a gentle stream of helium for 4 h. TLC analysis (system II) showed complete conversion of the starting material (R_F 0.43, system II) in compound 3d. The solvent was evaporated and the catalyst was removed by short column chromatography (40 g silica gel, *n*-hexane-/diethyl ether, 1:1, v/v, 250 ml), to afford homogeneous 3d (1.5 g, 1.35 mmol); Rf 0.53 (system II).

3,4,5,6-Tetra-*O*-benzyl-2-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (3e)

To a solution of 3d (1.5 g, 1.35 mmol) in dichloromethane/methanol (50.0 ml, v/v, 1:1) was added a solution of acetylchloride in methanol (0.5 M, 5.0 ml) and the reaction was stirred for 2 h at 20°C, TLC analysis (system I) indicated complete conversion of 3d in 3e. The reaction was stopped with water (1.0 ml), and the solution was diluted with dichloromethane (100 ml). The organic layer was washed with aqueous sodium bicarbonate (75 ml, 10%, w/v), water (75 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The oily residue was purified by column chromatography (50 g silica gel, *n*-hexane/diethyl ether, 1:1, v/v, 100 ml), to afford 3e (1.4 g, 1.31 mmol); $[\alpha]_D^{20}$ = +16° (c 1 $CHCl_3$); Rf 0.57 (system I); ^1H NMR ($CDCl_3$) δ 2.15 (bs, 1 H, 1-OH), 3.32-3.57 (m, 5 H), 3.63-3.65 (dd, 1 H, H-2', $J_{2',3'}$ 3.2 Hz), 3.74-3.87 (m, 2 H), 4.08-4.10 (m, 2 H), 4.32-4.37 (m, 2 H), 4.45-4.69 (m, 8 H, 4 x CH_2, benzyl), 4.75-4.97 (m, 8 H, 4 x CH_2, benzyl), 5.36-5.37 (d, 1 H, H-1', $J_{1',2'}$ 1.77 Hz), 7.05-7.42 (m, 40 H, $H_{arom.}$ 8 x benzyl); ^{13}C NMR ($CDCl_3$) δ 65.8, 71.6, 71.9, 72.1, 74.5, 75.0, 75.2, 75.6 and 75.7 (8 x CH_2 benzyl and C-6'), 71.8, 72.3, 73.5, 74.8, 78.8, 79.0, 80.8, 81.6 and 83.5 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5 and C-6), 98.7 (C-1'), 127.1-128.9 (40 x $CH_{arom.}$ benzyl), 138.1, 138.4, 138.5, 138.6 and 138.8 (8 x $C_{arom.}$ benzyl).

3,4,5,6-Tetra-*O*-benzyl-D-*myo*-inositol (1a)

A solution of 3e (0.26 g, 0.24 mmol) in acetic acid and 3% hydrochloric acid (15 ml, 9:1, v/v) was heated at 100°C for 3 h. The reaction mixture was cooled (20°C), neutralized with triethylamine and evaporated with *n*-hexane and toluene (4 x 25 ml). The oily residue was diluted with dichloromethane (50 ml), washed with water (25 ml), aqueous sodium bicarbonate (25 ml, 10%, w/v), water (25 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The oil was purified by column chromatography (25 g silica, *n*-hexane/diethyl ether, 1:3, v/v, 400 ml), to furnish 1a. Yield 27.1 mg (0.05 mmol); $[\alpha]_D^{20}$ -22.0° (c 1 $CHCl_3$); Rf 0.18 (system I); ^1H NMR ($CDCl_3$) δ 3.25-3.44 (m, 3 H, H-1, H-3, H-5), 3.71-3.78 (t, 1 H, H-4), 3.86-3.92 (t, 1 H, H-6), 4.07-4.08 (t, 1 H, H-2), 4.57-4.84 (m, 8 H, 4 x CH_2, benzyl), 7.12-7.43 (m, 20 H, $H_{arom.}$ 4 x benzyl); ^{13}C NMR ($CDCl_3$) δ 69.2, 71.5, 79.6, 81.0, 81.2 and 82.7 (C-1, C-2, C-3, C-4, C-5 and C-6), 71.9 75.0, 75.2 and 75.3 (4 x CH_2, benzyl), 127.1-127.9 (20 x $CH_{arom.}$ benzyl), 137.6 and 138.2 (4 x $C_{arom.}$ benzyl).

1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-D-*myo*-inositol (1b)

The same acidic hydrolysis of the α-glycosidic bond was carried out with compound 3b as for 3e resulting in the isolation of the title compound.
$[\alpha]_D^{20}$ - 2.3° (c 1, $CHCl_3$).

1,2-Di-*O*-palmitoyl-*sn*-glycero-benzyloxy (*N*,*N*-diisopropylamino) phosphoramidite (5)

To a cooled (0°C) and stirred solution of *bis*(*N*,*N*-diisopropylamino)chlorophosphine (2.5 g, 10.0 mmol) in dry ether (20 ml), was added dropwise a mixture of benzyl alcohol (0.97 ml, 10.0 mmol) and triethylamine (1.40 ml, 10.0 mmol) in ether (5 ml) under an atmosphere of nitrogen. After 30 min at 20°C, cold (0°C) *n*-hexane (30 ml) was added, followed by removal of the precipitated salts by filtration. The filtrate was concentrated *in vacuo* and the remaining oil was redissolved in dry dichloromethane to give a 1 M stock solution. ^{31}P NMR

(CH_2Cl_2) : δ 123.3.
To a solution of 1,2-di-*O*-palmitoyl-*sn*-glycerol (1.0 g, 1.76 mmol) in dry dichloromethane (4.0 ml) was added *bis*(*N*,*N*-diisopropylamino)benzyloxyphosphine (1 M, 2 ml) and 1*H*-tetrazole (100 mg, 1.42 mmol). After 30 min, ^{31}P NMR indicated the reaction to be complete. The solution was diluted with dichloromethane (100 ml), washed with triethyl ammonium bicarbonate (1 M, 3 x 30 ml), dried ($MgSO_4$), filtered, concentrated and purified by flash column chromatography (100 g silica gel, *n*-hexane/triethylamine, 97.5:2.5, v/v), to yield homogeneous 5 (1.34 g, 1.66 mmol); ^{31}P NMR (CH_2Cl_2) δ 148.9 and 149.2.

3,4,5,6-Tetra-*O*-benzyl-2-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero)-D-*myo*-inositol benzylphosphate (6a)
To a solution of **3e** (0.9 g, 0.84 mmol) and **5** (0.97 g, 1.14 mmol) in dichloromethane (5 ml) was added 1*H*-tetrazole (92.4 mg, 1.32 mmol) in acetonitrile (5 ml) and the mixture was stirred for 30 min at 20°C. ^{31}P NMR spectroscopy showed *inter alia* the presence of the phosphite-triester (δ 142.03 and 142.19). To the cooled (0°C) reaction mixture *t*-butyl hydroperoxide (0.5 ml, 1.0 mmol) was added and stirring was continued for 90 minutes. The solution was diluted with dry dichloromethane (200 ml), washed with triethyl ammonium bicarbonate (1 M, 3 x 75 ml), dried ($MgSO_4$) and concentrated *in vacuo* to give crude **6a**. Purification by column chromatography (100 g silica gel, eluens n-hexane/diethyl ether, 2:1, v/v, 500 ml, followed by *n*-hexane/diethyl ether, 1:1, v/v, 500 ml) afforded pure **6a** (1.2 g , 0.67 mmol); Rf 0.45 (system II); ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3, acetyl), 22.6, 24.8, 29.1, 29.2, 29.3, 29.5, 29.6, 31.9 and 33.9 (28 x CH_2, palmitoyl), 61.5, 65.6, 68.3, 69.3, 71.3, 71.5, 72.8, 74.5, 74.8, 75.1 and 75.5 (9 x CH_2 benzyl, C-6', C-1'' and C-3'' glycerol), 69.1, 71.8, 73.4 73.7, 74.1, 74.4, 78.1, 78.6, 79.2, 80.3 and 82.4 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5, C-6 and C-2'' glycerol), 98.7 (C-1'), 127.1-128.6 (45 x $CH_{arom.}$ benzyl), 137.9, 138.2, 138.4 and 138.7 (9 x $C_{arom.}$ benzyl); ^{31}P NMR (CH_2Cl_2) δ -0.63 and -0.75.

3,4,5,6-Tetra-*O*-benzyl-2-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero)-D-*myo*-inositol phosphate (6b)
Compound **6a** (300 mg, 0.18 mmol) was dissolved in *t*-butylamine (10 ml) and refluxed for 24 h at 50°C. TLC analysis (dichloromethane/methanol, 9:1, v/v) showed complete conversion of **6a** into the deprotected phosphate analogue **6b**. The solution was diluted with toluene (25 ml), concentrated and coevaporated with toluene (2 x 10 ml) to afford **6b** (*t*-butylammonium-salt). Rf (system IV) 0.49; ^{13}C NMR ($CDCl_3$) δ 14.08 (2 x CH_3, palmitoyl), 22.6, 24.7, 29.1, 29.3, 29,7, 31.9 and 33.9 (28 x CH_2, palmitoyl), 67.6, 71.3, 71.6, 72.0, 72.8, 74.6, 75.4 and 75.7 (8 x CH_2 benzyl, C-1'' and C-6'), 62.9 (d, C-3'', J_{cp} 73.3 Hz), 70.2, 71.0, 72.6, 74.4, 78.8, 80.7, 81.1 and 83.1 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5 and C-6), 98.1 (C-1'), 126.9-128.2 (40 x $CH_{arom.}$ 8 x benzyl), 137.5, 137.7, 138.2 and 138.4 (8 x $C_{arom.}$ benzyl), 173.2 and 173.4 (2 x $C_{carb.}$ palmitoyl); ^{31}P NMR ($CDCl_3$) δ -1.36.
Compound **6b** was passed over a column (5 x 0.5 cm) of SP-Sephadex C-25 (Na^+-form) in dichloromethane to give, after concentration of the appropriate fractions, the sodium-salt of **6b**. ^{13}C NMR ($CDCl_3$) δ 14.0 (2 x CH_3, palmitoyl), 22.6, 24.7, 29.1, 29.3, 29.7, 31.9, 33.9 and 34.1 (28 x CH_2, palmitoyl); 62.7 (d, C-3''), 67.3, 71.7, 71.8, 73.0, 74.8, 75.5 and 76.0 (8 x CH_2 benzyl, C-1'' and C-6'), 70.0, 70.5, 71,1, 72.5, 74.6, 77.1, 78.8, 81.0 and 82.9 (C-2', C-3', C-4', C-5', C-2'', C-1, C-2, C-3, C-4, C-5 and C-6), 97.4 (C-1'), 126.8-128.6 (40 x $CH_{arom.}$ benzyl), 137.5, 137.7, 138.2, 138.5 and 138.6 (8 x $C_{arom.}$ benzyl), 173.2 and 173.4 (2 x $C_{carb.}$ palmitoyl); ^{31}P NMR ($CDCl_3$) δ -1.12.

1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphoryl)-2-*O*-α-D-mannopyranosyl-D-*myo*-inositol (6c)
A solution of **6b** (240 mg, 0.14 mmol) in a mixture of *t*-butanol and water (24.5/0.5 , v/v, 25 ml) was hydrogenated in the presence of 10% palladium on charcoal (400 mg) for 72 h. The catalyst was removed by filtration and washed with *t*-butanol/water (49/1, v/v, 2 x 50 ml). The combined filtrates were evaporated to dryness, to give **6c** (67 mg, 0.07 mmol) as a solid. ^1H, NMR (DMSO) δ 1.08-1.11 (ds, 6 H, 2 x CH_3 palmitoyl), 1.32-1.64 (bs, 56 H, 28 x CH_2 palmitoyl), 1.96 (m, 5 H), 3.01-3.80 (m, 18 H), 4.82 (ds, 2 H), 5.55 (s, 1 H, H-1'); ^{13}C NMR (DMSO) δ 13.8 (2 x CH_3, palmitoyl), 22.0, 24.4, 28.4, 28.6, 29.0, 31.2 and 33.4 (28 x CH_2, palmitoyl), 61.2, 62.4 and 62.7 (C-6', C-1'' and C-3''), 66.8, 67.5, 68.4, 70.4, 70.7, 72.2, 72.4, 72.8, 73.1, 73.5 and 75.6 (C-2', C-3', C-4', C-5', C-1, C-2, C-3, C-4, C-5, C-6 and C-2''), 100.4 (C-1'), 172.2 and 172.4 (2 x $C_{carb.}$ palmitoyl); ^{31}P NMR (DMSO) δ 2.92.
Anal. Calc. for $C_{47}H_{88}O_{18}PNa$: P, 3.11. Found: P, 2.90.

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