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TETRAHEDRON

Stereoselective Synthesis of *myo*-Inositol-1,3,4,5-tetrakisphosphate Analogues from 6-deoxy D-Inositol Precursors.

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Abstract: The synthesis of 6-deoxy-D-*myo*-inositol-1,3,4,5-tetrakisphosphates is described. The access to opticaly pure Ins(1,3,4,5)P_4 analogues was carried out from deoxy *myo* inositol precursors derived from D-galactose. Modification of Ins(1,3,4,5)P_4 analogues by lipophilic substituents has been investigated in order to produce neutral phosphate derivatives aimed to be incorporated in cell membrane for *in vivo* evaluation.

The involvement of *myo* inositol polyphosphates in signal transduction *via* the polyphosphoinositide pathway has justified the need for the synthesis of molecules that would somehow interfere with, or modulate, the processes of cellular signalling.[1] Synthesis of structurally-modified analogues offers the prospect of pharmacological intervention in this ubiquitous metabolism where the second messenger D-*myo*-inositol-1,4,5-trisphosphate [Ins(1,4,5)P_3] is deactivated to D-*myo*-inositol-1,4-bisphosphate [Ins(1,4)P_2] or to D-*myo*-inositol-1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] which is subsequently degraded to D-*myo*-inositol-1,3,4-trisphosphate [Ins(1,3,4)P_3].[2] Ins(1,3,4,5)P_4 metabolite is produced from the [Ins(1,4,5)P_3] second messenger by specific cytosolic 3-kinase. Its biological function as another second messenger involved in Ca^{2+} haemostasis at the plasma membrane helping to control entry of extracellular Ca^{2+} into the cell, has not been unambiguously resolved.[2] However binding sites for Ins(1,3,4,5)P_4 have been identified in a range of tissues.[3] In support of this hypothesis, a sensitive Ca^{2+} permeable channel has been characterized from endothelial cells and Ins(1,3,4,5)P_4-binding proteins proposed as Ins(1,3,4,5)P_4-receptor have been purified from pig and rat cerebelum.[4] Results of these experiments emphasized the extreme specificity for 1,3,4,5 configuration of

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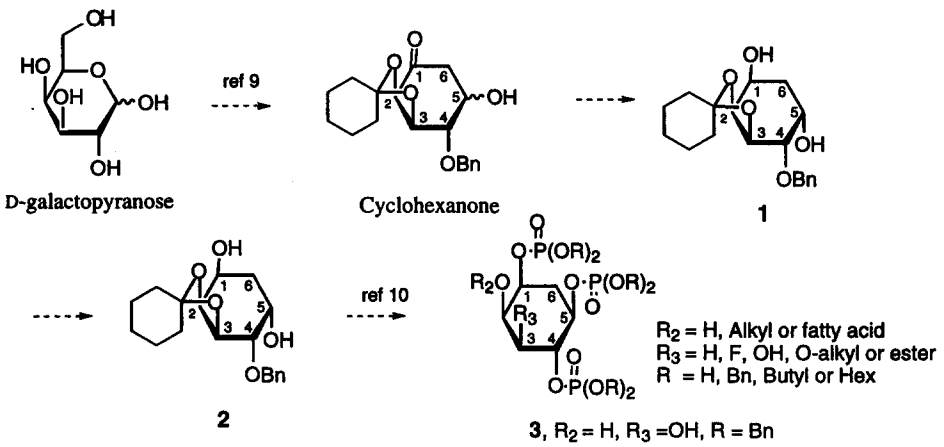
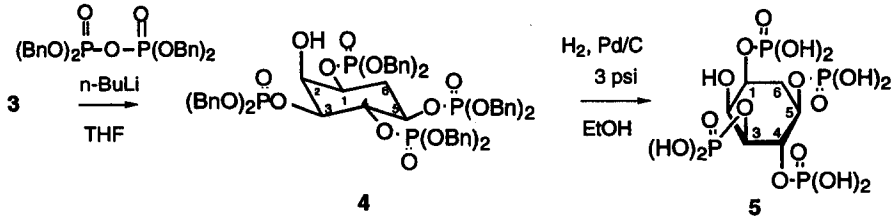
phosphate groups. The identification of a specific binding region of guanosine triphosphatase-activating protein (GAP^{IP4BP}) stimulating activity against *Ras* [5], strongly increased the interest of synthetic natural and analogue derivatives of Ins tetrakisphosphate.[6] Furthermore, Ins(1,2,4,5)P_4[7] has been regarded as connected to Ins(1,3,4)P_3 with a charge phosphate at 2-position in comparison with 2-neutral analogues synthesized previously.[8] With respect to these considerations it was obvious, that preparation of 6-deoxy derivatives analogues of Ins(1,3,4,5)P_4 were of current interest. The strategy has been elaborated from deoxy inositol precursors previously synthesized from the D-galactose,[9,10] already used for the preparation of chiral *myo*-inositol-trisphosphate analogues (**Scheme 1**).



Scheme 1

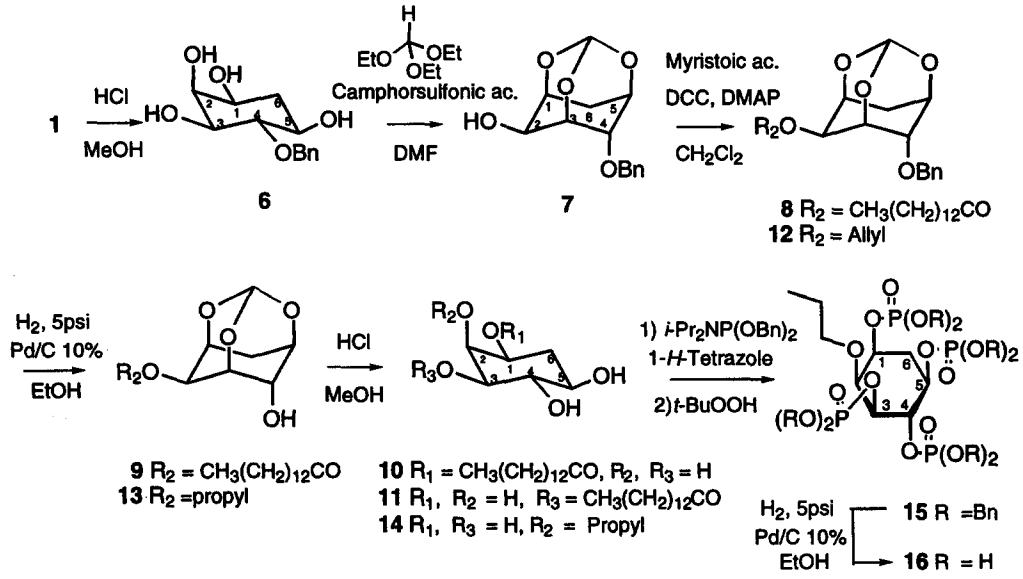
RESULTS AND DISCUSSION

The synthesis of the 6-deoxy Ins(1,3,4,5)P_4 **5** was firstly attempted by the selective equatorial 3-OH phosphorylation of the protected 6-deoxy-myo-inositol-1,4,5-tris(dibenzyl)phosphate **3**,[10] already described , using the pyrophosphate method,[11] in the presence of 1.2eq. of tetrabenzylpyrophosphate reagent and n-BuLi (**Scheme 2**). Usual hydrogenolysis of the resulting tertrakis(dibenzyl)phosphate intermediate **4**, in the presence of a catalytic amount of palladium on charcoal (Pd/C 10%) produced the targeted 6-deoxy Ins(1,3,4,5)P_4 **5**, with no migration of phosphate groups, in 70% overall yield isolated as octa-tris(hydroxymethyl)aminomethane salt, (TRIS salt).



Scheme 2

After the success accounted in our previous paper on the transmembrane incorporation of lipophilic derivatives of Ins(1,4,5)P_3,[10] the transformation of the tetrakisphosphate derivatives into lipophilic analogues seemed attractive. Two types of these analogues could be envisaged, by substitution on 2-hydroxy or on phosphate groups. In both cases, the strategy required the prior preparation of a 2-*O*-protected 6-deoxy-*myo*-inositol precursor which could be prepared from the 4-*O*-benzyl-2,3-*O*-cyclohexylidene-6-deoxy-*myo* -inositol **1**[9] *via* the orthoformate **7** [6k,11,12] (**Scheme 3**). The diol **1**[9] was converted into the tetrol **6** under acidic treatment before reacting with triethylorthoformate in *N,N*-dimethylformamide in the presence of a catalytic amount of camphor sulfonic acid (10%) affording the orthoformate **7** in 97% yield. Myristoylation of **7** using DCC in dichloromethane in the presence of a catalytic amount of DMAP gave the intermediate **8** in 90% yield. Catalytic hydrogenolysis of the benzylether of **8** followed by acidic treatment of compound **9** with methanolic/HCl solution to remove the ketal protecting groups induced an intramolecular migration of the myristoyl group from axial 2 position to equatorial 1 or 3 position leading to a mixture of 1- and 3-*O*-myristate **10** and **11** in 54 and 36% yields respectively.

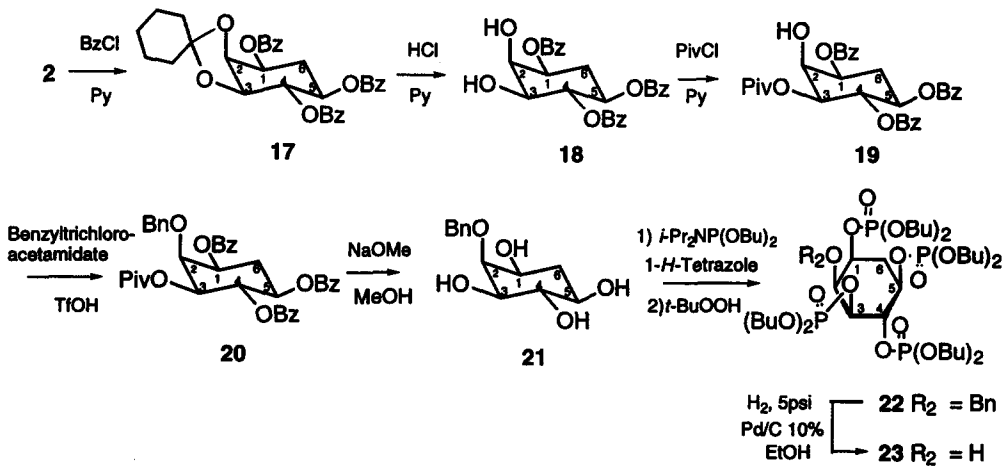


Scheme 3

Thus introduction of an ether group on 2 position was attempted from the orthoformate **7** *via* the 2-*O*-allyl derivative **12** (NaH, 1.5 eq.; AllBr, 1.5 eq.; DMF : 55%). Hydrogenation of compound **12** in the presence of Pd/C gave **13** (94%) by hydrogenolysis of the benzylether and reduction of the allyl- to propyl-ether. Hydrolysis of orthoformate **13** by methanolic HCl solution afforded the 2-*O*-propyl 1,3,4,5-tetrol **14** in 98% yield. Compound **14** was submitted to the phosphorylation-deprotection procedure, in the presence of bisbenzyloxy(diisopropylamino)phosphine reagent, to give the 6-deoxy-2-*O*-propyl Ins(1,3,4,5)P_4 **16** in 65%

overall yield isolated as octa-TRIS salt. This latter derivative could be of interest to determine the influence of the 2-hydroxy group in the degradation process of Ins(1,3,4,5)P_4.

Another strategy was established to produce protected phosphate analogues from the 3,4-*O*-cyclohexylidene-6-deoxy-*myo*-inositol 2[9] (**Scheme 4**).



Scheme 4

The tribenzoylation of triol **2** was carried out, in 96% yield, in the presence of benzoyl chloride in pyridine to give the intermediate **17** which was hydrolyzed in acidic conditions into the diol **18** (90%) without ester migration. Selective protection of 3-OH of compound **18** was achieved by trimethylacetylchloride (1.3 eq.) in pyridine in 80% yield. Benzylation of the resulting free 2-OH of alcohol **19** was performed, in 70% yield, using benzyltrichloroacetamidate in the presence of trifluoromethanesulfonic acid.[13] Saponification of the tetraester intermediate **20**, under basic medium, afforded the tetrol **21** in 86% yield, which was allowed to be phosphorylated in the presence of bisbutyloxy(diisopropylamino)phosphine and tetrazole, followed by oxydation with *t*-BuOOH, leading to the tetrakis(dibutyl)phosphate **22** in 55% yield. Hydrogenation, in the presence of Pd/C 10% in AcOEt, of intermediate **22** furnished the lipophilic tetrakis(dibutyl)phosphate **23**.

CONCLUSION

In conclusion of this work, we have illustrated the potentiality of deoxy cyclitol precursors, stereoselectively produced from D-galactose,[9] to be used for the synthesis of optically pure D-Ins(1,3,4,5)P_4 analogues. The strategy previously elaborated for the access to deoxy *myo* inositol trisphosphate analogues,[10] could be easily extended to a variety of 6-deoxy Ins(1,3,4,5)P_4 derivatives, which might be modified at the 2-hydroxy position or at the phosphate moieties using the selectively protected cyclitol intermediates described. The quantity of material available allowed the study of their interaction with rapidly expanding range of Ins(1,3,4,5)P_4-binding proteins. Therefore, preliminary success encountered *in vivo* in the incorporation of lipophilic analogues of $InsP_3$ into the cell membrane, encouraged the investigation of lipophilic Ins(1,3,4,5)P_4 on Ca^{2+}-mobilization. Data on biological evaluation are under investigation and will be published elsewhere.

Phosphatidylinositol analogues represent now the logical attractive targets for chemical development from deoxy cyclitol precursors and will be presented in the next paper.

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EXPERIMENTAL PART

^1H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometra WP 200, AC 200, AC 250; chemical shifts are expressed in parts per million (ppm) referenced to residual chloroform (7.27 ppm). Coupling constants (J) are given in Hertz (Hz). Multiplicities are recorded as s or bs (singlet or broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The $[\alpha]_D$ were recorded on Perkin-Elmer 241-MC sodium absorbtion at 20°C. Mass spectra (m/z (% base peak) were recorded on Atlas CH_4 or AEI MS9 spectrometra. Melting points were determined on a C. REICHERT microscope apparatus and are uncorrected. Elemental analysis were carried out at the "Laboratoire de Microanalyse de l'I.C.S.N." (CNRS, Gif/yvette). All solvents were freshly distilled prior to use by standard methods.[14] Flash chromatography was performed on silica-gel Merck 60 230-400 mesh. Thin layer chromatography was performed on precoated plates of silica gel PF_{254} neutralized with sodium bicarbonate. All crystallizations were obtained from AcOEt/pentane if not specified. All extractions were followed by addition of magnesium sulfate to the organic layer and filtration.

For general procedure for phosphorylation and deprotection process see the previous paper.

D-6-Deoxy-*myo*-inositol-1,3,4,5-tetrakis(dibenzyl)phosphate 4

1,4,5-tris(dibenzyl)phosphate **3** was phosphorylated using the method B in the presence of tetrabenzylpyrophosphate (1.2 eq.) to give 1,3,4,5-tetrakis(dibenzyl)-phosphate **4** (70%): $[\alpha]_D$ +1° (c 0.98, $CHCl_3$); ^1H NMR (400MHz; $CDCl_3$): δ : 4.75 (t, 1H, H-4; $J_{4\text{-}3}=J_{4\text{-}5}=8$); 4.5 (bs, 1H, H-2); 4.35 (m, 1H, H-1); 4.20 (m, 1H, H-5); 4.10-4.32 (m, 16H, H-3, $C\underline{H}_2O$); 2.55 (dt, 1H, H-6eq, $J_{6eq\text{-}6ax}=12$; $J_{6eq\text{-}1}=J_{6eq\text{-}5}=4$); 2.35 (q, 1H, H-6ax; $J_{6eq\text{-}6ax}=J_{6ax\text{-}1}=J_{6ax\text{-}5}=12$); ^{13}C NMR (63MHz; $CDCl_3$): δ: 78.50 (C-2); 77.02 (C-4); 74.70 (C-3); 75.35 ($\underline{C}H_2Ph$); 73.23, 71.82 (C-1, C-5); ^{31}P NMR (81MHz; $CDCl_3$): δ ppm: -1.52; -1.38; -1.21; -1.09; (P_1, P_3, P_4, P_5); (Found C, 61.90; H, 5.15; P, 10.31; $C_{62}H_{64}O_{17}P_4$ requires C, 61.79; H, 5.35; P, 10.28).

D-6-Deoxy -*myo*-inositol-1,3,4,5-tetrakisphosphate 5

Tetrakis(dibenzyl)phosphate **4** dissolved in the minimun amount of EtOH 95% was hydrogenated for 2h., under 4-5 psi, in the presence of the same amount of Pd/C 10%. The catalyst was removed by filtration on Whatman paper and washing with water. Tris(hydroxymethyl)aminomethane (TRIS, 2 eq. per phosphate) was added and the aqueous solution was concentrated *in vacuo* then lyophilized.The tetraphosphate **5** was precipitated as octa-TRIS salt; $[\alpha]_D$ +0° (c 0.75, H_2O); (Found C, 30.21; H, 7.35; N, 7.52; $C_{38}H_{104}O_{41}N_8P_4$, $4H_2O$ requires C, 29.92; H, 7.40; N, 7.35).

D-4-*O*-Benzyl-6-deoxy-*myo*-inositol 6

Diol **1** (1g, 2.9 mmol.) was treated by aq. solution of HCl 1M for 1h. After evaporation to dryness the tetrol **52** was isolated and crystallized from isopropanol/pentane (95%); m.p.130-131°C; $[\alpha]_D$=+2° (c 1,

CH_3OH); ^1H NMR (200MHz; $CDCl_3$): δ: 3.7 (ddd, 1H, H-1, J_{1-2}=4, J_{1-6ax}=10, J_{1-6ax}=4); 3.5 (m, 1H, H-2); 3.43 (m, 1H, H-4); 3.40 (m, 1H, H-5); 3.33 (m, 1H, H-3); 2.10 (m, 1H, H-6ax); 1.90 (m, 1H, H-6eq); (Found C, 61.38; H, 7.26; O, 31.80; $C_{13}H_{18}O_5$ requires C, 61.40; H, 7.14; O, 31.46).

D-4-*O*-Benzyl-1,3,5-*O*-orthoformyl-6-deoxy-*myo*-inositol 7

To a solution of tetrol **6** (470 mg, 1.85 mmol.) in dry DMF (5 ml) was added triethyl orthoformate (0.55 ml, 3.3 mmol.) and *p*-toluene sulfonic acid monohydrate (76 mg). The solution was stirred under argon 12h. at 60°C before neutralization with sodium bicarbonate aq. solution. The mixture was then filtered on celite and the organic layer was evaporated *in vacuo*. The residue was diluted with methanol and treated with charcoal, 15 min. at 60°C and filtered on celite. The filtrate was concentrated and the orthoformate **7** was crystallized from $CHCl_3$ (97%); m.p.104-106°C; $[\alpha]_D$=+9° (c 1,31, CH_2Cl_2); ^1H NMR (200MHz; $CDCl_3$): δ: 5.50 (sl, 1H, H-7); 4.2 0(d, 2H, CH_2O); 4.26 (t, 1H, H-4, J_{4-3}=J_{4-5}=2); 4.19 (m, 2H, H-1, H-5); 4.08 (t, 1H, H-3, J_{3-4}=J_{3-2}=2), 3.84 (t, 1H, H-2, J_{2-3}=J_{2-1}=2), 2.60 (m, 1H, H-6ax); 2.0 (m, 1H, H-6eq); ^{13}C NMR (50MHz; $CDCl_3$): δ: 104.0 (C-7); 73.3, 72.8, 71.4, 67.6, 64.8 (C-1, C-2, C-3, C-4, C-5); 72.0 ($\underline{C}H_2Ph$); 27.5 (C-6); (Found C, 62.59; H, 6.37; O, 31.12; $C_{14}H_{16}O_5$,1/4 H_2O requires C, 62.56; H, 6.19; O, 31.23).

D-4-*O*-Benzyl-1,3,5-*O*-orthoformyl-2-*O*-myristoyl-6-deoxy-*myo*-inositol 8

To the alcohol **7** (264 mg, 1 mmol.) dissolved in dry CH_2Cl_2 (20 ml) was added DCC (309 mg, 1.5 mmol.), DMAP (20 mg) and myristoic acid (342 mg, 1.54 mmol.). After 4h. of stirring at r.t., the mixture was filtered on celite and the filtrate was concentrated. The residue was chromatographed on silica gel to give the crystalline monoester **8** (90%); m.p. 88-90°C; $[\alpha]_D$ 0° (c 1, CH_2Cl_2); ^1H NMR (200MHz; $CDCl_3$): δ: 5.6 (bs, 1H, H-7); 5.05 (bs, 1H, H-2); 4.60 (dd, 2H, CH_2Ph); 4.35 (m, 1H, H-4); 4.30 (m, 1H, H-3); 4.20 (m, 2H, H-3, H-5); 2.61 (m, 1H, H-6ax); 2.12 (m, 1H, H-6eq); ^{13}C NMR (50MHz; $CDCl_3$): δ: 173.4 (C=O); 103.94 (C-7); 72.4, 70.36; 68.26, 67.68, 66.63 (C-1, C-2, C-3, C-5); 71.61 ($\underline{C}H_2Ph$); 14.06 ($\underline{C}H_3$); (Found C, 65.68; H, 6.73; P, 7.47; $C_{69}H_{83}O_{16}P_3$ requires C, 65.70; H, 6.73; P, 7.37).

D-6-Deoxy-2-*O*-myristoyl-1,3,5-*O*-orthoformyl-*myo*-inositol 9

Compound **8** dissolved in the minimun amount of EtOH 95% was hydrogenated for 4 h., under 3 psi, in the presence of the same amount of Pd/C 10%. The catalyst was removed by filtration on Whatman paper and the filtrate was concentrated *in vacuo*. The alcohol **9** was crystallized from CH_3OH/H_2O in quantitative yield. m.p.99-101°C; $[\alpha]_D$=-4° (c 1, $CHCl_3$); ^1H NMR (200MHz; $CDCl_3$): δ: 5.6 (bs, 1H, H-7); 5.05 (bs, 1H, H-2); 4.6 (m, 1H, H-1); 4.20 (m, 3H, H-3, H-4, H-5); 2.60 (m, 1H, H-6ax); 2.11 (m, 1H, H-6eq); ^{13}C NMR (50MHz; $CDCl_3$): δ: 174.2 (C=O); 103.69 (C-7); 72.1, 70.36, 69.1, 68.4, 66.6, 65.7 (C-1, C-2, C-3, C-4, C-5); 14.1 ($\underline{C}H_3$); (Found C, 65.37; H, 9.56; O, 24.92; $C_{21}H_{36}O_6$ requires C, 65.60; H, 9.44; O, 24.97).

D-1-*O*-Myristoyl-6-deoxy-*myo*-inositol 10 and D-3-*O*-myristoyl-6-deoxy-*myo*-inositol11

2-*O*-myristoyl orthoformate **9** was treated by a methanolic solution of HCl 1M, 2h. at r.t.. After neutralization of the acidic mixture with sodium bicarbonate aq. solution and evaporation to dryness, the residue was chromatographed on silica gel. First the 1-*O*-myristoyl **10** was eluted (54%) and then the 3-*O*-myristoyl **11** (36%). Both esters were crystallized from CH_3OH/H_2O.

10: m.p.142-144°C; $[\alpha]_D$ -13° (c 1, C_5H_5N); ^1H NMR (200MHz; C_5D_5N): δ: 5.40 (m, 1H, H-1); 5.11 (bs, 4H, OH); 4.85 (bs, 1H, H-2); 4.6 (t, 1H, H-4; $J_{4-3}=J_{4-5}=9$); 4.20 (m, 1H, H-5); 4.05 (dd, 1H, H-3; $J_{3-2}=2$, $J_{3-4}=9$); 2.9 (q, 1H, H-6ax; $J_{6ax-6eq}=J_{6ax-1}=J_{6ax-5}=12$); 2.60 (m, 1H, H-6eq); ^{13}C NMR (50MHz; C_5D_5N): δ: 173.3 (C=O); 76.7; 74.79, 74.37, 70.96, 68.63 (C-1, C-2, C-3, C-4, C-5); 14.30 (CH_3); (Found C, 63.94; H, 10.19; O, 25.35; $C_{20}H_{38}O_6$ requires C, 64.14; H, 10.22; O, 25.63).

11: m.p. 136-137°C; $[\alpha]_D$ -35° (c 1, C_5H_5N); ^1H NMR (250MHz; $C_5D_5N + D_2O$): δ: 5.90 (bs, 4H, OH); 5.45 (dd, 1H, H-3; $J_{3-4}=10$; $J_{3-2}=2$); 4.9 (sl, 1H, H-2); 4.80 (t, 1H, H-4; $J_{4-3}=J_{4-5}=10$); 4.20 (m, 2H, H-1, H-5); 2.90 (q, 1H, H-6ax; $J_{6ax-6eq}=J_{6ax-1}=J_{6ax-5}=12$); 2.6 (m, 3H, H-6eq, $CH_2C=O$); 1.00 (t, 3H, CH_3); ^{13}C NMR (50MHz; C_5D_5N): δ: 173.87 (C=O); 76.83, 73.92, 72.72, 71.32, 68.39 (C-1, C-2, C-3, C-4, C-5); 14.38 (CH_3); (Found C, 63.95; H, 9.98; O, 25.48; $C_{20}H_{38}O_6$ requires C, 64.14; H, 10.22; O, 25.63).

D-2-*O*-Allyl-4-*O*-benzyl-6-deoxy-1,3,5-*O*-orthoformyl-*myo*-inositol 12

To a solution of orthoformate **7** (700 mg, 2.65 mmol.) in DMF (5 ml) was added sodium hydride (95 mg, 3.97 mmol.) and allyl bromide (0.35ml, 3.9 mmol.). The mixture was stirred for 1h. before addition of MeOH (5 ml) and the stirring was maintained for another 1h.. After extraction with CH_2Cl_2, the organic layer was concentrated *in vacuo* The residue was chromatographed on silicagel to give the allylether **12** in 55% yield; $[\alpha]_D$=+ 11° (c 0.55, CH_2Cl_2); ^1H NMR (200MHz; $CDCl_3$): δ: 6.13 (m, 1H, =CH-); 5.73 (bs, 1H, H-7); 5.4 (d, 2H, CH_2=); 4.40 (m, 4H, H-1, H-2, H-5, CH_2O); 4.20 (m, 1H, H-3); 3.76 (m, 1H, H-4); 2.6 (m, 1H, H-6ax); 2.0 (m, 1H, H-6eq); (Found C, 67.25; H, 6.63; $C_{17}H_{20}O_5$ requires C, 67.09; H, 6.62).

D-6-Deoxy-1,3,5-*O*-orthoformyl-2-*O*-propyl-*myo*-inositol 13

Compound **12** dissolved in the minimun amount of AcOEt was hydrogenated for 1h., under 4 psi, in the presence of the same amount of Pd/C 10%. The catalyst was removed by filtration on Whatman paper and the filtrate was concentrated *in vacuo* The alcohol **13** was crystallized (94%): m.p.161-163°C; $[\alpha]_D$=+ 10° (c 0.9, CH_2Cl_2); ^1H NMR (200MHz; $CDCl_3$): δ: 5.56 (bs, 1H, H-7); 4.26 (m, 1H, CH_2O); 3.70 (ddd, 1H, H-1, $J_{1-2}=4$, $J_{1-6ax}=8$, $J_{1-6eq}=2$); 3.67 (ddd, 1H, H-5, $J_{5-4}=6$, $J_{5-6ax}=8$, $J_{5-6eq}=2$); 3.66 (dd, 1H, H-3, $J_{3-4}=7$, $J_{3-2}=4$); 3.60 (t, 1H, H-2, $J_{2-1}=J_{2-3}=4$); 3.40 (dd, 1H, H-4, $J_{4-3}=7$, $J_{4-5}=6$); 2.56 (ddd, 1H, H-6ax, $J_{6ax-1}=8$, $J_{6ax-6eq}=12$, $J_{6ax-5}=2$); 2.06 (m, 1H, H-6eq); 1.66 (m, 2H, CH_2); 0.96 (t, 2H, CH_3); (Found C, 55.51; H, 7.23; $C_{10}H_{16}O_5$ requires C, 55.54; H, 7.46)

D-6-Deoxy-2-*O*-propyl-*myo*-inositol 14

Orthoformate **13** was treated by a methanolic solution of HCl 1M, 2h. at r.t.. After neutralization of the acidic mixture with aq. sodium bicarbonate solution and evaporation to dryness, the residue was chromatographed on silicagel and the tetrol **14** was crystallized from hexane; m.p. 138-140°C; $[\alpha]_D$ + 6° (c 1.3, CH_3OH); ^1H NMR (200MHz; CH_3OD): δ: 3.7 (ddd, 1H, H-1, $J_{1-2}=4$, $J_{1-6ax}=10$, $J_{1-6ax}=3$); 3.59 (m, 1H, H-2); 3.50 (q, 2H, CH_2O); 3.44 (m, 1H, H-5); 3.33 (m, 1H, H-3); 3.29 (m, 1H, H-4); 2.06 (ddd, 1H, H-6ax, $J_{6ax-1}=10$, $J_{6ax-6eq}=12$, $J_{6ax-5}=8$); 1.86 (m, 1H, H-6eq); 1.41 (m, 2H, CH_2); 0.91 (t, 2H, CH_3); (Found C, 49.98; H, 8.80; $C_9H_{18}O_5$ .1/2 H_2O requires C, 50.22; H, 8.90).

D-6-Deoxy-2-*O*-propyl-*myo*-inositol-1,3,4,5-tetrakis(dibenzyl)phosphate 15

Tetrol **14** was phosphorylated using the method A in the presence of bisbenzyloxy-(diisopropylamino)phosphine reagent to give the 1,3,4,5-tetrakis(dibenzyl)phosphate **15** (65%);

$[\alpha]_D$ +3°(c 0.77, CH_2Cl_2); ^1H NMR (200MHz; $CDCl_3$): δ: 4.86 (dt, 1H, H-1, J_{1-2}=4, J_{1-6ax}=12, J_{1-6ax}=4), 4.20 (t, 1H, H-2, J_{2-1}=J_{2-3}=4); 4.1 (m, 2H, H-3, H-4); 4.0 (ddd, 1H, H-5, J_{5-4}=8, J_{5-6ax}=12, J_{5-6eq}=6); 3.53 (q, 2H, CH_2O); 2.46 (ddd, 1H, H-6ax, J_{6ax-1}=12, $J_{6ax-6eq}$=>20, J_{6ax-5}=12); 2.26 (m, 1H, H-6eq); 1.46 (m, 2H, CH_2); 0.83 (t, 2H, CH_3); ^{13}C NMR (50MHz; $CDCl_3$): δ: 77.8 (C-2); 75.9, 73.6, 73.3, 73.3 (C-1, C-3, C-4, C-5); 75.6 ($\underline{C}H_2Ph$); 31.9 (C-6); 69.6, 23.5, 10.6 (propyl); (Found C, 62.46; H, 5.49; P, 9.90; $C_{65}H_{69}O_{17}P_4$ requires C, 62.65; H, 5.58; P, 9.94).

D-6-Deoxy-2-*O*-propyl-*myo*-inositol-1,3,4,5-tetrakisphosphate 16

Tetrakis(dibenzyl)phosphate **15** dissolved in the minimun amount of EtOH 95% was hydrogenated for 2 h., under 4-5 psi, in the presence of the same amount of Pd/C 10%. The catalyst was removed by filtration on Whatman paper. Tris(hydroxymethyl)aminomethane (TRIS, 2 eq. per phosphate) was added and the aqueous solution was concentrated *in vacuo*. After lyophilization the tetraphosphate **16** was precipitated as a octa-TRIS-salt; $[\alpha]_D$ +0° (c 1.2, H_2O);^1H NMR (250MHz D_2O):δ: 4.36 (m 1H, H-5); 4.20 (m, 1H, H-1); 4.10 (m, 2H, H-2, H-3); 3.8 (m, 1H, H-4); 2.26 (m, 1H, H-6eq); 2.00 (m, 1H, H-6eq); (Found C, 31.20; H, 7.41; N, 7.28; $C_{41}H_{109}O_{41}N_8P_4$+ $4H_2O$ requires C, 31.44; H, 7.53; N, 7.15).

D-1,4,5-Tri-*O*-benzoyl-2,3-*O*-cyclohexylidene-6-deoxy-*myo*-inositol 17

To a solution of triol **1** (1,22g, 5 mmol.) dissolved in dry pyridine, was added benzoyl chlroride (2,32 ml, 20 mmol.) and the mixture was stirred for 3h. at r. t.. After extraction with CH_2Cl_2, the organic layer was concentrated *in vacuo*. The residue was chromatographed on silica gel to give crystalline **17** (96%); m.p. 200°C; $[\alpha]_D$ -40° (c 0.65, $CHCl_3$); S.M. (I.C; isobutanol; m/z): 557 $[MH]^+$; ^1H NMR (250MHz; $CDCl_3$): δ: 5.85 (dd, 1H, H-4; J_{4-3}=8; J_{4-5}=10); 5.55 (m, 1H, H-1); 5.30 (m, 1H, H-5); 4.65 (t, 1H, H-2; J_{2-3}=J_{2-1}=4); 4.45 (dd, 1H, H-3); 2.50 (m, 2H, H-6ax H-6eq); ^{13}C NMR (63MHz; $CDCl_3$): δ: 165.76 (3C=O); 111.78 (O-C-O); 76.62 (C-4); 75,14, 74.17 (C-2, C-3); 69.55, 67.58 (C-1, C-5); 29.88 (C-6); (Found C, 71.42; H, 5.90; $C_{33}H_{32}O_8$ requires C, 71.21; H, 5.80).

D-1,4,5-Tri-*O*-benzoyl-6-deoxy-*myo*-inositol 18

Tribenzoyl **17** was treated by a methanolic solution of HCl 37% (3 ml), 12h. at r.t.. After neutralization of the acidic mixture with sodium bicarbonate aq. solution and evaporation to dryness, the residue was chromatographed on silicagel to give the crystalline diol **18** (90%); m.p. 101-103°C; $[\alpha]_D$ -18° (c 1.65, $CHCl_3$); S.M. (I.C; isobutanol; m/z): 477 $[MH]^+$; ^1H NMR (200MHz; $CDCl_3$): δ: 5.85 (t, 1H, H-4; J_{4-3}=J_{4-5}=10); 5.40 (m, 1H, H-5); 5.25 (m, 1H, H-1); 4.40 (sl, 1H, H-2); 3.90 (dd, 1H, H-3; J_{3-2}=2.5, J_{3-4}=10); 3.70 (bs, 2H, 2O$\underline{H}$); 2.50 (m, 2H, H-6ax, H-6eq); ^{13}C NMR (63MHz; $CDCl_3$): δ: 167.41; 165.89; 165.69 (3 C=O); 75.04 (C-4); 71.40; 71.26 (C-2, C-3); 69.61; 69.56 (C-1, C-5); 29.00 (C-6); (Found C, 66.46; H, 5.24; $C_{27}H_{24}O_8$,1/2 H_2O requires C, 66.79; H, 5.19).

D-1,4,5-Tri-*O*-benzoyl-6-deoxy-3-*O*-trimethylacetyl-*myo*-inositol 19

A solution of diol **18** (400 mg, 0.84 mmol.) in dry pyridine under argon, was cooled to 0°C before addition of pivaloyl chloride (0.135 ml, 1.09 mmol.). The mixture was stirred 4h. at r.t. and extracted with CH_2Cl_2. The organic phase was concentrated *in vacuo* and the residue chromatographed on silica gel to give

crystalline **19** (80%); m.p. 208-210°C; $[\alpha]_D$=-38° (c 1, $CHCl_3$); ^1H NMR (200MHz; $CDCl_3$): δ: 6.15 (t, 1H, H-4; $J_{4-3}=J_{4-5}=10$); 5.4 (m, 2H, H-1 et H-5); 5.30 (dd, 1H, H-3; $J_{3-2}=2.5$, $J_{3-4}=10$); 4.05 (bs, 1H, H-2); 2.60 (m, 3H, O<u>H</u>; H-6ax, H-6eq); 1.05 (s, 9H, (CH_3)); ^{13}C NMR (50MHz; $CDCl_3$): δ: 177.32 (<u>C</u>=$O_{pivaloyl}$); 165.91; 165.77; 165.47 (3C=$O_{benzoyl}$); 71.38; 71.17; 69.90; 69.46; 69.15 (C-1,C-2, C-3, C-4, C-5); 39.00 [<u>C</u>$(CH_3)_3$]; 28.97 (C-6); 27.00 [$(CH_3)_3$]; (Found C, 68.37; H, 5.85; $C_{32}H_{32}O_9$ requires C, 68.56; H, 5.75).

D-1,4,5-Tri-*O*-benzoyl-2-*O*-benzyl-6-deoxy-3-*O*-trimethylacetyl-*myo*-inositol 20

To a solution of alcohol **19** (230 mg, 0.5 mmol.) dissolved in CH_2Cl_2 (5 ml) was added under argon cyclohexane (10 ml), benzyl-2,2,2-trichloroacetamidate (380 mg, 1.5 mmol.) and trifluoromethanesulfonic acid (0.1 ml). After 6h. of stirring and neutralization with aq. sodium bicarbonate solution, the mixture was extracted with CH_2Cl_2. The organic layer was concentrated *in vacuo* and the residue chromatographed on silicagel to give the crystalline benzylether **20** (70%); m.p. 128-129°C; $[\alpha]_D$ -13° (c 1.25, $CHCl_3$); S.M. (I.C; isobutanol; m/z): 651 $[MH]^+$; ^1H NMR (250MHz; $CDCl_3$): δ: 6.15 (t, 1H, H-4; $J_{4-3}=J_{4-5}=10$); 5.35 (m, 3H, H-1, H-3, H-5); 4.80 (dd, 2H, C<u>H</u>$_2$Ph); 4.35 (bs, 1H, H-2); 2.55 (m, 2H, H-6ax, H-6eq); 1.05 [s, 9H, (CH_3)]; ^{13}C NMR (63MHz; $CDCl_3$): δ: 177.20 (<u>C</u>=$O_{pivaloyl}$); 165.66 (3C=$O_{benzoyl}$); 77.10 (C-2); 75.35 (CH_2Ph); 71.67 (C-3, C-4); 69.98; 69.36 (C-1, C-5); 38.79 [<u>C</u>$(CH_3)_3$]; 29.62 (C-6); 26.93 [$(CH_3)_3$]; (Found C, 71.57; H, 5.95, O,22.52 $C_{39}H_{38}O_9$ requires C, 71.98; H, 5.89; O, 22.13).

D-2-*O*-Benzyl-6-deoxy-*myo*-inositol 21

Tribenzoate **20** (300 mg, 0.46 mmol.) was treated by a sodium hydroxide (600 mg) solution in methanol (10 ml), 2h. at reflux. After neutralization of the mixture with HCl aq. solution and evaporation to dryness, the residue was chromatographed on silicagel to give the crystalline tetrol **21** from CH_3OH/H_2O (86%); m.p. 138-140°C; $[\alpha]_D$=+16° (c 1.7, CH_3OH); ^1H NMR (200MHz; C_5D_5N): δ: 6.00 (m, 4H, O<u>H</u>); 5.05 (dd, 2H, C<u>H</u>$_2$Ph); 4.25 (t, 1H, <u>H</u>-4; $J_{4-3}=J_{4-5}=9$); 4.15 (bs, 1H, H-2); 3.95 (ddd, 1H, H-1; $J_{1-2}=3$; $J_{1-6eq}=4$; $J_{1-6ax}=12$); 3.80 (m, 2H, H-3, H-5); 2.45 (q, 1H, H-6ax; $J_{6ax-1}=J_{6ax-5}=J_{6ax-6eq}=12$); 2.3 (m, 1H, H-6eq); ^{13}C NMR (50MHz; C_5D_5N): δ: 83.84 (C-2); 77.11 (C-4); 75.50 (CH_2Ph); 74.68 (C-3); 71.47 (C-5); 68.91 (C-1); 38.17 (C-6); (Found C, 61.11; H, 7.12; $C_{13}H_{18}O_5$ requires C, 61.40; H, 7.14).

D-2-*O*-Benzyl-6-deoxy-*myo*-inositol-1,3,4,5-tetrakis(dibutyl)phosphate 22

Tetrol **21** was phosphorylated using the phosphorylation method A in the presence of bisbutyloxy(diisopropylamino)phosphine to give 1,3,4,5-tetrakis(dibutyl)phosphate **22** (55%); $[\alpha]_D$=+7° (c 1, $CHCl_3$); ^1H NMR.(400MHz; $CDCl_3$): δ: 4.90 (dd, 2H, CH_2Ph); 4.75 (t, 1H, H-4; $J_{4-3}=J_{4-5}$= 9); 4.5 (bs, 1H, H-2); 4.35 (m, 1H, H-1); 4.20 (m, 1H, H-5); 4.10 (m, 17H, H-3, CH_2O); 2.55 (dt, 1H, H-6eq, $J_{6eq-6ax}=12$; $J_{6eq-1}=J_{6eq-5}=4$); 2.35 (q, 1H, H-6ax; $J_{6eq-6ax}=J_{6ax-1}=J_{6ax-5}=12$); ^{13}C NMR (63MHz; $CDCl_3$): δ: 78.00 (C-2); 76.81 (C-4); 75.56 (C-3); 75.23 (CH_2Ph); 73.00, 71.73 (C-1, C-5); 67.90 (8<u>C</u>H_2O); 31.96 (C-6 et 8<u>C</u>H_2CH_2O); 18.38 (8<u>C</u>H_2CH_3); 13.30 [8<u>C</u>$H_3(CH_2)_3$]; ^{31}P NMR (81MHz; $CDCl_3$): δ: -1.32; -0.86 (P_1, P_3, P_4, P_5); (Found C, 53.05; H, 8.51; P, 11.82; $C_{45}H_{86}O_{17}P_4$ requires C, 52.83; H, 8.47; P, 12.11).

D-6-Deoxy-*myo*-inositol-1,3,4,5-tetrakis(dibutyl)phosphate 23

Tetrakis(dibenzyl)phosphate **22** dissolved in the minimun amount of AcOEt was hydrogenated for 2h., under 4-5 psi, in the presence of the same amount of Pd/C 10%. The catalyst was removed by filtration on

Whatman paper and the filtrate was concentrated to give the tetrakisphosphate **23** quantitatively; $[\alpha]_D$ +1° (c 0.7, $CHCl_3$); ^1H NMR (250MHz; $CDCl_3$): δ: 4.75 (q, 1H, H-4; $J_{4-3}=J_{4-5}=9$); 4.6 (bs, 1H, H-2); 4.35 (m, 2H, H-1, H-5); 4.10 (m, 17H, H-3, $C\underline{H}_2O$); 3.2 (bs, 1H, O$\underline{H}$); 2.55 (dt, 1H, H-6eq, $J_{6eq-6ax}=12$; $J_{6eq-1}=J_{6eq-5}=4$); 2.35 (q, 1H, H-6ax; $J_{6eq-6ax}=J_{6ax-5}=J_{6ax-1}=12$); ^{13}C NMR (50MHz; $CDCl_3$): δ: 76.91 (C-4); 76.39 (C-3); 73.18 (C-2); 71.92; 69.72 (C-1, C-5); 67.90, 68.15 (8$\underline{C}H_2O$); 32.27 (8$\underline{C}H_2CH_2O$); 31.41 (C-6); 18,65 (8$\underline{C}H_2CH_3$); 13.53 [8$\underline{C}H_3(CH_2)_3$]; ^{31}P NMR (81MHz; $CDCl_3$):δ : -1.69, -1.43, -1.21, -0.97 (P_1, P_3, P_4, P_5); (Found C, 49.02; H, 8.70; P, 12.98; $C_{38}H_{80}O_{17}P_4$ requires C, 48.92; H, 8.64; P, 13.28).

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