Synthesis of *myo*-inositol 4,6-*cyclic*,1,5-trisphosphate, a conformationally restricted analogue of *myo*-inositol 1,4,5-trisphosphate

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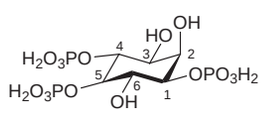
***myo*-Inositol 4,6-*cyclic*,1,5-trisphosphate 2 has been prepared starting from *myo*-inositol. The suitably protected intermediates, obtained by judicious selective protections and deprotections, have been phosphorylated. Final one-pot deprotection gives the expected, conformationally restricted, analogue of *myo*-inositol 1,4,5-trisphosphate. The physico-chemical behaviour of this derivative could explain its biological inactivity.**

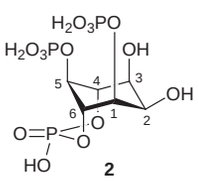
The second-messenger role played by 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** is now well established. Its interaction with the inositol trisphosphate receptors (IP_3-R) induces mobilisation of the intracellular calcium stores.[1,2] Structure–activity relationship (SAR) studies developed around compound **1**, permit the elucidation of some structural requirements for its agonist activity towards the IP_3-R. In particular, these SAR studies demonstrate the crucial importance of the vicinal phosphates in positions 4 and 5, the enhancing effect of the third phosphate in position 1, and the importance of the unphosphorylated hydroxy group in position 6.[3,4] Our researches in this field concern the synthesis and the physico-chemical properties of inositol phosphates (I*P*s) and related compounds.[5–14] Our studies have shown a possible correlation between the physico-chemical and the pharmacological properties of I*P*s. Thus, the ionisation state of the phosphate groups, and particularly that of the phosphate in position 5, seems of prime importance for binding to the receptors and for calcium mobilisation.[5] For polyphosphorylated inositols the evolution of the phosphate ionisation state *versus* the pH involves a complex set of reactions implying numerous microequilibria.[11]



1

It is particularly interesting to note that the activity increases simultaneously with the loss of the last acidic proton of Ins(1,4,5)P_3 **1**.[5] This last proton is localised mainly between the phosphates at C-4 and -5, and, therefore, stabilises the vicinal bisphosphate, allowing the two negatively charged groups to stay together. However, as the last proton is neutralised, the two strongly negatively charged groups will probably repel each other, leading to new orientations of the phosphate groups. The hydroxy group in position 6 seems also to be involved in these intramolecular interactions, as we have shown by comparing the physico-chemical behaviour of 6-deoxy-6-fluoro-Ins(1,4,5)P_3 with that of the parent compound **1**.[14]

It has been shown that the cyclohexane ring of Ins(1,4,5)P_3 stays in a chair conformation where the phosphoester oxygens (CHOP) occupy equatorial positions at any pH.[15] However, owing to their σ bonds, the phosphate groups could occupy large conformational areas around the medium plane of the carbon backbone. Not enough SAR studies have been developed to allow us to define precisely the location of the phosphates themselves. To enable there to be a large distance between the vicinal ionised phosphates we proposed the synthesis of compound **2** where the phosphoester oxygens in positions **4** and **5** stay nearly antiparallel. Such a structure differs apparently a great deal from the more stable conformation, since the cyclohexane chair conformation of Ins(1,4,5)P_3 **1** is inverted for the analogue **2**. Nevertheless, it is possible to superimpose the three phosphates of the cyclic derivative **2** with those of the parent compound **1** without the need for significant internal energy increase. In this molecular modelling analysis the hydroxy groups in positions 2 and 3 of compound **2** do not fit with the corresponding functions of Ins(1,4,5)P_3; however, these hydroxy groups seem not to be essential for the activity towards the IP_3-R. Moreover, as shown in preliminary molecular modelling studies, the internal energy difference between the two chair conformations is not very large (4–6 kcal mol^{-1}†). It cannot be excluded that conformational changes can occur at the receptor site. The supramolecular coordination energy could, in such a case, compensate for the possible increase in ligand internal energy.



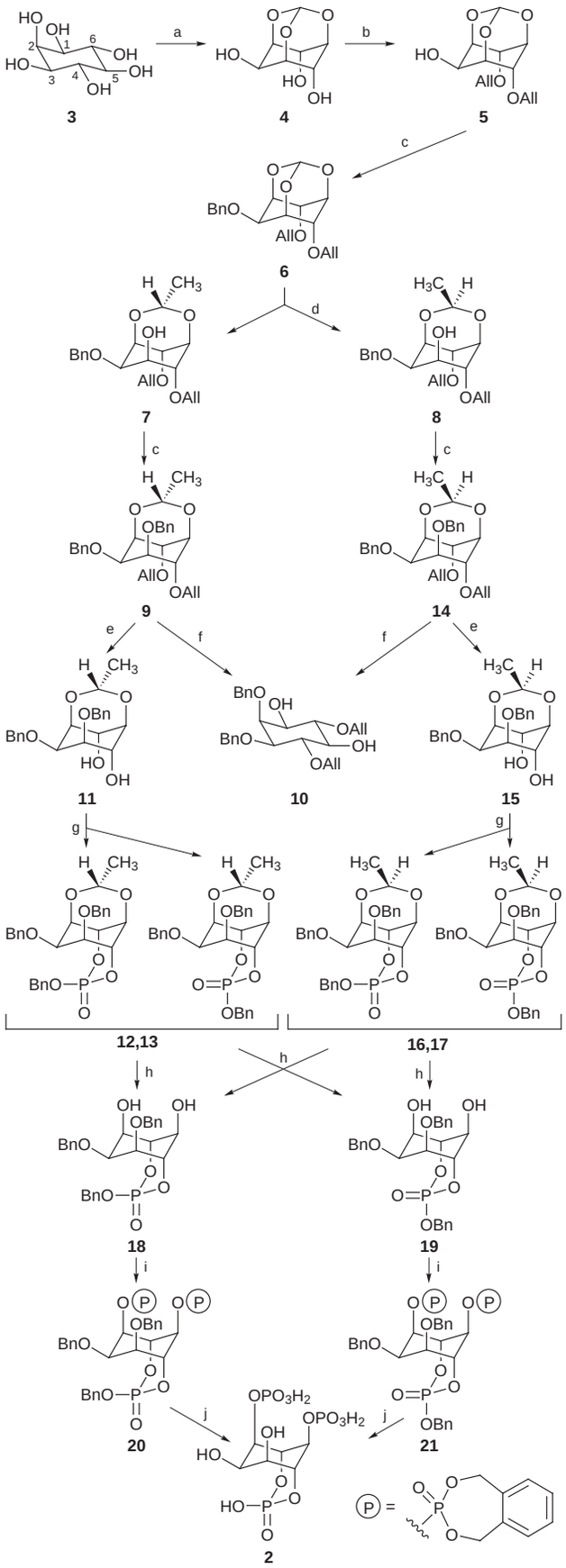
2

We report here the synthesis (±)-*myo*-inositol 4,6-*cyclic*,1,5-trisphosphate **2**.

Synthesis

The starting material for this synthesis was *myo*-inositol **3**, which was converted into the *meso*-orthoester **4** as previously described.[16] The two axial alcohols of compound **4** were protected as allyl ethers by reaction with allyl bromide to give the *meso*-compound **5** in 44% yield [triallyl derivative (26% yield) and traces of an unsymmetrical diallyl derivative were also formed] (Scheme 1). The last equatorial hydroxy function of

† 1 cal = 4.185 J.



Scheme 1 Synthesis of *myo*-inositol 4,6-*cyclic*,1,5-trisphosphate. *Reagents and conditions:* a, $HC(OEt)_3$, *p*-TsOH, DMF; b, NaH, AllBr, DMF, rt; c, NaH, BnBr, DMF, rt; d, $AlMe_3$, CH_2Cl_2, 0 °C, then rt; e, $RhCl(PPh_3)_3$, DABCO, aq. ethanol, reflux; then $Hg(OAc)_2$, aq. THF; f, c. HCl, reflux; g, $(Et_2N)_2POBn$, 1*H*-tetrazole, CH_2Cl_2; then MCPBA, CH_2Cl_2; h, TFA, aq. THF, EtOH, reflux; i, *o*-xylylene *N*,*N*-diethyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2; then MCPBA, CH_2Cl_2; j, H_2, Pd/C (10%), aq. MeOH, c-$C_6H_{11}NH_2$. All = allyl, Bn = benzyl.

compound **5** was protected with a benzyl group, leading to the totally protected *meso*-derivative **6**. The orthoester **6** was regioselectively opened by reduction with trimethylaluminium, which furnished two epimeric ethylidene derivatives **7** and **8** differing in their orientation of the methyl group. These epimers were obtained as racemates as were all the following intermediates and the final compound. Only one of these epimers was expected according to the mechanism proposed in the literature.[17] These two epimers were easily separated by chromatography. The stereochemistry of isomer **7** was established by means of a nuclear Overhauser effect spectroscopy (NOESY) experiment which showed an NOE effect between the acetalic proton and proton 4-H of the inositol ring. In addition no NOE was observed between the methyl group and the inositol protons. This observation is in agreement with the structure proposed by Gilbert *et al.*[17] for the selective opening of 2,4,6-tribenzyl-*myo*-inositol orthoformate. On the other hand, the NOESY experiment run with the epimer **8** showed an NOE effect between the acetalic proton and the inositol proton 6-H. The synthesis was continued on both epimers separately. For the isomer **7**, the liberated alcohol was protected as its benzyl ether to give compound **9**. Hydrolysis of the ethylidene protective group led to the monocyclic diol **10**. Treatment of the totally protected inositol **9** with a catalytic amount of tris(triphenylphosphine)rhodium(I) chloride in the presence of DABCO permitted the selective removal of the allylic protective groups, leading to the diaxial diol **11**. This diol **11** was phosphitylated by means of benzyloxybis(diethylamino)phosphine and 1*H*-tetrazole to give a cyclic phosphite, which was oxidised with MCPBA to afford two separable cyclic phosphoesters **12** and **13** differing in their stereochemistry of the phosphate group. The same procedure conducted on the epimer **8** also gave two cyclic phosphoesters, **16** and **17** (*via* compounds **14** and **15**). Hydrolysis of the ethylidene group of compound **14** gave the same monocyclic diol **10** as obtained from derivative **9**. The synthesis can be pursued on all four separated isomers **12**, **13**, **16** and **17**. Thus, acidic hydrolysis of the ethylidene protective group on S_P-phosphates **12** and **16** gave the diol **18** and the same deprotection of the derivatives R_P-phosphates **13** and **17** led to the analogue **19**. The two diols **18** and **19** were phosphorylated by the phosphite method with *o*-xylylene *N*,*N*-diethyl phosphoramidite and 1*H*-tetrazole followed by oxidation with MCPBA to give the protected phosphates **20** and **21**, respectively. Final deprotection by hydrogenolysis over Pd/C conducted on benzyl ethers **20** and **21** gave the expected racemic product **2**, which was stored as its stable cyclohexylammonium salt.

Binding properties

The product was tested on adrenal cortex microsomes.[18] No competitive inhibition of [^{3}H]Ins(1,4,5)P_3 binding was observed with the racemic cyclic compound **2** at any of the tested concentrations (10^{-11} to 10^{-5} M).

Discussion

The NMR titrations of the phosphate groups of Ins(1,4,5)P_3 and the cyclic derivative **2** show marked differences in their deprotonation processes. Thus, as expected, the chemical shift of the cyclic phosphate **2** remains unchanged during the titration as it contains only one strongly acidic proton which is totally dissociated over the studied pH range. For compounds **1** and **2**, the chemical-shift variations for the phosphate in position 1 show opposite effects for high pH-values; P1 for compound **1** is shielded whereas that for compound **2** shows an additional deshielding. The phosphate P5 for compound **1** presents a plateau between pH 6.5 and 8.5, which plateau is totally absent for the equivalent phosphate of derivative **2** (Figs. 1 and 2, respectively). These differences enlighten the conformational restrictions of the analogue **2**. In particular, the

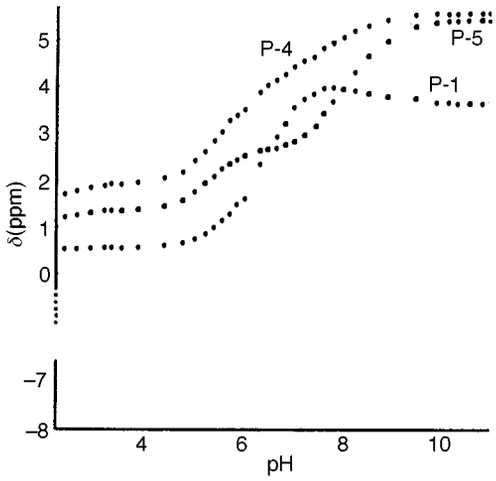


Fig. 1 ^{31}P NMR (water containing 10% D_2O) titration curves of *myo*-inositol 1,4,5-trisphosphate performed at 37 °C; medium: 0.2 M KCl

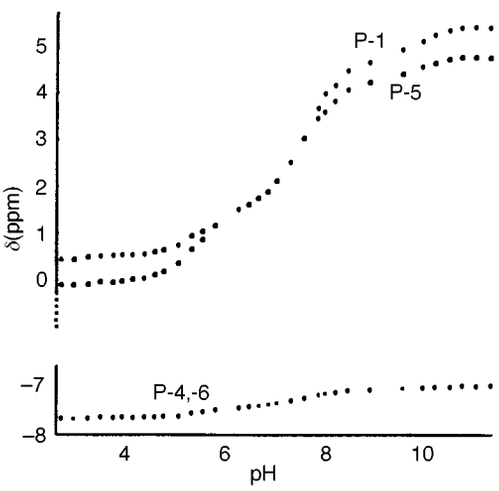


Fig. 2 ^{31}P NMR (water containing 10% D_2O) titration curves of *myo*-inositol 4,6-*cyclic*,1,5-trisphosphate performed at 37 °C; medium: 0.2 M KCl

neighbouring phosphate or hydroxy group can no longer participate in the ionisation state of the phosphate P5.[5] As a recent publication reports that no conformational changes were observed for the cyclohexyl ring for low phosphorylated inositol (mono-, bis- and tris-phosphates)[15] one can also consider that the rigidified analogue was unable to fit into the receptor site; the possible superimposition of the phosphates of compounds **1** and **2** leading to the wrong spacial arrangement. All these observations could explain the lack of activity of the cyclic compound **2**. The physico-chemical analyses for compound **2** will be reported in detail elsewhere. More work is in progress to enable us to understand the intramolecular interactions in these types of inositol derivatives.

Experimental

General methods

Mps were measured on a Mettler FP62 apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer; coupling constants *J* are given in Hz.

meso-4,6-Di-*O*-allyl-*myo*-inositol 1,3,5-*O*-orthoformate 5

Sodium hydride (60% oil dispersion; 2.37 g, 59.2 mmol) was added at 0 °C to a solution of *myo*-inositol orthoformate[16] **4** (5 g, 26.3 mmol) in anhydrous DMF (15 ml). The mixture was stirred for 20 min at 0 °C and allyl bromide (4.78 ml, 55.2 mmol) was slowly added. The mixture was warmed to room temperature (rt) and, after being stirred for 3 h, was treated with water (5 ml). The solvents were removed *in vacuo* and the residue was treated with a mixture of water (80 ml) and dichloromethane (160 ml). The organic layer was washed with brine and dried over sodium sulfate. The oil obtained after filtration and evaporation of the mixture was purified by chromatography on a silica gel column eluted with diethyl ether–hexane (1 : 2). Compound **5**: R_f (diethyl ether–hexane 1 : 2) 0.18 was obtained as an oil (3.15 g, 44%); $\delta_H(CDCl_3)$ 5.84 (M part of an ABMXX′, J_{MX} 17.2, $J_{MX'}$ 10.4, $J_{MA} = J_{MB} = 5.5$, 2 H, 2 × $OCH_2CH{=}CH_2$), 5.43 (d, *J* 1.3, 1 H, HCO_3), 5.25 (X part of an ABMXX′, J_{MX} 17.2, $J_{XX'} = J_{XA} = J_{XB} = 1.6$, 2 H, 2 × OCH_2-$CH{=}CH_{trans}$), 5.25 (X′ part of an ABMXX′, $J_{MX'}$ 10.4, $J_{XX'} = J_{X'A} = J_{X'B} = 1.6$, 2 H, 2 × $OCH_2CH{=}C_{cis}$H), 4.36 (tt, $J_{H5H6} = J_{H5H4} = 3.3$, $J_{H5H1} = J_{H5H3} = 1.6$, 1 H, 5-*H*), 4.23 (t, *J* 3.8, 2 H, 4- and 6-H), 4.15 (dt, *J* 3.4 and 1.8, 2 H, 1- and 3-H) and 4.1–3.9 [m, 5 H, containing at 4.04 (AB part of an ABMXX′, J_{AB} 12.7, $J_{AM} = J_{BM} = 5.6$, $J_{AX} = J_{AX'} = J_{BX} = J_{BX'} = 1.5$, $\Delta\delta$ 0.04, 4 H, 2 × $OCH_2CH{=}CH_2$ and 2-H_2].

Triallyl derivative (26%), R_f (diethyl ether–hexane 1 : 2) 0.36 and traces of unsymmetrical 2,4-diallyl derivative, R_f (diethyl ether–hexane 1 : 2) 0.16 were also formed.

meso-4,6-Di-*O*-allyl-2-*O*-benzyl-*myo*-inositol 1,3,5-*O*-orthoformate 6

The alcohol **5** (3.15 g, 11.4 mmol) was dissolved in anhydrous DMF (80 ml), the solution was cooled to 0 °C and sodium hydride (60%; 915 mg, 22.9 mmol) was added. After stirring of the mixture for 20 min, benzyl bromide (2.72 ml, 22.9 mmol) was added. The flask was warmed to rt and the contents were stirred overnight. The mixture was precipitated with water (4 ml) and extracted with ethyl acetate (2 × 100 ml). The organic layer was washed successively with water (2 × 100 ml) and brine (100 ml), dried over sodium sulfate, filtered and evaporated. The crude product was chromatographed on a silica gel column eluted with diethyl ether–hexane (1 : 4). The ether **6** was obtained as an oil (4.05 g, 99%), $\delta_H(CDCl_3)$ 7.5–7.2 (m, 5 H, $OCH_2C_6H_5$), 5.84 (M part of an ABMXX′, J_{MX} 17.2, $J_{MX'}$ 10.4, $J_{MA} = J_{MB} = 5.5$, 2 H, 2 × $OCH_2CH{=}CH_2$), 5.54 (d, *J* 1.3, 1 H, HCO_3), 5.26 (X part of an ABMXX′, J_{MX} 17.2, $J_{XX'} = J_{XA} = J_{XB} = 1.6$, 2 H, 2 × $OCH_2CH{=}CH_{trans}$), 5.19 (X′ of an ABMXX′, $J_{MX'}$ 10.4, $J_{XX'} = J_{X'A} = J_{X'B} = 1.5$, 2 H, 2 × OCH_2-$CH{=}C_{cis}$H), 4.41 (tt, $J_{H5H6} = J_{H5H4} = 3.5$, $J_{H5H1} = J_{H5H3} = 1.6$, 1 H, 5-*H*), 4.4–4.2 (m, 4 H, 1-, 3-, 4- and 6-H), 4.03 (AB part of an ABMXX′, J_{AB} 12.8, $J_{AM} = J_{BM} = 5.5$, $J_{AX} = J_{AX'} = J_{BX} = J_{BX'} = 1.6$, $\Delta\delta$ 0.08, 4 H, 2 × $OCH_2CH{=}CH_2$) and 3.98 (t, *J* 3.0, 1 H, 2-*H*).

(±)-4,6-Di-*O*-allyl-2-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol 7 and 8

meso-4,6-Di-*O*-allyl-2-*O*-benzyl-*myo*-inositol 1,3,5-*O*-orthoformate **6** was dissolved in anhydrous dichloromethane (50 ml) under argon. The solution was cooled to 0 °C, a solution of trimethylaluminium (2.0 M in hexane; 11.9 ml, 23.9 mmol) was slowly added and the reaction mixture was then allowed to return to rt. After 5 h of stirring at rt, TLC showed remaining starting material; further trimethylaluminium was therefore added (11.9 ml, 23.9 mmol) and the mixture was stirred overnight before being poured into a mixture of aq. sodium potassium tartrate (400 ml; 500 g l^{-1}) and saturated aq. ammonium chloride (400 ml). This mixture was vigorously stirred for 1 h. The product was extracted with dichloromethane (3 × 200 ml). The organic layer was dried over sodium sulfate, filtered and evaporated. Column chromatography on silica gel eluted with diethyl ether–hexane (1 : 4 then 1 : 2) permitted the separation of the two ethylidene epimers: compound **7**, an oil, R_f (diethyl ether–hexane 1 : 2) 0.45 (1.72 g, 48%) and epimer **8**, an oil, R_f (diethyl ether–hexane 1 : 2) 0.25 (0.95 g, 26%); $\delta_H(CDCl_3)$ for **7**: 7.5–7.2 (m, 5 H, $OCH_2C_6H_5$), 5.95 (M part of an ABMXX′, J_{MX} 15.9, $J_{MX'}$ 10.4, $J_{MA} = J_{MB} = 5.5$, 1 H, $OCH_2CH{=}CH_2$), 5.86

(M part of an ABMXX′, J_{MX} 17.2, $J_{MX'}$ 10.4, J_{MA} 6.8, J_{MB} 5.5, 1 H, OCH_2CH=CH_2), 5.4–5.1 [m, 5 H, containing at 5.54 (q, J 4.7, 1 H, $CHCH_3$) and 2 × OCH_2CH=CH_2], 4.69 (AB, J_{AB} 11.0, $\Delta\delta$ 0.28, 2 H, $OCH_2C_6H_5$), 4.48 (ddd, J_{H1H2} 6.2, J_{H1H6} 4.0, J 1.6, 1 H, 1-*H*), 4.31 (dt, J_{H3H2} 7.7, J_{H3H4} = J_{H3OH} = 6.6, 1 H, 3-*H*), 4.3–3.9 [m, 7 H, containing at 4.15 (dd, J_{H2H3} 7.8, J_{H2H1} 6.6, 1 H, 2-*H*), at 3.92 (td, J_{H6H5} = J_{H6H1} = 4.0, 1 H, 6-*H*), 5-*H* and 2 × OCH_2CH=CH_2], 3.73 (d, J_{H4H3} 6.4, 1 H, 4-*H*), 3.98 (d, J_{H3OH} 6.7, 1 H, D_2O-exchangeable, *OH*) and 1.12 (d, J 4.9, 3 H, CH_3); $\delta_H(C_6D_6)$ for **8**: 7.5–7.1 (m, 5 H, $OCH_2C_6H_5$), 5.84 (M part of an ABMXX′, J_{MX} 17.2, $J_{MX'}$ 10.4, J_{MA} 5.1, J_{MB} 4.9, 1 H, OCH_2CH=CH_2), 5.83 (M part of an ABMXX′, J_{MX} 17.2, $J_{MX'}$ 10.4, J_{MA} 5.1, J_{MB} 4.9, 1 H, OCH_2CH=CH_2), 5.29 (X part of an ABMXX′, J_{MX} 17.2, $J_{XX'}$ = J_{XA} = J_{XB} = 1.6, 1 H, OCH_2CH=$CH_{trans}H$), 5.26 (X part of an ABMXX′, J_{MX} 17.2, $J_{XX'}$ = J_{XA} = J_{XB} = 1.6, 1 H, OCH_2CH=$CH_{trans}H$), 5.19 (q, J 4.7, 1 H, $CHCH_3$), 5.12 (X′ part of an ABMXX′, $J_{MX'}$ 10.4, $J_{XX'}$ = $J_{X'A}$ = $J_{X'B}$ = 1.5, 1 H, OCH_2CH=$CH_{cis}H$), 5.09 (X′ part of an ABMXX′, $J_{MX'}$ 10.4, $J_{XX'}$ = $J_{X'A}$ = $J_{X'B}$ = 1.5, 1 H, OCH_2CH=$CH_{cis}H$), 4.67 (dq, J_{H1H6} 5.3, J_{H1H2} = J = 1.6, 1 H, 1-*H*), 4.63 (AB, J_{AB} 11.3, $\Delta\delta$ 0.47, 2 H, $OCH_2C_6H_5$), 4.62 (ddd, J_{H3H2} 5.3, J_{H3H4} 1.4, J_{H3OH} 8.2, 1 H, 3-*H*), 4.45 (td, J_{H5H6} = J_{H5H4} = 3.8, J 1.6, 1 H, 5-*H*), 4.36 (dd, J_{H2H3} 5.3, J_{H2H1} 1.4, 1 H, 2-*H*), 4.30 (ddd, J_{H6H5} 5.3, J_{H6H1} 3.8, J 1.4, 1 H, 6-*H*), 4.26 (dd, J_{H4H5} 3.8, J_{H4H3} 1.4, 1 H, 4-*H*), 4.1–3.6 [m, 5 H, containing at 3.99 (d, J_{H3OH} 8.2, 1 H, D_2O-exchangeable, *OH*) and 2 × OCH_2CH=CH_2] and 1.26 (d, J 4.7, 3 H, CH_3).

(±)-4,6-Di-*O*-allyl-2,3-di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol 9

The (±)-4,6-di-*O*-allyl-2-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol **7** (1.72 g, 4.57 mmol) was dissolved in anhydrous DMF (40 ml). The solution was cooled to 0 °C. Sodium hydride (60% oil suspension; 370 mg, 9.14 mmol) was added. After stirring of the mixture for 20 min, benzyl bromide (1.09 ml, 9.14 mmol) was added. The mixture was stirred at rt overnight. Water (2 ml) was added and the product was extracted with ethyl acetate (3 × 100 ml). The organic layer was washed successively with water (2 × 100 ml) and brine (100 ml), dried over sodium sulfate, filtered and evaporated. The crude product was chromatographed on a silica gel eluted with diethyl ether–hexane (1:9) to give the benzylated derivative **9** (2.0 g, 94%) [Found: C, 66.7; H, 7.0. Calc. for $C_{28}H_{34}O_6$ (M_r 466.57): C, 72.1; H, 7.4%]; $\delta_H(CDCl_3)$ 7.5–7.2 (m, 10 H, 2 × $OCH_2C_6H_5$), 5.96 (M part of an ABMXX′, J_{MX} 17.2, $J_{MX'}$ 10.4, J_{MA} = J_{MB} = 5.3, 1 H, OCH_2CH=CH_2), 5.84 (M part of an ABMXX′, J_{MX} 17.4, $J_{MX'}$ 10.4, J_{MA} 6.0, J_{MB} 5.5, 1 H, OCH_2CH=CH_2), 5.4–5.1 [m, 5 H, containing at 5.38 (q, J 4.9, 1 H, $CHCH_3$) and 2 × OCH_2CH=CH_2], 4.67 (AB, J_{AB} 11.5, $\Delta\delta$ 0.29, 2 H, $OCH_2C_6H_5$), 4.63 (AB, J_{AB} 11.5, $\Delta\delta$ 0.07, 2 H, $OCH_2C_6H_5$), 4.49 (ddd, J_{H1H2} 6.1, J_{H1H6} 4.0, J 1.8, 1 H, 1-*H*), 4.3–3.8 (m, 9 H, 2-, 3-, 4-, 5- and 6-H, 2 × OCH_2CH=CH_2) and 1.27 (d, J 4.8, 3 H, CH_3).

(±)-4,6-Di-*O*-allyl-2,3-di-*O*-benzyl-*myo*-inositol 10

Compound **9** or **14** (see below) (15 mg) was dissolved in methanol (2 ml), conc. HCl (0.2 ml) was added and the mixture was refluxed for 45 min. Neutralisation with $NaHCO_3$, filtration, and evaporation to dryness gave the crude product. Purification was made by preparative thick-layer chromatography using ethyl acetate–hexane (1:1) as developer to give diol **10** (80%), $\delta_H(CDCl_3)$ 7.5–7.2 (m, 10 H, 2 × $OCH_2C_6H_5$), 6.1–5.8 (m, 2 H, 2 × OCH_2CH=CH_2), 5.4–5.1 (m, 4 H, 2 × OCH_2CH=CH_2), 4.81 (AB, J_{AB} 11.5, $\Delta\delta$ 0.24, 2 H, $OCH_2C_6H_5$), 4.62 (s, 2 H, $OCH_2C_6H_5$), 4.5–4.2 (m, 4 H, 2 × OCH_2CH=CH_2), 3.98 (t, J_{H2H3} = J_{H2H1} = 2.6, 1 H, 2-*H*), 3.71 (t, J_{H4H3} = J_{H4H5} = 9.5, 1 H, 4-*H*), 3.51 (t, J_{H6H1} = J_{H6H5} = 9.3, 1 H, 6-*H*) and 3.4–3.3 [m, 3 H, containing at 3.33 (dd, J_{H3H4} 9.8, J_{H3H2} 2.4, 1 H, 3-*H*), 1- and 5-*H*; after D_2O exchange, at δ 3.37 (dd, J_{H1H6} 9.5, J_{H1H2} 2.7, 1 H, 1-*H*) and at δ 3.35 (t, J_{H5H6} = J_{H5H4} = 8.8, 1 H, 5-*H*)], 3.65 (s, 1 H, D_2O-exchangeable, O*H*) and 2.39 (d, J 6.2, 1 H, D_2O exchangeable, O*H*).

(±)-4,6-Di-*O*-allyl-2,3-di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol 14

Same procedure as for epimer **9**, with (±)-4,6-di-*O*-allyl-2-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol **8** (950 mg, 2.52 mmol), sodium hydride (60%; 200 mg, 5.04 mmol), benzyl bromide (600 µl, 5.04 mmol) and chromatography on silica gel [diethyl ether–hexane (1:4)], gave the benzylated product **14** (1.1 g, 94%) [Found: C, 71.8; H, 7.4. Calc. for $C_{28}H_{34}O_6$ (M_r 466.57): C, 72.1; H, 7.4%]; $\delta_H(CDCl_3)$ 7.6–7.2 (m, 10 H, 2 × $OCH_2C_6H_5$), 6.0–5.7 (m, 2 H, 2 × OCH_2CH=CH_2), 5.49 (q, J 4.8, 1 H, $CHCH_3$), 5.4–5.1 (m, 4 H, 2 × OCH_2CH=CH_2), 4.76 (s, 2 H, $OCH_2C_6H_5$), 4.68 (AB, J_{AB} 12.4, $\Delta\delta$ 0.03, 2 H, $OCH_2C_6H_5$), 4.61 (ddd, J 5.2, 4.0 and 1.2, 1 H, 6-*H*), 4.6–4.5 (m, 1 H, 1-*H*), 4.4–4.3 (m, 1 H, 5-*H*), 4.2–3.8 (m, 7 H, 2-, 3- and 4-*H* and 2 × OCH_2CH=CH_2) and 1.28 (d, J 4.8, 3 H, CH_3).

(±)-2,3-Di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol 11

(±)-4,6-Di-*O*-allyl-2,3-di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol **9** (1.0 g, 2.14 mmol) and DABCO (96 mg, 0.86 mmol) were dissolved in aq. ethanol (10% water; 30 ml). The catalyst $RhCl(PPh_3)_3$ (280 mg, 0.30 mmol) was added and the mixture was refluxed for 3 h. TLC showed the total transformation of the starting material. The reaction mixture was poured in water (100 ml) and extracted with ethyl acetate (2 × 100 ml). The organic layer was dried over sodium sulfate, filtered and evaporated. The crude product (1.23 g) was taken in a mixture of THF (8 ml) and water (2.5 ml). Under vigorous stirring, aq. mercury(II) acetate (1.5 g, 4.7 mmol in 4 ml) was rapidly added to the mixture. After 20 min the mixture was diluted with diethyl ether (40 ml). The organic layer was separated, dried over sodium sulfate, filtered and evaporated. Chromatography on a silica gel column eluted with diethyl ether–hexane (2:1) gave (±)-2,3-di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol **11** (670 mg, 81%), mp 174 °C [Found: C, 68.2; H, 7.0. Calc. for $C_{22}H_{26}O_6$ (M_r 386.44): C, 68.4; H, 6.8%]; $\delta_H(CDCl_3)$ 7.6–7.2 (m, 10 H, 2 × $OCH_2C_6H_5$), 5.65 (q, J 4.9, 1 H, $CHCH_3$), 4.65 (AB, J_{AB} 11.5, $\Delta\delta$ 0.07, 2 H, $OCH_2C_6H_5$), 4.60 (AB, J_{AB} 11.7, $\Delta\delta$ 0.13, 2 H, $OCH_2C_6H_5$), 4.35 (t, J 4.9, 1 H, 5-*H*), 4.3–4.0 (m, 5 H, 1-, 2-, 3-, 4- and 6-H), 3.60 (d, J 4.2, 1 H, D_2O-exchangeable, O*H*), 3.48 (d, J 6.4, 1 H, D_2O-exchangeable, O*H*) and 1.25 (d, J 4.7, 3 H, CH_3).

(±)-2,3-Di-*O*-benzyl-1,5-*O*-ethylidne-*myo*-inositol 15

Same procedure as for compound **11**, with (±)-4,6-di-*O*-allyl-2,3-di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol **14** (245 mg, 0.525 mmol), DABCO (25 mg, 0.22 mmol), $RhCl(PPh_3)_3$ (67 mg, 0.08 mmol), mercury(II) acetate (436 mg, 1.37 mmol), furnished title compound **15** (128 mg, 65%), $\delta_H(CDCl_3)$ 7.6–7.2 (m, 10 H, 2 × $OCH_2C_6H_5$), 5.26 (q, J 4.9, 1 H, $CHCH_3$), 4.84 (t, J 4.9, 1 H, 5-*H*), 4.67 (AB, J_{AB} 11.7, $\Delta\delta$ 0.08, 2 H, $OCH_2C_6H_5$), 4.57 (AB, J_{AB} 12.3, $\Delta\delta$ 0.05, 2 H, $OCH_2C_6H_5$), 4.34 (d, J 4.2, 1 H, 4-*H*), 4.3–4.1 [m, 3 H, containing at δ 4.42 (br s, 1 H, 6-*H*), at δ 4.15 (br s, 1 H, 1-*H*), and 1 H, D_2O-exchangeable, O*H*], 4.01 (d, J 4.4, 1 H, 2-*H*), 3.95 (dd, J 4.2 and 1.5, 1 H, 3-*H*), 3.62 (br s, 1 H, D_2O-exchangeable, O*H*) and 1.09 (d, J 4.8, 3 H, CH_3).

(±)-2,3-Di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate 12 and 13

The diol **11** (400 mg, 1.04 mmol) and 1*H*-tetrazole (220 mg, 3.10 mmol), dried *in vacuo*, were dissolved in anhydrous dichloromethane (12 ml). Benzyloxybis(diethylamino)phosphine (570 mg, 2.0 mmol) was added and the mixture was stirred at rt for 6 h. The flask was then cooled to −78 °C and a solution of MCPBA (790 mg, 3.2 mmol) in dichloromethane (5 ml) was added. After 1 h, the mixture was diluted with ethyl acetate (30 ml) and washed successively with 10% aq. sodium

hydrogen sulfite (2 × 50 ml), 5% aq. sodium hydrogen carbonate (2 × 50 ml) and brine (2 × 50 ml). The organic layer was separated, dried over sodium sulfate, filtered and evaporated. Chromatography on a silica gel column eluted with diethyl ether–hexane (2:1) gave the two epimeric cyclic phosphate triesters **12** and **13** (461 mg, global yield 83%). Compound **12**: R_f (diethyl ether–hexane 2:1) 0.35 [Found: C, 64.4; H, 5.9; P, 5.3. Calc. for $C_{29}H_{31}O_8P$ (M_r 538.53): C, 64.7; H, 5.8; P, 5.8%]; $^1H\{^{31}P\}$ $\delta_H(CDCl_3)$ 7.6–7.2 (m, 15 H, 3 × $OCH_2C_6H_5$), 6.05 (q, *J* 4.7, 1 H, C*H*CH_3), 5.12 (s, 2 H, $POCH_2C_6H_5$), 4.8–4.6 [m, 6 H, containing at δ 4.79 (AB, J_{AB} 11.7, $\Delta\delta$ 0.06, 2 H, $OCH_2C_6H_5$), at δ 4.63 (AB, J_{AB} 11.5, $\Delta\delta$ 0.02, 2 H, $OCH_2C_6H_5$), and 4- and 5-*H*], 4.57 (td, $J_{H6H5} = J_{H6H1} = 4.6$, *J* 1.8, 1 H, 6-*H*), 4.51 (t, $J_{H1H6} = J_{H1H2} = 3.7$, 1 H, 1-*H*), 4.36 (dd, J_{H2H3} 7.5, J_{H2H1} 4.2, 1 H, 2-*H*), 4.31 (d, J_{H3H2} 7.3, 1 H, 3-*H*) and 1.24 (d, *J* 4.8, 3 H, CH_3). $^{31}P\{^1H\}$ $\delta_P(CDCl_3)$ −8.42.

Compound **13**: R_f (diethyl ether–hexane 2:1) 0.22 (Found: C, 60.7; H, 6.0; P, 7.0); $^1H\{^{31}P\}$ $\delta_H(CDCl_3)$ 7.6–7.1 (m, 15 H, 3 × $OCH_2C_6H_5$), 6.00 (q, *J* 4.8, 1 H, C*H*CH_3), 4.99 (AB, J_{AB} 11.7, $\Delta\delta$ 0.06, 2 H, $POCH_2C_6H_5$), 4.8–4.6 [m, 5 H, containing at δ 4.78 (td, $J_{H6H5} = J_{H6H1} = 4.4$, *J* 2.4, 1 H, 6-*H*), and $OCH_2C_6H_5$, 4- and 5-*H*], 4.51 (AB, J_{AB} 11.9, $\Delta\delta$ 0.04, 2 H, $OCH_2C_6H_5$), 4.42 (t, $J_{H1H6} = J_{H1H2} = 4.1$, 1 H, 1-*H*), 4.19 (dd, J_{H2H3} 7.5, J_{H2H1} 4.5, 1 H, 2-*H*), 4.04 (d, J_{H3H2} 7.5, 1 H, 3-*H*) and 1.24 (d, *J* 4.8, 3 H, CH_3); $^{31}P\{^1H\}$ $\delta_P(CDCl_3)$ −8.40.

(±)-2,3-Di-*O*-benzyl-1,5-*O*-ethylidene-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate 16 and 17

Same procedure as for epimers **12** and **13**, with the diol **15** (128 mg, 0.33 mmol), 1*H*-tetrazole (70 mg, 0.99 mmol), benzyloxybis(diethylamino)phosphine (164 mg, 0.58 mmol) and MCPBA (270 mg, 0.93 mmol). Thick-layer chromatography of the crude product gave two additional cyclic phosphate triesters (115 mg, global yield 65%). Compound **16**: R_f (diethyl ether–hexane 4:1) 0.40; $^1H\{^{31}P\}$ $\delta_H(CDCl_3)$ 7.6–7.2 (m, 15 H, 3 × $OCH_2C_6H_5$), 5.36 (q, *J* 4.8, 1 H, C*H*CH_3), 5.23 (td, $J_{H6H5} = J_{H6H1} = 5.1$, *J* 2.4, 1 H, 6-*H*), 5.13 (s, 2 H, $POCH_2C_6H_5$), 4.84 (t, $J_{H5H4} = J_{H5H6} = 4.0$, 1 H, 5-*H*), 4.8–4.6 [m, 6 H, containing at δ 4.72 (AB, J_{AB} 11.7, $\Delta\delta$ 0.10, 2 H, $OCH_2C_6H_5$), at δ 4.70 (s, 2 H, $OCH_2C_6H_5$), and 1- and 4-*H*], 4.19 (dt, J_{H3H2} 5.1, *J* 1.1, 1 H, 3-*H*), 4.14 (dd, J_{H2H3} 5.1, J_{H2H1} 2.2, 1 H, 2-*H*) and 1.22 (d, *J* 4.8, 3 H, CH_3); $^{31}P\{^1H\}$ $\delta_P(CDCl_3)$ −9.31.

Compound **17**: R_f (diethyl ether–hexane 4:1) 0.11; $^1H\{^{31}P\}$ $\delta_H(C_6D_6)$ 7.6–7.2 (m, 15 H, 3 × $OCH_2C_6H_5$), 5.1–4.9 [m, 3 H, containing at δ 5.07 (d, *J* 0.9, 2 H, $POCH_2C_6H_5$) and 5-*H*], 4.87 (q, *J* 4.7, 1 H, C*H*CH_3), 4.81 (td, $J_{H6H5} = J_{H6H1} = 4.9$, *J* 2.5, 1 H, 6-*H*), 4.7–4.4 [m, 7 H, containing at δ 4.54 (AB, J_{AB} 12.1, $\Delta\delta$ 0.17, 2 H, $OCH_2C_6H_5$), at δ 4.41 (d, *J* 2.7, 2 H, $OCH_2C_6H_5$), 1-, 4- and 6-*H*], 4.21 (dd, J_{H2H3} 5.1, J_{H2H1} 2.2, 1 H, 2-*H*), 4.04 (dt, J_{H3H2} 5.1, *J* 0.9, 1 H, 3-*H*) and 1.18 (d, *J* 4.7, 3 H, CH_3); $^{31}P\{^1H\}$ $\delta_H(CDCl_3)$ −8.29.

(±)-2,3-Di-*O*-benzyl-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate 18 and 19

A mixture of esters **12** and **13** (460 mg, 0.85 mmol) was dissolved in THF (0.3 ml) and ethanol (95%; 3.0 ml). A water–TFA mixture (2:1; 0.9 ml) was then added and the mixture was refluxed for 2 h. The solvents were evaporated off and the crude product was purified by silica gel thick-layer chromatography with development first with diethyl ether–hexane (2:1) and then twice with ethyl acetate–hexane (1:1). Two fractions were isolated, corresponding to the two isomeric (±)-2,3-di-*O*-benzyl-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphates **18** and **19**, with a 52% global yield. Compound **18**: R_f (ethyl acetate–hexane 1:1) 0.43 (124 mg) [Found: C, 61.0; H, 5.9; P, 6.0. Calc. for $C_{27}H_{29}O_8P$ (M_r 512.50): C, 63.3; H, 5.7; P, 6.0]; $^1H\{^{31}P\}$ $\delta_H(C_6D_6)$ 7.5–7.1 (m, 15 H, 3 × $OCH_2C_6H_5$), 5.07 (AB, J_{AB} 11.7, $\Delta\delta$ 0.05, 2 H, $POCH_2C_6H_5$), 4.85 (br s, 1 H, 5-*H*), 4.77 (q, *J* 2.9, 1 H, 6-*H*), 4.60 (q, *J* 2.7, 1 H, 4-*H*), 4.52 (t, *J* 3.4, 1 H, 1-*H*), 4.45 (t, *J* 3.7, 1 H, 1-*H*), 4.39 (s, 2 H, $OCH_2C_6H_5$), 4.25 (AB, J_{AB} 11.3, $\Delta\delta$ 0.08, 2 H, $OCH_2C_6H_5$) and 4.15 (t, *J* 3.5, 1 H, 3-*H*); $^{31}P\{^1H\}$ $\delta_P(CDCl_3)$ −8.73.

Compound **19**: R_f (ethyl acetate–hexane 1:1) 0.15 (125 mg); $^1H\{^{31}P\}$ $\delta_H(CDCl_3)$ 7.5–7.2 (m, 15 H, 3 × $OCH_2C_6H_5$), 5.06 (s, 2 H, $POCH_2C_6H_5$), 4.85 (q, *J* 3.1, 1 H, 6-*H*), 4.81 (q, *J* 3.0, 1 H, 4-*H*), 4.8–4.6 [m, 3 H, containing at δ 4.71 (AB, J_{AB} 11.5, $\Delta\delta$ 0.02, 2 H, $OCH_2C_6H_5$) and 5-*H*], 4.56 (AB, J_{AB} 11.7, $\Delta\delta$ 0.04, 2 H, $OCH_2C_6H_5$), 4.5–4.3 (m, 1 H, 1-*H*), 4.2–4.1 (m, 2 H, 2- and 3-*H*), 4.08 (d, J_{H5OH} 11.3, 1 H, D_2O-exchangeable, O*H*) and 3.00 (d, J_{H1OH} 1.5, 1 H, D_2O-exchangeable, O*H*); $^{31}P\{^1H\}$ $\delta_P(CDCl_3)$ −7.90.

The same procedure applied to a mixture of epimeric ethylidene ethers of **16** and **17** led to the same diols **18** and **19**.

(±)-2,3-Di-*O*-benzyl-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate 1,5-di-*O*-(*o*-xylylene phosphate) 20

(±)-2,3-Di-*O*-benzyl-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate **18** (105 mg, 0.205 mmol), 1*H*-tetrazole (115 mg, 1.64 mmol) and *o*-xylylene *N*,*N*-diethylphosphoramidite (196 mg, 0.82 mmol), dried *in vacuo*, were dissolved in anhydrous dichloromethane (2 ml). After being stirred for 2 h at rt, the mixture was cooled at −78 °C and MCPBA (323 mg, 1.31 mmol) as a solution in dichloromethane (3 ml) was added. The mixture was allowed to warm to rt. After 30 min, ethyl acetate (50 ml) was added. The organic layer was washed successively with 10% aq. sodium hydrogen sulfite (2 × 50 ml), then with 5% aq. sodium hydrogen carbonate (2 × 50 ml) and brine (50 ml). The organic layer was dried over sodium sulfate and evaporated. The crude product was chromatographed on a silica gel column eluted with ethyl acetate–hexane (1:1) to give 143 mg (yield 79%) (±)-2,3-di-*O*-benzyl-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate 1,5-di-*O*-(*o*-xylylene phosphate) **20** (143 mg, 79%), $^1H\{^{31}P\}$ $\delta_H(CDCl_3)$ 7.5–6.8 (m, 23 H, 3 × $OCH_2C_6H_5$ and 2 × $OCH_2C_6H_4CH_2O$), 5.44 (t, *J* 3.2, 1 H, 5-*H*), 5.29 (t, *J* 4.2, 1 H, 1-*H*), 5.19 (s, 2 H, $POCH_2C_6H_5$), 5.08 (q, *J* 3.3, 1 H, 4-*H*), 5.02 (AB, J_{AB} 13.5, $\Delta\delta$ 0.29, 2 H, $POCH_2C_6H_4CH_2O$), 4.98 (q, *J* 3.2, 1 H, 6-*H*), 4.88 (AB, J_{AB} 13.9, $\Delta\delta$ 0.86, 2 H, $POCH_2C_6H_4CH_2O$), 4.81 (AB, J_{AB} 10.8, $\Delta\delta$ 0.18, 2 H, $OCH_2C_6H_5$), 4.76 (AB, J_{AB} 13.4, $\Delta\delta$ 0.58, 2 H, $POCH_2C_6H_4CH_2O$), 4.72 (s, 2 H, $OCH_2C_6H_5$), 4.66 (AB, J_{AB} 13.3, $\Delta\delta$ 0.10, 2 H, $POCH_2C_6H_4CH_2O$), 4.46 (t, *J* 4.2, 1 H, 2-*H*) and 4.38 (t, *J* 3.7, 1 H, 3-*H*); $^{31}P\{^1H\}$ $\delta_H(CDCl_3)$ −0.93, −2.05 and −9.77; *m*/*z* (EI^+) 876.9 (M^+).

(±)-2,3-Di-*O*-benzyl-*myo*-inositol 4,6-*cyclic*-*O*-benzyl phosphate 1,5-di-*O*-(*o*-xylylene phosphate) 21

The same procedure applied to diol **19** (105 mg, 0.205 mmol) and by means of 1*H*-tetrazole (115 mg, 1.64 mmol), *o*-xylylene *N*,*N*-diethylphosphoramidite (196 mg, 0.82 mmol), MCPBA (323 mg, 1.31 mmol) led to the isomeric trisphosphate **21** (152 mg, 85%); R_f (CH_2Cl_2–MeOH 95:5) 0.34; $^1H\{^{31}P\}$ $\delta_H(CDCl_3)$ 7.5–6.5 (m, 23 H, 3 × $OCH_2C_6H_5$ and 2 × $OCH_2C_6H_4CH_2O$), 5.93 (t, *J* 2.7, 1 H, 5-*H*), 5.56 (t, *J* 3.5, 1 H, 1-*H*), 5.4–5.2 [m, 16 H, containing at δ 5.17 (s, 2 H, $POCH_2C_6H_5$), at δ 4.98 (AB, J_{AB} 13.5, $\Delta\delta$ 0.51, 2 H, $POCH_2C_6H_4CH_2O$), at δ 4.91 (AB, J_{AB} 13.9, $\Delta\delta$ 0.93, 2 H, $POCH_2C_6H_4CH_2O$), at δ 4.80 (AB, J_{AB} 14.3, $\Delta\delta$ 0.70, 2 H, $POCH_2C_6H_4CH_2O$), at δ 4.54 (s, 2 H, $OCH_2C_6H_5$), at δ 4.46 (AB, J_{AB} 10.8, $\Delta\delta$ 0.30, 2 H, $OCH_2C_6H_5$), at δ 4.22 (t, *J* 3.4, 1 H, 3-*H*) and 2-, 4- and 6-*H*]; $^{31}P\{^1H\}$ $\delta_P(CDCl_3)$ −1.26, −2.03 and −9.16.

(±)-*myo*-Inositol 4,6-*cyclic*,1,5-trisphosphate 2

The phosphotriester **20** or **21** (140 mg, 0.159 mmol) as a solution in a 3:1 methanol–water mixture (40 ml) was hydrogenolysed under pressure (5 atm) in the presence of 10% of Pd/C (0.1 g) and cyclohexylamine (93.2 μl, 0.81 mmol) for 12 h at 20 °C. The Pd/C catalyst was filtered off and the filtrate was evaporated to give the crude product (164 mg). One part of this crude product (64 mg) was purified on an ion-exchange chromatography column (Q Sepharose Fast Flow) eluted with

an aq. ammonium hydrogen carbonate gradient (0 to 0.8 M) to give pure compound **2** (49 mg), $^{1}H\{^{31}P\}$ $\delta_H(D_2O$; pH 1.0) 5.27 (t, *J* 2.9, 1 H, 5-*H*), 4.91 (q, *J* 2.9, 1 H, 6-*H*), 4.8–4.6 [m, 2 H, containing at δ 4.62 (t, *J* 4.0, 1 H, 1-*H*) and 4-*H*], 4.33 (t, *J* 4.2, 1 H, 2-*H*) and 4.13 (t, *J* 3.5, 1 H, 3-*H*); $^{31}P\{^{1}H\}$ $\delta_P(D_2O)$ 0.79 (s, 1 P, *P*-1), 0.27 (s, 1 P, *P*-5) and −7.22 (s, 1 P, *P*-4, -6); *m*/*z* (FAB) 402.1 (M^+) and 400.9 [M − H].

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