A Conformationally Restricted Cyclic Phosphate Analogue of Inositol Trisphosphate: Synthesis and Physicochemical Properties

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The design and total synthesis of DL-6-deoxy-6-(hydroxymethyl)-*scyllo*-inositol 1:7-cyclic 2,4-trisphosphate (**4**), a conformationally restricted cyclic phosphate analogue of the second messenger inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], is described. The protected inosose 2,4,6/3,5-pentahydroxy-3,5-bis-*O*-(*p*-methoxybenzyl)-2,4,6-*O*-methylidynecyclohexanone (**7**) was obtained from *myo*-inositol orthoformate in two steps, and Wittig methylenation and then hydroboration−oxidation using 9-BBN−H/OH^-/H_2O_2 gave the axial hydroxymethyl derivative **9**. A series of protection/deprotection steps provided the diol **13**, which was converted into two cyclic phosphate esters **14a** and **14b**, epimeric at phosphorus, by reaction with (benzyloxy)bis(*N*,*N*-diisopropylamino)phosphine /1*H*-tetrazole followed by *m*-CPBA. Two other hydroxyl groups were then exposed and phosphorylated, and total deprotection gave racemic **4**. NMR studies confirmed that in **4** the phosphate group equivalent to the 4-phosphate of Ins(1,4,5)P_3 is held in the positive gauche orientation and that the inositol ring maintains a chair conformation from pH 2 to 12. Investigation of the acid−base properties of **4** using potentiometric and ^{31}P NMR techniques showed that, over the physiological pH range, **4** behaves as a diprotic acid and that the ionization of the phosphate group equivalent to the 5-phosphate of Ins(1,4,5)P_3 is enhanced. In biological assays, **4** appears to behave as a weak full agonist at the platelet Ins(1,4,5)P_3 receptor, and the possible interpretation of this result is discussed.

1. Introduction

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] functions as an intracellular signaling molecule by interacting with a family of receptor-operated Ca^{2+} channels to mobilize nonmitochondrial Ca^{2+} in many cell types.[1] Ins(1,4,5)P_3 receptors (IP_3Rs) are now known to be tetrameric with a central ion channel, and the four subunits may be of different subtypes, allowing the existence of heterotetramers.[2] It is currently assumed that each IP_3R subunit has a positively charged pocket containing basic amino acid residues that engage in ionic interactions with the negative charges on the three phosphate groups of the Ins(1,4,5)P_3 molecule,[3] although no direct information is yet available on the three-dimensional structure of the binding site, nor of the receptor-bound conformation of Ins(1,4,5)P_3. Many analogues of Ins(1,4,5)P_3 have been synthesized[4] and to date all molecules behaving as agonists at Ins(1,4,5)P_3 receptors have contained a structure equivalent to the vicinal *trans*-4,5-bisphosphate of Ins(1,4,5)P_3, although isosteres such as phosphorothioate[5] may be tolerated to some extent.

Previously, we demonstrated that linking of the vicinal phosphates in Ins(1,4,5)P_3 to give the 1-phosphate-4,5-pyrophosphate analogue **2** abolishes Ca^{2+}-releasing activity.[6] Although **2** may be regarded as a conformationally restricted analogue of Ins(1,4,5)P_3, it differs from Ins(1,4,5)P_3 in two important ways. First, the formation of the cyclic pyrophosphate in **2** reduces the negative charge on the equivalents of *both* the 4- and 5-phosphate groups in Ins(1,4,5)P_3. Physicochemical studies by some of us have shown that the affinity of Ins(1,4,5)P_3 for its receptor is correlated with the degree of ionization of the phosphate group at position 5, suggesting that binding affinity will be maximized for a fully ionized 5-phosphate.[7] Second, it is probable that **2** mimics a high-energy conformation of Ins(1,4,5)P_3, unlikely to exist in appreciable amounts in solution or at the IP_3R binding site, due to electrostatic repulsion between the two vicinal phosphates. The remaining pyrophosphate oxygens would therefore be incorrectly placed to interact with the appropriate basic amino acid residues in the Ins(1,4,5)P_3 binding pocket. We therefore set out to design a

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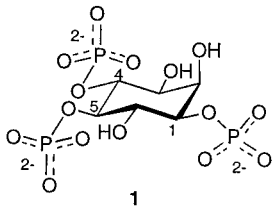
different conformationally restricted Ins(1,4,5)P_3 analogue that would mimimize these difficulties by constraining only the 4-phosphate group, preferably in an orientation that would mimic a low-energy conformation of the 4,5-bisphosphate in Ins(1,4,5)P_3. It would be interesting to see whether such an analogue could retain some of the binding affinity of Ins(1,4,5)P_3 or show novel activity in its interaction with the Ins(1,4,5)P_3 receptor or Ins(1,4,5)P_3-metabolizing enzymes.



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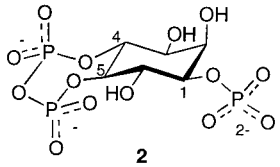

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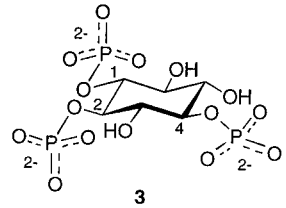

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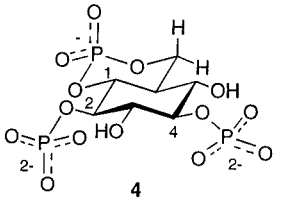
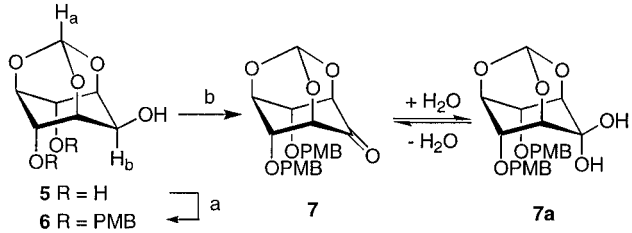


4

To constrain the 4-phosphate group it would be necessary to tether it to the carbon atom at position 3 of the inositol ring, to give a cyclic phosphate. This would, in turn, require the removal of the 3-hydroxyl group, and its replacement with a methylene bridge. This strategy is valid because it had previously been shown that 3-deoxy-Ins(1,4,5)P_3 is highly active,[8,9] and it is also known that the Ins(1,4,5)P_3 receptor can accommodate slightly increased bulk around the 3-position.[10] In contrast, steric bulk at position 6 is poorly tolerated,[11] and deletion of the 6-hydroxyl group leads to a 70-fold reduction in activity,[12] affirming the importance of retaining the 6-hydroxyl group in the target structure. We had previously shown that the *scyllo* analogue of Ins(1,4,5)P_3 [*scyllo*-Ins(1,2,4)P_3, **3**][13] in which the relative stereochemistry is all-trans, showed high potency, approaching that of Ins(1,4,5)P_3 itself. The implication of this, that it was not necessary to retain the *myo*-inositol configuration in the target molecule, would greatly simplify the synthetic strategy. This conjecture was later borne out by the finding that the then newly discovered adenophostins showed remarkably high potency at the IP_3R, while lacking an equivalent to the axial 2-hydroxyl group of Ins(1,4,5)P_3.[14]

Finally, our own molecular modeling investigations of Ins(1,4,5)P_3 showed that conformers of Ins(1,4,5)P_3 in which the P4−O4−C4−H4 torsion angle was positive and synclinal were energetically more favorable than those having the antiperiplanar conformation. This suggested that the cyclohexane and dioxaphosphorinane rings of the target molecule should be fused in a trans sense, to mimic a low-energy conformation. Taken together then, all these considerations suggested the initial target **4**. Retrosynthetic analysis of **4** led to a symmetrical precursor which, in turn, could be synthesized from *myo*-inositol orthoformate. This strategy would initially lead to racemic **4** but should be capable of modification to give optically pure material by resolution of an intermediate should this prove necessary. We report here a synthetic route to racemic **4** and an examination of its inframolecular acid−base properties. Some of the synthetic work has been presented in Communication form.[15]

Scheme 1[a]



[a] Key: (a) NaH (2.1 equiv), PMBCl (2.0 equiv), DMF; (b) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C and then Et_3N. PMB = *p*-methoxybenzyl. $^5J_{Ha-Hb}$ = 0.9 Hz.

2. Results and Discussion

2.1 Synthesis. Reaction of *myo*-inositol orthoformate (**5**)[16] with 2.1 equiv of *p*-methoxybenzyl chloride and 2.3 equiv of sodium hydride gave the symmetrical 4,6-disubstituted alcohol **6** as the highly crystalline major product, easily recognizable from the NMR spectra by its plane of symmetry. With careful workup and chromatography, it was possible to increase the yield of **6** to around 40% (Scheme 1).

Swern oxidation of **6** using DMSO/oxalyl chloride was expected to give the ketone **7**. However, after aqueous workup, an IR spectrum of the product showed two bands at 3540 and 3440 cm^{-1}, suggesting two alcohol groups, together with a band at 1760 cm^{-1}, corresponding to the expected ketone. The ^{13}C NMR spectrum of the product showed the unusual feature of a quaternary carbon resonating at δ 88.67, corresponding to $C(OH)_2$, confirming that **7** had been partially converted into the *gem*-diol **7a**. After the mixture of products was refluxed in toluene, with azeotropic removal of water, pure ketone **7** was isolated in 92% yield. The highly crystalline *gem*-diol **7a** could also be isolated by allowing a solution of the ketone in dioxane with a few drops of water added to stand for a few days. The ease of hydration of ketone **7** may be attributable to strain effects in its rigid cagelike structure, resulting in destabilization of sp^2-hybridized carbon relative to sp^3, and since our preliminary report[15] a similar phenomenon has been reported for a related compound.[17] Ketone **7** also reacted with methanol at room temperature to give a single hemiketal.

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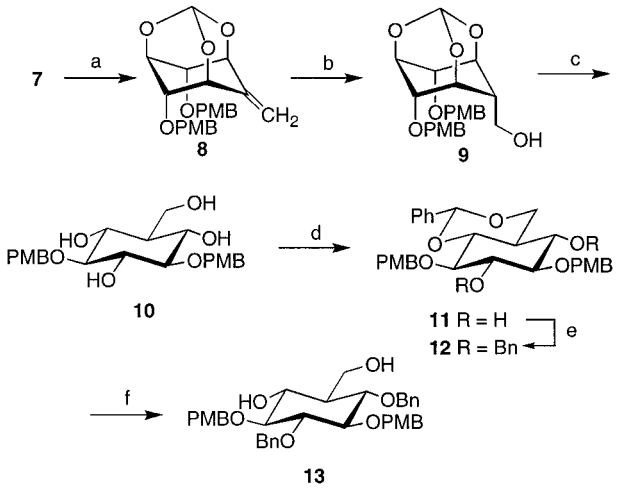
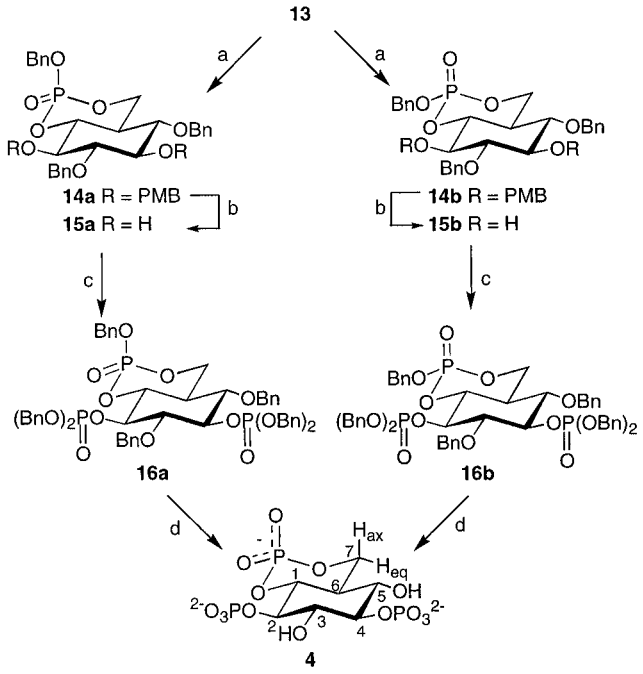
Scheme 2[a]



[a] Key: (a) CH_3PPh_3Br, *t*-BuOK, THF, reflux; (b) (i) 9BBN-H, THF, 50 °C, (ii) H_2O_2, OH^-; (c) (i) 1 M HCl/MeOH 1:10, 50 °C, (ii) $NH_{3(aq)}$; (d) $C_6H_5CH(OMe)_2$, DMF, *p*-TsOH; (e) NaH, BnBr, DMF; (f) 1 M HCl/THF/MeOH 1:5:5, reflux. PMB = *p*-methoxybenzyl. Bn = benzyl. Asymmetrical compounds are racemic.

Scheme 3[a]



[a] Key (a) (i) $BnOP(NPr^i_2)_2$, 1*H*-tetrazole, (ii) *m*-CPBA, −78 °C; (b) DDQ, CH_2Cl_2, H_2O; (c) (i) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, (ii) *m*-CPBA, −78 °C; (d) Na/liq NH_3. PMB = *p*-methoxybenzyl. Bn = benzyl. All compounds are racemic.

The next step was the conversion of ketone **7** into the symmetrical alkene **8** by Wittig methylenation using methylenetriphenylphosphorane (Scheme 2). In the first attempts, using methyltriphenylphosphonium bromide, potassium *tert*-butoxide as base and heating to 50 °C for 2 h, the yields were consistently low (30 to 50%). Further investigations showed that the intermediate oxaphosphetane, visible in the ^{31}P NMR spectrum as a single resonance at δ_P −68.9, was unusually stable. A sample of this material could be kept for several days at 4 °C with only slight decomposition. When the reaction was repeated, but with heating to reflux for 2 h after adding ketone to Wittig reagent, **8** was obtained in 91% yield.

The alkene **8** was converted into alcohol **9** by hydroboration/oxidation using 9-BBN-H followed by alkaline hydrogen peroxide solution, and a single product was obtained in 97% yield after chromatography. The strategy exploits the fact that one diastereotopic face of alkene **8** is exposed while the other is very hindered by the two *p*-methoxybenzyl groups, allowing only one orientation of approach by the bulky 9-BBN-H molecule. Hydroboration using borane−THF was less successful, giving mainly **9**, but accompanied by various other minor products, which were not identified. It was possible to deduce the stereochemistry of **9** as follows: It has been noted that the ^1H NMR spectra of *myo*-inositol orthoformate and many of its derivatives show a long-range five-bond coupling (typically around 1 Hz) between the methylidyne proton and the axial proton at C-2.[18] Such a coupling was observed in the ^1H NMR spectrum of **6** (Scheme 1). Now the corresponding signal in the spectrum of the hydroboration/oxidation product was a sharp singlet (δ 5.58). The fact that there was no coupling to the equivalent proton of H-2 was evidence that this proton was no longer axial and that the product therefore had the desired structure **9**.

Mild acid treatment removed the orthoformate ester without significant loss of *p*-methoxybenzyl groups to give the tetrol **10**. To maximize the yield of **10**, it was necessary to add aqueous ammonia after the acid hydrolysis stage, to cleave a persistent formate ester intermediate resulting from partial hydrolysis of the cage. The ^1H NMR spectrum of **10** showed only axial-axial ^3J couplings between ring protons, confirming the previously deduced stereochemistry of the precursor **9** beyond doubt.

Two protection steps were now used to produce the fully protected **12**. The racemic benzylidene acetal **11** was prepared in 93% yield by the reaction of tetrol **10** with benzaldehyde dimethyl acetal in dry DMF with a catalytic amount of PTSA at 70 °C. High yields and short reaction times were made possible by continuous removal of formed methanol using an air condenser attached to a filter pump. Benzylation under standard conditions then gave fully protected **12**. This is a versatile intermediate[19] because either the benzylidene or *p*-methoxybenzyl groups can be removed chemoselectively or the benzylidene ring can be reduced regioselectively in either direction.

The benzylidene acetal was removed selectively by mild acid hydrolysis, giving diol **13**, which was reacted with 1.2 equiv of the bifunctional phosphitylating agent (benzyloxy)bis(*N*,*N*-diisopropylamino)phosphine[20] in the presence of 3 equiv of 1*H*-tetrazole to give two cyclic phosphite triesters, epimeric at phosphorus as expected (Scheme 3). These intermediates were visible as two spots close together on the TLC plate, and also as two distinct signals at δ_P 125.0 and δ_P 130.4 in the ^{31}P NMR spectrum. These signals disappeared and were replaced by peaks at δ_P −4.6 and −7.5 after addition of *m*-CPBA. It was possible to separate the two epimeric cyclic phosphate

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triesters by column chromatography, and they were isolated as crystalline solids.

The configurations at phosphorus of the two epimers were determined by examination of their ^{31}P and ^{1}H NMR chemical shifts, $^{3}J_{HCOP}$ coupling constants and P═O stretching frequencies. Epimers **14a** and **14b** can be regarded as esters of 2-oxo-1,3,2-dioxaphosphorinanes, and studies have been published on simple related compounds.[21−23] In all previous studies on isomeric pairs of phosphorinanes (see ref 23 and references therein) the axially substituted epimer has an upfield ^{31}P chemical shift. This identifies the less polar epimer (δ_P −7.5) as **14a** and the more polar isomer (δ_P −4.6) as the equatorially substituted **14b**. The P═O stretching frequency in the IR spectrum of **14b** is 21 cm^{-1} lower than that for **14a** ($\nu_{P=O}$ 1287 cm^{-1}), confirming this assignment.

The *p*-methoxybenzyl ethers of **14a** and **14b** were cleaved with DDQ, giving the corresponding diols **15a** and **15b**. TLCs taken during the reaction suggested that a competing reaction was loss of the benzyl protecting group on the cyclic phosphate, but the diols were isolated in moderate yields. A closer examination of the ^{1}H NMR spectra of **14a**, **14b**, **15a**, and **15b** revealed further evidence for the assigned configurations at phosphorus. In the equatorially protected epimers **14b** and **15b**, H-1 and H-7_{ax} are deshielded, as expected for protons in a 2,4-diaxial relationship with a P═O group. These protons therefore resonate downfield of the corresponding protons in **14a** and **15a**, while the H-7_{eq} protons have similar chemical shifts in all four molecules (approximately δ 4.4).

The diols **15a** and **15b** were next phosphitylated using the monofunctional phosphitylating agent bis(benzyloxy)(*N*,*N*-diisopropylamino)phosphine[24] with 1*H*-tetrazole. This step involves the formation of a phosphite triester at a position vicinal to an existing phosphate triester, and in each case we were able to observe in the ^{31}P NMR spectrum a $^{5}J_{PP}$ spin−spin coupling of 1.2 Hz between the phosphorus atoms of neighboring phosphate and phosphite groups. To the best of our knowledge, such a long-range P(III)−P(V) coupling has not previously been reported, although it is known in vicinal P(III)−P(III) systems. Oxidation with *m*-CPBA and purification gave crystalline **16a** and **16b**, neither of which showed any phosphorus−phosphorus couplings.

The axially protected **16a** was deprotected using sodium in liquid ammonia, and purification by ion-exchange chromatography on Q-Sepharose Fast Flow gave the target compound **4** in 78% yield as the triethylammonium salt. The structure was confirmed by ^{1}H, ^{31}P, and ^{13}C NMR. A $^{1}H-^{1}H$ COSY NMR spectrum allowed all the proton resonances to be assigned unequivocally. The equatorially protected **16b** was also deblocked with equal success.

The proton-coupled ^{31}P NMR spectrum of **4** (Figure 1) shows clearly the two different classes of phosphate esters and the expected heteronuclear spin couplings, with the phosphorus atoms in the unconstrained phosphate groups at positions 2 and 4 appearing as doublets with $^{3}J_{HP}$ approximately 7 Hz. The signal from the cyclic phosphate phosphorus atom (δ_P −2.76) is also a doublet, with a large coupling constant ($^{3}J_{P-Heq}$ = 22.5 Hz). The predicted $^{3}J_{HP}$ coupling constant for a dihedral angle of 180° is approximately 23 Hz,[25] and so we can conclude that the angle $H_{eq}COP$ is close to 180°. This suggests that the dioxaphosphorinane ring adopts a chair conformation as shown in Figure 1.

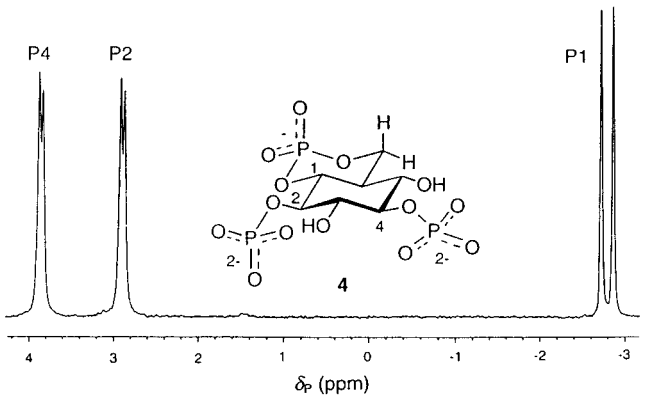

Figure 1. 162 MHz ^{31}P NMR spectrum of **4** (^{1}H-coupled, Na^+ salt, D_2O, pH ∼8).

2.2 Physicochemistry. We now proceeded to investigate the physicochemical properties of **4** by the use of potentiometric and NMR investigations. Previous studies[26−28] have shown drastic changes in the binding profile and Ca^{2+} mobilization of Ins(1,4,5)P_3 as well as of other inositol phosphates as a function of pH. Such alterations can be of biological significance since intracellular pH varies in subcellular compartments following, for instance, phospholipase C activation or trophic factor action.[29] Thus, a knowledge of the acid−base properties of an inositol phosphate may contribute to a better understanding of the events occurring at a molecular level. These properties are usually expressed as overall protonation or dissociation constants which characterize a molecule as a whole, but a detailed investigation of the ionization state of each individual phosphate group provides a more accurate picture of the distribution of charges in the molecule at any given pH. The protonation process has therefore to be expressed in terms of microprotonation equilibria, which are characterized by their related microconstants. These equilibria are influenced in a complex way by various aspects of the molecular structure, such as the nature and orientation of neighboring functional groups. We have previously determined the microprotonation scheme for various bis and tris phosphates[7,30] and analyzed the protonation of tetrakis phosphates[31] at an "inframolecular level", i.e., at a level referring to a unique donor site. The conformationally restricted analogue **4** is unique, however, in

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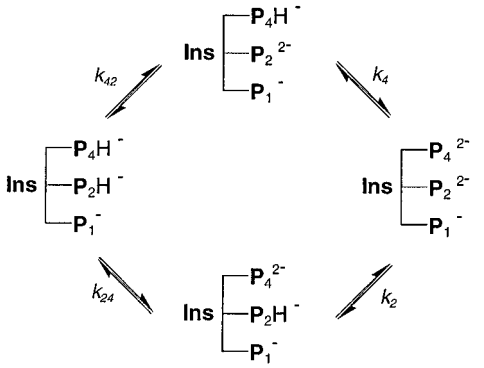


Figure 2. Microprotonation states of **4.**

that it combines a cyclic phosphate diester and phosphate monoesters together in the same molecule. Since the ionization of the 5-phosphate group appears to be so crucial in Ca^{2+} mobilizing activity, and the overall ionization of the crucial 4,5-bisphosphate system is clearly different than that of two individual phosphate groups, we felt it of particular interest to study a system where a weakly acidic function [represented by one pK_a of the 4-phosphate in Ins(1,4,5)P_3] had effectively been removed and the conformational access of this phosphate group to its vicinal partner was highly restricted.

A sample of **4** (triethylammonium salt) was first converted into the free acid and then titrated with potassium hydroxide solution. The study was performed in a 0.2 mol·dm^{-3} KCl solution at 37 °C, conditions that roughly mimic those encountered within the cell. When the pH was decreased from 10 to 3, the fully deprotonated **4**, bearing five negative charges, gained only two protons, leading to a diprotonated species. Thus, in the pH range considered, **4** behaves as a difunctional ligand characterized by the macroscopic stepwise protonation equilibria

$$H_{y-1}L^{(5-y+1)-} + H^+ \rightleftarrows H_yL^{(5-y)-}$$

and the related K_y constants, where $y = 1$ and 2 stand for the first and second step, respectively. The microprotonation diagram is depicted in Figure 2. Note that the second protonation of the phosphates P2 and P4 and the protonation of P1 occur below pH 3, well outside the physiological range, and the related constants were therefore not considered in this study.

In previous studies[30] we have shown that, in a number of favorable cases, the analytical concentration of the coexisting microspecies can ultimately be obtained from ^{31}P NMR spectroscopy carried out over a large pH range. If the observed chemical shift for the phosphorus resonance δ_i^{obs} depends on the electronic effects accompanying the variations in protonation state, then the protonated fraction $f_{i,p}$ of a phosphate group in position i on the inositol ring can be calculated by eq 1,

$$f_{i,p} = \frac{\delta_i^{obs} - \delta_{i,d}}{\delta_{i,p} - \delta_{i,d}} \quad (1)$$

where $\delta_{i,p}$ and $\delta_{i,d}$ correspond respectively to the chemical shifts of the protonated and deprotonated fractions of the phosphates in position i. The previous condition can be checked by the superimposition of the $\bar{p} = f(pH)$ curves

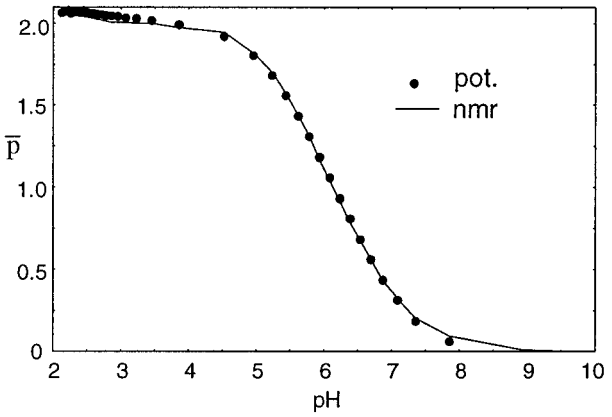


Figure 3. Mean number of protons bound per molecule of **4** ($\bar{p}$) vs pH calculated from potentiometric (●) and ^{31}P NMR (—) measurements.

Table 1[a]

y	log $K_y \pm \sigma$	i	log $k_i \pm \sigma$	ii'	log $k_{ii'} \pm \sigma$
1	6.70 ± 0.01	4	6.10 ± 0.01	42	6.20 ± 0.02
	6.76 ± 0.03				
2	5.60 ± 0.01	2	6.57 ± 0.01	24	5.73 ± 0.02
	5.64 ± 0.04				

[a] Logarithms of the macro- and microprotonation constants for **4** in 0.2 M KCl at 37 °C. The macroconstants given in italic were determined from the ^{31}P-NMR data. log k_i and log $k_{ii'}$ represent a general designation for, respectively, the logarithms of the first and second stepwise microprotonation constants. The uncertainties are estimates of the standard deviation as calculated by Superquad, Hypnmr, and Enzfitter (Elsevier-Biosoft) for the macro- and microconstants.

calculated from both the potentiometric and NMR data according to eqs 2 and 3, respectively.

$$\bar{p} = \frac{C_H - [H^+] + [OH^-]}{C_L} \quad (2)$$

$$\bar{p} = \sum_{i=1}^{i=N} f_{i,p} \quad (3)$$

In these equations, $\bar{p}$ is the mean number of protons bound per molecule of **4**, while C_H and C_L correspond to the analytical concentrations of the acid and **4**, respectively. Figure 3 shows the excellent superimposition of both $\bar{p} = f(pH)$ curves for **4**, thus allowing further interpretation of the δ_i^{obs} in terms of microprotonation constants.

As shown earlier,[7] $f_{i,p}$ can be expressed as a function of the macro- and microprotonation constants and the proton concentration. For instance, the fractions of protonation of phosphate P2 ($f_{2,p}$) are defined as

$$f_{2,p} = \frac{k_{24}k_2[H^+]^2 + k_2[H^+]}{k_{24}k_2[H^+]^2 + (k_2 + k_4)[H^+] + 1} \quad (4)$$

The equation is solved by nonlinear regression, introducing $K_1 = k_2 + k_4$ obtained by potentiometry or NMR experiments. It can be seen that the logarithms of the macroprotonation constants (Table 1) determined by both techniques are in good agreement.

The ^{31}P NMR titration curves and the corresponding protonation fractions of **4** are shown in Figure 4. These curves appear monophasic for P2 and P4, indicating the

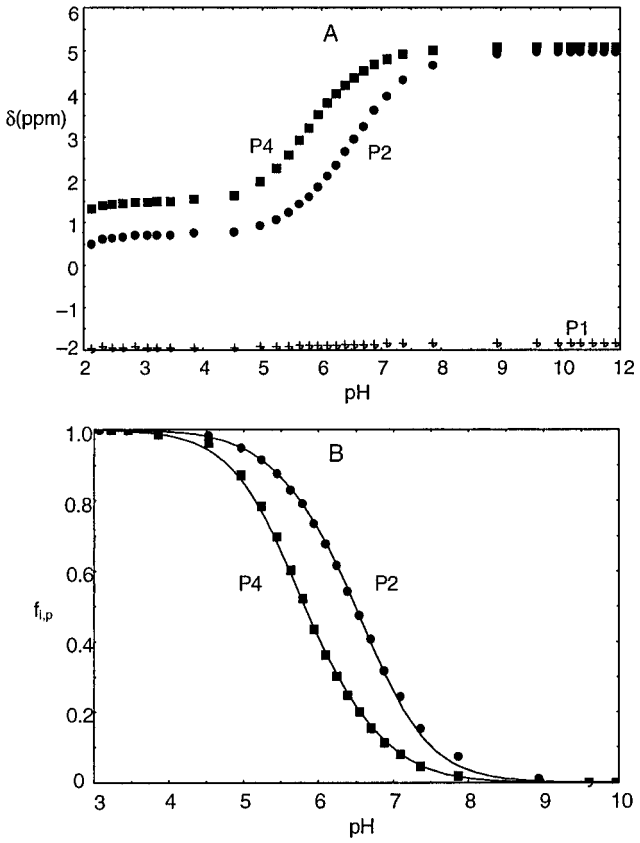


Figure 4. Chemical shifts δ from a ^{31}P NMR titration for **4** (A) and the corresponding protonation fraction curves $f_{i,p}$ (B) as a function of pH in 0.2 M KCl at 37 °C ($H_2O-10\%D_2O$). The least-squares fit of $f_{i,p}$ vs pH according to type 4 equations is shown in solid line.

relative independence of the two phosphate groups, while the P1 chemical shifts do not change significantly, affirming that the cyclic phosphate remains monocharged over all the studied pH range. The determination of the microconstants (Table 1) and the derived $k_2/k_4 = 2.95$ ratio reveal that P2 is more basic than P4. This confirms the importance of the presence of neighboring OH groups which tend to decrease the basic character of a phosphate either through an inductive field effect or via changing the local dielectric constant of the solvent.[7,30] Thus, the higher basicity of phosphate P2 seems due to the presence of a phosphate ester oxygen, rather than through the stabilization of the negative charge of P1 which appears not to be affected by the deprotonation of P2. Calculation of the interactivity parameters allows the quantification of the basicity changes at one site when the other site takes up a proton. For **4** this parameter is defined as $\Delta\log k_{2-4} = \log k_2 - \log k_{42} = \log k_4 - \log k_{24} = 0.37$. This value is not too far from $\Delta\log k_{1-4} = 0.07$ for Ins(1,4)P_2 compared to $\Delta\log k_{4-5} = 2.12$ for Ins(4,5)P_2.[7] Such a result confirms that P2 and P4 tend to behave independently during the protonation process.

It should also be noticed that the $\delta_i^{obs} = f(\text{pH})$ curves of **4** appear very straightforward with regard to those of Ins(1,4,5)P_3.[7] In particular, the unexpected initial downfield shift of P1 (equivalent of P4 for **4**) occurring for Ins(1,4,5)P_3 with the addition of the first equivalent of protons is not observed. This may be related to the *scyllo*-inositol structure of **4** and the difference in the configuration of one vicinal hydroxyl group in the two molecules.

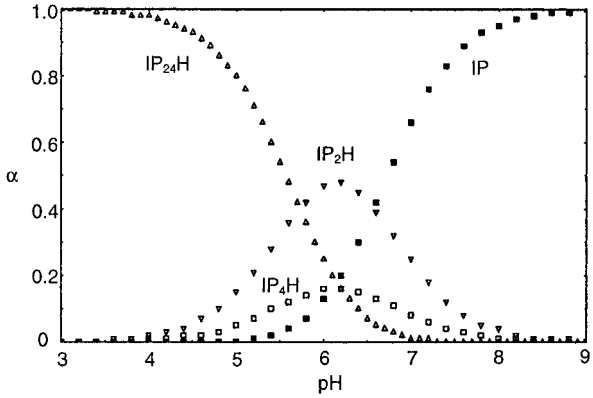


Figure 5. Distribution curves of the protonated microspecies of **4** in 0.2 M KCl at 37 °C plotted against pH.

Figure 5 shows the distribution of the various microprotonated species as a function of pH, providing a direct observation of the protonation state of the P2 and P4 phosphate groups. It is noteworthy that, in the ligand concentration used in this study, and at physiological pH (7.5), phosphate P2 is only 10% protonated, whereas in Ins(1,4,5)P_3, the P5 phosphate (the equivalent of P2 in **4**) is protonated to the extent of about 40%.[7] As discussed above, it has been shown that for Ins(1,4,5)P_3 specific binding to brain membrane receptors increases with pH and especially with the ionization of the P5 phosphate. Thus, the higher dissociation of the equivalent phosphate group in **4** would therefore be expected to enhance binding to the receptor at physiological pH.

^{1}H NMR titration curves (Figure 6) were also obtained, to follow possible conformational changes over the studied pH range. On going from the lowest to the highest pH limits ($2.0 < \text{pH} < 12$) the H-1, H-2, H-3, and H-4 resonances are shifted upfield by about 0.15 ppm while the rest of the signals are almost unaffected. As we observed previously,[30] the ^{1}H resonances of the *myo*-inositol ring may be influenced by the ionization of both the distal and adjacent phosphates. In addition, the coupling pattern as well as the $^3J_{H-H}$ coupling constants of the ring protons remain unchanged from pH 2 to 12. They undoubtedly correspond to an equatorial chair conformation slightly distorted by the cyclization of the P1 phosphate.

2.3 Conclusions. We have described the design and synthesis of a novel conformationally restricted cyclic phosphate analogue of Ins(1,4,5)P_3. NMR studies confirm that in **4** the phosphate group corresponding to P4 of Ins(1,4,5)P_3 is held in the positive gauche orientation and the inositol ring maintains a chair conformation over the entire pH range studied. One consequence of introducing the cyclic phosphate has been to simplify the acid−base properties of the analogue so that **4** behaves essentially as a diprotic acid under physiological conditions, with the equivalent of P4 in Ins(1,4,5)P_3 being singly charged over the physiological pH range. Another effect has been to enhance the acidity of the equivalent of P5 in Ins(1,4,5)P_3. Finally, the *scyllo*-inositol structure of **4** appears to have slightly modified the behavior of the equivalent of the 1-phosphate in Ins(1,4,5)P_3.

So far, the biological properties of **4** are less clear. When **4** was examined for Ca^{2+} mobilizing ability at platelet Ins(1,4,5)P_3 receptors using saponin-permeabilized platelets loaded with $^{45}Ca^{2+}$, it appeared to behave

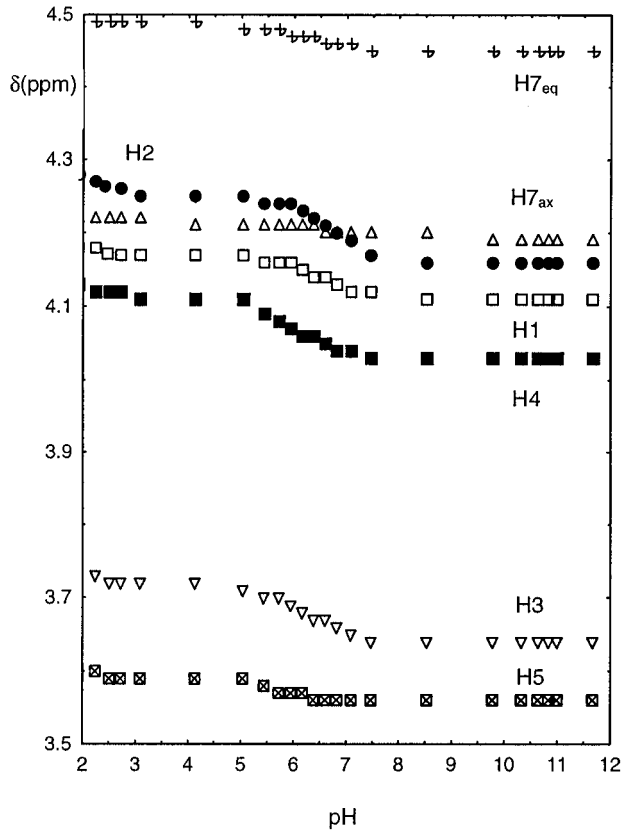
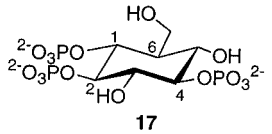


Figure 6. Chemical shifts δ from a ^{1}H NMR titration for **4** as a function of pH in 0.2 M KCl at 37 °C (D_2O). The H6 resonance appearing at about 2.3 ppm has been omitted.

as a full agonist, being able to release all of the Ins(1,4,5)-P_3 -sensitive Ca^{2+} pool, but was 40−70-fold weaker than Ins(1,4,5)P_3 itself. Assuming that only one enantiomer of **4** is active, this would imply an approximately 30-fold reduction in potency compared to Ins(1,4,5)P_3. Binding studies in rat cerebellar membranes showed that the affinity of **4** for the cerebellar Ins(1,4,5)P_3 receptor was much reduced (200 to 300-fold) compared to Ins(1,4,5)-P_3. However, we have now shown that 6-deoxy-6-(hydroxymethyl)-*scyllo*-inositol 1,2,4-trisphosphate [6-CH_2-OH-*scyllo*-Ins(1,2,4)P_3, (**17**)], an analogue of **4** in which the dioxaphosphorinane ring has been opened to give a phosphate monoester with an adjacent hydroxymethyl group, shows remarkably high activity in the same assays, being equipotent to Ins(1,4,5)P_3 itself, despite being racemic.[19]



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While it is possible then that the cyclic phosphate of **4** might be able to mimic the 4-phosphate of Ins(1,4,5)P_3, it is difficult to be certain at this stage whether the apparent behavior of **4** as weak full agonist might not originate from partial enzymatic hydrolysis of **4** by nonspecific phosphodiesterases to give **17** during the biological assays. Because of the high activity of **17**, this is particularly difficult to assess. The Ins(1,4,5)P_3-like activity of **17** does, however, confirm that the assumptions discussed above regarding the design of **4** were valid; the *scyllo*-inositol structure and added methylene group are clearly no obstacle to potent activity.

In summary, we have designed an efficient synthetic route to the first example of a conformationally restricted inositol polyphosphate analogue which is, we believe, a unique example of a molecule possessing both phosphate monoester and six-membered cyclic phosphodiester groups. Investigation of microprotonation equilibria has shown clear differences in the analogue as compared to Ins-(1,4,5)P_3. While the biological activity of **4** may be complex and is under current examination, it seems likely that the approaches outlined here will find further application in the phosphoinositide field among the wealth of newly discovered inositol phosphates and phospholipids.

3. Experimental Section

Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica 60 F_{254}) with detection by UV light or with phosphomolybdic acid in methanol followed by heating. Flash chromatography was performed on silica gel (Sorbsil C60). Dichloromethane was distilled over phosphorus pentoxide and stored in the presence of 4 Å molecular sieves. DMF was purchased in anhydrous form and stored over 4 Å sieves. THF was distilled from sodium benzophenone ketyl under a nitrogen atmosphere. ^{31}P NMR shifts were measured in ppm relative to external 85% phosphoric acid and are positive when downfield from this reference. FAB mass spectra were recorded at the EPSRC Mass Spectrometry Service Centre, Swansea, and at the University of Bath using *m*-nitrobenzyl alcohol as the matrix. Microanalysis was carried out by the Microanalysis Service, University of Bath. Melting points (uncorrected) were determined using a Reichert-Jung hot stage microscope apparatus. Ion-exchange chromatography was performed on an LKB−Pharmacia medium-pressure ion exchange chromatograph using Q-Sepharose Fast Flow and gradients of triethylammonium bicarbonate (TEAB) as eluent. Quantitative analysis of phosphate was performed using a modification of the Briggs phosphate assay.[32,33]

Potentiometric and NMR determinations were carried out as previously described.[7,30] The experiments were performed in two steps in which the same initial solution of **4** of about 3 $\times 10^{-3}$ mol·dm^{-3} was successively subjected to potentiometric and ^{31}P NMR or ^{1}H NMR titrations. The processing of the pH measurements allowed the total concentration of the ligand and the acid as well as the macroscopic protonation constants (by using SUPERQUAD[34]) to be determined. ^{31}P NMR spectra were recorded at 81.015 MHz on a Bruker AC 200 Fourier transform spectrometer. Field-frequency lock was achieved using 10% D_2O. The HypNMR program[35] was used to check the potentiometrically determined protonation constants. The ^{1}H NMR titration was performed on a Bruker 300 DPX spectrometer on 0.45 mL of solution in D_2O instead of 2 mL for the ^{31}P NMR titration. Phosphorus resonances of **4** were assigned by performing phosphorus−proton and proton−proton 2D correlation experiments at pH = 6.8.

4,6-Bis-*O*-(*p*-methoxybenzyl)-1,3,5-*O*-methylidyne-*myo*-inositol (6). To a solution of *myo*-inositol orthoformate[16] (**5**) (15.0 g, 78 mmol) in dry DMF (300 mL) at 0 °C was added sodium hydride (7.2 g of a 60% dispersion in oil, 180 mmol). The mixture was stirred for 20 min at 0 °C, and then *p*-methoxybenzyl chloride (22.5 mL, 166 mmol) was added. The mixture was stirred for a further 2 h at rt, after which TLC

(32) Briggs, A. P. *J. Biol. Chem.* **1922**, *53*, 13−16.
(33) Lampe, D.; Liu, C.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 907−912.
(34) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195−1200.
(35) Frassineti, C.; Ghelli, S.; Gans, P.; Sabatini, A.; Moruzzi, M. S.; Vacca, A. *Anal. Biochem.* **1995**, *231*, 374−382.

(dichloromethane/ethyl acetate 2:1) showed a major product at R_f 0.49 and minor products (mono- and tri-*O*-*p*-methoxybenzylated material) at R_f 0.22 and 0.64. Water (10 mL) was added carefully to quench the reaction. Solvents were evaporated in vacuo, and the residue was partitioned between water (200 mL) and dichloromethane (400 mL). The organic layer was washed with brine (200 mL), dried ($MgSO_4$), and evaporated to give an oil which was purified by flash chromatography (dichloromethane/ethyl acetate 5:1) giving, in order of elution, the tri-*O*-*p*-methoxybenzylated ether, di-*O*-*p*-methoxybenzylated material, and, finally, monosubstituted products. Recrystallization of the second fraction from ethyl acetate/hexane gave **6** (13.5 g, 31.4 mmol, 40% yield): mp 120−121 °C (from ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.26 (1 H, d, J = 11.3 Hz, D_2O ex), 3.79 (6 H, s), 4.16 (1 H, br d, J = 11.2 Hz, D_2O ex gives br s), 4.18−4.20 (2 H, m) 4.32 (2 H, t, J = 3.4 Hz), 4.40 (1 H, tt, J = 3.4 Hz, 1.8 Hz), 4.48, 4.56 (4 H, AB, J_{AB} = 11.0 Hz) 5.46 (1 H, d, J = 0.9 Hz), 6.81 (4 H, d, J = 8.9 Hz), 7.17 (4 H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 55.14, 61.12, 67.74, 71.21, 72.90, 73.38, 103.24, 113.75, 129.27, 129.53, 159.29; MS *m*/*z* (positive ion FAB, rel intensity) 431 [$(M + H)^+$, 12%], 309 [$(M - C_7H_6OCH_3)^+$, 15%], 121 [$(C_7H_6OCH_3)^+$, 100%]. Anal. Calcd for $C_{23}H_{26}O_8$ (430.45): C, 64.18; H, 6.09. Found: C, 63.9; H, 6.05.

2,4,6/3,5-Pentahydroxy-3,5-bis-*O*-(*p*-methoxybenzyl)-2,4,6-*O*-methylidynecyclohexanone (7). Dry dichloromethane (40 mL) was placed into a 250 mL flask under an atmosphere of N_2. A solution of oxalyl chloride in dry dichloromethane (12.8 mL of a 2 M solution, 25.6 mmol) was added, and the flask was cooled to −60 °C using a chloroform/solid CO_2 bath. A solution of anhydrous DMSO (3.6 mL, 51 mmol) in dry dichloromethane (5 mL) was added dropwise over 5 min (*care! rapid evolution of gas*) and stirring was continued for 5 min. A solution of **6** (10.0 g, 23.2 mmol) in dry dichloromethane (30 mL) was added dropwise over 5 min and stirring continued for an additional 20 min, maintaining a temperature of −55 to −60 °C. Triethylamine (15 mL) was added dropwise over 2 min, and the reaction was stirred for a further 5 min before allowing it to warm to rt. The mixture was stirred with water (100 mL) for 10 min, and then dichloromethane (200 mL) was added. The organic layer was separated, and the aqueous layer re-extracted with a further 200 mL of dichloromethane. The combined organic layers were then washed successively with saturated NaCl, 1% HCl, water, 10% $NaHCO_3$, and water (200 mL of each), dried ($MgSO_4$), and evaporated to give a white solid consisting of a mixture of ketone **7** and its hydrated *gem*-diol (**7a**). The mixture was dissolved in toluene (300 mL) and refluxed with azeotropic removal of water in a Dean−Stark apparatus for 3 h. The toluene was removed by evaporation in vacuo, and the residue was recrystallized from ethyl acetate/hexane to give ketone **7** (9.14 g, 21.3 mmol, 92%): mp 125−126 °C (from ethyl acetate/hexane); IR (KBr disk) $\nu_{C=O}$ 1760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 3.78 (6 H, s), 4.38−4.42 (2 H, m), 4.47−4.56 (3 H, m), 4.52 (4 H, br s), 5.63 (1 H, s), 6.81 (4 H, d, J = 8.2 Hz), 7.16 (4 H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 55.22, 68.88, 71.18, 76.37, 77.97, 102.64, 113.85, 128.90, 129.50, 159.47, 199.23; MS *m*/*z* (positive ion FAB, relative intensity) 447 [$(M + H_2O + H)^+$, 0.3%], 429 [$(M + H)^+$, 1.2%], 121 [$(C_7H_6OCH_3)^+$, 100%]; MS *m*/*z* (negative ion FAB, relative intensity) 580 [$(M + NBA - H)^-$, 75%], 427 [$(M - H)^-$, 30%], 322 (80), 303 (100), 287 (60%). Anal. Calcd for $C_{23}H_{24}O_8$ (428.44): C, 64.48; H, 5.65. Found: C, 64.6; H, 5.66.

2-*C*-Hydroxy-4,6-bis-*O*-(*p*-methoxybenzyl)-1,3,5-*O*-methylidyne-*myo*-inositol (*gem*-diol 7a). The ketone **7** (400 mg, 0.924 mmol) was dissolved in dioxane (4 mL), and water (0.4 mL) was added. The solution was left at room temperature for 3 days, and then water was added dropwise until crystals began to appear. After another day at room temperature, the crystals were filtered off and were found to consist of pure *gem*-diol **7a** (315 mg, 0.706 mmol, 76%): mp 129−131 °C (from ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 3.79 (6H, s), 3.82 (1H, s, D_2O ex), 4.12−4.15 (2H, m), 4.41−4.60 (7 H, m), 4.97 (1 H, s, D_2O ex), 5.50 (1 H, s), 6.80 (4 H, d, J = 8.6 Hz), 7.12 (4 H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 55.25, 67.51, 71.42, 73.32, 73.56, 88.67, 102.30, 113.95, 128.74, 129.76, 159.58; MS *m*/*z* (positive ion FAB, relative intensity) 447 [$(M + H)^+$, 2%], 121 [$(C_7H_6OCH_3)^+$, 100%]. Anal. Calcd for $C_{23}H_{26}O_9$ (446.45): C, 61.88; H, 5.87. Found: C, 61.8; H, 5.89.

2,4-Bis-*O*-(*p*-methoxybenzyl)-6-methylene-1,3,5-*O*-methylidynecyclohexane-1,3,5/2,4-pentol (8). Methyltriphenylphosphonium bromide (6.13 g, 17.2 mmol), previously dried in vacuo at 70 °C, was suspended in dry THF (20 mL) under N_2 at 0 °C. Potassium *tert*-butoxide (16.3 mL of a 1 M solution in THF, 16.3 mmol) was added. The resulting yellow suspension was allowed to reach rt and was then stirred at rt for 10 min. A solution of ketone **7** (3.50 g. 8.17 mmol) in dry THF (30 mL) was added. [At this stage, a ^{31}P NMR spectrum of a sample taken from the yellow suspension showed the presence of an oxaphosphetane intermediate (δ_P −68.9 ppm). This signal could still be observed in the NMR sample after several days at 4 °C.] The mixture was refluxed for 2 h, after which time its color had darkened to orange and TLC (ethyl acetate/hexane 1:1) showed the reaction to be complete, with the product at R_f 0.52. The solvent was removed by evaporation in vacuo, the residue was taken up in ether (100 mL), and the solution was washed with brine (100 mL), dried ($MgSO_4$), and evaporated to give a clear brown oil. Purification by flash chromatography (ethyl acetate/hexane 1:2) gave the alkene **8** as a white crystalline solid (3.17 g, 7.42 mmol, 91%): mp 95−97 °C (from ethanol); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 3.80 (6 H, s), 4.23 (2 H, t, J = 3.6 Hz), 4.31 (1 H, tt, J = 3.6 Hz, 1.7 Hz), 4.40 (2 H, dd, J = 3.6 Hz, 1.7 Hz), 4.54, 4.58 (4 H, AB, J_{AB} = 11.8 Hz), 5.25 (2 H, s), 5.57 (1 H, s), 6.82−6.87 (4 H, m), 7.22−7.26 (4 H, m); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 55.19, 68.94, 73.55, 74.26, 71.05, 103.73, 113.75, 129.43, 114.27, 129.82, 137.15, 159.32; MS *m*/*z* (positive ion FAB, relative intensity) 427 [$(M + H)^+$, 1%], 305 [$(M - C_7H_6OCH_3)^+$, 6%], 121 [$(C_7H_6OCH_3)^+$, 100%]. Anal. Calcd for $C_{24}H_{26}O_7$ (426.47): C, 67.59; H, 6.15. Found: C, 67.3; H, 6.15.

(1,3,5/2,4,6)-6-(Hydroxymethyl)-1,3,5-*O*-methylidyne-2,4-bis-*O*-(*p*-methoxybenzyl)cyclohexane-1,2,3,4,5-pentol (9). The alkene **8** (6.0 g, 14.0 mmol, previously dried at 60 °C in vacuo) was placed in a dry three-neck 250 mL flask, and 9-BBN−H (60 mL of a 0.5M solution in THF, 30 mmol) was added under an atmosphere of N_2 at rt. The temperature was increased to 50 °C, and the mixture was stirred under N_2 for 2 h. The mixture was allowed to cool to room temperature and then further cooled to 0 °C in an ice bath. Ethanol (20 mL), 6 M NaOH (5 mL), and 30% H_2O_2 (10 mL) were added dropwise (*care! exothermic reaction with rapid evolution of gas*), and then the temperature was increased to 50 °C. After being stirred at 50 °C for 30 min, the mixture was cooled to room temperature and the aqueous layer was saturated with K_2CO_3. The organic layer was removed, dried ($MgSO_4$), and evaporated in vacuo to give a colorless oil which was purified by column chromatography, giving **9** as a white solid (6.04 g, 13.6 mmol, 97%): mp 81−82 °C (from ethanol or ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.58 (1 H, t, J = 5.9 Hz, D_2O ex), 2.97 (1 H, br t, J = 8.2 Hz), 3.80 (6 H, s), 4.08 (2 H, dd, J = 8.2 Hz, 5.9 Hz, D_2O ex gives d, J = 8.2 Hz), 4.27 (2 H, br s), 4.36 (2 H, br s), 4.49, 4.59 (4 H, AB, J_{AB} = 10.8 Hz), 4.53 (1 H, partly obscured by AB system), 5.58 (1 H, s), 6.79−6.82 (4 H, m), 7.15−7.26 (4 H, m); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 42.88, 55.17, 59.89, 68.50, 69.02, 73.06, 71.41, 103.84, 113.78, 129.29, 129.63, 159.26; MS *m*/*z* (positive ion FAB, relative intensity) 445 [$(M + H)^+$, 1.2%], 323 [$(M - C_7H_6OCH_3)^+$, 3.1%], 121 [$(C_7H_6OCH_3)^+$, 100%]; MS *m*/*z* (negative ion FAB, relative intensity) 597 [$(M + NBA)^-$, 100%], 323 [$(M - C_7H_6OCH_3)^-$, 90%]. Anal. Calcd for $C_{24}H_{28}O_8$ (444.48): C, 64.85; H, 6.35. Found: C, 65.1; H, 6.47.

(1,3,5/2,4,6)-6-(Hydroxymethyl)-2,4-bis-*O*-(*p*-methoxybenzyl)cyclohexane-1,2,3,4,5-pentol (10). Alcohol **9** (2.32 g, 5. 22 mmol) was dissolved in methanol (100 mL) and heated to 50 °C. Then 1 M HCl (10 mL) was added and the mixture stirred at 50 °C for 30 min. Excess concentrated ammonia solution was added and the mixture was allowed to cool. Stirring was continued at room temperature for a further 30 min, and the solvents were removed by evaporation in vacuo.

The residue was extracted with hot ethyl acetate (2 × 100 mL), and the combined extracts were evaporated in vacuo to give a white solid which was purified by flash chromatography ($CHCl_3$/MeOH 100:0−50:50), giving tetrol **10** (1.98 g, 4.56 mmol, 87%): mp 136−137 °C (from ethyl acetate/hexane); ^{1}H NMR (d_6-DMSO, 400 MHz, ^{1}H−^{1}H COSY) δ 1.25 (1 H, tt, J = 10.7 Hz, 2.1 Hz, C-6-H), 3.09 (2 H, t, J = 9.3 Hz, 9.3 Hz, C-2-H and C-4-H), 3.20 (1 H, td, J = 9.2 Hz, 5.4 Hz, D_2O ex gives t, J = 9.2 Hz, C-3-H), 3.29 (2 H, ddd, J = 10.7 Hz, 9.3 Hz, 5.9 Hz, D_2O ex gives dd, J = 10.7 Hz, 9.3 Hz, C-1-H and C-5-H), 3.69 (2 H, dd, J = 5.4 Hz, 2.1 Hz, D_2O ex gives d, J = 2.1 Hz, CH_2OH), 3.73 (6 H, s, 2 × OCH_3), 4.27 (1 H, t, J = 5.4 Hz, D_2O ex, CH_2OH), 4.71 (4 H, s, CH_2OPMB), 4.74 (2 H, d, J = 5.9 Hz, D_2O ex, C-1-OH and C-5-OH), 4.92 (1 H, d, J = 5.4 Hz, D_2O ex, C-3-OH), 6.83−6.87 (4 H, m, C_6H_4OMe), 7.29−7.36 (4 H, m, C_6H_4OMe); ^{13}C NMR (d_6-DMSO, 67.8 MHz) δ 48.13, 55.49, 57.54, 69.00, 73.87, 73.88, 85.90, 113.78, 129.80, 132.07, 158.91; MS *m*/*z* (positive ion FAB, relative intensity) 433 [(M − H)$^+$, 2.0%], 313 [(M − $C_7H_6OCH_3$)$^+$, 7.0%], 121 [($C_7H_6OCH_3$)$^+$, 100%]; MS *m*/*z* (negative ion FAB, relative intensity) 587 [(M + NBA)$^-$, 100%], 433 [(M − H)$^-$, 100%], 313 [(M − $C_7H_6OCH_3$)$^-$, 20%]. Anal. Calcd for $C_{23}H_{30}O_8$ (434.49): C, 63.58; H, 6.96. Found: C, 63.4; H, 6.94.

DL-(1,3,5/2,4,6)-1,7-*O*-Benzylidene-6-(hydroxymethyl)-2,4-bis-*O*-(*p*-methoxybenzyl)cyclohexane-1,2,3,4,5-pentol (11). The tetrol **10** (2.00 g, 4.60 mmol) was dissolved in dry DMF (10 mL) in a 100 mL round-bottomed flask. A catalytic amount of toluene-*p*-sulfonic acid (50 mg) was added, followed by benzaldehyde dimethyl acetal (0.80 mL, 5.33 mmol). The flask was fitted with a 250 mm air condenser connected to a filter pump, and the solution was stirred at 65−75 °C under reduced pressure for 1 h, after which TLC (ethyl acetate) showed the reaction to be complete. The solution was cooled to room temperature and triethylamine (1 mL) added. After the solution was stirred for 30 min at room temperature, the solvents were removed by evaporation in vacuo. The residue was taken up in dichloromethane (100 mL), washed with brine (50 mL), dried ($MgSO_4$), and evaporated in vacuo to give a solid which was purified by column chromatography (ethyl acetate/chloroform 1:1), giving the diol **11** as a white solid (2.24 g, 4.29 mmol, 93%): mp 158−160 °C (from ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.92 (1 H, qd, J = 11 Hz, 4.4 Hz), 2.36 (1 H, br s, D_2O ex), 2.69 (1 H, br s, D_2O ex), 3.22 (1 H, br dd, D_2O ex gives dd, J = 11.0 Hz, 8.8 Hz), 3.33 (1 H, dd, J = 8.8 Hz, 8.6 Hz), 3.50−3.66 (3 H, m), 3.68 (1 H, dd, J = 11.1 Hz, 11.1 Hz), 3.78 (6 H, br s), 4.49 (1 H, dd, J = 11.2 Hz, 4.4 Hz), 4.61, 4.97 (2 H, AB, J_{AB} = 11.2 Hz), 4.63, 4.96 (2 H, AB, J_{AB} = 11.0 Hz), 5.53 (1 H, s), 6.84−6.92 (4 H, m), 7.24−7.32 (4 H, m), 7.34−7.54 (5 H, m); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 39.51, 55.27, 69.39, 69.41, 74.68, 80.03, 82.03, 84.33, 74.62, 74.75, 101.10, 113.97, 114.09, 125.98, 128.27, 128.87, 129.74, 129.87, 130.47, 130.62, 138.06, 159.42, 159.45; MS *m*/*z* (positive ion FAB, relative intensity) 523 [(M + H)$^+$, 1.0%], 522 [M$^+$, 2.0%], 401 [(M − $C_7H_6OCH_3$)$^+$, 2.0%], 121 [($C_7H_6OCH_3$)$^+$, 100%]; MS *m*/*z* (negative ion FAB, rel intensity) 1043 [(2M − H)$^-$, 30%], 828 (10%), 688 (10%), 675 [(M + NBA)$^-$, 100%], 521 [(M − H)$^-$, 100%], 401 [(M − $C_7H_6OCH_3$)$^-$, 40%]. Anal. Calcd for $C_{30}H_{34}O_8$ (522.60): C, 68.95; H, 6.56. Found: C, 68.8; H, 6.53.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-5,7-*O*-benzylidene-6-(hydroxymethyl)-2,4-bis-*O*-(*p*-methoxybenzyl)cyclohexane-1,2,3,4,5-pentol (12). The diol **11** (1.00 g, 1.91 mmol) was dissolved in dry DMF, and sodium hydride (250 mg of a 60% dispersion in oil, 6.25 mmol) was added. The suspension was stirred for 20 min at room temperature, and then benzyl bromide (0.50 mL, 4.60 mmol) was added and stirring was continued for 2 h, after which TLC (ethyl acetate/hexane 1:1) showed the reaction to be complete. Excess NaH was carefully destroyed by dropwise addition of water, and the solvents were removed by evaporation in vacuo. The residue (which had a very low solubility in ether) was taken up in dichloromethane (50 mL), washed with brine (2 × 50 mL), dried ($MgSO_4$), and evaporated in vacuo to give a white solid which was washed with pentane and then recrystallized from hot ethanol, yielding **12** (1.26 g, 1.79 mmol, 94%) as colorless crystals: mp 135−137 °C (from ethanol); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.98 (1 H, qd, J = 11 Hz, 4.4 Hz), 3.21 (1 H, dd, J = 10.8 Hz, 9.3 Hz), 3.45−3.73 (5 H, m), 3.76 (3 H, s), 3.78 (3 H, s), 4.42 (1 H, dd, J = 11.2 Hz, 4.4 Hz), 4.51−4.97 (8 H, m), 5.48 (1 H, s), 6.77−6.81 (4 H, m), 7.20−7.50 (19 H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 39.69, 55.21, 69.31, 74.93, 75.20, 75.62, 76.06, 77.94, 80.03, 82.77, 83.19, 85.70, 100.95, 113.73, 113.84, 125.91, 127.60, 127.78, 127.96, 128.05, 128.18, 128.38, 128.51, 128.77, 129.59, 129.83, 130.58, 130.74, 138.00, 138.04, 138.64, 159.20, 159.23; MS *m*/*z* (positive ion FAB, relative intensity) 703 [(M + H)$^+$, 0.5%], 702 [M$^+$, 0.7%], 611 [(M − C_7H_7)$^+$, 0.3%], 581 [(M − $C_7H_6OCH_3$)$^+$, 3.5%], 121 [($C_7H_6OCH_3$)$^+$, 100%], 91 [(C_7H_7)$^+$, 22%]; MS *m*/*z* (negative ion FAB, rel intensity) 855 [(M + NBA)$^-$, 100%], 611 [(M − C_7H_7)$^-$, 40%], 581 [(M − $C_7H_6OCH_3$)$^-$, 20%]. Anal. Calcd for $C_{44}H_{46}O_8$ (702.84): C, 75.19; H, 6.60. Found: C, 75.2; H, 6.62.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-6-(hydroxymethyl)-2,4-bis-*O*-(*p*-methoxybenzyl)cyclohexane-1,2,3,4,5-pentol (13). Compound **12** (1.00 g, 1.42 mmol) was dissolved in a mixture of THF (25 mL) and methanol (25 mL). Then 1 M HCl (5 mL) was added and the solution was refluxed for 30 min, after which TLC (ethyl acetate) showed that no starting material remained. Excess $NaHCO_3$ (1 g) was added, and the mixture was allowed to cool to rt with stirring before the solvents were removed by evaporation in vacuo. The residue was taken up in dichloromethane (100 mL), washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), and evaporated in vacuo to give a solid which was purified by flash chromatography, yielding the diol **13** (720 mg, 1.17 mmol, 82%): mp 116−117.5 °C (from ethanol); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.65 (1 H, tdd, J = 10 Hz, 4.5 Hz, 3.0 Hz), 2.27 (1 H, br t, J = 5.1 Hz, 5.1 Hz, D_2O ex), 2.77 (1 H, d, J = 1.5 Hz, D_2O ex), 3.34−3.51 (4 H, m), 3.63 (1 H, t, J = 9.3 Hz, 9.3 Hz), 3.73 (1 H, br m, D_2O ex gives dd, J = 11.2 Hz, 4.5 Hz), 3.77 (3 H, s), 3.78 (3 H, s), 3.89 (1 H, br m, D_2O ex gives dd, J = 11.2 Hz, 3.0 Hz), 4.59−4.97 (8 H, m), 6.79−6.87 (4 H, m), 7.18−7.23 (4 H, m), 7.29−7.38 (10 H, m); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 46.07, 55.20, 60.22, 70.37, 77.52, 83.20, 84.80, 85.95, 75.10, 75.20, 75.43, 75.57, 113.80, 114.03, 127.63, 127.84, 127.99, 128.41, 129.37, 129.53, 130.49, 130.60, 138.14, 138.45, 159.14, 159.37; MS *m*/*z* (positive ion FAB, rel intensity) 615 [(M + H)$^+$, 0.5%], 614 [M$^+$, 0.4%], 523 [(M − C_7H_7)$^+$, 0.2%], 493 [(M − $C_7H_6OCH_3$)$^+$, 2.8%], 121 [(C_7H_6-OCH_3)$^+$, 100%], 91 [(C_7H_7)$^+$, 20%]; MS *m*/*z* (negative ion FAB, rel intensity) 767 [(M + NBA)$^-$, 60%], 613 [(M − H)$^-$, 100%), 493 [(M − $C_7H_6OCH_3$)$^-$, 35%]. Anal. Calcd for $C_{37}H_{42}O_8$ (614.74): C, 72.29; H, 6.89. Found: C, 72.3; H, 6.88.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-5,7-*O*-(benzyloxyphosphoryl)-6-(hydroxymethyl)-2,4-bis-*O*-(*p*-methoxybenzyl)-cyclohexane-1,2,3,4,5-pentol (Epimers 14a and 14b). (Benzyloxy)bis(*N*,*N*-diisopropylamino)phosphine[20] (285 mg, 0.84 mmol) was placed in a dry round-bottomed flask, and dry dichloromethane (5 mL) was added, followed by 1*H*-tetrazole (150 mg, 2.14 mmol). The suspension was stirred for 10 min and then cooled to 0 °C. The diol **13** (430 mg, 0.70 mmol, previously dried in vacuo at 60 °C) was added, and stirring was continued at 0 °C for 2 h. ^{31}P NMR spectroscopy now showed signals at δ_P 125.0 and 130.4 ppm, corresponding to the two cyclic phosphite triester invertomers. The mixture was cooled to −78 °C, and *m*-CPBA (240 mg, 1.4 mmol) was added. The clear solution was now allowed to warm to room temperature and then diluted with ethyl acetate (50 mL), washed with 10% Na_2SO_3, 1 M HCl, saturated $NaHCO_3$ and brine (50 mL of each), dried ($MgSO_4$), and evaporated in vacuo to give a colorless oil. Purification by flash chromatography (ethyl acetate/hexane 1:1) gave the two epimeric cyclic phosphate triesters **14a**, R_f 0.30 (246 mg, 0.32 mmol), and **14b**, R_f 0.18 (214 mg, 0.28 mmol), corresponding to a total yield of 0.60 mmol (86% from **13**).

14a: mp 130−132 °C (from ethyl acetate/hexane); IR: (KBr disk) $\nu_{P=O}$ 1287 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.19 (1 H, qd, J = 11 Hz, 4.5 Hz), 3.08 (1 H, dd, J = 11.2 Hz, 9.2 Hz), 3.40 (1 H, t, J = 9.3 Hz), 3.63 (1 H, t, J = 9.4 Hz), 3.66 (1 H, t, J = 9.4 Hz), 3.76 (1 H, dd, J = 11.3 Hz, 11.3 Hz), 3.768 (3 H, s), 3.773 (3 H, s), 4.04 (1 H, dd, J = 11.2 Hz, 9.2 Hz), 4.41 (1 H, ddd, J = 24.2 Hz, 11.4 Hz, 4.5 Hz), 4.41 (1 H, d, J = 11.2

Hz, part of a broad AB system), 4.68−4.93 (7 H, m), 5.06, 5.11 (2 H, ABX, J_{AB} = 11.7 Hz, J_{HP} = 7.7 Hz, 7.7 Hz), 6.79−6.83 (4 H, m), 7.15−7.36 (19 H, m); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 40.28 ($^3J_{CP}$ = 5.5 Hz), 55.20, 68.93 ($^2J_{CP}$ = 5.5 Hz), 70.26 ($^2J_{CP}$ = 7.7 Hz), 75.02, 75.59, 76.01, 76.29, 81.22 (J_{CP} = 7.7 Hz), 82.30 (J_{CP} = 2.2 Hz), 82.60 (J_{CP} = 7.7 Hz), 84.93, 113.77, 113.83, 127.58, 127.65, 127.97, 128.13, 128.38, 128.56, 128.67, 128.72, 129.50, 129.76, 130.15, 130.24, 135.44 ($^3J_{CP}$ = 7.7 Hz), 137.30, 138.25, 159.30; ^{31}P NMR ($CDCl_3$, 162 MHz) −7.49 (dt, $^3J_{HP}$ = 24.2 Hz, 7.7 Hz, 7.7 Hz); MS *m*/*z* (positive ion FAB, relative intensity) 767 [$(M + H)^+$, 1.2%], 675 [$(M - C_7H_7)^+$, 1.2%], 645 [$(M - C_7H_6OCH_3)^+$, 1.4%], 121 [$(C_7H_6OCH_3)^+$, 100%], 91 [$(C_7H_7)^+$, 28%]; MS *m*/*z* (negative ion FAB, rel intensity) 919 [$(M + NBA)^-$, 80%], 765 [$(M - H)^-$, 30%), 675 [$(M - C_7H_7)^-$,100%], 645 [$(M - C_7H_6OCH_3)^-$, 30%], 187 [$C_7H_7OPO_3H)^-$, 80%], 97 [$(H_2PO_4)^-$, 45%]. Anal. Calcd for $C_{44}H_{47}O_{10}P$ (766.82): C, 68.92; H, 6.18. Found: C, 69.1; H, 6.11.

14b: mp 101−102.5 °C (from ethanol); IR (KBr disk) $\nu_{P=O}$ 1266 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.17 (1 H, qd, J = 11.2 Hz, 4.8 Hz), 3.18 (1 H, dd, J = 11.0 Hz, 9.0 Hz), 3.44 (1 H, t, 9.0 Hz), 3.51 (1 H, t, J = 9.1 Hz), 3.60 (1 H, t, J = 9.0 Hz), 3.76 (3 H, s), 3.78 (3 H, s), 4.03 (1 H, td, J = 11.2 Hz, 3.9 Hz), 4.32 (1 H, br dd, J = 11.2 Hz, 9.2 Hz), 4.39 (1 H, ddd, J = 20.4 Hz, 11.2 Hz, 4.7 Hz), 4.45−4.90 (8 H, m), 5.08, 5.13 (2 H, ABX, J_{AB} = 11.9 Hz, J_{HP} = 9.9 Hz, 9.9 Hz), 6.78−6.84 (4 H, m), 7.17−7.38 (19 H, m); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 40.41 ($^3J_{CP}$ = 6.6 Hz), 55.19, 55.22, 69.47 ($^2J_{CP}$ = 5.5 Hz), 70.40 ($^2J_{CP}$ = 6.6 Hz), 75.12, 75.59, 76.06, 76.47, 81.14 (J_{CP} = 5.5 Hz), 82.29, 82.65 (J_{CP} = 7.7 Hz), 85.04, 113.65, 113.85, 127.63, 127.71, 127.86, 128.17, 128.26, 128.35, 128.62, 128.65, 129.50, 129.72, 130.18, 130.26, 135.45 ($^2J_{CP}$ not readable), 137.28, 138.30, 159.22, 159.30; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −4.56 (dtd, $^3J_{HP}$ = 20.3 Hz, 9.9 Hz, 3.9 Hz); MS *m*/*z* (positive ion FAB, relative intensity) 767 [$(M + H)^+$, 2.0%], 675 [$(M - C_7H_7)^+$, 1.0%], 645 [$(M - C_7H_6OCH_3)^+$, 4.0%], 121 [$(C_7H_6OCH_3)^+$, 100%], 91 [$(C_7H_7)^+$, 22%]; MS *m*/*z* (negative ion FAB, relative intensity) 919 [$(M + NBA)^-$, 20%], 765 [$(M - H)^-$, 10%), 675 [$(M - C_7H_7)^-$,100%], 645 [$(M - C_7H_6OCH_3)^-$, 12%], 187 [$C_7H_7OPO_3H)^-$, 70%], 97 [$(H_2PO_4)^-$, 45%]. Anal. Calcd for $C_{44}H_{47}O_{10}P$ (766.82): C, 68.92; H, 6.18. Found: C, 69.1; H, 6.13.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-5,7-*O*-(benzyloxyphosphoryl)-6-(hydroxymethyl)cyclohexane-1,2,3,4,5-pentol (Epimer 15a). To a solution of **14a** (300 mg, 0.39 mmol) in dichloromethane (10 mL) were added water (1 mL) and DDQ (355 mg, 1.56 mmol). The mixture was stirred at rt for 2.5 h, after which TLC (ethyl acetate/ hexane 2:1) showed the reaction to be complete. Dichloromethane (60 mL) was added, and the organic layer was washed with a 10% Na_2SO_3 solution (3 × 50 mL), a saturated $NaHCO_3$ solution, and brine (50 mL of each), dried ($MgSO_4$), and evaporated in vacuo to give a yellow oil which was purified by column chromatography (ethyl acetate/pentane 3:2), giving the diol **15a** (137 mg, 0.260 mmol, 66%): mp 145−149 °C (from ethyl acetate/ hexane); R_f 0.45 (ethyl acetate/dichloromethane 1:1), R_f 0.31 (ethyl acetate/ hexane 2:1); IR (KBr disk) $\nu_{P=O}$ 1280 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, ^{1}H−^{1}H COSY) δ 2.07 (1 H, qd, J = 11 Hz, 10.7 Hz, 4.4 Hz, C-6-H), 2.83 (1 H, d, J = 1.96 Hz, D_2O ex, C-2-OH), 3.07 (1 H, dd, J = 10.7 Hz, 9.3 Hz, C-1-H), 3.20 (1 H, t, J = 9.3 Hz, C-3-H), 3.55 (1 H, d, J = 2.9 Hz, D_2O ex, C-4-OH), 3.63−3.70 (2 H, m, C-2-H and C-4-H), 3.81 (1 H, t, J = 11.2 Hz, C-7-H_{ax}), 3.86 (1 H, dd, J = 10.7 Hz, 9.3 Hz, C-5-H), 4.43 (1 H, ddd, J = 24.4 Hz, 11.2 Hz, 4.4 Hz, C-7-H_{eq}), 4.48−5.13 (6 H, AB systems, CH_2OBn), 7.25−7.38 (15 H, m); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ 39.60 ($^3J_{CP}$ = 3.6 Hz), 69.15 ($^2J_{CP}$ = 5.5 Hz), 70.45 ($^2J_{CP}$ = 7.4 Hz), 74.35, 75.17, 74.70 ($^3J_{CP}$ = 7.4 Hz), 75.85, 76.69 (J_{CP} unreadable), 80.90 (J_{CP} = 7.3 Hz), 81.32, 127.99, 128.06, 128.13, 128.28, 128.39, 128.52, 128.59, 128.72, 128.85, 135.28 ($^2J_{CP}$ = 7.3 Hz), 137.59, 138.17; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −7.27 (1 P, dt, $^3J_{HP}$ = 24.2 Hz, 8.1 Hz); MS *m*/*z* (positive ion FAB, relative intensity) 527 [$(M + H)^+$, 22%], 435 [$(M - C_7H_7)^+$, 3%], 391 (10), 181 (6), 91 [$(C_7H_7)^+$, 100%]; MS *m*/*z* (negative ion FAB, relative intensity) 679 [$(M + NBA)^-$, 90%], 525 [$(M - H)^-$, 100%), 435 [$(M - C_7H_7)^-$, 90%], 187 [$C_7H_7OPO_3H)^-$, 40%], 97 [$(H_2PO_4)^-$, 20%]. Anal. Calcd for $C_{28}H_{31}O_8P$ (526.52): C, 63.87; H, 5.93. Found: C, 63.7; H, 6.00.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-5,7-*O*-(benzyloxyphosphoryl)-6-(hydroxymethyl)cyclohexane-1,2,3,4,5-pentol (Epimer 15b). The *p*-methoxybenzyl groups were removed from **14b** (260 mg, 0.34 mmol) using the same procedure as for **14a**. Purification by column chromatography (ethyl acetate/ dichloromethane 1:1) gave **15b** (125 mg, 0.237 mmol, 70%): mp 160−162 °C (from ethyl acetate/hexane); R_f 0.25 (ethyl acetate/dichloromethane 1:1), R_f 0.15 (ethyl acetate/hexane 2:1); IR (KBr disk) $\nu_{P=O}$ 1263 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.13 (1 H, qd, J = 11 Hz, 4.9 Hz), 2.93 (1 H, d, J = 2.4 Hz, D_2O ex), 3.13 (1 H, dd, J = 10.7 Hz, 9.3 Hz), 3.25 (1 H, t, J = 9.3 Hz), 3.50 (1 H, br s, D_2O ex), 3.63−3.69 (2 H, br m), 4.08 (1 H, td, J = 11.2 Hz, J_{HP} = 4.4 Hz), 4.17 (1 H, dd, J = 10.8 Hz, 10.7 Hz), 4.42 (1 H, ddd, J = 20.0 Hz, 10.7 Hz, 4.9 Hz), 4.51−5.12 (6 H, AB systems), 7.25−7.40 (15 H, m); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ 40.13 ($^3J_{CP}$ = 7.4 Hz), 70.16 ($^2J_{CP}$ = 7.4 Hz), 70.86 ($^2J_{CP}$ = 7.4 Hz), 74.81, 75.39 (J_{CP} = 7.3 Hz), 75.54, 76.56, 77.13, 80.08, 75.39 (J_{CP} = 5.5 Hz), 81.94, 128.32, 128.48, 128.55, 128.72, 128.87, 128.90, 129.05, 135.41 ($^3J_{CP}$ = 7.3 Hz), 137.90, 138.56; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −4.43 (1P, br dtd, $^3J_{HP}$ = 20 Hz, 8 Hz, 4 Hz); MS *m*/*z* (positive ion FAB, relative intensity) 527 [$(M + H)^+$, 20%], 435 [$(M - C_7H_7)^+$, 4%], 91 [$(C_7H_7)^+$, 100%]; MS *m*/*z* (negative ion FAB, rel intensity) 1051 [$(2M - H)^-$, 4%], 960 (5), 679 [$(M + NBA)^-$, 40%], 525 [$(M - H)^-$, 80%), 435 [$(M - C_7H_7)^-$, 100%], 187 [$C_7H_7OPO_3H)^-$, 50%], 97 [$(H_2PO_4)^-$, 22%]. Anal. Calcd for $C_{28}H_{31}O_8P$ (526.52): C, 63.87; H, 5.93. Found: C, 63.6; H, 6.01.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-5,7-*O*-(benzyloxyphosphoryl)-2,4-bis-*O*-[bis(benzyloxy)phosphoryl]-6-(hydroxymethyl)cyclohexane-1,2,3,4,5-pentol (Epimer 16a). To a solution of bis(benzyloxy)(*N*,*N*-diisopropylamino-)phosphine[24] (345 mg, 1.00 mmol) in dry dichloromethane (3 mL) was added 1*H*-tetrazole (140 mg, 2.00 mmol). The mixture was stirred at rt for 20 min, and then the diol **15a** (130 mg, 0.247 mmol) was added. Stirring was continued for 30 min, after which ^{31}P NMR showed signals at δ 143 (1 P, s, phosphite at C-4), 142 (1 P, d, $^5J_{PP}$ = 1.2 Hz, phosphite at C-2), −7.9 (1 P, d, $^5J_{PP}$ = 1.2 Hz, cyclic phosphate). The mixture was cooled to −78 °C, *m*-CPBA (345 mg, 2.00 mmol) was added, and the cooling bath was removed. The mixture was allowed to reach rt and then diluted with ethyl acetate (50 mL). The clear solution was washed with 10% Na_2SO_3, 1 M HCl, saturated $NaHCO_3$, and brine (50 mL of each), dried ($MgSO_4$), and evaporated in vacuo, giving a solid residue. Purification by column chromatography (ethyl acetate/dichloromethane 1:2) afforded **16a** (192 mg, 0.183 mmol, 74%) as a white solid: mp 171−172.5 °C (from ethanol); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.20 (1 H, qd, J = 11 Hz, 4.4 Hz), 3.21 (1 H, dd, J = 11.0 Hz, 9.0 Hz), 3.58 (1 H, t, J = 9.2 Hz, 9.2 Hz), 3.68 (1 H, dd, J = 11.2 Hz, 11.0 Hz), 4.10 (1 H, dd, J = 11.2 Hz, 9.4 Hz), 4.32 (1 H, ddd, J = 24.2 Hz, 11.0 Hz, 4.4 Hz), 4.33 (1 H, d, J = 11.4 Hz, part of a broad AB system), 4.50−4.68 (2 H, m), 4.72−5.10 (13 H, m), 6.95−7.42 (35 H, m); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 39.0 ($^3J_{CP}$ = 5.5 Hz), 69.04 ($^2J_{CP}$ = 5.5 Hz), 69.40, 69.49, 69.60, 69.68, 74.16, 75.04, 78.20, 78.75, 79.38, 80.81, 127.36, 127.44, 127.84, 127.89, 128.09, 128.17, 128.23, 128.26, 128.36, 128.39, 128.51, 128.67, 128.77, 135.51, 135.63, 136.75, 137.54 (many signals showed J_{CP} couplings that were unreadable due to complex or overlapping multiplets); ^{31}P NMR ($CDCl_3$, 162 MHz) δ −8.33 (1 P, dt, J = 24.1 Hz, 7.6 Hz), −1.81, −1.65 (2 P, overlap to give m in ^{1}H-coupled spectrum); MS *m*/*z* (positive ion FAB, relative intensity) 1047 [$(M + H)^+$, 5%], 181 (10), 91 [$(C_7H_7)^+$, 100%]; MS *m*/*z* (negative ion FAB, rel intensity) 1199 [$(M + NBA)^-$, 8%], 1045 [$(M - H)^-$, 3%] 955 [$(M - C_7H_7)^-$, 55%], 277 [$(BnO)_2PO_2^-$, 100%], 187(38%), 97 [$H_2PO_4^-$, 10%]. Anal. Calcd for $C_{56}H_{57}O_{14}P_3$ (1046.98): C, 64.24; H, 5.49. Found: C, 63.9; H, 5.46.

DL-(1,3,5/2,4,6)-1,3-Di-*O*-benzyl-5,7-*O*-(benzyloxyphosphoryl)-2,4-bis-*O*-[bis(benzyloxy)phosphoryl]-6-(hydroxymethyl)cyclohexane-1,2,3,4,5-pentol (Epimer 16b). Compound **15b** (110 mg, 0.209 mmol) was phosphorylated using the procedure described for **15a**. Purification by

flash chromatography (ethyl acetate/dichloromethane 1:3) afforded **16b** (190 mg, 0.182 mmol, 87%) as a white solid: mp 136−138 °C (from ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.24 (1 H, qd, J = 11 Hz, 4.6 Hz), 3.33 (1 H, t, J = 9.8 Hz, 9.8 Hz), 3.62 (1 H, dd, J = 9.2 Hz, 8.9 Hz), 4.00 (1 H, br t, J = 11 Hz), 4.31 (1 H, ddd, J = 21.4 Hz, 11.0 Hz, 4.6 Hz), 4.39 (1 H, d, J = 11.3 Hz, part of a broad AB system), 4.44 (1 H, br dd, J = 10.7 Hz, 10.1 Hz), 4.61 (1 H, td, J = 9.2 Hz, 8.9 Hz), 4.67−5.27 (14 H, m), 6.99−7.40 (35 H, m); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ 39.3 ($^3J_{CP}$ = 5.5 Hz), 68.88 ($^2J_{CP}$ = 3.7 Hz), 69.17 ($^2J_{CP}$ = 5.5 Hz), 69.54 ($^2J_{CP}$ = 5.5 Hz), 69.59 ($^2J_{CP}$ = 5.5 Hz), 70.82 ($^2J_{CP}$ = 7.4 Hz), 74.18, 74.34, 75.31, 77.14, 78.82, 79.44, 81.03, 127.27, 127.32, 127.72, 127.89, 128.00, 128.13, 128.22, 128.33, 128.42, 128.60, 128.71, 134.88 ($^3J_{CP}$ = 7.3 Hz), 135.54 ($^3J_{CP}$ = 7.3 Hz), 135.66, 135.73, 135.83, 136.76, 137.65 (many signals showed J_{CP} couplings that were unreadable due to complex or overlapping multiplets); ^{31}P NMR ($CDCl_3$, 162 MHz) δ −5.28 (1 P, dtd, J = 21.4 Hz, 7.6 Hz, 2 Hz), −1.58, −1.51 (2 P, overlap to give m in ^{1}H-coupled spectrum); MS *m*/*z* (positive ion FAB, relative intensity) 1047 [$(M + H)^+$, 70%], 181 (12), 91 [$(C_7H_7)^+$, 100%]; MS *m*/*z* (negative ion FAB, relative intensity) 1212 (65), 1199 [$(M + NBA)^-$, 30%], 1046 (50), 955 [$(M - C_7H_7)^-$, 100%], 277 [$(C_6H_5O)_2PO_2^-$, 60%]. Anal. Calcd for $C_{56}H_{57}O_{14}P_3$ (1046.98): C, 64.24; H, 5.49. Found: C, 64.4; H, 5.55.

DL-(1,3,5/2,4,6)-6-(Hydroxymethyl)cyclohexane-1,2,3,4,5-pentol 1:7-Cyclic 2,4-Trisphosphate [*DL*-6-deoxy-6-(hydroxymethyl)-*scyllo*-inositol 1:7-Cyclic 2,4-Trisphosphate] (4). Ammonia (∼100 mL) was condensed into a three-necked flask at −78 °C. An excess of sodium was added to dry the liquid ammonia, and the blue-black solution was stirred at −78 °C for 30 min. A small volume of ammonia (∼30 mL) was then distilled into a second three-necked flask and kept at −78 °C. Sodium was added until the solution remained blue-black for 10 min. Either compound **16a** or **16b** (95 mg, 91 μmol) was dissolved in anhydrous dioxane (2 mL) and added to the vigorously stirring sodium−liquid ammonia mixture. After 60−90 s the reaction was quenched by careful addition of methanol until the blue color disappeared, followed by deionized water (10 mL). Ammonia and solvents were then removed by evaporation in vacuo. The residue was dissolved in deionized water (500 mL) and purified by ion-exchange chromatography on Q Sepharose Fast Flow Resin, eluting with a gradient of triethylammonium bicarbonate buffer (0 to 1 M), pH 8.0. The glassy triethylammonium salt of **4** eluted between 500 and 650 mM TEAB and was quantified by phosphate assay (yield 78−82%): ^{1}H NMR (D_2O, 400 MHz ^{1}H−^{1}H COSY, Na^+ salt, pH 8) δ 1.94 (1 H, qd J = 11 Hz, 4.4 Hz, C-6-H), 3.32 (1 H, dd, J = 10.7 Hz, 9.3 Hz, C-5-H), 3.41 (1 H, dd, J = 9.3 Hz, 8.8 Hz, C-3-H), 3.80 (1 H, td, J = 8.8 Hz, 7.8 Hz, C-4-H), 3.85−3.98 (3 H, m, C-1-H, C-2-H, C-7-H_{ax}), 4.21 (1 H, ddd, J = 22.9 Hz, 11.3 Hz, 4.4 Hz, C-7-H_{eq}); ^{13}C NMR (D_2O, 100.4 MHz, Na^+ salt, pH 8) δ 41.67 ($^3J_{CP}$ = 3.6 Hz, *C*-6), 69.15 ($^2J_{CP}$ = 5.4 Hz, *C*-7), 70.58, 74.37 (J_{CP} = 3.7 Hz), 77.57 (J_{CP} = 5.5 Hz, 5.5 Hz), 78.14 (J_{CP} = 7.3 Hz, 5.5 Hz), 80.21 (J_{CP} = 5.5 Hz); ^{31}P NMR (D_2O, 162 MHz, ^{1}H-coupled, Na^+ salt, pH 8) δ −2.76 (1 P, d, J_{HP} = 22.5 Hz, P1), 2.99 (1 P, d, J_{HP} = 7.1 Hz, P2), 3.96 (1 P, d, J_{HP} = 6.8 Hz, P4); MS *m*/*z* (negative ion FAB, relative intensity) 831 [$(2M - H)^-$, 20%], 415 [$(M - H)^-$, 90%], 159-(100), 97 [$H_2PO_4^-$, 83%]; accurate mass negative ion FAB *m*/*z* calcd for $C_7H_{14}O_{14}P_3^-$, 414.960, found 414.962.

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