Synthesis and inhibitory properties of (1*R*,2*R*,4*R*,6*R*)-6-*O*-(2-hydroxyethyl)cyclohexane-1,2,4,6-tetraol derivatives: mechanistic probes for the inositol monophosphatase reaction[1]

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The phosphate derivatives 2, 3 and 4 of 6-*O*-(2-hydroxyethyl)cyclohexane-1,2,4,6-tetraol have been designed to inhibit inositol monophosphatase, the putative target for lithium therapy, by interacting simultaneously with both cofactor metal ions at the active site of the enzyme. The compounds have been synthesised, *via* the known key common intermediate cyclohexene oxide, from cyclohexane-1,4-diol in moderate yield, and have been tested for activity in standard enzyme assays. Each compound serves as a competitive inhibitor and displays the expected inhibitory properties. Indeed, compound 4 and the cyclic phosphate 3 of 6-*O*-(2-hydroxyethyl)cyclohexane-1,2,4,6-tetraol are, respectively, the most potent examples of a primary alkyl phosphate inhibitor and a phosphate monoanion inhibitor yet reported for the enzyme. The stereochemistry of the most potent inhibitor, (1*R*,2*R*,4*R*,6*R*)-2 as deduced from the X-ray crystal structure of a synthetic precursor, provides useful mechanistic insight into the action of the enzyme and the mode of inhibitor binding.

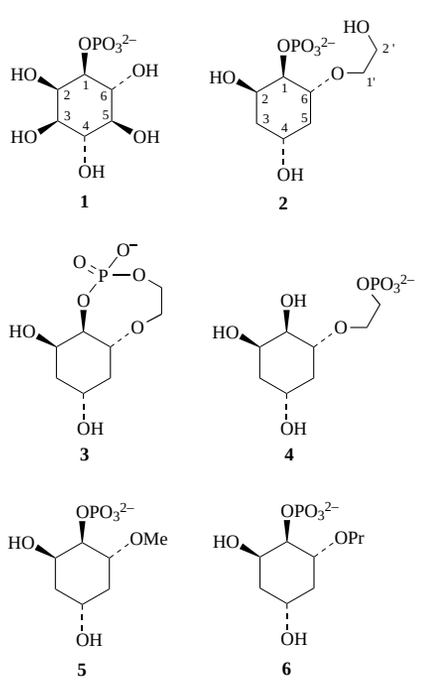
The action of inositol monophosphatase (IMPase, EC 3.1.3.25) in mammalian brain cells is to provide inositol for the biosynthesis of the key secondary messenger precursor, phosphatidylinositol 4,5-bisphosphate. Phosphatidylinositol 4,5-biophosphate is hydrolysed by phosphatidylinositidase C, in response to receptor occupation, to give both diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3) each of which mediate signal transduction through specific interactions with their own targets.[2–4] DAG activates protein kinase C,[5,6] which modulates the activity of certain proteins by phosphorylating specific serine and threonine residues,[7] while Ins 1,4,5-P_3 causes the release of calcium ions from an intracellular store, an action which also modulates the activity of certain proteins.[8]

Brain cells vary in their ability to take-up free inositol [3,9] and the major pathway for replenishing intracellular inositol levels appears to depend on a series of phosphatases which exist to sequentially hydrolyse Ins 1,4,5-P_3 and other inositol polyphosphates *via* the bisphosphates to give inositol 1- and 4-monophosphates, the substrates for IMPase. In the only other available route, D-Ins 3-P (L-Ins 1-P), also a substrate for IMPase, is produced in *de novo* biosynthesis from glucose 6-phosphate through the action of inositol synthase.[3] Thus, IMPase plays a pivotal role in being able to process each of these inositol monophosphates to give the inositol required for the resynthesis of the secondary messengers.

The effect of blocking the action of the enzyme with inhibitor Li^+ cations leads to the accumulation of inositol monophosphates and to the depletion of free inositol in brain cells[10,11] and since these results were first described, several groups have suggested that the enzyme might be the target for lithium ion therapy in the treatment of manic depression. Many of these groups have probed the kinetics of inhibition by lithium[12–18] and there is now a substantial body of evidence to show that the activity of the enzyme would be very low in the presence of therapeutic concentrations of Li^+ ion (*ca.* 1 mmol dm^{-3}). Furthermore, the sensitivity of the enzyme to Li^+ has been shown to be acutely dependent upon the concentration of phosphate dianion, a reaction product that is present in brain cells at high concentration, indicating that the efficacy of Li^+ as an inhibitor is likely to be greater in cells than was originally thought.[18] Subsequent studies have defined how Li^+ ions interact with the enzyme in causing inhibition (see below).

IMPase catalyses the hydrolysis of a range of phosphate esters including both enantiomers of *myo*-inositol 1-phosphate (Ins 1-P, **1**) and *myo*-inositol 4-phosphate (Ins 4-P),[14] ethane-1,2-diol phosphate[19] and 2′-ribonucleoside[13,19] and 2′-ribofuranoside phosphates.[20] The activity of IMPase shows an absolute requirement for divalent metal ions,[13] such as Mg^{2+}, and it is now known that two Mg^{2+} ions bind at each active-site of the homodimer.[19,21,22] The reinterpretation of kinetic data [18,23,24] taking account of the requirement for two Mg^{2+} ions suggests that one metal ion (Mg^{2+} 1) binds to the enzyme before the substrate and the second metal ion (Mg^{2+} 2) binds after the substrate.[25] Lithium ion appears to replace Mg^{2+} 2 in a phosphate product complex in its uncompetitive inhibition of the enzyme.[25]

Elegant synthetic transformations of Ins 1-P **1** revealed that



the 3-OH and 5-OH groups are not necessary for binding or catalysis.[26–28] Further probing showed that the 4-OH and 2-OH groups were important for binding, whereas the 6-OH group was essential for catalysis (Fig. 1). Deletion[26,27] or alkylation[28] of the 6-OH group in Ins 1-P **1** leads to tight binding competitive inhibitors of IMPase, revealing the pivotal role of the 6-OH group in the catalytic hydrolysis step. While ^{18}O-phosphate ligand exchange studies established that the enzyme did not operate *via* a substituted enzyme mechanism, but rather, that water displaced the phosphate ester group directly,[18,29] it was only recently that proposals emerged on how this might be achieved.

On the basis of kinetic data for hydrolysis of different substrates, data for ^{18}O-phosphate ligand exchange and for inhibition,[18,30,31] together with the X-ray crystal coordinates of a Gd^{3+} sulfate complex of the enzyme[32] and the results of extensive modelling studies, we proposed a three-dimensional structure for the active complex containing both Mg^{2+} ions in which the second ion to bind, Mg^{2+} 2, coordinates to and activates the attacking nucleophilic water molecule [Fig. 2(*a*)].[19,25] According to this model, the role of the catalytic 6-OH group of Ins 1-P **1** is to hydrogen-bond to the nucleophile so that it is properly positioned to attack the phosphate phosphorus atom *via* the adjacent displacement of the inositol moiety.[19,25] This mechanism differs significantly in detail from a proposal which was put forward by the Merck, Sharp and Dohme group[22,24,33] (at the same time as our own) which was derived largely from X-ray crystal data for different enzyme metal ion complexes [Fig. 2(*b*)]. Nevertheless, at a structural level, the positions of the substrate binding groups and the metal ions within the active complex are virtually identical in the two models. The major difference is that in the Merck model, metal ion one (Mg^{2+} 1), which is deeply buried in the active site of the enzyme, coordinates to and activates the attacking nucleophilic water such that it would replace the inositol moiety with inversion of configuration at the phosphorus atom.[22] (Note that a full comparison of the two mechanisms depicted in Fig. 2 is given in ref. 25.)

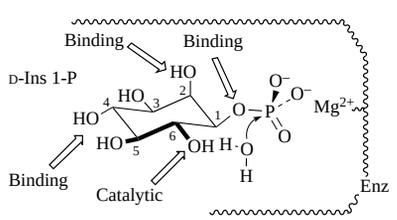


Fig. 1 Proposed roles of the flanking 2- and 6-hydroxy groups in binding to the enzyme and in facilitating catalysis. The active site nucleophile is a water molecule.

Given that there is chemical precedent for both types of mechanism, adjacent displacement with pseudorotation and direct inline displacement with inversion,[34] ongoing work in our laboratory has been concerned with determining which of the two possible sites is occupied by the nucleophile. Recent theoretical studies on the mechanism of phosphoryl transfer indicate that the transition state energy differences for adjacent displacement and inline displacement mechanisms are small.[35] Hence it is not possible, *a priori*, to expect one mechanism to be favoured over the other. In the absence of information on the stereochemical course of the reaction with respect to phosphorus, our attentions have been focussed on the design of structural probes for the coordination sphere of Mg^{2+} 2.

According to our proposed mechanism,[25] extension of the 6-OH group by an ethylene bridge, as in compound **2**, places the 2-OH group of the 'pendant arm' into the position of the nucleophilic water molecule. Here we describe the synthesis of both enantiomers of **2** and some closely related inositol monophosphate analogues (compounds **3**–**6**) including the phosphate **4** and the cyclic phosphate **3**. The inhibitory properties of these materials provides useful mechanistic insight into the action of IMPase, as discussed below.

Results and discussion

The synthesis of the potential inhibitors for inositol monophosphatase started with the commercially available *cis*/*trans*-mixture of cyclohexane-1,4-diol **7** (Scheme 1). The required stereogenic centres at 1-C, 2-C, 4-C and 6-C of compounds **2**–**6** were then constructed in a sequence of seven steps following a route communicated by Baker *et al.*[28] Accordingly, cyclohexane-1,4-diol **7** was heated at 240 °C in the presence of a catalytic amount of 65% sulfuric acid[36] to give racemic cyclohex-3-enol which was collected by distillation at 165 °C. Benzylation of the 1-OH group gave the required racemic ether **8**[37] which was treated with *m*-chloroperoxybenzoic acid (MCPBA) in dichloromethane to give a 2:3 mixture of the *trans*-epoxide **9** and *cis*-epoxide **10**.[38] These isomers could be seperated by column chromatography, as had been observed previously,[38] but it was more convenient to separate the diastereoisomers at a later stage (see below). Accordingly epoxides **9** and **10**, as a mixture, were converted to the allyl alcohols **13** and **14** using a three-step method first developed by Sharpless and Lauer.[39] The oxirane rings were opened with phenyl

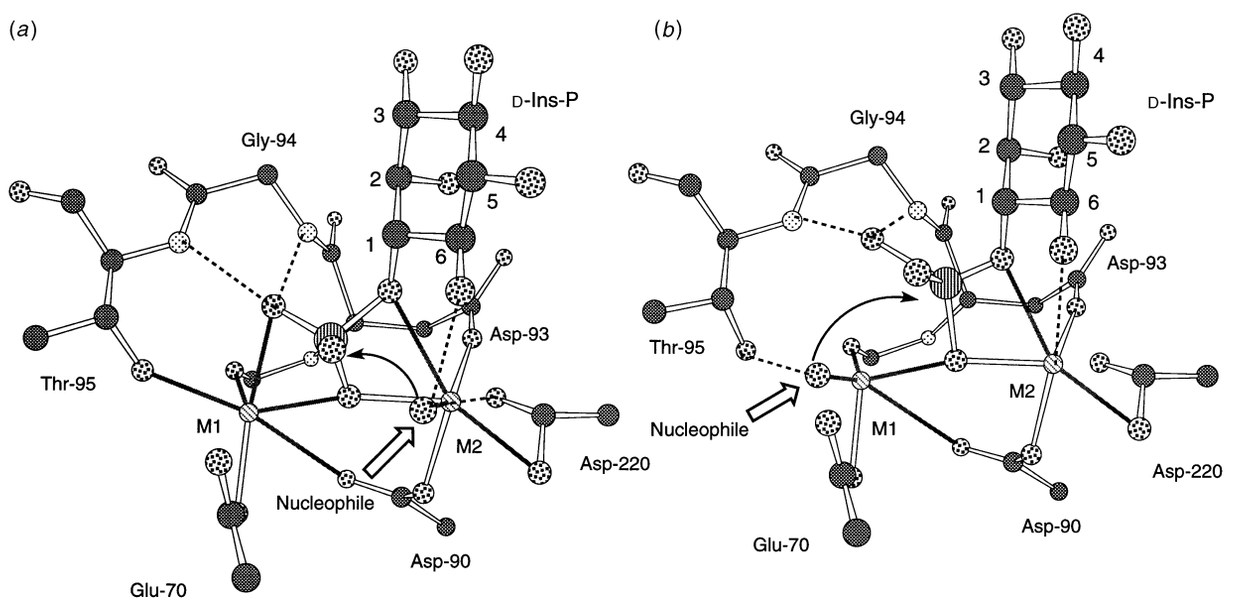
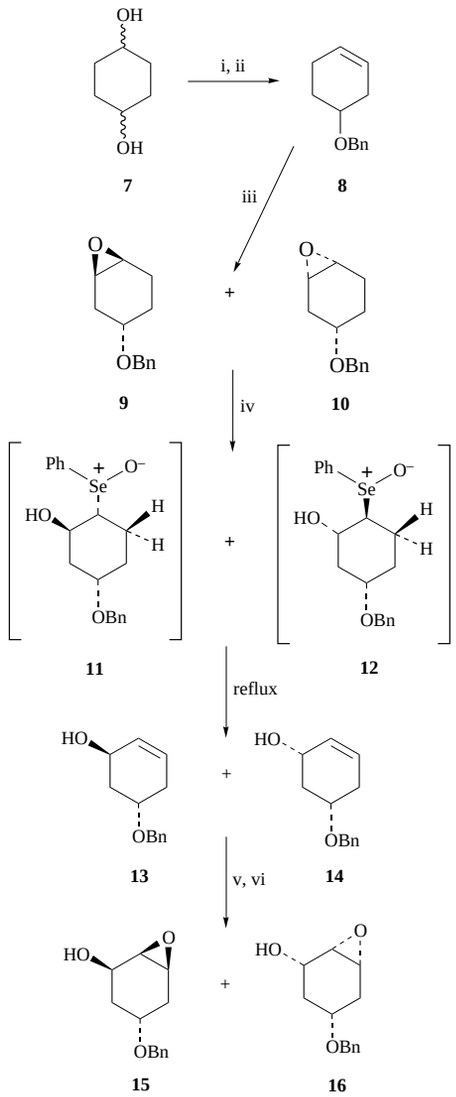


Fig. 2



Scheme 1 *Reagents and conditions:* i, H_2SO_4 (60%; cat.), 240 °C, 50%; ii, BnBr, NaH, DMF, 96%; iii, MCPBA, CH_2Cl_2, 83%; iv, $(PhSe)_2$, $NaBH_4$, EtOH, then THF, H_2O_2, then reflux, 50% overall; v, MCPBA, CH_2Cl_2, 81% of **15** and**16**; vi, separate isomers, 33% of **15**

selenide (generated *in situ* from diphenyl diselenide and $NaBH_4$) in ethanol to give exclusively the 5-benzyloxy-2-phenylselanylcyclohexanols,[28] which were oxidised to the selenoxides **11** and **12** using 30% H_2O_2. Upon prolonged heating at *ca.* 80 °C each phenyl selenoxide partook in an E_i *syn*-elimination to give a 2:3 mixture of the *trans*- and *cis*-allylic alcohols **13** and **14**, respectively, in 50% yield[28,39] (Scheme 1). The long reaction times required for the elimination process[39] and the moderate yield obtained for the allylic alcohols **13** and **14** may reflect the fact that the 6-C hydrogen atoms cannot adopt an ideal *syn*-periplanar orientation with respect to the leaving phenyl selenoxide group.

Utilising the known *cis*-directing effect of hydroxy groups on the epoxidation of an adjacent olefinic double bond by MCPBA, the allylic alcohols **13** and **14** were converted to a 2:3 mixture of the required epoxy alcohols **15** and **16** without incident[40] (Scheme 1). The two isomers were readily separated by column chromatography on silica and the major product, the unwanted *cis*-epoxy alcohol **16**, was discarded.

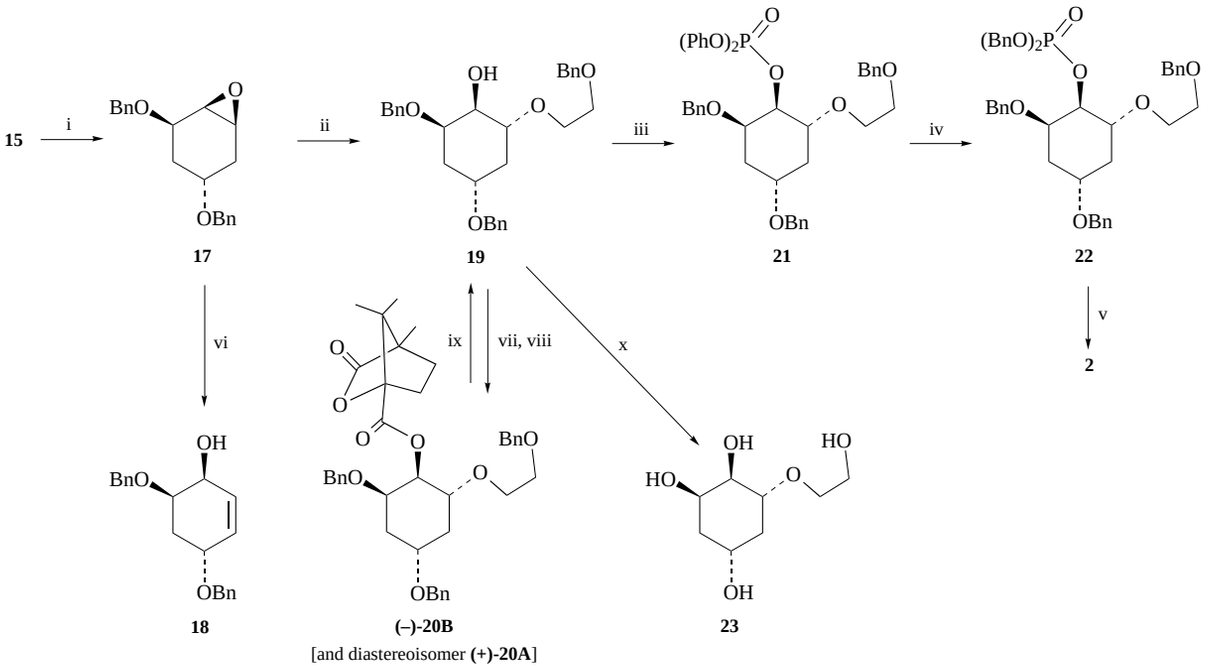
Benzylation of the *trans*-epoxy alcohol **15** by a modification of the protocol communicated by Baker *et al.*[28] gave the required racemic 1,5-dibenzyloxy-2,3-epoxycyclohexane **17** in 5% overall yield from diol **7** (Scheme 2). This pivotal compound was now suitably protected and functionalised for reaction with nucleophiles.

It was envisaged that reaction of the epoxide **17** with a range of suitably derivatised oxygen nucleophiles would facilitate the introduction of the required functionality at the 6-C position of the inhibitors **2**–**6**. Attempts to open the oxirane ring using 2-benzyloxyethanol in the presence of alumina failed. Since an analogous procedure had been sucessfully utilised for the addition of more simple alcohols to epoxide **17**, we assume that the 1,2-arrangement of the Lewis bases in the nucleophile prevented reaction from occurring. Generation of 2-benzyloxyethoxide using NaH and subsequent reaction with the epoxide in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) at 100 °C[41] led to elimination of the oxirane ring to give the allyl alcohol **18** but none of the required ether **19**. Weakly acidic conditions, for example using camphorsulfonic acid, gave no reaction at all.[42] However, in the presence of a catalytic amount of a strong Lewis acid, $BF_3{\cdot}Et_2O$, and the nucleophile, 2-benzyloxyethanol, the oxirane ring opened smoothly to give the required racemic 2-benzyloxyethyl ether **19** (Scheme 2).[43] Note that the reaction could be carried out successfully either in the absence of solvent or in the presence of a small quantity of dry toluene, and that the NMR spectra for the 2-benzyloxyethyl ether **19** were in keeping with those expected for the required regio- and stereo-specificity for the ring opening reaction. The structure of compound **19** was later confirmed to be correct when the X-ray crystal structure of a camphanate ester derivative **20** was solved[1] (see below).

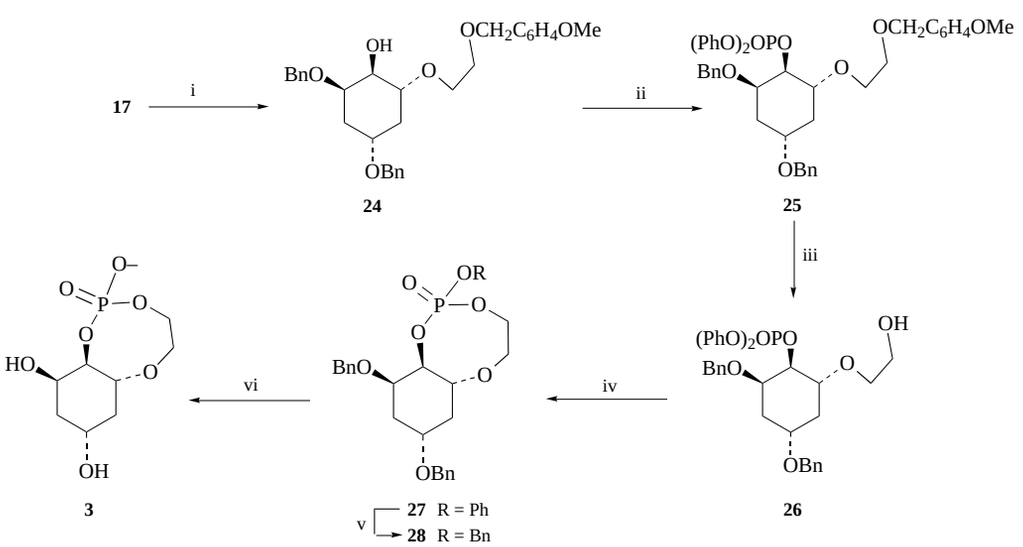
Under optimised reaction conditions, the 2-benzyloxyethyl ether **19**, a precursor to inhibitor **2**, could be obtained in 50% yield. Phosphorylation[44] of the cyclitol ether **19** was achieved with $ClPO(OPh)_2$ in 95% yield. Subsequent transesterificaton[44] of the diphenyl phosphate triester **21** in the presence of 2 equiv. of sodium benzyloxide in THF gave the fully protected dibenzyl phosphate triester **22**, which displayed all of the correct spectral and analytical properties. Reductive cleavage of all of the benzyl protecting groups was accomplished by dissolving metal reduction[45] using sodium in liquid ammonia. After work-up by ion exchange chromatography and treatment with cyclohexylamine, the racemic potential inhibitor **2** was obtained as its bis(cyclohexylammonium) salt in 68% yield. The compound was tested for biological activity as described below.

In order to prepare the cyclic phosphate **3**, the racemic epoxide **17** was treated with 2-(*p*-methoxybenzyloxy)ethanol (itself prepared from ethane-1,2-diol and *p*-methoxybenzyl chloride) in the presence of boron trifluoride to give the required 2-(*p*-methoxybenzyloxy)ethyl ether **24** in 50% yield (Scheme 3).[1] Phosphorylation to give the diphenyl phosphate triester **25** was achieved in 95% using $ClPO(OPh)_2$, as before. The *p*-methoxybenzyl protection was removed using dichlorodicyanoquinone (DDQ)[46] to give the alcohol **26** in 80% yield and this was treated with sodium hydride to effect phospholactonisation. The cyclic phenyl phosphate triester **27** was obtained in 50% yield and displayed the expected spectral and analytical properties. Treatment of the cyclic phenyl phosphate **27** with sodium benzyloxide gave the cyclic benzyl phosphate triester **28** as a mixture of diastereoisomers in 57% yield (Scheme 3). The diastereoisomerism arises because there are two possible configurations at phosphorus relative to the fixed stereochemistry of the carbocycle. Since the intended product, cyclic phosphate diester **3**, contains a prochiral phosphorus centre, the unseparated diastereoisomers of the cyclic benzyl phosphate **28** were subjected to treatment with sodium in liquid ammonia. The cyclic phosphate diester **3** was converted to its cyclohexylammonium salt using the procedure described previously.

While 2-benzyloxyethanol (Scheme 2) and 2-(*p*-methoxybenzyloxy)ethanol (Scheme 3) had reacted moderately well with the epoxide **17**, we expected that the analogous reaction with a monophosphorylated ethane-1,2-diol **29** on the route to inhibitor **4** might prove troublesome. However, alternative preparative strategies were considered and all of them required several



Scheme 2 *Reagents and conditions:* i, BnBr, NaH, THF, 80%; ii, $BnOCH_2CH_2OH$, $BF_3{\cdot}OEt_2$, 50%; iii, $ClPO(OPh)_2$, Et_3N, DMAP, CH_2Cl_2, 95%; iv, BnONa (2 equiv.), THF, 70%; v, Na, $NH_{3(liq)}$, −78 °C, then Amberlite IR 118H and cyclohexylamine, 68%; vi, NaH, TMEDA, $BnOCH_2CH_2OH$ 100 °C, 20%; vii, (−)-(1*S*,4*R*)-camphanoyl chloride, Et_3N, DMAP, CH_2Cl_2, 90%; viii, separate diastereoisomers, 40% of each; ix, KOH, EtOH, 90%; x, Na, $NH_{3(liq)}$, −78 °C, 57%

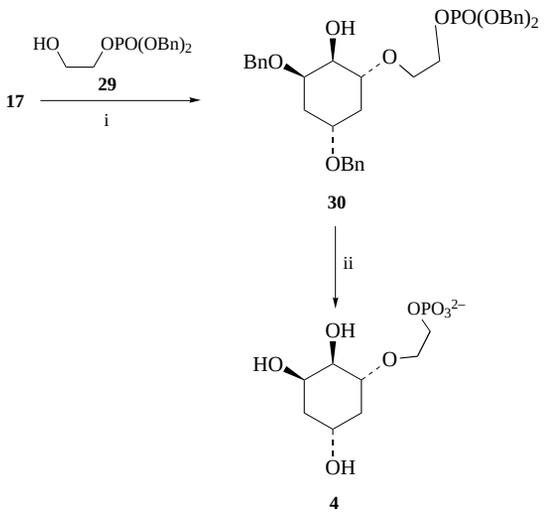


Scheme 3 *Reagents and conditions:* i, $HOC_2H_4OCH_2C_6H_4OMe$, $BF_3{\cdot}OEt_2$, 50%; ii, $ClPO(OPh)_2$, Et_3N, DMAP, CH_2Cl_2, 95%; iii, DDQ, CH_2Cl_2, 80%; iv, NaH, THF, −78 °C, 50%; v, BnONa (1 equiv.), THF, −78 °C, 60%; vi, Na, $NH_{3(liq)}$, −78 °C, then Amberlite IR 118H and cyclohexylamine, 60%

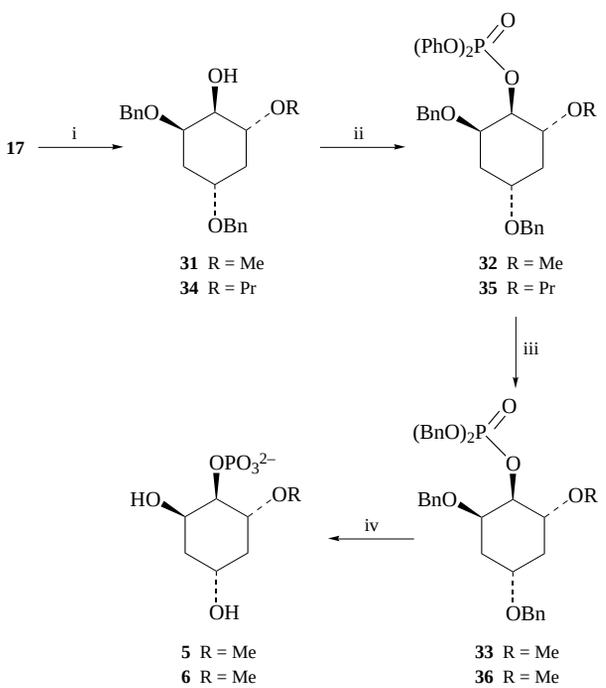
protection and deprotection steps to be performed after the precious epoxide **17** had been ring-opened. For example, we knew compound **24** could be prepared in 50% yield from epoxide **17**. Benzylation of the secondary alcohol in **24** should proceed in a reasonable yield, possibly 80%, but this material would still need to be deprotected with DDQ and then phosphorylated and then transesterified before deprotonation. Since on the basis of previous experience (Scheme 3), this latter route seemed likely to yield only 10–15% of the fully protected phosphorylated inhibitor **4** precursor, we opted to prepare the pre-phosphorylated nucleophile 2-dibenzyloxyphosphoryloxyethanol **29**. This material was obtained from *p*-methoxybenzyloxyethanol by phosphorylation, followed by DDQ-promoted deprotection of the *p*-methoxybenzyl group.

When epoxide **17** was treated with compound **29** in the presence of boron trifluoride, the desired racemic ether **30** was obtained in 13% yield after chromatographic purification on silica and displayed the expected properties and spectral data (Scheme 4). The low yield is ascribed to partial decomposition of the acid-labile phosphate group in both the reactant **29** and the product **30** induced by the Lewis acidic catalyst. Reductive deprotonation of all of the benzyl groups was achieved in one step in the usual way, and after ion exchange chromatography and treatment with cyclohexylamine, the salt of the potential inhibitor **4** was obtained in 60% yield as a racemate.

To prepare inhibitors **5** and **6**, the racemic epoxide **17** was treated with methanol and propan-1-ol, respectively, in the presence of $BF_3{\cdot}OEt_2$ (Scheme 5). In each case the desired ether products **31** and **34** were obtained in 70% yield and showed the expected spectral and analytical properties. These compounds were taken through sequential phosphorylation, transphosphoesterification and deprotection and were then converted to their respective racemic cyclohexylammonium salts **5** and **6** as described above, and as summarised in Scheme 5.



Scheme 4 *Reagents and conditions:* i, $BF_3{\cdot}OEt_2$, 13%; ii, Na, $NH_{3(liq)}$, −78 °C, then Amberlite IR 118H and cyclohexylamine, 60%



Scheme 5 *Reagents and conditions:* i, MeOH or PrOH, $BF_3{\cdot}OEt_2$, 70%; ii, $ClPO(OPh)_2$, Et_3N, DMAP, CH_2Cl_2, 95%; iii, BnONa (2 equiv.), THF, 60%; iv, Na, $NH_{3(liq)}$, −78 °C, then Amberlite IR 118H and cyclohexylamine, 60%

In order to determine the inhibitory potential of the compounds **2**–**6**, the concentration of each of the materials was varied and for each concentration the initial rates of reaction were obtained for a range of substrate concentrations in standard enzyme assays, as described previously.[18] The initial rate data was fitted using non-linear regression analysis[18] and the values of K_{App} and V_{App} were plotted graphically to determine the mode of inhibition and the values of K_i.[18] (Table 1).

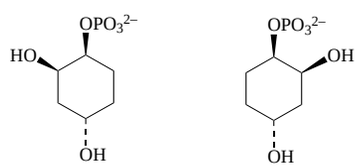
The racemic 6-methyl ether **5** behaved as a competitive inhibitor and showed a K_i value of 2.5 μmol dm^{-3} which is almost identical to the value obtained for the 6-deoxy analogue.[26] The racemic 6-propyl ether analogue **6** proved to be a slightly more potent competitive inhibitor and displayed a K_i value of 1.2 μmol dm^{-3}.[1] This finding was expected and accords with earlier results which showed that the presence of a large lipophilic side-chain appended to 6-C enhanced inhibitor efficacy.[28,47] In the light of this analysis it might have been expected that the potency of the racemate of the 6-hydroxyethyl ether **2** with its hydrophilic side-chain should be very much lower than the isosteric hydrophobic propyl ether **6**. However, our previous modelling studies had shown that groups attached to the cyclitol at the 6-position can access either of two quite distinct and contrasting regions of the active site.[25] These are a lipophilic pocket formed by Val-40 and Leu-42 and, to a lesser extent, Trp-219 and Ile-216 [the space occupied by the adenine moiety in the substrate 2′-AMP (and used previously by Merck in the design of inhibitors)[28]] or the hydrophilic site near the Mg^{2+} 2 ion normally occupied by a nucleophilic water molecule according to our hypothesis [Fig. 2(*a*)].

When tested, the racemic hydroxyethyl ether **2** behaved as a competitive inhibitor and displayed a K_i value of 1.8 μmol dm^{-3}.[1] The compound showed no tendency to to serve as a substrate and was completely stable to hydrolysis, as determined by monitoring enzyme incubations by 500 MHz ^{1}H NMR spectroscopy. Furthermore, the compound did not undergo transesterification to give compound **4**. This pleasing result accords with the idea that the hydroxyethyl arm can access the coordination sphere of the Mg^{2+} 2 ion, although clearly more information was required to confirm this notion.

On the basis of the earlier modelling work[25] it was possible to predict that, if the hydroxyethyl ether **2** was able to bind with its side-chain in contact with Mg^{2+} 2, then the (1*R*,2*R*,4*R*,6*R*) enantiomer should be a much better inhibitor than its (1*S*,2*S*,4*S*,6*S*) antipode. In order to evaluate these theoretical predictions it was necessary to prepare the individual enantiomers of compound **2** and test their biological activities.

In order to effect resolution, the 1-hydroxy group of the racemic 2-benzyloxyethyl ether **19** was derivatised with (−)-(1*S*,4*R*)-camphanoyl chloride in excellent yield using a literature protocol.[48] The diastereoisomeric camphanate esters (+)-**20A** {$[a]_D$ 24.5 (*c* 0.09, MeOH)} and (−)-**20B** {$[a]_D$ −31.5 (*c* 0.09, MeOH)} were separated by column chromatography on silica and attempts were made to obtain suitable crystals for X-ray crystallography. Camphanate ester (+)-**20A**, the least polar dextrorotatory diastereoisomer, gave suitable crystals and analysis by X-ray diffraction, using the known absolute configuration of the camphanoyl moiety as a stereochemical reference, defined the absolute configuration of the cyclitol moiety as (1*S*,2*S*,4*S*,6*S*).[1] The separated diastereoisomers were then each saponified to give (+)-(1*S*,2*S*,4*S*,6*S*)-2-benzyloxyethyl ether **19** and (−)-(1*R*,2*R*,4*R*,6*R*)-2-benzyloxyethyl ether **19** and each compound was converted to its deprotected phosphate ester derivative, (+)-(1*S*,2*S*,4*S*,6*S*)-**2** and (−)-(1*R*,2*R*,4*R*,6*R*)-**2**, respectively.

When the compounds were tested as inhibitors, both displayed competitive inhibition. The K_i value of (+)-(1*S*,2*S*,4*S*,6*S*)-hydroxyethyl ether **2** was 60 μmol dm^{-3} while that for the (−)-(1*R*,2*R*,4*R*,6*R*)-hydroxyethyl ether **2** was 120-fold lower at 0.5 μmol dm^{-3} (Table 1, entries 2 and 3).[1] The relative magnitudes of these values are in accord with theoretical predictions[25] and indicates that the hydroxyethyl side chain can bind in the coordination sphere for Mg^{2+} 2. It is interesting to note that the (−)-antipode of the 6-deoxy analogue **37**, which

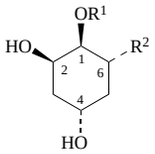


(–)-37 (inhibitor) **(+)-37** (substrate)

possesses the same relative configuration as (−)-**2**, was found to be much more potent than its (+)-antipode (Table 1, entries 8 and 9).[26] Presumably this compound behaves as an inhibitor because there is no 6-hydroxy group available to hydrogen-bond to the nucleophilic water molecule in directing it to attack the phosphorus atom.

In order to investigate further the hypothesis that the side-

Table 1 Inhibition constants for substituted cyclohexane-1,2,4,6-tetraols



Entry	Compound	R^1	R^2	K_i/μmol dm^{-3}	Mode of inhibition
1	(±)-**2**	PO_3^{2-}	OC_2H_4OH	1.8	competitive
2	(+)-(1*S*,2*S*,4*S*,6*S*)-**2**	PO_3^{2-}	OC_2H_4OH	60.0	competitive
3	(−)-(1*R*,2*R*,4*R*,6*R*)-**2**	PO_3^{2-}	OC_2H_4OH	0.5	competitive
4	(±)-**3**	$-P(O)(O^-)(O^-)OCH_2CH_2O-$		160.0	competitive
5	(±)-**4**	H	$OC_2H_4OPO_3^{2-}$	8.5	competitive
6	(±)-**5**	PO_3^{2-}	OMe	2.5	competitive
7	(±)-**6**	PO_3^{2-}	OPr	1.2	competitive
8	(−)-(1*S*,2*R*,4*S*)-**37**	PO_3^{2-}	H	3.0	competitive[a]
9	(+)-(1*R*,2*S*,4*R*)-**37**	PO_3^{2-}	H	weak substrate	weak substrate[a]

[a] Ref. 26.

chain of the hydroxyethyl ether **2** can displace the nucleophilic water molecule, we prepared the theoretical intramolecular transesterification product, potential inhibitor **4**. In designing this compound we reasoned that it should only be possible to maintain all of the interactions of the peripheral ring hydroxy groups with the enzyme and, simultaneously, all of the interactions of the phosphate group with the enzyme bound metal ions if the bridging ester oxygen atom in the phosphonatooxyethyl group of compound **4** could interact with Mg^{2+} 2. Evidence in support of this arrangement would then be provided by a low observed K_i value. In the event, when compound **4** was tested as a racemate, it proved to be a good competitive inhibitor and possessed a K_i value of 8.5 μmol dm^{-3} (Table 1, entry 5) only five times higher than the racemate of the isomeric 6-hydroxyethyl ether 1-phosphate **2** (see Table 1, entry 1). This is the lowest K_i value, by far, for any known primary alkyl phosphate inhibitor for inositol monophosphatase and we believe that its potency can be ascribed to its similar binding mode to that of compound **2**.

Finally, in spite of the weight of evidence which now suggests that hydrophilic side-chains appended to the 6-position of the cyclitol can interact with Mg^{2+} 2, all of the information comes from compounds which contain a flexible side-chain. In order to fix the position of the side-chain and maintain the key interactions of the peripheral oxygen atoms with the enzyme, the constrained bicyclic phosphate diester **3** was prepared. Note that the compound is an analogue which resembles the transition state for the hypothetical intramolecular transesterification of compound **2** to **4**. Compound **3** differs substantially from other compounds in the series in being a monoanion, which we knew would adversely effect binding.[19,13] However, when tested, the bicyclic phosphate diester **3** served as a moderate competitive inhibitor and displayed a K_i value of 160 μmol dm^{-3}.[1] This is a remarkably low value (Table 1, entry 4) and the compound is by far the most potent monoanionic inhibitor for IMPase known.

The results presented here are in accord with the predictions of our earlier molecular modelling work[25] and provided support for the structural detail of the active complex [Fig. 2(*a*)]. The results suggest that there is a water molecule bound to Mg^{2+} 2 in the vicinity of the position we have ascribed to the nucleophilic water molecule (or hydroxide), but do not prove that this molecule is the nucleophile. The results do not rule out the alternative in-line mechanism depicted in Fig. 2(*b*) but, if such a mechanism is followed, it is extremely difficult to understand why the replacement of the 6-OH group in a substrate by an alkoxy group or by a hydrogen atom should give a tight-binding non-hydrolysable compound. Other evidence, we believe, favours the location of the nucleophile on Mg^{2+} 2, rather than on Mg^{2+} 1, and this has been summarised previously.[25] Our own current efforts are focused on the determination of the stereochemical course, the results of which, we hope, will resolve the present mechanistic ambiguities.

In terms of future inhibitor design, the results reported here indicate that two different environments in two different locations within the active site, one lipophilic (Val-40 and Leu-42) and one hydrophilic (the coordination sphere of Mg^{2+} 2), can be accessed by side-chains attached to the 6-C position of the cyclitol.[25] A major challenge will be to access both at the same time. Furthermore, the finding that the bicyclic phosphate diester monoanion **3** has moderate affinity for the enzyme bodes well for the future design of less highly charged inhibitors.

Experimental

NMR Spectra were recorded on a Bruker AM-300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz; ^{31}P, 121.5 MHz), a Varian Gemini spectrometer (^{1}H, 200 MHz; ^{13}C, 50.3 MHz), a Varian Gemini spectrometer (^{1}H, 300 MHz; ^{13}C, 75.4 MHz; ^{31}P, 121.5 MHz) and a Varian Unity Plus 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125.6 MHz; ^{31}P, 202.5 MHz). ^{1}H NMR Spectra were referenced internally to ^{2}HOH (δ 4.68) and C^2HCl_3 (δ 7.27). ^{13}C NMR Spectra were referenced to C^2HCl_3 (δ 77.5) and ^{31}P NMR to external H_3PO_4 (δ 0). *J* Values are given in Hz. Infrared spectra were recorded using a Perkin-Elmer 1710 FT-IR spectrometer. The samples were prepared as Nujol mulls or thin films between sodium chloride discs. Absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Melting points were measured using an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on an Optical Activity Ltd. AA-1000 polarimeter using 10 cm path length cells at room temperature and are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra were recorded on a VG AutoSpec. Major fragments are given as percentages of the base peak intensity. UV–VIS optical densities were measured on a Cam Spec M302 spectrophotometer. Solvents and common reagents were purified according to the method of Perrin and Armarego.[49] Analytical thin layer chromatography (TLC) was carried out on 0.25 mm precoated silica gel plates (MN SIL G/UV$_{254}$), and compounds were visualised by UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid or aqueous potassium permanganate. Light petroleum refers to the fraction boiling at 40–60 °C. Inositol 1-phosphates were prepared from *myo*-inositol as described previously,[18,50] while other substrates were prepared as described below. Amberlite IR 118H ion exchange resin was obtained from British Drug Houses (Poole, Dorset, UK). Phosphorylating agents were obtained from the Aldrich Chemical Co. Ltd. (Gillingham, Dorset, UK). All other chemicals were of analytical grade or were recrystallised or redistilled before use.

Enzyme

Bovine brain inositol monophosphatase was purified from a recombinant *Escherichia coli* strain[51] as described previously in a routine yield of 20%.[18] Purity was assessed using polyacrylamide gel electrophoresis as described previously.[18] Enzyme activity assays were performed using a colorimetric assay developed by Itaya and Ui[52] employing molybdic acid and malachite green. Rate determinations were performed at 37 °C in triplicate in assay buffer A containing KCl (300 mmol dm^{-3}), $MgCl_2$ (2 mmol dm^{-3}) and Tris·HCl at pH 7.8 (50 mmol dm^{-3}). Background phosphatase activity was assessed in each experiment by performing parallel assays in the presence of Li^+ ion in buffer B (buffer B is buffer A plus 150 mmol dm^{-3} LiCl). Rate data were analysed and processed graphically and by using non-linear regression analysis as described previously.[18]

Colorimetric assay. Colorimetric assay reagent: malachite green (1.5 g) was dissolved in hydrochloric acid (5 mol dm^{-3}; 25 cm^3) and diluted with water (750 cm^3). To this solution was added ammonium molybdate (10.5 g) in hydrochloric acid (5 mol dm^{-3}; 225 cm^3) and the solution stirred at room temperature for 10 min. The solution was filtered by gravity and stored in the dark for periods of up to one month.

Incubation samples contained the following: assay buffer A (210 mm^3), substrate (Ins 1-P) at various concentrations in assay buffer (30 mm^3), inhibitor at various concentrations in assay buffer (30 mm^3) [in the absence of an inhibitor, this addition was substituted by assay buffer (30 mm^3)] and enzyme solution (activity pre-determined for the requirements of individual experiments) (30 mm^3).

The assay solutions were incubated at 37 °C and the reaction was quenched by the addition of colorimetric assay reagent (2.0 cm^3) at the required time (relative to the addition of the enzyme solution). The colour was allowed to develop over a period of 30 min, and the absorbance at 660 nm was measured in a 10 mm pathlength cuvette. Phosphate concentrations were determined by comparison of absorbance value to a preconstructed standard curve prepared using known phosphate concentrations.

(±)-Cyclohex-3-enol

Cyclohex-3-enol was prepared from cyclohexane-1,4-diol **7** (23 g, 0.2 mol) according to the method of Godek *et al.*[36] Distillation afforded a colourless liquid (9.8 g, 50%), bp 165 °C (lit.,[36] 165 °C); δ_H(200 MHz; C^2HCl_3) 1.5–1.7 (1 H, m, secondary-H), 1.8–2.2 (3 H, m, secondary-H), 2.3–2.45 (2 H, m, secondary-H), 3.85–4.0 (1 H, m, 1-H) and 5.5–5.7 (2 H, m, 1-H and 2-H); δ_C(50.3 MHz; C^2HCl_3) 24.2, 31.3 and 34.8 (2-C, 5-C and 6-C), 67.4 (1-C) and 124.6 and 127.2 (3-C and 4-C).

(±)-4-Benzyloxycyclohexene 8

Cyclohex-3-enol (9.8 g, 0.1 mol) was dissolved in dry DMF (100 cm^3), the reaction mixture was cooled in an ice bath and NaH (60% dispersion in oil; 4.8 g, 0.12 mol) was added under a nitrogen atmosphere. After 30 min, benzyl bromide (14.3 cm^3, 20.5 g, 0.12 mol) was added through a septum. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 3 h. Water was carefully added until all the NaH was destroyed. Water (100 cm^3) was added, and the mixture was extracted with diethyl ether (3 × 100 cm^3). The combined diethyl ether phases were dried ($MgSO_4$) and the solvents were removed under reduced pressure. The residual oil was chromatographed on silica (gradient column: first light petroleum, then light petroleum–ethyl acetate, 15:1) to give racemic benzyl ether **8** as a colourless liquid (18 g, 96%); δ_H(200 MHz; C^2HCl_3) 1.65–1.85 (1 H, m, secondary-H), 2.0–2.3 (4 H, m, secondary-H), 2.4–2.6 (1 H, m, secondary-H), 3.65–3.8 (1 H, m, 4-H), 4.6–4.7 (2 H, m, OCH_2Ph), 5.6–5.8 (2 H, m, 1-H and 2-H) and 7.2–7.5 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 24.6, 28.4 and 32.3 (3-C, 5-C and 6-C), 70.4 and 74.3 (4-C and OCH_2Ph), 124.9, 127.4, 127.9, 128.1, 128.3, 128.9 and 130.0 (1-C, 2-C, Ar-CH) and 139.6 (Ar-C quaternary); *m/z* (EI) 188 (9%, M^+) and 91 (100, $[C_7H_7]^+$). (Note, this compound has been prepared previously from 4-benzyloxycyclohexanol in 2 steps in 78% overall yield.[37])

(±)-4-Benzyloxy-1,2-epoxycyclohexane 9 and 10 (mixture of diastereoisomers)

Using a modified literature procedure,[38] a solution of MCPBA (60% pure, 16.8 g, 58 mmol) in CH_2Cl_2 (250 cm^3), dried over $MgSO_4$, was filtered into a flask containing 4-benzyloxycyclohexene **8** (9 g, 48 mmol) cooled in an ice bath, and the mixture then stirred for 12 h. The solution was washed with $NaHSO_3$ (10% solution; 100 cm^3) and $NaHCO_3$ (saturated; 2 × 100 cm^3). The organic phase was dried ($MgSO_4$), the solvent was evaporated under reduced pressure and the residue chromatographed on silica (light petroleum–ethyl acetate, 5:1) to give a colourless oil (8.1 g, 83%), a 2:3 mixture of **9** (*trans*) and **10** (*cis*).

For **9** (*trans*): δ_H(200 MHz; C^2HCl_3) 1.5–1.7 (2 H, m, secondary-H), 1.85–2.3 (4 H, m, secondary-H), 3.2 (2 H, s, 1-H and 2-H), 3.5–3.6 (1 H, m, 4-H), 4.45–4.55 (2 H, d, $^2J_{HH}$12.0, OCH_2Ph) and 7.2–7.5 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 21.1, 24.0 and 31.1 (3-C, 5-C and 6-C), 52.2 and 52.7 (1-C and 2-C), 70.5 and 71.1 (4-C and OCH_2Ph), 128.0 and 128.9 (Ar-CH) and 139.2 (Ar-C quaternary).

For **10** (*cis*): δ_H(200 MHz; C^2HCl_3) 1.4–2.4 (6 H, m, 3-H, 5-H and 6-H), 3.1 (2 H, s, 1-H and 2-H), 3.3 (1 H, m, 4-H), 4.55 (2 H, s, OCH_2Ph) and 7.2–7.5 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 24.3 and 31.3 (3-C, 5-C and 6-C), 51.5 and 52.4 (1-C and 2-C), 70.4 and 73.5 (4-C, OCH_2Ph), 128.0 and 128.9 (Ar-CH) and 139.1 (Ar-C quaternary); *m/z* (EI) 204 (25%, M^+) and 91 (100, $[C_7H_7]^+$). (Note, epoxides **9** and **10** have been reported previously together with limited ^{1}H NMR spectroscopic data.[38])

(±)-5-Benzyloxycyclohex-2-enol 13 and 14 (mixture of diastereoisomers)

Using a modification of the procedure of Sharpless and Lauer[39] diphenyl diselenide (3.7 g, 12 mmol) was dissolved in ethanol (60 cm^3). Under a stream of nitrogen $NaBH_4$ (950 mg, 24.3 mmol) was added in batches (the yellow solution turned colourless when addition was complete). 4-Benzyloxy-1,2-epoxycyclohexane **9** and **10** (a 2:3 mixture, 4.35 g, 21.3 mmol) was then added and the mixture stirred at room temperature for 30–45 min. THF (30 cm^3) was added, followed by the dropwise (over 30 min) addition of H_2O_2 (22.7 cm^3 of a 30% solution). The reaction mixture was then heated under reflux for 6–7 h. The solvents were removed under reduced pressure, and the residual brown oil was partitioned between water (100 cm^3) and diethyl ether (100 cm^3). The water phase was extracted with diethyl ether (2 × 100 cm^3). The diethyl ether phases were combined and dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The residual oil was chromatographed on silica (light petroleum–ethyl acetate, 2:1) to give a colourless oil (2.1 g, 49%), a 2:3 mixture of **13** (*trans*) and **14** (*cis*).

For **13** (*trans*): δ_H(200 MHz; C^2HCl_3) 1.9–2.2 [4 H, m, 2 × (4-H and 6-H)], 3.8–3.9 (1 H, m, tertiary-H), 4.3–4.45 (1 H, m, tertiary-H), 4.6 (2 H, s, OCH_2Ph), 5.75–5.85 (2 H, s, 2-H and 3-H) and 7.2–7.6 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 32.3 and 37.4 (4-C and 6-C), 65.7 (C tertiary) 71.8 and 71.4 (C tertiary and OCH_2Ph), 128.1, 129.0 and 129.4 (2-C, 3-C, Ar-CH) and 139.2 (Ar-C quaternary).

For **14** (*cis*): δ_H(200 MHz; C^2HCl_3) 1.7–2.05 [4 H, m, 2 × (4-H and 6-H)], 3.9 (1 H, m, tertiary-H), 4.15 (1 H, m, tertiary-H), 4.6 (2 H, d, $^2J_{HH}$ 12.2, OCH_2Ph), 5.95 (2 H, s, 2-H, 3-H) and 7.2–7.6 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 25.0 and 28.8 (4-C and 6-C), 66.0 (C tertiary), 70.9 and 72.2 (C tertiary and OCH_2Ph), 128.1, 128.2, 128.9, 130.8 and 133.4 (2-C, 3-C, Ar-CH) and 139.0 (Ar-C quaternary). [Note, compound (−)-**14** has been prepared previously from 5-benzyloxycyclohex-2-en-1-one in 90% yield, and compound (+)-**13** has been prepared

previously from (−)-**14** in 71% yield *via* the Mitsunobu reaction, and their ^{1}H NMR spectroscopic data have been reported.[40]]

(±)-5-Benzyloxy-2,3-epoxycyclohexanol 15 and 16 (mixture of diastereoisomers)

A solution of MCPBA (60% purity; 2.5 g, 8.8 mmol) in CH_2Cl_2 (100 cm^3) was dried ($MgSO_4$) and filtered into a flask containing 5-benzyloxycyclohex-2-enol **13** and **14** (a 2:3 mixture, 1.5 g, 7.3 mmol) at 0 °C. The resulting solution was stirred for 12 h and then washed with $NaHSO_3$ (10%; 100 cm^3) followed by $NaHCO_3$ (2 × 100 cm^3). The organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residual oil was chromatographed on silica (light petroleum–ethyl acetate, 1:1) to give a white solid **15** (530 mg, 33%, higher R_f, *trans*) and a colourless oil **16** (770 mg, 48%, lower R_f, *cis*).

For **15** (*trans*): mp 64–65 °C (Found: C, 70.7; H, 7.2. Calc. for $C_{13}H_{16}O_3$: C, 70.9; H, 7.3%); δ_H(200 MHz; C^2HCl_3) 1.5–1.65 (1 H, m, secondary-H), 1.95–2.1 (3 H, m, 3 × secondary-H), 3.35–3.45 (2 H, m, 2-H and 3-H), 3.65–3.75 (1 H, m, tertiary-H), 4.25-4.4 (1 H, m, tertiary-H), 4.48 (1 H, d, $^2J_{HH}$ 12.0, OCH_2Ph), 4.52 (1 H, d, $^2J_{HH}$ 12.0, OCH_2Ph), 7.25–7.4 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 29.7 and 33.0 (6-C and 4-C), 54.6 and 56.0 (2-C and 3-C), 65.3 (1-C), 70.6 (O*C*H$_2$Ph), 71.1 (5-C), 127.9, 128.1 and 128.9 (Ar-CH) and 138.4 (Ar-C quaternary).

For **16** (*cis*): δ_H(200 MHz; C^2HCl_3) 1.4–1.65 (2 H, m, 4-H and 6-H), 1.8–1.95 (2 H, m, 4-H and 6-H), 3.4–3.5 (2 H, m, 2-H and 3-H), 3.7–3.8 (1 H, m, tertiary-H), 3.95–4.05 (1 H, m, tertiary-H), 4.65 (2 H, s, OCH_2Ph), 7.25–7.4 (5 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 22.3 and 28.4 (4-C and 6-C), 55.7 and 56.1 (2-C and 3-C), 64.4 (1-C), 70.4 (O*C*H$_2$Ph), 72.8 (5-C), 127.7, 127.8 and 128.4 (Ar-CH) and 138.8 (Ar-C quaternary). [Note, compound (+)-**15** has been prepared previously from (+)-**13** in 99% yield, and ^{1}H NMR spectroscopic data have been reported.[40]]

***trans*-(±)-1,5-Dibenzyloxy-2,3-epoxycyclohexane 17†**

5-Benzyloxy-2,3-epoxycyclohexanol **15** (1.5 g, 6.8 mmol) was dissolved in dry DMF (50 cm^3) and set under an atmosphere of N_2. NaH (60% dispersion in oil; 324 mg, 8.1 mmol) was then added, and the mixture stirred at room temperature for 30 min. Benzyl bromide (0.96 cm^3, 1.4 g, 8.1 mmol) was added through the septum, and stirring was continued at room temperature for 4 h. The reaction was carefully quenched with water (5 cm^3), aqueous NaCl (saturated; 50 cm^3) was added, and the aqueous phase was extracted with diethyl ether (3 × 100 cm^3). The combined organic layers were dried ($MgSO_4$) and the solvent removed under reduced pressure. The residual oil was chromatographed on silica (light petroleum–ethyl acetate, 5:1) to give the bis(benzyl ether) **17** as a colourless oil (1.7 g, 81%) (Found: C, 76.7; H, 7.2. Calc. for $C_{20}H_{22}O_3$: C, 77.4; H, 7.1%); δ_H(200 MHz; C^2HCl_3) 1.6–1.7 (1 H, m, secondary-H), 1.95–2.1 (3 H, m, 3 × secondary-H), 3.29–3.31 (1 H, m, tertiary-H), 3.4–3.42 (1 H, m, tertiary-H), 3.67–3.75 (1 H, m, tertiary-H), 4.1–4.21 (1 H, m, tertiary-H), 4.4 (2 H, s, OCH_2Ph), 4.7 (2 H, s, OCH_2Ph), 7.2–7.5 (10 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 29.1 and 29.7 (4-C and 6-C), 53.0 and 53.8 (2-C and 3-C), 70.6 and 71.1 (2 × O*C*H$_2$Ph), 71.6 and 72.6 (1-C and 5-C), 128.0, 128.2, 128.3 and 128.9 (Ar-CH) and 138.9 and 139.1 (Ar-C quaternary); *m/z* (EI) 311 (1%, $[M + H]^+$), 219 (2, $[M - C_7H_7]^+$) and 91 (100, $[C_7H_7]^+$). (Note that previous reports of the compound did not provide spectroscopic data for comparison.[28,47])

(±)-2,4-Di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)cyclohexane-1,2,4,6-tetraol 19

Epoxide **17** (1.5 g, 4.8 mmol) and 2-benzyloxyethanol (1.5 g, 10 mmol) were cooled in an ice bath. Three drops of $BF_3{\cdot}OEt_2$ in diethyl ether were then added with stirring, and stirring at room temperature was continued for 30 min. The reaction mixture was paritioned between water (50 cm^3) and diethyl ether (50 cm^3). The diethyl ether phase was dried ($MgSO_4$), evaporated to dryness, and excess 2-benzyloxyethanol was distilled off in a Kugelrohr apparatus under reduced pressure. The residual oil was chromatographed on silica (light petroleum–ethyl acetate, 2:1) to give alcohol **19** as a colourless oil (1.1 g, 50%) (Found: C, 75.0; H, 7.6. $C_{29}H_{34}O_5$ requires C, 75.3; H, 7.4%); ν_{max}(neat)/cm^{-1} 3475s, 2948s, 2873s and 1094s; δ_H(200 MHz; C^2HCl_3) 1.25–1.48 (2 H, m, 2 × secondary-H), 2.22–2.40 (1 H, m, secondary-H), 2.39–2.56 (1 H, m, secondary-H), 3.5–4.0 (8 H, m, OC$_2$H$_4$O and 4 × H tertiary), 4.5 (2 H, s, OCH_2Ph), 4.55–4.65 (4 H, m, 2 × OCH_2Ph) and 7.2–7.5 (15 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 34.9 and 36.3 (3-C and 5-C), 69.7 and 70.2 (OC$_2$H$_4$O), 71.1 (O*C*H$_2$Ph), 72.2 (4-C), 72.7 (O*C*H$_2$Ph), 73.7 (O*C*H$_2$Ph), 76.4 and 76.7 (2-C and 6-C), 78.3 (1-C), 128.1, 128.2, 128.3, 128.8, 128.9 and 128.9 (Ar-CH) and 139.1 and 139.4 (Ar-C quaternary); *m/z* (EI) 371 (8%, $[M - C_7H_7]^+$), 281 {10, $[M + H - (2 \times C_7H_7)]^+$}, 105 (42, $[PhCO]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-1-*O*-Diphenoxyphosphoryl-2,4-di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)cyclohexane-1,2,4,6-tetraol 21

Alcohol (±)-**19** (1.16 g, 2.5 mmol), 4-dimethylaminopyridine (DMAP) (32 mg, 0.26 mmol) and dry Et_3N (1.04 cm^3, 757 mg, 7.5 mmol) were dissolved in dry CH_2Cl_2 (60 cm^3) under an atmosphere of N_2. $ClPO(OPh)_2$ (0.78 cm^3, 1.02 g, 3.8 mmol) was added, and the mixture was stirred at room temperature for 3–4 h. The mixture was washed with water (60 cm^3) and the organic phase dried ($MgSO_4$). The solvent was removed under reduced pressure and the residual oil chromatographed on silica (light petroleum–ethyl acetate, 5:1) to give phosphate triester (±)-**21** as a colourless oil (1.65 g, 95%); δ_H(300 MHz; C^2HCl_3) 1.4–1.53 (2 H, m, 2 × secondary-H), 2.24–2.34 (1 H, m, secondary-H), 2.43–2.54 (1 H, m, secondary-H), 3.46–3.52 (2 H, m, OC$_2$H$_4$O), 3.6–3.8 (3 H, m, OC$_2$H$_4$O and tertiary-H), 3.8–3.95 (1 H, m, tertiary-H), 4.08–4.12 (1 H, m, tertiary-H), 4.5–4.55 (6 H, m, 3 × OCH_2Ph), 4.55–4.62 (1 H, m, 1-H) and 7.1–7.4 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 33.9 and 35.6 (3-C and 5-C), 69.5 and 69.7 (OC$_2$H$_4$O), 70.5 (O*C*H$_2$Ph), 70.9 (4-C), 72.0 and 73.1 (O*C*H$_2$Ph), 75.1 and 75.2 (2-C and 6-C), 82.9 (1-C, $^2J_{CP}$ 6.3), 119.9, 120.0, 120.1, 125.0, 125.1, 127.3, 127.4, 128.1, 128.3 and 129.5 (Ar-CH) and 138.2 and 138.3 (Ar-C quaternary), 150.6 (Ar-C quaternary, $^2J_{CP}$ 7.8) and 150.7 (Ar-C quaternary, $^2J_{CP}$ 9.1); δ_P(121.5 MHz; C^2HCl_3) −12.1; *m/z* (FAB-MS) 695 (8%, M^+), 341 (29, $[C_{21}H_{25}O_4]^+$), 251 {810 $[(PhO)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$). (These data were identical to those obtained for the two resolved antipodes of compound **21** which were fully characterised, see below.)

(±)-2,4-Di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-1-*O*-dibenzyloxyphosphorylcyclohexane-1,2,4,6-tetraol (±)-22

To a stirred solution of diphenyl phosphate (±)-**21** (1.39 g, 2 mmol) in dry THF (50 cm^3) under an atmosphere of N_2 was added NaH (60% dispersion in oil; 153 mg, 4 mmol) followed by benzyl alcohol (0.41 cm^3, 432 mg, 4 mmol). The mixture was stirred at room temperature for a further 4 h and then quenched carefully with water (1 cm^3). The solvent was removed under reduced pressure and the residual oil partitioned between water (100 cm^3) and CH_2Cl_2 (100 cm^3). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residual oils were chromatographed on silica (light petroleum–ethyl acetate, 2:1) to give phosphate triester (±)-**22** as a white solid (1.1 g, 75%), mp 57–59 °C; ν_{max}(Nujol)/cm^{-1} 1271m, 1094m and 1023m; δ_H(200 MHz; C^2HCl_3) 1.25–1.46 (2 H, m, 2 × secondary-H), 2.13–2.35 (1 H, m, secondary-H), 2.42–2.55 (1 H, m, secondary-H), 3.4–3.6 (2 H, m, OC$_2$H$_4$O), 3.6–3.9 (4 H, m, OC$_2$H$_4$O, 2 × tertiary-H), 4.05–4.2 (2 H, m, 1-H and tertiary-H), 4.4–4.6 (6 H, m, 3 × OCH_2Ph), 5.0–5.1 (4 H, m, 2 × POCH_2Ph) and 7.2–7.4 (25 H, m, Ar-H); δ_C(50.3 MHz;

† The *trans* prefix refers to the relative configuration of the two benzyloxy substituents.

C^2HCl_3) 34.6 and 36.1 (3-C and 5-C), 69.6 ($POCH_2Ph$, $^2J_{CP}$ 7.7), 69.8 ($POCH_2Ph$, $^2J_{CP}$ 8.3), 69.9 and 70.3 (OC_2H_4O), 70.3 (OCH_2Ph), 71.1 (OCH_2Ph), 71.7 (4-C), 72.8 and 73.6 (OCH_2Ph), 75.8 (C-tertiary), 75.85 (C-tertiary, $^3J_{CP}$ 6.6), 82.0 (1-C, $^2J_{CP}$ 6.2), 128.0, 128.1, 128.4, 128.8, 128.9 and 129.0 (Ar-CH) and 139.0 (Ar-C quaternary); δ_P(121.5 MHz, C^2HCl_3) −1.4; *m/z* (EI) 723 (4%, $[M + H]^+$), 631 (7, $[M - C_7H_7]^+$), 353 {15, $[M - (PhCH_2O)_2PO_2H_2 - C_7H_7]^+$}, 279 {10, $[(PhCH_2O)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$). (Note that satisfactory microanalyses and accurate mass spectra were obtained on the resolved enantiomers of this compound, see below.)

(±)-6-*O*-(2-Hydroxyethyl)-1-*O*-phosphonatocyclohexane-1,2,4,6-tetraol bis(cyclohexylammonium) salt (±)-2

Under an atmosphere of N_2, gaseous ammonia (15–20 cm^3) was condensed at −78 °C, and sodium metal (206 mg, 9 mmol) was added. A solution of (±)-**22** (723 mg, 1 mmol) in dry THF (1–2 cm^3) was added to the blue solution through a septum. After stirring at −78 °C for 30 min, methanol (0.5 cm^3) was added and the solution was allowed to warm to room temperature. The solvents were removed under reduced pressure and the residual white solid was subjected to chromatography on Amberlite IR-118H ion exchange resin, eluting with water. The acid fractions containing the product were collected, freshly distilled cyclohexylamine (0.1 cm^3) added and stirring at room temperature continued for 4 h. The aqueous solution was extracted with diethyl ether (3 × 50 cm^3) to remove excess of cyclohexylamine, and lyophilised. The crude white solid was recrystallised from water–acetone to give racemic phosphate **2** as a white solid (330 mg, 70%), mp >200 °C (decomp.); δ_H(500 MHz; ^2H_2O) 1.1–1.2 (2 H, m, 2 × 4-H of Cha‡), 1.26–1.4 (8 H, m, 4 × 2-H and 4 × 3-H of Cha), 1.4–1.47 (1 H, m, 5-H), 1.54–1.6 (1 H, m, 3-H), 1.6–1.66 (2 H, m, 2 × 4-H of Cha), 1.73–1.86 (4 H, m, 4 × 3-H of Cha), 1.9–2.01 (4 H, m, 2 × 2-H of Cha), 2.03–2.14 (1 H, m, 3-H), 2.27–2.32 (1 H, m, 5-H), 3.1–3.2 (2 H, m, 2 × 1-H of Cha), 3.6–3.8 (5 H, m, OC_2H_4O and 6-H), 3.95–4.0 (1 H, m, 1-H), 4.0–4.05 (1 H, m, 4-H) and 5.0–5.04 (1 H, m, 2-H); δ_C(125.6 MHz; ^2H_2O) 24.2 (3-C of Cha), 24.7 (4-C of Cha), 30.7 (2-C of Cha), 37.4 (5-C), 37.7 (3-C), 50.7 (1-C of Cha), 61.2 (OCH_2CH_2OH), 64.8 (4-C), 68.1 (2-C), 71.3 (OCH_2CH_2OH), 76.4 (6-C, $^3J_{CP}$ 6.0) and 77.3 (1-C, $^2J_{CP}$ 6.5); δ_P(202.5 MHz; ^2H_2O) 7.05; *m/z* (FAB-MS) 349 (3.5%, $[M + 2K + H]^+$), 311 (1, $[M + K + 2H]^+$), 273 (1, $[M + 3H]^+$) and 133 (100), where M is the molecular weight of the phosphate dianion; *m/z* (CI) 471 (2%, $[M + H]^+$), 372 (3, $[M - C_6H_{14}N + 2H]^+$) and 100 (100, $[C_6H_{14}N]^+$).

(±)-2,4-Di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-1-*O*-[(1*S*,4*R*)-camphanoyl]cyclohexane-1,2,4,6-tetraol and its resolution into (+)-20A and (−)-20B

The racemic tetraol **19** (1.5 g, 3.25 mmol) was dissolved in dry CH_2Cl_2 (50 cm^3). DMAP (79 mg, 0.65 mmol) and dry Et_3N (0.9 cm^3, 656 mg, 6.5 mmol) were added and the mixture was cooled in an ice bath. (−)-(1*S*,4*R*)-Camphanoyl chloride (1.04 g, 4.8 mmol) was added and stirring was continued until the reaction was complete.[48] The organic layer was washed with water (50 cm^3) and dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The residual oil was chromatographed on silica (CH_2Cl_2–ethyl acetate, 15:1) to give two white solids (0.92 g of each diastereoisomer, 90% overall yield).

For (+)-(1*S*,2*S*,4*S*,6*S*)-**20A** (higher R_f): mp 76–78 °C (HRMS: found $[M - C_7H_7]^+$, 551.2648. $C_{32}H_{39}O_8$ requires 551.2645); $[\alpha]_D$ +24.5 (*c* 0.09, CH_3OH); ν_{max}(Nujol)/cm^{-1} 1735s and 1810s; δ_H(300 MHz; C^2HCl_3) 0.9 (3 H, s, camph-CH_3), 1.0 (3 H, s, camph-CH_3), 1.1 (3 H, s, camph-CH_3), 1.4–1.7 (4 H, m, camph-CH_2 and 2 × secondary-H), 1.75–2.05 (2 H, m, secondary-H and camph-CH_2), 2.2–2.4 (1 H, m, secondary-H), 2.45–2.6 (1 H, m, camph-CH_2), 3.55–3.95 (6 H, m, OC_2H_4O and 2 × tertiary-H), 4.0–4.1 (1 H, m, tertiary-H), 4.45–4.6 (6 H, m, 3 × OCH_2Ph), 5.0 (1 H, dd, $^3J_{HH}$ 9.3, $^3J_{HH}$ 2.5, 1-H) and 7.2–7.4 (15 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 10.3 and 17.2 (2 × camph-CH_3), 29.5 and 31.2 (camph-C_2H_4), 34.5 and 36.0 (3-C and 5-C), 54.7 and 55.3 (camph-C), 69.5 and 70.2 (OC_2H_4O), 71.2 (OCH_2Ph), 71.6 (4-C), 72.5 and 73.7 (OCH_2Ph), 74.8 and 74.9 (2-C and 6-C), 78.1 (1-C), 91.7 (camph-C), 128.1, 128.2, 128.3, 128.6, 128.7, 128.9 and 129.0 (Ar-CH), 138.6, 138.8 and 139.0 (Ar-C quaternary) and 167.6 and 178.8 (camph-CO); *m/z* (EI) 642 (1%, M^+), 551 (18, $[M - C_7H_7]^+$), 181 (17, $[C_{10}H_{13}O_3]^+$) and 91 (100, $[C_7H_7]^+$). This compound was subjected to X-ray crystallographic analysis to determine the absolute configuration for the cyclitol C-atoms.[1]

For (−)-(1*R*,2*R*,4*R*,6*R*)-**20B** (lower R_f): mp 52–54 °C (HRMS: found $[M - C_7H_7]^+$, 551.2656. $C_{32}H_{39}O_8$ requires 551.2645); $[\alpha]_D$ −31.5 (*c* 0.09, MeOH); ν_{max}(Nujol)/cm^{-1} 1740s and 1811s; δ_H(300 MHz; C^2HCl_3) 0.95 (3 H, s, camph-CH_3), 1.0 (3 H, s, camph-CH_3), 1.1 (3 H, s, camph-CH_3), 1.4–1.7 (4 H, m, camph-CH_2 and 2 × secondary-H), 1.7–2.05 (2 H, m, camph-CH_2 and secondary-H), 2.2–2.35 (1 H, m, secondary-H), 2.45–2.6 (1 H, m, camph-CH_2), 3.5–3.95 (6 H, m, OC_2H_4O and 2 × tertiary-H), 4.0–4.1 (1 H, m, tertiary-H), 4.45–4.55 (6 H, m, 3 × OCH_2Ph), 4.95 (1 H, dd, $^3J_{HH}$ 9.5, $^4J_{HH}$ 2.95, 1-H) and 7.2–7.4 (15 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 10.2 (camph-CH_3), 17.2 (2 × camph-CH_3), 29.4 and 31.2 (camph-C_2H_4), 34.2 and 36.1 (3-C and 5-C), 54.6 and 55.3 (camph-C), 69.8 and 70.2 (OC_2H_4O), 71.2 (OCH_2Ph), 71.5 (4-C), 72.2 and 73.8 (OCH_2Ph), 74.4 and 74.9 (2-C and 6-C), 78.3 (1-C), 91.7 (camph-C), 128.2, 128.5, 128.6, 128.9, 128.95, 129.1, 129.3 and 129.5 (Ar-CH), 138.5, 138.6 and 138.9 (Ar-C quaternary) and 167.6 and 178.8 (camph-CO); *m/z* (FAB-MS) 643 (1%, $[M + H]^+$), 181 (7, $[C_{10}H_{13}O_3]^+$) and 91 (100, $[C_7H_7]^+$); *m/z* (EI) 551 (7%, $[M - C_7H_7]^+$), 181 (13, $[C_{10}H_{13}O_3]^+$) and 91 (100, $[C_7H_7]^+$).

2,4-Di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)cyclohexane-1,2,4,6-tetraol (+)-(1*S*,2*S*,4*S*,6*S*)-19 and (−)-(1*R*,2*R*,4*R*,6*R*)-19

To a stirred solution of the separated camphanate ester (+)-**20A** (960 mg, 1.5 mmol) in ethanol (30 cm^3) was added potassium hydroxide pellets (840 mg, 15 mmol). The mixture was stirred at room temperature overnight.[48] The solvent was removed under reduced pressure, and the residual oil was partitioned between water (50 cm^3) and CH_2Cl_2 (50 cm^3). The CH_2Cl_2 phase was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residual oil was chromatographed on silica (light petroleum–ethyl acetate, 2:1) to give (+)-(1*S*,2*S*,4*S*,6*S*)-**19** as a colourless oil (624 mg, 90%) (Found: C, 74.9; H, 7.9. $C_{29}H_{34}O_5$ requires C, 75.3; H, 7.4%); $[\alpha]_D$ +26.6 (*c* 0.165, MeOH); δ_H(200 MHz; C^2HCl_3) 1.25–1.45 (2 H, m, 2 × secondary-H), 2.25–2.4 (1 H, m, secondary-H), 2.4–2.55 (1 H, m, secondary-H), 3.5–4.0 (8 H, m, OC_2H_4O and 4 × H tertiary), 4.5 (2 H, s, OCH_2Ph), 4.55–4.65 (4 H, m, 2 × OCH_2Ph) and 7.2–7.5 (15 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 34.9 and 36.3 (3-C and 5-C), 69.7 and 70.2 (OC_2H_4O), 71.1 (OCH_2Ph), 72.2 (4-C), 72.7 (OCH_2Ph), 73.7 (OCH_2Ph), 76.4 and 76.7 (2-C and 6-C), 78.3 (1-C), 128.1, 128.2, 128.3, 128.8, 128.9 and 128.9 (Ar-CH) and 139.1 and 139.4 (Ar-C quaternary); *m/z* (CI) 371 (<1%, $[M - C_7H_7]^+$).

The procedure was repeated using (−)-**20A** to give the required enantiomeric alcohol (−)-(1*R*,2*R*,4*R*,6*R*)-**19** (620 mg, 90%) (Found: C, 74.95; H, 7.55. Calc. for $C_{29}H_{34}O_5$: C, 75.3; H, 7.4%); $[\alpha]_D$ −25.1 (*c* 0.165, MeOH); *m/z* (EI) 371 (8%, $[M - C_7H_7]^+$), 281 {10, $[M + H - (2 \times C_7H_7)]^+$}, 105 (42, $[PhCO]^+$) and 91 (100, $[C_7H_7]^+$). [All NMR spectroscopic data were identical to those obtained for the (+)-antipode of **19**.]

2,4-Di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-1-*O*-diphenoxyphosphorylcyclohexane-1,2,4,6-tetraol (+)-(1*S*,2*S*,4*S*,6*S*)-21 and (−)-(1*R*,2*R*,4*R*,6*R*)-21

The resolved enantiomer (+)-(1*S*,2*S*,4*S*,6*S*)-**19** (580 mg, 1.25

‡ Cha = cyclohexylammonium.

mmol), DMAP (16 mg, 0.13 mmol) and dry Et_3N (0.52 cm^3, 578 mg, 3.75 mmol) were dissolved in dry CH_2Cl_2 (30 cm^3) with stirring under an atmosphere of N_2. $ClPO(OPh)_2$ (0.39 cm^3, 510 mg, 1.9 mmol) was added, stirring at room temperature was continued for a further 3–4 h and the mixture was worked-up as described for the racemic compound to give (+)-(1*S*,2*S*,4*S*,6*S*)-**21** as a colourless oil (825 mg, 95%) (HRMS: found $[M + H]^+$, 695.2747. $C_{41}H_{44}O_8P$ requires 695.2774); $[\alpha]_D$ +10.1 (*c* 0.105, CH_3OH); δ_H(300 MHz; C^2HCl_3) 1.4–1.55 (2 H, m, 2 × secondary-H), 2.25–2.35 (1 H, m, secondary-H), 2.45–2.55 (1 H, m, secondary-H), 3.48–3.52 (2 H, m, OC_2H_4O), 3.6–3.8 (3 H, m, OC_2H_4O and tertiary-H), 3.8–3.95 (1 H, m, tertiary-H), 4.09–4.12 (1 H, m, tertiary-H), 4.5–4.55 (6 H, m, 3 × OCH_2Ph), 4.55–4.62 (1 H, m, 1-H) and 7.1–7.4 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 33.9 and 35.6 (3-C and 5-C), 69.5 and 69.7 (OC_2H_4O), 70.5 (OCH_2Ph), 70.9 (4-C), 72.0 and 73.1 (OCH_2Ph), 75.1 and 75.2 (2-C and 6-C), 82.9 (1-C, $^2J_{CP}$ 6.3), 119.9, 120.0, 120.1, 125.0, 125.1, 127.3, 127.4, 128.1, 128.3 and 129.5 (Ar-CH) and 138.2 and 138.3 (Ar-C quaternary), 150.6 (Ar-C quaternary, $^2J_{CP}$ 7.8) and 150.7 (Ar-C quaternary, $^2J_{CP}$ 9.1); δ_P(121.5 MHz; C^2HCl_3) −12.1; *m/z* (FAB-MS) 695 (7%, M^+), 341 (26, $[C_{21}H_{25}O_4]^+$), 251 {8, $[(PhO)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$).

The procedure was repeated using (−)-(1*R*,2*R*,4*R*,6*R*)-**19** to give the required enantiomeric diphenyl phosphate (−)-(1*R*,2*R*,4*R*,6*R*)-**21** (822 mg, 95%) (Found: C, 71.1; H, 6.4. $C_{41}H_{43}O_8P$ requires C, 70.9; H, 6.25%); $[\alpha]_D$ −9.3 (*c* 0.105, MeOH); (FAB-MS) 695 (2%, $[M + H]^+$), 341 (4, $[C_{21}H_{25}O_4]^+$), 251 (10, $[Ph_2PO_2H_2]^+$) and 91 (100, $[C_7H_7]^+$). [All NMR spectroscopic data were identical to those obtained for the (+)-antipode of **21**.]

2,4-Di-*O*-benzyl-6-*O*-(2-benzyloxyethyl)-1-*O*-dibenzyloxyphosphorylcyclohexane-1,2,4,6-tetraol (+)-(1*S*,2*S*,4*S*,6*S*)-22 and (−)-(1*R*,2*R*,4*R*,6*R*)-22

To a stirred solution of (+)-(1*S*,2*S*,4*S*,6*S*)-**21** (784 mg, 1.13 mmol) in dry THF (25 cm^3) under an atmosphere of N_2 was added NaH (60% dispersion in oil; 91 mg, 2.26 mmol) followed by benzyl alcohol (0.23 cm^3, 244 mg, 2.26 mmol). The mixture was stirred at room temperature for a further 4 h and was worked up as described for the racemic compound to give (+)-(1*S*,2*S*,4*S*,6*S*)-**22** as a white solid (596 mg, 73%), mp 56–59 °C (HRMS: found $[M + H]^+$, 723.3108. $C_{43}H_{48}O_8P$ requires 723.3087) (Found: C, 71.6; H, 6.65. $C_{43}H_{47}O_8P$ requires C, 71.45; H, 6.55%); $[\alpha]_D$ +17.3 (*c* 0.3, MeOH); ν_{max}(Nujol)/cm^{-1} 1271m, 1094m and 1023m; δ_H(200 MHz; C^2HCl_3) 1.25–1.45 (2 H, m, 2 × secondary-H), 2.15–2.35 (1 H, m, secondary-H), 2.4–2.55 (1 H, m, secondary-H), 3.4–3.6 (2 H, m, OC_2H_4O), 3.6–3.9 (4 H, m, OC_2H_4O, 2 × tertiary-H), 4.05–4.2 (2 H, m, 1-H and tertiary-H), 4.4–4.6 (6 H, m, 3 × OCH_2Ph), 5.0–5.1 (4 H, m, 2 × $POCH_2Ph$) and 7.2–7.4 (25 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) and 34.6 and 36.1 (3-C and 5-C), 69.6 ($POCH_2Ph$, $^2J_{CP}$ 7.7), 69.8 ($POCH_2Ph$, $^2J_{CP}$ 8.3), 69.9 and 70.3 (OC_2H_4O), 70.3 (OCH_2Ph), 71.1 (OCH_2Ph), 71.7 (4-C), 72.8 and 73.6 (OCH_2Ph), 75.8 (C-tertiary), 75.85 (C-tertiary, $^3J_{CP}$ 6.6), 82.0 (1-C, $^2J_{CP}$ 6.2), 128.0, 128.1, 128.4, 128.8, 128.9 and 129.0 (Ar-CH) and 139.0 (Ar-C quaternary); δ_P(121.5 MHz, C^2HCl_3) −1.4; *m/z* (EI) 723 (2%, $[M + H]^+$), 631 (5, $[M - C_7H_7]^+$), 353 {14, $[M - (PhCH_2O)_2PO_2H_2 - C_7H_7]^+$}, 279 {8, $[(PhCH_2O)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$).

The procedure was repeated for (−)-(1*R*,2*R*,4*R*,6*R*)-**21** to give the required enantiomeric dibenzyl phosphate (−)-(1*R*,2*R*,4*R*,6*R*)-**22** (592 mg, 73%), mp 56–59 °C (Found: C, 69.75; H, 6.7. $C_{43}H_{47}O_8P·H_2O$ requires C, 69.7; H, 6.67%); $[\alpha]_D$ −16.2 (*c* 0.3, MeOH); ν_{max}(Nujol)/cm^{-1} 1271m, 1094m and 1023m; *m/z* (EI) 723 (2%, $[M + H]^+$), 631 (1, $[M - C_7H_7]^+$), 353 {10, $[M - (PhCH_2O)_2PO_2H_2 - C_7H_7]^+$}, 279 {8, $[(PhCH_2O)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$). [All NMR spectroscopic data were identical to those obtained for the (+)-antipode of **22**.]

6-*O*-(2-Hydroxyethyl)-1-*O*-phosphonatocyclohexane-1,2,4,6-tetraol bis(cyclohexylammonium) salt (+)-(1*S*,2*S*,4*S*,6*S*)-2 and (−)-(1*R*,2*R*,4*R*,6*R*)-2

Under an atmosphere of N_2, gaseous ammonia (15–20 cm^3) was condensed at −78 °C, and sodium metal (103 mg, 4.5 mmol) was added. To the blue solution was added a solution of (+)-(1*S*,2*S*,4*S*,6*S*)-**22** (318 mg, 0.44 mmol) in dry THF (1–2 cm^3) through a septum. After stirring at −78 °C for 30 min, methanol (0.5 cm^3) was added with caution and the mixture was worked up as described for the racemic compound to give (+)-(1*S*,2*S*,4*S*,6*S*)-**2** as a white solid (145 mg, 70%), mp >200 °C (decomp.); $[\alpha]_D$ +16.7 (*c* 0.155, MeOH); δ_H(500 MHz; ^2H_2O) 1.1–1.2 (2 H, m, 2 × 4-H of Cha), 1.28–1.4 (8 H, m, 4 × 2-H and 4 × 3-H of Cha), 1.4–1.48 (1 H, m, 5-H), 1.55–1.6 (1 H, m, 3-H), 1.6–1.68 (2 H, m, 2 × 4-H of Cha), 1.75–1.85 (4 H, m, 4 × 3-H of Cha), 1.92–2.02 (4 H, m, 4 × 2-H of Cha), 2.05–2.14 (1 H, m, 3-H), 2.28–2.32 (1 H, m, 5-H), 3.1–3.2 (2 H, m, 2 × 1-H of Cha), 3.62–3.8 (5 H, m, OC_2H_4O and 6-H), 3.95–4.0 (1 H, m, 1-H), 4.0–4.05 (1 H, m, 4-H) and 5.0–5.03 (1 H, m, 2-H); δ_C(125.6 MHz; ^2H_2O) 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 37.3 (5-C), 37.6 (3-C), 50.7 (1-C of Cha), 61.1 (OCH_2CH_2OH), 64.8 (4-C), 68.0 (2-C), 71.3 (OCH_2CH_2OH), 76.5 (6-C, $^3J_{CP}$ 6.2) and 77.0 (1-C, $^2J_{CP}$ 6.5); δ_P(202.5 MHz; ^2H_2O) 7.05; *m/z* (FAB-MS) 349 (3.5%, $[M + 2K + H]^+$), 311 (1, $[M + K + 2H]^+$), 273 (1, $[M + 3H]^+$) and 133 (100), where M is the molecular weight of the phosphate dianion.

The procedure was repeated for (−)-(1*R*,2*R*,4*R*,6*R*)-**22** to give the required enantiomeric phosphate (−)-(1*R*,2*R*,4*R*,6*R*)-**2** (141 mg, 70%), mp >200 °C (decomp.); $[\alpha]_D$ −14.5 (*c* 0.115, MeOH); *m/z* (CI) 471 (1%, $[M + H]^+$), 372 (2, $[M - C_6H_{14}N + 2H]^+$) and 100 (100, $[C_6H_{14}N]^+$). [All NMR spectroscopic data were identical to those obtained for the (+)-antipode of **2**.]

(±)-2,4-Di-*O*-benzyl-6-*O*-methylcyclohexane-1,2,4,6-tetraol 31

Following the procedure used for compound **19**, methanol (0.39 cm^3, 308 mg, 9.6 mmol) was reacted with epoxide **17** (1.5 g 4.8 mmol) to give alcohol **31** as a colourless oil (1.0 g, 63%) (Found: C, 72.9; H, 7.5. Calc. for $C_{21}H_{26}O_4$: C, 73.65; H, 7.65%) (HRMS: found $[M]^+$, 342.1838. $C_{21}H_{26}O_4$ requires 342.1831); ν_{max}(neat)/cm^{-1} 3485s, 2927s, 1453s, 1364s and 1093s; δ_H(200 MHz; C^2HCl_3) 1.2–1.45 (2 H, m, 2 × secondary-H), 2.45 (1 H, m, secondary-H), 2.45–2.6 (1 H, m, secondary-H), 3.45 (3 H, s, OCH_3), 3.3–3.6 (2 H, m, 2 × tertiary-H), 3.65–3.85 (1 H, m, tertiary-H), 3.9–4.0 (1 H, m, tertiary-H), 4.5 (2 H, s, OCH_2Ph), 4.6 (2 H, s, OCH_2Ph) and 7.25–7.4 (10 H, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 34.3 and 35.0 (3-C and 5-C), 57.5 (OCH_3), 71.1 (OCH_2Ph), 72.0 (4-C), 72.4 (OCH_2Ph), 75.8 and 76.8 (2-C and 6-C), 78.8 (1-C), 128.0, 128.1, 128.2, 128.7, 128.8, 130.0 and 130.2 (Ar-CH) and 138.8 and 138.9 (Ar-C quaternary); *m/z* (EI) 342 (8%, M^+), 250 (37, $[M - C_7H_7 - H]^+$), 235 (11, $[M - C_7H_7O]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-2,4-Di-*O*-benzyl-1-*O*-diphenoxyphosphoryl-6-*O*-methylcyclohexane-1,2,4,6-tetraol 32

Following the procedure used for compound **21**, alcohol **31** (0.89 g, 2.6 mmol) was reacted with DMAP (65 mg, 0.53 mmol), dry Et_3N (0.54 cm^3, 394 mg, 3.9 mmol) and $ClPO(OPh)_2$ (0.8 cm^3, 1.05 g, 3.9 mmol) in CH_2Cl_2 (50 cm^3) to give phosphate triester **32** as a colourless oil (1.36 g, 91%) (HRMS: found $[M + H]^+$, 575.2204. $C_{33}H_{36}O_7P$ requires 575.2199); δ_H(200 MHz; C^2HCl_3) 1.35–1.5 (2 H, m, 2 × secondary-H), 2.2–2.4 (1 H, m, secondary-H), 2.4–2.6 (1 H, m, secondary-H), 3.3 (3 H, s, OCH_3), 3.6–3.85 (2 H, m, 2 × tertiary-H), 4.0–4.1 (1 H, m, tertiary-H), 4.4 (2 H, s, OCH_2Ph), 4.5 (2 H, s, OCH_2Ph), 4.5–4.6 (1 H, m, 1-H) and 7.25–7.4 (20 H, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 34.5 and 35.3 (3-C and 5-C), 57.7 (OCH_3), 71.2 (OCH_2Ph), 71.5 (4-C), 72.7 (OCH_2Ph), 75.8 (C tertiary, $^3J_{CP}$ 3), 76.5 (C tertiary, $^3J_{CP}$ 5.1), 83.2 (1-C, $^2J_{CP}$ 6.3), 120.5, 120.6, 120.7, 125.6, 125.7, 128.0,

128.1, 128.2, 128.8, 128.9, 130.1 and 130.2 (Ar-CH), 138.8 and 138.9 (Ar-C quaternary), 150.4 (Ar-C quaternary, $^2J_{CP}$ 8.1) and 150.5 (Ar-C quaternary, $^2J_{CP}$ 8.6); δ_P(121.5 MHz; C^2HCl_3) −12.0; *m/z* (EI) 575 (20%, $[M + H]^+$), 341 {15, $[M - (PhO)_2PO]^+$}, 251 {58, $[(PhO)_2PO_2H_2]^+$}, 233 {13, $[(PhO)_2PO]^+$} and 91 (100, $[C_7H_7]^+$).

(±)-2,4-Di-*O*-benzyl-1-*O*-dibenzyloxyphosphoryl-6-*O*-methylcyclohexane-1,2,4,6-tetraol 33

Following the procedure used for compound **22**, phosphate triester **32** (1.2 g, 2.1 mmol) was reacted with benzyl alcohol (0.41 cm^3, 433 mg, 4 mmol) and NaH (60% dispersion in oil; 160 mg, 4 mmol) in THF (100 cm^3) to give phosphate triester **33** as a colourless oil (785 mg, 62%) (HRMS: found $[M + H]^+$, 603.2520. $C_{35}H_{40}O_7P$ requires 603.2512); ν_{max}(neat)/cm^{-1} 1273s, 1106s, 1023s, 931s, 740s and 696s; δ_H(500 MHz; C^2HCl_3) 1.3–1.4 (2 H, m, 2 × secondary-H), 2.18–2.23 (1 H, m, secondary-H), 2.43–2.46 (1 H, m, secondary-H), 3.35 (3 H, s, OCH_3), 3.6–3.65 (1 H, m, tertiary-H), 3.65–3.75 (1 H, m, 4-H), 3.98–4.03 (1 H, m, tertiary-H), 4.28–4.32 (1 H, m, 1-H), 4.4–4.5 (4 H, s, 2 × OCH_2Ph), 4.95–5.1 (4 H, m, 2 × $POCH_2$Ph) and 7.25–7.5 (20 H, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 34.5 and 35.2 (C secondary), 57.8 (OCH_3), 69.5 ($POCH_2$Ph, $^2J_{CP}$ 5.1), 69.7 ($POCH_2$Ph, $^2J_{CP}$ 5.5), 71.2 (OCH_2Ph), 71.6 (4-C), 72.8 (OCH_2Ph), 75.7 (C tertiary), 76.6 (C tertiary, $^3J_{CP}$ 6.0), 81.8 (1-C, $^2J_{CP}$ 6.3), 128.0, 128.1, 128.3, 128.8, 128.9, 129.0 and 129.0 (Ar-CH) and 138.9 (Ar-C quaternary); δ_P(121.5 MHz; C^2HCl_3) −1.25; *m/z* (EI) 603 (15%, $[M + H]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-6-*O*-Methyl-1-*O*-phosphonatocyclohexane-1,2,4,6-tetraol bis(cyclohexylammonium) salt 5

Following the procedure used for compound **2**, phosphate triester **33** (500 mg, 0.83 mmol) was treated with sodium metal (152 mg, 6.6 mmol) in liquid ammonia (20 cm^3) at −78 °C to give phosphate **5** as a white solid (271 mg, 74%), mp >200 °C (decomp.) (HRMS: found $[M - C_6H_{14}N - PO_4]^+$, 145.0869. $C_7H_{13}O_3$ requires 145.0865); δ_H(500 MHz; ^2H_2O) 1.1–1.2 (2 H, m, 2 × 4-H of Cha), 1.25–1.35 (8 H, m, 4 × 2-H and 4 × 3-H of Cha), 1.35–1.45 (1 H, m, 5-H), 1.55–1.6 (1 H, m, 3-H), 1.6–1.65 (2 H, m, 2 × 4-H of Cha), 1.72–1.82 (4 H, m, 4 × 3-H of Cha), 1.9–2.0 (4 H, m, 4 × 2-H of Cha), 2.05–2.1 (1 H, m, 3-H), 2.35–2.4 (1 H, m, 5-H), 3.1–3.15 (2 H, m, 2 × 1-H of Cha), 3.43 (3 H, s, OCH_3), 3.58–3.62 (1 H, m, 6-H), 3.94–3.98 (1 H, m, 4-H), 3.96–4.02 (1 H, m, 1-H) and 4.3–4.33 (1 H, m, 2-H); δ_C(125.6 MHz; ^2H_2O) 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 36.1 (5-C), 37.4 (3-C), 50.7 (1-C of Cha), 57.0 (OCH_3), 64.8 (4-C), 67.6 (2-C), 76.8 (1-C, $^2J_{CP}$ 5.5) and 77.5 (6-C, $^3J_{CP}$ 6.7); δ_P(121.2 MHz; ^2H_2O) 3.0; *m/z* (FAB-MS) 319 (1%, $[M + 2K + H]^+$), 281 (8, $[M + K + 2H]^+$), 243 (7, $[M + 3H]^+$) and 147 (100), where M is the molecular weight of the phosphate dianion; *m/z* (CI) 145 (26, $[C_7H_{13}O_3]^+$) and 127 (100).

(±)-2,4-Di-*O*-benzyl-6-*O*-propylcyclohexane-1,2,4,6-tetraol 34

Following the procedure used for compound **19**, propan-1-ol (0.72 cm^3, 577 mg, 9.6 mmol) was reacted with epoxide **17** (1.5 g, 4.8 mmol) to give alcohol **34** as a colourless oil (1.26 g, 71%) (HRMS: found $[M + H]^+$, 371.2240. $C_{23}H_{31}O_4$ requires 371.2222); δ_H(200 MHz; C^2HCl_3) 0.9 (3 H, t, $OCH_2CH_2CH_3$), 1.2–1.4 (2 H, m, 2 × secondary-H), 1.5–1.7 (2 H, m, $OCH_2CH_2CH_3$), 2.3–2.4 (1 H, m, secondary-H), 2.4–2.55 (1 H, m, secondary-H), 3.35–3.6 (4 H, m, $OCH_2CH_2CH_3$ and 2 × H tertiary), 3.6–3.8 (1 H, m, tertiary-H), 3.95–4.0 (1 H, m, tertiary-H), 4.5 (2 H, s, OCH_2Ph), 4.6 (2 H, s, OCH_2Ph), 7.2–7.5 (10 H, Ar-H); δ_C(75.5 MHz; C^2HCl_3) 11.1 ($OCH_2CH_2CH_3$), 23.8 ($OCH_2CH_2CH_3$), 34.6 and 35.9 (3-C and 5-C), 71.1 (OCH_2Ph), 71.7 ($OCH_2CH_2CH_3$), 72.2 (4-C), 72.5 (OCH_2Ph), 76.1 and 76.7 (2-C and 6-C), 77.2 (1-C), 128.0, 128.1, 128.2, 128.7, 128.8 and 130.0 (Ar-CH) and 138.8 and 138.9 (Ar-C quaternary); *m/z* (EI) 371 (10%, $[M + H]^+$), 279 (23, $[M - C_7H_7]^+$), 264 (10, $[M - C_7H_7O]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-2,4-Di-*O*-benzyl-1-*O*-diphenoxyphosphoryl-6-*O*-propylcyclohexane-1,2,4,6-tetraol 35

Following the procedure used for compound **21**, alcohol **34** (1.1 g, 3 mmol) was treated with DMAP (73 mg, 0.6 mmol), dry Et_3N (0.63 cm^3, 455 mg, 4.5 mmol) and $ClPO(OPh)_2$ (0.93 cm^3, 1.2 g, 4.5 mmol) in dry CH_2Cl_2 (100 cm^3) to give phosphate triester **35** as a colourless oil (1.63 g, 90%) (HRMS: found $[M + H]^+$, 603.2523. $C_{35}H_{40}O_7P$ requires 603.2512); δ_H(200 MHz; C^2HCl_3) 0.85 (3 H, t, $OCH_2CH_2CH_3$), 1.35–1.6 (4 H, m, $OCH_2CH_2CH_3$ and 2 × secondary-H), 2.2–2.3 (1 H, m, secondary-H), 2.4–2.5 (1 H, m, secondary-H), 3.3–3.5 (2 H, m, $OCH_2CH_2CH_3$), 3.7–3.85 (2 H, m, 2 × tertiary-H), 4.05–4.1 (1 H, m, tertiary-H), 4.35–4.55 (4 H, m, 2 × OCH_2Ph), 4.5–4.6 (1 H, m, 1-H) and 7.1–7.4 (20 H, m, Ar-H); δ_C(75.5 MHz; C^2HCl_3) 11.1 ($OCH_2CH_2CH_3$), 23.7 ($OCH_2CH_2CH_3$), 34.4 and 36.0 (3-C and 5-C), 71.1 (OCH_2Ph), 71.5 (4-C), 72.3 ($OCH_2CH_2CH_3$), 72.7 (OCH_2Ph), 75.0 (C tertiary, $^3J_{CP}$ 5.9), 75.9 (C tertiary), 83.4 (1-C, $^2J_{CP}$ 6.2), 120.5, 120.6, 120.7, 125.7, 125.7, 127.9, 128.0, 128.1, 128.2, 128.8, 128.9 and 130.1 (Ar-CH), 138.8 and 138.9 (Ar-C quaternary), 150.5 (Ar-C quaternary, $^2J_{CP}$ 8.2) and 150.6 (Ar-C quaternary, $^2J_{CP}$ 8.8); δ_P(121.5 MHz; C^2HCl_3) −12.2; *m/z* (EI) 603 (30%, $[M + H]^+$), 261 {22, $[(M + H) - (PhO)_2PO_2H_2 - C_7H_7]^+$}, 251 {66, $[(PhO)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$).

(±)-2,4-Di-*O*-benzyl-1-*O*-dibenzyloxyphosphonyl-6-*O*-propylcyclohexane-1,2,4,6-tetraol 36

Following the procedure used for compound **22**, phosphate triester **35** (1.5 g, 2.5 mmol) was reacted with benzyl alcohol (0.49 cm^3, 510 mg, 4.75 mmol) and NaH (60% dispersion in oil; 190 mg, 4.75 mmol) in dry THF (100 cm^3) to give the phosphate triester **36** as a colourless oil (945 mg, 60%) (Found: C, 69.85; H, 6.8. $C_{37}H_{43}O_7P$ requires C, 70.45; H, 6.85%) (HRMS: found M^+, 631.2810. $C_{37}H_{44}O_7P$ requires 631.2825); δ_H(500 MHz; C^2HCl_3) 0.8 (3 H, t, $OCH_2CH_2CH_3$), 1.38–1.42 (2 H, m, 2 × secondary-H), 1.47–1.53 (2 H, m, $OCH_2CH_2CH_3$), 2.13–2.17 (1 H, m, secondary-H), 2.38–2.42 (1 H, m, secondary-H), 3.4–3.5 (2 H, m, $OCH_2CH_2CH_3$), 3.7–3.8 (2 H, m, 2 × tertiary-H), 4.08–4.12 (1 H, m, tertiary-H), 4.34–4.38 (1 H, m, 1-H), 4.4–4.6 (4 H, m, 2 × OCH_2Ph), 5.0–5.15 (4 H, m, 2 × $POCH_2$Ph) and 7.2–7.4 (20 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 11.0 ($OCH_2CH_2CH_3$), 23.8 ($OCH_2CH_2CH_3$), 34.5 and 35.9 (3-C and 5-C), 69.6 ($POCH_2$Ph, $^2J_{CP}$ 5.5), 69.7 ($POCH_2$Ph, $^2J_{CP}$ 5.8), 71.1 (OCH_2Ph), 71.6 (4-C), 72.2 ($OCH_2CH_2CH_3$), 72.8 (OCH_2Ph), 75.1 (C tertiary, $^3J_{CP}$ 6.6), 75.7 (C tertiary), 82.0 (1-C, $^2J_{CP}$ 6.4), 127.6, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5 and 128.6 (Ar-CH) and 136.3, 136.4, 136.5 and 139.0 (Ar-C quaternary); δ_P(121.5 MHz; C^2HCl_3) −1.25; *m/z* (EI) 631 (1%, $[M + H]^+$), 539 (4, $[M - C_7H_7]^+$), 279 {11, $[(BnO)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$).

(±)-1-*O*-Phosphonato-6-*O*-propylcyclohexane-1,2,4,6-tetraol bis(cyclohexylammonium) salt 6

Following the procedure used for compound **2**, phosphate triester **36** (630 mg, 1 mmol) was reacted with sodium metal (184 mg, 8 mmol) in liquid ammonia (20 cm^3) at −78 °C to give phosphate **6** as a white solid (337 mg, 72%), mp >200 °C (decomp.) (HRMS: found $[M + 2K + H]^+$, 347.0077. $C_9H_{18}K_2O_7P$ requires 347.0064, where M^+ is the molecular weight of the free acid); δ_H(500 MHz; ^2H_2O) 0.8 (3 H, t, $OCH_2CH_2CH_3$), 1.1–1.2 (2 H, m, 2 × 4-H of Cha), 1.25–1.35 (8 H, m, 4 × 2-H and 4 × 3-H of Cha), 1.35–1.45 (1 H, m, 5-H), 1.55–1.65 (5 H, m, $OCH_2CH_2CH_3$, 2 × 4-H of Cha and 3-H), 1.75–1.83 (4 H, m, 4 × 3-H of Cha), 1.9–2.0 (4 H, m, 4 × 2-H of Cha), 2.07–2.13 (1 H, m, 3-H), 2.37–2.42 (1 H, m, 5-H), 3.1–3.2 (2 H, m, 2 × 1-H of Cha), 3.58–3.68 (2 H, m, $OCH_2CH_2CH_3$), 3.7–3.75 (1 H, m, 6-H), 3.96–4.0 (1 H, m, 1-H), 4.0–4.04 (1 H, m, 4-H) and 4.3–4.38 (1 H, m, 2-H); δ_C(125.6 MHz; ^2H_2O) 10.1 ($OCH_2CH_2CH_3$), 22.8 ($OCH_2CH_2CH_3$), 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 37.1 (5-C), 37.6 (3-C), 50.7 (1-

C of Cha), 64.9 (4-C), 67.7 (2-C), 72.2 ($OCH_2CH_2CH_3$), 75.9 (1-C, $^2J_{CP}$ 6.8) and 77.4 (6-C, $^3J_{CP}$ 5.0); δ_P(121.4 MHz; ^2H_2O) 3.0; *m/z* (FAB-MS) 347 (95%, $[M + 2K + H]^+$), 309 (60, $[M + K + 2H]^+$), 271 (20, $[M + 3H]^+$) and 157 (100), where M is the molecular weight of the phosphate dianion.

(±)-2,4-Di-*O*-benzyl-6-*O*-(2-dibenzyloxyphosphoryloxyethyl)-cyclohexane-1,2,4,6-tetraol 30

Following the procedure used for compound **19**, epoxide **17** (465 mg, 1.5 mmol) was treated with 2-hydroxyethyl dibenzyl phosphate **29** (966 mg, 3 mmol) to give, after chromatographic work up on silica (ethyl acetate–light petroleum, 3:1), phosphate triester **30** as a colourless oil (120 mg, 13%) (HRMS: found $[M + H]^+$, 633.2590. $C_{36}H_{42}O_8P$ requires 633.2617) (Found: $[M + 2H - C_7H_7]^+$, 543.2129. $C_{29}H_{36}O_8P$ requires 543.2148); δ_H(500 MHz; C^2HCl_3) 1.25–1.3 (2 H, m, 2 × secondary-H), 2.25–2.28 (1 H, m, secondary-H), 2.41–2.44 (1 H, m, secondary-H), 3.48–3.52 (1 H, m, 1-H), 3.52–3.59 (1 H, m, tertiary-H), 3.63 (2 H, m, OCH_2CH_2OP), 3.68–3.72 (1 H, m, 4-H), 3.78–3.82 (1 H, m, OCH_2CH_2OP), 3.92–3.96 (1 H, m), 4.1–4.2 (1 H, m, OCH_2CH_2OP), 4.4–4.7 (4 H, m, 2 × OCH_2Ph), 5.0–5.1 (4 H, m, 2 × $POCH_2Ph$) and 7.2–7.4 (20 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 34.7 and 35.9 (3-C and 5-C), 67.8 (OC_2H_4OP, J_{CP} 5.6), 66.8 (OC_2H_4OP, J_{CP} 5.7), 69.8 (2 × $POCH_2Ph$, $^2J_{CP}$ 4.8 and 5.4), 71.0 (OCH_2Ph), 72.0 (4-C), 72.6 (OCH_2Ph), 76.0, 76.7 and 78.3 (1-C, 2-C and 6-C), 128.1, 128.4, 128.8, 128.9, 129.0 and 129.1 (Ar-CH) and 136.3, 139.0 and 139.2 (Ar-C quaternary); δ_P(121.4 MHz; C^2HCl_3) −0.27; *m/z* (EI) 633 (100%, $[M + H]^+$), 543 (46, $[M + 2H - C_7H_7]^+$), 453 (5, $[M + 3H - 2 \times C_7H_7]^+$) and 279 {7, $[(BnO)_2PO_2H_2]^+$}.

(±)-6-*O*-(2-Phosphonatooxyethyl)cyclohexane-1,2,4,6-tetraol bis(cyclohexylammonium) salt 4

Following the procedure used for compound **2**, phosphate triester **30** (107 mg, 0.17 mmol) was reacted with sodium metal (32 mg, 1.4 mmol) in liquid ammonia (10 cm^3) at −78 °C to give phosphate **4** as a white solid (50 mg, 63%), mp >200 °C (decomp.); δ_H(500 MHz; ^2H_2O) 1.1–1.2 (2 H, m, 2 × 4-H of Cha), 1.25–1.35 (9 H, m, 4 × 2-H, 4 × 3-H of Cha and 5-H), 1.4–1.5 (1 H, m, 3-H), 1.55–1.65 (2 H, m, 2 × 4-H of Cha), 1.75–1.8 (4 H, m, 4 × 3-H of Cha), 1.9–2.0 (4 H, m, 4 × 2-H of Cha), 1.98–2.02 (1 H, m, 3-H), 2.3–2.4 (1 H, m, 5-H), 3.0–3.1 (2 H, m, 2 × 1-H of Cha), 3.4–3.5 (1 H, m, 1-H), 3.5–3.6 (1 H, m, 6-H), 3.6–3.7 (1 H, m, OC_2H_4OP), 3.7–3.8 (1 H, m, OC_2H_4OP), 3.8–3.95 (2 H, m, OC_2H_4OP), 4.0–4.05 (1 H, m, 4-H) and 4.1–4.2 (1 H, m, 2-H); δ_C(75.5 MHz; ^2H_2O) 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 37.7 (5-C), 38.5 (3-C), 50.7 (1-C of Cha), 64.3 (4-C), 64.3 (OCH_2CH_2OP, $^3J_{CP}$ 5.6), 69.0 (2-C), 69.5 (OCH_2CH_2OP, $^2J_{CP}$ 5.2), 74.4 (1-C) and 77.0 (6-C); δ_P(121.4 MHz; ^2H_2O) −0.41 (br); *m/z* (FAB-MS) 349 (5%, $[M + 2K + H]^+$), 311 (10, $[M + K + 2H]^+$), 273 (21, $[M + 3H]^+$) and 154 (100), where M is the molecular weight of the phosphate dianion.

(±)-2,4-Di-*O*-benzyl-6-*O*-[2-(*p*-methoxybenzyloxy)ethyl]cyclohexane-1,2,4,6-tetraol 24

Following the procedure used for compound **19**, epoxide **17** (1.5 g, 4.8 mmol) and 2-(*p*-methoxybenzyloxy)ethanol (1.75 g, 9.6 mmol) were reacted with $BF_3{\cdot}Et_2O$ (3 drops) to give, after chromatographic work up on silica (ethyl acetate–light petroleum, 1:2), alcohol **24** as a colourless oil (1.1 g, 46%); δ_H(300 MHz; C^2HCl_3) 1.25–1.45 (2 H, m, 2 × secondary-H), 2.25–2.4 (1 H, m, secondary-H), 2.5–2.55 (1 H, m, secondary-H), 3.5–3.9 (10 H, m, OC_2H_4O, OCH_3 and 3 × tertiary-H), 3.9–4.0 (1 H, m, tertiary-H), 4.4–4.55 (4 H, m, 2 × OCH_2Ph), 4.55–4.65 (2 H, m, OCH_2Ph), 6.9 (2 H, d, $^3J_{HH}$ 8.6, $OCH_2C_6H_4OCH_3$) and 7.2–7.4 (12 H, m, Ar-H and $OCH_2C_6H_4OCH_3$); δ_C(50.3 MHz; C^2HCl_3) 34.9 and 36.3 (3-C and 5-C), 55.7 (OCH_3), 69.7 and 69.9 (OC_2H_4O), 71.1 (OCH_2Ph), 72.2 (4-C), 72.7 (OCH_2Ph), 73.4 ($OCH_2C_6H_4OCH_3$), 76.4 and 76.7 (2-C and 6-C), 78.3 (1-C), 114.3 (Ar-CH *ortho* of $OCH_2C_6H_4OCH_3$), 128.0, 128.1, 128.8, 128.9 and 130.0 (Ar-CH *meta* of $OCH_2C_6H_4OCH_3$, Ar-C quaternary of $OCH_2C_6H_4OCH_3$ and Ar-CH), 139.0 and 139.2 (Ar-C quaternary of $OCH_2C_6H_5$) and 159.7 (Ar-C quaternary of $OCH_2C_6H_4OCH_3$); *m/z* (EI) 492 (4%, M^+), 401 (2, $[M - C_7H_7]^+$), 371 (3, $[M - C_8H_9O]^+$) and 121 (100, $[C_8H_9O]^+$).

(±)-2,4-Di-*O*-benzyl-1-*O*-diphenoxyphosphoryl-6-*O*-[2-(*p*-methoxybenzyloxy)ethyl]cyclohexane-1,2,4,6-tetraol 25

Following the procedure used for compound **21**, alcohol **24** (980 mg, 2.0 mmol), DMAP (49 mg, 0.4 mmol), Et_3N (0.42 cm^3, 303 mg, 3.0 mmol) and $ClPO(OPh)_2$ (0.62 cm^3, 810 mg, 3.0 mmol) were reacted in dry CH_2Cl_2 (100 cm^3) to give the desired phosphate triester as a colourless oil (1.38 g, 95%) (HRMS: found $[M + H]^+$, 725.3426. $C_{47}H_{50}O_5P$ requires 725.3396); δ_H(300 MHz; C^2HCl_3) 1.35–1.45 (1 H, m, secondary-H), 1.5–1.65 (1 H, m, secondary-H), 2.35–2.45 (1 H, m, secondary-H), 2.55–2.7 (1 H, m, secondary-H), 3.55–3.65 (2 H, m, OC_2H_4O), 3.7–4.05 (7 H, m, OC_2H_4O, OCH_3 and 2 × tertiary-H), 4.0–4.3 (1 H, m, tertiary-H), 4.35–4.5 (6 H, m, 3 × OCH_2Ph), 4.5–4.6 (1 H, m, 1-H, tertiary-H), 6.95 (2 H, d, $^3J_{HH}$ 8.6, $OCH_2C_6H_4OCH_3$) and 7.2–7.5 (22 H, m, $OCH_2C_6H_4OCH_3$ and Ar-H); δ_C(75.4 MHz; C^2HCl_3) 34.1 and 35.7 (3-C and 5-C), 55.3 (OCH_3), 69.5 and 69.6 (OC_2H_4O), 70.7 (OCH_2Ph), 71.1 (4-C), 72.2 (OCH_2Ph), 72.9 ($OCH_2C_6H_4OCH_3$), 75.3 (C tertiary, $^3J_{CP}$ 6.0), 75.4 (C tertiary, $^3J_{CP}$ 6.3), 83.0 (1-C, $^2J_{CP}$ 7.0), 113.7 (Ar-CH *ortho* of $OCH_2C_6H_4OCH_3$), 120.1, 120.2, 120.3, 125.2, 125.3, 127.5, 127.6, 128.3, 128.4, 129.3, 129.6 and 130.4 (Ar-CH, Ar-C quaternary of $OCH_2C_6H_4OCH_3$ and Ar-CH *meta* of $OCH_2C_6H_4OCH_3$), 138.4 and 138.5 (Ar-C quaternary of $OCH_2C_6H_5$), 150.3 (Ar-C quaternary, $^2J_{CP}$ 8.5), 150.4 (Ar-C quaternary, $^2J_{CP}$ 8.8) and 159.3 (Ar-C quaternary of $OCH_2C_6H_4OCH_3$); δ_P(121.5 MHz; C^2HCl_3) −12.1; *m/z* (EI) 725 (2%, $[M + H]^+$), 633 (30, $[M - C_7H_7]^+$), 603 (5, $[M - C_8H_9O]^+$), 497 (5, $[M - C_7H_7 - C_8H_8O_2]^+$), 341 (30, $[C_{21}H_{25}O_4]^+$), 251 {65, $[(PhO)_2PO_2H_2]^+$}, 136 (30, $[C_8H_8O_2]^+$), 121 (92, $[C_8H_9O]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-2,4-Di-*O*-benzyl-1-*O*-diphenoxyphosphoryl-6-*O*-(2-hydroxyethyl)cyclohexane-1,2,4,6-tetraol 26

p-Methoxybenzyl ether **25** (1.3 g, 1.8 mmol) was dissolved in CH_2Cl_2 (50 cm^3) containing water (2.5 cm^3) at room temperature. DDQ (409 mg, 1.8 mmol) was added and stirring was continued for 2 h. The reaction mixture was washed with $NaHCO_3$ (50 cm^3), the organic phase dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude oil was chromatographed on silica (ethyl acetate–light petroleum, 3:1) to give alcohol **26** as a white solid (880 mg, 81%), mp 64–66 °C (Found: C, 67.4; H, 6.35. $C_{34}H_{37}O_8P$ requires C, 67.55; H, 6.15%); δ_H(500 MHz; C^2HCl_3) 1.6–1.7 (2 H, m, 2 × secondary-H), 2.4–2.47 (1 H, m, secondary-H), 2.6–2.67 (1 H, m, secondary-H), 3.68–3.72 (1 H, m, HOC_2H_4O), 3.77–3.8 (2 H, m, HOC_2H_4O), 3.87–3.91 (1 H, m, HOC_2H_4O), 3.95–4.0 (1 H, m, 4-H), 4.0–4.05 (1 H, m, tertiary-H), 4.16–4.19 (1 H, m, tertiary-H), 4.55–4.7 (4 H, m, 2 × OCH_2Ph), 4.75–4.79 (1 H, m, 1-H) and 7.2–7.4 (20 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 34.1 and 35.3 (3-C and 5-C), 62.1 ($HOCH_2CH_2O$), 71.2 (OCH_2Ph), 71.4 ($HOCH_2CH_2O$), 71.5 (4-C), 72.6 (OCH_2Ph), 75.3 (2 × C tertiary), 83.2 (1-C, $^2J_{CP}$ 6.4), 115.8, 120.5, 120.6, 120.7, 125.9, 128.0, 128.2, 128.3, 128.9, 129.0, 130.0 and 130.3 (Ar-CH), 138.7 and 138.8 (Ar-C quaternary of $OCH_2C_6H_5$) and 151.1 (Ar-C quaternary, $^2J_{CP}$ 6.5); δ_P(121.5 MHz; C^2HCl_3) −12.3; *m/z* (EI) 605 (1%, $[M + H]^+$), 574 (1, $[M + H - CH_3O]^+$), 251 {28, $[(PhO)_2PO_2H_2]^+$} and 91 (100, $[C_7H_7]^+$).

(±)-10,12-Dibenzyloxy-3-oxo-3-phenoxy-2,4,7-trioxa-3λ⁵-phosphabicyclo[6.4.0]dodecane 27‡

To a stirred solution of alcohol **26** (785 mg, 1.31 mmol) in dry

‡ NMR Assignments are made using the inositol numbering system.

THF (200 cm^3) at −78 °C was added NaH (60% dispersion in oil; 56 mg, 1.4 mmol) under an atmosphere of N_2. The cold bath was removed and, after the reaction had reached room temperature, methanol (5 cm^3) was added. After 1 h, the solvents were removed under reduced pressure and the residual oil was partitioned between water and diethyl ether. The organic phase was dried ($MgSO_4$), the solvent was evaporated under reduced pressure and the residual oil was chromatographed on silica (ethyl acetate–light petroleum, 1:1) to give the cyclic phosphate triester **27** as a colourless oil (318 mg, 48%) (HRMS: found $[M]^+$, 510.1800. $C_{28}H_{31}O_7P$ requires 510.1807. Found: $[M - C_7H_7]^+$, 419.1631. $C_{21}H_{24}O_7P$ requires 419.1623); δ_H(500 MHz; C^2HCl_3) 1.2–1.3 (1 H, m, secondary-H), 1.4–1.6 (1 H, m, secondary-H), 2.17–2.23 (1 H, m, secondary-H), 2.37–2.42 (1 H, m, secondary-H), 3.6–3.65 (1 H, m, OCH_2CH_2OP), 3.65–3.72 (2 H, m, tertiary-H), 3.79–3.82 (1 H, m, tertiary-H), 4.0–4.15 (2 H, m, $CHOCH_2CH_2OP$), 4.35–4.38 (1 H, m, 1-H), 4.3–4.5 (5 H, m, OCH_2CH_2OP and 2 × OCH_2Ph) and 7.05–7.35 (15 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 35.1 and 37.9 (3-C and 5-C), 69.8 (OC_2H_4OP, J_{CP} 6.0), 71.3 (OCH_2Ph), 71.5 (4-C), 73.1 and 73.2 (OCH_2Ph and OC_2H_4OP), 75.7 (C tertiary, $^3J_{CP}$ 8.8), 79.9 (C tertiary), 84.45 (1-C, $^2J_{CP}$ 6.3), 120.7, 120.8, 125.6, 128.0, 128.1, 128.8, 129.0 and 130.1 (Ar-CH), 138.7 (Ar-C quaternary of $OCH_2C_6H_5$) and 151.6 (Ar-C quaternary, $^2J_{CP}$ 7.2); δ_P(121.5 MHz; C^2HCl_3) −6.8; *m/z* (EI) 510 (8%, M^+), 419 (2, $[M - C_7H_7]^+$), 296 (30, $[M - 2 \times C_7H_7O]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-3,10,12-Tribenzyloxy-3-oxo-2,4,7-trioxa-3λ^5-phosphabicyclo[6.4.0]dodecane 28‡

Following the procedure used for compound **22**, cyclic phosphate triester **27** (300 mg, 0.59 mmol) was dissolved in dry THF (50 cm^3) and treated with benzyl alcohol (0.058 cm^3, 60.5 mg, 0.56 mmol) and NaH (60% dispersion in oil; 22.4 mg, 0.56 mmol). Chromatographic work-up on silica (ethyl acetate–light petroleum, 3:1) yielded cyclic phosphate triester **28** as a colourless oil which consisted of a mixture of 2 diastereoisomers (ratio 2:1) (176 mg, 57%) (HMRS: found M^+, 524.1961. $C_{29}H_{33}O_7P$ requires 524.1964. Found: $[M - C_7H_7]^+$, 433.1424. $C_{22}H_{26}O_7P$ requires 433.1416); δ_H(500 MHz; C^2HCl_3) 1.2–1.6 (2 H, both isomers, m, 2 × secondary-H), 2.2–2.3 (1 H, minor isomer, m, secondary-H), 2.3–2.4 (1 H, major isomer, m, secondary-H), 2.4–2.5 (1 H, both isomers, m, secondary-H), 3.6–3.9 (3 H, both isomers, m, OC_2H_4OP and tertiary-H), 3.9–4.2 (4 H, both isomers, m, OC_2H_4OP and 2 × tertiary-H), 4.3–4.6 (5 H, both isomers, m, 2 × $OCH_2C_6H_5$ and 2-H), 5.1–5.2 (2 H, both isomers, m, $POCH_2C_6H_5$) and 7.2–7.5 (15 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 34.9 (major isomer, C secondary), 35.4 (minor isomer, C secondary), 37.1 (minor isomer, C secondary), 37.8 (major isomer, C secondary), 67.8, 68.3, 69.2, 69.3, 69.8, 71.1 and 71.2 (both isomers, C secondary), 71.3 and 71.4 (both isomers, C tertiary), 72.9 and 73.3 (both isomers, C secondary), 75.8, 76.0, 76.2 and 79.6 (both isomers, C tertiary), 83.8 (major isomer, 1-C, $^2J_{CP}$ 6.5), 84.25 (minor isomer, 1-C, $^2J_{CP}$ 6.5), 127.9, 128.0, 128.1, 128.6, 128.8, 128.9, 129.0 and 129.1 (both isomers, Ar-CH) and 136.5 and 138.8 (both isomers, Ar-C quaternary); δ_P(121.5 MHz; C^2HCl_3) −3.3 (major isomer) and −0.9 (minor isomer); *m/z* (EI) 524 (5%, M^+), 433 (35, $[M - C_7H_7]^+$), 310 (7, $[M - 2 \times C_7H_7O]^+$) and 91 (100, $[C_7H_7]^+$).

(±)-3-Oxido-3-oxo-2,4,7-trioxa-3λ^5-phosphabicyclo[6.4.0]-dodecane-10,12-diol 3‡

Following the procedure used for compound **2**, the mixture of diastereoisomeric cyclic phosphate triesters **28** (150 mg, 0.28 mmol) was reacted with sodium metal (39 mg, 1.7 mmol) in liquid ammonia (20 cm^3) at −78 °C to give cyclic phosphate diester **3** as a white solid (59 mg, 60%) (HRMS: found $[M + H]^+$, 354.1678. $C_{14}H_{29}NO_7P$ requires 354.1682); δ_H(500 MHz; ^2H_2O) 1.1–1.2 (1 H, m, 4-H of Cha), 1.25–1.35 (4 H, m, 2 × 3-H and 2 × 2-H of Cha), 1.35–1.45 (1 H, m, 5-H), 1.47–1.52 (1 H, m, 3-H), 1.6–1.65 (1 H, m, 4-H of Cha), 1.75–1.8 (2 H, m, 2 × 3-H of Cha), 1.9–2.0 (2 H, m, 2 × 2-H of Cha), 2.05–2.12 (1 H, m, 3-H), 2.22–2.29 (1 H, m, 5-H), 3.08–3.16 (1 H, m, 1-H of Cha), 3.78–3.84 (1 H, m, 6-H), 3.88–4.05 (6 H, m, $CHOCH_2CH_2OP$, 1-H and 4-H) and 4.12–4.16 (1 H, m, 2-H); δ_C(75.4 MHz; ^2H_2O) 24.1 (3-C of Cha), 24.5 (4-C of Cha), 30.6 (2-C of Cha), 38.3 (5-C), 39.2 (3-C), 50.7 (1-C of Cha), 63.9 (4-C), 66.3 (OC_2H_4OP), 69.2 (2-C), 69.9 (OC_2H_4OP), 77.2 (6-C) and 81.0 (1-C, $^2J_{CP}$ 7.0); δ_P(121.4 MHz; ^2H_2O) −0.84; *m/z* (CI) 354 (9%, $[M + H]^+$), 255 (4, $[M + 2H - C_6H_{14}N]^+$), 237 (16, $[M + 2H - C_6H_{14}N - H_2O]^+$) and 100 (100, $[C_6H_{14}N]^+$).

(±)-6-*O*-(2-Hydroxyethyl)cyclohexane-1,2,4,6-tetraol 23

A solution of alcohol **19** (462 mg, 1 mmol) in dry THF (1 cm^3) was added to a blue solution of sodium metal (138 mg, 6 mmol) in liquid ammonia (15 cm^3) at −78 °C. The mixture was stirred for 30 min, quenched with methanol (1 cm^3) and then allowed to warm to room temperature. The solvents were removed under reduced pressure and the residual white solid extracted with ethanol (2 × 10 cm^3). Removal of ethanol under reduced pressure yielded tetraol **23** as a white solid (110 mg, 57%), mp >150 °C (decomp.); δ_H(300 MHz; ^2H_2O) 1.1–1.25 (1 H, m, secondary-H), 1.35–1.45 (1 H, m, secondary-H), 1.92–2.0 (1 H, m, secondary-H), 2.25–2.42 (1 H, m, secondary-H), 3.35–3.52 (3 H, m, CH_2CH_2OH and 6-H), 3.52–3.7 (3 H, m, OCH_2CH_2OH and 1-H), 3.38–3.9 (1 H, m, 4-H) and 3.98–4.02 (1 H, m, 2-H); δ_C(125.6 MHz; ^2H_2O) 37.4 (5-C), 38.4 (3-C), 61.1 (OCH_2CH_2OH), 64.1 (4-C), 68.9 (2-C), 70.3 (OCH_2CH_2OH), 74.2 (1-C) and 76.5 (6-C); *m/z* (CI) 193 (70%, $[M + H]^+$), 175 (15, $[M + H - H_2O]^+$), 157 (61, $[M + H - 2 \times H_2O]^+$), 139 (22, $[M + H - 3 \times H_2O]^+$), 113 (32, $[M + H - 2 \times H_2O - C_2H_4O]^+$) and 33 (100).

(±)-2,4-Di-*O*-benzylcyclohex-5-ene-1,2,4-triol 18

Epoxide **17** (310 mg, 1 mmol) was added to a mixture of dry THF (2 cm^3), dry TMEDA (4 cm^3) and 2-benzyloxyethanol (228 mg, 1.5 mmol). NaH (60% suspension in oil; 40 mg, 1.0 mmol) was added and the mixture was heated at 110 °C for 30 min. The mixture was allowed to cool to room temperature, water was added very carefully and the reaction was partitioned between water (20 cm^3) and diethyl ether (20 cm^3). The organic phase was dried ($MgSO_4$), the solvent was removed under reduced pressure and the residue was chromatographed on silica (ethyl acetate–light petroleum, 1:2) to give triol **18** as a colourless oil (60 mg, 20%); δ_H(300 MHz; C^2HCl_3) 1.88–1.94 (1 H, m, 3-H), 2.22–2.31 (1 H, m, 3-H), 3.92–3.98 (1 H, m, 2-H), 4.1–4.18 (1 H, m, 4-H), 4.2–4.25 (1 H, m, 1-H), 4.5–4.65 (4 H, m, 2 × OCH_2Ph), 5.8–5.85 (1 H, m, 5-H), 5.95–6.0 (1 H, m, 6-H), 7.2–7.4 (10 H, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.9 (3-C), 65.8 (1-C), 70.8, 71.4, 71.6 and 75.0 (OCH_2Ph, 2-C and 4-C), 127.9, 128.0, 128.1, 128.6, 128.7, 130.3 and 130.4 (Ar-CH, 5-C and 6-C) and 138.2 and 138.7 (Ar-C quaternary); *m/z* (CI) 293 (30, $[M + H - H_2O]^+$) and 91 (100, $[C_7H_7]^+$).

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