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Removal of the phosphate group in mechanism-based inhibitors of inositol monophosphatase leads to unusual inhibitory activity

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Inositol monophosphatase is widely held to be the therapeutic target for inhibition by lithium ion in the treatment of bipolar disorder. In a continued effort to improve the bioavailability of alternative inhibitors, we have designed and tested two new series of compounds; phosphonates and product-like mimics. Phosphonate substrate mimics were competitive inhibitors of reduced potency as compared to phosphate based inhibitors. Product mimics however, showed various inhibitory modes of action. The 6-butylamino derivative **6p** was an uncompetitive inhibitor when acting alone (K_i = 0.3 mM) but displayed non-competitive inhibition in the presence of inorganic phosphate. This compound represents a new lead in the search for a viable replacement for lithium ion therapy.

Introduction

The chronic and debilitating condition of manic depression (bipolar disorder) affects up to 340 million people worldwide.[1] It remains a severe problem as there is no cure and the current treatments with lithium salts have serious limitations due to the high toxicity of these compounds.[2] Inositol monophosphatase (IMPase, E.C. 3.1.3.25) is widely believed to be the therapeutic target of lithium ion and as such has been the focus of much effort to discover a more attractive therapy for the condition.[3–9]

IMPase is a key enzyme in a brain secondary messenger system.[10] Its purpose is to provide free *myo*-inositol for the biosynthesis of the second messenger precursor phosphatidylinositol-4,5-bisphosphate.[10,11] In response to extra-cellular stimuli, phosphatidylinositol-4,5-bisphosphate is hydrolysed to 1,2-diacylglycerol and inositol-1,4,5-trisphosphate. These so called second messengers give rise to protein kinase C activation and intracellular calcium release respectively.[10–12] It is thought that over-activity of this cellular response mechanism leads to the violent mood swings characteristic of manic-depressive illness.[13] Once inositol-1,4,5-trisphosphate has been released it is rapidly dephosphorylated by a cascade of enzymes to give ultimately, *myo*-inositol.[10] IMPase catalyses the final step of this dephosphorylation sequence and is responsible for the hydrolysis of monophosphates of D-*myo*-inositol in the 1, 3, 4 or 6 positions.[14] Suppression of IMPase activity reduces the pool of *myo*-inositol available in the brain and therefore slows down the resynthesis of the second messenger precursors.[13]

Previous work has lead to a detailed understanding of the structure and mode of action of IMPase. The enzyme is a 58 kDa homodimer with a requirement of 2 Mg^{2+} ions per active site.[3–8] Kinetic data indicated that one metal ion ($Mg^{2+}1$) binds before binding of the substrate and the second ($Mg^{2+}2$) binds after (Fig. 1).[3–8] A water molecule associated with $Mg^{2+}1$ deep within the active site acts as a nucleophile and attacks the phosphate group of the substrate D-inositol-1-phosphate (D-Ins-1-P) **1** and a second water molecule, coordinated to $Mg^{2+}2$ and hydrogen bonded to the 6-OH group of **1** acts as a general acid, protonating the inositolate group as it leaves.[15] Deletion of this 6-OH group removes any substrate activity from related compounds and it is known that the 4-OH and 2-OH groups along with the 1-O atom provide strong interactions for the binding of the substrate to the active site.[16]

Many mechanism-based inhibitors of IMPase have been synthesized and evaluated during the accumulation of the above

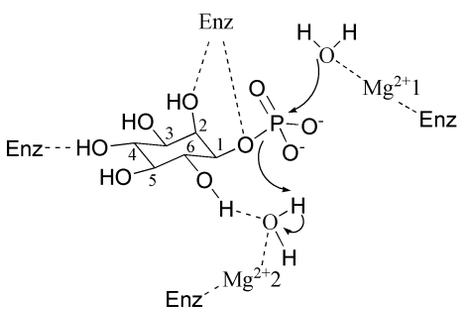
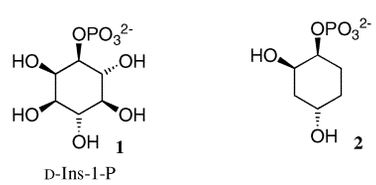
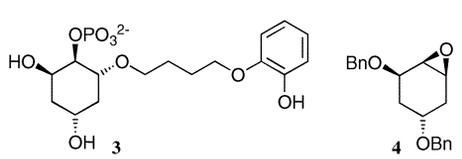


Fig. 1 Schematic representation of the hydrolysis of D-Ins-1-P by IMPase. Key interactions of the substrate with the active site are shown.

knowledge. Substrate analogues of cyclitol phosphate **1** with deleted 3-OH and 5-OH groups bind to the enzyme more tightly than the natural substrates, and if the 6-OH group is either deleted or alkylated, the resulting molecule is a tight binding competitive inhibitor of IMPase.[9,16,17] For example compound **2** is such an inhibitor and displays an IC_{50} value of 3 μM. Similarly, compound **3** is the most potent known inhibitor of IMPase (IC_{50} = 40 nM).[9] Modeling studies have suggested that the long lipophilic side chain of inhibitors such as **3** enhance binding to the enzyme by interaction with a lipophilic pocket near the active site bounded by Val40 and Leu42.[5c] It is now relatively easy to prepare such compounds and we have used epoxide **4** as a common precursor to all such inhibitors.[18]





These materials have proved invaluable as probes for elucidation of the mechanism of action of IMPase but they are of no use *in vivo*. In common with all known organic phosphate

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inhibitors of IMPase there are bioavailability limitations that preclude their use as drugs.[9] These inhibitors possess a double negative charge at physiological pH which renders them too hydrophilic to cross the lipophilic blood–brain barrier in order to have a therapeutic effect.[19] The aim of the work described here was to design and prepare 'reduced charge' mechanism based inhibitors of IMPase. Guided by data available for the importance of binding interactions it was reasoned that we could either modify or delete the phosphate group of IMPase inhibitors in known materials in order to retain good inhibitory activity against the enzyme.

Results and discussion

Two approaches towards overcoming the problems of poor bioavailability were contemplated. Firstly, modeling studies derived from X-ray data have indicated that one of the oxygen atoms of the phosphate group of D-Ins-1-P does not form a direct interaction with the active site.[5c] Moreover, it is evident that the P–O bond points directly towards the lipophilic pocket bounded by Val40 and Leu42 (illustrated in Fig. 2).[5c] It seemed that the preparation of various phosphonate derivatives with alkyl groups attached to both the phosphorus atom and to the 6-position of the inositol ring would give inhibitors with a single negative charge at physiological pH. In addition, if the 6-alkoxy group could be replaced with an alkylamino group at this position then such compounds would be neutral overall. These compounds are illustrated as general structure **5** in Fig. 3.

The second approach was entirely different and uses knowledge of the mechanism of action of Li^+ as an inhibitor of IMPase. At its therapeutic concentrations (~1 mM) Li^+ is an uncompetitive inhibitor and acts synergistically with inorganic phosphate (P_i).[3] It replaces $Mg^{2+}2$ at the active site of IMPase in an enzyme–product complex and prevents the release of P_i, thereby preventing access by substrate molecules. It was proposed to prepare compounds that would mimic the *myo*-inositol product of the reaction catalysed by the enzyme. Such molecules would be inositol analogues with similar structural features to previously prepared inhibitors, except, with the phosphate group absent. The presence of a good lipophilic side chain at the 6-position of the inositol ring was expected to compensate for the binding energy loss that would inevitably arise from removal of the phosphate group from such compounds. It was anticipated that, like Li^+, these compounds might act synergistically with the product P_i since they would not need to displace it from the active site in order to bind. It is known that P_i is a product inhibitor of the enzyme with K_i = 0.3 mM (at pH 8.0[3]) and that brain phosphate levels are ~3 mM. Therefore the enzyme is largely P_i-bound under physiological conditions in the cell, which would aid these compounds *in vivo* should they transport effectively to the brain. At the outset of this work *myo*-inositol itself was the only known product inhibitor of IMPase and as one would expect, it has a very large K_i of 400 mM.[3] Since no good leads were available for this approach, it was not expected to devise a tight binding inhibitor straight away but rather to produce compounds displaying sufficient potency as inhibitors of IMPase, and the required cooperative effects with P_i to validate the design strategy. The product-like inhibitors envisaged are illustrated as general structure **6** in Fig. 3.

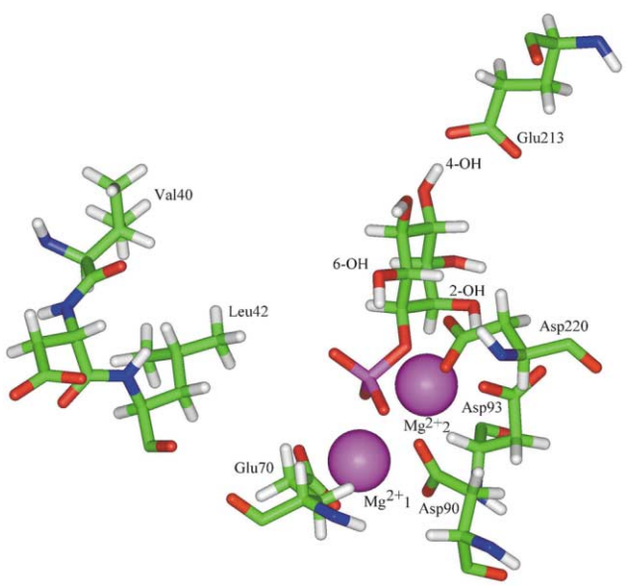
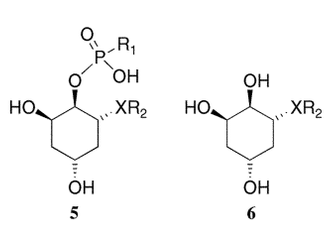


Fig. 2 Model of Ins-1-P bound at the active site of IMPase. The binding interactions between the 4-OH and 2-OH groups of Ins-1-P with Glu213 and Asp93 of the enzyme can be clearly seen. The hydrophobic loop containing Val40 and Leu42 is also illustrated and one can also clearly see that both the 6-OH group and one P–O bond of Ins-1-P point directly towards this exploitable area of the active site.



R_1 and R_2 = alkyl, X = O or NH

Fig. 3 Proposed reduced charge inhibitors of IMPase.

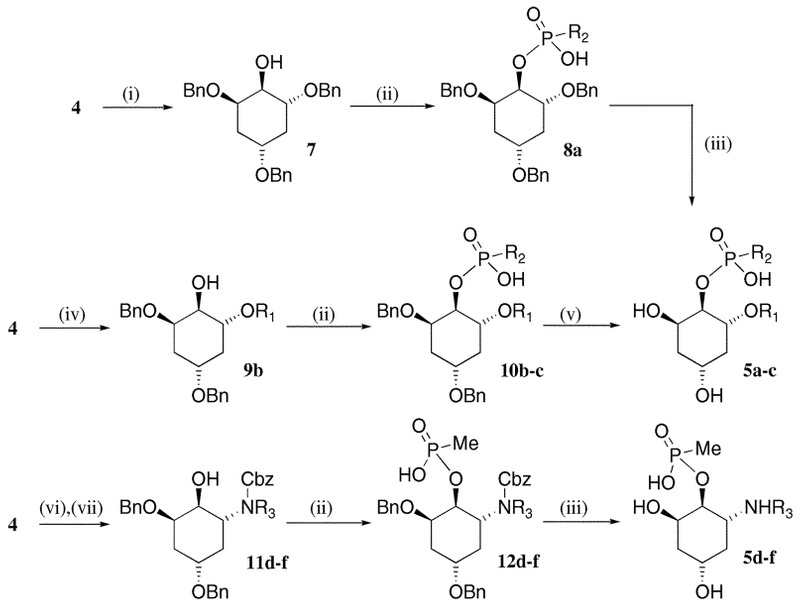
Synthesis of phosphonate inhibitors

As with our previous work, the enantiomerically pure epoxide **4** was used as the common intermediate for the preparation of all the compounds in this work. It is available in multigram quantities using an 11 step synthesis from (−)-quinic acid.[18] Scheme 1 shows how the phosphonate compounds were prepared.

The simplest of these compounds to prepare were the phosphonates bearing a 6-hydoxy **5a** or 6-propyloxy group **5b** and **5c**. These compounds differ in both the identity of the alkoxy group at the C-6 and the alkyl group attached to phosphorus. Firstly the epoxide **4** was stereoselectively ring opened with benzyl alcohol using ytterbium(III) triflate as a Lewis acid catalyst as reported previously.[18,20] The resulting alcohol **7** was then transformed into phosphonate **8a** by treatment with methyl phosphonic dichloride. The intermediate phosphonyl chloride was hydrolysed in a mixture of *t*-butanol–water (1 : 1; v/v) at 50 °C to give the acid.[21] The phosphonic acid **5a** was isolated in 86% yield after removal of the benzyl groups using sodium in liquid ammonia, and conversion of the 1,2,4,6 tetrol phosphonic acid into its cyclohexylammonium salt.

The 6-propyloxy alcohol **9b** was obtained by ring opening of epoxide **4** with 1-propanol as described above. The phosphonates **10b** and **10c** were obtained (52% and 23% respectively) in a similar manner to above using respectively methyl and ethyl phosphonic dichlorides as phosphonylating agents. Debenzylation of these phosphonic acids, was however achieved using catalytic hydrogenolysis using 10% palladium on activated charcoal, and the required 6-alkoxy phosphonates **5b** and **5c** were isolated after transformation into their cyclohexylammonium salts in 41% and 98% yield respectively.

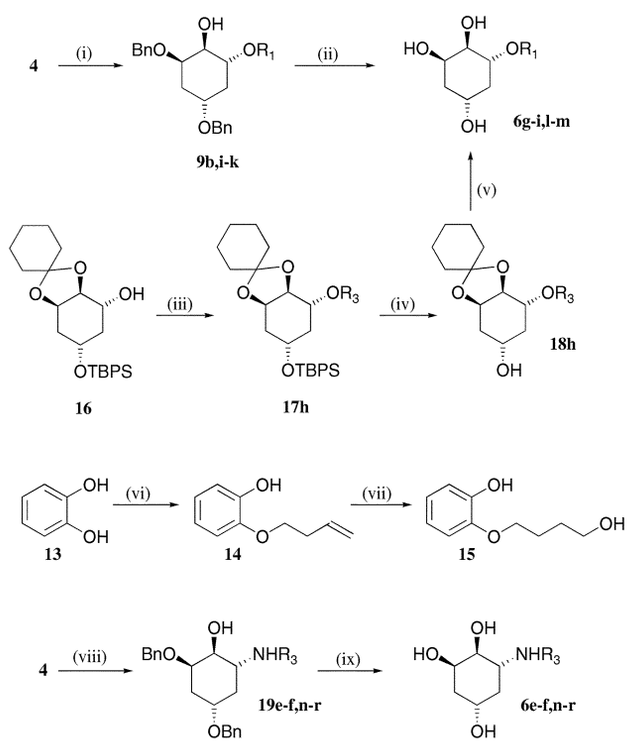
The preparation of the phosphonate compounds possessing a 6-alkylamino side chain **5d–f** was slightly more complicated as the amino functionality needed protection prior to the phosphonylation step. The ring opening of epoxide **4** proved slightly more problematic than had previously been encountered due to the bulkiness of some of the amines used in this step. The reaction with methylamine was carried out as had been previously communicated[20] and involved heating a mixture of **4**, methylamine in water and ytterbium(III) triflate in a sealed vessel.



a R_1 = H, R_2 = Me, **b** R_1 = *n*-Pr, R_2 = Me, **c** R_1 = *n*-Pr, R_2 = Et, **d** R_3 = Me, **e** R_3 = *n*-hexyl, **f** R_3 = 2-phenylethyl

Scheme 1 Synthesis of phosphonate inhibitors of IMPase. *Reagents and conditions:* (i) BnOH, $Yb(OTf)_3$, 1,2-dichloroethane, reflux; (ii) R_2POCl_2, TEA, DMAP, DCM, then *t*-BuOH/H_2O, 50 °C; (iii) Na or Ca in NH_3(liq.), then MeOH; (iv) R_1OH, $Yb(OTf)_3$, 1,2-dichloroethane, reflux. v. H_2, Pd–C, MeOH; (vi) R_3NH_2, $Yb(OTf)_3$, various solvents; (vii) BnOCOCl, TEA, DCM.

After protection of the resulting secondary amine with benzyl chloroformate, alcohol **11d** was isolated in 78% yield. For the preparation of alcohol **11e** hexylamine was used to open the epoxide **4**, but under the above conditions or using a solution of hexylamine in 1,2-dichloroethane none of the required amine was isolated, and under forcing conditions the amine reacted with 1,2-dichloroethane. However, treatment of epoxide **4** with hexylamine and ytterbium(III) triflate in a refluxing mixture of toluene–THF (3 : 1) gave the desired secondary amine, which was Cbz protected to give alcohol **11e** in 68% yield. For the synthesis of alcohol **11f**, the epoxide **4** was ring opened with 2-phenylethylamine and ytterbium(III) triflate in refluxing 1,2-dichloroethane, and the resulting secondary amine Cbz protected to give alcohol **11f** in 70% yield. These three protected amines were then phosphonylated using methylphosphonic dichloride under the same conditions as before. Phosphonate **12d** was carried forward to the next step without isolation, whereas **12e** and **12f** were isolated in 84% and 90% yield respectively. The resulting phosphonic acids **12d–f** were deprotected using dissolving metal reduction since catalytic hydrogenolysis proved slow and unreliable for amine bearing compounds of this type. The 6-methylamino phosphonate **5d** was isolated in 36% yield after reaction of the intermediate phosphonate **12d** with sodium in liquid ammonia to facilitate global deprotection. Similarly, the 6-hexylamino phosphonate **5e** was isolated in 44% yield from phosphate **12f**. However, in the case of phosphonate **12f**, in order to avoid possible Birch reduction of the phenyl group in the 2-phenylethyl side chain, calcium in liquid ammonia was used for the deprotection step.[22,23] 6-(2-Phenylethyl)amino phosphonate **5f** was isolated in 47% yield.



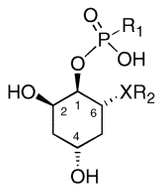
Scheme 2 Synthesis of product-like inhibitors of IMPase. *Reagents and conditions:* (i) R_1OH, $Yb(OTf)_3$, 1,2-dichloroethane, reflux; (ii) H_2, Pd–C, MeOH; (iii) NaH, *n*-hexyl iodide, DMF, 0 °C; (iv) TBAF, THF. v. TFA(cat.), MeOH; (vi) 4-bromo-1-butene, acetone, reflux; (vii) BH_3·THF, Et_2O, then $NaOH/H_2O_2$; (viii) R_3NH_2, $Yb(OTf)_3$, various solvents; (ix) TMSBr, $CHCl_3$, 50 °C.

Synthesis of product-like inhibitors

The synthesis of the product mimics is shown in Scheme 2. The preparation and initial evaluation of some these compounds as inhibitors of IMPase was the subject of an earlier communication.[24]

Two series of compounds were prepared; one had a 6-alkyloxy side chain and the other a 6 amino or 6-alkylamino side chain. We have previously demonstrated that 6-alkylamino side chains are tolerated at the active site of IMPase; 6-amino- and 6-methylamino-phosphate derivatives having been shown to be substrates of IMPase.[15,20] Compound **6i** is merely the potent inhibitor **3** without a phosphate group attached at C-1. It was hoped that since the phosphate group had been removed then the pendant arm of **3**, above all others, would compensate for the binding energy that would be lost through this change. Compounds **6l** and **6m** were designed with an amino group at the terminus of the 6-alkyloxy group. It was hoped that this group, which would be positively charged at physiological pH, would replace the $Mg^{2+}2$ ion at the active site of IMPase and

Table 1 Inhibitory constants and modes of action of substrate-like inhibitors of IMPase



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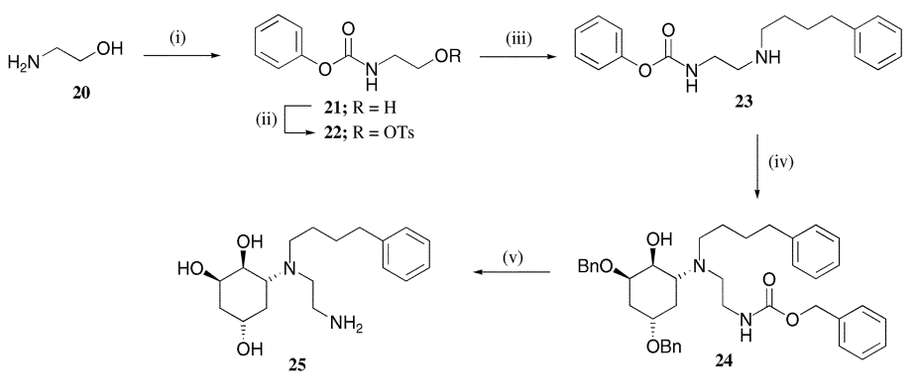
Compound	Phosphonate (R_1)	Side chain on C-6 (XR_2)	Mode of action	K_i or IC_{50} (mM)
myo-Inositol 1-P				0.1 (K_M)
5	Hydroxyl	OH	Substrate	0.025 [16c] (K_M)
5a	Methyl	OH	—	260 ± 16
5b	Methyl	$O(CH_2)_2CH_3$	Competitive	0.04 ± 0.004 (K_i)
5c	Ethyl	$O(CH_2)_2CH_3$	Competitive	0.16 ± 0.016 (K_i)
5d	Methyl	$NHCH_3$	—	>8
5e	Methyl	$NH(CH_2)_5CH_3$	—	1.3 ± 0.2
5f	Methyl	$NH(CH_2)_2Ph$	—	>54



Scheme 3 Synthesis of product-like inhibitor of IMPase bearing a diamino functionality. *Reagents and conditions:* (i) BnOCOCl, TEA, DMAP, 1,4-dioxane–water (1 : 1; v/v), 0 °C to RT; (ii) TsCl, DMAP, TEA, DCM; (iii) 4-phenylbutylamine, 50 °C, closed system, then K_2CO_3, $CHCl_3$; (iv) **4**, $Yb(OTf)_3$, toluene–THF (3 : 1; v/v), reflux. v. TMSBr, $CHCl_3$, 50 °C.

that this would mimic the action of Li^+. The 6-alkylamino derivatives **6e–f** and **6n–r** were designed with varying alkyl chain lengths in order to gain a structure activity relationship should these compounds be successful as inhibitors of IMPase.

The synthesis of the 6-alkyloxy derivatives involved simple ring opening of epoxide **4**, under standard conditions, with *n*-propanol, alcohol **15** (prepared from catechol as shown in Scheme 2) and Cbz-protected ethanolamine and propanolamine to give compounds **9g**,**i–k** in 65%,[18] 72%, 71% and 52% yield, respectively. Deprotection was achieved using catalytic hydrogenolyisis over palladium–charcoal in excellent yields (84–95%).

In an effort to simplify the synthetic route to these 6-alkyloxy derivatives we decided to investigate the direct alkylation of the 4-silyl ether **16**; an intermediate in the synthesis of the epoxide **4**.[18] Treatment of 4-silyl ether **16** with *n*-hexyl iodide in the presence of sodium hydride gave the fully protected tetrol **17h** in 64% yield. Sequential removal of the silyl and cyclohexylidene ketal protecting groups with TBAF in THF and TFA in MeOH respectively, gave the 6-hexyloxy 1,2,4-triol **6h** in 63% yield. This route, which is considerably shorter and higher yielding offers an efficient way of synthesizing these and further 6-alkyloxy derivatives.

The 6-amino and 6-alkylamino derivatives, of general structure **19**, were prepared in the same manner as that outlined above for the 6-alkylaminophosphate derivatives (**5d–f**) starting from epoxide **4**. Removal of the benzyl groups was however achieved by heating the amino alcohols **19** at 50 °C with trimethylsilyl bromide in chloroform, and yields ranged from 54% to 88%.

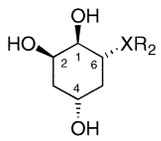
In order to investigate whether the introduction of a secondary alkyl group at C-6 would increase binding due to the lipophilic pocket at that site we decided to synthesize the diaminotriol **25**, Scheme 3.

Ethanolamine **20** was Cbz protected in 80% yield using benzyl chloroformate in the presence of DMAP. The free alcohol **21** was converted into the corresponding tosylate in 88% using tosyl chloride in the presence of DMAP and TEA. Nucleophilic substitution of the tosyl group with 4-phenylbutylamine gave the secondary amine **23** in 45% yield. Although we were concerned that ring opening of epoxide **4** would be difficult, due the added steric bulk of the secondary amine, the reaction proceeded efficiently under the standard conditions of epoxide ring opening to give the diamino alcohol **24** in 58% yield. Global deprotection using either hydrogenolysis or dissolving metal reduction proved unfruitful. However when the diamino alcohol was treated with TMS-bromide the resulting diamino triol **25** was isolated in 38% yield.

Biological evaluation

Phosphonates. For the testing of the phosphonate compounds **5a–f** as inhibitors of IMPase, the colorimetric assay of Itaya *et al.* was used.[3,25] This employs a malachite green/molybdic acid colorimetric reagent that is sensitive to the release of P_i that occurs upon turnover of the substrate. Using this assay **5a** (260 μM) was shown to be a competitive inhibitor of IMPase but with reduced potency relative to a phosphate derivative (K_M = 25 μM [16c]), Table 1. It was also noted that this compound may be a substrate for the enzyme. Incubation of **5a** with the enzyme under standard assay conditions and following the progress of the reaction by ^1H and ^{31}P NMR spectroscopy showed (due to no change in the NMR spectrum) that the com-

Table 2 Inhibitory constants and modes of action of product-like inhibitors of IMPase



6 or 25

Compound	Side chain (XR_2)	Mode of action	K_i or IC_{50} (mM)
myo-Inositol	—	Non-competitive	400 (K_i)[3]
6g	$O(CH_2)_2CH_3$	—	~150 (IC_{50})
6h	$O(CH_2)_5CH_3$	—	~10 (IC_{50})
6i	$O(CH_2)_4O$-[(2-OH)C_6H_4]	Competitive	4 ± 0.6 (K_i)
6l	$O(CH_2)_2NH_2$	—	5 ± 1 (IC_{50})
6m	$O(CH_2)_3NH_2$	—	10 ± 2 (IC_{50})
6n	NH_2	—	>50 (IC_{50})
6o	$NHCH_2CH_3$	—	>50 (IC_{50})
6p	$NH(CH_2)_3CH_3$	Uncompetitive (in absence of P_i)	0.3 ± 0.03 (K_i)
6e	$NH(CH_2)_5CH_3$	Uncompetitive	0.3 ± 0.04 (K_i)
6q	$NH(CH_2)_7CH_3$	—	4 ± 0.4 (IC_{50})
6f	$NH(CH_2)_2Ph$	—	6 ± 0.6 (IC_{50})
6r	$NH(CH_2)_4Ph$	Non-competitive	9 ± 0.9 (K_i)
25	$H_2N(CH_2)_2N(CH_2)_4Ph$	—	9 ± 0.9 (IC_{50})

pound displayed no substrate activity. The methyl and ethyl phosphates **5b** (K_i = 40 μM) and **5c** (K_i = 160 μM) were also shown to be competitive inhibitors of IMPase but again with reduced potency relative to a phosphate derivative with a 6-propyloxy side chain (K_i = 0.85 μM [17,26]). It seemed therefore that increasing the length of the alkyl chain directly attached to the phosphorus in these compounds had a detrimental effect on their inhibitory activity. Attempts to make further homologues of this type were complicated due to problems encountered in separating the final products from contaminating phosphonic acids. The crude materials, when tested, did not show any significant inhibitory activity. The phosphonates **5d–f** possessing the 6-alkylamino functionality and hence no net charge were even more disappointing as inhibitors of IMPase. Neither the 6-methylamino derivative **5d** nor the 6-(2-phenylethyl)amino derivative **5f** gave any measurable inhibition of the enzyme. Indeed the latter did not retard the turnover of substrate even at concentrations up to 50 mM. Of these, only the 6-hexylamino derivative **5e** was active against IMPase and this had a relatively poor IC_{50} of 1.3 mM. The results are summarized in Table 1. We therefore concluded that reduction of the charge on IMPase by modification of the phosphate group to a phosphonate was not likely to lead to an effective inhibitor of IMPase.

Product mimics. The product mimics prepared in this work proved much more interesting as inhibitors of IMPase. Because it was anticipated that any inhibitor showing promise would be tested as an inhibitor in the presence of P_i (in order to assess whether the two would act cooperatively) it was necessary to use a different assay. The colorimetric assay is sensitive to a maximum concentration of 100 μM P_i [25] and our needs required assay conditions containing concentrations 10–30 times higher than this. The assay used was the radiochemical assay of Gee *et al.*[3,14] employing tritium labeled D-inositol-1-phosphate as the substrate. The concentration of D-Ins-1-P was 0.2 mM. For each compound in this series an IC_{50} was calculated and for promising compounds a full K_i determination was performed to assess the mode of action of the inhibitor. The results are summarized in Table 2.

All inhibitors tested showed a marked improvement in affinity for the enzyme as compared to the natural product *myo*-inositol. The 6-alkyloxy derivatives were the least promising of these compounds, yet bound to the enzyme approximately 2 orders of magnitude more tightly than *myo*-inositol itself. Interestingly, the double reciprocal plots (1/*v versus* 1/[S]) for **6i** possessing the catechol derived side chain of **3** showed that it differed in its mode of action. *myo*-Inositol is known to be a non-competitive inhibitor of IMPase but the double reciprocal plots for **6i** clearly intersected on the y-axis showing this compound to be a competitive inhibitor of IMPase,[27] Fig. 4. This result demonstrates that this compound blocks the binding site from access by the substrate.

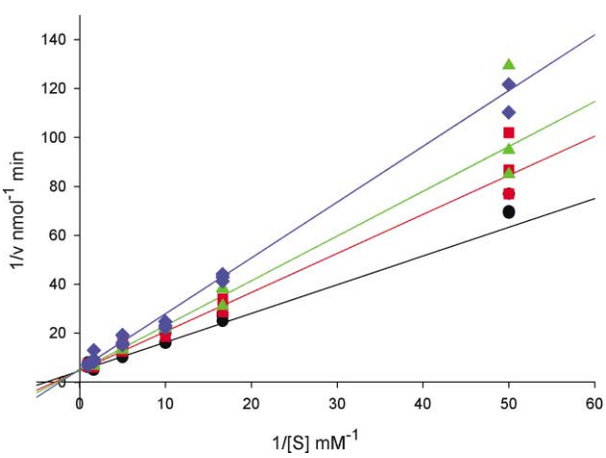


Fig. 4 Double reciprocal plots of initial rate *versus* [Ins-1-P] at **6i** concentrations of 0 (●), 1 mM (red ■), 2 mM (green ▲) and 3 mM (blue ◆). Experiments were performed at 25 °C and at pH 8.0. Intersection of the lines on the *y*-axis indicates that **6i** is a competitive inhibitor of IMPase.

The two 6-alkyloxy compounds containing an amino group in the pendant arm **6l** and **6m** showed a lower affinity for the enzyme than **6i**. Two further 6-alkyloxy derivatives containing simple alkyl chains, 6-propyloxy **6g** and 6-hexyloxy **6h**, were also prepared and tested but were found to be of lower affinity (IC_{50} ~150 mM and ~10 mM respectively). In addition the relatively low water solubility of these compounds precluded accurate IC_{50} measurements.[24] Interestingly, **6l** showed a 30-fold increase in potency as compared to **6g**, indicating that replacement of the terminal CH_3 by an NH_2 group enhances binding almost certainly through its interaction with the $Mg^{2+}2$ binding site.

The 6-alkylamino derivatives showed more potency as inhibitors of IMPase as well as having a different mode of action. The 6-(4-phenylbutyl) derivative **6r** when tested as an inhibitor gave double reciprocal plots that intersect on the *x*-axis, Fig. 5. This is clearly indicative of non-competitive inhibition and is

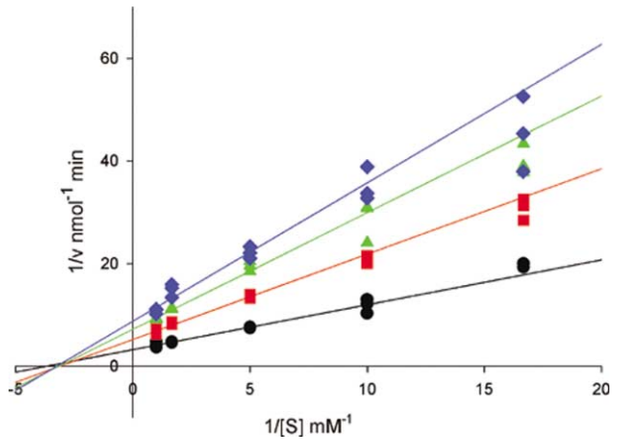


Fig. 5 Double reciprocal plots of initial rate *versus* [Ins-1-P] at **6r** concentrations of 0 (●), 5 mM (red ■), 10 mM (green ▲) and 15 mM (blue ◆). The plots intersect on the *x*-axis indicating non-competitive inhibition. Experiments were performed at 25 °C and at pH 8.0.

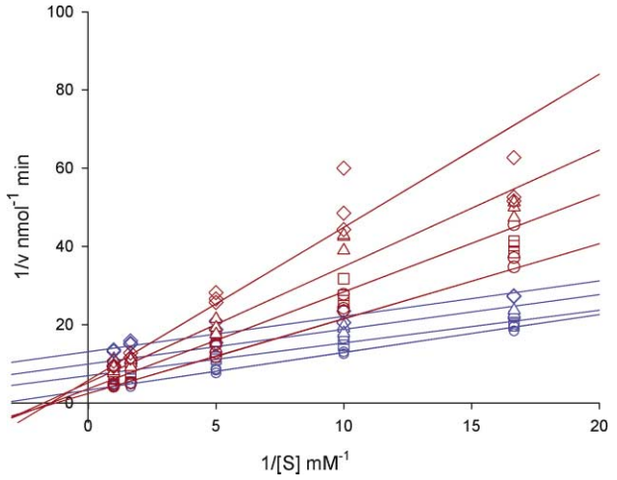


Fig. 6 Double reciprocal plots of initial rate *versus* [Ins-1-P] at **6p** concentrations of 0 (○), 0.25 mM (□), 0.5 mM (△) and 0.75 mM (◊). The parallel line pattern is clearly indicative of uncompetitive inhibition. Also shown are the same measurements {**6p** concentrations of 0 (○), 0.25 mM (□), 0.5 mM (△) and 0.75 mM (◊)} in the presence of 1 mM Pi. Under these conditions the plots now intersect on the *x*-axis indicating a change of behaviour to non-competitive inhibition. Experiments were performed at 25 °C and at pH 8.0.

the same mode of action as shown by the natural product of IMPase. This indicates that **6r** does not compete with Ins-1-P for the active site of IMPase yet it does lower the k_{cat} of the reaction. Presumably this compound is acting as a substrate for the enzyme in the reverse direction and hence inhibits in a non-competitive fashion.

The diamino derivative **25** had an IC_{50} value (9 mM) marginally lower than the 6-(4-phenylbutyl) derivative **6r** (13 mM). Presumably the 4-phenylbutyl group binds with the Val40–Leu42 lipophilic pocket and the primary amino group in the Mg^{2+} site. This could be investigated further using other analogues. It would also be interesting to see whether replacement of oxygen in the 2-aminopropyloxy compound **6l** by nitrogen would lead to a better inhibitor than either **6l** or the diamino derivative **25**.

The most interesting of all of these product-like inhibitors proved to be the 6-butylamine and 6-hexylamine derivatives **6p** and **6e**, with sub-millimolar IC_{50} values. Moreover upon measurement of their K_i values it became apparent that they again had a different mode of action as inhibitors of IMPase. The double reciprocal plots at different inhibitor concentrations showed a parallel line pattern indicating that these compounds were uncompetitive inhibitors of IMPase—both with K_i = 0.3 mM. This suggests that these compounds have effectively no affinity at all for the free enzyme and only bind to the enzyme substrate complex, not affecting k_{cat}/K_m.[27] The butylamine derivative **6p** was then assessed as an inhibitor in the presence of P_i and showed a change in mode of action; the double reciprocal plots now showed a non-competitive inhibition pattern, see Fig. 6. Presumably this change in behaviour is due to the secondary amine functionality of **6p**, which will be positively charged under the assay conditions (pH 8.0) and therefore have no affinity for an active site designed for the anionic inositol monophosphates. Only when the requirement for an anion to be bound is fulfilled does it bind to the enzyme active site. Under these circumstances it is presumably acting as a substrate for the enzyme in the reverse direction and so, like inositol itself will act as a non-competitive inhibitor. The K_i for **6p** was then measured at concentrations of 1, 2 and 3 mM P_i to see if the two were cooperative in their inhibition of IMPase. The results are shown in Fig. 7 and are inconclusive. Certainly the presence of P_i affects the magnitude of K_i for **6p** but it seems less effective rather than more effective. Moreover, from 1–2 mM P_i the K_i of **6p** gets worse but then improves again as the concentration of P_i increases to 3 mM.

It seems from the data shown in Table 2 that as the 6-alkylamino side chain is reduced in length then the more potent the inhibitor (albeit **6p** and **6e** are of the same potency within experimental error). Consequently the 6-amino and 6-ethyl-

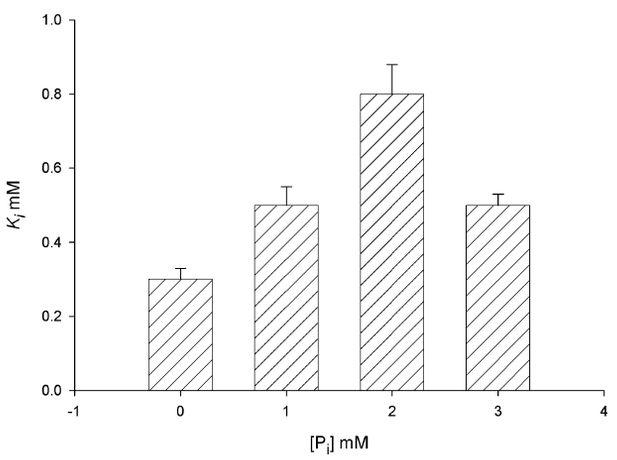


Fig. 7 K_i for **6p** at P_i concentrations of 0, 1, 2 and 3 mM. Note that at $[P_i] = 0$ **6p** displays uncompetitive inhibition and at 1–3 mM it is a non-competitive inhibitor.

amino derivatives **6n** and **6o** (of general structure **6**) were prepared but these were completely ineffective as inhibitors of IMPase with IC_{50} values > 50 mM. This indicates that at least a 4-carbon chain length seems to be necessary on the 6-amino group for effective inhibition of the enzyme.

Conclusion

Phosphonate inhibitors of IMPase are far less effective than their phosphate counterparts, although at K_i = 40 μM, **5b** is amongst the most potent mono-anionic inhibitors of IMPase yet produced.[9] Introduction of a 6-amino or 6-alkylamino group on these materials further reduces their potency as inhibitors of IMPase.

The product-like inhibitor series were not tight binding inhibitors of IMPase but were 2–3 orders of magnitude more effective as product inhibitors than *myo*-inositol. No conclusive evidence that these compounds might cooperatively inhibit IMPase with P_i was found but the K_i of **6p** did seem to vary somewhat with the P_i concentration indicating that this might be possible. The change in the mode of action of this inhibitor in the presence of P_i is certainly encouraging. These compounds therefore represent good lead structures from which more potent inhibitors of this type might be derived. Given that they

have a very different nature to the phosphate inhibitors prepared in our previous work, it is certainly possible that they might have very different pharmacological properties and future work will aim at investigating their transport properties *in vivo*.

Experimental

Elemental microanalyses were performed in the departmental micro-analytical laboratory using a Carlo Erba EA1110 CHNS elemental analyzer. NMR spectra were recorded on a Bruker AC-300 spectrometer (^{1}H, 300 MHz; ^{31}P, 121.5 MHz), Bruker AV-300 spectrometer (^{13}C, 75.4 MHz), Bruker AMX-400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) and a Bruker DRX-500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz). Chemical shifts are described in parts per million downfield shift from $SiMe_4$ and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = double of doublets, sep = septet, m = multiplet, and br = broad), coupling constant (*J*/Hz) and assignment (numbering according to the IUPAC nomenclature for the compound). ^{1}H-NMR were referenced internally on CH_3OH (δ 3.35), ^{2}HOH (δ 4.68) or $CHCl_3$ (δ 7.27). ^{13}C-NMR were referenced on CH_3OH (δ 49.0), C^2HCl_3 or (δ 77.0). ^{31}P NMR spectra were referenced to an external capillary containing 1 mol dm^{-3} solution of H_3PO_4 in deuterated water (δ 0.00). Infra-red spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. The samples were prepared as KBr discs, Nujol mulls, solutions in chloroform or thin films between sodium chloride discs. The frequencies (ν) as absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Mass spectra and accurate mass (HRMS) measurements were recorded on a VG ProSpec or VG ZabSpec spectrometer (EI and CI), Micromass LCT TOF spectrometer (ES) and VG ZabSpec spectrometer [LSIMS (modern version of FAB)]. LSIMS spectra were recorded using *m*-nitrobenzyl alcohol as a matrix. Chemical ionization (CI) spectra were recorded using ammonia as a reagent gas. Electrospray (ES) spectra were recorded using MeOH or acetonitrile as the mobile phase. Major fragments were given as percentages of the base peak intensity (100%). Melting points were taken on an Electrothermal Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured at room temperature (25 °C) on Optical Activity AA-2001 polarimeter using a 5, 10 or 20 cm path length cell and are given in 10^{-1} deg cm^2 g^{-1}. Flash chromatography was performed according to the method of Still *et al*.[28] using Fluka silica gel 60 (3570 μm mesh). Analytical thin layer chromatography (TLC) was carried out on 0.25 mm precoated silica gel glass plates (Whatman K6F) and compounds were visualised using UV fluorescence, ethanolic phosphomolybdic acid, ninhydrin or iodine. Preparative cation exchange column chromatography was performed using Amberlite X 131 WET (H^+ ion-exchange resin, 120 × 25 mm), eluting with distilled water at a rate of 1 drop every 3 seconds. RP HPLC was carried out using a Dionex Summit System. Analytical RP HPLC was performed on a Phenomenex Luna C18 (2) 10 μm column (250 × 4.6 mm). Preparative RP HPLC was performed on a Phenomenex Luna C18 (2) 10 μm column (250 × 21.2 mm).

myo-Inositol-phosphatase was supplied by Sigma and was used according to the manufacturers instructions. (2-^{3}H)-*myo*-inositol-1-phosphate was supplied by Tocris-Cookson Ltd. Amberlite X 131 WET ion exchange resin and phosphorylating reagents were obtained from Aldrich Chemical Co. Ltd. (Gillingham, Dorset, UK). The solvents were used either distilled or of Analar quality and petroleum ether refers to that portion boiling between 40 and 60 °C. Solvents were dried according to literature procedures.[29] MeOH was dried using magnesium turnings, dichloromethane and TEA were distilled over calcium hydride and THF was distilled over sodium–benzophenone.

(+)-(1*S*,2*R*,4*R*,6*R*)-2,4-Bis(benzyloxy)-1,6-epoxycyclohexane 4

This compound was prepared as previously described.[18]

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4,6-Tris(benzyloxy)-cyclohexanol 7

To a stirred solution of the epoxide (+)-**4** (300 mg, 0.968 mmol) and benzyl alcohol (2.00 g, 19.4 mmol) in 1,2-dichloroethane (30 cm^3) was added Yb(III)(OTf)$_3$ (120 mg, 0.194 mmol) and the solution was heated under reflux for 16 h. After cooling, the solvent was removed under reduced pressure and the excess benzyl alcohol was removed by distillation *in vacuo* using a Kugelrohr apparatus. The residue was then purified by flash silica column chromatography (petroleum ether–ethyl acetate; 1 : 1) to give the alcohol **7** as a colourless oil (359 mg, 89%); (HRMS: found: [M + Na]$^+$, 441.2031. $C_{27}H_{30}O_4Na$ requires 441.2042); $[\alpha]_D$ −33.0 (*c* 0.98 in MeOH); ν_{max}(thin film)/cm^{-1} 3562, 3460, 2930, 2867, 1952, 1876, 1810, 1605, 1586, 1496 and 1454; δ_H(300 MHz; C^2HCl_3) 1.37–1.49 (2 H, m, 3-H and 5-H), 2.34–2.42 (1 H, m, 3-H), 2.50–2.55 (1 H, m, 5-H), 2.58 (1 H, d, J_{HH} 4.5, OH), 3.60–3.82 (3 H, m, 1 H, 4-H and 6-H), 3.97–4.00 (1 H, m, 2-H), 4.47–4.74 (6 H, m, PhCH_2) and 7.28–7.39 (15 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.91 and 35.34 (3-C and 5-C), 70.60, 71.64 and 71.93 (PhCH_2), 71.59 (1-C), 75.54, 76.22 and 76.89 (2-C, 4-C and 6-C), 127.59, 127.62, 127.72 and 128.39 (Ar-CH) and 138.40 and 138.50 (Ar-C quaternary); *m*/*z* (TOF ES$^+$) 441 (100%, [M + Na]$^+$).

(−)-(1*R*,2*R*,4*R*,6*R*)-2,4,6-Trihydroxycyclohexyl cyclohexylammonium 1-methylphosphonate [cyclohexylammonium salt of 5a]

To a stirred solution of alcohol (−)-**7** (142 mg, 0.340 mmol) in dry dichloromethane (10 cm^3) under argon was added dry TEA (237 mm^3, 1.70 mmol) and DMAP (42 mg, 0.340 mmol) and the solution was cooled to 5 °C. Methyl phosphonic dichloride (226 mg, 1.70 mmol) was added and the solution was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue was redissolved in *t*-butanol–water, 1 : 1 (10 cm^3) and the solution was heated at 50 °C for 2 h. After cooling, the solvent was removed under reduced pressure and the residue was partitioned between 10% aqueous sodium bicarbonate (10 cm^3) and diethyl ether (10 cm^3). The separated aqueous layer was extracted with diethyl ether (10 cm^3) then acidified to pH 1 by addition of concentrated HCl and then the resulting white precipitate was extracted with ethyl acetate (3 × 20 cm^3). The pooled organic extracts were dried ($MgSO_4$), filtered and evaporated to give the intermediate protected phosphonate **8a** as a colourless oil. This was then dissolved in dry THF (2 cm^3).

Anhydrous ammonia was condensed at −78 °C into a dry three-necked flask fitted with a cold-finger condenser until approximately 10 cm^3 had condensed. To this stirred liquid ammonia was added freshly cut sodium (75 mg, 3.28 mmol) and the solution turned a deep blue colour. The solution of the protected phosphonate **8a** in dry THF was then added *via* a cannula (plus washings) and the mixture stirred at −78 °C for 30 min. MeOH (2 cm^3) was then added and the resulting colourless solution was allowed to stand at room temperature for 16 h to allow the volatile solvents to evaporate. The solid white residue was dissolved in water (2 cm^3) and passed down a column of Amberlite IR 118H cation exchange resin (pre-loaded with H^+ ions, eluting with water). To the pooled, acidic column fractions was added a 1% solution of cyclohexylamine in water until the solution reached pH 6 and the solution was lyophilised. The resulting white powder was crystallised from MeOH–diethyl ether to give the phosphonate **5a** as a white crystalline solid (95 mg, 86%), mp >200 °C (decomp.) (HRMS: found: [M + Na]$^+$, 249.0499. $C_7H_{15}O_6NaP$ requires 249.0504); $[\alpha]_D$ −25.6 (*c* 0.5 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3405 br, 2938, 2860, 1646, 1540 and 1456; δ_H(300 MHz; ^2H_2O) 0.96–1.06 (1 H,

m, 1 × 4-H of Cha), 1.09–1.31 (4 H, m, 2 × 2-H of Cha and 2 × 3-H of Cha), 1.36–1.50 (3 H, m, 1 × 4-H of Cha, 3-H and 5-H), 1.61–1.64 and 1.77–1.83 (4 H, m, 2 × 2-H of Cha and 2 × 3-H of Cha), 1.93–2.01 (1 H, m, 3-H), 2.07–2.13 (1 H, m, 5-H), 2.90–3.05 (1 H, m, 1-H of Cha), 3.67–3.79 (2 H, m, 1-H and 6-H), 3.88 (1 H, tt, J_{HH} 4.5 and 11.5, 4-H) and 4.04–4.10 (1 H, m, 2-H); δ_C(75 MHz; ^2H_2O) 14.55 (d, J_{CP} 137, PCH_3), 26.26 (3-C of Cha), 26.75 (4-C of Cha), 32.79 (2-C of Cha), 40.46 (5-C), 42.35 (3-C), 52.78 (1-C of Cha), 66.17 (4-H), 69.14 (d, J_{CP} 5, 6-C), 70.91 (2-C) and 81.55 (d, J_{CP} 6, 1-C); δ_P(121 MHz; ^2H_2O) 27.23; *m*/*z* (TOF ES$^+$) 303 (25%, $[M - H + 2K]^+$), 287 (10, $[M - H + Na + K]^+$), 271 (100, $[M - H + 2Na]^+$) and 249 (40%, $[M + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-(*O*^{1}-Methylphosphonyl)-2,4-bis(benzyloxy)-6-propyloxycyclohexanol 10b

To a stirred solution of (−)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis(benzyloxy)-6-propyloxycyclo-hexanol **9b**[18] (200 mg, 0.541 mmol) in dry dichloromethane (10 cm^3) under argon was added dry TEA (150 mm^3, 1.08 mmol) and DMAP (66 mg, 0.541 mmol) and the solution was cooled to 5 °C (ice–water bath). A solution of methyl phosphonic dichloride (359 mg, 2.70 mmol) in dry dichloromethane (10 cm^3) was then added *via* a cannula. After stirring for 16 h at room temperature the solvent was removed under reduced pressure and the residue was redissolved in 1 : 1 water : *tert*-butanol (10 cm^3). This solution was then heated at 60 °C for 2 h. After cooling the solution was diluted with 10% sodium hydrogen carbonate solution (10 cm^3) and then extracted with diethyl ether (2 × 20 cm^3). The separated aqueous layer was acidified to pH 1 by dropwise addition of concentrated HCl and the resulting white precipitate was extracted with ethyl acetate (3 × 20 cm^3). The pooled organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the protected phosphonate **10b** as a white solid (125 mg, 52%); mp 76–78 °C (HRMS: found: $[M + Na]^+$, 471.1905. $C_{24}H_{33}O_6NaP$ requires 471.1912); $[\alpha]_D$ −35 (*c* 0.16 in CH_2Cl_2); ν_{max}(KBr disc)/cm^{-1} 3437 br, 2960, 2858, 1737, 1495, 1455, 1368 and 1368; δ_H(300 MHz; C^2HCl_3) 0.84 (3 H, t, J_{HH} 7, $OCH_2CH_2CH_3$), 1.27–1.59 (4 H, m, 1 × 3-H, 1 × 5-H, $OCH_2CH_2CH_3$), 1.47 (3 H, d, J_{HP} 18, PCH_3), 2.12–2.23 (1 H, m, 1 × 3-H), 2.34–2.44 (1 H, m, 1 × 5-H), 3.43 (2 H, ddt, J_{HH} 7, 9.5 and 22, $OCH_2CH_2CH_3$), 3.58–3.77 (2 H, m, 6-H and 4-H), 3.97–4.02 (1 H, m, 2-H), 4.19 (1 H, dt, J_{HH} 3 and 8, 1-H), 4.43 (2 H, ABq, J_{HH} 11, 4-C OCH_2Ph), 4.58 (2 H, ABq, J_{HH} 12, 2-C OCH_2Ph) and 7.19–7.32 (10 H, m, Ar-H); δ_C(125 MHz; C^2HCl_3), 10.51 ($OCH_2CH_2CH_3$), 12.07 (d, J_{CP} 146, PCH_3), 23.23 ($OCH_2CH_2CH_3$), 34.56 (3-C), 35.66 (5-C), 70.58 (4-C OCH_2Ph), 71.36 (4-C), 71.79 ($OCH_2CH_2CH_3$), 72.88 (2-C OCH_2Ph), 74.93 (d, J_{CP} 3, 6-C), 76.06 (2-C), 79.55 (d, J_{CP} 4, 1-C), 127.5, 127.6, 128.3 and 128.4 (Ar-CH) and 138.6 and 138.8 (2 × Ar-C quaternary); δ_P(121 MHz; C^2HCl_3) 33.90; *m*/*z* (ES$^+$) 471 (100%, $[M + Na]^+$) and 449 (15, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-(*O*^{1}-Ethylphosphonyl)-2,4-bis(benzyloxy)-6-propyloxycyclohexanol 10c

This compound was prepared in a manner identical with that described for the phosphonate **10b** using ethyl phosphonic dichloride (397 mg, 2.71 mmol) as the phosphonylating agent to give phosphonate **10c** as a colourless oil which solidified upon standing (58 mg, 23%); mp 53–56 °C (HRMS: found: $[M + Na]^+$, 485.2053. $C_{25}H_{35}O_6NaP$ requires 485.2069); $[\alpha]_D$ −21.8 (*c* 0.17 in CH_2Cl_2); ν_{max}(KBr disc)/cm^{-1} 3431 br, 2919, 1649, 1495, 1459 and 1362; δ_H(300 MHz; C^2HCl_3) 0.90 (3 H, t, J_{HH} 7.5, $OCH_2CH_2CH_3$), 1.11–1.88 (9 H, m, PCH_2CH_3, $OCH_2CH_2CH_3$, 1 × 3-H and 1 × 5-H), 2.49–2.29 and 2.43–2.47 (2 × 1 H, 2 × m, 1 × 3-H and 1 × 5-H), 3.53 (2 H, m, $OCH_2CH_2CH_3$), 3.69–3.82 (2 H, m, 6-H and 4-H), 4.09 (1 H, m, 2-H), 4.35 (1H, d t, *J* 2.5 and 9, 1-H), 4.50 (2 H, ABq, J_{HH} 12, $PhCH_2$), 4.62 (2 H, ABq, J_{HH} 12, $PhCH_2$) and 7.26–7.38 (10 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 6.42 (d, J_{CP} 7, PCH_2CH_3), 10.43 ($OCH_2CH_2CH_3$), 19.33 (d, J_{CP} 144, PCH_2CH_3), 23.08 ($OCH_2CH_2CH_3$), 34.30 and 35.65 (5-C and 3-C), 70.52 ($PhCH_2O$), 71.25 (4-C), 72.08 ($OCH_2CH_2CH_3$), 72.75 ($PhCH_2O$), 75.06 (d, J_{CP} 5, 6-C), 76.09 (2-C), 79.02 (d, J_{CP} 4, 1-C), 127.49, 127.55, 127.56, 128.25, 128.34 (Ar-CH) and 138.4 and 138.7 (Ar-C quaternary); δ_P(121 MHz; C^2HCl_3) 36.19; *m*/*z* (ES$^+$ TOF) 485 (100%, $[M + Na]^+$).

(−)-(1*R*,2*R*,4*R*,6*R*)-6-Propyloxy-2,4-dihydroxycyclohexyl cyclohexylammonium 1-methylphosphonate [cyclohexylammonium salt 5b]

To a stirred solution of protected phosphonate **10b** (88 mg, 0.197 mmol) in ethanol (10 cm^3) was added a drop of acetic acid followed by 10% palladium on activated charcoal (20 mg) and the mixture was stirred under an atmosphere of hydrogen for 72 h. The solution was filtered through a thin pad of Celite to remove the catalyst and then the solvent was removed under reduced pressure. The resulting residue was dissolved in water (1 cm^3) and the solution passed through a column of Amberlite IR 118H cation exchange resin (H^+ form, eluting with water). The acidic fractions were pooled and a 1% solution of freshly distilled cyclohexylamine in water was added until the solution reached pH 7. The solvent was then lyophilised to give phosphonate **5b** as a white powder (29.6 mg, 41%); mp >200 °C (decomp.) (HRMS: found: $[M + Na]^+$, 291.0983. $C_{10}H_{21}O_6NaP$ requires 291.0973); $[\alpha]_D$ −33.6 (*c* 0.23 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3379 br, 2937, 2860, 1633, 1535 and 1448; δ_H(300 MHz; ^2H_2O) 0.71 (3 H, t, J_{HH} 7, $OCH_2CH_2CH_3$) 0.97–1.49 (13 H, m, 3-H, 5-H, $OCH_2CH_2CH_3$, PCH_3 and 6 × Cha-H), 1.56–1.66 and 1.76–1.84 (4 H, m, 4 × Cha-H), 1.91–1.98 and 2.18–2.23 (2 × 1 H, 2 × m, 3-H and 5-H), 2.96 (1 H, m, 1-H of Cha), 3.37–3.56 (3 H, m, $OCH_2CH_2CH_3$ and 6-H), 3.79–3.89 (2 H, m, 1-H and 4-H) and 4.02–4.10 (1 H, m, 2-H); δ_C(75 MHz; ^2H_2O), 12.24 ($OCH_2CH_2CH_3$), 14.13 (d, J_{CP} 138, PCH_3), 24.92 ($OCH_2CH_2CH_3$), 26.26 (3-C of Cha), 26.74 (4-C of Cha), 32.78 (2-C of Cha), 40.31 (3-C), 40.43 (5-C), 52.81 (1-C of Cha), 66.16 (4-C), 70.75 (2-C), 75.05 ($OCH_2CH_2CH_3$), 77.22 (d, J_{CP} 5.5, 6-C) and 81.15 (1-C); δ_P(121 MHz; ^2H_2O) 30.07; *m*/*z* (ES TOF) 291 (100%, $[M + Na]^+$) and 269 (10, $[M + H]^+$).

(−)-(1*R*,2*R*,4*R*,6*R*)-6-Propyloxy-2,4-dihydroxycyclohexyl cyclohexylammonium 1-ethylphosphonate [cyclohexylammonium salt 5c]

This compound was prepared in a manner identical with that described for the phosphonate **5b** using the protected phosphonate **10c** (46 mg, 98.7 μmol) to give a crude oil which was crystallised from MeOH–diethyl ether to afford phosphonate **5c** as a white powder (34 mg, 98%); mp >200 °C (decomp.) (HRMS: found: $[M + Na]^+$, 305.1137. $C_{11}H_{23}O_6NaP$ requires 305.1130); ν_{max}(KBr disc)/cm^{-1} 3435 br, 2942, 2865, 1679, 1643, 1529 and 1448; $[\alpha]_D$ −23.3 (*c* 0.18 in MeOH); δ_H(500 MHz; ^2H_2O), 0.84 (3 H, t, J_{HH} 7.5, $OCH_2CH_2CH_3$), 0.94–1.12 (4 H, m, PCH_2CH_3 and 1 × 4-H of Cha), 1.17–1.30 (3 H, m, 5-H and 2 × 3-H of Cha), 1.43–1.59 (5 H, m, PCH_2CH_3, $OCH_2CH_2CH_3$ and 1 × 4-H of Cha), 1.64–1.74 (2 H, m, 2 × 3-H of Cha), 1.86–1.92 (2 H, m, 4 × 2-H of Cha), 1.98–2.05 (1 H, m, 3-H), 2.24–2.30 (1 H, m, 5-H), 2.96–3.09 (1 H, m, 1-H of Cha), 3.55 (2 H, t, J_{HH} 6.5, $OCH_2CH_2CH_3$), 3.59 (1 H, dt, J_{HH} 10 and 4, 6-H), 3.85–3.95 (2 H, m, 1-H and 4-H) and 4.15 (1 H, br s, 2-H); δ_C(125 MHz; ^2H_2O) 9.28 (d, J_{CP} 6, PCH_2CH_3), 10.77 ($OCH_2CH_2CH_3$), 22.51 (d, J_{CP} 164, PCH_2CH_3), 25.01 ($OCH_2CH_2CH_3$), 26.31 (3-C of Cha), 26.81 (4-C of Cha), 32.86 (2-C of Cha), 40.31 (3-C), 40.45 (5-C), 52.92 (1-C of Cha), 66.42 (4-C), 71.16 (2-C), 75.05 ($OCH_2CH_2CH_3$), 77.56 (d, J_{CP} 3, 6-C) and 80.53 (d, J_{CP} 6, 1-C); δ_P(121 MHz; ^2H_2O) 33.60; *m*/*z* (ES TOF) 305 (100%, $[M + Na]^+$) and 283 (10, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-(*N*-benzyloxycarbonyl)-(*N*-methyl)-6-aminocyclohexanol 11d

A mixture of epoxide (+)-**4** (583 mg, 1.88 mmol) and Yb(III)-(OTf)$_3$ (42 mg, 0.67 mmol) was suspended in a solution of methylamine in water (20 cm^3, 40% w/v) and the resulting solution was carefully heated at 60 °C in a sealed flask for 12 h. The reaction mixture was concentrated under reduced pressure and then dissolved in a 1 M hydrochloric acid solution (20 cm^3) and extracted with diethyl ether (2 × 30 cm^3). To the resulting aqueous layer was added, dropwise, a 4 M NaOH solution until the pH was 14. The solution was then extracted with ethyl acetate (3 × 50 cm^3) and the pooled organic fractions were washed with brine (100 cm^3), dried ($MgSO_4$), filtered and then concentrated under reduced pressure to give the intermediate amine as a yellow oil (569 mg, 89%). A portion of this material (318 mg, 1.32 mmol) was dissolved in dry dichloromethane (40 cm^3) and to this stirred solution was added DMAP (22 mg, 0.180 mmol) and TEA (202 mm^3, 1.45 mmol). After cooling to 5 °C benzyl chloroformate (581 mm^3, 1.45 mmol) was added and stirring continued at room temperature for 16 h. The solution was concentrated under reduced pressure to give an oil which was dissolved in ethyl acetate (30 cm^3) and successively washed with 1 M HCl (20 cm^3), water (20 cm^3) and brine (20 cm^3). The resulting organic layer was dried ($MgSO_4$), filtered and concentrated to give an oil that was purified by flash silica column chromatography (petroleum ether–ethyl acetate; 4 : 1) to give the fully protected *N*-methylamino alcohol **11d** as a white solid (470 mg, 78%); mp 102–103 °C (Found: H, 7.05; C, 73.3; N, 2.95. $C_{29}H_{33}NO_5$ requires H, 7.0; C, 73.25; N, 2.95%); (HRMS: found: [M + Na]$^+$, 498.2258. $C_{29}H_{33}NO_5Na$ requires 498.2256); $[a]_D$ −29.1 (*c* 0.12 in $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3500 br, 3165, 2921, 2850, 1689, 1494, 1400, 1344, 1149, 1088, 1026, 733 and 692; δ_H(300 MHz; C^2HCl_3) 1.30–1.36 (1 H, m, 5-H), 1.50–1.58 (1 H, m, 3-H), 1.65 (1 H, br s, OH), 1.98–2.06 (1 H, m, 5-H), 2.31–2.46 (2 H, m, 3-H and 6-H), 2.87 (3 H, s, N-CH_3), 3.56 (1 H, m, 1-H), 3.84–3.90 (1 H, m, 4-H), 3.95–4.00 (1 H, m, 2-H), 4.42–4.64 (4 H, m, 2 × OCH_2Ph), 5.10–5.26 (2 H, m, CO-OCH_2Ph) and 7.27–7.34 (15 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 28.33 (N-CH_3), 34.00 and 34.16 (3-C and 5-C), 54.02 (6-C), 71.30 (O*C*H$_2$Ph), 71.70 and 76.66 (2 × O*C*H$_2$Ph), 71.30 (4-C), 71.88 (2-C), 76.87 (1-C), 127.69, 127.78 and 128.52 (Ar-CH), 136.88, 138.11 and 138.43 (3 × Ar-C quaternary) and 157.4 (*C*O-OCH_2Ph); *m*/*z* (ES$^+$ TOF) 514.2 (75%, [M + K]$^+$) and 498.2 (100, [M + Na]$^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-(*N*-benzyloxycarbonyl)-(*N*-hexyl)-6-aminocyclohexano1 11e

This compound was prepared in a manner identical with that described for the *N*-methylamino alcohol **11d** using epoxide (+)-**4** (260 mg, 0.83 mmol) and *n*-hexylamine (111 mm^3, 0.83 mmol) to give initially the unprotected hexylamino alcohol [see compound **19e** for full experimental details and data] and then the *N*-Cbz protected *N*-hexylamino alcohol **11e** as a pale yellow oil (68% overall yield) (Found: H, 7.95; C, 74.9; N, 2.6. $C_{34}H_{43}NO_5$ requires H, 7.95; C, 74.85; N, 2.55%); (HRMS: found: [M + Na]$^+$, 568.3045. $C_{34}H_{43}NO_5Na$ requires 568.3039); $[a]_D$ −27.2 (*c* 0.11 in $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3325 br, 3091, 3029, 2926, 2865, 1689, 1452, 1416, 1350, 1262, 1041, 1114, 739 and 693; δ_H(300 MHz; C^2HCl_3) 0.88 (3 H, s, CH_3) 1.05–1.39 (8 H, m, 6 of $CH_2(CH_2)_4CH_3$, 3-H and 5-H), 1.40–1.67 [3 H, m, 2 of $CH_2(CH_2)_4CH_3$and OH], 2.10–2.46 [3 H, m, 1 of CH_2-$(CH_2)_4CH_3$, 3-H and 5-H], 2.98–3.29 [2 H, m, 1 of CH_2-$(CH_2)_4CH_3$ and 6-H], 3.51–3.84 (2 H, m, 1-H and 4-H), 3.90 (1 H, s, 2-H), 4.47–4.70 (4 H, m, 2 × O*C*H$_2$Ph), 5.02–5.27 (2 H, m, CO-O*C*H$_2$Ph) and 7.15–7.42 (15 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 12.01, 20.57, 24.76, 28.12 and 29.45 [$CH_2(CH_2)_4CH_3$], 31.93 (3-C), 33.5 (5-C), 41.20–42.70 (br, 6-C), 43.20–45.10 [$CH_2(CH_2)_4CH_3$], 67.34 (CO-O*C*H$_2$Ph), 68.76 and 69.72 (2 × O*C*H$_2$Ph), 69.89 and 70.03 (2-C and 4-C), 74.92 (1-C), 125.62, 125.77, 125.81, 126.43 and 126.47 (Ar-CH), 134.89, 136.16 and 136.46 (Ar-C, quaternary) and 155.10 (CO); *m*/*z* (ES$^+$ TOF) 568.3 (100%, [M + Na]$^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-(*N*-benzyloxycarbonyl)-(*N*-2-phenylethyl)-6-aminocyclohexano1 11f

This compound was prepared in a manner identical with that described for the *N*-methylamino alcohol **11d** using epoxide (+)-**4** (222 mg, 0.536 mmol) and a mixture of 2-phenylethylamine in 1,2-dichloroethane (40 cm^3, 1 : 1, v/v) to give initially the unprotected hexylamino alcohol [see compound **19f** for full experimental details and data] and then the *N*-2-phenylethylamino alcohol **11f** as a colourless oil (53% overall yield); (Found: H, 7.0; C, 76.5; N, 2.5. $C_{36}H_{39}NO_5$ requires H, 6.95; C, 76.45; N, 2.5%); (HRMS: found: [M + Na]$^+$, 588.2729. $C_{36}H_{39}NO_5Na$ requires 588.2726); $[a]_D$ −78.6 (*c* 0.12 in $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3440 br, 3090, 3050, 3020, 2938, 2856, 1691 br, 1500, 1413, 1276, 1102, 1075, 1032, 738 and 699; δ_H(300 MHz; C^2HCl_3) 1.27–1.71 (2 H, m, 3-H and 5-H), 2.16 (1 H, br s, OH), 2.36–2.51 (2 H, m, 3-H and 5-H), 2.76–3.13 (3 H, m, 1 of N-C*H*$_2$CH$_2$Ph and N-CH$_2$C*H*$_2$Ph), 3.27–3.38 (4 H, m, 1 of N-C*H*$_2$CH$_2$Ph, 6-H, 1-H and 4-H), 3.39–4.02 (1 H, m, 2-H), 4.27–4.71 (4 H, m, 2 × OCH_2Ph), 5.01–5.37 (2 H, m, CO-OCH_2Ph) and 7.04–7.52 (20 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 34.71 (N-CH_2CH_2Ph), 35.11 and 35.30 (3-C and 5-C), 36.71 (N-*C*H$_2$CH$_2$Ph), 55.42 (6-C), 67.44 (CO-O*C*H$_2$Ph), 70.87 and 71.81 (2 × O*C*H$_2$Ph), 72.06 (4-C), 72.11 (2-C), 76.83 (1-C), 127.78, 127.96, and 128.98 (Ar-CH), 136.86, 138.27, 138.62, 139.43 (Ar-C quaternary) and 157.31 (*C*O-OCH_2Ph); *m*/*z* (ES$^+$ TOF) 588.3 (100%, [M + Na]$^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Methylamino-2,4-dihydroxycyclohexyl cyclohexylammonium 1-methylphosphonate [cyclohexylammonium salt 5d]

To a stirred solution of the 6-methylamino compound **11d** (200 mg, 0.421 mmol) in dry dichloromethane (10 cm^3) under argon was added dry TEA (293 mm^3, 2.12 mmol) and DMAP (51 mg, 0.421 mmol) and the solution was cooled to 5 °C. Methyl phosphonic dichloride (280 mg, 2.105 mmol) was added and the solution was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue was redissolved in *t*-butanol–water (10 cm^3, 1 : 1, v/v) and the resulting solution was heated at 50 °C for 2 h. After cooling, the solvent was removed under reduced pressure and the residue partitioned between 10% sodium hydrogen carbonate solution (15 cm^3) and diethyl ether (15 cm^3). The separated aqueous layer was further extracted with diethyl ether (15 cm^3) then acidified to pH 1 by addition of concentrated hydrochloric acid. The resulting white precipitate was extracted into ethyl acetate (3 × 20 cm^3) and the pooled organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The intermediate protected phosphonic acid **12d** was isolated as a white foam and dissolved in dry THF (5 cm^3). Anhydrous ammonia was condensed at −78 °C (dry ice–acetone bath) into a three necked flask fitted with a cold-finger condenser until approximately 10 cm^3 had collected. Freshly cut sodium metal (82 mg, 3.58 mmol) was added to the stirred liquid ammonia and the solution turned a deep blue colour. The solution of **12d** in THF was then added *via* a cannula and the resulting solution was stirred under argon at −78 °C for 30 min. MeOH (2 cm^3) was added (causing the solution to become colourless) and the solution allowed to stand at room temperature for 16 h. The volatile solvents were then removed under reduced pressure to give a white powder which was dissolved in water (2 cm^3) and was passed through a column of Amberlite IR 118H cation exchange resin (H^+ form, eluting with water). The acidic fractions were pooled and pH adjusted to 6 by dropwise addition of a 1% solution of freshly distilled cyclohexylamine in water. The solution was then lyophilised and the resulting white solid was

crystallised from MeOH–diethyl ether to give the *N*-methyl methylphoshonate **5d** as a white crystalline solid (51 mg, 36%); mp >200 °C (decomp.) (HRMS: found: $[M + H]^+$, 240.1008. $C_8H_{19}NO_5P$ requires 240.1001); $[\alpha]_D$ −27.3 (*c* 1.0 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3415 br, 2937, 1634, 1540 and 1456; δ_H(300 MHz; ^2H_2O) 0.98–1.24 (8 H, m, H-4 of Cha, 2 × 2-H of Cha, 2 × 3-H of Cha and PCH_3), 1.33–1.51 (3 H, m, 4-H of Cha, 3-H and 5-H), 1.57–1.72 (2 H, m, 2 × 3-H of Cha), 1.76–1.88 (2 H, m, 2 × 2-H of Cha), 1.98–2.08 (1 H, m, 3-H), 2.23–2.09 (1 H, m, 5-H), 2.60 (3 H, s, NCH_3), 2.92–3.04 (1 H, m, 1-H of Cha), 3.32–3.41 (1 H, m, 6-H), 3.91–4.00 (1 H, m, 4-H), 4.05–4.12 (1 H, m, 1-H) and 4.16–4.22 (1 H, m, 2-H); δ_C(75 MHz; ^2H_2O) 14.86 (d, J_{PC} 136.5, PCH_3), 26.27, 26.76 and 32.79 (3 × CH_2 of Cha), 32.43 ($NHCH_3$), 35.68 (3-C), 40.12 (5-C), 57.91 (d, J_{CP} 5, 6-C), 65.63 (4-C), 69.97 (2-C) and 76.33 (d, J_{CP} 5.5, 1-C); δ_P(121 MHz; ^2H_2O) 27.24; *m*/*z* (TOF ES$^+$) 284 (15%, $[M - H + 2Na]^+$), 262 (70, $[M + Na]^+$) and 240 (100, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-(*O*^{1}-Methylphosphonyl)-2,4-bis(benzyloxy)-*N*-benzyloxycarbonyl-6-hexylamino-cyclohexanol 12e

This compound was prepared in a manner identical with that described for the phosphonate **10b** using *n*-hexylamino alcohol **11e** (340 mg, 0.624 mmol) and methyl phosphonic dichloride as the phosphorylating agent to give the fully protected *N*-hexyl methylphoshonate **12e** as a colourless oil (327 mg, 84%); (Found: H, 7.45; C, 67.5; N, 2.25. $C_{35}H_{46}NO_7P$ requires H, 7.45; C, 67.4; N, 2.25%); (HRMS: found: $[M + Na]^+$, 646.2908. $C_{35}H_{46}NO_7PNa$ requires 646.2910); $[\alpha]_D$ −26.7 (*c* 0.12 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3447 br, 3096, 3061, 3031, 2922, 2856, 2617 br, 1756, 1691, 1500, 1419, 1353, 1195, 1095, 1065, 754 and 694; δ_H(300 MHz; C^2HCl_3) 0.81–0.94 [3 H, m, $CH_2(CH_2)_4CH_3$], 1.11–1.78 [13 H, m, $CH_2(CH_2)_4CH_3$, P-CH_3, 3-H and 5-H], 2.19–2.30 (2 H, m, 3-H, 5-H), 2.72–3.68 [3 H, m $CH_2(CH_2)_4CH_3$ and 6-H], 3.68–3.75 (1 H, m, 4-H), 4.07–4.19 (1 H, m, 2-H), 4.43–4.82 (5 H, m, 2 × OCH_2Ph and 1-H), 4.99–5.36 (2 H, m, CO-OCH_2Ph), 7.23–7.32 (15 H, m, Ar-H) and 10.46 (I H, br, OH); δ_C(75.4 MHz; C^2HCl_3) 16.48, 25.0, 28.95, 31.20 and 32.41 [$CH_2(CH_2)_4CH_3$], 34.00 (P-CH_3), 35.26 (3-C), 37.46 [$CH_2(CH_2)_4CH_3$], 37.78 (5-C), 54.13–61.12 (br, 6-C), 69.41 (CO-OCH_2Ph), 73.06 and 74.26 (2 × OCH_2Ph), 75.60 (4-C), 77.08 (2-C), 77.45 (1-C), 130.03, 130.35, 130.42, 130.57, 130.78 and 130.88 (Ar-CH), 139.18, 141.03 and 141.68 (Ar-C quaternary) and 158.11 (*C*O-OCH_2Ph); δ_P(121 MHz; C^2HCl_3) 33.17; *m*/*z* (ES$^+$ TOF) 646.2 (100%, $[M + Na]^+$) and 528.2 (45, $[M - CH_4PO_3]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-(*O*^{1}-Methylphosphonyl)-2,4-bis(benzyloxy)-*N*-benzyloxycarbonyl-6-(2-phenylethyl)amino-cyclohexanol 12f

This compound was prepared in a manner identical with that described for the phosphonate **10b** using the 2-phenylethyl-amino alcohol **11f** (305 mg, 0.540 mmol) and methyl phosphonic dichloride as the phosponylating agent to give the fully protected *N*-(2-phenylethyl) methylphoshonate **12f** as a yellow oil (312 mg, 90%); (Found: H, 6.6; C, 69.1; N, 2.2. $C_{37}H_{42}NO_7P$ requires H, 6.6; C, 69.05; N, 2.2%); (HRMS: found: $[M + Na]^+$, 666.2612. $C_{37}H_{42}NO_7PNa$ requires 666.2597); $[\alpha]_D$ −31.8 (*c* 0.14 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3346–3548 br, 3096, 3063, 2922, 2856, 2617 br, 1756, 1691, 1500, 1419, 1353, 1195, 1095, 1065, 754 and 694; δ_H(300 MHz; C^2HCl_3) 1.08–1.48 (5 H, m, P-CH_3, 3-H and 5-H), 1.98–2.07 (2 H, m, N-CH_2CH_2Ph), 2.21–2.33 (2 H, m, 3-H and 5-H), 2.77–2.87 (2 H, m N-CH_2-CH_2Ph), 3.50–3.56 (1 H, m, 6-H), 3.69–3.74 (1 H, m, 4-H), 3.76–3.83 (1 H, m, 4-H), 4.07–4.11 (1 H, m, 2-H), 4.17–4.77 (5 H, m, 2 × OCH_2Ph and 1-H), 5.13–5.41 (2 H, m, CO-OCH_2Ph), 6.67 (1 H, br s, OH) and 7.22–7.35 (20 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 31.73 (P-CH_3), 33.22 and 34.84 (3-C and 5-C), 35.67 (N-CH_2CH_2Ph), 45.39 (N-CH_2CH_2Ph), 54.63 (6-C), 67.59 (CO-OCH_2Ph), 71.37 and 73.62 (2 × OCH_2Ph), 72.89 (4-C), 73.00 (2-C), 76.45 (1-C), 127.27, 128.40, and 129.55 (Ar-CH), 137.37, 139.23, 139.42, 139.65 (Ar-C quaternary) and 159.31 (*C*O-OCH_2Ph); δ_P(121 MHz; C^2HCl_3) 33.85; *m*/*z* (ES$^+$ TOF) 704.5 (20%, $[M - H + Na + K]^+$), 688.5 (25, $[M - H + 2Na]^+$), 682.4 (50, $[M + K]^+$), 666.5 (100, $[M + Na]^+$), 496 (25, $[M - CH_4PO_3 - C_7H_7 + K]^+$) and 480.4 (40, $[M - CH_4PO_3 - C_7H_7 + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Hexylamino-2,4-dihydroxycyclohexyl 1-methylphosphonate 5e

To a stirred blue solution of freshly cut sodium (84 mg, 3.65 mmol) in liquid ammonia (15 cm^3) was added dropwise a solution of the fully protected *N*-hexyl methylphoshonate **12e** (252 mg, 0.405 mmol) in dry THF. After stirring for 1 h, 2 drops of MeOH were added and the mixture was left to evaporate. The resulting yellow solid was washed thoroughly with MeOH and the filtrate concentrated under reduced pressure to give a yellow solid which was dissolved in water and loaded onto a cation exchange chromatography column (Amberlyst 131 WET resin, H^+ form, 4.5 g). The column was eluted with water and the acidic fractions were combined and concentrated under reduced pressure to give a pale yellow solution that was lyophilised. The resulting crude white solid was recrystallised from hexane–MeOH (8 : 1) to give triol **5e** as a white solid (55 mg, 44%); mp >166 °C (decomp.); (Found: H, 9.15; C, 50.55; N, 4.55. $C_{13}H_{28}NO_4P$ requires H, 9.1; C, 50.5; N, 4.55%); (HRMS: found: $[M + H]^+$, 293.1756. $C_{13}H_{29}NO_4P$ requires 310.1783); $[\alpha]_D$ −33.6 (*c* 0.12 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3445 br, 3092, 3058, 3028, 2956, 2867, 1690, 1349, 1265, 1026, 1133 and 1042; δ_H(300 MHz; $C^2H_3O^2H$) 0.89 (3 H, s, CH_3), 1.15–1.89 [11 H, m, 6 of $CH_2(CH_2)_4CH_3$, P-CH_3, 3-H and 5-H], 1.92–2.64 [4 H, m, 2 of $CH_2(CH_2)_4CH_3$, 3-H and 5-H], 2.82–2.43 [3 H, m, $CH_2(CH_2)_4CH_3$and 6-H], 3.42–3.66 (1 H, m, 4-H), 3.71–3.79 (1 H, m, 2-H) and 3.92–4.06 (1 H, m, 1-H); δ_C(75.4 MHz; $C^2H_3O^2H$) 14.24 [$CH_2(CH_2)_4CH_3$], 17.56 (d, $^1J_{P\text{-}C}$ 145.0, P-CH_3), 23.36, 27.18, 27.28 and 32.31 [$CH_2(CH_2)_4CH_3$], 35.65 (3-C), 39.40 (5-C), 46.17 [$CH_2(CH_2)_4CH_3$], 58.23 (6-C), 64.35 and 64.45 (2-C and 4-C) and 71.34 (1-C); δ_P(121 MHz; C^2HCl_3) 28.10; *m*/*z* (ES$^+$ TOF) 310.2 (100%, $[M - H]^+$) and 214.2 (50, $[M - CH_3PO_3H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-(2-Phenylethyl)amino-2,4-dihydroxy-cyclohexyl 1-methylphosphonate 5f

To a stirred blue solution of calcium turnings (94 mg, 2.35 mmol) in liquid ammonia (15 cm^3) was added dropwise a solution of the fully protected *N*-(2-phenylethyl) methyl-phoshonate **12f** (150 mg, 0.235 mmol) in dry THF. After 1 h, 2 drops of MeOH were added and the reaction mixture left to evaporate. The resulting yellowish solid was then washed thoroughly with MeOH and the filtrate concentrated under reduced pressure to give a yellow solid which was dissolved in water (2 cm^3) and then loaded on to a cation exchange chromatography column (Amberlyst 131 WET resin, 4.5 g). The column was eluted with water and the acidic fraction concentrated under reduced pressure to give a pale yellow solution that was lyophilised. The crude white solid was recrystallised from hexane–MeOH (8 : 1) to give the triol **5f** as a white solid (36 mg, 47%); mp >145 °C (decomp.); (Found: H, 7.4; C, 54.8; N, 4.25. $C_{15}H_{24}NO_5P$ requires H, 7.35; C, 54.7; N, 4.25%); (HRMS: found: $[M + Na]^+$, 352.1288. $C_{15}H_{24}NO_5NaP$ requires 352.1290); $[\alpha]_D$ −24.5 (*c* 0.13 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3447 br, 3096, 3063, 3031, 2922, 2856, 2617 br, 1756, 1691, 1500, 1419, 1353, 1195, 1095, 1065, 754 and 694; δ_H(300 MHz; $C^2H_3O^2H$) 1.33 (3 H, d, $^2J_{H\text{-}H}$ 16.7, P-CH_3), 1.44–162 (2 H, m, 3-H and 5-H), 2.93–3.10 (2 H, m CH_2CH_2Ph), 3.24–3.37 (1 H, m, 6-H), 3.41–3.58 (2 H, m CH_2CH_2Ph), 3.95–4.08 (1 H, m, 4-H), 4.18–4.24 (1 H, m, 2-H), 4.24–4.32 (1 H, m, 1-H) and 7.31–7.39 (20 H, m, Ar-H); δ_C(75.4 MHz; $C^2H_3O^2H$) 14.82 (d, $^1J_{P\text{-}C}$ 138.0, P-CH), 34.39 and 36.41 (3-C and 5-C), 40.23 (CH_2CH_2Ph), 48.79 (N-CH_2CH_2Ph), 56.62 (6-C), 65.79 (4-C), 70.29 (2-C), 76.32

(1-C), 130.06, 131.49 and 131.75 (Ar-CH) and 139.03 (Ar-C quaternary); δ_P(121 MHz; C^2HCl_3) 27.93; *m/z* (ES^+ TOF) 703.3 (10%, $[(2 \times M) - H + 2 Na]^+$), 681.3 (100, $[(2 \times M) + Na]^+$), 352.1 (30, $[M + Na]^+$), 330.2 (25, $[M + H]^+$) and 257.1 (15, $[M - CH_4PO_3 + Na]^+$).

(−)-(1*S*,2*R*,4*R*,6*R*)-6-Propyloxycyclohexane-1,2,4-triol 6g

To a stirred solution of (−)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis(benzyloxy)-6-propyloxycyclohexanol **9b**[18] (636 mg, 1.72 mmol) in MeOH (20 cm^3) was added 5% palladium on activated carbon (182 mg, 0.086 mmol) followed by a small drop of acetic acid. The mixture was stirred under an atmosphere of hydrogen for 16 h then the catalyst was removed by filtration through a small plug of Celite. The solvent was removed under reduced pressure to give the triol **6g** as an off-white solid (320 mg, 98%); mp 86–89 °C (HRMS: found: $[M + Na]^+$, 213.1107. $C_9H_{18}O_4Na$ requires 213.1103); $[a]_D$ −72.1 (*c* 1.38 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3432 br, 2930, 1470 and 1451; δ_H(300 MHz, ^2H_2O) 0.81 (3 H, t, J_{HH} 7.5, CH_3), 1.17 (1 H, q, J_{HH} 11, 3-H), 1.39–1.55 (3 H, m, 5-H and CH_3CH_2), 1.99–2.04 (1 H, m, 5-H), 2.31–2.36 (1 H, m, 3-H), 3.39–3.59 (4 H, m, 1-H, 6-H and OCH_2), 3.90 (1 H, ddd, J_{HH} 4, 11 and 15.5, 4-H) and 4.02–4.03 (1 H, m, 2-H); δ_C(75 MHz, ^2H_2O) 10.87 (CH_3), 23.56 (CH_3CH_2), 38.72 and 39.44 (3-C and 5-C), 65.05, 69.95, 75.19 and 77.01 (1-C, 2-C, 4-C and 6-C), 72.67 (OCH_2); *m/z* (ES^+ TOF) 213 (100%, $[M + Na]^+$).

(−)-(1*R*,2*R*,4*S*,6*R*)-1,2-Cyclohexyldienedioxy-4-(*tert*-butyl-diphenylsilyloxy)-6-*n*-hexyloxy)cyclohexane 17h

A stirred solution of (−)-(1*S*,2*R*,4*S*,6*R*)-1,2-cyclohexylidene-dioxy-4-(*tert*-butyl-diphenylsilyloxy)cyclohexan-6-ol **16**[18] (1.12 g, 2.37 mmol) in dry DMF (20 cm^3) was cooled to 0 °C in an ice–water bath. NaH (270 mg, 6.77 mmol) was added in small portions under argon followed by dropwise addition of *n*-hexyl iodide (3.56 cm^3, 23.7 mmol). The reaction mixture was gradually brought to room temperature and stirred for 12 h. The resulting solution was diluted with water (40 cm^3) and then extracted with ethyl acetate (3 × 50 cm^3). The organic layers were pooled and washed with brine (40 cm^3), dried ($MgSO_4$), filtered and then concentrated under reduced pressure. The resulting yellow oil was purified by flash silica column chromatography (petroleum ether–ethyl acetate; 4 : 1) to give the 6-hexyloxycyclohexane **17h** as a colourless oil (834 mg, 64%); (Found: H, 9.2; C, 74.2. $C_{34}H_{50}O_4Si$ requires H, 9.15; C, 74.15%); (HRMS: found: $[M + Na]^+$, 573.3378. $C_{34}H_{50}O_4Si$ Na requires 573.3376); $[a]_D$ −33.7 (*c* 0.14 in $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3085, 3063, 3028, 2932, 2857, 1462, 1363, 1112, 1058, 940, 823, 739 and 702; δ_H(300 MHz; C^2HCl_3) 0.88 [3 H, t, $^3J_{H\text{-}H}$ 7.2, $CH_2(CH_2)_4CH_3$], 1.05 [6 H, s, 6 of $C(CH_3)_3$], 1.07 [3 H, s, 3 of $C(CH_3)_3$], 1.24–2.30 [22 H, m, cyclohexylidene, $CH_2(CH_2)_4CH_3$, 2 × 3-H and 2 × 5-H], 3.05–4.35 [6 H, m, $CH_2(CH_2)_4CH_3$, 1-H, 2-H, 4-H and 6-H], 7.32–7.45 (6 H, m, Ar-H) and 7.64–7.77 (4 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 16.65 [$CH_2(CH_2)_4$-CH_3], 21.10 [$SiC(CH_3)_3$], 25.21, 26.51, 27.69, 28.30, 29.53, 32.47, 34.20, 35.80, 37.69, 37.80, 38.75 and 40.55 (C × 5, cyclohexylidene, $CH_2(CH_2)_4CH_3$, $C(CH_3)_3$, 3-C and 5-C), 68.86 (4-C), 71.17 [$CH_2(CH_2)_4CH_3$], 74.00 (2-C), 75.87 (C-6), 81.69 (1-C), 119.39 (C quaternary, cyclohexylidene), 129.95, 130.16 and 132.19 (Ar-CH) and 138.36 and 138.75 (Ar-C quaternary); *m/z* (ES^+ TOF) 573.4 (100%, $[M + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Hexyloxycyclohexane-1,2,4-triol 6h

To a stirred solution of the 6-hexyloxy silyl ether **17h** (802 mg, 1.46 mmol) in DCM (30 cm^3) was added, dropwise, TBAF (1.6 cm^3, 1.60 mmol). After 5 h, the resulting solution was diluted with DCM (30 cm^3) and water (40 cm^3). The aqueous layer was then back extracted with DCM (3 × 40 cm^3). The organic layers were pooled and washed with brine (50 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting oil (alcohol) **18h** was dissolved in MeOH (75 cm^3) and TFA (0.15 cm^3) added. The solution was stirred for 6 days and then the solvent removed under reduced pressure to give a dirty white solid which was recrystallised from petroleum ether–ethyl acetate (5 : 1) to give the 6-hexyloxy triol **6h** as a white solid (163 mg, 63%), mp 84–85 °C; (Found: H, 10.5; C, 72.1. $C_{12}H_{24}O_4$ requires H, 10.4; C, 72.05%); (HRMS: found: $[M + Na]^+$, 255.1517. $C_{12}H_{24}O_4Na$ requires 255.1572); $[a]_D$ −27.8 (*c* 0.14 in $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3396 br, 2921, 2867–2921, 1456, 1374, 1070, 1170 and 1018; δ_H(300 MHz; C^2HCl_3) 0.94 (3 H, m, $^3J_{H\text{-}H}$ 6.4, *n*-hexyl), 1.21–1.43 (6 H, m, *n*-hexyl) 1.48–1.72 (4 H, m, 2 of *n*-hexyl, 5-H and OH), 2.01 (1 H, br, OH), 2.10–2.23 (1 H, m, 3-H), 2.25–2.40 (2 H, m, 3-H and 5-H), 2.71 (1 H, br, OH), 3.32–3.39 (1 H, m, 1 of *n*-hexyl), 3.55–3.69 (3 H, m, 1 of *n*-hexyl, 1-H and 6-H) and 4.00–4.15 (1 H, m, 4-H) and 4.16–4.20 (1 H, m, 2-H); δ_C(75.4 MHz; C^2HCl_3) 12.48, 21.05, 24.27, 28.46 and 30.09 (*n*-hexyl), 34.32 and 36.68 (3-C and 5-C), 62.55 (*n*-hexyl), 63.95 (4-C), 65.87 (6-C), 72.18 (2-C) and 73.38 (1-C); *m/z* (FAB^+) 233.2 (100%, $[M + H]^+$) and 197.2 (15, $[M - C_3H_8 - OH + Na]^+$).

2-(3-Butenyloxy)-phenol 14[30]

To a stirred mixture of catechol **13** (6.00 g, 54.5 mmol) and potassium carbonate (7.53 g, 54.5 mmol) in acetone (150 cm^3) was added 4-bromo-1-butene (1.85 cm^3, 18.2 mmol) and potassium iodide (50 mg). This mixture was stirred and heated under reflux for 2 days. After cooling the solvent was carefully removed under reduced pressure and the residue was partitioned between diethyl ether (200 cm^3) and 1 M HCl (200 cm^3). The separated organic layer was washed with water (200 cm^3) and brine (200 cm^3) and then dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow solid that was purified by flash silica column chromatography (petroleum ether–ethyl acetate; 4 : 1) to give alcohol **14** as a colourless oil (1.09 g, 37%); bp 96–98 °C/4 mmHg; ν_{max}(thin film)/cm^{-1} 3528 br, 3081, 2934, 1643 and 1597; δ_H(300 MHz, C^2HCl_3) 2.54–2.61 (2 H, m, $CH_2CH_2CH{=}CH_2$), 4.11 (2 H, t, J_{HH} 7, OCH_2), 5.13–5.24 (2 H, m, $CH_2{=}CH$), 5.66 (1 H, s, ArCO*H*), 5.83–5.96 (1 H, m, $CH_2{=}CH$), 6.83–6.96 (4 H, m, Ar-CH); δ_C(75 MHz; C^2HCl_3) 34.50 (OCH_2CH_2), 68.93 (OCH_2), 112.98, 115.46, 120.93, and 122.54 (Ar-CH), 118.36 ($CH{=}CH_2$), 135.01 ($CH_2{=}CH$), 146.84 and 146.86 (Ar-C quaternary); *m/z* (EI^+) 164 (34%, M^+), 110 (73, $[C_6H_4(OH)_2]^+$) and 55 (100%, $CH_2CH_2CH{=}CH_2^+$).

2-(4-Hydroxybutyloxy)-phenol 15

To a stirred solution of alcohol **14** (227 mg, 1.38 mmol) in dry THF (10 cm^3), under argon, was added borane–THF complex (1 M solution in THF, 0.914 cm^3) and the mixture stirred for 1 h. Sodium hydroxide solution (1 M, 2.74 cm^3) was then added and when effervescence had ceased hydrogen peroxide solution (1 M, 2.74 cm^3) was added and the solution stirred for 3 h. 1 M HCl (10 cm^3) was then added and the separated aqueous layer was extracted with diethyl ether (2 × 20 cm^3). The pooled ethereal extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to a yellow oil that was purified by flash silica column chromatography (hexane–ethyl acetate; 1 : 1) to give diol **15** as a white crystalline compound (169 mg, 67%); mp 82–84 °C (HRMS: found: $[M + Na]^+$, 205.0387, $C_{10}H_{14}O_3Na$ requires 205.0841); ν_{max}(KBr disc)/cm^{-1} 3461, 3066, 2963, 2865 and 1597; δ_H(300 MHz; C^2HCl_3) 1.63 (1 H, br s, CH_2OH), 1.73–1.82 and 1.90–1.99 (4 H, m, $OCH_2CH_2CH_2$), 3.76 (2 H, t, J_{HH} 6, OCH_2), 4.10 (2 H, t, J_{HH} 6, OCH_2), 5.91 (1 H, br s, ArCO*H*) and 6.80–6.96 (Ar-H); δ_C(75 MHz; $C^2H_3O^2H$) 28.75 and 32.02 ($OCH_2CH_2CH_2$), 64.53 and 71.69 [$OCH_2(CH_2)_2$-CH_2OH], 116.06, 118.26, 122.74 and 124.11 (Ar-CH) and 149.71 and 150.13 (Ar-C quaternary); *m/z* (EI^+) 182 (12%, M^+) and 110 (100, $[C_6H_4(OH)_2]^+$).

(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-[4-(2-hydroxyphenyloxy)-butyloxy]cyclohexanol 9i

To a stirred solution of diol **15** (134 mg, 0.738 mmol) in 1,2-dichloroethane (5 cm^3) was added the epoxide (+)-**4** (76 mg, 0.246 mmol) as a solution in 1,2-dichloroethane (5 cm^3). Yb(III)$(OTf)_3$ (31 mg, 0.049 μmol) was then added and the solution was heated under reflux for 1 h. After cooling the solvent was removed under reduced pressure and the residue was dissolved in diethyl ether (20 cm^3) and washed with water (2 × 20 cm^3) and brine (20 cm^3). The ethereal solution was dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a brown oil that was purified by flash silica column chromatography (petroleum ether–ethyl acetate; 1 : 1) to give the alcohol **9i** as a colourless oil (87 mg, 72%); (HRMS: found: $[M + Na]^+$, 515.2401, $C_{30}H_{36}O_6Na$ requires 515.2410); $[\alpha]_D$ −7.66 (*c* 1.11 in MeOH); ν_{max}(thin film)/cm^{-1} 3538 br, 3425 br, 3086, 3060, 3024, 2943, 2880, 1740, 1602 and 1509; δ_H(300 MHz; C^2HCl_3) 1.27–1.44 (2 H, m, 3-H and 5-H), 1.75–1.96 [4 H, m, $OCH_2(CH_2)_2$], 2.42–2.50 (2 H, m, 3-H and 5-H), 3.52–3.74 (5 H, 1-H, 2-H, 6-H and OCH_2CH_2), 3.95–3.96 (1 H, m, 4-H), 4.08 (2 H. t. J_{HH} 6, OCH_2CH_2), 4.51 (2 H, ABq, J_{HH} 12, $ArCH_2$), 4.60 (2 H, s, $ArCH_2$), 5.91 (1 H, br s, ArCO*H*) and 6.62–6.95 and 7.26–7.39 (14 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 25.27 and 25.87 [$OCH_2(CH_2)_2$], 33.06 and 34.51 (3-C and 5-C), 67.88 and 68.09 [$OCH_2(CH_2)_2CH_2O$], 69.70 and 71.10 ($PhCH_2O$), 70.69, 74.58, 75.41 and 76.18 (1-C, 2-C, 4-C and 6-C), 111.08, 113.85, 119.10, 120.56, 126.70, 126.79 and 127.50 (Ar-CH), 138.50, 139.00 and 143.40 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 515 (100%, $[M + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-(*N*-benzyloxy-carbonyl-2-aminoethyl)cyclohexanol 9j

To a stirred solution of epoxide (+)-**4** (261 mg, 0.842 mmol) in 1,2-dichloroethane (30 cm^3) was added Yb(III)$(OTf)_3$ (25 mg, 0.32 mmol) followed by *N*-benzyloxycarbonyl-ethanolamine[31] (378 mg, 1.94 mmol) and the resulting mixture heated under reflux for 20 h. The reaction mixture was concentrated under reduced pressure and then dissolved in 1 M HCl solution (20 cm^3). To this solution was added, dropwise, a 4 M sodium hydroxide solution until the pH was 14. The solution was then extracted with ethyl acetate (3 × 50 cm^3) and the pooled organic fractions were washed with brine (50 cm^3), dried ($MgSO_4$), filtered and then concentrated under reduced pressure to give a light pale yellow oil which was purified by flash silica column chromatography (ethyl acetate–MeOH; 9 : 1 with addition of 0.5 cm^3 of TEA per 10 cm^3 of solvent) to give alcohol **9j** as a colourless oil (302 mg, 71%); (Found: H, 7.0; C, 71.3; N, 2.8. $C_{30}H_{35}O_6$ requires H, 6.7; C, 71.25; N, 2.75%); (HRMS: found: $[M + Na]^+$, 528.2359. $C_{30}H_{35}O_6Na$ requires 528.2362); $[\alpha]_D$ −20.7 (*c* 0.12 in $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3410 br, 3086, 3056, 3030, 2932, 2861, 1711, 1562, 1454, 1362, 1258, 1217, 1093, 1028, 752 and 697; δ_H(300 MHz; C^2HCl_3) 1.22–1.46 (2 H, m, 3-H and 5-H), 2.29–2.44 (2 H, m, 3-H and 5-H), 2.56 (1 H, br, OH), 3.32–3.41 (2 H, m, OCH_2CH_2NH), 3.44–3.63 (3 H, m, 1-H, 2-H and 6-H), 3.72–3.79 (2 H, m, OCH_2CH_2NH), 3.91–3.97 (1 H, m, 2-H), 4.47 (1 H, A of first AB system, $^2J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.51–4.55 (2 H, B of first AB system and A of second AB system, 2 × 1 of OCH_2Ph), 4.57 (1 H, B of second AB system, $^2J_{H\text{-}H}$ 12.0, 1 of OCH_2Ph), 5.08 (2 H, s, CO-OCH_2Ph), 5.46 (1 H, br, NH) and 7.24–7.41 (15 H, m, Ar-H); δ_C(75.5 MHz; C^2HCl_3) 32.71 and 34.46 (3-C and 5-C), 40.56 (OCH_2CH_2NH), 65.90 (OCH_2CH_2NH), 67.82 (CO-OCH_2Ph), 69.88 (OCH_2Ph), 70.65 (4-C), 71.03 (OCH_2Ph), 74.31 (6-C), 75.54 (2-C), 76.88 (1-C), 126.87, 127.00, 127.09, 127.28, 127.69 and 127.73 (Ar-CH), 135.97, 137.11 and 137.48 (Ar-C quaternary) and 156.16 (CO); *m*/*z* (ES^+ TOF) 528.2 (100%, $[M + Na]^+$) and 327.3 (15, $[M - C_{10}H_{12}NO_2]^+$).

(*N*-Benzyloxycarbonyl)-3-aminopropanol

To a stirred solution of 3-aminopropanol (7.65 cm^3, 100 mol) in 1,4-dioxane–water (1 : 1, v/v, 200 cm^3) was added DMAP (50 mg, 4 mmol), benzyl chloroformate (16 cm^3, 110 mmol) and TEA (13.9 cm^3, 100 mmol) and the reaction mixture stirred for 12 h. The solvents were removed under reduced pressure to give a thick solution which was diluted with DCM (150 cm^3) and water (50 cm^3). The aqueous layer was then back-extracted with DCM (3 × 50 cm^3). The pooled organic fractions were then successively washed with 0.1 M solution of HCl (100 cm^3), water (100 cm^3), and brine (100 cm^3), dried ($MgSO_4$) and filtered. Removal of the solvents under reduced pressure gave a white solid which was recrystallised from petroleum ether–ethyl acetate (3 : 1) to give the Cbz protected 3-aminopropanol as a white solid (19.2 g, 92%); mp 51–52 °C (lit.,[32] 51–52 °C); (Found: H, 7.3; C, 63.2; N, 6.7. Calc. for $C_{11}H_{15}O_3$: H, 7.25; C, 63.15; N, 6.7%); (HRMS: found: $[M + Na]^+$, 232.0956. Calc. for $C_{11}H_{15}O_3Na$: 232.0950); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3325 br, 3057, 3043, 3029, 2955, 2873, 1682, 1536, 1453, 1327, 1265, 1143, 1024, 750, 725 and 697; δ_H(300 MHz; C^2HCl_3) 1.69 (2 H, qt, $^3J_{H\text{-}H}$ 5.9, $NCH_2CH_2CH_2OH$), 2.62 (1 H, br, OH), 3.35 (2 H, q, $^3J_{H\text{-}H}$ 6.3, $NCH_2CH_2CH_2OH$), 3.66 (2 H, t, $^3J_{H\text{-}H}$ 6.3, $NCH_2CH_2CH_2OH$), 5.09 (3 H, s, OCH_2Ph and NH) and 7.25–7.36 (5 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 31.82 ($NCH_2CH_2CH_2OH$), 37.01 ($NCH_2CH_2CH_2OH$), 58.85 ($NCH_2CH_2CH_2OH$), 66.15 (CO-OCH_2Ph), 127.38, 127.45 and 127.82 (Ar-CH), 135.72 (Ar-C quaternary) and 156.12 (CO); *m*/*z* (ES^+ TOF) 232.1 (100%, $[M + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-(*N*-benzyloxy-carbonyl-3-aminopropyl)cyclohexanol 9k

This compound was prepared in a manner identical with that described for (2-aminoethyl) alcohol **9j** using (*N*-benzyloxy-carbonyl)-3-aminopropanol (284 mg, 1.36 mmol) to give alcohol **9k** as a colourless oil (279 mg, 52%); (Found: H, 7.2; C, 71.7; N, 2.7. $C_{31}H_{37}NO_6$ requires H, 7.2; C, 71.65; N, 2.7%); (HRMS: found: $[M + Na]^+$, 542.2533. $C_{31}H_{37}NO_6Na$ requires 542.2519); $[\alpha]_D$ −22.1 (*c* 0.12 in $CHCl_3$); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3410 br, 3086, 3053, 3030, 2932, 2861, 1711, 1562, 1454, 1362, 1258, 1217, 1093, 1028, 752 and 697; δ_H(300 MHz; C^2HCl_3) 1.25–1.42 (2 H, m, 3-H and 5-H), 1.74 (2 H, t, $^3J_{H\text{-}H}$ 5.7, $OCH_2CH_2CH_2NH$), 2.31–2.43 (2 H, m, 3-H and 5-H), 2.73 (1 H, d, $^3J_{H\text{-}H}$ 5.9, OH), 3.24–3.55 (5 H, m, $OCH_2CH_2CH_2NH$ and 1-H), 3.67–3.75 (2 H, m, 4-H and 6-H), 3.90–3.96 (1 H, m, 2-H), 4.45 (1 H, A of first AB system, $^2J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.51 (1 H, B of first AB system, $^2J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.53 (1 H, A of second AB system, $^2J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph), 4.57 (1 H, B of second AB system, $^2J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph), 5.07 (2 H, s, CO-OCH_2Ph), 5.47 (1 H, br, NH) and 7.25–7.33 (15 H, m, Ar-H); δ_C(75.5 MHz; C^2HCl_3) 31.98 and 35.73 (3-C and 5-C), 37.23 ($OCH_2CH_2CH_2NH$), 41.03 ($OCH_2CH_2CH_2NH$), 68.58 ($OCH_2CH_2CH_2NH$), 69.71 (CO-OCH_2Ph), 72.73 (OCH_2Ph), 73.64 (4-C), 73.98 (OCH_2Ph), 77.25, 78.49 and 79.40 (1-C, 2-C and 6-C), 129.75, 129.86, 130.07 and 130.56 (Ar-CH), 137.16, 139.16 and 139.91 (Ar-C quaternary) and 157.15 (CO); *m*/*z* (ES^+ TOF) 542.3 (100%, $[M + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-[4-(2-Hydroxyphenyloxy)butyloxy]cyclohexane-1,2,4-triol 6i

To a stirred solution of alcohol **9i** (372 mg, 0.755 mmol) in MeOH (20 cm^3) was added 5% palladium on activated charcoal (318 mg) and a drop of acetic acid. The mixture was stirred vigorously under an atmosphere of hydrogen gas for 16 h and then filtered through a pad of Celite. The pad was further washed with MeOH (10 cm^3) and water (20 cm^3), and the pooled filtrate concentrated under reduced pressure. The residue was redissolved in a small amount of water (~5 cm^3) and lyophilised to give the 6-[4-(2-hydroxyphenyloxy)butyloxy] triol

6i as a white powder (222 mg, 84%); mp 92–97 °C (decomp.); (HRMS: found: $[M + Na]^+$ 335.1468, $C_{16}H_{24}O_6Na$ requires 335.1471); $[\alpha]_D$ −48.5 (*c* 0.6 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3412 br, 2937, 2867, 1614, 1599 and 1498; δ_H(300 MHz; ^2H_2O) 1.12–1.20 and 1.40–1.55 (2-H, m, 3-H and 5-H), 1.65–1.85 (4 H, m, $OCH_2(CH_2)_2CH_2O$), 2.00–2.12 (1 H, m, 5-H), 2.30–2.40 (1 H, m, 3-H), 3.40–4.12 (8-H, m, $OCH_2(CH_2)_2CH_2O$, 1-H, 2-H, 4-H and 6-H), 6.80–7.05 (4-H, m, Ar-H); δ_C(75 MHz; ^2H_2O) 23.19, 23.80, 35.66 and 36.39 (3-C, 5-C and OCH_2CH_2-CH_2CH_2O), 61.96, 66.87, 72.14 and 74.08 (1-C, 2-C, 4-C and 6-C), 66.80 and 67.29 ($OCH_2CH_2CH_2CH_2O$), 111.91, 113.64, 118.84 and 119.65 (Ar-CH) and 143.21 and 144.48 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 357 (9%, $[M + 2Na - H]^+$) and 335 (100, $M + Na]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-(2-Aminoethyloxy)cyclohexane-1,2,4-triol 6l

To a stirred solution of alcohol **7j** (172 mg, 0.340 mmol) in ethanol (20 cm^3) was added 10% palladium on activated charcoal (180 mg) and hydrogen gas was bubbled through the mixture for 5 min followed by vigorous stirring for 18 h under a constant hydrogen pressure. The reaction mixture was then filtered through Celite and the resulting solution concentrated under reduced pressure to give the 6-(2-aminoethyloxy) triol **6l** as a colourless oil (63 mg, 97%); (Found: H, 9.0; C, 50.3; N, 7.35. $C_8H_{17}NO_4$ requires H, 8.95; C, 50.25; N, 7.3%); (HRMS: found: $[M + H]^+$, 192.1227. $C_8H_{18}NO_4$ requires 192.1236); $[\alpha]_D$ −17.3 (*c* 0.10 in MeOH); ν_{max}(thin film)/cm^{-1} 3382 br, 2931, 2859, 1457, 1343, 1215, 1074 and 1022; δ_H(300 MHz; $C^2H_3O^2H$) 1.19–1.35 (1 H, m, 5-H), 1.39–1.48 (1 H, m, 3-H), 2.04–2.13 (1 H, m, 3-H), 2.28–2.37 (1 H, m 5-H), 3.05–3.23 (2 H, m, OCH_2-CH_2NH), 3.47 (1 H, dd, $^3J_{H\text{-}H}$ 9.1, 2.8, 1-H), 3.53–3.61 (1 H, m, 6-H), 3.64–3.71 (1 H, m, 4-H), 3.85–3.99 (2 H, m, OCH_2-CH_2NH) and 4.01–4.07 (1 H, m, 2-H); δ_C(75.5 MHz; $C^2H_3O^2H$) 28.59 and 39.91 (3-C and 5-C), 40.30 (OCH_2CH_2NH), 64.43 (4-C), 65.37 (OCH_2CH_2NH), 69.42 (6-C), 75.41 (C-2) and 77.45 (1-C); *m*/*z* (ES^+ TOF) 192.1 (100%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-(3-Aminopropyloxy)cyclohexane-1,2,4-triol 6m

This compound was prepared in a manner identical with that described for the 6-(2-aminoethyloxy) triol **6l** using alcohol **7k** (173 mg, 0.333 mmol) to give 6-(3-aminopropyloxy) triol **6m** as a colourless oil (65 mg, 95%); (Found: H, 9.35; C, 52.7; N, 6.85. $C_9H_{19}NO_4$ requires H, 9.35; C, 52.65; N, 6.8%); (HRMS: found: $[M + H]^+$, 206.1388. $C_9H_{20}NO$ requires 206.1392); $[\alpha]_D$ −16.5 (*c* 0.11 in MeOH); ν_{max}(thin film)/cm^{-1} 3396 br, 2944, 2854, 1454, 1343, 1216, 1072 and 1022; δ_H(300 MHz; $C^2H_3O^2H$) 1.13–1.48 (2 H, m, 3-H and 5-H), 1.54–1.82 (2 H, m, OCH_2CH_2-CH_2NH_2), 2.03–2.15 (1 H, m, 3-H), 2.26–2.35 (1 H, m 5-H), 2.65–3.04 (2 H, m, $OCH_2CH_2CH_2NH_2$), 3.26–3.73 (3 H, m, $OCH_2CH_2CH_2NH_2$ and 6-H), 3.78–3.84 (1 H, m, 1-H) and 3.89–4.04 (2 H, m, 2-H and 4-H); δ_C(75.5 MHz; $C^2H_3O^2H$) 31.12 ($OCH_2CH_2CH_2NH_2$), 39.04 and 40.04 (3-C and 5-C), 40.42 ($OCH_2CH_2CH_2NH_2$), 65.02 (4-C), 68.92 (OCH_2CH_2-CH_2NH_2), 70.05 (6-C), 76.07 (C-2) and 77.80 (1-C); *m*/*z* (ES^+ TOF) 206.1 (100%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-hexylaminocyclohexanol 19e

To a solution of epoxide (+)-**4** (260 mg, 0.839 mmol) in toluene–THF mixture (3 : 1, 3 cm^3) was added Yb(III)(OTf)$_3$ (25 mg, 0.040 mmol) followed by hexylamine (111 mm^3, 0.84 mmol) and the resulting mixture was heated under reflux for 18 h. The solvents were removed under reduced pressure and the residue dissolved in a 1 M hydrochloric acid solution (20 cm^3) and extracted with diethyl ether (2 × 30 cm^3). To the resulting aqueous layer was added, dropwise, a 4 M sodium hydroxide solution until the pH was 14. The solution was then extracted with ethyl acetate (3 × 50 cm^3) and the pooled organic fractions were washed with brine (50 cm^3), dried ($MgSO_4$), filtered and then concentrated under reduced pressure to give a light pale yellow oil which was purified by flash silica column chromatography (ethyl acetate–MeOH; 9 : 1, with addition of 0.5 cm^3 of TEA per 10 cm^3 of solvent mixture) to give the 6-hexylamino alcohol **19e** as a colourless oil (310 mg, 90%); (Found: H, 9.1; C, 75.95; N, 3.4. $C_{26}H_{37}NO_3$ requires H, 9.05; C, 75.85; N, 3.4%); (HRMS: found: $[M + H]^+$, 412.2855. $C_{26}H_{38}NO_3$ requires 412.2852); $[\alpha]_D$ −26.1 (*c* 0.13 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3380 br, 3091, 3060, 3020, 2957, 2828, 1493, 1359, 1096, 1027, 734 and 665; δ_H(300 MHz; C^2HCl_3) 0.83 [3 H, t, $^3J_{H\text{-}H}$ 6.8, $CH_2(CH_2)_4CH_3$], 1.09–1.73 [11 H, m, $CH_2(CH_2)_4$-CH_3, 3-H, 5-H and NH], 2.35–2.50 (2 H, m, 3-H and 5-H), 2.60–2.75 [2 H, m, $CH_2(CH_2)_4CH_3$], 2.68–2.79 (3 H, m, 1 of 1-H, 6-H and OH), 3.72–3.78 (1 H, m, 4-H), 3.90–3.94 (1 H, m, 2-H), 4.46–3.69 (4 H, m, 2 × OCH_2Ph) and 7.19–7.32 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 14.26 [$CH_2(CH_2)_4CH_3$], 22.81, 26.71, 27.24 and 31.96 [$CH_2(CH_2)_4CH_3$], 34.09 and 35.71 (3-C and 5-C), 42.12 [$CH_2(CH_2)_4CH_3$], 56.54 (6-C), 70.78 and 71.97 (2 × OCH_2Ph), 72.48 (4-C), 74.86 (2-C), 76.55 (1-C), 127.80 and 128.60 (Ar-CH) and 138.68 and 138.88 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 412.3 (100%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-(2-phenylethyl)-aminocyclohexanol 19f

To a solution of epoxide (+)-**4** (222 mg, 0.536 mmol) in a mixture of 2-phenylethylamine and 1,2-dichloroethane (40 cm^3, 1 : 1, v/v) was added Yb(III)(OTf)$_3$ (111 mg, 0.179 mmol) and the resulting mixture was heated under reflux for 20 h. The reaction mixture was concentrated under reduced pressure and then dissolved in 1 M HCl (20 cm^3). The solution was washed with diethyl ether (2 × 30 cm^3) and the separated aqueous layer was adjusted to pH 14 by dropwise addition of 4 M NaOH solution. The solution was then extracted with ethyl acetate (3 × 50 cm^3) and the pooled organic fractions were washed with brine (50 cm^3), dried ($MgSO_4$), filtered and then concentrated under reduced pressure to give a light yellow oil. Traces of 2-phenylethylamine were removed by Kugelrohr distillation to give the 6-(2-phenylethyl)amino alcohol **19f** as a pale yellow oil (231 mg, 75%); (Found: H, 7.75; C, 78.0; N, 3.25. $C_{28}H_{33}NO_3$ requires H, 7.7; C, 77.95; N, 3.25%); (HRMS: found: $[M + H]^+$, 432.2537. $C_{28}H_{34}NO_3$ requires 432.2539); $[\alpha]_D$ −25.7 (*c* 0.12 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3303–3602 br, 3085, 3055, 2933, 2807, 1500, 1359, 1135, 1032, 749 and 699; δ_H(300 MHz; C^2HCl_3) 1.09–1.45 (3 H, m, NH and 3-H and 5-H), 2.31–2.49 (3 H, m, 1 of N-CH_2CH_2Ph, 3-H and 5-H), 2.74–2.87 (4 H, m, 1 of N-CH_2CH_2Ph, OH and 2 × N-CH_2CH_2Ph), 3.03–3.09 (1 H, m, 6-H), 3.36–3.41 (1 H, dd, $^3J_{H\text{-}H}$ 9.1 and 3.0, 1-H), 3.67–3.78 (1 H, m, 4-H), 3.89–3.93 (1 H, m, 2-H), 4.43 (1 H, A of an AB system, $^3J_{H\text{-}H}$ 11.5, 1 of OCH_2Ph), 4.52 (43 (1 H, B of an AB system, $^3J_{H\text{-}H}$ 11.5, 1 of OCH_2Ph), 4.55 (1 H, m, A of an AB system, $^3J_{H\text{-}H}$ 12.2, 1 of OCH_2Ph), 4.64 (1 H, B of an AB system, $^3J_{H\text{-}H}$ 12.2, 1 of OCH_2Ph), and 7.19–7.38 (15 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.48 and 30.90 (3-C and 5-C), 48.11 (N-CH_2CH_2Ph), 56.17 (6-C), 60.52 (N-CH_2CH_2Ph), 70.53 and 71.75 (2 × OCH_2Ph), 72.15 (4-C), 74.54 (2-C), 76.25 (1-C), 126.63, 127.65 and 128.87 (Ar-CH) and 138.41, 138.59 and 139.87 (Ar-C quaternary); *m*/*z* (FAB) 432 (38%, $[M + H]^+$), 416 (44, $[M - OH + 2H]^+$), 295 (65, $[M - OH - PhCH_2CH_2NH + 2H]^+$), 181 (100, $[2 \times C_7H_7 - H]^+$) and 105 (25, $[PhCH_2CH_2]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-aminocyclohexanol 19n

To a stirred mixture of epoxide (+)-**4** (500 mg, 1.6 mmol) and ammonia solution (50 cm^3), containing a few drops of ethanol to aid solvation, was added Yb(III)(OTf)$_3$ (300 mg, 0.48 mmol). The flask was sealed and heated to 70 °C for 12 h. After cooling,

the flask was opened and the reaction mixture was extracted with ethyl acetate (2 × 50 cm^3). The pooled organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to an off-white solid which was purified by flash silica column chromatography (petroleum ether–ethyl acetate; 10 : 1) to give the 6-amino alcohol **19n** as white crystals (459 mg, 87%); mp 81–83 °C; (HRMS: found: $[M + H]^+$, 328.1906. $C_{20}H_{26}NO_3$ requires 328.1913); $[\alpha]_D$ −10.6 (*c* 0.67 in EtOAc); ν_{max}(KBr disc)/cm^{-1} 3853, 3748, 3304 br, 2929, 2867, 2362, 1648, 1543, 1459, 1364, 1106 and 695; δ_H(300 MHz; C^2HCl_3) 1.13–1.24 and 1.30–1.40 (2 × 1 H, 2 × m, 3-H and 5-H), 1.75–1.85 (3 H, br s, OH and NH_2), 2.17–2.25 and 2.29–2.37 (2 × 1 H, 2 × m, 3-H and 5-H), 2.81–2.90 (1 H, m, 6-H), 3.14 (1 H, dd, J_{HH} 3 and 9, 1-H), 3.62–3.72 (1 H, m, 4-H), 3.82–3.85 (1 H, m, 2-H), 4.41 (2 H, Abq, J_{HH} 12, OCH_2Ph), 4.52 (2 H, ABq, J_{HH} 11.5, OCH_2-Ph) and 7.19–7.31 (10 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.61 and 38.26 (3-C and 5-C), 49.82 (6-C), 70.36 and 71.32 (2 × O*C*H_2Ph), 71.85, 76.57 and 76.94 (1-C, 2-C and 4-C), 127.32, 127.36, 127.52, 128.32 and 128.38 (Ar-CH) and 138.28 and 138.58 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 350 (100%, $[M + Na]^+$) and 328 (85%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-ethylaminocyclohexanol 19o

To a stirred mixture of epoxide (+)-**4** (520 mg, 1.68 mmol) and ethylamine (70% solution in water, 70 cm^3) was added Yb(III)-$(OTf)_3$ (200 mg, 0.33 mmol). The flask was sealed and heated to 65 °C for 48 h. After cooling, the flask was opened and the excess ethylamine gas was allowed to escape by bubbling with nitrogen gas for several hours. The aqueous layer was then extracted with ethyl acetate (3 × 50 cm^3). The pooled organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the ethylamino alcohol **19o** as an oil (620 mg, 95%); (HRMS: found: M^+, 355.2129. $C_{22}H_{29}NO_3$ requires 355.2147); $[\alpha]_D$ −23.6 (*c* 0.19 in EtOAc); ν_{max}(thin film)/cm^{-1} 3302 br, 3088, 3063, 3030, 2903, 2867, 1737 and 1657; δ_H(300 MHz; C^2HCl_3) 1.05 (3 H, t, J_{HH} 7, NHCH$_2$CH_3), 1.07–1.38 (2 H, m, 3-H and 5-H), 1.97–2.53 (5 H, m, 6-H, 3-H, 5-H, OH and NH), 2.68–2.77 (2 H, m, NHCH_2CH$_3$), 3.31 (1 H, dd, J_{HH} 3.5 and 10, 1-H), 3.67 (1 H, tt, J_{HH} 4 and 11, 4-H), 3.84–3.87 (1 H, m, 2-H), 4.44 (2 H, Abq, J_{HH} 12, OCH_2Ph), 4.51 (2 H, ABq, J_{HH} 11.5, OCH_2Ph) and 7.19–7.31 (10 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 15.51 (NHCH$_2$*C*H$_3$), 33.86 and 35.33 (3-C and 5-C), 41.12 (NH*C*H$_2$CH$_3$), 56.28 (6-C), 70.60 and 71.78 (2 × O*C*H$_2$Ph), 72.19, 74.53 and 76.44 (1-C, 2-C and 4-C), 127.64, 127.74, 127.94, 128.46 and 128.91 (Ar-CH) and 138.44 and 138.65 (Ar-C quaternary); *m*/*z* (EI^+) 356 (3%, $[M + H]^+$), 264 (65, $[M - C_7H_7]^+$), 248 (10, $[M - C_7H_7O]^+$) and 91 (100, $C_7H_7^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-butylaminocyclohexanol 19p

To a solution of epoxide (+)-**4** (300 mg, 0.968 mmol) in 1,2-dichloroethane was added Yb(III)$(OTf)_3$ (25 mg, 0.040 mmol) followed by butylamine (3 cm^3, 50 mmol) and the resulting mixture was heated under reflux for 20 h. The reaction mixture was concentrated under reduced pressure and then dissolved in a 1 M hydrochloric acid solution (20 cm^3) and extracted with diethyl ether (2 × 30 cm^3). To the resulting aqueous layer was added, dropwise, a 4 M sodium hydroxide solution until the pH was 14. The solution was then extracted with ethyl acetate (3 × 50 cm^3) and the pooled organic fractions were washed with brine (50 cm^3), dried ($MgSO_4$), filtered and then concentrated under reduced pressure to give a light yellow oil. Traces of butylamine were removed by Kugelrohr distillation to give the 6-butylamino alcohol **19p** as a yellow oil (266 mg, 72%); (Found: H, 8.7; C, 75.2; N, 3.65. $C_{24}H_{33}NO_3$ requires H, 8.65; C, 75.15; N, 3.65%); (HRMS: found: $[M + H]^+$, 384.2541. $C_{24}H_{34}NO_3$ requires 384.2539); $[\alpha]_D$ −34.7 (*c* 0.14 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3450 br, 3093, 3061, 3028, 2952, 2802, 1506, 1454, 1361, 1136, 1029, 754 and 697; δ_H(300 MHz; C^2HCl_3) 0.91 (3 H, m, $^3J_{H\text{-}H}$ 7.2, NCH$_2$CH$_2$CH$_2$CH_3), 1.14–1.28 (1 H, m, 5-H), 1.30–1.52 (6 H, m, NCH$_2$CH_2CH_2CH$_3$, 3-H and NH), 2.34–2.55 (4 H, m, 3-H, 5-H, OH and 1 of NCH_2CH$_2$CH$_2$CH$_3$), 2.72–2.74 (2 H, m, 1 of NCH_2CH$_2$CH$_2$CH$_3$ and 6-H), 3.39 (1 H, dd, $^3J_{H\text{-}H}$ 9,6 and 3.1, 1-H), 3.70–3.77 (1 H, m, 4-H), 3.90–3.95 (1 H, m, 2-H), 4.46 (1 H, A of an AB system, $^3J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.53 (1 H, A of an AB system, $^3J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.54 (1 H, m, B of an AB system, $^3J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph), 4.61 (1 H, B of an AB system, $^3J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph) and 7.25–7.33 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 13.85 (NCH$_2$CH$_2$CH$_2$*C*H$_3$), 20.30 and 31.54 (NCH$_2$-*C*H$_2$*C*H$_2$CH$_3$), 33.71 and 34.54 (3-C and 5-C), 46.29 (N*C*H$_2$-CH$_2$CH$_2$CH$_3$), 56.54 (6-C), 70.66 and 71.83 (2 × O*C*H$_2$Ph), 72.04 (4-C), 73.89 (2-C), 76.11 (1-C), 127.65, 127.78 and 128.46 (Ar-CH) and 138.62 and 138.89 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 406.2 (5%, $[M + Na]^+$) and 384.2 (100, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-octylaminocyclohexanol 19q

This compound was prepared and purified in a manner identical with that described for the 6-butylamino alcohol **19p** using (+)-**4** (257 mg, 0.831 mmol) and *n*-octylamine (138 mm^3, 0.83 mmol) to give the 6-octylamino alcohol **19q** as a colourless oil (310 mg, 85%); (Found: H, 9.45; C, 76.6; N, 3.2. $C_{28}H_{41}NO_3$ requires H, 9.4; C, 76.5; N, 3.2%); (HRMS: found: $[M + H]^+$, 440.3170. $C_{28}H_{42}NO_3$ requires 440.3165); $[\alpha]_D$ −31.7 (*c* 0.12 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3398 br, 3089, 3060, 3030, 2926, 2854, 1479, 1357, 1095 br, 1026, 738, 696 and 664; δ_H(300 MHz; C^2HCl_3) 0.84 (3 H, m, $^3J_{H\text{-}H}$ 6.8, *n*-octyl), 1.06–1.45 (15 H, m, 12 of *n*-octyl, 3-H, 5-H, and NH), 1.58 (1 H, s, OH), 2.31–2.46 [3 H, m, 1 of N-CH_2(CH$_2$)$_6$CH$_3$, 3-H and 5-H], 2.65–2.75 [2 H, m, 1 of N-CH_2(CH$_2$)$_6$CH$_3$ and 6-H], 3.31 (1 H, dd, $^3J_{H\text{-}H}$ 9,6 and 3.1, 1-H), 3.67–3.71 (1 H, m, 4-H), 3.85–3.89 (1 H, m, 2-H), 4.43 (1 H, A of first AB system, $^3J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.49 (1 H, B of first AB system, $^3J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.50 (1 H, m, A of second AB system, $^3J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph), 4.57 (1 H, B of second AB system, $^3J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph) and 7.24–7.33 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 14.16, 22.70, 27.42, 29.31, 29.55, 30.55 and 31.88 [N-CH$_2$(*C*H$_2$)$_6$*C*H$_3$], 33.90 and 35.55 (3-C and 5-C), 46.97 [N-*C*H$_2$(CH$_2$)$_6$CH$_3$], 56.37 (6-C), 70.60 and 71.78 (2 × O*C*H$_2$Ph), 72.29 (4-C), 74.70 (2-C), 76.38 (1-C), 127.65, 127.72 and 128.46 (Ar-CH) and 138.50 and 138.69 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 440.2 (100%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-(4-phenylbutyl)-aminocyclohexanol 19r

This compound was prepared and purified in a manner identical with that described for the 6-butylamino alcohol **19p** using (+)-**4** (260 mg, 0.838 mmol) and 4-phenylbutylamine (130 mm^3, 0.84 mmol) to give the 6-(4-phenylbutyl)amino alcohol **19r** as a colourless oil (369 mg, 96%); (Found: H, 8.15; C, 78.5; N, 3.05. $C_{30}H_{37}NO_3$ requires H, 8.1; C, 78.4; N, 3.05%); (HRMS: found: $[M + H]^+$, 460.2855. $C_{30}H_{38}NO_3$ requires 460.2852); $[\alpha]_D$ −36.1 (*c* 0.10 in $CHCl_3$); ν_{max}(CH_2Cl_2)/cm^{-1} 3399 br, 3085, 3062, 3027, 2933, 2855, 1497, 1361, 1092, 1026, 754 and 664; δ_H(300 MHz; C^2HCl_3) 1.08–1.28 (1 H, m, 5-H), 1.35–1.43 (1 H, m, 3-H), 1.46–1.70 [6 H, m, N*H*CH$_2$(CH_2)$_2$CH$_2$Ph and OH], 2.35–2.44 (2 H, m, 3-H and 5-H), 2.45–2.53 [1 H, m, 1 of NHCH_2-(CH$_2$)$_2$CH$_2$Ph], 2.58–2.63 [2 H, t, $^3J_{H\text{-}H}$ 7.6, NHCH$_2$(CH$_2$)$_2$-CH_2Ph], 2.68–2.79 [1 H, m, 1 of NHCH_2(CH$_2$)$_2$CH$_2$Ph and 6-H], 3.33 (1 H, dd, $^3J_{H\text{-}H}$ 9.7 and 3.1, 1-H), 3.68–3.75 (1 H, m, 4-H), 3.90–3.95 (1 H, m, 2-H), 4.46 (1 H, A of first AB system, $^2J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.52 (1 H, B of first AB system, $^2J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 4.53 (1 H, A of second AB system, $^2J_{H\text{-}H}$ 11.7, 1 of OCH_2Ph), 4.62 (1 H, B of second AB system, $^2J_{H\text{-}H}$ 11.8, 1 of OCH_2Ph), 7.15–7.17 (3 H, m, Ar-H) and 7.24–

7.36 (12 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.23 and 30.03 [$NCH_2(CH_2)_2CH_2Ph$], 33.88 and 35.40 (3-C and 5-C), 35.85 [$NCH_2(CH_2)_2CH_2Ph$], 46.73 [$NCH_2(CH_2)_2CH_2Ph$], 56.40 (6-C), 70.63 and 71.80 (2 × OCH_2Ph), 72.25 (4-C), 74.56 (2-C), 76.36 (1-C), 125.77, 127.68, 127.76, 128.35 and 128.48 (Ar-CH) and 138.49, 138.68 and 142.45 (Ar-C quaternary); *m*/*z* (ES^+) 482.4 (5%, $[M + Na]^+$) and 460.4 (100, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Hexylaminocyclohexane-1,2,4-triol 6e

To a solution of 6-hexylamino alcohol **19e** (168 mg, 0.409 mmol) in chloroform (20 cm^3) was added trimethylsilyl bromide (270 mm^3, 2.05 mmol), dropwise, under argon. The resulting solution was stirred and heated at 50 °C under an argon atmosphere for 36 h. After cooling, the solution was concentrated under reduced pressure and the residue was dissolved in 0.2 M HCl. This solution was washed with ethyl acetate (2 × 30 cm^3) and then adjusted to pH 14 by dropwise addition of 4 M NaOH. The solution was then extracted with ethyl acetate (3 × 50 cm^3) and the pooled organic extracts were washed with brine (70 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the 6-hexylamino triol **6e** as a colourless oil (68.2 mg, 72%); mp 90–91 °C; (Found: H, 10.9; C, 62.4; N, 6.05. $C_{12}H_{25}NO_3$ requires H, 10.9; C, 62.3; N, 6.05%); (HRMS: found: $[M + H]^+$, 232.1905. $C_{12}H_{26}NO_3$ requires 232.1913); $[\alpha]_D$ −18.6 (*c* 0.16 in CH_3OH); ν_{max}($CHCl_3$)/cm^{-1} 3385 br, 2993, 2859, 1457, 1344, 1222, 1076 and 1017; δ_H(300 MHz; $C^2H_3O^2H$) 0.77 [3 H, m, $^3J_{H\text{-}H}$ 6.4, $NCH_2(CH_2)_4CH_3$], 1.02–1.14 (1 H, m, 5-H), 1.18–1.34 [6 H, m, 6 of $NCH_2(CH_2)_4CH_3$], 1.36–1.53 [3 H, m, 2 of NCH_2-$(CH_2)_4CH_3$ and 3-H], 2.01–2.12 (1 H, m, 5-H), 2.17–2.24 (1 H, m, 3-H), 2.32–2.43 [1 H, m, 1 of $NCH_2(CH_2)_4CH_3$], 2.53–2.66 [1 H, m, 1 of $NCH_2(CH_2)_4CH_3$], 2.67–2.74 (2 H, m, 6-H), 3.36 (1 H, dd, $^3J_{H\text{-}H}$ 9.3 and 2.1, 1-H), 3.88–3.96 (1 H, m, 4-H) and 4.01–4.07 (1 H, m, and 2-H); δ_C(75.4 MHz; $C^2H_3O^2H$) 11.72 (NCH_2-$(CH_2)_4CH_3$), 20.71, 25.01, 26.89 and 29.72 ($NCH_2(CH_2)_4CH_3$), 34.46 and 37.32 (3-C and 5-C), 44.10 ($NCH_2(CH_2)_4CH_3$), 52.95 (6-C), 62.53 (4-C), 68.08 (2-C) and 71.33 (1-C); *m*/*z* (ES^+ TOF) 232.0 (100%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-(2-Phenylethyl)aminocyclohexane-1,2,4-triol 6f

To a solution of 6-(2-phenylethyl)amino alcohol **19f** (164 mg, 0.380 mmol) in MeOH (20 cm^3) was added 10% palladium on activated charcoal (180 mg) and hydrogen gas was bubbled through the mixture for 5 min followed by vigorous stirring for 10 h under a constant hydrogen pressure. The reaction mixture was then filtered through Celite and the resulting solution concentrated under reduced pressure to give a pale yellow oil. This whole procedure was repeated twice for the reaction to go to completion. The 6-(2-phenylethyl)amino triol **6f** was isolated as a colourless oil (62 mg, 65%); (Found: H, 8.5; C, 67.0; N, 5.6. $C_{14}H_{21}NO_3$ requires H, 8.4; C, 66.9; N, 5.55%); (HRMS: found: $[M + Na]^+$, 274.1425. $C_{14}H_{21}NO_3Na$ requires 274.1419); $[\alpha]_D$ −54.9 (*c* 0.17 in CH_3OH); ν_{max}(thin film)/cm^{-1} 3365 br, 3091, 3058, 3023, 2939, 2850, 1576, 1451, 1315, 1080, 1127, 756 and 704; δ_H(300 MHz; $C^2H_3O^2H$) 1.09–1.22 (2 H, dt, $^3J_{H\text{-}H}$ 11.5 and 7.2, 3-H), 1.39–1.48 (1 H, dt, $^3J_{H\text{-}H}$ 11.2 and 2.6, 5-H), 2.05–2.13 (1 H, m, 5-H), 2.17–2.24 (1 H, m, 3-H), 2.71–2.91 (4 H, m, N-CH_2CH_2Ph), 2.95–3.02 (1 H, m, 6-H), 3.29–3.32 (1 H, m, 1-H), 3.91–4.01 (1 H, m, 4-H and 2-H) and 7.15–7.30 (5 H, m, Ar-H); δ_C(75.4 MHz; $C^2H_3O^2H$) 37.37 and 39.41 (3-C and 5-C), 41.59 (N-CH_2CH_2Ph), 49.64 (N-CH_2CH_2Ph), 56.79 (6-C), 66.13 (4-C), 71.22 (2-C), 75.92 (1-C), 128.13, 130.34 and 130.48 (Ar-CH) and 141.43 (Ar-C quaternary); *m*/*z* (ES^+ TOF) 274.1 (15%, $[M + Na]^+$) and 252.1 (100, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Aminocyclohexane-1,2,4-triol 6n

This compound was prepared in a manner identical with that described for the 6-hexylamino triol **6e** using 6-amino alcohol **19n** (85 mg, 0.260 mmol). The 6-amino triol **6p** was isolated as off white sticky solid (23 mg, 60%); (HRMS: found: $[M + H]^+$, 148.0975. $C_6H_{14}NO_3$ requires 148.0974); $[\alpha]_D$ −64.7 (*c* 0.38 in CH_3OH); ν_{max}(KBr disc)/cm^{-1} 3373 br, 3049, 2942, 1622 and 1499; δ_H(300 MHz; ^2H_2O) 1.38–1.55 (2 H, m, 3-H and 5-H), 2.09 (1 H, dq, J_{HH} 4 and 7, 5-H), 2.24 (1 H, ddd, J_{HH} 4, 7 and 12, 3-H), 3.34 (1 H, dt, J_{HH} 4 and 10, 6-H), 3.59 (1 H, dd, J_{HH} 3 and 10, 1-H), 3.99 (1 H, tt, J_{HH} 4.5 and 11.5, 4-H) and 4.06 (1 H, dd, J_{HH} 3 and 6.5, 2-H); δ_C(75.4 MHz; ^2H_2O) 35.93 and 38.05 (3-C and 5-C), 48.93 (6-C), 63.16, 68.33 and 71.40 (1-C, 2-C and 4-C); *m*/*z* (EI^+) 148 (40%, M^+).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Ethylaminocyclohexane-1,2,4-triol 6o

This compound was prepared in a manner identical with that described for the 6-hexylamino triol **6e** using 6-ethylamino alcohol **19o** (100 mg, 0.282 mmol). The 6-ethylamino triol **6o** was isolated as an off white sticky solid (39 mg, 78%); (HRMS: found: $[M + H]^+$, 176.1280. $C_8H_{18}NO_3$ requires 176.1287); $[\alpha]_D$ −36.3 (*c* 0.20 in CH_3OH); ν_{max}(KBr disc)/cm^{-1} 3302 br, 3055, 2952, 2980, 2814, 1720 and 1638; δ_H(300 MHz; ^2H_2O) 1.23 (3 H, t, J_{HH} 7.5, NCH_2CH_3), 1.34–1.55 (2 H, m, 3-H and 5-H), 2.06–2.12 (1 H, m, 5-H), 2.31–2.36 (1 H, m, 3-H), 3.10 (2 H, ddt, J_{HH} 7.5 20 and 41, NCH_2CH_3), 3.31 (1 H, m, 6-H), 3.65 (1 H, dd, J_{HH} 3 and 10.5, 1-H), 3.99 (1 H, tt, J_{HH} 4.5 and 11.5, 4-H) and 4.06 (1 H, m, 2-H); δ_C(75.4 MHz; ^2H_2O) 15.79 (NCH_2CH_3), 38.94 (NCH_2CH_3), 43.17 and 45.17 (3-C and 5-C), 59.86 (6-C), 68.63, 73.76 and 76.07 (1-C, 2-C and 4-C); *m*/*z* ($ES\ TOF^+$) 198 (10%, $[M + Na]^+$) and 176 (100, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Butylaminocyclohexane-1,2,4-triol 6p

This compound was prepared in a manner identical with that described for the 6-hexylamino triol **6e** using 6-butylamino alcohol **19p** (228 mg, 0.592 mmol). The 6-butylamino triol **6p** was isolated as a pale yellow solid (92 mg, 76%); mp 87–89 °C; (Found: H, 10.45; C, 59.15; N, 6.9. $C_{10}H_{21}NO_3$ requires H, 10.4; C. 59.1; N, 6.9%); (HRMS: found: $[M + H]^+$, 204.1594. $C_{10}H_{22}NO_3$ requires 204.1600); $[\alpha]_D$ −21.4 (*c* 0.13 in CH_3OH); ν_{max}($CHCl_3$)/cm^{-1} 3382 br, 2931, 2859, 1457, 1343, 1215, 1074 and 1022; δ_H(300 MHz; $C^2H_3O^2H$) 0.96 (3 H, m, $^3J_{H\text{-}H}$ 6.4, $NCH_2CH_2CH_2CH_3$), 1.33–1.62 (4 H, m, $NCH_2CH_2CH_2CH_3$, 3-H and 5-H), 1.67–1.86 (2 H, m, $NCH_2CH_2CH_2CH_3$), 2.09–2.21 (1 H, m, 5-H), 2.31–2.43 (1 H, m, 3-H), 3.01–3.25 (2 H, m, $NCH_2CH_2CH_2CH_3$), 3.32–3.47 (2 H, m, 6-H), 3.64–3.73 (1 H, m, 1-H) and 4.00–4.15 (2 H, m, 4-H and 2-H); δ_C(75.4 MHz; $C^2H_3O^2H$) 15.19 ($NCH_2CH_2CH_2CH_3$), 22.07 and 30.44 (NCH_2-$CH_2CH_2CH_3$), 37.00 and 41.41 (3-C and 5-C), 46.93 (NCH_2-$CH_2CH_2CH_3$), 57.96 (6-C), 66.00 (4-C), 71.20 (2-C) and 73.69 (1-C); *m*/*z* (ES^+ TOF) 406.2 (5%, $[M + Na]^+$) and 384.2 (100%, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-Octylaminocyclohexane-1,2,4-triol 6q

This compound was prepared in a manner identical with that described for the 6-hexylamino triol **6e** using the 6-octylamino alcohol **19q** (174 mg, 0.396 mmol) to give the 6-octylamino triol **6q** as a thick pale yellow oil (55.5 mg, 54%); (Found: H, 11.3; C, 64.9; N, 5.45. $C_{14}H_{29}NO_3$ requires H, 11.25; C, 64.85; N, 5.4%); (HRMS: found: $[M + H]^+$, 260.2229. $C_{14}H_{30}NO_3$ requires 260.2226); $[\alpha]_D$ −16.7 (*c* 0.23 in $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3393 br, 2930, 2867, 1454, 1343, 1214, 1076 and 1021; δ_H(300 MHz; C^2HCl_3) 0.86 (3 H, m, $^3J_{H\text{-}H}$ 6.3, *n*-octyl), 1.16–1.36 (11 H, m, 10 of *n*-octyl and 5-H), 1.38–1.58 (3 H, m, 2 of *n*-octyl and 3-H), 1.96–2.36 (3 H, m, 3-H, 5-H and NH), 2.41–2.54 [1 H, m, 1 of N-$CH_2(CH_2)_6CH_3$], 2.67–2.89 [2 H, m, 1 of N-$CH_2(CH_2)_6CH_3$and 6-H], 3.14–3.70 (4 H, m, 3 × OH and 4-H) and 3.94–4.22 (2 H, m, 1-H and 2-H); δ_H(75.4 MHz; C^2HCl_3) 13.94 [N-$CH_2(CH_2)_6CH_3$], 22.49 and 27.15 [2 of N-CH_2-$(CH_2)_6CH_3$], 28.10 (5-C), 29.14 and 29.35 [2 of N-CH_2-$(CH_2)_6CH_3$], 29.91 (3-C), 31.67 [1 of N-$CH_2(CH_2)_6CH_3$], 36.32

[N-$CH_2(CH_2)_6CH_3$, due to folding of octyl chain], 38.66 [1 of N-$CH_2(CH_2)_6CH_3$], 46.43 and 53.45 [N-$CH_2(CH_2)_6CH_3$], 55.68, 55.91 and 59.82 (6-C), 65.23 and 65.56 (4-C), 67.50 and 67.71 (2-C) and 70.27 and 73.44 (1-C); *m/z* (ES^+ TOF) 274.2 (40%, $[M - C_2H_4 - OH + 2Na]^+$) and 260.2 (100, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-(4-Phenylbutyl)aminocyclohexane-1,2,4-triol 6r

This compound was prepared in a manner identical with that described for the 6-hexylamino triol **6e** using the 6-(4-phenylbutyl)amino alcohol **19r** (161 mg, 0.351 mmol) to give the 6-(4-phenylbutyl)amino triol **6r** as a white amorphous solid (86 mg, 88%), mp 91–92 °C; (Found: H, 9.05; C, 68.9; N, 5.0. $C_{16}H_{25}NO_3$ requires H, 9.0; C, 68.8; N, 5.0%); (HRMS: found: $[M + H]^+$, 280.1924. $C_{16}H_{26}NO_3$ requires 280.1913); $[\alpha]_D$ −50.4 (*c* 0.06 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3315 br, 3086, 3056, 3026, 2930, 2856, 1602, 1453, 1092, 1026, 747 and 699; δ_H(300 MHz; C^2HCl_3) 1.13–1.24 (1 H, m, 5-H), 1.41–1.54 [3 H, m, 2 of N-$CH_2(CH_2)_2CH_2Ph$ and 3-H], 1.96–2.18 (6 H, m, 3-H, 5-H, NH and 3 × OH), 2.46–2.54 [1 H, m, 1 of N-$CH_2(CH_2)_2CH_2Ph$], 2.60 [2 H, t, $^3J_{H\text{-}H}$ 6.0, N-$CH_2(CH_2)_2CH_2Ph$], 2.76–2.84 [2 H, m, 1 of N-$CH_2(CH_2)_2CH_2Ph$ and 6-H], 3.29–3.33 (1 H, m, 1-H), 4.01–4.10 (1 H, m, 4-H), 4.14–4.17 (1 H, m, 2-H), 7.15–7.19 (3 H, m, Ar-H) and 7.25–7.30 (2 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.62 and 30.60 [N-$CH_2(CH_2)_2CH_2Ph$], 36.33 [N-$CH_2(CH_2)_2CH_2Ph$], 39.06 and 40.07 (3-C and 5-C), 47.03 [N-$CH_2(CH_2)_2CH_2Ph$], 56.51 (6-C), 66.52 (4-C), 68.08 (2-C), 74.43 (1-C), 125.35 and 128.91 (Ar-CH) and 142.84 (Ar-C quaternary); *m/z* (ES^+ TOF) 302.1 (10%, $[M + Na]^+$), 280.2 (100, $[M + H]^+$) and 262.2 (5, $[M - OH]^+$).

(*N*-Benzyloxycarbonyl)-2-aminoethanol 21

To a solution of ethanolamine (6 cm^3, 100 mmol) in 1,4-dioxane–water (1 : 1, 200 cm^3) was added DMAP (50 mg, 4 mmol), benzyl chloroformate (16 cm^3, 110 mmol) and TEA (13.9 cm^3, 110 mmol) and the reaction mixture stirred for 12 h. The solvents were removed under reduced pressure to give a thick solution which was diluted with DCM (150 cm^3) and water (50 cm^3). After separation, the aqueous layer was back-extracted with DCM (3 × 50 cm^3). The pooled organic layers were then successively washed with 0.1 M solution of HCl (100 cm^3), water (100 cm^3), and brine (100 cm^3), dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure and the resulting white crystalline solid was recrystallised from petroleum ether–ethyl acetate (3 : 1) to give *N*-Cbz 2-aminoethanol **21** as white crystals (15.6 g, 80%); mp 61–62 °C (lit.,[31] 61–62 °C); (Found: H, 6.8; C, 61.8; N, 7.2. Calc. for $C_{10}H_{13}NO_3$: H, 6.7; C, 61.55; N, 7.15%) (HRMS: found: $[M + Na]^+$, 218.0802. Calc. for $C_{10}H_{13}NO_3Na$: 218.0793); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3370 br, 3250, 3140, 3030, 2950, 2850, 1692, 1546, 1453, 1276, 1150, 747 and 696; δ_H(300 MHz; C^2HCl_3) 2.85 (1 H, br OH), 3.30 (2 H, q, $^3J_{H\text{-}H}$ 5.2, NCH_2CH_2OH), 3.65 (2 H, m, NCH_2CH_2OH), 5.07 (2 H, s, OCH_2Ph), 5.39 (1 H, br, NH) and 7.25–7.32 (5 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 43.74 (NCH_2CH_2OH), 62.37 (NCH_2CH_2OH), 67.16 (OCH_2Ph), 128.35, 128.43 and 128.79 (Ar-CH), 136.64 (Ar-C quaternary) and 157.41 (CO); *m/z* (FAB^+) 218 (50%, $[M + Na]^+$), 199 (15, $[M - H_2O - H + Na]^+$), 176 (100, $M - H_2O - H]_+$), 105 (30, $[M - C_7H_7 + H]^+$) and 92 (25, $C_7H_8O^+$)

(*N*-Benzyloxycarbonyl)-2-aminoethyl *p*-toluenesulfonate 22

A solution of alcohol **21** (3.79 g, 19.4 mmol) and DMAP (50 mg, 4 mmol) in dry DCM (50 cm^3) was cooled to 0 °C in an ice–water bath and then tosyl chloride (4.08 g, 21.4 mmol) was added, dropwise, under argon followed by TEA (2.98 cm^3, 21.4 mmol). The reaction mixture was gradually brought to room temperature and stirred for 5 h. The solvents were removed under reduced pressure to give a white residue which was dissolved in ethyl acetate (50 cm^3) and washed with water (50 cm^3). The aqueous layer was then back extracted with ethyl acetate (3 × 50 cm^3). The combined organic fractions were washed with brine (50 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting oil was purified by flash silica column chromatography (petroleum ether–ethyl acetate; gradient from 1 : 2 to 2 : 1) to give the tosylate **22** as a white solid (5.97 g, 88%); mp 51–52 °C (lit.,[33] 51–52 °C); (Found: H, 5.55; C, 58.5; N, 4.05. $C_{17}H_{19}NO_5S$ requires H, 5.5; C, 58.45; N, 4.0%); (HRMS: found: $[M + Na]^+$, 372.0893. $C_{17}H_{19}NO_5NaS$ requires 372.0882); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3390 br, 3091, 3060, 3030, 2950, 2850, 1716, 1597, 1455, 1358, 1259, 1189, 1176, 1096, 1010, 915, 754, 698 and 663; δ_H(300 MHz; C^2HCl_3) 2.42 (3 H, br, tosyl), 3.45 (2 H, q, $^3J_{H\text{-}H}$ 5.4, NCH_2CH_2O), 4.08 (2 H, t, $^3J_{H\text{-}H}$ 5.0, NCH_2CH_2O), 5.05 (3 H, s, OCH_2Ph and NH), 7.25–7.38 (7 H, m, Ar-H) and 7.78 (2 H, d, $^3J_{H\text{-}H}$ 8.2, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 22.58 (CH_3), 41.20 (NCH_2CH_2O), 67.89 (NCH_2CH_2O), 70.04 (OCH_2Ph), 128.87, 128.97, 129.15, 129.50 and 130.91 (Ar-CH), 135.50, 138.12 and 148.12 (Ar-C quaternary) and 156.17 (CO); *m/z* (ES^+ TOF) 372.2 (100%, $[M + Na]^+$).

N-(4-Phenylbutyl)-*N*-(benzyloxycarbonyl)-ethane-1,2-diamine 23

A sealed round bottom flask containing a stirred solution of the tosylate **22** (5.02 g, 14.4 mmol) in phenebutylamine (23.2 cm^3, 144 mmol) was heated at 50 °C for 12 h. Chloroform (150 cm^3) and K_2CO_3 (2.20 g, 15.9 mmol) were then added and the resulting solution was stirred for a further 3 h. The solution was diluted with chloroform (30 cm^3) and washed with water (150 cm^3), saturated solution of NH_4Cl (120 cm^3), water (120 cm^3) and brine (120 cm^3). The resulting organic layer was dried ($MgSO_4$), filtered and then concentrated under reduced pressure to give a light yellow which was purified by distillation *in vacuo* at 80 °C using a Kugelrohr apparatus to give the diamine **23** as a colourless oil (2.11 g, 45%); (Found: H, 8.1; C, 73.65; N, 8.6. $C_{20}H_{26}N_2O_2$ requires H, 8.05; C, 73.6; N, 8.6%); (HRMS: found: $[M + H]^+$, 327.2054. $C_{20}H_{27}N_2O_2$ requires 327.2073); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3326 br, 2091, 3062, 3033, 2961, 2849, 1706, 1537, 1454, 1258, 1181, 1122, 1030, 1006, 754 and 697; δ_H(300 MHz; C^2HCl_3) 1.42–1.71 [4 H, m, $CH_2(CH_2)_2CH_2Ph$], 2.48–2.69 [6 H, m, NH, $CONHCH_2CH_2$ and $CH_2(CH_2)_2CH_2Ph$], 3.22 (2 H, t, $^3J_{H\text{-}H}$ 6.2, $CONHCH_2CH_2$), 5.08 (2 H, s, OCH_2Ph) and 7.12–7.38 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 28.18 and 28.56 [$CH_2(CH_2)_2CH_2Ph$], 34.80 [$CH_2(CH_2)_2CH_2Ph$], 39.26 [$CH_2(CH_2)_2CH_2Ph$], 48.01 ($CONHCH_2CH_2$), 48.51 ($CONHCH_2CH_2$), 65.47 (OCH_2Ph), 124.46, 127.10, 127.18, 127.51, 128.69 and 128.73 (Ar-CH), 136.13 and 141.44 (Ar-C quaternary) and 156.91 (CO); *m/z* (ES^+ TOF) 327.2 (100%, $[M + H]^+$) and 300.2 (40, $[M - C_2H_4 + 2H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-{*N*-(4-phenylbutyl)-*N*-[(*N*′-benzyloxycarbon-yl)-2-aminoethyl]amino}cyclohexanol 24

This compound was prepared in a manner identical with that described for amino alcohol **19e** using diamine **23** (1.28 g, 3.93 mmol). The resulting oil was purified by flash silica column chromatography (petroleum ether–ethyl acetate; 6 : 1) to give the alcohol **24** as a colourless oil (290 mg, 58%); (Found: H, 7.7, C; 73.6, N; 4.4. $C_{40}H_{48}N_2O_5$ requires H, 7.6; C, 75.45; N, 4.4%); (HRMS: found: $[M + H]^+$, 637.3645. $C_{40}H_{49}N_2O_5$ requires 637.3641); $[\alpha]_D$ −26.8 (*c* 0.12 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3395 br, 3089, 3052, 3024, 2954, 2851, 1724, 1526, 1454, 1242, 1094, 742 and 697; δ_H(300 MHz; C^2HCl_3) 1.15–1.75 [6 H, m, 4 of $CH_2(CH_2)_2CH_2Ph$, 3-H and 5-H], 2.11–2.20 (1 H, m, 5-H), 2.26–2.34 (1 H, m, 3-H), 2.44–2.74 [7 H, m, $CH_2(CH_2)_2CH_2Ph$, NCH_2CH_2NH and OH], 2.88–2.95 (1 H, m, 6-H), 3.17–3.23 (2 H, m, NCH_2CH_2NH), 3.36–3.40 (1 H, m, 1-H), 3.70–3.76 (1 H, m, 4-H), 3.93–4.01 (1 H, m, 2-H), 4.45 (1 H, A of first AB system, $^2J_{H\text{-}H}$ 11.9, OCH_2Ph), 4.50 (1 H, B of AB system,

$^2J_{H\text{-}H}$ 11.9, OCH_2Ph), 4.60 (1 H, A of second AB system, $^2J_{H\text{-}H}$ 12.1, OCH_2Ph), 4.73 (1 H, B of second AB system, $^2J_{H\text{-}H}$ 12.1, OCH_2Ph), 5.09 (2 H, s, CO-OCH_2Ph), 5.28 (1 H, br, NH) and 7.13–7.37 (20 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.58, 29.91, 30.32 [$CH_2(CH_2)_2CH_2$Ph], 35.74 and 36.61 (3-C and 5-C), 40.91 [$CH_2(CH_2)_2CH_2$Ph], 50.15 (NCH_2CH_2NH), 51.02 (NCH_2CH_2NH), 58.87 (6-C), 67.43 (CO-OCH_2Ph), 71.51 (OCH_2Ph), 72.73 (4-C), 73.48 (OCH_2-Ph), 73.82 (2-C), 76.66 (1-C), 126.58, 128.33, 128.44, 128.89 and 129.18 (Ar-CH), 136.17, 138.42, 138.89 and 142.31 (Ar-C quaternary) and 156.55 (CO-OCH_2Ph); *m/z* (ES^+ TOF) 659.4 (100%, $[M + Na]^+$), 637.4 (30, $[M + H]^+$).

(−)-(1*S*,2*R*,4*S*,6*R*)-6-[*N*-(4-phenylbutyl)-*N*-(2-aminoethyl)-amino]cyclohexane-1,2,4-triol 25 [hydrochloride salt]

This compound was prepared in a manner identical with that described for the 6-hexylamino triol **6e** using alcohol **24** (285 mg, 0.45 mmol) to give a crude oil which dissolved in concentrated HCl (10 cm^3) and stirred for 1 h. Removal of the solvent under reduced pressure gave a yellow oil which was dissolved in MeOH (1 cm^3). The solution was added dropwise to diethyl ether (250 cm^3), under stirring, and the resulting white solid was filtered, washed with diethyl ether (100 cm^3) and dried under high vacuum to give the diamine triol hydrochloride **25** as a white solid (55 mg, 38%); mp 93–94 °C (Found: H 8.75; C, 60.3; N, 7.85. $C_{18}H_{31}ClN_2O_3$ requires H, 8.7; C, 60.25; N, 7.8%); (HRMS: found: $[M - Cl]^+$, 323.2324. $C_{18}H_{31}N_2O_3$ requires 323.2335); $[a]_D$ −25.9 (*c* 0.10 in MeOH); ν_{max}(KBr disc)/cm^{-1} 3396 br, 3089, 3056, 2967, 2854, 1506, 1454, 1246, 1094, 742 and 698; δ_H(300 MHz; $C^2H_3O^2H$) 1.48–1.91 [6 H, m, 4 of $CH_2(CH_2)_2CH_2$Ph, 3-H and 5-H], 2.11–2.19 (1 H, m, 5-H), 2.29–2.34 (1 H, m, 3-H), 2.68–2.73 [2 H, m, $CH_2(CH_2)_2CH_2$Ph], 2.98–3.14 [1 H, m, 1 of $CH_2(CH_2)_2CH_2$Ph], 3.32–4.18 [9 H, m, 1 of $CH_2(CH_2)_2CH_2$Ph, NCH_2CH_2NH, 6-H, 1-H, 4-H and 2-H], and 7.19–7.37 (5 H, m, Ar-H); δ_C(75.4 MHz; $C^2H_3O^2H$) 25.38 and 29.41 [$CH_2(CH_2)_2CH_2$Ph], 32.89 and 35.80 (3-C and 5-C), 36.09 [$CH_2(CH_2)_2CH_2$Ph], 40.27 ($NCH_2CH_2NH_3$), 49.00 [$CH_2(CH_2)_2CH_2$Ph], 53.72 (NCH_2CH_2NH), 61.80 (6-C), 64.98 (4-C), 70.12 (2-C), 70.38 (1-C), 127.04 and 129.48 (Ar-CH) and 141.18 (Ar-C quaternary); *m/z* (ES^+ TOF) 323.3 (100%, $[M - Cl]^+$), 306.3 (70, $[M - Cl - NH_3]^+$) and 280.3 (30, $[M - Cl - C_2H_7N + 2H]^+$).

Enzyme

Recombinant bovine-brain *myo*-inositol-phosphatase was supplied by Sigma and was used according to the manufacturers instructions. Enzyme activity assays were performed using either a colorimetric assay developed by Itaya and Ui[25] employing molybdic acid and malachite green or by a radiolabelled assay developed by Gee *et al.*[14] Rate determinations were performed at 25 °C in triplicate in assay buffer A containing 50 mM KCl, 100 mM Tris.HCl and 3 mM $MgCl_2$ (pH 8.0). 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 mM LiCl). Rate data were analysed and processed graphically and by using non-linear regression analysis as described previously.[3]

Colorimetric assay. Colorimetric assay reagent: malachite green (1.5 g) was dissolved in 5 M hydrochloric acid (225 cm^3) and the solution was 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.

Assays (100 mm^3), which were initiated by addition of enzyme solution (10 mm^3, 0.04 units), contained 50 mM KCl, 100 mM Tris.HCl, 3mM $MgC1_2$, enzyme, substrate (2′AMP) at various concentrations in the assay buffer and inhibitor at various concentrations in the assay buffer (pH 8.0), were incubated at 25 °C and the reaction was quenched by the addition of colorimetric reagent (800 mm^3) at the required time followed 1 minute later by 34% trisodium citrate solution (100 mm^3). Absorbance at 660 nm was measured in 10 mm pathlength cuvette. Phosphate concentrations were determined by comparison of absorbance value to a preconstructed standard curve prepared using known phosphate concentrations.

Radiolabelled assay. Assays (50 mm^3), which were initiated by addition of enzyme (5 mm^3) contained 50 mM KCl, 100 mM Tris.HCl, 3 mM $MgCl_2$, substrate {(±)-[2-^{3}H]-Ins-1-P, 500 dpm nmol^{-1}} at varying concentrations in the assay buffer, inhibitor at varying concentrations in the assay buffer and K_2HPO_4 (at 0, 1, 2 or 3 mM in the assay buffer) were incubated at 25 °C and the reaction was quenched after 20 min by addition of 1 M NaOH (5 mm^3). The solution was then diluted with water (100 mm^3) and applied to a pre-equilibrated DOWEX-1X2-400 anion exchange column (OH^- form, 200 mg). The column was eluted by micro-centrifugation and the eluates (plus washings) were emulsified with scintillation fluid. The radioactivity in each sample was determined by liquid scintillation counting using a Packard Tri-Carb 2500 TR scintillation counter.

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References

1 Mental Health Review and WHO, *WHO Fact Sheet N 130*, 1996, pp. 1–3.
2 J. M. Baraban, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 5738–5739.
3 A. P. Leech, G. R. Baker, J. K. Shute, M. A. Cohen and D. Gani, *Eur. J. Biochem.*, 1993, **212**, 693–704.
4 R. Bone, J. P. Springer and J. R. Atack, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 10031–10035.
5 (*a*) A. G. Cole and D. Gani, *J. Chem. Soc., Chem. Commun.*, 1994, 1139–1141; (*b*) A. G. Cole and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2685–2694; (*c*) A. G. Cole, J. Wilkie and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2695–2707.
6 J. Wilkie, A. Cole and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2709–2727.
7 J. Wilkie and D. Gani, *J. Chem. Soc., Perkin Trans. 2*, 1996, 783–787.
8 (*a*) S. J. Pollack, J. R. Atack, M. R. Knowles, G. McAllister, C. I. Ragan, R. Baker, S. R. Fletcher, L. L. Iverson and H. B. Broughton, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 5766–5770; (*b*) R. Bone, L. Frank, J. P. Springer, S. J. Pollack, S. A. Osborne, J. R. Atack, M. R. Knowles, G. McAllister, C. I. Ragan, H. B. Broughton, R. Baker and S. R. Fletcher, *Biochemistry*, 1994, **33**, 9468–9471.
9 C. M. J. Faroux and S. Freeman, *J. Enzyme Inhib.*, 1999, **14**, 97–108.
10 D. Gani, C. P. Downes, I. Batty and J. Bramham, *Biochim. Biophys. Acta*, 1993, **1177**, 253–269.
11 B. V. L. Potter and D. Lampe, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1933–1972.
12 M. J. Berridge and R. F. Irvine, *Nature*, 1989, **341**, 197–205.
13 R. Spector and A. V. Lorenzo, *Am. J. Physiol.*, 1975, **228**, 1510–1518.
14 N. S. Gee, C. I. Ragan, K. J. Watling, S. Aspley, R. G. Jackson, G. G. Reid, D. Gani and J. K. Shute, *Biochem. J.*, 1988, **249**, 883–889.
15 D. J. Miller, M. W. Beaton, J. Wilkie and D. Gani, *ChemBioChem*, 2000, **1**, 262–271.
16 (*a*) R. Baker, P. D. Leeson, N. J. Liverton and J. J. Kulagowski, *J. Chem. Soc., Chem. Commun.*, 1990, 462–464; (*b*) R. Baker, J. J. Kulagowski, D. C. Billington, P. D. Leeson, I. C. Lennon and N. Liverton, *J. Chem. Soc., Chem. Commun.*, 1989, 1383–1385; (*c*) R. Baker, C. Carrick, P. D. Leeson, I. C. Lennon and N. J. Liverton, *J. Chem. Soc., Chem. Commun.*, 1991, 298–300.
17 J. Schulz, J. Wilkie, M. W. Beaton, D. J. Miller and D. Gani, *Biochem. Soc. Trans.*, 1998, **26**, 315–322.
18 J. Schulz, M. W. Beaton and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 2000, 943–954.

19 D. Voet and J. G. Voet, *Biochemistry*, J. Wiley, New York, 1990.
20 M. W. Beaton and D. Gani, *Tetrahedron Lett.*, 1998, **39**, 8549–8552.
21 H. A. C. Montgomery and J. H. Turnbull, *J. Chem. Soc.*, 1958, 1963–1966.
22 J. R. Hwu, V. Chua, J. E. Schroeder, R. E. Barrans Jr, K. P. Khoudary, N. Wang and J. M. Wetzel, *J. Org. Chem.*, 1986, **51**, 4731–4733.
23 J. R. Hwu, Y. S. Wein and Y. J. Leu, *J. Org. Chem.*, 1996, **61**, 1493–1499.
24 M. B.-U. Surfraz, D. J. Miller, D. Gani and R. K. Allemann, *Tetrahedron Lett.*, 2003, **41**, 7677–7679.
25 K. Itaya and M. Ui, *Clin. Chim. Acta*, 1966, **14**, 361–366.
26 J. Schulz and D. Gani, *Tetrahedron Lett.*, 1997, **38**, 111–114.
27 R. A. Copeland, *Enzymes*, Wiley-VCH, New York, 1996.
28 W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923–2925.
29 W. L. F. Armarego and D. D. Perrin, *Purification of Laboratory Chemicals*, Fourth Edition, Butterworth and Heinemann, Oxford, 1998.
30 S. Cabbidu, W. Marongiu, S. Melis and F. Sotgiu, *J. Organomet. Chem.*, 1976, **116**, 275–279.
31 Y. Hamada, M. Shibata, T. Sigiura, S. Kato and T. Shioiri, *J. Org. Chem.*, 1987, **52**, 1252–1255.
32 D. E. DeMong and R. M. Williams, *Tetrahedron Lett.*, 2001, **42**, 185–187.
33 S. Ginsburg and I. B. Wilson, *J. Am. Chem. Soc.*, 1964, **86**, 4716–4720.