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Structure of (±)-1,2;4,5-di-*O*-cyclohexylidene *myo*-inositol and synthesis of *myo*-inositol 3-phosphate via its phosphorylation with (*2R,4S,5R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one [1]

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Abstract

The crystal structure of (±)-1,2;4,5-di-*O*-cyclohexylidene *myo*-inositol, refined to R = 2.9%, shows interestingly disordered (flip-flop) hydrogen bonding. The higher reactivity of the 1/3 positions (rather than 4/6) has been evaluated using semi-empirical calculations. This diol has been phosphorylated with (*2R,4S,5R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one and the diastereoisomer derived from phosphorylation at the 3-position was isolated by crystallisation. Deprotection with TFA/H_2O then H_2/Pd-C afforded *myo*-inositol 3-phosphate in only four steps from *myo*-inositol. © 1997 Elsevier Science Ltd.

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1. Introduction

A key metabolic enzyme, inositol monophosphatase (IMPase), catalyses the hydrolysis of *myo*-inositol monophosphates to *myo*-inositol. In contrast to typical phosphatases, IMPase does not proceed *via* a phospho-enzyme intermediate [2]. Atack et al. [3]

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have proposed an in-line displacement hydrolysis with inversion of stereochemistry at phosphorus, whereas Wilkie et al. [4] have proposed an adjacent association and pseudorotation mechanism with retention. Stereochemical studies using *myo*-inositol [^{16}O, ^{17}O]-thiophosphate as substrate in the presence of $H_2{}^{18}O$ will distinguish between these mechanisms [2]. Towards this goal a synthesis of *myo*-inositol 3-phosphate is presented, the route to which could be applied to the preparation of this isotopically labelled analogue.

All isomeric *myo*-inositol monophosphates can be prepared either from a naturally occurring optically active precursor or by resolution of a protected *myo*-inositol intermediate [5,6]. The latter approach typically entails selective protection of the hydroxyl groups, resolution of enantiomers, phosphorylation and removal of protecting groups. The shortest reported synthesis of D-*myo*-inositol 1- and 3-phosphate from *myo*-inositol has utilised D- and L-camphor dimethyl acetals as both the resolving agent and protecting group [7].

A common protecting group for *myo*-inositol is cyclohexylidene and the bisacetal **1** [8] has been used in many studies. Diol **1** can be further protected by the addition of benzyl [8,9], allyl [8,9] and *tert*-butyldiphenylsilyl [10] groups selectively at the 1/3 position. Here, the crystal structure of **1** has been solved, partly in an attempt to explain the higher reactivity at 1/3 compared to the 4/6 positions. The optically active phosphorylating agent (*2R,4S,5R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (**2**) [11] and its P = S analogue, have previously been used in the syntheses of [^{16}O, ^{17}O, ^{18}O]-phosphate and [^{16}O, ^{18}O]-thiophosphate monoesters [12]. However, they have never been utilised as a combined phosphorylating and resolving agent, and here the reaction of diol **1** with **2** is evaluated.

2. Results and discussion

(±)-1,2;4,5-Di-*O*-cyclohexylidene-*myo*-inositol (**1**) was synthesised using the method described by Dreef et al. [13], and its X-ray structure was solved at 293 and 150 K. The structure of compound **1** revealed all hydrogen atoms and was refined to R = 2.9% for observed reflections at 293 K, and to 4.6% at 150 K. Figs. 1 and 2 show the molecular geometry and the crystal packing of **1**. Tables 1 and 2 give the atomic coordinates of **1** at 293 and 150 K, respectively. The inositol ring adopts a distorted chair conformation, with the oxygen at C-02 in an expected

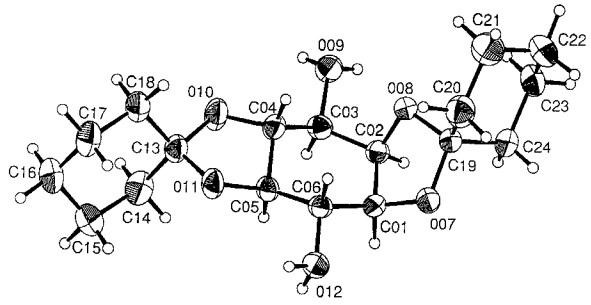


Fig. 1. ORTEPII diagram [14] of **1** at 293 K showing the labelling scheme for non-H atoms. Thermal ellipsoids are drawn at the 50% probability level. The D-1,2;4,5-di-*O*-cyclohexylidene *myo*-inositol enantiomer is shown.

axial position and the other five oxygen atoms in equatorial positions. Although the C–C bond lengths (range 1.487–1.541 Å) and C–O bond lengths (range 1.417–1.435 Å) are comparable with those of *myo*-inositol [16], the ring angles (range 106.6–118.5°) deviate from those of *myo*-inositol [16] (mean angle 110.7°) and a perfect chair [17] (111°).

The torsion angles of the *cis* ring junction O-07–C-01–C-02–O-08 [−35.7(1)° at 293 K] and the *trans* ring junction O-10–C-04–C-05–O-11 [−42.0(1)°], compared with 56° for the perfect chair, indicate twisting of the inositol ring to accommodate the five-membered ring acetal linkages. The asymmetry

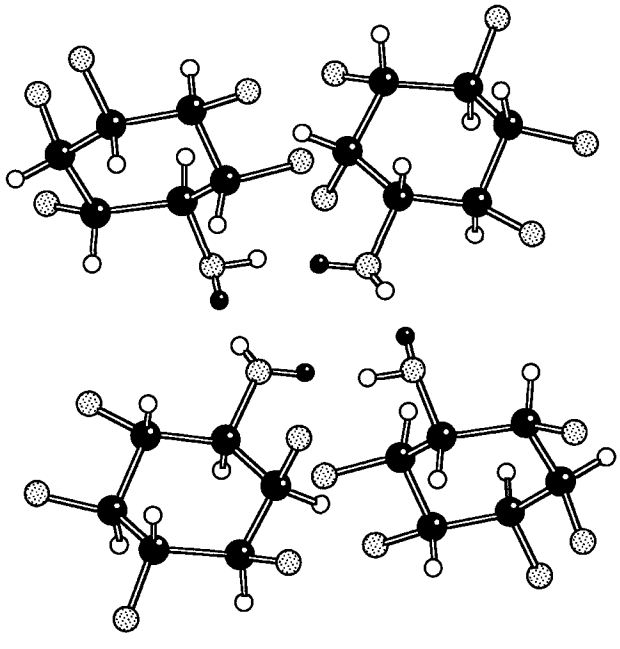


Fig. 2. Crystal packing diagram of **1** [15] at 293 K showing the inositol moieties of four molecules which participate in flip-flop hydrogen bonding around the centre of symmetry at (0, 0.5, 0). Carbon atoms are black, oxygen atoms stippled, and hydrogen atoms including H-51 and H-52 white, but H-53 and H-54 are black.

Table 1
Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (Å$^2 \times 10^3$) for **1** at 293 K. U(eq) is defined as one third of the trace of the orthogonalised U_{ij} tensor

	x	y	z	U(eq)
C-04	120(1)	3461(1)	2978(1)	34(1)
C-05	144(1)	2274(1)	3558(1)	32(1)
C-06	1278(1)	1846(1)	3809(1)	31(1)
C-01	1779(1)	1672(1)	2740(1)	33(1)
C-02	1534(1)	2674(1)	1917(1)	33(1)
C-03	432(1)	3271(1)	1868(1)	33(1)
O-10	−965(1)	3839(1)	3048(1)	50(1)
O-11	−466(1)	2573(1)	4441(1)	40(1)
O-12	1342(1)	732(1)	4376(1)	38(1)
O-07	2927(1)	1697(1)	2866(1)	46(1)
O-08	2358(1)	3528(1)	2216(1)	36(1)
O-09	452(1)	4376(1)	1302(1)	41(1)
C-13	−1344(1)	3305(1)	3996(1)	39(1)
C-14	−2324(2)	2544(2)	3691(2)	51(1)
C-15	−2866(2)	2083(2)	4654(2)	61(1)
C-16	−3123(2)	3106(2)	5390(2)	59(1)
C-17	−2122(2)	3819(2)	5726(2)	53(1)
C-18	−1582(2)	4295(2)	4771(2)	48(1)
C-19	3302(1)	2823(1)	2456(1)	38(1)
C-20	4013(2)	3470(2)	3282(2)	53(1)
C-21	4480(2)	4626(2)	2853(2)	65(1)
C-22	5055(2)	4393(2)	1852(2)	70(1)
C-23	4331(2)	3751(2)	1027(2)	58(1)
C-24	3889(2)	2585(2)	1460(2)	48(1)
H-51	−152(20)	4622(31)	1090(26)	51(6)
H-52	1152(27)	801(32)	4975(19)	51(6)
H-53	719(27)	4267(34)	728(21)	51(6)
H-54	880(25)	266(29)	4094(28)	51(6)

Table 2
Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (Å$^2 \times 10^3$) for **1** at 150 K. U(eq) is defined as one third of the trace of the orthogonalised U_{ij} tensor

	x	y	z	U(eq)
C-04	86(2)	3472(3)	2971(2)	18(1)
C-05	122(2)	2299(3)	3574(2)	18(1)
C-06	1282(2)	1875(3)	3829(2)	18(1)
C-01	1770(2)	1681(3)	2757(2)	17(1)
C-02	1512(2)	2677(3)	1924(2)	18(1)
C-03	392(2)	3273(3)	1870(2)	18(1)
O-10	−1029(2)	3837(2)	3031(2)	25(1)
O-11	−480(2)	2610(2)	4459(1)	20(1)
O-12	1369(2)	768(2)	4417(2)	22(1)
O-07	2941(2)	1699(2)	2878(2)	24(1)
O-08	2353(2)	3539(2)	2227(2)	19(1)
O-09	410(2)	4379(2)	1291(2)	22(1)
C-13	−1389(2)	3319(3)	3999(2)	21(1)
C-14	−2375(3)	2527(3)	3718(3)	24(1)
C-15	−2897(3)	2079(3)	4702(3)	31(1)
C-16	−3155(3)	3135(3)	5429(3)	29(1)
C-17	−2148(3)	3883(3)	5734(3)	26(1)
C-18	−1632(3)	4331(3)	4752(3)	25(1)
C-19	3320(2)	2829(2)	2459(2)	20(1)
C-20	4066(3)	3477(3)	3280(3)	26(1)
C-21	4530(3)	4642(3)	2839(3)	32(1)
C-22	5070(3)	4422(3)	1816(3)	33(1)
C-23	4306(3)	3759(3)	997(3)	28(1)
C-24	3881(3)	2587(3)	1449(3)	24(1)
H-51	−247(29)	4607(51)	1124(44)	8(8)
H-52	953(40)	771(52)	4929(33)	8(8)
H-53	716(35)	4300(43)	703(27)	8(8)
H-54	950(35)	257(37)	4083(37)	8(8)

parameters [18] of the inositol ring (Table 3) show that one approximate rotation axis through the midpoints of C-01–C-02 and C-04–C-05 is retained. Twisting of the inositol ring is also indicated by the opening of torsion angle C-03–C-04–C-05–C-06 to 72.0(2)° and the compression of the opposite torsion angle C-06–C-01–C-02–C-03 to −35.8(2)°.

Both cyclohexylidene rings differ only slightly from ideal chair conformations [17]. However, the two acetal five-membered rings have different conformations. The axial–equatorial linked ring adopts a distorted half-chair with C-02 above and O-08 below the plane of C-01, O-07, C-19 with ΔC_2(2–8) 7.6°. The equatorial–equatorial linked ring adopts an envelope in which C-05 is below a plane through O-11, C-13, O-10, C-04 with ΔC_s 1.6°.

The conformation of the *myo*-inositol ring in the X-ray structure is qualitatively consistent with that observed in solution by ^{1}H NMR spectroscopy. The $J_{ax,ax}$ coupling constants of $J_{3,4}$ 9.5, $J_{4,5}$ 9.6, and $J_{5,6}$ 10.4 Hz are consistent with dihedral angles close to 180°, observed in the crystal structure as −177,

Table 3
Asymmetry parameters [18] for inositol ring of **1** at 293 and 150 K

293 K		150 K	
$\Delta C_s(1) = 15.4$	$\Delta C_2(1–2) = 3.7$	$\Delta C_s(1) = 15.3$	$\Delta C_2(1–2) = 4.1$
$\Delta C_s(2) = 10.4$	$\Delta C_2(2–3) = 25.2$	$\Delta C_s(2) = 9.7$	$\Delta C_2(2–3) = 24.1$
$\Delta C_s(3) = 25.1$	$\Delta C_2(3–4) = 28.5$	$\Delta C_s(3) = 24.3$	$\Delta C_2(3–4) = 27.8$

ΔC_s = Mirror-related asymmetry parameter.
ΔC_2 = Two-fold related asymmetry parameter.

−169, and +178°, respectively. The $J_{ax,eq}$ coupling constants of $J_{1,2}$ 5.2 and $J_{2,3}$ 4.6 Hz are compatible with the observed dihedral angles of −38 and +46°. The $J_{ax,ax}$ coupling constant $J_{1,6}$ 5.9 Hz is consistent only with a dihedral angle significantly less than 180°, as observed in the crystal structure (+157°). However, by using the equation $^3J_{ab} = J^{180}\cos^2\phi - 0.28$ [19] and J^{180} 10.4 Hz, a coupling constant of $J_{1,6}$ 5.9 Hz suggests a H–C-6–C-1–H dihedral angle of 140.5°, supporting a flattening of the *myo*-inositol chair in solution. This result is consistent with the distortion observed when the crystal structure was optimised using semi-empirical calculations in MOPAC93 [20] with PM3 and AM1 parameter sets, which gave H–C-6–C-1–H torsion angles of 132–142°.

The structure shows the unusual existence of two half-occupied hydrogen atom sites (H-51 or H-53 attached to O-9; H-52 or H-54 attached to O-12) on both the 3- and 6-hydroxyl groups at both temperatures. All four sites are a covalent bond distance from one oxygen atom and a hydrogen-bond distance from an oxygen in a symmetry-related molecule, with O · · · O contact distances of 2.75–2.77 Å (Table 4). These intermolecular hydrogen bonds produce tetramers (Fig. 2) about the centres of symmetry at (0, 0.5, 0) and (0, 0, 0.5). Incompatible hydrogen atom sites (H-51 with H-54 or H-52 with H-53) lead to impossibly close O–H · · · H–O interactions with H · · · H contacts as short as 1.13 Å. Such coordinated hydrogen atom disorder is comparable with a phenomenon seen in neutron diffraction studies [22] of β-cyclodextrins, termed flip-flop hydrogen bonding. If the disorder is dynamic, the hydrogen atoms flip from one position to the other in a concerted mechanism which is favoured due to the entropic contribution of two equivalent or nearly equivalent states [23], and only a time-averaged equilibrium is observed. Confirmation of dynamic disorder, as opposed to statistical disorder in which the hydrogen bonds are ordered one way in one unit cell and the opposite way in another, was obtained for β-cyclodextrin in two ways. Calorimetric measurements indicated an exothermic reaction at 227 K which corresponds to ordering of hydrogen bonds, and neutron diffraction studies at 173 K showed the disappearance of flip-flop hydrogen bonds [24]. However, **1** yielded no such evidence. Differential scanning calorimetry on a crystalline powder of **1** recorded no exothermic reaction between 293 and 103 K, and the low temperature (150 K) X-ray data for **1** still showed the flip-flop effect. Thus the disorder may be statistical throughout the temperature range, or dynamic disorder at room temperature may change to statistical disorder below 103 K, or if ordering occurs, it must take place below 103 K.

The reaction of **1** with NaH and **2** occurs selectively at the 1/3 position beginning with deprotonation of O-9. The selectivity could be attributed to differences in stability of the O-9- and O-12-deprotonated anions, or easier access of **2** to the O-9-position of the anion. Semi-empirical calculations with full optimisation of geometry were carried out using MOPAC93 [20] in both the AM1 and the PM3 parameter sets for **1** with hydrogen atom sites H-51 and H-52 occupied and again with H-53 and H-54, as well as the four monoanions resulting from removal of each OH hydrogen atom in turn. The calculated heats of formation (Table 5) show that the free molecule of **1** is 12–18 kJ mol^{-1} more stable with hydrogen atom sites H-53 and H-54 occupied than with H-51 and H-52. The anions resulting from deprotonation of O-9 are ca. 14–22 kJ mol^{-1} less stable than those from O-12, irrespective of the site occupied by the surviving H atom. Near-neighbour intramolecular contacts to O-9 and O-12 (Fig. 3) are fairly similar, any difference in openness slightly favouring O-12. The angles X · · · O–C involving the designated atoms and the C–O bonds are ≤ 70°, suggesting that a clear hemisphere is available in the imme-

Table 4
Geometry of flip-flop hydrogen bonding in **1** at 293 and 150 K with estimated standard deviations in parentheses. The O–H vectors have been extended to a bond distance of 0.967 Å [21]

	$d_{O-H\cdots O}$ (Å)		$\alpha_{O-H\cdots O}$ (°)		Symmetry [a]
	150 K	293 K	150 K	293 K	
O-09–H-51 · · · O-12	1.86(4)	1.85(2)	152(5)	159(4)	(−X, 0.5 + Y, 0.5 − Z)
O-09–H-53 · · · O-12	1.77(3)	1.79(2)	176(5)	171(4)	(X, 0.5 − Y, −0.5 + Z)
O-12–H-52 · · · O-09	1.80(3)	1.81(2)	162(5)	164(4)	(X, 0.5 − Y, 0.5 + Z)
O-12–H-54 · · · O-09	1.85(3)	1.83(2)	155(5)	164(4)	(−X, −0.5 + Y, 0.5 − Z)

[a] Symmetry transformation to be applied to acceptor atom.

Table 5
Energies in kJ mol^{-1} calculated by semi-empirical molecular orbital AM1 and PM3 methods for **1** and its anions with respect to hydroxy H atom site occupation

		ΔH_f	
H(O-9) Site	H(O-12) Site	AM1	PM3
H-51	H-52	−1224.0	−1113.1
H-53	H-54	−1235.8	−1131.1
H-51	–	−1254.6	−1149.8
–	H-52	−1233.8	−1135.6
H-53	–	−1254.6	−1157.1
–	H-54	−1233.1	−1139.4

diate vicinity of the O atoms for the approach of molecule **2**. Thus the available evidence suggests that the observed selectivity cannot be attributed to either OH acidity or steric effects. The greater reactivity of the OH group at position 3 in analogous isopropylidene derivatives has recently been examined by Chung et al. [25], who also dismissed steric effects as the controlling factor and instead suggested its possible involvement in intramolecular hydrogen bonding. This explanation is unlikely to apply to the present reaction sequence. No intramolecular O–H · · · O hydrogen bonding occurs in the crystal structure. The O–H · · · O contacts that could be generated in solution by C–O bond rotation are closer around O-9 than O-12. Such contacts should impede the initial deprotonation step and could have no influence thereafter.

The synthesis of *myo*-inositol 3-phosphate (**6**) is shown in Scheme 1. Diol **1** was reacted with **2** in the presence of NaH in DMF. The reaction proceeds with retention of configuration at phosphorus [11] to give mainly the two diastereoisomers **3** and **4**. Attempts to separate diastereoisomers **3** and **4** by flash chromatography were unsuccessful, although it did remove starting material **2**. Slow crystallisation from

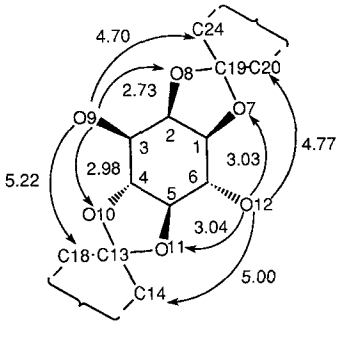
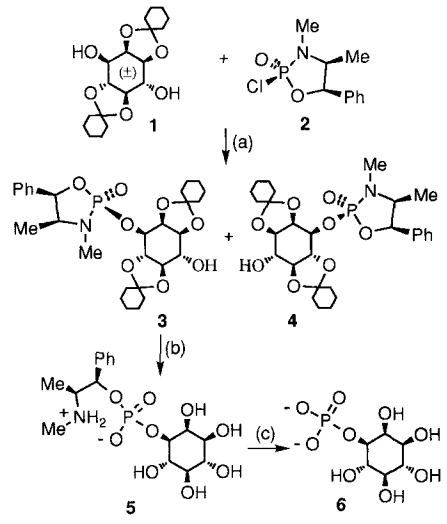


Fig. 3. Spatial distances (Å) at 293 K of O-9 and O-12 of **1** from nearby atoms.



Scheme 1. Synthesis of *myo*-inositol 3-phosphate (**6**). (a) NaH, DMF; (b) CF_3COOH, H_2O–THF; (c) H_2, Pd–C.

acetone–hexane gave a 7.5:1 ratio (^{31}P NMR) of diastereoisomers **3** and **4**. Subsequent recrystallisation gave **3** in 17% yield, which was fully characterised by ^{31}P, ^{1}H, and ^{13}C NMR spectroscopy, elemental analysis, and mass spectrometry. Diastereoisomer **4**, which was enriched in the filtrate, could not be purified by crystallisation. However, it was separated from **3** on an analytical reverse phase HPLC column.

Treatment of **3** with aqueous trifluoroacetic acid cleaved the P–N bond with inversion of configuration at phosphorus [26]. The cyclohexylidene ring was also removed under these conditions to give zwitterion **5**, δ_P −0.54 ppm, in quantitative yield.

Hydrogenolysis of **5** using 10% palladium-on-carbon under an atmosphere of hydrogen (50 psi) cleaved the substituted benzyl ester to give *myo*-inositol 3-phosphate (**6**), isolated as its cyclohexylammonium salt in 95% yield. ^{31}P NMR spectroscopy showed a single peak at δ_P 4.68 ppm, and ^{1}H and ^{13}C NMR spectra were in agreement with reported data [7]. Optical rotation measurements confirmed the formation of the 3-phosphate, rather than the 1-phosphate. Ballou and Pizer [27] showed that the optical rotation of **6** changes in sign when the pH of the solution is adjusted from acidic [pH 2, +9.3°] to basic [pH 9, −3.2°]. Values obtained here were +8.9° at pH 1.63 and −3.6° at pH 11.4, confirming the synthesis of **6**.

In summary, the use of a chiral phosphorylating agent enables both the phosphorylation and resolution to be achieved in one step, providing a short synthesis of *myo*-inositol 3-phosphate in only four steps

from *myo*-inositol. Extensions to this study include the synthesis of *myo*-inositol 3-thiophosphate from the corresponding P=S phosphorylating agent to ultimately probe the stereochemical course of IMPase.

3. Experimental

General.—NMR spectra were recorded on a Bruker AC-250 spectrometer: ^{1}H (250.1 MHz), ^{31}P (101.3 MHz), and ^{13}C (62.9 MHz); or a Varian Unity 500 spectrometer: ^{1}H (499.9 MHz). The ^{1}H NMR spectra were referenced to tetramethylsilane unless otherwise stated, ^{31}P NMR spectra were referenced to 85% phosphoric acid, and ^{13}C NMR spectra were referenced to $CDCl_3$ or Me_2SO-d_6 in D_2O. All ^{13}C and ^{31}P NMR spectra were ^{1}H decoupled unless otherwise stated. Infrared spectra were recorded on a FT-IR Mattson 3000 Series spectrometer. Mass spectra were recorded using a VG 7070E instrument: FAB spectra were recorded with a 3-nitrobenzyl alcohol or PEG matrix, while CI mass spectra were recorded using NH_3 as carrier gas. Melting points were measured on a Kofler Reichert-Jung hot stage. Elemental analyses were measured by Butterworths Laboratories, Middlesex. Flash column chromatography [31] was performed using Sorbsil C60 40/60H silica gel. TLC was performed using plastic-backed Kieselgel 60 silica gel plates containing a fluorescent indicator. Spots were visualised under 254 nm UV light or with the aid of iodine. Optical rotation was measured on an AA-100 polarimeter, Optical Activity Ltd. THF was dried by heating under reflux over Na-benzophenone. (*2R,4S,5R*)-2-Chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-one (**2**) was prepared by the method of Cooper et al. [11] from (−)-ephedrine and phosphorus oxychloride.

Crystal structure determination of **1** *at 293 K* [3]. —$C_{18}H_{28}O_6$, M = 340.4. Monoclinic, a = 12.445(2), b = 11.050(2), c = 12.652(3) Å, $\beta = 94.03(2)°$, V = 1735.6(6) Å^3 (by least-squares analysis of setting angles of 25 reflections, $9.4 \leq \theta \leq 13.8°$, $\lambda = 0.71073$ Å), space group $P2_1/c$, Z = 4, $D_x = 1.303$ Mg m^{-3}. Rectangular plate crystal with dimensions 0.65 × 0.60 × 0.08 mm, μ(Mo-Kα) = 0.10 mm^{-1}.

Enraf–Nonius CAD4 diffractometer, ω–2θ scan technique, with ω scan width = 0.9 + 0.35 tan θ, ω scan speed 0.4–2.8° min^{-1}, graphite-monochromated Mo-Kα radiation: 6192 reflections measured ($2 \leq \theta \leq 25°$, for $-14 \leq h \leq 0$, $-13 \leq k \leq 13$, $-14 \leq l \leq 15$), 3039 unique (merging R = 0.024 after correction for Lorentz and polarisation factors), giving 2171 with $F_o > 4\sigma(F_o)$. Linear crystal decay, ca. 3.5% corrected during processing.

Direct methods followed by full-matrix least-squares refinement on F_o^2 using SHELXL-93 [28] with all non-hydrogen atoms anisotropic and all hydrogen atoms located from difference electron density maps. The disorder of the two hydroxy groups was accommodated by refining the two possible H-atom positions on each group with occupancy factors that added to 1. All four sites were restrained to a common O–H distance with esd 0.02 Å and given a common temperature factor. Comparison of the weighted R values for the best ordered model and this disordered model gave a ratio of 1.047:1, far in excess of the requirement for significance at the 99% level by Hamilton's test [29]. The weighting scheme was $w = 1/[\sigma^2(F_o^2) + (0.0278P)^2 + 0.3664P]$ where $P = (F_o^2 + 2F_c^2)/3$. Final discrepancy indices are R = 0.029 for observed reflections, $wR(F^2) = 0.080$ for all reflections. Final maximum shift/esd was < 0.001, and maximum/minimum features on a difference electron density map were 0.18 and −0.13 e Å^{-3}.

Crystal structure determination of **1** *at 150 K* [3]. —Cell dimensions: a = 12.273(2), b = 11.037(5), c = 12.620(4) Å, $\beta = 94.92(2)°$, V = 1703.2(10) Å^3 (by least-squares analysis of 2θ values of 32 reflections obtained by measurements at $\pm\omega$, $28 \leq \theta \leq 30°$, $\lambda = 0.71073$ Å), $D_x = 1.328$ Mg m^{-3}, μ(Mo-Kα) = 0.10 mm^{-1}.

Stoe Stadi-4 diffractometer equipped with an Oxford Cryosystems open-flow cryostat [30], ω–2θ scan technique, graphite-monochromated Mo-Kα radiation: 2169 reflections measured ($2.9 \leq \theta \leq 25.0°$ for $-14 \leq h \leq 10$, $0 \leq k \leq 11$, $0 \leq l \leq 13$), 2165 unique (merging R = 0.038 after correction for Lorentz and polarisation factors), giving 1523 with $F_o > 4\sigma(F_o)$. Linear crystal decay, ca. 1.7% corrected during processing.

Non-hydrogen atom coordinates were taken from the refinement at 293 K, followed by full-matrix least-squares refinement on F_o^2 using SHELXL-93 [28] with all non-hydrogen atoms anisotropic and all hydrogen atoms located from difference electron den-

[3] Tables of fractional atomic coordinates, bond lengths, bond angles, torsion angles, and the list of thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. These tables may be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

sity maps. The same two-fold hydroxy group disorder was found as at 293 K and treated in the same manner. Temperature factors were kept fixed for hydrogen atoms H-25 and H-30. The weighting scheme was $w = 1/[\sigma^2(F_o^2) + (0.0994P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$. Final R and $wR(F^2)$ values are 0.046 (observed data), 0.134 (all data). Final maximum shift/esd was < 0.001, and maximum/minimum features on a difference electron density map were 0.28 and -0.31 e Å^{-3}.

*(±)-1,2;4,5-Di-*O*-cyclohexylidene-*myo*-inositol* (**1**).—Diol **1** was prepared by the method of Dreef et al. [13] from *myo*-inositol, 1,1-diethoxycyclohexane, and *p*-toluenesulfonic acid which crystallised from acetone–hexane to form rectangular plate crystals: mp 176–179 °C, lit. 172–174 °C [13]; ^{1}H NMR (250.1 MHz, $CDCl_3$, assigned with the aid of a COSY spectra, D_2O shake, and decoupling experiments): δ 1.4–1.8 (m, 20 H, CH_2), 2.70 (d, 1 H, $J_{OH,H}$ 8.9 Hz, 3-OH), 3.15 (d, 1 H, $J_{OH,H}$ 3.0 Hz, 6-OH), 3.30 (dd, 1 H, $J_{5,4}$ 9.4, $J_{5,6}$ 10.6 Hz, H-5), 3.82 (dd, 1 H, $J_{4,3} \sim J_{4,5} \sim 9.75$ Hz, H-4), 3.83–3.90 (m, 1 H, H-6) (gives on D_2O shake dd, $J_{6,1}$ 6.2, $J_{6,5}$ 10.2 Hz, H-6), 3.99 (dd, 1 H, $J_{3,2}$ 4.5, $J_{3,4}$ 9.3 Hz, H-3), 4.06 (dd, 1 H, $J_{1,2} \sim J_{1,6} \sim 5.7$ Hz, H-1), 4.46 (dd, 1 H, $J_{2,1} \sim J_{2,3} \sim 4.8$ Hz, H-2).

D-*1,2;4,5-Di-*O*-cyclohexylidene-3-[(2′*S*,4′*S*,5′*R*)-3′,4′-dimethyl-5′-phenyl-1′,3′,2′-oxazaphospholidin-2′-one]-*myo*-inositol* (**3**).—Diol **1** (2.0 g, 5.88 mmol) and 95% NaH (0.155 g, 6.47 mmol) in dry DMF (10 mL) was stirred for 1 h at 0 °C under argon. To this mixture was added dropwise a soln of **2** (1.44 g, 5.88 mmol) in dry DMF (6 mL). The mixture was stirred for 2 h, and over this time the temperature was warmed to 20 °C. The reaction mixture was concd in vacuo. ^{31}P NMR ($CDCl_3$) analysis of the crude reaction mixture showed the presence of four diastereoisomers at δ 19.4 (**3**, 43%), 19.1 (**4**, 40%), 18.9 and 18.7 (4-/6-phosphorylation, total 12%), together with starting material **2** (δ 20.9, 5%). Flash chromatography of the residue eluting with EtOAc, separated products from starting material. Slow crystallisations from acetone–hexane afforded **3** (0.669 g, 17%): mp 212.5–214 °C; R_f (1:1 EtOAc–hexane) 0.12; IR (KBr): ν 3410 (OH), 1240 cm^{-1} (P=O); ^{1}H NMR (499.9 MHz, $CDCl_3$, assigned with the aid of COSY spectra and decoupling experiments): δ 0.80 (d, 3 H, J_{HH} 6.6 Hz, $CHCH_3$), 1.3–1.8 (m, 20 H, $10 \times CH_2$), 2.60 (d, 1 H, J_{HH} 3.0 Hz, 6-OH), 2.75 (d, 3 H, J_{PH} 10.4 Hz, NCH_3), 3.39 (dd, 1 H, J_{HH} 9.5, J_{HH} 10.7 Hz, 5-H), 3.75 (ddq, 1 H, J_{PH} 23.6, $J_{CHCH_3,CH_3} \sim J_{CHCH_3,CHPh} \sim 6.6$ Hz, $CHCH_3$), 3.89 (ddd, 1 H, J_{HH} 10.7, J_{HH} 9.6, $J_{6,6\text{-OH}}$ 3.0 Hz, 6-H), 4.0–4.1 (m, 2 H, 1-H and 4-H), 4.61 (t, 1 H, $J_{2,1} \sim J_{2,3} \sim 4.7$ Hz, 2-H), 4.87 (ddd, 1 H, $J_{3,2}$ 4.5, $J_{3,4}$ 9.6, J_{PH} 10.7 Hz, 3-H), 5.64 (dd, 1 H, J_{PH} 3.7, $J_{CHPh,CHCH_3}$ 6.5 Hz, $CHPh$), 7.3–7.4 (m, 5 H, Ph); ^{13}C NMR (assignments made with the aid of a DEPT spectrum): δ 14.1 (d, CCH_3, J_{PC} 1.9 Hz), 23.8, 24.0, 24.2, 24.4, 25.3, 35.1, 36.6, 36.8, 38.4 ($10 \times CH_2$, 1 overlapping), 29.2 (d, NCH_3, J_{PC} 4.9 Hz), 59.6 (d, $CHCH_3$, J_{PC} 13.9 Hz), 75.2 (d, J_{PC} 6.2 Hz, inositol CH), 75.5 (inositol CH), 75.8 (d, J_{PC} 6.7 Hz, inositol CH), 76.9 (inositol CH), 78.1 (inositol CH), 81.2 (d, J_{PC} 2.3 Hz, inositol CH), 81.7 (*C*HPh), 111.3, 113.6 (C-1 of cyclohexylidene), 126.5 (arom CH), 128.6 (arom CH), 136.5 (d, J_{PC} 6.7 Hz, C-1 of Ph); ^{31}P NMR: δ 19.9; MS (CI): observed accurate mass 550.257 $[M + H]^+$, $C_{28}H_{41}NO_8P$ requires 550.257 $[M + H]^+$. Anal. Calcd for $C_{28}H_{40}NO_8P \cdot 0.5H_2O$: C, 60.2; H, 7.4; N, 2.5. Found C, 60.5; H, 7.3; N, 2.6.

*(1′R,2′S)-*P*-(2′-Methylamino-1′-phenylpropyl)-myo-inositol 3-phosphate* (**5**).—Diastereoisomer **3** (1.45 g, 2.64 mmol) in THF (3.1 mL) was stirred with CF_3COOH (0.95 mL, 12.41 mmol) and H_2O (2.5 mL) for 15 min at 20 °C. The crude product was concd in vacuo to afford zwitterion **5** (1.05 g, 97.9%), mp 150–152 °C; IR (KBr): ν 3500–3000 (OH), 1210 cm^{-1} (P=O); ^{1}H NMR (250.1 MHz, D_2O, referenced to acetone at 2.22 ppm, assigned with the aid of a COSY spectrum and decoupling experiments): δ 1.18 (d, 3 H, J_{HH} 6.8 Hz, $CHCH_3$), 2.78 (s, 3 H, NCH_3), 3.24 (t, 1 H, $J_{5,4} \sim J_{5,6} \sim 9.3$ Hz, 5-H), 3.45 (dd, 1 H, $J_{1,6}$ 10.0, $J_{1,2}$ 2.7 Hz, 1-H), 3.55–3.65 (m, 2 H, 6-H and $CHCH_3$), 3.71 (t, 1 H, $J_{4,3} \sim J_{4,5} \sim 9.6$ Hz, 4-H), 3.93 (ddd, 1 H, $J_{3,4}$ 10.2, J_{PH} 7.5, $J_{3,2}$ 2.7 Hz, 3-H), 4.11 (t, 1 H, $J_{2,1} \sim J_{2,3} \sim 2.7$ Hz, 2-H), 5.56 (dd, 1 H, J_{PH} 9.0, J_{HH} 2.9 Hz, $CHPh$), 7.4–7.5 (5 H, m, Ph); ^{13}C NMR (referenced to Me_2SO-d_6 at 39.7 ppm, assignments made with the aid of a DEPT spectrum): δ 18.3 ($CHCH_3$), 38.9 (NCH_3), 68.9 (d, J_{PC} 6.1 Hz, $CHCH_3$), 79.3 (inositol CH), 80.0 (inositol CH), 80.1 (inositol CH), 80.8 (inositol CH), 82.6 (inositol CH), 84.9 (d, J_{PC} 5.2 Hz, inositol CH), 85.2 (d, J_{PC} 6 Hz, *C*HPh), 135.1 (arom CH), 137.4 (arom CH), 137.5 (arom CH), 144.4 (C-1 of Ph); ^{31}P NMR: δ −0.54; MS (positive ion FAB): observed accurate mass 408.1413 $[M + H]^+$, $C_{16}H_{27}NO_9P$ requires 408.1423 $[M + H]^+$.

Cyclohexylammonium D-myo-*inositol 3-phosphate* (**6**).—A soln of zwitterion **5** (0.081 g, 0.20 mmol) in

MeOH–H_2O (8:2, 30 mL) with palladium-on-carbon (40 mg, 10%) was shaken in a Parr pressure reaction-apparatus under an atmosphere of hydrogen (50 psi) for 16 h at 20 °C. The catalyst was removed by filtration through Celite and the product was evaporated in vacuo. The residue was dissolved in water (4 mL) and stirred with cyclohexylamine (2 mL) for 4 h at 20 °C. Extraction with ether removed excess cyclohexylamine and the aq soln was concd in vacuo, yielding a solid, which was recrystallised from water–acetone to give the bis(cyclohexylammonium) salt of **6** (0.087 g, 95%): mp 191–193 °C, lit. 192–193 °C [32]; ^{1}H NMR (250.1 MHz, D_2O, referenced to acetone at 2.22 ppm, assigned with the aid of a COSY spectrum and decoupling experiments): δ [1.1–1.4 (m, 10 H), 1.59 (d, 2 H, J_{HH} 11.8 Hz), 1.73 (s, 4 H), 1.91 (s, 4 H), 3.08 (s, 2 H), 2 × cyclohexylammonium], 3.27 (t, 1 H, $J_{5,4} \sim J_{5,6} \sim 8.8$ Hz, 5-H), 3.5–3.6 (m, 2 H, 1-H and 6-H), 3.69 (t, 1 H, $J_{4,3} \sim J_{4,5} \sim 9.4$ Hz, 4-H), 3.84 (br t, 1 H, $J_{3,4} \sim J_{PH} \sim 9.9$ Hz, 3-H), 4.16 (br s, 1 H, 2-H); ^{13}C NMR (62.9 MHz, referenced to Me_2SO-d_6 at 39.7 ppm, assignments made with the aid of a DEPT spectrum): δ 26.7 (4 × CH_2, C-3 and C-5 of cyclohexylamine), 27.2 (2 × CH_2, C-4 of cyclohexylamine), 33.3 (4 × CH_2, C-2 and C-6 of cyclohexylamine), 53.2 (2 × CH, C-1 of cyclohexylamine), 73.7 (inositol CH), 74.6 (d, J_{PC} 2.9 Hz, inositol CH), 75.2 (d, J_{PC} 4.3 Hz, inositol CH), 75.2 (inositol CH), 77.2 (d, J_{PC} 5.2 Hz, inositol CH), 77.3 (inositol CH); ^{31}P NMR (^{1}H decoupled): δ 4.68 (s); ^{31}P NMR (^{1}H coupled): δ 4.68 (d, J_{PH} 7.8 Hz).

The mono(cyclohexylammonium) salt of **6** was prepared as above, but with the addition of less cyclohexylamine: MS (positive ion FAB): observed accurate mass 259.0215 $[M + H]^+$, $C_6H_{12}NO_9P$ requires 259.0219 $[M + H]^+$. Anal. Calcd for $C_{12}H_{26}NO_9P \cdot H_2O$: C, 38.2; H, 7.5; N, 3.7. Found: C, 38.4; H, 7.5; N, 4.3.

A soln of the mono(cyclohexylammonium) salt of **6** in water (3 mL) was applied to a cation-exchange column (Dowex 50-X8, mesh 20–50, 50 mL, Na^+ form) which was eluted with water (150 mL). The water was evaporated under vacuum to give the free acid of **6** as a gum (16.2 mg), which was dissolved in water (2 mL). The optical rotation was measured at 19.5 °C: $[\alpha]_D$ +8.9° (pH 1.63), lit. +9.3° (pH 2) [25]. Cyclohexylamine (28.5 μL, 4 equiv) was added to give $[\alpha]_D$ −3.56° (pH 11.14), lit. −3.2° (pH 9) [26], lit. −3.4° (pH 9) [7], lit. −2.8° (pH 9) [32], lit. −3.45° (pH 9) [33], lit. −4.9 ± 1.0 (pH 9) [34].

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