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Note

The synthesis of 2-*O*-alkyl-*myo*-inositol 1-phosphates as competitive inhibitors of inositol monophosphatase

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The receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate is now firmly established as a transmembrane signalling system which gives rise to two second messengers, namely, diacylglycerol (DAG) and 1D-*myo*-inositol 1,4,5-trisphosphate (1,4,5-IP_3). 1,4,5-IP_3 has been shown[1] to activate specific receptors on intracellular membranes and to mediate the mobilisation of intracellular calcium, whilst DAG binds to, and activates, protein kinase C. 1,4,5-IP_3 is converted into 1D-*myo*-inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP_4), which itself may play an important role in regulating the influx of calcium into the cell from the external medium[2]. A complex metabolic cycle exists in which both 1,4,5-IP_3 and 1,3,4,5-IP_4 are recycled to free inositol via a highly specific series of dephosphorylations. The final dephosphorylation step in this pathway is mediated by inositol monophosphatase. This enzyme, which has been purified to homogeneity from bovine brain[3], plays a pivotal role in regulating the supply of inositol for the synthesis of phosphatidylinositol in the central nervous system, as peripheral inositol cannot penetrate the blood–brain barrier.

It has been found[4] that the enzyme accepts both enantiomers of *myo*-inositol 1-phosphate and both enantiomers of *myo*-inositol 4-phosphate as substrates. Deletion of the flanking 2- or 6-hydroxyl group from *myo*-inositol 1-phosphate leads to compounds that are competitive inhibitors of monophosphatase activity (K_i 40 μM)[4]. We now report the synthesis of a series of racemic 2-*O*-alkyl-*myo*-inositol 1-phosphates together with the biological results, and draw conclusions on the nature of the monophosphatase enzyme.

The racemic 2-*O*-alkyl-*myo*-inositol 1-phosphates (**6**) were synthesized as shown in Scheme 1. Racemic 1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**1**) was pre-

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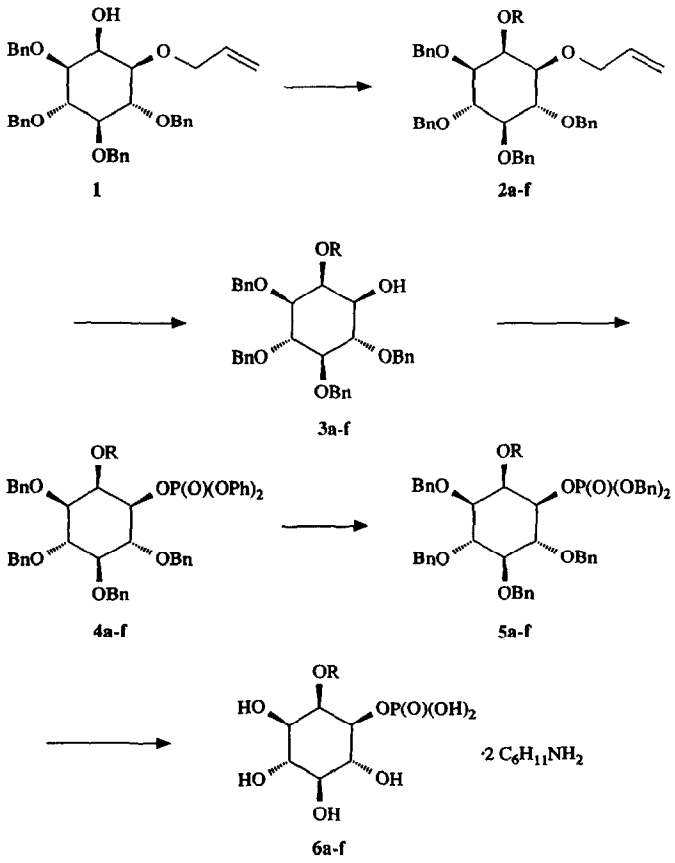


Scheme 1. **a**, R = Me; **b**, R = Et; **c**, R = cyclopropylmethyl; **d**, R = Bu; **e**, R = hexyl; **f**, R = cyclohexylmethyl.

pared via 1,2-*O*-cyclohexylidene-*myo*-inositol by an amalgamation of reported procedures[5,6]. Alkylation of **1** with the appropriate alkyl halide followed by removal of the allyl protecting group[7] gave the 2-*O*-alkyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositols (**3**) in yields ranging from 46% to 78%. Phosphorylation of **3** gave the diphenyl phosphates **4** (98–100%). Transesterification of **4**, using the anion of benzyl alcohol in tetrahydrofuran[8], gave the dibenzyl phosphates **5** (31–75%). Hydrogenolysis of **5** cleanly cleaved all of the benzyl groups to give the 2-*O*-alkyl derivatives (**6**), which were isolated as the crystalline biscyclohexylammonium salts. These compounds all gave satisfactory elemental analyses (Table I) and their structures were confirmed by ^{1}H NMR spectroscopy (see Experimental section).

All analogues synthesized were found to be competitive inhibitors of inositol monophosphatase, the most potent being the ethyl analogue **6b** (K_i 40 μM), with affinity decreasing only slowly up to the hexyl analogue **6e** (K_i 200 μM), although the cyclohexylmethyl analogue **6f** ($K_i > 600$ μM) is significantly less active (Table II).

TABLE I

Microanalytical data and melting points for the racemic 2-*O*-alkyl-*myo*-inositol 1-phosphates **6a–f**

Compound	Mp (deg)	Formula	Anal. (%)					
			Calcd			Found		
			C	H	N	C	H	N
6a	209–216	$C_{19}H_{41}N_2O_9P \cdot 2.4H_2O$	44.25	8.95	5.43	44.35	8.94	5.15
6b	232–238	$C_{20}H_{43}N_2O_9P \cdot 2.4H_2O$	45.34	9.09	5.29	45.44	9.04	5.12
6c	236–239	$C_{22}H_{45}N_2O_9P \cdot 1.1H_2O$	49.63	8.94	5.26	49.59	9.00	5.12
6d	238–242	$C_{22}H_{47}N_2O_9P \cdot 1.0H_2O$	49.61	9.27	5.26	49.79	8.99	5.10
6e	244–254	$C_{24}H_{51}N_2O_9P \cdot 2.4H_2O$	49.20	9.60	4.78	49.31	9.48	4.40
6f	256–270	$C_{25}H_{51}N_2O_9P \cdot 2.0H_2O$	50.83	9.39	4.74	50.56	9.30	4.46

These results may be compared with those obtained[4] for 2-deoxy-*myo*-inositol 1-phosphate (**7**), for which it was found that one enantiomer, (+)-**7**, was a weak substrate showing little inhibitory potency whilst (−)-**7** was a competitive inhibitor of inositol monophosphatase displaying no substrate activity. It can be seen that the data support the hypothesis that only one flanking hydroxyl group in *myo*-inositol 1-phosphate is required for binding to inositol monophosphatase whilst the other group is involved in the mechanism of phosphate hydrolysis (Fig. 1). Thus, compounds **6a–f** are inhibitors of the enzyme, as the mechanistic hydroxyl group in one of the enantiomers has been replaced by an alkoxy group. The mechanism of hydrolysis must involve the H atom of the hydroxyl group since none of the alkoxy analogues were substrates for the enzyme, as demonstrated by the non-release of inorganic phosphate. However, no additional binding is gained by these analogues over the 2-deoxy analogue, so it seems likely that the alkyl group does not interact significantly with the active site, although there is obviously considerable space in this region.

EXPERIMENTAL

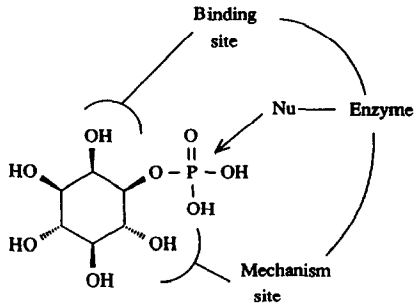
General methods.—Melting points were determined on a Büchi capillary melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker AM360 spectrometer. Fast atom bombardment mass spectra were obtained on a VB70-250 instrument. Analytical TLC was carried out on pre-coated Silica Gel 60 F_{254} plates (Merck) with UV detection. Column chromatography was

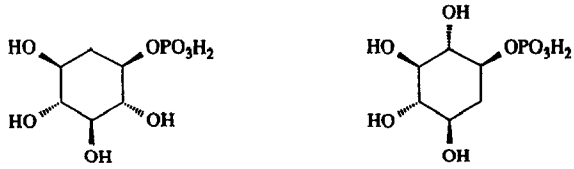
TABLE II

Inhibitory constants of inositol monophosphatase for the racemic 2-*O*-alkyl-*myo*-inositol 1-phosphates **6a–f**

Compound	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**
K_i (μM) [a]	70	40	110	150	210	> 600

[a] These values were determined using standard methods[3,9].





(+)-7 (-)-7

Fig. 1. Proposed recognition mode of *myo*-inositol 1-phosphate by the inositol monophosphatase enzyme, together with the enantiomers of 2-deoxy-*myo*-inositol 1-phosphate [(+)- and (−)-7].

performed on Silica Gel 60 (220–440 mesh, Fluka) under low pressure. Solutions were evaporated on a Büchi rotary evaporator under reduced pressure. Light petroleum refers to that fraction having a boiling range of 60–80°.

Detailed experimental protocols will be given below for only one analogue (R = hexyl). All other analogues were synthesized in an analogous fashion.

1-O-*Allyl*-*3,4,5,6*-*tetra*-O-*benzyl*-myo-*inositol* (**1**).—This compound was prepared via 1,2-*O*-cyclohexylidene-*myo*-inositol by an amalgamation of reported procedures[5,6].

1-O-*Allyl*-*3,4,5,6*-*tetra*-O-*benzyl*-*2*-O-*hexyl*-myo-*inositol* (**2e**).—A solution of **1** (2.04 g, 3.52 mmol) in anhyd DMF (15 mL) was added to a stirred suspension of NaH (80% dispersion in oil; 0.317 g, 10.6 mmol) in anhyd DMF (10 mL) under N_2, and the mixture was stirred at room temperature for 40 min and then cooled in an ice bath before adding 1-iodohexane (3.12 mL, 21.1 mmol). This mixture was stirred in the dark at room temperature under N_2 for 3 days. Saturated aq NH_4Cl (5 mL) was then added and the mixture was partitioned between diethyl ether and water. The ether layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with 10–20% EtOAc–light petroleum) to afford **2e** as an oil (2.19 g, 94%). ^1H NMR ($CDCl_3$): δ 7.36–7.25 (m, 20 H, 4 Ph), 5.92 (m, 1 H, C–CH=C), 5.29 (dd, 1 H, *J* 17.2 and 1.7 Hz, C–C=CH), 5.16 (dd, 1 H, *J* 10.3 and 1.6 Hz, C–C=CH), 4.89 (dd, 2 H, *J* 10.6 and 3.3 Hz, PhCH_2O), 4.85 (s, 2 H, PhCH_2O), 4.79 (t, 2 H, *J* 10.4 Hz, PhCH_2O), 4.70 (s, 2 H, PhCH_2O), 4.13 (dt, 2 H, *J* 5.4 and 1.5 Hz, OCH_2C=C), 3.99 (t, 1 H, *J*

9.5 Hz, CHOBn), 3.95 (t, 1 H, *J* 9.5 Hz, CHOBn), 3.85 (fine t, 1 H, H-2), 3.73 (t, 2 H, *J* 6.6 Hz, OCH_2-pentyl), 3.42 (t, 1 H, *J* 9.3 Hz, CHOBn), 3.32 (dd, 1 H, *J* 9.8 and 2.3 Hz, CHO), 3.20 (dd, 1 H, *J* 9.8 and 2.3 Hz, CHO), 1.60 (m, 2 H, O–C–CH_2), 1.37 (m, 2 H, O–C–C–CH_2), 1.31–1.26 (m, 4 H, CH_2CH_2Me), 0.88 (t, 3 H, *J* 6.9 Hz, CH_3).

3,4,5,6-Tetra-O-benzyl-2-O-hexyl-myo-inositol (**3e**).—To a stirred solution of **2e** (2.18 g, 3.28 mmol) in 1:9 H_2O–EtOH (75 mL) was added tris(triphenylphosphine)rhodium(I) chloride (0.213 g, 0.23 mmol) and 1,4-diazabicyclo[2.2.2]octane (0.077 g, 0.69 mmol). The mixture was then heated at reflux under N_2 for 18 h. The solvent was evaporated and a solution of the residue in 3:1:1 THF–AcOH–H_2O (45 mL) was stirred under N_2 at room temperature for 18 h, then heated at reflux for 3 days. The solvents were evaporated and the residue was dissolved in 1:3 M HCl(aq)–THF and boiled under reflux for 18 h. The solvents were evaporated and the residue was partitioned between CH_2Cl_2 and H_2O. The aqueous layer was re-extracted with $CHCl_3$, and the two organic extracts were combined, dried ($MgSO_4$), and evaporated. Column chromatography of the residue (elution with 30–35% EtOAc–light petroleum) yielded **3e** as a colourles oil (1.39 g, 68%). ^{1}H NMR ($CDCl_3$): δ 7.34–7.28 (m, 20 H, 4 Ph), 4.93–4.78 (m, 6 H, 3 PhCH_2O), 4.70 (s, 2 H, PhCH_2O), 4.00–3.91 (m, 2 H, 2 CHOBn), 3.85 (fine t, 1 H, H-2), 3.74 (t, 1 H, *J* 9.4 Hz, CHOBn), 3.60 (m, 1 H, H-1), 3.45 (t, 2 H, *J* 9.3 Hz, OCH_2-pentyl), 3.41 (m, 1 H, H-3), 2.24 (d, 1 H, *J* 7.0 Hz, OH), 1.58 (m, 2 H, O–C–CH_2), 1.30 [m, 6 H, $(CH_2)_3$Me], 0.89 (t, 3 H, *J* 6.6 Hz, CH_3).

3,4,5,6-Tetra-O-benzyl-2-O-hexyl-myo-inositol 1-(diphenyl phosphate) (**4e**).—To a solution of **3e** (1.39 g, 2.22 mmol) and 4-dimethylaminopyridine (0.023 g, 0.19 mmol) in anhyd CH_2Cl_2 (30 mL) was added triethylamine (1.02 mL, 7.32 mmol), then diphenyl chlorophosphate (0.46 mL, 2.22 mmol). The resulting solution was stirred under N_2 for 3 days. Water was then added and the aqueous layer was re-extracted with CH_2Cl_2. The two organic extracts were combined, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with 25–30% EtOAc–light petroleum), to give **4e** as a colourless oil (1.86 g, 98%). ^{1}H NMR ($CDCl_3$): δ 7.31–7.11 (m, 30 H, 6 Ph), 4.91–4.74 (m, 6 H, 3 PhCH_2O), 4.66 (m, 2 H, PhCH_2O), 4.46 (m, 1 H, H-1), 4.11 (fine t, 1 H, H-2), 4.06 (t, 1 H, *J* 9.5 Hz, CHOBn), 4.00 (t, 1 H, *J* 9.5 Hz, CHOBn), 3.75 (q, 1 H, *J* 8.6 Hz, OC*H*-pentyl), 3.49 (m, 1 H, OC*H*-pentyl), 3.48 (t, 1 H, *J* 9.3 Hz, CHOBn), 3.42 (dd, 1 H, H-3), 1.63–1.50 (m, 2 H, O–C–CH_2), 1.27 [m, 6 H, $CH_2)_3$Me], 0.88 (t, 2 H, *J* 6.4 Hz, CH_3).

3,4,5,6-Tetra-O-benzyl-2-O-hexyl-myo-inositol 1-(dibenzyl phosphate) (**5e**).—To a stirred suspension of NaH (80% dispersion in oil; 0.130 g, 4.33 mmol) in anhyd THF under N_2 was added benzyl alcohol (0.45 mL, 4.35 mmol), then a solution of **4e** (1.846 g, 2.15 mmol) in anhyd THF. The mixture was stirred at room temperature under N_2 for 3 h. Saturated aq NH_4Cl (5 mL) was then added and the mixture was partitioned between diethyl ether and water. The aqueous layer was re-extracted with more ether, and the two ether extracts were combined, dried

($MgSO_4$), and evaporated. Column chromatography of the residue (elution with 30–40% EtOAc–light petroleum) afforded **5e** as a colourless oil (1.343 g, 70%). ^{1}H NMR ($CDCl_3$): δ 7.38–7.17 (m, 30 H, 6 Ph), 5.00–4.70 (m, 10 H, 5 PhCH_2O), 4.60 (m, 2 H, PhCH_2O), 4.19 (m, 1 H, H-1), 4.12 (fine t, 1 H, H-2), 3.99 (m, 2 H, 2 C*H*OBn), 3.74 (m, 1 H, OC*H*-pentyl), 3.65 (m, 1 H, OC*H*-pentyl), 3.44 (t, 1 H, *J* 9.2 Hz, C*H*OBn), 3.35 (dd, 1 H, H-3), 1.65–1.50 (m, 2 H, O–C–CH_2), 1.25 [m, 6 H, (CH_2)$_3$Me], 0.86 (t, 3 H, *J* 6.7 Hz, CH_3).

*2-O-Hexyl-*myo*-inositol 1-phosphate, dicyclohexylammonium salt* (**6e**).—A mixture of **5e** (0.661 g, 0.747 mmol) and 10% Pd–C (0.224 g) in 4:1 EtOH–H_2O (200 mL) was hydrogenated at 50 psi for 18 h. The mixture was filtered, the solid was washed with EtOH–H_2O and then H_2O, and the combined filtrates were evaporated. The residue was passed down a column (1 × 7 cm) of Dowex 50-X8-200 (H^+) resin by elution with water. The acidic fractions were combined, treated with excess of cyclohexylamine, stirred for 2 h, and then washed with diethyl ether (3 × 25 mL). The remaining aqueous phase was freeze-dried to leave **6e** (0.352 g, 87%) as a white solid, mp 244–254° (from H_2O–Me_2CO). ^{1}H NMR (D_2O): δ 3.99 (fine t, 1 H, H-2), 3.95–3.91 (m, 2 H, H-1 and OC*H*-pentyl), 3.74 (t, 1 H, *J* 9.5 Hz, C*H*OBn), 3.70 (m, 1 H, OC*H*-pentyl), 3.64–3.55 (m, 2 H, H-3 and C*H*OBn), 3.30 (t, 1 H, *J* 8.9 Hz, C*H*OBn), 3.16 (m, 2 H, CHN), 1.98 (m, 4 H), 1.64 (m, 2 H), 1.60 (m, 2 H, O–C–CH_2), 1.38–1.32 (m, 14 H), 1.19 (m, 2 H), 0.88 (t, 3 H, *J* 7.0 Hz, CH_3). Mass spectrum (FAB): 343 $[M - H]^-$.

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