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6-DEOXY-6-HYDROXYMETHYL *SCYLLO*-INOSITOL 1,2,4-TRISPHOSPHATE: A POTENT AGONIST AT THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR

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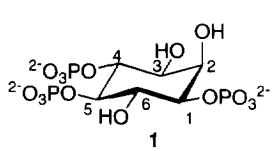
Abstract: The synthesis of racemic 6-deoxy-6-hydroxymethyl *scyllo*-inositol 1,2,4-trisphosphate is described. This compound is a highly potent agonist at the platelet D-*myo*-inositol 1,4,5-trisphosphate receptor, and it binds to the rat cerebellar receptor with an affinity equal to that of the natural ligand. These results suggest that the 5″-hydroxymethyl group of adenophostin A may contribute to its unusual potency. Copyright © 1996 Elsevier Science Ltd

The binding of many hormones, neurotransmitters and growth factors to their extracellular receptors results in the production of the second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, (**1**)] *via* activation of phosphoinositidase C. Ins(1,4,5)P_3 interacts with a family of Ins(1,4,5)P_3 -receptor-operated Ca^{2+} channels to mobilise non-mitochondrial intracellular Ca^{2+} stores in a vast array of cell types.[1] Many analogues of Ins(1,4,5)P_3 have been synthesised in recent years, and the various alterations that have been made to the structure of the Ins(1,4,5)P_3 molecule[2] have often resulted in a reduction in activity. However, the adenophostins A (**2**) and B (**2a**), isolated from cultures of *Penicillium brevicompactum*,[3] and radically different from Ins(1,4,5)P_3 in structure, have been reported to possess Ca^{2+}-mobilising potencies much higher than Ins(1,4,5)P_3 itself.[4] We are currently engaged in the synthesis of analogues[5,6] with the aim of establishing the minimum structural requirements for this activity. In the adenophostins, there is no equivalent to the 2-hydroxyl group of Ins(1,4,5)P_3, with this position being occupied by the pyranoside oxygen, and this is in accordance



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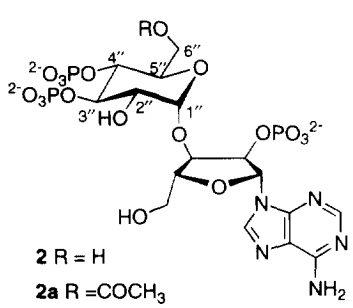
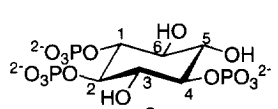


2 R = H

2a R = $COCH_3$



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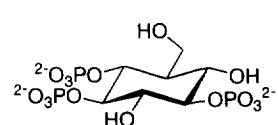
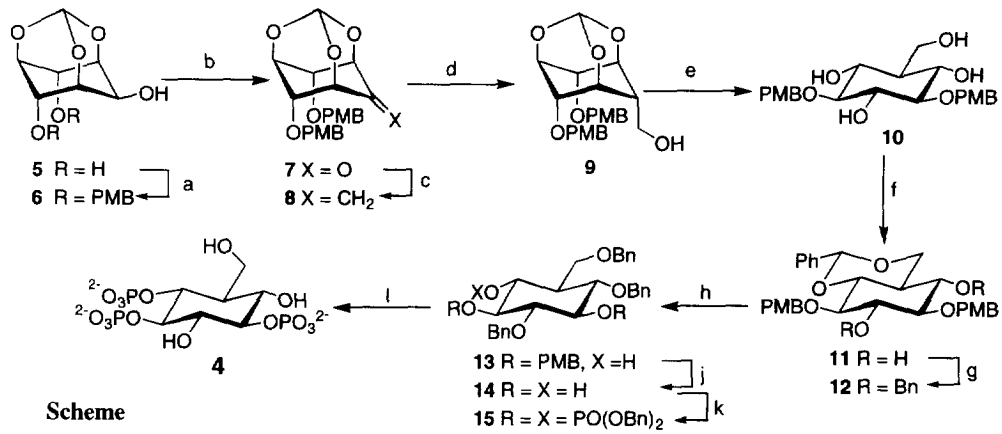


4

Figure 1

with studies that have shown the minimal importance of this feature of Ins(1,4,5)P_3 for recognition by its receptor.[7] Thus, one of the most potent synthetic Ins(1,4,5)P_3 receptor agonists to-date is *scyllo*-Ins(1,2,4)P_3 (**3**),[8,9] which differs from Ins(1,4,5)P_3 solely in the orientation of this hydroxyl group, and shows only slightly reduced potency relative to Ins(1,4,5)P_3. The effect of placing increasingly bulky groups at the equatorial 3-position of D-*myo*-Ins(1,4,5)P_3, has been investigated by ourselves[10] and others[11,12] and the results show that affinity for the Ins(1,4,5)P_3 receptor dramatically falls off with increasing molecular volume of the substituent. It might therefore seem odd that bulky structures at the 5″-position in the adenophostins, which presumably occupies a similar position at the receptor to the 3-position of Ins(1,4,5)P_3, should be compatible with high potency.[13] We have therefore synthesised **4**, an analogue of *scyllo*-Ins(1,2,4)P_3, which bears an equatorial hydroxymethyl group at this position, analogous to the 5″-hydroxymethyl group of adenophostin A, and compared its biological activity with D-*myo*-Ins(1,4,5)P_3 and DL-*scyllo*-Ins(1,2,4)P_3.



Scheme

Reagents and conditions: a) NaH (2.1equiv), PMBCl (2.0 equiv), DMF, 40%; b) DMSO, $(COCl)_2$, CH_2Cl_2, -60°C then Et_3N, 92%; c) CH_3PPh_3Br, *t*-BuOK, THF, reflux, 91% d) i) 9-BBN, THF, 50°C ii) H_2O_2, OH^-, 97%; e) 1M HCl / MeOH 1:10, 50°C, 30 min, 87%; f) $C_6H_5CH(OMe)_2$, DMF, *p*-TsOH, 70°C, -MeOH, 93%; g) NaH, BnBr, DMF, 94%; h) $Me_3N{\bullet}BH_3$, $AlCl_3$, 4Å sieves, THF, 0°C, 23 h, 65%; j) 1M HCl / EtOH 1:2, reflux, 87%; k) i) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2 ii) *m*-CPBA, -78°C, 85%; l) Na/liq NH_3, -78°C, 71%.
PMB = *p*-methoxybenzyl; Bn = benzyl; All asymmetrical compounds are racemic.

The key protected intermediate **12**[14] was synthesised from *myo*-inositol orthoformate **5**[15] as shown in the **Scheme**. Regioselective reduction of the benzylidene acetal using borane-trimethylamine complex / aluminium chloride[16] gave the alcohol **13** in 65% yield. The *p*-methoxybenzyl groups were removed by acid hydrolysis giving triol **14** (87%). Phosphitylation with bis-(benzyloxy)-*N*,*N*-diisopropylaminophosphine / 1*H*-tetrazole,[17] followed by oxidation of phosphites with *m*-CPBA gave the fully-protected trisphosphate triester **15** (85%). Deprotection with sodium in liquid ammonia[18] gave racemic **4**, which was purified by ion-exchange chromatography on Sepharose Q Fast Flow resin, isolated as the pure triethylammonium salt in 71% yield, and quantified by total phosphate assay.

The ability of **4** to release $^{45}Ca^{2+}$ from permeabilised rabbit platelets[19] was examined. The results, shown in **Figure 2** demonstrate that **4** is equal in potency to Ins(1,4,5)P_3 in this assay, despite being racemic. Furthermore, **4** was significantly more active than racemic *scyllo*-Ins(1,2,4)P_3 in the same assay.

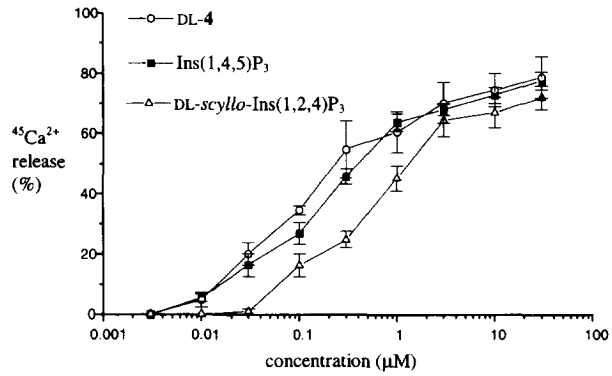


Figure 2 $^{45}Ca^{2+}$ release from permeabilised rabbit platelets induced by Ins(1,4,5)P_3 (**1**), DL-*scyllo*-Ins(1,2,4)P_3 (**3**), and DL-6-deoxy-6-hydroxymethyl-*scyllo*-Ins(1,2,4)P_3 (**4**). Permeabilised platelets preloaded with $^{45}Ca^{2+}$ were treated with Ins(1,4,5)P_3 or analogues for 3 minutes at 4°C. Release of $^{45}Ca^{2+}$ was terminated by rapid filtration and is given as a percentage of maximal $^{45}Ca^{2+}$ releasable upon treatment of platelets with 75μM ionomycin. The values are the mean ± S.E.M. of six separate experiments, each performed in triplicate.

When **4** was tested for inhibition of specific [^{3}H]Ins(1,4,5)P_3 binding to rat cerebellar membranes,[20] the results (see **Table 1**) were in full agreement with the $^{45}Ca^{2+}$ release data. Racemic **4** was equipotent to Ins(1,4,5)P_3, and more potent than racemic *scyllo*-Ins(1,2,4)P_3. Presumably, only one enantiomer of **4** is responsible for the observed activity, and this should be one of the most potent synthetic Ins(1,4,5)P_3 analogues yet identified.

Table 1 Binding and $^{45}Ca^{2+}$ release data* for Ins(1,4,5)P_3 (**1**), DL-*scyllo*-Ins(1,2,4)P_3 (**3**) and DL-6-deoxy-6-hydroxymethyl-*scyllo*-Ins(1,2,4)P_3 (**4**).

Compound	Binding (IC_{50}/μM)	$^{45}Ca^{2+}$ release (EC_{50}/μM)
Ins(1,4,5)P_3	0.04 ± 0.01	0.40 ± 0.11
DL-*scyllo*-Ins(1,2,4)P_3	0.15 ± 0.02	1.67 ± 0.35
4	0.027 ± 0.01	0.44 ± 0.26

*Displacement of specific [^{3}H]Ins(1,4,5)P_3 binding from rat cerebellar membranes, and $^{45}Ca^{2+}$ release from permeabilised rabbit platelets were used to determine the IC_{50} and EC_{50} values respectively. Both studies were performed at 4°C. Each result is given as the mean ± S.E.M. for at least three experiments.

The observation that racemic **4** is equipotent with Ins(1,4,5)P_3 implies that the CH_2OH component, which is not present in Ins(1,4,5)P_3 itself, is tolerated by the Ins(1,4,5)P_3 receptor despite the additional steric bulk. The presence of an analogous structure in adenophostin A is in accordance with this finding. The results also suggest that, at least in *scyllo*-analogues of Ins(1,4,5)P_3, replacement of the secondary hydroxyl group at this position

with an hydroxymethyl group *enhances* potency at the Ins(1,4,5)P_3 receptor. This motif could therefore be of great interest in the design of Ins(1,4,5)P_3 receptor ligands. The interaction of **4** with a purified Ins(1,4,5)P_3 3-kinase preparation is currently under examination, and the enantiomers of **4** are being synthesised *via* the optical resolution of intermediate **13**.

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