Synthesis of a Conformationally Restricted Cyclic Phosphate Analog of Inositol Trisphosphate

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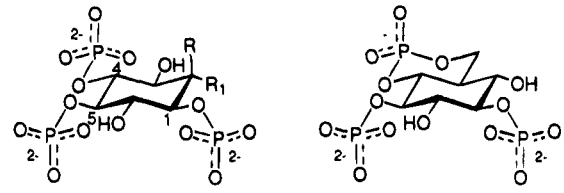
The binding of many hormones, neurotransmitters, and growth factors to their receptors results in production of the second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1** (Figure 1)] *via* activation of phosphoinositidase C. Ins(1,4,5)P_3 interacts with a family of intracellular receptor-operated Ca^{2+} channels to mobilize nonmitochondrial Ca^{2+} in many cell types,[1] and the synthesis of structurally-modified analogs offers the prospect of pharmacological intervention in this ubiquitous signaling pathway. Many such analogs have been synthesized,[2] including some that appear to behave as partial agonists.[3] However, their structural uniformity means that they provide limited insight into the nature of the Ins(1,4,5)P_3 binding site. Recently, the adenophostins A and B, isolated from cultures of *Penicillium brevicompactum,* have been reported to behave as exceptionally potent Ins(1,4,5)P_3 receptor agonists,[4] although they bear little apparent resemblance to Ins(1,4,5)P_3. Nevertheless, they contain a motif that mimics a feature also found in Ins(1,4,5)P_3, namely the vicinal *trans*-4,5-bisphosphate in a six-membered ring, something common to all agonists at the Ins(1,4,5)P_3 receptor.[5] This conformationally mobile system (or isostere[6]) is regarded as essential for binding and Ca^{2+}-release, and little work has yet focused upon modification of this region of Ins(1,4,5)P_3.[7] Thus, little is known about the way in which these two phosphate groups interact with the receptor in the series of events that leads to the opening of the integral ion channel.



1 R = OH, R_1 = H

1a R = H, R_1 = OH

2

Figure 1. D-*myo*-Inositol 1,4,5-trisphosphate (**1**), *scyllo*-inositol 1,2,4-trisphosphate (**1a**), and cyclic phosphate analog (**2**). Numbering is based on *myo*-inositol.

One way to study the interaction of a ligand with its receptor is to synthesize conformationally restrained analogs and examine the effect on biological activity. Apart from a study of the naturally occurring inositol 1:2-cyclic,4,5-trisphosphate,[8] which mobilizes Ca^{2+}, and the synthetic inositol 1-phosphate-4,5-pyrophosphate,[9] which was inactive, this approach has not yet been explored for Ins(1,4,5)P_3. We therefore decided to constrain a single component of the vicinal pair using a ring. This results in reduction of charge and, since the binding affinity of Ins(1,4,5)P_3 correlates with the ionization state of the 5-phosphate,[10] the more cautious approach is to focus on position 4. Deletion of the hydroxyl at position 3, we reasoned, should be well tolerated, as 3-deoxy-Ins(1,4,5)-P_3 is highly active.[7,11] We therefore designed cyclic phosphate **2** (Figure 1), in which the phosphate group equivalent to the 4-phosphate of Ins(1,4,5)P_3 is tethered *via* a methylene group to the equivalent carbon of position 3 in Ins(1,4,5)P_3. Also, the hydroxyl group at the equivalent position 2 is equatorial rather than axial. It is unlikely that this inversion of stereochemistry would significantly reduce activity, as *scyllo*-inositol 1,2,4-trisphosphate (**1a**, Figure 1) is almost equipotent with Ins(1,4,5)P_3.[12] Finally, the 3- and 6-OH groups of Ins(1,4,5)P_3 may engage in intramolecular hydrogen-bonding to the 4- and 5-phosphates respectively,[13] and we therefore chose to constrain the 4-phosphate group so as to mimic this conformation. This requires that the two rings of **2** be fused in a *trans* sense.

The synthesis (Scheme 1) starts with *myo*-inositol orthoformate[14] **3**. Reaction of **3** with 2.1 equiv of *p*-methoxybenzyl chloride and 2.3 equiv of sodium hydride gave the 4,6-disubsituted alcohol **4** as the major product[15] (40%), and this was converted to ketone **5** by Swern oxidation using DMSO/oxalyl chloride (92%). **5** was found

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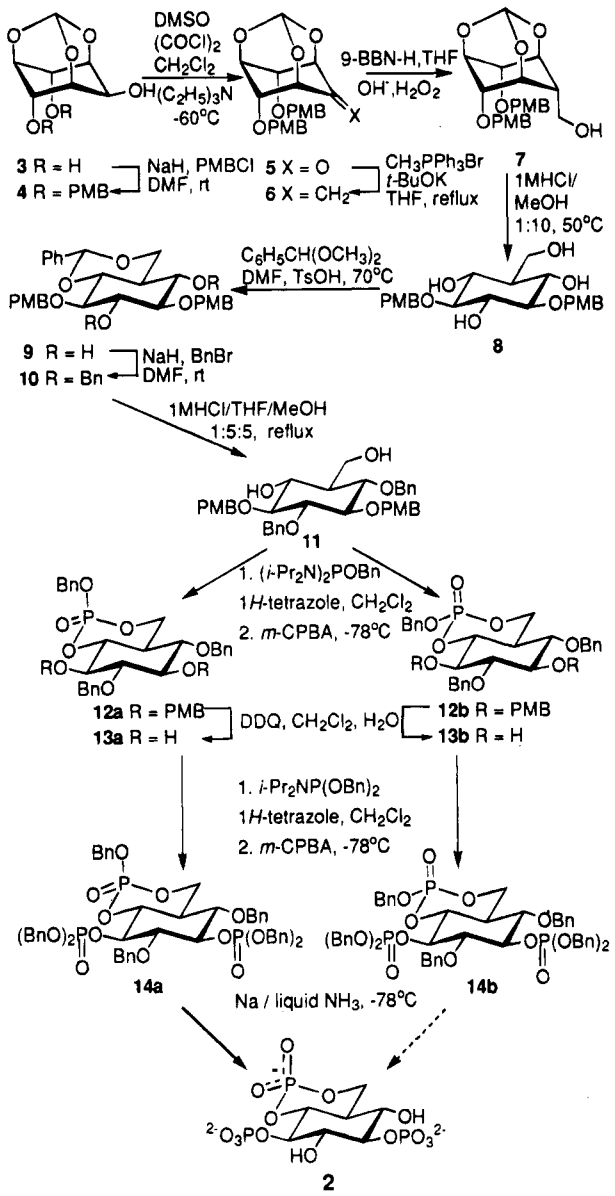
Scheme 1[a]



[a] Bn = benzyl, PMB = *p*-methoxybenzyl. All asymmetrical compounds are racemic.

to hydrate spontaneously to the *gem*-diol, and this process could be reversed by azeotropic removal of water. It also reacted with methanol to form a hemiacetal. The high reactivity of **5** may be due to strain effects, resulting in destabilization of sp^2-hybridized carbon relative to sp^3. Wittig methylenation of **5** gave alkene **6** in 91% yield via an unusually stable oxaphosphetane intermediate, observable by ^{31}P NMR spectroscopy as a single peak at δ_P −68.9 ppm. Heating to reflux was necessary to decompose this intermediate. Note that cyclobutanone- and norbornanone-derived oxaphosphetanes also show high stability.[16] **6** was then hydroborated using 9-BBN-H, giving exclusively the kinetic product **7**, with an axial $-CH_2OH$ group, in 97% yield. The very high regio- and stereoselectivity can be attributed to steric hindrance by the orthoformate and *p*-methoxybenzyl groups, allowing only one orientation of approach by 9-BBN-H. The orthoformate was removed to give tetrol **8** (87%), and benzylidenation gave the racemic diol **9** (93%). Benzylation of **9** to give fully-protected **10** (94%) and subsequent removal of the benzylidene acetal provided the key diol **11** (82%).

Phosphitylation using (benzyloxy)bis(diisopropylamino)phosphine[17] and 1*H*-tetrazole gave two cyclic phosphite triesters which were not isolated, but were clearly observable by ^{31}P NMR at δ_P 125.0 and 130.4 ppm. Oxidation with *m*-CPBA yielded the two cyclic phosphate triesters **12a** (46%) and **12b** (40%) with axial and equatorial benzyl groups, respectively. These epimers could be separated by flash chromatography as crystalline solids or used as a mixture for the subsequent stages. The configuration at phosphorus of **12a** and **12b** was determined by examination of their ^{31}P and ^1H NMR chemical shifts, $^3J_{HCOP}$ coupling constants, and P=O stretching frequencies.[18] The *p*-methoxybenzyl groups were removed from **12a** and **12b** with DDQ, and the diols **13a** and **13b**, respectively, were then phosphitylated using bis(benzyloxy)(diisopropylamino)phosphine[19] with 1*H*-tetrazole. This step forms a phosphite triester at a position vicinal to a phosphate triester and in each case we observed in the ^{31}P NMR spectrum an unusual $^5J_{PP}$ coupling of 1.2 Hz between these P atoms. To the best of our knowledge, such a long-range P(III)−P(V) coupling has not been previously reported, although it is known in vicinal P(III)−P(III) systems.[20] Oxidation using *m*-CPBA gave crystalline **14a** and **14b**. Deprotection of either epimer would, in principle, yield cyclic phosphate **2**, and this target was reached by deprotection of **14a** using sodium in liquid ammonia.[21] Racemic **2** was purified by ion-exchange chromatography as the triethylammonium salt, and quantified by phosphate assay (78% yield).

Racemic **2** was examined for Ca^{2+} mobilizing activity using saponin-permeabilized platelets[22] loaded with $^{45}Ca^{2-}$. It behaved as a full agonist, although with an EC_{50} around 40-fold higher than Ins(1,4,5)P_3. Full biological results will be published elsewhere.

Thus, we have shown that a 4-phosphate modified analog of Ins(1,4,5)P_3 can retain the ability to mobilize intracellular Ca^{2+} in spite of both conformational restriction and charge reduction at the highly sensitive 4,5-bisphosphate. **2** therefore joins a small class of structurally diverse natural[4] and synthetic[5,23] Ins(1,4,5)P_3 mimics, demonstrating new directions for chemical modification of this second messenger.

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Supporting Information Available: Experimental procedures and characterization data (16 pages).

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