Pentagon IP_3: Synthesis of a Ring-Contracted Mimic of a Second Messenger

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Intracellular Ca^{2+} mobilization mediated by the second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1** (Figure 1)] is the prime response to phosphoinositidase C activation *via* stimulation of an extracellular G-protein coupled receptor in a vast array of cell types.[1] Understanding the subtleties of the polyphosphoinositide signaling pathway has been a fundamental biological aim since the discovery of the Ca^{2+} releasing activity of Ins(1,4,5)P_3 in 1983.[2] Since 1986, there has been an intensive chemical focus on the synthesis of inositol polyphosphates and on understanding the structure-recognition parameters at the Ins(1,4,5)P_3 receptor and other binding proteins.[3] The synthesis of structurally-modified Ins(1,4,5)P_3 analogs offers the prospect of pharmacological intervention in such signaling pathways.

All reported approaches to structural modification of Ins(1,4,5)P_3 resulting in compounds possessing biological activity have focused on the introduction of conservative perturbations at phosphorus (e.g., phosphorothioates, phosphonates, etc.) or by hydroxyl group deletion, reorientation, alkylation, or replacement by isosteres and other groups in the six-membered cyclitol ring.[4,5] Despite numerous single and multiple modifications, the fundamental requirement of a six-membered ring for activity has not yet been addressed. Some recently described compounds that do not contain cyclohexyl structures are the synthetic benzene 1,2,4-trisphosphate, in which replacement of the cyclohexyl ring of Ins(1,4,5)P_3 by benzene results in loss of Ca^{2+} mobilizing activity but conferment of weak antagonist activity,[6] and the naturally occurring adenophostins A and B.[7] The adenophostins, isolated from cultures of *Penicillium brevicompactum*, are potent agonists with little apparent resemblance to Ins(1,4,5)P_3. Nevertheless, the key feature for their recognition by the Ca^{2+} mobilizing receptor is clearly the glucose 3,4-bisphosphate/2-hydroxyl triad, analogous to the 4,5-bisphosphate/6-hydroxyl motif in Ins(1,4,5)P_3. Thus, their Ca^{2+} mobilizing structural elements are still couched in a six-membered ring, with the pyranoside oxygen acting as a surrogate for C-2 of Ins(1,4,5)P_3.

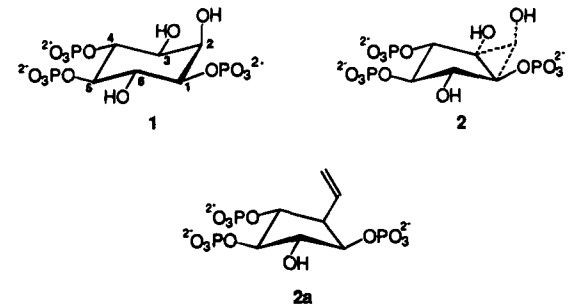

Figure 1. D-*myo*-Inositol 1,4,5-trisphosphate (**1**) and ring-contracted analogs.

Since recent studies have demonstrated that the 2- and 3-positions of Ins(1,4,5)P_3 are surprisingly tolerant to modification without dramatic loss of activity,[5] we envisaged that a contracted structure such as **2** should also fulfil the recognition requirements of the Ins(1,4,5)P_3 receptor. We report here the synthesis of a "pentagon IP_3", (1*R*,2*R*,3*S*,4*R*,5*S*)-3-hydroxy-1,2,4-trisphospho-5-vinylcyclopentane (**2a**), an optically active Ins(1,4,5)P_3 mimic, potent in intracellular Ca^{2+} mobilization, but possessing a *five-membered* cyclic core structure obtained essentially by deletion of the 2-position carbon atom of **1** with its associated hydroxyl group (Figure 1). Molecular modeling studies of **2a** (see supplementary material) indicate a good overlay of essential recognition elements for activity with those of Ins(1,4,5)P_3.

Our route (Scheme 1) illustrates the applicability of recently reported carbohydrate ring contraction methodology.[8] The key protected 5-vinyl pyranoside[9] **8** was synthesized from methyl α-D-glucopyranoside in five steps. Thus, stannylene-mediated benzylation of methyl 4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside[10] (**4**) gave the chromatographically separable 2- and 3-substituted ethers **5a** and **5b** in a ratio of 1:4.4. The major product **5b** was *p*-methoxybenzylated under standard conditions, and stereospecific cleavage of the *p*-methoxybenzylidene acetal was achieved using $LiAlH_4-AlCl_3$.[11] Swern oxidation with DMSO/oxalyl chloride, followed by Wittig methylenation, gave the vinyl carbohydrate **8**, and zirconium-mediated "Cp_2Zr" ring contraction of **8** gave the protected vinyl carbocycle **9b** with the desired stereochemistry and regiochemical protection in 46% yield. A small amount of the minor diastereoisomer **9a** (<5%)

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(9) All products after **4** are new compounds, and their structures were determined by ^1H, ^{13}C, and ^{31}P NMR spectroscopy, mass spectrometry, and combustion analysis. Full details are described in the supplementary material.

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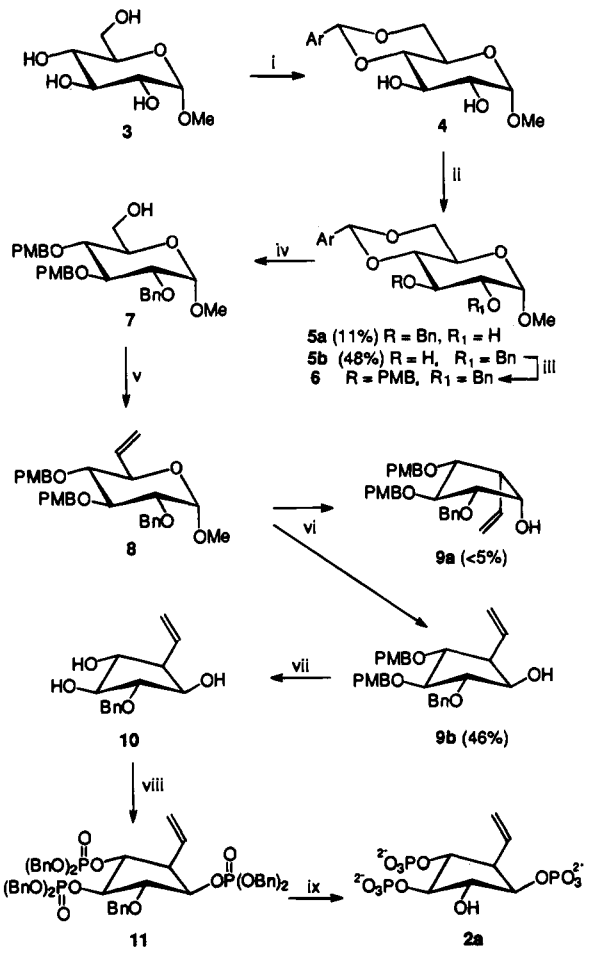
Scheme 1[a]



[a] (i) $ArCH(OCH_3)_2$, *p*-toluenesulfonic acid, DMF, 70 °C, -MeOH, 81%; (ii) (a) *n*-Bu_2SnO, *n*-Bu_4NI, CH_3CN, 4-Å molecular sieves, Δ, 2 h, (b) BnBr, Δ, 16 h; (iii) NaH, PMBCl, DMF, room temperature, 87%; (iv) $LiAlH_4$, $AlCl_3$, THF, N_2, Δ, 3 h, 73%; (v) (a) DMSO, $(COCl)_2$, CH_2Cl_2 then Et_3N, −60 °C, (b) CH_3PPh_3Br, KOBu^t, THF, 75% from **7**; (vi) "Cp_2Zr"/THF then $BF_3{\cdot}Et_2O$, −78 °C to room temperature; (vii) MHCl/EtOH 1:2, Δ, 3 h, 87%; (viii) (a) $Pr^i_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, (b) MCPBA, −78 °C, 82%; (ix) Na/NH_3, −78 °C, 58%; Ar = *p*-methoxyphenyl; Bn = benzyl, PMB = *p*-methoxybenzyl.

was also isolated. The relative stereochemistries of **9a** and **9b** were confirmed by phase-sensitive 2D-NOESY and NOE difference NMR spectroscopy. Removal of the *p*-methoxybenzyl protecting groups from **9b** furnished the triol **10** in 87% yield. Phosphitylation using bis(benzyloxy)(diisopropylamino)-phosphine,[12] followed by oxidation of phosphites with *m*-chloroperoxybenzoic acid (MCPBA), gave the fully protected trisphosphate **11**. ^{31}P NMR spectroscopy of the intermediate trisphosphite triester showed an unusually high $^5J_{PP}$ coupling of 6.7 Hz [*cf.* 2.9 and 3.4 Hz for precursors of $Ins(4,5)P_2$[13] and $Ins(1,4,5)P_3$,[14] respectively]. Deprotection in one step using sodium in liquid ammonia,[15] followed by ion-exchange chromatography of the crude product on Sepharose Q fast flow resin, gave the target trisphosphate **2a**, which was isolated as the triethylammonium salt and quantified by phosphate assay.

Trisphosphate **2a** was examined for heparin-sensitive Ca^{2+} mobilizing activity at the platelet $Ins(1,4,5)P_3$ receptor using fluorescence techniques and also using saponin-permeabilized platelets[16] loaded with $^{45}Ca^{2+}$. It was found to be a relatively potent full agonist with an EC_{50} some 65-fold higher than $Ins(1,4,5)P_3$, testifying to its functional recognition by this receptor; **2a** was also active in Jurkat T lymphocytes. Full biological results will be published elsewhere.

The presence of a vinyl group in **2a** and **9b** provides versatility and opportunities for elaboration of these novel structures into those possessing alkyl, aldehyde, hydroxyalkyl, and other side chains in order to explore further which functionalities are tolerated in a pentagon IP_3. Since the 2-position of $Ins(1,4,5)P_3$ is remarkably amenable to large structural modifications with minimal loss of activity, and molecular modeling studies suggest that the pseudoaxial vinyl group of **2a** lies in a closely equivalent position in space to the 2-OH of $Ins(1,4,5)P_3$, we expect that compounds containing such modifications will retain activity. This work is now in progress.

We have thus demonstrated for the first time that *myo*-inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} mobilization does not necessarily require a cyclohexyl (or equivalent) structural motif. Potent Ca^{2+}-releasing activity can be achieved with a smaller ring polyphosphate that retains crucial recognition elements of $Ins(1,4,5)P_3$, namely three appropriately oriented phosphates and a pseudo-6-hydroxyl group. This observation opens a new chapter in the design of potential receptor agonists, antagonists, and enzyme inhibitors to interfere with the polyphosphoinositide pathway of signal transduction.

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Supplementary Material Available: ^1H, ^{13}C, and ^{31}P NMR spectral data, specific rotations, mass spectral, elemental analysis, molecular modeling, and NOE data (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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