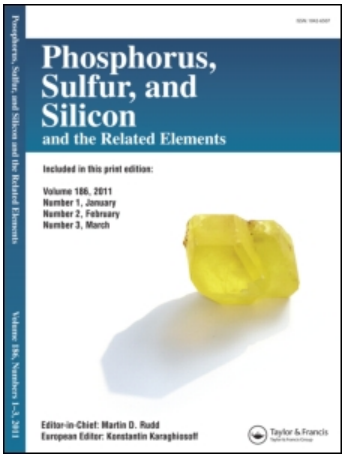

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SYNTHETIC MODULATORS OF THE POLYPHOSPHOINOSITIDE PATHWAY OF SIGNAL TRANSDUCTION

BARRY V. L. POTTER*, ANDREW M. RILEY, STEPHEN J. MILLS, DAVID J. JENKINS, CHANGSHENG LIU & DETHARD LAMPE
Department of Medicinal Chemistry, School of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK

Abstract Syntheses of regiochemically and structurally modified analogues of D-*myo*-inositol 1,4,5-trisphosphate are described to give second messenger mimics, enzyme inhibitors and receptor antagonists, demonstrating new leads for the design of agents to interfere with a key pathway of signal transduction.

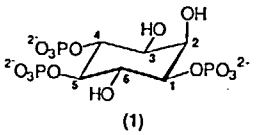
The second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** mobilizes intracellular Ca^{2+} as a response to phospholipase C activation by stimulation of an extracellular G-protein coupled receptor in many cell types.[1,2] Intensive biological interest has followed the discovery of the Ca^{2+} releasing activity of Ins(1,4,5)P_3 in 1983.[3] Additionally, chemical investigations have aimed at synthesis of inositol polyphosphates and understanding structure-recognition parameters at the Ins(1,4,5)P_3 receptor and other binding proteins.[4-6] The key feature for Ca^{2+}-mobilizing activity is the *trans* 4,5-bisphosphate motif of Ins(1,4,5)P_3. The synthesis of structurally-modified Ins(1,4,5)P_3 analogues offers the prospect of pharmacological intervention in this signalling pathway. We have approached this challenge in four different areas:

(a) Regioisomers of Ins(1,4,5)P_3 with Ca^{2+}-mobilizing activity

Although Ins(1,4,5)P_3 is the most well known mobilizer of intracellular Ca^{2+}, we reasoned that the regioisomeric *myo*-inositol trisphosphates D-Ins(1,4,6)P_3 and L-Ins(1,3,4)P_3 should also be D-Ins(1,4,5)P_3 mimics, although they do not formally possess the crucial 4,5-bisphosphate group. We thus synthesized both enantiomers of Ins(1,4,6)P_3 and Ins(1,3,4)P_3.[7,8] In Ca^{2+} mobilization neither synthetic L-Ins(1,4,6)P_3 **2** nor D-Ins(1,3,4)P_3 **3** [the naturally occurring metabolite of Ins(1,3,4,5)P_4] showed activity. By contrast, the enantiomers D-Ins(1,4,6)P_3 **4** and L-Ins(1,3,4)P_3 **5** were both relatively potent full agonists, thus abolishing some previous structure-activity dogma. L-Ins(1,3,4)P_3 also mobilises Ca^{2+} in *Limulus* photoreceptors.[8]

(b) Inositol phosphorothioates as enzyme inhibitors and receptor antagonists

Inositol phosphorothioates are well established as analogues of Ins(1,4,5)P_3 with *inter alia* resistance against degradation by metabolic enzymes. We noted earlier that phosphorothioate substitution generally increases the affinity of an analogue for 5-phosphatase.[9] For design of potent and selective non-Ca^{2+}-mobilizing inhibitors of this



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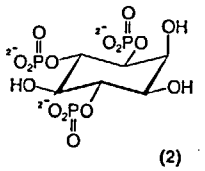

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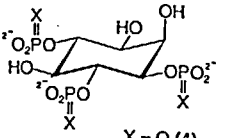
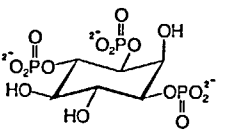


X = O (4)
X = S (7)



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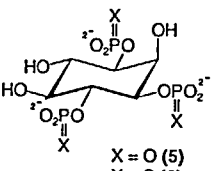

X = O (5)
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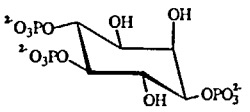

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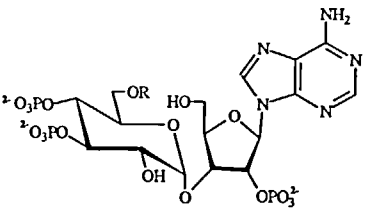
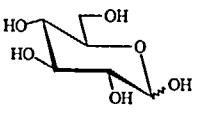
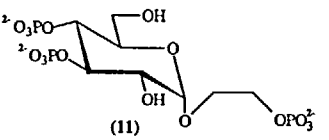


(8) R=H
(9) R=$COCH_3$

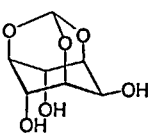


(10)

6 steps

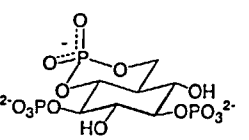


(11)



(12)

12 steps



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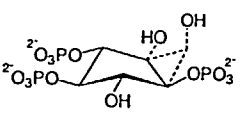

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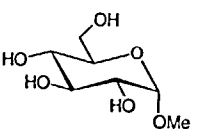
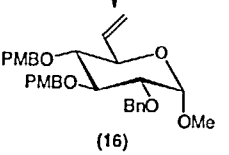


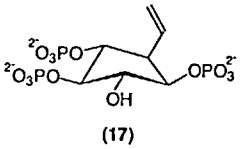
(15)

6 steps



(16)

4 steps



(17)

enzyme, we thus reasoned that phosphorothioate substitution of weak polyphosphate-based non-Ca^{2+}-mobilizing 5-phosphatase inhibitors should be appropriate. Indeed, the trisphosphorothioates of Ins(1,3,5)P_3, L-Ins(1,4,5)P_3 and L-*chiro*-Ins(1,4,6)P_3 are all sub-micromolar inhibitors with no Ca^{2+}-mobilizing activity or action on the other metabolic enzyme 3-kinase.[10,11] Trisphosphorothioate analogues **6** and **7** of L-Ins(1,3,4)P_3 and D-Ins(1,4,6)P_3 respectively proved to be low intrinsic activity partial agonists at the Ins(1,4,5)P_3 receptor[12] providing leads for small molecule antagonist design. Another trisphosphorothioate, L-*chiro*-Ins(2,3,5)PS_3, and its parent trisphosphate are moderate inhibitors of phosphoinositide 3-kinase[13] and represent new leads for development of inhibitors in this newly emerging signal transduction pathway.

(c) A carbohydrate polyphosphate mimic of Ins(1,4,5)P_3 and adenophostin A

Structural modification of Ins(1,4,5)P_3 has generally consisted of phosphate alteration or hydroxyl group deletion, reorientation, alkylation, or replacement by isosteres and other groups in the cyclitol ring.[4-6] Much success has been achieved in understanding structure activity profiles for Ins(1,4,5)P_3 analogues. The recently reported adenophostins A and B,[14] **8** and **9** respectively, isolated from *Penicillium brevicompactum*, are agonists with little apparent resemblance to Ins(1,4,5)P_3 and yet possess a Ca^{2+}-mobilizing potency some 100 fold *greater* than Ins(1,4,5)P_3. A structural rationalisation of this exceptional potency is presently lacking. The adenophostins are thus targets for chemical modification and we have made the first step in this direction with the synthesis of the polyphosphorylated carbohydrate derivative (2-hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate **11**.[15] Our route from D-glucose **10** employed a regioselective dibenzylation of allyl α-D-glucopyranoside, and elaboration to provide a key triol for phosphorylation. After deblocking and purification, trisphosphate **11** was found to release intracellular Ca^{2+}. Its potency was not comparable to that reported for **7** and was some 10 fold weaker than Ins(1,4,5)P_3. The adenosine motif is thus important for the extreme potency of the adenophostins and **11** represents the first synthetic carbohydrate polyphosphate mimic of Ins(1,4,5)P_3.

(d) Ins(1,4,5)P_3 mimics with extreme structural dissimilarity

Synthesis of conformationally restrained analogues has not yet been explored for Ins(1,4,5)P_3. We therefore decided to constrain one of the vicinal bisphosphates using a ring. Since binding affinity correlates most closely with the ionization state of the 5-phosphate,[16] the more cautious approach was to focus on position 4. Deletion of the 3-hydroxyl should be well tolerated, as 3-deoxy-Ins(1,4,5)P_3 is highly active.[6] We therefore synthesized the cyclic phosphate **13**, 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. The hydroxyl group at the equivalent position 2 is equatorial rather than axial, but this is not significant, as D-*scyllo*-inositol 1,2,4-trisphosphate is almost equipotent with Ins(1,4,5)P_3.[17] The 3- and 6-OH groups of Ins(1,4,5)P_3 may hydrogen bond to the 4- and 5-phosphates respectively, and we therefore constrained the 4-phosphate in such a way as to mimic this conformation. An efficient synthetic route to **13** started from *myo*-inositol orthoformate **12** in some 12 steps.[18] Racemic **13** was examined for Ca^{2+}-mobilizing activity and behaved as a full agonist, although with

an EC_{50} around 40 fold higher than Ins(1,4,5)P_3. Thus, we have shown that a conformationally restricted analogue of Ins(1,4,5)P_3, even at the supposedly crucial 4,5-bisphosphate group, can retain activity, despite reduction of charge.

The fundamental requirement of a six-membered ring for Ins(1,4,5)P_3 activity has not yet been addressed. Since the 2- and 3-positions are surprisingly tolerant to modification,[4-6] we envisaged that a contracted structure such as **14** should also fulfil receptor recognition requirements. We thus synthesized a related "pentagon IP_3", (1*R*, 2*R*, 3*S*, 4*R*, 5*S*)-3-hydroxy-1,2,4-trisphospho-5-vinylcyclopentane **17**[19] with a *five-membered* cyclic core structure. Molecular modelling studies of **17** indicate a good overlay of essential recognition elements for activity with those of Ins(1,4,5)P_3. The 5-vinyl pyranoside **16** was synthesized from methyl α-D-glucopyranoside **15** in 5 steps. After zirconium mediated ring contraction, the major diastereoisomer was partially deblocked, phosphorylated and completely deblocked. The purified trisphosphate **17** was examined for Ca^{2+}-mobilizing activity and was a relatively potent agonist with an EC_{50} some 65-fold higher than Ins(1,4,5)P_3. We have thus demonstrated for the first time that Ins(1,4,5)P_3 receptor mediated Ca^{2+}-mobilization does not necessarily require a cyclohexyl (or equivalent) structural motif. Potent activity can be achieved with a smaller ring polyphosphate retaining key recognition elements of Ins(1,4,5)P_3.

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