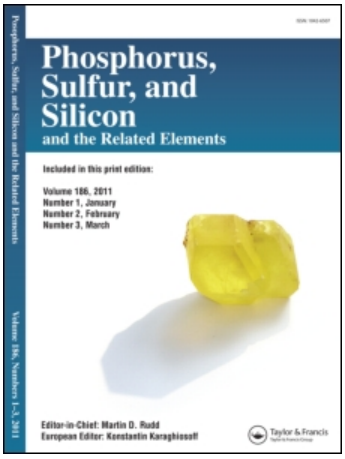

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Synthetic Analogues of the Second Messenger D-*MYO* Inositol 1,4,5 Trisphosphate as Receptor Agonists and Inhibitors of the Enzymes of the Polyphosphoinositide Pathway of Signal Transduction

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SYNTHETIC ANALOGUES OF THE SECOND MESSENGER D-*MYO* INOSITOL 1,4,5 TRISPHOSPHATE AS RECEPTOR AGONISTS AND INHIBITORS OF THE ENZYMES OF THE POLYPHOSPHOINOSITIDE PATHWAY OF SIGNAL TRANSDUCTION

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Abstract Novel synthetic probes of the polyphosphoinositide pathway of signal transduction are discussed.

INTRODUCTION

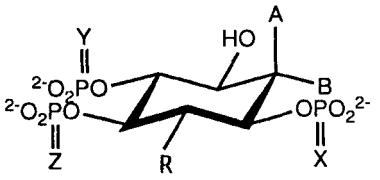
D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** is a ubiquitous intracellular messenger that couples stimulation of cell surface receptors to the release of intracellular Ca^{2+} [1]. An Ins(1,4,5)P_3 receptor has been purified[2], reconstituted[3], an amino acid sequence determined from the cDNA[4,5] and a sequence near to the N-terminus is responsible for Ins(1,4,5)P_3 binding[6]. Because of the fundamental importance of the polyphosphoinositide signalling system it is desirable to have access to pharmacological tools for intervention at Ins(1,4,5)P_3 receptors and inhibition of the metabolic enzymes acting upon this second messenger[7,8]. Ins(1,4,5)P_3 antagonists and enzyme inhibitors may have therapeutic potential[9]. Few such tools have yet been identified and there are significant difficulties intrinsic to a drug design strategy based upon Ins(1,4,5)P_3.

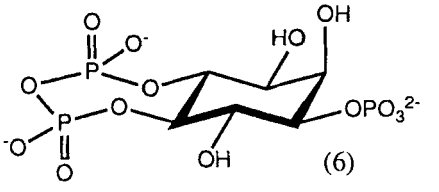
Synthetic approaches to inositol polyphosphates addressed the problems of selective protection, phosphorylation, resolution and deblocking[10,11]. The design and synthesis of almost any inositol polyphosphate analogue can now be envisaged[10-12]. The first example was *myo*-inositol 1,4,5-trisphosphorothioate [Ins(1,4,5)PS_3] **2**[13]. Further analogues have since been synthesized, i.e. inositol 1,4-bisphosphate-5-phosphorothioate **3**[14], inositol 1-phosphorothioate-4,5-bisphosphate **4**[15] and inositol 1-phosphate-4,5-bisphosphorothioate **5**[16]. Their resistance to enzymic degradation[17] makes them valuable pharmacological tools. We focus here on inositol phosphorothioates and other novel agonists and enzyme inhibitors synthesized by our group. We have also recently synthesised an interesting pyrophosphate analogue of Ins(1,4,5)P_3, **6**[16].

Numerous synthetic routes to Ins(1,4,5)P_3 have now been devised[10-12]. Our initial route[18] exploited methodology used to synthesize *myo*-inositol 4,5-bisphosphate and involved phosphorylation of the precursor, L-1,2,4-tri-*O*-benzyl-*myo*-inositol[19]. After deblocking and purification, the resulting Ins(1,4,5)P_3 was fully active at mobilising Ca^{2+} and binding to the cerebellar Ins(1,4,5)P_3 receptor. Ins(1,4,5)PS_3 Was synthesized by a modification of the procedure[13]. Ins(1,4,5)PS_3 binds with high affinity to Ins(1,4,5)P_3 receptor sites in brain[17,20] and liver[21]. It is a full Ca^{2+}-releasing agonist, being only some 3-4 fold less potent than Ins(1,4,5)P_3 in a variety of cells, such as in *Xenopus* oocytes[22,23], permeabilised Swiss 3T3

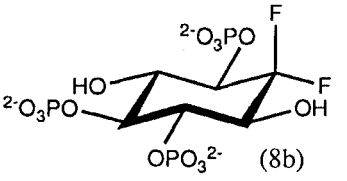
cells[22,24], GH_3 cells[24], hepatocytes[25], pancreatic[26-28] and parotid[29] acinar cells, skeletal muscle triads[30], mouse lacrimal cells[31] and SH-SY5Y cells[7]. It is resistant to dephosphorylation by 5-phosphatase[17,25] and can produce a sustained cellular calcium transient[7,25]. Ins(1,4,5)PS_3 is a potent competitive inhibitor of 5-phosphatase[32], but does not compete with Ins(1,4,5)P_3 for 3-kinase[25-33]. Applications of inositol phosphorothioates have been reviewed[34].

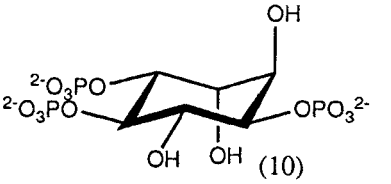
Applications of inositol phosphorothioates detailed above have been extended by the commercial availability of the metabolically stable radioligand, [^{35}S]-Ins(1,4,5)PS_3. [^{35}S]-D-Ins(1,4,5)PS_3 Was prepared by a modification to the chemical synthesis of unlabelled material[13]. Characterisation of its interaction with cerebellar membranes shows that D-[^{35}S]-Ins(1,4,5)PS_3 binds with high affinity to the Ins(1,4,5)P_3 receptor[35], labels more sites than does [^{3}H]-Ins(1,4,5)P_3, and may label different conformations of the receptor with equal affinity, whereas Ins(1,4,5)P_3 induces or stabilises some of the sites in a high affinity form.





A=OH; B=H; R=OH; X=Y=Z=O (1)
X=Y=Z=S (2)
X=Y=O; Z=S (3)
X=S; Y=Z=O (4)
X=O; Y=Z=S (5)
A=H; B=F; R=OH; X=Y=Z=O (7ab)
A=B=F; R=OH; X=Y=Z=O (8a)
A=OH; B=H; R=H; X=Y=Z=O (9)





(a) Fluorinated analogues **8ab** Inositol ring-modified and phosphate-modified analogues have been synthesized[10,11] to understand the role of recognition motifs of Ins(1,4,5)P_3 in receptor binding, specificity and stimulation. We synthesised 2-deoxy-2-fluoro-*scyllo* Ins(1,4,5)P_3 **7ab** and 2,2-difluoro-2-deoxy Ins(1,4,5)P_3 **8ab** and studied their interaction with the Ca^{2+}-releasing receptor and Ins(1,4,5)P_3 5-phosphatase and 3-kinase[36]. Unexpectedly, DL-(**8ab**), was not a substrate for 5-phosphatase. We suspected that L-(**8b**) might act as a 5-phosphatase inhibitor and we resolved DL-(**8ab**) into its enantiomers[37] and studied their differential interactions with the three Ins(1,4,5)P_3 binding proteins[38]. D-(**8a**) was a full agonist and released Ca^{2+} with kinetics similar to Ins(1,4,5)P_3 and with an EC_{50} of 0.21μM. Fluoro-substitution at the 2-position therefore makes D-(**8a**) an excellent analogue of Ins(1,4,5)P_3 and essentially equipotent to it. D-(**8a**) was a substrate for Ins(1,4,5)P_3 3-kinase and inhibited Ins(1,4,5)P_3 phosphorylation potently [apparent K_i, 10.2μM]. L-(**8b**) inhibited phosphorylation of Ins(1,4,5)P_3 [K_i, 11.9μM] and is

a potent 3-kinase inhibitor. 5-Phosphatase is relatively non-selective for inositol phosphates, but 3-kinase is very specific[7], posing problems in inhibitor design. L-(**8b**) represents a lead in the search for a small molecule 3-kinase inhibitor.

Ins(1,4,5)P_3 and D-(**8a**) were comparable substrates for 5-phosphatase but treatment of L-(**8b**) with 5-phosphatase did not liberate inorganic phosphate and racemic DL-(**8ab**) was apparently not a substrate[36] but an inhibitor with a K_i of 19μM. Fluorination at the 2-position of L-Ins(1,4,5)P_3 has led to the production of a small molecule 3-kinase inhibitor and a moderately potent 5-phosphatase inhibitor which is a very poor agonist for Ca^{2+} release.

(b) 6-Deoxy-*myo*-inositol 1,4,5-trisphosphate **9** A key structure-activity aspect concerns the potential role of the 6-OH of Ins(1,4,5)P_3, adjacent to the crucial vicinal 4,5-bisphosphate, in determining the affinity and specificity of Ins(1,4,5)P_3 for its receptor and the metabolic enzymes 3-kinase and 5-phosphatase. We have thus deleted the 6-OH of Ins(1,4,5)P_3 to generate 6-deoxy Ins(1,4,5)P_3 **9**[39]. **9** has an EC_{50} for Ca^{2+} release of 6.4μM and is a full agonist. Deletion of the 6-OH makes **9** approximately 70-fold less potent than Ins(1,4,5)P_3, suggesting a lower affinity for the receptor[39]. **9** Inhibited 5-phosphatase [K_i, 76μM] suggesting that the 6-OH may play a key role in the catalytic mechanism of this enzyme.

(c) L-*Chiro*-inositol 2,3,5-trisphosphate **10** Generation of a 3-kinase inhibitor could arise by modification of the 3-OH of Ins(1,4,5)P_3. We developed a concise synthetic route to L-*Chiro*-inositol 2,3,5-trisphosphate **10**, i.e. Ins(1,4,5)P_3 with an inverted 3-OH group, from L-quebrachitol[40]. **10** Was a potent agonist for the release of intracellular Ca^{2+} with an EC_{50} some 5-10 fold higher than Ins(1,4,5)P_3. It was not a substrate for 3-kinase (K_i, 7.1μM), nor surprisingly for 5-phosphatase (K_i, 7.7μM), and released Ca^{2+} in a sustained fashion similar to Ins(1,4,5)PS_3.

We have already noted that deletion of the 6-OH of Ins(1,4,5)P_3 generates a moderately potent 5-phosphatase inhibitor in **9**[39] of this enzyme. However, the finding that L-*chiro*-Ins(2,3,5)P_3 is a potent inhibitor was unexpected and poses the question as to how a single change in orientation of a remote OH group can have such a radical effect. Whether this is the result of subtle conformational changes induced in the molecule relative to Ins(1,4,5)P_3, or of non-productive binding of **10** to the enzyme present intriguing prospects for further investigation.

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