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SYNTHESIS OF L-*CHIRO*-INOSITOL 1,4,6-TRISPHOSPHOROTHIOATE, A POTENT AND SELECTIVE INHIBITOR OF *MYO*-INOSITOL 1,4,5-TRISPHOSPHATE 5-PHOSPHATASE

Changsheng Liu[1], Stephen T Safrany[2], Stefan R Nahorski[2] and Barry V L Potter[1]*

**[1]School of Pharmacy and Pharmacology and Institute for Life Sciences
University of Bath
Claverton Down, Bath BA2 7AY, UK**

**[2]Department of Pharmacology and Therapeutics
University of Leicester
Leicester LE1 9HN, UK**

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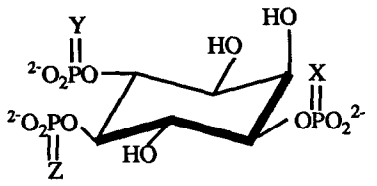
Abstract: L-*chiro*-inositol 1,4,6-trisphosphate and trisphosphorothioate have been synthesized from L-quebrachitol; the trisphosphorothioate is the most potent inhibitor of Ins(1,4,5)P_3 5-phosphatase yet discovered.

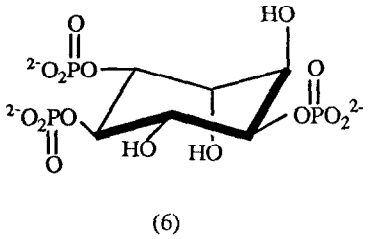
It is now generally accepted that D-*myo*-inositol 1,4,5-trisphosphate Ins(1,4,5)P_3 **(1)** (Fig. 1), released by receptor-mediated phospholipase C-catalysed cleavage of phosphatidylinositol 4,5-bisphosphate, is the second messenger linking the spatially separated events of receptor stimulation and release of intracellular calcium from internal stores[1,2]. Ins(1,4,5)P_3 is metabolised *via* two pathways[3]: deactivation by a 5-phosphatase to Ins(1,4)P_2 or phosphorylation by a 3-kinase to Ins(1,3,4,5)P_4. The function of the latter still remains controversial and Ins(1,3,4,5)P_4 may gate a plasma membrane Ca^{2+} channel[4]. Ins(1,4,5)P_3 acts through an intracellular receptor which has been isolated[5], cloned and sequenced[6,7] and reconstituted[8].



X=Y=Z=O	(1)	X=Y=O, Z=S	(4)
X=Y=Z=S	(2)	X=O, Y=Z=S	(5)
X=S, Y=Z=O	(3)		

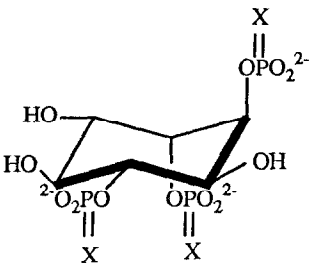
Figure 1

We have sought to develop synthetic routes to inositol phosphates[9] and especially to prepare non-hydrolysable analogues such as phosphorothioates[9,10,11]. Our synthesis of *myo*-inositol 1,4,5-trisphosphorothioate [Ins(1,4,5)PS_3] **(2)**[12] (Fig. 1) has provided an analogue that is a potent releaser of calcium[13-15] and yet is resistant to phosphatase-catalysed deactivation[16]. Other biologically potent Ca^{2+}-mobilising synthetic phosphorothioate analogues include *myo*-inositol 1-phosphorothioate 4,5-bisphosphate **(3)**[17], *myo*-inositol 1,4-bisphosphate 5-phosphorothioate [Ins(1,4,5)P_3-5S] **(4)**[18,19] and *myo*-inositol 1-phosphate 4,5-bisphosphorothioate **(5)**[20]. It is clear that such analogues offer considerable potential for investigation and modification of the complex metabolism of Ins(1,4,5)P_3 and this has been recognized by other groups[21,22].



(6)

Figure 2



X=O (7)

X=S (8)

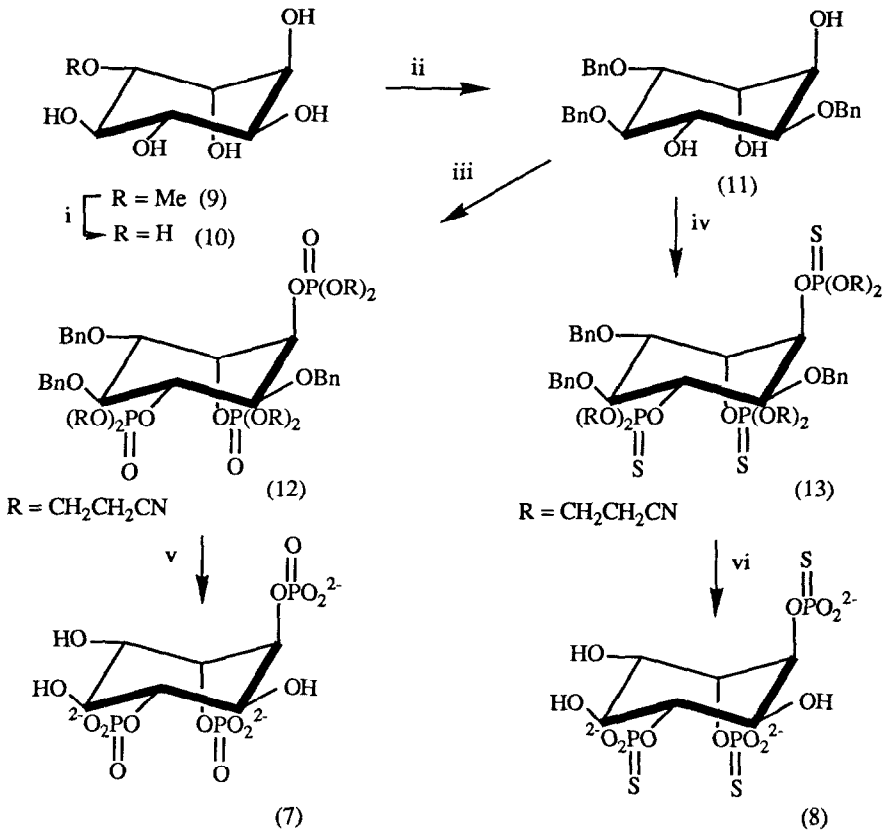
A current challenge lies in the development of potent and selective inhibitors for the metabolic enzymes, Ins(1,4,5)P_3 5-phosphatase and 3-kinase, which are not active in intracellular Ca^{2+} release. We proposed earlier DL-Ins(1,4,5)PS_3 (K_i 1.7μM)[15] and DL-Ins(1,4,5)P_3-5S (K_i 6.8μM)[15] as potent 5-phosphatase inhibitors and noted that phosphorothioate substitution in an analogue apparently markedly increased affinity for 5-phosphatase[23]. However, although Ins(1,4,5)PS_3 and Ins(1,4,5)P_3-5S are much more potent than the commonly used 5-phosphatase inhibitor, 2,3-bisphosphoroglycerate[24] (K_i 350μM[24], 978μM[23]), they suffer from the disadvantage that both are highly potent agonists. Our recent synthesis[25,26] of L-*chiro*-inositol 2,3,5-trisphosphate **(6)** (Fig. 2),

itself a potent Ca^{2+} mobilising agonist, and its evaluation[27] as a 5-phosphatase and 3-kinase inhibitor has demonstrated the usefulness of employing cyclitols other than those of the *myo*-configuration in synthesis. We report here syntheses of L-*chiro*-inositol 1,4,6-trisphosphate [L-chr Ins(1,4,6)P_3] (**7**) and the corresponding trisphosphorothioate [L-chr Ins(1,4,6)PS_3] (**8**) (Fig. 2) and demonstrate their potency and selectivity as Ins(1,4,5)P_3 5-phosphatase inhibitors.

L-Quebrachitol (**9**) (Scheme) was demethylated with HI and the resulting L-*chiro*-inositol (**10**) was regiospecifically tri-benzylated[25,26] via tin-mediated alkylation to give L-*chiro*-2,3,5-tri-*O*-benzyl-inositol (**11**). (**11**) Was polyphosphorylated on the free hydroxyl groups using bis(2-cyanoethyl)diisopropylaminophosphine/tetrazole followed by oxidation of the resulting trisphosphite either with t-BuOOH to give the fully protected trisphosphate (**12**) or with sulfur in pyridine to yield the protected trisphosphorothioate (**13**). Treatment respectively with sodium in liquid ammonia yielded the free trisphosphate (**7**) and phosphorothioate (**8**) (Scheme), which were purified by ion-exchange chromatography on Q-Sepharose, eluting with a gradient of triethylammonium bicarbonate buffer.

The ability of L-chr-Ins(1,4,6)P_3 (**7**) and L-chr-Ins(1,4,6)PS_3 (**8**) to mobilise Ca^{2+} from intracellular stores was examined using electrically permeabilised human neuroblastoma cells[15]. While Ins(1,4,5)P_3 released Ca^{2+} potently (EC_{50} 0.12μM), neither (**7**) nor (**8**) mobilised Ca^{2+} or antagonised Ins(1,4,5)P_3-induced Ca^{2+} mobilisation at concentrations up to 30μM. (**7**) And (**8**) were also ineffective at inhibiting [^{3}H]-Ins(1,4,5)P_3 phosphorylation by crude rat brain Ins(1,4,5)P_3 3-kinase at concentrations of 250μM and 30μM respectively [K_m for Ins(1,4,5)P_3 0.6μM].

Although (**7**) and (**8**) did not interact with the Ins(1,4,5)P_3 receptor nor with Ins(1,4,5)P_3 3-kinase they competitively inhibited the dephosphorylation of [^{3}H]-Ins(1,4,5)P_3 by human erythrocyte membrane Ins(1,4,5)P_3 5-phosphatase with K_i values of 44μM and 0.3μM respectively [K_m for Ins(1,4,5)P_3 19μM]. No inorganic phosphate or phosphorothioate was



Reagents and conditions:

(i) 47% aq. HI; (ii) (a) Bu_2SnO, Bu_4NI/MeCN, (b) BnCl reflux; (iii) (a) $Pr^i{}_2NP(OCH_2CH_2CN)_2$ tetrazole in CH_2Cl_2, (b) 70% *tert*-BuOOH; (iv) (a) $Pr^i{}_2NP(OCH_2CH_2CN)_2$,tetrazole in CH_2Cl_2 (b) Sulphur in pyridine; (v), (vi) (a) Na/liq NH_3, (b) H_2O

Scheme

released, as monitored by colorimetric assays[23], when (7) and (8) were incubated with $Ins(1,4,5)P_3$ 5-phosphatase. Full biological results will be published elsewhere. Phosphorothioate substitution has thus resulted in a 150 fold increase in affinity for $Ins(1,4,5)P_3$ 5-phosphatase.

The reasons for the inhibitory potency of (7) and (8) towards $Ins(1,4,5)P_3$ 5-phosphatase are not clear, but are presumably reflected in the marked non-specificity of this enzyme for inositol polyphosphates[3,9,28]. For example, $Ins(1,4,5)P_3$ 5-phosphatase from bovine aorta is inhibited moderately potently by $Ins(1,3,5)P_3$, an inositol polyphosphate which does not possess a *trans*-diequatorial- vicinal 4,5-bisphosphate[28]. The L-*chiro* analogues reported here possess a *trans*-vicinal bisphosphate, albeit *trans*-diaxial and at the L-*chiro*-1,6 positions, not D-*myo*-4,5. Presently, we have no information available concerning the conformation of these analogues in solution but this and molecular modelling studies will obviously be of importance to establish whether the 1,4 and 5-phosphate groups of $Ins(1,4,5)P_3$ can potentially be mimicked by the 4,1 and 6 phosphates and phosphorothioates of (7) and (8) respectively.

With a K_i of 300nM, L-chr-$Ins(1,4,6)PS_3$ is by far the most potent, selective $Ins(1,4,5)P_3$ 5-phosphatase inhibitor yet described. While a further challenge lies in synthesizing a membrane-permeable precursor of (8), it will now be important to evaluate the biological activity of L-chr-$Ins(1,4,6)PS_3$ to potentiate agonist-induced and $Ins(1,4,5)P_3$-mediated Ca^{2+} mobilisation in a variety of cells.

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