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# *MYO*-INOSITOL 1,4,6-TRISPHOSPHOROTHIOATE AND *MYO*-INOSITOL 1,3,4-TRISPHOSPHOROTHIOATE: NEW SYNTHETIC $Ca^{2+}$- MOBILISING PARTIAL AGONISTS AT THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR

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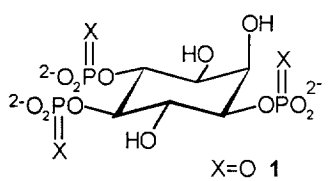
**Abstract:** Syntheses of *myo*-inositol 1,4,6-trisphosphorothioate and 1,3,4-trisphosphorothioate from *myo*-inositol are described; these trisphosphorothioates, derived from structure-activity considerations of *myo*-inositol 1,3,4,6-tetrakisphosphate, are low intrinsic activity partial agonists at the platelet Ins(1,4,5)$P_3$ receptor.

Receptor-mediated phospholipase C-catalysed cleavage of phosphatidylinositol 4,5-bisphosphate releases D-*myo*-Inositol 1,4,5-trisphosphate Ins(1,4,5)$P_3$ (**1**), which has emerged within the last decade as a second messenger linking the spatially separated events of receptor stimulation and release of intracellular calcium from internal stores.[1,2]

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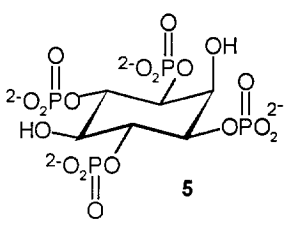
X=S **2**

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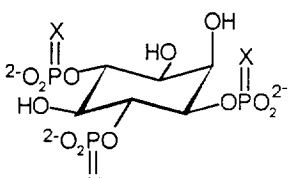


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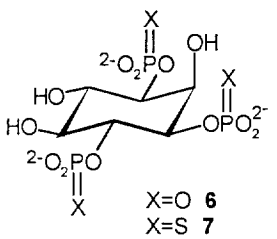



X=O **6**
X=S **7**

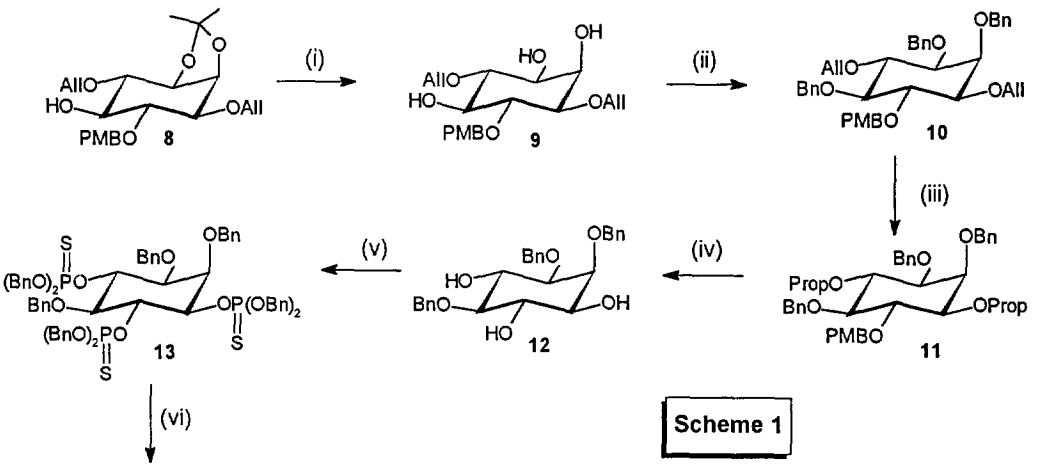
**Figure 1**

Ins(1,4,5)$P_3$ acts through an intracellular endoplasmic reticular receptor which has been isolated,[3] cloned and sequenced[4,5] and reconstituted;[6] Ins(1,4,5)$P_3$ is metabolised *via* two pathways;[7] deactivation by a 5-phosphatase to Ins(1,4)$P_2$ or phosphorylation by a 3-kinase to the tetrakisphosphate 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.[8]

As part of our studies on structure-activity relationships in inositol tris- and tetrakisphosphates[9] we are synthesising *myo*-inositol polyphosphates and their analogues, such as inositol 1,4,5-trisphosphorothioate (**2**), as potential enzyme inhibitors and receptor antagonists. Structure-activity studies performed to date[7,9,10] on Ins(1,4,5)$P_3$ analogues have confirmed the key rôle of the vicinal 4,5-bisphosphate system in mediating intracellular $Ca^{2+}$ release. The observations[11,12] that the naturally occurring tetrakisphosphate *myo*-inositol 1,3,4,6-tetrakisphosphate [Ins(1,3,4,6)$P_4$] (**5**) possesses $Ca^{2+}$- mobilising activity, despite the apparent absence of a 4,5-bisphosphate motif, was therefore of considerable interest. We have rationalised this[9] by invoking two alternative receptor binding conformations for Ins(1,3,4,6)$P_4$, in which the 1,6-vicinal bisphosphate mimics the normal 4,5-bisphosphate of Ins(1,4,5)$P_3$.

These conformations would predict that the trisphosphates D-Ins(1,4,6)$P_3$ (**3**) and L-Ins(1,3,4)$P_3$ (**6**) should show $Ca^{2+}$- mobilising activity, and we[13] and others[14] have reported such activities. Because of the unusual biological properties of phosphorothioate-substituted Ins(1,4,5)$P_3$-like compounds, we therefore undertook the preparation of the racemic phosphorothioate analogues of these compounds, and we report here syntheses of racemic inositol 1,4,6-trisphosphorothioate (**4**) and inositol 1,3,4-trisphosphorothioate (**7**) and demonstrate their $Ca^{2+}$-mobilising activity in permeabilised platelets.

Ins(1,4,6)$PS_3$ (**4**) was synthesised according to **Scheme 1**. The intermediate **8** was synthesised according to Mills *et al.*[13] The triol **9** was exposed by treatment with MHCl-methanol (1:9) at 50°C for 0.5h (86% yield). The triol was then alkylated with benzyl bromide in DMF to give the fully protected intermediate **10** in 85% yield. The allyl groups were isomerised using potassium *t*-butoxide in DMSO to provide the bis(cis-propenyl) ether **11**, and the 1,4,6-triol was then exposed using MHCl-ethanol (1:2) at reflux for 3h (84% yield). **12** was phosphitylated with bis(benzyloxy)(diisopropylamino)phosphine[15] in dichloromethane, and the resulting trisphosphite oxidised within 5min by a dry sulphur/pyridine-DMF mixture. For this improved sulphoxidation (with respect to sulphur in pyridine)[16] to occur, the dichloromethane was evaporated and DMF-pyridine (2:1) (3mL) was added, followed by 1.5 equivalents of sulphur per phosphite group. The small excess of sulphur was filtered off and the solvents evaporated *in vacuo* to give a syrup which was purified by flash chromatography, giving the totally protected trisphosphorothioate intermediate **13** in 81% yield. A single-step deprotection with sodium in liquid ammonia,[17] followed by purification of the product by ion-exchange chromatography on Q-Sepharose Fast Flow resin, using a gradient (0 - 1M) of triethylammonium bicarbonate as eluent, gave pure *myo*-inositol




**Scheme 1**

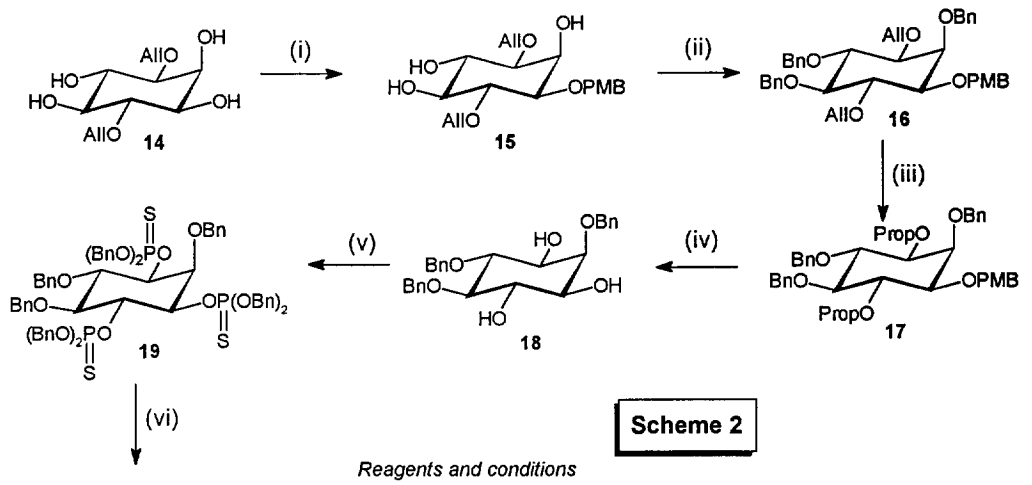
*Reagents and conditions*

i MeOH - 1M HCl ( 9 :1 v / v ), 50°C, 45mins;
ii, BnBr, NaH, DMF;iii, Bu$^t$OK–DMSO, 50°C,4hrs;iv, EtOH - 1MHCl ( 2:1 v/v ), reflux 3hrs;v, (a) $(BnO)_2PNPr^i_2$ ( 6 equiv.), tetrazole( 12 equiv.), (b) $S_8$, pyridine, DMF; vi, Na-liq. $NH_3$.
All = allyl; PMB = *p* – methoxybenzyl; Bn = benzyl;Prop. = *cis*–prop–1–enyl.
All compounds are racemic.

1,4,6-trisphosphorothioate (**4**) (40% yield) as the glassy triethylammonium salt, which was quantified by the Briggs phosphate assay.

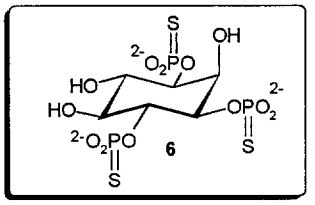
The synthesis of Ins(1,3,4)$PS_3$ is shown in **Scheme 2**. The intermediate tetraol **14** was synthesised according to Gigg *et al*,[18] and stannylation using dibutyltin oxide in the presence of caesium fluoride,[19] followed by alkylation *in situ* with *p*-methoxybenzyl chloride gave the 3-*O*-*p*-methoxybenzylated derivative **15** as the major product (65% yield). Benzylation of **15** then gave the fully protected intermediate **16**. The allyl groups were isomerised using potassium *t*-butoxide in DMSO at 50°C, giving the bis(*cis*-propenyl) ether **17** which was then deprotected in one step using MHCl-ethanol (1:2) at reflux to give the triol **18** in 90% yield. Phosphitylation and sulphoxidation as described for **12** gave the totally protected trisphosphorothioate **19**. Deprotection using sodium in liquid ammonia followed by ion-exchange chromatography of the product gave pure *myo*-inositol 1,3,4-trisphosphorothioate (**7**) as the triethylammonium salt (61% yield).

Racemic **4** and **7** were evaluated as $Ca^{2+}$- mobilising agonists in permeabilised platelets, relative to Ins(1,4,5)$P_3$. Rabbit platelets were isolated and washed according to Murphy *et al*,[20] then permeabilised with saponin and loaded with $^{45}Ca^{2+}$. The permeabilised platelets were stimulated with Ins(1,4,5)$P_3$ and analogues for 3 min. at




**Scheme 2**

*Reagents and conditions*




i, $Bu_2SnO$ (1.0 equiv.),CsF (2.5 equiv.),PMBCl (2.2 equiv.),
ii. BnBr, NaH, DMF
iii, $Bu^tOK$–DMSO 50°C,4hrs; iv, EtOH– 1M HCl ( 2 :1 v / v ), reflux 3hrs; )
v,(a) $(BnO)_2PNPr_2^i$ tetrazole ( 12 equiv.) in $CH_2Cl_2$, (b) $S_8$, pyridine, DMF;
vi, Na-liq. $NH_3$.
All = allyl; PMB = *p* – methoxybenzyl; Bn = benzyl; Prop. = *cis*–prop–1–enyl.
All compounds are racemic.

4°C, then the remaining cell-associated $^{45}Ca^{2+}$ was determined by rapid filtration. The percentage $^{45}Ca^{2+}$ release induced by each compound was expressed relative to that induced by 30μM ionomycin.

While Ins(1,4,5)$P_3$ released $Ca^{2+}$ potently with an $EC_{50}$ of 0.4 μM, both DL-Ins(1,4,6)$PS_3$ and DL-Ins(1,3,4)$PS_3$ were apparently very low intrinsic activity partial agonists (**Figure 2**). Indeed, in support of this we have already demonstrated[21] that DL-Ins(1,4,6)$PS_3$ can inhibit Ins(1,4,5)$P_3$ - induced $Ca^{2+}$ release in a concentration-dependent fashion. These data support our notion that structural perturbation linked to phosphorothioate substitution can provide low intrinsic activity partial agonists.[22,23] It is likely that D-Ins(1,4,6)$PS_3$ and L-Ins(1,3,4)$PS_3$ are the active components of the racemates. The present data provide the most encouraging current leads towards development of small molecule Ins(1,4,5)$P_3$ receptor antagonists, and resolution of the precursors required for the synthesis of these chiral analogues is in progress.

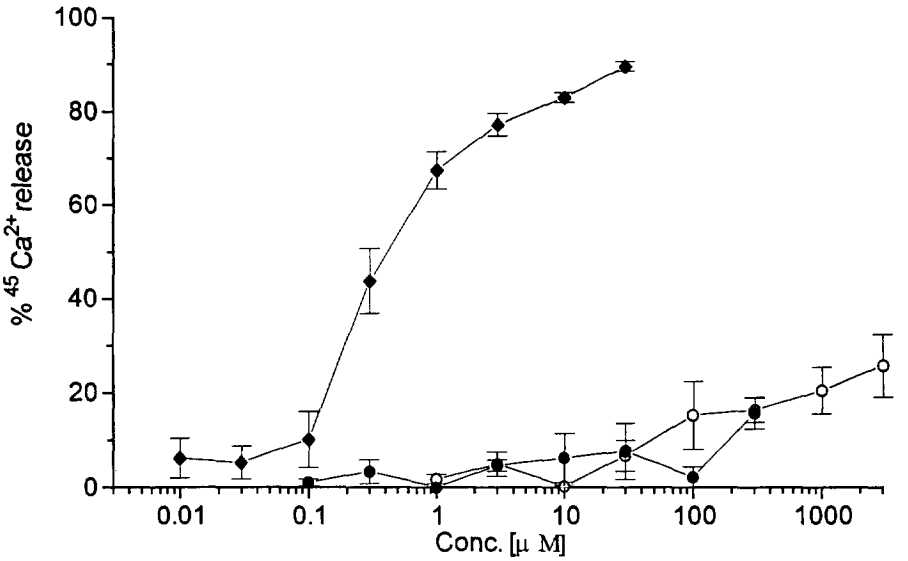



**Figure 2:** Dose-response curves for $Ins(1,4,5)P_3$ (♦), DL-$Ins(1,4,6)PS_3$ (•) and DL-$Ins(1,3,4)PS_3$ (o), showing ability to release $^{45}Ca^{2+}$ from permeabilised rabbit platelets. Each point is mean ± δ SE (n=3).

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