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MYO-INOSITOL 1,4,6-TRISPHOSPHATE: A NEW SYNTHETIC Ca^{2+}-MOBILISING INOSITOL PHOSPHATE

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Abstract: The synthesis of *myo*-inositol 1,4,6-trisphosphate from *myo*-inositol is described; this novel trisphosphate is a potent Ca^{2+}-mobilising agonist at the Ins(1,4,5)P_3 receptor and is derived from structure-activity considerations of *myo*-inositol 1,3,4,6-tetrakisphosphate.

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 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]. 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].

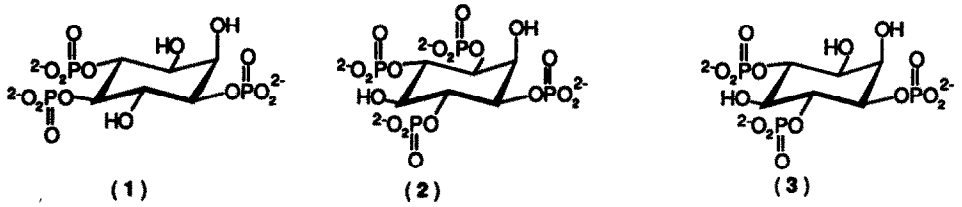


Figure 1

As part of an ongoing programme aimed to study structure-activity relationships in inositol tris- and tetrakisphosphates[9] we have been engaged in the synthesis of *myo*-inositol polyphosphates and their analogues as potential enzyme inhibitors and receptor antagonists. An important concept in the structure-activity studies performed to date[7,9,10] on Ins(1,4,5)P_3 analogues is the key role of the vicinal 4,5-bisphosphate system in mediating intracellular Ca^{2+} release. Most interesting, therefore, were the observations[11,12] that the naturally occurring tetrakisphosphate *myo*-inositol 1,3,4,6-tetrakisphosphate [Ins(1,3,4,6)P_4] (2), possesses Ca^{2+}-mobilising activity, despite the apparent absence of a 4,5-bisphosphate motif. We have rationalised this[9] by invoking two alternative receptor binding conformations (5) and (6) for Ins(1,3,4,6)P_4, where the 1,6-vicinal bisphosphate mimics the normal 4,5-bisphosphate in the Ins(1,4,5)P_3 binding conformation (4) (Fig 2).

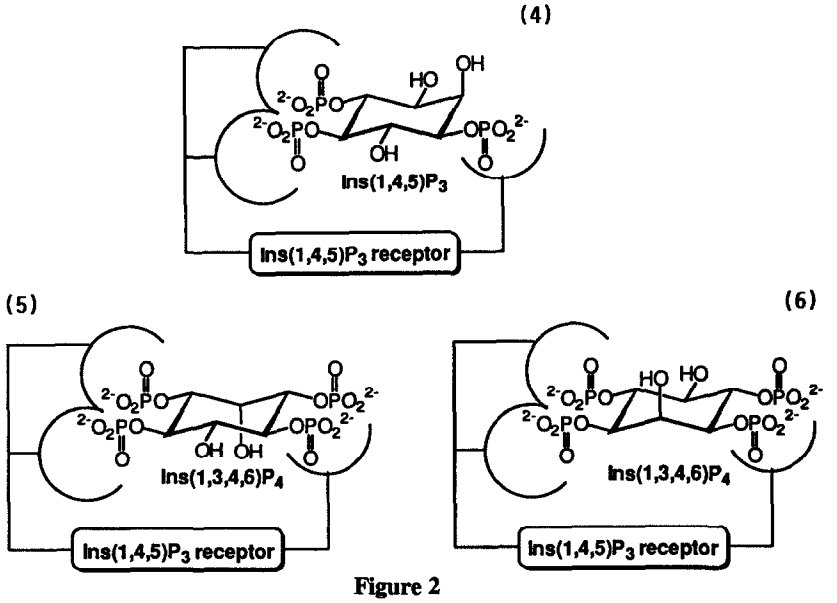


Figure 2

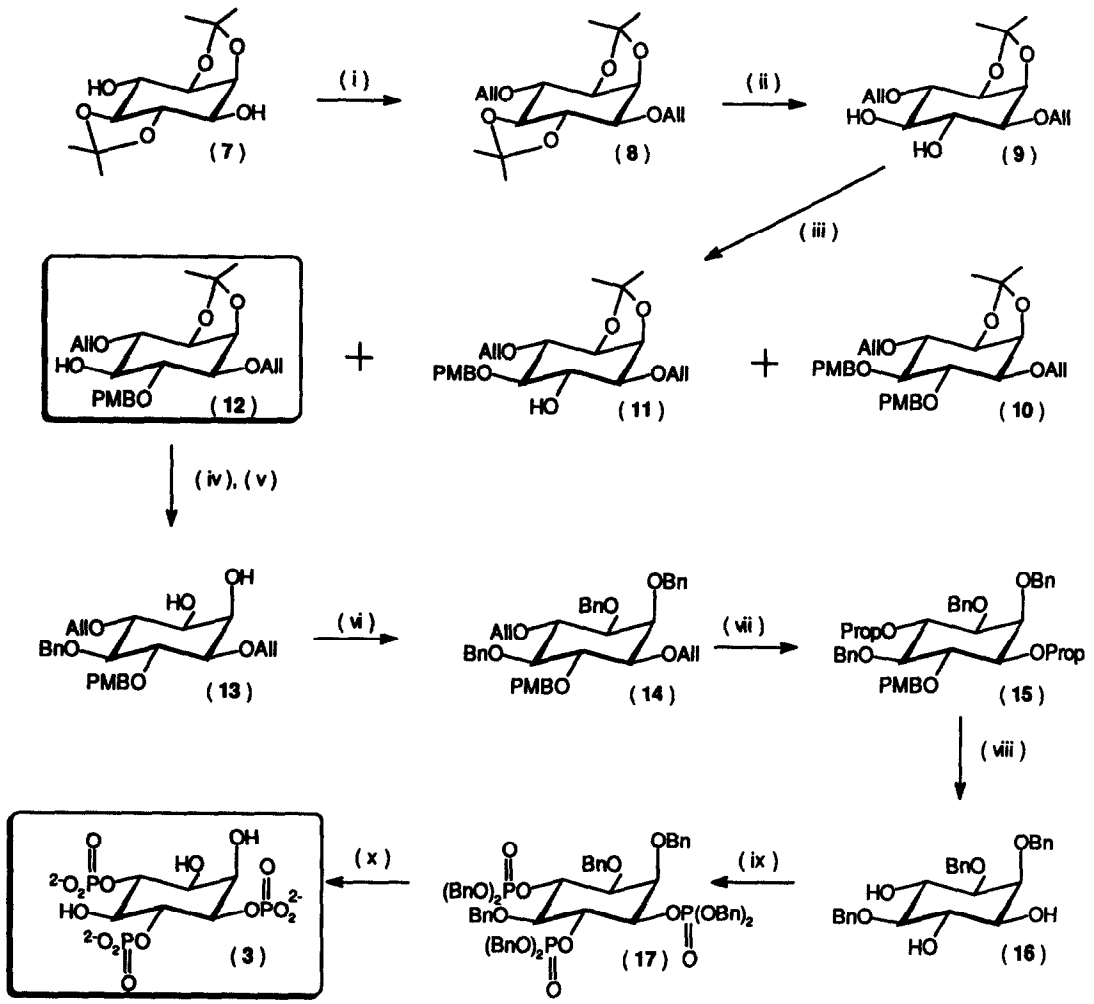
Since a 1-phosphate group and an equatorial 6-OH are thought to be responsible for enhanced receptor binding[7,13] it seems most likely that conformation (5) is the active one, especially since an axial -OH group at the 3-position as in L-*chiro*-Ins(2,3,5)P_3 only results on an approximate 10-fold decrease in Ca^{2+} mobilising activity[14], relative to Ins(1,4,5)P_3.

Conformation (**5**) would predict that the novel trisphosphate Ins(1,4,6)P_3 (**3**) should show Ca^{2+} mobilising activity. We therefore undertook the preparation of this compound and we report here the synthesis of racemic (**3**) and demonstrate its Ca^{2+}-mobilising activity in permeabilised platelets.

Ins(1,4,6)P_3 was synthesised from *myo*-inositol according to the Scheme.* The intermediate diol (**7**) was prepared from *myo*-inositol in three steps according to Gigg *et al*[15]. This diol was alkylated with allyl bromide in DMF to give fully protected (**8**). The less stable *trans*-diequatorial ketal was removed using a catalytic amount of *p*-toluene sulphonic acid and 1 equivalent of ethane 1,2-diol in dichloromethane in order to expose the vicinal diol of (**9**). Stannylation of (**9**) using dibutyltin oxide and a quaternary ammonium halide[16] followed by alkylation (*in situ*) with *p*-methoxybenzyl chloride gave, as a minor product, the di-*O*-*p*-methoxybenzyl derivative (**10**), (14% yield), and as major products the 5-*O*-*p*-methoxybenzylated derivative (**11**), (31% yield) and predominantly the desired 6-*O*-*p*-methoxybenzylated derivative (**12**), (52% yield), the latter of which was used to prepare the key 2,3,5-tri-*O*-benzyl triol (**16**). All three of these products were readily separated by flash column chromatography.

The 5-hydroxyl group of (**12**) was first benzylated and the *cis*-ketal of the resulting product was removed using methanol-MHCl (9:1) to expose the 2,3-*cis*-diol of (**13**). Benzylation of this diol on both hydroxyl groups and isomerisation of the allyl protecting groups to their *cis*-prop-1-enyl ethers using potassium *t*-butoxide in DMSO[17] gave fully protected (**15**), which could be deprotected in one step with acid to give the required precursor (**16**) for phosphorylation to *myo*-inositol 1,4,6-trisphosphate. The triol (**16**) was phosphitylated with bis(benzyloxy)(diisopropylamino)phosphine[18] and the resulting trisphosphite oxidised with *t*-butyl-hydroperoxide to give the syrupy, totally protected trisphosphate (**17**), (85% yield). A one step deprotection with sodium in liquid ammonia[19], followed by purification of the crude product by ion exchange chromatography on Q-Sepharose fast flow, using a

* All new compounds showed satisfactory spectroscopic properties.



Scheme

Reagents and conditions

i, AllBr, NaH, DMF; ii, PTSA (cat),ethane 1,2 diol,(1.0 equiv.),CH_2Cl_2; iii,Bu_2SnO (1.0 equiv.), Bu_4NI,CH_3CN,PMBCl (2 equiv.), reflux 24hrs; iv, BnBr,NaH,DMF; v, MeOH - 1M HCl (9 :1 v/ v), 50°C, 45mins; vi, BnBr, NaH, DMF; vii, Bu^tOK–DMSO, 50°C,4hrs; viii, EtOH – 1M HCl (2 :1 v/ v), reflux 3hrs; ix, (a) $(BnO)_2PNPr_2^i$ (6 equiv.),tetrazole (12 equiv.) in CH_2Cl_2, (b) H_2O, then Bu^tOOH (80%, Fluka); x,Na–liq. NH_3.
All = allyl; PMB = *p* – methoxybenzyl; Bn = benzyl; PTSA = *p* – toluenesulphonic acid; Prop. = *cis*-prop–1–enyl. All compounds are racemic.

gradient of triethylammonium bicarbonate as eluant, gave pure *myo*-inositol 1,4,6-trisphosphate (3) in 60% yield as its glassy triethylammonium salt.

Racemic (3) was evaluated as a Ca^{2+} mobilising agonist in permeabilised platelets, relative to Ins(1,4,5)P_3 and Ins(1,3,4,6)P_4. 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 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.

The dose response curves are shown in Fig 3 and the EC_{50} values (mean ± SE Mean) were 0.18 ± 0.1μM [Ins(1,4,5)P_3], 2.07 ± 0.08μM [Ins(1,4,6)P_3] and 9.67 ± 0.5μM [Ins(1,3,4,6)P_4]. Therefore Ins(1,4,6)P_3 is some 11 fold less potent than Ins(1,4,5)P_3, but five fold *more potent* than Ins(1,3,4,6)P_4 in its ability to mobilise calcium.

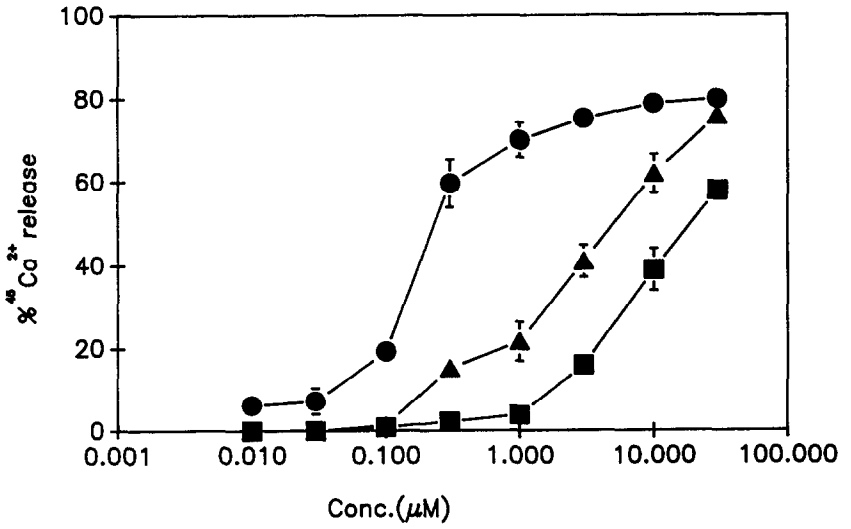


Figure 3: The dose-response curves of Ins(1,4,5)P_3 and its analogues for their ability to release $^{45}Ca^{2+}$ from permeabilised rabbit platelets. Each point is mean ± δ SE; Mean of 3 determinations. Ins(1,4,5)P_3 (●), Ins(1,4,6)P_3 (▲), Ins(1,3,4,6)P_4 (■).

This is consistent with our proposed model (Fig 2) and presumably only reflects activity of the D-enantiomer of Ins(1,4,6)P_3. This considerable enhancement of potency in a totally synthetic relative of the naturally occurring Ins(1,3,4,6)P_3 represents one of the first examples of rational structure-based molecular design in the inositol phosphate field.

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