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TOTAL SYNTHESIS OF *MYO*-INOSITOL-1-PHOSPHATE-4,5-PYROPHOSPHATE, A NOVEL SECOND MESSENGER ANALOGUE, VIA *MYO*-INOSITOL-1-PHOSPHATE-4,5-BISPHOSPHOROTHIOATE

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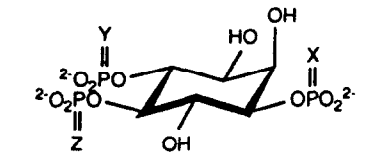
Abstract: The synthesis of the novel analogues of *myo*-inositol 1,4,5-trisphosphate, *myo*-inositol 1-phosphate 4,5-bisphosphorothioate and *myo*-inositol 1-phosphate 4,5-pyrophosphate is reported; the latter was prepared via desulphurisation and intramolecular coupling.

INTRODUCTION

It is now generally accepted that D-*myo*-inositol 1,4,5-trisphosphate (IP_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]. IP_3 is metabolised *via* two pathways[3]: deactivation by a 5-phosphatase to 1,4-IP_2 or phosphorylation by a 3-kinase to 1,3,4,5-IP_4. The function of the latter still remains controversial and IP_4 may gate a plasma membrane Ca^{2+} channel[4]. IP_3 acts through an intracellular receptor which has been isolated[5], cloned and sequenced[6,7] and reconstituted[8].

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 (IPS_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] and *myo*-inositol 1,4-bisphosphate 5-phosphorothioate **(4)**[18,19]. It is clear that such analogues offer considerable potential for investigation and modification of the complex metabolism of IP_3 and this has been recognized by other groups[20,21].



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)

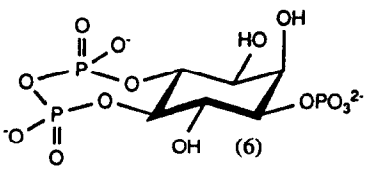
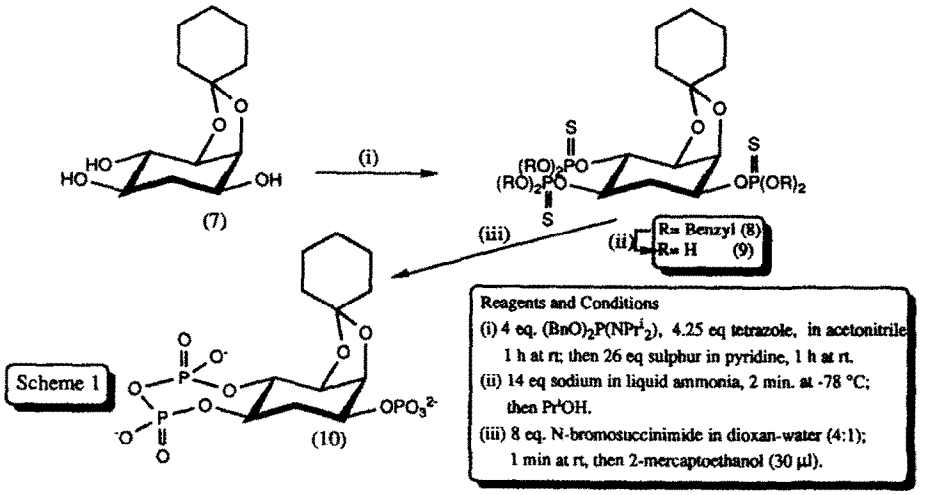


Figure 1

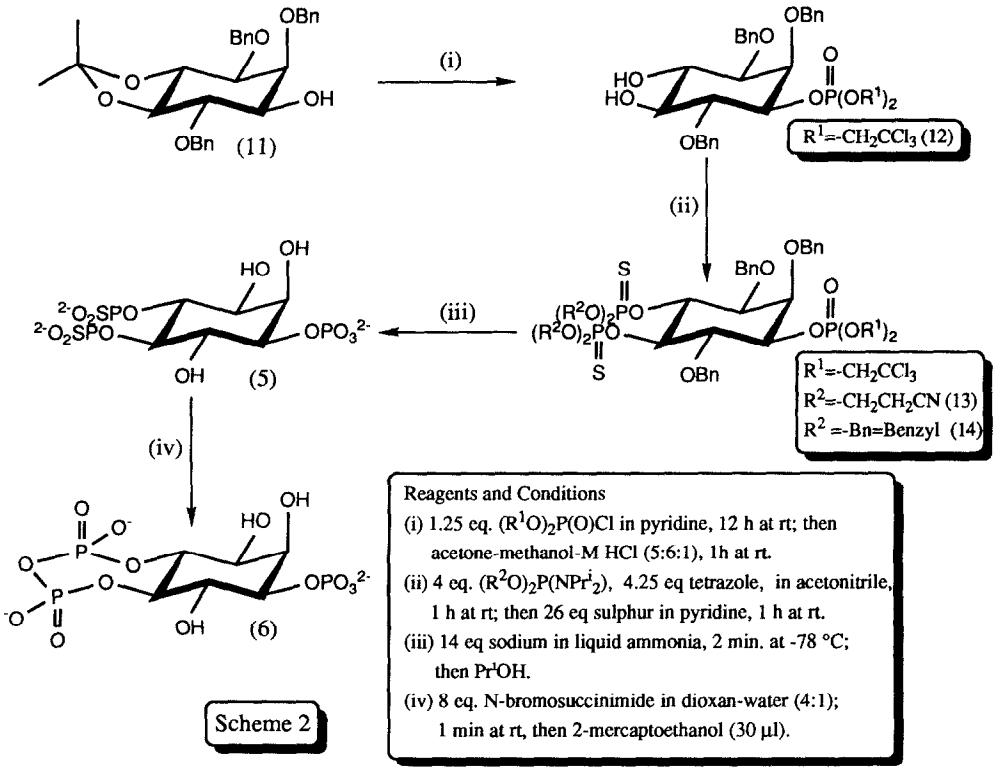
In structure-activity studies, the vicinal 4,5-bisphosphate moiety of IP_3 has been identified as being crucial for intracellular Ca^{2+} release[3,9-11]. Clearly, chemical modification at this locus may be one of the keys for designing potential IP_3 receptor antagonists. An obvious synthetically-challenging target is the pyrophosphate (**6**) where the 4,5-phosphate groups have been linked together. As well as comprising a novel modification to the IP_3 structure, formation of the pyrophosphate produces a less polar analogue that can potentially be converted to IP_3 enzymatically in cells. We have previously reported a new route to such seven-membered pyrophosphates by desulphurisation of a vicinal bisphosphorothioate using N-bromosuccinimide (NBS)[22]. We report here the total synthesis of (**6**) via the novel phosphorothioate analogue *myo*-inositol 1-phosphate 4,5-bisphosphorothioate (**5**).

Initial experiments on the feasibility of using polyphosphorothioate derivatives of inositol to prepare cyclic pyrophosphates by our desulphurisation method[22] were performed on (**7**), a protected precursor of D-6-deoxy-*myo*-inositol 1,4,5-trisphosphate[23]. Thus, D-2,3-*O*-cyclohexylidene-6-deoxy-*myo*-inositol[24] (**7**) (Scheme 1) was converted into its trisphosphorothioate ester (**8**) using bisbenzyl-diisopropylaminophosphine-tetrazole[25], followed by oxidation of the resulting trisphosphite using sulphur in pyridine[12]. Deprotection using sodium in liquid ammonia[26] removed the benzyl protecting groups and purification of the crude product on DEAE Sepharose using a gradient of triethylammonium bicarbonate (TEAB) gave D-2,3-*O*-cyclohexylidene-6-deoxy-*myo*-inositol-1,4,5-trisphosphorothioate (**9**) as its triethylammonium salt. This compound has the advantage that on NBS-mediated desulphurisation there are no neighbouring vicinal hydroxyl groups to facilitate intramolecular cyclisation to form cyclic 5-membered phosphates. Furthermore, we expected that conformational restriction of the cyclohexane

ring, due to the presence of the 2,3-ketal might aid the ring closure of the *trans*-vicinal 4,5-phosphates. Consequently, the 1-phosphorothioate group should be desulphurised to the corresponding phosphate and we expected intramolecular coupling of the activated 4,5-bisphosphorothioate to form the cyclic pyrophosphate. Indeed, desulphurisation of **(9)** using NBS gave D-2,3-*O*-cyclohexylidene-6-deoxy-*myo*-inositol-1-phosphate-4,5-cyclic pyrophosphate **(10)** in > 90% yield, demonstrating the feasibility of our approach in the inositol series. **(10)** Was purified by ion-exchange chromatography. ^{31}P nmr spectroscopy of **(10)** (109.4 MHz, D_2O pH9, ext. H_3PO_4 ref.) showed clearly a peak at δ -1.84 ppm assignable to the 1-phosphate group and an AB system with δ -12.52 and -13.02 ppm, $^2J_{pp}$ 16.8 Hz, assignable to the 4,5-pyrophosphate system.



To avoid complications due to potential cyclisation of an activated 1-phosphorothioate group during desulphurisation of IPS_3 (2) we have designed a synthesis of *myo*-inositol 1-phosphate 4,5-bisphosphorothioate **(5)** as a precursor of **(6)**. (±)-2,3,6-tri-*O*-benzyl-1[bis(2,2,2-trichloroethyl)phospho]-*myo*-inositol **(12)** was synthesised as described[19,27] from the protected mono-alcohol **(11)**[28]. Bisphosphitylation of **(12)** with either bis(2-cyanoethyl)diisopropylaminophosphine[29] or bisbenzyldiisopropylaminophosphine[25] gave the corresponding 4,5-bisphosphites, which were oxidised to the respective 4,5-bisphosphorothioates **(13)** and **(14)** with sulphur in pyridine (Scheme 2). Deprotection by



Reagents and Conditions

(i) 1.25 eq. $(R^1O)_2P(O)Cl$ in pyridine, 12 h at rt; then acetone-methanol-M HCl (5:6:1), 1h at rt.

(ii) 4 eq. $(R^2O)_2P(NPr^i_2)$, 4.25 eq tetrazole, in acetonitrile, 1 h at rt; then 26 eq sulphur in pyridine, 1 h at rt.

(iii) 14 eq sodium in liquid ammonia, 2 min. at -78 °C; then Pr^iOH.

(iv) 8 eq. N-bromosuccinimide in dioxan-water (4:1); 1 min at rt, then 2-mercaptoethanol (30 µl).

Scheme 2

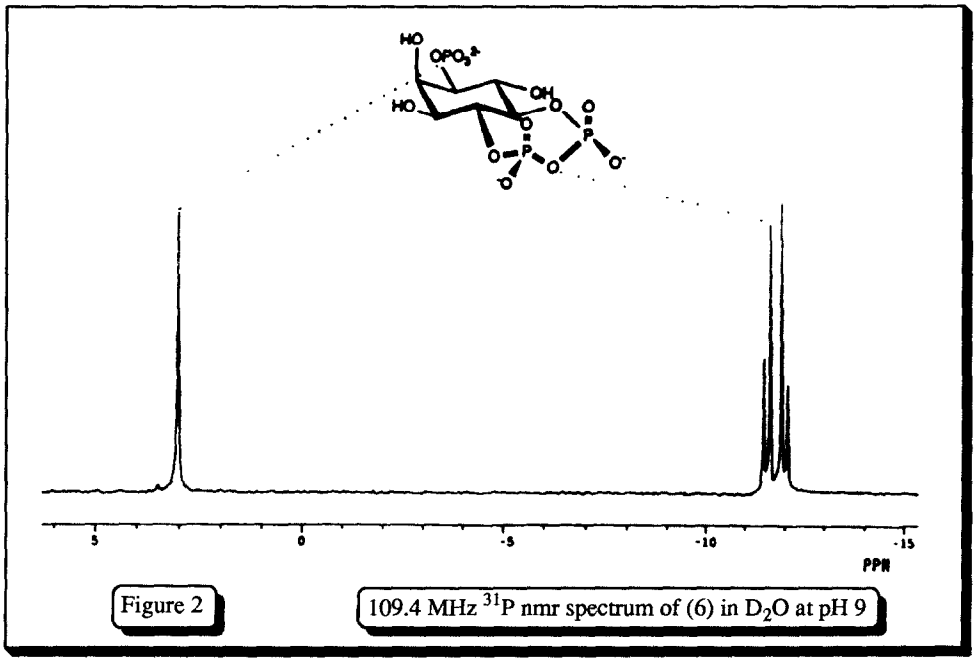


Figure 2

109.4 MHz ^{31}P nmr spectrum of (6) in D_2O at pH 9

sodium in liquid ammonia as above gave crude DL-*myo*-inositol-1-phosphate-4,5-bisphosphorothioate (**5**) which was purified by ion-exchange chromatography on either DEAE Sephadex A-25 or Q-Sepharose to give the pure triethylammonium salt of (**5**), quantified by Briggs phosphate assay[30], in 83% yield.

DL-(**5**) Was a potent agonist for intracellular Ca^{2+} mobilisation in permeabilised SH-SY5Y neuroblastoma cells[31]. It mobilised Ca^{2+} with a potency slightly less than IP_3 and similar to IPS_3 (**2**). Having a 5-phosphorothioate group, (**5**) is resistant to degradation by IP_3-5-phosphatase and stimulated a persistant release of Ca^{2+} like IPS_3 [3,31]. It inhibited IP_3-5 phosphatase potently with a K_i of 1.3 $\pm$ 0.3 μM similar to IPS_3 [32] suggesting that D-(**5**) would have a submicromolar K_i for this enzyme. Full biological details will be published elsewhere.

(**5**) Was desulphurised with NBS and gave the crude pyrophosphate (**6**) in high yield with some evidence of phosphate migration (24%) and straight desulphurisation to IP_3 (7%). (**6**) Was purified by anion exchange chromatography on Q-Sepharose as above to give the triethylammonium salt (67% yield). It exhibited a ^{31}P n.m.r spectrum (Figure 2) showing clearly the presence of the 1-phosphate (δ 3.04 ppm) and the 4,5-pyrophosphate [δ -11.61 (pos. 4) and -12.02 ppm (pos. 5), $^2J_{pp}$ 16.8 Hz], the latter resonating as the expected AB system. Biological evaluation of (**6**) is in progress.

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