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# SYNTHESIS OF *MYO*-INOSITOL 1,4,5-TRISPHOSPHATE 3-PHOSPHOROTHIOATE AS AN INHIBITOR OF *MYO*-INOSITOL 1,3,4,5-TETRAKISPHOSPHATE 3-PHOSPHATASE

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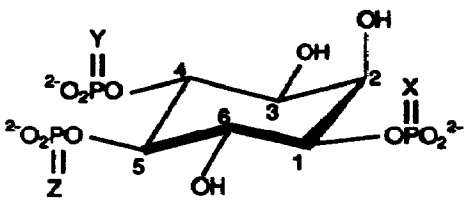
**Abstract:** *The synthesis of racemic* myo-*inositol 1,4,5-trisphosphate 3-phosphorothioate from* myo-*inositol is described using a protection/deprotection sequence employing allyl, benzyl and* p-*methoxybenzyl groups to facilitate selective 3-position thiophosphorylation.*

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[8] and Ins(1,3,4,5)$P_4$ may gate a plasma membrane $Ca^{2+}$ channel[9].




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

Figure 1

As part of an ongoing programme aimed to study structure-activity relationships in inositol tris- and tetrakisphosphates[7,10] we have been engaged in the synthesis of *myo*-inositol polyphosphates and their analogues as potential enzyme inhibitors and receptor antagonists. In particular, phosphorothioate analogues of Ins(1,4,5)$P_3$ either with multiple or specific substitutions eg (2) - (5) (Figure 1) have emerged as potent inhibitors of the metabolic enzyme Ins(1,4,5)$P_3$ 5-phosphatase[11].

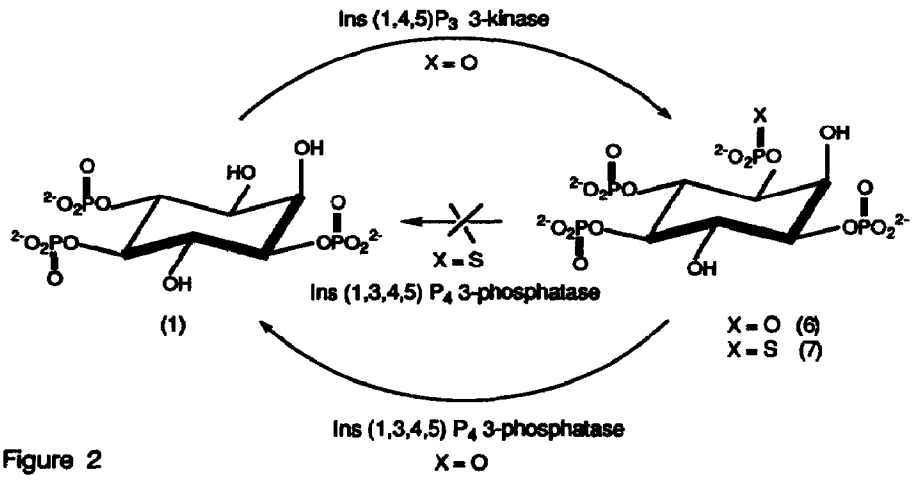
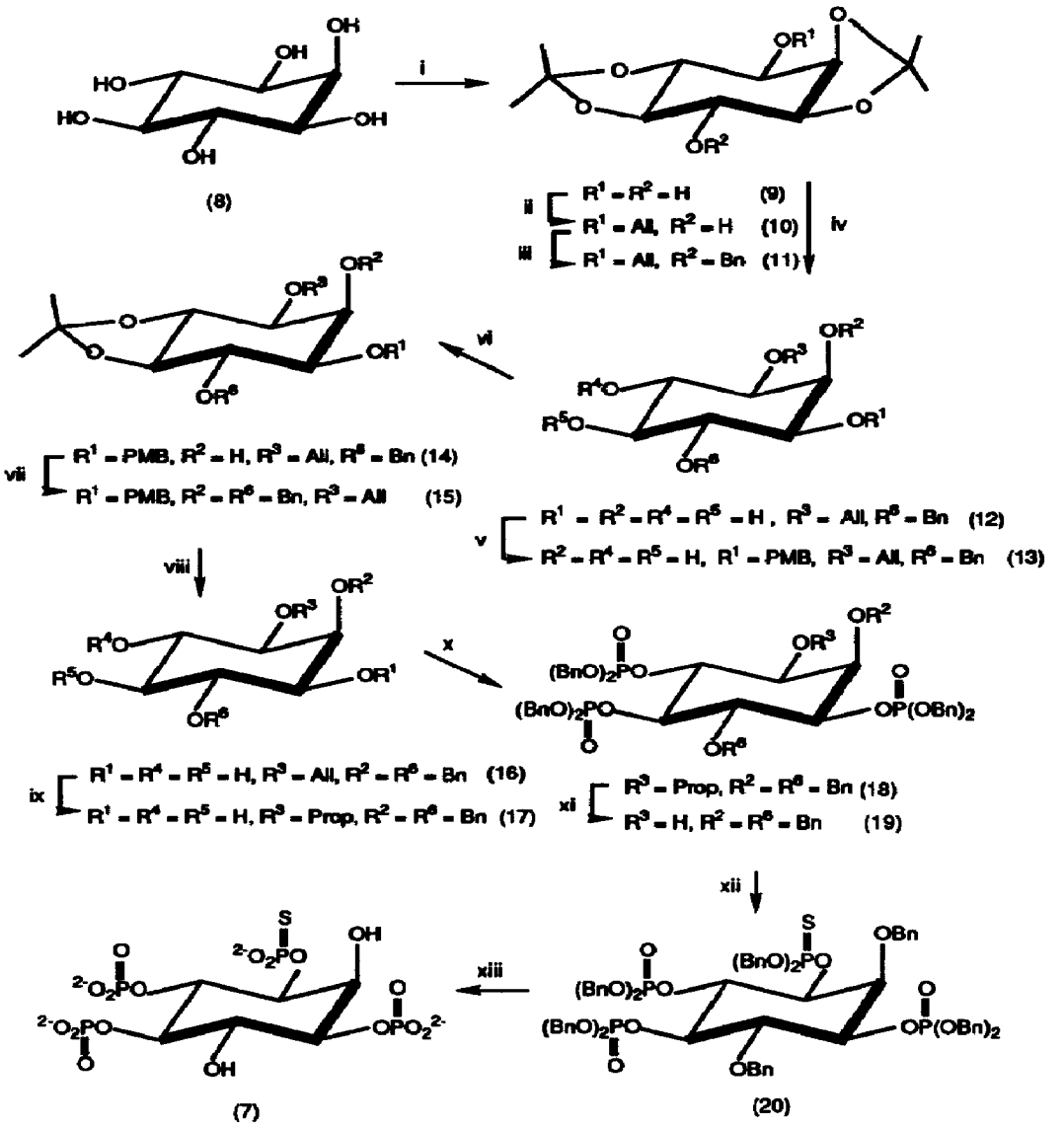



Figure 2

A complication in studies designed to investigate the potential role of Ins(1,3,4,5)$P_4$ in $Ca^{2+}$ homeostasis has been the existence of the enzyme Ins(1,3,4,5)$P_4$ 3-phosphatase[12] which converts the poor $Ca^{2+}$ mobiliser Ins(1,3,4,5)$P_4$ (6) into the potent Ins(1,4,5)$P_3$ (Figure 2). While endogenous inhibitors of this enzyme, Ins(1,3,4,5,6)$P_5$ and Ins$P_6$ potently inhibit Ins(1,3,4,5)$P_4$ 3-phosphatase activity[13,14], an inhibitor based upon Ins(1,3,4,5)$P_4$ would facilitate investigations of the potential biological importance of this molecule independent of the 3-phosphatase catalysed conversion into Ins(1,4,5)$P_3$. To this end, we have designed *myo*-inositol 1,4,5-trisphosphate 3-phosphorothioate [Ins(1,3,4,5)$P_4$-3S] (7) as a novel, specifically phosphorothioate-substituted Ins(1,3,4,5)$P_4$ analogue with potential as an Ins(1,3,4,5)$P_4$ 3-phosphatase inhibitor and we report here the synthesis of racemic (7).

Conversion of the inositol 1,2:4,5 diketal[15] (9), prepared from *myo*-inositol (8), to the 3-*O*-allyl (10) and 3-*O*-allyl-6-*O*-benzyl (11) derivatives was achieved by treatment of (9) first with allyl bromide/barium oxide and barium hydroxide (63% yield) and then (10) with benzyl chloride/sodium hydride respectively (100% yield) (Scheme). Removal of the isopropylidene groups by treatment of (11) with *p*-toluene sulfonic acid in ethyl acetate/acetone/water afforded (12) (88% yield). Regioselective introduction of a 1-*O*-*p*-methoxybenzyl ether in (12) was achieved by treatment with dibutyltin oxide in refluxing toluene, followed by caesium fluoride/*p*-methoxybenzyl bromide to give (13) in 74% yield. After reintroduction of the 4,5-*O*-isopropylidene ketal by use of 2-methoxypropene and *p*-toluene sulfonic acid giving (14) (90% yield) the remaining 2-hydroxyl group was benzylated to produce (15) (94% yield). The isopropylidene group and the 1-*O*-*p*-methoxybenzyl ether were successively cleaved by treatment of (15) with refluxing hydrochloric acid to produce the triol 3-*O*-allyl-2,6-di-*O*-benzyl-*myo*-inositol (16)[16] in 90% yield. The allyl group of (16) was isomerized to *cis*-propenyl using KOBu$^t$ to give the key intermediate (17). Phosphitylation of (17) was effected using bisbenzyloxy(diisopropylamino)phosphine-tetrazole in dichloromethane[17] to afford the corresponding trisphosphite which was smoothly oxidised with *tert*-BuOOH to the fully protected trisphosphate (18) in 80% overall yield from (17). The propenyl group of (18) was removed, avoiding phosphate migration, by use of trifluoroacetic acid to give the trisphosphate (19). [Racemic (19) has been prepared previously *via* a different route[18]]. This was converted to the protected 3-phosphite as above and the crude intermediate sulfoxidised using sulfur in




**Scheme** Reagents and conditions:
(i) (a) $CH_3C(CH_3O)_2CH_3$, PTSA, reflux, (b) BzCl, pyridine, (c) NaOH, reflux; (ii) allyl bromide, NaH; (iii) BnCl, NaH; (iv ) PTSA, ethyl acetate/acetone/water; (v) (a) dibutyltin oxide, reflux, (b) CsF, (*p*-MeO) BnCl; (vi) 2-methoxypropene, PTSA; (vii) BnCl, NaH; (viii) M HCl, reflux; (ix) *tert*-BuOK; (x) (a) $Pr^i_2NP(OBn)_2$, tetrazole in $CH_2Cl_2$, (b) 70% *tert*-BuOOH; (xi) $CF_3COOH$ in EtOH; (xii) (a) $Pr^i_2NP(OBn)_2$, tetrazole in $CH_2Cl_2$, (b) sulfur in pyridine; (xiii) (a) Na/liq, $NH_3$, (b) $H_2O$
All compounds are racemic.

pyridine to the fully protected (20). Benzyl protecting groups of (20) were subsequently removed by treatment with sodium in liquid ammonia[19] to provide crude (7) which was subjected to ion-exchange chromatography on Q-Sepharose eluting with a gradient of triethylammonium bicarbonate buffer, to afford pure (7) in 68% yield. As expected, (7) exhibited three singlets at δ -0.42, 0.23 and 0.61ppm in the $^{1}H$-decoupled $^{31}P$ NMR spectrum, corresponding to phosphate groups and a singlet at δ 49.89ppm corresponding to the unique 3-*O*-phosphorothioate.

Racemic Ins(1,3,4,5)$P_4$-3S was found to be a $Ca^{2+}$ mobilising agonist in permeabilised neuroblastoma cells with a potency [$EC_{50}$ = 4.7μM][20] similar to Ins(1,3,4,5)$P_4$ when assayed in the presence of $InsP_6$ as 3-phosphatase inhibitor [$EC_{50}$ = 2.5μM]. Ins(1,3,4,5)$P_4$-3S will complement Ins(1,3,4,5)$P_4$-5S, for which a synthetic route has been published[21] and be an important tool for the identification of potentially exclusive Ins(1,3,4,5)$P_4$ second messenger functions, since its resistance to 3-phosphatase action will preclude the inconvenient artefact of steady state Ins(1,4,5)$P_3$ generation. It has already found use in demonstrating that Ins(1,3,4,5)$P_4$ can independently mobilise intracellular $Ca^{2+}$ *via* the Ins(1,4,5)$P_3$ receptor[20].

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