Synthesis of *myo*-Inositol 1,4-Bisphosphate-5-phosphorothioate

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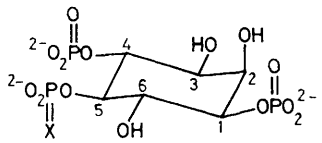
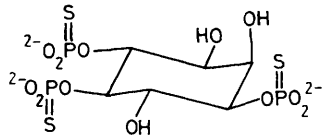
Synthesis of *myo*-insoitol 1,4,5-triphosphate and *myo*-inositol 1,4,-bisphosphate-5-phosphorothioate, a phosphatase-resistant analogue of a biological second messenger, have been accomplished using a novel combination of P^{III} and P^{V} chemistry and a new deprotection method for the 2,2,2-trichloroethyl group.

It is now generally accepted that D-*myo*-inositol 1,4,5-trisphosphate (IP_3) (**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 calcium from cellular internal stores.[1] IP_3 is metabolised *via* two pathways: deactivation by a 5-phosphatase[2] to 1,4-IP_2 and phosphorylation by a 3-kinase to 1,3,4,5-IP_4,[3] whose function remains controversial.

We have sought to develop synthetic routes to inositol phosphates[4,5] and especially to prepare novel non-hydrolysable analogues such as phosphorothioates.[6] Our recent synthesis of *myo*-inositol 1,4,5-trisphosphorothioate (IPS_3) (**2**)[7] has provided an analogue that is a potent releaser of calcium[8,9] and yet is resistant to phosphatase-catalysed deactivation.[10] It is clear that such analogues offer considerable potential for investigation and modification of the complex metabolism of IP_3.



(1) X = O
(3) X = S



(2)

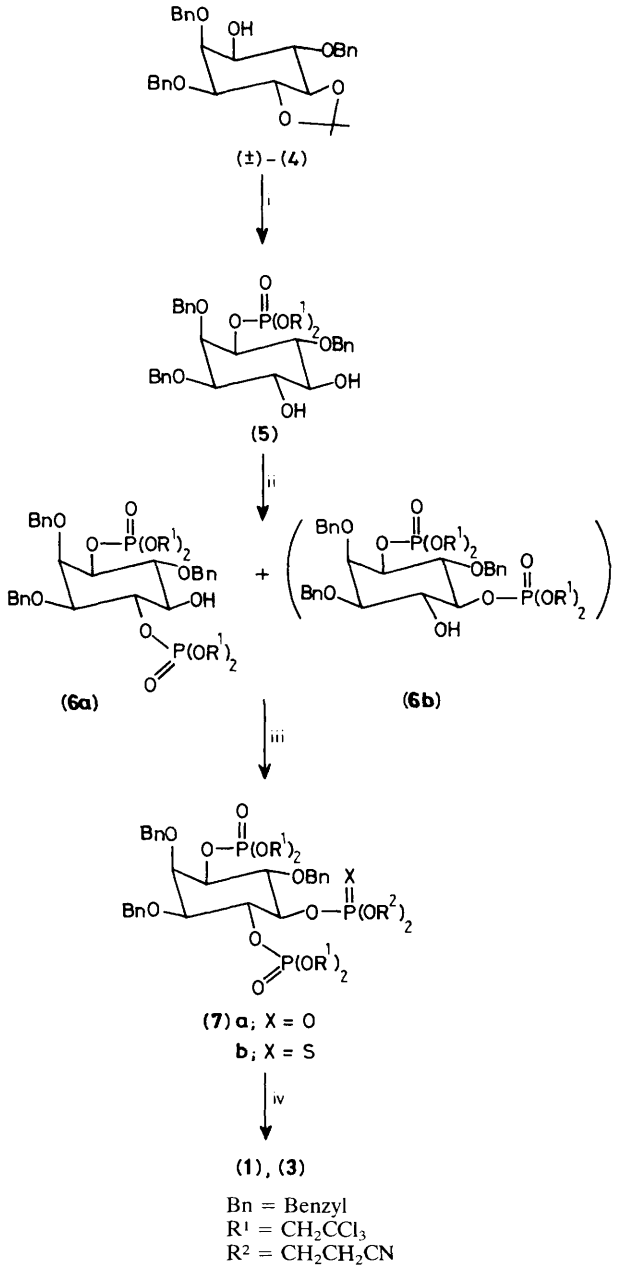
IPS_3 has all three phosphates replaced by phosphorothioates. We wished to devise a synthetic route to the novel analogue *myo*-inositol 1,4-bisphosphate-5-phosphorothioate (**3**), which has only the 5-phosphate position substituted by phosphorothioate and will be nearer in structure to the natural messenger whilst still enjoying the advantages of metabolic stability.

(±)-2,3,6-Tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol (**4**)[11] was phosphorylated in 90% yield at the free hydroxyl group using bis(2,2,2-trichloroethyl)phosphorochloridate to 2,3,6-tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol-1-(bis-2,2,2-trichloroethyl)phosphate (m.p. 114–115 °C) (Scheme 1). The isopropylidene group was removed quantitatively to give the corresponding diol (m.p. 141–143 °C) (**5**), which was phosphorylated to the mixed bisphosphate triesters (**6a**) and (**6b**) possessing 1,4- and 1,5-substitution patterns, respectively. It was clearly not possible to bisphosphorylate (**5**) under these conditions, presumably on account of the steric crowding which would accompany the formation of *trans*-vicinal bis(bis-2,2,2-trichloroethylphosphate) triesters. Only a small amount of pyridine was used in this phosphorylation, which was carried out in dichloromethane, since the normal use of pyridine as solvent was found to result in the formation of the five-membered cyclic phosphate, which decomposed on work-up to give a mixture of phosphate diesters at the 4- and 5-positions. Trituration of the mixed bisphosphate triesters with petrol after rapid chromatography on silica (to avoid cyclisation) afforded one bisphosphate in crystalline form† [m.p. 151–153 °C; δ_P ($CDCl_3$) (proton decoupled) −6.59, −7.94 p.p.m., 40% yield].

The structure of the crystalline bisphosphate could not readily be deduced from its complex ^1H n.m.r. spectrum on account of overlap of benzyl $-CH_2-$ protons and the complex ABX systems of the four trichloroethyl groups with three of the inositol ring protons. The latter, however, were readily located from the 2D J-resolved ^1H n.m.r. spectrum, which also distinguished those protons experiencing heteronuclear coupling to phosphorus. A 2D ^1H n.m.r. COSY spectrum was then sufficient to deduce the ring connectively and assign the structure to the 1,4-bisphosphate triester (**6a**).

Compound (**6a**) was then phosphitylated at the free 5-hydroxyl using our P^{III} approach[4,5] and oxidised either to 2,3,6-tri-*O*-benzyl-*myo*-inositol-1,4-bis(bis-2,2,2-trichloroethylphosphate)-5-(bis-2-cyanoethylphosphate) (**7a**) or the corresponding 5-(bis-2-cyanoethylphosphorothioate) (**7b**) using t-butyl hydroperoxide or sulphur in pyridine, respectively.

† Intermediates up to and including this stage gave satisfactory elemental analyses.



Scheme 1. *Reagents and conditions:* i, (a) $(CCl_3CH_2O)_2POCl$ (1.25 equiv.), pyridine, 12 h, (b) H_3O^+; ii, $(CCl_3CH_2O)_2POCl$ (2 equiv.), pyridine (4 equiv.), CH_2Cl_2, 6 h; iii, (a) $(NCCH_2CH_2O)P(NPr^i_2)Cl$ (1.2 equiv.), $EtNPr^i_2$ (1 equiv.), MeCN, 1 h, (b) $NCCH_2CH_2OH$, tetrazole (2.5 equiv. of each), MeCN, 1 h, (c) for X = O, Bu^tOOH (3 M solution in toluene), 0 °C, 15 min; X = S, sulphur in pyridine, 16 h; iv, Na in liq. NH_3. All compounds are racemic.

Deblocking of protecting groups was accomplished using sodium in liquid ammonia, which removed the cyanoethyl groups by β-elimination and reductively removed the benzyl groups and the 2,2,2-trichloroethyl groups, the latter cleavage presumably involving initial C–Cl bond cleavage. Although several methods have been devised for deblocking of 2,2,2-trichloroethyl groups[12] none is entirely satisfactory. To the best of our knowledge, the use of sodium in liquid ammonia has not previously been reported and therefore represents a new procedure for this purpose. *myo*-Inositol 1,4,5-trisphos-

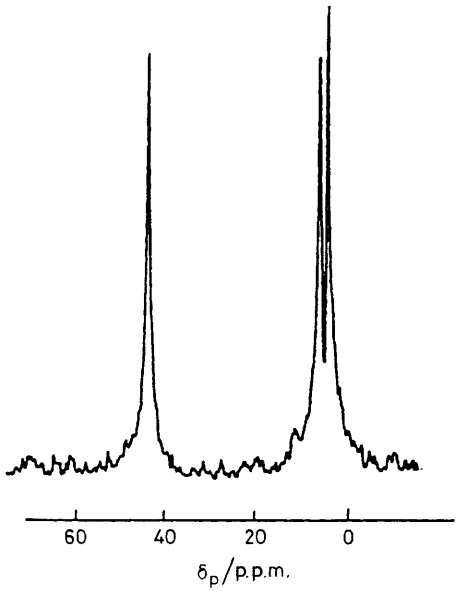


Figure 1. 24.15 MHz ^{31}P N.m.r. spectrum of *myo*-inositol 1,4-bisphosphate-5-phosphorothioate (*ca.* 70 mM solution in D_2O, pH 8). N.m.r. parameters were: sweep width, 10 kHz; pulse width, 8 μs; collected over 4 K; no. of transients, 1000; broad band proton decoupling; referenced to external H_3PO_4.

phate (**1**) and 1,4-bisphosphate-5-phosphorothioate (**3**) were obtained as triethylammonium salts by anion exchange chromatography in *ca.* 44% yield based upon (**6a**). The ^{31}P n.m.r. spectrum of (**3**) (Figure 1) clearly shows the presence of two phosphate groups [δ_P (D_2O) 2.62, 4.64 p.p.m.] and one phosphorothioate group (δ_P 44.98 p.p.m.) in the molecule; *m/z* [fast atom bombardment (FAB)] 435 $(M-H)^-$. DL-IP_3 prepared by this route was equipotent as previously synthesized material[5] at releasing calcium from permeabilized Swiss 3T3 cells[9] and displacing [^{3}H]-IP_3 from a specific IP_3 binding site in cerebellum.[13] *myo*-Inositol 1,4-bisphosphate-5-phosphorothioate was found to be very similar to IPS_3 in calcium release and 5-phosphatase resistance experiments. These novel biological properties will be reported in detail elsewhere.

We thank Dr. D. L. Turner for advice concerning the 2D n.m.r. experiments and Professor S. R. Nahorski for biological testing. This work was supported by the S.E.R.C., the Research Corporation Trust, and in part by Merck Sharp and Dohme. B. V. L. P. is a Lister Institute Research Fellow.

Received, 11th July 1988; Com. 8/02760I

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