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Synthesis of (±)-*myo*-inositol 1,4,5-trisphosphate and the novel analogue (±)-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate

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ABSTRACT

Novel routes to *myo*-inositol 1,4,5-trisphosphate and a phosphorothioate analogue involving mixed P(V) and P(III) chemistry have been developed. Phosphorylation of 2,3,6-tri-*O*-benzyl-*myo*-inositol 1-[di-(2,2,2-trichloroethyl) phosphate] with bis(2,2,2-trichloroethyl) phosphorochloridate gave a mixture of the 1,4- and 1,5-bisphosphate derivatives from which the 1,4-bis[di-(2,2,2-trichloroethyl) phosphate] **9** crystallised. Phosphitylation of HO-5 in **9** followed by oxidation yielded the 1,4-bis[di-(2,2,2-trichloroethyl) phosphate] 5-[di-(2-cyanoethyl) phosphate] which was deblocked using sodium in liquid ammonia to give (±)-*myo*-inositol 1,4,5-trisphosphate. Phosphitylation of HO-5 in **9** followed by sulphoxidation generated the 1,4-bis[di-(2,2,2-trichloroethyl) phosphate] 5-[di-(2-cyanoethyl) thiophosphate] which was deblocked to give (±)-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate. Removal of the 2,2,2-trichloroethyl group, using sodium in liquid ammonia, represents a new method for removing this protecting group.

INTRODUCTION

1D-*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 that stimulates the release of intracellular calcium from internal stores[1,2]. IP_3 is metabolised by two pathways[3], namely, deactivation by a 5-phosphatase to give 1,4-IP_2 or phosphorylation by a 3-kinase to give 1,3,4,5-IP_4; the function of the latter route is still controversial[4]. IP_3 acts through an intracellular receptor which has been isolated[5], cloned and sequenced[6,7], and reconstituted[8].

We have sought routes for the synthesis of inositol phosphates[9] and non-hydrolysable analogues such as phosphorothioates[9–11]. The synthesis[12] of (±)-*myo*-ino-

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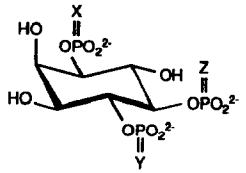
sitol 1,4,5-trisphosphorothioate (IPS_3, **2**) has provided an analogue that is a potent releaser of calcium[13–15] but resistant to phosphatase-catalysed deactivation[16]. Such analogues offer considerable potential for investigation and modification of the complex metabolism of IP_3.

(±)-*myo*-Inositol 1,4-bisphosphate 5-phosphorothioate (**3**) is much nearer in structure to IP_3 whilst still resistant to metabolism. We have reported the synthesis[17] of the related analogue *myo*-inositol 4,5-bisphosphate 1-phosphorothioate (**4**) and now describe a route to (±)-**3**, employing mixed P(III) and P(V) chemistry using intermediates reported in the preceding paper[18]. A preliminary account of this work has appeared[19]. Since this work was completed, the synthesis of (±)-**3** has been reported by another group[20].



1 X=Y=Z=O
2 X=Y=Z=S
3 X=Y=O, Z=S
4 X=S, Y=Z=O

EXPERIMENTAL

TLC was performed on Silica Gel 60F (Merck) with detection by UV light or by spraying with methanolic phosphomolybdic acid or 5% palladium chloride in M HCl followed by heating. Flash-column chromatography was performed on silica gel (SORBSIL C60). ^1H NMR spectra (internal Me_4Si) were recorded with Bruker AM-300, Jeol JMN-GX270, and JMN-GX400 spectrometers. ^{13}C NMR spectra (internal 1,4-dioxane) were run as DEPT experiments on the Bruker AM300 and Jeol JMN GX270 spectrometers. ^{31}P NMR spectra (external aq 85% phosphoric acid) were recorded with Jeol FX-90Q, Jeol GX270, Jeol JMN-GX400, and Bruker AM-300 spectrometers. The 2D correlation experiments (COSY, *J*-resolved) were acquired using standard pulse sequences[21] on a Bruker AM300 spectrometer. Mass spectra were recorded at the S.E.R.C. Mass Spectrometry Service Centre, Swansea or by the Mass Spectrometry Service, University of Bath. FAB-mass spectra were carried out using *m*-nitrobenzyl alcohol or glycerol as the matrix. Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler Block. Ion-exchange chromatography was performed on an LKB-Pharmacia automated medium pressure liquid chromatography system equipped with a 'GP250 plus' gradient programmer, using columns packed with Q-Sepharose Fast Flow or DEAE Sephadex A-25 resin and eluting with a gradient of triethylammonium hydrogen carbonate (TEAB) buffer pH 8.0. Quantitative analysis of phosphate was performed using the Briggs assay[22].

*(±)-2,3,6-Tri-*O*-benzyl-*myo*-inositol 1-[di-(2,2,2-trichloroethyl) phosphate]*[18] (**7**). —To a stirred solution of (±)-2,3,6-tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol[23] (**5**; 24.5 mg, 50 μmol) in dry pyridine (5 mL) was added bis(2,2,2-trichloroethyl) phosphorochloridate (24 mg, 62.5 μmol) during 2 min at room temperature. The mixture was stirred for 12 h at 20° when TLC (ether–pentane, 1:1) showed complete conversion of **5** (R_F 0.4) into the 1-[di-(2,2,2-trichloroethyl) phosphate] **6** (R_F 0.5). The reaction was quenched by the addition of water (0.5 mL), and the solution was kept at 20° for 1 h, diluted with more water (30 mL), and extracted with ether. The extract was washed with ice-cold M HCl (40 mL), satd aq KCl (40 mL), and satd aq $NaHCO_3$ (40 mL), then dried ($MgSO_4$), and the solvent was evaporated in vacuo. A solution of the residue in acetone–MeOH–M HCl (5:6:1, 50 mL) was kept at 20° for 1 h when TLC (ether–pentane, 2:1) showed complete conversion of **6** (R_F 0.90) into **7** (R_F 0.25). Anhyd NaOAc (100 mg) was added, the solvents were evaporated, water (50 mL) and ether (50 mL) were added to the residue, and the ether layer was washed with satd aq KCl (2 × 50 mL), dried ($MgSO_4$), and concentrated. Recrystallisation of the residue from EtOAc–pentane (3:10) gave **7** (36 mg, 45 μmol, 90%), mp 141–143°. FAB-mass spectrum: *m/z* 793 {[M(^{37}Cl) + H]$^+$, 3.5%; plus (M + H)$^+$ with 1,3-^{37}Cl}, 361 {[HOPO-(OCH_2CCl_3)$_2$]$^+$, 0.5}, 181 [($PhCHCH_2Ph$)$^+$, 18], 91 [($PhCH_2$)$^+$, 100]. NMR data ($CDCl_3$): ^{1}H (270 MHz), δ 2.90 (bs, 1 H, OH), 2.97 (bs, 1 H, OH), 3.25 (dd, 1H, ^3J 2.0 and 9.5 Hz, H-3), 3.49 (t, 1 H, ^3J 9.1 Hz, H-6), 4.00 (dt, 2 H, ^3J 9.5 and 11.7 Hz, H-4,5), 4.30–4.96 (m, 12 H, 3 PhCH_2, 2 OCH_2CCl_3, and H-1,2), 7.24–7.41 (m, 15 H, 3 Ph); ^{13}C (67.8 MHz), δ 72.14 (d), 72.26 (t, PhCH_2 or OCH_2CCl_3), 74.79 (d), 75.09 (t, PhCH_2 or OCH_2CCl_3), 76.90 (t, PhCH_2 or OCH_2CCl_3), 79.24 (d), 79.79 (d), 94.60 (s, OCH_2CCl_3), 127.60, 127.70, 127.90, 128.05, 128.28, 128.51, 128.57 (15 C, d, Ph), 137.27, 138.01, 138.11 (3 C, s, Ph); ^{31}P (121.5 MHz), δ −6.75 ($^3J_{P,H}$ 6.7 Hz).

Anal. Calcd for $C_{31}H_{33}Cl_6O_9P$: C, 46.92; H, 4.19. Found: C, 47.00; H, 4.15.

*(±)-2,3,6-Tri-*O*-benzyl-*myo*-inositol 1,4-bis[di-(2,2,2-trichloroethyl) phosphate]*[18] (**9**).—A solution of **7** (70 mg, 87 μmol) in dry pyridine (0.2 mL, 2.5 mmol) was added slowly to a stirred solution of bis(2,2,2-trichloroethyl) phosphorochloridate (70 mg, 185 μmol) in dry CH_2Cl_2 (15 mL) and stirring was continued for 6 h. TLC (ether–pentane, 2:1) then showed the conversion of **7** (R_F 0.14) into a major product (R_F 0.26). The solution was stirred with water (0.2 mL) for 30 min, then washed with M HCl (10 mL) and satd aq NaCl (10 mL), dried ($MgSO_4$), and concentrated. Short-column chromatography (1:2 ether–pentane, then 1:1 ether–pentane) of the residue gave a mixture (73.5 mg, 65% yield) of **8** and **9**. Expeditious chromatography reduced the formation of cyclic phosphate and consequent phosphate migration. The mixture was triturated with pentane and the solid was recrystallised from EtOAc–pentane to give **9** (25 mg, 22 μmol, 29%), mp 149–151°. FAB-mass spectrum: *m/z* 1135 {[M(^{37}Cl) + H]$^+$, 5%; also (M + H)$^+$ with 1,3-^{37}Cl}, 1044 [(M − CH_2Ph)$^+$, 2], 954 [(M − $2CH_2Ph$)$^+$, 2], 361 {[$HOPO(OCH_2CCl_3)_2$]$^+$, 0.5}, 181 [($PhCH_2CH_2Ph$)$^+$, 10], 91 [($PhCH_2$)$^+$, 100].

NMR data ($CDCl_3$): ^1H (300 MHz), δ 3.48 (dd, 1 H, ^3J 2.6 and 9.8 Hz, H-3), 3.73 (t, 1 H, ^3J 9.2 Hz, H-5), 4.01 (dd, 1 H, ^3J 9.2 and 9.7 Hz, H-6), 4.31–4.72 (m, 12 H, CH_2Ph, 4 CH_2CCl_3, and H-1,2), 4.69, 5.08 (ABq, 2 H, J 11.1 Hz, CH_2Ph), 4.83 (dd, 1 H, ^3J 9.2 and 9.8 Hz, H-4), 4.83, 4.91 (ABq, 2 H, J 11.5 Hz, CH_2Ph), 7.26–7.43 (m, 15 H, 3 Ph); ^{31}P (121.5 MHz), δ −6.58 ($J_{P,H}$ 8.6 Hz), −7.9 ($J_{P,H}$ 6.5 and 8.8 Hz).

Anal. Calcd for $C_{35}H_{35}Cl_{12}O_{12}P_2$: C, 37.04; H, 3.11. Found: C, 36.91; H, 3.08.

(±)-myo-*Inositol 1,4,5-trisphosphate* (**1**).—A solution of **9** (0.0909 g, 80 μmol) and *N*,*N*-diisopropylethylamine (0.0104 g, 80 μmol) in dry acetonitrile (0.5 mL) was treated with chloro(2-cyanoethoxy)diisopropylaminophosphine[24] (0.0273 g, 100 μmol) for 1 h at room temperature. ^{31}P NMR spectroscopy then showed complete reaction (δ 150.3, −5.4). 2-Cyanoethanol (0.0071 g, 100 μmol) and tetrazole (0.0070 g, 100 μmol) were added, the mixture was left for 1 h (δ 138.5, −5.4), then cooled in ice, and *tert*-butyl hydroperoxide[24] (2 mL of a 3 M solution in toluene, 6 mmol) was added. The mixture was allowed to warm to room temperature, the solvents were evaporated, and a solution of the oily residue in a small volume of dry dioxane was added to Na (0.2 g, 8.7 mmol) in liquid ammonia (30 mL). After 15 min, the reaction was quenched with EtOH until the blue colour had dissipated, the ammonia was evaporated, and the residue was dissolved in water (100 mL). Dowex (H^+) resin was added until the solution was acidic, the resin was removed, and the filtrate was neutralised with triethylamine (~ 1 mL), then concentrated at 0.1 mmHg. The residue was eluted from a column (2.5 × 14 cm) of DEAE Sephadex using a gradient of triethylammonium hydrogen carbonate, typically 100 → 1000 mM and 600 mL of each starting concentration. Fractions (~ 100) were collected at 1 per 8 min (or 250 drops) and assayed qualitatively using the Briggs phosphate test[22]. Elution at ~ 700 mM buffer gave **1**. After evaporation of solvent, proton-decoupled ^{31}P NMR spectroscopy of the residue showed resonances at δ 3.1, 4.6, and 4.8 with $^3J_{P,H}$ 8.2, 7.3, and 8.6 Hz, respectively. The spectrum was in accord with that for material from natural origin[26]. The yield (49 μmol, 62%) was calculated using a quantitative Briggs test. FAB-mass spectrum: *m/z* 419 [$(M - H)^-$, 100%], 401 (8), 321 (13), 159 (18), 97 (20), 91 (60). NMR data (D_2O, pH 8): ^1H, δ 3.7 (dd, 1 H, ^3J 9.8 and 2.9 Hz, H-3), 3.9 (t, 1 H, ^3J 9.2 Hz, H-6), 4.0 (dt, 1 H, ^3J 9.1 and 2.5 Hz, H-1), 4.0 (q, 1 H, ^3J 8.8 Hz, H-5), 4.2 (q, 1 H, ^3J 8.9 Hz, H-4), 4.3 (t, 1 H, ^3J 2.6 Hz, H-2); ^{13}C (75.33 MHz), δ 70.9 (s, C-2), 73.3 (s, C-3), 73.8 (s, C-6), 77.6 (s, C-1), 79.1 (s, C-4), 80.7 (s, C-5).

*(±)-2,3,6-Tri-O-benzyl-*myo*-inositol 5-[di-(2-cyanoethyl) thiophosphate], 1,4-bis[di-(2,2,2-trichloroethyl) phosphate]* (**13**).—To a solution of **9** (182 mg, 160 μmol) in *N*,*N*-diisopropylethylamine (45 μL, 250 μmol) and acetonitrile (2 mL) at 20° was added chloro(2-cyanoethoxy)diisopropylaminophosphine (53 mg, 224 μmol) with stirring to give the mixed phosphate/phosphoramidite **10**. ^{31}P NMR data (MeCN, 36.2 MHz): δ −5.79, −5.59 (2 s, Cl_3CCH_2OP), 151.63, [s, $NCCH_2CH_2OP(N^iPr_2)$] and other reagent peaks. Addition of tetrazole (21 mg, 300 μmol) and 2-cyanoethanol (22 μL, 300 μmol) then gave the mixed phosphate/phosphite **11**.

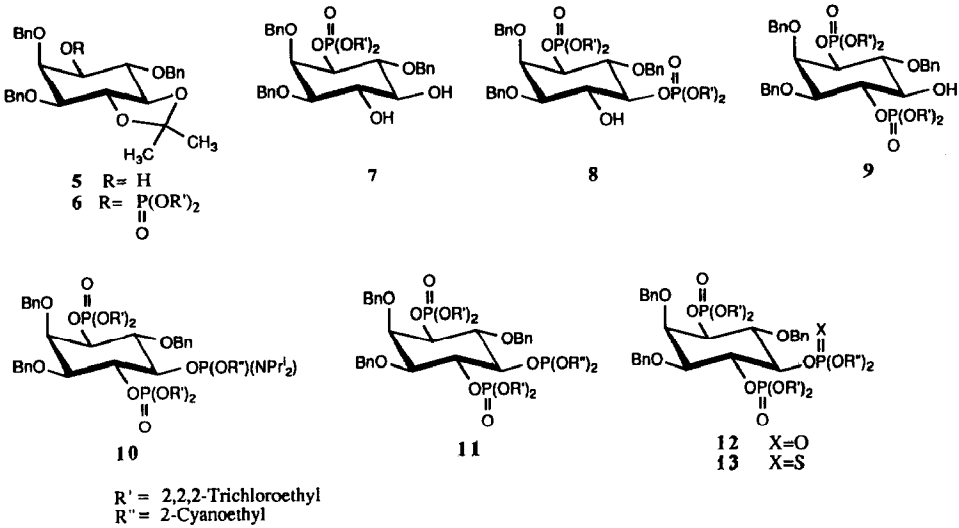
^{31}P NMR data (MeCN, 36.2 MHz): δ −5.45, −5.65 (2 s, Cl_3CCH_2OP), 141.4 (s, $NCCH_2CH_2OP$) and other reagent peaks. Oxidation with sulphur (0.5 g, 16 mmol) in pyridine (5 mL) followed by column chromatography (pentane–EtOAc, 1:2) of the product on silica gel (15 g) gave **13** (94 mg, 70 μmol, 44%), R_F 0.53. FAB-mass spectrum: *m/z* 1341 {[M(^{37}Cl) + H]$^+$, 6%; also (M + H)$^+$ with 2,3,5–7^{37}Cl}, 1306 [(M − Cl)$^+$, 1], 1249 [(M − H − CH_2Ph)$^+$, 1.2], 91 [(CH_2Ph)$^+$, 100]. NMR data ($CDCl_3$): ^{1}H (270 MHz), 2.70–2.78 (m, 4 H, OCH_2CH_2CN), 3.57 (dd, 1 H, ^3J 2.0 and 11.3 Hz, H-3), 3.66–3.80 (m, 2 H, H-6 and H-1), 4.00–4.75 (m, 18 H, 1.5 CH_2Ph, 4 OCH_2CCl_3, 2 OCH_2CH_2CN, and H-2,4,5), 4.80–5.00 (m, 3 H, 1.5 CH_2Ph), 7.26–7.44 (m, 15 H, 3 Ph); ^{13}C (67.80 MHz), δ 18.46 (dt, *J* 8.9 Hz, $POCH_2CH_2CN$), 19.33 (dt, $J_{C,P}$ 8.9 Hz, $POCH_2CH_2CN$), 62.62 (dt, $J_{C,P}$ 4.4 Hz, OCH_2CH_2CN), 62.89 (t, $POCH_2CH_2CN$), 72.36 (d), 74.60 (d), 75.77 (t, OCH_2), 76.77 (d), 76.87 (t, OCH_2), 77.20 (t, OCH_2), 77.62, 79.08, 79.17 (3 C, d, Ins-*C*), 94.55 (s, CCl_3), 126.14, 127.70, 128.09, 128.22, 128.51, 128.74, (d, CH, Ph), 136.42, 137.69 (s, C, Ph); ^{31}P (109.37 MHz), δ −5.98 ($^3J_{P,H}$ 6.7 Hz), −5.92 ($^3J_{P,H}$ 6.7 Hz), 67.00 ($^3J_{P,H}$ 6.7 Hz).

(±)-myo-*Inositol 1,4-bisphosphate 5-phosphorothioate* (**3**).—Ammonia (50 mL) was condensed onto Na (0.3 g) and the mixture was stirred for 5 min. The ammonia (40 mL) was then distilled onto Na (0.2 g, 9 μmol), giving a deep blue colour. To this mixture at −78° was added a solution of **13** (80 mg, 60 μmol) in dry dioxane (0.2 mL) during 5 min. The mixture was stirred for 2 min, the reaction was quenched by the addition of 2-propanol until the blue colour was lost, and the solution was allowed to warm to room temperature, then concentrated in vacuo. A solution of the solid residue in water (50 mL) was mixed with Dowex (H^+) resin until neutral, then filtered, and basified with triethylamine (pH 9). The salt was further purified by chromatography on DEAE Sephadex-A25, using triethylammonium hydrogen carbonate buffer (0.1 → 1 M) to give **3** (19.6 mg, 26 μmol, 88%), eluted at ~ 750 mM buffer. NMR data (D_2O, pH 9): ^{1}H (300 MHz), δ 3.70 (dd, 1 H, ^3J 2.6 and 9.5 Hz, H-3), 3.90 (m, 2 H), 4.10–4.20 (m, 2 H), 4.30 (t, 1 H, ^3J 2.6 Hz, H-2); ^{31}P (109.4 MHz), δ 3.25 ($^3J_{P,H}$ 6.7 Hz, P-1 or P-4), 4.35 ($^3J_{P,H}$ 6.7 Hz, P-1 or P-4), 43.24 ($^3J_{P,H}$ 6.7 Hz, P-5).

RESULTS AND DISCUSSION

Phosphorylation of (±)-2,3,6-tri-*O*-benzyl-*myo*-inositol-1-[di-(2,2,2-trichloroethyl) phosphate] (**7**) with bis(2,2,2-trichloroethyl) phosphorochloridate in dichloromethane and pyridine gave a mixture (65%) of the 1,5- (**8**) and the 1,4-bisphosphate (**9**) derivatives[18], from which **9** (29%) crystallised leaving a ~ 2:1 mixture of **8** and **9** (^{1}H and ^{31}P NMR data).

The identification of **9** by ^{1}H NMR spectroscopy was complicated by the overlapping of the signals of interest, i.e., the inositol ring protons, by AB and ABX systems from the benzyl and phosphotriester moieties, respectively (Fig. 1). For the signal at δ 3.48, the ^3J values are 2.6 and 9.8 Hz indicative of *eq–eq* or



eq–ax and *ax–ax* protons. Since H-2 is the only ring proton that is equatorial, H-1 and H-3 are the only ring protons that will have *ax–eq* and *ax–ax* couplings. Applying this information to the ^1H–^1H COSY (Fig. 2), it is plausible to assume that, if the signal at δ 3.48 is due to H-1, there are two possible assignments, arising from the fact that the first cross-peak encountered (δ 4.41) could be the result of correlation with H-2 or H-6. The resulting two sequences are as follows: δ 3.48, H-1; 4.41, H-2; 4.34, H-3; 4.01, H-4; 3.73, H-5; 4.83, H-6; 3.48, H-1; and 3.48, H-1; 4.41, H-6; 4.34, H-5; 4.01, H-4; 3.73, H-3; 4.83, H-2; 3.48, H-1. Similarly, by assuming that the signal at δ 3.48 is for H-3, there are another two plausible sequences possible, namely, δ 3.48, H-3; 4.41, H-4; 4.34, H-5; 4.01, H-6; 3.73, H-1; 4.83, H-2; 3.48, H-3; or 3.48, H-3; 4.41, H-2; 4.34, H-1; 4.01, H-6; 3.73, H-5; 4.83, H-4; 3.48, H-3.

In order to identify the correct sequence, a 2D *J*-resolved ^1H NMR spectrum was obtained (Fig. 3) in which the couplings to ^{31}P were not phase modulated. The signal (dd) at δ 3.48 has two vicinal homonuclear couplings of 2.6 (*eq–eq* or *eq–ax*) and 9.8 Hz (*ax–ax*). As there is no evidence of any heteronuclear coupling, it is possible to assign the signal to H-3. The signal at δ 3.73 (apparent t) has two $^3J_{ax,ax}$ couplings of 9.2 Hz and that at δ 4.01 (dd) has $^3J_{ax,ax}$ 9.2 and 9.7 Hz. The signal (ddd) at δ 4.34 has $^3J_{H,H}$ values of 2.6 (*eq–eq* or *eq–ax*) and 9.7 Hz (*ax–ax*), respectively, in f_1 and $^3J_{P,H}$ 8.8 Hz in f_2. Thus, it is possible to assign the signal at δ 4.34 to H-1 and that (apparent t) at δ 4.41 with two ^3J values of 2.6 Hz to H-2.

Finally, an assignment of the key ^1H resonance at δ 4.83 can be made. As with H-1, this proton resonance (ddd) had $J_{ax,ax}$ values of 9.2 and 9.8 and $^3J_{P,H}$ 8.5 Hz. Therefore, the sequence of the ^1H–^1H COSY (Fig. 2) is as follows: δ 3.48, H-3;

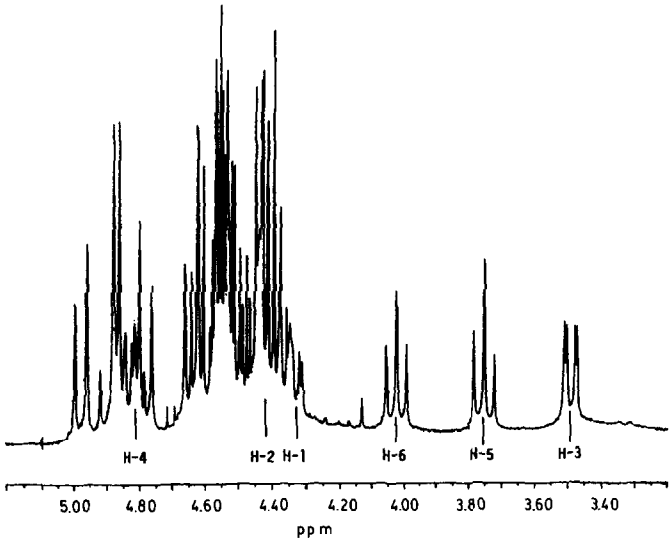


Fig. 1. 300-MHz ^{1}H NMR spectrum for a solution of **9** in $CDCl_3$.

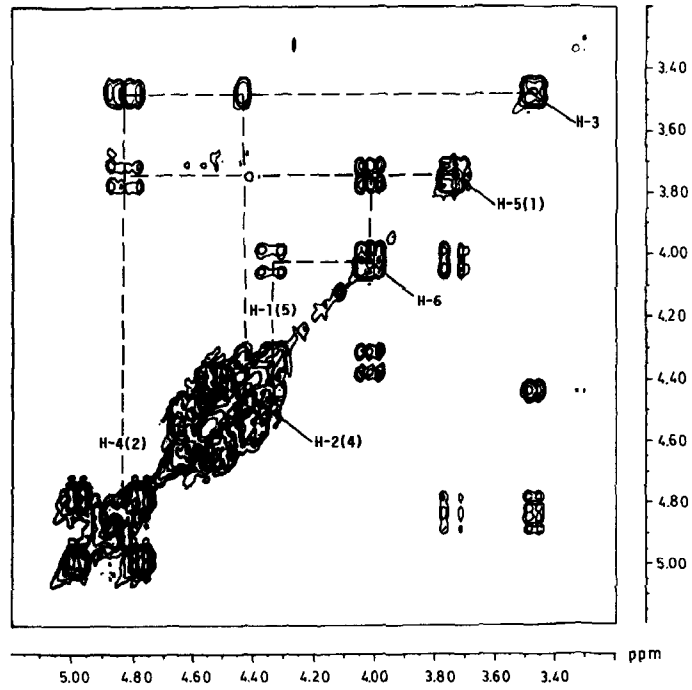


Fig. 2. 2D ^{1}H–^{1}H COSY contour map of a solution of **9** in $CDCl_3$: 32 scans for each of 256 experiments with 512 data points; spectral width, 525 Hz; sine-bell apodisation in each domain with diagonal symmetrisation.

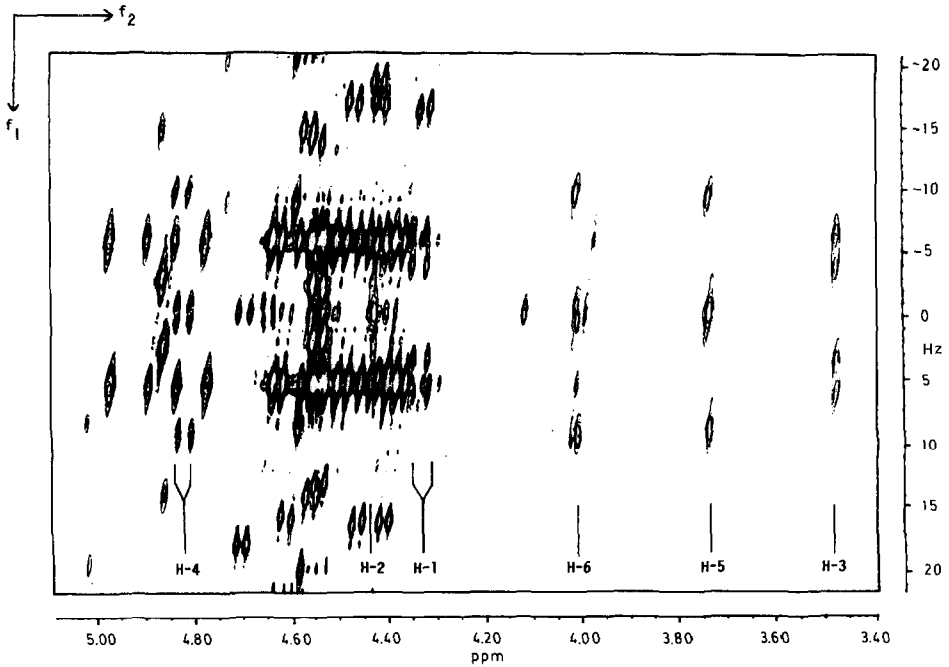


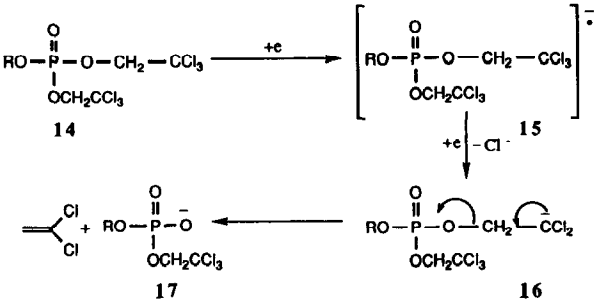
Fig. 3. 2D ^{1}H–^{1}H Homonuclear *J*-resolved correlated ^{1}H NMR spectrum of a solution of **9** in $CDCl_3$: 16 scans for each of the 64 experiments with 128 data points; spectral width, 20 Hz in the f_1 domain, 316 Hz in the f_2 domain; sine-bell apodisation in each dimension with subsequent tilting and symmetrisation.

4.41, H-2; 4.34, H-1; 4.01, H-6; 3.73, H-5; 4.83, H-4; 3.48, H-3. The phosphorus coupling observed for H-1 and H-4 enables the unambiguous assignment of **9** as (±)-2,3,6-tri-*O*-benzyl-*myo*-inositol 1,4-bis[di-(2,2,2-trichloroethyl) phosphate].

Using a method similar to that reported[24] in the synthesis of IP_3, the 1,4-bis-phosphate **9** was phosphitylated with chloro(2-cyanoethoxy)diisopropyl-aminophosphine to give the phosphoramidite **10**. Reaction of **10** in situ with 2-cyanoethanol–tetrazole gave the phosphite **11**, which was not isolated but oxidised to the trisphosphate **12** with *tert*-butyl hydroperoxide. In contrast to 1,2,3,4-tetra-*O*-benzyl-*myo*-inositol 4,5-bis[di-(2-cyanoethyl) phosphite], where an unusual $^5J_{P,P}$ coupling was noted[25], no such coupling was observed for the mixed P(III)/P(V) species **11**, indicating that the two P(III) centres are required for such coupling.

Deprotection of **12**, using sodium in liquid ammonia, gave (±)-*myo*-inositol 1,4,5-trisphosphate (**1**), which was isolated as the triethylammonium salt by chromatography on DEAE Sephadex A-25. Compound **1** was identical biologically to material prepared by the previous route[24] in so far as it bound to the cerebellar IP_3 receptor and mobilised Ca^{2+} from intracellular stores of GH_3 cells with a potency similar to that of IP_3, and it possessed spectral properties in accord with those reported for material of natural origin[26].

To the best of our knowledge, this method for the removal of the 2,2,2-trichloroethyl group is novel. This group has been used to protect carboxylic acids[27], and the phosphate in nucleotides[28] and, as the trichloroethoxycarbonyl group, to protect hydroxyl and amino groups[29]. The 2,2,2-trichloroethyl group can be removed using zinc dust in acetic acid[28], zinc–copper couple in *N*,*N*-dimethylformamide[28], zinc–*p*-toluenesulphonic acid in pyridine[28,30], zinc with silver carbonate in acetic acid[31], electrolysis in methanol[32] or in *N*,*N*-dimethylformamide[33], tetrabutylammonium fluoride[34], sodium borohydride under the catalysis of selenium in *N*,*N*-dimethylformamide[35], sodium naphthalene in hexamethylphosphoric triamide[36], or Pd-catalysed hydrogenolysis in aqueous ethanol[37]. Preliminary experiments on the deprotection of 2,2,2-trichloroethoxymethyl ethers, using lithium in liquid ammonia, have been reported[38]. However, the application of most of these methods to molecules containing phosphorothioate triesters could lead to desulphuration. The new method involves treatment with sodium in liquid ammonia and the mechanism proposed is **14** → **15** → **16** → **17**. The procedure also removes benzyl groups and avoids potential problems in catalytic hydrogenolysis caused by poisoning of the catalyst by phosphorothioates. Sodium in liquid ammonia also removes 2-cyanoethyl groups by β-elimination[24].



The mixed phosphate/phosphite **11** was also oxidised with sulphur in pyridine to give the mixed phosphate/phosphorothioate **13**, which was deblocked with sodium in liquid ammonia to give (±)-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate (**3**). The ^{31}P NMR spectrum of **3** (Fig. 4) shows clearly two resonances at δ_p 3.25 and 4.35 ppm, assignable to the two phosphate groups and a resonance at δ_p 43.24 ppm assignable to the single 5-phosphorothioate group.

Racemic **3** is a full agonist at the IP_3 receptor and a potent mobiliser of intracellular Ca^{2+}, being only some 7-fold less potent than IP_3 in permeabilised SH-SY5Y neuroblastoma cells (EC_{50} values: IP_3, 0.11 μM; IP_3-5S, 0.8 μM)[15] and more potent than IPS_3. If, following the precedent[13,14] for L-IP_3, L-IP_3-5S is inactive in Ca^{2+} mobilisation, then D-IP_3-5S should be only a few-fold less potent than IP_3. Racemic **3** is resistant to IP_3 5-phosphatase-catalysed cleavage and is a potent inhibitor of this enzyme (K_i 6.8 μM), but, apparently, is a substrate for

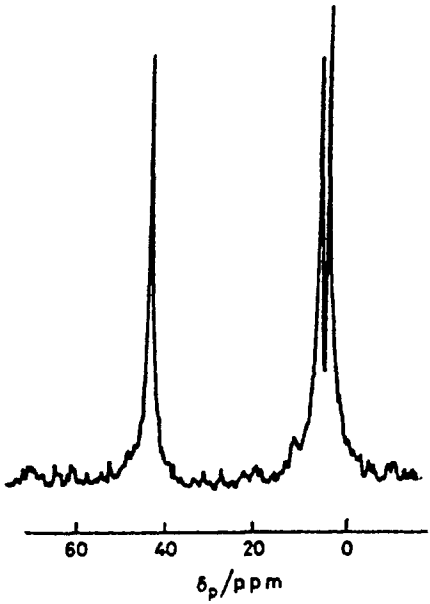


Fig. 4. 24.15-MHz ^{31}P NMR spectrum of **3** (ca. 70 mM solution in D_2O, pH 8).

IP_3-3-kinase[15]. These latter properties indicate that (±)-**3** is a potentially valuable probe of the polyphosphinositide pathway of signal transduction.

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