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Synthesis of Potent Ins(1,4,5)P_3 5-Phosphatase Inhibitors by Modification of *myo*-Inositol 1,3,4,6-Tetrakisphosphate

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Abstract—Three *myo*-inositol tetrakisphosphate analogues were synthesised based upon *myo*-inositol 1,3,4,6-tetrakisphosphate: 2,5-di-*O*-methyl *myo*-inositol-1,3,4,6-tetrakisphosphate **19** and its phosphorothioate derivative **22**, together with *myo*-inositol 1,3,4,6 tetrakisphosphorothioate **25**. These compounds were prepared by phosphitylating 2,5-di-*O*-methyl-*myo*-inositol and 2,5-di-*O*-benzyl-*myo*-inositol followed by oxidation with *t*-butylhydroperoxide or sulfoxidation at room temperature using sulfur in a mixed solvent of DMF and pyridine. Sulfoxidation was complete within 15 min; however, without DMF, the reaction was much slower, and required overnight. When evaluated against Ins(1,4,5)P_3 5-phosphatase, 3-kinase and for Ca^{2+} release at the Ins(1,4,5)P_3 receptor, only weak activity was observed for Ca^{2+} release. **22** and **25** are potent 5-phosphatase inhibitors and **25** is a moderate inhibitor of 3-kinase. Thus, we have synthesised potent enzyme inhibitors, which do not mobilise Ca^{2+} and devised conditions for quick, clean and inexpensive sulfoxidation of inositol polyphosphite intermediates.

Introduction

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3 **1**] is the hydrophilic second messenger derived from the enzymatic cleavage of the minor membrane lipid, phosphatidylinositol 4,5-bisphosphate, in response to a primary ligand stimulation.[1,2] Ins(1,4,5)P_3 interacts specifically with a tetrameric Ins(1,4,5)P_3 receptor-operated Ca^{2+} channel to mobilise Ca^{2+} from non-mitochondrial stores.[1,2] Design of receptor agonists, antagonists and enzyme inhibitors for inositol phosphates as biological tools, has been a serious enterprise for synthetic chemists for about 15 years.[3] A study by Jiang et al.[4] revealed the first three-dimensional structure of the type-1 Ins(1,4,5)P_3 receptor using cryo-electron microscopy. The structure of the Ins(1,4,5)P_3 receptor was resolved at 24 Å in which the Ins(1,4,5)P_3 receptor channel takes the form of an uneven dumbbell with one end significantly bigger than the other. The Ins(1,4,5)P_3 receptor can be sub-divided into three regions: first, the amino terminal Ins(1,4,5)P_3 binding domain, second, the central modulatory region and third, the carboxy terminal channel region. The first X-ray crystal structure of the Ins(1,4,5)P_3 binding domain of mouse type-1 Ins(1,4,5)P_3 receptor with bound Ins(1,4,5)P_3 at a resolution of 2.2 Å has also been published recently.[5] Data from the crystallographic studies illustrate the interactions of specific arginine and lysine residues with the three phosphate groups of Ins(1,4,5)P_3. Knowing the critical interactions between Ins(1,4,5)P_3 and its receptor we should be able to design effective new ligands.

Two enzymes, Ins(1,4,5)P_3 3-kinase and 5-phosphatase deactivate Ins(1,4,5)P_3 and switch off the Ca^{2+} signal.[6] 5-Phosphatase (type I)[7a] metabolises Ins(1,4,5)P_3 to give *myo*-inositol 1,4-bisphosphate Ins(1,4)P_2 **2**, which has no Ca^{2+} releasing activity. The crystal structure of an inositol polyphosphate 5-phosphatase domain of synaptojanin (from *Schizosaccharmyces pombe* synaptojanin, type II), bound to Ins(1,4)P_2 and Ca^{2+} at 1.8 Å resolution has been reported[8] and reveals the interactions between critical amino acid residues of the 5-phosphatase and the product of dephosphorylation, Ins(1,4)P_2. A number of disease states associated with malfunctioning of 5-phosphatase (group II) have been discovered over the last decade. One of these is Lowe's oculocerebrorenal syndrome (OCRL), an X-linked disorder which affects the brain, eye lens and kidney development, and its associated gene product bears a 71% similarity to group II 5-phosphatase.[9] A deficiency

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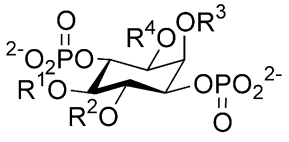
or over expression of 5-phosphatase and other related enzymes from Ins(1,4,5)P_3 pathway would result in unbalanced signalling products, so the design of specific inhibitors and activators to counteract these effects will make attractive targets for drug design of the future.

3-Kinase phosphorylates Ins(1,4,5)P_3 at the 3-hydroxyl to give D-*myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4 **3**]. This compound is then dephosphorylated to give D-*myo*-inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3 **4**] and further phosphorylation of compound **4** provides higher inositol polyphosphates such as Ins(1,3,4,6)P_4 **5**, Ins(1,3,4,5,6)P_5 **6** and Ins(1,2,3,4,5,6)P_6 **7** (Fig. 1). One such tetrakisphosphate, Ins(1,3,4,6)P_4 **5** can mobilise Ca^{2+} in *Xenopus* oocytes[10] and neuroblastoma cells[11] albeit with a much weaker potency than for Ins(1,4,5)P_3. Ins(1,3,4,6)P_4 is a partial agonist at the Ins(1,4,5)P_3 receptor of SH-SY5Y human neuroblastoma cells[11] releasing only 85% of the total Ca^{2+} pool and is the only naturally occurring inositol polyphosphate that apparently acts as a partial agonist at the Ins(1,4,5)P_3 receptor. Data from our previous studies[12] illustrate that Ins(1,3,4,6)P_4 is a good 5-phosphatase inhibitor having a K_i value of 7.7 μM. We predict from this work that the tetrakisphosphorothioate analogue **25** should give a 5-phosphatase inhibitor of sub-micromolar potency.[12] Thus, *myo*-inositol 1,3,4,6-tetrakisphosphorothioate makes an attractive target for a potent 5-phosphatase inhibitor. *scyllo*-Inositol 1,2,4,5-tetrakisphosphorothioate, is a high intrinsic activity partial agonist at the Ins(1,4,5)P_3 receptor that has been synthesised and evaluated.[13] It is a potent inhibitor of 5-phosphatase (K_i 0.3 μM); however, its ability to release Ca^{2+} makes it less useful as a 5-phosphatase inhibitor and a tool for investigating Ca^{2+} signalling. Generally, if a phosphate is replaced with a phosphorothioate group in inositol phosphate compounds, the potency of inhibition for 5-phosphatase increases.[3] We report here the synthesis of three potential 5-phosphatase inhibitors, based upon Ins(1,3,4,6)P_4, which are poorly recognised by 3-kinase and are devoid of Ca^{2+} release. We also report a simple method for the improved preparation of phosphorothioate derivatives by sulfoxidation of a suitable P(III) intermediate.

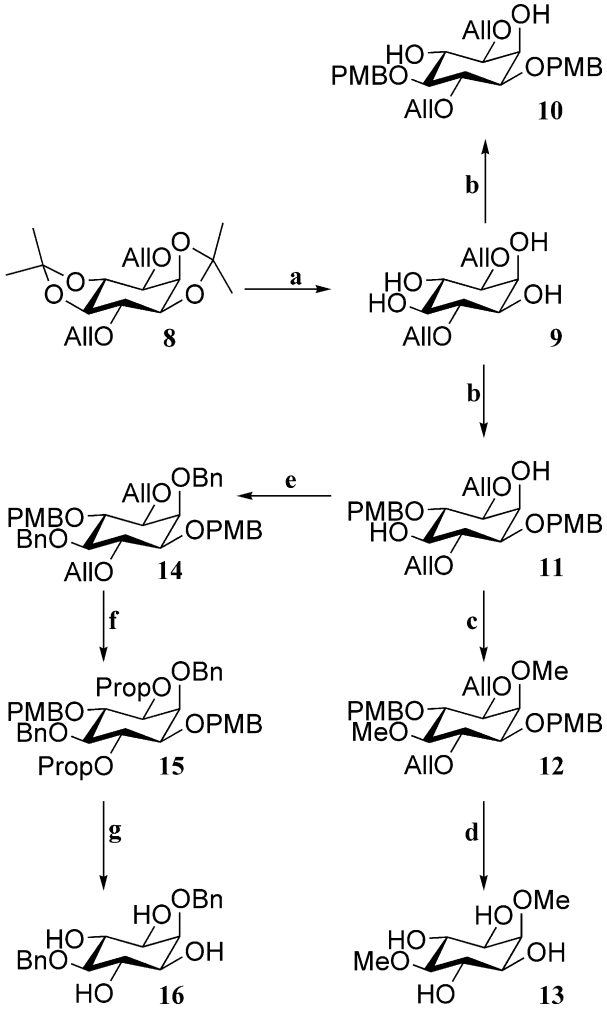


1 $R^1 = PO_3^{2-}$; $R^2 = R^3 = R^4 = H$; Ins(1,4,5)P_3
2 $R^1 = R^2 = R^3 = R^4 = H$; Ins(1,4)$P_2$
3 $R^1 = R^4 = PO_3^{2-}$; $R^2 = R^3 = H$; Ins(1,3,4,5)P_4
4 $R^4 = PO_3^{2-}$; $R^1 = R^2 = R^3 = H$; Ins(1,3,4)P_3
5 $R^2 = R^4 = PO_3^{2-}$; $R^1 = R^3 = H$; Ins(1,3,4,6)P_4
6 $R^1 = R^2 = R^4 = PO_3^{2-}$; $R^3 = H$; Ins(1,3,4,5,6)P_5
7 $R^1 = R^2 = R^3 = R^4 = PO_3^{2-}$; Ins(1,2,3,4,5,6)$P_6$

Figure 1.

Results and Discussion

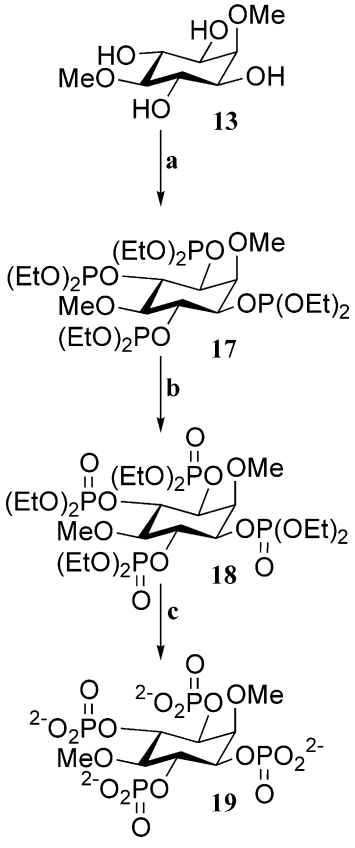
The tetraols **13** and **16** were prepared from the same intermediate **11** via DL-1,4-di-*O*-allyl-*myo*-inositol **9** (Scheme 1). Compound **9** was prepared by treating compound **8** with 80% acetic acid at reflux temperature for 30 min, after which the solvent was evaporated and the remaining solid was recrystallised. It was envisaged that selective *p*-methoxybenzylation at the 3- and 6-positions would give a protected 1,3,4,6-intermediate which could be alkylated at the 2- and 5-positions then deprotected to provide the 1,3,4,6-tetrol. A mixture of dibutyltin oxide and DL-1,4-di-*O*-allyl-*myo*-inositol **9** was heated in toluene under reflux to form the *cis*-2,3 and the *trans*-5,6-dibutylstannylene derivative. The toluene was evaporated and DMF was added to the syrup together with CsF and *p*-methoxybenzyl chloride and the reaction was then stirred overnight. TLC showed two products, DL-1,4-di-*O*-allyl-3,5-di-*O*-*p*-methoxybenzyl-*myo*-inositol **10** and DL-1,4-di-*O*-



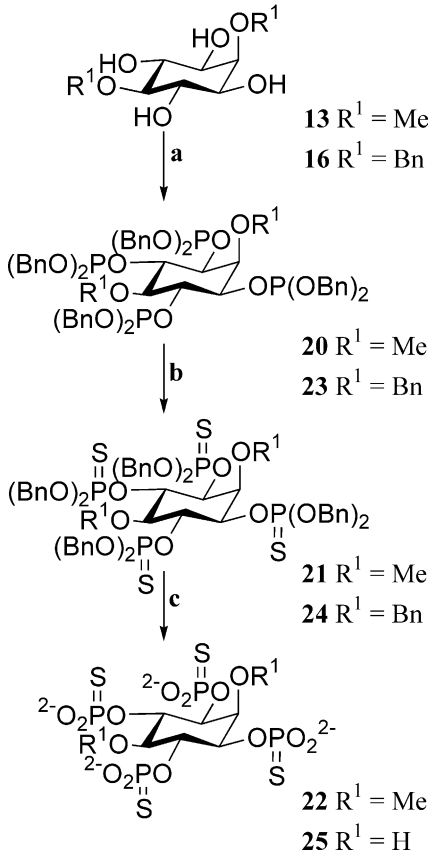
Scheme 1. (a) 80% HOAc, reflux, 30 min; (b) $Bu_2Sn{=}O$, toluene, reflux, 2.5 h, then DMF, CsF (5 equiv), *p*-methoxybenzyl chloride; (c) NaH, DMF, MeI; (d) Pd/C (10%), reflux, EtOH, PTSA; (e) BnBr, NaH, DMF; (f) KO^{t}Bu, DMF, 85 °C, 2 h; (g) CH_2Cl_2–TFA (10:1). All, allyl; Bn, benzyl; Me, methyl; PMB, *p*-methoxybenzyl; Prop, *cis*-prop-1-enyl.

allyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol **11**. Diol **11** was methylated with methyl iodide using sodium hydride as the base in DMF to give DL-1,4-di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-2,5-di-*O*-methyl-*myo*-inositol **12** and isolated as a crystalline solid. The allyl and *p*-methoxybenzyl protective groups were then removed in one step by refluxing compound **12** in a mixture of ethanol–water with palladium on carbon and a catalytic amount of acid. The palladium on carbon was filtered off over a pad of Celite, the solvents were evaporated and the crude mixture was recrystallised from ethanol to give 2,5-di-*O*-methyl-*myo*-inositol **13**. We found that compound **13** decomposed between 266 and 268 °C where previously, a melting point of 270 °C was reported.[14] Compound **13** was used to synthesise 2,5-di-*O*-methyl-*myo*-inositol 1,3,4,6-tetrakisphosphate **19** and the corresponding phosphorothioate analogue **22** (Schemes 2 and 3).

2,5-Di-*O*-benzyl-*myo*-inositol **16** was prepared in three steps from DL-1,4-di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol **11**. Compound **11** was benzylated with benzyl bromide and sodium hydride in DMF. Workup and purification gave the fully protected compound **14** as a crystalline solid in 92% yield. Several methods were attempted to deprotect the allyl and *p*-methoxybenzyl groups simultaneously, but none of these was satisfactory. However, success was achieved when the allyl groups were isomerised to the *cis*-prop-1-enyl ether derivative **15**, by using freshly sublimed potassium *t*-butoxide in dry DMF at 85 °C for 2 h, which rendered the prop-1-enyl ether susceptible to acidic deprotection. The R_f value of the 1,4-di-*O*-*cis*-prop-1-enyl derivative **15** was identical to that of compound **14**, but treatment of a small quantity of the mixture with acid gave a more polar derivative indicated by TLC. The compound was extracted from the mixture, purified by flash chromatography then recrystallised from hexane to give compound **15** in 83% yield. The ^{1}H NMR data showed the presence of 1,4-di-*O*-*cis*-prop-1-enyl groups because the coupling constants for the protons across the double bond of $CH_3HC{=}CH{-}O$ were 6.2 and 6.4 Hz, respectively, whereas a *trans*-prop-1-enyl ether would be ca. 15 Hz. The *cis*-prop-1-enyl ether and the *p*-methoxybenzyl group are both acid sensitive, and treatment of the derivative with 10% TFA in dichloromethane cleaved both groups whilst leaving the benzyl ethers at position 2 and 5 intact. The only product detected by TLC resulted from deprotection of the *p*-methoxybenzyl group, which stained purple in the presence of phosphomolybdic acid (PMA). The solvents were evaporated then co-evaporated with water and ethanol. A white solid precipitated from the solution, which was filtered off and washed with water, acetone and finally ether to remove any impurities then crystallised from DMF-ethanol to give 2,5-di-*O*-benzyl-*myo*-inositol[15] **16**. The ^{1}H NMR spectrum of 2,5-di-*O*-benzyl-*myo*-inositol **16** showed a similar symmetrical pattern to 2,5-di-*O*-methyl-*myo*-inositol and a mass spectrum of compound



Scheme 2. (a) $(EtO)_2P$–Cl, diisopropylethylamine, DMF; (b) *t*-BuOOH; (c) TMSBr, CH_2Cl_2, room temperature, 16 h, then purification on Q-Sepharose Fast Flow ion exchange resin. Et, ethyl; Me, methyl.



Scheme 3. (a) Bis(benzyloxy)diisopropylaminophosphine, 1*H*-tetrazole, DMF, 1 h; (b) S_8, pyridine; (c) Na/NH_3, then purification on Q-Sepharose Fast Flow ion exchange resin. Bn, benzyl; Me, methyl.

16 could not be obtained due to its inherent insolubility. 2,5-Di-*O*-benzyl-*myo*-inositol 16 was used to prepare *myo*-inositol 1,3,4,6-tetrakisphosphorothioate [Ins(1,3,4,6)PS_4 25].

2,5-Di-*O*-methyl-*myo*-inositol 13 was dissolved in dry DMF and dry *N,N*-diisopropylethylamine. The mixture was cooled, diethoxychlorophosphine added dropwise and was stirred for 45 min to give the 1,3,4,6-tetrakisphosphite intermediate 17. Oxidation of the P(III) intermediate was carried out using *t*-butylhydroperoxide to give compound 18. Purification by flash chromatography was unnecessary since aqueous work up provided the pure compound as a syrup. The eight ethyl groups were removed from the protected tetrakisphosphate using a two-step deprotection method. Compound 18 was dissolved in dry dichloromethane and bromotrimethylsilane was added to the mixture under nitrogen and stirred overnight. In this reaction, the eight ethyl groups of compound 18 were replaced by trimethylsilyl functions in quantitative yield by ^{31}P NMR, with the release of bromoethane as the by-product. The solvents were evaporated and water was added to the residue in order to hydrolyse the temporary trimethylsilyl moieties. The crude product was purified by ion exchange chromatography on Q-Sepharose Fast Flow using a gradient of 200–1000 mM TEAB buffer and the compound eluted at ca. 500 mM buffer then isolated as its glassy triethylammonium salt.

The phosphorothioate derivatives 22 and 25 were prepared using a different P(III) reagent. A mixture of bis(benzyloxy)diisopropylaminophosphine and 1*H*-tetrazole in DMF was stirred for 1 h to give a tetrazolide intermediate.[16] The formation of the complex in DMF was slower than in dichloromethane, with the appearance of two peaks at $\delta = +126.12$ and $\delta = +127.47$ ppm in the ^{31}P NMR spectrum. 2,5-Di-*O*-methyl-*myo*-inositol 13 was then added to the tetrazolide intermediate[16] and the mixture was stirred for a further 2 h. The ^{31}P NMR spectrum at this stage showed two peaks at $\delta = +141.26$ and $\delta = +139.45$ ppm, which indicated the phosphitylated *myo*-inositol derivative intermediate 20. However, at high resolution only two doublets (corresponding to phosphites at positions C-1, C-6, C-3 and C-4) were observed in the ^{31}P NMR spectrum, as part of an AB spin coupling pattern where $^5J_{PP} = 3.9$ Hz, due to the symmetrical nature of the molecule, where C-1 = C-3 and C-4 = C-6. Sulfoxidation of this intermediate with sulfur in pyridine–DMF gave the protected intermediate 21 within 15 min. The sulfur was filtered off and the solvent was carefully evaporated in vacuo below 30 °C. The protected phosphorothioate 21 was purified by flash chromatography and isolated in 82% yield to give a syrup. The octabenzyl derivative 21 was deprotected with sodium in liquid ammonia then purified on Q-Sepharose Fast Flow ion exchange resin to afford compound 22 (25% yield) which eluted at ca. 800 mM TEAB buffer and was characterised as a triethylammonium salt (Scheme 3).

Compound 16 was phosphitylated in a similar way to compound 13. Intermediate 23 showed a similar AB spin coupling pattern to compound 17, where $^5J_{PP} = 4.3$ Hz. Sulfoxidation was carried out using sulfur in a mixed solvent of pyridine–DMF, and stirred for 10 min. The remaining sulfur was filtered off, the solvents were evaporated in vacuo and the residue was purified by flash chromatography to give the decabenzyl derivative 24 in 71% yield. This is an efficient method for the synthesis of phosphorothioates from phosphites and the reaction time of 10 min is much faster than previously reported.[17] Deprotection of compound 24 with sodium in liquid ammonia followed by purification by ion exchange chromatography gave Ins(1,3,4,6)PS_4 25 in 46% yield.

Biological evaluation was carried out for compounds 19, 22 and 25 for the inhibition of Ins(1,4,5)P_3 5-phosphatase (from human erythrocyte ghosts), Ins(1,4,5)P_3 3-kinase (rat brain supernatant) and for Ca^{2+} release at the Ins(1,4,5)P_3 receptor of electrically permeabilised SH-SY5Y human neuroblastoma cells.[12] All three compounds were poor agonists at the Ins(1,4,5)P_3 receptor mobilising less than 15% of the maximum pool of Ca^{2+} at 30 μM. Only Ins(1,3,4,6)PS_4 25 significantly inhibited Ins(1,4,5)P_3 3-kinase with a K_i value of 46 μM, compared with >100 and 105 μM for compounds 19 and 22 respectively. However, these compounds were potent inhibitors of Ins(1,4,5)P_3 5-phosphatase, where compound 19 had a K_i value of 15.9 μM, while the phosphorothioate derivatives 22 and 25 had K_i values of 1.4 and 1.9 μM, respectively. The phosphorothioate compound 22 is 75-fold more potent for the inhibition of 5-phosphatase over 3-kinase, and very poor for Ca^{2+}-release. For comparison, Ins(1,3,4,6)P_4 had a K_i value of 7.7 μM for 5-phosphatase inhibition, a K_i value of 150 μM for 3-kinase and an EC_{50} value of 19.6 μM for Ca^{2+} release. Thus, methylation of the free hydroxyl groups and replacement with phosphorothioate moieties delivered a potent 5-phosphatase inhibitor 22 which did not release Ca^{2+} and only interacted weakly with 3-kinase.

In conclusion, we synthesised *myo*-inositol-2,5-di-*O*-methyl 1,3,4,6-tetrakisphosphorothioate 22 and *myo*-inositol 1,3,4,6-tetrakisphosphate. These were better 5-phosphatase inhibitors (K_i for compounds 22 and 25 1.4 and 1.9 μM) than their corresponding tetrakisphosphates [K_i for compound 19 and Ins(1,3,4,6)P_4 was 15.9 and 7.7 μM]. However, changing the trisphosphate motif to give a trisphosphorothioate increases the potency of 5-phosphatase inhibition and is more significant compared to the two tetrakisphosphorothioate compounds 22 and 25.[12,17] For example, L-*chiro*-Ins(2,3,5)P_3 has a K_i value of 7.7 μM [the same as Ins(1,3,4,6)P_4], whereas the corresponding trisphosphorothioate has a K_i value of 0.23 μM. Compared to L-*chiro*-Ins(2,3,5)PS_3, tetrakisphosphorothioate 25 is 8-fold less potent for 5-phosphatase inhibition at 1.9 μM.[17] From a previous study,[18] Ins(1,3,4,5)P_4 was found to be the most potent 5-phosphatase inhibitor with an IC_{50} value of 0.15 μM. This compound is a better fit at the active site of 5-phosphatase compared to the tetrakisphosphorothioates 22 and 25 although compounds 22 and 25 are potent inhibitors of the enzyme.

Sulfoxidation of phosphites is slow using a mixture of sulfur in pyridine.[17] However, the addition of DMF speeds up the rate significantly. DMF presumably accelerates the rate-determining step by increasing the polarisation of charge between sulfur atoms via dipole–dipole interactions between the solvent and the atoms in the S_8 ring. We have thus succesfully synthesised two new tetrakisphosphorothioate compounds (**22** and **25**) and a tetrakisphosphate (**19**) based upon the Ins(1,3,4,6)P_4 structure and discussed their inhibition of 5-phosphatase. We have also developed a simple, faster method for sulfoxidation of inositol polyphosphites than previously reported,[17] and this is now our method of choice for preparing inositol phosphorothioates. These compounds will be of use in investigating the polyphosphoinositide pathway of signal transduction.

Experimental

Thin-layer chromatography (TLC) was performed on pre-coated plates (Merck TLC aluminium sheets silica 60 F_{254}): the product was visualised by spraying with phosphomolybdic acid in MeOH, followed by heating. Flash chromatography refers to the procedure developed by Still and coworkers[19] and was carried out on sorbsil C60 silica gel. The NMR spectra for the nuclei ^{31}P, ^{1}H and ^{13}C were recorded on a Jeol FX-90Q, GX270, GX400 or GLI EX400 spectrometers. Chemical shifts were measured in parts per million (ppm) relative to tetramethylsilane (Me_4Si) in $CDCl_3$; deuterium oxide (D_2O) and dimethyl sulfoxide-d_6 (Me_2SO-d_6) were also used. The ^{31}P NMR shifts were measured in ppm relative to external 85% phosphoric acid. Coupling constants *J*, were measured in hertz (Hz). Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block, using two glass plates. Microanalysis was carried out by the University of Bath Microanalysis Service. Mass spectra were recorded by the University of Bath Mass Spectrometry Service using +ve and −ve Fast Atom Bombardment (FAB) with 3-nitrobenzylalcohol (NBA) as the matrix. Ion exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion Exchange Chromatograph using Q-Sepharose and gradients of triethylammonium bicarbonate (TEAB) as eluent. Fractions containing phosphate and phosphorothioate were assayed by a modification of the Briggs phosphate test.[17] The phosphitylating reagent was prepared by adding benzyl alcohol to *N*,*N*-diisopropylaminodichlorophosphine[20] in the presence of triethylamine at −78 °C. The resulting product[21] was then purified by flash chromatography (hexane-triethylamine, 10:1) in 96% yield.

DL-1,4-Di-*O*-allyl-*myo*-inositol (9). DL-3,6-Di-*O*-allyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **8** (17 g, 50 mmol) was dissolved in 80% HOAc (200 mL) and the mixture was heated under reflux for 30 min. The mixture was then cooled and the acetic acid was evaporated in vacuo to give a solid then co-evaporated with water (100 mL) to remove any acetic acid. The remaining solid was recrystallised from ethanol to give the title compound **9**. Yield (10.92 g, 84%); mp 136–137 °C (from ethanol); (lit.[22] 137–139 °C). ^{1}H NMR (Me_2SO-d_6, 270 MHz) δ 2.96 (1H, dd, J = 2.4, 9.7 Hz, H-1), 3.01 (1H, dt, J = 4.6, 8.8 Hz, H-5), 3.16–3.28 (2H, m, H-3 and H-4), 3.48 (1H, dt, J = 4.8, 9.3 Hz, H-6), 3.85 (1H, br s, H-2), 3.97–4.28 (4H, m, 2×O–CH_2CH=CH_2), 4.53 (1H, d, J = 6.2 Hz, D_2O ex, OH), 4.62 (1H, d, J = 3.7 Hz, D_2O ex, OH), 4.67 (1H, d, J = 4.8 Hz, D_2O ex, OH), 4.69 (1H, d, J 4.8 Hz, D_2O ex, OH), 5.01–5.33 (4H, m, 2×O–CH_2CH=CH_2), 5.83–6.00 (2H, m, 2×O–CH_2CH=CH_2).

DL-1,4-Di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol (11) and DL-1,4-di-*O*-allyl-3,5-di-*O*-*p*-methoxybenzyl-*myo*-inositol (10). A mixture of DL-1,4-di-*O*-allyl-*myo*-inositol **9** (5.2 g, 20 mmol) and dibutyltin oxide (12.5 g, 50 mmol) was suspended in toluene (400 mL) then heated under reflux for 2.5 h whilst removing water in a Dean and Stark apparatus. The solution was cooled and the toluene was evaporated under reduced pressure. The crystalline tin complex was dried at 130 °C under reduced pressure for a further 0.5 h. DMF (150 mL) and CsF (15.19 g, 100 mmol) were added to the tin complex and the suspension was stirred vigorously under nitrogen. *p*-Methoxybenzyl chloride (12.53 g, 60 mmol) was added dropwise and the reaction was monitored by TLC (ether). After 24 h at room temperature, TLC revealed two products, R_f = 0.40 and 0.56. The DMF was evaporated in vacuo and the residue was partitioned between a saturated aqueous solution of sodium hydrogen carbonate (300 mL) and dichloromethane (300 mL). The solution was stirred for 30 min and the precipitated tin derivatives were filtered off over a bed of Celite and washed with dichloromethane (2×100 mL). The organic layer was separated and washed with water, saturated brine and water again (200 mL of each). The dichloromethane solution was dried over magnesium sulfate and evaporated to give an oil. The mixture was purified by flash chromatography (ether–light petroleum, 2:1) to give the title compounds, **11**, (2.41 g, 24%), mp 115–117 °C (from ethyl acetate–hexane), (anal. calcd for $C_{28}H_{36}O_8$ C, 67.17; H, 7.25; found: C, 66.9; H, 7.28), and **10** (1.2 g, 12%), mp 94–95 °C (from ethyl acetate–hexane), (anal. calcd for $C_{28}H_{36}O_8$ C, 67.17; H, 7.25; found: C, 67.1; H, 7.20); (**11**) ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.62 (2H, br s, D_2O ex, 2×OH), 3.24 (1H, dd, J = 2.6, 9.5 Hz, H-3 or H-1), 3.30 (1H, dd, J = 2.6, 9.5 Hz, H-3 or H-1), 3.41 (1H, t, J = 9.3 Hz, H-5), 3.68 (1H, t, J = 9.3 Hz, H-4 or H-6), 3.76 (1H, t, J = 9.5 Hz, H-4 or H-6), 3.77 (3H, s, CH_2PhO*Me*), 3.79 (3H, s, CH_2PhO*Me*), 4.04–4.42 (5H, m, 2×O–CH_2CH=CH_2 and H-2), 4.58–4.85 (4H, m, 2×CH_2PhOMe), 5.14–5.33 (4H, m, 2×O–CH_2CH=CH_2), 5.87–6.05 (2H, m, 2×O–CH_2CH=CH_2), 6.84–6.90 (4H, m, 2×CH_2*Ph*OMe), 7.24–7.31 (4H, m, 2×CH_2*Ph*OMe); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 55.2, 71.61, 72.29, 74.30, 75.15, 67.75, 74.21, 79.20, 79.40, 80.21, 80.27, 113.80, 116.79, 117.38, 129.44, 129.64, 130.00, 134.67, 135.28, 159.32; (−ve ion FAB) m/z 653 $(M+NBA)^-$, 499 $(M-H)^-$, 379 $(M-CH_2PhOMe)^-$; (**10**) ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.48 (1H, br s, D_2O ex, OH), 2.57 (1H, br s, D_2O ex, OH), 3.13 (1H, dd, J = 2.75, 9.7 Hz, H-3 or H-1), 3.25 (1H, t, J = 9.3 Hz, H-5), 3.33 (1H, dd, J = 2.7, 9.5 Hz,

H-3 or H-1), 3.76 (1H, t, $J=9.5$ Hz, H-4 or H-6), 3.79 (3H, s, CH_2PhOMe), 3.80 (3H, s, CH_2PhOMe), 3.94 (1H, t, $J=9.5$ Hz, H-4 or H-6), 4.06–4.43 (5H, m, $2\times OCH_2CH=CH_2$ and H-2), 4.60–4.85 (4H, m, $2\times CH_2PhOMe$), 5.16–5.34 (4H, m, $2\times CH_2CH=CH_2$), 5.87–6.05 (2H, m, $2\times OCH_2CH=CH_2$), 6.85–6.90 (4H, m, $2\times CH_2PhOMe$), 7.25–7.32 (4H, m, $2\times CH_2PhOMe$); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 55.23, 67.17, 71.26, 72.00, 72.42, 74.43, 75.08, 78.78, 79.46, 80.56, 82.41, 113.84, 116.57, 117.74, 129.48, 129.61, 130.06, 130.84, 134.50, 135.28, 159.32; (−ve ion FAB) m/z 653 $(M+NBA)^-$, 499 $(M-H)^-$, 460 $(M-CH_2CH=CH_2)^-$, 379 $(M-CH_2PhOMe)^-$.

DL-1,4-Di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-2,5-di-*O*-methyl-*myo*-inositol (12). A mixture of sodium hydride (0.48 g, 20 mmol) and DL-1,4-di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol **11** (2.0 g, 4 mmol) was stirred in DMF (50 mL) at room temperature. Methyl iodide (1.25 mL, 20 mmol) was added dropwise and the mixture was stirred for 3 h after which TLC (ether) showed a new product, $R_f=0.80$. The excess sodium hydride was destroyed with methanol (10 mL) and the solvents were evaporated in vacuo. The product was taken up in ether (100 mL) washed with water, brine and water again (50 mL of each). The organic layer was dried over magnesium sulfate, evaporated and purified by flash chromatography (ether–hexane, 2:1) to give compound **12**. Yield (1.91 g, 90%); mp 93-94 °C (from hexane), (anal. calcd for $C_{30}H_{40}O_8$ C, 68.15; H, 7.73; found: C, 68.4; H, 7.73); ^1H NMR ($CDCl_3$, 270 MHz) δ 3.04 (1H, t, $J=9.25$ Hz, H-5), 3.14 (1H, dd, $J=2.4$, 9.9 Hz, H-3 or H-1), 3.33 (1H, dd, $J=2.2$, 9.9 Hz, H-3 or H-1), 3.60 (3H, s, Ins-OMe), 3.64 (3H, s, Ins-OMe), 3.66–3.73 (3H, m, H-2, H-4, H-6), 3.78 (3H, s, CH_2PhOMe), 3.79 (3H, s, CH_2PhOMe), 4.04–4.39 (4H, m, $2\times OCH_2CH=CH_2$), 4.40–4.85 (4H, m, $2\times CH_2PhOMe$), 5.14–5.32 (4H, m, $2\times OCH_2CH=CH_2$), 5.85–6.07 (2H, m, $2\times OCH_2CH=CH_2$), 6.85–6.90 (4H, m, $2\times CH_2PhOMe$), 7.25–7.32 (4H, m, $2\times CH_2PhOMe$); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 55.10, 61.05, 61.20, 71.76, 72.58, 74.27, 75.33, 77.53, 80.04, 80.14, 81.21, 81.24, 85.36, 113.61, 116.34, 116.73, 129.15, 129.65, 130.40, 131.07, 134.44, 135.89, 159.04, 159.09; (−ve ion FAB) m/z 681 $(M+NBA)^-$, 513 $(M-Me)^-$.

2,5-Di-*O*-methyl-*myo*-inositol (13). A mixture of DL-1,4-di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-2,5-di-*O*-methyl-*myo*-inositol **12** (1.056 g, 2 mmol) and palladium on activated charcoal, (10% Fluka, 0.30 g) and toluene-*p*-sulfonic acid (0.10 g, 0.52 mmol) was dissolved in a mixed solvent of ethanol (55 mL) and water (5 mL) then refluxed for 24 h after which, TLC (chloroform–methanol, 3:1) showed a single product $R_f=0.2$. The solution was filtered through Celite and recrystallised from ethanol to give compound **13**. Yield (0.321 g, 77%); mp 266–268 °C (from ethanol); (lit.[14] 270 °C); (anal. calcd for $C_8H_{16}O_6$ C, 46.13; H, 7.75; found: C, 46.1; H, 7.70); ^1H NMR (Me_2SO-d_6, 270 MHz) δ 2.65 (1H, t, $J=9.0$ Hz, H-5), 3.19 (2H, ddd, $J=2.6$, 5.3, 9.9 Hz, H-3 and H-1), 3.35 (1H, br s, H-2), 3.36 (2H, dt, $J=4.95$, 10.1 Hz, H-4 and H-6), 3.44 (6H, s, $2\times OMe$), 4.57 (2H, d, $J=5.1$ Hz, D_2O ex, 2×OH), 4.67 (2H, d, $J=4.8$ Hz, D_2O ex, 2×OH); ^{13}C NMR (Me_2SO-d_6, 68 MHz) δ 51.29, 59.90, 72.16, 72.58, 83.48, 85.72.

DL-1,4-Di-*O*-allyl-2,5-di-*O*-benzyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol (14). A mixture of DL-1,4-di-*O*-allyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol **11** (2.0 g, 4.0 mmol) and sodium hydride (480 mg, 20 mmol) was stirred in dry DMF (20 mL). Benzyl bromide (1.19 mL, 10 mmol) was added dropwise to the stirred solution at room temperature. After 2 h, TLC (ether–hexane, 2:1) showed a new product, $R_f=0.60$. Methanol (5 mL), was added to destroy the excess sodium hydride and the solvent was evaporated in vacuo. The resulting syrup was partitioned between water and ether, then washed with brine and water (100 mL of each). The organic layer was dried over magnesium sulfate and the solvent was evaporated to give a syrup. Flash chromatography (ether–hexane, 2:1) removed the remaining benzyl bromide to give compound **14**. Yield (2.94 g, 92%); mp 91–92 °C (from hexane); (anal. calcd for $C_{42}H_{48}O_8$ C, 74.07; H, 7.11; found: C, 74.3; H, 7.04); ^1H NMR ($CDCl_3$, 270 MHz) δ 3.21 (1H, dd, $J=2.0$, 9.9 Hz, H-3 or H-1), 3.27 (1H, dd, $J=2.0$, 9.9 Hz, H-3 or H-1), 3.37 (1H, t, $J=9.3$, H-5), 3.77 (3H, s, CH_2PhOMe), 3.79 (3H, s, CH_2PhOMe), 3.90 (1H, t, $J=9.3$ Hz, H-4 or H-6), 3.96 (1H, t, $J=9.9$ Hz, H-4 or H-6), 3.97 (1H, br s, H-2), 4.00–4.12 (2H, m, $OCH_2CH=CH_2$), 4.27–4.43 (2H, m, $OCH_2CH=CH_2$), 4.53, 4.59 (2H, AB, $J=11.4$ Hz, CH_2PhOMe or CH_2Ph), 4.71, 4.80 (2H, AB, $J=10.3$ Hz, CH_2PhOMe or CH_2Ph), 4.84 (4H, br s, $2\times CH_2PhOMe$ or $2\times CH_2Ph$), 5.12–5.33 (4H, m, $2\times OCH_2CH=CH_2$), 5.84–6.03 (2H, m, $2\times OCH_2CH=CH_2$), 6.81 (2H, d, $J=8.6$ Hz, CH_2PhOMe), 6.86 (2H, d, $J=8.6$ Hz, CH_2PhOMe), 7.21–7.41 (14H, m, $2\times CH_2PhOMe$ and $2\times CH_2Ph$); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 55.23, 71.58, 72.52, 73.95, 74.44, 74.53, 75.44, 75.86, 80.50, 80.66, 81.28, 81.44, 83.65, 113.71, 116.57, 127.24, 127.47, 127.76, 127.86, 128.08, 128.31, 129.09, 129.77, 130.61, 131.13, 134.99, 135.51, 138.95, 139.01, 158.12; (−ve ion FAB) m/z 833 $(M+NBA)^-$.

DL-2,5-Di-*O*-benzyl-3,6-di-*O*-*p*-methoxybenzyl-1,4-di-*O*-*cis*-prop-1-enyl-*myo*-inositol (15). A mixture of DL-1,4-di-*O*-allyl-2,5-di-*O*-benzyl-3,6-di-*O*-*p*-methoxybenzyl-*myo*-inositol **14** (2.38 g, 3.38 mmol) and sublimed potassium *t*-butoxide (1.57 g, 14 mmol) in dry DMF (40 mL) was stirred at 85 °C for 2 h under an atmosphere of nitrogen. TLC (ether–hexane 2:1) showed one product $R_f=0.60$ which was the same as starting material. The solution was then cooled, water (100 mL) was added and the product extracted with dichloromethane (4×100 mL). The organic layer was dried over magnesium sulfate, filtered and purified by flash chromatography (ether–hexane, 2:1) to give compound **15**. Yield (1.90 g, 83%); mp 108–110 °C (from hexane); (anal. calcd for $C_{42}H_{48}O_8$ C, 74.07; H, 7.11; found: C, 74.3; H, 7.07); ^1H NMR ($CDCl_3$, 270 MHz): δ 1.64 (3H, dd, $J=1.5$, 7.0 Hz, $OCH=CHCH_3$), 1.66 (3H, dd, $J=1.65$, 7.1 Hz, $OCH=CHCH_3$), 3.32 (1H, dd, $J=2.6$, 9.7 Hz, H-3 or H-1), 3.42 (1H, t, $J=9.3$ Hz, H-5), 3.51 (1H, dd, $J=2.2$, 9.7 Hz, H-3 or H-1), 3.77 (3H, s, CH_2PhOMe), 3.79 (3H, s, CH_2PhOMe), 3.98 (1H, br s, H-2), 4.02 (1H, t, $J=9.5$ Hz, H-4 or H-6), 4.14 (1H, t, $J=9.9$ Hz, H-4 or

H-6), 4.35 (1H, dq, $J=6.8$ Hz, OCH=C*H*CH_3), 4.44 (1H, dq, $J=6.8$ Hz, OCH=C*H*CH_3), 4.51–4.82 (8H, m, CH_2PhOMe and CH_2Ph), 6.08 (1H, dd, $J=1.65$, 6.2 Hz, OC*H*=CHCH$_3$), 6.26 (1H, dd, $J=1.65$, 6.4 Hz, OC*H*=CHCH$_3$), 6.84–6.90 (4H, m, CH$_2$*Ph*OMe), 7.22–7.41 (14H, m, CH$_2$*Ph*OMe and CH$_2$*Ph*); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 9.33, 9.40, 55.23, 72.29, 74.46, 75.30, 75.73, 75.99, 78.42, 80.36, 82.57, 82.92, 84.39, 98.14, 100.77, 113.71, 127.36, 127.59, 127.82, 128.11, 128.27, 129.24, 129.96, 130.35, 130.80, 138.59, 138.75, 145.75, 147.77, 159.18; (−ve ion FAB) *m*/*z* 833 $(M+NBA)^-$, 559 $(M-PMB)^-$.

2,5-Di-*O*-benzyl-*myo*-inositol (16). 2,5-Di-*O*-benzyl-3,6-di-*O*-*p*-methoxybenzyl-1,4-di-*O*-*cis*-prop-1-enyl-*myo*-inositol **15** (0.42 g, 0.62 mmol) was stirred in a mixture of dichloromethane–trifluoroacetic acid [20 mL, (10:1)] at room temperature overnight. TLC (ether) showed the presence of a *p*-methoxybenzyl derivative only, and the orange-red solution was evaporated to dryness. The mixture was co-evaporated with water then ethanol (10 mL of each) to remove traces of acid, after which a fine white solid precipitated from the solution. The solid was filtered off and washed successively with water, acetone and finally ether (20 mL of each) to give **16**. Yield (0.17 g, 76%); mp 271–273 °C (from DMF–ethanol); (lit.[15] 270–272 °C); (anal. calcd for $C_{20}H_{24}O_6$ C, 66.67; H, 6.67; found: C, 66.4; H, 6.64); ^{1}H NMR (Me_2SO-d_6, 270 MHz) δ 3.03 (1H, t, $J=9.2$ Hz, H-5), 3.33 (2H, ddd, $J=2.6$, 4.8, 9.7 Hz, H-3 and H-1), 3.60 (2H, dt, $J=5.1$, 9.3 Hz, H-4 and H-6), 3.73 (1H, t, $J=2.75$ Hz, H-2), 4.75 (2H, d, $J=4.8$ Hz, D_2O ex, OH-1 and OH-3), 4.78 (4H, s, 2×CH_2Ph), 4.83 (2H, d, $J=5.1$ Hz D_2O ex, OH-4 and OH-6), 7.20–7.43 (10H, m, 2×CH$_2$*Ph*); ^{13}C NMR (Me_2SO-d_6, 68 MHz) δ 73.75, 74.17, 72.19, 73.07, 81.83, 84.23, 127.01, 127.53, 127.92, 127.98, 139.92, 140.01.

2,5-Di-*O*-methyl-1,3,4,6-tetrakis(diethoxyphospho)-*myo*-inositol (18). A mixture of 2,5-di-*O*-methyl-*myo*-inositol **13** (0.104 g, 0.5 mmol) and dry diisopropylethylamine (0.7 mL, 4 mmol) was dissolved in dry DMF (5 mL) and kept under nitrogen at −78 °C. Diethoxychlorophosphine (0.58 mL, 4 mmol), (90–95%) was added dropwise to the stirred solution and left for 45 min. The mixture was oxidised with *t*-butylhydroperoxide (1 mL, 7.3 mmol) at −78 °C and stirred for a further 30 min at room temperature. The DMF was evaporated in vacuo and the remaining syrup was partitioned between water (50 mL) and dichloromethane (50 mL). The organic layer was washed with 10% sodium metabisulfite solution, brine (20 mL of each) and finally water (2×20 mL). The organic layer was dried over magnesium sulfate and evaporated to give compound **18** $R_f=0.40$ (chloroform–methanol, 3:1). Yield (0.286 g, 76%); ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.26–1.39 (24H, m, 8×P(O)OCH$_2$C*H*$_3$), 3.23 (1H, t, $J=9.4$ Hz, H-5), 3.57 (3H, s, O*Me*), 3.63 (3H, s, O*Me*), 4.12–4.27 (18H, 8×P(O)OCH_2CH$_3$, H-3 and H-1), 4.40 (1H, br s, H-2), 4.74 (2H, q, $J=9.5$, H-4 and H-6); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 16.05, 16.15, 59.64, 61.78, 63.89, 63.99, 64.09, 64.22, 64.31, 64.44, 64.51, 75.70, 76.28, 76.41, 76.51, 78.00, 80.79; ^{31}P NMR ($CDCl_3$, 109 MHz) δ −2.63 (q, $J=7.5$ Hz, *P*(O)OCH$_2$CH$_3$), −3.15 (q, $J=7.5$ Hz *P*(O)OCH$_2$CH$_3$); (+ve ion FAB) *m*/*z* 753 $(M+H)^+$, Accurate mass spectrum requires: $(M+H)^+=753.2182$; found: 753.2231.

2,5-Di-*O*-methyl-*myo*-inositol 1,3,4,6-tetrakisphosphate (19). 2,5-Di-*O*-methyl-1,3,4,6-tetrakis(diethoxyphospho)-*myo*-inositol **18** (0.25 g, 0.33 mmol) was dissolved in dry dichloromethane (5 mL) and kept under a blanket of nitrogen at room temperature. Trimethylsilyl bromide (0.70 mL, 5.3 mmol) was added dropwise and the solution was stirred for 16 h at room temperature. Evaporation gave a syrupy residue which was dissolved in water (5 mL) then stirred for 1 h to give compound **19** in quantitative yield by ^{31}P NMR. A small portion was purified by ion exchange chromatography, using a buffer gradient of 200–1000 mM of TEAB at a flow rate of 5 mL/min. Yield (43.5 μmol) which eluted at 500 mM buffer; ^{1}H NMR (D_2O, 270 MHz) δ 3.51 (3H, br s, O*Me*), 3.57 (4H, br s, O*Me* and H-5), 3.97 (1H, br s, H-2), 4.06 (2H, t, $J=9.5$ Hz, H-3 and H-1), 4.28 (2H, q, $J=9.4$ Hz, H-4 and H-6); ^{13}C NMR (D_2O, 68 MHz) δ 59.64, 61.39, 73.97, 75.66, 75.76, 76.21, 80.01, 81.60; ^{31}P NMR (D_2O, 162 MHz) δ 0.00 (1H, d, $J=9.9$ Hz, 2P), −0.58 (1H, d, $J=8.0$ Hz, 2P); (−ve ion FAB) *m*/*z* 527 $(M-H)^-$; Accurate mass spectrum requires: $(M-H)=526.9520$; found: 526.9520.

2,5-Di-*O*-methyl-1,3,4,6-tetrakis[di(benzyloxyphosphorothio)]-*myo*-inositol (21). A mixture of bis(benzyloxy)-diisopropylaminophosphine (0.69 g, 2 mmol) and 1*H*-tetrazole (350 mg, 5 mmol) was stirred in DMF (5 mL) for 1 h. 2,5-Di-*O*-methyl-*myo*-inositol **13** (0.052 g, 0.25 mmol) was added to the mixture and stirred for a further 2 h. TLC (ether–petroleum ether, 2:1) showed a major product $R_f=1.00$ for the phosphite. Sulfur (0.096 g, 3 mmol) and dry pyridine (2 mL) were added and the mixture stirred for 15 min after which TLC (ether–petroleum ether, 2:1) showed a new product $R_f=0.60$. The excess sulfur was filtered off and the solvents were evaporated in vacuo to give a syrup. Compound **21** was purified by flash chromatography (ether–petroleum ether, 2:1) and isolated as a syrup. Yield (0.27 g, 82%); (anal. calcd for $C_{64}H_{68}O_{14}P_4S_4$ C, 58.53; H, 5.22; found: C, 58.3; H, 5.21); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.23 (1H, t, $J=9.3$ Hz, H-5), 3.31 (3H, s, O*Me*), 3.50 (3H, s, O*Me*), 4.33 (2H, dt, 2.0, 9.9 Hz, H-3 and H-1), 4.61 (1H, br s, H-2), 5.00–5.14 (18H, m, 8×P(S)O–CH_2Ph, H-4 and H-6), 7.20–7.37 (40H, m, 8×P(S)O–CH$_2$*Ph*); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 61.29, 61.33, 69.47, 70.09, 76.02, 77.00, 77.25, 79.10, 79.30, 80.66, 127.69, 127.82, 127.89, 127.95, 128.08, 128.24, 135.18, 135.28, 135.41, 135.48, 135.57, 135.67, 135.80; ^{31}P ($CDCl_3$, 162 MHz) δ + 69.11 (dtt, $J=8.0$, 9.9, 9.9 Hz, 8×*P*(S)OCH_2Ph), + 67.09 (dtt, $J=7.9$, 9.9, 9.9 Hz, *P*(S)OCH_2Ph); (+ve ion FAB) *m*/*z* 1314 $(M+H)^+$.

2,5-Di-*O*-methyl-*myo*-inositol 1,3,4,6-tetrakisphosphorothioate (22). Ammonia (80 mL) was distilled into a three-neck flask and small slithers of freshly cut sodium metal (0.80 g, 34.8 mmol) were added until the solution remained blue. The dry-ice condenser was moved across to the reaction flask and ammonia (40 mL) was gently transferred to the flask by heating. Small slithers of

sodium (0.40 g, 17.4 mmol) were added to the ammonia until the colour remained blue. 2,5-Di-*O*-methyl-1,3,4,6-tetrakis[di(benzylphosphorothio)]-*myo*-inositol **21** (0.10 g, 76 μmol) in dry dioxan (1 mL) was added to the sodium in liquid ammonia. The reaction was left for 2 min and quenched with methanol (20 mL). The ammonia was then evaporated in a stream of nitrogen. MilliQ water was then added to the residue and evaporated to dryness in vacuo. The deprotected phosphorothioate **22** was purified by ion exchange chromatography using a buffer gradient of 0–1000 mmol and eluted at ca. 800 mmol. Yield (19.2 μmol, 25%); ^{1}H NMR (D_2O, 270 MHz) δ 3.32 (1H, t, J = 9.5 Hz, H-5), 3.60 (3H, s, O*Me*), 3.64 (3H, s, O*Me*), 4.15 (1H, t, J = 2.4 Hz, H-2), 4.23 (2H, ddd, J = 2.3, 10.3, 10.3 Hz, H-3 and H-1), 4.60 (2H, q, J = 10.0, H-4 and H-6); ^{13}C NMR (D_2O, 68 MHz) δ 59.57, 61.65, 74.07, 76.44, 80.14, 82.02; ^{31}P NMR (D_2O, 109 MHz) δ + 46.7 (d, J = 10.1 Hz, 2P), + 48.8 (d, J = 10.1 Hz, 2P); (−ve ion FAB) m/z 591 $(M-H)^-$; Accurate mass spectrum requires: $(M-H)^-$ = 590.8662; found: 590.8608.

2,5-Di-*O*-benzyl-1,3,4,6-tetrakis[di(benzyloxyphosphorothio)]-*myo*-inositol (24). A mixture of bis(benzyloxy)-diisopropylaminophosphine (0.69 g, 2 mmol) and 1*H*-tetrazole (0.35 g, 5 mmol) in DMF (2 mL) was stirred for 1 h. 2,5-Di-*O*-benzyl-*myo*-inositol **16** (0.90 g, 0.25 mmol) was added to the mixture and stirred for a further 1 h. TLC (ether–petroleum ether, 1:2) showed a major product R_f = 1.00 for the phosphite intermediate. Sulfur (0.096 g, 3 mmol) and dry pyridine (2 mL) were added and stirred for 10 min after which, TLC (ether–petroleum ether, 1:2) showed a new product R_f = 0.40. The excess sulfur was filtered off and the solvents were evaporated in vacuo at ambient temperature. Compound **24** was purified by flash chromatography (ether–petroleum ether, 1:2) and isolated as a syrup. Yield (0.26 g, 71%); (anal. calcd for $C_{76}H_{76}O_{14}P_4S_4$ C, 62.29; H, 5.23; found: C, 62.5; H, 5.32); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.59 (1H, t, J = 9.15 Hz, H-5), 4.42–4.48 (3H, m, H-1, H-2 and H-3), 4.78–5.07 (20H, m, 8×P(S)O–C*H*$_2$Ph and 2×C*H*$_2$Ph), 5.38 (2H, dt, J = 9.15, 12.5 Hz, H-4 and H-6), 6.86–7.37 (50H, 8×P(S)O–CH$_2$*Ph* and 2×CH$_2$*Ph*); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 69.99, 70.19, 70.25, 70.54, 70.74, 74.01, 75.83, 76.51, 76.93, 77.84, 79.30, 127.01, 127.27, 127.53, 127.66, 128.21, 128.37, 128.57, 128.63, 128.73, 128.80, 128.86, 128.93, 135.74, 135.87, 135.93, 136.06, 136.13, 136.23, 136.36, 136.49, 138.75, 138.85; ^{31}P NMR ($CDCl_3$, 109 MHz) δ + 66.78 (dtt, J = 9.3, 9.5 Hz, 2P), + 69.57 (dtt, J = 9.7, 11.4 Hz, 2P).

***myo*-Inositol 1,3,4,6-tetrakisphosphorothioate (25).** Ammonia (80 mL) was distilled into a three-neck flask and small slithers of freshly cut sodium metal (800 mg, 34.8 mmol) were added until the solution remained blue. The dry-ice condenser was moved across to the reaction flask and ammonia (40 mL) was transferred to the flask by gentle heating. Small slithers of sodium (400 mg, 17.4 mmol) were added to the ammonia until the colour remained blue. A solution of 2,5-di-*O*-benzyl-1,3,4,6-tetrakis[di(benzylphosphorothio)]-*myo*-inositol **24** (0.059 g, 76 μmol) in dry dioxan (1 mL) was added to the sodium in liquid ammonia. The reaction was left for 2 min and quenched with methanol (20 mL). The ammonia was evaporated in a stream of nitrogen and MilliQ water was then added to the residue then evaporated to dryness. The deprotected phosphorothioate **25** was purified by ion exchange chromatography using a buffer gradient of 0–1000 mmol and eluted at ca. 800 mmol. Yield (18.5 μmol, 46%); ^{1}H NMR (D_2O, 270 MHz) δ 3.61 (1H, t, J = 9.0 Hz, H-5), 4.16 (1H, dt, J = 2.95, 9.5 Hz, H-1 and H-3), 4.51 (2H, q, J = 9.9, H-4 and H-6), 4.99 (1H, br s, H-2); ^{31}P NMR (D_2O, 109 MHz) δ + 42.4 (d, J = 12.25 Hz, 2P), + 45.09 (d, J = 9.8 Hz, 2P); (−ve ion FAB) m/z 563 $(M-H)^-$; accurate mass spectrum requires: $(M-H)^-$ = 562.8295; found: 562.8314.

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References and Notes

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