Synthesis of Selective Non-Ca^{2+}-Mobilizing Inhibitors of D-*myo*-Inositol 1,4,5-Trisphosphate 5-Phosphatase

Dethard Lampe, Changsheng Liu, and Barry V. L. Potter*

Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

Received November 5, 1993®

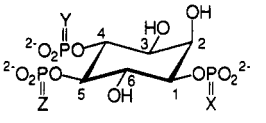
Syntheses of novel phosphorothioate-based non-Ca^{2+}-releasing inhibitors of *myo*-inositol 1,4,5-trisphosphate 5-phosphatase are described. 1L-*myo*-Inositol 1,4,5-trisphosphorothioate, *myo*-inositol 1,3,5-trisphosphorothioate, and 1L-*chiro*-inositol 1,4,6-trisphosphorothioate have been synthesized from 1L-2,3,6-tri-*O*-benzyl-1-*O*-(*cis*-prop-1-enyl)-*myo*-inositol, 2,4,6-tris-*O*-(*p*-methoxybenzyl)-*myo*-inositol orthoformate and 1L-2,3,5-tri-*O*-benzyl-*chiro*-inositol, respectively. 1L-2,3,5-Tri-*O*-benzyl-*chiro*-inositol was also used to prepare 1L-*chiro*-inositol 1,4,6-trisphosphate. The phosphorothioates did not mobilize intracellular Ca^{2+} but were highly potent inhibitors of Ins(1,4,5)P_3 5-phosphatase, and *myo*-inositol 1,3,5-trisphosphorothioate and 1L-*chiro*-inositol 1,4,6-trisphosphorothioate were selective for this enzyme.

Introduction

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], 1 (Figure 1) is a ubiquitous second messenger, which couples agonist stimulation of a wide variety of cell surface receptors to the mobilization of intracellular calcium.[1,2] The gene coding for the Ins(1,4,5)P_3 receptor has now been cloned,[3,4] and the ability of this transmembrane protein to gate calcium in response to Ins(1,4,5)P_3 has been demonstrated.[5] Realization of the fundamental cellular role played by Ins(1,4,5)P_3 and the acceptance of the polyphosphoinositide signal transduction mechanism has led to a massive increase in biological[1,2] and, more recently, chemical[6,7] effort to unravel the details of this complex pathway. The basic chemical problems intrinsic to inositol polyphosphate synthesis have been solved, and Ins(1,4,5)P_3 has now been synthesized by many groups.[6-8] Chemical emphasis in this field is now focusing upon the synthesis of novel structurally modified inositol phosphate analogues as potential enzyme inhibitors and receptor antagonists to facilitate pharmacological intervention in this signaling pathway.

A number of Ins(1,4,5)P_3 analogues have now been synthesized, but only few have been shown to be biologically potent, and many have not yet been evaluated.[6-8] Reports on analogues specifically ring-modified at the 2-position,[10-13] the 3-position,[14] and the 6-position[15,16] and on the synthesis of an active 5-methylene phosphonate analogue[17] have appeared as well as details of multiple modifications.[18] We have reasoned that, by virtue of their metabolic stability, phosphorothioate analogues (Figure 1) will prove to be of significant importance in this field.[9,19] We previously reported the first synthesis of *myo*-inositol 1,4,5-trisphosphorothioate **2** [Ins(1,4,5)PS_3],[20] a potent inhibitor of human erythrocyte membrane Ins(1,4,5)P_3 5-phosphatase,[21,22] and the specifically modified 5-phosphorothioate analogues **3** [Ins(1,4,5)P_3-5S],[23] 1-phosphorothioate 4,5-bisphosphate **4**,[24] and 1-phosphate 4,5-bisphosphorothioate **5**.[25] These highly potent Ca^{2+}-mobilizing analogues are already finding numerous biological applications.[7,9,19,26,27] Phosphorothioate analogues have also been synthesized by other groups.[28-31]

There is a great need for synthetic small molecule tools for pharmacological intervention in the polyphosphoinositide pathway of cellular signaling.[9,27] This is especially pertinent in relation to the highly complex metabolism of inositol polyphosphates.[32] Inhibitors of the metabolic enzymes Ins(1,4,5)P_3 5-phosphatase and 3-kinase are thus of considerable current interest. Especially valuable for metabolic studies would be small molecule inhibitors that selectively and potently block the enzyme activity and do not interact with the Ins(1,4,5)P_3 receptor. Such compounds have not been prepared before. Thus, our aim in this work was to develop potent 5-phosphatase inhibitors, unable to release Ca^{2+}, by improving the binding properties of compounds known to be moderately potent, non-Ca^{2+}-mobilizing inhibitors. We report here the synthesis of three such highly potent and novel Ins(1,4,5)P_3 5-phosphatase inhibitors. Part of this work has been published in preliminary form.[33]



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

Figure 1. Structures of Ins(1,4,5)P_3 and phosphorothioate analogues.

Results and Discussion

Ins(1,4,5)P_3 5-phosphatase is relatively nonspecific and recognizes not only Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, which are the natural substrates of this enzyme, but also, albeit weakly, synthetic inositol phosphates such as L-Ins(1,4,5)P_3, Ins(1,3,5)P_3, and Ins(2,4,5)P_3.[34] Unlike Ins(1,4,5)P_3, Ins(2,4,5)P_3, and Ins(1,3,4,5)P_4, however, L-Ins(1,4,5)P_3 [= D-Ins(3,5,6)P_3] and Ins(1,3,5)P_3 are competitive inhibitors of this enzyme with K_i values of 39 and 45 μM, respectively.[34] In another study using human erythrocyte 5-phosphatase, L-Ins(1,4,5)P_3 was found to inhibit the enzyme with a K_i of 124 μM.[22] Until then, the best known inhibitor was 2,3-bisphosphoglycerate (K_i = 350 μM)[35] [but see ref 22, K_i = 978 μM].

The few structure–activity studies which have been performed show that the vicinal 4,5-bisphosphate moiety of Ins(1,4,5)P_3 is essential for Ca^{2+}-releasing activity,[6,7,19,27] the 1-phosphate group being thought to provide enhanced affinity for the receptor. Cooke *et al.*[22] first observed that

* Abstract published in *Advance ACS Abstracts*, March 1, 1994.

0022-2623/94/1837-0907$04.50/0

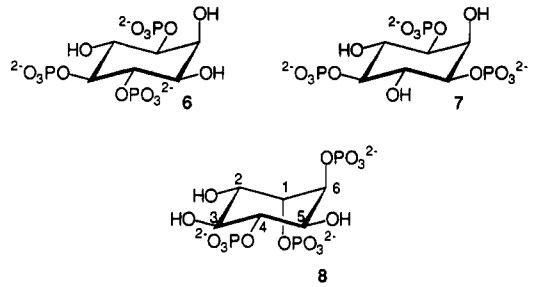
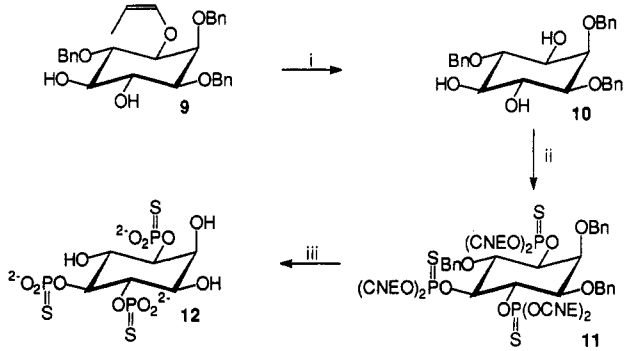


Figure 2. Structures of non-Ca^{2+}-mobilizing weak Ins(1,4,5)P_3 5-phosphatase inhibitors.

Scheme 1[a]



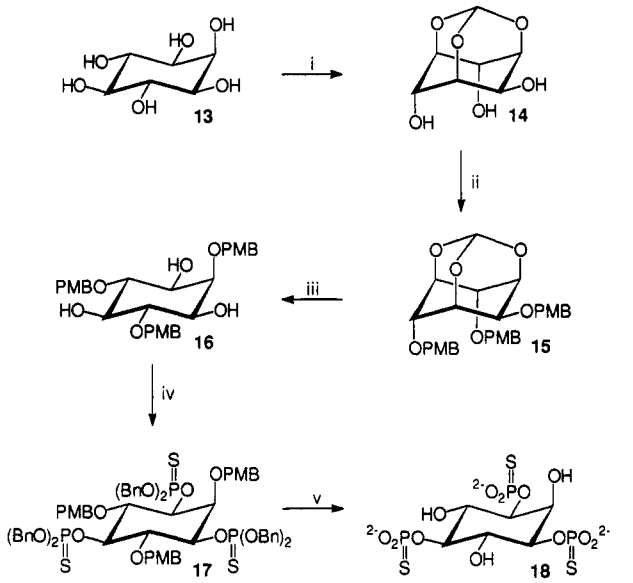
[a] Reagents and conditions: (i) H^+; (ii) $(NCCH_2CH_2O)_2PNPr^i_2$/1*H*-tetrazole, then S_8/pyridine; (iii) Na/liquid NH_3. Bn, benzyl; CNE, 2-cyanoethyl.

the trisphosphorothioate analogue of Ins(1,4,5)P_3, Ins(1,4,5)PS_3 **2** (Figure 1), acted as a potent competitive inhibitor of human erythrocyte membrane Ins(1,4,5)P_3 5-phosphatase, and subsequently Ins(1,4,5)PS_3 was found to have a K_i of 1.7 μM.[21] Thus, substitution of phosphate groups by phosphorothioates apparently results in increased binding properties of the analogue [K_m for Ins(1,4,5)P_3, 40 μM]. Ins(1,4,5)PS_3, however, mobilizes intracellular Ca^{2+},[36,37] and this was considered to disadvantage this compound as a specific Ins(1,4,5)P_3 5-phosphatase inhibitor.

Considering the effect of phosphorothioate replacement on Ins(1,4,5)P_3, we reasoned that phosphorothioate analogues of L-Ins(1,4,5)P_3 **6** and Ins(1,3,5)P_3 **7** (Figure 2) would prove to be more potent inhibitors than the parent compounds. Additionally, since we had also found that the analogue L-*chiro*-inositol 1,4,6-trisphosphate [L-*chiro*-Ins(1,4,6)P_3] **8** (Figure 2) was a weak inhibitor of 5-phosphatase,[27,33] it also seemed appropriate to synthesize the corresponding phosphorothioate of this non *myo*-inositol based analogue.

L-Ins(1,4,5)P_3 **6** is a weak Ins(1,4,5)P_3 5-phosphatase inhibitor[22,34] and is only a very weak mobilizer of intracellular Ca^{2+}. In order to synthesize L-*myo*-inositol 1,4,5-trisphosphorothioate (Scheme 1), the L-enantiomer of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol[38] was isomerized to the corresponding 1-*O*-(*cis*-prop-1-enyl) derivative **9**[24] and was deprotected to give 1D-1,2,4-tri-*O*-benzyl-*myo*-inositol **10**.[39] The melting point (116–118 °C) and optical rotation ($[\alpha]^{18}_D = -10.1°$) measured for this previously described triol were in good agreement with the reported values (mp 118–120 °C; $[\alpha]^{18}_D = -9.0°$).[39] Compound **10** was then tris-thiophosphorylated using bis(2-cyanoethoxy)(diisopropylamino)phosphine[40]/tetrazole, followed by oxidation of the resulting trisphosphite triester with sulfur in pyridine to give the fully protected 1L-2,3,6-tri-*O*-benzyl-1,4,5-tris[bis(2-cyanoethoxy)thiophospho]-*myo*-inositol, **11**. Deblocking with sodium/liquid ammonia[20] furnished

Scheme 2[a]



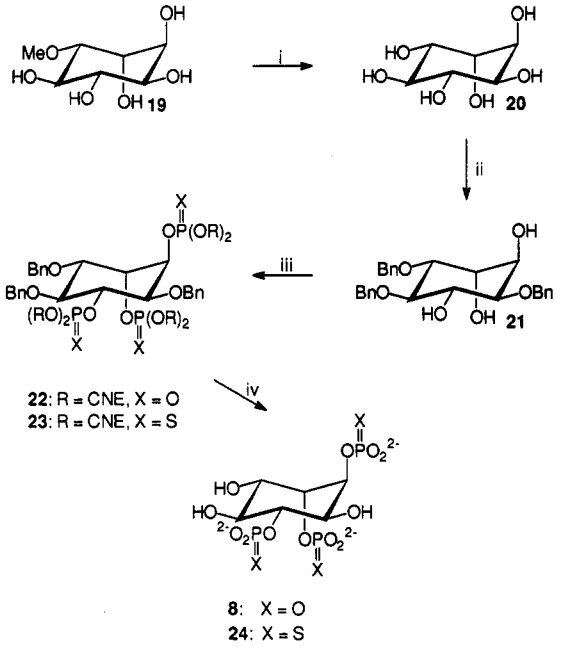
[a] Reagents and conditions: (i) $HC(OEt)_3/H^+$/DMF; (ii) PMBCl/NaH/DMF; (iii) 2 M HCl/MeOH (1:20); (iv) $(BnO)_2PNPr^i_2$/1*H*-tetrazole, then S_8/pyridine; (v) Na/liquid NH_3. PMB, *p*-methoxybenzyl; Bn, benzyl.

1L-*myo*-inositol 1,4,5-trisphosphorothioate [1L-Ins(1,4,5)PS_3], **12**, which was purified by ion-exchange chromatography on DEAE Sephadex, eluting with a gradient of triethylammonium bicarbonate and was quantified by Briggs phosphate assay.[41] The ^{31}P-NMR spectrum of **12** showed, as expected, signals at 44.8, 44.7, and 42.1 ppm for the three phosphorothioate groups.

Ins(1,3,5)P_3 **7** is a weak inhibitor of Ins(1,4,5)P_3 5-phosphatase.[34] Selective protection of the 1-, 3-, and 5-hydroxyl groups of *myo*-inositol **13** can be effected by converting *myo*-inositol into its orthoformate ester **14** by Billington's modification[42] of the method devised by Lee and Kishi,[43] *i.e.*, the use of DMF rather than DMSO as solvent for the reaction. The remaining free hydroxyl groups of **14** (Scheme 2) were protected as *p*-methoxybenzyl ethers using *p*-methoxybenzyl chloride/sodium hydride in DMF. The ortho ester was removed by treatment of the fully protected **15** with dilute hydrochloric acid yielding the desired 2,4,6-tris-*O*-(*p*-methoxybenzyl)-*myo*-inositol **16**. Triol **16** was thiophosphorylated with bis(benzyloxy)(diisopropylamino)phosphine[44]/tetrazole followed by sulfoxidation to give the fully protected trisphosphorothioate **17**, which was then deprotected with sodium in liquid ammonia[20] to give *myo*-inositol 1,3,5-trisphosphorothioate [Ins(1,3,5)PS_3] **18** as the triethylammonium salt after ion-exchange chromatography and quantified by Briggs phosphate assay.[41] ^{31}P-NMR spectroscopy of this symmetrical trisphosphorothioate showed, as expected, two signals at 47.7 and 46.6 ppm integrating for one and two P-nuclei, respectively. Both signals were doublets with J = 10.1 Hz in the ^{1}H-coupled spectrum.

We[45] and others[46,47] have reported the synthesis of inositol polyphosphate analogues based upon *chiro*-inositol. (Note the different conventional numbering of the *myo*- and *chiro*-inositol systems in Figures 1 and 2.) In particular, L-*chiro*-inositol 2,3,5-trisphosphate [L-*chiro*-Ins(2,3,5)P_3] is a potent Ca^{2+}-mobilizing Ins(1,4,5)P_3 3-kinase and 5-phosphatase inhibitor.[45,48] During the synthesis of L-*chiro*-Ins(2,3,5)P_3, we prepared L-2,3,5-tri-*O*-benzyl-*chiro*-inositol **21**, which we have now used to prepare L-*chiro*-inositol 1,4,6-trisphosphate and its trisphosphorothioate analogue L-*chiro*-inositol 1,4,6-trisphos-

Scheme 3[a]



[a] Reagents and conditions: (i) HI; (ii) Bu_2SnO (4 equiv), Bu_4NI (4 equiv), MeCN, BnCl (5 equiv); (iii) $(NCCH_2CH_2O)_2PNPr^i_2$/1*H*-tetrazole, then either *t*-BuOOH or S_8/pyridine; (iii) Na/liquid NH_3. Bn, benzyl; CNE, 2-cyanoethyl.

phorothioate [L-*chr*-Ins(1,4,6)PS_3]. Thus, L-quebrachitol **19** (Scheme 3) was demethylated with HI, and the resulting L-*chiro*-inositol **20** was regiospecifically tribenzylated *via* tin-mediated alkylation to give L-2,3,5-tri-*O*-benzyl-*chiro*-inositol **21**.[45] Compound **21** was polyphosphorylated on the free hydroxyl groups using bis(2-cyanoethoxy)(diisopropylamino)phosphine[40]/tetrazole followed by oxidation of the resulting triphosphite triester either with t-BuOOH to give the fully protected trisphosphate **22** or with sulfur in pyridine to yield the protected phosphorothioate **23**. Treatment respectively with sodium in liquid ammonia[20] yielded either the free trisphosphate **8** or the trisphosphorothioate **24**, which were purified by ion-exchange chromatography on DEAE-Sephadex, eluting with a gradient of triethylammonium bicarbonate buffer and quantified by Briggs phosphate assay.[41]

All three potential Ins(1,4,5)P_3 5-phosphatase inhibitors, L-*myo*-inositol 1,4,5-trisphosphorothioate **12**, *myo*-inositol 1,3,5-trisphosphorothioate **18**, and L-*chiro*-inositol 1,4,6-trisphosphorothioate **24** have been pharmacologically evaluated[49] and are highly potent inhibitors of Ins(1,4,5)-P_3 5-phosphatase from human erythrocyte membrane, with submicromolar K_i values (K_i values determined as the mean of at least three experiments as previously described[21,22]) of 0.50, 0.43, and 0.3 μM, respectively.[49] L-*chr*-Ins(1,4,6)P_3 had a K_i of 44 μM.[33] None of the phosphorothioates, nor L-*chr*-Ins(1,4,6)P_3, mobilized Ca^{2+} from intracellular stores in electrically permeabilized human neuroblastoma cells, although Ins(1,4,5)P_3 released Ca^{2+} potently under similar conditions (EC_{50} 0.12 μM).

All analogues were also assayed for their interactions with Ins(1,4,5)P_3 3-kinase.[49] Whereas L-Ins(1,4,5)PS_3 is recognized surprisingly well by the enzyme (K_i = 1.46 μM; K_i's determined as previously described[21]), Ins(1,3,5)PS_3 shows hardly any interaction (K_i 247 μM), and L-*chr*-Ins-(1,4,6)P_3 and L-*chr*-Ins(1,4,6)PS_3 were ineffective at inhibiting [^{3}H]Ins(1,4,5)P_3 phosphorylation by crude rat brain Ins(1,4,5)P_3 3-kinase at concentrations of 250 and 30 μM, respectively [K_m for Ins(1,4,5)P_3 0.6 μM]. Thus, while all three analogues are potent 5-phosphatase inhibitors, Ins(1,3,5)PS_3 and L-*chr*-Ins(1,4,6)PS_3 have the advantage of being selective for this enzyme. L-*chiro*-Ins(1,4,6)PS_3, in particular, is more than 1 order of magnitude more potent than (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenesulfonate), a recently reported selective Ins(1,4,5)P_3 5-phosphatase inhibitor.[50] Full biological details will be published elsewhere.

It is not yet clear why phosphorothioate substitution results in such a marked increase in affinity for Ins(1,4,5)-P_3 5-phosphatase, although the larger size and greater hydrophobicity of sulfur over oxygen together with the lower pK_a of the phosphorothioate group[51] must invariably play a part. Moreover, the inhibitors we have described here possess a diverse range of phosphorothioate substitution patterns. Their potent activity underlines the marked lack of specificity of Ins(1,4,5)P_3 5-phosphatase in its binding of inositol polyphosphates and analogues. While it is not certain precisely how these inhibitors bind to the enzyme, they are nevertheless key leads and the elucidation of their mode of action provides an important future challenge.

Conclusion

In conclusion, phosphorothioate substitution has provided sub-micromolar inhibitors of Ins(1,4,5)P_3 5-phosphatase of the highest potency currently available. Moreover, Ins(1,3,5)PS_3 and L-*chr*-Ins(1,4,6)PS_3, by virtue of their lack of affinity for 3-kinase emerge as selective non-Ca^{2+}-mobilizing inhibitors of Ins(1,4,5)P_3 5-phosphatase. Clearly, such compounds will be of considerable use in unravelling aspects of the polyphosphoinositide pathway of cellular signaling. The recent demonstration that the Lowe's oculocerebrorenal syndrome gene encodes a protein highly homologous to inositol polyphosphate 5-phosphatase and that this affliction may be an inborne error of inositol phosphate metabolism,[52] as well as the demonstration that lymphocytes infected *in vivo* with HIV virus show a defect in inositol polyphosphate-mediated signal transduction associated with 5-phosphatase, together with a concomitant abnormality in intracellular Ca^{2+} regulation,[53] underline the need for such agents.

Experimental Section

Chemicals were purchased from Aldrich, Fluka, and Lancaster. Diethyl ether was dried over sodium wire and distilled. Dichloromethane, triethylamine, and dimethylformamide were dried over calcium hydride, distilled, and stored over 4-Å molecular sieves. Pyridine was dried by refluxing with sodium hydroxide pellets, followed by distillation, and stored over 5-Å sieves. TLC and high-performance TLC (HPTLC) were performed on precoated plates (Merck TLC aluminum sheets silica 60 F_{254}, Art. no. 5554, and Merck HPTLC plates silica 60 F_{254}, Art. no. 5635). Products were visualized by spraying phosphomolybdic acid in methanol followed by heating. Flash chromatography refers to the method of Still *et al.*[54] and was carried out using Sorbsil C60 silica gel.

^{1}H- and ^{13}C-NMR spectra were recorded on either JEOL JNM GX-270 NMR or JEOL EX-400 NMR spectrometers. Chemical shifts were measured in ppm relative to tetramethylsilane (TMS). ^{31}P-NMR spectra were recorded on a JEOL FX-90Q spectrometer. ^{31}P chemical shifts were measured in ppm relative to external 85% H_3PO_4. *J* values are given in hertz. Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block. Microanalysis was carried out at Butterworth Laboratories Ltd. and by the University of Bath microanalysis service. Mass spectra were recorded at the SERC Mass Spectrometry Service Centre, Swansea, and at the University of Bath. Optical rotations were measured using a Optical Activity Ltd. AA-10 polarimeter. Ion-exchange chromatography was per-

formed on an LKB-Pharmacia Medium Pressure Ion Exchange Chromatograph using DEAE Sephadex or DEAE Sepharose with gradients of triethylammonium bicarbonate (TEAB) as eluent.

Column fractions containing inositol polyphosphate analogues were assayed for phosphate by a modification of the Briggs test[41] as follows: A molybdate solution (12.5 g of ammonium molybdate dissolved in 250 mL of water and 35 mL of concentrated H_2SO_4), a hydroquinone solution (0.5 g of hydroquinone dissolved in 100 mL of water and a drop of concentrated H_2SO_4), and a sulfite solution (20% w/v sodium sulfite in water) were prepared. Aliquots (usually 250 μL) of the fractions to be assayed were transferred into test tubes, and concentrated H_2SO_4 (3 drops) was added to these samples. The samples were heated at 150 °C for 1 h. After cooling, water (500 μL), molybdate solution (500 μL), hydroquinone solution (250 μL), and sulfite solution (250 μL) were added to each of the test tubes. The samples were then boiled for 7 s and allowed to cool. Phosphate-containing fractions could be identified by their blue color. For quantitative analysis, samples containing known amounts of potassium dihydrogen phosphate were coassayed with samples of unknown phosphate contents. After being processed as above, the test tube fractions were transferred to 10-mL volumetric flasks, and water was added to give 10 mL of solution. The UV absorbance at 340 nm was recorded using 3-mL quartz cells. The concentration of the unknown samples was calculated from a standard curve derived from the absorbances of the reference samples.

Compounds containing phosphorothioates were additionally assayed by a modification of the Ellman test[55] for sulfhydryl groups as follows. To 250-μL aliquots of the ion-exchange column fractions was added 1 mL of a buffered solution of Ellman's reagent [100 mL of 10 mM Tris buffer, pH 8, containing 40 mg of 5',5-dithiobis(2-nitrobenzoic acid)]. The fractions containing phosphorothioates were identified by their deep yellow color.

(Diisopropylamino)dichlorophosphine was prepared by the method of Tanaka *et al.*[56] by adding 2 equiv of diisopropylamine to a solution of PCl_3 in dry ether at −78 °C. The crude product (δ_P 166.4) was purified by distillation under reduced pressure, and reaction with 2 equiv of benzyl alcohol in the presence of 2 equiv of triethylamine afforded bis(benzyloxy)(diisopropylamino)phosphine[44] (δ_P 145.24) which could be purified by flash chromatography.

Bis(2-cyanoethoxy)(diisopropylamino)phosphine[40] was synthesized according to the procedure of Bannwarth and Trzeciak[44] and purified by flash chromatography.

1L-(−)-2,3,6-Tri-*O*-benzyl-1,4,5-tris[bis(2-cyanoethoxy)-thiophospho]-*myo*-inositol (11). Bis(2-cyanoethoxy)(diisopropylamino)phosphine (1.27 g, 4.74 mmol) was added to a solution of 1L-(−)-2,3,6-tri-*O*-benzyl-*myo*-inositol **10**[38] (142 mg, 316 μmol) and tetrazole (399 mg, 5.69 mmol) in dichloromethane (15 mL). The mixture was stirred at room temperature for 1 h, after which TLC (chloroform/acetone, 9:1) showed complete conversion of the starting material (R_f 0.24) into a product (R_f 0.38) and ^{31}P-NMR spectroscopy showed three phosphite triester peaks at 141.1, 140.5, and 139.7 ppm. Dry pyridine (5 mL) and sulfur (500 mg, 15.6 mmol) were added, and the suspension was stirred overnight. TLC showed conversion of the triphosphite into a new product (R_f 0.37). The solvent was evaporated, and the residue was chromatographed on silica gel to give **11** (332 mg, 314 μmol, 99%) as a syrup. $[\alpha]^{17}_D$: −15.9° (c = 3.5 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.95–2.41 (4 H, m, CH_2CH_2CN), 2.58–2.62 (2 H, m, CH_2CH_2CN), 2.73–2.82 (6 H, m, CH_2CH_2CN), 3.64 (1 H, dd, J = 9.9, 2.8 Hz, C-3-H), 3.68–3.89 (2 H, m, CH_2-CH_2CN), 3.98–4.55 (14 H, m, CH_2CH_2CN, C-2-H, C-6-H, C-5-H, C-1-H), 4.60, 4.78 (2 H, AB, J = 11.7 Hz, CH_2Ph), 4.88 (2 H, AB, CH_2Ph), 4.81, 4.95 (2 H, AB, J = 11.6 Hz, CH_2Ph), 5.19 (1 H, q, J = 9.5 Hz, C-4-H), 7.34–7.43 (15 H, m, CH_2Ph). ^{13}C NMR ($CDCl_3$, 68 MHz): δ 18.36, 18.52, 18.81, 18.94, 19.23, 19.40, 19.53 (7 t, CH_2CH_2CN), 62.44, 62.63, 62.73, 62.79, 62.86, 62.99, 63.09 (7 t, CH_2CH_2CN), 72.16, 73.92, 75.22 (3 t, CH_2Ph), 74.57, 77.42, 77.78, 78.69, 78.78, 79.63 (6 d, inositol ring C), 116.38, 116.44, 116.60, 116.73, 116.96, 117.03 (6 s, CN), 125.88, 127.24, 127.53, 127.73, 127.83, 127.99, 128.44, 128.60 (8 d, CH_2Ph), 137.17, 137.88, 138.21 (3 s, CH_2Ph). ^{31}P NMR ($CDCl_3$, 36 MHz): δ 66.70, 66.43, 66.25. MS: m/z (+ve ion FAB) 1057 [$(M + H)^+$, 5.4], 91 (100). MS: m/z (−ve ion FAB) 1001 [$(M - CH_2CH_2CN)^-$, 30], 219 (100).

1L-(+)-*myo*-Inositol 1,4,5-Trisphosphorothioate (12). Ammonia was condensed into a three-necked flask at −78 °C. An excess of sodium was added to dry the liquid ammonia, which was then distilled into a second three-necked flask and kept at −78 °C. Sodium was added until the solution remained blue. **11** (150 mg, 142 μmol) was dissolved in dry dioxane (1 mL) and added to the sodium/liquid ammonia mixture. After the reaction mixture was stirred for 15 min, the reaction was quenched until colorless by adding ethanol to the mixture. The ammonia was evaporated, and the crude product was purified by ion-exchange chromatography on DEAE Sephadex A-25 eluting with a gradient of triethylammonium bicarbonate buffer (0.1–1 M), pH 8.0. The triethylammonium salt of **12** eluted at a buffer concentration of *ca.* 800 mM. Yield: 129 μmol (91%). $[\alpha]^{17}_D$: +28° (pH 8, c = 0.5 in H_2O). ^{1}H NMR (D_2O, pH 8; 400 MHz) δ 3.76 (1 H, dd, J = 9.5, 2.8 Hz, C-3-H), 3.98 (1 H, t, J = 9.5 Hz, C-6-H), 4.16–4.34 (2 H, m, C-1,5-H), 4.39 (1 H, t, J = 2.8 Hz, C-2-H), 4.54 (1 H, m, C-4-H). ^{31}P NMR (D_2O; pH 8; 36 MHz): δ 44.82, 44.69, 42.06. MS: m/z (−ve ion FAB) 467 [$(M - H)^-$; 100]. MS: m/z 466.882 $(M - H)^-$ (calcd for $C_6H_{14}O_{12}P_3S_3$, 466.886).

2,4,6-Tris-*O*-(*p*-methoxybenzyl)-*myo*-inositol Orthoformate (15). Sodium hydride (5.33 g of a 60% dispersion, 113 mmol) was added to a stirred solution of **14**[42] (5 g, 26 mmol) in anhydrous DMF (250 mL) at room temperature. The mixture was stirred for 20 min, after which *p*-methoxybenzyl chloride (17.6 mL, 20.36 g, 130 mmol) was added. The suspension was stirred overnight, after which TLC (hexane/ethyl acetate, 1:1) showed a product (R_f 0.41). The reaction was quenched with water (10 mL), the solvents were evaporated, and the residue was partitioned between chloroform (200 mL) and water (50 mL). The organic phase was washed with brine, dried over magnesium sulfate, and evaporated to give a syrup which was chromatographed on silica gel (eluent ether/hexane, 2:1) to give **15** (11.2 g, 20.3 mmol, 78%). Mp: 47–48 °C (from ethanol/water). ^{1}H NMR ($CDCl_3$, 270 MHz): δ 3.76 (3 H, s, OCH_3), 3.79 (6 H, s, 2 OCH_3), 4.00 (1 H, br s, C-5-H), 4.24 (2 H, br s, 2 C-H), 4.29 (2 H, br s, 2 C-H), 4.35–4.54 (5 H, m, 2 CH_2Ph, C-2-H), 4.58 (2 H, s, CH_2Ph), 5.51 (1 H, s, O_3CH), 6.80 (4 H, d, J = 8.4 Hz, arom), 6.83 (2 H, d, J = 8.6 Hz, arom), 7.10 (4 H, d, J = 8.2 Hz, arom), 7.28 (2 H, d, J = 8.4 Hz, arom). ^{13}C NMR ($CDCl_3$, 68 MHz): δ 55.07 (q, OCH_3), 66.65 (d, 2 inositol ring C), 68.08 (d, inositol ring C), 70.48 (d, 2 inositol ring C), 71.00 (t, CH_2PhOMe), 73.66 (d, inositol ring C), 103.08 (d, O_3CH), 113.62 (d, 6 *Ph*OMe C), 129.12 (d, 4 *Ph*OMe C), 129.58 (d, 2 *Ph*OMe C), 134.57 (s, 3 *Ph*OMe C), 159.16 (s, 3 *Ph*OMe C). MS: m/z (+ve ion FAB) 551 [$(M + H)^+$, 2], 429 [$(M - CH_2C_6H_4OCH_3)^+$, 6], 121 [$(CH_2C_6H_4OCH_3)^+$, 100]. MS: m/z (−ve ion FAB) 703 [$(M + NBA)^-$, 25], 535 (30), 322 (55), 188 (100). Anal. ($C_{31}H_{34}O_9$) C, H.

2,4,6-Tris-*O*-(*p*-methoxybenzyl)-*myo*-inositol (16). **15** (5.6 g, 10.2 mmol) was heated under reflux in a mixture of 2 M HCl (10 mL) and methanol (200 mL) for 30 min, when TLC (hexane/ethyl acetate, 1:2) showed conversion of the starting material (R_f 0.67) into a product (R_f 0.35). The solution was cooled to room temperature and adjusted to pH 8 with aqueous ammonia. The solvents were evaporated, and the residue was extracted with ethyl acetate (2 × 200 mL). The combined extracts were evaporated and the crude product was chromatographed on silica gel using ether → ether/ethyl acetate (1:1) as eluent to give **16** (4.7 g, 8.7 mmol, 85%). Mp: 110–112 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$, 270 MHz): δ 2.45 (2 H, d, J = 5.7 Hz, D_2O ex, 2 OH), 2.66 (1 H, br s, D_2O ex, OH), 3.40–3.55 (3 H, m, C-1,3,5-H), 3.61 (2 H, t, J = 9.25 Hz, C-4,6-H), 3.77 (6 H, s, 2 OCH_3), 3.80 (3 H, s, OCH_3), 3.90 (1 H, t, J = 3.0 Hz, C-2-H), 4.73 (2 H, s, CH_2Ph), 4.76 (4 H, s, 2 CH_2Ph), 6.87 (4 H, d, J = 8.6 Hz, CH_2PhOMe), 6.89 (2 H, d, J = 8.6 Hz, CH_2PhOMe), 7.24 (2 H, d, J = 8.6 Hz, CH_2PhOMe), 7.29 (4 H, d, J = 8.6 Hz, CH_2PhOMe). ^{13}C NMR ($CDCl_3$, 68 MHz): δ 55.14 (q, OCH_3), 72.33 (d, 2 C, inositol ring C), 74.47 (t, CH_2Ph), 74.79, 78.95 (2 d, 2 C, inositol ring C), 81.48 (d, 2 C, inositol ring C), 113.78 (d, 2 *Ph*OMe C), 113.85 (d, 4 *Ph*OMe C), 129.45 (d, 2 *Ph*OMe C), 129.61 (d, 4 *Ph*OMe C), 130.58 (s, *Ph*OMe), 159.22 (s, *Ph*OMe). MS: m/z (+ve ion FAB) 539 [$(M - H)^+$, 1.5], 419 (5), 121 [$(CH_2C_6H_4$-$OCH_3)^+$, 100]. MS: m/z (−ve ion FAB) 706 (36), 693 [$(M + NBA)^-$, 57], 539 [$(M - H)^-$, 100], 419 [$(M - CH_2C_6H_4OCH_3)^-$, 38]. Anal. ($C_{30}H_{36}O_9$) C, H.

2,4,6-Tris-*O*-(*p*-methoxybenzyl)-1,3,5-tris[bis(benzyloxy)-thiophospho]-*myo*-inositol (17). A mixture of **16** (541 mg, 1 mmol) and tetrazole (1.26 g, 18 mmol) was stirred at room temperature in dichloromethane (30 mL). Bis(benzyloxy)-

(diisopropylamino)phosphine (6.2 g, 15 mmol) was added and stirring continued for 1 h, when ^{31}P NMR showed two signals at 141.7 and 140.3 ppm integrating for 1 and 2 P, respectively. Pyridine (5 mL) and sulfur (500 mg) were added and stirring continued overnight. TLC (ethyl acetate/hexane, 2:1) showed one product (R_f 0.81). The solution was partitioned between chloroform and saturated aqueous $NaHCO_3$, the organic phase was dried over magnesium sulfate, and the solvents were evaporated. The residue was chromatographed on silica gel to give **17** (835 mg, 61%) as a syrup. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 3.70 (6 H, 2 s, OCH_3), 3.76 (3 H, s, OCH_3), 4.07 (2 H, t, J = 9.5 Hz, C-4,6-H), 4.43 (2 H, td, J = 10.3, 2.2 Hz, C-1,3-H), 4.60–5.10 (20 H, m, 9 CH_2Ph, C-2-H, C-5-H), 6.71 (4 H, d, J = 8.6 Hz, CH_2PhOMe), 6.80 (2 H, d, J = 8.8 Hz, CH_2PhOMe), 6.98 (4 H, dd, J = 7.6, 1.9 Hz, CH_2PhOMe), 7.11–7.35 (32 H, m, CH_2PhOMe and CH_2Ph). ^{13}C NMR ($CDCl_3$, 68 MHz): δ 54.16 (q, OCH_3), 68.56, 68.73, 68.89, 69.02, 72.71, 74.14 (6 t, CH_2Ph), 76.12 (d, 1 inositol ring C), 76.54 (d, 2 inositol ring C), 76.70 (d, 2 inositol ring C), 78.91 (d, 1 inositol ring C), 112.38 (d, 4 *Ph*OMe C), 112.54 (d, 2 *Ph*OMe C), 126.89, 126.95, 127.21, 127.27, 127.34, 127.43, 127.56, 127.66, 127.76, 128.05 (10 d, CH_2Ph and *Ph*OMe), 129.35 (s, 2 *Ph*OMe C), 129.67 (s, 1 *Ph*OMe C), 134.54, 134.67, 134.80, 134.93 (4 s, CH_2Ph), 157.73 (s, *Ph*OMe C). ^{31}P NMR ($CDCl_3$, 36 MHz): δ 68.1 (1 P), 66.8 (2 P). MS: m/z (+ve ion FAB) 1370 [$(M + H)^+$, 0.17], 1247 (0.3), 121 [$(CH_2C_6H_4OCH_3)^+$, 100], 91 [$(CH_2C_6H_5)^+$, 37]. MS: m/z (−ve ion FAB) 1277 [$(M - C_7H_7)^-$, 10], 293 [$(OP(S)(OCH_2C_6H_5)_2^-$, 100], 95 (55). MS: m/z 1370.360 $(M + H)^+$ (calcd for $C_{72}H_{77}O_{15}P_3S_3$, 1370.364).

***myo*-Inositol 1,3,5-Trisphosphorothioate (18).** Ammonia was condensed into a three-necked flask at −78 °C. An excess of sodium was added to dry the liquid ammonia, which was then distilled into a second three-necked flask and kept at −78 °C. Sodium was added until the solution remained blue. Compound **17** (237 mg, 173 μmol) was dissolved in dry dioxane (2 mL) and added to the sodium in liquid ammonia. After the reaction mixture was stirred for 15 min, the reaction was quenched with ethanol. Ammonia and ethanol were evaporated, and the crude product was purified by ion-exchange chromatography on DEAE Sepharose eluting with a gradient of triethylammonium bicarbonate buffer (0.1–1 M), pH 8.0. The triethylammonium salt of **18** eluted between 450 and 530 mM. Yield: 157 μmol (91%). ^{1}H NMR (D_2O, 270 MHz): δ 3.87 (2 H, t, J = 9.3 Hz, C-4-H, C-6-H), 4.08 (1 H, dt, J = 10.3, 9.2 Hz, C-5-H), 4.19 (2 H, td, J = 10.3, 2.5 Hz, C-1-H, C-3-H), 4.62 (1 H, br s, C-2-H). ^{13}C NMR (D_2O, 68 MHz): δ 69.63 (1 C, d, C-2), 70.83 (2 C, d, C-4, C-6), 74.29 (2 C, dd, J_{C-O-P} = 6.6 Hz, C-1, C-3), 78.41 (1 C, dd, J_{C-O-P} = 6.6 Hz, C-5). ^{31}P NMR (D_2O, 36 MHz): δ 47.74 (1 P, d, J = 10.1 Hz, 5-P), 46.55 (2 P, d, J = 10.1 Hz, 1-P, 3-P). MS: m/z (−ve ion FAB) 466 [100, $(M - H)^-$], 432 (15), 238 (15), 113 (17), 95 [$(PSO_2)^-$, 40]. MS: m/z 466.886 $(M - H)^-$ (calcd for $C_6H_{14}O_{12}P_3S_3$, 466.886).

1L-(−)-2,3,5-Tri-*O*-benzyl-1,4,6-tris[bis(2-cyanoethoxy)-phospho]-*chiro*-inositol (22). To a mixture of L-2,3,5-tri-*O*-benzyl-*chiro*-inositol **21** (45 mg, 0.1 mmol) and 1*H*-tetrazole (63 mg, 0.9 mmol) in dry CH_2Cl_2 (3 mL) was added bis(2-cyanoethoxy)(diisopropylamino)phosphine (200 mg, 0.9 mmol). The mixture was stirred at room temperature for 1 h. t-BuOOH in water (60%) (0.5 mL) was added, and the resulting solution was stirred overnight, washed with saturated aqueous $NaHCO_3$ (10 mL), dried over $MgSO_4$, and concentrated. Flash column chromatography of the residue gave **22** (76 mg, 75 μmol, 75%) as an oil. $[\alpha]^{20}_D$: −22° (c = 3.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.35–2.53 (2 H, m, CH_2), 2.60–2.80 (10 H, m, CH_2), 3.87 (1H, t, J = 9.5 Hz, CH), 3.90–4.25 (15 H, m, 6 × CH_2, 3 × CH), 4.67, 5.07 (2 H, AB q, J = 10.1 Hz, CH_2Ph), 4.77, 4.78 (2 H, AB q, J = 11.1 Hz, CH_2Ph), 4.87, 4.92 (2 H, AB q, J = 11.3 Hz, CH_2Ph), 5.05–5.15 (2 H, m, CH), 7.28–7.49 (15 H, m, CH_2Ph). ^{31}P NMR (D_2O, 36 MHz): δ −3.43, −3.07, and −2.44. MS: m/z (+ve ion FAB) 1009 [$(M + H)^+$, 1], 917 (1), 372 (1), 181 (10), 144 (6), 91 (100).

1L-(−)-*chiro*-Inositol 1,4,6-Trisphosphate (8). To liquid ammonia (40 mL) was added a solution of **22** (70 mg, 69 μmol) in dry dioxane (2 mL), followed by Na (0.1 g, 4.3 mmol) in small pieces. The solution was stirred for 5 min, the reaction was quenched with EtOH, and the ammonia was evaporated in a stream of N_2. Ion-exchange chromatography of the residue on DEAE Sephadex A-25, using a gradient from 0 to 1 M TEAB (pH 8) gave **8** (52 μmol, 75%). $[\alpha]^{20}_D$: −11° (c = 0.5 in H_2O, pH 8). ^{1}H NMR (D_2O, 400 MHz): δ 3.72 (1 H, t, J = 9.7 Hz, CH), 3.81 (1 H, dd, J = 2.6, 9.8 Hz, CH), 3.83 (1 H, dd, J = 2.3, 9.6 Hz, CH), 4.05 (1 H, q, J = 8.3 Hz, CH), 4.39–4.47 (2 H, m, 2 CH). ^{31}P NMR (D_2O, 36 MHz): δ 1.89, 2.65, 3.31. MS: m/z (−ve ion FAB) 419 [$(M - H)^-$, 12], 177 (6), 159 (7), 97 (7). MS: m/z 418.957 $(M - H)^-$ (calcd for $C_6H_{14}O_{15}P_3$, 418.955).

1L-(−)-2,3,5-Tri-*O*-benzyl-1,4,6-tris[bis(2-cyanoethoxy)-thiophospho]-*chiro*-inositol (23). To a mixture of **21** (90 mg, 0.2 mmol) and 1*H*-tetrazole (0.25 g, 3.6 mmol) in dry CH_2Cl_2 (5 mL) was added bis(2-cyanoethoxy)(diisopropylamino)phosphine (0.53 g, 2.4 mmol). The mixture was stirred at room temperature for 1 h, and the solvent was evaporated *in vacuo*. Dry pyridine (5 mL) was added to the residue, followed by sulfur (0.1 g), and the resulting mixture was stirred at room temperature overnight. The pyridine was evaporated. Flash column chromatography of the residue gave **23** (0.19 g, 90%). $[\alpha]^{20}_D$: −25° (c = 3.0 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.35–2.54 (4 H, m, CH_2), 2.62–2.82 (8 H, m, CH_2), 3.89 (1 H, t, J = 9.2 Hz, CH), 3.90–4.35 (15 H, m, 6 × CH_2 and 3 × CH), 4.67, 5.05 (2 H, ABq, J = 11.4 Hz, CH_2Ph), 4.70, 4.76 (2 H, AB q, J = 10.8 Hz, CH_2Ph), 4.82, 4.89 (2 H, ABq, J = 11.2 Hz, CH_2Ph), 5.08–5.23 (2 H, m, CH), 7.26–7.47 (15 H, CH_2Ph). ^{31}P NMR ($CDCl_3$, 36 MHz): δ 68.39, 67.49, 67.26. MS: m/z (+ve ion FAB) 1057 [$(M + H)^+$, 2], 965 (1), 437 (1), 181 (5), 91 (100).

1L-(−)-*chiro*-Inositol 1,4,6-Trisphosphorothioate (24). To liquid ammonia (40 mL) was added a solution of **23** (60 mg, 57 μmol) in dry dioxane (2 mL), followed by Na (0.1 g, 4.3 mmol) in small pieces. The solution was stirred for 5 min, the reaction was quenched with EtOH, and the ammonia was evaporated in a stream of N_2. Ion-exchange chromatography of the residue on DEAE Sephadex A-25, using a gradient from 0 to 1 M TEAB (pH 8), gave **24** (40 μmol, 70%). $[\alpha]^{20}_D$: −11° (c = 0.5 in H_2O, pH 8). ^{1}H NMR (D_2O, 400 MHz): δ 3.77 (1 H, t, J = 9.2 Hz, CH), 3.89–3.92 (1 H, m, CH), 3.94–3.96 (1 H, m, CH), 4.28–4.76 (3 H, m, 3 CH). ^{31}P NMR (D_2O, 36 MHz): δ 44.22, 44.49, and 45.09. MS: m/z (−ve ion FAB) 467 [$(M - H)^-$, 100], 451 (20), 433 (27), 211 (30), 113 (28), 95 (31). MS: m/z 466.887 $(M - H)^-$ (calcd for $C_6H_{14}O_{12}P_3S_3$, 466.886).

Acknowledgment. We thank the SERC (Molecular Recognition Initiative) and The Wellcome Trust (Prize Fellowship to D.L.) for financial support. B.V.L.P. is a Lister Institute Fellow.

References

(1) Berridge, M. J. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu. Rev. Biochem.* **1987**, *56*, 159–193.
(2) Berridge, M. J. Inositol trisphosphate and calcium signalling. *Nature (London)* **1993**, *361*, 315–325.
(3) Furuichi, T.; Yoshikawa, S.; Miyawaki, A.; Wada, K.; Maeda, N.; Mikoshiba, K. Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P_{400}. *Nature (London)* **1989**, *342*, 32–38.
(4) Mignery, G. A.; Newton, C. L.; Archer, B. T., III; Südhof, T. C. Structure and Expression of the Rat Inositol 1,4,5-Trisphosphate Receptor. *J. Biol. Chem.* **1990**, *265*, 12679–12685.
(5) Ferris, C. D.; Huganir, R. L.; Supattapone, S.; Snyder, S. H. Purified inositol 1,4,5-trisphosphate receptor mediates calcium flux in reconstituted lipid vesicles. *Nature (London)* **1989**, *342*, 87–89.
(6) Billington, D. C. Recent Developments in the Synthesis of *myo*-Inositol Phosphates. *Chem. Soc. Rev.* **1989**, *18*, 83–122.
(7) Potter, B. V. L. Recent Advances in the Chemistry and Biochemistry of Inositol Phosphates of Biological Interest. *Nat. Prod. Rep.* **1990**, *7*, 1–24.
(8) *Inositol Phosphates and Derivatives - Synthesis, Biochemistry and Therapeutic Potential*; Reitz, A. B., Ed.; American Chemical Society: Washington, DC, 1991.
(9) Potter, B. V. L. Synthetic Second Messenger Analogues. In *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven Press Ltd.: New York, 1993; pp 383–416.
(10) Hirata, M.; Yanaga, F.; Koga, T.; Ogasawara, T.; Watanabe, Y.; Ozaki, S. Stereospecific Recognition of Inositol 1,4,5-Trisphosphate Analogs by the Phosphatase, Kinase, and Binding Proteins. *J. Biol. Chem.* **1990**, *265*, 8404–8407.
(11) Hirata, M.; Watanabe, Y.; Ishimatsu, T.; Ikebe, T.; Kimura, Y.; Yamaguchi, K.; Ozaki, S.; Koga, T. Synthetic Inositol Trisphosphate Analogs and Their Effects on Phosphatase, Kinase, and the Release of Ca^{2+}. *J. Biol. Chem.* **1989**, *264*, 20303–20308.
(12) Sawyer, D. A.; Potter, B. V. L. Total Synthesis of Fluorinated Analogues of Inositol and Inositol 1,4,5-Trisphosphate. *J. Chem. Soc., Perkin Trans. 1* **1992**, 923–932.

(13) Safrany, S. T.; Sawyer, D. A.; Nahorski, S. R.; Potter, B. V. L. Synthetic D- and L-Enantiomers of 2,2-Difluoro-2-deoxy-*myo*-inositol 1,4,5-Trisphosphate Interact Differently With *myo*-inositol 1,4,5-Trisphosphate Binding Proteins: Identification of a Potent Small Molecule 3-Kinase Inhibitor. *Chirality* **1992**, *4*, 415–422.
(14) Seewald, M. J.; Aksoy, I. A.; Powis, G.; Fauq, A. H.; Kozikowski, A. P. Synthesis of D-3-Deoxy-*myo*-Inositol 1,4,5-Trisphosphate and its Effect on Ca^{2+} Release in NIH 3T3 cells. *J. Chem. Soc., Chem. Commun.* **1990**, 1638–1639.
(15) Ley, S. V.; Parra, M.; Redgrave, A. J.; Sternfeld, F. Microbial oxidation in synthesis: preparation of *myo*-inositol phosphates and related cyclitol derivatives from benzene. *Tetrahedron* **1990**, *46*, 4995–5026.
(16) Safrany, S. T.; Wojcikiewicz, R. J. H.; Strupish, J.; Nahorski, S. R.; Dubreuil, D.; Cleophax, J.; Gero, S. D.; Potter, B. V. L. Interaction of synthetic D-6-deoxy-*myo*-inositol 1,4,5-trisphosphate with the Ca^{2+}-releasing D-*myo*-inositol 1,4,5-trisphosphate receptor, and the metabolic enzymes 5-phosphatase and 3-kinase. *FEBS Lett.* **1991**, *278*, 252–256.
(17) Falck, J. R.; Abdali, A.; Wittenberger, S. J. Total Synthesis of the 5-Methylenephosphate Analogue of D-*myo*-Inositol 1,4,5-Trisphosphate. *J. Chem. Soc., Chem. Commun.* **1990**, 953–955.
(18) Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Nahorski, S. R.; Wilcox, R. A. Synthesis of 1D-3-Deoxy-, 1D-2,3-Dideoxy-, and 1D-2,3,6-Trideoxy-*myo*-inositol 1,4,5-Trisphosphate from Quebrachitol, Their Binding Affinities, and Calcium Release Activity. *J. Am. Chem. Soc.* **1993**, *115*, 4429–4434.
(19) Nahorski, S. R.; Potter, B. V. L. Molecular recognition of inositol polyphosphates by intracellular receptors and metabolic enzymes. *Trends Pharmacol. Sci.* **1989**, *10*, 139–144.
(20) Cooke, A. M.; Gigg, R.; Potter, B. V. L. *myo*-Inositol 1,4,5-Trisphosphorothioate: A Novel Analogue of a Biological Second Messenger. *J. Chem. Soc., Chem. Commun.* **1987**, 1525–1526.
(21) Safrany, S. T.; Wojcikiewicz, R. J. H.; Strupish, J.; McBain, J.; Cooke, A. M.; Potter, B. V. L.; Nahorski, S. R. Synthetic Phosphorothioate-Containing Analogues of Inositol 1,4,5-Trisphosphate Mobilize Intracellular Ca^{2+} Stores and Interact Differentially with Inositol 1,4,5-Trisphosphate 5-Phosphatase and 3-Kinase. *Mol. Pharmacol.* **1991**, *39*, 754–761.
(22) Cooke, A. M.; Nahorski, S. R.; Potter, B. V. L. *myo*-Inositol 1,4,5-trisphosphorothioate is a potent competitive inhibitor of human erythrocyte 5-phosphatase. *FEBS Lett.* **1989**, *242*, 373–377.
(23) Noble, N. J.; Cooke, A. M.; Potter, B. V. L. Synthesis of (±)-*myo*-inositol 1,4,5-trisphosphate and the novel analogue (±)-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate. *Carbohydr. Res.* **1992**, *234*, 177–187.
(24) Lampe, D.; Mills, S. J.; Potter, B. V. L. Total Synthesis of The Second Messenger Analogue D-*myo*-Inositol 1-Phosphorothioate 4,5-Bisphosphate: Optical Resolution of DL-1-*O*-Allyl-2,3,6-tri-*O*-Benzyl-*myo*-inositol and Fluorescent Labelling of *myo*-Inositol 1,4,5-Trisphosphate. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2899–2906.
(25) Noble, N. J.; Dubreuil, D.; Potter, B. V. L. Total synthesis of *myo*-inositol-1-phosphate-4,5-pyrophosphate, a novel second messenger analogue, via *myo*-inositol-1-phosphate-4,5-bisphosphorothioate. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 471–476.
(26) Wakui, M.; Potter, B. V. L.; Petersen, O. H. Pulsatile intracellular calcium release does not depend on fluctuations in inositol trisphosphate concentration. *Nature (London)* **1989**, *339*, 317–320.
(27) Potter, B. V. L. Synthesis and Biology of Inositol Polyphosphates and Analogues. In *Trends in Drug Research, Proceedings of the 9th Noordwijkerhoot-Camerino Symposium*; Claasen, V., Ed.; Elsevier: Amsterdam, 1993; Vol. 20, pp 185–214.
(28) Dreef, C. E.; Mayr, G. W.; Jansze, J.-P.; Roelen, H. C. P. F.; van der Marel, G. A.; van Boom, J. H. An expeditious synthesis of biologically important *myo*-inositol phosphorothioates. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 239–242.
(29) Schultz, C.; Metschies, T.; Jastorff, B. Synthesis of thiophosphate analogues of DL-*myo*-inositol 1.2-cyclic phosphate. *Tetrahedron Lett.* **1988**, *29*, 3919–3920.
(30) Metschies, T.; Schultz, C.; Jastorff, B. Synthesis of DL-*myo*-inositol 1-phosphate and its thiophosphate analogue. *Tetrahedron Lett.* **1988**, *29*, 3921–3922.
(31) Lin, G.; Tsai, M. D. Phospholipids Chiral at Phosphorus. 18. Stereochemistry of Phosphatidylinositide-Specific Phospholipase C. *J. Am. Chem. Soc.* **1989**, *111*, 3099–3101.
(32) Shears, S. B. Metabolism of the inositol phosphates produced upon receptor activation. *Biochem. J.* **1989**, *260*, 313–324.
(33) Liu, C.; Safrany, S. T.; Nahorski, S. R.; Potter, B. V. L. Synthesis of L-*chiro*-inositol 1,4,6-trisphosphorothioate, a potent and selective inhibitor of *myo*-inositol 1,4,5-trisphosphate 5-phosphatase. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1523–1528.
(34) Polokoff, M. A.; Bencen, G. H.; Vacca, J. P.; deSolms, S. J.; Young, S. D.; Huff, J. R. Metabolism of Synthetic Inositol Trisphosphate Analogs. *J. Biol. Chem.* **1988**, *263*, 11922–11927.
(35) Downes, C. P.; Mussat, M. C.; Michell, R. H. The inositol trisphosphate phosphomonoesterase of the human erythrocyte membrane. *Biochem. J.* **1982**, *203*, 169–177.
(36) Strupish, J.; Cooke, A. M.; Potter, B. V. L.; Gigg, R.; Nahorski, S. R. Stereospecific mobilisation of intracellular Ca^{2+} by inositol 1,4,5-trisphosphate - Comparison with inositol 1,4,5-trisphosphorothioate and inositol 1,3,4-trisphosphate. *Biochem. J.* **1988**, *253*, 901–905.
(37) Taylor, C. W.; Berridge, M. J.; Brown, K. D.; Cooke, A. M.; Potter, B. V. L. DL-*myo*-Inositol 1,4,5-trisphosphorothioate mobilizes intracellular calcium in Swiss 3T3 cells and *Xenopus* oocytes. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 626–632.
(38) Desai, T.; Fernandez-Mayoralas, A.; Gigg, J.; Gigg, R.; Jaramillo, C.; Payne, S.; Penades, S.; Schnetz, N. Preparation Of Optically Active *myo*-Inositol Derivatives as Intermediates for the Synthesis of Inositol Phosphates. In *Inositol Phosphates and Derivatives–Synthesis, Biochemistry, and Therapeutic Potential*; Reitz, A. B., Ed.; American Chemical Society: Washington, DC, 1991; pp 86–102.
(39) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. The Allyl Group for Protection in Carbohydrate Chemistry. Part 20. Synthesis of 1L-*O*-Methyl-*myo*-inositol [(+)-Bornesitol] by Resolution of (±)-1,2,4-Tri-*O*-benzyl-*myo*-inositol. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1757–1762.
(40) Uhlmann, E.; Engles, J. Chemical 5'-phosphorylation of oligonucleotides valuable in automated DNA synthesis. *Tetrahedron Lett.* **1986**, *27*, 1023–1026.
(41) Briggs, A. P. A modification of the Bell-Doisy phosphate method. *J. Biol. Chem.* **1922**, *53*, 13–16.
(42) Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M.; Vacca, J. P.; DeSolms, S. J.; Huff, J. R. The Total Synthesis of *myo*-Inositol Phosphates *via myo*-Inositol Orthoformate. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1423–1429.
(43) Lee, H. W.; Kishi, Y. Synthesis of mono and unsymmetrical bis ortho esters of *scyllo*-inositol. *J. Org. Chem.* **1985**, *50*, 4402–4404.
(44) Bannwarth, W.; Trzeciak, A. A Simple and Effective Chemical Phosphorylation Procedure for Biomolecules. *Helv. Chim. Acta* **1987**, *70*, 175–186.
(45) Liu, C.; Nahorski, S. R.; Potter, B. V. L. Synthesis from quebrachitol of 1L-*chiro*-inositol 2,3,5-trisphosphate, an inhibitor of the enzymes of 1D-*myo*-inositol 1,4,5-trisphosphate metabolism. *Carbohydr. Res.* **1992**, *234*, 107–115.
(46) Carless, H. A. J.; Busia, K. Total synthesis of *chiro*-inositol 2,3,5-trisphosphate: a *myo*-inositol 1,4,5-trisphosphate analogue from benzene *via* photo-oxidation. *Tetrahedron Lett.* **1990**, *31*, 1617–1620.
(47) Carless, H. A. J.; Busia, K. Synthesis of racemic 6-deoxy-6-fluoro-*chiro*-inositol 2,3,5-trisphosphate. *Carbohydr. Res.* **1992**, *234*, 207–215.
(48) Safrany, S. T.; Wilcox, R. A.; Liu, C.; Potter, B. V. L.; Nahorski, S. R. 3-Position modification of myo-inositol 1,4,5-trisphosphate: consequences for intracellular Ca^{2+} mobilisation and enzyme recognition. *Eur. J. Pharmacol.* **1992**, *226*, 265–272.
(49) Safrany, S. T. Phosphoinositide Signalling in a Neuroblastoma Cell Line. Ph.D. Thesis, University of Leicester, 1993.
(50) Kozikowski, A. P.; Ognyanov, V. I.; Chen, C.; Fauq, A. H.; Safrany, S. T.; Wilcox, R. A.; Nahorski, S. R. Deoxygenated Inositol 1,4,5-Trisphosphate Analogues and Their Interaction with Metabolic Enzymes. (1*R*,2*R*,4*R*)-Cyclohexane-1,2,4-tris(methylenesulfonate): A Potent Selective 5-Phosphatase Inhibitor. *J. Med. Chem.* **1993**, *36*, 3035–3038.
(51) Jaffe, E. K.; Cohn, M. ^{31}P Nuclear Magnetic Resonance Spectra of the Trisphosphate Analogues of Adenine Nucleotides; Effect of pH and Mg^{2+} Binding. *Biochemistry* **1978**, *17*, 652–657.
(52) Attree, O.; Olivios, I. M.; Okabe, I.; Bailey, L. C.; Nelson, D. L.; Lewis, R. A.; McInnes, R. R.; Nussbaum, R. L. The Lowe's oculocerebrorenal syndrome gene encodes a protein highly homologous to inositol polyphosphate 5-phosphatase. *Nature (London)* **1992**, *358*, 239–242.
(53) Nye, K. E.; Riley, G. A.; Pinching, A. J. The defect seen in the phosphatidyl-inositol hydrolysis pathway in HIV-infected lymphocytes and lymphoblastoid cells is due to inhibition of the inositol 1,4,5-trisphosphate 1,3,4,5-tetrakisphosphate 5-phosphomonoesterase. *Clin. Exp. Immunol.* **1992**, *89*, 89–93.
(54) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(55) Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.
(56) Tanaka, T.; Tamatsukuri, S.; Ikehara, M. New approach to the synthesis of deoxynucleoside-phosphoramide derivatives. *Tetrahedron Lett.* **1986**, *27*, 199–202.