

# Synthesis of Selective Non-Ca<sup>2+</sup>-Mobilizing Inhibitors of D-*myo*-Inositol 1,4,5-Trisphosphate 5-Phosphatase

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Syntheses of novel phosphorothioate-based non-Ca<sup>2+</sup>-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<sup>2+</sup> but were highly potent inhibitors of Ins(1,4,5)P<sub>3</sub> 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<sub>3</sub>], 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.<sup>1,2</sup> The gene coding for the Ins(1,4,5)P<sub>3</sub> receptor has now been cloned,<sup>3,4</sup> and the ability of this transmembrane protein to gate calcium in response to Ins(1,4,5)P<sub>3</sub> has been demonstrated.<sup>5</sup> Realization of the fundamental cellular role played by Ins(1,4,5)P<sub>3</sub> and the acceptance of the polyphosphoinositide signal transduction mechanism has led to a massive increase in biological<sup>1,2</sup> and, more recently, chemical<sup>6,7</sup> 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<sub>3</sub> has now been synthesized by many groups.<sup>6-8</sup> 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<sub>3</sub> analogues have now been synthesized, but only few have been shown to be biologically potent, and many have not yet been evaluated.<sup>6-8</sup> Reports on analogues specifically ring-modified at the 2-position,<sup>10-13</sup> the 3-position,<sup>14</sup> and the 6-position<sup>15,16</sup> and on the synthesis of an active 5-methylene phosphonate analogue<sup>17</sup> have appeared as well as details of multiple modifications.<sup>18</sup> We have reasoned that, by virtue of their metabolic stability, phosphorothioate analogues (Figure 1) will prove to be of significant importance in this field.<sup>9,19</sup> We previously reported the first synthesis of *myo*-inositol 1,4,5-trisphosphorothioate 2 [Ins(1,4,5)PS<sub>3</sub>],<sup>20</sup> a potent inhibitor of human erythrocyte membrane Ins(1,4,5)P<sub>3</sub> 5-phosphatase,<sup>21,22</sup> and the specifically modified 5-phosphorothioate analogues 3 [Ins(1,4,5)P<sub>3</sub>-5S],<sup>23</sup> 1-phosphorothioate 4,5-bisphosphate 4,<sup>24</sup> and 1-phosphate 4,5-bisphosphorothioate 5.<sup>25</sup> These highly potent Ca<sup>2+</sup>-mobilizing analogues are already finding numerous biological applications.<sup>7,9,19,26,27</sup> Phosphorothioate analogues have also been synthesized by other groups.<sup>28-31</sup>

There is a great need for synthetic small molecule tools

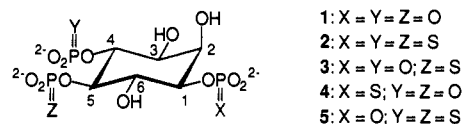


Figure 1. Structures of Ins(1,4,5)P<sub>3</sub> and phosphorothioate analogues.

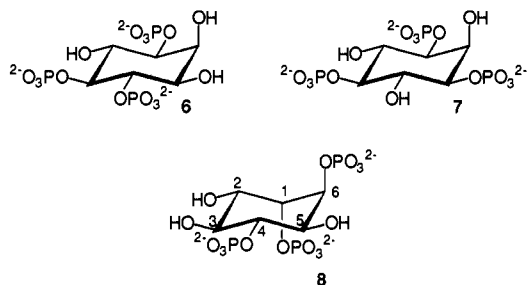
for pharmacological intervention in the polyphosphoinositide pathway of cellular signaling.<sup>9,27</sup> This is especially pertinent in relation to the highly complex metabolism of inositol polyphosphates.<sup>32</sup> Inhibitors of the metabolic enzymes Ins(1,4,5)P<sub>3</sub> 5-phosphatase and 3-kinase 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<sub>3</sub> 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<sup>2+</sup>, by improving the binding properties of compounds known to be moderately potent, non-Ca<sup>2+</sup>-mobilizing inhibitors. We report here the synthesis of three such highly potent and novel Ins(1,4,5)P<sub>3</sub> 5-phosphatase inhibitors. Part of this work has been published in preliminary form.<sup>33</sup>

## Results and Discussion

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

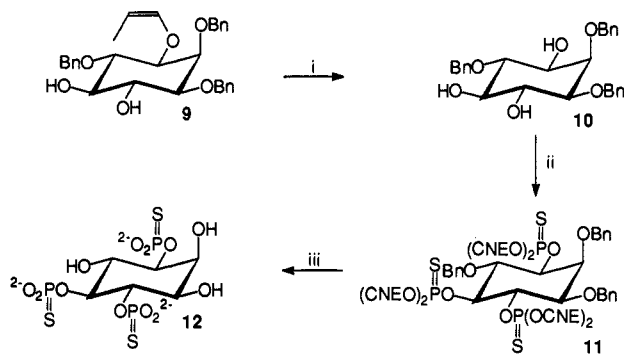
The few structure-activity studies which have been performed show that the vicinal 4,5-bisphosphate moiety of Ins(1,4,5)P<sub>3</sub> is essential for Ca<sup>2+</sup>-releasing activity,<sup>6,7,19,27</sup> the 1-phosphate group being thought to provide enhanced affinity for the receptor. Cooke *et al.*<sup>22</sup> first observed that

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**Figure 2.** Structures of non- $\text{Ca}^{2+}$ -mobilizing weak  $\text{Ins}(1,4,5)\text{P}_3$  5-phosphatase inhibitors.

#### Scheme 1<sup>a</sup>



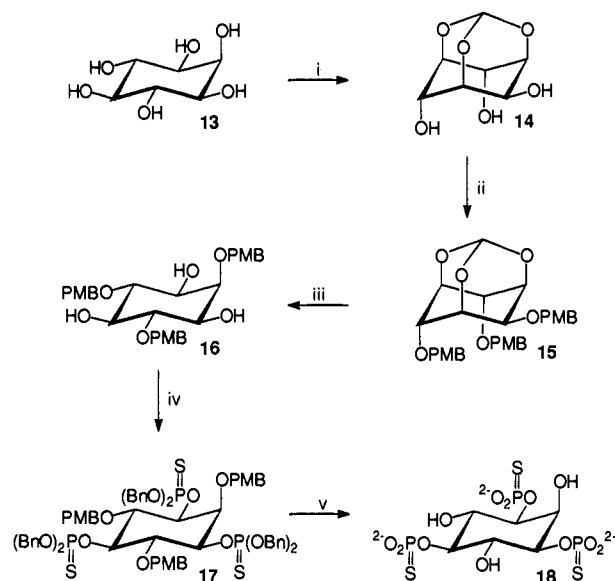
<sup>a</sup> Reagents and conditions: (i)  $\text{H}^+$ ; (ii)  $(\text{NCCH}_2\text{CH}_2\text{O})_2\text{PNPr}^+_2/1\text{H-tetrazole}$ , then  $\text{S}_8/\text{pyridine}$ ; (iii)  $\text{Na}/\text{liquid NH}_3$ . Bn, benzyl; CNE, 2-cyanoethyl.

the trisphosphorothioate analogue of  $\text{Ins}(1,4,5)\text{P}_3$ ,  $\text{Ins}(1,4,5)\text{PS}_3$  **2** (Figure 1), acted as a potent competitive inhibitor of human erythrocyte membrane  $\text{Ins}(1,4,5)\text{P}_3$  5-phosphatase, and subsequently  $\text{Ins}(1,4,5)\text{PS}_3$  was found to have a  $K_i$  of  $1.7 \mu\text{M}$ .<sup>21</sup> Thus, substitution of phosphate groups by phosphorothioates apparently results in increased binding properties of the analogue [ $K_m$  for  $\text{Ins}(1,4,5)\text{P}_3$ ,  $40 \mu\text{M}$ ].  $\text{Ins}(1,4,5)\text{PS}_3$ , however, mobilizes intracellular  $\text{Ca}^{2+}$ ,<sup>36,37</sup> and this was considered to disadvantage this compound as a specific  $\text{Ins}(1,4,5)\text{P}_3$  5-phosphatase inhibitor.

Considering the effect of phosphorothioate replacement on  $\text{Ins}(1,4,5)\text{P}_3$ , we reasoned that phosphorothioate analogues of  $\text{L-Ins}(1,4,5)\text{P}_3$  **6** and  $\text{Ins}(1,3,5)\text{P}_3$  **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-chr-Ins*(1,4,6) $\text{P}_3$ ] **8** (Figure 2) was a weak inhibitor of 5-phosphatase,<sup>27,33</sup> it also seemed appropriate to synthesize the corresponding phosphorothioate of this non *myo*-inositol based analogue.

*L-Ins*(1,4,5) $\text{P}_3$  **6** is a weak  $\text{Ins}(1,4,5)\text{P}_3$  5-phosphatase inhibitor<sup>22,34</sup> and is only a very weak mobilizer of intracellular  $\text{Ca}^{2+}$ . In order to synthesize *L-myoinositol* 1,4,5-trisphosphorothioate (Scheme 1), the *L*-enantiomer of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol<sup>38</sup> was isomerized to the corresponding 1-*O*-(*cis*-prop-1-enyl) derivative **9**<sup>24</sup> and was deprotected to give 1D-1,2,4-tri-*O*-benzyl-*myo*-inositol **10**.<sup>39</sup> The melting point ( $116\text{--}118^\circ\text{C}$ ) and optical rotation ( $[\alpha]^{18}_D = -10.1^\circ$ ) measured for this previously described triol were in good agreement with the reported values (mp  $118\text{--}120^\circ\text{C}$ ;  $[\alpha]^{18}_D = -9.0^\circ$ ).<sup>39</sup> Compound **10** was then tris-thiophosphorylated using bis(2-cyanoethoxy)(diisopropylamino)phosphine<sup>40</sup>/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)thiophosphol]-*myo*-inositol, **11**. Deblocking with sodium/liquid ammonia<sup>20</sup> furnished

#### Scheme 2<sup>a</sup>

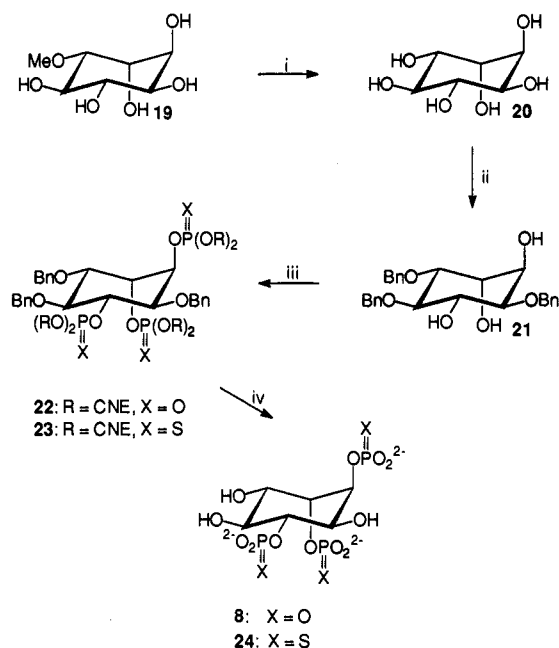


<sup>a</sup> Reagents and conditions: (i)  $\text{HC}(\text{OEt})_3/\text{H}^+/\text{DMF}$ ; (ii)  $\text{PMBCl}/\text{NaH}/\text{DMF}$ ; (iii)  $2 \text{ M HCl}/\text{MeOH}$  (1:20); (iv)  $(\text{BnO})_2\text{PNPr}^+_2/1\text{H-tetrazole}$ , then  $\text{S}_8/\text{pyridine}$ ; (v)  $\text{Na}/\text{liquid NH}_3$ . PMB, *p*-methoxybenzyl; Bn, benzyl.

1L-*myo*-inositol 1,4,5-trisphosphorothioate [*L-Ins*(1,4,5)- $\text{PS}_3$ ] **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.<sup>41</sup> The  $^{31}\text{P}$ -NMR spectrum of **12** showed, as expected, signals at 44.8, 44.7, and 42.1 ppm for the three phosphorothioate groups.

$\text{Ins}(1,3,5)\text{P}_3$  **7** is a weak inhibitor of  $\text{Ins}(1,4,5)\text{P}_3$  5-phosphatase.<sup>34</sup> 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<sup>42</sup> of the method devised by Lee and Kishi,<sup>43</sup> 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<sup>44</sup>/tetrazole followed by sulfidation to give the fully protected trisphosphorothioate **17**, which was then deprotected with sodium in liquid ammonia<sup>20</sup> to give *myo*-inositol 1,3,5-trisphosphorothioate [*Ins*(1,3,5) $\text{PS}_3$ ] **18** as the triethylammonium salt after ion-exchange chromatography and quantified by Briggs phosphate assay.<sup>41</sup>  $^{31}\text{P}$ -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  $J = 10.1 \text{ Hz}$  in the  $^1\text{H}$ -coupled spectrum.

We<sup>45</sup> and others<sup>46,47</sup> 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-chr-Ins*(2,3,5) $\text{P}_3$ ] is a potent  $\text{Ca}^{2+}$ -mobilizing  $\text{Ins}(1,4,5)\text{P}_3$  3-kinase and 5-phosphatase inhibitor.<sup>45,48</sup> During the synthesis of *L-chr-Ins*(2,3,5) $\text{P}_3$ , 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<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i) HI; (ii) Bu<sub>2</sub>SnO (4 equiv), Bu<sub>4</sub>Ni (4 equiv), MeCN, BnCl (5 equiv); (iii) (NCCH<sub>2</sub>CH<sub>2</sub>O)<sub>2</sub>PNPr<sub>1/2</sub>/1*H*-tetrazole, then either *t*-BuOOH or S<sub>8</sub>/pyridine; (iii) Na/liquid NH<sub>3</sub>, Bn, benzyl; CNE, 2-cyanoethyl.

phorothioate [*L*-*chr*-Ins(1,4,6)PS<sub>3</sub>]. Thus, *L*-quebrachitol 19 (Scheme 3) was demethylated with HI, and the resulting *L*-*chiro*-inositol 20 was regioselectively tribenzylated *via* tin-mediated alkylation to give *L*-2,3,5-tri-*O*-benzyl-*chiro*-inositol 21.<sup>45</sup> Compound 21 was polyphosphorylated on the free hydroxyl groups using bis(2-cyanoethoxy)(diisopropylamino)phosphine<sup>40</sup>/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<sup>20</sup> 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.<sup>41</sup>

All three potential Ins(1,4,5)P<sub>3</sub> 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<sup>49</sup> and are highly potent inhibitors of Ins(1,4,5)-P<sub>3</sub> 5-phosphatase from human erythrocyte membrane, with submicromolar *K*<sub>i</sub> values (*K*<sub>i</sub> values determined as the mean of at least three experiments as previously described<sup>21,22</sup>) of 0.50, 0.43, and 0.3 μM, respectively.<sup>49</sup> *L*-*chr*-Ins(1,4,6)P<sub>3</sub> had a *K*<sub>i</sub> of 44 μM.<sup>33</sup> None of the phosphorothioates, nor *L*-*chr*-Ins(1,4,6)P<sub>3</sub>, mobilized Ca<sup>2+</sup> from intracellular stores in electrically permeabilized human neuroblastoma cells, although Ins(1,4,5)P<sub>3</sub> released Ca<sup>2+</sup> potently under similar conditions (EC<sub>50</sub> 0.12 μM).

All analogues were also assayed for their interactions with Ins(1,4,5)P<sub>3</sub> 3-kinase.<sup>49</sup> Whereas *L*-Ins(1,4,5)PS<sub>3</sub> is recognized surprisingly well by the enzyme (*K*<sub>i</sub> = 1.46 μM; *K*<sub>i</sub>'s determined as previously described<sup>21</sup>), Ins(1,3,5)PS<sub>3</sub> shows hardly any interaction (*K*<sub>i</sub> 247 μM), and *L*-*chr*-Ins(1,4,6)P<sub>3</sub> and *L*-*chr*-Ins(1,4,6)PS<sub>3</sub> were ineffective at inhibiting [<sup>3</sup>H]Ins(1,4,5)P<sub>3</sub> phosphorylation by crude rat brain Ins(1,4,5)P<sub>3</sub> 3-kinase at concentrations of 250 and 30 μM, respectively [*K*<sub>m</sub> for Ins(1,4,5)P<sub>3</sub> 0.6 μM]. Thus,

while all three analogues are potent 5-phosphatase inhibitors, Ins(1,3,5)PS<sub>3</sub> and *L*-*chr*-Ins(1,4,6)PS<sub>3</sub> have the advantage of being selective for this enzyme. *L*-*chiro*-Ins(1,4,6)PS<sub>3</sub>, in particular, is more than 1 order of magnitude more potent than (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methanesulfonate), a recently reported selective Ins(1,4,5)P<sub>3</sub> 5-phosphatase inhibitor.<sup>50</sup> 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<sub>3</sub> 5-phosphatase, although the larger size and greater hydrophobicity of sulfur over oxygen together with the lower p*K*<sub>a</sub> of the phosphorothioate group<sup>51</sup> 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<sub>3</sub> 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<sub>3</sub> 5-phosphatase of the highest potency currently available. Moreover, Ins(1,3,5)PS<sub>3</sub> and *L*-*chr*-Ins(1,4,6)PS<sub>3</sub>, by virtue of their lack of affinity for 3-kinase emerge as selective non-Ca<sup>2+</sup>-mobilizing inhibitors of Ins(1,4,5)P<sub>3</sub> 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 inborn error of inositol phosphate metabolism,<sup>52</sup> 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<sup>2+</sup> regulation,<sup>53</sup> 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 pre-coated plates (Merck TLC aluminum sheets silica 60 F<sub>254</sub>, Art. no. 5554, and Merck HPTLC plates silica 60 F<sub>254</sub>, 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.*<sup>54</sup> and was carried out using Sorbsil C60 silica gel.

<sup>1</sup>H- and <sup>13</sup>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). <sup>31</sup>P-NMR spectra were recorded on a JEOL FX-90Q spectrometer. <sup>31</sup>P chemical shifts were measured in ppm relative to external 85% H<sub>3</sub>PO<sub>4</sub>. *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 an 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<sup>41</sup> as follows: A molybdate solution (12.5 g of ammonium molybdate dissolved in 250 mL of water and 35 mL of concentrated H<sub>2</sub>SO<sub>4</sub>), a hydroquinone solution (0.5 g of hydroquinone dissolved in 100 mL of water and a drop of concentrated H<sub>2</sub>SO<sub>4</sub>), and a sulfite solution (20% w/v sodium sulfite in water) were prepared. Aliquots (usually 250  $\mu$ L) of the fractions to be assayed were transferred into test tubes, and concentrated H<sub>2</sub>SO<sub>4</sub> (3 drops) was added to these samples. The samples were heated at 150 °C for 1 h. After cooling, water (500  $\mu$ L), molybdate solution (500  $\mu$ L), hydroquinone solution (250  $\mu$ L), and sulfite solution (250  $\mu$ L) 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<sup>56</sup> for sulfhydryl groups as follows. To 250- $\mu$ L 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.*<sup>56</sup> by adding 2 equiv of diisopropylamine to a solution of PCl<sub>3</sub> in dry ether at -78 °C. The crude product ( $\delta_P$  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<sup>44</sup> ( $\delta_P$  145.24) which could be purified by flash chromatography.

Bis(2-cyanoethoxy)(diisopropylamino)phosphine<sup>40</sup> was synthesized according to the procedure of Bannwarth and Trzeciak<sup>44</sup> 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<sup>38</sup> (142 mg, 316  $\mu$ mol) 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 <sup>31</sup>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  $\mu$ mol, 99%) as a syrup.  $[\alpha]^{17}_D$ : -15.9° ( $c$  = 3.5 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.95–2.41 (4 H, m, CH<sub>2</sub>CH<sub>2</sub>CN), 2.58–2.62 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>CN), 2.73–2.82 (6 H, m, CH<sub>2</sub>CH<sub>2</sub>CN), 3.64 (1 H, dd,  $J$  = 9.9, 2.8 Hz, C-3-H), 3.68–3.89 (2 H, m, CH<sub>2</sub>-CH<sub>2</sub>CN), 3.98–4.55 (14 H, m, CH<sub>2</sub>CH<sub>2</sub>CN, C-2-H, C-6-H, C-5-H, C-1-H), 4.60, 4.78 (2 H, AB,  $J$  = 11.7 Hz, CH<sub>2</sub>Ph), 4.88 (2 H, AB, CH<sub>2</sub>Ph), 4.81, 4.95 (2 H, AB,  $J$  = 11.6 Hz, CH<sub>2</sub>Ph), 5.19 (1 H, q,  $J$  = 9.5 Hz, C-4-H), 7.34–7.43 (15 H, m, CH<sub>2</sub>Ph). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz):  $\delta$  18.36, 18.52, 18.81, 18.94, 19.23, 19.40, 19.53 (7 t, CH<sub>2</sub>CH<sub>2</sub>CN), 62.44, 62.63, 62.73, 62.79, 62.86, 62.99, 63.09 (7 t, CH<sub>2</sub>CH<sub>2</sub>CN), 72.16, 73.92, 75.22 (3 t, CH<sub>2</sub>Ph), 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<sub>2</sub>Ph), 137.17, 137.88, 138.21 (3 s, CH<sub>2</sub>Ph). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 36 MHz):  $\delta$  66.70, 66.43, 66.25. MS:  $m/z$  (+ve ion FAB) 1057 [(M + H)<sup>+</sup>, 5.4], 91 (100). MS:  $m/z$  (-ve ion FAB) 1001 [(M - CH<sub>2</sub>CH<sub>2</sub>CN)<sup>-</sup>, 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  $\mu$ mol) 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  $\mu$ mol (91%).  $[\alpha]^{17}_D$ : +28° (pH 8,  $c$  = 0.5 in H<sub>2</sub>O). <sup>1</sup>H NMR (D<sub>2</sub>O, pH 8; 400 MHz)  $\delta$  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). <sup>31</sup>P NMR (D<sub>2</sub>O; pH 8; 36 MHz):  $\delta$  44.82, 44.69, 42.06. MS:  $m/z$  (-ve ion FAB) 467 [(M - H)<sup>-</sup>, 100]. MS:  $m/z$  466.882 (M - H)<sup>-</sup> (calcd for C<sub>6</sub>H<sub>14</sub>O<sub>12</sub>P<sub>3</sub>S<sub>3</sub>, 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<sup>42</sup> (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). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  3.76 (3 H, s, OCH<sub>3</sub>), 3.79 (6 H, s, 2 OCH<sub>3</sub>), 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<sub>2</sub>Ph, C-2-H), 4.58 (2 H, s, CH<sub>2</sub>Ph), 5.51 (1 H, s, O<sub>3</sub>CH), 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). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz):  $\delta$  55.07 (q, OCH<sub>3</sub>), 66.65 (d, 2 inositol ring C), 68.08 (d, inositol ring C), 70.48 (d, 2 inositol ring C), 71.00 (t, CH<sub>2</sub>PhOMe), 73.66 (d, inositol ring C), 103.08 (d, O<sub>3</sub>CH), 113.62 (d, 6 PhOMe C), 129.12 (d, 4 PhOMe C), 129.58 (d, 2 PhOMe C), 134.57 (s, 3 PhOMe C), 159.16 (s, 3 PhOMe C). MS:  $m/z$  (+ve ion FAB) 551 [(M + H)<sup>+</sup>, 2], 429 [(M - CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>)<sup>+</sup>, 6], 121 [(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>)<sup>+</sup>, 100]. MS:  $m/z$  (-ve ion FAB) 703 [(M + NBA)<sup>-</sup>, 25], 535 (30), 322 (55), 188 (100). Anal. (C<sub>31</sub>H<sub>34</sub>O<sub>9</sub>) 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  $\times$  200 mL). The combined extracts were evaporated and the crude product was chromatographed on silica gel using ether  $\rightarrow$  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). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.45 (2 H, d,  $J$  = 5.7 Hz, D<sub>2</sub>O ex, 2 OH), 2.66 (1 H, br s, D<sub>2</sub>O 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<sub>3</sub>), 3.80 (3 H, s, OCH<sub>3</sub>), 3.90 (1 H, t,  $J$  = 3.0 Hz, C-2-H), 4.73 (2 H, s, CH<sub>2</sub>Ph), 4.76 (4 H, s, 2 CH<sub>2</sub>Ph), 6.87 (4 H, d,  $J$  = 8.6 Hz, CH<sub>2</sub>PhOMe), 6.89 (2 H, d,  $J$  = 8.6 Hz, CH<sub>2</sub>PhOMe), 7.24 (2 H, d,  $J$  = 8.6 Hz, CH<sub>2</sub>PhOMe), 7.29 (4 H, d,  $J$  = 8.6 Hz, CH<sub>2</sub>PhOMe). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz):  $\delta$  55.14 (q, OCH<sub>3</sub>), 72.33 (d, 2 C, inositol ring C), 74.47 (t, CH<sub>2</sub>Ph), 74.79, 78.95 (2 d, 2 C, inositol ring C), 81.48 (d, 2 C, inositol ring C), 113.78 (d, 2 PhOMe C), 113.85 (d, 4 PhOMe C), 129.45 (d, 2 PhOMe C), 129.61 (d, 4 PhOMe C), 130.58 (s, PhOMe), 159.22 (s, PhOMe). MS:  $m/z$  (+ve ion FAB) 539 [(M - H)<sup>+</sup>, 1.5], 419 (5), 121 [(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>-OCH<sub>3</sub>)<sup>+</sup>, 100]. MS:  $m/z$  (-ve ion FAB) 706 (36), 693 [(M + NBA)<sup>-</sup>, 57], 539 [(M - H)<sup>-</sup>, 100], 419 [(M - CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>)<sup>-</sup>, 38]. Anal. (C<sub>30</sub>H<sub>36</sub>O<sub>9</sub>) 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 <sup>31</sup>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<sub>f</sub>* 0.81). The solution was partitioned between chloroform and saturated aqueous NaHCO<sub>3</sub>, 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. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 3.70 (6 H, s, OCH<sub>3</sub>), 3.76 (3 H, s, OCH<sub>3</sub>), 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<sub>2</sub>Ph, C-2-H, C-5-H), 6.71 (4 H, d, *J* = 8.6 Hz, CH<sub>2</sub>PhOMe), 6.80 (2 H, d, *J* = 8.8 Hz, CH<sub>2</sub>PhOMe), 6.98 (4 H, dd, *J* = 7.6, 1.9 Hz, CH<sub>2</sub>PhOMe), 7.11–7.35 (32 H, m, CH<sub>2</sub>PhOMe and CH<sub>2</sub>Ph). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 54.16 (q, OCH<sub>3</sub>), 68.56, 68.73, 68.89, 69.02, 72.71, 74.14 (6 t, CH<sub>2</sub>Ph), 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 PhOMe C), 112.54 (d, 2 PhOMe C), 126.89, 126.95, 127.21, 127.27, 127.34, 127.43, 127.56, 127.66, 127.76, 128.05 (10 d, CH<sub>2</sub>Ph and PhOMe), 129.35 (s, 2 PhOMe C), 129.67 (s, 1 PhOMe C), 134.54, 134.67, 134.80, 134.93 (4 s, CH<sub>2</sub>Ph), 157.73 (s, PhOMe C). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 36 MHz): δ 68.1 (1 P), 66.8 (2 P). MS: *m/z* (+ve ion FAB) 1370 [(M + H)<sup>+</sup>, 0.17], 1247 (0.3), 121 [(CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>3</sub>)<sup>+</sup>, 100], 91 [(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)<sup>+</sup>, 37]. MS: *m/z* (-ve ion FAB) 1277 [(M - C<sub>7</sub>H<sub>7</sub>)<sup>-</sup>, 10], 293 [(OP(S)(OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>)<sup>-</sup>, 100], 95 (55). MS: *m/z* 1370.360 (M + H)<sup>+</sup> (calcd for C<sub>77</sub>H<sub>77</sub>O<sub>15</sub>P<sub>3</sub>S<sub>3</sub>, 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%). <sup>1</sup>H NMR (D<sub>2</sub>O, 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). <sup>13</sup>C NMR (D<sub>2</sub>O, 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*<sub>C-O-P</sub> = 6.6 Hz, C-1, C-3), 78.41 (1 C, dd, *J*<sub>C-O-P</sub> = 6.6 Hz, C-5). <sup>31</sup>P NMR (D<sub>2</sub>O, 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)<sup>-</sup>], 432 (15), 238 (15), 113 (17), 95 [(PSO<sub>2</sub>)<sup>-</sup>, 40]. MS: *m/z* 466.886 (M - H)<sup>-</sup> (calcd for C<sub>6</sub>H<sub>14</sub>O<sub>12</sub>P<sub>3</sub>S<sub>3</sub>, 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<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub> (10 mL), dried over MgSO<sub>4</sub>, and concentrated. Flash column chromatography of the residue gave 22 (76 mg, 75 μmol, 75%) as an oil. [α]<sub>D</sub><sup>20</sup>: -22° (*c* = 3.0 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 2.35–2.53 (2 H, m, CH<sub>2</sub>), 2.60–2.80 (10 H, m, CH<sub>2</sub>), 3.87 (1 H, t, *J* = 9.5 Hz, CH), 3.90–4.25 (15 H, m, 6 × CH<sub>2</sub>, 3 × CH), 4.67, 5.07 (2 H, AB q, *J* = 10.1 Hz, CH<sub>2</sub>Ph), 4.77, 4.78 (2 H, AB q, *J* = 11.1 Hz, CH<sub>2</sub>Ph), 4.87, 4.92 (2 H, AB q, *J* = 11.3 Hz, CH<sub>2</sub>Ph), 5.05–5.15 (2 H, m, CH), 7.28–7.49 (15 H, m, CH<sub>2</sub>Ph). <sup>31</sup>P NMR (D<sub>2</sub>O, 36 MHz): δ -3.43, -3.07, and -2.44. MS: *m/z* (+ve ion FAB) 1009 [(M + H)<sup>+</sup>, 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<sub>2</sub>. 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%). [α]<sub>D</sub><sup>20</sup>: -11° (*c* = 0.5 in H<sub>2</sub>O, pH 8).

<sup>1</sup>H NMR (D<sub>2</sub>O, 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). <sup>31</sup>P NMR (D<sub>2</sub>O, 36 MHz): δ 1.89, 2.65, 3.31. MS: *m/z* (-ve ion FAB) 419 [(M - H)<sup>-</sup>, 12], 177 (6), 159 (7), 97 (7). MS: *m/z* 418.957 (M - H)<sup>-</sup> (calcd for C<sub>6</sub>H<sub>14</sub>O<sub>15</sub>P<sub>3</sub>, 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<sub>2</sub>Cl<sub>2</sub> (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%). [α]<sub>D</sub><sup>20</sup>: -25° (*c* = 3.0 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 2.35–2.54 (4 H, m, CH<sub>2</sub>), 2.62–2.82 (8 H, m, CH<sub>2</sub>), 3.89 (1 H, t, *J* = 9.2 Hz, CH), 3.90–4.35 (15 H, m, 6 × CH<sub>2</sub> and 3 × CH), 4.67, 5.05 (2 H, ABq, *J* = 11.4 Hz, CH<sub>2</sub>Ph), 4.70, 4.76 (2 H, AB q, *J* = 10.8 Hz, CH<sub>2</sub>Ph), 4.82, 4.89 (2 H, ABq, *J* = 11.2 Hz, CH<sub>2</sub>Ph), 5.08–5.23 (2 H, m, CH), 7.26–7.47 (15 H, CH<sub>2</sub>Ph). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 36 MHz): δ 68.39, 67.49, 67.26. MS: *m/z* (+ve ion FAB) 1057 [(M + H)<sup>+</sup>, 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<sub>2</sub>. 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%). [α]<sub>D</sub><sup>20</sup>: -11° (*c* = 0.5 in H<sub>2</sub>O, pH 8). <sup>1</sup>H NMR (D<sub>2</sub>O, 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). <sup>31</sup>P NMR (D<sub>2</sub>O, 36 MHz): δ 44.22, 44.49, and 45.09. MS: *m/z* (-ve ion FAB) 467 [(M - H)<sup>-</sup>, 100], 451 (20), 433 (27), 211 (30), 113 (28), 95 (31). MS: *m/z* 466.887 (M - H)<sup>-</sup> (calcd for C<sub>6</sub>H<sub>14</sub>O<sub>12</sub>P<sub>3</sub>S<sub>3</sub>, 466.886).

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