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

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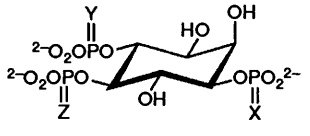
Two routes for the synthesis of the *myo*-inositol 1,4,5-triphosphate analogue *myo*-inositol 1-phosphorothioate 4,5-bisphosphate have been devised. DL-2,3,6-Tri-*O*-benzyl-1-*O*-(*cis*-prop-1-enyl)-*myo*-inositol was prepared from DL-1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol and phosphorylated to the protected 4,5-biphosphate. Removal of the propenyl group generated DL-1,2,4-tri-*O*-benzyl-5,6-bis[bis(2-cyanoethoxy)phospho]-*myo*-inositol which was thiophosphorylated to give DL-2,3,6-tri-*O*-benzyl-1-*O*-[bis(2-cyanoethoxy)thiophospho]-4,5-bis[bis(2-cyanoethoxy)phospho]-*myo*-inositol. Deblocking afforded racemic *myo*-inositol 1-phosphorothioate 4,5-bisphosphate, which was reacted with the iodoketone of a fluorescent label to generate a fluorescently labelled inositol 1,4,5-trisphosphate analogue. DL-1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol was resolved into its enantiomers by means of the crystalline 4,5-biscamphanate ester. 1D-(+)-1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol was used to prepare D-*myo*-inositol 1-phosphorothioate 4,5-bisphosphate in a fashion analogous to the racemic modification *via* 1D-(+)-2,3,6-tri-*O*-benzyl-1-*O*-(*cis*-prop-1-enyl)-4,5-bis[bis(2-cyanoethoxy)phospho]-*myo*-inositol.

In a second route, DL-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol was converted into the corresponding 4,5-dibutyrate. Removal of the isopropylidene group gave DL-1,4,-di-*O*-benzyl-5,6-di-*O*-butyryl-*myo*-inositol which was converted into the corresponding 1-*O*-*p*-methoxybenzyl ether *via* the 1,2-*O*-dibutylstannylidene derivative. Benzylation of the 2-position and removal of the butyrates yielded DL-1-*O*-*p*-methoxybenzyl-2,3,6-tri-*O*-benzyl-*myo*-inositol which was phosphorylated to DL-1-*O*-*p*-methoxybenzyl-2,3,6-tri-*O*-benzyl-4,5-bis[bis(2-cyanoethoxy)phospho)]-*myo*-inositol. Removal of the *p*-methoxybenzyl group gave the key intermediate DL-1,2,4-tri-*O*-benzyl-5,6-bis[bis(2-cyanoethoxy)phospho]-*myo*-inositol, which could be further elaborated to *myo*-inositol 1-phosphorothioate 4,5-bisphosphate.

D-*myo*-Inositol 1,4,5-triphosphate [Ins(1,4,5)P_3], **1** is a ubiquitous second messenger, which couples agonist stimulation of a wide variety of cell surface receptors to the mobilisation 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] Realisation 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, latterly, chemical[6–8] effort to unravel the details of this complex pathway. Ins(1,4,5)P_3 has now been synthesised by many groups and

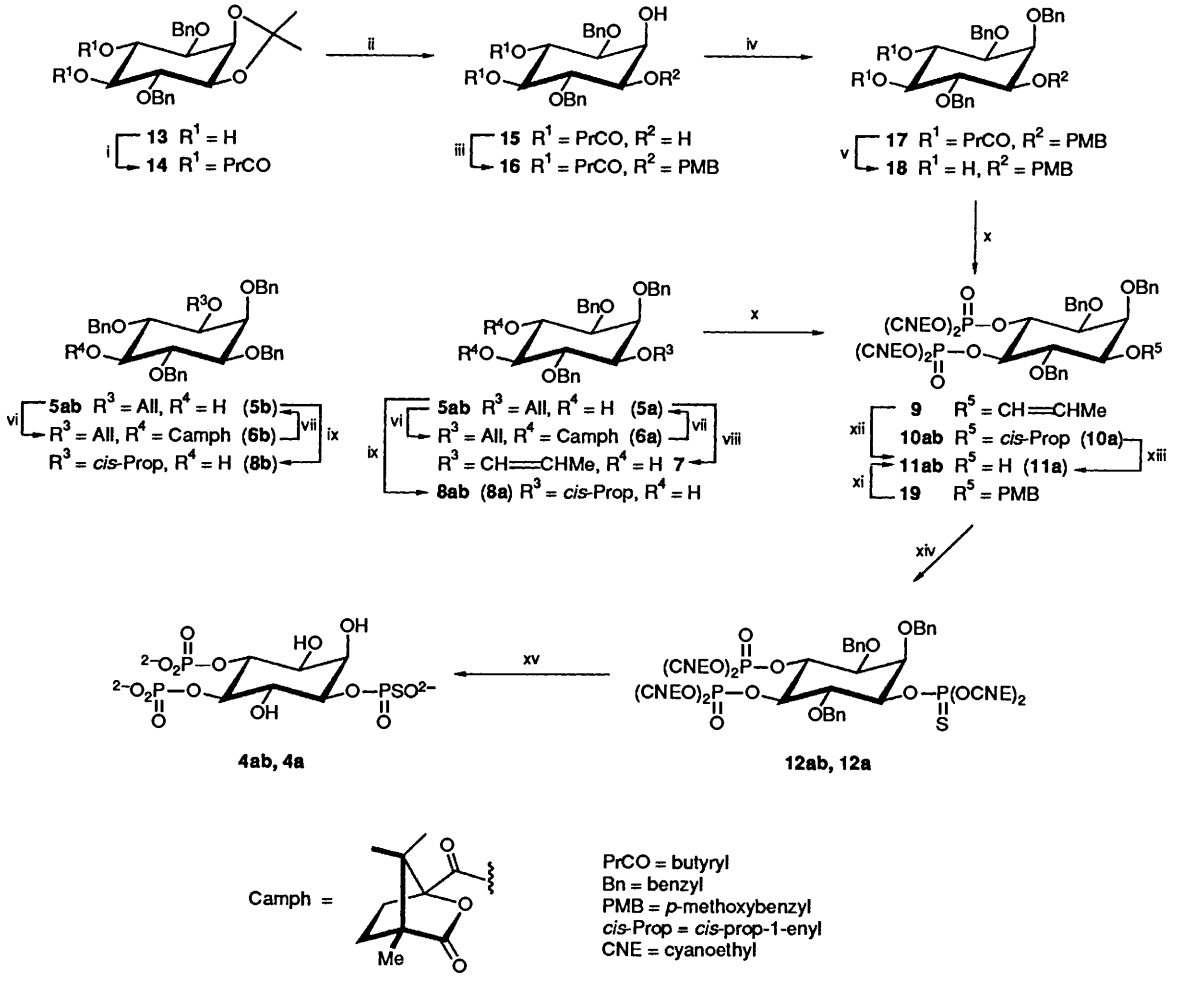


1 X = Y = Z = O
2 X = Y = Z = S
3 X = Y = O; Z = S
4 Y = Z = O; X = S

chemical emphasis in this field must now focus upon the synthesis of novel structurally modified inositol phosphate analogues, as potential enzyme inhibitors and receptor antagonists to facilitate pharmacological intervention in this signalling pathway.

Few biologically potent Ins(1,4,5)P_3 analogues have yet been synthesised[6–8] although recent reports on analogues modified at the 2-position,[9,10] the 3-position,[11] the 6-position[12] and on the synthesis of an active 5-methylene phosphonate analogue[13] have appeared. We have reasoned that, by virtue of their metabolic stability, phosphorothioate analogues will prove to be of significant importance in this field.[14,15] We previously reported the first synthesis of the trisphosphorothioate **2** [Ins(1,4,5)PS_3],[16] a potent inhibitor of human erythrocyte Ins(1,4,5)P_3 5-phosphatase,[17] and the specifically modified 5-phosphorothioate analogue **3** [Ins(1,4,5)P_3-5S].[18] Both of these highly potent analogues are already finding numerous biological applications.[7,14,15,19,20] Phosphorothioate analogues have also been synthesised by another group.[21]

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,[14] the 1-phosphate group being thought to provide enhanced affinity for the receptor. Semisynthetic Ins(1,4,5)P_3 analogues with modifications at the 1-phosphate position have been prepared from the deacylated polyphosphoinositide phospholipid and are biologically potent.[22] Other groups have recently addressed the problem of attaching reporter groups to Ins(1,4,5)P_3[23,24] or related compounds,[25] and Ins(1,4,5)P_3 to affinity matrices.[10,25,26] We propose here that introduction of the nucleophilic sulfur of a 1-phosphorothioate group into the Ins(1,4,5)P_3 molecule should permit the facile attachment of reporter groups to Ins(1,4,5)P_3, such as photoaffinity labels, spin labels and fluorescent probes. Fluorescent labelling methodology has already shown its versatility in the nucleic acid field,[27] where it is already



Scheme 1 *Reagents and conditions:* i, Butyric anhydride, pyridine, dimethylaminopyridine (DMAP); ii, 2 mol dm^{-3}–HCl–H_2O–methanol (1:2:5); iii, (a) Bu_2SnO–toluene–reflux, then (b) dry DMF–KI, *p*-methoxybenzyl chloride–CsF; iv, DMF–NaH–BnBr; v, NaOH–MeOH; vi, (−)-camphanic acid chloride, pyridine; vii, NaOH–MeOH; viii, tris(triphenylphosphine)rhodium(I) chloride DABCO, EtOH–toluene–water v/v/v 7:3:1; ix, Bu^tO_2K, dimethyl sulfoxide; x, bis(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine, tetrazole, CH_2Cl_2 then Bu^tO_2H; xi, DDQ, H_2O–CH_2Cl_2 (15:1); xii, HgO–$HgCl_2$ in 10:1 v/v acetone:water; xiii, 1 mol dm^{-3} HCl–MeOH v/v 1:5; xiv, bis(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine, tetrazole, CH_2Cl_2 then S_8–pyridine; xv, Na–liq. NH_3

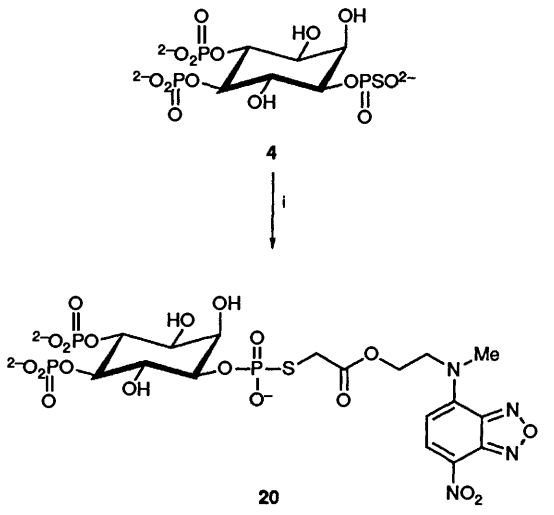
established as a valuable alternative to the use of radioactivity. Consequently, we have devised syntheses of the novel Ins-(1,4,5)P_3 analogue *myo*-inositol 1-phosphorothioate 4,5-bisphosphate **4** [Ins(1,4,5)P_3-1S] in racemic and optically active form by two different routes and demonstrate its use in the fluorescent labelling of Ins(1,4,5)P_3 (Scheme 1). A preliminary account of this work has already appeared.[28]

Results and Discussion

Isomerisation of the 1-*O*-allyl group of DL-1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol[29] DL-**5ab*** to the corresponding prop-1-enyl derivative DL-**7** (mixture of *ca.* 5:1 *cis*:*trans* prop-1-enyl isomers) was accomplished using tris(triphenylphosphine)-rhodium(I) chloride catalyst in the presence of diazabicyclo-[2.2.2]octane (DABCO) in 7:3:1 ethanol–benzene–water.[30] Phosphitylation of the 4,5-vicinal diol was effected using bis-(2-cyanoethyl)-*N*,*N*-diisopropylaminophosphine,[31] followed by oxidation of the protected inositol P^{III} bisphosphite to the P^V bisphosphate with Bu^tO_2H[16] to give the bisphosphate DL-**9**. Removal of the prop-1-enyl protecting group using HgO–$HgCl_2$[32] gave DL-**11ab**, which was phosphitylated in the same fashion and the product oxidized to the fully protected inositol 1-monophosphorothioate 4,5-bisphosphate DL-**12ab** using sulfur in pyridine. Deblocking of all protecting groups was accomplished using sodium in liquid ammonia[16] to give the Ins(1,4,5)P_3 analogue DL-**4ab**, which was purified by ion-exchange chromatography on DEAE Sephadex A-25. DL-**4ab** was eluted at *ca.* 800 mmol dm^{-3} triethylammonium hydrogen carbonate (TEAB) buffer. ^{31}P NMR spectroscopy showed clearly that the product possessed a single phosphorothioate group (δ_p, 42.1) and two phosphate groups (δ_p, 4.8, 5.0).

The protected key intermediate **11** was also prepared in optically active form by resolution of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol DL-**5ab** *via* its 4,5-bis(−)-ω-camphanate derivative DL-**6ab**. The biscamphanate of the protected D-isomer readily crystallised initially from a diastereoisomeric mixture, however the biscamphanates of both the D-**5a** and L-**5b** protected inositols, **6a** and **6b** respectively, could be crystallised under appropriate conditions. The ^1H NMR resonances of the camphanate methyl groups were used as an initial guide to the efficiency of this resolution. After base-deblocking of the camphanate moieties both enantiomers of 1-*O*-allyl-2,3,6-tri-*O*-

* In this paper, where compounds have been optically resolved, a suffix **ab** refers to the racemic modification and suffixes of **a** and **b** denote D- and L-enantiomers respectively.



Scheme 2 *Reagents and conditions:* i, IANBD (1.1 equiv.), EtOH

benzyl-*myo*-inositol were obtained. The absolute configuration of these enantiomers was assigned by comparison of our physical data with those for one of them in the literature[29] and by conversion of one of them (L-**5b**) to the known triol D-(−)-1,2,4-tri-*O*-benzyl-*myo*-inositol[29] by isomerisation of the allyl group and subsequent removal of the *cis*-prop-1-enyl group with acid. D-1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol **5a** was isomerised to the *cis*-1-prop-1-enyl derivative **8a** using KOBu^t–DMSO[33] and the product was phosphorylated to give the corresponding optically active protected 4,5-bisphosphate D-**10a**. Removal of the propenyl group with acid and thiophosphorylation of the resulting alcohol D-**11a** generated D-2,3,6-tri-*O*-benzyl-1-*O*-[bis(2-cyanoethoxy)thiophospho]-4,5-bis[bis(2-cyanoethoxy)-phospho]-*myo*-inositol which was deblocked to give D-*myo*-inositol 1-phosphorothioate 4,5-bisphosphate **4a**.

The key intermediate DL-**11** was also prepared *via* a different route commencing with the known DL-1,2-*O*-isopropylidene-3,6-di-*O*-benzyl-*myo*-inositol[29] DL-**13**. This was converted to the 4,5-di-*O*-butyrate DL-**14** and the isopropylidene group was removed with acid. Selective tin-mediated alkylation[34] of the equatorial 1-hydroxy of the resulting diol DL-**15** group by *p*-methoxybenzyl chloride was achieved *via* the corresponding 1,2-dibutylstannylene derivative to give DL-1-*O*-*p*-methoxybenzyl-3,6-di-*O*-benzyl-*myo*-inositol DL-**16**. Benzylation of the free 2-hydroxy group afforded fully protected DL-**17** and removal of the butyrates gave the key intermediate DL-1-*O*-*p*-methoxybenzyl-2,3,6-tri-*O*-benzyl-*myo*-inositol DL-**18**. which was phosphorylated to the protected 4,5-bisphosphate DL-**19**. The *p*-methoxybenzyl group was removed using 2,3-chloro-5,6-dicyano-1,4-benzoquinone (DDQ)[35] to give DL-**11**, which was converted into final product DL-**4ab** as above. Compound **4ab** was a potent mobiliser of intracellular Ca^{2+} from permeabilised cells.[36]

Nitrobenzoxadioazole (NBD) derivatives, such as the iodoacetate 4-{*N*-[2-(iodoacetoxy)ethyl]-*N*-methylamino}-7-nitro-2,1,3-benzoxadiazole (IANBD), are unique in having long-wavelength fluorescein-like fluorescence spectral properties, but with high environmental sensitivity of the quantum yield coupled with a relatively small molecular size.[37] Such a probe seems ideal for preliminary studies of the interactions of a fluorescently tagged Ins(1,4,5)P_3 with the intracellular receptor and the metabolic enzymes 5-phosphatase and 3-kinase. Reaction of the phosphorothioate analogue DL-**4ab** with IANBD proceeded smoothly to give the adduct **20**, which was purified by ion-exchange chromatography, and was eluted at *ca.* 800 mmol dm^{-3} TEAB. ^{31}P NMR spectroscopy (Fig. 1) showed clearly that the 1-phosphorothioate group (δ_p 42.1) had been converted into the *S*-alkyl phosphorothiolate (δ_p 19.9; $^3J_{POCH} = {}^3J_{PSCH} = 9.5$ Hz). The adduct **20** exhibited a UV spectrum consistent with the presence of an NBD chromophore and when excited at 460 nm showed the expected fluorescence at 540 nm.

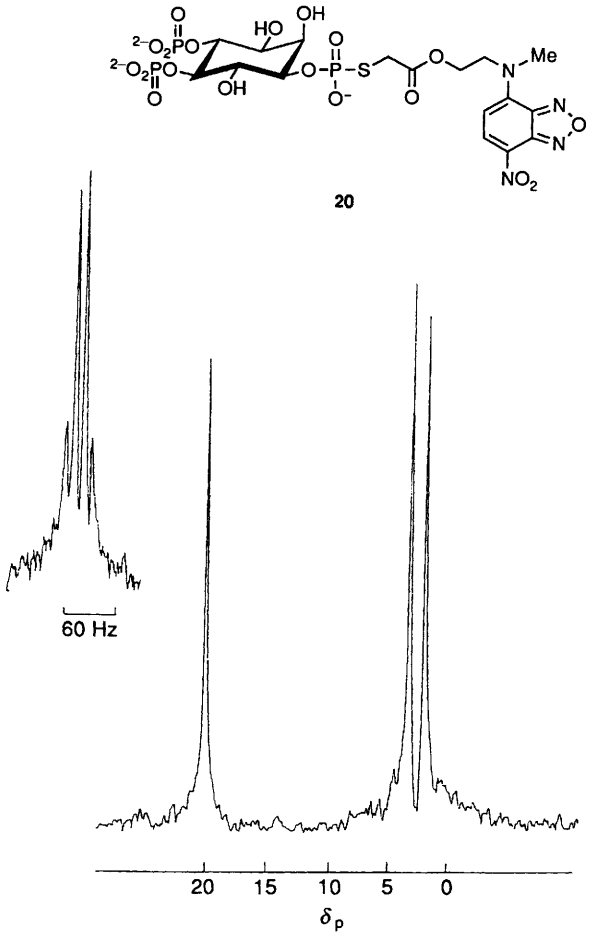
Compound **20** was potent at releasing ATP-sequestered intracellular Ca^{2+} from permeabilised cells and was recognised by the Ins(1,4,5)P_3 receptor. Biological results for **4** and **20** will be reported elsewhere. The synthesis of **20** thus provides the first example of a biologically active Ins(1,4,5)P_3 analogue labelled with a fluorescent reporter group, which should be of considerable utility in probing the interactions of this second messenger with proteins. These studies are now in progress.

Experimental

Materials and Methods.—TLC and HPTLC were performed on pre-coated plates (Merck TLC aluminium sheets silica 60 F_{254}, Art. no. 5554 and Merck HPTLC plates silica 60 F_{254}, Art. no. 5635). Products were visualised by spraying phosphomolybdic acid in methanol followed by heating. Flash chromatography refers to the method of Still *et al.*[38] and was carried out using Sorbsil C60 silica gel.

^{1}H and ^{13}C NMR spectra were recorded on either Bruker AM-300 or JEOL JNM GX-270 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 NMR chemical shifts were measured in ppm relative to external 85% H_3PO_4. *J* Values are given in Hz. Melting points (uncorrected) were determined using a Reichert–Jung Thermo Galen Kofler block. Microanalysis was carried out at Butterworth Laboratories Ltd. and the University of Bath microanalysis service. Mass spectra were recorded at the SERC Mass Spectrometry Service Centre and at the University of Bath Mass Spectrometry Service. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Ion-exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion-Exchange Chromatograph using DEAE Sephadex or DEAE Sepharose and gradients of triethylammonium hydrogen carbonate (TEAB) as eluent. Compounds containing phosphates were assayed quantitatively by the Briggs phosphate test.[39] Compounds containing phosphorothioates were assayed by a modification of the Ellman test[40] for sulfydryl groups as follows. To aliquots (250 mm^3) of the ion-exchange column fractions was added a buffered solution of Ellman's reagent [1 cm^3; 100 cm^3 of 10 mmol dm^{-3} Tris buffer, pH 8, containing 40 mg of 5,5′-dithio-bis(2-nitrobenzoic acid)]. The fractions containing phosphorothioates were identified by their deep yellow colour.

(±)-3,6-*Di*-O-*benzyl*-4,5-*di*-O-*butyryl*-1,2-O-*isopropylidine*-myo-*inositol* **14**.—A mixture of 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-*myo*-inositol **13**[29] (1.69 g, 4.2 mmol), pyridine (30 cm^3, 0.37 mol), butyric anhydride (4 cm^3, 24.4 mmol) and dimethylaminopyridine (DMAP) (50 mg, 4.1 mmol) was stirred at room temp. The reaction was followed by TLC ($CHCl_3$–ethyl acetate, 1:1) and after 2 h showed a single product (R_f = 0.70). The solution was diluted with methanol (10 cm^3), the mixture was stirred for 30 min and the solvents evaporated. A solution of the residue in dichloromethane (100 cm^3) was washed with saturated brine, ice-cold 1 mol dm^{-3} hydrochloric acid, a saturated solution of sodium hydrogen carbonate and water (50 cm^3 each). The organic layer was dried ($MgSO_4$), filtered and evaporated. The oily residue was purified by flash chromatography ($CHCl_3$–ethyl acetate, 1:1) to give a syrup which could not be crystallised. Yield 2.03 g (88%); δ_H($CDCl_3$; 300 MHz)



20

Fig. 1 36.2 MHz broad band ^{1}H-decoupled ^{31}P NMR spectrum of **20** in D_2O [15 mmol dm^{-3} solution of **20** in 50 mmol dm^{-3} TEAB, 5 mmol dm^{-3} ethylenediaminetetraacetic acid (EDTA), pH 7.7].^{31}P NMR parameters were: sweep width, 10 kHz; pulse width, 9 μs; collected over 8 K; no. of transients, 2500; referenced to external H_3PO_4. The insert shows part of the ^{1}H-coupled spectrum for the resonance at 19.9 ppm

0.92 (6 H, q, *J* 7.4, $COCH_2CH_2CH_3$), 1.34, 1.53 (6 H, 2 s, CMe_2), 1.60 (4 H, tq, *J* 7.4, $COCH_2CH_2CH_3$), 2.23 (4 H, t, *J* 7.4, $COCH_2CH_2CH_3$), 3.74–3.80 (2 H, m, 1-H, 6-H), 4.21 (1 H, t, *J* 6.0, 3-H), 4.37 (1 H, dd, *J* 6.0, 3.7, 2-H), 4.66, 4.77 (2 H, AB, J_{AB} 12.0, $CH_2C_6H_5$), 4.69 (2 H, AB, $CH_2C_6H_5$), 5.03 (1 H, t, *J* 8.3, 5-H), 5.44 (1 H, t, *J* 8.7, 4-H) and 7.24–7.34 (10 H, m, $CH_2C_6H_5$); *m/z* (CI) 558 [(M + NH_4)$^+$, 100%], 541 [(M + H)$^+$, 65], 453 (20), 433 (27), 108 (41) and 91 (51) (Found: M$^+$, 541.2801. $C_{31}H_{41}O_8$ requires (M + H)$^+$, 541.2801).

(±)-1,4-*Di*-O-*benzyl*-5,6-*di*-O-*butyryl*-myo-*inositol* **15**.—Compound **14** (2.03 g, 3.7 mmol) was heated under reflux for 1 h in 2 mol dm^{-3} hydrochloric acid (5 cm^3), water (10 cm^3) and methanol (25 cm^3). The solution was cooled, sodium hydrogen carbonate (2 g) was added, the solvents were evaporated and the product extracted with dichloromethane (3 × 50 cm^3). The organic layer was washed with brine, saturated sodium hydrogen carbonate, brine and water (50 cm^3 each). The organic layer was dried ($MgSO_4$), filtered and evaporated to give an oil. A minimum quantity of ethyl acetate was added to dissolve the oil and light petroleum was added. After scratching, crystals appeared which were filtered to give **15** (1.8 g, 97%); R_f = 0.48 ($CHCl_3$–ethyl acetate, 1:1); m.p. 78–79 °C (Found: C, 67.2; H, 7.5. Calc. for $C_{28}H_{36}O_8$: C, 67.18; H, 7.25%); δ_H($CDCl_3$; 300 MHz) 0.90 (6 H, q, *J* 7.5, $COCH_2CH_2CH_3$), 1.57 (4 H, tq, *J* 7.4, $COCH_2CH_2CH_3$), 2.17–2.23 (4 H, m, $COCH_2CH_2CH_3$), 2.61–2.62 (2 H, br s, D_2O ex, 2 OH), 3.47 (1 H, dd, *J* 9.8, 2.7, 3-H), 3.57 (1 H, dd, *J* 9.5, 2.8, 1-H), 3.89 (1 H, t, *J* 9.6, 4-H), 4.19 (1 H, t, *J* 2.7, 2-H), 4.55, 4.63 (2 H, AB, J_{AB} 12.0, $CH_2C_6H_5$), 4.70, 4.72 (2 H, AB, J_{AB} 11.5, $CH_2C_6H_5$), 5.07 (1 H, t, *J* 9.8, 5-H), 5.46 (1 H, t, *J* 9.9, 6-H) and 7.24–7.37 (10 H, m, $CH_2C_6H_5$); *m/z* (CI) 518 [(M + NH_4)$^+$, 100%], 501 [(M + H)$^+$, 6], 428 (7), 303 (8), 108 (20), 91 (7) and 44 (7) [Found: (M + NH_4)$^+$, 518.2754. Calc. for $C_{28}H_{40}O_8N$ (M + NH_4)$^+$ 518.2754].

(±)-1-O-*p-Methoxybenzyl*-3,6-*di*-O-*benzyl*-4,5-*di*-O-*butyryl*-myo-*inositol* **16**.—Compound **15** (1.8 g, 3.6 mmol) and dibutyltin oxide (1.076 g, 4.2 mmol) were heated at reflux in toluene using a Dean-and-Stark apparatus for 3 h. The reaction mixture was cooled and the toluene was evaporated to give a syrup which was dried under vacuum for 2 h. Caesium fluoride (1.367 g, 9 mmol) was added to the syrup, which was dried for a further hour. Dry DMF (20 cm^3) was added to the syrup under an atmosphere of nitrogen together with dry potassium iodide (1.195 g, 7.2 mmol) and *p*-methoxybenzyl chloride (1.127 g, 7.2 mmol) at room temp. After 24 h at room temp. the reaction was complete and TLC ($CHCl_3$–ethyl acetate, 2:1) showed a product with R_f = 0.60. The solvents were evaporated under reduced pressure and the residue was extracted with ethyl acetate (100 cm^3), washed with water (50 cm^3) and stirred with sodium hydrogen carbonate solution (10% w/v) for 30 min, washed with water again, dried ($MgSO_4$) filtered and evaporated. The product was chromatographed on silica gel ($CHCl_3$–ethyl acetate, 2:1). The resulting syrup was dissolved in a minimum amount of ether and light petroleum was added. After scratching for a few minutes crystals appeared. Yield 1.9 g (85%); m.p. 104–106 °C (Found: C, 69.7; H, 7.4. Calc. for $C_{36}H_{44}O_9$: C, 69.66; H, 7.14); δ_H($CDCl_3$; 300 MHz) 0.88 (6 H, q, *J* 7.3, $COCH_2CH_2CH_3$), 1.55 (4 H, tq, *J* 7.4, $COCH_2CH_2CH_3$), 2.08–2.21 (4 H, m, $COCH_2CH_2CH_3$), 2.50 (1 H, s, D_2O ex, OH), 3.38 (1 H, dd, *J* 9.7, 2.5, 3-H or 1-H), 3.40 (1 H, dd, *J* 10.0, 2.6, 1-H or 3-H), 3.80 (3 H, s, OCH_3), 4.01 (1 H, t, *J* 9.6, 6-H), 4.15 (1 H, t, *J* 2.6, 2-H), 4.50–4.86 (6 H, m, $CH_2C_6H_5$), 5.03 (1 H, t, *J* 9.9, 5-H), 5.47 (1 H, t, *J* 10.0, 4-H), 6.81–6.86 (2 H, m, $CH_2C_6H_4OMe$) and 7.20–7.36 (12 H, m, $CH_2C_6H_5$ and $CH_2C_6H_4OMe$).

(±)-2,3,6-*Tri*-O-*benzyl*-4,5-*di*-O-*butyryl*-1-O-p-*Methoxybenzyl*-myo-*inositol* **17**.—Compound **16** (1.3 g, 2 mmol) was added to dry DMF (20 cm^3) and sodium hydride (0.2 g, 8 mmol). Benzyl bromide (0.5 cm^3, 4.2 mmol) was added and the reaction was stirred at room temp. for 2 h after which TLC ($CHCl_3$–ethyl acetate, 2:1) showed a single new product, R_f = 0.80. Methanol (5 cm^3) was added dropwise to destroy excess sodium hydride. Dichloromethane was added and the solution was washed with brine and water (50 cm^3 each), dried ($MgSO_4$), filtered and evaporated to give **17** as a solid. Yield 1.33 g (89%); m.p. 88–90 °C (from light petroleum) (Found: C, 72.85; H, 7.2. Calc. for $C_{43}H_{50}O_9$: C, 72.66; H, 7.09%); δ_H($CDCl_3$; 300 MHz) 0.87 (6 H, q, *J* 7.5, $COCH_2CH_2CH_3$), 1.56 (4 H, tq, *J* 7.4, $COCH_2CH_2CH_3$), 2.08–2.21 (4 H, m, $COCH_2CH_2CH_3$), 3.35 (1 H, dd, *J* 10.2, 2.2, 1-H or 3-H), 3.37 (1 H, dd, *J* 9.8, 2.3, 3-H or 1-H), 3.80 (3 H, s, OCH_3), 3.96 (1 H, t, *J* 2.2, 2-H), 4.06 (1 H, t, *J* 9.6, 6-H), 4.42–4.89 (8 H, m, $CH_2C_6H_5$), 5.05 (1 H, t, *J* 9.7, 5-H), 5.57 (1 H, t, *J* 10.0, 4-H), 6.82–6.85 (2 H, m, $CH_2C_6H_4OMe$) and 7.18–7.35 (17 H, m, $CH_2C_6H_5$ and $CH_2C_6H_4OMe$); *m/z* (CI) 728 [(M + NH_4)$^+$, 22%], 137 (20), 121 (100), 105 (9) and 91 (12) [Found: (M + NH_4)$^+$, 728.3799. Calc. for $C_{43}H_{54}O_9N$ (M + NH_4)$^+$, 728.3799].

(±)-2,3,6-*Tri*-O-*benzyl*-1-O-p-*methoxybenzyl*-myo-*inositol* **18**.—A mixture of **17** (1 g, 1.4 mmol) and sodium hydroxide (0.6 g, 20 mmol) in methanol (25 cm^3) was heated at reflux for 30 min. The solution was cooled and TLC ($CHCl_3$–ethyl acetate, 2:1) showed a product (R_f = 0.30). The reaction mixture was

neutralised with carbon dioxide and partitioned between water (20 cm^3) and dichloromethane (50 cm^3), washed with brine and water (20 cm^3 each) and dried ($MgSO_4$). The dichloromethane solution was evaporated and the oily residue chromatographed on silica gel ($CHCl_3$–ethyl acetate, 2:1). The resulting oil was dissolved in the minimum amount of ethyl acetate and light petroleum was added. After scratching, crystals appeared. Yield 710 mg (88%); m.p. 93–94 °C (Found: C, 73.75; H, 6.7. Calc. for $C_{35}H_{38}O_7$: C, 73.66; H, 6.71%); δ_H($CDCl_3$; 300 MHz); 2.67 (2 H, br s, D_2O ex, 2 OH), 3.17 (1 H, dd, *J* 9.7, 2.3, 3-H), 3.35 (1 H, dd, *J* 9.7, 2.3, 1-H), 3.42 (1 H, t, *J* 9.1, 5-H), 3.80 (3 H, s, OCH_3), 3.89 (1 H, t, *J* 9.4, 6-H), 4.02 (1 H, t, *J* 2.3, 2-H), 4.02 (1 H, t, *J* 9.3, 4-H), 4.52, 4.58 (2 H, AB, J_{AB} 12.0, $CH_2C_6H_5$), 4.78, 4.86 (2 H, AB, J_{AB} 11.3, $CH_2C_6H_5$), 4.76, 4.96 (2 H, AB, J_{AB} 11.9, $CH_2C_6H_5$), 6.83–6.88 (2 H, m, $CH_2C_6H_4OMe$) and 7.22–7.40 (17 H, m, $CH_2C_6H_5$ and $CH_2C_6H_4OMe$); *m/z* (CI) 588 [$(M + NH_4)^+$, 1%], 479 (3), 449 (9), 121 (100) and 91 (9) [Found: $(M + NH_4)^+$, 588.2961. Calc. for $C_{34}H_{42}NO_7$ $(M + NH_4)^+$, 588.2961].

(±)-2,3,6-*Tri*-O-*benzyl*-4,5-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-1-O-p-*methoxybenzyl*-myo-*inositol* **19**.—A mixture of **18** (100 mg, 0.175 mmol), tetrazole (49 mg, 0.7 mmol) and bis(2-cyanoethoxy)diisopropylaminophosphine (276 mg, 1 mmol) was stirred at room temp. in dry dichloromethane (5 cm^3) for 1 h. The reaction mixture was cooled to −78 °C and 70% *tert*-butylhydroperoxide (1 cm^3, 7.29 mmol) was added dropwise and the solution stirred for a further 30 min at room temp. The mixture was diluted with dichloromethane (40 cm^3) and washed twice with 10% sodium metabisulfite (2 × 20 ml), sodium hydrogen carbonate (2 × 20 cm^3), water (20 cm^3) and brine (20 cm^3). The dichloromethane solution was dried ($MgSO_4$), filtered and chromatographed on silica gel (ethyl acetate) to give the title compound as a syrup. Yield (110 mg, 67%). R_f = 0.22 (ethyl acetate); δ_H($CDCl_3$; 300 MHz) 2.60–2.80 (8 H, m, OCH_2CH_2CN), 3.42 (1 H, dd, *J* 9.8, 2.0, 1-H or 3-H), 3.78 (3 H, s, OCH_3), 3.73–5.14 (21 H, m, 5 Ins C-H, 4 CH_2Ph and 4 OCH_2CH_2CN), 6.78–6.80 (2 H, m, $CH_2C_6H_4OMe$) and 7.11–7.55 (17 H, m, $CH_2C_6H_5$ and $CH_2C_6H_4OMe$); δ_P($CDCl_3$; 121 MHz) −5.97 and −6.15; *m/z* FAB$^+$ 943 [$(M + H)^+$, 10%], 288 (100), 121 (95) and 91 (100).

(±)-1,2,4-*Tri*-O-*benzyl*-5,6-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-myo-*inositol* **11ab** *from* **19**.—A mixture of **19** (75 mg, 0.079 mmol) and DDQ (27 mg, 0.118 mmol) was stirred in dichloromethane (5 cm^3) and water (0.33 cm^3) for 1 h. The reaction was diluted with dichloromethane (40 cm^3) and filtered to remove the precipitate. The dichloromethane solution was washed with 10% sodium metabisulfite (3 × 20 cm^3), sodium hydrogen carbonate solution (20 cm^3) and brine (20 cm^3), dried ($MgSO_4$), filtered and the dichloromethane was evaporated to give an oil. The oily residue was chromatographed on silica gel (ether–pentane, 3:1) to give the title compound; yield (55 mg, 84%); R_f = 0.20 (ethyl acetate); δ_H($CDCl_3$; 300 MHz) 2.63–2.83 (8 H, m, OCH_2CH_2CN), 3.55 (1 H, dd, *J* 9.9, 1.7, 3-H), 3.74–4.98 (19 H, m, 5 Ins C-H, 3 $CH_2C_6H_5$ and 4 OCH_2CH_2CN) and 7.18–7.40 (15 H, m, $CH_2C_6H_5$); δ_P($CDCl_3$; 121 MHz) −2.88 and −2.99; *m/z* FAB$^+$ 823 [$(M + H)^+$, <1%], 728 (13), 581 (15), 328 (29), 147 (53) and 107 (100).

(±)-2,3,6-*Tri*-O-*benzyl*-1-O-(*prop*-1-*enyl*)-myo-*inositol* **7**.—A solution of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol **5ab**[29] (1.55 g, 3.16 mmol) and DABCO (71 mg, 0.65 mmol) in a mixture of ethanol–toluene–water (7:3:1, v/v/v) was heated. When the solution had reached reflux temperature, tris(triphenylphosphine)rhodium chloride (202 mg, 0.22 mmol) was added and the mixture was heated under reflux for 30 min. After cooling, the mixture was diluted with water and extracted twice with ether. The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. Chromatography on silica gel (ether–hexane, 2:1) gave **7** as a mixture of *ca.* 5:1 *cis*:*trans* prop-1-enyl isomers. Yield 1.27 g (82%); δ_H($CDCl_3$; 300 MHz) 1.60 (0.5 H, dd, *J* 6.8, 1.55, *trans*-CH=CH-CH_3), 1.72 (2.5 H, dd, *J* 6.8, 1.6, *cis*-CH=CH-CH_3), 2.71 (1 H, d, *J* 2.0, D_2O ex, OH), 2.74 (1 H, d, *J* 1.8, D_2O ex, OH), 3.26 (1 H, dd, *J* 9.7, 2.4, 3-H), 3.50 (1 H, dd, *J* 9.3, 9.3, 5-H), 3.61 (1 H, dd, *J* 9.7, 2.3, 1-H), 3.97 (1 H, dd, *J* 9.4, 9.4, 6-H), 4.08 (1 H, dd, *J* 9.5, 9.5, 4-H), 4.13 (1 H, dd, *J* 2.4, 2.4, 2-H), 4.50–5.02 (7 H, m, CH=C*H*-CH_3 and 3 $CH_2C_6H_5$), 6.11–6.19 (1 H, m, C*H*=CH-CH_3) and 7.29–7.46 (15 H, m, $CH_2C_6H_5$).

(±)-2,3,6-*Tri*-O-*benzyl*-1-O-(cis-*prop*-1-*enyl*)-myo-*inositol* **8ab**.—A solution of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol **5ab**[29] (2 g, 4.08 mmol) and freshly sublimed potassium *tert*-butoxide (2.28 g, 20 mmol) in dry DMSO (50 cm^3) was stirred for 3 h at 50 °C when HPTLC (ether) showed complete conversion of the starting material (R_f = 0.78) into a single product (R_f = 0.80). Water (50 cm^3) was added to the brown solution, which was then extracted with ether (2 × 100 cm^3). The combined organic layers were dried ($MgSO_4$) and evaporated to dryness to give **8ab** (1.97 g, 4.02 mmol, 98%); m.p. 101–103 °C (from ethanol–water) (Found: C, 73.3; H, 6.95. Calc. for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99%); δ_H($CDCl_3$; 270 MHz) 1.67 (3 H, dd, *J* 6.9, 1.65, CH=CH-CH_3), 2.76 (1 H, d, *J* 2.4, D_2O ex, OH), 2.79 (1 H, d, *J* 2.0, D_2O ex, OH), 3.21 (1 H, dd, *J* 9.7, 2.4, 3-H), 3.42 (1 H, ddd, *J* 9.2, 9.2, 2.2, D_2O shake gives dd, 9.2, 9.2, 5-H), 3.55 (1 H, dd, *J* 9.7, 2.4, 1-H), 3.92 (1 H, dd, *J* 9.5, 9.5, 6-H), 4.03 (1 H, ddd, *J* 9.5, 9.5, 2.0, D_2O shake gives dd, *J* 9.5, 9.5, 4-H), 4.07 (1 H, dd, *J* 2.4, 2.4, 2-H), 4.49 (1 H, dq, *J* 6.7, 6.7, CH=C*H*-CH_3), 4.49, 4.56 (2 H, AB, J_{AB} 11.6, $CH_2C_6H_5$), 4.70, 4.88 (2 H, AB, J_{AB} 11.6, $CH_2C_6H_5$), 4.77, 4.88 (2 H, AB, J_{AB} 11.6, $CH_2C_6H_5$), 6.08 (1 H, dd, *J* 6.2, 1.65, C*H*=CH-CH_3) and 7.23–7.42 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 9.44 (q, CH=CH-*C*H_3), 74.44, 75.35 (2 t, *C*$H_2C_6H_5$), 72.07, 74.24, 74.96, 79.43, 80.28, 83.23 (6 d, inositol ring C), 100.97 (d, CH=*C*H-CH_3), 127.50, 127.66, 127.79, 128.18, 128.28, 128.41, 128.48 (7 d, $CH_2C_6H_5$), 137.60, 138.43 (2 s, $CH_2C_6H_5$) and 145.34 (d, *C*H=CH-CH_3); *m/z* FAB$^+$ 489 [$(M - H)^+$, 0.5%], 399 (3), 181 (15) and 91 (100); *m/z* FAB$^-$ 979 [$(2M - H)^-$, 30%], 643 [$(M + NBA)^-$, 60], 489 [$(M - H)^-$, 100], 399 (20) and 381 (20).

(±)-2,3,6-*Tri*-O-*benzyl*-4,5-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-1-O-(cis-*prop*-1-*enyl*)-myo-*inositol* **10ab**.—A solution of bis-(2-cyanoethoxy)(diisopropylamino)phosphine (7 g, 26 mmol) in dichloromethane (50 cm^3) was added to a solution of **8ab** (1.24 g, 2.6 mmol) and tetrazole (2.19 g, 31.2 mmol) in dichloromethane (50 cm^3). The mixture was stirred at room temp. for 1 h (δ_P 140.70, 140.47); 10% water in THF (20 cm^3) was added and stirring continued for 30 min. 2,6-Lutidine (2 cm^3) followed by *tert*-butyl hydroperoxide (20 cm^3, 70% in water) was then added and stirring continued overnight. The solution was washed with saturated aqueous sodium hydrogen carbonate (2 × 100 cm^3) and dried ($MgSO_4$). The solvents were evaporated and the residue chromatographed on silica gel with 0–10% ethyl acetate in hexane and then 0–10% ethanol in ethyl acetate. The product was recrystallised from ethanol to give **10ab** (1.43 g, 64%); m.p. 118–120 °C (from ethanol); R_f (ethyl acetate:ethanol 9:1) = 0.76 (Found: C, 58.5; H, 5.55; N, 6.5. Calc. for $C_{42}H_{48}N_4O_{12}P_2$: C, 58.47; H, 5.61; N, 6.49%); δ_H($CDCl_3$; 270 MHz) 1.63 (3 H, dd, *J* 6.8, 1.6, CH=CH-CH_3), 2.16–2.39 (4 H, m, CH_2CH_2CN), 2.65–2.69 (4 H, m, CH_2CH_2CN), 3.50 (1 H, dd, *J* 9.5, 2.0, 3-H), 3.66 (1 H, dd, *J* 9.7, 2.0, 1-H), 3.89–4.31 (10 H, m, CH_2CH_2CN, 2-H, 6-H), 4.38–4.99 (9 H, m, $CH_2C_6H_5$, 4-H, 5-H, CH=C*H*-CH_3), 6.06 (11 H, dd, *J* 6.8, 1.6, C*H*=CH-CH_3) and 7.26–7.40 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 9.34 (q, CH=CH-*C*H_3), 18.97,

19.10, 19.23, 19.33 (4 t, CH_2CH_2CN), 62.11, 62.70 (2 t, CH_2CH_2CN), 71.97, 74.70 (2 t, $CH_2C_6H_5$), 73.76, 74.83, 78.10, 78.52, 79.60, 82.48 (6 d, inositol ring C), 102.04 (d, CH=CH-CH_3) 116.60, 116.83, 116.93 (3 s, CN), 126.85, 127.31, 127.60, 127.70, 127.92, 128.12, 128.22, 128.34, 128.51 (9 d, $CH_2C_6H_5$), 137.01, 137.85, 138.21 (3 s, $CH_2C_6H_5$) and 144.66 (d, CH=CH-CH_3); δ_P($CDCl_3$; 36 MHz) −3.57, −3.70; *m/z* FAB$^+$ 863 [(M + H)$^+$, 1.3%], 771 [(M − C_7H_7)$^+$, 0.3], 181 (6), 144 (8) and 91 [$(C_7H_7)^+$, 100]; *m/z* FAB$^-$ 808 [(M − CH_2CH_2CN)$^-$, 55%], 718 (12), 203 (100), 150 (85) and 97 (70) [Found: (M + H)$^+$, 863.2822. Calc. for $C_{42}H_{49}O_{12}N_4P_2$ (M + H)$^+$, 863.2822].

(±)-2,3,6-*Tri*-O-*benzyl*-4,5-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-1-O-(*prop*-1-*enyl*)-myo-*inositol* **9**.—Phosphorylation of **7** in an analogous fashion to that for **8ab** as above gave **9** (mixture of *cis*- and *trans*-isomers): δ_H($CDCl_3$; 270 MHz) 1.51 (0.5 H, dd, *J* 6.8, 1.6, *trans*-CH=CH-CH_3), 1.63 (2.5 H, dd, *J* 6.8, 1.6, *cis*-CH=CH-CH_3), 2.10–2.45 (4 H, m, CH_2CH_2CN), 2.56–2.76 (4 H, m, CH_2CH_2CN), 3.50 (1 H, dd, *J* 9.5, 2.0, 3-H), 3.66 (1 H, dd, *J* 9.7, 2.0, 1-H), 3.89–4.31 (10 H, m, CH_2CH_2CN, 2-H, 6-H), 4.38–4.99 (9 H, m, $CH_2C_6H_5$, 4-H, 5-H, CH=CH-CH_3), 6.03–6.13 (1 H, m, CH=CH-CH_3) and 7.26–7.40 (15 H, m, $CH_2C_6H_5$).

(±)-1,2,4-*Tri*-O-*benzyl*-5,6-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-myo-*inositol* **11ab** *from* **10ab** *and* **9**.—(*a*) A solution of mercury(II) chloride (300 mg, 1.1 mmol) in acetone–water (10:1 v/v, 4 cm^3) was added dropwise with stirring to a mixture of **9** (949 mg, 1.1 mmol) and yellow mercury(II) oxide (300 mg) in acetone–water (9:1 v/v, 10 cm^3). After the addition was complete, stirring was continued for a further 5 min. The mercury(II) oxide was removed by filtration through Celite, the solvents evaporated and the residue taken up in ethyl acetate (50 cm^3). The solution was washed with semisaturated aqueous potassium iodide solution (50 cm^3), dried and evaporated. Chromatography on silica gel using hexane ⟶ ethyl acetate ⟶ ethyl acetate–ethanol (9:1 v/v) gave the pure title compound (786 mg, 87%) as a syrup.

(*b*) A solution of **10ab** (470 mg, 0.54 mmol) in 1 mol dm^{-3} HCl–methanol (1:5, 30 cm^3) was heated under reflux for 30 min when TLC (ethyl acetate–ethanol, 9:1) showed complete conversion of the starting material (R_f 0.76) into a single product (R_f 0.71). After cooling, the mixture was treated with an excess of sodium hydrogen carbonate and the solvents were evaporated. The residue was extracted with ether (2 × 50 cm^3) and the solvent was evaporated to give **11ab** (426 mg, 95%) as a syrup which could not be crystallised: δ_H($CDCl_3$; 300 MHz) 2.14–2.46 (4 H, m, CH_2CH_2CN), 2.60–2.72 (5 H, m, CH_2CH_2CN, OH, OH D_2O ex), 3.53 (1 H, dd, *J* 9.9, 2.1, 3-H), 3.62 (1 H, ddd, *J* 9.9, 7.3, 2.4, D_2O ex gives dd, *J* 9.9, 2.4, 1-H), 3.86 (1 H, dd, *J* 9.4, 9.4, 6-H), 4.06 (1 H, dd, *J* 2.3, 2.3, 2-H), 3.90–4.37 (8 H, m, CH_2CH_2CN), 4.43 (1 H, q, *J* 9.1, 5-H), 4.56, 4.67 (2 H, AB, J_{AB} 11.3, $CH_2C_6H_5$), 4.75, 4.93 (2 H, AB, J_{AB} 11.6, $CH_2C_6H_5$), 4.79, 4.92 (2 H, AB, J_{AB} 11.4, $CH_2C_6H_5$), 4.83 (1 H, q, *J* 9.3, 4-H) and 7.22–7.41 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 18.65, 18.78, 18.84 (3 t, CH_2CH_2CN), 62.02, 62.08, 62.66 (3 t, CH_2CH_2CN), 71.97, 74.05, 74.86 (3 t, $CH_2C_6H_5$), 71.55, 75.80, 77.65, 78.40, 79.23, (5 d, inositol ring C), 116.64, 116.86, 116.99 (3 s, CN), 127.08, 127.50, 127.57, 127.63, 127.86, 128.15, 128.22, 128.31 (8 d, $CH_2C_6H_5$), 136.91, 137.85 and 138.04 (3 s, $CH_2C_6H_5$); δ_P($CDCl_3$; 36 MHz) −5.52 and −5.65; *m/z* FAB$^+$ 823 [(M + H)$^+$, 1.3%], 222 (8), 181 (6), 144 (8) and 91 [$(C_7H_7)^+$, 100]; *m/z* FAB$^-$ 768 [(M − CH_2CH_2CN)$^-$, 45%], 203 (100), 150 (95) and 97 (80) [Found: (M + H)$^+$, 823.2509. Calc. for $C_{39}H_{45}O_{12}N_4P_2$ (M + H)$^+$ 823.2509].

(±)-2,3,6-*Tri*-O-*benzyl*-1-O-[*bis*(2-*cyanoethoxy*)*thiophospho*]-4,5-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-myo-*inositol* **12ab**.—Bis(2-cyanoethoxy)diisopropylaminophosphine (670 mg, 2.5 mmol) was added to a solution of **11ab** (412 mg, 0.5 mmol) and tetrazole (210 mg, 3 mmol) in dichloromethane (25 cm^3). The mixture was stirred at room temp. for 1 h. Dry pyridine (5 cm^3) and sulfur (320 mg, 10 mmol) was added and the solution stirred for another 24 h. The solvent was evaporated and the residue chromatographed on silica gel [eluent hexane ⟶ ethyl acetate ⟶ ethyl acetate–ethanol (9:1 v/v)] to give the pure compound as a syrup (405 mg, 79%) after evaporation of the solvent. δ_H($CDCl_3$; 270 MHz) 2.03–2.47 (6 H, m, CH_2CH_2CN), 2.49–2.79 (6 H, m, CH_2CH_2CN), 3.61 (1 H, dd, *J* 9.9, 1.8, 3-H), 3.82–4.46 (14 H, m, CH_2CH_2CN, 1-H, 6-H), 4.40 (1 H, br s, 2-H), 4.52 (1 H, q, *J* 9.2, 5-H), 4.67, 4.75 (2 H, AB, J_{AB} 11.3, $CH_2C_6H_5$), 4.87 (2 H, AB, $CH_2C_6H_5$), 4.85 (1 H, q, *J* 8.6, 4-H), 4.84, 4.92 (2 H, AB, J_{AB} 11.7, $CH_2C_6H_5$) and 7.27–7.44 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 18.78, 18.91, 19.14, 19.20, 19.27 (5 t, CH_2CH_2CN), 62.11, 62.18, 62.66 (3 t, CH_2CH_2CN), 72.33, 74.34, 77.26 (3 t, $CH_2C_6H_5$), 74.63, 75.25, 78.04, 78.36, 78.46, 79.17 (6 d, inositol ring C), 116.41, 116.51, 116.57, 116.83, 116.96 (5 s, CN), 126.40, 127.60, 127.76, 128.05, 128.18, 128.31, 128.48 (7 d, $CH_2C_6H_5$), 136.86, 137.72 and 137.85 (3 s, $CH_2C_6H_5$); δ_P($CDCl_3$; 36 MHz) 66.83, −2.83 and −3.10; *m/z* FAB$^+$ 1025 [(M + H)$^+$, 1.3%], 181 (6), 144 (10) and 91 [$(C_7H_7)^+$, 100]; *m/z* FAB$^-$ 970 [(M − CH_2CH_2CN)$^-$, 45%], 219 (30), 203 (100), 150 (90) and 97 (80) [Found: (M + H)$^+$, 1025.2475. Calc. for $C_{45}H_{52}O_{14}N_6P_3S$ (M + H)$^+$, 1025.2475].

(±)-myo-*Inositol* 1-*Phosphorothioate* 4,5-*Bisphosphate* **4ab**.—Ammonia was condensed into a three-neck flask at −78 °C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three-neck flask and kept at −78 °C. Sodium was added until the solution remained blue. Compound **12ab** (120 mg, 117 μmol) was dissolved in dry dioxane (2 cm^3) and added to the sodium–liquid ammonia mixture. After stirring for 15 min the reaction was quenched by adding ethanol to the mixture, which became colourless. The ammonia was evaporated and the crude product taken up in water. The aqueous solution was treated with Dowex resin (H^+) until a pH of 6 was reached. The resin was filtered off and washed well with water. A few drops of triethylamine were added to the filtrate which was then evaporated to dryness. The crude product was purified by ion-exchange chromatography on DEAE Sephadex A-25 eluting with a gradient of triethylammonium hydrogen carbonate buffers (0.1–1 mol dm^{-3}), pH 8.0. The triethylammonium salt of **4ab** eluted at approx. 800 mmol dm^{-3} and after evaporation of TEAB the product was obtained as a glass. Yield 54 μmol (46%); δ_H(D_2O; pH 8; 300 MHz), 3.62 (1 H, dd, *J* 9.8, 2.5, 3-H), 3.78 (1 H, dd, *J* 9.5, 9.5, 6-H), 4.01 (1 H, q, *J* 9.2, 5-H), 4.20–4.11 (2 H, m, 2-H, 1-H) and 4.25 (1 H, q, *J* 9.4, 4-H); δ_P(D_2O; pH 8; 36 MHz) 42.13, 4.58 and 3.50; *m/z* FAB$^+$ 538 [(M + Et_3NH)$^+$, 10%], 436 (M$^+$, 3) and 102 (Et_3NH^+, 100) (Found: M$^+$, 538.0678. Calc. for $C_{12}H_{31}O_{14}NP_3S$ (M + Et_3NH)$^+$ 538.0678).

S-{2-[N-*Methyl*-N-(7-*nitro*-2,1,3-*benzoxadiazol*-4-*yl*)*amino*]-*ethoxycarbonylmethyl*}-(±)-myo-*inositol* 1-*Phosphorothioate* 4,5-*Bisphosphate* **20**.—A mixture of **4ab** (30 μmol) and 4-{*N*-[2-(iodoacetoxy)ethyl]-*N*-methylamino}-7-nitro-2,1,3-benzoxadiazole (15 mg, 33 μmol) in ethanol was shielded from light and stirred for 2 h at 0 °C. The product was purified by ion-exchange chromatography on DEAE sephadex A-25 eluting with a gradient of triethylammonium hydrogen carbonate buffers (0.1–1 mol dm^{-3}), pH 8.0. **20** Eluted at *ca.* 800 mmol dm^{-3} and was obtained as a dark orange glass after evaporation of TEAB. Yield 17 μmol (57%); δ_P(D_2O; pH 7.7; 36 MHz; ^{1}H-coupled) 1.72 (d, *J* 8.5), 3.01 (d, *J* 8.5) and 19.86 (q, *J* 9.1); *m/z* (+ve ion FAB) 815 [(M + Et_3NH)$^+$, 3%], 799 [(M + Et_3NH −O)$^+$,

5], 714 (M^+, 12), 698 [$(M - O)^+$, 14], 596 [$(Ins(1,4,5)P_3S - CH_2CO_2 + Et_3NH)^+$, 27] and 102 ($Et_3NH^+$, 100).

Bis-(+)-ω-*Camphanate of* 1D-(+)-1-O-*Allyl*-2,3,6-*tri*-O-*benzyl*-myo-*inositol* **6a**.—A mixture of (±)-1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol (3.432 g, 7 mmol) and (−)-ω-camphanic acid chloride (6.067 g, 28 mmol) in dry pyridine (50 cm^3) was stirred for 12 h at room temp. The solution was cooled in ice–water, water (0.5 cm^3) was added, and the solution was stirred for another 1 h at room temp., after which HPTLC (ether–light petroleum, 1:1) showed two products (R_f 0.52 and 0.42). Ether (100 cm^3) and dichloromethane (50 cm^3) were added and the organic phase was washed successively with saturated aqueous potassium chloride, ice-cold 1 mol dm^{-3} hydrochloric acid, saturated aqueous potassium chloride and saturated aqueous sodium hydrogen carbonate (200 cm^3 each) and then dried ($MgSO_4$). Evaporation of the solvents gave a syrup, which was taken up in ether (40 cm^3) and kept at −20 °C overnight. The crystals formed (1.2 g) were filtered off, the mother liquor evaporated, and the residue was dissolved in a mixture of ether (20 cm^3) and methanol (5 cm^3) to give more crystals (1 g). Overall yield: 2.2 g (2.6 mmol, 74%) of **6a**; R_f 0.42; m.p. 142–143 °C (from ethyl acetate–hexane); $[\alpha]_D^{21}$ +19.4 (*c* 5 in $CHCl_3$) (Found: C, 70.4; H, 6.75. Calc. for $C_{50}H_{58}O_{12}$: C, 70.57; H, 6.87%); δ_H($CDCl_3$; 300 MHz) 0.747 (3 H, s, CH_3), 0.753 (3 H, s, CH_3), 0.91 (3 H, s, CH_3), 0.95 (3 H, s, CH_3), 1.03 (6 H, s, CH_3), 1.59–1.67 (2 H, m, CH_2), 1.77–1.88 (4 H, m, CH_2), 2.27–2.39 (2 H, m, CH_2), 3.44 (1 H, dd, *J* 9.7, 2.1, 3-H), 3.55 (1 H, dd, *J* 10.7, 2.1, 1-H), 3.96–4.09 (2 H, m, $CH_2CH{=}CH_2$), 4.14 (1 H, dd, *J* 2.0, 2.0, 2-H), 4.17 (1 H, dd, *J* 9.6, 9.6, 6-H), 4.45, 4.59 (2 H, AB, J_{JB} 11.5, $CH_2C_6H_5$), 4.85, 4.87 (2 H, AB, J_{AB} 12.1, $CH_2C_6H_5$), 4.62, 5.02 (2 H, AB, J_{AB} 11.3, $CH_2C_6H_5$), 5.18 (1 H, ddt, *J* 10.4, 1.4, 1.4, *cis*-$CH_2CH{=}CH_2$), 5.27 (1 H, ddt, *J* 17.2, 1.6, 1.6, *trans*-$CH_2CH{=}CH_2$), 5.34 (1 H, dd, *J* 9.6, 9.6, 5-H), 5.76 (1 H, dd, *J* 9.9, 9.9, 4-H), 5.83 (1 H, ddt, *J* 17.2, 10.4, 5.7, $CH_2CH{=}CH_2$) and 7.20 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 16.28, 16.54, 16.64 (3 q), 28.80, 30.76 (2 t), 54.00, 54.78 (2 s), 71.39, 71.84, 74.15 (3 t, $CH_2CH{=}CH_2$ and $CH_2C_6H_5$), 72.82, 73.37, 74.08, 78.07, 78.23, 80.18 (6 d, inositol ring C), 91.05 (s), 117.45 (t, $CH_2CH{=}CH_2$), 126.85, 127.24, 127.34, 127.708, 127.83, 127.96, 128.18, 128.28, 128.39 (9 d, $CH_2C_6H_5$), 134.12 (d, $CH_2CH{=}CH_2$), 137.04, 138.17 (2 s, $CH_2C_6H_5$), 166.36, 166.62 and 177.91 (3 s); *m/z* FAB^+ 851 [$(M + H)^+$, 7%], 181 (13), 109 (8) and 91 (100).

Bis-(−)-ω-*Camphanate of* 1L-(−)-1-O-*Allyl*-2,3,6-*tri*-O-*benzyl*-myo-*inositol* **6b**.—The mother liquor left from the crystallisation of **6a** was kept at −20 °C for several days when a solid had formed at the bottom of the flask. The supernatant was filtered off and the solid dissolved in hot ether. After leaving the solution in the fridge for two days crystals had formed which were collected to give **6b** (2.4 g, 2.8 mmol, 80%); R_f 0.52; m.p. 174–177 °C (from ethyl acetate–hexane); $[\alpha]_D^{18}$ −25.0 (*c* 4.2 in $CHCl_3$) (Found: C, 70.7; H, 6.8. Calc. for $C_{50}H_{58}O_{12}$: C, 70.57; H, 6.87%); δ_H($CDCl_3$; 270 MHz) 0.81 (3 H, s, CH_3), 0.82 (3 H, s, CH_3), 0.96 (3 H, s, CH_3), 0.98 (3 H, s, CH_3), 1.04 (6 H, s, CH_3), 1.57–1.65 (2 H, m, CH_2), 1.76–1.98 (4 H, m, CH_2), 2.16–2.26 (2 H, m, CH_2), 3.39 (1 H, dd, *J* 9.9, 2.0, 3-H), 3.56 (1 H, dd, *J* 9.9, 2.0, 1-H), 4.01 (2 H, d, *J* 5.3, $CH_2CH{=}CH_2$), 4.05 (1 H, dd, *J* 1.5, 1.5, 2-H), 4.16 (1 H, dd, *J* 9.8, 9.8, 6-H), 4.47, 4.54 (2 H, AB, J_{AB} 11.7, $CH_2C_6H_5$), 4.63, 4.95 (2 H, AB, J_{AB} 11.2, $CH_2C_6H_5$), 4.86, 4.86 (2 H, AB, J_{AB} 12.4, $CH_2C_6H_5$), 5.18 (1 H, ddt, *J* 10.4, 1.4, 1.4, *cis*-$CH_2CH{=}CH_2$), 5.27 (1 H, ddt, *J* 17.2, 1.6, 1.6, *trans*-$CH_2CH{=}CH_2$), 5.34 (1 H, dd, *J* 9.6, 9.6, 5-H), 5.76 (1 H, dd, *J* 9.9, 9.9, 4-H), 5.83 (1 H, ddt, *J* 17.2, 10.4, 5.7, $CH_2CH{=}CH_2$) and 7.22–7.42 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 16.28, 16.44, 16.54 (3 q), 28.70, 31.10 (2 t), 53.58, 53.74, 54.56, 54.62 (4 s), 71.42, 71.97, 72.26, 74.79 (4 t, $CH_2CH{=}CH_2$ and $CH_2C_6H_5$), 72.26, 73.56, 74.21, 77.81, 78.49, 80.02 (6 d, inositol ring C), 90.66 (s), 117.16 (t, $CH_2CH{=}CH_2$), 126.95, 127.42, 127.53, 127.66, 127.86, 128.12, 128.31 (7 d, $CH_2C_6H_5$), 134.21 (d, $CH_2CH{=}CH_2$), 137.23, 138.17, 138.24 (3 s, $CH_2C_6H_5$), 166.65, 177.87 and 178.00 (3 s); *m/z* as **6a**.

1D-(+)-1-O-*Allyl*-2,3,6-*tri*-O-*benzyl*-myo-*inositol* **5a**.—The (+)-biscamphanate **6a** (1.26 g, 1.48 (mmol) was dissolved in methanol (100 cm^3) containing NaOH (1.3 g). The solution was heated under reflux for 1 h when TLC (ether) showed complete conversion of the starting material (R_f 0.79) to a single product (R_f 0.59). After cooling, the solution was neutralised with solid CO_2. Water (100 cm^3) was added and the solution extracted twice each with chloroform (100 cm^3). The organic layers were dried ($MgSO_4$) and the solvent evaporated to give **5a** (703 mg, 1.44 mmol, 97%); m.p. 97–98 °C (lit.,[42] 98 °C) (Found: C, 73.4; H, 6.9. Calc. for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99%); $[\alpha]_D^{18}$ +21.5 (*c* 4 in $CHCl_3$), [lit.,[42] $[\alpha]_D$ +20 ($CHCl_3$)]; δ_H($CDCl_3$; 270 MHz) 2.56, 2.60 (2 H, 2 br s, D_2O ex, OH), 3.20 (1 H, dd, *J* 9.7, 2.2, 3-H), 3.28 (1 H, dd, *J* 9.7, 2.2, 1-H), 3.42 (1 H, dd, *J* 9.3, 9.3, 5-H), 3.87 (1 H, dd, *J* 9.4, 9.4, 6-H), 4.04 (1 H, dd, *J* 9.5, 9.5, 4-H), 4.06 (1 H, dd, *J* 2.5, 2.5, 2-H), 4.10 (2 H, ddd, *J* 5.3, 1.4, 1.4, $CH_2CH{=}CH_2$), 4.55, 4.61 (2 H, AB, J_{AB} 11.7, $CH_2C_6H_5$), 4.75, 4.96 (2 H, AB, J_{AB} 11.9, $CH_2C_6H_5$), 4.79, 4.89 (2 H, AB, J_{AB} 11.2, $CH_2C_6H_5$), 5.19 (1 H, ddt, *J* 10.4, 1.4, 1.4, *cis*-$CH_2CH{=}CH_2$), 5.31 (1 H, ddt, *J* 17.2, 1.6, 1.6, *trans*-$CH_2CH{=}CH_2$), 5.91 (1 H, ddt, *J* 17.2, 10.4, 5.3, $CH_2CH{=}CH_2$) and 7.25–7.42 (15 H, m, $CH_2C_6H_5$); δ_C($CDCl_3$; 68 MHz) 71.45, 72.26, 74.02, 75.35 (4 t, $CH_2CH{=}CH_2$ and $CH_2C_6H_5$), 72.20, 73.53, 74.60, 79.95, 80.76, 80.83 (6 d, inositol ring C), 116.73 (t, $CH_2CH{=}CH_2$), 127.37, 127.63, 127.70, 127.79, 127.96, 128.12, 128.38, 128.44 (8 d, $CH_2C_6H_5$), 134.67 (d, $CH_2CH{=}CH_2$), 137.85 and 138.76 (2 s, $CH_2C_6H_5$); *m/z* (70 eV EI) 399 [$(M - C_7H_7)^+$, 2%], 307, 181 (5), 131 (10), 109 (5) and 91 [$(C_7H_7)^+$, 100%]; *m/z* (CI, Isobutane) 491 $(M + H)^+$, 399 (10), 309, 181 (20), 131 (20), 107 (100), 91 (80) and 69 (20).

1L-(−)-1-O-*Allyl*-2,3,6-*tri*-O-*benzyl*-myo-*inositol* **5b**.—The (−)-biscamphanate **6b** (2.37 g, 2.79 mmol) in methanol (200 cm^3) containing NaOH (2.4 g) was heated under reflux for 1 h. Work-up as for **6a** gave **5b** (1.36 g, 2.78 mmol, 100%); m.p. 96–98 °C (from ethanol) (lit.,[29] 96–98 °C) (Found: C, 73.5; H, 6.95. Calc. for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99%); $[\alpha]_D^{19}$ −21.9 (*c* 4.3 in $CHCl_3$) [lit.,[29] $[\alpha]_D^{26}$ −20.5 (*c* 1 in $CHCl_3$)]. Mass spectral and NMR spectroscopic data were identical to **5a**.

1D-(+)-2,3,6-*Tri*-O-*benzyl*-1-O-(cis-*prop*-1-*enyl*)-myo-*inositol* **8a**.—Compound **5a** (840 mg, 1.71 mmol) and freshly sublimed potassium *tert*-butoxide (778 mg, 6.84 mmol) in dry DMSO (30 cm^3) was stirred for 3 h at 50 °C. The solution was worked up as described for the racemic compound to give **8a** (815 mg, 1.66 mmol, 97%); m.p. 116–118 °C (from ethanol–water) (Found: C, 73.5; H, 7.0. Calc. for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99%); $[\alpha]_D^{11}$ +40.6 (*c* 4 in $CHCl_3$). Mass spectra and NMR spectroscopic data were identical to **8ab**.

1L-(−)-2,3,6-*Tri*-O-*benzyl*-1-O-(cis-*prop*-1-*enyl*)-myo-*inositol* **8b**.—Compound **5b** (546 mg, 1.11 mmol) and freshly sublimed potassium *tert*-butoxide (505 mg, 4.44 mmol) in dry DMSO (20 cm^3) was stirred for 3 h at 50 °C. The solution was worked up as described for the racemic compound to give **8b** (536 mg, 1.09 mmol, 98%); m.p. 117–119 °C (from ethyl acetate–hexane) (Found: C, 73.6; H, 7.0. Calc. for $C_{30}H_{34}O_6$ C, 73.45; H, 6.99%); $[\alpha]_D^{19}$ −41.1 (*c* 4.3 in $CHCl_3$). Mass spectra and NMR spectroscopic data were identical to **8ab**.

Elucidation of the Absolute Configuration of **5b**.—Compound **5b** was first isomerised to the *cis*-prop-1-enyl compound **8b** which was then deprotected to the known triol 1D-(−)-1,2,4-tri-

O-benzyl-*myo*-inositol. Compound **8b** (413 mg, 843 μmol) in MeOH–1 mol dm^{-3} HCl (5:1, 10 cm^3) was heated under reflux for 30 min after which TLC (ether) showed complete conversion of the starting material (R_f 0.90) to a single product (R_f 0.59). The solution was allowed to cool and an excess of $NaHCO_3$ was added. The solvent was evaporated and the residue was extracted with chloroform, dried and the solvent evaporated to give 1D-(−)-1,2,4-(tri-*O*-benzyl-*myo*-inositol (329 mg, 731 μmol, 87%); m.p. 116–118 °C (from ethanol–water) (lit.,[29] 118–120 °C; lit.,[41] 117–119 °C for the enantiomer); $[\alpha]_D^{18}$ −10.1 (*c* 2.5 in $CHCl_3$) (lit.,[29] −9.0; lit.,[41] +15.5 for the enantiomer); $\delta_H(CDCl_3$; 400 MHz) 2.3–2.7 (3 H, br s, D_2O ex, 3 OH), 3.27 (1 H, dd, *J* 9.7, 2.6, 1-H), 3.46 (1 H, dd, *J* 9.1, 9.1, 5-H), 3.52 (1 H, dd, *J* 9.5, 2.6, 3-H), 3.68 (1 H, dd, *J* 9.2, 9.2, 4-H), 4.01 (1 H, dd, *J* 9.5, 9.5, 6-H), 4.07 (1 H, dd, *J* 2.6, 2.6, 2-H), 4.58, 4.68 (2 H, AB, J_{AB} 11.7, $CH_2C_6H_5$), 4.83, 4.87 (2 H, AB, J_{AB} 11.5, $CH_2C_6H_5$), 4.72, 4.92 (2 H, AB, J_{AB} 11.3, $CH_2C_6H_5$) and 7.28–7.33 (15 H, m, $CH_2C_6H_5$).

1D-(+)-2,3,6-*Tri*-O-*benzyl*-4,5-*bis*[*bis*(2-*cyanoethoxy*)*phospho*]-1-O-(cis-*prop*-1-*enyl*)-myo-*inositol* **10a**.—To a mixture of **8a** (348 mg, 0.71 mmol) and tetrazole (615 mg, 8.8 mmol) in dry dichloromethane (20 cm^3) was added bis(2-cyanoethoxy)-diisopropylaminophosphine (1.9 g, 7.1 mmol). After stirring at room temp. for 1 h water in THF (10% v/v) was added and the solution was stirred for another 30 min. 2,6-Lutidine (0.5 cm^3) and *tert*-butyl hydroperoxide (5 cm^3) was then added and stirring continued overnight. Work-up as for compound **8** gave **10a** (485 mg, 0.57 mmol, 80%); m.p. 131–132 °C (from ethanol) (Found: C, 58.6; H, 5.65; N, 6.5. Calc. for $C_{42}H_{48}N_4O_{12}P_2$: C, 58.47; H, 5.61; N, 6.49%); $[\alpha]_D^{19}$ +11.5 (*c* = 3.5 in $CHCl_3$). Mass spectra and NMR spectroscopic data were identical to those for **8ab**.

1D-(+)-2,3,6-*Tri*-O-*benzyl*-4,5-*bis*[*bis*-(2-*cyanoethoxy*)*phospho*]-myo-*inositol* **11a**.—Compound **10a** (200 mg, 232 μmol) was heated under reflux with 1 mol dm^{-3} HCl–methanol (1:5; 10 cm^3) and work-up as for the racemic compound gave **11a** (183 mg, 96%) as a syrup; $[\alpha]_D^{15}$ +8.5 (*c* 4.5 in $CHCl_3$); the chiral material has been alluded to in preliminary form[42] with $[\alpha]_D$ +5 ($CHCl_3$). Mass spectra and NMR spectroscopic data were identical to those for **11ab**.

1D-(+)-2,3,6-*Tri*-O-*benzyl*-1-O-[*bis*-(2-*cyanoethoxy*)*thiophospho*]-4,5-*bis*-[*bis*-(2-*cyanoethoxy*)*phospho*]-myo-*inositol* **12a**.—Compound **11a** (152 mg, 185 μmol) was phosphitylated and sulfoxidised analogously to the racemic compound to give **12a** (140 mg, 137 μmol, 74%) after column chromatography. $[\alpha]_D^{16}$ +10.5 (*c* 2.5 in $CHCl_3$). Mass spectra and NMR spectroscopic data were identical to those for **12ab**.

1D-(−)-myo-*Inositol* 1-*Phosphorothioate* 4,5-*Bisphosphate* **4a**.—Compound **12a** (50.6 mg, 49.4 μmol) was deprotected as for the racemic compound to give pure **4a** (21.5 μmol, 44%) after ion-exchange chromatography; $[\alpha]_D^{21}$ −42.7 (*c* 0.19 in H_2O, pH 9.4). Mass spectra and NMR spectroscopic data were identical to those for **4ab**.

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