Tetrahedron Vol. 47, No. 23, pp. 3895-3908, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

Synthesis of D- and L-*myo*-Inositol 1-phosphorothioate, Substrates for Inositol Monophosphatase

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(*Received in UK* 14 *February* 1991)

Abstract: The D- and L- enantiomers of *myo*-inositol 1-phosphorothioate have been synthesized from 2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol in 6 steps, both compounds are substrates for inositol monophosphatase. D-glucopyranose 6-phosphorothioate did not serve as a substrate for the enzyme inositol synthase in an alternative synthesis of L-*myo*-inositol 1-phosphorothioate.

In recent years many aspects of the metabolic pathways associated with the release of calcium from intracellular stores during signalling processes have been defined.[1] An important and key step involves the hydrolysis of inositol 1-phosphate to give free inositol.[2] The free inositol is recycled to provide the precursor, phosphatidylinositol 4,5-bisphosphate, for the two secondary messengers, diacylglycerol and inositol 1,4,5-trisphosphate.

The hydrolysis is catalysed by the lithium sensitive phosphoesterase inositol monophosphatase. The enzyme is capable of hydrolysing both enantiomers of *myo*-inositol 1-phosphate and *myo*-inositol 4-phosphate although the kinetic parameters, V_{max} and K_m for each substrate are different The modes of inhibition by lithium cation are similar for the 1- and 4-phosphates, both show apparent uncompetitive inhibition, but, the K_i value for inositol 4-phosphate (0.11 mM) is 7-fold lower than that for the 1-phosphate.[3,*cf.*4]

Recently we suggested that the enzyme might operate *via* a substituted enzyme mechanism in which the phosphate group of the substrate was first transferred to an enzyme bound nucleophile, and then, in a subsequent step, to water.[5] Based on the observation of the burst-phase formation of [^{14}C]-inositol from [^{14}C]-inositol 1-phosphate at high [Li^+], together with the established uncompetiive mode of lithium cation inhibition, we further suggested that Li^+ might act by retarding

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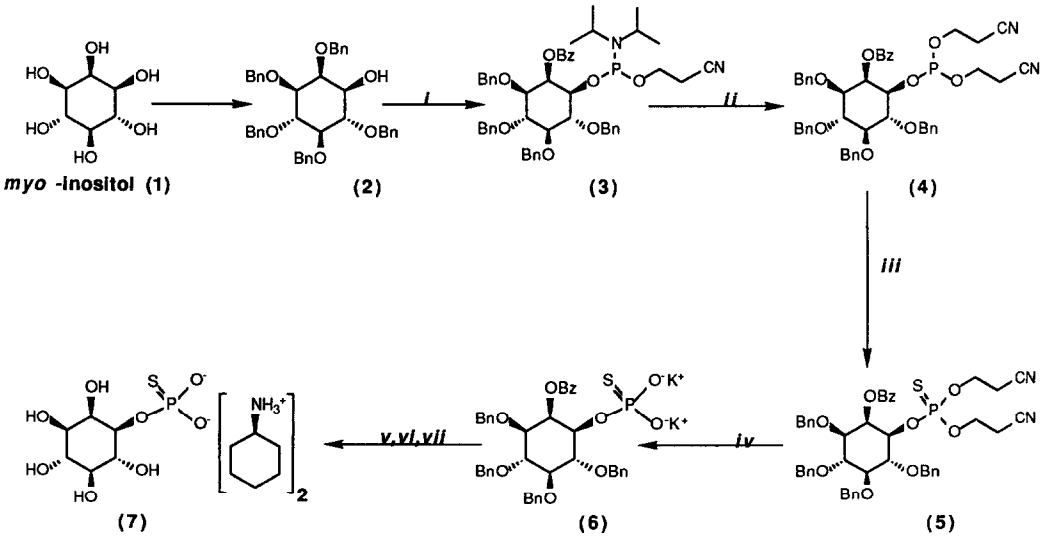
the rate of conversion of the phosphoryl enzyme to free enzyme, relative to the rate of phosphorylated enzyme formation.

In order to further probe the mechanism of the phosphatase, we sought substrates which might display more pronounced differences in the rates of formation and break-down of the putative phosphorylated enzyme intermediate.

Here we report on the synthesis of both enantiomers of *myo*-inositol 1-phosphorothioate from inositol. In an alternative synthesis of the L-enantiomer, D-glucopyranose 6-phosphorothioate did not serve as a substrate for inositol synthase.

Racemic 2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol (2) was prepared from *myo*-inositol (1) in six steps using literature procedures.[6] Reaction of the alcohol (2) in dichloromethane with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite in the presence of diisopropylethylamine under argon gave a solution of the pentakis-*O*-benzyl *myo*-inositol 1-phosphoramidite ester (3). This was treated *in situ* with 3-hydroxypropionitrile and 1*H*-tetrazole to afford the phosphite triester (4). The 146 MHz ^{31}P-nmr spectrum showed a new signal at 139 ppm and the ^{1}H-nmr and mass spectra of the compound were consistent with the expected structure. Without purification, the triester was dissolved in pyridine and was treated with sulphur, essentially using the method of Burgers and Eckstein[7] to give the pentakis-*O*-benzyl *myo*-inositol 1-phosphorothioate triester (5) in 75 % overall yield from the alcohol.



i) $iPr_2NClPOCH_2CH_2CN$, iPr_2NEt, CH_2Cl_2, *ii)* $HOCH_2CH_2CN$, 1H-Tetrazole, CH_3CN, *iii)* S_8, Pyridine, *iv)* MeOK, MeOH, *v)* Na, NH_3, *vi)* Amberlite IR 118 [H^+], H_2O, *vii)* $C_6H_{13}N$

Scheme 1

The phosphorothioate triester was converted to the dipotassium salt (6) quantitatively *via* base catalysed β-elimination of the phosphate moiety from both cyanoethyl groups. Reductive cleavage of the five benzyl protecting groups with sodium and ammonia in tetrahydrofuran gave the disodium salt of *myo*-inositol 1-phosphorothioate[8] which was purified by ion exchange chromatography on Amberlite IR118H and then converted to the crystalline *bis*-cyclohexylammonium salt (7), in 70 % overall yield from the protected inositol (2).

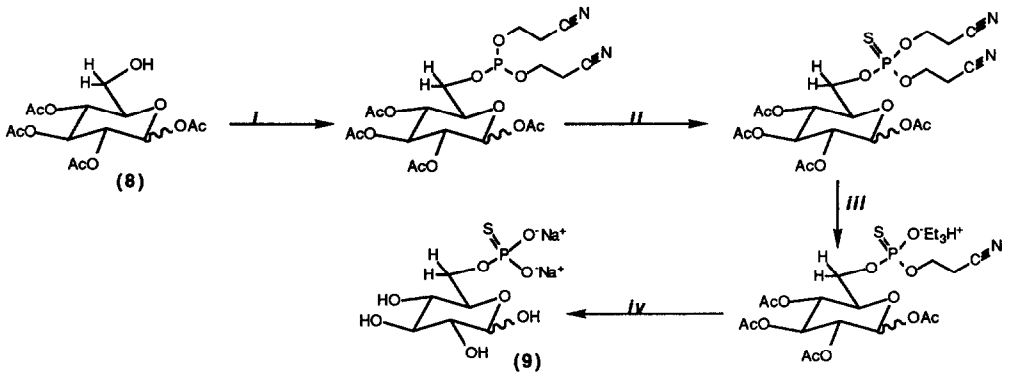
To synthesise the enantiomers of the phosphorothioate (7), the racemic 2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol (2) was resolved by preparing, chromatographically separating, and then saponifying the diastereomeric (1S,4R)-camphanate ester derivatives using established procedures.[6] Treatment of each separate enantiomer in the manner outlined above gave the *bis*-cyclohexylammonium salt of the chiral inositol 1-phosphorothioates in 57 % overall yield from the resolved alcohols.

In order to test the phosphorothioates as inhibitors for inositol monophosphatase, the compounds were added at various concentrations to standard activity assay incubations[2] containing inositol 1-phosphate (1 mM). The rates of phosphate ester hydrolysis were deduced and analysis of the double reciprocal plots indicated that each of the enantiomers acted as a competitive inhibitor, K_i = 1 mM for each antipode.

In order to test the phosphorothioates as substrates for the enzyme, the phosphorothioates, together with Mg^{2+} ions (2 mM) and ammonium bicarbonate buffer (20 mM), were incubated at 37 °C and at pH 7.8, with pure inositol monophosphatase. After 18 hours, the protein in each incubation was denatured with ethanol and was then removed by filtration. Examination of the lyophilised filtrate by ^{1}H-nmr spectroscopy indicated that each of the phosphorothioates were converted to inositol, but more slowly than inositol 1-phosphate. The phosphorothioate ester hydrolyses were inhibited by lithium cation, and control experiments which contained the phosphorothioates but no enzyme, showed no reaction or decomposition at all. Thus, both of the phosphorothioates were substrates and would be suitable as probes for a kinetic and mechanistic study of the phosphatase.

To facilitate the introduction of labels into the inositol and phosphorothioate moieties of the new substrates, and specifically to allow the synthesis of the ^{14}C-compound and compounds containing a chiral phosphorothioate group, a new synthesis was examined. If glucopyranose 6-phosphorothioate could be converted to inositol 1-phosphorothioate by inositol synthase, an enzyme which usually catalyses the analogous reaction[9] with phosphate substrates, then the synthetic problems would be reduced to preparing suitably labelled glucopyranose 6-

phosphorothioates, Scheme 2. Note that, secondary alcohols, for example, the free 1-hydroxyl group of protected inositol, react very slowly with Lowe's (chiral) thiophosphorylating agent, however primary alcohols, for example, the 6-hydroxyl group of glucose, react quickly.[10]



i) $iPr_2NP(OCH_2CH_2CN)_2$, 1*H*-Tetrazole, CH_3CN, *ii)* S_8, Pyridine; *iii)* 3:1 Pyridine·Et_3N, *iv)* MeONa, MeOH

Scheme 2

Accordingly, 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (8) was prepared by the method of Stacey[11] and was converted to D-glucopyranose 6-phosphorothioate disodium salt (12) as outlined in Scheme 2. All spectral and analytical data for the compound and the intermediates was consistent with the expected structures.

Incubation of compound (12) with partially purified inositol synthase from bovine testes at pH 7.7 and at 37 °C in the presence of a catalytic amount of NAD, surprisingly, did not give inositol 1-phosphorothioate (see experimental section). Under similar conditions D-glucopyranose 6-phosphate was rapidly converted to L-*myo*-inositol 1-phosphate. This is a curious result and suggests that the phosphate moiety serves an important role in the synthase reaction Nevertheless, the finding excludes the possibility of using the synthase in the synthesis of inositol 1-phosphorothioate as outlined in Scheme 2.

In summary, the enantiomers of inositol 1-phosphorothioate have been synthesized and have been shown to be slow substrates, and therefore suitable probes for the inositol monophosphatase reaction. The future preparation of labelled phosphorothioate substrates will need to follow the chemical synthesis, Scheme 1, since D-glucopyranose 6-phosphorothioate is not a substrate for inositol synthase.

Experimental:

Melting points were determined using an electrothermal melting point apparatus and are uncorrected. ^{1}H-, ^{13}C- and ^{31}P-nmr spectra were recorded on Jeol FX90Q, Jeol JNM-GX270 and Bruker AM-360 instruments as indicated. Chloroform, TMS or the sodium salt of 3-(trimethylsilyl)-propionic acid-2,2,3,3-$^{2}H_4$ were used as reference standards for ^{1}H-nmr spectra, chloroform or methanol were used to reference ^{13}C-nmr spectra and external 85 % H_3PO_4 was used to reference ^{31}P-nmr spectra. Mass spectra were obtained using AEI-MS30 or VG 7-70 spectrometers. Microanalysis facilities were provided on a service basis by University College London, UK. Specific rotations were determined on an Optical Activity Ltd. AA-100 polarimeter using 5 cm path-length cells. Petroleum ether refers to the fraction boiling at 60-80 °C.

(±)-*cis*--1,2-*O*-cyclohexylidene *myo*-inositol: *myo*-Inositol (10 g, 55.6 mmol) was suspended in cyclohexanone (100 ml), toluene (50 ml) and DMF (10 ml). The mixture was refluxed in a Dean-Stark apparatus for 1 h to dry the reagents. *p*-Toluene sulphonic acid (75 mg) was added and the reaction was refluxed for 18 h when no further water was collected. The clear solution was cooled to 35 °C and toluene (50 ml), petroleum ether (50 ml) and ethanol (25 ml) were added. To this solution was added *p*-toluene sulphonic acid (0.6 g). A precipitate began to form immediately and the solution was allowed to stand at 4 °C for 2 h. Triethylamine (0.6 ml) was added and the mixture was allowed to stand 24 h at -20 °C. The suspension was filtered and the resulting paste was heated on a steam bath for 1 h in ethanol (200 ml) and triethylamine (1 ml). The solution was cooled and the crystalline product was collected by filtration (10.6 g, 40.8 mmol, 74%), m.p. 181-183 °C (dec., phase transition 160-163 °C); ν_{max} (nujol) 3000-3600 sb (OH), 1240 s, 1140 s, 1020 m cm^{-1}; δ_H (270 MHz; D_2O) 1.10-1.80 (10H, m, C_6H_{10}-C*H*$_2$), 3.27 (1H, t, $J_{5,6}$ 10.4 Hz, $J_{4,5}$ 9.3 Hz, 5-*H*), 3.58 (1H, dd, $J_{5,6}$ 10.4 Hz, $J_{1,6}$ 7.8 Hz, 6-*H*), 3.65 (1H, t, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 9.3 Hz, 4-*H*), 3.85 (1H, dd, $J_{2,3}$ 4.1 Hz, $J_{3,4}$ 9.8 Hz, 3-*H*), 4.05 (1H, dd, $J_{1,2}$ 4.8 Hz, $J_{1,6}$ 7.8 Hz, 1-*H*), 4.48 (1H, t, $J_{1,2}$ 4 8 Hz, $J_{2,3}$ 4.1 Hz, 2-*H*); δ_C (22.5 MHz; D_2O) 21.4, 21.7, 22.4, 32.7, 35.6 (C_6H_{10}-*C*H$_2$) 67 8, 70 4, 70.9, 73.3, 73.8, 76.3 (Ins-*C*H) 109.3 (C_6H_{10}-*C*(OR)$_2$); *m/z* (FAB, glycerol) 261 ($M+H^+$, 100%), 217 (4), 109 (3), 99 (40), 81 (9).

(±)-*cis*-1,2-*O*-cyclohexylidene-3,4,5,6-tetrakis-*O*-benzyl *myo*-inositol: To (±)-*cis*-1,2-*O*-cyclohexylidene *myo*-inositol (7.8 g, 30 mmol) were added benzyl chloride (80 ml, 70 mmol) and KOH (47.6 g, 85 mmol). The resulting suspension was refluxed under dinitrogen for 16 h. Toluene (50 ml) and water (150 ml) were added and after stirring for 15 min the phases were separated. The aqueous phase was extracted with toluene (50 ml) and the pooled organic phases were washed

with water (3 x 50 ml, until further washings were neutral), saturated brine (50 ml) and then dried over magnesium sulphate. The solvent was removed *in vacuo* to give the crude product as a oil in essentially quantitative recovery. A sample was purified by column chromatography on silica (10% ethyl acetate, petroleum ether) and was recrystallised from ethanol, m.p. 84-86 °C; (Found: C, 77.3; H, 7.2 $C_{40}H_{44}O_6$ requires: C, 77.4; H, 7.1 %); ν_{max} ($CHCl_3$) 2950 m, 1500 w, 1460 m, 1360 m, 1080 s, 930 m, 700 s cm^{-1}; δ_H (270 MHz; $CDCl_3$) 1.2-1.8 (10H, m, C_6H_{10}-*H*), 3.41 (1H, dd, $J_{5,6}$ 9.7 Hz, $J_{4,5}$ 8.4 Hz, 5-*H*), 3.68 (1H, dd, $J_{3,4}$ 8.6 Hz, $J_{2,3}$ 3.8 Hz, 3-*H*), 3.82 (1H, dd, $J_{5,6}$ 9.7 Hz, $J_{1,6}$ 7 1 Hz, 6-*H*), 3.94 (1H, t, $J_{4,5}$ 8.4 Hz, $J_{3,4}$ 8.6 Hz, 4-*H*), 4.19 (1H, dd, $J_{1,6}$ 7.0 Hz, $J_{1,2}$ 5.6 Hz, 1-*H*), 4 27 (1H, dd, $J_{1,2}$ 5.6 Hz, $J_{2,3}$ 3.8 Hz, 2-*H*), 4.7-5.0 (8H, m, Bz-CH_2), 7 2-7 4 (20H, m, Ar-*H*); δ_C (22.5 MHz; $CDCl_3$) 23.9, 24.2, 25.3, 35.1, 37.5 (C_6H_{10}-CH_2), 73.2, 73.9 (Bz-CH_2), 74.2 (Ins-*C*H), 74 8, 75.1 C_6H_{10}-CH_2), 77.4, 78.9, 81.2, 82.6, 82.9 (Ins-*C*H), 110.6 (*C*[OR]$_2$), 127.6, 127.8, 128 0, 128.4, 139.0 (Ar-*C*); *m/z* (CI, NH_3) 638 (M+NH_4^+, 1 %), 621 (M+H^+, 2), 529 (5), 423 (8), 181 (42), 91 (100).

(±)-3,4,5,6-tetrakis-*O*-benzyl *myo*-inositol: The crude (±)-*cis*-1,2-*O*-cyclohexylidene-3,4,5,6-tetrakis-*O*-benzyl *myo*-inositol was passed through a column of silica eluting with 30 % ethyl acetate, petroleum ether, to remove polar impurities, and the solvents were removed *in vacuo*. The resulting oil was refluxed in acetic acid (120 ml) and water (25 ml) for 2 h. The solvents were removed under reduced pressure and the residual oil was azeotropically dried using toluene. The crude product was subjected to suction chromatography on silica (80 x 40 mm) eluting with 20-40 % ethyl acetate in petroleum ether. The fractions containing the product were concentrated *in vacuo* to give a white solid which was recrystallised from methanol (10.4 g, 19.3 mmol, 75 %, two steps), m.p. 114-115 °C; (Found: C, 75.4; H, 6 7. $C_{34}H_{36}O_6$ requires: C, 75.5, H, 6.7 %); ν_{max} ($CHCl_3$) 3600 mb, 3010 m, 1500 m, 1460 m, 1370 m, 1140 s, 1070 vs, 700 s cm^{-1}, δ_H (270 MHz; $CDCl_3$) 1.6 (1H, s, RO*H*), 3.45 (3H, m, Ins-*H*), 3.84 (1H, t, Ins-*H*), 3.95 (1H, t, Ins-*H*), 4.21 (1H, m, 2-*H*), 4 7-5.0 (8H, m, Bz-CH_2), 7.2-7.4 (20H, m, Ar-*H*); δ_C (22.5 MHz, $CDCl_3$) 69.5, 72.1 (Ins-*C*H), 72 9, 75.7, 76.0 (Bz-CH_2), 80 3, 81.6, 81 8, 83.4 (Ins-*C*H), 127 6, 127.9, 128.0, 128.5, 128 6, 139.9 (Ar-*C*), *m/z* (CI, NH_3) 558 (M+NH_4, 1 %), 540 (M^+, 1), 449 (8), 359 (7), 269 (11), 181 (42), 91 (100)

(±)-1-*O*-allyl-3,4,5,6-tetrakis-*O*-benzyl *myo*-inositol: Following the method of David *et al.*,[12] (±)-3,4,5,6-tetrakis-*O*-benzyl *myo*-inositol (2.70 g, 5 mmol) in dry benzene (100 ml) was treated with dibutyl tin oxide (1.24 g, 5 mmol) The mixture was refluxed for 24 h under dinitrogen in a Dean-Stark apparatus. The volume of benzene was reduced to one half by distillation and the reaction was cooled to 60 °C. Tetrabutyl ammonium bromide (1.61 g, 5 mmol) and allyl bromide (1.3 ml, 15 mmol) were added and the reaction was stirred for 36 h when all the starting material had been converted to product as judged by tlc. Water (1 ml) was added and stirring was continued for 1 h. The solvents were removed *in vacuo* and the residual oil was purified twice by silica

column chromatography to give the pure product as an oil, (2.79 g, 4.8 mmol, 96 %), ν_{max} (neat) 3700-3300 bm, (OH) 2600-2300 s, 1500 m, 1460 s, 1370 s, 1080 bvs, 740 vs, 700 vs; δ_H (270 MHz; $CDCl_3$) 3.30 (1H, dd, J 9.7 Hz, J 2.7 Hz, Ins-*H*), 3.41 (1H, dd, J 9.5 Hz, J 2.5 Hz, Ins-*H*), 3.45 (1H, t, J 9.5 Hz, Ins-*H*), 3.96 (1H, t, J 9.8 Hz, Ins-*H*), 3.99 (1H, t, J 9.7 Hz, Ins-*H*), 4.19 (1H, d, $J_{1'\text{-}2'}$ 5.5 Hz,1'-H_2), 4.23 (1H, t, J 3 Hz, 2-*H*), 4.70-4.90 (8H, m, Bz-H_2), 5.18 (1H, dd, $J_{3'\ cis\text{-}2'}$ 10.4 Hz, $J_{3'\ cis\text{-}3'\ trans}$ 1.6 Hz, 3'-H_{cis}), 5.30 (1H, dd, $J_{3'\ trans\text{-}2'}$ 17.1 Hz, $J_{3'\ trans\text{-}3'\ cis}$ 1.6 Hz, 3'-H_{trans}), 5.93 (1H, ddt, $J_{2'\text{-}3'\ trans}$ 17.1 Hz, $J_{2'\text{-}3'\ cis}$ 10.4 Hz, $J_{2'\text{-}1'}$ 5.5 Hz, 2'-*H*), 7.2-7.4 (20H, m, Ar-*H*); δ_C (22.5 MHz; $CDCl_3$) 67.8 (2-*C*H), 72.0, 72.9, 76.0 (Bz-CH_2 & 1'-CH_2), 79.7, 80.0, 81.3, 83.2 (Ins-*C*H), 117.5 (3'-*C*), 127.6, 127.9, 128.1, 128.4 138.8 (Ar-*C*), 134.8 (2'-*C*); *m/z* (CI, NH_3) 599 ($M+NH_4^+$, 100 %), 581 ($M+H^+$, 22), 508 (13), 489 (21), 108 (50), 91 (42), 35 (97).

(±)-1-*O*-allyl-2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol: To a solution of (±)-1-*O*-allyl-3,4,5,6-tetrakis-*O*-benzyl *myo*-inositol (10.0 g, 17 mmol) in anhydrous DMF (400 ml) under dinitrogen was added sodium hydride (1.5 g, 55 % dispersion in oil, 34 mmol) and benzyl bromide (3.7 ml, 34 mmol). The reaction was stirred for 24 h until complete as judged by tlc. Water (50 ml) was added, cautiously, and the solvents were removed *in vacuo*. The residual oil was partitioned between water (100 ml) and dichloromethane (250 ml) and the organic phase collected. The aqueous phase was extracted with dichloromethane (3 x 50 ml) and the pooled organic phases were washed with saturated brine (250 ml) and dried over magnesium sulphate. The solvent was removed *in vacuo* and the residue was chromatographed on silica (15 % ethyl acetate in petroleum ether). The product was recrystallised from ethanol (11.4 g, 17 mmol, 97 %), m.p. 60-62 °C; (Found: C, 78.6; H, 6.9. $C_{44}H_{46}O_6$ requires: C, 78.8; H, 6.9 %); ν_{max} ($CHCl_3$) 3010 m, 2880 m, 1500 m, 1460 m, 1370 s, 1080 vs, 700 s cm^{-1}; δ_H (360 MHz; $CDCl_3$) 3.25 (1H, dd, J 9.8 Hz, J 2.3 Hz, Ins-*H*), 3.36 (1H, dd, J 9.9 Hz, J 2.3 Hz, Ins-*H*), 3.45 (1H, t, J 9.2 Hz, Ins-*H*), 4.0-4.1 (5H, m, 3 x Ins-H and 1'-H_2), 4.6-4.9 (10H, m, Bz-CH_2), 5.16 (1H, dd, $J_{3'\ cis\text{-}2'}$ 10.3 Hz, $J_{3'\ cis\text{-}3'\ trans}$ 1 6 Hz, 3'-H_{cis}), 5.30 (1H, dd, $J_{3'\ trans\text{-}2'}$ 17.3 Hz, $J_{3'\ trans\text{-}3'\ cis}$ 1.6 Hz, 3'-H_{trans}), 5.90 (1H, ddt, $J_{2'\text{-}3'\ trans}$ 17.3 Hz, $J_{2'\text{-}3'\ cis}$ 10.3 Hz, $J_{2'\text{-}1'}$ 5.3 Hz, 2'-*H*), 7.2-7.4 (25H, m, Ar-*H*); δ_C (22.5 MHz; $CDCl_3$) 71.8, 73.0, 74.3, 75.8 (Bz-CH_2 and 1'-CH_2), 74.9 (2-*C*H), 81.0, 81.2, 81.9, 84.0 (Ins-*C*H), 116.7 (2'-*C*H), 127.4, 127.7, 127.9, 128.1, 128.2, 128.3, 128.6, 139 2 (Ar-*C*), 135.2 (3'-CH_2); *m/z* (ACE) 671 (M+H, 20 %), 181 (16), 91 (100).

(±)-2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol (2): (±)-1-*O*-allyl-2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol (6.3 g, 9.4 mmol), Wilkinson's catalyst (0.9 g, 1 0 mmol) and DABCO (0.34 g, 3.0 mmol) were refluxed in 90 % aqueous ethanol (100 ml) with under dinitrogen for 3 h. The suspension was hot filtered and the pad was washed with absolute ethanol. Removal of the solvents *in vacuo* gave an oil which was refluxed in acetic acid (150 ml), water (50 ml) and THF (100 ml) for 4h. The solvents were removed *in vacuo* and the residue was chromatographed on silica (0-35 % ethyl

acetate in petroleum ether in 5 % steps). Recrystallisation of the combined product fractions from ethanol gave white crystals (5.5 g, 8.7 mmol, 93 %), m.p. 91-93 °C; (Found C, 77.9; H, 6.7. $C_{41}H_{42}O_6$ requires: C, 78.1; H, 6.7 %); ν_{max} ($CHCl_3$) 3570 bw (OH), 3020 m, 2880 m, 1505 m, 1460 s, 1370 s, 1140 s, 1070 vs, 700 vs cm^{-1}; δ_H (360 MHz; $CDCl_3$) 2.21 (1H, bs, RO*H*), 3.4-3.5 (3H, m, Ins-*H*), 3.80 (1H, t , J 9.4 Hz, Ins-*H*), 4.04 (2H, m, Ins-*H*), 4.6-5.0 (10H, m, Bz-CH_2), 7.1-7.3 (25H, m, Ar-*H*); δ_C (22.5 MHz; $CDCl_3$) 72.7 (Ins-*C*H), 73.2, 74.9, 75.5, 75.8, 75.9 (Bz-*C*H_2), 77.5, 81.4, 82.1, 82.4, 83.8 (Ins-*C*H), 127.6, 127.7, 127.9,128.1, 128.4, 128.5, 138.9 (Ar-*C*); *m/z* (ACE) 649 (M+NH_4, 17 %), 632 (M+H, 26), 540 (10), 181 (16), 91 (100).

(±)-2,3,4,5,6-pentakis-*O*-benzyl myo-inositol bis(2-cyanoethyl) phosphorothioate (5): To 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.4 ml, 6 mmol) at 0 °C under argon were added simultaneously dropwise the alcohol **(2)** (2.5 g, 4 mmol) and N,N-diisopropyl ethylamine (1 ml, 6 mmol), each in dichloromethane (15 ml). The reaction was stirred for 4 h and 3-hydroxypropionitrile (1.3 ml, 18 mmol) was added. The reaction mixture was stirred for a further 30 min and 1*H*- tetrazole (0.4 g, 6 mmol) in anhydrous acetonitrile (15 ml) was added. The reaction was stirred for a further 16 h and the solvent was removed *in vacuo*. The residue was partitioned between diethyl ether (100 ml) and water (50 ml). The aqueous fraction was extracted with diethyl ether (3 x 75 ml) and the pooled organic phases were washed with saturated brine (100 ml), dried with magnesium sulphate and reduced in volume *in vacuo*. The residual oil was dissolved in pyridine (100 ml) and sulphur (1.9 g, 60 mmol) was added. After 15 min the solvent was removed and the residue was extracted with toluene (15 ml). The sulphur precipitate was removed on a pad of glass wool, the solvent was removed *in vacuo* and the oil was reextracted with toluene (5 ml). The filtrate was purified by chromatography on a short silica column (0-40% ethyl acetate, petroleum ether in steps of 5 %). Recrystallisation from methanol yielded white crystals (2.4g, 2.9 mmol, 73 %), m.p. 97-98 °C; (Found C, 67.7; H, 5.9; N, 3.3; P, 3.9, S, 4.0. $C_{47}H_{49}N_2O_8PS$ requires: C, 67.8; H, 5.9; N, 3.4; P; 3.7; S, 3.85 %); ν_{max} ($CHCl_3$) 3050 w, 2900 w, 2270 vw (CN), 1505 m, 1460 m, 1370 m, 1080 vs, 1030 vs, 950 m, 800 m (PS), 700 m cm^{-1}; δ_H (360 MHz, $CDCl_3$) 2.22-2.58 (4H, m, 2'-*H*), 3.49 (1H, dd, $J_{2,3}$ 2.2 Hz, $J_{3,4}$ 9.8 Hz, 3-*H*), 3.53 (H, t, $J_{4,5\ \&5,6}$ 9 1 Hz, 5-*H*), 3.83-4.10 (6H, m, 1'-*H* and 4&6-*H*), 4.29 (1H, t, $J_{1,2}$ 2.5 Hz, $J_{2,3}$ 2.2 Hz, 2-*H*), 4.34 (1H, td, $J_{1,2}$ 2 5 Hz, $J_{1,6\ \&\ 1,P}$ 10.0 Hz, 1-*H*), 4.71-4.99 (10H, m, Bz-CH_2), 7.2-7.4 (25H, m, Ar-*H*), δ_C (22.5 MHz, $CDCl_3$) 18.9, 19.2 (2d, $J_{2'-P}$ 4 Hz, 2'-*C*), 62.4 (1'-*C*), 73.0, 74.9, 75.3, 75.6, 75 8 (Bz-*C*H_2), 76.5, 79.7 (t, J_{1-P} -5.4 and -6.7 Hz), 80.5, 81 4, 83.3 (Ins-*C*H), 116.4 (3'-*C*H), 127.6, 127.7, 128 3, 138.2, 138 5, 138.8 (Ar-*C*); δ_P (145 MHz; $CDCl_3$) 66.7 ppm; *m/z* (CI, NH_3) 850.8 (M+NH_4, 1 %), 833 (M+H, 1), 307 (1), 201 (7), 181 (16), 105 (32), 91 (100).

(±)-*myo*-inositol 1-phosphorothioate biscyclohexylammonium salt (7): To the triester **(5)** (0.832 g, 1 mmol) in dry methanol (10 ml) at 4 °C under argon was added a freshly prepared

solution of potassium methoxide (4 mmol) in dry methanol (10 ml). After stirring for 4 h, the solvents were removed *in vacuo*. The solid residue was redissolved in dry methanol (50 ml) and again, the solvents were removed *in vacuo*. The crude monoester dipotassium salt **(6)** which showed the expected nmr spectral parameters, was extracted into dry THF (3 x 30 ml) and was added slowly dropwise to a solution of sodium (50 mg) in liquid ammonia (100 ml) at -78 °C under argon with stirring. When the blue colouration of the solution disappeared, further small portions of sodium were added, followed by the monoester **(6)** until the colour persisted. After the final addition of **(6)**, the reaction was stirred for a further 30 min and then methanol (10 ml) was added, the ammonia was allowed to evaporate and the solvents were then removed *in vacuo*. The residue was dissolved in water (50 ml) which was removed *in vacuo* and was then partitioned between water (100 ml) and ether (50 ml). The aqueous layer was separated, reduced in volume to ~10 ml *in vacuo* and then subjected to chromatography on Amberlite IR-118H$^+$ ion-resin. The acidic eluant fractions containing the phosphorothioate were pooled and were treated with freshly distilled cyclohexylamine (3 ml) with stirring. The excess cyclohexylamine was removed by extracting the aqueous solution with ether (3 x 50 ml) and the dissolved ether was removed *in vacuo*. The aqueous solution was lyophilised and the residue was recrystallised from aqueous acetone to yield an amorphous white solid, (0.3 g, 0.63 mmol, 63 %), m.p. 165-170 °C (dec.); (Found C, 43.7; H, 8.5; N, 5.5; P, 6.2 S, 6.4. $C_{18}H_{39}N_2O_8PS.H_2O$ requires: C, 43.9; H, 8.4; N, 5.7; P; 6.3; S, 6.5 %); ν_{max} (nujol mull) 3500-2500 bs, 1608 m, 1523 m, 1114 s, 1040 s, 979 s, 811 s, 722 s; δ_H (360 MHz; D_2O) 1.10-2 00 (20H, m, $C_6H_{11}NH_3$-C*H*$_2$), 3.12 (2H, m, $C_6H_{11}NH_3$-C*H*), 3.33 (1H, t, $J_{5,6}$ 9.3 Hz, $J_{4,5}$ 9.0 Hz, 5-*H*), 3.56 (1H, dd, $J_{2,3}$ 2.8 Hz, $J_{3,4}$ 9.8 Hz, 3-*H*), 3.61 (1H, t, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 9.0 Hz, 4-*H*), 3.74 (1H, t, $J_{1,6}$ 9.9 Hz, $J_{5,6}$ 9.3 Hz, 6-*H*), 4.07 (1H, td, $J_{1,P}$ 11.0 Hz, $J_{1,6}$ 9.9 Hz, $J_{1,2}$ 2 7 Hz, 1-*H*), 4.27 (1H, t, $J_{1,2}$ 2 7 Hz, $J_{2,3}$ 2.8 Hz, 2-*H*); δ_C (67 MHz; D_2O) 22.0, 22.4, 28.5 (CHA-*C*H$_2$), 48.5 (CHA-*C*H) 69.0, 69.7, 70.3, 70.5, 72.6, 72.9 (Ins-*C*H); δ_P (146 MHz; D_2O) 44.9 ppm; *m/z* (FAB, GTA) 567 (M+H+G, 1 %), 475 (M+H, 2), 376 (M+2H-CHA, 6), 234 (8), 192 (21), 100 (100).

(±)-2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol (1S,4R)-camphanate: To the alcohol **(2)** (5.1 g, 8 mmol), triethylamine (2 ml, 15 mmol) and DMAP (0.16 g, 1.3 mmol) in anhydrous dichloromethane (100 ml) at 0 °C under an atmosphere of nitrogen, was added dropwise (-)-(1S,4R)-camphanoyl chloride (3.5 g, 16 mmol) in dichloromethane (20 ml). The reaction was stirred at 0 °C for 1 h and then at 25 °C for 24 h. The solution was washed with water (3 x 50 ml) and saturated sodium bicarbonate (2 x 50 ml) and then dried over magnesium sulphate. The solvent was removed *in vacuo* to give a white solid in quantitative recovery. The ^{1}H-nmr spectrum showed that only the two product diastereomeric esters were present.

Hplc analysis of camphanoyl esters on a Hewlett Packard hplc, using an analytical 5 μ silica column and eluting with 4% methyl t-butyl ether in dichloromethane at 1 ml min^{-1} gave retention times of 3.7 min for ester A-(+) and 4.8 min for ester B-(-).

Chromatographic resolution of the diastereomers:- The crude reaction mixture was applied to a silica column (200 x 90 mm) in dichloromethane. The products were eluted with 2000 ml of 0%, 9000 ml of 1 % and 3000 ml of 3 % diethyl ether in dichloromethane collecting 250 ml fractions. Ester A was eluted in fractions 18-37 and ester B in fractions 30-56. The fractions were assayed by hplc and tlc. The pure fractions were pooled and the solvent was removed *in vacuo* (ester **A**, 3.25g, 4 mmol, 50 %; **B**, 3.24g, 4 mmol, 50 %; mixture 0.85g). The mixed fraction residue was purified by further silica column chromatography and the pure ester residues by recrystallisation from ethyl acetate and petroleum ether.

Ester **A**, m.p. 146-148 °C; $[\alpha]_D$ +12.0° ± 0.4° (c 0.5, $CHCl_3$); (Found: C, 75.4; H, 6.6. $C_{51}H_{54}O_9$ requires: C, 75.5; H, 6.7 %); ν_{max} ($CHCl_3$) 2960 w, 1790 vs, 1740 s, 1500 w, 1460 m, 1370 s, 1320 m, 1280 s, 1080 vs, 700s cm^{-1}; δ_H (360 MHz; $CDCl_3$) 0.90, 0.99, 1.08 (9H, 3s, Camp Me-*H*), 1 63 (1H, m, Camp-CH_2), 1.80 (2H, m, Camp-CH_2), 2.70 (1H, m, Camp-CH_2), 3.57 (2H, m, Ins-*H*), 4.13 (3H, m, Ins-*H*), 4.64-4.98 (11H, m, Bz-CH_2 and Ins-*H*), 7.2-7.4 (25H, m, Ar-*H*); δ_C (22.5 MHz; $CDCl_3$) 9 8, 16 8, 17.0 (Camp Me-*C*), 29.2, 31.0 (Camp-*C*H_2), 54.3 (Camp 4°-*C*),73 3, 75.4, 75.8, 76.0 (Bz-*C*H_2), 76.4, 79.4, 81.2, 81.7, 83.8 (Ins-*C*H), 127.4, 127.7, 127.9, 128 1, 128.5, 128.6, 139.8 (Ar-*C*),167 (Camp-*C*O); *m/z* (ACE) 829 ($M+NH_4$, 20 %), 216 (44), 91 (100).

Ester **B**, m.p. 161-164 °C; $[\alpha]_D$ -17.6° ± 0.4° (c 0.5, $CHCl_3$); (Found: C, 75.3; H, 6 8 $C_{51}H_{54}O_9$ requires: C, 75.5; H, 6.7 %); δ_H (360 MHz; $CDCl_3$) 0.83, 0.96, 1 07 (9H, 3s, Camp Me-*H*), 1.63 (1H, m, Camp-CH_2), 1.80 (2H, m, Camp-CH_2), 2.70 (1H, m, Camp-CH_2), 3.57 (2H, m, Ins-*H*), 4.13 (3H, m, Ins-*H*), 4.64-4.98 (11H, m, Bz-CH_2 and Ins-*H*), 7.2-7.4 (25H, m, Ar-*H*);
ν_{max}, δ_C and *m/z* data identical to ester **A**

(+)- and (-)-2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol (2A and 2B): A suspension of the appropriate camphanoyl ester (A or B, 7.3 g, 9 mmol) in 170 ml absolute alcohol, with KOH (5.0 g, 90 mmol) was stirred for 20 h at 25 °C to produce a clear solution The solvent was removed *in vacuo* and the products were partitioned between water (300 ml) and diethyl ether (1200 ml). The ethereal layer was washed with water (3 x 300 ml), saturated brine (300 ml) and then dried over magnesium sulphate. The solvent was removed *in vacuo* to give a solid residue which was recrystallised from hexane (yield **2A**, 4.5 g, 7.1 mmol, 79 %; **2B**, 4.6 g, 7.3 mmol, 81 %). Alcohol **2A**, m.p. 62-64 °C, $[\alpha]_D$ +9.3° ± 0.7° (c 0.3, $CHCl_3$); (Found: C, 78.2; H, 6.75. $C_{41}H_{42}O_6$ requires: C, 78.1; H, 6.7 %). Alcohol **2B**, m.p. 63-65 °C; $[\alpha]_D$ -8.7° ± 0.7° (c 0 3, $CHCl_3$); (Found. C, 78 2; H, 6 8. $C_{41}H_{42}O_6$ requires: C, 78.1, H, 6.7 %). All other data was the same as for the racemic compound.

(+)- and (-)-2,3,4,5,6-pentakis-*O*-benzyl *myo*-inositol bis(2-cyanoethyl) phosphorothioate (5A and 5B): The separated enantiomers of the alcohol (**2A** and **2B**) were phosphorylated using the methodology described (*vide supra*) to yield oils which resisted

crystallisation. Triester **5A**: (2.20 g, 2.6 mmol, 66 %), $[\alpha]_D$ +1.4°± 0.4° (c 1, $CHCl_3$); Triester **5B**. (1.81 g, 2.2 mmol, 55 %), $[\alpha]_D$ -2.2°± 0.4° (c 1, $CHCl_3$). All other spectroscopic and analytical data were identical to the racemic compound.

(D)- and (L)- *myo*-inositol 1-phosphorothioate biscyclohexylammonium salt (7A and B): The appropriate triester (**5A** or **5B**, 0.87 g, 1.04 mmol) was treated as for the racemate (*vide supra*) to yield the salts. The D-antipode **7A**, (0.29 g, 0.62 mmol, 60 %), [$\alpha]_D$ -2.5° ± 0.2° (c 2.5, water, pH 6.5), and the L-antipode **7B**, (0.35 g, 0.76 mmol,73 %), $[\alpha]_D$ +1.8° ± 0.2° (c 2.5, water, pH 6.5). All other spectroscopic and analytical data were identical to the racemic compound.

Purification of *myo*-inositol synthase. The partial purification of *myo*-inositol synthase was achieved using the methodology of Sherman and coworkers.[9] Decapsulated bovine testis (335 g) were homogenised at 4 °C in 670 ml 154 mM KCl and 0.1 mM dithiothreitol (DTT), using a Waring blender. The cell debris was removed by centrifugation (30 000 x g, 4 °C, 50 min) and the supernatent liquid was cleared by ultracentrifugation (70 000 x g, 4 °C, 250 min). The supernatant solution was heat treated at 60 °C for 2 min, cooled on ice to 4 °C and then fractionated with ammonium sulphate. The 20-40 % of saturation ammonium sulphate fraction contained all of the synthase activity and was resuspended in 50 ml buffer A (50 mM Tris.HCl, pH 8.0, 4 °C, 1mM NH_4Cl, 0.5 mM DTT, 0.2 mM EDTA) and was dialysed overnight against 100 volumes of buffer A The suspension was then cleared by ultracentrifugation (*vide supra*) and was then applied to a column of DEAE-cellulose (Whatman DE-52, 200 x 26 mm) pre-equilibrated in buffer A at a flow rate of 20 ml h^{-1} at 4 °C. After washing the column with 5 column volumes of buffer A, the bound synthase was eluted with a 4 column volume linear gradient of 0-0.5 M KCl in buffer A. The synthase activity eluted at 0.12-0.2 M KCl. The active fractions were concentrated by ultrafiltration (Amicom YM30) to 80 ml and then dialysed against 8000 ml of homogenisation solution.

***myo*-Inositol synthase assay.** During the purification, fractions were routinely assayed for activity using the periodate-cleavage molybdic acid method of Barnett *et al*.[13]
By this method, the DEAE-cellulose purified enzyme solution had an activity of 3.6 µmol h^{-1} ml^{-1} using 5.3 mM D-glucopyranose 6-phosphate (G6P) as substrate.
The activity of the synthase was also measured enzymically using glucose 6-phosphate dehydrogenase (*ex Leuconostoc mesenteroides*, Sigma) which allows the determination of the unreacted substrate. A 50 µl sample of the inositol synthase incubation after a given time, was added at 25 °C to 100 mM NAD^+ (50 µl), 100 µl of a cocktail containing glucose 6-phosphate dehydrogenase (10 unit ml^{-1}), BSA (1 mg ml^{-1}) and 50 mM Tris.HCl at pH 8.0, and 800 µl Tris.HCl pH 8.0 The final $\Delta O.D_{340nm}$ after a 10 min incubation of the sample against a control containing no inositol synthase gave the residual concentration of G6P.

1,2,3,4-tetra-*O*-acetyl-ß-D-glucopyranose bis(2-cyanoethyl)phosphite (9): To the alcohol **(8)** (1.04 g 3 mmol) and 1*H*-tetrazole (0.21 g, 3 mmol) in dry acetonitrile (25 ml) under dinitrogen, was added N,N-diisopropyl bis(2-cyanoethyl)phosphamidite (1.08 g, 4 mmol) in dry acetonitrile (25 ml) and the reaction was stirred at 25 °C for 16 h. Ethyl acetate (100 ml) and saturated brine (50 ml) were added and the organic phase was separated, dried over magnesium sulphate and then concentrated *in vacuo* . The resulting oil was purified by short column silica chromatography (CCl_4-$CHCl_3$-pyridine, 75:15:10) to give a colourless oil (1.34 g, 2.6 mmol, 87 %).ν_{max} ($CHCl_3$) 2260 w (CN), 1780 vs, 1380 s, 1260 s, 1090 vs (POR), and 1050 vs (POR) cm^{-1}; δ_H (270 MHz; $CDCl_3$) 2.01, 2.04, 2.06, 2.11 (12H, 4s, Ac-*H*), 2.7 (4H, td, $J_{1',2'}$ 6.2 Hz, $J_{2',P}$ -2.3 Hz, 2'-*H*), 3.8-4.1 (7H, m, 1'-*H* , 6-*H*, 5-*H*), 5.10 (1H, dd, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 9. Hz, 2-*H*), 5.13 (1H, t, $J_{2,3\ \&\ 3,4}$ 9.5 Hz, 3-*H*), 5.30 (1H, t, $J_{3,4\&\ 4,5}$ 9.5 Hz, 4-*H*), 5.7 (1H, d, $J_{1,2}$ 8 Hz, 1-*H*); δ_C (22.5 MHz; $CDCl_3$) 20.3 (Ac-CH_3), 20.0, 20.5 (d, $J_{2',P}$ 4.3 Hz, 2'-*C*), 57.3 (d, $J_{1',P}$ -9.7 Hz, 1'-*C*), 57.1 (d, $J_{1',P}$ -8.8 Hz, 1'-*C*), 61.0 (d, $J_{6,P}$ -12.1 Hz, 6-*C*), 67.8 (3-*C*), 70.0 (2-*C*), 72.6 (4-*C*), 73.6 (d, $J_{5,P}$ 5.4 Hz, 5-*C*), 91.5 (1-*C*), 117.4 (3'-*C*N), 168.7, 169.0, 169.2, 169.8 (Ac-*C*O); δ_P (36.2 MHz; $CDCl_3$) 139 ppm; *m/z* (FAB, GTA) 406 (M^+-Ac-CH_2CH_2CN, 48 %), 244 (100), 169 (31), 127 (29).

1,2,3,4-tetra-*O*-acetyl-ß-D-glucopyranose bis(2-cyanoethyl)phosphorothioate (10): To the phosphite **(9)** (1.34 g, 2.6 mmol) in pyridine (75 ml), sulphur (1.00 g, 31 mmol) was added. The reaction was stirred for 15 min and then the solvent was removed *in vacuo* to give a yellow oil The oil was extracted with CCl_4-$CHCl_3$ (1:1, 10 ml) and filtered to remove the sulphur. The filtrate was purified by short column silica chromatography (CCl_4: $CHCl_3$: pyridine, 70:20:10) to give a clear, colourless oil(1.27 g, 2.3 mmol, 88 %). (Found C, 43.7; H, 5.2; N, 5.3. $C_{20}H_{27}N_2O_{12}PS$ requires: C, 43.6; H, 4.9; N, 5.1 %); ν_{max} ($CHCl_3$) 2280 w (CN), 1770 vs (CO), 1380 m, 1230 s,1090 vs, 1050 vs, 850 m (PS) cm^{-1}; δ_H (360 MHz; $CDCl_3$) 2.01, 2.03, 2.07, 2.12 (12H, 4 x s, Ac-*H*), 2.77 (2H, t, $J_{1',2'}$ 6.3 Hz, 2'-*H*), 2.78 (2H, t, $J_{1',2'}$ 6.2 Hz, 2'-*H*), 3.88 (1H, dtd, $J_{4,5}$ 10 Hz, $J_{5,6}$ 3 5 Hz, $J_{5,P}$ - 1.6 Hz, 5-*H*), 4.21 (2H, m, 6-*H*), 4.28 (4H, dt, $J_{1',P}$ 9.9 Hz, $J_{1',2'}$ 6.2 Hz, 1'-*H*), 5.10 (1H, t, $J_{2,3\ \&\ 3,4}$ 9 4 Hz, 3-*H*), 5.13 (1H, dd, $J_{2,3}$ 9.4 Hz, $J_{1,2}$ 8.1 Hz, 2-*H*), 5.25 (1H, t , $J_{4,5\ \&\ 3,4}$ 9.4 Hz, 4-*H*), 5.72 (1H, d, $J_{1\text{-}2}$ 8.2 Hz, 1-*H*) ; δ_C (90.6 MHz; $CDCl_3$) 19.5 (d, $J_{2',P}$ 8.5 Hz, 2'-*C*), 20.6 (Ac-CH_3), 20.8 (d, $J_{2',P}$ 7.1 Hz, 2'-*C*), 62.8 (d, $J_{1',P}$ -4.4 Hz, 1'-*C*), 66.2 (d, $J_{1',P}$ -4.3 Hz, 6-*C*), 67.6 (3-*C*), 70.2 (2-*C*, 72.8 (4-*C*), 73.1 (d, $J_{5,P}$ 10.1 Hz, 5-*C*), 91.8 (1-*C*), 116.5 (3'-*C*N), 170.1, 169.6, 169.3, 169.1 (*C*O); δ_P (36.2 MHz; $CDCl_3$) 67.4 ppm; *m/z* (FAB, GTA) 491 (M-Ac+H^+, 52 %), 329 (100),276 (38), 221 (24).

1,2,3,4-tetra-*O*-acetyl-ß-D-glucopyranose (2-cyanoethyl)phosphorothioate triethyl ammonium salt (11): To the triester **(10)** (1.27 g, 2.3 mmol) in dry pyridine (75 ml), triethylamine (25 ml) was added. The solution was stirred at 25 °C for 15 h and the solvent was removed *in vacuo* . The residual traces of triethylamine were removed azeotropically using pyridine (3 x 50 ml)

and then toluene (3 x 50 ml) to yield the product as an oil, in essentially quantitative recovery, which was used in the next step without purification. δ_H (270 MHz; $CDCl_3$) 1.3 (9H, q, Et-*H*), 1.9-2.1 (12H, 4s, Ac-*H*), 2.75 (2H, 2t, 2'-*H*), 3.1 (6H, t, Et-*H*), 3.9 (1H, m, 5-*H*), 4.1 (3H, m, 6-*H* and 1'-*H*), 5.1 (2H, m, 2-*H* and 3-*H*), 5.2 (1H, t, 4-*H*) 5.7 (1H, d, 1-*H*); δ_C (22.5 MHz; $CDCl_3$) 8.5 (Et-*C*H_3), 19.4 (d, $J_{2',P}$ 6.7 Hz, 2'-*C*), 20 5 (Ac-*C*H_3), 20.5 (d, $J_{2',P}$ 5.4 Hz, 2'-*C*), 45.9 (Et-*C*H_2), 60.7 (t, $J_{1',P}$ -4.0 Hz, 1'-*C*), 64.4 (d, $J_{6,P}$ -4.0 Hz, 6-*C*), 68.2 (3-*C*), 70.5 (2-*C*), 73.0 (4-*H*), 74.0 (2 x d, J_{5-P} 9.4 Hz and 8.1 Hz, 5-*C*), 91.8 (1-*C*), 117.5 (3'-*C*N), 168.3, 168.7, 169.0, 169.4 (4 x *C*O); δ_P (36.2 MHz, $CDCl_3$) 58.8 ppm.

D-glucopyranose 6-phosphorothioate disodium salt (12): To the crude oil **(11)** in methanol (100 ml), with stirring under argon at 0 °C, was added potassium methoxide (0.4 g, 5.7 mmol) in 10 ml methanol. The reaction was then stirred at 4 °C for 4 days when the precipitation of the white crystalline product from the orange coloured solution was judged complete. The crystals were isolated by centrifugation and were washed repeatedly with anhydrous methanol and diethyl ether and then dried *in vacuo*. The crude product was purified by ion exchange chromatography on DEAE-Sephadex A25 eluting with a 10-500 mM gradient of triethylamine hydrogen carbonate, pH 8.3. The fractions containing the product were pooled and solvent was removed *in vacuo*. Cations were exchanged for sodium by ion exchange chromatography on Dowex 50-X8 and the disodium salt was isolated by trituration from water with ethanol (0.37 g, 1 1 mmol, 48 %), m.p.85 °C (dec.), (Found C, 22.0; H, 4.3. $C_6H_{11}O_8PSNa_2.0.5\ H_2O$ requires: C, 21.9; H, 3.7 %); δ_H (360 MHz; D_2O) 3.27 (1H, dd, $J_{2,1}$ 8.0 Hz, $J_{2,3}$ 8.9 Hz, 2α-*H*), 3.49 (1H, t, $J_{3,2\ \&\ 3,4}$ 9.2 Hz, 3α-*H*), 3.56 1(H, m, 4-*H*, 2β-*H*), 3.73 (1H, t, $J_{3\text{-}4\ \&3\text{-}2}$ 9.5 Hz, 3β-*H*), 3.92 (1H, m, 5-*H*), 4.07 (1H, m, 6-*H*), 4.65 (1H, d, $J_{1\text{-}2}$ 8.0 Hz, 1α-*H*), 5.23 (1H, d, $J_{1\text{-}2}$ 4 Hz, 1β-*H*); δ_C (90 6 MHz; D_2O) 65.7 (*C*-6), 71.7 (*C*-2β), 73 6 (d, $J_{5,P}$ - 7 8 Hz, *C*-5), 74.2 (*C*-4), 74.9 (*C*-3β), 76.9 (*C*-2α), 77.8 (*C*-3α), 94.8 (*C*-1β), 98.7 (*C*-1α); δ_P (145.8 MHz; D_2O) 47.3; *m/z* (FAB, GTA) 343 ([M+Na]$^+$, 49 %), 321 ([M+H]$^+$, 17), 251 (70), 159 (100), 137 (40) All nmr assignments were made on the basis of DEPT, COSY and ^1H -^{13}C correlation spectra. The compound acted as a substrate for glucose 6-phosphate dehydrogenase, potentially allowing the dehydrogenase to be used in assays for following the conversion of the compound to inositol 1-phosphorothioate, *vide infra*.

Incubation of *myo*-inositol synthase and glucopyranose 6-phosphorothioate. The substrate analogue, glucopyranose 6-phosphorothioate (0.16 g, 500 μmol, 10 mM), was incubated with *myo*-inositol synthase (45 ml, 160 units) and NAD^+ (45 mg, 68 μmol, 1.5 mM) at 37 °C for 12 h under the assay conditions, *vide supra*. After this time there was no indication that the analogue had been converted to any product as judged using the G6P dehydrogenase assay.
Nevertheless, the synthase incubation was quenched with 3 volumes of 95 % ethanol and cooled to 4 °C. The solvent was removed *in vacuo* and the residue was suspended in water (1 ml) The

suspension was filtered and the cations in the filtrate were exchanged for Li^+ on a column of Amberlite IR118 (Li^+). The eluent was concentrated *in vacuo* and was subjected to preparative tlc (3 times) on cellulose developing the plates with 1M aqueous ammonium acetate-ethanol (30:70), conditions which were previously optimised for the purification of inositol 1-phosphorothioate (from mixtures containing the glucopyranose 6-phosphorothioate and nicotine nucleotides) using the synthetic material. No inositol 1-phosphorothioate was detected either by staining the chromatograms with silver nitrate[14] or by high field ^{1}H-nmr spectroscopic analysis of the water solubilised cellulose tlc bands under conditions where a 5 % conversion would have been detected easily. The same result was obtained in prolonged incubations (75 h) of the synthase with glucopyranose 6-phosphorothioate. Note that in a similar incubation of G6P with inositol synthase, both the chemical and the enzymic assays showed that >85 % of the starting material had been converted to inositol 1-phosphate after 7 h.

We thank the SERC and Merck, Sharp and Dohme for a CASE studentship to G.R.B A preliminary account of some of this work was communicated recently.[15]

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