Synthesis of naturally occurring phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] and its diastereoisomers

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The chemical synthesis of naturally occurring phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] **2**, in which the 1- and 2-hydroxy functions of the glycerol moiety are esterified with stearic and arachidonic acid, respectively, is described. The synthesis of three diastereoisomers **41**, **42** and **43** of PtdIns(3,4,5)P_3 is also reported.

Introduction

The identification in 1983 by Berridge and his co-workers[1] of D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** as a cellular second messenger is one of the seminal discoveries of modern biology. More recently, 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] **2**, which is usually referred to as phosphatidylinositol 3,4,5-trisphosphate, has also been shown to act as a key second messenger[2] in cell regulation.

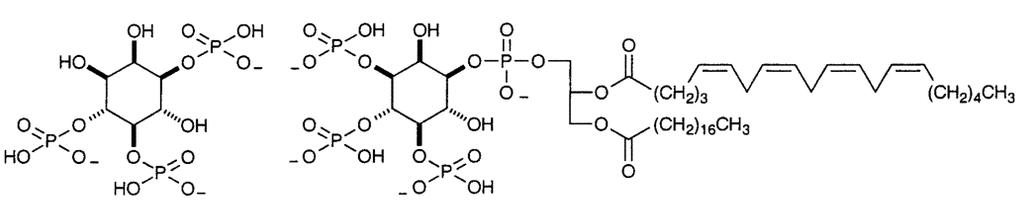
In our original synthesis of Ins(1,4,5)P_3 **1**, we adopted a strategy[3] that depended solely on the use of acid-labile and base-labile protecting groups. Indeed, unlike most subsequent workers in this field,[4] we particularly avoided the use of benzyl and related protecting groups that are removable by hydrogenolysis. This strategy is particularly suitable for the synthesis of naturally occurring PtdIns(3,4,5)P_3 **2** as the olefinic double bonds in the arachidonoyl residue are clearly susceptible to catalytic hydrogenation. Prior to the preliminary publication[5,6] of the present work, only the synthesis of PtdIns(3,4,5)P_3 analogues containing saturated fatty acid residues in the glycerol moiety had been reported.[7–13] Following our preliminary publication,[6] Watanabe and Nakatomi published[14,15] an alternative synthesis of naturally occurring PtdIns(3,4,5)P_3 **2**. We now report the full experimental details of our synthesis of PtdIns(3,4,5)P_3 **2** and three of its diastereoisomers.

Results and discussion

Our synthesis involved four main stages. In the first stage, the preparation of the required enantiomeric 1,2-di-*O*-acylglycerol derivatives was undertaken.[5] The second stage involved the preparation of suitably protected enantiomeric *myo*-inositol 1-phosphates. In stage three, the glycerol and inositol building blocks were coupled together and the 3-, 4- and 5-hydroxy functions of the inositol residues in the coupled products were phosphorylated. In the final stage, all of the protecting groups were removed to give PtdIns(3,4,5)P_3 **2** and its diastereoisomers.

The first main stage of the synthesis is indicated in outline in Scheme 1. Enantiomerically pure 2,3-*O*-isopropylidene-*sn*-glycerol[16] **3** was treated (Scheme 1a) with a slight excess of stearoyl chloride (R^1COCl) in the presence of triethylamine and a catalytic quantity of 4-(dimethylamino)pyridine (DMAP) in dichloromethane to give its 1-*O*-stearoyl derivative (step i). The product was treated with trifluoroacetic acid (TFA) in 2,2,2-trifluoroethanol–triethyl borate (1 : 8 v/v) solution at room temperature to give 1-*O*-stearoyl-*sn*-glycerol[17] **4** as a crystalline solid in good isolated yield. Under these acidic conditions, acyl migration, which can lead to racemization, is almost completely avoided. Other workers[18] had previously unblocked 2,3-*O*-isopropylidene-1-*O*-palmitoylglycerol with boric acid in 2-methoxyethanol at 100 °C. The 1-*O*-stearoyl derivative **4** was then allowed to react with a 10% excess of 9-phenylxanthen-9-ol[19] (PxOH) in glacial acetic acid at room temperature to give 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **5** as the major product. This compound **5** was treated with a ≈20% excess of arachidonic acid (R^2CO_2H) in the presence of 2,6-dichlorobenzoyl chloride[20] and 1-methylimidazole in dichloromethane at room temperature (Scheme 1a, step iv). Finally, the 9-phenylxanthen-9-yl protecting group[21] was removed under very mild conditions by treatment with dichloroacetic acid in the presence of pyrrole,[22] which is a very efficient carbocation scavenger, in dichloromethane solution at room temperature. In this way, 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6**, which is the glycerol building block required for the synthesis of naturally occurring PtdIns(3,4,5)P_3 **2**, was obtained. The overall yield for the five steps was ≈48%. In the same way, 1,2-*O*-isopropylidene-*sn*-glycerol[23] **7** was converted in five steps (Scheme 1b and Experimental section) into 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8**.

The enantiomeric purity of 2-*O*-arachidonyl-1-*O*-stearoyl-*sn*-glycerol **6** was estimated by converting it (Scheme 2) into its 3-*O*-(−)-camphanyl ester **10** and then determining the diastereoisomeric purity of this product by ^{13}C NMR spectroscopy. The region of the ^{13}C NMR spectrum containing the resonance signals of the glycerol carbon atoms of compound **10** is illustrated in Fig. 1a. The most downfield signal at δ 68.58 may be assigned to the resonance of *C*-2 of the glycerol moiety.



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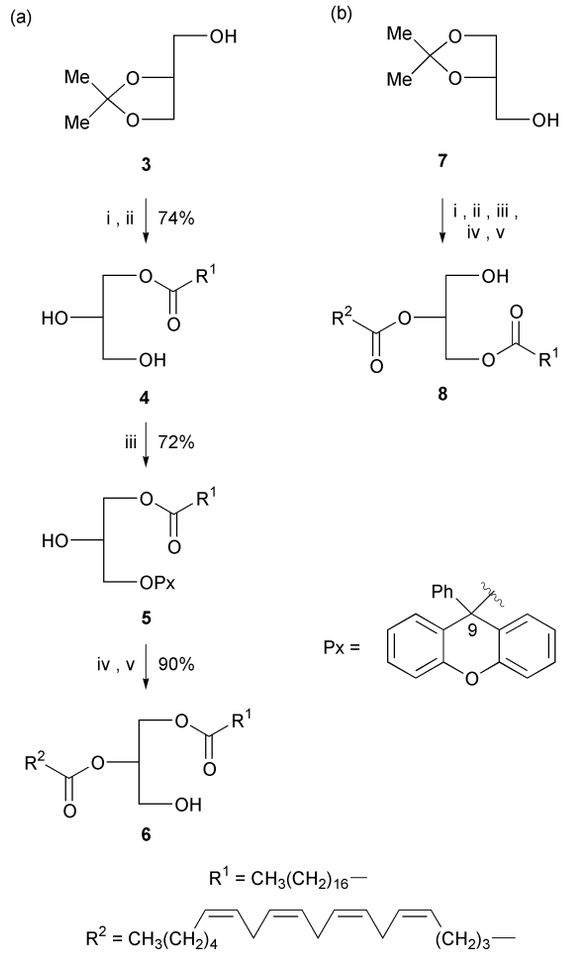


Scheme 1 *Reagents and conditions*: i, stearoyl chloride (R^1COCl), Et_3N, DMAP, 0 °C to room temp., 50 min; ii, CF_3CO_2H (TFA), CF_3CH_2OH, $B(OEt)_3$, room temp., 5 h; iii, 9-phenylxanthen-9-ol (PxOH), AcOH, room temp. to <35 °C, ≈15 mmHg; iv, arachidonic acid (R^2COCl), 2,6-$Cl_2C_6H_3COCl$, 1-methylimidazole, CH_2Cl_2, room temp., 80 min; v, Cl_2CHCO_2H, pyrrole, room temp., 5 min.

The corresponding region of the ^{13}C NMR spectrum of the 3-*O*-(−)-camphanyl ester of racemic 2-*O*-arachidonoyl-1-*O*-stearoylglycerol is illustrated in Fig. 1b. The signal at δ 68.73 in Fig. 1b is assigned to the C-2 resonance of the diastereoisomeric 1-*O*-(−)-camphanyl ester **11** of 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8**. From a close examination of Fig. 1a, it may be estimated that 2-*O*-arachidonoyl-3-*O*-(−)-camphanyl-1-*O*-stearoyl-*sn*-glycerol **10** is contaminated with ≈2% of the other diastereoisomer **11**. It follows from this that the enantiomeric excess of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6** must be at least 96%. In the same way, the enantiomeric excess of 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8** was estimated to be ≈90%.

The second stage of the synthesis initially involved the conversion (Scheme 3a) of *myo*-inositol **12** into 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol **21** and its enantiomer **22**. *myo*-Inositol **12** was first allowed to react with *p*-anisoyl chloride in pyridine solution (step i) to give its penta-anisoyl derivative **13**, which was isolated as a pure crystalline solid in 72% yield. Under the reaction conditions, acylation of the axially disposed 2-hydroxy function occurred only to a negligible extent. Acid-catalysed reaction of compound **13** with the enol ether[24] **14**, followed by deacylation (steps ii and iii) gave the 2-*O*-Mthp (4-methoxytetrahydropyran-4-yl) derivative[24] **15** as a crystalline solid in 81% isolated yield. Reaction between compound **15** and Markiewicz reagent[25] **16**, in the presence of imidazole and triethylamine in hexamethylphosphoric triamide (HMPA) solution (step iv), gave the

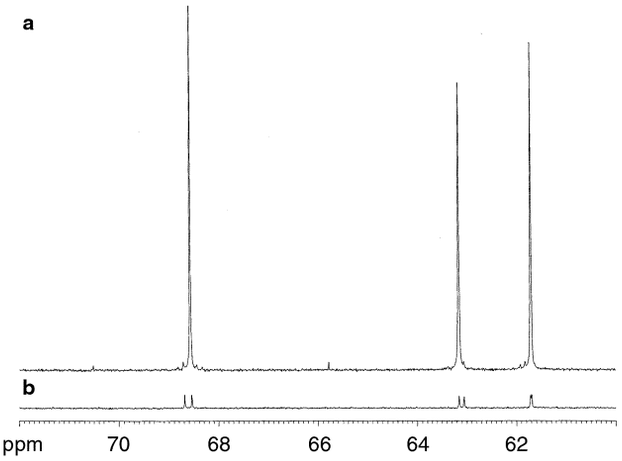
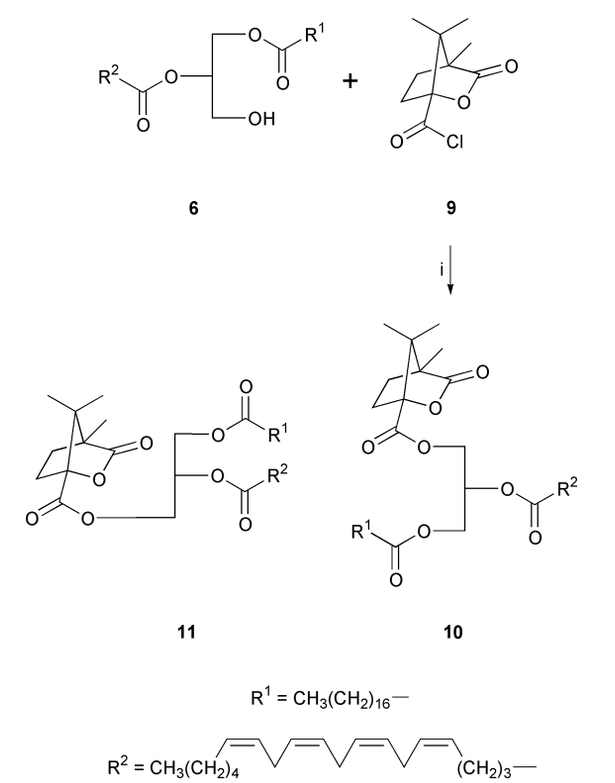
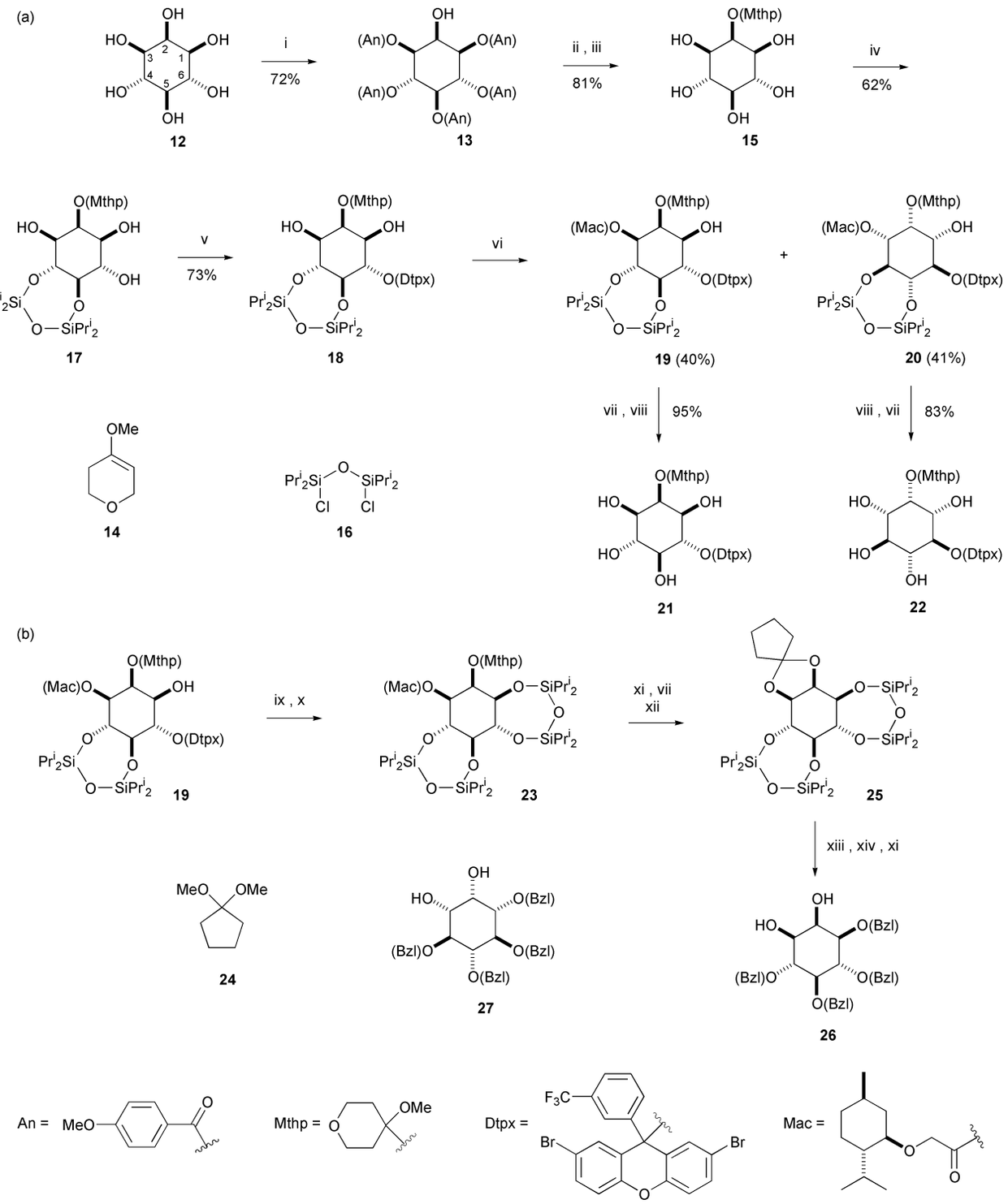


Fig. 1 Region (δ 60–70) of ^{13}C NMR spectra (in $CDCl_3$) of (a) tris-*O*-acylglycerol derivative **10** and (b) an equimolar mixture of compound **10** and its diastereoisomer **11**.



Scheme 2 *Reagents and conditions*: i, 1-methylimidazole, CH_2Cl_2, room temp.

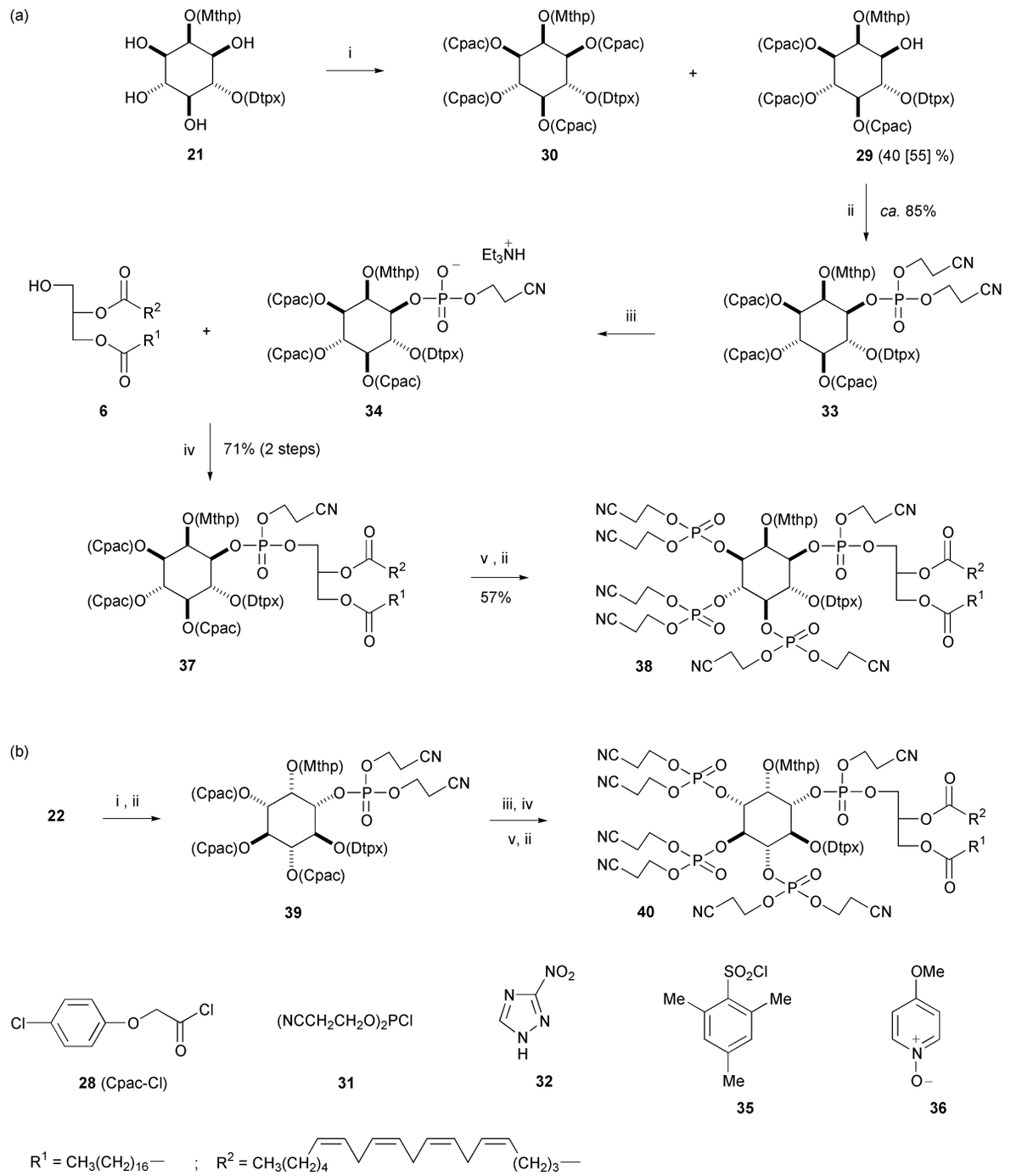
4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivative **17**, which was isolated as a crystalline solid in ≈62% yield. When the latter compound **17** was treated with 2,7-dibromo-9-chloro-9-[3-(trifluoromethyl)phenyl]xanthene[26] (DtpxCl) in pyridine–acetonitrile solution (step v), the 6-*O*-Dtpx derivative **18** was obtained and isolated in 73% yield. This compound was allowed to react with (−)-menthoxyacetyl chloride (MacCl) in the presence of 1*H*-tetrazole in pyridine solution (step vi). Following fractionation of the products, each of the pure diastereoisomers **19** and **20** was isolated in a yield of ≈40%. These diastereoisomers may readily be distinguished by NMR spectroscopy: the signals at δ 80.25 and 79.74, respectively, in the ^{13}C NMR spectra (in $CDCl_3$) of diastereoisomers **19** and **20**, may be assigned to the resonances of the carbon atom (*i.e. C*-3) directly attached to the (−)-menthoxyacetoxy residue. An examination of the two ^{13}C NMR spectra revealed that neither diastereoisomer was detectably contaminated with the other. Following treatment with ethanolic methylamine (step vii)



Scheme 3 *Reagents and conditions*: i, AnCl, C_5H_5N, 0 °C to room temp., 18 h; ii, **14**, $Ph_3P{\cdot}HBr$, room temp., 40 h; iii, NaOMe, MeOH, THF, reflux, 30 min; iv, **16**, imidazole, Et_3N, HMPA, room temp., 60 h; v, DtpxCl, C_5H_5N, MeCN, room temp., 80 min; vi, MacCl, 1*H*-tetrazole, DMAP, MeCN, C_5H_5N, room temp., 30 min; vii, $MeNH_2$, EtOH, room temp., 90 min; viii, Et_4NF, MeCN, room temp., 30 min; ix, $ClCH_2CO_2H$, pyrrole, CH_2Cl_2, room temp., 15 min; x, **16**, imidazole, DMF, 80 °C, 7 h; xi, $TsOH{\cdot}H_2O$, CH_2Cl_2–MeOH (1 : 1 v/v), room temp., 2 h; xii, **24**, $TsOH{\cdot}H_2O$, room temp., 1 h; xiii, Et_4NF, Et_4NOH, MeCN, aq. EtOH, reflux, 16 h; xiv, $PhCH_2Cl$, NaH, DMF, room temp., 2.5 h.

and tetraethylammonium fluoride (TEAF) (step viii), diastereoisomer **19** was converted into 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol **21** in 95% yield. An important piece of evidence in support of the enantiomeric purity of compound **21** is that its precursor **19** was diastereoisomerically almost pure. It was not possible to obtain satisfactory optical rotation data for compound **21**. However, the circular dichroism (CD) spectrum of compound **21** and that of its enantiomer **22**, which was prepared in the same way (Scheme 3a) from the L-inositol derivative **20**, are virtually exact mirror images of each other.

Although it seemed likely that the enantiomeric excesses of compounds **21** and **22** were both very high, it was by no means clear which enantiomer was which. This was established (Scheme 3b) by relating them to the known 1,4,5,6-tetra-*O*-benzyl-D- and -L-*myo*-inositol[27] **26** and **27**. The (−)-menthoxyacetyl derivative **19** was treated first with chloroacetic acid in the presence of pyrrole in dichloromethane solution (Scheme 3b, step ix) and then with Markiewicz reagent **16** under rather forcing conditions (step x) to give the bis(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivative **23**. After the Mthp and Mac groups had been removed by treatment with



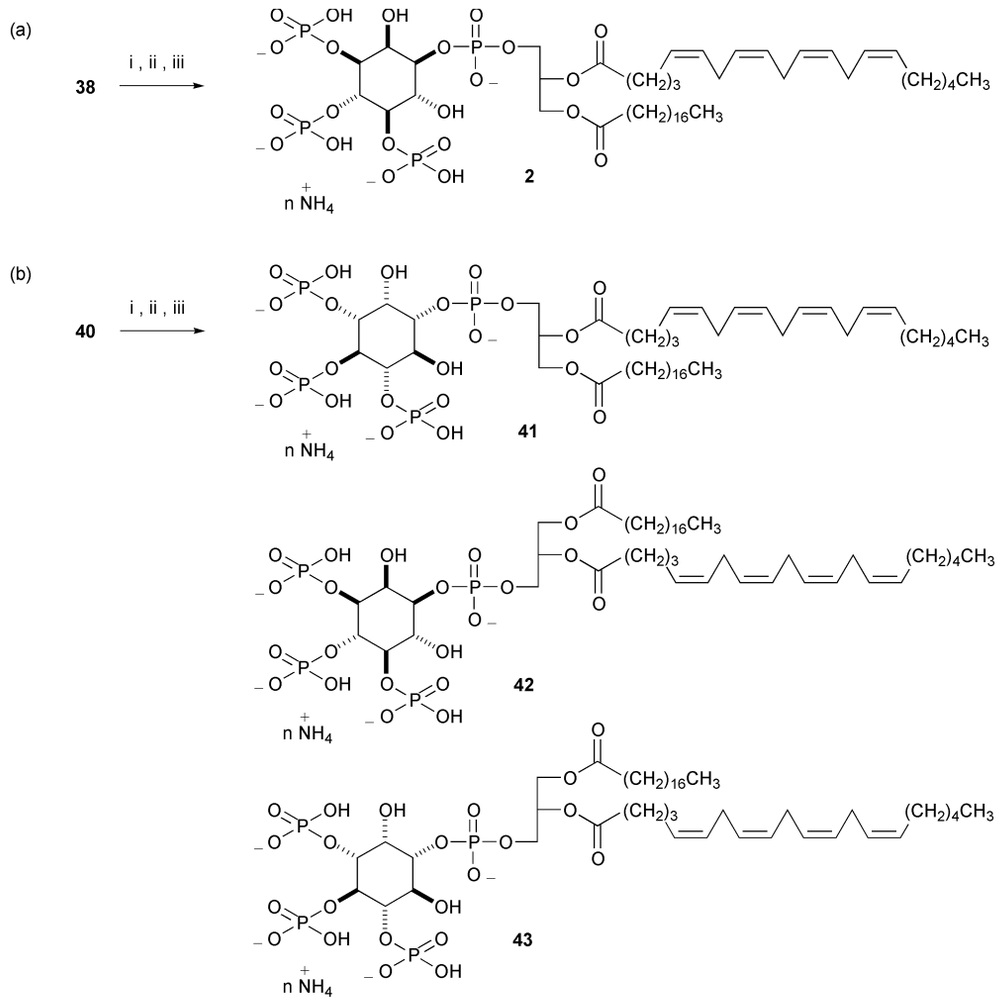
Scheme 4 *Reagents and conditions*: i, CpacCl **28**, 1*H*-tetrazole, DMAP, MeCN, C_5H_5N, room temp., 95 min; ii, (a) **31**, **32**, C_5H_5N, MeCN, room temp., 1 h; (b) 70% *t*-BuO_2H, room temp., 90 min; iii, Et_3N, MeCN, room temp., 16 h; iv, **35**, **36**, MeCN, C_5H_5N, room temp. 1 h; v, $N_2H_4{\cdot}H_2O$, MeCN, room temp., 90 min.

toluene-4-sulfonic acid (PTSA) in methanol–dichloromethane and ethanolic methylamine, respectively, the 2,3-diol system was protected (as a cyclopentylidene acetal[28]) by treatment with 1,1-dimethoxycyclopentane **24** in the presence of PTSA (step xii) to give compound **25**. Following the removal of the two 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl groups (step xiii), the 1-, 4-, 5- and 6-hydroxy functions were benzylated by treatment with benzyl chloride and sodium hydride in dimethylformamide (DMF) (step xiv). Finally, the cyclopentylidene protecting group was removed by acid-catalyzed methanolysis to give 1,4,5,6-tetra-*O*-benzyl-D-*myo*-inositol **26**. The identity of enantiomer **26** was clear from its specific rotation ($[a]_D^{20}$ +22.7†), which was close to the value reported[27] in the literature. In the same way (Scheme 3b), diastereoisomer **20**, the precursor of enantiomer **22**, was converted into 1,4,5,6-tetra-*O*-benzyl-L-*myo*-inositol **27** ($[a]_D^{20}$ −21.7).

The rest of the second stage of the synthesis of naturally occurring PtdIns(3,4,5)P_3 **2** (*i.e.*, the conversion of 6-*O*-Dtpx-2-*O*-Mthp-D-*myo*-inositol **21** into the protected D-*myo*-inositol 1-phosphate building block **34**) and the third stage (*i.e.*, the coupling reaction and the phosphorylation of the 3-, 4- and 5-hydroxy functions of the inositol moiety in the coupled product) are indicated in outline in Scheme 4a.

Reaction between 6-*O*-Dtpx-2-*O*-Mthp-D-*myo*-inositol **21** and (4-chlorophenoxy)acetyl chloride (CpacCl) **28** in the presence both of DMAP and 1*H*-tetrazole in acetonitrile–pyridine solution gave a mixture of the 3,4,5-tris-*O*-(4-chloro-

† Throughout this paper, $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}.



Scheme 5 *Reagents and conditions*: i, $(Me_2N)_2C{=}NH$ (TMG), Me_3SiCl, MeCN, room temp., 16 h; ii, NH_3, aq. MeOH, room temp.; iii, (a) AcOH–water (2 : 1 v/v), room temp., 90 min; (b) aq. NH_3, MeOH, room temp.

phenoxy)acetyl and 1,3,4,5-tetrakis-*O*-(4-chlorophenoxy)-acetyl derivatives[29] **29** and **30**. Following fractionation of the products, the desired tris[(4-chlorophenoxy)acetate] **29** was isolated in 40% yield. However, the starting material **21** could be regenerated from the tetrakis[(4-chlorophenoxy)acetate] **30** by treating it with alcoholic methylamine. Thus the effective yield of the desired tris[(4-chlorophenoxy)acetate] **29** was 55%, based on the 6-*O*-Dtpx-2-*O*-Mthp-D-*myo*-inositol **21** consumed. Phosphorylation of the tris[(4-chlorophenoxy)acetate] **29** with di(2-cyanoethyl) phosphorochloridite[30] **31** (see Experimental section) in the presence of 3-nitro-1,2,4-1*H*-triazole **32** in pyridine–acetonitrile, followed by oxidation of the resulting phosphite triester with *tert*-butyl hydroperoxide[31] (Scheme 4, step ii) gave the 1-[di(2-cyanoethyl) phosphate] **33** in ≈85% isolated yield. This material was treated with triethylamine in acetonitrile (step iii) to give the triethylammonium salt of the corresponding 1-[(2-cyanoethyl) phosphate] **34**.

In the third step of the synthesis, the triethylammonium salt **34** was allowed to react with 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6** and mesitylene-2-sulfonyl chloride **35** in the presence of 4-methoxypyridine 1-oxide[32] **36** (Scheme 4a, step iv) to give the fully protected coupled product **37** in 71% overall yield for steps iii and iv. In order to complete this stage of the synthesis, the (4-chlorophenoxy)acetyl groups were removed from the coupled product **37** by hydrazinolysis (step v). Under the reaction conditions, both the stearoyl and arachidonoyl residues remained completely intact. The released 3-, 4- and 5-hydroxy functions were then phosphorylated by treatment first with di(2-cyanoethyl) phosphorochloridite **31**, followed by oxidation of the intermediate trisphosphite with *tert*-butyl hydroperoxide to give the fully protected PtdIns(3,4,5)P_3 **38**. This material was isolated from the products in ≈57% overall yield for the three steps (v, iia and iib), starting from the tris[(4-chlorophenoxy)acetyl] derivative **37**.

In the same way, 6-*O*-Dtpx-2-*O*-Mthp-L-*myo*-inositol **22** was converted into the L-enantiomer of its tris[(4-chlorophenoxy)acetate] (*i.e.*, the enantiomer of compound **29**), which in turn was converted (Scheme 4, step ii) into the corresponding 1-[di(2-cyanoethyl) phosphate] **39**. Following treatment with triethylamine in acetonitrile (step iii), the product (*i.e.*, the enantiomer of triethylammonium salt **34**) was then coupled with 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6**. Following the same three-step procedure (steps v, iia and iib), the initial coupling product, which corresponded to the tris[(4-chlorophenoxy)acetyl] derivative **37**, was converted into the L-*myo*-inositol analogue **40** of the fully protected PtdIns(3,4,5)P_3 **38**. The yields obtained in the various steps are indicated in the Experimental section. Two additional diastereoisomers of the fully protected PtdIns(3,4,5)P_3 **38** were prepared by first coupling both intermediate phosphodiesters (*i.e.*, **34** and its enantiomer) with 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8** and then following the same three-step procedure (Scheme 4, steps v, iia and iib). The preparative details and the yields obtained are again indicated in the Experimental section.

The three-step procedure followed in the unblocking of the fully protected PtdIns(3,4,5)P_3 **38** is indicated in outline in Scheme 5. Arguably the most critical step was the removal of the seven 2-cyanoethyl protecting groups. In order to ensure that the 3-, 4- and 5-phosphate residues were completely unblocked, a modification of Evans' procedure,[33] in which

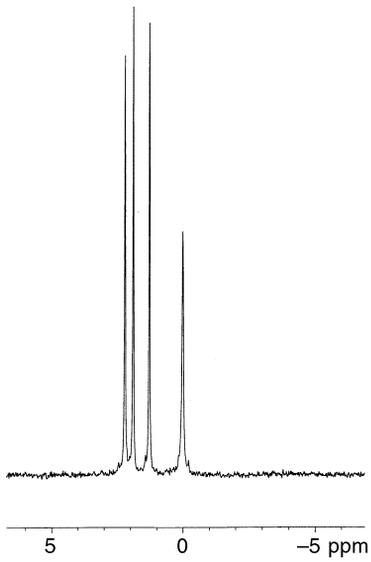


Fig. 2 ^{31}P NMR spectrum (in CD_3OD–D_2O) of the ammonium salt of PtdIns(3,4,5)P_3 **2**.

chlorotrimethylsilane was added to a solution of fully protected PtdIns(3,4,5)P_3 **38** and N^1,N^1,N^3,N^3-tetramethylguanidine (TMG) in acetonitrile solution, was adopted. After subsequent treatment with aq. methanolic ammonia (step ii), the products were kept in acetic acid–water solution (step iii) in order to remove the acid-labile 2-*O*-Mthp and 6-*O*-Dtpx groups. The ammonium salt of PtdIns(3,4,5)P_3 **2** was then isolated as an off-white hygroscopic solid in ≈96% yield. The material was characterized on the basis of ^{1}H and ^{31}P NMR and MALDI-TOF mass spectroscopic data; as can be seen from Fig. 2, its ^{31}P NMR spectrum displays four well resolved resonance signals. Other diastereoisomers of fully protected PtdIns(3,4,5)P_3 **38** (*i.e.*, **40** and the two diastereoisomers derived from 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8**) were unblocked by the same three-step procedure (Scheme 5 and Experimental section) to give products **41**, **42** and **43**.

The biological activity of this synthetic natural PtdIns-(3,4,5)P_3 **2** has been clearly demonstrated in a number of biological studies.[34–37] Alessi *et al.*[34] showed that, at very low concentration, natural PtdIns(3,4,5)P_3 **2** was much more effective in activating PtdIns(3,4,5)P_3-dependent protein kinase 1 than is the corresponding lipid in which both the arachidonoyl and stearoyl residues were replaced by saturated (*i.e.* palmitoyl) residues. These workers[34] further reported that the two diastereoisomers **41** and **43** derived from L-*myo*-inositol were inactive. These conclusions were essentially confirmed by Stokoe, Stephens *et al.*[36] in corresponding studies.

Experimental

Mps were measured with a Büchi melting-point apparatus and are uncorrected. ^{1}H NMR spectra were measured at 360 MHz with a Bruker AM 360 spectrometer. ^{13}C NMR spectra were measured at 90.6 MHz with the same spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane, and *J*-values are given in Hz; ^{31}P NMR spectra were measured at 145.8 MHz with the same spectrometer with 85% orthophosphoric acid being used as external standard. Mass spectra were obtained using Kratos MS890 and Voyager MALDI-TOF spectrometers. Optical rotations were measured with a Perkin-Elmer 141 polarmeter. Merck silica gel 60 F_{254} plates (Art 5715 and 5642) were used for TLC and were developed in the solvent systems indicated below. Merck silica gel 60 (Art 7729 and 9385) was used for short-column chromatography and Merck silanized silica gel (Art 7719) was used for reversed-phase column chromatography. Acetonitrile, pyridine and triethylamine were dried by heating with calcium hydride, under reflux, for 3–5 h and were then distilled; 1-methylimidazole, TMG, DMF and HMPA were dried by heating with calcium hydride at ≈100 °C and were then distilled under reduced pressure; methanol was dried by heating under reflux with magnesium methoxide (generated *in situ* from magnesium turnings and methanol) and was then distilled; diethyl ether was dried over sodium wire and was then distilled; THF was dried by heating, under reflux, over sodium–potassium alloy and benzophenone and was then distilled; dichloromethane was dried over phosphorus pentaoxide and was then distilled; toluene was dried by heating, under reflux, with sodium metal and was then distilled. All solvents were stored over 4 Å molecular sieves in sealed containers. Petroleum spirit refers to the fraction with distillation range 60–80 °C, unless stated otherwise.

1-*O*-Stearoyl-*sn*-glycerol 4; $R^1 = C_{17}H_{35}$

Triethylamine (4.15 cm^3, 29.8 mmol), followed by stearoyl chloride (6.03 cm^3, 17.9 mmol), was added to a rapidly stirred solution of 2,3-*O*-isopropylidene-*sn*-glycerol **3** (1.96 g, 14.8 mmol) and DMAP (0.182 g, 1.5 mmol) in dry dichloromethane (40 cm^3) at 0 °C (ice–water-bath). After 5 min, the reactants were allowed to warm to room temperature. After a further period of 45 min, water (0.64 cm^3) was added and the products were concentrated under reduced pressure. The residue was stirred with hexane (150 cm^3) and the insoluble material was removed by filtration. Silanized silica gel (50 g) was added to the filtrate and the resulting mixture was evaporated under reduced pressure. Acetonitrile–water (1 : 1 v/v; 600 cm^3) was added to the residue which was then filtered through a large sintered glass funnel. The filtrate was discarded. The residue was then washed with acetonitrile (600 cm^3) and, after toluene (200 cm^3) had been added, the washings were concentrated under reduced pressure. The crude 1-*O*-stearoyl-2,3-*O*-isopropylidene-*sn*-glycerol (6.09 g) thereby obtained was dissolved in triethyl borate (80 cm^3) at room temperature, and 2,2,2-trifluoroethanol (10 cm^3) and TFA (10 cm^3) were added. The reactants were stirred at room temperature. After 5 h, the products were evaporated under reduced pressure. Dry toluene (50 cm^3) was added and the resulting solution was re-evaporated under reduced pressure. The process was repeated and the residue was dissolved in methanol (200 cm^3). Water (200 cm^3; the first 20 cm^3 dropwise) was added. The colourless solid precipitate was collected by filtration, washed with water and then dissolved in diethyl ether (600 cm^3). The dried ($MgSO_4$) solution was evaporated under reduced pressure and the residue was crystallized twice from hexane to give the title compound (3.955 g, 74%) (Found: C, 69.7; H, 12.0. $C_{21}H_{42}O_4$·0.2 H_2O requires C, 69.45; H 11.80%), mp 67.5–68.5 °C (lit.,[18] 72.5–73 °C); $[a]_D^{20}$ +3.66 (*c* 4, C_5H_5N) {lit.,[18] $[a]_D^{25}$ +3.55 (*c* 5.24, C_5H_5N)}; δ_H ($CDCl_3$) 0.87 (3 H, t, *J* 6.8), 1.25 (28 H, m), 1.60 (2 H, m), 2.34 (2 H, t, *J* 7.6), 2.60 (1 H, br), 2.97 (1 H, br), 3.57 (1 H, dd, *J* 5.9 and 11.5), 3.69 (1 H, dd, *J* 3.7 and 11.5), 3.92 (1 H, m), 4.16 (2 H, m); δ_C ($CDCl_3$) 14.19, 22.76, 24.97, 29.20, 29.33, 29.43, 29.53, 29.68, 29.73, 29.77, 31.99, 34.23, 63.42, 65.18, 70.33, 174.49.

3-*O*-Stearoyl-*sn*-glycerol

1,2-*O*-Isopropylidene-*sn*-glycerol **7** (1.00 g, 7.56 mmol) was converted into 3-*O*-stearoyl-*sn*-glycerol (1.49 g, 55%) (Found: C, 69.9; H, 11.9. $C_{21}H_{42}O_4$ requires C, 70.35; H, 11.81%) by the same two-step procedure (see the above preparation of 1-*O*-stearoyl-*sn*-glycerol); it had mp 68.5 °C; $[a]_D^{19}$ −3.41 (*c* 3.99, C_5H_5N). The ^{1}H and ^{13}C NMR spectra of 3-*O*-stearoyl-*sn*-glycerol were identical with the corresponding spectra (see above) of the 1-*O*-stearoyl enantiomer **4**.

3-*O*-(9-Phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol 5; $R^1 = C_{17}H_{35}$

A solution of 1-*O*-stearoyl-*sn*-glycerol **4** (2.87 g, 8.0 mmol) and PxOH (2.42 g, 8.8 mmol) in glacial acetic acid (100 cm^3) was evaporated under reduced pressure (bath temperature <35 °C). The residue was re-dissolved in glacial acetic acid (2 × 50 cm^3) and the resulting solution was re-evaporated. A solution of the residue in hexane (100 cm^3) was washed with saturated aq. sodium hydrogen carbonate (2 × 50 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure. The residue was stirred with hexane (30 cm^3) and the insoluble material was removed by filtration. The filtrate was applied to a column of silica gel (100 g). The column was then eluted with hexane–ethyl acetate (100:0 to 74:26 v/v) containing ≈0.1% pyridine. Early fractions, which were eluted with hexane–ethyl acetate (96:4 to 94:6 v/v), were evaporated under reduced pressure to give putative 2,3-bis-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol (1.05 g); R_f 0.75 [hexane–diethyl ether (1:1 v/v)]. Later fractions, which were eluted with hexane–ethyl acetate (86:14 to 77:23 v/v), were combined, washed successively with 0.2 mol dm^{-3} sodium acetate buffer (pH 5) and saturated aq. sodium hydrogen carbonate, dried ($MgSO_4$) and evaporated under reduced pressure to give the title compound as a colourless oil (3.58 g, ≈72%); R_f 0.48 [hexane–diethyl ether (1:1 v/v)] ; $[\alpha]_D^{20}$ +4.1 (*c* 2.02, toluene); δ_H ($CDCl_3$) 0.80 (3 H, m), 1.18 (28 H, m), 1.46 (2 H, m), 2.15 (2 H, t, *J* 7.6), 2.30 (1 H, br), 2.94 (2 H, m), 3.85 (1 H, m), 4.07 (2 H, m), 6.94 (2 H, m), 7.0–7.3 (11 H, m); δ_C ($CDCl_3$) 14.57, 23.13, 25.26, 29.56, 29.68, 29.80, 29.90, 30.05, 30.10, 30.14, 32.36, 34.55, 64.17, 65.92, 69.49, 76.11, 116.80, 123.04, 124.05, 126.85, 127.16, 128.31, 129.74, 129.93, 148.88, 151.69, 174.39.

1-*O*-(9-Phenylxanthen-9-yl)-3-*O*-stearoyl-*sn*-glycerol

3-*O*-Stearoyl-*sn*-glycerol (1.401 g, 3.9 mmol) was converted into 1-*O*-(9-phenylxanthen-9-yl)-3-*O*-stearoyl-*sn*-glycerol (1.84 g, ≈76%) by the same procedure [see the above preparation of 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **5**; $R^1 = C_{17}H_{35}$)]; it has $[\alpha]_D^{20}$ −4.1 (*c* 3.03, toluene). The ^1H and ^{13}C NMR spectra of 1-*O*-(9-phenylxanthen-9-yl)-3-*O*-stearoyl-*sn*-glycerol were virtually identical with the corresponding spectra of the above enantiomer.

2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycerol 6; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$

1-Methylimidazole (1.38 cm^3, 17.3 mmol), a solution of arachidonic acid (1.053 g, 3.46 mmol) in dichloromethane (5 cm^3), and 2,6-dichlorobenzoyl chloride (0.99 cm^3, 6.9 mmol) were added in turn to a stirred solution of 3-*O*-(9-phenylxanthen-9-yl)-1-*O*-stearoyl-*sn*-glycerol **5**; $R^1 = C_{17}H_{35}$ (1.774 g, ≈2.9 mmol) in dichloromethane (20 cm^3) at room temperature. A heavy, colourless precipitate was obtained after 2 min. After 80 min, water (0.21 cm^3, 11.7 mmol) and then triethylamine (1.20 cm^3, 8.6 mmol) were added. The products were evaporated under reduced pressure and the residue was triturated with diethyl ether–hexane (1:5 v/v; 3 × 30 cm^3). The insoluble material was removed by decantation and the supernatant was washed with saturated aq. sodium hydrogen carbonate (100 cm^3). The aqueous layer was back-extracted with hexane (50 cm^3). The combined organic layers were washed first with 0.2 mol dm^{-3} sodium acetate buffer (pH ≈ 5; 2 × 100 cm^3) and then with an aqueous solution of sodium chloride and sodium hydrogen carbonate (50 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure. A solution of the residue in hexane was applied to a column of silica gel (100 g), which was then eluted with hexane–diethyl ether (98:2 v/v). The appropriate fractions of the eluate were evaporated under reduced pressure to give an oily residue (2.57 g); R_f 0.43 [hexane–diethyl ether (9:1 v/v)]. This material was dissolved in dry dichloromethane (45 cm^3), and pyrrole (1.95 cm^3, 28 mmol) and dichloroacetic acid (0.47 cm^3, 5.7 mmol) were added to the stirred solution at room temperature. After 5 min, the reaction solution was shaken with 0.2 mol dm^{-3} sodium acetate buffer (pH ≈ 5; 150 cm^3). The layers were separated and the aqueous layer was extracted with dichloromethane (50 cm^3). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure, ensuring that all of the excess of pyrrole was removed. The residue was triturated with hexane–acetic acid (99.95:0.05 v/v; 3 × 10 cm^3). The supernatant was applied to a column of silica gel (100 g) which was then eluted with hexane–diethyl ether (100:0 to 40:60 v/v) containing ≈0.05% acetic acid. The appropriate fractions, which were eluted with hexane–diethyl ether (80:20 to 45:55 v/v), were combined, and evaporated under reduced pressure to give the *title compound* (1.68 g, ≈90%) as an oily residue (HRMS Found: M^+, 644.5360. $^{12}C_{41}{}^1H_{72}{}^{15}O_5$ requires *M*, 644.53795); R_f 0.43 [diethyl ether–hexane (1:1 v/v)]; $[\alpha]_D^{22}$ −0.4 (*c* 3.42, toluene); δ_H ($CDCl_3$) 0.88 (6 H, m), 1.25–1.45 (34 H, m), 1.61 (2 H, m), 1.72 (2 H, m), 2.06 (2 H, dd, *J* 6.9 and 13.8), 2.13 (2 H, dd, *J* 7.2 and 13.6), 2.34 (4 H, dt, *J* 7.6 and 16.6), 2.82 (6 H, m), 3.72 (2 H, d, *J* 5.1), 4.22 (1 H, dd, *J* 5.8 and 11.9), 4.33 (1 H, dd, *J* 4.3 and 12.0), 5.09 (1 H, m), 5.37 (8 H, m); δ_C ($CDCl_3$) 14.33, 14.37, 22.83, 22.95, 24.97, 25.12, 25.85, 26.71, 27.46, 29.37, 29.52, 29.57, 29.62, 29.73, 29.88, 29.92, 29.95, 31.76, 32.18, 33.86, 34.32, 61.58, 62.33, 72.41, 127.75, 128.05, 128.30, 128.51, 128.82, 128.99, 129.23, 130.71, 173.46, 174.08.

2-*O*-Arachidonoyl-3-*O*-stearoyl-*sn*-glycerol 8; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$

1-*O*-(9-Phenylxanthen-9-yl)-3-*O*-stearoyl-*sn*-glycerol (1.255 g, ≈2.04 mmol) was allowed to react with arachidonic acid (0.746 g, 2.45 mmol), 2,6-dichlorobenzoyl chloride (0.70 cm^3, 4.9 mmol) and 1-methylimidazole (0.98 cm^3, 12.1 mmol) in dichloromethane (12 cm^3) solution, as in the above preparation of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol, to give its 2-*O*-arachidonoyl derivative (1.794 g). This material (0.853 g) was treated as above with pyrrole (0.65 cm^3, 9.4 mmol) and dichloroacetic acid (0.156 cm^3, 3.2 mmol) in dichloromethane (15 cm^3) to give the *title compound* (0.578 g, ≈92% overall yield for the two steps). The ^1H and ^{13}C NMR spectra of 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol were closely similar to the corresponding spectra of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6**; $R^1 = C_{17}H_{25}$, $R^2 = C_{19}H_{31}$.

Determination of the diastereoisomeric excesses of 2-*O*-arachidonoyl-1(and 3)-*O*-stearoyl-*sn*-glycerol 6 and 8; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$

(a) 1-Methylimidazole (0.121 cm^3, 1.5 mmol) and then (−)-camphanyl chloride **9** (0.111 g, 0.5 mmol) were added to a stirred solution of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6** (0.156 g, 0.24 mmol) in dry dichloromethane (3 cm^3) at room temperature. After 45 min, hexane (50 cm^3) was added and the resulting solution was washed with saturated aq. sodium hydrogen carbonate (2 × 50 cm^3). The organic layer was separated and washed first with 0.2 mol dm^{-3} sodium acetate buffer (pH ≈ 5; 50 cm^3) and then with an aqueous solution of sodium acetate and sodium chloride (50 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure and the residue was fractionated by chromatography on silica gel (10 g). The column was eluted with hexane–diethyl ether (100:0 to 76:24 v/v); the appropriate fractions, which were eluted with hexane–diethyl ether (90:10 to 80:20 v/v) were combined, and evaporated under reduced pressure to give 2-*O*-arachidonoyl-3-*O*-(−)-camphanyl-1-*O*-stearoyl-*sn*-glycerol **10**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$ (0.184 g, ≈92%), R_f 0.44 [hexane–diethyl ether (1:1 v/v)]; δ_H ($CDCl_3$) 0.83 (6 H, m), 0.92 (3 H, s), 1.02 (3 H, s), 1.07 (3 H, s), 1.25 (34 H, m), 1.61 (5 H, m), 1.87

(1 H, m), 2.02 (5 H, m), 2.33 (5 H, m), 2.78 (6 H, m), 4.13 (1 H, dd, *J* 5.8 and 11.9), 4.27 (2 H, m), 4.45 (1 H, dd, *J* 4.0 and 11.9), 5.33 (9 H, m); δ_C ($CDCl_3$) 9.64, 14.04, 14.09, 16.61, 16.67, 22.53, 22.65, 24.62, 24.79, 25.56, 25.57, 25.59, 26.41, 27.17, 28.85, 29.06, 29.23, 29.25, 29.28, 29.33, 29.43, 29.58, 29.62, 29.66, 30.64, 31.47, 31.89, 33.43, 33.93, 54.13, 54.69, 61.71, 63.16, 68.58, 90.83, 127.76, 128.00, 128.22, 128.52, 128.64, 128.98, 130.39, 166.99, 172.43, 173.07, 177.71. An expansion of this ^{13}C NMR spectrum revealed (Fig. 1a) two signals in the region of δ 68 [68.58 (98%) and 68.72 (2%)], assigned to the *C*-2 resonances of 2-*O*-arachidonoyl-3-*O*-(−)-camphanyl-1-*O*-stearoyl-*sn*-glycerol **10**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$ and 2-*O*-arachidonoyl-1-*O*-(−)-camphanyl-3-*O*-stearoyl-*sn*-glycerol **11**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$, respectively. The enantiomeric excess of the 1-*O*-stearoyl isomer was thereby estimated to be at least 96%.

(b) In the same way, 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$, was converted into its 1-*O*-(−)-camphanyl derivative **11**. Examination of the ^{13}C NMR spectrum ($CDCl_3$) of this product revealed resonance signals at δ 68.68 (5%) and 68.83 (95%), respectively. The enantiomeric excess of 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8** was thereby estimated to be at least 90%. The ^{13}C NMR spectrum ($CDCl_3$) of a 1:1 mixture of the two diasterioisomers (Fig. 1b), prepared by the action of (−)-camphanyl chloride **9** on the racemic modification of 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol, revealed two resonance signals of equal intensity in the region of δ 68.

1,3,4,5,6-Penta-*O*-(*p*-anisoyl)-*myo*-inositol 13

myo-Inositol **12** (19.61 g, 0.109 mol) was added to a stirred solution of *p*-anisoyl chloride (96.32 g, 0.565 mol) in dry pyridine (380 cm^3) at 0 °C (ice–water-bath). After 1 h, the reactants were allowed to warm to room temperature. After a further period of 18 h, water (20 cm^3) was added and the products were evaporated under reduced pressure. A solution of the residue in chloroform (2 dm^3) was washed first with saturated aq. sodium hydrogen carbonate (700 + 300 cm^3) and then with 4 mol dm^{-3} hydrochloric acid (350 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure and re-dissolved in boiling dichloromethane (≈2.5 dm^3). Petroleum spirit (≈1 dm^3) was added until the solution became slightly turbid. After the solution had been kept at room temperature for 14 h, the *title compound* was collected by filtration as a colourless crystalline precipitate (66.72 g, 72%) (Found, in material recrystallized from hexane–ethyl acetate: C, 64.7; H, 4.8. $C_{46}H_{42}O_{16}$ requires C, 64.94; H 4.98%), mp 234–236 °C; R_f 0.55 [chloroform–methanol (95:5 v/v)]; δ_H ($CDCl_3$ + CD_2Cl_2) 3.67, 3.68, 3.72 (15 H, 3 s), 4.71 (1 H, t, *J* 2.2), 5.45 (2 H, dd, *J* 2.4 and 10.4), 5.80 (1 H, t, *J* 9.9), 6.21 (2 H, t, *J* 10.2), 6.68 (6 H, m), 6.75 (4 H, m), 7.73 (6 H, m), 7.85 (4 H, m); δ_C ($CDCl_3$ + CD_2Cl_2) 55.21, 55.26, 68.62, 69.63, 70.81, 71.65, 113.38, 113.56, 121.17, 121.35, 131.59, 131.64, 131.79, 163.36, 163.63, 165.03, 165.10.

2-*O*-(4-Methoxytetrahydropyran-4-yl)-*myo*-inositol 15

Triphenylphosphine hydrobromide[38] (1.60 g, 4.7 mmol) was added to a stirred suspension of 1,3,4,5,6-penta-*O*-(*p*-anisoyl)-*myo*-inositol **13** (39.58 g, 46.5 mmol) and 4-methoxy-3,6-dihydro-2*H*-pyran[24] **14** (≈85%; 62.4 g, ≈0.465 mol) in dichloromethane (460 cm^3) at room temperature. After 40 h, triethylamine (5.0 cm^3) was added and the products were concentrated under reduced pressure (water pump, bath temperature <25 °C). Diethyl ether was added to the yellow oily residue until the precipitation of a colourless solid commenced. After 5 min, additional diethyl ether (≈500 cm^3) followed by petroleum spirit (30–40 °C; ≈350 cm^3) were then added to complete the precipitation process. The precipitate was collected by filtration‡ and dissolved in boiling ethyl acetate–triethylamine (99.9:0.1 v/v). Activated charcoal (≈5 g) was added to the boiling solution which, after 5 min, was filtered. Petroleum spirit was added to the hot filtrate until it became slightly turbid. After 18 h at room temperature, the colourless crystalline precipitate (44.73 g) was collected by filtration; mp 219–221 °C; R_f 0.43 [chloroform–methanol (98:2 v/v)]. This material (73.32 g, prepared in two batches) was dissolved in methanol–THF (1:4 v/v; 1.2 dm^3) and the stirred solution was heated under gentle reflux. Methanolic sodium methoxide (25% w/w; 8.8 cm^3, ≈38 mmol) was added with continued heating and stirring. After 30 min, the products were allowed to cool. The resulting colourless precipitate was collected by filtration, washed with diethyl ether and then dissolved in the minimum quantity of water (≈20 cm^3). Aq. triethylammonium hydrogen carbonate (2 mol dm^{-3}; 4 cm^3) was added and the resulting solution was evaporated under reduced pressure. Crystallization of the residue from methanol–water gave the *title compound* (18.27 g, 81% overall yield) (Found: C, 48.85; H, 7.5. $C_{12}H_{22}O_8$ requires C, 48.97; H, 7.53%), mp 187 °C; R_f 0.21 [chloroform–methanol (7:3 v/v)]; δ_H [$(CD_3)_2SO$–D_2O] 1.75 (4 H, m), 2.95 (1 H, t, *J* 8.9), 3.16 (5 H, m), 3.38 (4 H, m), 3.67 (2 H, m), 4.01 (1 H, br s); δ_C [$(CD_3)_2SO$] 34.63, 48.49, 64.42, 71.05, 73.03, 73.46, 75.49, 93.36.

2-*O*-(4-Methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol 17

Triethylamine (94 cm^3, 0.67 mol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[25] **16** (26.83 cm^3, 84 mmol) were added to a mechanically stirred mixture of finely powered 2-*O*-(4-methoxytetrahydropyran-4-yl)-*myo*-inositol **15** (20.57 g, 69.9 mmol), imidazole (22.84 g, 0.335 mol) and HMPA (350 cm^3) at room temperature. After 60 h, the products were partitioned between ethyl acetate (1.5 dm^3) and half-saturated aq. sodium hydrogen carbonate (1.0 dm^3). The separated organic phase was washed with half-saturated aq. sodium hydrogen carbonate (0.5 dm^3) and the combined aqueous layers were back-extracted with ethyl acetate (200 cm^3). The combined organic layers were washed successively with water (3 × 1.0 dm^3) and saturated brine (0.5 dm^3), and were then dried ($MgSO_4$), and evaporated under reduced pressure. Crystallization of the gummy residue from hexane gave the *title compound* (23.56 g, 62.8%) (Found, in material recrystallized from methanol–water: C, 52.89; H, 9.12. $C_{24}H_{48}O_9Si_2 \cdot 0.5\ H_2O$ requires C, 52.81; H 9.05%); mp 133–134 °C; R_f 0.49 (ethyl acetate); δ_H ($CDCl_3$) 1.04 (28 H, m), 1.82 (1 H, m), 1.96 (3 H, m), 2.54 (1 H, d, *J* 0.8), 2.71 (1 H, d, *J* 3.6), 3.36 (3 H, s), 3.46 (3 H, m), 3.69 (4 H, m), 3.81 (1 H, m), 3.91 (2 H, m), 4.40 (1 H, t, *J* 2.4); δ_C ($CDCl_3$) 12.13, 12.19, 12.82, 17.22, 17.26, 17.29, 17.35, 17.36, 17.39, 17.40, 33.26, 34.53, 49.66, 64.72, 64.96, 70.95, 71.66, 72.28, 74.60, 76.37, 78.30, 99.81.

6-*O*-{2,7-Dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol 18

A mixture of 2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol **17** (10.62 g, 19.8 mmol), 2,7-dibromo-9-chloro-9-[3-(trifluoromethyl)phenyl]xanthene[26] (DtpxCl) (20.49 g, 39.5 mmol) and pyridine (10 cm^3) were stirred at room temperature for 20 min. Acetonitrile (70 cm^3) was then added over a period of 1 h to the stirred reactants. Water (1.4 cm^3) and triethylamine (6.0 cm^3, 43 mmol) were added and the products were evaporated under reduced pressure. A solution of the residue in ethyl acetate (500 cm^3) was washed with water (2 × 250 cm^3) and the aqueous layer was back-extracted with ethyl acetate (100 cm^3). The

‡ 4-Methoxy-3,6-dihydro-2*H*-pyran **14** (≈70%) was recovered by distillation of the filtrate.

combined organic layers were washed with brine (100 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. Acetonitrile (≈250 cm^3) was added and the crystalline precipitate (3.14 g), believed to be the 1-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl} derivative, was collected by filtration. The filtrate was evaporated and the residue was chromatographed on silica gel. The column was eluted with hexane–diethyl ether (100 : 0 to 0 : 100 v/v). More of the putative isomeric 1-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]-xanthen-9-yl} derivative, which had R_f 0.55 [dichloromethane–methanol (98 : 2 v/v)], was eluted first. The *title compound* (14.88 g, 73%) [Found: $(M - H)^-$ (FAB), 1017.1849. $^{12}C_{44}{}^{1}H_{56}{}^{79}Br^{81}Br^{19}F_3{}^{16}O_{10}{}^{28}Si_2$ requires *m/z*, 1017.1710] had R_f 0.25 [dichloromethane–methanol (98 : 2 v/v)]; δ_H ($CDCl_3$) 1.12 (28 H, m), 1.48 (1 H, m), 1.76 (3 H, m), 2.58 (1 H, br), 2.81 (1 H, d, *J* 8.0), 2.91 (3 H, s), 3.18 (1 H, dt, *J* 2.6 and 8.3), 3.44 (1 H, d, *J* 8.6), 3.60 (3 H, m), 3.78 (2 H, m), 3.89 (1 H, t, *J* 8.6), 3.97 (1 H, t, *J* 8.4), 4.08 (1 H, t, *J* 2.3), 7.02 (1 H, d, *J* 8.7), 7.09 (1 H, d, *J* 8.8), 7.37 (7 H, m), 7.80 (1 H, d, *J* 8.4); δ_C ($CDCl_3$) included the following signals: 12.46, 12.60, 12.68, 13.45, 17.28, 17.34, 17.36, 17.41, 17.54, 17.67, 17.78, 18.02, 32.98, 34.36, 48.97, 64.58, 64.79, 71.33, 71.51, 72.18, 75.94, 76.56, 77.83, 79.00, 99.53.

6-*O*-{2,7-Dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-3-*O*-(−)-menthoxyacetyl-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D- and -L-*myo* inositol 19 and 20

Dry pyridine (80 cm^3) was added slowly to a stirred mixture of (−)-MacCl (6.30 g, 27.1 mmol) and freshly sublimed 1*H*-tetrazole (1.98 g, 28.3 mmol) in dry acetonitrile (15 cm^3) at room temperature. The resulting solution was added rapidly to a mixture of 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)-phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol **18** (14.62 g, 14.3 mmol) and DMAP (4.94 g, 40.4 mmol). After 45 min, when TLC [hexane–diethyl ether (1 : 1 v/v)] revealed that the proportions of starting material (R_f 0.12) and the putative bis[(−)-menthoxyacetate] (R_f 0.64) were approximately equal, water (1 cm^3) was added. The products were evaporated under reduced pressure and the residue was re-evaporated with toluene (3 × 50 cm^3). Ethyl acetate (250 cm^3) was added, and after filtration the solution was washed with saturated aq. sodium hydrogen carbonate (2 × 150 cm^3). The combined aqueous layers were back-extracted with ethyl acetate (100 cm^3). Finally, the combined organic layers were washed successively with water (2 × 100 cm^3) and brine (100 cm^3), and were then dried ($MgSO_4$), and evaporated under reduced pressure.

When the residual glass was dissolved in acetonitrile, colourless crystals of *6-O-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]-xanthen-9-yl}-3-O-(−)-menthoxyacetyl-2-O-(4-methoxytetrahydropyran-4-yl)-4,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-L-myo-inositol* **20** were obtained (5.96 g, 34.2%) (Found, in material recrystallized from acetonitrile–diethyl ether: C, 55.2; H, 6.25. $C_{56}H_{77}Br_2F_3O_{12}Si_2$ requires C, 55.35; H, 6.39%); mp 217 °C; $[a]_D^{25}$ −25.3 (*c* 2.00, ethyl acetate); R_f 0.38 [hexane–diethyl ether (1 : 1 v/v)]: δ_H ($CDCl_3$) 0.73–1.35 (42 H, m), 1.47 (2 H, m), 1.62 (3 H, m), 1.77 (1 H, m), 1.99 (1 H, m), 2.25 (1 H, m), 2.76 (1 H, d, *J* 8.5), 2.80 (3 H, s), 3.12 (1 H, dt, *J* 4.1 and 10.5), 3.23 (1 H, dt, *J* 2.6 and 8.8), 3.51 (2 H, m), 3.64 (2 H, m), 3.82 (1 H, m), 4.05 (5 H, m), 4.89 (1 H, m), 7.00 (1 H, d, *J* 8.7), 7.08 (1 H, d, *J* 8.7), 7.26–7.45 (7 H, m), 7.81 (1 H, d, *J* 7.5); δ_C ($CDCl_3$) included the following signals: 12.51, 12.59, 12.67, 13.40, 16.10, 17.23, 17.27, 17.43, 17.69, 17.79, 18.02, 20.98, 22.25, 23.14, 25.31, 31.47, 33.30, 34.27, 34.33, 39.65, 48.05, 48.75, 64.49, 64.80, 65.27, 70.68, 71.76, 72.08, 73.65, 75.78, 78.15, 78.64, 79.74, 99.30.

The mother liquors from the above crystallizations (from both acetonitrile and acetonitrile–diethyl ether) were combined, evaporated under reduced pressure, and the residue was fractionated by chromatography on silica gel. The column was eluted with hexane–ethyl acetate (100 : 0 to 66 : 34 v/v). Appropriate fractions, eluted with hexane–ethyl acetate (80 : 20 to 74 : 26 v/v), were combined, and evaporated under reduced pressure to give 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-3-*O*-(−)-menthoxyacetyl-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-*myo*-inositol **19** as a colourless foam (4.47 g, 25.6%); R_f 0.45 [hexane–diethyl ether (1 : 1 v/v)]; $[a]_D^{25}$ −16.2 (*c* 2.00, ethyl acetate); δ_H ($CDCl_3$) 0.74–1.39 (42 H, m), 1.48 (2 H, m), 1.63 (3 H, m), 1.74 (1 H, m), 2.01 (1 H, m), 2.23 (1 H, m), 2.70 (1 H, d, *J* 8.5), 2.80 (3 H, s), 3.10 (1 H, dt, *J* 4.2 and 10.6), 3.22 (1 H, dt, *J* 2.6 and 8.8), 3.50 (2 H, m), 3.63 (2 H, m), 3.81 (1 H, m), 4.05 (5 H, m), 4.84 (1 H, m), 7.00 (1 H, d, *J* 8.7), 7.08 (1 H, d, *J* 8.7), 7.30–7.45 (7 H, m), 7.80 (1 H, d, *J* 7.6); δ_C ($CDCl_3$) included the following signals: 12.52, 12.63, 12.69, 13.43, 14.13, 16.13, 17.21, 17.26, 17.28, 17.43, 17.70, 17.81, 18.03, 20.97, 22.23, 23.16, 25.32, 26.92, 31.55, 33.41, 34.29, 34.34, 39.83, 48.04, 48.68, 64.50, 64.80, 65.65, 70.68, 71.70, 72.36, 73.58, 75.78, 78.23, 78.67, 80.25, 99.31.

Later fractions, which were eluted from the column with hexane–ethyl acetate (73 : 27 to 66 : 34 v/v), were concentrated under reduced pressure. The residue was crystallized from acetonitrile to give more L-*myo*-inositol-derived diastereoisomer **20** [1.33 g; total yield of this diastereoisomer: 7.29 g (41.7%)]. The mother liquor was concentrated and rechromatographed to give more D-*myo*-inositol-derived diastereoisomer **19** [2.62 g; total yield of this diastereoisomer, 7.09 g (40.5%)].

6-*O*-{2,7-Dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 21

A solution of 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]-xanthen-9-yl}-3-*O*-(−)-menthoxyacetyl-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-*myo*-inositol **19** (7.09 g, 5.8 mmol) in 8 mol dm^{-3} ethanolic methylamine (40 cm^3) was stirred at room temperature for 90 min and was then evaporated under reduced pressure. The residue was dissolved in a 1.0 mol dm^{-3} solution of TEAF in acetonitrile (25 cm^3) at room temperature. After 30 min, the products were concentrated under reduced pressure and the residue was fractionated between ethyl acetate (300 cm^3) and half-saturated aq. sodium hydrogen carbonate (200 cm^3). The organic layer was separated, washed successively with water (2 × 100 cm^3) and brine (50 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by chromatography on silica gel (200 g). The column was eluted first with dichloromethane–acetonitrile (100 : 0 to 50 : 50 v/v). Subsequent elution with dichloromethane–methanol (96 : 4 to 89 : 11 v/v) and evaporation of the appropriate fractions gave the title compound (4.33 g, ≈95%) as a colourless foam; R_f 0.42 [dichloromethane–ethanol (9 : 1 v/v)]; δ_H [$(CD_3)_2SO$] 1.55–1.75 (4 H, m), 3.05 (3 H, s), 3.10 (1 H, m), 3.30 (5 H, m), 3.50 (1 H, t, *J* 7.5), 3.56 (2 H, m), 3.99 (1 H, m), 4.19 (1 H, d, *J* 4.7), 4.27 (1 H, d, *J* 5.7), 4.52 (1 H, d, *J* 4.1), 4.70 (1 H, m), 7.08 (1 H, d, *J* 2.3), 7.16 (1 H, d, *J* 2.4), 7.20 (2 H, t, *J* 9.0), 7.41 (1 H, d, *J* 8.0), 7.48 (3 H, m), 7.60 (1 H, d, *J* 7.6), 8.07 (1 H, br s); δ_C [$(CD_3)_2SO$] included the following signals: 34.32, 34.44, 48.37, 64.27, 71.14, 73.45, 73.67, 75.12, 76.76, 98.33.

6-*O*-{2,7-Dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol 22

A suspension of crystalline 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-3-*O*-(−)-menthoxyacetyl-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-L-*myo*-inositol **20** (1.215 g, 1.0 mmol) in a 1.0 mol dm^{-3} solution of TEAF in acetonitrile (4 cm^3) was heated, under gentle reflux, for *ca*. 2 min until a homogeneous

solution was obtained. The cooled products were evaporated under reduced pressure and the residue was dissolved in ethyl acetate (100 cm^3). The solution was washed first with water (2 × 30 cm^3) and then with an aqueous solution containing both sodium chloride and sodium hydrogen carbonate (30 cm^3). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the residue was dissolved in absolute ethanol (5 cm^3) at room temperature. Ethanolic methylamine (8.0 mol dm^{-3}; 5 cm^3) was then added. After 40 min, the products were evaporated under reduced pressure and fractionated by chromatography on silica gel as in the above preparation of the enantiomeric inositol derivative **21**. The title compound **22** (0.646 g, ≈83%) was obtained as a colourless glass; its ^1H and ^{13}C NMR spectra were identical with the corresponding spectra of its enantiomer.

1,4,5,6-Tetra-*O*-benzyl-D-*myo*-inositol 26

Chloroacetic acid (0.37 g, 3.9 mmol) was added to a stirred solution of 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]-xanthen-9-yl}-3-*O*-(−)-menthoxyacetyl-2-*O*-(4-methoxytetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-*myo*-inositol **19** (0.243 g, 0.02 mmol) and pyrrole (0.20 cm^3, 2.9 mmol) in dry dichloromethane (10 cm^3) at room temperature. After 15 min, triethylamine (2.0 cm^3) and dichloromethane (50 cm^3) were added and the products were washed with saturated aq. sodium hydrogen carbonate (100 cm^3). The aqueous layer was separated, and back-extracted with dichloromethane (50 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by chromatography on silica gel (10 g). The column was eluted with hexane–ethyl acetate (100 : 0 to 50 : 50 v/v): the appropriate fractions, which were eluted with hexane–ethyl acetate (70 : 30 to 60 : 40 v/v), were combined, and evaporated under reduced pressure to give a colourless glass (0.132 g); R_f 0.26 [hexane–ethyl acetate (7 : 3 v/v)].

This material (0.132 g) and imidazole (0.049 g, 0.72 mmol) were dissolved in dry DMF (1.0 cm^3) at room temperature and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane **16** (0.115 cm^3, 0.36 mmol) was added to the well stirred solution. The reactants were heated at 80 °C. After 7 h, the products were cooled and ethyl acetate (100 cm^3) was added. The resulting solution was washed successively with half-saturated aq. sodium hydrogen carbonate (2 × 100 cm^3), water (3 × 100 cm^3) and brine (50 cm^3). The combined aqueous layers were back-extracted with ethyl acetate (50 cm^3). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure. Crystallization from acetonitrile gave colourless crystals of compound **23** (0.119 g), mp 160–162 °C; R_f 0.46 [hexane–diethyl ether (4 : 1 v/v)].

This material (0.423 g, obtained from more than one experiment) and PTSA monohydrate (≈0.005 g) were dissolved in dichloromethane–methanol (1 : 1 v/v; 5 cm^3) at room temperature. After 2 h, triethylamine (2 cm^3) was added and the products were evaporated under reduced pressure. The residue was dissolved in ethanolic methylamine (8 mol dm^{-3}, 10 cm^3). After 45 min, the products were concentrated under reduced pressure and the residue was recrystallized from acetonitrile to give colourless crystals (0.155 g); R_f 0.26 [hexane–diethyl ether (4 : 1 v/v)]. A second crop (0.098 g) was obtained following chromatography of the mother liquors.

The product (0.202 g) and PTSA monohydrate (≈0.005 g) were dissolved in 1,1-dimethoxycyclopentane (1.0 cm^3) at room temperature. After 1 h, triethylamine (0.2 cm^3) was added and the products were concentrated under reduced pressure. The residue was dissolved in hexane (30 cm^3) and silica gel (2 g) was added to the stirred solution. After 1 min, the products were filtered and the residue was washed with hexane–diethyl ether (95 : 5 v/v; 100 cm^3). The filtrate and washings were evaporated under reduced pressure and the residue (compound **25**) was dissolved in ethanol (2 cm^3). An acetonitrile solution of TEAF (1 mol dm^{-3}, 80 mm^3) and aq. tetraethylammonium hydroxide (20% w/v; 49 mm^3) were added and the reactants were heated, under gentle reflux, for 16 h. The products were neutralized by bubbling in carbon dioxide gas and were then concentrated. The residual gum was triturated with diethyl ether (3 × 10 cm^3) and the supernatant liquid was discarded. The remaining material was evaporated from acetonitrile solution (3 × 5 cm^3) and was then dissolved in DMF (2 cm^3). This solution was added carefully to sodium hydride (60% dispersion in mineral oil; 0.389 g, 9.7 mmol) at 0 °C (ice–water-bath) and benzyl chloride (0.55 cm^3, 4.8 mmol) was added dropwise over a period of 1 min to the stirred resulting mixture. After 2.5 h, ethanol was added dropwise until the effervescence ceased. Ethyl acetate (100 cm^3) was then added. The resulting mixture was washed successively with half-saturated aq. sodium hydrogen carbonate (2 × 50 cm^3), water (3 × 50 cm^3) and brine (50 cm^3); it was then dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by silica gel chromatography. The column was eluted with hexane–diethyl ether (100 : 0 to 80 : 20 v/v): the appropriate fractions, which were eluted with hexane–diethyl ether (96 : 4 to 80 : 20 v/v), were evaporated under reduced pressure. The residue was dissolved in dichloromethane–methanol (1 : 1 v/v; 6 cm^3) at room temperature. Water (0.5 cm^3) and PTSA monohydrate (0.02 g) were added. After 2 h, triethylamine (0.2 cm^3) was added and the products were concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 cm^3). The resulting solution was washed successively with half-saturated aq. sodium hydrogen carbonate (2 × 50 cm^3) and brine; it was then dried ($MgSO_4$), and evaporated under reduced pressure. Crystallization of the residue from cyclohexane gave the title compound (0.132 g, 19% overall yield for the 8 steps) (Found: C, 75.35; H, 6.6. Calc. for $C_{34}H_{36}O_2$: C, 75.53; H, 6.71%), mp 144–145 °C (lit.,[27] 142.5 °C); R_f 0.08 [hexane–diethyl ether (1 : 1 v/v)]; $[a]_D^{20}$ +22.7 (*c* 1.18, chloroform) [lit.,[27] +23.4 (*c* 0.22, chloroform)]; δ_H ($CDCl_3$) 2.45 (1 H, d, *J* 4.3), 2.53 (1 H, br s), 3.48 (3 H, m), 3.84 (1 H, t, *J* 8.5), 3.97 (1 H, t, *J* 9.5), 4.21 (1 H, m), 4.72 (3 H, m), 4.84 (2 H, dd, *J* 3.4 and 10.7), 4.94 (3 H, m), 7.31 (20 H, m).

1,4,5,6-Tetra-*O*-benzyl-L-*myo*-inositol 27

In the same way, 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)-phenyl]xanthen-9-yl}-3-*O*-menthoxyacetyl-2-*O*-(4-methoxy-tetrahydropyran-4-yl)-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-L-*myo*-inositol **20** was converted into 1,4,5,6-tetra-*O*-benzyl-L-*myo*-inositol **27** in 24% overall yield for the 8 corresponding steps. The title compound (Found: C, 75.0; H, 6.7%) had mp 144–145 °C, $[a]_D^{20}$ −21.7 (*c* 1.05, chloroform); its ^1H NMR spectrum was identical with that of its D-enantiomer **26** (see above).

3,4,5-Tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 29

CpacCl (2.81 cm^3, 18.0 mmol) was added to a stirred suspension of 1*H*-tetrazole (1.33 g, 19.0 mmol) in dry acetonitrile (15 cm^3) at room temperature. Pyridine (7.5 cm^3) was then added slowly. The resulting yellow–orange solution was added to 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol **21** (3.12 g, 4.0 mmol) and the reactants were stirred at room temperature for 1 h. DMAP (2.66 g, 21.8 mmol) was then added and the reactants were stirred at room temperature for a further period of 35 min. Water (0.33 cm^3) was added and the products were concentrated under reduced pressure. The residue was suspended in ethyl acetate (200 cm^3) and the mixture was filtered through a bed of Celite. The filtrate was washed first with saturated aq. sodium hydrogen carbonate

(2×100 cm^3) and then with brine–0.20 mol dm^{-3} sodium acetate buffer (pH 5; 100 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure and the residue was fractionated by chromatography on silica gel. The column was washed with hexane–dichloromethane–triethylamine (0:99.5:0.5 to 49.5:50:0.5 v/v) and was then eluted with hexane–dichloromethane–triethylamine–acetonitrile (49.5:50:0.5:0.0 to 44.5:45:0.5:10 v/v). Early fractions, which were eluted with a solvent mixture containing 1–5% acetonitrile, were combined, and evaporated under reduced pressure to give a product (1.99 g) with R_f 0.48 [diethyl ether–hexane (4:1 v/v)] which was believed to be the tetrakis(4-chlorophenoxy)acetyl] derivative **30**. Later fractions, which were eluted with a solvent mixture containing 6–9% acetonitrile, were combined and evaporated under reduced pressure to give the title compound **29** (2.10 g, ≈40%) as a pale yellow glass, R_f 0.34 [diethyl ether–hexane (4:1 v/v)]; δ_H ($CDCl_3$) 1.54 (2 H, m), 1.75 (2 H, m), 2.53 (1 H, d, *J* 7.5), 3.08 (3 H, s), 3.52 (3 H, m), 3.70 (2 H, m), 3.78 (1 H, t, *J* 8.1), 4.37 (5 H, m), 4.47 (2 H, s), 4.99 (1 H, dd, *J* 2.6 and 9.3), 5.29 (1 H, t, *J* 8.3), 5.39 (1 H, t, *J* 8.9), 6.74 (6 H, m), 7.01 (1 H, d, *J* 2.4), 7.15 (9 H, m), 7.39 (4 H, m), 7.52 (1 H, d, *J* 7.6), 7.70 (1 H, br s); δ_C ($CDCl_3$) included the following signals: 33.59, 34.35, 49.39, 64.67, 64.90, 65.28, 69.10, 71.20, 71.54, 71.59, 72.95, 74.85, 100.10.

The above putative tetrakis[(4-chlorophenoxy)acetyl] derivative **30** (3.77 g, obtained from two separate experiments) was dissolved in ethanol (50 cm^3) at room temperature and ethanolic methylamine (8 mol dm^{-3}; 50 cm^3) was added to the stirred solution. After 90 min, the products were concentrated under reduced pressure and fractionated by chromatography on silica gel. The column was first washed with dichloromethane–acetonitrile (100:0 to 50:50 v/v). The appropriate fractions, which were eluted with dichloromethane–methanol (96:4 to 89:11 v/v), were combined, and evaporated under reduced pressure to give 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)-phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol **21** (1.53 g), identical (TLC, ^{1}H and ^{13}C NMR) with the starting material **21** used in the preparation of the above 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl] derivative **29**. Thus the yield of the latter compound **29** can be estimated to be ≈55%, based on consumed starting material **21**.

3,4,5-Tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol

6-*O*-{2,7-Dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol **22** (3.11 g, 4.0 mmol) was allowed to react with 1*H*-tetrazole (1.33 g, 19.0 mmol), CpacCl (2.81 cm^3, 18.0 mmol) and DMAP (2.66 g, 21.8 mmol) in pyridine (7.5 cm^3) and acetonitrile (15 cm^3) as in the above preparation of the corresponding D-enantiomer **29**. The products were worked up and fractionated to give the title compound (2.62 g, 51%; identical TLC, ^{1}H and ^{13}C NMR spectra with the above D-enantiomer **29**) and the corresponding putative tetrakis-*O*-[(4-chlorophenoxy)acetyl] derivative **30**.

Di(2-cyanoethyl) phosphorochloridite 31

A solution of triethylamine (84.5 cm^3, 0.61 mol) in 3-hydroxypropionitrile (40.3 cm^3, 0.59 mol) was added dropwise over a period of 20 min to a stirred solution of chlorotrimethylsilane (76.6 cm^3, 0.60 mol) in dry diethyl ether (500 cm^3) at 0 °C (ice–water-bath). After a further period of 2 h, the products were filtered. The filtrate was concentrated under reduced pressure and then distilled to give 3-(trimethylsilyloxy)propionitrile (80.92 g) as a colourless liquid, bp 78–80 °C/21 mmHg.

The latter compound (8.63 g, ≈60 mmol) was dissolved in acetonitrile (30 cm^3) at room temperature and phosphorus trichloride (2.39 cm^3, 27.4 mmol) was added to the stirred solution. After 2 days, the products were concentrated under reduced pressure (water-pump, followed by oil-pump) to give a colourless oil (6.18 g). This material was estimated by ^{31}P NMR spectroscopy {δ_P ($CDCl_3$) 139.1 [≈20%, assigned to the phosphorus resonance of tri(2-cyanoethyl) phosphite], 165.7 (≈75%, assigned to the phosphorus resonance of di(2-cyanoethyl) phosphorochloridite], 179.5 (≈5%, assigned to the phosphorus resonance of 2-cyanoethyl phosphorodichloridite)} to contain ≈75 mol% of the title compound. This material was used without further purification.

3,4,5-Tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 1-phosphate di(2-cyanoethyl) ester 33

A solution of the above di(2-cyanoethyl) phosphorochloridite reagent **31** (≈75 mol%; 1.45 g) in dry acetonitrile (6 cm^3) was added to a stirred solution of 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]-xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol **29** (3.842 g, 3.0 mmol) and 3-nitro-1,2,4-1*H*-triazole **32** (0.820 g, 7.2 mmol) in dry pyridine (24 cm^3) at room temperature. After 1 h, water (0.216 cm^3, 12 mmol) was added. The products were concentrated under reduced pressure and the residue was re-dissolved in acetonitrile (20 cm^3). *tert*-Butyl hydroperoxide (70%; 1.64 cm^3, 12 mmol) was added to the stirred solution. After 90 min, the products were concentrated to ≈one-third volume under reduced pressure and the residue was dissolved in ethyl acetate (200 cm^3). The solution was washed with saturated aq. sodium hydrogen carbonate (2×100 cm^3) and the combined aqueous washings were extracted with ethyl acetate (100 cm^3). The combined organic layers were washed successively with sodium acetate buffer (pH 5; 0.1 mol dm^{-3}; 100 cm^3) and brine (50 cm^3), and were then dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by chromatography on silica gel. The column was washed with hexane–dichloromethane (1:1 v/v) and was then eluted with hexane–dichloromethane–ethanol (50:50:0 to 46:46:8 v/v); the appropriate fractions, which were eluted with solvent mixtures containing 5–7% ethanol, were combined, and evaporated under reduced pressure to give the title compound as an off-white glass (3.765 g, ≈85%); $[\alpha]_D^{23}$ +10.7 (*c* 4.01, toluene); R_f 0.57 [dichloromethane–ethanol (9:1 v/v)]; Found (M − H − acrylonitrile) (FAB) 1414. $^{12}C_{59}{}^{1}H_{49}{}^{79}Br^{81}Br^{35}Cl_3{}^{19}F_3{}^{14}N^{16}O_{18}{}^{31}P$ requires *m*/*z*, 1414; δ_H ($CDCl_3$) 1.60–1.80 (4 H, m), 2.69 (2 H, t, *J* 6.2), 2.73 (2 H, m), 3.19 (3 H, s), 3.50 (2 H, m), 3.79 (2 H, m), 4.00–4.32 (7 H, m), 4.4–4.55 (5 H, m), 4.82 (1 H, s), 4.96 (1 H, m), 5.13 (1 H, m), 5.26 (1 H, t, *J* 7.2), 6.74 (6 H, m), 7.07 (2 H, m), 7.20 (8 H, m), 7.40 (3 H, m), 7.53 (2 H, m), 7.80 (1 H, br); δ_P ($CDCl_3$) −1.6.

3,4,5-Tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol 1-phosphate di(2-cyanoethyl) ester 39

3,4,5-Tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol **29** (2.615 g, 2.04 mmol), the above di(2-cyanoethyl) phosphorochloridite reagent **31** (≈75 mol%; 0.98 g) and 3-nitro-1,2,4-1*H*-triazole **32** (0.562 g, 4.9 mmol) were allowed to react together in dry acetonitrile (4 cm^3) and pyridine (12 cm^3) solution as in the above preparation of the D-enantiomer **33** . After 80 min, water (0.147 cm^3, 8.2 mmol) was added. The products were then treated with *tert*-butyl hydroperoxide (70%; 1.14 cm^3, ≈8.3 mmol) and worked up and chromatographed as above to give the title compound as a colourless glass (2.312 g, ≈77%); $[\alpha]_D^{20}$ −10.8 (*c* 3.99, toluene); R_f 0.57 [dichloromethane–ethanol (9:1 v/v)];

its ^{1}H and ^{31}P NMR spectra ($CDCl_3$) were identical with those of the above D-enantiomer.

Product of the coupling reaction between 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[(3-trifluoromethyl)phenyl]-xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 1-phosphate (2-cyanoethyl) ester triethylammonium salt 34 and 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol 6; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$

A solution of 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 1-phosphate di-(2-cyanoethyl) ester **33** (1.76 g, 1.20 mmol) in triethylamine–acetonitrile (1:2 v/v; 12 cm^3) was stirred at room temperature. After 16 h, the products were concentrated under reduced pressure and the residue was dissolved in dry acetonitrile (10 cm^3), and 4-methoxypyridine 1-oxide[39] **36** (1.50 g, 12.0 mmol) and 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6**; $R^1 = C_{17}H_{35}$, $R^2 = C_{17}H_{31}$ (1.52 g, 2.4 mmol) were added. The resulting solution was evaporated under reduced pressure. The residue was re-dissolved in dry acetonitrile (10 cm^3) and the solution was re-evaporated. After this process had been repeated once more, the residue was redissolved in dry acetonitrile (4 cm^3). A solution of mesitylene-2-sulfonyl chloride **35** (1.31 g, 6.0 mmol) in dry pyridine (8 cm^3) was added dropwise over a period of 30 min to the stirred solution at room temperature. After a further period of 30 min, water (0.22 cm^3) was added and the products were concentrated under reduced pressure. The residue was dissolved in ethyl acetate (200 cm^3) and the resulting solution was washed with saturated aq. sodium hydrogen carbonate (2 × 100 cm^3). The aqueous washings were back-extracted with ethyl acetate (100 cm^3). The combined organic layers were washed successively with 0.1 mol dm^{-3} sodium acetate buffer (pH 5; 2 × 100 cm^3) and brine (50 cm^3) and were then dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by chromatography on silica gel. The column was eluted with hexane–dichloromethane–ethanol (50:50:0 to 48.5:48.5:3 v/v); the appropriate fractions, which were eluted with solvent mixtures containing 1.5–2.5% ethanol, were evaporated under reduced pressure to give the product **37**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$ (1.75 g, 71%); R_f 0.44 [dichloromethane–ethanol (95:5 v/v)]: $[\alpha]_D^{22}$ +3.3 (*c* 3.84, ethyl acetate); δ_H ($CDCl_3$) 0.88 (6 H, m), 1.10–1.45 (34 H, m), 1.58–1.76 (8 H, m), 2.07 (4 H, m), 2.30 (4 H, m), 2.67 (2 H, m), 2.81 (6 H, m), 3.17 (3 H, s), 3.49 (2 H, m), 3.76 (2 H m), 4.05–4.30 (9 H, m), 4.38–4.54 (5 H, m), 4.79 (1 H, m), 5.0–5.42 (12 H, m), 6.72 (6 H, m), 7.0–7.22 (10 H, m), 7.36 (3 H, m), 7.50 (2 H, m), 7.77 (1 H, m); δ_P ($CDCl_3$) −0.89, −0.73.

Fully protected 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol 3,4,5-trisphosphate 38; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$

Hydrazine monohydrate (0.189 cm^3, 3.9 mmol) was added to a stirred solution of compound **37** (1.746 g, 0.86 mmol) in acetonitrile (8 cm^3) at room temperature. After 90 min, acetone (8 cm^3) was added. After a further period of 2 min, the products were evaporated under reduced pressure and hexane (100 cm^3) was added. The crystalline precipitate was removed by filtration and washed with hexane. The combined filtrate and washings were concentrated under reduced pressure. The residue was fractionated by chromatography on silica gel. The column was eluted with ethyl acetate–hexane (2:8 to 10:0 v/v); the appropriate fractions, which were eluted with 100% ethyl acetate, were combined, and evaporated under reduced pressure to give a colourless gum (1.137 g). This material was evaporated with dry acetonitrile (3 × 10 cm^3) and then dissolved in dry pyridine (4.5 cm^3). To the resulting solution, which was maintained at room temperature, was added 3-nitro-1,2,4-1*H*-triazole **32** (0.768 g, 6.7 mmol), followed by di(2-cyanoethyl) phosphorochloridite reagent **31** (≈75 mol%; 1.316 g) in acetonitrile (8 cm^3) solution. After 1 h, water (0.10 cm^3) was added and the products were evaporated under reduced pressure. The residue was re-dissolved in acetonitrile (10 cm^3) at 0 °C (ice–water-bath) and *tert*-butyl hydroperoxide (70%; 1.08 cm^3, ≈7.8 mmol) was added to the stirred solution. After 5 min, the products were allowed to warm to room temperature and, after 1 h, water was added until the solution became turbid. The products were then applied directly to a column of silanized silica gel. The column was eluted with acetonitrile–water (2:8 to 10:0 v/v): the appropriate fractions, which were eluted with 100% acetonitrile, were combined, and evaporated under reduced pressure. The residue was rechromatographed on a standard silica gel column which was eluted with dichloromethane–propan-2-ol–acetic acid (99.7:0:0.3 to 89.7:10:0.3 v/v): the appropriate fractions, which were eluted with 6–9% propan-2-ol, were combined, and evaporated under reduced pressure to give the title product as a colourless gum (1.027 g, ≈57% overall yield for the three steps); R_f 0.26 [dichloromethane–ethanol (19:1 v/v)]; $[\alpha]_D^{20}$ +25.9 (*c* 5.1, ethyl acetate); δ_P ($CDCl_3$) −3.68, −3.37, −2.01, −1.78, −1.75, −1.72, −1.68.

1-*O*-(2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] 2

The above fully protected 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho-D-*myo*-inositol 3,4,5-trisphosphate **38**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$ (0.254 g, 0.12 mmol) was co-evaporated with dry toluene (3 × 5 cm^3) under reduced pressure and then dissolved in dry acetonitrile (3 cm^3). TMG (0.27 cm^3, 2.2 mmol) and chlorotrimethylsilane (0.22 cm^3, 1.7 mmol) were added and the mixture was stirred at room temperature. After 16 h, the products were evaporated under reduced pressure (oil-pump) and the residue was co-evaporated with dry toluene (3 × 5 cm^3). The residue was triturated with hexane (3 × 15 cm^3) and the resulting mixture was filtered in an atmosphere of argon. The slightly turbid filtrate was concentrated under reduced pressure. The residual colourless glass obtained was dissolved in conc. aq. ammonia (*d* 0.88)–methanol (5.5:94.5 v/v; 0.86 cm^3), and the solution obtained was evaporated under reduced pressure. The colourless residue was dissolved in acetic acid–water (2:1 v/v; 6 cm^3) and the resulting solution was stirred at room temperature. After 90 min, the products were evaporated under reduced pressure and the residue was co-evaporated with ethanol (3 × 10 cm^3). The powdery residue was triturated first with acetonitrile and then with ethyl acetate. An aqueous solution of this material was passed through an Amberlite IR-120 (plus) cation-exchange resin column (H^+ form; 20 cm × 2.5 cm diameter). The acidic eluate was carefully neutralized with methanolic ammonia and the resulting solution was evaporated under reduced pressure. The residue was co-evaporated with dry ethanol to give the ammonium salt of 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol [PtdIns(3,4,5)P_3] **2** as an off-white hygroscopic powder (0.146 g, ≈96% yield, assuming that the product is an unhydrated hepta-ammonium salt); δ_H (CD_3OD–D_2O) 0.86 (6 H, m), 1.30 (32 H, m), 1.55 (2 H, m), 1.68 (2 H, m), 2.05 (4 H, m), 2.27–2.47 (4 H, m), 2.78 (6 H, m), 3.90 (1 H, t, *J* 9.5), 4.0–4.15 (6H, m), 4.23 (1 H, dd, *J* 8.2 and 11.6), 4.41 (3 H, m), 5.34 (8 H, m); δ_P (CD_3OD–D_2O) 0.02, 1.30, 1.91, 2.22 [Found: $(M - H)^-$ (MALDI-TOF) 1125.49. $^{12}C_{47}{}^{1}H_{85}{}^{16}O_{22}{}^{31}P_4$ requires *m*/*z*, 1125.45].

Products of other coupling reactions between protected D- and L-*myo*-inositol 1-phosphate (2-cyanoethyl) esters and enantiomeric di-*O*-acylglycerol derivatives

(a) A fully protected phosphatidylinositol derivative (0.639 g, 69%) was prepared in the same way as the above fully protected

natural diastereoisomer **37**; $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$ from 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[(3-trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 1-phosphate di(2-cyanoethyl) ester **33** (0.658 g, 0.45 mmol) and 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8** (0.578 g, 0.90 mmol); it had $[\alpha]_D^{20}$ −0.3 (*c* 4.3, ethyl acetate) and δ_P ($CDCl_3$) −1.16, −0.63.

(b) A fully protected phosphatidylinositol derivative (0.834 g, 68%) was prepared in the same way as above from 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol 1-phosphate di(2-cyanoethyl) ester **39** (0.871 g, 0.59 mmol) and 2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycerol **6** (0.765 g, 1.19 mmol); it had $[\alpha]_D^{21}$ +0.7 (*c* 4.6, ethyl acetate) and δ_P ($CDCl_3$) −1.16, −0.61.

(c) A fully protected phosphatidylinositol derivative (0.692 g, 75%) was prepared in the same way as above from 3,4,5-tris-*O*-[(4-chlorophenoxy)acetyl]-6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl}-2-*O*-(4-methoxytetrahydropyran-4-yl)-L-*myo*-inositol 1-phosphate di(2-cyanoethyl) ester **39** (0.663 g, 0.45 mmol) and 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **8** (0.580 g, 0.90 mmol); it had $[\alpha]_D^{20}$ −1.5 (*c* 3.6, ethyl acetate) and δ_P ($CDCl_3$) −0.87, −0.71.

Other fully protected 1-*O*-(2-*O*-arachidonoyl-1(or 3)-*O*-stearoyl-*sn*-glycero-3-phospho)-D (or L)-*myo*-inositol 3,4,5-trisphosphates

The diastereoisomers of the fully protected 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol 3,4,5-trisphosphate **38** were prepared from the products of the other coupling reactions between protected D- and L-*myo*-inositol 1-phosphates (2-cyanoethyl) esters and enantiomeric di-*O*-acylglycerol derivatives described above.

(a) The coupling-reaction product (0.639 g, 0.43 mmol) described above under heading (a) was subjected to the same three-step process [*i.e.*, treatment with (i) hydrazine hydrate, (ii) di(2-cyanoethyl) phosphorochloridite and (iii) *tert*-butyl hydroperoxide] described above in the preparation of the natural diastereoisomer **38** to give fully protected 1-*O*-(2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycero-1-phospho)-D-*myo*-inositol 3,4,5-trisphosphate (0.206 g, 31%); $[\alpha]_D^{20}$ +17.8 (*c* 4.1, ethyl acetate); δ_P ($CDCl_3$) −3.65, −3.33, −2.14, −1.80, −1.75, −1.52.

(b) In the same way, the coupling reaction product (0.692 g, 0.47 mmol) described above under heading (b) was converted into fully protected 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-L-*myo*-inositol 3,4,5-trisphosphate (0.238 g, 33%); $[\alpha]_D^{20}$ −16.4 (*c* 3.5, ethyl acetate); δ_P ($CDCl_3$) −3.67, −3.35, −2.00, −1.86, −1.80, −1.77, −1.71.

(c) In the same way, the coupling reaction product (0.644 g, 0.44 mmol) described above under heading (c) was converted into fully protected 1-*O*-(2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycero-1-phospho)-L-*myo*-inositol 3,4,5-trisphosphate (0.273 g, 40%); $[\alpha]_D^{20}$ −26.8 (*c* 3.5, ethyl acetate); δ_P ($CDCl_3$) −3.68, −3.37, −2.00, −1.81, −1.78, −1.75, −1.73, −1.69.

Other diastereoisomers of 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] 2

The three above fully protected diastereoisomers of 1-*O*-(2-*O*-arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol 3,4,5-trisphosphate were unblocked by the same three-step process [*i.e.*, treatment with (i) TMG and chlorotrimethylsilane, (ii) methanolic ammonia and (iii) acetic acid–water (2:1 v/v) used in the unblocking of fully protected PtdIns(3,4,5)P_3 **38** itself].

(a) 1-*O*-(2-*O*-Arachidonoyl-3-*O*-stearoyl-*sn*-glycero-1-phospho)-D-*myo*-inositol 3,4,5-trisphosphate **42** (0.077 g, ≈78% yield, assuming that the product is an unhydrated hepta-ammonium salt) was thereby obtained from its fully protected precursor (0.206 g, 0.10 mmol); δ_P (CD_3OD–D_2O) 1.60, 2.51, 3.05, 3.33.

(b) 1-*O*-(2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycero-3-phospho)-L-*myo*-inositol 3,4,5-trisphosphate **41** (0.137 g, ≈97% yield, assuming that the product is an unhydrated hepta-ammonium salt) was thereby obtained from its fully protected precursor (0.236 g, 0.11 mmol); δ_P (CD_3OD–D_2O) 4.01, 5.10, 5.68, 5.98.

(c) 1-*O*-(2-*O*-Arachidonoyl-3-*O*-stearoyl-*sn*-glycero-1-phospho)-L-*myo*-inositol 3,4,5-trisphosphate **43** (0.159 g, ≈97% yield, assuming that the product is an unhydrated hepta-ammonium salt) was thereby obtained from its fully protected precursor (0.273 g, 0.13 mmol); δ_P (CD_3OD–D_2O) 1.62, 2.83, 3.48, 3.73.

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