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SYNTHESIS OF 1-*O*-STEAROYL-2-*O*-ARACHIDONOYL-*sn*-GLYCER-3-YL-D-*myo*-INOSITOL 3,4,5-TRISPHOSPHATE AND ITS STEREOISOMERS

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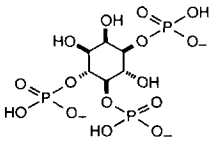
Abstract: The chemical synthesis of PtdIns(3,4,5)P_3 **2** and three of its stereoisomers is described.

The identification of D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** as a cellular second messenger[1] has had a major impact on modern biology. In recent years, this seminal discovery has stimulated considerable activity in the chemical synthesis of inositol phosphates[2]. As it is now believed to be a second messenger in its own right in phosphoinositide-mediated signal transduction, 1-*O*-stearoyl-2-*O*-arachidonoyl-*sn*-glycer-3-yl-D-*myo*-inositol 3,4,5-trisphosphate[3,4] [PtdIns(3,4,5)P_3] **2** is currently also of great interest to biologists in the field. In order to make it more accessible, we have undertaken the chemical synthesis of PtdIns(3,4,5)P_3 **2**. We have also undertaken the synthesis of three of its stereoisomers.



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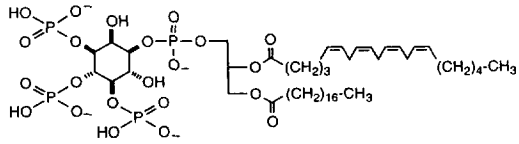

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The synthesis of analogues of PtdIns(3,4,5)P_3 **2** with identical saturated fatty acids in the glyceryl moiety has been reported[5-10]. However, we are unaware of any previous report relating to the synthesis of the naturally-occurring material. Presumably one reason for this is that the unsaturated arachidonoyl residue would not have survived the conditions commonly used[2] to remove all of the protecting groups from the inositol moiety. The protecting groups that were used in our synthesis[11] of Ins(1,4,5)P_3 **1** were all removable under either mildly basic or mildly acidic conditions. A similar strategy has proved to be particularly suitable in the synthesis of PtdIns(3,4,5)P_3 **2**.

The procedure used for the preparation of the partially-protected *myo*-inositol building block **18** required for the synthesis of PtdIns(3,4,5)P_3 **2** is indicated in outline in Scheme 1. Reaction between *myo*-inositol **3** and *p*-anisoyl chloride **4** in pyridine solution (Scheme 1a) gave the penta-(*p*-anisoyl) derivative[12] **5** in 72% isolated yield. Compound **5** was allowed to react with 5,6-dihydro-4-methoxy-2*H*-pyran[13] **6** in the presence of triphenylphosphine hydrobromide[14] in dichloromethane and the products were treated with sodium methoxide in methanol - THF to give 2-*O*-(4-methoxytetrahydropyran-4-yl)-*myo*-inositol **7** which was isolated as a crystalline solid in 81% overall yield[15]. When compound **7** was reacted with the Markiewicz reagent[16] **8** in hexamethylphosphoric triamide (HMPA) solution, the 4,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl) deriv-

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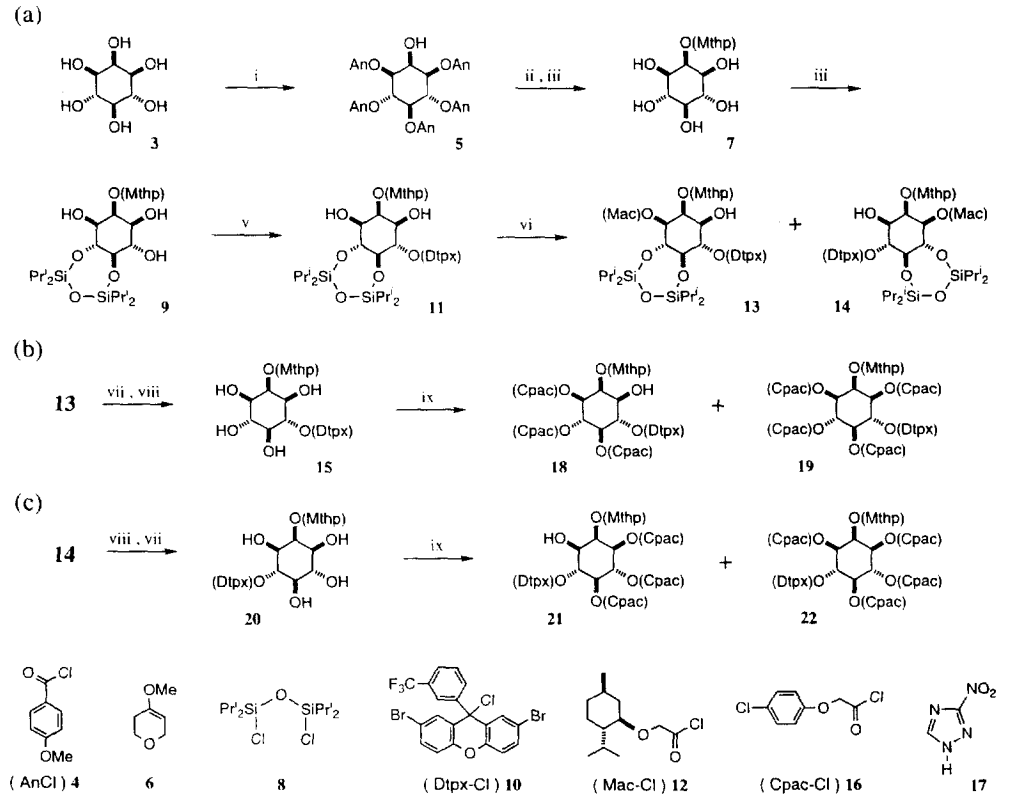
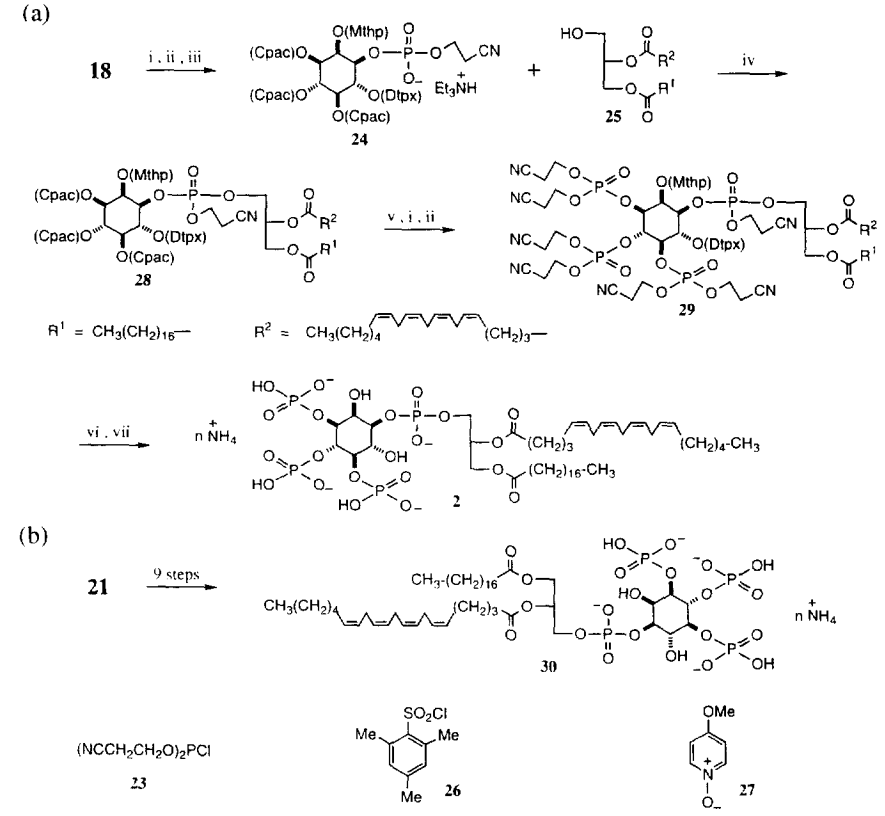


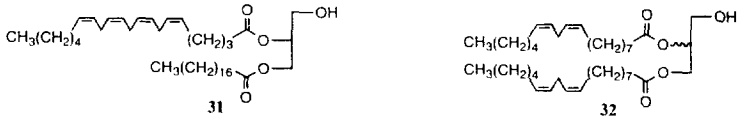
Scheme 1 *Reagents and conditions* : i , AnCl **4** , C_5H_5N , 0°C to room temp., 18 h ; ii , **6** , $Ph_3P.HBr$, CH_2Cl_2 , 40 h , room temp.; iii , NaOMe , MeOH , THF , reflux , 30 min ; iv , **8** , imidazole , Et_3N , HMPA , room temp., 18 h ; v , Dtpx-Cl **10** , C_5H_5N , MeCN ; vi , Mac-Cl **12** , 1*H*-tetrazole , 4-dimethylaminopyridine (DMAP) , MeCN , C_5H_5N , room temp., 45 min ; vii , $MeNH_2$, EtOH , room temp., 1.5 h ; viii , Et_4NF , MeCN , room temp., 30 min ; ix , Cpac-Cl **16** , 3-nitro-1,2,4-1*H*-triazole **17** , DMAP , MeCN , C_5H_5N , room temp.

-ative **9** was obtained as the major product, and was isolated in 62% yield[17]. The latter compound **9** reacted regioselectively with 2,7-dibromo-9-chloro-9-[3-(trifluoromethyl)phenyl]xanthene (Dtpx-Cl)[18] **10** to give its 6-*O*-(Dtpx) derivative[19] **11** which was isolated in 74% yield. Reaction between (-)-menthoxyacetyl chloride **12** and the racemic material **11**, followed by fractionation of the products gave the diastereoisomerically-pure menthoxyacetates **13** and **14** in 40.7 and 41.8% isolated yields[20], respectively. When compound **13** was treated first with 8M-ethanolic methylamine and then with tetraethylammonium fluoride in acetonitrile, the 1,3,4,5-tetraol **15** was obtained (Scheme 1b) in *ca.* 95% isolated yield[21]. When this compound **15** was allowed to react with (4-chlorophenoxy)acetyl chloride (Cpac-Cl) **16** in the presence of 3-nitro-1,2,4-1*H*-triazole **17** and 4-(dimethylamino)pyridine (DMAP) in acetonitrile - pyridine, a mixture of the required inositol building block[23] **18** and the corresponding tetra-(4-chlorophenoxy)acetate **19** was obtained. Compounds **18** and **19** were isolated in 59 and 22% yield, respectively. Tetraol **15** was recovered in 76% yield when the tetra-(4-chlorophenoxy)acetate **19** was treated with ethanolic methylamine.

The inositol building block **18** reacted readily (Scheme 2a) with di-(2-cyanoethyl) phosphorochloridite[24] **23** in the presence of 3-nitro-1,2,4-1*H*-triazole **17** to give the di-(2-cyanoethyl) ester of its 1-*phosphite* which was immediately treated with *tert*-butyl hydroperoxide[25] to give the di-(2-cyanoethyl) *phosphate*. Further



Scheme 2 *Reagents and conditions* : i , $(NCCH_2CH_2O)_2PCl$ **23** , **17** , C_5H_5N , MeCN , room temp., 1 h ; ii , 70 % t-BuO_2H , room temp., 1.5 h ; iii , Et_3N , MeCN , room temp., 18 h ; iv , **26** , **27** , MeCN , C_5H_5N , room temp.; v , $N_2H_4.H_2O$, MeCN , room temp., 1.5 h ; vi , $(Me_2N)_2C{=}NH$ (TMG) , Me_3SiCl , MeCN , room temp., 18 h ; vii , a , acetic acid - water (2 : 1 v/v) , room temp., 1.5 h , b , NH_3 , MeOH.



treatment with triethylamine in dry acetonitrile gave the triethylammonium salt of the corresponding mono-(2-cyanoethyl) phosphate[26] **24**. The latter phosphodiester **24**, 1-*O*-stearoyl-2-*O*-arachidonoyl-*sn*-glycerol[27] **25** (2.0 mol equiv.), mesitylene-2-sulfonyl chloride **26** (5.0 mol equiv.) and 4-methoxypyridine-1-oxide[28] **27** (10.0 mol equiv.) were allowed to react together in acetonitrile - pyridine to give the fully-protected phosphotriester[29] **28** in *ca.* 61% overall yield for the four steps starting from building block **18.** This fully-protected phosphotriester **28** was treated first with a small excess of hydrazine hydrate in acetonitrile and the resulting 3,4,5-triol was phosphorylated [again by phosphitylation with di-(2-cyanoethyl) phosphorochloridite **23**, followed by oxidation with *tert*-butyl hydroperoxide] to give the hepta-(2-cyanoethyl) tetrakisphosphate[30] **29** in *ca.* 55% yield for the three steps. All of the protecting groups were then removed under very mild conditions by a two-step procedure. First, the hepta-(2-cyanoethyl) tetrakisphosphate **29** was treated with N^1, N^1, N^3, N^3-tetramethylguanidine (TMG) and chlorotrimethylsilane[31] in dry acetonitrile at room temperature

to remove all seven 2-cyanoethyl protecting groups. The remaining 2-*O*-(4-methoxytetrahydropyran-4-yl) and 6-*O*-{2,7-dibromo-9-[3-(trifluoromethyl)phenyl]xanthen-9-yl} protecting groups were then easily removed by treatment with acetic acid - water (2 : 1 v/v) at room temperature. The resulting completely unprotected PtdIns(3,4,5)P_3 **2** was converted into its ammonium salt, and isolated as an off-white hygroscopic powder[32] in virtually quantitative yield, based on the fully-protected material **29**; its ^{31}P NMR spectrum is illustrated in Figure 1.

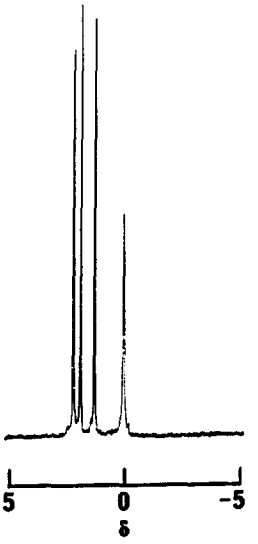


Fig. 1 ^{31}P NMR Spectrum [161.98 MHz, CD_3OD - D_2O (1 : 1 v/v)] of NH_4^+ salt of PtdIns(3,4,5)P_3 **2**

The enantiomeric inositol building block **21** (Scheme 1c) was converted by the same nine step procedure (Scheme 2b), involving the same enantiomer of 1-*O*-stearoyl-2-*O*-arachidonoylglycerol **25**, into the ammonium salt of the diastereoisomer **30**[33] of PtdIns(3,4,5)P_3 **2**. In order to complete the synthesis of the other two possible *myo*-inositol derived stereoisomers of PtdIns(3,4,5)P_3 **2**, the phosphodiester **24** and its enantiomer were coupled in turn with 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **31**. The overall yields of all three stereoisomers of PtdIns(3,4,5)P_3 were similar to that of PtdIns(3,4,5)P_3 **2** itself. Finally, the *racemic* phosphodiester **24** was coupled with *racemic*-1,2-di-*O*-linoleoylglycerol[27] **32** leading, after a corresponding series of transformations (Scheme 2a) to the ammonium salt of the dilinoleoyl analogue of PtdIns(3,4,5)P_3, obtained as a mixture of diastereoisomers[34].

Both synthetic PtdIns(3,4,5)P_3 **2** and the corresponding diastereoisomer derived from 2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol **31** (*i.e.* the enantiomer of **30**) were substantially more effective than the synthetic dipalmitoyl analogue of PtdIns(3,4,5)P_3 **2** in the activation[3] of protein kinase B. However, such biological activity was not shown by the other two stereoisomers (*i.e.* **30** and the enantiomer of **2**). Thus biological activity in the system under consideration appears to depend on the absolute stereochemistry of the inositol moiety, and on the nature of the acyl residues rather than on the configuration at *C*-2 of the glycerol moiety.

Acknowledgement: We thank the Wellcome Trust for generous financial support.

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12. Compound **5** has m.p. 234-236°C [Found : C, 64.74; H, 4.80. $C_{46}H_{42}O_{16}$ requires : C, 64.94; H, 4.98%]; δ_H [$CDCl_3$ - CD_2Cl_2] 3.67, 3.68, 3.72 (15 H, 3s), 4.71 (1 H, t, *J* 2.2), 4.45 (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).

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15. Compound **7** has m.p. 187°C [Found : C, 48.85; H, 7.49. $C_{12}H_{22}O_8$ requires : C, 48.97; H, 7.53%]; δ_C [$(CD_3)_2SO$] 34.63, 48.49, 64.42, 71.05, 73.03, 73.46, 75.49, 98.36.

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17. Compound **9** has m.p. 131-132°C [Found : 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%]; δ_C [$CDCl_3$] includes the following signals assigned to the resonances of the inositol carbon atoms: 70.95, 71.66, 72.28, 74.60, 76.37, 78.20. The sites of attachment of the 1,1,3,3-tetraisopropyldisiloxan-1,3-diyl protecting group follow from the structure of compound **11**[19].

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19. The ^1H NMR spectrum [$CDCl_3$] of compound **11** includes the following signals: 2.53 (1 H, m), 2.77 (1 H, d), 3.16 (1 H, m), 3.43 (1 H, m), 4.07 (1 H, t, *J* 2.6, assigned to H-2). When D_2O is added, the signals at δ 2.53 and 2.77 (assigned to the resonances of the hydroxy protons) disappear, and the signals at δ 3.16 and 3.43 (assigned to the resonances of H-1 and H-3) collapse to double-doublets (*J* 2.6 and 8.6, and 2.1 and 8.9, respectively). It is clear from the COSY spectrum of compound **11** that H-1 and H-3 are both adjacent to H-2.

20. Compound **13** has R_f 0.45 [ether - hexane (1 : 1 v/v)], $[\alpha]_D^{25}$ -16.2° (*c* 2, EtOAc]; δ_C [$CDCl_3$] includes the following signals assigned to the resonances of methine carbon atoms attached to one oxygen atom: 70.68, 71.70, 72.36(*), 73.58, 78.23, 78.67, 80.25(*). Compound **14** has m.p. 217°C [Found: C, 55.23; H, 6.25. $C_{56}H_{77}Br_2F_3O_{12}Si_2$ requires: C, 55.35; H, 6.39%]; R_f 0.38 [ether - hexane (1 : 1 v/v)]; $[\alpha]_D^{25}$ - 25.3° (*c* 2, EtOAc); δ_C [$CDCl_3$] includes the following signals assigned to the resonances of the methine carbon atoms attached to one oxygen atom: 70.68, 71.76, 72.08(*), 73.65, 78.15, 78.64, 79.74(*). It is apparent both from ^1H and ^{13}C NMR spectroscopic data that **13** and **14** are diastereoisomers and not regioisomers. Thus only two [indicated by (*)] of the above seven methine carbon resonance signals in **13** and **14** differ by more than 0.1 ppm. The position of the menthoxyacetyl group in **13** (i.e. whether it is on *O*-3 or *O*-1) is not firmly established but the 1-hydroxy function of **11** is believed to be more hindered than its 3-hydroxy function.

21. Compound **15** has R_f [CH_2Cl_2 - EtOH (9 : 1 v/v)] 0.42; δ_H [$(CD_3)_2SO$] includes the following signals assigned to exchangeable protons : 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). The absolute stereochemistry of compound **15** was determined by converting its precursor **13** in several steps into the known 1,4,5,6-tetra-*O*-benzyl-D-*myo*-inositol, m.p. 145°C [lit.[22], m.p. 142.5°C], $[\alpha]_D^{22}$ +22.7° (*c* 1.18, $CHCl_3$) [lit.[22], $[\alpha]_D$ +23.4° (*c* 4.5, $CHCl_3$)]. In the same way, compound **14** was converted into 3,4,5,6-tetra-*O*-benzyl-D-*myo*-inositol, m.p. 144-145°C [lit.[22], m.p. 143°C], $[\alpha]_D^{20}$ -21.7° (*c* 1.05, $CHCl_3$)] [lit.[22], $[\alpha]_D$ -25.1° (*c* 5.2, $CHCl_3$)]. The circular dichroism spectra of enantiomers **15** and **20** were virtually mirror images of each other.

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23. Compound **18** has R_f 0.34 [ether - hexane (4 : 1 v/v)]; δ_H [$CDCl_3$] includes the following signals: 2.53 (1 H, d, *J* 7.5, assigned to 1-OH), 3.78 (1 H, t, *J* 8.1, assigned to H-6), 4.99 (1 H, dd, *J* 2.6 and 9.3, assigned to H-3), 5.29 (1 H, t, *J* 8.3, assigned to H-5), 5.39 (1 H, t, *J* 8.9, assigned to H-4); δ_C [$CDCl_3$] includes the following signals assigned to the resonances of the inositol ring carbon atoms: 69.10, 72.95, 74.85, 76.73, 77.08, 77.44. It is clear from the COSY spectrum of compound **18** that H-4 is coupled with both H-3 and H-5, and thus that the (4-chlorophenoxy)acetyl residues are attached to three adjacent hydroxy functions. The tetra-(4-chlorophenoxy)acetyl derivative **19** has R_f 0.48 [ether - hexane (4 : 1 v/v)].

24. Di-(2-cyanoethyl) phosphorochloridite **23** was prepared by stirring a solution of 1-cyano-2-(trimethylsilyloxy)ethane (8.63 g, 60 mmol) and phosphorus trichloride (2.39 ml, 27.4 mmol) in dry acetonitrile (30 ml) at room temperature for 48 h. The products were concentrated under reduced pressure (oil-pump, room temperature) to give a colourless oil which was estimated by ^{31}P NMR spectroscopy [$CDCl_3$] to contain di-(2-cyanoethyl) phosphorochloridite **23** (75%; δ_P 165.7), 2-cyanoethyl phosphorodichloridite (5%; δ_P 179.5) and tri-(2-cyanoethyl) phosphite (20%; δ_P 139.1).

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29. Fully-protected phosphotriester intermediate **28** has R_f 0.44 [CH_2Cl_2 - EtOH (95 : 5 v/v)]; $[\alpha]_D^{22}$ +3.3° (*c* 3.84, EtOAc); δ_P [$CDCl_3$] -0.89, -0.73.

30. The hepta-(2-cyanoethyl) tetrakisphosphate **29** has R_f 0.26 [CH_2Cl_2 - EtOH (95 : 5 v/v)]; $[\alpha]_D^{20}$ +25.9° (*c* 5.12, EtOAc); δ_P [$CDCl_3$] -3.68, -3.37, -2.01, -1.81, -1.78, -1.75, -1.72, -1.68.

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32. The ammonium salt of PtdIns(3,4,5)P_3 has δ_P [CD_3OD - D_2O (1:1 v/v)] 0.02, 1.30, 1.91, 2.22 (see Fig. 1); found : M-1 (negative ion FAB) 1125. Calc. for $^{12}C_{47}{}^{1}H_{85}{}^{16}O_{22}{}^{31}P_4$: 1125.5.

33. The ammonium salt of **30** has δ_P [CD_3OD - D_2O (1 : 1 v/v)] 1.60, 2.51, 3.05, 3.33.

34. The ammonium salt of the dilinoleoyl analogue of PtdIns(3,4,5)P_3 has δ_P [CD_3OD - D_2O (1 : 1 v/v)] 1.62, 2.50, 3.00, 3.34.

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