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SYNTHESIS OF 1-*O*-(1,2-DI-*O*-PALMITOYL-*SN*-GLYCERO-3-PHOSPHO)-D-*MYO*-INOSITOL 4,5-*BIS*PHOSPHATE: AN ANALOGUE OF NATURALLY OCCURRING (Ptd)Ins(4,5)P_2

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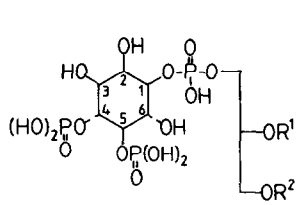
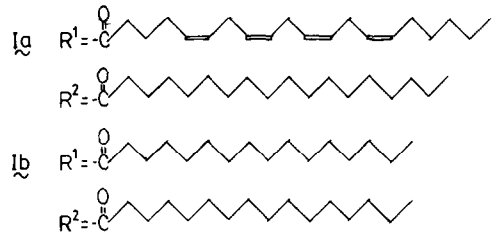
Abstract: Optically active 2,3,6-tri-*O*-benzyl-4,5-di-*O*-(*trans*-prop-1-enyl)-D-*myo*-inositol and 1,2-di-*O*-palmitoyl-*sn*-glycerol were coupled using mono- and bifunctional phosphitylating reagents to yield, after final removal of all benzyl-protecting groups the chiral title compound.

The naturally occurring triphosphoinositide Ia[1] [(Ptd)Ins(4,5)P_2] is believed to play a pivotal role in receptor-mediated Ca^{2+} mobilization. Receptor activation coupled with the action of a specific phospholipase C leads to phosphodiesteratic cleavage of phosphatidyl inositol 4,5-bisphosphate (Ia) into the corresponding diglyceride (1,2-DG) and *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. The two species thus released by receptor-initiated cleavage may function as second messengers. Thus 1,2-DG was found[2] to be involved in the activation of protein kinase C, and Ins(1,4,5)P_3 in binding to a receptor, presumably a component of the endoplasmic reticulum, resulting in the discharge of Ca^{2+} from intracellular stores into the cytosol[3].

In order to get a deeper insight into the biological function and biosynthetic pathways of (Ptd)Ins(4,5)P_2, we report here a convenient route to the preparation of an optically active analogue of (Ptd)Ins(4,5)P_2 (*i.e.* compound **Ib**).

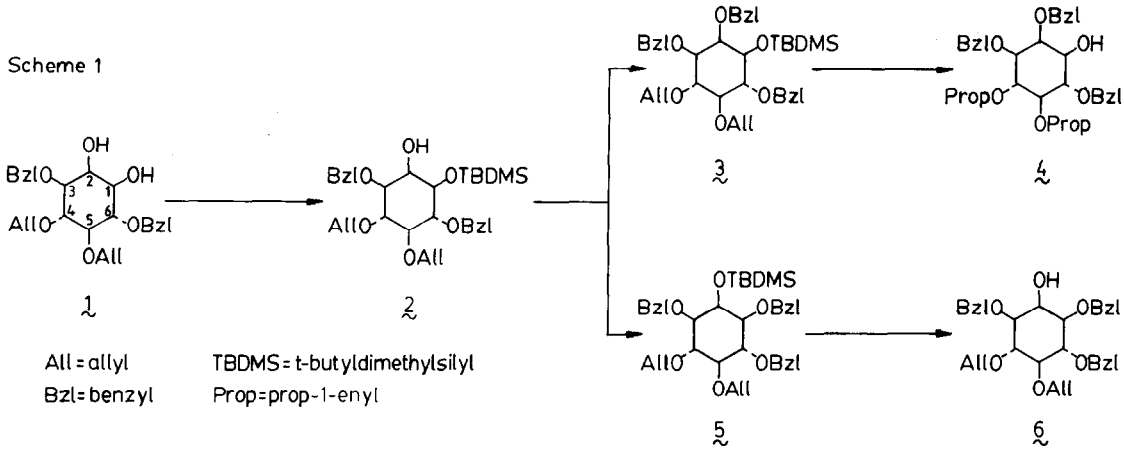




Diastereoisomers of **Ib** were prepared earlier[4] in a reasonable yield by a non-regioselective phosphorylation of racemic 3,6-di-*O*-benzyl-4,5-bis-*O*-(dianilinophosphoryl)-*myo*-inositol with racemic 1,2-di-*O*-stearoyl-glycero-3-phosphate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride, and subsequent removal of all protective groups.

In our approach (see Scheme 1[5]) we started from optically active 4,5-di-*O*-allyl-3,6-di-*O*-benzyl-D-*myo*-inositol (1), which was prepared according to the procedure of Ozaki *et al.*[6]. Treatment of 1 with excess *t*-butyldimethylsilyl chloride in pyridine for 16 h at 50°C gave, after purification by short-column chromatography, the crystalline silyl derivative 2 {$[\alpha]_D^{20}$ -7.7°, c 1, $CHCl_3$; m.p. 58.5-60°C (from pentane)} in a yield of 87%. Benzylation of 2 (10 mmol) in DMF (50 ml) with benzyl bromide (11.5 mmol) and sodium hydride (15 mmol) showed, after work-up, the presence (TLC-analysis) of the two positional isomers 3 and 5 (ratio 4:1), the separation of which could easily be effected by short-column chromatography. The

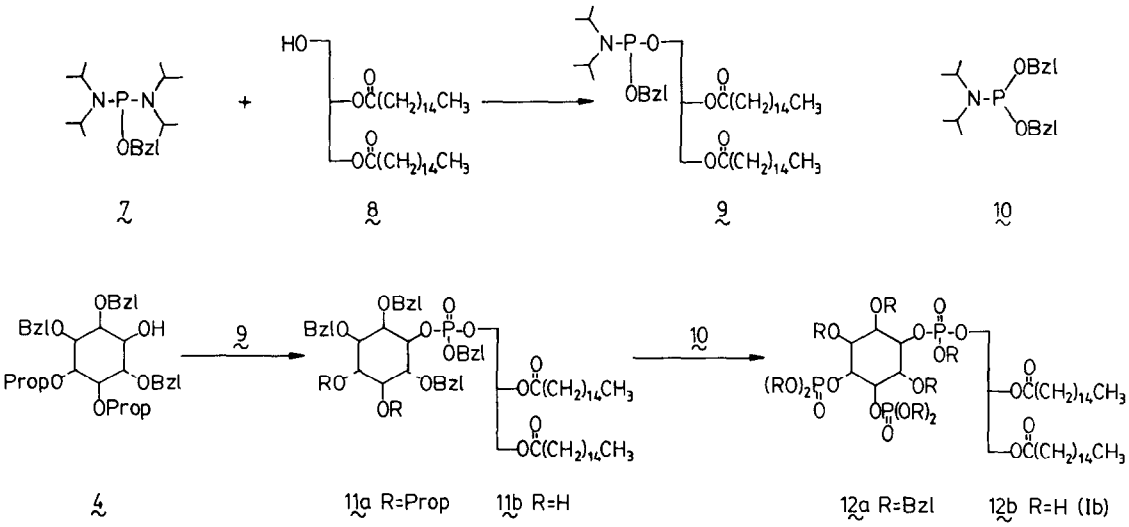
Scheme 1



structural assignment of the minor product 5 ($[\alpha]_D^{20}$ -4.7°, c 1, $CHCl_3$; m.p. 35.5-36°C; 19% yield) was, apart from spectroscopic data (^1H- and ^{13}C-NMR), corroborated as follows. Complete removal of the *t*-butyldimethylsilyl group from the axially orientated hydroxyl group at C-2 in 5 to provide 6 ($[\alpha]_D^{20}$ + 0.3°, c 1, $CHCl_3$; m.p. 73-74°C) proceeded most conveniently with tetra-*n*-butylammonium fluoride[7]/dioxane at elevated temperature (*i.e.*, 1 h at 50°C). On the other hand, deblocking of the silyl group from the equatorial hydroxyl in 3 with *n*-Bu_4NF proceeded, as expected, rapidly at 20°C. Further, regioselective benzylation of the 1,2-*O*-stannylated[8] derivative of starting compound 1 with benzyl bromide in the presence of *n*-Bu_4NI[9] gave an optically active *myo*-inositol derivative (yield 76%) having identical physical properties as 6 obtained by de-silylation of 5. The required compound 3 ($[\alpha]_D^{20}$ +7.9, c 1, $CHCl_3$), which was isolated in a yield of 78%, was now converted into the key intermediate 4 by the following two-step one-pot procedure. Isomerization[10] of the allyl-groups into the isomeric *trans*-prop-1-enyl groups could be effected smoothly using a catalytic amount of 1,5-cyclooctadiene-*bis*[methyldiphenylphosphine]iridium hexafluorophosphate[11] (activated under H_2 for 2 min) in $ClCH_2CH_2Cl$, followed by removal of the *t*-butyldimethylsilyl group by treatment with *n*-Bu_4NF at room temperature. In this way, crystalline 4 {$[\alpha]_D^{20}$ -6.2°, c 1, $CHCl_3$; m.p. 86.5-88°C (from pentane)} was isolated in a yield of 90%.

The next stage in the synthesis of **Ib** consisted of the introduction of one phosphodiester and two phosphomonoester functions. The formation of the phosphodiester linkage between 1,2-*O*-diacyl-sn-glycerol **8**[12] and the *myo*-inositol derivative 4 *via* a phosphite coupling approach[13] is outlined in Scheme 2[5]. In the first step, treatment of 1,2-di-*O*-palmitoyl-*sn*-glycerol (**8**, 2.5 mmol) in CH_2Cl_2 (10 ml) with the bifunctional phosphitylating reagent **7**[14] (2.75 mmol) in CH_2Cl_2 (5 ml) and in the presence of 1*H*-tetrazole (1.37 mmol in 2.7 ml CH_3CN) for 30 min at 20°C afforded, after work-up and purification by column chromatography[15], homogeneous **9** (δ_p 148.96 and 149.14 ppm) in a yield of 92%. Subsequent coupling of **9** (1.2 mmol) with 4 (1 mmol) in the presence of 1*H*-tetrazole (1.32 mmol) gave an intermediate phosphite-triester (δ_p 140.64 ppm). The latter was oxidised *in situ* with *t*-butyl hydroperoxide[16] for 45 min at 0°C. Work-up of the reaction-mixture gave **11a** (δ_p -1.24 ppm). Acidic hydrolysis (0.1 N HCl/MeOH; 15 min at 20°C) of the *trans*-prop-1-enyl groups in **11a** afforded compound **11b** (δ_p -1.15 and -1.24 ppm) in an overall yield of 72% based on 4. 1*H*-

Scheme 2



Tetrazole-mediated phosphitylation (see Scheme 2[5]) of **11b** (0.5 mmol) with the monofunctional phosphitylating reagent **10**[17] (1.5 mmol) afforded an intermediate containing two phosphite-triesters and one phosphate-triester (δ_p 142.55, 141.58 and -1.00 ppm). Oxidation of the latter two phosphite-triester intermediates, as mentioned before, gave after purification by short column chromatography the fully benzyl-protected derivative **12a** (δ_p -1.15, -1.27 and -1.36 ppm) in a yield of 83%. Finally, hydrogenolysis (H_2/Pd(C)/$CHCl_3$-MeOH) of compound **12a** for 24 h under pressure furnished **12b** (δ_p 0.59, 0.26 and 0.09 ppm), which was isolated as the cyclohexylammonium-salt[4] in an excellent yield.

In conclusion, the approach described in this paper promises to be a convenient protocol for the synthesis of biologically important analogues of (Ptd)Ins(4,5)P_2. In this respect it is also of interest to note that **Ib** [R^1= R^2= $C(O)(CH_2)_{14}CH_3$] could be completely converted with phospholipase A_2 into the corresponding lyso-(Ptd)Ins(4,5)P_2 [*i.e.* **Ib** R^1= H and R^2= $C(O)(CH_2)_{14}CH_3$]. The above lyso-derivative may also give access to analogues of (Ptd)Ins-(4,5)P_2 having specific labels (*e.g.* fluorescence or photoaffinity) at the *sn*-2-position of the glyceride moiety. These probes may *inter alia* be useful to study in detail the transport of phosphoinositides between the intracellular membranes by phospholipid transfer proteins [18].

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14. Reagent 7 was prepared by dropwise addition of benzyl alcohol (10 mmol) and triethylamine (10 mmol) to a cooled (0°C) solution of *bis*(*N*,*N*-diisopropylamino)chlorophosphine (10 mmol) in ether. After 30 min at 20°C, cold (0°C) *n*-hexane (30 ml) was added, followed by removal of $Et_3N.HCl$ by filtration. The filtrate was concentrated *in vacuo* and the remaining oil was redissolved in CH_2Cl_2 to give a 1 M stock solution of 7 (δ_p 123.94 ppm).

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