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THE SYNTHESIS OF DIASTEREOMERS OF PHOSPHOROTHIOATE ANALOGUE OF DIPALMITOYLPHOSPHATIDYLINOSITOL

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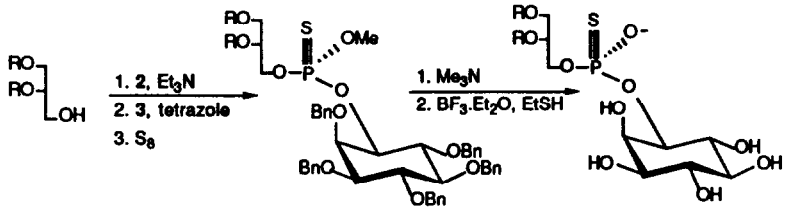
Abstract: The synthesis of diastereomers of 1,2-dipalmitoyl-sn-glycero-3-thiophospho-1'--inositol (DPPsI) has been accomplished via the phosphitylation and chromatographic separation of phosphorothioate triesters.

Chemical synthesis of inositol phospholipids and of myo-inositol phosphates has been the subject of considerable interest, due to the recent results on the role of inositol phospholipids and phosphates in cellular signalling pathways[1].

Numerous methods of the synthesis of the key representatives of inositol phospholipids diacylphosphatidylinositol, and of its analogues have been lately described[2], including the phosphorothioate analogue (DPPsI)[3]. In view of the documented stereospecificity of phospholipases towards phosphorothioate analogues of phospholipids[4], the diastereomers of DPPsI may prove useful antimetabolites blocking the receptor-mediated inositol phosphate metabolism. We now describe the method for chemical synthesis of individual diastereomers of DPPsI.

Thus, 1,2-dipalmitoyl-sn-glycerol 1 was undergone the reaction with chloro-N,N-diisopropylaminomethoxyphosphine 2 in the presence of triethylamine in CH_2Cl_2 as described recently[5]. The resulting phosphoramidite was further condensed with D-(-)-2,3,4,5,6-pentabenzyl-*myo*-inositol[6] (3) in THF-acetonitrile in the presence of tetrazole[8]. The product was sulfurized with elemental sulfur and the mixture of phosphorothioates 4 was deprotected using sequentially trimethylamine (to demethylate phosphorothioate function) and BF_3-ether in ethyl mercaptane[9] (to deprotect inositol hydroxyl functions). Finally, pure DPPsI (5) was obtained by chromatography on silica gel as the mixture of diastereomers.



The attempts at separating this mixture by means of chromatographic techniques were unsuccesful. The total yield of isolated 5 was 47% (with respect to 3). Using the above scheme we have carried out the synthesis of natural DPPI with 61% total yield[10]. In this case, benzyl protective groups were removed by hydrogenolysis.

The separation of diastereomers 5 was finally achieved at the stage of the fully protected triesters 4 using column chromatography on silica gel and CCl_4-acetone (100:1) as eluting solvent (R_F: 4a 0.48, 4b 0.43, CCl_4-acetone, 40:1[11]). Separated diastereomers **4a,b** were deprotected as described above yielding pure **5a** (δ 57.45 ppm) and **5b** (δ 57.05 ppm), respectively. Both diastereomers were fully characterized by their ^1H NMR spectra[12] and by their hydrolysis reactions catalyzed by phospholipase A_2 (PLA_2) and C. ^1H NMR spectra of **5a** and **5b**[12] and of DPPI[10] indicated that these compounds possess virtually identical conformation in the unaggregated state. The hydrolysis of 5a in the presence of

PLA_2 from bee venom in mixed micellar system (Figure 1) gave rise to palmitic acid and lyso-phospholipid **6** as opposed to **5b** for which very little of such products could be detected by TLC.

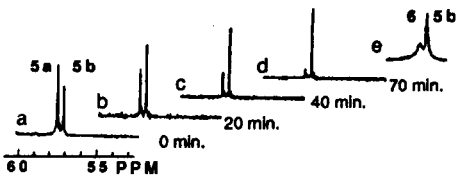


Figure 1. ^{31}P NMR spectra of the reaction mixture of (Sp + Rp)-DPPsI (10 mg) with PLA_2; other conditions : 50 mM HEPES.Na buffer, pH 7.2, 0.25 mM EDTA, 2 mM $CaCl_2$, 4% Triton X-100, 20% D_2O, temperature 310 K; each spectrum was acquired during 10 min.; curve e is an expansion of the signal in the spectrum d.

The time course of the enzymatic reaction is shown in Figure 1. The decrease in the intensity of the low field signal (**5a**) is accompanied by the increase of the other resonance due to coincidence of the chemical shifts of the signal from the undigested substrate **5b** and the lyso-compound **6** (spectrum 1e). In accordance with the previously reported results, phospholipase C from Bacillus cereus catalyzed the hydrolysis of **5b**, while no reaction was obtained with **5a**. Therefore, based on the previously determined enzyme stereospecificities[4], the configuration Rp is assigned to **5b** and Sp to **5a**.

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6. **3** {$[\alpha]_D^{20}$ -13.1°, (c3.8, $CHCl_3$)} was obtained from D-3,4,5,6-tetrabenzyl-*myo*-inositol [7] as described by R.Gigg, and C.D.Warren, *J.Chem.Soc. (C)*, 2367 (1969)
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10. DPPI: {$[\alpha]_D^{20}$ +9.3° (c0.3, $CHCl_3$), m.p. 181° (dec.), lit.$[\alpha]_D^{20}$ +7.48°, B.A,Klyashchitskii, E.G.Zhelvakova, V.I.Shvets, R.P.Evstigneeva, and N.A.Preobrazhenskii, *Tetrahedron Lett.*, 587 (1970); ^1H NMR, 300 MHz, CD_3OD): δ 5.26 (m, H-2), 4.45 (dd, H-1, $J_{1,2}$ 3.3 Hz), 4.208 (t, H-2', $J_{1',2'} = J_{2,3'}$ 2.8 Hz), 4.213 (dd, H-1, $J_{1,2}$ 6.7 Hz), 4.079 (m, H-3), 3.913 (ddd, H-1', $J_{1',2'}$ 2.7 Hz, $J_{1',P}$ 8.2 Hz, $J_{1',6'}$ 9.6 Hz), 3.796 (t, H-6', $J_{6,1'} = J_{6,5'}$ 9.7 Hz, $J_{6,P}$ 1 Hz), 3.656 (t, H-4', $J_{3',4'} = J_{4',5'}$ 9.5 Hz), 3.397 (dd, H-3'), 3.225 (t, H-5'), 2.344 (t, H-2", J 7.4 Hz), 2.319 (t, H-2", J 7.5 Hz), 1.62 (m, H-3"), 1.29 (m, CH_2), 0.893 (t, CH_3)
11. **4a:** δ_{31P} 71.03 ppm; **4b:** δ_{31P} 70.5 ppm
12. ^1H NMR (CD_3OD); **5a:** δ 5.26 (ddt, H-2, $J_{2,3}$ 5.0 Hz), 4.44 (dd, H-1, $J_{1,2}$ 3.3 Hz), 4.256 (t, H-2', $J_{1',2'} + J_{2',3'}$ 2.8 Hz), 4.213 (dd, H-1, $J_{1,2}$ 6.9 Hz), 4.129 (m, H-3, $J_{2,3}$ 5.1 Hz), 3.80 (t, H-6', $J_{6,1'} = J_{6,5'}$ 9.6 Hz, $J_{6,P}$ 1 Hz), 3.644 (t, H-4', $J_{3',4'} = J_{4',5'}$ 9.5 Hz), 3.39 (dd, H-3'), 3.237 (t, H-5'), 2.345 (t, H-2" J 7.4 Hz) 2.317 (t, H-2", J 7.5 Hz), 1.62 (m, H-3"), 1.29 (m, CH_2), 0.893 (t, CH_3); **5b:** δ 5.267 (ddt, H-2, $J_{2,3}$ 5.0 Hz), 4.445 (dd, H-1, $J_{1,2}$ 3.3 Hz), 4.274 (t, H-2', $J_{1',2'} = J_{2',3'}$ 2.5 Hz, $J_{2',P}$<1.0 H), 4.203 (dd, H-1, $J_{1,2}$ 6,7 Hz), 4.134 (m, H-3, $J_{2,3}$ 5.3 Hz J 7.7 Hz), 3.79 (t, H-6', $J_{6,1'} = J_{6,5'}$ 9.3 Hz, $J_{6,P}$ 1 Hz), 3.637 (t, H-4', $J_{3',4'} = J_{4',5'}$ 9.4 Hz), 3.39 (dd, H-3'), 3.222 (t, H-5'), 2.342 (t, H-2" J 7.4 Hz), 2.316 (t, H-2", J 7.6 Hz), 1.62 (m, H-3"), 1.29 (m, CH_2), 0.893 (t, CH_3)
13. The change in relative priority of glyceryl substituent in DPPsC with respect to DPPsI results in the inverse configuration assignment of PLA2 hydrolyzable isomer of DPPsI as compared to DPPsC

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