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SYNTHESIS OF THREONINE PHOSPHOGLYCERIDES

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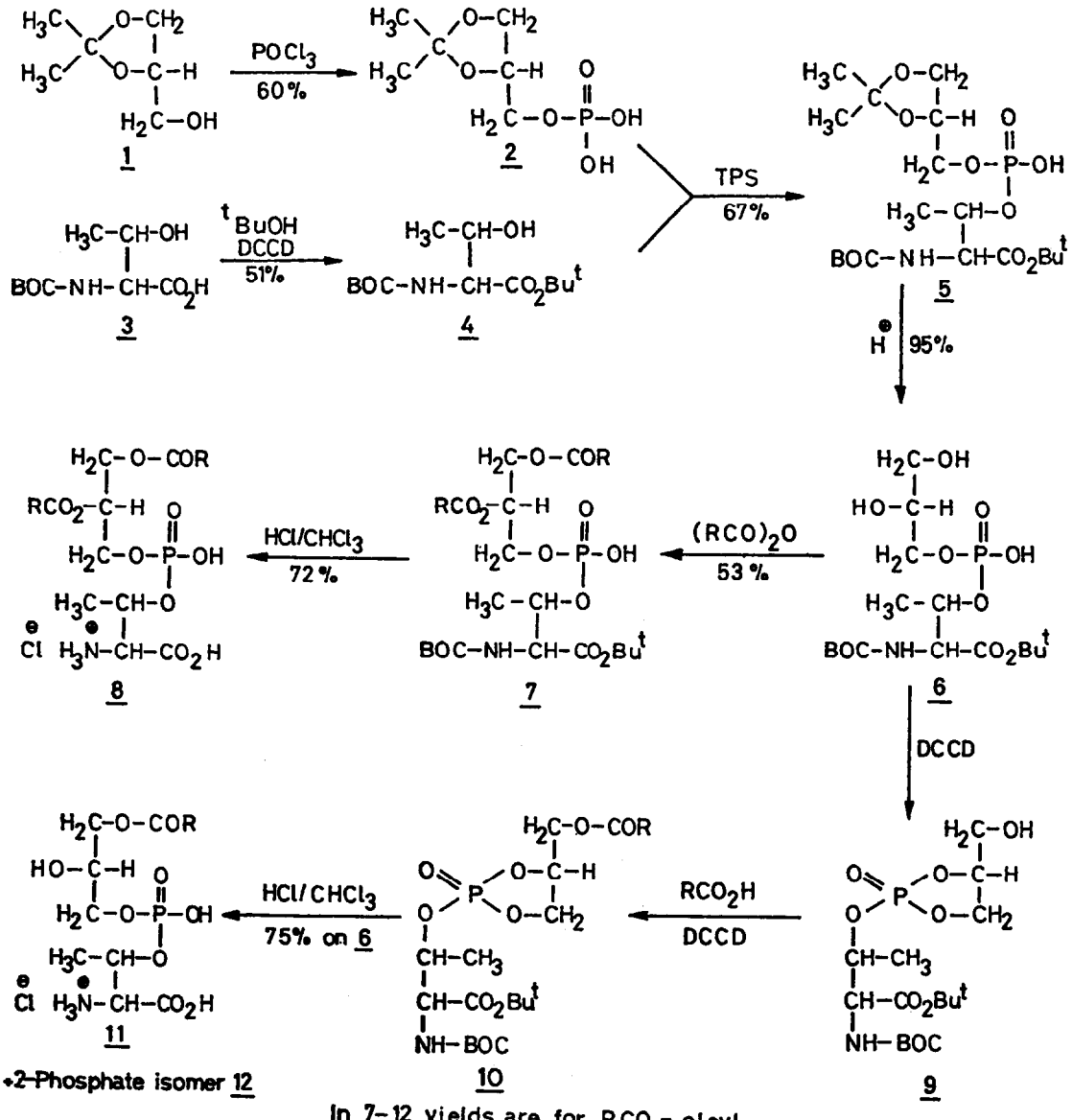
Interest in the biochemical properties of mono- and diacyl threonine phosphoglycerides has led us to explore a practical route to this class of phospholipids. As we wanted to vary the fatty acid residue, we were particularly interested in a synthesis that would permit the introduction of the acyl groups towards the end of the reaction sequence. This would also minimise the exposure of labile polyunsaturated fatty acids.

Relevant published work is limited to the synthesis of racemic serine phosphoglycerides via a multi-step procedure by de Haas and his coworkers (1), and to a similarly laborious preparation of ethanolamine phosphoglycerides by Billimoria and Lewis (2). Both, however, introduce the fatty acid at the beginning of a long sequence.

The route that we have found convenient for the synthesis of both mono- and diacyl phosphoglycerides of L-threonine, and subsequently of other β-hydroxy-α-amino acids, is shown in the scheme.

1,2-Isopropylidene-sn-glycerol, 1 (6) was phosphorylated with $POCl_3$ in quinoline (7) to give 1,2-isopropylidene-sn-glycero-3-phosphoric acid 2, isolated in 60% yield as the crystalline trihydrate of its barium salt (R_F, 0.3; m.p. $> 300^{\circ}$, dec.). The latter was dissolved in water and passed through a column of Amberlite IR-120 ion-exchange resin in the pyridinium form to effect quantitative conversion into the pyridinium salt (R_F, 0.3); n.m.r. in $CDCl_3$, τ: -3.4 (s, 1H), 1.2 (bs, 1H), 2.2 (m, 5H), 6.0 (m, 5H) 8.6 (s, 3H) and 8.65 (s, 3H).



•2-Phosphate isomer 12

In 7-12 yields are for RCO = oleyl.

N-^{t}Butyloxycarbonyl-L-threonine, 3, prepared in 90% yield by Schnabel's method (3), was treated with O-^{t}butyl-N,N'-dicyclohexyl-isourea (4,5) to give ^{t}butyl N-^{t}butyloxycarbonyl-L-threoninate 4, as a crystalline solid in 51% yield, R_F 0.85; m.p. 93-4°; $[\alpha]_D^{27}$ -28.1° (c, 2 in methanol); n.m.r. in CCl_4, τ: 8.52 (s, 9H), 8.55 (s, 9H), 8.8 (d, 3H; J=7Hz); i.r. in mujol: ν_{max} 1730, 1680 cm.$^{-1}$. The L-serine and L-hydroxyproline analogues were prepared in a similar manner.

Condensation of ester 4 with 1,2-isopropylidene-sn-glycero-3-phosphoric acid, 2 by Khorana's method (8) using 2,4,6-triisopropylbenzenesulphonyl chloride (TPS) in dry pyridine (12) furnished the protected phosphate diester 5 which was isolated in 67% yield as the crystalline cyclohexylammonium salt, R_F (in $CHCl_3$:MeOH, 3:1) 0.45; m.p. 133-4°; $[\alpha]_D^{27}$ -1.5° (c, 10 in methanol); n.m.r. in $CDCl_3$, τ: 2.5 (b.s., 3H), 6.2 (m, 5H), 8.52 (s, 9H), 8.56 (s, 9H), 8.65 (s, 3H) and 8.70 (s, 3H); i.r. in nujol, ν_{max}: 3400, 2180, 1720, 1190 and 950 $cm.^{-1}$.

Selective removal of the isopropylidene blocking group in 5 was effected smoothly by treatment with 80% acetic acid for 10 mins. at 50°. The resultant O-(sn-glycero-3-phosphoryl)-N-^{t}butyloxycarbonyl-L-threonine ^{t}butyl ester 6 was isolated in 95% yield as the cyclohexylammonium salt, R_F (in $CHCl_3$:MeOH, 3:1) 0.2; $[\alpha]_D^{27}$ -4.66° (c, 5 in methanol). The latter was readily converted into the pyridinium salt, a viscous gum, $[\alpha]_D^{28}$ -4.05° (c, 10 in methanol), n.m.r. in D_2O, τ: 1.6 (m, 5H), 6.2 (m, 5H), 8.52 (s, 9H), 8.57 (s, 9H), 8.63 (d, 3H; J=7Hz); i.r. in $CHCl_3$, ν_{max}: 3300, 1740, 1690.

Acylation of 6 with the anhydrides (9) of various saturated or unsaturated fatty acids in the presence of a large excess of the corresponding tetraethylammonium salts (10, 11) furnished protected diacyl phosphoglycerides 7 which were deprotected by treatment with hydrogen chloride in chloroform (1) to give hydrochloride salts of the various diacyl-phospholipids 8.

Preparation of the di-oleyl phosphoglyceride 8, (RCO=oleyl) is a typical example. Heating 6 in CCl_4 with ten mols. of tetraethyl-ammonium oleate and ten mols. of oleic anhydride (9) at 75° for 24 hrs. gave, after chromatography on silica in $CHCl_3$-MeOH, a 53% yield of the protected dioleyl phosphoglyceride 7 (RCO=oleyl) as a waxy solid, R_F (in $CHCl_3$:MeOH, 3:1) 0.7; $[\alpha]_D^{25}$ + 7.28° (c, 2 in $CHCl_3$). Deprotection of the latter with HCl/$CHCl_3$ at 0° furnished a 72% yield of pure dioleyl phosphatidyl-L-threonine hydrochloride, 8 (RCO=oleyl) as a white amorphous solid, R_F, 0.55; m.p. 161-2° (dec.); $[\alpha]_D^{25}$ + 20.8° (c, 5 in methanol); n.m.r. in $CDCl_3$, τ: 4.9 (t, 4H), 6.1 (m, 5H), 7.8-9.2 (complex, 66H).

Alternatively, 6 could be converted quantitatively into the cyclic phosphate 9 by treatment with one mol. of N,N'-dicyclohexylcarbodiimide (DCCD) in CCl_4. Addition of a further 1.1 mol. of DCCD and 1.1 mol. of fatty acid to the reaction mixture led, after 17 hrs. at 20°, to the quantitative 1-monoacylation of 9 to give 10. The latter was treated with HCl/$CHCl_3$ to remove the protecting groups from threonine, and the cyclic phosphate was hydrolysed with aqueous pyridine to give a mixture of the isomeric phosphates 11 and 12 in good yield. This was resolved into the components by gradient elution chromatography with $CHCl_3$/MeOH on silica.

Treatment of the cyclic phosphate 9 in this fashion with e.g., oleic acid and subsequent deprotection furnished a 1:1 mixture of the isomeric 1-oleyl phosphoglycerides 11 and 12 (RCO= oleyl in both) as the hydrochlorides in 75% yield (based on 6). This white solid had R_F 0.4, 0.45; m.p. 148-50° (dec.); n.m.r. in $CDCl_3$, τ: 4.75 (t, 3H), 6.0 (m, 5H), 7.8-9.2 (complex, 36H). Chromatographic resolution yielded pure O-(1-oleyl-sn-glycero-3-phosphoryl)-L-threonine 11, (RCO=oleyl) as the hydrochloride, R_F 0.40; m.p. 149-50°; n.m.r. as before; $[\alpha]_D^{25}$ + 3.5° (c, 2 in 50% chloroform, methanol).

The assignment of structures 8, 11 and 12 was confirmed by enzymatic hydrolyses. The acyl group of the faster moving (R_F 0.45) component of the mixture 11 and 12 (RCO=oleyl) was completely cleaved by phospholipase A from Crotalus adamanteus venom, indicating that this was the 2-phosphate 12 and that optical integrity at C_2 in the glycerol moiety was preserved during synthesis. The diacyl phosphoglyceride 8 (RCO=oleyl) was converted by the same enzyme quantitatively into the slower moving (R_F 0.40) monoacyl compound 11 (RCO=oleyl) confirming that the latter was the 3-phosphate.

Satisfactory microanalyses were obtained for all the compounds described. R_F values were measured by t.l.c. on silica plates in the system butanol:acetic acid:water, 12:3:5 unless stated otherwise.

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12) After this work, submitted by J. Moore to the University of East Anglia in part fulfilment of the requirements for the degree of Master of Sciences, was completed, a publication (13) appeared describing the use of TPS in the esterification of phosphatidic acids with various N-protected ethanolamines.

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