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A SHORT, GENERAL SYNTHESIS OF GLYCEROPHOSPHOLIPIDS

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The biologically important monoalkyl esters[1] (I) of 1,2-diacyl-sn-glycero-3-phosphoric acid[2] (II) are prepared mostly by multistep syntheses[3] in which the phosphate ester bonds are created by the reaction of phosphoryl chlorides with alcohols, or alkyl iodides with silver phosphate derivatives. The direct esterification of (II) (scheme 1) provides a short, general and potentially useful route and we have exploited it successfully for the preparation of several glycerophospholipids*. This was possible by the choice of 2,4,6-triisopropylbenzenesulphonyl chloride[6] (TPS) in the presence of pyridine, as the condensing agent. Phosphorylation proceeded smoothly and little pyrophosphate was obtained even in the presence of a two-fold excess of the alcohol component. Essentially complete conversions are obtained and in the three examples cited hereunder, chromatographically pure condensation products were isolated in 71-93% yield (based on the phosphatidic acid).

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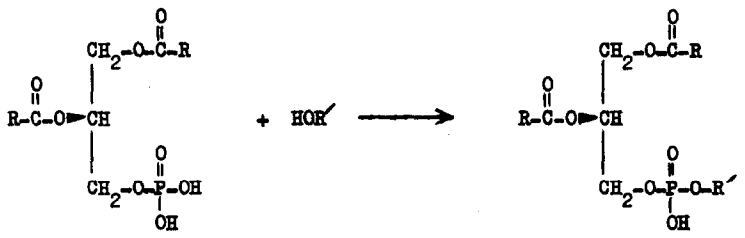
* The condensation of a phosphatidic acid with glycerol using DCC has been described earlier[4]. However, a large amount of pyrophosphate is formed as a by-product using this condensing agent[4,5].

Scheme 1



(II) (III) (I)

a, R′ = $-CH_2CH_2N(CH_3)_2$

b, R′ = $-CH_2CH_2NH\,C(C_6H_5)_3$

c, R′ = $-CH_2CH_2NH_2$

d, R′ = $-CH_2CH_2N(CO\cdot O\cdot CH_2C_6H_5)CH_3$

e, R′ = $-CH_2CH_2NHCH_3$

In a typical experiment, a pyridine-chloroform solution of 1,2-dioctadecanoyl-sn-glycero-3-phosphoric acid[7] (II, R = $nC_{17}H_{35}$) (1 molar equivalent), N,N-dimethylamino-ethanol[8] (IIIa) (2 molar equivalents) and TPS[9] (2-3 molar equivalents) was stirred at 20-22° for 3 hours. Water was added to destroy the unreacted TPS or mixed phosphoric-sulphonic anhydride and the mixture evaporated to dryness at 40°C, under reduced pressure. The residue was purified by chromatography over SilicAR CC-7 (ex. Mallinkrodt). Pure N,N-dimethyl-O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine (Ia) was obtained in 80% yield; m.p. 163-64°, $[\alpha]_D^{22}$ + 5.9° (c 1.0, ethanol-free dry chloroform); lit.,[10] m.p. 169-170°, $[\alpha]_D$ + 5.4° (c 5.7 ethanol-free dry chloroform).

In general, reversible blocking of additional groups in poly-functional alcohols will be necessary before the TPS mediated condensation can be effected. Thus, N-tritylethanol-amine* (IIIb), the phosphatidic acid II (R=n-$C_{17}H_{35}$) and TPS, after reaction in chloroform-pyridine for 4 hours and work up, gave a 93% yield of N-trityl-O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine[11] (Ib); m.p. 113-114°, $[\alpha]_D^{22}$ + 6.3° (c, 2, dry ethanol-free

* Prepared by the action of tritylbromide on ethanolamine in the presence of triethylamine

chloroform). Subsequent hydrogenolysis (H_2/Pd-C) as described earlier[11] gave O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine[12,13,14,11] (Ic), m.p. 187-88°, $[\alpha]_D^{22}$ + 6.2° (C,4); lit.[13] m.p. 188-89°, $[\alpha]_D$ + 6.2° (C, 3.4, chloroform, acetic acid; 9:1)*.

Similarly, the condensation of N-methyl-N-carbobenzoxy-ethanolamine[5](IIId) with II, yielded (71%), N-carbobenzoxy-N-methyl-O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine† (Id), m.p. 53°, $[\alpha]_D$ + 3.8° (C,2 ethanol-free dry chlorform). Hydrogenolysis (H_2/Pd-C) of Id yielded N-methyl-O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine (Ie), m.p. 176°, $[\alpha]_D^{22}$ + 7.5° (C,2, ethanol-free dry chloroform); lit.[15], m.p. 178-179° $[\alpha]_D^{25}$ + 7.5° (C, 5.7 chloroform).

By choosing protecting groups which do not require hydrogenation for their removal, it should be possible to prepare glycerophospholipids containing unsaturated fatty acyl residues. This work, together with the preparation of phosphatidylserines and other phospholipids is in hand.

The utility of the approach to the syntheses discussed above will depend on the availability of phosphatidic acids. Several satisfactory methods for their preparation are available[3], and a timely publication[16] describing the direct acylation of glycerophosphoric acid has provided an excellent new method. Considering this development and our experience of glycerophospholipid syntheses by the existing routes, we conclude that the TPS mediated condensation of phosphatidic acids with the appropriate alcohols (Scheme 1) offers an efficient, simple and general route for the preparation of several glycerophospholipids required for biochemical and biophysical investigations.

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