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A CONVENIENT SYNTHESIS OF PHOSPHATIDYLETHANOLAMINES

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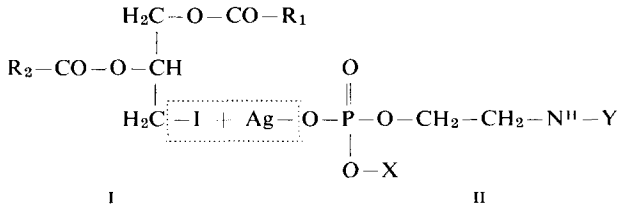
Silver-*t*-butyl-(N-*t*-butyloxycarbonyl-2-aminoethyl) phosphate was synthesized. Coupling of this compound with 1-palmitoyl-2-oleoyl-glycerol-3-iodohydrin, followed by removal of both protecting groups, gave, after recrystallization (without chromatography) pure 1-palmitoyl-2-oleoyl-3-phosphatidylethanolamine in 55% yield.

Introduction

Synthetic techniques for phosphatidylethanolamines and phosphatidylserines are greatly needed, since there exists neither an adequate isolation method for gram quantities of chromatographically pure material nor a partial synthesis starting from natural material. In connection with work in this laboratory on the role of phosphatidylethanolamine and/or phosphatidylserine in the mechanism of the visual process [1]), fully characterized and pure specimens of these phosphatides were needed. A simplified and convenient synthesis of phosphatidylethanolamines was developed, which will be reported here.

A suitable pattern for the synthesis of phosphatidylethanolamines with, if desired, two different fatty acids is the reaction between 1,2 diacyl-glycerol-3-iodohydrins (I) and the silver salt of an O-ethanolamine phosphate (II), the amino and residual phosphoric acid function of which are adequately protected (scheme 1).

With Ag-phenyl-(N-benzyloxycarbonyl)-ethanolamine phosphate [2]) (IIa) only phosphatides containing saturated fatty acids could be synthesized, since removal of the protecting groups could be effected only by catalytic hydrogenolysis. The use of Ag-benzyl-(2-phthalimido-ethyl) phosphate [3]) (IIb) permitted the synthesis of phosphatidylethanolamines containing unsaturated fatty acids, but in this case removal of the phthaloyl group was cumbersome. The introduction of Ag-(*p*-nitro)-benzyl-(N-*t*-butyloxycarbonyl-2-aminoethyl) phosphate [4]) (IIc) appeared to be a considerable improvement, since the *t*-butyloxycarbonyl group could be easily removed. Finally the use of Ag-benzyl-(trityl-2-aminoethyl) phosphate (IId) was reported in a short communication [5]).



I II

	X	Y
a) R_1: $-C_{15}H_{31}$ R_2: $-C_{17}H_{33}$	a) phenyl	benzyloxycarbonyl
	b) benzyl	phthaloyl
	c) p-NO_2-benzyl	t-butyloxycarbonyl
	d) benzyl	trityl
	e) t-butyl	t-butyloxycarbonyl

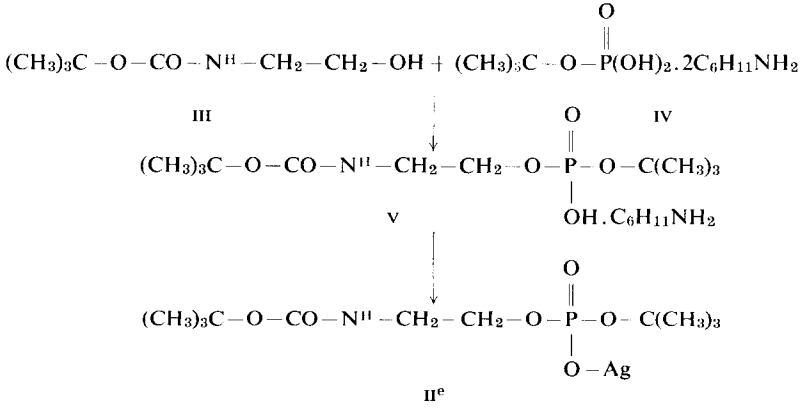
Scheme 1. Syntheses of phosphatidylethanolamines using protected silver O-ethanolamine phosphates.

Critical consideration of the phosphatidylethanolamine synthesis employing IIc shows that (a) the synthesis of IIc numbers three steps, (b) the protecting groups, after coupling of IIc and I, have to be removed by two separate reactions, (c) chromatographic purification of the product is necessary.

The use of silver-t-butyl-(N-t-butyloxycarbonyl-2-aminoethyl) phosphate (IIe), which could be synthesized in two steps, enabled the simultaneous, easy removal of both protecting groups after condensation with the diacylglycerol-iodohydrin (I). A pure endproduct was obtained, which did not require chromatographic purification.

Results

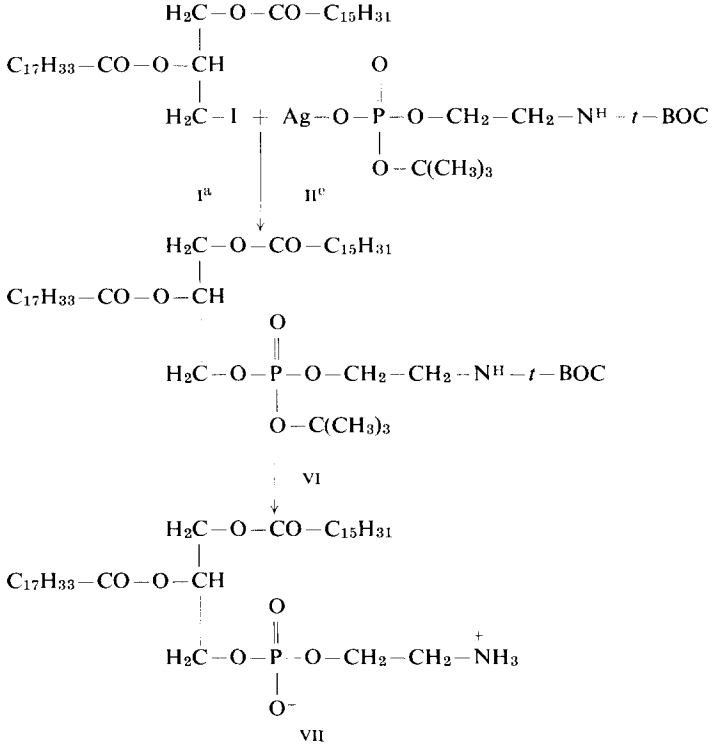
Coupling of t-butyloxycarbonyl-ethanolamine (III) with di-cyclohexyl-



Scheme 2. Synthesis of Ag-t-butyl-(N-t-butyloxycarbonyl-2-aminoethyl) phosphate.

ammonium *t*-butyl phosphate (IV) was carried out in pyridine with trichloroacetonitrile as a transphosphorylating agent, according to a method of Cramer and Weimann[6]). The phosphoric acid diester V could be isolated and purified as the cyclohexylammonium salt. A nearly quantitative conversion to Ag-*t*-butyl-(N-*t*-butyloxycarbonyl-2-aminoethyl) phosphate (IIe) was effected by percolation of V over a cation exchange resin in the Ag^+-form. The synthesis of IIe is depicted in scheme 2.

Since all phosphatidylethanolamine syntheses were carried out in a similar manner, we shall only describe the synthesis of 1-palmitoyl-2-oleoyl-3-phosphatidylethanolamine (scheme 3).



Scheme 3. Synthesis of 1-palmitoyl-2-oleoyl-3-phosphatidylethanolamine.

In spite of numerous attempts, the yield of the silver salt-iodohydrin coupling reaction between a diacyl-glycerol-3-iodohydrin and silver salt IIe in boiling benzene or toluene never exceeded 60%. Steric hindrance caused by the *t*-butyl group is probably involved. The resulting phosphoric acid triester, *t*-butyl-N-*t*-butyloxycarbonyl-phosphatidylethanolamine (VI), was not further purified, since the protecting groups appeared to be extremely

labile, already partially splitting off during thin layer chromatography on SiO_2 with a basic eluent. In addition the (apolar) impurities appeared to be easily removable during recrystallization of the endproduct.

Removal of both acid-labile protecting groups was effected by treatment of VI with gaseous, dry hydrogen chloride in dry ether or chloroform. The product, a hydrogen chloride adduct of phosphatidylethanolamine (VII), was obtained as an entirely colourless foam and was converted to the free phosphatide, 1-palmitoyl-2-oleoyl-3-phosphatidylethanolamine (VII), by treatment with a weakly anionic exchange resin. After recrystallization a relatively hard, fatty wax resulted, which was freely soluble in chloroform, but in absolute ether only after addition of a few drops of methanol. The product did not show contaminations on thin layer chromatography. Fatty acid-containing intermediates and the endproduct were checked for their fatty acid composition by gas–liquid chromatography of the fatty acid methyl esters. A correct 1:1 ratio of palmitic and oleic acid was invariably found.

EXPERIMENTAL PART

General methods

Preparation of fatty acid methyl esters for gas–liquid chromatography was carried out with boron trifluoride by the method of Metcalfe et al.[7]). Gas–liquid chromatography and thin layer chromatography were carried out following established procedures[4,8]). A Perkin–Elmer 141 polarimeter was used for determining optical rotation. Elementary analyses were carried out in the analytical laboratories of the Department of Chemistry of the State University of Groningen and of the Department of Organic Chemistry of the Municipal University of Amsterdam.

Materials

The synthesis of di-cyclohexylammonium *t*-butyl phosphate (IV) was described by Cramer et al.[9]). *t*-Butyloxycarbonyl-ethanolamine (III) and 1-palmitoyl-glycerol-3-iodohydrin were prepared as described previously[4,8]). Trichloroacetonitrile was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin and the fatty acids from the Hormel Institute, Austin, Minnesota. Fatty acid chlorides were prepared with oxalyl chloride.

Silver t-butyl-(N-t-butyloxycarbonyl-2-aminoethyl) phosphate (IIe)

1.75 g of di-cyclohexylammonium *t*-butyl phosphate[9]) (IV, 5 mmol) was suspended in 25 ml of dry pyridine and 2.4 g of N-*t*-butyloxycarbonyl-ethanolamine[4]) (III, 15 mmol) and 7.2 g of trichloroacetonitrile (50 mmol)

were added. The mixture was kept in a closed vessel at 90–100° for 4 hr. The resulting brown reaction mixture was diluted with 100 ml of water and continuously extracted with ether for 1 hr. To the aqueous layer 2 ml of cyclohexylamine was added and the solvent was removed in a rotating evaporator below 40°. The residue was once recrystallized from dimethylformamide, containing 2% of cyclohexylamine. The precipitate was collected at 4°, washed with acetone and ether, yielding 1.2 g (60%) of the cyclohexylammonium salt V with m.p. 185–187 °C.

Found:	C 52.1	H 9.5	N 7.1	P 7.5
$C_{17}H_{37}N_2O_6P$ (396.5):	C 51.5	H 9.4	N 7.1	P 7.8

A column of Amberlite IR 120 H^+ (15 g wet weight) was prepared in 50% ethanol and converted into the Ag^+-form by percolating a solution of 15 g of silver nitrate in 50% ethanol. After washing out the adhering electrolyte, 1.2 g of V was applied on the column and eluted with 50% ethanol (100 ml). The eluate was concentrated and the residue recrystallized from absolute ethanol. The silver salt IIe was obtained as a purely white, light-sensitive powder with m.p. 158–160 °C. Yield 1.06 g (95%). The conversion to IIe was carried out in dim red light.

Found:	C 32.8	H 6.0	N 3.7	P 7.6
$C_{11}H_{23}NO_6PAg$ (404.2):	C 32.7	H 5.7	N 3.5	P 7.7

1-Palmitoyl-2-oleoyl-glycerol-3-iodohydrin (Ia)

This compound was synthesized, with minor modifications, as described earlier[8]) for analogous substances. Yield: 80% (calculated on 1-palmitoyl-glycerol-3-iodohydrin), m.p. 10.5–11 °C, $[\alpha]_D^{22} = +3.46°$ (c 9 in chloroform).

Found:	C 63.0	H 9.8
$C_{37}H_{69}IO_4$ (704.8):	C 63.0	H 9.9

1-Palmitoyl-2-oleoyl-3-phosphatidylethanolamine (VII)

Silver *t*-butyl-(N-*t*-butyloxycarbonyl-2-aminoethyl) phosphate (IIe, 1.5 g, 3.7 mmol) and glycerol-iodohydrin Ia (2.2 g, 3.1 mmol) were introduced into 200 ml of freshly distilled, anhydrous benzene and the suspension was boiled under reflux in a nitrogenous atmosphere for 2 hr. After removal of the precipitated silver iodide the reaction mixture was washed with potassium bicarbonate solution and water. After drying on sodium sulphate, 2.4 g of a colourless oil was obtained. This oil, mainly consisting of phosphoric acid triester VI, was dissolved in 100 ml of anhydrous ether and dry hydrogen chloride was led through this solution at 0 °C for 2 hr. The solvent was evaporated in vacuo below 40 °C and the remaining solid, containing the hydrogen chloride adduct of VII (phosphatidyl-ethanolammonium chloride), was dissolved in 50 ml of a mixture of ether, ethanol and water (4:2:1 v/v)

and percolated over a column of Amberlite IR 45 (OH^-). After evaporation, the residue was crystallized once from chloroform–acetone to yield 1.24 g (55% calculated on Ia) of the desired product. M.p. 194–196 °C, $[\alpha]_D^{22} = +6.35°$ (c 3 in chloroform).

Found:	C 64.1	H 10.6	N 2.0	P 4.4
$C_{39}H_{76}NO_8P.H_2O$ (736.0):	C 63.5	H 10.6	N 1.9	P 4.2

The specific position of the fatty acids in the phosphatide molecule was fully confirmed by enzymatic hydrolysis with highly purified pancreatic phospholipase A (E.C.3.1.1.4). We are indebted to Drs. A. J. Slotboom, Department of Biochemistry, Laboratory of Organic Chemistry, State University of Utrecht, for carrying out these enzymatic hydrolysis experiments.

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