A Convenient Synthesis of Mixed-Acid Glycerophosphocholines

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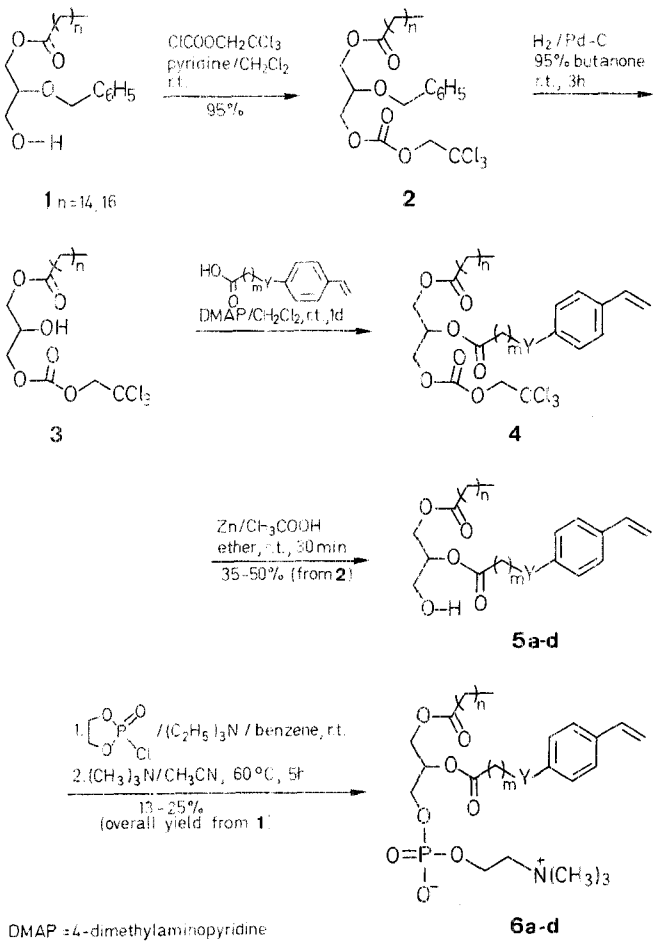
A convenient and non-enzymatic method for the synthesis of mixed-acid glycerophosphocholines is reported, using 1-*O*-acyl-2-*O*-benzyl-glycerol-3-*O*-(β,β,β-trichloroethyl)carbonate as the starting material.

Mixed-acyl chain containing glycerophosphocholines (asymmetric glycerophosphocholines), having two different fatty acids attached to carbons 1 and 2 of the glycerol backbone, are major structural components of biological membranes.[1,2] Although symmetric glycerophosphocholines are easily synthesized, only an enzymatic method is available for the synthesis of asymmetric ones. Phospholipase A_2 hydrolyzes the acyl moiety in the 2 position of a symmetric chain containing sn-glycero-phosphocholine to give the 1-*O*-acyl-sn-glycero-3-phosphocholine (lysolipid), followed by acylating with a fatty acid anhydride to afford the desired mixed-acid phospholipid.[3-8] Without enzymes, several methods have been reported for the synthesis of a mixed-acid glycerophosphocholine: the preparations started with 1-acyl-3-iodo-3-deoxyglycerol[9] (the desired isomer was formed with 97% regioselectivity[10]), 1-*O*-trityl-3-chloro-3-deoxyglycerol,[11] 2-*O*-allyl-3-benzyl-glycerol[12] or 1-acyl-2-bromo-2-deoxyglycero-3-phosphocholine.[13]

As a part of our study on model membrane synthesis, we have prepared polymerizable glycerophosphocholines having styrene group(s)[14-16] and synthesized the corresponding polymerized vesicles[17-19] as carriers of biologically active compounds. Our present intention is to prepare a mixed-acid glycerolipid having a polymerization-sensitive styrene group at the 2 position of glycerol. Known preparation methods are rather complicated and often include reaction conditions that are not mild and chromatographic separations. Therefore they are not adequate for the synthesis of a labile or functionalized glycerophosphocholine. We describe here a new and convenient method to prepare mixed-acid glycerophosphocholines using benzyl and β,β,β-trichloroethyloxycarbonyl[20] groups as protecting groups for the hydroxy groups at the 2 and 3 positions. This method, including the preparation of 1-*O*-acyl-2-*O*-benzyl-rac-glycerol-3-*O*-(β,β,β-trichloroethyl)carbonate as a starting compound, needs no chromatographic purification until the final step.

The reaction of 1-*O*-acyl-2-*O*-benzyl-rac-glycerol[21] **1** with β,β,β-trichloroethyl carbonochloridate yields 1-*O*-acyl-2-*O*-benzyl-rac-glycerol-3-*O*-(β,β,β-trichloroethyl)carbonate **2**. **2** was debenzylated by H_2 (1 atm)/10% Pd-carbon to give 1-*O*-acyl-rac-glycerol-3-*O*-(β,β,β-trichloroethyl)carbonate **3**. **3** was then acylated with a fatty acid anhydride in the presence of *N*,*N*-dimethylaminopyridine to afford a mixed-acid compound **4**, followed by removing the trichloroethyloxycarbonyl group by Zn/acetic acid to give a mixed-acid compound **5**. If necessary for determining the structure, **5** could be chromatographed on silica gel. Proton decoupled ^{13}C-NMR spectrum of the purified **5a** and **5b** gave four signals due to oxygen-neighbored carbons (72.1, 68.0, 62.1, 61.4) and two signals due to ester carbonyl carbons (173.66, 173.31), supporting that no acyl migration occurred during the chromatgraphic purification. Unpurified **5** was phosphocholinated by a known method using 2-chloro-2-oxo-1,3,2-dioxaphospholane[22] and trimethylamine to give the desired mixed-acyl chain containing rac-glycerophosphocholine **6a–e** after chromatographic purification.



Two carboxylic acids containing a styrene group, i.e., 11-(*p*-vinylphenoxy)undecanoic acid (m = 10, R = —O—) and 9-(*p*-vinylbenzoyl)nonanoic acid (m = 8, R = —CO—), were used as fatty acids in the present work, because we are interested in polymerizable phospholipids, and because these special fatty acids make the analysis of products easier than with normal saturated fatty acids. The results and analytical data are summarized in the Table.

^{13}C-NMR spectra gave only two resonance signals in the carbonyl region. This NMR spectral evidence, together with the fast-atom bombardment mass spectral and microanalytical results, supports that one saturated fatty acid and one styrene containing fatty acid are introduced per glycerol and are attached to the corresponding desired position of the glycerol backbone. Acyl group migration[23] from the β-position to the α- or γ-position, resulting in the formation of 1,3-diacylglycerophosphocholine, was not found within the accuracy of the analytical methods used (less than 5%).

When sn-glycerol derivatives are required, the present procedure is applicable also to 1-*O*-acyl-2-*O*-benzyl-sn-glycerol.[24]

The polymerizable glycerophosphocholines **6** formed vesicles and gave stable microcapsules (polymerized vesicles) after photopolymerization. Details will be published elsewhere.

1-*O*-Acyl-2-*O*-benzyl-rac-glycerol-3-(β,β,β-trichloroethyl)carbonate (2); General Procedure:

A solution of 1-*O*-acyl-2-*O*-benzyl-rac-glycerol (**1**; 9.4 mmol) and dry pyridine (10 mmol) in dry dichloromethane (50 ml) is cooled in an ice bath. Trichloroethyl carbonochloridate (Aldrich) (2.1 g, 9.9 mmol) in

Table. Mixed-Acid Glycerophosphocholines **6** Prepared

Product **6**	–Y–	n	m	Yield[a] (%)	Molecular Formula[b]	FAB MS[c] $[M+H]^+$	UV (MeOH)[d] $\lambda_{max}(\varepsilon_{max})$	IR (KBr)[e] $\nu_{C=O}(cm^{-1})$	Proton Decoupled ^{13}C-NMR[f] ($CDCl_3$/TMS) δ(ppm)
a	–O–	16	10	22	$C_{45}H_{80}NO_9P$ (810.1)	810	258 (1.7×10^4)	1730	54.31 ($N(CH_3)_3$); 63.1, 70.6 (glycerol); 111.32, 136.24 (vinyl); 130.19, 127.28, 114.42, 158.89 (phenyl); 173.14, 173.48 (carbonyl)
b	–O–	14	10	25	$C_{43}H_{76}NO_9P$ (782.0)	782	258 (1.6×10^4)	1725	54.25 ($N(CH_3)_3$); 63.0, 70.4 (glycerol); 111.25, 136.18 (vinyl); 130.13, 127.22, 114.40, 158.84 (phenyl); 173.04, 173.40 (carbonyl)
c	–CO–	16	8	13	$C_{44}H_{76}NO_9P$ (794.1)	794	276 (1.5×10^4)	1720, 1680	54.39 ($N(CH_3)_3$); 63.0, 63.7, 70.6 (glycerol); 126.26, 128.40, 136.18, 141.84 (phenyl); 173.14, 173.48, 199.72 (carbonyl)
d	–CO–	14	8	17	$C_{42}H_{72}NO_9P$ (766.0)	766	276 (1.6×10^4)	1725, 1680	54.35 ($N(CH_3)_3$); 62.8, 63.6, 70.6 (glycerol); 126.37, 128.47, 135.96, 142.04 (phenyl); 173.34, 173.75, 200.31 (carbonyl)

[a] Overall yield based on **1**.
[b] Satisfactory microanalyses for N ±0.3.
[c] Recorded with a JEOL JMS-OIG-2 spectrometer.
[d] λ_{max} in nm (ε_{max} in liter/mol. cm); recorded with a Shimadzu UV-240 spectrophotometer.
[e] Recorded with on Hitachi 260-50 spectrophotometer.
[f] Recorded with a JEOL-FX 100 spectrometer.

dichloromethane (15 ml) is then added dropwise. After stirring overnight at room temperature, the reaction mixture is diluted with ether (150 ml) and washed with dilute hydrochloric acid, water, and 5% aqueous sodium carbonate solution. After being dried with sodium sulfate, the organic phase is concentrated, and the residue is dried *in vacuo* over P_4O_{10} at room temperature to yield **2a** (n = 16; 96%) or **2b** (n = 14; 94%).

Mixed-acid glycerophosphocholines 6; General Procedure:
2 (3.2 mmol) in 95% butanone (80 ml) is debenzylated by H_2 (1 atm)/10% Pd–C (0.4 g) at room temperature for 3 h. The reaction mixture is then filtered and evaporated to dryness. Dry benzene is added to the residue and solvents are removed by evaporation under reduced pressure using a rotatory evaporator to remove residual water.

To a solution of **3** obtained above and *N,N*-dimethylaminopyridine (0.1 g, 0.8 mmol) in dichloromethane (40 ml) is added the acid anhydride of a fatty acid containing a styrene group (3.8 mmol) dissolved in dichloromethane (80 ml). After stirring for 1 d at room temperature in the dark, the mixture is diluted with ether (0.4 l), washed with dilute hydrochloric acid, water, 5% aqueous sodium carbonate solution and water, and dried with sodium sulfate. Solvents are evaporated to give crude **4**.

The residue obtained above is dissolved in acetic acid/ether (15 ml/15 ml) and treated with activated zinc (3.0 g). After 30 min, the reaction mixture is diluted with ether/chloroform (300 ml/100 ml) and filtered. The filtrate is concentrated and dried *in vacuo* over P_2O_5 at room temperature to give crude **5** in 35–50% yield based on **2**. A sample of crude **5** can be purified to obtain spectroscopic and physical data by chromatography on silica gel (benzene/ether, 9:1, as eluent); m.p. of **5a** and **5b**: 60–61 °C and 53–55 °C, respectively.

To a solution of crude **5** (1.0 mmol), triethylamine (0.12 g, 1.2 mmol) and *N,N*-dimethylaminopyridine (12 mg, 0.1 mmol) in dry benzene (20 ml) is added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.17 g, 1.2 mmol), followed by stirring overnight and then filtration. The filtrate is evaporated to dryness, and the residue, dissolved in dry acetonitrile (30 ml), is allowed to react with dry trimethylamine (20 ml) in a pressure bottle at 60 °C for 5 h. After the reaction mixture is evaporated to dryness, and the residue is chromatographed on silica gel (chloroform/methanol/water, 65:25:4, as eluent). Elutions having the same Rf value on TLC as purified egg yolk lecithins (Sigma) are collected and evaporated to dryness. The product is freeze-dried from benzene/methanol (5:1) to afford **6** in 13–25% yield based on **1**.

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