Facile and Useful Synthesis of Enantiomeric Phosphatidylcholines

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The synthesis of optically active phosphatidylcholines (D- and L-4) containing two of the same fatty acid moieties in a molecule is described. Optically pure D-enantiomers (D-4) were obtained from 2,3-di-*O*-acyl-*sn*-glycerol (D-1) in high yield by phosphorylation with phosphorus oxychloride and subsequent treatment with choline tosylate (11a). L-Enantiomers (L-4) were also prepared in a similar manner from 1,2-di-*O*-acyl-*sn*-glycerol (L-1). The whole procedure is easy and useful for the synthesis of enantiomeric phosphatidylcholines.

Key words L-phosphatidylcholine; D-phosphatidylcholine; 1,2-di-*O*-acyl-*sn*-glycerol; 2,3-di-*O*-acyl-*sn*-glycerol; lyposome; lecithine

Various liposomes have been expected to play an important role in drug delivery systems. For example, a temperature-sensitive liposome has been studied as a targeting system in connection with localized hyperthermia.[1] In these investigations, L-phosphatidylcholines (L-**4**) containing two of the same fatty acid moieties in the molecule, such as 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphorylcholine[2] (L-**4a**) and 1,2-di-*O*-stearoyl-*sn*-glycero-3-phosphorylcholine (L-**4c**), were used, and the stability of L-series of liposome (natural type) against various enzymes was investigated. In order to determine the degradation rate of these liposomes, it is essential to use the D-series of liposome (unnatural type) consisting of D-phosphatidylcholines (D-**4**) as reference compounds, because they are free from enzymatic degradation.[3a,b] For the synthesis of D-enantiomers (D-**4**), however, only a complicated and laborious multi-step synthesis[3] involving inefficient enzymatic synthesis has been reported to date. Under these circumstances, we intended to explore a more simple and useful synthesis of optically active D-phosphatidylcholines (D-**4**).

In the case of the total synthesis of L-phosphatidylcholine (L-**4**), only a few methods for the introduction of a phosphorylcholine moiety have been published (Chart 1). Eibl *et al.* described[4] that phosphorylation of L-diacylglycerol (L-**1**) with 2-bromoethyldichlorophosphate and subsequent quaternization with trimethylamine gave L-**4** (Eq. 1). Nguyen *et al.* used[5] 2-chloro-2-oxo-1,3,2-dioxa-

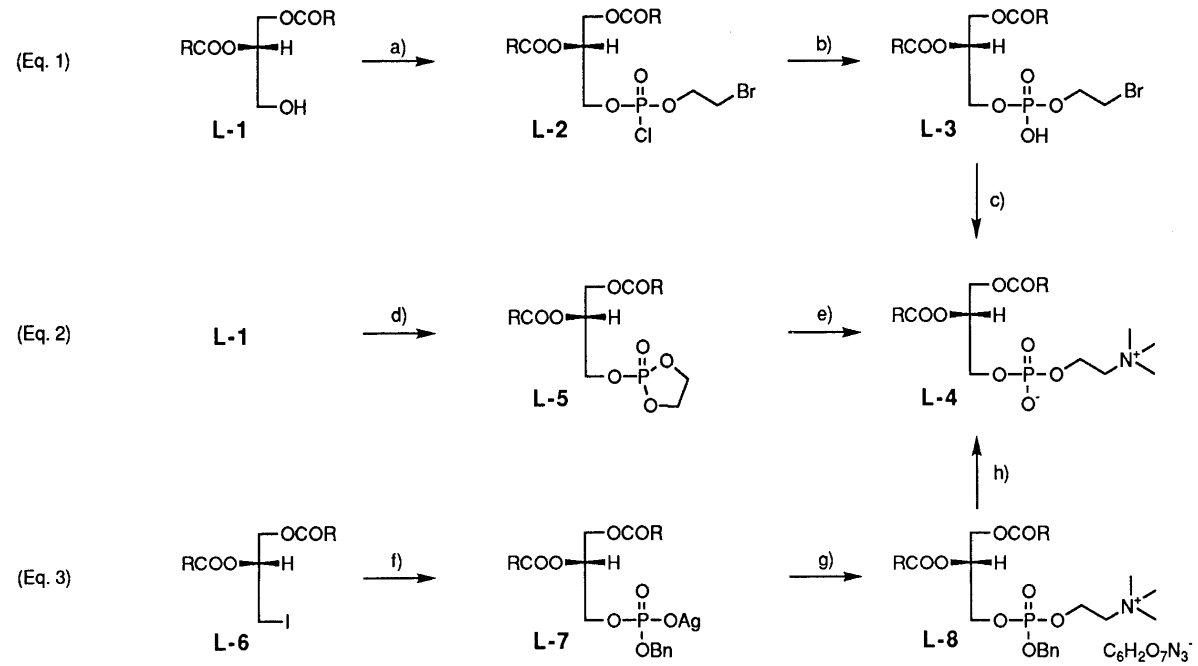

Chart 1

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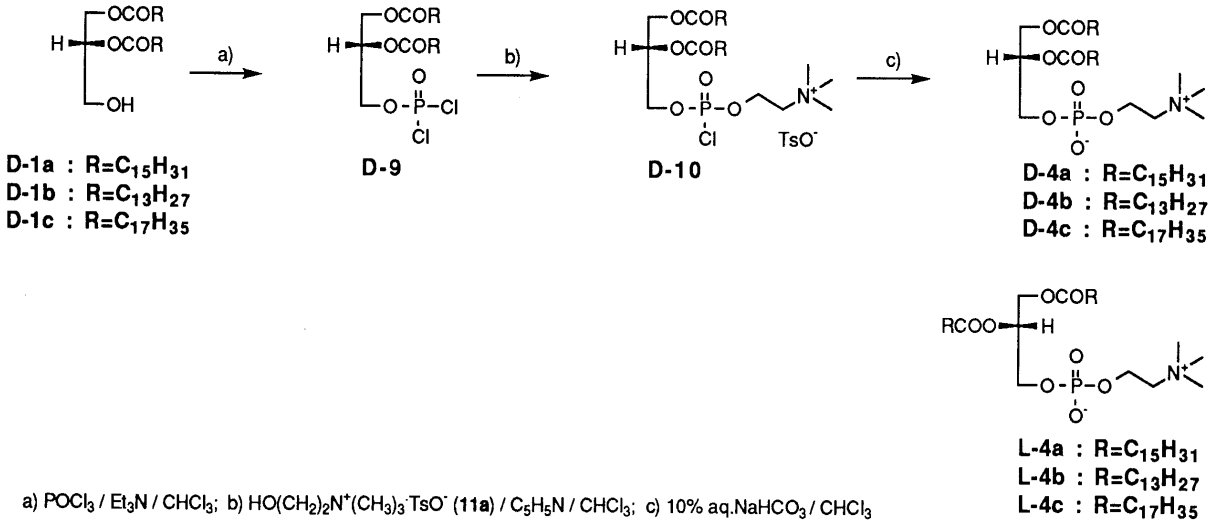

Chart 2

phospholane in place of 2-bromoethyldichlorophosphate for the phosphorylation of diacylglycerol (Eq. 2). However, these methods require a sealed tube or a pressure bottle to complete the quaternization reaction. An alternative preparation of L-**4**, avoiding the quaternization with trimethylamine, was reported by Haas *et al.*[3b] (Eq. 3). Namely, the treatment of silver 1,2-di-*O*-acyl-*sn*-glycero-3-benzylphosphate (L-**7**), obtained from 1,2-diacyloxy-3-iodopropane (L-**6**) by four-step reactions, with 2-bromoethyltrimethylammonium picrate and subsequent debenzylation afforded L-**4**. However, this method requires many tedious processes to obtain L-**4** from the starting compound (L-**6**).

Since these methods seem to be impractical for obtaining substantial amounts of D-**4**, we tried to combine the diacylglycerol (D-**1**) with the phosphorylcholine moiety by an alternative procedure which consists of phosphorylation of D-**1** with phosphorus oxychloride to give 2,3-di-*O*-acyl-*sn*-glycerol-3-dichlorophosphates (D-**9**), followed by treatment with choline tosylate (**11a**)[6] to afford D-**4** (Chart 2).

The starting compounds, 2,3-di-*O*-acyl-*sn*-glycerols (D-**1**), containing two of the same fatty acid moieties in the molecule, were prepared from commercially available 2,3-di-*O*-isopropyliden-*sn*-glycerol according to the known method.[7]

The phosphorylation of 2,3-di-*O*-palmitoyl-*sn*-glycerol (D-**1a**) with phosphorus oxychloride in the presence of triethylamine afforded 2,3-di-*O*-palmitoyl-*sn*-glycerol-1-dichlorophosphate (D-**9a**) in a quantitative yield. The coupling reaction of dichlorophosphate (D-**9a**) and choline tosylate (**11a**) was performed in chloroform containing pyridine to give 2,3-di-*O*-palmitoyl-*sn*-glycerol-1-chlorophosphorylcholine tosylate (D-**10a**), and the subsequent hydrolysis of monochlorophosphorylcholine (D-**10a**) with a 10% aqueous solution of sodium hydrogen carbonate gave optically pure 2,3-di-*O*-palmitoyl-*sn*-glycerol-1-phosphorylcholine (D-**4a**) in 57% yield.

Replacement of dichlorophosphate (D-**9a**) with the various choline salts (**11a**—**11e**) shown in Table 1 was investigated in order to examine the influence of various counter anions of choline, and the results are listed in Table 1. Although three kinds of choline salts (**11a**, **11b** and **11c**) gave D-**4a** in good yields (entries 1,2 and 3), the mesylate (**11d**) and chloride (**11e**) resulted in fairly low yields (entries 4 and 5). The low yields were probably caused by the poor solubility of the choline salts (**11d** and **11e**) in the solvent used.

Table 1. Yields of D-**4a** with the Reaction of D-**9a** and Various Choline Salts

Entry	Choline salt	Anion	Yield of D-**4a** (%)
1	Choline tosylate (**11a**)	TsO^-	57.2
2	Choline benzenesulfonate (**11b**)	$PhSO_3^-$	59.9
3	Choline methylsulfate (**11c**)	$MeSO_4^-$	65.4
4	Choline mesylate (**11d**)	MsO^-	9.5
5	Choline chloride (**11e**)	Cl^-	1.4

We next tried to carry out the three-step reactions described above by a one-pot procedure. After various conditions were examined, a one-pot synthesis of D-**4** was established; a mixture of D-**1a** in chloroform was added dropwise to a mixture of phosphorous oxychloride and triethylamine in chloroform, and after stirring, choline tosylate (**11a**) in pyridine was added to the mixture. Purification by silica gel chromatography gave D-**4a**. The one-pot method was also successfully applicable to the synthesis of D-**4b** and D-**4c** as well as L-phosphatidylcholines (L-**4**) (Table 2). Optical rotations of each enantiomer thus obtained showed that these compounds are highly optically pure.

In conclusion, various optically pure 2,3-di-*O*-acyl-*sn*-glycero-1-phosphorylcholines (D-**4**) having two of the same fatty acid moieties in the molecule were prepared from 2,3-di-*O*-acyl-*sn*-glycerols (D-**1**) in good yields. The synthetic method was applicable also to the preparation of their L-enantiomers (L-**4**). A one-pot procedure involving three consecutive reactions was established. The method thus developed was superior to previously known methods since it is applicable for large-scale preparation of enantiomeric phosphatidylcholines avoiding the use of a sealed tube or a pressure bottle.

Table 2. Yields and Physicochemical Data of Phosphatidylcholines

Compd. No.	R	Yield (%)	mp (°C)		$[\alpha]_d$ (°) (°C, *c*, solvent)[a]		Formula	Analysis (%) Calcd (Found)		
			Found	Reported	Found	Reported		C	H	N
D-**4a**	n-$C_{15}H_{31}$	51.8	218—220		−6.4 (20, 2.0, C)	−6.0[3c] (23, —, 90%C–M)	$C_{40}H_{80}NO_8P$	65.45 (65.42	10.98 10.74	1.91 1.90)
D-**4b**	n-$C_{13}H_{27}$	62.0	219—220		−7.0 (20, 2.0, C)		$C_{36}H_{72}NO_8P$	63.78 (64.04	10.70 10.21	2.07 1.97)
D-**4c**	n-$C_{17}H_{35}$	60.7	219—220		−6.3 (20, 2.0, C)		$C_{44}H_{88}NO_8P$	66.88 (66.67	11.23 10.92	1.77 1.75)
L-**4a**	n-$C_{15}H_{31}$	61.3	219—220	230.5—231.5[9]	+5.9 (20, 2.0, C)	+6.6[9] (23, 4.2, 50%C–M)	$C_{40}H_{80}NO_8P$	65.45 (65.75	10.98 10.86	1.91 1.90)
L-**4b**	n-$C_{13}H_{27}$	60.5	218—220	234—235[9]	+6.6 (20, 2.0, C)	+7.0[9] (—, 3.9, 50%C–M)	$C_{36}H_{72}NO_8P$	63.78 (63.76	10.70 10.65	2.07 2.00)
L-**4c**	n-$C_{17}H_{35}$	57.0	218—221	237—237.5[9]	+6.0 (20, 2.0, C)	+6.1[9] (26, 4.2, 50%C–M)	$C_{44}H_{88}NO_8P$	66.88 (66.79	11.23 11.24	1.77 1.72)

a) C, $CHCl_3$; M, MeOH.

Experimental

All melting points were determined on a Mettler apparatus type FP-61 and are uncorrected. IR spectra and optical rotations were measured by means of a JASCO IRA-2 spectrophotometer and JASCO DIP-4 automatic polarimeter, respectively. ^{1}H-NMR spectra were obtained on a JEOL GSX 270 spectrometer with tetramethylsilane as an internal standard. Wakogel C-200 was used for silica gel column chromatography. All reagents and solvents were of commercial quality.

2,3-Di-*O*-palmitoyl-*sn*-glycero-1-dichlorophosphate (D-9a) A mixture of D-**1a** (1.1 g, 2 mmol) in $CHCl_3$ (15 ml) was added dropwise at 0 °C to a mixture of $POCl_3$ (3.1 g, 20 mmol) and Et_3N (10.2 g, 100 mmol) in $CHCl_3$ (60 ml) over a period of 1.5 h, and the mixture was further stirred for 3 h at room temperature, then concentrated *in vacuo*. The resulting residue was suspended in Et_2O (50 ml) and the suspension was filtered. The filtrate was evaporated and dried *in vacuo* to give dichlorophosphate (D-**9a**) as a waxy solid in a quantitative yield. mp 32—53 °C, IR (KBr) ν cm^{-1}: 2940, 2860, 1755, 1475, 1165. ^{1}H-NMR ($CDCl_3$) δ ppm: 0.88 (t, 6H, $-CH_3$), 1.26 (s, 48H, $-(CH_2)_{12}-$), 1.62 (m, 4H, $-COCH_2CH_2-$), 2.32 (m, 4H, $-COCH_2-$), 3.66 (m, 2H, $-CH_2OPO_3$), 4.22, 4.35, (m, 2H, $-CH_2OCO-$), 5.23 (m, 1H, CH).

Reaction of D-9a with Various Choline Salts[8] A mixture of choline tosylate (**11a**) (7.3 mmol) in pyridine (70 ml) was added dropwise to a mixture of dichlorophosphate (D-**9a**) (6.9 g, 10.2 mmol) in $CHCl_3$ (105 ml) at 0 °C over a period of 0.5 h with stirring and then the mixture was stirred at room temperature for 6 h. After 10% aqueous $NaHCO_3$ (35 ml) was added, the mixture was stirred at room temperature for 0.5 h and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography using $ClCH_2CH_2Cl$–MeOH–H_2O (60 : 30 : 1, v/v) as an eluent. Crystallization from $CHCl_3$–acetone (1 : 1, v/v) gave 2,3-di-*O*-palmitoyl-*sn*-glycero-1-phosphorylcholine (D-**4a**). Other choline salts (**11b**—**11e**) were treated in a similar manner to that described above. The yields of D-**4a** are shown in Table 1.

2,3-Di-*O*-palmitoyl-*sn*-glycero-1-phosphorylcholine (D-4a) by One-pot Procedure A mixture of D-**1a** (6.3 g, 41 mmol) in $CHCl_3$ (240 ml) was added dropwise at 0—5 °C to a mixture of phosphorous oxychloride (6.3 g, 41 mmol) and triethylamine (20.5 g, 203 mmol) in $CHCl_3$ (120 ml) over a period of 1 h, and the mixture was stirred for 1 h at room temperature, then cooled in an ice bath. To this mixture was added dropwise a solution of choline tosylate (16.2 g 58.5 mmol) in pyridine (570 ml) at 0—5 °C over a period of 1.5 h with stirring. After stirring for 3 h at room temperature, the mixture was treated with 10% aqueous $NaHCO_3$ (270 ml). The separated organic layer was evaporated at 50 °C *in vacuo* to give an oily residue. During the evaporation, ethanol (total 2000 ml) was added to the organic layer in several portions to curb inconvenient foaming. A suspension of the residue in $CHCl_3$ (500 ml) was filtered and the filtrate was evaporated *in vacuo* to obtain a brownish paste (40.4 g) which was chromatographed on a silica gel column using $ClCH_2CH_2Cl$–MeOH–H_2O (60 : 30 : 1, v/v) as an eluent. The eluate containing the desired product was collected and concentrated *in vacuo* to give a colorless oil. The oil was solidified from $CHCl_3$–acetone (1 : 1, v/v, 160 ml), filtered and dried to give the desired product (D-**4a**) as colorless crystalline powder. Yield, 15.7 g (53%). mp 218—220 °C, $[\alpha]_D$: −6.4° ($CHCl_3$, c = 2.0). Other diacylphosphatidylcholines were prepared in a similar manner. The yields and physicochemical data are listed in Table 2.

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