METHOD OF SYNTHESIZING SPHINGOMYELINS FROM CERAMIDES

E. N. Zvonkova, B. I. Mitsner,
A. S. Bushnev, K. I. Éller,
S. A. Soldatova, and R. P. Evstigneeva

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One of the important lipid components of biological membranes consists of the sphingomyelins. In order to determine the significance of phospholipids of this type in the functioning of a membrane a detailed study of the features of their structure is necessary – in particular, the study of such an important specific element of it as the sphingosine base which, together with a fatty-acid residue, forms a lipophilic moiety of the sphingomyelin molecule and also makes a definite contribution to the polar region of the molecule in view of the presence of a hydroxy group at the C_3 atom.

The present paper describes a general method for the synthesis of sphingomyelins (Va-f) from acylated sphingosine bases – ceramides (II a-f) – which enables the sphingomyelins to be obtained with yields of 40-50% on the starting material. In all cases, the acylating component was stearic acid, and the compounds (Va-f) differed in the nature of the sphingosine bases. The use of the ceramides (II) as starting compounds for the construction of molecules of complex sphingolipids has advantages since it gives freedom of the choice of the method of synthesizing the required sphingosine base. In addition to this, ceramides of the natural D-erythro configuration can be obtained from accessible natural sources by saponifying

TABLE 1. N-Stearoyl Derivatives of Sphingosine Bases – Ceramides (IIa-f)

Compound (II)	Sphingosine base	Empirical formula	Yield, %	mp, °C	R_f (system 1)	Literature ref.
a	rac-Sphinganine	$C_{36}H_{73}NO_3$	83,2	106–107	0,44	[10]
b*	Sphinganine	$C_{36}H_{73}NO_3$	82,6	108–109	0,44	—
c†	L-Sphinganine	$C_{36}H_{73}NO_3$	80,2	107–108	0,44	—
d	threo-rac-Sphinganine	$C_{36}H_{73}NO_3$	91,1	96–97	0,42	—
e	rac-Sphingenine	$C_{36}H_{71}NO_3$	93,5	93–94	0,47	[11]
f	rac-Eicososphingenine	$C_{38}H_{75}NO_3$	78,1	93–94	0,45	—

* $[\alpha]_D^{20}$ +5.3° (c 0.9; chloroform–methanol, 13 : 2);
† $[\alpha]_D^{20}$ –4.9° (c 0.9; chloroform–methanol, 13 : 2).

TABLE 2. 3-Benzoylceramides (IIIa-f)

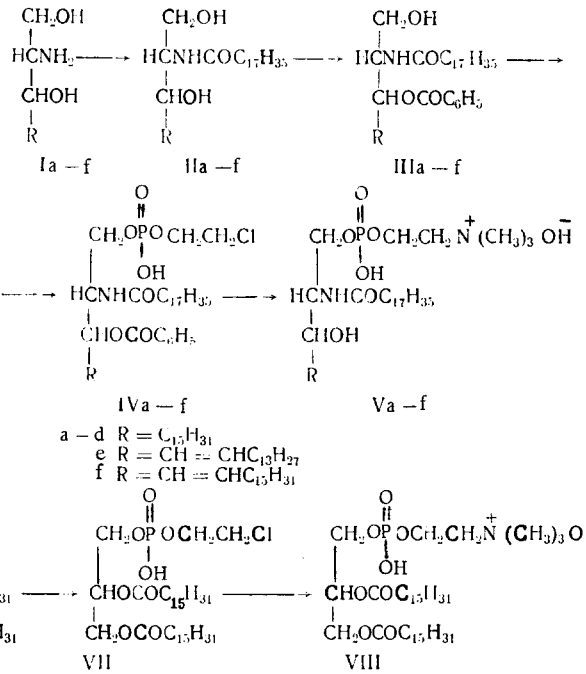
Compound (III)	Sphingosine base	Empirical formula	Yield, %	mp, °C	R_f (system 2)	Literature ref.
a	rac-Sphinganine	$C_{43}H_{77}NO_4$	73,3	76–77	0,62	[7]
b*	Sphinganine	$C_{43}H_{77}NO_4$	67,1	79–80	0,62	
c†	L-Sphinganine	$C_{43}H_{77}NO_4$	66,7	79–80	0,62	
d	threo-rac-Sphinganine	$C_{43}H_{77}NO_4$	59,6	61–62	0,46	—
e	rac-Sphingenine	$C_{43}H_{75}NO_4$	72,3	82–83	0,59	[12]
f	rac-Eicososphingenine	$C_{45}H_{79}NO_4$	71,2	74–75	0,54	—

* $[\alpha]_D^{20}$ +29.3° (c 1; chloroform);
† $[\alpha]_D^{20}$ –28.4° (c 1; chloroform).

M. V. Lomonosov Moscow Institute of Fine Chemical Technology. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 553-558, September-October, 1974. Original article submitted May 31, 1973.

sphingolipid fractions [1]. Thus, for the present work we used the N-stearoyl derivatives of Table 1: rac-sphinganine (Ia), sphinganine (Ib), and its optical antipode (Ic), the synthesis of these compounds being performed by a stereospecific method [2], rac-sphingenine (Ie) and rac-eicososphingenine (If), obtained from hexadecenoyl- and octadecenoylacetoacetic esters [3] and [4], respectively, and threo-rac-sphinganine (Id) synthesized from the hydrochloride of ethyl threo-2-amino-3-hydroxyoctadecanoate [5].

The proposed method is based on a three-stage scheme for synthesizing the sphingomyelins (V), consisting in the conversion of the initial ceramides (II) into their 3-benzoyl derivatives (III) [6], the phosphorylation of the latter with β-chloroethyl phosphorodichloridate, and the quaternization of the phosphoric acid diesters (IV) obtained under the action of trimethylamine [7]



The 3-benzoylceramides (IIIa-f) were obtained from the corresponding ceramides (IIa-f) by their successive tritylation, benzoylation, and detritylation, which were performed without isolation in a single stage according to the method that we have developed for sphinganine derivatives [6]. We established that this method permits the synthesis with high yields of 3-benzoylceramides belonging to the unnatural threo series (IIId) and including unsaturated sphingosine bases (IIIe, f) (Table 2). At the same time, for the latter compounds this variant of the synthesis is apparently the optimum one because of the low stability of the intermediate trityl derivatives.

The phosphorylation of the 3-benzoylceramides (IIIa-f) was performed with β-chloroethyl phosphorodichloridate in chloroform in the presence of pyridine. We have previously investigated the conditions for the phosphorylation of these compounds with various chlorides of substituted phosphoric acids [8]. The optimum conditions found for β-chloroethyl phosphorodichloridate (fivefold excess of phosphorylating agent at −10°C for 45 min) permit the corresponding diesters (IVa-f) (Table 3) to be synthesized with yields of 80-85%, which, in its turn, makes unnecessary operations for the purification of the phosphates (IVa-f) chromatographically or by crystallization of their barium salts.

The preparations of the sphingomyelins (Va-f) from the 3-benzoyl-1-[(β-chloroethoxy)hydroxyphosphinyl]ceramides (IVa-f) under the action of trimethylamine took place without resinification on heating in benzene in a sealed tube at 60°C for 50 h. After the benzoyl group had been removed with sodium methoxide, the corresponding sphingomyelins (Va-f) were isolated in the form of hydrates after treatment with a mixture of cation- and anion-exchange resins. The sphingomyelins obtained (Table 4) have similar chromatographic mobilities, close to that of a natural sample, and their spectral characteristics and melting points are identical with those described in the literature [9].

In order to compare the behavior of the 3-benzoylceramides (III) and diglycerides in the phosphorylation reaction, we studied the reaction of dipalmitoylglycerol (VI) with β-chloroethyl phosphorodichloridate under the conditions used for the phosphorylation of the 3-benzoylceramides (III). In this case, as well,

TABLE 3. 3-Benzoyl-1-[(β-chloroethoxy)hydroxyphosphinyl]-ceramides (IVa-f)

Compound (IV)	Sphingosine base	Empirical formula	Yield, %	mp, °C	R_f (system 3)	Literature ref.
a	rac-Sphinganine	$C_{45}H_{81}ClNO_7P$	86,2	97—98	0,35	[8]
b*	Sphinganine	$C_{45}H_{81}ClNO_7P$	82,2	101—102	0,35	—
c†	L-Sphinganine	$C_{45}H_{81}ClNO_7P$	81,4	100—101	0,35	—
d	threo-rac-Sphinganine	$C_{45}H_{81}ClNO_7P$	77,8	65—66	0,33	—
e	rac-Sphingenine	$C_{45}H_{79}ClNO_7P$	85,0	95—97	0,36	[9]
f	rac-Eicososphingenine	$C_{47}H_{83}ClNO_7P$	81,2	98—99	0,37	—

* $[\alpha]_D^{20}$ −5.0° (c 0.36; chloroform),
† $[\alpha]_D^{20}$ +4.4° (c 0.36; chloroform).

TABLE 4. Sphingomyelins (Va-f)

Compound (V)	Sphingosine base	Empirical formula	Yield, %	mp, °C	R_f (system 4)	Literature ref.
a	rac-Sphinganine	$C_{41}H_{87}N_2O_7P$	66,4	215—217	0,24	13
b*	Sphinganine	$C_{41}H_{87}N_2O_7P$	67,0	220—222	0,24	[9]
c†	L-Sphinganine	$C_{41}H_{87}N_2O_7P$	69,0	219—221	0,24	—
d	threo-rac-Sphinganine	$C_{41}H_{87}N_2O_7P$	60,3	226—227	0,24	—
e	rac-Sphingenine	$C_{41}H_{85}N_2O_7P$	64,8	212—213	0,24	[9]
f	rac-Eicososphingenine	$C_{43}H_{89}N_2O_7P$	65,2	220—222	0,24	—

* $[\alpha]_D^{20}$ +20.5° (c 0.5; chloroform−methanol, 1:1),
† $[\alpha]_D^{20}$ +20.8° (c 0.5; chloroform−methanol, 1:1).

the diester (VII) was isolated in practically quantitative yield. In the quaternization of the 1-[(β-chloroethoxy)hydroxyphosphinyl]dipalmitoylglycerol (VII), in addition to the phosphatidylcholine (VIII), considerable amounts (about 30%) of lysophosphatidylcholine were formed, which makes a stage of chromatographic purification necessary in the synthesis of phosphatidylcholines by this method.

EXPERIMENTAL

The IR spectra were taken on a Perkin-Elmer model 257 instrument. The purity of the substances isolated was checked by thin-layer chromatography on L 5/40μ silica gel in the following solvent systems: 1) chloroform−methanol−acetone (10:1:1); 2) chloroform−methanol−acetone (40:1:1); 3) chloroform−methanol−acetone (4:1:1); and 4) chloroform−methanol−acetone−water (8:6:6:1). The results of elementary analysis of all the compounds corresponded to the calculated figures.

The Ceramides (IIa-f). A solution of 0.011 mole of stearoyl chloride in 27 ml of tetrahydrofuran and 100 ml of a saturated solution of sodium acetate were added simultaneously to a solution of 0.01 mole of a sphingosine base (Ia-f) in 120 ml of tetrahydrofuran and 30 ml of 1 N acetic acid. The mixture was stirred at 20°C for 2 h, and then 300 ml of water was added, and the precipitate was separated off, washed on the filter with water, dried, and crystallized from 300 ml of ethanol (see Table 1).

3-Benzoylceramides (IIIa-f). A mixture of 5.2 mmole of a ceramide (II) and 3 g of chlorotriphenylmethane in 90 ml of dry pyridine was stirred at 20°C for 20 h and at 35°C for 3 h, the moment of completion of the reaction being determined by thin-layer chromatography from the absence of the ceramide (II) in the reaction mixture. Then 1.2 g of freshly distilled benzoyl chloride was added. After 3 h, the reaction mixture was poured into 150 ml of ice water, the substance was extracted with chloroform (3 × 100 ml), the combined organic extract was washed with 150 ml of 2% hydrochloric acid solution and with water, evaporated to small volume, and 75 ml of chloroform previously saturated with hydrogen chloride was added. After 20 min, the solution was washed with water to pH 7, dried with sodium sulfate, and evaporated in vacuum; the residue was deposited on a column containing 120 g of silicic acid. The substance was eluted with chloroform, the fractions containing it were combined, the solvent was driven off in vacuum, and the residue was crystallized from hexane (see Table 2).

3-Benzoyl-1-[(β-chloroethoxy)hydroxyphosphinyl]ceramides (IVa-f). With stirring and cooling to −10°C, a solution of 3 mmole of a 3-benzoylceramide (III) in 60 ml of chloroform was added dropwise over 45 min to a solution of 1.9 g of β-chloroethyl phosphorodichloridate [14] in 25 ml of dry chloroform and 5 ml of dry pyridine. Stirring at the same temperature was continued for 15 min, and then a mixture of 12

ml of water and 12 ml of pyridine was added and the resulting mixture was left at 20°C for 3 h. After this, 120 ml of water and 120 ml of chloroform were added, the organic layer was separated off, and the aqueous layer was extracted with chloroform (3× 100 ml). The chloroform extract was washed with 1 N hydrochloric acid to pH 2 and with water to pH 5, the solvent was driven off, and the residue was crystallized from a mixture of chloroform and acetone (1:7) (see Table 3).

The Sphingomyelins (Va-f). A mixture of 2 mmole of a 3-benzoylceramide phosphate (IV), 12 ml of trimethylamine, and 12 ml of benzene was heated at 60°C in a sealed tube for 50 h. The tube was opened, the solvent and the excess of trimethylamine were driven off, the residue was dissolved in 15 ml of methanol, and 3 ml of 1 N sodium methoxide was added. After 16 h, the reaction mixture was acidified with 1 N hydrochloric acid, 60 ml of chloroform was added, the chloroform layer was separated off, and the aqueous methanolic layer was extracted with chloroform (3× 30 ml). The organic extract was washed with 20 ml of 1 N hydrochloric acid and with a mixture of methanol and water (1:1) to pH 5. The solvent was driven off in vacuum, the residue was dissolved in 100 ml of a mixture of chloroform, methanol, and water (7:7:1), and the resulting solution was passed through a column containing a mixture of 15 g each of the resins Amberlite IRC-50 (H^+ form) and Amberlite IRC-45 (OH^- form). The column was washed additionally with 100 ml of the same mixture, the solvent was driven off from the eluate in vacuum, and the residue was crystallized from a mixture of methanol and acetone (1:4) (see Table 4).

1-[(β-Chloroethoxy)hydroxyphosphinyl]-2,3-dipalmitoylglycerol (VII). With stirring at −10°C, a solution of 2 g of dipalmitoylglycerol (VI) in 76 ml of chloroform was added over 45 min to a solution of 2.9 ml of β-chloroethyl phosphorodichloridate in 6.3 ml of pyridine and 32 ml of chloroform. Mixing was continued at the same temperature for 15 min and then a mixture of 26 ml of pyridine and 26 ml of water was added and the resulting mixture was left at 20°C for 3 h. After the addition of 200 ml of water and 200 ml of chloroform, the organic layer was separated off, the aqueous layer was extracted with chloroform (3× 40 ml), and the organic layer was washed with 5% sulfuric acid to pH 2 and with water to pH 5. The solvent was driven off in vacuum and the residue was crystallized from a mixture of chloroform and acetone (1:7). Yield 2.03 g (80.5%), $C_{37}H_{72}ClO_8P$, mp 73-74°C, R_f 0.40 (system 3).

IR spectrum, cm^{-1}: 2700-2600 (P−OH), 1740 (C=O), 1170 (P=O), 1090, 1030 (P−O−C).

2,3-Dipalmitoyl-1-[(β-trimethylammonioethoxy)hydroxyphosphinyl] glycerol Hydroxide; A Phosphatidylcholine (VIII). A mixture of 1 g of the phosphate (VII) in 6 ml of trimethylamine and 6 ml of benzene was heated in a sealed tube at 60°C for 50 h. The tube was opened, the solvent and the excess of trimethylamine were driven off, the residue was deposited on a column containing 30 g of silicic acid previously washed free from cations [8], and the substance was eluted with chloroform−methanol (1:1). The fractions containing the substance were combined and evaporated, the residue was dissolved in 100 ml of a mixture of chloroform, methanol, and water (7:7:1), and the resulting solution was passed through a column containing a mixture of 10 g each of the resins Amberlite IRC-50 (H^+ form) and Amberlite IR-45 (OH^- form). The column was washed additionally with 100 ml of the same mixture, the solvent was driven off from the eluate at 10 mm, and the residue was crystallized from chloroform−acetone (1:20). Yield 0.54 g (53.2%), mp 232-235°C [15], R_f 0.34 (system 4).

SUMMARY

1. A general method is proposed for the synthesis of sphingomyelins from N-acylated sphingosine bases – ceramides – and compounds (IVa-f) differing by the nature of the sphingosine bases, have been obtained as examples.

2. Dipalmitoyl phosphatidylcholine has been synthesized by a similar method.

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