Pergamon

Tetrahedron Vol. 51, No. 4, pp. 1177-1186, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4020/95 $9.50+0.00

0040-4020(94)01013-7

Synthesis of 2-Modified 1,3-Diacylglycerols

Regine Haftendorn and Renate Ulbrich-Hofmann*

Department of Biochemistry/Biotechnology, Martin-Luther
University, D-06099 Halle, Germany

Abstract: 1,3-Diacylglycerols modified in 2-position were synthesized by combining enzymatic and chemical methods. The syntheses started from the regioisomerically pure 1,3-dilauroylglycerol or 1,3-dimyristoylglycerol obtained from glycerol and vinyl esters of carboxylic acids by means of lipase from *Mucor mihei*. The 2-hydroxyl group in the glycerol derivatives was subsequently substituted by phosphate as well as its disodium salt, by sulfate, phosphocholine, the benzyloxy, the succinimidyl or the amino group.

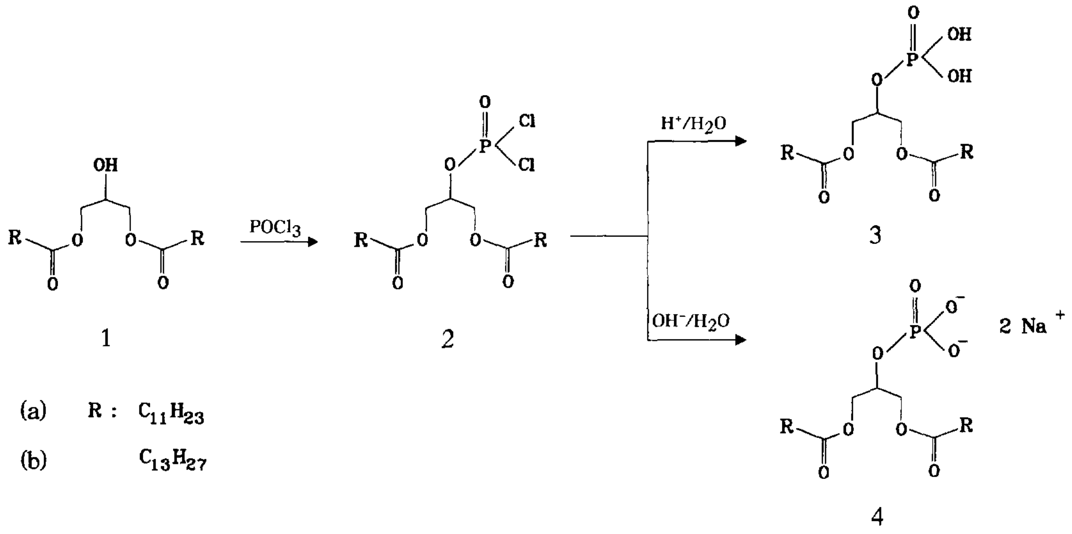
INTRODUCTION

1,3-Diacylglycerols modified in the 2-position are expected to possess interesting properties from different points of view. They are analogues of naturally occurring glycerol derivatives such as glycerolipids and glycerophospholipids and might have various pharmacological effects.[1,2] Bearing polar or ionic groups in the 2-position, the compounds become amphiphilic and are presumably able to form self-organized structures such as reverse micelles or liquid crystals.[2] They should be biodegradable and are therefore non-polluting alternatives for some of the conventional detergents. With the aim to study such analogues of natural phospholipids, we have synthesized different regioisomerically pure 1,3-dilauroylglycerols and 1,3-dimyristoylglycerols modified in 2-position.

RESULTS AND DISCUSSION

The synthetic routes were based on the elegant enzymatic method for the production of 1,3-diacylglycerols[3]. 1,3-Dilauroylglycerol (1a) and 1,3-dimyristoylglycerol (1b) were synthesized from glycerol adsorbed on silica gel and vinyl laurate or vinyl myristate by means of 1,3-selective lipase (*Mucor mihei*). Starting from these regioisomerically pure compounds, various substituents of different polarity were introduced into the 2-position of the glycerol derivatives.

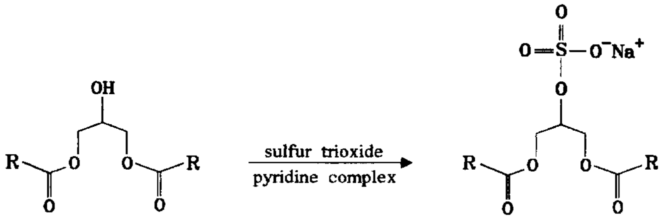
Scheme 1 shows the synthetic route for 1,3-dilauroylglycerol 2-phosphate (3a) and 1,3-dimyristoylglycerol 2-phosphate (3b) as well as their disodium salts (4a, 4b). The 1,3-diacylglycerols (1a, 1b) were treated with phosphorus oxychloride to yield the 1,3-diacylglycerol 2-dichlorophosphates (2a, 2b) that were hydrolyzed by acid or alkali.



Scheme 1

All four derivatives could be obtained in TLC-pure form by column chromatography on silica gel. They are analogues to the naturally occurring phosphatidic acids which play an essential part in the biosynthesis of glycerophospholipids and triacylglycerols. First investigations in our laboratory showed that the synthesized compounds possess a remarkable tendency to form reverse micelles.

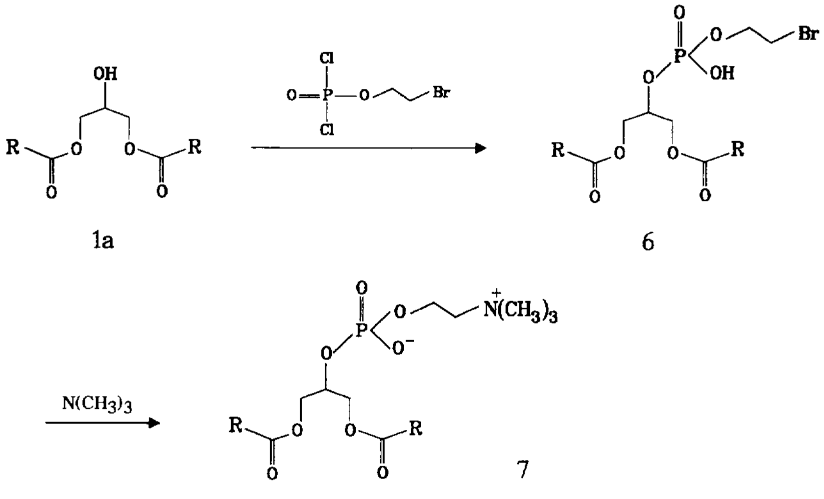
Comparable properties are expected if the sulfuric acid residue is introduced into the 2-position of 1,3-diacylglycerols. In order to study the significance of the polar head group for the formation of reverse micelles, 1,3-dilauroylglycerol 2-sulfate (5) was synthesized by solving (1a) in dry tetrahydrofuran and adding an excess of the sulfur trioxide pyridine complex according to the procedure of Sandler and Karo[4] (scheme 2).



1a 5

Scheme 2

Other interesting compounds for various purposes are 1,3-diacylglycero-2-phosphocholines, which are analogues of natural neutral 1,2-diacylglycerophospholipids such as phosphatidylcholine. For the introduction of the phosphocholine group into 1,2-diacylglycerols, a great number of methods were described,[5-7] though two procedures are preferred. The method of Hirt and Berchtold[8] improved by Eibl and Niksch[9] uses 2-bromoethyl dichlorophosphate and substitutes bromine by trimethylamine. In the second method,[10] 1,2-diacylglycerols are treated with 2-chloro-2-oxo-1,3,2-dioxaphospholanes to give cyclic phosphates, which are converted to the corresponding phospholipids with trimethylamine. We succeeded in using a modified procedure of the first method according to Hansen et al.,[11] which allowed to perform the reaction in anhydrous diethyl ether instead of chloroform, thereby reducing reaction time and avoiding side-reactions (scheme 3).

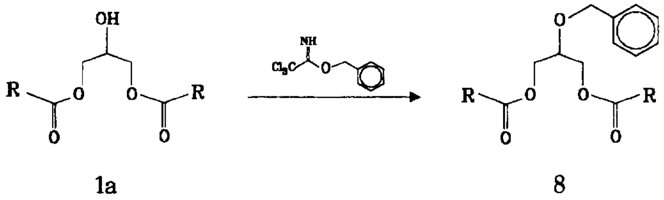


1a 6 7

Scheme 3

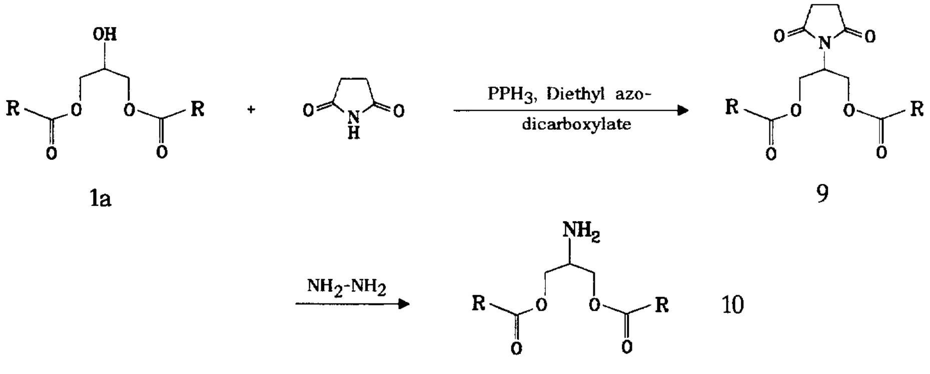
1,3-Dilauroylglycerol (1a) was phosphorylated by means of 2-bromoethyl dichlorophosphate in boiling diethyl ether. Then the resulting intermediate (6) was converted to the 1,3-dilauroylglycero-2-phosphocholine (7) by use of trimethylamine. First observations revealed that it possesses inhibitory effects on phospholipase D, whose natural function is the cleavage of 1,2-diacylglycerophospholipids to phosphatidic acid and the polar head group, e. g. choline.

In Scheme 4 the synthesis of a relatively unpolar derivative of 1,3-diacylglycerol is shown. The preparation of 2-benzyl 1,3-dilauroylglycerol (8) succeeded by acid-catalyzed ether synthesis with benzyl trichloroacetimidate in the presence of boron trifluoride etherate.[12]



Scheme 4

Finally, an amino derivative was synthesized from 1,3-dilauroylglycerol (1a) (Scheme 5). The synthesis of 2-amino-1,3-dilauroylpropan-1,3-diol (10) was carried out in a two-step reaction under neutral conditions. 2-Succinimidyl-1,3-dilauroylpropan-1,3-diol (9) was prepared by the triphenylphosphine-catalyzed reaction of (1a) with succinimide according to Mitsunobu.[13] The isolated product was converted by hydrazinolysis[14] to the 2-amino-1,3-dilauroylpropan-1,3-diol (10).



Scheme 5

All these new compounds could be obtained in pure form and satisfactory yields. The results demonstrate that the combination of enzymatic and chemical synthesis offers the opportunity to create interesting compounds.

EXPERIMENTAL

1,3-Dilauroylglycerol and 1,3-dimyristoylglycerol (**1a**, **1b**) were synthesized by the method of Berger et al.[3] The lipase (*mucor mihei*) used for these syntheses as well as benzyl trichloroacetimidate were gifts from Prof. Schneider, Bergische Universität-GH-Wuppertal. All solvents were dried and distilled before use. Phosphorus oxychloride was distilled before use with the fraction of b.p. 106 - 107°C being collected. 2-Bromoethyl dichlorophosphate was prepared according to Hirt and Berchtold.[8] All other materials were purchased from Fluka, Buchs (Switzerland) and used without further purification. Column chromatography was performed on silica gel 60 (230 - 400 mesh), Merck, Darmstadt (Germany). Thin-layer chromatography was performed on aluminium sheets silica 60 F_{254} (Merck, Darmstadt, Germany) with visualizing the spots by bromothymol blue.[15] Melting points were measured with a Boetius melting point apparatus and are uncorrected. NMR spectra were recorded on the Bruker spectrometers ARX 500, WM 250, WB 200, or AC 80.

1,3-Diacylglycerol 2-phosphate (3a, 3b)

Fresh distilled phosphorus oxychloride (0.92 g, 6 mmol) was dissolved in 10 ml of tetrahydrofuran and stirred at 0°C for 30 min. After the addition of pyridine (1,96 g, 25 mmol) dissolved in 25 ml of tetrahydrofuran, a solution of 1,3-diacylglycerol (a: 1,82 g, 4 mmol; b: 2,56 g, 5 mmol) in 30 ml of tetrahydrofuran was added dropwise during a period of 10 min. The reaction mixture was stirred at 0°C for 1 hour, then at room temperature for 18 hours. In both cases, thin layer chromatography showed complete conversion of diacylglycerol to 1,3-diacylglycerol 2-dichlorophosphate. Subsequently, the reaction mixture was filtered to remove the precipitated pyridinium hydrochloride. To hydrolyze the phosphatidic acid dichloride, 40 ml of ice-water was added and the reaction mixture was vigorously stirred for 1 hour. The clear solution was treated with 50 ml of a mixture of chloroform:methanol (2:1). The phases were separated, and the organic layer was extracted twice each with 30 ml of water acidified to pH 5.0 by HCl. The aqueous layers were reextracted with 50 ml of chloroform:water (2:1) each. The combined organic extracts were dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by chromatography on silica gel using chloroform:methanol:water (65:35:5) as eluent. The yield was 1.2 g (56%) for 1,3-dilauroylglycerol 2-phosphate and 2.3 g (85%) for 1,3-dimyristoylglycerol 2-phosphate.

3a: mp 123°-125°; R_f 0.39 (chloroform/methanol/water, 65:35:5, v/v/v). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 6H, CH_3, $^3J(HH)$ 7.0 Hz); 1.24 (m, 32H, $-(CH_2)_n-$); 1.58 (m, 4H, $COCH_2C\underline{H}_2$); 2.30 (t, 4H, $COCH_2$, $^3J(HH)$ 7.6 Hz); 4.19-4.26 (m, 4H, CH_2 of glycerol); 4.56-4.60 (m, 1H, CHOP)

^{13}C NMR (125.71 MHz, $CDCl_3$): δ 14.81 (CH_3); 23.45 ($CH_3\underline{C}H_2$); 25.52 ($\underline{C}H_2(CH_2)_2OCOR$); 29.90-30.52 ($-(CH_2)_n-$); 32.63 ($CH_3CH_2\underline{C}H_2$); 34.81 ($\underline{C}H_2CH_2OCOR$); 63.20 ($\underline{C}H_2OCOR$); 72.51 (CHOP); 174.23 (C=O)

^{31}P NMR (32.44 MHz, $CHCl_3$; D_2O-capillary, [H_3PO_4 (0 ppm)]): 0.47 ppm

3b: mp 131°-133° R_f 0.36 (chloroform/methanol/water, 65:35:5, v/v/v), ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 0.86 (t, 6H, CH_3, $^3J(HH)$ 6.3 Hz); 1.23 (m, 36H, $-(CH_2)_n-$); 1.55 (m, 4H, $COCH_2C\underline{H}_2$); 2.28 (t, 4H, $COCH_2$, $^3J(HH)$ 7.2 Hz); 4.23-4.53 (m, 5H, CH_2 of glycerol and CHOP)

^{13}C NMR (20.15 MHz, $CDCl_3$): δ 14.09 (CH_3); 22.68 ($CH_3\underline{C}H_2$); 24.93 ($\underline{C}H_2(CH_2)_2OCOR$); 29.15-29.65 ($-(CH_2)_n-$); 31.94 ($CH_3CH_2\underline{C}H_2$); 34.13 ($\underline{C}H_2CH_2OCOR$); 65.08 ($\underline{C}H_2OCOR$); 68.47 (CHOP); 173.89 (C=O)

^{31}P NMR (32.44 MHz, $CHCl_3$; D_2O-capillary, [H_3PO_4 (0 ppm)]): 0.52 ppm

1,3-Diacylglycerol 2-disodiumphosphate (4a, 4b)

A solution of pyridine (3.96 g, 50 mmol) and 1,3-diacylglycerol (a: 9.12 g, 20 mmol; b: 10.26 g, 20 mmol) in 120 ml of tetrahydrofuran was added dropwise to a solution of fresh distilled ice-cold phosphorus oxychloride (3.83 g, 25 mmol) in 20 ml of tetrahydrofuran. The reaction mixture was stirred at 0°C for 90 min and at room temperature for 20 hours. In both cases, thin layer chromatography showed complete conversion of the 1,3-diacylglycerol to 1,3-diacyl-glycerol 2-dichlorophosphate. Subsequently, 4 N NaOH was added dropwise until neutrality. To remove non-lipid by-products, 100 ml of ethanol:37% hydrochloric acid (10:1), 200 ml of chloroform and 100 ml of water were added to the reaction mixture. The layers were separated, and the chloroform solution was washed with 30 ml of water acidified to pH 5.0 by HCl. The organic layer was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dissolved in 100 ml of tetrahydrofuran. After the addition of 150 ml of ethanol, a solution of 40% aqueous sodium acetate was added dropwise until precipitation stopped. The solid product was separated, dried with P_4O_{10} and purified by chromatography on silica gel using chloroform:methanol:water (65:35:5) as eluent. The yield was 5.9 g (55%) for disodium 1,3-dilauroyl-glycerol 2-phosphate and 7.1 g (56%) for disodium 1,3-dimyristoylglycerol 2-phosphate.

4a: mp 145°-146°, R_f 0.37 (chloroform/methanol/water, 65:35:5, v/v/v), ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 0.86 (t, 6H, CH_3, $^3J(HH)$ 6.4 Hz); 1.24 (m, 32H, $-(CH_2)_n-$); 1.54 (m, 4H, $COCH_2C\underline{H}_2$); 2.29 (t, 4H, $COCH_2$, $^3J(HH)$ 7.3 Hz); 4.23-4.32 (m, 5H, CH_2 of glycerol and CHOP)

^{13}C NMR (20.15 MHz, $CDCl_3$): δ 14.09 (CH_3); 22.68 ($CH_3\underline{C}H_2$); 24.93 ($\underline{C}H_2(CH_2)_2OCOR$); 29.15-29.65 ($-(CH_2)_n-$); 31.94 ($CH_3CH_2\underline{C}H_2$); 34.13 ($\underline{C}H_2CH_2OCOR$); 65.08 ($\underline{C}H_2OCOR$); 68.47 (CHOP); 173.89 (C=O)

^{31}P NMR (32.44 MHz, $CHCl_3$; D_2O-capillary, [H_3PO_4 (0 ppm)]): 7.1 ppm

4b: mp 142°-144°, R_f 0.34 (chloroform/methanol/water, 65:35:5, v/v/v), ^1H NMR (200.13 MHz, $CDCl_3$): δ 0.86 (t, 6H, CH_3, $^3J(HH)$ 6.4 Hz); 1.23 (m, 36H, $-(CH_2)_n-$); 1.54 (m, 4H, $COCH_2C\underline{H}_2$); 2.28 (t, 4H, $COCH_2$, $^3J(HH)$ 7.3 Hz); 4.21-4.38 (m, 5H, CH_2 of glycerol and CHOP)

^{13}C NMR (20.15 MHz, $CDCl_3$): δ 14.09 (CH_3); 22.72 ($CH_3\underline{C}H_2$); 24.96 ($\underline{C}H_2(CH_2)_2OCOR$); 29.45-29.85 ($-(CH_2)_n-$); 31.99 ($CH_3CH_2\underline{C}H_2$); 34.21 ($\underline{C}H_2CH_2OCOR$); 65.19 ($\underline{C}H_2OCOR$); 69.03 (CHOP); 173.93 (C=O)

^{31}P NMR (32.44 MHz, $CHCl_3$; D_2O-capillary, [H_3PO_4 (0 ppm)]): 3.7 ppm

1,3-Dilauroylglycerol 2-sulfate (5)

10 mmol (1,59g) of sulfur trioxide pyridine complex was added to a solution of 5 mmol (2.28 g) 1,3-dilauroylglycerol in 50 ml of anhydrous tetrahydrofuran and the reaction mixture was stirred for 3 hours at room temperature. The mixture was filtered, and the organic layer was concentrated under reduced pressure. The residue was dissolved in 20 ml of n-butanol, and 4 N NaOH was added dropwise to adjust pH 7. Subsequently, the mixture was filtered to remove the precipitate, and the solvent was evaporated under reduced pressure. The residue was dissolved in 20 ml of dry acetone and filtered. Crystallisation from anhydrous methanol led to the sodium salt of 1,3-dilauroylglycerol 2-sulfate.

mp 151°-153°, R_f 0.6 (chloroform/methanol/water, 65:35:5, v/v/v), ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 6H, CH_3, $^3J(HH)$ 7.0 Hz); 1.25 (m, 32H, $-(CH_2)_n-$); 1.57 (m, 4H, $COCH_2C\underline{H}_2$); 2.31 (t, 4H, $COCH_2$, $^3J(HH)$ 7.7 Hz); 4.27-4.36 (m, 4H, CH_2 of glycerol); 4.70-4.74 (m, 1H, CHOS)

^{13}C NMR (125.71 MHz, $CDCl_3$): δ 14.81 (CH_3); 23.45 ($CH_3\underline{C}H_2$); 25.52 ($\underline{C}H_2(CH_2)_2OCOR$); 30.02-30.52 ($-(CH_2)_n-$); 32.63 ($CH_3CH_2\underline{C}H_2$); 34.81 ($\underline{C}H_2CH_2OCOR$); 62.62 ($\underline{C}H_2OCOR$); 73.84 (CHOP); 174.92 (C=O)

1,3-Dilauroylglycero-2-phosphocholine (7)

2-Bromoethyl dichlorophosphate (2.71 g, 11.2 mmol) was dissolved in 100 ml of diethyl ether cooling in an ice-bath. After the addition of dry pyridine (15.3 g, 0.1 mmol) in 20 ml of diethyl ether, the phosphorylation mixture was stirred at room temperature. A solution of 1,3-dilauroylglycerol (1.82 g, 4 mmol) in 25 ml of diethyl ether was added dropwise. Stirring was continued at room temperature overnight and subsequently under reflux for 8 hours. Thin layer chromatography indicated complete reaction of the 1,3-dilauroylglycerol. The dichlorophosphates were hydrolyzed by addition of 40 ml of ice-water under vigorous stirring. Then the solvent was removed by evaporation. The residue was dissolved in 50 ml of chloroform:methanol (2:1) and extracted twice with 15 ml of water acidified to pH 5.0 by HCl. The organic layer was dried with Na_2SO_4 and the solvent was evaporated under reduced pressure yielding 2′-bromoethyl 1,3-dilauroylglycero-2-phosphate which was used without further purification. It was dissolved in 50 ml of chloroform and 70 ml of acetonitril:isopropanol (1:1). 2.36 g (40 mmol) of 33% trimethylamine in ethanol were added under vigorous stirring and the reaction mixture was kept at 50°C in a firmly closed vessel overnight. Thin layer chromatography indicated the complete conversion of the bromoethyl phosphate into the phosphatidylcholine. The solvent was removed under reduced pressure. The residue was dissolved in 60 ml of chloroform and extracted with the following mixtures: twice with 60 ml of formic acid:methanol (5:7), twice with 60 ml of 0.1 M sodium acetate:methanol (5:7) and with 60 ml of 1 M NaCl: methanol (5:7). The remaining organic solution was dried with Na_2SO_4, and the solvent was removed by evaporation. The product was dried with P_4O_{10} under reduced pressure and purified by column chromatography on silica gel using a gradient elution with chloroform:methanol:water (9:1:0, 8:1:0.1, 7:2:0.2, 6.5:3.5:0.5). The yield was 0.63 g (20%).

R_f 0.24 ($CHCl_3/CH_3OH/NH_3$ 25%, 65:35:5, v/v/v), ^1H NMR (200.13 MHz, $CDCl_3$): δ 0.86 (t, 6H, CH_3, $^3J(HH)$ 6.4 Hz); 1.23 (m, 32H, $-(CH_2)_n-$); 1.56 (m, 4H, $COCH_2C\underline{H}_2$); 2.23 (t, 4H, $COC\underline{H}_2$); 3.34 (s, 9H, $N^+(CH_3)_3$); 3.76 (m, 2H, CH_2-N); 4.21-4.23 (m, 4H, CH_2 of glycerol); 4.31 (m, 2H, P-O-CH_2); 4.55 (m, 1H, CHOP)

^{13}C NMR (20.149 MHz, $CDCl_3$): δ 14.06 (CH_3); 22.67-31.91 ($-(CH_2)_n-$); 34.19 ($\underline{C}H_2OCOR$); 54.48 ($N^+(CH_3)_3$); 62.99 (CH_2 of glycerol); 66.49 (CH_2-N); 70.44 (CHOP); 173.44 (C=O)

^{31}P NMR (32.44 MHz, $CHCl_3$; D_2O-capillary, [H_3PO_4 (0 ppm)]): -1.91 ppm

2-Benzyl 1,3-dilauroylglycerol (8)

10 mmol (4.56 g) of 1,3-dilauroylglycerol was dissolved in 60 ml of absolute carbon tetrachloride, treated with 12 mmol (3.15 g) of benzyl trichloroacetimidate under nitrogen at 0°C. Then 1 ml of boron trifluoride etherate was added and the mixture was stirred at 4°C for 2 hours and at room temperature for 24 hours. The precipitate was removed and the organic layer was washed with 20 ml of a saturated sodium hydrogen carbonate solution and 20 ml of a saturated sodium chloride solution. Evaporation of the solvent under reduced pressure resulted in a yellow viscous oil, which was purified by chromatography on silica gel with petroleum ether/diethyl ether (2:1) to yield 2.67g (50%) of a colorless oil.

R_f 0.49 (petroleum ether/diethyl ether, 2:1, v/v)

^1H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 6H, CH_3); 1.24 (m, 32H, $-(CH_2)_n-$); 1.6 (m, 4H, $COCH_2C\underline{H}_2$); 2.26 (t, 4H, $COC\underline{H}_2$); 3.8 (m, 1H, CHO); 4.1-4.3 (m, 4H, OCH_2); 4.7 (s, 2H, o-CH_2Ar); 7.3 (m, 5H, aromat)

^{13}C NMR (62.896 MHz, $CDCl_3$): δ 13.98 (CH_3); 22.53-31.79 ($-(CH_2)_n-$); 34.05 ($\underline{C}H_2OCOR$); 62.86 (CH_2 of glycerol); 71.96 (OCH); 74.34 (O-CH_2-Ar); 127.67 (aromat. CH, para); 127.72 (aromat. CH, ortho); 128.29 (aromat. CH, meta); 137.70 (O-CH_2-$\underline{C}$=); 173.41 (C=O)

2-Succinimidyl-1,3-dilauroylpropan-1,3-diol (9)

5.5 mmol (0.96 g) of diethyl azodicarboxylate in 10 ml of diethyl ether was added dropwise to a solution of 5 mmol (2.28 g) of 1,3-dilauroylglycerol, 5 mmol (1.31 g) of triphenylphosphine and 5 mmol (0.5 g) succinimide in 100 ml of diethyl ether. After stirring at room temperature for 24 hours the precipitated triphenylphosphine oxide was removed and the organic layer was concentrated under reduced pressure. The product was purified by chromatography on silica gel with diethyl ether/hexane (3:2) to yield 1.4 g (52%).

m.p. 55°-56° R_f 0.25 (diethyl ether/hexane, 3:2, v/v), ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.87 (t, 6H, $C\underline{H}_3$); 1.24 (m, 32H, $-(CH_2)_n-$); 1.6 (m, 4H, $COCH_2C\underline{H}_2$); 2.26 (t, 4H, $COCH_2$); 2.68 (m, 4H, CH_2 of imid); 4.36-4.56 (5H, CH-N and OCH_2)

^{13}C NMR (62.896 MHz, $CDCl_3$): δ 13.96 (CH_3); 22.53-31.76 ($-(CH_2)_n-$); 33.85 ($\underline{C}H_2COO$); 50.48 (CH-N); 60.12 (CH_2 of glycerol); 173.11 (C=O); 176.78 (N-C=O, imid)

2-Amino-1,3-dilauroylpropan-1,3-diol (10)

5 ml of 1 M hydrazine hydrate in ethanol was added to a solution of 2 mmol (1.07 g) of (9) in 10 ml of 96% ethanol and the reaction mixture was stirred under reflux for 90 min. The organic layer was concentrated under reduced pressure. The residue was dissolved in 15 ml of water and acidified with glacial acetic acid to pH 5.0. After removal of the precipitate, the solvent was evaporated under reduced pressure. The product was crystallized from chloroform yielding 0,49 g (54%).

mp 201°C dec., R_f 0.78 (chloroform/methanol, 95:5, v/v), ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.85 (t, 6H, CH_3); 1.25 (m, 32H, $-(CH_2)_n-$); 1.6 (m, 4H, $COCH_2C\underline{H}_2$); 2.3 (t, 4H, $COCH_2$); 2.9-37 (2H of amino); 4.1 (m, 4H, OCH_2); 4.4 (m, 1H, $C\underline{H}_2-NH_2$)

^{13}C NMR (62.896 MHz, $CDCl_3$). δ 13.9 (CH_3); 22.5-31.4 ($-(CH_2)_n-$); 33.8 ($\underline{C}H_2COO$); 47.4 (CH-N); 62.3 (CH_2 of glycerol); 173.5 (C=O)

Acknowledgement: We wish to thank Prof. Dr. M. Schneider and his coworkers for the gift of lipase and benzyl trichloroacetimidate. Furthermore, we are grateful to the Department of Chemistry, Martin-Luther University Halle, for the NMR spectra. The financial support of this research by the Bundesministerium für Forschung und Technologie, Bonn, is thankfully acknowledged.

REFERENCES

1. Nuhn, P.; Kertscher, H.P.; Rüger, H.J. *Die Pharmazie* 1982, *37*, 681-701.
2. Eibl, H. *Angew. Chem.* 1984, *96*, 247-262; *Angew. Chem. Int. Ed. Engl.* 1984, *23*, 257-272.
3. Berger, M.; Laumen, K.; Schneider, M. P. *J. Amer. Oil Chem. Soc.* 1992, *69*, 955-960.
4. Sandler, R.; Karo, W.: Monoalkylsulfates. In *Organic Functional Group Preparations, Vol.3;* Academic Press: New York, 1972; pp. 114-141.
5. Paltauf, F.; Hermetter, A. *Prog. Lipid Res.* 1994, *3*, 239-328.

6. Baer, E.; Kindler, A. *Biochemistry* 1962, *1*, 518-520.

7. Eibl, H. *Proc. Natl. Acad. Sci. USA.* 1978, *75*, 4074-4077.

8. Hirt, R.; Berchtold, R. *Pharm. Acta Helv.* 1958, *33*, 349-356.

9. Eibl, H.; Niksch, A. *Chem. Phys. Lipids* 1978, *22*, 1-8.

10. Thuong, N.; Thuong, H.P.; Chabrier, P. *Bull. Soc. Chim. France* 1975, 2336-2338.

11. Hansen, W. J.; Murari, R.; Wedmid, Y.; Baumann, W. J. *Lipids* 1982, *17*, 453-459.

12. Wessel, H. P. *J. Chem. Soc. Perkin I* 1985, 2247-2250.

13. Mitsunobu, O. *Synthesis* 1981, 1-28.

14. Grassmann, W.; Schulte-Uebbing, E. *Chem. Ber.* 1953, *83*, 244-247.

15. Jatzkewitz, H.; Mehl, E. *Hoppe-Seylers Z. Physiol. Chem.* 1960, *320*, 251-253.

(*Received in Germany* 29 *August* 1994; *revised* 4 *November* 1994; *accepted* 12 *November* 1994)