Synthesis of cationic ether lipids of alkyl type with short-chain substituents at the 2-position of the glycerol backbone

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A series of cationic ether lipids with short-chain substituents at the 2-position and various cationic groups attached directly or through a spacer group to the glycerol backbone was synthesized.

Key words: cationic lipids, spacer group, platelet activating factor.

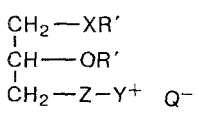
In recent years, the syntheses of various analogs of the natural lipids and modified forms of artificial structure have been extensively developed. The lipids of the alkyl type with a positively charged group (cationic lipids) belong to the latter.[1–3] The interest in this class of compounds arises from the great variety of their biological activity. Hence, they show promise for application as mediators for transfection of biologically active compounds (polynucleotides, peptides, hormones, *etc.*) in cells of various types,[1,2,4,5] and also in liposomology.[6]

Cationic lipids have been found to inhibit metastasis and growth of different tumor cells because of their effect on the activity of membrane-bound proteinkinase C and diacylglycerolkinase.[7–9]

Positively charged alkyl-type lipids have been shown to inhibit replication of the HIV-1 virus. As a result, virus mutants that do not effect human T-cell growth are formed.[10]

Among this class of lipids are the effective antagonists of lipid biocontroller, *e.g.*, the platelet activating factor (PAF). It offered possibilities for creating chemotheurapeutic drugs of a new type based on cationic lipids.[11–13]

In order to elucidate the interrelations between the structure and the type of physiological activity as well as to study the mechanism of this effect, different positively charged lipids of the following general formula have been synthesized:



A study of the artificial lipids of this type allows one to formulate the basic requirements for their structure to obtain biological activity of a definite type. In most cases, long-chain hydrocarbon residue ($R' = C_{10} \div C_{20}$) must be present at the C(1) atom, attached to the glycerol backbone by the ether, thioether, or amide bond. The short-chain alkoxyl group ($R'' = C_1 \div C_4$) at C(2) is also necessary, except when the cationic lipids are used for transfection ($R' = R'' = C_{10} \div C_{20}$). The spacer group (Z) may be absent or be the connecting group of alkyl, acyl, or amide type of the 1—8 carbon atoms length. The cationic head (Y^+) is, as a rule, the ammonium (or sulfonium) group with small (C_1—C_3) substituents of the alkyl type, or with the heteroatom involved in heterocyclic systems (pyridine, thiazoline, *etc.*). The counter ion (Q^-) may be of any accessible type (Hal^-, Ac^-, *etc.*).[7–13]

Previously, we synthesized some positively charged lipids containing long-chain alkyl substituents at C(1) and C(2) atoms of the glycerol backbone; the lipids may be used for transfection of the genetic material into cells of different type.[14]

In a continuation of studies in the area of cationic lipids and their activity, we describe in the present work the synthesis of compounds containing the short-chain alkoxyl substituents at C(2) and positively charged groups of different structure attached directly to the glycerol backbone (compounds **1a,b**; and **2**) or through the spacer group (compounds **3a,b**; and **4a,b**).

To obtain lipids **1a,b** (Scheme 1) the starting *rac*-1,2-di-*O*-alkylglycerols (**5a,b**) were transformed to tosylates (**6a,b**); the latter were subjected to pyridine quaternization on heating. The yields of cationic lipids **1a** and **1b** were 93 and 96 %, respectively, at the final stage of the synthesis.

In the synthesis of cationic lipid **2**, starting *rac*-3-bromo-2-*O*-ethyl-1-*O*-octadecyl-3-deoxyglycerol (**7**), prepared from the corresponding tosylate **6b** at reflux with LiBr, was reacted with *N,N*-dimethylaminoethanol

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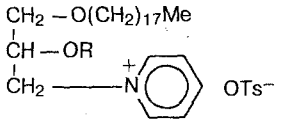
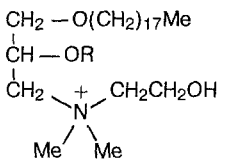
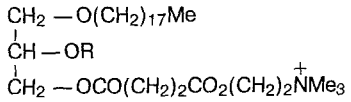


1a,b



2



3a,b; 4a,b

1a, 3a, 4a: R = Me
1b, 2, 3b, 4b: R = Et
3a,b: X^- = I^-
4a,b: X^- = TsO^-

in methylethylketone. The yield of compound **2** at the final stage of the synthesis was 30 %.

To obtain lipids **3a,b** and **4a,b** the succinic acid moiety was used as a spacer group (Scheme 1). For this purpose the starting compounds **5a,b** were acylated with succinic anhydride. The resulting succinyl derivatives (**8a,b**) were transformed to acyl chlorides (**9a,b**) without further purification. The reaction of the latter with *N,N*-dimethylaminoethanol yielded compounds (**10a,b**) in *ca.* 90 % yield (with respect to starting **5a,b**), quaternization of which with MeI or MeOTs gave the desired positively charged lipids **3a,b** and **4a,b** in a form of iodides or tosylates, respectively.

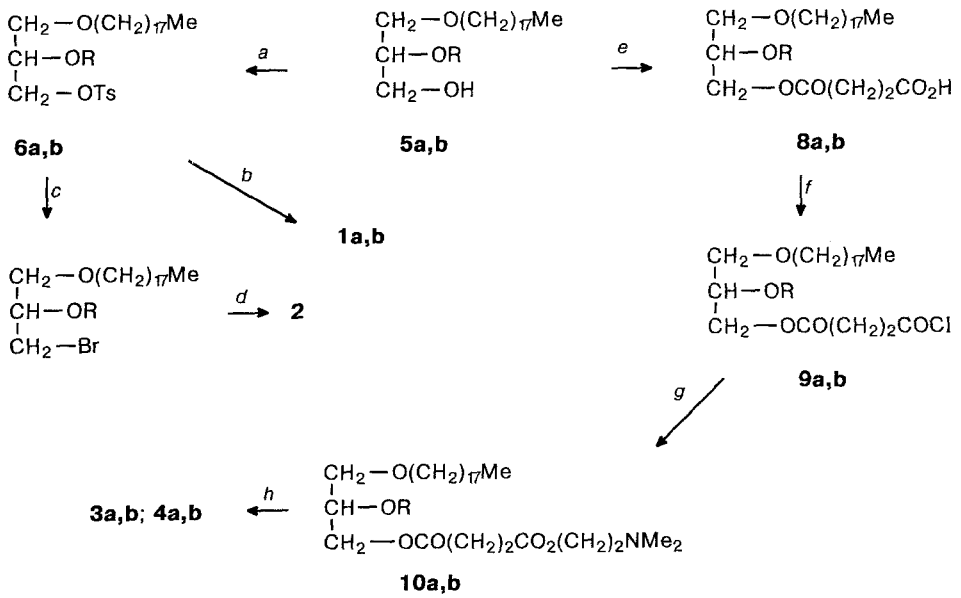
Purities and structures of the synthesized compounds were confirmed by TLC, IR, ^1H NMR, and mass spectra and elemental analysis.

Experimental

Succinic anhydride was purified by sublimation *in vacuo* (3—5 Torr). TsCl was recrystallized from hexane in the presence of $SOCl_2$. Starting compounds **5a,b** were synthesized using previously developed procedures.[8,15]

SiO_2 plates (Silufol) were used for monitoring the reaction by TLC, unless otherwise noted. TLC was carried out in the following systems: hexane—ether, 4:1 (A); chloroform—methanol—water, 65:25:4 (B); toluene—ether, 7:1 (C); hexane—ether—methanol—AcOH, 70:30:5:1 (D); hexane—ether—triethylamine—ethyl acetate, 20:40:1:(E) (volume ratios). Removal of solvents in isolation of the reaction products was performed with rotor evaporator at a residual pressure of 10—15 Torr and at 40 °C bath temperature. The compounds were dried over P_2O_5 *in vacuo* at 3—5 Torr at least for 6 h at 35—40 °C.

Scheme 1



5a, 6a, 8a—10a : R = Me
5b, 6b, 7, 8b—10b : R = Et

Reagents and conditions: *a.* TsCl, Py, 12 h, 20 °C; *b.* Py, 6 h, 110 °C; *c.* LiBr, MeC(O)Et, 5 h, 100 °C; *d.* $HO(CH_2)_2NMe_2$, MeC(O)Et, 31 h, 110 °C; *e.* succinic anhydride, $N(Et)_3$, $CHCl_3$, 24 h, 50 °C; *f.* $SOCl_2$, CCl_4, 12 h, 18 °C; *g.* Py, CCl_4, $HO(CH_2)_2NMe_2$, 12 h, 20 °C; *h.* MeI and MeC(O)Et, or MeOTs and MePh, 5 h, 40 °C.

Chromatography was performed on L 100/160 μm silica gel (Chemapol). Melting points were determined with Boetius instrument.

The IR spectra were recorded with a Shimadzu IR-435 spectrophotometer (as suspension in nujol for crystalline compounds and in thin film for low-melting compounds). The ^{1}H NMR spectra were recorded with a pulsed FT Bruker WM-500 spectrometer (500 MHz) in $CDCl_3$ for the neutral lipids and in a $CDCl_3$—CD_3OD (1:1) mixture for positively charged lipids. Mass spectra were obtained with time-of-flight mass spectrometer MSBKh (californium-252 plasma desorption). Accelerating voltage was ±5 or ±20 kV.

***rac*-2-*O*-Methyl-1-*O*-octadecyl-3-*O*-tosylglycerol (6a).** To a solution of compound **5a** (0.23 g, 0.63 mmol) in 3 mL of anhydrous Py, TsCl (0.13 g, 0.72 mmol) was added. The mixture was stirred for 12 h at 20 °C, then 15 mL of $CHCl_3$ was added to the reaction mixture. The organic phase was washed with 5% aqueous HCl (15 mL) and water to pH 7 and dried over anhydrous Na_2SO_4. Yield 0.31 g (96 %). R_f 0.56 (A). IR (film), ν/ cm^{-1}: 3030 (C_{arom}—H); 2950 (C—H); 1610, and 1475 (C—C_{arom}); 1380 (S=O); 1150—1050 (C—O—C); 718 [$(CH_2)_x$]. Mass spectrum, *m/z* (I_{rel} (%)): 513.7 $[M+H]^+$ (100).

***rac*-2-*O*-Ethyl-1-*O*-octadecyl-3-*O*-tosylglycerol (6b)** was obtained analogously to **6a** from compounds **5b** (0.19 g, 0.53 mmol) and TsCl (0.11 g, 0.61 mmol). Yield 0.27 g (97 %). R_f 0.63 (A). IR (film), ν/ cm^{-1}: 3030 (C_{arom}—H); 2950 (C—H); 1615, and 1475 (C—C_{arom}); 1380 (S=O); 1160—1050 (C—O—C); 718 [$(CH_2)_x$]. Mass spectrum, *m/z* (I_{rel} (%)): 527.9 $[M+H]^+$ (100).

***rac*-2-Methyloxy-3-octadecyloxypropylpyridinium *p*-toluenesulfonate (1a).** A solution of starting material **6a** (0.31 g, 0.60 mmol) in 6 mL of anhydrous Py was heated for 6 h at 110 °C. Py was removed *in vacuo*, and the residue was recrystallized from ether. Yield 0.33 g (93 %). M.p. 64.5—65.0 °C. R_f 0.60 (B). Mass spectrum, *m/z* (I_{rel} (%)): 420.8 $[M-TsO]^+$ (100). Found (%): C, 68.55; H, 9.52; N, 2.09. $C_{34}H_{57}O_5NS$. Calculated (%): C, 68.99; H, 9.71; N, 2.37. ^{1}H NMR, δ: 0.86 (t, 3 H, $CH_2C\underline{H}_3$, J = 7 Hz); 1.25 (br.s, 30 H, $(C\underline{H}_2)_{15}Me$); 1.54 (br.quint, 2 H, $OCH_2C\underline{H}_2$); 2.33 (s, 3 H, $C_6H_4C\underline{H}_3$); 3.30 (s, 3 H, OMe); 3.45 (t, 2 H, OCH_2, J = 7.1 Hz); 3.49 (d.d, 1 H, $C\underline{H}_2OCH_2$, J = 5.5, 11 Hz); 3.59 (d.d, 1 H, $C\underline{H}_2OCH_2$, J = 4, 11 Hz); 3.79 (m, 1 H, C<u>H</u>OMe); 4.64 (d.d, 1 H, CH_2N, J = 8, 13 Hz); 4.89 (d.d, 1 H, CH_2N, J = 3, 13 Hz); 7.18 (m, 2 H), and 7.71 (m, 2 H, $C_6\underline{H}_4Me$); 8.05, 8.54, and 8.88 (all m, 5 H, NC_5H_5).

***rac*-2-Ethyloxy-3-octadecyloxypropylpyridinium *n*-toluenesulfonate (1b)** was obtained analogously to **1a** from compound **6b** (0.27 g, 0.51 mmol). Yield 0.30 g (96 %). M.p. 59—59.5 °C. R_f 0.62 (B). Mass spectrum, *m/z* (I_{rel} (%)): 434.9 $[M-TsO]^+$ (100). Found (%): C, 69.41; H, 9.89; N, 2.31. $C_{35}H_{59}O_5NS$. Calculated (%): C, 69.38; H, 9.81; N, 2.31. ^{1}H NMR δ: 0.86 (t, 3 H, $(CH_2)_{15}C\underline{H}_3$, J = 7 Hz); 1.0 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7 Hz); 1.21 (br.s, 30 H, $(C\underline{H}_2)_{15}Me$); 1.55 (br.quint, 2 H, $OCH_2C\underline{H}_2$); 2.34 (s, 3 H, C_6H_4Me); 3.43 (t, 2 H, $OC\underline{H}_2CH_2$, J = 7.2 Hz); 3.57 (d.d, 2 H, $C\underline{H}_2OCH_2$, J = 4, 11 Hz); 3.62 and 3.63 (two q, 2 H, $OC\underline{H}_2Me$, J = 7 Hz); 3.87 (m, 1 H, C<u>H</u>OEt); 4.60 (d.d, 1 H, CH_2N, J = 8, 13.8 Hz); 4.86 (d.d, 1 H, CH_2N, J = 3.2, 13.8 Hz); 7.16 (m, 2 H), and 7.70 (m, 2 H, $C_6\underline{H}_4Me$); 8.05, 8.55, and 8.88 (all m, 5 H, NC_5H_5).

***rac*-3-Bromo-2-*O*-ethyl-1-*O*-octadecyl-3-deoxyglycerol (7).** A mixture of tosylate **6b** (0.48 g, 0.92 mmol) and LiBr (0.34 g, 3.9 mmol) in 10 mL MeC(O)Et was refluxed for 5 h, and ether (10 mL) was added after cooling. The precipitate was filtered off and washed with ether (3.5 mL). The combined filtrate was passed through an Al_2O_3 layer (II Brockmann degree of activity, neutr.). The residue was chromatographed after removal of the solvent using toluene as the eluent. Yield 0.38 g (96 %). R_f 0.8 (C). Mass spectrum, *m/z* (I_{rel} (%)): 435.3 $[M]^+$ (100).

***rac*-(2-Ethyloxy-3-octadecyloxypropyl)(2-hydroxyethyl)dimethylammonium bromide (2).** A mixture of bromide 7 (0.38 g, 0.89 mmol) and $HO(CH_2)_2NMe_2$ (2 mL, 19.8 mmol) in 10 mL of MeC(O)Et was refluxed for 31 h. The residue was dried *in vacuo* after removal of the solvent. The product was chromatographed with a $CHCl_3$—MeOH (9:1) mixture. Yield 0.12 g (30 %). M.p. 91—91.5 °C. R_f 0.73 (B). Mass spectrum, *m/z* (I_{rel} (%)): 444.5 $[M-Br]^+$ (100), 430.4 $[M-Me+H]^+$ (48). ^{1}H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}C\underline{H}_3$, J = 6.5 Hz); 1.23 (t, 3 H, $OCH_2C\underline{H}_3$, J = 7 Hz); 1.27 (br.s, 30 H, $(C\underline{H}_2)_{15}Me$); 1.55 (br.quint, 2 H, $OCH_2C\underline{H}_2$); 3.29 (s, 6 H, NMe_2); 3.45 (t, 2 H, $OC\underline{H}_2CH_2$, J = 7 Hz); 3.54 (d, 2 H, $C\underline{H}_2OC_{18}H_{37}$, J = 4); 3.56 (m, 1 H, C<u>H</u>OEt); 3.61—3.7 (m, 2 H, $C\underline{H}_2OH$); 3.76 and 3.81 (two q, 2 H, $OC\underline{H}_2Me$, J = 7 Hz); and 3.99—4.15 (m, 4 H, CH_2NCH_2).

***rac*-3-*O*-(3-Carboxypropionyl)-2-*O*-methyl-1-*O*-octadecylglycerol (8a).** A mixture of lipid **5a** (0.8 g, 2.23 mmol), succinic anhydride (0.25 g, 2.46 mmol), and NEt_3 (0.05 mL, 0.36 mmol) in 10 mL of anhydrous $CHCl_3$ was stirred at 50 °C for 24 h. After removal of solvent 1.02 g of compound was obtained, the latter was used at the next stage without further purification. R_f 0.35 (D). Mass spectrum, *m/z* (I_{rel} (%)): 459.4 $[M+H]^+$ (100), 481.8 $[M+Na]^+$(67). IR (in nujol), ν/cm^{-1}: 3350 (O—H); 2850 (C—H); 1720 (C=O in COOR); 1560 (C=O in COOH); 1465 (C—H); 1150,1100,1050 (C—O—C and C—OH); and 718 [$(CH_2)_x$].

***rac*-3-*O*-(3-Carboxypropionyl)-2-*O*-ethyl-1-*O*-octadecylglycerol (8b)** was obtained analogously to **8a** from lipid **5b** (0.89 g, 2.38 mmol), 0.26 g (2.56 mmol) of succinic anhydride, and NEt_3 (0.06 mL, 0.41 mmol). The synthesized compound (1.13 g) was used at the next stage without further purification. R_f 0.36 (D). Mass spectrum, *m/z* (I_{rel} (%)): 473.7 $[M+H]^+$ (100), 495.5 $[M+Na]^+$ (57). IR (in nujol), ν/cm^{-1}: 3310 (O—H); 2850 (C—H); 1730 (C=O in COOR); 1560 (C=O in COOH); 1460 (C—H); 1150, 1100, 1060 (C—O—C and C—OH); and 718 [$(CH_2)_x$].

***rac*-3-*O*-(β-Chloropropionyl)-2-*O*-methyl-1-*O*-octadecylglycerol (9a).** A mixture of non-purified **8a** (1.02 g) and $SOCl_2$ (3.3 mL, 0.05 mmol) in 15 mL of anhydrous CCl_4 was kept for 12 h at 18 °C. An excess of $SOCl_2$ and CCl_4 was removed *in vacuo* (10—15 Torr). Acyl chloride **9a** (1.07 g) was obtained. IR (thin film), ν/cm^{-1}: 2850 (C—H); 1780 (C=O in COCl); 1720 (C=O in COOR); 1465 (C—H); 1150 (C—O—C); and 718 [$(CH_2)_x$].

***rac*-3-*O*-(β-Chloropropionyl)-2-*O*-ethyl-1-*O*-octadecylglycerol (9b)** was obtained analogously to **9a** from non-purified **8b** (1.13 g) and $SOCl_2$ (3.4 mL, 0.05 mmol) in 15 mL of anhydrous CCl_4. Acyl chloride **9b** (1.17 g) was used at the next stage without further purification. IR (thin film), ν/cm^{-1}: 2865 (C—H); 1785 (C=O in COCl); 1730 (C=O in COOR); 1460 (C—H); 1150 (C—O—C); and 718 [$(CH_2)_x$].

***rac*-{2-[3-(2-Methyloxy-3-octadecyloxypropoxycarbonyl)-propionyloxy]ethyl}dimethylamine (10a).** To a stirred solution of $HO(CH_2)_2NMe_2$ (1.7 mL, 17 mmol) in 3.6 mL of anhydrous Py and 5 mL of anhydrous CCl_4, a solution of acyl chloride **9a** (0.53 g) in 3 mL of anhydrous CCl_4 was added dropwise at 20 °C. The reaction mixture was kept for 12 h at 20 °C, $CHCl_3$ (25 mL) was added and the mixture was washed with 1 % aqueous HCl and with water to pH 7. After drying

over Na_2SO_4 the solvent was removed *in vacuo* and the residue was chromatographed using the system E. Yield 0.54 g (91 % on **5a**). R_f 0.72 (Al_2O_3 plates, Alufol, E). Mass spectrum, *m/z* (I_{rel} (%)): 530.9 $[M+H]^+$ (100), 517.4 $[M-Me+H]^+$(58). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}CH_3$, J = 7.2 Hz); 1.24 (br.s, 30 H, $(CH_2)_{15}Me$); 1.54 (br.quint, 2 H, $CH_2OCH_2CH_2$); 2.24—2.74 (m, 4 H, $OCO(CH_2)_2COO$); 2.31 (s, 6 H, NMe_2); 2.64 (m, 2 H, CH_2N); 3.43 (s, 3 H, OMe); 3.44 (t, 2 H, CH_2OCH_2, J = 5.9 Hz); 3.47 (d.d, 1 H, CH_2OCH_2, J = 5, 10.5 Hz); 3.49 (d.d, 1 H, CH_2OCH_2, J = 4.5, 10.5 Hz); 3.52—3.59 (m, 1 H, CHOMe); 4.11 (d.d, 1 H, CH_2OCO, J = 6, 12 Hz); 4.20 (t, 2 H, CH_2CH_2N, J = 6 Hz); and 4.25 (d.d, 1 H, CH_2OCO, J = 4.8, and 12 Hz).

***rac*-{2-[3-(2-Ethyloxy-3-octadecyloxypropoxycarbonyl)propionyloxy]ethyl}dimethylamine (10b)** was synthesized analogously to **10a** from $HO(CH_2)_2NMe_2$ (1.8 mL, 18 mmol) in anhydrous Py (3.8 mL) and acyl chloride **9b** (0.59 g). Yield 0.58 g (90 % on compound **5b**). R_f 0.74 (Al_2O_3 plates, Alufol, E). Mass spectrum, *m/z* (I_{rel} (%)): 544.5 $[M+H]^+$ (100) and 530.8 $[M-Me+H]^+$ (67). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}CH_3$, J = 7 Hz); 1.18 (t, 3 H, OCH_2CH_3, J = 7.1 Hz); 1.24 (br.s, 30 H, $(CH_2)_{15}Me$); 1.54 (br.quint, 2 H, $CH_2OCH_2CH_2$); 2.18—2.75 (m, 4 H, $OCO(CH_2)_2COO$); 2.28 (s, 6 H, NMe_2); 2.60 (m, 2 H, CH_2N); 3.44 (t, 2 H, CH_2OCH_2, J = 7 Hz); 3.48 (d, 2 H, CH_2OCH_2, J = 5.3 Hz); 3.62 (q, 2 H, OCH_2Me, J = 7.1 Hz); 3.64 (m, 1 H, CHOEt); 4.10 (d.d, 1 H, CH_2OCO, J = 6, 11.9 Hz); 4.19 (t, 2 H, CH_2CH_2N, J = 6 Hz); and 4.21 (d.d, 1 H, CH_2OCO, J = 4.2, 11.9 Hz).

***rac*-{2-[3-(2-Methyloxy-3-octadecyloxypropoxycarbonyl)-propionyloxy]ethyl}trimethylammonium iodide (3a).** A mixture of amine **10a** (0.36 g, 0.67 mmol) and MeI (0.06 mL, 1.01 mmol) in 10 mL of acetone was kept for 5 h at 40 °C. After removal of solvent, the residue was recrystallized from ether at 4 °C. Yield 0.42 g (93 %). R_f 0.67 (B). M.p. 80—81 °C. Found (%): C, 53.96; H, 8.82; N, 2.24. $C_{31}H_{62}O_6NI$. Calculated (%): C, 54.26; H, 9.01; N, 2.21. Mass spectrum, *m/z* (I_{rel} (%)): 544.7 $[M]^+$ (100). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}CH_3$, J = 6.9 Hz); 1.24 (br.s, 30 H, $(CH_2)_{15}Me$); 1.55 (br.quint, 2 H, $CH_2OCH_2CH_2$); 2.69 (m, 4 H, $OCO(CH_2)_2COO$); 3.25 (s, 9 H, NMe_3); 3.44 (s, 3 H, OMe); 3.45 (t, 2 H, CH_2OCH_2, J = 7 Hz); 3.50 (d, 2 H, CH_2OCH_2, J = 5.5 Hz); 3.57 (m, 1 H, CHOMe); 3.77 (m, 2 H, CH_2N); 4.11 (d.d, 1 H, CH_2OCO, J = 6, 11.5 Hz); 4.24 (d.d, 1 H, CH_2OCO, J = 3.8, 11.5 Hz); and 4.56 (m, 2 H, CH_2CH_2N).

***rac*-{2-[3-(2-Ethyloxy-3-octadecyloxypropoxycarbonyl)propionyloxy)]ethyl}trimethylammonium iodide (3b)** was synthesized analogously to **3a** from compound **10b** (0.39 g, 0.72 mmol) and MeI (0.07 mL, 1.08 mmol). Yield 0.45 g (91 %). R_f 0.68 (B). M.p. 74.5—75 °C. Found (%): C, 56.33; H, 9.44; N, 2.05. $C_{32}H_{64}O_6NI$. Calculated (%): C, 56.05; H, 9.41; N, 2.04. Mass spectrum, *m/z* (I_{rel} (%)): 558.7 $[M]^+$(100). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}CH_3$, J = 7 Hz); 1.18 (t, 3 H, OCH_2CH_3, J = 7 Hz); 1.25 (br.s, 30 H, $(CH_2)_{15}Me$); 1.54 (br.quint, 2 H, $CH_2OCH_2CH_2$); 2.69 (m, 4 H, $OCO(CH_2)_2COO$); 3.26 (s, 9 H, NMe_3); 3.44 (t, 2 H, CH_2OCH_2, J = 7 Hz); 3.48 (d, 2 H, CH_2OCH_2, J = 5.7 Hz); 3.61 and 3.62 (two quart, 2 H, OCH_2Me, J = 7 Hz); 3.64 (m, 1 H, CHOEt); 3.76—3.89 (m, 2 H, CH_2N); 4.10 (d.d, 1 H, CH_2OCO, J = 6, 11.8 Hz); 4.22 (d.d, 1 H, CH_2OCO, J = 4.3, 11.8 Hz); and 4.47—4.73 (m, 2 H, CH_2CH_2N).

***rac*-{2-[3-(2-Methyloxy-3-octadecyloxypropoxycarbonyl)-propionyloxy]ethyl}trimethylammonium *p*-toluenesulfonate (4a).** A mixture of amine **10a** (0.21 g, 0.40 mmol) and MeOTs (0.09 g, 0.49 mmol) in 10 mL of anhydrous toluene was kept for 5 h at 40 °C. After removal of the solvent the residue was recrystallized from ether at 4 °C. Yield 0.22 g (76 %). R_f 0.66 (B). M.p. 71—71.5 °C. Found (%): C, 63.44; H, 9.43; N, 1.96. $C_{38}H_{69}O_9NS$. Calculated (%): C, 63.74; H, 9.71; N, 1.96. Mass spectrum, *m/z* (I_{rel} (%)): 544.8 $[M]^+$ (100). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}CH_3$, J = 7 Hz); 1.24 (br.s, 30 H, $(CH_2)_{15}Me$); 1.56 (br.quint, 2 H, $CH_2OCH_2CH_2$); 2.35 (s, 3 H, $CH_3C_6H_4$); 2.64 (m, 4 H, $OCO(CH_2)_2COO$); 3.19 (s, 9 H, NMe_3); 3.44 (s, 3 H, OMe); 3.45 (t, 2 H, CH_2OCH_2, J = 7 Hz); 3.49 (d, 2 H, CH_2OCH_2, J = 5.4 Hz); 3.57 (m, 1 H, CHOMe); 3.69 (m, 2 H, CH_2N); 4.12 (d.d, 1 H, CH_2OCO, J = 6, 12 Hz); 4.26 (d.d, 1 H, CH_2OCO, J = 3.6 and 12 Hz); 4.54 (m, 2 H, CH_2CH_2N); 7.17 and 7.72 (two m, 2 H, C_6H_4Me).

***rac*-{2-[3-(2-Ethyloxy-3-octadecyloxypropoxycarbonyl)propionyloxy]ethyl}trimethylammonium *p*-toluenesulfonate (4b)** was synthesized analogously to **4a** from compound **10b** (0.19 g, 0.35 mmol) and MeOTs (0.08 g, 0.43 mmol). Yield 0.20 g (78 %). R_f 0.69 (B). M.p. 65.5—66 °C. Found (%): C, 64.20; H, 9.69; N, 2.13. $C_{39}H_{71}O_6NS$. Calculated (%): C, 64.16; H, 9.80; N, 1.92. Mass spectrum, *m/z* (I_{rel} (%)): 558.7 $[M]^+$ (100). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{15}CH_3$, J = 7.2 Hz); 1.18 (t, 3 H, OCH_2CH_3, J = 6.8 Hz); 1.25 (br.s, 30 H, $(CH_2)_{15}Me$); 1.55 (br.quint, 2 H, $CH_2OCH_2CH_2$); 2.35 (s, 3 H, $CH_3C_6H_4$); 2.66 (m, 4 H, $OCO(CH_2)_2COO$); 3.20 (s, 9 H, NMe_3); 3.44 (t, 2 H, CH_2OCH_2, J = 6.9 Hz); 3.47 (d, 2 H, CH_2OCH_2, J = 5 Hz); 3.62 and 3.64 (two q, 2 H, OCH_2Me, J = 7.0 Hz); 3.66 (m, 1 H, CHOEt); 3.67—3.71 (m, 2 H, CH_2N); 4.11 (d.d, 1 H, CH_2OCO, J = 5.8 and 11.7 Hz); 4.22 (d.d, 1 H, CH_2OCO, J = 4.6, 11.7 Hz); 4.44 (m, 2 H, CH_2CH_2N); 7.18 and 7.71 (two m, 2 H, C_6H_4Me).

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