Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

A convenient method for the synthesis of cationic glycerolipids with potential biological activity

M. A. Maslov, N. G. Morozova, and G. A. Serebrennikova*

M. V. Lomonosov Moscow State Academy of Fine Chemical Technology, 86 prosp. Vernadskogo, 117571 Moscow, Russian Federation. Fax: +7 (095) 434 8711. E.-mail: maslov@httos.mitht.msk.ru

Cationic ether lipids containing nitrogenous heterocyclic bases as the positively charged groups were synthesized. Treatment of methylthiomethyl ethers of dialkylglycerols with bromine afforded highly reactive α-bromo ethers. These products reacted with various nucleophilic reagents giving rise to a series of cationic alkylglycerols.

Key words: methylthiomethyl ethers, heterocyclic amines, cationic lipids, gene therapy

The success of the gene therapy largely depends on the development of a transporting agent that could accomplish efficient and specific delivery of a therapeutic gene (normally, a recombinant plasmid DNA encoding the protein with a potential therapeutic effect) to the target cells and would have a minor toxic action on the organism.[1] At present, the most effective systems for the delivery of genetic material are viral vectors. However, they suffer from a number of serious drawbacks, and this stimulates the development of alternative approaches. One of them is lipofection, *i.e.*, the delivery of DNA by cationic liposomes.[2—4] The complexes formed by cationic liposomes and therapeutic genes are already used in clinical trials for treatment of a number of diseases.[5—8]

Cationic liposomes have several advantages over the viral vectors, in particular, they are noninfectious, able to transfer DNA of any size and to protect it from the action of cell enzymes, stable during storage, and inexpensive.[2—4] However, the toxicity of cationic lipids, the cell nonspecificity of liposomes, and the relatively low degree of DNA penetration into the cell nucleus call for further development of the lipofection technique.

In addition, some cationic lipids were found to be effective antagonists of a potent lipid bioregulator, the platelet-activating factor (PAF), or to exhibit antitumor, antiviral (HIV-1), and antibacterial activities.[9,10]

Thus, the preparation of new positively charged lipids that may prove to be effective mediators of lipofection of eukaryotic cells or to display an extended spectrum of therapeutic action is a topical task of bioorganic chemistry.

In recent years, numerous positively charged glycerolipids usually containing an aliphatic ammonium group as

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the cationic head[11—14] and a short- or long-chain aliphatic substituent at the C(2) atom of the glycerol backbone[9,15] have been synthesized in order to study the structure—biological activity relationships for cationic lipids.

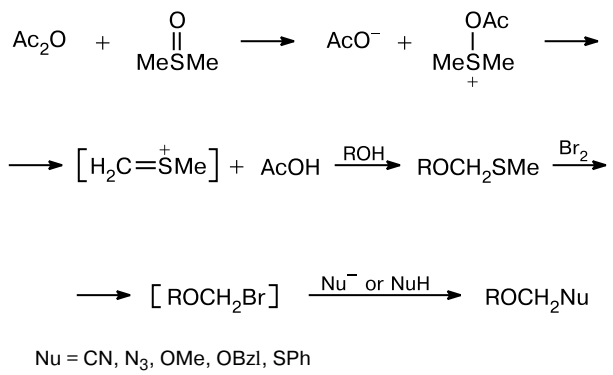
With the object of looking for new compounds with potential biological activities, we markedly extended the range of biologically active positively charged lipids.[16—19] This study deals with the development of a convenient method for the synthesis of positively charged ether glycerolipids containing nitrogenous heterocyclic bases as the cationic groups.

The introduction of a positively charged group into a lipid molecule is based most often on either alkylation of tertiary amines with 3-bromo-3-deoxy-, 3-*O*-methanesulfonyl-, and 3-*O*-toluenesulfonyl-1,2-dialkylglycerols[17,19—21] or alkylation of *O*-substituted 3-(dimethylamino)propane-1,2-diol derivatives with short-chain alkyl halides.[11,22] It should be noted that the yields of target substances in reactions of this type are usually relatively low. We developed an alternative approach to the synthesis of cationic glycerolipids based on the interaction of amines with methylthiomethyl ethers of diglycerides in the presence of bromine; this resulted in a substantially higher yields of the target cationic lipids.[16,18] Racemic 1,2-dialkylglycerols were used in the syntheses because the configuration of the chiral center appears to have no influence on the biological activity.[17]

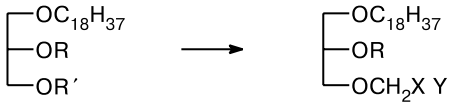
The methylthiomethyl ethers have found wide use for the protection of hydroxy[23—26] and carboxy[27] groups and for the preparation of modified nucleoside analogs.[28—30]

Being asymmetrical O,S-acetals, methylthiomethyl ethers readily react with bromine to give highly reactive α-bromo ethers; these can react with various nucleophilic reagents, which may provide a pathway to a number of modified compounds (Scheme 1). Thus using the methylthiomethyl ethers of 1,2-di-*O*-alkylglycerols (**2a—d**) with hydrocarbon groups of various lengths (Me, Et, All, *n*-$C_{18}H_{37}$) at the C(2) atom of the glycerol backbone, we prepared positively charged lipids **3** — **5** (Scheme 2).

Scheme 1

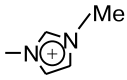
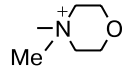


Scheme 2



1a—d: R′ = H, **2a—d:** R′ = CH_2SMe — **3a—d, 4a—c, 5a,b**

1—4: R = Me (**a**), Et (**b**), All (**c**), *n*-$C_{18}H_{37}$ (**d**); **5a,b:** R = *n*-$C_{18}H_{37}$

Compound	X	Y	Compound	X	Y
3a—d		Br^-	**5a**		—
4a—d		Br^-	**5b**		—

Reachents and conditions: (i) DMSO—Ac_2O—AcOH, benzene, 24 °C, 2—5 days; (ii) XH or X, Br_2, $ClCH_2CH_2Cl$, 24 °C, 10—30 min.

In most cases, methylthiomethyl (MTM) ethers are prepared by reactions of alcohols with a DMSO—Ac_2O mixture in the presence of AcOH (the Pummerer rearrangement)[23,24,28—30] (see Scheme 1). In this study, we tested two methods, namely, the reaction of the sodium alkoxide derived from alcohol **1d** with $MeSCH_2Cl$ in the presence of NaI[25] and $AgNO_3$[26] and the Pummerer rearrangement. The former procedure, *i.e.*, the reaction of sodium *rac*-1,2-di-*O*-octadecylglycerolate with $MeSCH_2Cl$ in the presence of NaI, required prolonged heating. Moreover, this reaction yielded large amounts of side products. The addition of $AgNO_3$[26] as an electrophilic catalyst did not change the reaction pattern, and the yield of the methylthiomethyl ether was only 30%.

According to the second approach, lipid **1d** was kept in a DMSO—Ac_2O—AcOH mixture (molar ratio 6.5 : 3.4 : 1). At the end, the reaction mixture contained only minor amounts of the starting compound and side products, the yield of the target product being 60%. Thus, this approach proved to be more effective.

rac-1,2-Dialkylglycerols **1a—d** were converted into the corresponding methylthiomethyl ethers **2a—d** on treatment with a DMSO—Ac_2O—AcOH mixture for 2—5 days (see Scheme 2). After chromatographic purification, the methylthiomethyl ethers were isolated in 50—61% yields as crystallizing oils stable for long at 4 °C. The ^1H NMR spectra of compounds **2a—d** were found to exhibit characteristic signals for the methylthiomethyl group, namely, the singlet for SMe-group protons with a chemical shift of 2.11—2.13 ppm and a singlet for OCH_2S-group protons at 4.64—4.65 ppm. The mass spectra of the resulting compounds contained peaks for the molecular ion $[M]^+$ and for the $[M - SMe]^+$ ion.

The cationic lipids **3** and **4** and tertiary amines **5** were prepared using a general experimental procedure accord-

ing to which a fivefold excess of the corresponding amine and a 1.2—1.5-fold excess of bromine were successively added with stirring to a solution of methylthiomethyl ether **2** in $ClCH_2CH_2Cl$; after removing the solvent and the amine *in vacuo*, lipids **3—5** were isolated by chromatography on silica gel. It should be noted that the use of *N*-methylmorpholine increased the reaction duration and required the use of larger amounts of Br_2. Apparently, this is related both to the structural features of the molecule and to the lower nucleophilicity of nitrogen. When using the methylthiomethyl ether of *rac*-2-*O*-allyl-1-*O*-octadecylglycerol (**2c**), we found that the nucleophilic displacement of the thiomethyl group by bromide is accompanied by competing bromination of the allyl group, which affords compounds containing the 2,3-dibromopropyl substituent at C(2). These compounds were isolated by chromatography and characterized by mass spectrometry: the spectrum of the brominated derivative of **3c** ($R = -CH_2CHBrCH_2Br$) exhibited a molecular ion with m/z 639.2, and the spectrum of dibrominated derivative of **4c** displays an ion with m/z 657.3, which is in line with the proposed structures.

The structures of cationic lipids **3—5** were confirmed by the data of mass spectrometry and ^1H NMR spectroscopy (see Experimental).

While examining the ^1H NMR spectra of compounds **3—5**, we noted differences in the signals of the protons of the OCH_2N groups. These protons are described as an AB system; in the general case, the spectrum of such a system consists of four different-intensity lines, arranged symmetrically with respect to to the midpoint. The positions and the integral intensities of the spectral lines depend on the coupling constant and the difference between the chemical shifts of the nuclei.[31] In the case of cationic lipids **4**, the intensities of the internal and terminal lines of the OCH_2N signal ($\nu_{AB} = 19.2$ Hz, $J_{AB} = 8.1$ Hz) differ insignificantly. On passing to the spectra of compounds **3**, the specific intensities of the terminal lines decrease, while those of the internal lines increase with a decrease in the difference between the chemical shifts ($\nu_{AB} = 8.3$ Hz) and an increase in the spin-spin coupling constant ($J_{AB} = 10.2$ Hz). The signal for the same group of protons in compounds **5** is also a pair of doublets; however, the intensities of the terminal signals are very low, while the intensity of the central signal (resulting from the overlap of two internal signals) is much higher.

Tertiary amines **5** can be protonated at certain pH values. Having acquired a positive charge, they can efficiently encapsulate a DNA molecule. It is quite probable that the action of these substances as pH-sensitive agents would markedly facilitate the endosomal release of the plasmid DNA and increase the efficiency of lipofection.[32]

Thus, we prepared glycerolipids **3—5**, containing alkyl substituents of various lengths at the glycerol C(2) atom and nitrogenous heterocyclic bases as positively charged groups, in high yields *via* the corresponding methylthiomethyl ethers.

Experimental

Distilled solvents and chemicals Py, AcOH, Ac_2O, and Br_2 produced in Russia were used. Commercial imidazole, benzimidazole, *N*-methylimidazole, *N*-methylmorpholine (Fluka) and DMSO (Merck) were used as received. Amines were distilled from CaH_2 and stored over calcined 4 Å molecular sieves. The solvents used for the reactions were distilled from P_2O_5. *rac*-1,2-Di-*O*-alkylglycerols (**1a—d**) were prepared using previously developed procedures.[33,34]

Thin layer chromatography was performed on Kieselgel 60 F_{254} plates (Merck) using the following solvent systems: petroleum ether—Et_2O, 8 : 1 (A); $CHCl_3$—MeOH, 50 : 1 (B) and 4 : 1 (C). The spots were visualized by a solution of $KMnO_4$ or by spraying with 50% H_2SO_4 followed by heating. Derivatives of tertiary amines and cationic lipids were identified using the Dragendorff reagent.[35] Chromatography was carried out on silica gel L 40/100 μm (Chemapol, Czechia). Melting points were determined on a Boetius hot stage (Germany). ^1H NMR spectra were recorded on a Bruker MSL-200 spectrometer (200 MHz) (Germany) in $CDCl_3$; the chemical shifts were measured in the δ scale relative to the $CHCl_3$ signal (δ, 7.24). The mass spectra of compounds **1** and **2** were recorded on a MSBKh time-of-flight mass spectrometer (Sumy, Ukraine) with ionization by the ^{252}Cf fission fragments; the ionizing voltage was ±5 or ±20 kV; the mass spectra of other compounds were recorded on a Vision 2000 laser-desorption ionization time-of-flight mass spectrometer (Thermo BioAnalysis Ltd.) (MALDI-TOF) using 2,5-dihydroxybenzoic acid as a matrix.

***rac*-2-*O*-Methyl-1-*O*-methylthiomethyl-3-*O*-octadecylglycerol (2a).** Dimethyl sulfoxide (5.8 mL) and a mixture of AcOH (1.2 mL) and Ac_2O (4.0 mL) were added successively to a solution of *rac*-2-*O*-methyl-1-*O*-octadecylglycerol (**1a**) (0.66 g, 1.84 mmol) in 2 mL of anhydrous benzene. The reaction mixture was kept for 96 h at ~25 °C, a saturated solution of Na_2CO_3 (100 mL) was added, and the mixture was stirred for 1.5 h. The product was extracted with benzene (4×30 mL). The combined extract was washed with a saturated solution of Na_2CO_3 (1×100 mL) and water (4×30 mL) and dried (Na_2SO_4). The residue obtained after removal of the solvent was chromatographed using a heptane—Et_2O (96 : 4) mixture as the eluent to give 0.47 g (61%) of compound **2a**, R_f 0.34 (A). MS, m/z (I_{rel} (%)): 418.5 $[M]^+$ (100), 371.1 $[M - SMe]^+$ (47). Calculated for $C_{24}H_{50}O_3S$: 418.35 $[M]^+$. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.9 Hz); 1.25 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.48—1.64 (m, 2 H, $OCH_2C\underline{H}_2$); 2.13 (s, 3 H, SCH_3); 3.42 (t, 2 H, $OC\underline{H}_2CH_2$, J = 6.8 Hz); 3.46 (s, 3 H, OCH_3); 3.47—3.56 (m, 3 H, $C\underline{H}OMe$, $C\underline{H}_2OC_{18}H_{37}$); 3.58 (dd, 1 H, $CH\underline{H}_aOCH_2S$, J = 4.5 Hz, J = 10.0 Hz) and 3.68 (dd, 1 H, $CH\underline{H}_bOCH_2S$, J = 3.8 Hz, J = 10.0 Hz); 4.65 (s, 2 H, OCH_2S).

***rac*-2-*O*-Ethyl-1-*O*-methylthiomethyl-3-*O*-octadecylglycerol (2b)** was prepared by the procedure described for compound **2a** using *rac*-2-*O*-ethyl-1-*O*-octadecylglycerol (**1b**) (0.93 g, 2.51 mmol), DMSO (8.3 mL), AcOH (1.6 mL) and Ac_2O (5.4 mL). The compound was purified by chromatography with a heptane—Et_2O (96 : 4) mixture as the eluent to give 0.54 g (50%) of compound **2b**, R_f 0.45 (A). MS, m/z (I_{rel} (%)):

432.1 $[M]^+$ (100), 385.4 $[M - SMe]^+$ (45). Calculated for $C_{25}H_{52}O_3S$: 432.36 $[M]^+$. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.18 (t, 3 H, $OCH_2C\underline{H}_3$, J = 6.8 Hz); 1.23 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.45—1.61 (m, 2 H, $OCH_2C\underline{H}_2$); 2.12 (s, 3 H, SCH_3); 3.41 (t, 2 H, $OC\underline{H}_2CH_2$, J = 6.6 Hz); 3.49—3.67 (m, 7 H, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OC\underline{H}_2CH_3$, $C\underline{H}_2OCH_2S$); 4.64 (s, 2 H, OCH_2S).

***rac*-2-*O*-Allyl-1-*O*-methylthiomethyl-3-*O*-octadecylglycerol (2c)** was prepared by the procedure described for compound **2a** using *rac*-2-*O*-allyl-1-*O*-octadecylglycerol (0.53 g, 1.39 mmol) (**1c**), DMSO (4.3 mL), AcOH (0.5 mL), and Ac_2O (3.0 mL). The product was purified by chromatography using a heptane—Et_2O (95 : 5) mixture as the eluent to give 0.34 g (55%) of compound **2c,** R_f 0.45 (A). MS, m/z (I_{rel} (%)): 444.5 $[M]^+$ (70), 397.5 $[M - SMe]^+$ (100). Calculated for $C_{26}H_{52}O_3S$: 444.36 $[M]^+$. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.23 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.44—1.60 (m, 2 H, $OCH_2C\underline{H}_2$); 2.12 (s, 3 H, SCH_3); 3.42 (t, 2 H, $OC\underline{H}_2CH_2$, J = 6.4 Hz); 3.49—3.68 (m, 5 H, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OAll$, $C\underline{H}_2OCH_2S$); 4.12 (dddd, 1 H, $OCH\underline{H}_aCH{=}CH_2$, J = 1.7 Hz, J = 1.7 Hz, J = 5.6 Hz, J = 7.6 Hz); 4.14 (dddd, 1 H, $OCH\underline{H}_bCH{=}CH_2$, J = 1.7 Hz, J = 1.7 Hz, J = 5.6 Hz, J = 7.6 Hz); 4.65 (s, 2 H, OCH_2S); 5.14 (dddd, 1 H, $OCH_2CH{=}CH\underline{H}_a$, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 10.2 Hz); 5.26 (dddd, 1 H, $OCH_2CH{=}CH\underline{H}_b$, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.2 Hz); 5.91 (dddd, 1 H, $OCH_2C\underline{H}{=}CH_2$, J = 5.6 Hz, J = 5.6 Hz, J = 10.2 Hz, J = 17.2 Hz).

***rac*-1-*O*-Methylthiomethyl-2,3-di-*O*-octadecylglycerol (2d)** was prepared by the procedure described for compound **2a** using *rac*-1,2-di-*O*-octadecylglycerol (**1d**) (2.14 g, 3.58 mmol), DMSO (11 mL), AcOH (1.4 mL), and Ac_2O (7.7 mL). The substance was purified by chromatography using petroleum ether as the eluent to give 1.40 g (60%) of compound **2d**, R_f 0.58 (A). MS, m/z (I_{rel} (%)): 655.7 $[M]^+$ (50), 609.9 $[M - SMe]^+$ (100). Calculated for $C_{41}H_{84}O_3S$: 656.61 $[M]^+$. ^1H NMR, δ: 0.85 (t, 6 H, 2 $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.23 (br.s, 60 H, 2 $(C\underline{H}_2)_{15}CH_3$); 1.45—1.61 (m, 4 H, 2 $OCH_2C\underline{H}_2$), 2.11 (s, 3 H, SCH_3); 3.37—3.63 (m, 9 H, 2 $OC\underline{H}_2CH_2$, $CH_2OC_{18}H_{37}$, $CHOC_{18}H_{37}$, $C\underline{H}_2OCH_2S$); 4.64 (s, 2 H, OCH_2S).

***rac*-*N*-Methyl-*N*´-[(2-methoxy-3-octadecyloxy)prop-1-yloxymethyl]imidazolium bromide (3a).** *N*-Methylimidazole (96.5 mL, 1.21 mmol) was added to a solution of methylthiomethyl ether **2a** (0.10 g, 0.24 mmol) in 2 mL of anhydrous $ClCH_2CH_2Cl$. Then Br_2 (15.0 μL, 0.29 mmol) was added and the mixture was stirred for 10 min at ~25 °C. The residue obtained after removing the solvent and traces of the amine (1 Torr, 40 °C, 4 h) was chromatographed using a $CHCl_3$—MeOH (95 : 5) mixture as the eluent to give 0.129 g (97%) of compound **3a**, R_f 0.43 (C). MS, m/z (I_{rel} (%)): 452.6 $[M - Br]^+$ (100). Calculated for $C_{27}H_{53}N_2O_3$: 453.41 $[M - Br]^+$. ^1H NMR, δ: 0.85 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.22 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.45—1.58 (m, 2 H, $OCH_2C\underline{H}_2$); 3.35—3.50 (m, 8 H, $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OC\underline{H}_3$); 3.68 (dd, 1 H, $CH\underline{H}_aO$, J = 5.5 Hz, J = 10.7 Hz); 3.83 (dd, 1 H, CHH_bO, J = 3.0 Hz, J = 10.7 Hz); 4.10 (s, 3 H, NCH_3); 5.79 and 5.83 (both d, each 1 H, OCH_2N, J = 10.2 Hz); 7.27—7.31 (m, 1 H); 7.42—7.46 (m, 1 H, CH=CH); 10.56—10.63 (m, 1 H, CH=N).

***rac*-*N*-Methyl-*N*´-[(2-ethoxy-3-octadecyloxy)prop-1-yloxymethyl]imidazolium bromide (3b)** was prepared by the procedure described for compound **3a** using compound **2b** (0.18 g, 0.42 mmol), *N*-methylimidazole (165.6 μL, 2.08 mmol), and Br_2 (25.7 μL, 0.5 mmol). The product was chromatographed using a $CHCl_3$—MeOH (95 : 5) mixture as the eluent to give 0.219 g (97%) of compound **3b**, R_f 0.45 (C). MS, m/z (I_{rel} (%)): 466.4 $[M - Br]^+$ (100). Calculated for $C_{28}H_{55}N_2O_3$: 467.42 $[M - Br]^+$. ^1H NMR, δ: (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.14 (t, 3 H, $OCH_2C\underline{H}_3$, J = 6.8 Hz); 1.21 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.43—1.56 (m, 2 H, $OCH_2C\underline{H}_2$); 3.32—3.65 (m, 7 H, $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OC\underline{H}_2CH_3$); 3.68 (dd, 1 H, $CH\underline{H}_aO$, J = 5.1 Hz, J = 10.7 Hz); 3.83 (dd, 1 H, $CH\underline{H}_bO$, J = 3.0 Hz, J = 10.7 Hz); 4.10 (s, 3 H, NCH_3); 5.78 and 5.81 (both d, each 1 H, OCH_2N, J = 10.2 Hz); 7.34—7.39 (m, 1 H) and 7.43—7.48 (m, 1 H, CH=CH); 10.46—10.52 (m, 1 H, CH=N).

***rac*-*N*-Methyl-*N*´-[(2-allyloxy-3-octadecyloxy)prop-1-yloxymethyl]imidazolium bromide (3c)** was prepared by the procedure described for compound **3a** using compound **2c** (0.85 g, 0.19 mmol), *N*-methylimidazole (76.5 μL, 0.96 mmol), and Br_2 (11.9 μL, 0.23 mmol). The product was chromatographed using a $CHCl_3$—MeOH (90 : 10) mixture as the eluent to give 0.47 g (45%) of compound **3c,** R_f 0.50 (C). MS, m/z (I_{rel} (%)): 479.6 $[M - Br]^+$ (100). Calculated for $C_{29}H_{55}N_2O_3$: 479.42 $[M - Br]^+$. ^1H NMR, δ: 0.85 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.22 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.41—1.59 (m, 2 H, $OCH_2C\underline{H}_2$); 3.35—3.50 (m, 4 H, $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$); 3.63—3.81 (m, 3 H, CH_2O, $C\underline{H}OAll$); 3.95—4.02 (m, 2 H, $OC\underline{H}_2CH{=}CH_2$); 4.08 (s, 3 H, NCH_3); 5.14 (dddd, 1 H, $OCH_2CH{=}CH\underline{H}_a$, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 10.7 Hz); 5.24 (ddt, 1 H, $OCH_2CH{=}CH\underline{H}_b$, J = 1.7 Hz, J = 1.7 Hz, J = 1.7 Hz, J = 17.3 Hz); 5.74—5.90 (m, 3 H, OCH_2N, $OCH_2C\underline{H}{=}CH_2$); 7.23—7.28 (m, 1 H) and 7.42—7.46 (m, 1 H, CH=CH); 10.59—10.65 (m, 1 H, CH=N).

***rac*-*N*-Methyl-*N*´-[2,3-di(octadecyloxy)prop-1-yloxymethyl]imidazolium bromide (3d)** was prepared by the procedure described for compound **2a** using compound **2d** (0.11 g, 0.167 mmol), *N*-methylimidazole (66.7 μL, 0.84 mmol), and Br_2 (10.3 μL, 0.2 mmol). The product was chromatographed using a $CHCl_3$—MeOH (95 : 5) mixture as the eluent to give 0.124 g (96%) of compound **3d**, R_f 0.65 (C), m.p. 78—79 °C. MS, m/z (I_{rel} (%)): 693.1 $[M - Br]^+$ (100). Calculated for $C_{44}H_{87}N_2O_3$: 691.67 $[M - Br]^+$. ^1H NMR, δ: 0.85 (t, 6 H, 2 $(CH_2)_{17}C\underline{H}_3$, J = 6.4 Hz); 1.23 (br.s, 60 H, 2 $(C\underline{H}_2)_{15}CH_3$); 1.42—1.58 (m, 4 H, 2 $OCH_2C\underline{H}_2$); 3.34—3.57 (m, 7 H, 2 $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OC_{18}H_{37}$); 3.68 (dd, 1 H, $CH\underline{H}_aO$, J = 5.2 Hz, J = 10.0 Hz); 3.79 (dd, 1 H, $CH\underline{H}_bO$, J = 3.2 Hz, J = 10.0 Hz); 4.09 (s, 3 H, NCH_3); 5.77; 5.82 (both d, each 1 H, OCH_2N, J = 10.2 Hz); 7.25—7.29 (m, 1 H) and 7.39—7.45 (m, 1 H, CH=CH); 10.65—10.71 (m, 1 H, CH=N).

***rac*-*N*-Methyl-*N*-[(2-methoxy-3-octadecyloxy)prop-1-yloxymethyl]morpholinium bromide (4a).** *N*-Methylmorpholine (173.3 μL, 1.57 mmol) was added to a solution of methylthiomethyl ether **2a** (0.13 g, 0.31 mmol) in 3 mL of anhydrous $ClCH_2CH_2Cl$. Then Br_2 (19.5 μL, 0.38 mmol) was added, the mixture was stirred for 10 min at ~25 °C, an additional portion of Br_2 (19.5 μL, 0.38 mmol) was added, and the mixture was stirred for 20 min. The residue obtained after the removal of the solvent and traces of the amine (1 Torr, 40 °C, 4 h) was chromatographed using a $CHCl_3$—MeOH (95 : 5) mixture as the eluent to give 0.160 g (92%) of compound **4a**, R_f 0.52 (C), m.p. 72—73 °C. MS, m/z (I_{rel} (%)): 471.8 $[M - Br]^+$ (100). Calculated for $C_{28}H_{58}NO_4$: 472.44 $[M - Br]^+$. ^1H NMR, δ: 0.84 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.22 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.43—1.59 (m, 2 H, $OCH_2C\underline{H}_2$); 3.35—3.48 (m,

8 H, $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$, $CHOC\underline{H}_3$); 3.52 (s, 3 H, NCH_3); 3.66—3.76 (m, 4 H, 2 $NCH_2C\underline{H}_2O$); 3.92 (dd, 1 H, $CH\underline{H}_aO$, J = 5.1 Hz, J =11.1 Hz); 3.97—4.05 (m, 4 H, 2 $NC\underline{H}_2CH_2O$); 4.16 (dd, 1 H, $CH\underline{H}_bO$, J = 2.5 Hz, J = 11.1 Hz); 5.31 and 5.40 (both d, each 1 H, OCH_2N, J = 8.1 Hz).

***rac*-*N*-Methyl-*N*-[(2-ethoxy-3-octadecyloxy)prop-1-yloxymethyl]morpholinium bromide (4b)** was prepared by the procedure described for compound **4a** using compound **2b** (0.15 g, 0.34 mmol), *N*-methylmorpholine (190 μL, 1.73 mmol), and Br_2 (42.8 μL, 0.82 mmol). The product was chromatographed using a $CHCl_3$—MeOH (95 : 5) mixture as the eluent to give 0.178 g (91%) of compound **4b**, R_f 0.60 (C), m.p. 78.5—80 °C. MS, m/z (I_{rel} (%)): 485.8 $[M - Br]^+$ (100). Calculated for $C_{29}H_{60}NO_4$: 486.45 $[M - Br]^+$. ^{1}H NMR, δ: 0.84 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.4 Hz); 1.16 (t, 3 H, $OCH_2C\underline{H}_3$, J = 6.8 Hz); 1.22 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.42—1.58 (m, 2 H, $OCH_2C\underline{H}_2$); 3.34—3.51 (m, 4 H, $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$); 3.53 (s, 3 H, NCH_3); 3.58 (q, 2 H, $OC\underline{H}_2CH_3$, J = 6.8 Hz); 3.62 (m, 1 H, $C\underline{H}OEt$); 3.69—3.71 (m, 4 H, 2 $NCH_2C\underline{H}_2O$); 3.92—4.05 (m, 5 H, 2 $NC\underline{H}_2CH_2O$, $CH\underline{H}_aO$); 4.13 (dd, 1 H, CHH_b, J = 3.0 Hz, J = 10.7 Hz); 5.31 and 5.40 (both d, each 1 H, OCH_2N, J = 8.1 Hz).

***rac*-*N*-Methyl-*N*-[(2-Allyloxy-3-octadecyloxy)prop-1-yloxymethyl]morpholinium bromide (4c)** was prepared by the procedure described for compound **4a** using compound **2b** (0.028 g, 0.065 mmol), *N*-methylmorpholine (35.6 μL, 0.323 mmol), and Br_2 (8 μL, 0.16 mmol). The product was chromatographed using a $CHCl_3$—MeOH (95 : 5) mixture as the eluent to give 0.013 g (35%) of compound **4c,** R_f 0.56 (C), m.p. 76—78 °C. MS, m/z (I_{rel} (%)): 497.8 $[M - Br]^+$ (100). Calculated for $C_{30}H_{60}NO_4$: 498.45 $[M - Br]^+$. ^{1}H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{17}C\underline{H}_3$, J = 6.5 Hz); 1.22 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$); 1.41—1.60 (m, 2 H, $OCH_2C\underline{H}_2$); 3.35—3.53 (m, 8 H, $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OAll$, NCH_3); 3.65—3.79 (m, 4 H, 2 $NCH_2C\underline{H}_2O$); 3.92—4.17 (m, 8 H, 2 $NC\underline{H}_2CH_2O$, $OC\underline{H}_2CH{=}CH_2$, CH_2O); 5.20—5.49 (m, 4 H, OCH_2N, $OCH_2CH{=}C\underline{H}_2$); 5.73—5.88 (m, 1 H, $OCH_2C\underline{H}{=}CH_2$).

***rac*-*N*-[2,3-Di(octadecyloxy)prop-1-yloxymethyl]imidazole (5a).** Imidazole (0.015 g, 0.22 mmol) and Br_2 (6.2 μL, 0.12 mmol) were added successively to a solution of methylthiomethyl ether **1d** (0.66 g, 0.1 mmol) in 3 mL of anhydrous $ClCH_2CH_2Cl$, and the mixture was stirred for 10 min. The residue obtained after removal of the solvent was chromatographed using $CHCl_3$ as the eluent to give 0.063 g (92%) of compound **5a**, R_f 0.71 (B), m.p. 70—71 °C. MS, m/z (I_{rel} (%)): 678.0 $[M]^+$ (25), 699.8 $[M + Na]^+$ (100). Calculated for $C_{43}H_{84}N_2O_3$: 676.65 $[M]^+$, 699.64 $[M + Na]^+$. ^{1}H NMR, δ: 0.87 (t, 6 H, 2 $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.23 (br.s, 60 H, 2 $(C\underline{H}_2)_{15}CH_3$); 1.43—1.61 (m, 4 H, 2 $OCH_2C\underline{H}_2$); 3.35—3.56 (m, 9 H, 2 $OC\underline{H}_2CH_2$, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OC_{18}H_{37}$, CH_2O); 5.31 and 5.37 (both d, each 1 H, OCH_2N, J = 11.9 Hz); 7.04—7.07 (m, 1 H) and 7.09—7.13 (m, 1 H, CH=CH); 7.73—7.77 (m, 1 H, CH=N).

***rac*-*N*-[2,3-Di(octadecyloxy)prop-1-yloxymethyl]benzimidazole (5b).** Bromine (9.6 μL, 0.186 mmol) was added to a solution containing methylthiomethyl ether **1d** (0.102 g, 0.16 mmol) and benzimidazole (0.046 g, 0.386 mmol) in 5 mL of anhydrous $CHCl_3$, and the mixture was stirred for 10 min. The residue obtained after removal of the solvent was chromatographed using $CHCl_3$ as the eluent to give 0.091 g (81%) of compound **5b**, R_f 0.78 (B), m.p. 85—87 °C. MS, m/z (I_{rel} (%)): 726.9 $[M]^+$ (33), 748.9 $[M + Na]^+$ (100). Calculated для $C_{47}H_{86}N_2O_3$: 726.66 $[M]^+$, 749.65 $[M + Na]^+$. ^{1}H NMR, δ: 0.86 (t, 6 H, 2 $(CH_2)_{17}C\underline{H}_3$, J = 6.8 Hz); 1.23 (br.s, 60 H, 2 $(C\underline{H}_2)_{15}CH_3$); 1.43—1.61 (m, 4 H, 2 $OCH_2C\underline{H}_2$); 3.28 (br.t, 4 H, 2 $OC\underline{H}_2CH_2$, J = 6.8 Hz); 3.35—3.55 (m, 5 H, $C\underline{H}_2OC_{18}H_{37}$, $C\underline{H}OC_{18}H_{37}$, CH_2O); 5.55 and 5.63 (both d, each 1 H, OCH_2N, J = 11.9 Hz); 7.26—7.33 (m, 2 H), 7.47—7.58 (m, 2 H) and 7.96—8.02 (m, 1 H, benzimidazole).

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