Synthesis of sulfur-containing cationic lipids of the 1,3-dioxolane type

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A series of cationic acetal lipids containing different spacer and cationic groups were synthesized starting from 1,2-*O*-hexadecylidene-3-thioglycerol.

Key words: cationic lipids, acetal lipids, 1,2-*O*-hexadecylidene-3-thioglycerol.

The preparation of phosphorus-free cationic glycerolipids that are characterized by different sets of hydrophobic components and cationic groups attached to the glycerol residue either directly or through spacer groups has been studied intensively in recent years.[1]

Cationic lipids show promise from the viewpoint of their possible use in genetic therapy[2] as well as in connection with a number of biological effects that they exhibit, namely, anti-HIV activity,[3] antitumor action,[4] and the antagonistic effect with respect to the known lipid bioregulator, the platelet-activating factor.[5]

This work was undertaken as part of our continuing studies[6,7] of cationic glycerolipids, in particular, acetal lipids.[8,9]

Previously, we have prepared cationic lipids of the 1,3-oxathiolane[8] and 1,3-dioxolane[9] series in which the quaternary ammonium group was attached directly to the carbon atom of 1-mercaptopropan-2-ol or propane-1,2-diol.

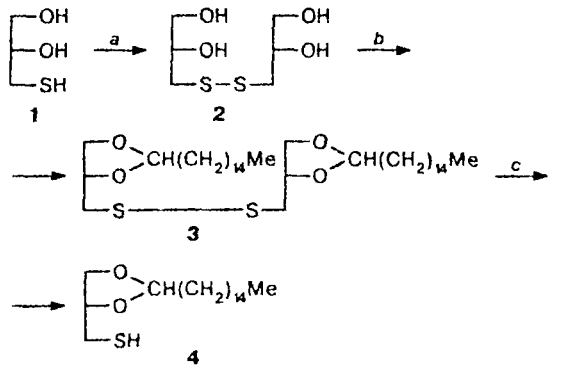
In this work, we synthesized positively charged lipids of the 1,3-dioxolane series in which the quaternary nitrogen atom is bonded to the sulfur atom through an alkyl or acyl spacer. Apparently, these differences in the structures of cationic lipids may be reflected in their biological properties and will allow one to extend the search for drugs with antitumor and anti-HIV activities.

3,3'-Dithiobisglycerol (**2**) was prepared by oxidation of 3-thioglycerol (**1**) with a 30% H_2O_2 solution according to a known procedure[10] and was used without additional purification (Scheme 1). Disulfide **2** was then converted into 3,3'-dithiobis(1,2-*O*-hexadecylideneglycerol) (**3**) by the reaction with palmitaldehyde in the presence of $BF_3 \cdot Et_2O$. Compound **3** was transformed into 1,2-*O*-hexadecylidene-3-thioglycerol (**4**), which is the key compound in the synthesis of cationic lipids, by the method of thiol-disulfide exchange with 1,4-dithiothreitol.[8]

Lipid (**5**) was synthesized according to Scheme 2.

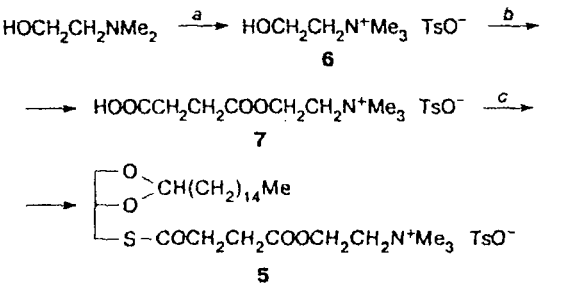
Choline tosylate (**6**), which was prepared by *N*-methylation of $Me_2NCH_2CH_2OH$ with methyl *p*-toluenesulfonate, was acylated with succinic anhydride in the presence of DMAP to form *O*-(3-carboxypropionyl)choline tosylate (**7**) in 92% yield. Condensation of compound **7** with thiol **4** in MeCN in the presence of dicyclohexylcarbodiimide afforded target product **5** in 54% yield.

Scheme 1



Reagents and conditions: *a*. 30% H_2O_2, 20 °C, 4 h; *b*. $Me(CH_2)_{14}CHO$, $BF_3 \cdot Et_2O$, anhydrous DMSO, PhMe, 20 °C, 4 h; *c*. $HSCH_2CH(OH)CH(OH)CH_2SH$, EtOH, hexane, 20 °C, 2 h.

Scheme 2



Reagents and conditions: *a*. TsOMe, PhMe, 30—40 °C, 2 h; *b*. $(CH_2CO)_2O$, PhMe, MeCN, Py, DMAP, 100 °C, 30 min; *c*. **4**, DCC, MeCN, 20 °C, 30 h.

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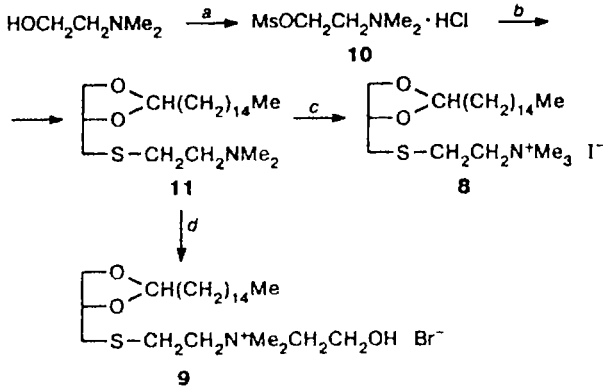
The synthesis of cationic lipids (**8** and **9**), which are characterized by the ethylene spacer between the nitrogen base and the sulfur atom, was carried out according to Scheme 3.

Scheme 3



Reagents and conditions: *a*. MsCl, anhydrous MeCN, 0 °C, 30 min; *b*. **4**, 0.5 *M* NaOH in EtOH, 20 °C, 6 h; *c*. MeI, anhydrous acetone, 57 °C, 30 min; *d*. $BrCH_2CH_2OH$, anhydrous MeCOEt, 100 °C, 2 h.

Mesylation of 2-dimethylaminoethanol gave *N*-(2-mesyloxyethyl)-*N*,*N*-dimethylamine hydrochloride (**10**). Alkylation of thiol **4** with mesyl derivative **10** in the presence of an ethanolic solution of NaOH gave amine **11** in 90% yield. Quaternization of **11** with MeI afforded the corresponding tetraalkylammonium iodide (**8**) in 94% yield, while quaternization of amine **11** with 2-bromoethanol gave bromide **9** in 48% yield. Compounds **3**, **4**, **5**, **8**, **9**, and **11** were obtained as mixtures of diastereomers, which was confirmed by the fact that the signals (triplets) from the acetal protons in the ^{1}H NMR spectra are doubled. The structures of the compounds synthesized were confirmed by the data of TLC, ^{1}H NMR and IR spectroscopy, mass spectrometry, and elemental analysis.

Experimental

The solvents were distilled before use. Succinic anhydride, MeI, $Me_2NCH_2CH_2OH$, $BF_3 \cdot Et_2O$, and DMSO were domestic reagents, *n*-hexadecanol and ethyl methyl ketone were from Reanal, 3-thioglycerol and 1,4-dithiotreitol were from Serva, DMAP and MsCl were from Fluka, and $BrCH_2CH_2OH$ was from Aldrich–Europe. Palmitaldehyde was prepared by oxidation of *n*-hexadecanol under the action of DMSO.[11] Methyl *p*-toluenesulfonate was synthesized by methanolysis of TsCl in an alkaline medium. TLC was carried out on plates with Al_2O_3 (activity II, Reanal) in the 14 : 7 : 1 $CHCl_3$–MeOH–H_2O system (*A*) (spots were visualized with iodine vapor) and on plates with SiO_2 (Silufol, Kavalier) in the 3 : 1 $CHCl_3$–light petroleum (*B*), 65 : 25 : 4 $CHCl_3$–MeOH–H_2O (*C*), and 65 : 25 : 4 $CHCl_3$–MeOH–H_2O (+ 1 drop of NH_4OH) (*D*) systems (spots were visualized by calcination). Column chromatography was performed on silica gel L 100/160 μm (Chemapol). The melting points were determined on a Boetius instrument. The IR spectra were recorded on a Shimadzu IR-435 spectrophotometer in a thin layer or as Nujol mulls. The ^{1}H NMR spectra were obtained on a Bruker MSL-200 pulse Fourier spectrometer (200 MHz) in $CDCl_3$ or in a 1 : 1 $CDCl_3$–CD_3OD mixture. The mass spectra were measured on a MSBKh time-of-flight spectrometer (Elektron) with ionization by nuclear fragments of californium-252. The accelerating voltage was ±5 or ±20 kV. The masses of ions that contain only ^{12}C are given.

3,3′-Dithiobis(1,2-*O*-hexadecylideneglycerol) (3). Anhydrous DMSO (40 mL), anhydrous toluene (40 mL), and $BF_3 \cdot Et_2O$ (4 mL) were added to a mixture of 3,3′-dithiobisglycerol (**2**) (2.20 g, 10.3 mmol) and palmitaldehyde (4.20 g, 17.5 mmol). The reaction mixture was stirred at 20 °C for 5 h. Then the major portion of the solvent was distilled off *in vacuo* (20 Torr) at 50 °C. The residue was dissolved in $CHCl_3$ (120 mL), washed with water (2×100 mL), and dried with Na_2SO_4. The solvent was distilled off *in vacuo*. The residue was extracted with methanol (3×30 mL) with heating (40 °C) to remove admixtures. Then the residue was dried *in vacuo* (1 Torr) at 40 °C for 2 h. Compound **3** was obtained in a yield of 3.45 g (51%), R_f 0.45 (*B*), m.p. 52–54 °C. MS, *m/z*: 658 $[M]^+$. IR, ν/cm^{-1}: 2880, 1455, 1420, 1380, 1150, 1120, 1100, 1040, 920, 720, 540. ^{1}H NMR, δ: 0.86 (t, 3 H, Me); 1.24 (m, 26 H, $(CH_2)_{13}$); 1.62 (m, 2 H, O_2CHCH_2); 2.85 (m, 2 H, $-CH_2S$); 3.54–4.40 (m, 3 H, CH_2O–CHO); 4.87 and 4.99 (both t, 1 H, OCHO, diastereomers).

1,2-*O*-Hexadecylidene-3-thioglycerol (4). 1,4-Dithiothreitol (0.52 g, 3.4 mmol) and concentrated NH_4OH (0.1 mL) were added with stirring to a solution of disulfide **3** (1.66 g, 2.5 mmol) in a mixture of ethanol (30 mL) and light petroleum (40 mL) at 25 °C. The reaction mixture was stirred for 2 h. Then $CHCl_3$ (75 mL) was added, and the reaction mixture was washed with water (2×50 mL). The emulsion that formed was separated by saturating with $MgSO_4$. The solvent was removed *in vacuo* at 30 °C. The residue was chromatographed (2 : 1 light petroleum–$CHCl_3$). After the removal of the solvent, the residue was dried *in vacuo* (1 Torr) under a stream of nitrogen at 30 °C for 2 h. Compound **4** was obtained in a yield of 0.85 g (51%), R_f 0.49 (*B*), m.p. 23–24 °C. Found (%): C, 69.06; H, 11.21. $C_{19}H_{38}O_2S$. Calculated (%): C, 69.03; H, 11.59. IR, ν/cm^{-1}: 2870, 1460, 1420, 1380, 1140, 1120, 1040, 720. ^{1}H NMR, δ: 0.84 (t, 3 H, Me); 1.26 (m, 26 H, $(CH_2)_{13}$); 1.62 (m, 2 H, O_2CHCH_2); 2.62 (m, 2 H, CH_2–SH); 3.56–4.21 (m, 3 H, OCH_2–CHO); 4.87 and 4.99 (both t, 1 H, OCHO, diastereomers).

Choline tosylate (6). A solution of methyl *p*-toluenesulfonate (100 g, 0.537 mol) in anhydrous toluene (300 mL) was added portionwise with stirring to a solution of $Me_2NCH_2CH_2OH$ (50 g, 0.561 mol) in anhydrous toluene (500 mL) at 30–40 °C. Then the reaction mixture was heated until the precipitate was dissolved (1 h, 100 °C) and kept at 20 °C for 20 h. The solvent was distilled *in vacuo* (20 Torr). The residue was dried *in vacuo* (1 Torr) at 55 °C for 5 h under a stream of nitrogen. Compound **6** was obtained in a yield of 149 g (100%), R_f 0.63 (*A*), m.p. 102–103 °C. IR, ν/cm^{-1}: 3400, 2900, 1650, 1630, 1600, 1490, 1470, 1370, 1190, 1120, 1080, 1030, 1000, 950, 810, 680, 560.

***O*-(3-Carboxypropionyl)choline tosylate (7).** A mixture of choline tosylate **6** (2.0 g, 7.3 mmol), succinic anhydride (2.0 g, 20 mmol), and a catalytic amount of DMAP in anhydrous toluene (30 mL), anhydrous MeCN (30 mL), and anhydrous Py (20 mL) was heated *in vacuo* (20 Torr) at 70 °C, and the mixture of the solvents (50 mL) was distilled off within 30 min.

The reaction mixture was kept at 20 °C for 20 h. The precipitate was filtered off, washed with anhydrous toluene, and dried *in vacuo* (1 Torr) at 60 °C for 1 h. Compound **7** was obtained in a yield of 2.51 g (92%), R_f 0.18 (*A*), m.p. 138—140 °C. Found (%): C, 51.06; H, 6.87; N, 3.95; S, 7.77. $C_{16}H_{25}NO_7S$. Calculated (%): C, 51.18; H, 6.71; N, 3.73; S, 8.54. IR, ν/cm^{-1}: 2740, 2650, 2500, 1730, 1710, 1500, 1480, 1460, 1400, 1380, 1330, 1220, 1150, 1110, 1020, 680. ^1H NMR, δ: 2.37 (s, 3 H, $-C_6H_4\underline{Me}$); 2.69 (m, 4 H, $COCH_2CH_2CO$); 3.17 (s, 9 H, $-N^+Me_3$); 3.69 (m, 2 H, $-CH_2N$); 4.54 (m, 2 H, $-OCH_2$); 7.34 (d, 2 H, 3,5-H_{Ar}); 7.66 (d, 2 H, 2,6-H_{Ar}).

***O*-[3-(1,2-Hexadecylidenedioxypropyl-3-thiocarbonyl)propionyl]choline tosylate (5).** Dicyclohexylcarbodiimide (2.10 g, 0.01 mol) was added to a solution of thiol **4** (0.60 g, 1.8 mmol) and acylcholine tosylate **7** (1.02 g, 2.7 mmol) in anhydrous MeCN (40 mL). The reaction mixture was kept at 20 °C for 30 h. The precipitate was filtered off. The filtrate was concentrated *in vacuo*. The residue was extracted with light petroleum (2×30 mL) to remove excess dicyclohexylcarbodiimide. Then the residue was extracted with anhydrous toluene (20 mL) to remove nonpolar admixtures. The residue was dried *in vacuo* (1 Torr) at 50 °C for 2 h. Compound **5** was obtained in a yield of 0.68 g (54%), R_f 0.61 (*C*), m.p. 225—230 °C. MS, *m/z*: 159, 218, 516 $[C_{28}H_{54}O_5NS]^+$. IR, ν/cm^{-1}: 2850, 1740, 1690, 1460, 1380, 1220, 1180, 1170, 1130, 1030, 720, 680. ^1H NMR, δ: 0.86 (t, 3 H, Me); 1.25 (m, 26 H, $(CH_2)_{13}$); 1.67 (m, 2 H, $O_2CH\underline{CH_2}$); 2.33 (s, 3 H, $\underline{Me}-C_6H_4$); 2.57—3.13 (m, 6 H, CH_2S, $COCH_2CH_2CO$); 3.17 (s, 9 H, $-N^+Me_3$); 3.67 (m, 2 H, $-CH_2N$); 3.43—4.18 (m, 3 H, OCH_2-CHO); 4.50 (m, 2 H, $OC\underline{H_2}CH_2N$); 4.81 and 4.94 (both t, 1 H, OCHO, diastereomers); 7.21 (d, 2 H, 3,5-H_{Ar}); 7.67 (d, 2 H, 2,6-H_{Ar}).

***N*-(2-Mesyloxyethyl)-*N*,*N*-dimethylamine hydrochloride (10).** A solution of MsCl (3.85 g, 33.6 mmol) in anhydrous MeCN (20 mL) was added dropwise with stirring to a solution of $Me_2NCH_2CH_2OH$ (3.0 g, 33.6 mmol) in anhydrous MeCN (20 mL) at 0 °C. The reaction mixture was kept at 20 °C for 20 h. The white precipitate that formed was filtered off, dissolved in $CHCl_3$ (70 mL), washed with water (25 mL), and dried with Na_2SO_4. The solvent was removed. The residue was dried *in vacuo* (1 Torr) at 40 °C for 2 h. Compound **10** was obtained in a yield of 3.40 g (49%), R_f 0.53 (*D*). MS, *m/z*: 168 $[C_5H_{14}O_3NS]^+$. IR, ν/cm^{-1}: 2880, 2600, 1460, 1410, 1350, 1175, 1070, 1055, 1030, 970, 925, 805, 730, 550, 515.

1,2-*O*-Hexadecylidene-3-(2-dimethylaminoethyl)thioglycerol (11). A mixture of thiol **4** (1.0 g, 3.0 mmol) and mesyl derivative **10** (2.0 g, 9.8 mmol) in 100 mL of a 0.5 *M* ethanolic NaOH solution was stirred at 25 °C for 6 h. The reaction mixture was filtered, the precipitate was washed with ethanol, and the solvent was distilled off *in vacuo*. The residue was extracted with a 1 : 1 toluene—light petroleum mixture (2×25 mL) at 40 °C. The extract was washed with water (2×20 mL) and dried with Na_2SO_4. The solvent was distilled off *in vacuo*, and the residue was dried *in vacuo* (1 Torr) at 50 °C for 2 h to give compound **11** (1.10 g, 90%), R_f 0.84 (*D*). MS, *m/z*: 190, 402 $[M+1]^+$. IR, ν/cm^{-1}: 2900, 2850, 2700, 1460, 1420, 1370, 1340, 1280, 1260, 1160, 1130, 1090, 1060, 1040, 1010, 940, 900, 850, 750, 720. ^1H NMR, δ: 0.85 (t, 3 H, Me); 1.26 (m, 26 H, $(CH_2)_{13}$); 1.60 (m, 2 H, $O_2CH\underline{CH_2}$); 2.22 (s, 6 H, $-NMe_2$); 2.51 (m, 2 H, CH_2N); 2.54—2.85 (m, 4 H, CH_2-S-CH_2); 3.52—3.98 (m, 2 H, $\underline{CH_2}-O-CH$); 4.09—4.28 (m, 1 H, CH—O); 4.86 and 4.97 (both t, 1 H, OCHO, diastereomers).

[2-(1,2-Hexadecylidenedioxypropyl-3-thio)ethyl]trimethylammonium iodide (8). A solution of compound **11** (0.52 g, 1.3 mmol) and MeI (1.14 g, 8 mmol) in anhydrous acetone (15 mL) was boiled for 15 min. The solvent was distilled off *in vacuo*. The residue was recrystallized from anhydrous acetone (5 mL). The precipitate that formed was filtered off, washed with ether (3 mL), and dried *in vacuo* (1 Torr) at 40 °C for 2 h. Compound **8** was obtained in a yield of 0.66 g (94%), R_f 0.63 (*C*), m.p. 210—212 °C. MS, *m/z*: 360, 417 $[C_{24}H_{50}O_2NS]^+$. IR, ν/cm^{-1}: 2850, 2800, 1460, 1420, 1370, 1250, 1160, 1130, 1100, 1030, 960, 930, 880, 850, 820, 720. ^1H NMR, δ: 0.82 (t, 3 H, Me); 1.24 (m, 26 H, $(CH_2)_{13}$); 1.58 (m, 2 H, $O_2CHC\underline{H_2}$); 2.72—3.20 (m, 4 H, CH_2-S-CH_2); 3.46 (s, 9 H, $-N^+Me_3$); 3.94 (m, 2 H, $-CH_2N$); 3.52—4.01 (m, 2 H, $C\underline{H_2}-O-CH$); 4.11—4.34 (m, 1 H, CH—O); 4.84 and 5.01 (both t, 1 H, OCHO, diastereomers).

[2-(1,2-Hexadecylidenedioxypropyl-3-thio)ethyl](2-hydroxyethyl)dimethylammonium bromide (9). A solution of compound **11** (0.22 g, 0.55 mmol) and $BrCH_2CH_2OH$ (0.27 g, 2.2 mmol) in anhydrous ethyl methyl ketone (5 mL) was boiled for 2 h. The solvent was distilled off *in vacuo*, and the residue was chromatographed (MeCN—MeOH, 15 : 1). The solvent was removed *in vacuo*, and the residue was dried *in vacuo* (1 Torr) at 40 °C for 2 h to give compound **9** (0.14 g, 48%), R_f 0.55 (*C*), m.p. 155—160 °C. MS, *m/z*: 358, 446 $[C_{25}H_{52}O_3NS]^+$. ^1H NMR, δ: 0.86 (t, 3 H, Me); 1.26 (m, 26 H, $(CH_2)_{13}$); 1.60 (m, 2 H, $O_2CHC\underline{H_2}$); 2.72—3.22 (m, 4 H, CH_2-S-CH_2); 3.40 (s, 6 H, Me_2N^+); 3.54—4.04 (m, 6 H, $C\underline{H_2}-O-CH$, $CH_2-N^+-CH_2$); 4.14 (m, 2 H, $C\underline{H_2}OH$); 4.24 (m, 1 H, CH—O); 4.85 and 5.00 (both t, 1 H, OCHO, diastereomers).

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