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Synthesis of cationic cholesterol-containing amphiphiles with an acid-labile linker

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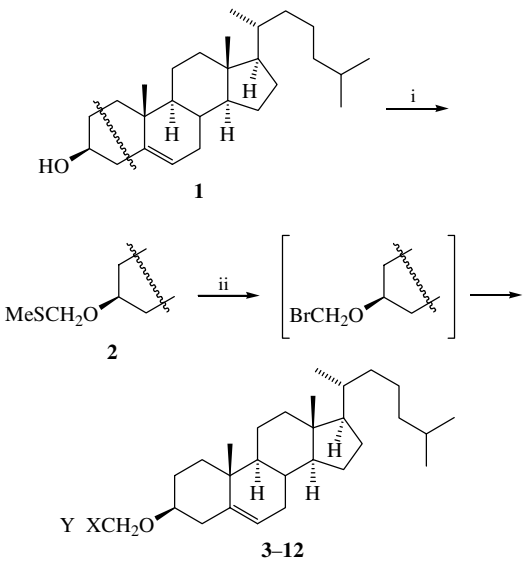
The synthesis of cationic amphiphiles from cholesterol methylthiomethyl ether has been carried out.

A promising branch of medicine, gene therapy, which is aimed at either correcting a hereditary disease or imparting new functions to cells, is being successfully developed to cure hereditary, infectious and oncological diseases. A prerequisite of gene therapy is that the therapeutic gene must be efficiently delivered into target cells (transfection) and its expression and prolonged functioning in the cells must be ensured. Considerable attention is given to the development of non-viral gene delivery systems. Of these, lipofection based on the delivery of the genetic material into cells by means of cationic liposomes[1–3] has a high therapeutic potential. Positively charged liposomes form complexes (lipoplexes) with molecules of oligo- and polynucleotides and thus protect the latter from degradation by cell enzymes and favour their transfer through cell membranes by endocytosis.

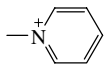
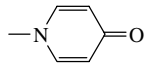
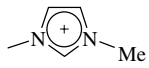
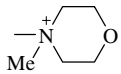
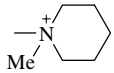
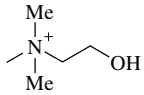
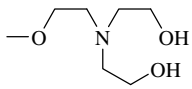
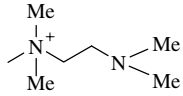
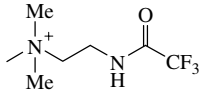
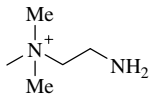
A cationic amphiphile molecule is a combination of three structural units: a hydrophobic component, a hydrophilic cationic domain and a linker connecting the two. The nature, size and mutual arrangement of structural domains in cationic lipids affect the structure and stability of DNA–liposome complexes, which further determine the delivery efficiency and the mechanisms of interaction with the cell.[4]

Endocytosis is accompanied by a noticeable increase in the environment acidity from a physiological value of pH 7.4 to 6.5–6.0 in endosomes and to 5.0 in primary or secondary lysosomes.[5] Therefore, incorporation of acid-labile bonds to the amphiphile structure favours the lipoplex destabilisation and facilitates the DNA release from the endosomal compartment to the cytoplasm, thus enhancing the transfection efficiency.[5–7] Furthermore, the use of biodegradable cationic amphiphiles lowers the cytotoxicity of cationic liposomes and makes them promising transfection agents.



Scheme 1 *Reagents and conditions*: i, DMSO–Ac_2O–AcOH, benzene, 24 °C, 3 days; ii, corresponding organic base, NBS, DCE, 24 °C, 10 min–10 h.

Table 1 Compounds **3–12**.

Compound	X	Y	Yield (%)
3		Br^-	67
4		—	52
5		Br^-	58
6		Br^-	62
7		Br^-	21
8		Br^-	67
9		—	46
10		Br^-	51
11		Br^-	60
12		Br^-	58

We have synthesised positively charged lipids **3–12**, in which the hydrophobic domain (cholesterol) is linked with the hydrophilic residue of an aliphatic or heterocyclic base by an acid-labile linker of the N,O-acetal type. It is known that N,O-acetals (1,1-aminoethers) readily undergo hydrolysis with dilute acids.[11–13] Syntheses of cationic lipids **3–12** are based on the use of

Table 2 Chemical shifts (δ) and coupling constants (J/Hz) of OCH_2X group protons in ^1H NMR spectra of lipids **3**–**12**.

Compound	Chemical shift	Compound	Chemical shift
3	6.04 (s, 2H)	**8**	4.92 (d, 1H, *J* 7.2 Hz), 4.99 (d, 1H, *J* 7.2 Hz)
4	5.13 (s, 2H)	**9**	4.71 (s, 2H)
5	5.68 (s, 2H)	**10**	4.80 (d, 1H, *J* 7.5 Hz), 4.84 (d, 1H, *J* 7.5 Hz)
6	4.90 (d, 1H, *J* 7.5 Hz), 4.97 (d, 1H, *J* 7.5 Hz)	**11**	4.68 (d, 1H, *J* 7.5 Hz), 4.71 (d, 1H, *J* 7.5 Hz)
7	5.06 (d, 1H, *J* 7.5 Hz), 5.21 (d, 1H, *J* 7.5 Hz)	**12**	4.68 (d, 1H, *J* 7.8 Hz), 4.71 (d, 1H, *J* 7.8 Hz)

cholesterol methylthiomethyl ether (Scheme 1). Methylthiomethyl ethers previously employed for the protection of hydroxy and carboxy groups[8–10] were used to synthesise cationic glycerolipids,[14–16] which were subsequently used for the transfection of the RGGN and HeLa cell lines.[17]

The synthesis started from cholesterol **1**, which was converted to methylthiomethyl ether **2** by treatment with a DMSO–acetic anhydride–acetic acid mixture (6.5:3.4:1 mol) for three days. The yield of methylthiomethyl ether was 76% after chromatographic purification; its physicochemical characteristics match published data.† Since methylthiomethyl ethers are non-symmetrical O,S-acetals, they react with bromine to give highly reactive α-bromoethers, which can react with nucleophilic reagents. However, the use of bromine in the reaction with cholesterol methylthiomethyl ether results in a side reaction, *viz.*, bromination of the double bond. Therefore, we converted the methylthiomethyl group to the α-haloether using *N*-bromosuccinimide (NBS).

Amphiphiles **3**–**12** were obtained by treatment of methylthiomethyl ether **2** with NBS followed by treatment with the corresponding heterocyclic base or amine in anhydrous dichloroethane. The reaction time depended on the nucleophilicity of the nitrogen atom in the amine molecule. After chromatographic purification, lipids **3**–**12** were isolated in 21–67% yields and characterised using NMR spectroscopy and mass spectrometry.‡

Cationic lipid **12** cannot be obtained in one stage due to the existence of two nucleophilic centres in the amine molecule, so it was synthesised from precursor **11**. For this purpose, the primary amino group in *N*,*N*-dimethylaminoethylamine was blocked by the trifluoroacetyl protection group, and the resulting derivative was treated with methylthiomethyl ether **2** to give cationic lipid **11**. The trifluoroacetyl group was then removed by treatment with sodium borohydride in methanol to give lipid **12** in 58% yield.

An analysis of the ^1H NMR spectra of compounds **3**–**12** revealed differences in the shape of proton signals from the OCH_2N group (Table 2). The OCH_2N proton signals for lipids **3**–**5** are singlets with an integral intensity equal to two protons. We observed the same signal shape for protons of the OCH_2O group in the spectrum of compound **9**, in which the amine is bound *via* the hydroxy group due to the spatial inaccessibility of the nitrogen atom. The signals of similar protons in compounds **6**–**8** and **10**–**11** are two doublets with the coupling constant J_{AB} 7.2–7.8 Hz. Since the resulting cationic amphiphiles contain asymmetric centres, the protons of the OCH_2N group are diastereotopic and thus magnetically non-equivalent. The methyl group at the adjacent nitrogen atom in compounds **6**–**8** and **10**–**11** differently interacts with each proton of the OCH_2N group. Therefore, the signal of the diastereotopic protons is resolved and two doublets appear in the NMR spectrum. In the spectra of compounds **3**–**5** containing no methyl substituent at the nitrogen atom of the OCH_2N group, the signals of these protons are not resolved and form a singlet.

Thus, we found that methylthiomethyl cholesterol ether **2** can be successfully used to synthesise cationic lipids containing various nitrogen bases.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2007.09.011.

† Compound **2**: ^1H NMR (Bruker MSL-200, 200 MHz, $CDCl_3$, $SiMe_4$ internal standard) δ: 0.65 (s, 3H, 18-Me), 0.86 (d, 6H, 26-Me, 27-Me, *J* 6.8 Hz), 0.89 (d, 3H, 21-Me, *J* 6.7 Hz), 1.01 (s, 3H, 19-Me), 1.02–1.60 (m, 21H, Chol), 1.75–2.05 (m, 5H, Chol), 2.16 (s, 3H, SMe), 2.28 (m, 2H, 4-CH_2), 3.50 (m, 1H, 3-CH), 4.64 (s, 2H, OCH_2S), 5.25–5.32 (m, 1H, 6-CH). MS (Finnigan MAT 900XL-TRAP with ESI ionization), *m*/*z*: $[M]^+$ 446.5, $[M – SMe]^+$ 400.1. Lit.:[18] ^1H NMR, τ: 9.34 (18-Me), 9.00 (19-Me), 7.91 (SMe), 6.50 (3-CH), 5.42 (OCH_2S), 4.67 (6-CH).

‡ A solution of NBS (0.355 mmol) in dichloroethane (2.4 ml) was added dropwise with stirring to a solution of methylthiomethyl ether **2** (0.338 mmol) and anhydrous amine (0.777 mmol) in anhydrous dichloroethane (1.5 ml). After the spot of ether **2** disappeared (TLC), dichloroethane and the amine were removed *in vacuo*. The target compounds were isolated by flash chromatography on a column with Kieselgel 60 (40–63 μm, Merck) using the following eluent systems: $CHCl_3$–MeOH (10:1) for quaternary amines **3**–**8**, **10**, **11**; $CHCl_3$–MeCOMe–aq. NH_3 (40:6:1) for **9**, and $CHCl_3$–MeOH–aq. NH_3 (20:5:1) for **12**. Compounds **4**, **5**, **7** and **10** were crystalline whereas the others were crystallising oils.

Compound **3**: ^1H NMR (Bruker AMX-400, 400 MHz, $CDCl_3$, $SiMe_4$ internal standard) δ: 0.60 (s, 3H, 18-Me), 0.78 (d, 6H, 26-Me, 27-Me, *J* 6.6 Hz), 0.84 (d, 3H, 21-Me, *J* 6.6 Hz), 0.93 (s, 3H, 19-Me), 1.00–1.60 (m, 21H, Chol), 1.68–2.01 (m, 5H, Chol), 2.20–2.34 (m, 2H, 4-CH_2), 3.35–3.54 (m, 1H, 3-CH), 5.25–5.33 (m, 1H, 6-CH), 6.04 (s, 2H, OCH_2N^+), 8.06–8.18 (m, 2H), 8.48–8.62 (m, 1H) and 9.06–9.17 (m, 2H, $C_5H_5N^+$). MS, *m*/*z*: 478.8 $[M – Br]^+$.

Compound **4**: mp 186–188 °C. ^1H NMR, δ: 0.60 (s, 3H, 18-Me), 0.78 (d, 6H, 26-Me, 27-Me, *J* 6.6 Hz), 0.83 (d, 3H, 21-Me, *J* 6.6 Hz), 0.92 (s, 3H, 19-Me), 0.93–1.60 (m, 21H, Chol), 1.65–1.98 (m, 5H, Chol), 2.07–2.27 (m, 2H, 4-CH_2), 3.14–3.27 (m, 1H, 3-CH), 5.13 (s, 2H, OCH_2N^+), 5.22–5.28 (m, 1H, 6-CH), 6.36–6.43 (m, 2H, CHN^+CH), 7.54–7.61 [m, 2H, CHC(O)CH]. MS, *m*/*z*: 493.7 $[M]^+$.

Compound **7**: mp 186–188 °C. ^1H NMR, δ: 0.60 (s, 3H, 18-Me), 0.79 (d, 6H, 26-Me, 27-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.92 (s, 3H, 19-Me), 0.98–1.53 (m, 23H, Chol, piperidine CH_2), 1.71–1.98 (m, 9H, Chol, 2 piperidine CH_2), 2.24–2.36 (m, 2H, 4-CH_2), 3.21 (s, 3H, N^+Me), 3.39–3.50 (m, 1H, 3-CH), 3.60–3.73 (m, 4H, $CH_2N^+CH_2$), 5.06 (d, 1H, OCH_aN^+, *J* 7.5 Hz) and 5.21 (d, 1H, OCH_bN^+, *J* 7.5 Hz), 5.31–5.35 (m, 1H, 6-CH). MS, *m*/*z*: 498.2 $[M – Br]^+$.

Compound **10**: mp 250–252 °C. ^1H NMR, δ: 0.60 (s, 3H, 18-Me), 0.78 (d, 6H, 26-Me, 27-Me, *J* 6.6 Hz), 0.83 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.98–1.63 (m, 21H, Chol), 1.68–1.99 (m, 5H, Chol), 2.21–2.41 (m, 2H, 4-CH_2), 2.95 (s, 6H, NMe_2), 3.14 (s, 6H, N^+Me_2), 3.51–3.63 (m, 1H, 3-CH), 3.80–3.89 (m, 2H, NCH_2), 3.95–4.05 (m, 2H, N^+CH_2), 4.80 (d, 1H, OCH_aN^+, *J* 7.5 Hz) and 4.84 (d, 1H, OCH_bN^+, *J* 7.5 Hz), 5.31–5.37 (m, 1H, 6-CH). MS, *m*/*z*: 515.0 $[M – Br]^+$.

Compound **11**: ^1H NMR, δ: 0.61 (s, 3H, 18-Me), 0.79 (d, 6H, 26-Me, 27-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.97–1.60 (m, 21H, Chol), 1.70–1.99 (m, 5H, Chol), 2.23–2.33 (m, 2H, 4-CH_2), 3.06 (s, 6H, N^+Me_2), 3.46–3.59 (m, 1H, 3-CH), 3.60 (t, 2H, NCH_2, *J* 6.2 Hz), 3.70 (t, 2H, N^+CH_2, *J* 6.2 Hz), 4.68 (d, 1H, OCH_aN^+, *J* 7.5 Hz) and 4.71 (d, 1H, OCH_bN^+, *J* 7.5 Hz), 5.30–5.35 (m, 1H, 6-CH). MS, *m*/*z*: 583.5 $[M – Br]^+$.

For ^1H NMR spectroscopic and mass spectrometric data for compounds **5**, **6**, **8**, **9** and **12**, see Online Supplementary Materials.

References

1 A. J. Kirby, P. Camilleri, J. B. F. N. Engberts, M. C. Feiters, R. J. M. Nolte, O. Soderman, M. Bergsma, P. C. Bell, M. L. Fielden, C. L. Garcia Rodriguez, P. Guedat, A. Kremer, C. McGregor, C. Perrin, G. Ronsin and M. C. P. van Eijk, *Angew. Chem., Int. Ed. Engl*., 2003, **42**, 1448.

2 B. Martin, M. Sainlos, A. Aissaoui, N. Oudrhiri, M. Hauchecorne, J.-P. Vigneron, J.-M. Lehn and P. Lehn, *Curr. Pharm. Des*., 2005, **11**, 375.

3 M. A. Maslov, E. V. Syicheva, N. G. Morozova and G. A. Serebrennikova, *Izv. Akad. Nauk, Ser. Khim*., 2000, 385 (*Russ. Chem. Bull., Int. Ed*., 2000, **49**, 385).

4 K. Ewert, N. L. Slack, A. Ahmad, H. M. Evans, A. J. Lin, C. E. Samuel and C. R. Safinya, *Curr. Med. Chem*., 2004, **11**, 133.

5 A. Aissaoui, B. Martin, E. Kan, N. Oudrhiri, M. Hauchecorne, J.-P. Vigneron, J.-M. Lehn and P. Lehn, *J. Med. Chem*., 2004, **47**, 5210.

6 X. Guo and F. C. Szoka, *Acc. Chem. Res*., 2003, **36**, 335.

7 M.-Z. Zhu, Q.-H. Wu, G. Zhang, T. Ren, D. Liu and Q.-X. Guo, *Bull. Chem. Soc. Jpn*., 2002, **75**, 2207.

8 P. M. Pojer and S. J. Angyal, *Aust. J. Chem*., 1978, **31**, 1031.

9 E. J. Corey and M. G. Bock, *Tetrahedron Lett*., 1975, **25**, 3269.

10 L. G. Wade, J. M. Gerdes and R. P. Wirth, *Chem. Lett*., 1978, **28**, 731.

11 *Comprehensive Organic Chemistry*, eds. D. Barton and W. D. Ollis, Pergamon Press, Oxford, 1979, vol. 2.

12 J. T. Shaw and K. A. Woerpel, *J. Org. Chem*., 1997, **62**, 442.

13 F. Sasse and D. Menche, *Nat. Chem. Biol*., 2007, **3**, 87.

14 I. D. Konstantinova, S. G. Zavgorodny, A. I. Miroshnikov, I. P. Ushakova and G. A. Serebrennikova, *Bioorg. Khim*., 1995, **21**, 66 (*Russ. J. Bioorg. Chem*., 1995, **21**, 58).

15 M. A. Maslov, N. G. Morozova and G. A. Serebrennikova, *Mendeleev Commun*., 2000, 65.

16 N. V. Plyavnik, M. A. Maslov and G. A. Serebrennikova, *Mendeleev Commun*., 2005, 26.

17 R. I. Zhdanov, O. V. Podobed, N. G. Kutsenko, O. A. Buneeva, T. A. Tsvetkova, S. O. Gur'ev, T. P. Lavrenova, G. A. Serebrennikova, I. D. Konstantinova and M. A. Maslov, *Dokl. Akad. Nauk*, 1998, **362**, 557 [*Dokl. Biochem. Biophys.* (*Engl. Transl.*), 1998, **362**, 164].

18 S. M. Ifzal and D. A. Wilson, *J. Chem. Soc. (C)*, 1969, 2168.

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