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Synthesis of novel cationic lipids with oxyethylene spacers at the linkages between hydrocarbon chains and pseudoglyceryl backbone

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Abstract

Four novel cationic lipids with *oligo*-oxyethylene units at various linkage regions between the pseudoglyceryl backbone and the hydrocarbon chains have been synthesized. The membrane-forming properties of these new lipids are briefly presented.

Cationic lipids have been attracting increasing attention recently owing to their possible applications in gene therapy.[1,2] The functional group that connects the head group and the hydrocarbon chains of these cationic lipid molecules plays an important role in their utilization in gene transfer events. For instance, DOTMA, which contains an ether linkage between the head group and the long alkyl chain is shown to have much greater in vivo transfection efficiency than the corresponding cationic lipid with an ester linkage (DOTAP).[3] For the last few years, we have been investigating the role of various molecular level modifications on the properties of membranes formed from different lipids.[4] An alternative concept in lipid molecular design is introduced herein by the incorporation of a hydrophilic spacer between the hydrocarbon chains and the pseudoglyceryl backbone of the cationic lipids. The importance of the hydrophilic spacer groups for the interaction with bio-reactive centres (such as enzymes) attached to various carriers is well known.[5] Recently, it has been shown that the hydrophilic polymer, polyoxyethylene glycol (PEG) brings about interesting biological and pharmaceutical effects when grafted onto the head group regions of some phospholipids.[6] However, the effects of insertion of *oligo*-oxyethylene units at the linkage site of the lipid has not been examined. Consequently, the properties of the resulting membranes from these types of lipids presently remain unknown. Herein we describe a convenient synthesis of the above type of cationic lipids **1–4** and briefly report their membrane forming properties.

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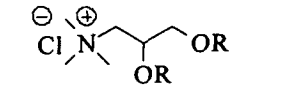
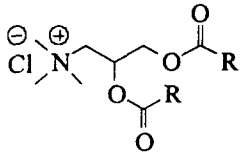
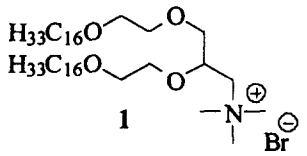


DOTMA: R = n-(9z)-$C_{18}H_{35}$



DOTAP: R = n-(9z)-$C_{17}H_{33}$



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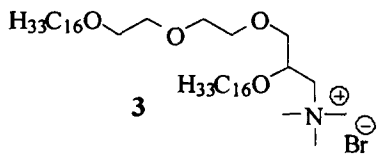

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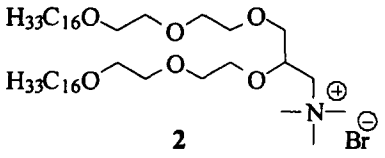

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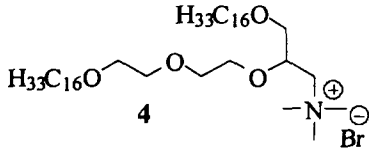


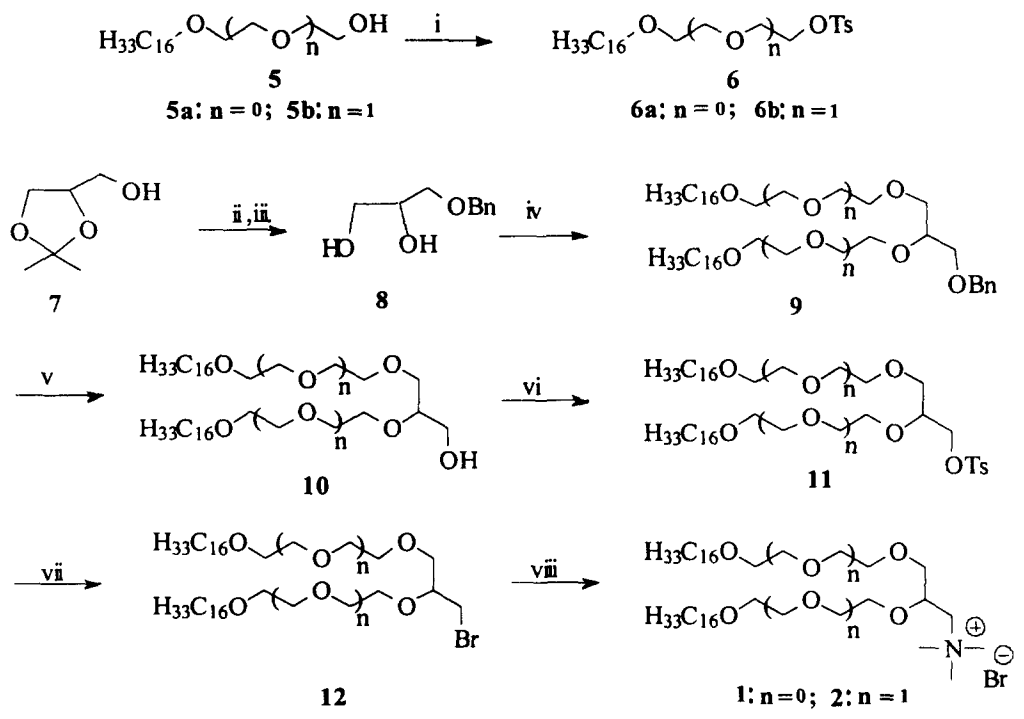
4

The synthesis of the first series of this class of molecule, which contains oxyethylene units in both the chains at the C-1 and C-2 positions of the backbone is outlined in Scheme 1. Ethylene glycol monohexadecyl ether **5a**, and diethylene glycol monohexadecyl ether **5b**, on reaction with tosyl chloride in the presence of pyridine in CH_2Cl_2, afforded the corresponding tosylates **6a–b**. Benzyl glycerol **8** was synthesized by slight modification of a literature procedure from 1,2-*O*-isopropylidene glycerol.[7] Benzyl glycerol **8**, on reaction with either tosylates **6a** or **6b** under NaH/benzene conditions, yielded **9a** or **9b**, respectively, in moderate yields. Debenzylation of **9** to the glycerol derivatives **10** was achieved conveniently by hydrogenolysis. The conversion of **10** to the primary bromide **12**, however, proved difficult. The bromination under Appel conditions (CBr_4/PPh_3 in CH_2Cl_2) was not satisfactory. Ultimately, success was attained by treating the corresponding tosylates **11a** or **11b** with LiBr in DMF. Substitution of the tosylate residue in **11** with NaBr in acetone was, in contrast, very sluggish. The bromides **12a** and **12b** were finally converted in good yields to the corresponding cationic lipids **1** and **2**, respectively, by reaction with trimethylamine in acetonitrile–toluene mixture in a sealed tube.

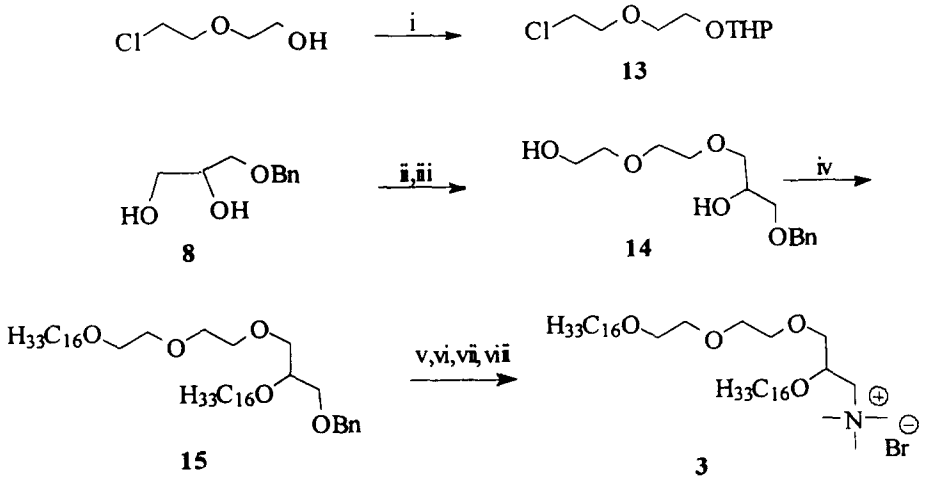
Scheme 2 depicts the synthesis of the mixed chain lipid **3** in which the hydrophilic dioxyethylene spacer has been introduced only at C-1 of the pseudoglyceryl backbone. The alcohol **14** was synthesized by modification of a literature procedure.[8] Chloroethoxyethanol was protected as a tetrahydropyranyl (THP) ether **13**. Benzyl glycerol was then selectively alkylated at the primary OH residue via a phase transfer catalyzed reaction. The yield of this reaction was found to be dependent upon the reaction time since the corresponding dialkylated derivative was also formed on prolonged reaction. Subsequent removal of the THP protecting group afforded **14**, which on reaction with hexadecyl chloride under similar phase transfer conditions, gave **15** in moderate yields. From **15**, the cationic lipid **3** was then prepared via removal of benzyl protecting group, conversion of the alcohol to bromide via the tosylate, and quaternization with trimethylamine in a similar manner as described in Scheme 1.

The synthesis of cationic lipid **4**, in which the oxyethylene unit remains at C-2 of the pseudoglyceryl backbone, has been achieved as summarized in Scheme 3. Benzyl glycerol **8** was refluxed with one equiv. of hexadecyl tosylate in the presence of NaH in THF to yield the monoalkylated derivative **16**. It was then treated with **6b** under the same conditions to furnish **17**. This, on hydrogenolysis, afforded the corresponding alcohol, which was finally converted to the desired lipid **4** following the same route as described in the previous cases. All the numbered intermediates and the final products were characterized by their IR, ^1H NMR and mass spectra.[9]

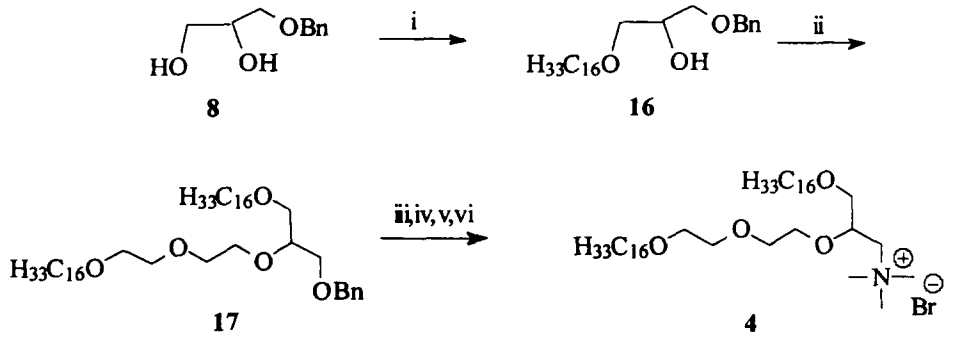
Sonication (10–15 min) of aqueous suspensions of each of the newly synthesized lipids above ≈50°C afforded stable translucent solutions. Existence of vesicle-like organizations in these suspensions was evident from the thermal studies. The thermal gel-to-liquid crystalline phase transition temperatures of vesicular **1**, **2** and **3** were found to be ca. 38, 39 and 31°C, respectively, on the basis of temperature-dependent anisotropy measurements using a fluorescent probe, DPH. The lipid **4**, however, showed a



Scheme 1. (i) *p*-TsCl, Py, CH_2Cl_2, rt, 25–50 h (82 and 68%); (ii) KOH, $PhCH_2Cl$, benzene, reflux, 23 h; (iii) *p*-TsOH, MeOH, CH_2Cl_2, rt, 5 h (overall 53% for steps ii and iii); (iv) **6**, NaH, benzene, reflux, 24 h (48 and 45%); (v) H_2, Pd/C, MeOH, EtOAc, 10 h (88 and 80%); (vi) *p*-TsCl, Py, CH_2Cl_2, rt, 27 h (87 and 98%); (vii) LiBr, DMF, 70°C, 8 h (81 and 84%); (viii) NMe_3, MeCN–toluene, 80°C, pressure tube, 24 h (74 and 68%)



Scheme 2. (i) DHP, conc. HCl, rt, 1 h (98%); (ii) **13**, aq. NaOH (50%), $Bu_4N^+HSO_4^-$, 65°C, 5 h; (iii) MeOH, CH_2Cl_2, conc. HCl, rt, 2 h (overall 34% for steps ii and iii); (iv) $C_{16}H_{33}Cl$, aq. NaOH (50%), $Bu_4N^+HSO_4^-$, 65°C, 5 days (53%); (v) H_2, Pd/C, MeOH, EtOAc, 12 h (85%); (vi) *p*-TsCl, Py, CH_2Cl_2, rt, 24 h (80%); (vii) LiBr, DMF, 70°C, 6 h (73%); (viii) NMe_3, MeCN–toluene, 80°C, pressure tube, 24 h (95%)



Scheme 3. (i) $C_{16}H_{33}OTs$, NaH, THF, reflux, 24 h (40%); (ii) **6b**, NaH, THF, reflux, 48 h (74%); (iii) H_2, Pd/C, MeOH, EtOAc, 12 h (79%); (iv) *p*-TsCl, Py, CH_2Cl_2, rt, 24 h (85%); (v) LiBr, DMF, 70°C, 20 h (59%); (vi) NMe_3, MeCN–toluene, 80°C, pressure tube, 24 h (72%)

broadened transition profile indicating the non-cooperative character of its melting due to mismatch in hydration at its C-1 and C-2 positions of the backbone.

In summary, we have been able to synthesize a series of novel cationic lipids, which will help in understanding the effect of modulation of hydration at the linkages of lipid molecules in model membranes. Subsequent synthetic extension to the corresponding phosphocholine lipids and elucidation of the precise effects of incorporation of *oligo*-oxyethylene spacers at the membrane level are currently underway in our laboratory. We are also exploring the transfection potential of these new cationic lipids.

Acknowledgements

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References

1. For general reviews, see: (a) Miller, A. D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1768. (b) Deshmukh, H. M.: Huang, L. *New J. Chem.* **1997**, *21*, 113. (c) Behr, J. P. *Acc. Chem. Res.* **1993**, *26*, 274.
2. (a) Bhattacharya, S.; Mandal, S. S. *Biochemistry* **1998**, *37*, 7764. (b) Wheeler, C. J.; Felgner, P. L.; Tsai, Y. J.; Marshall, J.; Sukhu, L.; Doh, S. G.; Hartikka, J.; Nietupski, J.; Manthorpe, M.; Nichols, M.; Plewe, M.; Liang, X.; Norman, J.; Smith, A.; Cheng, S. H. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 114–544.
3. (a) Song, Y. K.; Liu, F.; Chu, S.; Liu, D. *Human Gene Therapy* **1997**, *8*, 1585. (b) Felgner, J. H.; Kumar, R.; Sridhar, C. N.; Wheeler, C. J.; Tsai, Y. J.; Border, R.; Ramsey, P.; Martin, M.; Felgner, P. L. *J. Biol. Chem.* **1994**, *269*, 2550.
4. (a) Bhattacharya, S.; De, S. *J. Org. Chem.* **1998**, *63*, 7640. (b) Bhattacharya, S.; Ghosh, S.; Easwaran, K. R. K. *J. Org. Chem.* **1998**, *63*, 9232. (c) Bhattacharya, S.; De, S.; George, S. K. *J. Chem. Soc., Chem. Commun.* **1997**, 2287. (d) Ghosh, S.; Easwaran, K. R. K.; Bhattacharya, S. *Tetrahedron Lett.* **1996**, *37*, 5769. (e) Bhattacharya, S.; De, S. *J. Chem. Soc., Chem. Commun.* **1996**, 1283. (f) Bhattacharya, S.; De, S. *J. Chem. Soc., Chem. Commun.* **1995**, 651.
5. Zalipsky, S. *Advanced Drug Delivery Reviews* **1995**, *16*, 157.
6. For reviews, see: (a) Woodle, M. C.; Lasic, D. D. *Biochim. Biophys. Acta* **1992**, *1113*, 171. (b) Lasic, D. D.; Needham, D. *Chem. Rev.* **1995**, *95*, 2601.
7. Kates, M.; Chan, T. H.; Stanacev, N. Z. *Biochemistry* **1963**, *2*, 394.
8. Richard, A. B.; Bornislaw, P. C. *J. Org. Chem.* **1989**, *54*, 857.
9. All the new compounds exhibited spectral characteristics consistent with their given structures. Selected spectral data for the final compounds are as follows: (**1**) ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 6H, 2×-CH_3), 1.26 (s, 52H, 26×-CH_2-), 1.53–1.57 (m, 4H, 2×-O-CH_2-CH_2-), 3.41 (s, 9H, 3×-N^+CH_3), 3.43–3.[illegible] m, 14H, 7×-CH_2-O-), 3.90–3.92 (m,

1H, -O-CH_2-C*H*O-CH_2N^+-), 4.11–4.18 (m, 2H, -CHO-C*H*$_2N^+$-); IR: 1060–1150 cm^{-1} (br s, -C-O-C- str.); MALDI-TOF: calcd for $C_{42}H_{88}O_4N$: 671.2. Found: 672.2. (**2**) ^1H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 6H, 2×-C*H*$_3$), 1.26 (s, 52H, 26×-C*H*$_2$-), 1.55 (q, 4H, 2×-O-CH_2-C*H*$_2$-), 3.38 (s, 9H, 3×-N^+CH_3), 3.39–3.67 (m, 22H, 11×-C*H*$_2$-O-), 3.89–4.17 (m, 1H +2H, -O-CH_2-C*H*O-CH_2N^+-+-CHO-C*H*$_2N^+$-); IR: 1100 cm^{-1} (br s, -C-O-C- str.); MALDI-TOF: calcd for $C_{46}H_{96}O_6N$: 759.3. Found: 760.1. (**3**) ^1H NMR (500 MHz, $CDCl_3$) δ: 0.90 (t, 6H, 2×-C*H*$_3$), 1.27 (s, 52H, 26×-C*H*$_2$-), 1.55 (br s, 4H, 2×-O-CH_2-C*H*$_2$-), 3.41–3.72 (m, with a singlet at 3.49, 14H +9H, 7×-C*H*$_2$-O-+3×-N^+CH_3,), 3.97–4.14 (m, 1H +2H, -O-CH_2-C*H*O-CH_2N^+-+-CHO-C*H*$_2N^+$-); IR: 1100 cm^{-1} (br s, -C-O-C- str.); MALDI-TOF: calcd for $C_{42}H_{88}O_4N$: 671.2. Found: 672.0. (**4**) ^1H NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, 6H, 2×-C*H*$_3$); 1.25 (s, 52H, 26×-C*H*$_2$-), 1.54 (q, 4H, 2×-O-CH_2-C*H*$_2$-), 3.35 (s, 9H, 3×-N^+CH_3), 3.39–3.66 (m, 14H, 7×-C*H*$_2$-O-), 3.89–3.91 (m, 2H, -CHO-C*H*$_2N^+$-), 4.13 (m, 1H, -O-CH_2-C*H*O-CH_2N^+-); IR: 1070–1150 cm^{-1} (br, s, -C-O-C- str.); MALDI-TOF: calcd for $C_{42}H_{88}O_4N$: 671.2. Found: 671.6.