Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3147–3150
Bioorganic & Medicinal Chemistry Letters

Synthesis of carbamate-linked lipids for gene delivery

Dongliang Liu,[a,*] Jianjun Hu,[a] Weihong Qiao,[a] Zongshi Li,[a] Shubiao Zhang[b] and Lvbo Cheng[a]

[a]*State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China*
[b]*Department of Chemical Engineering, Dalian Nationalities University, Dalian 116600, China*

Received 28 January 2005; revised 3 April 2005; accepted 8 April 2005
Available online 3 May 2005

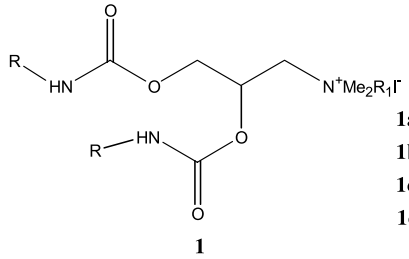
Abstract—Series of lipids **1a**–**d** and **2a**,**b**, with carbamate linkages between hydrocarbon chains and ammonium or tertiary amine head, which were pH sensitive, were synthesized for liposome-mediated gene delivery. The variable length of carbon chains and quaternary ammonium or neutral tertiary amine heads allowed to find the structure–function relationship of how these factors affect cationic lipids on gene delivery performance.

In 1987, Felgner et al. first reported the utilization of unnatural diether-linked cationic lipid (DOTMA) as a synthetic carrier to deliver gene into cells.[1] Since then, a number of published reports have described strategies for synthesis of versatile cationic lipids for gene delivery.[2] These studies covered diverse kinds of cationic lipids such as glycerol backbone based, cholesterol based, cationic peptides based, poly(ethylenimine) (PEI) based, poly(L-lysine) (PLL) based structures.[3] Cationic lipids, with prominent non-immunogenic character and low cellular toxicity in delivering gene, have engendered considerable interest by the gene therapy community.[4] It is generally believed that electrostatic interaction brings cationic lipids and polyanion DNA together to form DNA–liposome complexes. These complexes, once exposed to cells, are then taken up by the cells and the inserted gene expressed. Most of the spacers in the above mentioned synthesized lipids are ether, ester or amide bond, which are either too stable to be biodegraded thus cause cytotoxicity, such as ether linker, or liable to decompose in the circulation system such as the ester linker. Contrary to the above strategies, in this communication we developed six carbamate-linked lipids **1a**–**d** and **2a**,**b** having variable length of carbon chains and quaternary ammonium or neutral tertiary amine heads in the purpose to take advantage of the pH-sensitivity of the carbamate bond. It is familiar for chemists that compounds comprising carbamate bond is stable in the neutral circumstance and is liable to acid-catalyzed hydrolysis. An example of which is that di-*tert*-butyl dicarbonate ($O(Boc)_2$) is usually used as an excellent amino-protecting reagent for the formed carbamate can keep stable in the foregoing reaction and be acid-hydrolyzed in the acidic condition.[5] As well known, the pH value in cell is 1–2 lower than that of the circulation system, and it is expected that these carbamate-linked lipids can keep stable in the circulation system while decompose to release DNA after entering in-cell because of the pH decreasing from ex-cell to in-cell. Our idea is some what coincident to that of Boomer et al.'s, where they adopted a vinyl ether bond as linkage in the lipids.[6,7] In our work, dodecyl and octadecyl of carbon chain were selected for they represented two extremities of short and long. Quaternary ammonium heads containing ethyl or not were used to find if different cationic heads influence its binding with DNA phosphates. On the other side, iodide ion rather than chloride ion was adopted here as the counterion to see the gene delivery properties of this kind of ion though it may be expected that they will unlikely have significantly different gene delivery properties since the DNA phosphates will exchange for these counterions during the complexation step preceding transfection.

To acquire the above four cationic lipids, two intermediates **2a** and **b** were synthesized. Then **2** was converted to **1** through quaternization with suitable halogenated hydrocarbons. As well known cationic lipids can

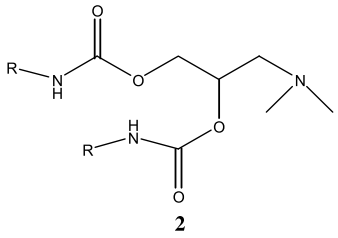
Keywords: Carbamate-linked; Lipids; Gene delivery; Structure–function relationship.
* Corresponding author. Tel.: +86 41188993902; fax: +86 41183683229; e-mail: dut-liu@163.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.04.010



1a: R_1=Me, R=$C_{12}H_{25}$
1b: R_1=Me, R=$C_{18}H_{37}$
1c: R_1=Et, R=$C_{12}H_{25}$
1d: R_1=Et, R=$C_{18}H_{37}$

1



2a R=$C_{12}H_{25}$
2b R=$C_{18}H_{37}$

2

combined well with the anionic DNA through electrostatic attraction to form DNA–liposome complex, yet cationic liposome may also cause cytotoxicity. Here we take neutral intermediates **2a** and **b** also as a kind of lipid vector and found they can form liposome through the Bangham method.[1,8]

Take **2a**, for example, a solution of **2a** (20 mg) in chloroform (1 ml) was evaporated to dryness under a stream of nitrogen, and residual solvent was removed under vacuum overnight. Liposome were prepared by resuspending the lipids in deionized water (2 ml) at above 30 °C and sonicating to clarity in a closed vial. The TEM photography of liposome formed by it was shown in Figure 1, and the applications of these neutral lipids as DNA carriers to deliver gene into cells in vitro and in vivo is now also in progress. It is expected that these lipids, if feasible, may reduce the cytotoxicity caused by their cationic counterparts.

The preparation of the initial intermediate 1-dimethylamino-2,3-propanediol **3** was mainly referenced to Alquist's route.[9] To prepare small amount of **3** in laboratory, we modified the reaction condition to atmosphere pressure.[10] Through distillation under reduced pressure, we got sufficient quantity of 1-dimethylamino-2,3-propanediol for the foregoing steps (Scheme 1).

Another intermediates alkyl isocyanates were prepared as follows: a saturated aqueous solution of Na_2CO_3

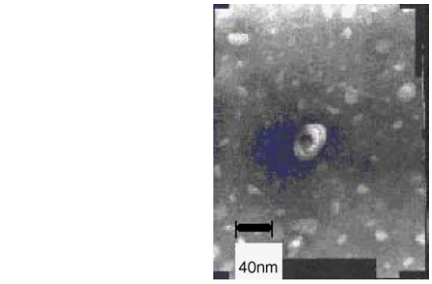
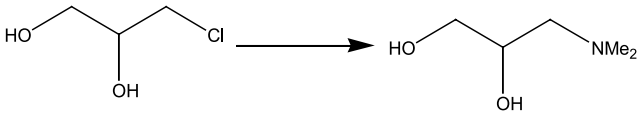
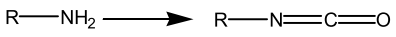


Figure 1. TEM photography of liposome formed by **2a**.



3

Scheme 1. Reagents and conditions: 1.3 equiv dimethylamine in aqueous solution, 1.5 equiv sodium hydroxide, 24 h, 33 °C, 60%.



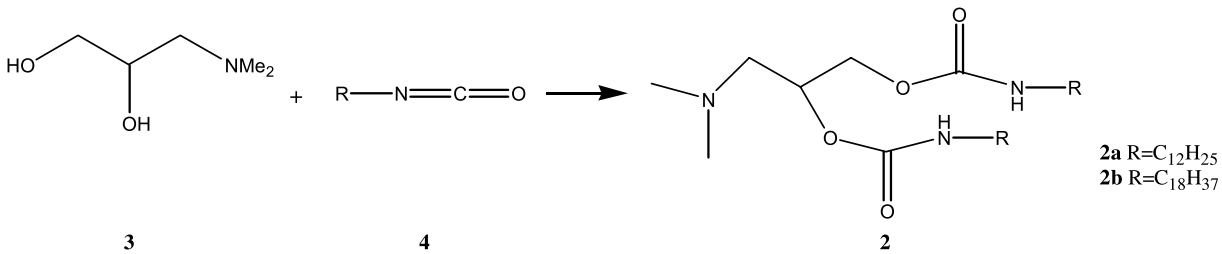
4

Scheme 2. Reagents and conditions: 5 equiv BTC in CH_2Cl_2 solution, saturated $NaCO_3$ aqueous solution, 2 h, room temperature, 60%. R = $C_{12}H_{25}$, $C_{18}H_{37}$. BTC: Bis-(trichloromethyl)-carbonate.

was added into a alkyl amine CH_2Cl_2 solution, after stirring for 5 min, a solution of BTC (1 N) in CH_2Cl_2 was added and the mixture was stirred vigorously for 2 h. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to afford the alkyl isocyanates, which was used for next step without further purification (Scheme 2).[11]

The neutral lipids **2** were synthesized by reactions of alkyl isocyanates with 1-dimethylamino-2,3-propanediol **3**. Reactions between **3** and the suitable isocyanates were carried out in toluene solution at 60–70 °C for 4 h, and Et_3N was used as the catalyst (Scheme 3).[12,13]

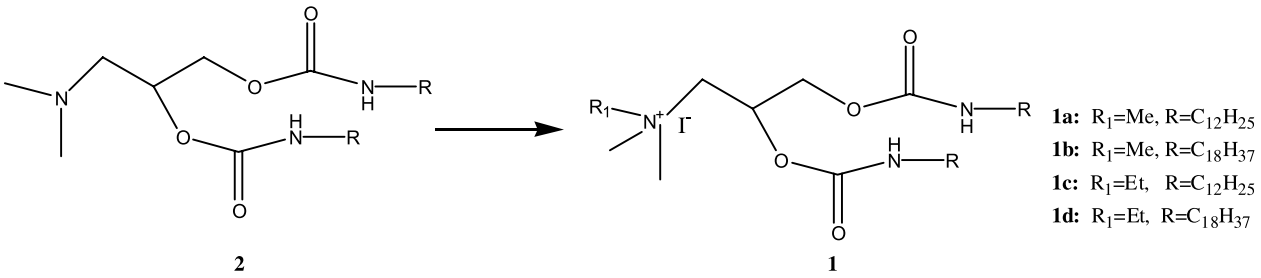
Cationic lipids **1** were acquired by quaternization of **2** with halogenated hydrocarbons.[1] A suitable halogenated hydrocarbon was condensed into a pressure apparatus containing a suitable **2**. The sealed vessel was heated behind a safety shield at 70 °C for 48 h. After



2a R=$C_{12}H_{25}$
2b R=$C_{18}H_{37}$

3 **4** **2**

Scheme 3. Reagents and conditions: 4 equiv **4**, 1 equiv Et_3N in toluene solution, 4 h, 60–70 °C, 43%.



Scheme 4. Reagents and conditions: 60 equiv CH_3I or C_2H_5I, 48 h, 70 °C, 95%.

cooling to 0 °C, the reaction vessel was opened, and the excess halogenated hydrocarbons was allowed to evaporate and then evaporated under reduced pressure. The crude residue was recrystallized from acetonitrile to afford **1** (Scheme 4).[14]

In summary, we have described the synthesis of series of carbamate-linked lipids **1a**–**d** and **2a**,**b** with the structural feature of having variable length of carbon chains and quaternary ammonium or neutral tertiary amine heads as well as iodide ions combined with them. Application of these carbamate-linked lipids as DNA carriers to deliver gene into cells in vitro and in vivo is now in progress.

References and notes

1. Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7413–7417.
2. For review of cationic lipid synthesis, see: (a) Miller, A. D. *Angew. Chem., Int. Ed.* **1998**, *37*, 1768–1785, and a recent review of ours; (b) Zhang, S. B.; Xu, Y. M.; Wang, B.; Qiao, W. H.; Liu, D. L.; Li, Z. S. *J. Controlled Release* **2004**, *100*, 165–180.
3. (a) Leventis, R.; Silvius, J. R. *Biochim. Biophys. Acta* **1990**, *1023*, 124–132; (b) Bennett, M. J.; Malone, R. W.; Nantz, M. H. *Tetrahedron Lett.* **1995**, *36*, 2207–2210; (c) Kikuchi, I. S.; Carmona-Ribeiro, A. M. *J. Phys. Chem. B* **2000**, *104*, 2829–2835; (d) Kim, A.; Lee, E. H.; Choi, S. H.; Kim, C. K. *Biomaterials* **2004**, *25*, 305–313; (e) Gao, X.; Huang, L. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 280–285; (f) Bottega, R.; Epand, R. M. *Biochemistry* **1992**, *31*, 9025–9030; (g) Walker, S.; Sofia, M. J.; Kakarla, R.; Kogan, N. A.; Wierichs, L.; Longley, C. B.; Bruker, K.; Axelrod, H. R.; Midha, S.; Babu, S.; Kahne, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1585–1590; (h) Smith, A. E. *Annu. Rev. Microbiol.* **1995**, *4*, 807–838; (i) Smith, L. C.; Duguid, J.; Wadhwa, M. S.; Logan, M. J.; Tung, C. H.; Edwards, V.; Sparrow, J. T. *Adv. Drug Delivery Rev.* **1998**, *30*, 115–131; (j) Rittner, K.; Benavente, A.; Bompard-Sorlet, A.; Heitz, F.; Divita, G.; Brasseur, R.; Jacobs, E. *Mol. Ther.* **2002**, *5*, 104–114; (k) El-Aneed, A. *J. Controlled Release* **2004**, *94*, 1–14; (l) Boussif, O.; Lezoualc'h, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7297–7301; (m) Lampel, P.; Eloma, M.; Ruponen, M.; Urtti, A.; Männistö, P. T.; Raasmaja, A. *J. Controlled Release* **2003**, *88*, 173–183; (n) Wu, G. Y.; Wu, C. H. *J. Biol. Chem.* **1987**, *262*, 4429–4432; (o) Ward, C. M.; Pechar, M.; Oupicky, D.; Ulbrich, K.; Seymour, L. W. *J. Gene Med.* **2002**, *4*, 536–547.
4. (a) Hood, J. D.; Bednarski, M.; Frausto, R.; Guccione, S.; Reisfeld, R. A.; Xiang, R.; Cheresh, D. A. *Science* **2002**, *296*, 2404–2407; (b) Cavazzana-Calvo, M.; Thrasher, A.; Mavilio, F. *Nature* **2004**, *427*, 779–781.
5. Kokotos, G.; Verger, R.; Chiou, A. *Chem. Eur. J.* **2000**, *6*, 4211–4217.
6. Boomer, J. A.; Thompson, D. H. *Chem. Phys. Lipids* **1999**, *99*, 145–453.
7. Boomer, J. A.; Thompson, D. H.; Sullivan, S. M. *Pharm. Res.* **2002**, *19*, 1292–1301.
8. Bangham, A. D.; Standish, M. M.; Watkins, J. C. *J. Mol. Biol.* **1965**, *13*, 238–253.
9. Alquist, F. N.; Slagh, H. R. US Pat. 2147226, **1939**.
10. For the purpose of increase of yield at atmosphere pressure, we carried out a $L_{4,3}$ orthogonal test; Liu, D. L.; Hu, J. J.; Qiao, W. H.; Li, Z. S.; Cheng, L. B.; Zhang, S. B., in preparation.
11. (a) Cotarca, L.; Delogu, P.; Nardelli, A.; Šunjić, V. *Synthesis* **1996**, *5*, 553–576; (b) Wang, X. Z.; Li, X. Q.; Shao, X. B.; Zhao, X.; Deng, P.; Jiang, X. K.; Li, Z. T.; Chen, Y. Q. *Chem. Eur. J.* **2003**, *9*, 2904–2913.
12. Moniruzzaman, M.; Goodbrand, B.; Sundararajan, P. R. *J. Phys. Chem. B* **2003**, *107*, 8416–8423.
13. Compound **2a**: ^{1}H NMR (400 M, $CDCl_3$) δ [5.82, 5.17, 5.07 (OCH, $2\times$NH)], [4.29 (d, J = 11.2, 1H), 4.22 (d, J = 11.2, 1H), OCH_2], [3.24, 3.13, 2.94, ($2\times NHCH_2$, NCH_2)], 2.72 (s, 6H, $N(CH_3)_2$), 1.48 (m, 4H, $2\times NHCH_2CH_2$), 1.23 (s, 36H, $2\times(CH_2)_9$), 0.86 (t, J = 5.6, 6.8, 6H, $2\times CH_3$); ^{13}C NMR (400 M, $CDCl_3$) δ 155.98 (C═O), 67.22 (OCH), 63.92 (OCH_2), 58.03 (NCH_2), 44.23 ($NHCH_2$), 41.44 ($N(CH_3)_2$), 32.11–17.70 ($(CH_2)_{10}$), 14.32 (CH_3); MS *m*/*z* 542 $[M+H]^+$. Compound **2b**:[1] H NMR (400 M, $CDCl_3$) δ [5.10, 4.87 (OCH, $2\times$NH)], 3.83 (d, J = 9.6, 2H, OCH_2), [3.15, 2.84, 2.76 ($2\times NHCH_2$, NCH_2)], 2.45 (s, 6H, $N(CH_3)_2$), 1.49 (m, 4H, $2\times NHCH_2$-CH_2), 1.25 (s, 60H, $2\times(CH_2)_{15}$), 0.86 (t, J = 4.6, 6.4, 6H, $2\times CH_3$); ^{13}C NMR (400 M, $CDCl_3$) δ 155.98 (C═O), 70.73 (OCH), 64.86 (OCH_2), 60.74 (NCH_2), 45.96 ($NHCH_2$), 41.34 ($N(CH_3)_2$), 32.12–22.89 ($(CH_2)_{16}$), 14.33 (CH_3); MS *m*/*z* 710 $[M+H]^+$.
14. Compound **1a**: ^{1}H NMR (400 M, $CDCl_3$) δ [5.98, 5.47, 5.39 (OCH, $2\times$NH)], 4.30–4.17 (OCH_2, NCH_2), 3.50 (s, 9H, $N(CH_3)_3$), 3.15 (d, J = 6.8, 4H, $2\times NHCH_2$), 1.51 (d, J = 6.4, 4H, $2\times NHCH_2CH_2$), 1.26 (s, 36H, $2\times(CH_2)_9$), 0.88 (t, J = 6.4, 6.4, 6H, $2\times CH_3$); ^{13}C NMR (400 M, $CDCl_3$) δ 155.78 (C═O), 66.76 (OCH), [63.27, 61.28 (OCH_2, NCH_2)], 55.08 ($N(CH_3)_3$), [41.58, 41.38 ($2\times NHCH_2$)], 32.09–22.86 ($(CH_2)_{10}$), 14.30 (CH_3); MS *m*/*z* 556 $[M-I]^+$, 127 $[I]^-$. Compound **1b**: ^{1}H NMR (400 M, $CDCl_3$) δ [5.86, 5.48, 5.32 (OCH, $2\times$NH)], [4.24, 3.79 (OCH_2, NCH_2)], 3.46 (s, 9H, $N(CH_3)_3$), 3.15 (d,

$J = 4.8$, 4H, $2 \times NHCH_2$), 1.51 (d, $J = 6.4$, 4H, $2 \times NHCH_2CH_2$), 1.26 (s, 60H, $2 \times (CH_2)_{15}$), 0.88 (t, $J = 6.4$, 6.8, 6H, $2 \times CH_3$); ^{13}C NMR (400 M, DMSO-d_6) δ 154.99 (C═O), 68.50 (OCH), [65.84, 61.38 (OCH_2, NCH_2)], 53.22 ($N(CH_3)_3$), 40.33 ($NHCH_2$), 31.33–22.14 ($(CH_2)_{16}$), 14.00 (CH_3); MS *m*/*z* 724 $[M-I]^+$, 127 $[I]^-$. Compound **1c**: ^1H NMR (400 M, CD_3CN) δ [5.86, 5.63, 5.34 (OCH, $2 \times NH$)], 4.21–4.06 (OCH_2, NCH_2), 3.33 (m, 2H, NCH_2CH_3), 3.13 (m, 4H, $2NHCH_2$), 3.00 (s, 6H, $N(CH_3)_2$), 1.43–0.85 ($2 \times NHCH_2CH_2$, $2(CH_2)_9$, NCH_2CH_3, hidden behind $2 \times (CH_2)_9$, $2 \times CH_3$); MS *m*/*z* 570 $[M-I]^+$, 127 $[I]^-$. Compound **1d**: ^1H NMR (400 M, DMSO-d_6) δ [5.23, 5.14, 4.89 (OCH, $2 \times NH$)], 3.55–3.15 (OCH_2, NCH_2, NCH_2CH_3, one peak hidden behind that of H_2O, 3.37), 3.02 (s, 6H, $N(CH_3)_2$), 2.76 (m, 4H, $2 \times NHCH_2$), 1.38–0.85 ($2 \times NHCH_2CH_2$, $2 \times (CH_2)_{15}$, NCH_2CH_3, hidden behind $2 \times (CH_2)_{15}$, $2 \times CH_3$); ^{13}C NMR (400 M, DMSO-d_6) δ 154.97 (C═O), 68.18 (OCH), [63.02, 61.32, 59.75 (OCH_2, NCH_2, NCH_2CH_3)], 50.61 (NCH_3), 40.33 ($NHCH_2$), 31.33–22.13 ($(CH_2)_{16}$), 13.99 (CH_3), 7.95 (NCH_2CH_3); MS *m*/*z* 738 $[M-I]^+$, 127 $[I]^-$.