Chinese Chemical Letters Vol. 12, No. 9, pp 779– 802, 2001
http://www.imm.ac.cn/journal/ccl.html

Design and Synthesis of Lipids Bearing Nucleosides as Gene Vectors

Man Zhou ZHU, Qi Hua WU, Qing Xiang GUO*

Department of Chemistry, University of Science and Technology of China, Hefei 230026

Abstract: Some novel lipids bearing nucleosides were designed and synthesized as gene vectors, and the structures of these compounds were characterized by UV, IR, ^{1}HNMR, ^{13}CNMR and elemental analysis.

Keywords: Gene vector, lipid, nucleoside, synthesis.

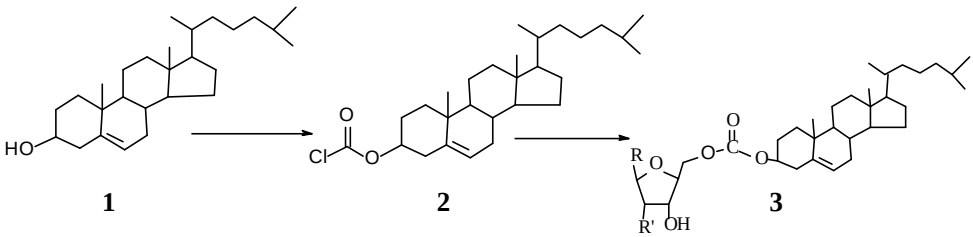
Delivery of exogenous DNA into cells of various origins plays an important role not only in basic research, but also in clinical application in gene therapy. Great efforts have been made towards the development of viral and nonviral vectors for these purposes[1~6]. However, at the present stage, none of the vectors can claim to be the panacea. Although most viral vectors can transfer gene efficiently and some of them can be used for gene transfer into tissue cells *in vivo*, they suffer from immunogenicity, toxicity, lack of tissue specificity, difficulty in large scale production and potential risk of inducing tumorigenic mutations and/or generating active viral particles through recombination[7]. Synthetic vectors such as liposomes have drawn increasing attention due to their simplicity, non-immunogenicity, low toxicity and commercial availability[8,9].

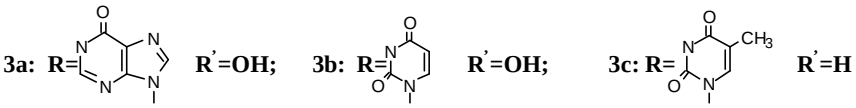
Cholesterol derivatives as gene vectors can increase the stability of liposomes perhaps by stabilizing the bilayers and their complexes with DNA to improve the transfection efficiency[10]. In 1991, Gao and Huang reported the cholesterol-based cationic lipid DC-chol[11]. Since then, several groups have been working in synthesis and application of the steroidal cationic lipids[12-17]. Two of the most efficacious cytofectins reported to date are cholesterol derivatives: polyamine lipid 67[18] and CTAP[19]. ATP with a cholesteryl group at the ã-phosphate can enter through membrane[20]. In this letter, we wish to report the synthesis of a series of lipids with a cholesteryl group as hydrophobic tail, nucleoside as head. It is hoped that as gene vectors these lipids may help DNA to enter the cells.

Cholestryl chloroformate (2) was prepared *via* the reaction of cholesterol with phosgene. To a stirred solution of 4.49g (0.01 mol) of cholestryl chloroformate in 60 mL anhydrous pyridine a mixture of 4g (0.015 mol) of inosine and 40 mL anhydrous pyridine was slowly added under ice-cooling. After stirring at room temperature for 2 days, the pyridine solution was concentrated under reduced pressure and the residue was neutralized with dilute hydrochloride. The resulting precipitate was collected by filtration, washed with water and dried to afford a solid, which was chromatographed

* E-mail: qxguo@ustc.edu.cn

from silica column with $CHCl_3$/MeOH. **3a** was obtained as white powder. **3b** and **3c** were prepared by the same procedure. The experimental study on the gene delivery *in vitro* with the synthetic lipids in this work is undergoing.





3a yield 63%. mp 199-200°C; UV (ETOH), λ_{max} (nm) 204, 250; IR (cm^{-1}, KBr) 3417 (O-H), 2939, 2867 (CH_2CH_2), 1738 (O-CO_2), 1698 (O=C-N), 1591, 1551, 1515, 1465, 1379, 1223, 1251, 1134, 1089; ^{1}H NMR (500MHz, DMSO-d_6 δ ppm) 8.35 (s, 1H, H-8''), 8.09 (d, 1H, H-2''), 5.85 (m, 1H, H-1'), 5.37 (t, 1H, J=2.5Hz, H-6), 5.12 (1H, H-3'), 4.82 (1H, H-2'), 4.40 (m, 1H, H-4'), 4.16 (m, 1H, H-3), 3.85 (m, 2H, H-5'), 1-2.5 (m, 28H, CH, CH_2), 1.08 (s, 3H, H-18), 0.99 (d, 3H, J=6.5Hz, H-21), 0.90 (d, 3H, J=2.2Hz, H-27), 0.84 (d, 3H, J=2.2Hz, H-26), 0.65 (s, 3H, H-19); ^{13}C NMR (DMSO-d_6 δ ppm) 156.4 (C-6''), 153.2 (O-CO_2), 148.2 (C-4''), 145.9 (C-2''), 139.2 (C-5), 138.7 (C-8''), 124.5 (C-6), 122.3 (C-5''), 86.9 (C-1'), 83.1 (C-4'), 77.4 (C-3'), 76.4 (C-3), 72.3 (C-2'), 61.1 (C-5'), 56.1 (C-14), 55.6 (C-17), 49.4 (C-9), 40.0 (C-13,C-4), 36-40 (C-10,C-1,C-24,C-16), 35.6 (C-22), 35.1 (C-8,C-20), 31.9 (C-7), 31.3 (C-2), 28.0 (C-12), 27.3 (C-25), 24.3 (C-15), 23.8 (C-23), 23.1 (C-27), 22.5 (C-26), 22.3 (C-11), 18.8 (C-18), 18.5 (C-21), 11.6 (C-19); Anal. calcd. for ($C_{38}H_{56}N_4O_7$) C, 67.02; H, 8.31; N, 8.23. Found: C, 67.21; H, 8.17; N, 8.05.

3b yield 84%. mp 198-199°C; UV λ_{max} (nm) 205, 260; IR (cm^{-1}, KBr) 3441 (O-H), 2935, 2867 (CH_3CH_2), 1742 (O-CO_2), 1705 (O=C-N), 1468, 1381, 1252, 1094; ^{1}H NMR (500MHz, DMSO-d_6 δ ppm) 7.83 (d, 1H, H-6''), 5.79 (d, 1H, H-5''), 5.70 (m, 1H, H-1'), 5.34 (t, 1H, J=2.5Hz, H-6), 4.98 (m, 1H, H-3'), 4.42 (m, 1H, H-2'), 4.40 (m, 1H, H-4'), 4.06 (m, 1H, H-3), 3.60 (m, 2H, H-5'), 1-2.5 (m, 28H, CH, CH_2), 1.07 (s, 3H, H-18), 0.99 (d, 3H, J=6.5Hz, H-21), 0.89 (d, 3H, J=2.2Hz, H-27), 0.85 (d, 3H, J=2.2Hz, H-26), 0.64 (s, 3H, H-19); ^{13}C NMR (DMSO-d_6 δ ppm) 162.58 (C-4''), 152.94 (O-CO_2), 150.50 (C-2''), 140.10 (C-5), 139.07 (C-6''), 122.01 (C-6), 102.02 (C-5''), 86.96 (C-1'), 85.87 (C-4'), 82.19 (C-3'), 77.26 (C-3), 71.35 (C-2'), 60.79 (C-5'), 56.0 (C-14), 55.48 (C-17), 49.4 (C-9), 40.1 (C-4,C-13), 36-40 (C-10,C-1,C-24,C-16), 34.84 (C-22), 33.81 (C-8,C-20), 31.21 (C-7), 30.89 (C-2), 27.94 (C-12), 27.04 (C-25), 24.3 (C-15), 23.21 (C-23), 22.98 (C-27), 22.06 (C-26), 22.01 (C-11), 18.64 (C-18), 18.32 (C-21),

11.71 (C-19); Anal. calcd. for ($C_{37}H_{56}N_2O_8$) C, 67.64; H, 8.61; N, 4.27. Found: C, 67.28; H, 8.43; N, 4.32.

3c yield 79%. mp 183-184°C; UV λ_{max} (nm) 210, 262; IR (cm^{-1}, KBr) 3441 (O-H), 2933, 2867 (CH_3CH_2), 1749 (O-CO_2), 1693 (O=C-N), 1469, 1382, 1274; ^1H NMR (500MHz, $CDCl_3$ δ ppm) 8.65 (s, 1H, H-3''), 7.38 (1H, H-6''), 6.31 (1H, H-1'), 5.34 (t, 1H, J=2.5Hz, H-6), 4.42 (m, 3H, H-5', H-3'), 4.20 (m, 1H, H-4'), 3.47 (m, 1H, H-3), 2.40 (m, 2H, H-2'), 1.92 (s, 3H, H-7''), 1-2.5 (m, 28H, CH, CH_2), 1.06 (s, 3H, H-18), 0.99 (d, 3H, J=6.5Hz, H-21), 0.90 (d, 3H, J=2.2Hz, H-27), 0.85 (d, 3H, J=2.2Hz, H-26), 0.63 (s, 3H, H-19); ^{13}C NMR ($CDCl_3$ δ ppm) 164.27 (C-4''), 154.41 (O-CO_2), 150.89 (C-2''), 146.99 (C-5), 135.83 (C-6), 111.34 (C-6''), 99.96 (C-5''), 85.23 (C-1'), 84.31 (C-4'), 71.36 (C-3'), 66.92 (C-5'), 56.30 (C-14), 56.03 (C-17), 46.28 (C-9), 42.76 (C-2'), 40.59 (C-4,C-13), 40.43 (C-16), 39.88 (C-24), 39.61 (C-1), 36.25 (C-10), 35.87 (C-22), 34.64 (C-8,C-20), 31.43 (C-7), 28.07 (C-2), 28.0 (C-12), 26.45 (C-25), 23.96 (C-15), 22.87 (C-7), 21.57 (C-23), 22.60 (C-26), 22.58 (C-27), 18.75 (C-11), 16.21 (C-18), 12.64 (C-21), 12.26 (C-19); Anal. calcd. for ($C_{38}H_{58}N_2O_7$) C, 69.68; H, 8.94; N, 4.28. Found: C, 69.73; H, 9.01; N, 3.98.

Acknowledgment

This research was supported by the National Natural Science Foundation of China.

References

1. A.Kasid, S. Morecki, P. Aebersold, K. Cornetta, K. Culver, S. Freeman, E. Director, M. T. Lotee, R. M. Blaese, W. F. Anderson, S. A. Rosenberg, *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 473.
2. W. F. Anderson, *Science* **1992**, *256*, 808.
3. R. C. Mulligan, *Science* **1993**, *260*, 926.
4. C. E. Dunbar, *Annu. Rev. Med.*, **1996**, *47*, 11.
5. P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, M. Danielsen, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 7413.
6. P. L. Felgner, G. M. Ringold, *Nature* **1989**,*337*, 387.
7. J. F. Engelhardt, X. Ye, B. Doranz, J. M. Wilson, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 6196.
8. A. D. Miller, *Angew. Chem. Int. Ed.*, **1998**, *37*, 1768.
9. H. Li, Q. X. Guo, *Ziran Zazhi* **1998**, *20*, 275.
10. N. S. Templeton, D. D. Lasic, P. M. Prederik, H. H. Strey, D. D. Roberts, G. N. Pavlakis, *Nature Biotechnology* **1997**, *15*, 651.
11. X. Gao, L. Huang, *Biochem. Biophys. Res. Commun.* **1991**, *179*, 280.
12. K. Takeuchi, M. Ishihara, C. Kawaura, M. Noji, T. Furuno, M.Nakanishi, *FEBS Lett.* **1996**, *397*, 207.
13. R. Okayama, M. Noji, M. Nakanishi, *FEBS Lett.* **1997**, *408*, 232.
14. J. P. Vigneron, N. Oudrhiri, M. Fauquet, L.Vergely, J. C. Bradley, M. Basseville, P. Lehn, J. M. Lehn, *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 9682.
15. F. X. Tang, J. A. Hughes, *Bioconjugate Chem.* **1999**, *10*, 791.
16. T. Ren, G. Zhang, F. Liu, D. Liu, *Bioorg. & Med. Chem. Lett.* **2000**, *10*, 1.
17. M.Z.Zhu, J.N.Wei, Q.H.Wu, X.Q.Zheng, Q.X.Guo *Synth. Commun.* **2000**, *30*, 2325.
18. E. R. Lee, J. Marshall, C. S. Siegel, C. Jiang, N. S. Yew, M. R. Nichols, J. B. Nietupski, R. J.

Ziegler, M. B. Lane, K. X. Wang, N. C. Wan, R. K. Scheule, D. J. Harris, A. E. Smith, S. H. Cheng, *Hum. Gene Ther.*, **1996**, *7*, 170.
19. R. G. Cooper, C. J. Etheridge, L. Stewart, J. Marshall, S. Rudginsky, S. H. Cheng, A. D. Miller, *Chem. Eur. J.*, **1998**, *4*, 137.
20. A. Kreimeyer, F. Andre, C. Gougette, T. Huynh-Dinh, *Angew. Chem. Int. Ed.* **1998**, *37*, 2853.

Received 23 February, 2001