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Synthesis of Novel Lipids Bearing Cholesteryl Group as Gene Vectors

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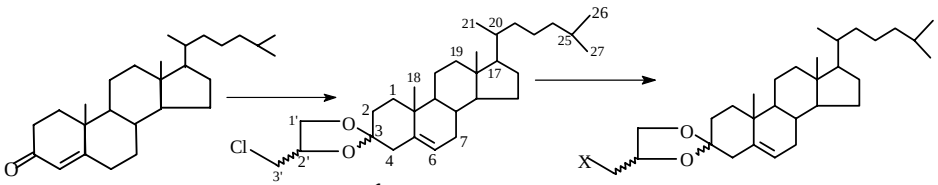
Abstract: Some cationic and neutral lipids bearing cholesteryl group were synthesized as gene vectors, and the structures of the compounds were characterized by IR, ^{1}HNMR, MS and elemental analysis.

Keywords: Cholesteryl derivatives, gene vector, lipid.

With the rapid developments in the human genome sequencing project and the discovery of disease gene, gene therapy will be one of the major methods to cure disease in the twenty-first century. One of the key points in gene therapy is to prepare appropriate vectors for gene delivery[1,2]. The strategy utilizing viral vectors are now well established and widely used[3-5]. At the present stage, a possibility still remains that infection by a viral particle disorders the normal cell function and/or produces unexpected deleterious viruses. In spite of lower efficiency, transfection with artificial liposomes would not entail such risks. Cationic liposomes have attracted organic chemists because this species, if the transfection efficiency is high enough and does not have notable cytotoxicity, would serve as clinically useful vector systems for the delivery of therapeutic nucleic acids to patients[6-8].

Cholesterol derivatives have been reported as gene vectors recently since they can increase the stability of liposomes perhaps by stabilizing the bilayers and their complexes with DNA to improve the transfection efficiency[9]. Two of the most efficaceous cytofectins reported to date are cholesterol derivatives, polyamine Lipid 67[10] and CTAP [11]. From the standpoint of gene therapy, a good vector should be stable outside the cells and degradable after entering cells[6,12]. In the present communication, we wish to report the synthesis of a series of novel cationic and neutral lipids with a cholesteryl group as hydrophobic tail, ketal as linker bond. In acidic environment inside of cells (pH=2~5), the ketals should be easy to break up resulting little toxicity, while in the neutral environment outside of cells (pH=7), they should be stable as gene carriers. These properties will be significant in gene delivery process.



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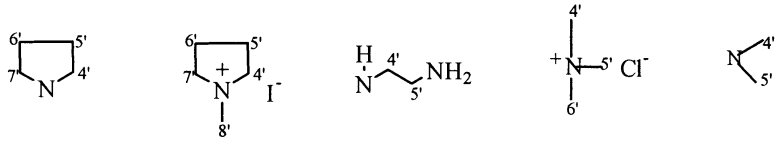

X= **2** **3** **4** **5** **6**

General Procedure

Compound **1**[12] (6.3 mmol) dissolved in pyrrolidine (10 mL) was refluxed for 6 hrs. After removal of the excess pyrrolidine, the residue was extracted with water and $CHCl_3$. The organic layer was separated by silica column with $CHCl_3$/MeOH (20:1 v/v) to give **2**. A mixture of compound **2** (1.95 mmol) and iodomethane (1mL) in $CHCl_3$/DMSO (1:1 v/v) binary solvents was stirred for 24 hrs in dark. After removal of solvents, **3** was crystallized from $CHCl_3$/MeOH solvents. A mixture of compound **1** (4.2 mmol) and ethylenediamine (10 mL) was refluxed for 10 hrs under nitrogen. After removal of the excess ethylenediamine, the residue was separated by silica gel column chromatography using $CHCl_3$/MeOH (8:1) as eluent to give **4**. A methanol solution of compound **1** (4.2 mmol) and trimethylamine (0.1 mol) in autoclave was heated at 120°C for 24 hrs. Getting rid of methanol and the residue was separated by silica gel, interestingly, a demethylation product **6** rather than **5** was obtained as product. Alternatively, the reaction was carried out at 80°C for 7 days, the same results were obtained. In other systems, the demethylation of N–methyl quaternary ammonium compounds *via* S_N2 displacement by Br^- or I^- in an alcohol solvent has been reported[13-15].

According to the mechanism for demethylation proposed in the literature[15,16], the surfactant molecules aggregated while the product formed. The halide ions may attack the carbon atom of the methyl in the ammonium cation at the interface between the aggregates and methanol, and the amine was eliminated as leaving group. In this way, **6** was produced.

2. mp. 104-106°C, yield 76%. ^{1}HMNR (500MHz,$CDCl_3$) δ 0.68 (s, 3H, H-19), 0.86 (d, J=2.2Hz, 3H, H-26), 0.87 (d, J=2.2Hz, 3H, H-27), 0.92 (d, J=6.5Hz, 3H, H-21), 1.01 (s, 3H, H-18), 1~2 (strong coupling 32H, CH_2, CH), 2.40 (br.s, 4H, H-4', H-7'), 2.43 (m, 1H, H-3'), 2.51 (m, 1H, H-3'), 3.55, 3.64 (41:59, m, 1H, H-1'), 4.08, 4.10 (59:41, m, 1H, H-1'), 4.28 (m, 1H, H-2'), 5.33 (t, J=2.5Hz, 1H, H-6). IR (KBr) ν 3440, 2939, 1374, 1095 cm^{-1}. Elemental Analysis ($C_{34}H_{57}NO_2$) cal. C 79.78, H

11.22, N 2.74. Found C 79.51, H 11.07, N 2.56. EI-MS: *m/z* 511 (0.08), 384 (0.08), 229 (0.21), 178 (2.95), 128 (4.59), 84 (100), 55 (6.54).

3. mp. 264 °C (decomposition), yield 63%. ^{1}HMNR (500MHz, $CDCl_3$) δ 0.68 (s, 3H, H-19), 0.86 (d, J=2.2Hz, 3H, H-26), 0.87 (d, J=2.2Hz, 3H,H-27), 0.92 (d, J=6.5Hz, 3H, H-21), 1.02 (s, 3H, H-18), 1~2 (strong coupling, 28H, CH_2, CH), 2.39 (m, 4H, H-5' and H-6'), 3.02 (br.s, 3H, H-8'), 3.35 (br.s, 6H, H-4', H-7' and H-3'), 3.76 (m, 1H, H-1'), 4.10 (m, 1H, H-1'), 4.18 (br.s, 1H, H-2'), 5.62 (br.s, 1H, H-6). IR (KBr) ν 3439, 2938, 1376, 1112cm^{-1}. Elemental Analysis ($C_{35}H_{60}NO_2I$) cal. C 64.30, H 9.25, N 2.14. Found C 64.57, H 9.43, N 2.11.

4. mp. 98-101°C, yield 56%. ^{1}HMNR (500MHz, $CDCl_3$) δ 0.67 (s,3H, H-19), 0.86 (d, J=2.2Hz, 3H, H-26), 0.87 (d, J=2.2Hz, 3H, H-27), 0.91 (d, J=6.5Hz, 3H, H-21), 1.02 (s, 3H, H-18), 1~2 (strong coupling 28H, CH_2, CH), 2.42 (t, J=6.3Hz, 2H, H-5'), 2.70 (m, 3H, H-3' and H-4'), 2.72 (m, 1H, H-3'), 3.65, 3.68 (52:48, m, 1H, H-1'), 4.07, 4.11 (48:52, m, 1H, H-1'), 4.25 (m, 1H, H-2'), 5.33 (t, J=2.5Hz, 1H, H-6'). IR (KBr) ν 3441, 2938, 2884, 1096 cm^{-1}. Elemental Analysis ($C_{32}H_{56}N_2O_2$) cal. C 76.75, H 11.27, N 5.59. Found C 76.48, H 11.15, N 5.72. EI-MS: *m/z* 470 (4.92), 384 (0.69), 229 (0.89), 211 (30.45), 156 (30.96), 113 (100), 55 (17.6).

6. mp.140-141°C, ^{1}HMNR (500MHz, $CDCl_3$) δ 0.68 (s, 3H, H-19), 0.86 (d, J=2.2 Hz, 3H, H-26), 0.88 (d, J=2.2Hz, 3H, H-27), 0.92 (d, J=6.5 Hz, 3H, H-21), 1.04 (s, 3H, H-18), 1~2 (strong coupling, 28H, CH_2, CH), 2.27 (s, 6H, H-4' H-5'), 2.35 (m, 1H, H-3'), 2.48 (m, 1H, H-3'), 3.62 (m, 1H, H-1'), 4.11 (m, 1H, H-1'), 4.24 (m, 1H, H-2'), 5.34 (t, J=2.5Hz, 1H, H-6). IR(KBr) ν 2936, 2884, 2867, 2766, 1380, 1365, 1099 cm^{-1}. Elemental Analysis ($C_{32}H_{55}NO_2$) cal. C 79.12, H 11.41, N 2.88. Found C 78.87, H 11.19, N 3.05. EI-MS: *m/z* 485 (0.10), 382 (0.13), 269 (0.15), 229 (0.37), 156 (2.93), 102 (11.10), 84 (7.79), 58 (100), 43 (5.39).

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