0.85–2.50 (49H, *m*, methine, methylene, and methyl protons), 2.94 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.87 (6H, *br* s, CH_2NH $COCH_2NCH_2CH_2CH_2NHCO$), 3.92 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.32 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 853.8 $[M + H]^+$ (100).

Lipid **IICXe** Yield: (resin: 1.1 mmol/g, 130.0 mg) 24.4 mg, 20%; IR: ν_{max} 3298, 3084, 2925, 2852, 1676, 1542, 1467, 1378, 1255, 1201, 1133 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.6 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.3 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (20H, *s*, methylene protons of fatty acid), 0.85–2.50 (49H, *m*, methine, methylene, and methyl protons), 2.93 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.88 (6H, *br* s, CH_2NH $COCH_2NCH_2CH_2CH_2NHCO$), 3.92 (2H, *br s*, $NHCOCH_2N$), 4.42 (1H, *br s*, H-3-Chol), 5.32 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 867.6 $[M + H]^+$ (100).

Lipid **IICXf** Yield: (resin: 1.1 mmol/g, 130.0 mg) 31.1 mg, 25%; IR: ν_{max} 3276, 2924, 2852, 1677, 1542, 1466, 1377, 1247, 1201 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.6 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.2 Hz, H-21-Chol), 0.97 (3H, *s*, H-19-Chol), 1.22 (22H, *s*, methylene protons of fatty acid), 0.85–2.50 (49H, *m*, methine, methylene, and methyl protons), 2.92 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.88 (6H, *br* s, CH_2NHCO $CH_2NCH_2CH_2CH_2NHCO$), 3.91 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.32 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 881.9 $[M + H]^+$ (100).

Lipid **IICXg** Yield: (resin: 1.1 mmol/g, 130.0 mg) 21.2 mg, 17%; IR: ν_{max} 3276, 2928, 2853, 1677, 1539, 1466, 1378, 1255, 1202, 1134 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.3 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (14H, *s*, methylene protons of fatty acid), 0.85–2.50 (57H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.37 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2CH_2CH_2NHCO$), 3.89 (2H, *br s*, $NHCOCH_2N$), 4.39 (1H, *br s*, H-3-Chol), 5.30, 5.39 (3H, *br s*, H-6-Chol and C*H*=C*H* in fatty acid), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 907.8 $[M + H]^+$ (100).

Lipid **IICXh** Yield: (resin: 1.1 mmol/g, 130.0 mg) 28.0 mg, 22%; IR: ν_{max} 3308, 2924, 2851, 1681, 1541, 1467, 1379, 1251, 1201, 1136 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.6 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.3 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (26H, *s*, methylene protons of fatty acid), 0.85–2.50 (49H, *m*, methine, methylene, and methyl protons), 2.99 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.20, 3.41 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2CH_2CH_2NHCO$), 3.96 (2H, *br s*, $NHCOCH_2N$), 4.42 (1H, *br s*, H-3-Chol), 5.33 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 909.7 $[M + H]^+$ (100).

Lipid **IICXi** Yield: (resin: 1.1 mmol/g, 130.0 mg) 19.7 mg, 15%; IR: ν_{max} 3297, 3070, 2925, 2852, 1681, 1548, 1467, 1378, 1253, 1202, 1135 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.3 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (28H, *s*, methylene protons of fatty acid), 0.85–2.50 (49H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.37 (6H, *br* s, CH_2NH $COCH_2NCH_2CH_2CH_2NHCO$), 3.89 (2H, *br s*, $NHCOCH_2N$), 4.30 (1H, *br s*, H-3-Chol), 5.33 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 923.9 $[M + H]^+$ (100).

Lipid **IICXj** Yield: (resin: 1.1 mmol/g, 130.0 mg) 20.8 mg, 16%; IR: ν_{max} 3306, 3077, 2924, 2851, 1677, 1535, 1467, 1379, 1255, 1201, 1134 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.3 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (30H, *s*, methylene protons of fatty acid), 0.85–2.50 (49H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.38 (6H, *br* s, CH_2NH $COCH_2NCH_2CH_2CH_2NHCO$), 3.89 (2H, *br s*, $NHCOCH_2N$), 4.30 (1H, *br s*, H-3-Chol), 5.32 (1H, *br s*, H-6-Chol), 7.65 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 937.8 $[M + H]^+$ (100).

Lipid **IICYa** Yield: (resin: 1.1 mmol/g, 130.0 mg) 42.0 mg, 36%; IR: ν_{max} 3298, 3055, 2930, 2853, 1679, 1539, 1467, 1378, 1254, 1202, 1136, 1048 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (12H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.17, 3.38 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.91 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.65 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 825.8 $[M + H]^+$ (100).

Lipid **IICYb** Yield: (resin: 1.1 mmol/g, 130.0 mg) 40.8 mg, 34%; IR: ν_{max} 3307, 2928, 2853, 1679, 1555, 1466, 1379, 1254, 1202, 1135, 1048 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (14H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.17, 3.38 (6H, *br* s, CH_2NH $COCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.90 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 839.8 $[M + H]^+$ (100).

Lipid **IICYc** Yield: (resin: 1.1 mmol/g, 130.0 mg) 49.5 mg, 41%; IR: ν_{max} 3302, 3062, 2926, 2852, 1679, 1631, 1540, 1467, 1378, 1254, 1202, 1135 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (16H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.17, 3.37 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.89 (2H, *br s*, $NHCOCH_2N$), 4.40 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 853.9 $[M + H]^+$ (100).

Lipid **IICYd** Yield: (resin: 1.1 mmol/g, 130.0 mg) 50.0 mg, 40%; IR: ν_{max} 3297, 2926, 2852, 1677, 1558, 1466, 1379, 1254, 1202, 1135, 1048 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (18H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.17, 3.40 (6H, *br* s, CH_2NH $COCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.90 (2H, *br s*, $NHCOCH_2N$), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 867.7 $[M + H]^+$ (100).

Lipid **IICYe** Yield: (resin: 1.1 mmol/g, 130.0 mg) 47.2 mg, 38%; IR: ν_{max} 3297, 3055, 2924, 2852, 1681, 1623, 1540, 1467, 1378, 1263, 1201, 1134 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz,

H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (20H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.17, 3.41 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.89 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 881.8 $[M + H]^+$ (100).

Lipid **IICYf** Yield: (resin: 1.1 mmol/g, 130.0 mg) 53.8 mg, 42%; IR: ν_{max} 3291, 2924, 2852, 1674, 1540, 1467, 1377, 1251, 1201 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (22H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.18, 3.40 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2(CH_2)_2CH_2NHCO$), 3.90 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 895.8 $[M + H]^+$ (100).

Lipid **IICYg** Yield: (resin: 1.1 mmol/g, 130.0 mg) 45.8 mg, 35%; IR: ν_{max} 3331, 2927, 2854, 1679, 1632, 1559, 1466, 1383, 1365, 1252, 1202, 1171 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (14H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.35 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.90 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.30, 5.39 (3H, *br s*, H-6-Chol and CH=CH in fatty acid), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 921.9 $[M + H]^+$ (100).

Lipid **IICYh** Yield: (resin: 1.1 mmol/g, 130.0 mg) 43.1 mg, 33%; IR: ν_{max} 3299, 2923, 2852, 1679, 1627, 1540, 1467, 1377, 1254 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (26H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.20, 3.41 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2(CH_2)_2CH_2NHCO$), 3.91 (2H, *br s*, $NHCOCH_2N$), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 923.9 $[M + H]^+$ (100).

Lipid **IICYi** Yield: (resin: 1.1 mmol/g, 130.0 mg) 40.7 mg, 31%; IR: ν_{max} 3306, 3062, 2924, 2851, 1679, 1540, 1467, 1378, 1254, 1202, 1135 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (28H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.35 (6H, *br* s, CH_2NH $COCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.91 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 937.9 $[M + H]^+$ (100).

Lipid **IICYj** Yield: (resin: 1.1 mmol/g, 130.0 mg) 34.0 mg, 25%; IR: ν_{max} 3291, 2923, 2852, 1678, 1540, 1467, 1378, 1254, 1202, 1135, 1052 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (30H, *s*, methylene protons of fatty acid), 0.85–2.50 (51H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.38 (6H, *br* s, CH_2NH $COCH_2NCH_2(CH_2)_2CH_2NHCO$), 3.91 (2H,

br s, NHCOCH_2N), 4.41 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 951.0 $[M + H]^+$ (100).

Lipid **IICZa** Yield: (resin: 1.1 mmol/g, 130.0 mg) 17.3 mg, 14%; IR: ν_{max} 3298, 2931, 2854, 1681, 1623, 1540, 1466, 1378, 1253, 1202, 1135, 1049 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (12H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.13, 3.35 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_4CH_2NHCO$), 3.90 (2H, *br s*, NHCOCH_2N), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 853.8 $[M + H]^+$ (100).

Lipid **IICZb** Yield: (resin: 1.1 mmol/g, 130.0 mg) 19.6 mg, 16%; IR: ν_{max} 3284, 2929, 2853, 1681, 1540, 1466, 1377, 1247, 1201, 1174, 1134, 1045 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (14H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.40 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_4CH_2NHCO$), 3.90 (2H, *br s*, NHCOCH_2N), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 867.8 $[M + H]^+$ (100).

Lipid **IICZc** Yield: (resin: 1.1 mmol/g, 130.0 mg) 25.1 mg, 20%; IR: ν_{max} 3297, 2928, 2853, 1681, 1541, 1467, 1377, 1253, 1202, 1134, 1050 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.88 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (16H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.40 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_4CH_2NHCO$), 3.90 (2H, *br s*, NHCOCH_2N), 4.41 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 881.9 $[M + H]^+$ (100).

Lipid **IICZd** Yield: (resin: 1.1 mmol/g, 130.0 mg) 18.3 mg, 14%; IR: ν_{max} 3323, 2928, 2854, 1682, 1633, 1559, 1466, 1383, 1358, 1252, 1202, 1170 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (18H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.41 (6H, *br* s, $CH_2NHCOCH_2NCH_2(CH_2)_4CH_2NHCO$), 3.89 (2H, *br s*, NHCOCH_2N), 4.41 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 895.8 $[M + H]^+$ (100).

Lipid **IICZe** Yield: (resin: 1.1 mmol/g, 130.0 mg) 24.7 mg, 19%; IR: ν_{max} 3269, 2926, 2852, 1679, 1541, 1467, 1377, 1252, 1134, 1045 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (20H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.39 (6H, *br* s, CH_2NH $COCH_2NCH_2(CH_2)_4CH_2NHCO$), 3.90 (2H, *br s*, NHCOCH_2N), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 909.8 $[M + H]^+$ (100).

Lipid **IICZf** Yield: (resin: 1.1 mmol/g, 130.0 mg) 17.6 mg, 13%; IR: ν_{max} 3297, 2927, 2852, 1679, 1540, 1467, 1377, 1252, 1202 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (22H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.40 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2(CH_2)_4CH_2NHCO$), 3.90 (2H, *br s*, $NHCOCH_2N$), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 923.0 $[M + H]^+$ (100).

Lipid **IICZg** Yield: (resin: 1.1 mmol/g, 130.0 mg) 19.1 mg, 14%; IR: ν_{max} 3290, 2930, 2854, 1681, 1540, 1466, 1378, 1202, 1133 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (14H, *s*, methylene protons of fatty acid), 0.85–2.50 (63H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.41 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2(CH_2)_4CH_2NHCO$), 3.91 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.31, 5.39 (3H, *br s*, H-6-Chol and C*H*=C*H* in fatty acid), 7.60 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 949.9 $[M + H]^+$ (100).

Lipid **IICZh** Yield: (resin: 1.1 mmol/g, 130.0 mg) 24.3 mg, 18%; IR: ν_{max} 3291, 2924, 2852, 1681, 1541, 1467, 1377, 1255, 1201, 1134, 1048 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (26H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.41 (6H, *br* s, CH_2NH $COCH_2$ $NCH_2(CH_2)_4CH_2NHCO$), 3.89 (2H, *br s*, $NHCOCH_2N$), 4.41 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 951.8 $[M + H]^+$ (100).

Lipid **IICZi** Yield: (resin: 1.1 mmol/g, 130.0 mg) 16.2 mg, 12%; IR: ν_{max} 3284, 3070, 2925, 2852, 1681, 1540, 1467, 1377, 1252, 1202, 1134, 1045 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (28H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.30 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.16, 3.40 (6H, *br* s, $CH_2NHCOCH_2$ $NCH_2(CH_2)_4CH_2NHCO$), 3.90 (2H, *br s*, $NHCOCH_2N$), 4.40 (1H, *br s*, H-3-Chol), 5.30 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 965.0 $[M + H]^+$ (100).

Lipid **IICZj** Yield: (resin: 1.1 mmol/g, 130.0 mg) 24.0 mg, 17%; IR: ν_{max} 3284, 2924, 2852, 1679, 1540, 1467, 1377, 1252, 1202, 1134, 1030 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18-Chol), 0.84 (6H, *d*, *J* = 6.5 Hz, H-26, 27-Chol), 0.89 (3H, *d*, *J* = 6.0 Hz, H-21-Chol), 0.98 (3H, *s*, H-19-Chol), 1.22 (30H, *s*, methylene protons of fatty acid), 0.85–2.50 (55H, *m*, methine, methylene, and methyl protons), 2.31 (2H, *br s*, $H_3N^+CH_2CH_2$), 3.15, 3.40 (6H, *br* s, CH_2NH $COCH_2$ $NCH_2(CH_2)_4CH_2NHCO$), 3.91 (2H, *br s*, $NHCOCH_2N$), 4.40 (1H, *br s*, H-3-Chol), 5.31 (1H, *br s*, H-6-Chol), 7.59 (3H, *br s*, NH_3^+); ESMS (+ve): *m/z* (% rel. intensity): 979.1 $[M + H]^+$ (100).

สรุปผลการศึกษาและวิจารณ์ผล

จากการสังเคราะห์ไขมันประจุบวกกลุ่มที่ 2 ซึ่งมีโครงสร้างไขมันส่วนหางแบบไม่สมมาตร ด้วยเทคนิคการสังเคราะห์บนวัฏภาคของแข็ง สังเคราะห์สารได้ทั้งหมด 180 ชนิด การหาเอกลักษณ์ของสารดังกล่าวอาศัยข้อมูลทาง IR ^{1}H NMR และ MS สเปกโทรสโกปี จากการทดสอบประสิทธิภาพของการพาดีเอ็นเอเข้าเซลล์ HEK293 เบื้องต้น พบไขมันประจุบวก 8 ชนิด ที่มีประสิทธิภาพดีกว่า Effectene^{TM} ซึ่งเป็นสารมาตรฐาน การศึกษาสภาวะที่ทำให้ไขมันประจุบวกทั้ง 8 ชนิดให้มีประสิทธิภาพสูงที่สุด พบว่าไขมันประจุบวกมีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ HEK293 และ PC3 ได้ดีกว่าสารมาตรฐานทั้งสามชนิดคือ Effectene^{TM}, DOTAP และ DC-Chol และไขมันประจุบวกนี้ไม่มีความเป็นพิษต่อเซลล์ จากการศึกษาลักษณะของไลโปโซมและไลโปเพล็กซ์ ที่เตรียมจากไขมันประจุบวก ทั้ง 8 ชนิด พบว่ามีลักษณะเป็น unilamellar และ multilamellar มีขนาด 50-150 และ 300-700 nm ตามลำดับ

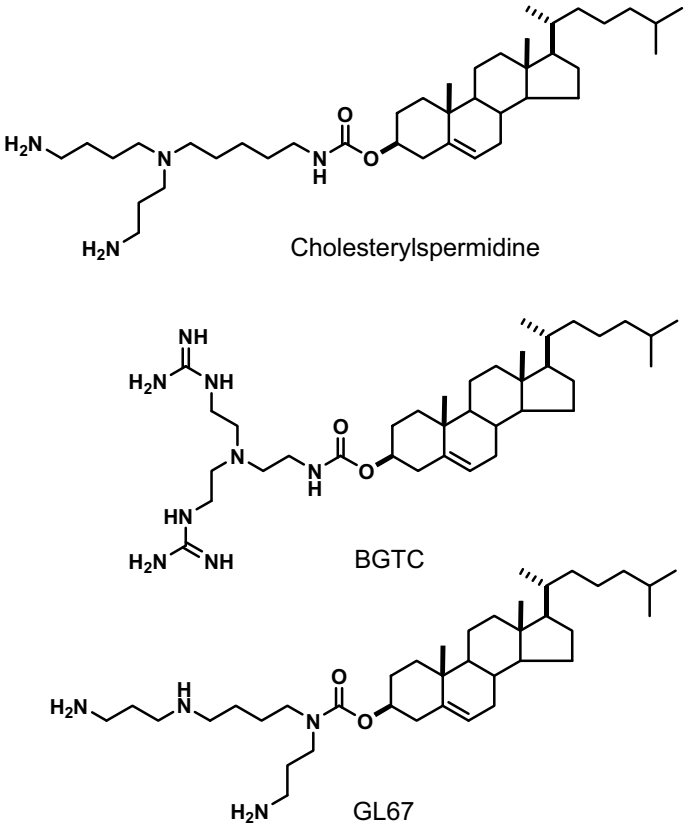
โครงการย่อยที่ 3
การสังเคราะห์ไขมันประจุบวกที่มีส่วนหัวประจุบวกแบบไม่สมมาตร

โครงการย่อยที่ 3
การสังเคราะห์ไขมันประจุบวกที่มีส่วนหัวประจุบวกแบบไม่สมมาตร

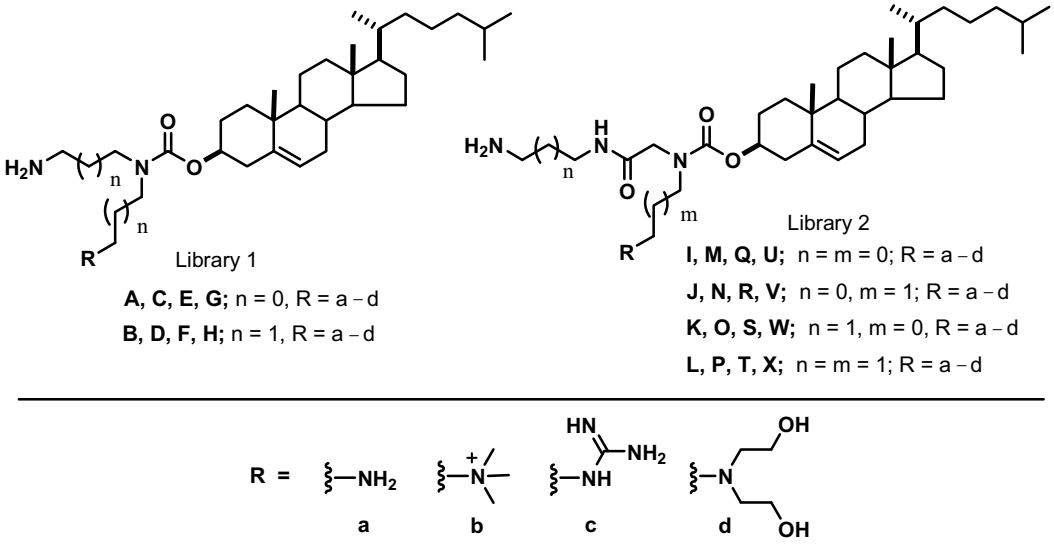
ผลการศึกษา

การสังเคราะห์ไขมันประจุบวก A-X

จากการรายงานการสังเคราะห์ไขมันประจุบวกที่ผ่านมาพบว่า ไขมันประจุบวกที่มี cholesterol เป็นส่วนหางนั้น จะมีส่วนหัวประจุบวกที่มีหมู่ฟังก์ชันด้านปลายเหมือนกัน[37-39] (แบบสมมาตร) ดังตัวอย่างรูปที่ 22 เนื่องจากยังไม่มีรายงานการสังเคราะห์ไขมันประจุบวกที่มี cholesterol เป็นส่วนไขมันส่วนหางที่ประกอบด้วยประจุบวกส่วนหัวแบบผสม ดังนั้นจึงเป็นสิ่งที่น่าสนใจในการสังเคราะห์และศึกษาประสิทธิภาพในการพาดีเอ็นเอเข้าเซลล์ของไขมันประจุบวกกลุ่มที่ 3 ซึ่งมีโครงสร้างดังแสดงในรูปที่ 23 โครงสร้างสารกลุ่มที่ 3 นี้ แบ่งออกเป็นสองกลุ่มย่อยคือ กลุ่มที่ 1 (Library 1) เป็นกลุ่มที่มีความยาวสายโซ่ส่วนหัวแบบสมมาตร และกลุ่มที่ 2 (Library 2) เป็นกลุ่มที่มีความยาวสายโซ่ส่วนหัวแบบไม่สมมาตร

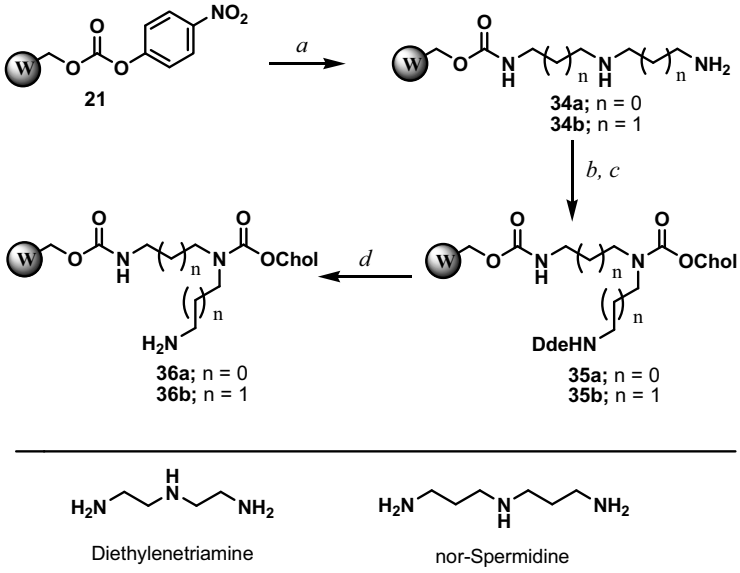


รูปที่ 23 ไขมันประจุบวกที่มีส่วนหัวประจุบวกแบบสมมาตร



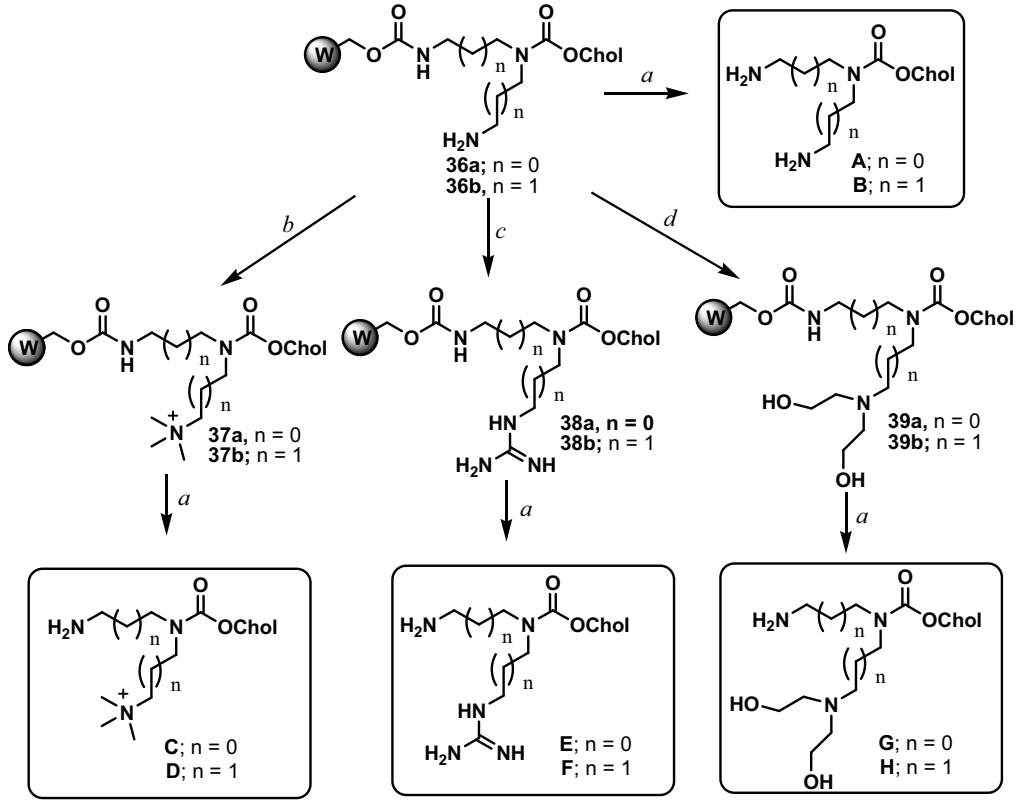
รูปที่ 24 โครงสร้างของไขมันประจุบวกกลุ่มที่ 3

การสังเคราะห์สารกลุ่มที่ 3 ได้แสดงใน Scheme 5 ถึง Scheme 8 เริ่มจากการสังเคราะห์ resin **36** ที่ใช้ในการสังเคราะห์ไขมันประจุบวก Library 1 (Scheme 5) โดยนำ active carbonate resin (**21**) มาทำปฏิกิริยากับ diethylenetriamine หรือ nor-spermidine ที่มากเกินพอในตัวทำละลาย CH_2Cl_2 เป็นเวลา 16 ชั่วโมงได้ resin **34** หลังจากการล้างจน resin สะอาด สามารถยืนยันว่ามีหมู่เอมีนอิสระอีกด้านหนึ่งโดยการทดสอบด้วยสารทดสอบ ninhydrin[35] ซึ่งในที่นี้จะให้สารละลายสีน้ำเงินเข้ม นำ resin **34** มาทำปฏิกิริยากับ Dde-OH ซึ่งเป็นหมู่ป้องกันที่เลือกทำปฏิกิริยากับ primary amine เท่านั้น ทำปฏิกิริยาข้ามคืน ล้างผลิตภัณฑ์ที่ได้ให้สะอาด และทำปฏิกิริยาต่อกับ cholesteryl chloroformate ได้ resin **35** กำจัดหมูป้องกัน Dde ออกด้วย N_2H_4/DMF ได้ผลิตภัณฑ์ resin **36**



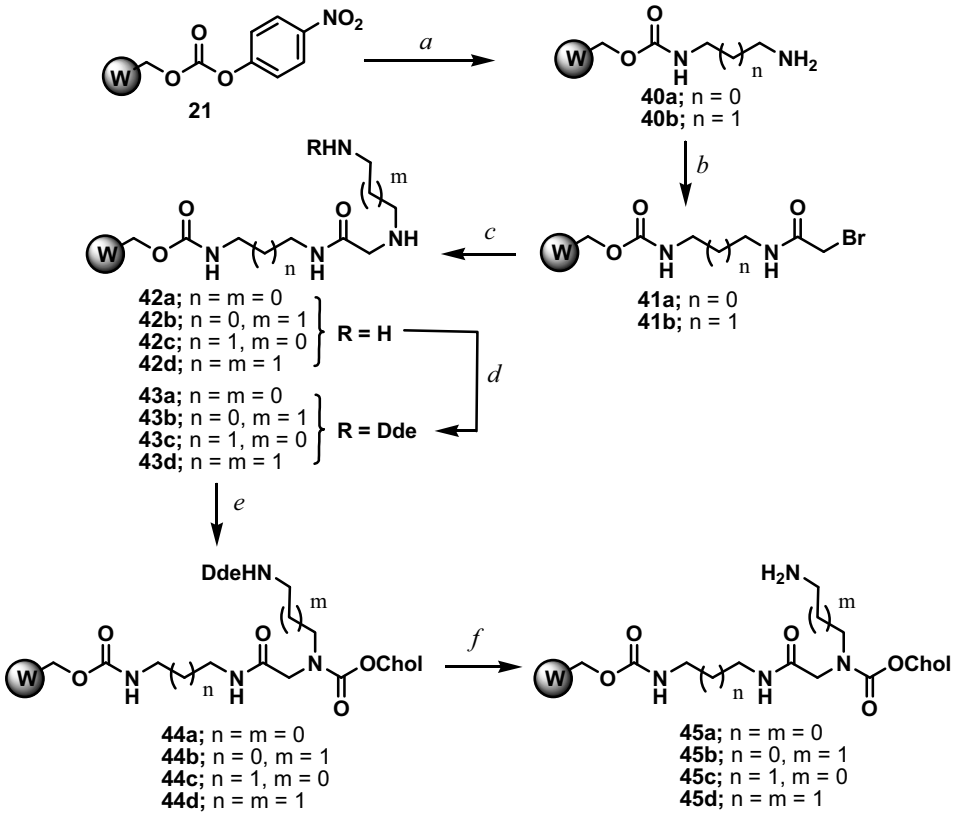
Scheme 5 *Reagents and conditions*: a) diethylenetriamine or nor-spermidine (excess), CH_2Cl_2, 6 h; b) Dde-OH (excess), CH_2Cl_2, DMF, 12 h; c) cholesteryl chloroformate (4 equiv), pyridine, CH_2Cl_2, 12 h; d) 5% N_2H_4/DMF, 2×30 min.

การสังเคราะห์ไขมันประจุบวก Library 1 แสดงใน Scheme 6 เพื่อเปรียบเทียบประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ของไขมันประจุบวกที่มีประจุบวกส่วนหัวแบบสมมาตรเปรียบเทียบกับแบบไม่สมมาตร จึงต้องทำการสังเคราะห์ไขมันประจุบวก **A** และ **B** เริ่มจากนำ resin **36** มาทำปฏิกิริยากับ 50% TFA/CH_2Cl_2 นาน 2 ช.ม. ได้ผลิตภัณฑ์ **A** และ **B** ตามต้องการ การสังเคราะห์ไขมันประจุบวกชนิดประจุบวกส่วนหัวแบบไม่สมมาตร (**C-H**) โดยการปรับเปลี่ยนหมู่ amine ของ resin **36** เป็นหมู่ trimethylamino, di(2-hydroxyethyl)amino และหมู่ guanidinyl การสังเคราะห์ไขมันประจุบวก **C** และ **D** ซึ่งมีประจุส่วนหัวเป็นหมู่ trimethylamino โดยนำ resin **36** ทำปฏิกิริยากับ methyliodide ในตัวทำละลาย DMF โดยมี DIEA เป็นเบส หลังจากทำปฏิกิริยากับ 50% TFA ได้ไขมันประจุบวก **C** และ **D** การสังเคราะห์ไขมันประจุบวกที่มีหมู่ guanidinyl (**E** และ **F**) และหมู di(2-hydroxyethyl)amino (**G** และ **H**) ก็ทำได้เช่นเดียวกันโดยการนำ resin **36** มาทำปฏิกิริยากับ *N,N′*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea และ 2-bromoethanol ตามลำดับและตามด้วยการ cleavage ด้วย 50% TFA/CH_2Cl_2



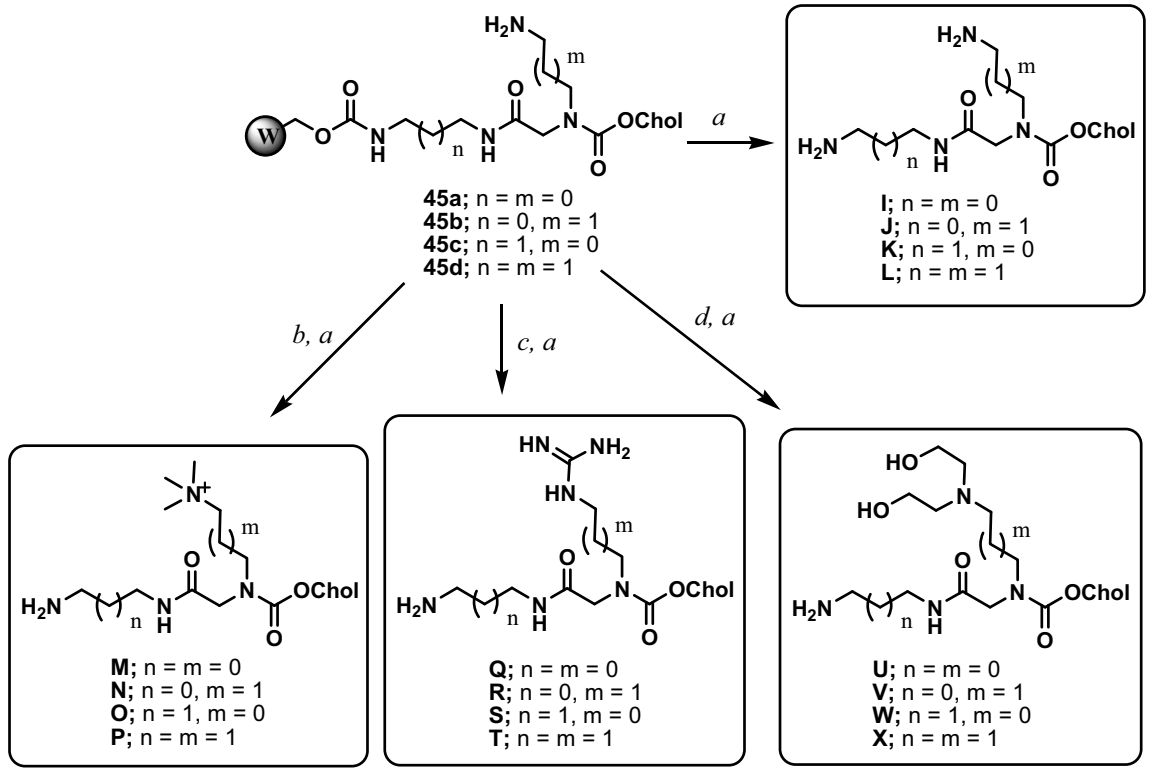
Scheme 6 *Reagents and conditions*. a) 50% TFA/CH_2Cl_2, 2 h; b) MeI (10 equiv), DIEA, DMF, 18 h; c) *N,N′*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (4 equiv), DIEA, DMF, 18 h.; d) 2-bromoethanol (8 equiv), DIEA, 18 h.

การสังเคราะห์สารกลุ่มที่ 3 Library 2 โดยที่มีประจุส่วนหัวแบบไม่สมมาตรและมีความยาวสายโซ่แบบไม่สมมาตร แสดงใน Scheme 7 และ 8 เริ่มจากการนำ resin **21** มาทำปฏิกิริยากับ 1,2-diamonoethane หรือ 1,3-diaminopropane ได้ resin **40** นำผลิตภัณฑ์ที่ได้ไปทำปฏิกิริยาต่อกับ



Scheme 7. *Reagents and conditions*: a) 1,2-diaminoethane or 1,3-diaminopropane (excess), CH_2Cl_2, 6 h.; b) bromoacetic acid (4 equiv), DIC (4 equiv), DMF, 12 h.; c) 1,2-diaminoethane or 1,3-diaminopropane (excess), DMF, 12 h.; d) Dde-OH (excess), CH_2Cl_2, DMF, 12 h.; e) cholesteryl chloroformate (4 equiv), pyridine (20 equiv), CH_2Cl_2, 12 h.; f) 5% N_2H_4/DMF, 2×30 min.

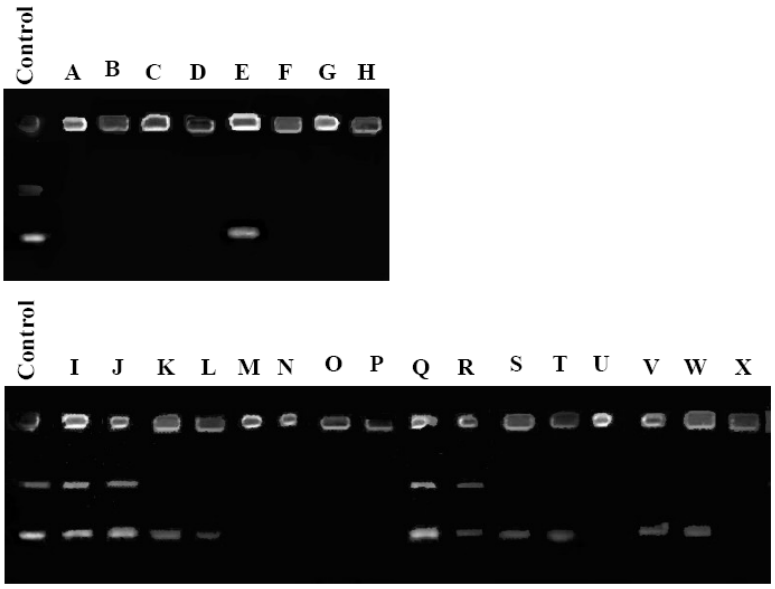
การสังเคราะห์ไขมันประจุบวก **I-X** แสดงดัง Scheme 8 ทำได้เช่นเดียวกันกับการสังเคราะห์ไขมันประจุบวก **A-H** ไขมันประจุบวกกลุ่มที่ 3 ที่สังเคราะห์ได้มีทั้งหมด 24 สารที่สังเคราะห์ได้มีเปอร์เซ็นต์ผลิตภัณฑ์ปานกลางถึงสูงมาก ทำการพิสูจน์โครงสร้างของสารที่สังเคราะห์ได้ ด้วยเทคนิค IR, NMR และ MS ข้อมูลทางสเปกโทรสโกปีที่ได้ตรงตามโครงสร้างที่สังเคราะห์



Scheme 8. *Reagents and conditions*. a) 50% TFA/CH_2Cl_2, 2 h; b) MeI (10 equiv), DIEA, DMF, 18 h; c) *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (4 equiv), DIEA, DMF, 18 h.; d) 2-bromoethanol (8 equiv), DIEA, 18 h.

การศึกษาการจับดีเอ็นเอของไขมันประจุบวก A-X

นำไขมันประจุบวก **A-X** ที่สังเคราะห์ได้มาทดสอบประสิทธิภาพในการจับดีเอ็นเอ ในการทดลองนี้ใช้วิธีการทางอิเล็กโตรโฟเรซีส เพื่อดูการหน่วงการเคลื่อนที่ของดีเอ็นเอ ผลการทดลองแสดงดังรูปที่ 25 พบว่า ไขมันประจุบวกในกลุ่มแรก (**A-H**) ที่มีสายโซ่แบบสมมาตร สามารถหน่วงการเคลื่อนที่ดีเอ็นเอได้ทุกสารยกเว้น ไขมันประจุบวก **E** สำหรับ ไขมันประจุบวกในกลุ่มที่สอง (**I-X**) ที่มีสายโซ่แบบไม่สมมาตรมีประสิทธิภาพการหน่วงดีเอ็นเอได้น้อยกว่าสารกลุ่มแรก สารในกลุ่มหลังนี้ ไขมันประจุบวก **M-P** หน่วงดีเอ็นเอได้ดีเมื่อส่วนหัวมีขั้วเป็นหมู่ trimethyl quaternary amine สำหรับไขมันประจุบวก **I-L** และ **Q-T** ที่มีส่วนหัวมีขั้วเป็นหมู่ amine และ guanidine, ตามลำดับ หน่วงการเคลื่อนที่ของดีเนเอได้ไม่สมบูรณ์ เป็นที่น่าสังเกตว่า ไขมันประจุบวกที่มีส่วนหัวมีขั้วเป็นหมู่ trimethyl quaternary amine (**C**, **D** และ **M-P**) สามารถจับกับดีเอ็นเอได้อย่างดี

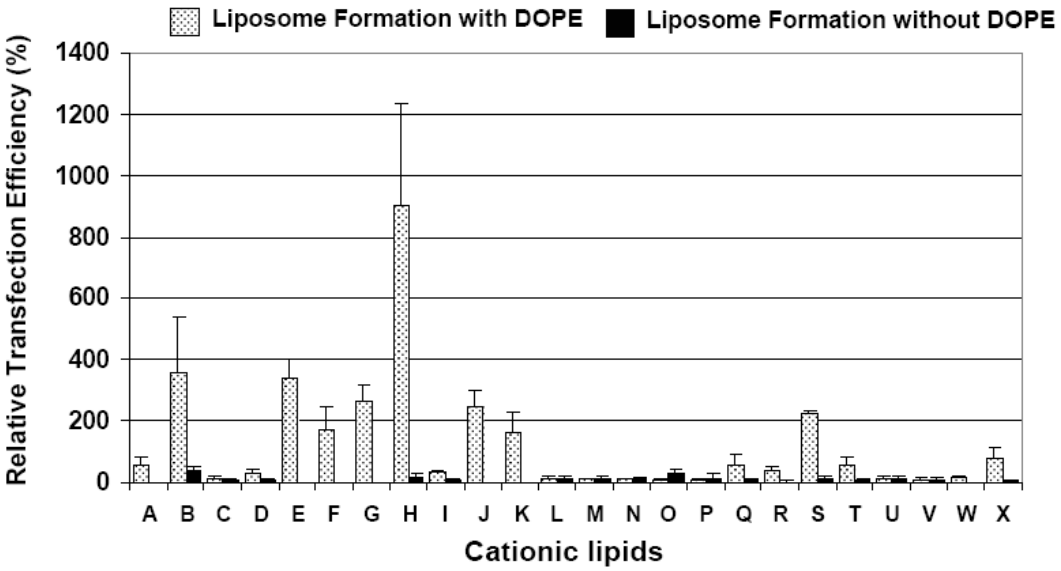


รูปที่ 25 การทดสอบการหน่วงการเคลื่อนที่ของดีเอ็นเอของไขมันประจุบวก **A-X**

การศึกษาการพาดีเอ็นเอเข้าสู่เซลล์ของไขมันประจุบวก A-X

การศึกษาเบื้องต้น

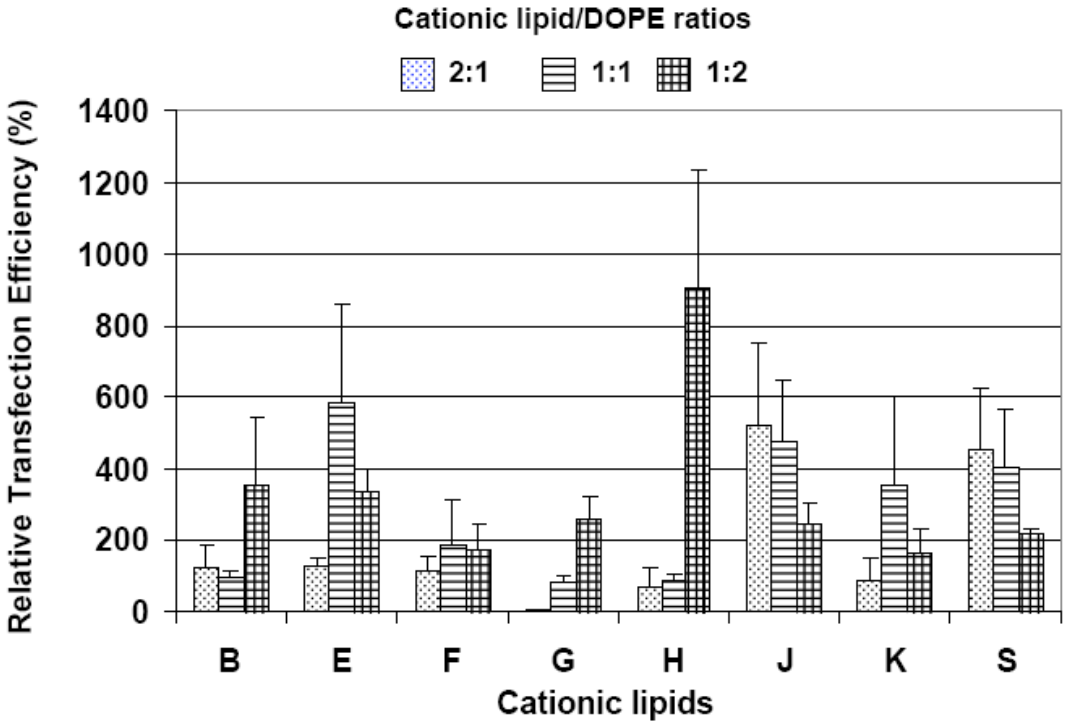
นำสารที่สังเคราะห์ได้ทั้งหมดมาศึกษาประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ การทดสอบนี้เตรียมไลโปโซมโดยการผสมไขมันประจุบวกต่อไขมันตัวช่วย (DOPE) ในอัตราส่วน 2:1 และนำไลโปโซมที่เตรียมได้มาผสมกับดีเอ็นเอ ในอัตราส่วนดีเอ็นเอต่อไลโปโซม 1:20 การศึกษาเบื้องต้นนี้ใช้เซลล์ human embryonic kidney (HEK) และ ดีเอ็นเอที่มี β-galactosidase เป็นยีนรายงานผล การทดลองนี้ใช้ Effectene^{TM} เป็นสารมาตรฐานและคำนวณให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์เท่ากับ 100% ผลการผลการทดลองแสดงดังรูปที่ 26 จากการทดลองแสดงให้เห็นว่าไขมันประจุบวก **B**, **E-H**, **J**, **K** และ **S** มีประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีกว่าสารมาตรฐาน Effectene^{TM} ซึ่งสารดังกล่าวนี้ส่วนมากเป็นไขมันประจุบวกกลุ่มที่ 1 ที่มีสายโซ่แบบสมมาตร นำไขมันประจุบวกทั้ง 8 สารนี้ไปศึกษาเพื่อหาสภาวะที่ทำให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีที่สุด



รูปที่ 26 ผลการพาดีเอ็นเอของไขมันประจุบวก **A-X** เข้าสู่เซลล์ HEK

การศึกษาอัตราส่วนของไขมันประจุบวกต่อไขมันตัวช่วย (DOPE)

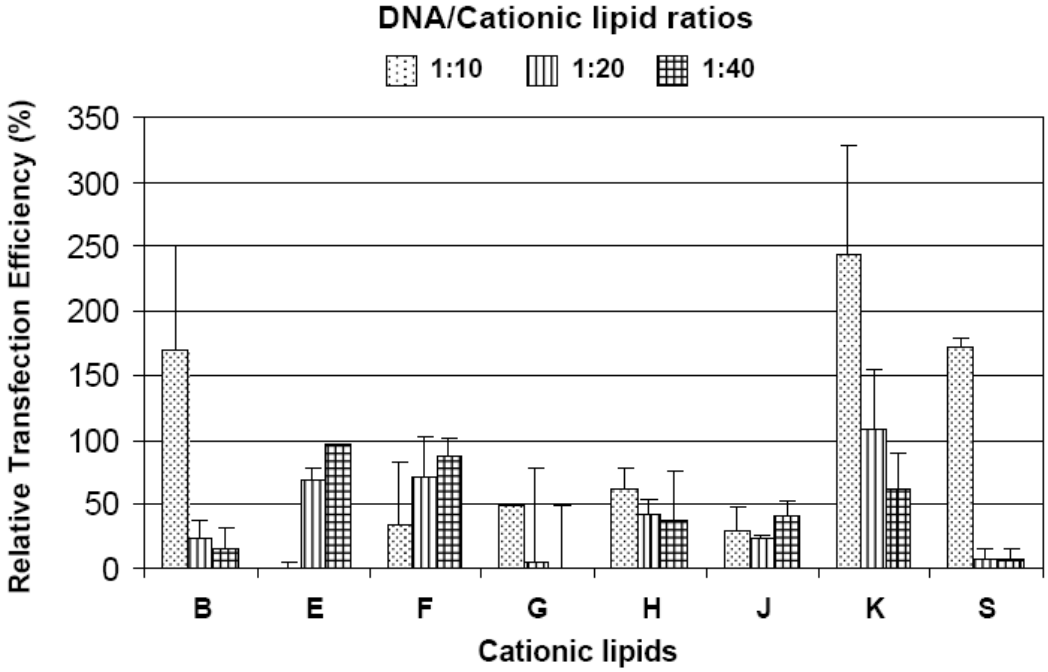
จากการศึกษาการพาดีเอ็นเอเข้าสู่เซลล์เบื้องต้น (รูปที่ 26) พบว่า ไขมันตัวช่วยเป็นส่วนสำคัญในการเตรียมไลโปโซมเพื่อให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ เพื่อศึกษาผลของไขมันตัวช่วยต่อประสิทธิภาพการพาดีเอ็นเอเข้าเซลล์ของไขมันประจุบวกทั้ง 8 ชนิด นำไขมันประจุบวกมาเตรียมไลโปโซม ที่มีอัตราส่วนไขมันประจุบวกต่อไขมันตัวช่วย 2:1, 1:1 และ 1:2 ในการทดลองนี้ใช้อัตราส่วนดีเอ็นเอต่อไลโปโซมเท่ากันคือ 1:20 และใช้ Effectene^{TM} เป็นสารมาตรฐานและคำนวณให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์เท่ากับ 100% ผลการทดลองแสดงดังรูปที่ 27 ไขมันประจุบวก **H** มีประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีที่สุด เมื่อเตรียมไลโปโซม ที่มีอัตราส่วนไขมันประจุบวกต่อไขมันตัวช่วย 1:2 ไขมันประจุบวก **J** และ **S** แสดงประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีที่สุดเมื่อใช้ อัตราส่วนไขมันประจุบวกต่อไขมันตัวช่วย 2:1 ในขณะที่ไขมันประจุบวก **E** และ **K** แสดงประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีที่สุดเมื่อใช้ อัตราส่วนไขมันประจุบวกต่อไขมันตัวช่วย 1:1 นำไขมันประจุบวกที่มีอัตราส่วนไขมันประจุบวกต่อไขมันตัวช่วยที่ทำให้มีประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีที่สุด ไปศึกษาสภาวะอื่นต่อไป



รูปที่ 27 การศึกษาผลของอัตราส่วนไขมันประจุบวกต่อไขมันตัวช่วยต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์

การศึกษาอัตราส่วนของดีเอ็นเอต่อไลโปโซม

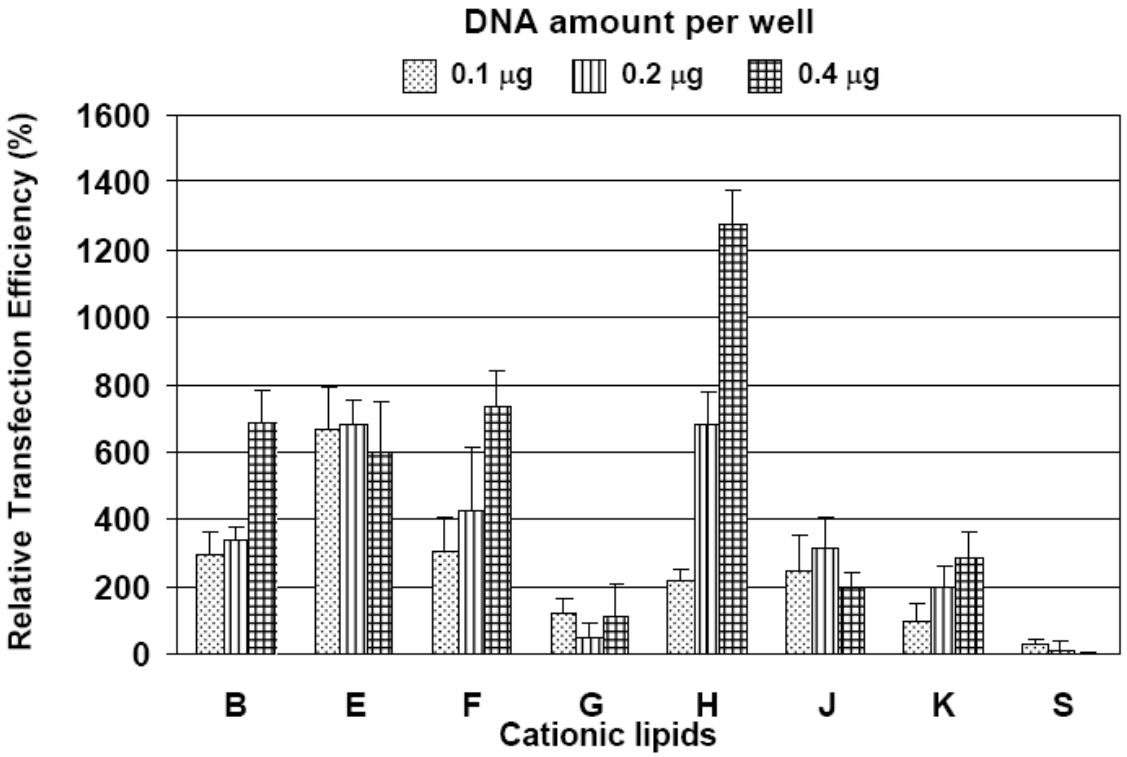
จากการศึกษาผลของอัตราส่วนของไขมันประจุบวกต่อไขมันตัวช่วยต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ (รูปที่ 27) พบว่า ไขมันประจุบวกแต่ละชนิดต้องการปริมาณไขมันตัวช่วยในปริมาณที่ไม่เท่ากัน จึงนำไลโปโซมที่มีประสิทธิภาพที่ดีที่สุดของไขมันประจุบวกแต่ละชนิด มาศึกษาผลของอัตราส่วนของดีเอ็นเอต่อไลโปโซมที่ทำให้ประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์สูงที่สุด ในการทดลองนี้ใช้ปริมาณดีเอ็นเอคงที่ๆ 0.1 mg/well และปรับเปลี่ยนอัตราส่วนของดีเอ็นเอต่อไลโปโซมที่ 1:10, 1:20 และ 1:40 และใช้ Effectene^{TM} เป็นสารมาตรฐานและคำนวณให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์เท่ากับ 100% ผลการทดลองแสดงดังรูปที่ 28 จากผลการทดลองพบว่าไขมันประจุบวก **B**, **G**, **H**, **K** และ **S** มีประสิทธิภาพในการพาดีเอ็นเอลดลงเมื่ออัตราส่วนดีเอ็นเอต่อไลโปโซมสูงขึ้น ในทางตรงกันข้าม ไขมันประจุบวก **E** และ **F** มีประสิทธิภาพในการพาดีเอ็นเอสูงขึ้นเมื่ออัตราส่วนดีเอ็นเอต่อไลโปโซมลดลง ผลที่ได้จากการทดลองนี้นำไปใช้ศึกษาต่อไป



รูปที่ 28 การศึกษาผลของอัตราส่วนดีเอ็นเอต่อไลโปโซมต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์

การศึกษาผลของปริมาณดีเอ็นเอ

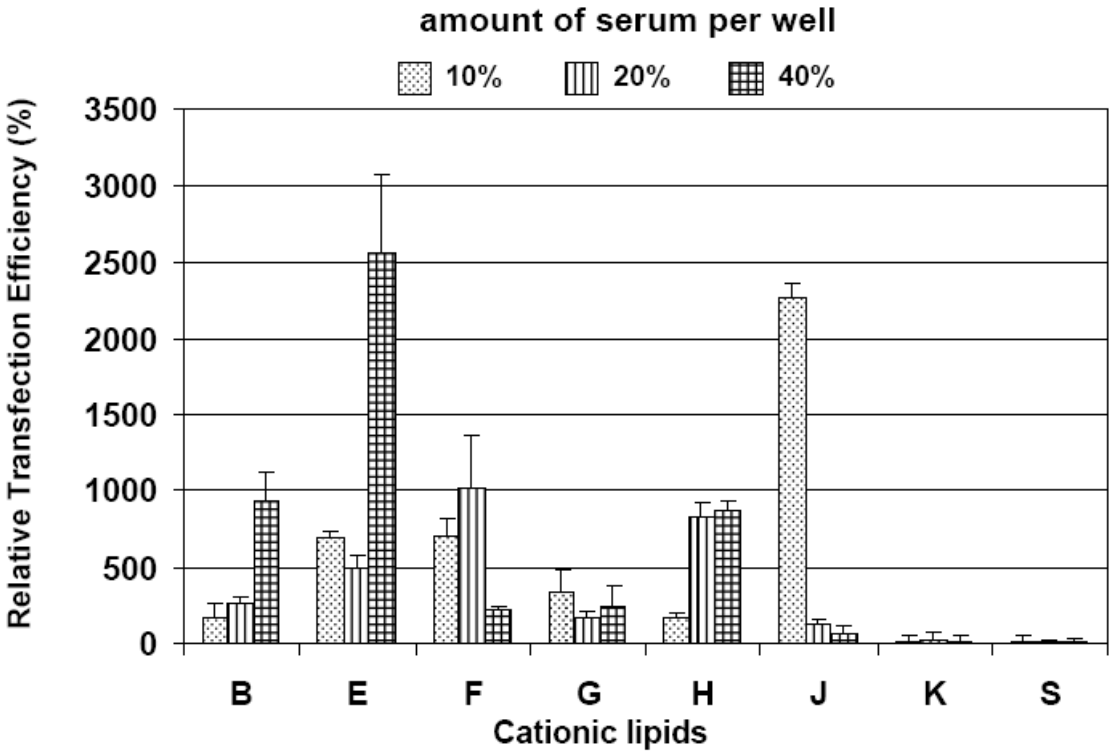
เพื่อศึกษาผลของปริมาณดีเอ็นเอต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ ได้นำสภาวะที่เหมาะสมที่ได้จากการทดลองที่ผ่านมา (รูปที่ 27 และ 28) มาใช้โดยปรับเปลี่ยนปริมาณดีเอ็นเอจาก 0.1 μg/well เป็น 0.2 μg/well และ 0.4 μg/well และใช้ Effectene^{TM} เป็นสารมาตรฐานและคำนวณให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์เท่ากับ 100% ผลการทดลองแสดงดังรูปที่ 29 จากผลการทดลองพบว่าไขมันประจุบวก **B**, **F**, **H** และ **K** มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ได้สูงขึ้นเมื่อใช้ปริมาณดีเอ็นเอมากขึ้น ประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ของไขมันประจุบวก **E**, **G**, **J** และ **S** ไม่เปลี่ยนแปลงตามปริมาณดีเอ็นเอ จากการทดลองนี้ไขมันประจุบวก **H** มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ได้สูงสุด



รูปที่ 29 การศึกษาผลของปริมาณดีเอ็นเอต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์

การศึกษาผลของ serum ต่อประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์

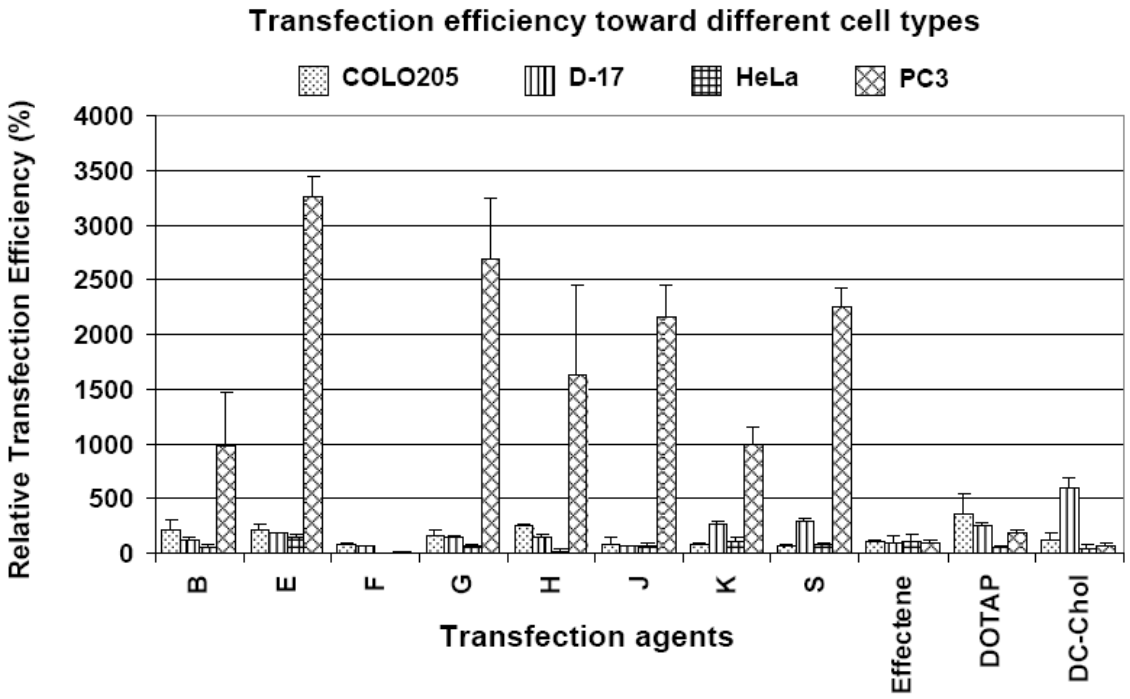
เพื่อศึกษาผลของปริมาณ serum ต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ ได้นำสภาวะที่เหมาะสมที่ได้จากการทดลองที่ผ่านมา (รูปที่ 27 - 29) มาใช้ โดยการทดลองนี้จะใช้สภาวะที่มีปริมาณของ serum 10, 20 และ 40% และใช้ Effectene^{TM} เป็นสารมาตรฐานและคำนวณให้มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์เท่ากับ 100% ผลการทดลองแสดงดังรูปที่ 30 ไขมันประจุบวก **B** และ **E** มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ได้ดีที่สุดเมื่อทำการทดลองในสภาวะที่มีปริมาณ serum 40% พบว่ามีประสิทธิภาพดีกว่าสารมาตรฐาน Effectene^{TM} ถึง 10 และ 26 เท่า ตามลำดับ ไขมันประจุบวก **J** มีประสิทธิภาพสูงสุดเมื่อทำการทดลองในสภาวะที่มี serum 10% และมีประสิทธิภาพดีกว่าสารมาตรฐาน Effectene^{TM} ถึง 23 เท่า แต่ไขมันประจุบวก **J** มีประสิทธิภาพลดลงเมื่อทำการทดลองในสภาวะที่มี serum 20 และ 40% ไขมันประจุบวก **H** มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ได้เท่ากันในสภาวะที่มี serum 20 และ 40% สำหรับไขมันประจุบวก **K** และ **S** ไม่สามารถพาดีเอ็นเอเข้าสู่เซลล์ได้ในสภาวะที่มี serum



รูปที่ 30 การศึกษาผลของ serum ต่อประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์

การศึกษาประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ชนิดต่างๆ

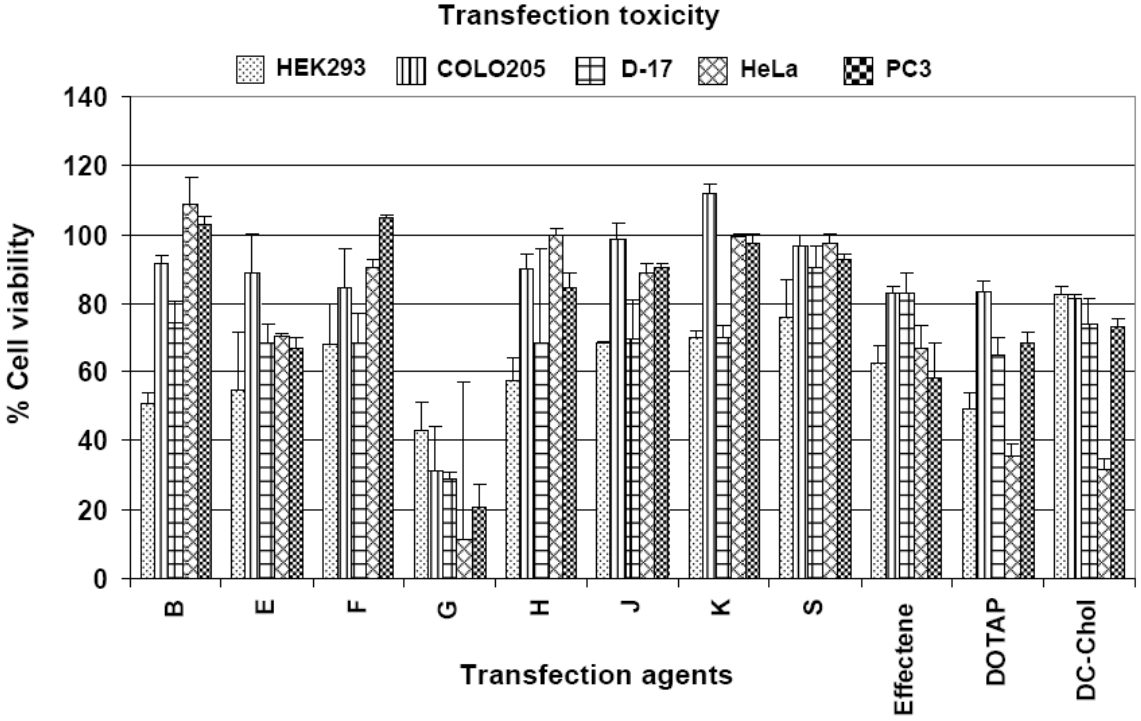
นำไขมันประจุบวกทั้ง 8 ชนิด มาศึกษาประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ชนิดต่างๆ โดยใช้สภาวะที่เหมาะสมที่ได้จากการทดลองที่ผ่านมา (รูปที่ 27 - 29) โดยการทดลองนี้จะใช้สภาวะไม่มี serum และใช้ Effectene^{TM}, DOTAP และ DC-Chol เป็นสารมาตรฐานและคำนวณให้ประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ของ Effectene^{TM} เท่ากับ 100% ผลการทดลองแสดงดังรูปที่ 31 จากผลการทดลองแสดงให้เห็นว่า ไขมันประจุบวกทุกชนิด ยกเว้นสาร **F** มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ PC3 ได้ดีกว่าสารมาตรฐานทั้ง 3 ชนิด โดยเฉพาะสาร E มีประสิทธิภาพการพาดีเอ็นเอเข้าสู่เซลล์ได้สูงที่สุด ซึ่งสูงกว่าสารมาตรฐานถึง 32 เท่า ไขมันประจุบวกทุกชนิดไม่สามารถพาดีเอ็นเอเข้าสู่เซลล์ COLO 205, D-17 และ HeLa สูงกว่า 500%



รูปที่ 31 การศึกษาประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ชนิดต่างๆ

การศึกษาความเป็นพิษต่อเซลล์

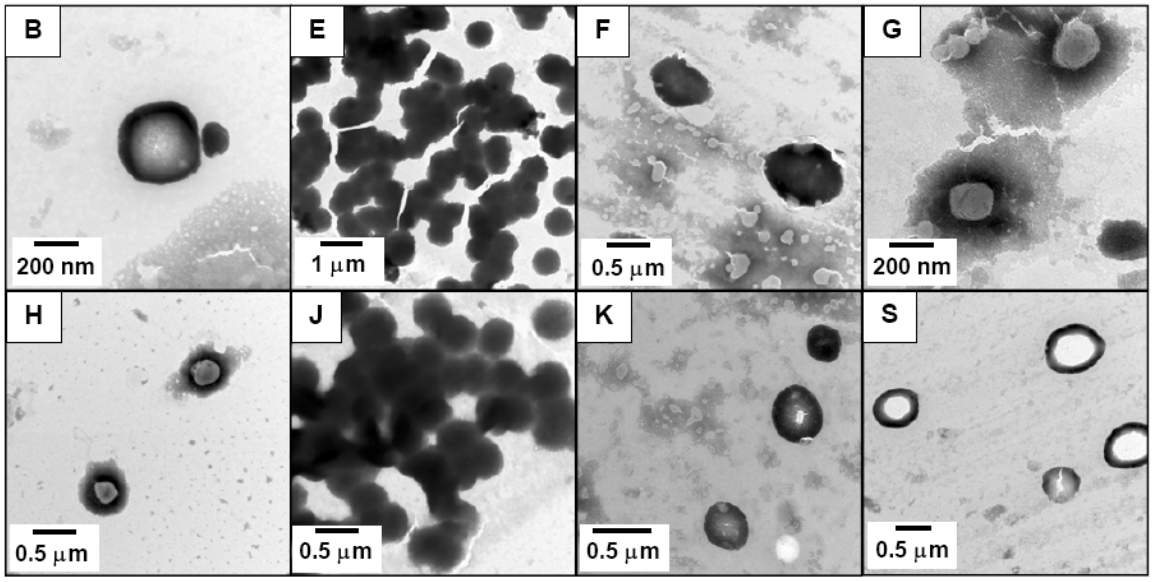
นำไขมันประจุบวกทั้ง 8 ชนิด มาศึกษาความเป็นพิษต่อเซลล์ โดยใช้วิธีการ MTT assay ชนิดต่างๆ ผลการทดลองแสดงดังรูปที่ 32 จากผลการทดลองแสดงให้เห็นว่า ไขมันประจุบวกทุกชนิดยกเว้นสาร **G** ไม่มีความเป็นพิษต่อเซลล์ที่ทดสอบ



รูปที่ 32 การศึกษาความเป็นพิษกับเซลล์ชนิดต่างๆ

การศึกษาสัญฐานวิทยาของไลโปโซม

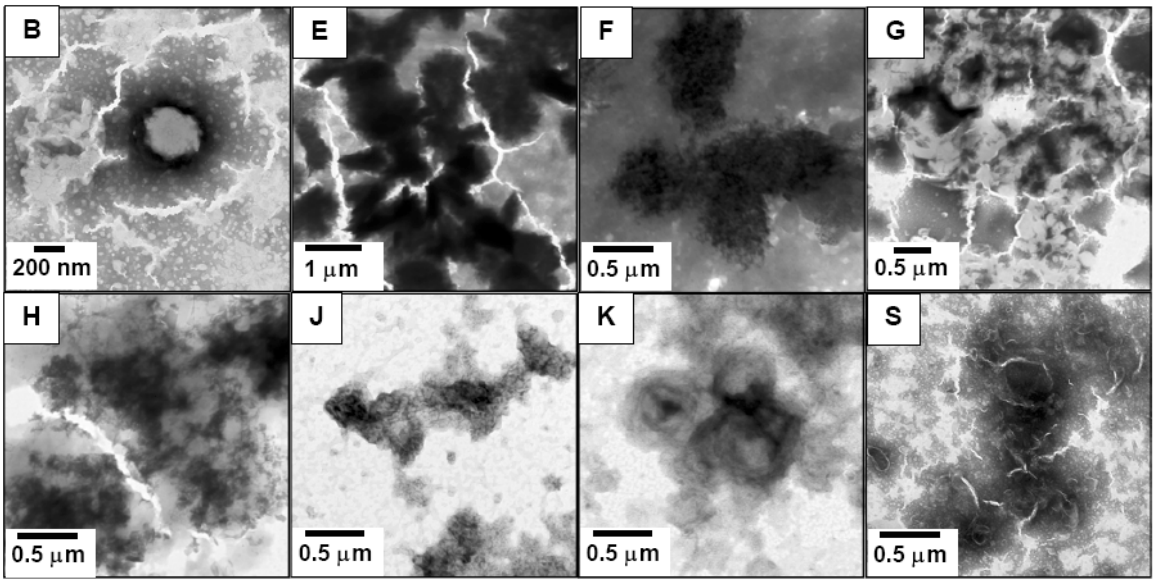
นำไขมันประจุบวกทั้ง 8 ชนิด เตรียมเป็นไลโปโซมและศึกษาสัญฐานวิทยา ด้วย transmission electron microscopy ผลที่ได้แสดงดังรูปที่ 33 ไลโปโซมที่เตรียมได้มีขนาด 200-700 nm



รูปที่ 33 การศึกษาสัญฐานวิทยาของไลโปโซม **B**, **E**, **F**, **G**, **H**, **J**, **K** และ **S** ด้วย transmission electron microscopy (TEM)

การศึกษาสัญฐานวิทยาของไลโปเพล็กซ์

นำไลโปโซมที่เตรียมจากไขมันประจุบวก **B**, **E**, **F**, **G**, **H**, **J**, **K** และ **S** มาผสมกับดีเอ็นเอ เตรียมเป็นไลโปเพล็กซ์ ผลที่ได้แสดงดังรูปที่ 34 ไลโปเพล็กซ์ที่เตรียมได้มีขนาด 300-1000 nm



รูปที่ 34 การศึกษาสัญฐานวิทยาของไลโปเพล็กซ์ **B**, **E**, **F**, **G**, **H**, **J**, **K** และ **S** ด้วย transmission electron microscopy (TEM)

วิธีการ

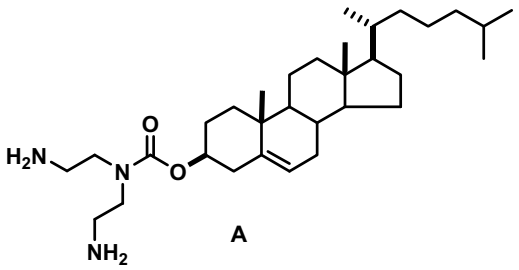
การสังเคราะห์ไขมันประจุบวก Library 1

การสังเคราะห์ resin 36a-b

นำ active carbamate resin **21** (1 equiv, 1.1 mmol/g) มาทำปฏิกิริยากับ diethylenetriamine หรือ nor-spermidine ที่มากเกินพอ ในตัวทำละลาย CH_2Cl_2 (5 ml) เขย่า resin นาน 6 ชม. ล้าง resin ที่ได้ให้สะอาดด้วยตัวทำละลาย CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 อย่างละ 3 ครั้ง ๆ ละ 10 มล. นำ resin ที่ได้ระเหยตัวทำละลายออกภายใต้ลดความดัน 2 ชม. เติมหมู่ป้องกัน Dde-OH (6 equiv) ในตัวทำละลายผสม CH_2Cl_2 และ DMF ทำปฏิกิริยาข้ามคืน ล้าง resin ให้สะอาด ทำ resin ให้แห้งและทำปฏิกิริยากับ cholesteryl chloroformate (4 equiv) ใช้ pyridine เป็นเบส ในตัวทำละลาย CH_2Cl_2 นาน 12 ชม. หลังจากนั้น กรอง และล้างด้วยตัวทำละลายได้ผลิตภัณฑ์ resin **35** กำจัดหมู่ป้องกัน Dde ออกจาก resin 35 โดยทำปฏิกิริยากับ 5% N_2H_4 ในตัวทำละลาย DMF นาน 30 นาที 2 ครั้ง ล้างให้ละอาดด้วยตัวทำละลาย CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 อย่างละ 3 ครั้ง ๆ ละ 10 มล. ได้ผลิตภัณฑ์ resin **36**

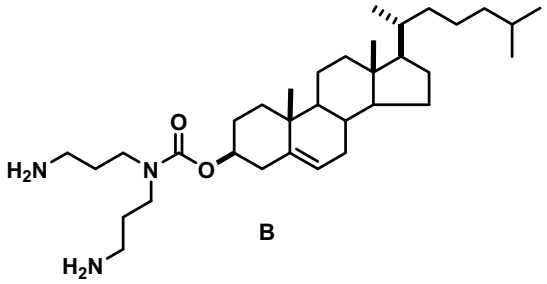
การสังเคราะห์ไขมันประจุบวก A และ B

นำ resin **36a** (214.5 mg) และ **36b** (123.9 mg) มาทำปฏิกิริยากับ 50% TFA ในตัวทำละลาย CH_2Cl_2 (1.5 ml) เขย่าปฏิกิริยานาน 2 ชม. เก็บส่วนสารละลาย ล้าง resin ด้วย CH_2Cl_2 รวมส่วนสารละลายเข้าด้วยกัน ระเหยตัวทำละลายออกด้วยแก๊สไนโตรเจน ได้ผลิตภัณฑ์ **A** และ **B** ตามลำดับ นำผลิตภัณฑ์ที่ได้ระเหยตัวทำละลายภายใต้ลดความดันนาน 2 ชม.



A

ไขมันประจุบวก A; Yield: (resin: 1.1 mmol/g, 214.5 mg) 45.8 mg, 38%; IR (CH_2Cl_2): ν_{max} 3436, 2918, 2849, 1773, 1682, 1541, 1507, 1463, 1381, 1202, 1021 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 9 drops of CD_3OD): δ 0.53 (s, 3H, CH_3-18), 0.765 and 0.769 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.80 (d, J = 6.4 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.91-2.26 (m, 30H, protons in cholesteryl skeleton), 3.05 (m, 4H, $(H_2NC\mathit{H}_2CH_2)_2N$), 3.38 (m, 4H, $(H_2NCH_2C\mathit{H}_2)_2N$), 3.48 (br s, 1H, H-3-Chol), 5.26 (br s, 1H, H-6-Chol), 8.00-8.4 (br s, 6H, $N\mathit{H}_3^+$); ^{13}C NMR ($CDCl_3$ + 9 drops of CD_3OD, 100 MHz): 11.6, 18.5, 18.9, 20.8, 22.3, 22.5, 23.7, 24.1, 27.6, 27.8, 28.0, 31.6, 31.7, 35.6, 35.8, 36.0, 36.3, 38.0, 39.3, 39.5, 42.1, 49.8, 56.0, 56.5, 76.7, 122.7, 139.4 (carbons in cholesteryl skeleton), 38.5 ($(H_2NC\mathit{H}_2CH_2)_2N$), 46.7 ($(H_2NCH_2C\mathit{H}_2)_2N$), 156.9 (C=O carbamoyl); MS (ES^+): *m/z*: 516 ($[M+H]^+$, 100%)

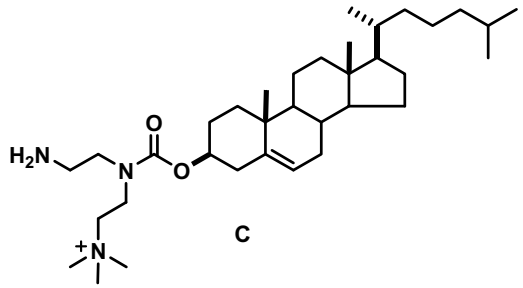


B

ไขมันประจุบวก B; Yield: (resin: 1.1 mmol/g, 123.9 mg) 52.4 mg, 70%; IR (CH_2Cl_2): ν_{max} 3355, 2918, 2845, 2280, 1675, 1600, 1509, 1468, 1429, 1202 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.811 and 0.815 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.2 Hz, 3H, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.90-2.28 (m, 32H, protons in cholesteryl skeleton and $(H_2NCH_2C\mathit{H}_2CH_2)_2N$), 2.88 (br s, 4H, $(H_2NC\mathit{H}_2CH_2CH_2)_2N$), 3.26 (br s, 4H, $(H_2NCH_2CH_2C\mathit{H}_2)_2N$), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.94 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.9, 24.2, 27.9, 28.1, 31.7, 35.8, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.2, 56.6, 75.8, 122.6, 139.4 (carbons in cholesteryl skeleton), 25.7 and 26.4 ($(H_2NCH_2C\mathit{H}_2CH_2)_2N$), 27.2 and 38.4 ($(H_2NC\mathit{H}_2CH_2CH_2)_2N$), 44.2 and 44.6 ($(H_2NCH_2CH_2C\mathit{H}_2)_2N$), 156.5 (C=O carbamoyl); MS (ES^+): *m/z* 544.7 ($[M+H]^+$, 100%).

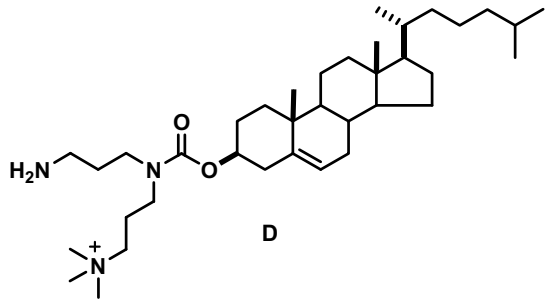
การสังเคราะห์ไขมันประจุบวก C และ D

นำ resin **36a** (148.3 mg) และ **36b** (148.3 mg) (1 equiv, 1.1 mmol/g) เติม methyl iodide (10 equiv, 83 และ 78 ml, ตามลำดับ) และ DIEA (10 equiv, 279 and 261 µl, ตามลำดับ) ในตัวทำละลาย DMF (2 มล.) เขย่าปฏิกิริยานาน 18 ชม. ล้าง resin ที่ได้ให้สะอาดด้วยตัวทำละลาย CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 อย่างละ 3 ครั้ง ๆ ละ 2 มล. นำ resin ที่ได้ทำปฏิกิริยากับ 50% TFA ในตัวทำละลาย CH_2Cl_2 (1.5 ml) เขย่าปฏิกิริยานาน 2 ชม. เก็บส่วนสารละลาย ล้าง resin ด้วย CH_2Cl_2 รวมส่วนสารละลายเข้าด้วยกัน ระเหยตัวทำละลายออกด้วยแก๊สไนโตรเจน ได้ผลิตภัณฑ์ **C** และ **D** ตามลำดับ นำผลิตภัณฑ์ที่ได้ระเหยตัวทำละลายภายใต้ลดความดันนาน 2 ชม.



C

ไขมันประจุบวก C; Yield: (resin: 1.1 mmol/g, 148.3 mg) 48.0 mg, 53%; IR (CH_2Cl_2): ν_{max} 3436, 2932, 2840, 1681, 1469, 1426, 1202, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.788 and 0.792 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.84 (d, J = 6.4 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.96-2.30 (m, 30H; protons in cholesteryl skeleton), 3.06 (s, 9H, $(C\mathit{H}_3)_3N^+$), 3.10 (br s, 2H, $H_2NC\mathit{H}_2CH_2N$), 3.48-3.77 (m, 6H, $CH_2C\mathit{H}_2NC\mathit{H}_2C\mathit{H}_2N(CH_3)_3$), 4.40 (br s, 1H, H-3-

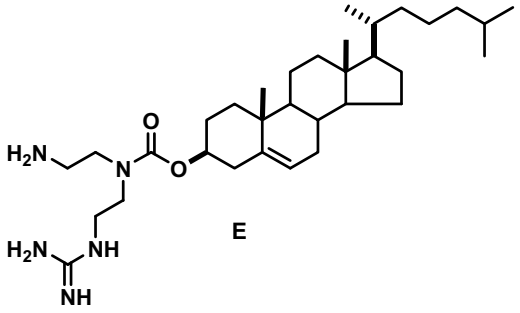


D

ไขมันประจุบวก D; Yield: (resin: 1.1 mmol/g, 139.3 mg) 57.4 mg, 64%; IR (CH_2Cl_2): ν_{max} 3436, 2931, 1677, 1468, 1423, 1379, 1202, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.797 and 0.801 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.85 (d, J = 6.3 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.94-2.28 (m, 32H, protons in cholesteryl skeleton, $CH_2CH_2CH_2NCH_2CH_2CH_2$), 2.89 (br s, 4H, $H_2NCH_2CH_2CH_2NCH_2CH_2CH_2N(CH_3)_3$), 3.02-3.03 (br s, 9H, $N(CH_3)_3^+$), 3.27 (br s, 4H, CH_2NCH_2), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 7.98 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.8, 122.6, 139.4 (carbons in cholesteryl skeleton), 25.7 and 26.4 ($CH_2CH_2NCH_2CH_2$), 37.0 ($H_2NCH_2CH_2CH_2N$), 38.4 ($CH_2CH_2CH_2N(CH_3)_3^+$), 44.3 ($NCH_2CH_2CH_2N(CH_3)_3$), 44.5 ($H_2NCH_2CH_2CH_2N$), 53.1 ($N(CH_3)_3$), 156.1 (C=O carbamoyl); MS (ES^+): *m/z* 587.1 ($[M+H]^+$, 100%).

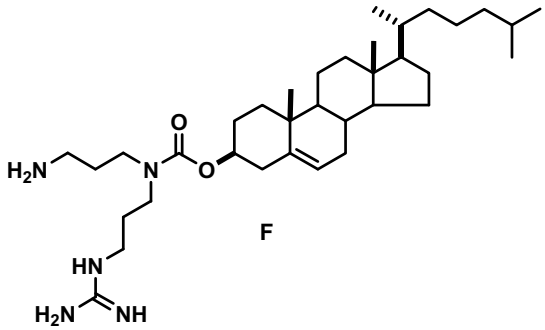
การสังเคราะห์ไขมันประจุบวก E และ F

นำ resin **36a** (148.4 mg) และ **36b** (145.3 mg) (1 equiv, 1.1 mmol/g) เติม *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (4 equiv, 190 and 186 mg, ตามลำดับ) และ DIEA (10 equiv, 111 and 109 µl, ตามลำดับ) ในตัวทำละลาย DMF (2 มล.) เขย่าปฏิกิริยานาน 18 ชม. ล้าง resin ที่ได้ให้สะอาดด้วยตัวทำละลาย CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 อย่างละ 3 ครั้ง ๆ ละ 2 มล. นำ resin ที่ได้ทำปฏิกิริยากับ 50% TFA ในตัวทำละลาย CH_2Cl_2 (1.5 ml) เขย่าปฏิกิริยานาน 2 ชม. เก็บส่วนสารละลาย ล้าง resin ด้วย CH_2Cl_2 รวมส่วนสารละลายเข้าด้วยกัน ระเหยตัวทำละลายออกด้วยแก๊สไนโตรเจน ได้ผลิตภัณฑ์ **E** และ **F** ตามลำดับ นำผลิตภัณฑ์ที่ได้ระเหยตัวทำละลายภายใต้ลดความดันนาน 2 ชม.



E

ไขมันประจุบวก E; Yield: (resin: 1.1 mmol/g, 148.4 mg) 42.1 mg, 46%; IR (CH_2Cl_2): ν_{max} 3355, 3182, 2934, 2278, 1779, 1674, 1509, 1468, 1432, 1376, 1203, 1138, 1018 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.821 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 5.7 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 1.00-2.30 (m, 30H, protons in cholesteryl skeleton), 3.07 (br s, 2H, $H_2NCH_2CH_2N$), 3.29 and 3.36 (br s, 4H, $H_2NCH_2CH_2NCH_2CH_2$), 3.51 (br s, 2H, $NCH_2CH_2NHC(NH)NH_2$)), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H; H-6-Chol), 8.01 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.7, 23.7, 24.2, 27.6, 27.9, 28.1, 31.7, 31.8, 35.7, 36.1, 36.4, 36.8, 38.0, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.6, 122.7, 139.3 (carbons in cholesteryl skeleton), 38.5 ($CH_2CH_2NCH_2CH_2$), 47.4 (CH_2NCH_2), 156.9 (C=O carbamoyl and C=N guanidine); MS (ES^+): *m/z* 558.6 ($[M+H]^+$, 100%).

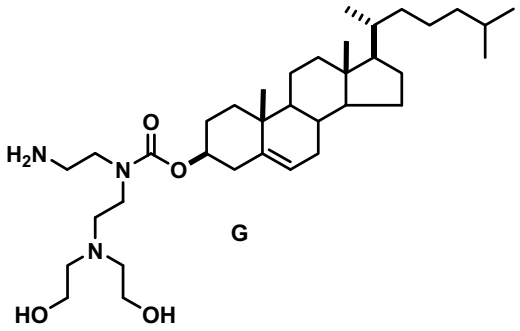


F

ไขมันประจุบวก F; Yield: (resin: 1.1 mmol/g, 145.3 mg) 52.4 mg, 56%; IR (CH_2Cl_2): ν_{max} 3350, 2918, 1673, 1509, 1465, 1429, 1203, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ = 0.62 (s, 3H, CH_3-18), 0.805 and 0.809 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.3 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 1.00-2.28 (m, 32H, protons in cholesteryl skeleton and $CH_2CH_2NCH_2CH_2$), 2.87 (br s, 2H, $H_2NCH_2CH_2CH_2N$), 3.08 (br s, 2H, $CH_2CH_2NHC(NH)NH_2$), 3.19 (br s, 2H, $H_2NCH_2CH_2CH_2N$), 3.25 (br s, 2H, $NCH_2CH_2CH_2NHC(NH)NH_2$), 4.38 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.97 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.8, 24.1, 27.9, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.5, 76.0, 122.6, 139.4 (carbons in cholesteryl skeleton), 25.7 and 26.4 ($CH_2CH_2NCH_2CH_2$), 37.3 ($H_2NCH_2CH_2CH_2N$), 38.5 ($NCH_2CH_2CH_2NHC(NH)NH_2$), 44.1 ($NCH_2CH_2CH_2NHC(NH)NH_2$), 44.6 ($H_2NCH_2CH_2CH_2N$), 157.2 (C=O carbamoyl and C=N guanidine); MS (ES^+): *m/z* 586.7 ($[M+H]^+$, 100%).

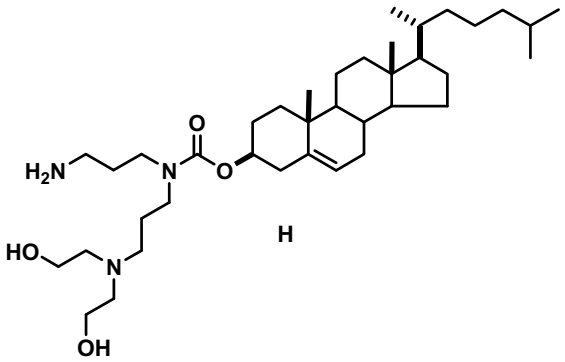
การสังเคราะห์ไขมันประจุบวก G และ H

นำ resin **36a** (152.8 mg) และ **36b** (159.3 mg) (1 equiv, 1.1 mmol/g) เติม 2-bromoethanol (8 equiv, 95 and 99 µl, ตามลำดับ) และ DIEA (8 equiv, 230 and 240 µl, ตามลำดับ) ในตัวทำละลาย DMF (2 มล.) เขย่าปฏิกิริยานาน 18 ชม. ล้าง resin ที่ได้ให้สะอาดด้วยตัวทำละลาย CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 อย่างละ 3 ครั้ง ๆ ละ 2 มล. นำ resin ที่ได้ทำปฏิกิริยากับ 50% TFA ในตัวทำละลาย CH_2Cl_2 (1.5 ml) เขย่าปฏิกิริยานาน 2 ชม. เก็บส่วนสารละลาย ล้าง resin ด้วย CH_2Cl_2 รวมส่วนสารละลายเข้าด้วยกัน ระเหยตัวทำละลายออกด้วยแก๊สไนโตรเจน ได้ผลิตภัณฑ์ **G** และ **H** ตามลำดับ นำผลิตภัณฑ์ที่ได้ระเหยตัวทำละลายภายใต้ลดความดันนาน 2 ชม.



G

ไขมันประจุบวก G; Yield: (resin: 1.1 mmol/g, 152.8 mg) 41.5 mg, 41%; IR (CH_2Cl_2): ν_{max} 3335, 2932, 2840, 2279, 1674, 1597, 1541, 1509, 1413, 1310, 1203, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.809 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 6.2 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.90-2.28 (m, 30H, protons in cholesteryl skeleton), 3.12 (br s, 4H, $CH_2CH_2NCH_2CH_2$), 3.36 (br s, 4H, CH_2NCH_2), 3.54 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.88 (br s, 4H, $N(CH_2CH_2OH)_2$), 4.37 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 8.12 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.6, 23.8, 24.1, 27.7, 27.9, 28.1, 31.7, 31.8, 35.7, 36.1, 36.4, 36.8, 38.0, 39.4, 39.6, 42.2, 49.9, 56.60, 56.69, 76.6, 122.9, 139.2 (carbons in cholesteryl skeleton), 38.5 ($CH_2CH_2NCH_2CH_2$), 46.9 ($CH_2CH_2NCH_2CH_2$), 55.5, 55.8, 56.1 ($N(CH_2CH_2OH)_2$), 157.0 (C=O carbamoyl); MS (ES^+): m/z 604.4 ($[M+H]^+$, 100%).



H

ไขมันประจุบวก H; Yield: (resin: 1.1 mmol/g, 159.3 mg) 64.1 mg, 58%; IR (CH_2Cl_2): ν_{max} 3383, 2932, 2840, 1676, 1468, 1432, 1202, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 7 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.80 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27),

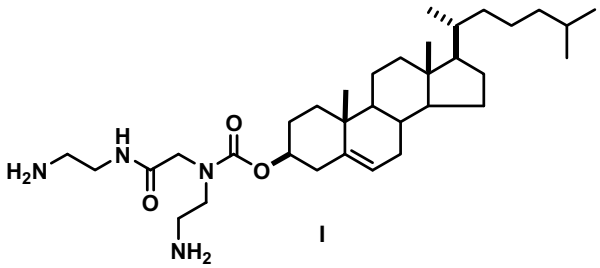
การสังเคราะห์ไขมันประจุบวก Library 2

การสังเคราะห์ resin 45a-d

นำ active carbamate resin **21** (1 equiv, 1.1 mmol/g) มาทำปฏิกิริยากับ 1,2-diamonoethane หรือ 1,3-diaminopropane ที่มากเกินพอในตัวทำละลาย CH_2Cl_2 (7 มล.) เขย่าปฏิกิริยาข้ามคืน ล้าง resin **40a** และ **40b** ที่ได้ด้วยตัวทำละลาย CH_2Cl_2, DMF และ CH_2Cl_2 3 ครั้ง ๆ ละ 5 มล. ตามลำดับ ทำ resin ให้แห้งภายใต้ลดความดัน นำ resin ที่แห้งแล้วมาทำปฏิกิริยากับ bromoacetic acid (4 equiv) โดยใช้ DIC (4 equiv) เป็น coupling agent ในตัวทำละลายผสม CH_2Cl_2 และ DMF (5 ml) ทำปฏิกิริยานาน 12 ชม. ล้าง resin ที่ได้ให้สะอาดดังที่กล่าวไว้ในตอนต้น ได้ resin **41a** และ **41b** นำ resin ที่ได้มาทำปฏิกิริยากับ1,2-diamonoethane หรือ 1,3-diaminopropane ที่มากเกินพอในตัวทำละลาย DMF (7 มล.) เขย่าปฏิกิริยานาน 12 ชม. ล้าง resin ที่ได้ให้สะอาดได้ผลิตภัณฑ์ resin **42a-42d** นำ resin ที่ได้มาทำปฏิกิริยาต่อกับ Dde-OH ตามด้วย cholesteryl chloroformate และกำจัดหมู่ป้องกัน Dde ตามลำดับ ซึ่งทำเช่นเดียวกับการสังเคราะห์ resin **36a** และ **36b** ได้ผลิตภัณฑ์ resin **45a-45d**

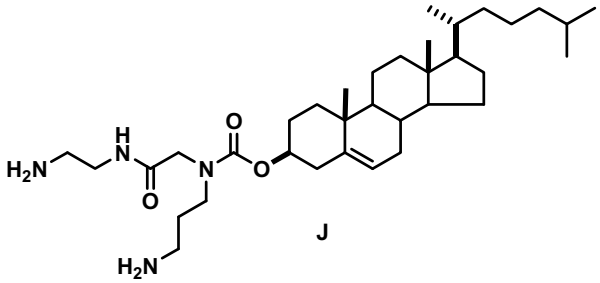
การสังเคราะห์ไขมันประจุบวก I, J, K และ L

นำ resin **45a** (177.0 mg), **45b** (208.0 mg), **45c** (142.0 mg) และ **45d** (261.3 mg) มาทำปฏิกิริยากับ 50% TFA ในตัวทำละลาย CH_2Cl_2 (1.5 ml) เขย่าปฏิกิริยานาน 2 ชม. เก็บส่วนสารละลาย ล้าง resin ด้วย CH_2Cl_2 รวมส่วนสารละลายเข้าด้วยกัน ระเหยตัวทำละลายออกด้วยแก๊สไนโตรเจน ได้ผลิตภัณฑ์ **I**, **J**, **K** และ **L** ตามลำดับ นำผลิตภัณฑ์ที่ได้ระเหยตัวทำละลายภายใต้ลดความดันนาน 2 ชม.



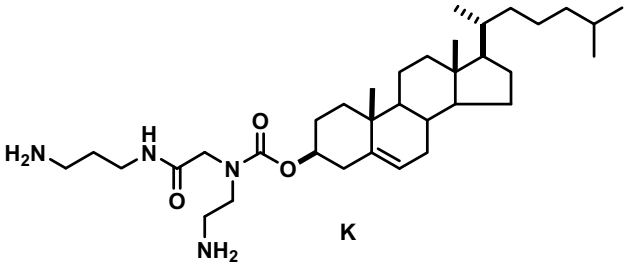
I

ไขมันประจุบวก I; Yield: (resin: 1.1 mmol/g, 177.0 mg) 46.3 mg, 42%; IR (CH_2Cl_2): ν_{max} 3346, 2918, 2279, 1675, 1597, 1509, 1413, 1310, 1203, 1124, 1015 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.794 and 0.797 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.84 (d, J = 6.2 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 0.92-2.27 (m, 28H, protons in cholesteryl skeleton), 3.04 (br s, 2H, $H_2NC\mathit{H}_2CH_2NHCO$), 3.11 (br s, 2H, $NHCOCH_2NCH_2C\mathit{H}_2NH_2$), 3.45 (br s, 2H, $H_2NCH_2C\mathit{H}_2NHCOCH_2N$), 3.54 (br s, 2H, $NHCOCH_2NC\mathit{H}_2CH_2NH_2$), 3.93 (br s, 2H, $NHCOC\mathit{H}_2N$), 4.37 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 8.05-8.40 (br s, 6H, $N\mathit{H}_3^+$); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.7, 27.8, 28.1, 31.7, 35.7, 36.0, 36.4, 36.7, 38.1, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.9, 122.7, 139.2 (carbons in cholesteryl skeleton), 36.9 ($H_2NCH_2\mathit{C}H_2NHCO$), 38.1 ($NHCOCH_2NCH_2\mathit{C}H_2NH_2$), 39.1 ($H_2N\mathit{C}H_2CH_2NHCO$), 47.5 ($NHCOCH_2N\mathit{C}H_2CH_2NH_2$), 51.0 ($NHCO\mathit{C}H_2N$), 156.0 (C=O carbamoyl), 173.0 (C=O amide); MS (ES^+): *m/z* 573.2 ($[M+H]^+$, 100%).



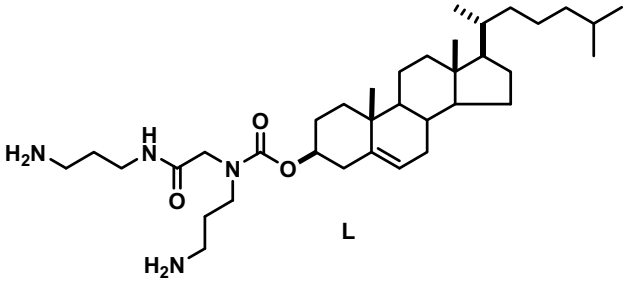
J

ไขมันประจุบวก J; Yield: (resin: 1.1 mmol/g, 208.0 mg) 69.0 mg, 51%; IR (CH_2Cl_2): ν_{max} 3428, 2918, 2834, 1674, 1462, 1376, 1202, 1130, 1018 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.816 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 6.2 Hz, 3H, CH_3-21), 0.96 (s, 3H; CH_3-19), 0.96-2.27 (m, 30H, protons in cholesteryl skeleton and $NHCOCH_2NCH_2C\mathit{H}_2CH_2$), 3.08 (br s, 4H, $C\mathit{H}_2CH_2NHCOCH_2NCH_2CH_2C\mathit{H}_2$), 3.47 (br s, 4H, $C\mathit{H}_2NHCOCH_2NC\mathit{H}_2$), 3.84 (br s, 2H, $NHCOC\mathit{H}_2N$), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 8.01 (br s, 6H, $N\mathit{H}_3^+$); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.7, 139.3 (carbons in cholesteryl skeleton), 30.0 ($NHCOCH_2NCH_2\mathit{C}H_2$), 37.0 ($H_2NCH_2\mathit{C}H_2NHCO$), 38.2 ($NHCOCH_2NCH_2CH_2\mathit{C}H_2$), 39.1 ($H_2N\mathit{C}H_2CH_2NHCO$), 45.8 ($NHCOCH_2N\mathit{C}H_2CH_2$), 52.2 ($NHCO\mathit{C}H_2N$), 156.0 (C=O carbamoyl), 173.0 (C=O amide); MS (ES^+): *m/z* 587.5 ($[M+H]^+$, 100%).



K

ไขมันประจุบวก K; Yield: (resin: 1.1 mmol/g, 142.0 mg) 37.9 mg, 42%; IR (CH_2Cl_2): ν_{max} 3338, 2918, 2851, 1676, 1594, 1535, 1460, 1202, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.81 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 4.6 Hz, 3H, CH_3-21), 0.97 (s, 3H, CH_3-19), 0.97-2.90 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2NHCO$), 2.94 (br s, 2H, $H_2NCH_2CH_2CH_2NHCO$), 3.05 (br s, 2H, $NHCOCH_2NCH_2CH_2NH_2$), 3.29 (br s, 2H, $H_2NCH_2CH_2CH_2NHCO$), 3.58 (br s, 2H, $NHCOCH_2NCH_2CH_2NH_2$), 3.93 (br s, 2H, $NHCOCH_2N$), 4.38 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.94 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.8, 35.7, 36.1, 36.4, 36.8, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.7, 139.4 (carbons in cholesteryl skeleton), 26.7 ($H_2NCH_2CH_2CH_2NHCO$), 38.3 ($H_2NCH_2CH_2CH_2NHCOCH_2NCH_2CH_2NH_2$), 52.4 ($NHCOCH_2N$), 156.5 (C=O carbamoyl), 172.5 (C=O amide); MS (ES^+): *m/z* 587.3 ($[M+H]^+$, 100%).

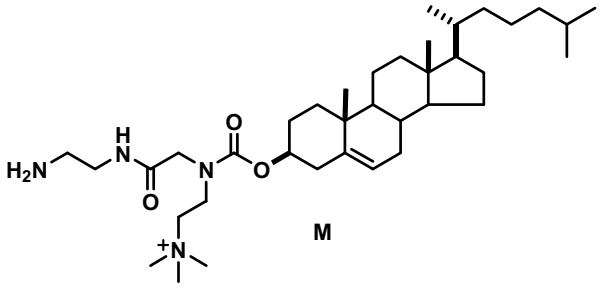


L

ไขมันประจุบวก L; Yield: (resin: 1.1 mmol/g, 261.3 mg) 70.9 mg, 41%; IR (CH_2Cl_2): ν_{max} 3299, 3064, 2935, 1682, 1541, 1468, 1379, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.813 (d, J = 6.4 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 6.0 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95-2.30 (m, 32H, protons in cholesteryl skeleton and $CH_2CH_2CH_2NHCOCH_2NCH_2CH_2CH_2$), 2.91 (br s, 4H, $H_2NCH_2CH_2CH_2NHCOCH_2NCH_2CH_2CH_2NH_2$), 3.27 (br s, 4H, $CH_2NHCOCH_2NCH_2CH_2$), 3.83 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 7.96 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.1, 31.8, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.9, 122.6, 139.4 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2NHCO$ and $CH_2NCH_2CH_2CH_2NH_2$), 37.1 ($H_2NCH_2CH_2CH_2NHCO$), 38.4 ($H_2NCH_2CH_2CH_2NHCO$), 47.5 ($CH_2NCH_2CH_2CH_2NH_2$), 50.5 ($NHCOCH_2N$), 156.5 (C=O carbamoyl), 171.6 (C=O amide); MS (ES^+): *m/z* 601.5 ($[M+H]^+$, 100%).

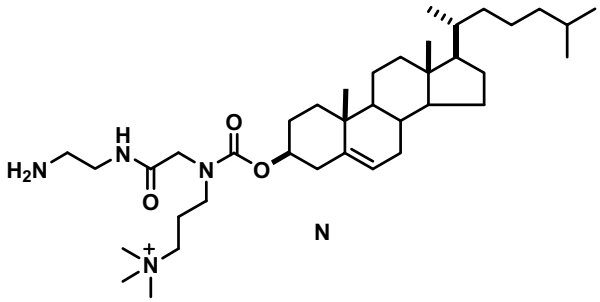
การสังเคราะห์ไขมันประจุบวก M, N, O และ P

การสังเคราะห์ไขมันประจุบวก **M-P** สามารถสังเคราะห์ได้จาก resin **45a-45d** โดยใช้วิธีการสังเคราะห์เช่นเดียวกับการสังเคราะห์ไขมันประจุบวก **C** และ **D** จาก resin **36a** และ **36b**



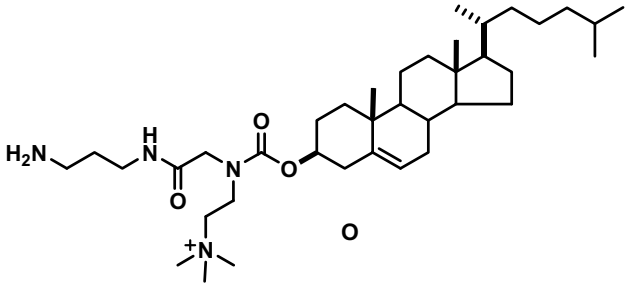
M

ไขมันประจุบวก M; Yield: (resin: 1.1 mmol/g, 192.0 mg) 76.1 mg, 59%; IR (CH_2Cl_2): ν_{max} 3436, 2934, 1678, 1541, 1468, 1378, 1202, 1131, 1023 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 10 drops of CD_3OD): δ 0.56 (s, 3H, CH_3-18), 0.749 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.80 (d, J = 6.2 Hz, 3H, CH_3-21), 0.88 (s, 3H, CH_3-19), 0.88-2.20 (m, 28H, protons in cholesteryl skeleton), 3.02-3.08 (m, 11H, $H_2NCH_2CH_2$ and $CH_2CH_2N(CH_3)_3$), 3.40 (br s, 2H, $CH_2N(CH_3)_3$), 3.50 (br s, 2H, $H_2NCH_2CH_2$), 3.64 (br s, 2H, $CH_2CH_2N(CH_3)_3$), 3.97 (br s, 2H, $NHCOCH_2$), 4.36 (br s, 1H, H-3-Chol), 5.21 (br s, 1H, H-6-Chol), 7.90-8.10 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 10 drops of CD_3OD, 100 MHz): 11.6, 18.4, 18.9, 20.3, 22.2, 22.5, 23.6, 24.0, 27.7, 27.8, 28.0, 31.6, 35.6, 35.9, 36.3, 36.4, 38.2, 39.3, 39.5, 42.1, 49.8, 55.9, 56.5, 76.5, 122.7, 139.1 (carbons in cholesteryl skeleton), 36.7 ($H_2NCH_2CH_2$), 38.0 ($CH_2N(CH_3)_3$), 39.1 ($H_2NCH_2CH_2$), 42.8 ($CH_2CH_2N(CH_3)_3$), 50.4 ($NHCOCH_2$), 53.4 and 53.5 ($CH_2CH_2N(CH_3)_3$), 156.1 (C=O carbamoyl), 170.6 (C=O amide); MS (ES^+): *m/z* 615.8 ($[M+H]^+$, 100%).



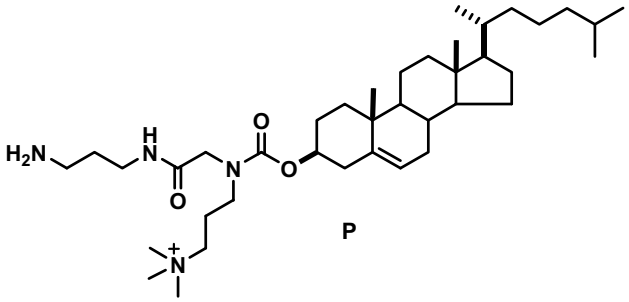
N

ไขมันประจุบวก N; Yield: (resin: 1.1 mmol/g, 173.1 mg) 62.7 mg, 52%; IR (CH_2Cl_2): ν_{max} 3334, 3042, 2930, 2851, 2279, 1677, 1594, 1541, 1462, 1203, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.795 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.84 (d, J = 5.3 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.95-2.27 (m, 30H, protons in cholesteryl skeleton and $CH_2CH_2CH_2N(CH_3)_3$), 3.03 and 3.07 (s, 11H, $H_2NCH_2CH_2NHCO$ and $N(CH_3)_3$, partially overlapping signal), 3.17 (br s, 2H, $CH_2N(CH_3)_3$), 3.44 (br s, 4H, $H_2NCH_2CH_2NHCO$ and $NCH_2CH_2CH_2N(CH_3)_3$), 3.91 (br s, 2H, $NHCOCH_2N$), 4.38 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 8.02 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 27.9, 28.1, 31.7, 35.6, 36.0, 36.4, 36.7, 38.2, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.6, 122.7, 139.1 (carbons in cholesteryl skeleton), 30.0 ($CH_2CH_2N(CH_3)_3$), 37.0 ($H_2NCH_2CH_2NHCO$), 38.2 ($CH_2N(CH_3)_3$), 39.2 ($H_2NCH_2CH_2NHCO$), 45.5 ($NCH_2CH_2CH_2N(CH_3)_3$), 52.3 ($NHCOCH_2N$), 53.3 ($N(CH_3)_3$), 156.1 (C=O carbamoyl), 170.6 (C=O amide); MS (ES^+): *m/z* 630.8 ($[M+H]^+$, 100%).



O

ไขมันประจุบวก O; Yield: (resin: 1.1 mmol/g, 177.8 mg) 48.4 mg, 39%; IR (CH_2Cl_2): ν_{max} 3333, 2918, 2840, 1678, 1538, 1509, 1456, 1202, 1121 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.809 (d, J = 5.8 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 4.8 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95-2.80 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2NHCO$), 2.83 (br s, 2H, $H_2NCH_2CH_2CH_2NHCO$), 2.93, 3.04 and 3.10 (br s, 9H, $N(CH_3)_3$), 3.27 (br s, 2H, $CH_2CH_2N(CH_3)_3$), 3.58 (br s, 2H, CH_2NHCO), 3.70 (br s, 2H, $CH_2CH_2N(CH_3)_3$), 4.01 (br s, 2H, $NHCOCH_2N$), 7.99 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.5, 122.7, 139.3 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2NHCO$), 38.3 ($H_2NCH_2CH_2CH_2NHCO$ and $NCH_2CH_2N(CH_3)_3$), 52.4 ($NHCOCH_2N$), 53.3 and 53.5 ($N(CH_3)_3$), 156.1 (C=O carbamoyl), 170.6 (C=O amide); MS (ES^+): *m/z* 629.6 ($[M+H]^+$, 100%).

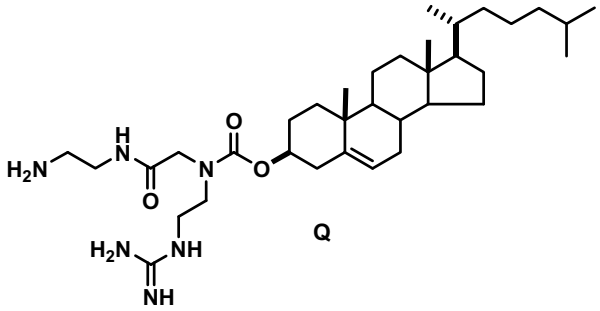


P

ไขมันประจุบวก P; Yield: (resin: 1.1 mmol/g, 186.1 mg) 61.8 mg, 47%; IR (CH_2Cl_2): ν_{max} 3313, 3042, 2934, 2274, 1678, 1541, 1468, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.80 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.85 (d, J = 6.1 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.98-2.30 (m, 32H, protons in cholesteryl skeleton, $H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2N(CH_3)_3$), 2.90 (br s, 4H, $H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2N(CH_3)_3$), 3.02, 3.07 and 3.10 (s, 9H, $N(CH_3)_3$, partially overlapping), 3.83 (br s, 4H, CH_2NHCO and $CH_2CH_2CH_2N(CH_3)_3$), 3.90 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 7.96 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.6, 23.7, 24.1, 27.9, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.9, 122.6, 139.4 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2N(CH_3)_3$), 37.1 ($H_2NCH_2CH_2CH_2$), 38.3 ($CH_2N(CH_3)_3$), 38.4 ($H_2NCH_2CH_2$), 47.5 ($CH_2CH_2CH_2N(CH_3)_3$), 50.9 ($NHCOCH_2N$), 53.2 ($N(CH_3)_3$), 156.4 (C=O carbamoyl), 171.5 (C=O amide); MS (ES^+): *m/z* 644.6 ($[M+H]^+$, 100%).

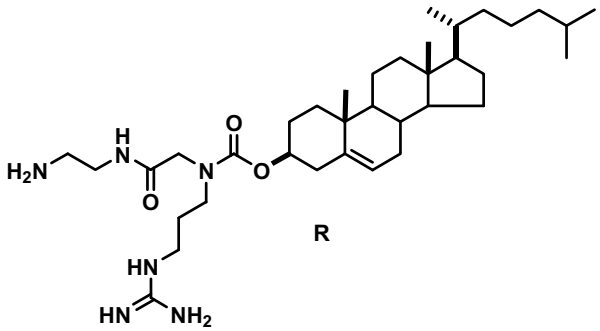
การสังเคราะห์ไขมันประจุบวก Q, R, S และ T

การสังเคราะห์ไขมันประจุบวก **Q-T** สามารถสังเคราะห์ได้จาก resin **45a-45d** โดยใช้วิธีการสังเคราะห์เช่นเดียวกับการสังเคราะห์ไขมันประจุบวก **E** และ **F** จาก resin **36a** และ **36b**



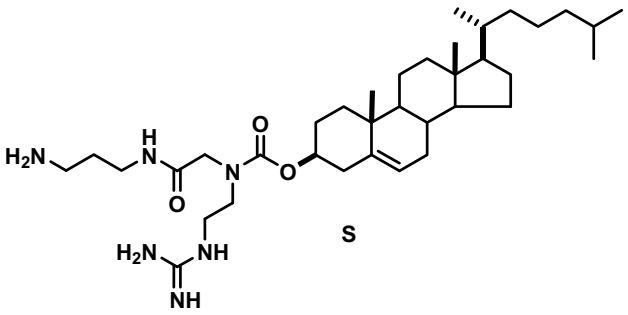
Q

ไขมันประจุบวก Q; Yield: (resin: 1.1 mmol/g, 159.5 mg) 50.5 mg, 47%; IR (CH_2Cl_2): ν_{max} 3347, 2920, 2850, 2279, 1672, 1597, 1509, 1465, 1311, 1203, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.796 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.84 (d, J = 5.8 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 0.93-2.28 (m, 28H, protons in cholesteryl skeleton), 3.02 (br s, 2H, $H_2NCH_2CH_2$), 3.30, 3.35 and 3.43 (br s, 6H, $H_2NCH_2CH_2NHCOCH_2NHCH_2CH_2NHC(NH)NH_2$), 3.93 (br s, 2H, $NHCOCH_2N$), 4.35 (br s, 1H, H-3-Chol), 5.27 (br s, 1H, H-6-Chol), 8.05 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 28.1, 31.7, 35.7, 36.0, 36.4, 36.7, 38.1, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.9, 123.6, 139.3 (carbons in cholesteryl skeleton), 36.9 ($H_2NCH_2CH_2$), 38.5 ($H_2NCH_2CH_2$), 47.5 ($CH_2CH_2NHC(NH)NH_2$), 51.0 ($CH_2CH_2NHC(NH)NH_2$), 156.5 (C=O carbamoyl and C=N guanidine, overlapping), 173.0 (C=O amide); MS (ES^+): *m/z* 615.4 ($[M+H]^+$, 100%).



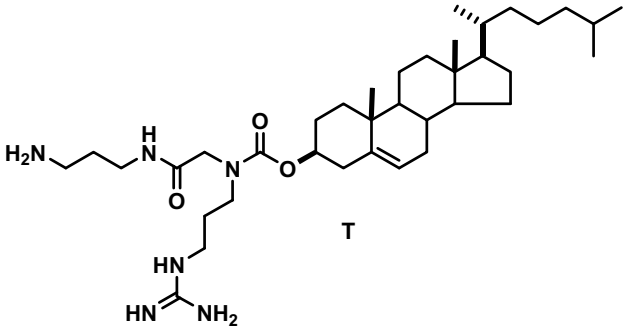
R

ไขมันประจุบวก R; Yield: (resin: 1.1 mmol/g, 173.4 mg) 51.4 mg, 43%; IR (CH_2Cl_2): ν_{max} 3327, 3070, 2918, 2834, 1674, 1574, 1467, 1432, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.82 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 4.4 Hz, 3H, CH_3-21), 0.98 (s, 3H, CH_3-19), 0.98-2.29 (m, 30H, protons in cholesteryl skeleton and $CH_2CH_2CH_2NHC(NH)NH_2$), 3.08 (br s, 2H, $H_2NCH_2CH_2$), 3.20 and 3.30 (br s, 4H, $H_2NCH_2CH_2NHCO$ and $CH_2CH_2CH_2NHC(NH)NH_2$), 3.47 (br s, 2H, $CH_2CH_2CH_2NHC(NH)NH_2$), 3.84 (br s, 2H, $NHCOCH_2N$), 4.40 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 8.04 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.2, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.7, 139.4 (carbons in cholesteryl skeleton), 30.0 ($CH_2CH_2CH_2NHC(NH)NH_2$), 37.0 ($H_2NCH_2CH_2NHCO$), 38.2



S

ไขมันประจุบวก S; Yield: (resin: 1.1 mmol/g, 161.7 mg) 36.3 mg, 33%; IR (CH_2Cl_2): ν_{max} 3327, 2918, 1673, 1535, 1462, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.805 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.85 (d, J = 5.0 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95-2.80 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2CH_2$), 2.92 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.26 and 3.33 (br s, 4H, $H_2NCH_2CH_2CH_2$ and $CH_2CH_2NHC(NH)NH_2$), 3.48 (br s, 2H, $CH_2CH_2NHC(NH)NH_2$), 3.92 (br s, 2H, $NHCOCH_2N$), 4.37 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 7.99 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.1, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.5, 76.2, 122.8, 139.2 (carbons in cholesteryl skeleton), 26.8 ($H_2NCH_2CH_2CH_2$), 38.2 ($H_2NCH_2CH_2CH_2$ and $CH_2CH_2NHC(NH)NH_2$)), 52.5 ($NHCOCH_2N$), 156.1 (C=O carbamoyl and C=N guanidine, overlapping), 170.5 (C=O amide); MS (ES^+): *m/z* 629.5 ($[M+H]^+$, 100%).

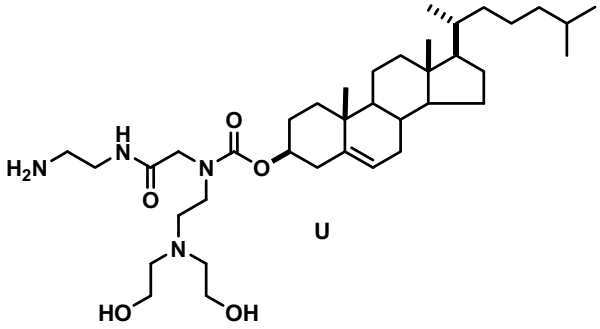


T

ไขมันประจุบวก T; Yield: (resin: 1.1 mmol/g, 170.3 mg) 62.1 mg, 52%; IR (CH_2Cl_2): ν_{max} 3320, 2933, 1678, 1543, 1468, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.82 (d, J = 6.4 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 5.9 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95-2.30 (m, 32H, protons in cholesteryl skeleton, $H_2NCH_2CH_2CH_2CH_2$ and $CH_2CH_2CH_2NHC(NH)NH_2$), 2.92 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.22 (br s, 2H, $CH_2NHC(NH)NH_2$), 3.28 (br s, 4H, $H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2NHC(NH)NH_2$), 3.83 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.91 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.1, 31.8, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.9, 122.6, 139.4 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2NHC(NH)NH_2$), 37.1 ($H_2NCH_2CH_2CH_2$), 38.3 $CH_2CH_2CH_2NHC(NH)NH_2$), 38.4 ($H_2NCH_2CH_2CH_2$), 47.5 ($CH_2CH_2CH_2NHC(NH)NH_2$), 50.5 ($NHCOCH_2N$), 156.5 (C=O carbamoyl and C=N guanidine, overlapping), 171.6 (C=O amide); MS (ES^+): *m/z* 643.3 ($[M+H]^+$, 100%).

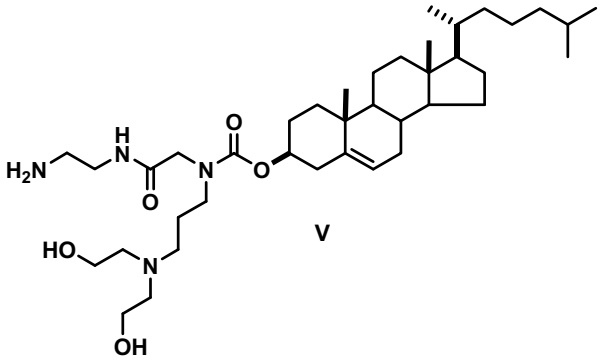
การสังเคราะห์ไขมันประจุบวก U, V, W และ X

การสังเคราะห์ไขมันประจุบวก **U**-**X** สามารถสังเคราะห์ได้จาก resin **45a**-**45d** โดยใช้วิธีการสังเคราะห์เช่นเดียวกับการสังเคราะห์ไขมันประจุบวก **G** และ **H** จาก resin **36a** และ **36b**



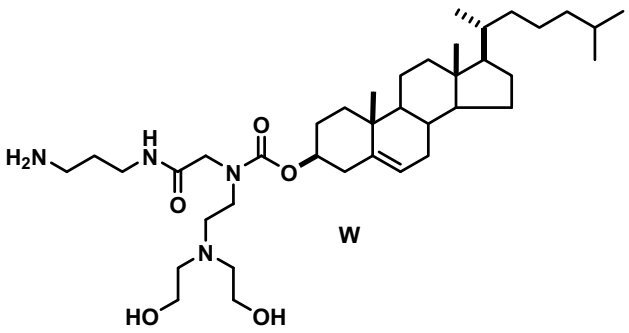
U

ไขมันประจุบวก U; Yield: (resin: 1.1 mmol/g, 185.7 mg) 71.8 mg, 53%; IR (CH_2Cl_2): ν_{max} 3334, 2933, 2279, 1678, 1541, 1509, 1432, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.818 and 0.821 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.1 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.93-2.32 (m, 28H, protons in cholesteryl skeleton), 3.10 (br s, 2H, $H_2NCH_2CH_2$), 3.37 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.51 (br s, 4H, $H_2NCH_2CH_2$ and $CH_2N(CH_2CH_2OH)_2$), 3.70 (br s, 2H, $CH_2CH_2N(CH_2CH_2OH)_2$), 3.87 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.99 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.31 (br s, 1H, H-6-Chol), 7.89 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.7, 23.8, 24.2, 27.7, 27.9, 28.1, 31.7, 35.8, 36.1, 36.4, 36.8, 38.1, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.4, 139.2 (carbons in cholesteryl skeleton), 37.2 ($H_2NCH_2CH_2$), 38.1 ($CH_2N(CH_2CH_2OH)_2$), 39.2 ($H_2NCH_2CH_2NHCO$), 47.5 ($CH_2CH_2N(CH_2CH_2OH)_2$), 50.5 ($NHCOCH_2N$), 55.3, 55.5 and 55.8 ($N(CH_2CH_2OH)_2$), 156.2 (C=O carbamoyl), 173.1 (C=O amide); MS (ES^+): *m/z* 661.5 ($[M+H]^+$, 100%).



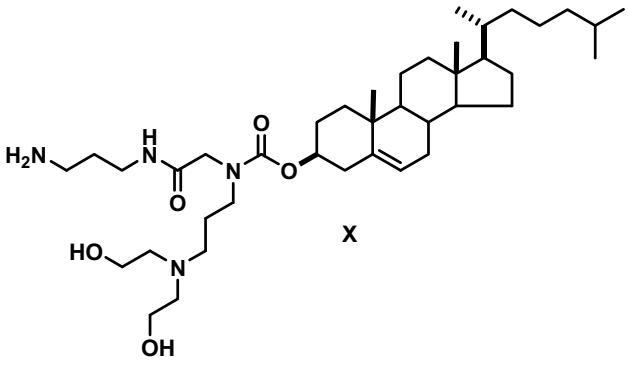
V

ไขมันประจุบวก V; Yield: (resin: 1.1 mmol/g, 199.3 mg) 61.6 mg, 42%; IR (CH_2Cl_2): ν_{max} 3315, 2934, 2840, 2274, 1677, 1541, 1467, 1429, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.792 (d, J = 5.6 Hz, 6H, CH_3-26 and CH_3-27), 0.85 (d, J = 5.3 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.97-2.26 (m, 30H, protons in cholesteryl skeleton and $CH_2CH_2CH_2N(CH_2CH_2OH)_2$), 3.05 (br s, 2H, $H_2NCH_2CH_2$), 3.27 (m, 10H, $CH_2NHCOCH_2NCH_2CH_2CH_2N(CH_2CH_2OH)_2$), 3.44 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.84 (br s, 2H, $NHCOCH_2N$), 4.38 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 8.02 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.1, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.1, 122.6, 139.4 (carbons in cholesteryl



W

ไขมันประจุบวก W; Yield: (resin: 1.1 mmol/g, 177.0 mg) 41.9 mg, 32%; IR (CH_2Cl_2): ν_{max} 3389, 3081, 2933, 1677, 1467, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.809 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 5.0 Hz, 3H, CH_3-21), 0.96 (s, 3H, CH_3-19), 0.96-2.28 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2C\mathit{H}_2CH_2$), 2.91 (br s, 2H, $H_2NC\mathit{H}_2CH_2CH_2$), 3.29 and 3.36 (br s, 4H, $N(C\mathit{H}_2CH_2OH)_2$), 3.50 (br s, 2H, $CH_2C\mathit{H}_2N(CH_2CH_2OH)_2$), 3.75 (br s, 2H, $H_2NCH_2CH_2C\mathit{H}_2$), 3.85 (br s, 2H, $C\mathit{H}_2CH_2N(CH_2CH_2OH)_2$), 3.92 (br s, 4H, $N(CH_2C\mathit{H}_2OH)_2$), 3.97 (br s, 2H, $NHCOC\mathit{H}_2N$), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.89 (br s, 3H, $N\mathit{H}_3^+$); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.1, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.5, 76.2, 122.8, 139.2 (carbons in cholesteryl skeleton), 26.8 ($H_2NCH_2\mathit{C}H_2CH_2$), 38.2 ($H_2N\mathit{C}H_2CH_2\mathit{C}H_2$ and $\mathit{C}H_2\mathit{C}H_2N(CH_2CH_2OH)_2$), 52.5 ($NHCO\mathit{C}H_2N$), 55.1 and 55.3 ($N(\mathit{C}H_2\mathit{C}H_2OH)_2$), 156.1 (C=O carbamoyl), 170.5 (C=O amide); MS (ES^+): m/z 675.5 ($[M+H]^+$, 100%).



X

ไขมันประจุบวก X; Yield: (resin: 1.1 mmol/g, 192.6 mg) 65.1 mg, 44%; IR (CH_2Cl_2): ν_{max} 3308, 3070, 2934, 1678, 1467, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.820 and 0.824 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.1 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.93-2.30 (m, 32H, protons in cholesteryl skeleton, $H_2NCH_2C\mathit{H}_2CH_2$ and $NCH_2C\mathit{H}_2CH_2N(CH_2CH_2OH)_2$), 2.93 (br s, 2H, $H_2NC\mathit{H}_2CH_2CH_2$), 3.10 (br s, 4H, $N(C\mathit{H}_2CH_2OH)_2$), 3.29 (br s, 2H, $H_2NCH_2CH_2C\mathit{H}_2$), 3.57 (br s, 4H, $C\mathit{H}_2CH_2C\mathit{H}_2N(CH_2CH_2OH)_2$), 3.87 (br s, 4H, $N(CH_2C\mathit{H}_2OH)_2$), 3.99 (br s, 2H, $NHCOC\mathit{H}_2N$), 4.39 (br s, 1H, H-3-Chol), 5.31 (br s, 1H, H-6-Chol), 7.80 (br s, 3H, $N\mathit{H}_3^+$); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.8, 18.6, 19.2, 21.0, 22.4,

สรุปผลการศึกษาและวิจารณ์ผล

จากการสังเคราะห์ไขมันประจุบวกกลุ่มที่ 3 ซึ่งมีประจุส่วนหัวแบบไม่สมมาตรด้วยเทคนิคการสังเคราะห์บนวัฏภาคของแข็ง สังเคราะห์สารได้ทั้งหมด 24 ชนิด การหาเอกลักษณ์ของสารดังกล่าวอาศัยข้อมูลทาง IR ^{1}H NMR และ MS สเปกโทรสโกปี จากการศึกษาประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ HEK293 พบว่า ไขมันประจุบวก 8 ชนิด มีประสิทธิภาพดีกว่าสารมาตรฐาน Effectene^{TM} นำไขมันประจุบวกทั้ง 8 ชนิดไปศึกษาหาสภาวะที่ทำให้มีประสิทธิภาพดีที่สุด พบว่าไขมันประจะบวก 7 ชนิด มีประสิทธิภาพในการพาดีเอ็นเอเข้าสู่เซลล์ PC3 ได้ดีกว่าสารมาตรฐานทั้ง 3 ชนิด คือ Effectene^{TM}, DC-Chol และ DOTAP ไขมันประจุบวกทั้ง 7 ชนิด ยกเว้น สาร G ไม่มีความเป็นพิษต่อเซลล์ที่ทดสอบ ไลโปโซมที่ 8 สารมีขนาด 300-1000 nm

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ภาคผนวก

ผลงานวิจัย

ผลงานตีพิมพ์ในวารสารวิชาการระดับนานาชาติ

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High transfection efficiency and low toxicity cationic lipids with aminoglycerol–diamine conjugate

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ABSTRACT

The solid phase synthesis of a library of aminoglycerol–diamine conjugate-based transfection agents having urea linkage between diverse length of diamines and various lengths of hydrophobic tails is described. These compounds were characterized and structure–activity relationships were determined for DNA binding and transfection ability when formulated as cationic liposomes. Cationic lipids with short spacer length and short hydrophobic tails bound to DNA and delivered DNA into HEK293 cells more efficient than those with longer ones. Transfection efficiency of some of the cationic liposomes was superior to that of the commercial transfection agents, Effectene™, DOTAP and DC-Chol. The lipids **6Ab** and **6Bb** did not require the helper lipid DOPE to produce high-efficiency transfection of human cells while displaying minimal cytotoxicity. This suggests that these newly described aminoglycerol-based lipids should be very promising in liposome-mediated gene delivery and illustrate the potential of solid phase synthesis method for non-viral vector discovery.

1. Introduction

Gene therapy represents an important advance in the alternative treatment of a variety of diseases of both genetic and acquired origin. One fundamental gene therapy technology is a gene delivery system. The delivery of genetic information into cells has a number of major applications, not only as an ideal method to treat diseases but also as an essential tool in many areas of molecular biology. The most efficient method for delivery of DNA into cells is the use of viral vectors.[1] However, there are growing concerns about both the short- and the long-term risks of viral vectors, the key problems being the immune response they provoke, which can even be lethal,[2] the limitation on the size of the DNA that can be introduced, and the difficulty of large-scale virus production. There have hence been great efforts to develop a range of non-viral vectors for gene therapy applications.[3] A broad range of non-viral delivery systems have been described to date, including microinjection,[4] electroporation,[5] and chemical-based systems such as calcium phosphate,[6] DEAE-dextran[7] and cationic lipid-mediated transfection.[8] Of all the non-viral vectors, cationic lipids have been shown to be the most promising method for in vivo applications, based on a combination of efficiency, stability and lack of toxicity.

Since Felgner and co-workers reported the application of cationic lipids in DNA delivery into living cells,[9] numerous cationic lipids have been synthesized.[10–15] Some of these have been used in gene therapy clinical trials,[11,12] while many others have established cationic lipids as the most common method for the transfection of cell lines in the laboratory. As shown in Figure 1, most of the cationic lipids are monocationic (e.g., *N*-(1-(2,3-dioleyloxy)propyl)-*N*,*N*,*N*-trimethylammonium chloride (DOTMA),[9] *N*-(1-(2,3-dimyristyloxypropyl)-*N*,*N*-dimethyl-(2-hydroxyethyl)ammonium bromide (DMRIE),[8] *N*-(1-(2,3-dioleoyloxypropyl)-*N*,*N*-dimethyl-(2-hydroxyethyl) ammonium bromide (DORIE),[16] *N*-(1-(2,3-dioleoyloxy)propyl)-*N*,*N*,*N*-trimethylammonium methylsulphate (DOTAP),[8] KL-1-14[17] and carbamate-linked lipid[18]) and polycationic heads (e.g., 2,3-dioleyloxy-*N*-(2-(sperminecarboxamido)ethyl)-*N*,*N*-dimethyl-1-propanamminium pentatrifluoroacetate (DOSPA)) which contain aminoglycerol as a core structure. The monocationic head of the above-mentioned compounds usually derived from the amino group while the diols are the attachment point of the hydrophobic tail via ether,[8,9,16] ester[8] and carbamate bond.[18] The requirement for high transfection efficiency of these lipids depends on the combination of cationic head, degree of hydrophobicity of the tails and the bond that links the hydrophobic tails. Co-lipid, for example, dioleoyl-L-α-phosphatidylethanolamine (DOPE), is also play an important role in the liposome formation for high activity.[16,19]

There are a number of reports on the synthesis of aminoglycerol-based cationic lipids having the variation of alkyl chain length with symmetric and asymmetric tails.[16,20] To our knowledge,

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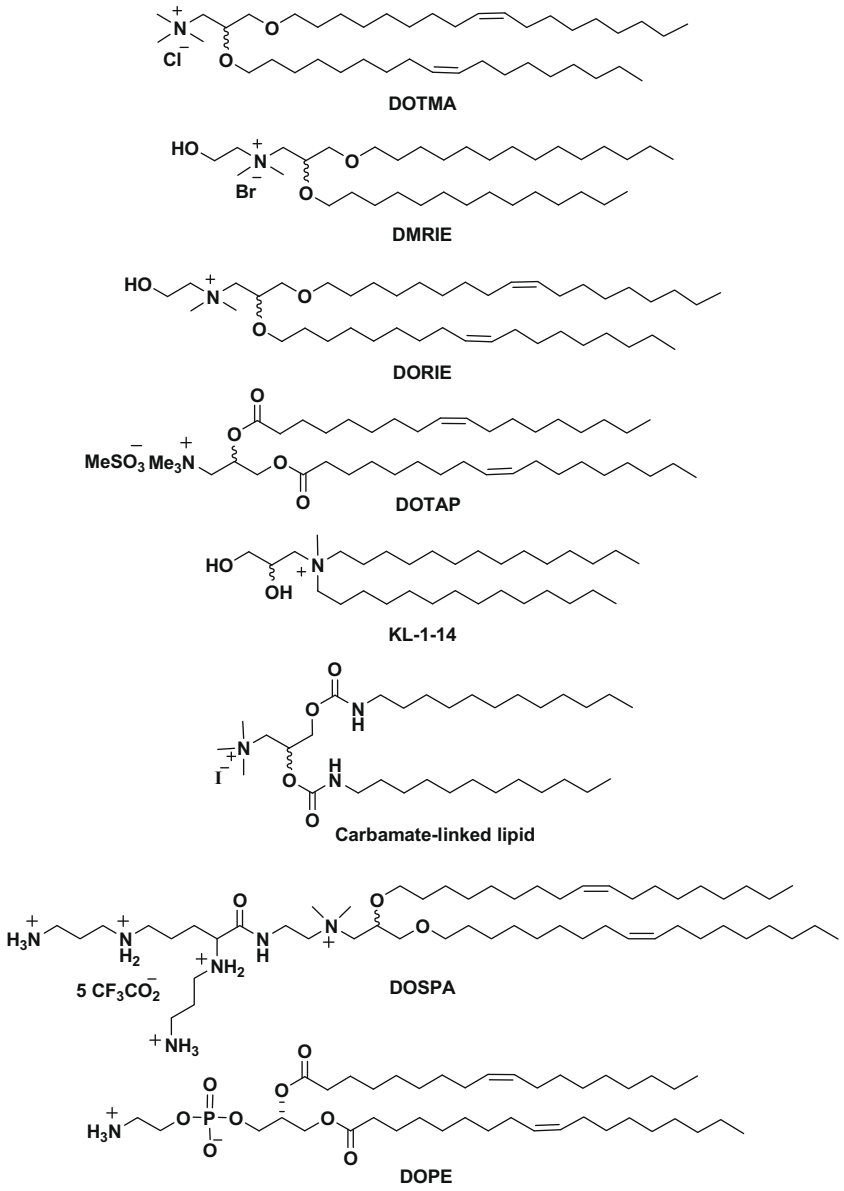


Figure 1. Structures of some of aminoglycerol-based cationic lipids and co-lipid, Dioleoyl-L-α-phosphatidylethanolamine (DOPE).

aminoglycerol-based cationic lipids having various lengths of spacer head have not been studied for transfection efficiency. Here, we designed and synthesized the monocationic lipids with various spacer lengths and various hydrophobic tails and studied for their structure–transfection activity relationships (SARs) (Fig. 2). The hydrophilic head was connected to the hydrophobic tail by the urea linkage. Since delivery of DNA into mammalian cells involves numerous steps,[21] it has been very difficult to generate SAR for transfection. In order to synthesize a wide variety of cationic lipids, we used solid phase synthesis technique that has been successfully synthesized these classes of compounds.[13–15]

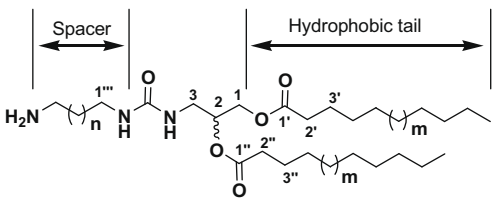
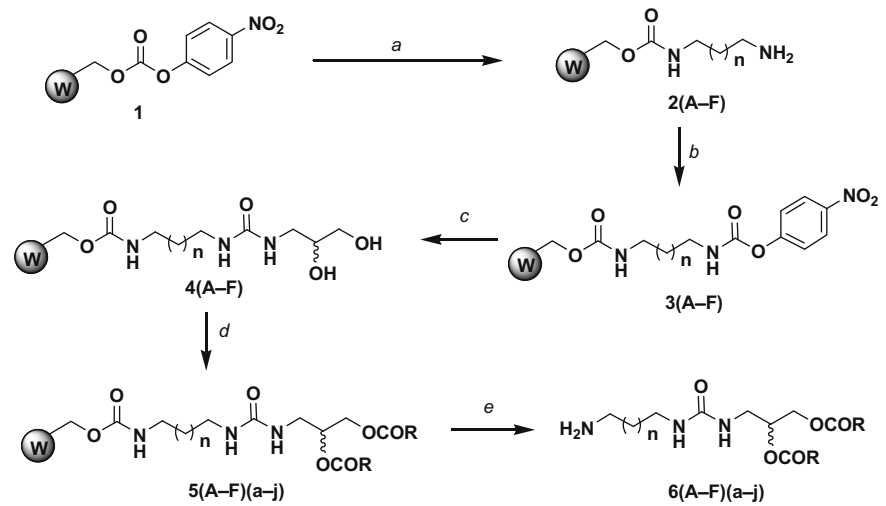


Figure 2. The common structure of monocationic lipids in this work.

2. Results and discussion

2.1. Synthesis of cationic lipids

The synthetic route for aminoglycerol–diamine conjugate cationic lipids is shown in Scheme 1. Starting from the active carbonate **1**,[22] various diamines (Fig. 3) were added to obtain the resin **2**. This allows free amine to link to aminoglycerol as a building block for hydrophobic tails to obtain monocationic head after cleavage at the final step. Thus, the resin **2A–F** was treated with 4-nitrophenylchloroformate in the presence of pyridine to give the active urethane resin **3A–F**. To this resin the leaving group, 4-nitrophenol, was replaced with aminoglycerol to provide the resin **4A–F** having urea linkage. To drive the reaction to completion, 1 M aminoglycerol in CH_2Cl_2/MeOH (9:1) was used. The diol groups of the resin **4A–F** were coupled with various fatty acids



Scheme 1. Reagents and conditions: (a) diamines (see Fig. 3), DMF, 12 h; (b) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2, 16 h; (c) (±)-3-amino-1,2-propanediol, CH_2Cl_2/MeOH (9:1), 12 h; (d) fatty acid (see Fig. 3), DIC, DMAP, CH_2Cl_2, DMF, 12 h; (e) 20% TFA/CH_2Cl_2, 2 h.

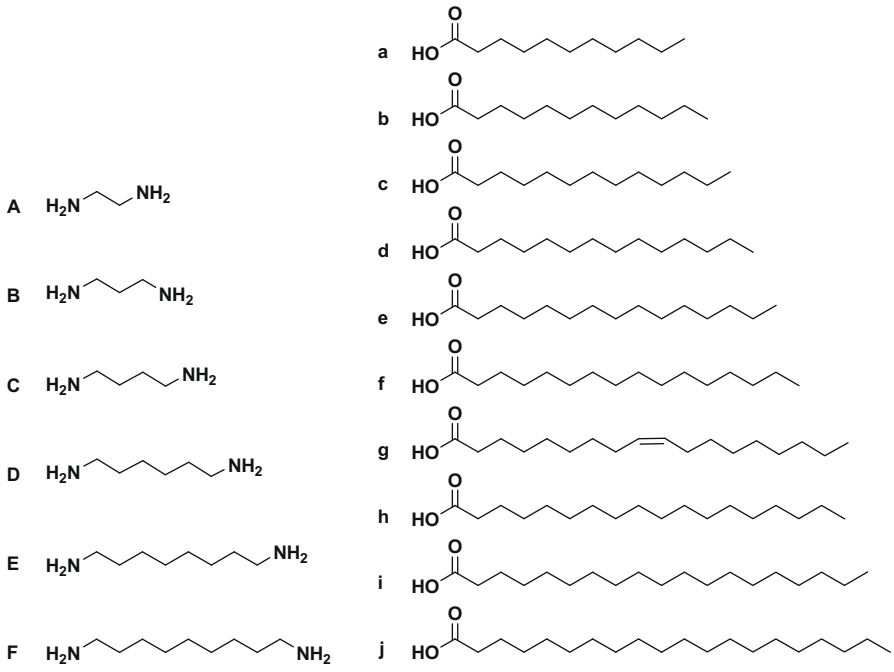


Figure 3. Diamines and long chain fatty acids used in Scheme 1.

using diisopropylcarbodiimide (DIC) as a coupling reagent and DMAP as a catalyst to generate the corresponding diesters **5(A–F)**(**a**–**j**). Treatment of the resin **5(A–F)**(**a**–**j**) with 20% (v/v) trifluoroacetic acid (TFA) in CH_2Cl_2 for 2 h gave rise to the desired products **6(A–F)**(**a**–**j**) in moderate to high yield (based on the original loading of Merrifield resin, 1.1 mmol/g) and high purity. Their structures were established by IR and ^{1}H NMR data and the observed molecular weights were in good agreement with the calculated values for all compounds.

2.2. DNA binding affinity

The relative DNA binding affinities of cationic lipids were evaluated to determine whether transfection activities correlated with DNA binding. Gel retardation assay was used to evaluate binding affinities. To perform this assay, cationic lipids were mixed with plasmid DNA at weight ratios of 1:10 (I) and 1:20 (II) (DNA/sample, w/w) and the lipoplexes were loaded onto an agarose gel. The result is shown in Figure 4. At both of the DNA/cationic lipid ratios of 1:10 and 1:20, nine compounds, **6Ba**, **6Bb**, **6Bc**, **6Ca**, **6Cb**, **6Cc**, **6Da**, **6Db** and **6Dc**, interacted sufficiently with DNA to retard migration through the gel matrix and four cationic lipids, **6Cd**, **6Ce**, **6Cf** and **6Dh**, partially bound with DNA. The results indicated that the chain length of the hydrophobic tail required for DNA binding were 11–13 carbons, with the spacer not exceeding 6 carbons. The cationic lipids **6Ea**–**6Fj** which contained 8 and 9 carbons spacer with any hydrophobic tail did not bind with DNA.

2.3. Transfection activity

To study structure–activity relationship, all the synthesized cationic lipids, both bound and not bound to DNA, were tested for DNA delivery to mammalian cell lines using β-galactosidase as a reporter gene. Figure 5 displays data generated from an assay

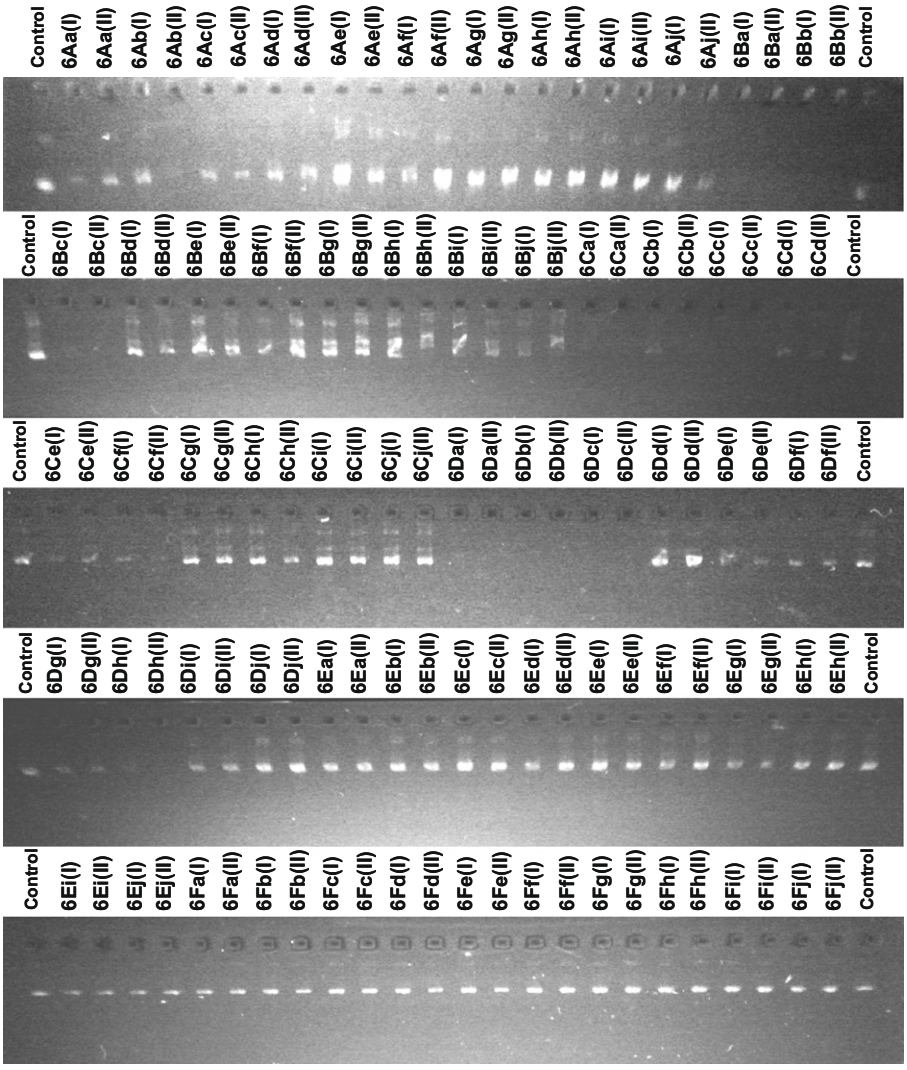


Figure 4. Gel retardation assay of DNA/cationic lipids complexes at a weight ratio of 1:10 (I) and 1:20 (II). Lanes marked "Control" contained DNA alone and was used as a control. The presence of a lower band indicated that the DNA has migrated and has not been bound by the transfection compound.

employing plasmid DNA (0.1 μg/well) at DNA/liposome ratios of 1:5, 1:10 and 1:20 (w/w). The liposome formation in the preliminary transfection activity screening was with and without DOPE. In the case of liposome formation with DOPE, cationic lipid/DOPE ratio was 2:1 (w/w). The majority of the lipids screened did not mediate transfection efficiency above 50% relative to the commercial reagent, Effectene™. The results have demonstrated that most of the cationic lipids with undecanoyl (C11) and lauroyl (C12) hydrophobic tails exhibited higher transfection efficiency than the longer tails. Cationic lipids with spacer length between cationic head and hydrophobic tail of 2–4 carbons showed high relative transfection activity than that of 8 and 9 carbons. Liposome formation with DOPE of compounds **6Aa** exhibited higher transfection activity than liposome without DOPE (Fig. 5). However, liposomes of the lipids **6Ab**, **6Bb** and **6Da** did not require DOPE for transfection activity. The cationic lipids **6Aa**, **6Ab**, **6Bb** and **6Da** which exhibited moderate transfection efficiency were then used for further optimization.

2.4. Transfection optimization

2.4.1. Effect of cationic lipid/DOPE ratios

The formulation of cationic liposome (cationic lipid and co-lipid ratios) have a crucial effect on the transfection efficiency.[23] Many studies on cells in culture clearly demonstrated that liposomes were composed of an equimolar mixture of co-lipid DOPE and cationic lipids mediated higher levels of transfection than those without DOPE or those with different helper lipids.[24] It has been suggested that DOPE play a role in facilitating the disassembly of the lipid-based DNA formulations after their internalization, and the escape of DNA from the endocytotic vesicle.[25] To evaluate the effect of DOPE on gene delivery, cationic lipids were formulated into cationic liposomes with different amounts of DOPE. In order to find out the most effective formulation, transfection with identical DNA/liposome weight ratio of 1:20 was used. Their ability to deliver a plasmid encoding β-galactosidase into human embryo kidney (HEK293) cells was studied (Fig. 6). All the selected lipids were not effective in any lipid/DOPE ratio, except the lipid **6Bb**, which was found to be most effective at a lipid/DOPE weight ratio of 1:2. The lipid **6Ab** was found to be effective transfection agent when the liposome was formed without DOPE (see Figs. 5 and 6). The lipid **6Da** exhibited similar transfection result when the liposome was formed without DOPE (Fig. 5) and with DOPE at a weight ratio (lipid/DOPE) of 1:2 (Fig. 6). In the case of lipid **6Aa**, the effective formulation for high transfection efficiency was lipid/DOPE ratio of 2:1. In conclusion, the optimal liposome formulation of each lipid, which was used for the next optimization, was as followed.

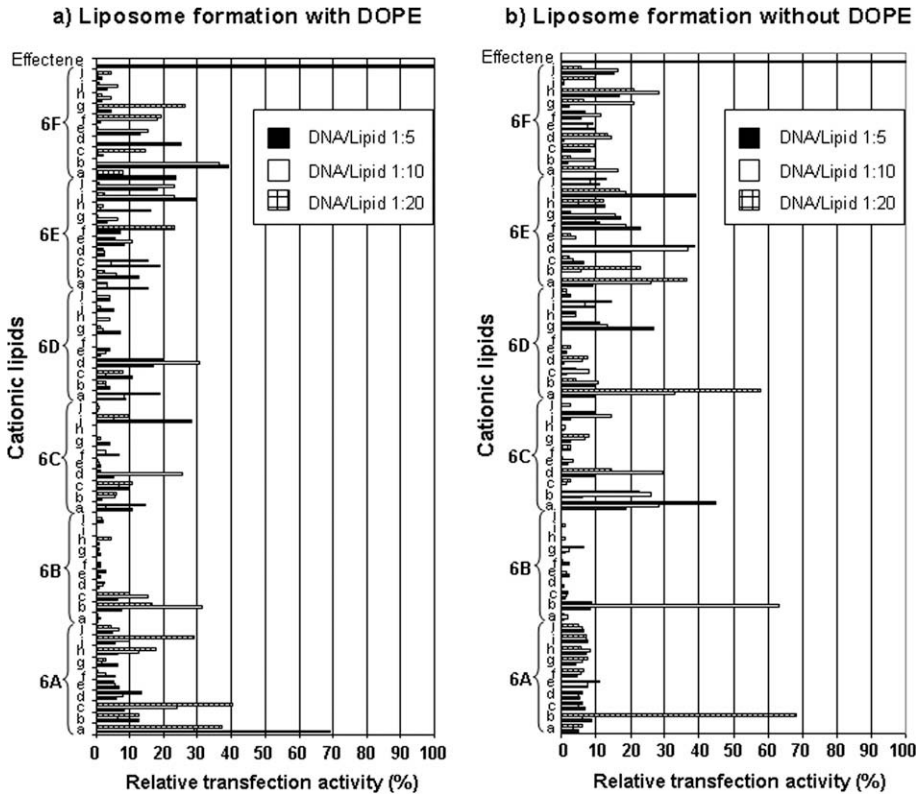


Figure 5. Transfection efficiencies of cationic lipids employing pCH110-encoding β-galactosidase (0.1 μg/well). The liposome formation was both (a) with DOPE and (b) without DOPE. The lipoplexes were used at DNA/lipids w/w ratios of 1:5, 1:10 and 1:20. The transfection efficiencies of the lipids were compared to that of commercially available reagent, Effectene™, which calculated as 100% transfection efficiency.

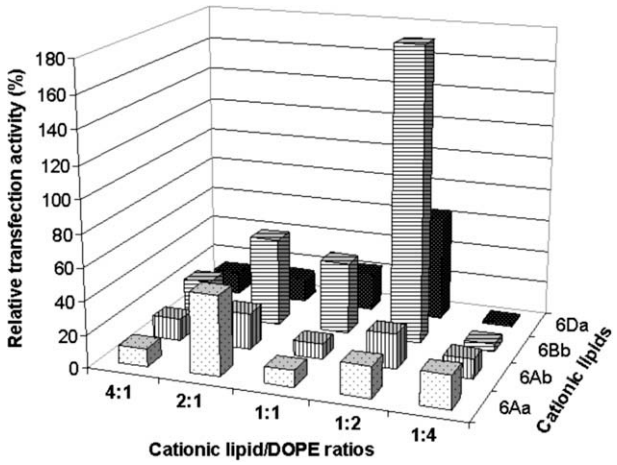


Figure 6. In vitro transfection efficiency of the lipids **6Aa**, **6Ab**, **6Ba** and **6Da** in HEK293 cells across the cationic lipid/DOPE weight ratios 4:1 to 1:4. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown).

Liposome which was composed of the lipid **6Bb** and **6Da** were prepared at lipid/DOPE ratios of 1:2 where as the ratio of the lipid **6Aa** was 2:1. Liposome of the lipid **6Ab**, as well as the lipid **6Bb**, was formed without helper lipid.

2.4.2. Effect of DNA/liposome ratios

After optimizing the lipid/DOPE ratio for selected lipids, all of them were tested by using the identical amount of DNA (0.1 μg/well) and varying the amount of lipid using the respective optimized lipid/DOPE ratio (Fig. 6). Thus, optimal positive/negative charge ratio of the cationic liposome to plasmid DNA for in vitro transfection was determined. Six DNA/cationic liposome ratios of 1:1, 1:5, 1:10, 1: 20, 1:40 and 1:80 (w/w) were prepared. The transfection efficiency of tested lipids was shown as % relative to that of the positive control, Effectene™, which was calculated as 100%. Transfection efficiency was found to increase at higher DNA/lipid ratio (Fig. 7). The lipid **6Bb** exhibited highest efficiency (270%) at DNA/lipid ratio of 1:40 without using DOPE as a co-lipid. However, liposome with lipid **6Bb**/DOPE at the weight ratio of 1:2 showed high activity (180%) at DNA/lipid ratio of 1:20. Interestingly, transfection efficiency of liposome with the lipid **6Bb** alone was superior to that contained DOPE, since most of the aminoglycerol-based lipid require helper lipid for high transfection efficiency.[16,19] The lipids **6Aa** and **6Da** exhibited high transfection efficiency at the DNA/lipid ratio of 1:40 whereas the lipid **6Ab** showed high activity at the DNA/lipid ratio of 1:80. The optimal DNA/lipid ratio for each lipoplex formulation was used for the next experiment.

2.4.3. Effect of variation of the amount of DNA

To see whether variation in the amount of the DNA affected the transfection efficiency of aminoglycerol-based cationic lipids, transfection experiments were performed using the optimal cationic liposome formulation (Fig. 6) and optimal lipoplex formation (Fig. 7) conditions. The amount of DNA was varied from 0.1, 0.2 and 0.4 μg/well. In all cases of tested lipids, transfection efficiencies increase at higher DNA amounts (Fig. 8). In the case of the lipid **6Bb**, liposome formation without DOPE showed much higher transfection efficiency than that with DOPE. This lipid exhibited 3.5-fold higher transfection efficiency than that of Effectene™. The lipids **6Ab** and **6Da** gave similar results. Thus, a 4-fold increase in transfection efficiency was observed when the amount of DNA was increased from 0.1 to 0.2 μg/well and from 0.2 to 0.4 μg/well.

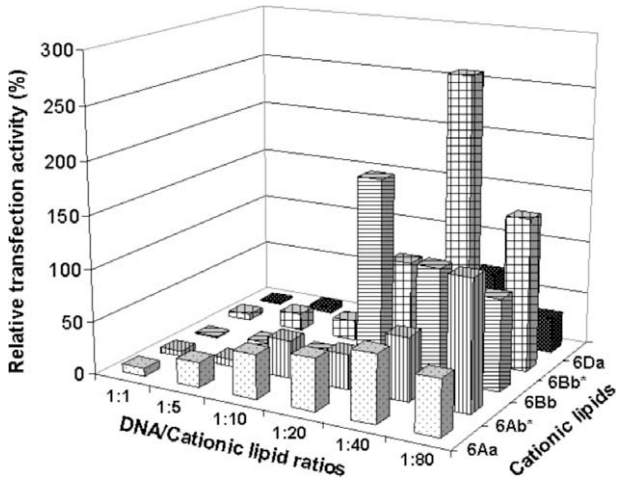


Figure 7. In vitro transfection efficiency of the lipids **6Aa**, **6Ab**, **6Ba** and **6Da** in HEK293 cells across the DNA/lipid weight ratios 1:1 to 1:80. The optimal liposome formations of each lipid from Figure 6 were used as described in the text. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown). The cationic lipid marked * displays the liposome formation without co-lipid, DOPE.

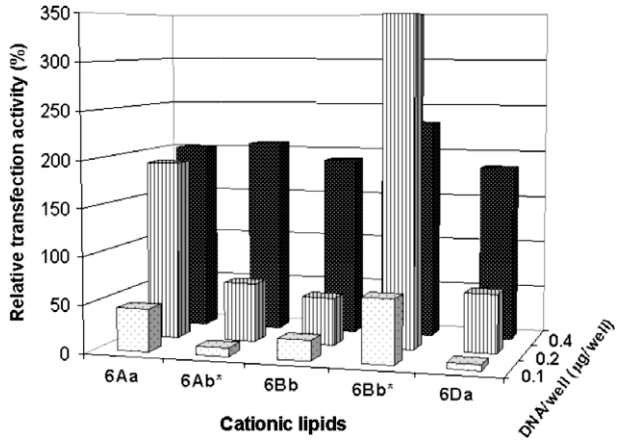


Figure 8. Effect of DNA amount for gene delivery. The optimal liposome formation (Fig. 6) and DNA/liposome complex (Fig. 7) of each lipid were used to mix with various amount of DNA from 0.1 to 0.4 μg. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown). The cationic lipid marked * displays the liposome formation without co-lipid, DOPE.

Similarly, in the case of the lipid **6Aa**, the relative transfection activity increased from 50% to 200% when the amount of DNA was increased from 0.1 to 0.2 μg/well. However, by using DNA 0.4 μg/well, there was little improvement in the transfection efficiency.

2.4.4. Effect of serum

One of the major drawbacks of cationic lipids for their in vivo use is the inhibition of the transfection efficiency of cationic liposomes in the presence of serum. Most of cationic lipids which exhibited high transfection activity in the absence of serum lost their efficiency when transfected in the presence of serum.[26] In order to investigate the effect of serum on gene transfection efficiencies of these cationic lipids, transfection experiments were therefore performed in the presence of 5% and 10% serum with the optimal condition of each lipid (optimal results from Figs. 6–8) and the results are shown in Figure 9. All lipids exhibited their

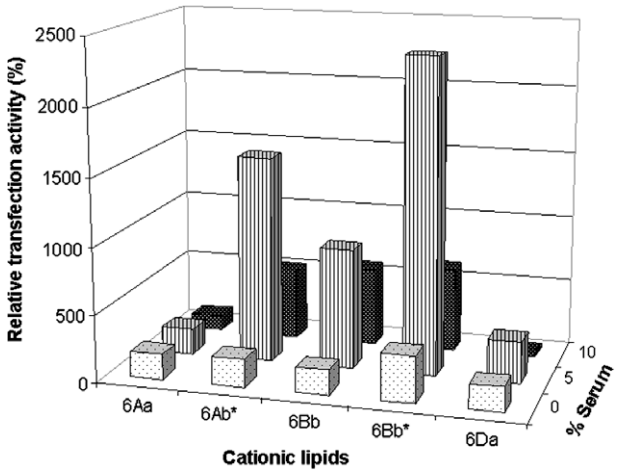


Figure 9. Effect of serum for transfection efficiency. The optimal liposome formation (Fig. 6), DNA/liposome complex (Fig. 7) and amount of DNA per well (Fig. 8) of each lipid were used to transfer gene to HEK293 cell at various amount of serum. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown). The cationic lipid marked * displays the liposome formation without co-lipid, DOPE.

transfection activity and the activity reached its maximum when 5% serum was present. Transfection efficiency was significantly decreased when the cells were tested in the presence of 10% serum. The lipids **6Bb** and **6Ab** showed approximately 20- and 15-fold higher transfection efficiency than Effectene™ under the 5% serum-containing condition.

2.4.5. Transfection efficiency towards different cell lines

To evaluate the transfection efficiency of the lipids **6Bb** and **6Ab** towards the mammalian cell lines, HEK293, PC-3 and HeLa cells, the optimum conditions for high transfection efficiency of the lipids **6Bb** and **6Ab** were used (optimal results from Figs. 6–8). Transfection experiment was performed under the 5% and 10% serum-containing condition since these lipids exhibited high transfection efficiency (Fig. 9). Three commercially available agents, Effectene™, DOTAP and DC-Chol, were tested under identical condition. As could be seen from Figure 10, transfection efficiency of two selected lipids, **6Ab** and **6Bb**, were higher than those of the commercially available agents against HEK293 and HeLa cell lines. However, lipids **6Ab** and **6Bb** could not transfer DNA into PC-3 cell.

2.5. Transfection toxicity

Two mostly concerned criteria for gene delivery carriers are transfection efficiency and their cytotoxicity. To assess the relationship between cytotoxicity and gene expression efficiency, the toxicity of the cationic lipids **6Ab** and **6Bb** on HEK293, PC-3 and HeLa cell lines using the optimal condition (From Figs. 6–8) were determined by measuring changes in cell metabolic activity (MTT assay). The results were shown as % cell viability as compared to the control cells in the presence of DNA (Fig. 11). Transfection efficiency and toxicity of cationic lipids have been found to depend on the cell lines. All the lipid-based transfection agents, DOTAP, DC-Chol, **6Ab** and **6Bb**, were not toxic to HEK293 cell and cell viability for the mentioned transfection agents was all above 90%. While the cytotoxicity of non-liposomal transfection agent, Effectene™ was relatively higher, the cell viability in HEK293, PC-3 and HeLa cells was only more than 50%. Lipids **6Ab** and **6Bb** exhibited higher transfection efficiency on HeLa cells but cell viability was only 50–60%. All the tested compounds including the commercially available agents were not efficient to deliver DNA into PC-3 cells.

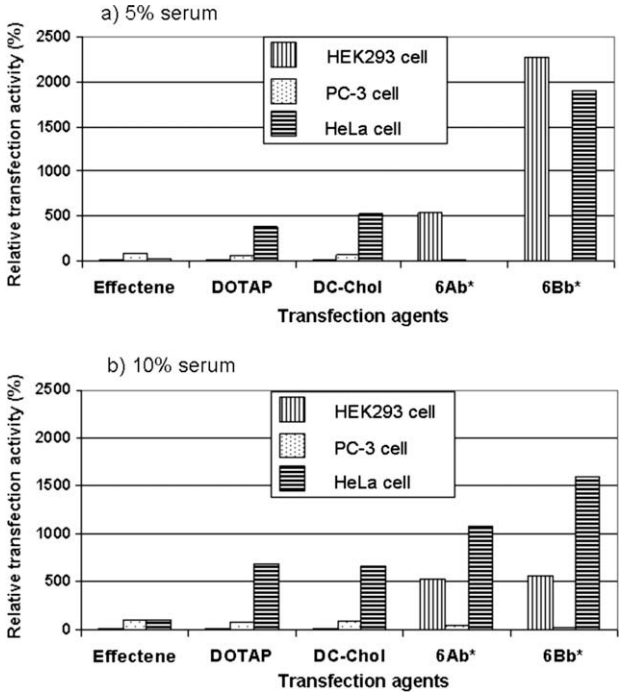


Figure 10. In vitro transfection efficiencies of lipids **6Ab** and **6Bb** on HEK293, PC-3 and HeLa cells in the presence of (a) 5% serum and (b) 10% serum. Cationic liposomes were prepared without co-lipid. The optimal weight ratios of DNA/liposome of lipids **6Ab** and **6Bb** were 1:80 and 1:40, respectively. Transfection efficiencies of the lipids were compared to those of the commercial reagents, Effectene™, DOTAP and DC-Chol. Transfection efficiency of Effectene™ against PC-3 cell was calculated as 100%. The cationic lipid marked * displays the liposome formation without co-lipid, DOPE.

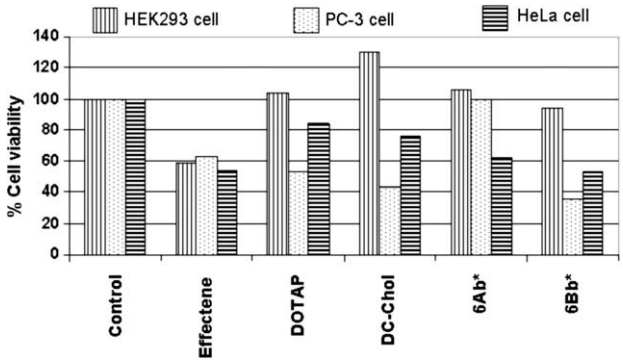


Figure 11. Effect of transfection compounds on cell metabolic activity. Liposomes of cationic lipid without DOPE were formed and added to DNA to form lipoplex. The total amounts per well of lipoplex of the lipids **6Ab** and **6Bb** as well as the commercially available agents, Effectene™, DOTAP and DC-Chol were 16 μg, 8 μg, 4 μg, 8 μg and 8 μg, respectively, which were added to HEK293, PC-3 and HeLa cells and incubated for 24 h. Cell metabolic activity was determined by an MTT assay.

This may result from the high toxicity of the transfection agent tested.

In conclusion we have demonstrated that combinatorial synthesis has allowed the discovery of highly efficient transfection agents with acceptable cytotoxicity. The transfection results suggest that aminoglycerol-based cationic lipid exhibited high transfection efficiency when the spacer head and length of hydrophobic tails are short (see Fig. 2). The lipids **6Ab** and **6Bb** effectively delivered DNA into HEK293 and HeLa cells even though the liposome was formed without the helper lipid, and this resulted in higher transfection activity than that of the commercially available transfection agents. The balance of hydrophilicity and hydrophobicity might play an important role for cationic lipid-based transfection agent to form liposome. Hence, the size of the liposome prepared from the effective lipids **6Ab** and **6Bb** were about 500 nm whereas lipids having longer hydrophobic tail, for example, **6Aj** and **6Bj**, did not form liposome, as observed under transmission electron microscopy (data not shown). We hope that the strategy illustrated here is useful for the development of cationic lipid-based gene delivery.

3. Experimental

3.1. General

IR spectra were recorded on a Perkin-Elmer Spectrum GX60237 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 spectrometer using a residual solvent signal as internal standard. Mass spectra were measured on a Finnigan LC-Q instrument. Starting materials and reagents were purchased from commercial suppliers and used without further purification.

3.2. General procedures for the preparation of compounds 6(A–F)(a–j)

3.2.1. Synthesis of the resins 2(A–F)

A solution of the individual 1 M diamine **A–F** (see Fig. 3) in CH_2Cl_2/DMF was added to the active carbonate resin **1**[21] (1.07 g) and the mixture was shaken for 12 h. The resin was successively washed with DMF (3× 15 mL) and CH_2Cl_2 (3× 15 mL) and dried to give the resin **2(A–F)**.

3.2.2. Synthesis of the active carbamate resins 3(A–F)

To the individual dried resins **2(A–F)** was added a solution of 4-nitrophenylchloroformate (0.95 g, 4.71 mmol) in CH_2Cl_2 (4 mL) and pyridine (2 mL, 24.8 mmol) was added dropwise to the mixture. The suspension was shaken at room temperature for 16 h or until negative Kaiser test.[27] The reaction mixture was filtered and the resin was successively washed with DMF (3×15 mL) and CH_2Cl_2 (3×15 mL) and dried to give the desired resins **3(A–F)**.

3.2.3. Synthesis of the resins 4(A–F)

The individual resins **3(A–F)** (550 mg) was pre-swollen in CH_2Cl_2 (5 mL) for 30 min and filtered. To this resin was added a solution of 1 M 3-amino-1,2-propanediol (250 mg) in CH_2Cl_2/DMF (5 mL) and the suspension was agitated at room temperature for 12 h. The reaction mixture was filtered and the resin was washed with DMF (3×15 mL) and CH_2Cl_2 (3×15 mL) and dried to give the resins **4(A–F)**.

3.2.4. Synthesis of the resins 5(A–F)(a–j)

The individual resins **4**(**A**–**F**) was divided into 10 equal parts and each part was treated with a solution of the long chain fatty acids **a–j** (6 equiv) (see Fig. 3), DIC (6 equiv) and DMAP (cat. amount) in CH_2Cl_2/DMF (5 mL). The reaction mixture was shaken at room temperature for 12 h. The reaction mixture was filtered and the resin was successively washed with DMF (3×15 mL) and CH_2Cl_2 (3×15 mL) and dried to give the desired resins **5(A**–**F**)(**a**–**j**).

3.2.5. Cleavage of aminoglycerol-based cationic lipids bound resins 5(A–F)(a–j)

A suspension of the individual resins **5**(**A**–**F**)(**a**–**j**) in CH_2Cl_2 (1.2 mL) was treated with neat TFA (0.3 mL) and gently agitated at room temperature for 2 h. The reaction mixture was filtered and the filtrate was collected. The resin was washed with CH_2Cl_2 (2× 1 mL) and the combined filtrate was evaporated under stream

of nitrogen gas and further dried under vacuum for 2 h to dryness to obtain the desired products **6(A–F)(a–j)**.

Compound ***6Aa*** *(59 mg from 115 mg of resin* ***4A****):* 90% yield; IR: ν_{max} 3375, 3064, 2925, 2855, 1681, 1573, 1507, 1466, 1264, 1203, 1140 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (br t, J = 6.5 Hz, 6H, H-11′ and H-11″), 1.23 (s, 24H, H-4′–H-9′ and H-4″–H-9″), 1.40 (br m, 4H, H-10′ and H-10″), 1.55 (br s, 4H, H-3′ and H-3″), 2.29 (m, 4H, H-2′ and H-2″), 3.17 (br s, 2H, H-2‴), 3.26 (br s, 2H, H-3), 3.47 (br s, 2H, H-1‴), 4.07 (dd, J = 12.1, 6.2 Hz, 1H, H-1a), 4.22 (br d, J = 9.8 Hz, 1H, H-1b), 5.05 (br s, 1H, H-2), 7.58 (br s, 3H, NH_3^+); MS (ES): *m/z*: 514 ($[M+H]^+$, 100%).

Compound ***6Ab*** *(61 mg from 108 mg of resin* ***4A****):* 95% yield; IR: ν_{max} 3376, 3058, 2924, 2854, 1677, 1570, 1466, 1433, 1262, 1203, 1138 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (br t, J = 6.5 Hz, 6H, H-12′ and H-12″), 1.23 (s, 28H, H-4′–H-10′ and H-4″–H-10″), 1.38 (br m, 4H, H-11′ and H-11″), 1.55 (br s, 4H, H-3′ and H-3″), 2.29 (m, 4H, H-2′ and H-2″), 3.19 (br s, 2H, H-2‴), 3.26 (br s, 2H, H-3), 3.48 (br s, 2H, H-1‴), 4.07 (dd, J = 12.1, 5.9 Hz, 1H, H-1a), 4.22 (br d, J = 9.9 Hz, 1H, H-1b), 5.05 (br s, 1H, H-2), 7.55 (br s, 3H, NH_3^+); MS (ES): *m/z*: 542 ($[M+H]^+$, 100%).

Compound ***6Ac*** *(49 mg from 87 mg of resin* ***4A****):* 86% yield; IR: ν_{max} 3372, 2922, 2852, 1737, 1667, 1569, 1466, 1203, 1180, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (br t, J = 6.5 Hz, 6H, H-13′ and H-13″), 1.23 (s, 32H, H-4′–H-11′ and H-4″–H-11″), 1.45 (br m, 4H, H-12′ and H-12″), 1.55 (br s, 4H, H-3′ and H-3″), 2.28 (m, 4H, H-2′ and H-2″), 3.14 (br s, 2H, H-2‴), 3.25 (br s, 2H, H-3), 3.46 (br s, 2H, H-1‴), 4.07 (dd, J = 11.9, 6.0 Hz, 1H, H-1a), 4.22 (br d, J = 9.8 Hz, 1H, H-1b), 5.04 (br s, 1H, H-2), 7.55 (br s, 3H, NH_3^+); MS (ES): *m/z*: 570 ($[M+H]^+$, 100%).

Compound ***6Ad*** *(51 mg from 92 mg of resin* ***4A****):* 85% yield; IR: ν_{max} 3376, 2918, 2850, 1683, 1574, 1467, 1429, 1202, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (br t, J = 6.6 Hz, 6H, H-14′ and H-14″), 1.23 (s, 36H, H-4′–H-12′ and H-4″–H-12″), 1.36 (br m, 4H, H-13′ and H-13″), 1.55 (br s, 4H, H-3′ and H-3″), 2.28 (m, 4H, H-2′ and H-2″), 3.17 (br s, 2H, H-2‴), 3.25 (br s, 2H, H-3), 3.47 (br s, 2H, H-1‴), 4.08 (dd, J = 12.0, 6.1 Hz, 1H, H-1a), 4.22 (br d, J = 9.7 Hz, 1H, H-1b), 5.05 (br s, 1H, H-2), 7.62 (br s, 3H, NH_3^+); MS (ES): *m/z*: 598 ($[M+H]^+$, 100%).

Compound ***6Ae*** *(47 mg from 84 mg of resin* ***4A****):* 81% yield; IR: ν_{max} 3367, 2919, 2850, 1734, 1678, 1568, 1467, 1429, 1272, 1202, 1135 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (br t, J = 6.5 Hz, 6H, H-15′ and H-15″), 1.23 (s, 40H, H-4′–H-13′ and H-4″–H-13″), 1.38 (br m, 4H, H-14′ and H-14″), 1.55 (br s, 4H, H-3′ and H-3″), 2.28 (m, 4H, H-2′ and H-2″), 3.16 (br s, 2H, H-2‴), 3.25 (br s, 2H, H-3), 3.47 (br s, 2H, H-1‴), 4.07 (dd, J = 12.0, 5.9 Hz, 1H, H-1a), 4.22 (br d, J = 9.7 Hz, 1H, H-1b), 5.04 (br s, 1H, H-2), 7.53 (br s, 3H, NH_3^+); MS (ES): *m/z*: 626 ($[M+H]^+$, 100%).

Compound ***6Af*** *(68 mg from 108 mg of resin* ***4A****):* 88% yield; IR: ν_{max} 3373, 2918, 2850, 1731, 1677, 1560, 1467, 1194, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (br t, J = 6.6 Hz, 6H, H-16′ and H-16″), 1.23 (s, 44H, H-4′–H-14′ and H-4″–H-14″), 1.35 (br m, 4H, H-15′ and H-15″), 1.55 (br s, 4H, H-3′ and H-3″), 2.29 (m, 4H, H-2′ and H-2″), 3.21 (br s, 2H, H-2‴), 3.27 (br s, 2H, H-3), 3.49 (br s, 2H, H-1‴), 4.08 (dd, J = 12.1, 5.9 Hz, 1H, H-1a), 4.22 (br d, J = 9.7 Hz, 1H, H-1b), 5.06 (br s, 1H, H-2), 7.50 (br s, 3H, NH_3^+); MS (ES): *m/z*: 654 ($[M+H]^+$, 100%).

Compound ***6Ag*** *(36 mg from 79 mg of resin* ***4A****):* 59% yield; IR: ν_{max} 3366, 2997, 2925, 2854, 1711, 1678, 1571, 1464, 1203, 1140 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (br t, J = 6.5 Hz, 6H, H-18′ and H-18″), 1.24 (s, 36H, H-4′–H-7′, H-12′–H-16′, H-4″–H-7″ and H-12″–H-16″), 1.40 (br m, 4H, H-17′ and H-17″), 1.55 (m, 4H, H-3′ and H-3″), 1.98 (br s, 8H, H-8′, H-11′, H-8″, H-11″), 2.29 (br t-like, 4H, H-2′ and H-2″), 3.13 (br s, 2H, H-2‴), 3.26 (br s, 2H, H-3), 3.48 (br s, 2H, H-1‴), 4.08 (dd, J = 12.0, 5.9 Hz, 1H, H-1a), 4.22 (br d, J = 9.8 Hz, 1H, H-1b), 5.05 (br s, 1H, H-2), 5.32 (br s, 4H, H-9′, H-10′, H-9″ and H-10″), 7.61 (br s, 3H, NH_3^+); MS (ES): *m/z*: 706 ($[M+H]^+$, 100%).

Compound ***6Ah*** *(72 mg from 98 mg of resin* ***4A****):* 94% yield; IR: ν_{max} 3376, 3054, 2925, 2853, 2677, 1679, 1569, 1422, 1265, 1203, 1138 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (br t, J = 6.6 Hz, 6H, H-18′ and H-18″), 1.23 (s, 52H, H-4′–H-16′ and H-4″–H-16″), 1.38 (br m, 4H, H-17′ and H-17″), 1.55 (br s, 4H, H-3′ and H-3″), 2.30 (m, 4H, H-2′ and H-2″), 3.21 (br s, 2H, H-2‴), 3.24 (br s, 2H, H-3), 3.49 (br s, 2H, H-1‴), 4.09 (dd, J = 12.0, 5.9 Hz, 1H, H-1a), 4.22 (br d, J = 9.7 Hz, 1H, H-1b), 5.07 (br s, 1H, H-2), 7.45 (br s, 3H, NH_3^+); MS (ES): *m/z*: 710 ($[M+H]^+$, 100%).

Compound ***6Ai*** *(79 mg from 98 mg of resin* ***4A****):* 99% yield; IR: ν_{max} 3392, 3054, 2980, 2926, 2853, 1679, 1566, 1422, 1265, 1202, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (br t, J = 6.5 Hz, 6H, H-19′ and H-19″), 1.23 (s, 56H, H-4′–H-17′ and H-4″–H-17″), 1.38 (br m, 4H, H-18′ and H-18″), 1.55 (br s, 4H, H-3′ and H-3″), 2.31 (m, 4H, H-2′ and H-2″), 3.24 (br s, 2H, H-2‴), 3.32 (br s, 2H, H-3), 3.50 (br s, 2H, H-1‴), 4.10 (dd, J = 12.0, 5.9 Hz, 1H, H-1a), 4.24 (br d, J = 9.8 Hz, 1H, H-1b), 5.08 (br s, 1H, H-2), 7.40 (br s, 3H, NH_3^+); MS (ES): *m/z*: 738 ($[M+H]^+$, 100%).

Compound ***6Aj*** *(82 mg from 105 mg of resin* ***4A****):* 92% yield; IR: ν_{max} 3368, 2917, 2849, 1736, 1659, 1563, 1465, 1429, 1264, 1176, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (br t, J = 6.6 Hz, 6H, H-20′ and H-20″), 1.23 (s, 60H, H-4′–H-18′ and H-4″–H-18″), 1.38 (br m, 4H, H-19′ and H-19″), 1.55 (br s, 4H, H-3′ and H-3″), 2.30 (m, 4H, H-2′ and H-2″), 3.25 (m, 4H, H-3 and H-2‴), 3.50 (br s, 2H, H-1‴), 4.01 (dd, J = 11.9, 5.9 Hz, 1H, H-1a), 4.24 (br d, J = 9.9 Hz, 1H, H-1b), 5.07 (br s, 1H, H-2), 7.40 (br s, 3H, NH_3^+); MS (ES): *m/z*: 766 ($[M+H]^+$, 100%).

Compound ***6Ba*** *(28 mg from 115 mg of resin* ***4B****):* 41% yield; IR: ν_{max} 3372, 2925, 2855, 1712, 1679, 1568, 1466, 1376, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-11′ and H-11″), 1.23 (s, 24H, H-4′–H-9′ and H-4″–H-9″), 1.40 (br m, 4H, H-10′ and H-10″), 1.56 (br m, 4H, H-3′ and H-3″), 1.84 (br s, 2H, H-2‴), 2.29 (m, 4H, H-2′ and H-2″), 3.04 (br s, 2H, H-3‴), 3.27 (br m, 4H, H-3 and H-1‴), 4.07 (dd, J = 12.1, 6.0 Hz, 1H, H-1a), 4.23 (br d, J = 9.9 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.75 (br s, 3H, NH_3^+); MS (ES): *m/z*: 528 ($[M+H]^+$, 100%).

Compound ***6Bb*** *(31 mg from 134 mg of resin* ***4B****):* 38% yield; IR: ν_{max} 3357, 2924, 2854, 1680, 1572, 1466, 1376, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-12′ and H-12″), 1.23 (s, 28H, H-4′–H-10′ and H-4″–H-10″), 1.38 (br m, 4H, H-11′ and H-11″), 1.56 (br m, 4H, H-3′ and H-3″), 1.84 (br s, 2H, H-2‴), 2.28 (m, 4H, H-2′ and H-2″), 3.04 (br s, 2H, H-3‴), 3.27 (br m, 4H, H-3 and H-1‴), 4.05 (dd, J = 12.1, 6.0 Hz, 1H, H-1a), 4.22 (br d, J = 9.7 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.73 (br s, 3H, NH_3^+); MS (ES): *m/z*: 556 ($[M+H]^+$, 100%).

Compound ***6Bc*** *(29 mg from 130 mg of resin* ***4B****):* 34% yield; IR: ν_{max} 3365, 2924, 2854, 1750, 1679, 1568, 1466, 1376, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-13′ and H-13″), 1.23 (s, 32H, H-4′–H-11′ and H-4″–H-11″), 1.39 (br m, 4H, H-12′ and H-12″), 1.56 (br m, 4H, H-3′ and H-3″), 1.83 (br s, 2H, H-2‴), 2.29 (m, 4H, H-2′ and H-2″), 3.05 (br s, 2H, H-3‴), 3.27 (br m, 4H, H-3 and H-1‴), 4.06 (dd, J = 12.1, 6.2 Hz, 1H, H-1a), 4.23 (br d, J = 9.6 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.73 (br s, 3H, NH_3^+); MS (ES): *m/z*: 584 ($[M+H]^+$, 100%).

Compound ***6Bd*** *(36 mg from 123 mg of resin* ***4B****):* 44% yield; IR: ν_{max} 3365, 2922, 2853, 1700, 1678, 1568, 1466, 1369, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-14′ and H-14″), 1.23 (s, 36H, H-4′–H-12′ and H-4″–H-12″), 1.38 (br m, 4H, H-13′ and H-13″), 1.56 (br m, 4H, H-3′ and H-3″), 1.84 (br s, 2H, H-2‴), 2.29 (m, 4H, H-2′ and H-2″), 3.05 (br s, 2H, H-3‴), 3.28 (br m, 4H, H-3 and H-1‴), 4.06 (dd, J = 12.1, 6.2 Hz, 1H, H-1a), 4.23 (br d, J = 9.5 Hz, 1H, H-1b), 5.02 (br s, 1H, H-2), 7.72 (br s, 3H, NH_3^+); MS (ES): *m/z*: 612 ($[M+H]^+$, 100%).

Compound ***6Be*** *(37 mg from 168 mg of resin* ***4A****):* 31% yield; IR: ν_{max} 3365, 3040, 2919, 2851, 1730, 1679, 1572, 1466, 1265, 1203, 1129 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-15′ and H-15″), 1.23 (s, 40H, H-4′–H-13′ and H-4″–H-13″), 1.38 (br m, 4H, H-14′ and H-14″), 1.56 (br m, 4H, H-3′ and H-3″), 1.81 (br s, 2H, H-2‴), 2.27 (m, 4H, H-2′ and H-2″), 3.01 (br s, 2H, 2H, H-3‴), 3.25 (br m, 4H, H-3 and H-1‴), 4.05 (dd, J = 11.9, 6.3 Hz, 1H, H-1a), 4.18 (br d, J = 10.0 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.90 (br s, 3H, NH_3^+); MS (ES): *m/z*: 640 ($[M+H]^+$, 100%).

Compound ***6Bf*** *(35 mg from 126 mg of resin* ***4B****):* 38% yield; IR: ν_{max} 3365, 2918, 2850, 1736, 1656, 1564, 1465, 1369, 1251, 1176, 1129 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-16′ and H-16″), 1.23 (s, 44H, H-4′–H-14′ and H-4″–H-14″), 1.38 (br m, 4H, H-15′ and H-15″), 1.56 (br m, 4H, H-3′ and H-3″), 1.83 (br s, 2H, H-2‴), 2.29 (m, 4H, H-2′ and H-2″), 3.04 (br s, 2H, H-3‴), 3.27 (br m, 4H, H-3 and H-1‴), 4.06 (dd, J = 12.1, 6.2 Hz, 1H, H-1a), 4.23 (br d, J = 9.7 Hz, 1H, H-1b), 5.02 (br s, 1H, H-2), 7.75 (br s, 3H, NH_3^+); MS (ES): *m/z*: 668 ($[M+H]^+$, 100%).

Compound ***6Bg*** *(30 mg from 133 mg of resin* ***4B****):* 29% yield; IR: ν_{max} 3357, 2924, 2854, 1704, 1679, 1568, 1464, 1376, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.5 Hz, 6H, H-18′ and H-18″), 1.24 (s, 28H, H-4′–H-6′, H-13′–H-16′, H-4″–H-6″ and H-13″–H-16″), 1.27 (s, 8H, H-7′, H-12′, H-7″ and H-12″), 1.38 (br m, 4H, H-17′ and H-17″), 1.56 (br m, 4H, H-3′ and H-3″), 1.98 (br m, 8H, H-8′, H-11′, H-8″ and H-11″), 2.30 (m, 4H, H-2′ and H-2″), 3.05 (br s, 2H, H-3‴), 3.28 (br m, 4H, H-3 and H-1‴), 4.07 (dd, 1H, J = 12.1, 6.1 Hz, H-1a), 4.23 (br d, 1H, J = 9.7 Hz, H-1b), 5.01 (br s, 1H, H-2), 5.32 (br s, 4H, H-9′, H-10′, H-9″ and H-10″), 7.74 (br s, 3H, NH_3^+); MS (ES): *m/z*: 720 ($[M+H]^+$, 100%).

Compound ***6Bh*** *(13 mg from 127 mg of resin* ***4B****):* 13% yield; IR: ν_{max} 3364, 2919, 2850, 1734, 1466, 1373, 1258, 1135 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-18′ and H-18″), 1.23 (s, 52H, H-4′–H-16′ and H-4″–H-16″), 1.36 (br m, 4H, H-17′ and H-17″), 1.56 (br m, 4H, H-4′ and H-4″), 1.77 (br m, 2H, H-2‴), 2.30 (m, 4H, H-3′ and H-3″), 2.94 (br s, 2H, H-3‴), 3.20 (m, 4H, H-3 and H-1‴), 4.07 (dd, J = 12.2, 6.1 Hz, 1H, H-1a), 4.29 (dd, J = 12.2, 4.1 Hz, 1H, H-1b), 5.04 (br s, 1H, H-2), 7.65 (br s, 3H, NH_3^+); MS (ES): *m/z*: 724 ($[M+H]^+$, 100%).

Compound ***6Bi*** *(34 mg from 117 mg of resin* ***4B****):* 36% yield; IR: ν_{max} 3360, 2919, 2850, 1736, 1655, 1422, 1265 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 6H, J = 6.6 Hz, H-19′ and H-19″), 1.23 (s, 56H, H-4′–H-17′ and H-4″–H-17″), 1.38 (br m, 4H, H-18′ and H-18″), 1.56 (br m, 4H, H-3′ and H-3″), 1.85 (br m, 4H, H-2′ and H-2″), 3.07 (br s, 2H, H-3‴), 3.26 (br m, 4H, H-3 and H-1‴), 4.07 (dd, 1H, J = 12.3, 6.2 Hz, H-1a), 4.23 (br d, 1H, J = 9.7 Hz, H-1b), 5.01 (br s, 1H, H-2), 7.66 (br s, 3H, NH_3^+); MS (ES): *m/z*: 752 ($[M+H]^+$, 100%).

Compound ***6Bj*** *(39 mg from 140 mg of resin* ***4B****):* 33% yield; IR: ν_{max} 3054, 2987, 2919, 2850, 1736, 1655, 1422, 1265 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 6H, J = 6.6 Hz, H-20′ and H-20″), 1.23 (s, 60H, H-4′–H-18′ and H-4″–H-18″), 1.38 (br m, 4H, H-19′ and H-19″), 1.38 (br m, 4H, H-19′ and H-19″), 1.56 (br m, 4H, H-3′ and H-3″), 1.85 (br s, 2H, H-2‴), 2.29 (m, 4H, H-2′ and H-2″), 3.06 (br s, 2H, H-3‴), 3.28 (br m, 4H, H-3 and H-1‴), 4.07 (dd, J = 12.1, 6.0 Hz, 1H, H-1a), 4.24 (br d, J = 9.8 Hz, 1H, H-1b), 5.02 (br s, 1H, H-2), 7.66 (br s, 3H, NH_3^+); MS (ES): *m/z*: 780 ($[M+H]^+$, 100%).

Compound ***6Ca*** *(28 mg from 124 mg of resin* ***4C****):* 37% yield; IR: ν_{max} 3372, 2925, 2854, 1741, 1679, 1568, 1466, 1376, 1203, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-11′ and H-11″), 1.23 (s, 24H, H-4′–H-9′ and H-4″–H-9″), 1.38 (br m, 4H, H-10′ and H-10″), 1.38 (br m, 4H, H-10′ and H-10″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.29 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-4‴), 3.07 (br s, 2H, H-1‴), 3.28 (br s, 2H, H-3), 4.05 (dd, J = 12.3, 6.6 Hz, 1H, H-1a), 4.23 (br d, J = 12.1 Hz, 1H, H-1b), 5.02 (br s, 1H, H-2), 7.81 (br s, 3H, NH_3^+); MS (ES): *m/z*: 542 ($[M+H]^+$, 100%).

Compound ***6Cb*** (30 mg from 131 mg of resin ***4C***): 36% yield; IR: ν_{max} 3357, 2924, 2854, 1738, 1678, 1568, 1466, 1196, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-12′ and H-12″), 1.23 (s, 28H, H-4′–H-10′ and H-4″–H-10″), 1.38 (br m, 4H, H-11′ and H-11″), 1.55 (m, 8H, H-2′, H-2″, H-2‴ and H-3‴), 2.28 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-4‴), 3.07 (br s, 2H, H-1‴), 3.27 (br s, 2H, H-3), 4.05 (dd, J = 12.2, 6.6 Hz, 1H, H-1a), 4.22 (br d, J = 12.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.80 (br s, 3H, NH_3^+); MS (ES): *m/z*: 570 ($[M+H]^+$, 100%).

Compound ***6Cc*** *(33 mg from 143 mg of resin* ***4C****):* 35% yield; IR: ν_{max} 3350, 2924, 2854, 1738, 1679, 1568, 1466, 1378, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.4 Hz, 6H, H-13′ and H-13″), 1.23 (s, 32H, H-4′–H-11′ and H-4″–H-11″), 1.38 (br m, 4H, H-12′ and H-12″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.28 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-4‴), 3.08 (br s, 2H, H-1‴), 3.28 (br s, 2H, H-3), 4.05 (dd, J = 12.4, 6.6 Hz, 1H, H-1a), 4.24 (br d, J = 12.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.81 (br s, 3H, NH_3^+); MS (ES): *m/z*: 598 ($[M+H]^+$, 100%).

Compound ***6Cd*** *(27 mg from 140 mg of resin* ***4C****):* 28% yield; IR: ν_{max} 3372, 2922, 2852, 1739, 1678, 1561, 1466, 1377, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-14′ and H-14″), 1.23 (s, 36H, H-4′–H-12′ and H-4″–H-12″), 1.38 (br m, 4H, H-13′ and H-13″), 1.60 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.30 (m, 4H, H-2′ and H-2″), 2.94 (br s, 2H, H-4‴), 3.10 (br s, 2H, H-1‴), 3.27 (br s, 2H, H-3), 4.06 (dd, J = 12.2, 6.6 Hz, 1H, H-1a), 4.23 (br d, J = 12.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.83 (br s, 3H, NH_3^+); MS (ES): *m/z*: 626 ($[M+H]^+$, 100%).

Compound ***6Ce*** *(33 mg from 140 mg of resin* ***4C****):* 33% yield; IR: ν_{max} 3357, 2919, 2850, 1736, 1678, 1568, 1467, 1378, 1266, 1202, 1135 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-15′ and H-15″), 1.23 (s, 40H, H-4′–H-13′ and H-4″–H-13″), 1.38 (br m, 4H, H-14′ and H-14″), 1.56 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.29 (m, 4H, H-2′ and H-2″), 2.93 (br s, 2H, H-4‴), 3.08 (br s, 2H, H-1‴), 3.34 (br s, 2H, H-3), 4.05 (dd, J = 12.1, 6.5 Hz, 1H, H-1a), 4.22 (br d, J = 12.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.75 (br s, 3H, NH_3^+); MS (ES): *m/z*: 654 ($[M+H]^+$, 100%).

Compound ***6Cf*** *(28 mg from 114 mg of resin* ***4C****):* 33% yield; IR: ν_{max} 3365, 2919, 2850, 1738, 1678, 1564, 1467, 1378, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-16′ and H-16″), 1.23 (s, 44H, H-4′–H-14′ and H-4″–H-14″), 1.38 (br m, 4H, H-15′ and H-15″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.29 (m, 4H, H-2′ and H-2″), 2.93 br s, (2H, H-4‴), 3.08 (br s, 2H, H-1‴), 3.08 (br s, 2H, H-3), 3.28 (br s, 2H, H-3), 4.05 (dd, J = 12.2, 6.5 Hz, 1H, H-1a), 4.24 (br d, J = 12.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.77 (br s, 3H, NH_3^+); MS (ES): *m/z*: 682 ($[M+H]^+$, 100%).

Compound ***6Cg*** *(31 mg from 140 mg of resin* ***4C****):* 27% yield; IR: ν_{max} 3357, 2925, 2854, 1738, 1679, 1568, 1464, 1377, 1203, 1129 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.3 Hz, 6H, H-18′ and H-18″), 1.24 (s, 28H, H-4′–H-6′, H-13′–H-16′, H-4″–H-6″ and H-13″–H-16″), 1.27 (s, 8H, H-7′, H-12′, H-7″ and H-12″), 1.38 (br m, 4H, H-17′ and H-17″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 1.98 (br m, 8H, H-8′, H-11′, H-8″ and H-11″), 2.29 (m, 4H, H-2′ and H-2″), 2.93 (br s, 2H, H-4‴), 3.08 (br s, 2H, H-1‴), 3.31 (br s, 2H, H-3), 4.05 (dd, J = 12.1, 7.0 Hz, 1H, H-1a), 4.23 (br d, J = 9.2 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 5.32 (m, 4H, H-8′, H-9′, H-8″ and H-9″), 7.83 (br s, 3H, NH_3^+); MS (ES): *m/z*: 734 ($[M+H]^+$, 100%).

Compound ***6Ch*** *(37 mg from 114 mg of resin* ***4C****):* 40% yield; IR: ν_{max} 3054, 2927, 2854, 1679, 1422, 1265, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-18′ and H-18″), 1.23 (s, 52H, H-4′–H-16′ and H-4″–H-16″), 1.38 (br m, 4H, H-17′ and H-17″), 1.55 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.30

(m, 4H, H-2′ and H-2″), 2.98 (br s, 2H, H-4‴), 3.13 (br s, 2H, H-1‴), 3.30 (br s, 2H, H-3), 4.04 (dd, J = 12.3, 6.5 Hz, 1H, H-1a), 4.22 (br d, J = 12.0 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.57 (br s, 3H, NH_3^+); MS (ES): m/z: 738 ($[M+H]^+$, 100%).

Compound ***6Ci*** *(48 mg from 110 mg of resin* ***4C****):* 51% yield; IR: ν_{max} 3054, 2926, 2860, 1740, 1678, 1421, 1265, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-19′ and H-19″), 1.23 (s, 56H, H-4′–H-17′ and H-4″–H-17″), 1.38 (br m, 4H, H-18′ and H-18″), 1.60 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.29 (m, 4H, H-2′ and H-2″), 2.94 (br s, 2H, H-4‴), 3.09 (br s, 2H, H-1‴), 3.27 (br s, 2H, H-3), 4.05 (dd, J = 12.6, 6.6 Hz, 1H, H-1a), 4.25 (br d, J = 9.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.71 (br s, 3H, NH_3^+); MS (ES): m/z: 766 ($[M+H]^+$, 100%).

Compound ***6Cj*** *(16 mg from 128 mg of resin* ***4A****):* 15% yield; IR: ν_{max} 3054, 2926, 2854, 1679, 1422, 1265, 1203, 1129 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-20′ and H-20″), 1.24 (s, 60H, H-4′–H-18′ and H-4″–H-18″), 1.38 (br m, 4H, H-19′ and H-19″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-3‴), 2.30 (m, 4H, H-2′ and H-2″), 2.95 (br s, 2H, H-4‴), 3.10 (br s, 2H, H-1‴), 3.30 (br s, 2H, H-3), 4.05 (dd, J = 12.3, 6.5 Hz, 1H, H-1a), 4.23 (br d, J = 12.1 Hz, 1H, H-1b), 5.01 (br s, 1H, H-2), 7.73 (br s, 3H, NH_3^+); MS (ES): m/z: 794 ($[M+H]^+$, 58%).

Compound ***6Da*** *(21 mg from 125 mg of resin* ***4D****):* 27% yield; IR: ν_{max} 3343, 2925, 2855, 1740, 1679, 1572, 1466, 1380, 1202, 1129 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-11′ and H-11″), 1.23 (s, 24H, H-4′–H-9′ and H-4″–H-9″), 1.35 (br m, 4H, H-10′ and H-10″), 1.36 (br s, 2H, H-4‴), 1.47 (br m, 2H, H-2‴), 1.57 (br m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.65 (br m, 2H, H-5‴), 2.29 (m, 4H, H-2′ and H-2″), 2.98 (br s, 2H, H-6‴), 3.10 (br t-like, 2H, H-1‴), 3.31 (br d, J = 5.5 Hz, 2H, H-3), 4.09 (dd, J = 12.0, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 2.8 Hz, 1H, H-1b), 5.00 (br s, 1H, H-2), 7.65 (br s, 3H, NH_3^+); MS (ES): m/z: 570 ($[M+H]^+$, 100%).

Compound ***6Db*** *(23 mg from 133 mg of resin* ***4D****):* 26% yield; IR: ν_{max} 3350, 2925, 2854, 1730, 1678, 1571, 1466, 1373, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.7 Hz, 6H, H-12′ and H-12″), 1.23 (s, 28H, H-4′–H-10′ and H-4″–H-10″), 1.35 (br m, 4H, H-11′ and H-11″), 1.38 (br s, 2H, H-4‴), 1.48 (br m, 2H, H-2‴), 1.57 (br m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.65 (m, 2H, H-5‴), 2.29 (m, 4H, H-2′ and H-2″), 2.98 (br s, 2H, H-6‴), 3.10 (br t-like, 2H, H-1‴), 3.31 (br d, J = 5.6 Hz, 2H, H-3), 4.09 (dd, J = 12.0, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.0, 2.9 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.64 (br s, 3H, NH_3^+); MS (ES): m/z: 598 ($[M+H]^+$, 100%).

Compound ***6Dc*** *(21 mg from 124 mg of resin* ***4D****):* 24% yield; IR: ν_{max} 3357, 2924, 2854, 1730, 1679, 1572, 1466, 1378, 1251, 1202, 1141 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.7 Hz, 6H, H-13′ and H-13″), 1.23 (s, 32H, H-4′–H-11′ and H-4″–H-11″), 1.38 (m, 4H, H-12′ and H-12″), 1.38 (br s, 2H, H-4‴), 1.48 (m, 2H, H-2‴), 1.57 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.65 (m, 2H, H-5‴), 2.29 (m, 4H, H-2′ and H-2″), 2.98 (br s, 2H, H-6‴), 3.10 (br t-like, 2H, H-1‴), 3.32 (br d, J = 5.5 Hz, 2H, H-3), 4.09 (dd, J = 12.0, 6.2 Hz, 1H, H-1a), 4.25 (dd, J = 12.0, 2.8 Hz, 1H, H-1b), 5.00 (br s, 1H, H-2), 7.69 (br s, 3H, NH_3^+); MS (ES): m/z: 626 ($[M+H]^+$, 100%).

Compound ***6Dd*** *(28 mg from 126 mg of resin* ***4D****):* 31% yield; IR: ν_{max} 3350, 2918, 2850, 1678, 1568, 1466, 1428, 1202, 1138 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-14′ and H-14″), 1.23 (s, 36H, H-4′–H-12′ and H-4″–H-12″), 1.32 (br s, 2H, H-4‴), 1.37 (m, 4H, H-13′ and H-13″), 1.48 (m, 2H, H-2‴), 1.57 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.65 (m, 2H, H-5‴), 2.30 (m, 4H, H-2′ and H-2″), 2.98 (br s, 2H, H-6‴), 3.11 (br t-like, 2H, H-1‴), 3.31 (br d, 2H, J = 5.5 Hz, 2H, H-3), 4.09 (dd, J = 12.1, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 4.1 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.63 (br s, 3H, NH_3^+); MS (ES): m/z: 654 ($[M+H]^+$, 100%).

Compound ***6De*** *(24 mg from 126 mg of resin* ***4D****):* 25% yield; IR: ν_{max} 3357, 2918, 2850, 1732, 1678, 1564, 1467, 1266, 1202, 1138 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.7 Hz, 6H, H-15′ and H-15″), 1.23 (s, 40H, H-4′–H-13′ and H-4″–H-13″), 1.35 (m, 4H, H-14′ and H-14″), 1.38 (br s, 2H, H-4‴), 1.49 (m, 2H, H-2‴), 1.57 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.66 (m, 2H, H-5‴), 2.29 (m, 4H, H-2′ and H-2″), 2.99 (br s, 2H, H-6‴), 3.11 (br t-like, 2H, H-1‴), 3.31 (br d, J = 5.6 Hz, 2H, H-3), 4.10 (dd, J = 12.1, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 2.9 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.61 (br s, 3H, NH_3^+); MS (ES): m/z: 682 ($[M+H]^+$, 100%).

Compound ***6Df*** *(27 mg from 135 mg of resin* ***4D****):* 25% yield; IR: ν_{max} 3357, 2917, 2850, 1734, 1679, 1564, 1467, 1258, 1202, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-16′ and H-16″), 1.23 (s, 44H, H-4′–H-14′ and H-4″–H-14″), 1.33 (m, 4H, H-15′ and H-15″), 1.37 (br s, 2H, H-4‴), 1.49 (m, 2H, H-2‴), 1.56 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.66 (m, 2H, H-5‴), 2.29 (m, 4H, H-2′ and H-2″), 3.00 (br s, 2H, H-6‴), 3.11 (br t-like, 2H, H-1‴), 3.32 (br d, J = 5.5 Hz, 2H, H-3), 4.10 (dd, J = 12.1, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 2.8 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.57 (br s, 3H, NH_3^+); MS (ES): m/z: 710 ($[M+H]^+$, 100%).

Compound ***6Dg*** *(24 mg from 139 mg of resin* ***4D****):* 21% yield; IR: ν_{max} 3365, 2925, 2854, 1745, 1679, 1572, 1465, 1378, 1202, 1139 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.6 Hz, 6H, H-18′ and H-18″), 1.24 (s, 28H, H-4′–H-6′, H-13′–H-16′, H-4″–H-6″ and H-13″–H-16″), 1.27 (s, 8H, H-7′, H-12′, H-7″ and H-12″), 1.34 (m, 4H, H-17′ and H-17″), 1.37 (br s, 2H, H-4‴), 1.48 (m, 2H, H-2‴), 1.57 (m, 4H, H-3′ and H-3″), 1.65 (m, 2H, H-5‴), 1.99 (m, 8H, H-8′, H-11′, H-8″ and H-11″), 2.30 (m, 4H, H-2′ and H-2″), 2.99 (br s, 2H, H-6‴), 3.11 (br t-like, 2H, H-1‴), 3.31 (br d, J = 5.5 Hz, 2H, H-3), 4.10 (dd, J = 12.1, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 2.8 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 5.32 (m, 4H, H-9′, H-10′, H-9″ and H-10″), 7.66 (br s, 3H, NH_3^+); MS (ES): m/z: 762 ($[M+H]^+$, 100%).

Compound ***6Dh*** *(28 mg from 126 mg of resin* ***4D****):* 26% yield; IR: ν_{max} 3357, 3047, 2922, 2852, 1679, 1568, 1466, 1265, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.9 Hz, 6H, H-18′ and H-18″), 1.24 (s, 52H, H-4′–H-16′ and H-4″–H-16″), 1.34 (br s, 2H, H-4‴), 1.38 (m, 4H, H-17′ and H-17″), 1.49 (m, 2H, H-2‴), 1.58 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.69 (m, 2H, H-5‴), 2.30 (m, 4H, H-2′ and H-2″), 2.99 (br s, 2H, H-6‴), 3.10 (br t-like, 2H, H-1‴), 3.32 (br d, J = 5.2 Hz, 2H, H-3), 4.10 (dd, J = 12.1, 5.7 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 6.1 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.55 (br s, 3H, NH_3^+); MS (ES): m/z: 766 ($[M+H]^+$, 100%).

Compound ***6Di*** *(30 mg from 131 mg of resin* ***4D****):* 26% yield; IR: ν_{max} 3333, 3054, 2922, 2852, 1738, 1678, 1568, 1422, 1265, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.7 Hz, 6H, H-19′ and H-19″), 1.23 (s, 56H, H-4′–H-17′ and H-4″–H-17″), 1.35 (m, 4H, H-18′ and H-18″), 1.38 (br s, 2H, H-4‴), 1.49 (m, 2H, H-2‴), 1.57 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.66 (m, 2H, H-5‴), 2.30 (m, 4H, H-2′ and H-2″), 2.99 (br s, 2H, H-6‴), 3.11 (br t-like, 2H, H-1‴), 3.32 (br d, J = 5.4 Hz, 2H, H-3), 4.10 (dd, J = 12.1, 6.1 Hz, 1H, H-1a), 4.24 (dd, J = 12.1, 2.8 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.58 (br s, 3H, NH_3^+); MS (ES): m/z: 794 ($[M+H]^+$, 100%).

Compound ***6Dj*** *(27 mg from 118 mg of resin* ***4D****):* 25% yield; IR: ν_{max} 3054, 2926, 2854, 1728, 1679, 1422, 1265, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.4 Hz, 6H, H-20′ and H-20″), 1.25 (s, 60H, H-4′–H-18′ and H-4″–H-18″), 1.35 (br s, 2H, H-4‴), 1.38 (m, 4H, H-19′ and H-19″), 1.51 (m, 2H, H-2‴), 1.58 (m, 4H, H-3′ and H-3″), 1.50–1.60 (m, 2H, H-3‴), 1.68 (m, 2H, H-5‴), 2.30 (m, 4H, H-2′ and H-2″), 3.01 (br s, 2H, H-6‴), 3.12 (br t-like, 2H, H-1‴), 3.32 (br d, J = 5.2 Hz, 2H, H-3), 4.11 (dd, J = 12.1, 6.1 Hz, 1H, H-1a), 4.25 (dd, J = 12.1, 6.1 Hz, 1H, H-1b),

4.99 (br s, 1H, H-2), 7.56 (br s, 3H, NH_3^+); MS (ES): *m/z*: 822 ([M+H]$^+$, 100%).

Compound ***6Ea*** *(18 mg from 122 mg of resin* ***4E****):* 22% yield; IR: ν_{max} 3357, 2925, 2854, 1734, 1678, 1572, 1466, 1369, 1202, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.4 Hz, 6H, H-11′ and H-11″), 1.23 (s, 32H, H-4′–H-9′, H-4″–H-9″ and H-3‴–H-6‴), 1.45 (m, 4H, H-10′ and H-10″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.30 (m, 4H, H-2′ and H-2″), 2.93 (br s, 2H, H-8‴), 3.08 (br t, 2H, H-1‴), 3.32 (br d, *J* = 4.8 Hz, 2H, H-3), 4.11 (dd, *J* = 12.0, 6.1 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.2 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.71 (br s, 3H, NH_3^+); MS (ES): *m/z*: 598 ([M+H]$^+$, 100%).

Compound ***6Eb*** *(17 mg from 134 mg of resin* ***4E****):* 86% yield; IR: ν_{max} 3357, 2925, 2854, 1734, 1678, 1571, 1466, 1377, 1203, 1139 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.4 Hz, 6H, H-12′ and H-12″), 1.23 (s, 36H, H-4′–H-10′, H-4″–H-10″ and H-3‴–H-6‴), 1.44 (br m, 4H, H-11′ and H-11″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.31 (m, 4H, H-2′ and H-2″), 2.93 (br s, 2H, H-8‴), 3.07 (br t-like, 2H, H-1‴), 3.31 (br d, *J* = 4.8 Hz, 2H, H-3), 4.10 (dd, *J* = 12.0, 6.0 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.5 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.80 (br s, 3H, NH_3^+); MS (ES): *m/z*: 626 ([M+H]$^+$, 100%).

Compound ***6Ec*** *(18 mg from 124 mg of resin* ***4E****):* 20% yield; IR: ν_{max} 3365, 2925, 2854, 1727, 1678, 1568, 1466, 1373, 1202, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.4 Hz, 6H, H-13′ and H-13″), 1.23 (s, 40H, H-4′–H-11′, H-4″–H-11″ and H-3‴–H-6‴), 1.45 (m, 4H, H-12′ and H-12″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.31 (m, 4H, H-2′ and H-2″), 2.94 (br s, 2H, H-8‴), 3.08 (br t-like, 2H, H-1‴), 3.32 (br d, *J* = 4.7 Hz, 2H, H-3), 4.11 (dd, *J* = 12.0, 5.9 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.3 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.70 (br s, 3H, NH_3^+); MS (ES): *m/z*: 654 ([M+H]$^+$, 100%).

Compound ***6Ed*** *(21 mg from 126 mg of resin* ***4E****):* 23% yield; IR: ν_{max} 3354, 2920, 2851, 1738, 1678, 1574, 1467, 1378, 1203, 1139 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.4 Hz, 6H, H-14′ and H-14″), 1.23 (s, 44H, H-4′–H-12′, H-4″–H-12″ and H-3‴–H-6‴), 1.44 (m, 4H, H-13′ and H-13″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.29 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-8‴), 3.07 (br t-like, 2H, H-1‴), 3.31 (br d, *J* = 5.3 Hz, 2H, H-3), 4.12 (dd, *J* = 12.1, 6.0 Hz, 1H, H-1a), 4.24 (br d, *J* = 11.6 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.72 (br s, 3H, NH_3^+); MS (ES): *m/z*: 682 ([M+H]$^+$, 100%).

Compound ***6Ee*** *(19 mg from 123 mg of resin* ***4E****):* 20% yield; IR: ν_{max} 3343, 2918, 2850, 1737, 1675, 1568, 1467, 1373, 1202, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.5 Hz, 6H, H-15′ and H-15″), 1.23 (s, 48H, H-4′–H-13′, H-4″–H-13″ and H-3‴–H-6‴), 1.43 (m, 4H, H-14′ and H-14″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.29 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-8‴), 3.06 (br t-like, 2H, H-1‴), 3.31 (br d, *J* = 5.2 Hz, 2H, H-3), 4.10 (dd, *J* = 12.0, 6.1 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.5 Hz, 1H, H-1b), 5.00 (br s, 1H, H-2), 7.83 (br s, 3H, NH_3^+); MS (ES): *m/z*: 710 ([M+H]$^+$, 100%).

Compound ***6Ef*** *(26 mg from 118 mg of resin* ***4E****):* 27% yield; IR: ν_{max} 3350, 2918, 2850, 1738, 1682, 1576, 1466, 1265, 1202, 1139 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.5 Hz, 6H, H-16′ and H-16″), 1.23 (s, 52H, H-4′–H-14′, H-4″–H-14″ and H-3‴–H-6‴), 1.47 (m, 4H, H-15′ and H-15″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.31 (m, 4H, H-2′ and H-2″), 2.96 (br s, 2H, H-8‴), 3.09 (br t-like, 2H, H-1‴), 3.33 (br d, *J* = 5.3 Hz, 2H, H-3), 4.12 (dd, *J* = 12.0, 5.9 Hz, 1H, H-1a), 4.23 (br d, *J* = 9.0 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.53 (br s, 3H, NH_3^+); MS (ES): *m/z*: 738 ([M+H]$^+$, 100%).

Compound ***6Eg*** *(21 mg from 131 mg of resin* ***4E****):* 18% yield; IR: ν_{max} 3350, 2925, 2854, 1738, 1679, 1571, 1464, 1377, 1202, 1138 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.2 Hz, 6H, H-18′ and H-18″), 1.24 (s, 36H, H-4′–H-6′, H-13′–H-16′, H-4″–H-6″, H-13″–H-16″ and H-3‴–H6‴), 1.27 (s, 8H, H-7′, H-12′, H-7″ and H-12″), 1.45 (m, 4H, H-17′ and H-17″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 1.98 (m, 8H, H-8′, H-11′, H-8″ and H-11″), 2.31 (m, 4H, H-2′ and H-2″), 2.93 (br s, 2H, H-8‴), 3.08 (br t-like, 2H, H-1‴), 3.32 (br d, *J* = 4.4 Hz, 2H, H-3), 4.41 (dd, *J* = 11.7, 5.8 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.5 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 5.33 (m, 4H, H-8′, H-9′, H-8″ and H-9″), 7.74 (br s, 3H, NH_3^+); MS (ES): *m/z*: 790 ([M+H]$^+$, 100%).

Compound ***6Eh*** *(27 mg from 136 mg of resin* ***4E****):* 23% yield; IR: ν_{max} 3300, 3054, 2925, 2853, 1740, 1678, 1568, 1466, 1417, 1265, 1202, 1133 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 5.9 Hz, 6H, H-18′ and H-18″), 1.23 (s, 60H, H-4′–H-16′, H-4″–H-16″ and H-3‴–H-6‴), 1.49 (m, 4H, H-17′ and H-17″), 1.59 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.33 (m, 4H, H-2′ and H-2″), 2.99 (br s, 2H, H-8‴), 3.11 (br t-like, 2H, H-1‴), 3.34 (br d, *J* = 6.5 Hz, 2H, H-3), 4.13 (dd, *J* = 12.1, 6.0 Hz, 1H, H-1a), 4.23 (br d, *J* = 8.9 Hz, 1H, H-1b), 4.97 (br s, 1H, H-2), 7.40 (br s, 3H, NH_3^+); MS (ES): *m/z*: 794 ([M+H]$^+$, 100%).

Compound ***6Ei*** *(25 mg from 125 mg of resin* ***4E****):* 22% yield; IR: ν_{max} 3350, 3054, 2922, 2851, 1736, 1670, 1422, 1265, 1200 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.5 Hz, 6H, H-19′ and H-19″), 1.23 (s, 64H, H-4′–H-17′, H-4″–H-17″ and H-3‴–H-6‴), 1.47 (m, 4H, H-18′ and H-18″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.32 (m, 4H, H-2′ and H-2″), 2.95 (br s, 2H, H-8‴), 3.09 (br t-like, 2H, H-1‴), 3.33 (br d, *J* = 5.1 Hz, 2H, H-3), 4.12 (dd, *J* = 11.9, 5.8 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.1 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.60 (br s, 3H, NH_3^+); MS (ES): *m/z*: 822 ([M+H]$^+$, 100%).

Compound ***6Ej*** *(24 mg from 119 mg of resin* ***4E****):* 22% yield; IR: ν_{max} 3357, 2928, 2853, 1735, 1679, 1574, 1422, 1265 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.5 Hz, 6H, H-20′ and H-20″), 1.23 (s, 68H, H-4′–H-18′, H-4″–H-18″ and H-3‴–H-6‴), 1.49 (m, 4H, H-19′ and H-19″), 1.59 (m, 8H, H-3′, H-3″, H-2‴ and H-7‴), 2.33 (m, 4H, H-2′ and H-2″), 2.98 (br s, 2H, H-8‴), 3.10 (br t-like, 2H, H-1‴), 3.34 (br d, *J* = 5.2 Hz, 2H, H-3), 4.13 (dd, *J* = 12.0, 5.8 Hz, 1H, H-1a), 4.24 (br d, *J* = 9.0 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.49 (br s, 3H, NH_3^+); MS (ES): *m/z*: 850 ([M+H]$^+$, 100%).

Compound ***6Fa*** *(38 mg from 155 mg of resin* ***4F****):* 37% yield; IR: ν_{max} 3350, 2925, 2855, 1735, 1678, 1564, 1466, 1373, 1202, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.5 Hz, 6H, H-11′ and H-11″), 1.23 (s, 34H, H-4′–H-9′, H-4″–H-9″ and H-3‴–H-7‴), 1.44 (m, 4H, H-10′ and H-10″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.29 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-9‴), 3.07 (br t-like, 2H, H-1‴), 3.32 (br s, 2H, H-3), 4.07 (dd, *J* = 12.0, 6.0 Hz, 1H, H-1a), 4.26 (br d, *J* = 8.5 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.60 (br s, 3H, NH_3^+); MS (ES): *m/z*: 612 ([M+H]$^+$, 100%).

Compound ***6Fb*** *(35 mg from 129 mg of resin* ***4F****):* 39% yield; IR: ν_{max} 3366, 2923, 2853, 1741, 1687, 1466, 1373, 1204, 1174, 1129 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.3 Hz, 6H, H-12′ and H-12″), 1.23 (s, 38H, H-4′–H-10′, H-4″–H-10″ and H-3‴–H-7‴), 1.44 (m, 4H, H-11′ and H-11″), 1.56 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.29 (m, 4H, H-2′ and H-2″), 2.91 (br s, 2H, H-9‴), 3.06 (br t-like, 2H, H-1‴), 3.31 (br s, 2H, H-3), 4.01 (dd, *J* = 12.1, 6.0 Hz, 1H, H-1a), 4.25 (br d, *J* = 9.0 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.70 (br s, 3H, NH_3^+); MS (ES): *m/z*: 640 ([M+H]$^+$, 100%).

Compound ***6Fc*** *(39 mg from 147 mg of resin* ***4F****):* 36% yield; IR: ν_{max} 3350, 2924, 2853, 1741, 1682, 1571, 1466, 1377, 1203, 1174, 1133 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.6 Hz, 6H, H-13′ and H-13″), 1.23 (s, 42H, H-4′–H-11′, H-4″–H-11″ and H-3‴–H-7‴), 1.44 (m, 4H, H-12′ and H-12″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.29 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-9‴), 3.06 (br t-like, 2H, H-1‴), 3.31 (br s, 2H, H-3), 4.06 (dd, *J* = 12.2, 6.0 Hz, 1H, H-1a), 4.25 (br d, *J* = 9.0 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.69 (br s, 3H, NH_3^+); MS (ES): *m/z*: 668 ([M+H]$^+$, 100%).

Compound **6Fd** *(31 mg from 133 mg of resin* **4F***):* 30% yield; IR: ν_{max} 3359, 3190, 2923, 2853, 1738, 1660, 1633, 1467, 1421, 1202, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.6 Hz, 6H, H-14′ and H-14″), 1.23 (s, 46H, H-4′–H-12′, H-4″–H-12″ and H-3‴–H-7‴), 1.42 (m, 4H, H-13′ and H-13″), 1.56 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.28 (m, 4H, H-2′ and H-2″), 2.90 (br s, 2H, H-9‴), 3.06 (br t-like, 2H, H-1‴), 3.30 (br s, 2H, H-3), 4.10 (dd, J = 12.0, 6.0 Hz, 1H, H-1a), 4.25 (br d, J = 8.5 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.83 (br s, 3H, NH_3^+); MS (ES): *m/z*: 696 ($[M+H]^+$, 100%).

Compound **6Fe** *(41 mg from 140 mg of resin* **4F***):* 37% yield; IR: ν_{max} 3365, 2918, 2850, 1737, 1686, 1660, 1406, 1376, 1202, 1177, 1133 cm^{-1}; ^1H NMR: δ 0.85 (t, J = 6.6 Hz, 6H, H-15′ and H-15″), 1.23 (s, 50H, H-4′–H-13′, H-4″–H-13″ and H-3‴–H-7‴), 1.42 (m, 4H, H-14′ and H-14″), 1.56 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.28 (m, 4H, H-2′ and H-2″), 2.90 (br s, 2H, H-9‴), 3.05 (br t-like, 2H, H-1‴), 3.30 (br s, 2H, H-3), 4.10 (dd, J = 12.1, 6.0 Hz, 1H, H-1a), 4.25 (br d, J = 8.5 Hz, 1H, H-1b), 5.00 (br s, 1H, H-2), 7.80 (br s, 3H, NH_3^+); MS (ES): *m/z*: 724 ($[M+H]^+$, 100%).

Compound **6Ff** *(38 mg from 142 mg of resin* **4F***):* 32% yield; IR: ν_{max} 3350, 2918, 2850, 1737, 1661, 1466, 1177, 1129 cm^{-1}; ^1H NMR: δ 0.85 (t, J = 6.5 Hz, 6H, H-16′ and H-16″), 1.23 (s, 54H, H-4′–H-14′, H-4″–H-14″ and H-3‴–H-7‴), 1.43 (m, 4H, H-15′ and H-15″), 1.56 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.28 (m, 4H, H-2′ and H-2″), 2.91 (br s, 2H, H-9‴), 3.06 (br t-like, 2H, H-1‴), 3.30 (br s, 2H, H-3), 4.11 (dd, J = 12.0, 6.0 Hz, 1H, H-1a), 4.24 (br d, J = 9.1 Hz, 1H, H-1b), 4.99 (br s, 1H, H-2), 7.84 (br s, 3H, NH_3^+); MS (ES): *m/z*: 752 ($[M+H]^+$, 100%).

Compound **6Fg** *(36 mg from 147 mg of resin* **4F***):* 28% yield; IR: ν_{max} 3350, 2925, 2854, 1735, 1675, 1464, 1376, 1202 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 6H, J = 6.7 Hz, H-18′ and H-18″), 1.24 (s, 38H, H-4′–H-6′, H-13′–H-16′, H-4″–H-6″, H-13″–H-16″ and H-3‴–H7‴), 1.27 (s, 8H, H-7′, H-12′, H-7″ and H-12″), 1.44 (m, 4H, H-17′ and H-17″), 1.58 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 1.99 (m, 8H, H-8′, H-11′, H-8″ and H-11″), 2.30 (m, 4H, H-2′ and H-2″), 2.92 (br s, 2H, H-9‴), 3.07 (br t-like, 2H, H-1‴), 3.32 (br s, 2H, H-3), 4.11 (dd, J = 12.0, 6.0 Hz, 1H, H-1a), 4.22 (br d, J = 9.0 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 5.32 (m, 4H, H-9′, H-10′, H-9″ and H-10″), 7.60 (br s, 3H, NH_3^+); MS (ES): *m/z*: 804 ($[M+H]^+$, 100%).

Compound **6Fh** *(47 mg from 142 mg of resin* **4F***):* 37% yield; IR: ν_{max} 3365, 2917, 2849, 1737, 1686, 1466, 1177, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (t, J = 6.6 Hz, 6H, H-18′ and H-18″), 1.22 (s, 62H, H-4′–H-16′, H-4″–H-16″ and H-3‴–H-7‴), 1.43 (m, 4H, H-17′ and H-17″), 1.56 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.28 (m, 4H, H-2′ and H-2″), 2.89 (br s, 2H, H-9‴), 3.06 (br t-like, 2H, H-1‴), 3.30 (br s, 2H, H-3), 4.11 (dd, J = 11.9, 6.0 Hz, 1H, H-1a), 4.26 (br d, J = 9.5 Hz, 1H, H-1b), 4.98 (br s, 1H, H-2), 7.79 (br s, 3H, NH_3^+); MS (ES): *m/z*: 808 ($[M+H]^+$, 100%).

Compound **6Fi** *(35 mg from 134 mg of resin* **4F***):* 28% yield; IR: ν_{max} 3350, 3054, 2924, 2851, 1736, 1686, 1466, 1422, 1265, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.5 Hz, 6H, H-19′ and H-19″), 1.23 (s, 66H, H-4′–H-17′, H-4″–H-17″ and H-3‴–H-7‴), 1.47 (m, 4H, H-18′ and H-18″), 1.60 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.31 (m, 4H, H-2′ and H-2″), 2.96 (br s, 2H, H-9‴), 3.09 (br t-like, 2H, H-1‴), 3.33 (br s, 2H, H-3), 4.09 (dd, J = 12.1, 6.0 Hz, 1H, H-1a), 4.23 (br d, J = 8.5 Hz, 1H, H-1b), 4.97 (br s, 1H, H-2), 7.47 (br s, 3H, NH_3^+); MS (ES): *m/z*: 836 ($[M+H]^+$, 100%).

Compound **6Fj** *(41 mg from 159 mg of resin* **4F***):* 27% yield; IR: ν_{max} 3365, 2916, 2845, 1740, 1699, 1679, 1463, 1265, 1200, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (t, J = 6.6 Hz, 6H, H-20′ and H-20″), 1.21 (s, 70H, H-4′–H-18′, H-4″–H-18″ and H-3‴–H-7‴), 1.43 (m, 4H, H-19′ and H-19″), 1.57 (m, 8H, H-3′, H-3″, H-2‴ and H-8‴), 2.27 (m, 4H, H-2′ and H-2″), 2.91 (br s, 2H, H-9‴), 3.08 (br t-like, 2H, H-1‴), 3.39 (br s, 2H, H-3), 4.10 (dd, J = 11.9, 5.9 Hz, 1H, H-1a), 4.26 (br d, J = 8.5 Hz, 1H,, H-1b), 5.01 (br s, 1H, H-2), 7.74 (br s, 3H, NH_3^+); MS (ES): *m/z*: 864 ($[M+H]^+$, 100%).

3.2.6. DNA binding affinity

DNA binding affinities of all samples were measured at two DNA/sample ratios (w/w), 1:10 and 1:20, by electrophoresis. DNA/sample complexes were formed at a ratio of 1:10 (w/w) by transferring of 12.5 μL (30 μg/μL) of sample into an Eppendorf tube. Each sample was further diluted with 37.5 μL of acetate buffer (20 mM, pH 7.4). To this solution, an aqueous solution of plasmid DNA (50 μL, 3 μg/25 mL) was added to each sample, and the solutions were successively mixed. The DNA/sample complex 1:20 (w/w) was prepared as above except that the 50 μL of stock sample (30 μg/μL) was used without dilution. DNA complexes were incubated at room temperature for 30 min. Bromophenol blue-free gel loading buffer (100 μL, 13.3% w/v sucrose in water) was added to 100 μL of this complex. The solution was inverted to mix and each sample (10 μL) was loaded onto a 1% agarose gel (0.5 × TBE buffer). The gel was run at 200 V, 400 mA for 2 h. DNA bands were viewed under UV light by ethidium bromide staining.

3.2.7. Liposome preparation

To a solution of DOPE (50 μL, 20 μg/μL in chloroform) in a 1.5 mL Eppendorf was added a solution of cationic lipid (50 μL, 20 μg/μL in ethanol) and shaken. The organic solvents were evaporated under a stream of nitrogen gas and further dried under high vacuum (>2 h). The resulting thin film was hydrated with 100 μL of phosphate-buffered saline (PBS, pH 7.4) at room temperature for 1 h. The mixture was vortexed for 1 min and sonicated (2 × 15 min) with 1 h rests between sonications in a bath-type sonicator, producing small unilamellar vesicles.[28] The liposomes were stored at 4 °C for 24 h prior to use.

3.2.8. Transfection procedure

HEK293 (human embryonic kidney cell), PC-3 (human prostate adenocarcinoma) and HeLa (human cervical adenocarcinoma) were grown in DMEM supplemented with 10% fetal calf serum (FCS), penicillin (100 U/mL), streptomycin (100 mg/mL) and L-glutamine (4 mM) at 37 °C, 5% CO_2. For transfection, the cells were seeded up to 1 × 10^4 cells/well in a 96-well plate, to give 50–70% confluence to be used on the next day. The growth medium was removed and the cells were washed with PBS and replaced with 100 μL of fresh serum-free DMEM. DNA (pCH110-encoding β-galactosidase)/cationic liposome complexes (lipoplexes) were prepared as follows. An appropriate volume of each cationic liposome (1 μg/μL) was added to the plasmid DNA (0.4 μL, 0.5 μg/μL) and the complex was incubated at room temperature for 30 min before being diluted with phosphate-buffered saline to make a final DNA concentration of 0.1 mg/10 mL. The lipoplexes (10 μL) were then added to the cells and left to incubate at 37 °C, 5% CO_2. The cells were then washed with PBS and fresh growth medium was added and further incubated for 48 h. For Effectene™ transfection, the method was carried out according to the manufacturer's instruction and the same ratios of plasmid DNA:Effectene™ were used.

After transfection, the cells were washed once with Dulbecco's phosphate-buffered saline (D-PBS) containing 0.1 g/L calcium and magnesium and then fixed with 100 μL fixative (2% formaldehyde, 0.05% glutaraldehyde in D-PBS) for 5 min at room temperature. The cells were washed and 100 μL of substrate/stain solution (1 mg/mL X-gal in stain solution; 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM $MgCl_2$) incubated at 37 °C for 2 h. The cells were washed with D-PBS and the blue cells were then counted under inverted microscope.

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Solid phase synthesis of novel asymmetric hydrophilic head cholesterol-based cationic lipids with potential DNA delivery

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ABSTRACT

Twenty-four asymmetric divalent head group cholesterol-based cationic lipids were designed and synthesized by parallel solid phase chemistry. These asymmetric head groups composed of amino functionality together with trimethylamino, di(2-hydroxyethyl)amino or guanidinyl groups. Spacers between cationic heads and linker were both equal and unequal in length. These lipids were subjected to evaluation for DNA binding affinities by gel retardation assay and were screened for their transfection efficiency on HEK293 cells. Cationic lipids with equal chain length exhibited high transfection efficiency when polar part contained asymmetric polar heads. In contrast, lipids with unequal chain length exhibited high transfection efficiency when polar part contained symmetric heads. According to the optimal formulation, seven lipids exhibited higher transfection efficiency than the commercially available transfection agents, Effectene™, DOTAP and DC-Chol, to deliver DNA into PC3 human prostate adenocarcinoma cells. 3β-[*N*-(*N*′-Guanidinyl)-2′-aminoethyl)-*N*-(2-aminoethyl)carbamoyl] cholesterol (**5**) bearing amino and guanidinyl polar heads exhibited highest transfection efficiency with minimal toxicity. The morphology of active liposomes was observed by transmission electron microscopy (TEM) and size of liposomes were around 200–700 nm.

1. Introduction

Gene therapy is an approach to treat genetic disorders, AIDS and other acquired genetic defects. This method is the replacement of a defective gene with a normal one. Various techniques involving viral and non-viral vectors have been developed to insert genetic material into recipient cells.[1] Much effort has been devoted to the development of non-viral delivery due to the disadvantages of viruses used for gene delivery which include generation of immune responses.[2] Consequently, non-viral vector was chosen for DNA delivery. Non-viral vectors can be classified into two major categories: physical methods (e.g., microinjection,[3] hydrodynamic,[4] particle bombardment,[5] electroporation,[6] ultrasound[7] and encapsulated microsphere[8]) and chemical methods (e.g., DEAE–dextran,[9] calcium phosphate,[10] cationic lipid,[11] cationic polymer[12] and cationic dendrimer[13]). Of all the non-viral chemical vectors, cationic lipid is the chemical transfection agent for delivery of nucleic acids using liposomes which hold great promise as a safe and non-immunogenic approach to gene delivery.[14]

Cationic lipids form liposomes when formulated in water under optimal conditions.[15] The surface of these liposomes is positively charged and is attracted electrostatically to the phosphate backbone of DNA, as well as to the negatively charged surface of the cell membrane. Lipoplexes, which are also known as liposome–DNA complex, play a central role in current approaches to gene delivery, serving as potent transfection vectors.[16] The mechanism of DNA intake is not exactly known but is believed to be related to endocytosis.[17] To achieve DNA delivery, lipoplex complexes need to bind the cell surface, across the membrane, release DNA into the cytoplasm, and finally transport the DNA into the nucleus.[18] Most cationic liposomes have a common neutral phospholipid in addition to the cationic lipid component. The phospholipid is needed for stabilizing most type of cationic lipids in a lipid bilayer and may provide the cell penetration function of cationic liposomes.[19] In this report the neutral phospholipid, dioleoylphosphatidyl ethanolamine (DOPE), was used as a helper lipid.

Since the key invention of cationic liposome in DNA delivery,[11] several cationic lipids having various cationic headgroups, linkers and hydrophobic tails were reported. Cholesterol-based cationic lipids[20] are among the most promising agents that are potentially and safely delivery gene into cells. Cholesterol was often used as a lipid anchor because of its lipid bilayer stabilizing activity[21] and minimal

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toxicity to the treated cells.[20] Cholesterol-based cationic lipids with variation of headgroups, mono-, di-, and polyvalent, have been synthesized and tested for their transfection efficiency.[20,22–24] Cholesterylspermidine,[22] BGTC[23] and Lipid 67[24] are samples of cholesterol-based cationic lipids bearing symmetric terminal polar groups (Fig. 1). To our knowledge, cholesterol-based lipid with unsymmetric terminal cationic heads has not been reported. We report here the synthesis of asymmetric hydrophilic head cholesterol-based cationic lipids and transfection efficiency evaluation. To study structure–transfection activity relationship, cationic lipids with symmetric polar head were synthesized and compared the transfection activity of those asymmetric lipids (Fig. 2).

2. Results and discussion

2.1. Synthesis

The synthesis of symmetric and asymmetric terminal hydrophilic head lipids (**1**–**2** and **3**–**8**, respectively) having symmetry spacer length (library 1, Fig. 2) was carried out as shown in Schemes 1 and 2. The key template **28** used for the synthesis of cationic lipids library 1 was synthesized as outlined in Scheme 1. The active carbonate resin **25**[25] was reacted with polyamine, diethylenetriamine or nor-spermidine, to afford the resin **26**. The primary amine was selectively protected with Dde-OH[26] and the secondary amine was capped with cholesteryl chloroformate to generate the resin **27**. The key intermediate **28** was obtained after treatment the resin **27** with 5% N_2H_4 in DMF. Treatment of the resin **28** with 50% TFA in CH_2Cl_2 gave the lipids **1** and **2**. The free primary amine on the resin **28** allowed different designs for the cationic head. Numbers of cationic heads, trimethyl quaternary amine,[11,20a] guanidinium[23,27] and amine having hydroxyethyl group[28,29] have been synthesized and evaluated for their transfection efficiency. Some of them exhibited remarkably high transfection efficiencies. Reaction of the resin **28** with methyl iodide in the presence of DIEA base for 18 h generated the resin **29**, which was treated with 50% TFA in CH_2Cl_2 to obtain the desired lipids **3** and **4**. The synthesis of the lipids **5** and **6** was accomplished by treatment the resin **28** with *N*′,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea[27] for 18 h followed by cleavage with 50% TFA in CH_2Cl_2. The lipids **7** and **8** were also synthesized by reaction of the resin **28** with 2-bromoethanol for 18 h followed by cleavage with TFA.

To investigate the effect of the chain length between cationic head and linker on the transfection efficiency, cationic lipids library 2 was synthesized (Fig. 2) as shown in Schemes 3 and 4. The resin **32**, which was prepared by reacting the active carbonate **25** with appropriate diamine, was coupled with bromoacetic acid

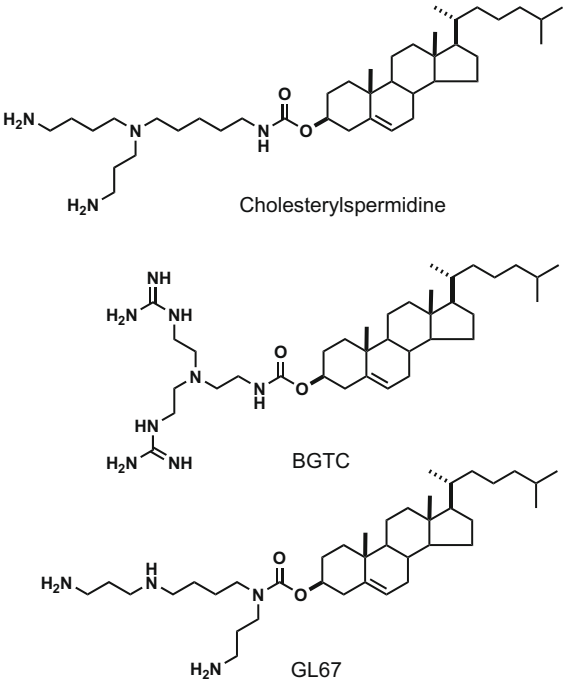
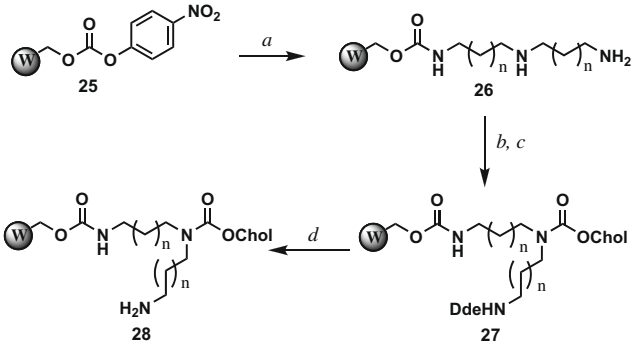


Figure 1. Structures of symmetrical edge-head cholesterol-based cationic lipids.



Scheme 1. Reagents and conditions: (a) diethylenetriamine or bis(3-aminopropyl)amine (excess), CH_2Cl_2, 6 h; (b) Dde-OH (excess), CH_2Cl_2, DMF, 12 h; (c) cholesteryl chloroformate (4 equiv), pyridine, CH_2Cl_2, 12 h; (d) 5% N_2H_4/DMF, 2 × 30 min.

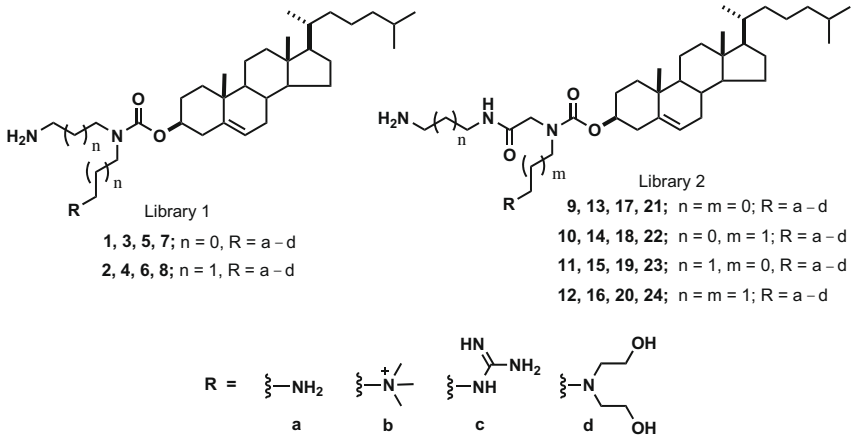
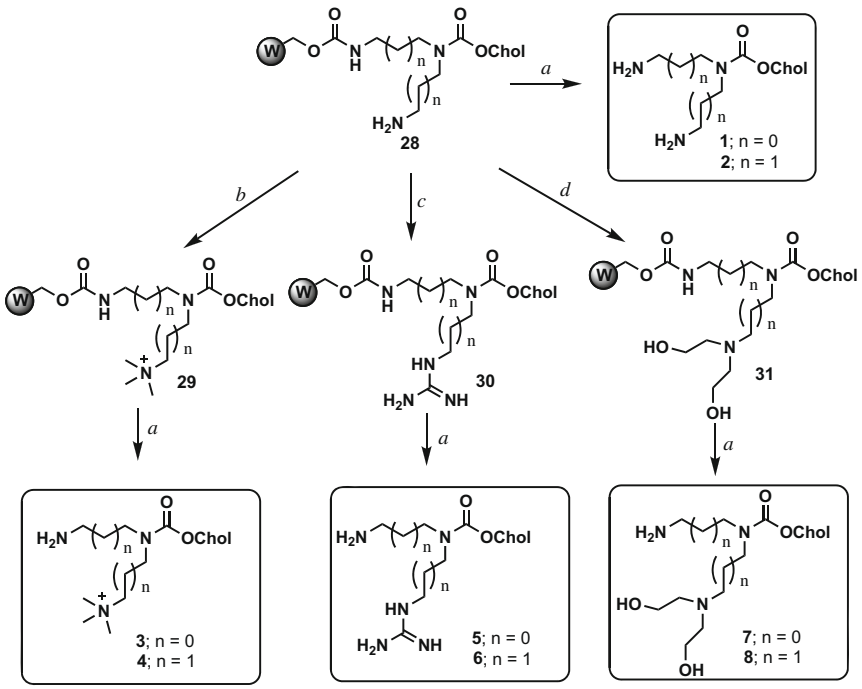
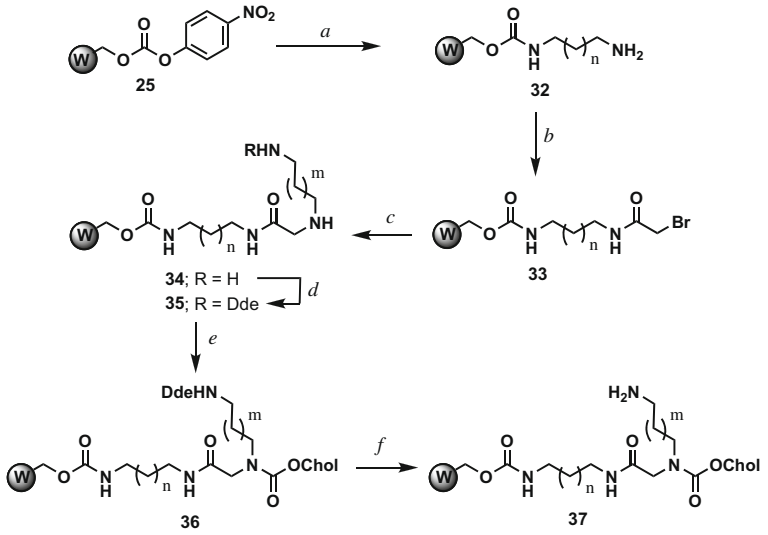


Figure 2. Structures of new asymmetrical polar head cholesterol-based cationic lipids.



Scheme 2. Reagents and conditions: (a) 50% TFA/CH_2Cl_2, 2 h; (b) MeI (10 equiv), DIEA, DMF, 18 h; (c) *N,N′*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (4 equiv), DIEA, DMF, 18 h; (d) 2-bromoethanol (8 equiv), DIEA, 18 h.

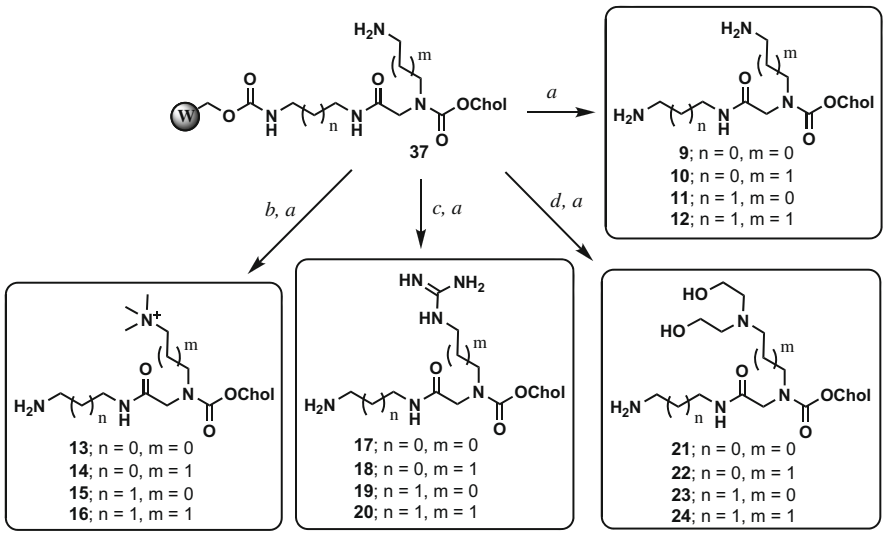


Scheme 3. Reagents and conditions: (a) 1,2-diaminoethane or 1,3-diaminopropane (excess), CH_2Cl_2, 6 h; (b) bromoacetic acid (4 equiv), DIC (4 equiv), DMF, 12 h; (c) 1,2-diaminoethane or 1,3-diaminopropane (excess), DMF, 12 h; (d) Dde-OH (excess), CH_2Cl_2, DMF, 12 h; (e) cholesteryl chloroformate (4 equiv), pyridine (20 equiv), CH_2Cl_2, 12 h; (f) 5% N_2H_4/DMF, 2 × 30 min.

to obtain the resin **33**, which was then reacted with different diamine to generate the resin **34**. The primary amine of the resin **34** was selectively protected with Dde-OH allowing the remaining amine to couple to the cholesterol tail. Thus, the resin **35** was reacted with cholesteryl chloroformate to provide the resin **36**. The Dde group was removed with 5% N_2H_4 in DMF to give the scaffold **37**. Cationic lipids **9**–**12** were obtained after TFA treatment (Scheme 4). The lipids **13**–**24** which contain various polar heads were prepared in the same manner as described for library 1. All synthesized lipids **1**–**24** were obtained in 32–70% yields (based on original loading of Merrifield resin and as TFA salts). Structure elucidation of these lipids was achieved by spectroscopic means (^1H and ^{13}C NMR, IR and mass spectra).

2.2. DNA binding affinity

The relative DNA binding affinities of cationic lipids were evaluated to determine whether transfection activities correlated with DNA binding. The DNA binding affinities were determined by gel retardation assay. Cationic lipids were mixed with plasmid DNA, known as lipoplex, at weight ratios of 1:20 (DNA/sample, w/w) and the lipoplexes were loaded on agarose gel (Fig. 3). The result indicated that most of lipids from library 1 interacted sufficiently with DNA to retard migration through the gel matrix except the lipid **5**. Lipids with equal chain length (library 1) were able to bind to DNA more sufficient than the lipids with unequal chain length (library 2) at the DNA/lipid weight ratio of 1:20. For library 2, the



Scheme 4. Reagents and conditions: (a) 50% TFA/CH_2Cl_2, 2 h; (b) MeI (10 equiv), DIEA, DMF, 18 h; (c) *N,N′*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (4 equiv), DIEA, DMF, 18 h; (d) 2-bromoethanol (8 equiv), DIEA, 18 h.

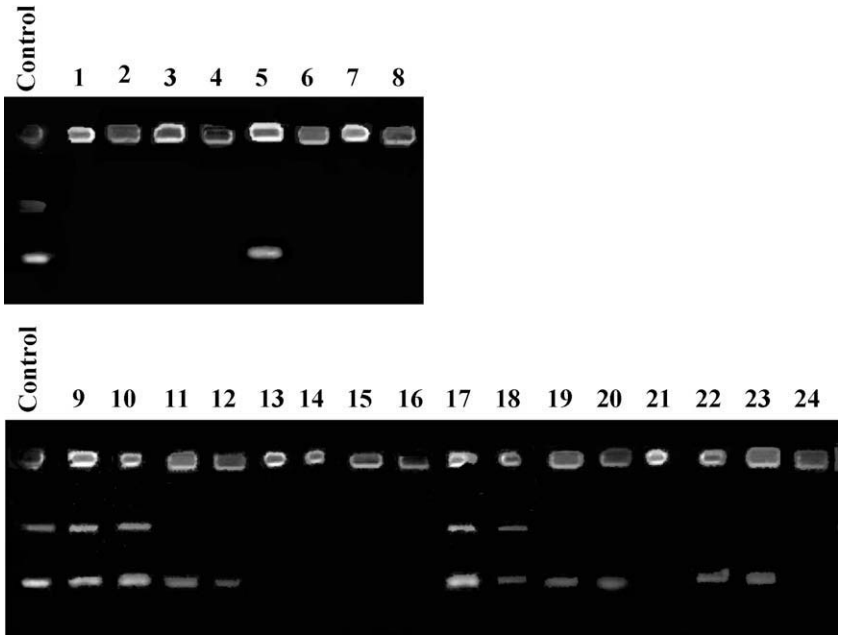


Figure 3. Gel retardation assay of DNA/cationic lipids complexes at a weight ratio of 1:20. Lanes marked 'Control' contained DNA alone and was used as a control. The presence of a lower band indicated that DNA has migrated and has not been bound by the transfection compound.

lipids **13–16** bearing trimethyl quaternary amine bound sufficiently with DNA whereas the lipids with the amino (**9–12**) and guanidinyl (**17–20**) head groups did not fully bind to DNA. It should be noted that the lipids **3–4** and **13–16** which possess permanent charge bound strongly to DNA to retard migration.

2.3. Transfection activity

2.3.1. Transfection screening

All the synthesized cationic lipids were tested for DNA delivery to HEK293 (Human embryonic kidney cell lines) using β-galactosidase as a reporter gene. Figure 4 shows the transfection screening results using plasmid DNA (0.1 μg/well) at DNA/liposome ratio 1:20 (w/w). The liposome formation in the preliminary transfection activity screening was with and without DOPE. In the case of liposome formation with DOPE, cationic lipid/DOPE ratio was 2:1 (w/w). The results indicated that eight lipids, **2**, **5–8**, **10–11** and **19**, exhibited relative transfection efficiency over 100% as compared to Effectene™ transfection (100%). Most the lipids in library 1 bearing unsymmetrical polar head and symmetrical chain length exhibited high transfection efficiency. In contrast, cationic lipid library 2 (**10** and **11**) with symmetric polar head and unsymmetric chain length showed higher transfection activity than Effectene™. From the screening result, eight lipids which exhibited higher transfection efficiency than the positive control were subjected to further optimization. To find out the optimized transfection efficiency of these lipids, the lipids/DOPE ratios, DNA/lipids ratios, and DNA per well were studied.

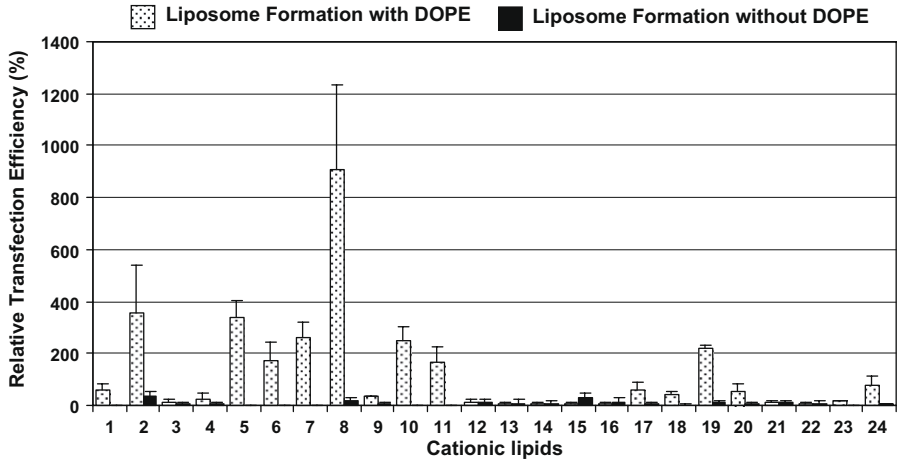


Figure 4. Transfection screening activity of synthesized cationic lipids employing pCH110-encoding β-galactosidase (0.1 μg/well). The liposome formation was both with DOPE and without DOPE. The lipoplexes was used at DNA/lipids (w/w) ratios of 1:20. The transfection efficiencies of the lipids were compared to that of commercially available reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown).

2.3.2. Optimization of cationic lipid/DOPE ratios

Helper lipid, DOPE, has been known to increase the transfection efficiency of cationic liposome to transfer and release DNA into the cytoplasm.[30] In order to find out the most effective formulations, transfections with equal DNA/lipid weight ratio (1:20) and varying the weight ratio of lipids (**2**, **5–8**, **10–11** and **19**)/DOPE were performed (Fig. 5). The optimized lipid/DOPE ratio was found to vary for each lipid. Among the compound tested, at a lipid/DOPE ratio of 1:2, the lipid **8** was found to be the most effective compound to deliver DNA into the HEK293 cells (900%). The transfection efficiency of the lipid **8** decreased dramatically when the weight ratio of lipid/DOPE decreased. The lipids **10** and **19** showed the maximum efficiency at a lipid/DOPE ratio of 2:1 whereas the lipids **5** and **11** exhibited the highest efficiency at a lipid/DOPE ratio of 1:1. The optimal formulation of each lipid was used for the next experiment.

2.3.3. Optimization of DNA/amount of cationic lipids ratios

By using the respective optimized lipid/DOPE ratio for each lipid, all the selected lipids were tested at the equal amount of DNA (0.1 μg/well) and varying the amount of lipid. Three DNA/cationic lipid ratios, 1:10, 1:20 and 1:40 (w/w), were prepared and evaluated for transfection efficiency and the result is shown in Figure 6. The transfection efficiency of the lipids **2**, **7**, **8**, **11** and **19** was found to decrease at lower DNA/lipid ratio. In contrast, the lipids **5** and **6** exhibited highest transfection efficiency when lower DNA/lipids ratio was used. The optimal DNA/cationic lipid ratio for each lipoplex formation was used for further optimization.

2.3.4. Optimization of the amount of DNA

To see whether variation in the amount of DNA affected the transfection efficiency of these lipids, the experiments were performed using the optimal lipid/DOPE (Fig. 5) and DNA/lipid (Fig. 6) ratios. The amounts of DNA used in the experiment were 0.1, 0.2 and 0.4 μg/well. The lipids **2**, **6**, **8** and **11** exhibited higher transfection efficiency when the amount of DNA increased (Fig. 7). The results have indicated that these lipids are more efficient at high DNA concentration. At the highest amount of DNA (0.4 μg/well), the lipid **8** exhibited the highest transfection activity.

2.3.5. The effect of serum

One of the major draw backs of cationic lipids for their in vivo use is the inhibition of the transfection efficiency of cationic liposomes in the presence of serum. Most of cationic lipids which

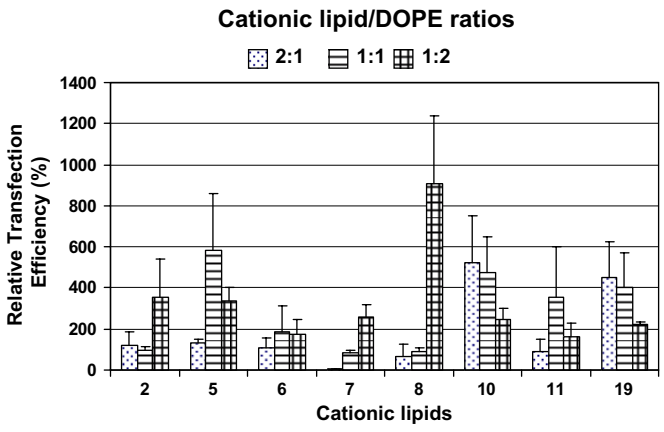


Figure 5. In vitro transfection efficiency of the selected lipids in HEK293 cells across the cationic lipid/DOPE weight ratio of 2:1, 1:1 and 1:2. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown).

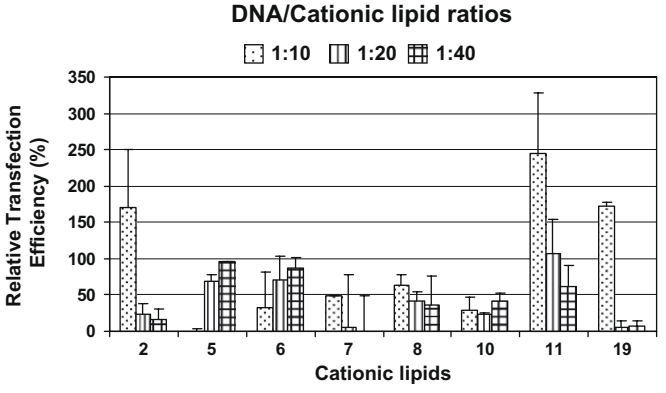


Figure 6. In vitro transfection efficiency of the selected lipids in HEK293 cells across the DNA/cationic lipid weight ratios of 1:10, 1:20 and 1:40 (w/w). The optimal liposome formation of each lipid from Figure 5 was used as described in the text. Transfection efficiency of the selected lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown).

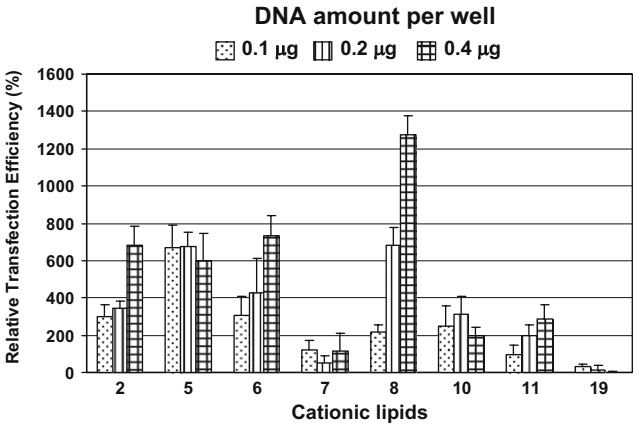


Figure 7. Effect of DNA amount for gene delivery. The optimal liposome formation (Fig. 5) and DNA/lipids complex (Fig. 6) of each lipid were used to mix with various amount of DNA from 0.1 to 0.4 μg. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency.

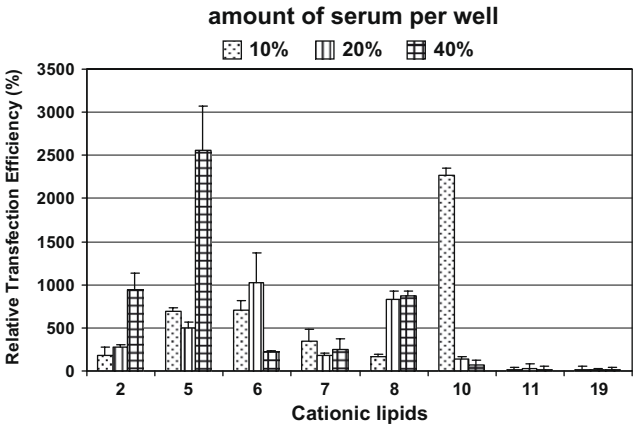


Figure 8. Effect of serum for transfection efficiency. The optimal liposome formation (Fig. 5), DNA/cationic lipid (Fig. 6) and amount of DNA per well (Fig. 7) of each lipid were used to transfer gene to HEK293 cell at various amount of serum. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency.

exhibited high transfection activity in the absence of serum lost their efficiency when transfected in the presence of serum.[20b,c] The selected lipids with the optimal condition of each lipid from Figures 5–7 were tested for the effect of serum on gene transfection efficiency. These experiments were carried out in the presence of 10%, 20% and 40% serum. The results are shown in Figure 8. The lipids **2** and **5** exhibited highest transfection efficiency (1000% and 2600%, respectively) when the experiment was performed under 40% serum. Transfection efficiency of the lipid **10** reached its maximum when 10% serum was present. Transfection efficiency of this lipid was significantly decreased when the cells were tested in the presence of 20% and 40% serum. At 20% and 40% serum, the lipid **8** showed similar activity to transfer DNA into cells. The lipids **11** and **19** could not deliver DNA into cells when the experiment was carried out under serum containing condition.

2.3.6. Transfection efficiency toward different cell lines

It is well-known that transfection agents have ability to specifically deliver DNA into different cell types. To evaluate the transfection efficiency of these lipids toward the different mammalian cell lines, COLO 205, D-17, HeLa and PC3 cells, the experiments were performed using optimum conditions of each lipid (Figs. 5–7). These four cells were chosen as representing cancers of importance to human health. Three commercially available transfection agents, Effectene™, DOTAP and DC-Chol, were tested under identical conditions for comparison. The experiment was carried out under serum-free condition. The transfection results are shown in Figure 9. All of lipids, except **6**, exhibited higher transfection efficiency to deliver DNA into PC3 cells than Effectene™, DOTAP and DC-Chol. Lipid **5** exhibited highest activity having relative transfection efficiency of 3200%. All the compounds tested could not reach their transfection efficiency of 500% against COLO 205, D-17 and HeLa cells. The results indicated that transfection efficiency of these newly synthesized lipids was cell dependent.

2.3.7. Transfection toxicity

Cytotoxicity of synthesized cationic lipids is very important for gene delivery. To assess the relationship between cytotoxicity and transfection efficiency, the toxicity of synthesized lipids on HEK293, COLO 205, D-17, HeLa and PC3 cell lines using optimal condition (Figs. 5–7) were determined by measuring changes in cell metabolic activity (MTT assay). The results were shown as % cell viability as compared to the control cells in the presence of

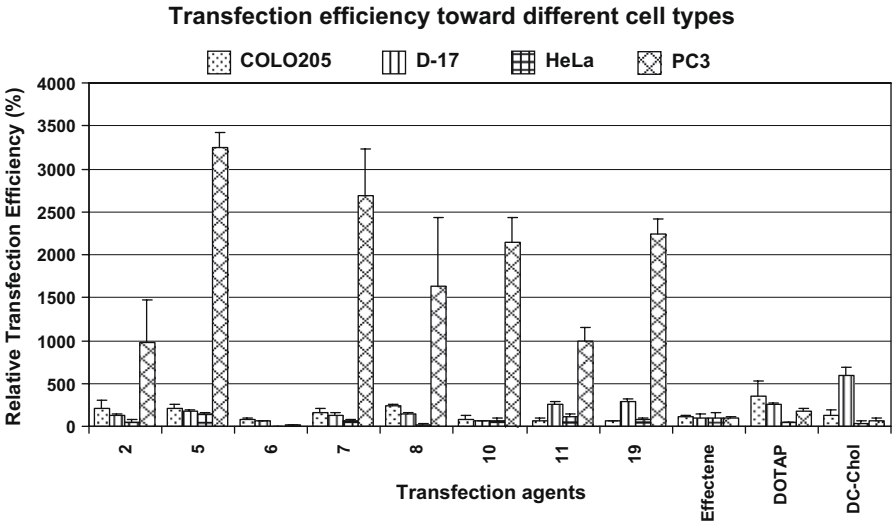


Figure 9. Transfection efficiency of selected lipids toward COLO 205, D-17, HeLa and PC3 cell using optimum conditions from Figures 5–7. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, DOTAP and DC-Chol. Transfection efficiency of Effectene™ for each cell line was calculated as 100%.

DNA (Fig. 10). Most of the lipids including the commercially available agents showed no cytotoxicity to all cell types. The lipid **7** was toxic to the cells tested and cell viability was lower than 50%. This result was contrast to the previous experiment which the lipid **7** exhibited relative transfection efficiency of 2700% against PC3 cells.

2.4. Transmission electron microscopy (TEM)

The morphology of the prepared liposome from the synthesized cationic lipids, which were used to study transfection optimization, was observed under transmission electron microscopy (TEM) after negative staining. The liposome of each lipid was prepared at its optimal lipid/DOPE ratio. The morphology of liposomes was shown in Figure 11. The diameter of the aggregates spanned from 200 to 700 nm. Most of the aggregates showed vesicle-like organizations. Morphology of liposomes/DNA complexes (lipoplexes) was also visualized under transmission electron microscopy as shown in Figure 12. Most of the lipoplexes were found to be large spherical aggregates which the sizes are within the range 300–1000 nm. In this study, sizes of lipoplexes prepared from these lipids are unlikely to play important role in modulating for transfection efficiency.

In summary, cholesterol-based cationic lipids have been of interest to many researchers for its use in gene delivery applications. Cholesterol-based cationic lipids can be easily synthesized with different polar head to enhance transfection efficiency. Toward this end, we have synthesized asymmetric divalent polar head groups cholesterol-based cationic lipids bearing amino group together with either trimethylamino, di(2-hydroxyethyl)amino or guanidinyl functionality. Based on the results reported here, new cationic lipids required neutral lipid, DOPE, to enhance transfection efficiency. It was found that cationic lipids library 1, which possessed equal spacer length, exhibited high transfection efficiency when bearing the asymmetric polar heads. However, when the unequal spacer length was introduced into cationic lipid library 2 the

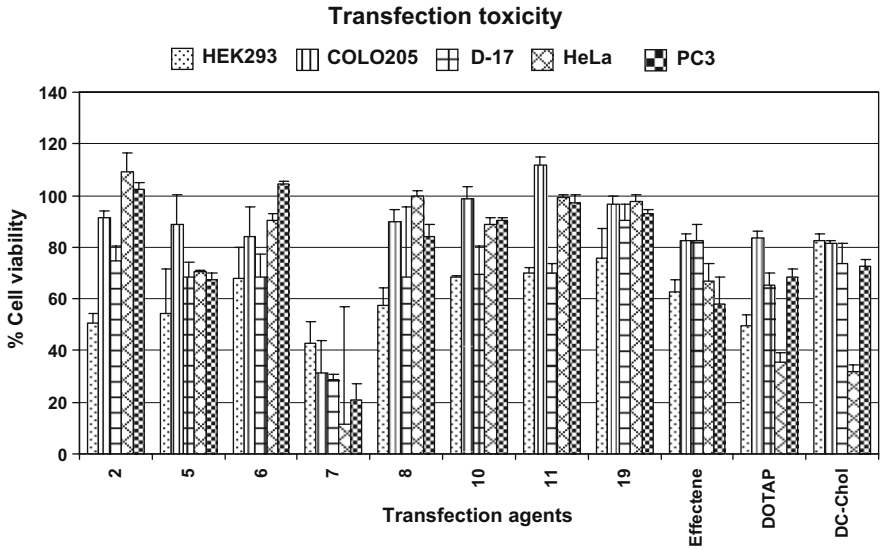


Figure 10. Effect of transfection synthesized lipids on cell metabolic activity. Liposomes of cationic lipid with helper lipid, DOPE, were formed and added to DNA to form lipoplexes. These complexes were added to HEK293, COLO 205, D-17, HeLa and PC3 cell. Cell metabolic activity was determined by a MTT assay.

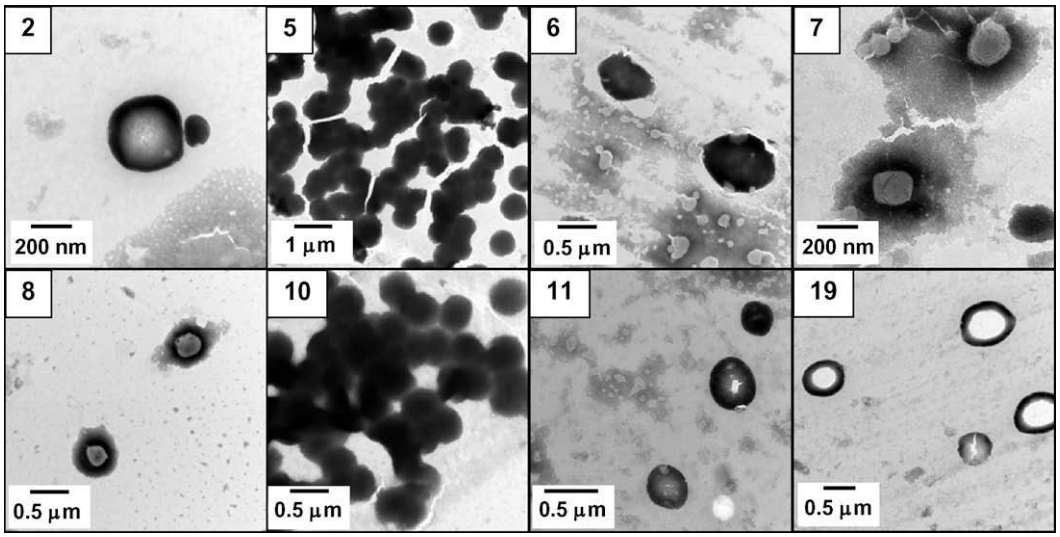


Figure 11. Transmission electron microscopic images of cationic liposomes **2**, **5**–**8**, **10**, **11** and **19**.

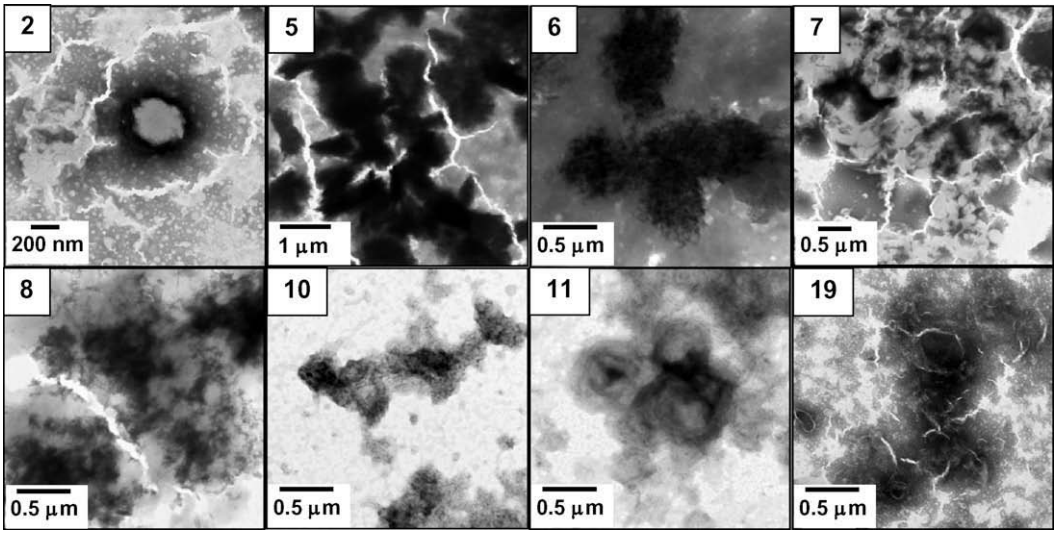


Figure 12. Transmission electron microscopic images of lipoplexes prepared from optimized liposomes (**2**, **5**–**8**, **10**, **11** and **19**)/DNA complexes.

transfection efficiency decreased. The optimal formulation (DOPE/lipid ratio, DNA/lipid ratio, amount of lipid) of each lipid for highest transfection efficiency was dependent on each compound. Seven cationic lipids exhibited greater transfection efficiency than commercially available transfection agents, Effectene™, DOTAP and DC-Chol, against PC3 (human prostate adenocarcinoma) cells. We have found that cationic lipid containing amino and guanidinyl polar headgroup with equal chain length (lipid **5**) is the most efficient one. The guanidinyl group might contribute to high transfection efficiency. It has been known that guanidinyl group processes several interesting features especially the high pK_a nature of guanidinyl group.[31] This makes it remain protonated at a broad range of pH and transfection efficiency should be insensitive to variations of pH during in vitro transfection. The optimal conditions for lipid **5** to exhibit highest transfection efficiency against PC3 cells included lipid/DOPE at weight ratio of 1:1, DNA/liposome ratio of 1:40 and the amount of DNA 0.1 μg/well. Most importantly, this lipid was compatible for high serum condition make it promising non-viral transfection vector for further in vivo study.

3. Experimental

3.1. General

NMR spectra were recorded on a Bruker AVANCE 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. All coupling constants (*J* values) were measured in hertz. ES mass spectra were recorded with a Finnigan LCQ mass spectrometer. Infra-red spectra were recorded on a Perkin–Elmer Spectrum GX60237. The size and morphology of the cationic liposomes were recorded on JEM-2100, JEOL electron microscope. Starting materials and reagents were purchased from commercial suppliers and used without further purification.

3.2. Solid phase synthesis of transfection agent

3.2.1. Synthesis of lipids library 1

To the active carbonate resin **25**[25] (1 equiv, 1.1 mmol/g) was added an excess of diethylenetriamine or bis(3-aminopropyl)amine in CH_2Cl_2 (10 mL). The suspension was shaken for 6 h. The resin was filtered and washed successively with CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 (3 × 10 mL each). The resulting resin **26** was dried under vacuum for 2 h and gave a positive ninhydrin test.[32] The solution of Dde-OH (excess) in CH_2Cl_2/DMF was added to the resin **26**. The reaction was shaken for 12 h. The resulting resin was filtered and washed successively with CH_2Cl_2, MeOH, DMF, MeOH and CH_2Cl_2 (3 × 10 mL each) to afford the negative ninhydrin test resin. After that, the resin was dried under vacuum for 2 h and reacted with cholesteryl chloroformate (4 equiv) in CH_2Cl_2 using pyridine (2 mL) as a base for 12 h. The resulting resin was filtered and washed successively with CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 (3 × 10 mL each) to give the desired resin **27**. The Dde-protecting group was removed with 5% N_2H_4 in DMF for 2 × 30 min to give free amino resin **28**. The resin **28** gave a positive ninhydrin test. The resin was washed successively with CH_2Cl_2 and MeOH (3 × 10 mL each) before cleavage at the last step. The dried resin **28** was treated with 50% TFA/CH_2Cl_2 for 2 h. The resin was filtered and the solution was collected. The solvents were removed under a stream of nitrogen and evaporated under reduced pressure to give the desired product **1** and **2**.

3.2.1.1. 3β-[*N,N*-(2,2′-Diaminoethyl)carbamoyl]cholesterol (1). Yield: (resin: 1.1 mmol/g, 214.5 mg) 45.8 mg, 38%; IR (CH_2Cl_2): ν_{max} 3436, 2918, 2849, 1773, 1682, 1541, 1507, 1463, 1381, 1202, 1021 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 9 drops of CD_3OD): δ 0.53 (s, 3H, CH_3-18), 0.765 and 0.769 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.80 (d, J = 6.4 Hz, 3H, CH_3-21), 0.91 (s, 3H, CH_3-19), 0.91–2.26 (m, 30H, protons in cholesteryl skeleton), 3.05 (m, 4H, ($H_2NCH_2CH_2$)$_2$N), 3.38 (m, 4H, ($H_2NCH_2CH_2$)$_2$N), 3.48 (br s, 1H, H-3-Chol), 5.26 (br s, 1H, H-6-Chol), 8.00–8.4 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 9 drops of CD_3OD, 100 MHz): 11.6, 18.5, 18.9, 20.8, 22.3, 22.5, 23.7, 24.1, 27.6, 27.8, 28.0, 31.6, 31.7, 35.6, 35.8, 36.0, 36.3, 38.0, 39.3, 39.5, 42.1, 49.8, 56.0, 56.5, 76.7, 122.7, 139.4 (carbons in cholesteryl skeleton), 38.5 (($H_2NCH_2CH_2$)$_2$N), 46.7 (($H_2NCH_2CH_2$)$_2$N), 156.9 (C=O carbamoyl); MS (ES$^+$): *m/z*: 516 ([*M*+H]$^+$, 100%).

3.2.1.2. 3β-[*N,N*-(3,3′-Diaminopropyl)carbamoyl]cholesterol (2). Yield: (resin: 1.1 mmol/g, 123.9 mg) 52.4 mg, 70%; IR (CH_2Cl_2): ν_{max} 3355, 2918, 2845, 2280, 1675, 1600, 1509, 1468, 1429, 1202 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.811 and 0.815 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.2 Hz, 3H, CH_3-21), 0.90 (s, 3H, CH_3-19), 0.90–2.28 (m, 32H, protons in cholesteryl skeleton and ($H_2NCH_2CH_2CH_2$)$_2$N), 2.88 (br s, 4H, (H_2NCH_2 CH_2CH_2)$_2$N), 3.26 (br s, 4H, ($H_2NCH_2CH_2CH_2$)$_2$N), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s,

1H, H-6-Chol), 7.94 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.9, 24.2, 27.9, 28.1, 31.7, 35.8, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.2, 56.6, 75.8, 122.6, 139.4 (carbons in cholesteryl skeleton), 25.7 and 26.4 (($H_2NCH_2CH_2CH_2)_2N$), 27.2 and 38.4 (($H_2NCH_2CH_2$-$CH_2)_2N$), 44.2 and 44.6 (($H_2NCH_2CH_2CH_2)_2N$), 156.5 (C=O carbamoyl); MS (ES^+): *m/z* 544.7 ($[M+H]^+$, 100%).

3.2.2. Synthesis of lipids 3 and 4

To the free amino resin **28** (1 equiv, 148.3 and 139.3 mg, 1.1 mmol/g) was added methyl iodide (10 equiv, 83 and 78 μL) and DIEA (10 equiv, 279 and 261 μL) in DMF (2 mL). The suspension was shaken for 18 h. The resin was washed successively with CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 (3 × 2 mL each) to afford resin **29**. The resin **29** was cleaved in the same manner of the synthesis of lipids **1** and **2** to give lipids **3** and **4**.

3.2.2.1. 3β-[*N*-((*N′*,*N′*,*N′*-Trimethyl)-2′-aminoethyl)-*N*-(2-aminoethyl)carbamoyl]cholesterol (3). Yield: (resin: 1.1 mmol/g, 148.3 mg) 48.0 mg, 53%; IR (CH_2Cl_2): ν_{max} 3436, 2932, 2840, 1681, 1469, 1426, 1202, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.788 and 0.792 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.84 (d, J = 6.4 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.96–2.30 (m, 30H; protons in cholesteryl skeleton), 3.06 (s, 9H, ($CH_3)_3N^+$), 3.10 (br s, 2H, $H_2NCH_2CH_2N$), 3.48–3.77 (m, 6H, $CH_2CH_2NCH_2$-$CH_2N(CH_3)_3$), 4.40 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 8.00–8.30 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.6, 18.5, 19.0, 20.9, 22.3, 22.6, 23.7, 24.1, 27.8, 28.0, 31.8, 35.6, 36.0, 36.4, 36.7, 39.3, 39.5, 42.1, 49.9, 56.0, 56.5, 76.6, 122.8, 139.1 (carbons in cholesteryl skeleton), 38.1 and 38.4 ($CH_2CH_2NCH_2CH_2$), 45.0 and 46.0 ($CH_2CH_2NCH_2CH_2$), 53.6 and 53.7 ($N(CH_3)_3$), 155.7 (C=O carbamoyl); MS (ES^+): *m/z* 559.4 ($[M+H]^+$, 100%).

3.2.2.2. 3β-[*N*-((*N′*,*N′*,*N′*-Trimethyl)-3′-aminopropyl)-*N*-(3-aminopropyl)carbamoyl]cholesterol (4). Yield: (resin: 1.1 mmol/g, 139.3 mg) 57.4 mg, 64%; IR (CH_2Cl_2): ν_{max} 3436, 2931, 1677, 1468, 1423, 1379, 1202, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.797 and 0.801 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.85 (d, J = 6.3 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.94–2.28 (m, 32H, protons in cholesteryl skeleton, $CH_2CH_2CH_2NCH_2CH_2CH_2$), 2.89 (br s, 4H, $H_2NCH_2CH_2CH_2NCH_2CH_2CH_2N(CH_3)_3$), 3.02–3.03 (br s, 9H, $N(CH_3)_3^+$), 3.27 (br s, 4H, CH_2NCH_2), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 7.98 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.8, 122.6, 139.4 (carbons in cholesteryl skeleton), 25.7 and 26.4 ($CH_2CH_2NCH_2CH_2$), 37.0 ($H_2NCH_2CH_2CH_2N$), 38.4 ($CH_2CH_2CH_2N(CH_3)_3^+$), 44.3 ($NCH_2CH_2CH_2N(CH_3)_3$), 44.5 (H_2NCH_2 CH_2CH_2N), 53.1 ($N(CH_3)_3$), 156.1 (C=O carbamoyl); MS (ES^+): *m/z* 587.1 ($[M+H]^+$, 100%).

3.2.3. Synthesis of lipids 5 and 6

The resin **28** (1 equiv, 148.4 and 145.3 mg, 1.1 mmol/g) was added a solution of *N,N′*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (4 equiv, 190 and 186 mg) and DIEA (4 equiv, 111 and 109 μL) in DMF (2 mL). The suspension was shaken for 18 h to give resin **30**. The resin was successively washed with CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 (3 × 2 mL each). The resin **30** was cleaved in the same manner for the lipids **1**–**2**.

3.2.3.1. 3β-[*N*-((*N′*-Guanidinyl)-2′-aminoethyl)-*N*-(2-aminoethyl)-carbamoyl]cholesterol (5). Yield: (resin: 1.1 mmol/g, 148.4 mg) 42.1 mg, 46%; IR (CH_2Cl_2): ν_{max} 3355, 3182, 2934, 2278, 1779, 1674, 1509, 1468, 1432, 1376, 1203, 1138, 1018 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.821 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 5.7 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 1.00–2.30 (m, 30H, protons in cholesteryl skeleton), 3.07 (br s, 2H, $H_2NCH_2CH_2N$), 3.29 and 3.36 (br s, 4H, $H_2NCH_2CH_2NCH_2CH_2$), 3.51 (br s, 2H, NCH_2CH_2NHC-$(NH)NH_2$)), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H; H-6-Chol), 8.01 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.7, 23.7, 24.2, 27.6, 27.9, 28.1, 31.7, 31.8, 35.7, 36.1, 36.4, 36.8, 38.0, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.6, 122.7, 139.3 (carbons in cholesteryl skeleton), 38.5 ($CH_2CH_2NCH_2CH_2$), 47.4 (CH_2NCH_2), 156.9 (C=O carbamoyl and C=N guanidine); MS (ES^+): *m/z* 558.6 ($[M+H]^+$, 100%).

3.2.3.2. 3β-[*N*-((*N′*-Guanidinyl)-3′-aminopropyl)-*N*-(3-aminoproyl)carbamoyl]cholesterol (6). Yield: (resin: 1.1 mmol/g, 145.3 mg) 52.4 mg, 56%; IR (CH_2Cl_2): ν_{max} 3350, 2918, 1673, 1509, 1465, 1429, 1203, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.805 and 0.809 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.3 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 1.00–2.28 (m, 32H, protons in cholesteryl skeleton and $CH_2CH_2NCH_2CH_2$), 2.87 (br s, 2H, $H_2NCH_2CH_2CH_2N$), 3.08 (br s, 2H, $CH_2CH_2NHC(NH)NH_2$), 3.19 (br s, 2H, $H_2NCH_2CH_2CH_2N$), 3.25 (br s, 2H, NCH_2CH_2 CH_2N $HC(NH)NH_2$), 4.38 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.97 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.8, 24.1, 27.9, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.5, 76.0, 122.6, 139.4 (carbons in cholesteryl skeleton), 25.7 and 26.4 ($CH_2CH_2NCH_2CH_2$), 37.3 ($H_2NCH_2CH_2CH_2N$), 38.5 (NCH_2CH_2 CH_2N $HC(NH)NH_2$), 44.1 ($NCH_2CH_2CH_2NHC(NH)NH_2$), 44.6 (H_2NCH_2 CH_2CH_2N), 157.2 (C=O carbamoyl and C=N guanidine); MS (ES^+): *m/z* 586.7 ($[M+H]^+$, 100%).

3.2.4. Synthesis of lipids 7 and 8

The resin **28** (1 equiv, 152.8 and 159.3 mg, 1.1 mmol/g) was added 2-bromoethanol (8 equiv, 95 and 99 μL) and DIEA (8 equiv, 230 and 240 μL) in DMF (2 mL) and the suspension was shaken for 18 h. The resulting resin **31** was successively washed with CH_2Cl_2, DMF, MeOH, DMF and CH_2Cl_2 (3 × 2 mL each). The resin **31** was cleaved in the same manner of the lipids **1** and **2** to give lipids **7** and **8**.

3.2.4.1. 3β-[*N*-(*N′*,*N′*-Di(2″-hydroxyethyl)-2′-aminoethyl)-*N*-(2-aminoethyl)carbamoyl]cholesterol (7). Yield: (resin: 1.1 mmol/g, 152.8 mg) 41.5 mg, 41%; IR (CH_2Cl_2): ν_{max} 3335, 2932, 2840, 2279, 1674, 1597, 1541, 1509, 1413, 1310, 1203, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.809 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 6.2 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.90–2.28 (m, 30H, protons in cholesteryl skeleton), 3.12 (br s, 4H, CH_2 $CH_2NCH_2CH_2$), 3.36 (br s, 4H, CH_2NCH_2), 3.54 (br s, 4H, $N(CH_2$ $CH_2OH)_2$), 3.88 (br s, 4H, $N(CH_2CH_2OH)_2$), 4.37 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 8.12 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.6, 23.8, 24.1, 27.7, 27.9, 28.1, 31.7, 31.8, 35.7, 36.1, 36.4, 36.8, 38.0, 39.4, 39.6, 42.2, 49.9, 56.60, 56.69, 76.6, 122.9, 139.2 (carbons in cholesteryl skeleton), 38.5 ($CH_2CH_2NCH_2CH_2$), 46.9 (CH_2CH_2 NCH_2 CH_2), 55.5, 55.8, 56.1 ($N(CH_2CH_2OH)_2$), 157.0 (C=O carbamoyl); MS (ES^+): *m/z* 604.4 ($[M+H]^+$, 100%).

3.2.4.2. 3β-[*N*-(*N′*,*N′*-Di(2″-hydroxyethyl)-3′-aminopropyl)-*N*-(3-aminopropyl)carbamoyl]cholesterol (8). Yield: (resin: 1.1 mmol/g, 159.3 mg) 64.1 mg, 58%; IR (CH_2Cl_2): ν_{max} 3383, 2932, 2840, 1676, 1468, 1432, 1202, 1132 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 7 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.80 (d, J = 6.5 Hz, 6H, CH_3-26

and CH_3-27), 0.85 (d, J = 6.3 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95–2.28 (m, 32H, protons in cholesteryl skeleton and CH_2CH_2N-CH_2CH_2), 2.88 (br s, 4H, H_2NCH_2 $CH_2CH_2NCH_2CH_2CH_2N$), 3.26 (br s, 8H, $CH_2NCH_2CH_2CH_2N$ ($CH_2CH_2OH)_2$), 3.84 (br s, 4H, $N(CH_2CH_2OH)_2$), 4.37 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.92 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 7 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.6, 23.8, 24.2, 27.9, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 50.0, 56.1, 56.6, 75.9, 122.6, 139.5 (carbons in cholesteryl skeleton), 25.7 ($H_2NCH_2CH_2CH_2N$), 26.4 ($CH_2CH_2CH_2$ $N(CH_2$ $CH_2OH)_2$), 37.1 ($H_2NCH_2CH_2CH_2N$), 38.3 ($CH_2CH_2CH_2$ $N(CH_2$ $CH_2OH)_2$), 44.3 ($CH_2CH_2CH_2N(CH_2CH_2OH)_2$), 44.5 ($H_2NCH_2CH_2$ CH_2N), 55.2, 55.3, 55.4 ($N(CH_2CH_2OH)_2$), 156.3 (C═O carbamoyl); MS (ES^+): m/z 632.8 ($[M+H]^+$, 100%).

3.2.5. Synthesis of lipids library 2

Active carbonate resin **25** (1 equiv) was added excess 1,2-diamonoethane or 1,3-diaminopropane in CH_2Cl_2. The suspension was shaken overnight. The resulting resin was washed with CH_2Cl_2, DMF and CH_2Cl_2 (three times each) to provide the corresponding resin **32**. Amino resin **32** (1 equiv, 1.1 mmol/g) was reacted with a solution of bromoacetic acid (4 equiv) and DIC in DMF (10 mL). The suspension was then shaken for 12 h. The resulting resin was filtered and washed successively with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, DMF and CH_2Cl_2 (3 × 10 mL each) to give the desired resin **33**. The excess of 1,2-diaminoethane or 1,3-diaminopropane in DMF was reacted with the resin **33** for 12 h to give the desired resin **34** after successively washed with DMF, CH_2Cl_2, MeOH, DMF and CH_2Cl_2 (3 × 10 mL each). The resin **34** was dried under vacuum for 2 h. The resin **34** was reacted with Dde-OH (excess) in CH_2Cl_2 for 12 h. The resin was filtered and washed successively with CH_2Cl_2 and MeOH (3 × 10 mL each) and dried under reduced pressure to give the desired resin **35**. A solution of cholesteryl chloroformate (4 equiv) in CH_2Cl_2 was added to the resin **35** and pyridine (2 mL) added in the reaction mixture. The suspension was shaken overnight. The resulting resin was filtered and washed successively with MeOH and CH_2Cl_2 (3 × 10 mL each) to afford the desired resin **36**. The Dde protecting groups was removed by treating resin **36** with 5% N_2H_4 in DMF (2 × 30 min). The resin was washed successively with DMF, CH_2Cl_2, MeOH, DMF and CH_2Cl_2 (3 × 10 mL each) to give the resin **37**.

Resin **37** was treated with 50% TFA in CH_2Cl_2 for 2 h to give symmetric polar head lipids **9–12**. The asymmetric cationic head lipids **13–16**, **17–20** and **21–24** were prepared from resin **37** using the same manner as the synthesis of lipids **3–4**, **5–6** and **7–8**, respectively.

3.2.5.1. 3β-[*N*-(2′-Aminoethyl)-*N*-(*N*-glycine(*N*-(2-aminoethyl) amide))carbamoyl]cholesterol (9). Yield: (resin: 1.1 mmol/g, 177.0 mg) 46.3 mg, 42%; IR (CH_2Cl_2): ν_{max} 3346, 2918, 2279, 1675, 1597, 1509, 1413, 1310, 1203, 1124, 1015 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.794 and 0.797 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.84 (d, J = 6.2 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 0.92–2.27 (m, 28H, protons in cholesteryl skeleton), 3.04 (br s, 2H, $H_2NCH_2CH_2NHCO$), 3.11 (br s, 2H, $NHCOCH_2NCH_2CH_2NH_2$), 3.45 (br s, 2H, $H_2NCH_2CH_2NHCOCH_2N$), 3.54 (br s, 2H, $NHCOCH_2NCH_2CH_2NH_2$), 3.93 (br s, 2H, $NHCOCH_2N$), 4.37 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 8.05–8.40 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.7, 27.8, 28.1, 31.7, 35.7, 36.0, 36.4, 36.7, 38.1, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.9, 122.7, 139.2 (carbons in cholesteryl skeleton), 36.9 ($H_2NCH_2CH_2NHCO$), 38.1 ($NHCOCH_2NCH_2CH_2NH_2$), 39.1 ($H_2NCH_2CH_2NHCO$), 47.5 ($NHCOCH_2N$ $CH_2CH_2NH_2$), 51.0 ($NHCOCH_2N$), 156.0 (C═O carbamoyl), 173.0 (C═O amide); MS (ES^+): m/z 573.2 ($[M+H]^+$, 100%).

3.2.5.2. 3β-[*N*-(3′-Aminopropyl)-*N*-(*N*-glycine(*N*-(2-aminoethyl)-amide))carbamoyl]cholesterol (10). Yield: (resin: 1.1 mmol/g, 208.0 mg) 69.0 mg, 51%; IR (CH_2Cl_2): ν_{max} 3428, 2918, 2834, 1674, 1462, 1376, 1202, 1130, 1018 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.816 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 6.2 Hz, 3H, CH_3-21), 0.96 (s, 3H; CH_3-19), 0.96–2.27 (m, 30H, protons in cholesteryl skeleton and $NHCOCH_2NCH_2CH_2CH_2$), 3.08 (br s, 4H, $CH_2CH_2NHCOCH_2NCH_2$-CH_2CH_2), 3.47 (br s, 4H, $CH_2NHCOCH_2$ NCH_2), 3.84 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 8.01 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.7, 139.3 (carbons in cholesteryl skeleton), 30.0 ($NHCOCH_2NCH_2CH_2$), 37.0 ($H_2NCH_2CH_2NHCO$), 38.2 ($NHCOCH_2N$-$CH_2CH_2CH_2$), 39.1 ($H_2NCH_2CH_2NHCO$), 45.8 ($NHCOCH_2NCH_2CH_2$), 52.2 ($NHCOCH_2N$), 156.0 (C═O carbamoyl), 173.0 (C═O amide); MS (ES^+): m/z 587.5 ($[M+H]^+$, 100%).

3.2.5.3. 3β-[*N*-(2′-Aminoethyl)-*N*-(*N*-glycine(*N*-(3-aminopropyl)-amide))carbamoyl]cholesterol (11). Yield: (resin: 1.1 mmol/g, 142.0 mg) 37.9 mg, 42%; IR (CH_2Cl_2): ν_{max} 3338, 2918, 2851, 1676, 1594, 1535, 1460, 1202, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.81 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 4.6 Hz, 3H, CH_3-21), 0.97 (s, 3H, CH_3-19), 0.97–2.90 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2NHCO$), 2.94 (br s, 2H, $H_2NCH_2CH_2CH_2NHCO$), 3.05 (br s, 2H, $NHCOCH_2NCH_2CH_2NH_2$), 3.29 (br s, 2H, $H_2NCH_2CH_2$-CH_2NHCO), 3.58 (br s, 2H, $NHCOCH_2NCH_2CH_2NH_2$), 3.93 (br s, 2H, $NHCOCH_2N$), 4.38 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.94 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.8, 35.7, 36.1, 36.4, 36.8, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.7, 139.4 (carbons in cholesteryl skeleton), 26.7 (H_2NCH_2-CH_2CH_2NHCO), 38.3 (H_2NCH_2 $CH_2CH_2NHCOCH_2NCH_2CH_2NH_2$), 52.4 ($NHCOCH_2N$), 156.5 (C═O carbamoyl), 172.5 (C═O amide); MS (ES^+): m/z 587.3 ($[M+H]^+$, 100%).

3.2.5.4. 3β-[*N*-(3′-Aminopropyl)-*N*-(*N*-glycine(*N*-(3-aminopropyl)-amide))carbamoyl]cholesterol (12). Yield: (resin: 1.1 mmol/g, 261.3 mg) 70.9 mg, 41%; IR (CH_2Cl_2): ν_{max} 3299, 3064, 2935, 1682, 1541, 1468, 1379, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.813 (d, J = 6.4 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 6.0 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95–2.30 (m, 32H, protons in cholesteryl skeleton and $CH_2CH_2CH_2NHCOCH_2NCH_2CH_2CH_2$), 2.91 (br s, 4H, $H_2NCH_2CH_2CH_2NHCOCH_2NCH_2CH_2CH_2NH_2$), 3.27 (br s, 4H, CH_2N-$HCOCH_2NCH_2CH_2$), 3.83 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 7.96 (br s, 6H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.1, 31.8, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.9, 122.6, 139.4 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2NHCO$ and CH_2NCH_2-$CH_2CH_2NH_2$), 37.1 ($H_2NCH_2CH_2CH_2NHCO$), 38.4 ($H_2NCH_2CH_2CH_2$-$NHCO$), 47.5 ($CH_2NCH_2CH_2CH_2NH_2$), 50.5 ($NHCOCH_2N$), 156.5 (C═O carbamoyl), 171.6 (C═O amide); MS (ES^+): m/z 601.5 ($[M+H]^+$, 100%).

3.2.5.5. 3β-[*N*-((*N*′,*N*′,*N*′-Trimethyl)-2′-aminoethyl)-*N*-(*N*-glycine (*N*-(2-aminoethyl)amide))carbamoyl] cholesterol (13). Yield: (resin: 1.1 mmol/g, 192.0 mg) 76.1 mg, 59%; IR (CH_2Cl_2): ν_{max} 3436, 2934, 1678, 1541, 1468, 1378, 1202, 1131, 1023 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 10 drops of CD_3OD): δ 0.56 (s, 3H, CH_3-18), 0.749 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.80 (d, J = 6.2 Hz, 3H, CH_3-21), 0.88 (s, 3H, CH_3-19), 0.88–2.20 (m, 28H, protons in cholesteryl skeleton), 3.02–3.08 (m, 11H, $H_2NCH_2CH_2$

and $CH_2CH_2N(CH_3)_3$), 3.40 (br s, 2H, $CH_2N(CH_3)_3$), 3.50 (br s, 2H, $H_2NCH_2CH_2$), 3.64 (br s, 2H, $CH_2CH_2N(CH_3)_3$), 3.97 (br s, 2H, $NHCOCH_2$), 4.36 (br s, 1H, H-3-Chol), 5.21 (br s, 1H, H-6-Chol), 7.90–8.10 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 10 drops of CD_3OD, 100 MHz): 11.6, 18.4, 18.9, 20.3, 22.2, 22.5, 23.6, 24.0, 27.7, 27.8, 28.0, 31.6, 35.6, 35.9, 36.3, 36.4, 38.2, 39.3, 39.5, 42.1, 49.8, 55.9, 56.5, 76.5, 122.7, 139.1 (carbons in cholesteryl skeleton), 36.7 ($H_2NCH_2CH_2$), 38.0 ($CH_2N(CH_3)_3$), 39.1 ($H_2NCH_2CH_2$), 42.8 ($CH_2CH_2N(CH_3)_3$), 50.4 ($NHCOCH_2$), 53.4 and 53.5 ($CH_2CH_2N(CH_3)_3$), 156.1 (C=O carbamoyl), 170.6 (C=O amide); MS (ES^+): *m/z* 615.8 ($[M+H]^+$, 100%).

3.2.5.6. 3β-[*N*-((*N*′,*N*′,*N*′-Trimethyl)-3′-aminopropyl)-*N*-(*N*-glycine-(*N*-(2-aminoethyl)amide))carbamoyl] cholesterol (14). Yield: (resin: 1.1 mmol/g, 173.1 mg) 62.7 mg, 52%; IR (CH_2Cl_2): ν_{max} 3334, 3042, 2930, 2851, 2279, 1677, 1594, 1541, 1462, 1203, 1132 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.795 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.84 (d, J = 5.3 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.95–2.27 (m, 30H, protons in cholesteryl skeleton and $CH_2CH_2CH_2N(CH_3)_3$), 3.03 and 3.07 (s, 11H, $H_2NCH_2CH_2NHCO$ and $N(CH_3)_3$, partially overlapping signal), 3.17 (br s, 2H, $CH_2N(CH_3)_3$), 3.44 (br s, 4H, $H_2NCH_2CH_2NHCO$ and $NCH_2CH_2CH_2N(CH_3)_3$), 3.91 (br s, 2H, $NHCOCH_2N$), 4.38 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 8.02 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 6 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 27.9, 28.1, 31.7, 35.6, 36.0, 36.4, 36.7, 38.2, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.6, 122.7, 139.1 (carbons in cholesteryl skeleton), 30.0 ($CH_2CH_2N(CH_3)_3$), 37.0 ($H_2NCH_2CH_2NHCO$), 38.2 (CH_2 $N(CH_3)_3$), 39.2 ($H_2NCH_2CH_2NHCO$), 45.5 (NCH_2CH_2 $CH_2N(CH_3)_3$), 52.3 ($NHCOCH_2N$), 53.3 ($N(CH_3)_3$), 156.1 (C=O carbamoyl), 170.6 (C=O amide); MS (ES^+): *m/z* 630.8 ($[M+H]^+$, 100%).

3.2.5.7. 3β-[*N*-((*N*′,*N*′,*N*′-Trimethyl)-2′-aminoethyl)-*N*-(*N*-glycine (*N*-(3-aminopropyl)amide))carbamoyl] cholesterol (15). Yield: (resin: 1.1 mmol/g, 177.8 mg) 48.4 mg, 39%; IR (CH_2Cl_2): ν_{max} 3333, 2918, 2840, 1678, 1538, 1509, 1456, 1202, 1121 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.809 (d, J = 5.8 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 4.8 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95–2.80 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2NHCO$), 2.83 (br s, 2H, $H_2NCH_2CH_2CH_2NHCO$), 2.93, 3.04 and 3.10 (br s, 9H, $N(CH_3)_3$), 3.27 (br s, 2H, $CH_2CH_2N(CH_3)_3$), 3.58 (br s, 2H, CH_2NHCO), 3.70 (br s, 2H, $CH_2CH_2N(CH_3)_3$), 4.01 (br s, 2H, $NHCOCH_2N$), 7.99 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.5, 122.7, 139.3 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2NHCO$), 38.3 ($H_2NCH_2CH_2CH_2NHCO$ and $NCH_2CH_2N(CH_3)_3$), 52.4 ($NHCOCH_2N$), 53.3 and 53.5 ($N(CH_3)_3$), 156.1 (C=O carbamoyl), 170.6 (C=O amide); MS (ES^+): *m/z* 629.6 ($[M+H]^+$, 100%).

3.2.5.8. 3β-[*N*-((*N*′,*N*′,*N*′-Trimethyl)-3′-aminopropyl)-*N*-(*N*-glycine-(*N*-(3-aminopropyl)amide))carbamoyl] cholesterol (16). Yield: (resin: 1.1 mmol/g, 186.1 mg) 61.8 mg, 47%; IR (CH_2Cl_2): ν_{max} 3313, 3042, 2934, 2274, 1678, 1541, 1468, 1202, 1133 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.80 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.85 (d, J = 6.1 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.98–2.30 (m, 32H, protons in cholesteryl skeleton, $H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2N(CH_3)_3$), 2.90 (br s, 4H, $H_2NCH_2CH_2CH_2$ and CH_2CH_2 $CH_2N(CH_3)_3$), 3.02, 3.07 and 3.10 (s, 9H, $N(CH_3)_3$, partially overlapping), 3.83 (br s, 4H, CH_2NHCO and $CH_2CH_2CH_2N(CH_3)_3$), 3.90 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 7.96 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.6, 23.7, 24.1, 27.9, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.9, 122.6, 139.4 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2N(CH_3)_3$), 37.1 ($H_2NCH_2CH_2CH_2$), 38.3 ($CH_2N(CH_3)_3$), 38.4 ($H_2NCH_2CH_2$), 47.5 ($CH_2CH_2CH_2N(CH_3)_3$), 50.9 ($NHCOCH_2N$), 53.2 ($N(CH_3)_3$), 156.4 (C=O carbamoyl), 171.5 (C=O amide); MS (ES^+): *m/z* 644.6 ($[M+H]^+$, 100%).

3.2.5.9. 3β-[*N*-(*N*′-Guanidinyl-2′-aminoethyl)-*N*-(*N*-glycine(*N*-(2-aminoethyl)amide))carbamoyl]cholesterol (17). Yield: (resin: 1.1 mmol/g, 159.5 mg) 50.5 mg, 47%; IR (CH_2Cl_2): ν_{max} 3347, 2920, 2850, 2279, 1672, 1597, 1509, 1465, 1311, 1203, 1132 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.796 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27), 0.84 (d, J = 5.8 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 0.93–2.28 (m, 28H, protons in cholesteryl skeleton), 3.02 (br s, 2H, $H_2NCH_2CH_2$), 3.30, 3.35 and 3.43 (br s, 6H, $H_2NCH_2CH_2NHCOCH_2NHCH_2CH_2NHC(NH)NH_2$), 3.93 (br s, 2H, $NHCOCH_2N$), 4.35 (br s, 1H, H-3-Chol), 5.27 (br s, 1H, H-6-Chol), 8.05 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.0, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 28.1, 31.7, 35.7, 36.0, 36.4, 36.7, 38.1, 39.4, 39.6, 42.2, 49.9, 56.0, 56.5, 76.9, 123.6, 139.3 (carbons in cholesteryl skeleton), 36.9 ($H_2NCH_2CH_2$), 38.5 ($H_2NCH_2CH_2$), 47.5 ($CH_2CH_2NHC(NH)NH_2$), 51.0 (CH_2CH_2NHC $(NH)NH_2$), 156.5 (C=O carbamoyl and C=N guanidine, overlapping), 173.0 (C=O amide); MS (ES^+): *m/z* 615.4 ($[M+H]^+$, 100%).

3.2.5.10. 3β-[*N*-(*N*′-Guanidinyl-3′-aminopropyl)-*N*-(*N*-glycine(*N*-(2-aminoethyl)amide)) carbamoyl]cholesterol (18). Yield: (resin: 1.1 mmol/g, 173.4 mg) 51.4 mg, 43%; IR (CH_2Cl_2): ν_{max} 3327, 3070, 2918, 2834, 1674, 1574, 1467, 1432, 1202, 1134 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.82 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.87 (d, J = 4.4 Hz, 3H, CH_3-21), 0.98 (s, 3H, CH_3-19), 0.98–2.29 (m, 30H, protons in cholesteryl skeleton and CH_2CH_2 $CH_2NHC(NH)NH_2$), 3.08 (br s, 2H, $H_2NCH_2CH_2$), 3.20 and 3.30 (br s, 4H, $H_2NCH_2CH_2$-NHCO and $CH_2CH_2CH_2NHC(NH)NH_2$), 3.47 (br s, 2H, $CH_2CH_2CH_2NHC(NH)NH_2$), 3.84 (br s, 2H, $NHCOCH_2N$), 4.40 (br s, 1H, H-3-Chol), 5.29 (br s, 1H, H-6-Chol), 8.04 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 3 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.2, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.7, 139.4 (carbons in cholesteryl skeleton), 30.0 (CH_2CH_2 $CH_2NHC(NH)NH_2$), 37.0 ($H_2NCH_2CH_2$-NHCO), 38.2 (CH_2CH_2 CH_2N $HC(NH)NH_2$), 39.1 ($H_2NCH_2CH_2NHCO$), 46.0 (CH_2CH_2 CH_2NHC $(NH)NH_2$)), 52.5 ($NHCOCH_2N$), 156.5 (C=O carbamoyl and C=N guanidine, overlapping), 173.0 (C=O amide); MS (ES^+): *m/z* 629.4 ($[M+H]^+$, 100%).

3.2.5.11. 3β-[*N*-(*N*′-Guanidinyl-2′-aminoethyl)-*N*-(*N*-glycine(*N*-(3-aminopropyl)amide)) carbamoyl]cholesterol (19). Yield: (resin: 1.1 mmol/g, 161.7 mg) 36.3 mg, 33%; IR (CH_2Cl_2): ν_{max} 3327, 2918, 1673, 1535, 1462, 1202, 1133 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.61 (s, 3H, CH_3-18), 0.805 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.85 (d, J = 5.0 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95–2.80 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2$), 2.92 (br s, 2H, $H_2NCH_2CH_2$), 3.26 and 3.33 (br s, 4H, $H_2NCH_2CH_2CH_2$ and $CH_2CH_2NHC(NH)NH_2$), 3.48 (br s, 2H, $CH_2CH_2NHC(NH)NH_2$), 3.92 (br s, 2H, $NHCOCH_2N$), 4.37 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 7.99 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.1, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.5, 76.2, 122.8, 139.2 (carbons in cholesteryl skeleton), 26.8 ($H_2NCH_2CH_2CH_2$), 38.2 ($H_2NCH_2CH_2CH_2$ and $CH_2CH_2NHC(NH)NH_2$)), 52.5 ($NHCO$ CH_2N), 156.1 (C=O carbamoyl and C=N guanidine, overlapping), 170.5 (C=O amide); MS (ES^+): *m/z* 629.5 ($[M+H]^+$, 100%).

3.2.5.12. 3β-[*N*-(*N*′-Guanidinyl-3′-aminopropyl)-*N*-(*N*-glycine (*N*-(3-aminopropyl)amide))carbamoyl]cholesterol (20). Yield: (resin: 1.1 mmol/g, 170.3 mg) 62.1 mg, 52%; IR (CH_2Cl_2): ν_{max} 3320, 2933, 1678, 1543, 1468, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.82 (d, J = 6.4 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 5.9 Hz, 3H, CH_3-21), 0.95 (s, 3H, CH_3-19), 0.95–2.30 (m, 32H, protons in cholesteryl skeleton, $H_2NCH_2CH_2CH_2$ and CH_2CH_2 $CH_2NHC(NH)NH_2$), 2.92 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.22 (br s, 2H, $CH_2NHC(NH)NH_2$), 3.28 (br s, 4H, $H_2NCH_2CH_2CH_2$ and CH_2CH_2 $CH_2NHC(NH)NH_2$), 3.83 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.91 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.2, 27.9, 28.1, 31.8, 35.7, 36.1, 36.4, 36.8, 38.3, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 75.9, 122.6, 139.4 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2$ and $CH_2CH_2CH_2NHC(NH)NH_2$), 37.1 (H_2NCH_2 CH_2CH_2), 38.3 $CH_2CH_2CH_2NHC(NH)NH_2$), 38.4 ($H_2NCH_2CH_2CH_2$), 47.5 ($CH_2CH_2CH_2NHC(NH)NH_2$), 50.5 (NHCO CH_2N), 156.5 (C=O carbamoyl and C=N guanidine, overlapping), 171.6 (C=O amide); MS (ES^+): m/z 643.3 ([M+H]$^+$, 100%).

3.2.5.13. 3β-[*N*-(*N*′,*N*′-Di(2″-hydroxyethyl)-2′-aminoethyl)-*N*-(*N*-glycine(*N*-(2-aminoethyl)amide))carbamoyl] cholesterol (21). Yield: (resin: 1.1 mmol/g, 185.7 mg) 71.8 mg, 53%; IR (CH_2Cl_2): ν_{max} 3334, 2933, 2279, 1678, 1541, 1509, 1432, 1203, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.818 and 0.821 (d, J = 6.5 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.1 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.93–2.32 (m, 28H, protons in cholesteryl skeleton), 3.10 (br s, 2H, $H_2NCH_2CH_2$), 3.37 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.51 (br s, 4H, $H_2NCH_2CH_2$ and $CH_2N(CH_2CH_2OH)_2$), 3.70 (br s, 2H, $CH_2CH_2N(CH_2CH_2OH)_2$), 3.87 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.99 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.31 (br s, 1H, H-6-Chol), 7.89 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.0, 20.9, 22.4, 22.7, 23.8, 24.2, 27.7, 27.9, 28.1, 31.7, 35.8, 36.1, 36.4, 36.8, 38.1, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.6, 122.4, 139.2 (carbons in cholesteryl skeleton), 37.2 ($H_2NCH_2CH_2$), 38.1 ($CH_2N(CH_2CH_2OH)_2$), 39.2 (H_2NCH_2 CH_2NHCO), 47.5 ($CH_2CH_2N(CH_2CH_2OH)_2$), 50.5 ($NHCOCH_2N$), 55.3, 55.5 and 55.8 ($N(CH_2CH_2OH)_2$), 156.2 (C=O carbamoyl), 173.1 (C=O amide); MS (ES^+): m/z 661.5 ([M+H]$^+$, 100%).

3.2.5.14. 3β-[*N*-(*N*′,*N*′-Di(2″-hydroxyethyl)-3′-aminopropyl)-*N*-(*N*-glycine(*N*-(2-aminoethyl)amide))carbamoyl] cholesterol (22). Yield: (resin: 1.1 mmol/g, 199.3 mg) 61.6 mg, 42%; IR (CH_2Cl_2): ν_{max} 3315, 2934, 2840, 2274, 1677, 1541, 1467, 1429, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.60 (s, 3H, CH_3-18), 0.792 (d, J = 5.6 Hz, 6H, CH_3-26 and CH_3-27), 0.85 (d, J = 5.3 Hz, 3H, CH_3-21), 0.94 (s, 3H, CH_3-19), 0.97–2.26 (m, 30H, protons in cholesteryl skeleton and CH_2CH_2 $CH_2N(CH_2CH_2$-$OH)_2$), 3.05 (br s, 2H, $H_2NCH_2CH_2$), 3.27 (m, 10H, $CH_2NHCOCH_2$-$NCH_2CH_2CH_2N(CH_2CH_2OH)_2$), 3.44 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.84 (br s, 2H, $NHCOCH_2N$), 4.38 (br s, 1H, H-3-Chol), 5.28 (br s, 1H, H-6-Chol), 8.02 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.5, 19.1, 20.9, 22.4, 22.6, 23.7, 24.1, 27.8, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.6, 76.1, 122.6, 139.4 (carbons in cholesteryl skeleton), 29.9 (CH_2-$CH_2CH_2N(CH_2CH_2OH)_2$), 37.0 (H_2NC H_2CH_2), 38.2 ($CH_2CH_2CH_2N$-$(CH_2CH_2OH)_2$), 39.1 ($H_2NCH_2CH_2$), 46.0 ($CH_2CH_2CH_2N(CH_2CH_2OH)_2$), 52.5 ($NHCOCH_2N$), 55.4 and 55.6 ($N(CH_2CH_2OH)_2$), 156.0 (C=O carbamoyl), 171.5 (C=O amide); MS (ES^+): m/z 675.7 ([M+H]$^+$, 100%).

3.2.5.15. 3β-[*N*-(*N*′,*N*′-Di(2″-hydroxyethyl)-2′-aminoethyl)-*N*-(*N*-glycine(*N*-(3-aminopropyl)amide))carbamoyl] cholesterol (23). Yield: (resin: 1.1 mmol/g, 177.0 mg) 41.9 mg, 32%; IR (CH_2Cl_2): ν_{max} 3389, 3081, 2933, 1677, 1467, 1202, 1133 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD): δ 0.62 (s, 3H, CH_3-18), 0.809 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27), 0.86 (d, J = 5.0 Hz, 3H, CH_3-21), 0.96 (s, 3H, CH_3-19), 0.96–2.28 (m, 30H, protons in cholesteryl skeleton and $H_2NCH_2CH_2CH_2$), 2.91 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.29 and 3.36 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.50 (br s, 2H, $CH_2CH_2N(CH_2CH_2OH)_2$), 3.75 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.85 (br s, 2H, $CH_2CH_2N(CH_2CH_2OH)_2$), 3.92 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.97 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.30 (br s, 1H, H-6-Chol), 7.89 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 5 drops of CD_3OD, 100 MHz): 11.7, 18.6, 19.1, 20.9, 22.4, 22.7, 23.8, 24.1, 27.9, 28.0, 28.1, 31.7, 35.7, 36.1, 36.4, 36.8, 38.2, 39.4, 39.6, 42.2, 49.9, 56.1, 56.5, 76.2, 122.8, 139.2 (carbons in cholesteryl skeleton), 26.8 ($H_2NCH_2CH_2CH_2$), 38.2 (H_2NCH_2 CH_2CH_2 and $CH_2CH_2N(CH_2CH_2OH)_2$), 52.5 ($NHCOCH_2N$), 55.1 and 55.3 ($N(CH_2CH_2OH)_2$), 156.1 (C=O carbamoyl), 170.5 (C=O amide); MS (ES^+): m/z 675.5 ([M+H]$^+$, 100%).

3.2.5.16. 3β-[*N*-(*N*′,*N*′-Di(2″-hydroxyethyl)-3′-aminopropyl)-*N*-(*N*-glycine(*N*-(3-aminopropyl)amide))carbamoyl]cholesterol (24). Yield: (resin: 1.1 mmol/g, 192.6 mg) 65.1 mg, 44%; IR (CH_2Cl_2): ν_{max} 3308, 3070, 2934, 1678, 1467, 1202, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 4 drops of CD_3OD): δ 0.63 (s, 3H, CH_3-18), 0.820 and 0.824 (d, J = 6.0 Hz, 6H, CH_3-26 and CH_3-27, overlapping signal), 0.86 (d, J = 6.1 Hz, 3H, CH_3-21), 0.93 (s, 3H, CH_3-19), 0.93–2.30 (m, 32H, protons in cholesteryl skeleton, $H_2NCH_2CH_2CH_2$ and $NCH_2CH_2CH_2N(CH_2CH_2OH)_2$), 2.93 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.10 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.29 (br s, 2H, $H_2NCH_2CH_2CH_2$), 3.57 (br s, 4H, $CH_2CH_2CH_2N(CH_2CH_2OH)_2$), 3.87 (br s, 4H, $N(CH_2CH_2OH)_2$), 3.99 (br s, 2H, $NHCOCH_2N$), 4.39 (br s, 1H, H-3-Chol), 5.31 (br s, 1H, H-6-Chol), 7.80 (br s, 3H, NH_3^+); ^{13}C NMR ($CDCl_3$ + 4 drops of CD_3OD, 100 MHz): 11.8, 18.6, 19.2, 21.0, 22.4, 22.7, 23.9, 24.2, 27.9, 28.1, 31.8, 35.8, 36.1, 36.5, 36.8, 38.3, 39.4, 39.7, 42.2, 49.9, 56.2, 56.6, 76.0, 122.6, 139.5 (carbons in cholesteryl skeleton), 26.9 ($H_2NCH_2CH_2CH_2$ and $NCH_2CH_2CH_2$ $N(CH_2CH_2OH)_2$), 37.3 ($H_2NCH_2CH_2CH_2$), 38.3 ($CH_2CH_2CH_2$ $N(CH_2CH_2OH)_2$), 38.4 (H_2NCH_2-CH_2CH_2), 50.5 ($NHCOCH_2N$), 55.4 ($N(CH_2CH_2OH)_2$), 156.5 (C=O carbamoyl), 171.5 (C=O amide); MS (ES^+): m/z 689.7 ([M+H]$^+$, 100%).

3.2.6. DNA binding affinities

The DNA binding ability of synthesized lipids was performed by gel retardation assay. The DNA/sample complexes 1/20 (w/w) were prepared by transferring 2.4 μL (10 μg/μL) of sample to an Eppendorf tube. Each sample was further diluted with 3 μL of PBS (phosphate-buffered saline) buffer. An aqueous solution of plasmid DNA (4 μL, 0.3 μg/μL) was added to each sample and the solutions were successively mixed by inverting several times. The DNA complexes were incubated at 25 °C for 30 min. Bromophenol blue-free gel-loading buffer (3 μL, 2 × 40% w/v sucrose in water) was added to the complexes. The solutions were mixed by inverting each tube and each sample (10 μL) was loaded onto a 1.0% agarose gel (0.5× TBE buffer). The gel was run at 135 V for 5 min and 50 V for 2 h at 400 mA. DNA bands were visualized by ethidium bromide staining.

3.2.7. Liposome preparation

Dioleoyl-L-α-phosphatidylethanolamine (DOPE) (Sigma) (1 μL, 50 μg/μL in CH_2Cl_2) and cationic lipid (2 μL, 50 μg/μL in abs. ethanol) were mixed (weight ratio 1:2). The organic solvents were evaporated under a stream of nitrogen and further dried under high vacuum (>2 h). The resulting thin film was hydrated with phosphate-buffer saline (PBS, pH 7.4, 100 μL) at room temperature for 1 h. The mixture was vortexed for one minute and sonicated (2 × 15 min) with 1 h rest between sonications using a bath-type sonicator. The liposomes were stored at 4 °C for 24 h prior to use. The liposome without DOPE was prepared as the same manner for DOPE-contained liposome excepted DOPE was not incubated.

3.2.8. Transfection procedure

Human embryonic kidney cells (HEK293), colorectal adenocarcinoma (COLO 205), canine osteosarcoma (D-17), human cervical adenocarcinoma (HeLa) and human prostate adenocarcinoma (PC3) were grown in DMEM medium supplemented with 10% fetal calf serum (FCS), penicillin (100 units/mL), streptomycin (100 mg/mL) and L-glutamine (4 mM) at 37 °C, 5% CO_2. For transfection, the cells were seeded up to 1×10^4 cells/well in a 96-well plate, to give 50–70% confluence to be used on the next day. The growth medium was removed and the cells were washed with PBS and replaced with 100 μL of fresh serum-free DMEM medium. DNA (pCH110-encoding β-galactosidase)/cationic liposome complexes (lipoplexes) were prepared as follows. An appropriate volume of each cationic liposome (1 μg/μL) was added to the plasmid DNA (0.4 μL, 0.5 μg/μL) and the complex was incubated at room temperature for 30 min before being diluted with phosphate-buffered saline to make a final DNA concentration of 0.1 mg/mL. The lipoplexes (10 μL) were then added to the cells and left to be incubated at 37 °C, 5% CO_2. The cells were then washed with PBS and fresh growth medium was added and further incubated for 48 h. For Effectene™ transfection, the method was carried out according to the manufacturer's instruction and the same ratios of plasmid DNA:Effectene™ were used. After transfection, the β-galactosidase activity per well was estimated by adding 100 μL of substrate solution (4 mg/mL of o-nitrophenyl-galactopyranoside (ONPG),[33] 0.2 M sodium phosphate (pH 7.3) and 2 mM magnesium chloride) to the lysate in a 96-well plate. Absorbance of the product *ortho*-nitrophenol at 405 nm was converted to% relative transfection by compared with positive control, Effectene™.

3.2.9. Transfection cytotoxicity

Cytotoxicities of the lipids **4**, **7**, **8**, **9**, **10**, **12**, **13** and **25** were assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay as described previously.[34] The experiment was performed in 96-well plates. The amount of cationic liposome and commercially available transfection agents, Effectene™, DOTAP and DC-Chol, per well was used the same as that used in the transfection experiments. After 24 h. incubation, the medium was removed and replaced with a phenol red-free medium (90 μL). MTT (3 mg/mL) was added (10 μL/well) to the cells, followed by MTT solubilization solution (Sigma) (100 μL) to dissolve the resulting crystals, and the absorbance was measured at 520 nm on a microplate reader. The change in metabolic activity was calculated as A_{520} with compound/A_{520} without compound.

3.2.10. Electron microscopy

The liposomes or their lipoplexes solution (10–20 μL) was dropped on the formvar grid and left it stands at room temperature for 1 min. The water was removed by touching the edge of the droplet to the edge of a filter paper, leaving the thin aqueous film on the grid. 1% Potassium phosphotungstate (PTA) (10 μL) was applied to the grid and left it stands for 1 min. The extra solution was similarly removed by touching to the edge of a filter paper. The negative stain liposomes were allowed to dry at room temperature for 1 night by placing onto a filter paper in a covered Petri dish. The samples were observed under transmission electron microscope operated at 200 kV.

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High Transfection Efficiency of Novel Cationic Lipids with Asymmetric Acyl-Cholesteryl Hydrophobic Tails

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Abstract

The ability of non-viral gene delivery system to overcome extracellular and intracellular barriers is a critical issue for the future clinical applications of gene therapy. In recent years much effort has been focused on the development of a variety of DNA carriers, and cationic liposomes have become the most common non-viral gene delivery system. One hundred and eighty novel cationic lipids with asymmetric acyl-cholesteryl hydrophobic tails were synthesized by parallel solid phase chemistry. The liposomes were prepared and gel retardation assay was used to study the binding efficiency between the prepared liposome and the DNA. Transfection efficiencies of the lipids were evaluated against various mammalian cells, HEK293, HeLa, D17, COLO 205 and PC3 cells. The lipids with acyl portion at the terminal part of the polyamine backbone exhibited high transfection efficiency than those with the acyl portion at the internal part of the backbone. These compounds also showed higher transfection efficiency and low cytotoxicity as compared with the commercially available agents, Effectene^{TM}, DOTAP and DC-Chol. The morphologies of liposomes and lipoplexes were observed under transmission electron microscopy (TEM).

Keywords: Cationic lipid; Liposome; Non-viral vector; DNA delivery; Asymmetric hydrophobic tail; Solid phase synthesis

Introduction

Over the past two decades, the progressive of gene delivery has been interested by many researchers. This approach is a new hope for treating of both genetic and acquired disease that offers promise for tomorrow's medicine.[1] In this process, the corrected exogeneous genes or portions of a gene are introduced into target cells. The major challenge of the use of gene therapy is to deliver the desired gene efficiently with the least toxicity and low immunogenicity. Viral vectors are the most efficient carrier to deliver DNA into cells.[1] Due to the toxicity, strong immunogenicity, and limitation to carry small DNA of this type of vectors, various researchers are trying to develop different kinds of non-viral vectors[2–6] for efficient gene delivery. Among these methods, cationic lipids have shown to be the most promise for in vivo applications, based on a combination of efficiency, stability and lack of toxicity. Since the first application of cationic lipids in DNA delivery,[7] numerous cationic lipids have been synthesized.[4]

While there are no absolute features for the structure of cationic lipids, cationic lipids are composed of three main parts, a cationic head, a hydrophobic tail and a linker that join hydrophilic head and hydrophobic tail. The polar head group is generally one or more cationic head consisting of guanidinium[8–10] or an amine[11–13] (primary, secondary, tertiary or quaternary). Linker represents any chemical part between hydrophilic head and hydrophobic tail; it is usually a biodegradable chemical bond[8–13] (ester, amide or carbamoyl) or a nondegradable ether bond.[14,15] Hydrophobic tail represents the nonpolar hydrocarbon which can be grouped into two categories, hydrocarbon and steroid. Most of the synthesized lipids contained double-chain hydrocarbon both saturated and unsaturated chains.[7,13,16] Cholesterol is the most frequently used as a steroidal tail, some of which bearing two cholesterol tails (Figure 1).[17,18] Cationic lipids with cholic acid and analogues as a hydrophobic tail have also been reported.[9]

In the present work, a library of 180 cationic lipids bearing the combination of various hydrocarbon chains and cholesterol as a hydrophobic tail were synthesized and evaluated for their transfection efficiency (Figure 2). Combinatorial solid phase synthesis was used to synthesize these lipids since this allows highly efficient production and purification of diverse libraries of compounds in the search of new therapeutic agents[19] as well as cationic lipids.[9,10,16] The synthesized lipids were evaluated for their transfection efficiency onto different cells including human embryonic kidney cells (HEK293), colorectal adenocarcinoma (COLO 205), canine osteosarcoma (D-17), human cervical adenocarcinoma (HeLa) and human prostate adenocarcinoma (PC3).

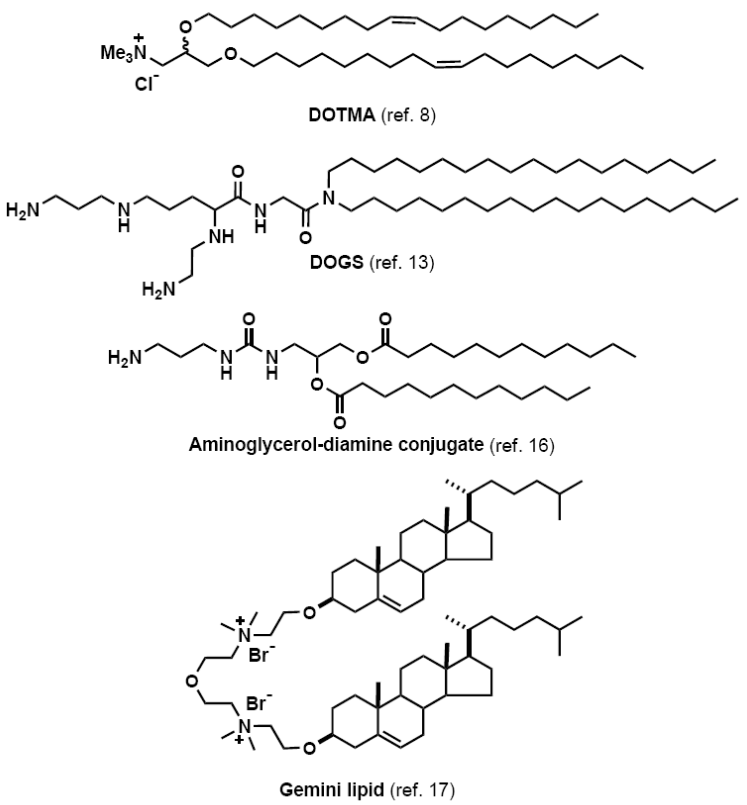


Figure 1. Cationic lipids with symmetric hydrophobic tails.

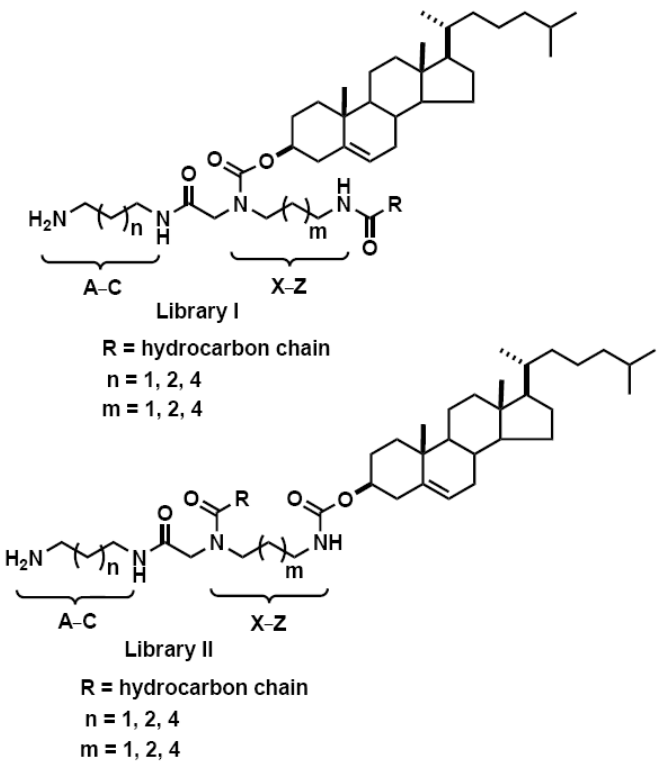
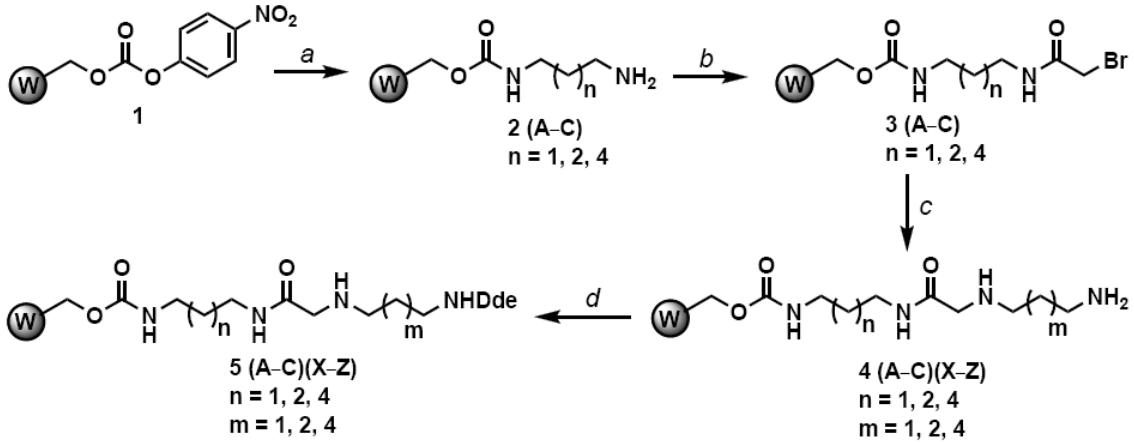


Figure 2. The structures of novel cationic lipids with asymmetric cholesterol-fatty acid tails.

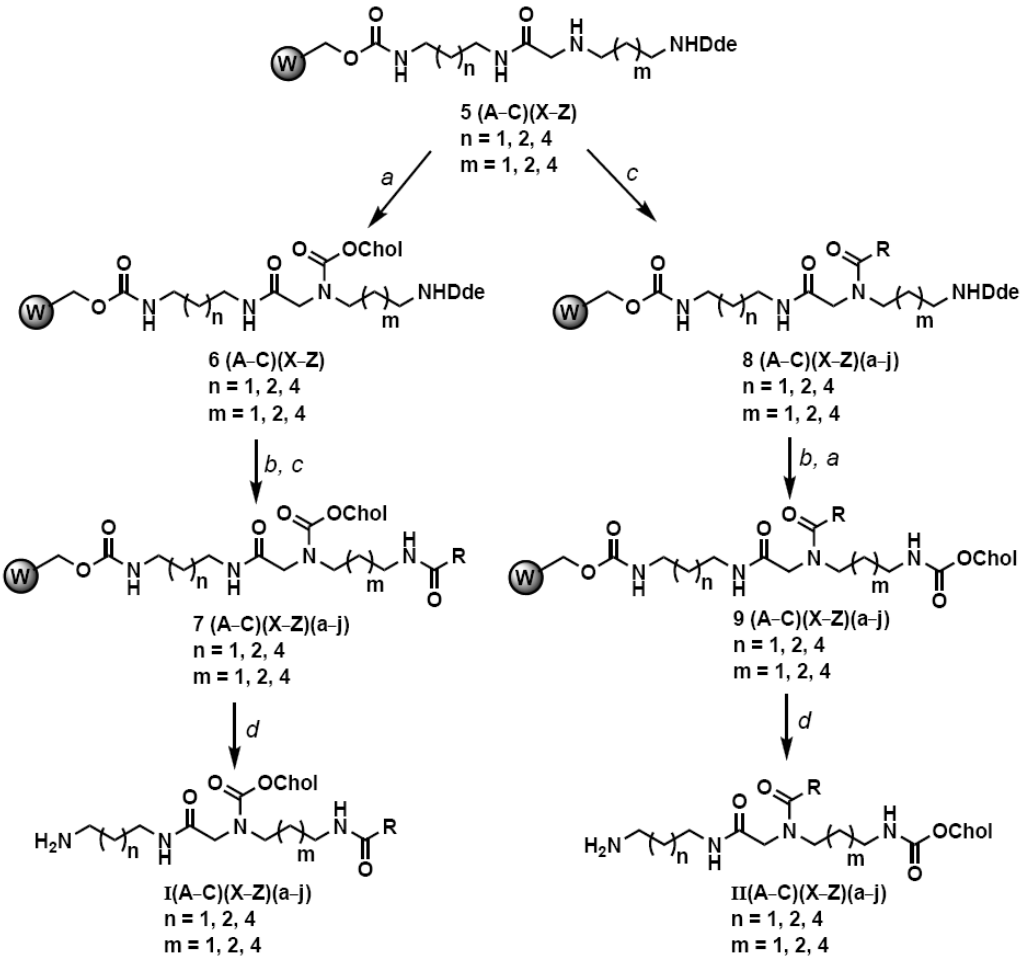
Results and Discussion

Synthesis

The key scaffold-bound resin **5**(**A–C**)(**X–Z**) was prepared from active carbonate resin **1**[16,20] following the synthetic pathway shown in Scheme 1. Thus, the resin **1** was reacted with diamines (**A**–**C**, Figure 3) to generate the resin **2**(**A–C**) which was further coupled with bromoacetic acid using diisopropylcarbodiimide (DIC) as a coupling agent to furnish the resin **3**(**A–C**). The completion of reaction was monitored by Kaiser test.[21] The bromo group on the resin **3** was replaced by diamines (**X**–**Z**) to obtain the resin **4**(**A–C**)(**X–Z**). To allow the different kind of hydrophobic tail attached to the scaffold resin, selective protection and deprotection of different amino group was needed. Dde protecting group was chosen since it selectively reacted with primary amine and was removed using mild condition.[22] Thus, the resin **4**(**A–C**)(**X–Z**) was treated with Dde-OH to generate the key resin **5**(**A–C**)(**X–Z**). Having the key scaffold resin **5**(**A–C**)(**X–Z**) in hand, the cationic lipids with asymmetric tails were ready to be synthesized. The cationic lipid library I was synthesized as followed (Scheme 2). The secondary amine of the resin **5**(**A–C**)(**X–Z**) was reacted with cholesteryl chloroformate to give the resin **6**(**A–C**)(**X–Z**). The Dde protecting group was removed by treatment with 5% N_2H_4 in DMF and the free primary amine was subsequently capped with a wide range of fatty acids (**a**–**j**) using DIC as a coupling agent in order to generate the corresponding resin **7**(**A–C**)(**X–Z**)(**a–j**). The final compounds (**I**(**A–C**)(**X–Z**)(**a–j**)) were obtained by treating the resin **7**(**A–C**)(**X–Z**)(**a–j**) with 50%TFA in CH_2Cl_2. The cationic lipids library II were also synthesized by the scaffold resin **5**(**A–C**)(**X–Z**) (Scheme 2). Thus, the fatty acids (**a**–**j**) were coupled to the free secondary amine to give the resin **8**(**A–C**)(**X–Z**)(**a–j**). The Dde protecting group was removed and the free primary amine was attached to the cholesterol tail. The final compounds (**II**(**A–C**)(**X–Z**)(**a–j**)) were obtained by treating the resin **9**(**A–C**)(**X–Z**)(**a–j**) with 50%TFA in CH_2Cl_2. All synthesized cationic lipids were obtained in moderate to good yields. The structures of these lipids were established by spectroscopic means.



Scheme 1. *Reagents and conditions*. a) diamines **A**–**C** (excess) (see Figure 3), CH_2Cl_2, 6 h; b) bromoacetic acid (4 equiv), DIC (4 equiv), DMF/CH_2Cl_2, 12 h; c) diamines **X**–**Z** (excess) (see Figure 3), CH_2Cl_2, 12 h; d) Dde-OH (excess), DMF/CH_2Cl_2, 12 h.



Scheme 2. *Reagents and conditions*. a) Cholesteryl chloroformate (4 equiv), pyridine (20 equiv), CH_2Cl_2, 12 h.; b) 5% N_2H_4/DMF, 2×30 min; c) Fatty acid (4 equiv, see Figure 3), DIC (4 equiv), CH_2Cl_2, 12 h.; d) 20% TFA/CH_2Cl_2, 2 h.

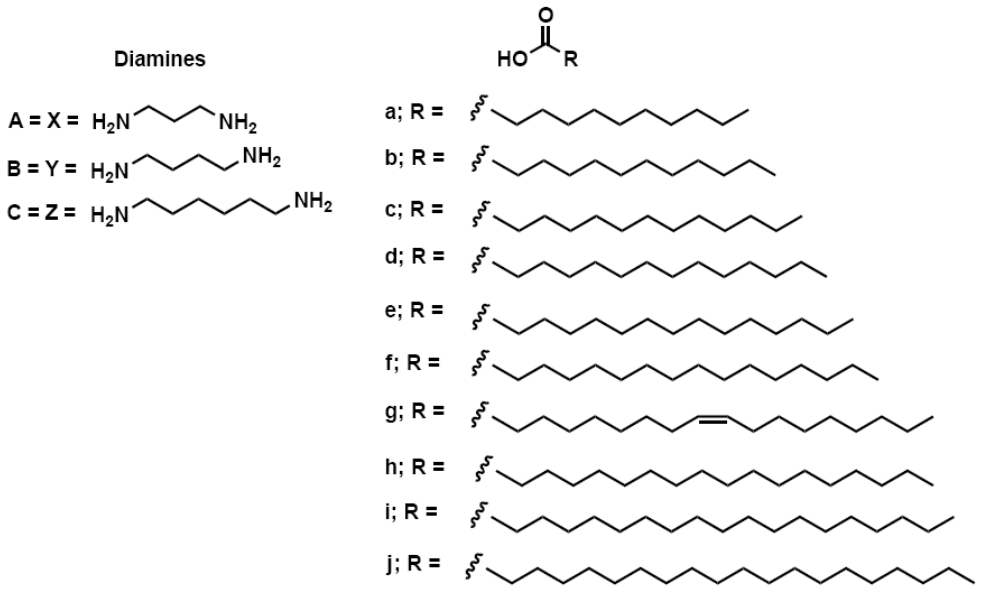


Figure 3. The structures of diamines and fatty acids used in Scheme 2.

DNA Binding Affinity

Gel retardation assay was performed to characterize the electrostatic binding interactions between the plasmid DNA and liposome.[16] Cationic liposomes were mixed with plasmid DNA, known as lipoplex, at weight ratios of 1:20 (DNA/sample) and the lipoplexes were loaded on agarose gel (Figure 4). The results indicated that most of lipids from library I, which acyl tail located at the terminal part of the polyamine backbone, interacted sufficiently with DNA to retard migration through the gel matrix. The spacers between cationic head and linker (amines **A**–**C**) and the length between the acyl and cholesteryl tails (amines **X**–**Z**, Figure 2) exerted influence for cationic lipids on binding with DNA. Thus, most of the lipids with three and six carbons spacer cationic head (**A** and **C**) with any length of spacer (**X**–**Z**) did not seem to bind to DNA to retard migration. Lipids with four carbons spacer head (**B**) and three and four carbons spacer between tails were able to retard plasmid DNA from the well whereas lipids with six carbons spacer between tails partially bound to DNA. It was interesting to note that lipids library II containing terminal cholesterol tail did not bind to DNA.

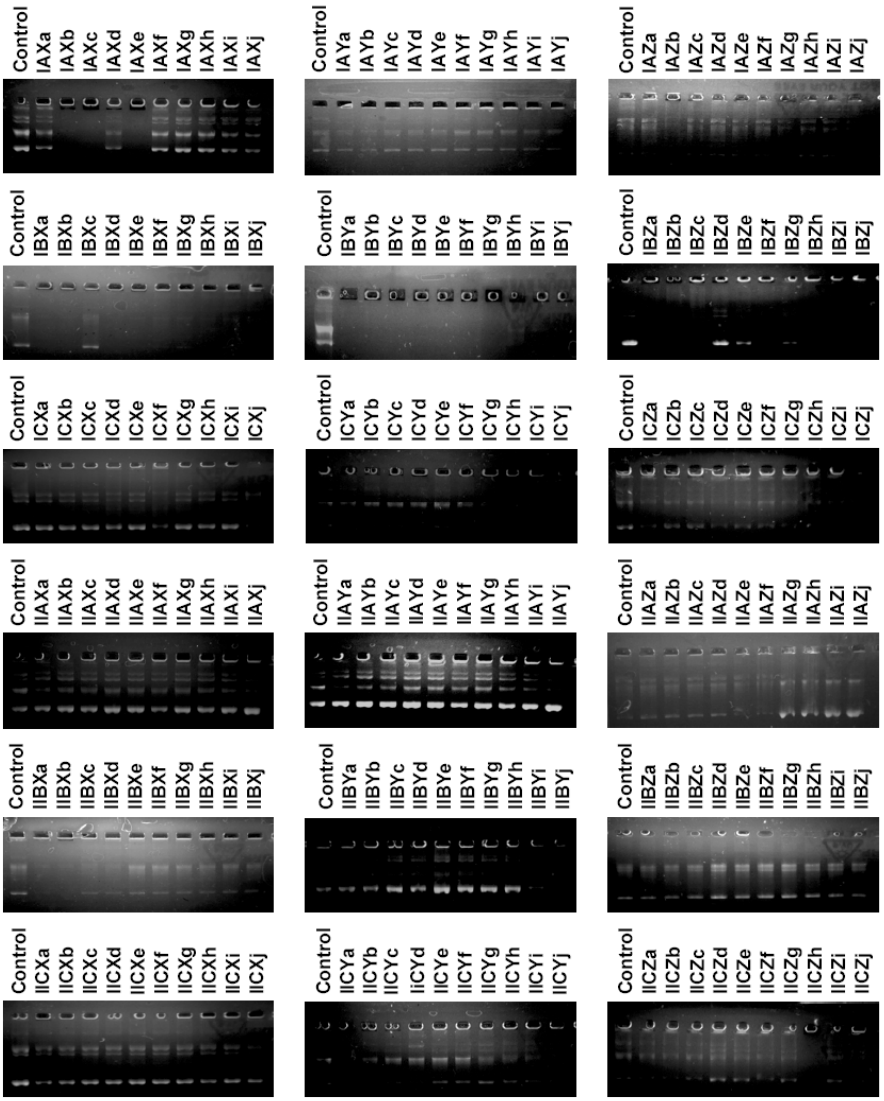


Figure 4. Gel retardation assay of DNA/cationic lipids complexes at a weight ratio of 1:20. Lanes marked "Control" contained DNA alone and was used as a control. The presence of a lower band indicated that DNA has migrated and has not been bound by the transfection compound.

Transfection Biology

Transfection Screening

All the synthesized cationic lipids were tested for transfection efficiency against HEK293 (Human embryonic kidney cell lines) using *β*-galactosidase as a reporter gene. The liposome formation both with and without DOPE was used in the preliminary transfection screening. The lipoplexes were prepared at DNA/liposome ratio (or N/P ratio) of 1:20 (w/w) using plasmid DNA at 0.1 μg/well. In the case of liposome formation with DOPE, cationic lipid/DOPE ratio was 2:1 (w/w). The results shown in Figure 5 indicated that eight lipids in library I, **IAXc**, **IAXe, IAYd, IAZc, IBYd, IBYg, IBZh,** and **IBZj**, exhibited relative transfection efficiency over 100% as compared with Effectene^{TM} transfection (100%). Only the lipids in library I bearing acyl tail at the terminal part of the polyamine scaffold and liposome formation with the helper lipid, DOPE, exhibited high transfection efficiency. The result was in agreement with binding assay, since most of the lipids which exhibited higher transfection efficiency than Effectene^{TM} completely bound to DNA, except the lipids **IAYd** and **IAZc**. From the screening results, the lipids which exhibited higher transfection efficiency than that of the positive control were subjected to further optimization.

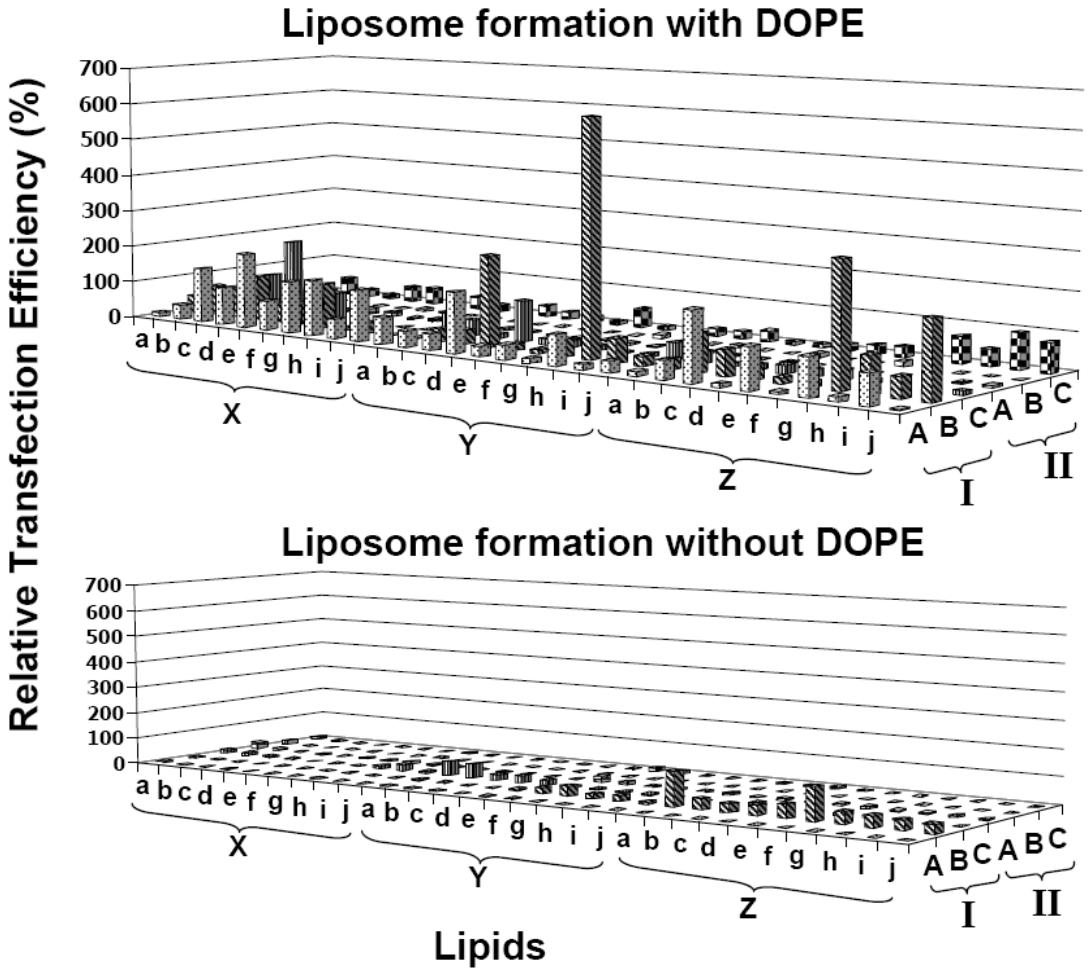


Figure 5. Transfection screening activity of synthesized cationic lipids employing pCH110-encoding β-galactosidase (0.1 μg/well). The liposome formation was both with and without DOPE. The lipoplexes was used at DNA/lipids (w/w) ratios of 1:20. The transfection efficiencies of the lipids were compared to that of commercially available reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

Transfection optimization

To find out the optimal formulation of these lipids for highest transfection efficiency, the lipids/DOPE ratios, DNA/lipids ratios, and the amount of DNA per well were studied. The helper lipid such as DOPE has been known to increase transfection efficiency in lipid mediated gene transfer applications.[23] To evaluate optimal liposome formulation, three lipids/DOPE weight ratios of 2:1, 1:1 and 1:2 were prepared. Each formulation was mixed with DNA to produce N/P weight ratio of 1:20 using 0.1 μg of DNA per well. The results shown in Figure 6 indicated that helper lipid has significant effect on lipids **IAXc** and **IBYd** to exhibit high transfection efficiency. The optimization lipid/DOPE ratios for **IAXc** and **IBYd** are 1:2 and 1:1, respectively. The transfection efficiency of lipid **IBYd** slightly reduced when the ratio of lipid/DOPE ratio decreased. However, the activity of this lipid was almost lost when liposome was prepared at high lipid/DOPE ratio.

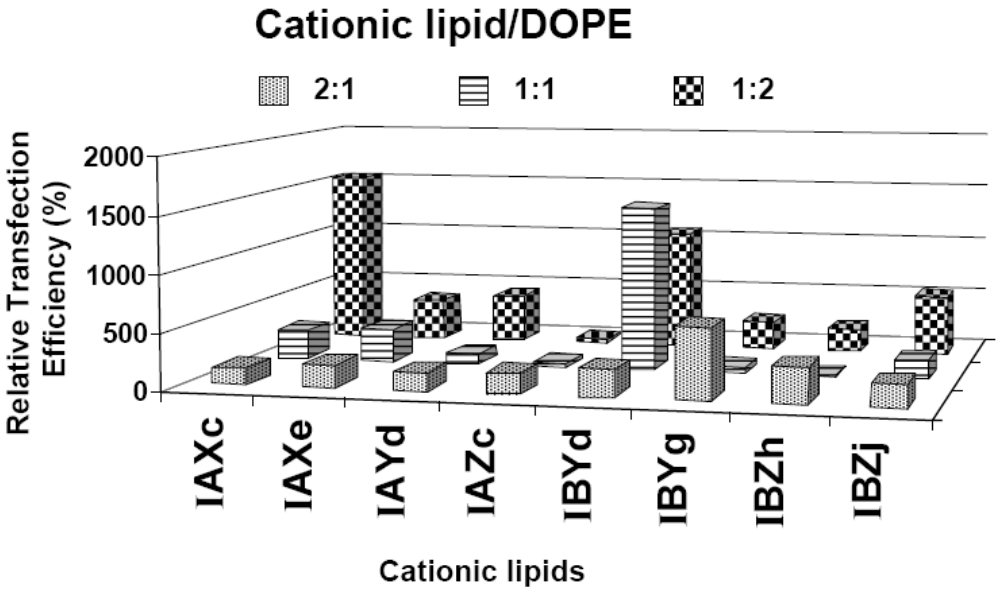


Figure 6. Transfection efficiency of the selected lipids against HEK293 cells across the cationic lipid/DOPE weight ratio of 2:1, 1:1 and 1:2. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, which calculated as 100% transfection efficiency (data not shown).

Transfection efficiency of cationic liposome was also depends on N/P ratio.[16,18,24] To find out the optimal ratio of each lipid, transfection experiments were performed against HEK293 cells using optimal lipid/DOPE ratio of each compound as shown in Figure 6. The optimal N/P ratios are shown in Figure 7. Formulations based on **IAXc**, **IAYd** and **IBYd** were found to be more effective as compared to **IAXe**, **IAZc**, **IBYg**, **IBZh** and **IBZj**. At N/P ratio of 1:40, the liposome **IBYd** exhibited relative transfection efficiency approximately 20 times higher than Effectene™. Transfection efficiency of this lipid decreased when N/P ratio increased. In contrast to the lipid **IBYd**, formulations based on **IAXc** and **IAYd** exhibited high transfection efficiency at high N/P ratio. The liposomes **IAXe**, **IAZc**, **IBYg** and **IBZj** showed the highest transfection efficiency at the N/P ratio of 1:20.

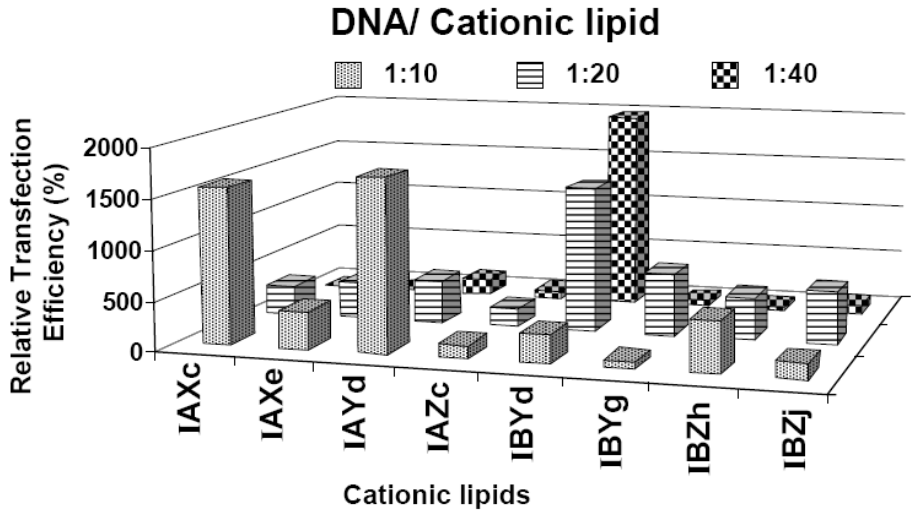


Figure 7. Transfection efficiency of the selected lipids against HEK293 cells across the DNA/cationic lipid weight ratios of 1:10, 1:20 and 1:40 (w/w). The optimal liposome formation of each lipid from Figure 6 was used as described in the text. Transfection efficiency of the selected lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

To see whether variation in the amount of DNA affected the transfection efficiency of these lipids, the experiments were performed using the optimal lipid/DOPE (Figure 6) and DNA/lipid (Figure 7) ratios. The amounts of DNA used in this experiment varied from 0.1, 0.2 and 0.4 μg per well. The transfection efficiency of each lipid is shown in Figure 8. The amount of DNA used for the formulation based on the lipid **IAYd** has no effected for transfection efficiency. In contrast, the efficiency of the lipids **IBYg** and **IBZh** decreased when the amount of DNA increased. The optimal amount of DNA for the lipids **IBYd** and **IBZj** to reach high efficiency was 0.2 μg per well.

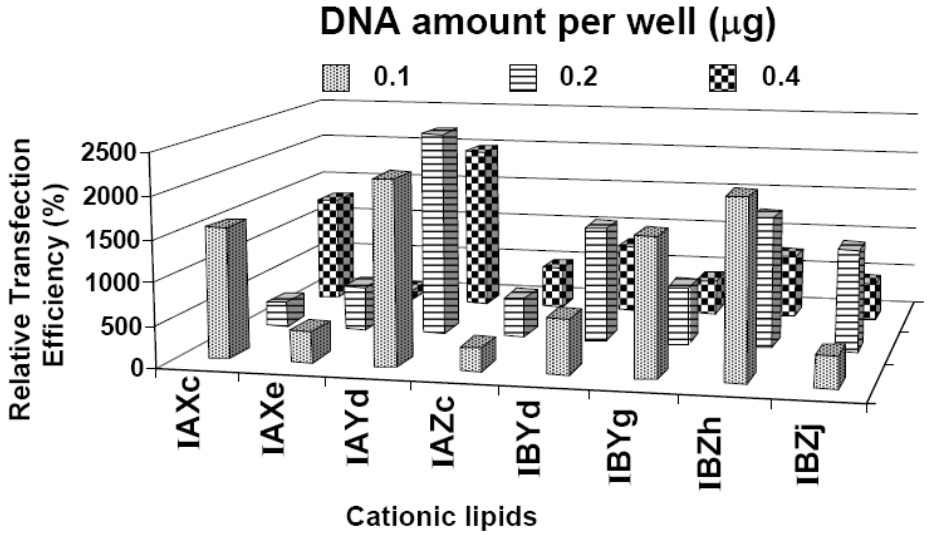


Figure 8. Effect of DNA amount for gene delivery. The optimal liposome formation (Figure 6) and DNA/lipids complex (Figure 7) of each lipid were used to mix with various amount of DNA from 0.1 to 0.4 μg. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

One of the major drawbacks of cationic lipids for their *in vivo* use is the inhibition of the transfection efficiency of cationic liposomes in the presence of serum. Most of cationic lipids which exhibited high transfection activity in the absence of serum lost their efficiency when transfected in the presence of serum.[15,17] The effect of serum on transfection activity of these lipids is shown in Figure 9. The result indicated that formulation based on the lipids **IAXc**, **IAYd**, **IBYg**, **IBZh** and **IBZj** dramatically reduced their transfection efficiency when the experiment performed in the presence of 10% serum. Further increase in the percentage of serum (20 and 40%) has no effect on transfection activity of these lipids. Transfection efficiency of the liposomes **IAZc** and **IBYd** was not reduced when the experiment was carried out in 10% serum.

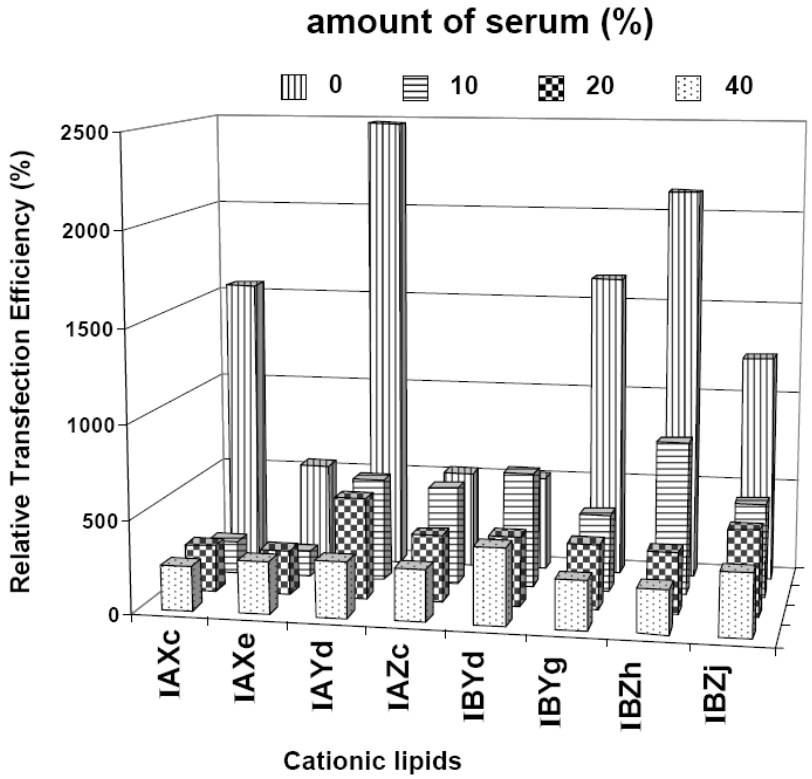


Figure 9. Effect of serum for transfection efficiency. The optimal liposome formation (Figure 6), DNA/cationic lipid (Figure 7) and amount of DNA per well (Figure 8) of each lipid were used to transfer gene to HEK293 cell at various amount of serum. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

Transfection efficiency toward different cell lines

It is well known that transfection agents have the ability to specifically deliver DNA into different cell types. To evaluate the transfection efficiency of these lipids toward the different mammalian cell lines, HEK293, COLO 205, D-17, HeLa and PC3 cells, the experiments were performed using optimum condition under serum-free condition. The transfection efficiency of these compounds was compared with the commercially available transfection agents, Effectene^{TM}, DOTAP and DC-Chol. The transfection efficiencies of different liposome formulations and commercially available agents against HEK293, COLO 205, D-17, HeLa and PC3 cells is shown in Figure 10. It was clearly shown that the synthesized lipids exhibited higher transfection efficiency than that of the commercial agents against PC3 and HEK293 cells. Formulation based on the lipids **IAXc**, **IAXe**, **IAYd**, **IBYg** and **IBZh** were found to be over 25-fold more efficient to deliver DNA into PC3 cells than the commercially available

agents. The least active formulations, **IAZc**, **IBYd** and **IBZj** were 10- to 13-fold higher transfection efficiency against PC3 cells than Effectene™.

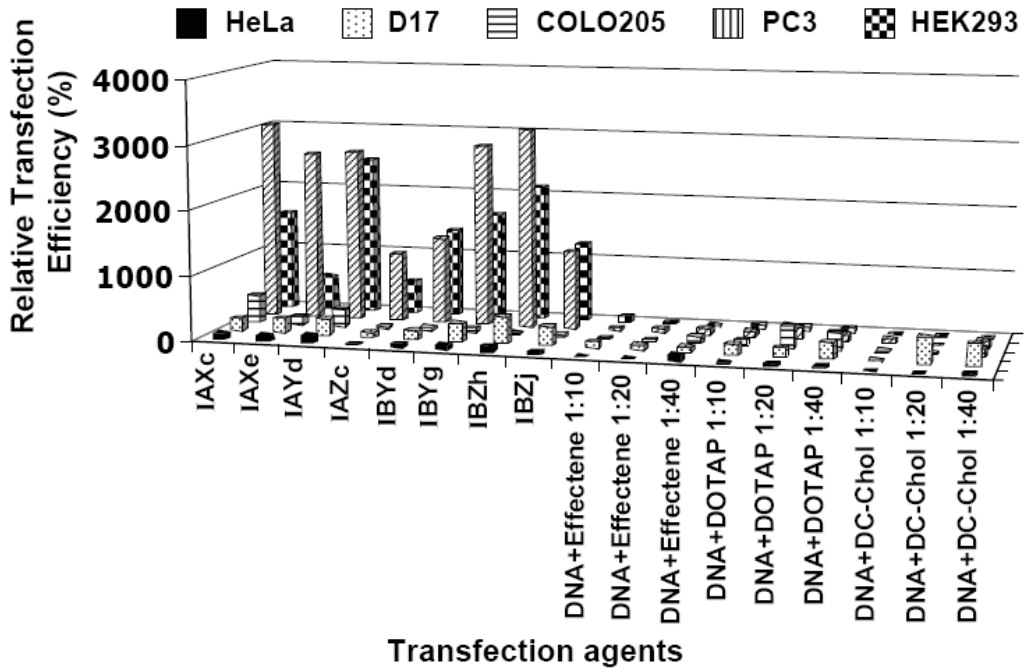


Figure 10. Transfection efficiency of selected lipids toward COLO 205, D-17, HeLa and PC3 cell using optimum conditions from Figures 6-8. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene™, DOTAP and DC-Chol. Transfection efficiency of Effectene™ for each cell line was calculated as 100%.

Transfection toxicity

Cytotoxicity of synthesized cationic lipids is very important for gene delivery. To assess the relationship between cytotoxicity and transfection efficiency, the toxicity of the synthesized lipids on HEK293, COLO 205, D-17, HeLa and PC3 cell lines using optimal conditions (Figures 6–8) were determined by measuring changes in cell metabolic activity (MTT assay). The % cell viability of the synthesized lipids and the commercial agents as compared to the control cells in the presence of DNA is shown in Figure 11. Most of the tested lipids which exhibited high transfection efficiency were found to be nontoxic to PC3 cells having cell viability over 80%. Less than 60% cell viability was observed with the lipid **IAXc** against all tested cells. The result was in contrast to our finding that the formulation based on the lipid **IAXc** exhibited exceptionally high transfection efficiency of 3000% against PC3 cells. All the tested lipids showed cell viability less than 70% against HEK293 cells. These results were also in contrast to the high transfection efficiency of these lipids. All the mentioned commercial agents were slightly more toxic than the tested lipids in the MTT assay. It is thus concluded that most of the tested lipids can produce high levels of transfection without inducing significant cell death, although there is a reduction in metabolic activity.

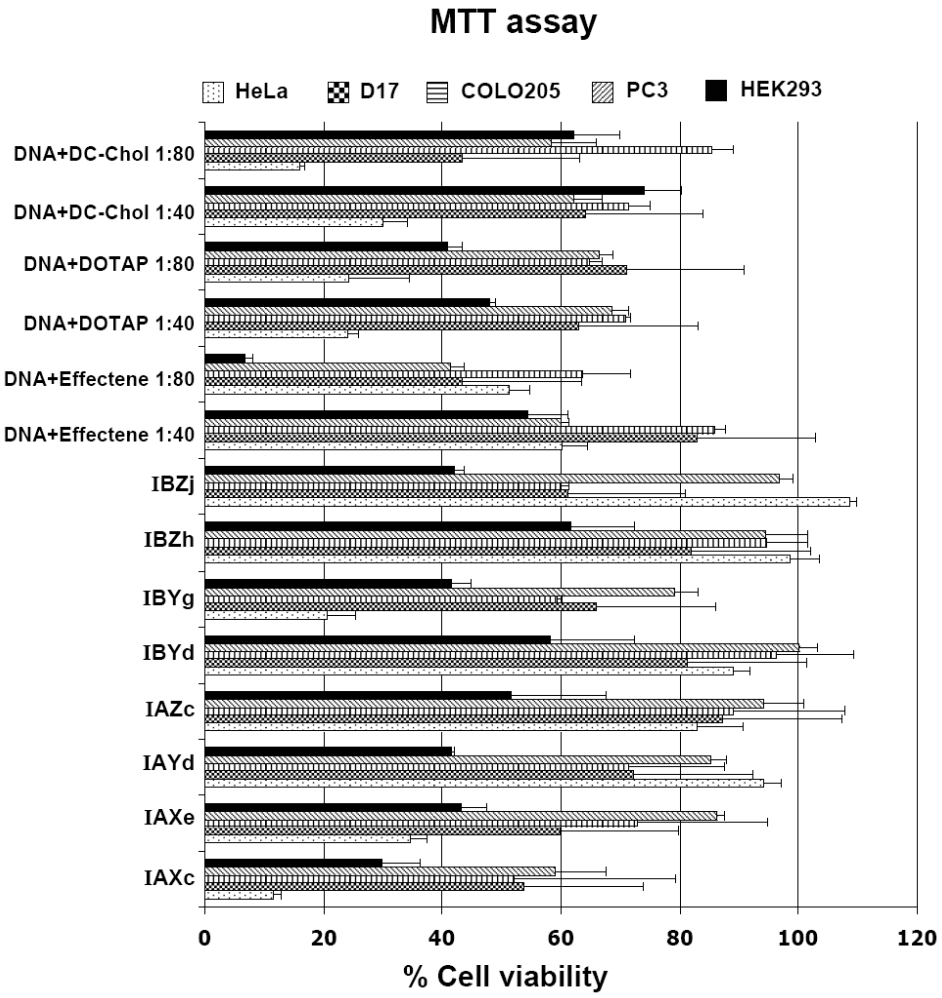


Figure 11. Effect of transfection synthesized lipids on cell metabolic activity. Liposomes of cationic lipid with helper lipid, DOPE, were formed and added to DNA to form lipoplexes. These complexes were added to HEK293, COLO 205, D-17, HeLa and PC3 cell. Cell metabolic activity was determined by a MTT assay.

Transmission electron microscopy (TEM)

Characterization of the morphologies of the liposomes and lipoplexes from the synthesized cationic lipids, which were used to study transfection optimization, was observed under transmission electron microscopy (TEM) after negative staining. The result gives a better understanding of the molecular assembly of DNA and liposome. Thus, it should help to establish correlations with their biological activity. The liposome of each lipid was prepared at its optimal lipid/DOPE ratio. The morphology of liposome is shown in Figure 12. The morphologies of the liposome based on the formulation of the lipids **IAXc**, **IAXe** and **IAYd** were found to be intermediated-sized unilamellar vesicles (IUVs) having diameter of 50–100 nm. Multilamellar vesicles (MLVs) were observed from the liposomes **IAZc**, **IBYd**, **IBYg**, **IBZh** and **IBZj** with diameter range 100–150 nm. Morphology of liposomes/DNA complexes (lipoplexes) was also visualized under transmission electron microscopy as shown in Figure 13. Most of the lipoplexes were found to be large spherical aggregates which the sizes are within the range 300–700 nm. In this study, the sizes of the lipoplexes prepared from these lipids are unlikely to play important role in modulating for transfection efficiency.

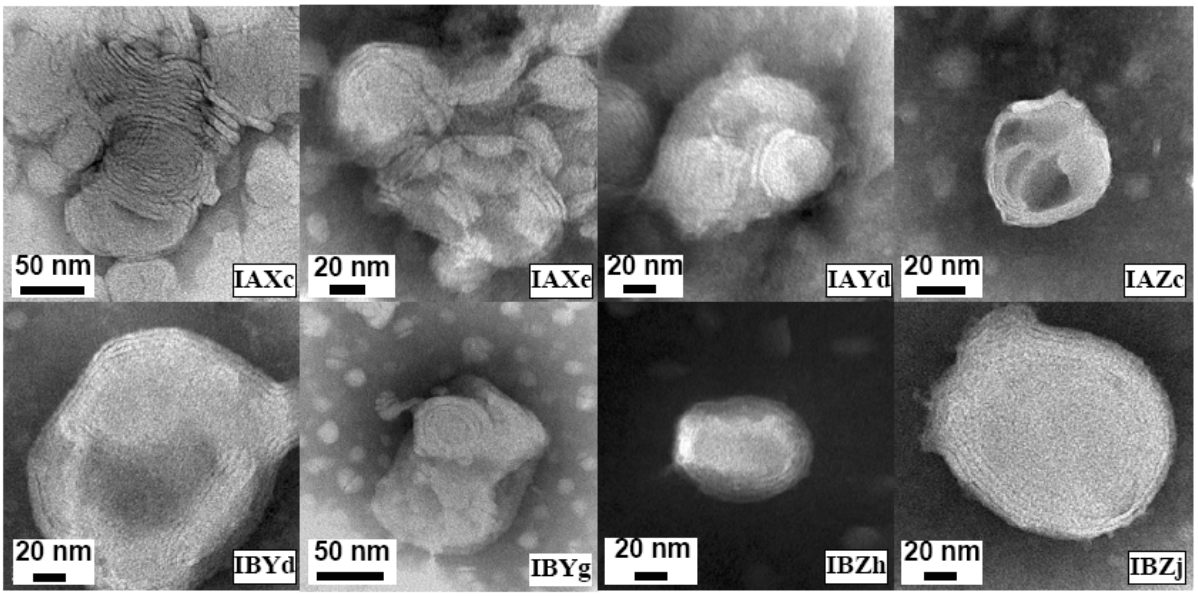


Figure 12. Transmission electron microscopic images of cationic liposomes **IAXc**, **IAXe, IAYd, IAZc, IBYd, IBYg, IBZh,** and **IBZj**.

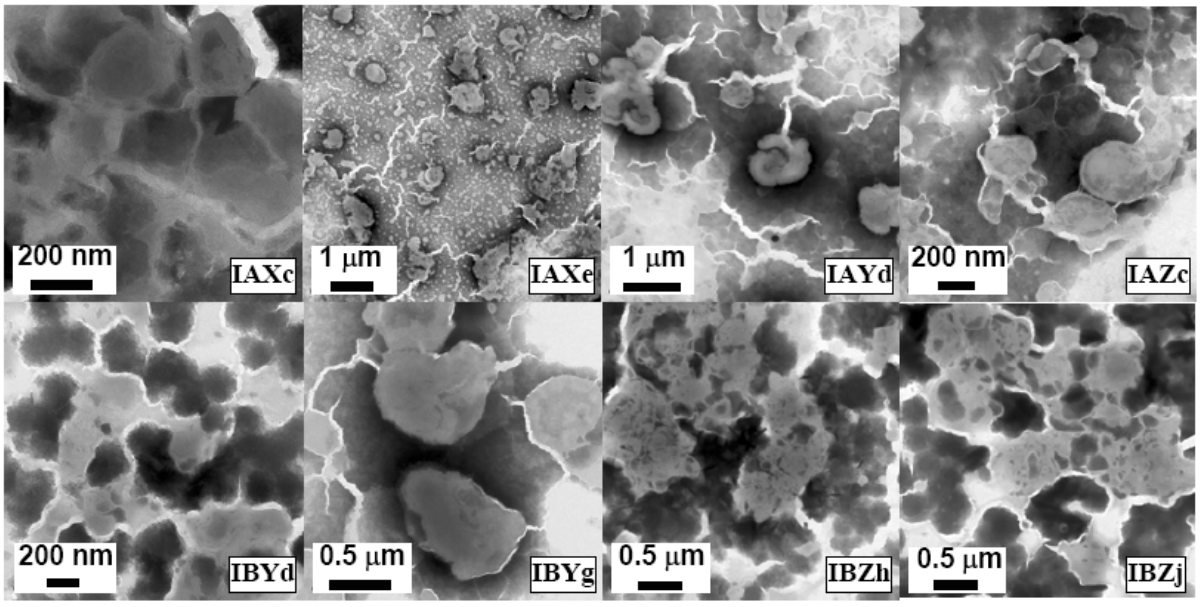


Figure 13. Transmission electron microscopic images of lipoplexes prepared from optimized liposomes (**IAXc**, **IAXe, IAYd, IAZc, IBYd, IBYg, IBZh,** and **IBZj**)/DNA complexes.

Conclusions

Cationic lipids with various hydrophilic heads and hydrophobic tails have been of interest to many scientists for their use in gene delivery applications. They can be synthesized by solid phase as well as conventional syntheses to obtain new analogues with high efficiency and minimal toxicity. Toward this end, we have demonstrated here the solid phase synthesis of novel cationic lipids having asymmetric cholesteryl-acyl hydrophobic tails. The lipids with acyl portion at the terminal part of the polyamine backbone exhibited higher transfection efficiency than those with the acyl portion at the internal part of the backbone. These compounds also exhibited higher transfection efficiency and lower cytotoxicity as compared

with the commercially available agents, Effectene^{TM}, DOTAP and DC-Chol. The generation of compounds with transfection abilities greater than those of widely used commercial agents suggests that this class of compound has significant potential for overcoming gene transfer difficulties in vitro and possibly in vivo.

Experimental Section

General information.

NMR spectra were recorded on a Bruker AVANCE 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. All coupling constants (*J* values) were measured in Hz. ES and HR–ESI–MS were recorded on a Finnigan LC-Q mass spectrometer and a Micromass Instrument type QTOF2 spectrometer, respectively. Infra-red spectra were recorded on a Perkin-Elmer Spectrum GX60237. The size and morphology of the cationic liposomes and lipoplexes were recorded on JEM-2100, JEOL electron microscope. Starting materials and reagents were purchased from commercial suppliers and used without further purification.

Synthesis

Solid phase synthesis of key scaffold resin 5(A–C)(X–Z) (Scheme 1)

Active carbonate resin **1**[20] (1 equiv) was added an excess 1,3-diamonopropane (**A**) or 1,4-diaminbutane (**B**) or 1,6-diaminohexane (**C**) in CH_2Cl_2. The suspension was shaken overnight. The resulting resin was washed with CH_2Cl_2, DMF and CH_2Cl_2 (3×10 mL, each) to provide the corresponding resin **2**(**A**–**C**). Amino resin **2**(**A**–**C**) (1 equiv, 1.1 $mmolg^{-1}$) was reacted with a solution of bromoacetic acid (4 equiv) and DIC in DMF (10 mL). The suspension was then shaken for 12 h. The resulting resin was filtered and washed successively with DMF, CH_2Cl_2, MeOH, CH_2Cl_2, DMF and CH_2Cl_2 (3 × 10 mL, each) to give the desired resin **3**(**A**–**C**). The excess of 1,3-diaminopropane (**X**) or 1,4-diaminobutane (**Y**) or 1,6-diaminohexane (**Z**) in DMF was reacted with the resin **3**(**A**–**C**) for 12 h to give the desired resin **4**(**A**–**C**)(**X**–**Z**) after successively washed with DMF, CH_2Cl_2, MeOH, DMF and CH_2Cl_2 (3 × 10 mL, each). The resin **4**(**A**–**C**)(**X**–**Z**) was dried under vacuum for 2 h. The resin **4**(**A**–**C**)(**X**–**Z**) was reacted with Dde-OH (excess) in CH_2Cl_2 for 12 h. The resin was filtered and washed successively with CH_2Cl_2 and MeOH (3 × 10 mL, each). The resulting resin was dried under reduced pressure to give the desired resin **5**(**A**–**C**)(**X**–**Z**).

Synthesis of lipids library I (Scheme 2)

Cholesteryl chloroformate (4 equiv) was dissolved in CH_2Cl_2 (5 mL) and the resulting solution was added to each Dde-protected amino resin **5**(**A**–**C**)(**X**–**Z**). Pyridine (2 mL) was gently added to the mixture and the suspensions were shaken overnight. The resins were filtered and washed successively with CH_2Cl_2, MeOH, and CH_2Cl_2 (3 × 10 mL, each) to give the resins **6**(**A**–**C**)(**X**–**Z**). Dde deprotection was carried out using 5% N_2H_4 in DMF (~10 mL) for two cycles of 30 minutes. The resulting resins were filtered and washed successively with CH_2Cl_2, DMF, MeOH, DMF, and CH_2Cl_2 (3 × 10 mL, each) to give the amino resins. The amino resins gave a positive ninhydrin test and were dried under vacuum before using in the next step. Each of the amino resins was splitted to 10 portions. Each portion of resin was added a solution of various long chain fatty acids (**a**–**j**) (4 equiv) (see Figure 3), DIC (4 equiv), in CH_2Cl_2/DMF (4:1). The suspensions were shaken overnight. The resins were washed with CH_2Cl_2, MeOH, DMF, MeOH and CH_2Cl_2 (3 × 10 mL, each) to give the resins **7**(**A**–**C**)(**X**–**Z**)(**a**–**j**). The resulted resins **7**(**A**–**C**)(**X**–**Z**)(**a**–**j**) were dried under vacuum before cleaving. A solution of 20% TFA/CH_2Cl_2 (1.5 mL) was added to each of the resins **7**(**A**–**C**)(**X**–**Z**)(**a**–**j**) and the suspensions were shaken for 2 h. The resins were filtered and the solutions were collected. The

solvents were removed under a stream of nitrogen and evaporated under reduced pressure to afford the desired products **I**(**A**–**C**)(**X**–**Z**)(**a**–**j**).

Synthesis of lipids library II (Scheme 2)
Each of the resins **5**(**A**–**C**)(**X**–**Z**) was splitted to 10 portions. Each portion of the resins was added a solution of various long chain fatty acids (**a**–**j**) (4 equiv) (see Figure 3), DIC (4 equiv) in CH_2Cl_2/DMF (4:1). The suspensions were shaken overnight. The resins were washed with CH_2Cl_2, MeOH, DMF, MeOH, and CH_2Cl_2 (3 × 10 mL, each) to give the resins **8**(**A**–**C**)(**X**–**Z**)(**a**–**j**). Dde deprotection was carried out using 5% N_2H_4 in DMF (~10 mL) for two cycles of 30 minutes. The resulting resins were filtered and washed successively with CH_2Cl_2, DMF, MeOH, and CH_2Cl_2 (3 × 10 mL, each) to give the free amino resins. The free amino resins gave a positive ninhydrin test and were dried under vacuum. Cholesteryl chloroformate (4 equiv) was dissolved in CH_2Cl_2, (1.5 mL) and the resulting solution was added to each of the resins **8**(**A**–**C**)(**X**–**Z**)(**a**–**j**). Pyridine (0.5 mL) was gently added to the mixture. The suspensions were shaken overnight. The resins were filtered and washed successively with CH_2Cl_2, MeOH, and CH_2Cl_2 (3 × 10 mL, each). A solution of 20% TFA/CH_2Cl_2 (1.5 mL) was added to each of the resins **9**(**A**–**C**)(**X**–**Z**)(**a**–**j**) and the suspensions were shaken for 2 h. The resins were filtered and the solutions were collected. The solvents were removed under a stream of nitrogen and evaporated under reduced pressure to afford the desired products **II**(**A**–**C**)(**X**–**Z**)(**a**–**j**).

Lipid IAXc Yield: (resin: 1.1 $mmolg^{-1}$, 130 mg) 31.0 mg, 27%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (br s, 3H; NH_3^+), 5.31 (br s, 1H; H-6-Chol), 4.30 (br s, 1H; H-3-Chol), 3.86 (br s, 2H; NHCOCH_2N), 3.19 and 3.29 (partially overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2CH$_2$CH_2NHCO), 2.95 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.40 (m, 43H; methyl, methylene and methine protons), 1.22 (s, 16H; methylene protons of long chain fatty acid), 0.97 (s, 3H; H-19-Chol), 0.88 (d, J = 6.0 Hz, 3H; H-21-Chol), 0.83 (d, J = 6.5 Hz, 6H; H-26, 27-Chol), 0.64 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.5, 161.1, 139.4, 122.7, 76.0, 56.6, 56.2, 52.4, 49.9, 42.2, 39.7, 39.4, 39.0, 38.4, 37.2, 36.9, 36.5, 36.4, 36.1, 35.8, 31.9, 29.7, 29.3, 28.2, 28.0, 27.9, 24.2, 23.9, 22.7, 22.6, 22.5, 21.0, 19.2, 18.7, 14.1, 11.8 ppm; IR: ν bar = 3290, 3070, 2930, 2851, 1681, 1541, 1468, 1379, 1202, 1136 cm^{-1}; HRMS: *m/z*: calcd for $C_{49}H_{89}N_4O_4^+$: 797.6878; found: 797.7023.
Lipid IAXe Yield: (resin: 1.1 $mmolg^{-1}$, 130 mg) 28.8 mg, 24%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 (br s, 3H; NH_3^+), 5.31 (br s, 1H; H-6-Chol), 4.40 (br s, 1H; H-3-Chol), 3.87 (br s, 2H; NHCO*C*H$_2$N), 3.21 and 3.30 (partially overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2CH$_2$CH_2NHCO), 2.97 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 35H; methyl, methylene and methane protons), 1.22 (s, 28H; methylene protons of long chain fatty acid), 0.97 (s, 3H; H-19-Chol), 0.88 (d, J = 6.0 Hz, 3H; H-21-Chol), 0.84 (d, J = 6.5 Hz, 6H; H-26, 27-Chol), 0.64 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 160.8, 139.4, 122.8, 76.6, 56.6, 56.2, 50.0, 42.3, 39.7, 39.5, 39.1, 38.9, 38.5, 38.4, 36.5, 36.4, 36.2, 35.8, 31.9, 31.8, 29.7, 29.3, 28.2, 28.1, 28.0, 24.2, 23.9, 22.8, 22.6, 22.5, 21.0, 19.2, 18.7, 14.1, 11.8 ppm; IR: ν bar = 3284, 3077, 2926, 2852, 1681, 1552, 1467, 1378, 1202, 1135, 1026 cm^{-1};HRMS: *m/z*: calcd for $C_{51}H_{93}N_4O_4^+$: 825.7191; found: 825.7294.
Lipid IAYd Yield: (resin: 1.1 $mmolg^{-1}$, 130 mg) 38.3 mg, 32%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (br s, 3H; NH_3^+), 5.31 (br s, 1H; H-6-Chol), 4.40 (br s, 1H; H-3-Chol), 3.84 (br s, 2H; NHCOCH_2N), 3.16 and 3.29 (partially overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2(CH$_2$)$_2$CH_2NHCO), 2.95 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 45H; methyl, methylene and methine protons), 1.22 (s, 18H; methylene protons of long chain fatty acid), 0.97 (s, 3H; H-19-Chol), 0.88 (d, J = 6.1 Hz, 3H; H-21-Chol), 0.83 (d, J = 6.6 Hz, 6H;

H-26, 27-Chol), 0.64 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.3, 171.3, 161.3, 139.5, 122.7, 76.6, 56.6, 56.2, 52.4, 49.9, 42.2, 39.7, 39.4, 39.0, 38.4, 37.2, 36.9, 36.5, 36.4, 36.1, 35.8, 31.9, 29.7, 29.3, 28.2, 28.0, 27.9, 24.2, 23.9, 22.7, 22.6, 22.5, 21.0, 19.2, 18.6, 14.0, 11.8 ppm; IR: ν bar = 3307, 3077, 2924, 2852, 1681, 1552, 1467, 1378, 1240, 1203, 1135 cm^{-1}; HRMS: *m/z*: calcd for $C_{51}H_{93}N_4O_4^+$: 825.7191; found: 825.7411.

Lipid IAZc Yield: (resin: 1.1 mmolg^{-1}, 130 mg) 24.1 mg, 20%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.90 (br s, 3H; NH_3^+), 5.32 (br s, 1H; H-6-Chol), 4.42 (br s, 1H; H-3-Chol), 3.85 (br s, 2H; NHCOCH_2N), 3.21 and 3.31 (partially overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2(CH$_2$)$_4$CH_2NHCO), 2.93 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 47H; methyl, methylene and methine protons), 1.22 (s, 18H; methylene protons of long chain fatty acid), 0.97 (s, 3H; H-19-Chol), 0.88 (d, *J* = 6.1 Hz, 3H; H-21-Chol), 0.84 (d, *J* = 6.6 Hz, 6H; H-26,27-Chol), 0.65 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.8, 160.7, 139.5, 122.8, 76.6, 56.7, 56.2, 52.4, 50.0, 42.3, 39.7, 39.5, 39.1, 38.9, 38.5, 36.5, 36.4, 36.2, 35.8, 31.9, 31.8, 29.7, 29.3, 28.2, 28.1, 28.0, 24.2, 23.8, 22.7, 22.6, 22.5, 21.0, 19.3, 18.7, 14.0, 11.8 ppm; IR: ν bar = 3276, 2925, 2853, 1681, 1552, 1467, 1432, 1378, 1202, 1137, 1019 cm^{-1}; HRMS: *m/z*: calcd for $C_{52}H_{95}N_4O_4^+$: 839.7347; found: 839.7473.

Lipid IBYd Yield: (resin: 1.1 mmolg^{-1}, 130 mg) 28.0 mg, 23%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (br s, 3H; NH_3^+),5.31 (br s, 1H; H-6-Chol), 4.41 (br s, 1H; H-3-Chol), 3.85 (br s, 2H; NHCOCH_2N), 3.21 (overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2(CH$_2$)$_2$CH_2NHCO), 2.94 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 47H; methyl, methylene and methine protons), 1.21 (s, 18H; methylene protons of long chain fatty acid), 0.97 (s, 3H; H-19-Chol), 0.87 (d, *J* = 6.2 Hz, 3H; H-21-Chol), 0.83 (d, *J* = 6.5 Hz, 6H; H-26,27-Chol), 0.64 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.8, 160.7, 139.5, 122.6, 75.7, 56.6, 52.7, 51.6, 49.9, 42.2, 39.6, 39.4, 39.0, 38.5, 38.4, 36.6, 36.5, 36.1, 35.7, 31.8, 31.7, 29.7, 29.3, 28.2, 28.1, 27.9, 24.2, 23.8, 22.7, 22.6, 22.5, 21.0, 19.2, 18.6, 14.0, 11.8 ppm; IR: ν bar = 3424, 3342, 2927, 2856, 1682, 1574, 1466, 1383, 1321, 1247, 1203, 1169, 1131 cm^{-1}; HRMS: *m/z*: calcd for $C_{52}H_{95}N_4O_4^+$: 839.7347; found: 839.7827.

Lipid IBYg Yield: (resin: 1.1 mmolg^{-1}, 130 mg) 29.5 mg, 23%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (br s, 3H; NH_3^+), 5.31 and 5.39 (br s, 3H; H-6-Chol and –C*H*=C*H*– in fatty acid), 4.43 (br s, 1H; H-3-Chol), 3.83 (br s, 2H; NHCOCH_2N), 3.22 (overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2(CH$_2$)$_2$CH_2NHCO), 2.94 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 55H; methyl, methylene and methine protons), 1.23 (s, 14H; methylene protons of long chain fatty acid), 0.97 (s, 3H; H-19-Chol), 0.88 (d, *J* = 6.2 Hz, 3H; H-21-Chol), 0.83 (d, *J* = 6.5 Hz, 6H; H-26,27-Chol), 0.64 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.8, 160.7, 139.5, 129.9, 129.6, 122.6, 75.7, 56.6, 52.4, 51.6, 49.9, 42.3, 39.7, 39.4, 39.1, 38.9, 38.5, 38.4, 36.5, 36.1, 35.7, 31.8, 29.7, 29.2, 28.1, 28.0, 27.9, 24.2, 23.8, 22.7, 22.6, 22.5, 21.0, 19.3, 18.6, 14.0, 11.8 ppm; IR: ν bar = 3342, 2966, 2929, 2856, 1675, 1632, 1574, 1464, 1384, 1325, 1247, 1203 cm^{-1}; HRMS: *m/z*: calcd for $C_{56}H_{101}N_4O_4^+$: 893.7347; found: 893.8056.

Lipid IBZh Yield: (resin: 1.1 mmolg^{-1}, 130 mg) 28.1 mg, 21%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88 (br s, 3H; NH_3^+), 5.32 (br s, 3H; H-6-Chol), 4.43 (br s, 1H; H-3-Chol), 3.84 (br s, 2H; NHCOCH_2N), 3.22 (overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2(CH$_2$)$_4$CH_2NHCO), 2.97 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 51H; methyl, methylene and methine protons), 1.22 (s, 26H; methylene protons of long chain fatty acid), 0.98 (s, 3H; H-19-Chol), 0.88 (d, *J* = 6.4 Hz, 3H; H-21-Chol), 0.83 (d, *J* = 6.6 Hz, 6H; H-26,27-Chol), 0.65 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.6, 160.6, 139.4, 122.7, 75.9, 56.6, 56.1, 52.8, 49.9, 42.2, 39.6, 39.4, 39.1, 38.9, 38.5, 38.4, 36.4, 36.1, 35.7, 31.8, 31.7, 29.6, 29.5, 29.3, 29.2, 28.1, 28.0, 27.9, 24.2, 23.8, 22.7, 22.6, 22.5, 20.9, 19.2, 18.6, 14.0, 11.7 ppm; IR: ν bar = 3322, 3084, 2924, 2852, 1681, 1552, 1467, 1432, 1378, 1203, 1136, 1023 cm^{-1}; HRMS: *m/z*: calcd for $C_{57}H_{105}N_4O_4^+$: 909.8130; found: 909.8722.

Lipid IBZj Yield: (resin: 1.1 mmolg^{-1}, 130 mg) 26.7 mg, 20%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 (br s, 3H; NH_3^+), 5.34 (br s, 3H; H-6-Chol), 4.43 (br s, 1H; H-3-Chol), 3.90 (br s, 2H; NHCOCH_2N), 3.11, 3.23 and 3.33 (partially overlapping, br s, 6H; CH_2NHCOCH$_2$NCH_2(CH$_2$)$_4$CH_2NHCO), 2.99 (br s, 2H; $^+$H$_3$NCH_2CH$_2$), 0.85–2.50 (m, 51H; methyl, methylene and methine protons), 1.22 (s, 18H; methylene protons of long chain fatty acid), 0.98 (s, 3H; H-19-Chol), 0.89 (d, J = 6.3 Hz, 3H; H-21-Chol), 0.84 (d, J = 6.5 Hz, 6H; H-26,27-Chol), 0.65 ppm (s, 3H; H-18-Chol); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.7, 170.6, 160.7, 138.4, 122.7, 76.6, 56.6, 56.1, 50.3, 50.0, 42.2, 39.8, 39.6, 39.5, 39.1, 38.6, 38.3, 36.5, 36.1, 35.7, 31.8, 31.8, 29.7, 29.3, 28.2, 27.9, 24.2, 23.8, 22.7, 22.6, 22.5, 21.0, 19.2, 18.6, 14.0, 11.8 ppm; IR: ν bar = 3333, 3070, 2924, 2852, 1680, 1553, 1467, 1432, 1378, 1203, 1137, 1028 cm^{-1}; HRMS: *m/z*: calcd for $C_{60}H_{111}N_4O_4^+$: 951.8599; found: 951.9085.

DNA binding affinities

The DNA binding ability of synthesized lipids was performed by gel retardation assay. The DNA/sample complexes 1/20 (w/w) were prepared by transferring 2.4 μL (10 μgμL^{-1}) of sample to an Eppendorf tube. Each sample was further diluted with 3 μL of PBS (phosphate buffered saline) buffer. An aqueous solution of plasmid DNA (4 μL, 0.3 μgμL^{-1}) was added to each sample and the solutions were successively mixed by inverting several times. The DNA complexes were incubated at 25 °C for 30 minutes. Bromophenol blue-free gel-loading buffer (3 μL, 2×40% w/v sucrose in water) was added to the complexes. The solutions were mixed by inverting each tube and each sample (10 μL) was loaded onto a 1.0% agarose gel (0.5×TBE buffer). The gel was run at 135V for 5 min and 50V for 2 h at 400 mA. DNA bands were visualized by ethidium bromide staining.

Liposome preparation

Dioleoyl-L-α-phosphatidylethanolamine (DOPE) (Sigma) (1 μL, 50 μgμL^{-1} in CH_2Cl_2) and cationic lipid (2 μL, 50 μgμL^{-1} in abs. ethanol) were mixed (weight ratio 1:2). The organic solvents were evaporated under a stream of nitrogen and further dried under high vacuum (> 2h). The resulting thin film was hydrated with phosphate buffer saline (PBS, pH 7.4, 100 μL) at room temperature for 1 h. The mixture was vortexed for one minute and sonicated (2×15 minutes) with 1 h rest between sonications using a bath-type sonicator. The liposomes were stored at 4 °C for 24 h prior to use. The liposome without DOPE was prepared as the same manner for DOPE-contained liposome excepted DOPE was not incubated.

Transfection procedure:

Human embryonic kidney cells (HEK293), colorectal adenocarcinoma (COLO 205), canine osteosarcoma (D-17), human cervical adenocarcinoma (HeLa) and human prostate adenocarcinoma (PC3) were grown in DMEM medium supplemented with 10% fetal calf serum (FCS), penicillin (100 unitsmL^{-1}), streptomycin (100 mgmL^{-1}) and l-glutamine (4 mM) at 37 °C, 5% CO_2. For transfection, the cells were seeded up to 1×10^4 cells/well in a 96-well plate, to give 50-70% confluence to be used on the next day. The growth medium was removed and the cells were washed with PBS and replaced with 100 μL of fresh serum-free DMEM medium. DNA (pCH110-encoding β-galactosidase)/cationic liposome complexes (lipoplexes) were prepared as follows. An appropriate volume of each cationic liposome (1 μgμL^{-1}) was added to the plasmid DNA (0.4 μL, 0.5 μgμL^{-1}) and the complex was incubated at room temperature for 30 min before being diluted with phosphate-buffered saline to make a final DNA concentration of 0.1 mg/mL. The lipoplexes (10 μL) were then added to the cells and left to be incubated at 37 °C, 5% CO_2. The cells were then washed with PBS and fresh growth medium was added and further incubated for 48 h. For Effectene^{TM} transfection, the method

was carried out according to the manufacturer's instruction and the same raios of plasmid DNA:Effectene^{TM} were used. After transfection, the β-galactosidase activity per well was estimated by adding 100 μL of substrate solution (4 mgmL^{-1} of o-nitrophenyl-galactopyranoside (ONPG),[25] 0.2 M soldium phosphate (pH 7.3) and 2 mM magnesium chloride) to the lysate in a 96-well plate. Absorbance of the product otho-nitrophenol at 405 nm was converted to % relative transfection by compared with positive control, Effectene^{TM}.

Transfection cytotoxicity

Cytotoxicities of the lipids **IAXc**, **IAXe**, **IAYd**, **IAZc**, **IBYd**, **IBYg**, **IBZh** and **IBZj** were assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay as described previously.[16] The experiment was performed in 96-well plates. The amount of cationic liposome and commercially available transfection agents, Effectene^{TM}, DOTAP and DC-Chol, per well was used the same as that used in the transfection experiments. After 24 h. incubation, the medium was removed and replaced with a phenol red-free medium (90 μL). MTT (3 mgmL^{-1}) was added (10 μL/well) to the cells, followed by MTT solubilization solution (Sigma) (100 μL) to dissolve the resulting crystals, and the absorbance was measured at 520 nm on a microplate reader. The change in metabolic activity was calculated as A_{520} with compound/A_{520} without compound.

Electron microscopy

The liposomes or their lipoplexes solution (10–20 μL) was dropped on the formvar grid and left it stands at room temperature for 1 min. The water was removed by touching the edge of the droplet to the edge of a filter paper, leaving the thin aqueous film on the grid. 1% Potassium phosphotungstate (PTA) (10 μL) was applied to the grid and left it stands for 1 min. The extra solution was similarly removed by touching to the edge of a filter paper. The negative stain liposomes were allowed to dry at room temperature for 1 night by placing onto a filter paper in a covered Petri dish. The samples were observed under transmission electron microscope operated at 200 kV.

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Legends

Figure 1. Cationic lipids with symmetric hydrophobic tails.

Figure 2. The structures of novel cationic lipids with asymmetric cholesterol-fatty acid tails.

Figure 3. The structures of diamines and fatty acids used in Scheme 2.

Figure 4. Gel retardation assay of DNA/cationic lipids complexes at a weight ratio of 1:20. Lanes marked "Control" contained DNA alone and was used as a control. The presence of a lower band indicated that DNA has migrated and has not been bound by the transfection compound.

Figure 5. Transfection screening activity of synthesized cationic lipids employing pCH110-encoding β-galactosidase (0.1 μg/well). The liposome formation was both with and without DOPE. The lipoplexes was used at DNA/lipids (w/w) ratios of 1:20. The transfection efficiencies of the lipids were compared to that of commercially available reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

Figure 6. Transfection efficiency of the selected lipids against HEK293 cells across the cationic lipid/DOPE weight ratio of 2:1, 1:1 and 1:2. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

Figure 7. Transfection efficiency of the selected lipids against HEK293 cells across the DNA/cationic lipid weight ratios of 1:10, 1:20 and 1:40 (w/w). The optimal liposome formation of each lipid from Figure 6 was used as described in the text. Transfection efficiency of the selected lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency (data not shown).

Figure 8. Effect of DNA amount for gene delivery. The optimal liposome formation (Figure6) and DNA/lipids complex (Figure7) of each lipid were used to mix with various amount of DNA from 0.1 to 0.4 μg. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency.

Figure 9. Effect of serum for transfection efficiency. The optimal liposome formation (Figure6), DNA/cationic lipid (Figure7) and amount of DNA per well (Figure8) of each lipid were used to transfer gene to HEK293 cell at various amount of serum. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene^{TM}, which calculated as 100% transfection efficiency.

Figure 10. Transfection efficiency of selected lipids toward COLO 205, D-17, HeLa and PC3 cell using optimum conditions from Figures 6-8. Transfection efficiency of the lipids was compared to that of the commercial reagent, Effectene^{TM}, DOTAP and DC-Chol. Transfection efficiency of Effectene^{TM} for each cell line was calculated as 100%.

Figure 11. Effect of transfection synthesized lipids on cell metabolic activity. Liposomes of cationic lipid with helper lipid, DOPE, were formed and added to DNA to form lipoplexes. These complexes were added to HEK293, COLO 205, D-17, HeLa and PC3 cell. Cell metabolic activity was determined by a MTT assay.

Figure 12. Transmission electron microscopic images of cationic liposomes **IAXc**, **IAXe, IAYd, IAZc, IBYd, IBYg, IBZh,** and **IBZj**.

Figure 13. Transmission electron microscopic images of lipoplexes prepared from optimized liposomes (**IAXc**, **IAXe, IAYd, IAZc, IBYd, IBYg, IBZh,** and **IBZj**)/DNA complexes.

Scheme 1. *Reagents and conditions*. a) diamines **A**–**C** (excess) (see Figure 3), CH_2Cl_2, 6 h; b) bromoacetic acid (4 equiv), DIC (4 equiv), DMF/CH_2Cl_2, 12 h; c) diamines **X**–**Z** (excess) (see Figure 3), CH_2Cl_2, 12 h; d) Dde-OH (excess), DMF/CH_2Cl_2, 12 h.

Scheme 2. *Reagents and conditions*. a) Cholesteryl chloroformate (4 equiv), pyridine (20 equiv), CH_2Cl_2, 12 h.; b) 5% N_2H_4/DMF, 2×30 min; c) Fatty acid (4 equiv, see Figure 3), DIC (4 equiv), CH_2Cl_2, 12 h.; d) 20% TFA/CH_2Cl_2, 2 h.

High Transfection Efficiency of Novel Cationic Lipids with Asymmetric Acyl-Cholesteryl Hydrophobic Tails

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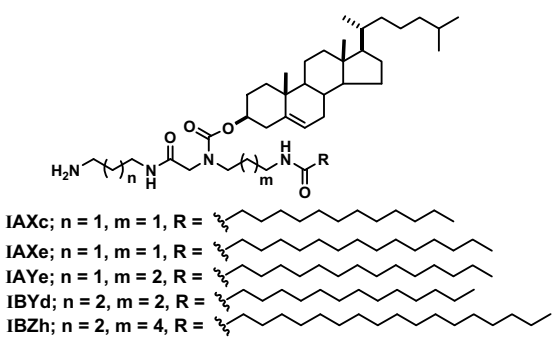

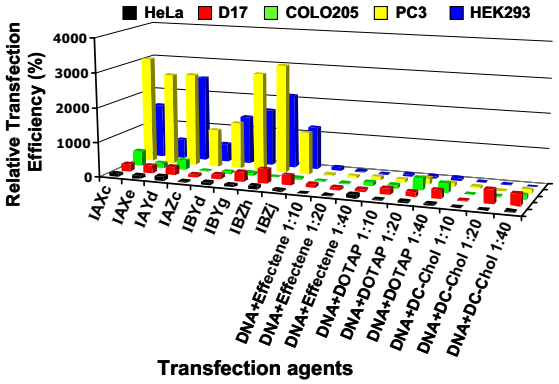

Cationic lipids with asymmetric acyl-cholesteryl hydrophobic tails were synthesized by solid phase chemistry. The transfection efficiency of these cationic lipids when formulated as liposome was investigated. Some of them exhibited higher transfection efficiency than the commercially available gene delivery reagent Effectene™, DOTAP and DC-Chol.