Supplementary Information

Effective Targeted Gene Delivery to Dendritic Cells via Synergetic Interaction of Mannosylated Lipid with DOPE and BCAT

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Lipid Precursor Synthesis Procedures

1,2,3,4,6 Penta-O-acetyl-α,β-D-Mannopyranoside (2). D-Mannose (10 g, 55.51 mmol) and pyridine (120 mL) were added to acetic anhydride (150 mL). After the reaction mixture was stirred at 22 °C for 20 h, the volatile species were co-evaporated with toluene (3 x 40 mL). The residue was extracted with ethyl acetate (3 x 50 mL) and washed with 2 N HCl (3 x 50 mL), water (50 mL), saturated $NaHCO_3$ (50 mL) and brine (40 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel flash chromatography using 2:1 hexane:EtOAc as eluent to give 1,2,3,4,6 penta-O-acetyl-α,β-D-mannopyranoside **2** (22.7 g, 99%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.07 (d, *J* = 1.5 Hz, 1H), 5.87 (d, *J* = 1.0 Hz, 1H), 5.47 (dd, *J* = 1.0, 3.0 Hz, 1H), 5.31 - 5.35 (m, 2H), 5.25 (m, 2H), 5.12 (dd, *J* = 3.6, 9.9 Hz, 1H), 4.25-4.33 (m, 2H), 4.15 (dd, *J* = 2.3, 12.0 Hz, 1H), 4.07 (dd, *J* = 2.5, 12.5 Hz, 1H), 3.799 (ddd, *J* = 2.5, 5.5, 8.0 Hz, 1H), 2.17 (s, 3H), 2.21 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H).

3'-Bromopropyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (3). BF_3•Et_2O (3.97 mL, 32.2 mmol) was added to a solution of 1,2,3,4,6-penta-O-acetyl-α,β-D-mannopyranoside (3.8 g, 9.20 mmol) and 3-bromo-1-propanol (0.99 mL, 11.04 mmol) in dry CH_2Cl_2. After the reaction mixture was stirred in the dark at 22 °C for 7 h, the reaction mixture was neutralized by adding saturated $NaHCO_3$. The resulting solution was washed with water (50 mL) and dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel flash chromatography using 2:1 hexane:EtOAc as eluent to give 3-bromopropryl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside **3** (1.4 g, 32%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.34-5.40 (m, 2H), 5.31 (m, 1H), 4.90 (d, *J* = 1.5 Hz, 1H), 4.34 (dd, *J* = 5.1, 12.3 Hz, 1H), 4.19 (dd, *J* = 2.1, 12.9 Hz,

1H), 4.05 - 4.07 (m, 1H), 3.94 - 4.01 (m, 1H), 3.57-3.67 (m, 3H), 2.23 (s, 3H), 2.18 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.0, 169.8, 169.7, 97.7, 69.5, 69.1, 68.7, 66.1, 65.6, 62.5, 32.0, 30.2, 20.9, 20.8; HRMS (ESI): $(M+H)^+$ *m:z* calc'd for $C_{17}H_{25}BrO_{10}$ = 468.0631, found 468.0873.

3'-Azidopropyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (4). NaN_3 (1,24 g, 19.18 mmol) was added to 3'-bromopropyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (1.5 g, 3.196 mmol) in DMF (10 mL). After the reaction mixture was stirred at 60 °C for 10 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with water (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel flash chromatography using 1.5:1 hexane:EtOAc as eluent to give 3'-azidopropyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside **4** (1.2 g, 87%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (m, 2H), 5.29 (m, 1H), 4.87 (d, *J* = 1.8 Hz, 1H), 4.32 (dd, *J* = 5.4, 12.3 Hz, 1H), 4.19 (dd, *J* = 3.0, 11.7 Hz, 1H), 4.00 - 4.05 (m, 1H), 3.83 - 3.91 (m, 1H), 3.57 - 3.62 (m, 1H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.21 (s, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.90- 1.99 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.2, 170.1, 169.9, 97.8, 69.6, 69.2, 68.8, 66.2, 65.0, 62.6, 48.3, 28.8, 21.1, 20.9; HRMS (ESI): $(M+H)^+$ *m:z* calc'd for $C_{17}H_{25}N_3O_{10}$ = 431.1540, found 431.1927.

3'-Azidopropyl-O-α-D-mannopyranoside (5). NaOMe (1.5 mL of 0.1 N, 27.76 mmol) was added to 3'-azidopropyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (1.2g, 2.278 mmol) in MeOH (15 mL). After stirring at 22 °C for 1 h, the reaction mixture was neutralized by adding Amberlite-H^+. The resin was filtered off and the solvent was evaporated to give 3'-azidopropyl-

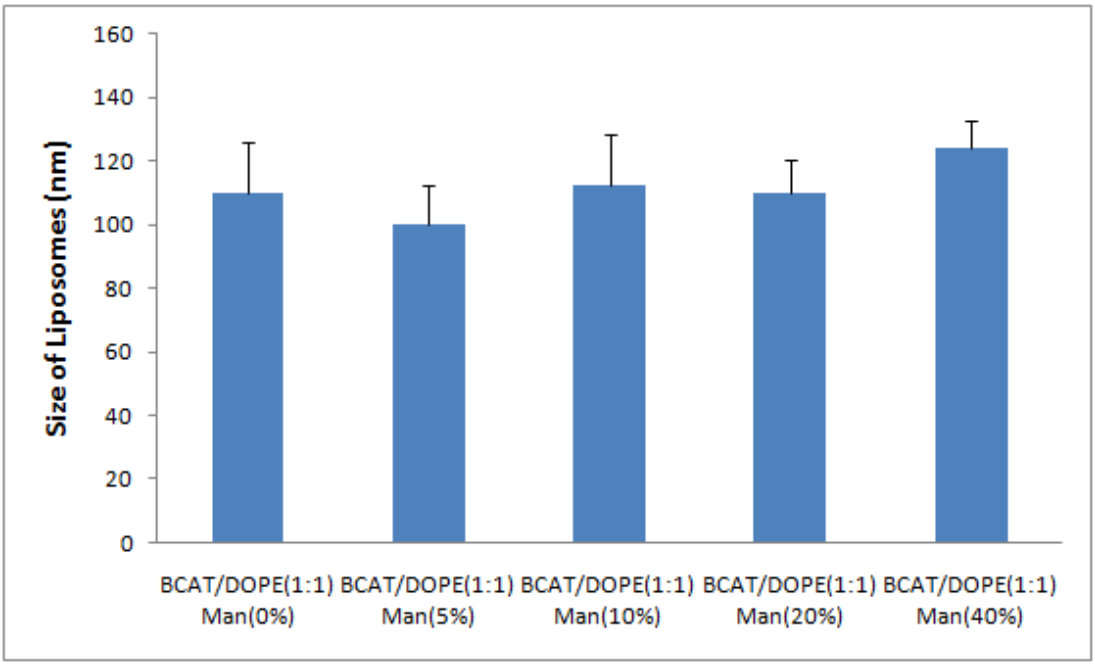
O-α-D-mannopyranoside **5** (0.6 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.82 (d, *J* = 1.2 Hz, 1H), 3.87-3.91 (m, 3H), 3.78 (dd, *J* = 5.6, 11.9 Hz, 1H), 3.73 (dd, *J* = 3.3, 9.3 Hz, 1H), 3.66 (m, 1H), 3.53 - 3.61 (m, 2H), 3.42-3.50 (m, 2H), 1.84 - 1.97 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.7, 101.7, 74.7, 72.6, 72.2, 68.6, 65.5, 62.8, 30.0; HRMS (ESI): $(M+H)^+$ *m:z* calc'd for $C_9H_{17}N_3O_6$ = 263.1117, found 263.1256.

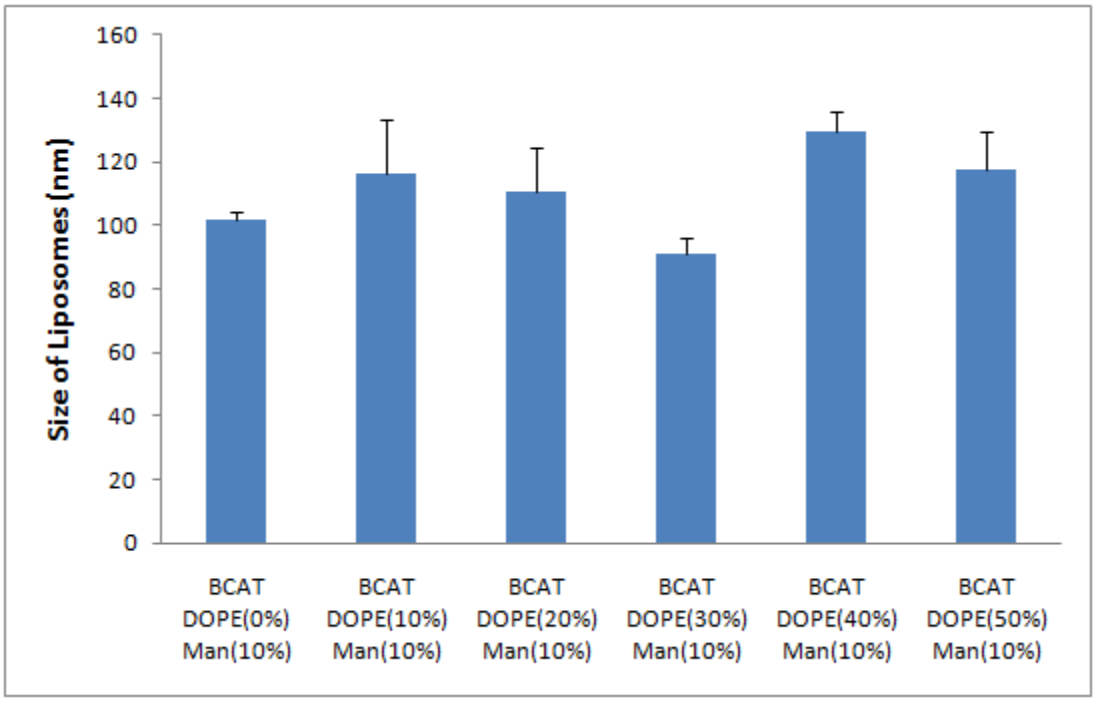
1-Allyl-3-*t*-butyl-dimethylsilyl-*rac*-glycerol (12). Imidazole (11.33 g, 166.46 mmol) was added to 1-allyloxy-propane-2,3-diol (10 g, 75.66 mmol) in DMF (50 mL). After dropwise addition of TBDMSCl (13.68 g, 90.79 mol) in DMF (50 mL) at 0 °C, the solution was stirred at 22 °C for 2 h. The mixture was washed with LiCl-saturated water (200 mL) and the resulting solution extracted with Et_2O (3 x 200 mL). The resulting organic phase was washed with water (3 x 100 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel flash chromatography using 5:1 hexane:EtOAc as eluent to give compound **12** (15.5 g, 83%) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (m, 1H), 5.14 (dd, *J* = 17, 2 Hz, 1H), 5.00 (dd, *J*= 11, 2 Hz, 1 H), 3.84 (q, 5 Hz, 1 H), 3.77 (d, *J* = 5 Hz, 2H), 3.63 (d, *J* = 5 Hz, 2 H), 3.14 (d, *J* = 5 Hz, 2 H), 2.64 (d, *J* = 5 Hz, 1 H), 0.893 (s, 9 H), 0.00 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$): 134.6, 117.2, 72.4, 70.95, 70.7, 64.1, 25.9, 18.3,- 5.38; HRMS (ESI): $(M+H)^+$ *m:z* calc'd for $C_{12}H_{26}O_3Si$ = 246.1651, found 246.2338.

1,2-Diallyl-3-*t*-butyl-dimethylsilyl-*rac*-glycerol (13). Compound **12** (2 g, 8.12 mmol) in THF (25 mL) was added dropwise to NaH (290 mg, 12.18 mmol) in THF (25 mL) at 0 °C. After the resulting mixture was stirred for 30 min at 22 °C, a solution of allyl bromide (1.96 g, 16.25 mmol) in THF (10 mL) was added dropwise and the reaction mixture was stirred for 14 h. The reaction

was quenched by the addition of saturated aqueous NH_4Cl solution (200 mL). The aqueous phase was extracted with Et_2O (3 x 100 mL) and the combined organic phases dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel flash chromatography using 10:1 hexane:EtOAc as eluent to give compound **13** (2.1 g, 90%) as a clear oil.^{1}H NMR (300 MHz, $CDCl_3$): 5.95 (m, 2H), 5.31 (dd, *J* = 17, 2 Hz, 2H), 5.15 (dd, *J*= 11, 2 Hz, 1 H), 4.16 (d, *J* = 6 Hz, 2 H), 4.02 (d, *J* = 5 Hz, 2H), 3.69 (d, *J* = 5 Hz, 2 H), 3.59-3.48 (m, 3H), 0.907 (s, 9 H), 0.07 (s, 6 H), ^{13}C NMR (75 MHz, $CDCl_3$): 135.6, 135.1, 117.0, 116.9, 79.0, 72.6, 71.65, 70.3, 63.2, 26.1, 18.5;-5.15; HRMS (ESI): $(M+H)^+$ *m:z* calc'd for $C_{15}H_{30}O_3Si$ = 286.1964, found 286.1856.

DLS Particle Size Analysis




Size of Liposomes (nm)
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140
120
100
80
60
40
20
0
BCAT DOPE(0%) Man(10%)
BCAT DOPE(10%) Man(10%)
BCAT DOPE(20%) Man(10%)
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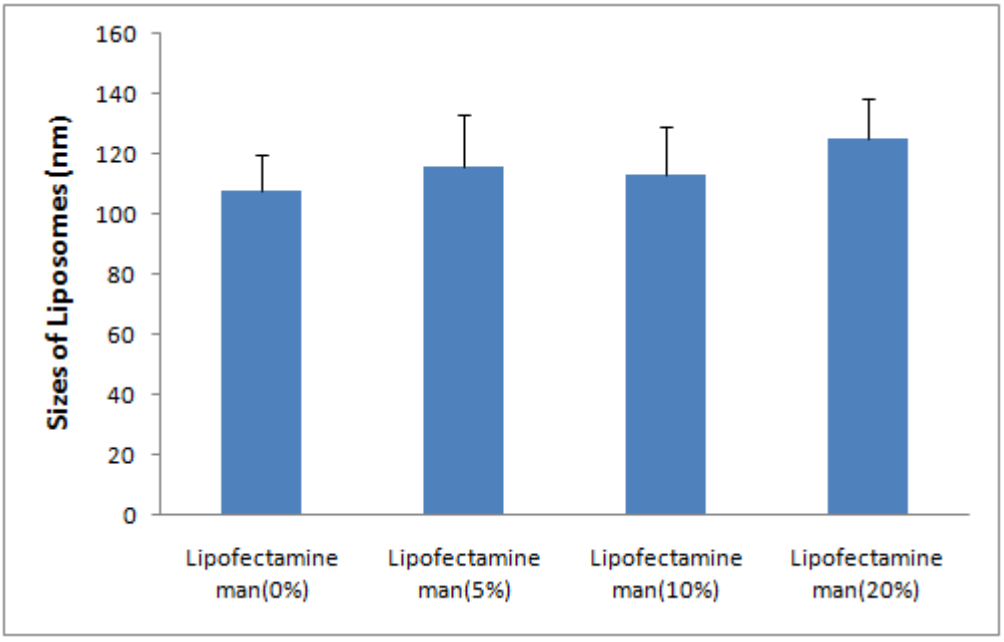
Sizes of Liposomes (nm)
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140
120
100
80
60
40
20
0
Lipofectamine man(0%)
Lipofectamine man(5%)
Lipofectamine man(10%)
Lipofectamine man(20%)