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Convenient synthesis of polycationic amphiphiles by the Fukuyama reaction

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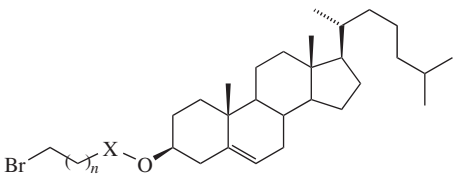
Cholesterol-containing spermine derivatives for gene delivery were synthesised *via* the Fukuyama reaction.

Natural polyamines such as putrescine, spermidine and spermine play a significant role in biological systems by affecting DNA replication and translation, membrane stability, and activity of kinases and topoisomerases.[1] The broad activity spectrum of polyamines gave impetus for creating various bioactive compounds on their basis, including synthetic ionophores,[2–4] antiviral[5] and antitumor[6] agents, endotoxin antagonists[7] and DNA condensing agents.[8,9]

The ability of natural polyamines to bind DNA molecules and pack it into small dense particles was used to create transporting agents for delivery of therapeutic nucleic acids (DNA, RNA and oligonucleotides) into cells in order to provide a therapeutic effect.[8–11] It was found that lipophilic derivatives of polyamines condense DNA more efficiently than polyamines[12] and the difference in the numbers of nitrogen atoms and methylene units between them determines the capability of polyamines to interact with DNA differently, causes DNA to change conformation[1,13] and affects the efficiency of nucleic acid transfer.[14]

Lipopolyamine molecules can be considered as a combination of two structural units, *viz.*, a hydrophobic substituent and a polyamine linked by a certain type of chemical bond. Cholesterol[1,13,15,16] and other steroids,[17] as well as long-chain hydrocarbon substituents,[18–22] are most commonly used as the hydrophobic domain. The polyamine component can be of natural or synthetic origin.[1,13–21] The covalent bonding of structural units is performed directly or *via* spacers of various length[17–19] *via* a carbamoyl[1,13,16] or amide[19,20] bond. The binding type determines the stability of the cationic amphiphile and its toxicity, which should be taken into account when developing carrier systems. It is known that alkyl lipids possess high stability and are hence more toxic than acyl lipids that are readily hydrolyzed by endogenous esterases in a cell. The carbamoyl group provides the most reasonable balance between amphiphile stability and toxicity.[10]

Despite considerable interest in the lipophilic derivatives of polyamines, syntheses of these compounds are often labor-intensive and involve multiple stages. For example, to obtain lipopolyamines, in which cholesterol is bound to the polycation *via* spacers of various lengths, a spacer group is first attached to the polyamine matrix and only then the resulting fragment is linked to the activated hydrophobic component.[7,15,16,19] The overall yield of the target compounds in such a synthesis varies from 14 to 25%.[7,16] A solid-phase method was proposed for synthesising cholesterol-containing polycationic amphiphiles using a 2-chlorotritylchloride resin, which allowed the yield of lipopolyamines to be increased to 87%.[23]



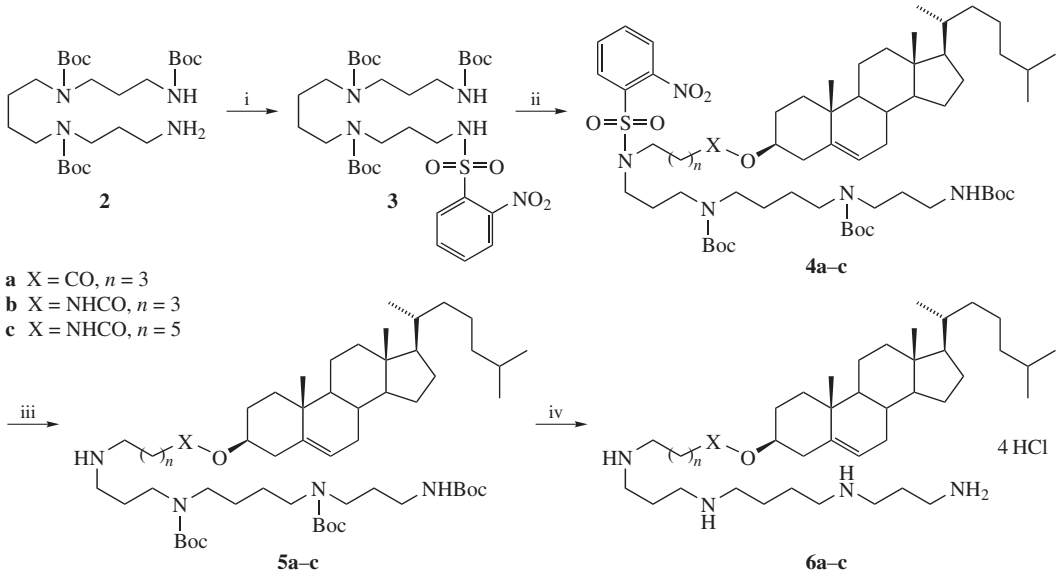
1a X = CO, *n* = 3
1b X = NHCO, *n* = 3
1c X = NHCO, *n* = 5

Here we present a convenient method for synthesising polycationic amphiphiles based on cell molecular components such as cholesterol and spermine. We searched for new DNA delivery agents possessing low cytotoxicity and high activity by varying the length and type of the spacer between the spermine and cholesterol. The synthetic approach is based on the Fukuyama reaction between 2-nitrobenzenesulfonamides, which are obtained from primary amines, and alkyl halides, followed by removal of the 2-nitrobenzenesulfonyl group to give a secondary amine.[24,25] To implement this approach, at the first stage, we obtained bromo derivatives of cholesterol **1a–c**† (see Online Supplementary Materials).

Lipopolyamines were synthesised from compound **2**, which was obtained from spermine by regioselective introduction/removal of protective groups by a known procedure.[19] N-Sulfonylation of compound **2** by treatment with 2-nitrobenzenesulfonyl chloride in triethylamine gave sulfonamide **3** in 88% yield.‡ The key step of the synthesis involved condensation of protected polyamine **3** with brominated derivatives of cholesterol **1a–c** in the presence of cesium carbonate. In a typical procedure, cesium carbonate (0.254 mmol) and the corresponding bromide (**1a–c**) (0.306 mmol) were successively added to a solution of compound **3** (0.254 mmol) in dry DMF (3 ml). The

† **1a**: mp 112–114 °C. **1b**: mp 92–94 °C. **1c**: mp 106–108 °C.

‡ **3**: molecular sieves 4 Å (1.0 g), triethylamine (0.239 ml, 1.716 mmol) and 2-nitrobenzenesulfonyl chloride (0.228 g, 1.02 mmol) were added to a cooled (0 °C) solution of compound **2** (0.431 g, 0.858 mmol) in dry CH_2Cl_2 (5 ml). The reaction mixture was stirred for 1 h at 24 °C. The molecular sieves were filtered off and washed with CH_2Cl_2; the solvent was removed *in vacuo*. The residue was chromatographed on a column with silica gel using a $CHCl_3$–MeOH mixture (50:1) as the eluent. Compound **3** was obtained (0.507 g, 88%). ^{1}H NMR (Bruker DPX-300, 300 MHz, $CDCl_3$, internal standard $SiMe_4$) δ: 1.27 [br. s, 31H, $3CMe_3$ and $NCH_2(CH_2)_2CH_2N$], 1.52–1.71 (m, 4H, $2NCH_2CH_2CH_2N$), 2.92–3.11 (m, 8H, $4NCH_2$), 3.11–3.30 (m, 4H, $2NHCH_2$), 7.59–7.71 (m, 2H), 7.71–8.89 (m, 1H) and 8.00–8.11 (m, 1H, C_6H_4). ^{13}C NMR, δ: 25.96, 28.54, 28.59, 28.93, 37.91, 41.03, 43.78, 44.23, 46.82, 47.05, 79.16, 79.74, 80.02, 125.25, 130.93, 132.68, 133.51, 148.29, 156.17. MS (MALDI-TOF Bruker Ultraflex spectrometer), *m/z*: 710.341 $[M + Na]^+$; calc. for $C_{31}H_{53}N_5NaO_{10}S$: 710.3411 $[M + Na]^+$.



Scheme 1 *Reagents and conditions*: i, 2-$NO_2C_6H_4SO_2Cl$, TEA, 24 °C; ii, bromides **1a–c**, Cs_2CO_3, DMF, 60 °C; iii, PhSH, K_2CO_3, DMF, 24 °C; iv, 4 N HCl, dioxane, 24 °C.

reaction mixture was stirred for 1 h at 60 °C, and the precipitate was filtered off using Celite® 545. Removal of the solvents *in vacuo* followed by chromatographic isolation gave compounds **4a–c** in 86–94% yields. The resulting compounds were characterized by mass-spectrometric data, which were shown to contain the expected molecular ion signals, and by ^{1}H and ^{13}C NMR spectroscopic data, in which aggregate signals of the polyamine and hydrophobic components were observed.§

Unblocking of amino groups in compounds **4a–c** started in early removal of 2-nitrobenzenesulfonyl protecting group on treatment with thiophenol in the presence of potassium carbonate to give secondary amines **5a–c** in high yields. The final removal of *tert*-butoxycarbonyl groups was achieved by treatment of compounds **5a–c** with 4 N HCl in dioxane for 2 h. After recrystallisation from a chloroform–ethanol mixture, lipophilic polyamines **6a–c** were obtained in 77, 78 and 99% yields, respectively. The structures of the resulting compounds were confirmed by NMR spectroscopic and mass-spectrometric data.¶

Thus, using the Fukuyama reaction, we synthesised polycationic amphiphiles based on spermine and cholesterol, which differ in the length of the spacer group (4 or 6 methylene links) and bonding type (ester or carbamoyl bond).

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§ **4a**: ^{1}H NMR, δ: 0.61 (s, 3H, 18-Me), 0.79 (d, 3H, 27-Me, *J* 6.5 Hz), 0.80 (d, 3H, 26-Me, *J* 6.5 Hz), 0.83 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.90–2.00 [m, 38H, Chol protons, $2CH_2(CH_2)_2CH_2$, $2NCH_2CH_2CH_2N$], 1.36 (br. s, 18H) and 1.39 (br. s, 9H, $3CMe_3$), 2.11–2.33 (m, 4H, 4-CH_2, CH_2COO), 2.95–3.12 (m, 8H, $4NCH_2$), 3.12–3.30 [m, 6H, CH_2NH, $CH_2N(CO_2)CH_2$], 4.51 (tt, 1H, 3-H, *J* 4.2 Hz, *J* 11.2 Hz), 5.01–5.26 (m, 1H, NHCO), 5.26–5.34 (m, 1H, 6-H), 7.50–7.59 (m, 1H), 7.59–7.70 (m, 2H) and 7.87–8.00 (m, 1H, C_6H_4). ^{13}C NMR, δ: 11.94, 18.80, 19.39, 21.11, 22.03, 22.64, 22.89, 23.90, 24.36, 25.61, 25.79, 27.87, 28.07, 28.29, 28.53, 31.94, 31.98, 34.00, 35.86, 36.26, 36.68, 37.07, 37.67, 38.22, 39.60, 39.82, 42.40, 43.94, 44.54, 45.19, 46.77, 47.07, 50.12, 56.23, 56.78, 74.02, 79.58, 122.73, 124.24, 130.80, 131.73, 133.50, 133.58, 139.71, 148.13, 155.50, 156.10, 172.51. MS, *m/z*: 1178.741 $[M + Na]^+$, calc. for $C_{63}H_{105}N_5NaO_{12}S$: 1178.7378 $[M + Na]^+$.

4b: ^{1}H NMR, δ: 0.61 (s, 3H, 18-Me), 0.79 (d, 3H, 27-Me, *J* 6.5 Hz), 0.80 (d, 3H, 26-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.90–2.00 [m, 38H, Chol, $2CH_2(CH_2)_2CH_2$, $2NCH_2CH_2CH_2N$], 1.36 (br. s, 18H) and 1.39 (br. s, 9H, $3CMe_3$), 2.11–2.33 (m, 2H, 4-CH_2), 2.91–3.12 (m, 10H, CH_2NHBoc, $4NCH_2$), 3.12–3.30 [m, 6H, $CH_2NHCOOChol$, $CH_2N(CO_2)CH_2$], 4.40 (tt, 1H, 3-H, *J* 4.2 Hz, *J* 11.3 Hz), 4.55–4.96 (m, 1H, NHCOOChol), 4.96–5.25 (m, 1H, NHBoc), 5.25–5.33 (m, 1H, 6-H), 7.50–7.59 (m, 1H), 7.59–7.70 (m, 2H) and 7.87–8.00 (m, 1H, C_6H_4). ^{13}C NMR, δ: 11.97, 18.84, 19.44, 21.17, 22.67, 22.92, 23.95, 24.40, 25.61, 26.01, 27.20, 27.57, 28.11, 28.30, 28.58, 32.01, 35.90, 36.31, 36.54, 36.69, 37.13, 37.80, 38.70, 39.63, 39.87, 40.36, 42.44, 44.16, 44.70, 45.57, 46.70, 50.15, 56.27, 56.82, 74.36, 79.66, 122.56, 124.27, 130.83, 131.77, 133.48, 133.61, 139.98, 148.18, 155.56, 156.16, 156.35. MS, *m/z*: 1193.762 $[M + Na]^+$, calc. for $C_{63}H_{106}N_6NaO_{12}S$: 1193.7487 $[M + Na]^+$.

4c: ^{1}H NMR, δ: 0.61 (s, 3H, 18-Me), 0.79 (d, 3H, 27-Me, *J* 6.5 Hz), 0.80 (d, 3H, 26-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.90–2.00 [m, 39H, Chol, $NHCH_2(CH_2)_2CH_2N$, $2NCH_2CH_2CH_2N$, $CH_2(CH_2)_4CH_2$], 1.37 (br. s, 18H) and 1.39 (br. s, 9H, $3CMe_3$), 2.11–2.35 (m, 2H, 4-CH_2), 2.92–3.13 (m, 10H, CH_2NHCOO, $4NCH_2$), 3.13–3.33 [m, 6H, $CH_2NHCOOChol$, $CH_2N(CO_2)CH_2$], 4.40 (tt, 1H, 3-H, *J* 4.3 Hz, *J* 11.3 Hz), 4.50–4.85 (m, 1H, NHCOOChol), 4.96–5.25 (m, 1H, NHBoc), 5.26–5.35 (m, 1H, 6-H), 7.51–7.59 (m, 1H), 7.59–7.70 (m, 2H) and 7.87–8.00 (m, 1H, C_6H_4). ^{13}C NMR, δ: 12.00, 18.87, 19.48, 21.20, 22.70, 23.00, 23.98, 24.43, 25.91, 26.31, 26.37, 27.60, 28.09, 28.14, 28.36, 28.60, 30.30, 32.05, 35.93, 36.34, 36.72, 37.16, 37.81, 38.74, 39.66, 39.90, 40.88, 42.47, 44.13, 44.67, 45.28, 46.82, 47.48, 50.19, 56.30, 56.85, 74.36, 79.65, 122.57, 124.29, 130.87, 131.73, 133.57, 133.68, 140.03, 148.20, 155.59, 156.18, 156.33. MS, *m/z*: 1221.124 $[M + Na]^+$, calc. for $C_{65}H_{110}N_6NaO_{12}S$: 1221.7800 $[M + Na]^+$.

¶ Compounds **6a–c** decompose above 300 °C.

6a: ^{1}H NMR (D_2O) δ: 0.61 (s, 3H, 18-Me), 0.77 (d, 3H, 27-Me, *J* 6.5 Hz), 0.79 (d, 3H, 26-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.90–2.00 [m, 42H, Chol protons, $NHCH_2(CH_2)_2CH_2N$, $2NCH_2CH_2CH_2N$, $CH_2(CH_2)_2CH_2$], 2.11–2.32 (m, 2H, 4-CH_2), 2.90–3.15 (m, 16H, CH_2COO, $7CH_2N$), 4.20–4.40 (m, 1H, 3-H), 5.20–5.35 (m, 1H, 6-H). MS (CI, 1100 LC/MS Agilent Technologies spectrometer), *m/z*: 671.5 $[M – 4HCl + H]^+$, calc. for $C_{42}H_{78}N_4O_2$: 670.6125 $[M – 4HCl]^+$.

6b: ^{1}H NMR (D_2O) δ: 0.61 (s, 3H, 18-Me), 0.77 (d, 3H, 27-Me, *J* 6.5 Hz), 0.79 (d, 3H, 26-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.90–2.00 [m, 42H, Chol protons, $NHCH_2(CH_2)_2CH_2N$, $2NCH_2CH_2CH_2N$, $CH_2(CH_2)_2CH_2$], 2.11–2.32 (m, 2H, 4-CH_2), 2.90–3.15 (m, 16H, $8CH_2N$), 4.15–4.40 (m, 1H, 3-H), 5.18–5.38 (m, 1H, 6-H). MS, *m/z*: 686.6 $[M – 4HCl + H]^+$, calc. for $C_{42}H_{79}N_5O_2$: 685.6234 $[M – 4HCl]^+$.

6c: ^{1}H NMR (D_2O) δ: 0.61 (s, 3H, 18-Me), 0.77 (d, 3H, 27-Me, *J* 6.5 Hz), 0.79 (d, 3H, 26-Me, *J* 6.5 Hz), 0.84 (d, 3H, 21-Me, *J* 6.5 Hz), 0.94 (s, 3H, 19-Me), 0.90–2.05 [m, 46H, Chol protons, $NHCH_2(CH_2)_2CH_2N$, $2NCH_2CH_2CH_2N$, $CH_2(CH_2)_4CH_2$], 2.13–2.33 (m, 2H, 4-CH_2), 2.91–3.15 (m, 16H, $8CH_2N$), 4.15–4.40 (m, 1H, 3-H), 5.18–5.38 (m, 1H, 6-H). MS, *m/z*: 714.6 $[M – 4HCl + H]^+$, calc. for $C_{44}H_{83}N_5O_2$: 713.6547 $[M – 4HCl]^+$.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2009.09.005.

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