Supplementary Material (ESI) for Chemical Communications

Synergistic effects in gene delivery – A structure-activity approach to the optimisation of hybrid dendritic-lipidic transfection agents

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SUPPLEMENTARY INFORMATION

Contents

1. DNA Binding and Biological Assays

1.1 Gel Retardation Studies

A solution of DNA (1 μg/10 μl) was prepared in 150 mM NaCl, 20 mM PIPES at pH 7.2. Varying amounts of polyamine were dissolved in buffer and added to make up solutions of the desired polyamine:DNA weight ratio. The mixtures were incubated at 4^{o}C for 20 minutes then 3 μl of loading dye was added. A 10 μl sample of each solution was then run on a 0.75 % agarose gel (70 V, 90 min). DNA was visualized using ethidium bromide.

1.2 Ethidium Bromide Exclusion

A solution of Calf Thymus DNA (0.5 mg/ml) was prepared in 150 mM NaCl, 20 mM PIPES at pH 7.4. Ethidium bromide (10 mg/ml) was diluted with 150 mM NaCl, 20 mM PIPES at pH 7.4 to give a final concentration of 0.1 mg/ml. Ethidium bromide (0.5 μg) was added to a 96 well plate along with PIPES buffer and then CT DNA (5 μg). This was incubated at room temperature for 15 minutes. Appropriate amounts of dendrimer were dissolved in PIPES buffer and added to the 96 well plate to achieve the desired weight ratio. The polyplexes and ethidium bromide were incubated at room temperature for 5 minutes before exciting at 540 nm and reading the fluorescence at 595 nm (Cary Eclipse, Palo Alto, CA). The fluorescence values were normalized to wells containing only DNA and ethidium bromide. Experiments were performed in triplicate.

1.3 Transfection

DNA/Dendrimer complexes were prepared at room temperature by dissolving 1.5 μg of DNA in 150 mM NaCl, 20 mM PIPES, pH 7.4. An equal volume of dendrimer solution was added so to achieve the desired DNA:dendrimer ratio. Polyplexes were then incubated at room temperature for 20 minutes. Cells (HEK293 and HeLa) were cultured in DMEM supplemented with 10 % horse serum and 1 % penicillin-streptomycin according to ATCC protocols and plated in 24-well plates at 10 x 10^4 cells/well at least 24 hours before transfection. Immediately before transfection the

medium was replaced with fresh serum-free medium and 50 µl of polyplex solution was added to each well (final DNA concentration, 0.5 µg plasmid/well). After 4 hours of transfection the medium was replaced with serum containing growth medium and cells were left for a further 20 hrs. Luciferase expression was carried out using the Promega luciferase assay system (Promega, Mdison, WI). Luciferase activity was measure in relative light units (RLU) using a Lumat LB 9507 luminometer (Berthold, GmbH, Germany). Results were normalized to total cell protein using the Pierce BCA protein assay kit (Pierce, Rockford, IL).

1.4 Cytotoxicity

Dendrimer solutions were prepared at room temperature by dissolving the required weight ratio in serum-free DMEM (1 ml) at room temperature. Dendrimers were then incubated at room temperature for 20 minutes. Cells (HEK293) were cultured in DMEM supplemented with 10 % horse serum and 1 % penicillin-streptomycin according to ATCC protocols and plated in 96-well plates at 2 x 10^4 cells/well at least 24 hours before transfection. Immediately before transfection the medium was replaced with fresh serum-free medium and 200 µl of varying dendrimer solution was added to each well. After 4 hours of transfection the medium was replaced with serum containing growth medium and cells were left for a further 20 hours. After this time Cell Titer Blue© (40 µl) was added to each well and the cells were incubated at 37°C for a further 2 hours. The absorbance of each well was then measured at 570 and 600 nm.

1.5 Dynamic Light Scattering

DNA/Dendrimer solutions were made up at the desired weight ranges in PBS buffered water (2 ml). The solutions were vortexed and then left to settle for 10 minutes before the sizes were measured on a Brookhaven Instruments Corporation 90Plus Particle Size Analyzer at 25^{o}C at 1 minute per run and 5 repeat runs for each sample.

1.6 Confocal Microscopy

DNA/Dendrimer/YOYO complexes were prepared at room temperature by dissolving 8 µg of DNA in 150 mM NaCl, 20 mM PIPES, pH 7.4 then adding 2 µl of YOYO (1 mg/ml in DMSO, Invitrogen) to the DNA. An equal volume of dendrimer solution was added so to achieve the desired DNA:dendrimer ratio (4:1, w:w). Polyplexes were then incubated at room temperature for 20 minutes. Cells (HEK293) were cultured in DMEM supplemented with 10% horse serum and 1% penicillin-streptomycin according to ATCC protocols and plated in 6-well plates at 200 x 10^4 cells/well at least 24 hours before transfection with a glass cover slip in each well. Immediately before transfection the medium was replaced with fresh serum-free medium and 50 µl of polyplex solution was added to each well (final DNA concentration, 2 µg plasmid/well). After the required transfection time the medium was removed, washed with PBS and the cells were fixed using formaldehyde and mounted on a viewing plate. The cells were then imaged on a confocal microscope (Olympus Model BX60) equipped with a 100x oil immersion lens using an Argon laser to visualize the dye.

2. Synthetic Methods and Characterisation Data

2.1 Synthesis of Boc-Protected Chol-G1

G1 with an amine group at the focal point (prepared according to M. A. Kostiainen, G. R. Szilvay, J. Lehtinen, D. K. Smith, M. B. Linder, A. Urtti and O. Ikkala, *ACS Nano* 2007, **1**, 103-113) (200 mg, 0.112 mmol) was dissolved in DCM (15 ml) and lauroyl chloride (200 mg, 0.447 mmol) was added drop wise over 30 seconds followed by Et_3N (77.8 µl, 0.559 mmol). The reaction was stirred for 16 h and monitored using TLC. The crude product was concentrated *in vacuo*. TLC showed impurity present so crude material was placed down a Sephadex LH-20 column being eluted with MeOH. Product fractions were collected and concentrated *in vacuo*. Product was obtained as a colourless oil/sticky solid (140 mg, 57%).

R_f 0.45 (95:5 DCM:MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.51-5.31 (m, N*H* amide, 3H); 5.26 (s, C*H*, 1H); 5.17-4.81 (m, N*H*BOC, N*H*COOCholesterol, 4H); 4.28 (s, C*H*, 1H) 3.61 (s, CCH_2O, 6H); 3.52 (s, OCH_2CH$_2$, 6H) 3.28-2.95 (br m, CH_2N, 36H); 2.45 (s, OCH$_2$CH_2, 6H); 2.26-2.08 (m, CH_2, 2H); 1.91-0.93 (m, CH_2CH$_2$N, (CH_3)$_3$C, CH_2CH$_2$NBOC, multiple CH_2's on cholesterol, 133H); 0.88 (s, CH_3, 3H); 0.80 (d, CH_3, J = 6.4 Hz, 3H); 0.75 (d, CH_3, J = 4.9 Hz, 6H); 0.56 (s, CH_3, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.21, 170.56 (*C*ONH (amide)); 156.10, 155.43, 154.80 (N*C*OO^t-Bu x9, multiple overlapping peaks); 139.67 (*C* alkene); 122.47 (*C*H alkene); 79.73, 79.03, 78.71 (*C*(CH$_3$)$_3$ x9, multiple overlapping peaks); 73.87 (*C*H) 69.69 (C*C*H$_2$O); 67.97 (O*C*H$_2$CH$_2$); 58.63 (*C*CH$_2$O); 56.60, 56.05 (*C*H/*C*H$_3$); 49.93 (*C*H/*C*H$_3$); 47.54, 46.55, 45.85, 45.58, 45.45, 44.19, 43.63, 42.61, 42.23, 39.65, 39.44, 38.93, 38.52, 38.00, 37.66, 37.26, 36.95, 36.49, 36.11 (*C*H$_2$ and OCH$_2$*C*H$_2$, multiple overlapping peaks); 35.72 (*C*H/*C*H$_3$); 33.31 (*C*H$_2$); 31.80 (*C*H/*C*H$_3$); 29.74 (*C*H/*C*H$_3$) 28.41, 28.18, 27.94 ((*C*H$_3$)$_3$CO x27, multiple overlapping peaks); 26.43, 25.96, 25.34, 25.06, 24.22, 23.74 (*C*H$_2$CH$_2$N, *C*H$_2$CH$_2$NH and *C*H$_2$'s multiple overlapping peaks); 22.79, 22.53 (*C*H/*C*H$_3$); 20.96 (*C*H$_2$); 19.30, 18.66, 11.81 (*C*H/*C*H$_3$). DEPT carried out. ESI-MS (m/z): Calc. Value for $C_{116}H_{211}N_{13}O_{26}$ 2203.99; found: 1192.7 (35%); 1124.8 (95%, $[M+2Na]^{2+}$); 1113.7 (30%, $[M+H+Na]^{2+}$); 1102.7 (22%, $[M+2H]^{2+}$); 1002.7 (70%).

HR-MS (ESI) Calc. Value for $[C_{116}H_{211}N_{13}O_{26} + 2Na]^{2+}$ 1124.7583; found 1124.7629. ν_{max} (solid): 3332*w* (N-H), 2970*m*, 2931*m* (C-H), 1685*s*, 1627*m* (C=O), 1508*m*, 1365*m*, 1246*s*, 1161*s*, 871*m*, 775*m*.

2.2 Synthesis of Chol-G1

Boc-protected Chol-G1 was dissolved in MeOH (5 ml) and stirred at room temperature. HCl gas was then bubbled through for 20 s and then the solution was left to stir for 1 h. The solution was then concentrated *in vacuo* and the product was obtained. Yield – quantitative.

^{1}H NMR (CD_3OD, 400 MHz) δ 5.36 (s, C*H*, 1H); 4.30 (s, C*H*, 1H) 3.76-2.92 (m, C*CH*$_2$O, O*CH*$_2$CH$_2$, C*H*$_2$NH, C*H*$_2$NH$_2$ and 6H on cholesterol, 54H); 2.70 (s, OCH$_2$C*H*$_2$, 6H); 2.75-1.76 (m, internal C*H*$_2$ on spermine and 7H from cholesterol, 31H); 1.60-1.07 (m, C*H*$_2$'s on cholesterol, 17H); 1.00 (s, C*H*$_3$, 3H); 0.88 (d, C*H*$_3$, J = 6.4 Hz, 3H); 0.84 (d, C*H*$_3$, J = 4.8 Hz, 6H); 0.65 (s, C*H*$_3$, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 174.75, 174.32, 173.93, 173.54 (*C*ONH (amide)); 141.31 (*C* alkene); 123.72 (*C*H alkene); 70.84 (*CH*^{A}) 70.19 (C*C*H$_2$O); 69.30 (O*C*H$_2$CH$_2$); 60.49 (*C*CH$_2$O); 58.23, 57.66 (*C*H/*C*H$_3$); 51.73 (*C*H/*C*H$_3$); 46.73, 46.13, 44.00, 43.61, 41.23, 40.81, 39.89, 38.49, 38.16, 37.89, 37.49, 37.23, 34.56, 33.33, 33.17, 29.47, 29.27, 28.16, 27.22, 27.02, 25.95, 25.45, 25.06, 24.71 (*C*H$_2$CH$_2$N, *C*H$_2$CH$_2$NH and *C*H$_2$'s multiple overlapping peaks and *C*H/*C*H$_3$ and OCH$_2$*C*H$_2$); 23.39, 23.14 (*C*H/*C*H$_3$); 22.30 (*C*H$_2$); 20.06, 19.42, 12.50 (*C*H/*C*H$_3$). ESI-MS (m/z): Calc. Value for $C_{71}H_{139}N_{13}O_8$ 1302.95; found: 1303.1 (5%, $[M+H]^+$); 652.1 (95%, $[M+2H]^{2+}$); 435.0 (40%, $[M+3H]^{3+}$). HR-MS (ESI) Calc. Value for $[C_{71}H_{139}N_{13}O_8 + 2H]^{2+}$ 652.0508; found 652.0509.

2.3 Synthesis of Boc-Protected Chol-G2

G2 with an amine group at the focal point (prepared according to M. A. Kostiainen, G. R. Szilvay, J. Lehtinen, D. K. Smith, M. B. Linder, A. Urtti and O. Ikkala, *ACS Nano* 2007, **1**, 103-113) (180 mg, 0.032 mmol) was dissolved in dry DCM (3 ml) and stirred at room temperature. The Et_3N (22 µl, 0.159 mmol) was added and then cholesteroyl chloroformate (57 mg, 0.127 mmol) was added over one minute to the

reaction mixture. The reaction was stirred for 24 h and followed by TLC. The crude mixture was concentrated *in vacuo* and purified by column chromatography (SiO_2 9:1 DCM:MeOH). The product containing fractions were combined and concentrated *in vacuo*. The product was obtained as a sticky white solid (100 mg, 52%).

R_f 0.54 (7:1 DCM:MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.71-6.48 (m, N*H* amide, 12H); 5.60-4.94 (m, N*H* carbamates and C*H* alkene, 11H); 4.32 (s, C*H*, 1H); 3.64 (s, CCH_2O and OCH_2CH$_2$ gen. 1 and gen. 2, 48H); 3.34-3.02 (m, CH_2N and CH_2NH, 108H); 2.51-2.45 (m, OCH$_2$CH_2 gen. 1 and gen. 2, 24H); 1.76-1.29 (m, CH_2CH$_2$N and C(CH_3)$_3$ and C*H* and CH_2 on cholesterol, 335H); 1.24-1.01 (m, C*H* and CH_2 on cholesterol, 12H); 0.95 (s, CH_3, 3H); 0.88 (d, CH_3, J = 5.8 Hz, 3H); 0.83 (d, CH_3, J = 6.1 Hz, 6H); 0.64 (s, CH_3, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.24, 170.86, 170.60 (*C*ONH amide); 156.25, 155.56 (*C*ONH carbamate); 139.98 (quaternary *C* alkene); 122.45 (*C*H alkene); 79.79, 79.09, 78.83 (*C*(CH$_3$)$_3$); 73.65 (*C*H); 69.55 (C*C*H$_2$O gen.1 and gen. 2); 67.94 (O*C*H$_2$CH$_2$ gen. 1 and gen. 2); 60.06 (*C*CH$_2$O gen. 2); 58.75 (*C*CH$_2$O gen. 1); 56.73, 56.20 (*C*H/*C*H$_3$ cholesterol); 53.54 (*C*H$_2$ cholesterol); 50.06 (*C*H/*C*H$_3$); 47.67, 46.61, 45.93, 45.63, 44.29, 43.70, 42.85, 42.37, 39.78, 39.57, 38.64, 38.16, 37.77, 37.51, 37.09, 26.64, 36.24 (*C*H$_2$N and *C*H$_2$NH spermine and OCH$_2$*C*H$_2$ gen. 1 and gen. 2 and *C*H$_2$ on cholesterol); 35.87 (*C*H/*C*H$_3$ cholesterol); 33.38 (*C*H$_2$ spermine); 31.96 (*C*H/*C*H$_3$ cholesterol); 29.84, 28.55 (C(*C*H$_3$)$_3$); 26.10, 25.48, 25.18, 24.39, 23.90 (*C*H$_2$CH$_2$N and *C*H$_2$CH$_2$NH and *C*H$_2$ on cholesterol); 22.91, 22.65, 19.46, 18.79, 11.96 (*C*H/*C*H$_3$ cholesterol). ESI-MS (m/z): Calc. Value for $C_{305}H_{562}N_{40}O_{80}$ 6069.95; found: 2041.05 (20%, $[M+3NH_4]^{3+}$); 1897.97 (20%); 1535.31 (100% $[M+4NH_4]^{4+}$); 950.63 (70%); 522.60 (100%, [Triboc spermine+Na]$^+$. ν_{max} (solid): 3671*w,* 3446*w,* 3349*w* (N-H), 2978*s*, 2934*s* (C-H), 1708*s*, 1631*m* (C=O), 1507*s*, 1479*w*, 1366*m*, 1168*s*, 909*w*.

2.4 Synthesis of Chol-G2

Boc-protected Chol-G2 was dissolved in MeOH (5 ml) and stirred at room temperature. HCl gas was then bubbled through for 20 s and then the solution was left to stir for 1 h. The solution was then concentrated *in vacuo* and the product was obtained. Yield – quantitative.

^{1}H NMR (MeOD, 400 MHz) δ 5.37 (s, CH, 1H) 4.32 (s, C*H*, 1H); 3.69 - 3.64 (m, CC*H*$_2$O and OC*H*$_2$CH$_2$ gen. 1 and gen. 2, 48H); 3.50-2.90 (m, C*H*$_2$N and C*H*$_2$NH, 108H); 2.68-2.52 (m, OCH$_2$C*H*$_2$ gen. 1 and gen. 2, 24H); 2.16-1.63 (m, C*H*$_2$CH$_2$NH and C*H*$_2$CH$_2$NH$_2$, 72H); 1.56-1.09 (m, C*H* and C*H*$_2$ on cholesterol, 28H); 1.00 (s, C*H*$_3$, 3H); 0.91 (d, C*H*$_3$, J = 6.1 Hz, 3H); 0.83 (d, C*H*$_3$, J = 6.4 Hz, 6H); 0.68 (s, C*H*$_3$, 3H). ^{13}C NMR (MeOD, 100 MHz) δ 174.46, 173.83, 173.59 (*C*ONH amide); 141.05 (quaternary *C* alkene); 123.64 (*C*H alkene); 73.65 (*C*H); 69.55 (C*C*H$_2$O gen.1 and gen. 2); 67.94 (O*C*H$_2$CH$_2$ gen. 1 and gen. 2); 60.06 (*C*CH$_2$O gen. 2); 58.75 (*C*CH$_2$O gen. 1); 56.73, 56.20 (*C*H/*C*H$_3$ cholesterol); 53.54 (*C*H$_2$ cholesterol); 50.06 (*C*H/*C*H$_3$); 47.67, 46.61, 45.93, 45.63, 44.29, 43.70, 42.85, 42.37, 39.78, 39.57, 38.64, 38.16, 37.77, 37.51, 37.09, 26.64, 36.24 (*C*H$_2$N and *C*H$_2$NH spermine and OCH$_2$*C*H$_2$ gen. 1 and gen. 2 and *C*H$_2$ on cholesterol); 35.87 (*C*H/*C*H$_3$ cholesterol); 33.38 (*C*H$_2$ spermine); 31.96 (*C*H/*C*H$_3$ cholesterol); 26.10, 25.48, 25.18, 24.39, 23.90 (*C*H$_2$CH$_2$N and *C*H$_2$CH$_2$NH and *C*H$_2$ on cholesterol); 22.91, 22.65, 19.46, 18.79, 11.96 (*C*H/*C*H$_3$ cholesterol). ESI-MS (m/z): Calc. Value for $C_{170}H_{346}N_{40}O_{26}$ 3366.82; found: 842.68 (20%, $[M+4H]^{4+}$); 674.35 (30%, $[M+5H]^{5+}$); 562.13 (80%, $[M+6H]^{6+}$); 481.96 (100%, $[M+7H]^{7+}$). HR-MS (ESI) Calc. Value for $[C_{170}H_{346}N_{40}O_{26} + 7H]^{7+}$ 481.6793; found 481.6794.

2.5 Synthesis of Boc-Protected Chol$_2$-G1

A solution of the relevant alkyne (**Boc-Protected G1-Alkyne**, 61.0 mg, 0.033 mmol) and azide (**Chol$_2$-PAMAM-Azide**, 42.0 mg, 0.036 mmol) precursors (see sections 2.7-2.10 for the synthesis of these precursors) was formed in a THF:H$_2$O (1:1) mixture, then copper sulfate (10 mol %) and sodium ascorbate (20 mol %) were added. The reaction was stirred for 24 h under N$_2$ and monitored by TLC. The reaction mixture was poured into brine (20 ml) and the resulting solution was extracted with EtOAc (3 x 20 ml). The combined organic was dried over MgSO$_4$, concentrated *in vacuo* then purified by column chromatography (SiO$_2$, 95:5 → 9:1 DCM:MeOH) to afford the desired product (70.5 mg, 71%) as a white sticky solid.

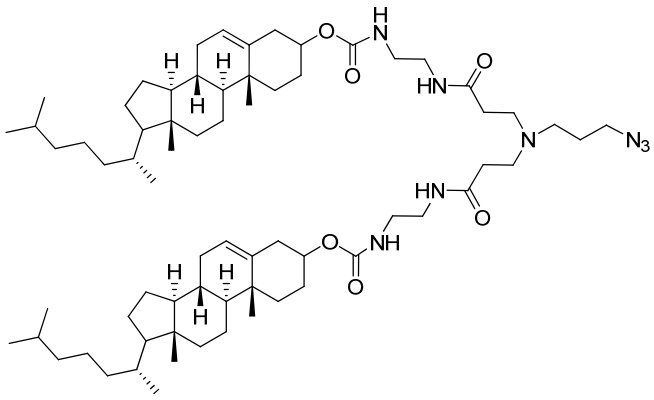
R_f 0.31 (9:1, DCM:MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (br s, C*H* triazole, 1H); 7.39 (s, N*H* amide spermine, 3H); 7.12 (s, N*H* amide PAMAM, 2H); 5.83 (br s, N*H* carbamate PAMAM, 2H); 5.31 (s, N*H* carbamate spermine and C*H*, 5H); 4.42-4.38 (m, C*H* and CH_2, 4H); 3.76-3.70 (m, OCH_2CH_2 and CCH_2O, 12H); 3.35-3.33 (m, CH_2^G, 4H); 3.27-3.11 (m, CH_2N and CH_2NH spermine, CH_2, 48H); 2.60 (s, CH_2, 4H); 2.41 (s, OCH_2CH_2, 6H); 2.35-0.64 (m, C*H*, CH_2 and CH_3 cholesterol, $C(CH_3)_3$ and CH_2 spermine, 191H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.40, 171.11 (*C*ONH amide); 160.12, 156.68, 156.14, 155.47 (*C*ONH carbamate); 143.62 (*C* triazole); 139.81 (*C* alkene); 125.93 (*C*H triazole); 122.59 (*C*H alkene); 79.61, 79.00 ($C(CH_3)_3$); 69.42 (CH^J); 69.42 (CCH_2O); 67.54 (OCH_2CH_2); 60.01 (CCH_2O); 56.73, 56.25, 49.99, 49.35, 47.46, 46.89, 46.44, 44.83, 44.27, 43.81, 42.35, 40.90, 39. 79, 38.69, 37.39, 37.03, 36.58, 36.24, 35.88, 34.39, 31.90, 28.97, 26.09, 25.55, 24.32, 23.94, 22.90, 22.62, 21.09, 19.43, 18.77, 11.93 (*C*H, CH_2 and CH_3 cholesterol and $C(CH_3)_3$, CH_2N and CH_2NH and CH_2 on spermine). ESI-MS (m/z): Calc. Value for $C_{160}H_{283}N_{21}O_{31}$ 2995.12; found: 1521.5 (100%, $[M+2Na]^{2+}$); 1510.1 (50%, $[M+H+Na]^{2+}$). HR-MS (ESI) Calc. Value for $[C_{158}{}^{13}C_2H_{283}N_{21}O_{31} + 2Na]^{2+}$ 1521.5530; found 1521.5479. ν_{max} (solid): 3445*w,* 3350*w*, 2977*w,* 2869*w* (C-H), 1674*s*, 1567*m*, 1509*m*, 1255*m*, 1166*s.*

2.6 Synthesis of $Chol_2$-G1

Boc-Protected $Chol_2$-G1 (70 mg) was dissolved in MeOH (5 ml) and stirred at room temperature. HCl gas was then bubbled through for 20 seconds and then the solution was left to stir for 1 hr. The solution was then concentrated *in vacuo* and the product (44 mg, 89 %) was obtained. Poor solubility and/or aggregation (depending on the solvent) limited the ability to determine ^{13}C NMR spectra and broadened the ^{1}H NMR. Yield – quantitative.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.64 (br s, C*H* triazole, 1H); 3.77-3.66 (m, OCH_2CH_2 and CCH_2O, 12H); 3.11-3.09 (m, CH_2N and CH_2NH spermine, CH_2, 36H); 2.48 (s, OCH_2CH_2, 6H); 2.08-1.77 (m, CH_2 spermine, 24H). MALDI-TOF (m/z): Calc. Value for $C_{115}H_{211}N_{21}O_{13}$ 2094.65; found: 2095.60 (100%, $[M+H]^+$).

2.7 Synthesis of $Chol_2$-PAMAM-Azide Click Chemistry Partner

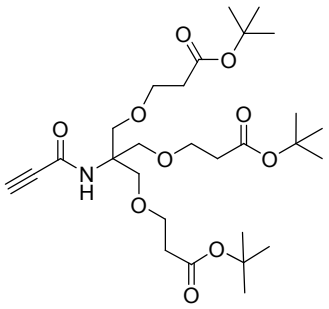


A PAMAM dendron with an azide group at the focal point (**PAMAM-Azide**) was first synthesised using literature methodology (J. W. Lee, J. H. Kim, B. K. Kim, J. H. Kim, W. S. Shin and S. H. Jin, *Tetrahedron* 2006, **62**, 9193-9200). **PAMAM-Azide** (300 mg, 0.92 mmol) was dissolved in anhydrous DCM (5 ml) and Et_3N (0.51 ml, 3.66 mmol) was added. Cholesterol chloroformate (1.01 g, 2.29 mmol) was dissolved in anhydrous DCM (5 ml) and added rapidly to the reaction vessel. The reaction was stirred under N_2 for 24 h and monitored by TLC. The reaction was concentrated *in vacuo* and the crude material was dissolved in DCM (10 ml) and washed with H_2O (2 x 10 ml). The organic layer was separated and the solvent removed *in vacuo*. The crude product was then purified by column chromatography (SiO_2, 95:5 DCM:MeOH → 9:1 DCM:MeOH). The purified product (600 mg, 57 %) was obtained as an off-white sticky solid.

R_f 0.43 (9:1, DCM:MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48 (s, N*H* amide, 2H); 5.76 (s, N*H* carbamate, 2H); 5.32 (s, C*H*, 2H); 4.44-4.39 (m, C*H*, 2H); 3.29 (t, CH_2, J = 5.5 Hz, 2H); 3.23 (s, CH_2, 8H); 2.62-0.64 (m, C*H*, CH_2 and CH_3, 98H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.27 (*C*O amide); 156.89 (*C*O carbamate); 139.72 (*C* alkene); 122.58 (*C*H alkene); 74.44 (*C*H_2); 56.68, 56.24, 50.87, 49.93, 49.08, 48.56, 42.29, 40.58, 39.74, 39.51, 38.67, 36.99, 36.52, 36.20, 35.84, 34.04, 31.83, 28.23, 27.96, 25.96, 24.27, 23.92, 22.85, 22.57, 21.05, 19.37, 18.72, 11.87 (*C*H, *C*H_2 and *C*H_3 from PAMAM and cholesterol). ESI-MS (m/z): Calc. Value for $C_{69}H_{116}N_8O_6$ 1152.91;

found: 1152.91 (100%, $[M]^+$). HR-MS (ESI) Calc. Value for $[C_{69}H_{116}N_8O_6 + H]^+$ 1153.9091; found 1153.9094. ν_{max} (solid): 3297*w*, 2954*s,2879m* (C-H), 2096*m* (N_3), 1718*s*, 1694*m*, 1507*s*, 1373*m*, 1217*s*, 1014*m*.

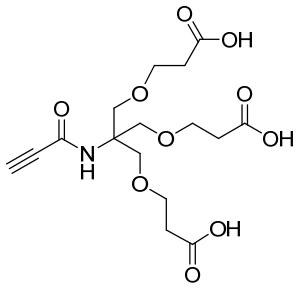
2.8 Synthesis of *t*-Bu-Ester-G1-Alkyne



Propiolic acid (427 mg, 6.10 mmol) was dissolved in DCM (61 ml) and then ***t*-Bu-Ester-G1-NH_2** (synthesised according to C. M. Cardona and R. E. Gawley, *J. Org. Chem.* 2002, **67**, 1411-1413) (3.70 g, 7.32 mmol) and DIPEA (1.27 ml, 7.32 mmol) were added. The mixture was cooled in an ice/water bath for 20 min before T3P (4.31 ml, 7.32 mmol) was injected over 5 minutes. The reaction was stirred at room temperature and monitored by TLC for 7 days. The reaction was then quenched with water (50 ml) and neutralised from pH 1 to pH 7 with base before the organic layer was removed. The organic layer was then washed once more by H_2O (50 ml) and then brine (60 ml) and the solvent was then removed *in vacuo.* The crude material was purified by chromatography (SiO_2, 4:1, Cyclohexane:EtOAc) with product-containing fractions being combined to give the pure product (1.50 g, 44.1 %) as a colourless oil.

R_f 0.10 (4:1 Cyclohexane/EtOAc, cerium stain). ^{1}H NMR ($CDCl_3$, 400MHz) δ 6.40 (s, N*H*, 1H); 3.67 (s, CCH_2O, 6H); 3.63 (t, OCH_2CH$_2$, J = 6.4 Hz, 6H); 2.67 (s, C*H* alkyne, 1H); 2.43 (t, CH_2CH$_2$O, J = 6.4 Hz, 6H); 1.43 (s, C(CH_3)$_3$, 27H). ^{13}C NMR ($CDCl_3$, 100MHz) δ 170.98 (*C*OO); 151.94 (*C*ONH); 80.64 (*C*(CH$_3$)$_3$); 78.11 (*C* alkyne); 71.87 (*C*H alkyne) 68.85 (C*C*H$_2$O); 67.17 (CH$_2$*C*H$_2$O); 60.89 (*C*CH$_2$O); 36.22 (*C*H$_2$CH$_2$O); 28.20 ((*C*H$_3$)$_3$C). ESI-MS (m/z): Calc. Value for $C_{28}H_{47}NO_{10}$ 557.32; found: 580.31 (100%, $[M+Na]^+$). HR-MS (ESI) Calc. Value for $[C_{28}H_{47}NO_{10} + Na]^+$ 580.3092; found 580.3108. ν_{max} (solid): 3247*w* (N-H), 2977*w* (C-H), 1737*s*, 1660*m*, 1479*m*, 1363*w*, 1115*m*, 1029*w*, 848*m.*

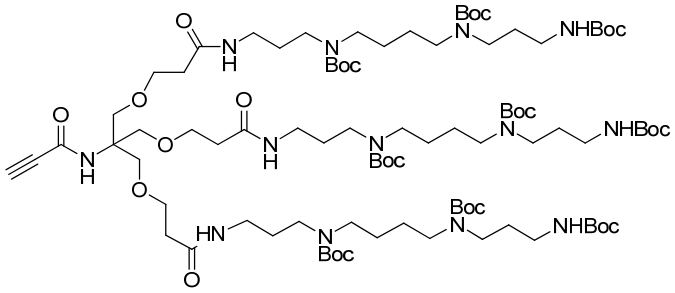
2.9 Synthesis of Acid-G1-Alkyne



***t*-Bu-Ester-G1-Alkyne** (1.50 g, 2.69 mmol) was dissolved in formic acid (50 ml) and stirred at room temperature for 24 h. The formic acid was removed under reduced pressure to yield the product (1.10 g, 100 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 3.41 (m, CC*H*$_2$O and OC*H*$_2$CH$_2$, 12H); 3.07 (s, C*H* alkyne, 1H); 2.25 (t, C*H*$_2$CH$_2$O, J = 6.12 Hz, 6H). ^{13}C NMR (MeOD, 100MHz) δ 175.49, 173.99 (*C*OOH); 154.46 (*C*ONH); 78.61 (*C* alkyne); 74.75 (*C*H alkyne) 69.52 (C*C*H$_2$O); 68.10 (CH$_2$*C*H$_2$O); 62.50 (*C*CH$_2$O); 35.71 (*C*H$_2$CH$_2$O). ESI-MS (m/z, positive): Calc. Value for $C_{16}H_{23}NO_{10}$ 389.36; found: 412.12 (100%, $[M+Na]^+$). HR-MS (ESI) Calc. Value for $[C_{16}H_{23}NO_{10} + Na]^+$ 412.1219; found 412.1237. ν_{max} (solid): 3252*w* (N-H), 2970*w* (C-H), 1728*s*, 1653*m*, 1365*m*, 1217*m*, 1107*w.*

2.10 Synthesis of Boc-Protected G1-Alkyne



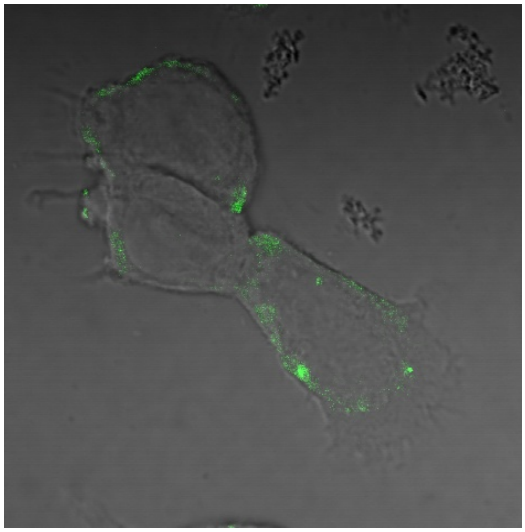
A solution of DCC (0.99 g, 0.949 mmol), HOBt (0.64 g, 0.949 mmol) and Et_3N (0.66 ml, 0.949 mmol) were dissolved in anhydrous THF (75 ml) and cooled on ice. The **Acid-G1-Alkyne** (500 mg 1.227 mmol) was added and stirred for 20 min followed by the addition of tri-Boc-spermine (synthesised according to I. S. Blagbrough and A. J. Geall, *Tetrahedron Lett.* 1998, **39**, 439-442) (2.39 g, 0.949 mmol). The reaction was

stirred under an N_2 atmosphere for 4 days and monitored by TLC. Following this period, the solvent was removed under reduced pressure and the crude residue was purified by column chromatography (SiO_2, 9:1 DCM:MeOH). The product-containing fractions were combined and dried under vacuum to yield the desired product (0.80 g, 36 %) as a colourless oil.

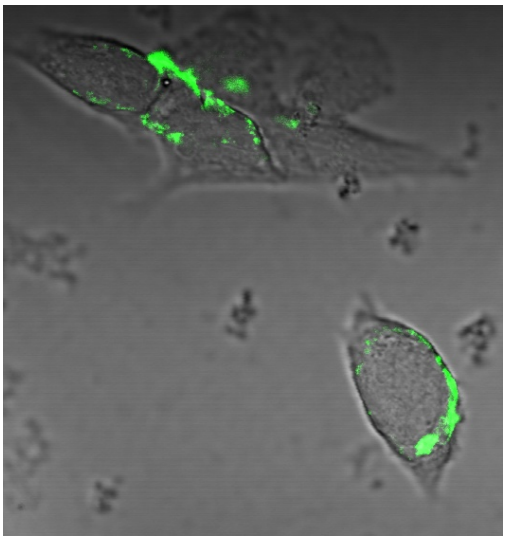
R_f 0.24 (9:1, DCM:MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (br s, N*H* amide, 4H); 5.30 (br s, N*H* carbamate, 3H); 3.70 (s, CC*H*$_2$O and OC*H*$_2$CH$_2$, 12H); 3.21-3.12 (m, C*H*$_2$N and C*H*$_2$NH spermine, 36H); 2.85 (br s, C*H* alkyne, 1H); 2.43 (s, OCH$_2$C*H*$_2$, 6H); 1.65-1.43 (m, C*H*$_2$ on spermine and C(C*H*$_3$)$_3$, 105H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.76, 170.99, 170.46 (*C*ONH (amide)); 155.83, 155.21, 151.86 (N*C*OO^{t-}Bu x9, multiple overlapping peaks); 79.26 (*C*(CH$_3$)$_3$ x9, multiple overlapping peaks); 78.56 (*C* alkyne); 72.43 (*C*H alkyne) 68.79 (C*C*H$_2$O); 67.58 (O*C*H$_2$CH$_2$); 60.63 (*C*CH$_2$O); 51.42, 47.31, 46.56, 46.12, 45.68, 45.27, 44.08, 43.58, 42.51, 37.78, 37.47, 37.13, 36.55, 35.84, 34.44, 32.95 (*C*H$_2$ and OCH$_2$*C*H$_2$, multiple overlapping peaks); 29.41 ((*C*H$_3$)$_3$CO x27, multiple overlapping peaks); 28.20, 25.76, 25.18 (*C*H$_2$CH$_2$N, *C*H$_2$CH$_2$NH, multiple overlapping peaks). ESI-MS (m/z): Calc. Value for $C_{91}H_{166}N_{13}O_{25}$ 1865.21; found: 1866.21 (10%, $[M+H]^+$); 944.60 (100%, $[M+H+Na]^{2+}$). HR-MS (ESI) Calc. Value for $[C_{91}H_{166}N_{13}O_{25} + H]^+$ 1866.2120; found 1866.2067. ν_{max} (solid): 3323w (C-H alkyne stretch), 2970w, 2872w (C-H), 1653s, 1363m, 1158s.

3. Additional Data

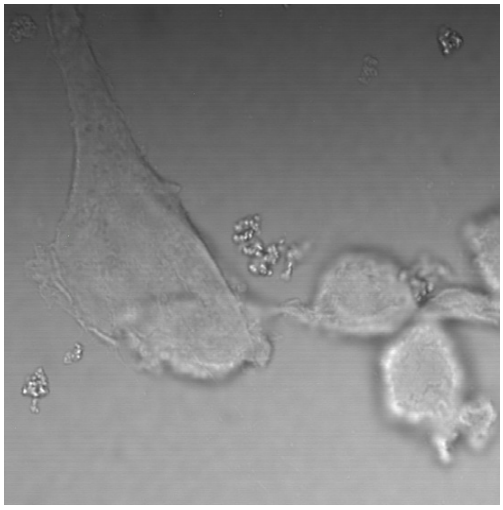
3.1 Confocal microscopy images showing time dependent uptake of labelled DNA into cells by G1-Chol in the presence of chloroquine



Chol-G1 30 minutes

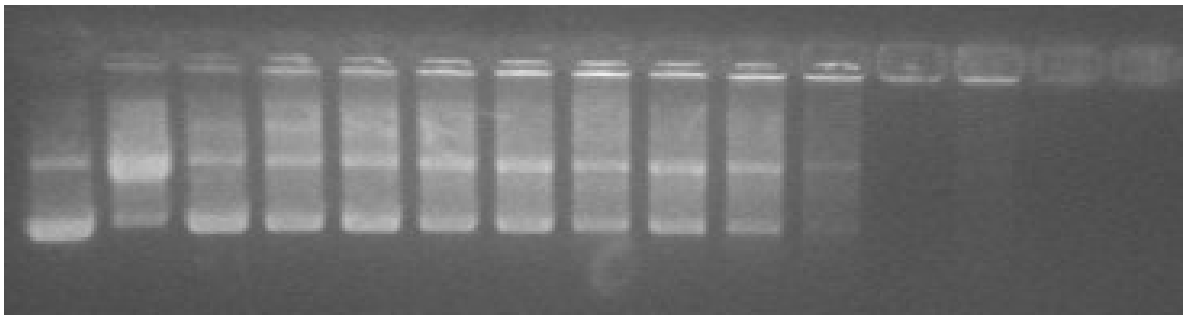


Chol-G1 2 hours

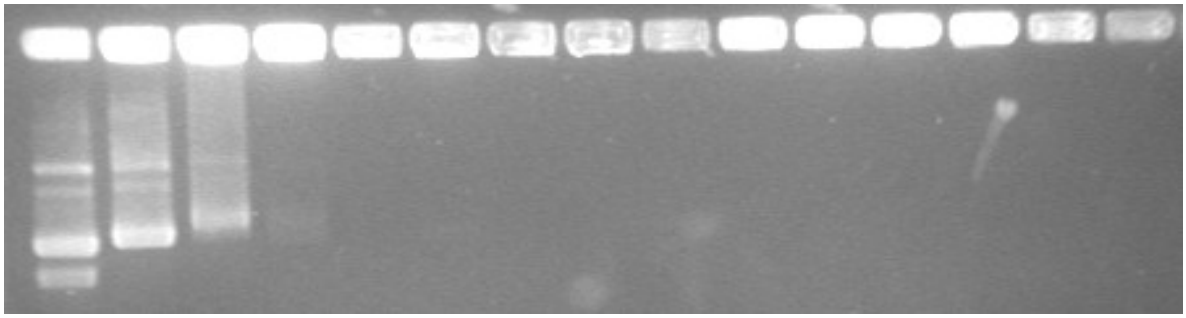


DNA/YOYO control in the absence of vector

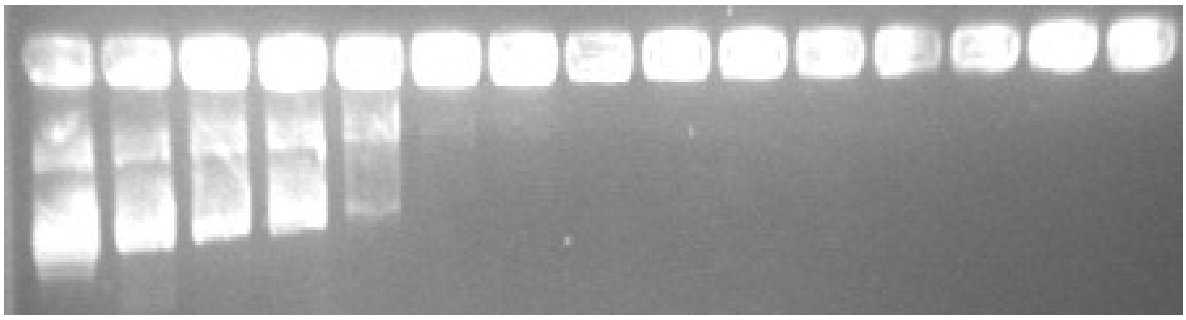
3.2 Gel Electrophoresis Data



Chol$_2$-G1
(Polyamine:DNA, w:w) lane 1, 0:1; lane 2, 0.05:1; lane 3, 0.1:1; lane 4, 0.15:1; lane 5, 0.2:1; lane 6, 0.25:1; lane 7, 0.3:1; lane 8, 0.35:1; lane 9, 0.4:1; lane 10, 0.5:1; lane 11, 0.6:1; lane 12, 0.8:1; lane 13, 1:1; lane 14, 1.5:1; lane 15, 2:1.



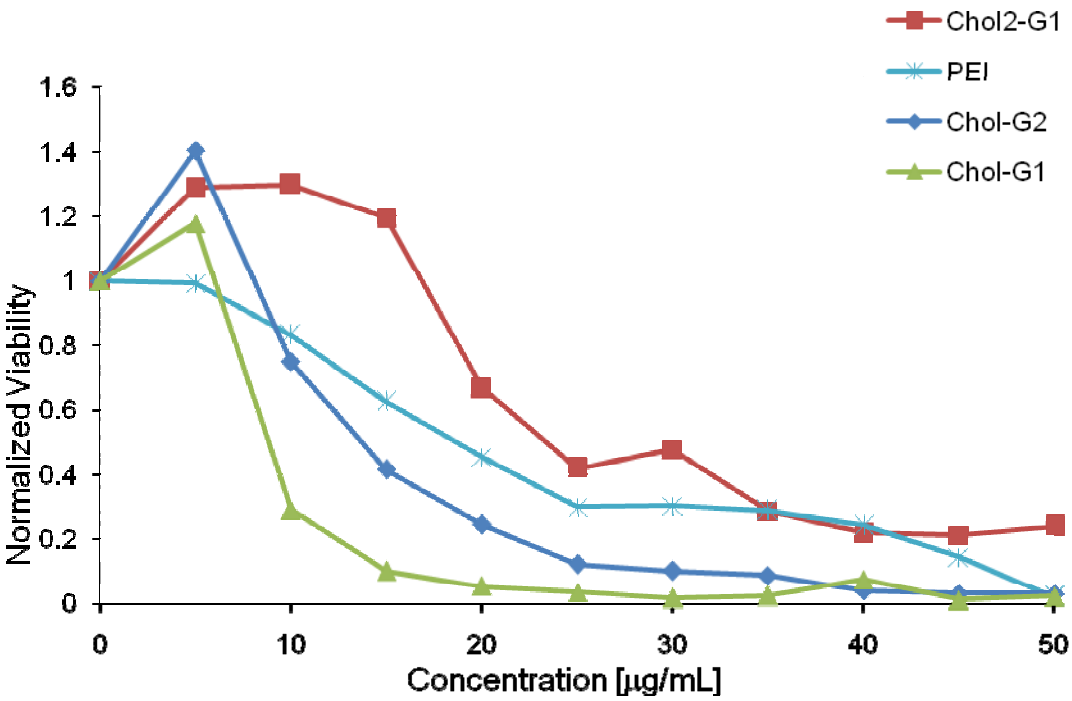
Chol-G1
(Polyamine:DNA, w:w) lane 1, 0:1; lane 2, 0.05:1; lane 3, 0.1:1; lane 4, 0.15:1; lane 5, 0.2:1; lane 6, 0.25:1; lane 7, 0.3:1; lane 8, 0.35:1; lane 9, 0.4:1; lane 10, 0.5:1; lane 11, 0.6:1; lane 12, 0.8:1; lane 13, 1:1; lane 14, 1.5:1; lane 15, 2:1.



Chol-G2

(Polyamine:DNA, w:w) lane 1, 0:1; lane 2, 0.1:1; lane 3, 0.2:1; lane 4, 0.3:1; lane 5, 0.4:1; lane 6, 0.5:1; lane 7, 0.6:1; lane 8, 0.7:1; lane 9, 0.8:1; lane 10, 0.9:1; lane 11, 1:1; lane 12, 1.5:1; lane 13, 2:1; lane 14, 2.5:1; lane 15, 3:1.

3.3 Toxicity data from cell titre blue assay



3.4 Transfection of HEK293 cells using Chol-G1 in the presence of chloroquine