Supplementary Information

Gene delivery with polycationic fullerene hexakis-adducts

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Table of Contents

Figure S15. TEM image of **G3NH$_3^+$**/pCMVLuc polyplexes (in water with 150 mM NaCl).

Gene transfer experiments.

Cell culture.

Polyplexes formation for pCMVLuc delivery.

Quantification of the luciferase gene expression.

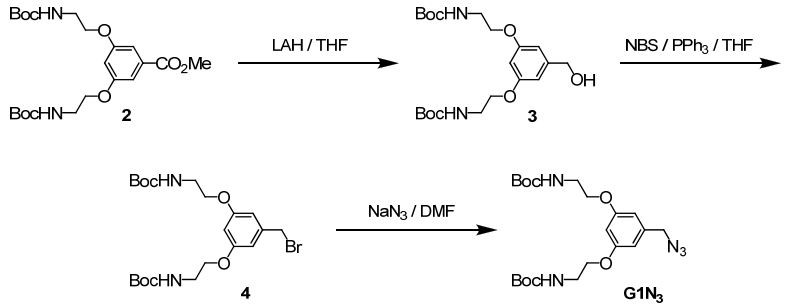
Figure S16. Delivery experiments of pCMVLuc at different N/P (Polyplexes prepared in 5% glucose solution).

Figure S17. Delivery experiments of pCMVLuc at different N/P (Polyplexes prepared in 150 mM NaCl solution).

Experimental section

General: Reagents and solvents were purchased as reagent grade and used without further purification. Compounds **1**[1] and **2**[2] were prepared according to a previously reported procedure. All reactions were performed in standard glassware under an inert Ar or N_2 atmosphere. Evaporation and concentration were done at water aspirator pressure and drying in vacuo at 10^{-2} Torr. Column chromatography: silica gel 60 (230-400 mesh, 0.040-0.063 mm) was purchased from E. Merck. Thin Layer Chromatography (TLC) was performed on glass sheets coated with silica gel 60 F_{254} purchased from E. Merck, visualization by UV light. IR spectra (cm^{-1}) were measured on an ATI Mattson Genesis Series FTIR instrument. NMR spectra were recorded on a Bruker AC 300 or AC 400 with solvent peaks as reference. Elemental analysis were performed by the analytical service at the Laboratoire de Chimie de Coordination (Toulouse, France).

Preparation of compound $G1N_3$.



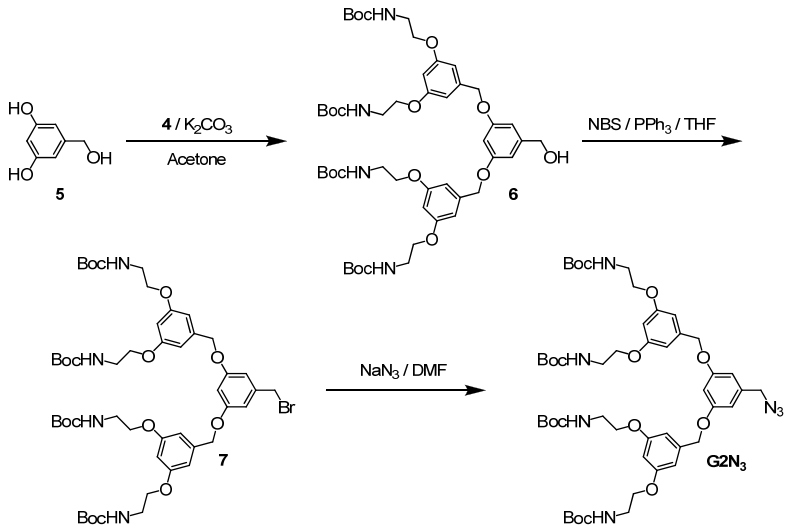
Compound 3. A 1 M LAH solution in THF (8.2 mL, 8.23 mmol) was added dropwise to a solution of **2** (3.74 g, 8.23 mmol) in dry THF (100 mL) at 0°C under argon. After 3 h, MeOH (1 mL) was slowly added then H_2O (several drops). The resulting mixture was filtered on Celite and evaporated. Column chromatography (SiO_2, CH_2Cl_2/AcOEt 1:1) gave **3** (3.52 g, 99%) as a white solid. IR (neat): 3347 (O-H), 3360 (N-H), 1689 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 6.53 (d, J = 2 Hz, 2H), 6.37 (t, J = 2 Hz, 1H), 4.95 (broad s, 2H), 4.63 (s, 2H), 4.01 (t, J = 5 Hz, 4H), 3.52 (m, 4H), 1.45 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.0, 156.0, 143.9, 105.5, 100.6, 79.7, 67.3, 65.0, 40.1, 28.5. Elemental analysis (%) calcd for $C_{21}H_{34}N_2O_7$: C 59.14, H 8.03, N 6.57; found: C 58.56, H 8.04, N 6.31.

[1] J. Iehl, J.-F. Nierengarten, *Chem. Eur. J.* **2009**, *15*, 7306-7309.
[2] A. J. Brouwer, S. J. E. Mulders, R. M. J. Liskamp, *Eur.J. Org. Chem*. **2001**, 1903-1915.

Compound 4. A solution of **3** (3.52 g, 8.26 mmol), PPh_3 (2.82 g, 10.08 mmol) and *N*-bromosuccinimide (1.91 g, 10.07 mmol) in THF (50 mL) was stirred at room temperature. After 15 min., H_2O was added to the mixture. The resulting aqueous layer was extracted with AcOEt (3 X). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography (SiO_2, cyclohexane/AcOEt 7:3) gave **4** (3.24 g, 80%) as a white solid. IR (neat): 3366 (N-H), 1677 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.54 (d, J = 2 Hz, 2H), 6.38 (t, J = 2 Hz, 1H), 4.94 (broad s, 2H), 4.39 (s, 2H), 4.00 (t, J = 5 Hz, 4H), 3.52 (m, 4H), 1.45 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): 159.8, 155.5, 139.9, 107.8, 101.4, 79.8, 67.3, 40.0, 33.3, 28.4. Elemental analysis (%) calcd for $C_{21}H_{33}BrN_2O_6$: C 51.54, H 6.80, N 5.72; found: C 51.27, H 6.59, N 5.57.

Compound G1N$_3$. A solution of **4** (0.92 g, 1.89 mmol) and NaN_3 (0.247 g, 3.8 mmol) in DMF (20 mL) was stirred at room temperature. After 12 h, H_2O was added to the mixture. The resulting aqueous layer was extracted with Et_2O (3 X). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography (SiO_2, cyclohexane/AcOEt 7:3) gave **G1N$_3$** (0.69 g, 81%) as a white solid. IR (neat): 3345 (N-H), 2098 (N_3), 1680 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.46 (d, J = 2 Hz, 2H), 6.41 (t, J = 2 Hz, 1H), 4.96 (broad s, 2H), 4.26 (s, 2H), 4.01 (t, J = 5 Hz, 4H), 3.52 (m, 4H), 1.45 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.2, 156.0, 138.0, 107.0, 101.3, 79.7, 67.5, 54.9, 40.2, 28.5. Elemental analysis (%) calcd for $C_{21}H_{33}N_5O_6$: C 55.86, H 7.37, N 15.51; found: C 55.50, H 7.09, N 15.11.

Preparation of compound $G2N_3$.



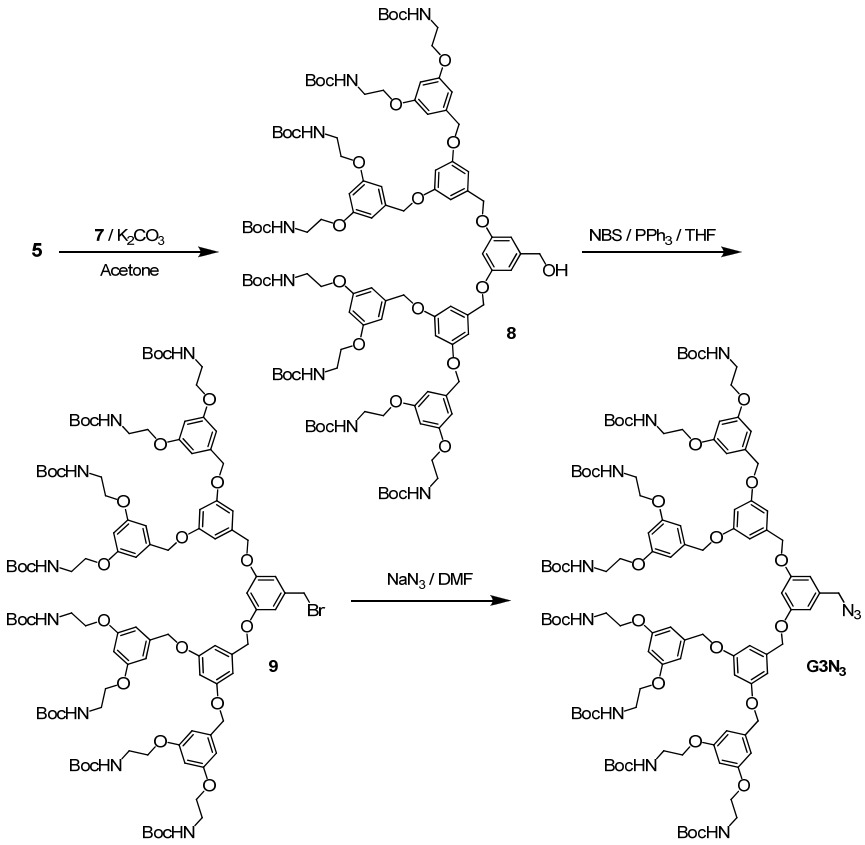
Compound 6. A mixture of **4** (3.24 g, 6.63 mmol), **5** (0.40 g, 3.87 mmol) and K_2CO_3 (1.59 g, 11.54 mmol) in acetone (55 mL) was heated under reflux. After 2 days, the mixture was filtered and evaporated. Column chromatography (SiO_2, CH_2Cl_2/AcOEt 8:2) gave **6** (2.67 g, 97%) as a white product. IR (neat): 3346 (O-H/N-H), 1686 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.61 (d, J = 2 Hz, 2H), 6.56 (d, J = 2 Hz, 4H), 6.50 (t, J = 2 Hz, 1H), 6.39 (t, J = 2 Hz, 2H), 5.00 (broad s, 4H), 4.97 (s, 4H), 4.64 (d, 2H), 4.00 (t, J = 5 Hz, 8H), 3.51 (m, 8H), 1.45 (s, 36H). Elemental analysis (%) calcd for $C_{49}H_{72}N_4O_{15}$: C 61.49, H 7.58, N 5.85; found: C 61.60, H 7.66, N 5.70.

Compound 7. As described for **4** from **6** (2.68 g, 2.80 mmol), PPh_3 (0.95 g, 3.63 mmol) and *N*-bromosuccinimide (0.65 g, 3.63 mmol) in THF (25 mL). Column chromatography (SiO_2, cyclohexane/AcOEt 7:3) gave **7** (2.08 g, 73%) as a white solid. IR (neat): 3347 (N-H), 1691 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.63 (d, J = 2 Hz, 2H), 6.56 (d, J = 2 Hz, 4H), 6.52 (t, J = 2 Hz, 1H), 6.40 (t, J = 2 Hz, 2H), 4.98 (broad s, 4H), 4.96 (s, 4H), 4.41 (s, 2H), 4.01 (t, J = 5 Hz, 8H), 3.52 (m, 8H), 1.45 (s, 36H). Elemental analysis (%) calcd for $C_{49}H_{71}BrN_4O_{14}$.0.6 CH_2Cl_2: C 55.69, H 6.81, N 5.24 ; found: C 55.76, H 6.86, N 5.25

Compound $G2N_3$. As described for **$G1N_3$** from **7** (600 mg, 0.59 mmol) and NaN_3 (76 mg, 1.17 mmol) in DMF (10 mL). Column chromatography (SiO_2, cyclohexane/AcOEt 7:3) gave **$G2N_3$** (521 mg, 90%) as a white solid. IR (neat): 3347 (N-H), 2100 (N_3), 1694 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.55-6.57 (m,7H), 6.40 (t, J = 2 Hz, 2H), 4.99 (broad s, 4H), 4.97

(s, 4H), 4.27 (s, 2H), 4.01 (t, *J* = 5 Hz, 8H), 3.52 (m, 8H), 1.45 (s, 36H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.2, 160.1, 156.0, 139.3, 137.8, 107.4, 106.1, 101.9, 101.0, 79.6, 70.0, 67.4, 54.9, 40.2, 28.5. Elemental analysis (%) calcd for $C_{49}H_{71}N_7O_{14}$: C 59.92, H 7.29, N 9.98; found: C 59.93, H 7.34, N 9.68.

Preparation of compound G3N$_3$.

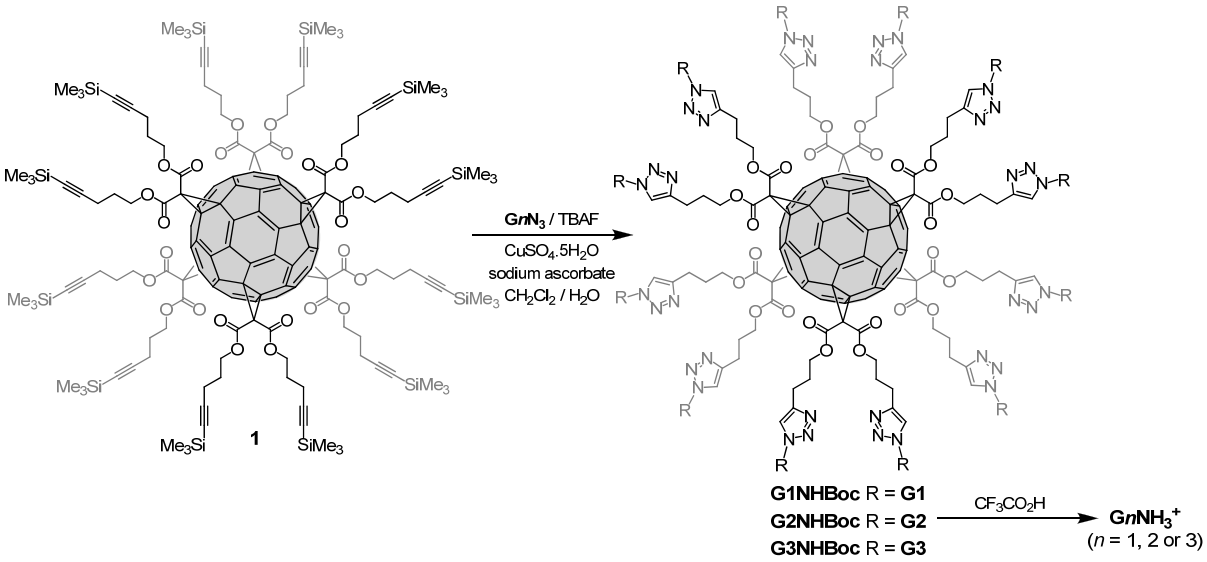


Compound 8. As described for **6** from **7** (1.47 g, 1.44 mmol), **5** (88 mg, 0.63 mmol) and K_2CO_3 (0.346 g, 2.51 mmol) in acetone (25 mL). Column chromatography (SiO_2, CH_2Cl_2/AcOEt 8:2) gave **8** (1.33 g, quantitative) as a white solid. IR (neat): 3341 (O-H/N-H), 1694 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.64 (d, *J* = 2 Hz, 4H), 6.59 (d, *J* = 2 Hz, 2H), 6.55 (d, *J* = 2 Hz, 8H), 6.52 (t, *J* = 2 Hz, 2H), 6.49 (t, *J* = 2 Hz, 1H), 6.38 (t, *J* = 2 Hz, 4H), 5.04 (broad s, 8H), 4.98 (s, 4H), 4.95 (s, 8H), 4.61 (d, 2H), 3.98 (t, *J* = 5 Hz, 16H), 3.50 (m, 16H), 1.44 (s, 72H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.1, 156.0, 143.9, 139.6, 139.5, 106.5, 106.2, 105.9, 101.7, 101.3, 101.0, 79.7, 70.1, 70.0, 67.4, 65.1, 40.2, 28.5. Elemental analysis (%) calcd for $C_{105}H_{148}N_8O_{31}$. 0.5 CH_2Cl_2: C 61.49, H 7.29, N 5.44; found: C 61.47, H 7.48, N 5.57.

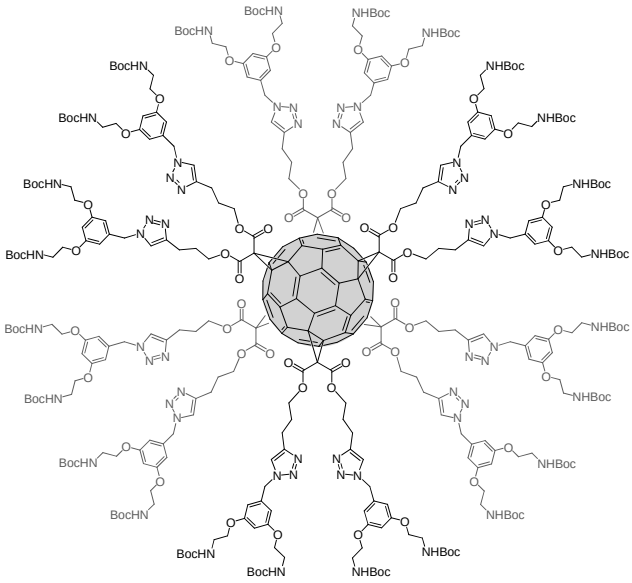
Compound 9. As described for **4** from from **8** (1.27 g, 0.63 mmol), PPh_3 (0.25 g, 0.94 mmol) and *N*-bromosuccinimide (0.17 g, 0.94 mmol) in THF (11 mL). Column chromatography (SiO_2, cyclohexane/AcOEt 1:1) gave **9** (0.87 g, 65%) as a white solid. IR (neat) 3356 (N-H), 1694 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.66 (d, *J* = 2 Hz, 4H), 6.63 (d, *J* = 2 Hz, 2H), 6.56-6.52 (m, 11H), 6.39 (t, *J* = 2 Hz, 4H), 5.03 (broad s, 8H), 4.97 (s, 4H), 4.95 (s, 8H), 4.41 (s, 2H), 3.99 (t, *J* = 5 Hz, 16H), 3.51 (m, 16H), 1.44 (s, 72H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.1, 156.0, 139.9, 139.4, 139.2, 108.4, 106.6, 106.1, 102.4, 101.7, 100.9, 79.7, 70.2, 70.0, 67.4, 40.2, 33.7, 28.5. Elemental analysis (%) calcd for $C_{105}H_{147}BrN_8O_{30}$: C 60.60, H 7.12, N 5.38; found: C 60.23, H 7.15, N 5.21.

Compound G3N$_3$. As described for **G1N$_3$** from **9** (871 mg, 0.42 mmol) and NaN_3 (54 mg, 0.84 mmol) in DMF (10 mL). Column chromatography (SiO_2, cyclohexane/AcOEt; 1:1) gave **G3N$_3$** (750 mg, 87%) as a white solid. IR (neat): 3352 (N-H), 2100 (N_3), 1694 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$): 6.66 (d, *J* = 2 Hz, 4H), 6.56-6.53 (m, 13H), 6.39 (t, *J* = 2 Hz, 4H), 5.02 (broad s, 8H), 4.98 (s, 4H), 4.95 (s, 8H), 4.26 (s, 2H), 3.99 (t, *J* = 5 Hz, 16H), 3.51 (m, 16H), 1.45 (s, 72H). ^{13}C NMR (75 MHz, $CDCl_3$): 160.3, 160.2, 160.1, 156.0, 139.5, 139.3, 137.8, 107.4, 106.5, 106.2, 102.1, 101.7, 101.0, 79.7, 70.2, 70.0, 67.4, 54.9, 40.2, 28.5. Elemental analysis (%) calcd for $C_{105}H_{147}N_{11}O_{30}$: C 61.72, H 7.25, N 7.54; found: C 61.42, H 7.43, N 7.31.

Preparation of G*n*NHBoc and G*n*NH_3^+.



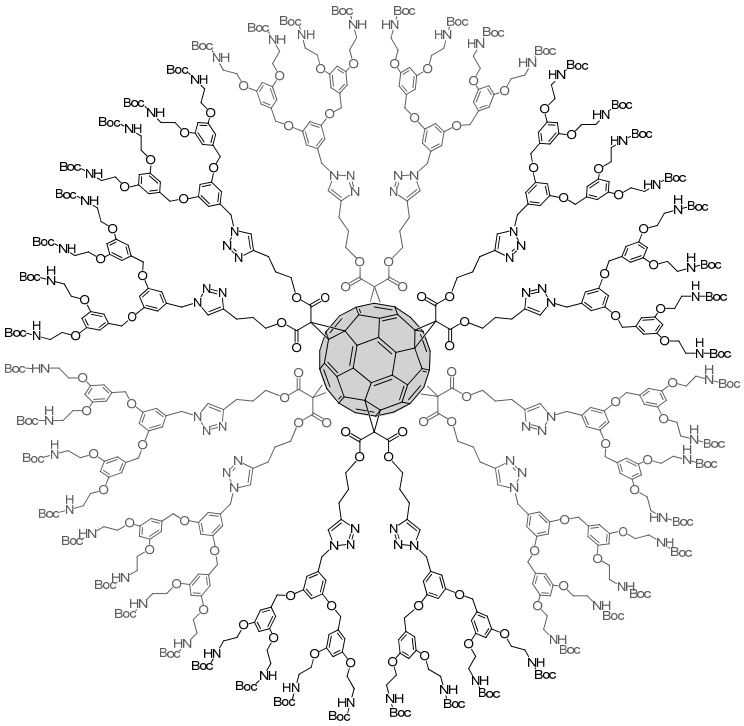
Compound G1NHBoc.



A 1 M TBAF solution in THF (0.7 mL, 0.7 mmol) was added to a mixture of **1** (51 mg, 0.02 mmol), **G1N$_3$** (294 mg, 0.65 mmol), $CuSO_4.5H_2O$ (0.8 mg, 0.005 mmol) and sodium ascorbate (2.3 mg, 0.015 mmol) in CH_2Cl_2/H_2O (1:1, 2.5 mL). Column chromatography (SiO_2, CH_2Cl_2 containing 5% methanol) followed by gel permeation chromatography (Biobeads SX-1, CH_2Cl_2) gave **G1NHBoc** (109 mg, 85%) as an orange glassy product. IR (neat): 3348 (N-H), 1743 (C=O ester), 1695 (C=O Boc). UV/Vis (CH_2Cl_2): 229 (253200), 278

(121100), 318 (sh, 53600), 334 (sh, 41700). ^{1}H NMR (300 MHz, $CDCl_3$): 7.52 (s, 12H), 6.37 (m, 36H), 5.38 (broad s, 24H), 5.20 (broad s, 24H), 4.30 (m, 24H), 3.92 (m, 48H), 3.46 (m, 48H), 2.74 (m, 24H), 2.07 (m, 24H), 1.43 (s, 216H). ^{13}C NMR (75 MHz, $CDCl_3$): 163.7, 160.2, 155.9, 146.7, 145.7, 141.2, 137.4, 121.6, 107.0, 101.0, 79.5, 69.2, 67.3, 66.4, 53.9, 45.6, 40.0, 28.5, 28.0, 22.1. Elemental analysis (%) calcd for $C_{390}H_{480}N_{60}O_{96}.6\ CH_2Cl_2$: C 59.06, H 6.16, N 10.43; found: C 58.90, H 6.08, N 9.35.

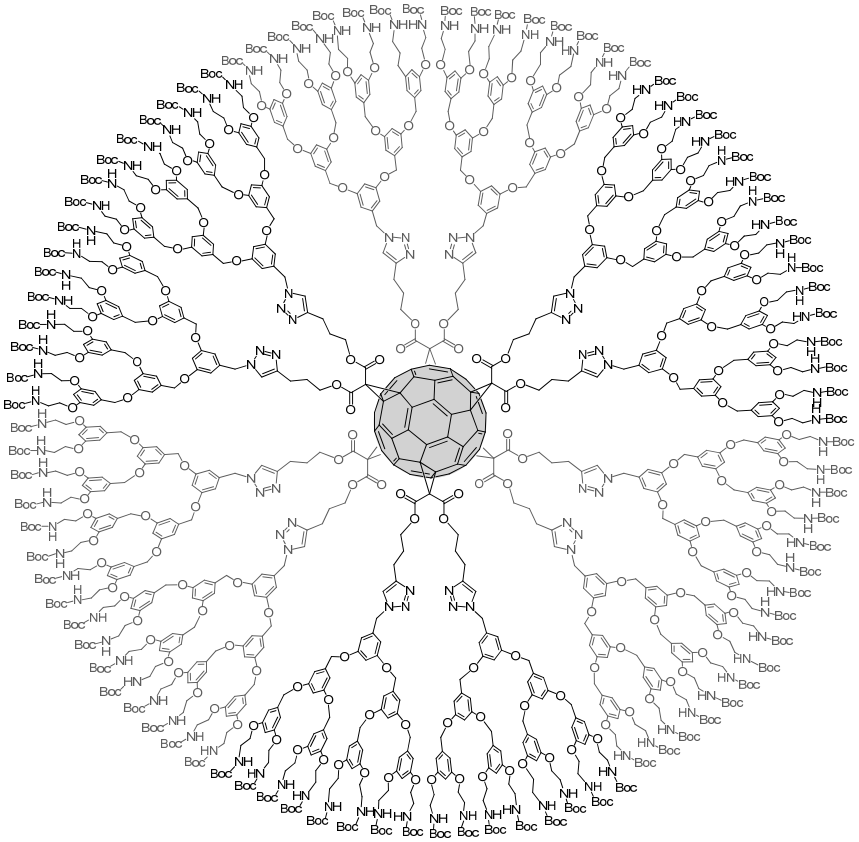
Compound G2NHBoc.



As described for **G1NHBoc** from **1** (85 mg, 0.03 mmol), **G2N$_3$** (550 mg, 0.04 mmol), $CuSO_4.5H_2O$ (0.5 mg, 0.0028 mmol), sodium ascorbate (2.3 mg, 0.008 mmol) and TBAF (0.4 mL, 0.4 mmol) in CH_2Cl_2/H_2O (1:1, 3 mL). Column chromatography (SiO_2, CH_2Cl_2 containing 3% methanol) followed by gel permeation chromatography (Biobeads SX-1, CH_2Cl_2) gave **G2NHBoc** (270 mg, 68%) as an orange glassy product. IR (neat): 3350 (N-H), 1740 (C=O ester), 1694 (C=O Boc). UV/Vis (CH_2Cl_2): 230 (405500), 281 (147600), 318 (sh, 44700), 334 (sh, 35000). ^{1}H NMR (300 MHz, $CDCl_3$): 7.44 (broad s, 12H), 6.44 (m, 84H), 6.32 (m, 24H), 5.27-5.32 (m, 72H), 4.78 (s, 48H), 4.28 (m, 24H), 3.91 (m, 48H), 3.45 (m, 48H), 2.70 (m, 24H), 2.04 (m, 24H), 1.41 (s, 432H). ^{13}C NMR (100 MHz, $CDCl_3$): 163.6, 160.2, 159.9, 156.0, 147.1, 145.8, 141.2, 139.0, 137.3, 121.6, 107.2, 106.1, 101.9, 100.7, 79.4,

69.9, 69.2, 67.2, 66.4, 53.8, 45.5, 40.0, 28.4, 28.1, 22.0. Elemental analysis (%) calcd for $C_{726}H_{936}N_{84}O_{192}.2\ CHCl_3$: C 61.79, H 6.68, N 8.31; found: C 61.40, H 6.80, N 8.13.

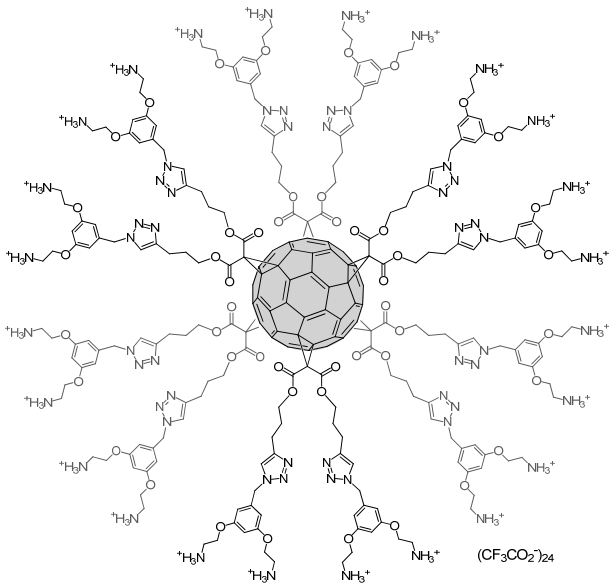
Compound G3NHBoc.



As described for **G1NHBoc** from **1** (57 mg, 0.02 mmol), **G3N$_3$** (550 mg, 0.27 mmol), $CuSO_4.5H_2O$ (0.4 mg, 0.0028 mmol), sodium ascorbate (1.1 mg, 0.006 mmol) and TBAF (0.3 mL, 0.3 mmol) in CH_2Cl_2/H_2O (1:1, 1 mL). Column chromatography (SiO_2, CH_2Cl_2) followed by gel permeation chromatography (Biobeads SX-1, CH_2Cl_2) gave **G3NHBoc** (451 mg, 89%) as an orange glassy product. IR (neat) 3363 (N-H), 1745 (C=O ester), 1695 (C=O Boc). UV/Vis (CH_2Cl_2): 231 (706500), 280 (228600), 318 (sh, 43100), 334 (sh, 33100). ^{1}H NMR (300 MHz, $CDCl_3$): 7.59 (broad s, 12H), 6.51 (m, 48H), 6.44 (m, 156H), 6.30 (m, 48H), 5.30 (s, 96H), 5.28 (s, broad, 96H), 4.76 (m, 48H), 4.70 (m, 24H), 4.33 (m, 24H), 3.88 (m, 192H), 3.42 (m, 192H), 2.74 (m, 24H), 2.11 (m, 24H), 1.40 (broad s, 864H). ^{13}C NMR (75 MHz, $CDCl_3$): 163.7, 160.3, 160.1, 159.9, 156.0, 146.9, 145.9, 141.1, 139.3, 139.0, 137.6, 121.8, 107.1, 106.4, 106.1, 101.7, 101.5, 100.6, 79.4, 69.7, 69.2, 67.2, 66.4, 53.8, 45.3, 43.5,

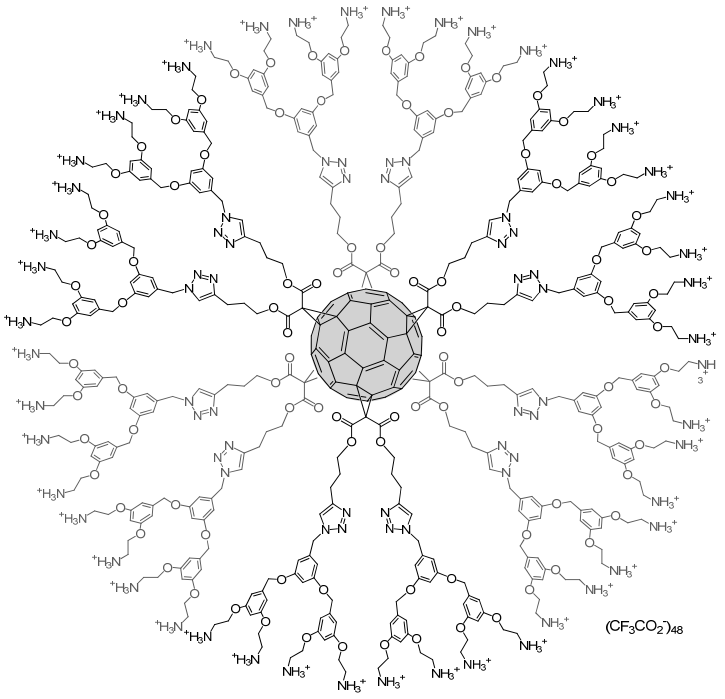
40.1, 28.5, 28.1, 22.2. Elemental analysis (%) calcd for $C_{1398}H_{1848}N_{132}O_{384}$: C 63.01, H 6.99, N 6.94; found: C 62.81, H 6.96, N 6.48.

Compound G1NH$_3^+$.



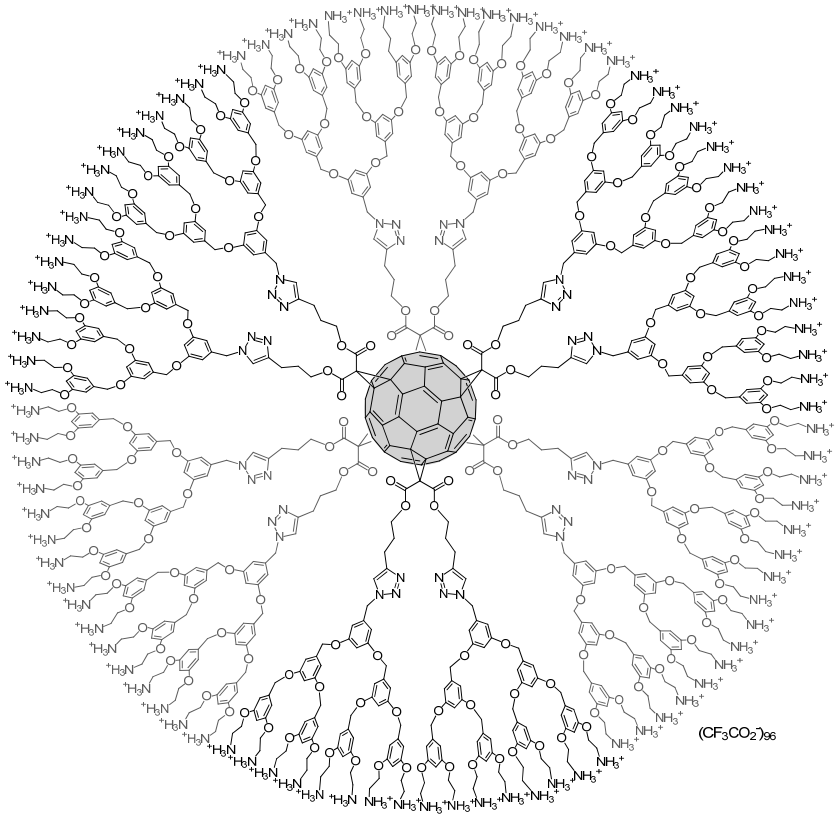
G1NHBoc (93 mg, 0.012 mmol) was dissolved in TFA (2 mL). After 4 h, the mixture was evaporated and dried under vacuum to afford **G1NH$_3^+$** as its trifluoroacetate salt (98 mg, quantitative). Orange glassy product. IR (neat): 1742 (C=O ester), 1671 (C=O TFA). UV/Vis (H_2O): 201 (576000), 275 (91500), 314 (sh, 45300), 332 (sh, 36200). ^{1}H NMR (300 MHz, D_2O): 7.67 (m, 12H), 6.52 (m, 12H), 6.46 (m, 24H), 5.35 (m, 24H), 4.12 (m, 72H), 3.33 (m, 48H), 2.57 (m, 24H), 1.84 (m, 24H). ^{13}C NMR (100 MHz, D_2O): 164.1, 162.4 (q, J = 36 Hz), 159.2, 146.1, 145.4, 141.3, 136.9, 123.7, 116.4 (q, J = 290 Hz), 107.6, 101.7, 69.2, 66.8, 64.1, 54.0, 46.3, 38.8, 27.0, 21.0.

Compound G2NH$_3^+$.



G2NHBoc (190 mg, 0.014 mmol) was dissolved in TFA (2 mL). After 4 h, the mixture was evaporated and dried under vacuum to afford **G2NH$_3^+$** as its trifluoroacetate salt (224 mg, quantitative). Orange glassy product. IR (neat): 1736 (C=O ester), 1671 (C=O TFA). UV/Vis (H_2O): 203 (1238300), 280 (136600), 318 (sh, 40100), 334 (sh, 31900). ^{1}H NMR (300 MHz, D_2O): 7.36 (m, 12H), 6.48-6.45 (m, 84H), 6.36 (m, 24H), 5.22 (m, 24H), 4.64 (m, 72H), 4.03 (m, 96H), 3.28 (m, 96H), 2.39 (m, 24H), 1.65 (m, 24H). ^{13}C NMR (100 MHz, D_2O) δ 163.7, 162.4 (q, J = 34 Hz), 160.2, 159.7, 159.0, 146.5, 145.4, 141.4, 139.1, 137.4, 122.8, 116.5 (q, J = 290 Hz), 107.1, 106.6, 101.7, 101.2, 66.6, 63.9, 53.6, 48.9, 46.4, 38.8, 27.1, 21.1.

Compound G3NH$_3^+$.



G3NHBoc (251 mg, 0.009 mmol) was dissolved in TFA (2 mL). After 4 h, the mixture was evaporated and dried under vacuum to afford **G3NH$_3^+$** as its trifluoroacetate salt (264 mg, quantitative). Orange glassy product. IR (neat) 1738 (C=O ester), 1671 (C=O TFA). UV/Vis (H_2O): 203 (2779600), 280 (247200), 318 (sh, 41400), 334 (sh, 29100). ^{1}H NMR (300 MHz, D_2O): 7.59 (broad s, 12H), 6.50-6.10 (m, 252H), 4.80 (broad s, 24H), 4.70 (broad s, 144H), 3.85 (m, 216H), 3.2 (m, 192H), 2.30 (m, 24H), 1.65 (m, 24H). ^{13}C NMR (100 MHz, D_2O): 163.6, 162.4 (q, J = 35 Hz), 159.8, 159.4, 158.9, 146.4, 145.2, 141.5, 139.9, 138.9, 137.4, 122.5, 116.5 (q, J = 292 Hz), 106.1, 106.1, 106.1, 101.1, 101.1, 101.1, 69.4, 69.0, 66.7, 65.5, 63.8, 53.4, 48.9, 38.8, 27.4, 21.0.

^{13}C NMR and IR spectra of fullerene hexa-adducts G*n*NHBoc and G*n*NH_3^+

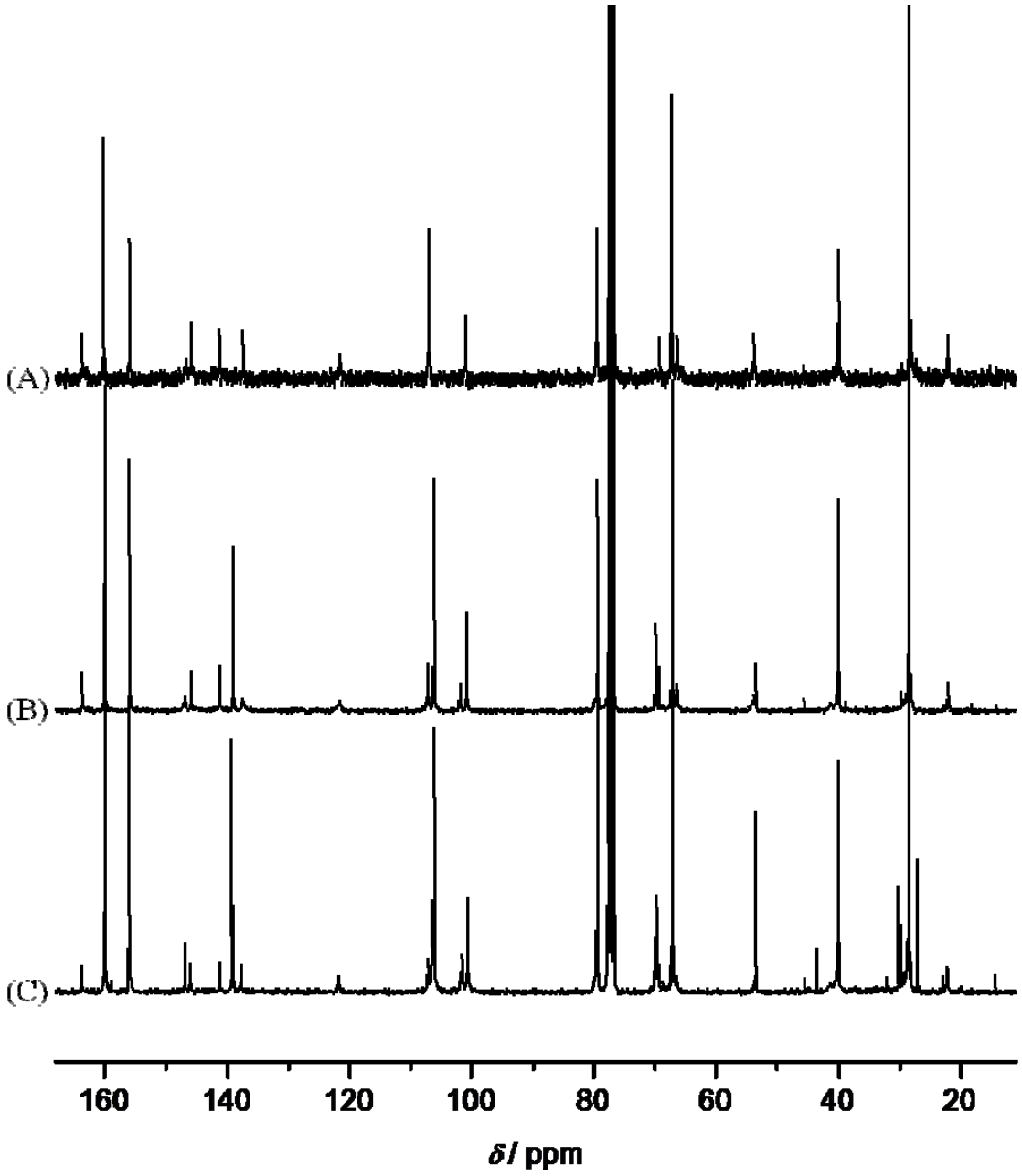


Figure S1A. ^{13}C NMR spectra ($CDCl_3$) of compounds **G1NHBoc** (A), **G2NHBoc** (B) and **G3NHBoc** (C).

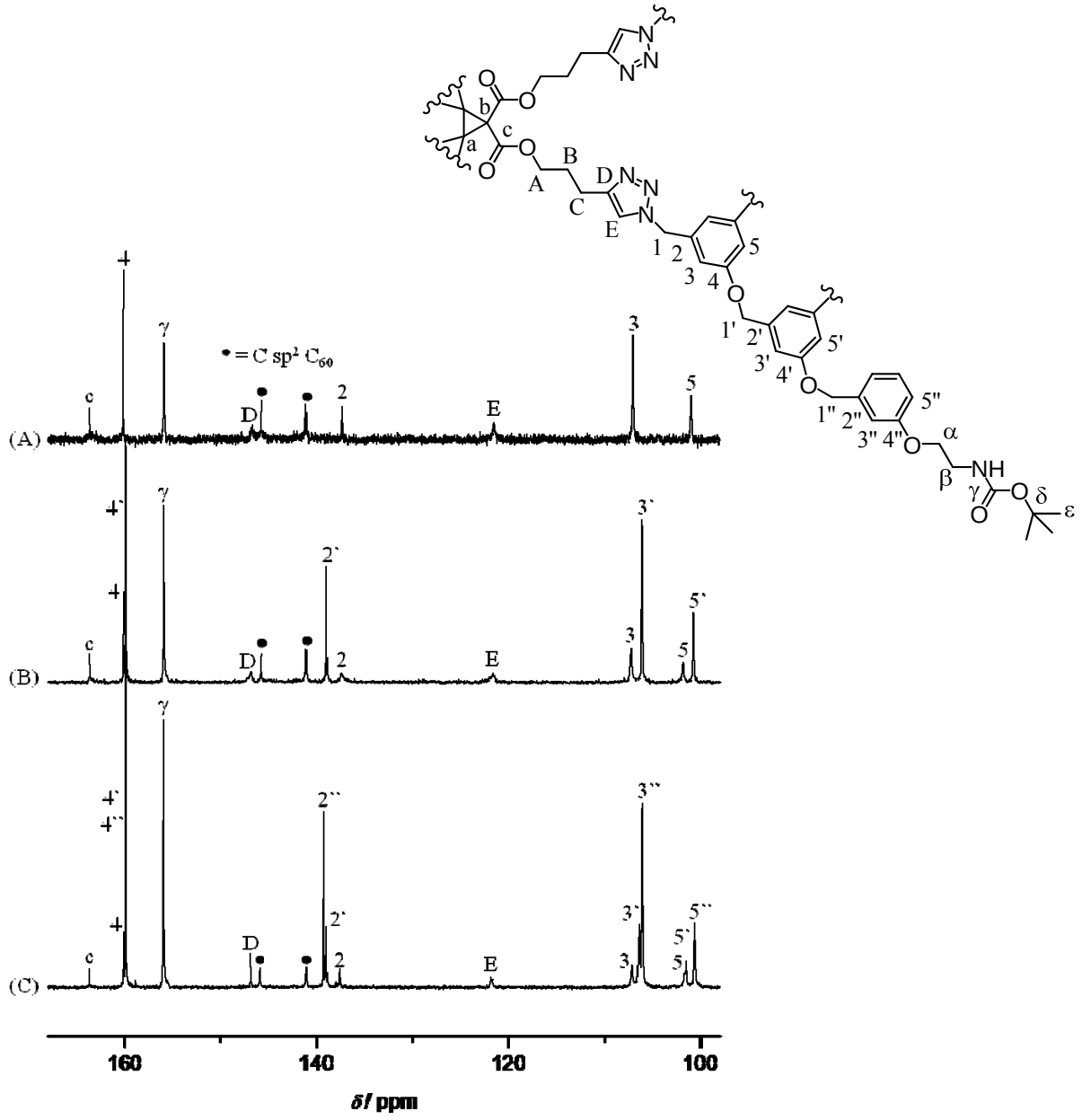


Figure S1B. ^{13}C NMR spectra ($CDCl_3$) of compounds **G1NHBoc** (A), **G2NHBoc** (B) and **G3NHBoc** (C).

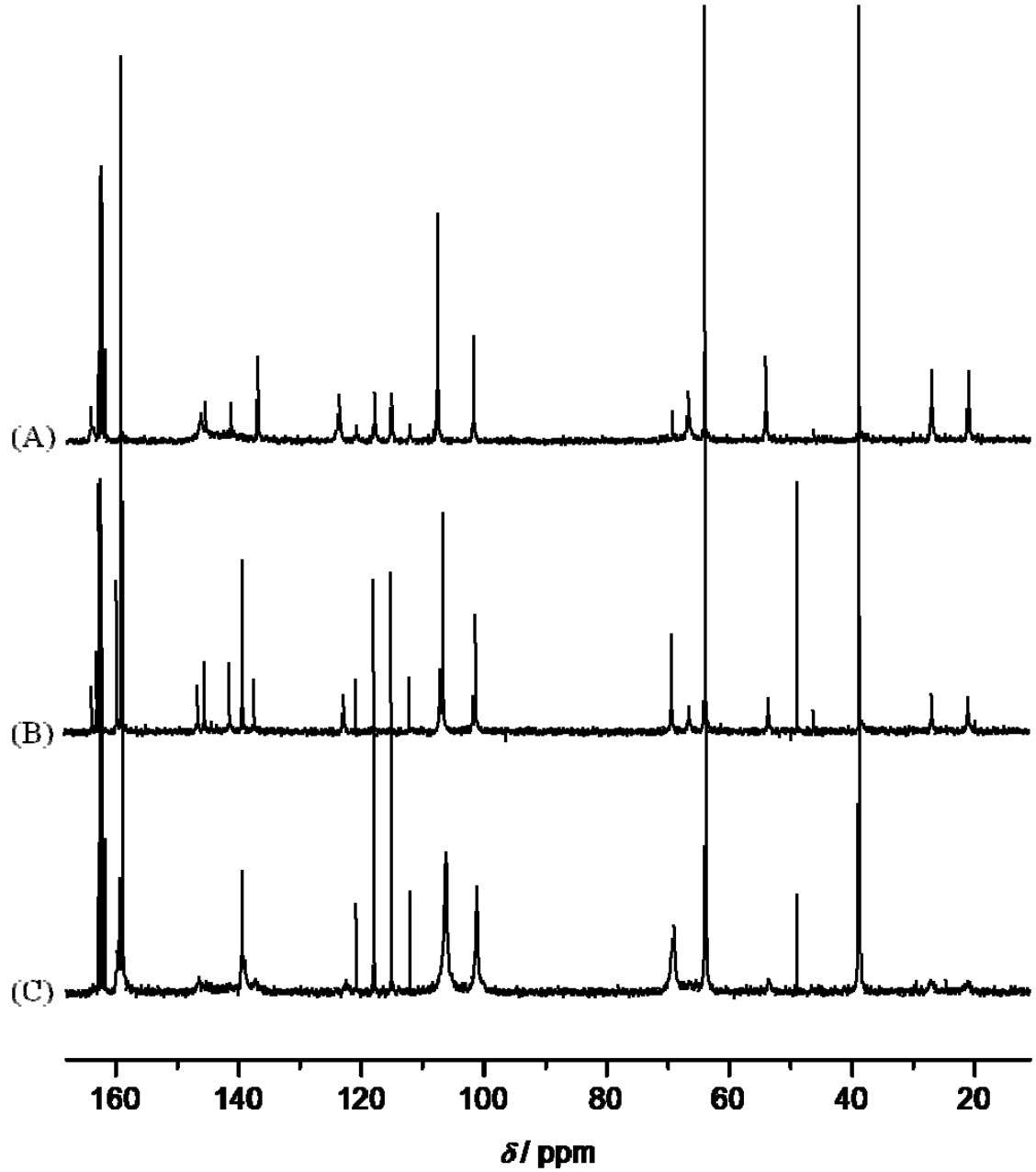


Figure S2A. ^{13}C NMR spectra (D_2O) of compounds **G1NH$_3^+$** (A), **G2NH$_3^+$** (B) and **G3NH$_3^+$** (C) (◊ = residual CH_3OH).

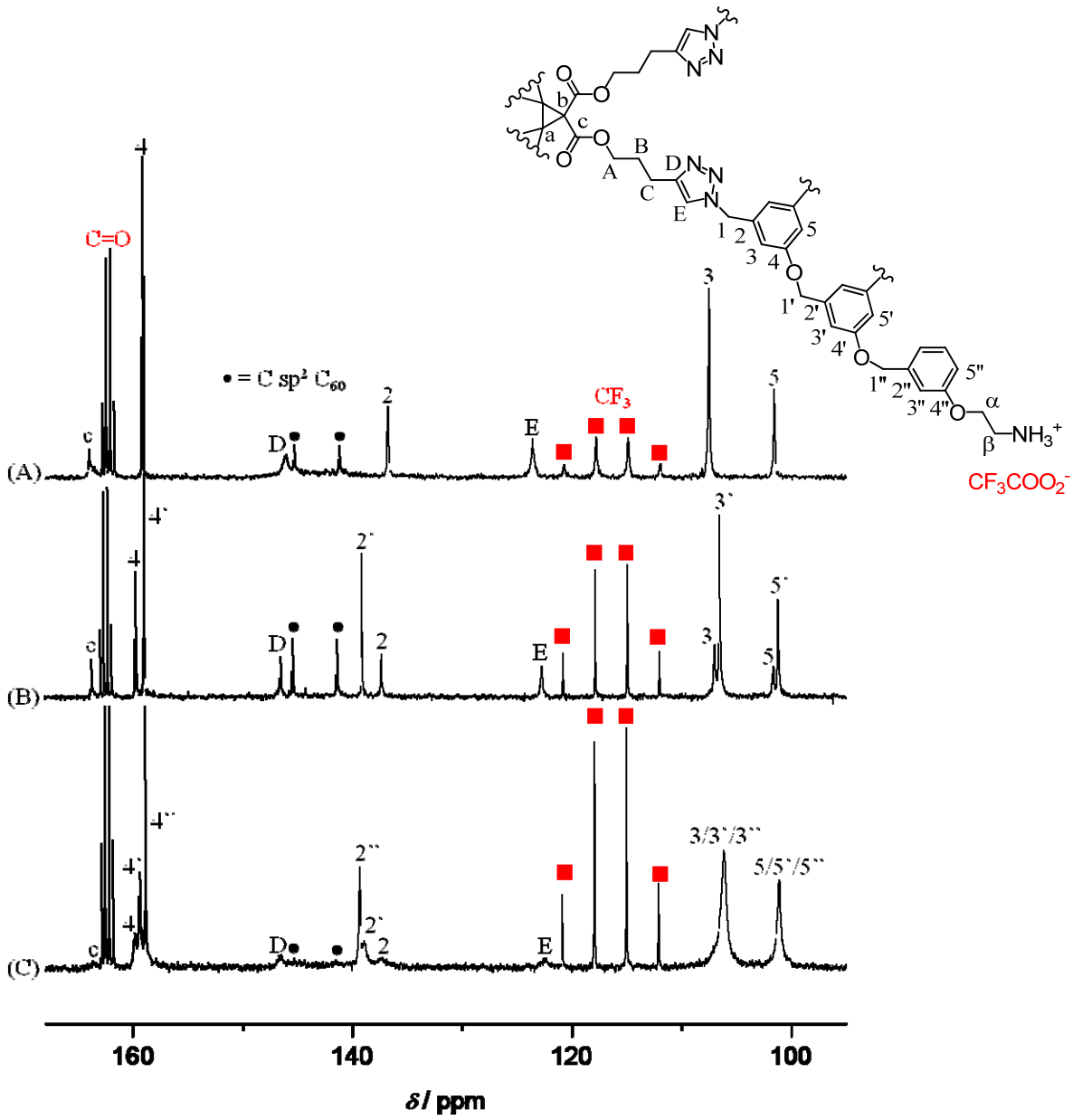


Figure S2B. ^{13}C NMR spectra (D_2O) of compounds **G1NH$_3^+$** (A), **G2NH$_3^+$** (B) and **G3NH$_3^+$** (C).

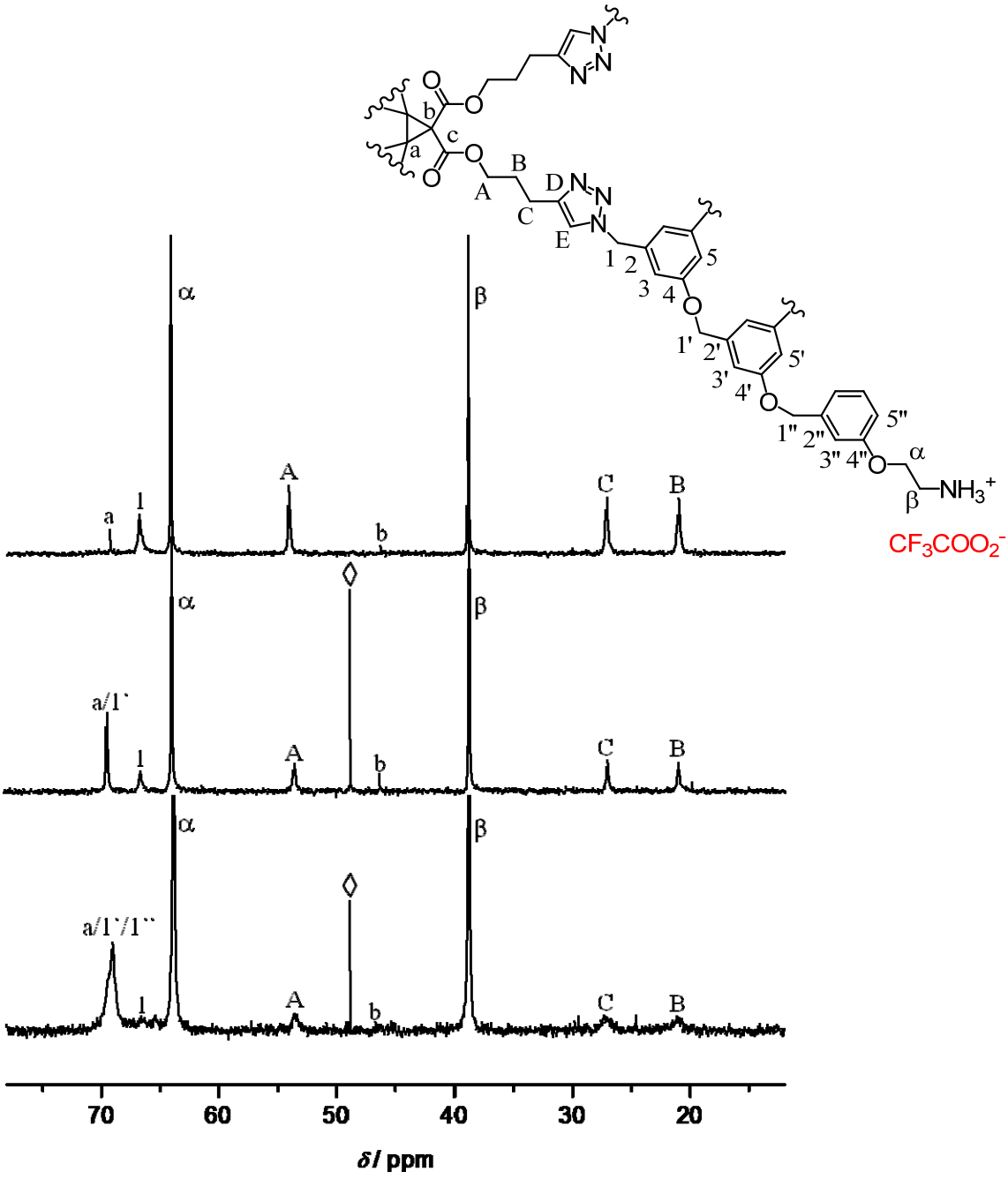


Figure S2C. ^{13}C NMR spectra (D_2O) of compounds **G1NH$_3^+$** (A), **G2NH$_3^+$** (B) and **G3NH$_3^+$** (C) (◊ = residual CH_3OH).

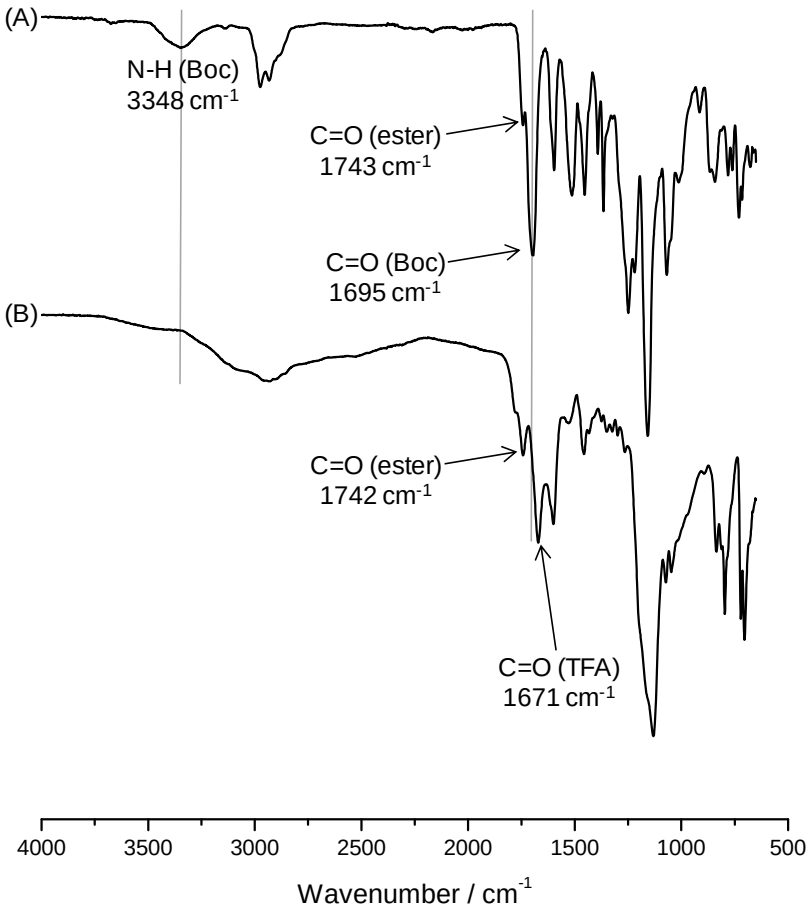


Figure S3. IR spectra of compounds **G1NHBoc** (A) and **G1NH$_3^+$** (B).

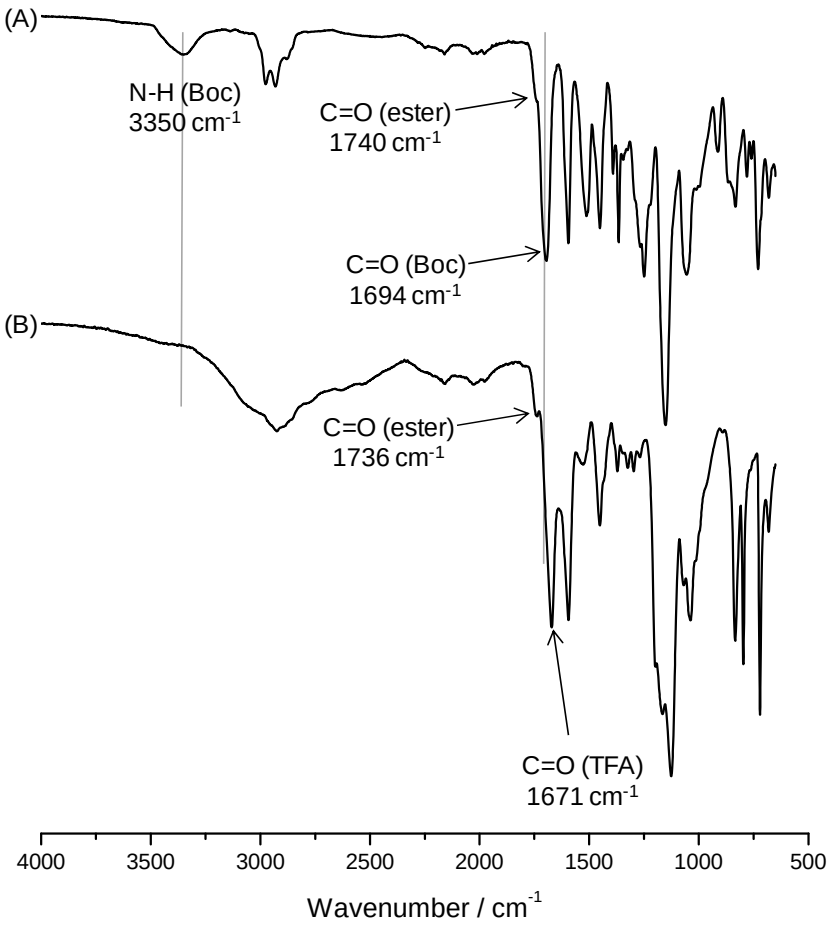


Figure S4. IR spectra of compounds **G2NHBoc** (A) and **G2NH$_3^+$** (B).

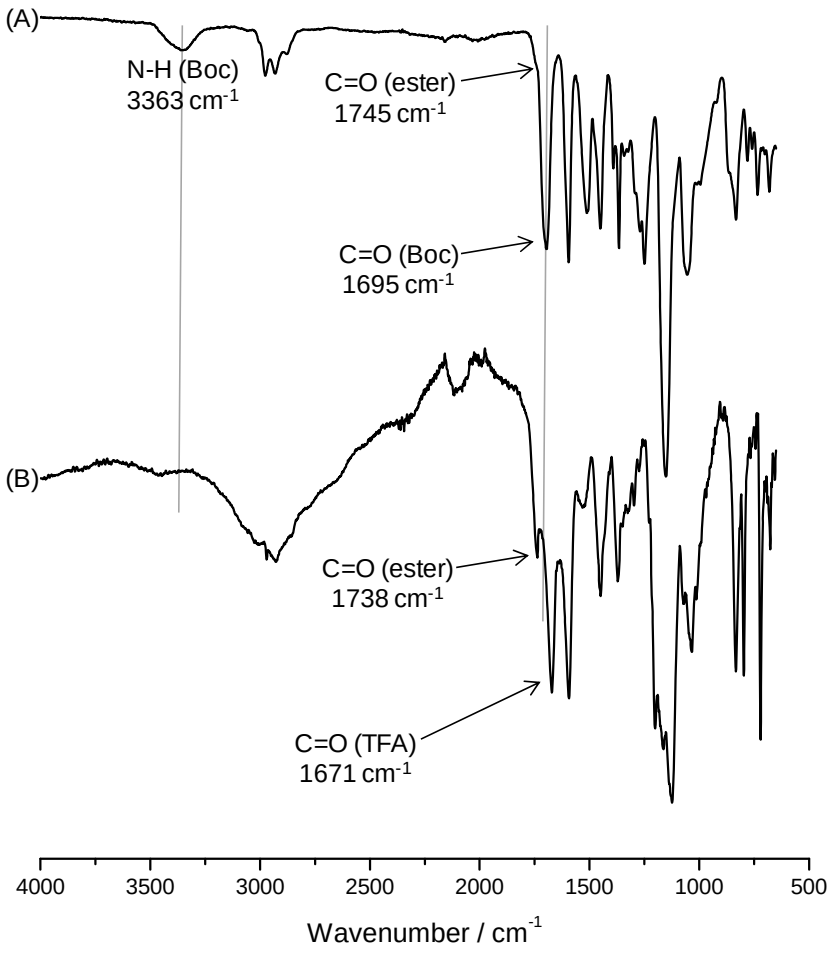


Figure S5. IR spectra of compounds **G3NHBoc** (A) and **G3NH$_3^+$** (B).

G*n*NH_3^+/pCMVLuc polyplexes

Agarose gel electrophoresis analysis.

Twenty microliters of water containing 5% glucose or 150 mM NaCl, 0.4 µg pCMVLuc and increasing amounts of cationic vectors G*n*NH_3^+ were subjected (30 min of complexation time) to electrophoresis in a 1% agarose gel containing 1 mM EDTA and 40 mM Tris acetate buffer and 0.5 µg/mL ethidium bromide, for 90 min at 100 V. DNA was visualized with an UV transilluminator at 254 nm.

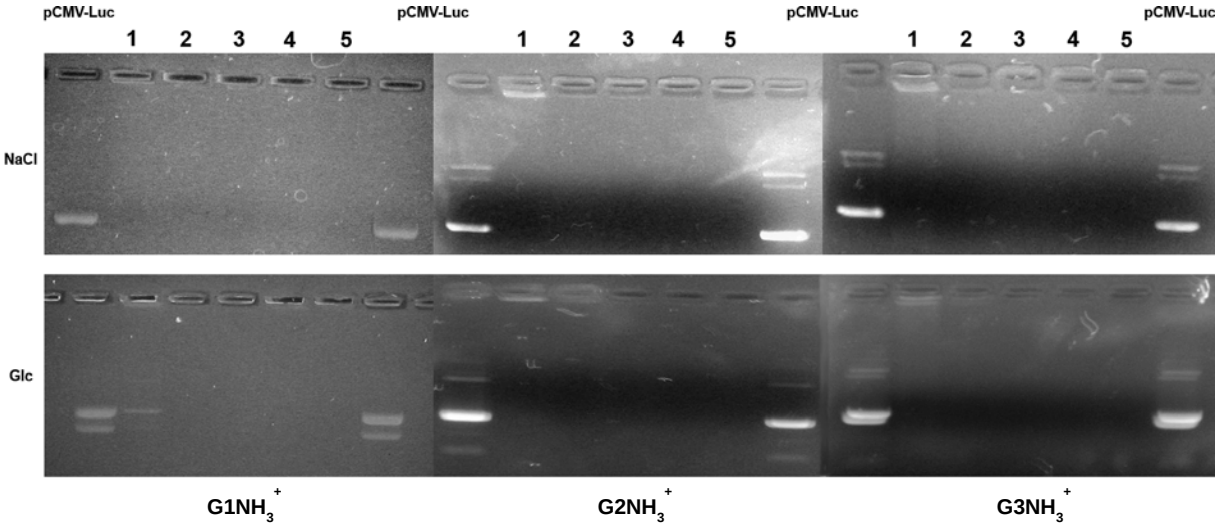


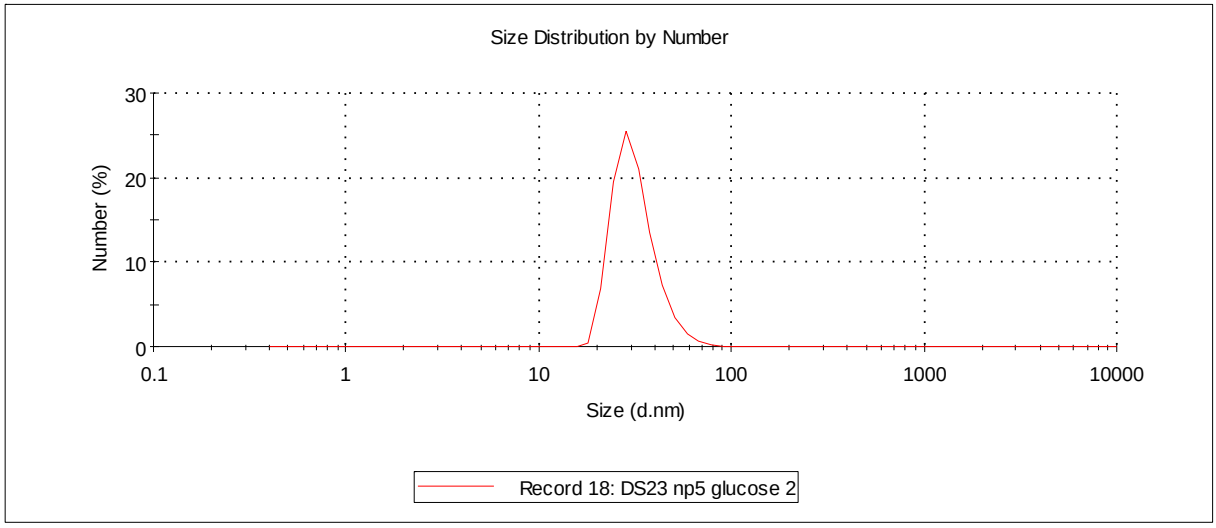
Figure S6. Agarose gels. Polyplexes were prepared at N/P = 1 to 5.

Size of particles, ς potential.

The hydrodynamic radii were determined *via* dynamic light scattering measurements using a Malvern nanoZS apparatus with the following specifications:

sampling time = 90 s; refractive index of medium (water with 5% glucose) = 1.340; refractive index of particles = 1.43; medium viscosity = 1.0140 cP temperature = 25°C. Data were analyzed using the multimodal number distribution software included with the instrument. ς potentials were measured with the same apparatus and with the following specifications: 20 measurements per sample; dielectric constant = 80; temperature = 25°C; beam mode F(Ka) = 1.5 (Smoluchowski model). Polyplexes (1 mL volume) were prepared as described for delivery experiment.

(A)



(B)

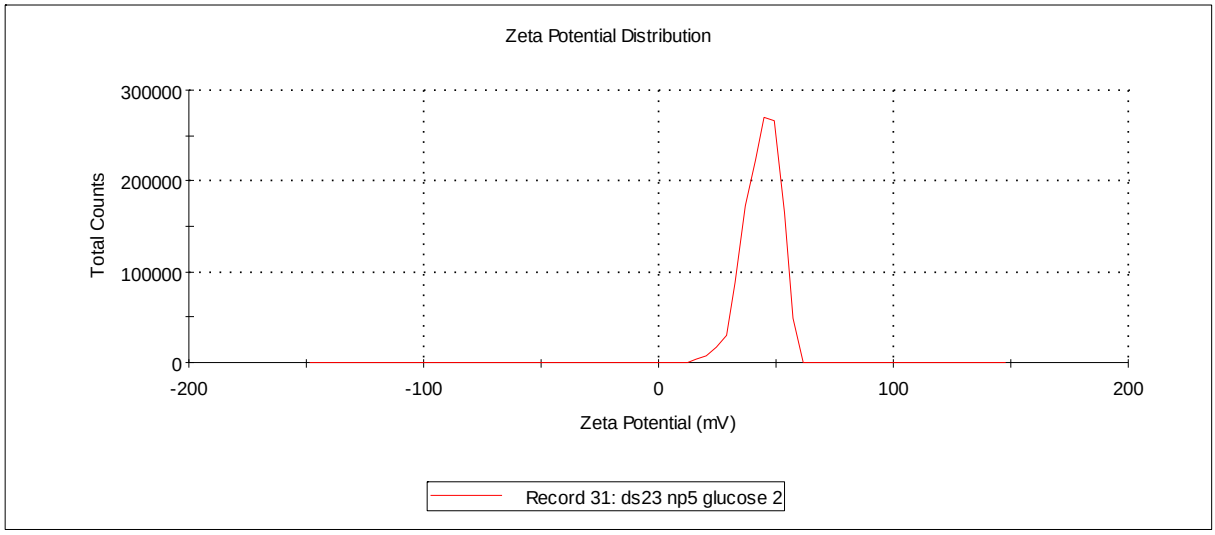
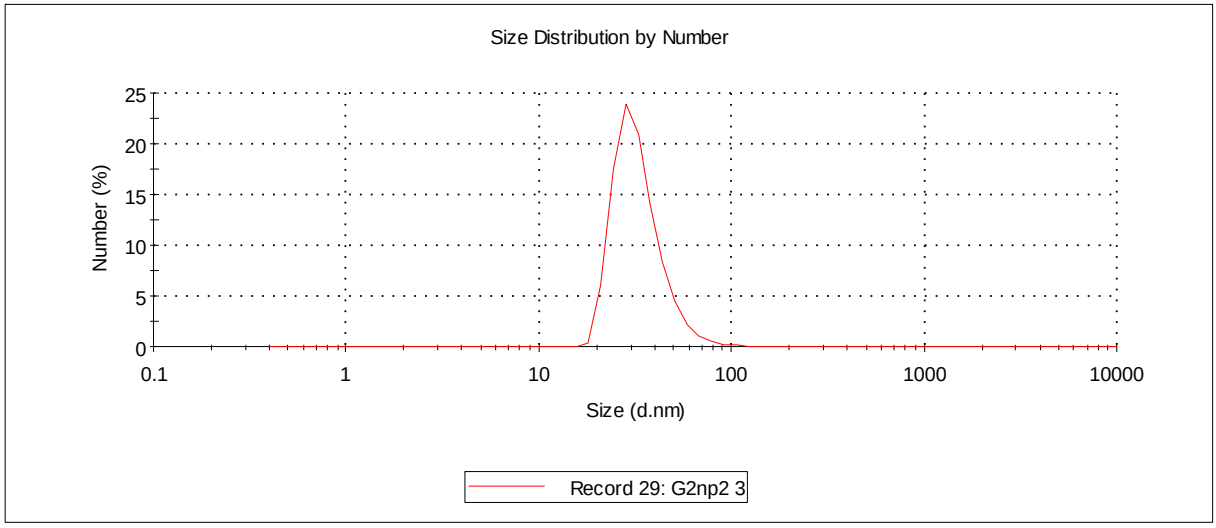


Figure S7. Size (A) and ς potential (B) of **G1NH$_3^+$**/pCMVLuc polyplexes at N/P 5.

(A)



(B)

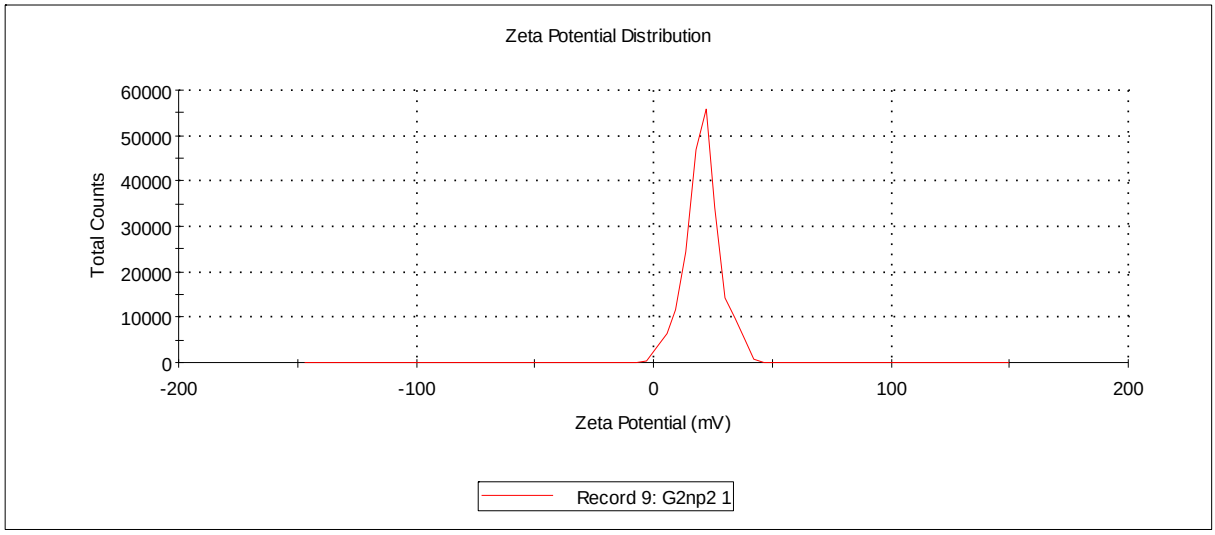
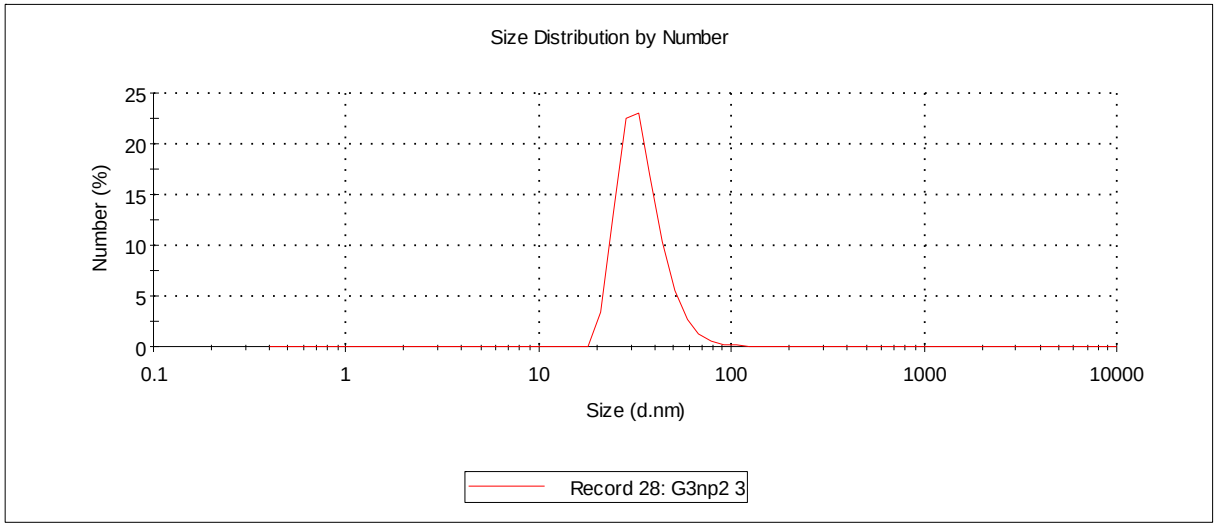


Figure S8. Size (A) and ς potential (B) of **G2NH$_3^+$**/pCMVLuc polyplexes at N/P 2.

(A)



(B)

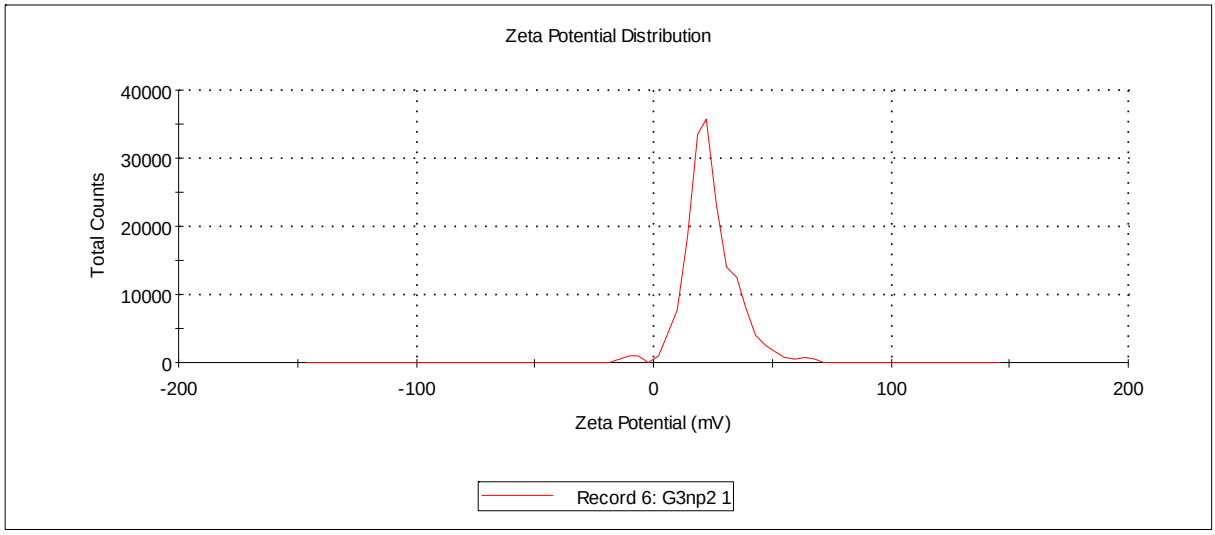


Figure S9. Size (A) and ς potential (B) of **G3NH$_3^+$**/pCMVLuc polyplexes at N/P 2.

Electron microscopy analysis.

Images were taken with a TEM Philips CM12 apparatus. Polyplexes were transferred onto ultrathin carbon film grids (Ted Pella, 1822-F, formvar removed) by placing the grid on top of 10 µL drop for 1 min. Grid with adherent particles was wicked from one side, placed on 100 µL water drop for 30 s for washing, wicked, placed for 1 min on 60 µL drop of freshly filtered 1.33% uranyle acetate, wicked again and air dried before imaging.

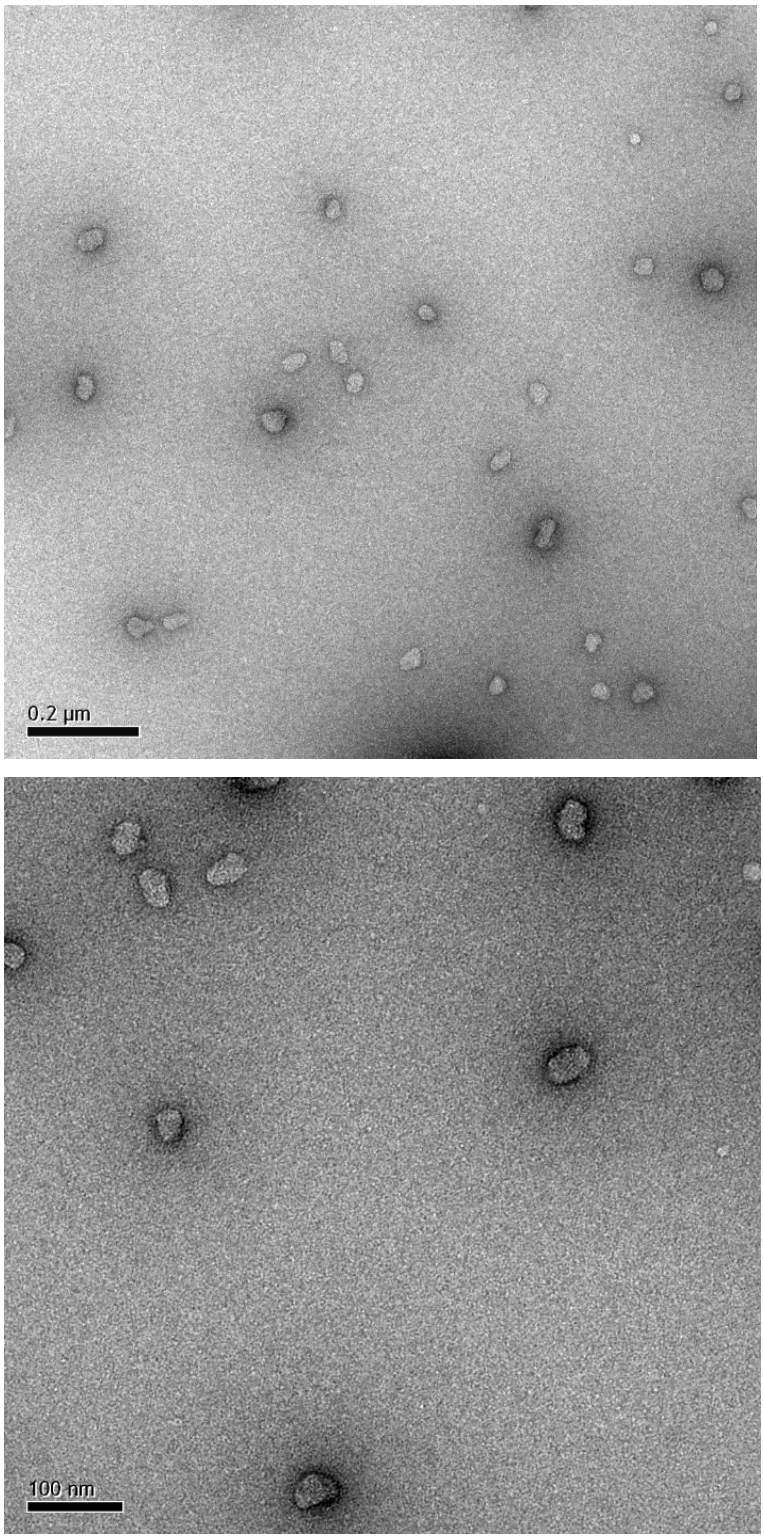


Figure S10. TEM images of **G1NH$_3^+$**/pCMVLuc polyplexes at N/P 5 in water with 5% glucose.

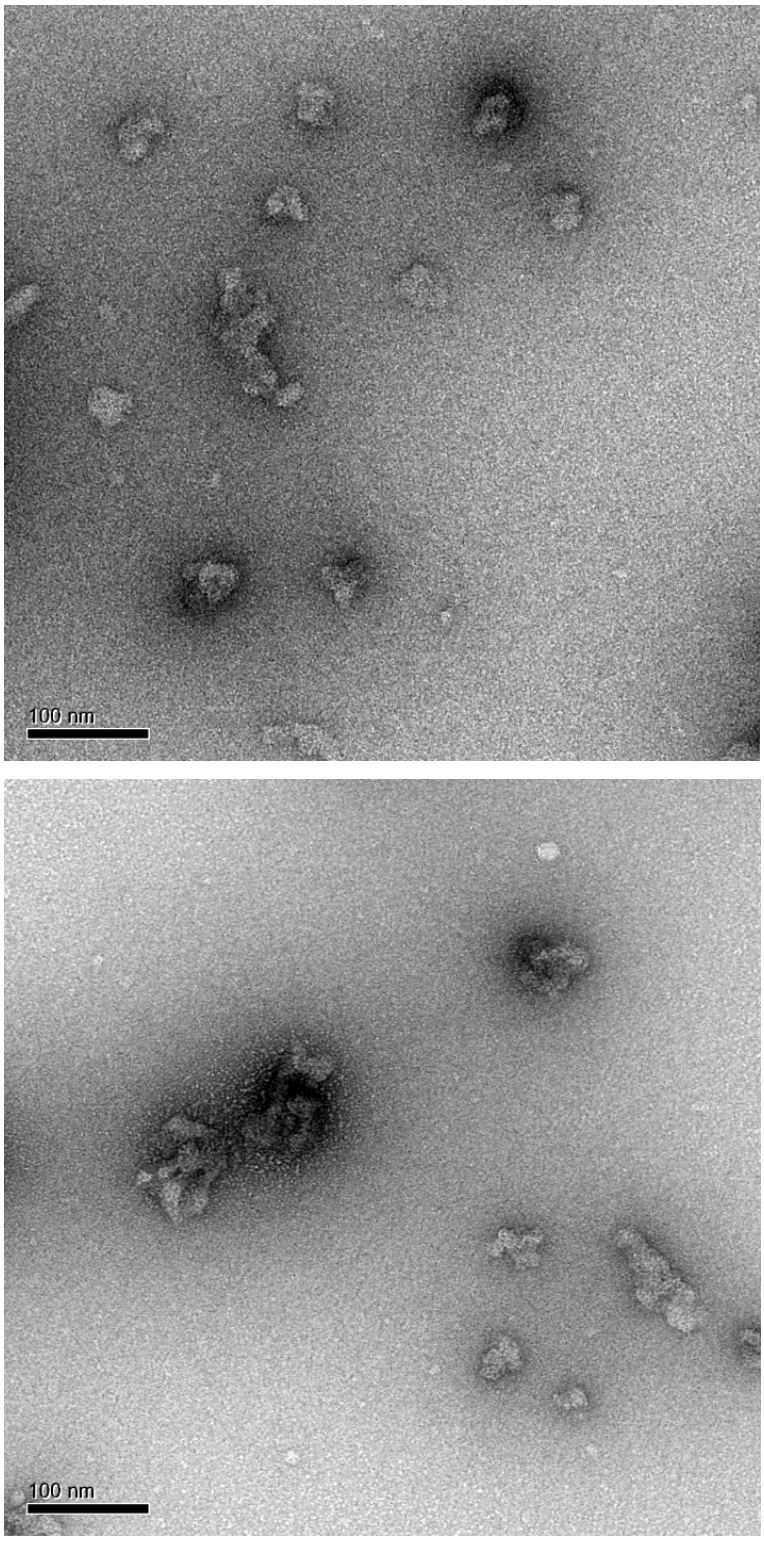


Figure S11. TEM images of **G2NH$_3^+$**/pCMVLuc polyplexes at N/P 3 in water with 5% glucose.

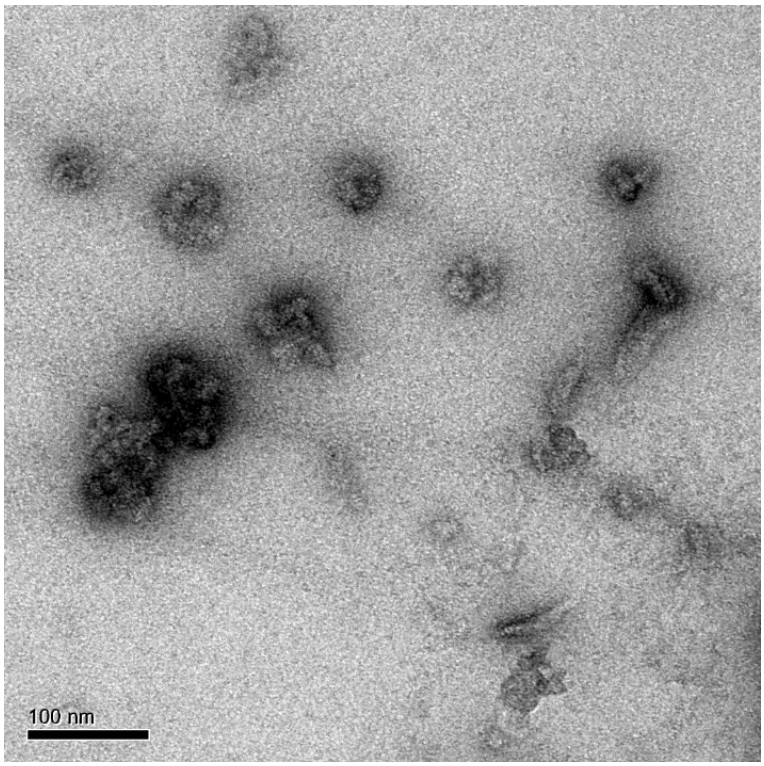


Figure S12. TEM image of **G3NH$_3^+$**/pCMVLuc polyplexes at N/P 5 in water with 5% glucose.

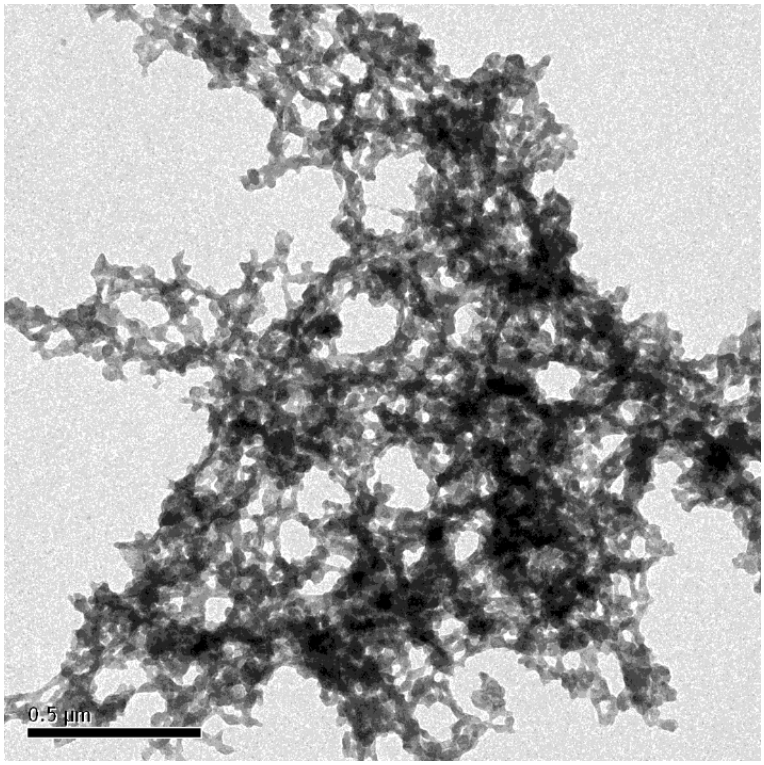


Figure S13. TEM image of **G1NH$_3^+$**/pCMVLuc polyplexes at N/P 5 in water with 150 mM NaCl.

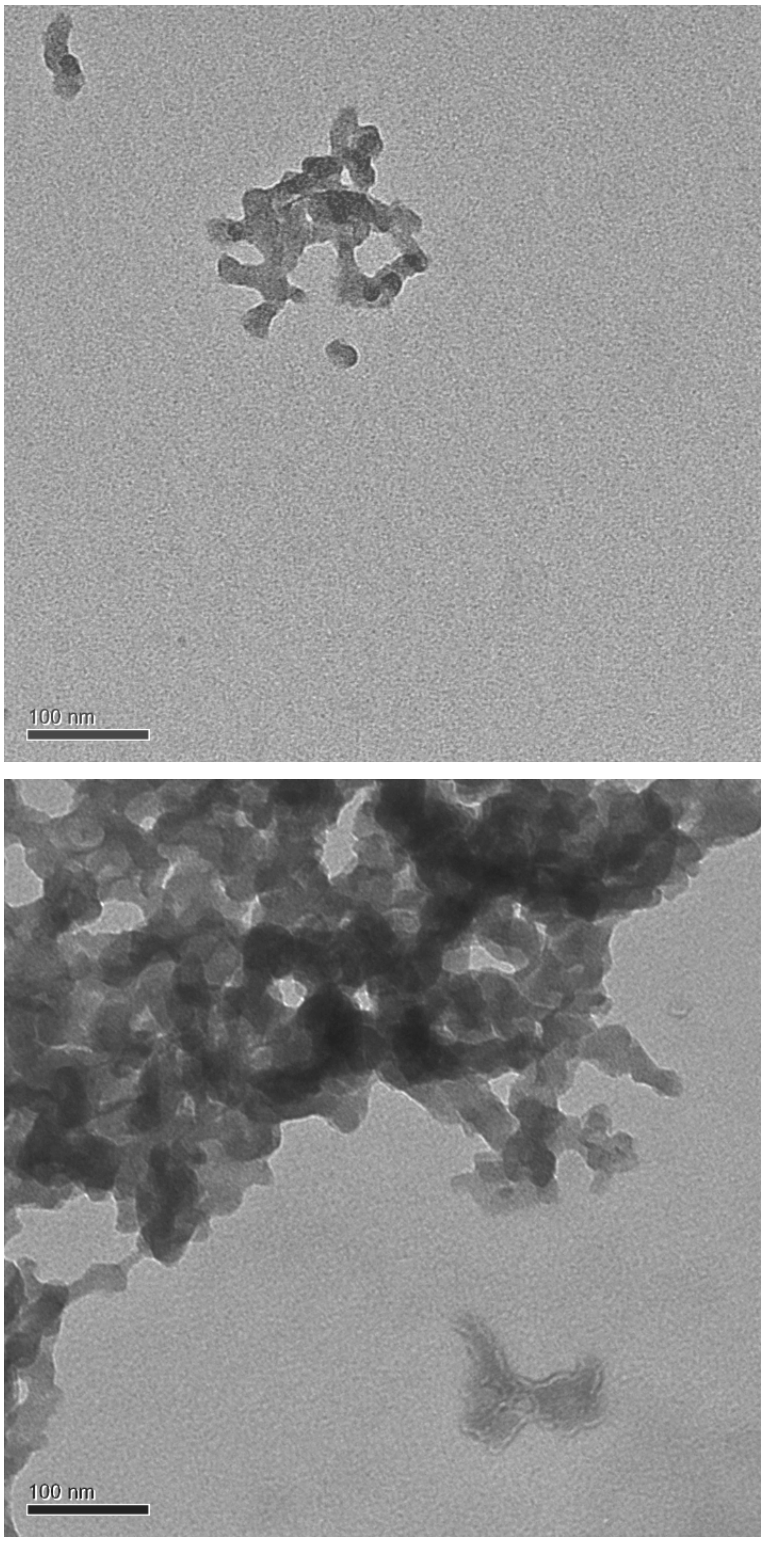


Figure S14. TEM images of **G2NH$_3^+$**/pCMVLuc polyplexes at N/P 4 in water with 150 mM NaCl.

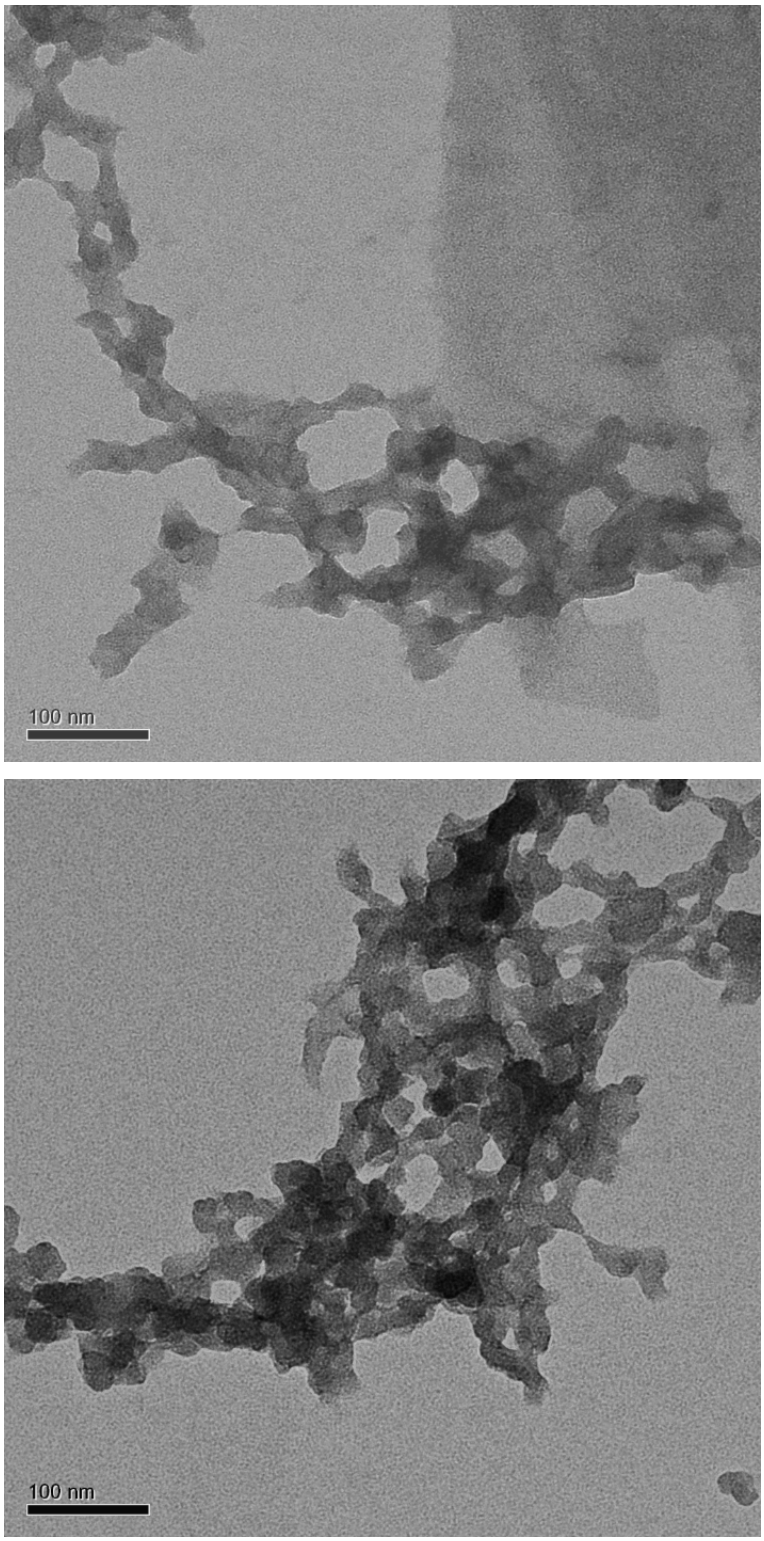


Figure S15. TEM images of **G3NH$_3^+$**/pCMVLuc polyplexes at N/P 6 in water with 150 mM NaCl.

Gene transfer experiments.

Cell culture.

HeLa cells were grown in Eagle's MEM supplemented with 10% FBS, L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 μg/mL). Cells were maintained at 37°C in a 5% CO_2 humidified atmosphere and all experiments were done in triplicates. The day before experiment, cells were seeded in 24-multiwell plates at 50.10^3 cells/well in fresh complete medium (1 mL).

Polyplexes formation for pCMVLuc delivery.

The procedure is for a 24-multiwell plates experiment. Typically, an aqueous solution of the $\mathbf{G}\boldsymbol{n}\mathbf{NH_3^+}$ (n = 1, 2 or 3) (volume depending on the desired N/P ratio) is diluted up to 50 μL in water containing 5% glucose or 150 mM NaCl. The solution is vortexed and left for 10 min. Separatly, an aqueous solution of pCMVLuc (corresponding to 2 μg of pCMVLuc) is diluted up to 50 μL in water containing 5% glucose or 150 mM NaCl. Then, the solution is vortexed and left for 10 min, after which the G_1, G_2 or G_3 solution is added to the pCMVLuc solution, and vigorously mixed (15 s). Finally, the polyplexes are incubated for 30 min at room temperature and added in each well by dilution with the cell medium without serum (1 mL). Four hours later, each well is completed with serum (0.1 mL). The gene expression profiles are analyzed 24 h after addition of polyplexes.

Quantification of the luciferase gene expression.

Luciferase gene expression was determined 24 h after delivery with a commercial kit, using manufacturer's protocol (Luciferase Assay System, Promega, Charbonnières, France). The luminescence was measured from 2 μL of lysate during 1 s with a luminometer (Centro LB960 XS; Berthold, Thoiry, France). Luciferase activity is expressed as the mean of light units integrated over 10 s (RLU) and normalized per mg of cell protein by using the BCA assay (Pierce, Brebières, France). The errors bars represent standard deviation derived from triplicate experiments.

JetSI™-ENDO is a transfection Reagent (Polyplus transfection), which is used according to manufacturer's instructions.

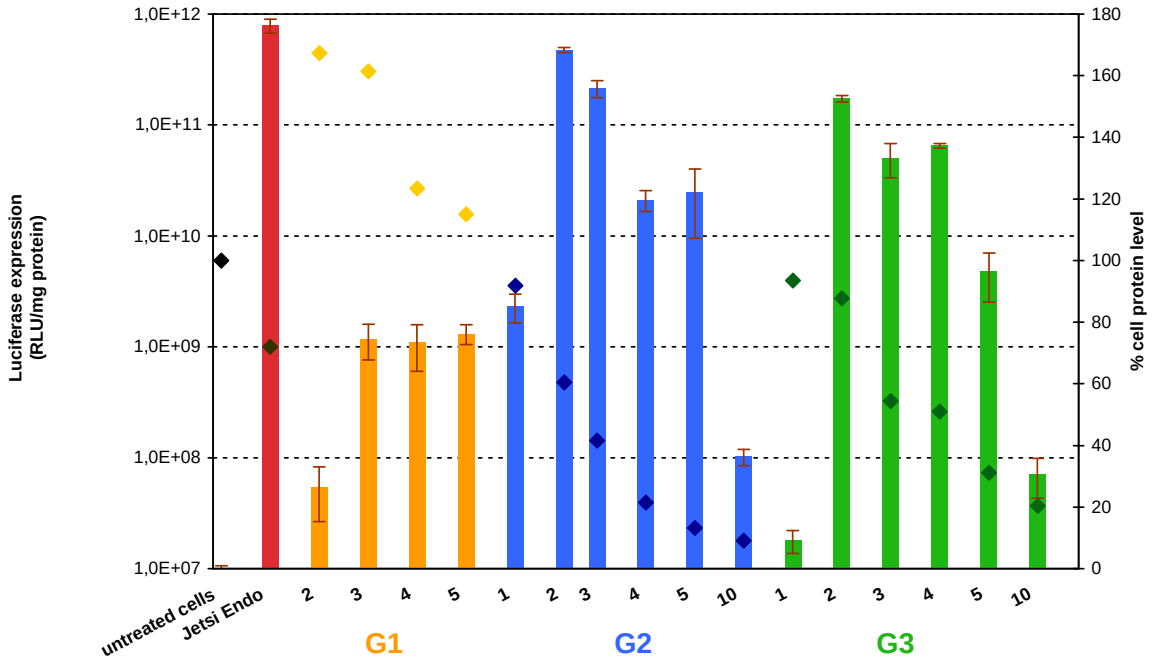


Figure S16. Delivery experiments of pCMVLuc at different N/P (Polyplexes prepared in 5% glucose solution).

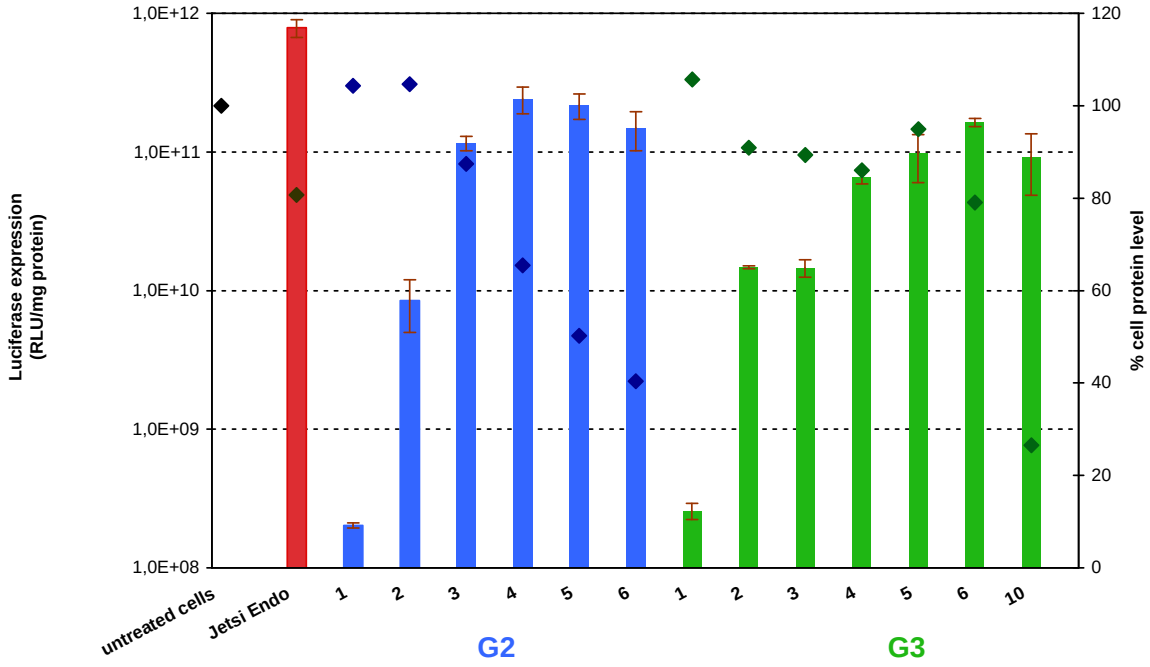


Figure S17. Delivery experiments of pCMVLuc at different N/P (Polyplexes prepared in 150 mM NaCl solution). N.B. **G1NH$_3^+$** has no efficiency under these conditions.