Cooperative Binding and Self-Assembling Behavior of Cationic Low-Molecular-Weight Dendrons with RNA Molecules

Jiehua Zhou[1], Jiangyu Wu[1,6], Xiaoxuan Liu[1], Fanqi Qu[1], Mu Xiao[2], Yi Zhang[2], Laurence Charles[3], Cheng-cai Zhang[4.5], Ling Peng[1, 6, *]

[1] College of Chemistry and Molecular Sciences, Wuhan University, Wuhan, 430072, P. R. China

[2] College of Life Sciences, Wuhan University, Wuhan, 430072, P. R. China

[3] JE 2421 TRACES, Faculté des Sciences et Techniques de St. Jérôme, Avenue Escadrille Normandie-Niemen, 13397 Marseille Cedex 13, France

[4] Laboratoire de Chimie Bactérienne, CNRS UPR 9043, 31 chemin Joseph Aiguier, 13402 Marseille cedex 20, France

[5] National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, 430070, P. R. China

[6] Département de Chimie, CNRS UMR 6114, 163, avenue de Luminy, 13288 Marseille, France

General Methods:

^{1}H NMR and ^{13}C NMR spectra were measured at 300 MHz and 75 MHz relatively on a Varian Mercury-VX300 spectrometer. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference. ^{1}H NMR spectra of RNA/dendrimer complexes were measured at 600 MHz on a Varian Inova-600 spectrometer. For dendrimers **6c**, **8c**, **10b-c**, **11**, **12a-c**, **13** and **14a-c**, positive mode electrospray mass spectra were obtained using API III Plus triple quadrupole mass spectrometer (Sciex, Thornhill, Canada), while for all other compounds, mass spectra were determined

either using ZAB-HF-3F mass spectrometer for FAB MS or Finnigan LCQ Advantage mass spectrometer for ESI MS. IR spectra were recorded with a PERKIN ELMER spectrophotometer. Freshly prepared sodium methoxide (MeONa) was used. Methyl acrylate and ethylenediamine were distilled before use. All other reagents and solvents were used without further purification from commercial sources. RNA oligonucleotide GCCUCUAAAAA was synthesized by Dharmacon Research, Inc. (Lafayette, IN). The RNA or DNA concentration was determined by measuring the absorbance at 260 nm. The dendrimers solution were prepared with an appropriate concentration in 50 mM Tris-HCl buffer (pH=7.6) and stored at 4°C. CD spectra were recorded on a Jasco J-810 spectropolarimeter. TEM studies were performed with a Hitachi H-7000FA electron microscope instrument.

3-[{2-[2-(2-Benzyloxy-ethoxy)-ethoxy]-ethyl}-(2-methoxycarbonyl-ethyl)-amino]-propionic acid methyl ester (5): To a solution of compound **4** (97.5 mg, 0.41 mmol) in MeOH (6 mL) was added 2 drops fresh sodium methoxide and methyl acrylate (0.5 mL), then the reaction was stirred at 25°C for 3 days. The reaction solution was concentrated under reduced pressure. Purification with flash chromatography (EtOAc/Petroleum ether 4/1) gave **5** as a pale yellow liquid (148 mg, 88.1%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22-7.40 (m, 5H), 4.55 (s, 2H), 3.55-3.73 (m, 14H), 3.51 (t, J = 6.3 Hz, 2H), 2.81 (t, J = 6.9 Hz, 4H), 2.66 (t, J = 6.0 Hz, 2H), 2.44 ppm (t, J = 6.9 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.3, 138.1, 128.2, 127.5, 127.4, 73.5, 71.0, 70.9, 70.0, 69.9, 53.8, 52.0, 50.5, 33.3 ppm; IR: υ = 1737.2 cm^{-1}; MS (FAB+): *m/z*: 412 $[M+H]^+$.

G_0-NH_2 (6a): To a solution of compound **5** (145 mg, 0.35 mmol) in MeOH (1 mL) was added ethylenediamine (5 mL), then the reaction was stirred at 25°C for 1 day. The reaction solution was concentrated *in vacuo* to gave **6a** as a pale yellow liquid (158 mg, 95.6%). ^{1}H NMR (300 MHz, D_2O): δ = 7.13-7.33 (m, 5H), 4.42 (s, 2H), 3.38-3.64 (m, 10H), 3.06 (t, J = 6.3 Hz, 4H), 2.64 (t, J = 7.2 Hz, 4H), 2.55 (t, J = 6.3 Hz, 6H), 2.24 ppm (t, J = 7.2 Hz, 4H); ^{13}C NMR (75 MHz, D_2O): δ = 174.8, 137.2, 128.7, 128.5, 128.3, 73.1, 69.9, 69.8, 69.1, 68.3, 52.1, 49.6, 41.8, 40.1, 33.0 ppm; IR: υ = 1647.1 cm^{-1}; MS (FAB+): *m/z*: 468.1 $[M+H]^+$.

G_0-NMe_2 (6b): To a solution of compound **5** (63.9 mg, 0.16 mmol) in MeOH (2.5 mL) was added N, N'-dimethylethylenediamine (2.5 mL). The reaction solution was stirred at 50°C for 5 days and the solvent was removed *in vacuo* to gave **6b** as a pale yellow oil (80.5 mg, 99.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.25-7.32 (m, 5H), 4.54 (s, 2H), 3.58-3.70 (m, 8H), 3.53 (t, J = 6.0 Hz, 2H), 3.24-3.32 (m, 4H), 2.77 (t, J = 6.6 Hz, 4H), 2.66 (t, J = 5.7 Hz, 2H), 2.38 (t, J = 6.3 Hz, 4H), 2.33 (t, J = 6.6 Hz, 4H), 2.21 ppm (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 138.1, 128.5, 128.0, 127.8, 73.6, 70.9, 70.6, 69.8, 69.2, 58.2, 56.7, 53.5, 50.7, 45.7, 45.2, 37.8, 36.7, 34.3 ppm; IR: υ = 1651.8 cm^{-1}; MS (ESI+): *m/z*: 524.1 $[M+H]^+$.

G_0-NMe_3^+ (6c): To a solution of compound **6b** (70.5 mg, 0.14 mmol) in CH_2Cl_2 (5 mL) was added MeI (153 mg, 1.08 mmol). The reaction was stirred at 25°C for 3 days. The solvent and the excess MeI were removed *in vacuo* to gave **6c** as a white solid (128 mg, 99.8%). ^{1}H NMR (300 MHz, D_2O): δ = 7.20 (m, 5H), 4.36 (s, 2H), 3.40-3.60 (m, 14H), 3.20-3.29 (m, 6H), 2.88-3.02 (m, 25H), 2.62 ppm (t, J = 7.5 Hz, 4H); ^{13}C NMR (75

MHz, D_2O): δ = 170.8, 137.6, 129.0, 128.8, 128.6, 73.0, 69.9, 69.8, 69.7, 69.1, 64.2, 61.7, 58.6, 53.7, 50.2, 33.8, 28.9 ppm; IR: υ = 1668.3 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{30}H_{58}N_5O_5 \cdot 3I$: 822.3 $[M\text{-}I]^+$, 347.7 $[M\text{-}2I]^{2+}$, 189.5 $[M\text{-}3I]^{3+}$, found: 822.2 $[M\text{-}I]^+$, 347.9 $[M\text{-}2I]^{2+}$, 189.5 $[M\text{-}3I]^{3+}$.

$G_{0.5}$ (7): To a solution of compound **6a** (111 mg, 0.24 mmol) in MeOH (6 mL) was added 2 drops fresh sodium methoxide and methyl acrylate (0.5 mL), then the reaction was stirred at 25°C for 3 days. The reaction solution was concentrated *in vacuo* to gave **7** as a pale yellow liquid(188 mg, 97.5%). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.14-7.30 (m, 5H), 4.46 (s, 2H), 3.42-3.64 (m, 22H), 3.12-3.23 (m, 4H), 2.76 (t, *J* = 6.6 Hz, 4H), 2.66 (t, *J* = 6.9 Hz, 10H), 2.44 (t, *J* = 6.0 Hz, 4H), 2.34 (t, *J* = 6.9 Hz, 8H), 2.29 ppm (t, *J* = 6.6 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.9, 172.1, 138.1, 128.4, 127.8, 127.7, 73.5, 70.8, 70.6, 69.7, 69.2, 53.3, 53.1, 52.1, 50.7, 50.6, 49.6, 37.6, 33.9, 33.1 ppm; IR: υ = 1736.1, 1655.4 cm^{-1}; MS (FAB+): *m/z*: 812 $[M+H]^+$.

G_1-NH_2 (8a): According to the same procedure described for the synthesis of compound **6a**, **8a** was obtained from compound **7**. ^1H NMR (300 MHz, D_2O): δ = 7.12-7.30 (m, 5H), 4.40 (s, 2H), 3.38-3.62 (m, 10H), 3.00-3.17 (m, 12H), 2.37-2.74 (m, 26H), 2.12-2.30 (m, 12H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 175.1, 174.8, 137.4, 128.8, 128.5, 128.4, 73.0, 69.8, 69.7, 69.0, 68.2, 51.9, 51.4, 49.4, 49.2, 41.6, 39.9, 36.9, 32.9, 32.7 ppm; IR: υ = 1646.8 cm^{-1}; MS (FAB+): *m/z*: 924 $[M+H]^+$.

G_1-NMe_2 (8b): According to the same procedure described for the synthesis of compound **6b**, **8b** was obtained from compound **7**. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.25-7.33 (m, 5H), 4.54 (s, 2H), 3.56-3.67 (m, 8H), 3.53 (t, *J* = 5.9 Hz, 2H), 3.31 (m,

8H), 3.23 (m, 2H), 2.77 (m, 4H), 2.72 (m, 8H), 2.53 (t, J = 6.0 Hz, 4H), 2.37-2.45 (m, 12H), 2.26-2.37 (m, 12H), 2.21 ppm (s, 24H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6, 138.0, 128.4, 127.8, 127.7, 73.5, 70.8, 70.6, 69.7, 69.3, 58.7, 53.1, 53.0, 50.6, 50.4, 46.0, 45.7, 45.2, 38.2, 37.5, 34.9, 34.1 ppm; IR: υ = 1648.4 cm^{-1}; MS (ESI+): *m/z*: 1036.7 $[M+H]^+$.

G_1-NMe_3^+ (8c): According to the same procedure described for the synthesis of compound **6c**, **8c** was obtained from compound **8b**. ^{1}H NMR (300 MHz, D_2O): δ = 7.25 (m, 5H), 4.41 (s, 2H), 3.45-3.60 (m, 34H), 3.24-3.36 (m, 14H), 2.86-3.01 (m, 45H), 2.60-2.80 ppm (m, 12H); ^{13}C NMR (75 MHz, D_2O): δ = 170.9, 170.6, 137.6, 129.0, 128.8, 128.6, 73.0, 70.0, 69.8, 69.2, 64.2, 61.7, 59.6, 58.7, 58.2, 53.7, 51.4, 49.4, 49.0, 33.9, 33.2, 29.0, 28.8 ppm; IR: υ = 1663.1 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{58}H_{118}N_{13}O_9.7I$: 887.8 $[M-2I]^{2+}$, 549.5 $[M-3I]^{3+}$, 380.4 $[M-4I]^{4+}$, 279.0 $[M-5I]^{5+}$, found: 887.9 $[M-2I]^{2+}$, 549.6 $[M-3I]^{3+}$, 380.2 $[M-4I]^{4+}$, 279.1 $[M-5I]^{5+}$.

$G_{1.5}$ (9): According to the same procedure described for the synthesis of compound **7**, **9** was obtained from compound **8a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22-7.38 (m, 5H), 4.58 (s, 2H), 3.52-3.80 (m, 34H), 3.18-3.38 (m, 12H), 2.64-2.98 (m, 30H), 2.50-2.64 (m, 12H), 2.28-2.50 ppm (m, 28H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.2, 172.5, 138.3, 128.5, 127.9, 127.7, 73.3, 70.7, 70.4, 69.5, 69.1, 53.0, 52.6, 52.3, 51.8, 51.7, 50.3, 50.0, 49.9, 49,4, 37.6, 37.3, 33.9, 33.6, 32.8, 32.7 ppm; IR: υ = 1735.5, 1648.8 cm^{-1}; MS (ESI+): *m/z*: 1613 $[M+H]^+$.

G_2-NH_2 (10a): According to the same procedure described for the synthesis of compound **6a**, **10a** was obtained from compound **9**. ^{1}H NMR (300 MHz, D_2O): δ =

7.15-7.30 (m, 5H), 4.44 (s, 2H), 3.41-3.64 (m, 10H), 3.04-3.30 (m, 28H), 2.59-2.80 (m, 46H), 2.38-2.55 (m, 12H), 2.14-2.36 ppm (m, 28H); ^{13}C NMR (75 MHz, D_2O): δ = 175.1, 174.7, 137.3, 128.8, 128.5, 128.4, 73.0, 69.8, 69.6, 69.0, 68.1, 51.8, 51.3, 49.2, 49.1, 48.9, 42.6, 41.8, 39.9, 36.8, 32.8, 32.7, 32.5 ppm; IR: υ = 1642.0 cm^{-1}; MS (ESI+): *m/z*: 1838 $[M+H]^+$.

G_2-NMe_2 (10b): According to the same procedure described for the synthesis of compound **6b**, **10b** was obtained from compound **9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30 (m, 5H), 4.54 (s, 2H), 3.50-3.68 (m, 10H), 3.18-3.40 (m, 28H), 2.60-2.82 (m, 28H), 2.39-2.60 (m, 30H), 2.29-2.39 (m, 28H), 2.25 ppm (s, 48H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 172.1, 137.8, 128.2, 127.6, 127.5, 73.6, 70.9, 70.6, 69.8, 69.3, 58.7, 53.0, 52.0, 50.9, 50.7, 45.8, 45.7, 38.3, 37.6, 37.3, 35.0, 34.8, 34.6 ppm; IR: υ = 1647.6 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{99}H_{193}N_{29}O_{17}$: 1031.8 $[M+2H]^{2+}$, 688.2 $[M+3H]^{3+}$, 516.4 $[M+4H]^{4+}$, found: 1031.8 $[M+2H]^{2+}$, 687.9 $[M+3H]^{3+}$, 516.2 $[M+4H]^{4+}$.

G_2-NMe_3^+ (10c): According to the same procedure described for the synthesis of compound **6c**, **10c** was obtained from compound **10b**. ^{1}H NMR (300 MHz, D_2O): δ = 7.24 (m, 5H), 4.41 (s, 2H), 3.40-3.62 (m, 40H), 3.20-3.40 (m, 40H), 2.92-3.08 (m, 109H), 2.60-2.80 ppm (m, 28H); ^{13}C NMR (75 MHz, D_2O): δ = 170.8, 170.6, 137.3, 129.0, 128.8, 128.6, 73.0, 69.9, 69.8, 69.2, 64.2, 61.5, 59.8, 58.8, 58.2, 53.7, 51.5, 49.8, 49.0, 33.9, 33.8, 33.2, 30.8, 29.0, 28.8 ppm; IR: υ = 1662.1 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{114}H_{238}N_{29}O_{17}$.15I: 920.7 $[M-4I]^{4+}$, 711.2 $[M-5I]^{5+}$, 571.5 $[M-6I]^{6+}$, 471.7 $[M-7I]^{7+}$, found: 920.7 $[M-4I]^{4+}$, 711.0 $[M-5I]^{5+}$, 571.6 $[M-6I]^{6+}$, 471.4 $[M-7I]^{7+}$.

$G_{2.5}$ (11): According to the same procedure described for the synthesis of compound **7**, **11** was obtained from compound **10a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30-7.40 (m, 5H), 4.54 (s, 2H), 3.56-3.76 (m, 58H), 3.24-3.35 (m, 28H), 2.69-2.95 (m, 62H), 2.51-2.60 (m, 28H), 2.32-2.48 ppm (m, 60H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3, 173.2, 172.7, 172.6, 138.3, 128.6, 128.0, 127.8, 73.4, 70.7, 70.4, 69.5, 69.1, 53.1, 52.7, 52.4, 51.9, 51.8, 50.3, 50.2, 49.9, 49,4, 37.7, 37.4, 34.0, 33.6, 32.9, 32.8 ppm; IR: υ = 1736.6 cm^{-1}, 1650.1; MS (ESI+): *m/z*: calcd for $C_{147}H_{257}N_{29}O_{49}$: 1607.9 $[M+2H]^{2+}$, 1072.3 $[M+3H]^{3+}$, Found: 1608.5 $[M+2H]^{2+}$, 1072.7 $[M+3H]^{3+}$.

G_3-NH_2 (12a): According to the same procedure described for the synthesis of compound **6a**, **12a** was obtained from compound **11**. ^{1}H NMR (300 MHz, D_2O): δ = 7.18-7.25 (m, 5H), 4.44 (s, 2H), 3.40-3.61 (m, 10H), 3.05-3.30 (m, 60H), 2.55-2.78 (m, 94H), 2.39-2.55 (m, 28H), 2.15-2.39 ppm (m, 60H); ^{13}C NMR (75 MHz, D_2O): δ = 174.5, 174.1, 137.5, 128.6, 128.3, 73.3, 70.1, 69.4, 68.6, 51.8, 50.7, 49.7, 43.2, 42.4, 40.5, 37.4, 33.5, 33.4 ppm; IR: υ = 1645.8 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{163}H_{321}N_{61}O_{33}$: 1222.2 $[M+3H]^{3+}$, 916.9 $[M+4H]^{4+}$, 733.7 $[M+5H]^{5+}$, 611.6 $[M+6H]^{6+}$, found: 1222.7 $[M+3H]^{3+}$, 917.1 $[M+4H]^{4+}$, 733.8 $[M+5H]^{5+}$, 611.7 $[M+6H]^{6+}$.

G_3-NMe_2 (12b): According to the same procedure described for the synthesis of compound **6b**, **12b** was obtained from compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32 (m, 5H), 4.55 (s, 2H), 3.58-3.68 (m, 10H), 3.06-3.46 (m, 60H), 2.65-2.88 (m, 60H), 2.42-2.64 (m, 62H), 2.31-2.42 (m, 60H), 2.26 ppm (s, 96H); ^{13}C NMR (75 MHz, $CDCl_3$): δ =172.3, 138.0, 128.4, 127.8, 73.6, 70.9, 70.6, 69.8, 69.3, 58.8, 53.1, 50.8,

45.9, 38.3, 37.7, 35.2, 34.8 ppm; IR: υ = 1646.0 cm^{-1}; MS (ESI+): calcd for $C_{195}H_{385}N_{61}O_{33}$: 1029.0 $[M+4H]^{4+}$, 823.4 $[M+5H]^{5+}$, 686.3 $[M+6H]^{6+}$, found: 1029.1 $[M+4H]^{4+}$, 823.3 $[M+5H]^{5+}$, 686.4 $[M+6H]^{6+}$.

G_3-NMe_3^+ (12c): According to the same procedure described for the synthesis of compound **6c**, **12c** was obtained from compound **12b**. ^1H NMR (300 MHz, D_2O): δ = 7.18-7.26 (m, 5H), 4.40 (s, 2H), 3.42-3.60 (m, 72H), 3.25-3.36 (m, 48H), 3.10-3.24 (m, 24H), 2.92-3.05 (m, 189H), 2.50-2.85 (m, 76H), 2.25-2.48 ppm (m, 32H); ^{13}C NMR (75 MHz, D_2O): δ = 174.2, 170.5, 137.5, 129.0, 128.8, 72.9, 69.8, 64.2, 59.7, 58.1, 53.6, 51.6, 49.2, 48.6, 33.8, 33.7, 33.2, 31.6, 28.8 ppm; IR: υ = 1661.6 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{226}H_{478}N_{61}O_{33}.31I$: 405.1 $[M-16I]^{16+}$, found: 404.8 $[M-16I]^{16+}$.

$G_{3.5}$ (13): According to the same procedure described for the synthesis of compound **7**, **13** was obtained from compound **12a**. ^1H NMR (300 MHz, $CDCl_3$): δ = 3.56-3.80 (m, 106H), 3.18-3.40 (m, 60H), 2.65-2.92 (m, 126H), 2.50-2.65 (m, 60H), 2.30-2.49 ppm (m, 124H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.2, 172.4, 138.0, 128.5, 127.9, 73.2, 70.3, 70.0, 68.8, 53.0, 52.5, 52.3, 51.8, 51.3, 50.2, 49.9, 49.8, 49.3, 37.6, 37.2, 33.9, 32.8, 32.7 ppm; IR: υ = 1734.8, 1647.4 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{291}H_{513}N_{61}O_{97}$: 1621.9 $[M+H+3Na]^{4+}$, 1284.5 $[M+5H]^{5+}$, 1297.7 $[M+2H+3Na]^{5+}$, found: 1621.8 $[M+H+3Na]^{4+}$, 1284.9 $[M+5H]^{5+}$, 1298.0 $[M+2H+3Na]^{5+}$.

G_4-NH_2 (14a): According to the same procedure described for the synthesis of compound **6a**, **14a** was obtained from compound **13**. ^1H NMR (300 MHz, D_2O): δ = 3.71-3.73 (m, 10H), 3.10-3.20 (m, 124H), 2.60-2.72 (m, 190H), 2.42-2.54 (m, 60H), 2.22-2.35 ppm (m, 124H); ^{13}C NMR (75 MHz, D_2O): δ = 175.0, 174.6, 137.6, 128.7,

128.4, 72.9, 69.7, 69.6, 69.4, 69.0, 68.2, 51.3, 50.0, 49.1, 43.2, 42.7, 41.8, 39.9, 38.7, 36.8, 32.8, 32.7 ppm; IR: υ = 1646.7 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{323}H_{641}N_{125}O_{65}$: 1464.2 $[M+5H]^{5+}$, 1220.4 $[M+6H]^{6+}$, 1046.2 $[M+7H]^{7+}$, 915.5$[M+8H]^{8+}$, found: 1463.6 $[M+5H]^{5+}$, 1220.1 $[M+6H]^{6+}$, 1046.1 $[M+7H]^{7+}$, 915.4 $[M+8H]^{8+}$.

G_4-NMe_2 (14b): According to the same procedure described for the synthesis of compound **6b**, **14b** was obtained from compound **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.55-3.65 (b, 10H), 3.14-3.40 (m, 124H), 2.62-2.90 (m, 124H), 2.48-2.60 (m, 40H), 2.37-2.48 (m, 84H), 2.27-2.37 (m, 124H), 2.23 ppm (s, 192H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6, 138.0, 128.5, 127.8, 73.6, 70.2, 69.2, 68.5, 58.4, 52.7, 51.7, 50.3, 50.2, 45.4, 37.9, 37.1, 36.1, 34.4, 34.2, 34.0 ppm; IR: υ = 1645.7 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{387}H_{769}N_{125}O_{65}$: 1643.8 $[M+5H]^{5+}$, 1370.0 $[M+6H]^{6+}$, 1174.4 $[M+7H]^{7+}$, 1027.8 $[M+8H]^{8+}$, found: 1643.3 $[M+5H]^{5+}$, 1369.6 $[M+6H]^{6+}$, 1174.1 $[M+7H]^{7+}$, 1027.5 $[M+8H]^{8+}$.

G_4-NMe_3^+ (14c): According to the same procedure described for the synthesis of compound **6c**, **14c** was obtained from compound **14b**. ^{1}H NMR (300 MHz, D_2O): δ = 3.42-3.58 (m, 108H), 3.25-3.38 (m, 96H), 3.05-3.18 (m, 56H), 2.90-3.05 (m, 381H), 2.55-2.80 (m, 112H), 2.38-2.54 (m, 40H), 2.25-2.48 ppm (m, 96H); ^{13}C NMR (75 MHz, D_2O): δ = 172.8, 172.4, 127.3, 126.2, 70.8, 67.7, 62.1, 57.6, 55.9, 51.4, 49.2, 47.1, 46.8, 46.6, 31.5 ppm; IR: υ = 1659.2 cm^{-1}; MS (ESI+): *m/z*: calcd for $C_{447}H_{949}N_{125}O_{65}\cdot 60I$: 802.5 $[M-18I]^{18+}$, 669.7 $[M-21I]^{21+}$, found: 802.7 $[M-18I]^{18+}$, 669.9 $[M-21I]^{21+}$.

Preparation of the *Candida* ribozyme Ca.LSU:

The *Candida* ribozyme Ca.LSU was prepared and labeled by *in vitro* transcription of the template DNA by T7 polymerase in the presence of [γ-^{32}P] GTP (3000 Ci mmol^{-1}, Perkin Elmer, Boston).[1]

Preparation of the *Candida* ribozyme Ca.L-11 DNA and RNA, and 5'-labeled RNA substrate:

The *Candida* ribozyme Ca.L-11, a truncated version of the Ca.LSU ribozyme which lacks the first 11 nt and ωG, was synthesized and purified as previously described.[1] Using primers Ca/L-11 T7 (taa tac gac tca cta tag gag gca aaa gta ggg ac) and Ca/R-1 (att gct cca aga aat cgc ttt), Ca.L-11 ribozyme DNA flanked by a T7 promoter at its 5' end was PCR amplified from the genomic DNA of C. *albicans*.

The amplified DNA was transcribed in *vitro* into Ca.L-11 ribozyme RNA by T7 RNA polymerase (MBI) for 3 h and then fractionated on a 5% polyacylamide/7 M urea gel. The Ca.L-11 RNA band was visualized by UV shadowing (254 nm), excised, electro-eluted and recovered by ethanol precipitation.

The substrate RNA oligonucleotide GCCUCUAAAAA was labeled at the 5'-end as previously described to yield [5'-^{32}P]-Ca/sub-11.[1]

Self-splicing assay of the *Candida* ribozyme Ca.LSU:

Splicing reactions were carried out in the presence of purified Ca.LSU, 10 μM GTP, 2 mM $MgCl_2$, 400 μM spermidine and the corresponding dendrimer in 50 mM Tris-HCl (pH 8.0) at 37°C for 40 min. The control sample was incubated on ice for 40 min. Then the reactions were stopped by adding an equal volume of loading buffer containing 100 mM EDTA and 8 M urea. Samples were then placed on ice until electrophoresis on a

5% polyacylamide-8 M urea gel that was then exposed to phosphor screens. Signals were detected and analyzed using a variable scanner (Typhoon 9200, Amersham Pharmcia Biotech). The data were plotted using the GraphPad Prism 4.0 program.

***Trans*-cleavage reaction catalyzed by the *Candida* ribozyme Ca.L-11:**

Each reaction (10 μL) contained 20 nM Ca.L-11 ribozyme, 0.1 mM GTP, 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 8.0), 50 nM [5'-^{32}P]-Ca/sub-11 and the corresponding dendrimer. Reactions were run at 37°C for 2.5 min and stopped by adding an equal volume of loading buffer containing 100 mM EDTA and 8 M urea. Samples were then placed on ice until electrophoresis on a 15% polyacylamide-8 M urea gel that was then exposed to X-ray film or phosphor screens. Signals on the phosphor screens were scanned and analyzed using a variable scanner (Typhoon 9200, Amersham Pharmcia Biotech). The data were plotted using the GraphPad Prism 4.0 program.

Mg^{2+} displacement experiments with the *Candida* ribozyme Ca.L-11:

Each mixture contained the ribozyme Ca.L-11, the indicated concentrations of $MgCl_2$, varying from 1 to 10 mM, and the corresponding dendrimer. After preincubation at 37°C for 5 min, the $MgCl_2$ solutions were compensated in the mixture in order to keep the final concentration of Mg^{2+} as 10 mM in each reaction. So each reaction contained 10 nM of ribozyme Ca.L-11, 10 mM Mg^{2+}, 0.1 mM GTP, 50 mM Tris-HCl (pH 8.0), 20 nM of [5'-^{32}P]-Ca/sub-11 and the corresponding dendrimers. Reactions were run at 37°C for 2.5 min, and then stopped by adding an equal volume of loading buffer containing 100 mM EDTA and 8 M urea. All samples were run on 15% polyacylamide-8 M urea gels and analyzed as described above.

GTP displacement experiments with the *Candida* ribozyme Ca.L-11:

Each mixture contained the ribozyme Ca.L-11, the indicated concentrations of GTP, varying from 0 to 100 μM, and the corresponding dendrimer. After preincubation at 37°C for 5 min, the GTP solutions were compensated in the mixture in order to keep the final concentration of GTP as 0.1 mM in each reaction. So each reaction contained 10 nM Ca.L-11 ribozyme, 10 mM Mg^{2+}, 0.1 mM GTP, 50 mM Tris-HCl (pH 8.0), 20 nM of [5'-^{32}P]-Ca/sub-11 and the corresponding dendrimers. Reactions were run at 37°C for 2.5 min, and then stopped by adding an equal volume of loading buffer containing 100 mM EDTA and 8 M urea. All samples were fractionated on 15% polyacylamide-8 M urea gels and analyzed as described above.

Preparation of the ribozyme/dendrimer complexes:

The dendrimers were diluted to an appropriate concentration in 50 mM Tris-HCl buffer (pH 7.6), with all solutions stored at 4°C. The Ca.L-11 RNA was diluted with H_2O. Both solutions were mixed at various N/P (= [total end amines in cationic dendrimer]/[RNA phosphates]) and incubated at 37°C for 10 min. The final concentration of Ca.L-11 RNA was adjusted to 5 ng μL^{-1} (25 ng/well).

Gel retardation experiments of the RNA/dendrimer complexes:

Each complex (5 μL) contained 25 ng Ca.L-11 RNA and the corresponding dendrimer was kept at 37°C in buffer solution for 10 min before loading on a 1% agarose gel for electrophoresis. The RNA bands were stained by ethidium bromide and then detected by a Kodak 290 digital camera.

Transmission electron microscope (TEM) Imaging:

Studies were performed with a Hitachi H-7000FA electron microscope instrument. 20 μL of a solution of Ca.L-11 RNA (5 ng μL^{-1}) were mixed with 30 μL of a solution of **G_4-NH_2** in 50 mM Tris-HCl buffer (23.1 ng μL^{-1}). After equilibration (3 min), 5 μL of this mixture were dropped on a standard carbon-coated copper TEM grid, and then allowed to evaporate (1 h at 30°C, ambient pressure). In the case of RNA-dendrimer solutions, the samples were premixed and allowed to equilibrate for 3 minute before placing on the grid. The grid was then stained with uranyl acetate (2% in water, pH 4.5) for 3 min. Imaging was performed immediately after air-dried for 20 min.

CD spectroscopic analysis of the RNA/dendrimer complexes:

Each sample for CD was prepared by adding the required volume of a solution of dendrimer, dissolved in the 50 mM Tris-HCl buffer (pH 7.6), to Ca.L-11 RNA solutions. Typically, the RNA final concentration was 50 ng μL^{-1}. Samples were incubated at 25°C for 30 min. The charge ratio N/P (= [total end amines in cationic dendrimer]/[RNA phosphates]) was varied between 0 and 5. CD spectra were recorded on a Jasco J-810 spectropolarimeter interfaced with a PC and analyzed the standard Jasco software package. A quartz cuvette of 1 cm path length was used. The CD spectra were obtained by scanning between 200 nm and 300 nm with 0.2 nm resolution with a scan rate of 200 nm min^{-1}. All measurements were carried out at 25°C.

NMR analysis of poly(rU)/dendrimer complexes:

The dendrimers were diluted to an appropriate concentration in D_2O and stored at 4°C. The poly(rU) was dissolved in D_2O. Both solutions were mixed at various N/P (= [total end amines in cationic dendrimer]/[RNA phosphates]) and incubated at 25°C for 10

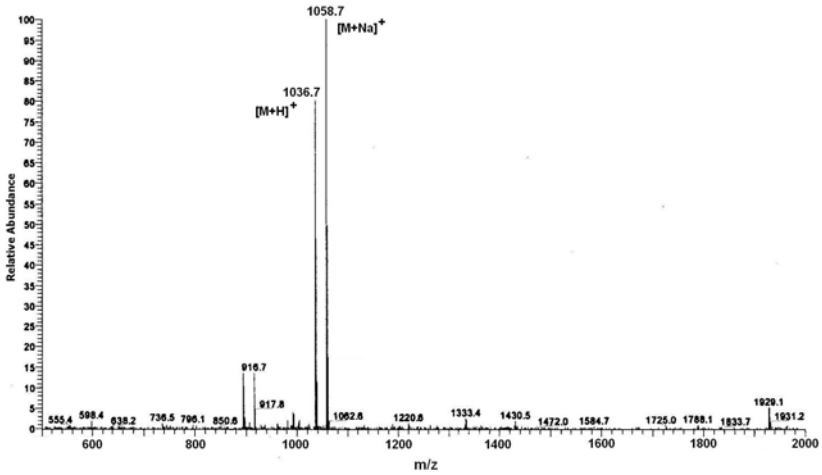
min. The NMR analysis were carried out at a fixed concentration of dendrimer and various concentrations of poly(rU). The spectra were measured at 600 MHz on a Varian Inova-600 spectrometer.

References

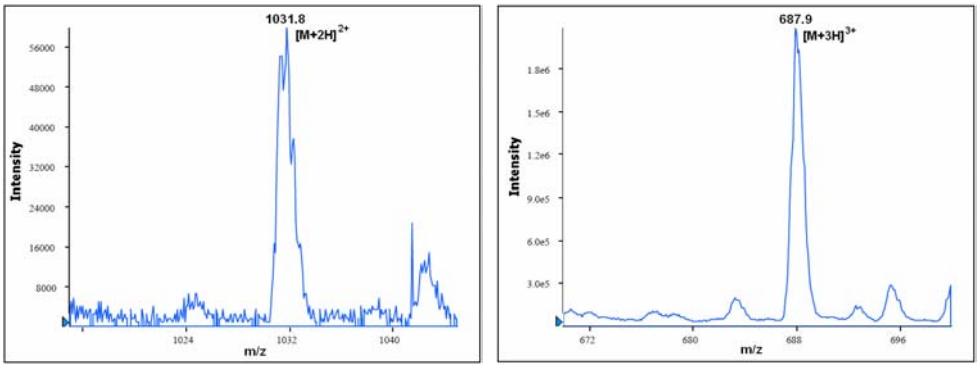
1 (*a*)Y. Zhang, M. J. Leibowitz, *Nucleic Acids Res.* 2001, **29**, 2644-2653; (*b*) M. Xiao, M. J. Leibowitz, Y. Zhang, *Nucleic Acids Res.* 2003, **31**, 3901-3908.

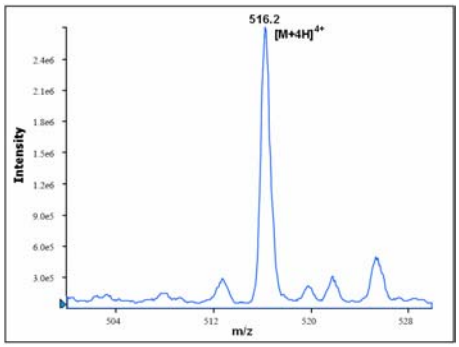
Figure S1: Mass spectra of A) **G_1-NMe_2**, B) **G_2-NMe_2**, C) **G_3-NMe_2**, and D) **G_4-NMe_2**.

A)

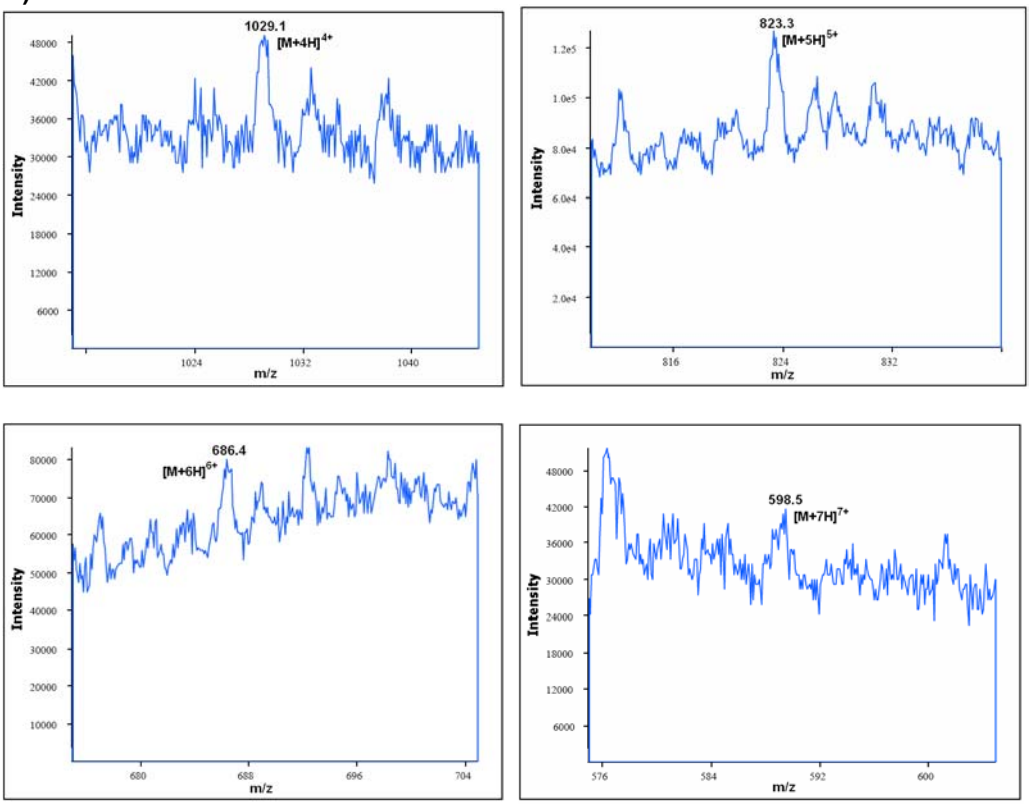


B)





C)


1029.1
[M+4H]4+
823.3
[M+5H]5+
686.4
[M+6H]6+
598.5
[M+7H]7+
Intensity
m/z

D)

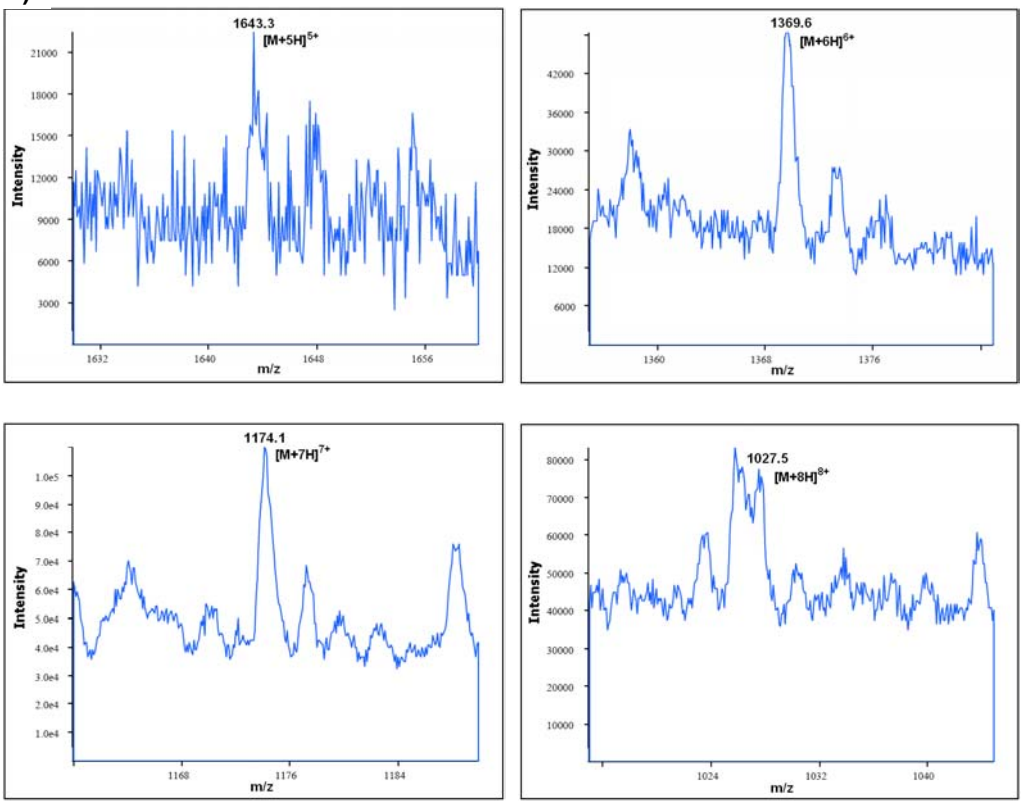

1643.3
[M+5H]5+
1369.6
[M+6H]6+
1174.1
[M+7H]7+
1027.5
[M+8H]8+
Intensity
m/z

Figure S2: GTP displacement experiments with the *Candida* ribozyme Ca.L-11. The indicated concentrations of GTP, varying from 0 to 100 μM, were the corresponding pre-incubated concentrations with dendrimers. The final concentrations of GTP were kept as 100 μM. The concentrations of different dendrimers G_2-NH_2, G_3-NH_2 and G_4-NH_2 were 10, 2.5 and 1.0 μM, respectively.

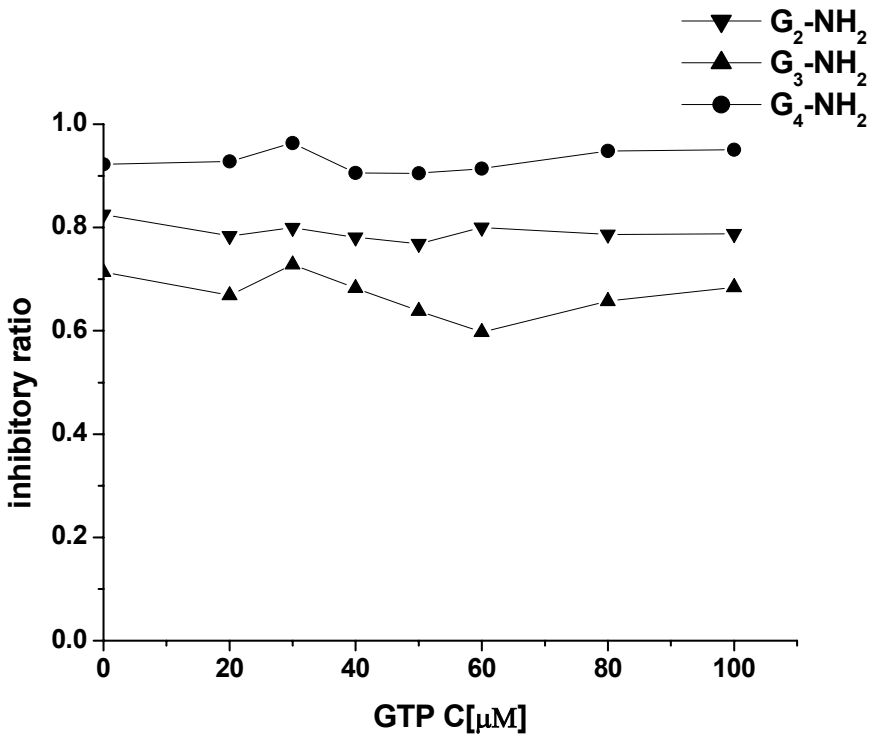
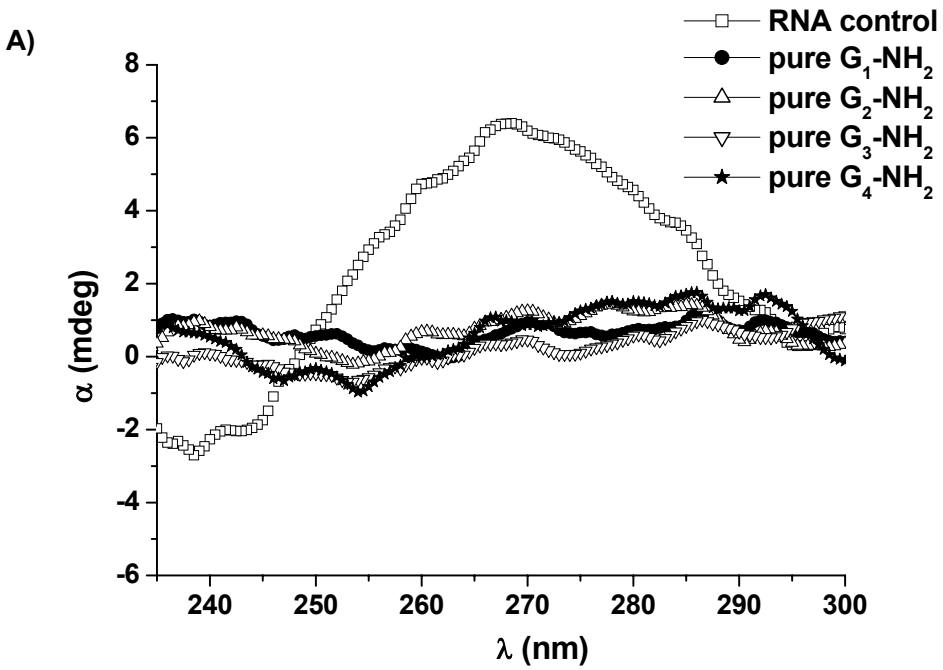
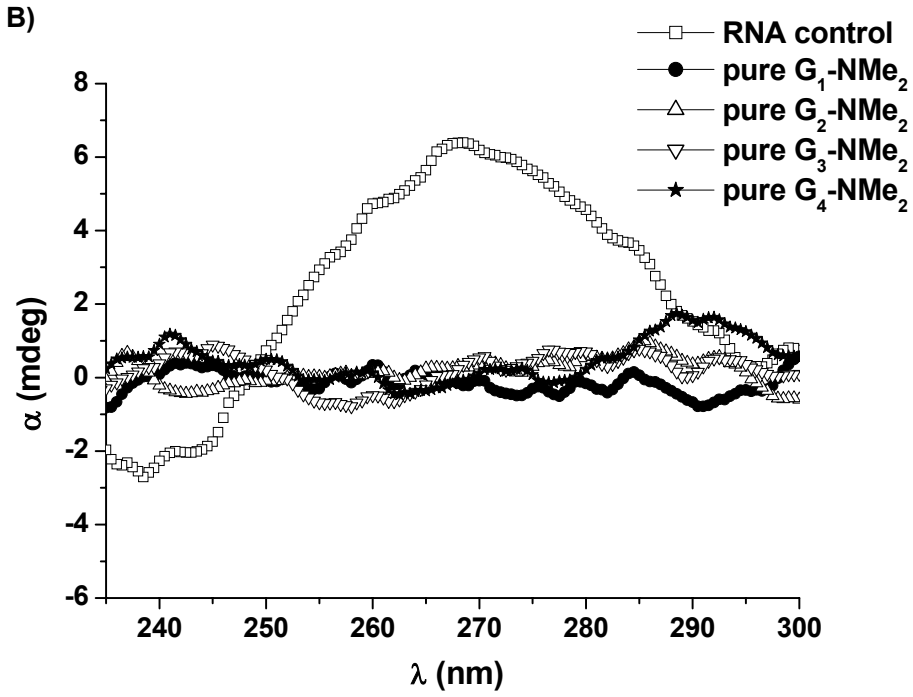
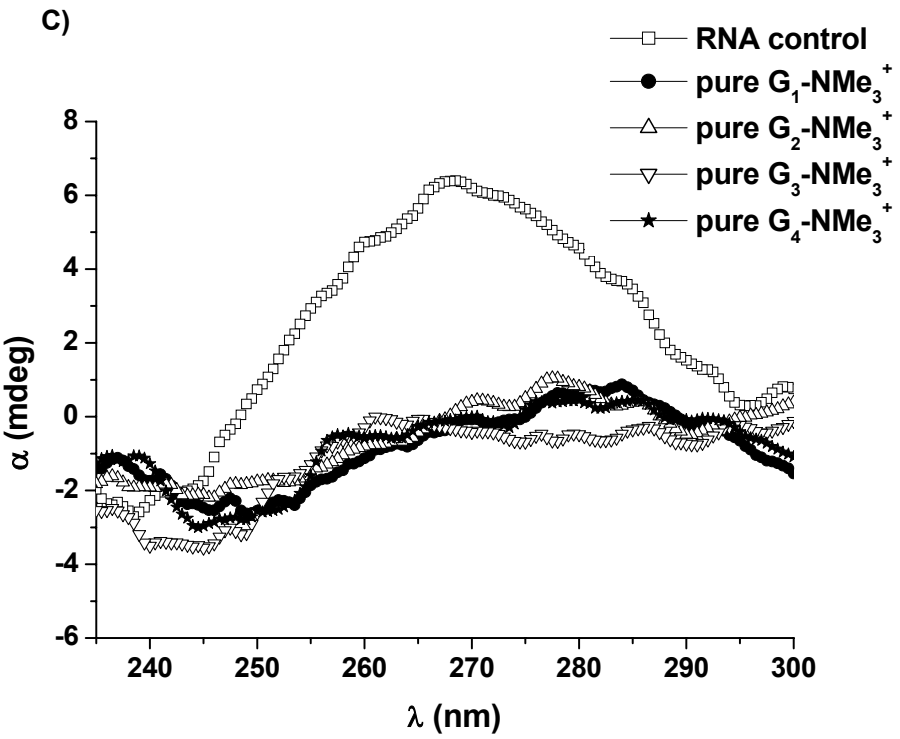


Figure S3: CD spectral analysis of pure RNA and pure G_n dendrimers: A) G_n-NH_2; B) G_n-NMe_2; C) G_n-NMe_3^+. (•)G_1, (□) G_2, (□)G_3, (□) G_4 and (□) RNA control.






C)
RNA control
pure G1-NMe3+
pure G2-NMe3+
pure G3-NMe3+
pure G4-NMe3+
α (mdeg)
λ (nm)
8
6
4
2
0
-2
-4
-6
240
250
260
270
280
290
300