Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


# Cationic poly(ester amide) dendrimers: alluring materials for biomedical applications

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**Supporting information**

**SI.1.2. Antiviral assays with Huh 5-2 cells.**

Huh5-2 cells were seeded at a density of $7 \cdot 10^3$ cells per well in a tissue culture-treated white 96-well view plate (Techno Plastic Products AG) in complete DMEM supplemented with 250 μg/mL G418. After incubation for 24 hours at 37°C, the medium was removed and twofold serial dilutions in complete DMEM (without G418) of the dendrimer/drug sytems were added in a total volume of 100 μL (final CPT concentration from 1 μM to 0 μM). Dendrimer free of drug experiments were performed using concentrations from 105 μg/mL to 0 μg/mL. After 3 days of incubation at 37°C, luciferease activity was determined using the Bright-Glo Luciferase Assay System (20 μL). The luciferease signal was measured using a Synergy HT 50 Multimode Reader (bioTek Instruments). Luciferase signal levels obtained in each assay were normalized using internal patterns previously determined in Huh 5-2 cells. The 50 % effective concentration (EC50) was defined as the concentration of compound that reduced the luciferease signal by 50 %.

**SI.2.2. Cytostatic assay.**

Huh5-2 cell lines were seeded at a density of $7 \cdot 10^3$ cells per well of a 96-well plate in complete DMEM with the appropriate concentration of G418). Serial dilutions of the dendrimer/drug systems in complete DMEM (without G418) were added 24 hours after seeding (final CPT concentration form 1 μM to 0 μM). Dendrimer free of drug experiments were performed using concentrations from 105 μg/mL to 0 μg/mL. Cells were allowed to proliferate for 3 days at 37ºC. Cell culture medium was removed and cell number was determined by Cell Titer 96 AQueous One Solution Cell Proliferation Assay. The 50% cytostatic concentration (CC50) was determined employing the dose-response equation (*i.e.* Hill equation). All experiments on Huh 5-2 cells were carried put in triplicate.

## SI.2. Protocols carried out for the pEGFP transfection and cellular uptake and internalization

### SI.2.1. In vitro pEGFP transfection.

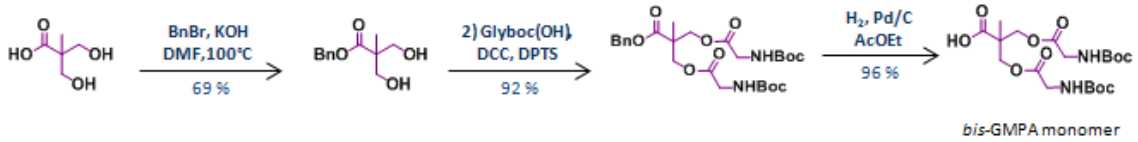
mMSCs were seeded at a density of $1 \times 10^4$ cells per well in 96-well plates 24 h prior to all experiments. 50 µL of dendriplexes consisting of 0.1–0.4 µg of pEGFP and the adjusted amount of dendrimer to obtain the desired dendrimer:pEGFP (w:w) ratios (10/1, 50/1, 100/1, 500/1) were prepared in SFM. After incubation at room temperature for 20 min, complexes were added in duplicate to each well and incubated for 4 h. EGFP expression was evaluated by fluorescence microscopy (Olympus IX81 Olympus, Spain) at 24-48 h.

### SI.2.2. Uptake and internalization.

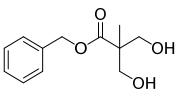
In order to determine the cellular uptake of the dendriplexes, pEGFP was Cy5-labeled using the Label IT Tracker Intracellular Nucleic Acid Localization Kit (Mirus) following the instructions recommend by the manufacturer. HeLa and mMSCs were seeded at a density of $5 \times 10^4$ cells per well in 24 multiwell culture plates on sterile coverslips and grown for 24 h, after which the medium was replaced with SFM with dendriplexes at ratios 50/1 and 100/1 (w/w). At different time points, cells were washed with PBS and fixed in paraformaldehyde 4%. To stain actin filaments, cells were first permeabilized in PBS-1% BSA-0.1% saponin and then incubated for 1 h at room temperature with AlexaFluor488-phalloidin (Thermo Fisher Scientific) diluted in the permeabilization solution (1:200) in the dark. After washing, the coverslips were mounted and the cell nuclei stained at the same time with a solution of Mowiol-DAPI (1:1000), and the cellular uptake and localization were visualized by confocal laser scanning microscopy using a 63X objective (Olympus FV10-i Oil Type, Olympus). Green fluorescence was observed under 499/520 nm (λexc/λem), DAPI under 359/461 nm (λexc/λem) and Cy5 under 645/664 nm (λexc/λem). Image treatment and quantification was carried out with FV10i-SW software (Olympus).

## SI.3.1. Synthesis of the *bis*-GMPA monomer.

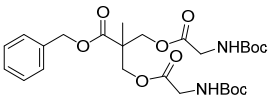
The *bis*-GMPA monomer was synthesized in 3 steps according to the following procedure (scheme SI.3.1).



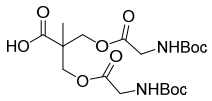

**Scheme SI.3.1.** Synthesis of the *bis*-GMPA monomer.



**(1).** *Bis*-MPA (20.00 g, 149.2 mmol, 1.00 eq.) was dissolved into DMF (100 mL). KOH (10.20 g, 179.0 mmol, 1.20 eq.) was added to it. The reaction was stirred at 100ºC during 1 hour. Then, BnBr (26.55 mL, 223.7 mmol, 2.00 eq.) was added drop wise. The reaction mixture was stirred overnight at 100ºC. A white precipitate appeared. It was filtered off. Water (450 mL) was added to the filtrate and the product was extracted three times with ethyl acetate (3 x 200 mL) and then washed three times with brine (3 x 150 mL) to give a yellow oil. The crude product was recristalized into toluene to get white crystals (23.20 g, 69 %). $^{1}$H (400 MHz, $CDCl_3$) δ (ppm): 1.02 (s, 3H, -$CH_3$), 2.82 (bs, -OH), 3.74 (d, J = 10.8 Hz, 2H, -C$\underline{H_2}$OH), 3.94 (d, J= 11.2 Hz, 2H, -C$\underline{H_2}$OH), 5.21 (s, 2H, Φ-C$\underline{H_2}$O-), 7.36 (m, 5H, $H_{ar}$). $^{13}$C (100 MHz, $CDCl_3$) δ (ppm): 17.1, 49.2, 66.7, 68.6, 127.9, 128.3, 128.6, 135.6, 175.7. MS ($ESI^+$) *m/z: 246.8* $[C_{12}H_{16}O_4,Na]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, nujol): 3352 (O-H st), 2923-2853 (C-H st and arC-H), 1701 (C=O st). EA (%): *Found*: C, 63.9; H, 7.3. *Calc. for* $C_{12}H_{16}O_4$: C, 64.3; H, 7.2.



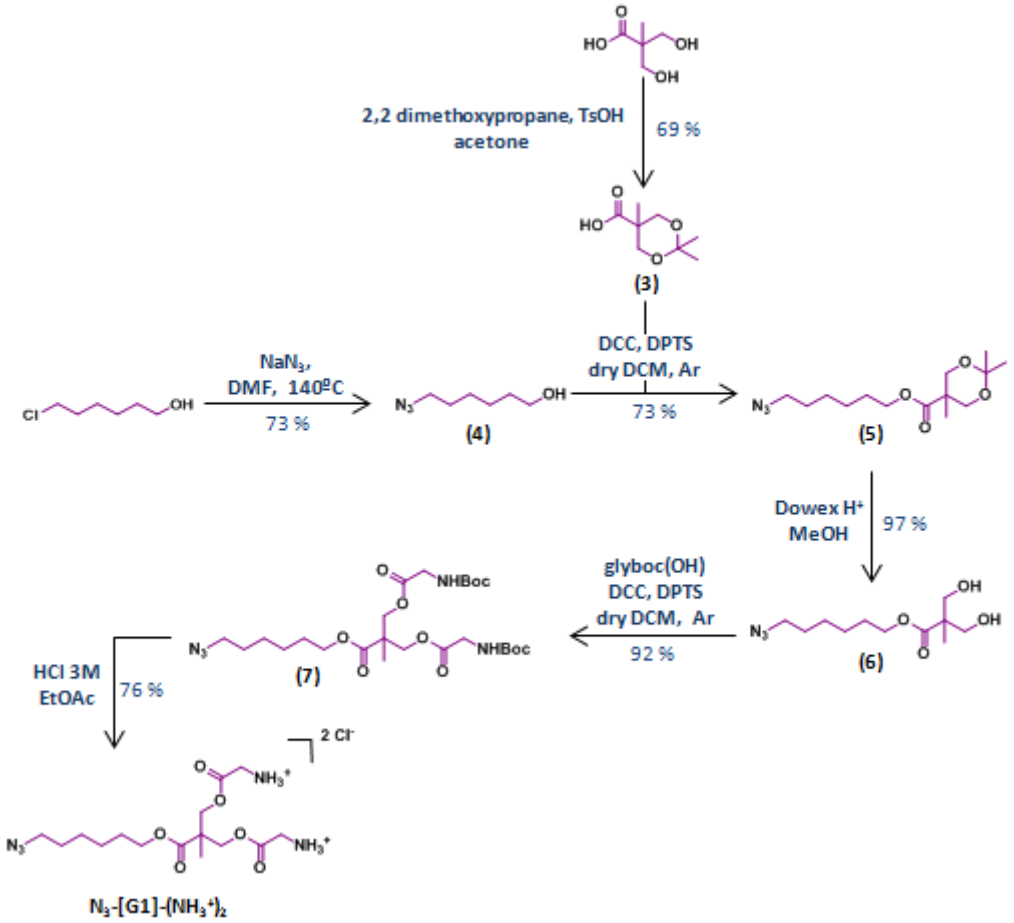
**(2).** (1) (10.00 g, 44.62 mmol, 1.00 eq.) was dissolved into dry DCM (230 mL). Glyboc(OH) (19.54 g, 111.56 mmol, 2.50 eq.) and DPTS (10.51 g, 35.70 mmol, 0.80 eq.) were added to it. The reaction mixture was stirred under argon atmosphere and cooled down to 0ºC. DCC (22.99 g, 111.56 mmol, 2.50 eq.) was dissolved into dry DCM (70 mL) and was added drop wise to the reaction mixture. The reaction mixture was stirred at room temperature overnight. The white precipitate, N,N'-dicyclohexylurea (DCU), was filtered off and the solvent was evaporated under vacuum to get a mixture of oil and solid. DCU was newly precipitated into a mixture of hexane and ethyl acetate (10:2) (240 mL) and was filtered off. The solvents were evaporated under reduce pressure. The crude powder was purified on silica gel (hexane : ethyl acetate = 8:2) to get a colorless oil (22.00 g, 92 %).$^{1}$H (300 MHz, $CDCl_3$) δ (ppm): 1.27 (s, 3H, -$CH_3$), 1.45 (s, 18 H, -$C(CH_3)_3$), 3.81 (d, J = 4Hz, 4H, -$CH_2$N-), 4.31 (ABq, J = 10.8 Hz, $\Delta\nu_{AB}$ = 22.4 Hz, 4H, -$CH_2$O-), 5.03 (bs, -NH), 5.17 (s, 2H, Φ-C$\underline{H_2}$O-), 7.36 (m, 5H, $H_{ar}$). $^{13}$C (75 MHz, $CDCl_3$) δ (ppm): 17.9, 28.3, 42.2, 46.3, 65.8, 67.0, 80.0, 128.3, 128.5, 128.6, 135.4, 155.7, 169.9, 172.1. HRMS ($ESI^+$) *m/z*: *561.2442, m/z calc. 561.2419* $[C_{26}H_{38}N_2O_{10},Na]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3379 (N-H st), 2980-2937-2878 (C-H st and C-$H_{ar}$ st), 1757 (C=O st aliphatic ester), 1720 (C=O st aromatic ester), 1690 (C=O st carbamate), 1512 (N-H δ), 1456 ($CH_2$, $CH_3$ δ), 1365 (C-N st), 1250 (CO-O st), 1153 ($\nu_{as}$ N-CO-O), 1132 (O-C-C st). EA (%): *Found:* C, 57.8; H, 7.2; N, 5.4. *Calc. for* $C_{26}H_{38}N_2O_{10}$: C, 58.0; H, 7.1; N, 5.2.



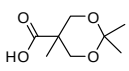
***bis*-GMPA monomer.** (2) (16.75 g, 31.10 mmol, 1.00 eq.) was dissolved into ethyl acetate (150 mL) under argon atmosphere. Pd/C (837 mg, 0.05 eq. in weight) was added carefully to the reaction mixture. Firstly, three cycles of argon - vacuum were made and secondly three cycles vaccum-$H_2$ were made. The reaction mixture was stirred overnight under $H_2$ pressure using a globe. Then, it was filtered on celite© with ethyl acetate flow. The solvent was evaporated under reduce pressure to give a colorless gel (13.39 g, 96 %). $^1$H (400 MHz, $CDCl_3$) δ (ppm): 1.26 (s, 3H, -$CH_3$), 1.43 (s, 18H, -$C(CH_3)_3$), 3.89 (d, J = 5.6 Hz, 4H, -$CH_2$N-), 4.32 (ABq, J = 11.2 Hz, $\Delta\nu_{AB}$ = 23.7 Hz, 4H, -$CH_2$O-), 5.27 (bs, -NH). $^{13}$C (100 MHz, $CDCl_3$) δ (ppm): 17.9, 28.3, 42.2, 45.9, 65.8, 80.2, 156.0, 170.1, 176.1. HRMS ($ESI^+$) *m/z*: *471.1952, m/z calc. 471.1949* $[C_{19}H_{32}N_2O_{10},Na]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3371 (N-H st), 2980-2937-2874 (C-H st), 1745 (C=O st aliphatic ester), 1699 (C=O st carbamate), 1522 (N-H δ), 1458 ($CH_2$, $CH_3$ δ), 1367 (C-N st), 1250 ($\nu_{as}$ CO-O), 1151 ($\nu_{as}$ N-CO-O), 1140 (O-C-C st). EA (%): *Found:* C, 50.6; H, 7.5; N, 5.9. *Calc. for* $C_{19}H_{32}N_2O_{10}$: C, 50.9; H, 7.2; N, 6.25.

## SI.3.2. Synthesis of the *bis*-GMPA dendron of first generation, $N_3$-[G1]-$(NH_3^+)_2$.

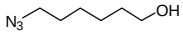
The *bis*-GMPA dendron of first generation was synthesized in 6 steps according to the following procedure (scheme SI.3.2).



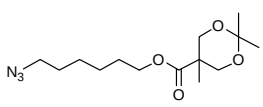

**Scheme SI.3.2.** Synthesis of the *bis*-GMPA dendron of generation 0, **$N_3$-[G1]-$(NH_3^+)_2$**.



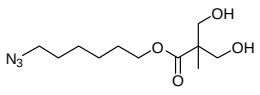
**(3).** *2,2-Bis*(hydroxymethyl)propionic acid (60.00 g, 447.33 mmol, 1.00 eq.) was dissolved in acetone (300 mL). 2,2 dimethoxypropane (82.51 mL, 670.99 mmol, 1.50 eq.) and $TsOH,H_2O$ (4.26 g, 22.37 mmol, 0.05 eq.) were added. The reaction mixture was stirred at room temperature during 2 hours. Then, the reaction was neutralized by adding a solution of $NH_3$/EtOH (1:1) (6 mL). The solvent was evaporated under vacuum. The white solid was dissolved in AcOEt (100 mL) and was washed twice with distillated water. The organic phase was dried over anhydrous $MgSO_4$. The solvent was evaporated under vacuum to get a white powder (54.07 g, 69 %). $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.21 (s, 3H, -$CH_3$), 1.41 (s, 3H, -$C(CH_3)_2$), 1.44 (s, 3H, -$C(CH_3)_2$'), 3.67 (d, J = 12 Hz, 2H, -$CH_2O$-), 4.19 (d, J = 12 Hz, 2H, -$CH_2O$-'), 9.95 (bs, COOH). $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.4, 21.9, 25.2, 41.8, 65.8, 98.3, 180.2. MS ($ESI^+$): *m/z (%) 196.9 (100)* $[C_8H_{14}O_4,Na]^+$, *174.0 (33)* $[C_8H_{14}O_4,H]^+$. FT-IR ($\nu_{max}$/$cm^{-1}$, nujol): 3126 (COO-H st), 2924 and 2854 (C-H st), 1722 (C=O st), 1460 (-$CH_2$, -$CH_3$ δ). EA (%): *Found*: C, 55.4; H, 8.4. *Calc. for* $C_8H_{14}O_4$: C, 55.2; H, 8.1%.



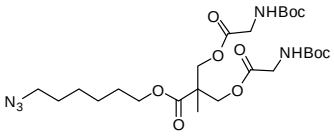
**(4).** 6-chlorohexan-1-ol (19.53 mL, 146 mmol, 1.00 eq.) was dissolved in DMF (60 mL). $NaN_3$ (19.99 g, 307 mmol, 2.10 eq.) was added. The reaction mixture was stirred at 140 ºC during 36 hours. Water (400 mL) was added. The product was extracted tree times using $Et_2O$ (3 x 200 mL). Organic phases were collected, put together, washed twice using brine (2 x 200 mL) and dried over anhydrous $MgSO_4$. Solvent was evaporated to give an orange oil. The crude product was purified on silica gel (hexane : ethyl acetate = 7:3) to give a light yellow oil (15.32 g, 73 %).$^1$H NMR (400 MHz, $CDCl_3$): δ 1.34 (m, -$CH_2$-C$\underline{H}_2$-C$\underline{H}_2$-$CH_2$-), 1.56 (m, 4H, -C$\underline{H}_2$-$CH_2$-$CH_2$-C$\underline{H}_2$-), 1.76 (bs, -OH), 3.24 (t, J = 6.8 Hz, 2H, $N_3$-$CH_2$-), 3.61 (t, J = 6.4 Hz, 2H, -C$\underline{H}_2$-OH). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 25.3, 26.4, 28.7, 32.5, 51.3, 62.6. MS ($ESI^+$): *m/z 166.0* $[C_6H_{13}N_3O_4,Na]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, nujol): 3339 (O-H st), 2936-2861 (C-H st), 2097 ($N_3$ st), 1456 ($CH_2$, $CH_3$ δ). EA (%): *Found:* C, 49.7; H, 9.2; N, 29.5. *Calc. for* $C_6H_{13}N_3O_1$: C, 50.3; H, 9.15; N, 29.35.



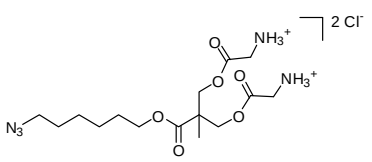
**(5).** (4) (12.68 g, 88.58 mmol, 1.00 eq.) was dissolved into dry dichloromethane (250 mL). (3) (16.97 g, 97.44 mmol, 1.10 eq.) and DPTS (10.43 g, 35.43 mmol, 0.40 eq.) were added. The reaction mixture was stirred under argon atmosphere and was cooled down to 0ºC. A solution of DCC (20.10 g, 97.44 mmol, 1.10 eq.) in dry dichloromethane (50 mL) was added drop wise. The reaction mixture was allowed to stir under argon atmosphere overnight at room temperature. The white precipitate, N,N'-dicyclohexylurea (DCU), was filtered off and the solvent was evaporated under vacuum to get a mixture of oil and solid. DCU was newly precipitated into hexane and filtered off. The solvent was evaporated under reduce pressure. The crude product was purified on silica gel (hexane : ethyl acetate = 8:2) to give a light yellow oil (25.68 g, 94 %). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.18 (s, 3H, -$CH_3$), 1.38 (s, 3H, -$C(C\underline{H}_3)_2$), 1.40 (m, 4H, -$CH_2$-C$\underline{H}_2$-C$\underline{H}_2$-$CH_2$-), 1.42 (s, 3H, -$C(C\underline{H}_3)_2$'), 1.61 (m, 2H, $N_3$-$CH_2$-C$\underline{H}_2$-) 1.67 (m, 2H, -C$\underline{H}_2$-$CH_2$-OC(O)-), 3.26 (t, J = 6.8 Hz, 2H, $N_3$-$CH_2$-), 3.64 (d, J = 11.6 Hz, 2H, -$CH_2O$-), 4.14 (t, J =6.6 Hz, 2H, -$CH_2OC(O)$-), 4.18 (d, J = 11.6 Hz, 2H, -$CH_2O$-). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 18.7, 22.7, 24.6, 25.4, 26.3, 28.4, 28.7, 41.8, 51.3, 64.6, 66.0, 98.0, 174.3. MS ($ESI^+$): *m/z* (%): *322.1 (15)* $[C_{14}H_{25}N_3O_4,Na]^+$, *300.0 (52)* $[C_{14}H_{25}N_3O_4,H]^+$, *272.0 (100)* $[C_{14}H_{25}N_1O_4,H]^+$.FTIR ($\nu_{max}$/$cm^{-1}$, nujol): 2939-2864 (C-H st), 2096 ($N_3$ st), 1731 (C=O st), 1455 ($CH_2$, $CH_3$ δ), 1259 (CO-O st), 1160 (O-C-C st). EA (%) : *Found:* C, 55.9; H, 8.7; N, 13.8. *Calc. for* $C_{14}H_{25}N_3O_4$: C, 56.2; H, 8.4; N, 14.0.



**(6).** Dowex 50 WX2 hydrogen form 50.100 (mesh) (12.50 g, 50 % mass equivalent) was washed by stirring it in MeOH and was recovered using filtration. (5) (25.68 g, 85.78 mmol, 1.00 eq.) was dissolved into MeOH (350 mL). Washed Dowex resin $H^+$ was added and the reaction was stirred during 5 hours. The resin was filtered off and the solvent was evaporated under vacuum to obtain a colorless oil (20.86 g, 97 %). $^1$H NMR (300 MHz, $CDCl_3$) δ (ppm). 1.05 (s, 3H, -$CH_3$), 1.39 (m, 4H, -$CH_2$-C$\underline{H_2}$-C$\underline{H_2}$-$CH_2$-), 1.61 (m, 2H, $N_3$-$CH_2$-C$\underline{H_2}$-) 1.67 (m, 2H, -C$\underline{H_2}$-$CH_2$-OC(O)-), 3.02 (bs, -OH), 3.25 (t, J = 6.9 Hz, 2H, N3-C$\underline{H_2}$-), 3.68 (dd, J = 11.2 Hz and J = 5.7 Hz, 2H, -C$\underline{H_2}$OH), 3.87 (dd, J = 11.2 Hz and J = 5.7 Hz, 2H, -C$\underline{H_2}$OH'), 4.14 (t, J = 6.6 Hz, 2H, -$CH_2$-C(O)-). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 17.0, 25.3, 26.2, 28.3, 28.6, 49.1, 51.2, 64.8, 67.9, 175.9. MS ($ESI^+$): *m/z*: *282.0* $[C_{11}H_{21}N_3O_4,Na]^+$, *232.0 (72)* $[C_{11}H_{21}NO_4,H]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, nujol): 3381 (O-H st), 2925-2855 (C-H st), 2097 ($N_3$ st); 1727 (C=O st), 1462 ($CH_2$,$CH_3$ δ), 1237 (CO-O st), 1131 (O-C-C st). EA (%): *Found:* C. 50.4; H, 8.2; N, 16.4. *Calc. for* $C_{11}H_{21}N_3O_4$: C. 50.95; H. 8.2; N. 16.2.



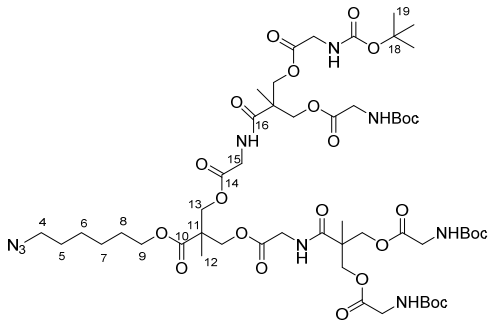
**(7).** (6) (3.69 g, 14.2 mmol, 1.00 eq.) was dissolved into dry dichloromethane (85 mL). Glyboc(OH) (6.23 g, 35.6 mmol, 2.50 eq.) and DPTS (3.35 g, 11.4 mmol, 0.80 eq.) were added to it. The reaction mixture was stirred under Argon atmosphere and cooled down to 0ºC. DCC DCC (7.33 g, 35.6 mmol, 2.50 eq.) was dissolved into dry dichloromethane (15 mL) and was added drop wise to the reaction mixture. It was stirred at room temperature under argon atmosphere during 2 days. The white precipitate, N,N'-dicyclohexylurea (DCU), was filtered off and the solvent was evaporated under vacuum to get a mixture of oil and solid. DCU was newly precipitated into a mixture of hexane and ethyl acetate (7:3) and filtered off. The solvent was evaporated under reduce pressure. The crude product was purified on silica gel (hexane : ethyl acetate = 8:2) to get a colorless and viscous oil (7.50 g, 92 %). $^1$H (300 MHz, $CDCl_3$) δ (ppm): 1.24 (s, 3H, -$CH_3$[G1]), 1.37 (m, 4H, -$CH_2$-C$\underline{H_2}$-C$\underline{H_2}$-$CH_2$-), 1.44 (s, 18H, -$C(CH_3)_3$), 1.60 (m, 2H, $N_3$-$CH_2$-C$\underline{H_2}$-) 1.64 (m, 2H, -C$\underline{H_2}$-$CH_2$-OC(O)-), 3.27 (t, J = 6.8 Hz, 2H, $N_3$-C$\underline{H_2}$-), 3.88 (d, J = 5.3 Hz, 4H, -$CH_2$N-[G1]), 4.12 (t, J = 6.6Hz, 2H, -$(CH_2)_5$C$\underline{H_2}$-OC(O)-), 4.32 (ABq, J = 11.1 Hz and $\Delta\nu_{AB}$ = 21.6 Hz, -C$\underline{H_2}$O-[G1]), 5.1 (bs, -NH). $^{13}$C (100 MHz, $CDCl_3$) δ (ppm): 18.0, 25.4, 26.3, 28.3, 28.7, 42.2, 46.2, 51.3, 65.2, 65.7, 80.0, 156.7, 170.0, 172.4. HRMS ($ESI^+$) *m/z*: *596.2927*, *m/z calc. 596.2902* $[C_{25}H_{43}N_5O_{10},Na]^+$. FT-IR ($cm^{-1}$, ATR): 3410-3339 (N-H st), 2984-2949-2872 (C-H st), 2093 ($N_3$ st), 1771-1757 (C=O st ester), 1722-1688 (C=O st carbamate), 1539-1510 (N-H δ), 1472 ($CH_2$, $CH_3$ δ), 1367 (C-N st), 1228 (CO-O st), 1161 ($\nu_{as}$ N-CO-O), 1128 (O-C-C st). EA (%): *Found:* C, 52.7; H, 7.6; N, 12.0. *Calc. for* $C_{25}H_{43}N_5O_{10}$: C, 52.35; H, 7.6; N, 12.2. SEC (*ref PMMA*): Mw 747 g.mol$^{-1}$; Đ: 1.01.



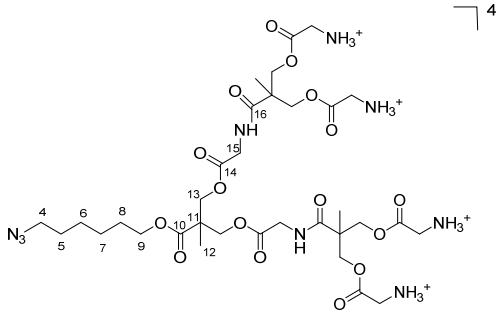
**$N_3$-[G1]-($NH_3^+$)$_2$.** (7) (4.54 g, 7.91 mmol, 1.00 eq.) was dissolved into AcOEt (15 mL). A dissolution of HCl 3M into AcOEt (15 mL) was added to it. It was stirred at room temperature during 4 hours. A white precipitate appeared. AcOEt (100 mL) was added to the reaction mixture and it was stirred at room temperature for 1 hour more. Then, it was stirred under vacuum to remove HCl vapors. Ethyl acetate was removed under reduce pressure. The white precipitate was washed by being newly dispersed into ethyl acetate and stirred during 15 min. Ethyl acetate was evaporated under reduce pressure to give a white powder (2.67 g, 76 %).$^1$H NMR (400 MHz, $CD_3OD$): δ 1.31 (s, 3H, -

$CH_3$[G1]), 1.43 (m, 4H, -$CH_2$-C<u>$H_2$</u>-C<u>$H_2$</u>-$CH_2$-), 1.61 (m, 2H, $N_3$-$CH_2$-C<u>$H_2$</u>-), 1.65 (m, 2H, -C<u>$H_2$</u>-$CH_2$-C(O)-), 3.89 (s, 4H, -C<u>$H_2$</u>N-[G1]), 4.16 (t, J = 6.4 Hz, 2H, -$(CH_2)_5$-C<u>$H_2$</u>-OC(O)-), 4.45 (ABq, J = 19.2 Hz, $\Delta\nu_{AB}$ = 7.0 Hz, 4H, -$CH_2$O-[G1]). *note: H-1 signal (~3.2 ppm) is overlapped with the residual $CH_3$ signal of $CD_3OD$ but the correlation between H-1 and H-2 is observed in $^1$H-$^1$H COSY (see Figure SI.1.1 as an example).* $^{13}$C NMR (100 MHz, $CD_3OD$): δ 17.9, 26.5, 27.3, 29.4, 29.7, 40.9, 47.5, 52.3, 66.6, 67.7, 168.3, 173.8. HRMS ($ESI^+$) *m/z*: *374.2042, m/z calc. 374.2034* $[C_{15}H_{29}N_5O_6,H]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3441-2505 (N-$H^+$), 2943-2860-2702-2625 (C-H st), 2097 ($N_3$ st), 1744 (C=O st ester), 1605-1574 (N-$H^+$ δ), 1466 ($CH_2$, $CH_3$ δ), 1420 (C-N st), 1224 (CO-O st), 1137 (O-C-C st). EA (%): *Found:* C, 40.1; H, 6.6; N, 14.2. *Calc. for* $C_{15}H_{29}Cl_2N_5O_6$: C, 40.4; H, 6.55; N, 15.7.

**<u>SI.3.1. Synthesis of the *bis*-GMPA dendrons.</u>**

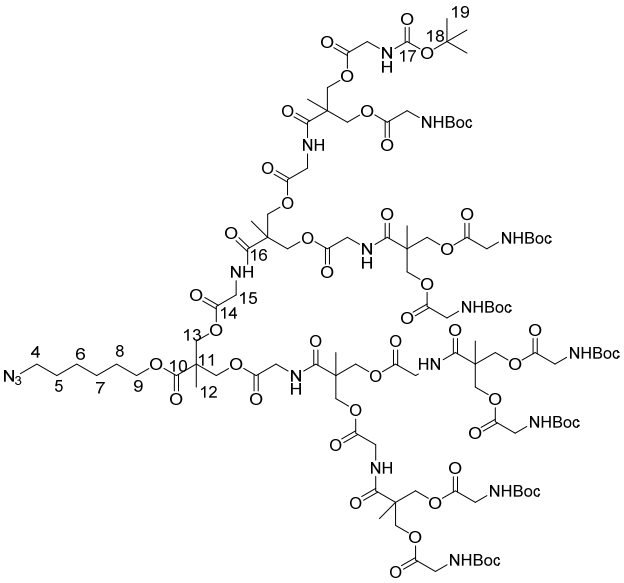



**$N_3$-[G2]-$(NHBoc)_4$ General procedure i)** $N_3$-[G1]-$(NH_3^+)_2$ (3.59 g, 8.04 mmol, 1.00 eq.); *Bis*-GMPA(monomer) (7.96 g, 17.70 mmol, 2.20 eq.); HOBt,n$H_2O$ (2.71 g, 17.70 mmol, 2.20 eq.); DMAP (2.42 g, 19.82 mmol, 2.80 eq.); DCC (3.65 g, 17.70 mmol, 2.20 eq.); dry DMF (40 mL); dry DCM (90 + 10 mL). The crude product was purified on silica gel (hexane : ethyl acetate = ramp from 5:5 to 2:8) to obtain a white product (7.64 g, 77 %). $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.27 (s, 3H, H-12[G1]), 1.29 (s, 6H, H-12[G2]), 1.41 (m, 4H, H-6 and H-7), 1.45 (m, 36H, H-19), 1.62 (m, J = 6.8 Hz, 2H, H-6), 1.67 (m, J = 6.8 Hz, 2H, H-8), 3.29 (t, 2H, J = 6.8Hz, H-4), 3.91 (d, J = 5.6Hz, 8H, H-15[G2]), 3.98 (d, J = 5.2Hz, 4H, H-15[G1]), 4.15 (t, J = 6.4 Hz, 2H, H-9), 4.17 (m, 2H, H-13[G1]), [4.17-4.38] (m, 10H, H-13[G1,2]), 5.29 (bs, -NH carbamate), 7.08 (bs, -NH amide). $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.8, 18.1, 25.4, 26.3, 28.3, 28.7, 41.0, 42.4, 46.1, 46.3, 51.3, 64.9, 65.3, 66.5, 80.1, 155.9, 169.6, 170.3, 172.5, 172.8. MS (MALDI$^+$) *m/z*: *1256* $[C_{53}H_{87}N_9O_{24},Na]^+$. FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3379 (N-H st), 2980-2943-2864 (C-H st), 2098 ($N_3$ st), 1742 (C=O ester st), 1699 (C=O st carbamate), 1676 (C=O st amide), 1520 (N-H δ), 1458 ($CH_2$, $CH_3$ δ), 1367 (C-N st), 1246 (CO-O st), 1155 ($\nu_{as}$ N-CO-O). EA (%): *Found:* C, 52.2; H, 7.5; N, 10.1. *Calc. for* $C_{53}H_{87}N_9O_{24}$: C, 51.6; H, 7.1; N, 10.2. SEC (*ref PMMA*): Mw 1581 g.$mol^{-1}$; Đ: 1.02.

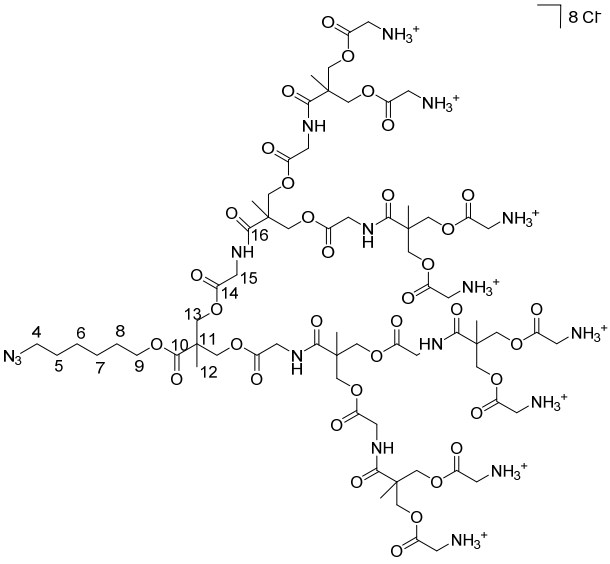



**$N_3$-[G2]-$(CH_3^+)_4$. General procedure ii)** $N_3$-[G2]-$(NHBoc)_4$ (6.00 g, 2.58 mmol, 1.00 eq.); HCl 3M into ethyl acetate (20 mL); ethyl acetate (10 mL). A white powder was obtained (3.77 g, 81 %). $^1$H NMR (400 MHz, $CD_3OD$): δ 1.27 (s, 3H, H-12[G1]), 1.37 (s, 6H, H-12[G1]), 1.43 (m, 4H, H-6 and H-7), 1.62 (m, 2H, H-5), 1.69 (m, 2H, H-8), 3.93 (m, 8H, H-15[G2]), 3.96 (m, 4H, H-15[G1]), 4.16 (t, J = 6.8 Hz, 2H, H-9), 4.32 (ABq, J = 11.6 Hz, $\Delta\nu_{AB}$ = 9.4 Hz, 4H, H-13[G1]), 4.45 (ABq, J = 11.2 HZ, 8H, H-13[G2]), 8.35 (bs, -NH amide). *note: H-4 signal (~3.2 ppm) is overlapped with the residual $CH_3$ signal of $CD_3OD$ but the correlation between H-4 and H-5 is observed in $^1$H-$^1$H COSY (see Figure SI.1.1 as an example).* $^{13}$C (100 MHz, $CD_3OD$): δ 17.8, 18.1, 26.5, 27.3, 29.4, 29.7, 41.1, 42.1, 47.3, 47.6, 52.3, 66.6, 67.2, 68.5, 168.2, 170.9, 174.0, 174.9. HRMS ($ESI^+$) *m/z*: *834.3842, m/z*

*calc. 834.3840* $[C_{33}H_{55}N_9O_{16},H]^+$.FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3222 (N-H st), 2941-2861 (C-H st and bs N-$H^+$ st), 2098 ($N_3$ st), 1747 (C=O st ester), 1662 (C=O st amide and N-$H^+$ δ), 1541 (N-H δ), 1478 ($CH_2$, $CH_3$ δ), 1407 (C-N st), 1246 (CO-O st), 1138 (O-C-C st). EA (%): *Found:* C, 39.9; H, 6.5; N, 12.9. *Calc. for* $C_{33}H_{59}Cl_4N_9O_{16}$: C, 40.5; H, 6.1; N, 12.9.

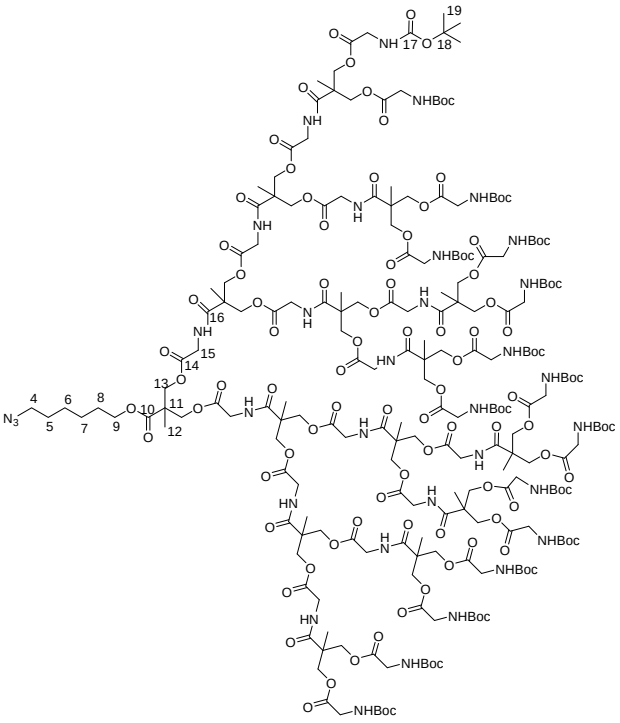


**$N_3$-[G3]-$(NHBoc)_8$ General procedure i)** $N_3$-[G2]-$(NH_3^+)_4$ (2.00 g, 2.04 mmol, 1.00 eq.); *Bis*-GMPA (monomer) (5.50 g, 12.25 mmol, 6.00 eq.); HOBt,$nH_2O$ (1.88 g, 12.25 mmol, 6.00 eq.); DMAP (1.40 mg, 11.43 mmol, 5.60 eq.); DCC (2.53 g, 12.25 mmol, 6.00 eq.); dry DMF (33 mL); dry DCM (56 + 10 mL). The crude product was purified on silica gel (DCM : MeOH = 95:5) to obtain a white solid (3.88 g, 74 %). $^1$H (400 MHz, $CDCl_3$) δ (ppm): 1.25 (s, 6H, H-12[G2]), 1,26 (s, 3H, H-12[G1]), 1.28 (s, 12H, H-12[G3]), 1.44 (m, 76H, H-6, H-7 and H-19), 1.61 (m, 4H, H-5), 1.66 (m, 2H, H-8), 3.27 (t, 2H, J = 6,8 Hz, H-4), 3.90 (d, J = 5.2 Hz, 16H, H-15[G3]), 3.95 (d, J = 5.2 Hz, 4H, H-15[G1]), 4.00 (d, J = 5.2 Hz, 8H, H-15[G2]), 4.13 (t, J = 6.4 Hz, 2H, H-9), [4.20-4.38] (m, 28H, H-13[G1,2,3]), 5.42 (bs, -NHBoc), 7.19 (bs, -NHCO). $^{13}$C (100 MHz, $CDCl_3$) δ (ppm): 17.8, 18.0, 18.4, 25.4, 26.3, 28.3, 28.7, 41.3, 42.3, 46.0, 46.1, 51.3, 65.3, 65.5, 66.2, 66.5, 80.0, 156.0, 169.5, 170.3, 172.6, 172.9. MS (MALDI$^+$, DHB) *m/z*: *2578.7* $[C_{109}H_{175}N_{17}O_{52},Na]^+$.FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3356 (N-H st), 2978-2928-2854 (C-H st), 2106 ($N_3$ st), 1742-1718 (C=O ester st), 1699 (C=O carbamate st), 1664 (C=O amide st), 1528 (N-H δ), 1458 ($CH_2$, $CH_3$ δ), 1367 (C-N st), 1250 (CO-O st), 1157 (N-CO-O st). EA (%): *Found:* C, 51.3; H, 7.3; N, 9.6. *Calc. for* $C_{109}H_{175}N_{17}O_{52}$: C, 51.2; H, 6.9; N, 9.3. SEC (*ref PMMA*): Mw 2966 g.$mol^{-1}$; Đ: 1.02.

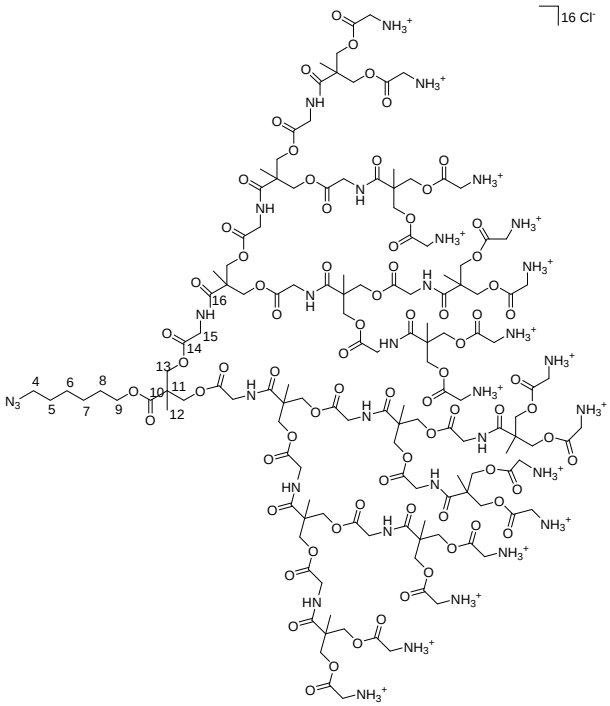


**$N_3$-[G3]-$(NH_3^+)_8$ General procedure ii)** $N_3$-[G3]-$(NHBoc)_8$ (1.50 g, 5.86x$10^{-1}$ mmol, 1.00 eq.); HCl 3M into ethyl acetate (10 mL); ethyl acetate (5 mL). The product was obtained as a white powder was obtained (1.12 g, 93 %). $^1$H NMR (400 MHz, $CD_3OD$) δ (ppm): 1.28 (s, 3H, H-12[G1]), 1.33 (s, 6H, H-12[G2]), 1,38 (s 12H, H-12[G3]), 1.43 (m, 4H, H-6 and H-7), 1.62 (m, 2H, H-5), 1.69 (m, 2H, H-8), 3.93 (s, 16H, H-15[G3]), 3.97 (s, 4H, H-15[G1]), 4.01 (s, 8H, H-15[G2]), 4.16 (t, J = 6.4 Hz, 2H, H-9), 4.32 (m, 14H, H-13[G1,2]), 4.45 (ABq, 16H, H-13[G3]). *note: H-4 signal (~3.2 ppm) is overlapped with the residual $CH_3$ signal of $CD_3OD$ but the correlation between H-4 and H-5 is observed in $^1$H-$^1$H COSY (see Figure SI.1.1).* $^{13}$C NMR (100 MHz, $CD_3OD$): δ 17.8, 18.1,

26.6, 27.4, 29.4, 29.8, 41.2, 42.2, 47.4, 47.7, 52.4, 66.5, 67.2, 67.8, 68.5, 168.3, 170.8, 171.1, 174.1, 175.0, 175.2. MS (MALDI$^+$, DHB) *m/z (%)*: *1775.2* $[C_{69}H_{111}N_{17}O_{36},Na]^+$. FTIR ($\nu_{max}$/cm$^{-1}$, ATR): 3342 (N-H st), 2984-2928 (C-H and bs N-H$^+$), 2091 ($N_3$ st), 1755 (C=O st ester), 1659 (C=O st amide and N-H$^+$ δ), 1541 (N-H δ), 1475 ($CH_2$, $CH_3$ δ), 1406 (C-N st), 1236 ($\nu_{as}$ CO-O), 1171 (O-C-C st). EA (%): *Found:* C, 39.7; H, 6.5; N, 11.5. *Calc. for* $C_{69}H_{119}Cl_8N_{17}O_{36}$: C, 40.5; H, 5.9; N, 11.6.



**$N_3$-[G4]-$(NHBoc)_{16}$ General procedure i)** $N_3$-[G3]-$(NH_3^+)_8$ (1.02 g, 4.99x10$^{-1}$ mmol, 1.00 eq.); *Bis*-GMPA (monomer) (3.14 g, 6.92 mmol, 14.00 eq.); HOBt,nH2O (1.07 g, 6.92 mmol, 14.00 eq.); DMAP (878 mg, 7.19 mmol, 14.4 eq.); DCC (1.44 g, 6.92 mmol, 14.00 eq.); dry DMF (7 mL); dry DCM (63 mL) The crude product was dissolved in ethyl acetate (30 mL) and hexane (70 mL) was added slowly under agitation until the formation of a gel. This operation was repeated twice. Then, it was purified on silica gel (DCM : MeOH = ramp from 95:5 to 9:1) to obtain a white solid (2.29 g, 88 %). $^1$H (400 MHz, $CDCl_3$) δ (ppm): [1.24-1.28] (m, 45H, H-12[G1,2,3,4]) 1.45 (s, 148H, H-6, H-7 and H-19), 1.61 (m, 4H, H-5 and H-8), 3.27 (t, 2H, J = 7,2 Hz, H-4), 3.88 (m, 32H, H-15[G4]), 3.98 (m, 28H, H-15[G1,2,3]), 4.11 (t, J = 6.4 Hz, 2H, H-9), [4.20-4.45] (m, 60H, H-13[G1,2,3,4]), 5.55 (bs, -NHBoc), 7.38 (bs, -NHCO). $^{13}$C (100 MHz, $CDCl_3$): δ [17.7-18.0], 25.3, 26.3, 28.3, 28.3, 41.3, 42.3, [46.0-46.1], 51.3, 65.3, [66.3-66.5], 80.0, 156.1, 169.6, [170.2-170.3], 172.6, [172.9-173.2]. MS (MALDI$^+$, DCTB) *m/z*: *5220.2* $[C_{221}H_{351}N_{33}O_{108},Na]^+$.FTIR ($\nu_{max}$/cm$^{-1}$, ATR): 3362 (N-H st), 2978-2930-2852 (C-H st), 2098 ($N_3$ st), 1749 (C=O st ester), 1695 (C=O st carbamate), 1668 (C=O st amide), 1520 (N-H δ), 1456 ($CH_2$, $CH_3$ δ), 1367 (C-N st), 1284 (CO-O st), 1155 (N-CO-O st).EA (%): *Found:* C, 52.2; H, 7.1; N, 8.8. *Calc. for* $C_{221}H_{351}N_{33}O_{108}$: C, 51.1; H, 6.8; N, 8.9. SEC (*ref PMMA*): Mw 4463 g.mol$^{-1}$; Đ: 1.04.



**$N_3$-[G4]-$(NH_3^+)_{16}$ General procedure ii)** $N_3$-[G4]-$(NHBoc)_{16}$ (708 mg, $1.36x10^{-1}$ mmol, 1.00 eq.); HCl 3M into ethyl acetate (10 mL); ethyl acetate (5 mL). The product was obtained as a white powder (506 mg, 89 %). $^1H$ (400 MHz, $CD_3OD$) δ (ppm): 1.23 (s, 3H, H-12[G1]), 1.28 (s, 6H, H-12[G2]), 1.33 (s, 12H, H-12[G3]) 1.38 (s, 24H, H-12[G4]), 1.43 (m, 4H, H-6 and H-7), 1.62 (m, 2H, H-5), 1.69 (m, 2H, H-8), 3.95 (s, 32H, H-15[G4]), 4.01 (m, 28H, H-15 [G1,2,3]), 4.16 (t, J = 6.4 Hz, 2H, H-9), 4.33 (m, 28H, H-13[G1,2,3]), 4.46 (m, 32H, H-13[G4]), 8.35 (bs, -NHCO). *note: H-4 signal (~3.2 ppm) is overlapped with the residual $CH_3$ signal of $CD_3OD$ but the correlation between H-4 and H-5 is observed in $^1H$-$^1H$ COSY (see Figure SI.3.1 as an example).* $^{13}C$ (100 MHz, $CD_3OD$) δ (ppm): [17-9-18.2], 26.6, 27.4, 29.4, 29.8, 41.2, 42.2, [47.3-47.7], 52.4, 66.4, [67.2-67.9], 68.5, 168.3, [170.8-171.3], 174.2, [174.9-175.3]. FTIR ($\nu_{max}/cm^{-1}$, ATR): 3318 (N-H st), 2986-2883-2615 (C-H and bs N-$H^+$), 2102 ($N_3$ st), 1744 (C=O ester st), 1655 (C=O st amide and N-$H^+$ δ), 1539 (N-H st), 1479 ($CH_2$, $CH_3$ δ), 1410 (C-N st), 1230 (CO-O st), 1176 (O-C-C st). EA (%): *Found:* C, 39.4; H, 6.5; N, 10.7. *Calc. for* $C_{141}H_{239}Cl_{16}N_{33}O_{76}$: C, 40.5; H, 5.8; N, 11.1.

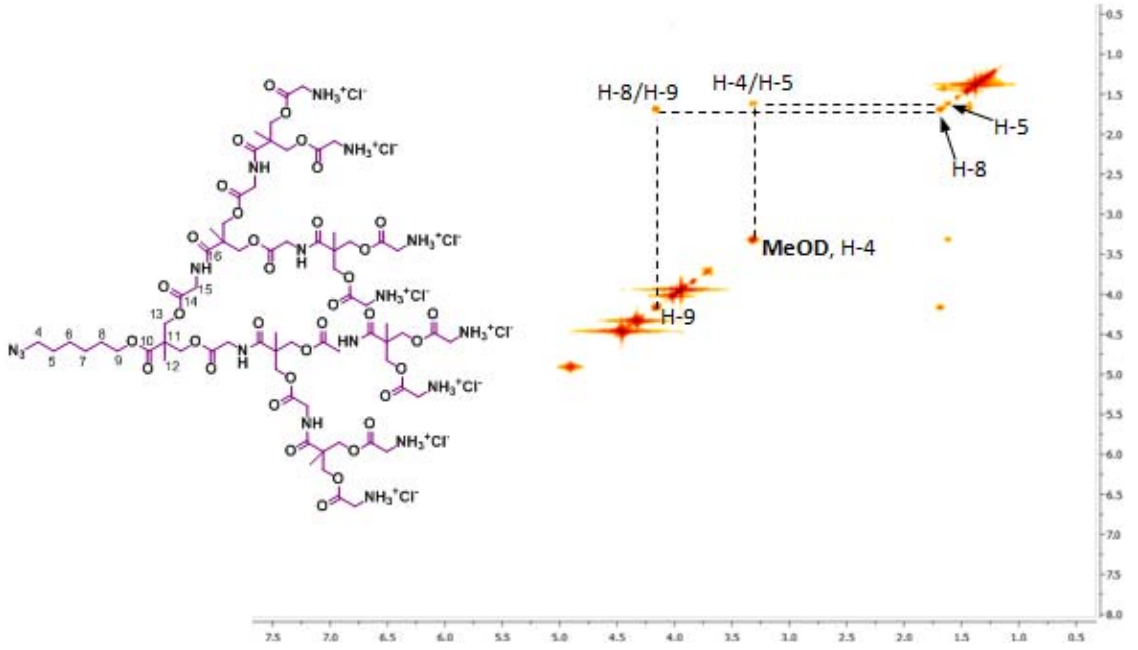



**Figure SI.3.1** $^1H$-$^1H$ COSY of **$N_3$-[G3]-$(NH_3^+)_8$** recorded at 400 MHz in $CD_3OD$. H-4 signal appears overlapped with residual methanol solvent peaks.

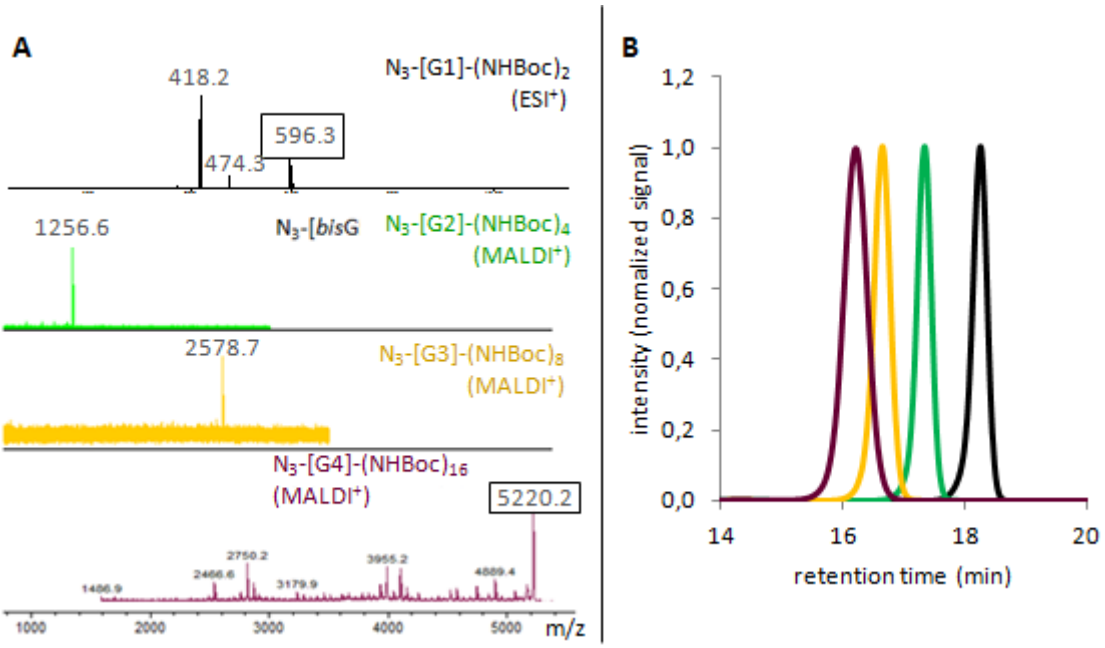
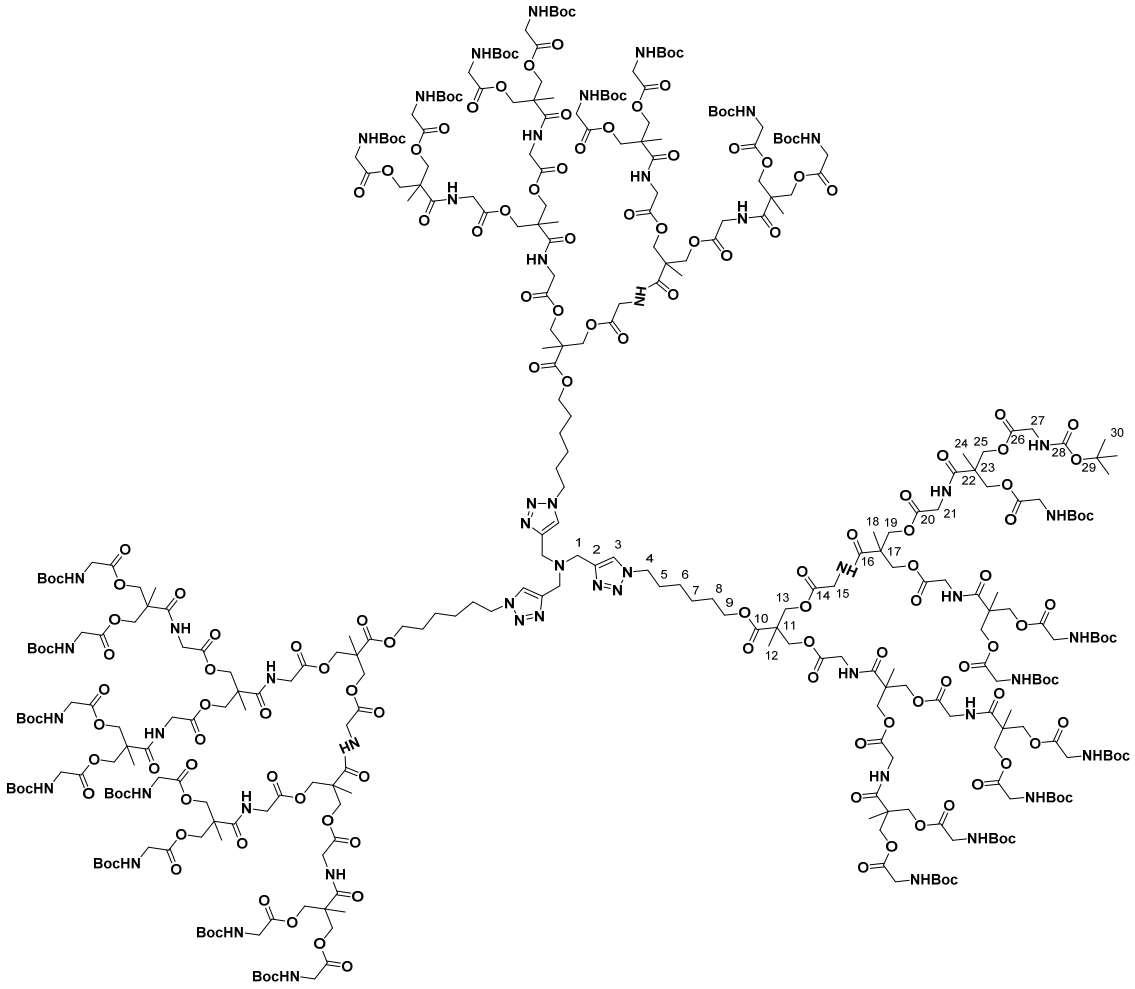



**Figure SI.3.2** MS spectra (A) and SEC chromatograms (B) of the *bis*-GMPA dendrons with terminal t-Boc protecting amino groups.

$ESI^+$ spectrum was recorded for the smallest bis-GMPA dendron. A peak that corresponds to $[M + Na]^+$ is observed together with some fragmentation peaks. $MALDI^+$-TOF spectra were recorded for the bis-GMPA dendrons of higher generations. Only one peak is observed for the $2^{nd}$ and $3^{rd}$ generation that correspond to $[M + Na]^+$ in each spectrum. In the case of the $4^{th}$ generation dendron, other peaks resulting from the fragmentation of the dendron are visible in addition to the $[M + Na]^+$ peak.

SEC chromatograms show only one monomodal and symmetrical peak for each dendron. The polydispersity of the dendrons was determined using poly(methyl methacrylate) (PMMA) as a reference and had values between 1.01 and 1.04.

## SI.4.1. Synthesis and characterization data of the *bis*-GMPA dendrimers.

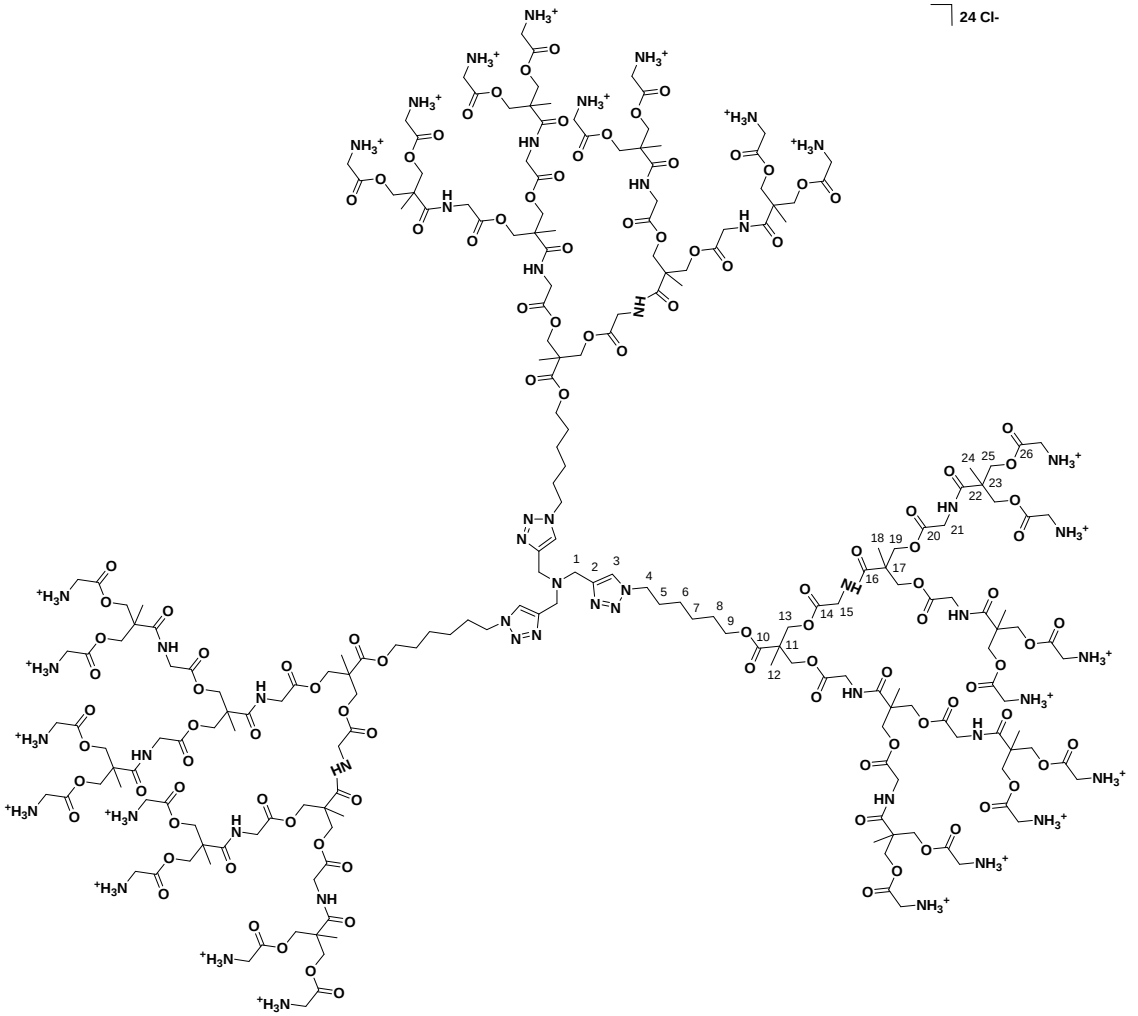


**Scheme SI.4.1.** Representation of **D[G3]-(NHBoc)$_{24}$**.

**D[G2]-(NHBoc)$_{24}$. General procedure iii-A)** Triporpargylamine amine (6.71 µL, 4.74x10$^{-2}$ mmol, 1.00 eq.); $N_3$-[G3]-(NHBoc)$_8$ (400 mg, 1.57x10$^{-1}$ mmol, 3.30 eq.); $CusO_4.5H_2O$ (17.8 mg, 7.11x10$^{-2}$ mmol, 1.50 eq.), (*L*)-ascorbate (28.2 mg, 1.42x10$^{-2}$ mmol, 3.00 eq.); TBTA (7.53 mg, 1.42x10$^{-2}$ mmol, 0.30 eq.); dry DMF (10 mL). The crude product was purified by precipitation into a mixture of hexane and ethyl acetate (4:6) and by dialysis against methanol to give a white powder (313 mg, 85 %). For $^1$H number, refer to the scheme SI2-1.1. $^1$H (400 MHz, $CDCl_3$) δ (ppm): [1.22-1.27] (m, 63H, H-12), 1.38 (m, 12H, H-6 and H-7), 1.42 (m, 216H, H-19), 1.63 (m, 6H, H-8), 1.93 (m, 2H, H-5), 3.70 (m, 6H, H-1), 3.88 (d, J = 5.2 Hz, 48H, H-15[G2]), 3.94 (d, J = 4.4 Hz, 6H, H-15[G0]), 3.98 (d, J = 4.0 Hz, H-15[G1]), 4.11 (m, 6H, H-9), [4.19-4.34] (m, 90H, H-13 and H-4), 5.48 (-NHBoc), 7.33 (-NHCO), 7.82 (s, 3H, H-3). $^{13}$C (100 MHz, $CDCl_3$) δ (ppm): [17.8-18.3], 25.2, 26.0, 28.3, 30.1, 41.3, 42.3, [46.0-46.1], 46.8, 50.2, [65.2-66.5], 80.0, 123.0, 156.1, 169.6, [170.3-171.1], 172.6, 172.9. *Note: The signals of the carbons C-1, C-2, C-3 and C-4 could*

*not be observed in the $^{13}C$ NMR spectrum. However, coupling signals between H-1 and C-1, H-2 and C-2 and H-4 and C-4 were observed in $^{1}H$-$^{13}C$ HSQC experiment.* FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3375 (N-H st), 2979-2927-2868 (C-H st), 1749 (C=O st ester), 1699 (C=O st carbamate), 1660 (C=O st amide), 1522 (N-H δ), 1457 ($CH_2$, $CH_3$ δ), 1366 (C-N st), 1287 (CO-O st), 1161 (N-CO-O st). EA (%): *Found:* C, 51.2; H, 6.9; N, 9.2. *Calc. for* $C_{336}H_{534}N_{52}O_{156}$: C, 51.75; H, 6.9; N, 9.3. SEC (*ref PMMA*): Mw 6895 g.$mol^{-1}$; Đ: 1.05.

**D[G4]-$(NHBoc)_{48}$. General procedure iii-A)** Triporpargylamine amine (3,45 µL, $2.33x10^{-2}$ mmol, 1.00 eq.); $N_3$-[G4]-$(NHBoc)_{16}$ (400 mg, $7.69x10^{-1}$ mmol, 3.30 eq.); $CusO_4.5H_2O$ (8.73 mg, $3.50x10^{-2}$ mmol, 1.50 eq.), (*L*)-ascorbate (13.9 mg, $7.00x10^{-2}$ mmol, 3.00 eq.); TBTA (3.71 mg, $7.00x10^{-3}$ mmol, 0.30 eq.); dry DMF (10 mL). The crude product was purified by precipitation into a mixture of hexane and ethyl acetate (5:5) (100 mL) and by dialysis against methanol to give a light yellow powder (287 mg, 78 %). For $^{1}H$ number, refer to the scheme SI2-1.1 at the previous page. $^{1}H$ (400 MHz, $CDCl_3$) δ (ppm): [1.22-1.28] (m, 135H, H-12), 1.43 (m, 444H, H-17, H-6 and H-7), 1.64 (m, 6H, H-8), 1.96 (m, 6H, H-5), 3.69 (m, 6H, H-1), 3.89 (m, 96H, H-15[G3]), 3.98 (m, 84H, H-15[G0,1,2]), 4.12 (m, 6H, H-9), [4.17-4.40] (m, 180H, H-13), 4.35 (m, 6H, H-4), 5.54 (-NHBoc), 7.70 (s, 3H, H-3). $^{13}C$ (100 MHz, $CDCl_3$) δ (ppm): [17.7-18.8], 28.3, 42.4, 42.4, 46.0, [65.3-66.1], 80.0, 156.1, 170.1, [169.6-173.1]. *Note: The signals of the carbons C-1 to C-9 could not be observed in the $^{13}C$ NMR spectrum and their $^{1}H$-$^{13}C$ correlations could not be observed in the $^{1}H$-$^{13}C$ HSQC spectrum.* FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3367 (N-H st), 2978-2927 (C-H st), 1749 (C=O st ester), 1699 (C=O st carbamate), 1675 (C=O st amide), 1525 (N-H δ), 1469 ($CH_2$, $CH_3$ δ), 1369 (C-N st), 1249 (CO-O st), 1159 (N-CO-O st). EA (%): *Found:* C, 52.0; H, 7.3; N, 8.0. *Calc. for* $C_{672}H_{1062}N_{100}O_{324}$: C, 51.3; H, 6.8; N, 8.9. SEC (*ref PMMA*): Mw 11822 g.$mol^{-1}$; Đ: 1.02.




**Scheme SI.4.2.** Representation of **D[G3]-($NH_3^+$)$_{24}$**.

**D[G3]-($NH_3^+$)$_{24}$. General procedure iii-B)** D[G3]-(NHBoc)$_{24}$ (154 mg, 7.38x10$^{-2}$ mmol, 1.00 eq.); HCl 3M into ethyl acetate (5 mL); ethyl acetate (5 mL). The product was obtained as a white powder (123 mg, quantitative yield). For $^1$H number, refer to the scheme SI2-1.2. (500 MHz, $CD_3OD$) δ (ppm): 1.28 (s, 9H, H-12$_{[G0]}$), 1.33 (s, 18H, H-12$_{[G1]}$), 1.38 (s, 36H, H-12$_{[G2]}$), 1.43 (m, 12H, H-6 and H-7), 1.68 (m, 6H, H-8), 1.99 (m, 6H, H-5), 3.95 (s, 48H, H-15$_{[G2]}$), 3.97 (s, 12H, H-15$_{[G0]}$), 4.01 (s, 24H, H-15$_{[G1]}$), 4.15 (m, 6H, H-9), 4.33 (m, 36H, H-13$_{[G0,1]}$), 4.46 (m, 48H, H-13$_{[G2]}$), 4.52 (m, 12H, H-1 and H-4), [8.26-8.34] (bs, N-H), 8.42 (s, 3H, H-3). $^{13}$C (125 MHz, $CD_3OD$) δ (ppm): [17.9-18.2], 26.4, 27.1, 29.4, 31.2, 41.2, 42.2, [47.4-47.9], 51.6, 66.4, [67.2-68.6], 128.7, 137.9, 168. 3, [170.8-174.1], [174.0-175.2]. FTIR ($\nu_{max}$/cm$^{-1}$, ATR): 3600-2600 (bs N-H$^+$ st), 2947-2633 (C-H st), 1749 (O-C=O st), 1655 (N-C=O st and N-H$^+$ δ), 1545 (N-H δ), 1473 ($CH_2$, $CH_3$ δ), 1379 (C-N st), 1221 (CO-O st).

**D[G4]-($NH_3^+$)$_{48}$. General procedure iii-B)** D[G4]-(NHBoc)$_{48}$ (163 mg, 1.04x10$^{-2}$ mmol, 1.00 eq.); HCl 3M into ethyl acetate (5 mL); ethyl acetate (5 mL). The product was obtained as a white powder (131 mg, quantitative yield). For $^1$H number, refer to the scheme SI2-1.2. $^1$H (500 MHz, $CD_3OD$) δ (ppm): 1.23 (s, 9H, H-12$_{[G0]}$), 1.28 (s, 18H, H-12$_{[G1]}$), 1.33 (s, 36H, H-12$_{[G2]}$), 1.38 (s, 72H, H-12$_{[G3]}$), 1.45 (m, 12H, H-6 and H-7), 1.68 (m, 6H, H-8), 1.99 (m, 6H, H-5), 3.96 (s, 96H, H-15$_{[G3]}$), 4.01 (m, 84H, H-15$_{[G0,1,2]}$), 4.15 (m, 6H, H-9), 4.33 (m, 84H, H-13$_{[G0,1,2]}$), 4.46 (m, 96H, H-13$_{[G3]}$), 4.56 (m, 12H, H-1 and H-4), [8.24-8.33] (bs, N-H), 8.44 (s, 3H, H-3). *Note: Some $^1$H*

*signals can be detected near 3.77 ppm, they result to the degradation of the bis-GMPA dendrons but they do not integrate for more than 5 % percent of the total of the bis-GMPA dendrons (Fig.SI2-2.3).* $^{13}$C (125 MHz, $CD_3OD$) δ (ppm): [17.9-18.2], 26.4, 27.1, 29.4, 31.2, 41.2, 42.3, [47.4-47.7], 51.6, [65.7-68.6], 128.9, 137.7, 168.3, [170.9-174.1], [175.0-177.0]. FTIR ($\nu_{max}$/$cm^{-1}$, ATR): 3600-2600 (bs N-$H^+$ st), 3391 (N-H st), 2935-2615 (C-H st), 1749 (C=O st ester), 1655 (C=O st and N-$H^+$ δ), 1545 (N-H δ), 1477 ($CH_2$, $CH_3$ δ), 1385 (C-N st), 1211 (CO-O st).

*Due to the high molecular weight of the dendrimers, their MALDI-TOF MS spectra could not be obtained properly so that to observe the molecular peak.*

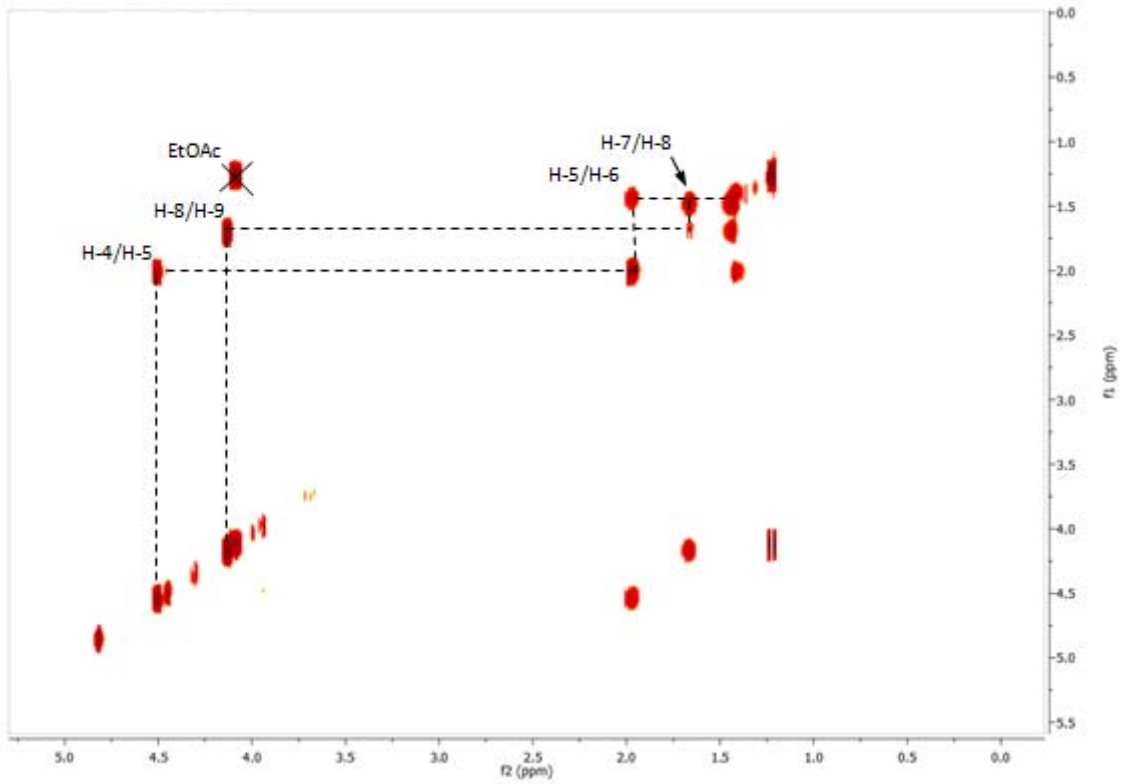



**Figure SI.4.1.** $^1$H-$^1$H COSY NMR spectrum of **D[G3]-($NH_3^+$)$_{24}$** recorded in $CD_3OD$ at 500 MHz. For the $^1$H number refer to scheme SI2-1.2 at the previous page.

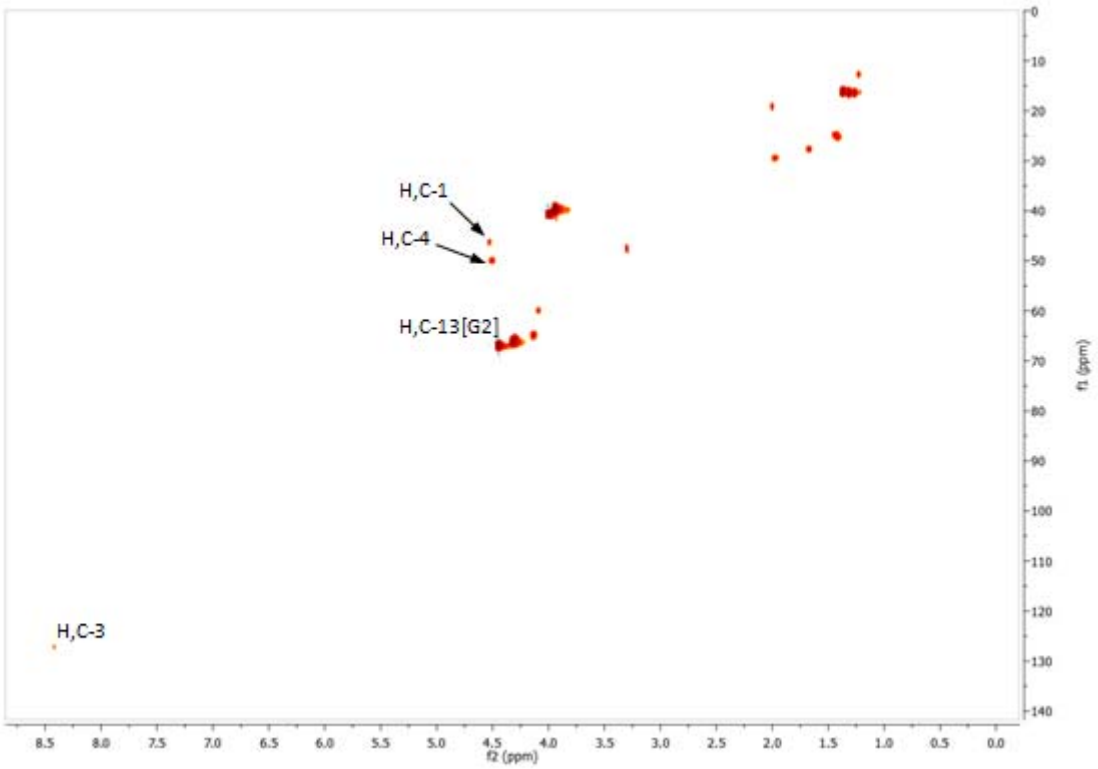



**Figure SI.4.2.** $^{1}$H-$^{13}$C HSQC NMR spectrum of **D[G3]-($NH_3^+$)$_{24}$** recorded in $CD_3OD$ at 500 MHz. For the $^{1}$H number refer to scheme SI2-1.2 two pages before.

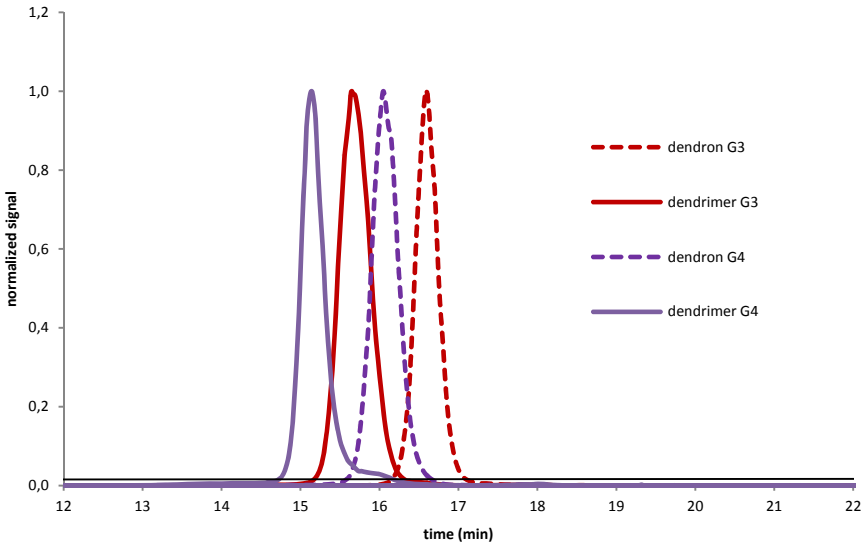



**Figure SI.4.3.** SEC chromatograms of the *bis*-GMPA dendrons and dendrimers of 3$^{rd}$ and 4$^{th}$ generation with *t*-Boc protected terminal amino groups.

## SI.5. Biocompatibility and degradability of the *bis*-GMPA dendrons and dendrimers.

### SI.5.1. Biocompatibility of the *bis*-GMPA dendrons and dendrimers.

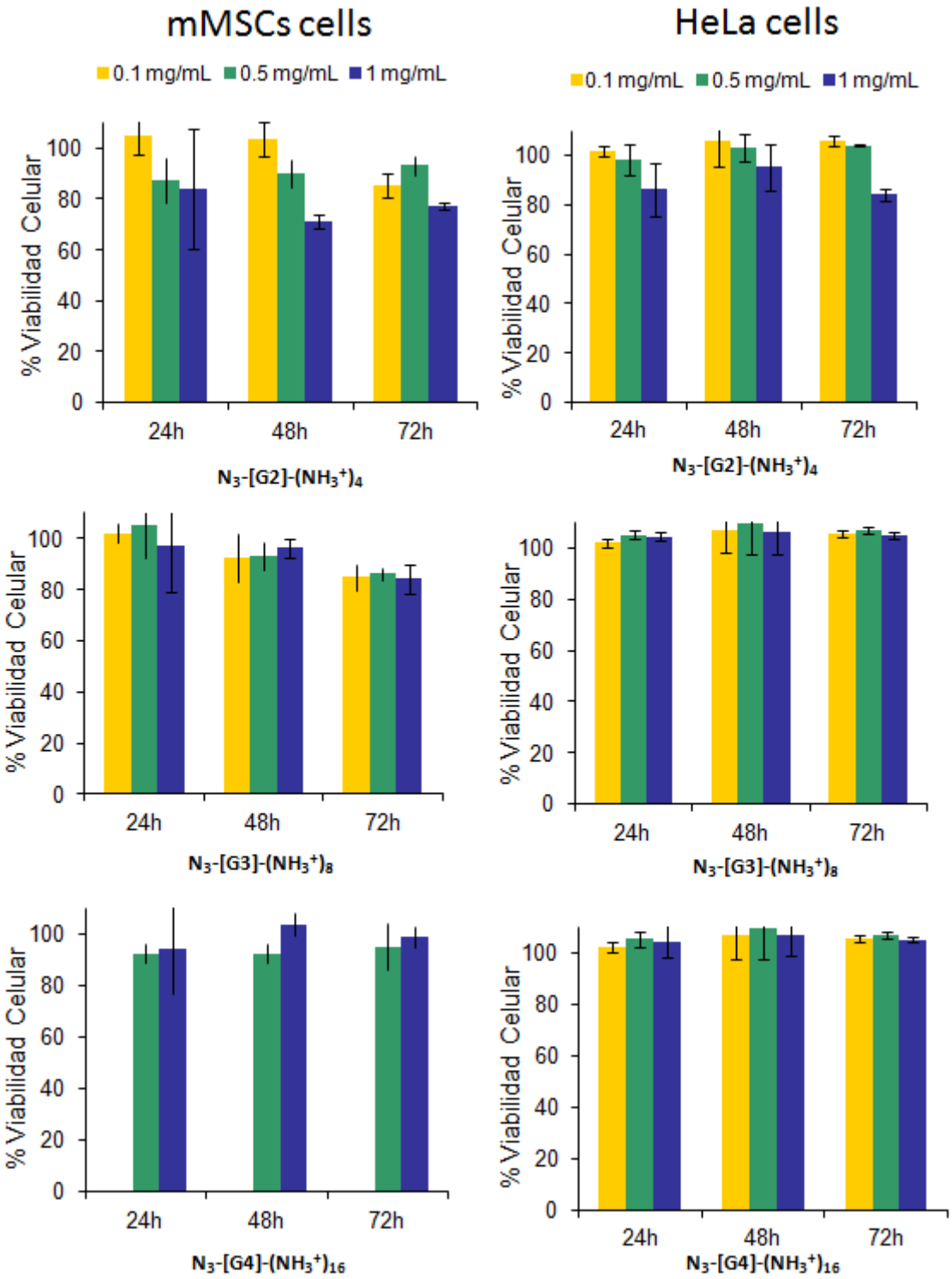



**Figure SI.5.1.1:** Biocompatibility of the *bis*-GMPA dendrons of 2nd, 3rd and 4th generation in mesenchymal cells (mMSCs) and HeLa cells.

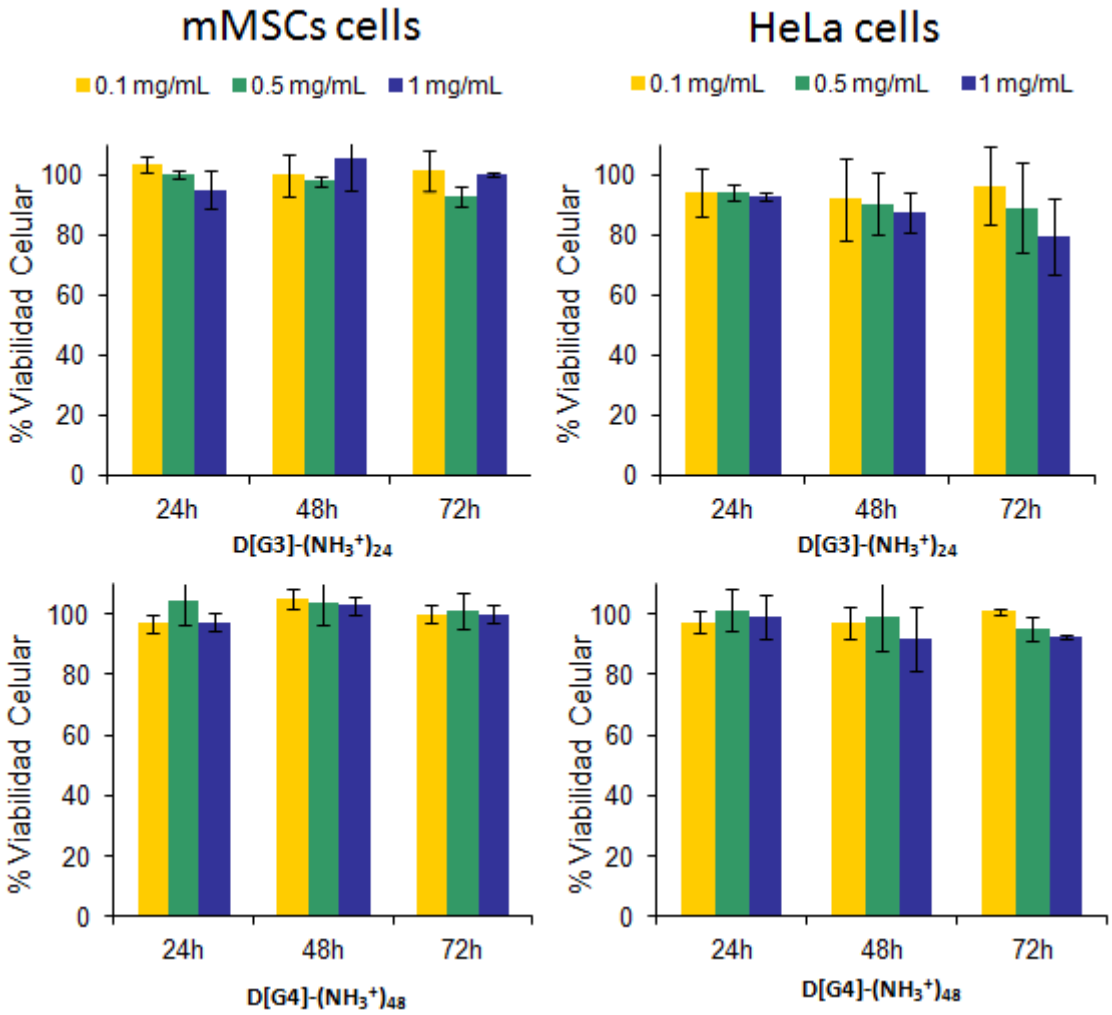



**Figure SI.5.1.2.** Biocompatibility of the *bis*-GMPA dendrimers of 3rd and 4th generation in mesenchymal cells (mMSCs) and HeLa cells.

**SI.5.2. Degradability of the *bis*-GMPA dendrons.**

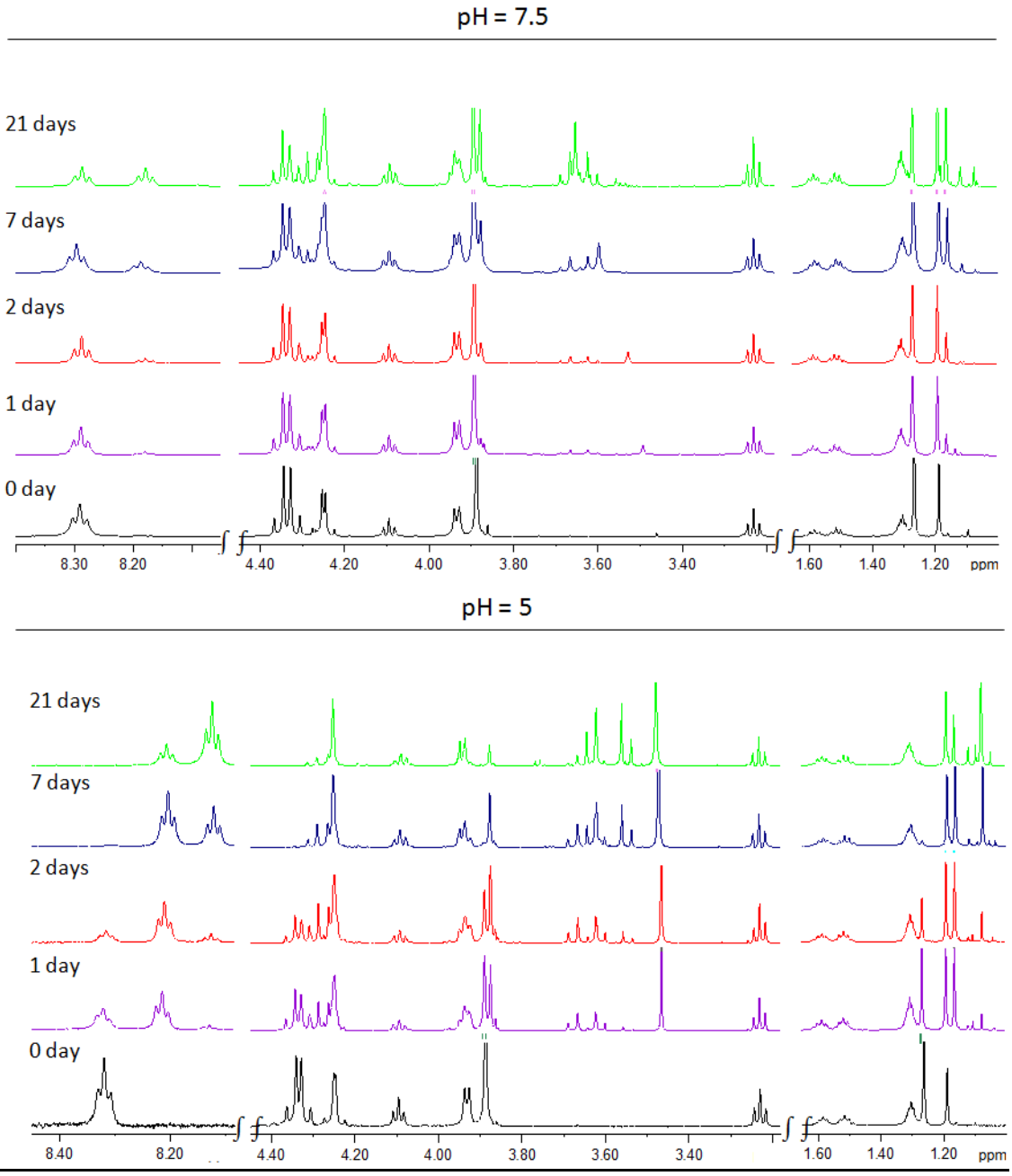



**Figure SI.5.2.1.** Degradation of the *bis*-GMPA dendron **$N_3$-[G2]-$(NH_3^+)_4$** in aqueous buffer at pH = 7.5 and 5.

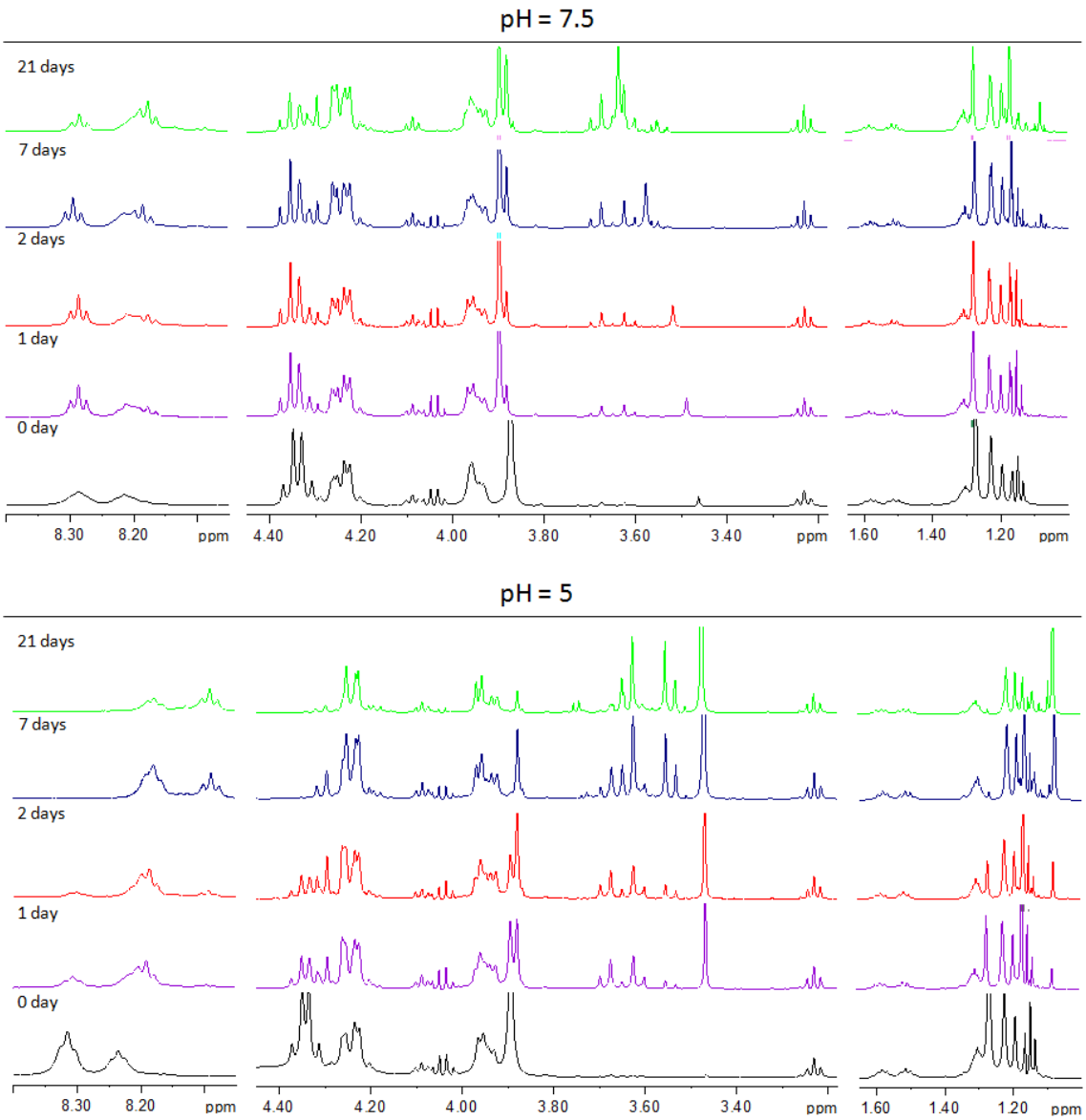



**Figure SI.5.2.2.** Degradation of the *bis*-GMPA dendron **$N_3$-[G3]-$(NH_3^+)_8$** in aqueous buffer at pH = 7.5, 5 and 11.

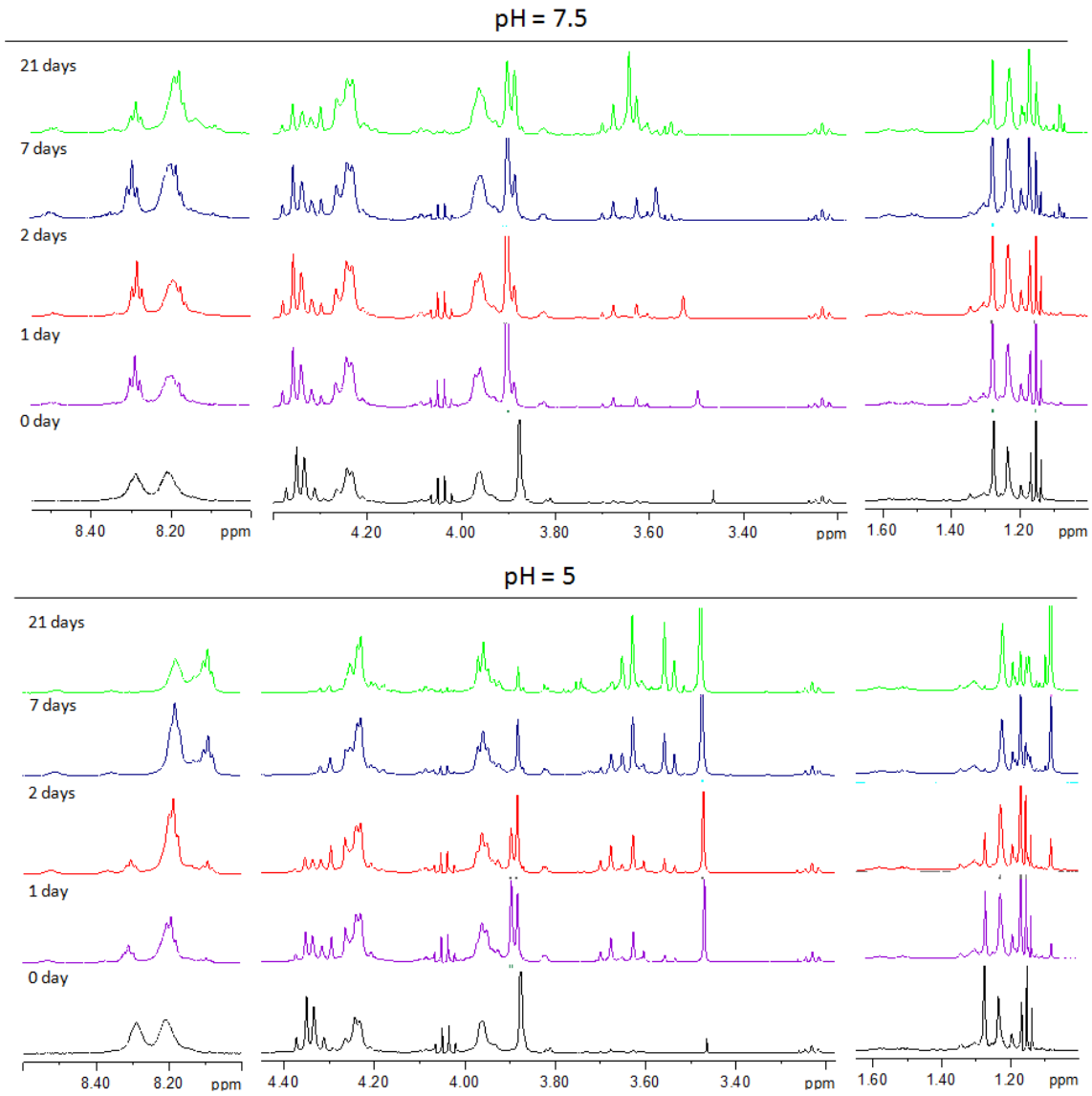



**Figure SI.5.2.3.** Degradation of the *bis*-GMPA dendron **$N_3$-[G4]-$(NH_3^+)_{16}$** in aqueous buffer at pH = 7.5 and 5.

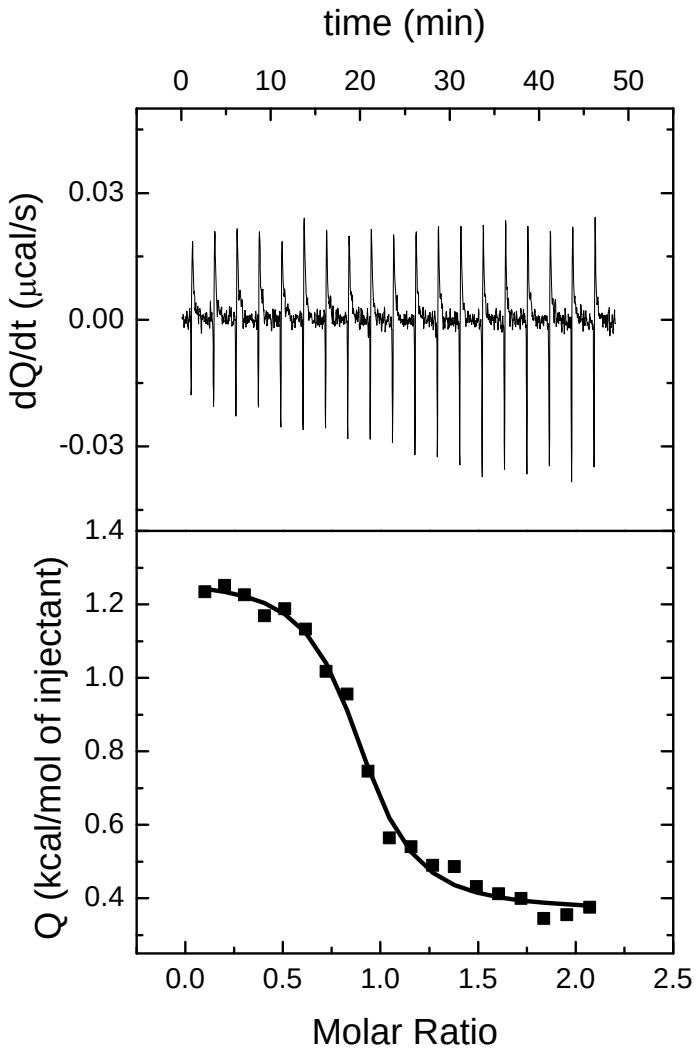



**Figure SI.6**. CPT/**D[G3]-($NH_3^+$)$_{24}$** interaction by isothermal titration calorimetry (ITC). Calorimetric titrations were performed by programming sequential injections of Camptothecin solution (100 μM) into **D[G3]-($NH_3^+$)$_{24}$** solution (10 μM) in the calorimetric cell. Upper plot shows the thermogram (thermal power to maintain a zero temperature difference between the reference and sample cells as a function of time) and lower plot shows the binding isotherm (normalized heat per injection as a function of molar ratio). From a simple analysis considering n identical and independent binding sites for CPT in a given **D[G3]-($NH_3^+$)$_{24}$** dendrimer, the equilibrium association constant ($K_a$ = 4.1 ± 0.6 · $10^6$ $M^{-1}$) and the interaction enthalpy ($\Delta H$= 0.9 ± 0.1 kcal·$mol^{-1}$) could be estimated.

## SI.7. Characterization of complexes formed between *bis*-GMPA dendrimers and pEGFP

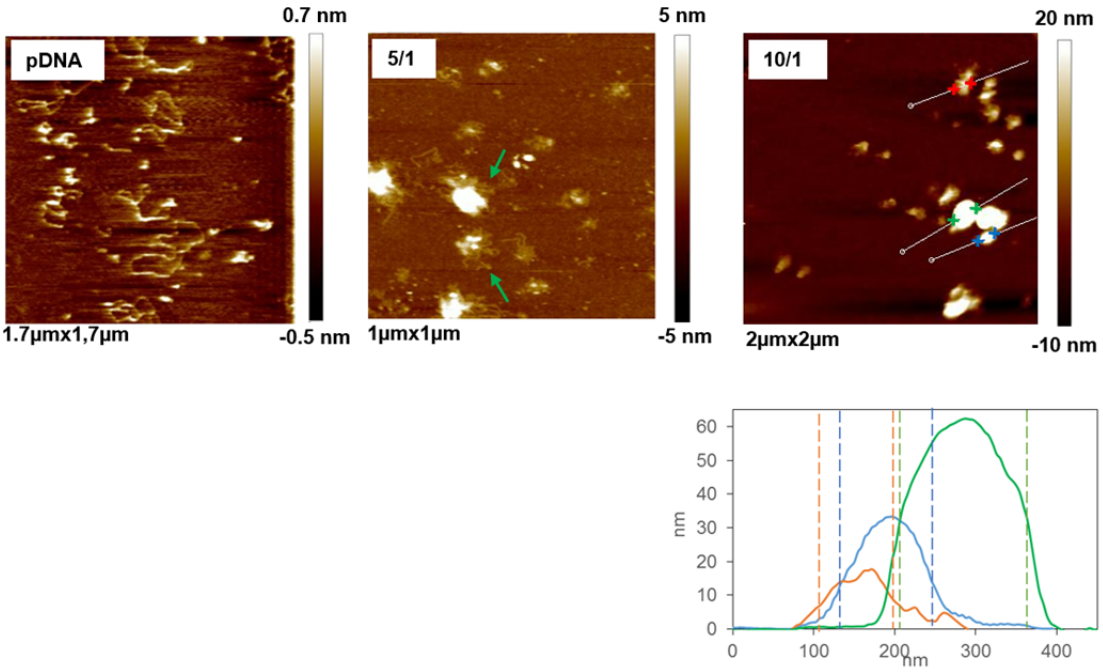



**Figure SI.7.** AFM images of complexes formed between D[G4]-$(NH_3^+)_{48}$ and pEGFP at different ratios. Morphology, free pDNA (green arrows) and sections size at 10/1 (red 100 nm, blue 118 nm and green 150 nm) can be observed.

**SI.8. siRNA transfection efficacy of commercial reagents.**

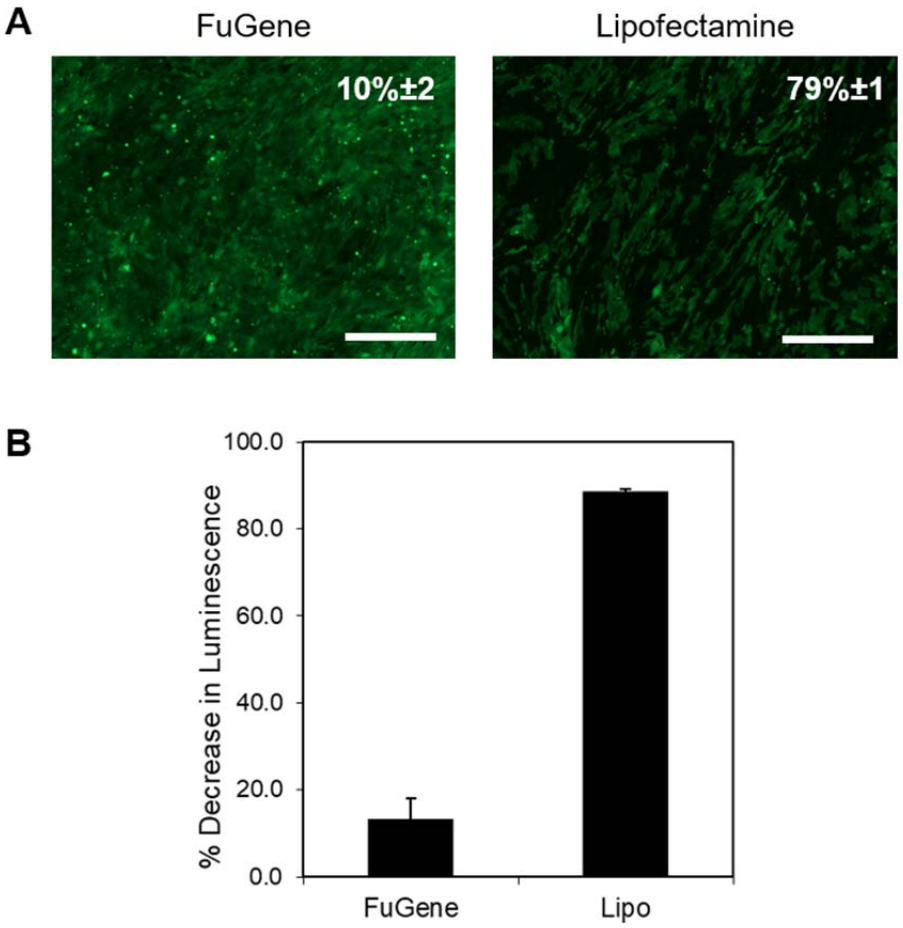



**Figure SI.8**. A. Reduction on GFP fluorescence after transfection of siGFP on DCK-GFP cells (scale = 100 µm). B. Reduction on luminescence after transfection of siLuc on SKOV3-Luc cells.