For Supporting Information:

New Synthetic Procedure for the Antiviral Sulfonate Carbosilane Dendrimer G2-S16 and its Fluorescein-Labelled Derivative for Biological Studies

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Experimental Section

General Considerations

All reactions were carried out under inert atmosphere and solvents were purified from appropriate drying agents. NMR spectra were recorded on a Varian Unity VXR-300 (300.13 (^{1}H), 75.47 (^{13}C) MHz) or on a Bruker AV400 (400.13 (1H), 100.60 (^{13}C) MHz). Chemical shifts (δ) are given in ppm. ^{1}H and ^{13}C resonances were measured relative to solvent peaks considering TMS = 0 ppm. Elemental analyses were performed on a LECO CHNS-932. Mass Spectra were obtained from an Agilent 6210 (ESI) and a Bruker Ultraflex III (MALDI-TOF). Compounds Karstedt's Pt catalyst, ESF ($C_2H_3SO_2F$), NaOH, trifluoroacetic anhydride were obtained from commercial sources. Compound $G_2Si(NH_2)_8$[1] was synthesized as published.

Synthesis of compounds

$G_2Si(SO_2F)_{16}$ (1). $(C_2H_3)SO_2F$ (ESF, 0.47 g, 4.28 mmol) was dropwise added to a solution of compound $G_2Si(NH_2)_8$ (0.40 g, 0.25 mmol) in THF (10 mL) and the mixture was stirred at r. T. for 16 h. The solvent and excess ESF (caution, toxic) was evaporated under vacuum, yielding **1** as pale yellow oil (0.76 g, 91%). ^{1}H NMR ($CDCl_3$): 3.54 (m, 32 H, C*H*$_2$SO$_2$F), 3.09 (m, 32 H, NC*H*$_2$CH$_2$SO$_2$F), 2.51 (m, 16 H, C*H*$_2$N), 1.59-1.29 (m, 40 H; SiCH$_2$C*H*$_2$CH$_2$Si and SiCH$_2$C*H*$_2$CH$_2$N), 0.55–0.42 (m, 64 H, C*H*$_2$Si), -0.02 (s, 48 H, Si*Me*$_2$), -0.07 ppm (s, 12 H, Si*Me*). ^{13}C NMR ($CDCl_3$): 57.5 (*C*H$_2$N), 49.3 (*C*H$_2$S, d, J = 13.7 Hz), 47.8 (*C*H$_2$N), 21.2 (SiCH$_2$*C*H$_2$CH$_2$N), 19.6, 18.5, 17.7 (Si*C*H$_2$*C*H$_2$*C*H$_2$Si), 12.1 (Si*C*H$_2$CH$_2$CH$_2$NH$_2$), -3.5 (Si*Me*$_2$), -4.9 ppm (Si*Me*). ^{19}F NMR ($CDCl_3$): 54.1. Elemental analysis calculated (%) for $C_{112}H_{244}F_{16}N_8O_{32}S_{16}Si_{13}$: C, 39.60; H, 7.24; N, 3.30; S, 15.10. Obt (%):C, 40.89; H, 7.99; N, 2.99; S, 14.50.

G2-S16 ($G_2Si(SO_3Na)_{16}$, 2). Compound **2** was obtained as a white solid from **1** (0.50 g, 0.15 mmol) and NaOH (0.28 g, 7.06 mmol) by reaction in MeOH at r. T. for 4 h. Afterwards, volatiles were removed under vacuum and compound **2** was purified by nanofiltration (MWCO = 1000), yielding **2** as white solid (0.46 g, 82%). ^{1}H-NMR (D_2O): -0.21 (br. s, 12 H, Si*Me*), -0.16 (s, 48 H, Si*Me*$_2$), 0.28 (m, 16 H, SiC*H*$_2$CH$_2$CH$_2$N), 0.43 (m, 48 H, Si*CH*$_2$CH$_2$CH$_2$Si), 1.21-1.31 (m, 40 H, SiCH$_2$C*H*$_2$CH$_2$Si and Si*C*H$_2$C*H*$_2$CH$_2$N), 2.28 (m, 16 H, CH$_2$CHC*H*$_2$N), 2.76 (m, 32 H, NC*H*$_2$CH$_2$SO$_3$), 2.86 (m, 32 H,

$NCH_2CH_2SO_3$). ^{13}C-NMR{^{1}H} (D_2O): -4.4 (Si*Me*), -3.5 (Si*Me*$_2$), 12.4 (Si*C*$H_2CH_2CH_2N$), 17.7-18.5 (CH_2), 20.1 (CH_2), 46.7 ($NCH_2CH_2SO_3$), 48.0 ($NCH_2CH_2SO_3$), 56.9 ($CH_2CH_2CH_2N$). ^{29}Si-NMR (D_2O): G_0-Si not observed, 1.1 (G_1-Si), 2.1 (G_2-Si). Elemental analysis calculated (%) for $C_{112}H_{244}N_8Na_{16}O_{48}S_{16}Si_{13}$ (3717.15): C, 36.19; H, 6.62; N, 3.01; S, 13.80; Exp. C, 35.73, H 6.43; N, 3.48; S, 13.01. These data are analogous to those observed in bibliography.[2]

$G_2Si(NHCOCF_3)(NH_2)_7$ (3). Trifluoroacetic anhydride (0.024 g, 0.12 mmol) was dropwise added to a suspension of dendrimer $G_2Si(NH_2)_8$ (0.200 g, 0.11 mmol) and K_2CO_3 (0.62 g, 4.5 mmol) in $CHCl_3$, and the mixture was stirred for 30 min at r. T. Afterwards, 2 mL of water were added to the mixture and the organic phase was separated and the aqueous phase was extracted with $CHCl_3$ 3 times. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under vacuum yielding compound **3** as colourless oil (0.19 g, 88%). Data for 3: ^{1}H NMR ($CDCl_3$): 3.26 (m, 2 H, C*H*$_2$NHCO), 2.60 (t, J = 7.0 Hz, 14 H, C*H*$_2$NH$_2$), 1.50 (m, 2 H, C*H*$_2$CH$_2$NHCO), 1.37 (m, 24 H, SiCH$_2$C*H*$_2$CH$_2$Si), 1.25 (m, 14 H, C*H*$_2$CH$_2$NH$_2$), 0.55–0.42 (m, 64 H, C*H*$_2$Si), -0.08 (s, 48 H, Si*Me*$_2$), -0.12 ppm (s, 12 H, Si*Me*). ^{13}C NMR ($CDCl_3$): 165.3 (C=O), 119.3 (q, $^1J_{CF}$ = 273.5 Hz), 45.5 (*C*H$_2$NH$_2$), 42.8 (*C*H$_2$NHCO), 28.2 (SiCH$_2$*C*H$_2$CH$_2$N), 19.3, 18.5, 17.6 (Si*C*H$_2$*C*H$_2$*C*H$_2$Si), 12.2 (Si*C*H$_2$CH$_2$CH$_2$NH$_2$), -3.4 (Si*Me*$_2$), -5.0 ppm (Si*Me*). ^{19}F NMR ($CDCl_3$): -75.8. Elemental analysis calculated (%) for $C_{82}H_{195}F_3N_8OSi_{13}$: C, 56.88; H, 11.35; N, 6.47. Obt.: C, 57.81; H, 11.28; N, 6.08.

$G_2Si(NHCOCF_3)(SO_2F)_{14}$ (4). ESF (0.143 g, 1.30 mmol) was dropwise added to a solution of compound 3 (0.150 g, 0.087 mmol) in dry THF (10 ml) and the mixture was stirred at r. T. for 16 h. The solvent and excess ESF (caution, toxic) were evaporated under vacuum yielding **4** as yellowish oil (0.25 g, 89%). ^{1}H NMR ($CDCl_3$): 3.54 (m, 30 H, NCH$_2$C*H*$_2$SO$_2$F), 3.26 (m, 2 H, C*H*$_2$NHCO), 3.09 (m, 30 H, NC*H*$_2$CH$_2$SO$_2$F), 2.51 (m, 14 H, C*H*$_2$N), 1.59–1.29 (m, 40 H, SiCH$_2$C*H*$_2$CH$_2$Si and C*H*$_2$CH$_2$N), 0.55–0.42 (m, 64 H; C*H*$_2$Si), -0.02 (s, 48 H, Si*Me*$_2$), -0.07 ppm (s, 12 H, Si*Me*). ^{13}C NMR ($CDCl_3$): 165.5 (C=O), 119.3 (q, $^1J_{CF}$ = 274.8 Hz), 57.5 (*C*H$_2$N), 49.2 (*C*H$_2$S, d, J = 13.9 Hz), 47.7 (*C*H$_2$N), 42.8 (*C*H$_2$NHCO), 21.2 (SiCH$_2$*C*H$_2$CH$_2$N), 19.6, 18.5, 17.7 (Si*C*H$_2$*C*H$_2$*C*H$_2$Si), 12.1 (Si*C*H$_2$CH$_2$CH$_2$NH$_2$), -3.5 (Si*Me*$_2$), -4.9 ppm (Si*Me*). ^{19}F NMR ($CDCl_3$): 54.1 (SF), -75.6 (CF_3). Elemental analysis calculated

(%) for $C_{110}H_{237}F_{17}N_8O_{29}S_{14}Si_{13}$: C, 40.37; H, 7.30; N, 3.42; S, 13.71. Obt.. (%):C, 42.11; H, 7.98; N, 3.40; S, 12.99.

$G_2Si(NH_2)(SO_3Na)_{14}$ (5). A solution of compound **4** (0.150 g, 0.046 mmol) and NaOH (0.082 g, 2.06 mmol) in MeOH (15 ml) were stirred at r. T. for 4 h. Afterwards, the solvent was evaporated under vacuum and the residue was diluted in water and purified by dialysis (MWCO = 1000 Da), yielding **5** as white solid (0.133 g, 84%). To confirm the presence of -NH_2 group a Kaiser test was performed. This compound was used without further treatment for next transformation. Data for **5**: ^{1}H-NMR (D_2O): 2.94 (m, 64 H, NC*H*$_2$C*H*$_2$SO$_3$), 2.42 (m, 16 H, $CH_2CH_2CH_2N$), 1.59 (m, 2H, $SiCH_2CH_2CH_2CH_2NH_2$), 1.44 (m, 14 H, $SiCH_2CH_2CH_2N$), 1.30 (m, 24 H, $SiCH_2CH_2CH_2Si$), 0.53 (m, 48H, $SiCH_2CH_2CH_2Si$), 0.39 (m, 16 H, $SiCH_2CH_2CH_2N$), −0.06 (s, 48 H, $SiMe_2$), −0.11 (br. s, 12 H, SiMe).

G2-S16-FITC ($G_2Si(NHFITC)(SO_3Na)_{14}$, 6). NEt_3 (0.04 mmol) was added to a DMF solution of compound **5** (0.100 g, 0.029 mmol) and fluorescein isothiocyanate (FITC, 0.016 g, 0.040 mmol). This mixture was stirred, protected from light at r. T. for 12 h. Volatiles were removed under vacuum and the residue was washed with ethanol and then purified by ultrafiltration (MWCO = 1000 Da), yielding compound **6** as orange solid (0.098 g, 88%). Data for 6: ^{1}H-NMR (D_2O): 7.59, 7.17, 6.97, 6.74, 6.65 (9H, m, FITC), 3.70 (m, 2H, $SiCH_2CH_2CH_2NH$), 3.39 (m, 28 H, $NCH_2CH_2SO_3$), 3.16 (m, 28 H, $NCH_2CH_2SO_3$), 3.00 (m, 14 H, $SiCH_2CH_2CH_2N$), 1.60 (m, 17H, $CH_2CH_2CH_2N$), 1.26 (m, 24 H, $SiCH_2CH_2CH_2Si$), 0.96 (m, 2 H, $SiCH_2CH_2CH_2NH$), 0.48 (m, 64 H, $SiCH_2CH_2CH_2Si$ and $SiCH_2CH_2CH_2N$), −0.07 (br. s, 60 H, Si*Me*$_2$ and Si*Me*). ^{13}C-NMR (D_2O): 146.8, 139.4, 135.2, 103.7 (*C*ar-FTIC), 57.1 ($SiCH_2CH_2CH_2N$), 49.1 ($NCH_2CH_2SO_3$), 43.0 ($NCH_2CH_2SO_3$), 40.2 ($SiCH_2CH_2CH_2NH$), 20.1, 19.1, 18.1 ($SiCH_2CH_2CH_2Si$), 12.3 ($SiCH_2CH_2CH_2N$), -3.0 ($SiMe_2$), -4.0 (SiMe). UV-Vis (H_2O): 500.80 nm. Elemental Analysis: Calcd. for $C_{129}H_{249}N_9Na_{14}O_{47}S_{15}Si_{13}$ (3846.29 g/mol): C, 40.28; H, 6.53; N, 3.28; S, 12.50; Obt.: C, 41.62; H, 7.66; N, 3.82; S, 11.26.

DOSY NMR experiments. These experiments were recorded on a Bruker AV400 at 298 K using the routine “dosy” and processed with TopSpin from Bruker. The experiments were done in a qualitative way, using gradient length δ = 120 ms (d20) and diffusion time Δ = 1500 μs.

MTT Assay. G2-S16 (**2**) and G2-S16-FITC (**6**) were screened by MTT assay (Sigma-Aldrich) using human PBMCs according to manufacturer's instructions. A range of concentrations between 0.5 μM and 50 μM was evaluated for each dendrimer. DMSO 20 μM were used as control of cell viability and death, respectively. Survival was adjusted compared to the untreated control, 80% cell viability was set as the minimum non-toxicity value.

Confocal Imaging of G2-S16-FITC (6) in PBMC. PBMCs were treated with 2 μM of G2-S16-FITC for 2 h and 24 h. Subsequently, stain protocol was performed to visualize the dendrimer. Briefly, PBMCs were fixed in 3.7-4% formaldehyde w/v (Panreac AppliChem, Darmstadt, Germany) for 10 min followed by two PBS washes. PBMCs were then incubated in 0.1% Triton X-100 for 5 min followed by a PBS wash. To avoid non-specific binding, they were blocked for 30 min in PBS-BSA 5% and incubated with anti-human CD4 at a 1:100 dilution, in PBS-BSA 2.5% for 1nh and washed three times with PBS-BSA 0.1%. After, samples were incubated with DAPI (Thermo Fisher Scientific, Waltham, MA, USA) to stain the nuclei. Samples were observed and imagines were obtained with a Zeiss LSM710 confocal microscope by using Zen 2015 software (Carl Zeiss Microimaging Inc., Thornwood, NY, USA).

References

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2. B. Rasines, J. Sánchez-Nieves, M. Maiolo, M. Maly, L. Chonco, J. L. Jiménez, M. A. Muñoz-Fernández, F. J. de la Mata and R. Gómez, Synthesis, structure and molecular modelling of anionic carbosilane dendrimers, *Dalton Trans.*, **41** (2012) 12733-12748.

Figures

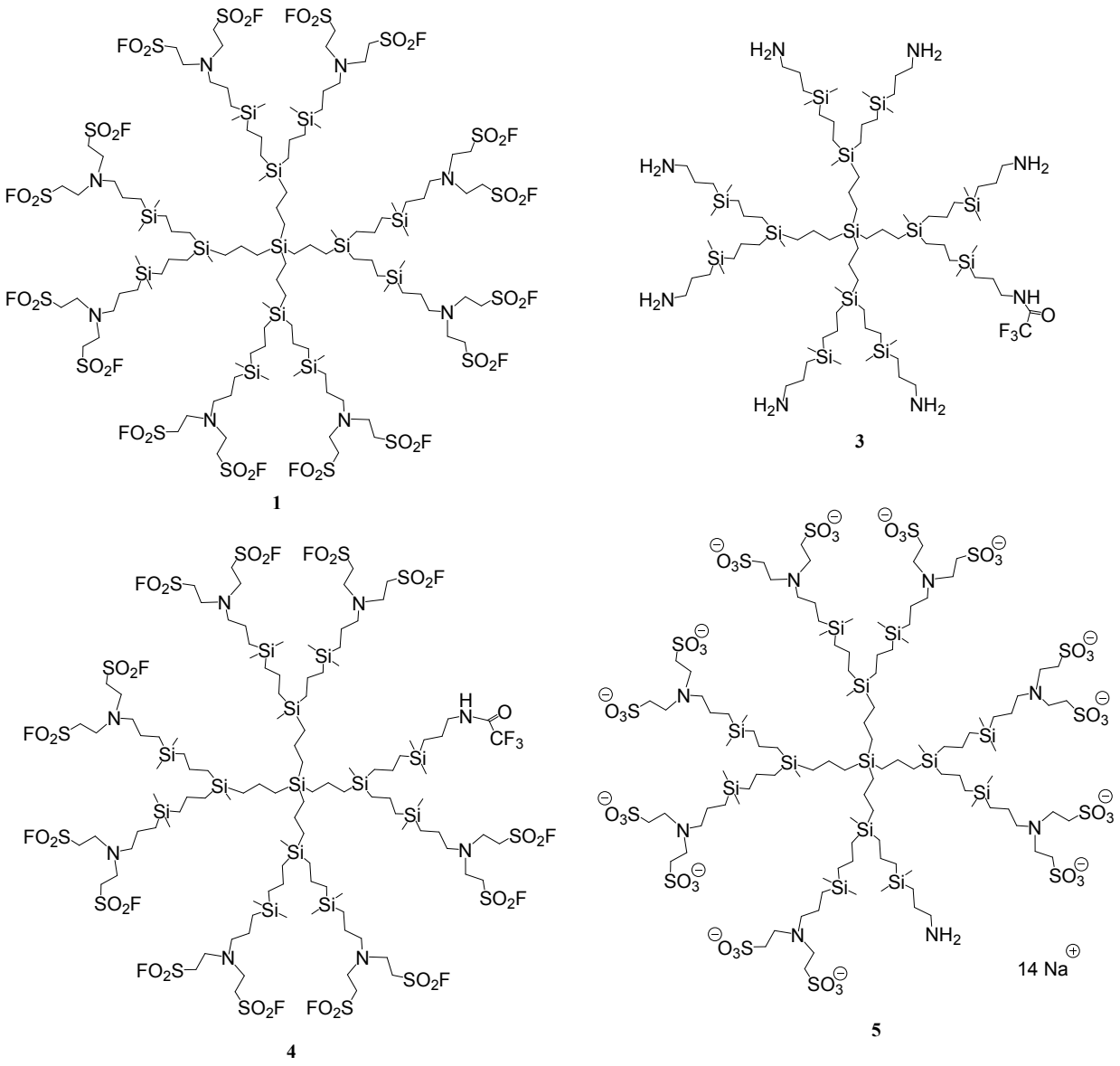

Figure S1. Drawing of dendrimer structures synthesized in this work.

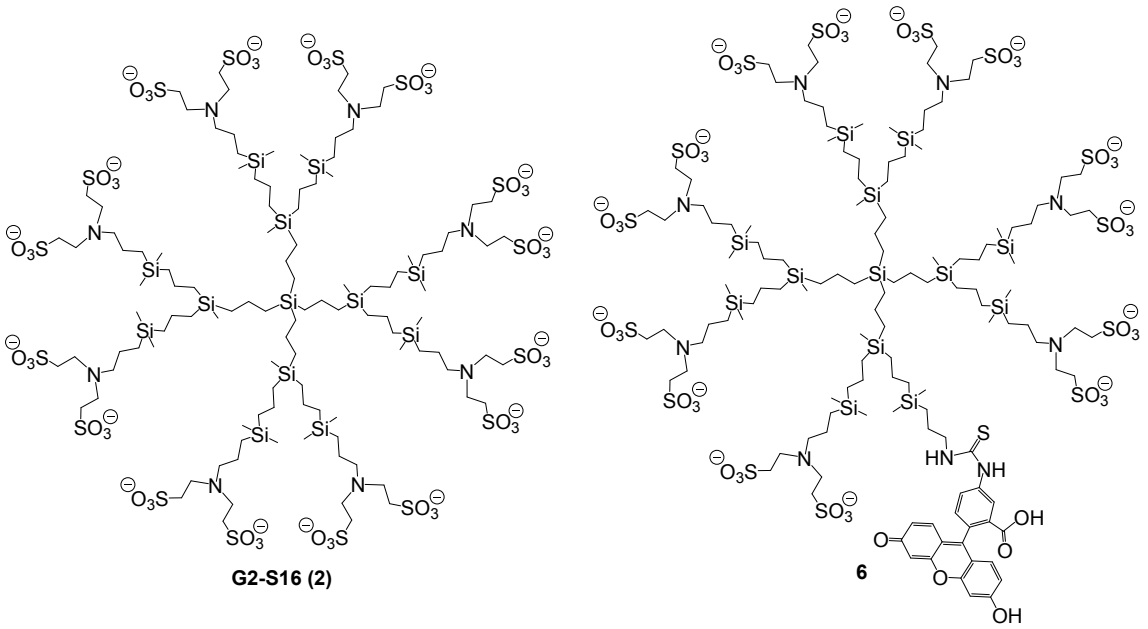

Figure S2. Drawing of anionic sulfonate carbosilane dendrimer G2-S16 (**2**) and corresponding fluorescein-labelled dendrimer G2-S16-FITC (**6**) (sodium cations are omitted for clarity).

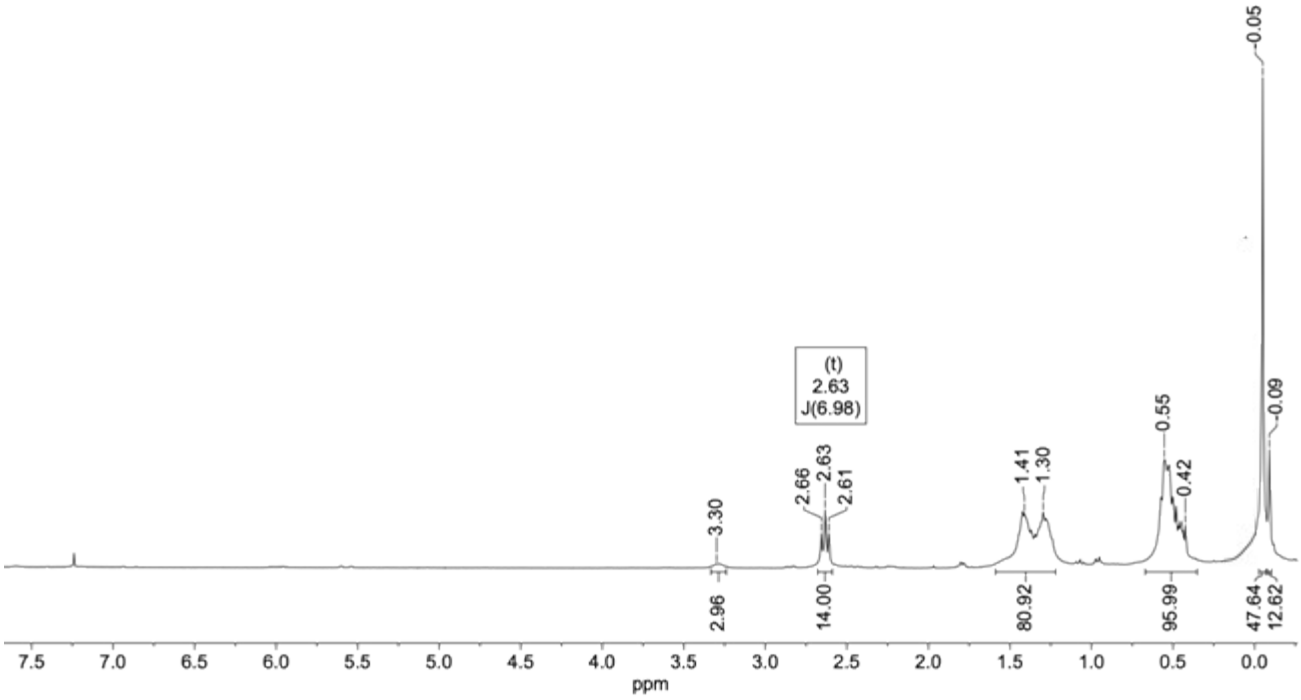

Figure S3. ^{1}H NMR spectrum of $G_2Si(NH_2)_7(NHTF)$ in $CDCl_3$ (**3**).

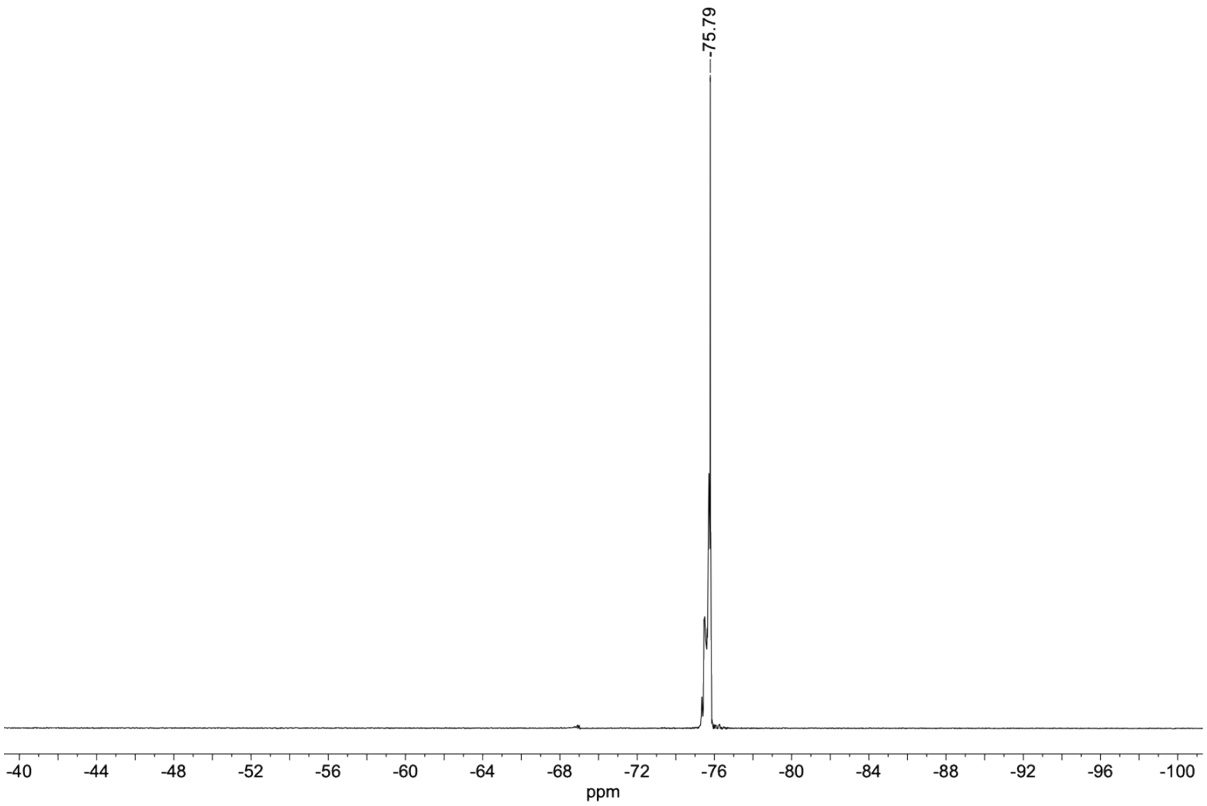

Figure S4. ^{19}F NMR spectrum of $G_2Si(NH_2)_7(NHTF)$ in $CDCl_3$ (**3**).

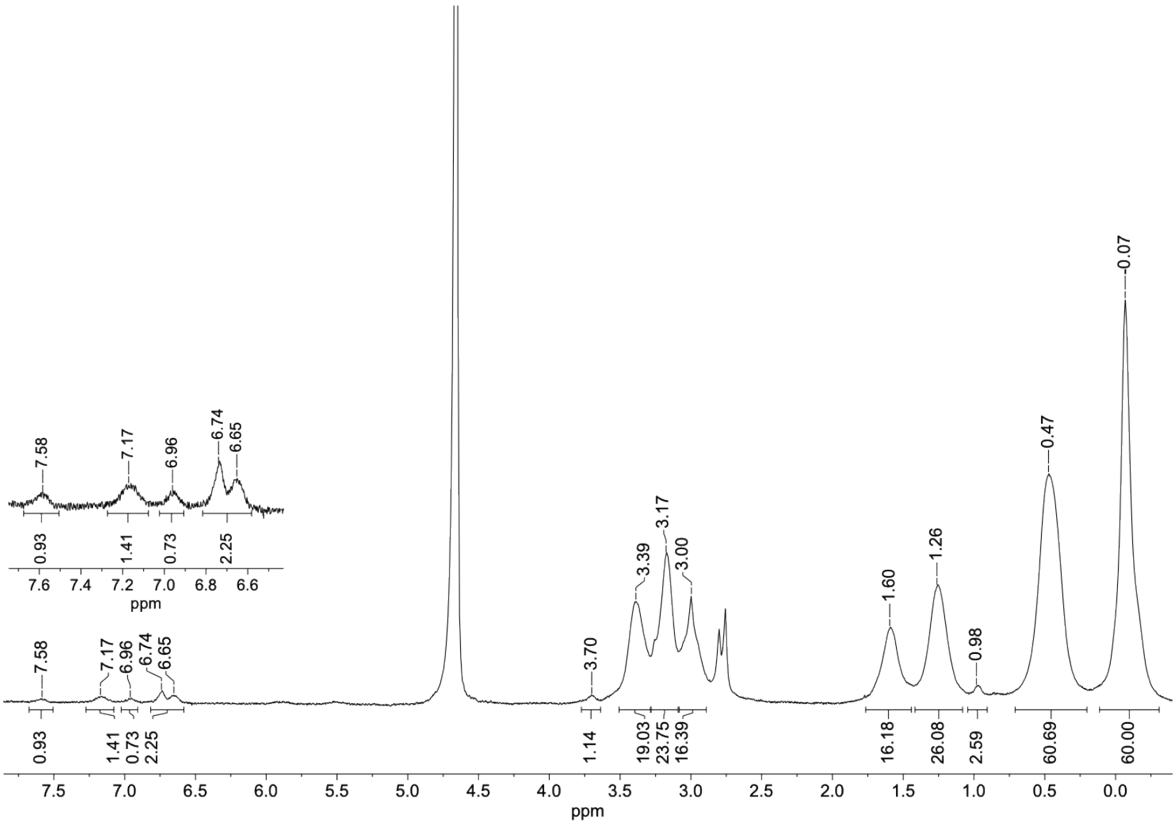

Figure S5. ^{1}H NMR spectrum of G2-S16-FITC in D_2O (**6**).

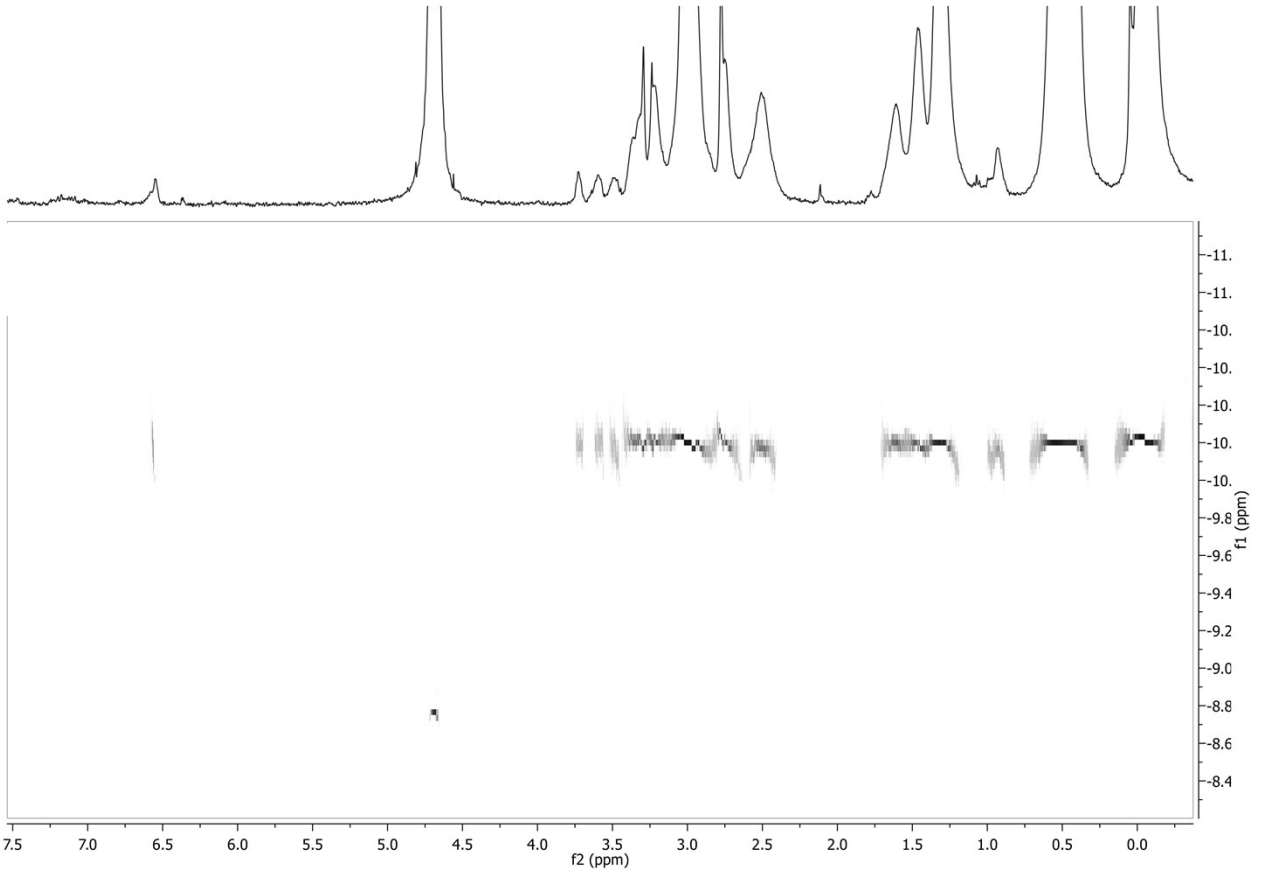

Figure S6. DOSY ^{1}H NMR spectrum of G2-S16-FITC in D_2O (**6**).

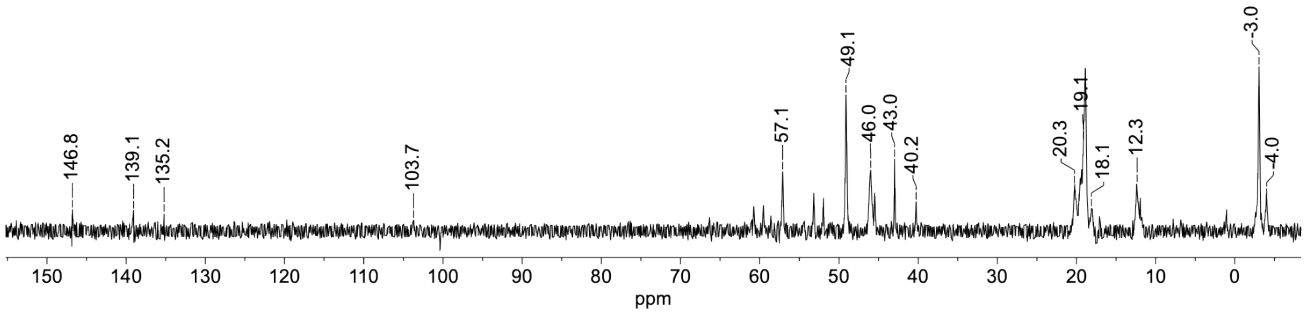

Figure S7. ^{13}C NMR spectrum of G2-S16-FITC in D_2O (**6**).

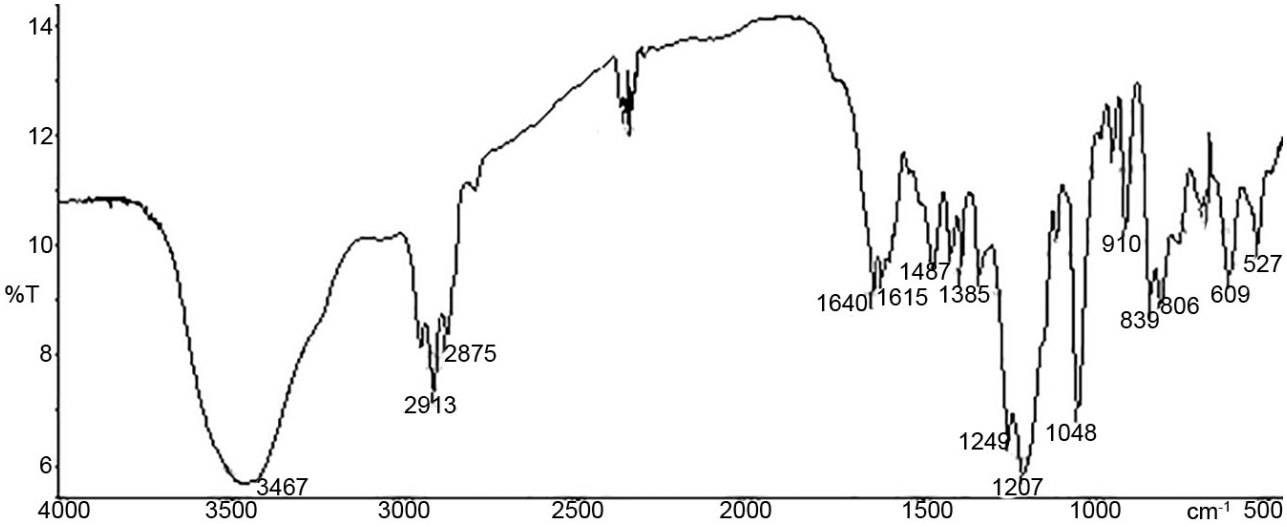

Figure S8. IR spectrum of G2-S16-FITC in KBr (**6**).

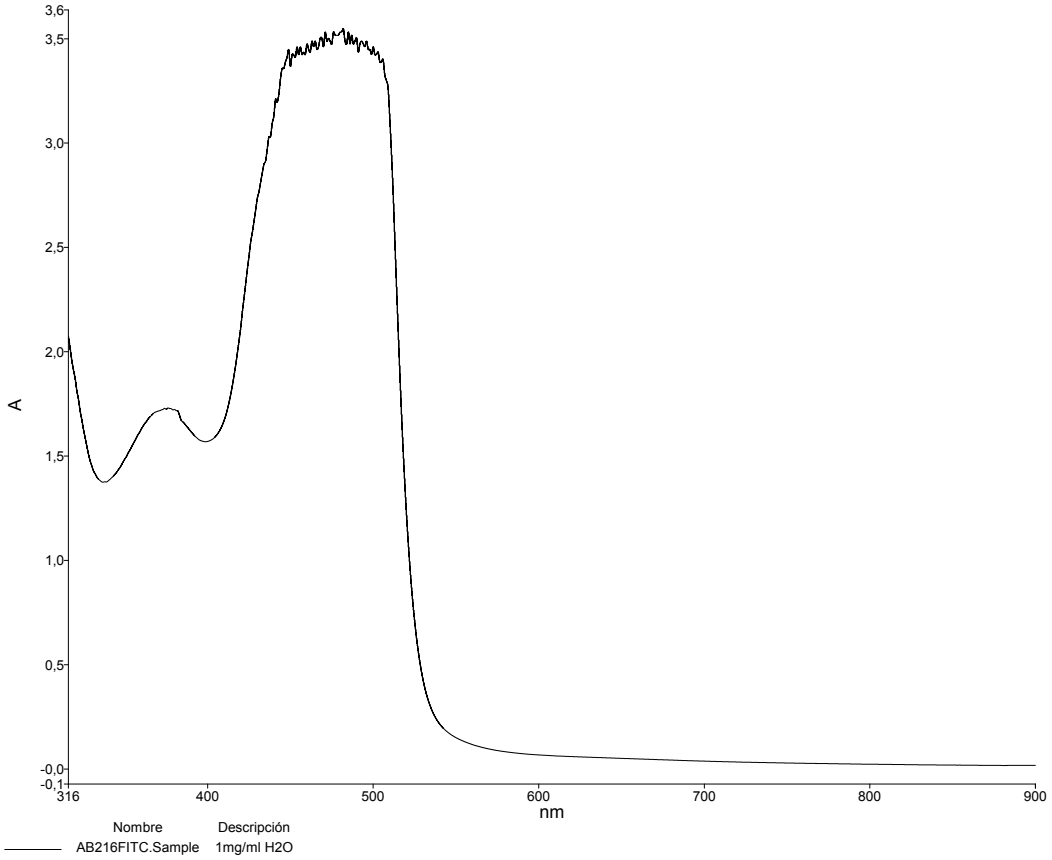

Figure S9. UVspectrum of G2-S16-FITC (**6**) (1 mg/ml in distilled H_2O).

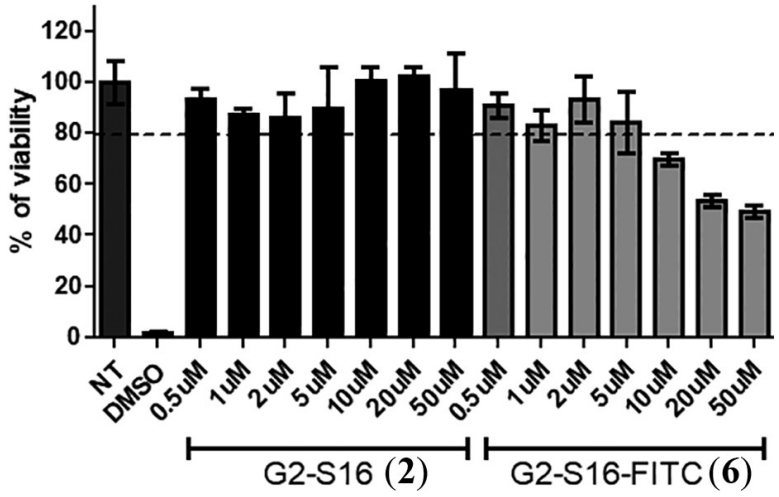

Figure S10. Biocompatibility of polyanionic carbosilane dendrimers in human PBMCs. Viability of human PBMCs was evaluated by MTT assay after 72 h of exposure to a range of G2-S16 (**2**) dendrimer concentrations; 80% of viability was set as the limit of toxicity for both G2-S16 (**2**) and G2-S16-FICT (**6**) carbosilane dendrimers studied. DMSO 10% was used as normal control. Data were represented as mean ± standard deviation of three independent experiments.