Electronic Supplementary Material (ESI) for Nanoscale.


# Electronic Supplementary Information (ESI)

## *New anionic poly(alkylideneamine) dendrimers as a microbicide against HIV-1 infection*

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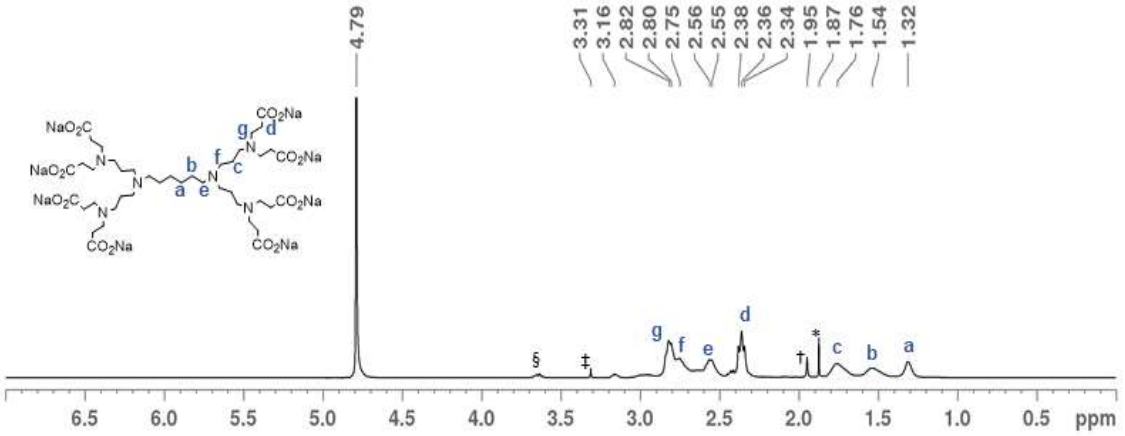


**Fig. S1.** $^1$H-NMR spectra of the carboxylate dendrimer G1C in $D_2O$ (*= $C_4H_8O$, †= $C_4H_8O_2$, ‡= $CH_3OH$, § = $(C_2H_5)_2O$).

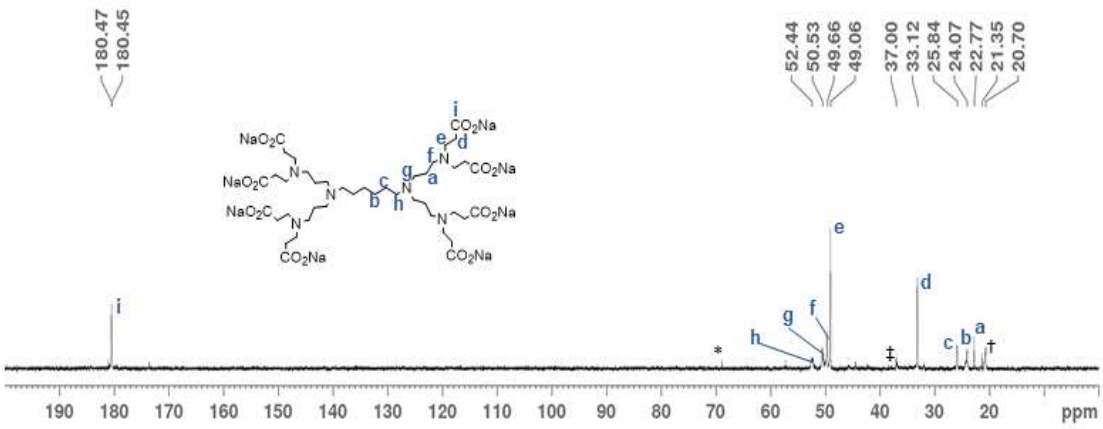


**Fig.S2.** $^{13}$C-NMR spectra of the carboxylate dendrimer G1C in $D_2O$ (†= $C_4H_8O_2$, ‡= $CH_3OH$, *= $C_4H_8O$).

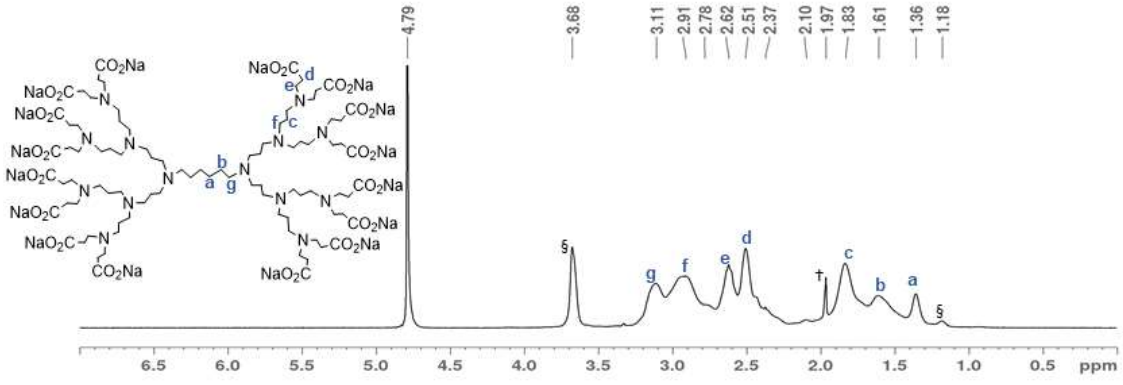


**Fig. S3.** $^1$H-NMR spectra of the carboxylate dendrimer G2C in $D_2O$ (§= $(C_2H_5)_2O$, †= $C_4H_8O_2$).

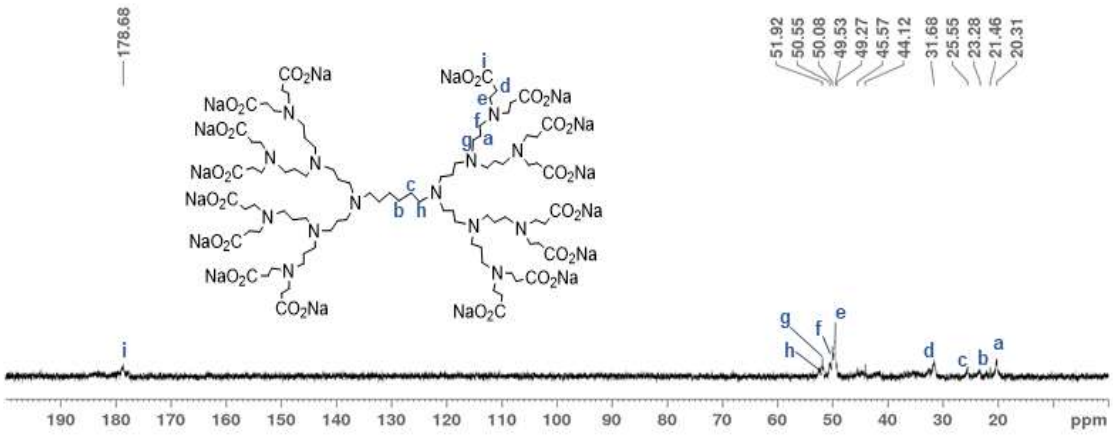


**Fig. S4.** $^{13}$C-NMR spectra of the carboxylate dendrimer G2C in $D_2O$.

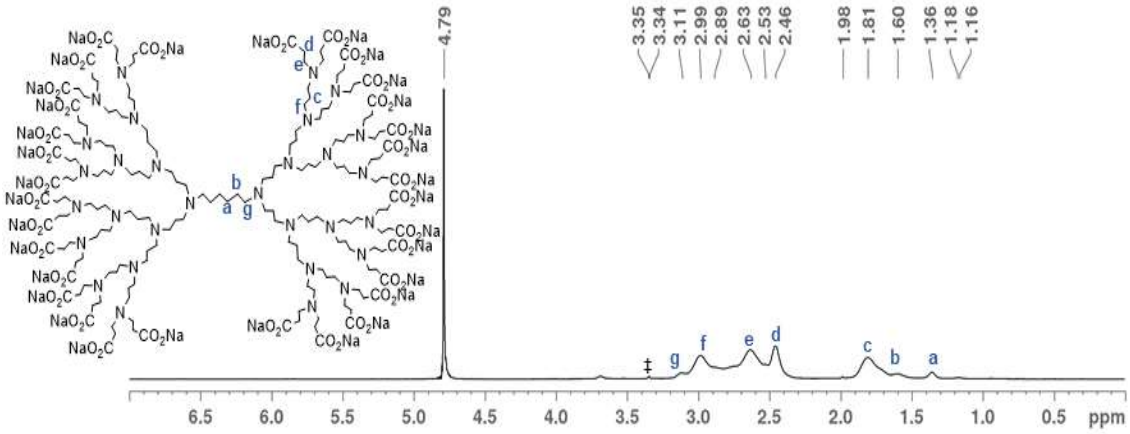


**Fig. S5.** $^{1}$H-NMR spectra of the carboxylate dendrimer G3C in $D_2O$. (‡= $CH_3OH$).

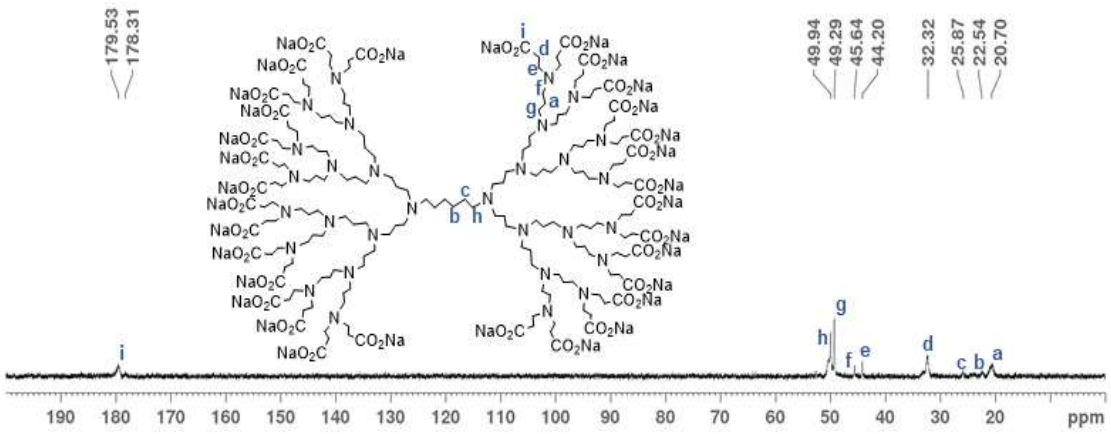


**Fig. S6.** $^{13}$C-NMR spectra of the carboxylate dendrimer G3C in $D_2O$.

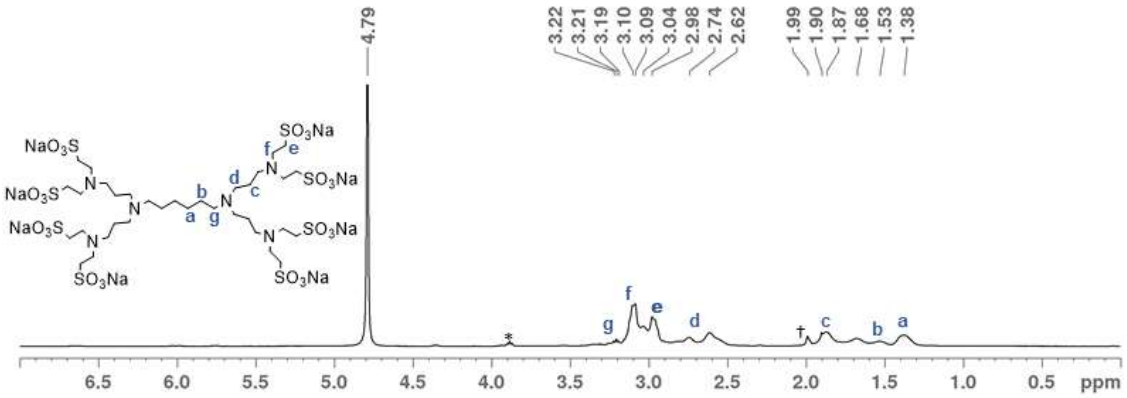


**Fig. S7.** $^{1}$H-NMR spectra of the sulfonate dendrimer G1S in $D_2O$ (†= $C_4H_8O_2$, *= $C_4H_8O$).

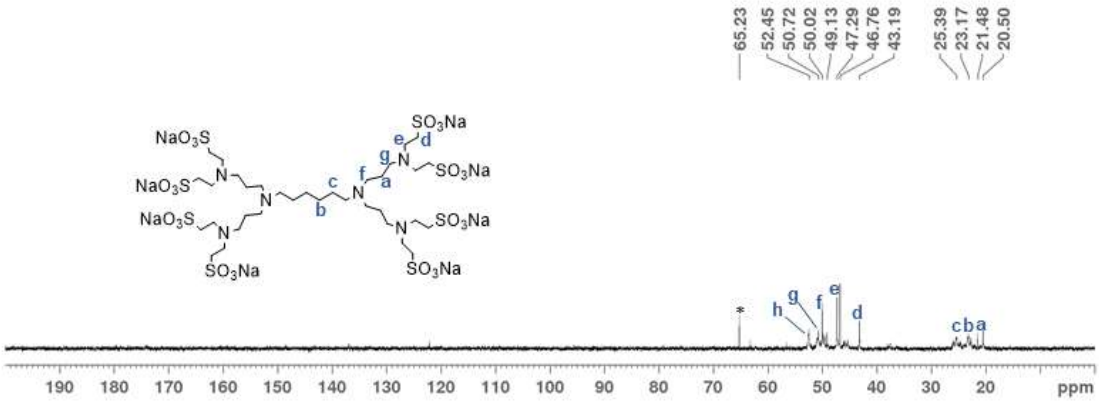


**Fig. S8.** $^{13}$C-NMR spectra of the sulfonate dendrimer G1S in $D_2O$ (*= $C_4H_8O$).

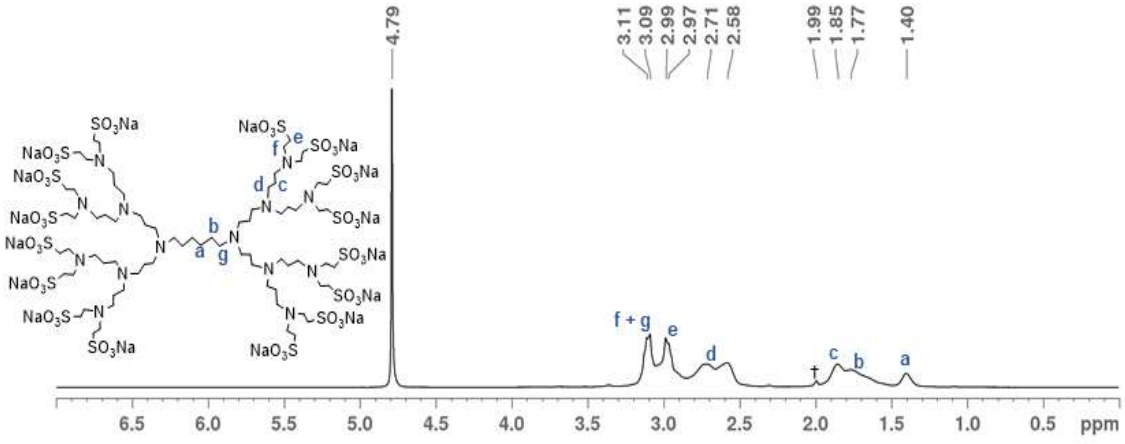


**Fig. S9.** $^{1}$H-NMR spectra of the sulfonate dendrimer G2S in $D_2O$ (†= $C_4H_8O_2$).

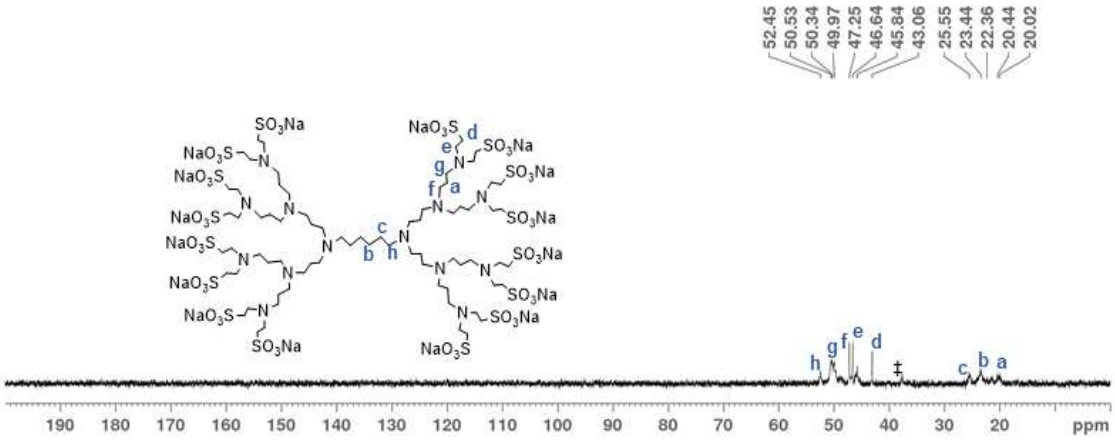


**Fig. S10.** $^{13}$C-NMR spectra of the sulfonate dendrimer G2S in $D_2O$ (‡= $CH_3OH$).

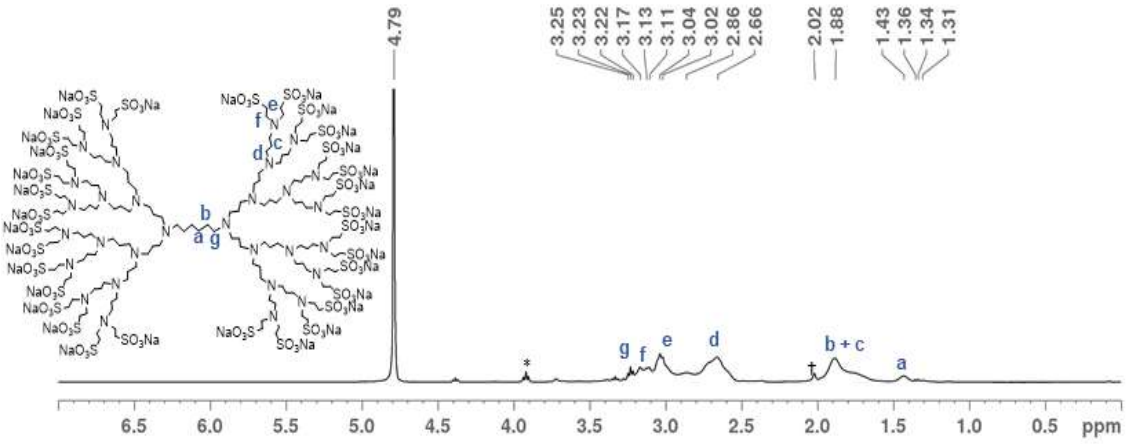


**Fig. S11.** $^{1}$H-NMR spectra of the sulfonate dendrimer G3S in $D_2O$ (†= $C_4H_8O_2$, *= $C_4H_8O$).

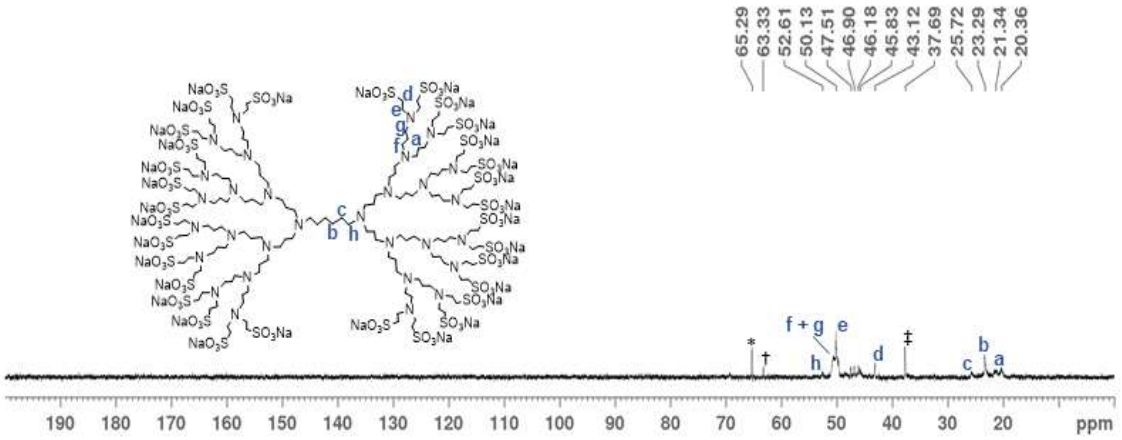


**Fig. S12.** $^{13}$C-NMR spectra of the sulfonate dendrimer G3S in $D_2O$ (‡= $CH_3OH$, †= $C_4H_8O_2$, *= $C_4H_8O$).

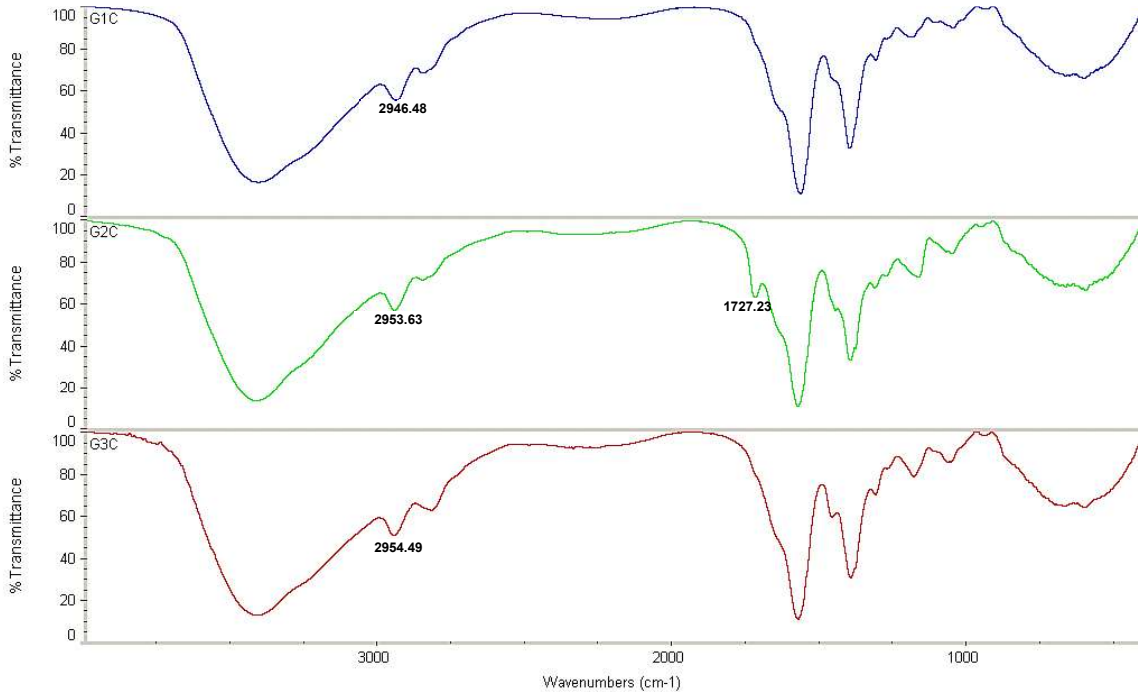


**Fig. S13.** FT-IR spectra of the carboxylate dendrimers from generation 1 to 3, G1C, G2C and G3C. The sample preparation was done using KBr pellets.

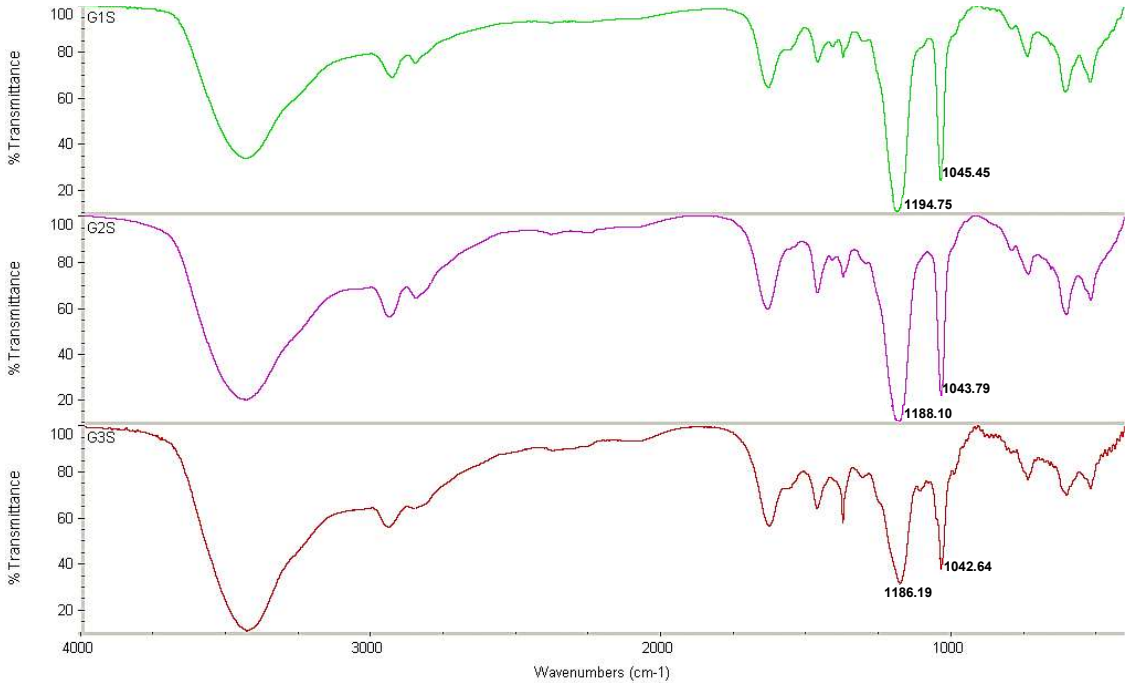


**Fig. S14.** FT-IR spectra of the sulfonate dendrimers from generation 1 to 3, G1S, G2S and G3S. The sample preparation was done using KBr pellets.

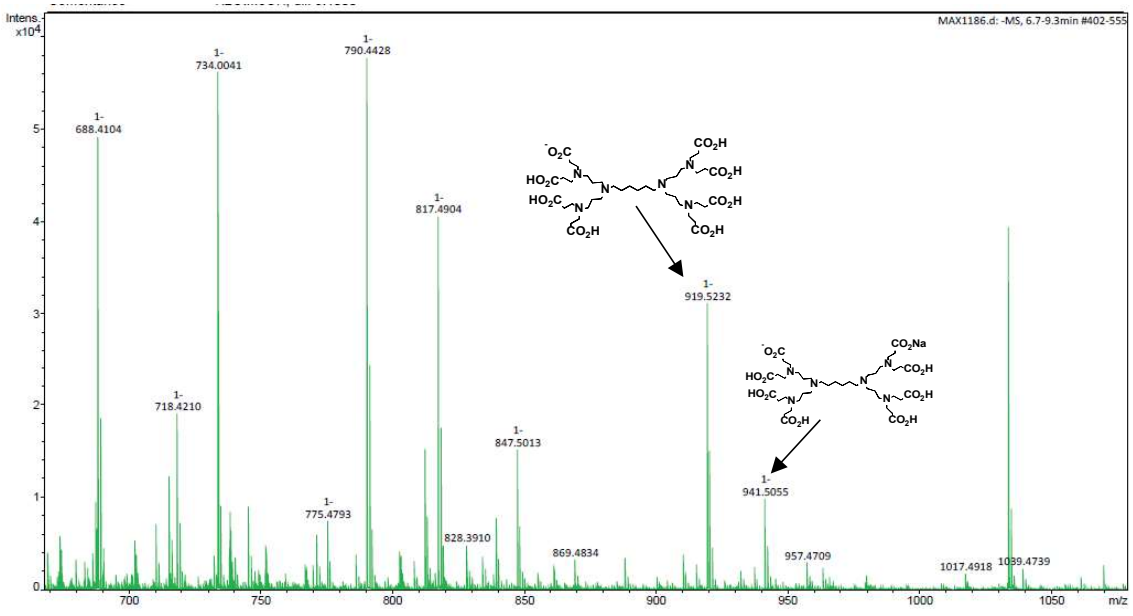


**Fig. S15. HRMS Q-TOF (ESI-)** mass spectrum of the G1C dendrimer.

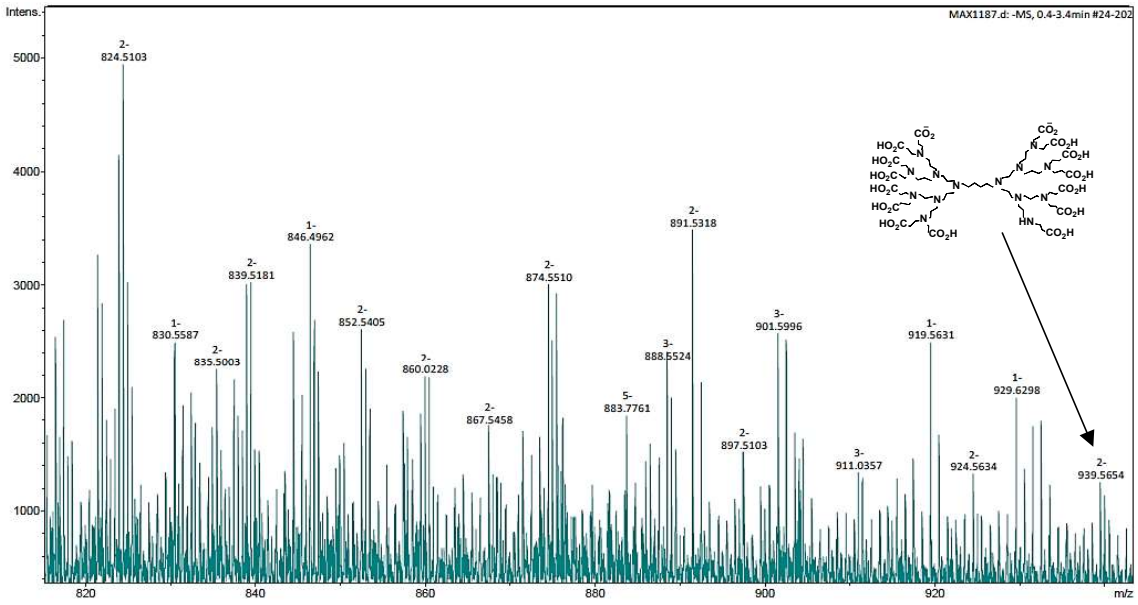


**Fig. S16. HRMS Q-TOF (ESI$^-$)** mass spectrum of the G2C dendrimer.

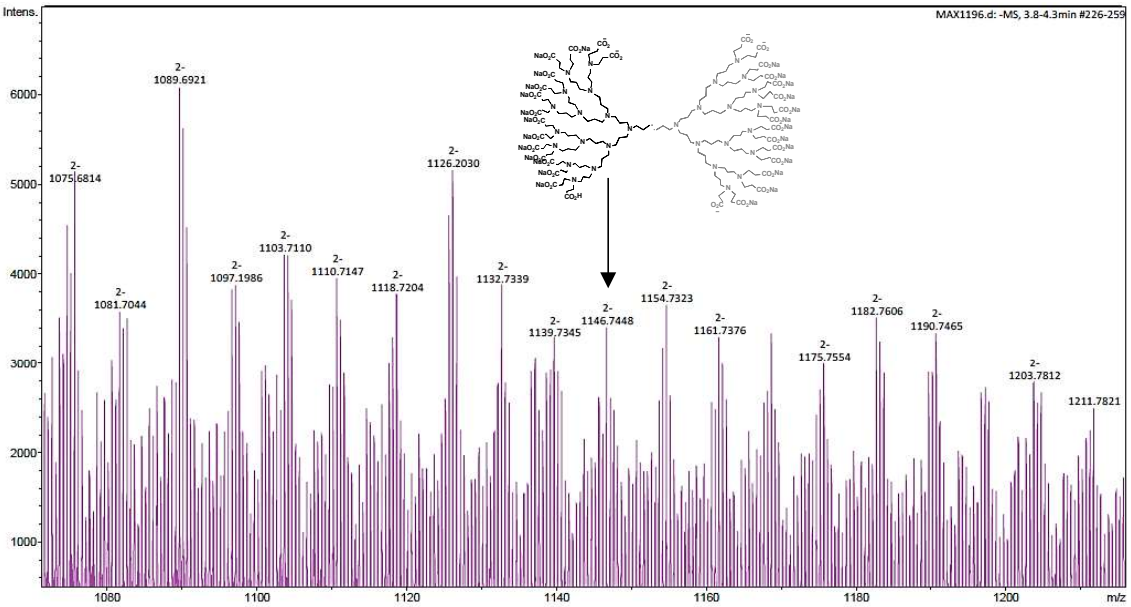


**Fig. S17. HRMS Q-TOF (ESI$^-$)** mass spectrum of the G3C dendrimer.

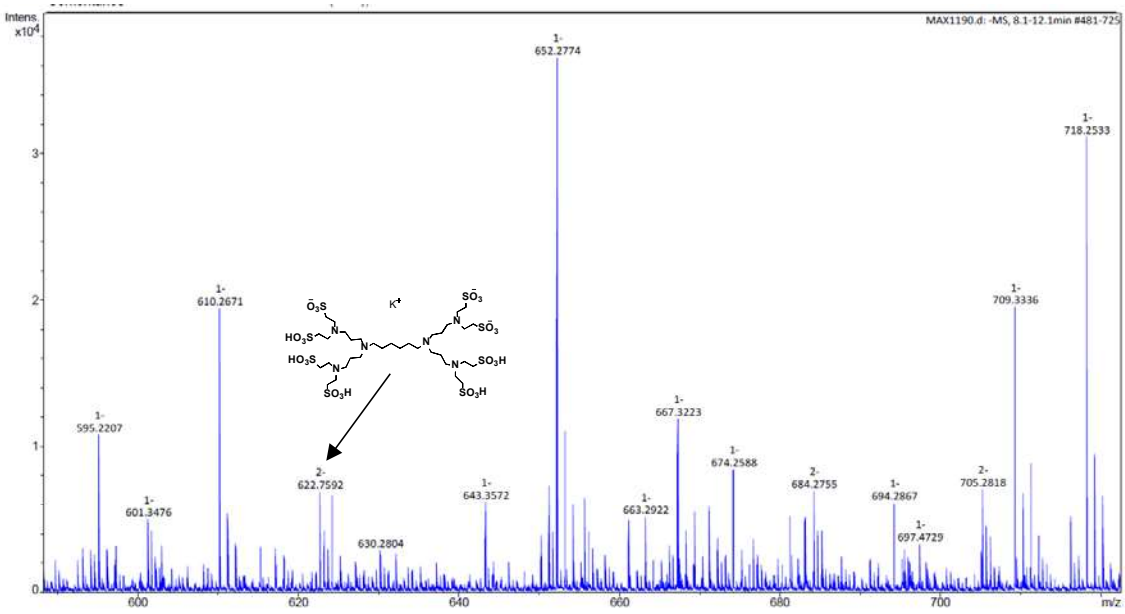


**Fig. S18. HRMS Q-TOF (ESI$^-$)** mass spectrum of the G1S dendrimer.

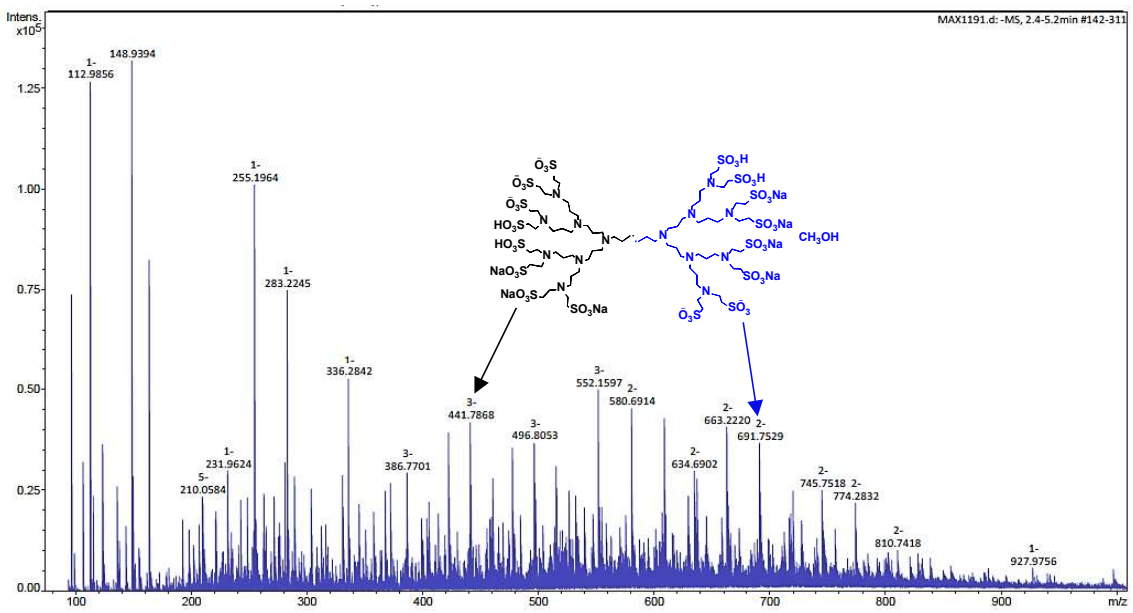


**Fig. S19. HRMS Q-TOF (ESI$^-$)** mass spectrum of the G2S dendrimer.

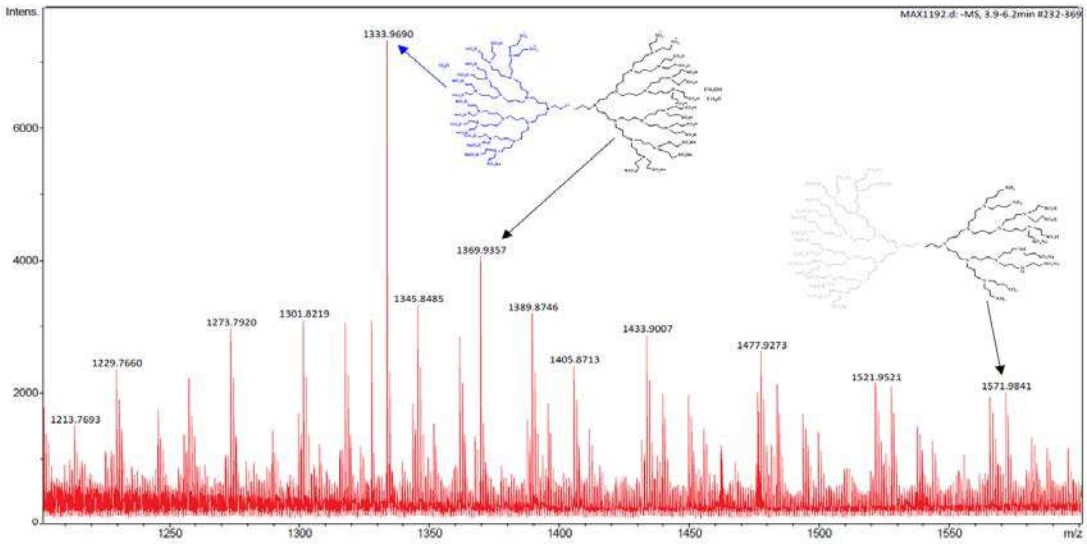


**Fig. S20. HRMS Q-TOF (ESI$^-$)** mass spectrum of the G3S dendrimer.

**Table S1.** Molecular weight of the carboxylate and sulfonate dendrimers.

| | G1C | G2C | G3C | G1S | G2S | G3S |
|---|---|---|---|---|---|---|
| **Molecular weight** | 1096.95 | 2306.08 | 4724.33 | 1385.33 | 2882.84 | 5877.86 |
| ***m/z* calculated** | 941.50<br>919.52 | 939.56 | 1146.98 | 622.10 | 441.74<br>691.13 | 1333.31<br>1369.32<br>1571.76 |
| ***m/z* found** | 941.51<br>$[M - 7Na + 6H]^{-1}$<br>919.52<br>$[M - 8Na + 7H]^{-1}$ | 939.57<br>$[M - C_3H_5O_2 - 16Na + 13H]^{-2}$ | 1146.74<br>$[M - C_{93}H_{154}N_{15}Na_{16}O_3 - 3Na + 1H]^{-2}$ | 622.76<br>$[M - 8Na + 5H + K]^{-2}$ | 441.79<br>$[M - C_{37}H_{74}N_7Na_8O_{24}S_8 - 5Na + 2H]^{-3}$<br>691.75<br>$[M - C_{37}H_{74}N_7Na_8O_{24}S_8 - 4Na + 2H + CH_3OH]^{-2}$ | 1333.97<br>$[M - C_{77}H_{154}N_{15}Na_{16}O_{48}S_{16} - 12Na + 10H + CH_3OH + 2H_2O]^{-2}$<br>1369.93<br>$[M - C_{77}H_{154}N_{15}Na_{16}O_{48}S_{16} - 13Na + 11H + H_2O]^{-2}$<br>1571.98<br>$[M - C_{77}H_{154}N_{15}Na_{16}O_{48}S_{16} - 8(C_2H_4NaO_3S) - 3Na + 5H]$ |

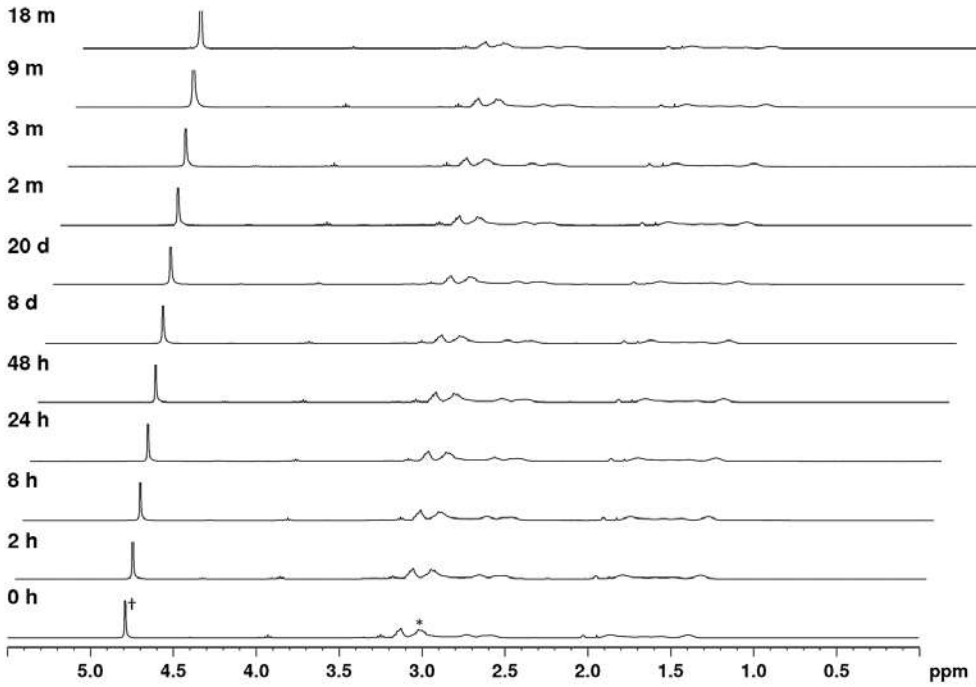


**Fig. S21.** $^{1}$H-NMR spectra of the anionic dendrimer with 8 sulfonate terminal groups (G1S). Stability studies were performed in $D_2O$ (probe temperature: 25 °C), and the dendrimer's solutions were kept at 4 °C a long time (until one year and half (18 months)). Abbreviations: h = hours, d = days, m = months, † = solvent peak, * = sulfonate terminal group ($–CH_2SO_3^-$).

### Zeta potential measurements with the carboxylate and sulfonate dendrimers

The zeta potential of carboxylate and sulfonate dendrimers was measured in nuclease-free water at a concentration of 1 mg/mL. As expected, G1C, G2C, and G3C carboxylate dendrimers displayed a negative charge surface of –31.9 mV, –43.5 mV, and –50.4 mV, respectively. The zeta potential of the G1S and G2S sulfonate dendrimers was negative too, presenting values of –45.1 mV and –44.3 mV, respectively. The positive charge of the G3S dendrimer (19.5 mV) suggests that the dendrimer could be only partially functionalized with the sulfonate groups, as it can be confirmed by the MS data where a half-molecule was found partially functionalized.

**Table S2.** Zeta potential data of the carboxylate and sulfonate dendrimers.

| | G1C | G2C | G3C | G1S | G2S | G3S |
|---|---|---|---|---|---|---|
| **Zeta potential (mV)[a]** | –31.9 ± 1.2 | –43.5 ± 1.4 | –50.4 ± 2.9 | –45.1 ± 4.4 | –44.3 ± 2.8 | 19.5 ± 1.2 |

[a] Data represent the mean ± SD of three independent experiments.

**Time-of-addition experiment**

TZM.bl cells were infected with R5-HIV-$1_{NLAD8}$ (15 ng/$10^6$ cells) isolate for 2 h at 37ºC. Dendrimers (25 μM), and the controls, T-20 (10 μM), TDF (1 μM) and RAL (1 μM), were added at different time points post-infection. HIV-1 infection was quantified after 48h by measuring luciferase activity.

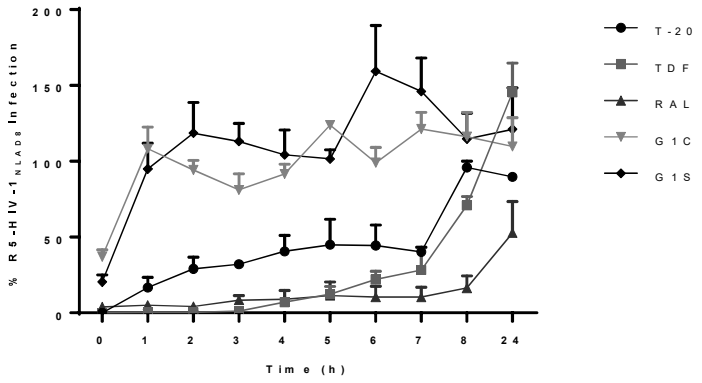


**Fig. S22. Time-of-addition experiment in the HIV-1 viral cycle.** TZM.bl cells were infected with R5-HIV-$1_{NLAD8}$ isolate for 2 h. G1C and G1S dendrimers (25 μM), T-20 (20 μM), TDF (1 μM) or RAL (1 μM) were added at various time points. Data represent the mean ± SD of three independent experiments performed in triplicate. Abbreviations: T-20 = enfuvirtide; TDF = Tenofovir Disoproxil Fumarate; RAL = Raltegravir.

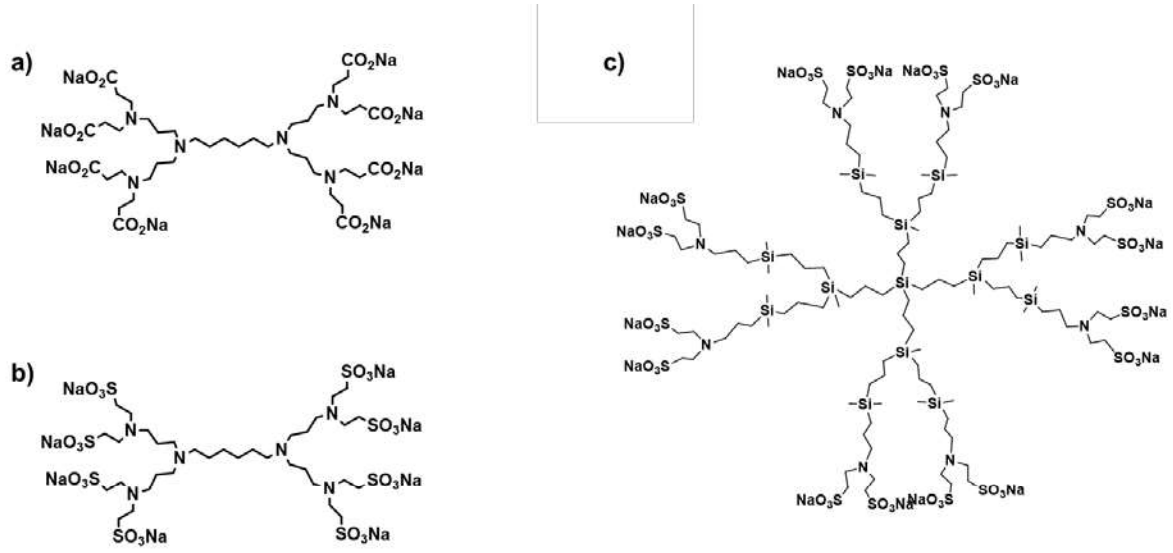


**Fig. S23.** Structure of G1C (a) and G1S (b) dendrimers and the reported G2-S16 dendrimer.[1,2]

## References


1 B. Rasines, J. Sánchez-Nieves, M. Maiolo, M. Maly, L. Chonco, J. L. Jiménez, M. Á. Muñoz-Fernández, F. J. de la Mata and R. Gómez, *Dalton Trans*, 2012, **41**, 12733.

2 L. Chonco, M. Pion, E. Vacas, B. Rasines, M. Maly, M. J. Serramía, L. López-Fernández, J. De La Mata, S. Alvarez, R. Gómez and M. A. Muñoz-Fernández, *J Control Release*, 2012, **161**, 949–958.