Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Biocompatible, multifunctional, and well-defined OEG-based dendritic platforms for biomedical applications

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1. Dendrons' purification and characterization

Flash chromatography was done using silica gel 60 (35-70 µm) from SDS or basic aluminium oxide (pH 10) from Sigma Aldrich. TLC was performed on Merck $60F_{254}$ silica foils or on Fluka aluminium oxide foils and visualized by potassium permanganate stains. Semi-preparative HPLC was carried out on a Waters chromatography system (2767 Sample Manager) with a 2489 UV/visible detector and ZQ 4000 mass detector. A reverse phase XBridge C_{18} column (5 µm, 19x100 mm) from Waters and linear gradients of CH_3CN into NH_4HCO_3 20 mM were used. The system was run at a flow rate of 16 mL/min over 5 min. For salts elimination, the samples were purified using reverse phase column (PoraPak Rxn from Waters) using a solution of 20% of CH_3CN in water as eluent. Analytical HPLC was performed using a Waters chromatography system (2695 Separation Module) with a 2998 photodiode array detector. The samples were run using a reverse phase XBridge BEH130 C_{18} column (3.5 µm, 4.6x100 mm) from Waters and linear gradients of CH_3CN with 0.036% trifluoroacetic acid (TFA) into H_2O with 0.045 % of TFA. The flow rate used was 1 mL/min over 8 min. HPLC-MS was performed using a Waters instrument (2795 Separations Module) with a 2996 photodiode array detector and ZQ 4000 mass detector; a reverse phase XBridge C_{18} column (3.5 µm, 4.6x50 mm) from Waters and linear gradients of CH_3CN with 0.07% formic acid into H_2O with 0.1% of formic acid. The system was run at a flow rate of 2 mL/min over 4.5 min. For electrospray high resolution mass spectrometry (HRMS), the samples were dissolved in CH_3CN: H_2O (1:10), introduced in an Automated Nanoeslectrospray (NanoMate, Advion BioSciences, Ithaca, NY, USA) and infused through LTQ-FT ultra mass spectrometer (Thermo Scientific). Mass spectra were also recorded on a MALDI Voyager DE RP time-of-flight (TOF) spectrometer (Applied Biosystems, Foster City, CA, USA) using α-cyano-4-hydroxycinnamic acid matrix (ACH, from Sigma Aldrich) and sinapinic acid matrix (from Fluka). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer and a Varian Inova 500 MHz spectrometer. Multiplicity of the carbons was assigned with gHSQC experiments. Standard abbreviations according to off-resonance decoupling are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, (bs) broad signal. The ^{13}C data is reported as the ppm on the δ scale.

Chemical shifts are reported in ppm and referenced to appropriate residual solvent peaks: proton ($CDCl_3$ 7.26 ppm, D_2O 4.70) and carbon ($CDCl_3$ 77.0 ppm).

2. Dynamic Light Scattering and ζ–Potential

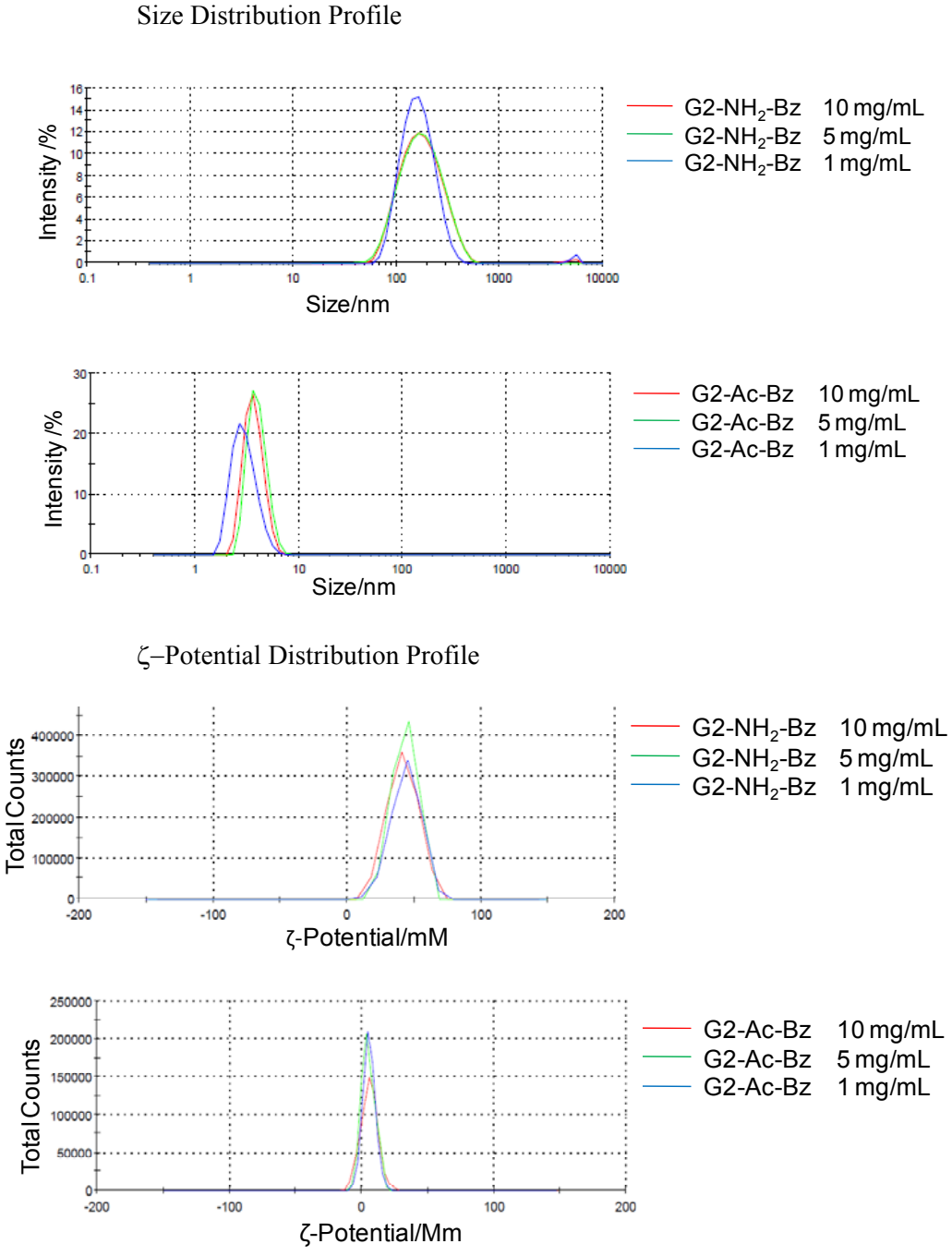


Figure 1. Size distribution profiles by intensity and ζ-Potential distribution profiles of the second generation dendrons at room temperature in PBS (0.01 M phosphate buffer, 0.154 M NaCl) measured by DLS.

3. RBC's morphology

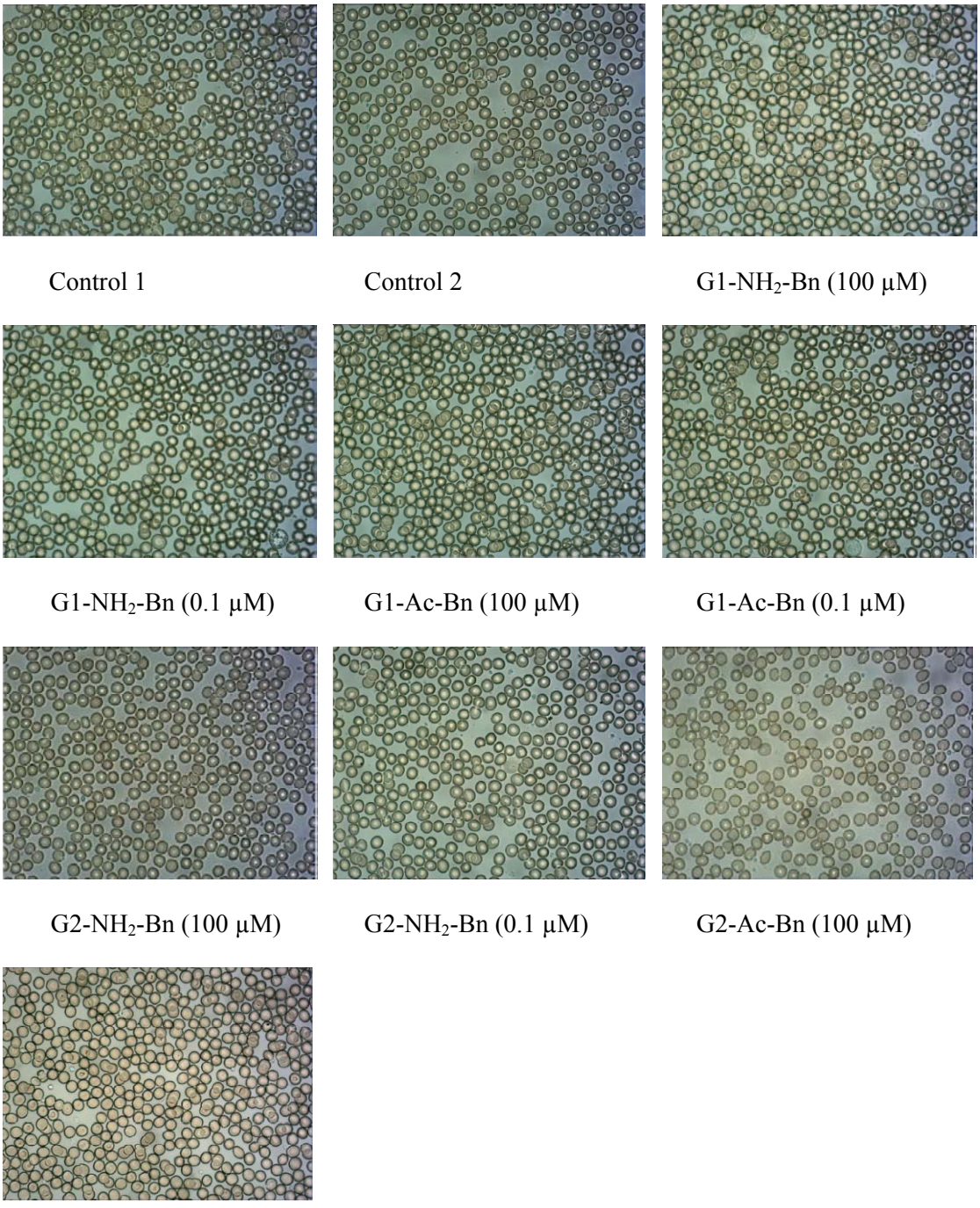


Figure 2. Micrographs of blood smear after incubation at 37 °C with the different dendrons during 15 min. The pictures were taken at 50x using an inverse phase microscopy Olympus Provis equipped with a digital camera VisiCam of 5 mega pixels (VWR International). Control 1: blood not exposed to dendrons and not incubated. Control 2: blood incubated but not exposed to dendrons.

4. Counting and size distribution of RBC, platelets and white blood cells

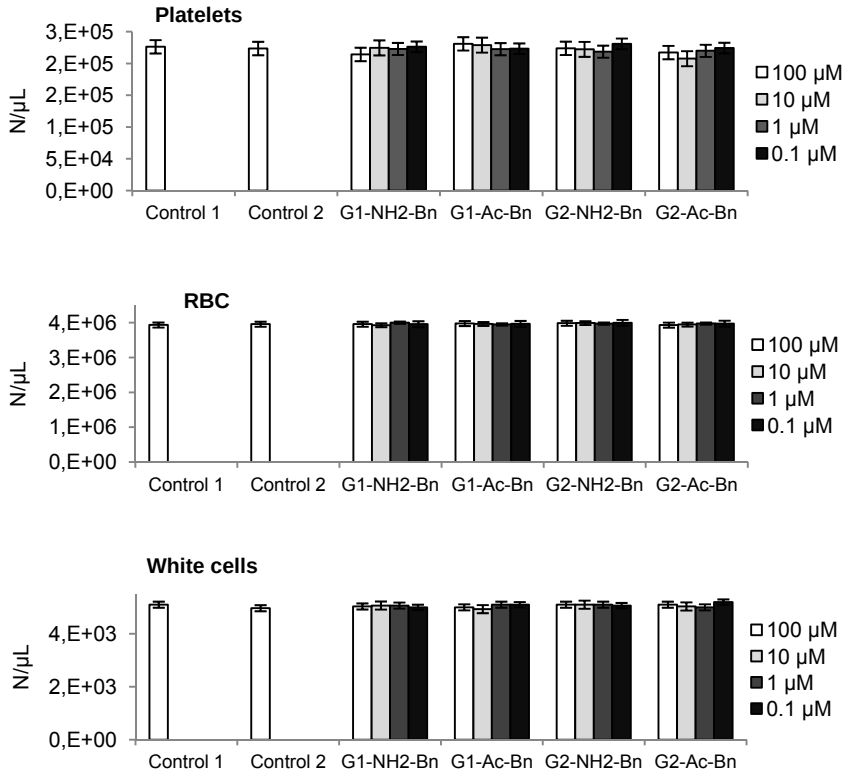


Figure 3. Counting of platelets, RBCs and white blood cells after incubation at 37 °C of the different dendrons during 15 min. Results are expressed in number of cells per µL of blood Control 1: blood not exposed to dendrons and not incubated. Control 2: blood incubated but not exposed to dendrons.

5. HPLC, MALDI-TOF spectrometry and NMR spectrometry of the tested dendrons

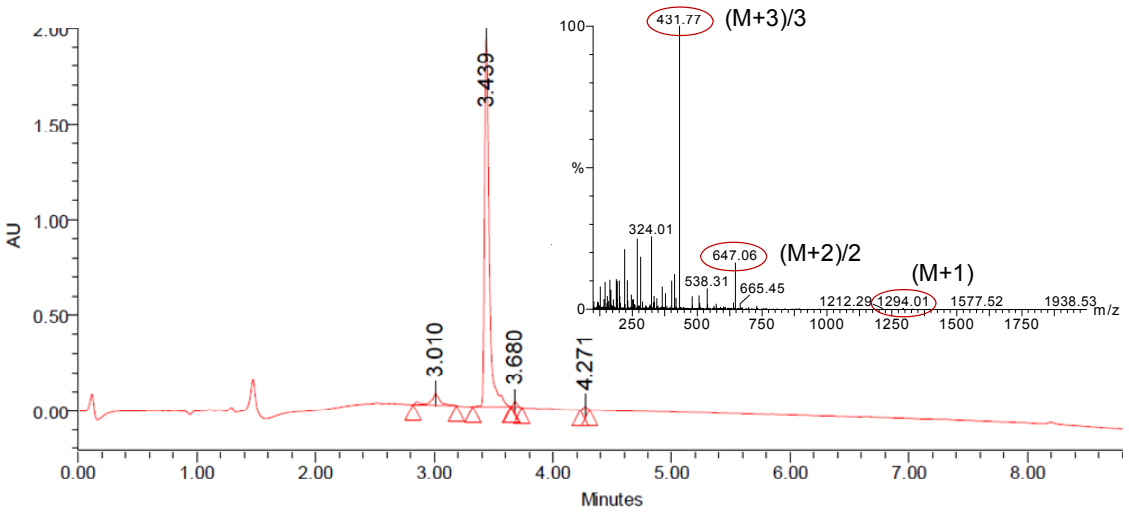


Figure 4. HPLC-MS profile of dendron **7** (**G1-NH$_2$-Bn**). Analytical HPLC: 5→100% of CH_3CN in H_2O over 8 min, t_R=3.44 min (92% at λ=210 nm).

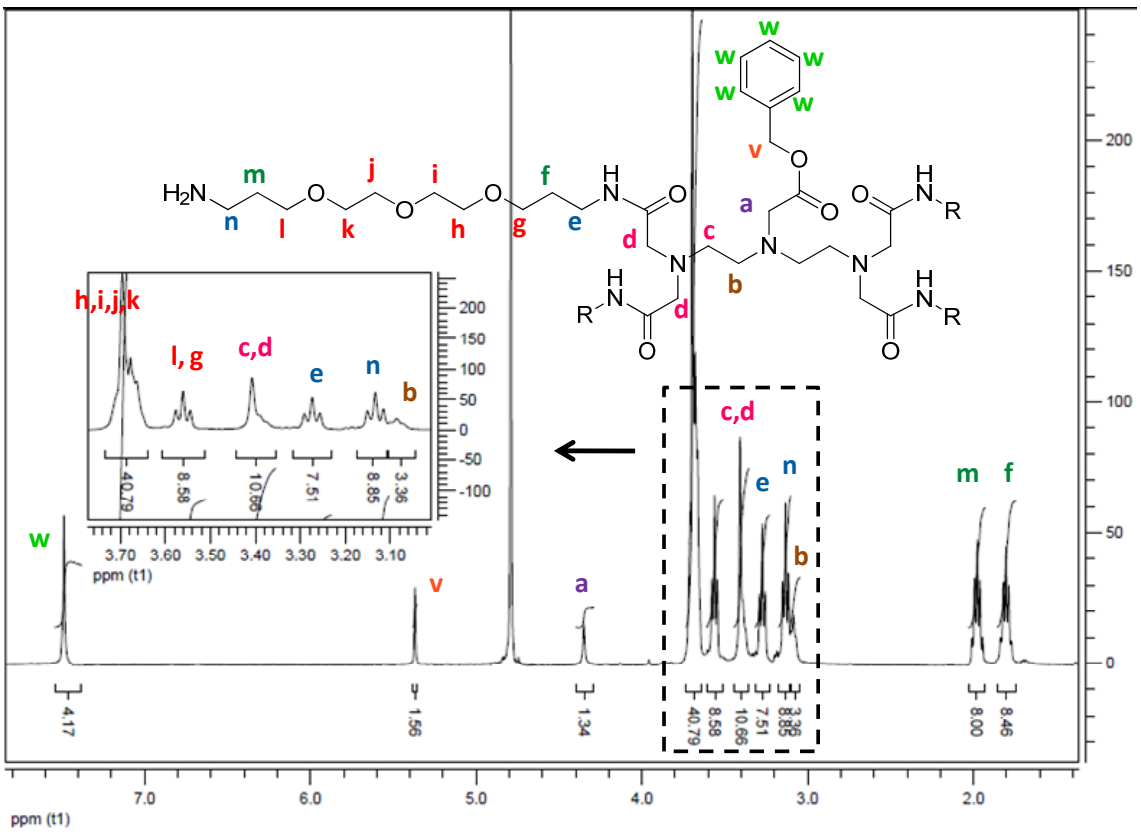
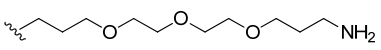


Figure 5. ^{1}H NMR spectrometry of first generation dendron **7** (**G1-NH$_2$-Bn**).

R represents: 

The signals assignation is equivalent for all branches.

^{1}H NMR (400 MHz, D_2O, 25 °C): δ=1.80 (m, 8 H), 1.98 (m, 8 H), 3.09 (m, 4 H), 3.13 (t, ^{3}J(H,H)=7.07 Hz, 8 H), 3.27 (t, ^{3}J(H,H)=6.90 Hz, 8 H), 3.41 (m, 12 H), 3.56 (t, ^{3}J(H,H)=6.41 Hz, 8 H), 3.69 (m, 40 H), 4.35 (s, 2 H), 5.37 (s, 2 H), and 7.49 (m, 5 H).

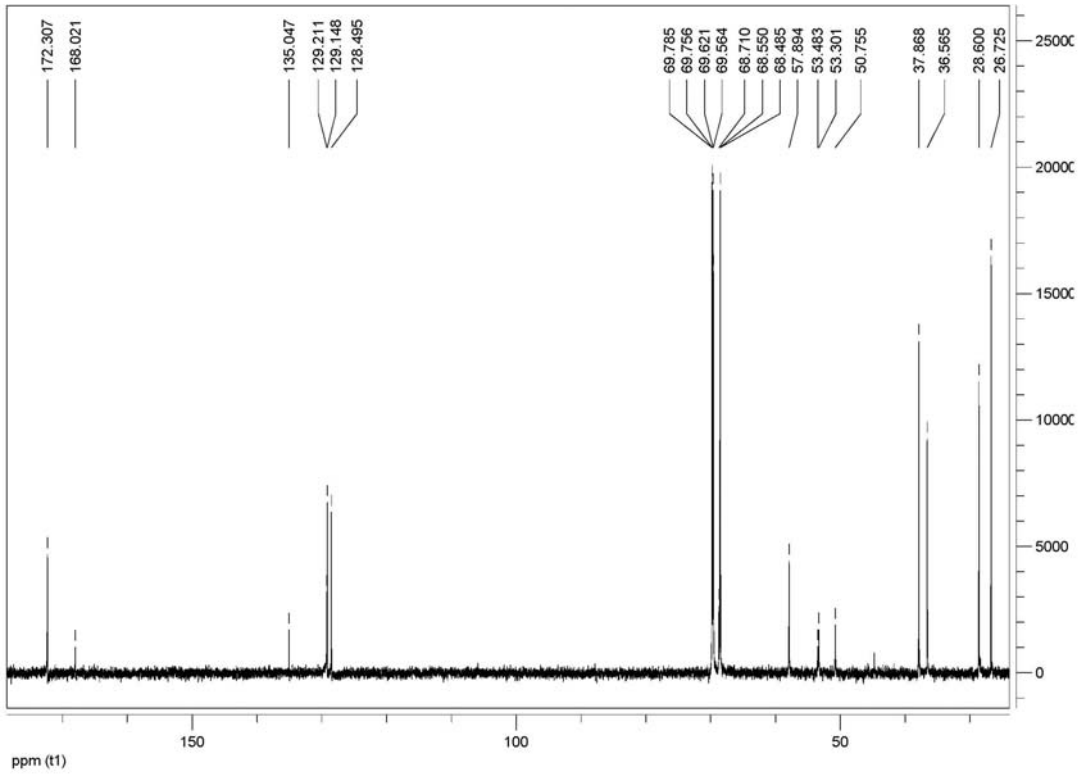


Figure 6. ^{13}C NMR spectrometry of first generation dendron **7** (**G1-NH_2-Bn**).

^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ= 26.73, 28.60, 36.57, 37.87, 50.76, 53.30, 53.48, 57.89, 68.49, 68.55, 68.71, 69.56, 69.62, 69.76, 69.79, 128.49, 129.14, 129.21, 135.05, 168.02, and 172.31.

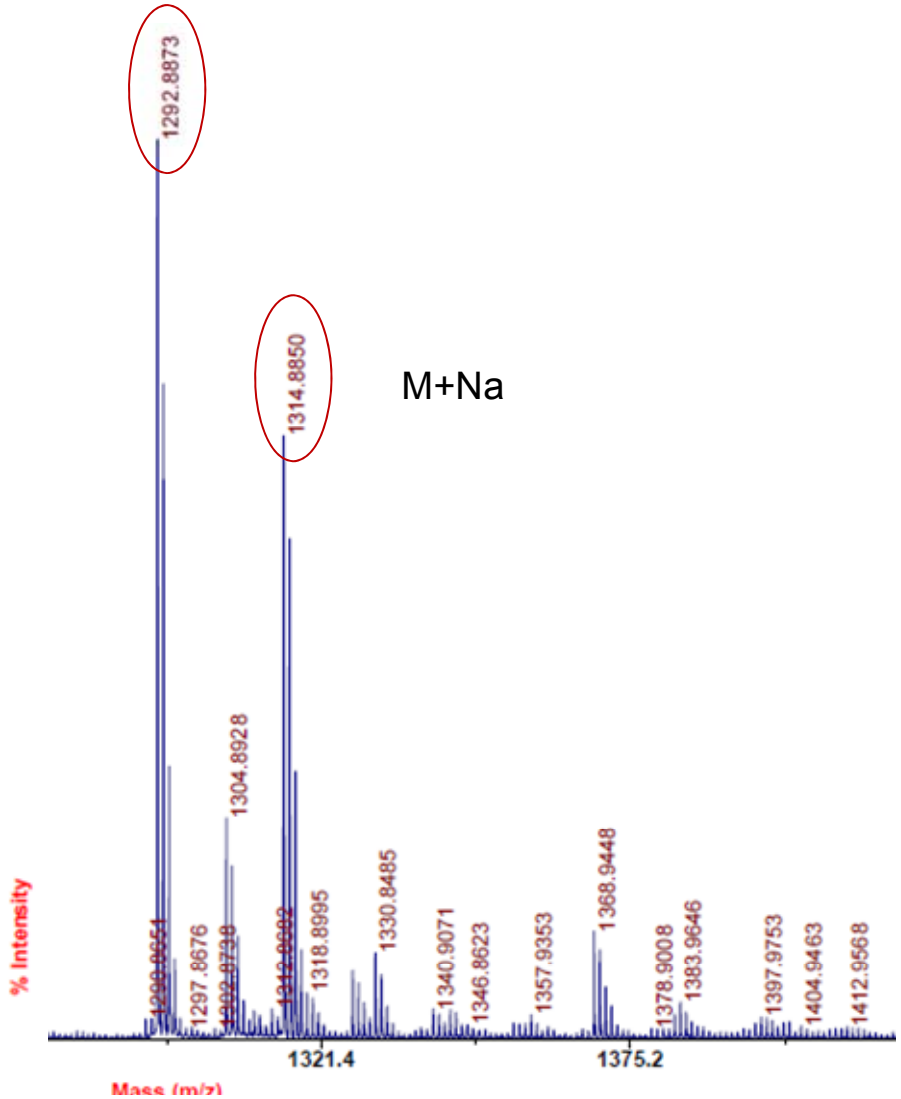


Figure 7. MALDI-TOF spectrometry of first generation dendron **7** (**G1-NH$_2$-Bn**).

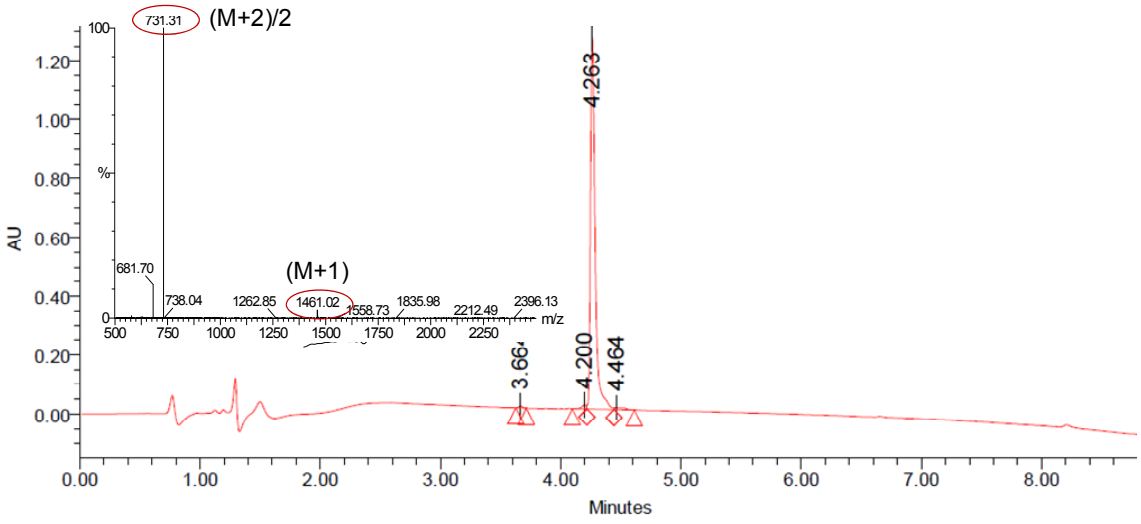


Figure 8. HPLC-MS profile of dendron **8** (**G1-Ac-Bn**). Analytical HPLC: 5→100% of CH_3CN in H_2O over 8 min, t_R=4.26 min (97% at λ=210 nm).

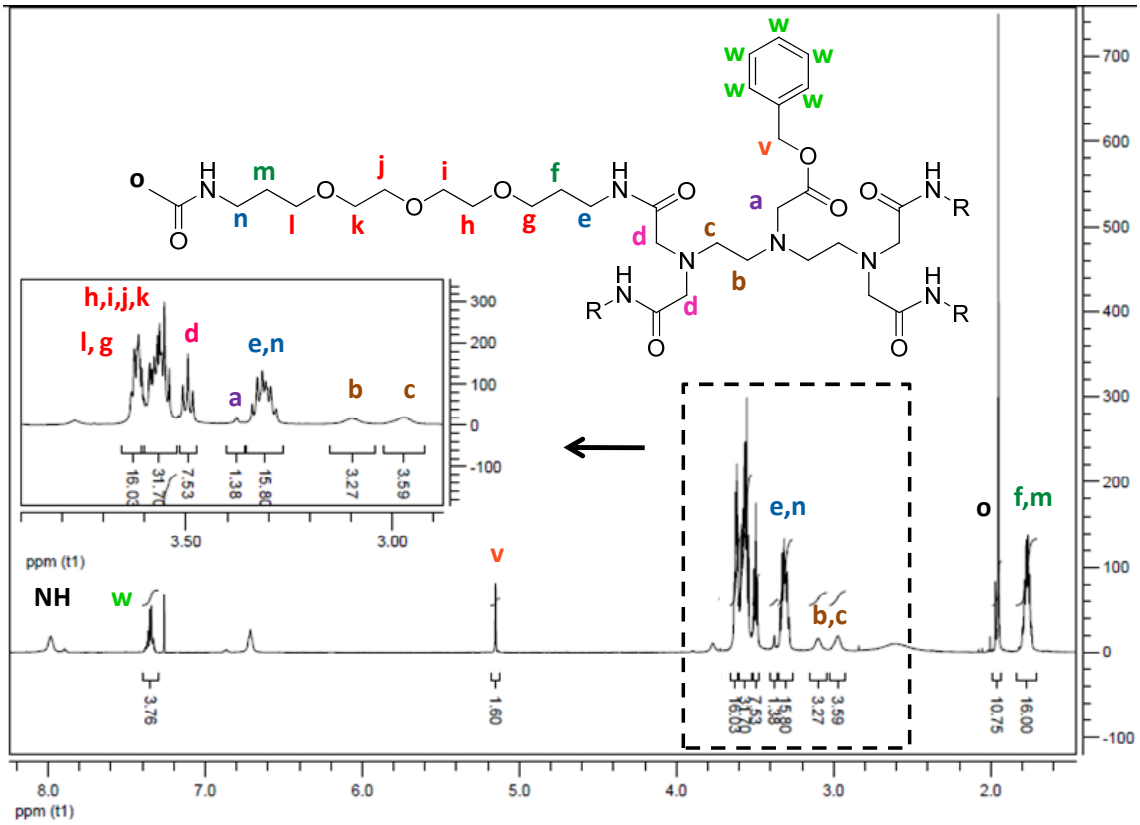
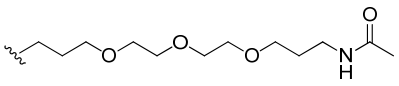


Figure 9. ^{1}H NMR spectrometry of first generation dendron **8** (**G1-Ac-Bn**).

R represents: 

The signals assignation is equivalent for all branches.

^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ=1.77 (m, 16 H), 1.95 (s, 12 H), 2.97 (bs, 4 H), 3.10 (bs, 4 H), 3.31 (m, 16 H), 3.38 (s, 2 H), 3.50 (t, ^{3}J(H,H)=6.02 Hz, 8 H), 3.53-3.60 (m, 32 H), 3.60-3.65 (m, 16 H), 5.15 (s, 2 H), 7.34 (m, 5 H), and 7.98 (bs, NH).

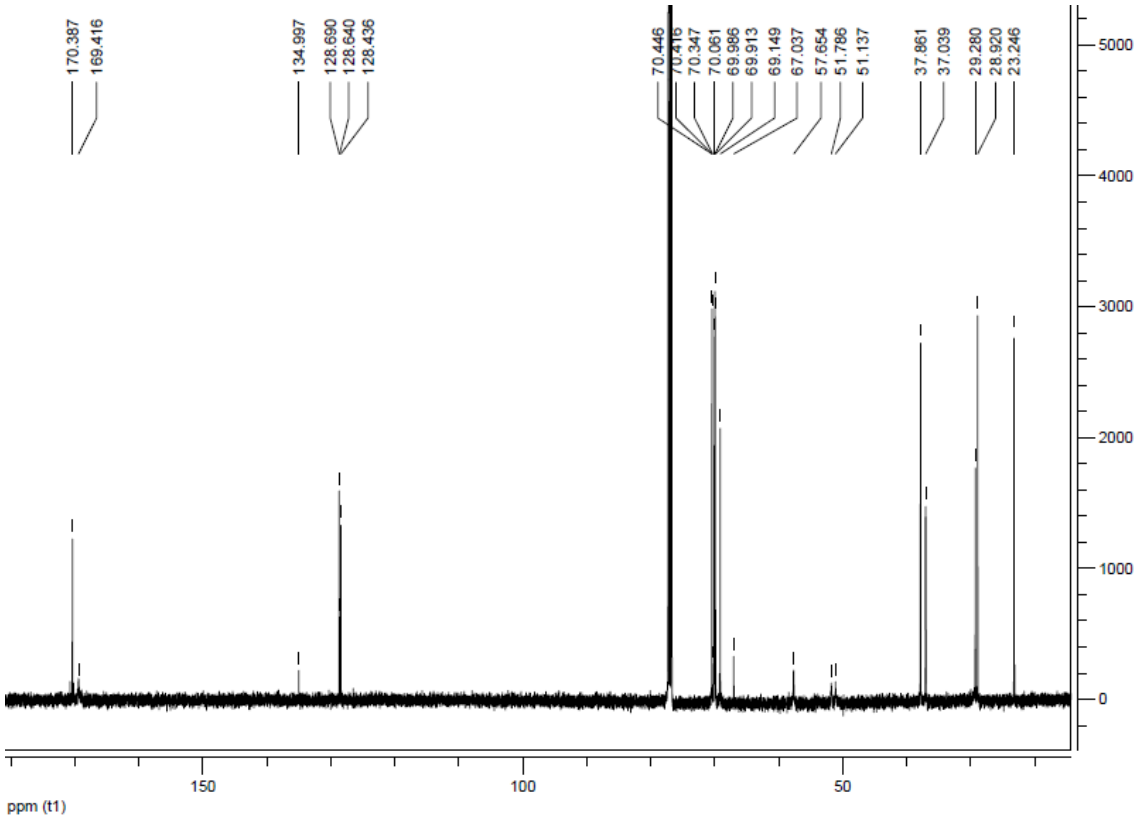


Figure 10. ^{13}C NMR spectrometry of first generation dendron **8** (**G1-Ac-Bn**).

^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ=23.25, 28.92, 29.30, 37.04, 37.90, 51.14, 51.79, 57.65, 67.04, 69.15, 69.91, 69.99, 70.06, 70.35, 70.42, 70.44, 128.44, 128.64, 128.69, 135.00, 169.42, and 170.39.

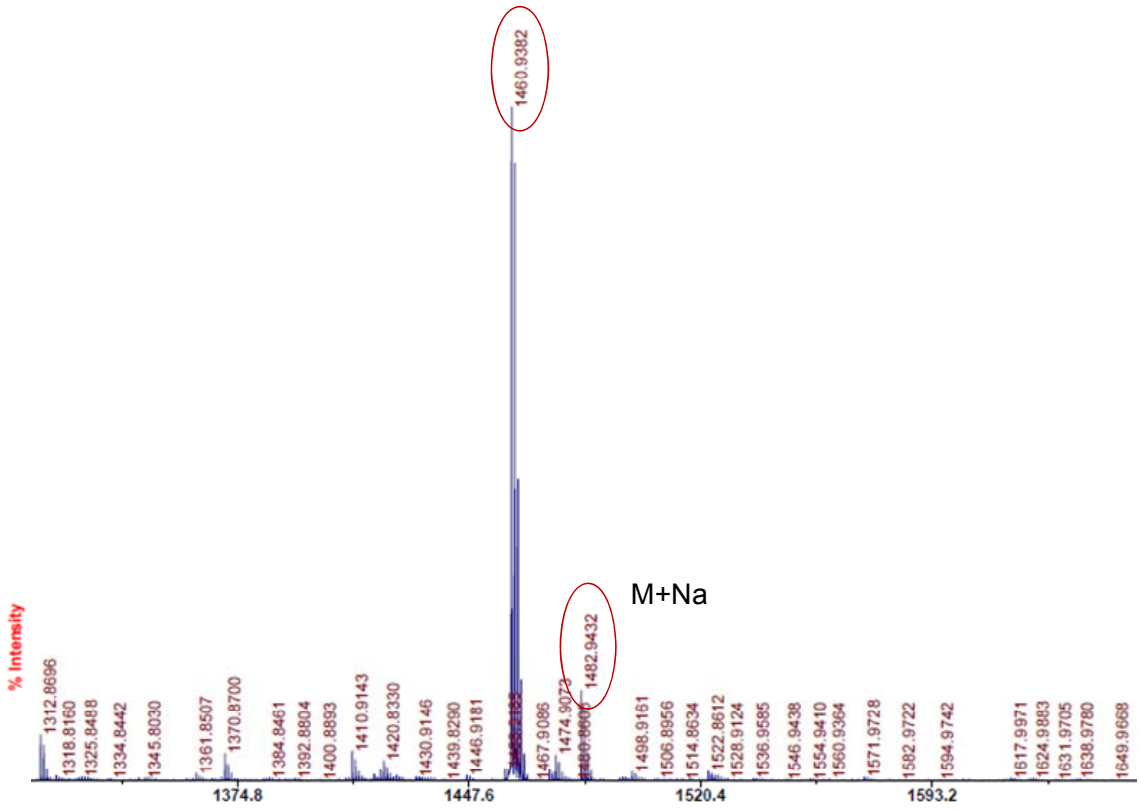


Figure 11. MALDI-TOF spectrometry of first generation dendron **8** (**G1-Ac-Bn**).

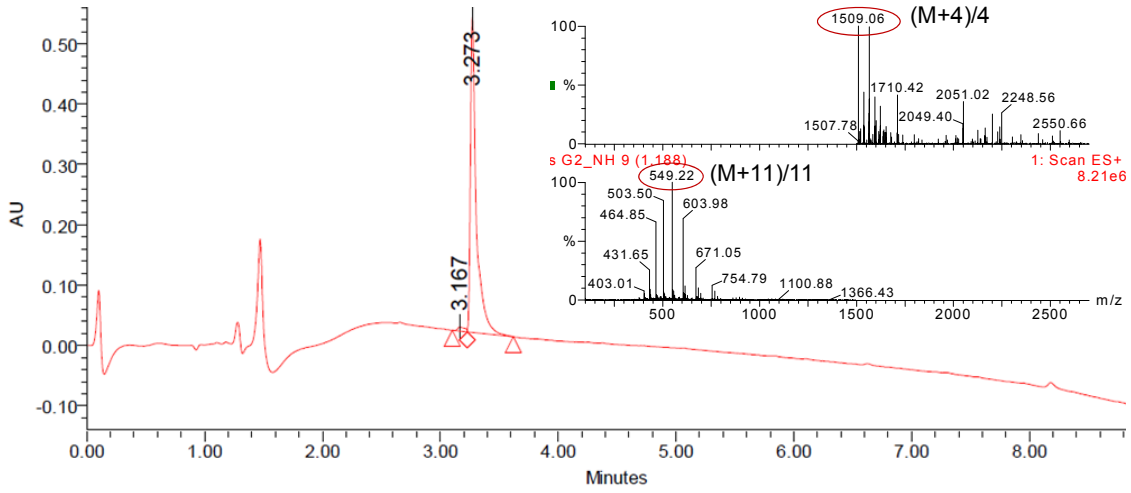


Figure 12. HPLC-MS profile of second generation dendron **13** (**G2-NH$_2$-Bn**). Analytical HPLC: 5→100% of CH_3CN in H_2O over 8 min, t_R=3.27 min (98% at λ=210 nm).

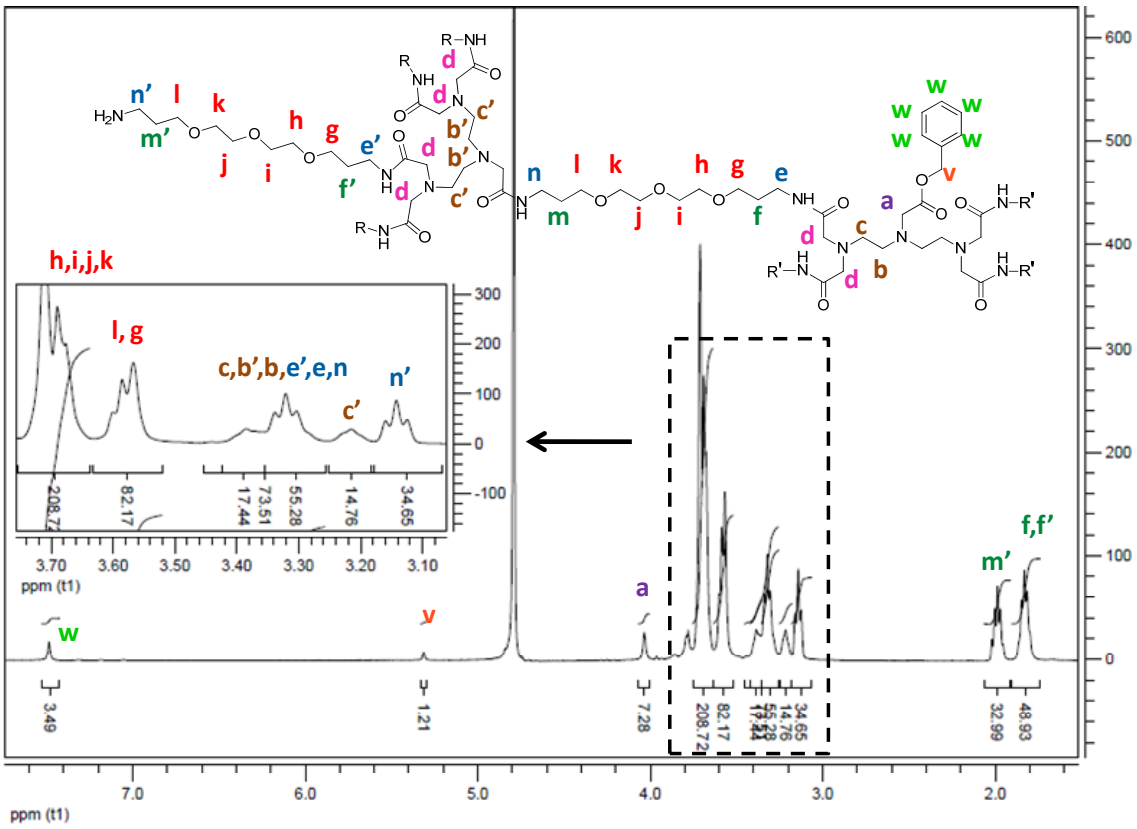
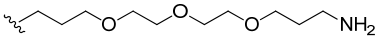


Figure 13. ^{1}H NMR spectrometry of second generation dendron **13** (**G2-NH$_2$-Bn**).

R represents: 

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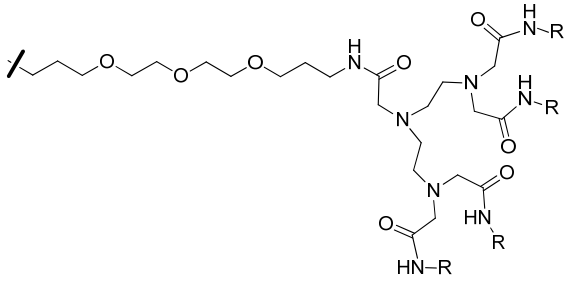


The signals assignation is equivalent for all branches.

^{1}H NMR (400 MHz, D_2O, 25 °C): δ=1.83 (m, 48 H), 1.98 (m, 32 H), 3.14 (t, ^{3}J(H,H)=6.92 Hz, 32 H), 3.22 (bs, 16 H), 3.32 (t, ^{3}J(H,H)=6.95 Hz, 54 H), 3.39 (bs, 16 H), 3.57 (m, 80 H), 3.65-3.75 (m, 208 H), 4.03 (s, 2 H), 5.31 (s, 2H), and 7.49 (m, 5 H).

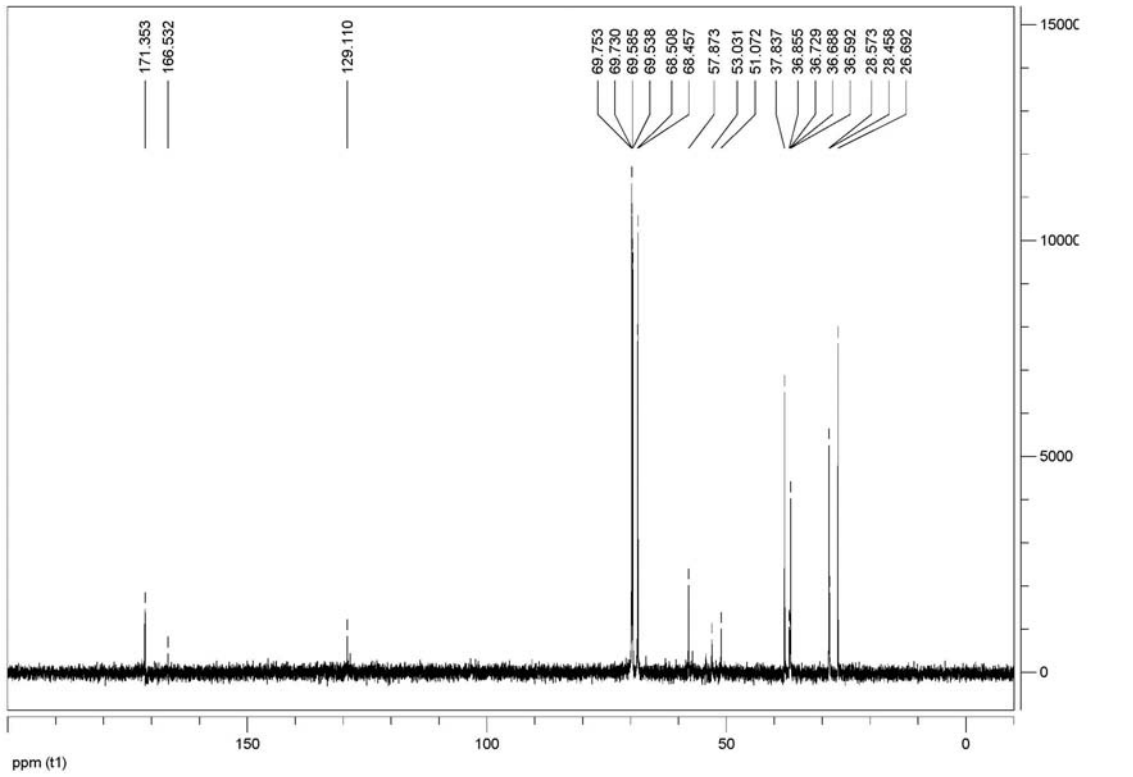


Figure 14. ^{13}C NMR spectrometry of second generation dendron **13** (**G2-NH$_2$-Bn**).

^{13}C NMR (100 MHz, D_2O, 25 °C): δ=26.69, 28.46, 28.57, 36.59, 36.86, 37.84, 51.07, 53.03, 57.87, 68.46, 68.51, 69.54, 69.59, 69.73, 69.75, 129.11, 166.53 and 171.35.

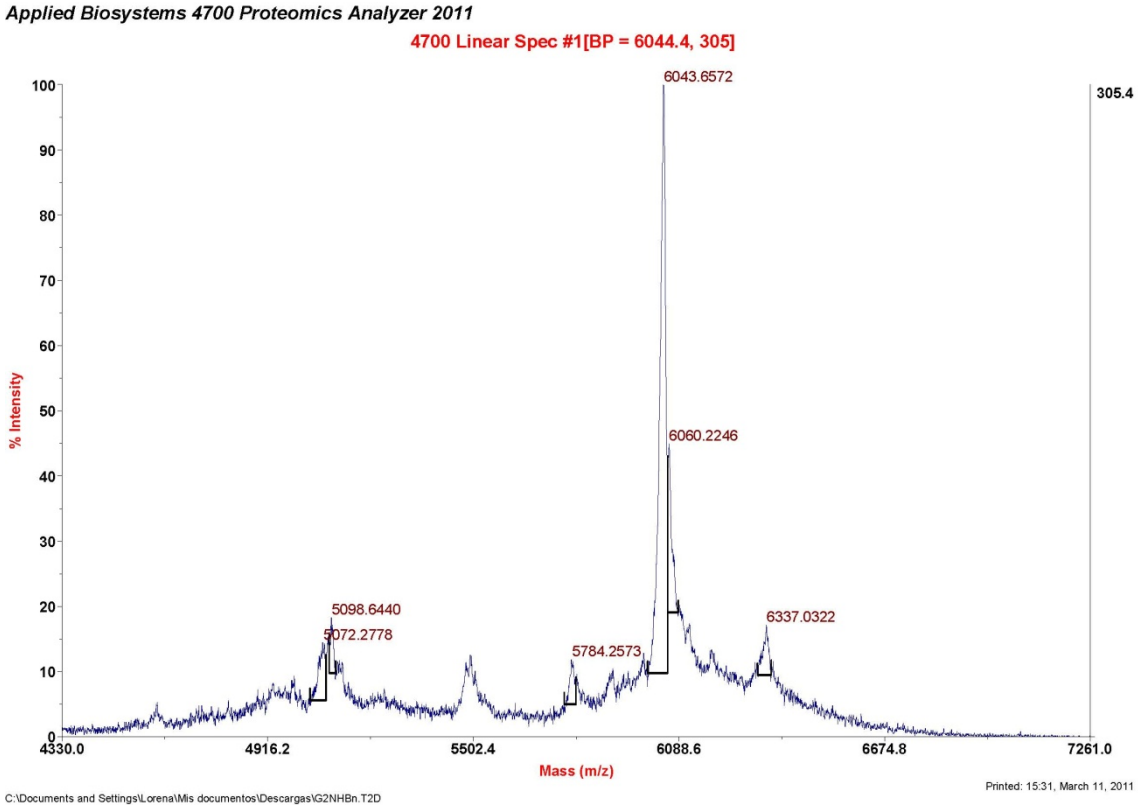


Figure 15. MALDI-TOF spectrometry of second generation dendron **13** (**G2-NH$_2$-Bn**).

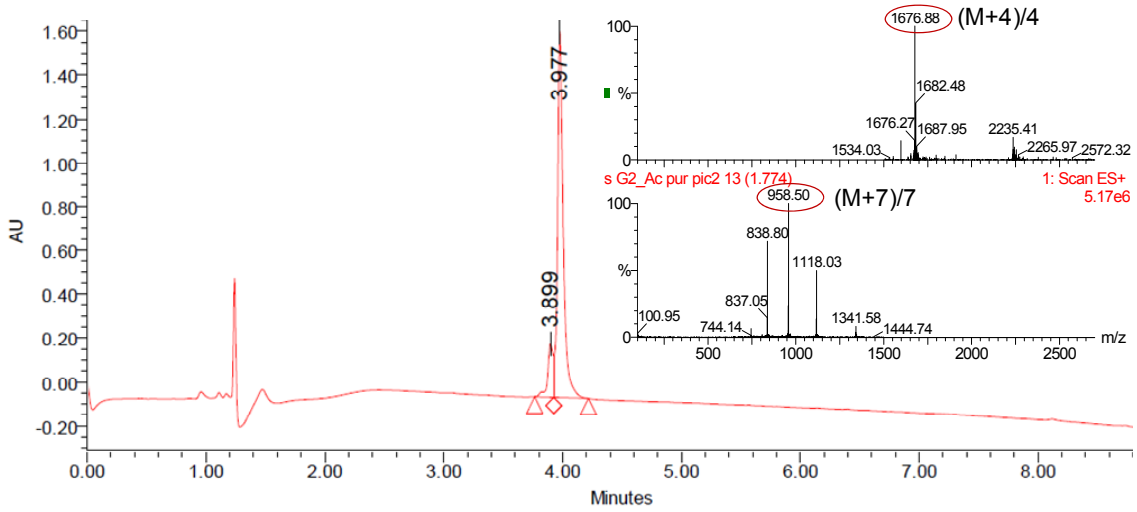


Figure 16. HPLC-MS profile of second generation dendron **14** (**G2-Ac-Bn**). Analytical HPLC: 5→100% of CH_3CN in H_2O over 8 min, t_R=3.98 min (88% at λ=210 nm).

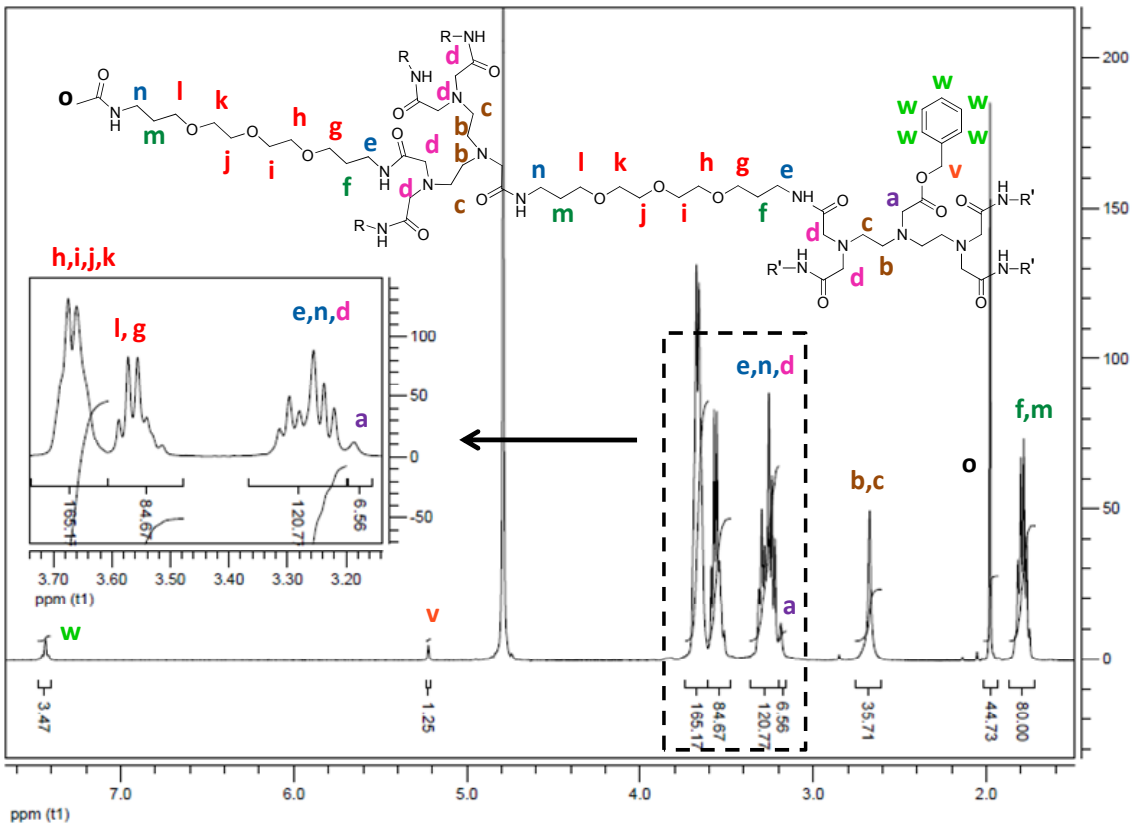
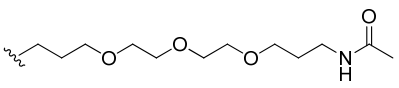


Figure 17. NMR spectrometry of second generation dendron **14** (**G2-Ac-Bn**).

R represents:



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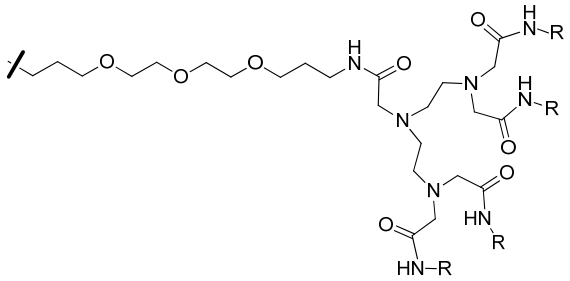

The signals assignation is equivalent for all branches.

^{1}H NMR (400 MHz, D_2O, 25 °C): δ=1.78 (m, 80 H), 1.98 (s, 48 H), 2.67 (s, 40 H), 3.19 (s, 2 H), 3.26 (m, 128 H), 3.56 (m, 80 H), 3.61-3.71 (m, 160 H), 5.22 (s, 2 H), and 7.43 (m, 5 H).

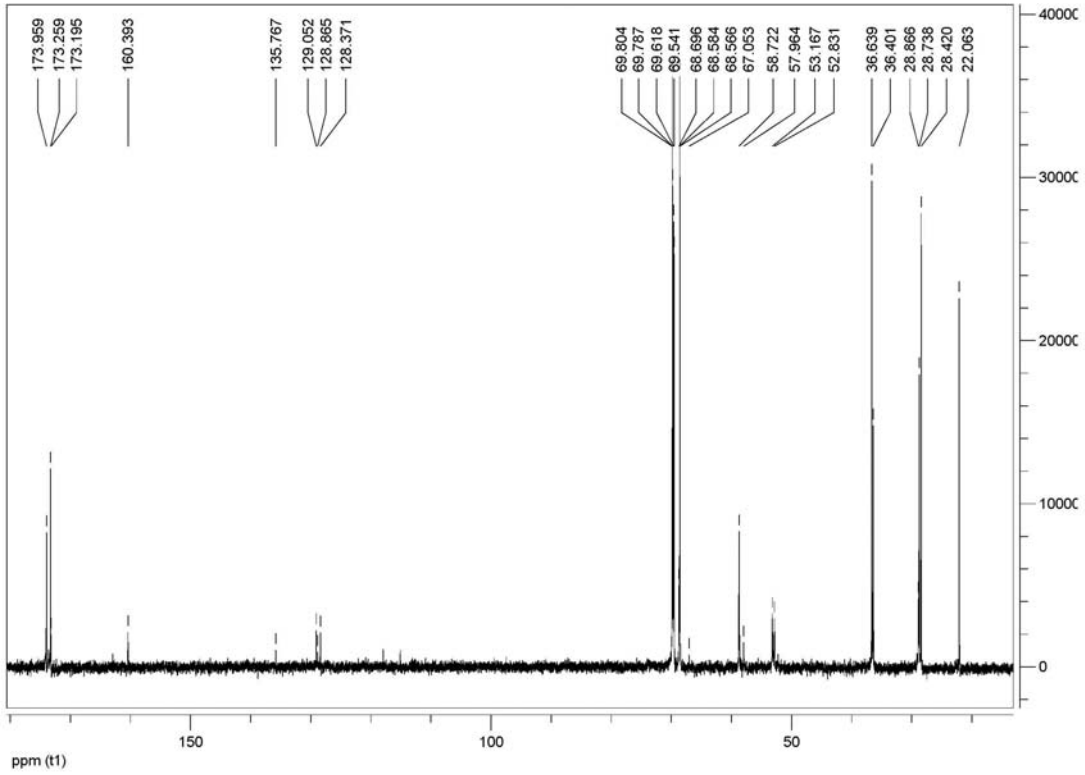

Figure 18. ^{13}C NMR spectrometry of first generation dendron **14** (**G2-Ac-Bn**).

^{13}C NMR (100 MHz, D_2O, 25 °C) δ: 22.01, 28.42, 28.74, 28.87, 36.40, 36.64, 52.83, 53.17, 57.96, 58.72, 67.05, 68.57, 68.58, 68.70, 69.54, 69.62, 69.79, 69.80, 128.37, 128.87, 129.05, 135.77, 160.39, 173.20, 173.26, and 173.96.

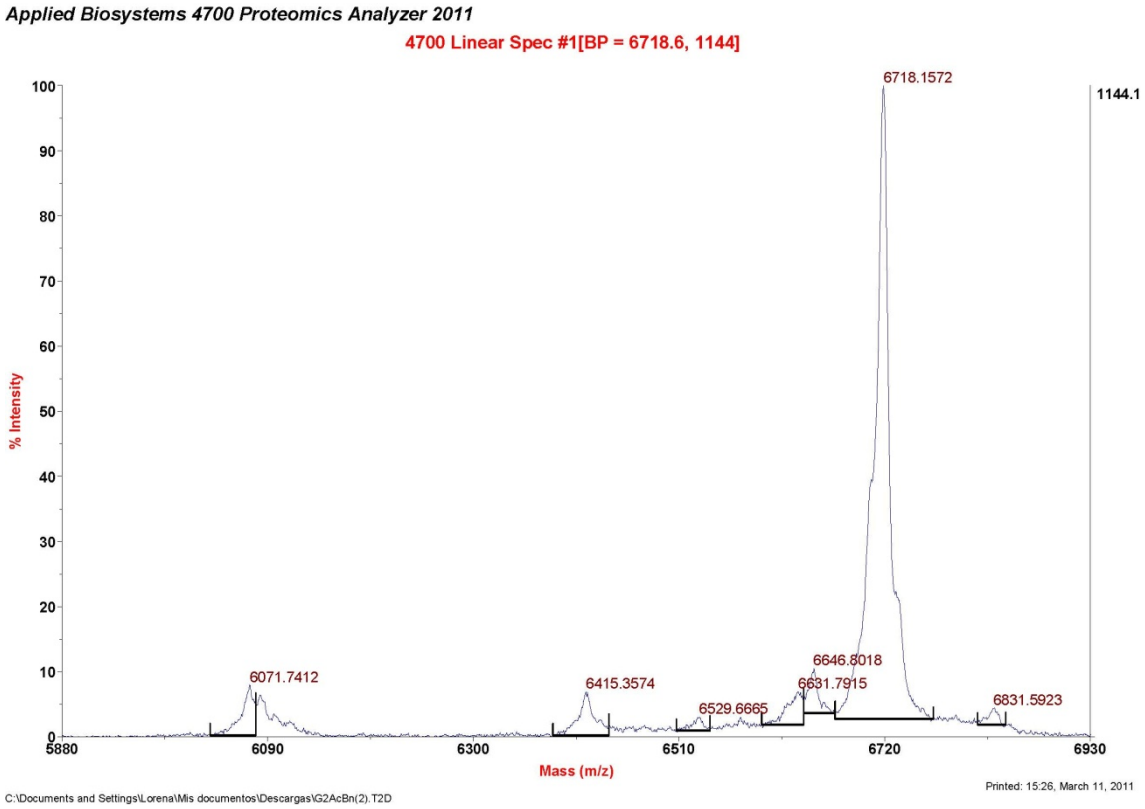

Figure 19. MALDI-TOF spectrometry of second generation dendron **14** (**G2-Ac-Bn**).