Supporting Information

Synthesis and characterization of fourth generation polyester-based dendrimers with cationic amino acids-modified crown as promising water soluble biomedical devices

Running Head: Water-soluble cationic dendrimers as promising biomedical devices

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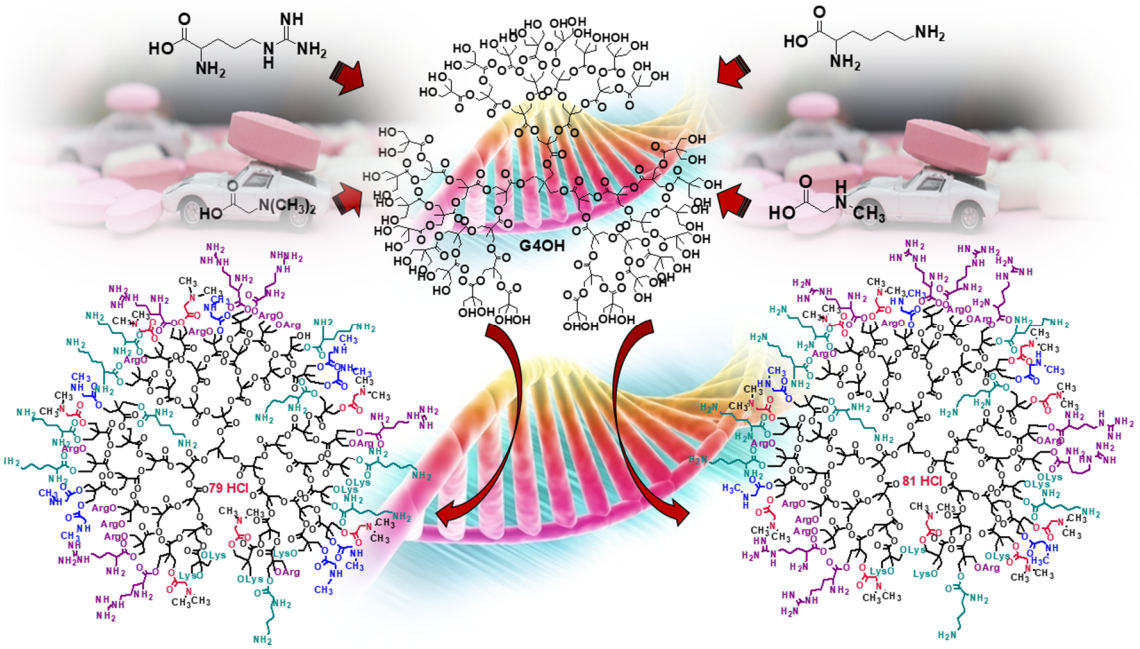
Graphical Abstract



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CONTENTS

Experimental procedure S1: Synthesis of *N*-BOC-amino acids 2b[1] and 2c[2]

General Procedure

A solution of the amino acid and base in the proper solvent system was cooled to 0 °C and treated with di-*tert*-butylcarbonate (1.1 equiv./amino group). The mixture was left overnight at room temperature under magnetic stirring. The disappearance of amino acid was checked by TLC using EtOAc containing a spatula tip of ninhydrin as the eluent. The mixture was concentrated at reduced pressure to half the volume, cooled to 0 °C and acidified with 10 % aq. $KHSO_4$ to pH = 2, extracted with EtOAc and dried (Na_2SO_4). The removal of the solvents at reduced pressure afforded the desired BOC protected amino acid.

*$^{\alpha}N$,$^{\varepsilon}N$-di-BOC-L-Lysine (**2b**).*[1] Resin (mixture of rotamers). Yield = 93 %.

FTIR (KBr, cm^{-1}): 3500-2400 (OH), 3374 (NH), 1713 (C=O acid + carbamate), 1529 (NH).

^{1}H NMR (300 MHz, $CDCl_3$,): δ ppm = 1.45 (s, 18H, C*H*$_3$ BOC), 1.30-2.00 (m, 6H, $^{\beta}$C*H*$_2$ $^{\gamma}$C*H*$_2$ $^{\delta}$C*H*$_2$), 3.11 (m, 2H, $^{\varepsilon}$C*H*$_2$NHBOC), 4.11-4.38 (m, 1H, $^{\alpha}$C*H*NHBOC), 4.70 and 6.30 (m, 1H, $^{\varepsilon}$N*H*), 5.27 (d, *J* = 7.9 Hz, 1H, $^{\alpha}$N*H*), 8.50 (br, 1H, O*H*).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ ppm = 22.42 (*C*H$_2$), 28.35 (*C*H$_3$ BOC), 28.42 (*C*H$_3$ BOC), 29.45 (*C*H$_2$), 32.10 (*C*H$_2$), 40.13 (major) and 41.24 (*C*H$_2$NH), 53.24 (major) and 54.58 (*C*HNH), 79.34 (major) and 80.86 (*C* BOC), 79.95 (major) and 81.48 (*C* BOC), 155.79 (major) and 156.88 (*C*=O carbamate), 156.33 (major) and 158.16 (*C*=O carbamate), 176.27 (*C*=O acid). NMR data were consistent with those of the literature.

*$^{\alpha}$N-BOC-Sarcosine (**2c**).*[2] Glassy off white solid (mixture of rotamers), mp = 88-90 °C. Yield = 94 %.

FTIR (KBr, cm^{-1}): 3500-2400 (OH), 1764 (C=O acid), 1751 (C=O carbamate).

^{1}H NMR (300 MHz, $CDCl_3$): δ ppm = 1.44 and 1.47 (two signals, 9H, C*H*$_3$ BOC), 2.94 (s, 3H, C*H*$_3$N), 3.95 and 4.03 (two signals, 2H, C*H*$_2$N).

^{13}C NMR (300 MHz, $CDCl_3$): δ ppm = 28.25 and 28.32, 35.56 and 35.64, 50.20 and 50.78, 80.67, 155.63 and 156.43, 175.03 and 175.13. NMR data were consistent with those of the literature.

TABLE S2 Some data about preparation of BOC-protected amino acids **2b**[1] and **2c**[2]

Amino acid [g, mmol]	**Solvent** v/v [mL]	**Base** [mmol]	***N*-BOC amino acid**	**Yield** [%]	**Physic state**
***L*-lysine** (2.00, 13.7)	Dioxane/H_2O 1/1 (40.0)	NaOH 1N (13.7)	**2b**[1]	93	Glassy solid
***N*-methylglycine** (1.00, 11.2)	H_2O (100.0)	Et_3N (33.6)	**2c**[2]	94	Glassy solid

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2. Yoon UC, Jin YX, Park CH, Park JH, Oh SW, Mariano PS, Cai X, Duesler E, J Am Chem Soc 2003;125: 10664-10671.

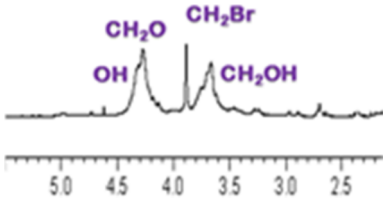

FIGURE S3 Significant part of ^{1}H NMR spectrum of **4**

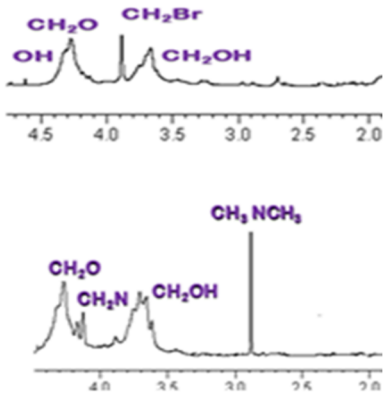

FIGURE S4 Comparison between significant portions of ^{1}H NMR spectra of **4** (top box) and of **5** (bottom box)

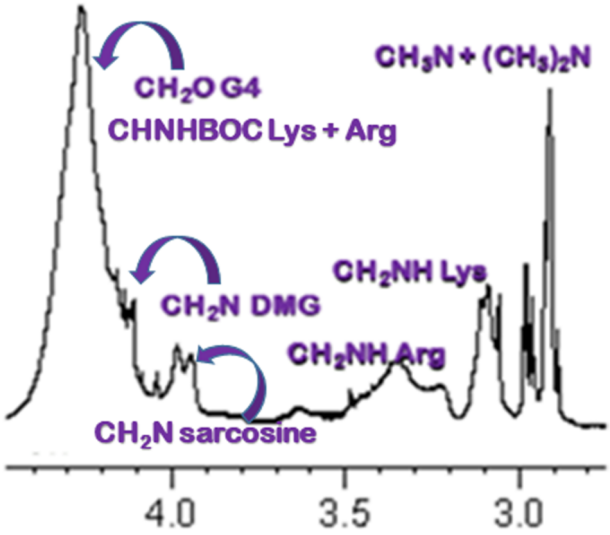

FIGURE S5 Significant portion of ^{1}H NMR spectrum of **6**

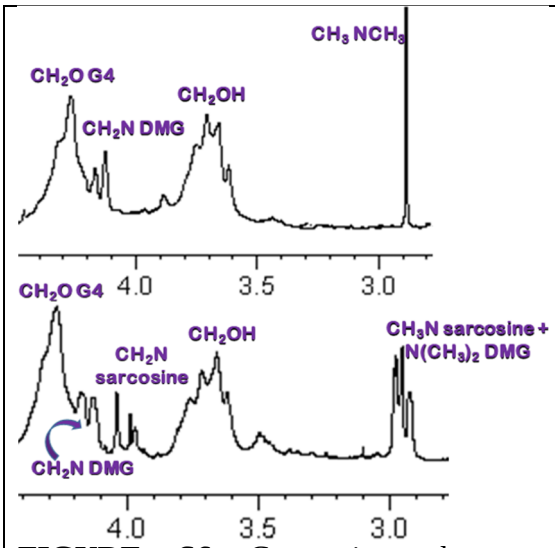

FIGURE S6 Comparison between significant portions of ^{1}H NMR spectra of **5** (top box) and of **7** (bottom box)

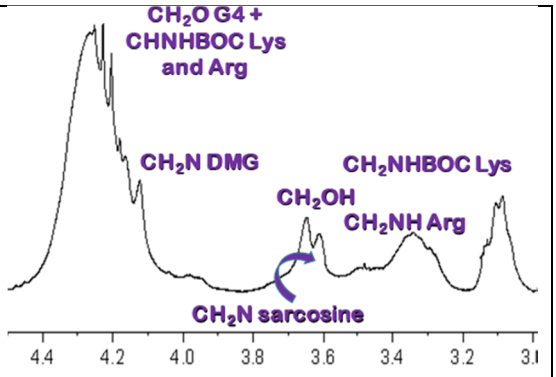

FIGURE S7 Significant portion of ^{1}H NMR spectrum of **8**

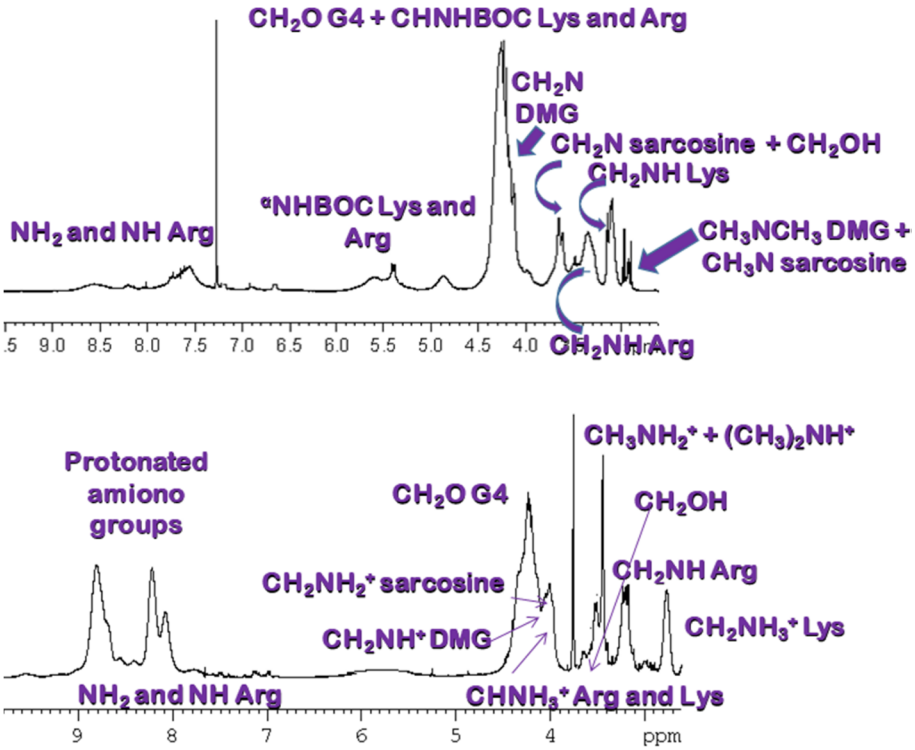

FIGURE S8 Significant portion of ^{1}H NMR spectra of **8** (top box) and of **10** (bottom box)

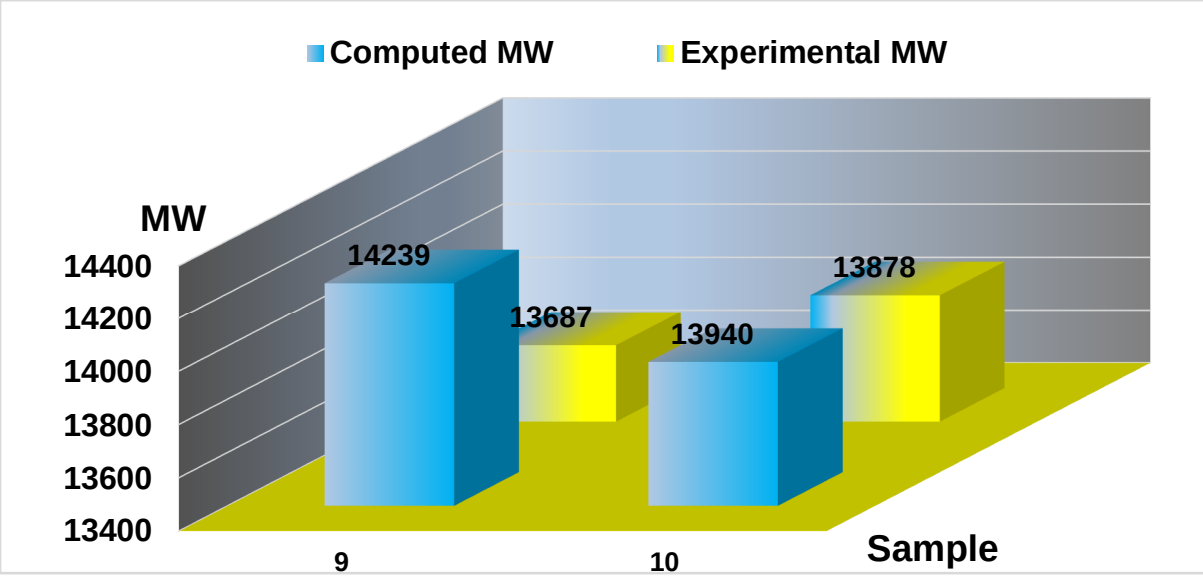

FIGURE S9 Graphic comparison between estimate MW (^{1}H NMR) and experimental MW by volumetric titration of compounds **9** and **10**

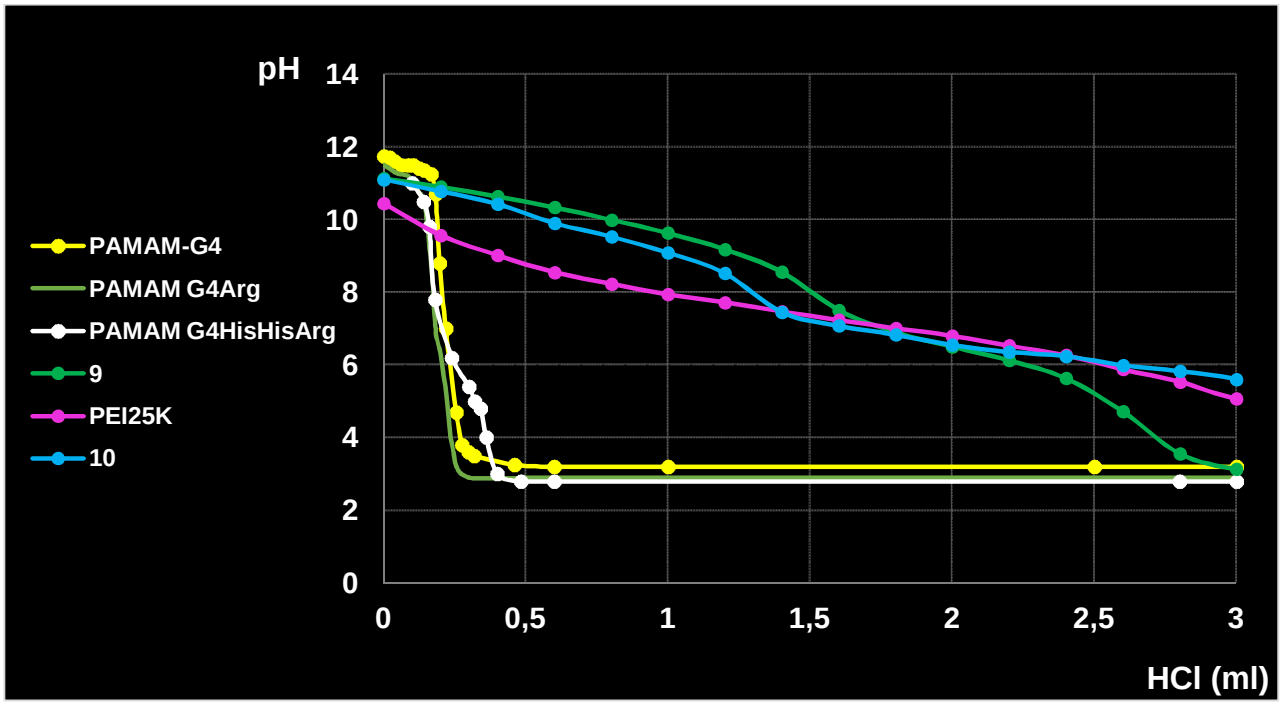

FIGURE S10 Titration curves of compounds **9** and **10** compared with G4-PAMAM derivatives and *b*-PEI25K

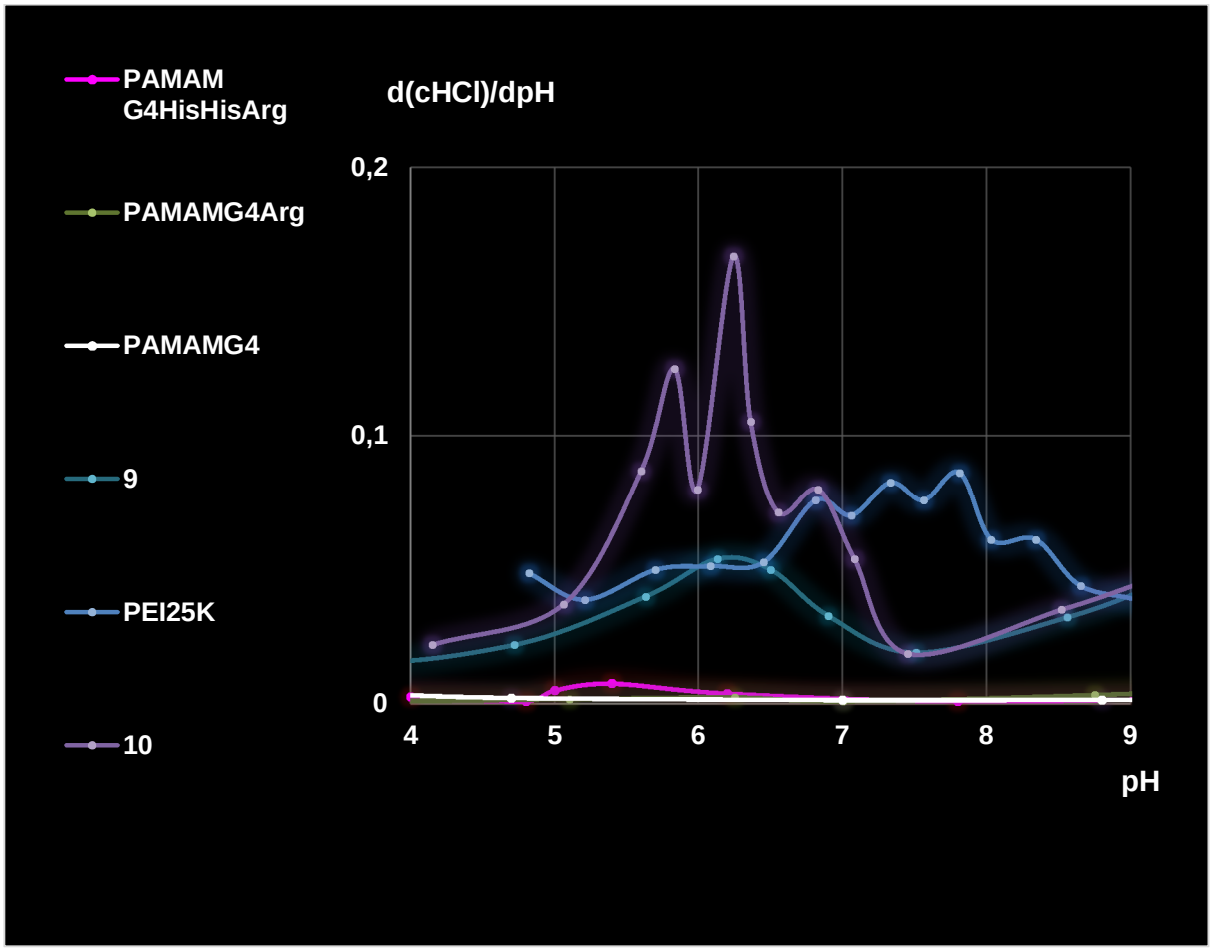

FIGURE S11 Buffer capacity values (β) computed by potentiometric titration of compounds **9** and **10** compared with G4-PAMAM derivatives and *b*-PEI25K

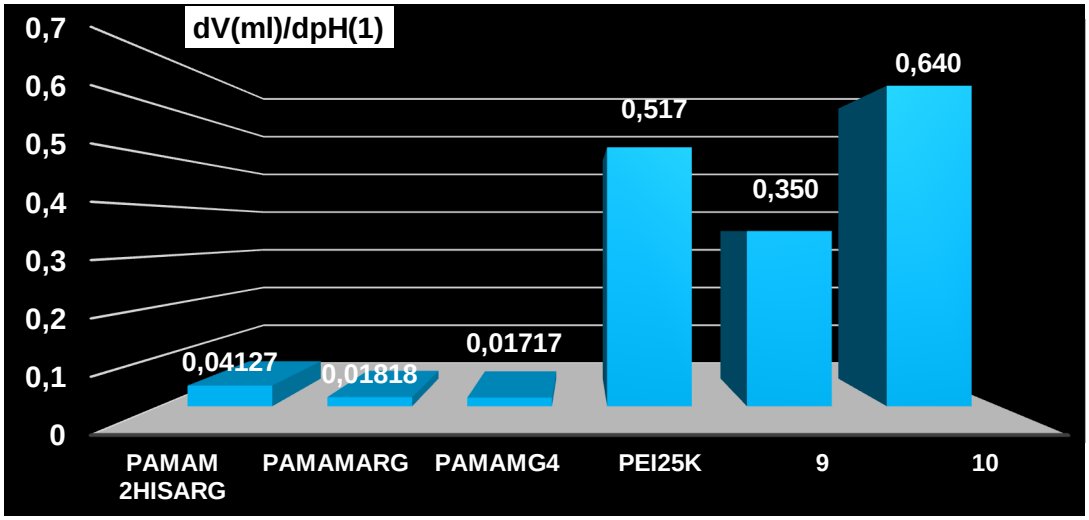

FIGURE S12 Average buffer capacity values ($\overline{\beta}$) of compounds **9** and **10** compared with G4-PAMAM derivatives and *b*-PEI25K

TABLE S13 Some useful analytical data and physicochemical properties of the most important reported compounds

Compound	Formula	MW	Required (%)	Found (%)	Error	Physical state
G4OH (1)[1]	$C_{230}H_{372}O_{138}$	5345.28	C 51.68 H 7.02	C 51.86 H 7.18	C +0.18 H +0.16	Fluffy white solid
3[2]	$C_2H_3BrO_2$	138.95	C 17.29 H 2.18 Br 57.51	C 17.45 H 2.52 Br 57.16	C +0.21 H +0.34 N -0.35	Off white low melting crystals[a]
4	$C_{250}H_{382}Br_{10}O_{148}$[b]	6554.69[b]	C 45.81 H 5.87 Br 12.19	C 46.02 H 6.06 Br 11.96	C +0.21 C +0.19 H -0.23	pale yellow viscous resin
5	$C_{270}H_{442}N_{10}O_{148}$[b]	6196.40[b]	C 52.33 H 7.19 N 2.26	C 52.65 H 7.16 N 1.96	H +0.32 C -0.03 H +0.30	white glassy solid
6	$C_{748}H_{1269}N_{135}O_{328}$[b]	17401.86[b]	C 51.63 H 7.35 N 10.87	C 52.00 H 7.36 N 10.96	C +0.37 H +0.01 N +0.09	off white spongy solid
7	$C_{322}H_{525}N_{15}O_{169}$[b]	73110.65[b]	C 52.91 H 7.24 N 2.87	C 53.15 H 7.56 N 2.96	C +0.24 H +0.32 N +0.09	off white spongy solid
8	$C_{754}H_{1277}N_{127}O_{329}$[b]	17385.94[b]	C 52.09 H 7.40 N 10.23	C 52.38 H 7.56 N 10.36	C +0.29 H +0.16 N +0.13	off white spongy solid
9	$C_{483}H_{944}N_{117}Cl_{81}O_{186}$[b]	14239.03[b] 13687[c]	C 40.74 H 6.68 Cl 20.17 N 11.51	C 41.11 H 6.56 Cl 19.96 N 11.63	C +0.37 H -0.12 Cl -0.21 N +0.12	Slightly Hygroscopic, off white spongy solid
10	$C_{474}H_{924}N_{111}Cl_{79}O_{185}$[b]	13939.79[b] 13878[c]	C 40.84 H 6.68 Cl 20.09 N 11.15	C 41.20 H 6.86 Cl 20.08 N 10.96	C +0.36 H +0.18 Cl -0.01 N -0.19	Slightly Hygroscopic, off white spongy solid

[a]mp = 48 °C, lit.[2b], mp = 50 °C.
[b]estimated by ^{1}H NMR spectra and confirmed by Elemental Analysis in order to avoid the routine well known but very expensive MALDI_TOF technique not available in our laboratory.
[c]by volumetric titration.

Analytical Data S14: *Compound **G4OH (1)**[1]*

Fluffy white solid (1.14 g, 0.2130 mmol). Isolated Yield = 98 %, mp 77 °C. FTIR (KBr, cm^{-1}): 3424 (OH), 1739 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.79 (s, 3H, CH_3 of *core*), 1.01 (s 72H, CH_3 of fourth generation), 1.16 (s, 36H, CH_3 of third generation), 1.18 (s, 18H, CH_3 of second generation), 1.22 (s, 9H, CH_3 of first generation), 3.28-3.48 (m, 96H, CH_2OH);

4.11-4.19 (m, 90H, CH_2O of dendrimer), 4.44 (br, 48H, OH). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ = 16.37, 16.67, 16.87 (two signals overlapped) and 17.11 (CH_3), 46.17, 46.23 (two signals overlapped) and 50.21(quaternary C), 63.64 (CH_2OH), 64.35, 64.86 and 65.29 (two signals overlapped) (CH_2O), 171.42 (two signals overlapped), 171.78 and 174.00 (C=O). MALDI-TOF: *m/z* calculated for $C_{230}H_{372}O_{138}Na_1$ [M^++Na^+]: 5368.27, found 5368.056. Anal. Cald. for $C_{230}H_{372}O_{138}$: C, 51.68; H, 7.02. Found: C, 51.86; H 7.18.

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2. a) Natelson S, Gottfried S, Org Synth 1934;23:37. DOI: 3710.15227/orgsyn.023.0037.

   b) Bryant GB, Logan TJ, Hill KR, Standish NW, J Org Chem 1960;25:1312-22.

^{1}H NMR (DMSO-d_6, 300 MHz)

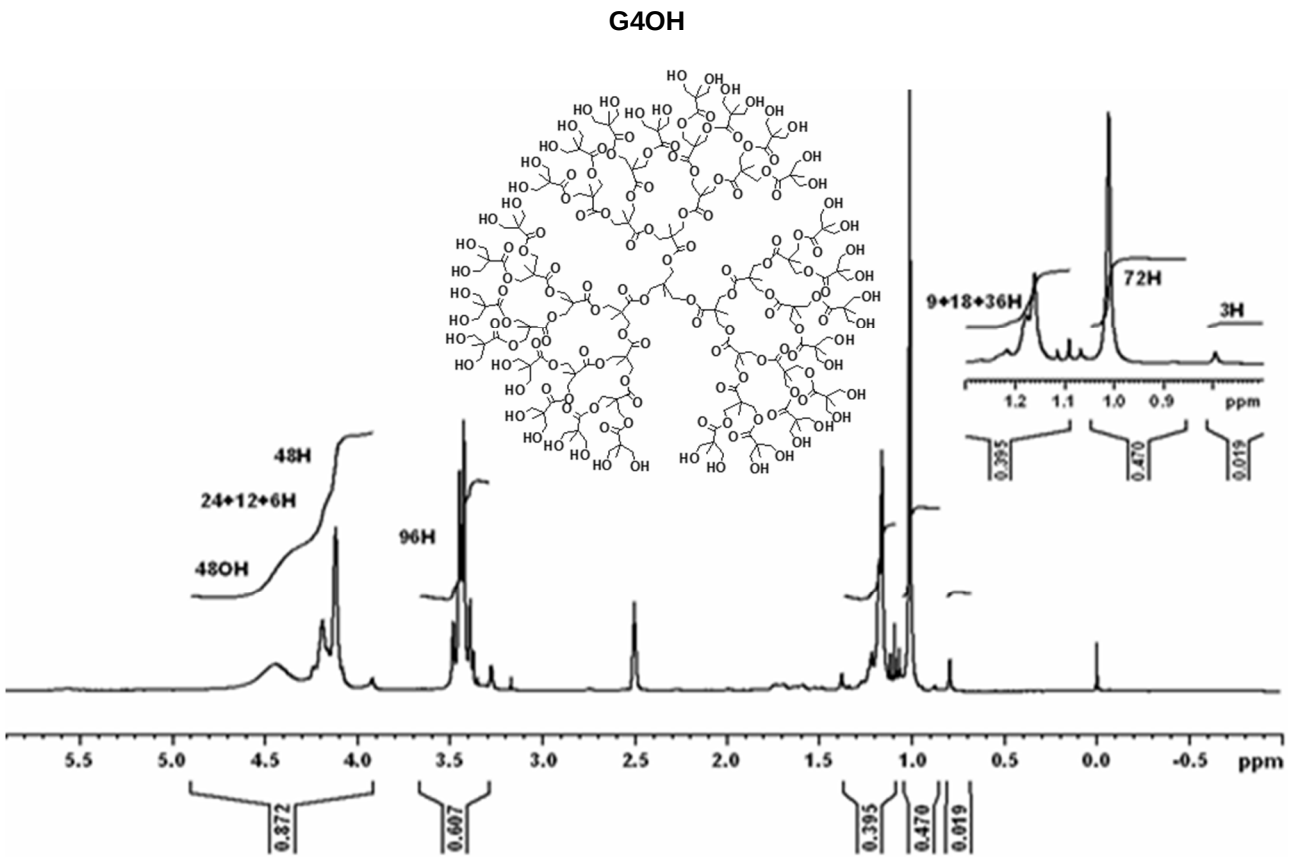

FIGURE S15 ^{1}H NMR spectrum of **G4OH** (**1**)

^{13}C NMR (DMSO-d_6, 75.5 MHz)

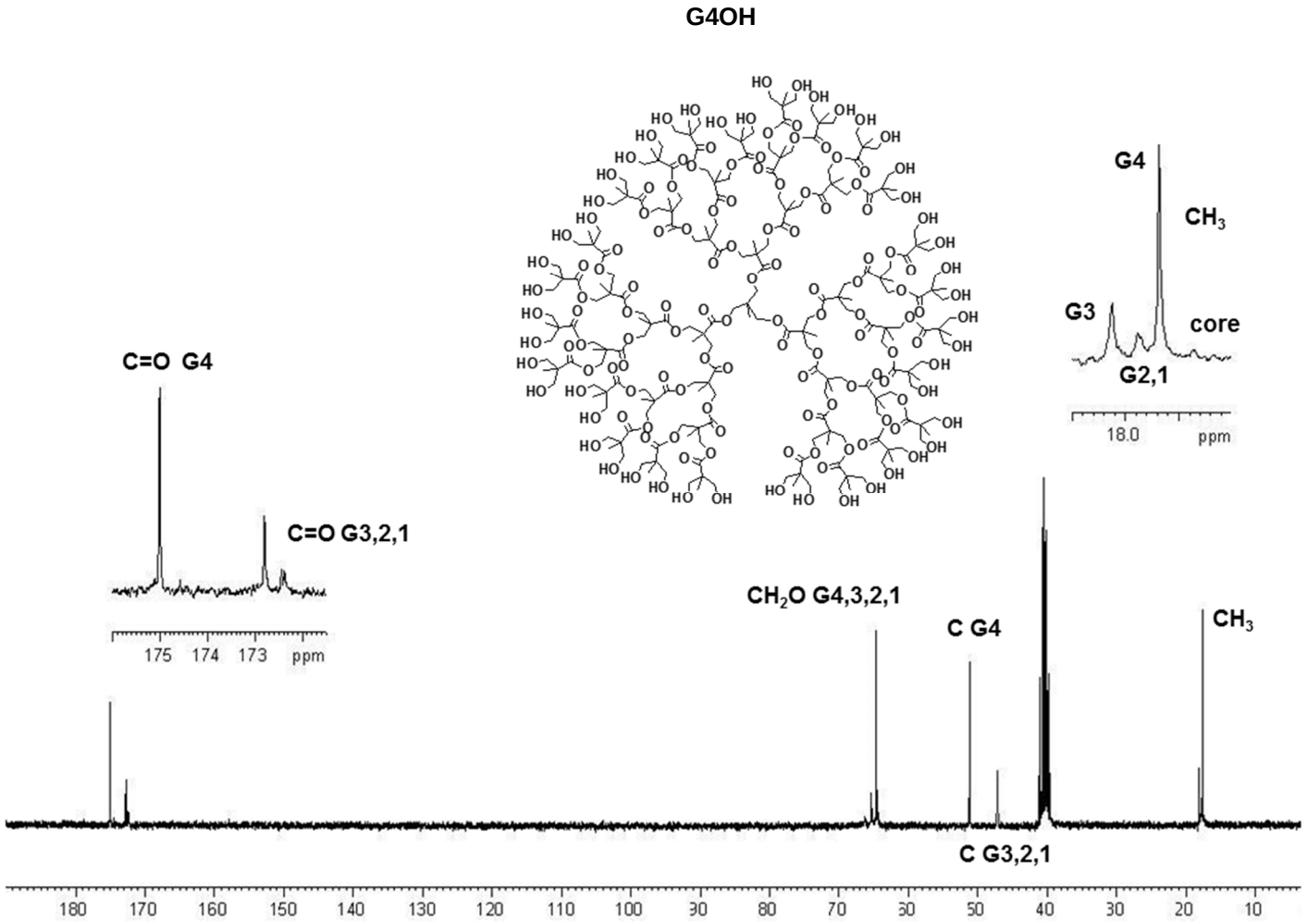

FIGURE S16 ^{13}C NMR spectrum of **G4OH** (**1**)

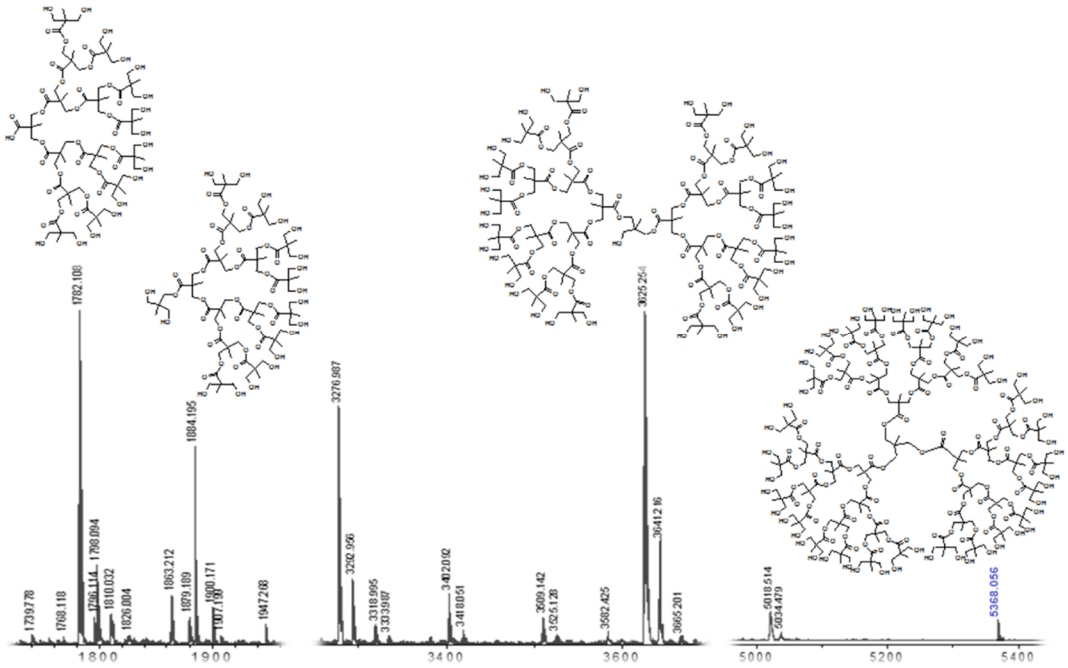

FIGURE S17 MALDI-TOF (*m/z* calculated for $C_{230}H_{372}O_{138}Na_1$ [M^++Na^+]: 5368.27, found 5368.056) of **G4OH** (**1**)