Supporting information

Star-shaped poly(L-lysine) with polyester *bis*-MPA dendritic core as potential degradable nano vectors for gene delivery

Contents

1 Experimental procedures

1.1 Initiator synthesis

Bis-MPA dendrimers generation 1, 2, and 3 were synthesised following literature procedures (Scheme S1).[1-3] Synthesis of both hydroxyl and amino terminated dendrimers can be performed in either dichloromethane (DCM) at room temperature or in ethyl acetate (EtOAc) at 50 °C.

1.1.1 Synthesis of acetonide protected *bis*-MPA

2,2-bis-(hydroxymethyl)propionic acid (*bis*-MPA) (100 g, 0.745 mol, 1eq.) was dissolved in acetone (650 mL). 2,2-Dimethoxypropane (2,2-DMP, 137.5 mL, 1.12 mol, 1.5 eq.) and p-toluenesulfonic acid (pTSA) monohydrate (0.85 g, 4.47 mmol, 0.006 eq.) were added to the reaction mixture. The reaction was carried out at room temperature overnight, and it was then quenched by adding a solution of NH_3/EtOH (1:1, 6 mL). The acetone was evaporated and the product was dissolved in dichloromethane (DCM). The organic phase was washed three times with deionized water, dried over $MgSO_4$, filtered and the solvent was evaporated giving acetonide-protected *bis*-MPA (78 g, 0.448 mol, 60.1%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.46 (s, 1H, -COO**H**), 4.13 (d, 2H, -C-C**H**$_2$-O-), 3.62 (d, 2H, -C-C**H**$_2$-O-), 1.35 (d, 6H, -C-C**H**$_3$), 1.21 (s, 3H, -C-C**H**$_3$). ^{13}C NMR (101 MHz, CDCl3) δ 180.24 (s), 98.47 (s), 66.02 (s), 41.86 (s), 25.52 (s), 21.92 (s), 18.52 (s).

1.1.2 Procedures for synthesis hydroxy terminated *bis*-MPA dendrimers

1.1.2.1 General procedure for the synthesis of acetonide-protected dendrimers

CsF was dried at 100 °C under constant vacuum overnight. In a round bottom flask, 1.5 equivalents of acetonide protected *bis*-MPA per hydroxyl group of the polyol were suspended in ethyl acetate (EtOAc) to a concentration of 2 M. The same number of equivalents of 1,1'-Carbonyldiimidazole (CDI) was slowly added, while heating the mixture at 50 °C and the reaction was carried out for 1 h. The formation of imidazole-activated acetonide protected *bis*-MPA was confirmed by ^{1}H-NMR ($CDCl_3$): 8.27 (s, 1H, N-CH=N), 7.54 (s, 1H, N-CH=CH), 7.08 (s, 1H, CH=CH-N)). Thereafter, one equivalent of pentaerythritol (PERT)/hydroxy dendrimer and 0.2 equivalents per hydroxyl group of CsF were added to the flask and the reaction was stirred overnight. Once the full conversion was confirmed with ^{1}H-NMR, the reaction was quenched through the addition of water (~3h). The organic phase was diluted and washed 3 times with 10% aqueous $NaHCO_3$, dried with $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure. (NMR spectra were not presented)

1.1.2.2 General procedure for acetonide deprotection

The acetonide protected *bis*-MPA dendrimer was added to a round bottom flask and dissolved in a large excess of methanol. 10 wt % of pTSA monohydrate was added. After one hour, the acetone formed was removed by rotary evaporation. Once full deprotection was confirmed by ^{1}H-NMR (disappearance of methyl signals at 1.34 and 1.41ppm), the solution was filtered through a column of Amberlyst A21. The excess solvent was evaporated, and the product was precipitated into diethyl ether. After decanting, solvent traces were removed by

vacuum to afford pure hydroxyl terminated dendrimer ((G1-G3)-OH). Obtained dendrimers were white solids, decreasing in solidity with the increasing generations.

G1-(8)-OH: ^{1}H NMR (400 MHz, MeOD) δ 4.24 (s, 8H, -C-C**H**$_2$-O-)^{PERT}, 3.71 (d, 8H, -C-C**H**$_2$-OH)$^{bis-MPA}$, 3.60 (d, 8H, C-C**H**$_2$-OH) $^{bis-MPA}$, 1.18 (s, 3H,-C-C**H**$_3$) $^{bis-MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 175.95, 65.92, 62.89, 52.01, 44.37, 44.27, 17.28.

G2-(16)-OH: ^{1}H NMR (400 MHz, MeOD) δ 4.37 (d, 8H -C-C**H**$_2$-O-)^{PERT}, 4.29 (d, 16H, -C-C**H**$_2$-O-)$^{G1-bis-MPA}$, 3.69 (d, 16, -C-C**H**$_2$-OH)$^{G2-bis-MPA}$, 3.60 (d, 16H, -C-C**H**$_2$-OH)$^{G2-bis-MPA}$, 1.34 (s, 12H, -C**H**$_3$)$^{G1-bis-MPA}$, 1.16 (s, 24H, -C-C**H**$_3$)$^{G2-bis-MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 175.99, 173.82, 66.13, 65.89, 63.67, 51.83, 48.09, 44.36, 18.27, 17.36.

G3-(32)-OH: ^{1}H NMR (400 MHz, MeOD) δ 4.31 (dd, 56H, -C-C**H**$_2$-O)$^{PERT/(G1-G2)-bis-MPA}$, 3.64 (dd, 10.4 Hz, 64H, -C-C**H**$_2$-OH)$^{G3-bis-MPA}$, 1.37 (s, 12H, -C-C**H**$_3$)$^{G1-bis-MPA}$, 1.32 (s, 24H, -C-C**H**$_3$)$^{G2-bis-MPA}$, 1.16 (s, 48H, -C-C**H**$_3$)$^{G3-bis-MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 175.93, 173.86, 173.40, 66.87, 66.14, 65.83, 51.80, 48.23, 47.93, 18.46, 18.20, 17.41.

1.1.3 Procedures for synthesis amino functional *bis*-MPA dendrimers

1.1.3.1 General procedure for the synthesis of Boc-protected dendrimers and hyper-branched polymers (Scheme S2)

Prior to the synthesis, CsF was dried at 100 °C under constant vacuum overnight. Boc-protected β-alanine was suspended in DCM at a concentration of 1 M. The same number of equivalents of 1,1'-Carbonyldiimidazole (CDI) was added slowly. The reaction was allowed to proceed for 1 hour and the formation of the imidazole-activated intermediate was confirmed with ^{1}H-NMR. The hydroxy dendrimer/hyperbranched polymer and CsF were added, and the reaction was left to stir overnight. Once the full conversion was confirmed with ^{1}H-NMR, the reaction was quenched by water addition (~3h). After 2-4 h, the reaction mixture was diluted with DCM and washed 3 times with 10% aqueous $NaHSO_4$, 3 times with 10% aqueous $NaHCO_3$, and once with brine. After drying, the solvent was evaporated under reduced pressure, which gave the pure Boc-protected polymers. (NMR spectra were not presented)

1.1.3.2 General procedure for Boc deprotection (Scheme S2)

The Boc-protected dendrimers were dissolved in a mixture of chloroform and trifluoroacetic acid (TFA) (1:1). The reaction was carried out for 2 h. Deprotection was confirmed by ^{1}H-NMR (disappearance of methyl signal at 1.42 ppm). The product was precipitated into diethyl ether. Deprotected dendrimer was purified by dissolving in methanol and precipitated twice into diethyl ether. After decanting of the diethyl ether, the excess solvent was removed in a vacuum oven at 50 °C to afford amino terminated dendrimers ((G1-G3)-NH_3^+.) The amino terminated dendrimers and the hyperbranched polymer were obtained as hygroscopic white solids.

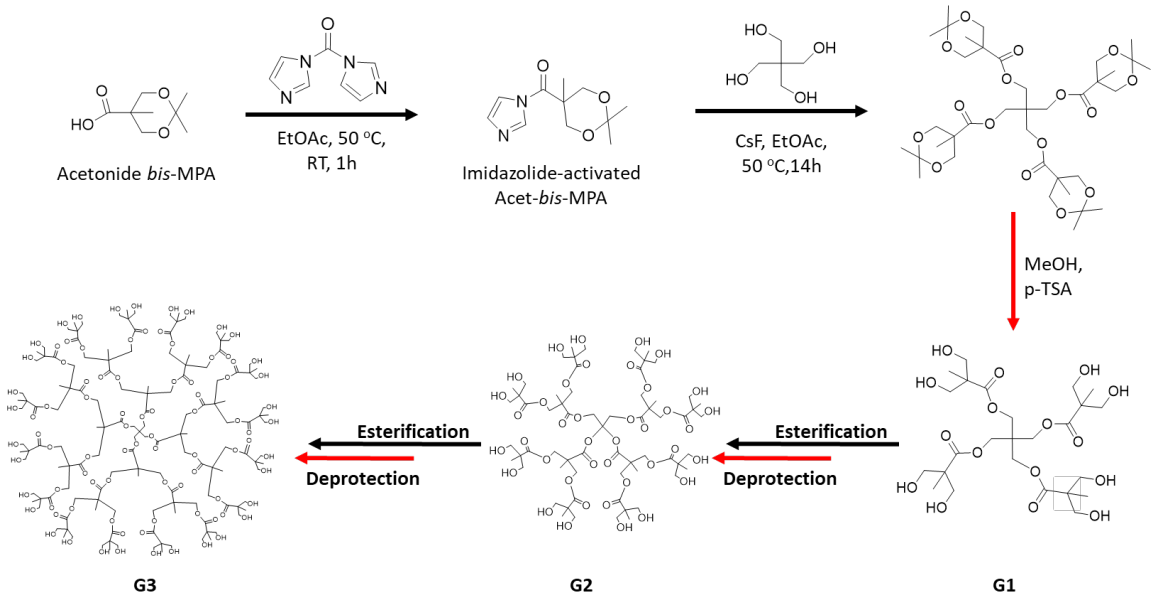
G1-(8)-NH_3^+: ^{1}H NMR (400 MHz, MeOD) δ 4.35 (q, 16H, -C-C**H**$_2$-O-)$^{bis-MPA}$, 4.22 (s, 8H, -C-C**H**$_2$-O-)^{PERT}, 3.24 (t, 16H, -CH_2-C**H**$_2$-NH_3^+)$^{β-ala}$, 2.81 (t, 16H, -CO-C**H**$_2$-CH_2-)$^{β-ala}$, 1.32 (s, 12H, -C-C**H**$_3$)$^{bis-MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 173.59, 171.90, 162.94, 66.69, 62.89, 47.84, 44.37, 36.26, 32.16, 18.04.

G2-(16)-NH_3^+: ^{1}H NMR (400 MHz, MeOD) δ 4.32 (dd, 13.3 Hz, 56H, -C-C**H**$_2$-O-)$^{bis\text{-}MPA/PERT}$, 3.25 (t, 32H, -CH_2-C**H**$_2$-NH_3^+)$^{β\text{-}ala}$, 2.82 (t, 32H, -CO-C**H**$_2$-CH_2-)$^{β\text{-}ala}$, 1.35 (s, 12H, -C-C**H**$_3$)$^{G1\text{-}bis\text{-}MPA}$, 1.29 (s, 24H, -C-C**H**$_3$)$^{G2\text{-}bis\text{-}MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 173.60, 173.60, 171.88, 163.22, 163.19, 119.68, 66.72, 66.31, 47.65, 36.29, 32.21, 18.18.

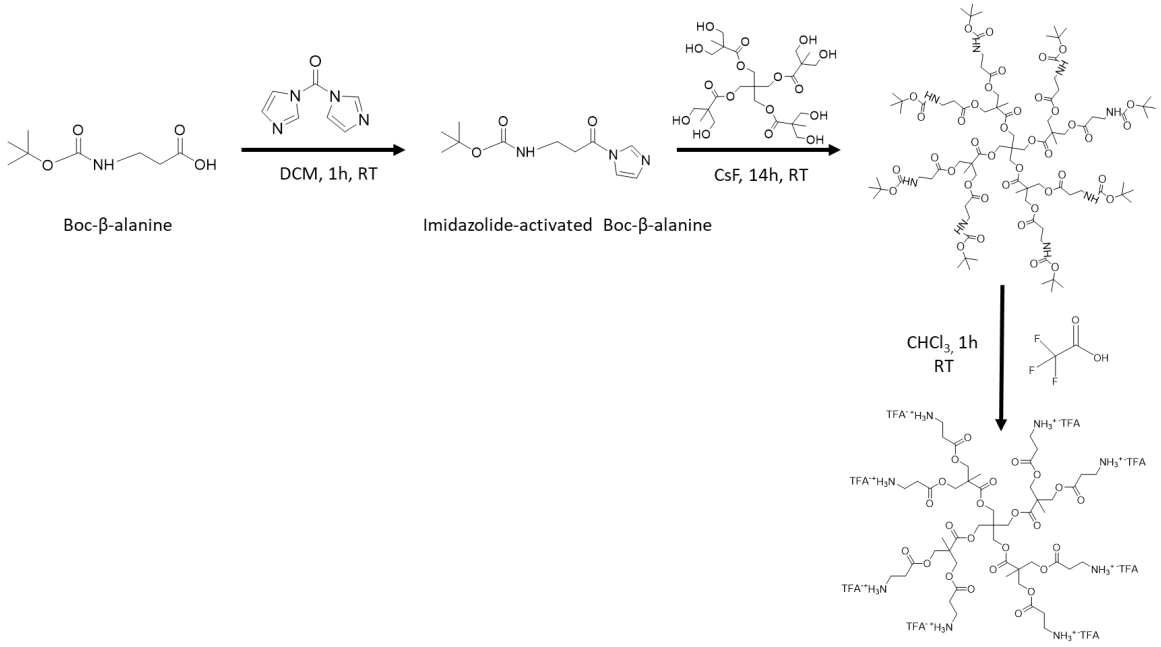
G2HB-(16)-NH_3^+: ^{1}H NMR (400 MHz, D_2O) δ 4.13 (d, 62H, -C**H**$_2$-O-)$^{PERT/EG}$, 3.65 (d, 16H, -C-C**H**$_2$-O-)$^{bis\text{-}MPA}$, 3.19 (s, 32H, -CH_2-C**H**$_2$-NH_3^+)$^{β\text{-}ala}$, 2.75 (s, 32H, -CO-C**H**$_2$-CH_2-)$^{β\text{-}ala}$, 1.19 (s, 36H, -C**H**$_3$)$^{bis\text{-}MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 173.39, 171.80, 163.13, 162.80, 119.63, 116.72, 66.89, 47.68, 36.30, 32.19, 18.04.

G3-(32)-NH_3^+: ^{1}H NMR (400 MHz, MeOD) δ 4.32 (dd, 122H, -C-C**H**$_2$-O-)$^{bis\text{-}MPA/PERT}$, 3.26 (t, 66H, -CH_2-C**H**$_2$-NH_3^+)$^{β\text{-}ala}$, 2.83 (t, 64H, -CO-C**H**$_2$-CH_2-)$^{β\text{-}ala}$, 1.38 (s, 12H, -C-C**H**$_3$)$^{G1\text{-}bis\text{-}MPA}$, 1.32 (s, 24H, -C-C**H**$_3$)$^{G2\text{-}bis\text{-}MPA}$, 1.29 (s, 48H, -C-C**H**$_3$)$^{G3\text{-}bis\text{-}MPA}$. ^{13}C NMR (101 MHz, MeOD) δ 173.57, 171.92, 163.78, 163.18, 119.68, 116.76, 66.69, 47.62, 36.31, 32.23, 18.26, 15.43.

Scheme S1. Synthesis of hydroxyl terminated *bis*-MPA dendrimers.



Scheme S2. Synthesis of amino functional *bis*-MPA dendrimers.



2 Results

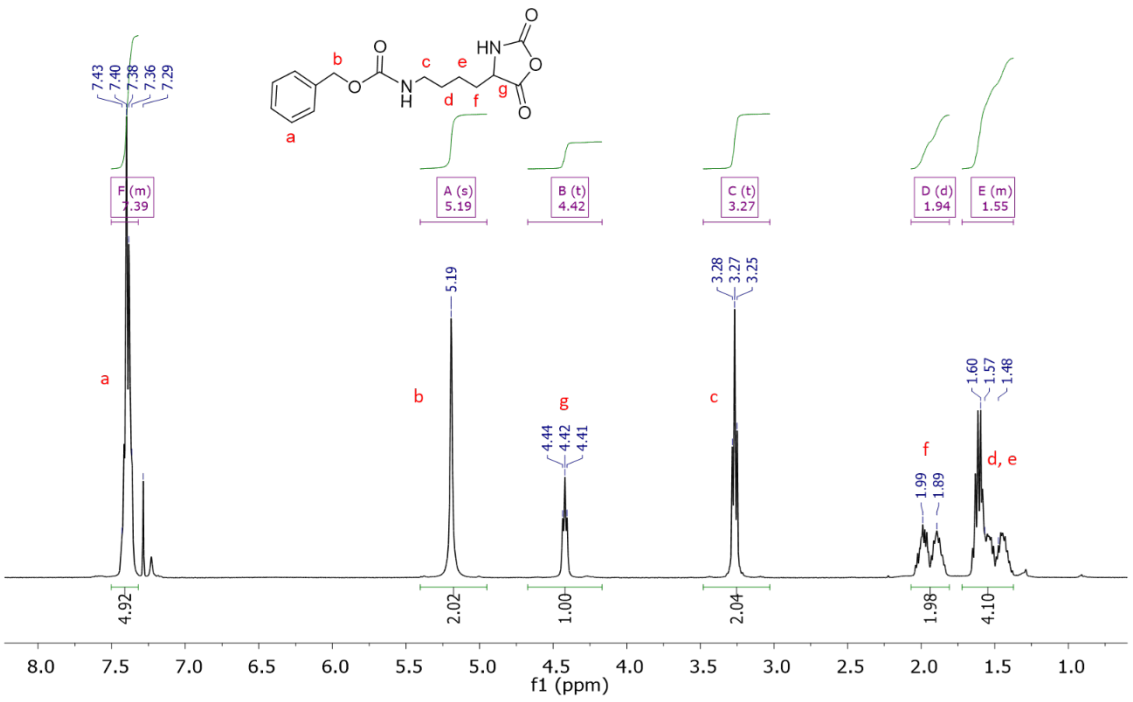


Figure S1. ^{1}H NMR spectrum of ε-carbobenzyloxy-L-lysine N-carboxyanhydride in $CDCl_3$.

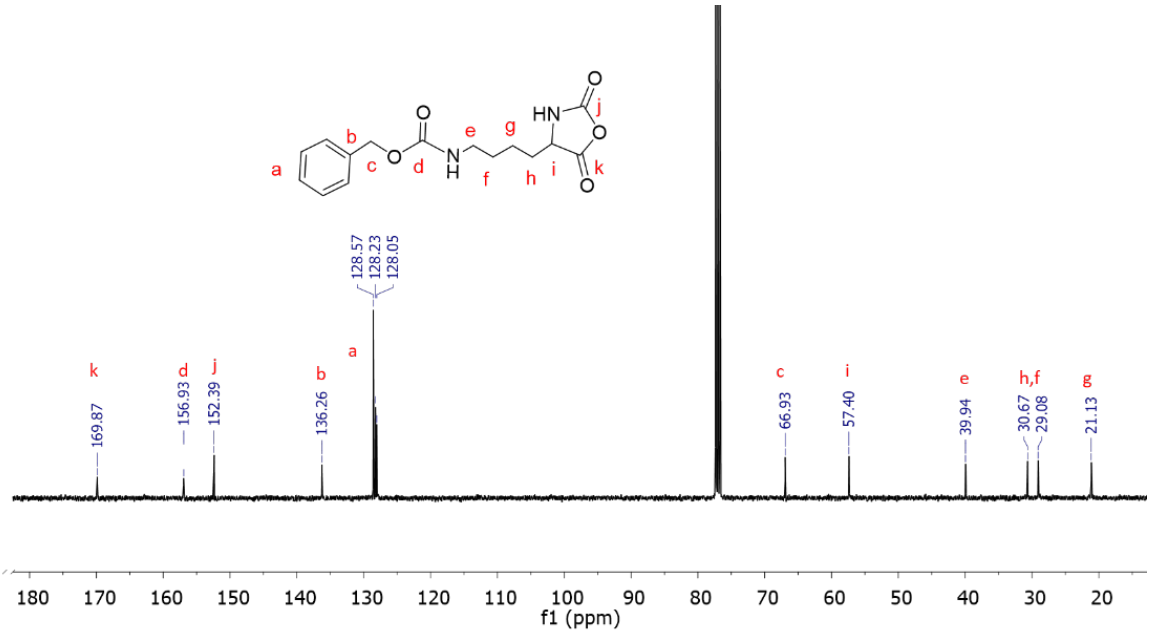


Figure S2. ^{13}C NMR spectrum of ε-carbobenzyloxy-L-lysine N-carboxyanhydride in $CDCl_3$.

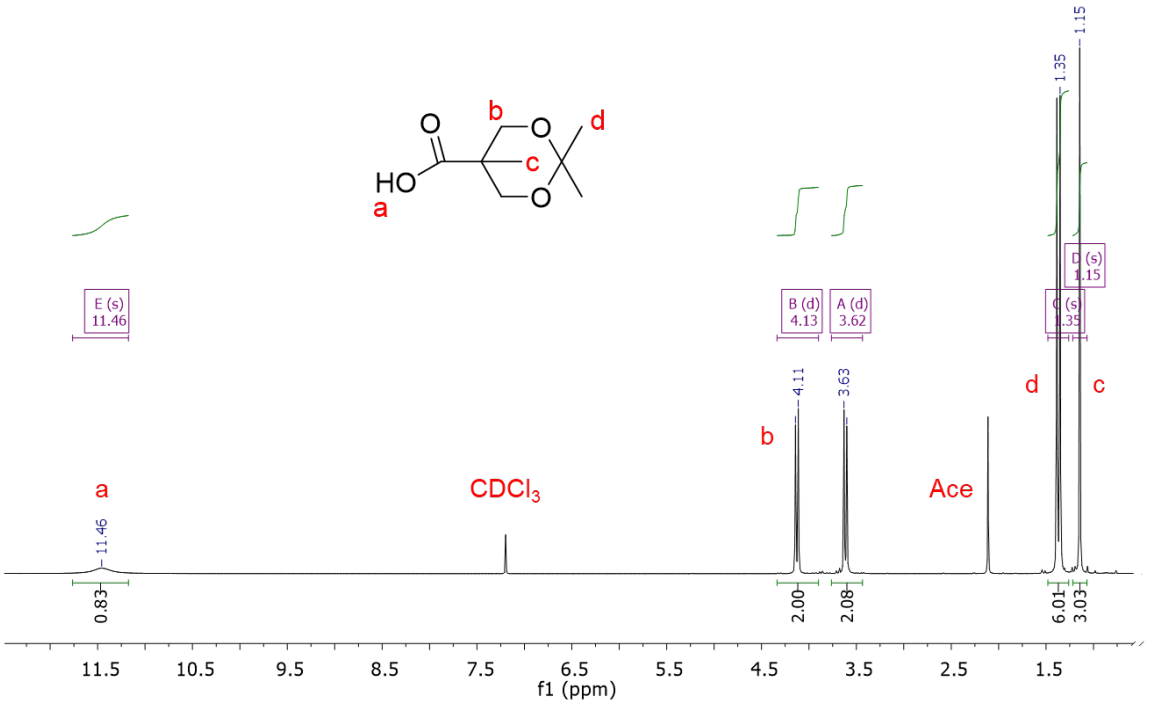


Figure S3. ^{1}H NMR spectrum of acetonide ptotected *bis*-MPA in $CDCl_3$.

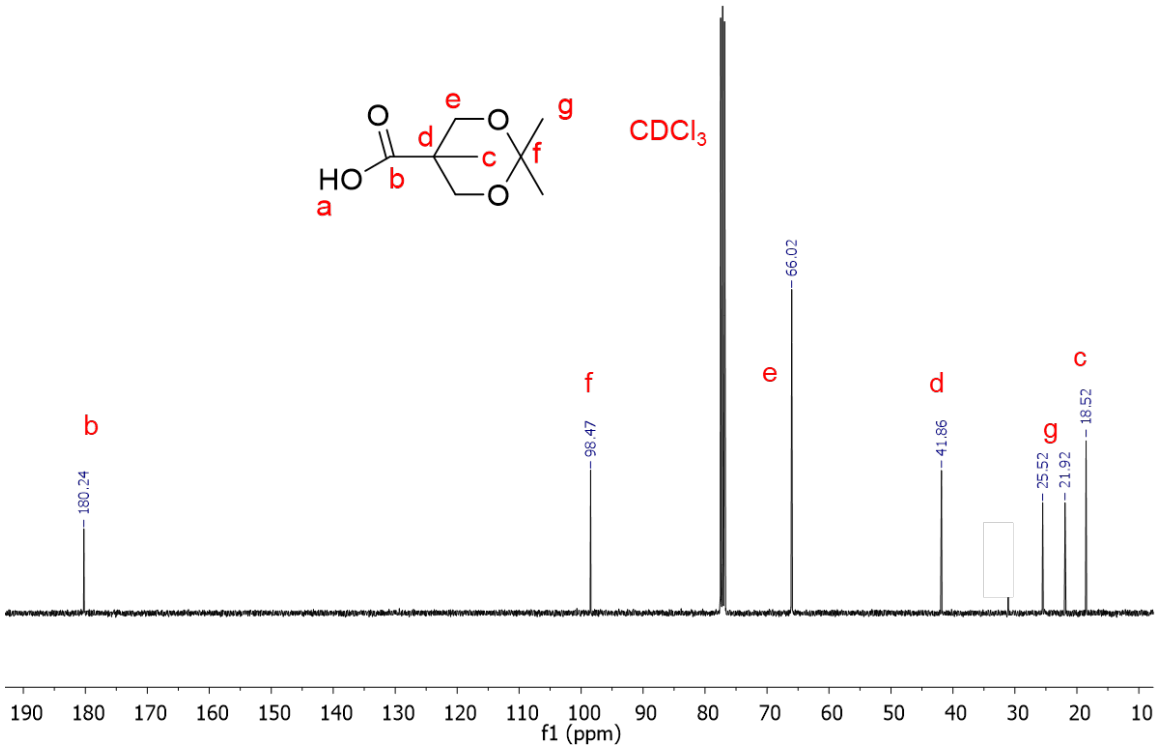


Figure S4. ^{13}C NMR spectrum of acetonide ptotected *bis*-MPA in $CDCl_3$.

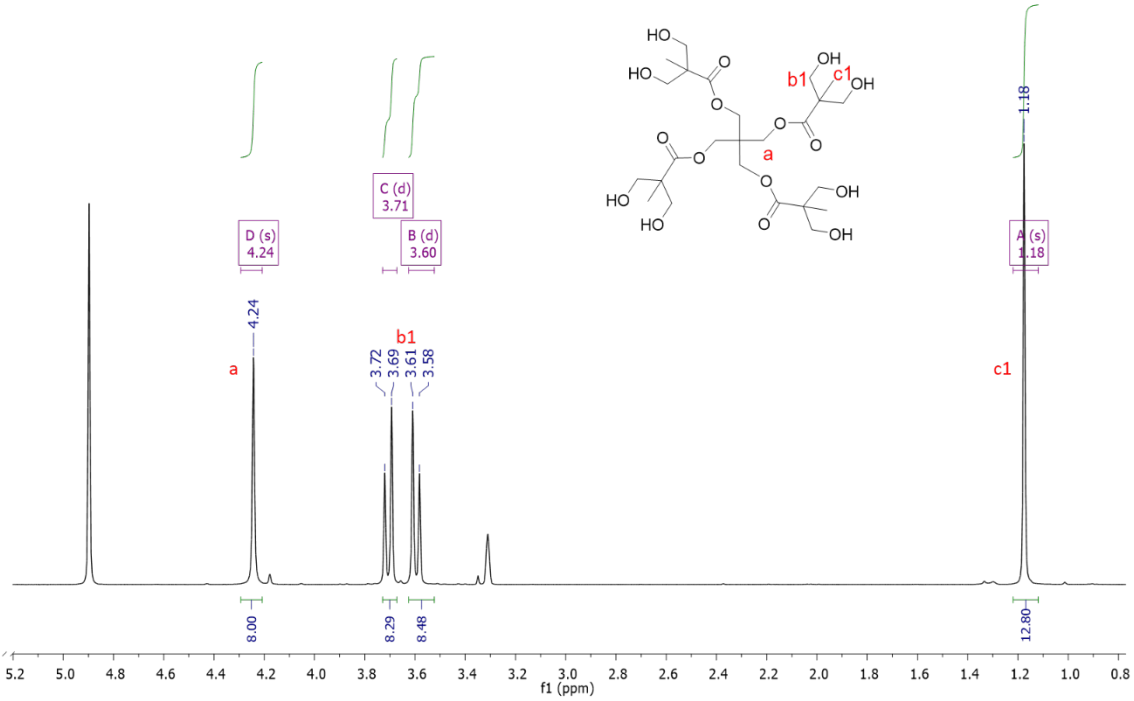


Figure S5. ^{1}H NMR spectrum of G1-(8)-OH in CD_3OD.

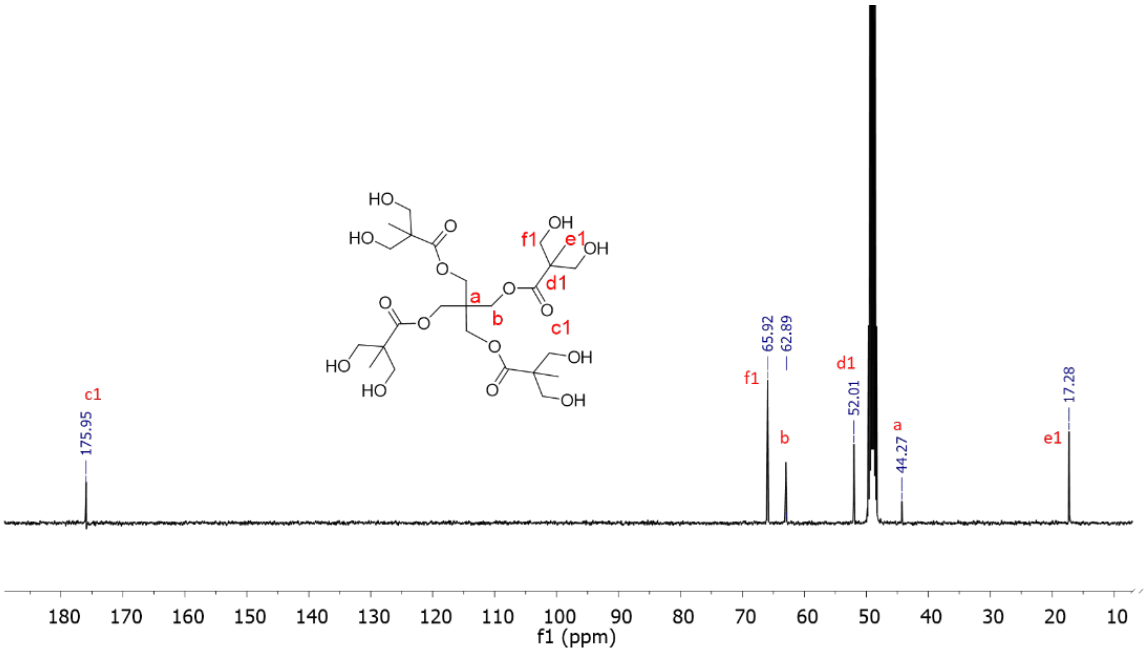


Figure S6. ^{13}C NMR spectrum of G1-(8)-OH in CD_3OD.

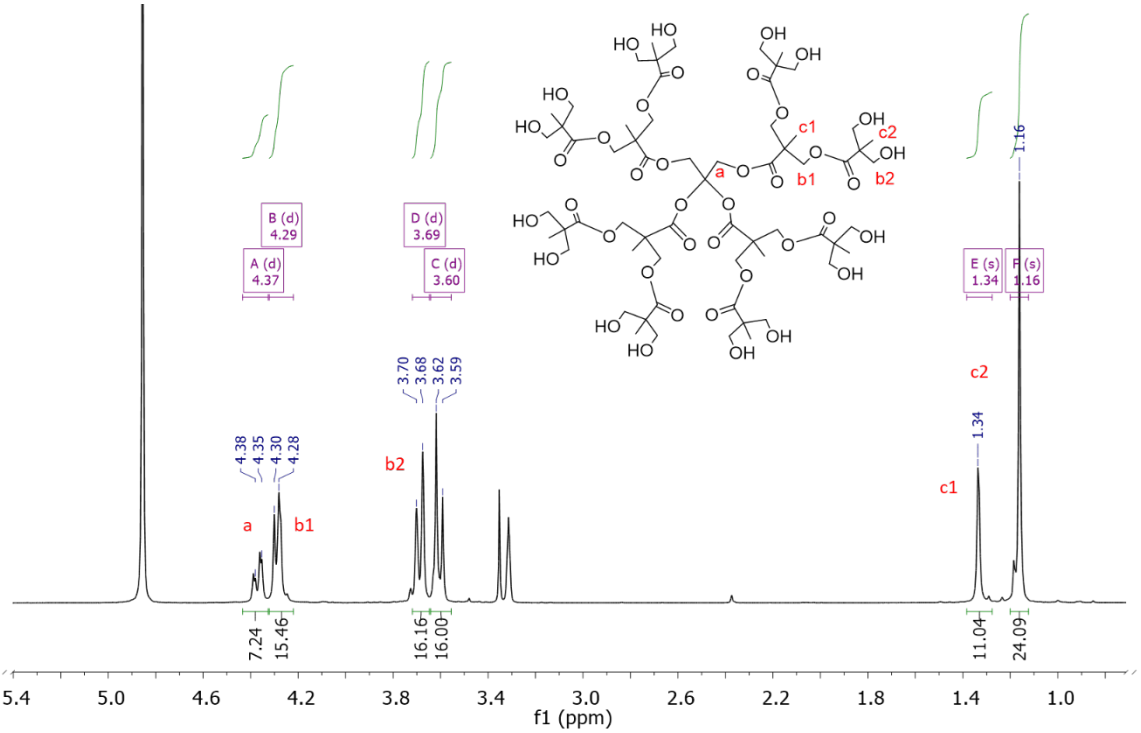


Figure S7. ^{1}H NMR spectrum of G2-(16)-OH in CD_3OD.

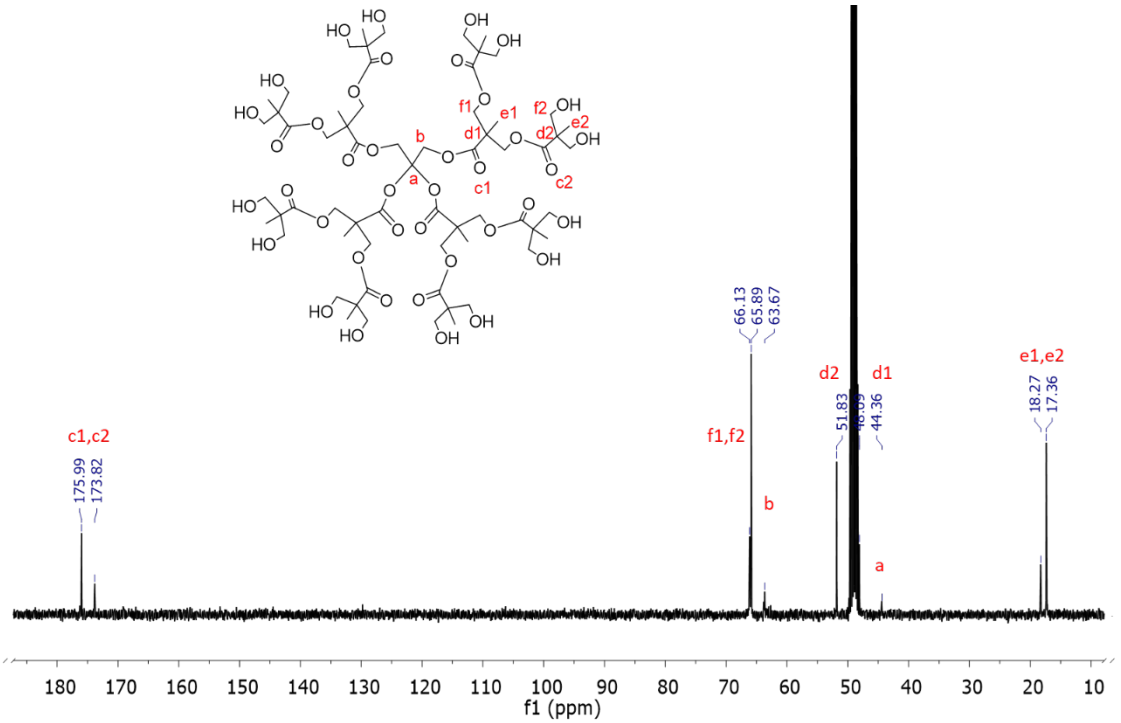


Figure S8. ^{13}C NMR spectrum of G2-(16)-OH in CD_3OD.

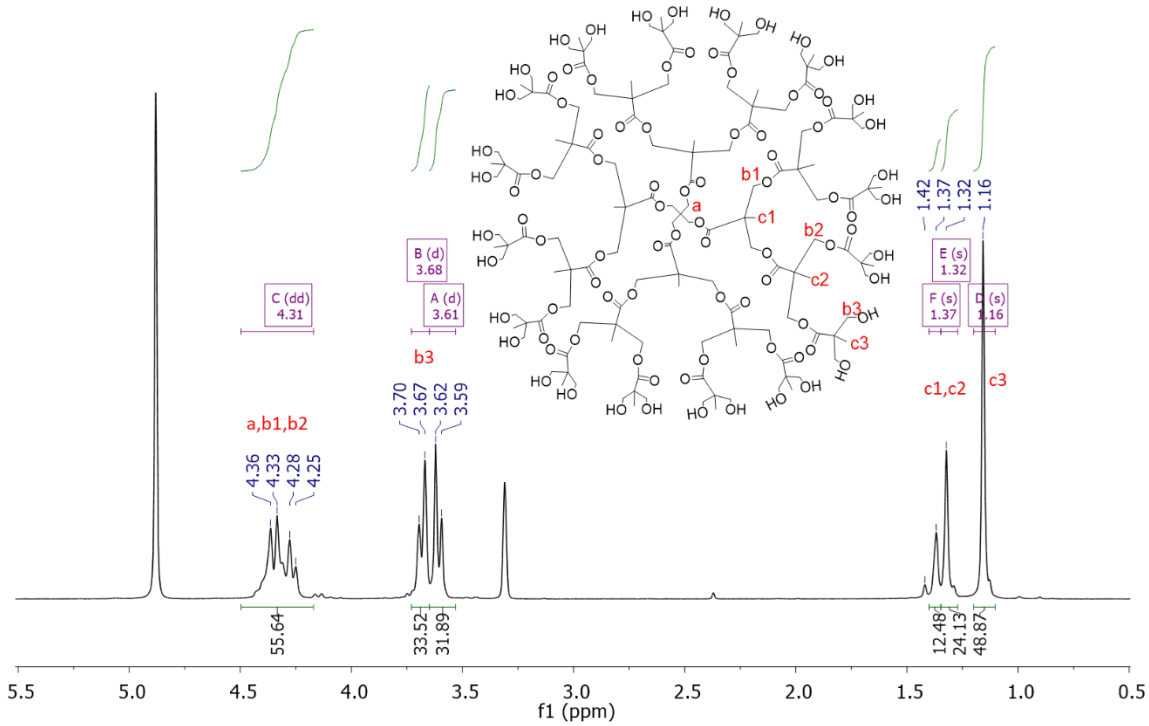


Figure S9. ^{1}H NMR spectrum of G3-(32)-OH in CD_3OD.

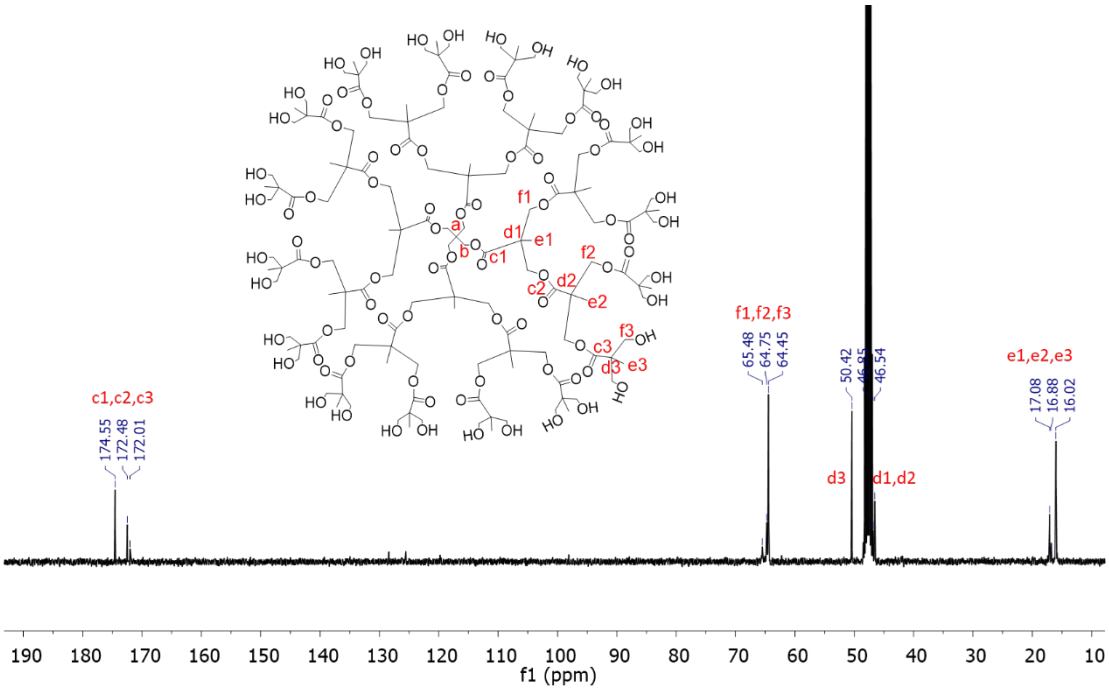


Figure S10. ^{13}C NMR spectrum of G3-(32)-OH in CD_3OD.

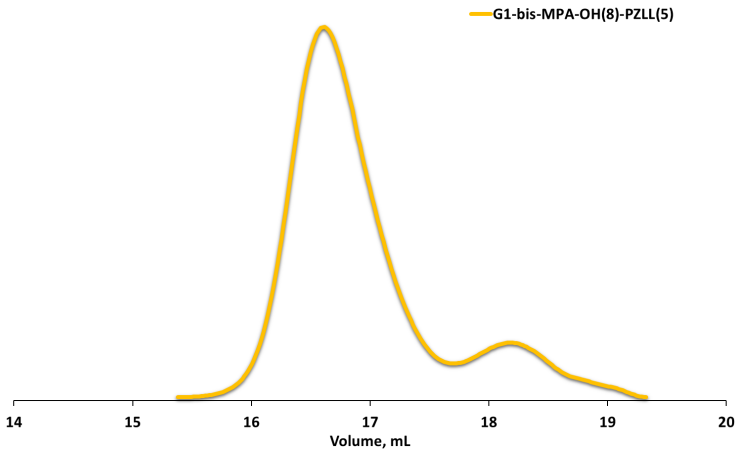


Figure S11. GPC trace of poly(ZLys) initiated from a G1-(8)-OH dendrimer (8 hydroxy groups) (HFIP, dRI detector).

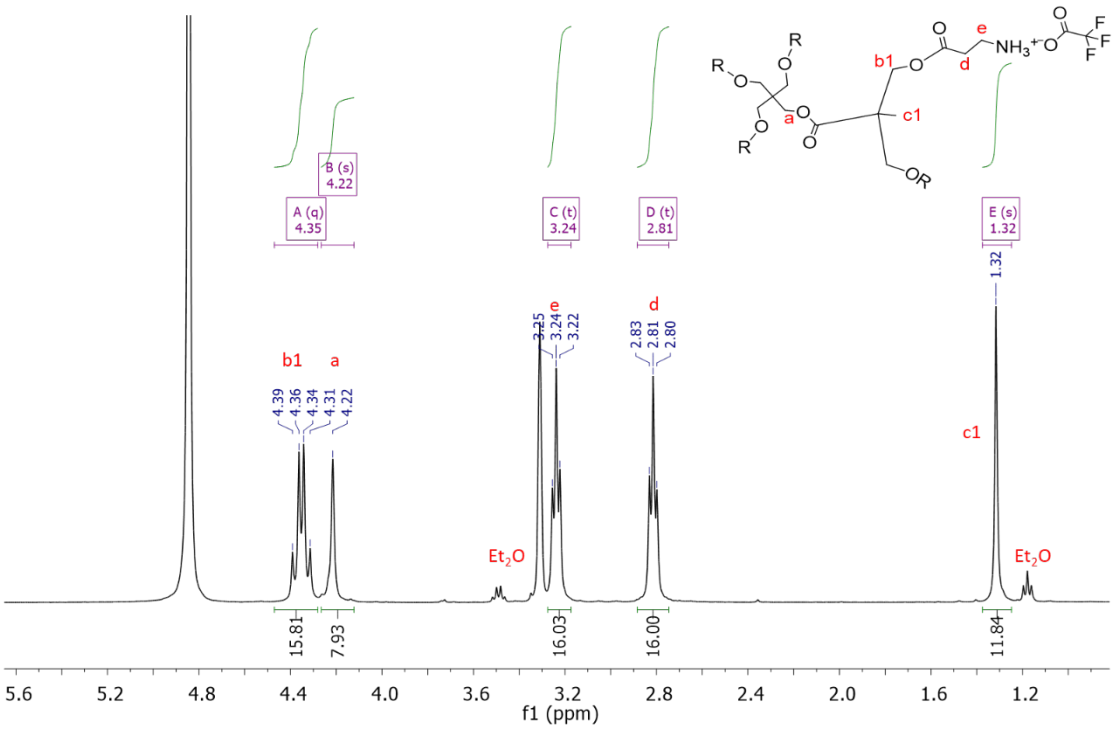


Figure S12. ^{1}H NMR spectrum of G1-(8)-NH_3^+ in CD_3OD.

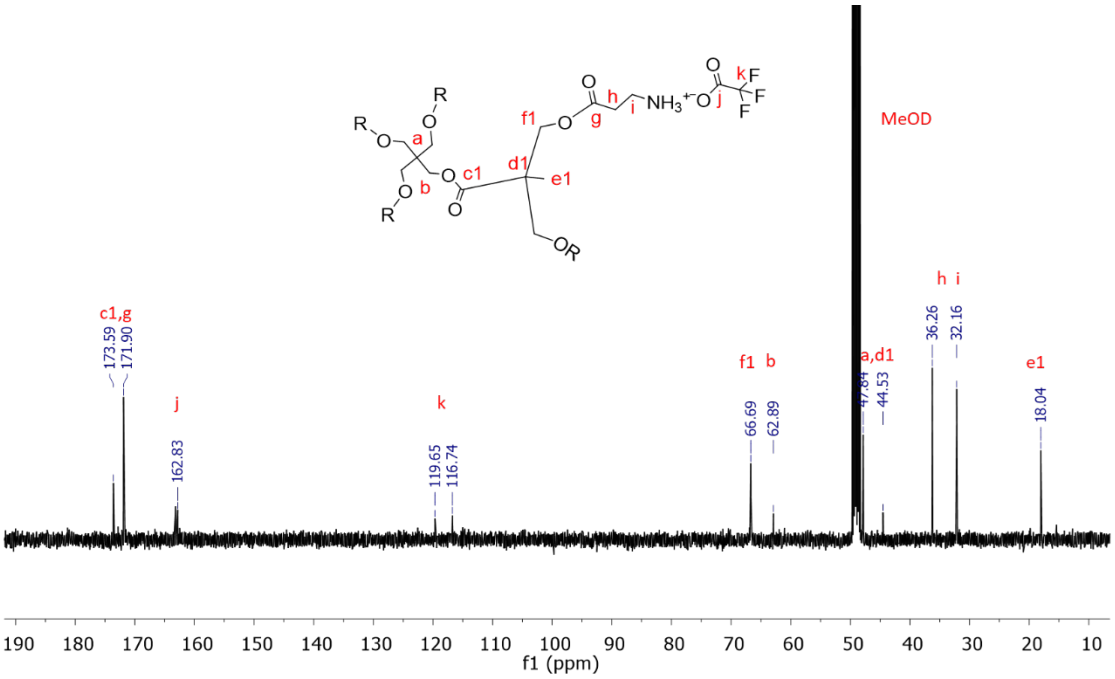


Figure 13. ^{13}C NMR spectrum of G1-(8)-NH_3^+ in CD_3OD.

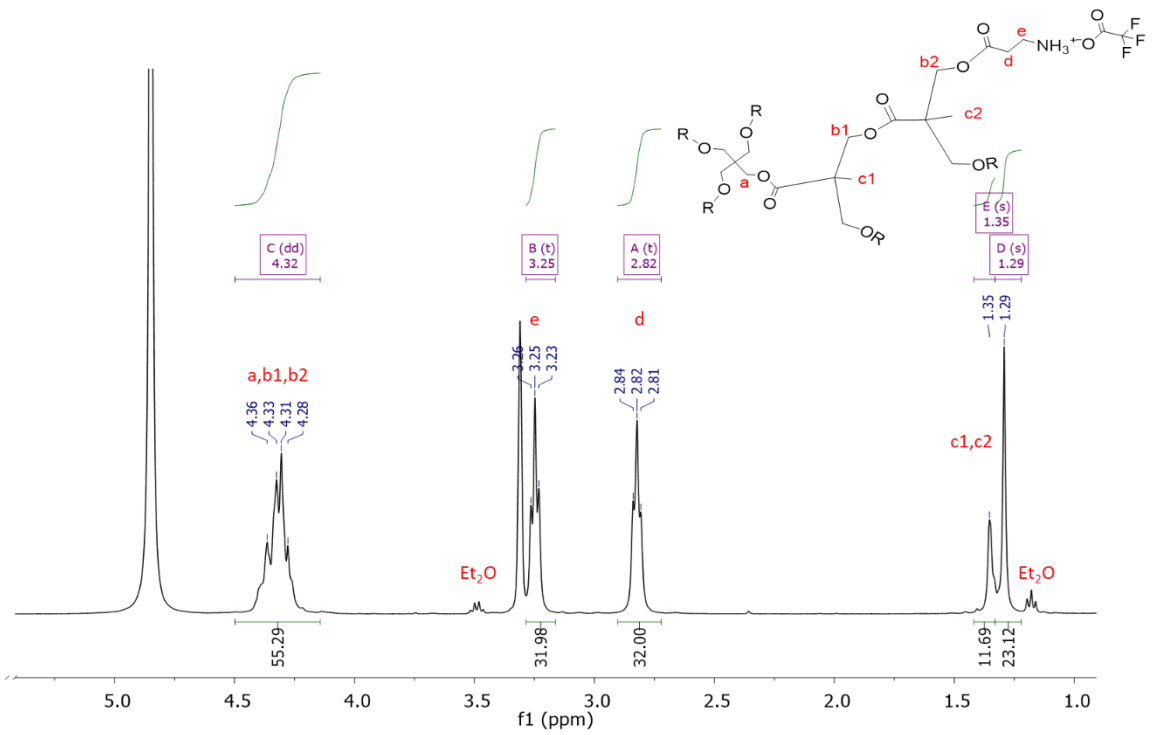


Figure S14. ^{1}H NMR spectrum of G2-(16)-NH_3^+ in CD_3OD.

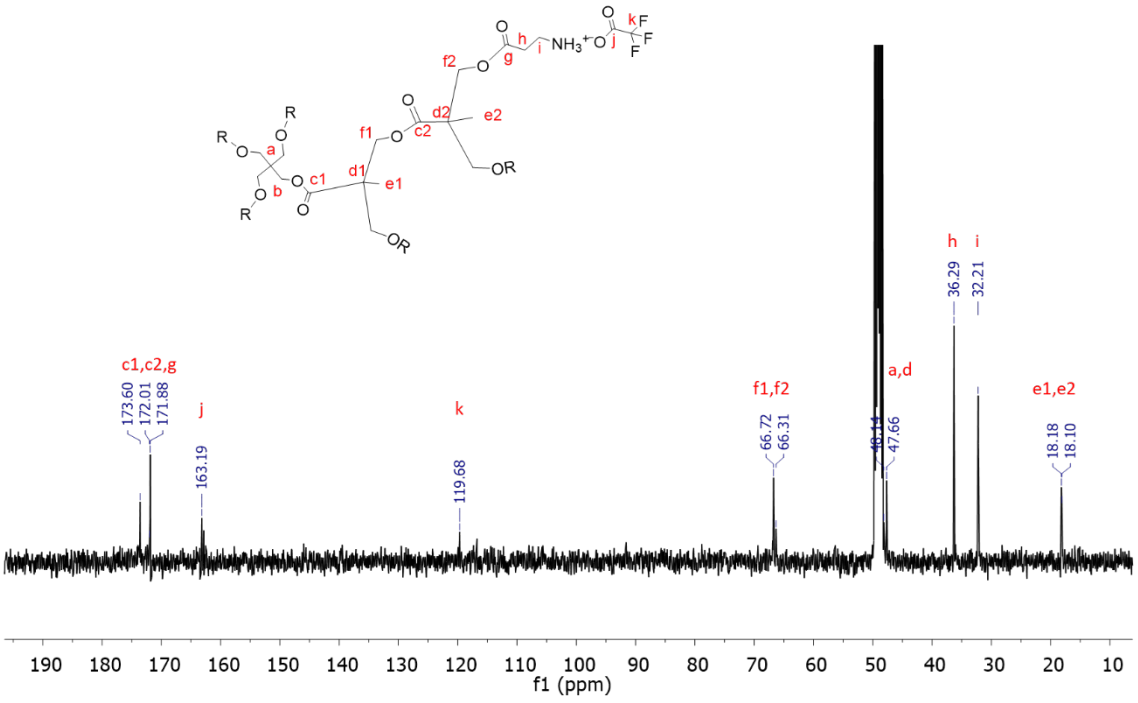


Figure S15. ^{13}C NMR spectrum of G2-(16)-NH_3^+ in CD_3OD.

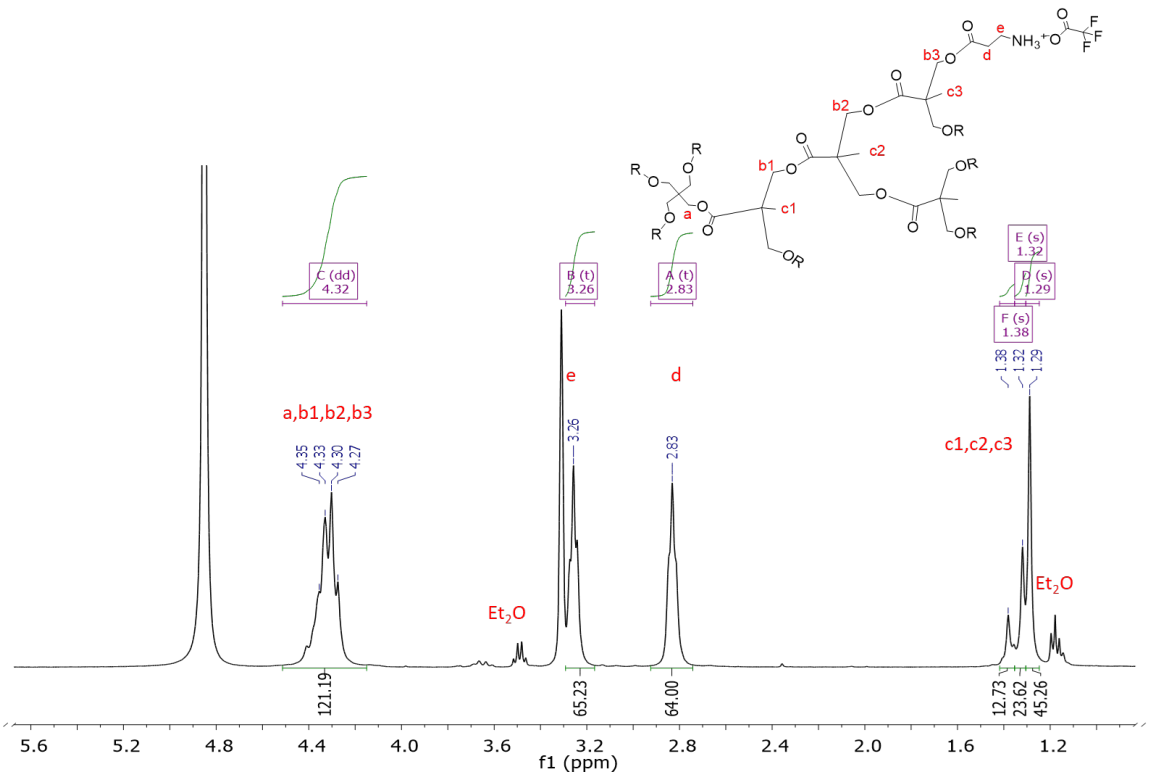


Figure S16. ^{1}H NMR spectrum of G3-(32)-NH_3^+ in CD_3OD.

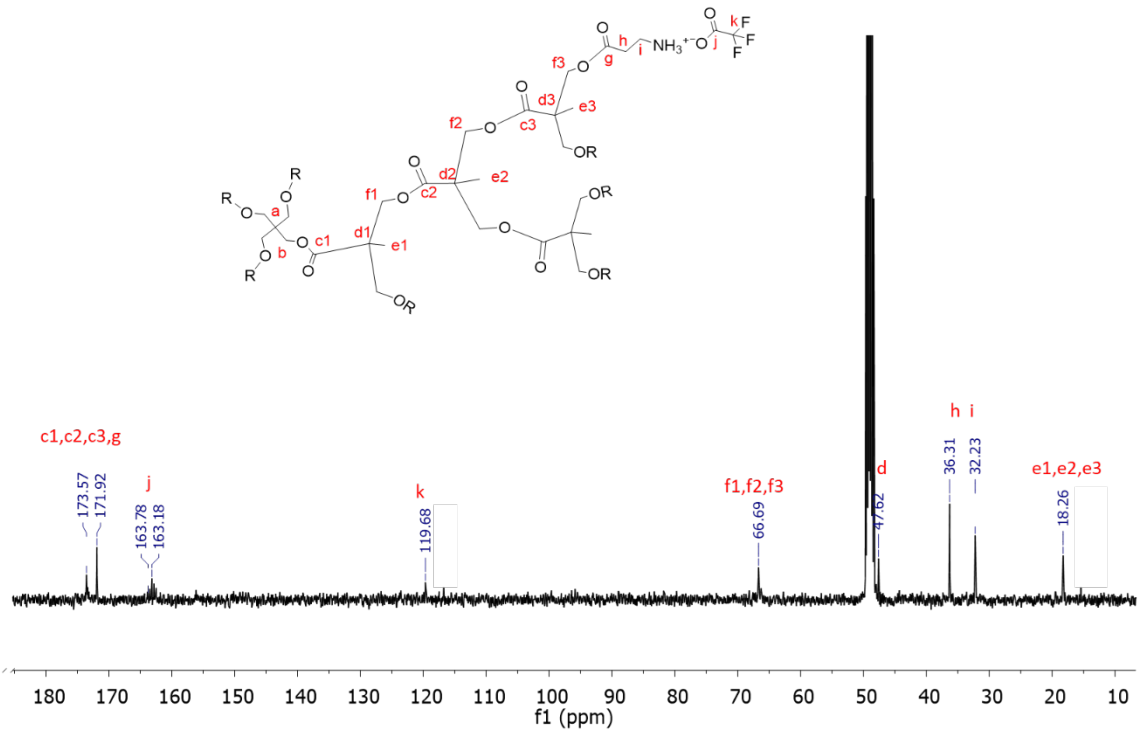


Figure S17. ^{13}C NMR spectrum of G3-(32)-NH_3^+ in CD_3OD.

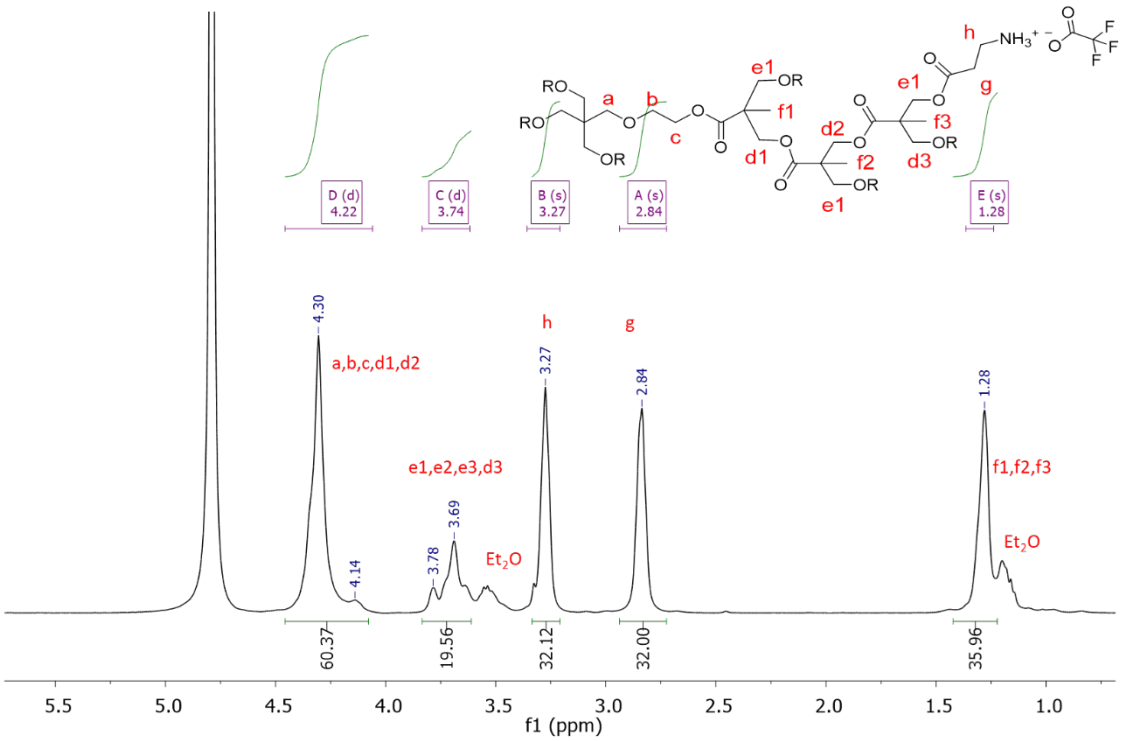


Figure S18. ^{1}H NMR spectrum of G2HB-(16)-NH_3^+ in CD_3OD.

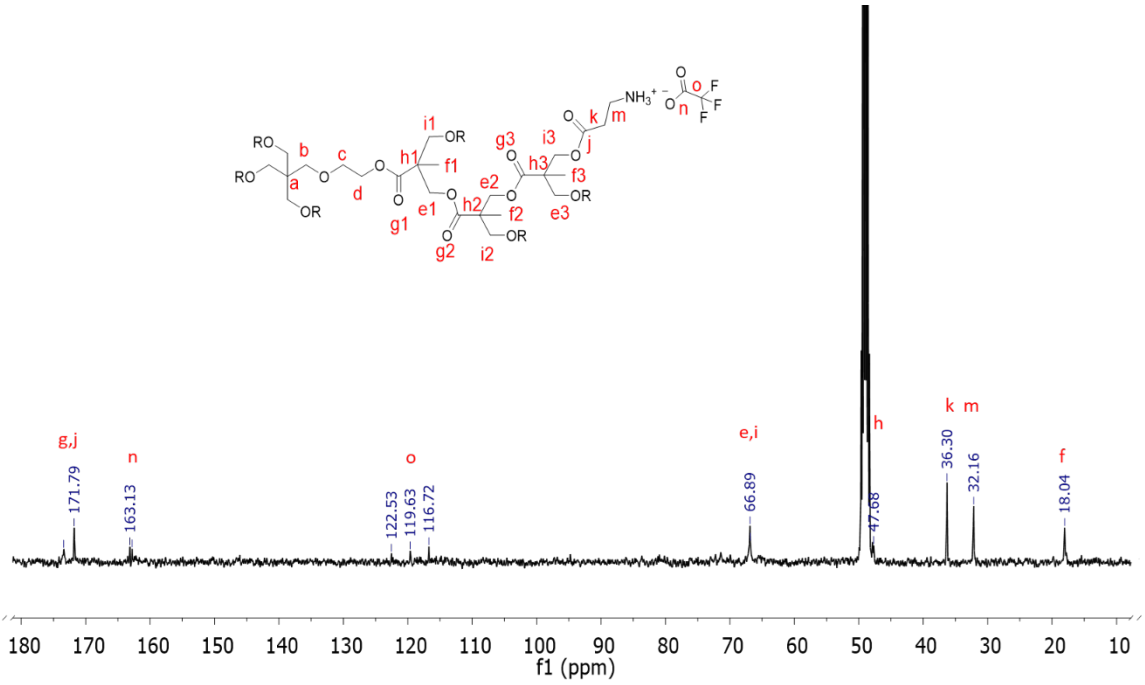


Figure S19. ^{13}C NMR spectrum of G2HB-(16)-NH_3^+ in CD_3OD.

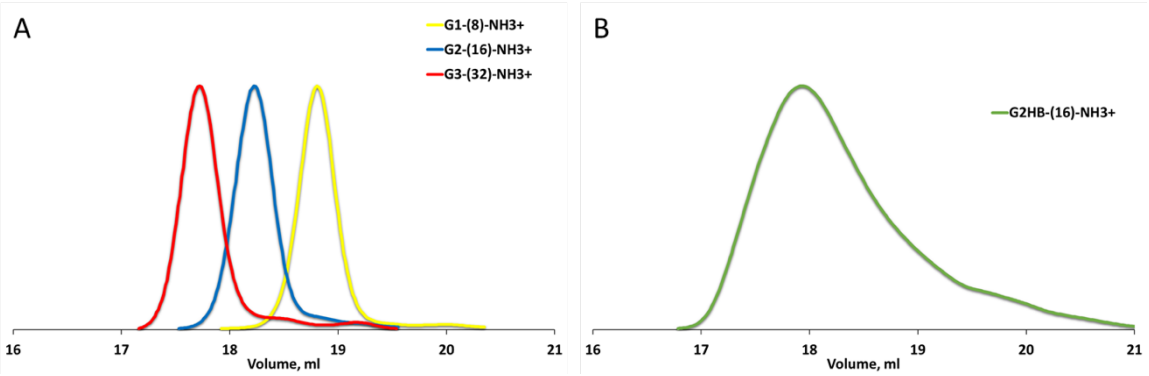


Figure S20. GPC traces of (A) ammonium trifluoroacetate terminated *bis*-MPA dendrimers generation 1-3 (HFIP, dRI detector) and (B) ammonium trifluoroacetate terminated second generation *bis*-MPA hyperbranched polymer.

Table S1. Ammonium trifluoroacetate terminated *bis*-MPA dendrimers generation 1-3 and second generation of *bis*-MPA hyper-branched polymer.

Sample	**No. of terminal amines**	$M_{n\ theory}$ **(g/mol)**[a]	M_n^{GPC} **(g/mol)**[b]	$Đ_M$ [b]
G1-(8)-NH_3^+	8	2 081	3 100	1.03
G2-(16)-NH_3^+	16	4 463	4 200	1.03
G2HB-(16)-NH_3^+	16	4 709	4 000	1.47
G3-(32)-NH_3^+	32	9 310	6 500	1.06
(a) Theoretical molecular weight of the dendrimers, b) GPC using HFIP as the mobile phase at a flow rate of 1 mL/min (PMMA standards).				

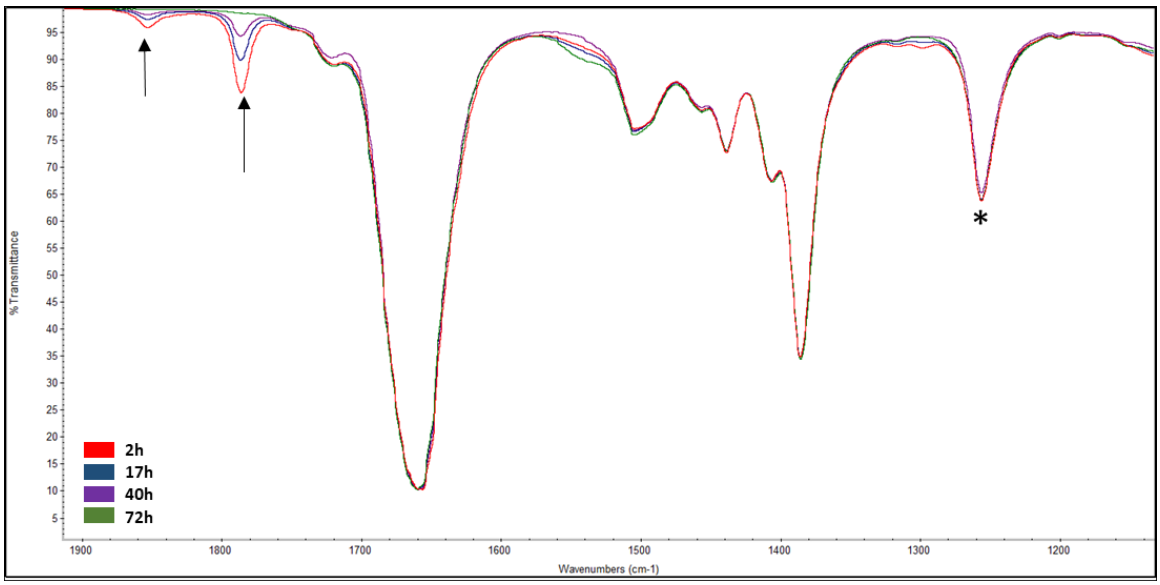


Figure S21. ATR-FTIR spectra of G1-(8)-$PZLL_5$ at different time points (2h, 17h, 40h, 72h). Arrows indicate NCA signature band reduction as polymerization progresses. Asterisk indicates unchanging band use for calculation of conversion.

Table S2. Intensity of corresponding NCA peaks obtained from FTIR spectra during the synthesis of G1-(8)-PZLL(40) at different time points.

I_{1786}[a]	I_{1255}[b]	Time, h	R	Remaining NCA[c], %	Conversion,%
192.60[d]		0	0.28	100	0
134.96		2	0.20	70	30
109.30	691.07	17	0.16	57	43
50.15		40	0.07	26	74
0.00		72	0.00	0	100

(a) Intensity of the anhydride band at 1786 cm^{-1} at different time intervals. (b) Intensity of L-Lysine finger print band at 1255 cm^{-1}that did not change during the polymerization. (c) Quantification of the remaining amount of NCA in the reaction calculated as the ratio between the intensity ratio at the given time divided by the intensity ratio at time 0. (d) Intensity of the anhydride band before adding the initiator ($\boldsymbol{I_0^{1786}}$).

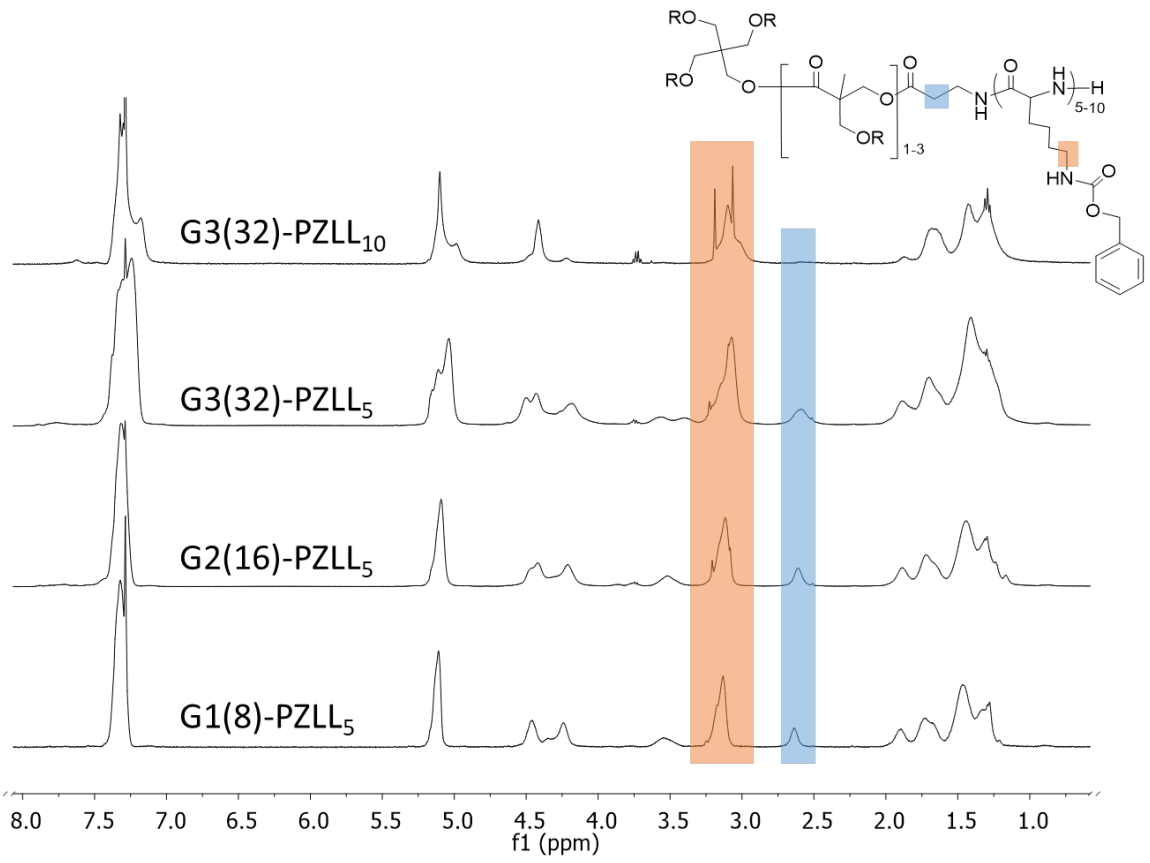


Figure S22. ^{1}H NMR spectra of star poly(ZLys) with arm length 5 and 10 in $CDCl_3$/d-TFA.

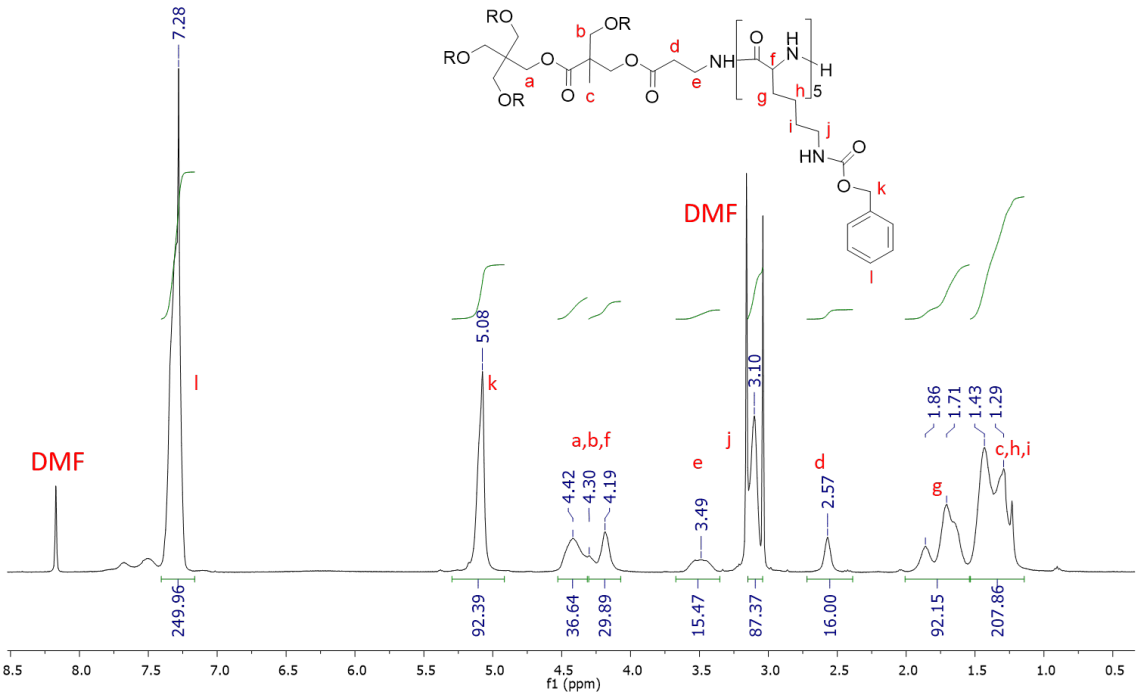


Figure S23. ^{1}H NMR spectrum of G1-(8)-PZLL$_5$ in $CDCl_3$/d-TFA.

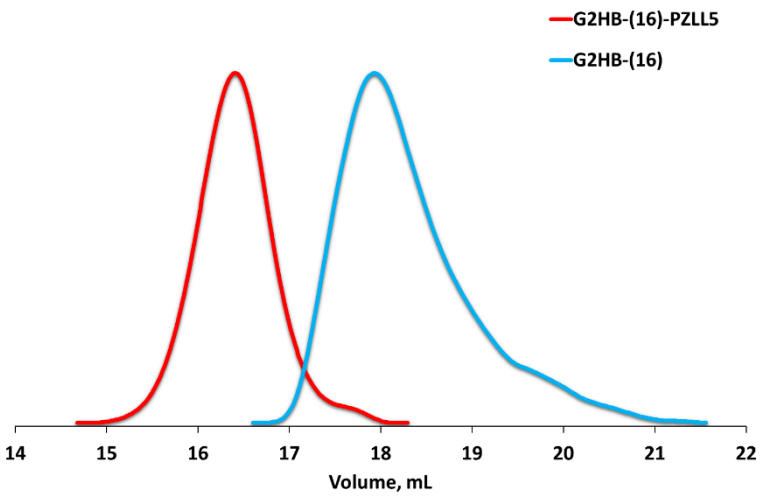


Figure S24. GPC chromatograms of amino terminated hyperbranched polymer and the corresponding polypeptide.

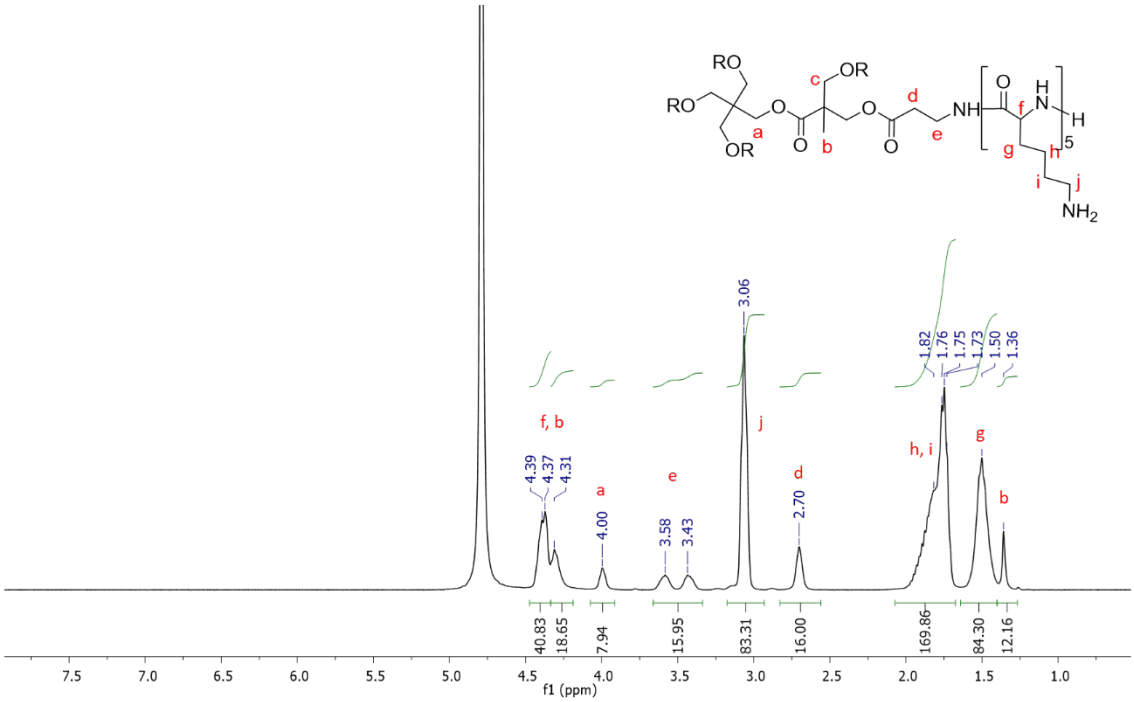


Figure S25. ^{1}H NMR spectrum of G1-(8)-PLL$_5$ in D_2O.

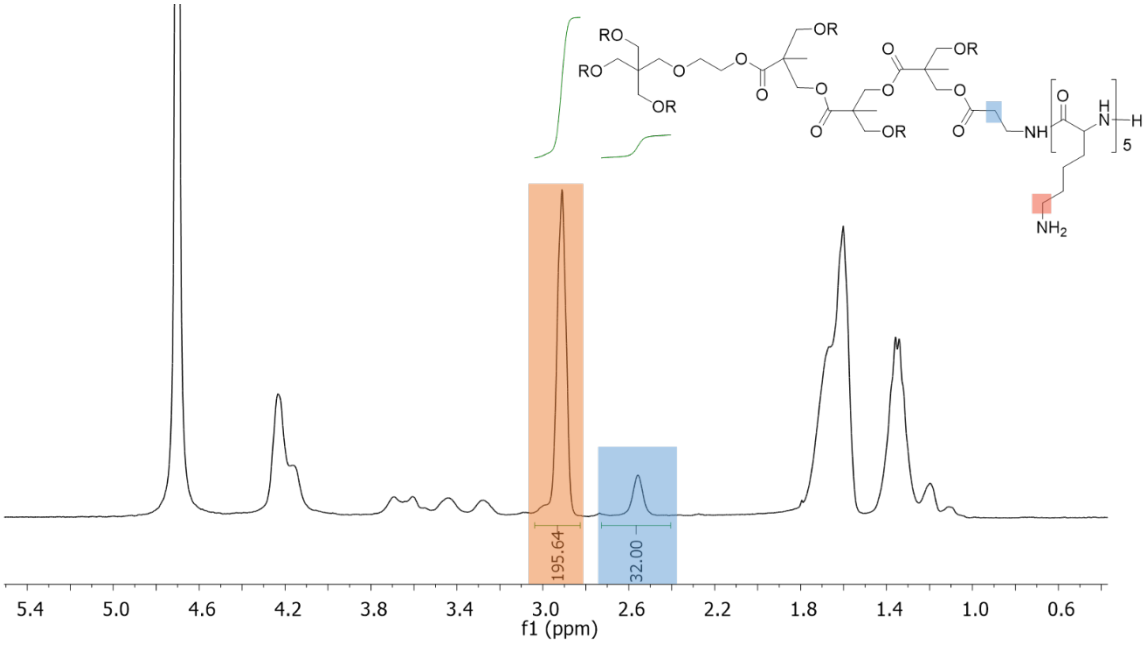


Figure S26. ^{1}H NMR spectrum of G2HB-(16)-PLL$_5$ in D_2O.

3 References

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