

# Synthesis, nanoprecipitation and pH sensitivity of amphiphilic linear-dendritic hybrid polymers and hyperbranched-polydendrons containing tertiary amine functional dendrons

Hannah E. Rogers,<sup>a</sup> Pierre Chambon,<sup>a</sup> Sam E. R. Auty,<sup>a</sup> Faye Y. Hern,<sup>a</sup>  
Andrew Owen<sup>b</sup> and Steve P. Rannard<sup>a\*</sup>

Electronic Supplementary Information

### Materials and suppliers

Anhydrous tertiary-butanol (**1**,  $\geq 99.5\%$ ), 1-dimethylamino-2-propanol (**7**,  $\geq 99\%$ ), 1-amino-2-propanol (**8**,  $\geq 98\%$ ),  $\alpha$ -bromoisobutyryl bromide (BiB, 98%), 4-(dimethylamino)pyridine (DMAP,  $\geq 99\%$ ), triethylamine (TEA,  $\geq 99\%$ ), dipropylentriamine (98%), propylene oxide ( $\geq 99.7\%$ ), 2-hydroxypropyl methacrylate (HPMA, 97%), ethylene glycol dimethacrylate (EGDMA, 98%), copper(I)chloride (Cu(I)Cl,  $\geq 99\%$ ), 2,2'-bipyridyl (bpy,  $\geq 99\%$ ) and ethyl  $\alpha$ -bromoisobutyrate (EBiB, 98%) were purchased from Aldrich. 2-(dimethylamino)ethyl acrylate (DMEA, 98%) was purchased from Alfa Aesar. 1,1'-Carbonyldiimidazole (CDI,  $\geq 97\%$ ) was purchased from Apollo Scientific Ltd. All purchased materials were used as received.

Anhydrous toluene, ethanol and methanol, neutral alumina for catalyst removal and silica gel used for column chromatography were purchased from Aldrich and used as received. All other solvents and reagents were analytical grade and purchased from Fisher.

### Analytical Equipment

Analytical thin layer chromatography (TLC) was performed on commercial Merck Plates coated with silica gel. Nuclear Magnetic Resonance (NMR) spectroscopy spectra were collected using a Bruker DPX-400 spectrometer.  $^1\text{H}$  NMR spectra were recorded at 400 MHz and  $^{13}\text{C}$  NMR spectra were recorded at 100 MHz. Deuterated chloroform ( $\text{CDCl}_3$ ), deuterated methanol ( $\text{CD}_3\text{OD}$ ) and deuterated water ( $\text{D}_2\text{O}$ ) containing tetramethylsilane (TMS) purchased from Sigma Aldrich were used as NMR solvents. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) and TMS was used as an internal standard for both  $^1\text{H}$  and  $^{13}\text{C}$  spectra. Electrospray mass spectrometry data (ES-MS) was recorded in the Mass Spectrometry Laboratory at the University of Liverpool. The data was obtained using a MicroMass LCT mass spectrometer using electron ionisation and direct infusion syringe pump sampling. All materials were diluted with methanol. Elemental analyses were obtained from a Thermo FlashEA 1112 series CHNSO elemental analyser. Size Exclusion Chromatography (SEC) was carried out using a Malvern Viscotek GPC Max connected to a Viscotek 270 Light Scattering detector, Viscometer and Refractive index (RI) triple detection system. HPLC grade THF (Fisher) containing 2% TEA

(Sigma Aldrich) was used as the eluent, with a flow rate of 1 mL/min. SEC Columns were mixed bed columns supplied by Viscotek. The column oven was set at 35°C. The obtained chromatograms were analysed using Malvern OmniSec software calibrated by a universal calibration calculation relative to a narrow linear polystyrene standard (105 kg/mol). Nanoparticle characterisation and pH autotitrations were carried out on a Malvern Zetasizer Nano ZS dynamic light scattering (DLS) instrument. Autotitrations were used to determine  $pK_a$  values and characterize nanoparticle dispersions through slow sodium hydroxide (NaOH, 0.25M) addition. Scanning electron microscopy (SEM) images were obtained using a Hitachi S-4800 FE-SEM. Aqueous nanoparticle samples (1 mg/mL) were either dropped onto a glass cover slide or silicon wafer mounted on an aluminium stub with a carbon tab, dried overnight at ambient temperature then Au coated at 20 mA for 2 minutes. Fourier-Transfer Infrared (FT-IR) measurements were carried out on a Bruker Alpha FT-IR Spectrometer and analysed using Bruker OPUS software.

### Synthesis

**G<sub>0</sub>-Br initiator, 11** - 1-dimethylamino-2-propanol, **7**, (1.12 g, 10.86 mmol, 1 eq.), TEA (1.54 g, 15.2 mmol, 1.4 eq.) and DMAP (132.7 mg, 1.086 mmol, 0.1 eq.) were added to a 250 mL 2 necked round-bottomed flask containing dichloromethane (DCM, 160 mL). The flask was deoxygenated under a positive nitrogen (N<sub>2</sub>) purge for 10 minutes. BiB (2.62 g, 1.4 mL, 11.4 mmol, 1.05 eq.) was added drop wise while the solution was stirring in an ice bath under a positive flow of N<sub>2</sub>. The reaction mixture was allowed to warm to room temperature and left stirring overnight. The organic phase was washed with saturated sodium hydrogen carbonate (NaHCO<sub>3</sub>) solution (3 x 160 mL) and the solution was subsequently dried with anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>). Yield: 2.38 g, yellow oil (84%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 1.27 (d, 3H), 1.89 (s, 6H), 2.17-2.55 (m, 8H), 5.07 (m, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  = 17.8, 31.1, 46.2, 56.1, 64.0, 70.7, 170.9. Calcd [MH]<sup>+</sup> (C<sub>9</sub>H<sub>18</sub>NO<sub>2</sub>Br) m/z = 252.15 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 252.0 Da. Anal. Calcd for C<sub>9</sub>H<sub>18</sub>NO<sub>2</sub>Br: C 42.86%; H 7.14%; N 5.55%. Found C 42.87%; H 7.20%; N 5.55%.

**G<sub>1</sub>-OH, 9** – DMEA (6.0 g, 42 mmol, 6 eq.) was added to a 50 mL round-bottomed 2 necked round-bottomed flask containing propan-2-ol (IPA, 12 mL). The flask was deoxygenated under a positive N<sub>2</sub> purge for 10 minutes. 1-amino-2-propanol, **8**,

(0.53 g, 7.0 mmol, 1 eq.) dissolved in IPA (12 mL) was added drop wise while the solution was stirring in an ice bath under a positive flow of N<sub>2</sub>. The final mixture was stirred for a further 10 minutes at 0°C before being allowed to warm to room temperature and left stirring for 48 hours. The solvent was removed and the product left to dry *in vacuo* overnight. Yield: 2.75 g, yellow oil (92%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 1.08 (d, 3H), 2.18-2.62 (m, 22H), 2.69 (m, 2H), 2.89 (m, 2H), 3.77 (m, 1H), 4.16 (m, 4H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 19.7, 32.6, 45.6, 49.5, 57.8, 62.1, 62.5, 63.8, 173.0. Calcd [MH]<sup>+</sup> (C<sub>17</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>) m/z = 361.5 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 362.3 Da. Anal. Calcd for C<sub>17</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>: C 56.43%; H 9.68%; N 11.62%. Found C 57.45%; H 9.77%; N 11.12%.

**G<sub>1</sub>-Br initiator, 12 – 9** (1.12 g, 10.86 mmol, 1 eq.), TEA (1.54 g, 15.2 mmol, 1.4 eq.) and DMAP (132.7 mg, 1.086 mmol, 0.1 eq.) were added to a 250 mL 2 necked round-bottomed flask containing DCM (160 mL). The flask was deoxygenated under a positive N<sub>2</sub> purge for 10 minutes. BiB (2.62 g, 1.4 mL, 11.4 mmol, 1.05 eq.) was added drop wise while the solution was stirring in an ice bath under a positive flow of N<sub>2</sub>. The reaction mixture was allowed to warm to room temperature and left stirring overnight. The organic phase was washed with saturated NaHCO<sub>3</sub> solution (3 x 160 mL) and then dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and the product left to dry *in vacuo* overnight. Yield: 1.06 g, yellow oil (67%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 1.22 (d, 3H), 1.89 (s, 6H), 2.24-2.69 (m, 22H), 2.83 (m, 4H), 4.20 (m, 4H), 5.0 (m, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 18.5, 33.0, 44.2, 50.3, 56.4, 58.7, 59.1, 59.7, 68.3, 69.2, 166.6, 172.0. Calcd: [MH]<sup>+</sup> (C<sub>21</sub>H<sub>40</sub>N<sub>3</sub>O<sub>6</sub>Br) m/z = 510.5 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 510.2 Da. Anal. Calcd for C<sub>21</sub>H<sub>40</sub>N<sub>3</sub>O<sub>6</sub>Br: C 49.41%; H 7.84%; N 8.24%. Found: C 49.41%; H 7.90%; N 8.23%.

**Synthesis of intermediate 3** – CDI (39.14 g, 0.241 mol.) was added to an oven-dried 500 mL 2-neck round-bottomed flask fitted with a reflux condenser, magnetic stirrer and a dry N<sub>2</sub> inlet. Anhydrous toluene (350 mL) was added and the flask was purged with N<sub>2</sub> for 10 minutes. The solution was stirred at 60°C and tertiary-butanol, **1** (35.7 g, 46 mL, 0.483 mol) added via a warm syringe. The mixture was left stirring at 60°C for 6 hours under a positive flow of N<sub>2</sub>. Dipropylenetriamine (16.077 g, 17.14 mL, 0.121 mol) was added drop wise, and the reaction was left stirring for a further 18

hours at 60°C under a positive flow of N<sub>2</sub>. Following this, the solution was allowed to cool to room temperature, and the pale yellow solution was filtered to remove any solid imidazole, and concentrated *in vacuo*. The resulting viscous oil was dissolved in DCM (300 mL) washed with distilled water (2 x 200 mL) and once with brine (150 mL). The organic layer was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. Yield: 38.0 g, white solid, (95%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.45 (s, 18H), 1.66 (m, 4H), 2.65 (t, 4H), 3.20 (m, 4H), 5.28 (s, br, -NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 28.7, 30.0, 39.3, 47.8, 79.5, 156.3. Calcd: [MH]<sup>+</sup> (C<sub>16</sub>H<sub>33</sub>N<sub>3</sub>O<sub>4</sub>) m/z = 332.3 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 332.3 Da. Anal. Calcd for C<sub>16</sub>H<sub>33</sub>N<sub>3</sub>O<sub>4</sub>: C 57.98%; H 10.04%; N 12.68%. Found: C 57.84%; H 10.45%; N 12.91%.

**Synthesis of intermediate 4 – 3** (20 g, 0.06 mol) was added to a 500 mL 2-necked round-bottomed flask fitted with a reflux condenser, magnetic stirrer and a dry N<sub>2</sub> inlet. The flask was degassed with dry nitrogen for 10 minutes, and dissolved in dry ethanol (200 mL). Whilst stirring, and maintaining the temperature at 30°C, propylene oxide (10.51g, 11.21mL, 0.181 mol) was added dropwise to the solution over a period of 10 minutes. Under a positive flow of dry N<sub>2</sub>, the reaction was left stirring at 30°C for 18 hours. After this time, the solvent and excess propylene oxide were removed *in vacuo*, and the crude product purified by liquid chromatography (silica gel, eluting with ethyl acetate:methanol (EtOAc:MeOH), 80:20). Yield: 19.90 g, pale yellow oil at ambient temperature, solidifying to an off white solid upon cooling (85%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.12 (d, 3H), 1.45 (s, 18H), 1.64 (m, 4H), 2.30 (d, 2H), 2.34-2.67 (d of m, 4H), 3.16 (m, 4H), 3.77 (m, 1H), 4.99 (s, br, -NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 27.6, 28.7, 38.8, 51.8, 60.9, 63.0, 79.5, 156.5. Calcd: [MH]<sup>+</sup> (C<sub>19</sub>H<sub>40</sub>N<sub>3</sub>O<sub>5</sub>) m/z = 390.3 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 390.3 Da. Anal. Calcd for C<sub>19</sub>H<sub>40</sub>N<sub>3</sub>O<sub>5</sub>: C 58.58%; H 10.09%; N 10.09%. Found: C 58.50%; H 10.19%; N 10.82%.

**1-[N, N-bis (2-aminopropyl)-amino]-2-propanol, 6** - To a 1000 mL round-bottomed flask, **4** (33.70g) was dissolved in EtOAc, (300 mL) and concentrated hydrochloric acid (HCl) (35.03 g, 30 mL, d=1.18, 12M) was added very slowly. CO<sub>2</sub> began to rapidly evolve. The reaction vessel was heated to 55°C and stirred vigorously for 5

hours before leaving overnight at room temperature. After removal of EtOAc *in vacuo*, <sup>1</sup>H NMR (D<sub>2</sub>O) confirmed complete decarboxylation and formation of **5**.

For the formation of **6**, the crude oil was dissolved very slowly in 4M NaOH (300 mL), and reduced by approximately half its volume on a rotary evaporator (60°C). A yellow oily substance formed on the surface of the NaOH solution. The mixture was extracted with chloroform (CHCl<sub>3</sub>) (2 x 300 mL), the organic layers combined, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated *in vacuo*. Yield: 15.27 g, pale yellow oil (94%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.11 (d, 3H), 1.60 (m, 4H), 2.10 (s, br, -OH), 2.32 (m, 2H), 2.39-2.67 (d of m, 4H), 2.75 (t, 4H), 3.79 (m, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 20.1, 30.7, 40.5, 52.1, 62.7, 63.9. Calcd: [MH]<sup>+</sup> (C<sub>9</sub>H<sub>23</sub>N<sub>3</sub>O) m/z = 189.3 Da. Found: CI-MS: [M+H]<sup>+</sup> m/z = 190.2 Da. Anal. Calcd for C<sub>9</sub>H<sub>23</sub>N<sub>3</sub>O: C 57.10%; H 12.25%; N 22.20%. Found: C 55.18%; H 12.15%; N 20.53%.

**G<sub>2</sub>-OH, 10** - DMEA (6.00 g, 42 mmol, 6 eq.) was added to a 50 mL round 2 necked round-bottomed flask containing IPA (12 mL). The flask was deoxygenated under a positive N<sub>2</sub> purge for 10 minutes. **6** (1.32 g, 6.984 mmol, 1 eq.) dissolved in IPA (12 mL) was added dropwise while the solution was stirring in an ice bath under a positive flow of N<sub>2</sub>. The final mixture was stirred for a further 10 minutes at 0°C, allowed to warm to room temperature and left stirring for 48 hours. The solvent was removed and the product left to dry *in vacuo* overnight. Yield: 5.37 g, yellow oil (99%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 1.13 (d, 3H), 1.67 (m, 4H), 2.26-2.65 (m, 50H), 2.77 (m, 8H), 3.87 (m, 1H), 4.17 (m, 8H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 20.0, 24.5, 32.2, 45.7, 48.9, 51.6, 52.2, 57.8, 62.1, 62.3, 63.6, 173.0. Calcd [MH]<sup>+</sup> (C<sub>37</sub>H<sub>75</sub>N<sub>7</sub>O<sub>9</sub>) m/z = 762.0 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 762.6 Da. Anal. Calcd for C<sub>37</sub>H<sub>75</sub>N<sub>7</sub>O<sub>9</sub>: C 58.27%; H 9.84%; N 12.86%. Found C 58.32%; H 9.92%; N 12.87%.

**G<sub>2</sub>-Br initiator, 13 – 10** (5.14 g, 6.749 mmol, 1 eq.), TEA (0.96 g, 9.45 mmol, 1.4 eq.) and DMAP (82.5 mg, 0.675 mmol, 0.1 eq.) were added to a 250 mL 2 necked round-bottomed flask containing DCM (160 mL). The flask was deoxygenated under a positive N<sub>2</sub> purge for 10 minutes. BiB (1.63 g, 0.88 mL, 7.09 mmol, 1.05 eq.) was added drop wise while the solution was stirring in an ice bath under a positive flow of N<sub>2</sub>. The reaction mixture was allowed to warm to room temperature and left stirring

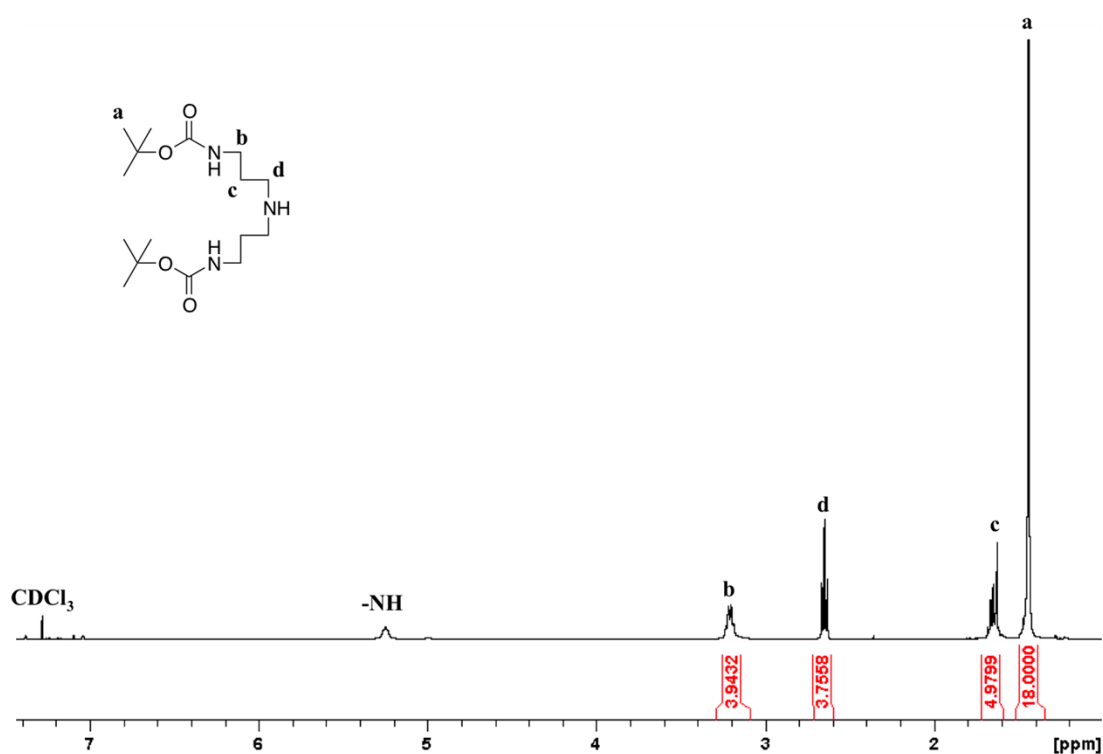
overnight. The organic phase was washed with saturated NaHCO<sub>3</sub> solution (3 x 160 mL). The solution was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and the product left to dry *in vacuo* overnight. Yield: 3.30 g, yellow oil (54%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 1.26 (d, 3H), 1.56 (m, 4H), 1.91 (s, 6H), 2.22-2.67 (m, 50H), 2.76 (m, 8H), 4.19 (m, 8H), 5.04 (m, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 17.9, 24.9, 30.7, 32.6, 45.8, 48.9, 51.7, 52.7, 56.2, 57.7, 58.6, 62.0, 71.1, 171.2, 172.7. Calcd [MH]<sup>+</sup> (C<sub>41</sub>H<sub>80</sub>N<sub>7</sub>O<sub>10</sub>Br) m/z = 911.0 Da. Found: ESI-MS: [MH]<sup>+</sup> m/z = 912.5 Da. Anal. Calcd for C<sub>41</sub>H<sub>80</sub>N<sub>7</sub>O<sub>10</sub>Br: C 54.01%; H 8.78%; N 10.76%. Found C 54.05%; H 8.85%; N 10.76%.

**Typical linear polymerisation of HPMA, 14 -17** - In a typical synthesis, targeting a number average degree of polymerisation (DP<sub>n</sub>) = 50 monomer units (*p*(HPMA)<sub>50</sub>), bpy (173.3 mg, 1.1096 mmol, 2 eq.), HPMA (4.0 g, 27.7 mmol, 50 eq.) and MeOH (35% v/v based on HPMA) were placed into a 50 mL round-bottomed flask. The solution was stirred and deoxygenated using a N<sub>2</sub> purge for 15 minutes. Cu(I)Cl (54.9 mg, 0.5548 mmol, 1 eq.) was added to the flask and left to purge for a further 5 minutes. **G<sub>2</sub>-Br, 13**, (0.505 g, 0.555 mmol, 1 eq.) was added to the flask under a positive flow of N<sub>2</sub>, and the solution was left to polymerise at 30°C. Reactions were terminated when >99% conversion was reached, as judged by <sup>1</sup>H NMR, by exposure to oxygen and addition of tetrahydrofuran (THF). The catalyst residues were removed by passing the mixture over a neutral alumina column. THF was removed under vacuum to concentrate the sample before precipitation into hexane and drying in the vacuum oven overnight.

**Typical synthesis of a hyperbranched polydendron using the copolymerisation of HPMA and EGDMA, 18-21** - In a typical synthesis, targeting a number average degree of polymerisation (DP<sub>n</sub>) = 50 monomer units within the primary chain, bpy (173.3 mg, 1.11 mmol, 2 eq.), HPMA (4 g, 27.7 mmol, 50 eq.), EGDMA (99.0 mg, 0.4993 mmol, 0.9 eq.) and MeOH (35% v/v based on HPMA) were placed into a 50 mL round-bottomed flask. The solution was stirred and deoxygenated using a N<sub>2</sub> purge for 15 minutes. Cu(I)Cl (54.9 mg, 0.5548 mmol, 1 eq.) was added to the flask and left to purge for a further 5 minutes. **G<sub>2</sub>-Br, 13**, (0.505 g, 0.555 mmol, 1 eq.) was added to the flask under a positive flow of N<sub>2</sub>, and the solution was left to polymerise at 30°C. Reactions were terminated when >99% conversion was reached, as judged

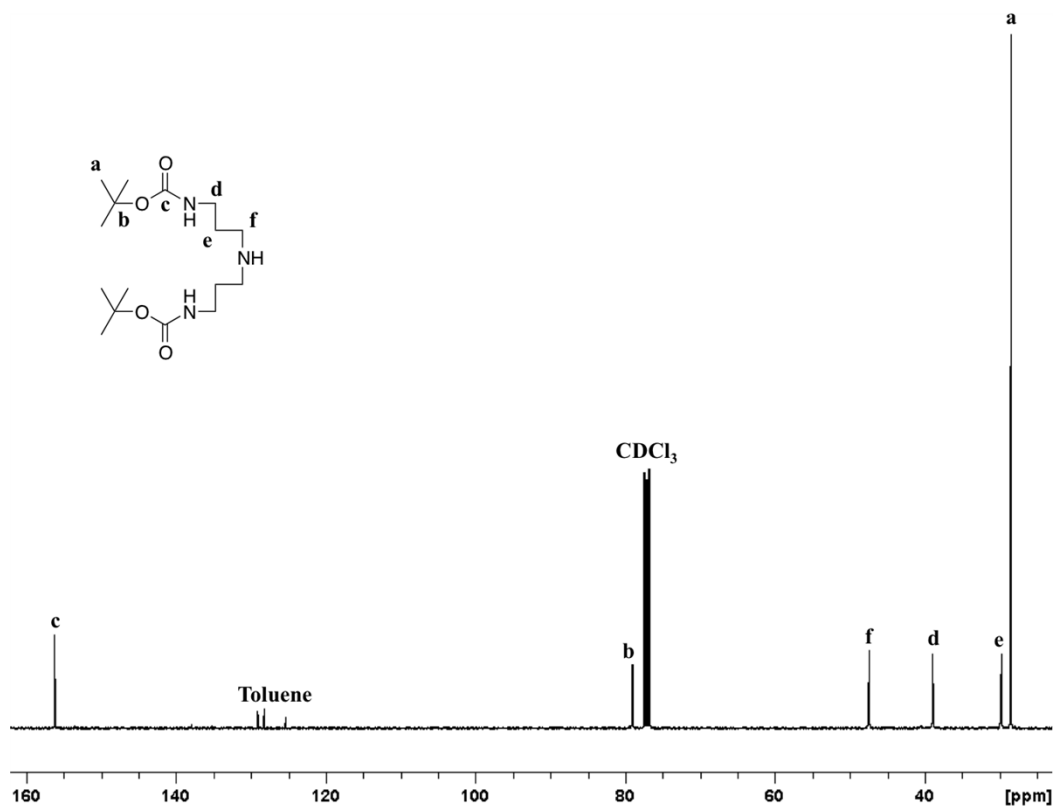
by  $^1\text{H}$  NMR, by exposure to oxygen and addition of THF. The catalyst residues were removed by passing the mixture over a neutral alumina column. THF was removed under vacuum to concentrate the sample before precipitation into hexane.

**Typical procedure for aqueous nanoprecipitation of  $p(\text{HPMA}_{50})$  and  $p(\text{HPMA}_{50}\text{-co-EGDMA}_x)$**  - The materials were dissolved in THF at a concentration of  $5 \text{ mg mL}^{-1}$ . 2 mL of this solution was then subjected to a rapid solvent switch through dropwise addition into 10 mL of water, to give a final polymer nanoparticle concentration of  $1 \text{ mg mL}^{-1}$  in water after THF removal by evaporation overnight.

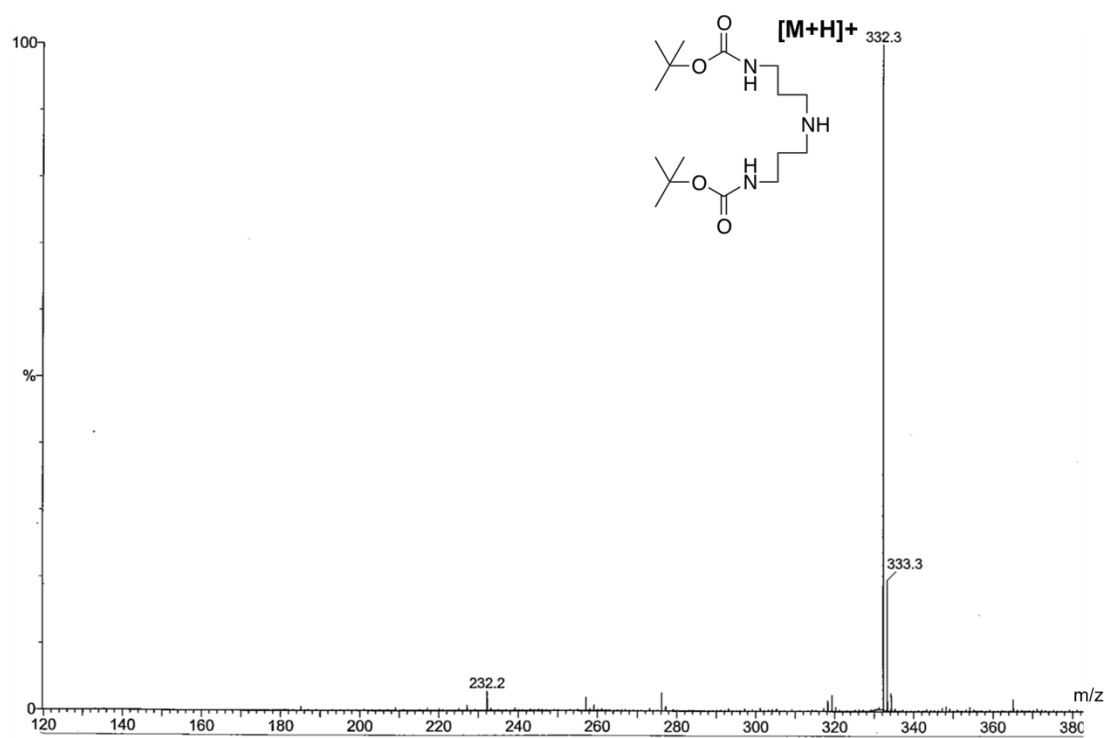


**Figure S1:**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of intermediate **3**

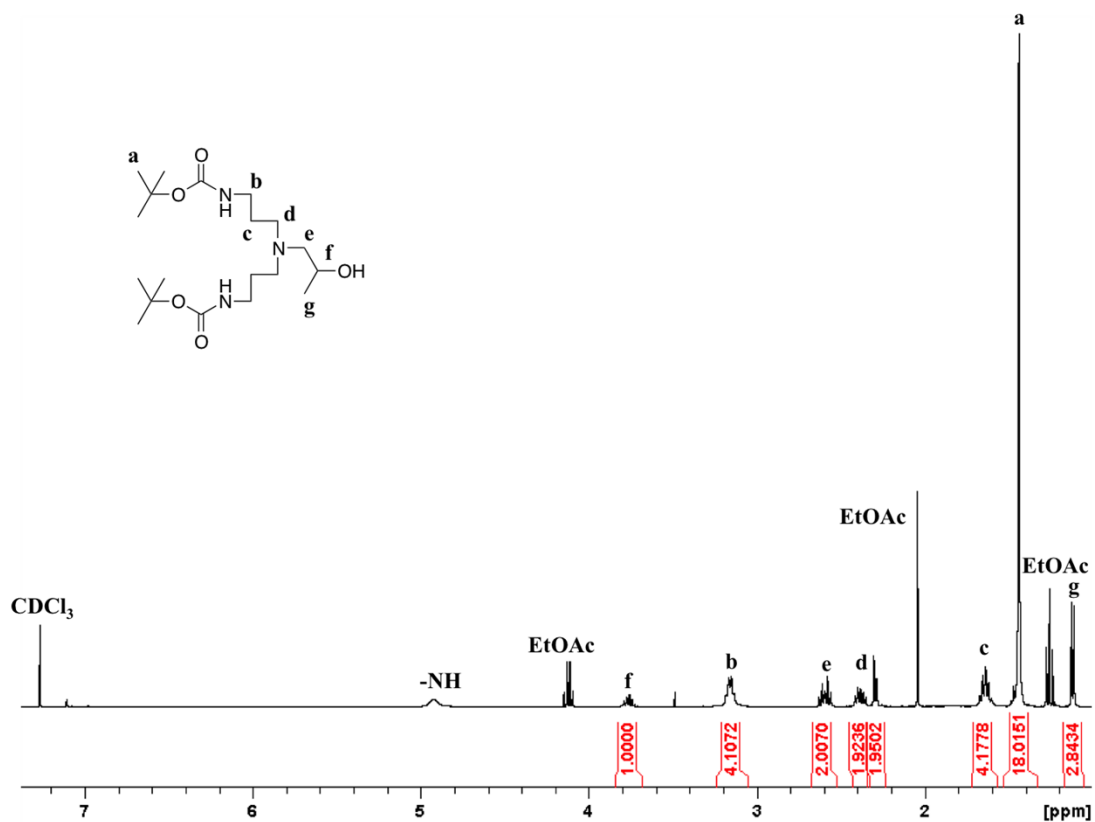




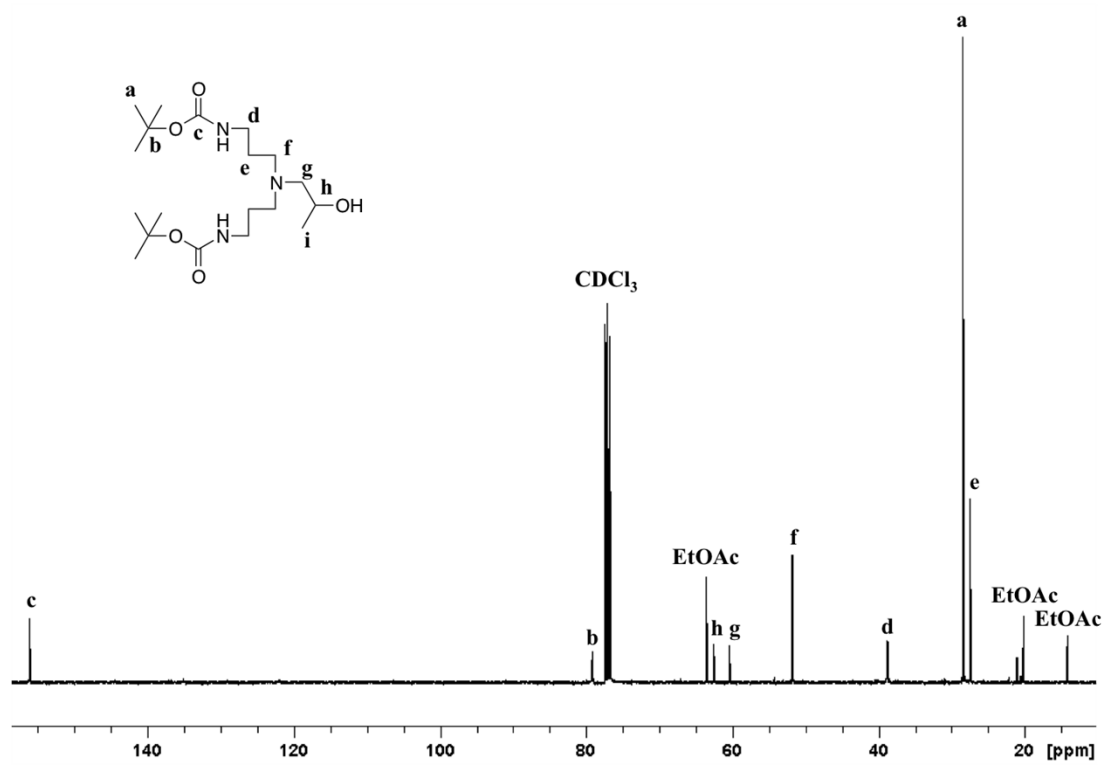
**Figure S2:**  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) spectrum of intermediate 3



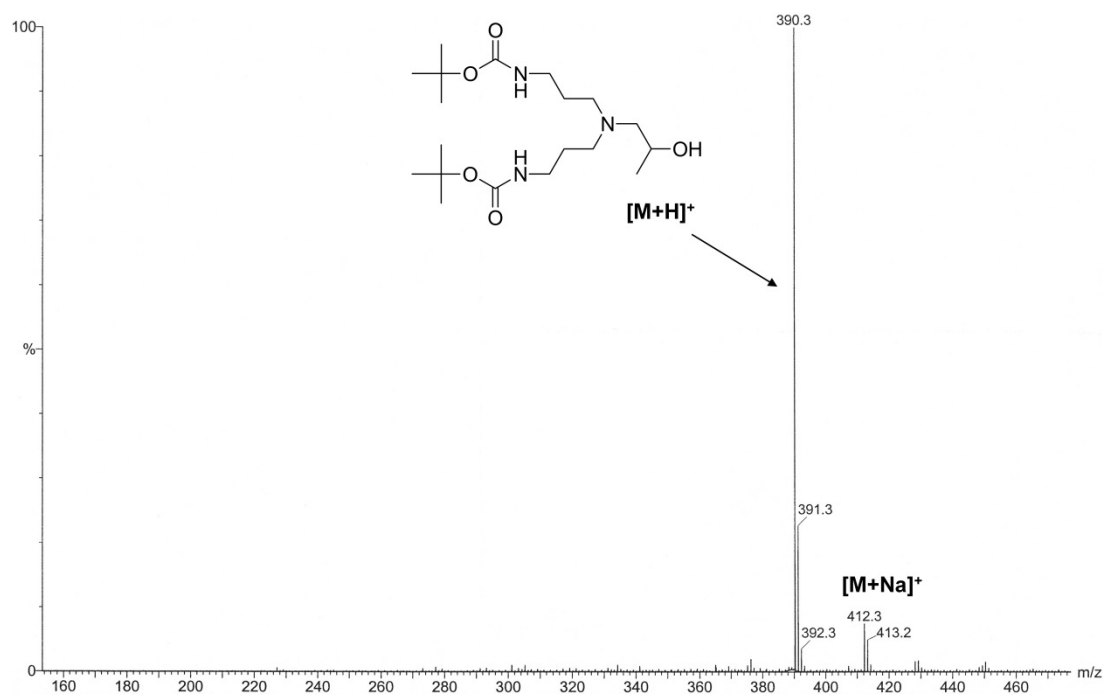
**Figure S3:** Electrospray mass spectrum of intermediate 3



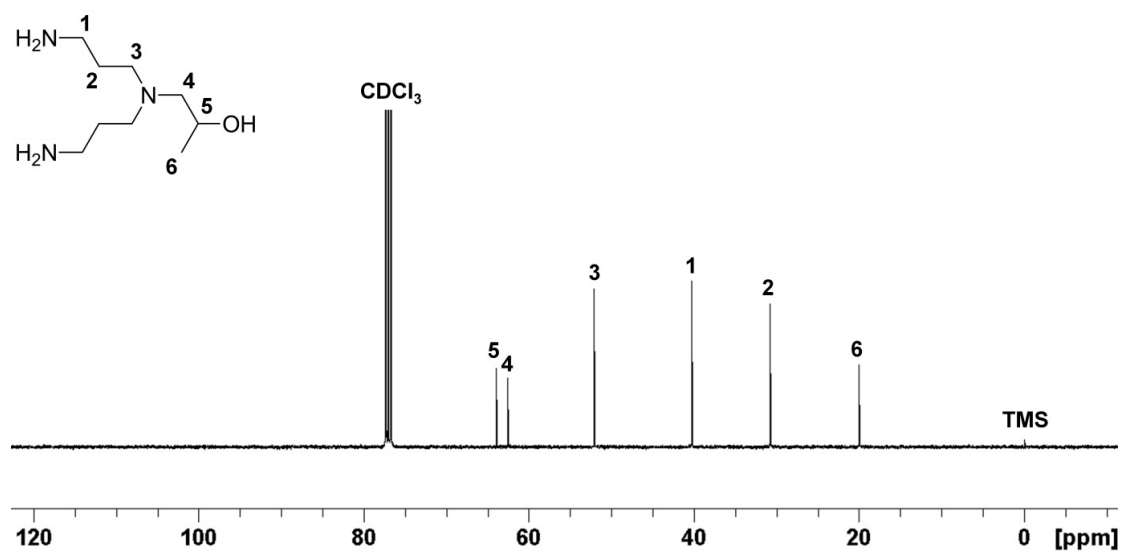
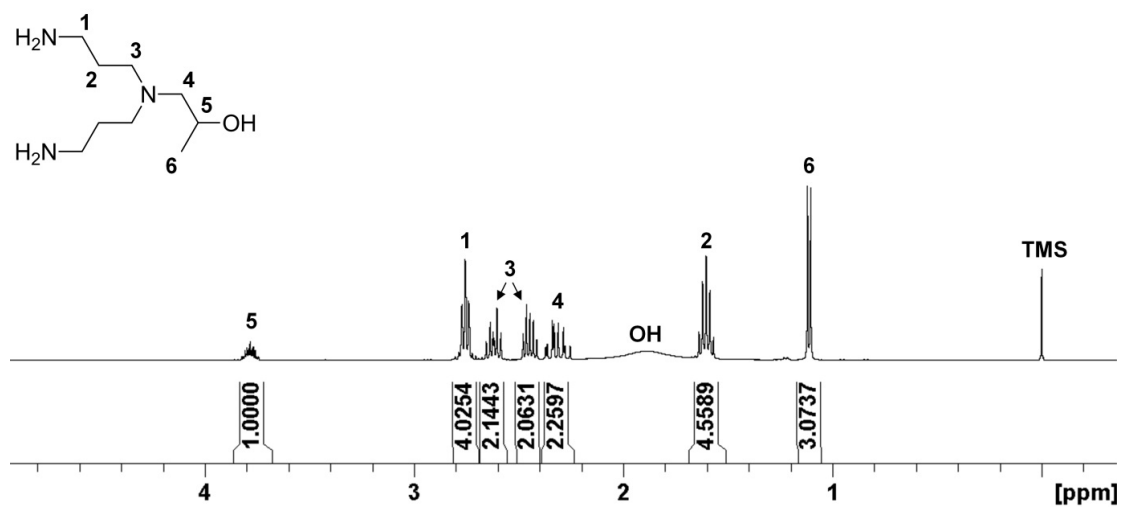
**Figure S4:** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) spectrum of intermediate 4

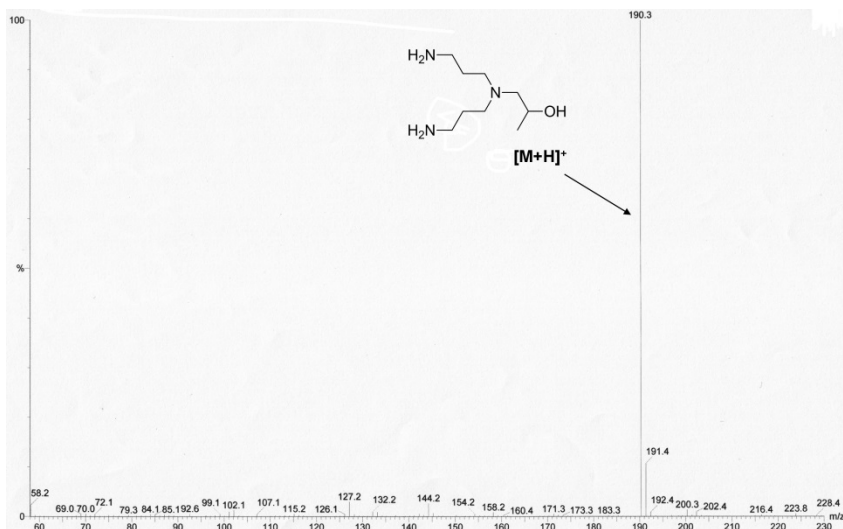


**Figure S5:** <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) spectrum of intermediate 4

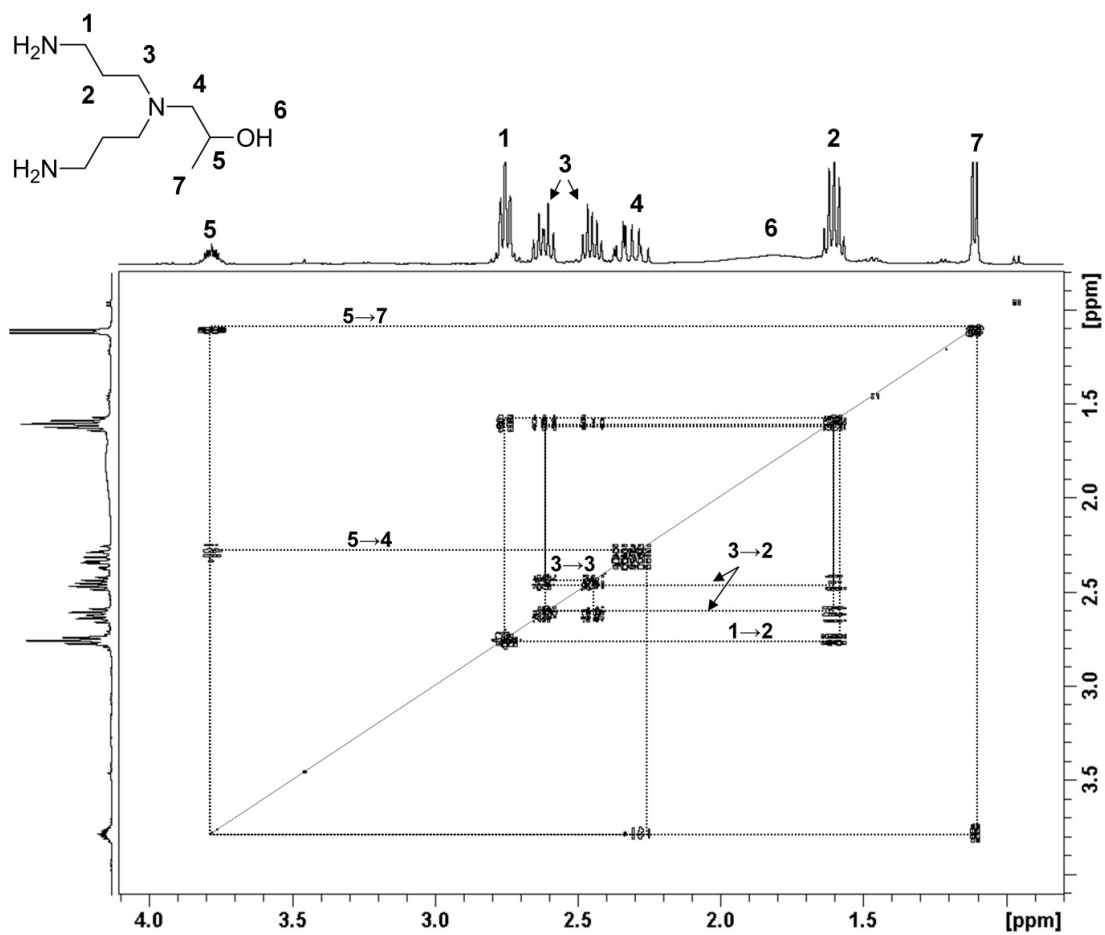


**Figure S6:** Electrospray mass spectrum of intermediate 4

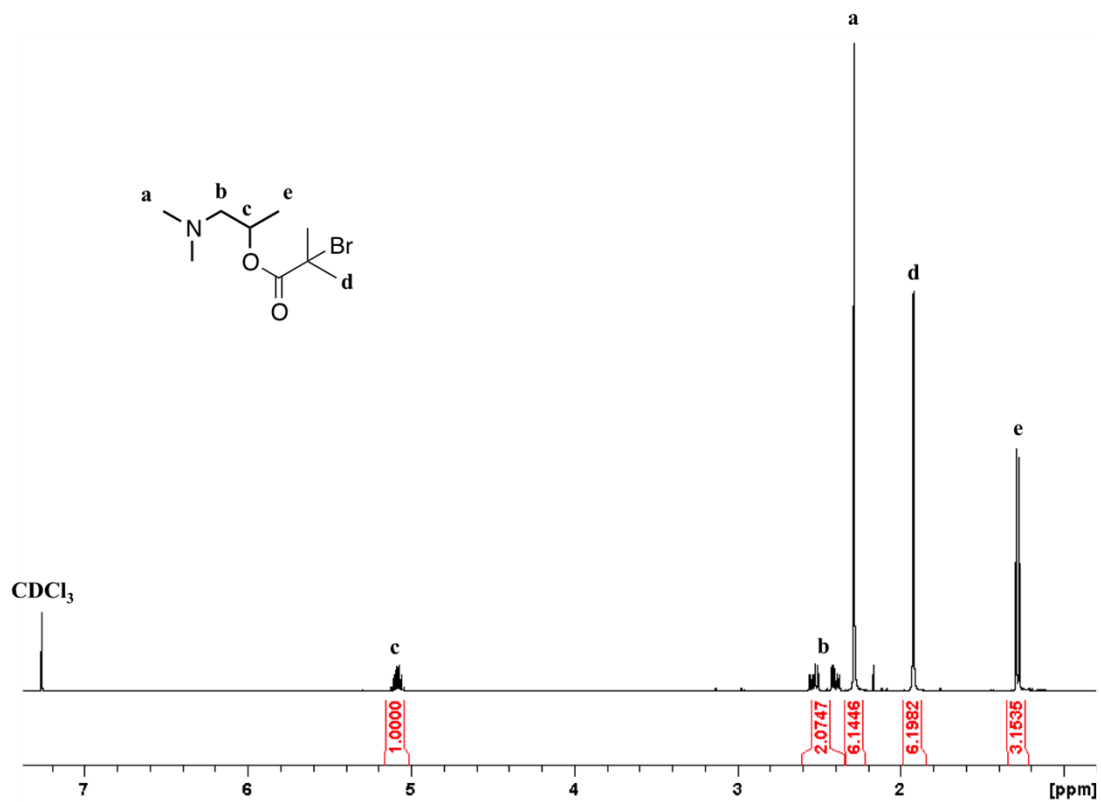




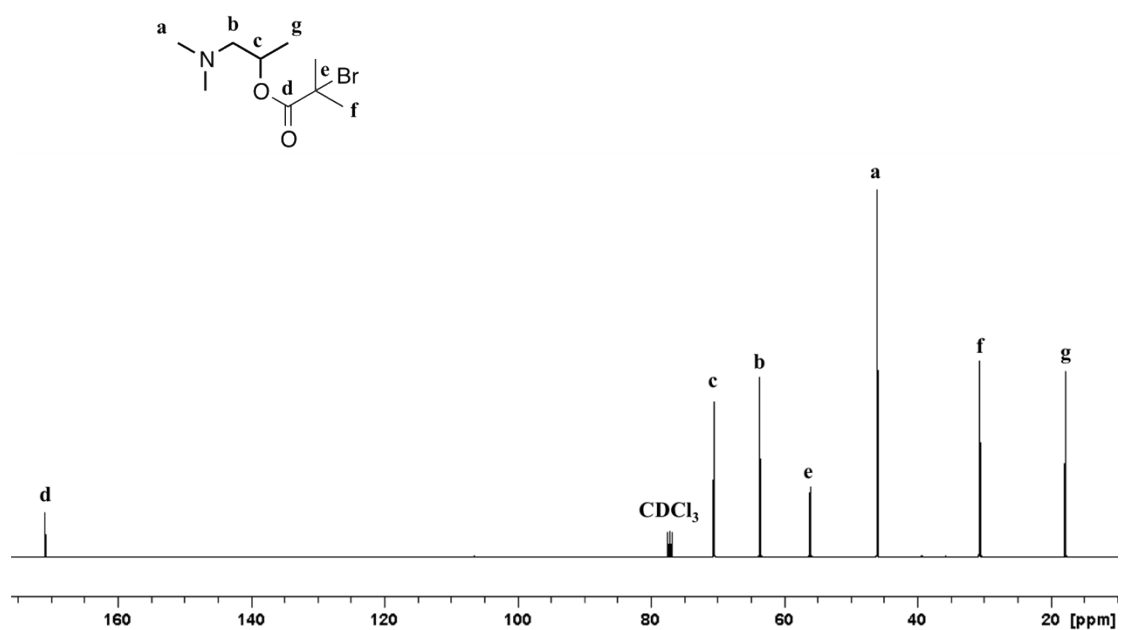
**Figure S9:** Chemical ionization mass spectrum of **6**



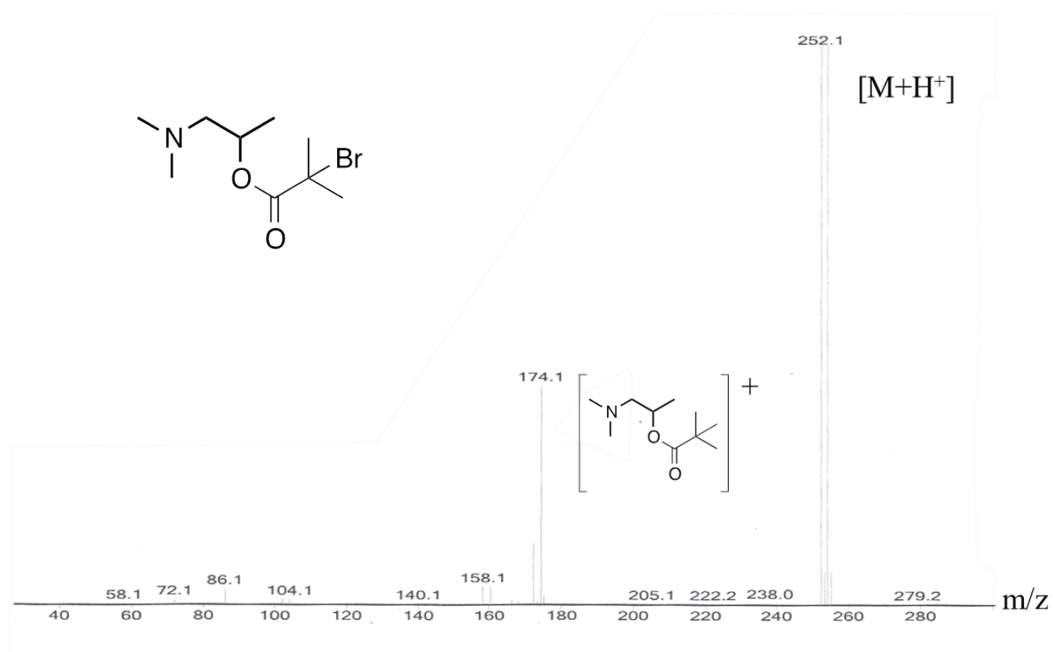
**Figure S10:** 2D  $^1\text{H}$ - $^1\text{H}$  COSY NMR spectrum ( $\text{CDCl}_3$ , 400 MHz) of **6**.



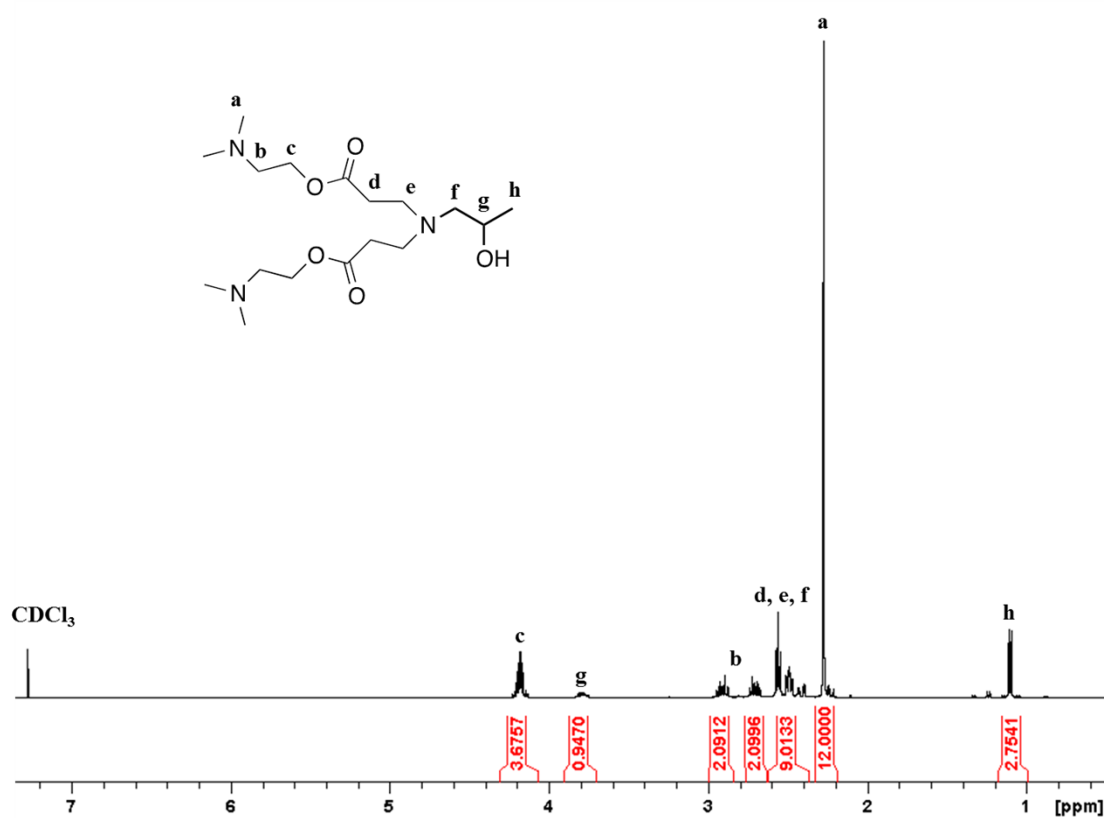
**Figure S11:** <sup>1</sup>H NMR spectrum (400MHz, CDCl<sub>3</sub>) of **G<sub>0</sub>-Br, 11**



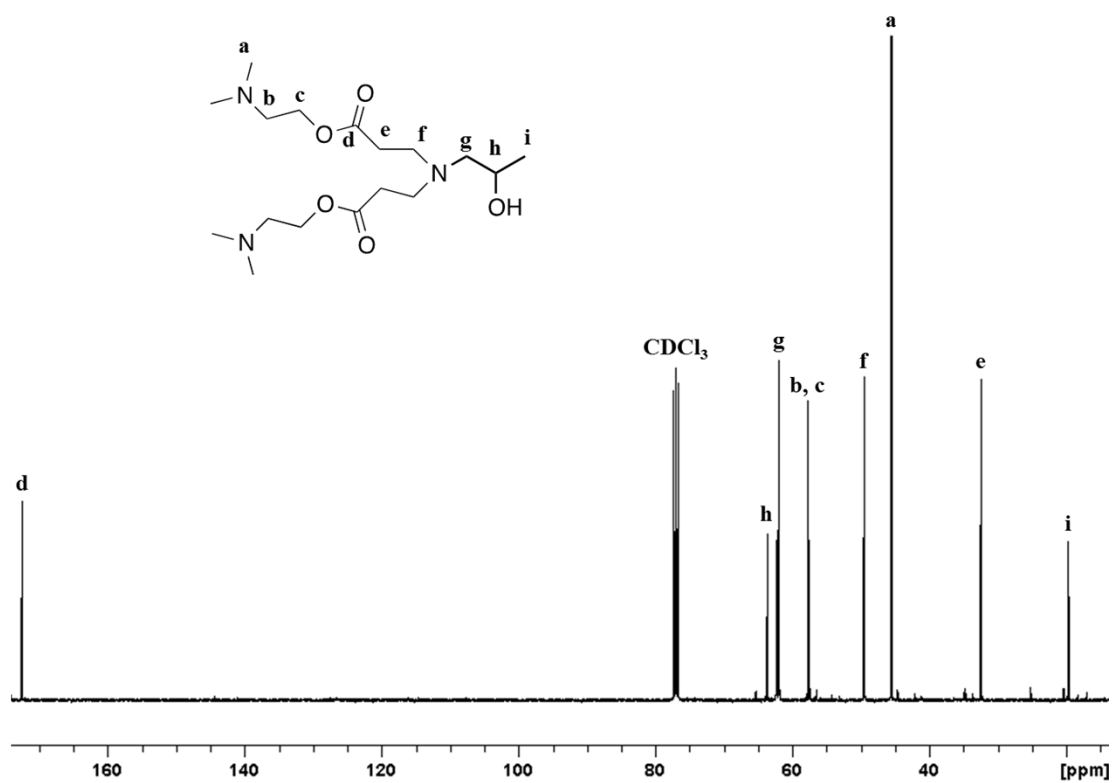
**Figure S12:** <sup>13</sup>C NMR spectrum (100MHz, CDCl<sub>3</sub>) of **G<sub>0</sub>-Br, 11**



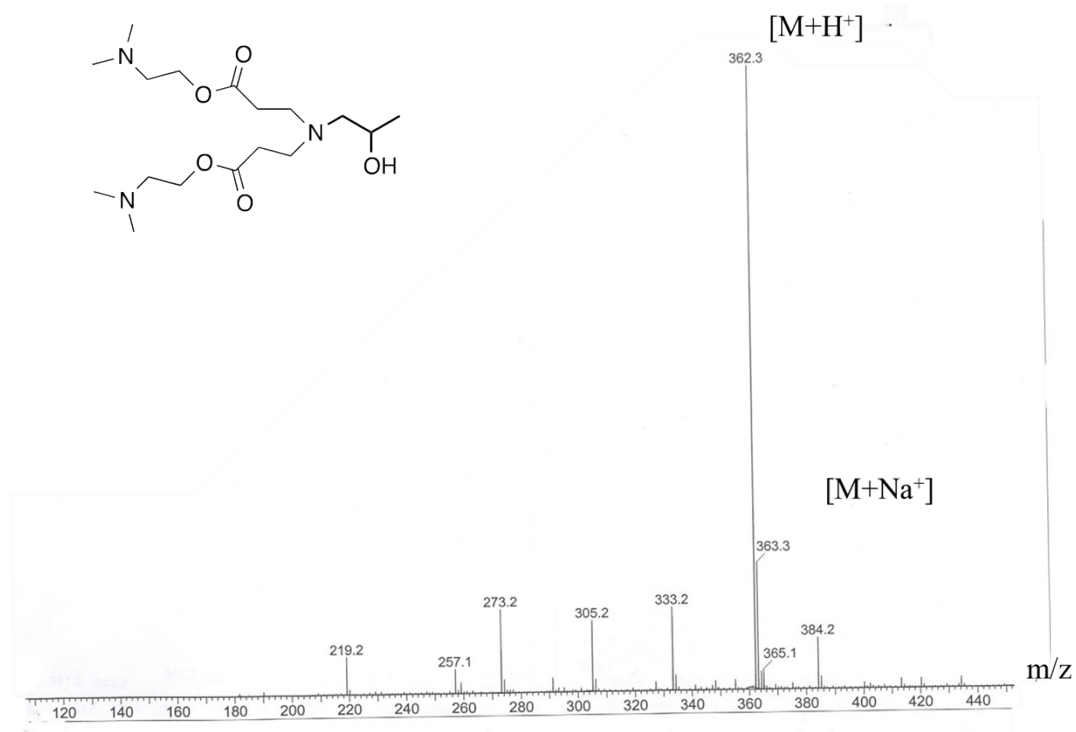
**Figure S13:** Electrospray mass spectrum of **G<sub>0</sub>-Br, 11**



**Figure S14:** <sup>1</sup>H NMR spectrum (400MHz, CDCl<sub>3</sub>) of **G<sub>1</sub>-OH, 9**



**Figure S15:** <sup>13</sup>C NMR spectrum (100MHz, CDCl<sub>3</sub>) of **G<sub>1</sub>-OH, 9**



**Figure S16:** Electrospray mass spectrum of **G<sub>1</sub>-OH, 9**



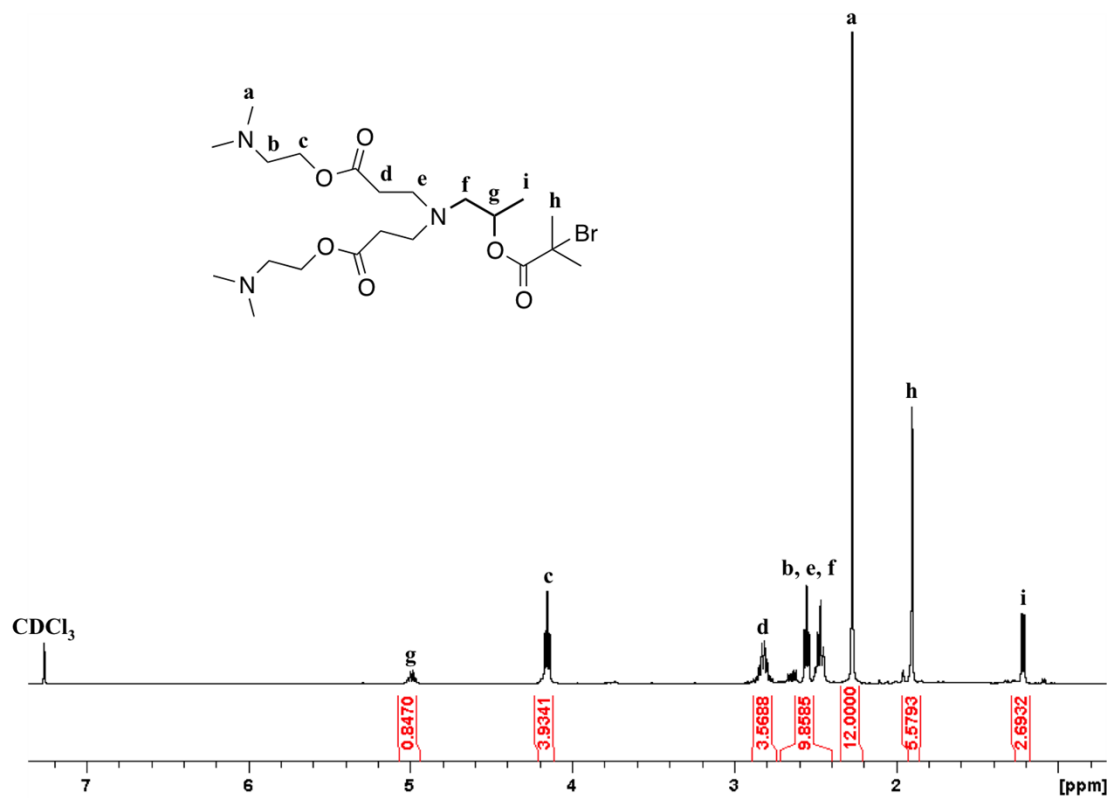


Figure S17: <sup>1</sup>H NMR spectrum (400MHz, CDCl<sub>3</sub>) of **G<sub>1</sub>-Br, 12**

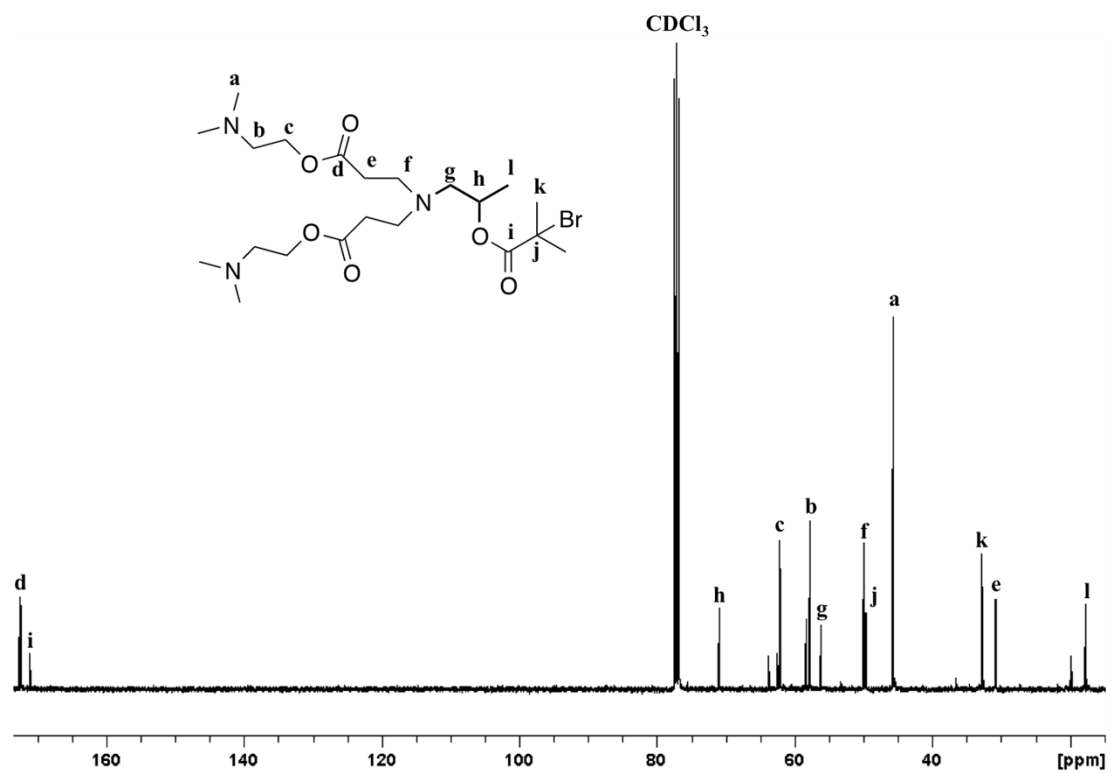
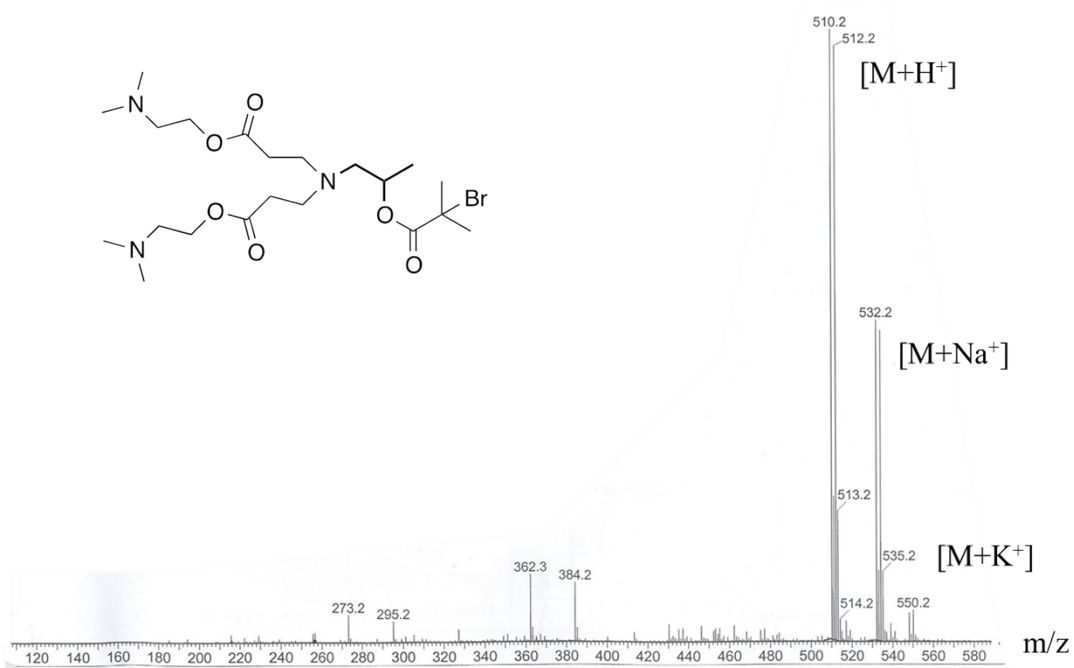
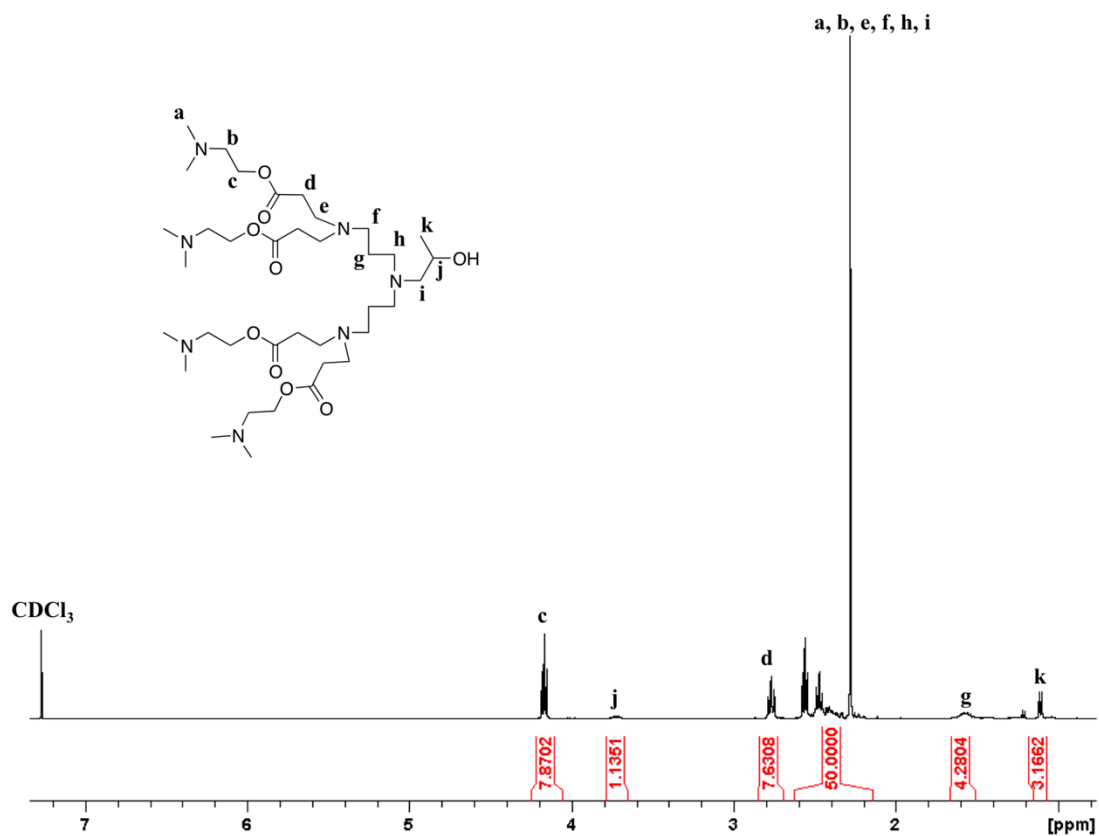


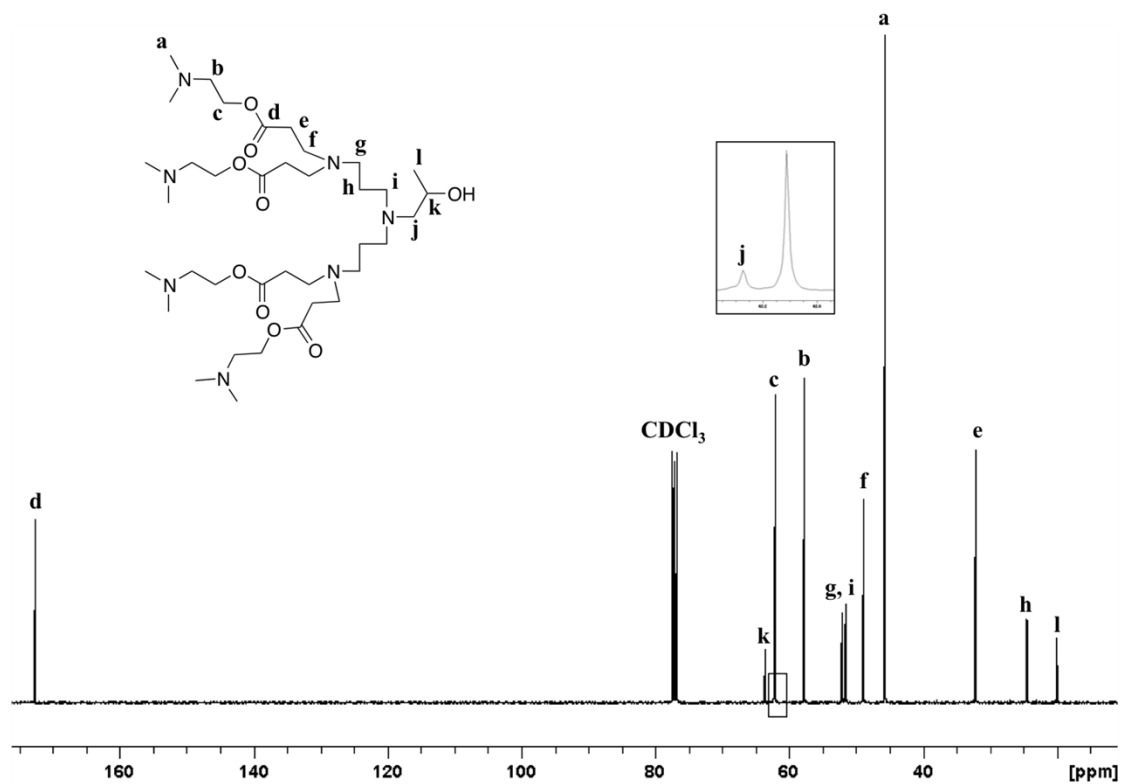
Figure S18: <sup>13</sup>C NMR spectrum (100MHz, CDCl<sub>3</sub>) of **G<sub>1</sub>-Br, 12**



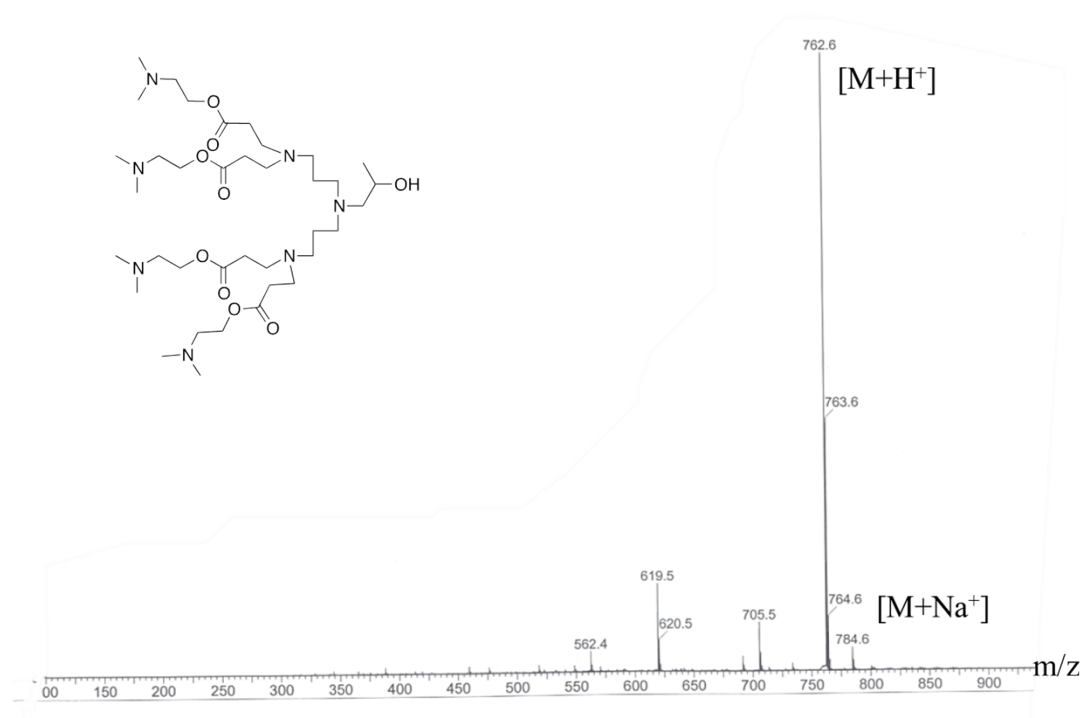
**Figure S19:** Electrospray mass spectrum of **G<sub>1</sub>-Br, 12**



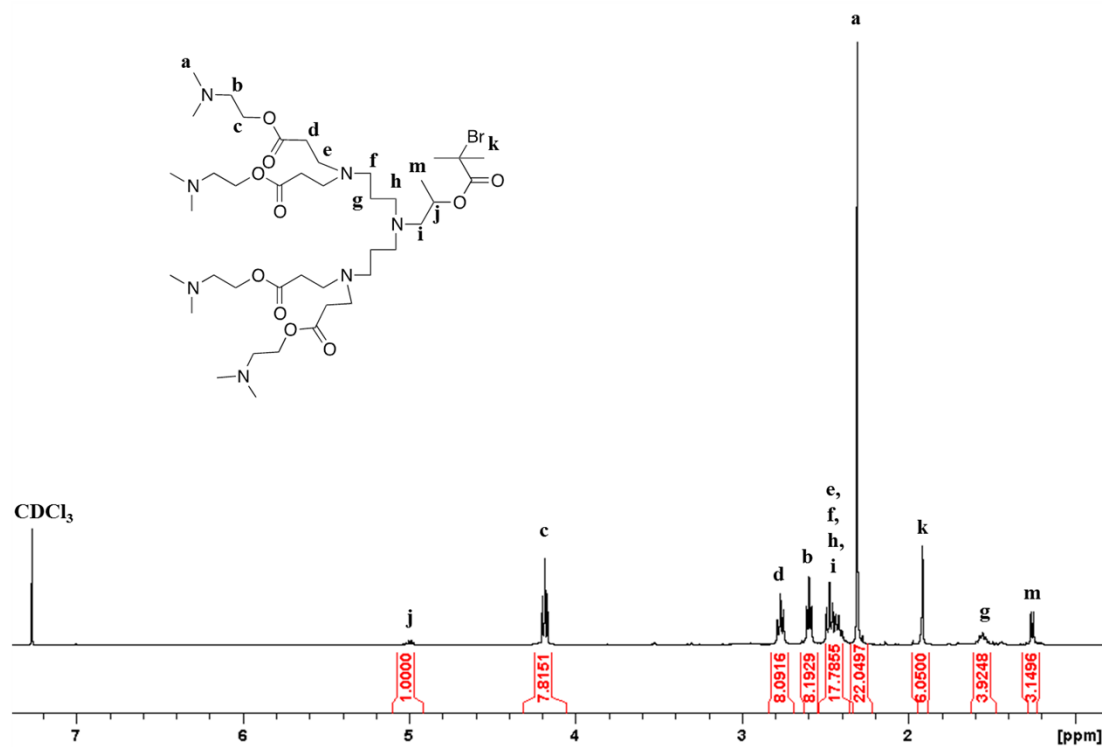
**Figure S20:** <sup>1</sup>H NMR spectrum (400MHz, CDCl<sub>3</sub>) of **G<sub>2</sub>-OH, 10**



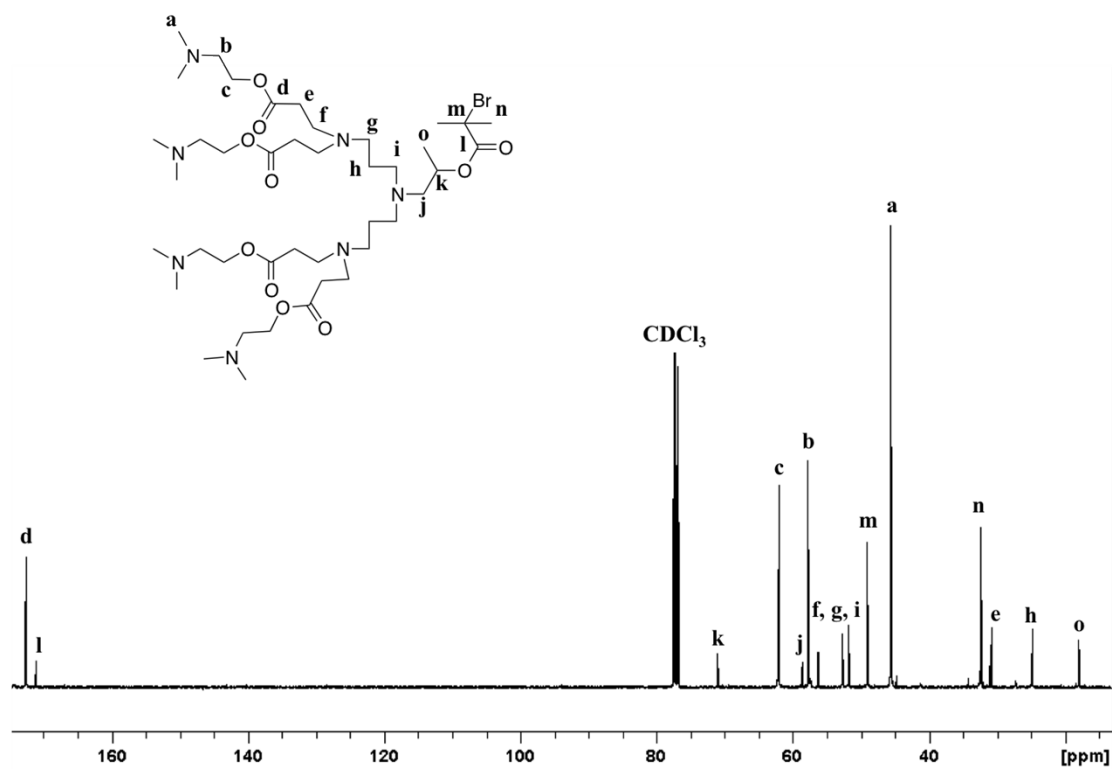
**Figure S21:** <sup>13</sup>C NMR spectrum (100MHz, CDCl<sub>3</sub>) of G<sub>2</sub>-OH, 10



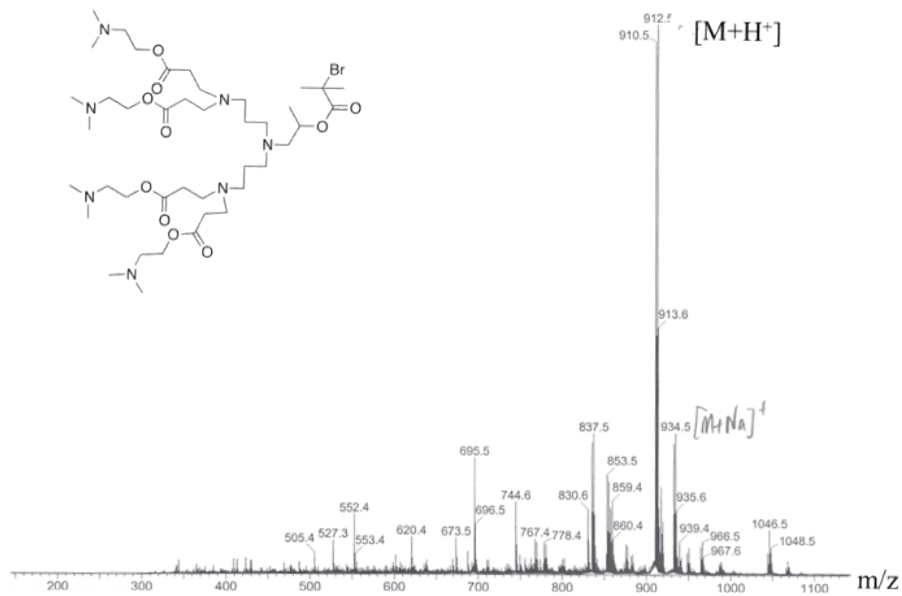
**Figure S22:** Electrospray mass spectrum of G<sub>2</sub>-OH, 10



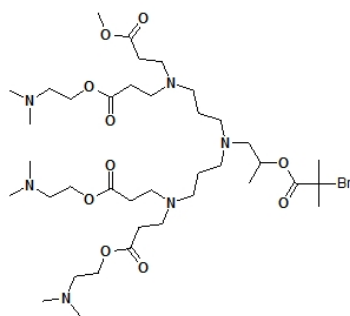
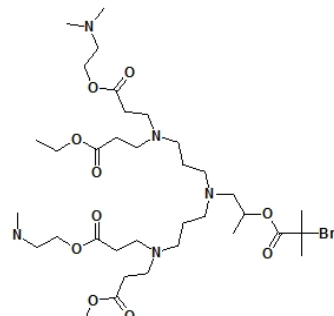
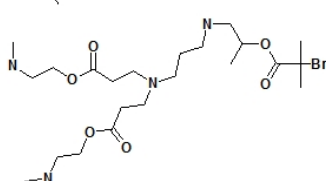
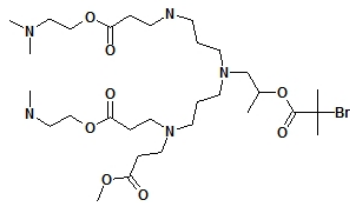
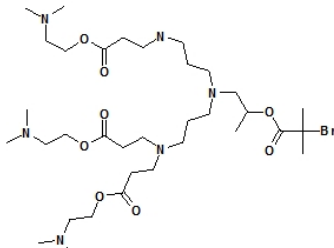
**Figure S23:** <sup>1</sup>H NMR spectrum (400MHz, CDCl<sub>3</sub>) of **G<sub>2</sub>-Br, 13**



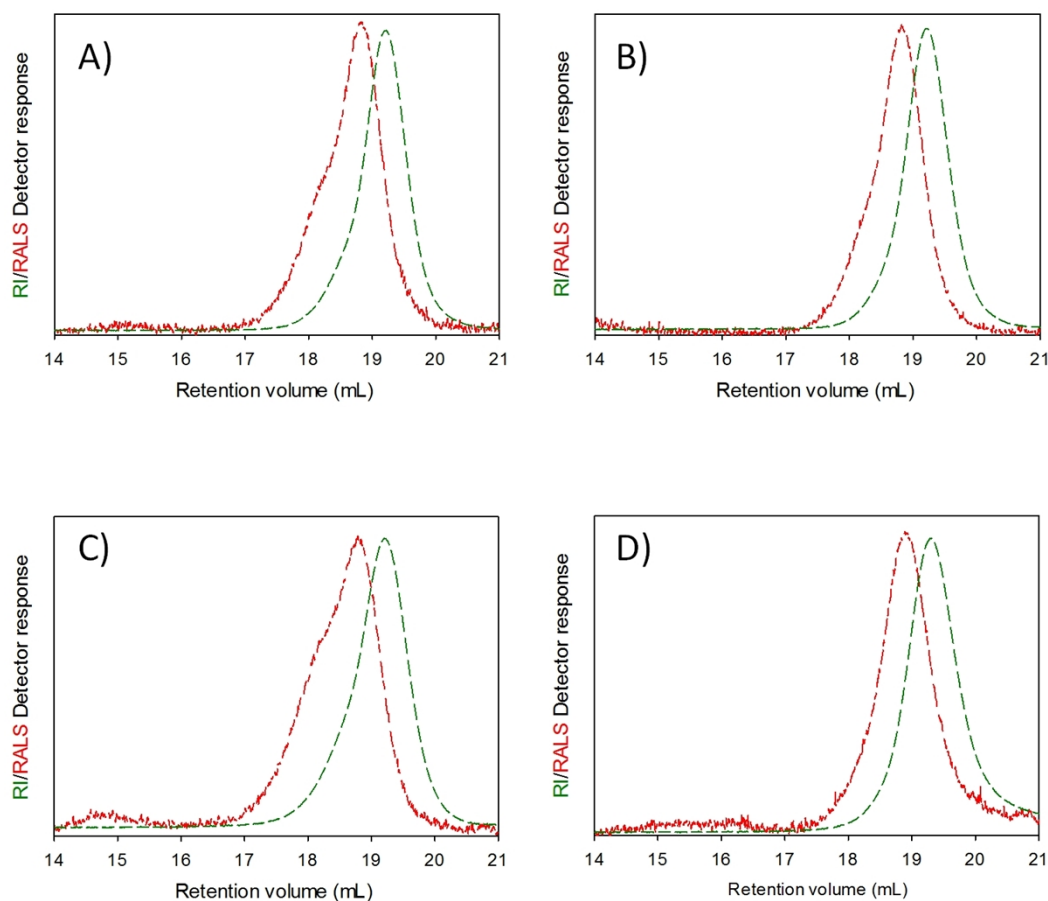
**Figure S24:** <sup>13</sup>C NMR spectrum (100MHz, CDCl<sub>3</sub>) of **G<sub>2</sub>-Br, 13**



**Figure S25:** Electrospray mass spectrum of G<sub>2</sub>-Br, 13

| Fragment Assignment   | Fragment MH <sup>+</sup> |
|---|--------------------------|
|    | 853 Da                   |
|    | 837 Da                   |
|  | 767 Da                   |
|  | 694 Da                   |
|  | 552 Da                   |

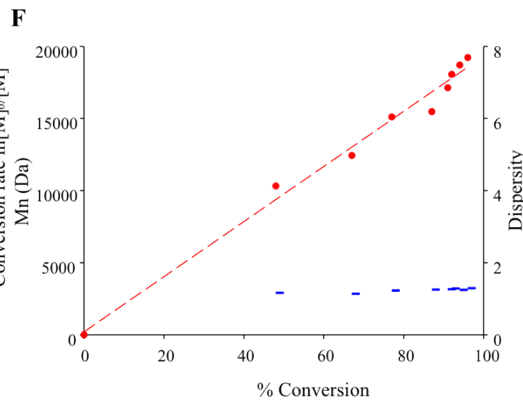
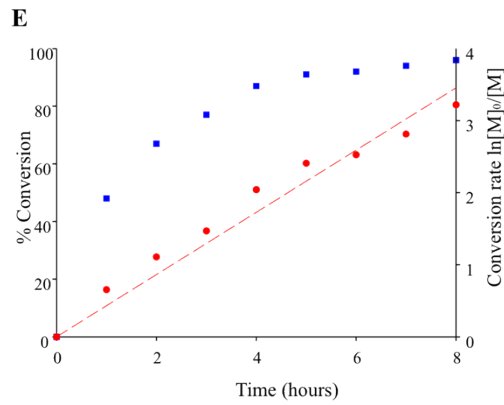
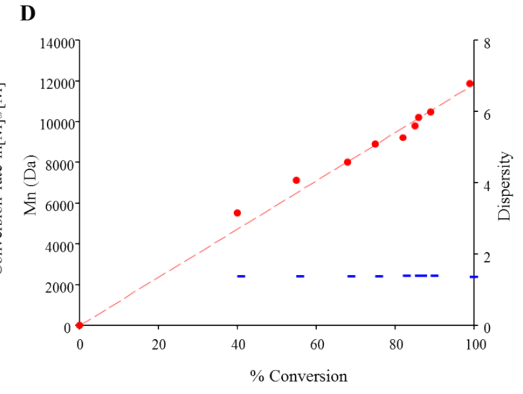
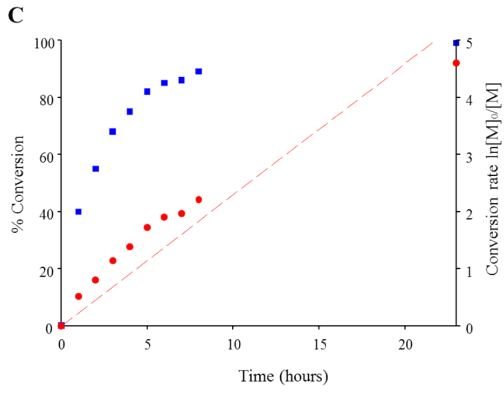
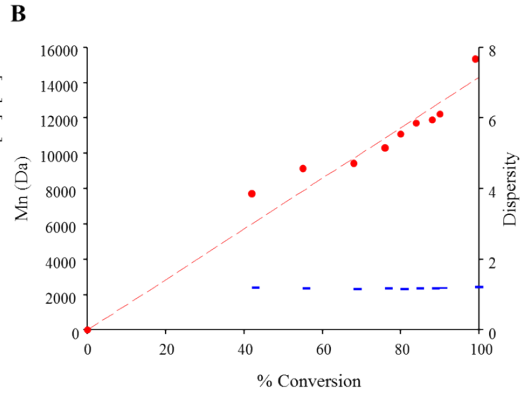
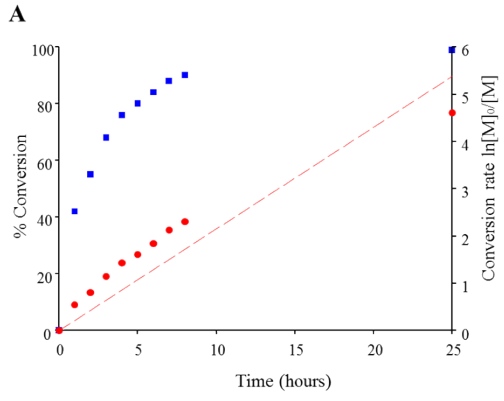
**Table S1:** Assignment of fragments from electrospray mass spectrum of **G<sub>2</sub>-Br, 13** (Figure S25)



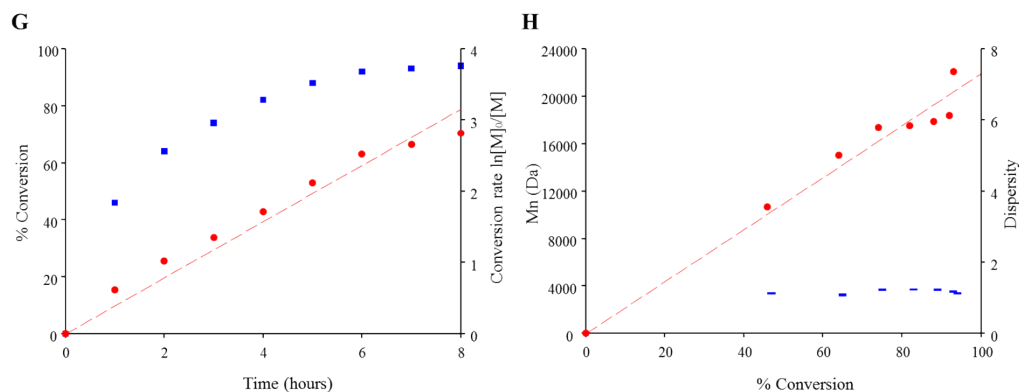
**Figure S26:** SEC Chromatograms of A) EBiB-*p*(HPMA<sub>50</sub>), **14**, B) G<sub>0</sub>-*p*(HPMA<sub>50</sub>), **15**, C) G<sub>1</sub>-*p*(HPMA<sub>50</sub>), **16**, D) G<sub>2</sub>-*p*(HPMA<sub>50</sub>), **17**

(NOTE 1: the RI and RALS chromatograms are not expected to overlay within each sample due to the different mode of detection and their relative relationship to concentration (RI) or light scattering intensity (RALS). The detectors will also not receive the sample at identical times after injection. The signals are internally consistent (see retention volumes for peaks and peak onsets))

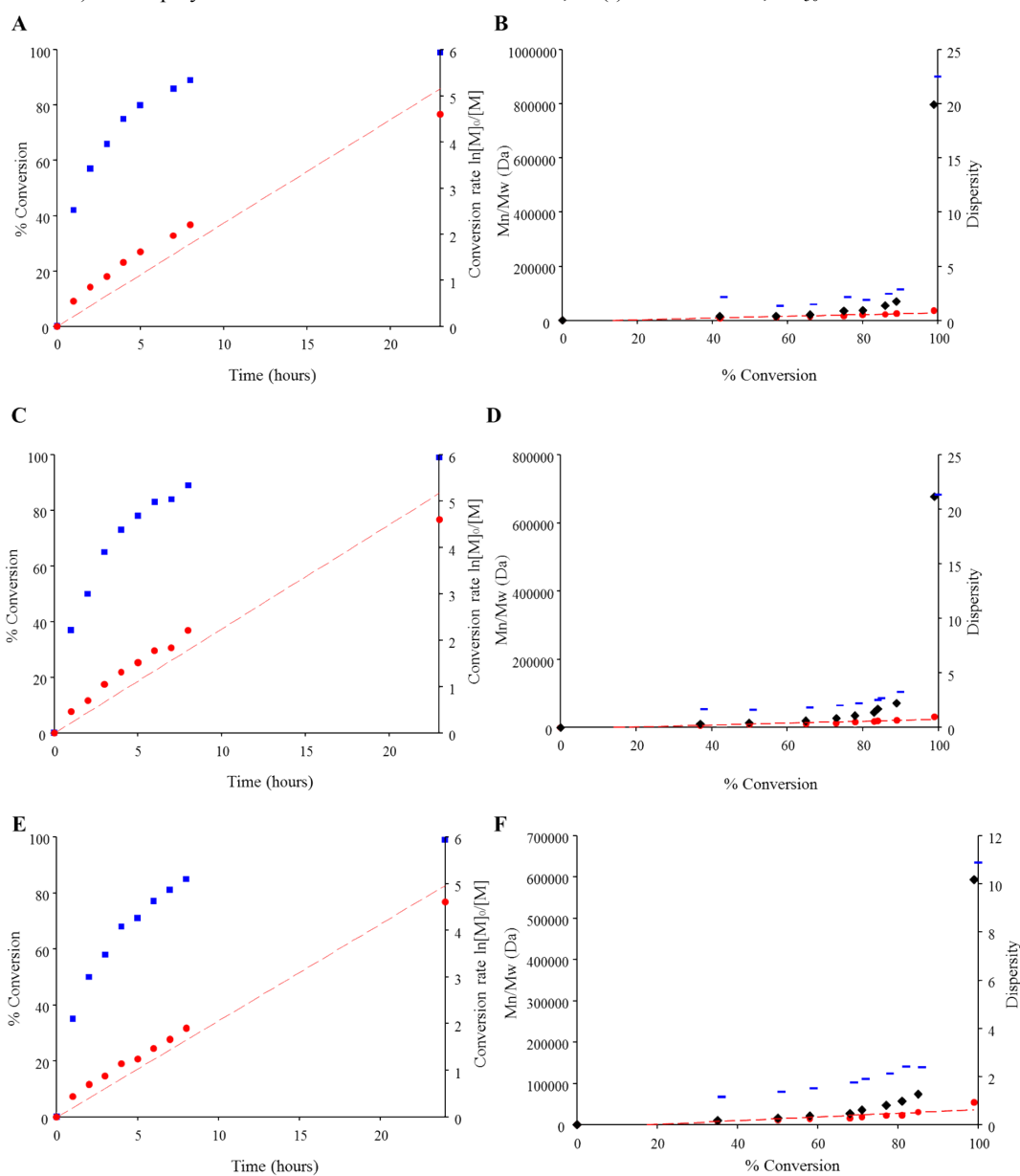
(NOTE 2: the shoulder present at lower retention volumes that is evident in the RALS signals is indicative of minor branching from previously reported low concentration divinyl impurities within the HPMA monomer – see A. Blanazs, J. Madsen, G. Battaglia, A. J. Ryan, S. P. Armes *J. Am. Chem. Soc.* 2011, 133, 16581– 16587)

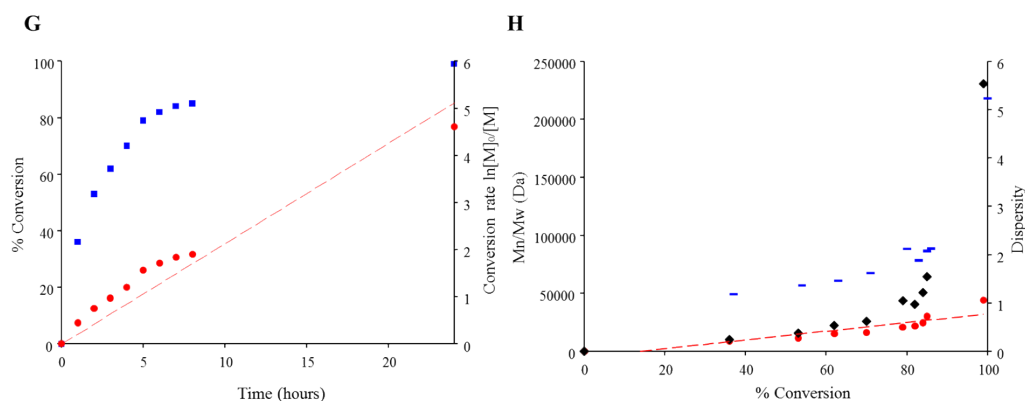






**Figure S27: A, C, E, G (EBIB-, G0-, G1-, G2- respectively):** ATRP kinetics (given by  $^1\text{H}$  NMR in MeOD) showing the evolution of the conversion rate  $\ln[M]_0/[M]$  (●) and the % monomer conversion (■) with the time of polymerisation of 2-HPMA. The molar ratios of polymerisation  $[\text{Cu(I)Br}]:[\text{bpy}]:[\text{initiator}]$  were 1:2:1 targeting a DP of 50, wt% v/v 56.0%. **B, D, F, H (EBIB-, G0-, G1-, G2- respectively):** Plot of number average molecular weight Mn (●) and polydispersity index (-) versus the % conversion obtained by GPC in THF/TEA 2% (PS standards) for the polymerisation of 2-HPMA wt% v/v 56.0%, Cu(I)Cl and initiator, DP<sub>50</sub>.

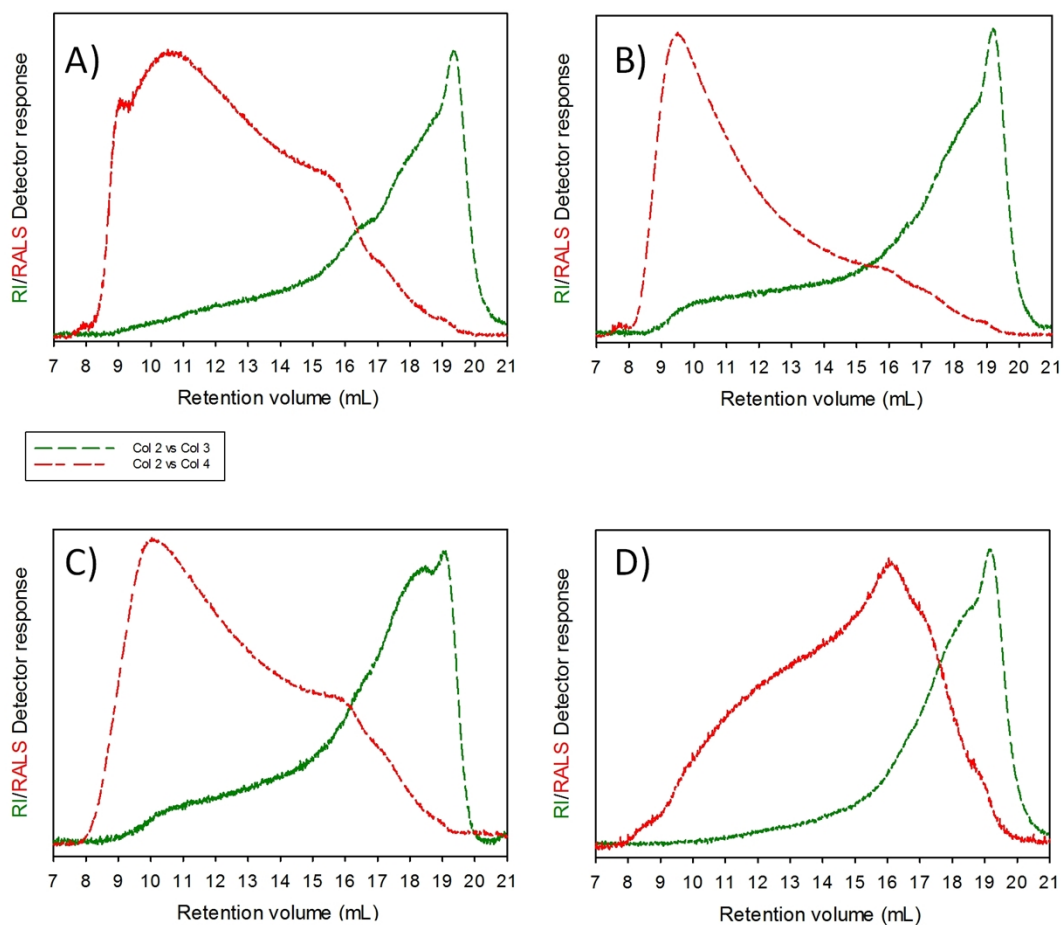




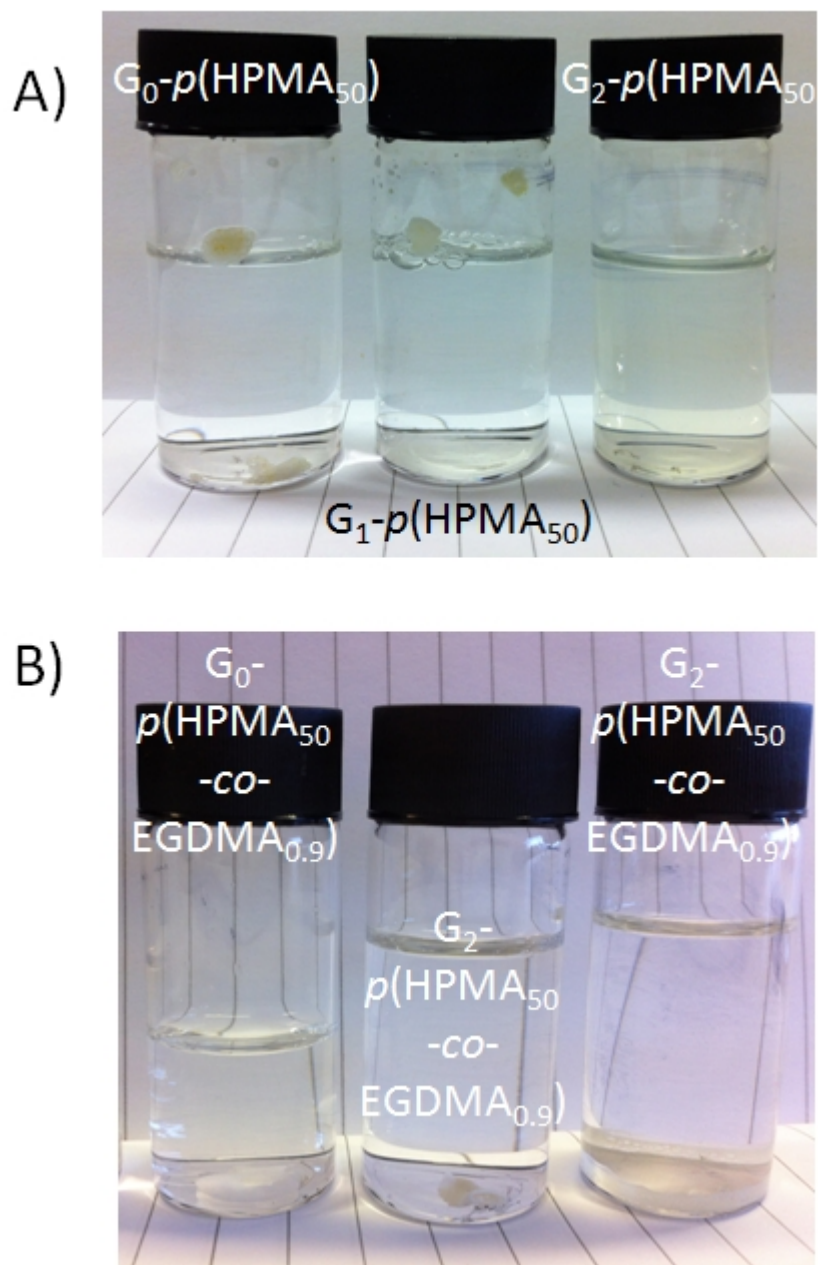
**Figure S28: A, C, E, G (EBiB-, G0-, G1-, G2- respectively):** ATRP kinetics (given by  $^1\text{H}$  NMR in MeOD) showing the evolution of the conversion rate  $\ln [M]_0/[M]$  (●) and the % monomer conversion (■) with the time of polymerisation of 2-HPMA-EGDMA. The molar ratios of polymerisation  $[\text{Cu(I)Br}]:[\text{bpy}]:[\text{initiator}]$  were 1:2:1 targeting a DP of 50, wt% v/v 56.0%. **B, D, F, H (EBiB-, G0-, G1-, G2- respectively):** Plot of number average molecular weight Mn (●), average molecular weight Mw (◆) and polydispersity index (-) versus the % conversion obtained by GPC in THF/TEA 2% (PS standards) for the polymerisation of 2-HPMA-EGDMA wt% v/v 56.0%, Cu(I)Cl and initiator,  $\text{DP}_{50}$ .

| Target polymer   | $\text{dn}/\text{dc}^a$ |
|--|-------------------------|
| EBiB- <i>p</i> (HPMA <sub>50</sub> ), <b>14</b>  | 0.0802                  |
| G <sub>0</sub> - <i>p</i> (HPMA <sub>50</sub> ), <b>15</b>                                   | 0.0853                  |
| G <sub>1</sub> - <i>p</i> (HPMA <sub>50</sub> ), <b>16</b>                                   | 0.0716                  |
| G <sub>2</sub> - <i>p</i> (HPMA <sub>50</sub> ), <b>17</b>                                   | 0.0658                  |
| -----  |                         |
| EBiB- <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.95</sub> ), <b>18</b>           | 0.0739                  |
| G <sub>0</sub> - <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.9</sub> ), <b>19</b> | 0.0825                  |
| G <sub>1</sub> - <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.9</sub> ), <b>20</b> | 0.0858                  |
| G <sub>2</sub> - <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.9</sub> ), <b>21</b> | 0.0758                  |
| -----  |                         |
| <sup>a</sup> THF eluent containing 2% TEA (v/v)  |                         |

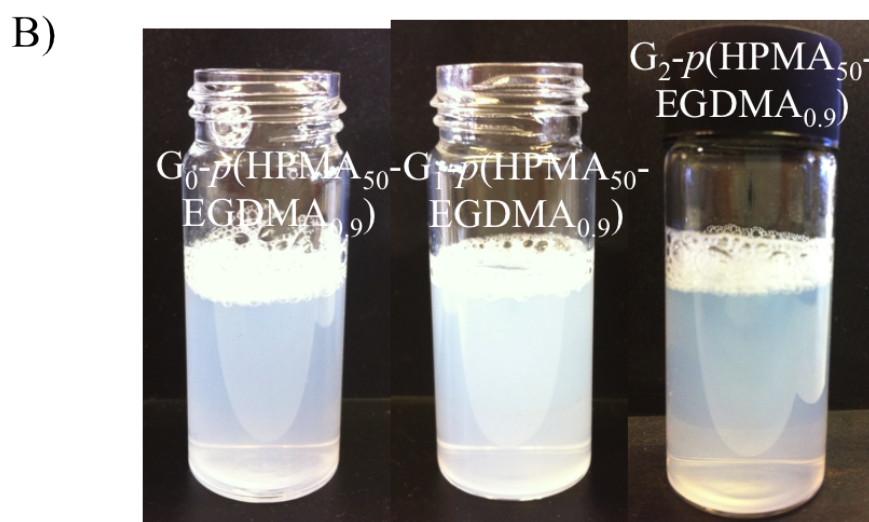
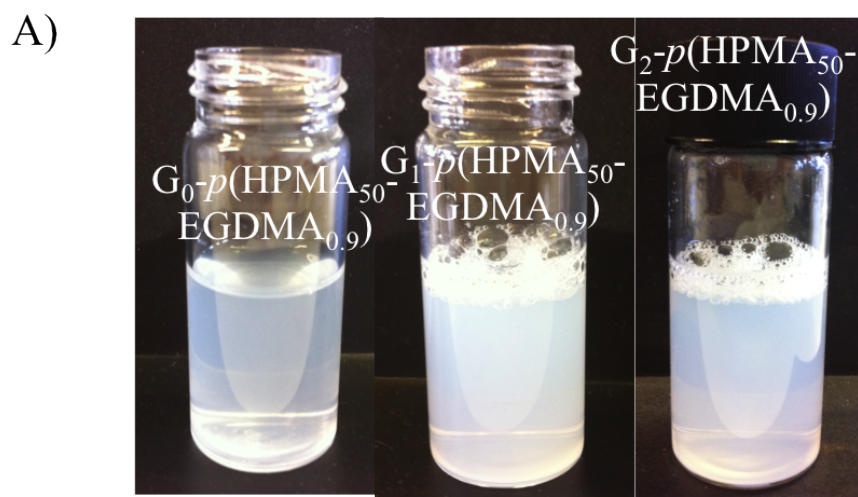
**Table S2:** Average  $\text{dn}/\text{dc}$  values utilised for triple detection SEC analyses (calculated from average kinetic values)



**Figure S29:** SEC Chromatograms of A) EBiB- $p$ (HPMA<sub>50</sub>-co-EGDMA<sub>0.95</sub>), **18**, B) G<sub>0</sub>- $p$ (HPMA<sub>50</sub>-co-EGDMA<sub>0.9</sub>), **19**, C) G<sub>1</sub>- $p$ (HPMA<sub>50</sub>-co-EGDMA<sub>0.9</sub>), **20**, D) G<sub>2</sub>- $p$ (HPMA<sub>50</sub>-co-EGDMA<sub>0.9</sub>), **21** (NOTE: the RI and RALS chromatograms are not expected to overlay within each sample due to the different mode of detection and their relative relationship to concentration (RI) or light scattering intensity (RALS). The detectors will also not receive the sample at identical times after injection. The signals are internally consistent (see retention volumes for peaks and peak onsets))



**Figure S30:** Solubility of linear and branched polymers in water at pH =2

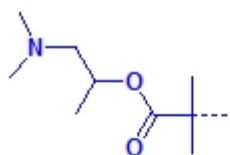


**Figure S31:** Stability of *hyp*-poldendrons to addition of salt A) nanoprecipitates in water at 1 mg/mL, and B) 24 hours after addition of 1 mL of 0.14 M NaCl to 10 mL of nanoprecipitate dispersion.

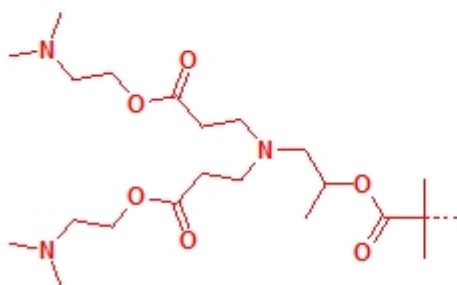
| <i>hyp</i> -Polydendron  | pH = 7.8 <sup>a</sup>  |       | + salt <sup>b</sup>    |       | 24 hrs <sup>c</sup>    |       |
|--|------------------------|-------|------------------------|-------|------------------------|-------|
|  | D <sub>z</sub><br>(nm) | PDI   | D <sub>z</sub><br>(nm) | PDI   | D <sub>z</sub><br>(nm) | PDI   |
| G <sub>0</sub> - <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.9</sub> ), <b>19</b> | 176                    | 0.193 | 237                    | 0.168 | 249                    | 0.153 |
| G <sub>1</sub> - <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.9</sub> ), <b>20</b> | 165                    | 0.097 | 163                    | 0.089 | 162                    | 0.104 |
| G <sub>2</sub> - <i>p</i> (HPMA <sub>50</sub> - <i>co</i> -EGDMA <sub>0.9</sub> ), <b>21</b> | 133                    | 0.081 | 160                    | 0.202 | 157                    | 0.130 |

<sup>a</sup> Initial pH of water  
<sup>b</sup> Addition of 0.14M NaOH (1 mL) to 1 mg/mL *hyp*-polydendron NP dispersion (10 mL)  
<sup>c</sup> 24 hours after salt addition

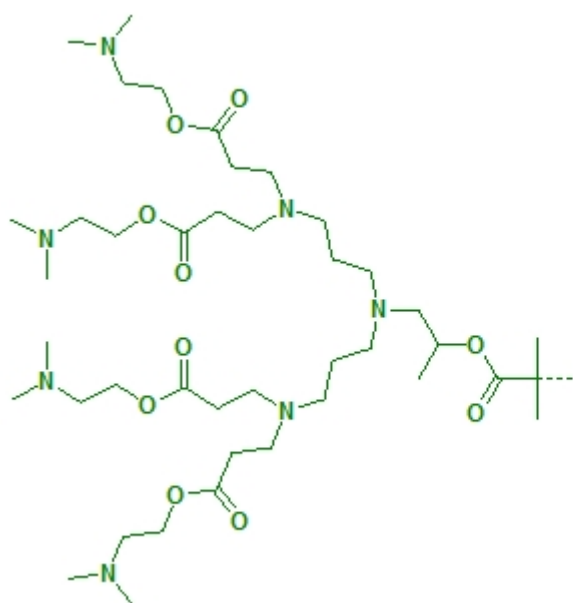
**Table S3:** Impact of salt addition to to *hyp*-polydendron nanoprecipitates (measured at 25 °C)



miLogP = 1.690

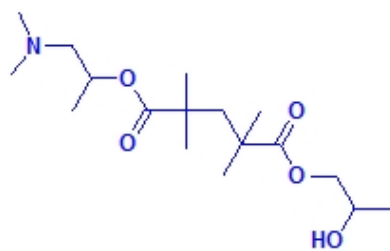


miLogP = 1.399

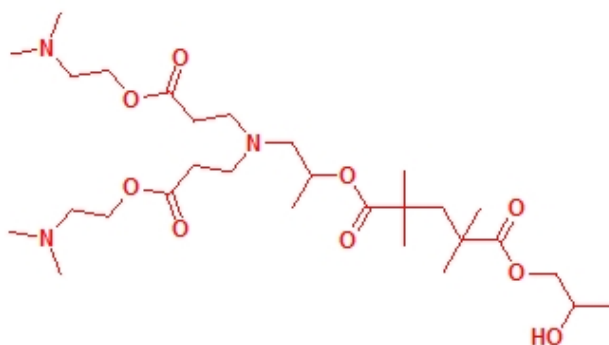


miLogP = 1.709

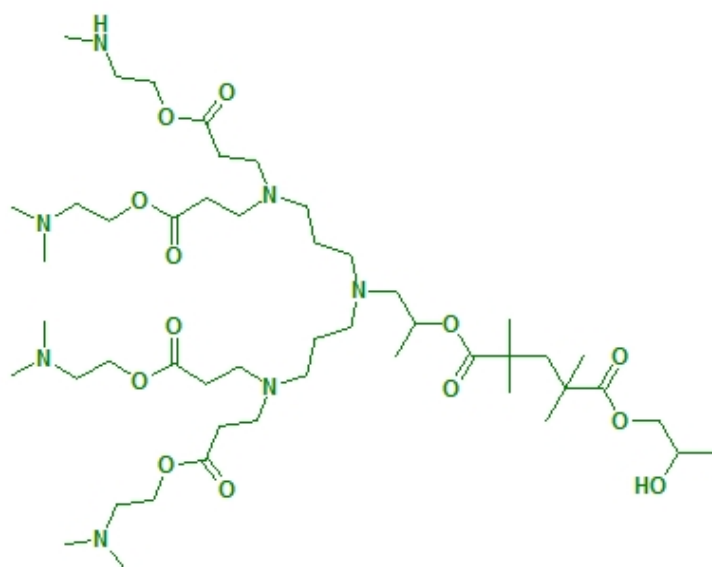
**Figure S32:** Estimation of logP for dendron end group fragments



miLogP = 2.749



miLogP = 2.458



miLogP = 2.768

**Figure S33:** Estimation of logP for dendron end group fragments



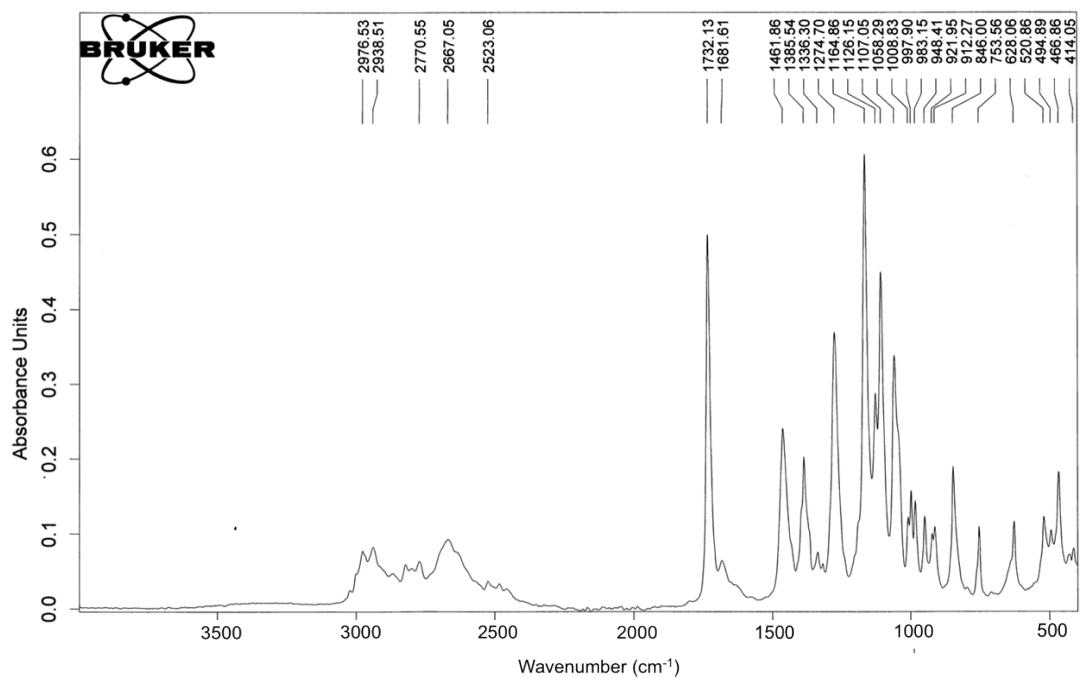


Figure S34: FT-IR spectrum of G<sub>0</sub>-Br, 11

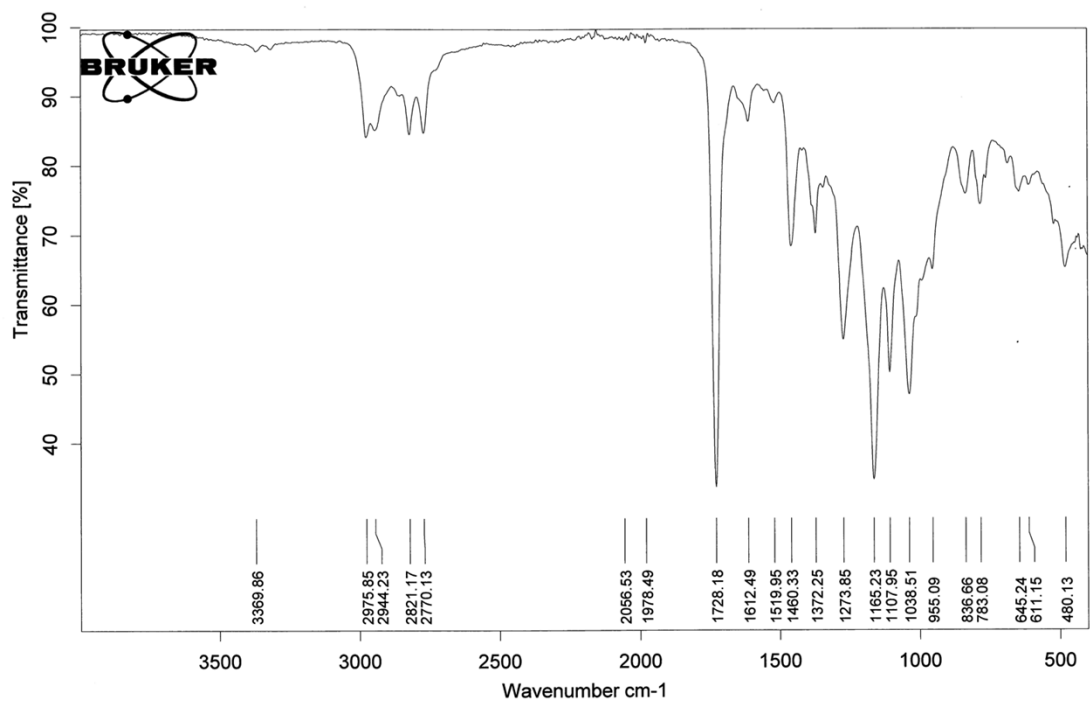
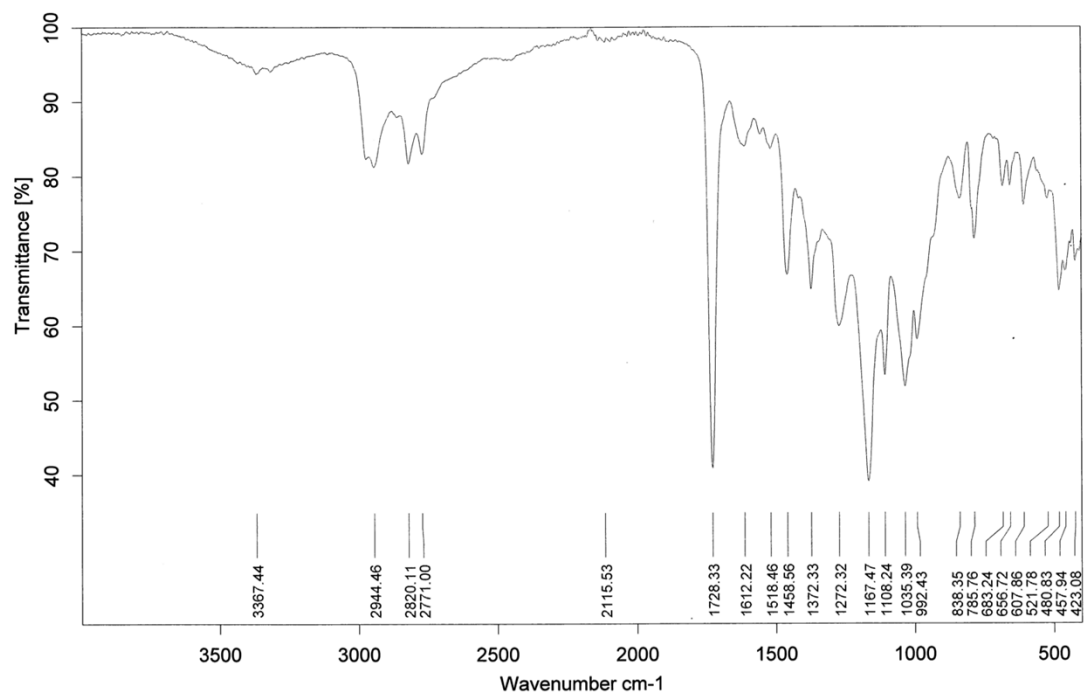


Figure S35: FT-IR spectrum of G<sub>1</sub>-Br, 12



**Figure S36:** FT-IR spectrum of G<sub>2</sub>-Br, 13