ELECTRONIC SUPPORTING INFORMATION

A New Proton-Sponge Polymer Synthesized by RAFT Polymerization for Intracellular Delivery of Biotherapeutics

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Synthesis of 3-azido-propan-1-ol

3-bromo-1-propanol (7 gr, 52.1 mmol) and sodium azide (5.24 gr (83.6 mmol) were dissolved in acetone/water mixture (5:1). The reaction was kept at 70 °C under refluxing for overnight. After cooled down, acetone was removed via rotary evaporator. The reaction solution was washed with dietylether (3x100 mL). The product was obtained as yellow liquid after removing of organic solvent. (2.14 g, 37%).

^{1}H NMR (D_2O, 298K, 400 MHz) δ(ppm) = 5.45 (1H), 3.95-3.60 (6H)
FT-IR ν: 2095 (N_3) cm^{-1}

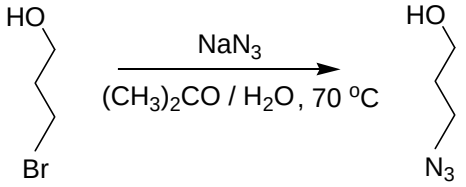

Figure S1. Synthesise of 3-azido-propan-1-ol.

As depicted in Figure S2, the reaction was confirmed by using ^{1}H NMR which showed a change in the chemical shift of protons after the reaction due to transformation of of the end group from bromine to azide.

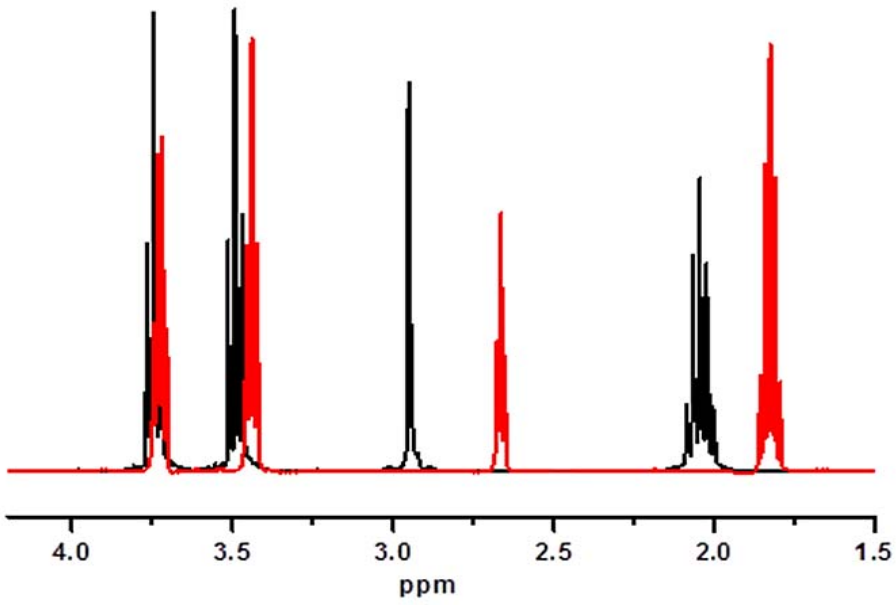

Figure S2. ^{1}H NMR spectra of 3-bromo-propan-1-ol (black) and 3-azido-propan-1-ol (red).

Moreover, this synthesis proved very successful and characterized by FITR showed the peak of azide appeared at 2095 cm^{-1} after reaction as shown in Figure S3.

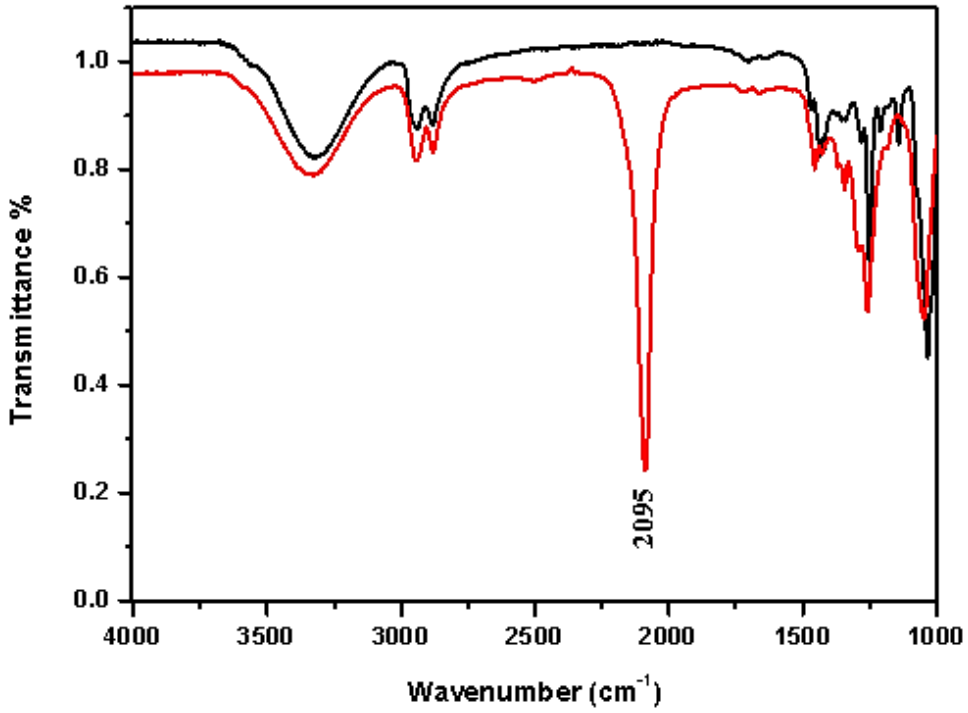

Figure S3. FITR spectra of 3-bromo-propan-1-ol (black) and 3-azido-propan-1-ol (red).

Synthesis of 3-azidopropyl acrylate

The obtained 3-azido-propan-1-ol (2.00 g, 1.8 mmol), TEA (4.2 mL, 2.4 mmol), hydroquinone (20 mg) and anhydrous diethyl ether (150 mL) was cooled with ice.. Acryloyl chloride (1.8 mL, 2.4 mmol) in 15 mL diethyl ether was added into the solution slowly. The reaction mixture was kept in the ice bath for 1 h and then stirred at room temperature overnight. The ammonium salts were removed by filtration and the residue was extracted sequentially with aqueous solution of hydrochloric acid (1/10 v/v, 2x 40 mL), water (2x 40 mL), 10 wt% aqueous NaOH (2x 40 mL) and water (2x 40 mL). The organic solvent was removed via rotary evaporator. The product was obtained as bright yellow liquid. (1.78 g, 88%).

^{1}H NMR ($CDCl_3$, 298 K, 400 MHz): δ =6.40 (dd, 1 H, J 1.4, 15.9 Hz, CH_2=CH), 6.11 (dd, 1 H, J 10.5, 6.9 Hz, CH_2=C*H*), 5.84 (dd, 1 H, J 1.4, 9.0 Hz, CH_2=CH), 4.24 (t, 2 H, J 6.2 Hz, O-CH_2), 3.40 (t, 2 H, J 6.7 Hz,CH_2N_3), 1.95 (m, 2 H, CCH_2C) ppm.

^{13}C NMR ($CDCl_3$, 298 K, 400 MHz):δ = 130.2 (*C*=O), 131.2 (*C*H_2=CH), 128.3 (CH_2=*C*H), 61.5 (O-*C*H_2), 48.3 (*C*H_2-N_3), 38.4 (C-*C*H_2-C) ppm. FT-IR *v*: 2095 (-N_3), 1725 (C=O) cm^{-1}.

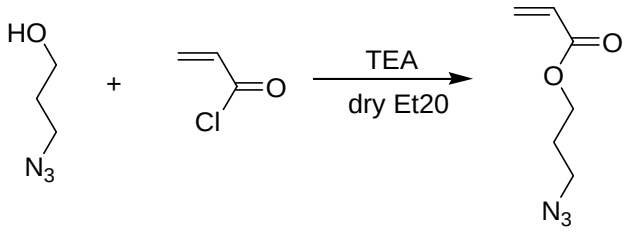

Figure S4. Schematic representation of the synthesised 3-azidopropyl acrylate.

This reaction is a typical esterification that was kept in anhydrous diethyl ether in the presence of triethylamine. Finally the pure product was obtained and that was confirmed by ^{1}H and ^{13}C NMR (Figure S5). The vinyl groups have peaks between δ5.8-6.4 and also formation of ester protons were observed at 4.2 ppm.

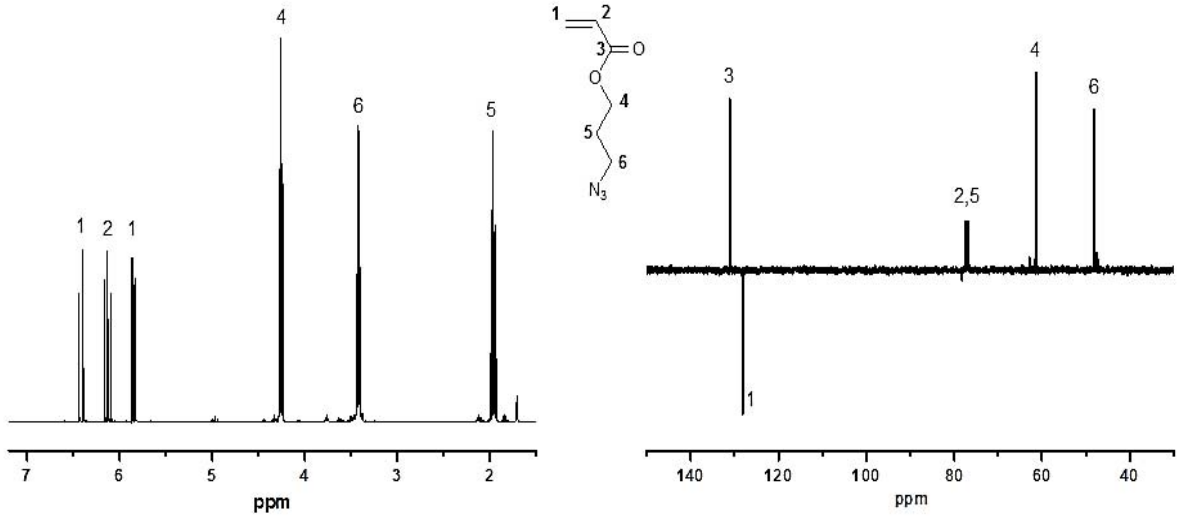

Figure S5. ^{1}H (left) and ^{13}C NMR (right) spectrum of 3-azidopropyl acrylate.

Additionally, FITR result demonstrated azide and carbon-oxygen double bond peaks at 2095 and 1725 cm^{-1}, respectively (Figure S6).

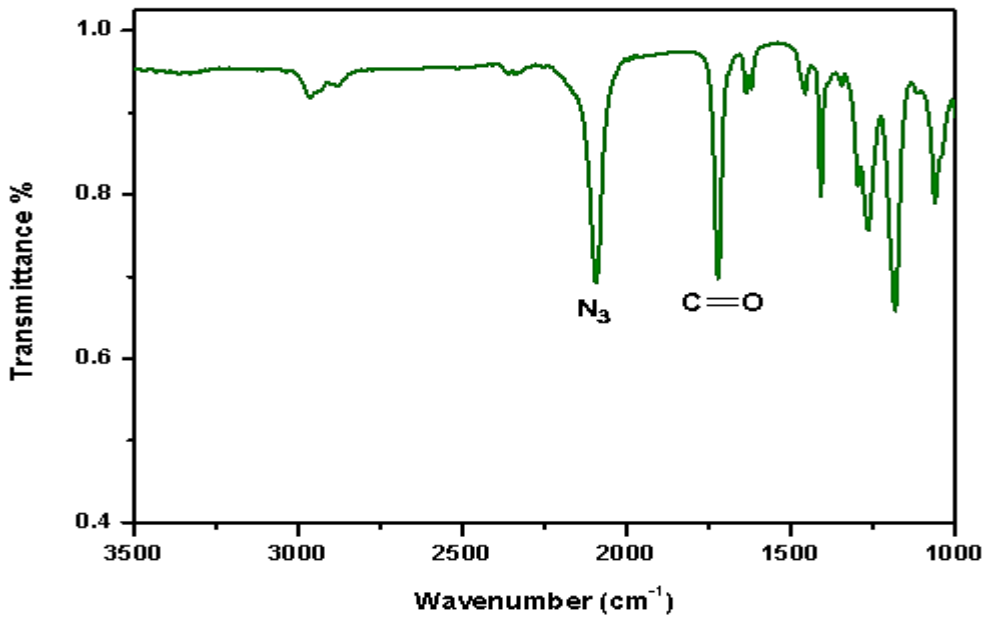

Figure S6. FITR spectra of 3-azidopropyl acrylate.

Synthesis of 1-(2′ -Propargyl) D-Mannose

1-(2′ -Propargyl) D-Mannose was prepared in according to the procedure reported by Mukhopadhyay *et al.*[42] The purification of reaction mixture was done by a silica column and the relevant fractions were collected and analysed.

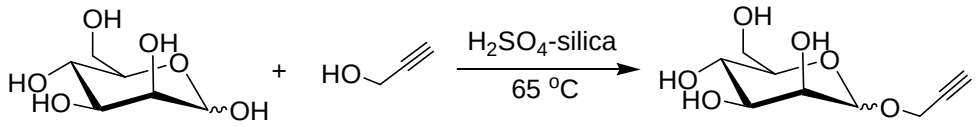

Figure S7. Schematic representation of the synthesised 1-(2′ -Propargyl) D-Mannose.

The reaction was basically followed by FITR that showed distinct bands of C≡C-H and C≡C bonds, Figure S8. Technically, it was expected to see a propargyl group peak at 2.5 ppm in NMR, but it was not seen because of the effect of hydroxyl groups.

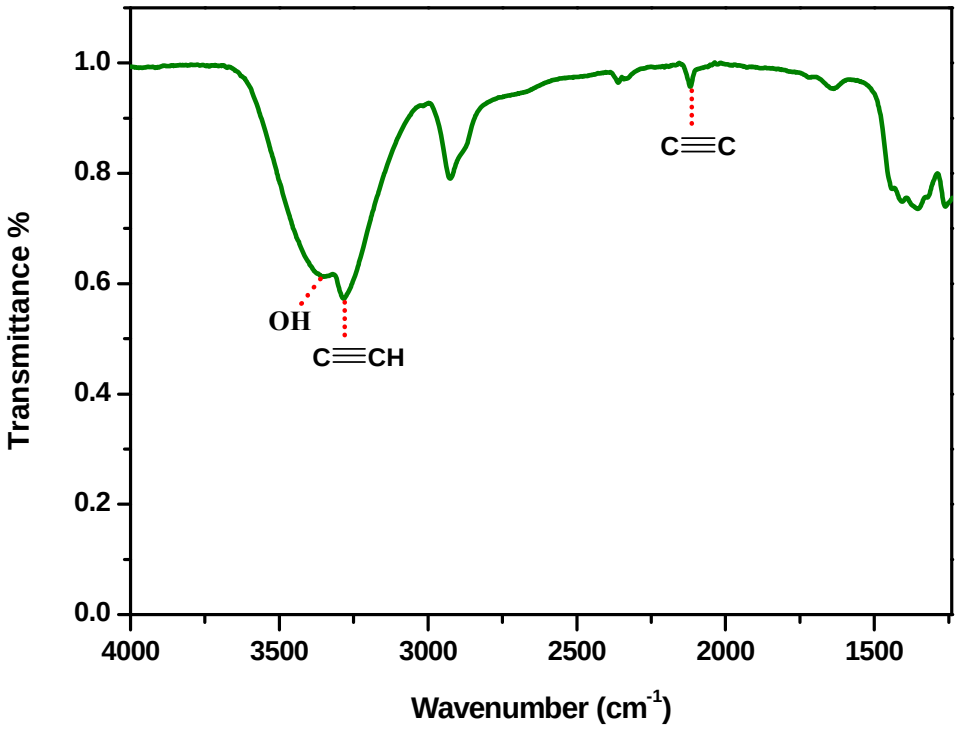

Figure S8. FITR spectra of 1-(2′ -Propargyl) D-Mannose.

Synthesis of D-Mannose glycomonomer via CuAAC reaction

1-(2′-propargyl) D-mannose (2.46 g, 12.6 mmol) and 3-azidopropyl acrylate (2.85 g, 11.8 mmol) were dissolved in MeOH/H_2O (2:1 vol/vol, 60 mL), aqueous solution of $CuSO_4 \cdot 5H_2O$ (246 mg, 0.9 mmol) and (+)-sodium L-ascorbate (284 mg, 1.2 mmol) were added into the reaction solution. The reaction mixture was stirred at ambient temperature for 24 h and then the methanol was removed under vacuum and residue mixture was freeze dried to remove water. The purification of the obtained product was done by silica gel column chromatography using dichloromethane-MeOH (8:1) as eluent. After the removing of solvent, the product was obtained as white (1.62 g, yield: 58.2%).

^{1}H NMR (D_2O, 298 K, 400 MHz): δ =8.07, 8.06 (s, overlaped, 1 H, NC*H*=C), 6.37 (dd, J=1.8, 15.5 Hz), 6.36 (dd, J=1.6, 15.7 Hz) (anomeric 1 H, CH_2=C), 6.14 (dd, J=10.4, 6.9 Hz), 6.13(dd, J=10.4, 7.0 Hz) (anomeric, 1 H, CH_2=C*H*C=O), 5.89 (dd, 1 H, J=1.5, 8.9 Hz, CH_2=C), 4.70-5.05 (m, CH_2-OH, H-1 of mannose , overlap with H_2O), 4.64 (d, 1 H, J=12.3 Hz, CH_2-OH), 4.55 (t, 2 H, J=6.9 Hz, CH_2-N), 4.19 (t, 2 H, J=6.0 Hz, C=O-O-CH_2), 3.40-3.92 (m, H residues of mannose), 2.30 (m, 2H, CH_2-CH_2-CH_2) ppm.

^{13}C NMR (D_2O, 298 K, 400 MHz): δ =146.4 (*C*=O), 145.4 (N-CH=*C*), 131.9 (*C*H_2=C), 129.2 (CH_2=*C*), 125.6 (N-*C*H=C), 100.8 (β anomeric, C 1 of mannose), 100.7 (α anomeric, C 1 of mannose), 78.4,75.2, 75.0, 72.5, 72.3, 72.0, 68.6, 68.4 (carbons of anomeric mannose), 63.0(*C*H_2-OH), 62.6 (C=O-O-*C*H_2), 60.7 (C-*C*H_2-O), 48.5 (CH_2-*C*H_2-N), 28.5(CH_2-*C*H_2-CH_2) ppm. FT-IR ν: 3350.6 (OH), 2901.6 (C=C-H), 1720.5 (C=O) cm^{-1}.

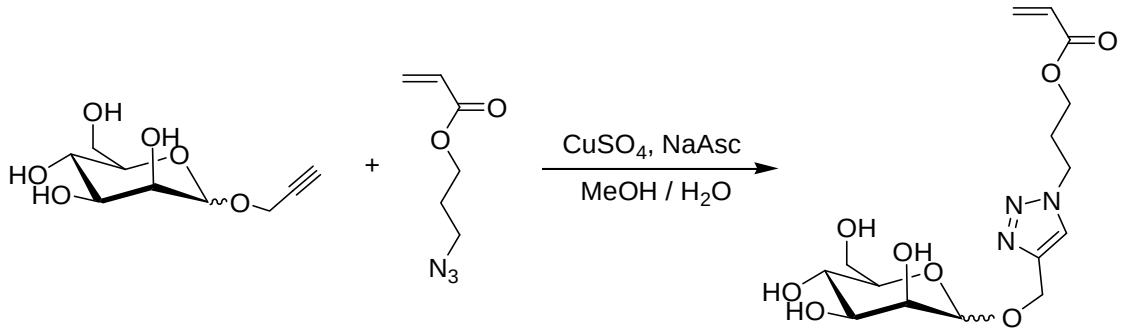

Figure S9. Schematic representation of the synthesised D-Mannose glycomonomer.

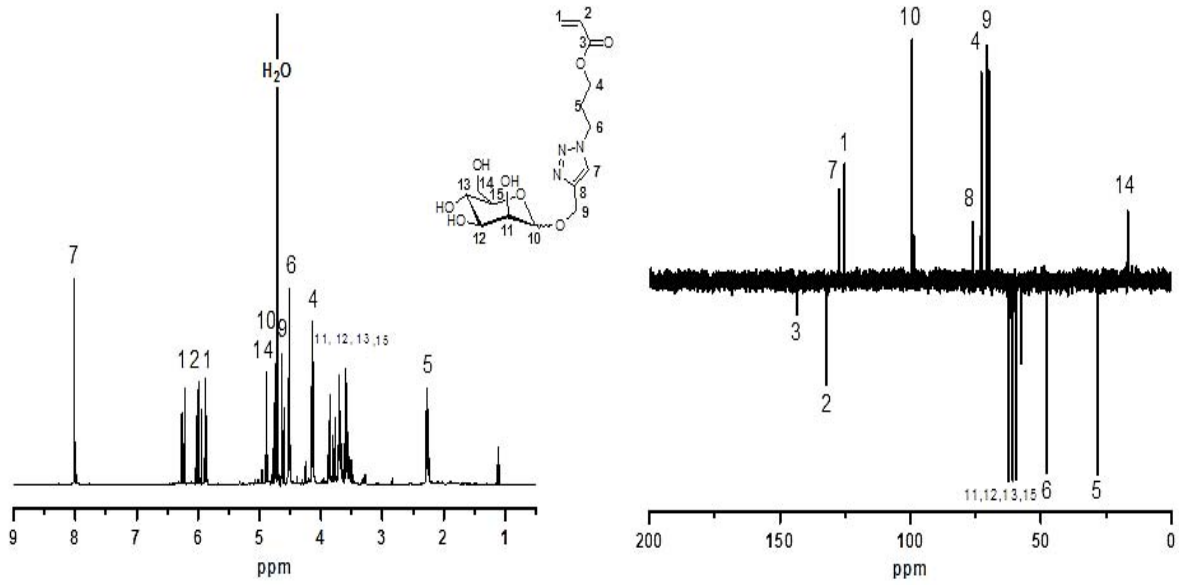

Figure 10. ^{1}H (left) and ^{13}C NMR (right) spectrum of D-Mannose glycomonomer.

Silica gel column was used with dichloromethane and methanol mixture as eluents for the purification. However, silica has a good solubility in methanol so that the ratio of it was kept low; however, this resulted in poor solubility of product in this solvent mixture. Therefore, an optimization was done using dichloromethane and methanol (10:1). The yield of reaction is calculated as 65%.

Mannose azide Synthesis

Mannose was activated at α anomeric position of the reducing terminus by 2-chloro-1,3-dimethylimidazolinium chloride (DMC), which then undergoes nucleophilic substitution by sodium azide, possibly via a 1,2-dehydro intermediate, Figure S11.

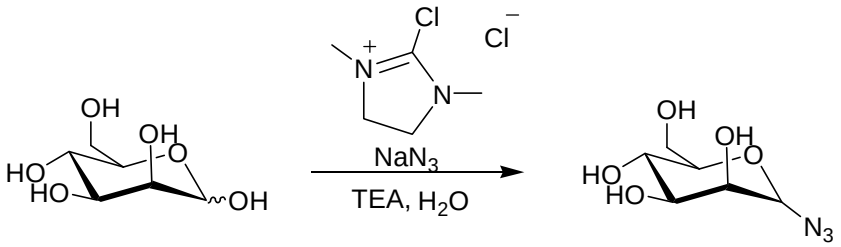

Figure S11. Synthesis of mannose azide.

D-mannose (2.38 g, 132 mmol), sodium azide (9 g, 132 mmol), and triethylamine (17.2 mL, 132 mmol) were dissolved in water (40 mL) and cooled down to 0 ºC. 2-Chloro-1,3-dimethylimidazolinium chloride (6.72 g, 396 mmol) was added and the mixture was stirred for an hour at 0 ºC. The solvent was removed under reduced pressure and EtOH (40 mL) was added. The sodium salts were removed by filtration and the residue impurities were removed through Amberlite IR-120 column, using EtOH as the eluent. The solvent was removed under reduced pressure, water (50 mL) was added and the extraction was done with dichloromethane (3 x 25 mL). After removing of the solvent, the solution was freeze-dried overnight to yield mannose azide (1.94 g, 76%) as an off- white solid.

^{1}H NMR (D_2O, 298K, 300 MHz) δ(ppm) = 5.45 (1H), 3.95-3.60 (6H)

^{13}C NMR (D_2O, 298K, 300 MHz) δ(ppm) = 89.72 (C1), 74.65, 69.86, 69.77, 66.40, 60.83 (C6) FT-IR ν: 2112 (N_3) cm^{-1}

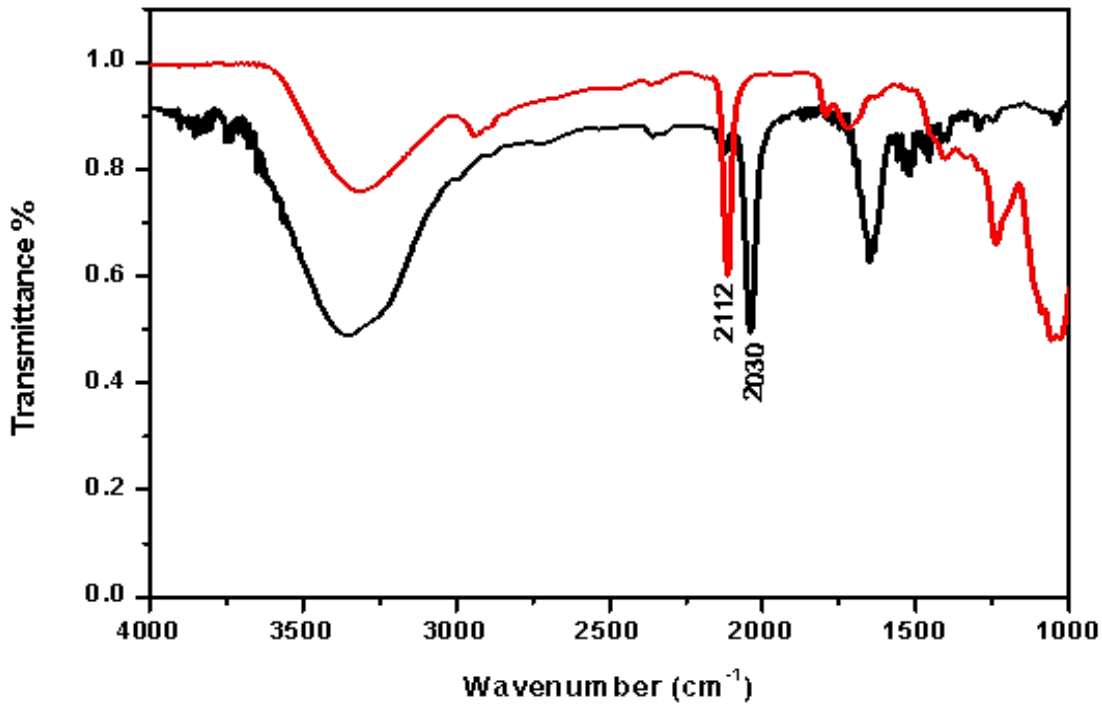

Figure S12. FITR spectra of mannose azide before (black) and after (red) an Amberlite column.

^{1}H and ^{13}C NMR confirmed the structure, as shown in Figure S13. Furthermore, ^{1}H NMR was used to assign the structure of D-Mannose azide as α-linked glycoside that was obtained at 5.4 ppm.

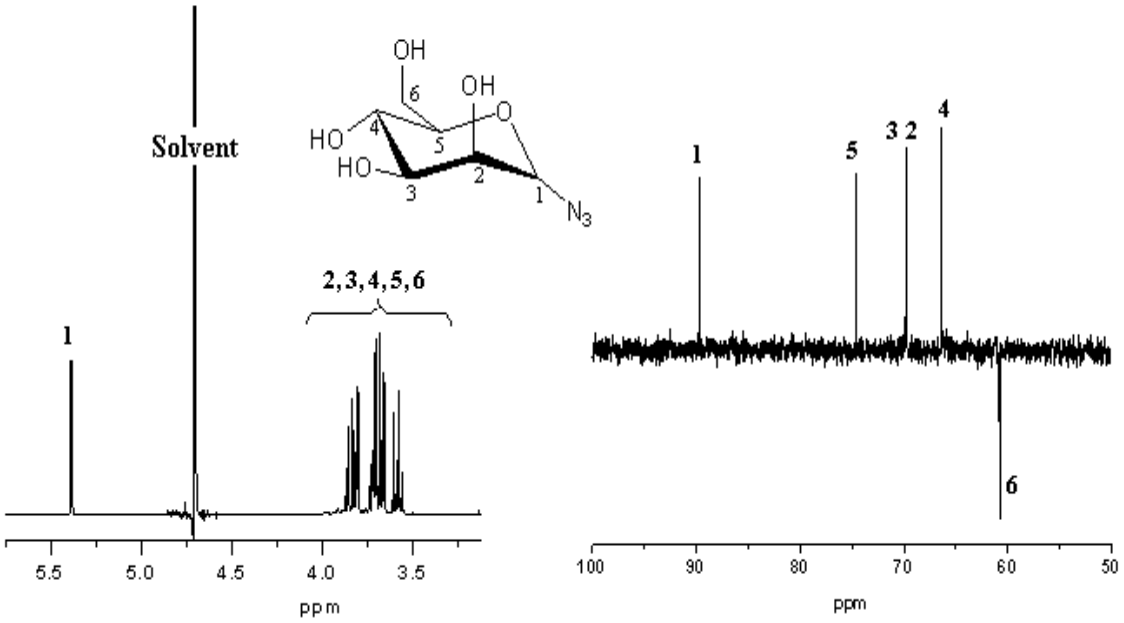

Figure S13. ^{1}H (left) and ^{13}C NMR (right) spectrum of mannose azide.

Synthesis of 1-(2′-Propargyl) D-Mannose

The solution of D-mannose (12 g, 65 mmol), propargyl alcohol (19.4 mL, 333 mmol) and H2SO4-silica (333 mg) was stirred at 65oC overnight and cooled to ambient temperature. Half of the product was transferred to a silica gel column and eluted with CHCl3-MeOH (8:1) to remove the excess propargyl alcohol. The product was obtained as white solid after drying under vacuum (3.4 g, yield: 54 %). FT-IR ν: 3347 (OH), 3285 (C≡C-H), 2118 (C≡C) cm^{-1}.

Protection of N-hydroxyethylethylenediamine

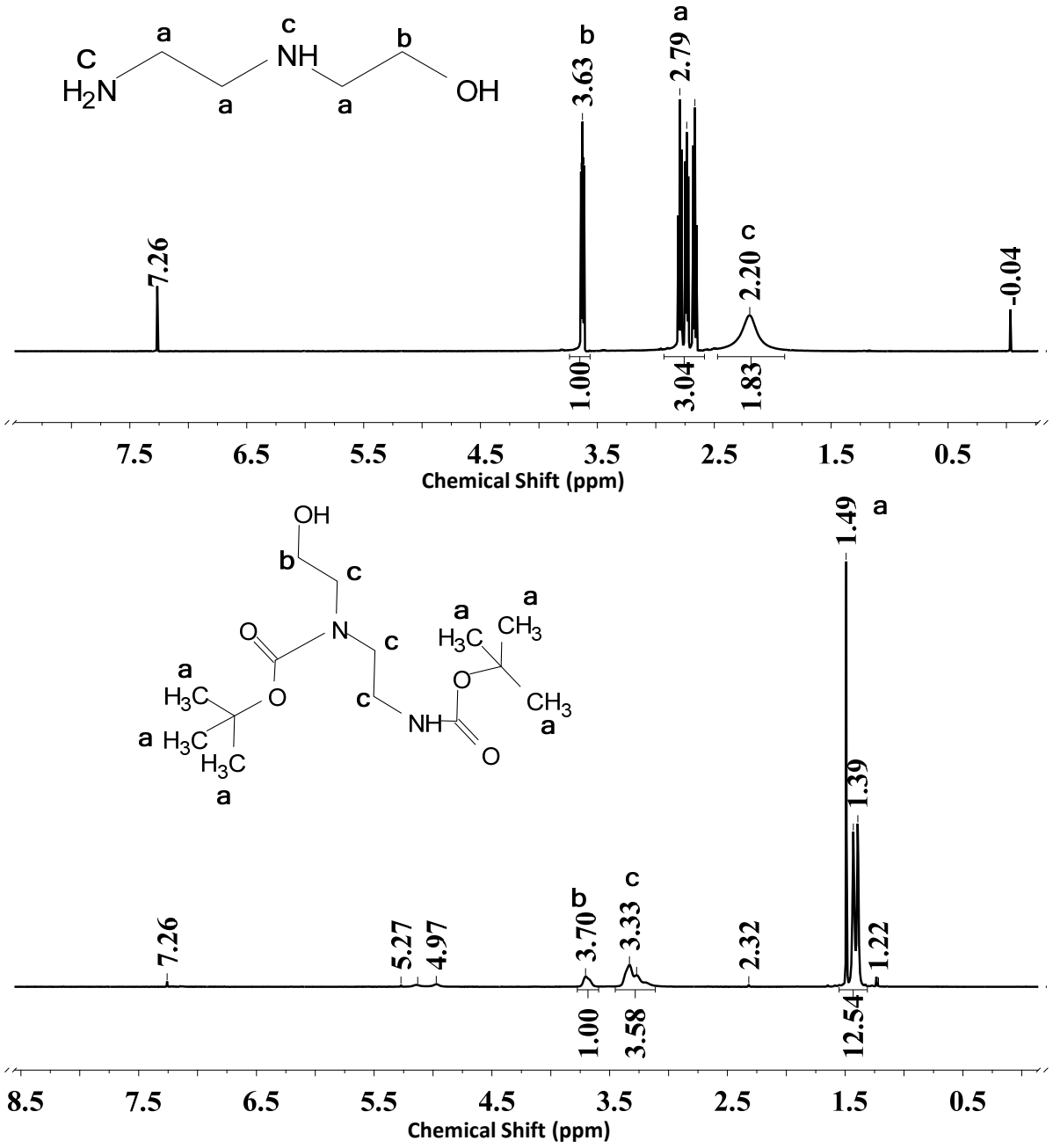

Figure S14. ^{1}H-NMR spectra of N-hydroxyethylethylenediamine before and after protection with Boc-group. The bottom spectrum was taken after purification.

Synthesis of methacrylated tert-butyl (2-((tert-butoxycarbonyl) amino) ethyl) (2-hydroxyethyl)carbamate

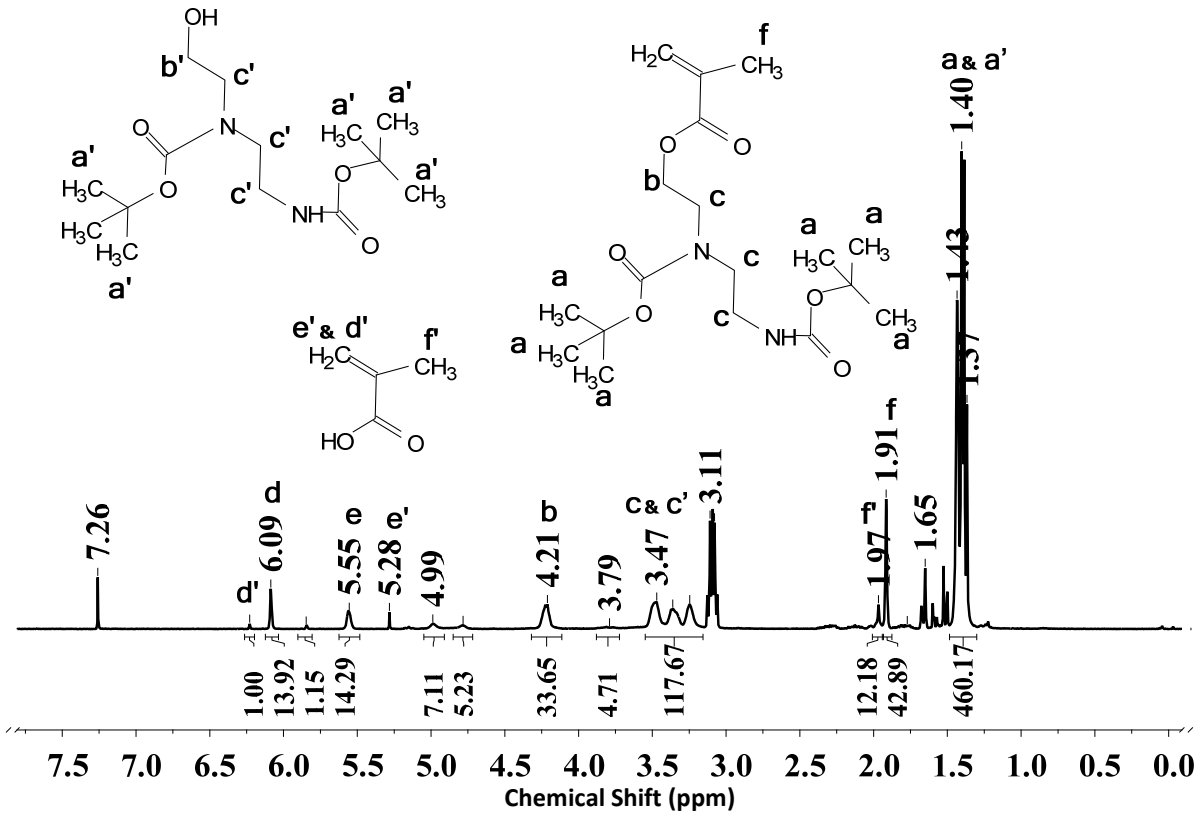

Figure S15. ^{1}H-NMR spectrum of methacrylated tert-butyl (2-((tert-butoxycarbonyl) amino) ethyl) (2-hydroxyethyl)carbamate before purification.

$$\text{Yield \%} = \frac{\frac{I_{4.22ppm}}{2}}{\frac{I_{3.78ppm} + I_{4.22ppm}}{2}} x100$$

$$\text{Yield \%} = \frac{\frac{3.74}{2}}{\frac{0.69 + 3.74}{2}} x100 = 84\%$$

Calculation of Monomer Conversion and Molecular Weight by Theoretical and NMR:

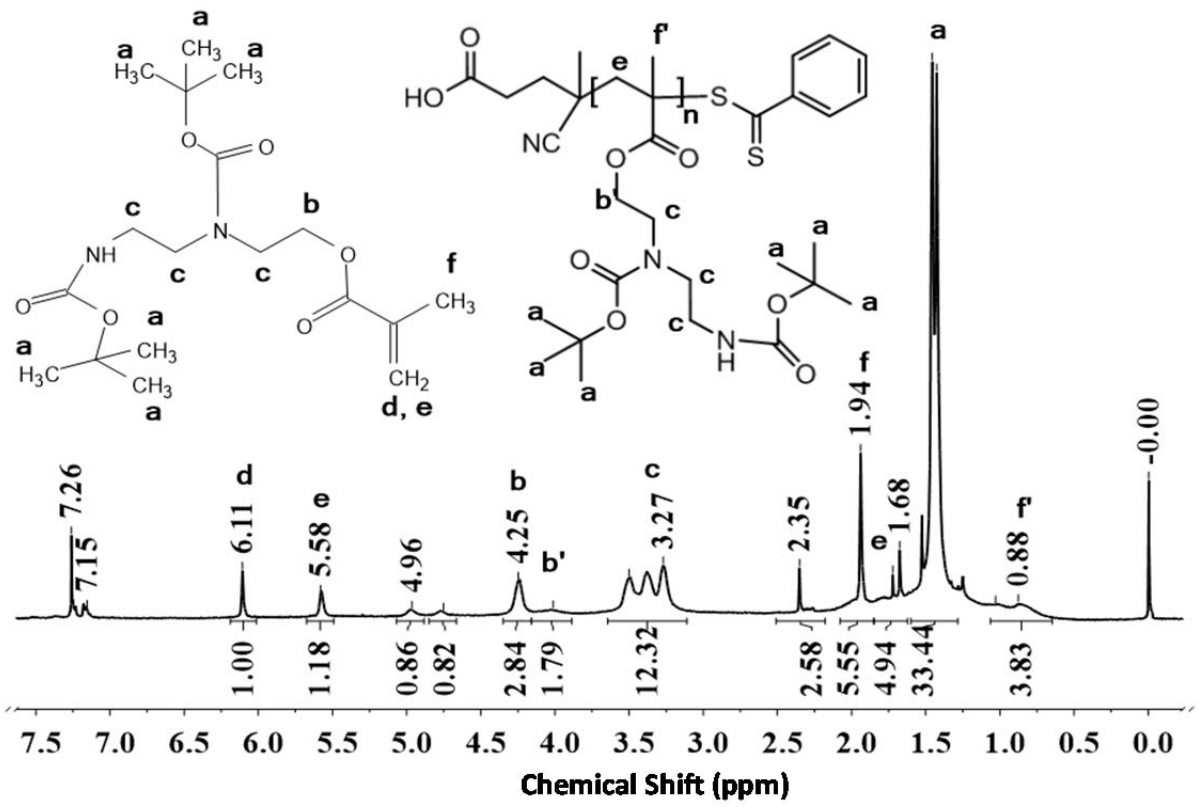

Figure S16. ^{1}H-NMR spectrum of polymerization mixture of 2-((tert-butoxycarbonyl) (2-((tert-butoxycarbonyl) amino) ethyl) amino) ethyl methacrylate $[M]_0$= 0.72 M and [M]/[R]/[I]:25/1/0.25. Reaction occurred at 65°C for 3h.

$$\text{Monomer Conv \%} = \frac{\frac{\left(I_{1H\text{ at }6.11ppm} + I_{1H\text{ at }5.58ppm}\right)}{2}}{\frac{\left(I_{2H\text{ at }4.24ppm} + I_{2H\text{ at }4.01ppm}\right)}{2}} \text{ x100}$$

$$\text{Monomer Conv \%} = \frac{\frac{(1.00 + 1.18)}{2}}{\frac{(2.84 + 1.79)}{2}} \text{ x100} = 47\%$$

$$M_{n_{(theo)}} = \frac{[M_0]}{[RAFT_0]} x Mw_{monomer} x\ Conv\ \% + Mw_{RAFT}$$

$$M_{n_{(theo)}} = \frac{0.72\ M}{0.0288\ M} x 372.46 \frac{g}{mol} x\ 0.47 + 279.38 \frac{g}{mol}$$

$$= 4656\ g/mol$$

Table S1 RAFT polymerization conditions of BocAEAEMA

Exp. No	Time (h)	[BocAEAEMA] (mol/L)	[CPDBA]x10^2 (mol/L)	[AIBN]x10^3 (mol/L)	$[BocAEAEMA]_0/[CPDBA]_0/[AIBN]_0$	Conversion %	Mn^{theo} (g/mol)	Mn^{NMR} (g/mol)	Mn^{GPC} (g/mol)	PDI
1	2	0.36	1.44	3.6	25/1/0.25	27	2795	ND	2560	1.14
	3	0.36	1.44	3.6	25/1/0.25	29	2980	ND	2840	1.11
	4	0.36	1.44	3.6	25/1/0.25	30	3073	ND	3160	1.12
	5	0.36	1.44	3.6	25/1/0.25	46	4563	ND	3600	1.14
	6	0.36	1.44	3.6	25/1/0.25	49	4842	ND	4250	1.13
	8	0.36	1.44	3.6	25/1/0.25	52	5121	ND	4900	1.14
	10	0.36	1.44	3.6	25/1/0.25	59	5773	ND	5050	1.15
2	1	0.72	2.9	7.3	25/1/0.25	27	2794	1520	4650	1.36
	2	0.72	2.9	7.3	25/1/0.25	37	3725	1760	3700	1.30
	3	0.72	2.9	7.3	25/1/0.25	47	4656	3140	3640	1.14
	4	0.72	2.9	7.3	25/1/0.25	64	6239	6340	4980	1.15
	5	0.72	2.9	7.3	25/1/0.25	66	6425	ND	5230	1.16
	6	0.72	2.9	7.3	25/1/0.25	77	7449	7350	5670	1.16
	8	0.72	2.9	7.3	25/1/0.25	80	7729	8630	6060	1.18
	10	0.72	2.9	7.3	25/1/0.25	86	8287	ND	6420	1.18

Table S1 (Continued) RAFT polymerization conditions of BocAEAEMA

Exp. No	Time (h)	[BocAEAEMA] (mol/L)	[CPDBA]x10^2 (mol/L)	[AIBN]x10^3 (mol/L)	$[BocAEAEMA]_0/[CPDBA]_0/[AIBN]_0$	Conversion %	Mn^{theo} (g/mol)	Mn^{NMR} (g/mol)	Mn^{GPC} (g/mol)	PDI
3	1	0.72	1.44	3.6	50/1/0.25	8	1769	2210	3040	1.15
	2	0.72	1.44	3.6	50/1/0.25	21	4190	3320	4060	1.18
	3	0.72	1.44	3.6	50/1/0.25	27	5308	6700	5320	1.22
	4	0.72	1.44	3.6	50/1/0.25	35	6797	12000	6960	1.25
	5	0.72	1.44	3.6	50/1/0.25	51	9777	ND	8170	1.28
4	1	0.72	0.72	1.8	100/1/0.25	11	4376	3240	4780	1.20
	2	0.72	0.72	1.8	100/1/0.25	28	10708	8610	5310	1.23
	3	0.72	0.72	1.8	100/1/0.25	37	14060	14400	8920	1.34
	4	0.72	0.72	1.8	100/1/0.25	39	14805	6870	13560	1.30
	5	0.72	0.72	1.8	100/1/0.25	57	21510	11400	14450	1.32
5	2	1.44	1.44	3.6	100/1/0.25	40	15178	ND	8500	1.50
	3	1.44	1.44	3.6	100/1/0.25	43	16295	ND	8900	1.70
	4	1.44	1.44	3.6	100/1/0.25	57	21510	ND	10240	1.50
	5	1.44	1.44	3.6	100/1/0.25	69	25979	ND	15200	1.40
	6	1.44	1.44	3.6	100/1/0.25	72	27097	ND	14900	1.55
	8	1.44	1.44	3.6	100/1/0.25	80	30076	ND	16300	1.41
	10	1.44	1.44	3.6	100/1/0.25	80	30076	ND	19000	1.31

GPC Chromatograms

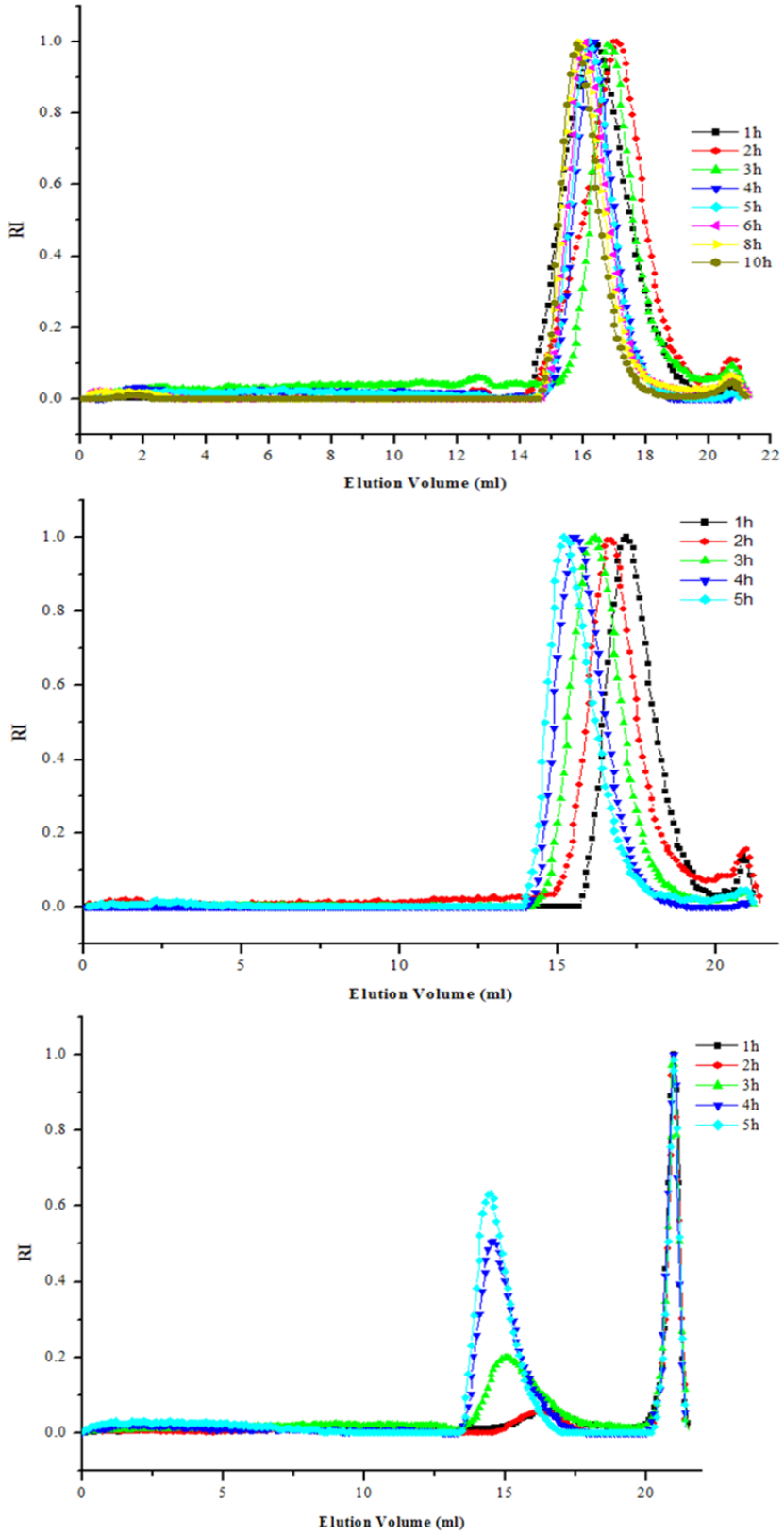

Figure S17. GPC chromatograms of polymerization mixtures. [Monomer]= 0.72 M and [M]/[R]/[I] ratios were top) 25/1/0.25, middle) 50/1/0.25 and bottom) 100/1/0.25.

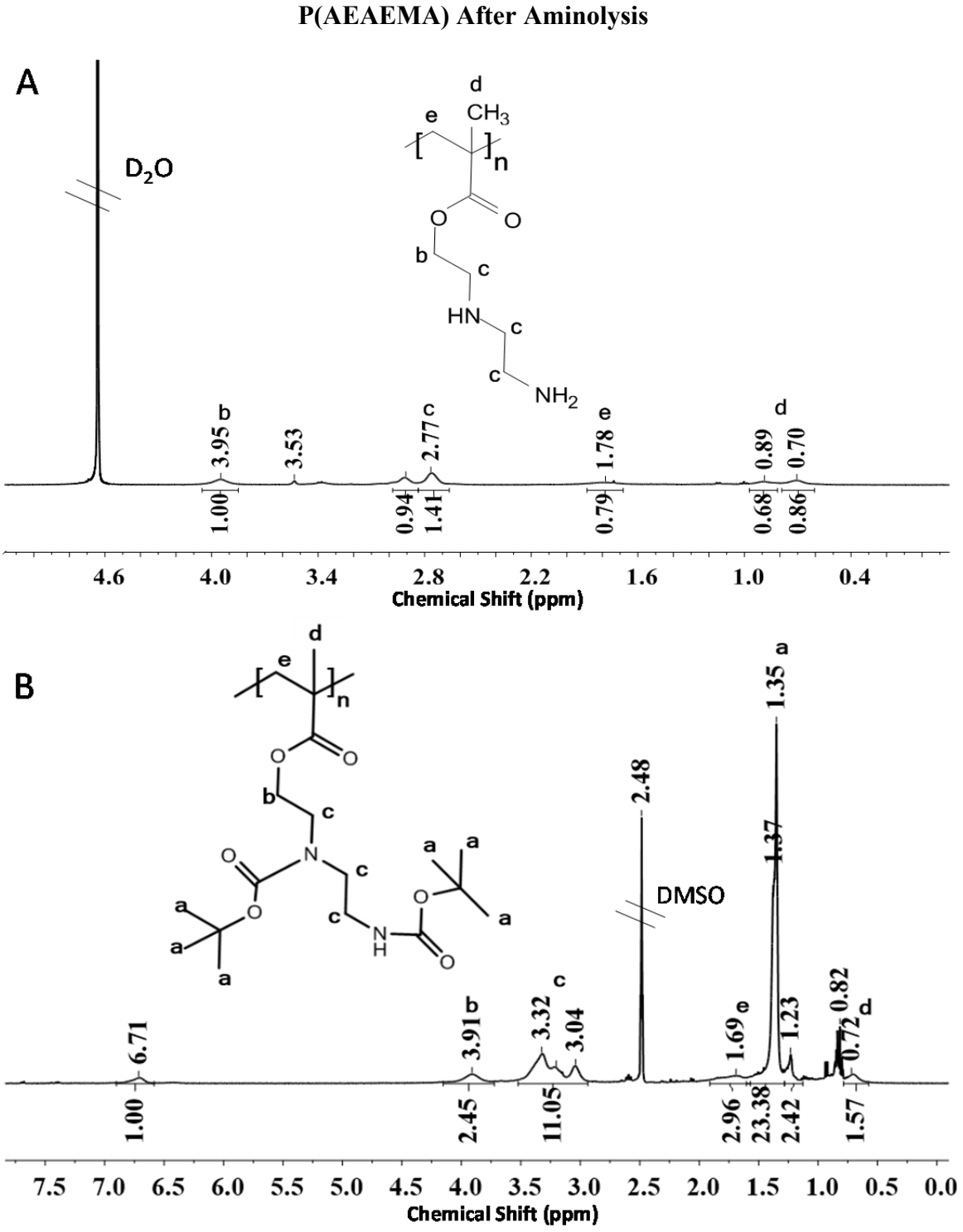

Figure S18. ^{1}H-NMR spectrum of (A) p(AEAEMA) ($M_{n,after\ deprotection}$= 5.5 kDa after aminolysis and dialysis; (B) p(BocAEAEMA) (M_n = 11 kDa) after aminolysis and precipitation in hexane.

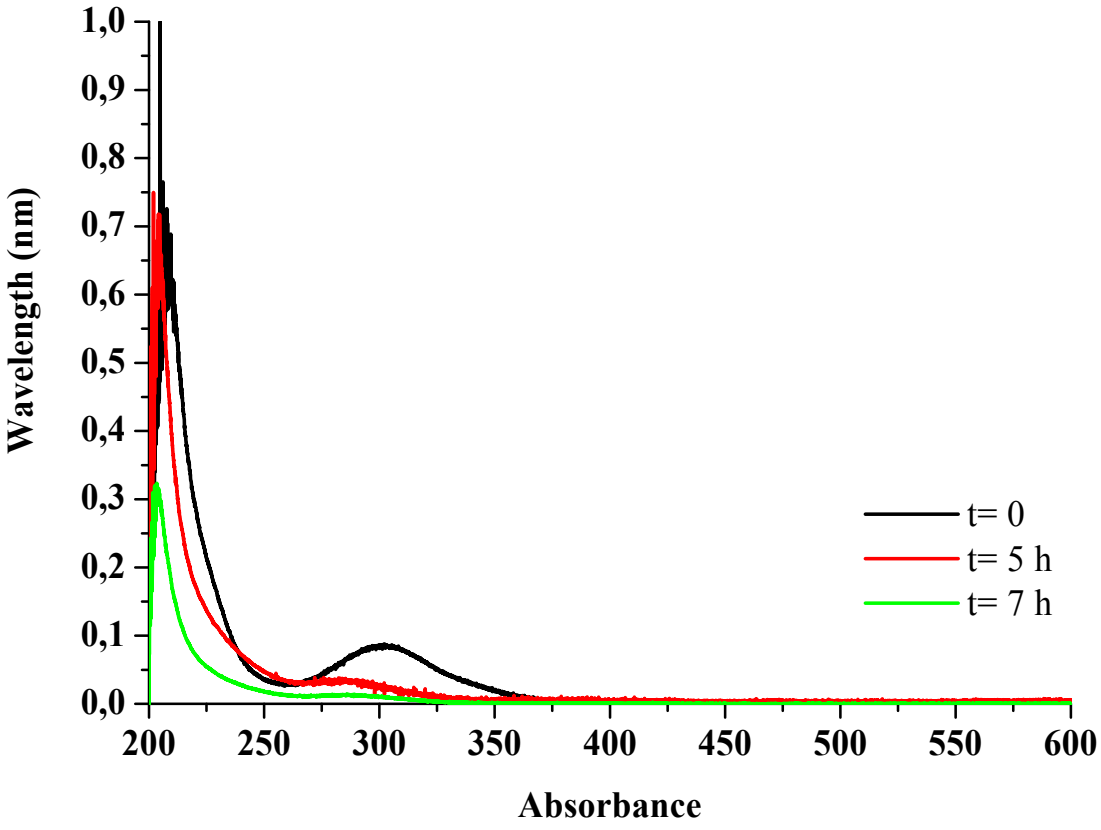

Figure S19. UV-vis analysis of aminolysis reaction of p(AEAEMA).