Electronic Supplementary Material (ESI) for Polymer Chemistry.

SUPPORTING INFORMATION

Controlling the Morphology of Glyco-nanoparticles in Water using Block Copolymer Mixtures: effect on cellular uptake

Aydan Dag[1, 2], Hongxu Lu[1], Martina Stenzel [1]*

[1] Centre for Advanced Macromolecular Design (CAMD), The University of New South Wales, Sydney NSW 2052, Australia

[2] Faculty of Pharmacy, Bezmialem Vakif University, Department of Pharmaceutical Chemistry, 34093 Fatih, Istanbul, Turkey

Synthetic Procedures

Synthesis of 3-(benzylthiocarbonothioylthio)propanoic acid (1)

3-(benzylthiocarbonothioylthio)propanoic acid, (**1**) was prepared according to published procedure [46]. 3-Mercapto propanoic acid (2.00 mL, 22.95 mmol) was added to a stirred suspension of K_3PO_4 (5.36 g, 25.24 mmol) in anhydrous THF (200 mL) and stirred for ten minutes. To this solution CS_2 (4.14 mL, 68.85 mmol) was added at 0 ºC drop by drop. The solution turned bright yellow. After stirring for two hours benzyl bromide (2.73 g, 22.95 mmol) was added and precipitation of KBr was observed. The reaction mixture was stirred for overnight the suspension was filtered and the cake was washed with CH_2Cl_2 (2x50 mL). After removing the solvent from the filtrate under reduced pressure the resulting yellow residue was added to a deionized water (50 mL) and extracted with CH_2Cl_2 (2x150 mL) and washed with saturated brine solution (2x50 mL). After drying the organic extracts over $MgSO_4$ the solvent was removed under reduced pressure to yield a yellow solid. The crude product was purified by column chromatography on silica using chloroform. ^{1}H NMR analysis of the yellow solid residue did not reveal the presence of substantial amount of any impurity, but one additional faint spot were observed by TLC (chloroform) analysis, the crude product was therefore purified by crystallization. The compound solved in minimum amount of CH_2Cl_2 and dropped to a warmed hexane solution by stirring and instant precipitation of compound was observed and keeped in refrigeration for one night. After decantation of solution canary yellow solid was dried in a vacuum oven at 25 ºC for 24 h.

(Yield: 4.95 g, 79.2%). IR (neat) *v*max/cm^{-1}: 3500-3000 (broad), 2916, 2586, 1695, 1407, 1272, 1203, 1069, 837, 798, 668; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.14-7.32 (m, 5H, Ph), 4.65 (s, 2H, C*H*$_2$Ph), 3.68 (2H, t, SC*H*$_2$CH$_2$), 2.86 (t, 2H, SCH$_2$C*H*$_2$); ^{13}C NMR (300 MHz, $CDCl_3$, δ): 222.8, 177.5, 134.8, 129.3, 128.7, 127.8, 41.7, 32.9, 30.9.

Synthesis of 1,2,3,4,6-penta-*o*-acetyl-D-mannose (AcMan) (2)

AcMan, **2**, was prepared in a same way according to published procedure. [47] Sulfuric acid (3 drops) was added at 0 ºC to a stirred mixture of acetic anhydride (26 mL, 0.277 mol) and D-mannose (5.00 g, 0.027 mol). The mixture was stirred for 1 h at 0 ºC and then allowed to warm to room temperature and stirred for 3 h. The mixture was then poured into ice-cold water (100 mL) and extracted with CH_2Cl_2. The extract was washed with water (3×100 mL) and then sat. aq. sodium hydrogencarbonate ($NaHCO_3$). The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to afford mannose pentaacetate (**2**) as a white solid. (Yield: 9.72 g, 89.6%) ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.12-6.11 (d, 1H, C*H* of sugar moiety), 5.39–5.34 (m, 2H, C*H* of sugar moiety), 5.29-5.26 (m, 1H, C*H* of sugar moiety), 4.37-4.05 (m, 3H, C*H*$_2$ of sugar and C*H* of sugar moiety), 2.19, 2.18, 2.11, 2.07, 2.02 (s, 15 H, COC*H*$_3$); ^{13}C (300 MHz, $CDCl_3$, δ): 170.61, 169.96, 169.71, 169.50, 168.03, 90.57, 70.58, 68.71, 68.31, 65.51, 62.08, 20.83 20.74, 20.68, 20.63, 20.61.

Synthesis of 2-(2',3',4',6'-tetra-*o*-acetyl-D-mannosyloxy) ethyl acrylate (AcManA) (3)

AcManA, **3**, was prepared according to similar published procedure.[12] AcMan (5.00 g, 12.80 mmol) and 2-hydroxyethyl acrylate, (HEA) (1.76 mL, 15.37 mmol), were added into a 250 mL two-neck round-bottom flask together with molecular sieves (4 Aº). Anhydrous CH_2Cl_2 (80 mL) was added to the stirred flask under nitrogen flow. The reaction started when boron trifluoride diethyl etherate ($BF_3O(Et)_2$) (7.90 mL, 64.04 mmol) was introduced over 30 min via a gastight syringe while maintaining the flow of nitrogen through the flask. After purging the solution for another 20 min, the flask was sealed and left stirring at room temperature for 36 h to allow maximum conversion. The reaction mixture was filtered through a sintered glass funnel pouring into ice-water (100 mL) and extracted. CH_2Cl_2 solution were washed (aqueous $NaHCO_3$, water), dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure, and the resulting oily light yellow mixture was purified by column chromatography using hexane/ethyl acetate (1/1) as eluent. The light yellow oil was obtained with the R_f value of 0.4. (Yield: 4.95 g, 86.5%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.51-6.45 (dd, 1H, CH=C*H*H), 6.23-6.14 (m, 1H, C*H*=CHH), 5.93-5.89 (dd, 1H, CH=CH*H*),

5.41-5.27 (m, 3H, sugar moiety C*H*), 4.90-4.88 (d, 1H, sugar moiety C*H*), 4.40-437 (t, 2H, sugar-OCH$_2$C*H*$_2$OC=O), 4.34-3.76 (m, 5H, C*H*$_2$ of sugar, sugar-OC*H*$_2$CH$_2$OC=O and C*H* of sugar moiety), 2.19, 2.12, 2.07, 2.02 (s, 12H, C*H*$_3$). ^{13}C (300 MHz, $CDCl_3$, δ): 170.64, 170.02, 169.87, 169.73, 165.91, 131.42, 128.00, 97.65, 69.45, 68.93, 68.67, 66.12, 66,05, 63.00, 62.44, 20.88 20.72, 20.70, 20.68.

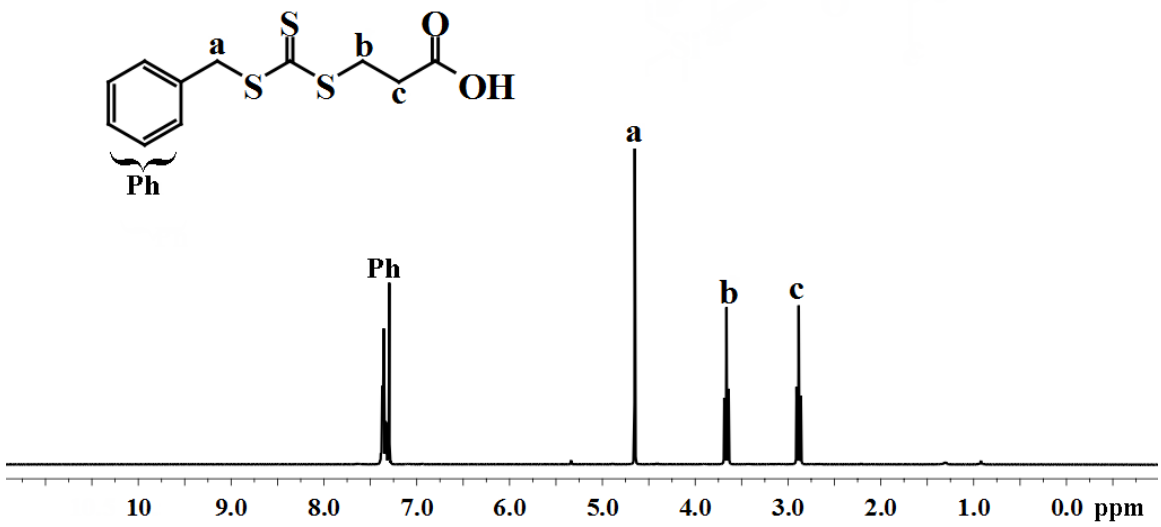

Figure S1: ^{1}H NMR spectrum of 3-(benzylthiocarbonothioylthio)propanoic acid (**1**) in $CDCl_3$.

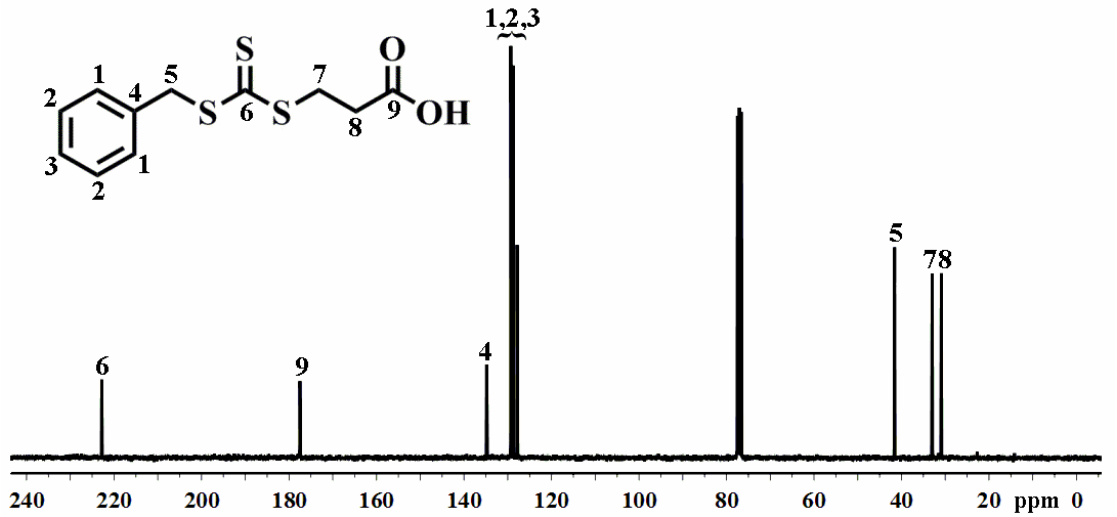

Figure S2: ^{13}C NMR spectrum of 3-(benzylthiocarbonothioylthio)propanoic acid (**1**) in $CDCl_3$.

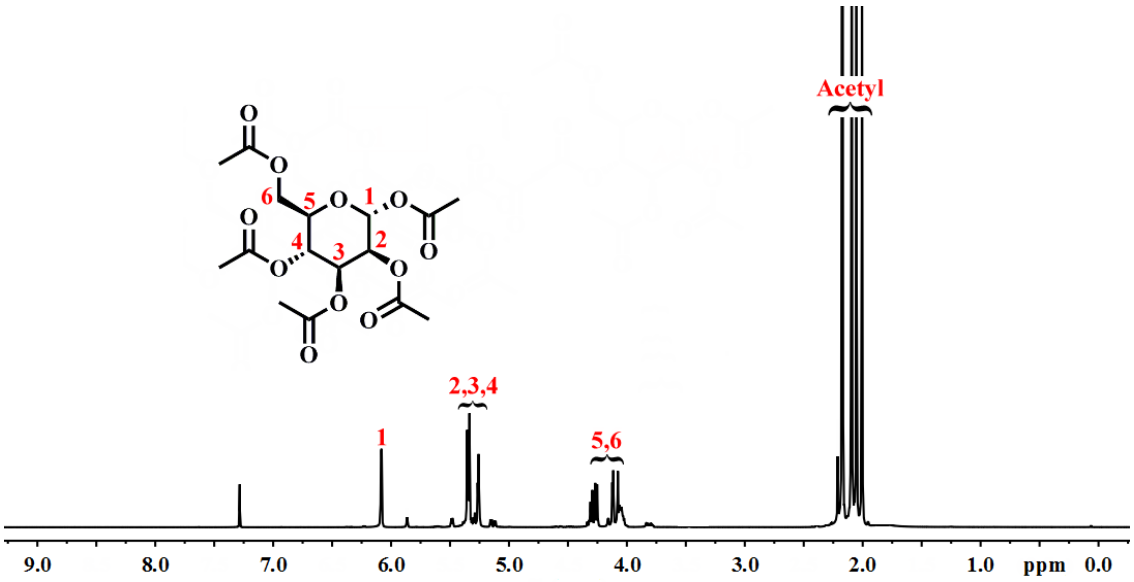

Figure S3: ^{1}H NMR spectrum of 1,2,3,4,6-penta-*o*-acetyl-D-mannose (AcMan) (**2**) in $CDCl_3$

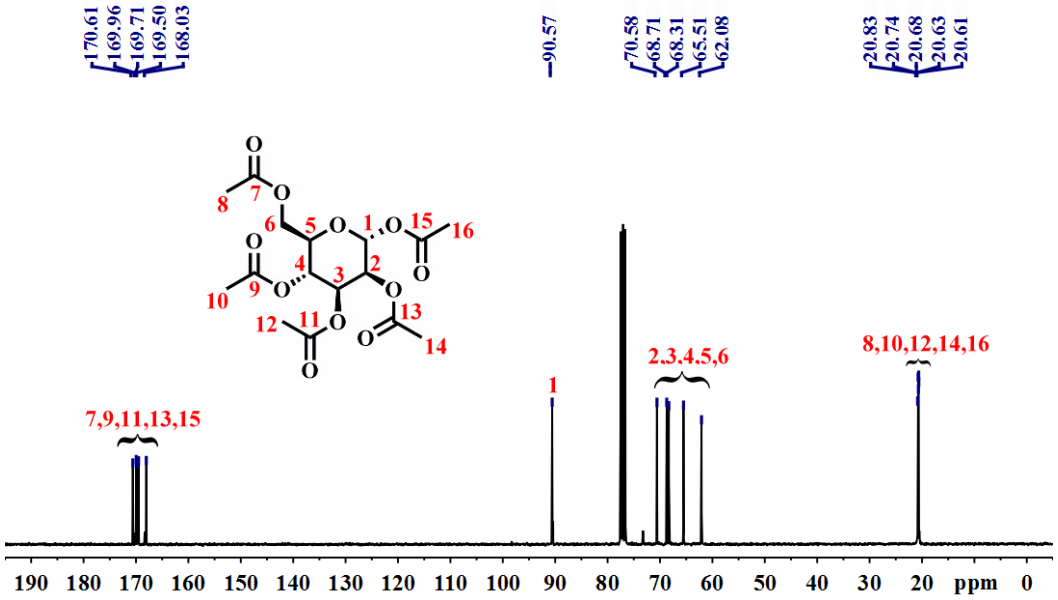

Figure S4: ^{13}C NMR spectrum of 1,2,3,4,6-penta-*o*-acetyl-D-mannose (AcMan) (**2**) in $CDCl_3$

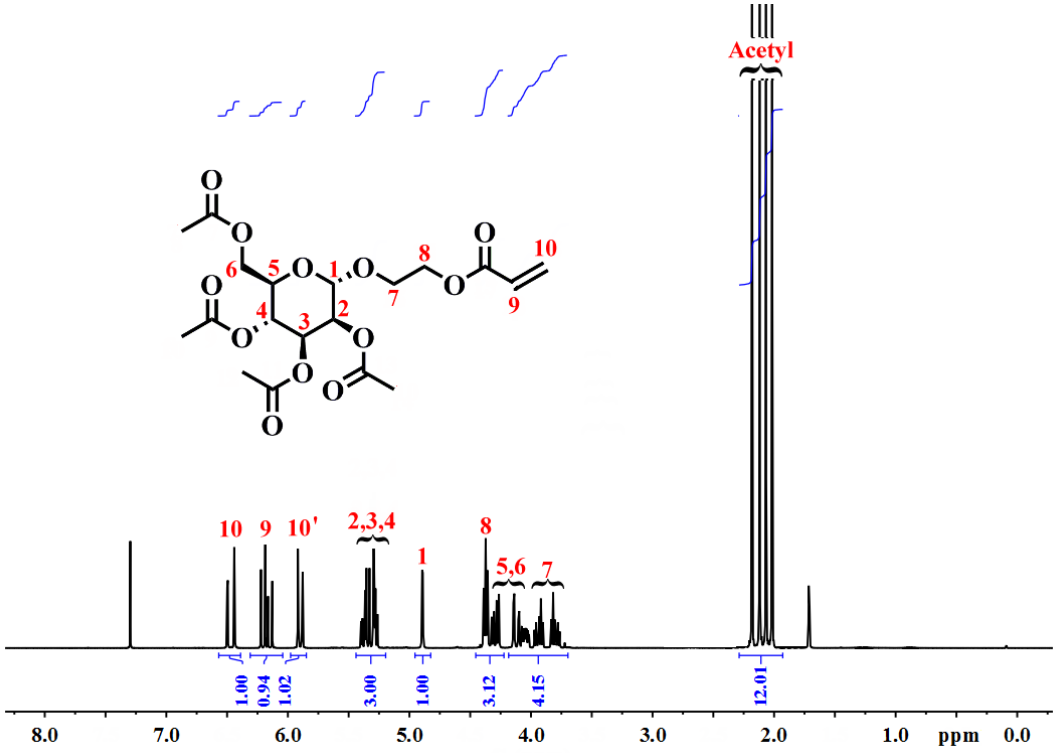

Figure S5: ^{1}H NMR spectrum of 2-(2',3',4',6'-tetra-*o*-acetyl-D-mannosyloxy) ethyl acrylate (AcManA) (**3**) in $CDCl_3$.

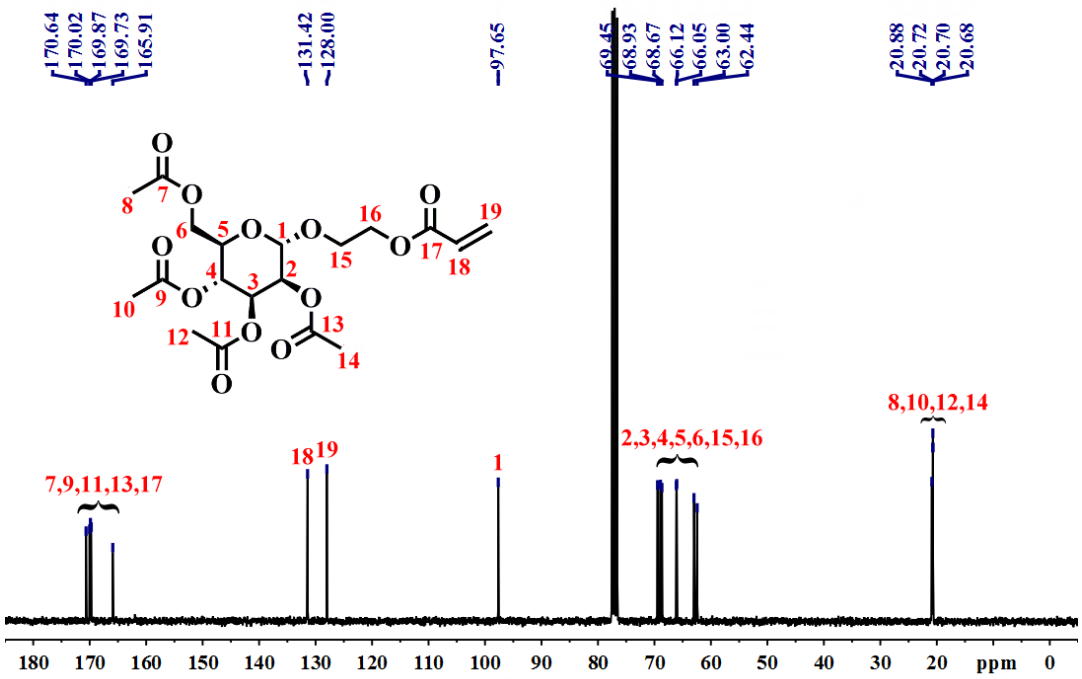

Figure S6: ^{13}C NMR spectrum of 2-(2',3',4',6'-tetra-*o*-acetyl-D-mannosyloxy) ethyl acrylate (AcManA) (**3**) in $CDCl_3$.

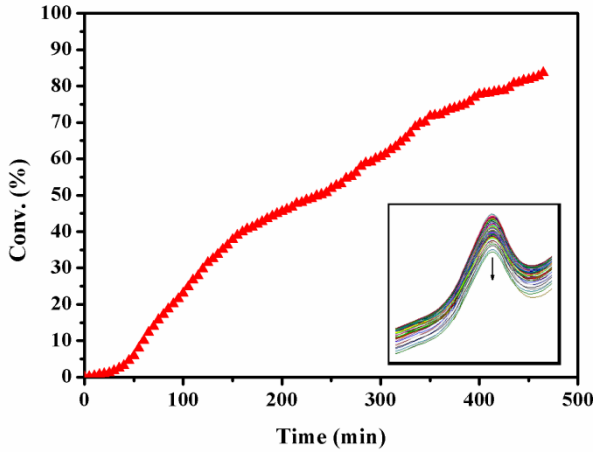

Figure S7: Conversion vs time plot of the RAFT polymerization of P(AcManA)

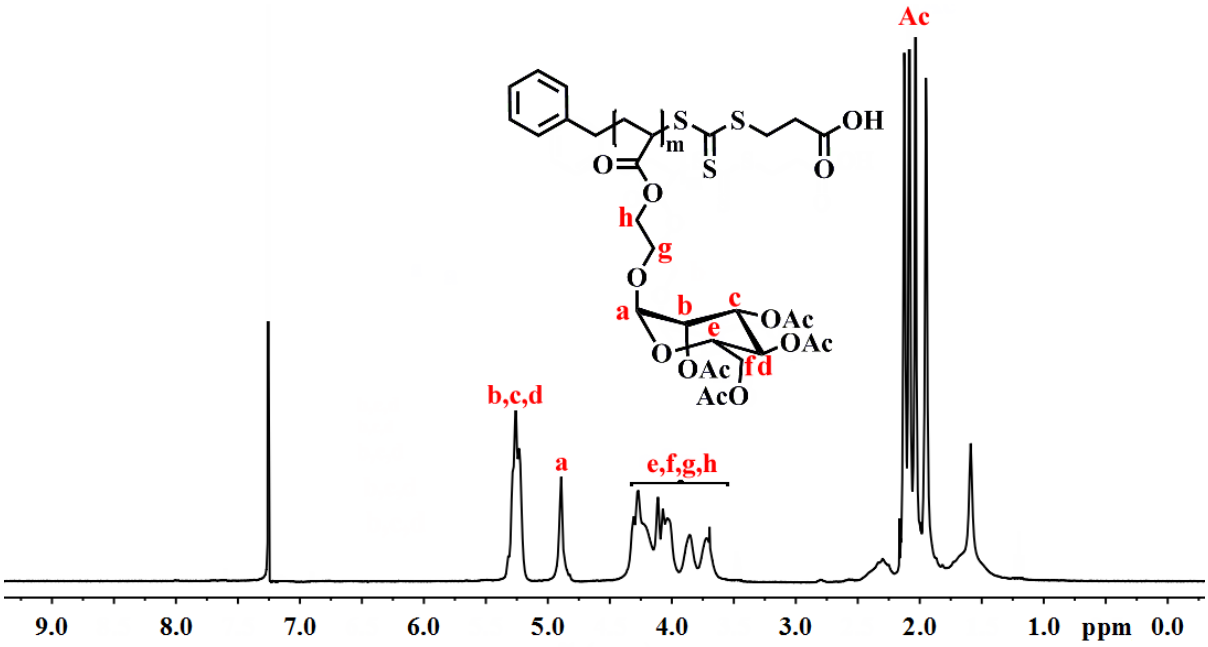

Figure S8: ^{1}H NMR spectrum of ($PacManA_{70}$) in $CDCl_3$.

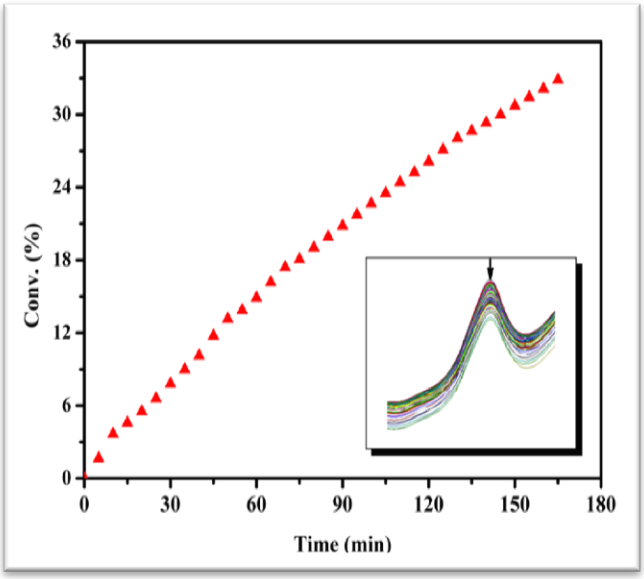

Figure S9: Conversion vs time plot of the RAFT polymerization of P(ManA$_{70}$-*b*-BA$_{151}$).

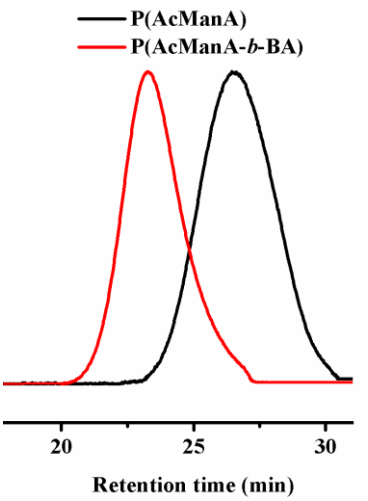

Figure S10: GPC traces of P(ManA$_{70}$) (black) and P(ManA$_{70}$-*b*-BA$_{151}$) (red) in DMAc at 50 ºC.

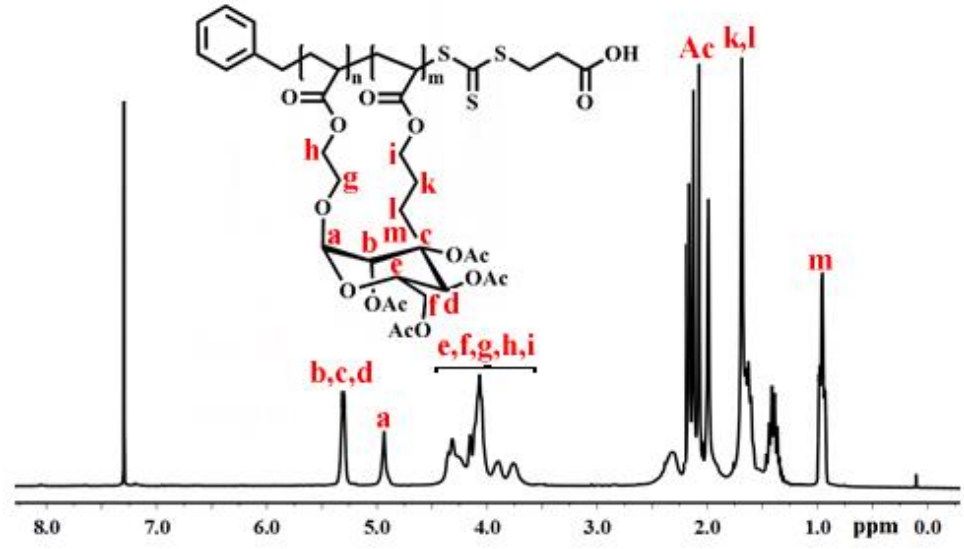

Figure S11: ^{1}H NMR spectrum of P(AcManA_{70}-*b*-BA_{151}) in $CDCl_3$.

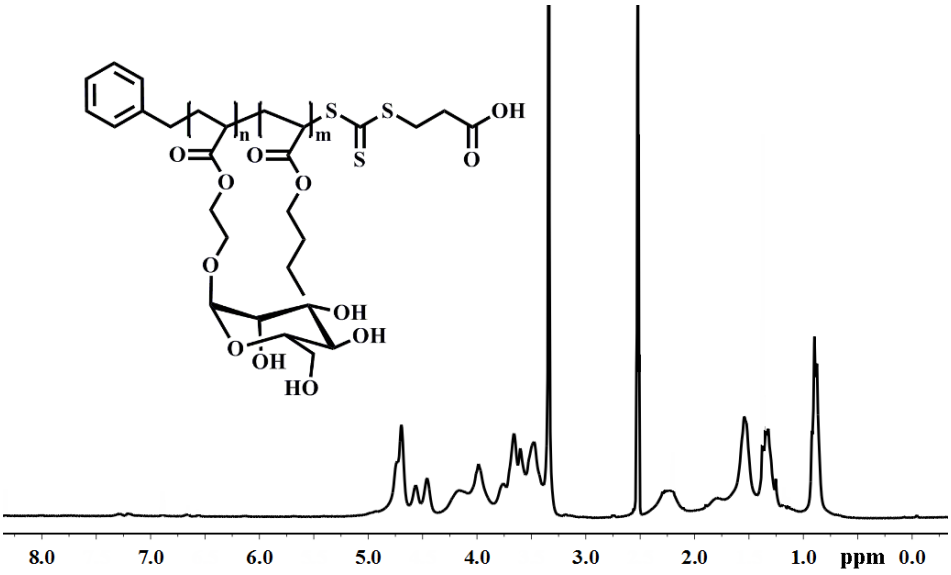

Figure S12: ^{1}H NMR spectrum of P(ManA_{70}-*b*-BA_{151}) in DMSO-d_6.

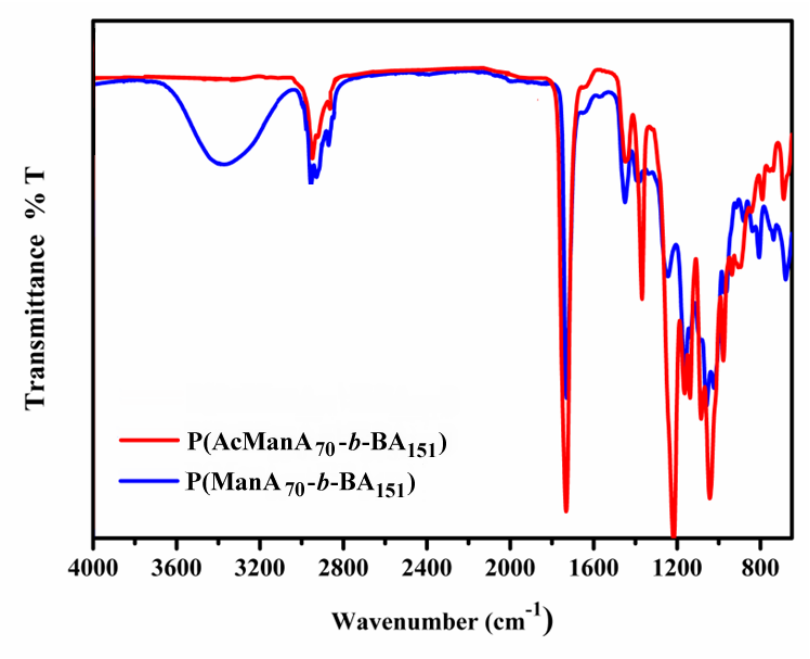

Figure S13: Comparison of the FT-IR spectra of diblock glycopolymer before and after deprotection

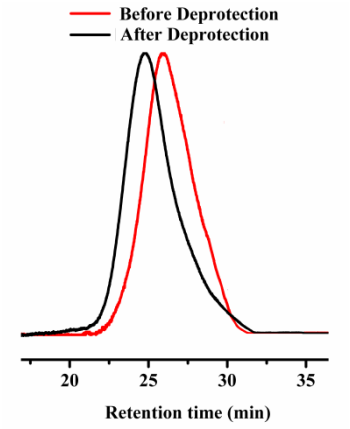

Figure S14: Evolution of the GPC traces of P(AcManA$_{70}$-*b*-BA$_{151}$) and P(ManA$_{70}$-*b*-BA$_{151}$) before and after deprotection.

Table S1. RAFT polymerization of POEGMEA

Entry	Time (h)	Conversion (%)[a]	$M_{n,th}$[b]	$M_{n,NMR}$[c]	GPC[d]	
					M_n	M_w/M_n
1	1	12	2800	3200	3300	1.10
2	2	20	4800	5100	6600	1.09
3	3	25	5500	6200	7500	1.12
4	4	32	7800	8900	8600	1.13
5	5	39	9000	9900	10200	1.13
6	6	51	11800	12550	12150	1.13
7	7	67	15300	16400	16550	1.13
8	9	83	15900	16650	16150	1.14

a) Obtained from ^{1}H NMR analysis; b) Calculated according to eq.=$[M]_0/[RAFT]$ x conv.% (isolated yield) x MW of OEGMA + MW of **1**; c) Calculated according to eq.=$[M]_0/[RAFT]$ x conv.% (from NMR) x MW of OEGMA + MW of **1**; d) Determined from DMAc GPC (relative to PS standarts).

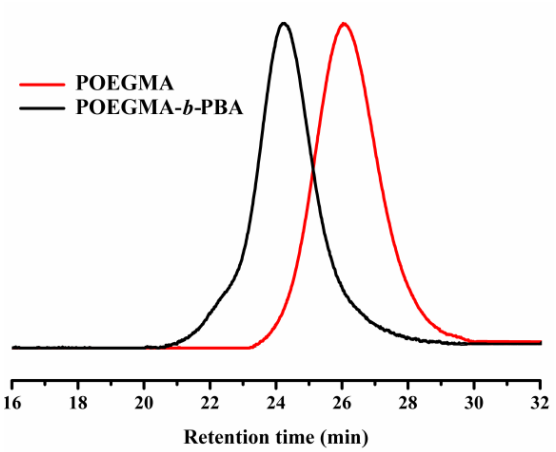

Figure S15: Evolution of the GPC traces of POEGMEA and P(OEGMEA-*b*-BA)

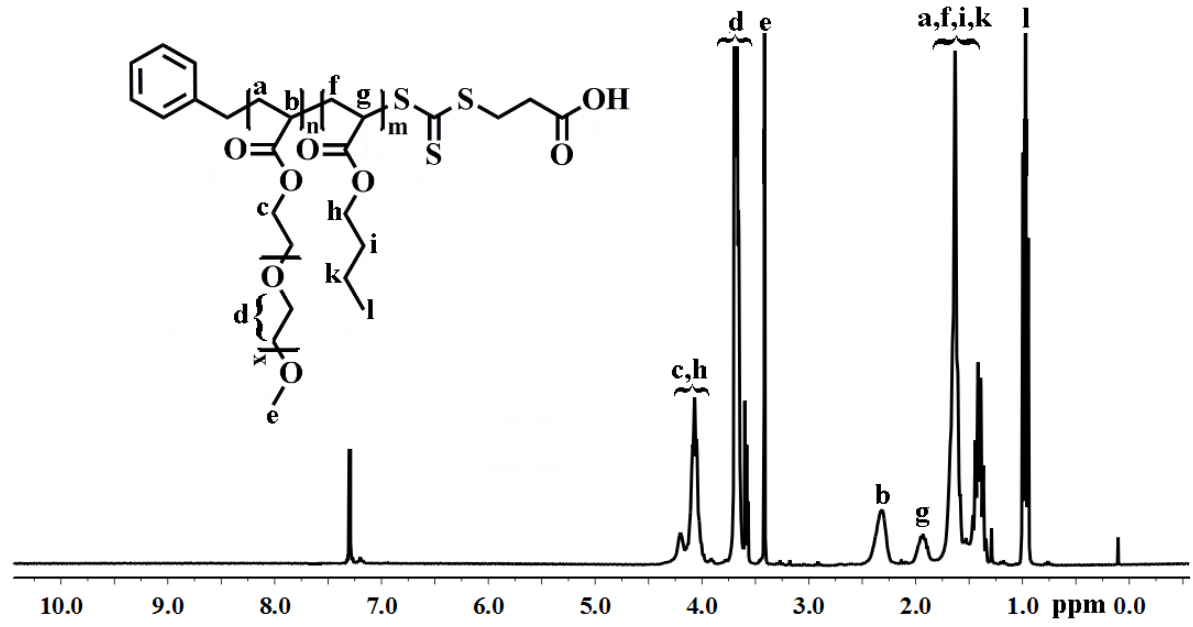

Figure S16: ^{1}H NMR spectrum of P(OEGMEA-*b*-BA) in $CDCl_3$.

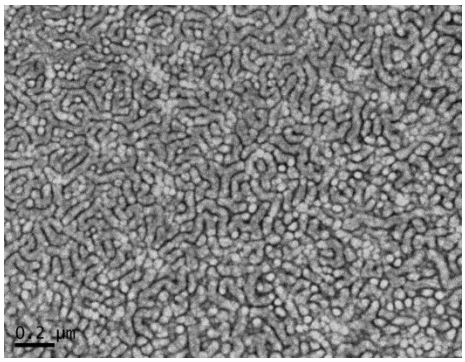

Figure S17: P(ManA_{70}-*b*-BA_{151})/P($OEGMEA_{20}$-*b*-BA_{142}) nanoparticles prepared in a mixing ratio of 70/30.

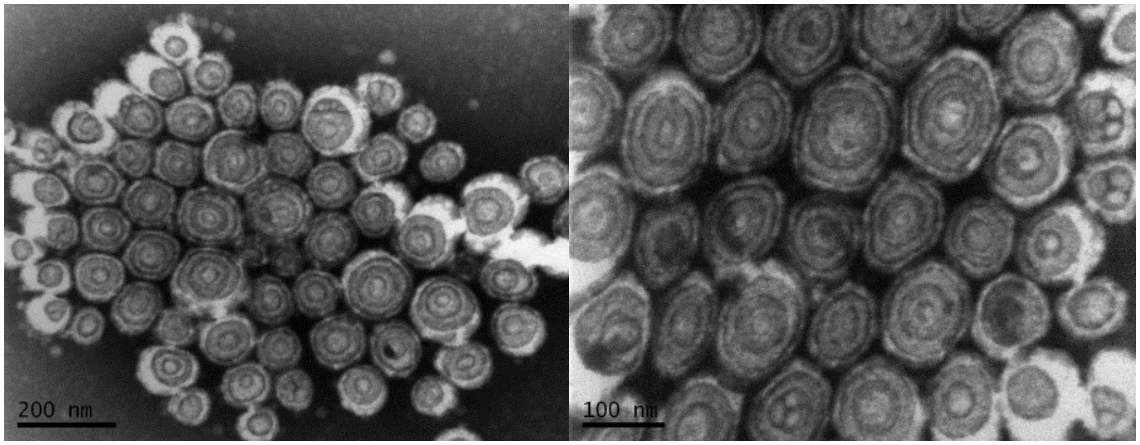

Figure S18: P(ManA$_{70}$-*b*-BA$_{151}$)/P(OEGMEA$_{20}$-*b*-BA$_{142}$) onion-like nanoparticles prepared in a mixing ratio of 10/90.

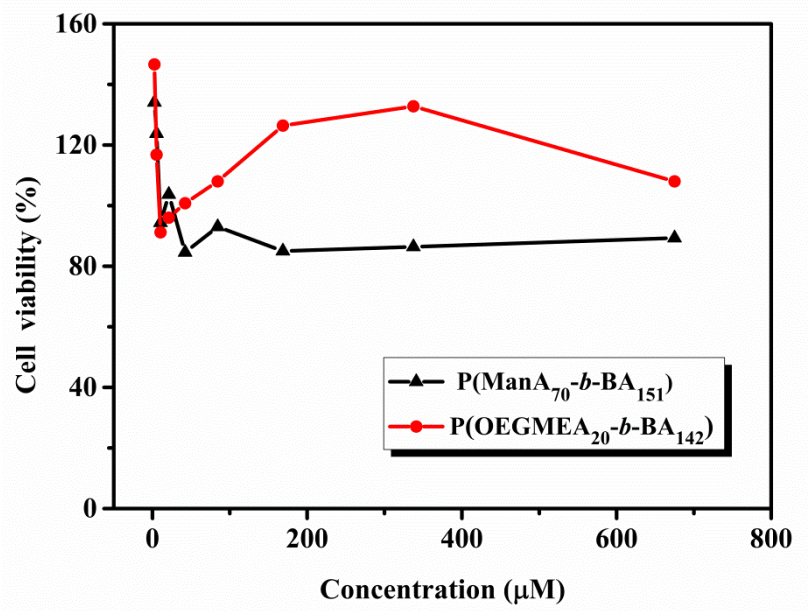

Figure S19: RAW 264.7 cell viability data after 24 h of treatment with P(ManA$_{70}$-*b*-BA$_{151}$) and P(OEGMEA$_{20}$-*b*-BA$_{142}$) micelles.

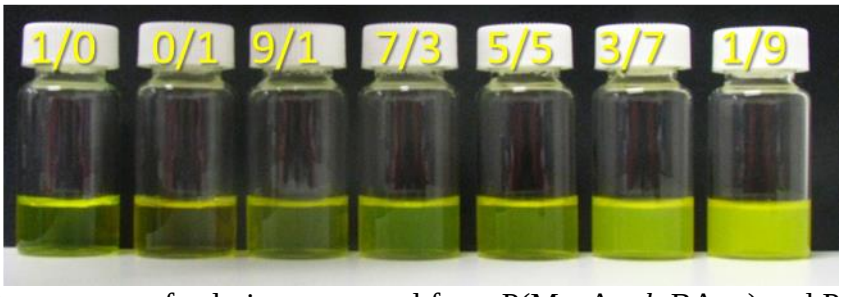

Figure S20: Appearance of solutions prepared from P(ManA$_{70}$-*b*-BA$_{151}$) and P(OEGMEA$_{20}$-*b*-BA$_{142}$) micelles