Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information:

Engineering mannosylated nanogels with membrane-disrupting properties

Ruben De Coen[a], Lutz Nuhn[b] and Bruno G. De Geest*[a]

[a.] *Department of Pharmaceutics, Ghent University, Belgium*

[b.] *Max Planck Institute for Polymer Research, Mainz, Germany*

Synthesis of pentafluorophenyl acrylate (PFPA).

Pentafluorophenyl acrylate was synthesized according to literature.[1] Pentafluorophenol (27.6 g, 149 mmol) was dissolved in a pre-dried round bottom flask of 500 mL equipped with stirring bar by the addition of 150 mL anhydrous dichloromethane (DCM) under inert atmosphere. The resulting clear solution was placed in an ice bath. Under continuous stirring, 1.05 equivalents of anhydrous triethylamine (21.8 mL, 156 mmol) was added dropwise to the cooled reaction mixture, followed by the dropwise addition of 1.03 equivalents of acryloyl chloride (12.8 mL, 153 mmol). After 30 min, the reaction was allowed to reach room temperature and stir for an additional 2 h. Monitoring of the reaction was performed by thin-layer chromatography (TLC; 80:20 Hexane:EtOAc; R_f = 0.70) until complete consumption of pentafluorophenol was observed. The reaction mixture was diluted with ethyl acetate, filtered and concentrated under vacuum. The resulting crude product (yellow oil) was purified by silica gel chromatography (80:20 Hexane:EtOAc) and yielded a colorless, clear oil that was characterized by ^{19}F-NMR and ^{1}H-NMR (vide infra) (79 % yield).

^{19}F-NMR (282 MHz, $CDCl_3$, **Figure S1**): δ (ppm): -152.7 (d; J = 16.9 Hz; 2F; o-C_6F_5); -158.1 (t; J = 21.6 Hz; 1F; p-C_6F_5); -162.5 (t; J = 19.2 Hz; 2F; m-C_6F_5).

^{1}H-NMR (500 MHz, $CDCl_3$, **Figure S2**), δ (ppm): 6.72 (dd; J = 17.4, 0.76 Hz, 1H; -CH=**CH_2**); 6.38 (dd; J = 17.2, 10.5 Hz; 1H; -**CH**=CH_2); 6.19 (dd; J = 10.5, 0.76 Hz; 1H; -CH=**CH_2**).

Synthesis of 2,3,4,6-Tetra-O-Acetyl-α-D-Mannosylethyl Acrylamide (TAManEAm).

1,2,3,4,6-penta-*O*-acetyl-α-D-mannose (11.7 g, 30.0 mmol) was dissolved in DCM (125 mL) prior to the dropwise addition of *N*-hydroxyethyl acrylamide (7.12 g, 60.0 mmol) under inert N_2 atmosphere. The solution was placed in an ice bath and 27.8 mL (225 mmol) boron trifluoride diethyl etherate was added dropwise over 45 min. The mixture was kept in an ice bath for 1 h before the reaction was allowed to reach room temperature. Monitoring via TLC (Hexane:EtOAc 20:80, R_f = 0.20) indicated complete consumption of 1, 2, 3, 4, 6-penta-*O*-acetyl-α-D-mannose after 36 h of reaction. The mixture was poured into ice water and extracted twice. The aqueous layer was extracted once with DCM and the organic layers were combined, washed (2 times with saturated sodium bicarbonate solution, 1 time with brine), dried over sodium sulfate and concentrated under vacuum. The resulting crude product (pale yellow oil) was purified by silica gel column chromatography (Hexane:EtOAc 20:80) and yielded a pale yellowish gum after concentration under vacuum (86.8 % yield).

^{1}H-NMR (300 MHz, $CDCl_3$, **Figure S3**), δ (ppm): 6.34 (t; J = 4.9 Hz; 1H; -CO-**NH**-); 6.27 (ddd; J = 17.0, 5.6, 1.7 Hz; 1H; -CH=**CH_2**); 6.12 (ddd; J = 16.9, 14.5, 9.9 Hz; 1H; -**CH**=CH_2); 5.62 (ddd; J = 10.1, 3.9, 1.7 Hz; 1H; -CH=**CH_2**); 5.31 – 5.16 (m; 3H; -**CH**-(OAc)-); 4.78 (d; J = 1.6 Hz; 1H; **α-CH**); 4.26 – 4.02 (m; 2H; -**CH_2**-OAc); 3.94 (ddd; J = 9.6, 5.6, 2.5 Hz; 1H; -**CH**-CH2-OAc); 3.79 (ddd; J = 9.5, 6.8, 3.4 Hz; 1H; -O-C**H**H-CH2-NH-); 3.65 - 3.41 (m; 3H; -O-CH**H**-C**H_2**-NH-); 2.11 (s; 3H; -**OAc**); 2.05 (s; 3H; -**OAc**); 2.01 (s; 3H; -O**Ac**); 1.96 (s; 3H; -**OAc**).

APT ^{13}C-NMR (75 MHz, $CDCl_3$, **Figure S4**), δ (ppm): 170.50 (-O-**CO**-CH_3); 169.9 (2 x -O-**CO**-CH_3); 169.57 (-O-**CO**-CH_3); 165.85 (-NH-**CO**-CH-CH_2); 130.5 (-NH-CO-**CH**-CH_2); 126.7 (-NH-CO-CH-**CH_2**); 97.63 (-α-**CH**-); 69.23 (-CH_2-CH-**CH**(OAc)-); 68.91 (-CH_2-**CH**-CH(OAc)-); 68.62 (-CH_2-CH-CH-**CH**(OAc)-); 67.37 (-CO-NH-CH_2-**CH_2**-); 66.01 (-α-CH-**CH**(OAc)-); 62.37 (-CH-**CH_2**-OAc); 39.00 (-CO-NH-**CH_2**-CH_2-); 20.73 (-O-CO-**CH_3**); 20.60 (O-CO-**CH_3**); 20.58 (2 x O-CO-**CH_3**).

ESI-MS: m/z $[M+H]^+$ = 446.1657 (theoretical), found = 446.1669

$[M+Na]^+$ = 468.1476 (theoretical), found = 468.1481

Synthesis of 2-(butylthiocarbonothioylthio)propanoic acid (PABTC).

The RAFT chain transfer agent 2-(butylthiocarbonothioylthio)propanoic acid (PABTC) was synthesized according to literature.[2] To a pre-dried 1 L uni-neck round bottom flask equipped with stirring bar and 150 mL anhydrous dichloromethane, 15.0 mL of 1-butanethiol (140 mmol, 1.00 Eq) and 21.2 mL of triethylamine (TEA, 152 mmol, 1.09 mmol) was added dropwise under inert atmosphere. This round bottom flask was placed in an ice bath on a stirring plate to cool the reaction to 0 °C. In a separate round bottom flask, 9.15 mL of carbondisulfide (152 mmol, 1.09 mmol) was dissolved in 150 mL anhydrous DCM under inert atmosphere. The content of the latter flask was dropped to the cooled reaction mixture containing 1-butanethiol and TEA. Upon addition, the reaction mixture developed a distinct yellow color. Once all reagent was added, the reaction mixture was allowed to warm to room temperature under continuous stirring. After 30 minutes, the content of a third round bottom flask containing 75.0 mL anhydrous DCM and 13.7 mL 2-bromopropanoic acid was added dropwise to the reaction mixture. After 2 h the reaction mixture was reduced under vacuum, diluted with cyclohexane and extracted subsequently with 10 % HCl aqueous solution, deionized water and brine. All organic phases were collected and dried over sodium sulfate before being concentrated in vacuum. The obtained crude yellow crystals were afterwards purified by recrystallization from hexane (65 % yield) prior to characterization by ^{1}H-NMR, APT ^{13}C-NMR and ESI-MS.

^{1}H-NMR (500 MHz, $CDCl_3$, **Figure S5**), δ (ppm): 11.5 - 8 (br; 1H; **H**O-C=O-CH-); 4.88 (q; J = 7.35 Hz; 2H; HO-C=O-C**H**-); 3.38 (t; J = 7.40 Hz; 2H; -C=S-S-C**H$_2$**-CH_2-); 1.70 (m; 2H; -S-CH_2-C**H$_2$**-CH_2-CH_3); 1.64 (d; J = 7.35 Hz; 3H; -C=O-CH-(C**H$_3$**)-S-) 1.45 (sextet; J = 7.72 Hz; 2H; -S-CH_2-CH_2-C**H$_2$**-CH_3); 0.95 (t; J = 7.35 Hz; 3H; -S-CH_2-CH_2-CH_2-C**H$_3$**).

APT ^{13}C-NMR (125 MHz, $CDCl_3$, **Figure S6**), δ (ppm): 176.86 (2C; HO-(**C=O**)-CH(CH_3)-S-(**C=S**)-S-); 47.55 (1C; HO-(C=O)-**CH**(CH_3)-S-(C=S)-S-); 37.29 (-S-(C=S)-S-**CH$_2$**-CH_2-CH_2-CH_3); 30.02 (1C; -S-(C=S)-S-CH_2-**CH$_2$**-CH_2-CH_3); 22.20 (1C; -S-(C=S)-S-CH_2-CH_2-**CH$_2$**-CH_3); 16.73 (1C; HO-(C=O)-CH(**CH$_3$**)-S-(C=S)-S-); 13.72 (1C; (-S-(C=S)-S-CH_2-CH_2-CH_2-**CH$_3$**).

ESI-MS: m/z $[M+H]^+$ = 239.0228 (theoretical), found = 239.067

$[M+Na]^+$ = 261.0048 (theoretical), found = 261.044

Synthesis of poly(Pentafluorophenyl Acrylate) (poly(PFPA)).

For a polymerization reaction of pentafluorophenyl acrylate aiming at a DP of 60, 4.76 g PFPA (20.0 mmol), 79.4 mg PABTC CTA (0.333 mmol), 10.9 mg AIBN (0.0666 mmol) were dissolved in 6.7 mL anhydrous 1,4-dioxane, to obtain a final monomer concentration of 2M. The solution was transferred to a Schlenk vial and degassed by 5 subsequent freeze-pump-thaw cycles before being placed inside a pre-heated oil bath of 80 °C under vacuum. After 2 h, the polymerization was quenched by cooling the vial in ice water and exposing the reaction to oxygen. Conversion was calculated by ^{19}F-NMR spectra of the reaction mixture in $CDCl_3$. The reaction mixture was purified by triple precipitation into ice-cold ethanol and dried for 24 h in a vacuum oven at 30 °C. The resulting purified polymer was used as macroCTA for the synthesis of the desired block copolymers. The macroCTA was analyzed using THF- SEC to determine the M_n, M_w and Đ and data can be found in the manuscript in **Table 1**. SEC-traces are depicted above in **Figure 1**. The theoretical M_n was calculated based on the conversion determined by ^{19}F-NMR.

^{19}F-NMR (282 MHz, $CDCl_3$, **Figure S7**): δ (ppm): -153.22 (br, 2F; o-C_6F_5); -156.79 (br, 1F; p-C_6F_5); -162.26 (br, 2F; m-C_6F_5).

Synthesis of p(PFPA-b-tetra-O-acetyl-α-D-mannosylethyl acrylamide) (p(PFPA-b-TAManEAm)).

For block copolymerization aiming at a DP of 65 for the second block , 890 mg 2,3,4,6-tetra-*O*-acetyl-α-D-mannosylethyl acrylamide (2.00 mmol), 410 mg poly(PFPA) macroCTA (0.0615 mmol), 1.00 mg AIBN (50 µL of a 0.0200 mg/µL stock solution, 0.0123 mmol) and 3.05 mL anhydrous 1, 4-dioxane were added to a Schlenk vial and degassed by 5 subsequent freeze-pump-thaw cycles before being placed inside a pre-heated oil bath of 80 °C under vacuum. After 60 minutes, the reaction was quenched by cooling the vial in ice water and exposing the reaction to oxygen. Conversion was calculated by ^{1}H-NMR spectra of the reaction mixture in $CDCl_3$. Purification of the reaction mixture was performed by triple precipitation into an ice-cold diethyl ether. The purified block copolymer was dried under vacuum at 30 °C for 24 h. The theoretical M_n was calculated based on the conversion determined by ^{1}H-NMR. The resulting

pure polymer was analyzed using THF- SEC to determine the M_n, M_w and Đ and data can be found below in **Table 1**. SEC-traces are depicted above in **Figure 1**.

^{1}H-NMR (300 MHz, $CDCl_3$, **Figure S8**): δ (ppm): 7.8 – 6.8 (1H; -CO-**NH**-); 5.40 (1H; -**CH**-(OAc)-); 5.10 (2H; 2 x -**CH**-(OAc)-); 4.61 (1H; **β-CH**); 4.15 (4H; -C**H**-**CH_2**-OAc, -O-C**H**H-CH2-NH-); 3.88 (1H; -O-C**H**H-CH2-NH-); 3.44 (2H; -O-CHH-**CH_2**-NH-); 3.08 (br; 1H; -CH_2-**CH**-CO-O-C_6F_5); 2.46 (1H; -CH_2-**CH**-CO-NH-); 2.25 – 1.5 (br; 16H; 4 x –**OAc**, -**CH_2**-CH-CO-O-C_6F_5, -**CH_2**-CH-CO-NH-).

^{19}F-NMR (282 MHz, $CDCl_3$): δ (ppm): -153.22 (br, 2F; **o**-C_6F_5); -156.79 (br, 1F; **p**-C_6F_5); -162.26 (br, 2F; **m**-C_6F_5).

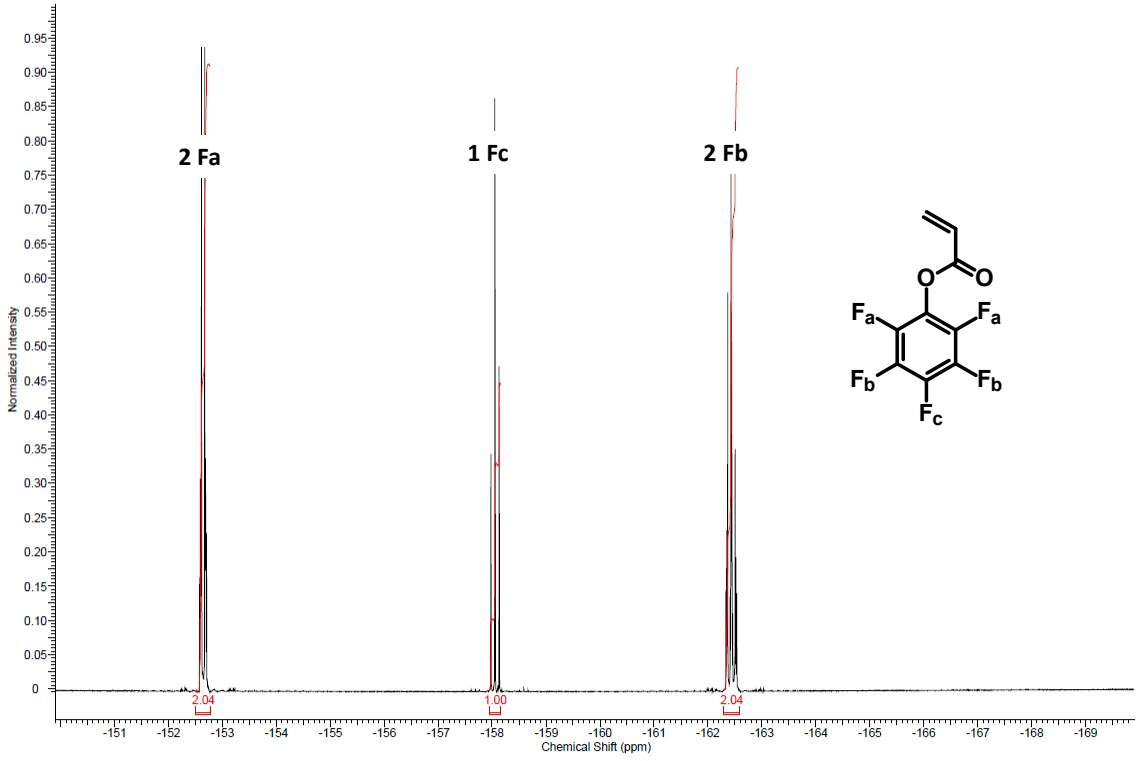

Figure S1. ^{19}F-NMR spectrum of pentafluorophenyl acrylate.

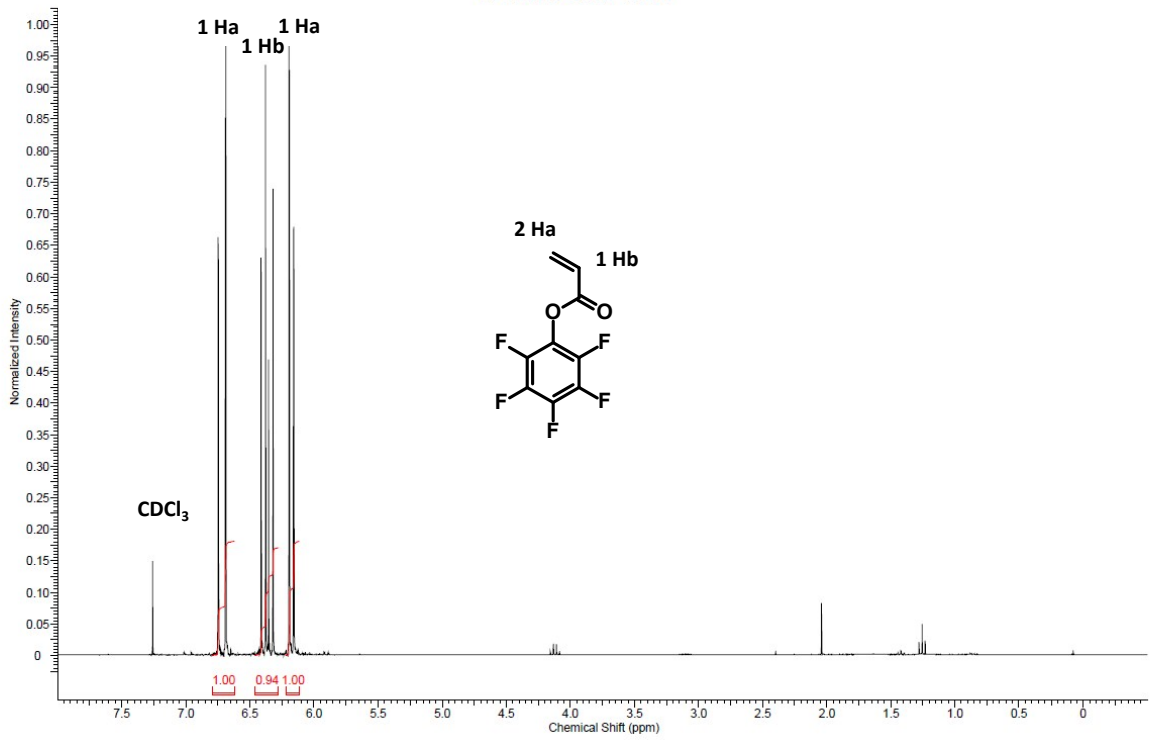

Figure S2. ^{1}H-NMR spectrum of pentafluorophenyl acrylate.

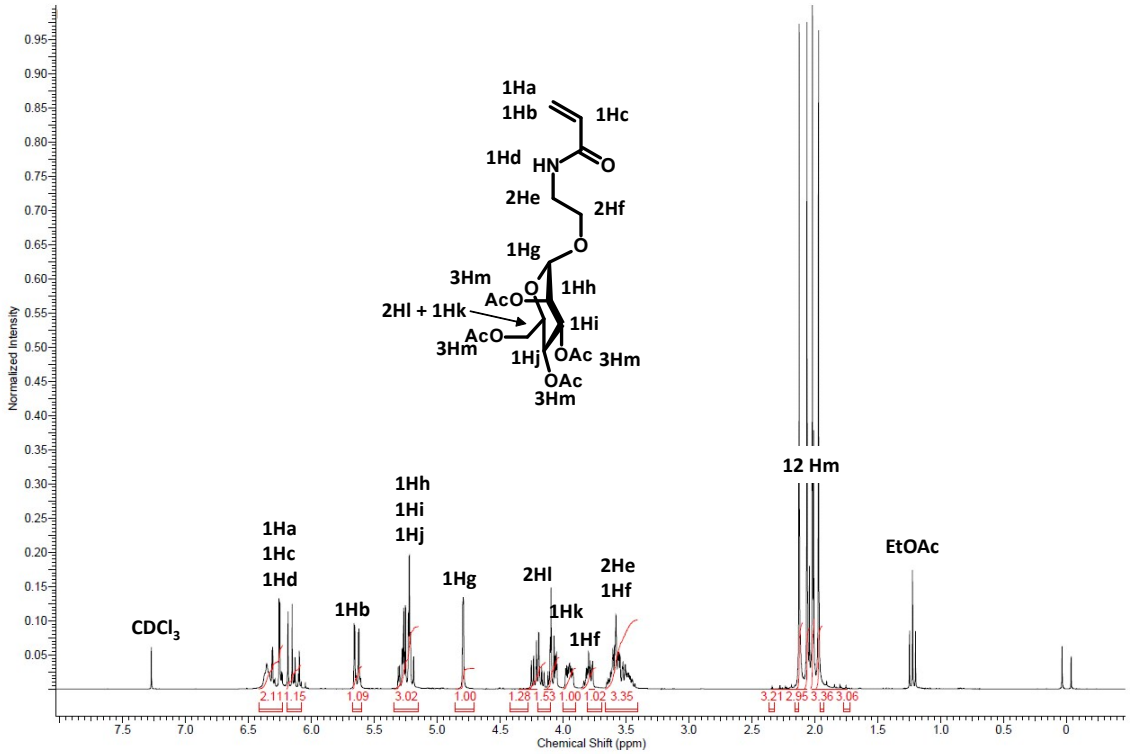

Figure S3. ^{1}H-NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-α-D-mannosylethyl acrylamide.

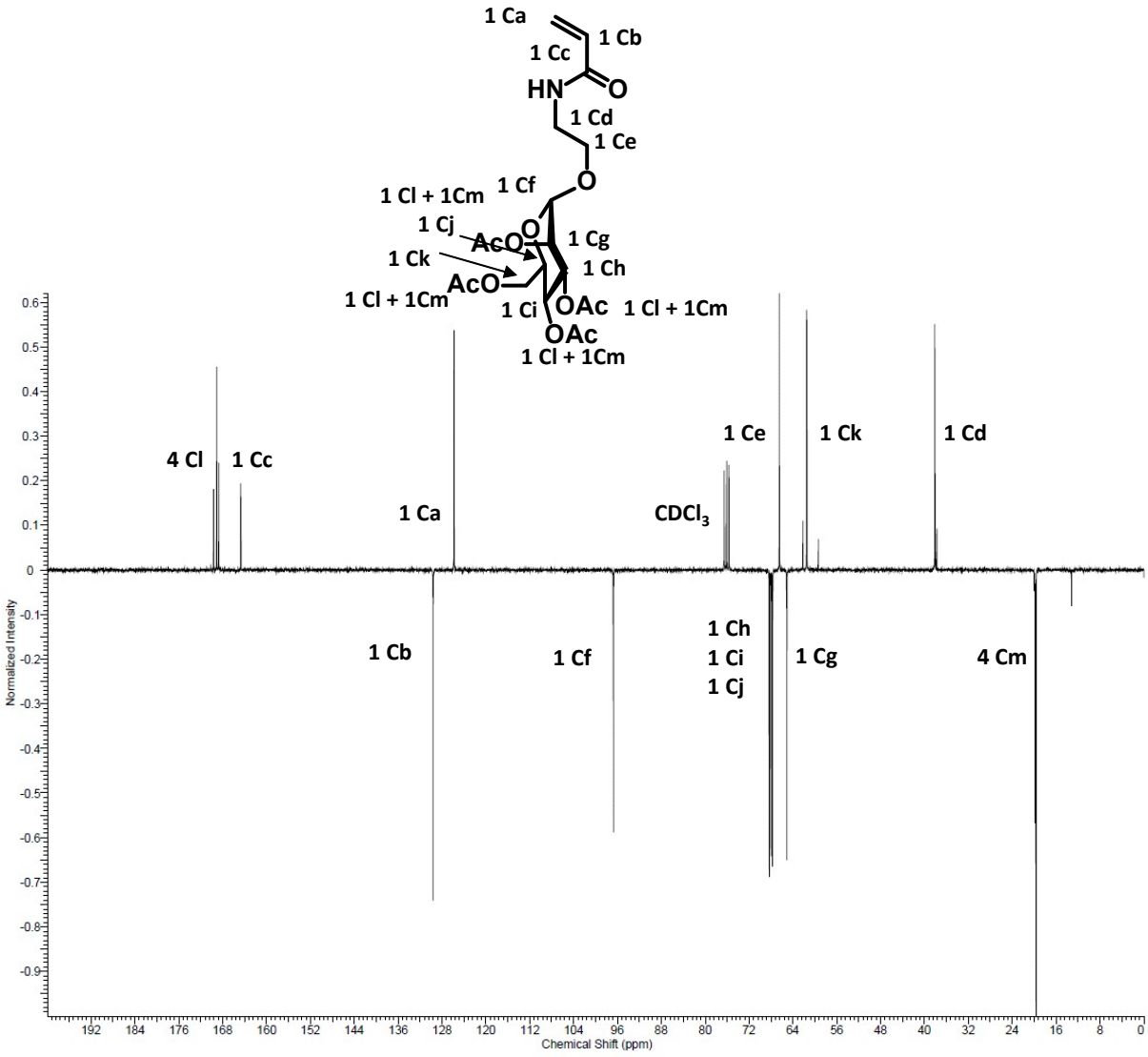

Figure S4. APT ^{13}C-NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-α-D-mannosylethyl acrylamide.

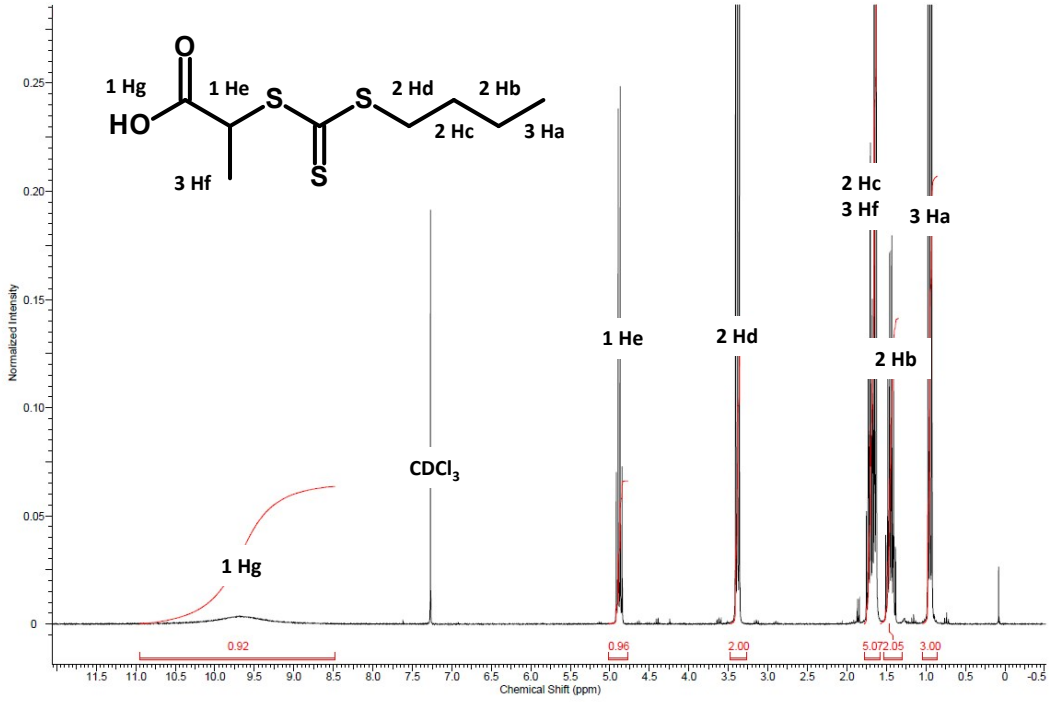

Figure S5. ^{1}H-NMR spectrum of 2-(butylthiocarbonothioylthio)propanoic acid.

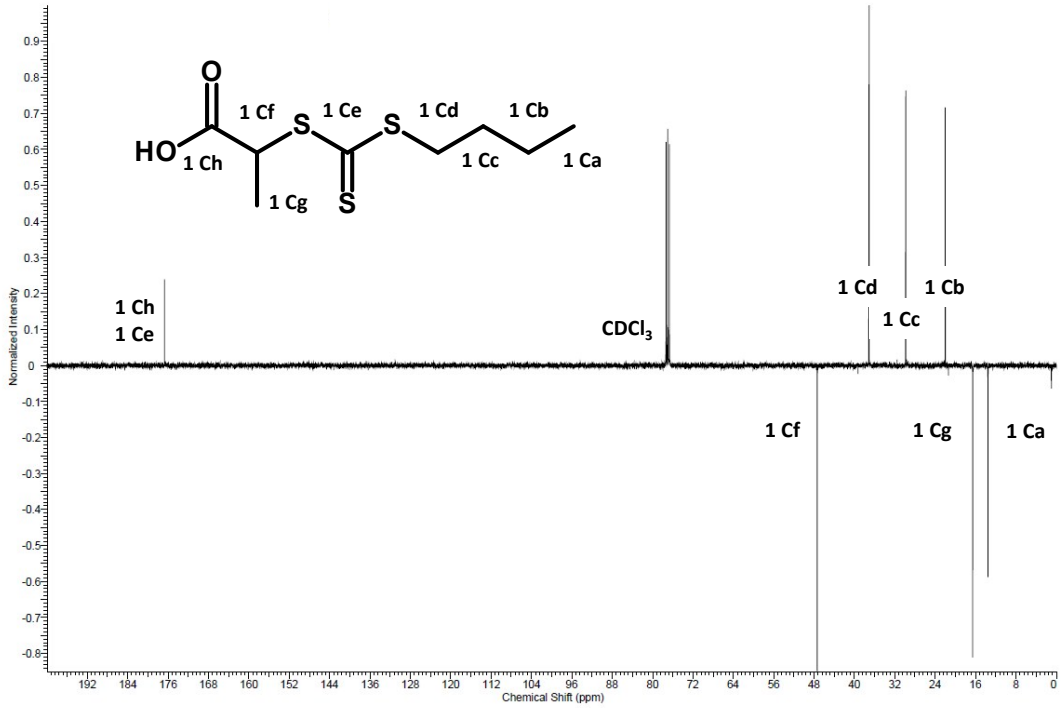

Figure S6. APT ^{13}C-NMR spectrum of 2-(butylthiocarbonothioylthio)propanoic acid.

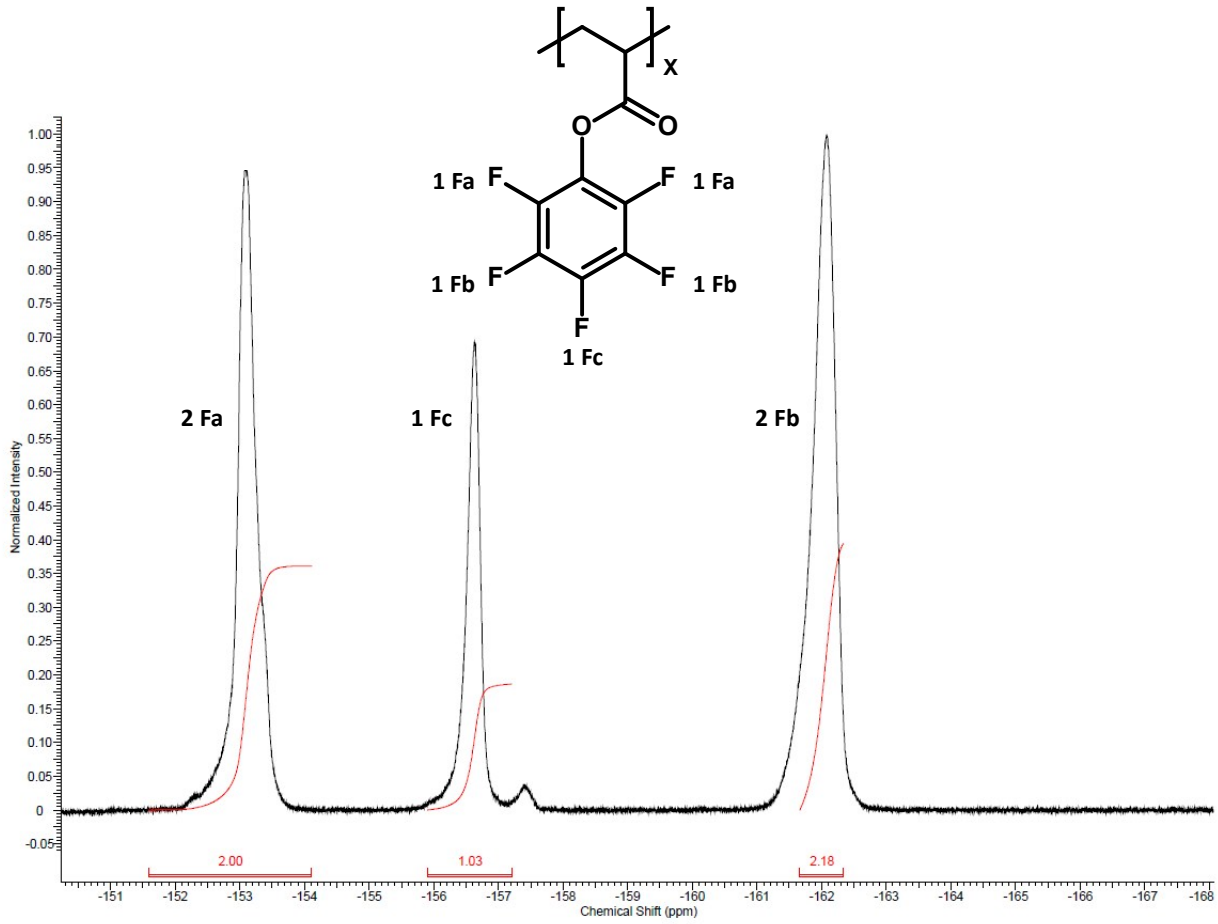

Figure S7. ^{19}F-NMR spectrum of purified poly(pentafluorophenyl acrylate).

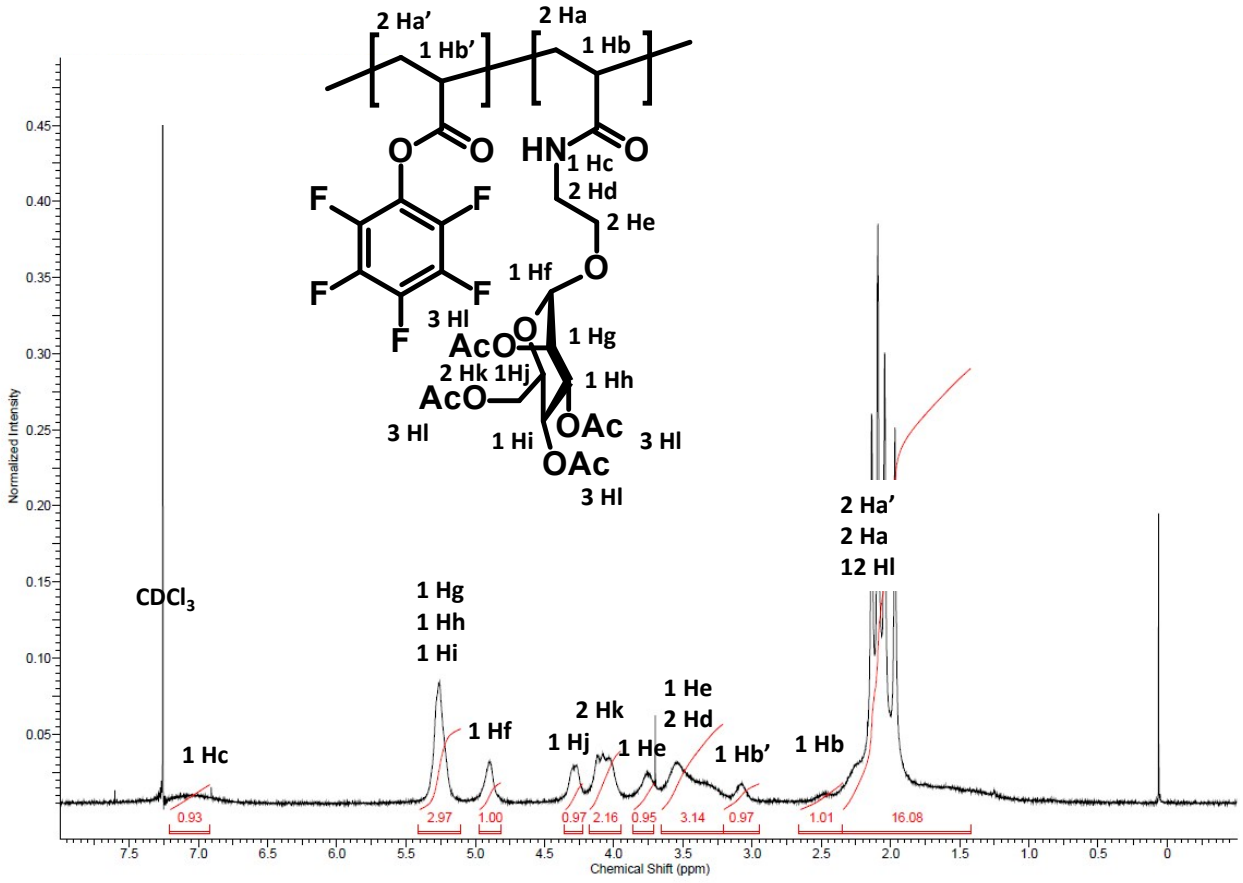

Figure S8. ^{1}H-NMR spectrum of purified block copolymer before end-group removal.

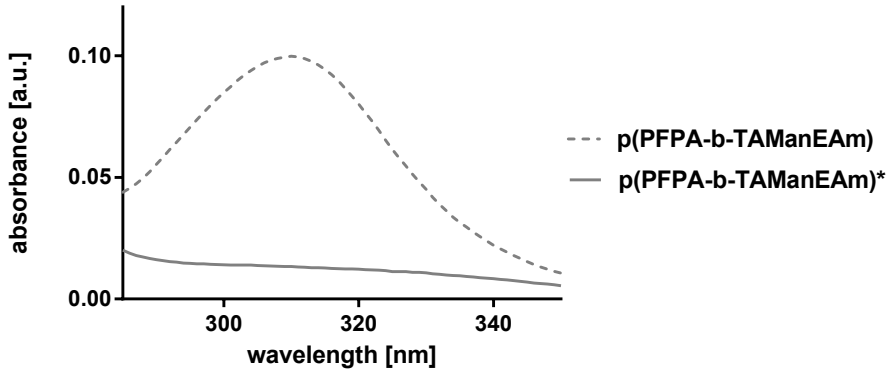

Figure S9. UV-Vis spectra of block copolymers before (dotted line) and after (full line) trithiocarbonate end-group removal by treatment with an excess of AIBN.

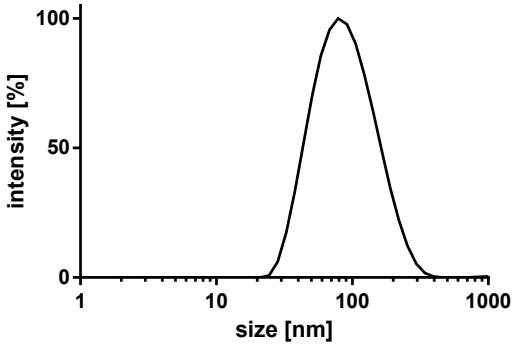

Figure S10. DLS spectrum of small sample that was taken to investigate self-assembly of block copolymers in DMSO. The block copolymers were dispersed at a concentration of 10 mg/mL and sonicated for 1 h prior to this measurement.

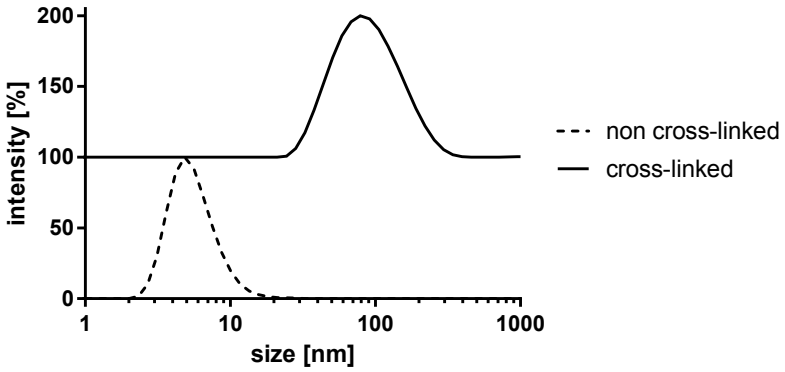

Figure S11. DLS spectra to illustrate effective cross-linking of the block copolymers. Two samples of a 10 mg/mL dispersion of block copolymers in DMSO were taken either before (dotted line) or after (full line) and subsequently diluted with chloroform to a final concentration of 2 mg/mL. While core-cross-linked micelles retained their morphology, non cross-linked block copolymers disassembled into unimers upon dilution, illustrating the need for cross-linking.

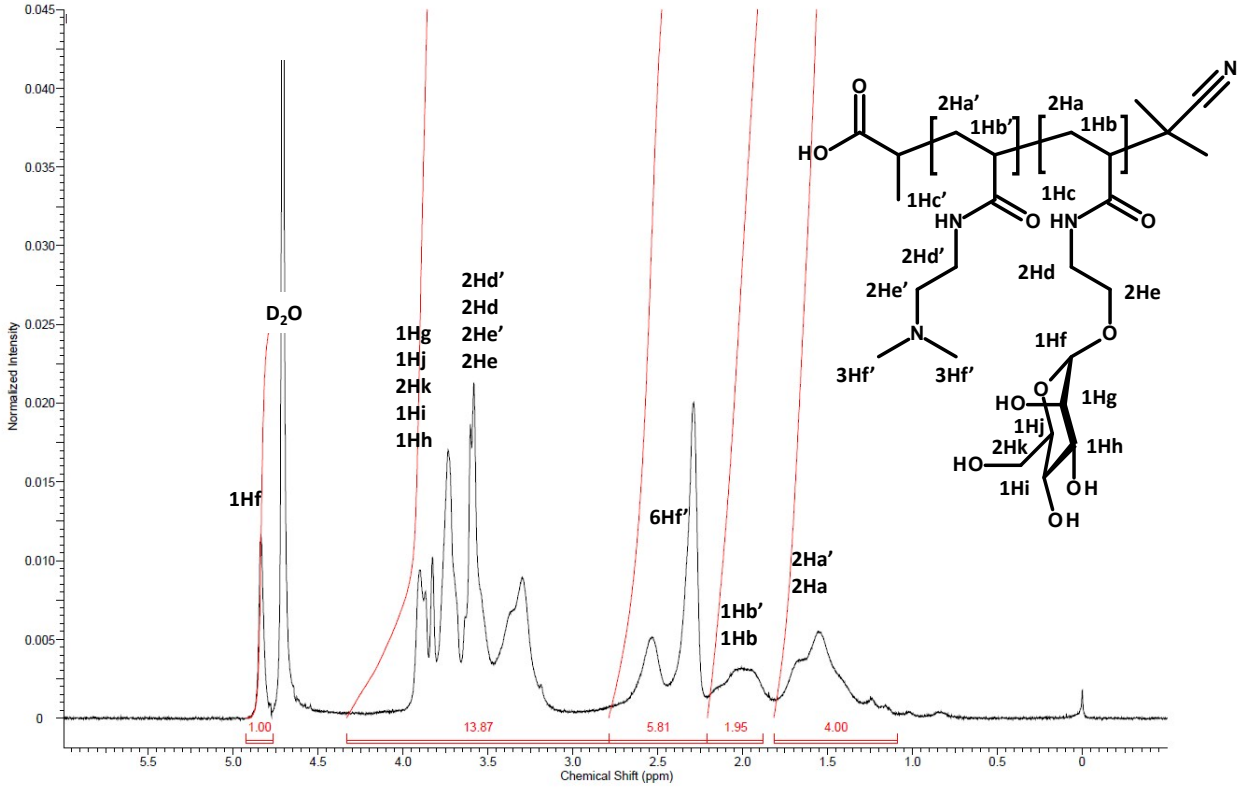

Figure S12. ^{1}H-NMR spectrum of DMAEA modified block copolymer in D_2O after deprotection. Notice the absence of acetyl protecting groups, illustrating complete deprotection of the mannosyl moieties.

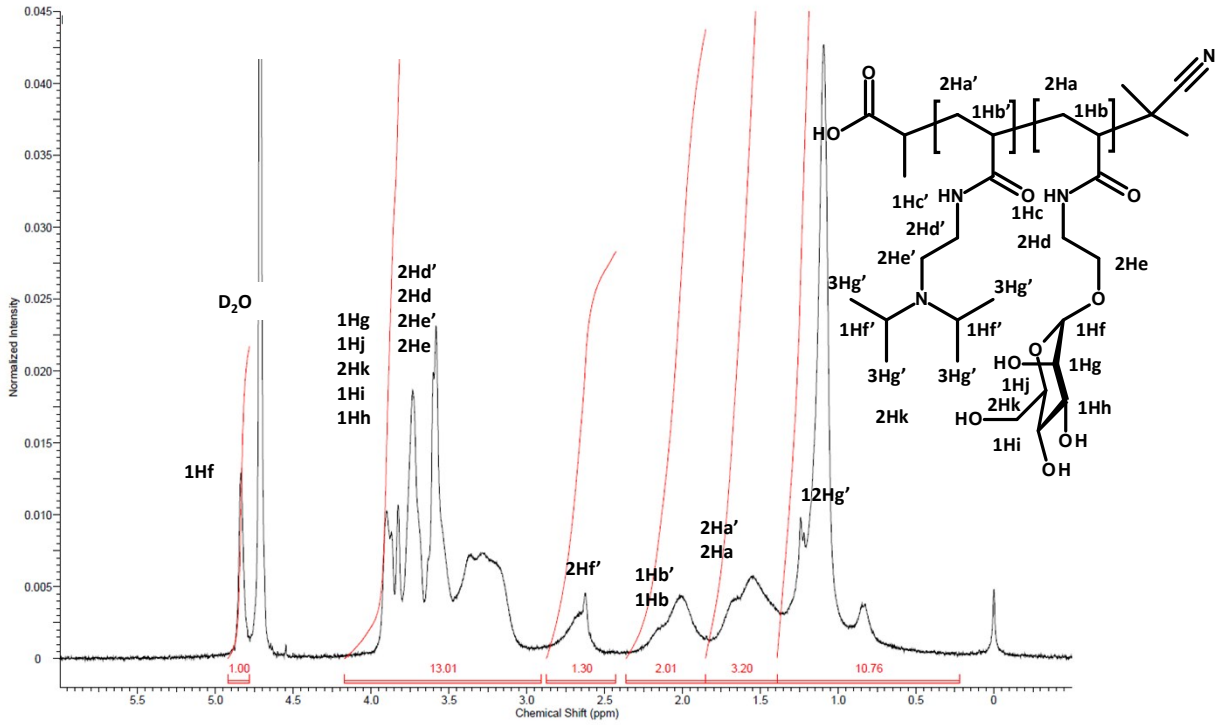

Figure S13. ^{1}H-NMR spectrum of DiPAEA modified block copolymer in D_2O after deprotection. Notice the absence of acetyl protecting groups, illustrating complete deprotection of the mannosyl moieties.

BIBLIOGRAPHY

(1) Nuhn, L.; Hirsch, M.; Krieg, B.; Koynov, K.; Fischer, K.; Schmidt, M.; Helm, M.; Zentel, R. *ACS Nano* **2012**, *6* (3), 2198.

(2) Li, D.; Li, H.; Wu, L. *Polym. Chem.* **2014**, *5* (6), 1930.