Electronic Supplementary Material (ESI) for Polymer Chemistry

Facile, Modular Transformations of RAFT Block Copolymers via Sequential Isocyanate and Thiol-Ene Reactions

Joel D. Flores,[1] Nicolas J. Treat,[1] Adam W. York[1] and Charles L. McCormick[1,2,*]

[1]Department of Polymer Science and [2]Department of Chemistry and Biochemistry

The University of Southern Mississippi, Hattiesburg, Mississippi, 39406 USA

Electronic Supporting Information

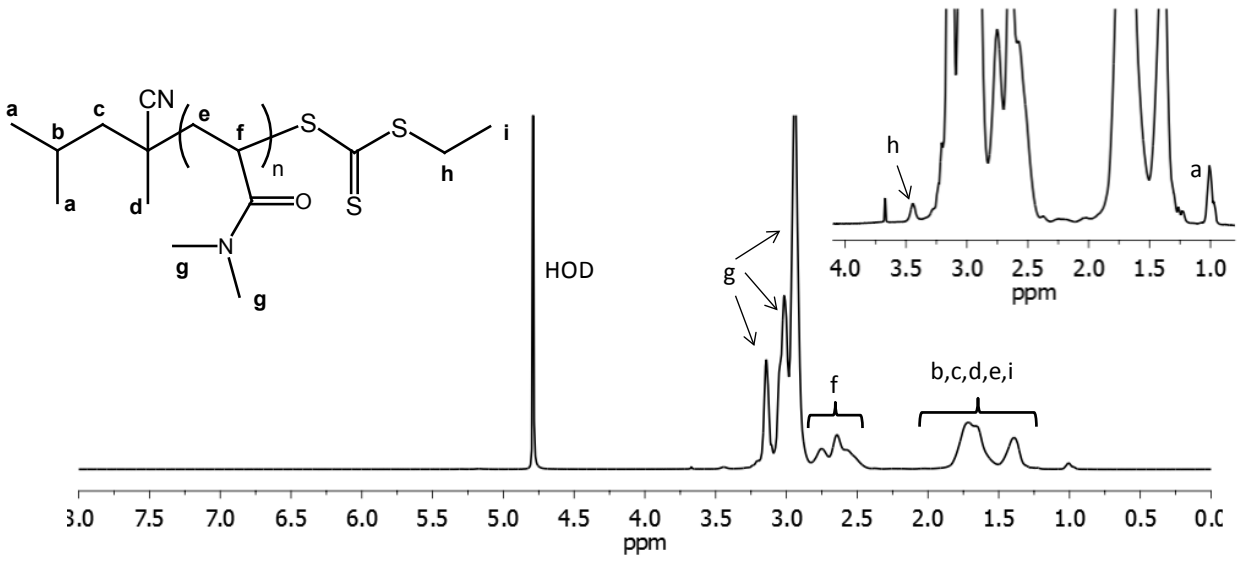

Figure S1. ^{1}H NMR spectrum (in D_2O) of PDMA-CEV macroCTA (**P1**). Degree of polymerization (DP) was calculated using peak areas of methyne proton (**f**) of the DMA backbone and the terminal methyl group (**a**, inset) of the RAFT CTA.

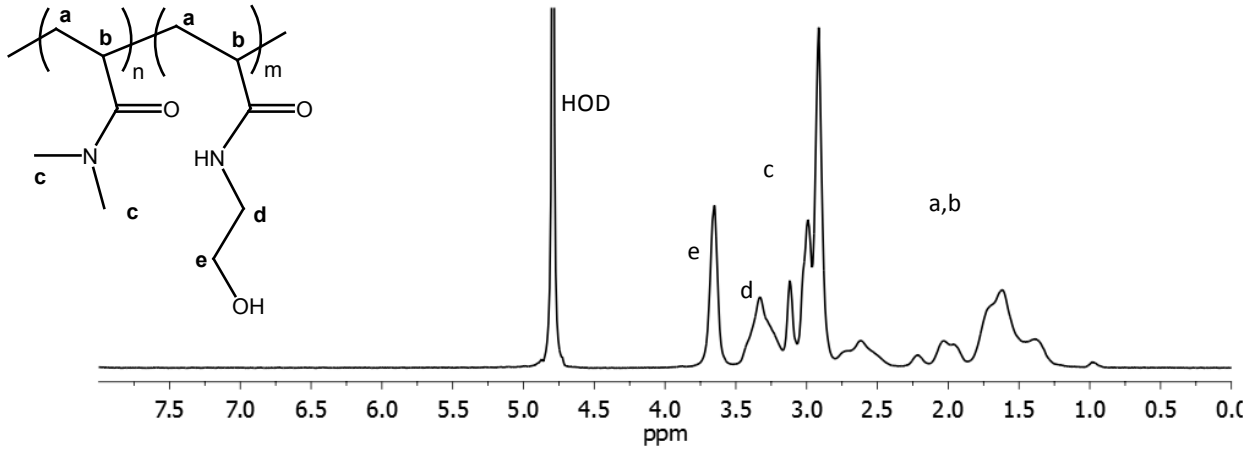

Figure S2. ^{1}H NMR spectrum (in D_2O) of PDMA-*b*-PHEA (**P2**) diblock copolymer.

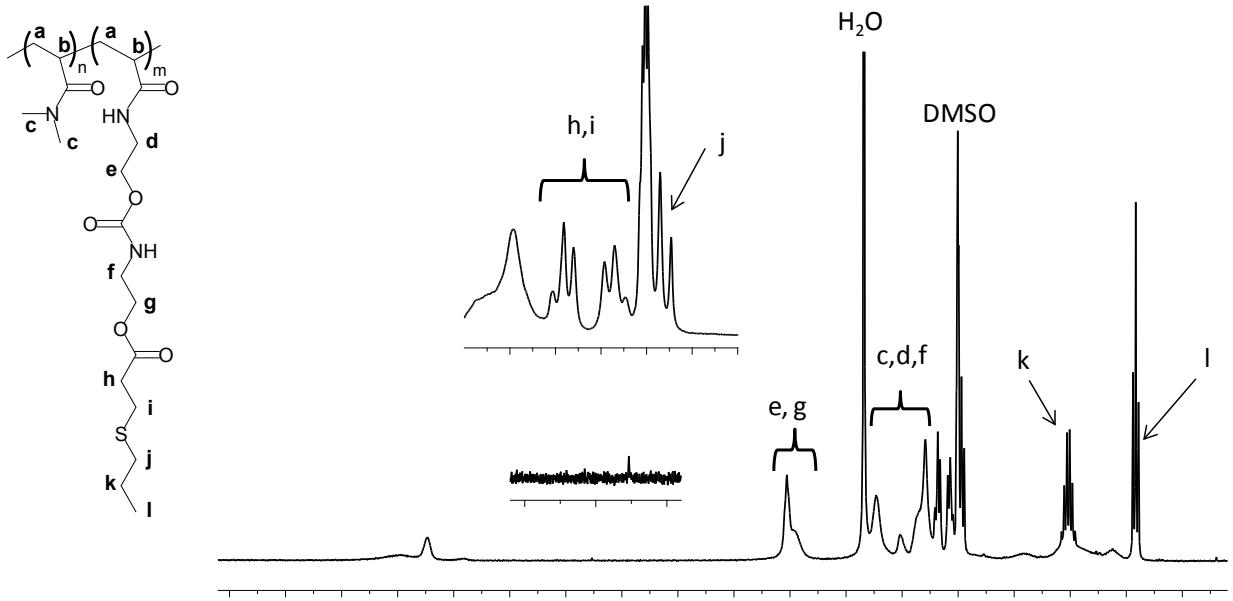

Figure S3. ^{1}H NMR spectrum of copolymer **P3a** in DMSO-d_6.

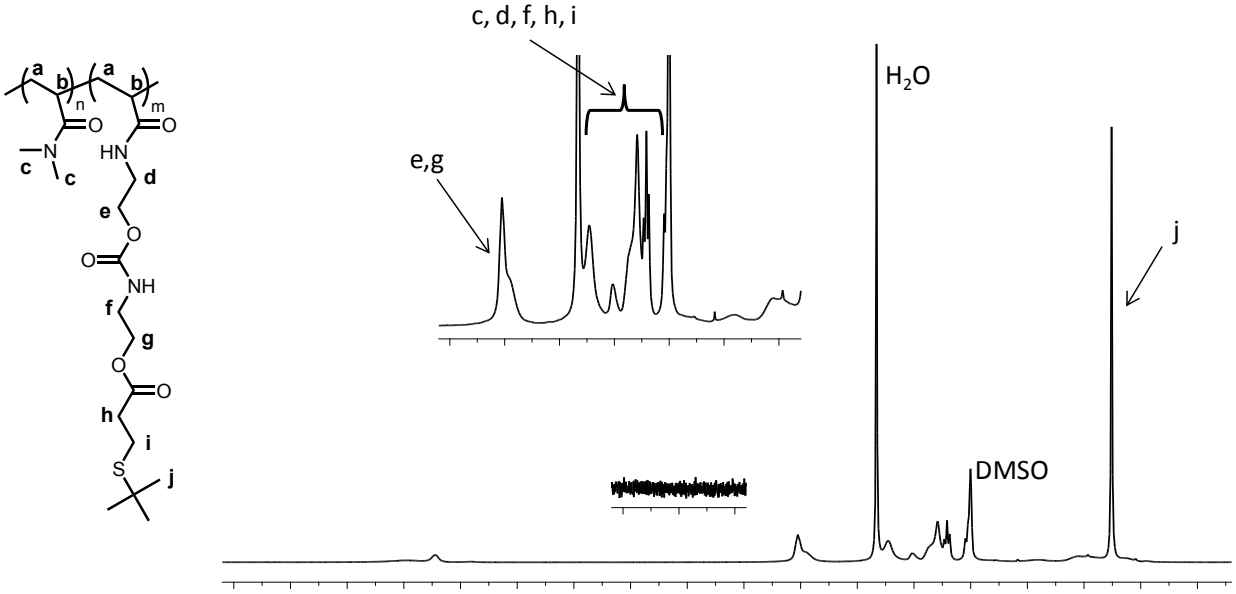

Figure S4. ^{1}H NMR spectrum of copolymer **P3b** in DMSO-d_6.

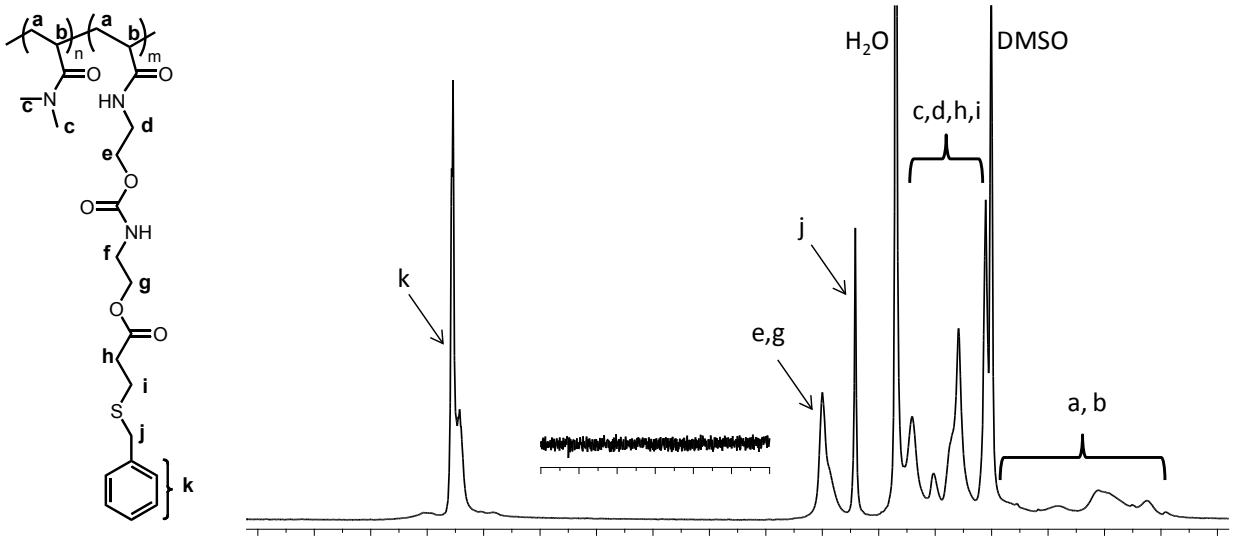

Figure S5. ^{1}H NMR spectrum of copolymer **P3c** in DMSO-d_6.

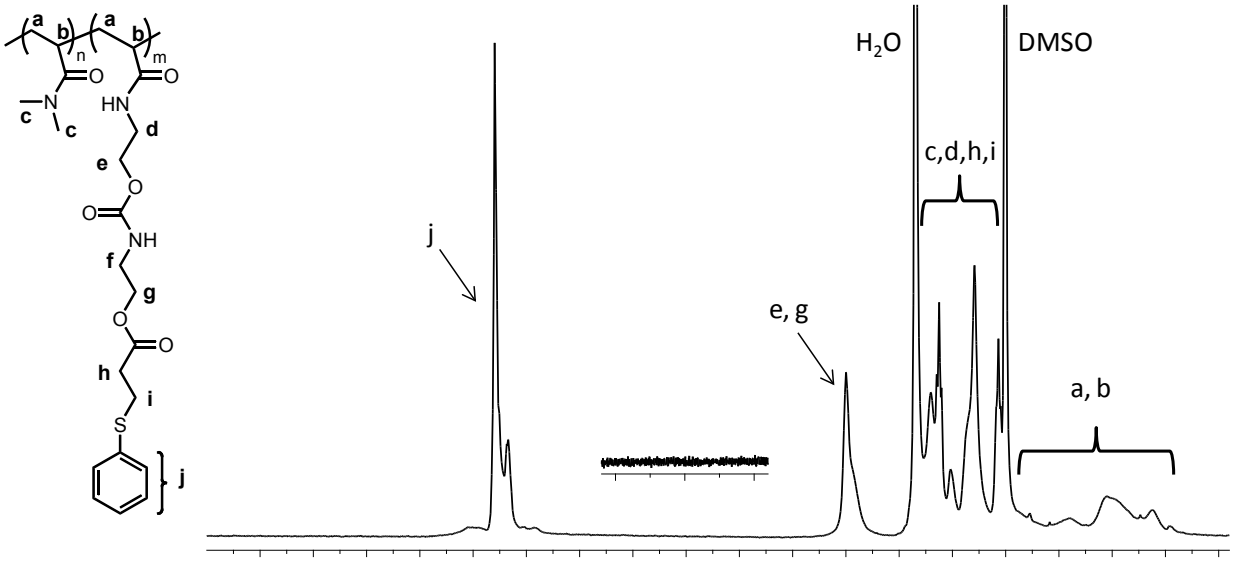

Figure S6. ^{1}H NMR spectrum of copolymer **P3d** in DMSO-d_6.

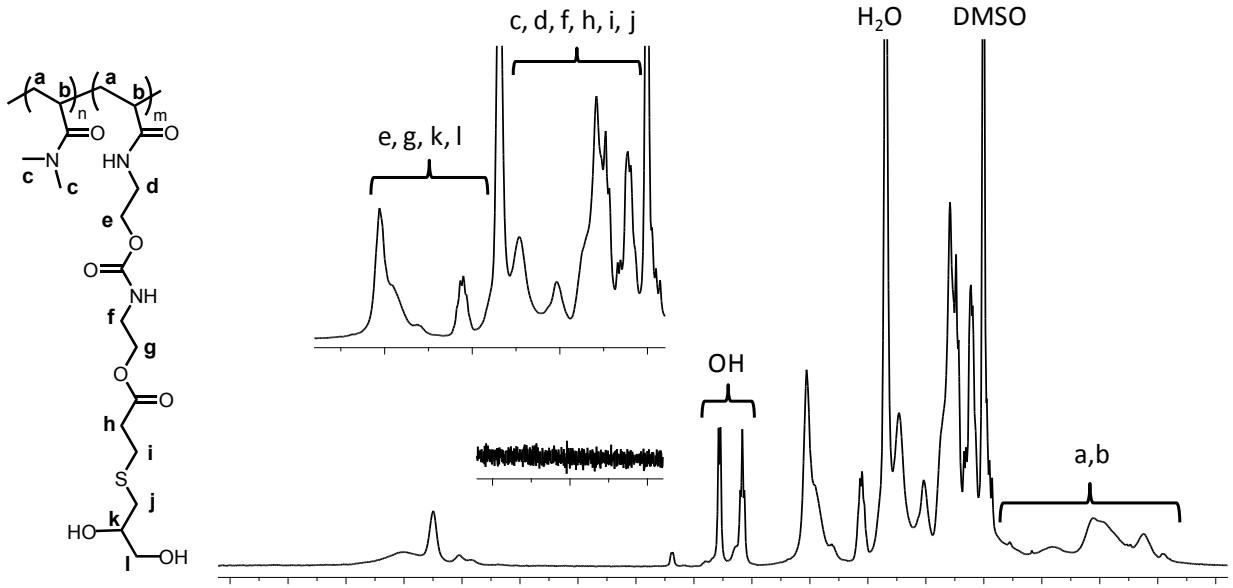

Figure S7. ^{1}H NMR spectrum of copolymer **P3f** in DMSO-d_6.

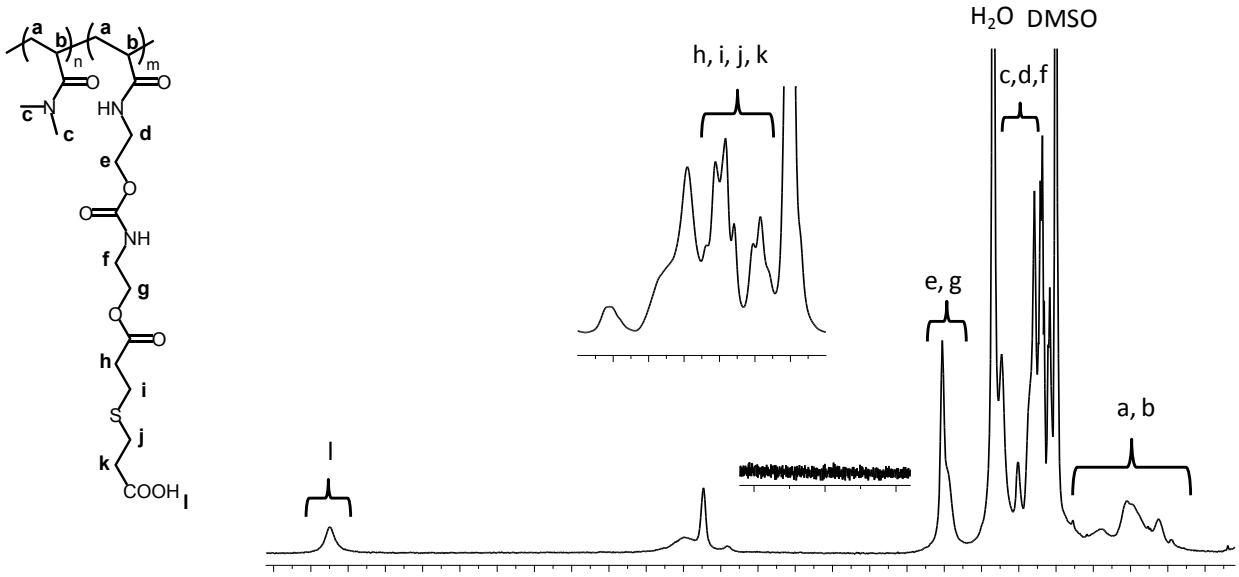

Figure S8. ^{1}H NMR spectrum of copolymer **P3g** in DMSO-d_6.

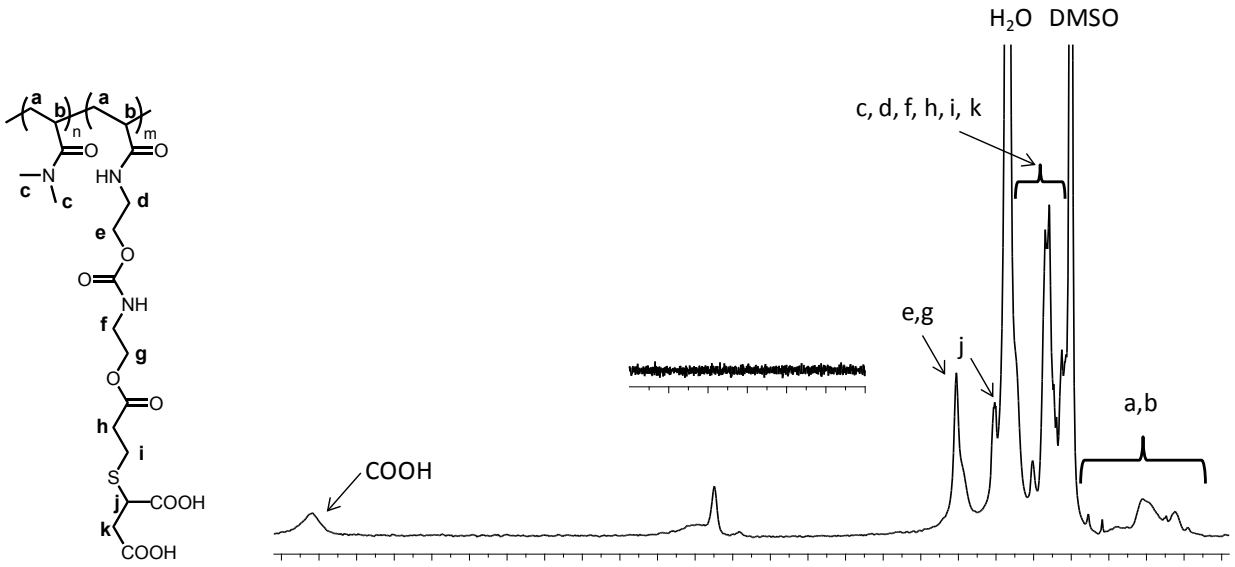

Figure S9. ^{1}H NMR spectrum of copolymer **P3h** in DMSO-d_6.

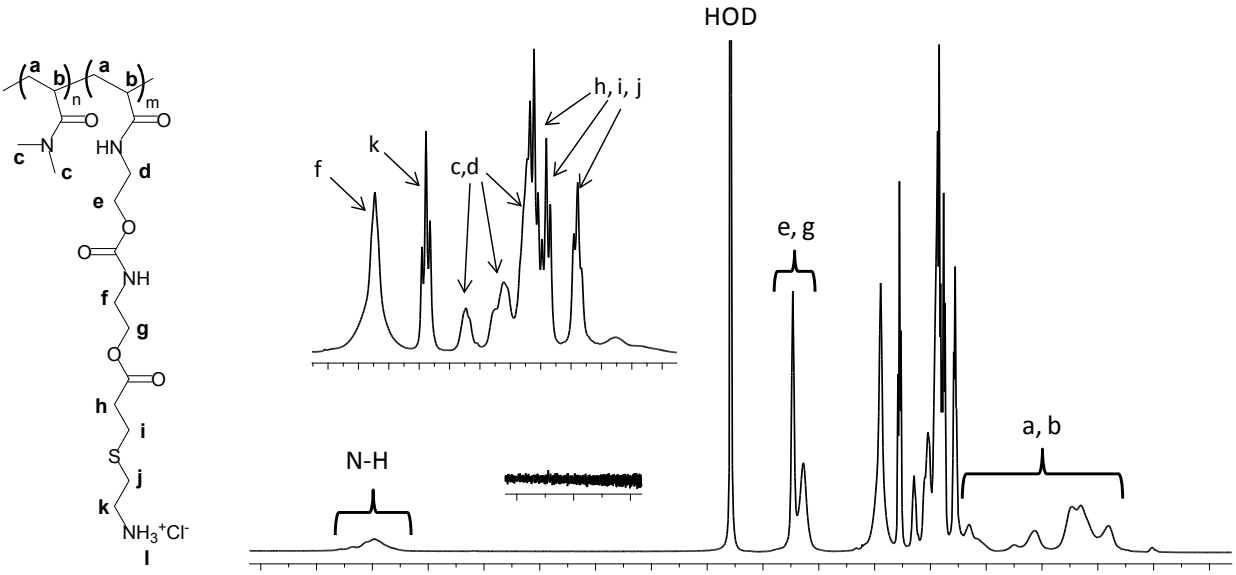

Figure S10. ^{1}H NMR spectrum of copolymer **P3i** in D_2O.

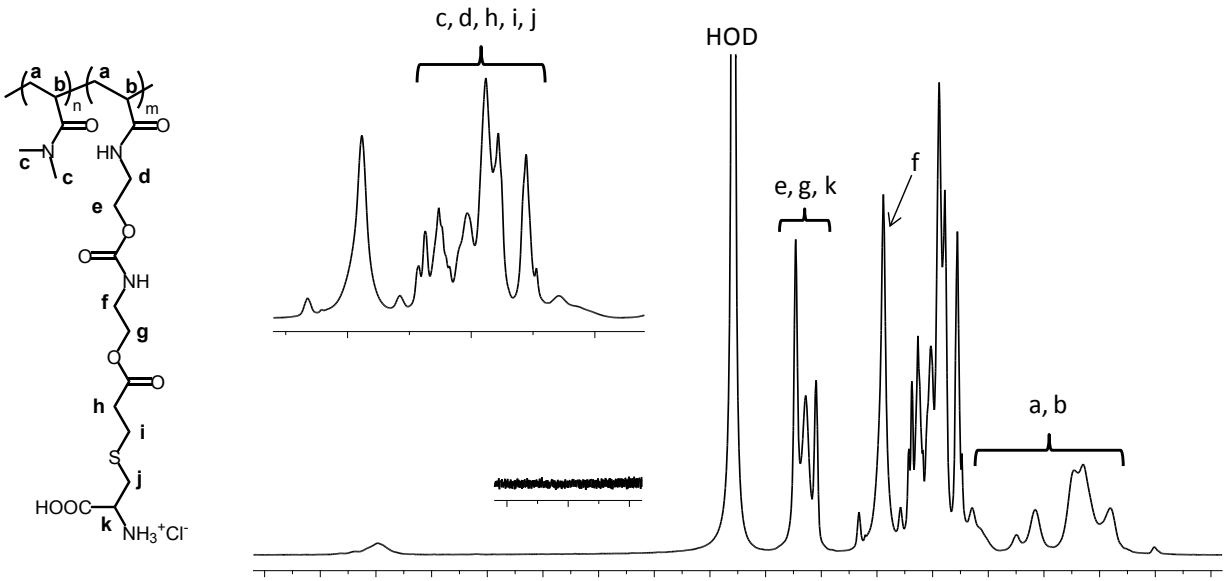

Figure S11. ^{1}H NMR spectrum of copolymer **P3j** in D_2O.

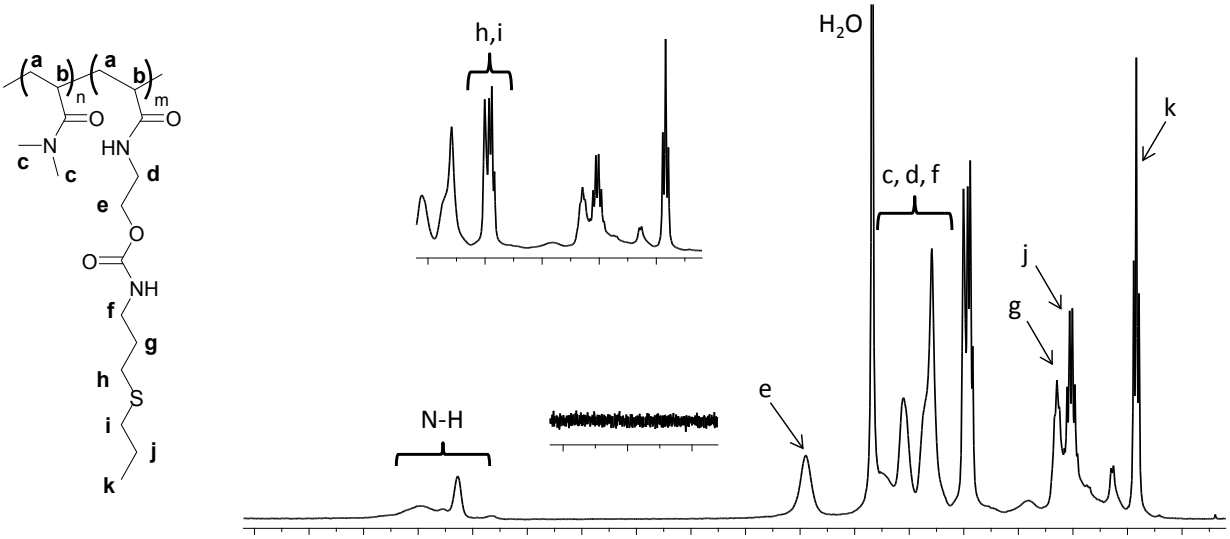

Figure S12. ^{1}H NMR spectrum of copolymer **P4a** in DMSO-d_6.

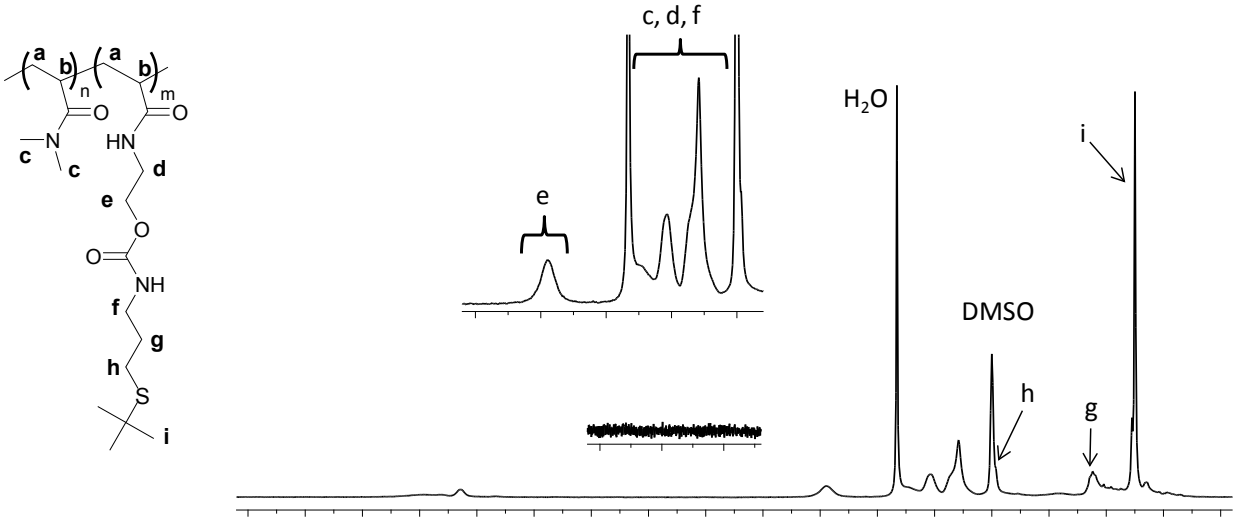

Figure S13. ^{1}H NMR spectrum of copolymer **P4b** in DMSO-d_6.

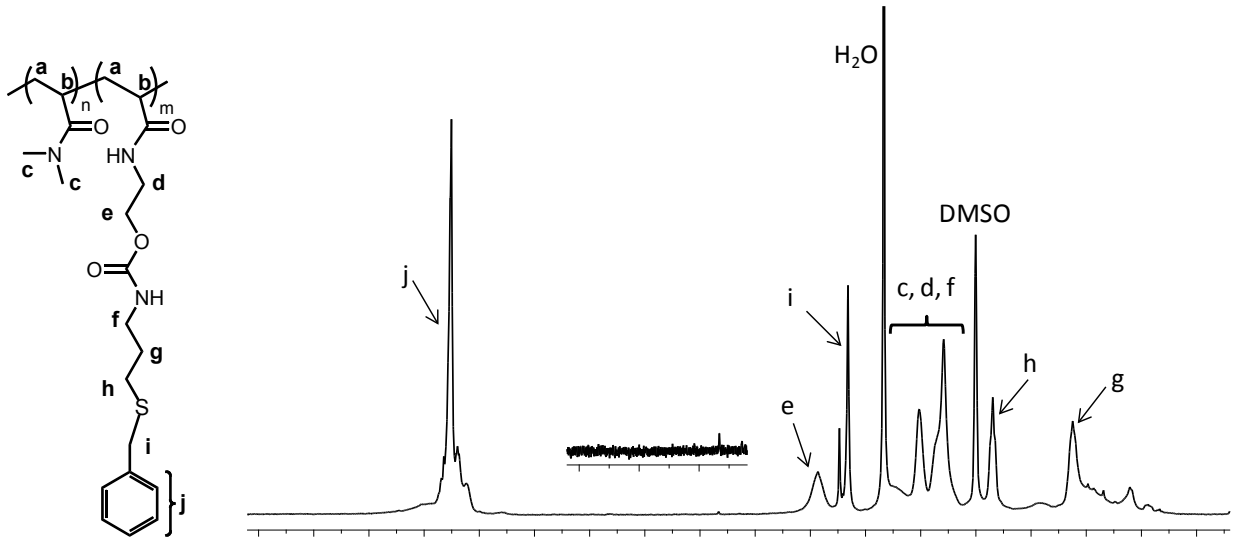

Figure S14. ^{1}H NMR spectrum of copolymer **P4c** in DMSO-d_6.

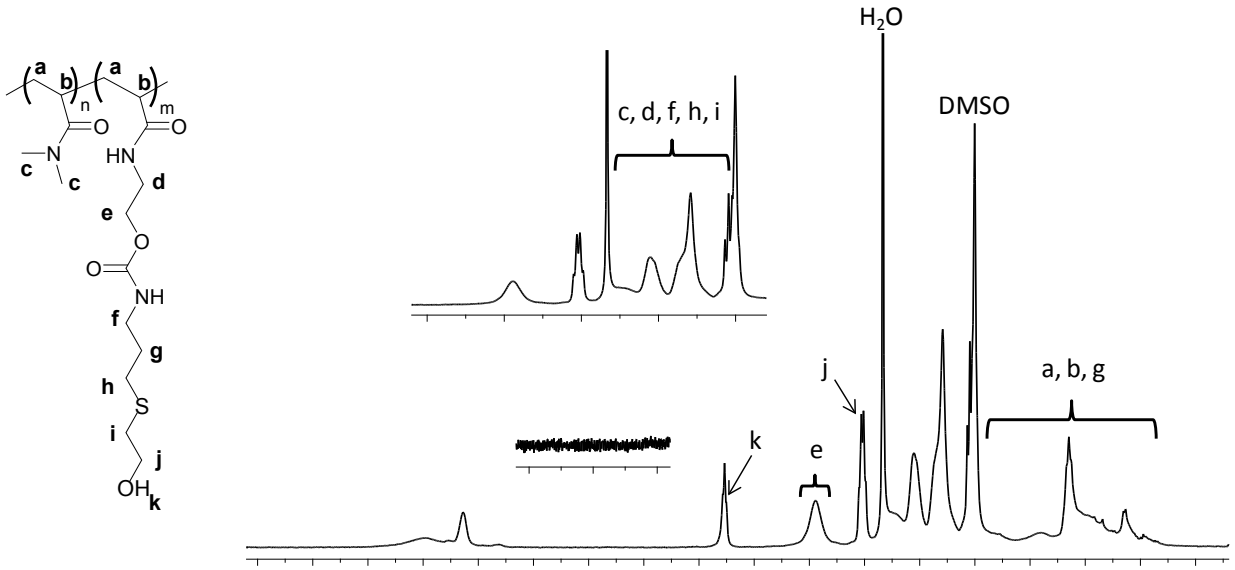

Figure S15. ^{1}H NMR spectrum of copolymer **P4e** in DMSO-d_6.

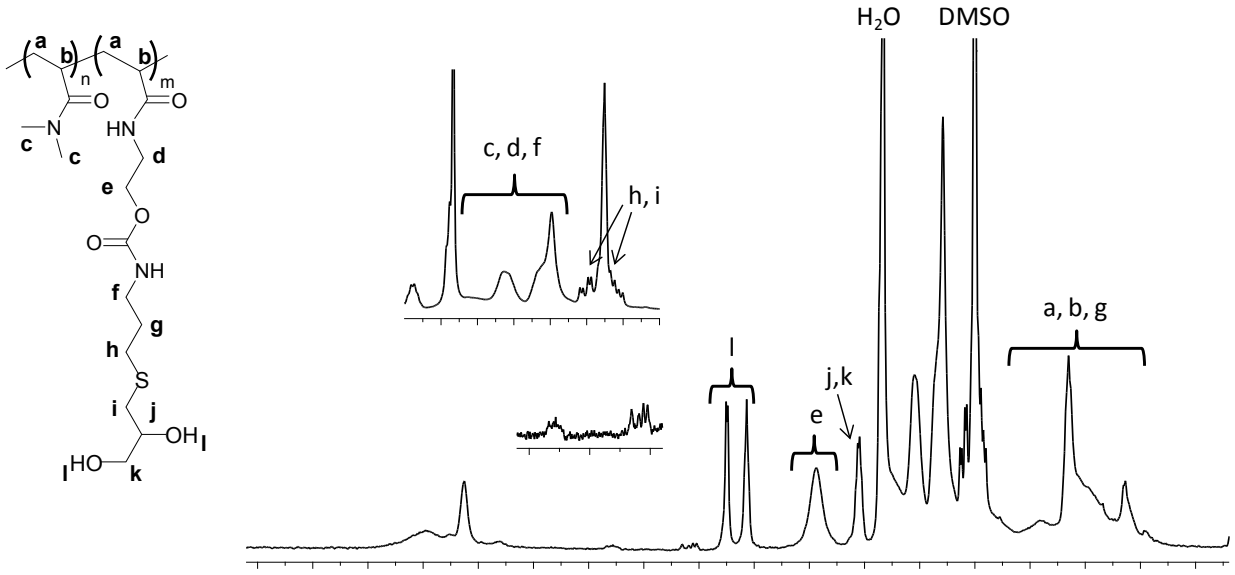

Figure S16. ^{1}H NMR spectrum of copolymer **P4f** in DMSO-d_6.

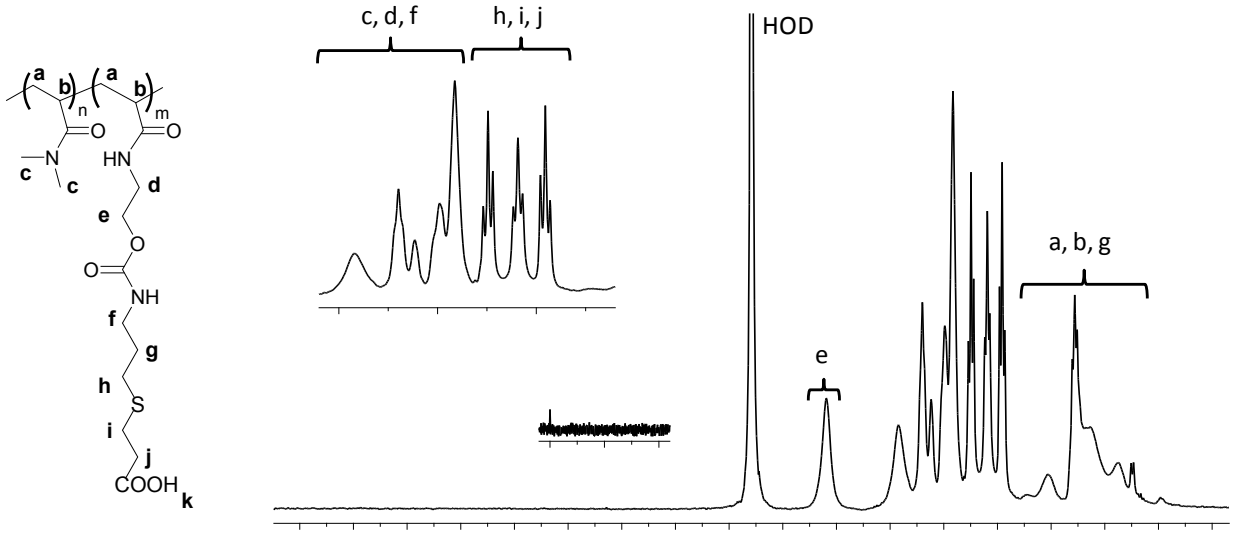

Figure S17. ^{1}H NMR spectrum of copolymer **P4g** in D_2O (at pH 7).

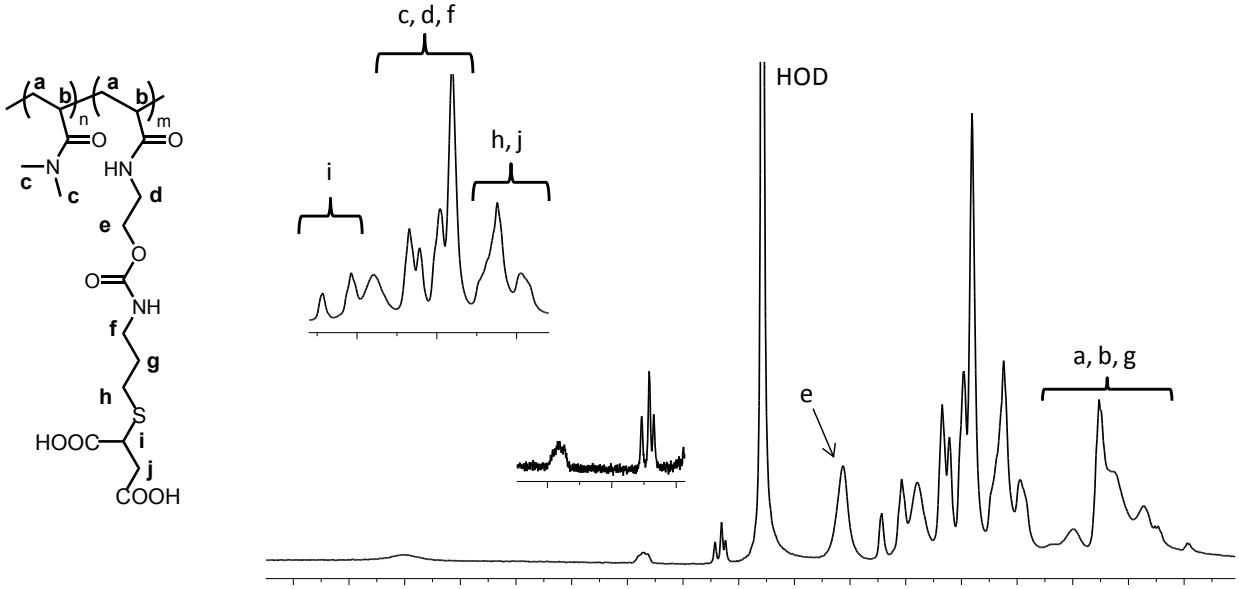

Figure S18. ^{1}H NMR spectrum of copolymer **P4h** in D_2O (at pH 7).

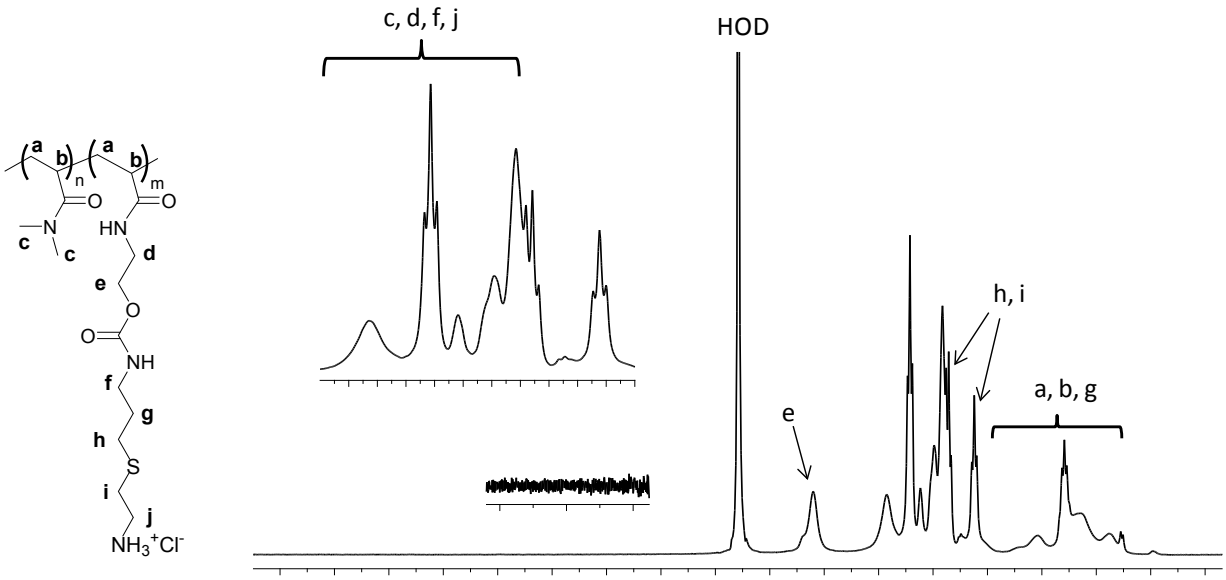

Figure S19. ^{1}H NMR spectrum of copolymer **P4i** in D_2O (at pH 5).

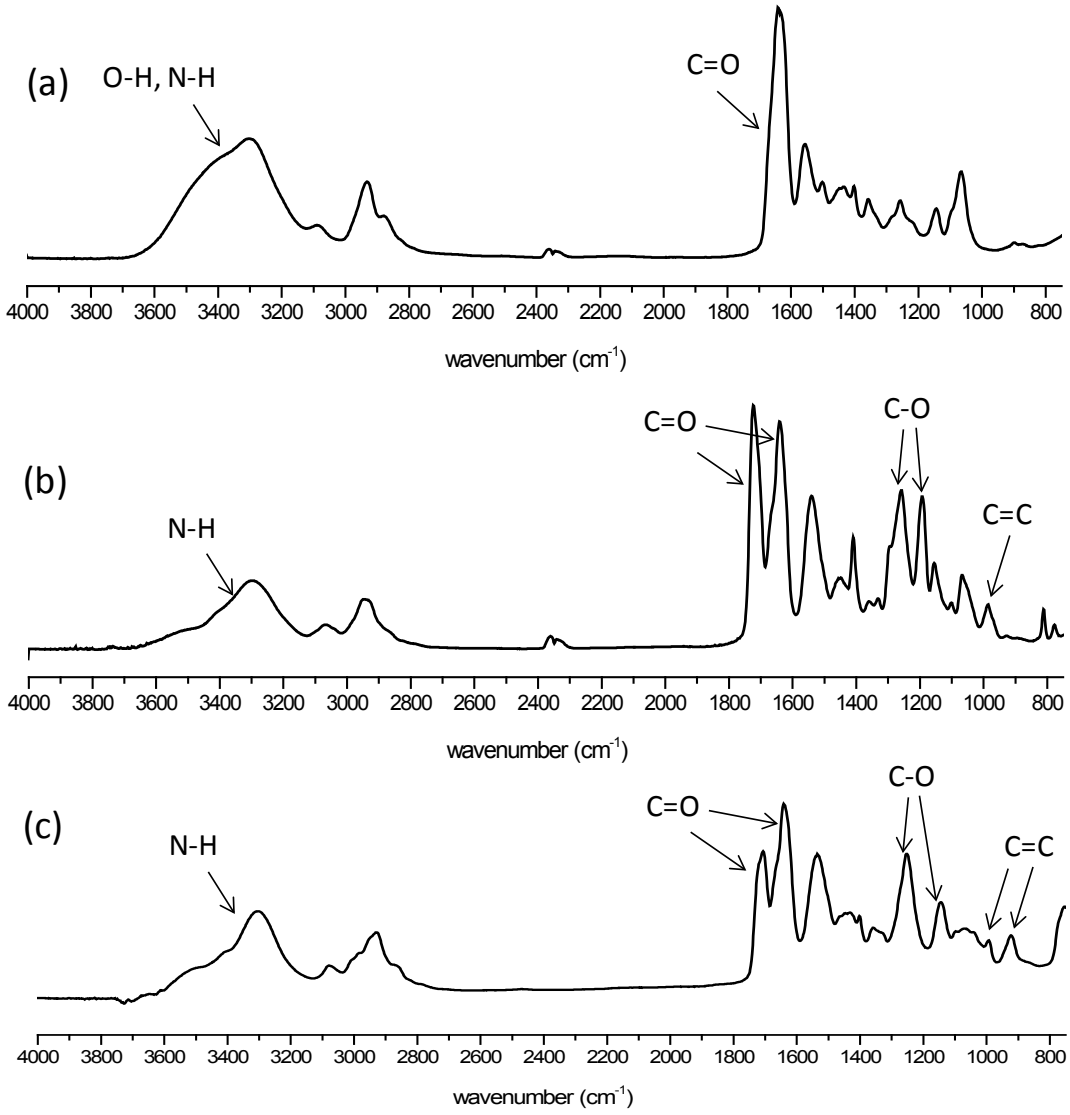

Figure S20. FT-IR absorption spectra (NaCl plate) of (a) $PDMA_n$-*b*-$PHEA_m$ (**P2**) diblock copolymer precursor, (b) $PDMA_n$-*b*-$PHEA(acrylate)_m$ (**P3)** copolymer, and (c) $PDMA_n$-*b*-$PHEA(allyl)_m$ (**P4)** copolymer. Characteristic bands associated to the reactions of hydroxyl groups with the isocyanate-containing alkenes are identified.

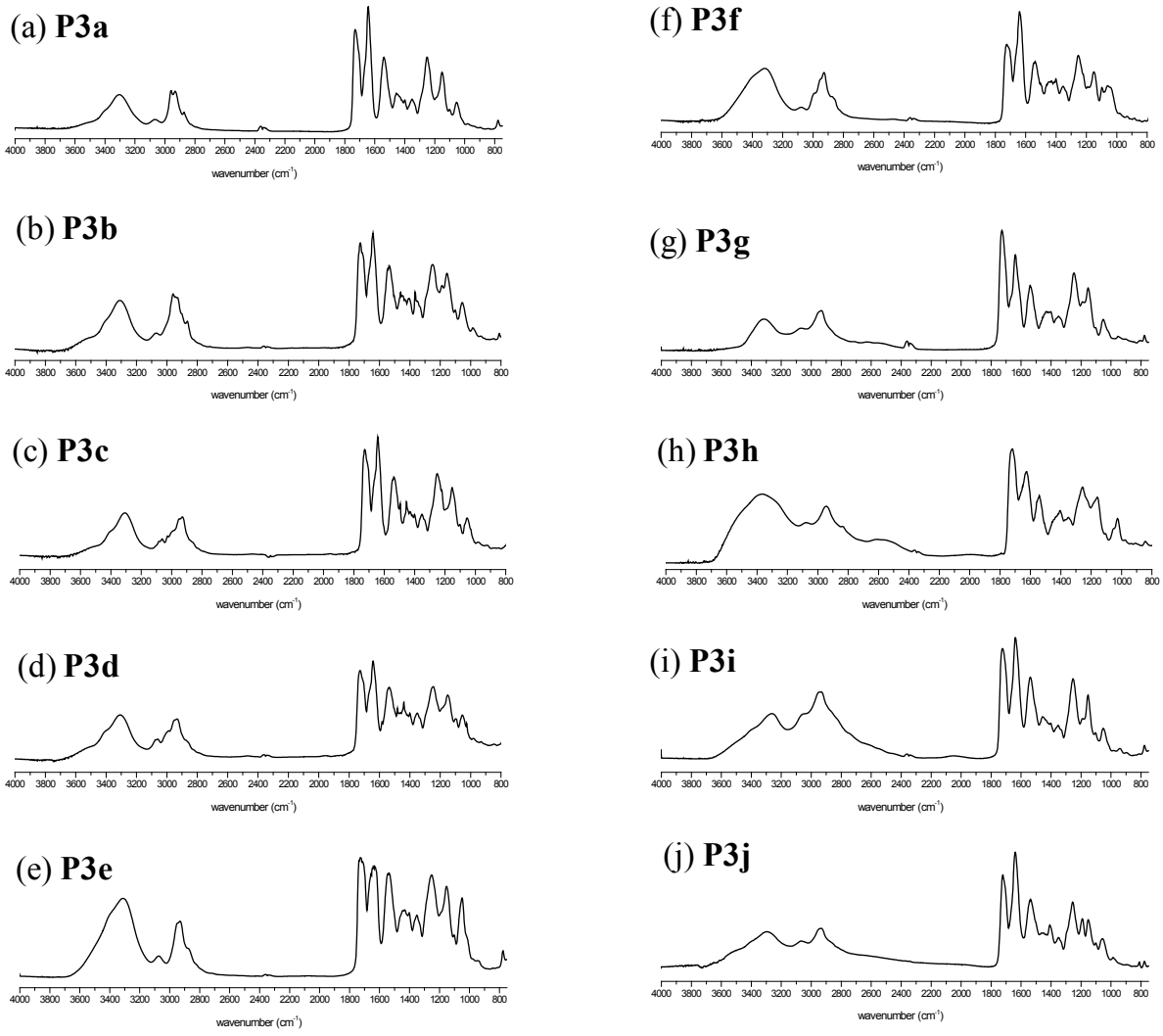

Figure S21. FT-IR absorption spectra (NaCl) of functionalized copolymers **P3a-P3j**.

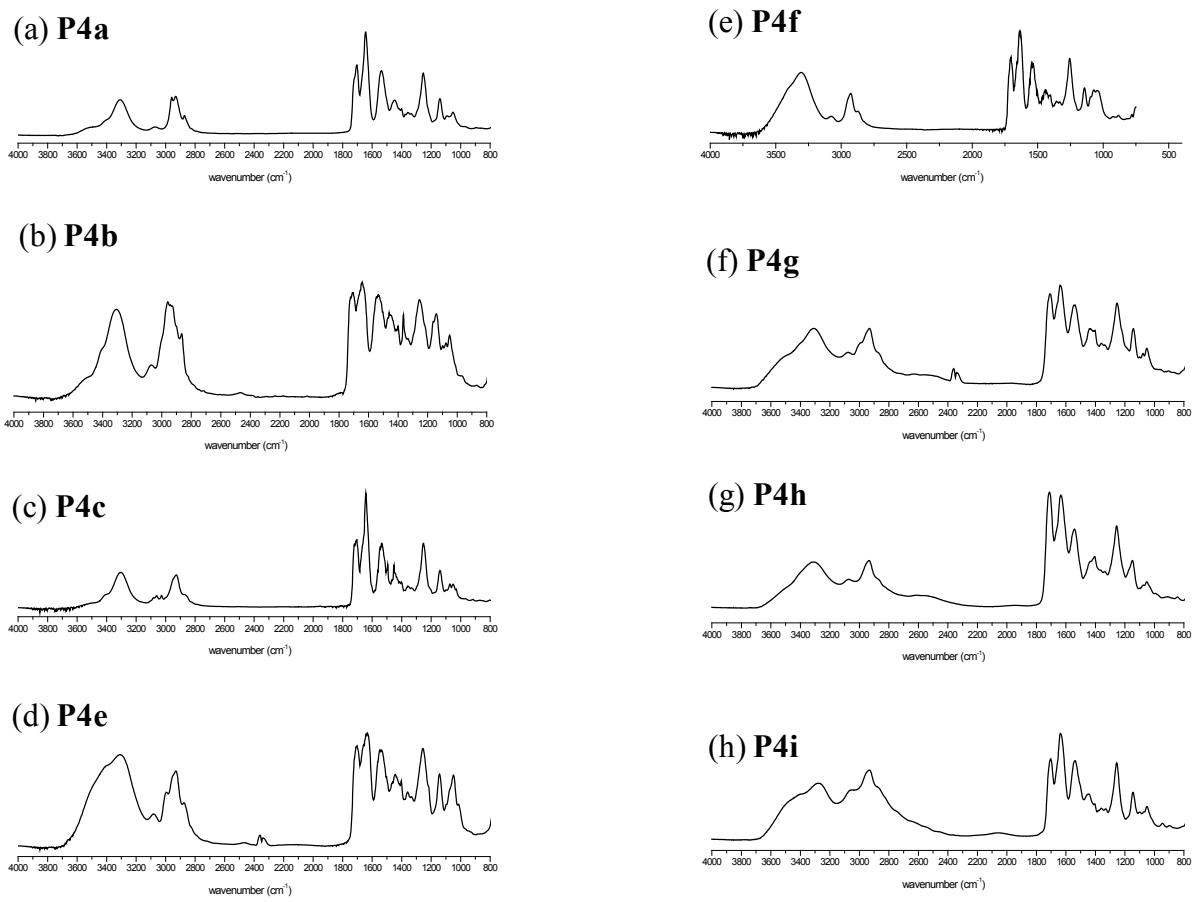

Figure S22. FT-IR absorption spectra (NaCl) of functionalized copolymers **P4a-P3c** and **P4e-P4i**. Conjugations of thiophenol (**P4d**) and *L*-cysteine (**P4j**) failed.

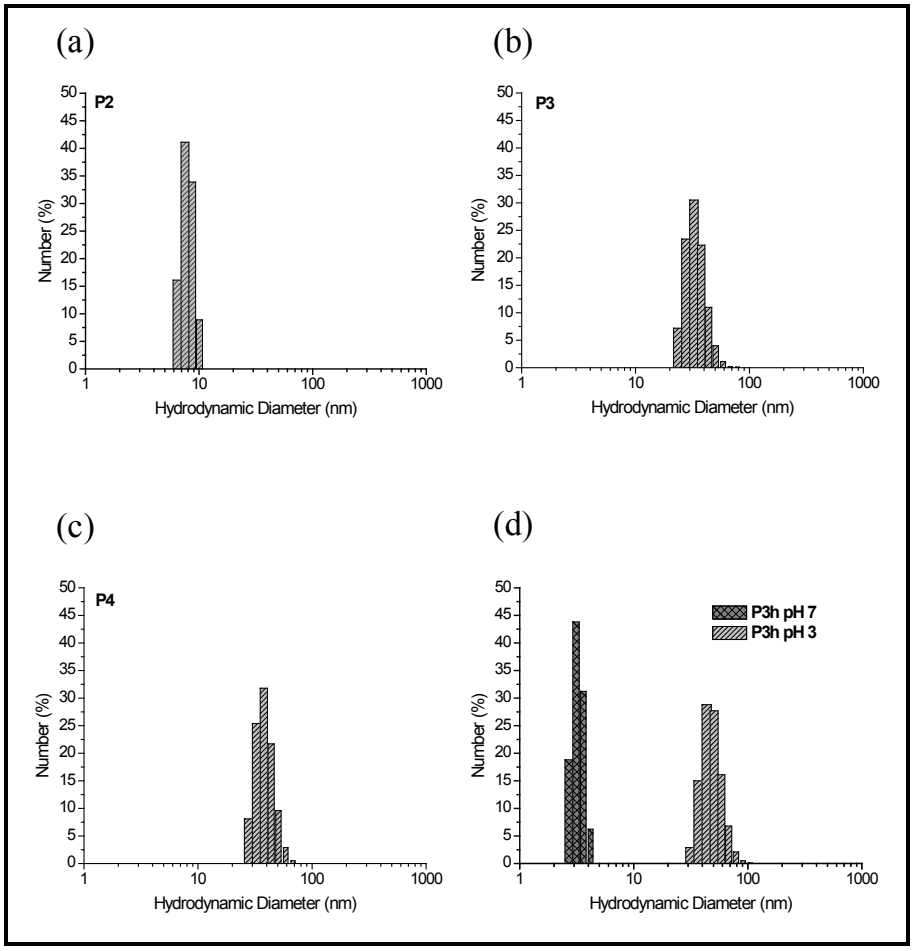

Figure S23. Size distributions as measured by DLS (in aqueous solutions) of (a) precursor copolymer **P2** (9.0 nm), (b) acrylate-functionalized copolymer **P3** (29.7 nm), (c) allyl-functionalized copolymer **P4** (37.8 nm), and (d) mercaptosuccinic acid-functionalized, pH-responsive copolymer **P3h** (2.9 nm at pH 7, 49.8 nm at pH 3) (1.0 mg mL^{-1} copolymer concentration).

*Procedure for Complexation of Copolymer **P4i** with tRNA*

Transfer RNA (tRNA) solution (1 μL, 20 μM) was pipetted into seven 200 μL centrifuge tubes. This was diluted with the appropriate amount of nuclease free water and phosphate buffer solution (2 μL, 82.5 mM, pH 7.4). Aliquots of copolymer solution were added into each tube for the corresponding N/P ratios. The final volume was 8.25 μL giving 20 mM phosphate buffer and approximately 2.5 μM tRNA concentrations. All samples were vortexed immediately and allowed to incubate for 30 minutes at room temperature. Agarose gel (1%) was prepared and pre run for 30 minutes prior to well loading. The running buffer was 1 X trisborate-EDTA, 8 M Urea. Each sample was diluted with 8.25 μL of 2 X trisborate-EDTA, 8 M Urea solution (no dye). The gel was allowed to run for 30 minutes (93 Volts) and was visualized through ethidium bromide staining (see Figure 6).

Procedure for Lectin-Binding Assay of Glycopolymer

The glycopolymer was prepared via free radical addition of sodium1-thio-*β*-*d*-glucose to an allyl-containing precursor copolymer ($PDMA_{112}$-*b*-$PHEA(allyl)_{23}$, Mn=15,200 PDI=1.21). The copolymer (200 mg, 0.30 mmol ene), thiol (650 mg, 3.0 mmol) and VA-044 (32 mg, 0.09 mmol) were dissolved in dioxane/water mixture. The pH of the solution was adjusted to 4-5 with 0.1 M HCl. After purging with N_2 for 1 hr at 0 °C, the mixture was heated at 40 °C for 24 hrs. The copolymer was purified by dialysis against acidic water for 3 days followed by lyophilization (Mn=17,800 PDI=1.25, conversion >99%).

To a solution of FITC-Con A in phosphate buffer (3 mL, 24 nM, pH 7.4) was added copolymer solution (2 μL, 8 mM). After mixing, the solution was equilibrated at room temperature for 15 minutes. The fluorescence emission intensity at 517 nm of the solution was then measured using 490 nm as the excitation wavelength. Additional aliquot of copolymer solution was added every 15 minutes and the incremental decrease in fluorescence intensity was monitored.

FITC-Con A has an intrinsic emission peak at 517 nm which is quenched upon binding of the glycopolymer. The relative change in fluorescence intensity of FITC-Con A as a function of glucose concentration was plotted (Figure S24a). The lectin-binding affinity or association constant (K_a) of the glucose- functionalized copolymer was estimated using Scatchard plot as described by the following equation:

$$\frac{[sugar]F_0}{\Delta F} = \frac{\frac{[sugar]F_0}{\Delta F_{max}} + F_0}{\Delta F_{max} K_a}$$

where [*sugar*] is the glucose concentration, F_0 is the initial fluorescence intensity and ΔF is the change in fluorescence intensity.[1-3] The obtained Ka value (7.5×10^4 M^{-1}) is comparable to those of other synthetic glycopolymers reported in the literature.[1-5]

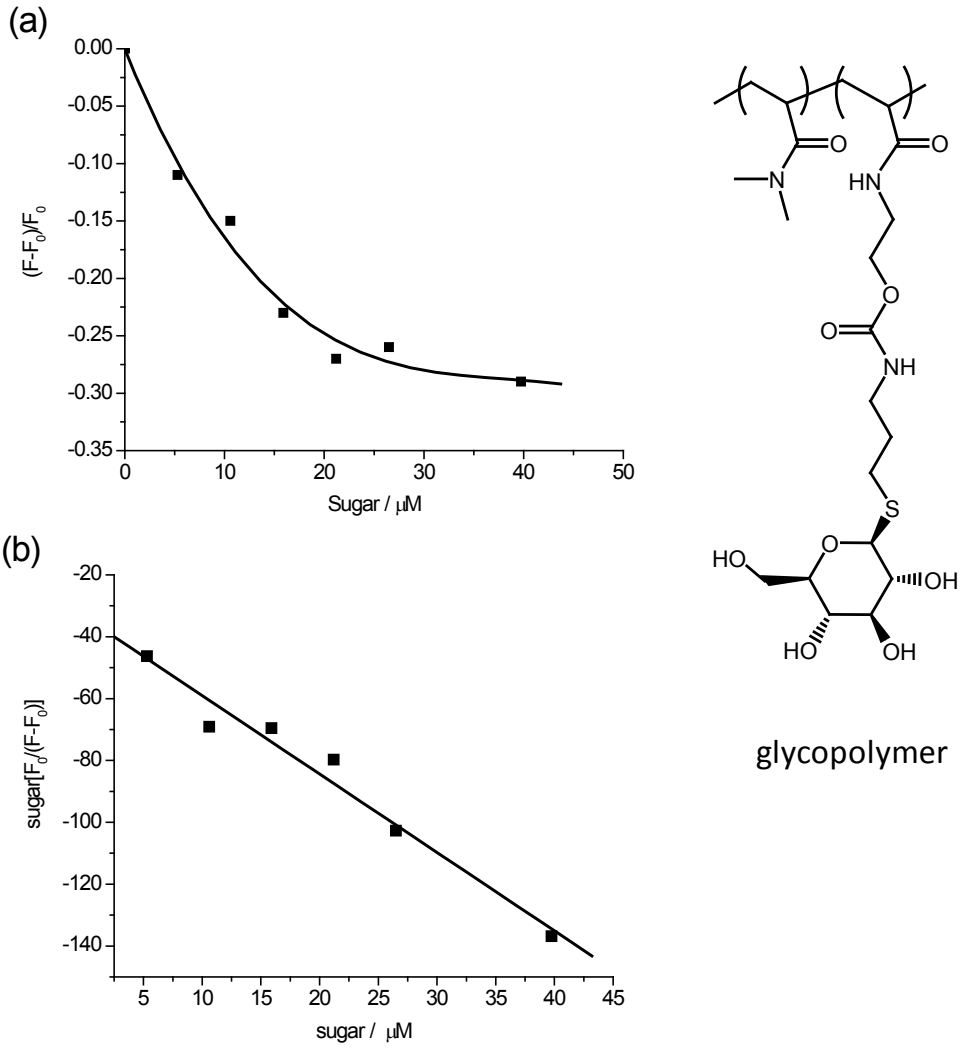

Figure S24. (a) Variation of fluorescence intensity from the binding of glycopolymer with fluorescently-labeled lectin (FITC-Con A) and (b) the resulting Scatchard plot for the estimation of the association constant, Ka (7.5 x 10^4 M^{-1}).

References:

1. Otsuka, I.; Hongo, T.; Nakade, H.; Narumi, A.; Sakai, R.; Satoh, T.; Kaga, H.; Kakuchi, T. *Macromolecules* **2007,** 40, 8930-8937.
2. Miura, Y.; Wada, N.; Nishida, Y.; Mori, H.; Kobayashi, K. *J. Polym. Sci., Part A: Polym. Chem.* **2004,** 42, 4598-4606.
3. Miura, Y.; Ikeda, T.; Kobayashi, K. *Biomacromolecules* **2003,** 4, 410-415.
4. Mandal, D. K.; Kishore, N.; Brewer, C. F. *Biochemistry* **1994,** 33, 1149-1156.
5. Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002,** 102, 555-578.