Supporting Information

Glycopolypeptide-Grafted Bioactive Polyionic Complex Vesicles (PICsomes) and Their Specific Polyvalent Interactions

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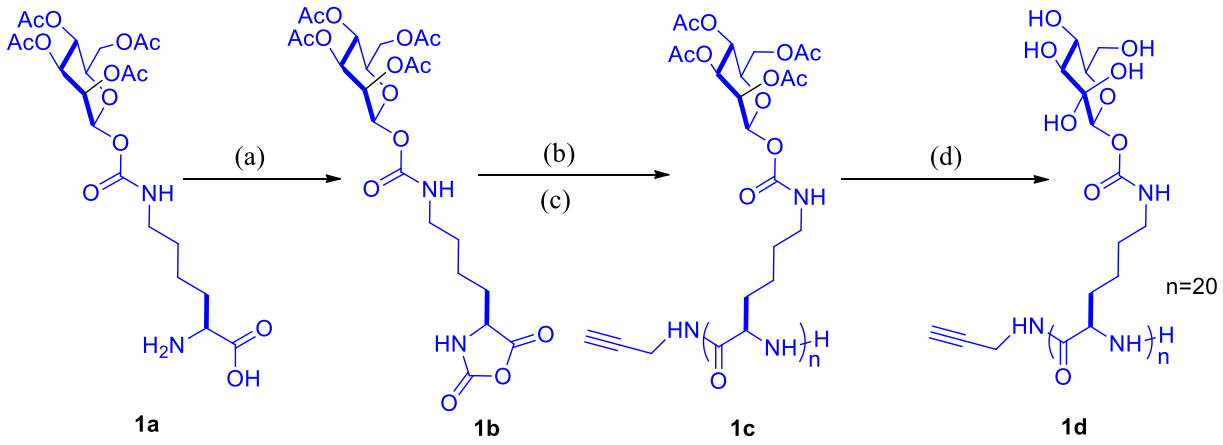
Supplementary Text

General Experimental Techniques and Apparatus:

Triphosgene. proton sponge (N,N´-tetramethylnapthalene) and peptide synthesis grade ultra-dry DMF were obtained from Aldrich. All other chemicals used were obtained from Merck, India. Diethyl ether, petroleum ether, ethyl acetate, dichloromethane and tetrahydrofuran were bought from Merck and dried by conventional methods and stored in the glove box. Ethyl acetate and dichloromethane were dried with P_2O_5 and CaH_2 and stored on activated molecular sieves 4Å after distillation. Tetrahydrofuran was passed through activated alumina and then dried on sodium wire and freshly distilled before use. n-Hexane was dried with sodium wire, distilled and stored on activated molecular sieves 4Å for use. All the other solvent drying procedures were followed from Purification of Laboratory Chemicals, 4th Edition by D.D. Perrin and W.L.F. Armarego.

(A) Synthesis of hydrophilic block (Pr-GP$_{20}$):

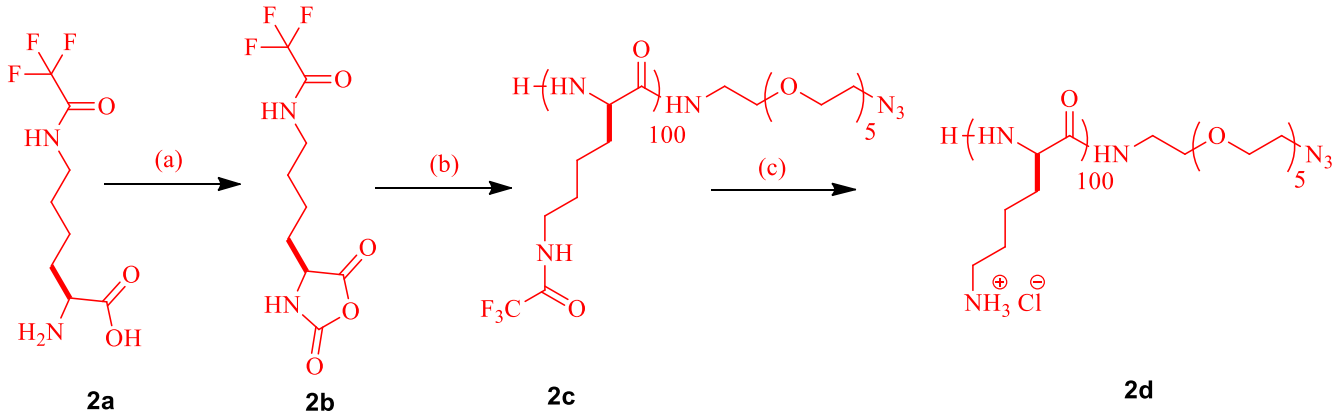


Scheme S1. (a) Triphosgene, α-pinene, dry THF,60^{o}C (b) Propargylamine, Dioxane (c) Proton Sponge (1.0 eq), 24 hrs, RT (d) THF, Hydrazine hydrate (25 equiv), 6 hrs.

(B) *General procedure for the synthesis of N-carboxyanhydride monomers:*

(a) Synthesis of N$^{\varepsilon}$-Trifluoroacetyl-*L*-lysine-*N-carboxyanhydride* ***(2b):*** To a solution of N$^{\varepsilon}$-trifluoroacetyl-*L*-lysine amino acid (500 mg, 1.9 mmol) in freshly distilled out tetrahydrofuran (10 ml) was added a solution of triphosgene (282 mg, 0.95 mmol) in anhydrous tetrahydrofuran (4 ml) under argon and the reaction mixture was heated to 50˚-55˚C. Subsequently α-pinene (0.4 ml,

2.85mmol) was added and the reaction mixture was allowed to stir for an additional 2 h. (Scheme S2). The reaction mixture was then cooled to room temperature and was then poured into dry hexane (400 ml) to afford a white precipitate. The white precipitate of NCA was filtered off by vacuum quickly and crystallized two more times by using ethyl acetate/petroleum ether mixtures. Finally the precipitate of N^{ε}-Trifluoroacetyl-*L*-lysine-N-carboxyanhydride was dried under vacuum and transferred into glove box. Final yield is 450 mg (90%).



Scheme S2. (a) Triphosgene, α- pinene, dry THF, 60°C (b) N_3-PEG-NH_2, DMF (c) THF, KOH (1.5eq)

N^{ε}-triflouroacetyl-*L*-lysine-N-carboxyanhydride (2b): ^{1}H NMR (400 MHz, Acetone-d_6): δ [ppm]: 1.48-1.68(m, 4H), 1.86-1.98 (m, 2H), 3.35-3.39 (dd, 2H), 4.54-4.57 (t, 1H), 7.96 (s, amide 1H), 8.49 (s, amide 1H); ^{13}C NMR (400 MHz, Acetone-d_6): δ [ppm]: 23.08, 32.25, 40.42, 58.59, 116.39, 118.67, 153.15, 157.84, 158.13, 172.27. FT-IR (cm^{-1}): 1858, 1785, 1775, 1265.

*(b)Synthesis of γ-Benzyl-L-glutamate- N-carboxyanhydride (**3a**):*

To a solution of γ-Benzyl-*L*-glutamate amino acid (500 mg, 2.10 mmol) in freshly distilled out tetrahydrofuran (10 ml) was added a solution of triphosgene (312.5mg, 1.05 mmol) in anhydrous tetrahydrofuran (4 ml) under argon and the reaction mixture was heated to 50˚-55˚C. Then α-pinene (0.5 ml, 3.16 mmol) was added and the reaction mixture was allowed to stir for 2 h. (Scheme 2). The reaction mixture was then cooled to room temperature and there after poured into dry hexane

(400 ml) to afford a white precipitate. The white precipitate of N-carboxyanhydrides was filtered off by vacuum quickly and crystallized two more times by using ethyl acetate/petroleum ether mixtures. Finally the precipitate of γ-Benzyl-L-glutamate- N-carboxyanhydride was dried under vacuum and transferred into glove box. Final yield is 425mg (85%).

γ-Benzyl-*L*-glutamate- N-carboxyanhydride (**3a**)**:** ^{1}H NMR (400 MHz, $CDCl_3$): δ[ppm]: 2.33-2.58(m, 2H), 2.85(t, 2H), 4.66(t, 1H), 5.40(s, 2H), 7.25(bs, 1H), 7.61-7.65(m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$): δ [ppm]: 26.7, 29.5, 56.7, 67.0, 128.2-128.7, 135.2, 152.2, 169.9, 172.3. FT-IR (cm^{-1}): 2890, 1858, 1785, 1775, 1495, 1250, 1056.

Circular Dichroism Measurements: Solutions of polymers were filtered through 0.22 μm syringe filters. Circular dichroism (CD) spectra (190−250 nm) of the block catiomer and aniomer (0.25 mg/mL in phosphate buffer: pH 7.4) were recorded (Jasco CD spectropolarimeter, J-815) in a cuvette with a 1 mm path length. All of the spectra were recorded for an average of three scans, and the spectra were reported as a function of milli degree θ versus wavelength.

Sample preparation for TEM, AFM and SEM

The 0.1 wt% solution of the PICsomes was spotted on carbon coated 400 mesh copper grid about 10 μL, kept for 15-20 min, the excess solvent was removed by touching the edge of the grid with Whatman filter paper, then grid was negatively stained by 0.2 wt% uranyl acetate for 10 sec, and excess solvent was removed. Grid was washed twice with deionized water to remove excess unbound uranyl acetate from the grid. Grids were dried in desiccators for 20h and analysed by transmission electron microscopy. AFM image of the polymersomes drop cast from aqueous solution (0.02 wt%) on silicon wafer and dried for 24 h. The sample for SEM was prepared by placing a small drop of the aqueous sample on silicon wafer and dried it for 24 h. Before imaging, the sample was gold coated for 30s and observed under SEM.

Supplementary Figures, Table and Movies:

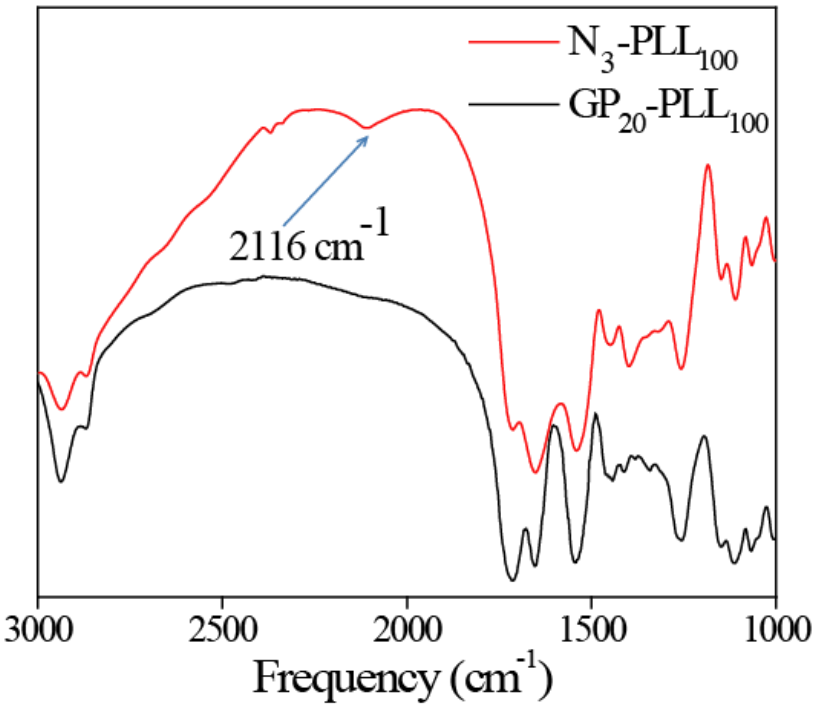


Figure S1. FTIR spectra of the synthesized block copolymer GP_{20}-PLL_{100} (catiomer) after "click" reaction (black). The red trace represents the FT-IR of N_3-PLL_{100}. The Azide peak at 2100 cm^{-1}, observed in N_3-PLL_{100}, is absent in GP_{20}-PLL_{100} indicating that the click reaction has proceeded to completion.

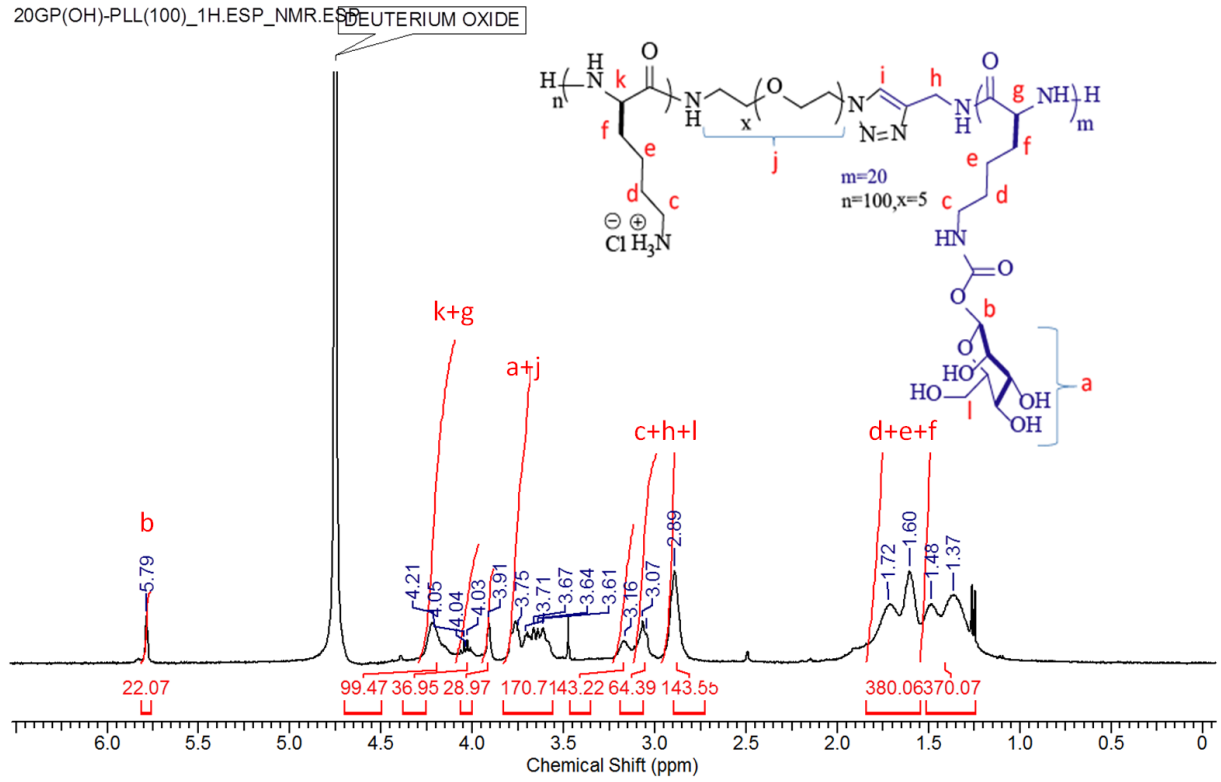


Figure S2. ^{1}HNMR spectrum of the synthesized GP_{20}-PLL_{100} (**4**) blocks copolymer (catiomer). The exact composition of the GP_{20}-*b*-poly(*L*-lysine)$_{100}$ was determined by comparing the relative intensity of resonance characteristic of anomeric protons in GP part at 5.7 ppm with the lysine methylenic proton in both GP and PLL part at 1-2 ppm and it was found to present in a equimolar ratio.

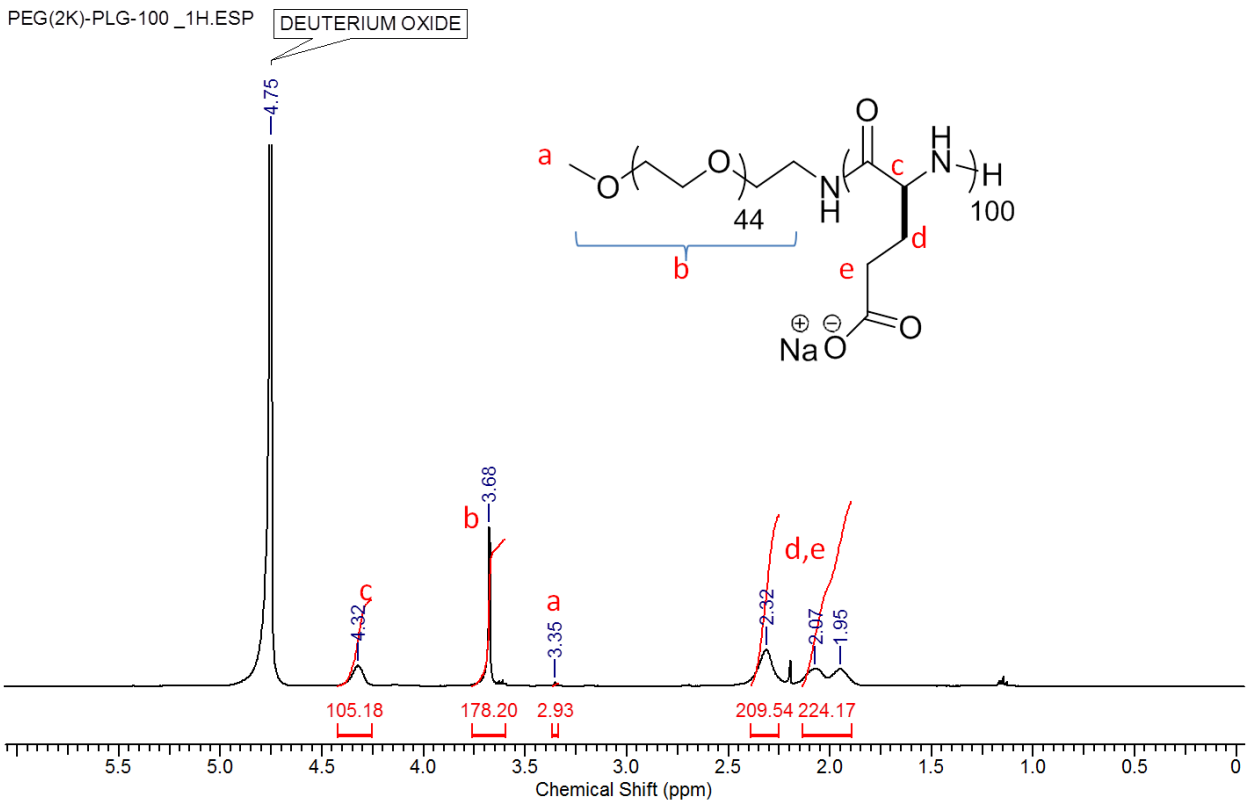


Figure S3. ^{1}HNMR spectrum of the synthesized PEG_{2k}-PLG_{100} **(3c)** block copolymer (aniomer)

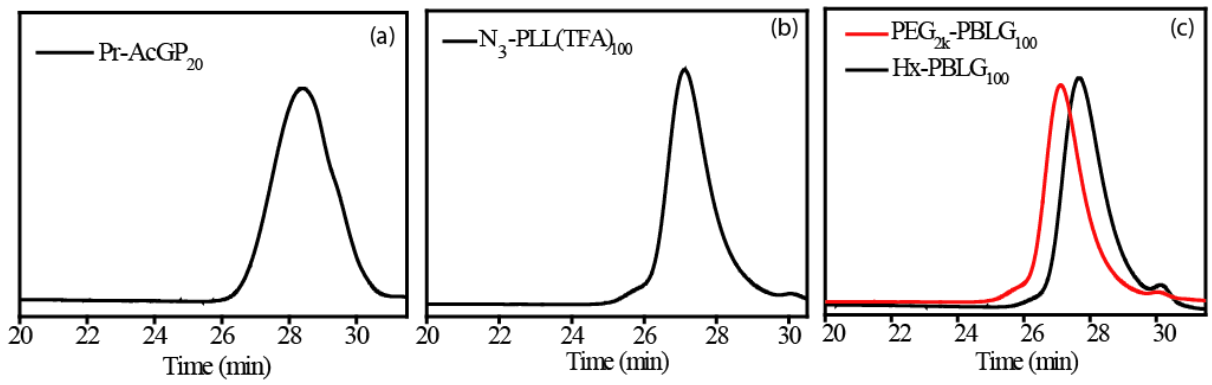


Figure S4. Size exclusion chromatogram of (a) Pr-$AcGP_{20}$ (b) N_3-$PLL(TFA)_{100}$ (c)PEG_{2k}-$PBLG_{100}$ and Hexyl-$PBLG_{100}$. (0.05 M LiBr in DMF as the eluent at 60 ˚C. GPC/LS samples were prepared at concentrations of 5 mg/ml. A constant flow rate of 1 mL/min was maintained)

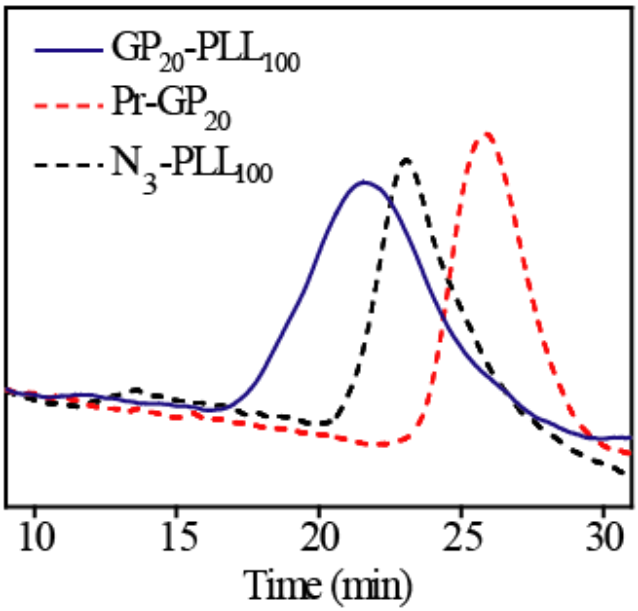


Figure S5. Aqueous GPC curves of the GP_{20}-PLL_{100} from reaction mixture overlaid with curves of parent polymers. (eluent:10 mM PBS buffer; pH 7.4, 100 mM NaCl; room temperature). GPC samples were prepared at concentrations of 2.5 mg/mL. A constant flow rate of 0.5mL/min was maintained.)

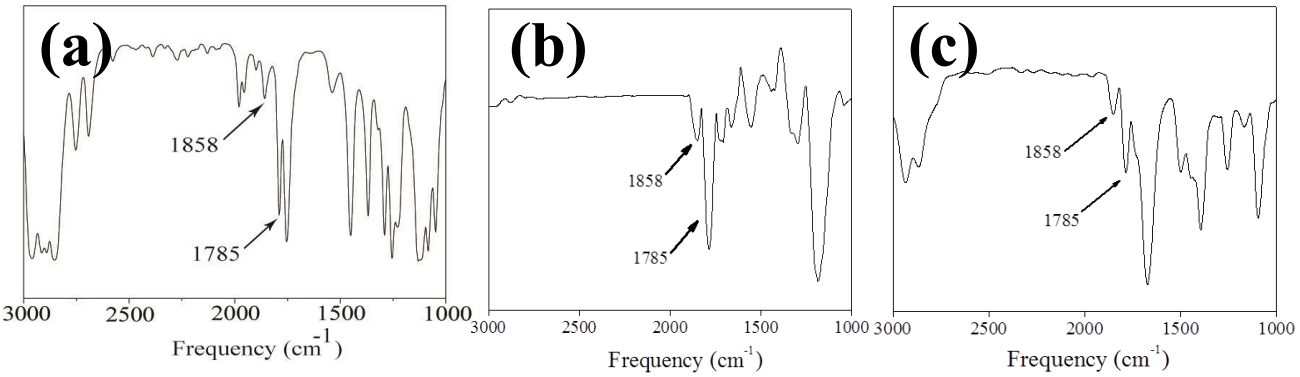


Figure S6. FTIR Spectra of (a) per-*O*-acetylated-D-glucose-*L*-lysine NCA (b) N-ε-Trifluoroacetyl-*L*-lysine NCA (c) γ-Benzyl-*L*-glutamate NCA: two unsymmetrical anhydride stretching of NCA ring at 1858 and 1785 cm^{-1} are characteristics of NCA formation.

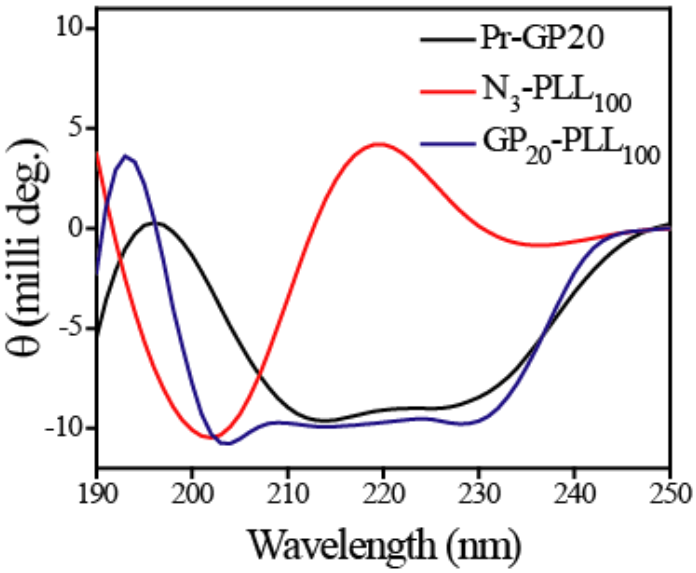


Figure S7. Circular dichroism spectra of parent polymers Pr-GP_{20}, N_3-PLL_{100} and block catiomer GP_{20}-PLL_{100} in 10 mM Phosphate buffer (pH 7.4)

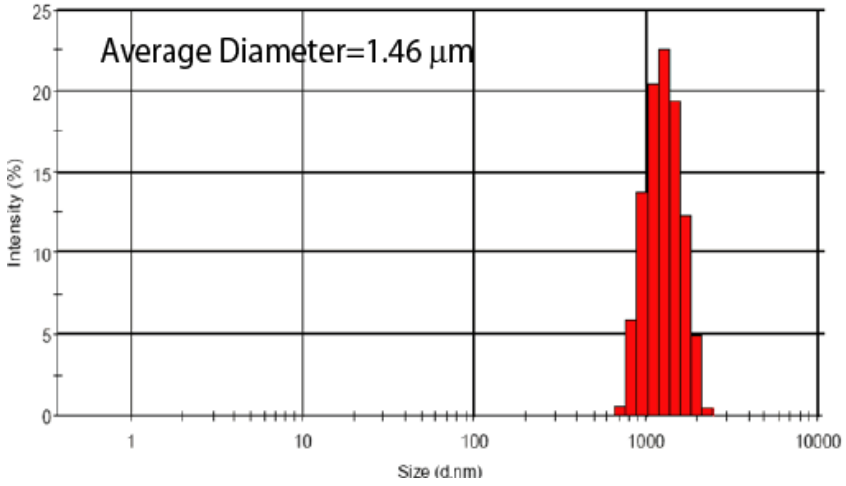


Figure S8: DLS measurement of GP-PICsomes formed by the self assembly of block aniomer (PEG_{2k}-PLG_{100}) and block catiomer (GP_{20}-PLL_{100}) in 50 mM phosphate buffer (pH 7.4).

Average diameter of GP-PICsomes (μm) with respect to time (days)						
NaCl Concentration	**I**	**II**	**III**	**IV**	**V**	**VI**
Without NaCl	1.5	1.46	1.43	1.43	1.40	1.36
75 mM	1.39	1.32	1.09	0.8	0.74	0.68
150 mM	0.56	0.34	-	-	-	-

Table S1: Changes in the size of GP-PICsomes with increasing salt concentration.

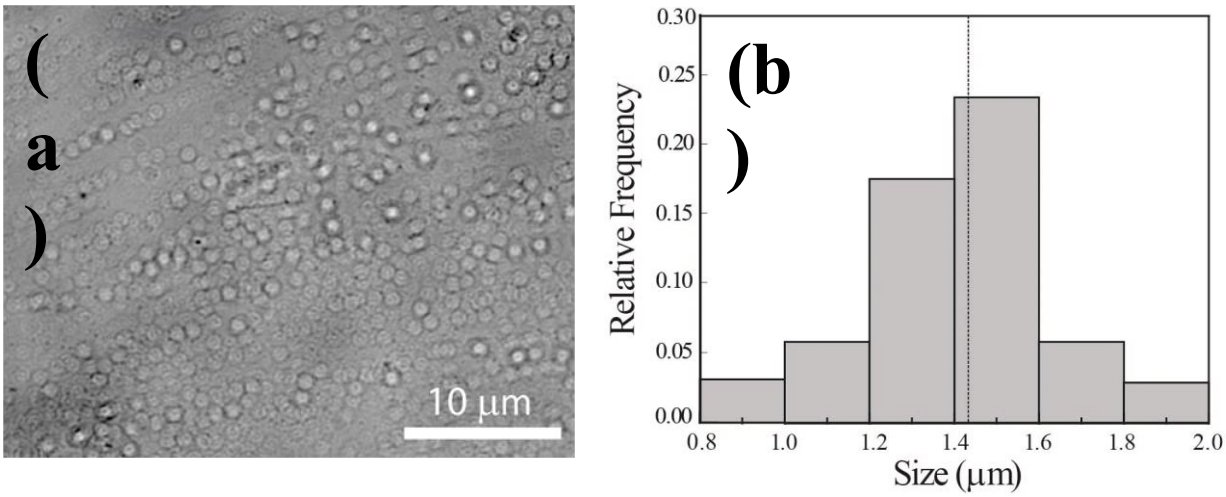


Figure S9: Optical microscopic Image and corresponding size distribution figure of GP-PICsomes (Average Diameter=1.43μm).

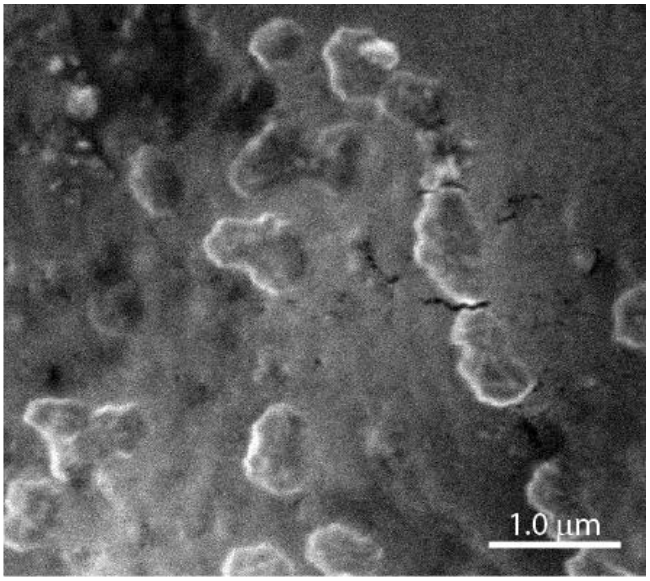


Figure S10. SEM image of aggregates formed by the self-assembly of homoaniomer (Hx-PLG_{100}) and block catiomer (GP_{20}-PLL_{100}) in 50 mM phosphate buffer (pH 7.4).

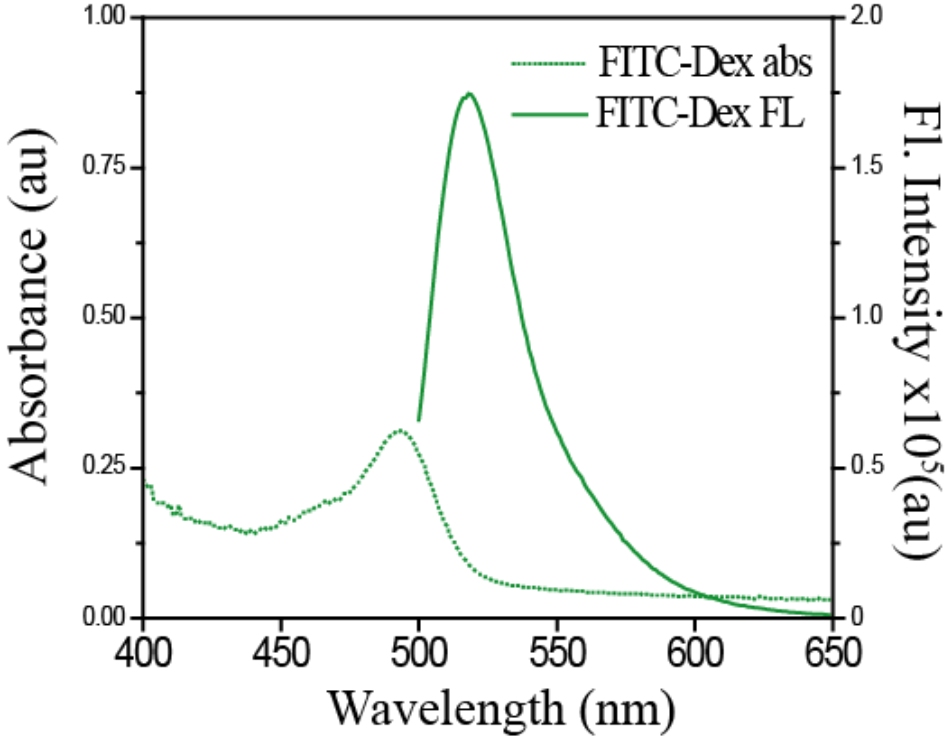


Figure S11. Absorption and emission spectra of FITC dextran loaded GP-PICsomes.

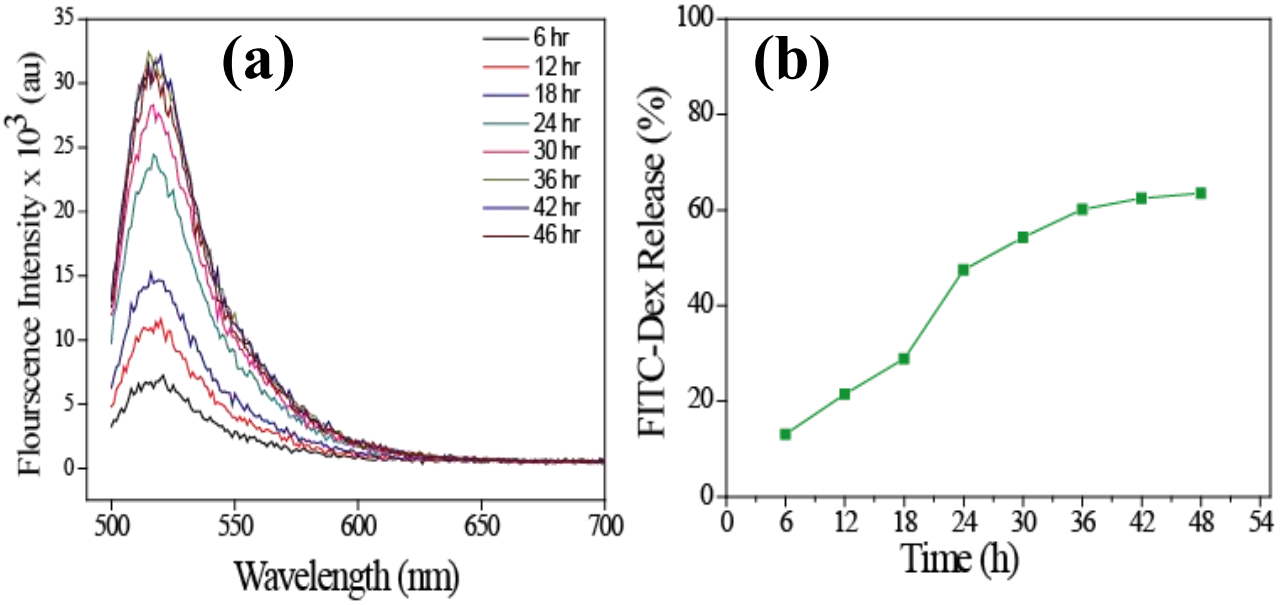


Figure S12. (a) Emission spectrum of FITC-Dextran in PBS released from the dialysis tube over a time period of 48 h (b) Plot of % of FITC-Dextran as a function of time.

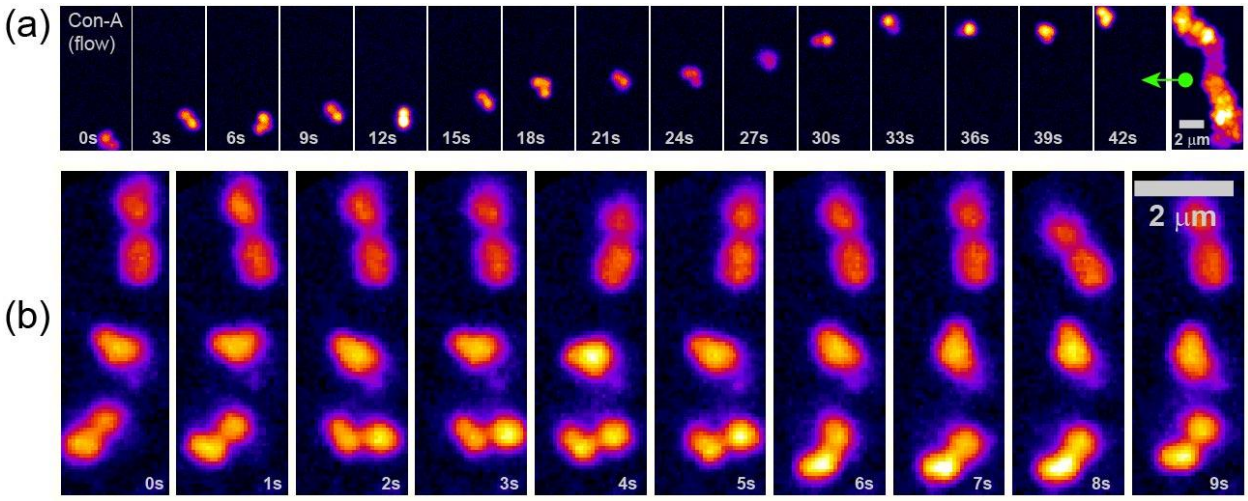


Figure S13. Fluorescence microscopy of GP-PICsomes (a) Dynamics of a small RhB-GP-PICsome aggregate in the presence of buffer containing 0.5mg/ml Con-A (weak unidirectional flow) represented by fifteen single-frame sequential snapshots along with its Maximum Projection image (right panel) of the same area. (b) Hindered motion of surface bound (partially anchored) RhB-GP-PICsome aggregates over a time span of 9s.

Captions for the Supplementary Movies:

Supplementary Movie 1: Wide field movie of a single RhB-GP-PICsome diffusing randomly in buffer solution. The movie is played at 10 fps although it was acquired at ~3.3 fps and the area shown is identical to that in Figure 5a (top panel).

Supplementary Movie 2: Random motion of another single Rh labelled GP-PICsome in buffer solution. The movie is played at 10 fps although it was acquired at 3.3 fps and the area shown is identical to that in Figure 5a (bottom panel).

Supplementary Movie 3: Movie of RhB-GP-PICsome in presence of Con-A, showing a small cluster undergoing tumbling motion in buffer solution. The movie is played at 10 fps although it was acquired at ~3.3 fps and the area shown is identical to that in Figure 5b.

Supplementary Movie 4: Small RhB-GP-PICsome aggregate in presence of unidirectional buffer flow. The movie is played at 10 fps although it was acquired at ~3.3 fps and the area shown is identical to that in Figure 5c.

Supplementary Movie 5: The dynamics of a larger RhB-GP-PICsome aggregate in the presence of a buffer flow. The movie is played at 10 fps although it was acquired at ~3.3 fps and the area shown is identical to that in Figure 5d.

Supplementary Movie 6: Movie of RhB-GP-PICsome aggregate-clusters while changing the Z focus during movie collection. The movie is played at 10 fps although it was acquired at ~6.6 fps and the area shown is a section of the time averaged image Figure 5f, from frame 30 to 50. The

movie shown here is a 17.5 micron X 17.5 micron area of the main movie with co-ordinates (24.50, 4.9). A small section was selected as the entire movie had a very large size.

Supplementary Movie 7: Deaggregation of a RhB GP-PICsome aggregate upon addition of excess mannose. The movie is played at 10 fps although it was acquired at ~3.3 fps and the area shown is identical to that in Figure 6a.

Supplementary Movie 8: Dynamics of a RhB-GP-PICsome aggregate-clusters incubated with excess galactose depicting its swivel motion by intermittent buffer flow. The movie is played at 10 fps although it was acquired at ~3.3 fps and the area shown is identical to that in Figure 6b (right panel).

Supporting information NMR spectra

(* peak in NMR indicates residual solvents peak)

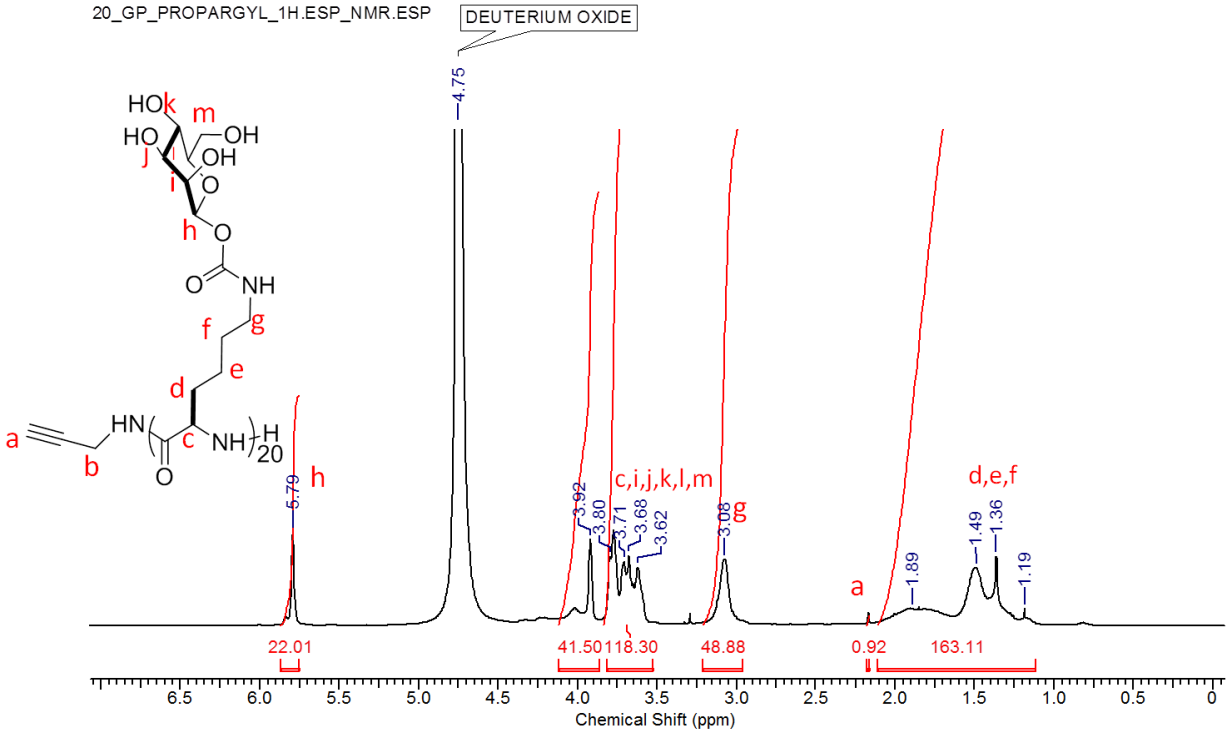


Figure S14. ^{1}H NMR ($CDCl_3$**,** 400 MHz) Spectrum of Pr-GP_{20} **(1d)**

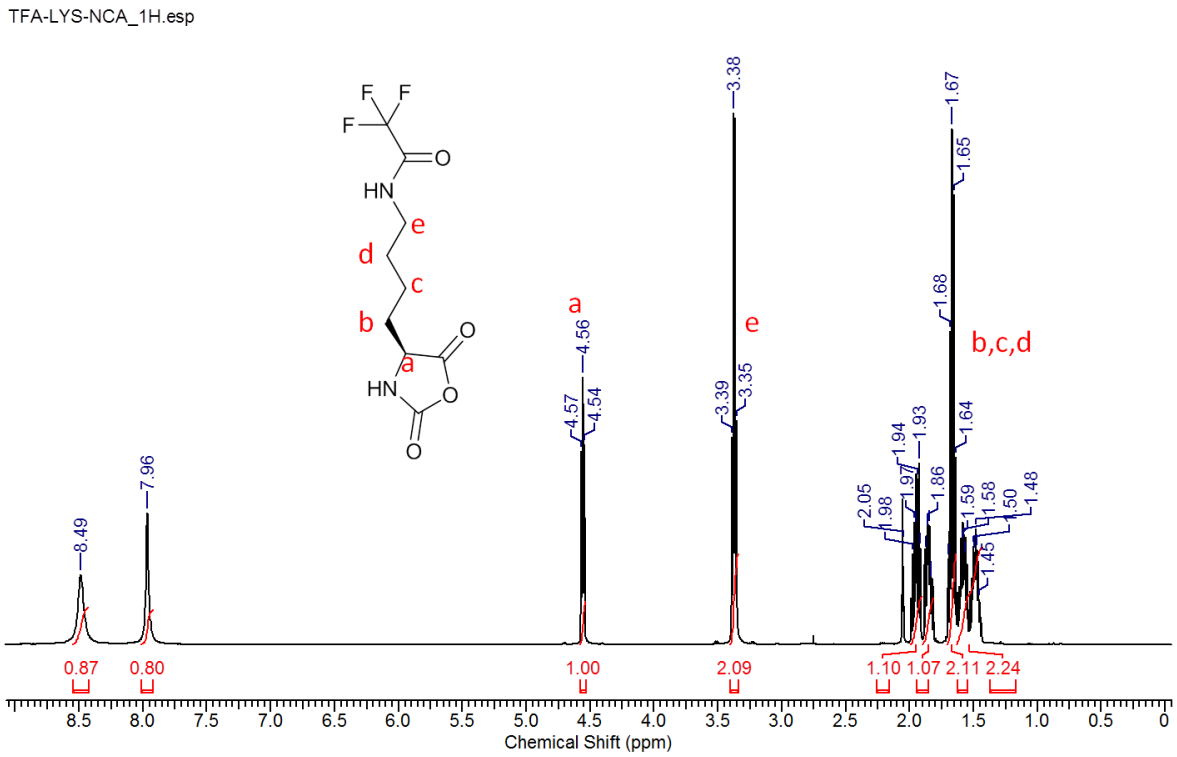


Figure S15a. ^{1}H NMR ($CDCl_3$**,** 400 MHz) Spectrum of TFA-Lys-NCA **(2b)**

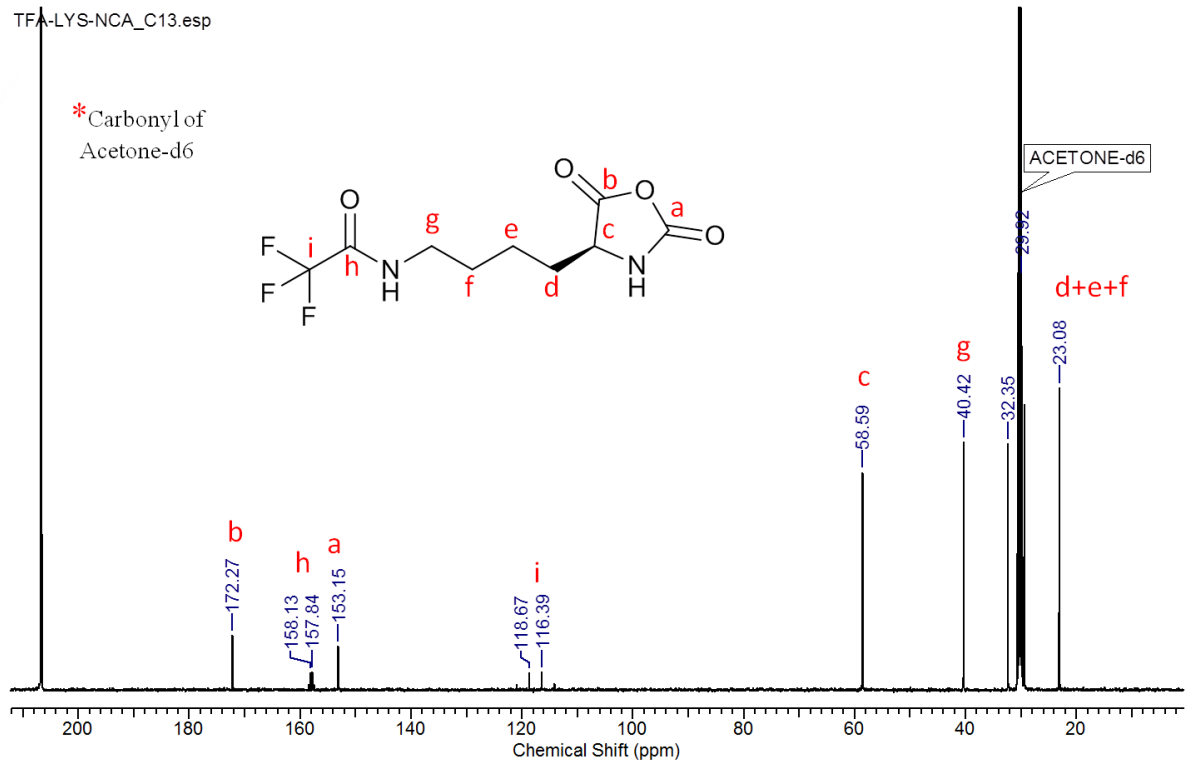


Figure S15b. C^{13}NMR ($CDCl_3$, 400 MHz) Spectrum of TFA-Lys-NCA **(2b)**

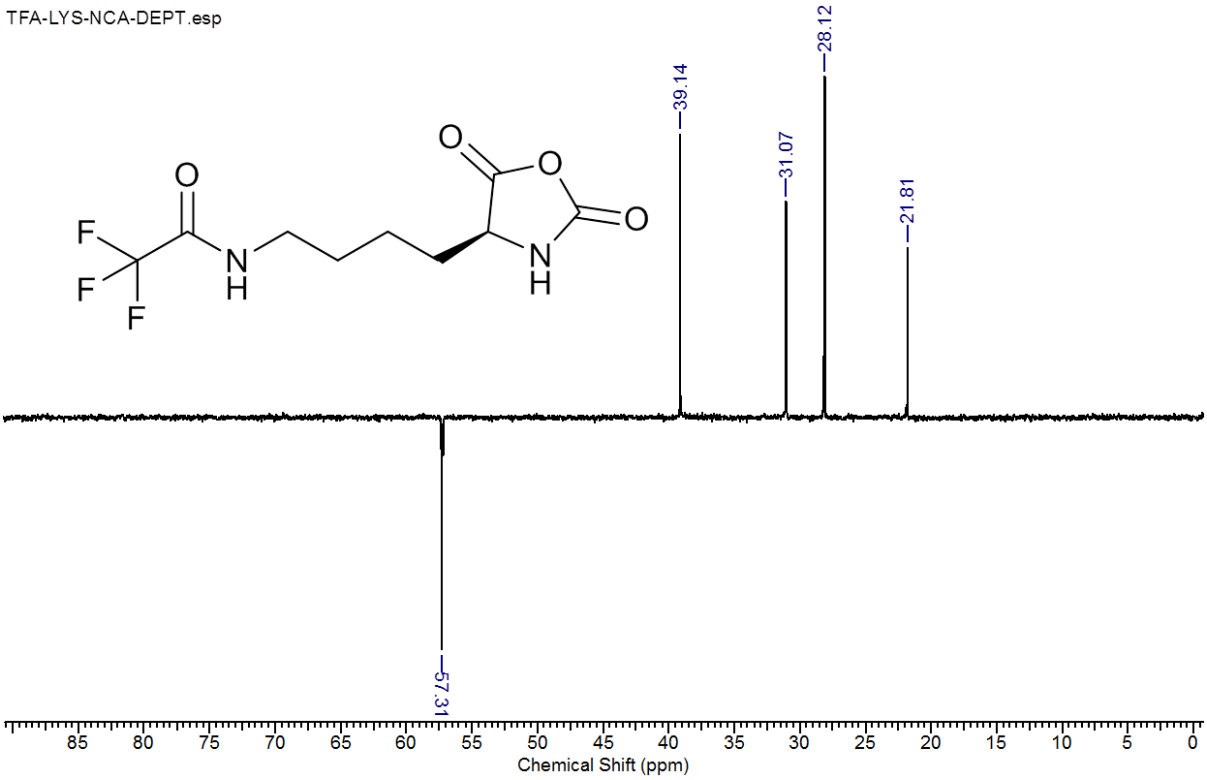


Figure S15c. DEPT NMR ($CDCl_3$, 400 MHz) TFA-Lys-NCA **(2b)**

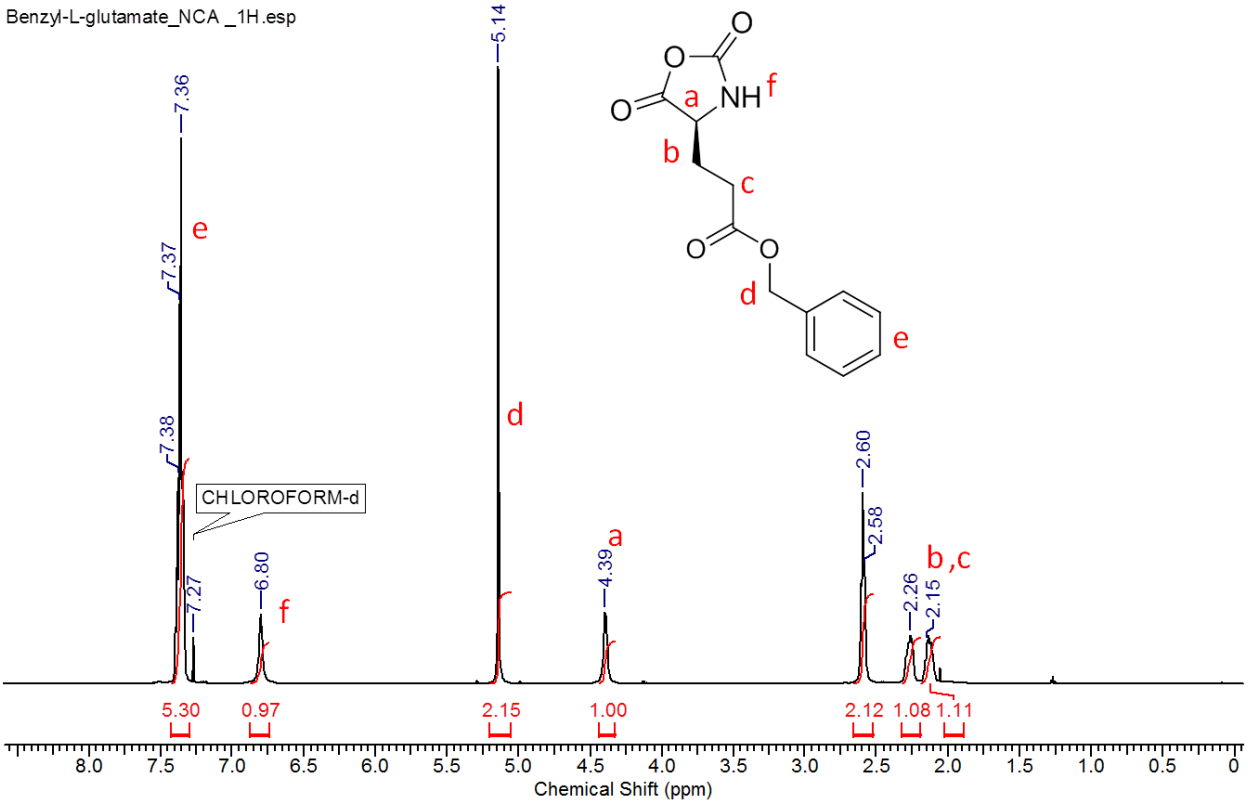


Figure S16a. ^{1}H NMR ($CDCl_3$, 400 MHz) Spectrum of Bn-Glu-NCA **(3a)**

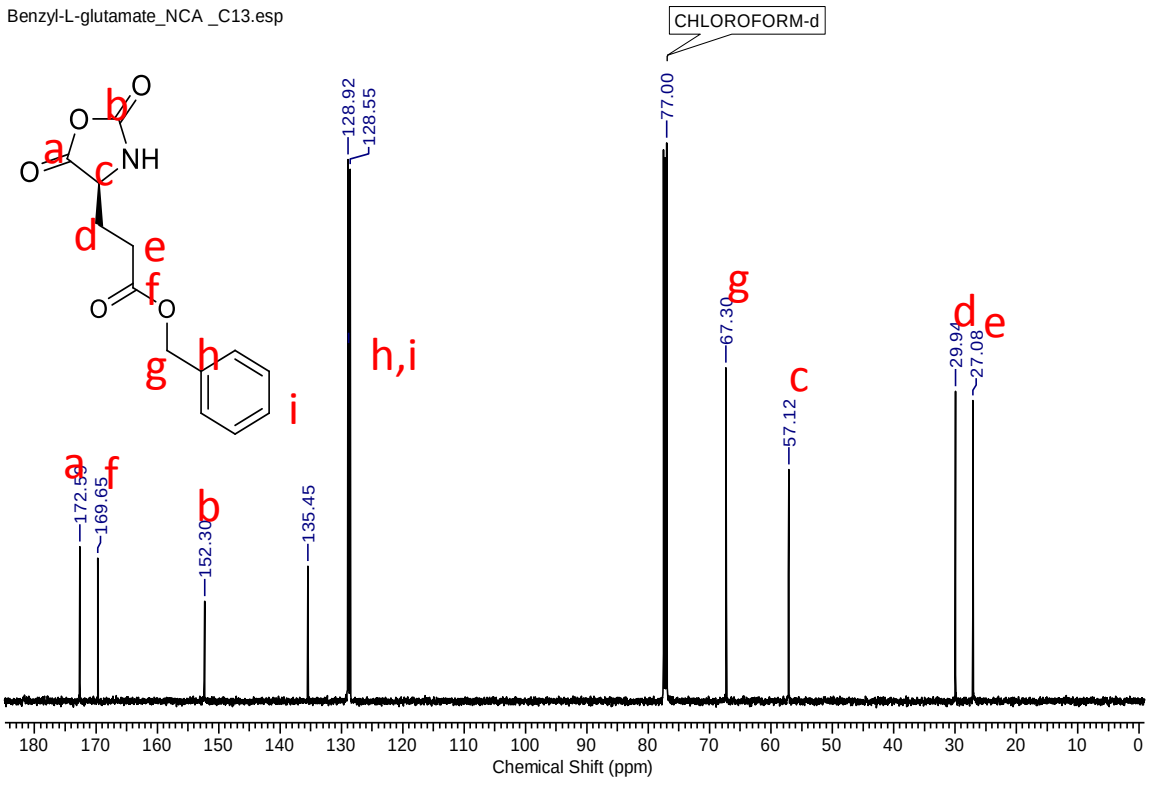


Figure S16b. C^{13}NMR ($CDCl_3$, 400 MHz) Spectrum of Bn-Glu-NCA **(3a)**

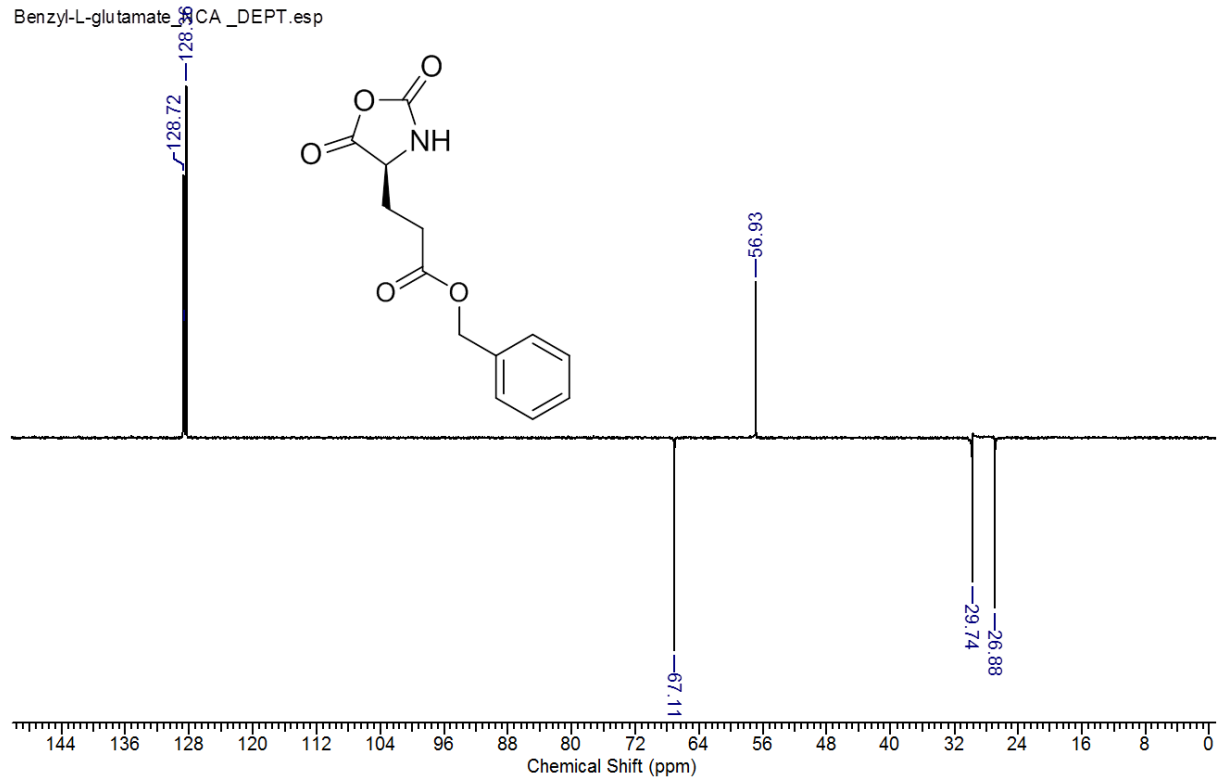


Figure S16c. DEPT NMR ($CDCl_3$**,** 400 MHz) Bn-Glu-NCA **(3a)**

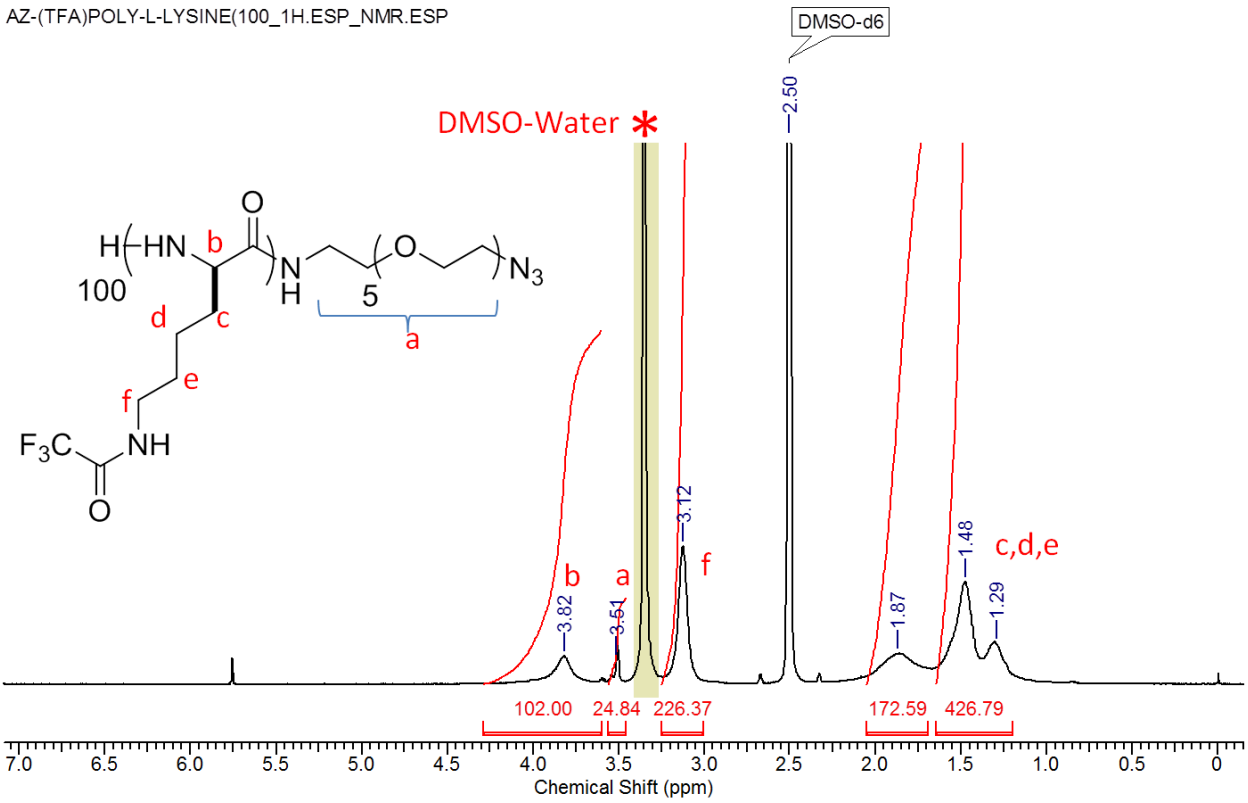


Figure S17.^{1}H NMR ($CDCl_3$**,** 400 MHz) Spectrum of N_3-PLL(TFA)$_{100}$ **(2c)**

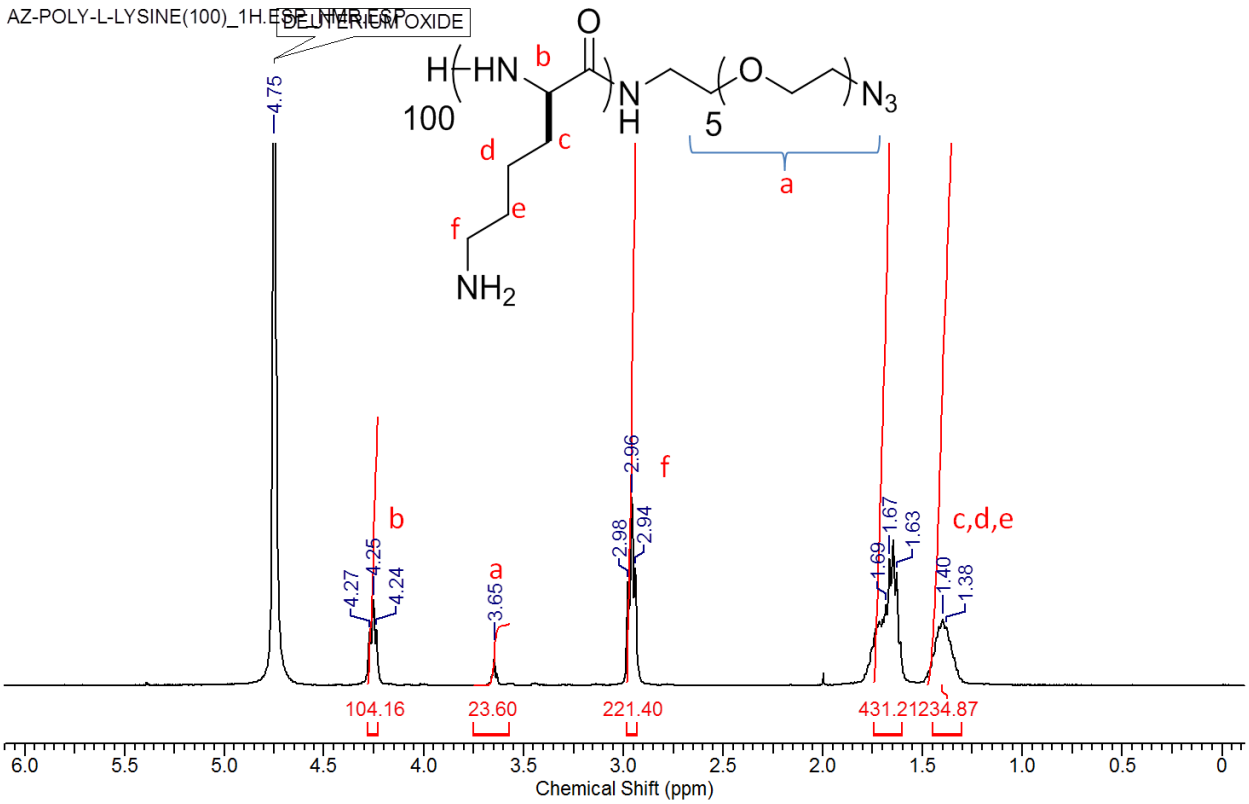


Figure S18. ^{1}H NMR ($CDCl_3$, 400 MHz) Spectrum of N_3-PLL_{100} **(2d)**

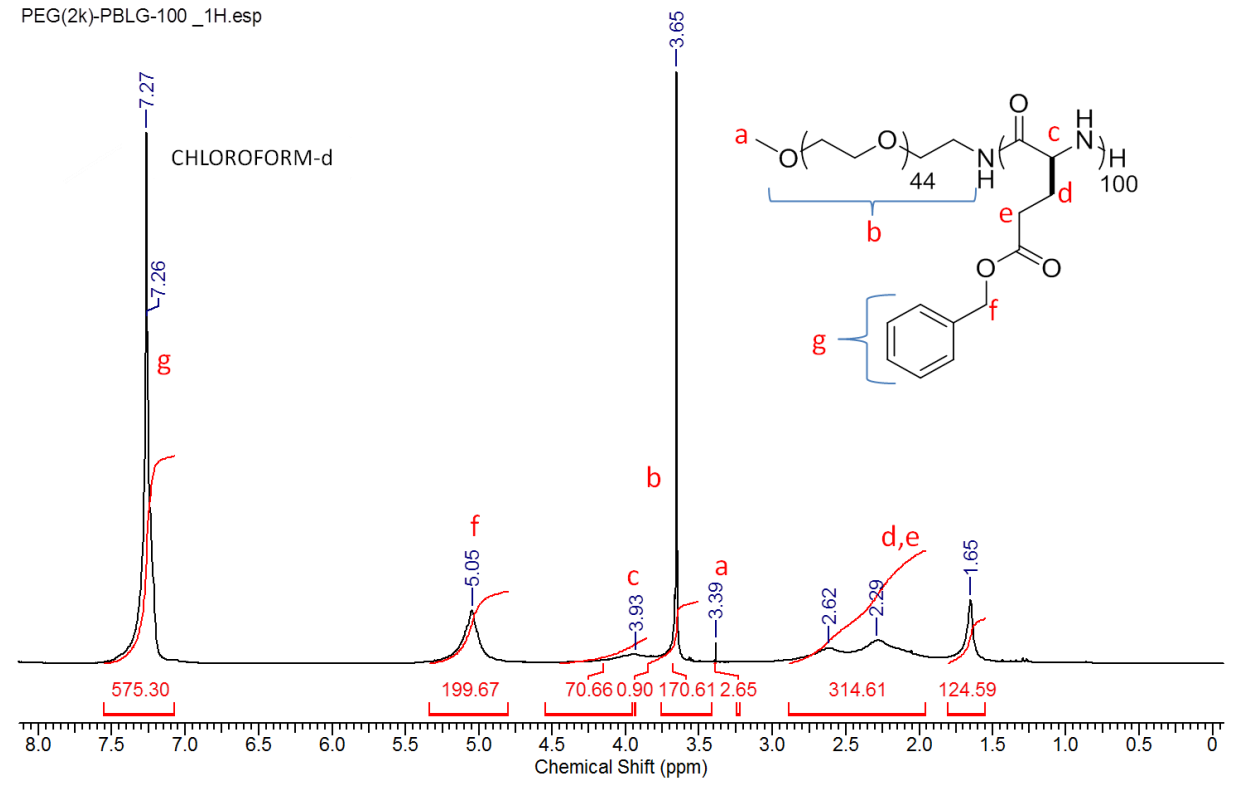


Figure S19. ^{1}H NMR ($CDCl_3$, 400 MHz) Spectrum of PEG_{2k}-$PBLG_{100}$ **(3b)**

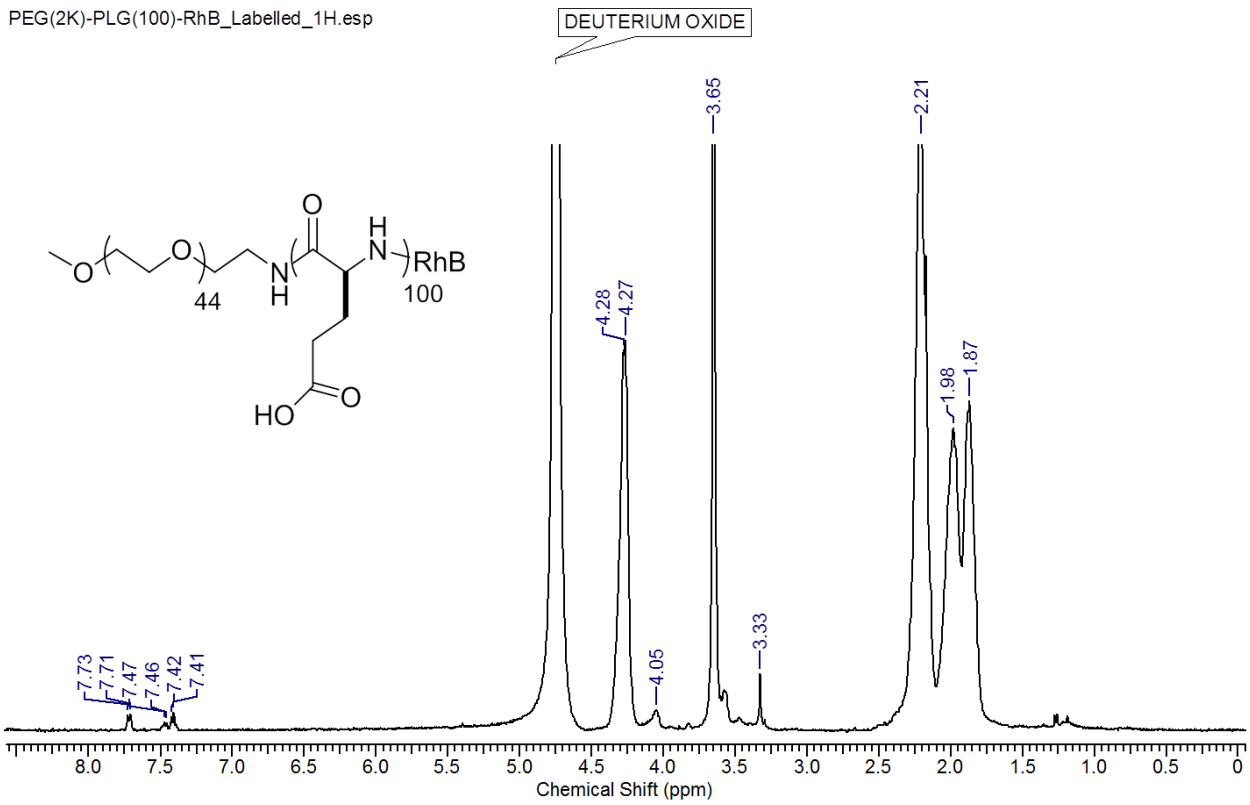


Figure S20. ^{1}H NMR ($CDCl_3$, 400 MHz) Spectrum of RhB-PEG_{2k}-$PBLG_{100}$ **(3d)**

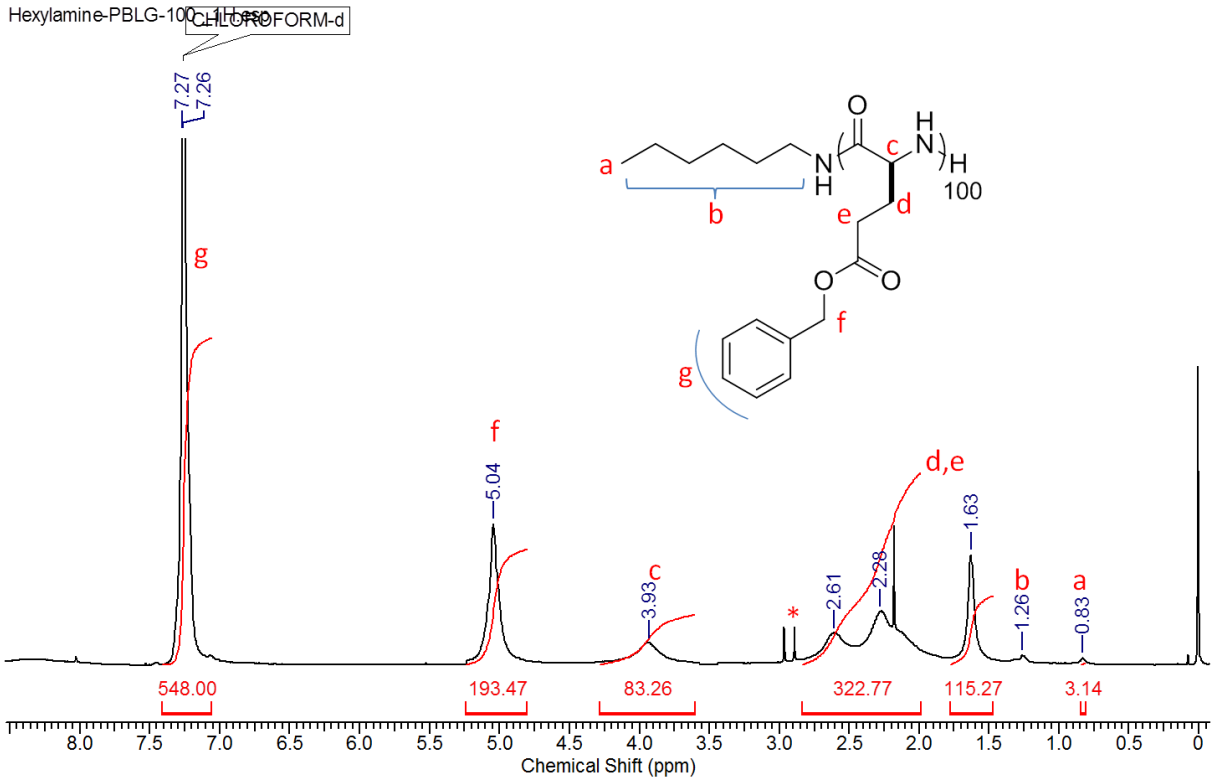


Figure S21. ^{1}H NMR ($CDCl_3$, 400 MHz) Spectrum of Hx-$PBLG_{100}$ **(5a)**

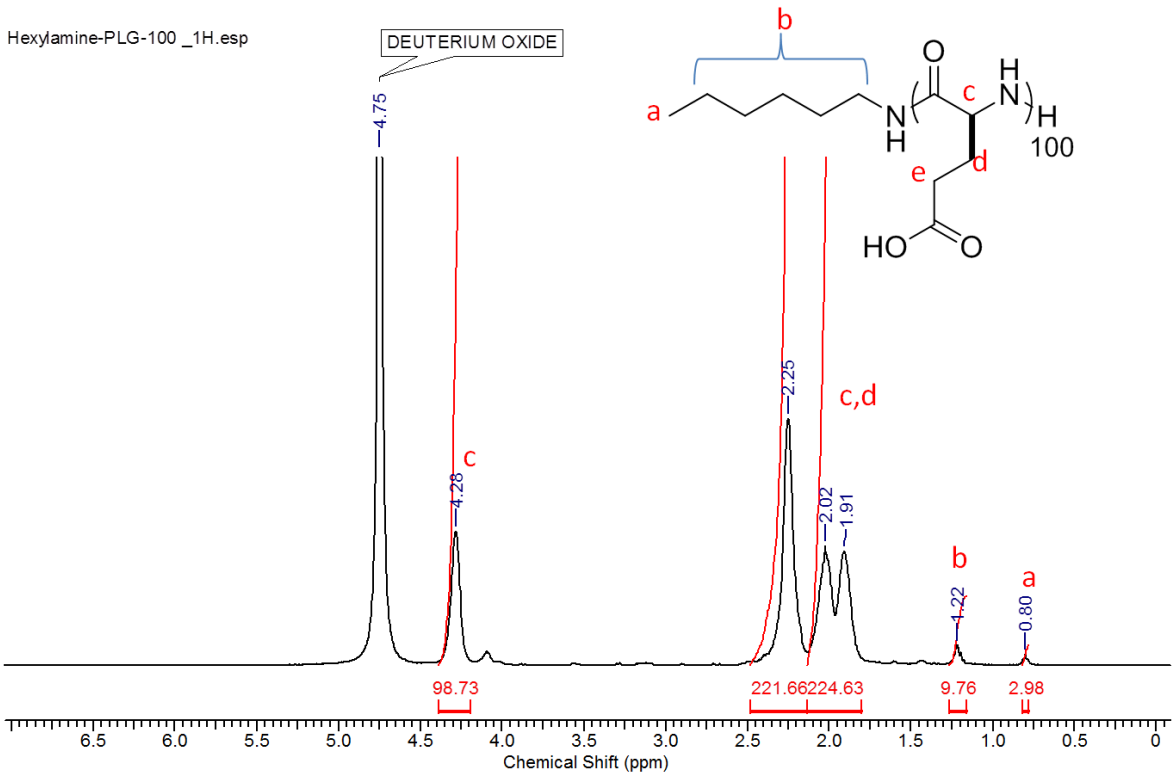


Figure S22. ^{1}H NMR ($CDCl_3$, 400 MHz) Spectrum of Hx-PLG_{100} **(5b)**