pH Induced Amphiphilicity-Reversing Schizophrenic Aggregation by Alternating Copolymers

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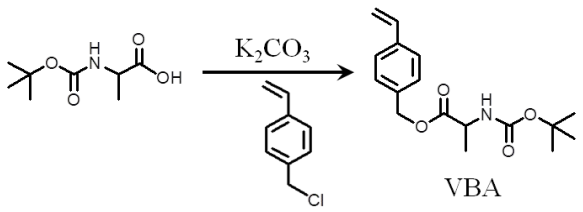
Synthesis of vinylbenzyl alanine (VBA). VBA was synthesized according to the literature report (Scheme S1).[1] Boc-L-alanine (3.78 g, 20 mmol) was dissolved in 100 mL DMF containing 1% water. 1.38 g of Na_2CO_3 was added into the solution under stirring. Afterwards, vinylbenzyl chloride (2.82 mL, 20 mmol) was added to the reaction mixture followed by stirring at 60 °C for 12 h. After removing the DMF by rotary evaporator the remaining residue was dissolved in ethyl acetate, washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The product, VBA was purified by column chromatography using 5% ethyl acetate in hexanes to a get a pale yellow viscous liquid (yield ~

80%). The ^{1}H NMR spectrum in Figure S1 confirmed the formation of VBA. ESI-MS (Figure S2): observed *m*/*z* for $[M + Na]^+$ = 327.50, calculated *m*/*z* for $[M + Na]^+$ = 328.15.



Scheme S1. Synthesis of VBA.

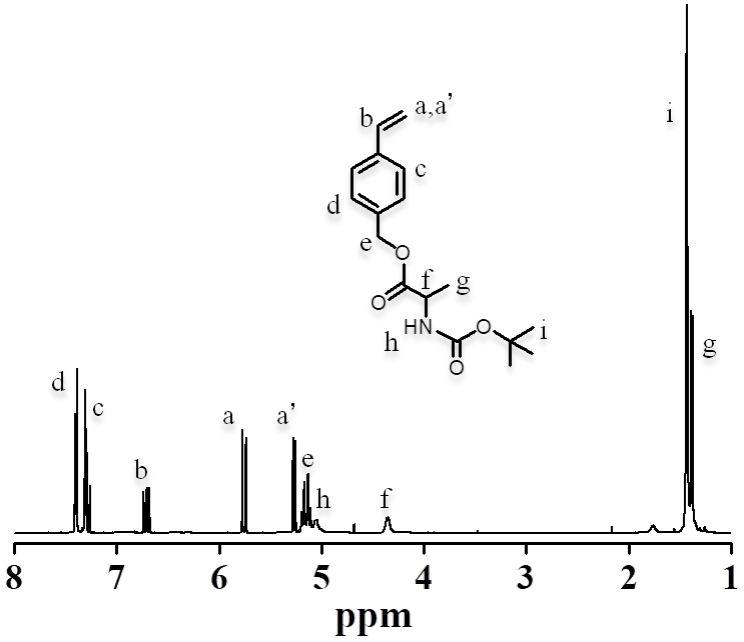


Figure S1. ^{1}H NMR spectrum of VBA in $CDCl_3$.

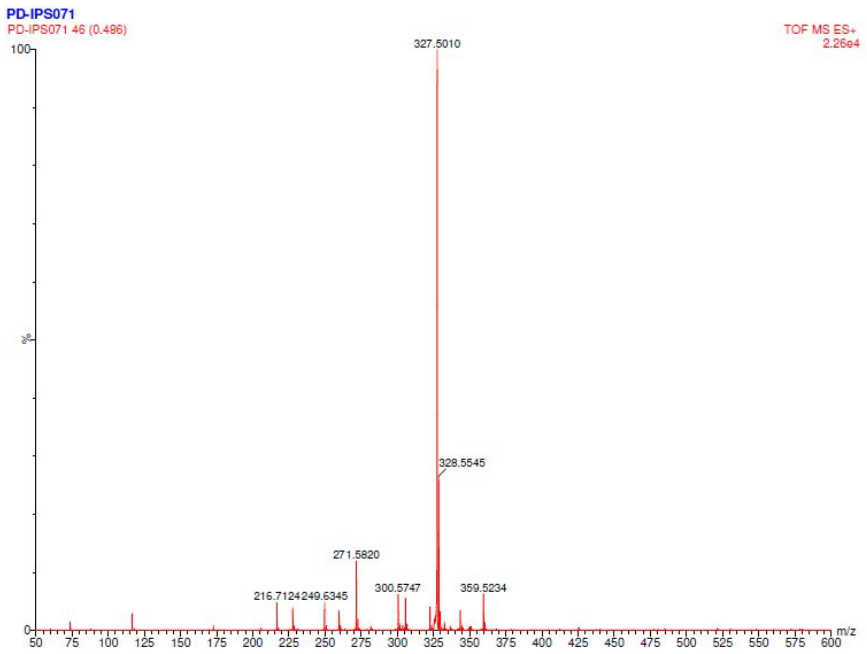
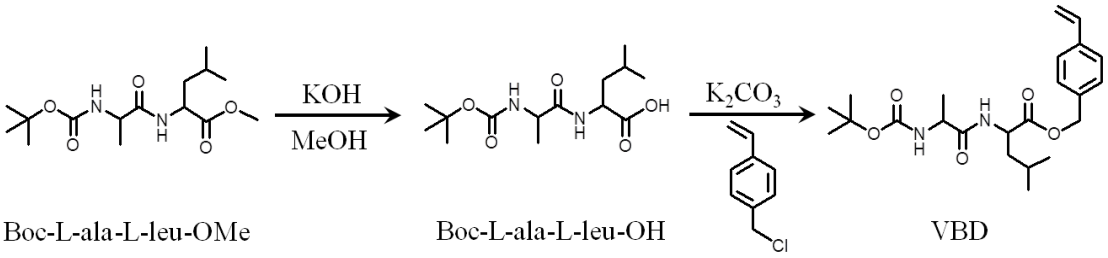


Figure S2. ESI-MS spectrum of VBA; observed *m*/*z* for $[M + Na]^+$ = 327.50, calculated *m*/*z* for $[M + Na]^+$ = 328.15.

Synthesis of vinylbenzyl dipeptide (VBD). At first, the dipeptide Boc-L-ala-L-leu-OMe was synthesized according to the conventional coupling reaction. L-Leucine methyl ester hydrochloride (3.0 g, 16.5 mmol) dissolve in 50 mL DMF was added into the mixture of Boc-L-alanine (2.84 g, 15 mmol) and triethylamine (3.13 mL, 22.5 mmol). The mixture was cooled at 0 °C for 1 h and then 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC•HCl, 2.88 g, 15 mmol) was added followed by the addition of 1-hydroxybenzotriazole (HOBt, 2.0 g, 15 mmol). The reaction was stirred continuously for 12 h at room temperature. After the evaporation of DMF, the reaction mixture was dissolved in ethyl acetate, washed successively with 1(N) HCl, saturated aqueous $NaHCO_3$ and brine solution. Then, it was dried over anhydrous Na_2SO_4, concentrated in vacuum and finally the product was purified by column chromatography using hexane:ethyl acetate (10:1) as eluent. The deprotection of ester

functionality was carried out by 4% KOH in methanol. After evaporation of methanol, aqueous $KHSO_4$ was added drop by drop until the solution reached pH ~ 5. Thereafter the product, Boc-L-ala-L-leu-OH was isolated by DCM through a separating funnel. Yield: 84%. ^{1}H NMR spectrum (Figure S3) shows the disappearance of peak at 3.7 ppm for the -CH_3 protons of ester group in the dipeptide.



Scheme S2. Synthesis route of VBD.

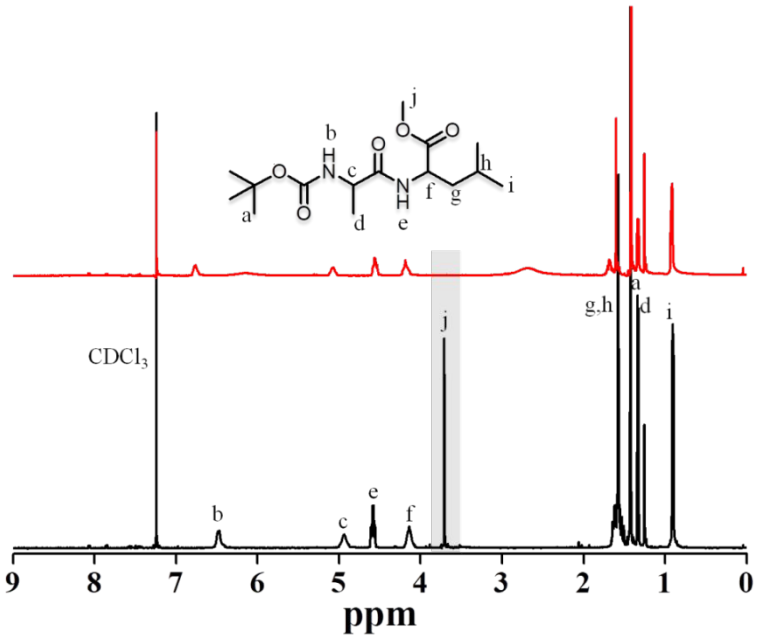


Figure S3. ^{1}H NMR spectra of Boc-L-ala-L-leu-OMe (black) and Boc-L-ala-L-leu-OH (red) in $CDCl_3$.

Next, the deprotected dipeptide Boc-L-ala-L-leu-OH (2.7 g, 8.92 mmol) was dissolved in 100 mL DMF. 1% water was added followed by the addition of 0.5 g of Na_2CO_3. Thereafter, vinylbenzyl chloride (1.26 mL, 8.92 mmol) was added to the reaction mixture. The reaction mixture was allowed for stirring at 60 °C for 1 day. After removing the DMF by rotary evaporator the remaining crude was dissolved in ethyl acetate, washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The product, VBD was purified by column chromatography using hexane:ethyl acetate (10:1) as the eluent. Yield: 78%. The entire synthetic route for the preparation of VBD is outlined in Scheme S2. The ^{1}H NMR spectrum in Figure S4 confirmed the formation of VBD. ESI-MS (Figure S5): observed *m*/*z* for $[M + Na]^+$ = 440.43, calculated *m*/*z* for $[M + Na]^+$ = 441.24 *m*/*z*.

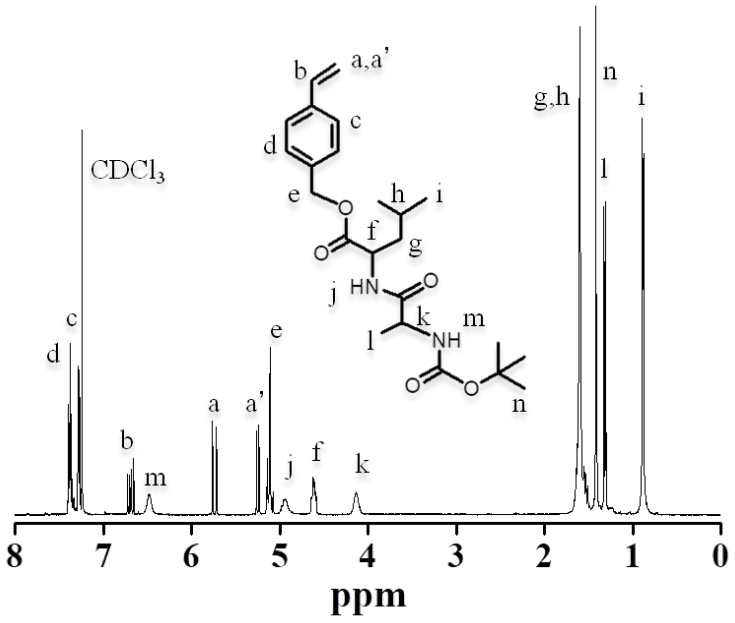


Figure S4. ^{1}H NMR spectrum of VBD in $CDCl_3$.

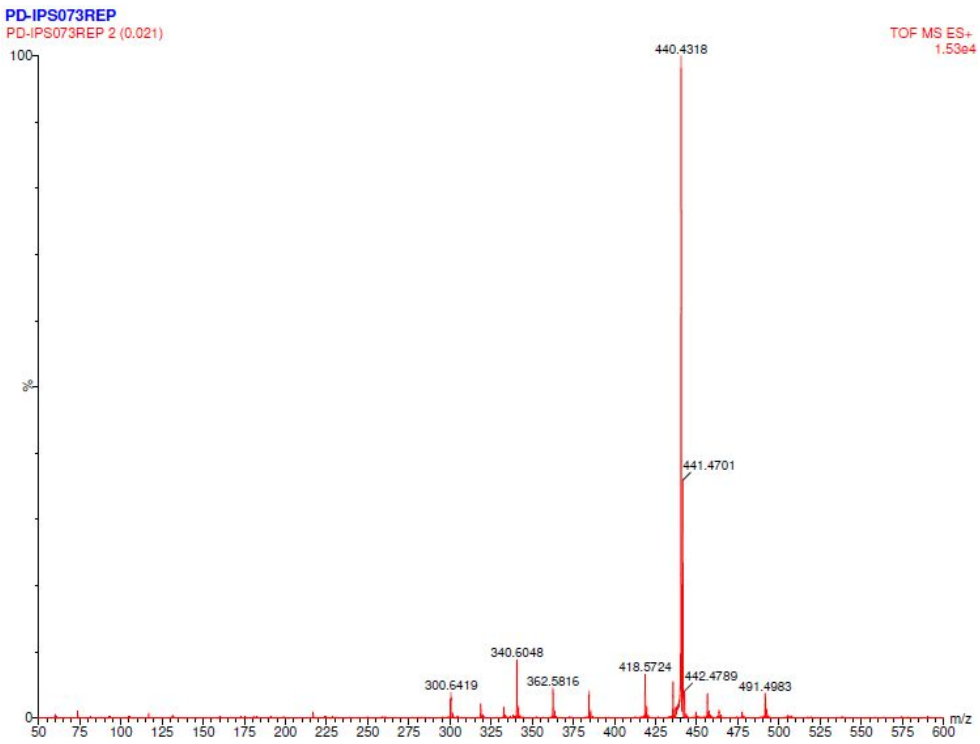
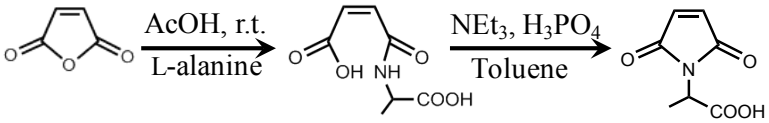


Figure S5. ESI-MS spectrum of VBD; observed *m*/*z* for $[M + Na]^+$ = 440.43, calculated *m*/*z* for $[M + Na]^+$ = 441.24 *m*/*z*.

Synthesis of *N*-maleoyl-L-alanine (NMA). *N*-maleoyl-L-alanine was synthesized by following earlier report (Scheme S3).[2] In a 250 mL double neck round bottom flask L-alanine (9.0 g, 10.2 mmol) was dissolved in 50 mL AcOH. A solution of maleic anhydride (10.0 g, 10.2 mmol) in 100 mL AcOH was added dropwise to the solution of L-alanine under stirring at room temperature. After 24 h the reaction mixture was distilled under reduced pressure to remove AcOH, and then washed with diethyl ether under stirring condition. The product was obtained as a white powder after dried under vacuum at 45 °C. The ^{1}H NMR spectrum of the product was recorded in DMSO-d_6 (Figure S6). Yield: 76 %.



NMA

Scheme S3. Synthetic route of NMA.

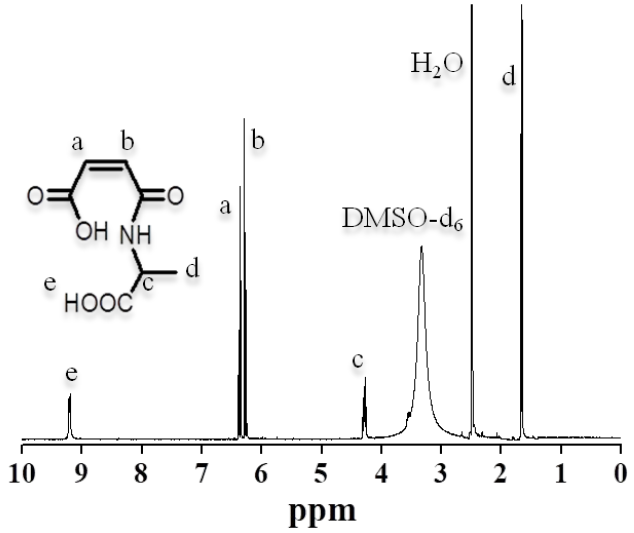


Figure S6. ^{1}H NMR spectrum of *N*-maleamic acid-L-alanine in DMSO-d_6.

The ring closing step was carried out with the help of Dean-Stark method. The white powder of *N*-maleamic acid-L-alanine (8.7 g, 46 mmol) was suspended into dry toluene (250 mL). After that 1.5 mL *ortho*-phosphoric acid and triethylamine (12.3 mL, 88 mmol) were added in sequence. The reaction mixture was refluxed at 120 °C for 4 h with vigorous stirring. After cooling to room temperature the remaining organic solution was evaporated. The obtained residue was extracted with ethyl acetate after neutralization with aqueous HCl to pH ~ 2. The organic phase was successively washed with water and brine solution, dried over anhydrous Na_2SO_4. The product was purified with the help of column chromatography using 30 % ethyl acetate/hexane mixture. Finally, the product NMA was obtained as a white powder. Yield: 65%. Figure S7 shows the ^{1}H NMR spectrum of NMA. ESI-MS (Figure S8): observed *m*/*z* for $[M + Na]^+$ is 191.74.

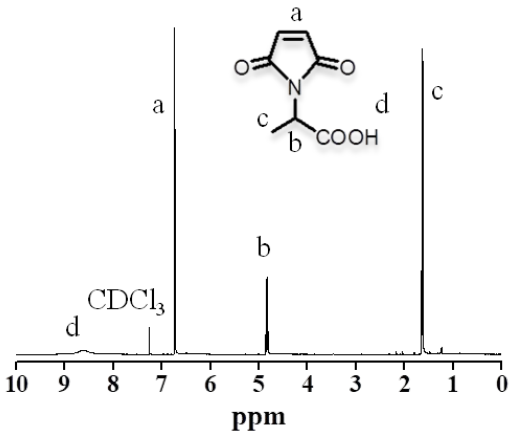


Figure S7. ^{1}H NMR spectrum of NMA in $CDCl_3$.

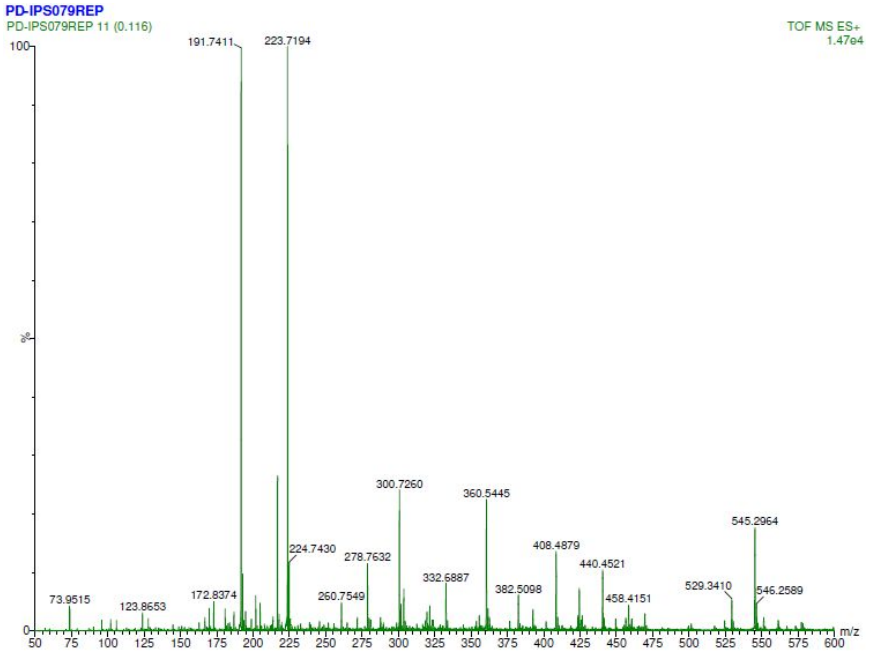


Figure S8. ESI-MS spectrum of NMA; observed *m*/*z* for $[M + Na]^+$ = 191.74, calculated *m*/*z* for $[M + Na]^+$ = 192.03 *m*/*z*.

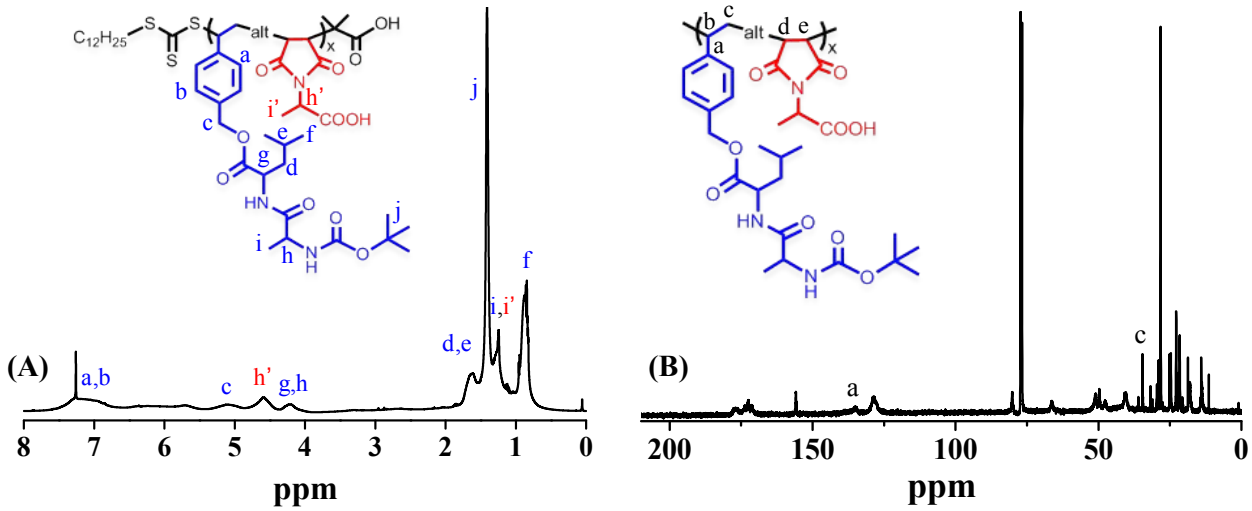


Figure S9. (A) ^{1}H NMR spectrum of **P4** in $CDCl_3$ and (B) ^{13}C NMR spectrum of **P4** in $CDCl_3$.

Determination of molecular weight by UV-vis spectroscopy. The detailed process[3,4] to determine the molecular weight of the copolymers ($M_{n,UV-vis}$) through UV-vis spectroscopy is as follows: Initially the average molar absorptivity (ε) of DMP solution was determined by measuring the absorbance in THF at room temperature. 3 mg of DMP (molecular weight (MW) = 364.63 g/mol) was dissolved in 3 mL of THF and from this solution 50 µL was taken out and was added in 3 mL of THF. The absorbance at 308 nm was determined to be 0.5895. From this result, ε was calculated as 13112 M^{-1} cm^{-1}. This process was again performed with solution of two different DMP concentrations: (1) From the stock of 3.1 mg of DMP in 3 mL of THF, 50 µL was taken out and diluted with 3 mL of THF. Absorbance value at 308 nm was found to be 0.5975. From this result, the ε value was evaluated as 12877 M^{-1} cm^{-1}. (2) From the stock of 3.2 mg of DMP in 3 mL of THF, 50 µL was taken out and diluted with 3 mL of THF. Absorbance at 308 nm was found to be 0.5996. From this result, the ε value was evaluated as 12511 M^{-1} cm^{-1}. The average ε value = 12833 M^{-1} cm^{-1} was used for further calculations. Next, the absorbance

values of the polymer solutions were measured at 308 nm and using ε = 12833 M^{-1} cm^{-1}, the molecular weights ($M_{n,UV-vis}$) of the copolymers were evaluated to be 9200, 25400, 40700, 15100 for the **P1**, **P2**, **P3** and **P4** copolymers, respectively.

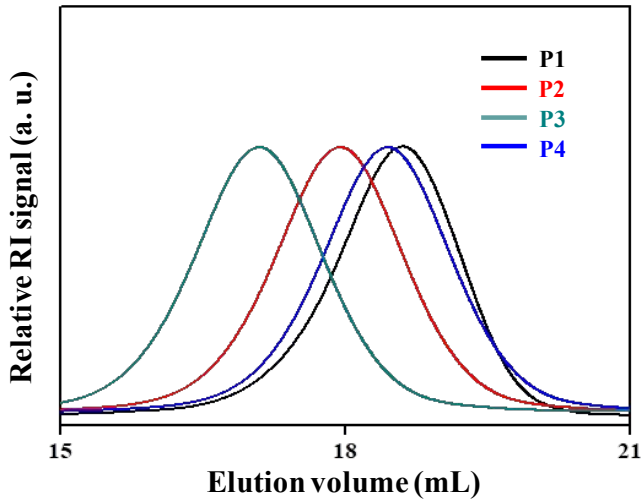


Figure S10. The GPC RI traces of alternating copolymers; **P1**, **P2**, **P3** and **P4**.

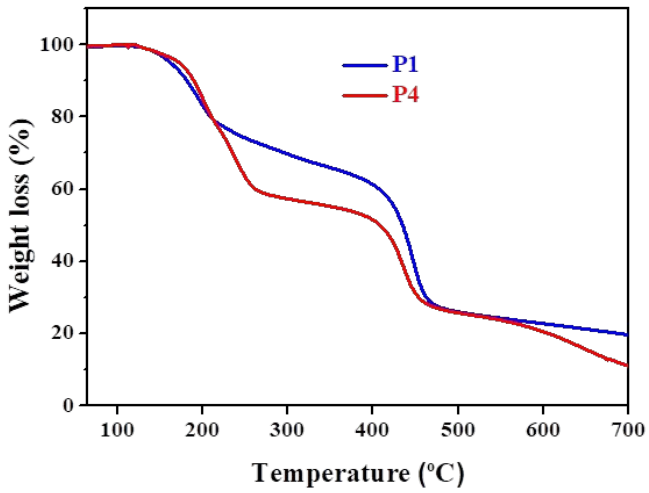


Figure S11. TGA profiles of **P1** and **P4** copolymers.

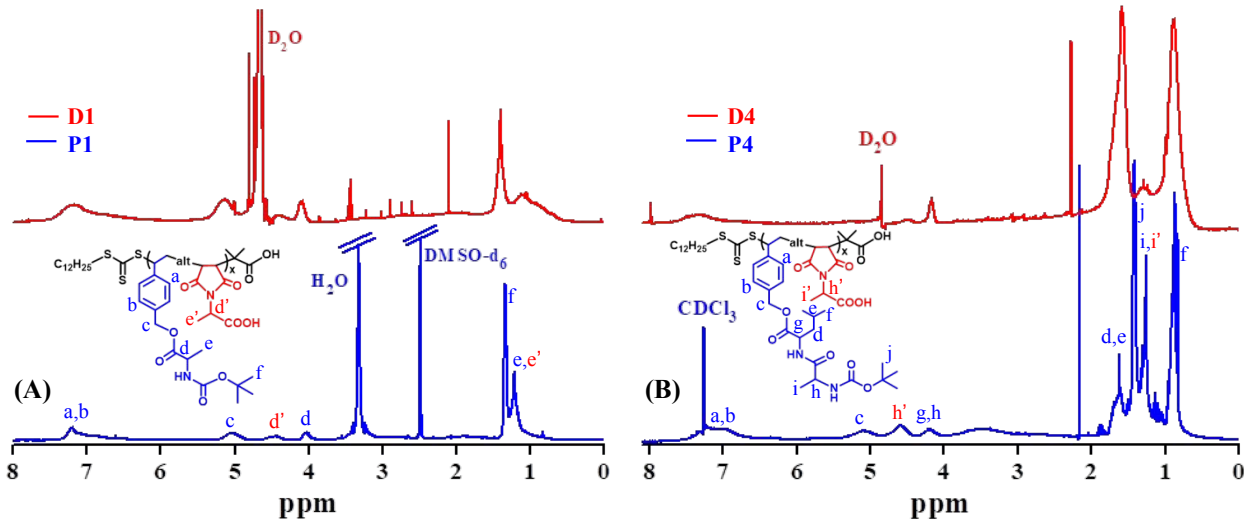


Figure S12. ^{1}H NMR spectral evidence of Boc deprotection of **P1 (A)** and **P4** copolymers (B).

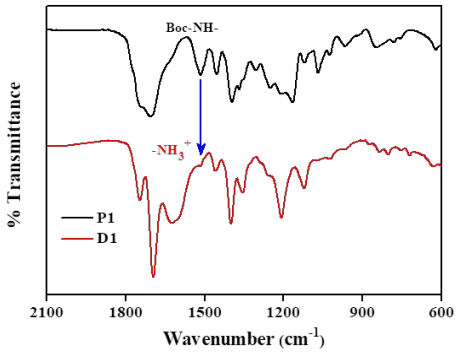


Figure S13. FT-IR spectroscopic evidence of Boc deprotection of **P1** copolymer.

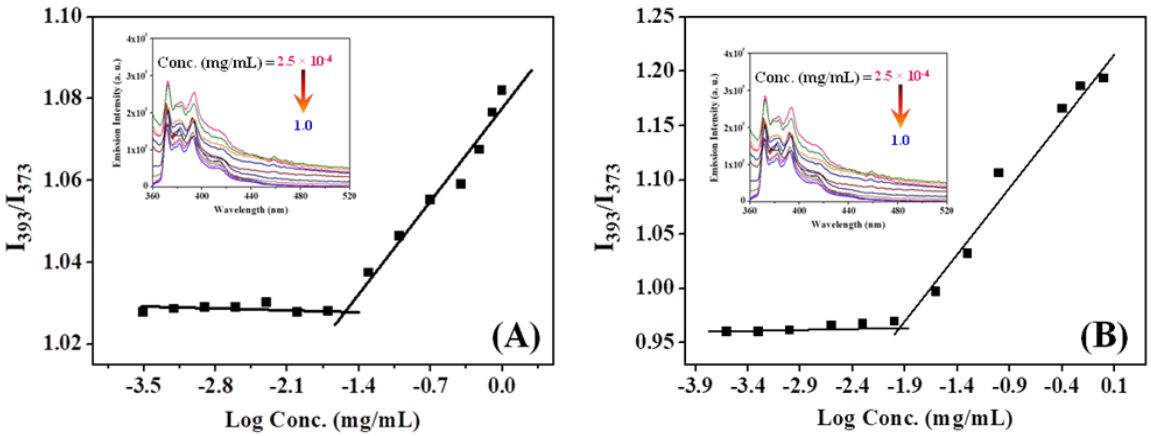


Figure S14. I_{393}/I_{373} ratios from pyrene emission spectra as a logerithimic function of the **D4** concentrations at pH ~ 3 (A) and pH ~ 8 (B).