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Supplementary Information

Novel Thermo-responsive Multiblock Architecture Composed of Sequential Peptide and Amino Acid-derived Vinyl Polymer: Toward Protein-mimicking Single-chain Folding

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Experimental Procedures

Materials

Solvents of analytical grade were used unless otherwise stated. *N*,*N*-Dimethlylformamide (DMF), *N*-methyl-2-pyrrolidone, diethyl ether, ethyl acetate, acryloylchloride, 2,2'-azobisisobutylonitrile (AIBN), magnesium sulfate anhydrous (anhydrous $MgSO_4$), DMSO-d_6, D_2O and sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) were purchased from Nacalai Tesque. LiBr was purchased from Wako Pure Chemical. H-Gly-OMe hydrochloride was purchased from Watanabe Chemical Industries. 2,2,5-Trimethyl-4-phenyl-3-azahexane-3-nitroxide, free radical (TIPNO) was purchased from Sigma Aldrich. Nile red was purchased from Tokyo Chemical Industry. DMF was used after purification with distillation. The others were used as received.

Measurements

^{1}H NMR spectra were acquired using a JEOL JNM-ECA500 (JEOL Resonance) spectrometer (500 MHz) (sample concentration: 1 wt%). The values of M_n and $Đ$ (M_w/M_n) of the block copolymers were determined by size exclusion chromatography (SEC) on a JASCO LC-net II/AD (JASCO Ltd.) equipped with a refractive index (RI) detector. The obtained polymers were analyzed in DMF (containing 10 mM LiBr) (flow rate: 0.6 mL/min, temperature: 40 ˚C, column: TSKgel α-4000). Poly(methyl methacrylate)s were purchased from GL Sciences Inc. and used as the calibration standards. Turbidity of aqueous polymer solutions was recorded at 600 nm on a V-650 spectrophotometer (JASCO Ltd.) equipped in a qurtz cell (1 cm path length) with a Peltier type thermostatic cell holder coupled with a controller PTC-423L (JASCO Ltd.) (sample concentration: 1 wt%, 1°C min^{-1}). CD spectra were recorded on a J-820 spectropolarimeter (JASCO Ltd.) under N_2 atmosphere. Experiments were performed in a quartz cell with a 1 mm path length over the range of 190-250 nm at 20°C (sample concentration: 0.05 wt%). The AFM image was collected at ambient temperature (ca. 20°C) on a SPM9700 (Shimazu Co.) operated by tapping using a silicon tip (MPP-11100, tip radius <12 nm). An aliquot (10 μL) of the aqueous solution of multiblock hybrid after thermal cycles (30 times) (0.05 wt%), which was used for CD analysis, was placed on freshly cleaved mica. After adsorption for 3 min, the excess solution was removed by absorption onto filter paper. The resultant substrate was washed three times with pure water (20 μL), and the sample was dried in a coverd container at room temperature. The scanning speed was at a line frequency of 1 Hz, and the original images were sampled at a resolution of 1024 x 1024 points. The TEM image was collected on a JEOL JEM2100F (JEOL Ltd.) at 200 kV accelerating voltage. After a small volume of the folded multiblock hybrid aqueous solution (0.05 wt%) was applied to a carbon-coated copper TEM grid for 20 min at room temperature, the excess solution was blotted with filter paper and stained with phosphotungstic acid aqueous solution (1 wt%). The sample was dried in a coverd container at room temperature. DLS measurements were performed in water (1 wt%) at 20°C on a DLS 7000 spectrometer (Otsuka Electric Ltd.) equipped with He-Ne laser (632.8 nm). Scattering light from the sample was analysed at a 90° angle from the incident light. The non-negatively constrained least-squares (NNLS) method was used for the correlation analysis to obtain the diameter probability distribution functions. Sample was filtered throught Cosmonice filter (Nacalai Tesque, pore size: 0.45 μm) to remove dust particles in solution prior to the DLS measurement. Fluorescence spectra were recorded on a JASCO FP-8300 spectrofluorometer (JASCO Ltd.). Samples were prepared as follows. Nile red 8 mg (25 μmol) was dissolved in acetone (100 μL) and then diluted with water (100 mL) ([Nile red]=250 μM). The Nile red aqueous solution (100 μL) was added into 3 mL of polymer solutions (1 wt%) with and without thermal cycles (20 to 80°C, 30 cycles), respectively. As a control, the Nile red aqueous solution (100 μL) was also added into pure water (3 mL). Experiments were performed in a quartz cell with a 1 cm path length over the range of 570-750 nm at 20°C (λ_{ex}=553 nm).

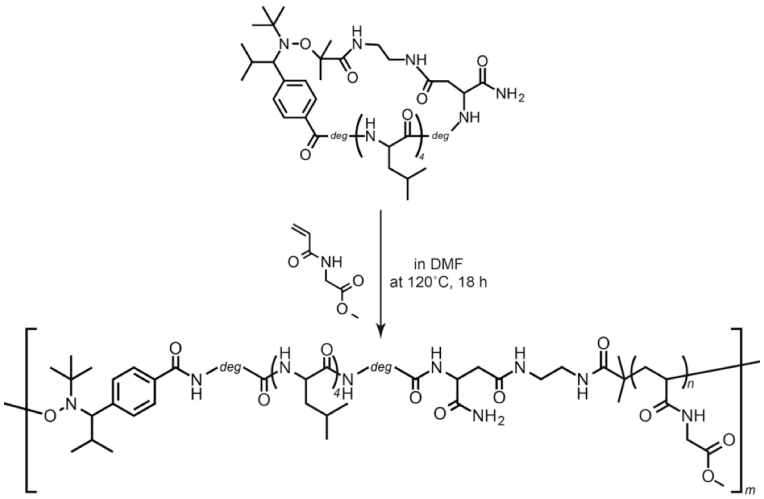
Synthesis of *N*-acryloyl-glycine *O*-methyl ester (NAGMe)

H-Gly-OMe hydrochloride 5.00 g (39.8 mmol) and TEA 12.2 mL (87.6 mmol) were dissolved in chloroform (200 mL) and cooled over an ice bath. Acryloyl chloride 3.87 mL (47.8 mmol) was diluted with chloroform (15 mL) and added dropwise to the amino acid solution. After the addition, the reaction solution was stirred for overnight at room temperature. The reaction mixture was washed five times with 1.5 M $MgSO_{4\,aq}$ (100 mL×5). The organic layer was collected and dried over anhydrous $NaSO_4$. The solution was concentrated in *vacuo* and then passed through a silica gel column using hexane/ethyl acetate (v/v=1/2) mixture solution as an eluent (R_f=0.33). The resultant solution was concentrated in *vacuo* to give a crude NAGMe as a pale yellow colored solid. To improve purity of the monomer, the pale yellow-colored NAGMe was dissolved to diethylether (20 mL) at 40˚C. Then it was slowly cooled to room temperature for an hour. Finally, the mixture was cooled under an ice bath for re-crystallization to give pure NAGMe as a white-colored solid.
Yield: 2.41 g (42.5 %).
^{1}H NMR (DMSO-d_6, TMS) (Fig. S6): 3.63 ppm (-C(O)OCH_3, 3H), 3.92 ppm (-NHCH_2C(O)O-, 2H), 5.63 and 6.12ppm (-CH=C$H_aH_{a'}$, 2H), 6.40 ppm (-CH=C$H_aH_{a'}$, 1H), 8.54 ppm (-CONH-, 1H).

Synthesis of multiblock hybrid [(Leu)$_4$-*b*-PNAGMe]$_m$ (Scheme S1)

Cyclic tetraleucine NMP initiator was synthesized as described previously.[33] NAGMe 0.286 g (2.0 mmol), cyclic tetraleucine peptide initiator 24.6 mg (0.020 mmol), and DMF (82.4 μL) were mixed together in a glass test tube (monomer

concentration: ca. 5 M). Note that the mixture could not form a homogeneous solution below the melting point of NAGMe. The heterogeneous mixture was deoxygenated using three freeze–pump–thaw cycles under dry N_2. The reaction mixture was placed in a preheated oil bath at 120°C for 18 h. At this temperature, the mixture could form a homogeneous solution. After polymerization, the reaction tube was removed from the oil bath, rapidly cooled in liquid N_2, and exposed to ambient air to stop the polymerization reaction. The resulting polymer was precipitated by dropping the polymer solution into a large excess of diethyl ether. After centrifugation, the obtained multiblock copolymer was further purified using a reprecipitation method from a DMF/diethyl ether system and dried to give a white-colored powder (conversion 86.4%). The chemical structure and molecular weight of the obtained polymer were evaluated by ^{1}H NMR, CD and SEC analyses.

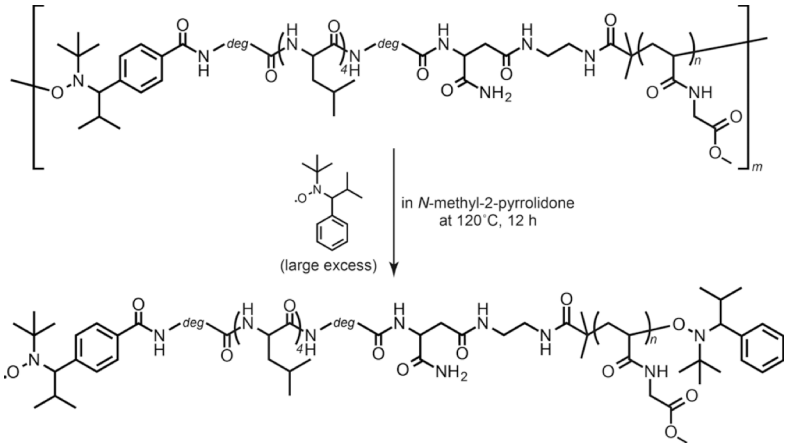


Scheme S1. Synthesis of multiblock copolymer composed of tetraleucine and Gly-derived vinyl polymer via NMP utilizing the TIPNO-based cyclic initiator.

SEC (DMF (containing 10 mM LiBr), 40°C, PMMA standards): M_n=80400, M_p=111900, $Đ$=2.89 (Fig. 2A, blue line).
^{1}H NMR (D_2O, DSS): 0.39 ppm (>CHCH(CH_3)$_2$, 3H, enantiomer), 0.90 ppm (Leu, δ-CH_3, 24H; >NC(CH_3)$_3$, 9H), 1.05-2.70 ppm (PNAGMe, 2*n*H, methylene of main chain; PNAGMe, *n*H, methine of main chain; >CHCH(CH_3)$_2$, 3H, enantiomer; >C(CH_3)$_2$, 6H; Leu, γ-C*H*, 4H; Leu, β-CH_2, 8H; >CHC*H*(CH_3)$_2$, 1H; Asp, β-CH_2, 2H), 3.78 ppm (PNAGMe, 3*n*H, methyl ester), 3.87-4.50 ppm (PNAGMe, 2*n*H, α-CH_2; -OC(=O)NHCH_2CH$_2$NH-, 2H; -OC(=O)NHCH$_2$CH_2NH-, 2H; -NHCH_2CH$_2$O-, 4H; -O(CH_2)$_2$O-, 8H; >C*H*CH(CH_3)$_2$, 1H; -NHCH$_2$CH_2O-, 4H; -OCH_2C(=O)-, 4H; Asp, α-C*H*, 1H; Leu, α-C*H*, 4H) (Fig. S1, blue line).

Fragmentation of multiblock hybrid [(Leu)$_4$-*b*-PNAGMe]$_m$ (Scheme S2)

Fragmentation of the multiblock polymer was carried out as follows: the multiblock copolymer (0.10 g, 1.25 μmol) and TIPNO (0.22 g, 1 mmol) were dissolved in *N*-methyl-2-pyrrolidone (1 mL), and the solution was poured into a glass test tube.



Scheme S2. Fragmentation of multiblock copolymer using radical crossover reaction in the presence of a large excess of TIPNO.

The solution was deoxygenated using three freeze–pump–thaw cycles under dry N_2. The fragmentation reaction was then performed in a preheated oil bath at 120°C for 12 h. The fragmented polymer was purified by repeated precipitation in diethyl ether and dried to give a pale brown-colored powder. The chemical structure and molecular weight of the original and fragmented polymer were evaluated by ^{1}H NMR, CD and SEC analyses. SEC analysis showed a marked decrease in both the molecular weight and polydispersity index after treatment (Fig. 2A). On the other hand, the ^{1}H NMR spectra before and after fragmentation (Fig. S1) did not change significantly, demonstrating cleavage of the polymer chain without altering the compositions of the PNAGMe/peptide blocks, namely, fragmentation from a multi- to a diblock structure.
SEC (DMF (containing 10 mM LiBr), 40°C, PMMA standards): M_n=12100, M_p=14300, $Đ$=1.25 (Fig. 2A, red line).
^{1}H NMR (D_2O, DSS): 0.39 ppm (>CHCH(CH_3)$_2$, 3H, enantiomer), 0.90 ppm (Leu, δ-CH_3, 24H; >NC(CH_3)$_3$, 9H), 1.05-2.70 ppm (PNAGMe, 2*n*H, methylene of main chain; PNAGMe, *n*H, methine of main chain; >CHCH(CH_3)$_2$, 3H, enantiomer; >C(CH_3)$_2$, 6H; Leu, γ-C*H*, 4H; Leu, β-CH_2, 8H; >CHC*H*(CH_3)$_2$, 1H; Asp, β-CH_2, 2H), 3.78 ppm (PNAGMe, 3*n*H, methyl ester), 3.87-4.50 ppm (PNAGMe, 2*n*H, α-CH_2; -OC(=O)NHCH_2CH_2NH-, 2H; -OC(=O)NHCH_2CH_2NH-, 2H; -NHCH_2CH_2O-, 4H; -O(CH_2)$_2$O-, 8H; >C*H*CH(CH_3)$_2$, 1H; -NHCH_2CH_2O-, 4H; -OCH_2C(=O)-, 4H; Asp, α-C*H*, 1H; Leu, α-C*H*, 4H) (Fig. S1, red line).

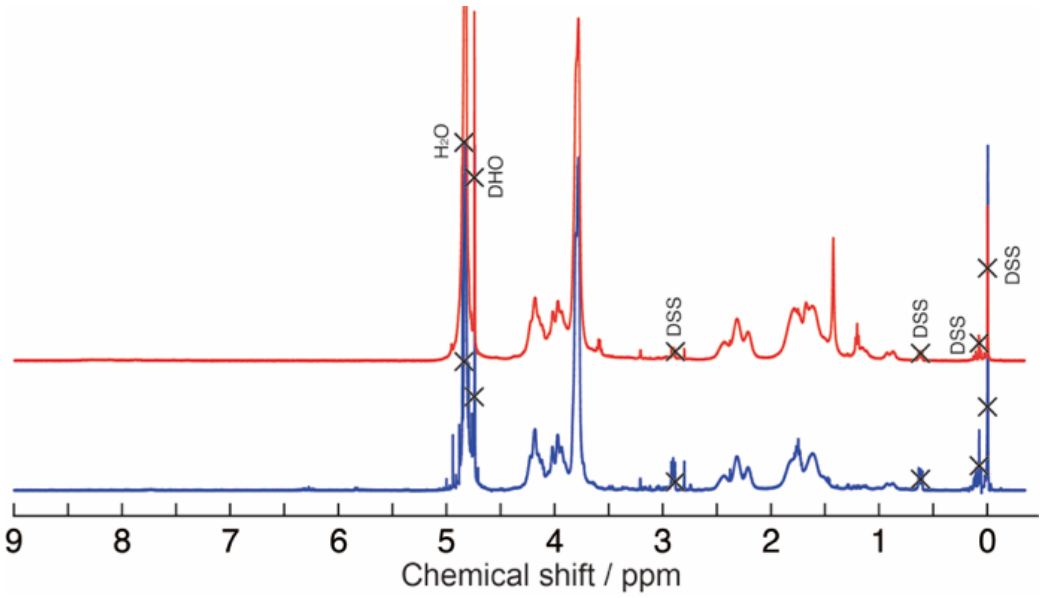


Fig. S1. ^{1}H NMR spectra of multiblock hybrid before (blue) and after (red) fragmentation in D_2O at 25°C.

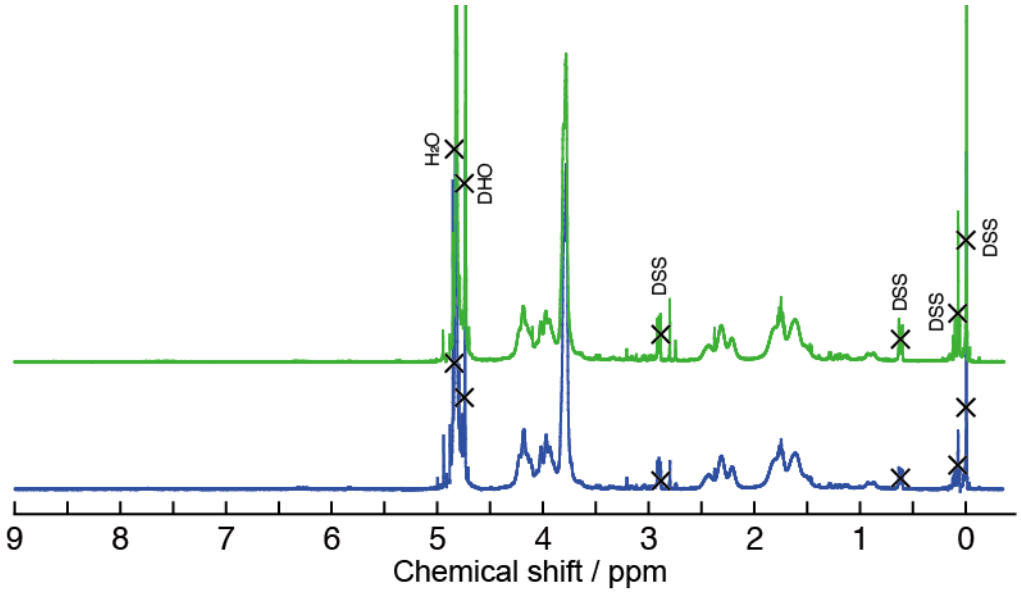


Fig. S2. ^{1}H NMR spectra of multiblock hybrid before (blue) and after (green) the thermal cycles (20 to 80°C, 30 cycles) in D_2O at 25°C.

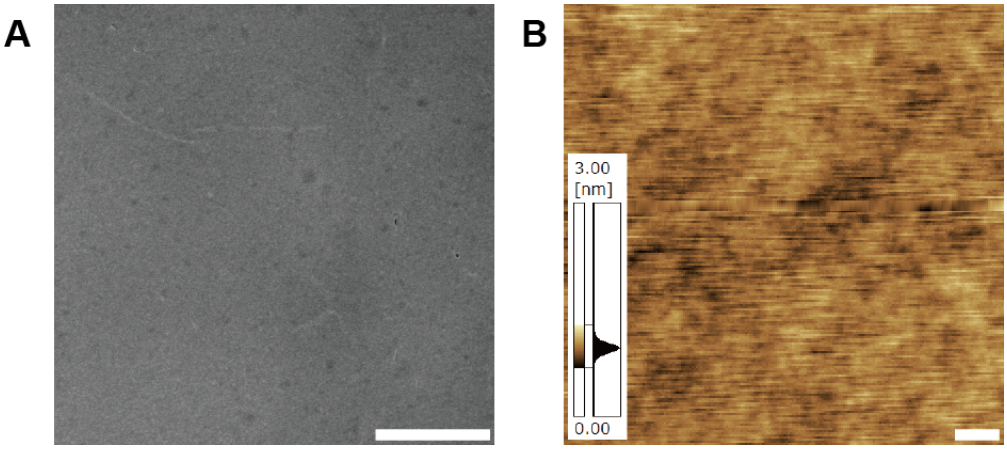


Fig. S3. TEM (A) and tapping-mode AFM (B) images of the expanded multiblock hybrid polymer before thermal cycle; scale bar=200 nm. [polymer]=0.05 wt%.

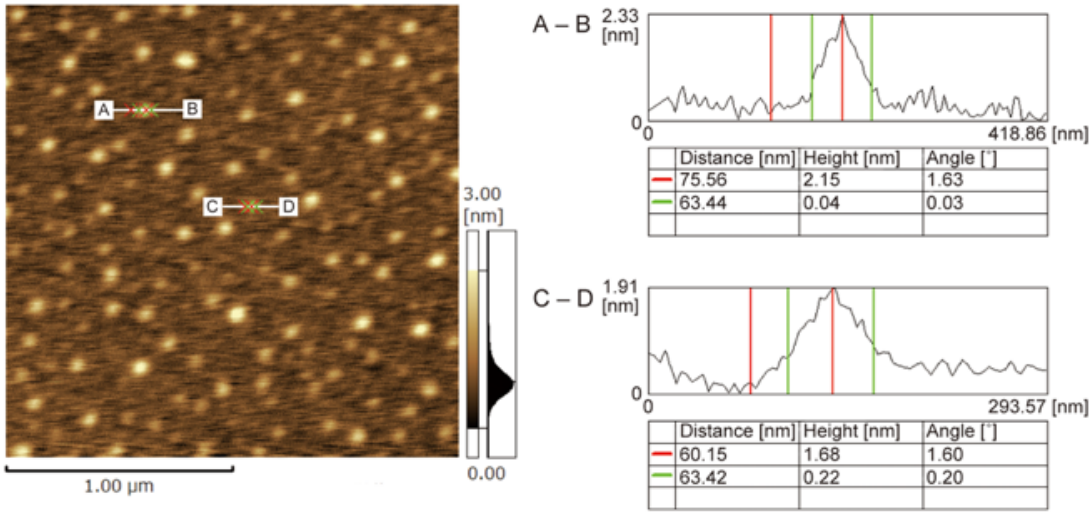


Fig. S4. AFM cross-sectional analysis of the folded multiblock hybrid polymer.

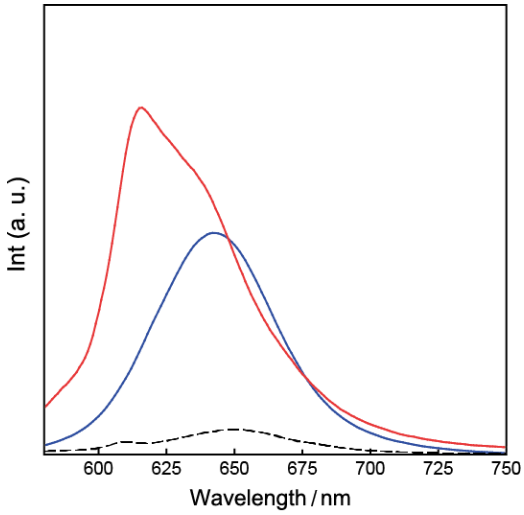


Fig. S5. Emission spectra of Nile red in pure water (dashed black line) and in aqueous solutions of the multiblock hybrid obtained before (blue line) and after (red line) the thermal cycles (20 to 80°C, 30 cycles). λ_{ex}=553 nm.

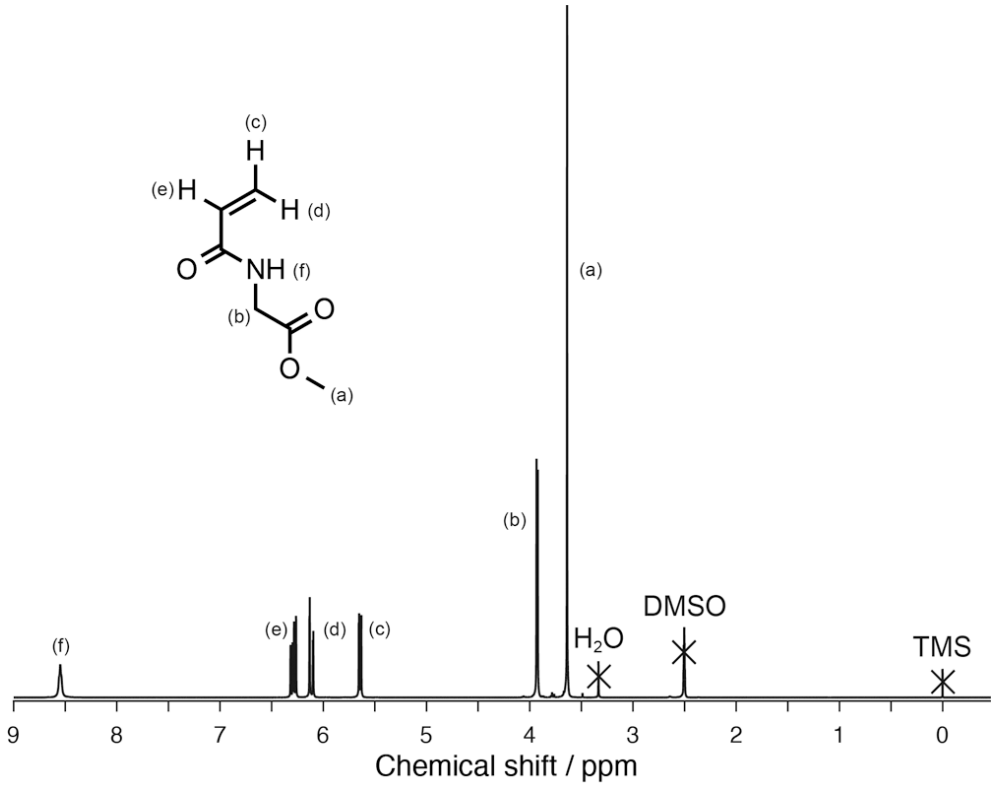


Fig. S6. ^{1}H NMR spectrum of NAGMe in DMSO-d_6 at 25°C.