Supporting Information

Programming the Photoelasticity of Hydrogels for Label-Free Mechanosensing by a Simultaneous Birefringence-Stress Reader (SiBRe)

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1. General

1.1 Materials

All optics were purchased from Thorlabs or Newport, and all reagents and solvents were obtained from TCI or Fujifilm Wako and used without further purification.

1.2 Instrumentation and Characterization

Column chromatography was performed by medium-pressure liquid chromatography (Yamazen). NMR experiments were performed at 25 °C using a 500 MHz spectrometer (JNM-ECA500, Jeol Resonance, Japan). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis was performed on a SpiralTOF mass spectrometer (JMS-S3000, Jeol Resonance, Japan).

2. Simultaneous Birefringence-Stress Reader (SiBRe)

The birefringence (Δn) and the true stress (σ) of the hydrogel samples were quantified simultaneously using a custom-built Simultaneous Birefringence-Stress Reader, shown schematically in Figure S1. The setup integrates an optical path for birefringence measurement integrated with a mechanical stage for stretching. Samples were mounted on a uniaxial stretching apparatus equipped with a digital force gauge with a resolution of 0.01 N. The optical path employed a 1064 nm laser of size 0.8 mm and power 2.5 mW as the light source incident on sample. To determine the birefringence, the sample's Jones matrix was measured. A series of 25 known input polarization states were generated using two voltage- and temperature-controlled liquid-crystal variable retarders (Thorlabs, LCC-1111-TC), forming a polarization-state generator.[36] For each input state, the output polarization after passing through the sample was measured with a polarimeter (Thorlabs, PAX-1000-IR2). The Jones matrix was calculated from 300 input/output polarization pairs to ensure accuracy, and the mean values of each matrix element were determined.[37] The variability was consistent across each sample; three independently prepared hydrogels of identical composition (N = 3) were measured to ensure sample-to-sample reproducibility, and their average and standard deviation were reported using error bars in the figures. Concurrently, the true stress (σ) was determined as follows. As the sample was stretched, the tensile force (F) was recorded by the digital force gauge. Measurements were performed in a step-wise manner. At each strain step, the system waited for the initial rapid relaxation to subside before simultaneously recording the tensile force (F) and the polarization state. Given that the optical acquisition required approximately 2 minutes, the recorded values represent an average over this duration. At each stretching step, the width (w) and thickness (d) of the hydrogel were measured with a micrometer, and the instantaneous cross-sectional area (A) was taken as $A = wd$. Thus,

$$\sigma = \frac{F}{A} = \frac{F}{wd} \tag{1}$$

Finally, the birefringence (Δn) was calculated from the linear retardation (δ_H) extracted from the Jones matrix, using the following equation:

$$\Delta n = \frac{\lambda \delta_H}{2\pi d} \tag{2}$$

where λ is the laser wavelength (1064 nm) and d is the sample thickness. As the samples were under uniaxial stress, only the linear retardation changed while the other retardance components showed no detectable change upon stretching.

Notes on System Performance and Limitations: First, a minimal pre-tension was applied to the hydrogel during mounting to prevent sagging and ensure proper alignment. This resulted in a slight non-zero birefringence at the initial state but did not affect the determination of the stress-optical coefficient (C), which is derived from the slope of the stress-birefringence curve. Second, the stress resolution is strictly determined by the force gauge precision ($\delta F = 0.01$ N) and the sample's cross-sectional area A, following the relationship $\delta \sigma = \delta F / A$. With the current sample geometry, the system achieves a stress resolution of ~1 kPa. While increasing the sample cross-sectional area would theoretically lower the detection limit (*i.e.*, improve sensitivity), the current A was optimized to balance measurement precision with the synthetic scalability and cost of the rationally designed force-transducing crosslinkers.

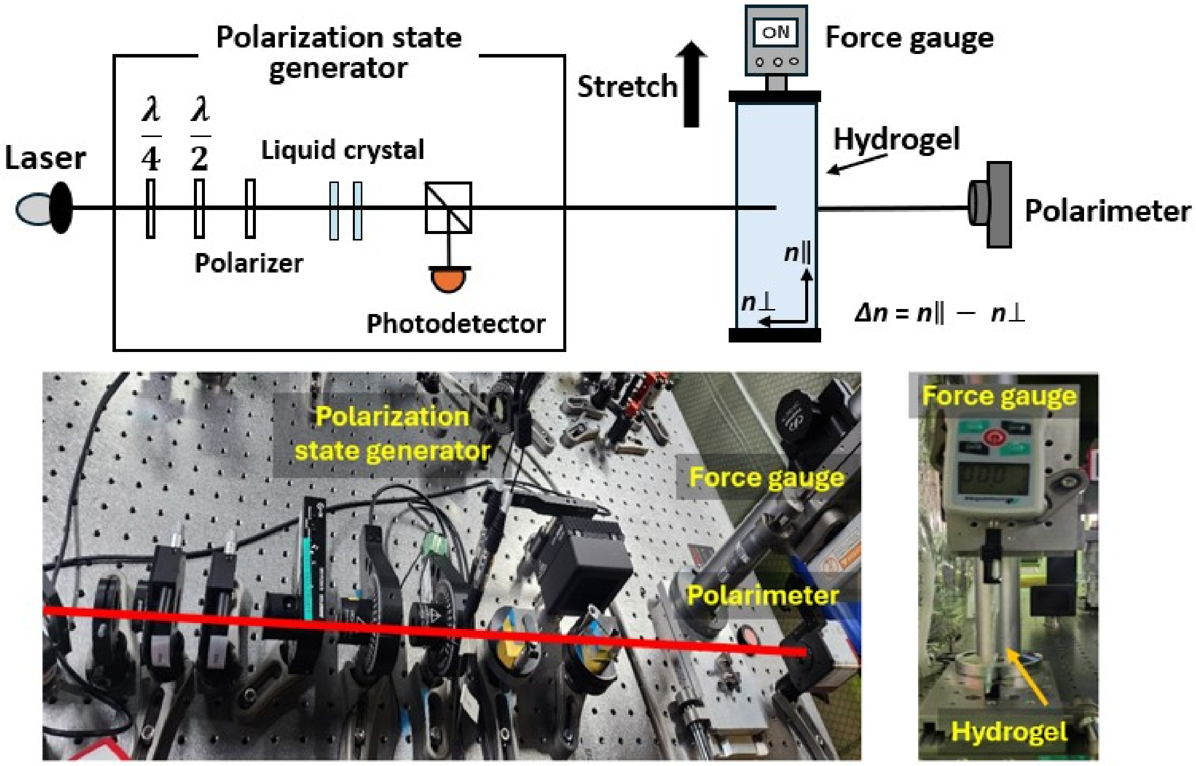
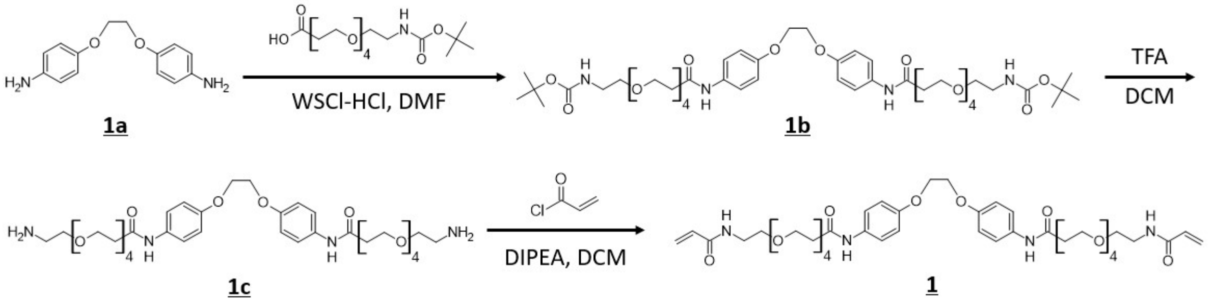


Figure S1. Schematic of the Simultaneous Birefringence-Stress Reader (SiBRe). A custom-built apparatus for concurrent measurement of stress and birefringence in a hydrogel sample. The system integrates a mechanical stretching stage equipped with a digital force gauge and an optical path. A 1064-nm laser beam passes through a polarization-state generator, the hydrogel sample, and a polarization-state analyzer, enabling real-time, co-registered quantification of true stress (σ) and birefringence (Δn).

3. Syntheses of force-transducing crosslinkers

Scheme S1. Synthesis of force-transducing crosslinker **1**.



Reagents and conditions: **1b**, (*N*-Boc-amino)-PEG4-carboxylic acid, WSCl·HCl, DMF, room temperature, overnight; **1c**, TFA, DCM, 4 °C to room temperature, 2.5 h; **1**, Acryloyl chloride, DIPEA, DCM, 4 °C to room temperature, 4.5 h.

Compound 1b

4,4'-[Ethane-1,2-diylbis(oxy)]dianiline (244 mg, 1.0 mmol, 1.0 eq.) was added to a mixture of the (*N*-Boc-amino)-PEG4-carboxylic acid (912 mg, 2.5 mmol, 2.5 eq.), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCl · HCl, 477 mg, 2.5 mmol, 2.5 eq.) in *N,N*-dimethylformamide (DMF, 10 mL) at room temperature and mixture was reacted for overnight. After the reaction, the mixture was diluted with dichloromethane (DCM, 200 mL) and washed with $NaHCO_3$ aq. (2×200 mL) and 0.1 M HCl aq. (2×200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by medium-pressure column chromatography (Yamazen silica column, 26×300 mm), eluting with a linear gradient of 0-100% methanol in DCM over 30 minutes at a flow rate of 10 mL/min to yield product **1b** (751 mg, 80% yield). Identification of purified **1b** was performed with ^1H, ^{13}C NMR, and MALDI-TOF MS.

1b: ^1H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.48 (d, J = 8.7 Hz, 4H), 6.88 (d, J = 7.0 Hz, 4H), 5.12 (s, 1H), 4.27 (s, 4H), 3.82 (t, J = 5.7 Hz, 4H), 3.69-3.49 (m, 28H), 3.29 (q, J = 4.9 Hz, 4H), 2.63 (t, J = 5.5 Hz, 4H), 1.38-1.50 (18H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 156.1, 155.1, 132.1, 121.7, 114.9, 79.2, 70.6, 70.6, 70.4, 70.3, 70.3, 70.2, 67.3, 66.9, 40.4, 37.8, 28.5;

MALDI-TOF MS (*m/z*): $[M + Na]^+$ calcd for $C_{46}H_{74}N_4O_{16}Na$, 961.50; found, 961.31.

Compound <u>1c</u>

To a solution of compound **<u>1b</u>** in DCM (5.0 mL) cooled to 4 °C, trifluoroacetic acid (TFA; 5.0 mL) was added. The mixture was stirred for 30 min at this temperature and then for 2 h at room temperature. After the reaction, the solvent was removed with N_2 gas. The product was purified with reverse phase medium-pressure column chromatography (Yamazen ODS column 26×300 mm), eluting with a linear gradient of 0-100% methanol in water over 30 minutes at a flow rate of 10 mL/min to afford **<u>1c</u>** (553 mg, 93% yield). Identification of purified **<u>1c</u>** was performed with ^{1}H, ^{13}C NMR, and MALDI-TOF MS.

<u>1c</u>: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.79 (s, 2H), 7.78 (s, 6H), 7.48 (d, J = 9.2 Hz, 4H), 6.88 (d, J = 9.2 Hz, 4H), 4.21 (s, 4H), 3.66 (t, J = 6.3 Hz, 4H), 3.56-3.47 (m, 28H), 2.94 (t, J = 5.7 Hz, 4H), 2.48 (t, J = 6.3 Hz, 4H);

^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 154.7, 133.2, 121.1, 115.0, 70.3, 70.2, 70.2, 70.1, 67.2, 67.2, 67.1, 39.1, 37.5;

MALDI-TOF MS (m/z): $[M + H]^+$ calcd for $C_{36}H_{59}N_4O_{12}$, 739.41; found, 739.34.

Crosslinker <u>1</u>

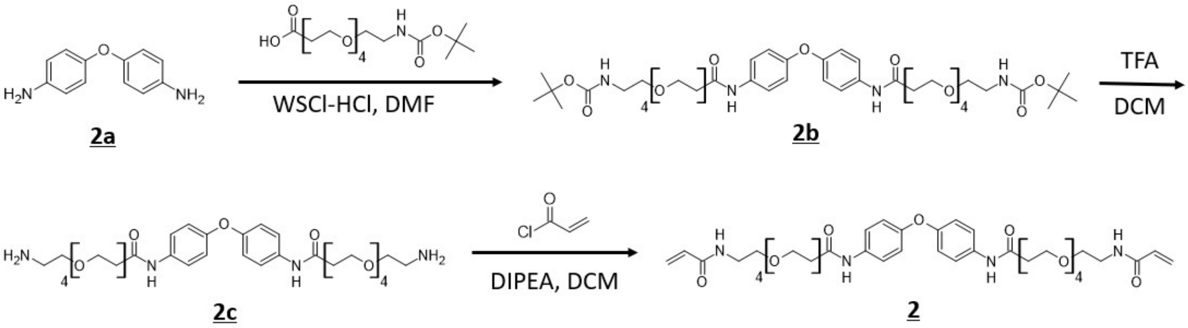
Acryloyl chloride (153 µL, 1.9 mmol, 2.5 eq.) in DCM (1 mL) was added to a mixture of **<u>1c</u>** (553 mg, 0.75 mmol, 1.0 eq.) and *N,N*-diisopropylethylamine (DIPEA; 606 µL, 3.8 mmol, 5.0 eq.) in DCM (10 mL) under ice bath and mixture was stirred for 30 min. After stirring for 4 hours, the mixture was diluted with DCM (200 mL) and washed with $NaHCO_3$ aq. (2×200 mL) and 0.1 M HCl aq. (2×200 mL). The organic layer was separated and removed solvent with evaporator. The product was purified with medium-pressure column chromatography (Yamazen silica column 26×300 mm) eluting with a linear gradient of 0-100% methanol in DCM over 30 minutes at a flow rate of 10 mL/min to afford crosslinker **<u>1</u>** (439 mg, 69% yield), which was identified by ^{1}H, ^{13}C NMR, and MALDI-TOF MS.

<u>1</u>: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.75 (s, 2H), 8.11 (s, 2H), 7.47 (d, J = 6.9 Hz, 4H), 6.88 (d, J = 9.2 Hz, 4H), 6.21 (dd, J = 17.2, 10.3 Hz, 2H), 6.04 (dd, J = 17.2 and 2.3 Hz, 2H), 5.53 (dd, J = 10.3 and 2.3 Hz, 2H), 4.21 (s, 4H), 3.65 (t, J = 6.3 Hz, 4H), 3.47-3.39 (m, 28H), 3.24 (q, J = 5.7 Hz, 4H), 2.49-2.47 (m, 4H);

^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 165.2, 154.6, 133.2, 132.3, 125.6, 121.0, 115.0, 70.3, 70.2, 70.2, 70.2, 70.1, 69.6, 67.2, 67.0, 39.2, 37.6;

MALDI-TOF MS (m/z): $[M + Na]^+$ calcd for $C_{42}H_{62}N_4O_{14}Na$, 869.41; found, 869.34.

Scheme S2. Synthesis of force-transducing crosslinker **2**.



Reagents and conditions: **2b**, (Boc-amino)-PEG4-carboxylic acid, WSCl·HCl, DMF, room temperature, overnight; **2c**, TFA, DCM, 4 °C to room temperature, 2.5 h; **2**, Acryloyl chloride, DIPEA, DCM, 4 °C to room temperature, 4.5 h.

Compound 2b

4,4'-Diaminodiphenyl ether (200 mg, 1.0 mmol, 1.0 eq.) was added to a mixture of the (*N*-Boc-amino)-PEG4-carboxylic acid (912 mg, 2.5 mmol, 2.5 eq.), WSCl ・ HCl (477 mg, 2.5 mmol, 2.5 eq.) in DMF 10 mL at room temperature and mixture was reacted overnight. After the reaction, the mixture was diluted with DCM (200 mL) and washed with $NaHCO_3$ aq. (2×200 mL) and 0.1 M HCl aq. (2×200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by medium-pressure column chromatography (Yamazen silica column, 26×300 mm), eluting with a linear gradient of 0-100% methanol in DCM over 30 minutes at a flow rate of 10 mL/min to yield product **2b** (653 mg, 73% yield). Identification of purified **2b** was performed with ^{1}H, ^{13}C NMR and MALDI-TOF MS.

2b: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.88 (s, 2H), 7.54 (d, J = 9.2 Hz, 4H), 6.89 (d, J = 9.2 Hz, 4H), 6.69 (s, 2H), 3.66 (t, J = 6.3 Hz, 4H), 3.48-3.44 (m, 24H), 3.33 (m, J = 6.0 Hz, 4H), 3.02 (q, J = 5.9 Hz, 4H), 2.49 (m, J = 6.3 Hz, 14H), 1.33 (s, 18H);

^{13}C NMR (125 MHz, DMSO-d_6) δ 169.5, 156.1, 152.9, 135.3, 121.2, 119.2, 78.1, 70.3, 70.2, 70.2, 70.2, 70.0, 69.7, 67.2, 40.7, 37.6, 28.8;

MALDI-TOF MS m/z: $[M + Na]^+$ calcd for $C_{44}H_{70}N_4O_{15}Na$, 917.47; found, 917.30.

Compound 2c

To a solution of compound **2b** in DCM (5.0 mL) cooled to 4 °C, TFA (5.0 mL) was added. The mixture was stirred for 30 min at this temperature and then for 2 h at room temperature. After the reaction, the solvent was removed with N_2 gas. The product was purified with reverse phase medium-pressure column chromatography (Yamazen ODS column 26×300 mm) eluting with a linear gradient of 0-100% methanol in water over 30 minutes at a flow rate of 10 mL/min to yield product **2c** (481 mg, 95% yield). Identification of purified **2c** was performed with ^{1}H, ^{13}C NMR, and MALDI-TOF MS.

2c: ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.52 (d, J = 9.2 Hz, 4H), 6.92 (d, J = 8.6 Hz, 4H), 3.81 (t, J = 5.7 Hz, 4H), 3.69-3.59 (m, 32H), 3.09 (t, J = 5.2 Hz, 4H), 2.62 (t, J = 6.0 Hz, 4H);

^{13}C NMR (125 MHz, Methanol-d_4) δ 171.0, 153.9, 134.0, 121.7, 118.6, 70.1, 70.0, 69.9, 69.6, 66.8, 66.5, 39.2, 36.8;

MALDI-TOF MS *m/z*: $[M + H]^+$ calcd for $C_{34}H_{55}N_4O_{11}$, 695.38; found, 695.31.

Crosslinker 2

Acryloyl chloride (138 µg, 1.7 mmol, 2.5 eq.) in DCM 1 mL was added to a mixture of **2c** (481 mg, 0.69 mmol, 1.0 eq.) and DIPEA (561 µL, 3.5 mmol, 5.0 eq.) in DCM (10 mL) under ice bath and mixture was stirred for 30 min. After stirring for 4 hours, the mixture was diluted with DCM (200 mL) and washed with $NaHCO_3$ aq. (2×200 mL) and 0.1 M HCl aq. (2×200 mL). The organic layer was separated and removed solvent with evaporator. The product was purified with medium-pressure column chromatography (Yamazen silica column 26×300 mm) eluting with a linear gradient of 0-100% methanol in DCM over 30 minutes at a flow rate of 10 mL/min to afford crosslinker **2** (332 mg, 60% yield), which was identified by ^{1}H, ^{13}C NMR, and MALDI-TOF MS.

2: ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.52 (d, J = 7.4 Hz, 4H), 6.91 (d, J = 8.6 Hz, 4H), 6.26-6.16 (m, 4H), 5.62 (dd, J = 9.7, 2.3 Hz, 2H), 3.79 (t, J = 5.4 Hz, 4H), 3.62-3.52 (m, 28H), 3.40 (t, J = 5.2 Hz, 4H), 2.59 (t, J = 5.4 Hz, 4H);

^{13}C NMR (125 MHz, Methanol-d_4) δ 170.9, 166.8, 153.9, 134.0, 130.7, 125.4, 121.7, 118.6, 70.2, 70.2, 70.1, 69.9, 69.1, 66.9, 39.1, 37.2;

MALDI-TOF MS *m/z*: $[M + Na]^+$ calcd for $C_{40}H_{58}N_4O_{13}Na$, 825.39; found, 825.32.

4. Preparation of hydrogels

All hydrogels were prepared by aqueous free-radical polymerization in custom-made molds constructed from two glass plates separated by silicone spacers of 3.0 mm thickness. Polymerization was initiated by adding ammonium persulfate (APS) and *N,N,N',N'*-tetramethylethylenediamine (TEMED) to final concentration of 1% and 0.05% (v/v), respectively. The reaction was allowed to proceed for at least 1 hour at room temperature. After polymerization, the resulting gels were removed from the molds and equilibrated in a large excess of deionized water for at least 1 hour to remove any unreacted monomers and soluble components before testing.

4.1 PAA gels with varying total polymer concentrations (% w/v)

To investigate the intrinsic photoelastic properties of the polyacrylamide (PAA) matrix, a series of standard PAA gels were prepared with total polymer concentrations ranging from 8% to 18% (w/v). For this series, the weight ratio of the monomer, acrylamide (AAm), to the cross-linker, *N,N′*-methylenebis(acrylamide) (Bis), was kept constant. Stock solutions of AAm and Bis were mixed with deionized water to achieve the desired final polymer concentrations and then polymerized as described above.

4.2. PAA gels with varying crosslinker functionality

To evaluate the topological effects, Bis ($f = 2$), Tris ($f = 3$) and Tetra ($f = 4$) were incorporated into 12% (w/v) PAA gels at various molar concentrations (26, 31, 39 μM). Appropriate volumes of AAm and Bis stock solutions were mixed to obtain the target ratios and then polymerized as described above.

4.3 PAA gels incorporating force-transducing crosslinkers

To create hydrogels with a programmable photoelastic response, a defined amount of the newly synthesized force-transducing crosslinker (**1** or **2**) was dissolved directly in a 10% (w/v) PAA precursor solution containing acrylamide (AAm) and the standard cross-linker, Bis. The final crosslinker concentration ranged from 1.0 mM to 10 mM. The resulting precursor solution was then polymerized as described in the general procedure.

5. Molecular dynamics (MD) simulations

Molecular dynamics (MD) simulations were performed to investigate the stable conformations of crosslinkers (**1** and **2**) in an aqueous environment using the Moldesk software package. Initial 3D structures of the crosslinkers were constructed, and partial atomic charges were assigned using the MOPAC PM7 semi-empirical method. Each crosslinker was then solvated in a periodic box of TIP3P water molecules, and the entire system was described using the AMBER force field. Prior to the dynamics run, the solvated system was subjected to energy minimization to remove any unfavorable steric contacts.

The MD simulation was carried out under the NVT (canonical) ensemble at a constant temperature of 300 K. The simulation consisted of an initial equilibration phase of 2,000 steps followed by a production phase of 10,000 steps. A time step of 0.5 fs was used throughout the simulation. During the production phase, system coordinates were saved every 200 steps (100 fs) for subsequent analysis. The conformation with the lowest-potential-energy along each trajectory was identified for structural evaluation.

6. Supplementary Figures

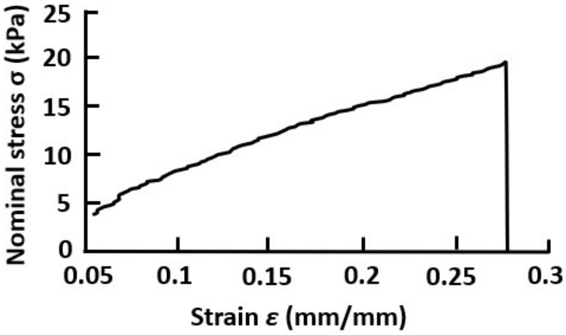


Figure S2. Nominal stress versus strain for 12% (w/v) PAA gel. The stress-strain relationship remains strictly linear up to the point of fracture. Consistent with reports on networks with high crosslinker (Bis) density, the limited extensibility causes the gel to fracture prior to reaching the non-linear hyperelastic regime.[26] This result validates the assumption of mechanical linearity within the specific experimental window used for optical interrogation.

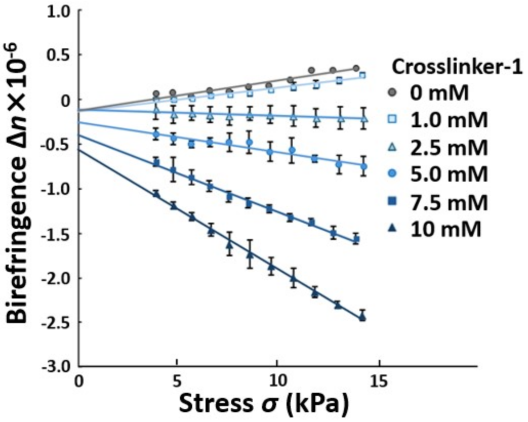


Figure S3. Concentration-dependent manner of the photoelastic response. The stress-birefringence relationships for hydrogels prepared with varying input concentrations of Crosslinker-1 (0 to 10 mM). The stress-optical coefficient (*C*), represented by the slope of the linear fit, changes systematically toward more negative values as the concentration of the force-transducing crosslinker increases. This linear correlation between the input concentration and the measured optical response supports the quantitative and uniform incorporation of the functional crosslinkers into the hydrogel network.

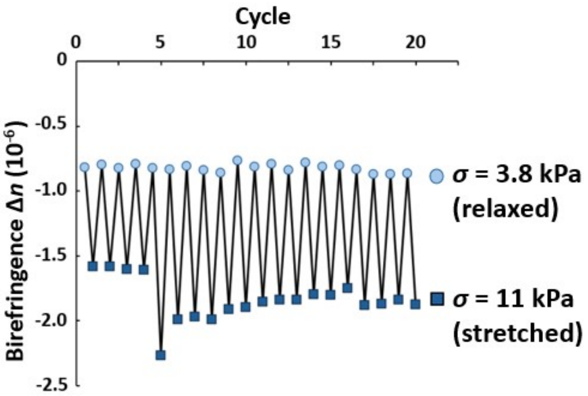


Figure S4. The photoelastic response of crosslinker-1-incorporated gel remained stable and consistent over 20 consecutive cycles of stretching and relaxation, indicating elastic and reversible deformation without detectable network fatigue or damage within the tested strain range.

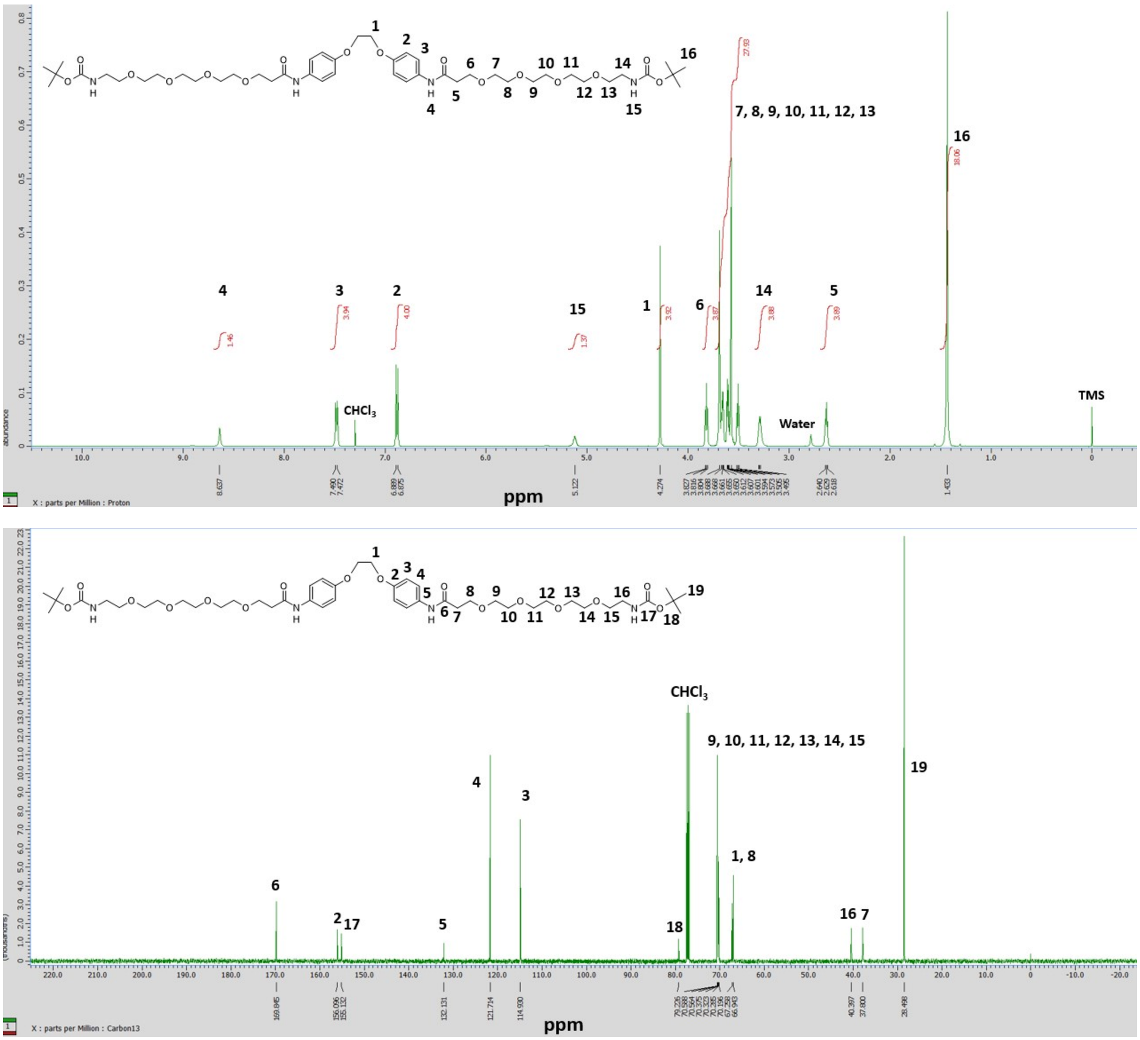


Figure S5. Identification of **<u>1b</u>**. (A)^{1}H NMR spectrum. (B)^{13}C NMR spectrum.

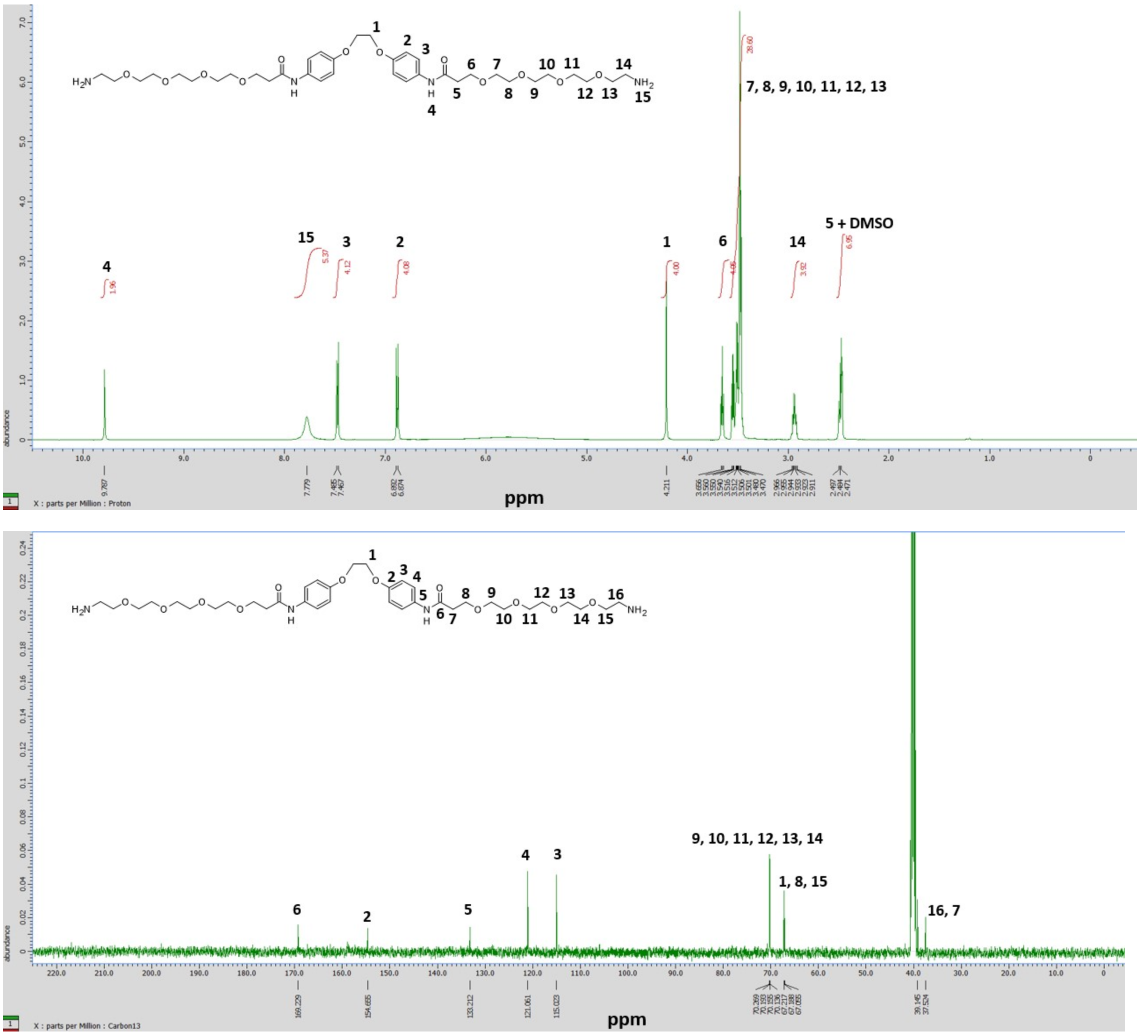


Figure S6. Identification of **<u>1c</u>**. (A)^{1}H NMR spectrum. (B)^{13}C NMR spectrum.

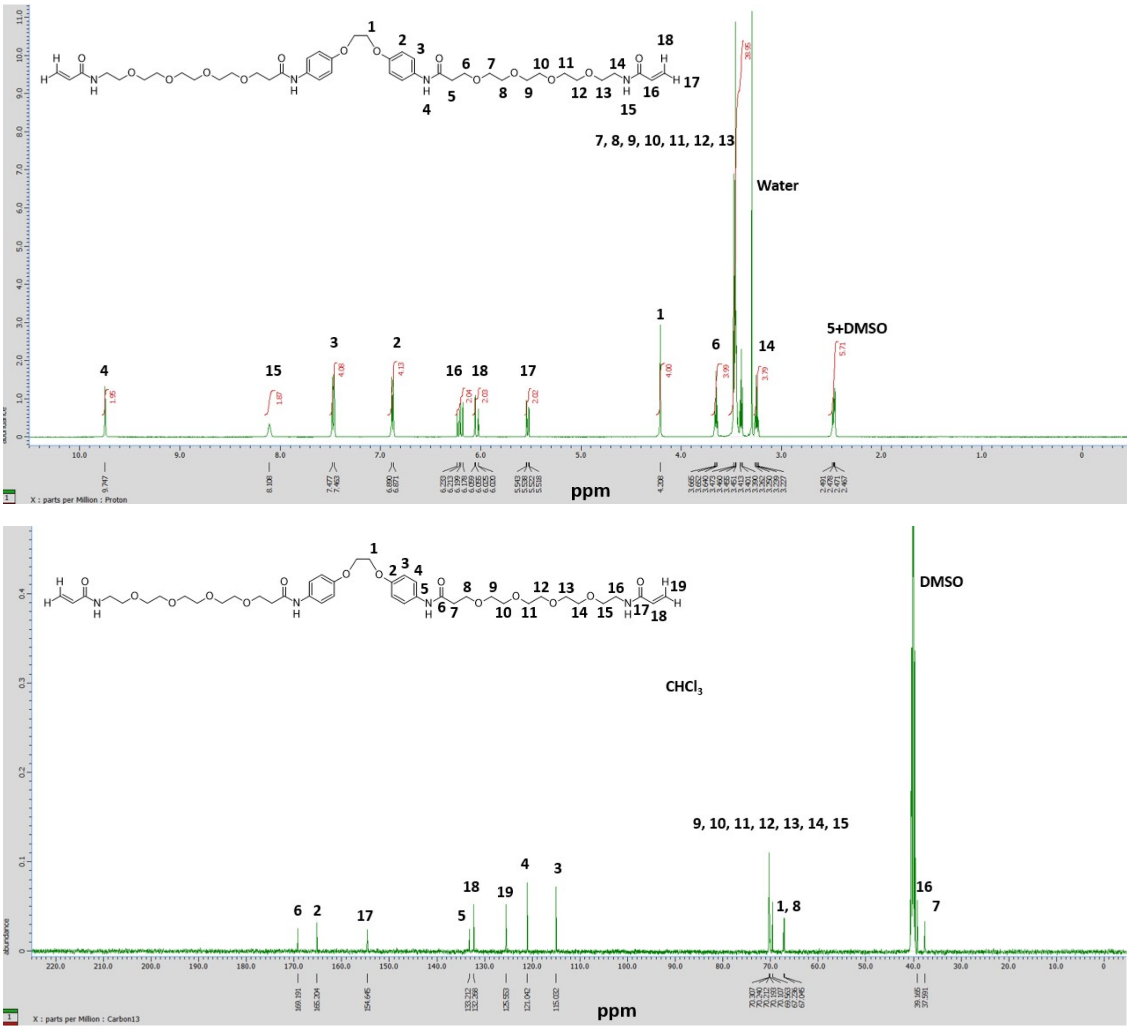


Figure S7. Identification of <u>**1**</u>. (A)^{1}H NMR spectrum. (B)^{13}C NMR spectrum.

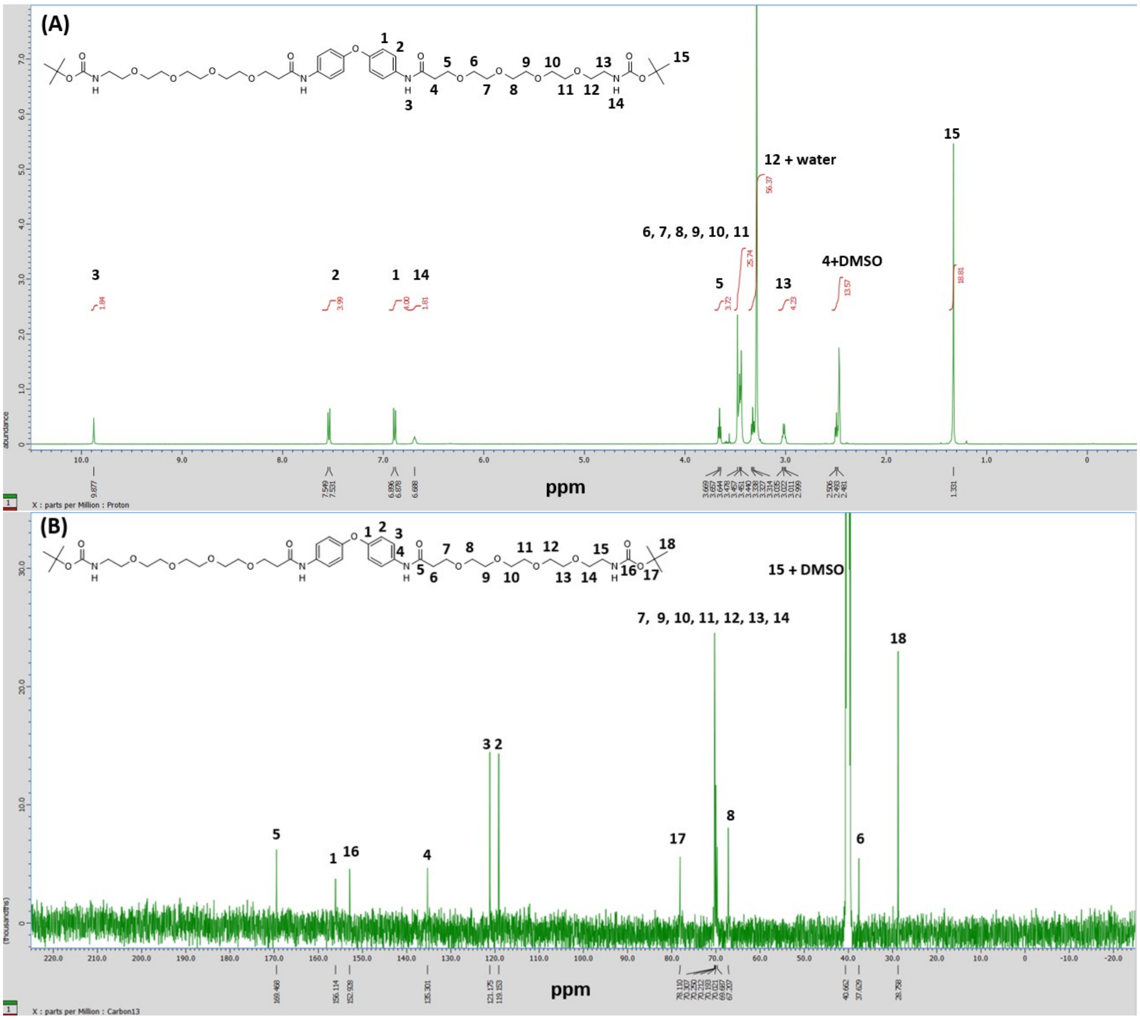


Figure S8. Identification of **2b**. (A)^{1}H NMR spectrum. (B)^{13}C NMR spectrum.

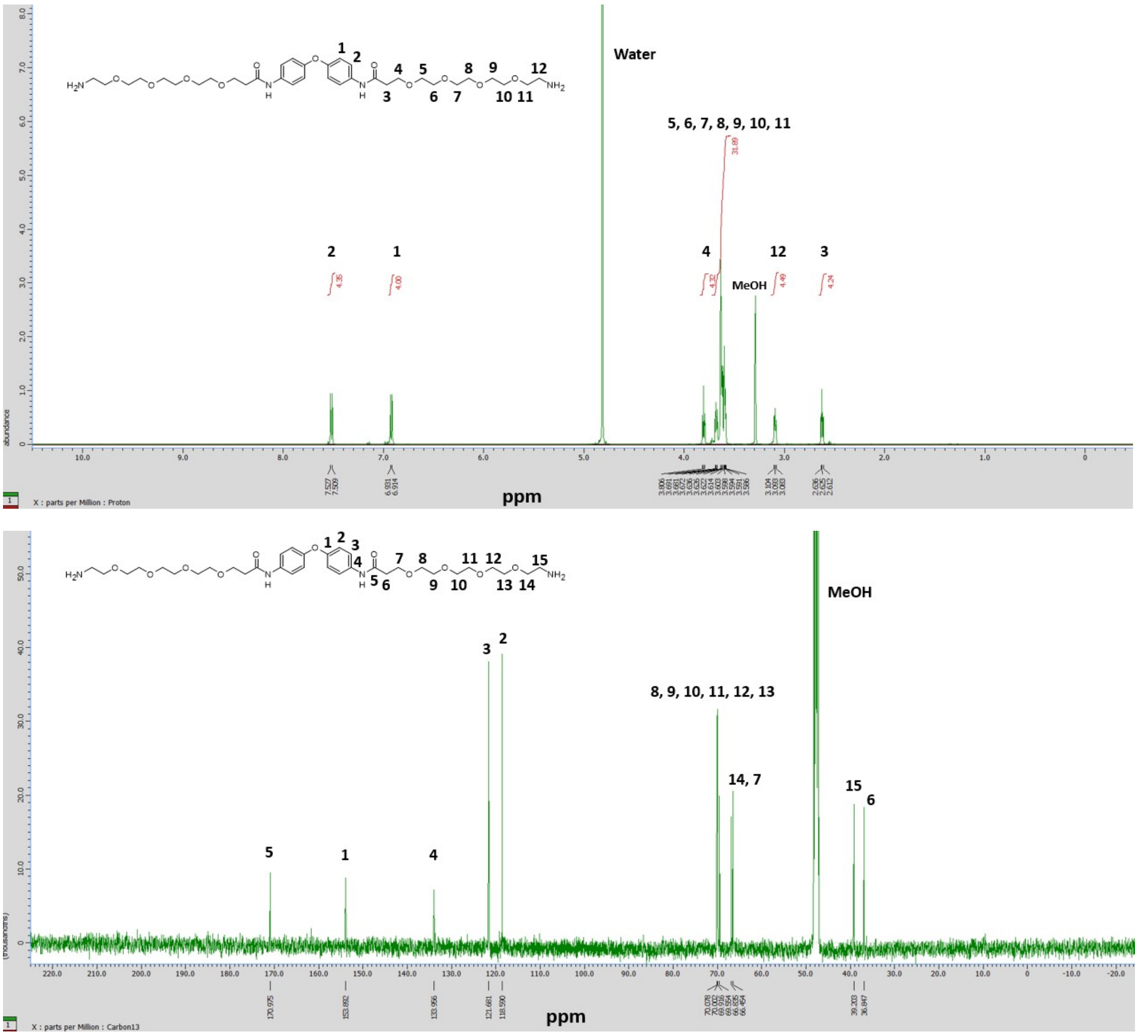


Figure S9. Identification of **2c**. (A)^{1}H NMR spectrum. (B)^{13}C NMR spectrum.

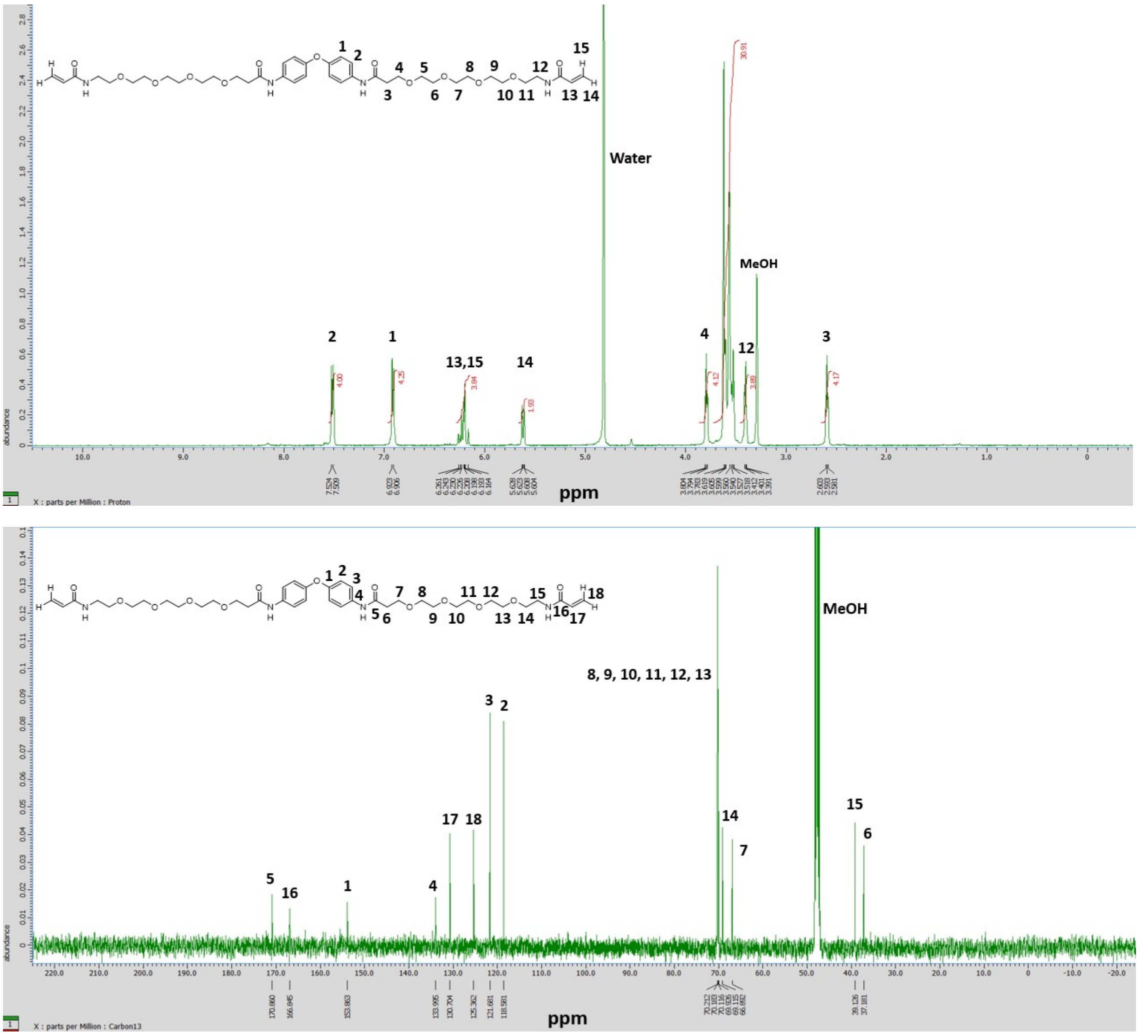


Figure S10. Identification of **2**. (A)^{1}H NMR spectrum. (B)^{13}C NMR spectrum.