Supporting Information for

A Degradable, Broad-Spectrum and Resistance-Resistant Antimicrobial Oligoguanidine as Disinfecting and Therapeutic Agent in Aquaculture

Guopu Huang,[#,a] Hui Shen,[#,b] Xianhui Chen,[a] Tong Wu,[a] Zhiyong Chen,[a] Yajie Chen,[a] Junfeng Song,[a] Qingyun Cai,[*,a] Yugang Bai,[*,a] Huangsheng Pu,[*,c] Xinxin Feng[*,a]

[a] *State Key Laboratory of Chem-/Bio-Sensing and Chemometrics, Hunan Provincial Key Laboratory of Biomacromolecular Chemical Biology, and School of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan, 410082, China*

[b] *Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, China*

[c] *College of Advanced Interdisciplinary Studies, National University of Defense Technology, Changsha 410073, China*

[#] *These authors contributed equally to this work.*

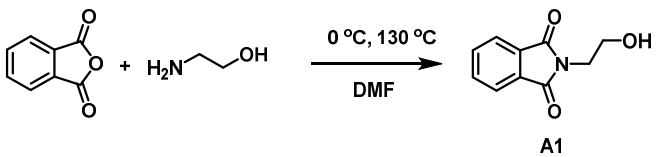
Instruments

Instrumentation. NMR spectra were recorded using a Bruker Avance II 400 MHz spectrometer equipped with an autosampler. The data was processed in MestReNova 6.1 and aligned/annotated in Adobe Illustrator CC or Microsoft Paint of Windows 10. Gel permeation chromatography (GPC) analyses were performed on a Waters system equipped with a Waters 515 isocratic pump and a Waters 2414 refractive index detector. Separations were performed at 40 °C using an aqueous solution of $NaNO_3$ (0.01 M) as the mobile phase. The molecular weight of polymers was determined based on a EasiVial PEO/PEG (Agilent) standard curve. The obtained data points were imported into Origin 2018, plotted and smoothed, and saved as vector image files (*.ai) for coloring and annotation in Adobe Illustrator CC. Scanning electron microscopy (SEM) experiments were conducted on a Hitachi S-4800 scanning electron microscope. Bacteria sample were gold-plated before they were observed on SEM. Zeta-potential analysis were conducted on a Malvern Zetasizer Nano ZSP dynamic light scattering (DLS) instrument. Flow cytometry studies were performed on a Becton Dickinson Accuri C6 Plus instrument. The data was processed in FlowJo, aligned and annotated in Adobe Illustrator CC.

Synthetic Protocols

Synthesis of ketal monomer A

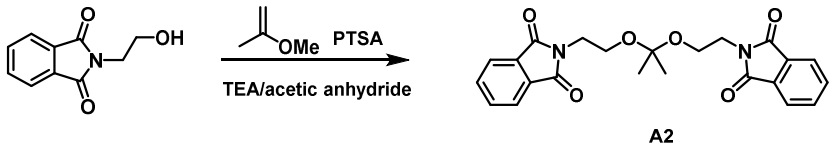
Synthesis of 2-(2-hydroxyethyl)isoindoline-1,3-dione (**A1**)[1]



Phthalic anhydride (25.00 g, 168.8 mmol, 1 equiv.) was dissolved (with stirring) in 50 ml of DMF in a round-bottom flask. Cooled the above mixture at 0 °C and then add dropwise

ethanolamine (10.00 g, 168.8 mmol, 1 equiv.) in it. Then after reaction mixture was refluxed at 130 °C for 3 h. Excess DMF was evaporated in vacuo and the residue was poured in ice-water mixture. The resulting mixture was filtered through a funnel and the crude product was washed with ice-water mixture several times and the resulting solid was dried in vacuum at 45 °C for 6h to afford product as a white solid (25.0 g, 77.5% yield). This crude product was used in the next step without further purification. Characterization results of the compound were consistent with previously reported data.[2] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 – 7.82 (m, 4H), 4.84 (t, J = 5.9 Hz, 1H), 3.64 (t, J = 5.6 Hz, 2H), 3.58 (dd, J = 11.4, 5.7 Hz, 2H).

Synthesis of 2,2'-((propane-2,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(isoin-doline-1,3-dione) (**A2**)[3]

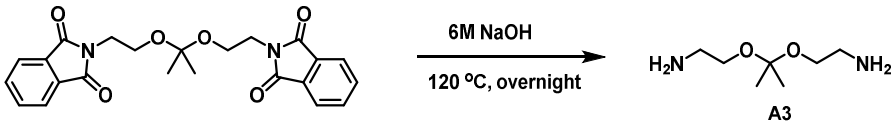


A2

The above product **A1** (10.00 g, 52.3 mmol, 1 equiv.) was added to DCM (150 mL), and the solution was cooled to 0 °C in an ice bath. 2-Methoxy propene (5 mL, 52.3 mmol, 1 equiv.) was slowly added into the solution, and *p*-toluenesulfonic acid (90 mg, 0.523 mmol, 0.01 equiv.) was further added. After being stirred for 1 h at 0 °C, the reaction mixture was heated to evaporate the solvent. When cooled to RT, trimethylamine (10 mL) and acetic anhydride (2.5 mL) were added, and the mixture was stirred overnight at RT. The crude product was precipitated from the solution using hexanes which was further purified by recrystallization with ethyl acetate for twice to obtain the final product as yellow solid (6.74 g, 61.0% yield). Characterization results of the compound were

consistent with previously reported data.[3] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.81 (m, 4H), 7.74 – 7.67 (m, 4H), 3.84 (t, *J* = 6.1 Hz, 4H), 3.62 (t, *J* = 6.1 Hz, 4H), 1.28 (s, 6H).

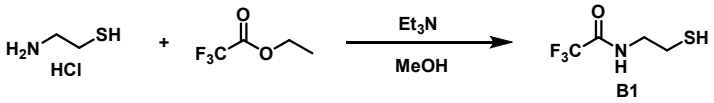
Synthesis of 2,2'-(propane-2,2-diylbis(oxy))bis(ethan-1-amine) (**A3**)



The above product **A2** (6.74 g, 16.0 mmol) was added to NaOH (6 M, 50 mL), and the mixture was stirred at 120 °C under refluxing overnight. The solution was extracted with 3 × 100 mL DCM and the organic phase was combined and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The product was obtained as amber-colored oil (2.20 g, 84.6% yield). Characterization results of the compound were consistent with previously reported data.[3] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.46 (t, *J* = 5.2 Hz, 4H), 2.85 (t, *J* = 5.2 Hz, 4H), 1.38 (s, 6H).

Synthesis of thioketal monomer B[4]

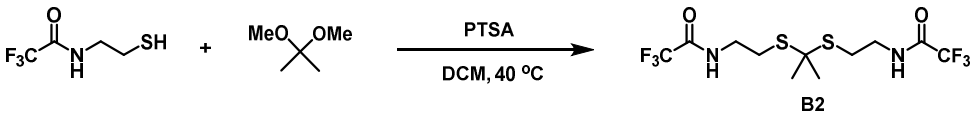
Synthesis of 2,2,2-trifluoro-N-(2-mercaptoethyl)acetamide (**B1**)



Ethyl trifluoroacetate (17.7 g, 124.4 mmol, 1.2 equiv.) was added dropwise to the solution of the cysteamine hydrochloride (8.0 g, 103.7 mmol, 1 equiv.) and TEA (15.7 g, 155.5 mmol, 2.5 equiv.) in 150 mL of MeOH. The resulting mixture was stirred overnight at room temperature. The solvent was evaporated. The residue was extracted with ethyl acetate (3 x 100 mL) and the organic layer was washed with brine. The organic layer was dried by anhydrous sodium sulfate. Evaporation in vacuum and purification by flash column chromatography (hexane/ethyl acetate = 3/1) gave the product **B1** (11.7 g, 65.0%

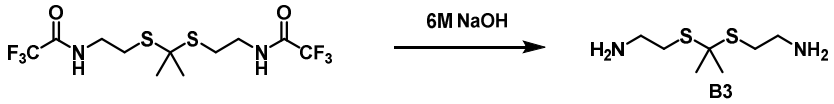
yield). Characterization results of the compound were consistent with previously reported data.[4] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (s, 1H), 3.59 (dd, *J* = 12.1, 6.0 Hz, 2H), 2.78 (dd, *J* = 13.9, 6.9 Hz, 2H), 1.44 (t, *J* = 8.5 Hz, 1H).

Synthesis of N,N'-((propane-2,2-diylbis(sulfanediyl))bis(ethane-2,1-diyl))-bis(2,2,2-trifluoroacetamide) (**B2**)



The above product **B1** (5.19 g, 30.0 mmol, 3 equiv.), 2,2-dimethoxypropane (1.04 g, 10.0 mmol, 1 equiv.), and *p*-toluenesulfonic acid monohydrate (60.0 mg, 0.300 mmol, 0.03 equiv.) were dissolved in DCM (30 mL) and the mixture was stirred for another 4 h at 40 °C. Evaporation in vacuum and purification by flash column chromatography (hexane/ethyl acetate = 3/1) gave the product as white solid (1.35 g, 35.0% yield). Characterization results of the compound were consistent with previously reported data.[4] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 2H), 3.63 (q, *J* = 6.3 Hz, 4H), 2.87 (t, *J* = 6.6 Hz, 4H), 1.64 (d, *J* = 9.4 Hz, 6H).

Synthesis of 2,2'-(propane-2,2-diylbis(sulfanediyl))bis(ethan-1-amine) (**B3**)



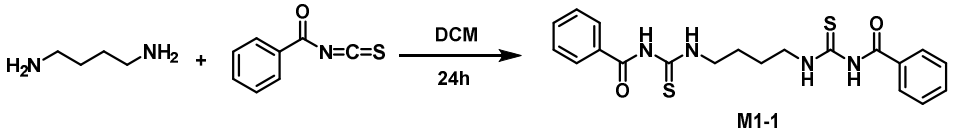
The above compound **B2** (4.30 g, 11.1 mmol) was dissolved in 30 mL 6 M NaOH to deprotect the trifluoroacetate groups. The crude mixture was stirred for 4 h and extracted with DCM (3 × 50 mL) to obtain product as amber oil (1.34 g, 67.0% yield). Characterization results of the compound were consistent with previously reported data.[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.93 (t, *J* = 6.5 Hz, 4H), 2.75 (t, *J* = 6.5 Hz, 4H), 1.63 (s, 6H), 1.46 (s, 4H).

Synthesis of S-methyl isothiourea monomer[5]

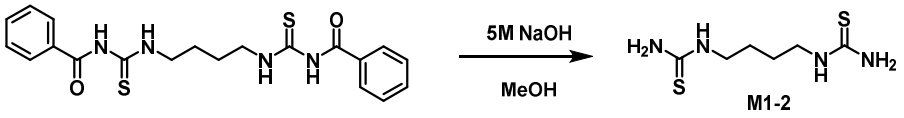
Synthesis of **M1** monomer

Synthesis of the bis(benzoylthiourea) (**M1-1**)



Benzoyl isothiocyanate (6.87 g, 47.7 mmol, 2.1 equiv.) was added dropwise to the solution of the1,4-butanediamine (2 g, 22.7 mmol, 1 equiv.) in 150 mL of DCM at 0 °C. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with DCM several times. The resulting solid was dried to afford the product as a white solid (7.23 g, 76.9 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (dd, *J* = 73.2, 68.1 Hz, 4H), 7.93 (d, *J* = 7.4 Hz, 4H), 7.63 (t, *J* = 7.4 Hz, 2H), 7.51 (t, *J* = 7.7 Hz, 4H), 3.68 (d, *J* = 5.3 Hz, 4H), 1.71 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.55, 168.41, 133.36, 132.74, 128.90, 44.81, 25.64. ESI-MS: Calculated for $C_{20}H_{22}N_4O_2S_2K^+$ ($[M+K]^+$): 453.12; found: 453.20.

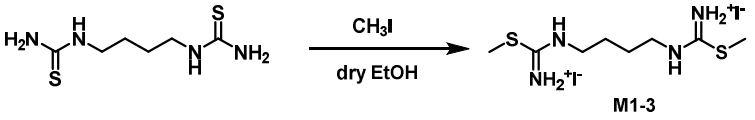
Synthesis of the bisthiourea (**M1-2**)



Aqueous sodium hydroxide (5 M, 14.0 mL, 69.8 mmol, 4 equiv.) was added dropwise to the solution of the above bis(benzoylthiourea) **M1-1** (7.23 g, 17.4 mmol, 1 equiv.) in 150 mL of MeOH. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with MeOH several times. The

resulting solid was dried to afford the product as a white solid (3.12 g, 86.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 – 6.83 (m, 6H), 3.34 (s, 4H), 1.43 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.57, 44.12, 40.63, 26.82. ESI-MS: Calculated for $C_6H_{15}N_4S_2^+$ ([M+H]$^+$): 207.07; found: 207.00.

Synthesis of the bis(S-methyl thiouronium iodide) (**M1-3**)

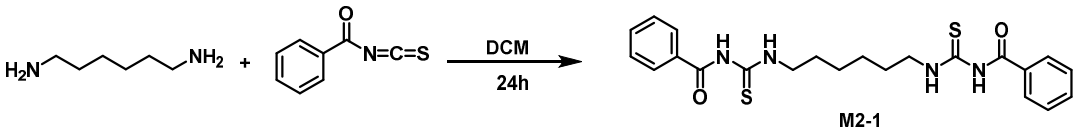


M1-3

CH_3I (5.14 g, 34.0 mmol, 2.25 equiv.) was added dropwise to the solution of the above bisthiourea **M1-2** (3.12 g, 15.1 mmol, 1 equiv.) in 150 mL of dry EtOH. The resulting mixture was stirred at 35 ^{o}C for 72 h. The reaction was filtered, and the crude product was washed with EtOH several times. The resulting solid was dried to afford the product as a white solid (2.50 g, 86.8% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 6H), 3.31 (s, 4H), 2.62 (s, 6H), 1.55 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.87, 43.74, 40.70, 40.56, 25.20, 14.31. High resolution ESI-MS: Calculated for $C_8H_{19}N_4S_2^+$ ([M+H]$^+$): 235.1045; obtained 235.1052.

Synthesis of **M2** monomer

Synthesis of the bis(benzoylthiourea) (**M2-1**)

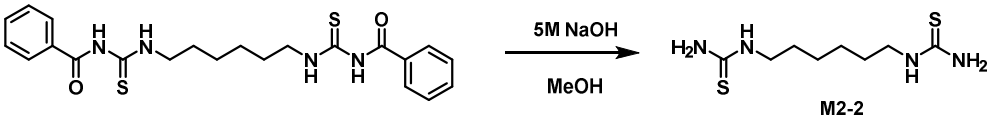


M2-1

Benzoyl isothiocyanate (5.21 g, 36.1 mmol, 2.1 equiv.) was added dropwise to the solution of the 1,6-diaminohexane (2 g, 17.2 mmol, 1 equiv.) in 150 mL of DCM at 0 ^{o}C. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered,

and the crude product was washed with DCM several times. The resulting solid was dried to afford the product as a white solid (4.5 g, 59.1 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 2H), 10.40 (s, 2H), 7.93 (d, *J* = 7.3 Hz, 4H), 7.63 (t, *J* = 7.4 Hz, 2H), 7.51 (t, *J* = 7.7 Hz, 4H), 3.62 (dd, *J* = 11.4, 6.4 Hz, 4H), 1.65 (d, *J* = 6.3 Hz, 4H), 1.39 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.46, 168.48 , 133.36, 132.73, 128.90, 45.14, 27.94, 26.54. ESI-MS: Calculated for $C_{22}H_{27}N_4O_2S_2^+$ ([M+H]$^+$): 443.15; found: 443.04.

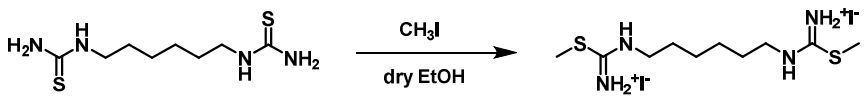
Synthesis of the bisthiourea (**M2-2**)



M2-2

Aqueous sodium hydroxide (5 M, 8.14 mL, 40.7 mmol, 4 equiv.) was added dropwise to the solution of the above bis(benzoylthiourea) **M2-1** (4.5 g, 10.2 mmol, 1 equiv.) in 150 mL of MeOH. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with MeOH several times. The resulting solid was dried to afford the product as a white solid (1.98 g, 83.2 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 – 6.72 (m, 6H), 3.34 (s, 4H), 1.44 (s, 4H), 1.26 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.57, 44.35, 29.25, 26.59. ESI-MS: Calculated for $C_8H_{18}N_4S_2$ (M): 234.10; found: 234.78.

Synthesis of the bis(S-methyl thiouronium iodide) (**M2-3**)



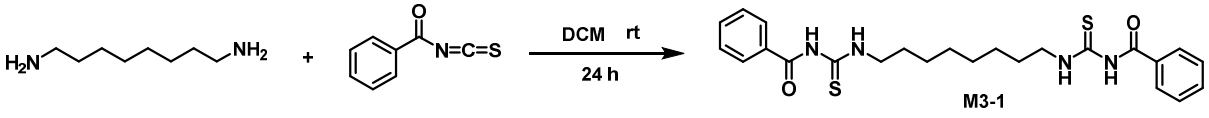
M2-3

CH_3I (2.87 g, 20.3 mmol, 2.25 equiv.) was added dropwise to the solution of the above bisthiourea **M2-2** (1.98 g, 8.45 mmol, 1 equiv.) in 150 mL of dry EtOH. The resulting

mixture was stirred at 35 °C for 72 h. The reaction was filtered, and the crude product was washed with EtOH several times. The resulting solid was dried to afford the product as a white solid (1.96 g, 44.8% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 6H), 3.28 (t, *J* = 7.0 Hz, 4H), 2.61 (s, 6H), 1.52 (s, 4H), 1.29 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.72, 44.18, 27.80, 26.12, 14.27. High resolution ESI-MS: Calculated for $C_{10}H_{23}N_4S_2^+$ ($[M+H]^+$): 263.1358; obtained 263.1359.

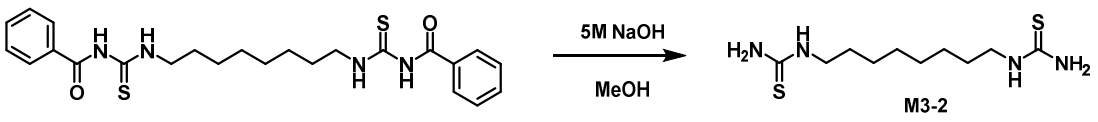
Synthesis of **M3** monomer

Synthesis of the bis(benzoylthiourea) (**M3-1**)



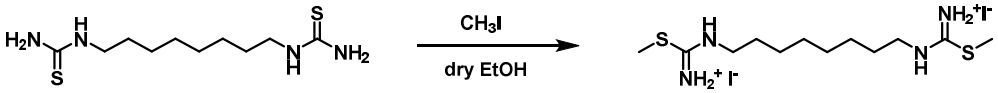
Benzoyl isothiocyanate (9.5 g, 58.2 mmol, 2.1 equiv.) was added dropwise to the solution of the1,8-diaminooctane (4 g, 27.7 mmol, 1 equiv.) in 150 mL of DCM at 0 oC. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with DCM several times. The resulting solid was dried to afford the product as a white solid (11.4 g, 87.4% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.25 (s, 2H), 10.89 (s, 2H), 9.40 – 9.39 (m, 1H), 7.93 (d, *J* = 7.7 Hz, 4H), 7.63 (t, *J* = 7.3 Hz, 2H), 7.51 (t, *J* = 7.5 Hz, 4H), 3.61 (dd, *J* = 12.4, 6.3 Hz, 4H), 1.64 (s, 4H), 1.34 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.44, 168.48, 133.36, 132.72, 128.90, 45.19, 29.02, 28.00, 26.80. ESI-MS: Calculated for $C_{24}H_{31}N_4O_2S_2^+$ ($[M+H]^+$): 471.18; found: 471.11.

Synthesis of the bisthiourea (**M3-2**)



Aqueous sodium hydroxide (5 M, 19.4 mL, 97.0 mmol, 4 equiv.) was added dropwise to the solution of the above bis(benzoylthiourea) **M3-1** (11.4 g, 24.2 mmol, 1 equiv.) in 150 mL of MeOH. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with MeOH several times. The resulting solid was dried to afford the product as a white solid (5.93 g, 93.2% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 – 6.81 (m, 6H), 3.32 (s, 4H), 1.44 (s, 4H), 1.26 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.55, 44.37, 29.21, 26.79. ESI-MS: Calculated for $C_{10}H_{23}N_4S_2^+$ ([M+H]$^+$): 263.13; found: 263.23.

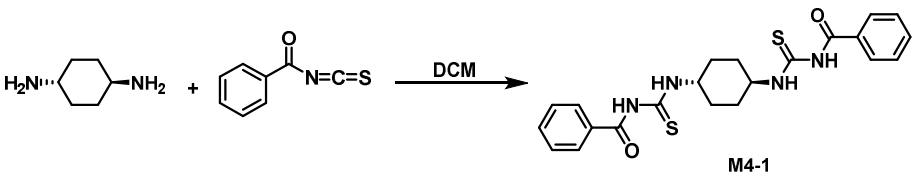
Synthesis of the bis(S-methyl thiouronium iodide) (**M3-3**)



CH_3I (7.7 g, 54.2 mmol, 2.25 equiv.) was added dropwise to the solution of the above bisthiourea **M3-2** (5.93 g, 22.6 mmol, 1 equiv.) in 150 mL of dry EtOH. The resulting mixture was stirred at 35 ^{o}C for 72 h. The reaction was filtered, and the crude product was washed with EtOH several times. The resulting solid was dried to afford the product as a white solid (9.60 g, 77.7% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (d, J = 149.0 Hz, 6H), 3.28 (t, J = 6.5 Hz, 4H), 2.61 (s, 6H), 1.52 (s, 4H), 1.27 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.72, 44.23, 28.94, 27.92, 26.48, 14.28. High resolution ESI-MS: Calculated for $C_{12}H_{27}N_4S_2^+$ ([M+H]$^+$): 291.1671; obtained 291.1674.

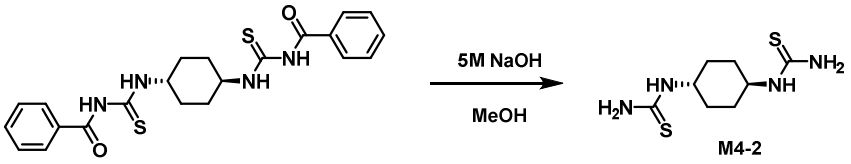
Synthesis of **M4** monomer

Synthesis of the bis(benzoylthiourea) (**M4-1**)



Benzoyl isothiocyanate (6.00 g, 36.8 mmol, 2.1 equiv.) was added dropwise to the solution of the trans-1,4-diaminocyclohexane (2.00 g, 17.5 mmol, 1 equiv.) in 150 mL of DCM at 0 ^{o}C. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with DCM several times. The resulting solid was dried to afford the product as a white solid (5.60 g, 72.6 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 2H), 10.85 (d, J = 7.7 Hz, 2H), 7.93 (d, J = 7.5 Hz, 4H), 7.64 (t, J = 7.4 Hz, 2H), 7.52 (t, J = 7.7 Hz, 4H), 4.23 (s, 2H), 2.14 (d, J = 6.1 Hz, 4H), 1.52 (dd, J = 19.7, 10.0 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.63, 168.70, 133.42, 132.69, 128.92, 53.18, 29.73. ESI-MS: Calculated for $C_{22}H_{25}N_4O_2S_2^+$ ([M+H]$^+$): 441.14; found: 441.11.

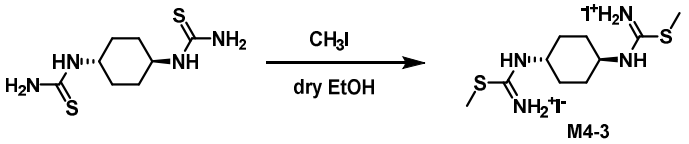
Synthesis of the bisthiourea (**M4-2**)



Aqueous sodium hydroxide (5 M, 10.2 mL, 50.8 mmol, 4 equiv.) was added dropwise to the solution of the above bis(benzoylthiourea) **M4-1** (5.60 g, 12.7 mmol, 1 equiv.) in 150 mL of MeOH. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with MeOH several times. The resulting solid was dried to afford the product as a white solid (2.57 g, 87.1 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 – 6.65 (m, 6H), 3.38 – 3.12 (m, 2H), 1.84 (d, J = 50.8 Hz,

4H), 1.19 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 182.51, 52.40, 31.23. ESI-MS: Calculated for $C_8H_{17}N_4S_2^+$ ([M+H]$^+$): 233.08; found: 233.00.

Synthesis of the bis(S-methyl thiouronium iodide) (**M4-3**)

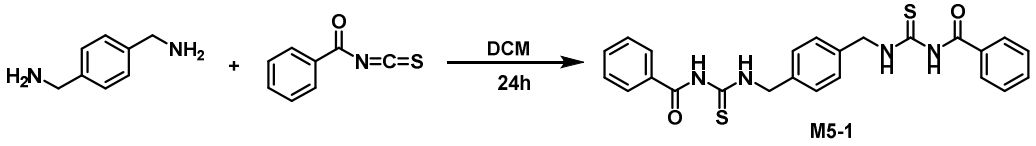


M4-3

CH_3I (3.76 g, 26.5 mmol, 2.4 equiv.) was added dropwise to the solution of the above bisthiourea **M4-2** (2.57 g, 11.1 mmol, 1 equiv.) in 150 mL of dry EtOH. The resulting mixture was stirred at 35 °C for 72 h. The reaction was filtered, and the crude product was washed with EtOH several times. The resulting solid was dried to afford the product as a white solid (4.66 g, 81.6 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (d, *J* = 133.6 Hz, 6H), 3.59 (s, 2H), 2.62 (s, 6H), 1.91 (d, *J* = 6.1 Hz, 4H), 1.46 – 1.34 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.15, 52.23, 29.86, 14.43. High resolution ESI-MS: Calculated for $C_{10}H_{21}N_4S_2^+$ ([M+H]$^+$): 261.1202 ; obtained 261.1211.

Synthesis of **M5** monomer

Synthesis of the bis(benzoylthiourea) (**M5-1**)

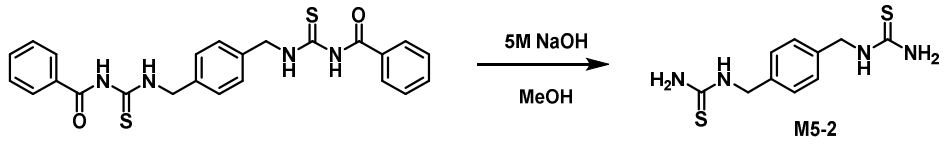


M5-1

Benzoyl isothiocyanate (6.54 g, 40.1 mmol, 2.1 equiv.) was added dropwise to the solution of the 1,4-(aminomethyl)benzene (2.6 g, 19.1 mmol, 1 equiv.) in 150 mL of DCM at 0 °C. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with DCM several times. The resulting solid was dried to afford the product as a white solid (7.77 g, 87.9 % yield). ^{1}H NMR (400 MHz,

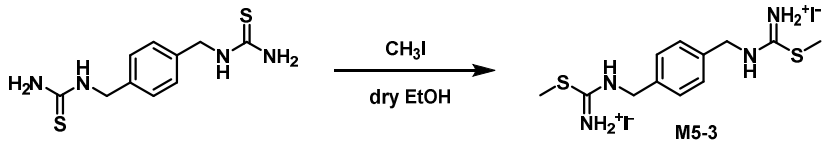
DMSO-d_6) δ 11.22 (s, 2H), 10.85 (s, 2H), 7.94 (d, *J* = 7.7 Hz, 4H), 7.64 (t, *J* = 7.4 Hz, 2H), 7.51 (t, *J* = 7.7 Hz, 4H), 7.40 (d, *J* = 7.1 Hz, 4H), 4.87 (d, *J* = 4.7 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.98, 168.51, 136.89, 133.41, 132.70, 128.93, 128.33, 48.36. ESI-MS: Calculated for $C_{24}H_{22}N_4O_2S_2$ (M): 462.12; found: 462.33.

Synthesis of the bisthiourea (**M5-2**)



Aqueous sodium hydroxide (5 M, 13.4 mL, 67.2 mmol, 4 equiv.) was added dropwise to the solution of the above bis(benzoylthiourea) **M5-1** (7.77 g, 16.8 mmol, 1 equiv.) in 150 mL of MeOH. The resulting mixture was stirred at room temperature for 24 h. The reaction was filtered, and the crude product was washed with MeOH several times. The resulting solid was dried to afford the product as a white solid (3.90 g, 91.3% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 2H), 7.25 (s, 4H), 7.06 (s, 4H), 4.59 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.91, 138.34, 127.79, 47.71. ESI-MS: Calculated for $C_{10}H_{14}N_4S_2$ (M): 254.07; found: 254.16.

Synthesis of the bis(S-methyl thiouronium iodide) (**M5-3**)

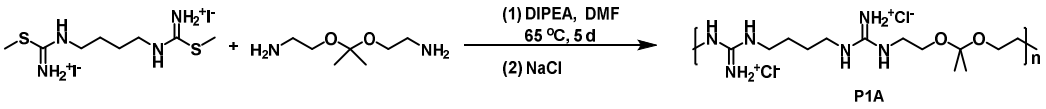


CH_3I (5.22 g, 36.8 mmol, 2.25 equiv.) was added dropwise to the solution of the above bisthiourea **M5-2** (3.90 g, 15.3 mmol, 1 equiv.) in 150 mL of dry EtOH. The resulting mixture was stirred at 35 °C for 72 h. The reaction was filtered, and the crude product was washed with EtOH several times. The resulting solid was dried to afford the product

as a white solid (5.00 g, 60.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (d, J = 256.2 Hz, 6H), 7.36 (s, 4H), 4.58 (s, 4H), 2.65 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 135.51, 128.30, 46.67, 14.29. High resolution ESI-MS: Calculated for $C_{12}H_{19}N_4S_2^+$ ([M+H]$^+$): 283.1045 ; obtained 283.1054.

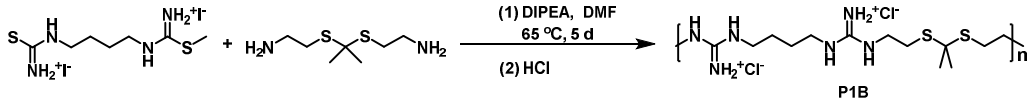
Synthesis of degradable polymer[6]

Synthesis of **P1A**



The thiouronium iodide monomer **M1** (50.0 mg, 0.102 mmol, 1 equiv.), ketal diamine **A** (16.5 mg, 0.102 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (71.0 µL, 0.408 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added NaCl solution (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (5.0 mg).

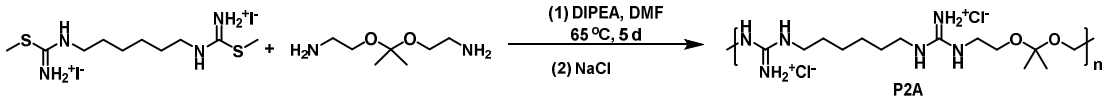
Synthesis of **P1B**



The thiouronium iodide monomer **M1** (50.0 mg, 0.102 mmol, 1 equiv.), thioketal diamine **B** (19.8 mg, 0.102 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (71.0 µL, 0.408 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The

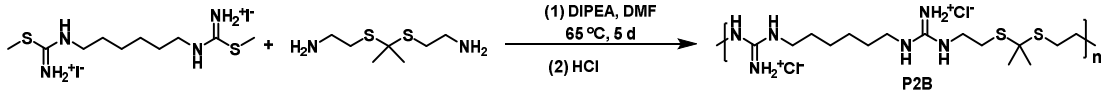
resulting mixture was added 1M HCl (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (6.0 mg).

Synthesis of **P2A**



The thiouronium iodide monomer **M2** (50.0 mg, 0.0965 mmol, 1 equiv.), ketal diamine **A** (15.6 mg, 0.0965 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (67.1 µL, 0.386 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added NaCl solution (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (10.0 mg).

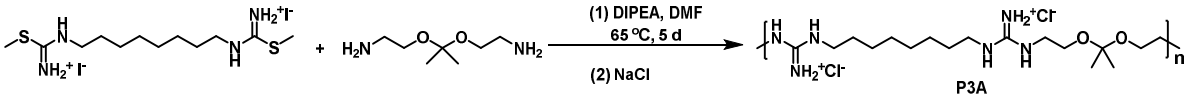
Synthesis of **P2B**



The thiouronium iodide monomer **M2** (50.0 mg, 0.0965 mmol, 1 equiv.), thioketal diamine **B** (18.7 mg, 0.0965 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (67.1 µL, 0.386 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added 1M HCl (for counterion exchange) and dialyzed against water

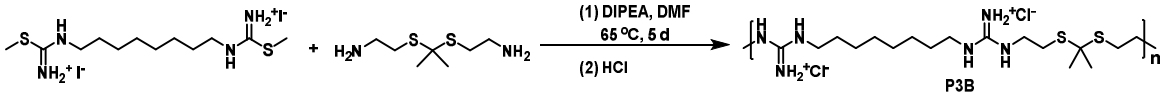
(MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (7.0 mg).

Synthesis of **P3A**



The thiouronium iodide monomer **M3** (50.0 mg, 0.0916 mmol, 1 equiv.), ketal diamine **A** (14.8 mg, 0.0916 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (63.7 µL, 0.366 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added NaCl solution (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (7.0 mg).

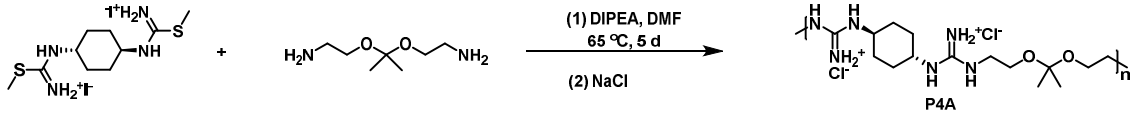
Synthesis of **P3B**



The thiouronium iodide monomer **M3** (50.0 mg, 0.0916 mmol, 1 equiv.), thioketal diamine **B** (17.8 mg, 0.0916 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (63.7 µL, 0.366 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added 1M HCl (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The

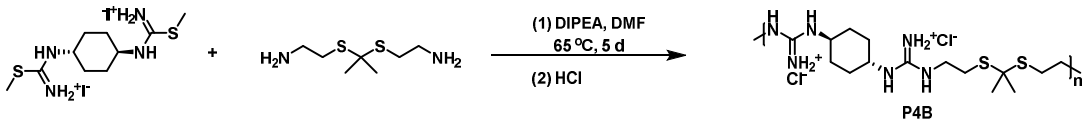
desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (8.0 mg).

Synthesis of **P4A**



The thiouronium iodide monomer **M4** (50.0 mg, 0.0969 mmol, 1 equiv), ketal diamine **A** (15.7 mg, 0.0969 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (67.4 µL, 0.388 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added NaCl solution (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (3.0 mg).

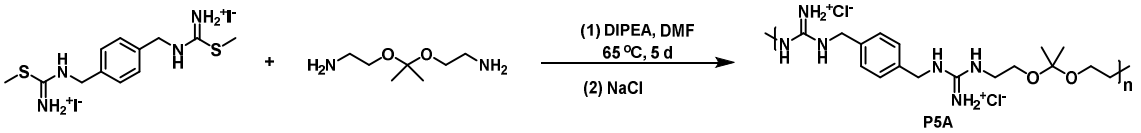
Synthesis of **P4B**



The thiouronium iodide monomer **M4** (50.0 mg, 0.0969 mmol, 1 equiv.), thioketal diamine **B** (18.8 mg, 0.0969 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (67.4 µL, 0.388 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added 1M HCl (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The

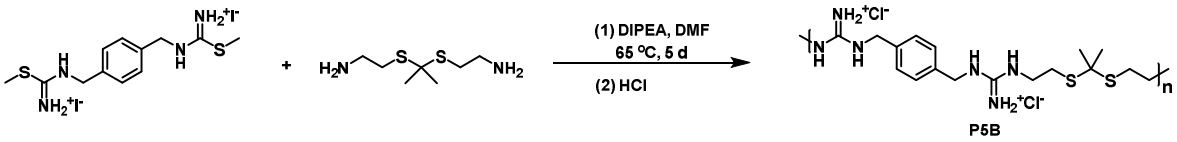
desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (5 mg).

Synthesis of **P5A**



The thiouronium iodide monomer **M5** (50.0 mg, 0.0929 mmol, 1 equiv.), ketal diamine **A** (15.1 mg, 0.0929 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (64.6 µL, 0.372 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added NaCl solution (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (6.0 mg).

Synthesis of **P5B**



The thiouronium iodide monomer **M5** (50.0 mg, 0.0929 mmol, 1 equiv.), thioketal diamine **B** (18.0 mg, 0.0929 mmol, 1 equiv.) and *N*,*N*-diisopropylethylamine (64.6 µL, 0.372 mmol, 4 equiv.) were added into a 7-mL glass vial containing anhydrous DMF as the solvent (2 mL). The vial was sealed, and the reaction was stirred at 65 °C for 5 d. The resulting mixture was added 1M HCl (for counterion exchange) and dialyzed against water (MWCO for membrane = 1 kDa) to remove small molecules and very short oligomers. The

desired product was obtained through lyophilization of the dialyzed solution. Product was a white solid (4.0 mg).

Additional Figures and Tables

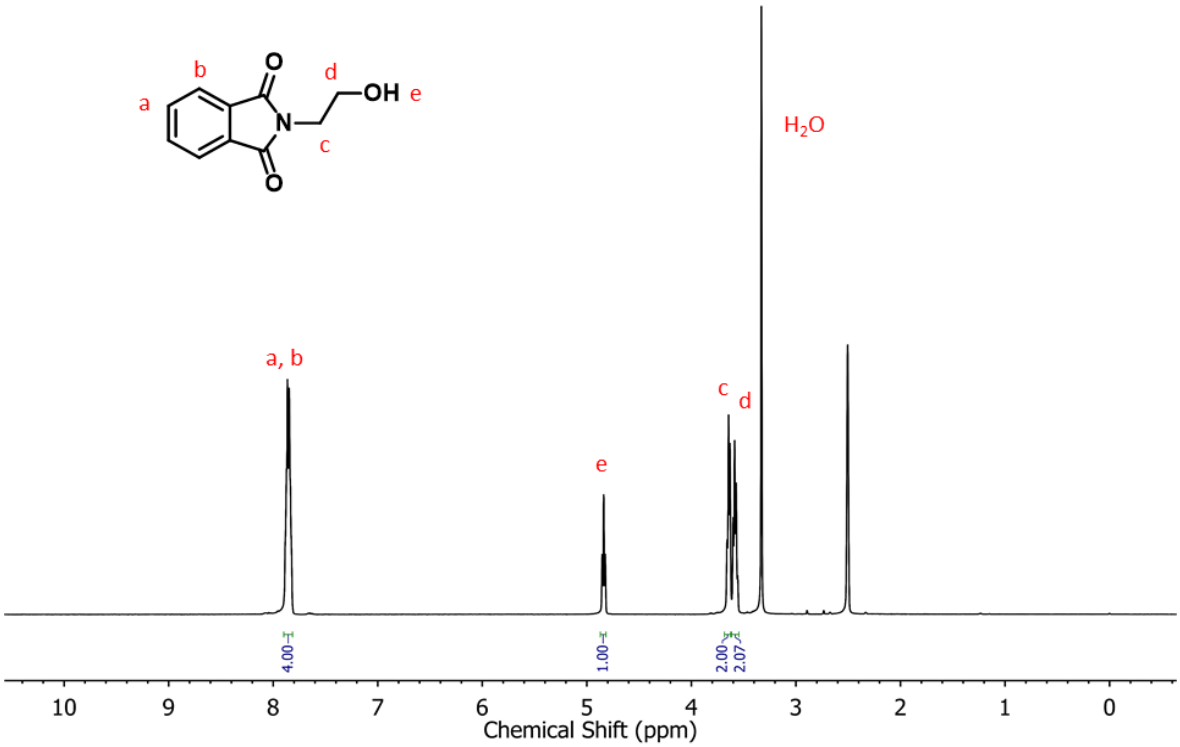


Fig. S1. ^{1}H NMR characterization of A1.

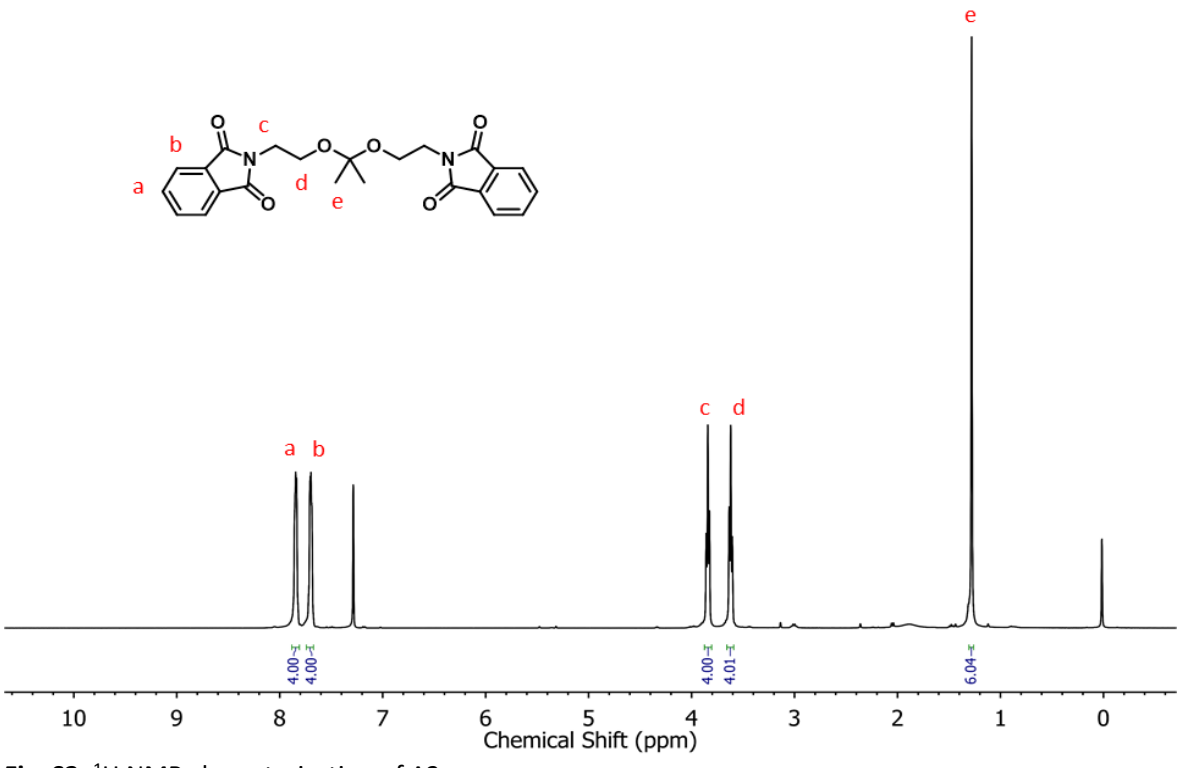


Fig. S2. ^{1}H NMR characterization of A2.

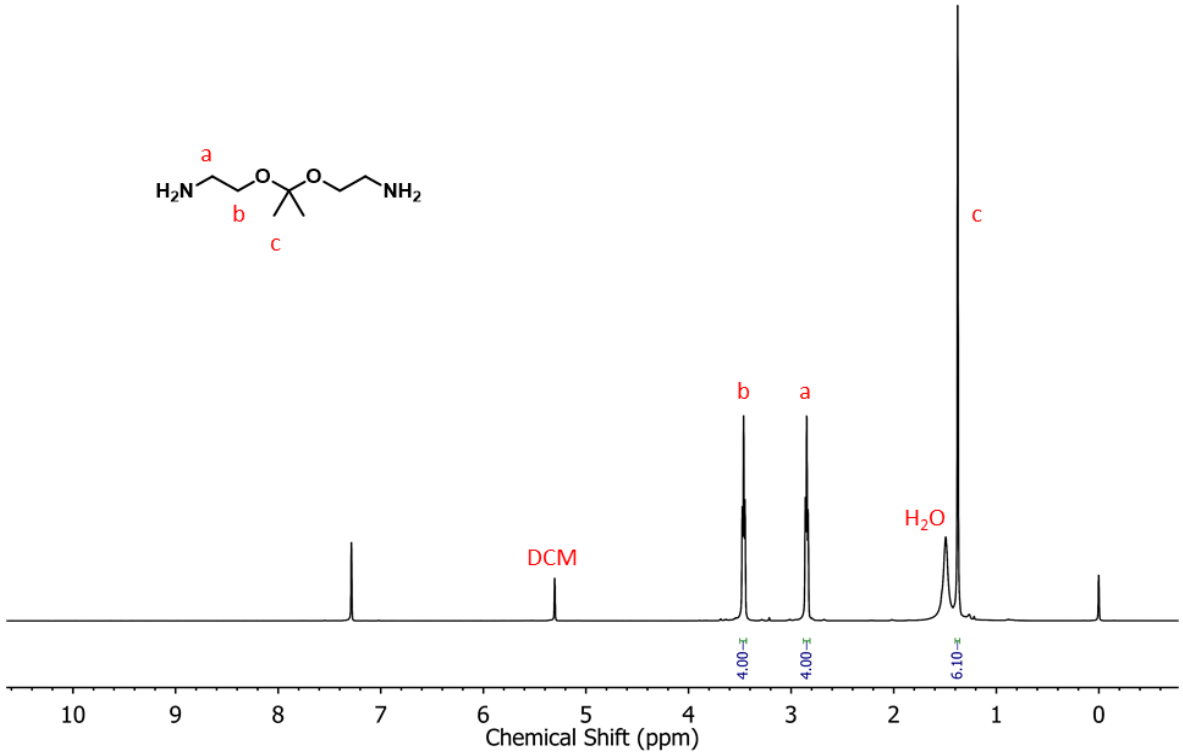


Fig. S3. ^{1}H NMR characterization of A3.

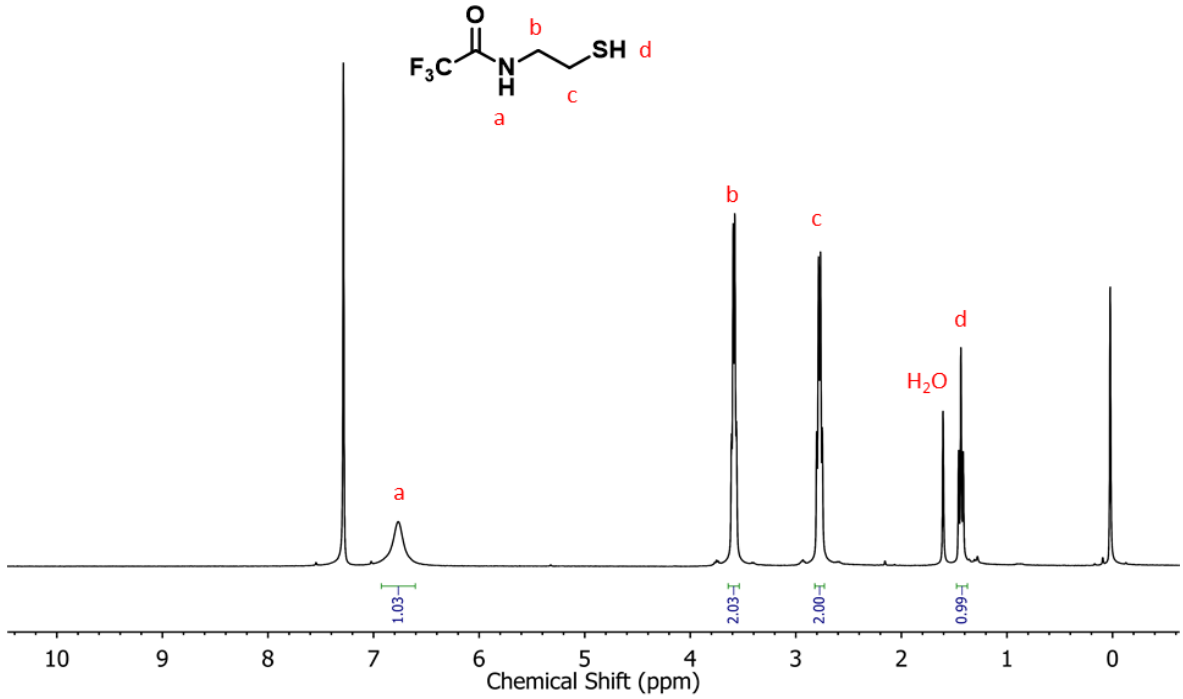


Fig. S4. ^{1}H NMR characterization of B1.

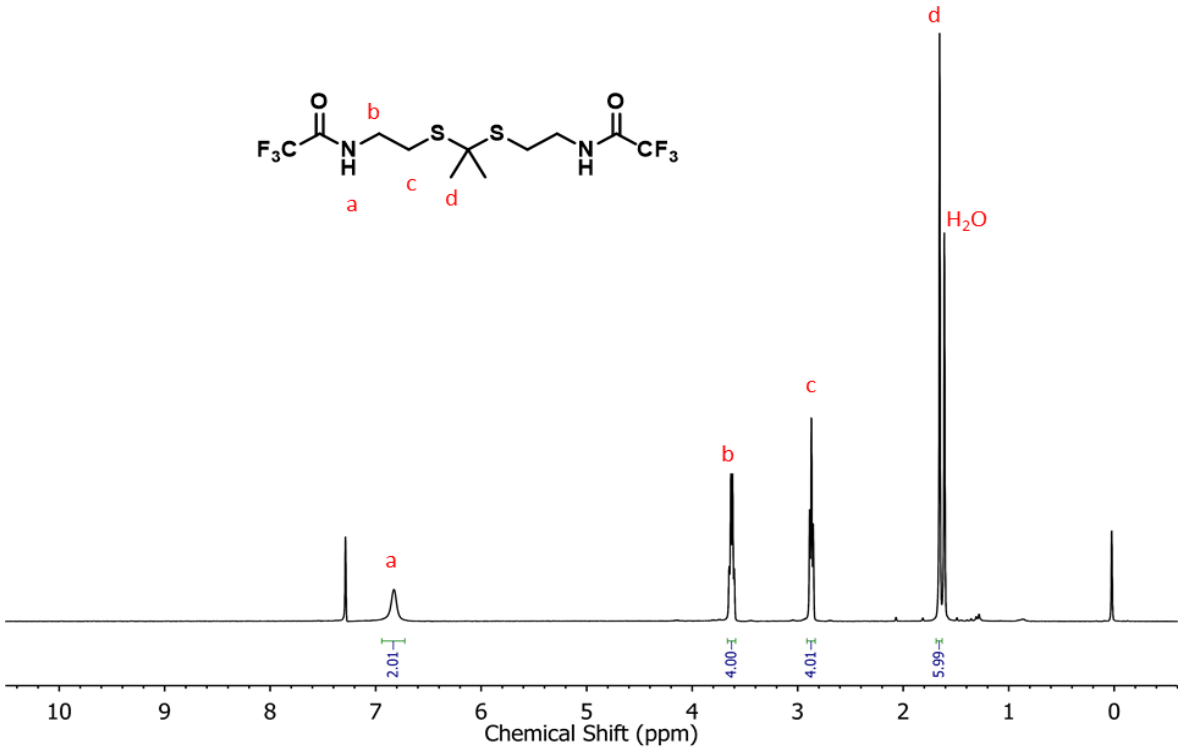


Fig. S5. ^{1}H NMR characterization of·B2.

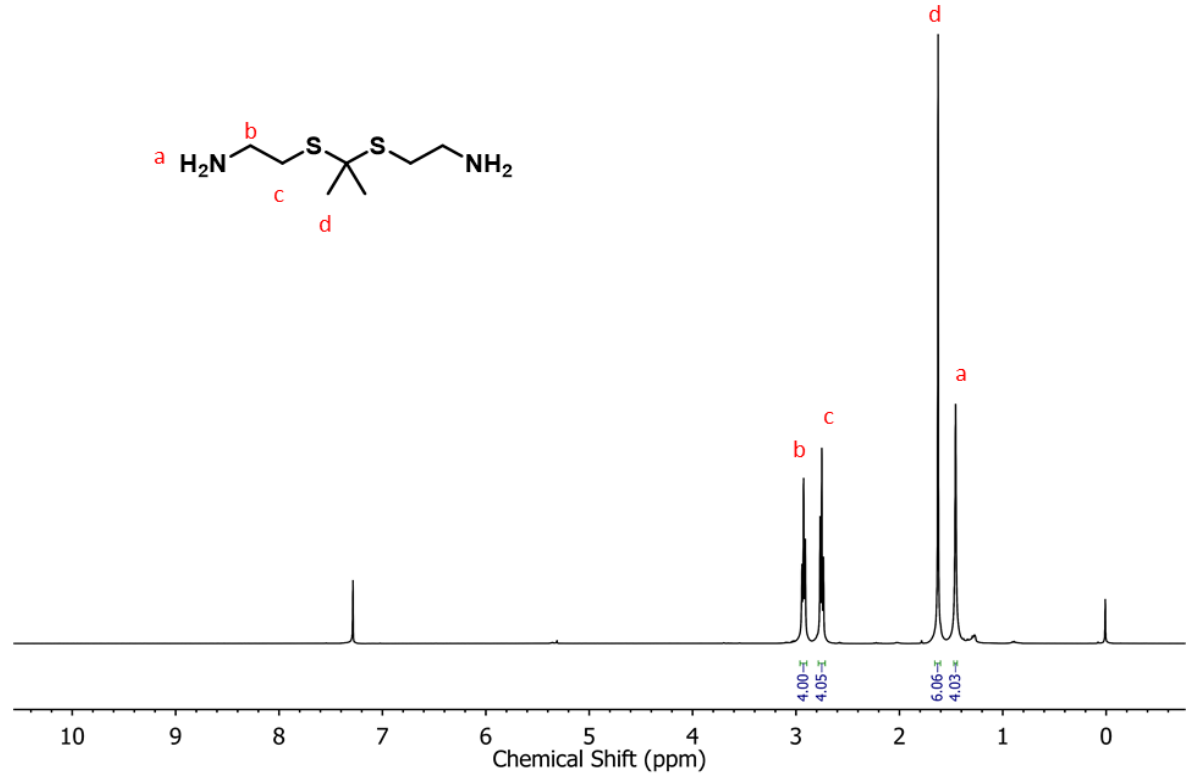


Fig. S6. ^{1}H NMR characterization of B3.

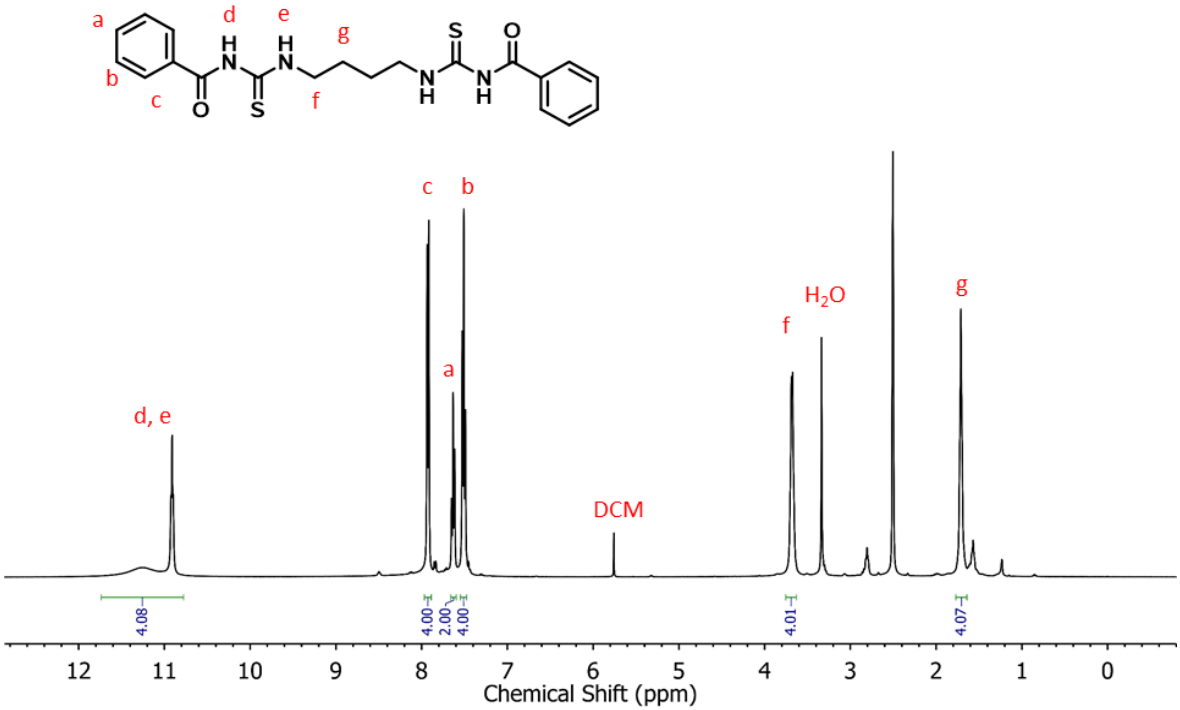


Fig. S7. ^{1}H NMR characterization of M1-1.

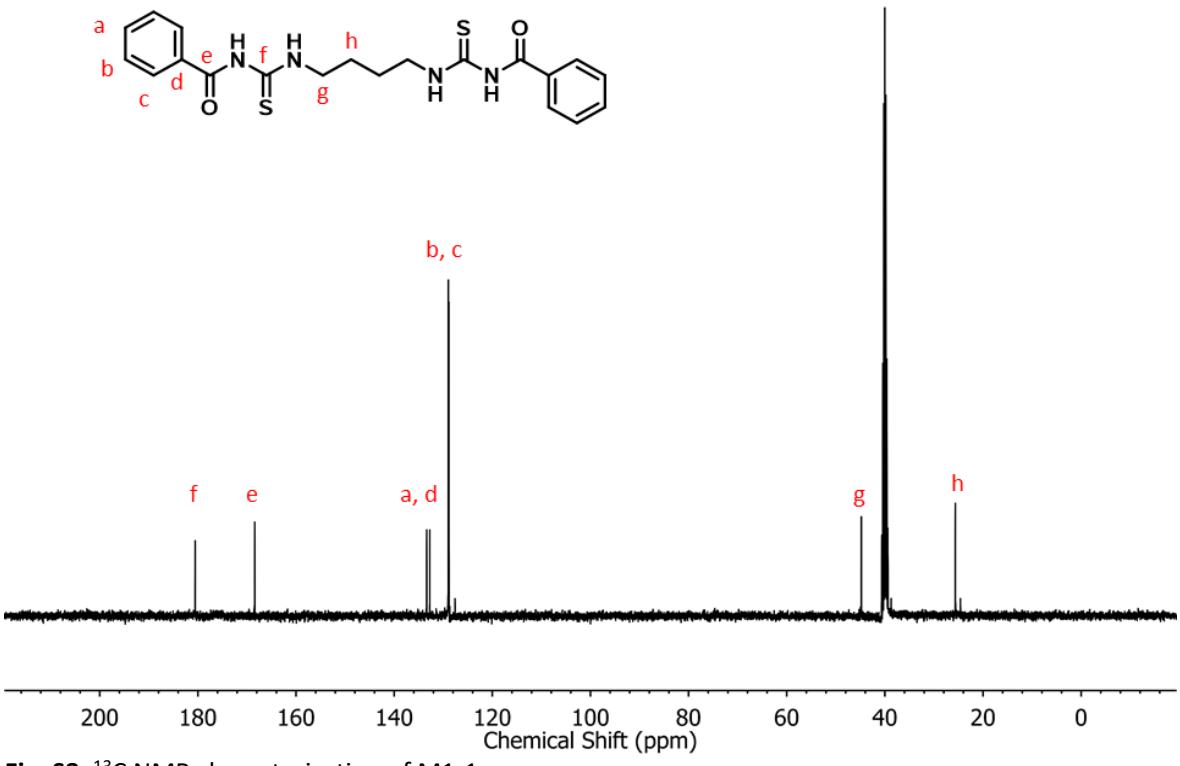


Fig. S8. ^{13}C NMR characterization of M1-1.

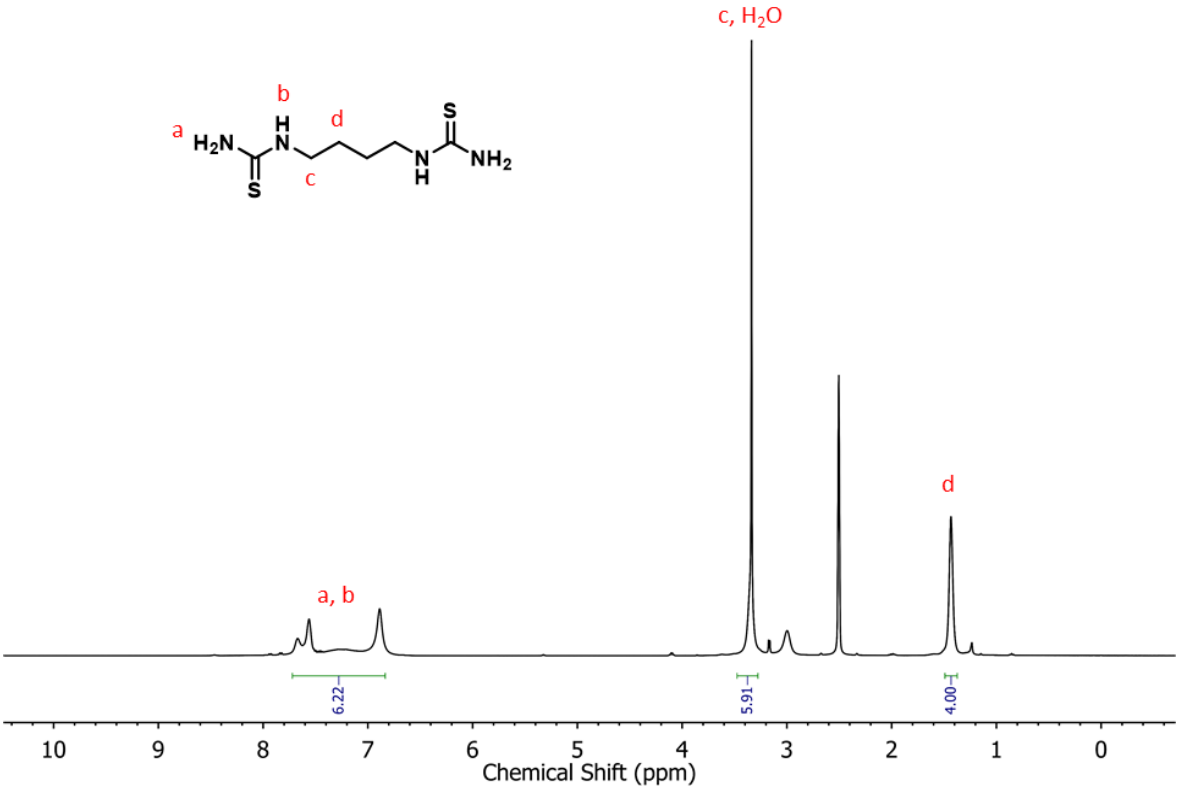


Fig. S9. ^{1}H NMR characterization of M1-2.

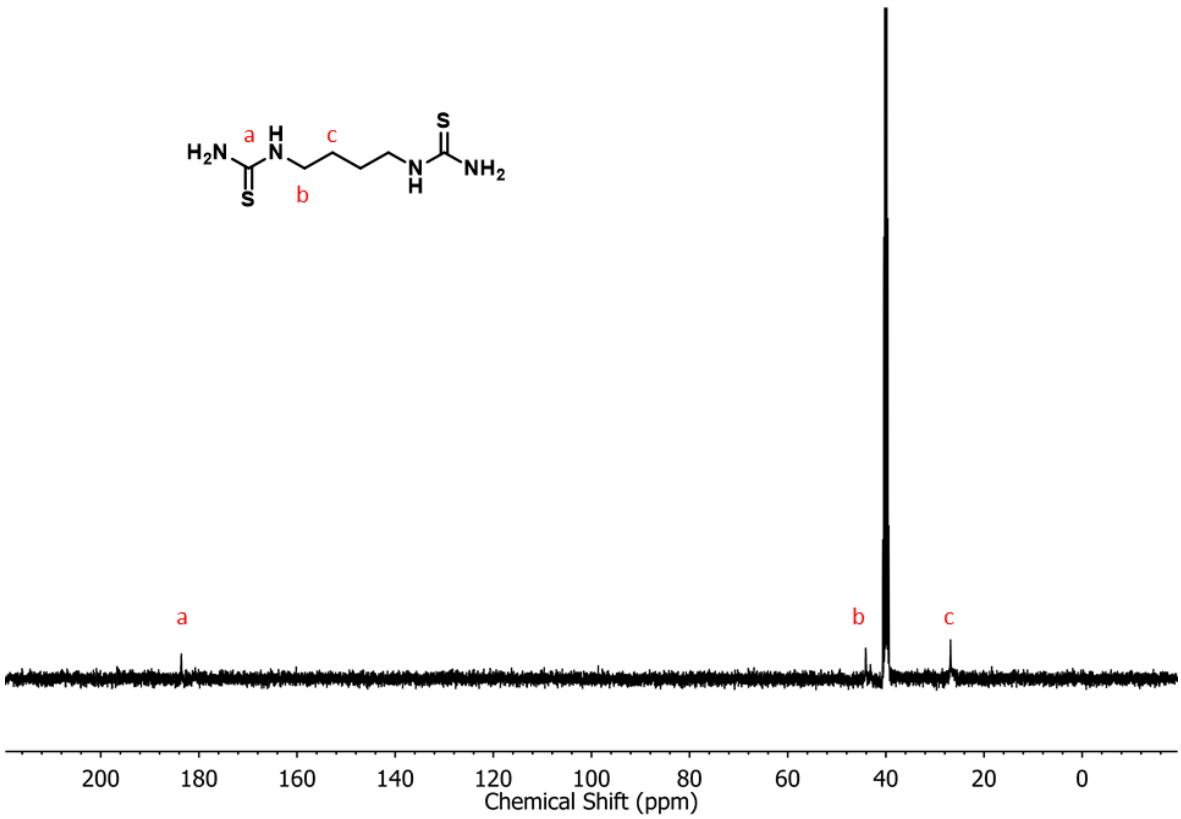


Fig. S10. ^{13}C NMR characterization of M1-2.

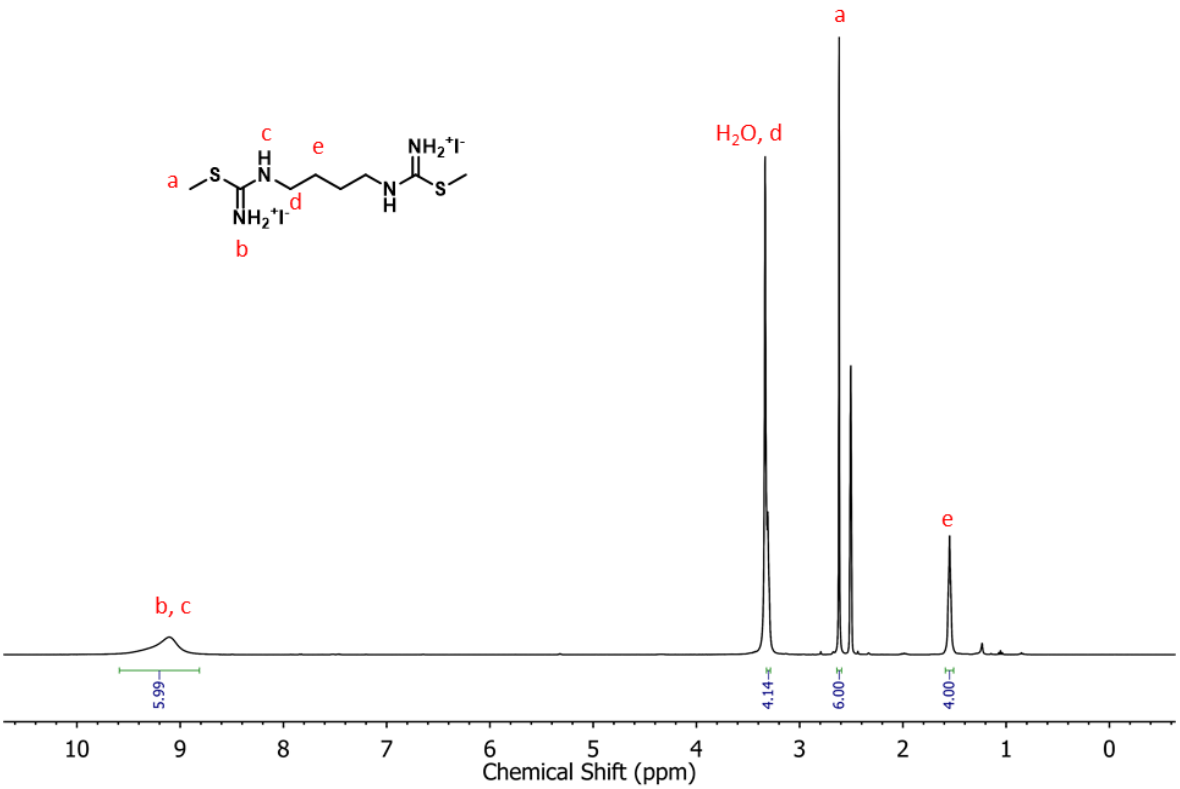


Fig. S11. ^{1}H NMR characterization of M1-3.

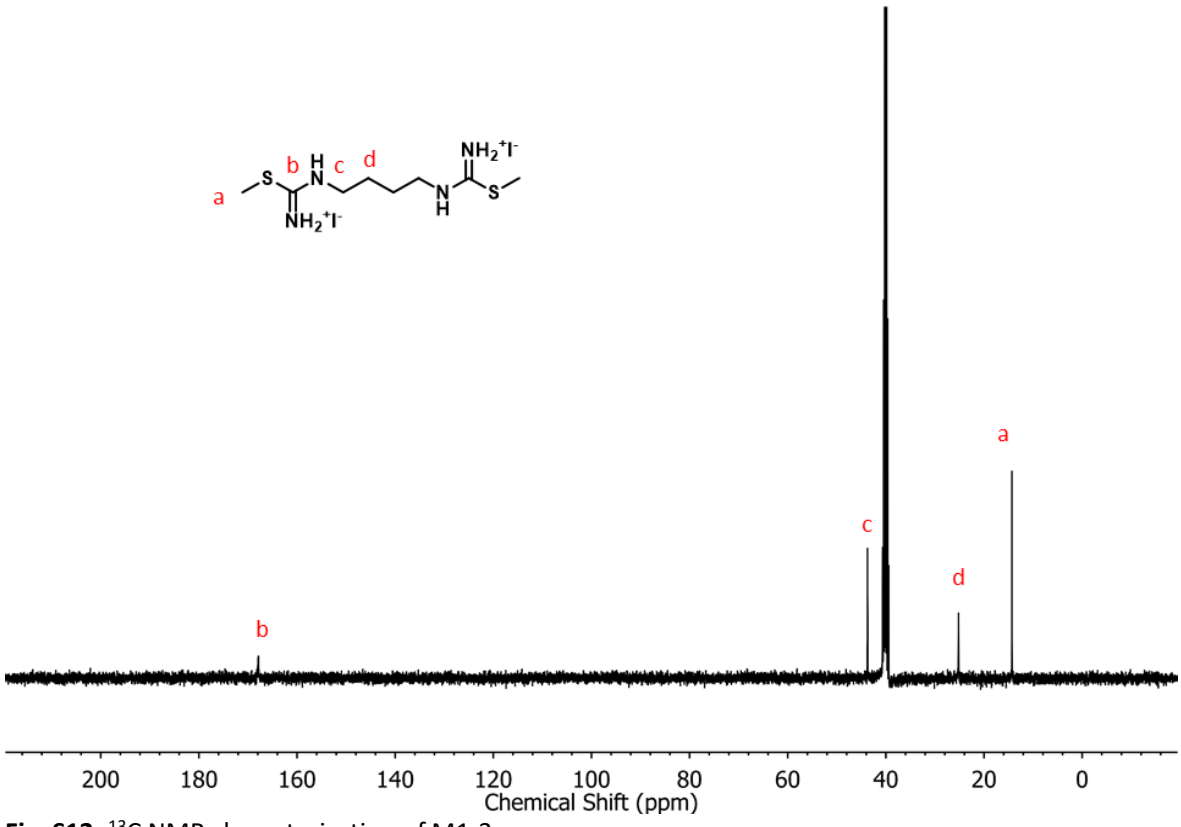


Fig. S12. ^{13}C NMR characterization of M1-3.

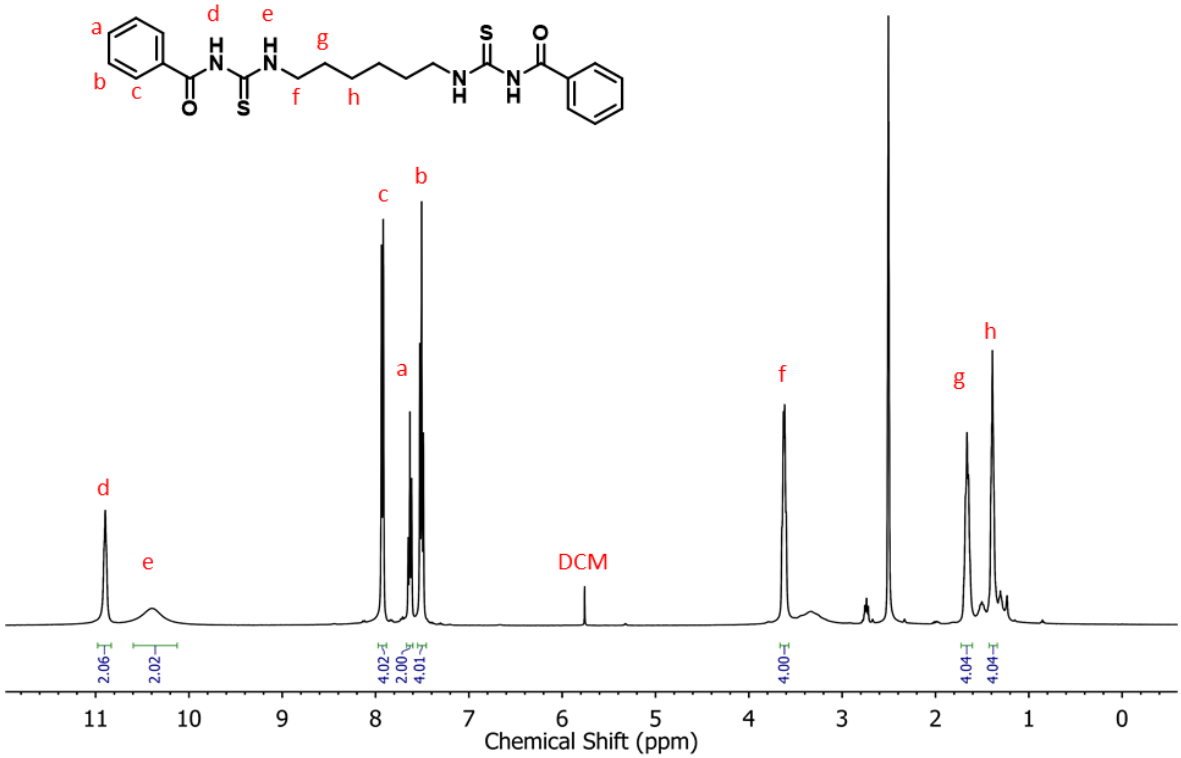


Fig. S13. ^{1}H NMR characterization of M2-1.

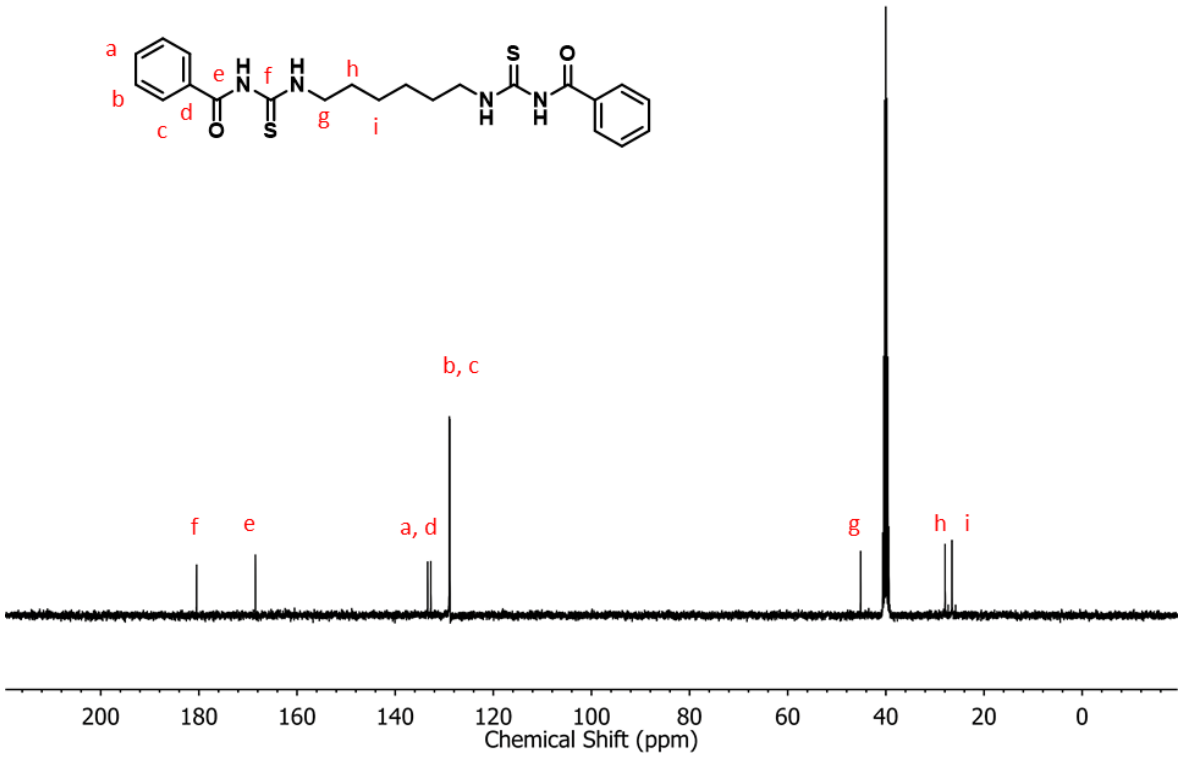


Fig. S14. ^{13}C NMR characterization of M2-1.

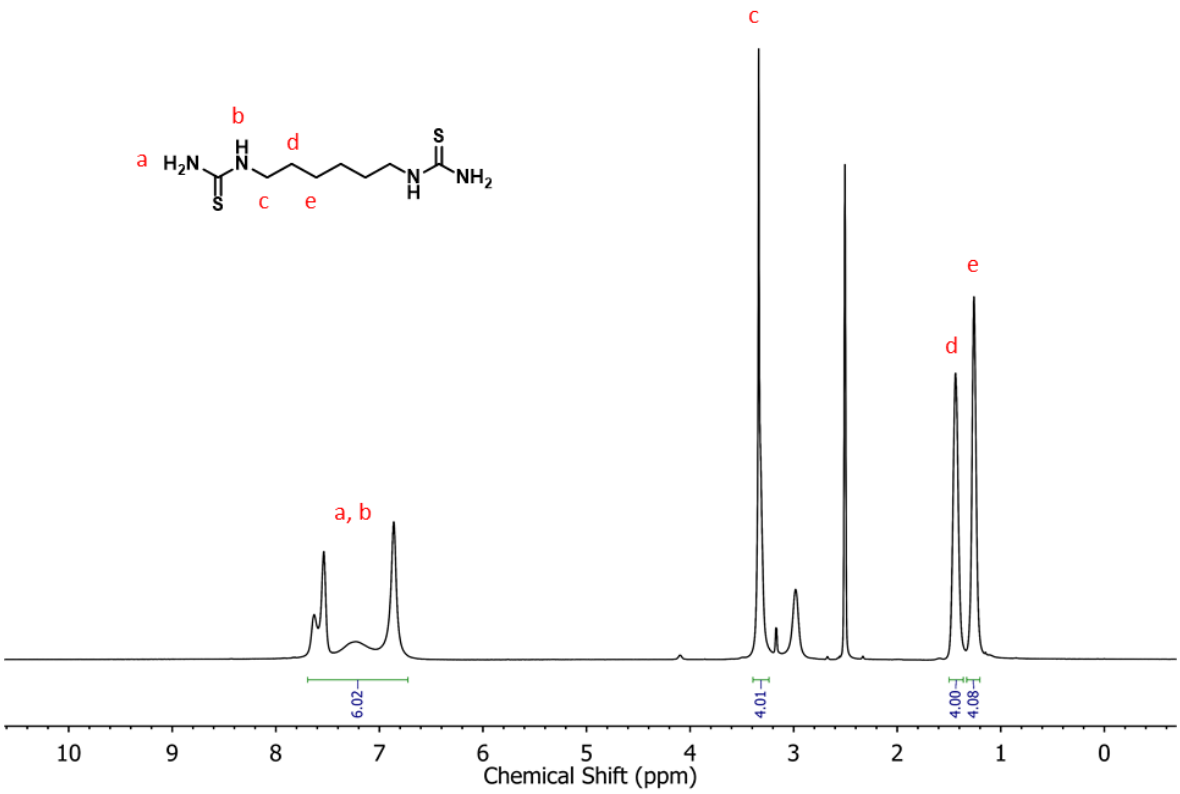


Fig. S15. ^{1}H NMR characterization of M2-2.

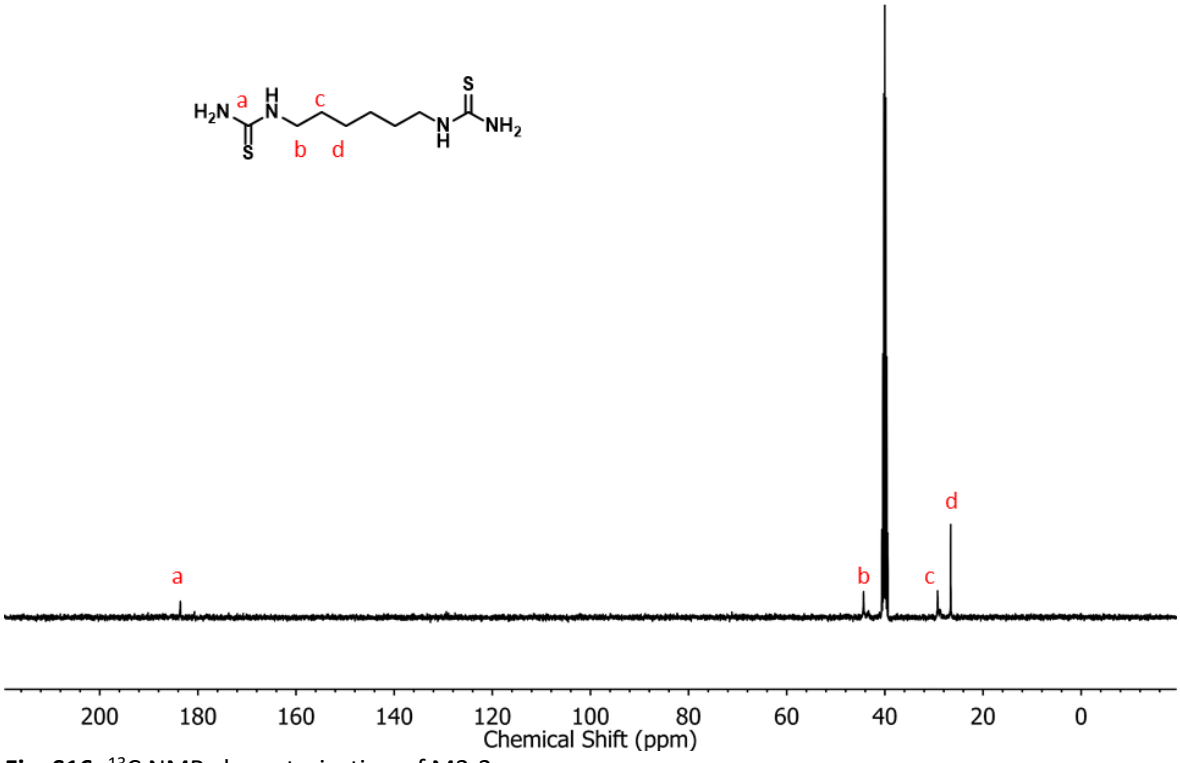


Fig. S16. ^{13}C NMR characterization of M2-2.

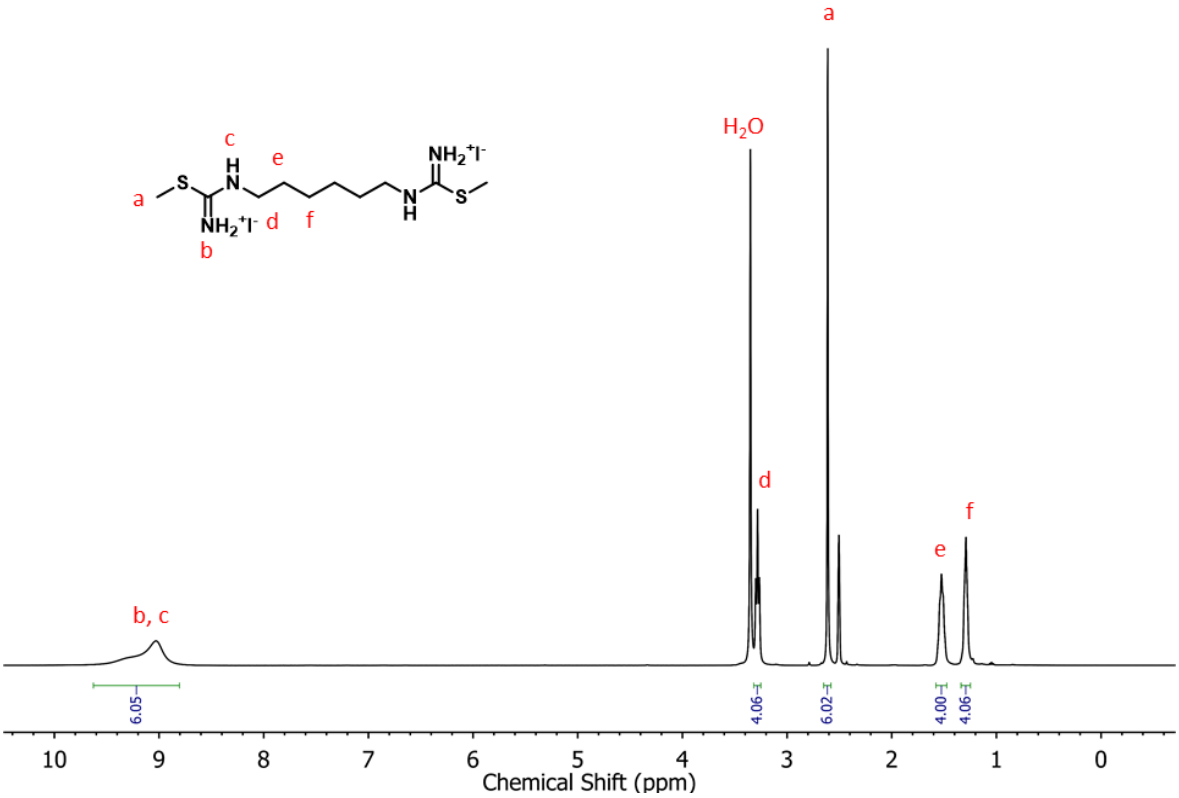


Fig. S17. ^{1}H NMR characterization of M2-3.

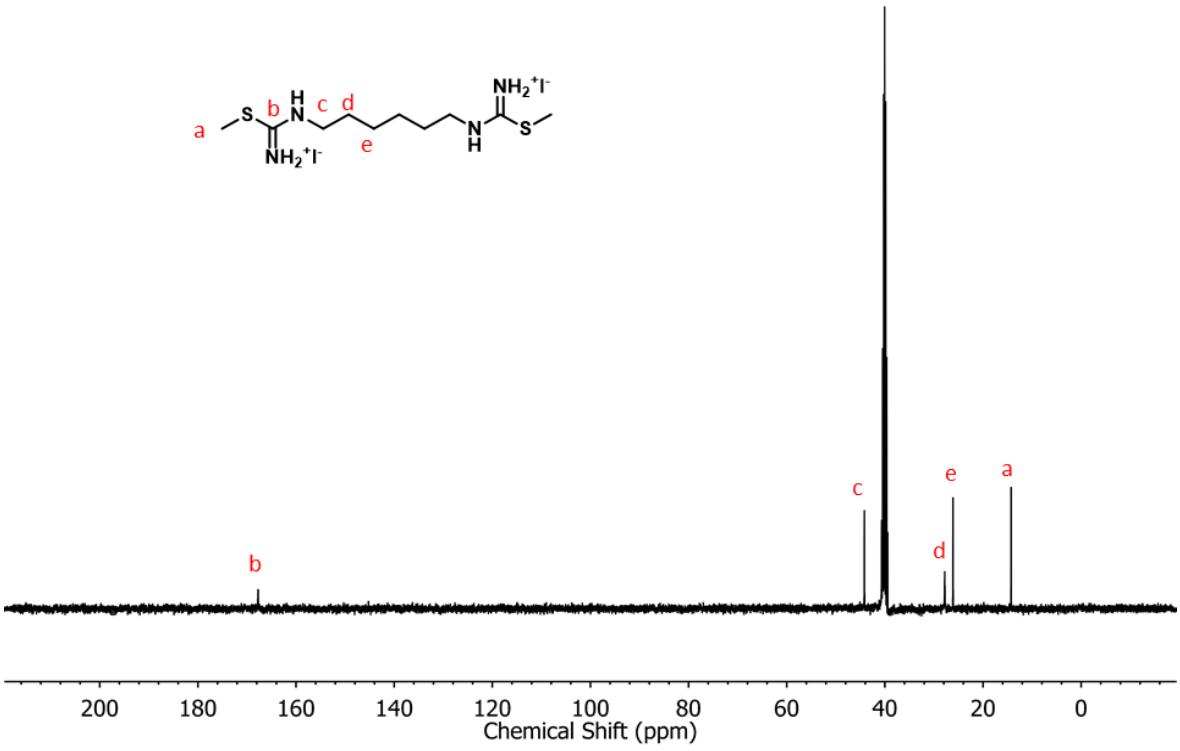


Fig. S18. ^{13}C NMR characterization of M2-3.

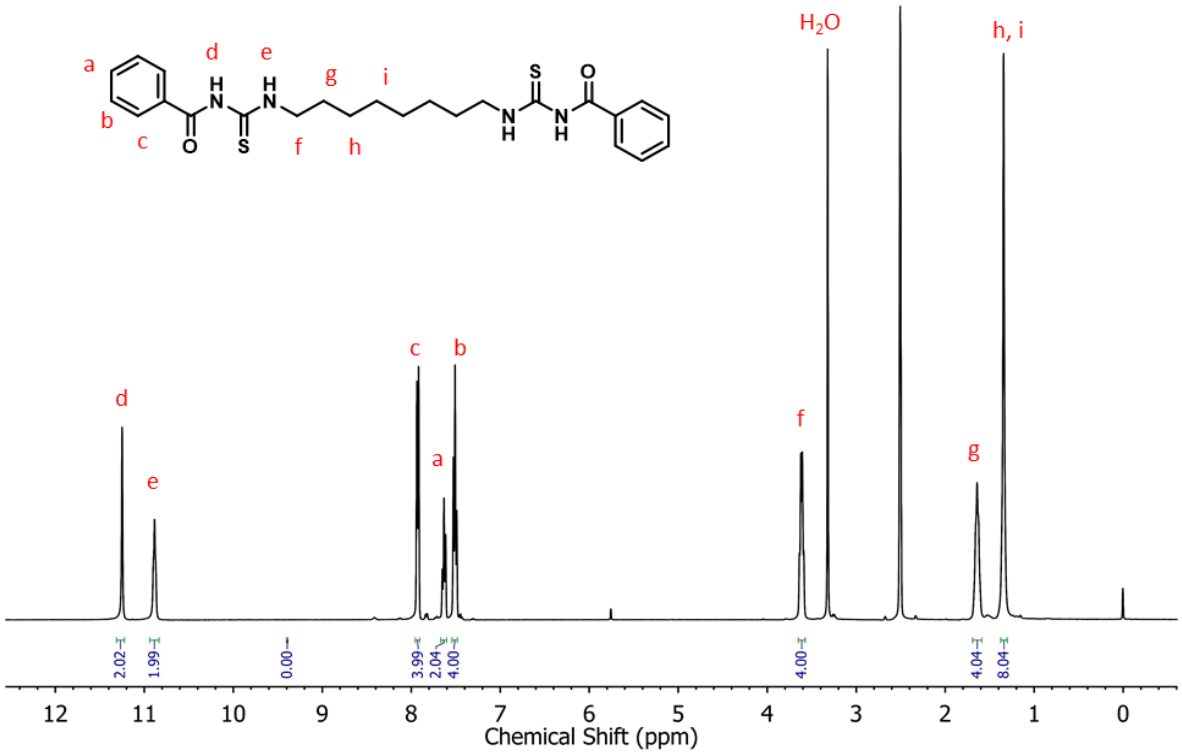


Fig. S19. ^{1}H NMR characterization of M3-1.

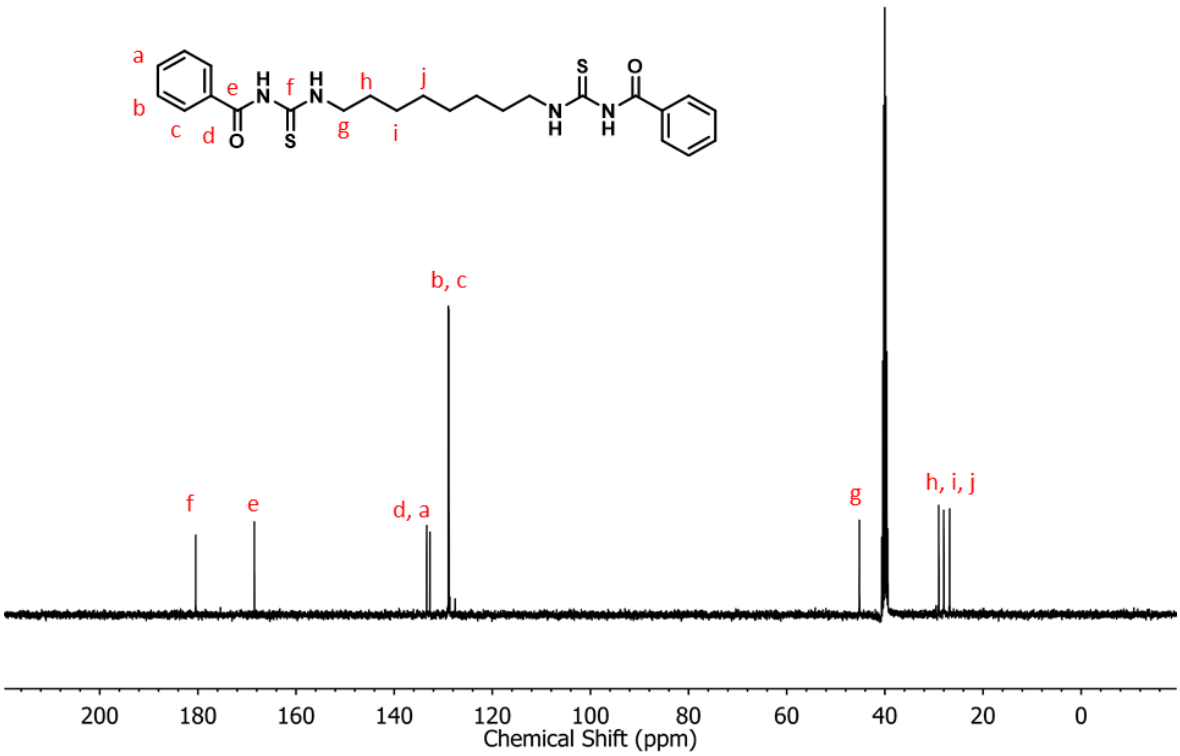


Fig. S20. ^{13}C NMR characterization of M3-1.

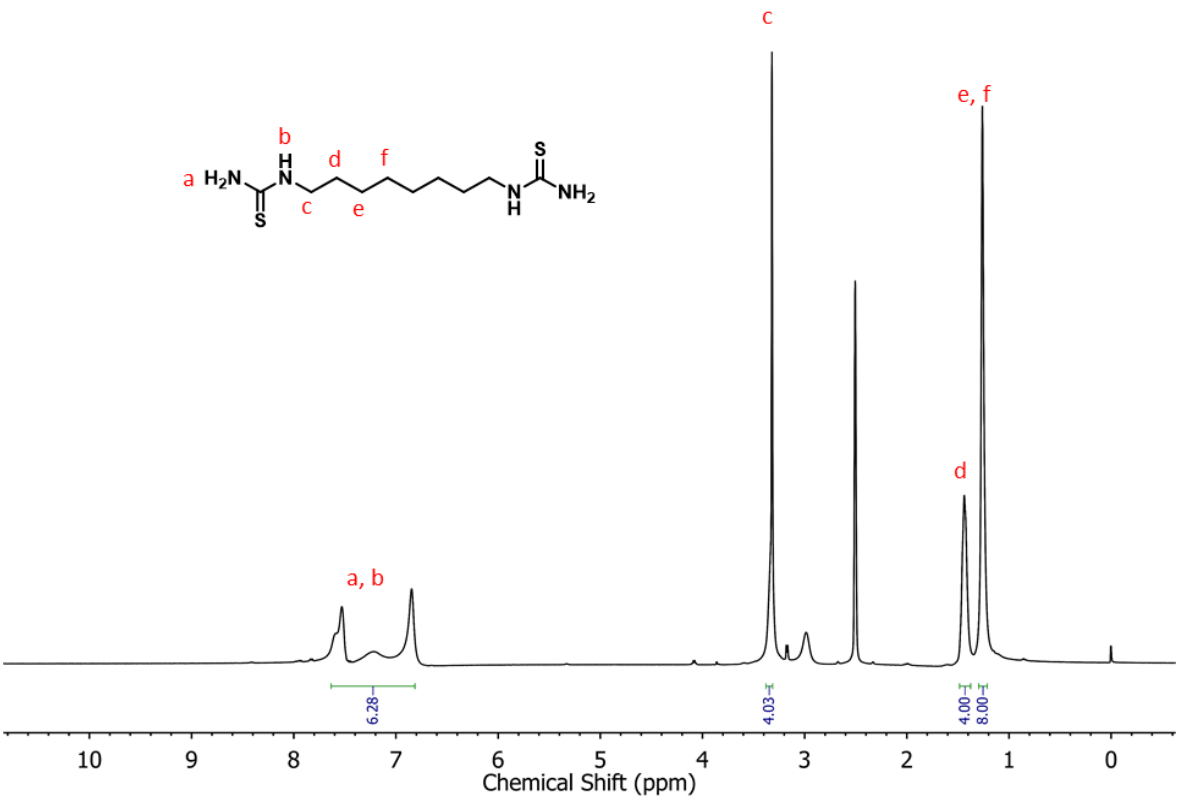


Fig. S21. ^{1}H NMR characterization of M3-2.

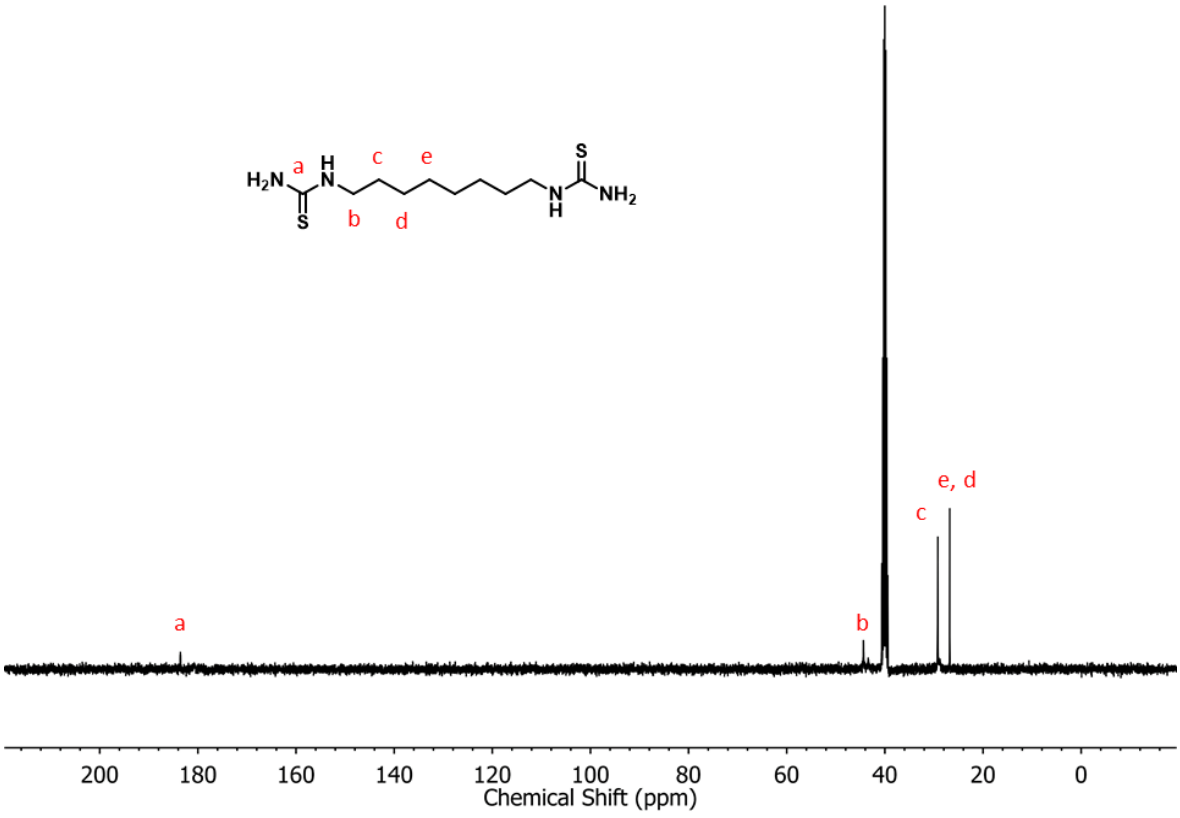


Fig. S22. ^{13}C NMR characterization of M3-2.

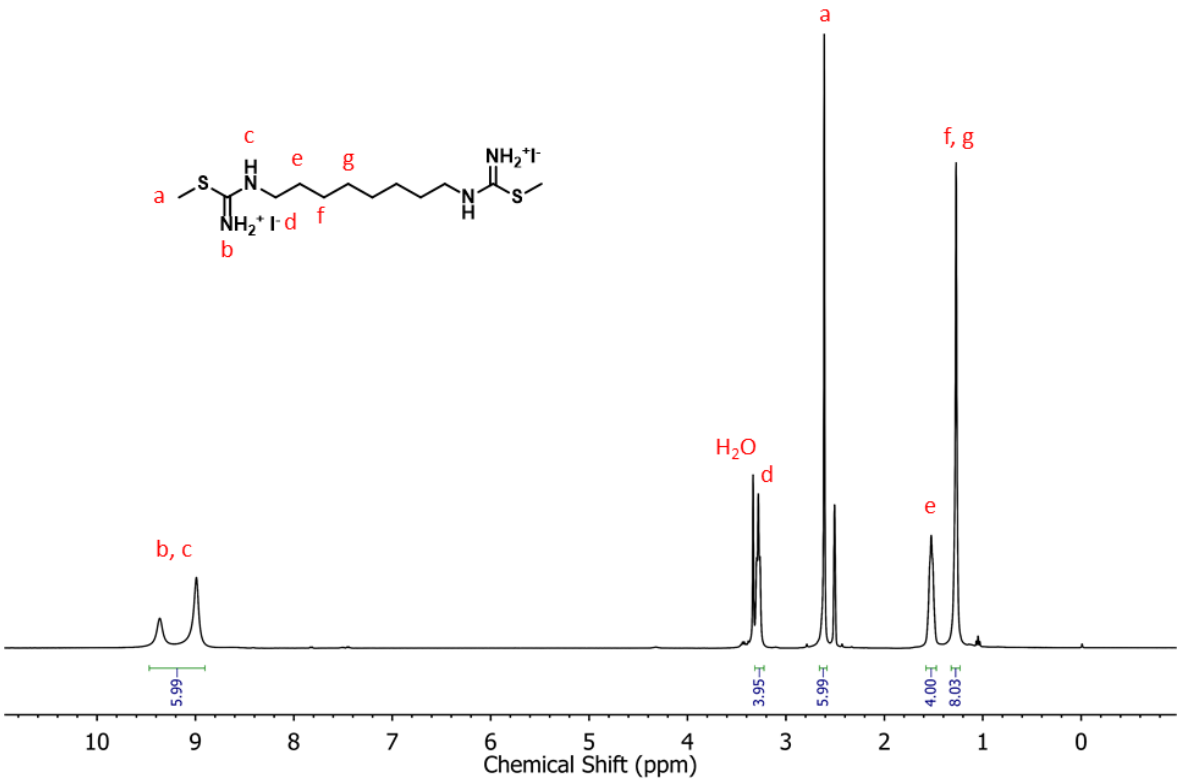


Fig. S23. ^{1}H NMR characterization of M3-3.

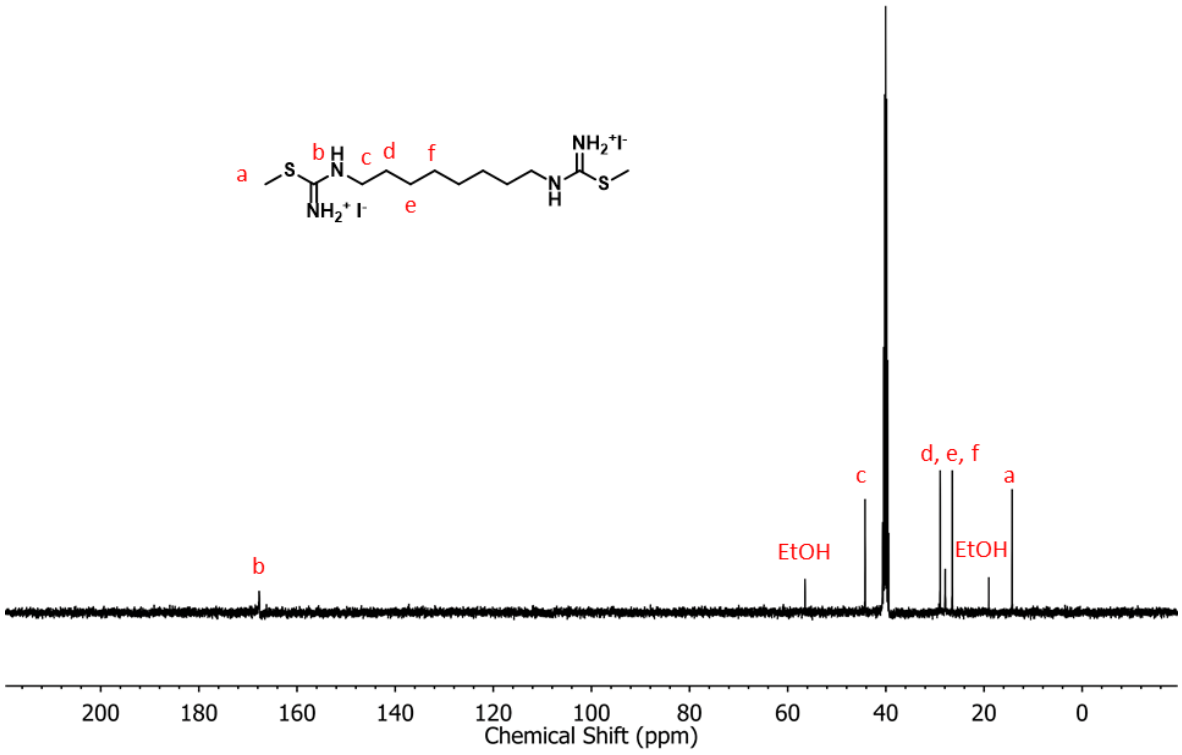


Fig. S24. ^{13}C NMR characterization of M3-3.

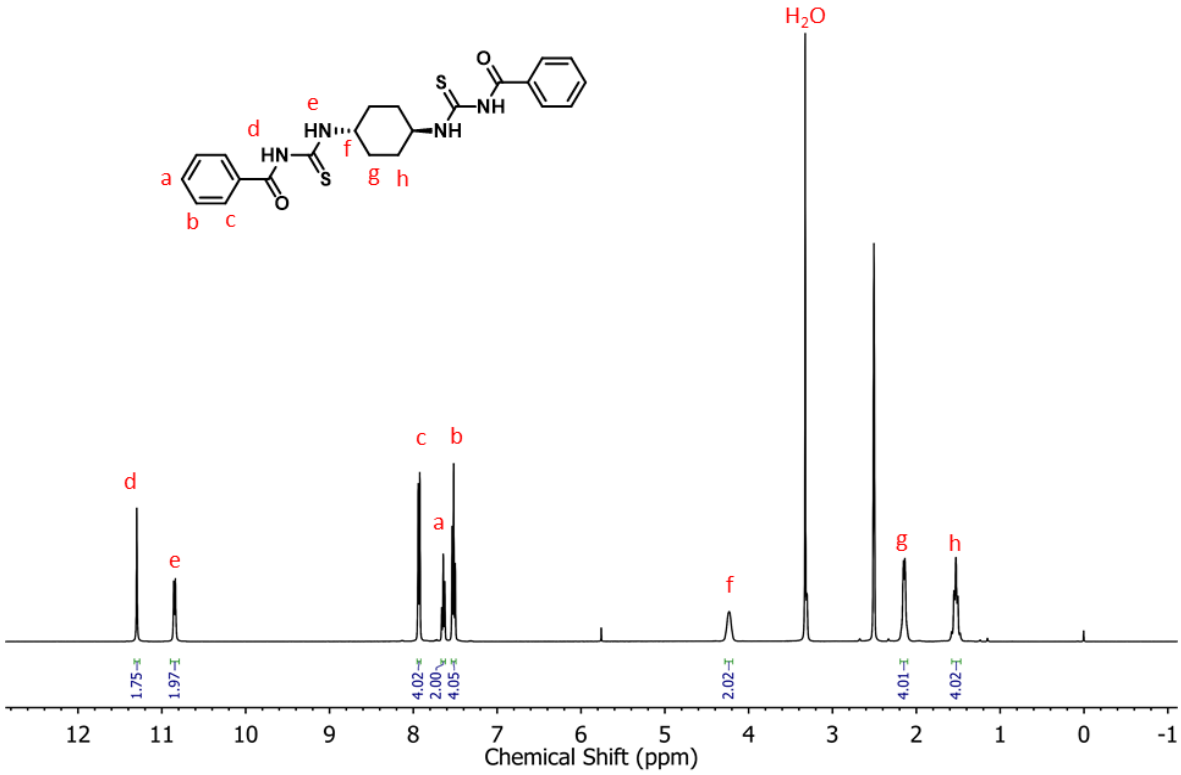


Fig. S25. ^{1}H NMR characterization of M4-1.

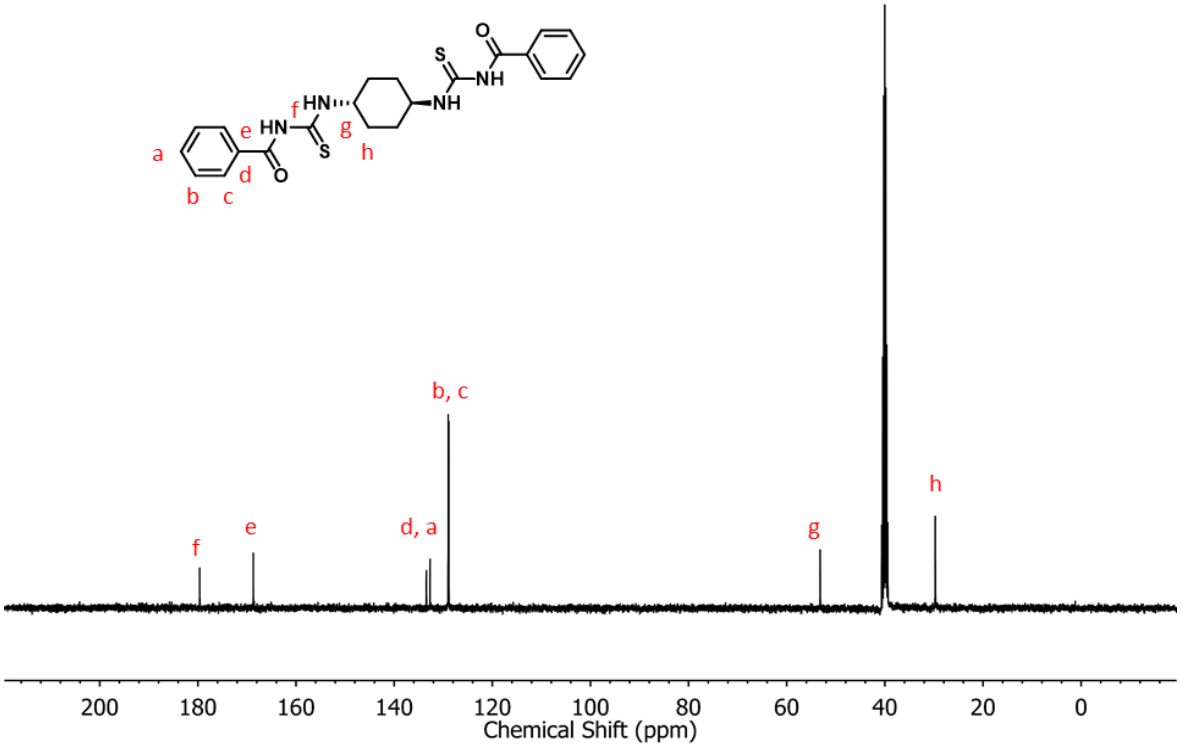


Fig. S26. ^{13}C NMR characterization of M4-1.

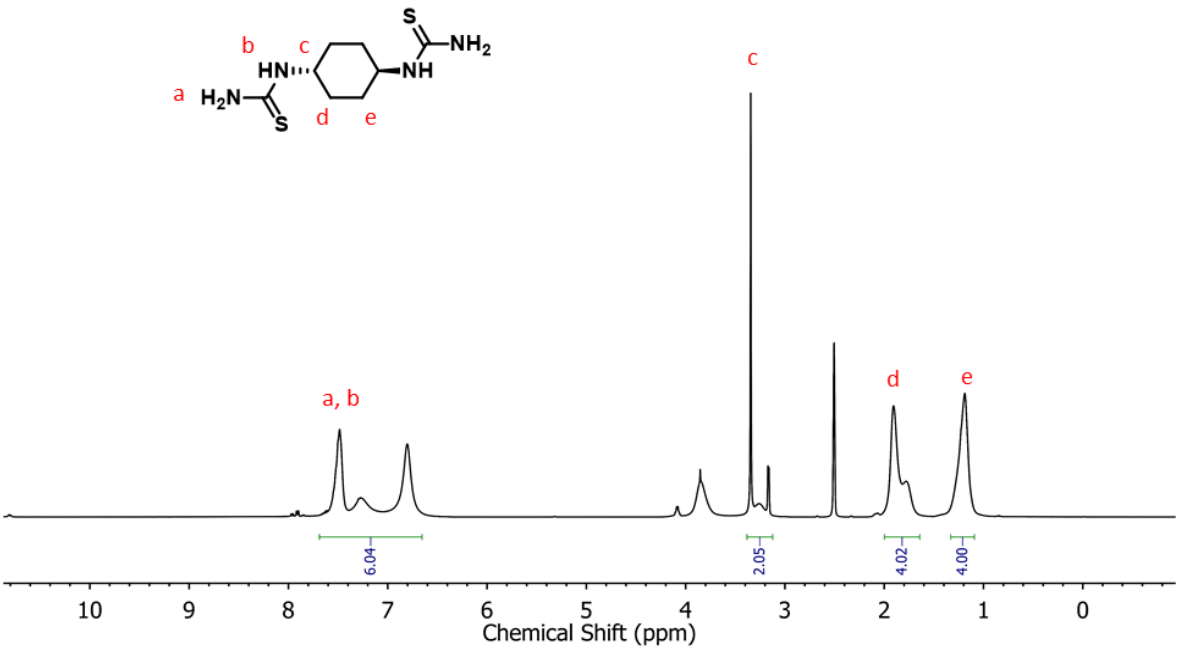


Fig. S27. ^{1}H NMR characterization of M4-2.

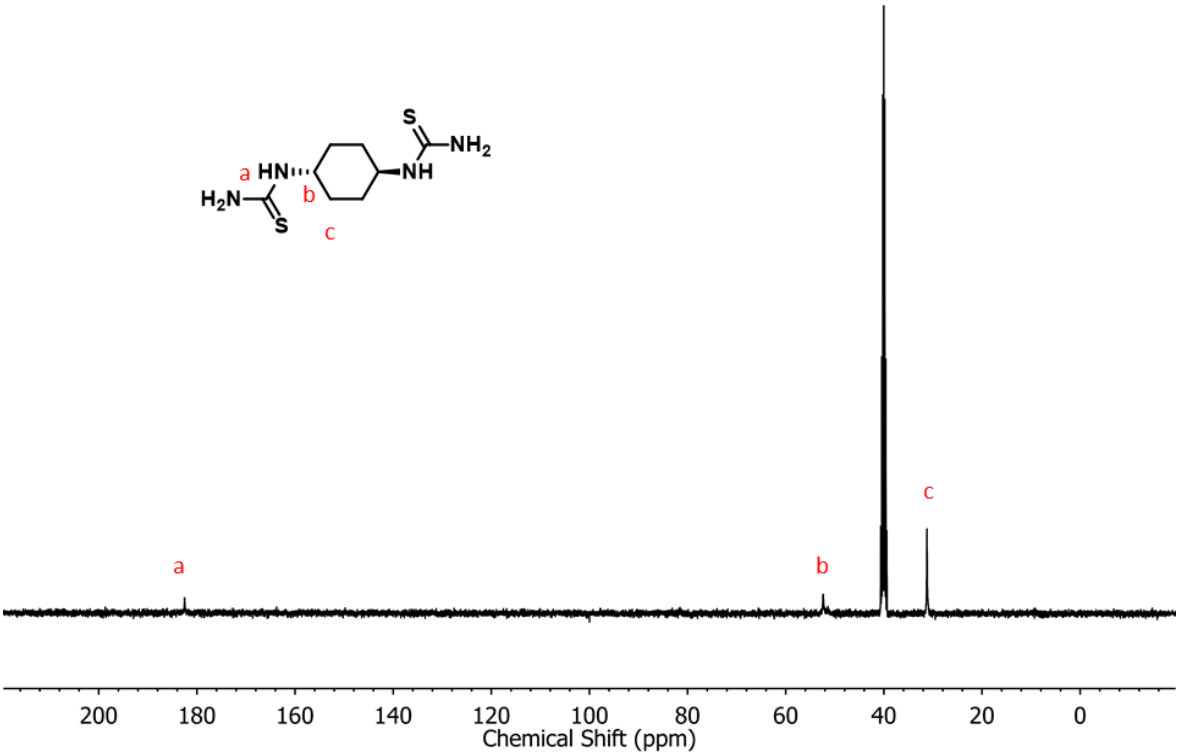


Fig. S28. ^{13}C NMR characterization of M4-2.

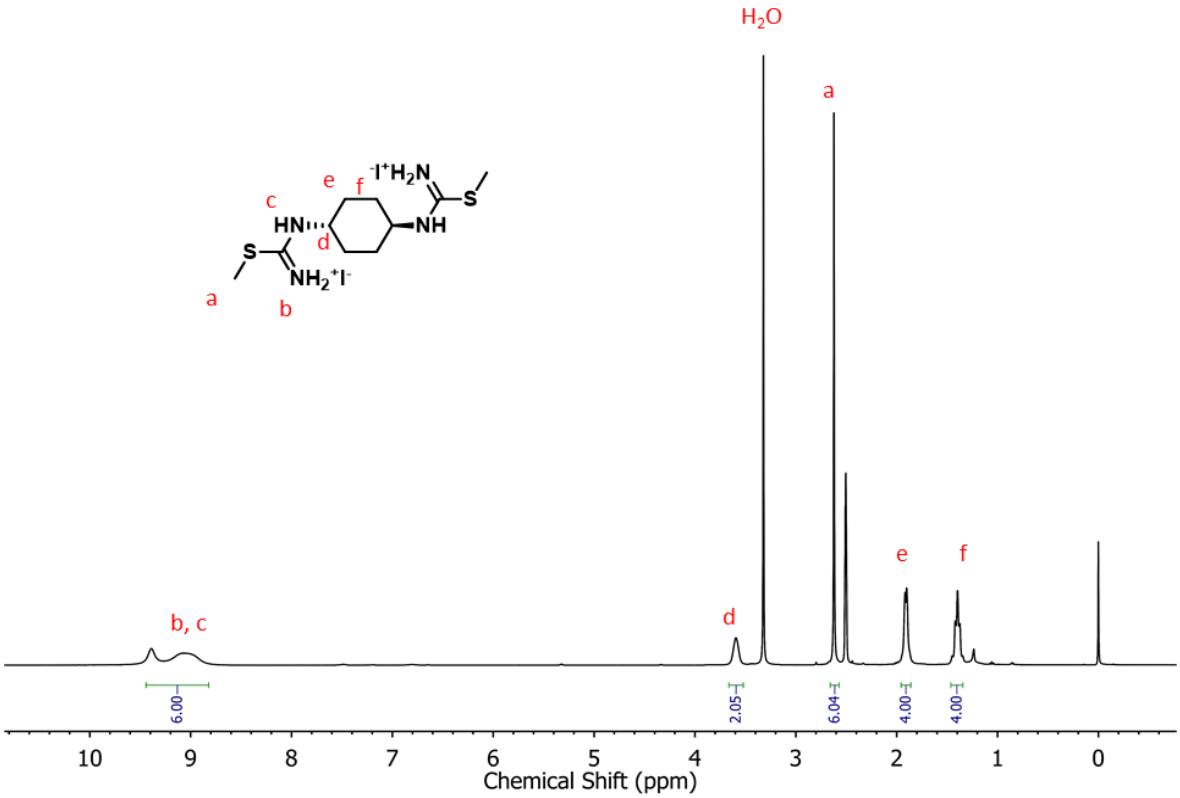


Fig. S29. ^{1}H NMR characterization of M4-3.

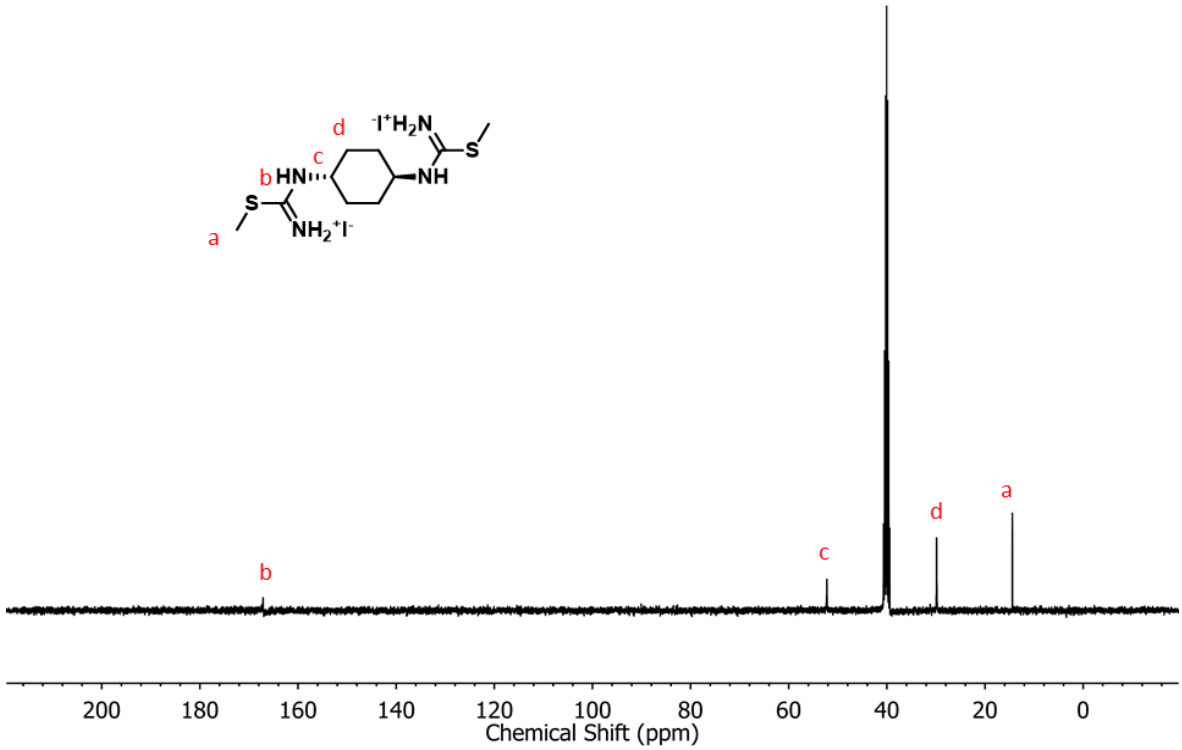


Fig. S30. ^{13}C NMR characterization of M4-3.

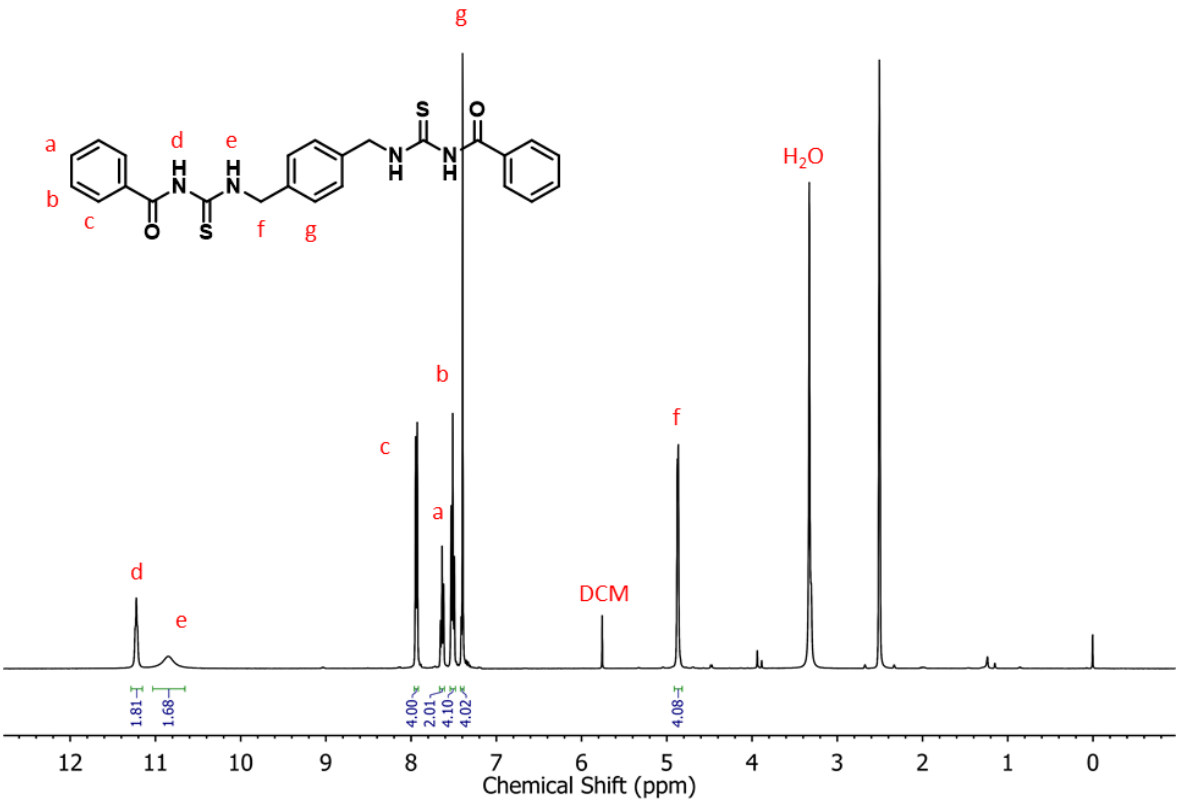


Fig. S31. ^{1}H NMR characterization of M5-1.

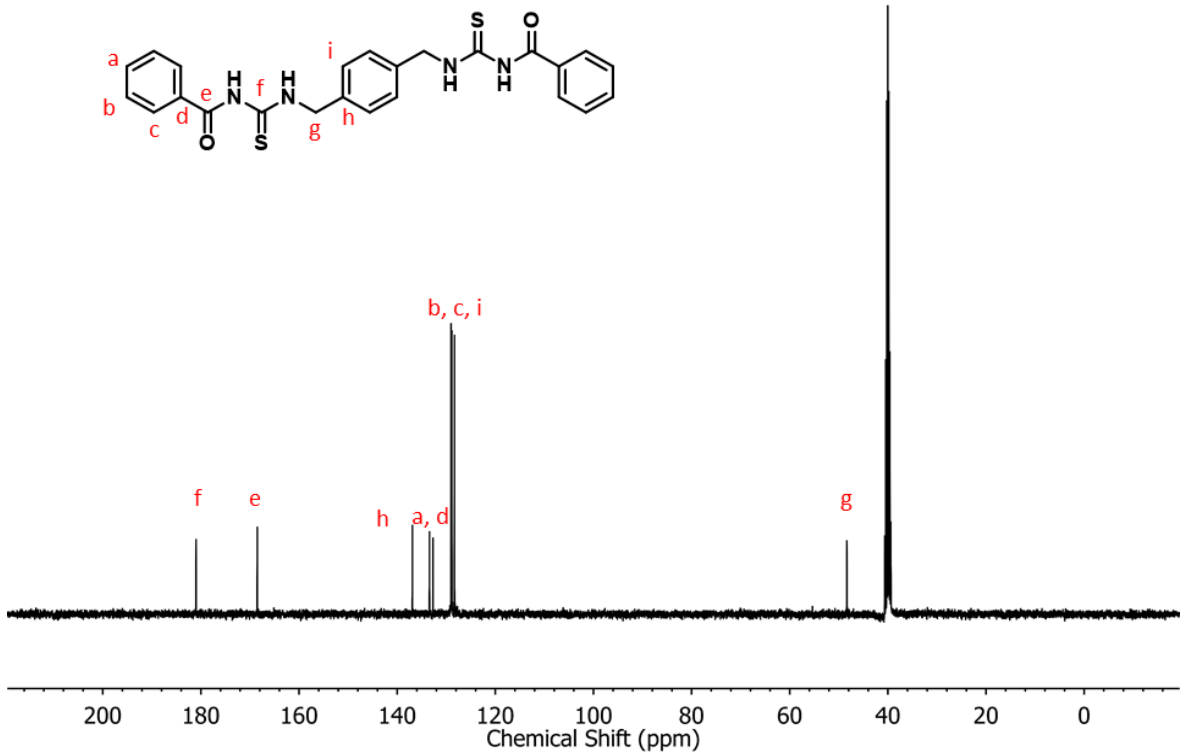


Fig. S32. ^{13}C NMR characterization of M5-1.

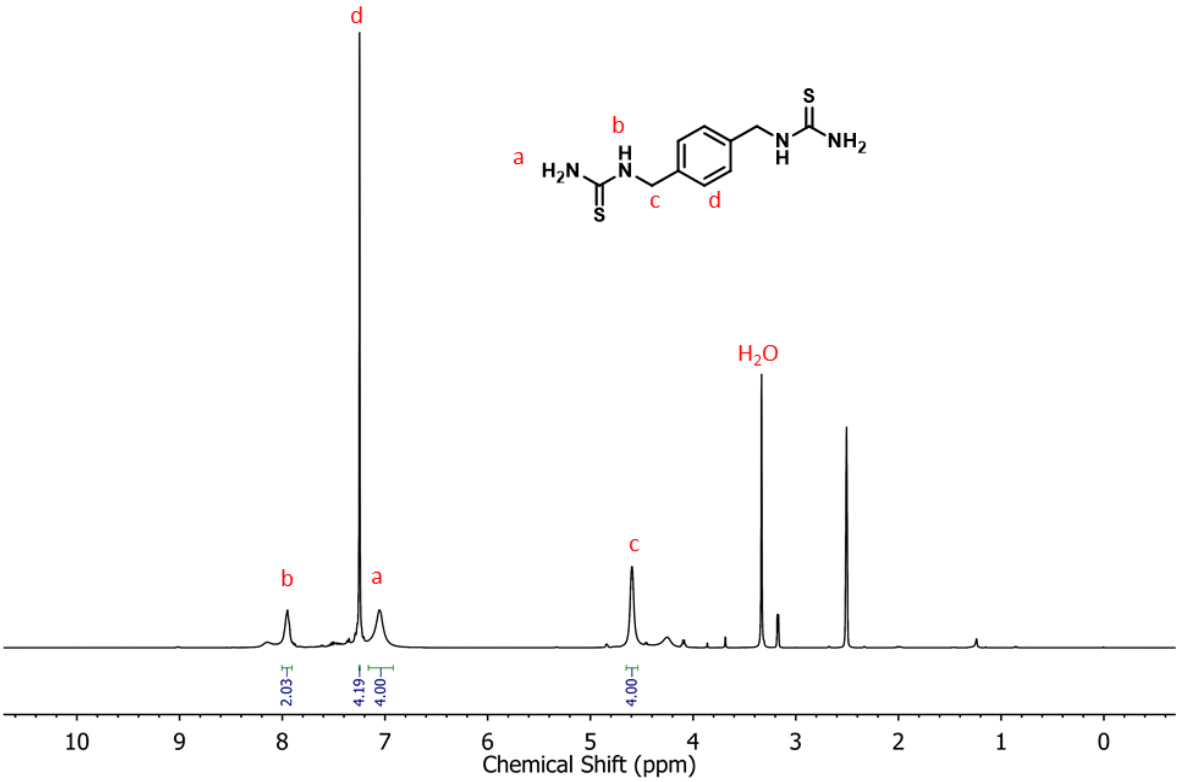


Fig. S33. ^{1}H NMR characterization of M5-2.

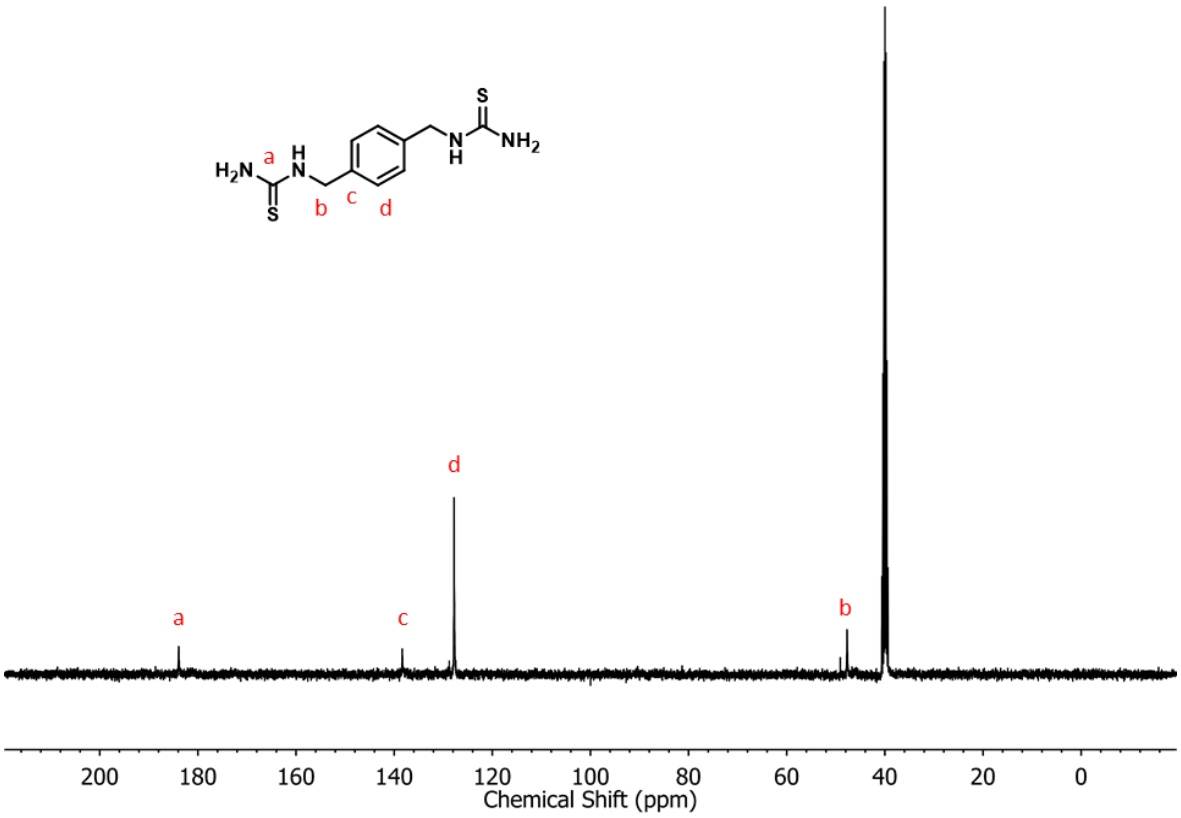


Fig. S34. ^{13}C NMR characterization of M5-2.

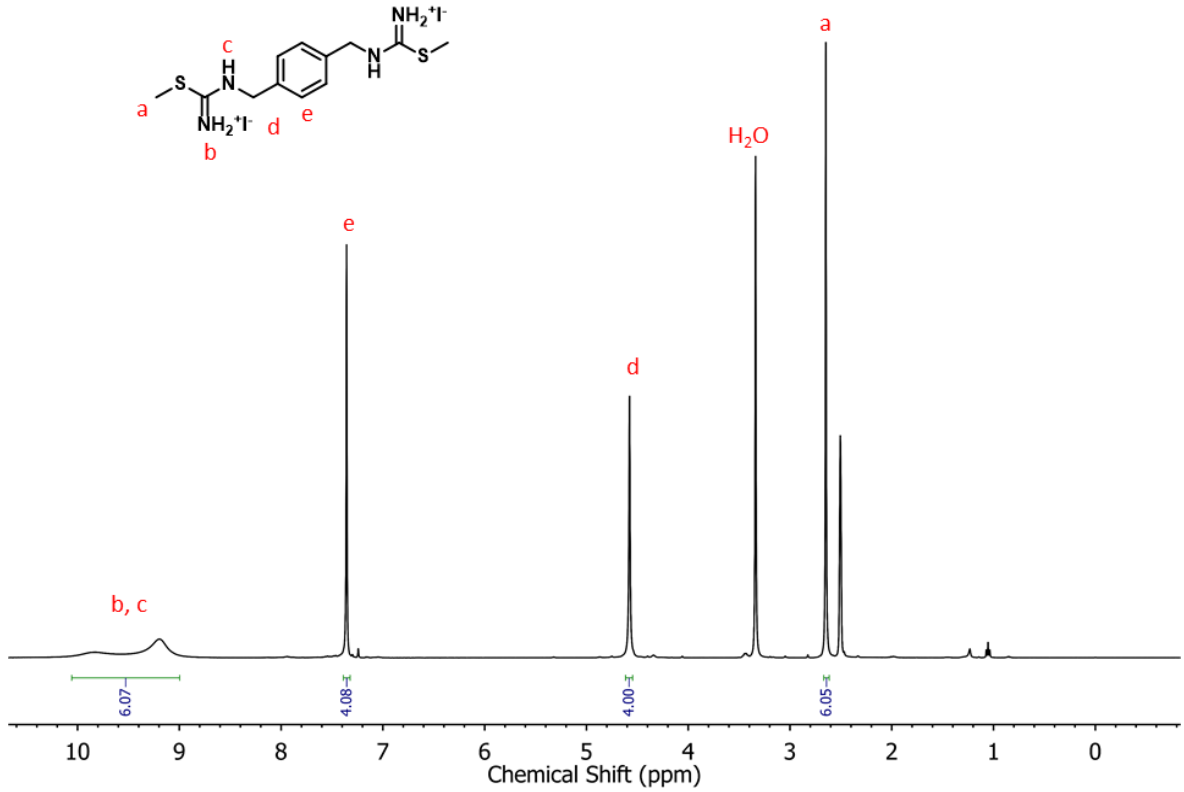


Fig. S35. ^{1}H NMR characterization of M5-3.

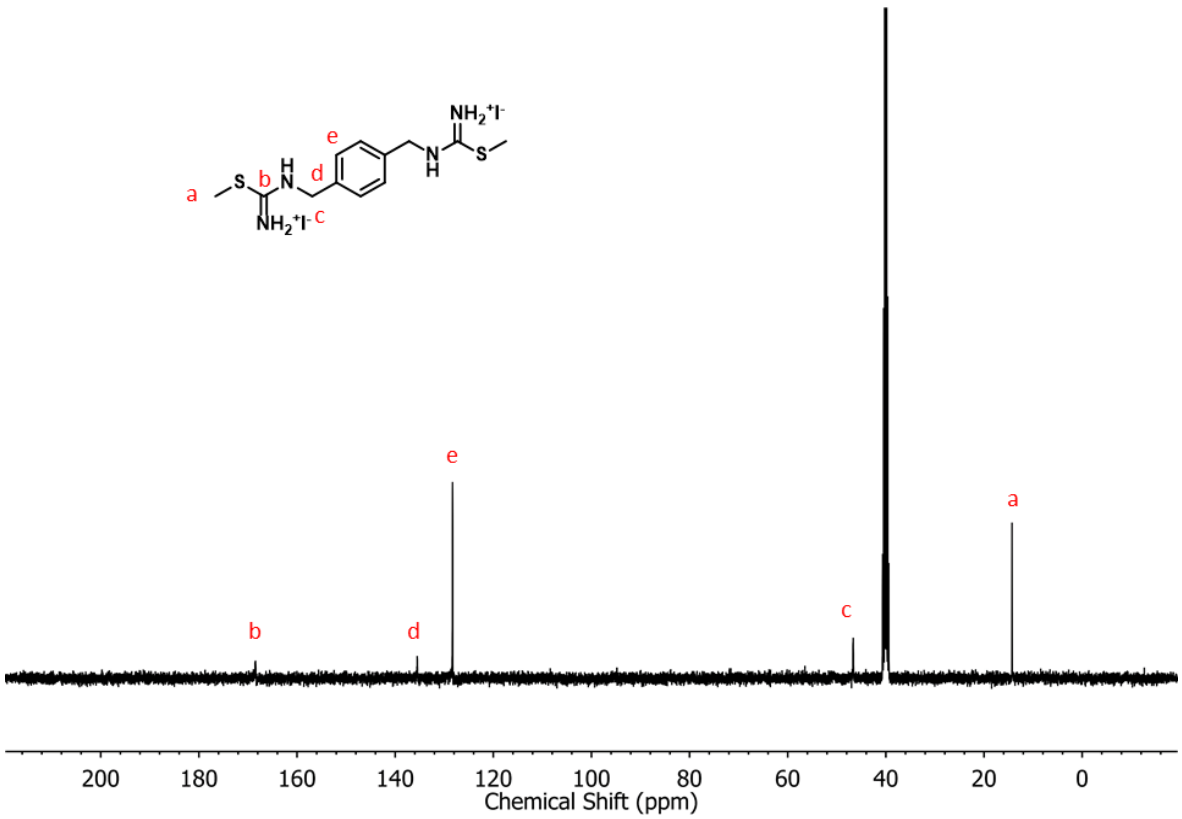


Fig. S36. ^{13}C NMR characterization of M5-3.

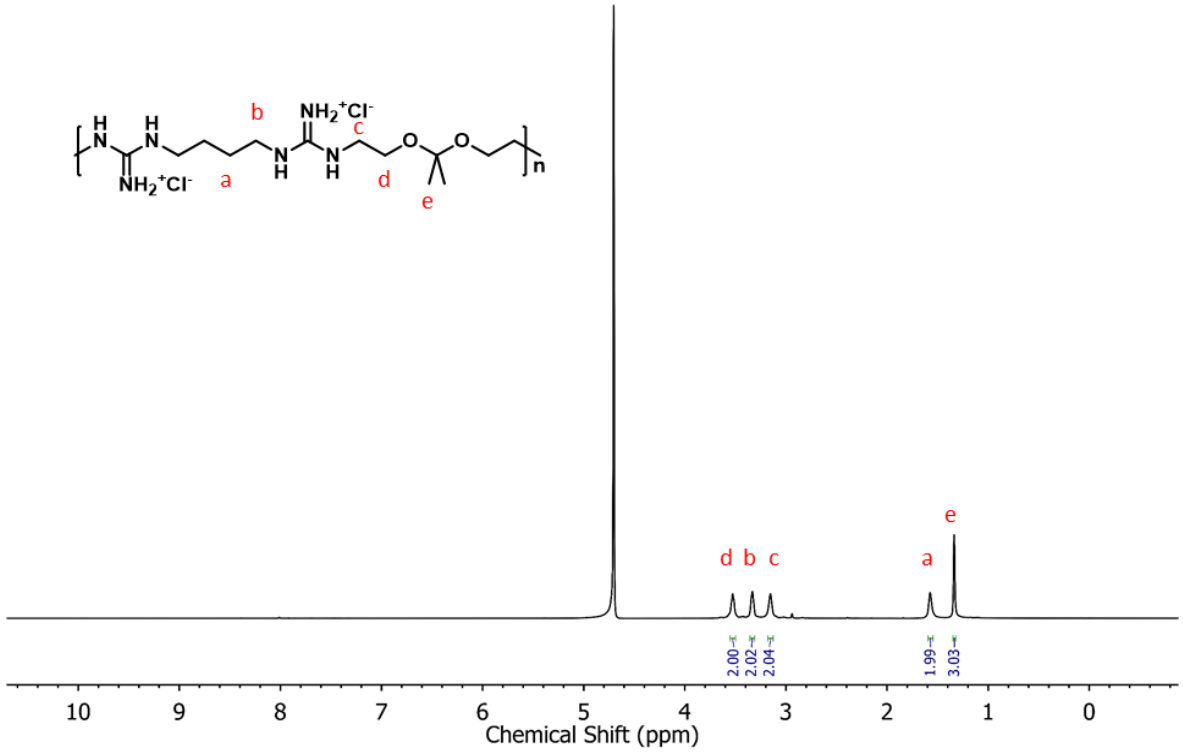


Fig. S37. ^{1}H NMR characterization of P1A.

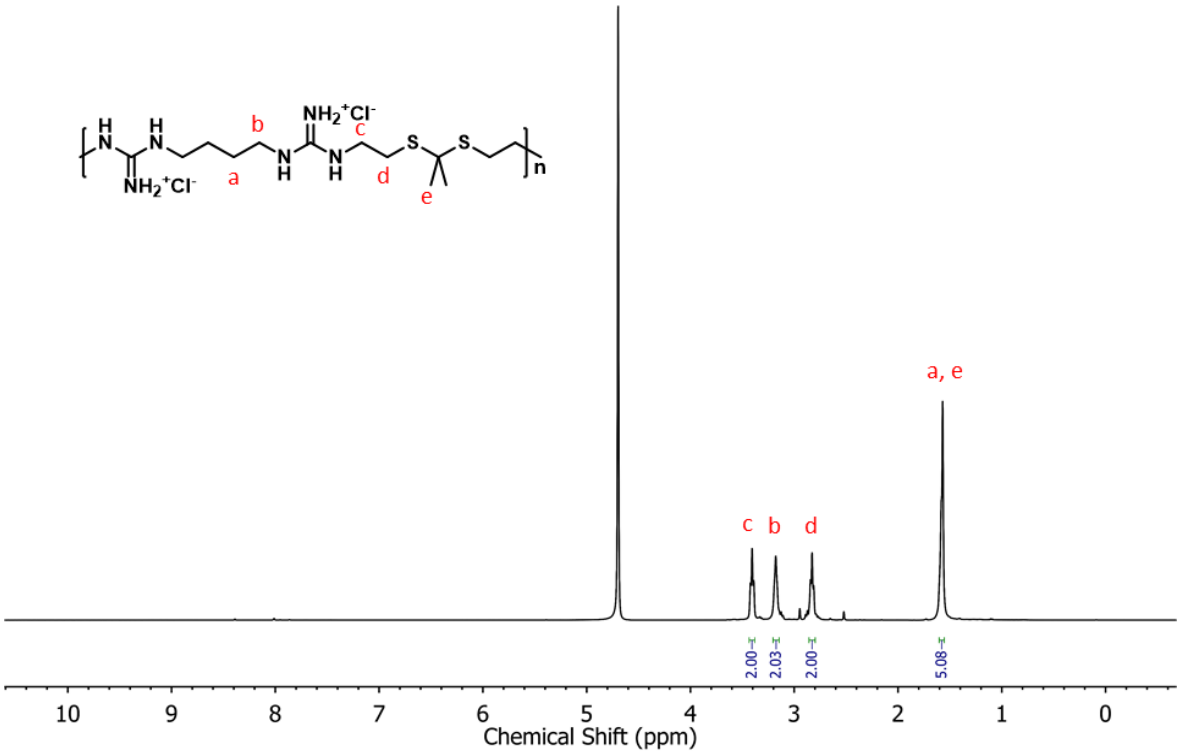


Fig. S38. ^{1}H NMR characterization of P1B.

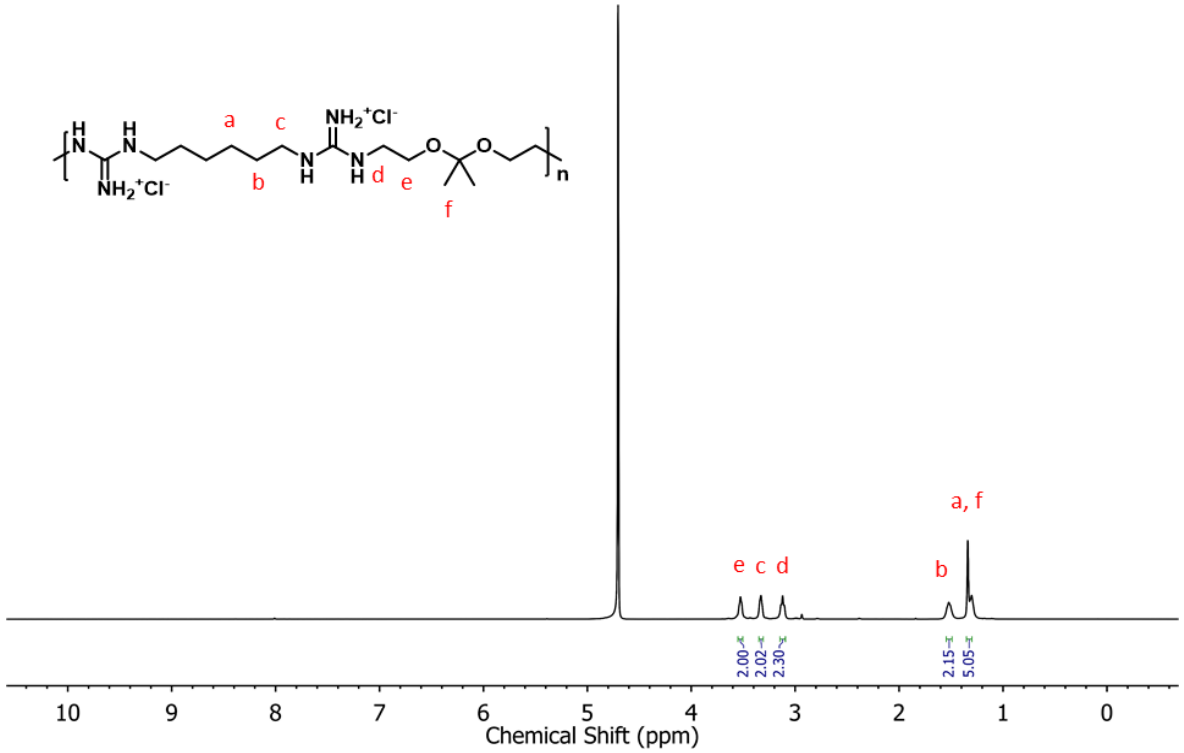


Fig. S39. ^{1}H NMR characterization of P2A.

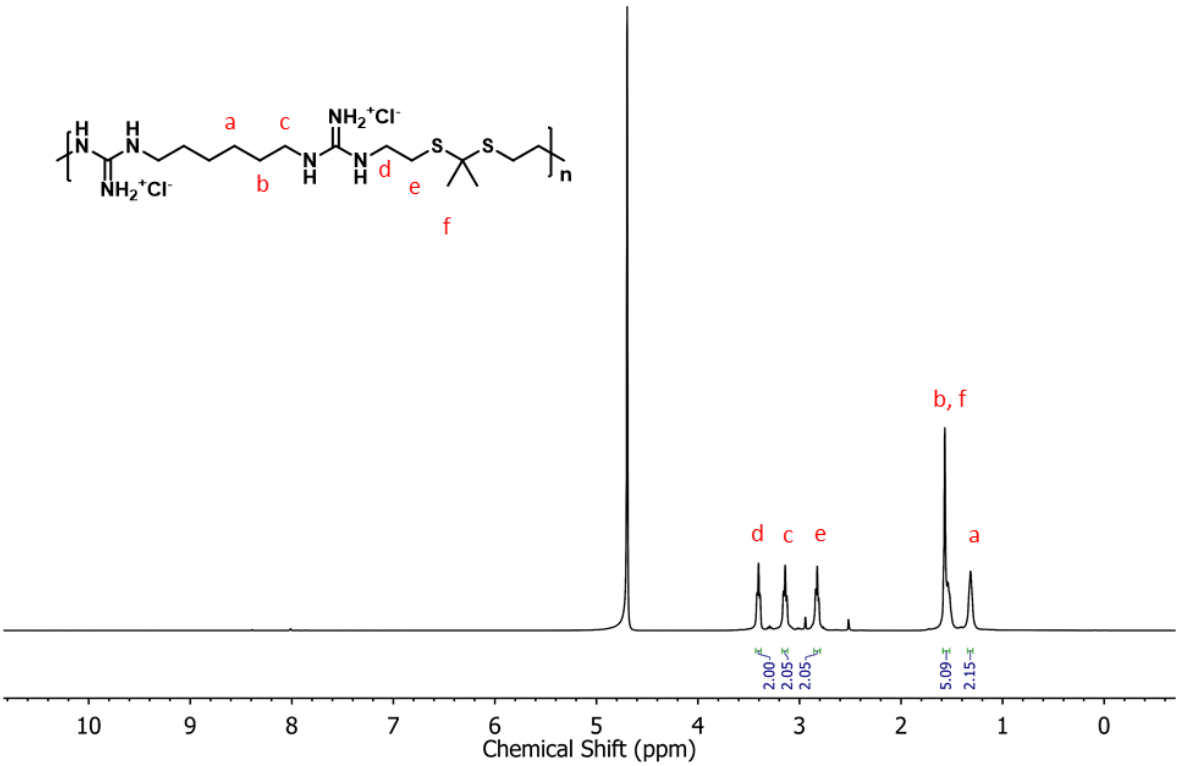


Fig. S40. ^{1}H NMR characterization of P2B.

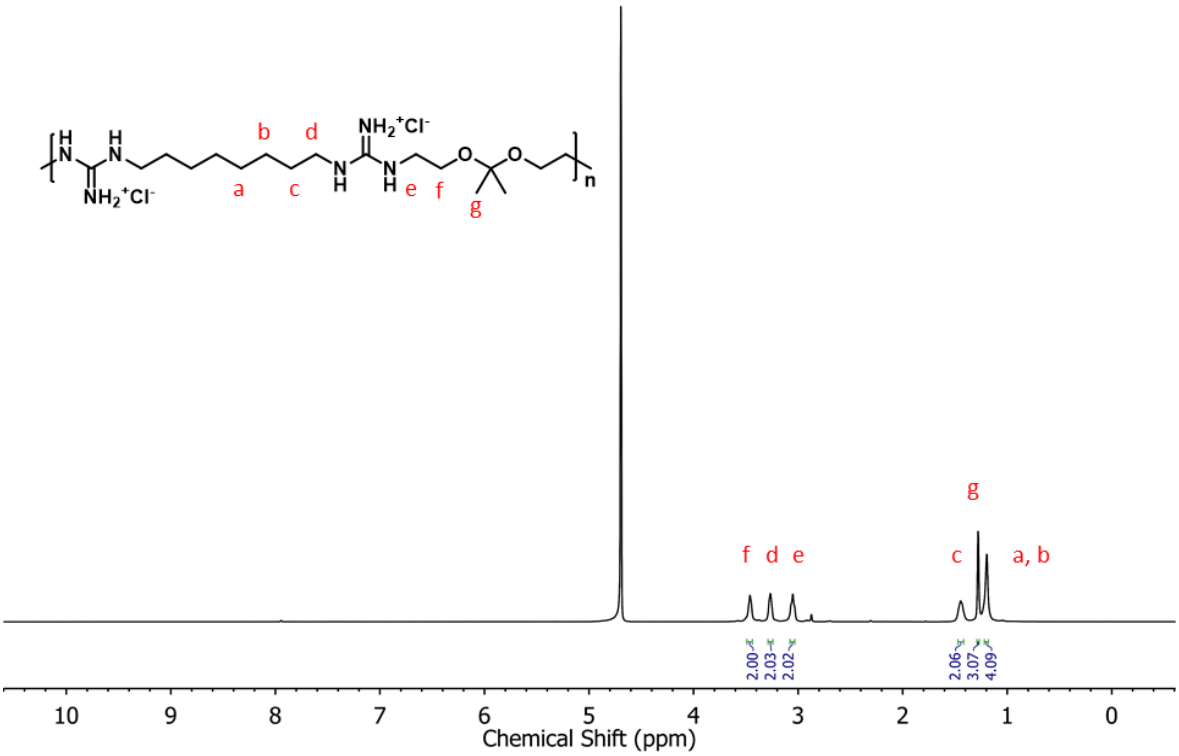


Fig. S41. ^{1}H NMR characterization of P3A.

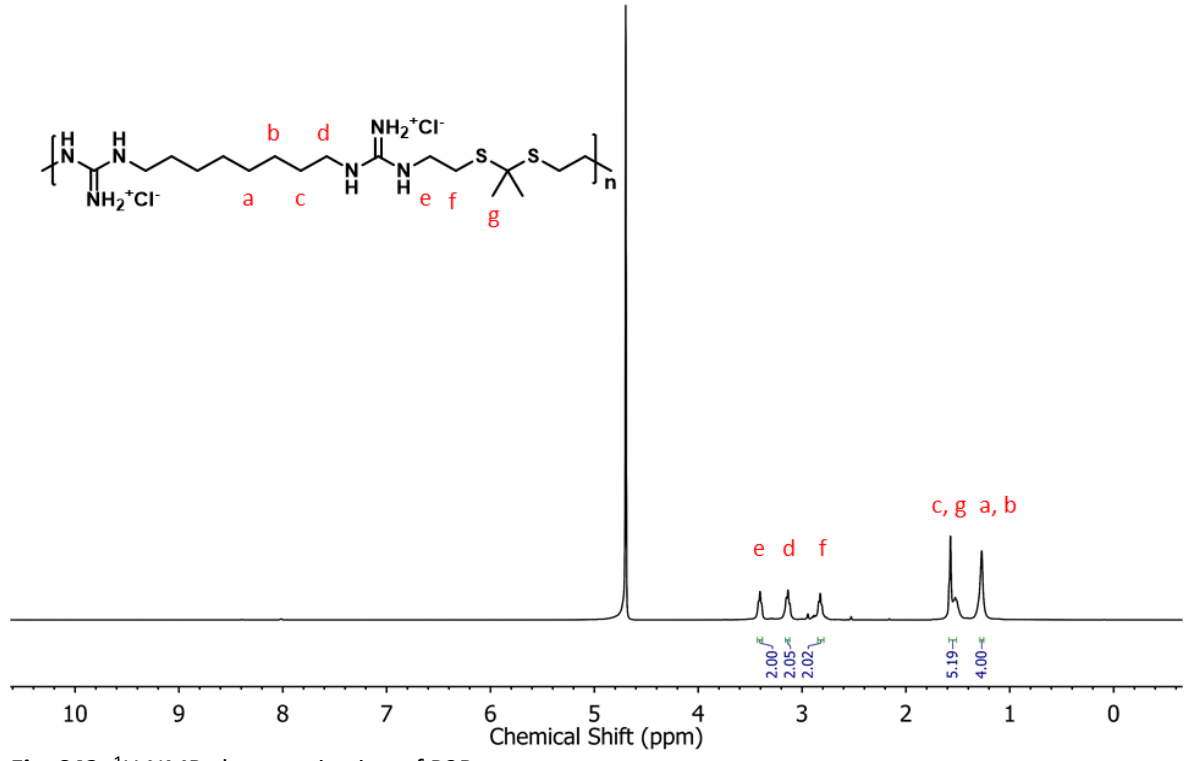


Fig. S42. ^{1}H NMR characterization of P3B.

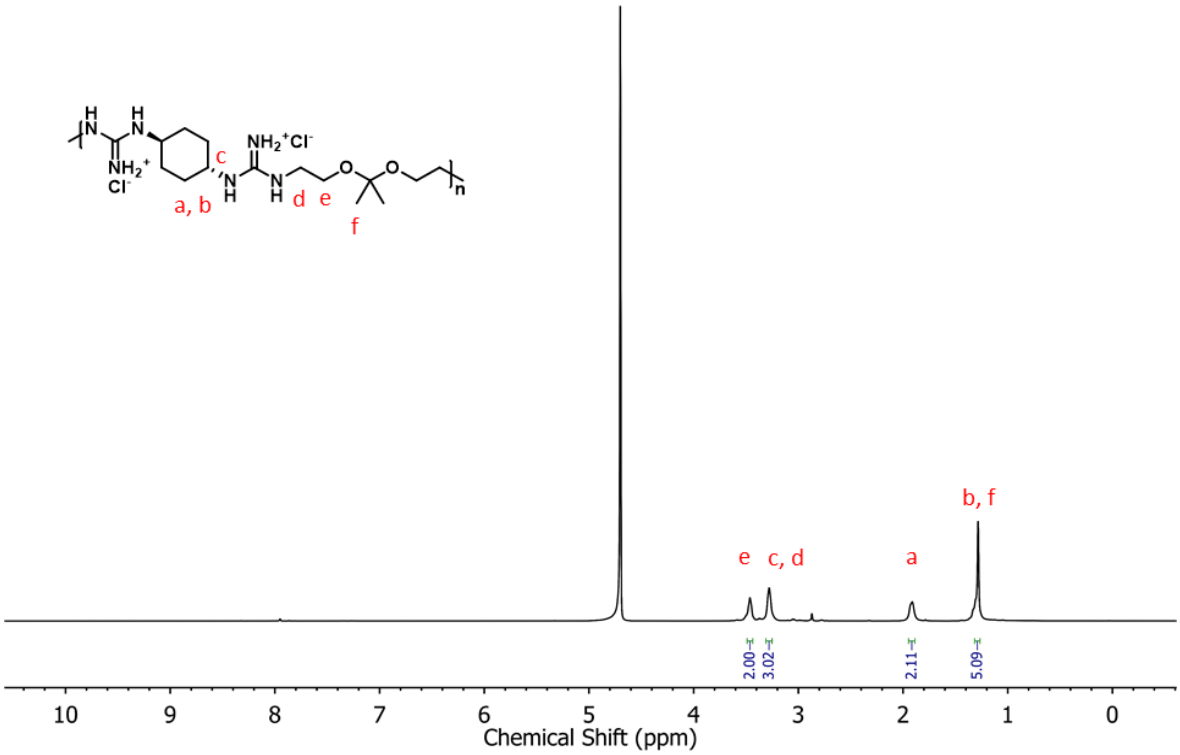


Fig. S43. ^{1}H NMR characterization of P4A.

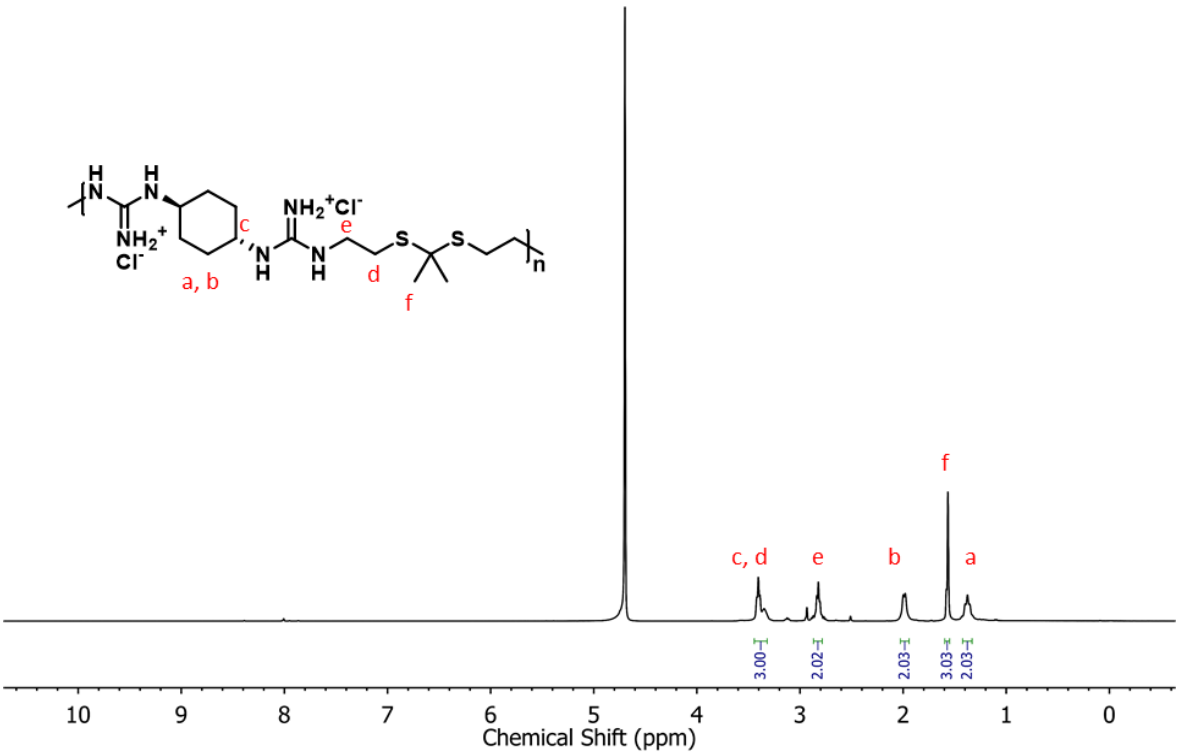


Fig. S44. ^{1}H NMR characterization of P4B.

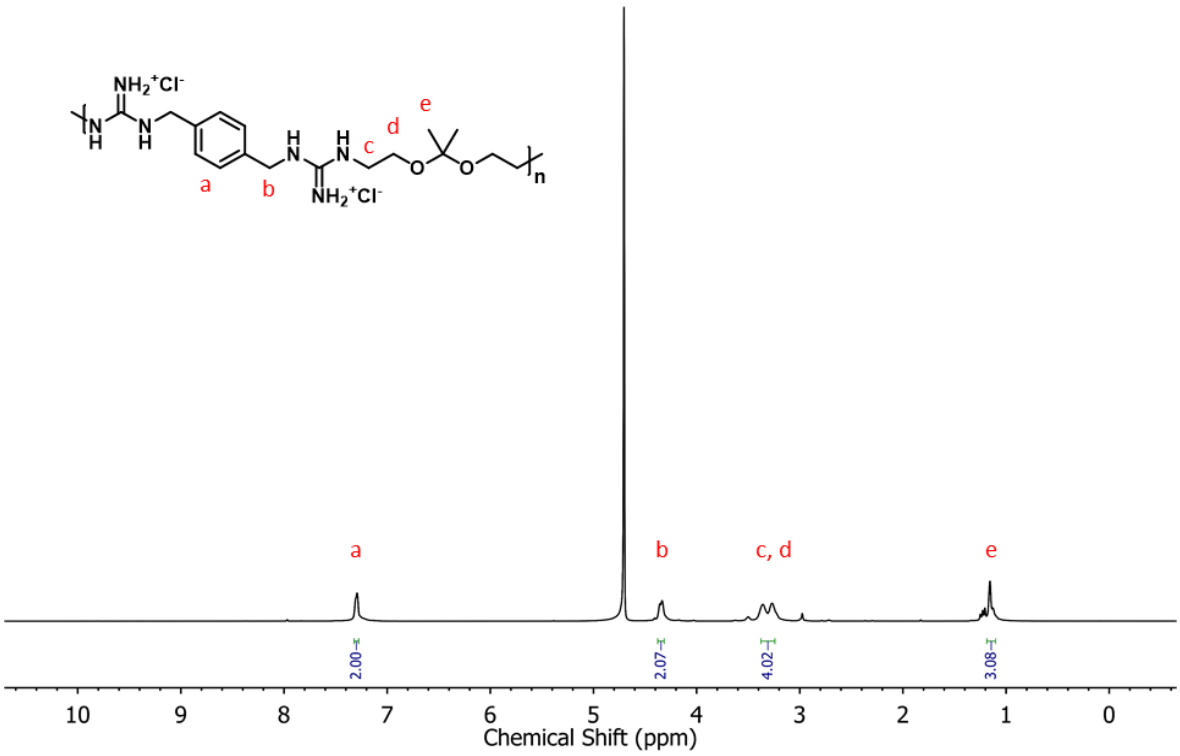


Fig. S45. ^{1}H NMR characterization of P5A.

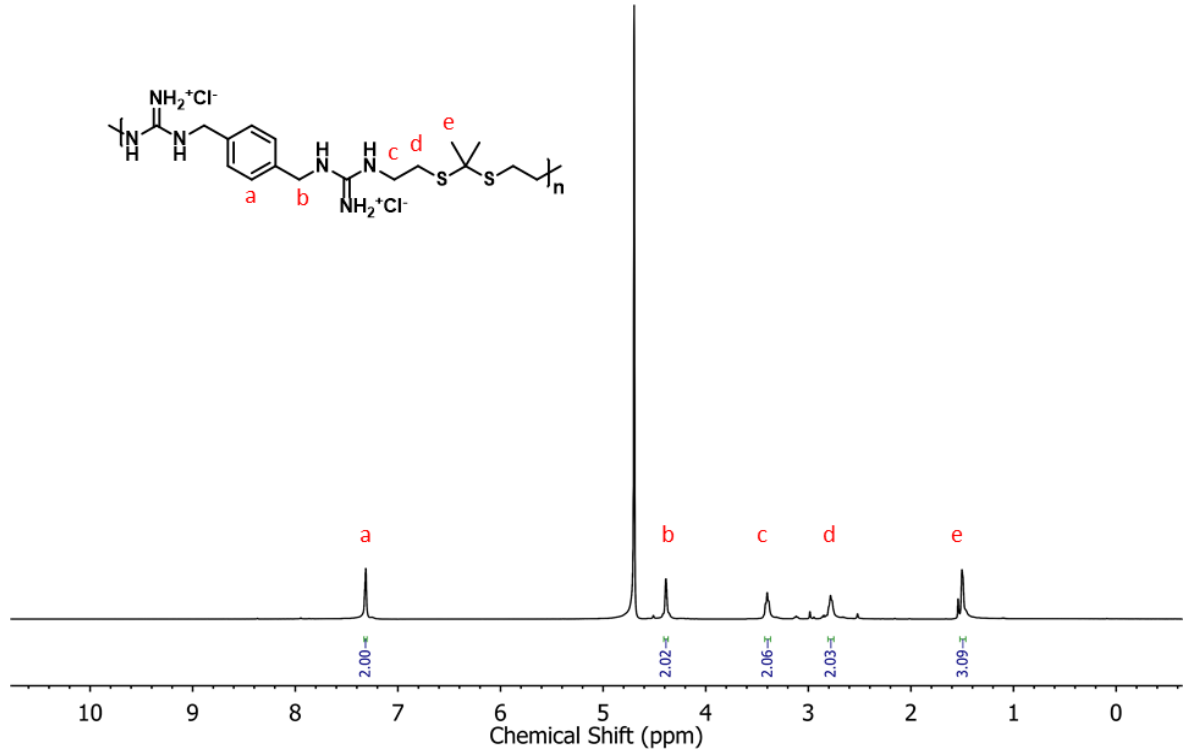


Fig. S46. ^{1}H NMR characterization of P5B.

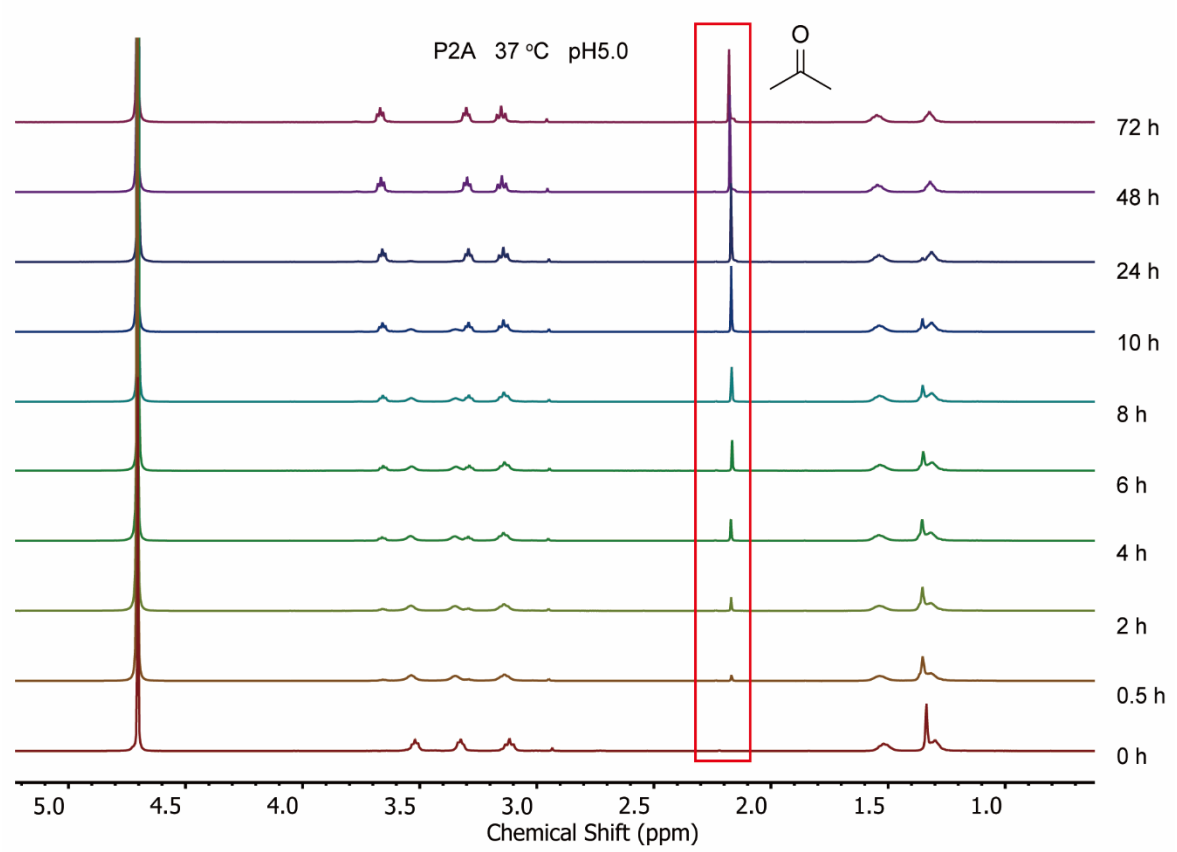


Fig. S47. [1]HNMR of P2A degradation in phosphate buffer solution (pH = 5.0) at different time.

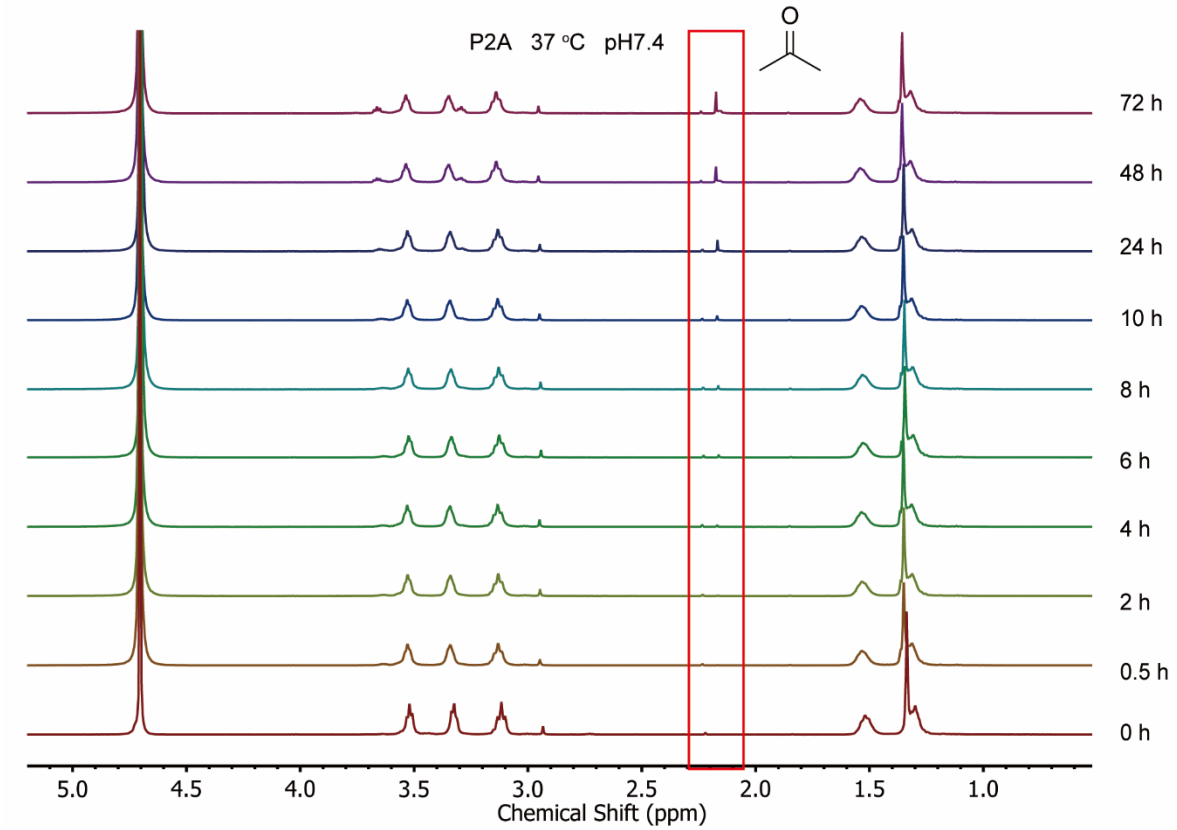


Fig. S48. [1]HNMR of P2A degradation in phosphate buffer solution (pH = 7.4) at different time.

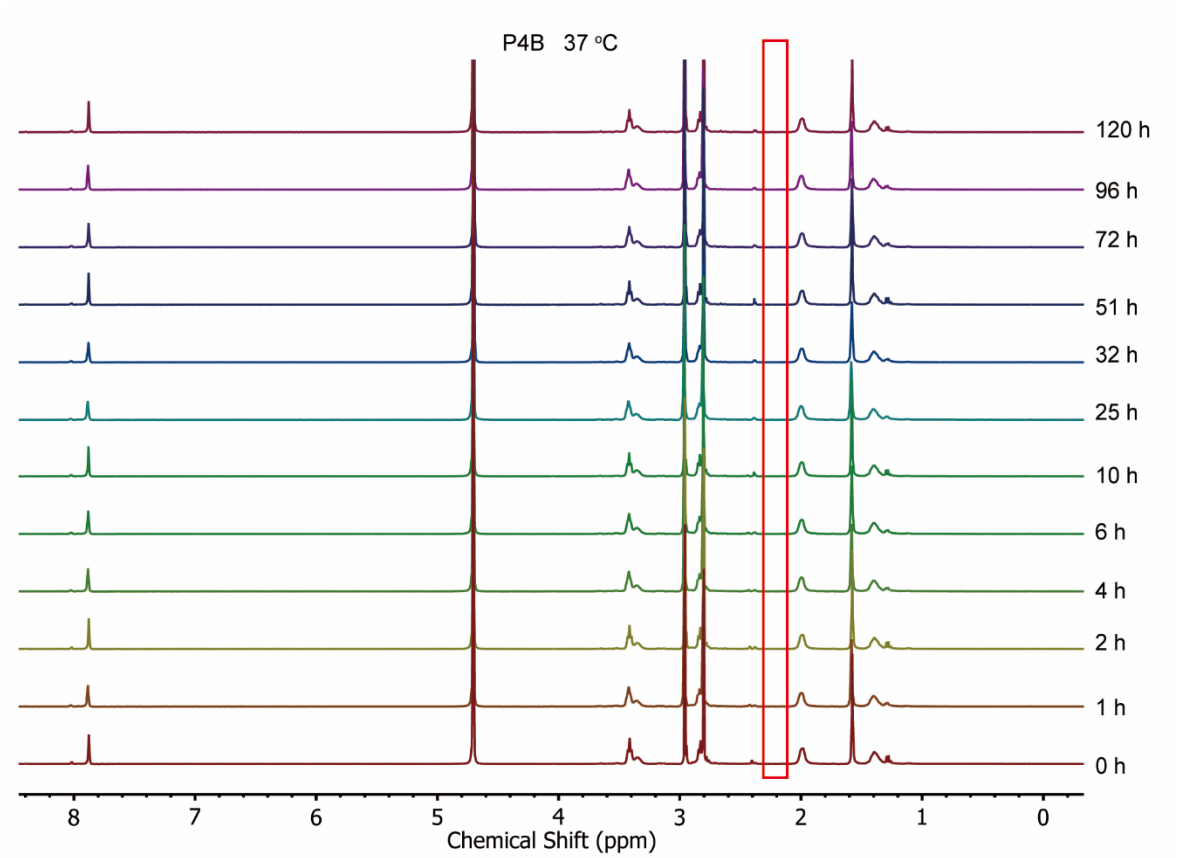


Fig. S49. ^{1}HNMR of P4B degradation in D_2O at 37 °C at different time.

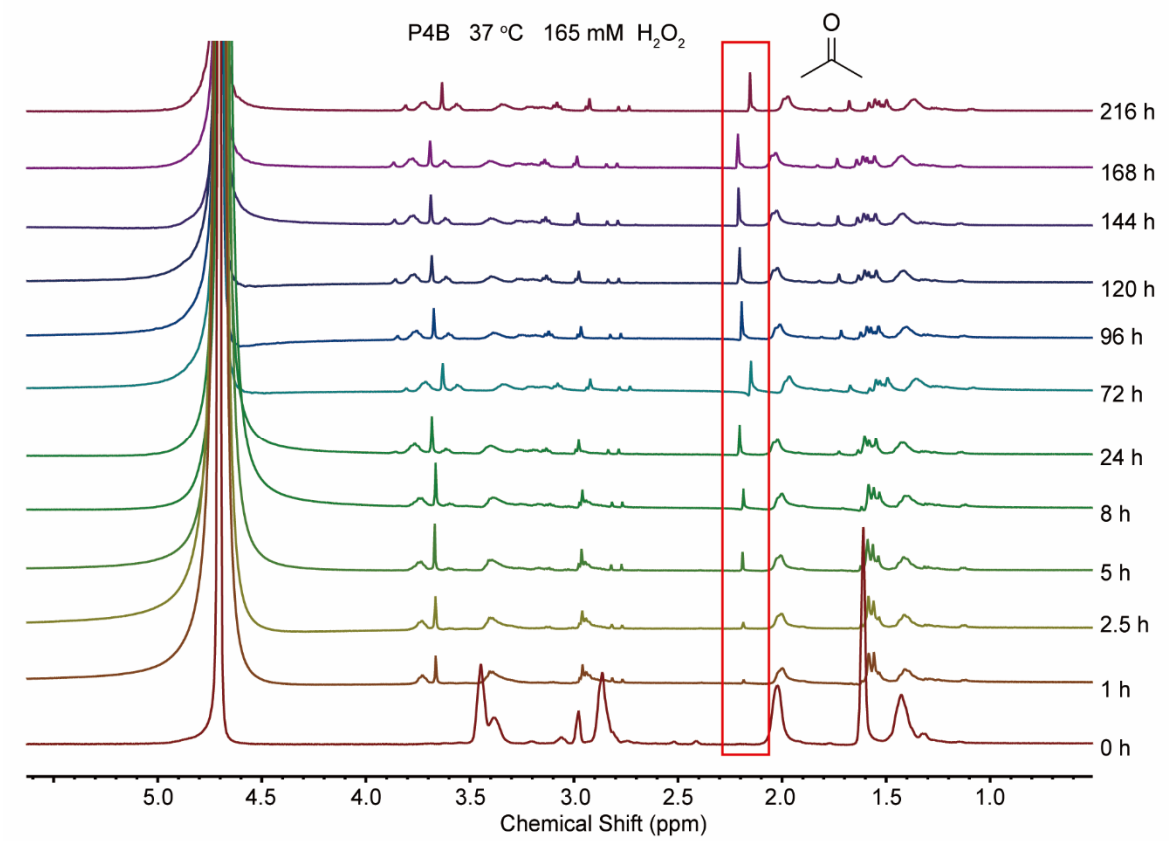


Fig. S50. ^{1}HNMR of P4B degradation in 165 mM H_2O_2 t 37 °C at different time.

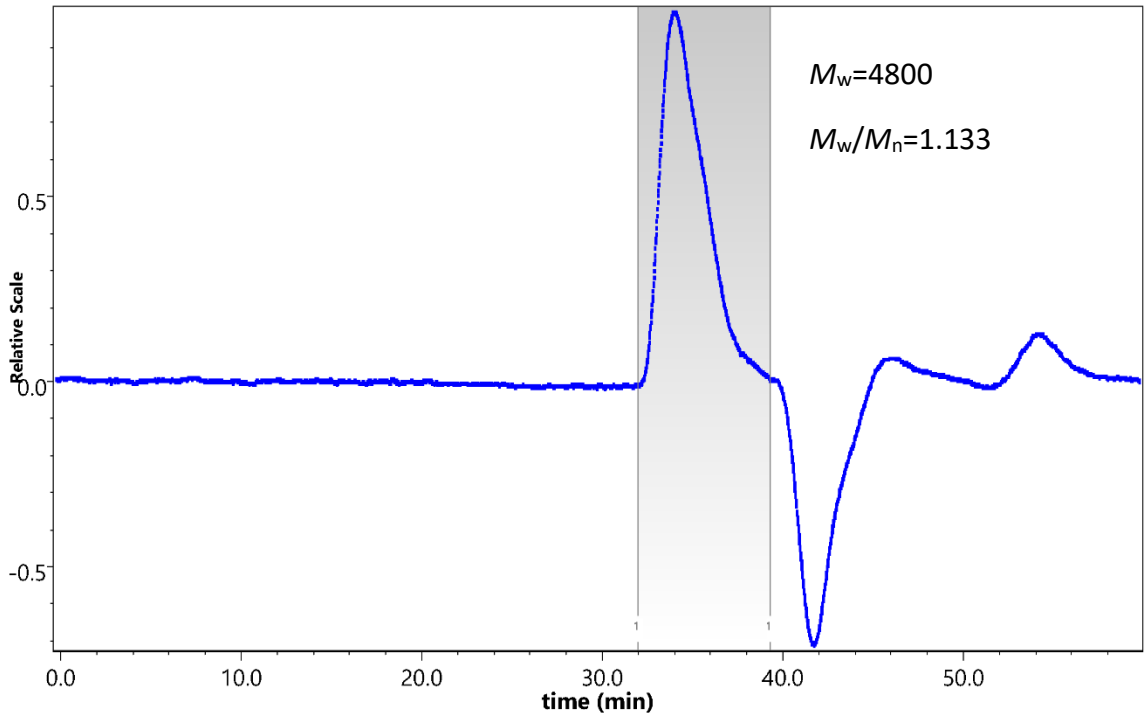


Fig. S51. Gel permeation chromatogram of P4B with marked molecular weight in Dalton. The negative peak is due to the solvent.

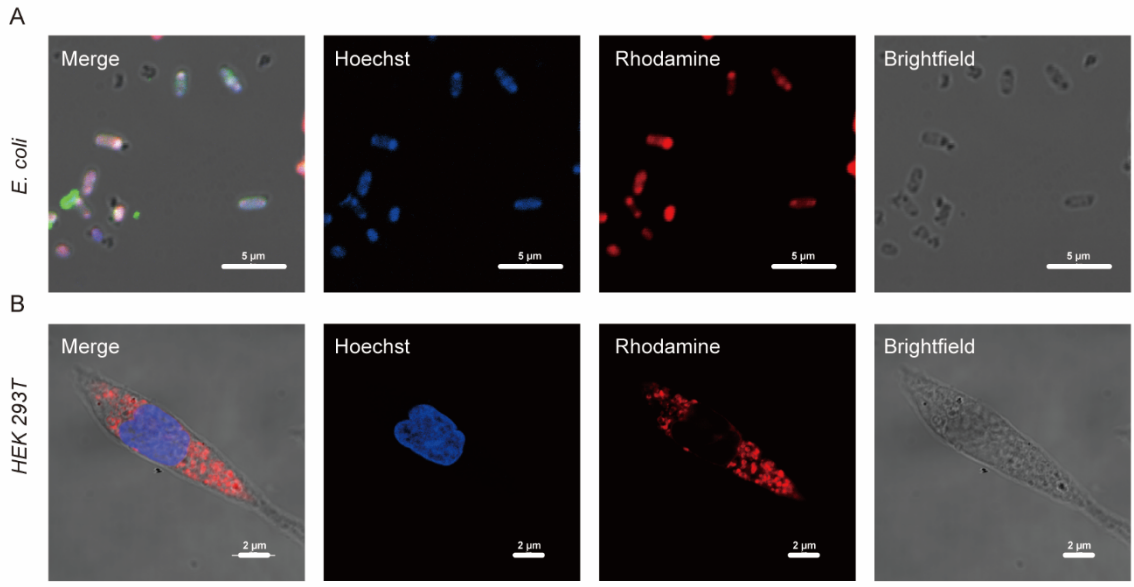


Fig. S52. (**A**) Confocal microscopic images showed that P4B stained both cell membrane and genomic DNA of *E. coli*, supporting the fulfillment of a dual mechanism of action design. Scale bar = 5 µm. (**B**) P4B could enter eukaryotic cells (HEK 293T) but it is excluded from the cell nucleus in (treatment time = 4 h). Scale bar = 2 µm.

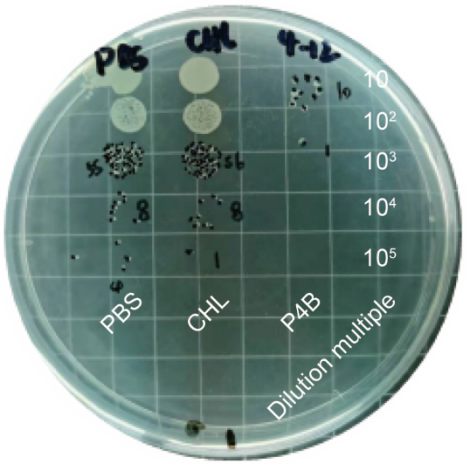


Fig. S53. Representative images of the agar plates for CFU determination in the zebrafish scratch wound model study after 9 h, under the treatment of PBS, 2 μg/mL chloramphenicol or 2 μg/mL P4B.

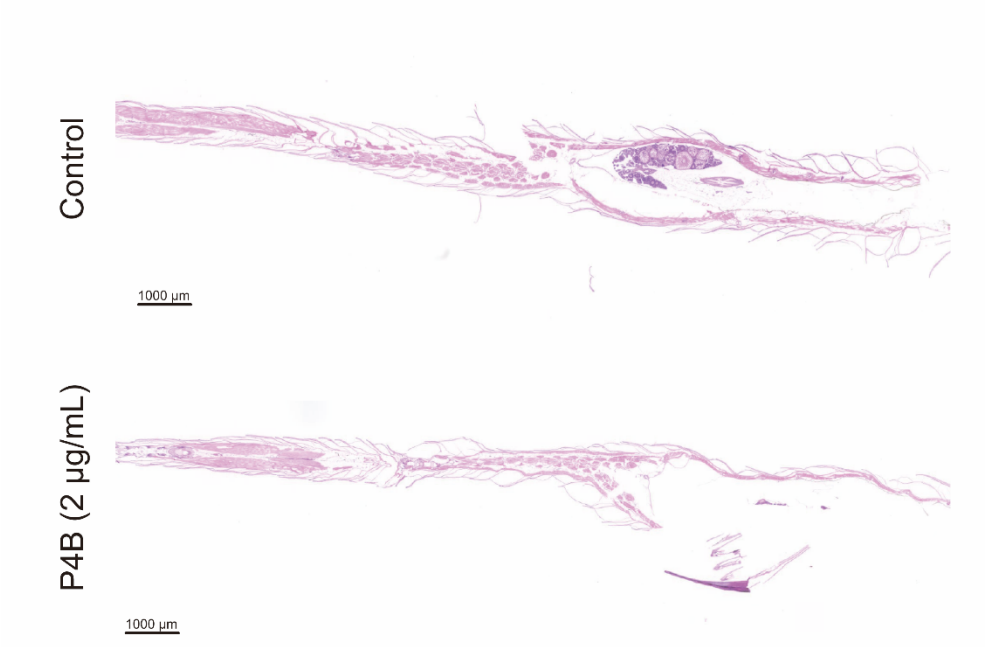


Fig. S54. H&E stain analysis of the zebrafish treated with PBS or 2 μg/mL P4B. Scale bar = 1000 μm

Table S1. Fold change of 32 FDA-approved antibiotics' MICs in combination with P4B of 1/4 MIC against *E.coli* K12, *B. subtilis* and *M. smegmatis*.

Number of drugs	Antibiotics	*E.coli* K12			*B. subtilis*			*M. smegmatis*		
		MIC	1/4 MIC P4B	MIC fold change	MIC	1/4 MIC P4B	MIC fold change	MIC	1/4 MIC P4B	MIC fold change
1	Spectinomycin	8	8	1	32	32	1	32	4	8
2	Gentamicin	0.5	<0.25	>2	<0.25	<0.25	1	0.5	1	1/2
3	Chloramphenicol	>32	>32	1	>32	>32	1	>32	>32	1
4	Rifampicin	16	16	1	<0.25	<0.25	1	32	8	4
5	Meropenem	<0.25	<0.25	1	<0.25	<0.25	1	32	8	4
6	Cefalexin	<0.25	<0.25	1	4	4	1	>32	>32	1
7	Cefotaxime	<0.25	<0.25	1	1	0.5	2	>32	32	>2
8	Bacitracin	>32	32	>2	4	32	1/8	>32	>32	1
9	Vancomycin	16	>32	1/2	0.5	<0.25	>2	4	4	1
10	Tigecycline	>32	>32	1	8	32	1/4	>32	>32	1
11	Clindamycin	>32	16	>2	1	2	1/2	4	2	2
12	Daptomycin	>32	>32	1	2	4	1/2	>32	>32	1
13	Erythromycin	>32	>32	1	1	<0.25	>4	8	4	2
14	Aztreonam	<0.25	<0.25	1	8	>32	1/4	>32	>32	1
15	Isoniazid	>32	>32	1	32	>32	1	8	4	2
16	Furazolidone	16	1	16	4	<0.25	>16	>32	32	>2
17	Metronidazole	>32	>32	1	>32	>32	1	>32	>32	1
18	Linezolid	>32	>32	1	2	<0.25	>8	1	0.5	2
19	Ampicillin	>32	32	>2	16	>32	1/2	>32	>32	1
20	Carbenicillin	4	4	1	8	8	1	>32	>32	1
21	Methicillin	>32	>32	1	<0.25	<0.25	1	>32	>32	1
22	Penicillin G	>32	>32	1	>32	>32	1	>32	>32	1
23	Valnemulin HCl	32	32	1	8	4	2	32	8	4

24	Colistin	<0.25	<0.25	1	16	4	4	8	16	1/2
25	Mupirocin	>32	>32	1	<0.25	<0.25	1	>32	>32	1
26	Ciprofloxacin	<0.25	<0.25	1	<0.25	<0.25	1	0.5	1	1/2
27	Clofazimine	4	8	1/2	32	32	1	32	8	4
28	Fusidic acid	0.5	0.5	1	>32	>32	1	>32	>32	1
29	Trimethoprim	0.5	0.5	1	2	1	2	8	8	1
30	Sulfadiazine	4	16	1/4	>32	>32	1	16	2	8
31	Dapsone	>32	>32	1	>32	>32	1	>32	2	>16
32	Doxycycline	2	2	1	<0.25	<0.25	1	<0.25	<0.25	1

Table S2. MIC of 7 disinfectants against *E. tarda*

Disinfectants	MIC (μg/mL)
Benzyldodecyldimethylammonium Bromide	2
Nano Silver	64
Povidone Iodine	2048
Sodium Percarbonate	>2048
Sodium Hypochlorite	128
Sodium Metaperiodate	256
Trichloroisocyanuric Acid	>128

Table S3. MIC (*E. tarda*), HC_{50} and IC_{50} of degradation product.

	Degradation Product		
	MIC	HC_{50}	IC_{50} (NIH/3T3)
μg/mL	>256	>512	>128

Reference

1 U. Patel, M. Shekh and R. Patel, *J. Chem. Pharm. Res.*, 2017, **9**, 12-19.

2 J. Singh, T. Singha, A. Naskar, M. Kundu, R. K. Harwansh, A. Mondal, T. Ghosh and T. K. Maity, *Pharmacologyonline*, 2011, **2**, 976-987.

3 H. He, Y. Bai, J. Wang, Q. Deng, L. Zhu, F. Meng, Z. Zhong and L. Yin, *Biomacromolecules*, 2015, **16**, 1390-1400.

4 N. Zheng, Z. Zhang, J. Kuang, C. Wang, Y. Zheng, Q. Lu, Y. Bai, Y. Li, A. Wang and W. Song, *ACS Appl. Mater. Interfaces*, 2019, **11**, 18224-18232.

5 Z. Chen, W. Huang, N. Zheng and Y. Bai, *Polym. Chem.*, 2020, **11**, 664-668.

6 Z. Chen, C. Zhou, Y. Xu, K. Wen, J. Song, S. Bai, C. Wu, W. Huang, Q. Cai, K. Zhou, H. Wang, Y. Wang, X. Feng and Y. Bai, *Biomaterials*, 2021, **275**, 120858-120867.