Substrate-Initiated Synthesis of Cell-Penetrating Poly(disulfide)s

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Supplementary Information

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1. Materials and Methods

As in ref. S1, Supporting Information. Briefly, reagents for synthesis were purchased from Fluka, Sigma-Aldrich, TCI and Across, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received. Egg yolk phosphatidylcholine (EYPC) and egg yolk phosphatidylglycerol (EYPG) were purchased from Avanti Polar Lipids.

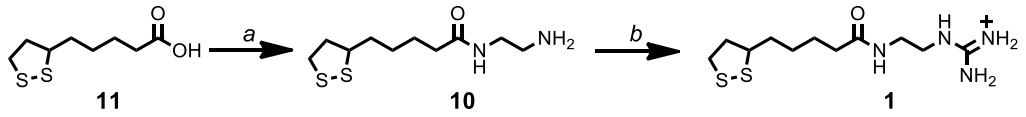
Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical TLC was performed on silica gel 60 (Fluka, 0.2 mm). Microwave reactions were performed using a Biotage Initiator^{TM} microwave synthesizer. Fluorescence measurements were performed with a FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon GmbH) or a FluoroMax-4 spectrofluorometer (Horiba Scientific) equipped with a stirrer and a temperature controller (25.0 ± 0.1 ºC). Fluorescence spectra were corrected using instrument-supplied correction factors. UV-Vis spectra were recorded on a JASCO V-650 spectrophotometer equipped with a stirrer and a temperature controller (25.0 ± 0.1 ºC) and are reported as maximal absorption wavelength λ in nm (extinction coefficient ε in $M^{-1}cm^{-1}$). Gel-Permeation Chromatography (GPC) analyses were performed using a JASCO LC- 2000Plus system equipped with quaternary pump (JASCO PU-2089), photodiode array (JASCO MD-2018 Plus) and fluorescence (JASCO FP-2020 Plus) detectors. The chromatographic column used was a Superdex 75 10/300 GL (flow 0.4 ml/min, eluent: 30% ACN in 0.1 M acetate buffer pH = 6.5). pH values were measured with a Consort C832 multi-parameter analyzer equipped with a VWR glass membrane pH electrode calibrated with Titrisol solution from Merck at pH 4.00 and 7.00. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak), br (broad). ^{1}H and ^{13}C NMR spectra were recorded (as indicated) either on a Bruker 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Proton spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (J) given in Hz, or multiplet (m). Proton signals with low deuterium exchange rates (half life ≥ 5 min) are marked "exchangeable". ^{1}H and ^{13}C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT-135, HSQC and HMBC). Multiplicity of ^{13}C signals were assigned with the aid of DEPT-135 and reported as s (*C*), d (*CH*), t (CH_2) and q (CH_3). ESI-MS for the characterization of new compounds was performed on a Finnigan MAT SSQ 7000 instrument or an ESI API 150EX and are reported as mass-per-charge ratio m/z (intensity in %, [assignment]). ESI-HRMS for the characterization of new compounds

were performed on a QSTAR Pulsar (AB/MDS Sciex) and are reported as mass-per-charge ratio m/z calculated and observed. Vesicles were prepared with a Mini-Extruder from Avanti Polar Lipids (pore size 100 nm).

Abbreviations. ACN: Acetonitrile; AcOEt: Ethyl acetate; Ac_2O: Acetic anhydride; AcOH: Acetic acid; BOC: *t*-Buthoxycarbonyl, BOC_2O: *t*-Buthoxycarbonyl anhydride; Calcd.: Calculated; CDI: 1,1'-Carbonyldiimidazole; CF: Carboxyfluorescein; DCC: *N*,*N*-Dicyclohexylcarbodiimide; DCM: Dichloromethane; DIEA: *N,N*-Diisopropylethylamine; DMF: *N*,*N*-Dimethylformamide; DTT: 1,4-dithio-DL-threitol; EYPC: Egg yolk phosphatidylcholine; EYPG: Egg yolk phosphatidylglycerol; Et_2O: Diethyl ether; EtOH: Ethanol; HBTU: *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; LUVs: Large unilamellar vesicles; MeOD: Methanol-d_4; MeOH: Methanol; μW: Microwave irradiation; NHS: *N*-Hydroxysuccinimide; NMM: *N*-Methylmorpholine; rt: room temperature; Pbf: 2,2,4,6,7-Pentamethyl-dihydrobensofuran-5-sulfonyl; TFA: Trifluoroacetic acid; TEOA: Triethanolamine; TIPS: triisopropylsilane; Tris: Tris(hydroxymethyl)aminomethane.

2. Synthesis

2.1 Propagators

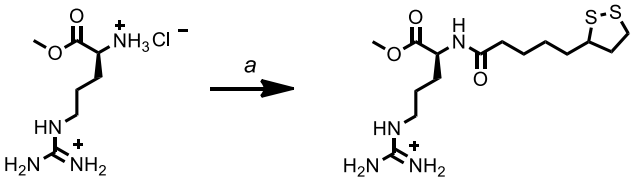


Scheme S1. a) Ethylenediamine, CDI, DCM, 0.5 h, rt, 61%. b) 1*H*-Pyrazole-1-carbox-amidine hydrochloride, DCM, 4 h, rt, 56%.

Compound 10. The procedure was adapted from ref. S2. Commercially available **11** (784 mg, 3.8 mmol) and CDI (812 mg, 5.0 mmol) were dissolved in 25 ml of anhydrous DCM. This solution was added dropwise to 7 ml of anhydrous DCM containing 2 ml (30 mmol) of ethylenediamine, kept at 0 °C. The reaction mixture was stirred 40 min at 0 °C and 30 min at room temperature, then it was washed with brine (3 × 20 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure yielding **10** as yellow oil (575 mg, 61%). Characterization is consistent with the data reported in the literature (ref. S2). IR (ATR, cm^{-1}): 3258 (m), 3138 (m),

2928 (m), 1637 (s), 1540 (m), 1433 (w), 1250 (w), 552 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 5.95 (s br, 1H), 3.60-3.53 (m, 1H), 3.29 (dt, ^{3}J (H,H) = 11.4 Hz, ^{3}J (H,H) = 11.4 Hz, 2H), 3.21-3.08 (m, 2H), 2.83 (t, ^{3}J (H,H) = 6.1 Hz, 2H), 2.49-2.42 (m, 1H), 2.22-2.17 (m, 2H), 1.95-1.86 (m, 1H), 1.76-1.59 (m, 4H), 1.54-1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.1 (s), 56.6 (d), 42.0 (t), 41.5 (t), 40.4 (t), 38.6 (t), 36.6 (t), 34.8 (t), 29.1 (t), 25.6 (t); MS (ESI, 0.1% HCOOH in MeOH): 271 (100, $[M+Na]^{+}$).

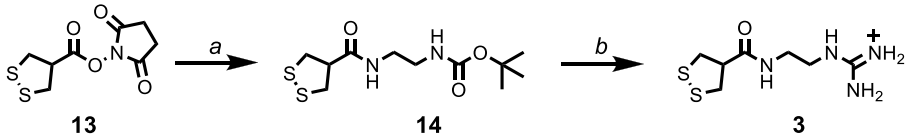
Compound 1. Compound **10** (419 mg, 1.7 mmol) was dissolved in anhydrous DCM (30 ml) and 1*H*-pyrazole-1-carbox-amidine hydrochloride (2.5 g, 1.7 mmol) was added. The suspension was stirred at rt for 4 h, until TLC control (DCM/CH_3OH 9:1) showed complete consumption of starting reagent **10**. Solvent was removed under reduced pressure and the residue dissolved in 1 ml of MeOH. Et_2O (10 ml) were added to induce precipitation. The solid was collected and washed with Et_2O (4 × 10 ml), yielding pure propagator **1** (309 mg, Cl salt, 56%) as a pale yellow solid. IR (ATR, cm^{-1}): 3258 (m), 3138 (m), 2928 (m), 1637 (s), 1540 (m), 1433 (w), 1250 (w), 552 (w); ^{1}H NMR (400 MHz, D_2O): 3.74-3.65 (m, 1H), 3.41-3.33 (m, 4H), 3.29-3.14 (m, 2H), 2.49 (dq, ^{2}J (H,H) = 12.8 Hz, ^{3}J (H,H) = 6.0 Hz, 1H), 2.27 (t, ^{3}J (H,H) = 7.2 Hz, 2H), 1.99 (dq, ^{2}J (H,H) = 12.8 Hz, ^{3}J (H,H) = 6.8 Hz, 1H), 1.81-1.60 (m, 4H), 1.41 (quint, ^{3}J (H,H) = 7.3 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): 176.9 (s), 158.9 (s), 57.5 (d), 42.1 (t), 41.3 (t), 39.4 (t, 2 carbons), 36.7 (t), 35.7 (t), 29.9 (t), 26.5 (t); MS (ESI, 0.1% HCOOH in MeOH): 291 (100, $[M]^{+}$); HRMS (ESI, +ve) calcd. for $C_{11}H_{23}N_4OS_2^{+}$: 291.1308, found: 291.1302.



Scheme S2. a) i) DIEA, DMF, ii) **11**, CDI, DMF, 3.5 h, rt, 84%.

Compound 2. To a solution of **11** (412 mg, 2.0 mmol) in anhydrous DMF (4 ml), CDI (324 mg, 2.0 mmol) was added and the mixture was stirred at rt under Ar atmosphere for 1 h. A solution of **12** (261 mg, 1.0 mmol) and DIEA (174 μL, 1.0 mmol) in anhydrous DMF (4 ml) was added through a cotton filter and then the mixture was stirred at rt under Ar atmosphere for 3.5 h. The

reaction mixture was added dropwise in Et_2O (40 ml). The emulsion was centrifuged (2 min, 4400 rpm) and the supernatant was discarded. The remained yellow oil was washed with DCM/Et_2O mixture (1:2, 5 × 10 ml). After drying the residue *in vacuo*, compound **2** was obtained as a pale yellow sticky solid (346 mg, Cl salt, 84%). IR (ATR, cm^{-1}): 3252 (br), 3144 (br), 2932 (s), 2856 (m), 1738 (s), 1640 (s), 1538 (s), 1435 (m), 1364 (w), 1210 (m), 1148 (w); ^{1}H NMR (500 MHz, CD_3OD): 4.44 (dd, ^3J (H,H) = 9.0 Hz, 5.0 Hz, 1H), 3.73 (s, 3H), 3.61-3.55 (m, 1H), 3.23-3.08 (m, 4H), 2.50-2.44 (m, 1H), 2.28 (t, ^3J (H,H) = 7.2 Hz, 2H), 1.95-1.86 (m, 2H), 1.78-1.60 (m, 7H), 1.52-1.42 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD): 176.3 (s), 173.7 (s), 158.6 (s), 57.6 (d), 53.1 (d), 52.8 (q), 41.9 (t), 41.3 (t), 39.3 (t), 36.5 (t), 35.8 (t), 29.9 (t), 29.8 (t), 29.7 (t), 26.7 (t), 26.4 (t); MS (ESI, MeOH): 377 (100, $[M]^+$), 360 (38, $[M\text{-}NH_3]^+$), 318 (50, $[M\text{-}COOMe]^+$); HRMS (ESI, +ve) calcd. for $C_{15}H_{29}N_4O_3S_2^+$: 377.1675, found: 377.1674.



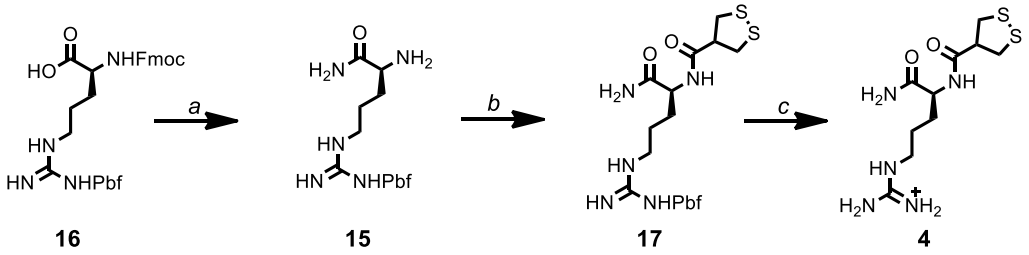
Scheme S3. a) *N*-Boc-ethylenediamine, DCM, 12 h, rt, 79%. (b) i) HCl in Et_2O, DCM, 1 h, rt. ii) 1*H*-pyrazole-1-carbox-amidine hydrochloride, K_2CO_3, MeOH, 3.5 h, rt, 84%.

Compound 13 was synthesized and purified according to procedures described in ref. S3.

Compound 14. Compound **13** (1.08 g, 4.4 mmol) was dissolved in 50 ml of anhydrous DCM. To this solution *N*-Boc ethylenediamine (0.70 g, 4.4 mmol) was added. The reaction mixture was stirred overnight and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, $CHCl_3$/MeOH 97:3, R_f 0.3) to give **14** as colorless solid (1.0 g, 79%). IR (ATR, cm^{-1}): 3224 (m), 3293 (m), 2973 (w), 2931 (w), 1680 (s), 1648 (s), 1531 (s), 1448 (m), 1365 (s), 1276 (s), 1249 (s), 1157 (s), 980 (m), 653 (s); ^{1}H NMR (500 MHz, CD_3OD): 3.39-3.35 (m, 2H), 3.28-3.25 (m, 4H), 3.19-3.13 (m, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): 174.4 (s), 158.6 (s), 80.2 (s), 53.3 (d), 43.5 (t), 40.9 (t), 40.8 (t), 28.8 (q); MS (ESI, 0.1% HCOOH in MeOH): 293 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_{11}H_{20}N_2O_3S_2Na^+$: 315.080x, found: 315.0812.

Compound 3. Compound **14** (198 mg, 0.68 mmol) was dissolved in 25 ml of anhydrous DCM and 5 ml of 1 M HCl in Et_2O were added. The reaction mixture was stirred for 4 h. Solution was concentrated to 8 ml, then the product was collected by filtration as a white solid. To a solution of

the obtained solid in MeOH (5 ml), 1*H*-pyrazole-1-carbox-amidine hydrochloride (99.2 mg, 0.68 mmol) and K_2CO_3 (95.2 mg, 0.69 mmol) were added. The reaction mixture was stirred for 4 h, then it was poured in 25 ml of Et_2O at 0 °C. The product was recovered by centrifugation (2 min, 4400 rpm), yielding propagator **3** as a pale yellow solid (122 mg, Cl salt, 67%). IR (ATR, cm^{-1}): 3255 (m), 3138 (m), 1644 (s), 1545 (s), 1249 (w); ^1H NMR (500 MHz, D_2O): 3.50-3.32 (m, 9H); ^{13}C NMR (125 MHz, D_2O): 175.8 (s), 157.7 (s), 51.9 (d), 42.8 (t), 41.2 (t), 39.0 (t); MS (ESI, 0.1% HCOOH in MeOH): 235 (100, $[M]^+$); HRMS (ESI, +ve) calcd. for $C_7H_{15}N_4OS_2^+$: 235.0682, found: 235.0681.



16 **15** **17** **4**

Scheme S4. a) i) Boc_2O, pyridine, NH_4HCO_3, ACN, 48 h, rt, ii) 30% piperidine in DCM, 10 min, rt, 98%. b) **13**, NMM, DCM, overnight, rt, 69%. c) TFA/H_2O/phenol/TIPS (88:5:5:2), 40 min, rt, dark, 59%.

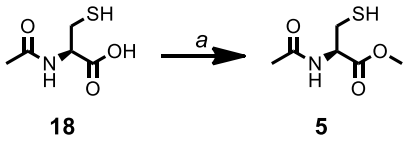
Compound 15. The procedure was adapted from ref. S4. To a solution of **16** (653 mg, 1.0 mmol) and BOC_2O (300 mg, 1.4 mmol) in ACN (5 ml), pyridine (50 µl, 0.6 mmol) and anhydrous NH_4HCO_3 (540 mg, 6.8 mmol) were added. The mixture was sealed and stirred at rt for 2 days. AcOEt (100 ml) was added to the mixture and the organic phase was washed with 1 M HCl (2 × 50 ml), water (50 ml) and brine (50 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The oily residue was washed with Et_2O by solid-liquid extraction. Resulting colorless solid was dried *in vacuo*. To a solution of the obtained solid in DCM (21 ml), piperidine (9 ml) was added and the mixture was stirred at rt for 10 min. After evaporating the solvent under reduced pressure at 30 ºC, the foamy solid was redissolved in DCM and purified by flash column chromatography (SiO_2, DCM/MeOH 97:3 to 9:1, R_f 0.1 in DCM/MeOH 19:1). The obtained solid was washed with Et_2O (5 x 10 ml) by solid-liquid extraction to yield **15** as a colorless powder (414 mg, 98%). Characterization was consistent with the data reported in the literature (ref. S4). IR (ATR, cm^{-1}): 3317 (br), 2948 (m), 2844 (w), 2799 (w), 2738 (m), 2525 (w), 1667 (m), 1614 (m), 1550 (s), 1455 (m), 1247 (m), 1090 (s), 814 (m), 733 (w), 660 (m); ^1H NMR (400 MHz, $CDCl_3$):

7.68 (s, br, 1H), 6.88 (s, br, 1H), 6.81 (br, 1H), 6.51 (s, br, 2H), 4.23 (s, br, 2H), 3.71-3.68 (m, 1H), 3.26-3.20 (m, 2H), 2.92 (s, 2H), 2.53 (s, 3H), 2.46 (s, 3H), 2.06 (s, 3H), 1.86-1.60 (m, 4H), 1.44 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 163.7 (s), 159.0 (s), 156.9 (s), 147.0 (s), 138.4 (s), 132.8 (s), 132.4 (s), 124.9 (s), 117.8 (s), 86.7 (s), 54.1 (d), 43.4 (t), 31.6 (t), 28.8 (q), 25.5 (t), 19.5 (q), 18.2 (q), 12.7 (q); MS (ESI, MeOH): 466 (100, $[M+K]^+$), 449 (5, $[M+Na]^+$), 426 (53, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_{19}H_{32}N_5O_4S^+$: 426.2169, found: 426.2186.

Compound 17. To a solution of **15** (755 mg, 1.8 mmol) in DCM (20 ml), **13** (439 mg, 1.8 mmol) was added and the mixture was stirred at rt under Ar atmosphere for 2 h. After NMM (97 μl, 0.9 mmol) was added, the mixture was stirred overnight, and then concentrated under reduced pressure and purified by flash column chromatography (SiO_2, DCM/MeOH 19:1 to 14:1 to 9:1, R_f 0.45 in DCM/Acetone 9:1). The product was washed with Et_2O (3 × 10 ml) by solid-liquid extraction and dried *in vacuo* to give **17** as a white solid (686 mg, 69%). IR (ATR, cm^{-1}): 3315 (br), 2972 (w), 2917 (w), 1653 (m), 1618 (m), 1546 (s), 1451 (m), 1407 (m), 1292 (w), 1242 (m), 1154 (w), 1092 (s), 993 (w) 851 (w), 811 (m), 732 (w), 665 (m); ^{1}H NMR (400 MHz, CD_3OD): 4.35 (dd, ^3J (H,H) = 9.0 Hz, 5.0 Hz, 1H), 3.41-3.15 (m, 7H), 3.00 (s, 2H), 2.57 (s, 3H), 2.51 (s, 3H), 2.08 (s, 3H), 1.86-1.78 (m, 1H), 1.70-1.49 (m, 3H), 1.46 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD): 174.4 (s), 160.0 (s), 139.5 (s), 134.5 (s), 133.7 (s), 126.2 (s), 118.6 (s), 101.6 (s), 87.8 (s), 54.3 (d), 53.1 (d), 44.1 (t), 43.9 (t), 43.5 (t), 41.5 (t), 30.7 (t), 28.9 (q), 19.7 (q), 18.5 (q), 12.7 (q); MS (ESI, MeOH): 581 (72, $[M+Na]^+$), 559 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_{23}H_{36}N_5O_5S_3^+$: 558.1875, found: 558.1873.

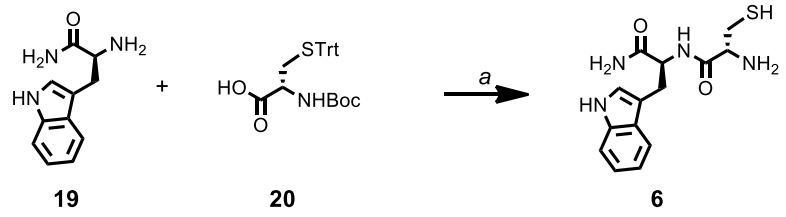
Compound 4. To a freshly prepared solution of TFA/H_2O/phenol/TIPS (88:5:5:2, 12 ml), **17** (540 mg, 0.97 mmol) was added under Ar atmosphere, protected from light. The reaction mixture was stirred at rt for 40 min and concentrated under reduced pressure at 30 °C to 3 ml. The mixture was purified by flash column chromatography (SiO_2, DCM/MeOH 49:1 to 4:1, R_f 0.1 in DCM/MeOH 9:1). The oily product was washed with Et_2O (5 × 10 ml) by solid-liquid extraction and dried *in vacuo* to yield **4** as a foamy solid (210 mg, TFA salt, 59%). IR (ATR, cm^{-1}): 3182 (br), 2925 (w), 1642 (s), 1533 (m), 1423 (m), 1178 (s), 1128 (s) 1047 (w), 837 (m), 801 (m), 721 (m); ^{1}H NMR (400 MHz, CD_3OD): 4.62 (s, br, 1H), 4.45 (dd, ^3J (H,H) = 8.4 Hz, 5.2 Hz, 1H), 3.43-3.19 (m, 7H), 1.93-1.84 (m, 1H), 1.76-1.60 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): 176.4 (s), 174.3 (s), 158.6 (s), 54.1 (d), 52.8 (d), 43.8 (t), 43.3 (t), 41.9 (t), 30.3 (t), 26.4 (t); MS (ESI, MeOH): 306 (100, $[M]^+$); HRMS (ESI, +ve) calcd. for $C_{10}H_{20}N_5O_2S_2^+$: 306.1052, found: 306.1061.

2.2 Initiators and Terminators



Scheme S5. a) $SOCl_2$, MeOH, 1.5 h, rt, 44%.

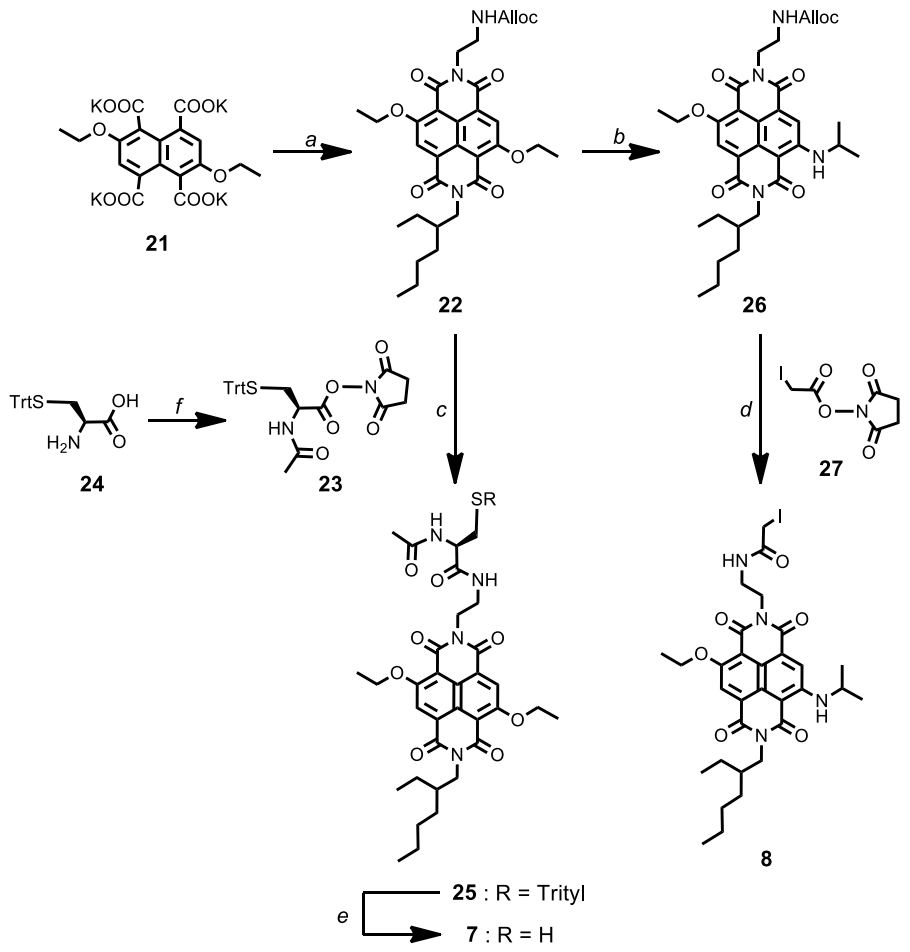
Compound 5. The procedure was adapted from ref. S5. Commercial *N*-acetyl cysteine (**18**, 2.32 g, 14.2 mmol) was dissolved in 50 ml of MeOH. $SOCl_2$ (1.2 ml, 16.5 mmol) was added dropwise and the resulting solution was stirred for 90 min at rt. Solvent was removed under reduced pressure and the resulting residue was dissolved in AcOEt (40 ml). The mixture was washed with brine (3 × 20 ml). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The compound was purified by flash column chromatography (SiO_2, $CHCl_3$/MeOH 95:5, R_f 0.3) to give **5** as a white solid (1.92 g, 76%). Characterization was consistent with the data reported in the literature (ref. S5). ^{1}H NMR (400 MHz, $CDCl_3$): 6.54 (s br, 1H), 4.88-4.84 (m, 1H), 3.76 (s, 3H), 2.99-2.95 (m, 2H), 2.04 (s, 3H), 1.35 (t, ^3J (H,H) = 9.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): 170.9 (s), 170.2 (s), 53.8 (d), 53.0 (q), 27.1 (t), 23.3 (q); MS (ESI, MeOH): 178 (100, $[M+H]^+$).



Scheme S6. a) i) HBTU, DIEA, DCM, 2 h, rt, ii) TIPS, TFA, DCM, 1.5 h, rt, dark, 44%.

Compound 6. The procedure was adapted from ref. S6. To a solution of **19** (251 mg, 1.1 mmol) and DIEA (539 μl, 3.2 mmol) in DCM (10 ml), a solution of **20** (534 mg, 1.2 mmol) and HBTU (478 mg, 1.3 mmol) in anhydrous DCM (10 ml) was added and stirred under Ar atmosphere at rt for 2 h.

The mixture was concentrated under reduced pressure and purified by flash column chromatography (SiO_2, DCM/MeOH 97:3, R_f 0.6 in DCM/MeOH 9:1) to give a crude product (486 mg). A portion of the obtained solid (48.6 mg, 0.075 mmol) was dissolved in DCM (3.5 ml) and TIPS (81.8 μl, 0.40 mmol) and TFA (187 μl, 2.4 mmol) were added sequentially. After the mixture was stirred under Ar atmosphere at 0 ºC, protected from light, for 10 min, toluene (5 ml) was added to the mixture and evaporated under reduced pressure at 34 °C. The resulting oil was dissolved in AcOEt and purified by flash column chromatography (SiO_2, AcOEt/MeOH 19:1 to 9:1, R_f 0.5 in AcOEt/MeOH 9:1). The resulting compound was dried *in vacuo* and dissolved in 3 N HCl in AcOEt (5 ml). After being stirred under Ar atmosphere at 0 ºC for 4 h, the mixture was evaporated under reduced pressure. Dissolving in AcOEt and evaporating were repeated five times to yield **6** as a white solid compound (25 mg, quant.). Characterization was consistent with the data reported in the literature (ref. S6). IR (ATR, cm^{-1}): 3312 (br), 2920 (w), 1659 (s), 1516 (m), 1415 (m), 1345 (m), 1232 (w), 1098 (m), 1011 (w), 742 (s); ^{1}H NMR (400 MHz, CD_3OD): 8.47 (d, ^3J (H,H) = 7.4 Hz, 1H, exchangeable), 7.66 (d, ^3J (H,H) = 7.9 Hz, 1H), 7.33 (d, ^3J (H,H) = 7.9 Hz, 1H), 7.17 (s, 1H), 7.11 – 7.07 (m, 1H), 7.04 – 7.00 (m, 1H), 4.74 (dd, ^3J (H,H) = 8.7 Hz, 5.7 Hz, 1H), 4.01 (t, ^3J (H,H) = 5.6 Hz, 1H), 3.32, (dd, ^2J (H,H) = 14.7 Hz, ^3J (H,H) = 5.7 Hz, 1H), 3.17 (dd, ^2J (H,H) = 14.7 Hz, ^3J (H,H) = 8.7 Hz, 1H), 3.02 (d, ^3J (H,H) = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): 174.4 (s) 173.2 (s), 168.4 (s), 138.2 (s), 128.8 (s), 124.9 (d), 122.6 (d), 120.0 (d), 119.5 (d), 112.5 (d), 56.0 (d), 55.8 (d), 29.3 (t), 26.7 (t); MS (ESI, MeOH): 307 (100, $[M+H]^+$). HRMS (ESI, +ve) calcd. for $C_{14}H_{19}N_4O_2S^+$: 307.1223, found: 307.1224.



Scheme S7. a) 2-Ethylhexylamine, *N*-allyloxycarbonyl-ethylenediamine, AcOH, H_2O, μW 120 °C, 2 h, 58%. b) DCM/2-*iso*-propylamine (1:1), overnight, rt, 95%. c) i) $(PPh_3)_4Pd$, PhH_3Si, DCM, 10 min, rt, ii) **23**, NMM, DCM, 1 h, rt, 46%. d) i) $(PPh_3)_4Pd$, PhH_3Si, DCM, 10 min, rt, ii) **27**, NMM, DCM, 0.5 h, rt, 55%. e) TIPS, TFA, DCM, 4 h, rt, dark, 93%. f) i) Ac_2O, water/1,4-dioxane (1:1), pH 9, overnight, 0 ºC to rt, ii) 1M HCl, pH 3, iii) NHS, DCC, AcOEt, overnight, rt, 29%.

Compound 21 was synthesized according to procedures described in ref. S7.

Compound 22. A solution of **21** (321 mg, 0.59 mmol) and KOH (315 mg, 5.5 mmol) in water (1.8 ml), 2-ethylhexylamine (391 μl, 2.4 mmol), *N*-allyloxycarbonyl-ethylenediamine (154 mg, 1.2 mmol),[S8] and AcOH (18 ml) were mixed in the sealed tube. The mixture was heated at 120 ° C for 1 h under microwave irradiation. The resulting solution was diluted with EtOAc (300 ml), washed with 1 M $KHSO_4$ (4 × 100 ml), water (2 × 100 ml), saturated $NaHCO_3$ (3 × 100 ml), water (2 × 100 ml) and brine (100 ml). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to apply to the flash column chromatography (SiO_2, DCM/acetone 19:1 to 14:1 to 9:1, R_f 0.3 in DCM/acetone 19:1, ×2). The resulting solid was washed with Et_2O (3 × 3 ml)

by solid-liquid extraction to yield **22** as a yellow solid (203 mg, 58%). IR (ATR, cm^{-1}): 3385 (w), 2960 (w), 2928 (w), 2861 (w), 1719 (w), 1697 (m), 1652 (s), 1573 (m), 1530 (w), 1451 (m), 1379 (w), 1276 (m), 1240 (m), 1206 (m) 1041 (w), 1014 (w), 936 (w), 871 (w), 790 (m), 737 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 8.23 (s, 1H), 8.19 (s, 1H), 5.86 (ddt, ^{3}J (H,H) = 17.0, 10.6, 5.7 Hz, 1H), 5.70 (t, ^{3}J (H,H) = 5.8 Hz, 1H), 5.26 (d, ^{3}J (H,H) = 17.0 Hz, 1H), 5.13 (d, ^{3}J (H,H) = 10.6 Hz, 1H), 4.52 (d, ^{3}J (H,H) = 5.2 Hz, 2H), 4.46 (q, ^{3}J (H,H) = 6.9 Hz, 2H), 4.45 (q, ^{3}J (H,H) = 6.9 Hz, 2H), 4.33 (t, ^{3}J (H,H) = 5.4 Hz, 1H), 4.05-3.94 (m, 2H), 3.64 (dt, ^{3}J (H,H) = 5.8 Hz, 5.4 Hz, 2H), 1.87-1.81 (m, 1H), 1.66 (t, ^{3}J (H,H) = 6.9 Hz, 3H), 1.65 (t, ^{3}J (H,H) = 6.9 Hz, 3H), 1.39-1.20 (m, 8H), 0.89 (t, ^{3}J (H,H) = 7.4 Hz, 3H), 0.86 (t, ^{3}J (H,H) = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 162.8 (s), 162.5 (s), 161.3 (s), 161.0 (s), 159.8 (s), 156.7 (s), 126.8 (d), 133.2 (d), 126.6 (d), 123.2 (s), 119.5 (d), 119.4 (d), 117.5 (t), 110.4 (s), 110.1 (s), 66.5 (t), 66.5 (t), 65.7 (t), 44.9 (t), 30.6 (t), 40.2 (t), 37.9 (d), 30.8 (t), 28.8 (t), 24.2 (t), 23.3 (t), 15.0 (q), 15.0 (q), 14.3 (q), 10.8 (q); MS (ESI, DCM/MeOH): 595 (100, $[M+H]^+$), 537 (27, [M-allyloxy-H]$^+$), 617 (7, $[M+Na]^+$); HRMS (ESI, +ve) calcd. for $C_{32}H_{40}N_3O_8^+$: 594.2809, found: 594.2810.

Compound 23. The procedure was adapted from ref. S9. Compound **24** (1.8 g, 5.0 mmol) was dissolved in water/1,4-dioxane (1:1, 20 ml) adjusting pH to 9~10 with 1 M NaOH. Ac_2O (940 μl, 10 mmol) was added at 0 ºC and the mixture was stirred overnight at rt. 1 M HCl was added to adjust pH to 3 and the crude compound was extracted in DCM (2 × 20 ml). The organic phase was dried with anhydrous Na_2SO_4, concentrated under reduced pressure and precipitated with Et_2O. The white solid was dried *in vacuo* and dissolved in AcOEt (120 ml). NHS (575 mg, 5.0 mmol) and DCC (1.0 g, 5.0 mmol) were added to the solution and the mixture was stirred at rt under Ar atmosphere overnight. The insoluble precipitate was filtered off and the filtrate was evaporated under reduced pressure. The solid product was washed with EtOH (2 × 50 ml) and Et_2O (3 × 10 ml) by solid-liquid extraction to yield **26** as a white powder (729 mg, 29%). Characterization was consistent with the data reported in the literature (ref. S9). IR (ATR, cm^{-1}): 1812 (w), 1784 (w), 1741 (s), 1651 (m), 1536 (w), 1490 (w), 1445 (w), 1366 (w), 1203 (s), 1158 (w), 1063 (m), 997 (w), 843 (w), 743 (m), 698 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 7.47-7.22 (m, 15H), 5.67 (d, ^{3}J (H,H) = 8.0 Hz, 1H), 4.71 (ddd, ^{3}J (H,H) = 8.0 Hz, 7.6 Hz, 5.4 Hz, 1H), 2.88 (dd, ^{2}J (H,H) = 12.8 Hz, ^{3}J (H,H) = 7.6 Hz, 1H), 2.83 (s, 4H), 2.88 (dd, ^{2}J (H,H) = 12.8 Hz, ^{3}J (H,H) = 5.4 Hz, 1H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 169.7 (s), 168.5 (s), 166.7 (s), 144.3 (s), 129.7 (d), 128.4 (d), 127.6 (d), 49.7 (d), 33.5 (t), 25.8 (t), 23.09 (q); MS (ESI, MeOH): 525 (100, $[M+Na]^+$).

Compound 25. To a solution of **22** (134 mg, 0.19 mmol) in anhydrous DCM (4 ml), $(PPh_3)_4Pd$ (56 mg, 0.048 mmol) and PhH_3Si (150 μl, 1.1 mmol) were added. The mixture was stirred at rt

under Ar atmosphere for 10 min and evaporated under reduced pressure at 25 ^{o}C and dried *in vacuo*. To a solution of the resulted solid in anhydrous DCM (5 ml), a solution of **23** (108 mg, 0.19 mmol) in DCM (5 ml) and NMM (21 μl, 0.19 mmol) were added sequentially. The mixture was stirred at rt under Ar atmosphere for 1 h, concentrated under reduced pressure at 25 °C and purified by flash column chromatography (SiO_2, DCM/Acetone 94:6 to 9:1, R_f 0.25 in DCM/acetone 9:1). The resulting solid was washed with Et_2O (3 × 3 ml) by solid-liquid extraction to yield **25** as a yellow solid (80 mg, 46%). IR (ATR, cm^{-1}): 3369 (w), 2934 (w), 1699 (m), 1655 (s), 1574 (s), 1494 (w), 1448 (s), 1379 (m), 1323 (s), 1272 (s), 1203 (s), 1109 (w), 1017 (m), 896 (w), 791 (m), 763 (m), 742 (m), 700 (s), 634 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 8.42 (s, 2H), 7.34-7.18 (m, 15H), 6.68 (t, ^{3}J (H,H) = 5.4 Hz, 1H), 5.82 (d, ^{3}J (H,H) = 8.0 Hz, 1H), 4.50 (q, ^{3}J (H,H) = 7.0 Hz, 2H), 4.49 (q, ^{3}J (H,H) = 7.0 Hz, 2H), 4.34 (t, ^{3}J (H,H) = 5.6 Hz, 2H), 4.21 (ddd, ^{3}J (H,H) = 8.0 Hz, 6.2 Hz, 5.8 Hz, 1H), 4.15-4.08 (m, 2H), 3.72-3.49 (m, 2H), 2.78 (dd, ^{2}J (H,H) = 13.0 Hz, ^{3}J (H,H) = 6.2 Hz, 1H), 2.45 (dd, ^{2}J (H,H) = 13.0 Hz, ^{3}J (H,H) = 5.8 Hz, 1H), 2.00-1.93 (m, 1H), 1.90 (s, 3H), 1.66 (t, ^{3}J (H,H) = 7.0 Hz, 3H), 1.65 (t, ^{3}J (H,H) = 7.0 Hz, 3H), 1.44-1.26 (m, 8H), 0.93 (t, ^{3}J (H,H) = 7.4 Hz, 3H), 0.88 (t, ^{3}J (H,H) = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 171.8 (s), 171.6 (s), 170.4 (s), 170.2 (s), 163.2 (s), 162.9 (s), 161.8 (s), 161.5 (s), 160.3 (s), 160.1 (s), 147.0 (s), 145.4 (s), 144.5 (s), 129.6 (d), 128.1 (d), 127.0 (d), 126.8 (s), 123.8 (s), 120.1 (d), 119.8 (d), 111.4 (s), 110.6 (s), 67.3 (t), 66.5 (t), 52.0 (d), 44.7 (t), 39.7 (t), 39.2 (t), 37.9 (d), 33.5 (t), 30.8 (t), 28.8 (t), 24.2 (t), 23.3 (q), 23.3 (t), 15.0 (q), 15.0 (q), 14.3 (q), 10.9 (q); MS (ESI, DCM/MeOH): 920 (100, $[M+Na]^+$), 897 (8, $[M+H]^+$), 493 (17, $[M-(Ac\text{-}Cys(Trt)\text{-}NH_2)+H]^+$); HRMS (ESI, +ve) calcd. for $C_{52}H_{57}N_4O_8S^+$: 897.3891, found: 897.3902.

Compound 7. To a solution of **25** (220 mg, 0.25 mmol) in DCM, TIPS (375 μl, 1.8 mmol) and TFA (750 μl, 9.8 mmol) were added sequentially under Ar atmosphere at rt for 4 h, protected from light. After the completion of the reaction, toluene (6 ml) and silica gel were added and the mixture was evaporated under reduced pressure below 35 °C. The obtained compound deposited on silica gel was applied to the flash column chromatography (SiO_2, DCM/acetone 4:1 to 3:1, R_f 0.25 in DCM/acetone 4:1) to yield **7** as a yellow solid (150 mg, 93%). IR (ATR, cm^{-1}): 3272 (w, br), 2929 (m), 2859 (w), 1799 (m), 1653 (s), 1574 (s), 1531 (w), 1449 (s), 1378 (m), 1323 (m), 1270 (s), 1202 (s), 1107 (m), 1059 (m), 1013 (m), 945 (w), 897 (w), 791 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 8.37 (s, 1H), 8.35 (s, 1H), 7.03 (t, ^{3}J (H,H) = 5.6 Hz, 1H), 6.56 (d, ^{3}J (H,H) = 8.0 Hz, 1H), 4.60 (ddd, ^{3}J (H,H) = 8.0 Hz, 6.0 Hz, 4.3 Hz, 1H), 4.51 (q, ^{3}J (H,H) = 7.1 Hz, 2H), 4.49 (q, ^{3}J (H,H) = 6.9 Hz, 2H), 4.38 (t, ^{3}J (H,H) = 5.4 Hz, 2H), 4.14-4.04 (m, 2H), 3.72 (dt, ^{3}J (H,H) = 5.6 Hz, 5.4 Hz, 2H), 2.99 (ddd, ^{2}J (H,H) = 13.8 Hz, ^{3}J (H,H) = 7.8 Hz, 4.3 Hz, 1H), 2.70 (ddd, ^{2}J (H,H) = 13.8 Hz, ^{3}J

(H,H) = 10.2 Hz, 6.0 Hz, 1H), 2.05 (s, 3H), 1.96-1.89 (m, 1H), 1.67 (t, ^3J (H,H) = 6.9 Hz, 3H), 1.65 (t, ^3J (H,H) = 7.1 Hz, 3H), 1.47 (dd, ^3J (H,H) = 10.2 Hz, 7.8 Hz, 1H), 1.40-1.27 (m, 8H), 0.92 (t, ^3J (H,H) = 7.4 Hz, 3H), 0.88 (t, ^3J (H,H) = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 170.2 (s), 170.0 (s), 163.1 (s), 162.7 (s), 161.8 (s), 161.2 (s), 160.3 (s), 160.0 (s), 127.5 (s), 126.5 (s), 123.6 (s), 123.5 (s), 120.0 (d), 119.6 (d), 111.1 (s), 110.2 (s), 66.5 (t), 54.1 (d), 44.6 (t), 40.0 (t), 39.1 (t), 37.9 (d), 30.8 (t), 28.8 (t), 26.9 (t), 24.1 (t), 23.4 (q), 23.2 (t), 15.0 (q), 14.9 (q), 14.3 (q), 10.8 (q); MS (ESI, DCM/MeOH): 655 (60, $[M+H]^+$), 614 (17, $[M-Ac+H]^+$), 510 (27, $[M-(Ac-Cys)+H]^+$), 493 (100, $[M-(Ac-Cys-NH_2)+H]^+$); HRMS (ESI, +ve) calcd. for $C_{33}H_{43}N_4O_8S^+$: 655.2976, found: 655.2973.

Compound 26. To a solution of **22** (26 mg, 0.044 mmol) in anhydrous DCM (2 ml), *iso*-propylamine (2 ml) was added. The mixture was stirred under Ar atmosphere at rt overnight. After evaporation at 25 °C, the resulting solid was dissolved in DCM and purified by the flash column chromatography (SiO_2, DCM/Acetone 97:3 to 96:4, R_f 0.3 in DCM/acetone 49:1). The resulting solid was washed with Et_2O (3 × 3 ml) by solid-liquid extraction to yield **26** as a purple solid (mixture of regio-isomers, 26 mg, 95%). IR (ATR, cm^{-1}): 3396 (w), 3265 (w), 2960 (m), 2930 (m), 2868 (w), 1728 (m), 1710 (m), 1665 (m), 1636 (s), 1582 (s), 1522 (m), 1497 (m), 1453 (s), 1380 (w), 1289 (s), 1237 (m) 1209 (m), 1138 (m), 1108 (w), 1061 (w), 996 (m), 929 (w), 891 (w), 788 (s), 744 (m), 652 (m); ^{1}H NMR (400 MHz, $CDCl_3$, 5/5 = regioisomeric equivalents): 9.80/9.77 (d, ^3J (H,H) = 8.0 Hz, 1H), 8.32/8.31 (s, 1H), 8.26 (s, 1H), 5.85 (ddt, ^3J (H,H) = 17.2 Hz, 10.4 Hz, 5.2 Hz, 1H), 5.31-5.28 (m, 1H), 5.25 (d, ^3J (H,H) = 17.2 Hz, 1H), 5.13 (d, ^3J (H,H) = 10.4 Hz, 1H), 4.51 (d, ^3J (H,H) = 5.2 Hz, 2H), 4.48-4.37 (m, 4H), 4.21-4.05 (m, 3H), 3.64-3.60 (m, 2H), 1.99-1.91 (m, 1H), 1.64 (t, ^3J (H,H) = 6.8 Hz, 3H), 1.45/1.44 (d, ^3J (H,H) = 6.0 Hz, 6H), 1.41-1.26 (m, 8H), 0.94/0.93 (t, ^3J (H,H) = 7.4 Hz, 3H), 0.89/0.88 (t, ^3J (H,H) = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, 2/1 = regioisomeric equivalents): 166.2/166.1 (s), 163.2/163.2 (s), 163.0/162.8 (s), 161.8/161.5 (s), 157.9/157.7 (s), 156.6 (s) 150.1/149.7 (s), 133.2/133.2 (d), 127.4/126.9 (s), 124.9/124.5 (s), 124.0/123.9 (s), 121.2/121.1 (s), 120.7/120.7 (d), 118.0/117.9 (d), 117.5/117.4 (t), 111.2/111.5 (s), 99.8/99.4 (s), 66.0/66.0 (t), 65.6 (t), 44.7 (t), 44.5/44.2 (t), 40.3/40.2 (t), 39.9 (t), 37.9/37.9 (d), 30.8/30.8 (t), 28.8/28.8 (t), 24.2/24.1 (t), 23.4/23.3 (d), 23.2 (t), 15.0 (q), 14.9 (q), 14.2 (q), 10.8/10.8 (q); MS (ESI, DCM/MeOH): 630 (12, $[M+Na]^+$), 608 (100, $[M+H]^+$), 550 (22, $[M-allyloxy+H]^+$); HRMS (ESI, +ve) calcd. for $C_{33}H_{43}N_4O_7^+$: 607.3126, found: 607.3111.

Compound 27 was synthesized according to procedures described in ref. S10.

Compound 8. To a solution of **26** (134 mg, 0.22 mmol) in anhydrous DCM (10 ml), $(PPh_3)_4Pd$ (140 mg, 0.11 mmol) and PhH_3Si (380 μl, 2.2 mmol) were added. The mixture was stirred at rt

under Ar atmosphere for 10 min. The solvent was evaporated under reduced pressure at 25 °C and dried *in vacuo*. To a solution of the resulted solid in anhydrous DCM (20 ml), **27** (170 mg, 0.44 mmol) and NMM (50 µl, 0.44 mmol) were added. After the mixture was stirred at rt under Ar atmosphere for 30 min, the reaction was quenched with 2 drops of AcOH and then, the mixture was concentrated under reduced pressure at 25 °C and purified by flash column chromatography (SiO_2, DCM/acetone 24:1, R_f 0.3 in DCM/acetone 24:1). The collected two regioisomers were evaporated under reduced pressure and washed with Et_2O (3 x 3 ml) to yield **8** as a purple solid (83 mg, 55%). IR (ATR, cm^{-1}): 2956 (m), 2928 (m), 2870 (w), 1702 (w), 1667 (m), 1639 (s), 1583 (s), 1453 (m), 1380 (w), 1292 (m), 1207 (m), 1014 (m), 790 (m), 653 (w); ^{1}H NMR (400 MHz, $CDCl_3$, 7/3 = regioisomeric equivalents): 9.79/9.77 (d, ^3J (H,H) = 8.0 Hz, 1H), 8.28/8.26 (s, 1H), 8.23/8.24 (s, 1H), 6.84/6.76 (t, ^3J (H,H) = 5.3 Hz, 1H), 4.43/4.42 (q, ^3J (H,H) = 6.8 Hz, 1H), 4.21-4.03 (m, 2H+1H), 3.72-3.68 (m, 2H), 3.65/3.65 (s, 2H), 1.97-1.89 (m, 1H), 1.63/1.64 (t, ^3J (H,H) = 7.0 Hz, 3H), 1.45/1.45 (d, ^3J (H,H) = 6.0 Hz, 6H), 1.40-1.28 (m, 8H), 0.93/0.93 (t, ^3J (H,H) = 7.4 Hz, 3H), 0.88/0.89 (t, ^3J (H,H) = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 7/3 = regioisomeric equivalents): 167.4/167.6 (s), 166.3/166.3 (s), 163.9/163.6 (s), 163.1/163.1 (s), 162.2 (s), 161.8 (s), 157.8 (s), 150.3 (s), 137.8 (s), 127.8 (s), 124.5 (s), 124.3 (s), 121.4 (s), 120.9 (d), 118.3 (d), 112.7 (s), 66.2/66.1 (t), 44.7/44.7 (d), 44.6 (t), 40.4/40.1 (t), 39.0 (t), 37.9/37.9 (d), 30.8 (t), 28.8 (t), 24.1/24.2 (t), 23.5 (q), 23.3/23.3 (t), 15.0/15.0 (q), 14.3/14.4 (q), 10.8/10.8 (q), -0.6/-0.6 (t); MS (ESI, DCM/MeOH): 692 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_{31}H_{40}IN_4O_6^+$: 691.1987, found: 691.1994.

3. Polymerization

3.1. General Procedure for Polymerization

Stock solutions of propagators **1-4** (500 mM in MeOH), initiators **5-6** (5 mM in appropriate buffer) and terminator **9** (500 mM in water) were freshly prepared and fluxed with Ar. Appropriate amounts of propagator solution were transferred to an Eppendorf tube and dried *in vacuo*. The solid propagators were dissolved in proper buffer solution (1.0 M TEOA, pH 7, or as specified), with or without initiators **5-6** (5 mM, or as specified) to give the final desired concentration of the polymerization mixture. For polymerization, the samples were kept at 25 °C with vigorous agitation. At fixed periods of time (1, 3, 5, 10, 15, 20 and 30 min), 20 µl of polymerization mixture

were taken and quenched with desired terminator solution (380 µl, 500 mM), yielding 5 mM overall guanidinium cations. This solution was tested in LUV assay (section 4.2) and GPC (section 5).

3.2. Dependence on Initiator Concentration (Fig. 4C)

The general procedure for polymerization was followed. Stock solutions of initiator **5**, at different concentrations, in 1 M TEOA buffer were freshly prepared and fluxed with Ar. Proper amount of initiator solution were used to dissolve propagator **1** in order to have 100 mM final concentration of guanidinium cations in the polymerization mixture. After 30 min, 20 µl of polymerization mixture were taken and quenched with terminator solution (380 µl, 500 mM), yielding 5 mM overall guanidinium cations. This solution was tested in LUV assay (section 4.2). The observed transport activity *Y* after 30 min polymerization time are reported in the following table.

Table S1. Transport activity *Y* of **1** with different concentration of initiator **5**

Initiator **5** (mM)	0	0.1	0.5	5	50	200	500
Activity (*Y*)	0.40	0.77	0.85	0.92	0.86	0.72	0.14

3.3. pH Dependence (Fig. 4B)

The general procedure for polymerization was followed. The buffers used for various pH were the following: Acetate buffer (pH=5.0), malate buffer (pH=6.0), TEOA buffer (pH=7.0; pH=7.5; pH=8.0), borate buffer (pH=8.5; pH=9.0). The solutions were freshly prepared and fluxed with Ar.

4. Polymerization Detected with Fluorogenic Vesicles

4.1. Vesicle Preparation

LUVs were prepared following the general procedures in ref. S11-S13. A thin lipid film was obtained by evaporating a solution of 25 mg EYPC or EYPG in 1 ml MeOH/$CHCl_3$ 1:1 on a rotary evaporator (rt) and then *in vacuo* overnight. The resulting film was hydrated with 1.0 ml buffer (50 mM CF, 10 mM Tris, 10 mM NaCl, pH 7.4) for more than 30 min, subjected to freeze-thaw cycles

(5×) and extrusions (15×) through a polycarbonate membrane (pore size, 100 nm). Extravesicular components were removed by gel filtration (Sephadex G-50) with 10 mM Tris, 107 mM NaCl, pH 7.4 buffer as eluent. Final conditions: ~5 mM EYPC; inside: 50 mM CF, 10 mM Tris, 10 mM NaCl, pH 7.4; outside: 10 mM Tris, 107 mM NaCl, pH 7.4.

4.2. The LUV Assay

EYPC-LUVs stock solutions (25 μl) were diluted with a buffer (10 mM Tris, 107 mM NaCl, pH 7.4) and placed in a thermostated fluorescence cuvette (25 °C) and gently stirred (total volume in the cuvette, ~2000 μl; final lipid concentration, ~62.5 μM). CF efflux was monitored at λ_{em} 517 nm (λ_{ex} 492 nm) as a function of time after addition of reaction mixtures (20 μl) at t = 50 s and 1.2% aqueous triton X-100 (40 μl, 0.024% final concentration) at t = 300 s. Fluorescence intensities were normalized to fractional emission intensity I(t) using equation (Eq.1):

$$I(t) = (I_t - I_0) / (I_\infty - I_0) \qquad (Eq.1)$$

where $I_0 = I_t$ just before reaction mixtures addition, $I_\infty = I_t$ at saturation after lysis (Fig. 2A, S1). Effective concentration for polymers or monomers EC_{50} and Hill coefficient n were determined by plotting the fractional activity Y (= I(t) at saturation just before lysis at t = ~290 s as a function of guanidinium concentration c and fitting them to the Hill equation (Eq.2)

$$Y = Y_0 + (Y_{MAX} - Y_0) / \{1 + (EC_{50} / c)^n\} \qquad (Eq.2)$$

where Y_0 is Y without polymer or monomer, Y_{MAX} is Y with an excess polymer or monomer at saturation, EC_{50} is the concentration of polymer or monomer required to reach 50% activity and n is the Hill coefficient.

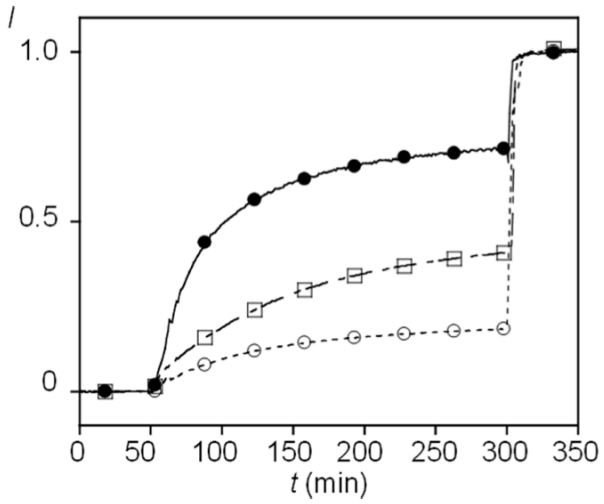


Figure S1. Change in CF emission intensity *I* during the addition of reaction mixtures (50 s, 25 μM final guanidinium concentration) and excess of triton X100 (300 s) to EYPC-LUVs⊃CF. Reaction mixtures: 200 mM **2**, before (○) and after polymerization with (●, 5 mM) and without **5** (□), pH 7.5, 1 min).

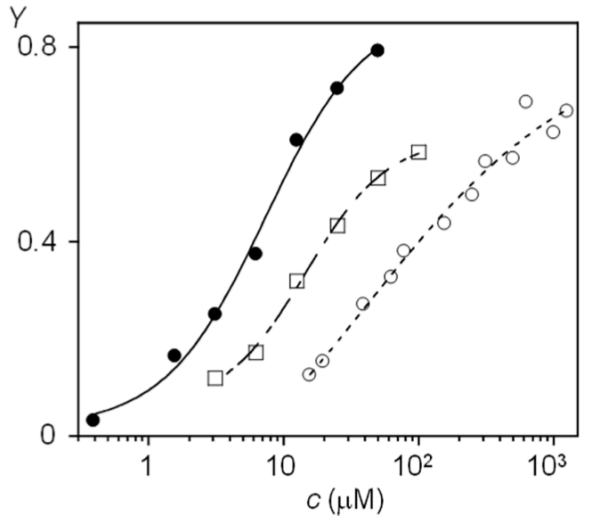


Figure S2. Transport activity *Y* of **2** before (○) and after polymerization with (●, 5 mM) and without **5** (□), with increasing concentration of guanidinium cations, in EYPC-LUVs⊃CF.

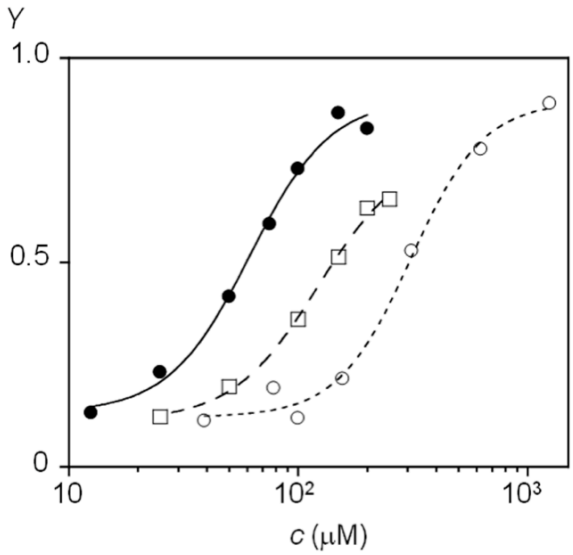


Figure S3. Transport activity *Y* of **2** before (○) and after polymerization with (●, 5 mM) and without **5** (□), with increasing concentration of guanidinium cations, in EYPG-LUVs⊃CF.

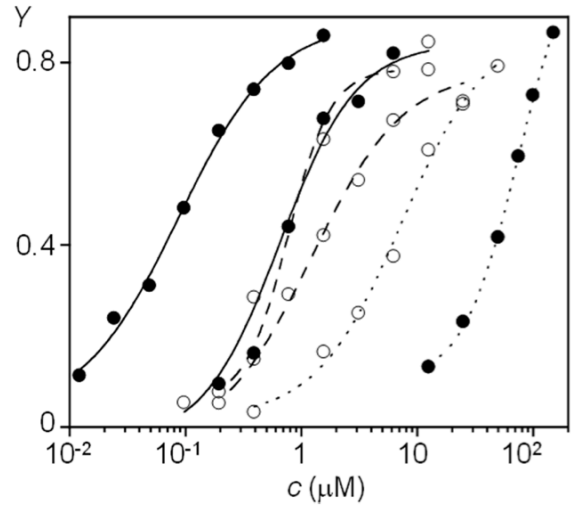


Figure S4. Transport activity *Y* of **2** after polymerization with **5** in EYPG LUVs (●) and EYPC LUVs (○) at lipid concentrations of 62.5 μM (dotted), 2.5 μM (dashed) and 0.25 μM (solid) with increasing concentration of guanidinium cations. *Comment:* Poor activity is observed in EYPG at high lipid concentration because of stoichiometric binding.[S14] High activity is observed in EYPG at low lipid concentration because of activation of the cationic polymer by ion pairing with the amphiphilic PG anion.[S11]

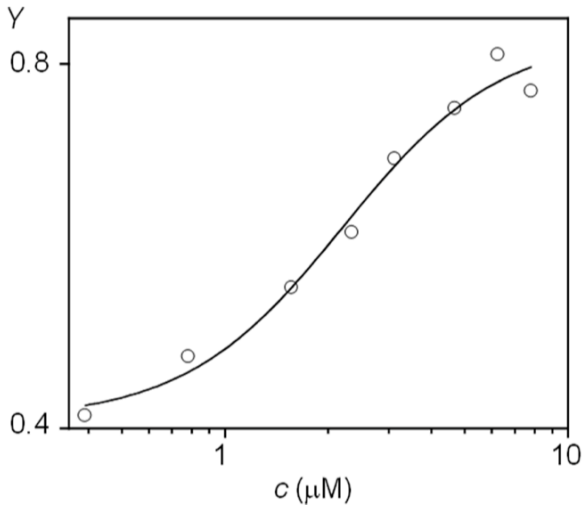


Figure S5. Transport activity *Y* of **2** after polymerization (2.5 μM final guanidinium concentration) with **5** in EYPC LUVs in the presence of increasing concentrations of pyrenebutyrate. *Comment:* Like other guanidinium-rich CPPs, substrate-initiated cell-penetrating poly(disulfide)s can be activated by ion pairing with amphiphilic counterions.[S11]

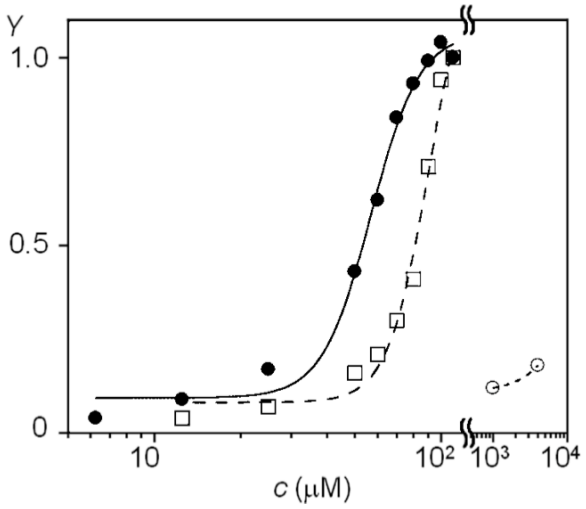


Figure S6. Transport activity *Y* of **3** (500 μM final guanidinium concentration) before (○) and after polymerization with (●, 5 mM) and without **5** (□), with increasing concentration of pyrenebutyrate, in EYPC-LUVs⊃CF. *Comment:* In the absence of counterion activators, substrate-initiated cell-penetrating poly(disulfide)s obtained from **3** were inactive in EYPC-LUVs⊃CF. Like other guanidinium-rich CPPs, substrate-initiated cell-penetrating poly(disulfide)s could be activated by ion pairing with amphiphilic counterions.[S11]

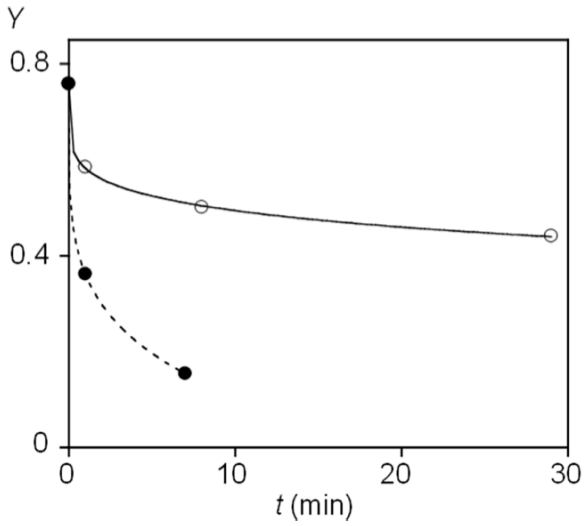


Figure S7. Transport activity *Y* of polymers of **2** during incubation with 2.5 mM (○) and 10 mM (●) of DTT. *Comment:* Depolymerization of cell-penetrating poly(disulfide)s under cytosolic conditions occurs within 5 min.

5. Polymerization Detected with Gel-Permeation Chromatography (GPC)

The column used was a Superdex 75 (10×300 mm), and 30% ACN in 0.1 M acetate buffer (pH = 6.5) was used as an eluent. After termination, the polymerization mixture was diluted with the eluent. The final concentrations of guanidinium cations in the samples were 5 mM ~ 7.5 mM. 100 μL of sample was loaded on the column and the flow rate was 0.4 ml/min from 0 to 40 min, 0.6 ml/min from 41 to 80 min. The absorbance at 333 nm was used to detect the five membered disulfide ring of the propagators and the one at 226 nm was for the amides in the polymers. The signals were normalized by the absorption of the excess terminators (Fig. 3B, 3C, 4D, S8). The tryptophan moiety was detected by the fluorescence emission at 350 nm (λ_{ex} 275 nm, Fig. S8).

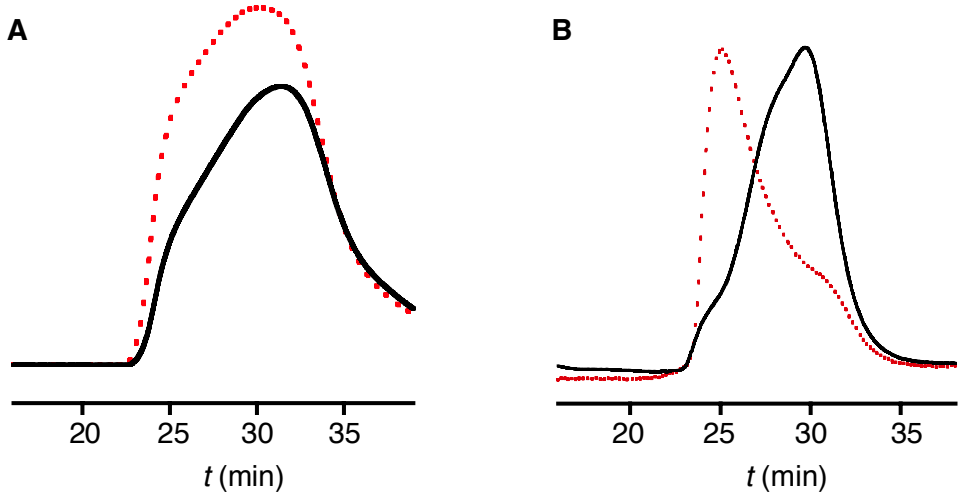


Figure S8. **A**, GPC chromatogram of **1** (100 mM) polymerized with **6** (5 mM, pH 7), detected at λ_{em} 350 nm (λ_{ex} 275 nm, solid, black) and λ_{abs} 226 nm (dashed, red). **B**, GPC chromatogram of **2** (200 mM) polymerized with **6** (5 mM, pH 7.5), detected at λ_{em} 350 nm (λ_{ex} 275 nm, solid, black) and λ_{abs} 226 nm (dashed, red); Sephadex G75, 30% acetonitrile in acetate buffer, pH 6.5. *Comment:* The detection at 350 nm demonstrates the incorporation of the Trp-labeled initiator **6**. Increasing Trp absorption with increasing polymer weight is consistent with the increasing Trp / polymer ratio in shorter polymers.

6. Polymerization Detected with FRET

To a mixture of a solution of **4** in DMF (100 mM, 10 μl) and a solution of **7** in $CHCl_3$ (2 mM, 20 ml), 1 vol.% DIEA in $CHCl_3$ (10 μl) was added and mixed under Ar atmosphere (25 mM of **4**, 1 mM of **7**, 0.25% DIEA and 25% DMF in $CHCl_3$ in the polymerization mixture). After 5 s, 30 s and 60 s, a solution of **8** in $CHCl_3$ (2 mM, 40 μl) was added to terminate the polymerization (12.5 mM of **4**, 0.5 mM of **7**, 1 mM of **8**, 0.125% DIEA and 12.5% DMF in $CHCl_3$ in the mixture). The mixture was diluted in $CHCl_3$ to measure the fluorescence. For the depolymerization, the mixture was diluted in a solution of DTT in $CHCl_3$ (10 mM) and incubated for 10 min at rt, and then the fluorescence was measured. The samples were excited at 445 nm, where compound **8** had almost no absorption (Fig. S9). The final concentrations in the cuvette were 62.5 μM of **4**, 2.5 μM of **7**, 5 μM of **8**, 6.25 ppm of DIEA and 625 ppm of DMF in $CHCl_3$ (Fig. 5).

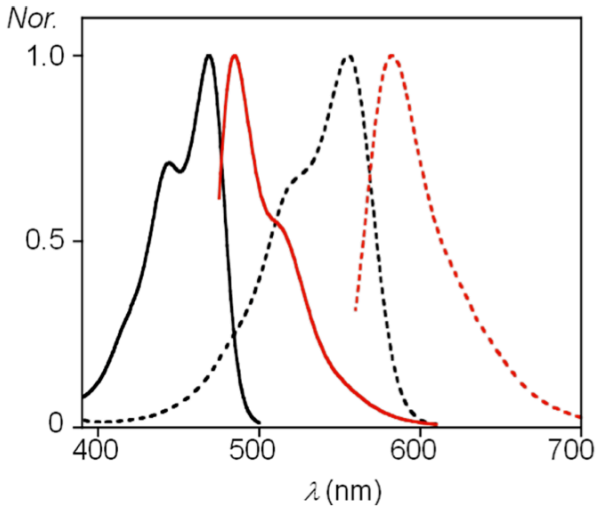


Figure S9. Normalized absorption (black) and emission (red) spectra of **7** (solid) and **8** (dashed) in $CHCl_3$ containing 625 ppm of DMF.

7. NMR Spectra

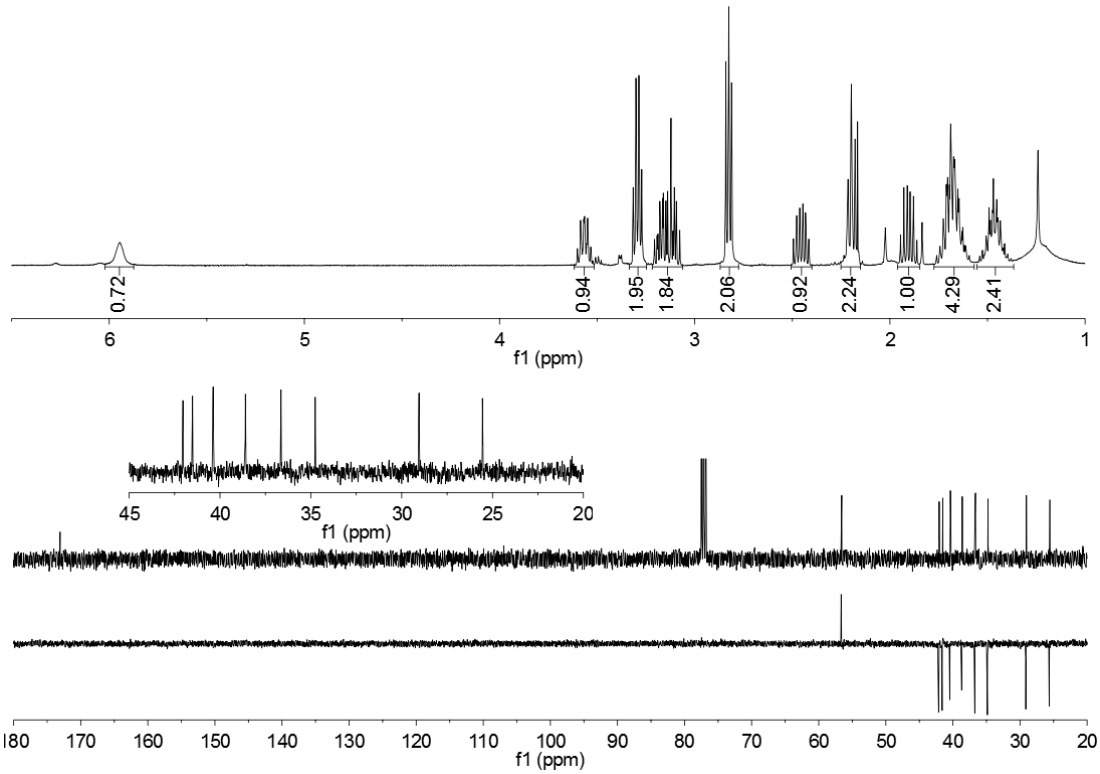


Figure S10. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **10** in $CDCl_3$.

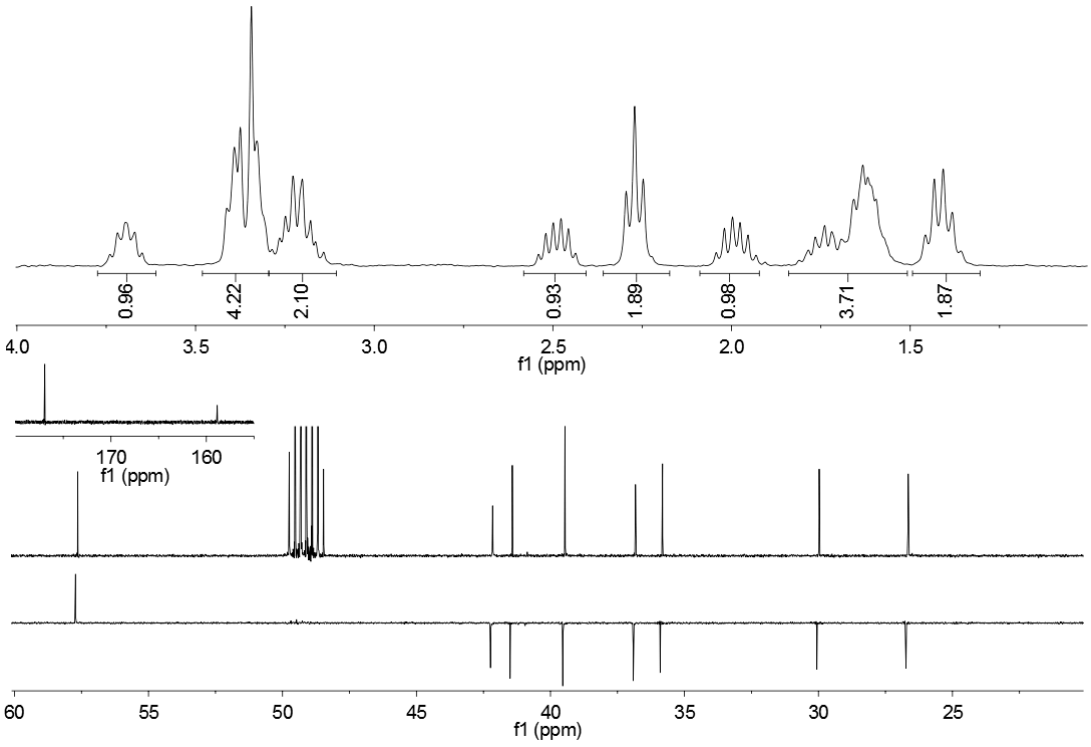


Figure S11. ^{1}H NMR spectrum of **1** in D_2O; ^{13}C NMR and DEPT-135 spectra of **1** in CD_3OD.

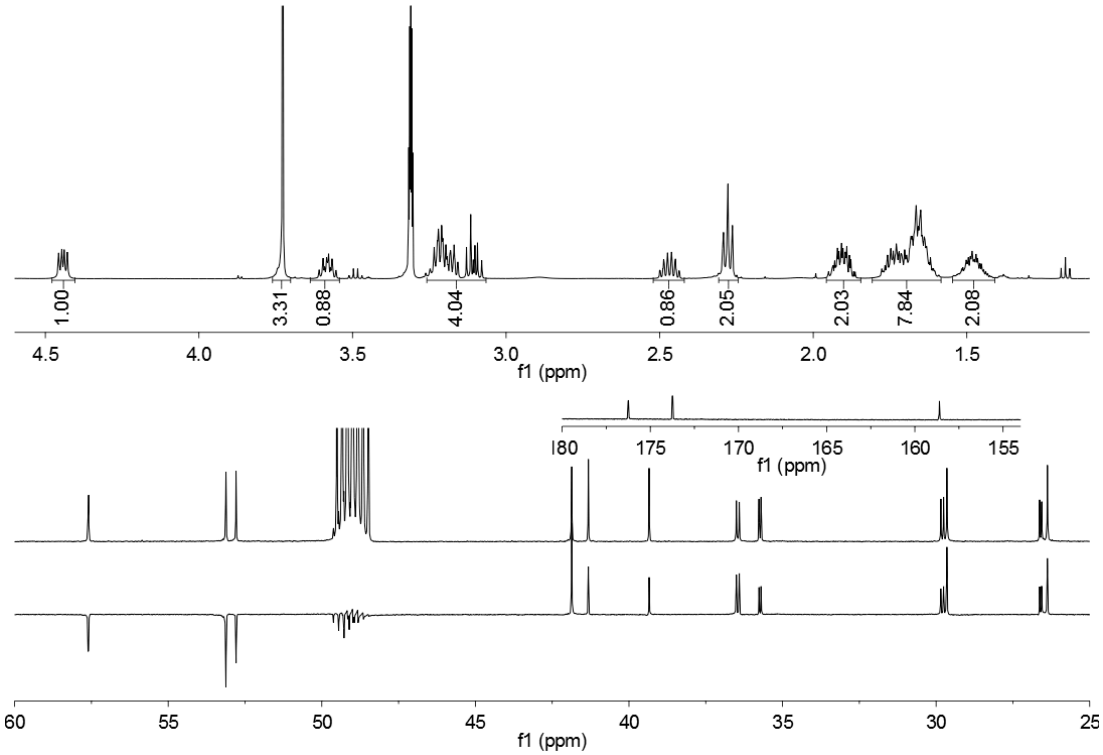


Figure S12. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **2** in CD_3OD.

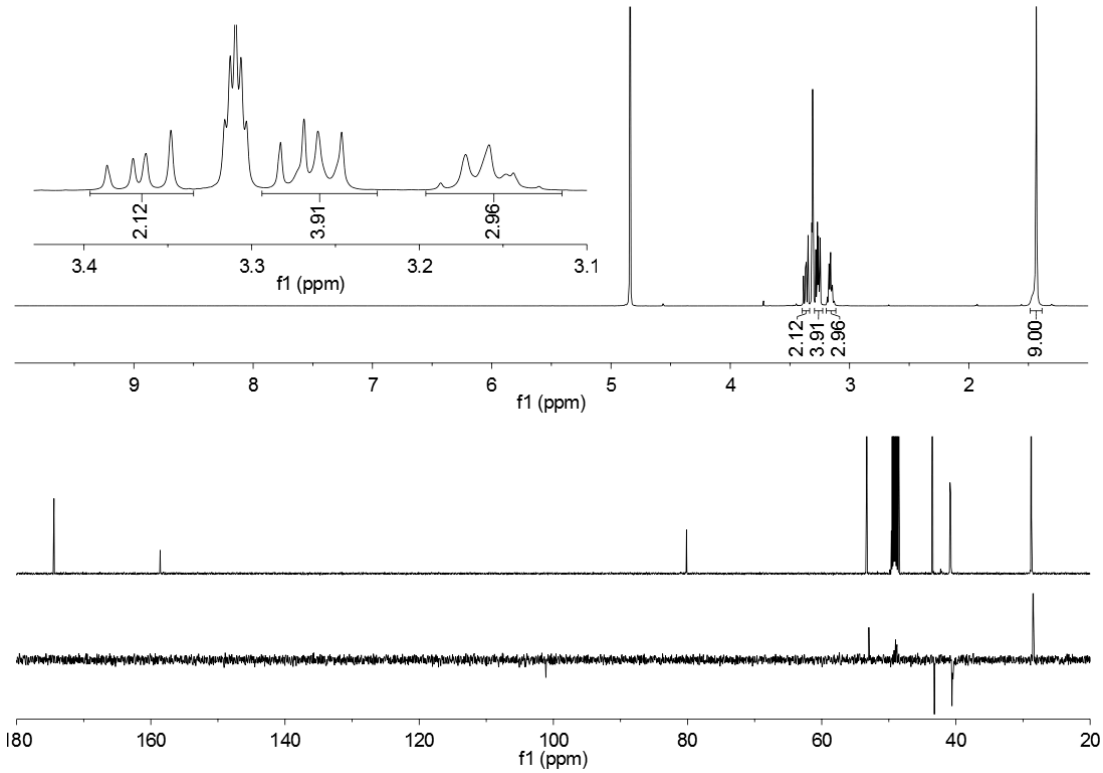


Figure S13. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **14** in CD_3OD.

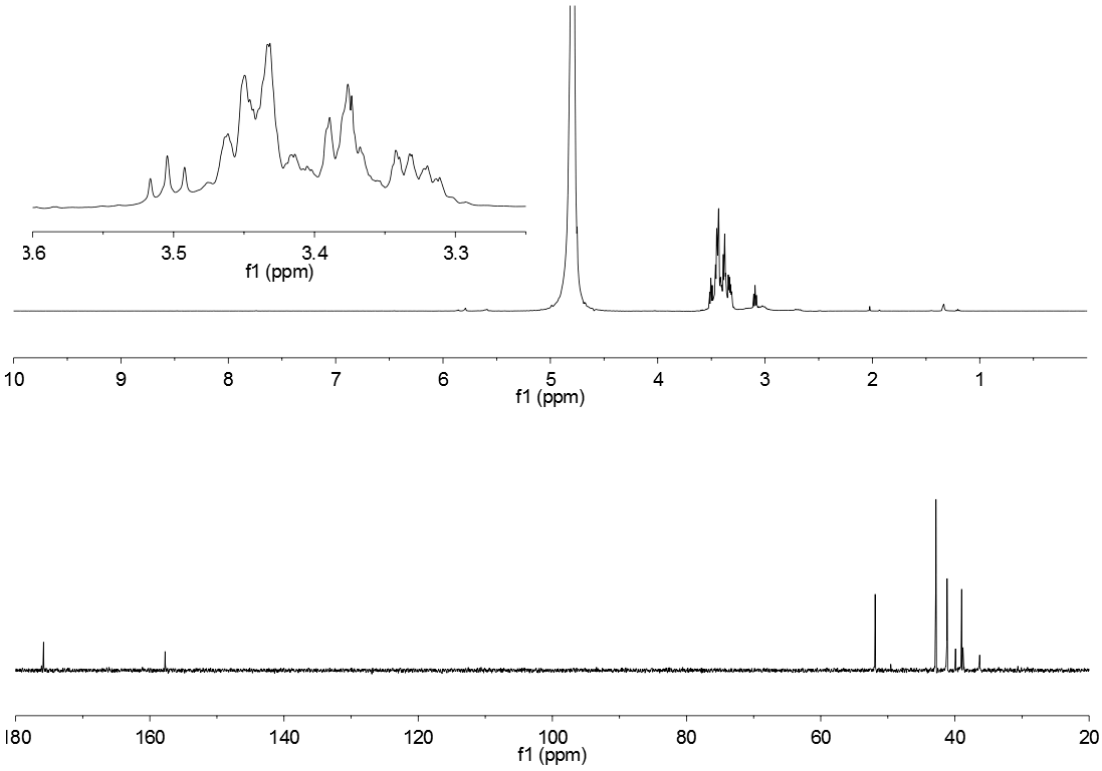


Figure S14. ^{1}H NMR and ^{13}C NMR spectra of **3** in D_2O.

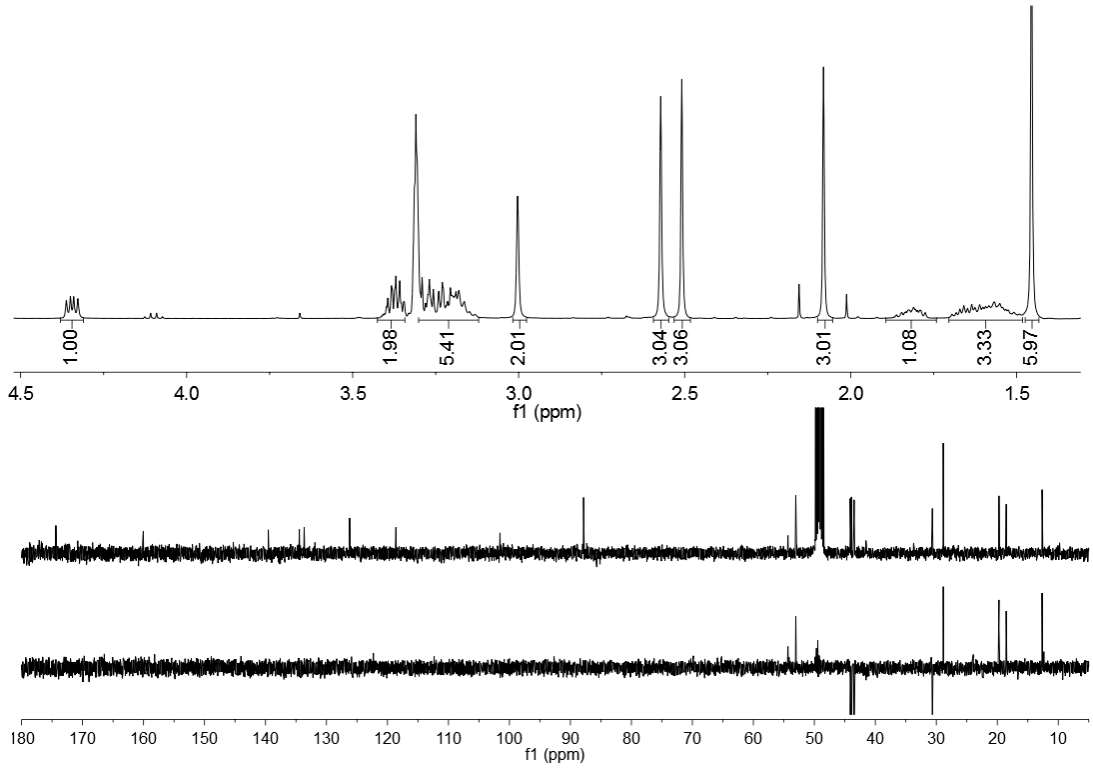


Figure S15. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **17** in CD_3OD.

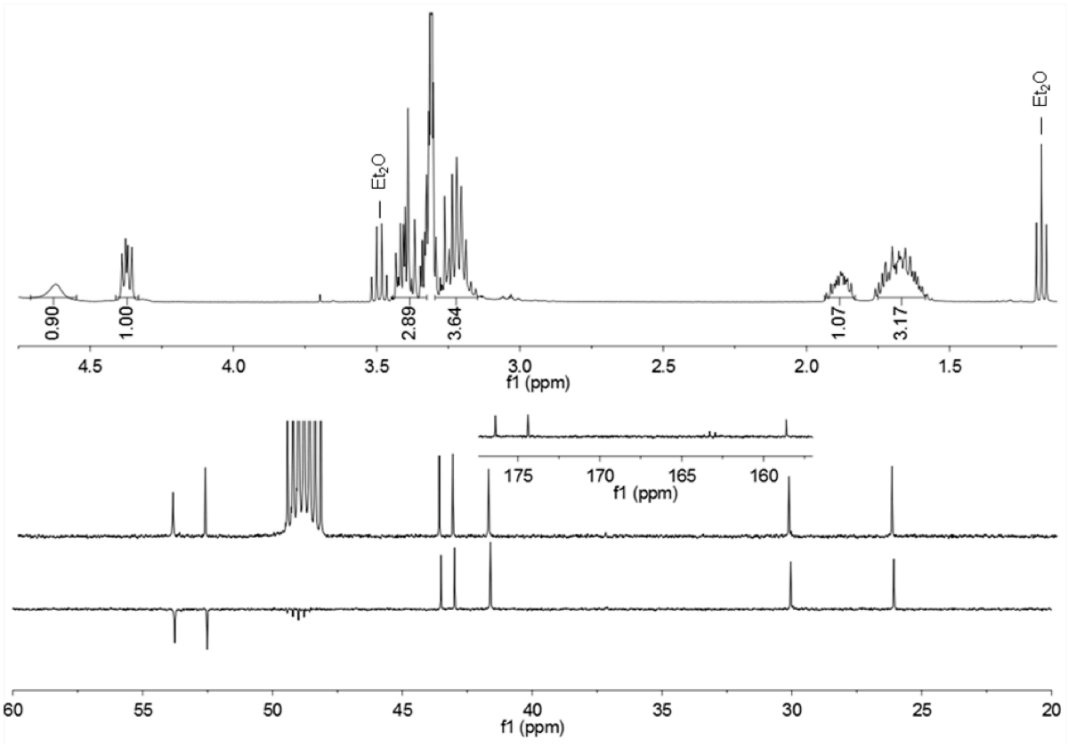


Figure S16. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **4** in CD_3OD

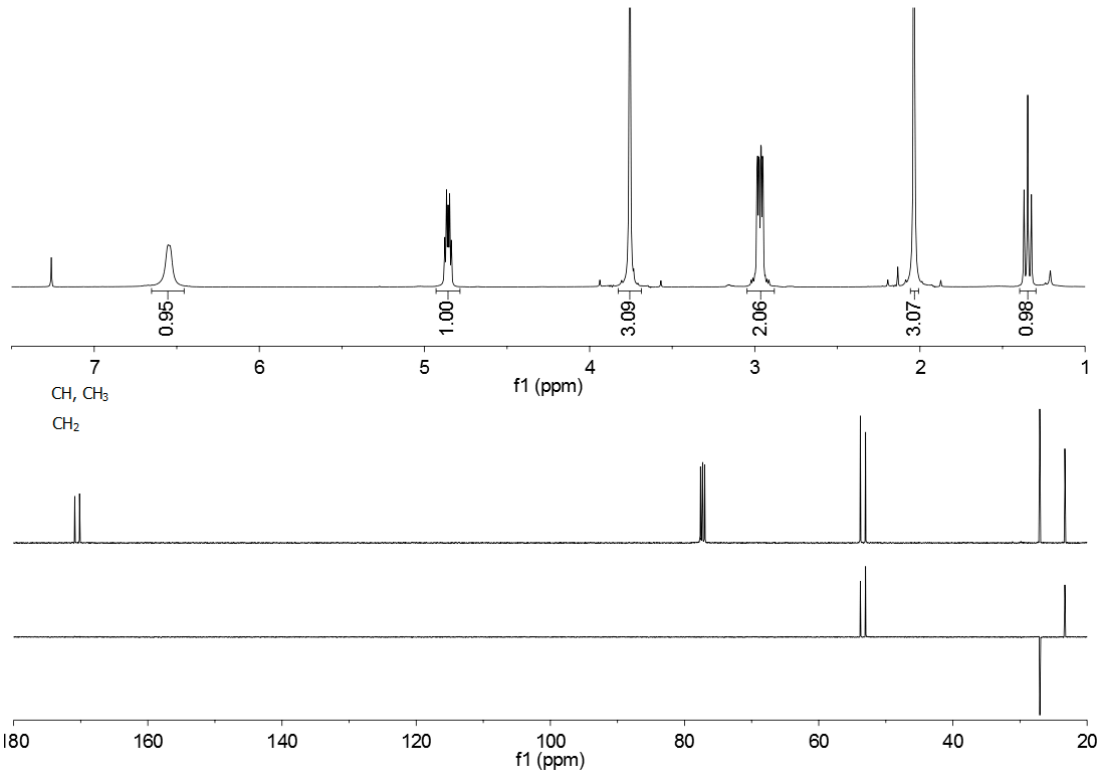


Figure S17. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **5** in $CDCl_3$.

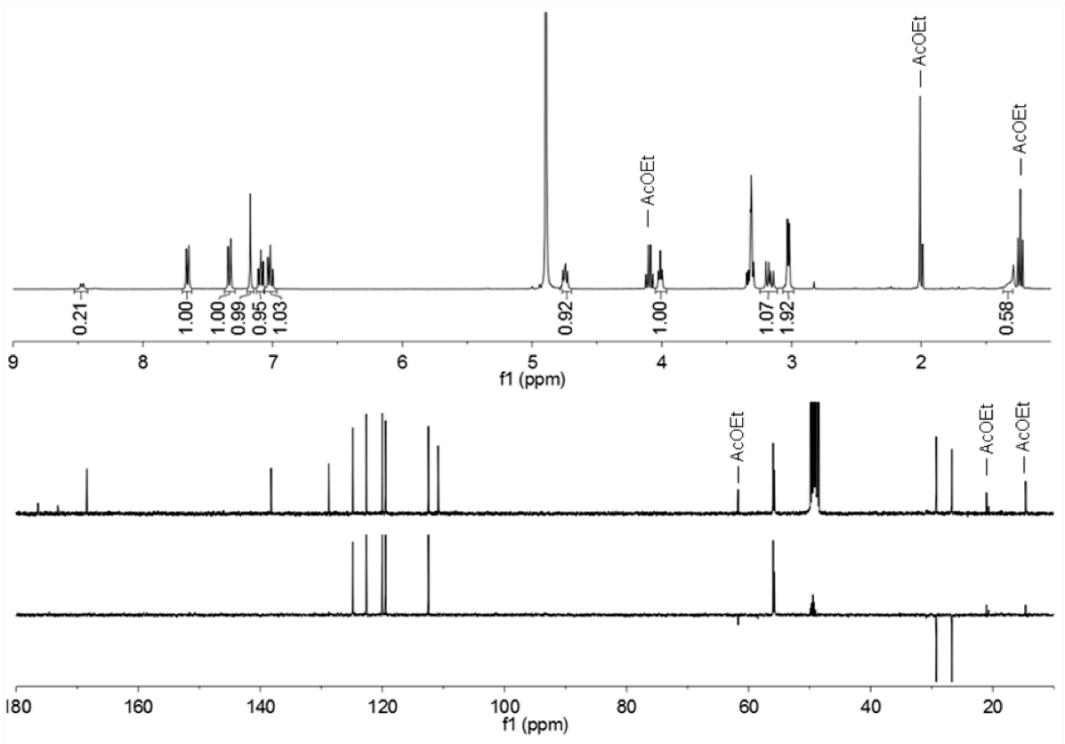


Figure S18. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **6** in CD_3OD.

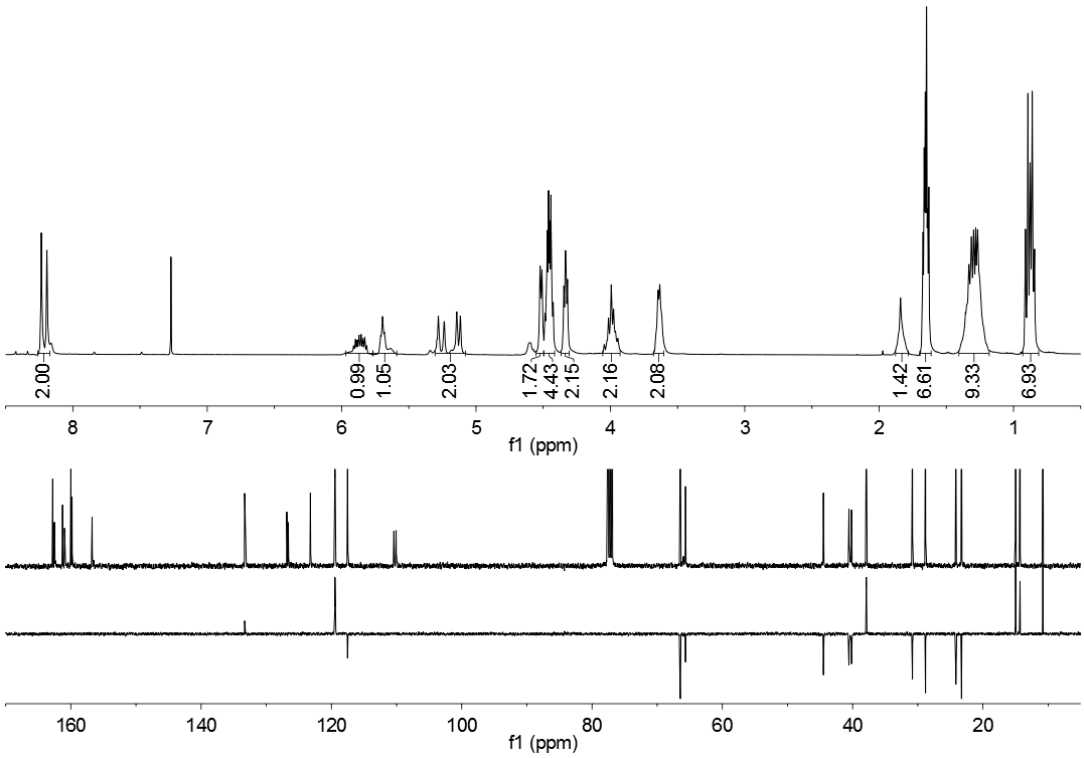


Figure S19. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **22** in $CDCl_3$.

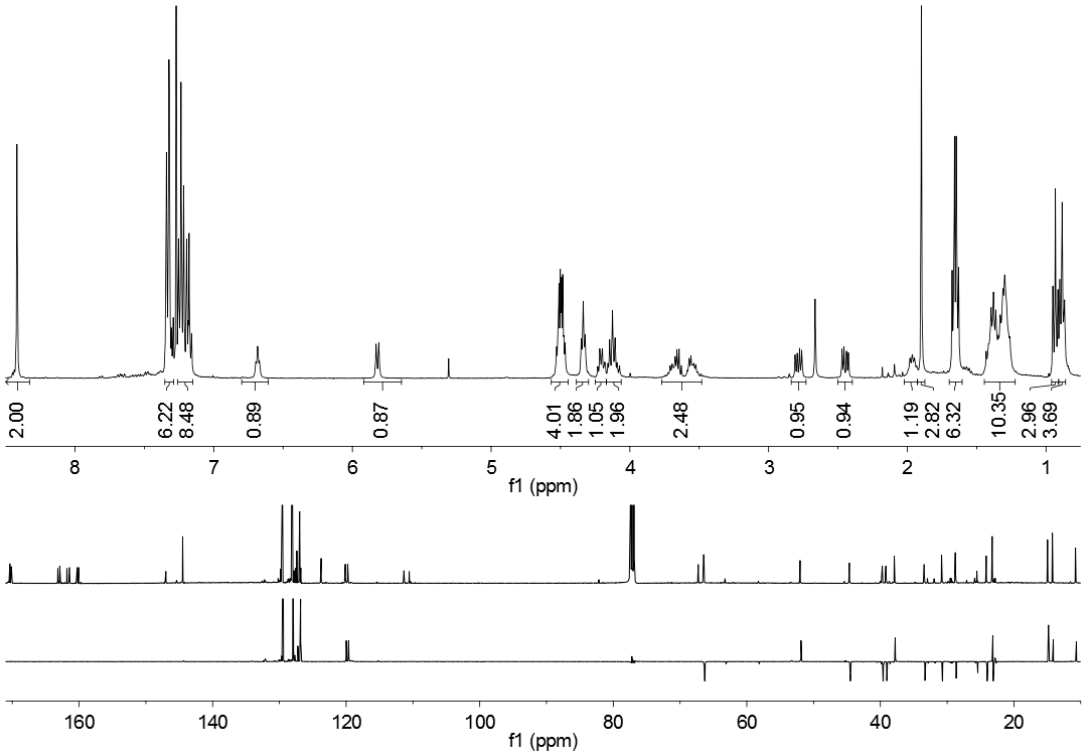


Figure S20. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **25** in $CDCl_3$.

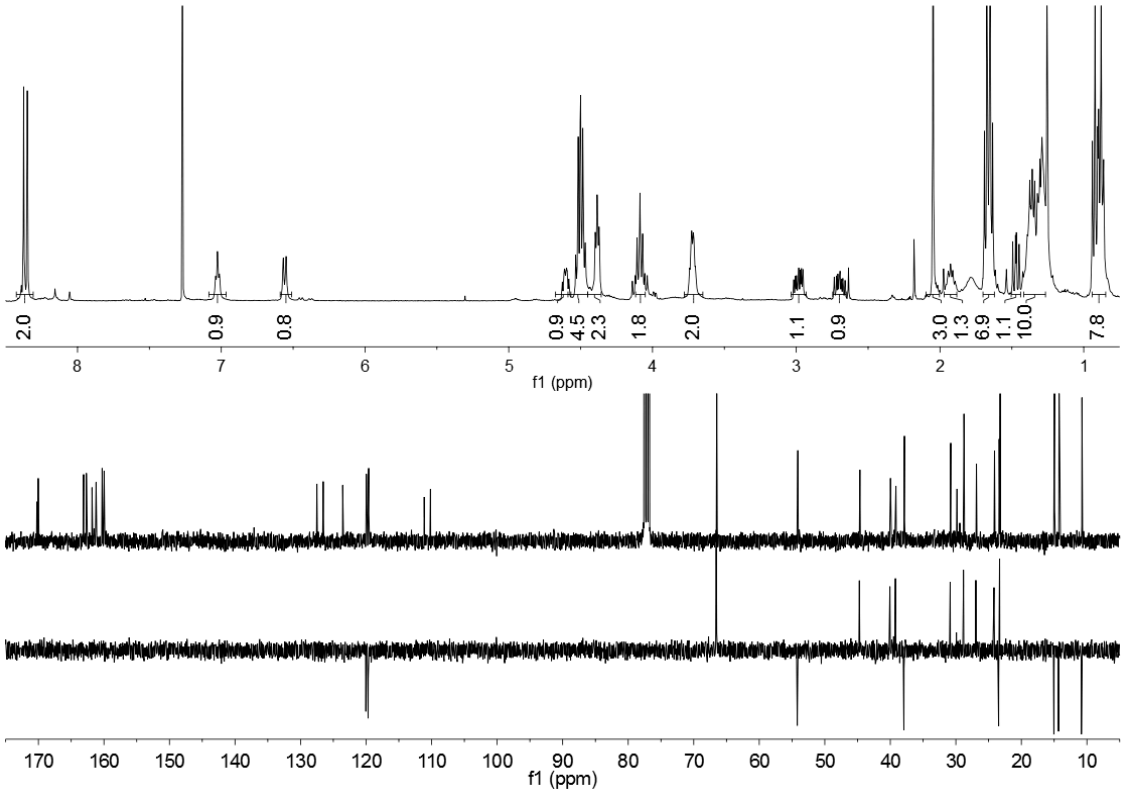


Figure S21. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **7** in $CDCl_3$.

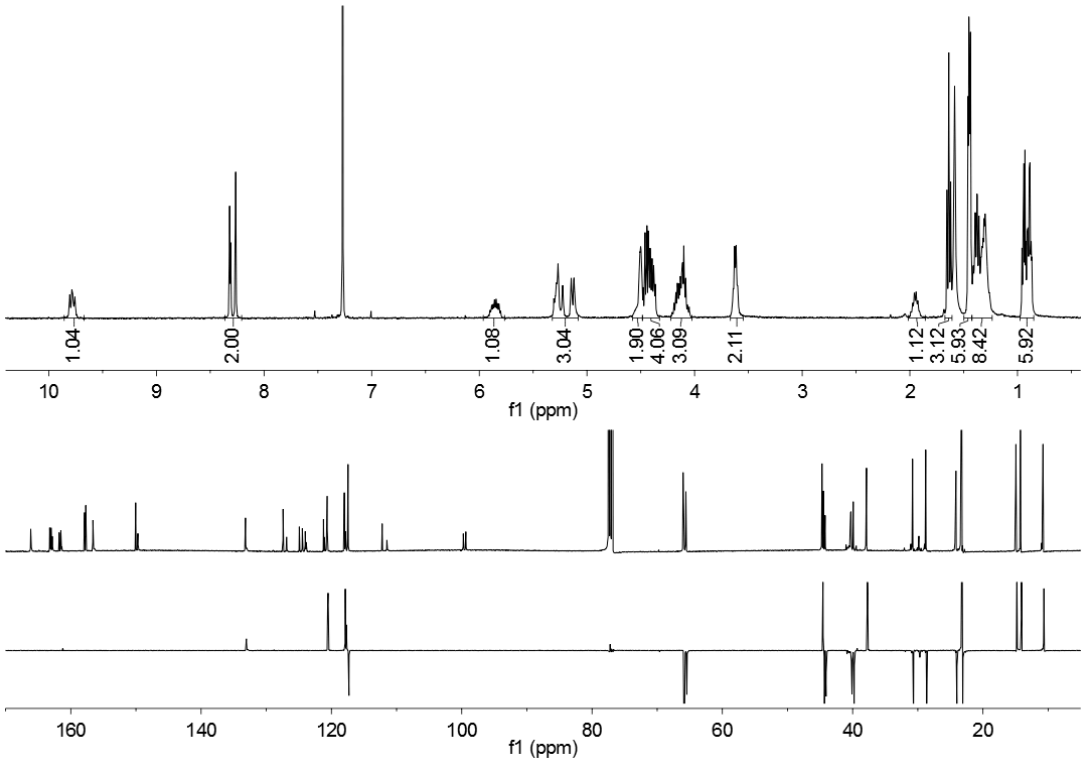


Figure S22. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **26** in $CDCl_3$.

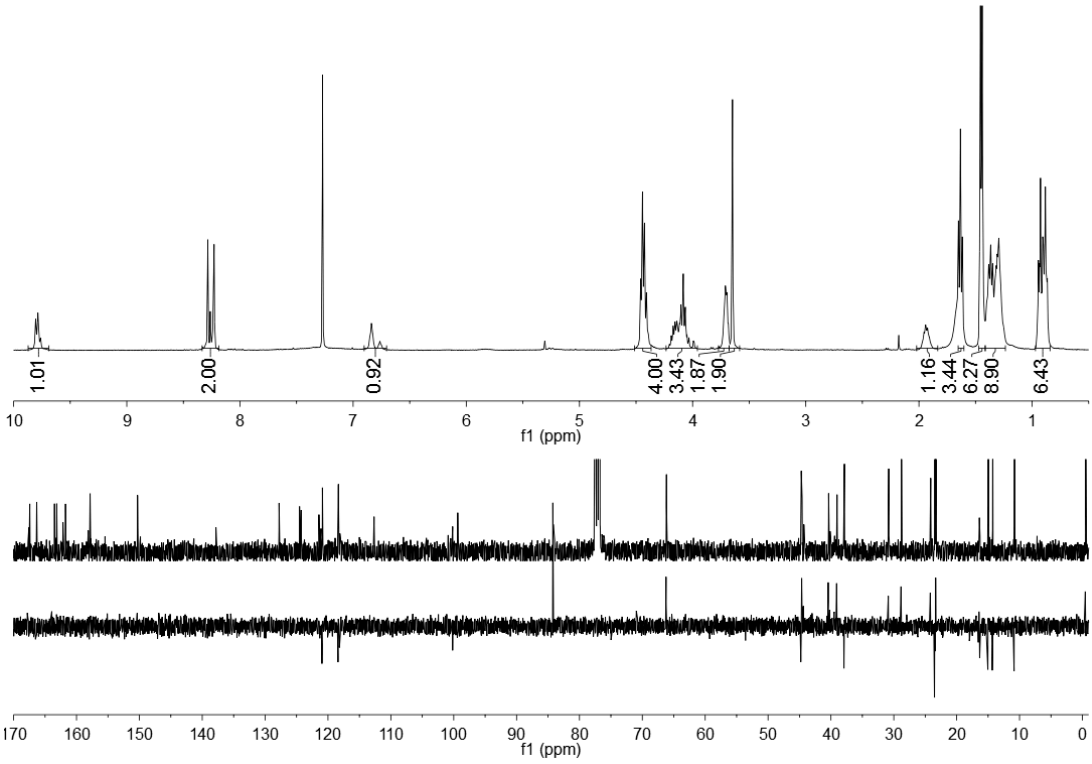


Figure S23. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **8** in $CDCl_3$.

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