Electronic Supplementary Material (ESI) for Polymer Chemistry

Cell-penetrating poly(disulfide)s: focus on substrate-initiated co-polymerization

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Supplementary Information

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1. Materials and Methods

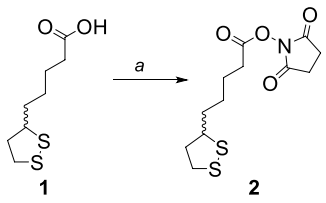
As in ref. S1, Supporting Information. Briefly, reagents for synthesis were purchased from Fluka, Sigma-Aldrich, TCI and Across, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received. Egg yolk phosphatidylcholine (EYPC) were purchased from Avanti Polar Lipids.

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). 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) was 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). HPLC was performed using Agilent 1100 series apparatus with a photo diode array detector. LC-MS were recorded using a Thermo Scientific Accela HPLC equipped with a Thermo C18 (5 cm x 2.1 mm, 1.9 μm particles) Hypersil gold column coupled with a LCQ Fleet three-dimensional ion trap mass spectrometer (ESI, Thermo Scientific) with a linear elution gradient from 95% H_2O 0.01% TFA to 90% MeCN 0.01% TFA in 4.0 minutes at a flow rate of 0.75 mL/min. 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

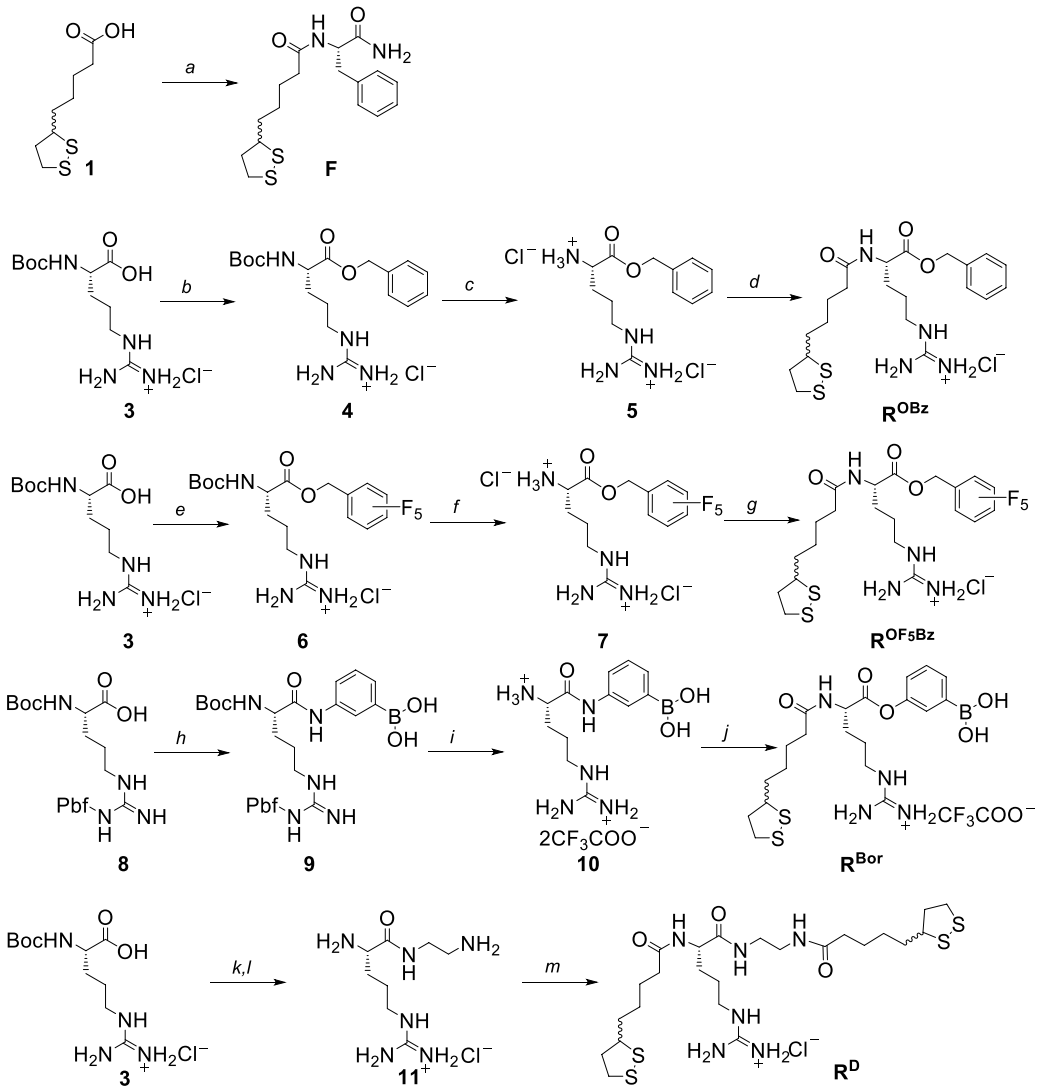
(quint) with coupling constants (*J*) given in Hz, or multiplet (m). ^{1}H and ^{13}C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT-135, HSQC and HMBC). Multiplicity of ^{13}C signals were assigned with the aid of DEPT-135 and reported as s (*C*), d (*CH*), t (CH_2) and q (CH_3). ^{19}F coupled ^{13}C signals are marked *C-F*. ESI-MS analysis were 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 analysis 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; BOC: *t*-Buthoxycarbonyl; Calcd: Calculated; CDI: 1,1'-Carbonyldiimidazole; CF: 5(6)-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; HATU: 2-(7-Aza-1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU: *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; LUVs: Large unilamellar vesicles; NHS: *N*-Hydroxysuccinimide; rt: Room temperature; Pbf: 2,2,4,6,7-Pentamethyl-dihydrobensofuran-5-sulfonyl; TFA: Trifluoroacetic acid; TEOA: Triethanolamine; TIPS: triisopropylsilane; quant: Quantitative yield.

2. Propagator Synthesis



Scheme S1. a) DCC, NHS, AcOEt, rt, 6 h, 90%.



Scheme S2. a) i) CDI, DMF, rt, 1h, ii) H-Phe-NH_2, rt, overnight, 68%; b) DIEA, benzyl bromide, DMF, rt, 6 h, 77%; c) 3 M HCl in AcOEt, rt, 1 h, quant; d) **2**, DIEA, DMF, rt, 3 h, 25%; e) 2,3,4,5,6-pentafluorobenzyl bromide, DIEA, DMF, rt., overnight, 67%; f) 3 M HCl in AcOEt, rt, 10 min, quant; g) **2**, DIEA, DMF, rt, 5 h, 30%; h) 3-aminophenylboronic acid, HATU, DMF, rt, 2 h, 96%; i) TFA/H_2O (95:5), rt, 3 h, 51%. j) **3**, HATU, TEA, DMF, rt, 14 h, 73%; k) i) CDI, DMF, rt, 1 h, ii) N-Boc-ethylene diamine, rt, overnight; l) 3 M HCl in AcOEt, rt, 2 h, 55% (2 steps); m) **2**, DIEA, DMF/H_2O (10:1), rt, 2.5 h, 80%.

Compound 2. To a solution of **1** (413 mg, 2.0 mmol) in AcOEt (50 ml), NHS (231 mg, 2.0 mmol) and DCC (413 mg, 2.0 mmol) were added. The mixture was stirred for 6 h at rt. The resulting mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was washed with EtOH (3 x 5 mL) through solid-liquid extraction and filtered yielding **2** (548 mg, 90%) as a yellowish solid. IR (ATR, cm^{-1}): 2935 (w), 2861 (w), 1809 (m), 1779 (m), 1726 (s), 1523 (w), 1410 (w), 1369 (m), 1322 (w), 1201 (s), 1147 (w), 1070 (s), 991(m), 936 (w), 896 (m), 878 (m), 810 (w), 729 (w), 654 (m); ^{1}H NMR (300 MHz, $CDCl_3$): 3.67 – 3.54 (m, 1H), 3.28 – 3.07 (m, 2H), 2.86 (s, 4H), 2.65 (t, ^{3}J (H,H) = 7.3 Hz, 2H), 2.55 – 2.44 (m, 1H), 2.00 – 1.89 (m, 1H), 1.88 – 1.67 (m, 4H), 1.67 – 1.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 169.2 (s), 168.4 (s), 56.1 (d), 40.2 (t), 38.5 (t), 34.4 (t), 30.8 (t), 28.3 (t), 25.6 (t), 24.4 (t); MS (ESI, DCM): 321 (53, $[M+NH_4]^+$), 304 (21, $[M+H]^+$); 189 (100, $[\text{Lipoic acid-OH}]^+$).

Propagator F. A solution of lipoic acid (206 mg, 1.0 mmol) and CDI (162 mg, 1.0 mmol) in anhydrous DCM (10 mL) was stirred for 1 h, and then a solution of **1** (164 mg, 1.0 mmol) in anhydrous DCM (10 mL) was added to the mixture. The resulting mixture was stirred overnight at rt and washed with brine (10 mL). The organic phase was collected and dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The yellowish compound was purified by flash column chromatography (DCM/MeOH 49:1 to 24:1, R_f 0.5 in DCM/MeOH 19:1) and the solid was re-dissolved in MeOH (1.5 mL) and precipitated with Et_2O to give **F** (241 mg, 68%). IR (ATR, cm^{-1}): 3363 (br), 3303 (br), 3186 (m), 2921 (m) 2854 (w), 1659 (s), 1637 (s), 1527 (s), 1496 (m), 1423 (m), 1260 (m) 1127 (w), 1032 (w), 930 (w), 808 (w), 737 (m); ^{1}H NMR (400 MHz, CD_3OD): 7.28 – 7.16 (m, 5H), 4.63 (dd, ^{3}J (H,H) = 9.6 Hz, 5.4 Hz, 1H), 3.51 – 3.44 (m, 1H), 3.15 (dd, ^{2}J (H,H) = 14.0 Hz, ^{3}J (H,H) = 5.4 Hz, 1H), 3.14 – 3.04 (m, 2H), 2.84 (dd, ^{2}J (H,H) = 14.0 Hz, ^{3}J (H,H) = 9.6 Hz, 1H), 2.45 – 2.37 (m, 1H), 2.14 (t, ^{3}J (H,H) = 7.4 Hz, 2H), 1.87 – 1.78 (m, 1H), 1.65 – 1.43 (m, 4H), 1.34 – 1.18 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): 176.6 (s), 175.9 (s), 138.8 (s), 130.4 (d), 129.6 (d), 127.9 (d), 57.7 (d), 55.7 (d), 41.4 (t), 39.5 (t), 39.2 (t), 38.8 (t), 35.9 (t), 29.8(t), 28.7 (t); MS (ESI, MeOH): 727 (16, $[2M+Na]^+$), 706 (41, $[2M+H]^+$), 375 (81, $[M+Na]^+$), 353 (100, $[M+H]^+$), 336 (72, $[M-NH_3+H]^+$); HRMS (ESI, +ve) calcd for $C_{17}H_{25}N_2O_2S_2^+$: 353.1352, found: 353.1351.

Compound 4. To a solution of **3** (329 mg, 1.0 mmol) and DIEA (1.0 mL, 6.0 mmol) in DMF (10 mL), benzyl bromide (513 µL, 3.0 mmol) was added. The mixture was stirred for 6

h at rt. The resulting mixture was concentrated *in vacuo*. The mixture was purified by flash column chromatography (AcOEt/MeOH 9:1, R_f 0.2 in AcOEt/MeOH 19:1) to give pure **4** (253 mg, 77%) as a colorless, hygroscopic solid. IR (ATR, cm^{-1}): 3291 (br), 3161 (m), 2978 (w), 1652 (s), 1510 (m), 1453 (m), 1366 (m), 1250 (m), 1157 (s), 1052 (m), 1018 (m), 860 (w), 745 (w), 698 (w); ^{1}H NMR (300 MHz, CD_3OD): 7.45 – 7.27 (m, 5H), 5.21 (d, ^2J (H,H) = 12.3 Hz, 1H), 5.16 (d, ^2J (H,H) = 12.3 Hz, 1H), 4.20-4.18 (m, 1H), 3.19 (t, ^3J (H,H) = 6.8 Hz, 2H), 2.00 – 1.78 (m, 1H), 1.78 – 1.56 (m, 3H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD): 173.9 (s), 158.7 (s), 158.4 (s), 137.4 (s), 129.7 (d), 129.50 (d), 129.47 (d), 80.9 (s), 68.1 (t), 54.9 (d), 42.0 (t), 29.8 (t), 28.9 (q), 26.6 (t); MS (ESI, MeOH): 365 (89, $[M+H]^+$), 309 (100, $[M+H\text{-tert-butyl}]^+$), 265 (73, $[M+H\text{-Boc}]^+$), 248 (52, $[M+H\text{-BocNH}_2]^+$); HRMS (ESI, +ve) calcd for $C_{18}H_{29}N_4O_4^+$: 365.2183, found: 365.2177.

Compound 5. To a solution of **4** (196 mg, 0.54 mmol) in AcOEt (7.5 mL), concentrated HCl (2.5 mL) was added. The reaction mixture was stirred for 2 h at rt. The resulting mixture was concentrated under reduced pressure. EtOAc (10 mL) was added and evaporated 5 times to yield **5** (142.5 mg, *quant*) as a yellowish solid. IR (ATR, cm^{-1}): 3322 (br), 3255 (m), 3154 (m), 2916 (m), 2621 (w), 1979 (br), 1739 (m), 1647 (s), 1497 (m), 1455 (m), 1395 (w), 1366 (w), 1292 (w), 1263 (m), 1209 (s), 1111 (m), 1025 (w), 978 (w), 942 (w), 908 (w), 825 (w), 744 (m), 697 (m), 589 (w), 571 (w); ^{1}H NMR (300 MHz, CD_3OD): 7.58 – 7.23 (m, 5H), 5.34 (d, ^2J (H,H) = 12.2 Hz, 1H), 5.31 (d, ^2J (H,H) = 12.2 Hz, 1H), 4.17 (t, ^3J (H,H) = 6.5 Hz, 1H), 3.24 (t, ^3J (H,H) = 7.0 Hz, 2H), 1.99 – 1.86 (m, 2H), 1.87 – 1.55 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): 168.6 (s), 157.2 (s), 134.9 (s), 128.5 (d), 128.4 (d), 67.9 (t), 52.3 (d), 40.3 (t), 27.3 (t), 24.3 (t); MS (ESI, MeOH): 265 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_{13}H_{21}N_4O_2^+$: 265.1659, found: 265.1666.

Propagator $\mathbf{R^{OBz}}$. Compound **5** (206 mg, 0.61 mmol) was dissolved in DIEA (320 µL) and DMF (10 mL), the solution was filtered through a cotton filter. To the solution, **2** (240 mg, 0.79 mmol) was added and the mixture was stirred for 6 h at rt. The product was precipitated by dropwise addition to 40 mL of Et_2O. The precipitate was washed with DCM/Et_2O 1:2 (5 x 30 mL) by solid-liquid extraction, yielding $\mathbf{R^{OBz}}$ (75.9 mg, 25%) as a brownish foamy solid. IR (ATR, cm^{-1}): 3202 (br), 2932 (m), 2861(w), 2398 (br), 1736 (m), 1636 (s), 1538 (m), 1498 (w), 1451 (m), 1381 (m), 1258 (m), 1182 (s), 1145 (m), 1073 (w), 1025 (w), 1001 (w), 967 (w), 914 (w), 741 (m), 697 (m), 591 (w); ^{1}H NMR (300 MHz,

CD_3OD): 7.46 – 7.27 (m, 5H), 5.21 (d, ^2J (H,H) = 12.4 Hz, 1H), 5.18 (d, ^2J (H,H) = 12.4 Hz, 1H), 4.49 (dd, ^3J (H,H) = 8.9 Hz, 4.9 Hz, 1H), 3.63 – 3.48 (m, 1H), 3.15-3.13 (m, 4H), 2.51 – 2.41 (m, 1H), 2.28 (t, ^3J (H,H) = 7.2 Hz, 2H), 2.08 – 1.81 (m, 2H), 1.81 – 1.54 (m, 7H), 1.54 – 1.31 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): 174.9 (s), 171.7 (s), 157.1 (s), 135.8 (d), 128.2 (d), 128.0 (d), 127.9 (d), 66.6 (t), 56.2 (d), 51.9 (d), 40.4 (t), 39.9 (t), 37.9 (t), 35.1 (t), 34.4 (t), 28.4 (t), 28.2 (t), 25.3 (t), 24.9 (t); MS (ESI, MeOH): 453 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{21}H_{33}N_4O_3S_2^+$: 453.1989, found: 453.1985.

Compound 6. To a solution of **3** (1.0 g, 3.2 mmol) in DIEA (3.9 mL, 22.5 mmol) and DMF (10 mL) was added perfluorinated benzyl bromide (930 µL, 6.4 mmol). The mixture was stirred at rt overnight. The resulting mixture was concentrated *in vacuo* and was purified by flash column chromatography (EtOAc/MeOH 1:1, R_f 0.3 in EtOAc/MeOH 4:1) to give pure **6** (1.20 g, 76%) as a colorless hygroscopic solid. IR (ATR, cm^{-1}): 3239 (br), 2935 (w), 2406 (w), 1743 (m), 1644 (s), 1522 (s) 1506 (s), 1456 (w), 1381 (w), 1307 (w), 1177 (m), 1129 (m), 1054 (s), 965 (s) 938 (s), 551 (m), 520 (m); ^{1}H NMR (300 MHz, $CDCl_3$): 5.41 – 5.20 (m, 2H), 4.16 – 4.07 (m, 1H), 3.21 (t, ^3J (H,H) = 6.8 Hz, 2H), 1.93 – 1.77 (m, 1H), 1.82 – 1.56 (m, 3H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, CD_3OD): 173.3 (s), 158.7 (s), 158.2 (s), 147.1 (s, *C-F*), 143.1 (s, *C-F*), 138.8 (s, *C-F*), 111.2 (s), 80.9 (s), 54.9 (t), 54.7 (d), 41.9 (t), 29.4 (t), 28.6 (q), 26.4 (t); MS (ESI, MeOH): 455 (100, $[M+H]^+$), 356 (50, $[M+H\text{-}BocNH_2]^+$); HRMS (ESI, +ve) calcd for $C_{18}H_{24}F_5N_4O_4^+$: 455.1712, found: 455.1699.

Compound 7. To a solution of **6** (780.1 mg, 1.59 mmol) in AcOEt (6.4 mL), concentrated HCl (1.56 mL) was added. The reaction mixture was stirred for 10 min at rt. The resulting mixture was concentrated *in vacuo* and co-evaporated with AcOEt (4 x 10 mL) to give pure **7** (690 mg, *quant*) as a yellow solid. IR (ATR, cm^{-1}): 3329 (w), 2946 (w), 2428 (w), 1750 (m), 1629 (s), 1506 (s), 1373 (w), 1309 (m), 1201 (m), 1131 (m), 1054 (m), 936 (s), 568 (m), 518 (s); ^{1}H NMR (300 MHz, D_2O): 5.36 (d, ^2J (H,H) = 12.4 Hz, 1H), 5.27 (d, ^2J (H,H) = 12.4 Hz, 1H), 4.16 (t, ^3J (H,H) = 6.4 Hz, 1H), 3.14 (t, ^3J (H,H) = 6.8 Hz, 2H), 1.98 – 1.82 (m, 2H), 1.78 – 1.42 (m, 2H); ^{13}C NMR (126 MHz, D_2O): 169.2 (s), 156.7 (s), 145.6 (s, *C-F*), 141.9 (s, *C-F*), 137.4 (s, *C-F*), 108.8 (t), 55.5 (t), 52.2 (d), 40.2 (t), 26.9 (t), 23.8 (t); MS (ESI, MeOH): 355 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{13}H_{16}F_5N_4O_2^+$: 355.1188, found: 355.1190.

Propagator $\mathbf{R^{OF_5Bz}}$. To a solution of **7** (690 mg, 1.76 mmol) in DMF (15 mL) and DIEA (1.2 mL) **2** was added (1.07 g, 3.53 mmol). The solution was stirred at rt for 5 h. The product was precipitated by dropwise addition to 40 mL of Et_2O. The precipitate was washed with Et_2O/DCM 2:1 (5 x 30 mL) by solid-liquid extraction to obtain $\mathbf{R^{OF_5Bz}}$ (286 mg, 30%) as a colorless solid. IR (ATR, cm^{-1}): 3250 (br), 2956 (w), 2428 (w), 1750 (m), 1629 (m), 1506 (s), 1373 (m), 1309 (m), 1201 (m), 1131 (s), 1054 (m), 936 (s), 568 (m), 519 (s); ^{1}H NMR (500 MHz, CD_3OD): 5.34 – 5.25 (m, 2H), 4.40 (dd, ^3J (H,H) = 9.3, 4.7 Hz, 1H), 3.77 – 3.51 (m, 1H), 3.26 – 3.05 (m, 5H), 2.50 – 2.40 (m, 1H), 2.30 – 2.21 (m, 2H), 1.95 – 1.82 (m, 2H), 1.79 – 1.54 (m, 8H), 1.52 – 1.41 (m, 2H), 1.37 – 1.32 (m, 4H); ^{13}C NMR (126 MHz, CD_3OD): 176.3 (s), 172.5 (s), 158.6 (s), 147.0 (s, *C-F*), 143.0 (s, *C-F*), 138.9 (s, *C-F*), 111.1 (s), 57.6 (d), 55.0 (t), 53.2 (d), 43.8 (t) , 41.8 (t), 41.3 (t), 39.3 (t), 36.4 (t), 35.7 (t), 35.7 (t), 29.8 (t), 29.3 (t), 26.5 (t), 26.4 (t); MS (ESI, MeOH): 543 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{21}H_2F_5N_4O_3S_2^+$: 543.1518, found: 543.1498.

Compound 9. Commercially available compound **8** (476 mg, 0.90 mmol) was dissolved in 10 mL of distilled DMF. To the resulting solution, a solution of HATU (319 mg, 0,84 mmol) in anhydrous DMF (3 mL) was added quickly. The reaction mixture was stirred for two minutes and a solution of 3-aminophenylboronic acid (108 mg, 0.70 mmol) in 2 mL of dry DMF was added. The resulting clear solution was stirred at rt for 2 h. The solvent was removed under reduced pressure and the residue was purified through silica gel flash chromatography (DCM/MeOH 19:1), yielding the desired product as a colorless solid (433 mg, 96%). IR (ATR, cm^{-1}): 3333 (w), 2978 (w), 1671 (m), 1618 (m), 1548 (m), 1346 (m), 1246 (m), 1159 (m), 1093 (m), 835 (s), 733 (m), 660 (m), 555 (s); ^{1}H NMR (400 MHz, CD_3OD): 7.78 (s br, 1H), 7.62 (s br, 1H), 7.34 – 7.31 (m, 2H), 4.18 (m, 1H), 3.22 – 3.17 (m, 2H), 2.96 (s, 2H), 2.56 (s, 3H), 2.49 (s, 3H), 2.05 (s, 3H), 1.80 – 1.60 (m, 4H), 1.44 (s, 15H); ^{13}C NMR (100 MHz, CD_3OD): 173.4 (s), 159.9 (s), 158.0 (s), 157.9 (s), 139.4 (s), 138.8 (s) 133.6 (s), 130.5 (d), 129.2 (d), 126.4 (d), 126.1 (2C, s), 122.9 (d), 118.5 (2C, s), 87.7 (s), 80.7 (s), 56.3 (d), 43.9 (t), 41.5 (t), 30.8 (t), 28.7 (2C, q), 27.1 (t), 19.6 (q), 18.4 (q), 12.5 (q); MS (ESI, MeOH): 646 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{30}H_{45}BN_5O_8S^+$: 646.3076, found: 646.3088.

Compound 10. Compound **9** (135 mg, 0.21 mmol) was dissolved in 5 mL of TFA/H_2O 19:1. The reaction mixture was stirred for 3 h at rt. The acid was removed by evaporation

under reduced pressure and the residue was treated with 15 mL of water. The resulting solution was washed with AcOEt (3 x 15 mL) and the aqueous phase was lyophilized to yield **10** (43.4 mg, 51%) as a colorless solid. IR (ATR, cm^{-1}): 3198 (s, br), 1673 (s), 1630 (m), 1564 (w), 1434 (w), 1350 (w), 1087 (s), 707 (w), 618 (w); ^{1}H NMR (400 MHz, CD_3OD): 7.86 (s, 1H), 7.68 (d, ^{3}J (H,H) = 7.6 Hz, 1H), 7.56 (d, ^{3}J (H,H) = 7.6 Hz, 1H), 7.38 (t, ^{3}J (H,H) = 7.6 Hz, 1H), 4.21 (t, ^{3}J (H,H) = 6.5 Hz, 1H), 3.26 (t, ^{3}J (H,H) = 6.5 Hz, 2H), 2.17 – 2.02 (m, 2H), 1.84 – 1.73 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): 168.8 (s), 158.3 (s), 137.7 (d), 135.0 (s), 131.7 (d), 129.5 (d), 127.0 (d), 124.1 (d), 54.4 (d), 41.4 (t), 29.4 (t), 24.7 (t); MS (ESI, CH_3OH): 294 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{12}H_{21}BN_5O_4^+$: 294.1732, found: 294.1728.

Propagator R^{Bor}. Lipoic acid **1** (46.0 mg, 0.22 mmol) was dissolved in 40 mL of anhydrous DMF. To this solution, HATU (76.9 mg, 0.20 mmol) and TEA (31 µL, 0.22 mmol) were added. The reaction mixture was stirred at rt for 10 min, then compound **10** (60.4 mg, 0.15 mmol) was added. The reaction mixture was stirred at rt for 14 h and the solvent was removed under reduced pressure. The residue was added dropwise to 30 mL of cold Et_2O. The resulting oily residue was collected by centrifugation, dissolved in 0.5 mL of MeOH and precipitated again by adding the solution to 20 mL of cold Et_2O. The precipitate was collected by centrifugation and washed with Et_2O by solid-liquid extraction. The residue was dried under vacuum, yielding $\mathbf{R^{Bor}}$ (52 mg, 73%) as a yellow foamy solid. IR (ATR, cm^{-1}): 3264 (s, br), 2936 (m), 1649 (s), 1542 (m), 1429 (m), 1342 (m), 1189 (s), 1134 (s), 837 (w), 800 (w), 708 (s); ^{1}H NMR (400 MHz, D_2O): 7.73 (br s, 1H), 7.62 (dt, ^{3}J (H,H) = 7.7 Hz, ^{4}J (H,H) = 1.1 Hz, 1H), 7.57 – 7.55 (m, 1H), 7.48 (t, ^{3}J (H,H) = 7.7 Hz, 1H), 4.44 (dd, ^{3}J (H,H) = 7.9 Hz, 6.5 Hz, 1H), 3.63 – 3.65 (m, 1H), 3.25 (t, ^{3}J (H,H) = 6.9 Hz, 2H), 3.19 – 3.07 (m, 2H), 2.44 – 2.34 (m, 3H), 2.00 – 1.54 (m, 9H), 1.42 – 1.35 (m, 2H); ^{13}C NMR (100 MHz, D_2O, *n/n*: stereoisomeric peaks): 177.09/177.07 (s), 172.5 (s), 156.7 (s), 135.9 (s), 135.3 (s), 131.0 (d), 128.9 (d), 127.2 (d), 124.7 (d), 56.49/56.47 (d), 54.1 (d), 40.5 (t), 40.2 (t), 38.0 (t), 35.1/35.0 (t), 33.69/33.65 (t), 28.0 (t), 27.8/27.7 (t), 24.92/24.88 (t), 24.5 (t); MS (ESI, CH_3OH): 482 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{12}H_{21}BN_5O_4^+$: 482.2061, found: 482.2065.

Compound 11. To a solution of **3** (330 mg, 1.0 mmol) in anhydrous DMF (5 mL), CDI (244 mg, 1.5 mmol) was added and the mixture was stirred at rt for 1 h. After completion of **3**,

N-Boc-ethylenediamine (315 mg, 2.0 mmol) was added to the mixture. After stirring the mixture overnight, 50 mL of Et_2O was added, and the colorless precipitate was collected by centrifugation (4400 rpm, 5 min). The crude product was purified by column chromatography (DCM/MeOH/H_2O 100:10:1) to give the product as a colorless solid. To the dried solid, freshly prepared 3 M HCl in AcOEt (20 mL) was added, and the mixture was stirred for 2 h. The resulting mixture was concentrated *in vacuo* and co-evaporated with AcOEt (>5 x 10 mL) to give pure **11** (181 mg, 55% 2 steps) as colorless crystals. IR (ATR, cm^{-1}): 3194 (br), 3051 (w), 2929 (w), 2369 (br), 2352 (br), 1730 (s), 1665 (s) 1594 (s), 1555 (m), 1435 (s), 1380 (w), 1317 (w), 1253 (m), 1223 (s), 1208 (s), 1141 (m), 1062 (s) 974 (m) 723 (m); ^{1}H NMR (400 MHz, D_2O): 3.94 (t, ^3J (H,H) = 6.6 Hz, 1H), 3.62 – 3.55 (m, 1H), 3.39 – 3.32 (m, 1H), 3.14 (t, ^3J (H,H) = 6.8 Hz, 2H), 3.10 – 3.01 (m, 2H), 1.87 – 1.78 (m, 2H), 1.60 – 1.52 (m, 2H); ^{13}C NMR (100 MHz, D_2O): 170.3 (s), 156.7 (s), 52.9 (d), 40.3 (t), 38.7 (t), 36.9 (t), 27.7 (t), 23.6 (t); MS (ESI, MeOH): 217 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_8H_{21}N_6O^+$: 217.1771, found: 217.1764.

Propagator R^D. A solution of **11** (89 mg, 0.27 mmol) and DIEA (209 μL, 1.1 mmol) in DMF/H_2O 10:1 (2.2 mL) was prepared and filtered through a cotton plug. To the filtrate, **2** (205 mg, 0.68 mmol) was added, and the mixture was stirred at rt for 2.5 h. Insoluble salts were filtered off, the filtrate was added to 40 mL of Et_2O, and the milky suspension was centrifuged (4400 rpm, 30 sec). The supernatant was discarded and Et_2O/DCM 3:1 (20 mL) was added to the solid residue. The mixture was shaken and centrifuged (4400 rpm, 30 sec). The supernatant was discarded, the solid was re-dissolved in MeOH/DCM 1:1 (2 mL), and 20 mL of Et_2O was added. The suspension was centrifuged (4400 rpm, 2 min) and the supernatant was discarded. The solid was washed with DCM/Et_2O 1:1 (5 x 5 mL) by solid-liquid extraction to yield $\mathbf{R^D}$ (138 mg, 80%) as a colorless solid. IR (ATR, cm^{-1}): 3253 (br), 3181 (br), 2928 (m), 2961 (w), 1778 (w), 1636 (s), 1530 (s), 1434 (m), 1364 (w), 1215 (m) 1071 (w), 813 (w); ^{1}H NMR (400 MHz, CD_3OD): 4.32 – 4.28 (m, 1H), 3.64 – 3.57 (m, 2H), 3.31 – 3.26 (m, 4H), 3.24 – 3.17 (m, 4H), 3.16 – 3.08 (m, 2H), 2.53 – 2.44 (m, 2H), 2.33 (t, ^3J (H,H) = 7.4 Hz, 2H), 2.23 (t, ^3J (H,H) = 7.4 Hz, 2H), 1.97 – 1.83 (m, 3H), 1.78 – 1.60 (m, 12H), 1.53 – 1.43 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD): 176.6 (s), 176.4 (s) 174.5 (s), 158.7 (s), 57.7 (d), 54.6 (d), 42.2 (t), 41.5 (t), 40.6 (t), 39.9 (t), 39.5 (t), 37.1 (t), 36.8 (t), 36.7 (t), 36.0 (t), 35.9 (t), 30.3 (t), 30.2 (t), 30.1 (t), 30.1 (t), 26.8 (t), 26.7 (t), 26.7 (t), 26.6 (t); MS

(ESI, MeOH): 616 (16, $[M+Na]^+$), 594 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd. for $C_{24}H_{45}N_6O_3S_4^+$: 593. 2431, found: 593.2417.

3. Co-Polymerization

3.1. General Procedure

The procedure described in ref. S2 was adapted and slightly modified to obtain each co-polymer. Stock solutions of propagators (400 mM in appropriate organic solvent), initiator (*N*-Acetyl cysteine methyl ester, 100 mM in appropriate organic solvent) and terminator (iodoacetamide, 500 mM in water) were freshly prepared and fluxed with N_2 gas. Appropriate amounts of $\mathbf{R}^{\mathbf{OMe}}$ were transferred to an Eppendorf tube and dried *in vacuo*. The solid propagators were mixed with or without a solution of a second propagator. Then the initiator, corresponding organic solvent (optional) and TEOA buffer (1 M, desired pH) were added adjusting the final desired concentration in the reaction mixture (200 mM of total propagators and 5 mM of initiators). The sample was kept at 25 °C with vigorous agitation. After polymerization, the mixture was diluted 20 times with the terminator solution, yielding 10 mM overall guanidinium cations. Specific conditions for each co-polymer are described in Table S1. The fresh mixture was tested in vesicle assay and GPC within a day, without further purification.

Table S1. Polymerization Conditions

Poly(disulfide)s	Propagator (mM)[a]	Initiator (mM)	TEOA *c* (M) / pH	Organic Solvent	Time (min)
Poly($\mathbf{R^{OMe}}$)s	200	5	0.9 / 7.5 0.8 / 7.0	10% MeOH 20% DMF	1 30
Poly($\mathbf{R^{D}}$)s	50	5 ~ 50	0.5 / 8.0	50% DMF	60
Poly($\mathbf{R^{OMe}}$/$\mathbf{R^{D}}$)s	200	5	0.9 / 7.5	10% MeOH	1
Poly($\mathbf{R^{OMe}}$/**F**)s	200	5	0.8 / 7.0	20% DMF	30
Poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$)s	200	5	0.7 / 7.0[b] 0.8 / 7.0[c]	30% DMF 20% DMF	30
Poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OF_5Bz}}$)s	200	5	0.8 / 7.0	20% DMF	30
Poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$)s	200	5	0.8 / 7.0[d] 0.75 / 7.0[c]	20% DMF 25% DMF	30

[a]Total concentration of propagators. [b]For co-polymers in Fig. 3a. [c]For co-polymers in Fig. 3c. [d]For co-polymers in Fig. 3b.

3.2. Purification and Characterization

The product mixture was purified by GPC (see section 4.3 for details). After lyophilization of the fraction, the resulting solid was dissolved in water and desalted through Sephadex G-25.

The purified co-polymers were added to a freshly prepared solution of DTT (10 mM in TEOA buffer (100 mM, pH 7.5)). The mixture was shaken for 20 min at 25 ºC (1000 rpm). Resulting solution was analyzed using LC-MS and HPLC (from 5% to 98% ACN in water with 0.1% TFA in 25 min, flow rate 0.5 mL/min, reverse phase column (C18, 150x3 mm, 5 μm particles, 110 Å pores). Each propagator was identified by LC-MS (Table S2) and quantified by HPLC (Fig. S1, Table S3). Mixtures of each component (1:1) were used as calibration standards (Fig. S1, Table S3).

The average molecular weight of propagators was calculated taking into account their contents in the co-polymer. The molecular weight and the degree of polymerization (DP) were estimated from GPC chromatogram of each poly(disulfide). The degrees of polymerization of purified poly(disulfide)s covered from 20 to 60 depending on the propagators (Table S3).

Table S2. Co-poly(disulfide) characterization by reductive depolymerisation and LC-MS

Name	Calcd $[M+H]^{+}$ [a]	Found $[M+H]^{+}$ [a]	Retention time[a] (min)
Poly($\mathbf{R^{OMe}}$/**F**)$_{8:1}$	355.1	354.9	2.51
Poly($\mathbf{R^{OMe}}$/**F**)$_{16:1}$	355.1	354.9	2.51
Poly($\mathbf{R^{OMe}}$/ $\mathbf{R^{OBz}}$)$_{8:1}$	455.2	455.3	2.58
Poly($\mathbf{R^{OMe}}$/ $\mathbf{R^{OBz}}$)$_{16:1}$	455.2	455.3	2.59
Poly($\mathbf{R^{OMe}}$/ $\mathbf{R^{OF5Bz}}$)$_{8:1}$	545.2	545.2	2.76
Poly($\mathbf{R^{OMe}}$/ $\mathbf{R^{OF5Bz}}$)$_{16:1}$	545.2	545.2	2.76
Poly($\mathbf{R^{OMe}}$/ $\mathbf{R^{Bor}}$)$_{8:1}$	484.2	484.2	2.15
Poly($\mathbf{R^{OMe}}$/ $\mathbf{R^{D}}$)$_{8:1}$	597.3	597.2	2.52

[a]For propagators other than $\mathbf{R^{OMe}}$. Data were obtained by using LC-MS. $\mathbf{R^{OMe}}$ was detected at 2.14~2.18 min of retention time and 379.2 was found as molecular ion mass (calcd. 379.2 for $[M+H]^{+}$).

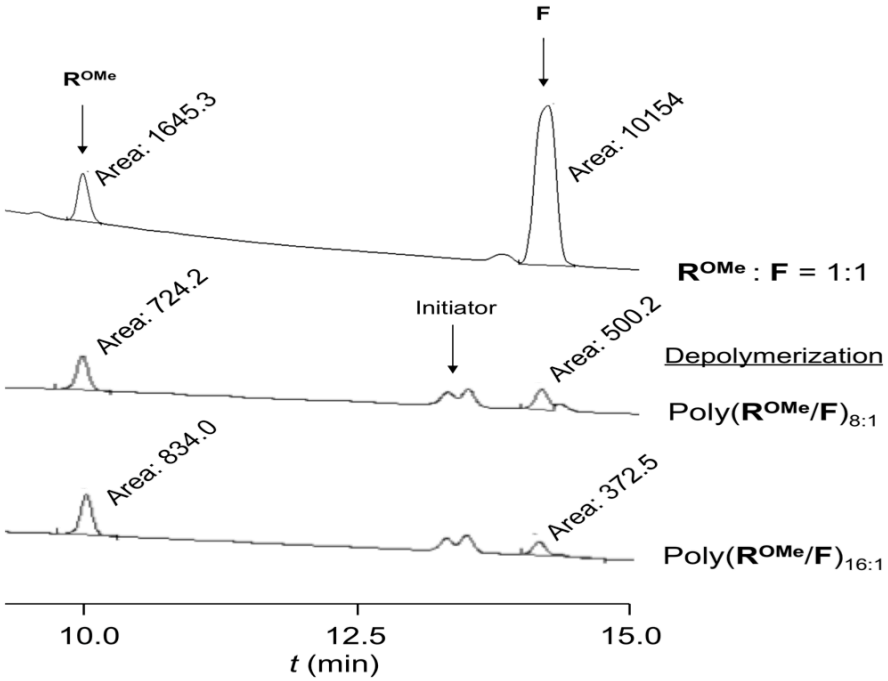


Fig. S1. HPLC chromatogram of depolymerized Poly($\mathbf{R}^{\mathbf{OMe}}$/**F**)s.

Table S3. Quantitative characterization of co-poly(disulfide)s after reductive depolymerisation

Name	**X** / $\mathbf{R}^{\mathbf{OMe}}$ [a]		$\mathbf{R}^{\mathbf{OMe}}$: **X** [b]	DP
	A	B		
Poly($\mathbf{R}^{\mathbf{OMe}}$/**F**)$_{8:1}$	6.17	0.69	8.9:1	31~45
Poly($\mathbf{R}^{\mathbf{OMe}}$/**F**)$_{16:1}$	6.17	0.45	13.8:1	30~42
Poly($\mathbf{R}^{\mathbf{OMe}}$/ $\mathbf{R}^{\mathbf{OBz}}$)$_{8:1}$	2.75	0.29	9.4:1	45~59
Poly($\mathbf{R}^{\mathbf{OMe}}$/ $\mathbf{R}^{\mathbf{OBz}}$)$_{16:1}$	2.75	0.13	20.4:1	48~64
Poly($\mathbf{R}^{\mathbf{OMe}}$/ $\mathbf{R}^{\mathbf{OF5Bz}}$)$_{8:1}$	1.68	0.37[c]	4.6:1	45~60
Poly($\mathbf{R}^{\mathbf{OMe}}$/ $\mathbf{R}^{\mathbf{OF5Bz}}$)$_{16:1}$	1.68	0.13[c]	13.0:1	44~58
Poly($\mathbf{R}^{\mathbf{OMe}}$/ $\mathbf{R}^{\mathbf{Bor}}$)$_{8:1}$	6.63	0.78	8.5:1	20~34
Poly($\mathbf{R}^{\mathbf{OMe}}$/ $\mathbf{R}^{\mathbf{D}}$)$_{8:1}$	1.33	0.33	4.0:1	20~29

[a]The values indicate the peak area of the functional propagator (**X**) divided by the peak area of $\mathbf{R}^{\mathbf{OMe}}$ in the 1:1 mixture of each component (A) and in the depolymerized mixture (B), analyzed by using HPLC (λ_{abs} = 214 nm). [b]The ratio between $\mathbf{R}^{\mathbf{OMe}}$ and functional propagators (**X**) in the purified co-polymers, calculated from the values **X** / $\mathbf{R}^{\mathbf{OMe}}$ A and B. [c] The ratio was calculated by LC-MS (λ_{abs} = 214 nm).

4. Properties of Cell-Penetrating Co-Poly(disulfide)s

4.1. Vesicle Preparation

LUVs were prepared following the general procedures in refs. S3-S5. A thin lipid film was obtained by evaporating a solution of 25 mg EYPC 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. Transport Activity in Fluorogenic Vesicles

EYPC-LUVs stock solutions (25 μL) were diluted with a buffer (10 mM Tris, 107 mM NaCl, pH 7.4), 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 S1):

$$I(t) = (I_t - I_0) / (I_\infty - I_0) \qquad \text{(Eq S1)}$$

where $I_0 = I_t$ just before reaction mixtures addition, $I_\infty = I_t$ at saturation after lysis. 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, t = ~290 s) as a function of guanidinium concentration c and fitting them to the Hill equation (Eq S2)

$$Y = Y_0 + (Y_{MAX} - Y_0) / \{1 + (EC_{50} / c)^n\} \qquad \text{(Eq S2)}$$

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.

Transport activities of propagators were measured as shown in Fig. S2 and Table S4. Their EC_{50} values are much higher than those of their polymers, indicating that enhanced transport activity of polymerization mixture reflects only the effect from the polymer.

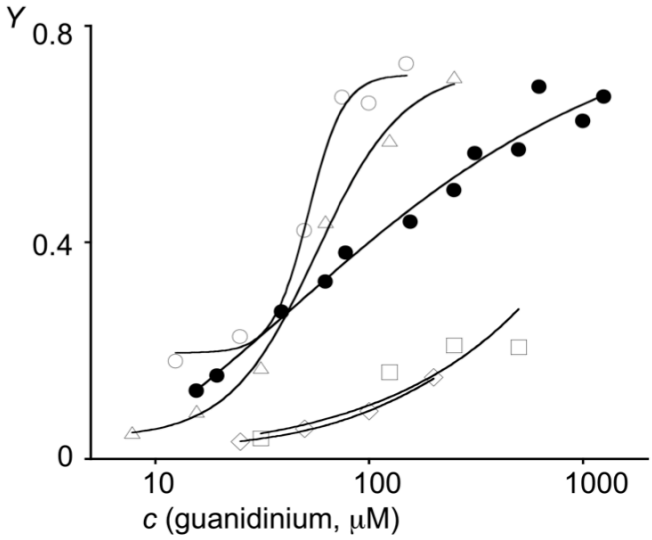


Fig. S2. Transport activity Y of monomeric propagators, $\mathbf{R}^{\mathbf{OMe}}$ (●), $\mathbf{R}^{\mathbf{D}}$ (○), **F** (□), $\mathbf{R}^{\mathbf{OBz}}$ (△) and $\mathbf{R}^{\mathbf{Bor}}$ (◇) in EYPC-LUVs⊃CF. **F** and $\mathbf{R}^{\mathbf{Bor}}$ precipitated at the higher concentration than given data.

Table S4. Transport activities of monomers

Monomers	$\mathbf{R}^{\mathbf{OMe}}$	$\mathbf{R}^{\mathbf{D}}$	$\mathbf{R}^{\mathbf{OBz}}$
Y_{max} (%)	81.8 ± 23.5	71.0 ± 3.6	72.0 ± 4.6
EC_{50} (μM) [a]	40.9 ± 80.1	51.9 ± 3.5	56.6 ± 6.2
n [b]	0.5 ± 0.5	5.3 ± 2.3	2.1 ± 0.5

[a]The concentration based on the guanidinium ions (μM), [b]Hill coefficient.

4.3. Gel-Permeation Chromatography

The chromatographic column used was a Superdex 75 10/300GL (10×300 mm) and 30% ACN in 0.1 M acetate buffer (pH = 6.5) was used as 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 detection at 333 nm was for the five membered disulfide ring of the propagators and the amides in the polymers were monitored at 220 nm.. The signals were normalized by the absorption of the excess terminators. Molecular weight of each polymer was obtained from the polymer band in the chromatogram in comparison with the molecular weight standards.

4.4. Summary of Results

Table S5. Poly(**R**$^{\mathbf{OMe}}$/**F**)s in Fig. 2

Name	EC_{50} (μM)[a]	Y_{max} (%)	Hill coefficient	M_w[b] (kDa)	M_n[c] (kDa)	PDI
Poly(**R**$^{\mathbf{OMe}}$)	9.9 ± 2.0	77.4 ± 5.8	1.5± 0.5	11.1	9.67	1.14
Poly(**R**$^{\mathbf{OMe}}$/**F**)$_{40:1}$	7.0 ± 0.7	91.5 ± 3.6	1.6 ± 0.3	13.0	11.1	1.13
Poly(**R**$^{\mathbf{OMe}}$/**F**)$_{20:1}$	5.9 ± 0.2	91.2 ± 1.8	2.0± 0.2	14.9	13.1	1.14
Poly(**R**$^{\mathbf{OMe}}$/**F**)$_{8:1}$	2.8 ± 0.1	84.9 ± 1.5	2.6 ± 0.2	18.1	16.2	1.12

[a]The concentration based on the guanidinium ions. [b]Mass average molecular weight. [c]Number average molecular weight.

Table S6. Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)s , Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)s and Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{Bor}}$)$_{8:1}$ in Fig. 3c

Name	EC_{50} (μM)[a]	Y_{max} (%)	Hill coefficient	M_w[b] (kDa)	M_n[c] (kDa)	PDI
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{16:1}$	3.52 ± 0.09	99.3 ± 1.5	1.8 ± 0.1	21.3	16.0	1.33
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{8:1}$	2.77 ± 0.08	104 ± 1.9	2.4 ± 0.2	23.4	17.9	1.31
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{16:1}$	2.30 ± 0.01	101 ± 2.6	2.0 ± 0.2	23.1	17.7	1.30
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{8:1}$	1.73 ± 0.01	105 ± 0.3	2.2 ± 0.0	25.1	19.1	1.32
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{Bor}}$)$_{8:1}$	0.55 ± 0.03	90.9 ± 1.3	1.6 ± 0.1	13.8	8.62	1.60

[a]The concentration based on the guanidinium ions. [b]Mass average molecular weight. [c]Number average molecular weight.

Table S7. Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{D}}$)s in Fig. 4

Name ($\mathbf{R}^{\mathbf{OMe}}$:$\mathbf{R}^{\mathbf{D}}$)[a]	EC_{50} (μM)[b]	Y_{max} (%)	Hill coefficient	M_w[c] (kDa)	M_n[d] (kDa)	PDI
Poly($\mathbf{R}^{\mathbf{OMe}}$)	8.0 ± 0.9	73.3 ± 2.4	1.0 ± 0.1	11.0	8.58	1.28
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{D}}$)$_{20:1}$	4.3 ± 1.4	71.9 ± 5.1	1.2 ± 0.4	12.3	9.18	1.34
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{D}}$)$_{8:1}$	3.8 ± 0.7	75.5 ± 4.8	1.6± 0.5	16.4	10.6	1.54
Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{D}}$)$_{4:1}$	3.9 ± 0.3	74.5 ± 3.2	2.0 ± 0.3	23.2	14.9	1.55

[a]The molar ratio in the polymerization mixture. [b]The concentration based on the guanidinium ions. [c]Mass average molecular weight. [d]Number average molecular weight.

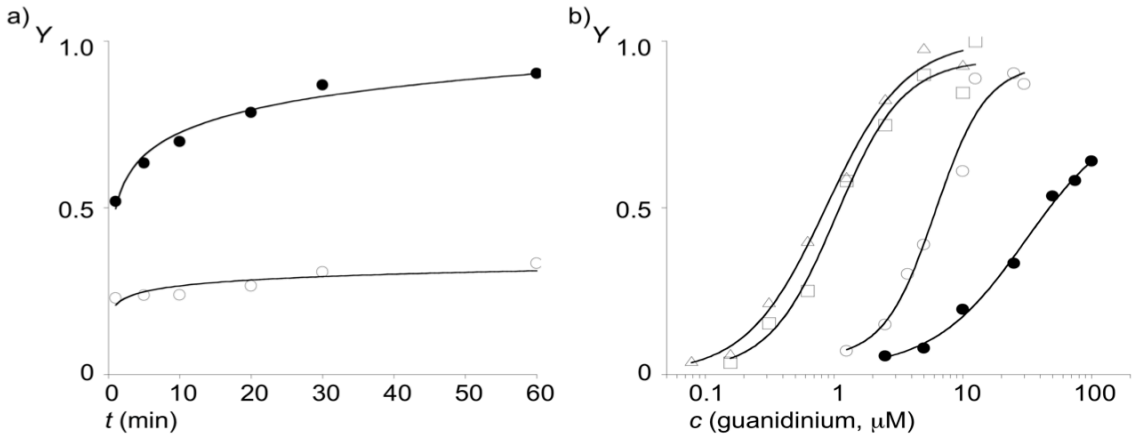


Fig. S3. (a) Time course of change in transport activity *Y* of poly($\mathbf{R}^{\mathbf{D}}$) with 5 mM (●) and 0 mM (○) initiator. (b) Dose response curves of poly($\mathbf{R}^{\mathbf{D}}$) with 0 mM (●), 5 mM (○), 50 mM (□) and 100 mM (△) of initiator in EYPC-LUVs⊃CF. Other conditions are given in Table S1.

Table S8. Transport activities of poly($\mathbf{R}^{\mathbf{D}}$)s

Initiator	0 mM[a]	5 mM[a]	50 mM[a]	100 mM[a]
Y_{max} (%)	79.1 ± 13.7	99.7 ± 9.4	94.8 ± 5.2	100.3 ± 5.5
EC_{50} (μM)[b]	30.9 ± 8.8	6.9 ± 1.0	1.0 ± 0.2	0.87 ± 0.12
n[c]	1.2 ± 0.4	1.6 ± 0.5	1.6 ± 0.5	1.4 ± 0.3

[a]Concentration of initiator during polymerization. [b]Guanidinium-based concentration (μM), [c]Hill coefficient.

5. NMR Spectra

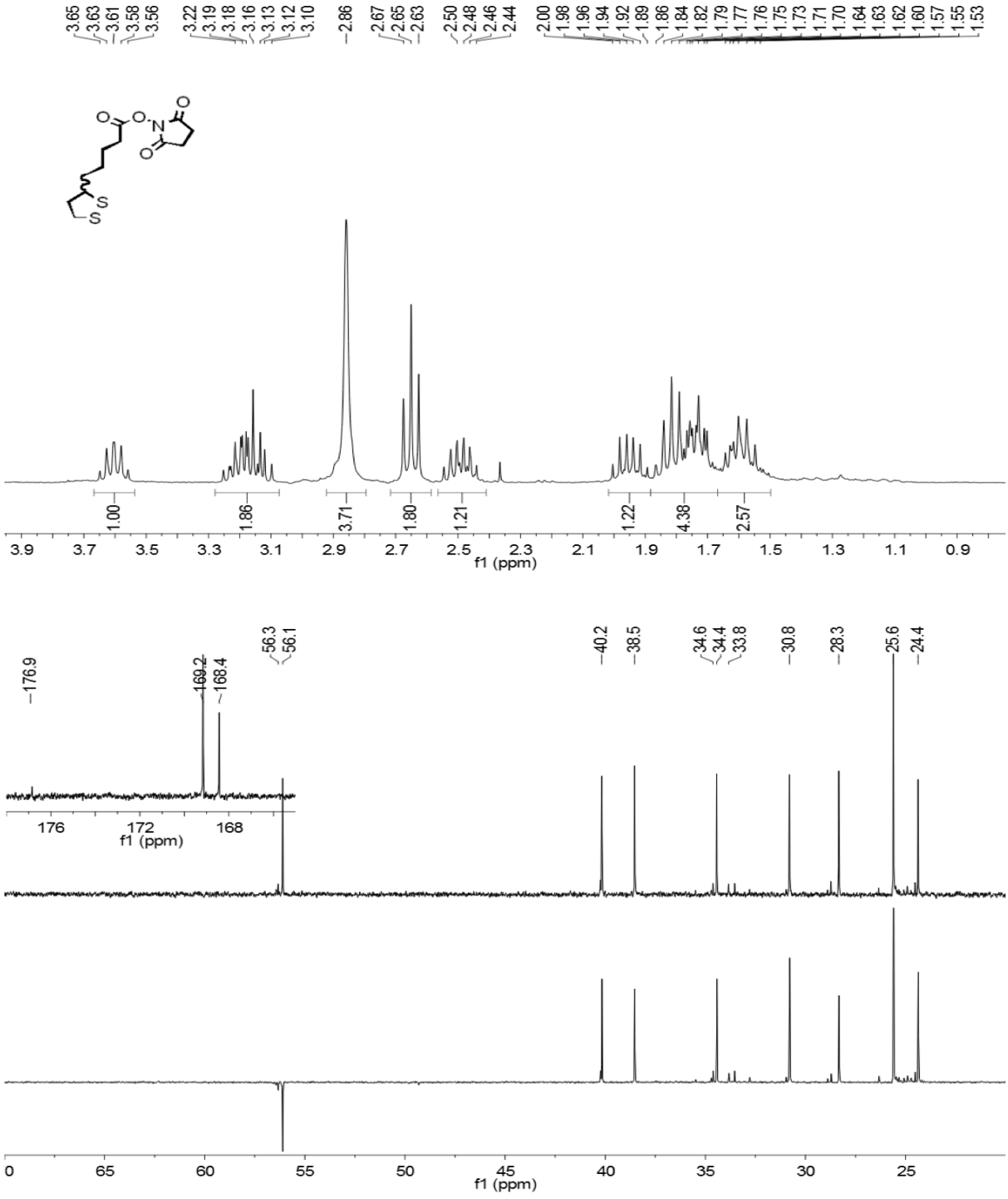


Fig. S4. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **2** in $CDCl_3$.

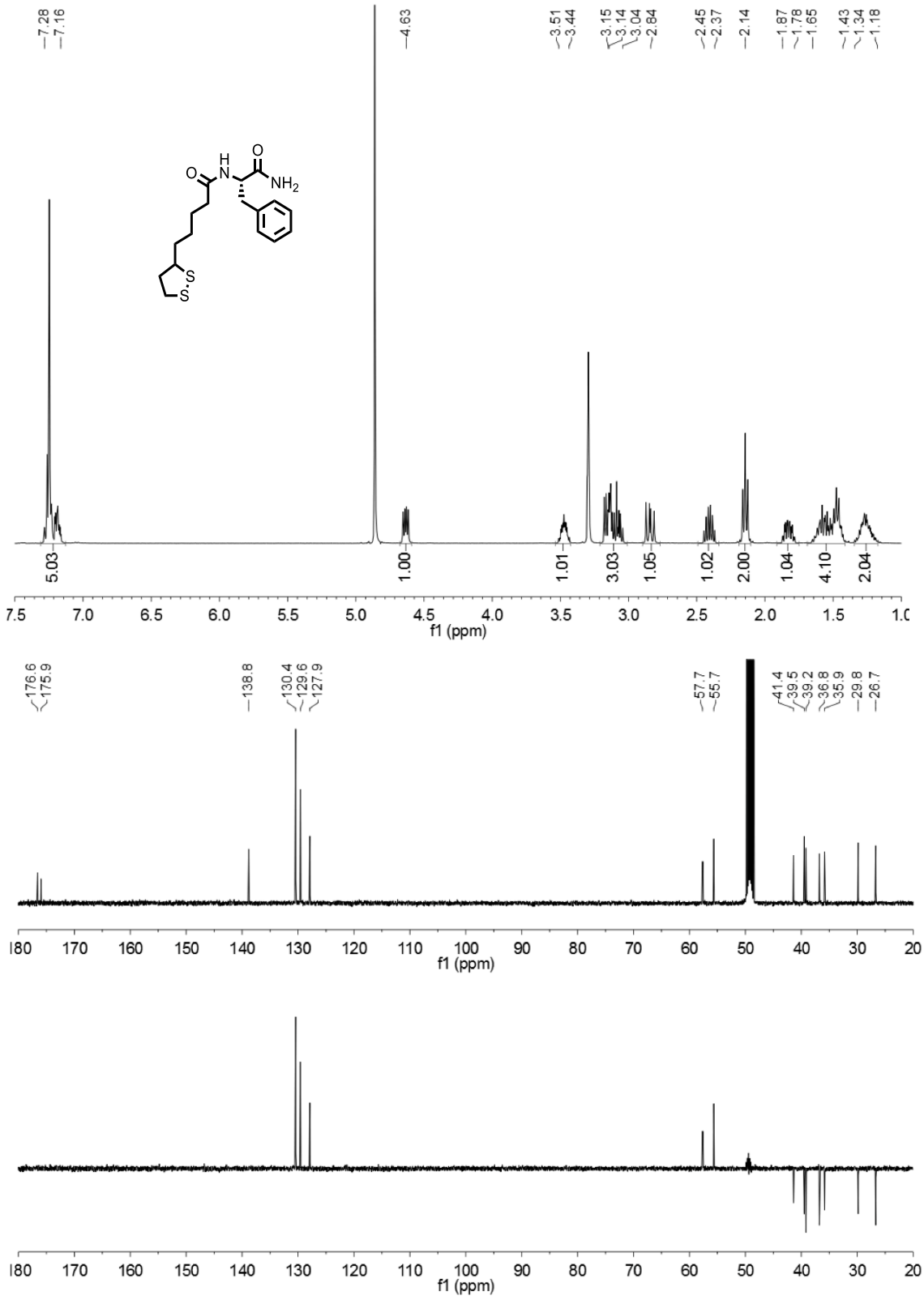


Fig. S5. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **F** in CD_3OD.

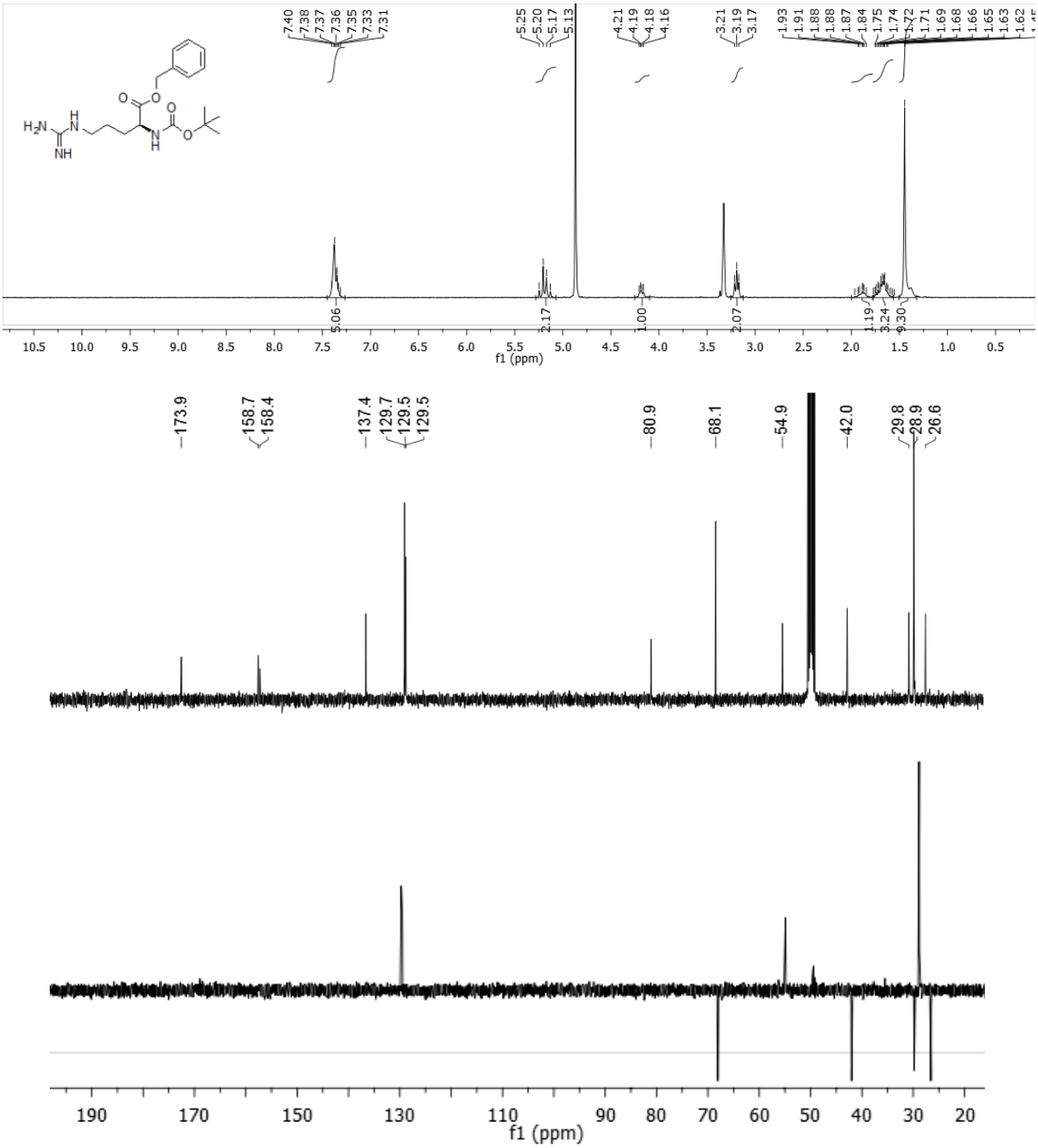


Fig. S6. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **4** in CD_3OD.

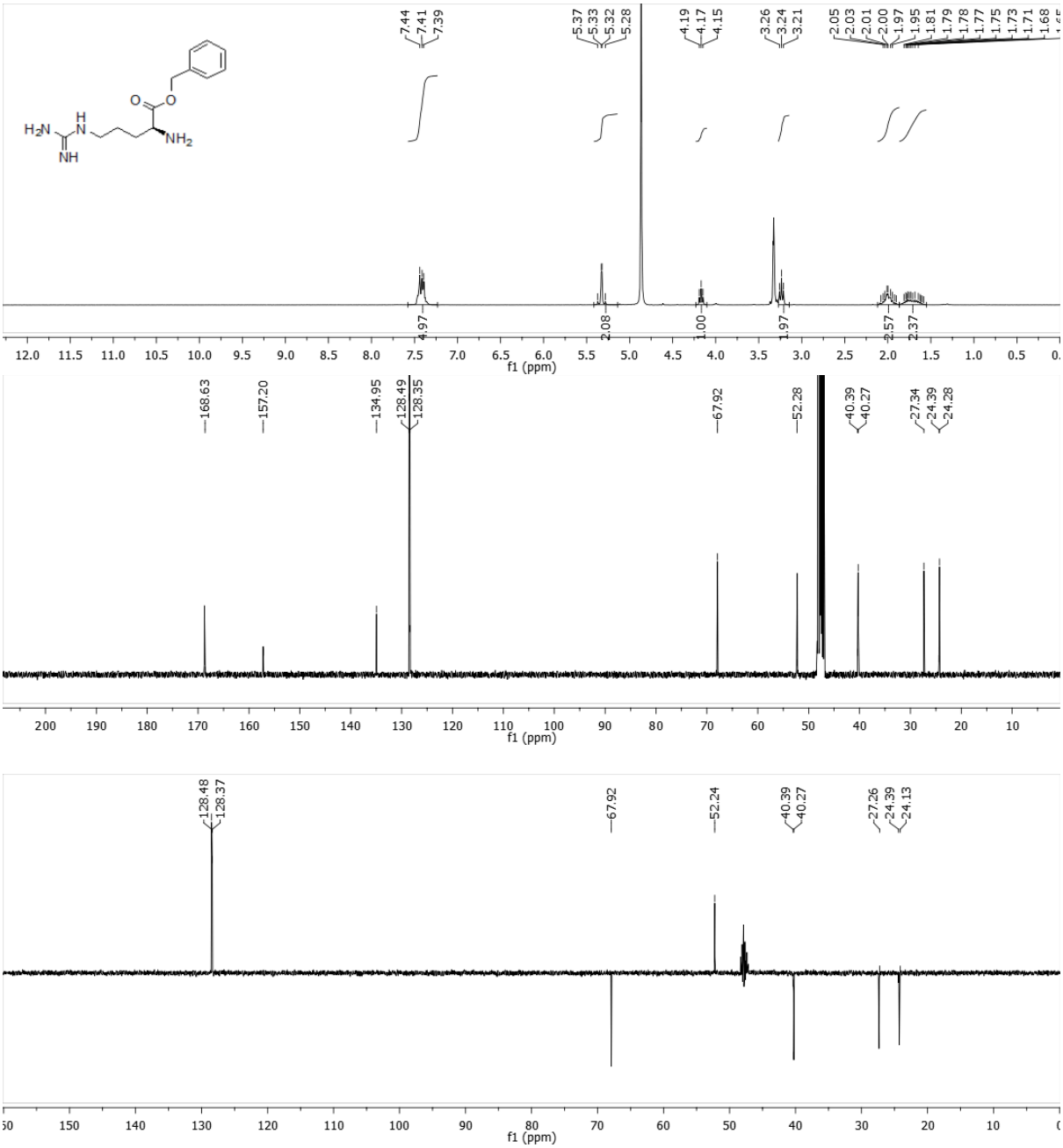


Fig. S7. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **5** in CD_3OD.

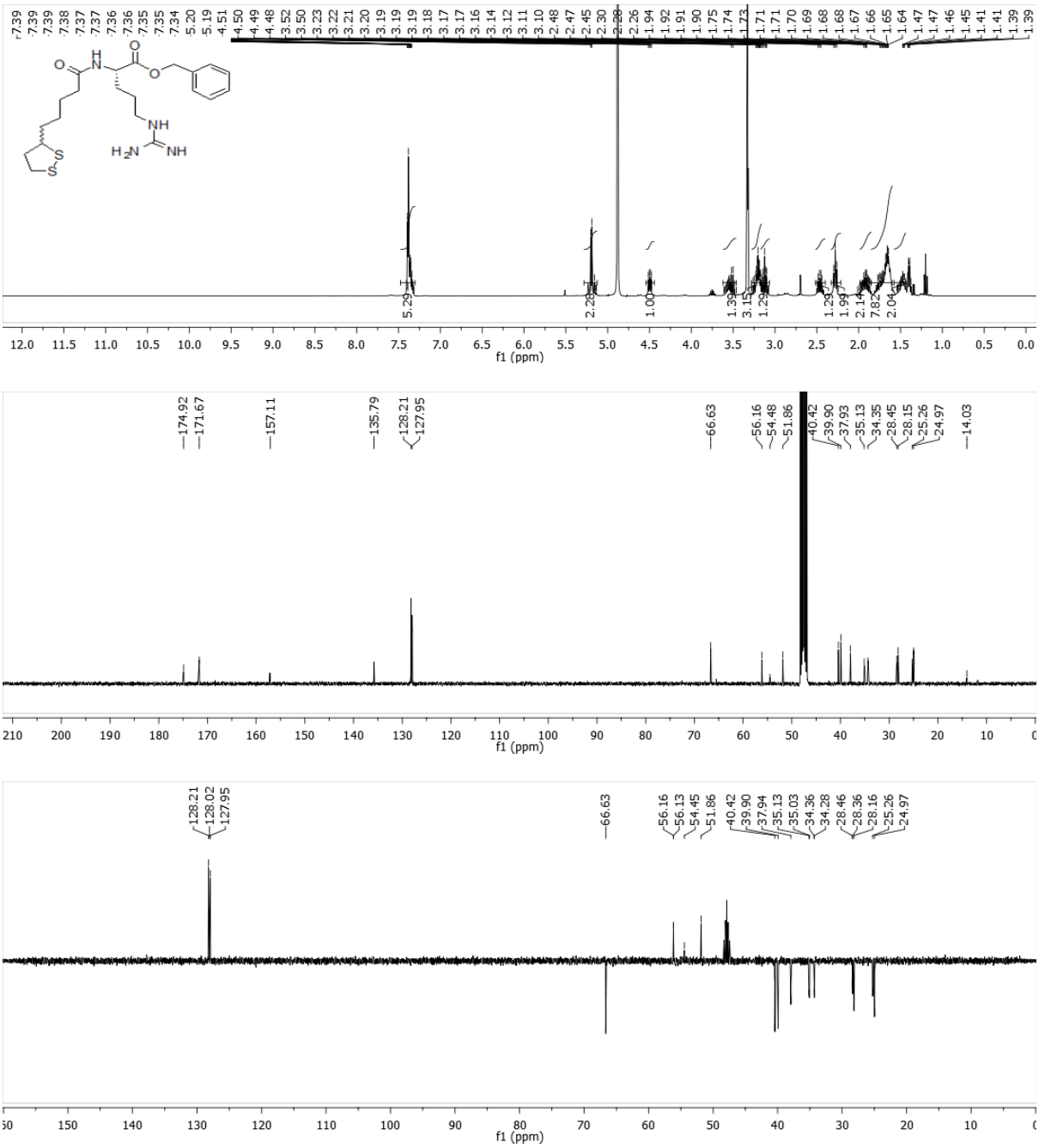


Fig. S8. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of $\mathbf{R^{OBz}}$ in CD_3OD.

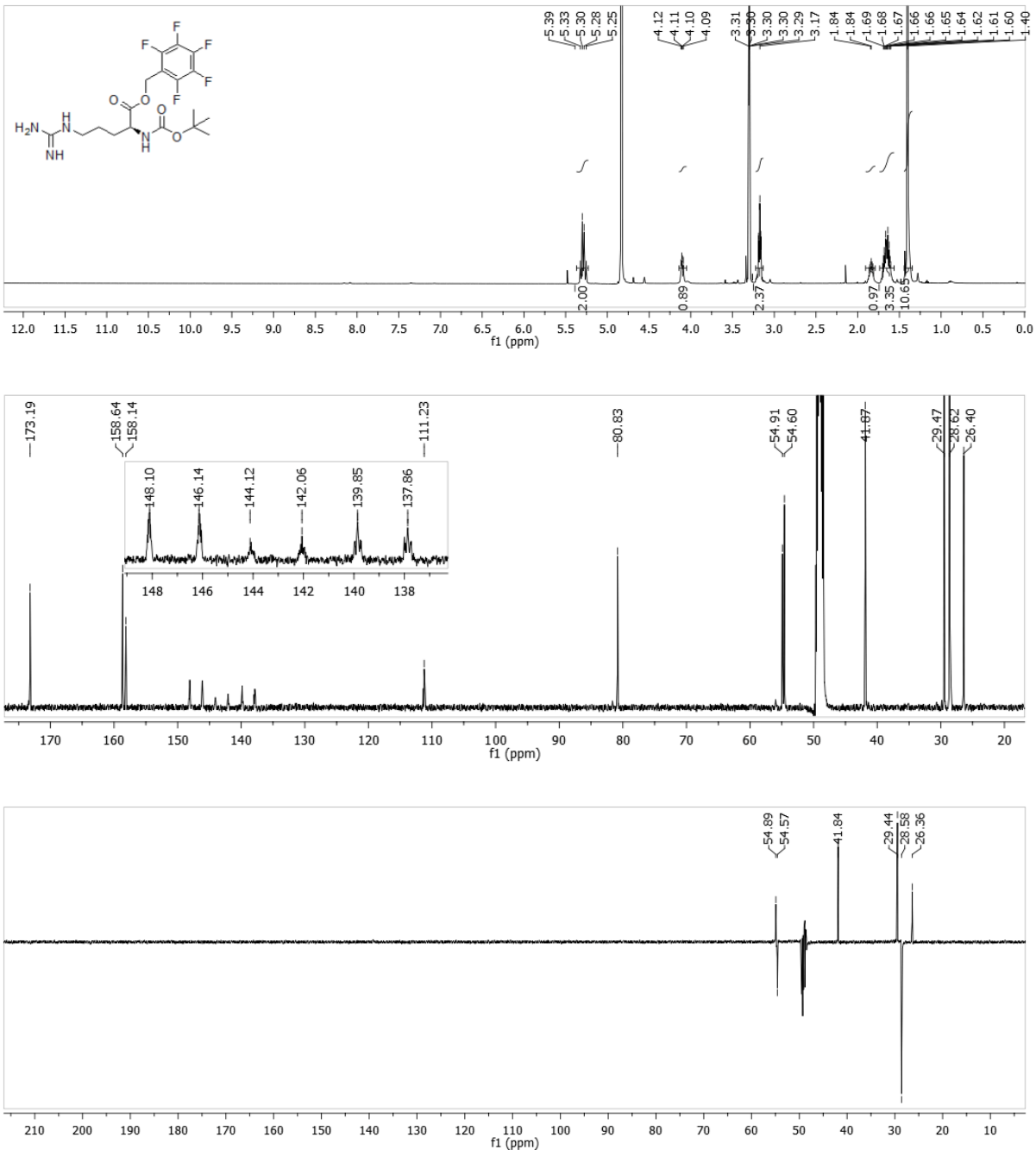


Fig. S9. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **6** in CD_3OD.

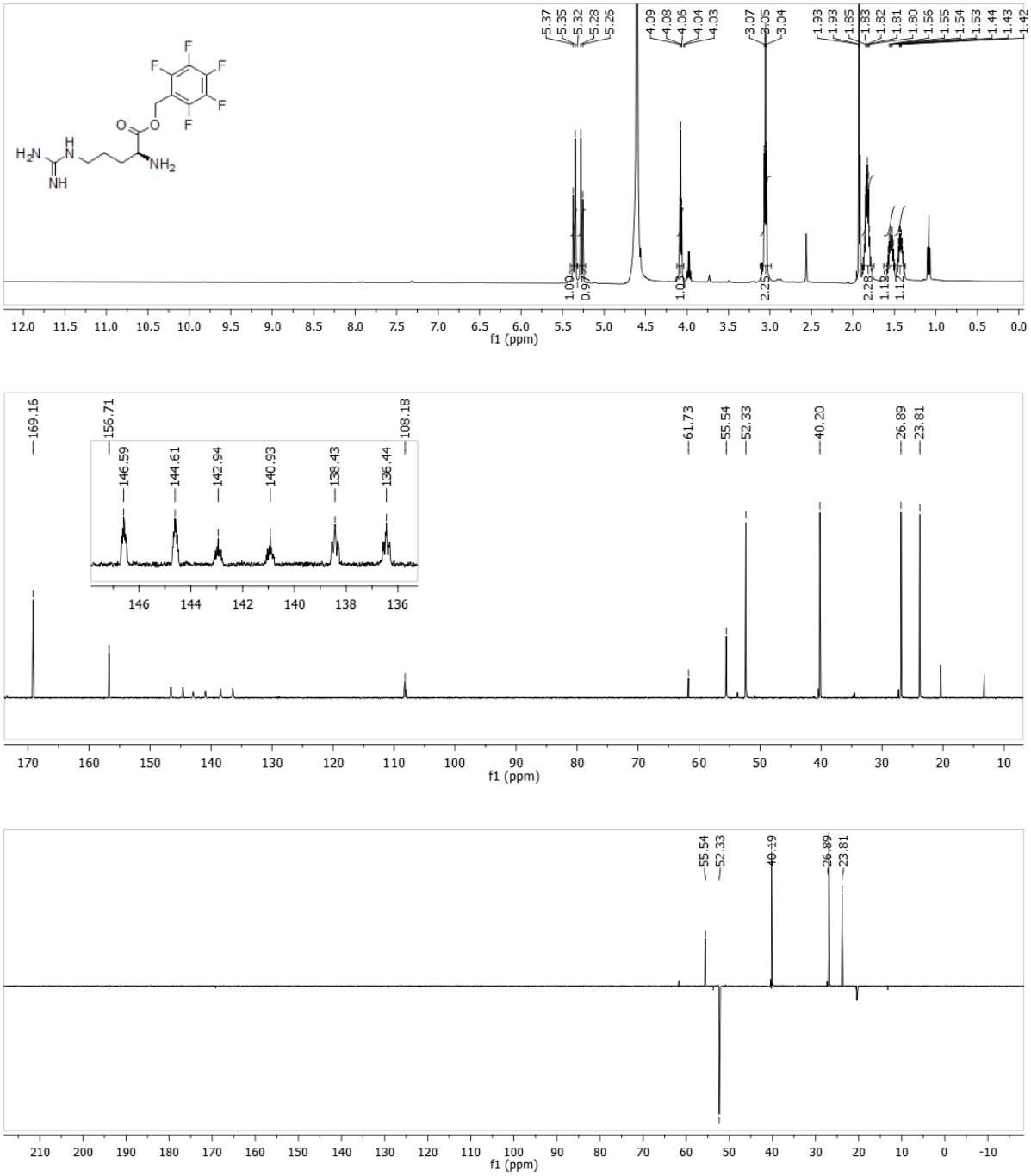


Fig. S10. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **7** in D_2O.

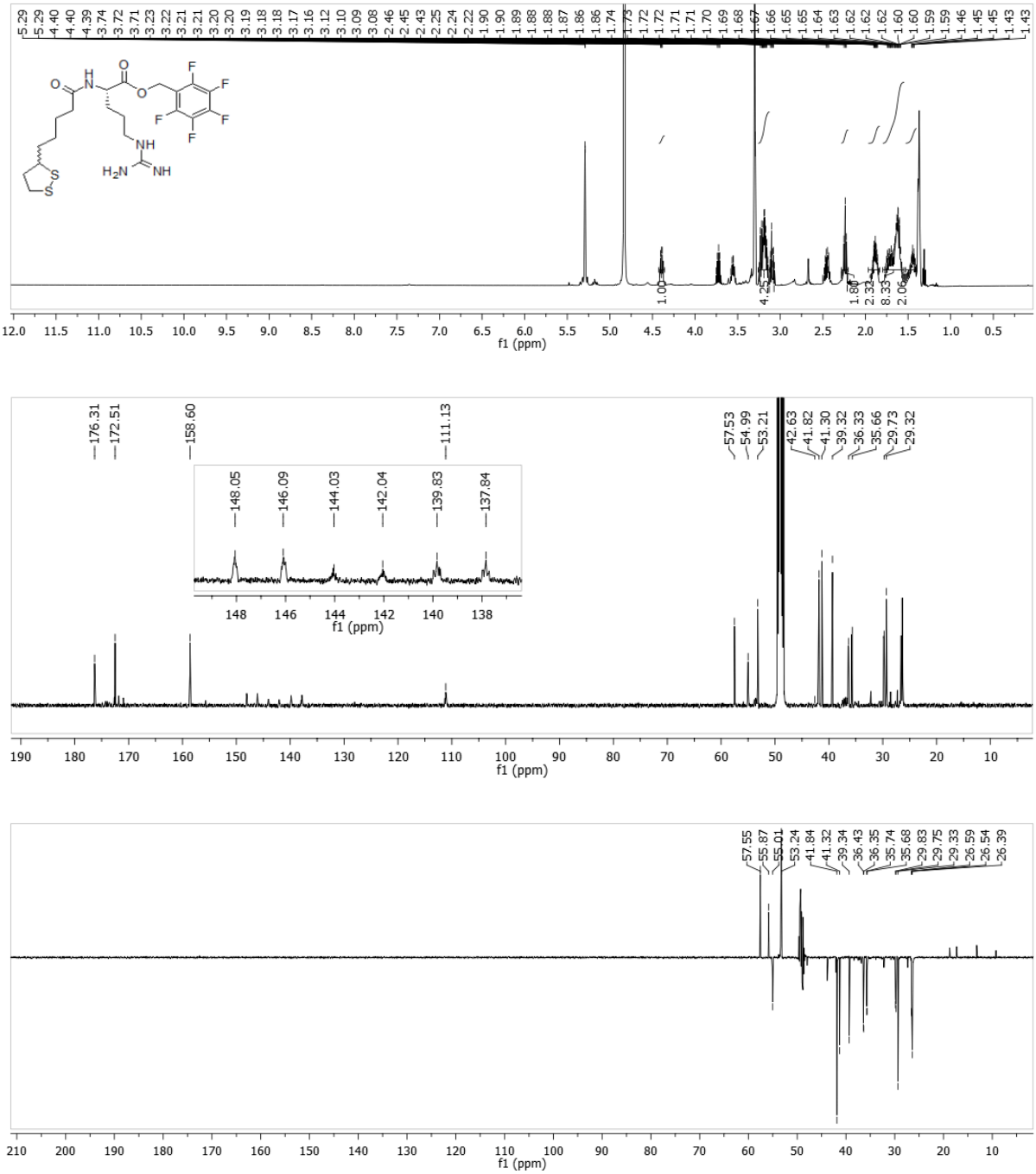


Fig. S11. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of $\mathbf{R}^{\mathbf{OF_5Bz}}$ in CD_3OD.

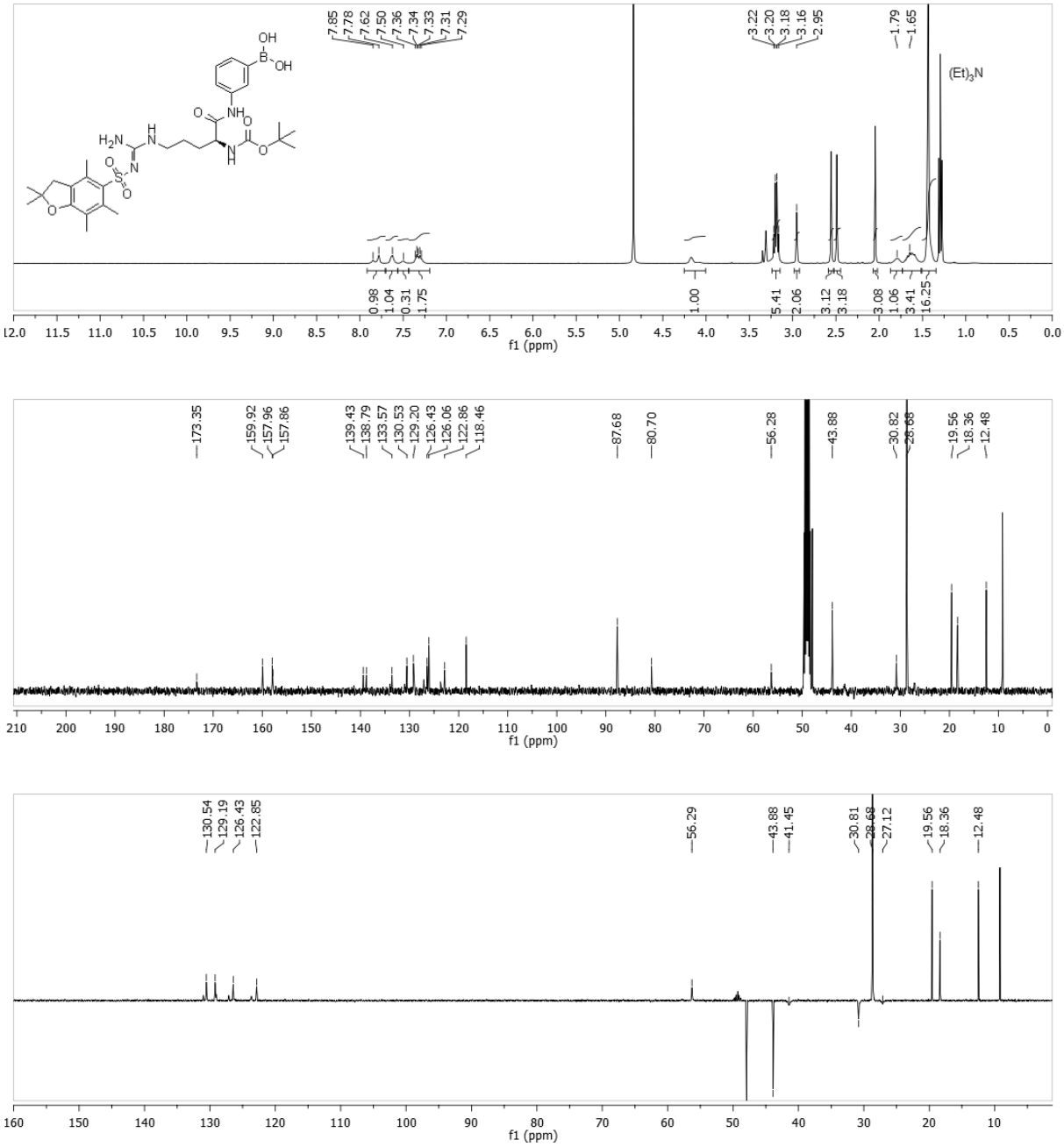


Fig. S12. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **9** in CD_3OD.

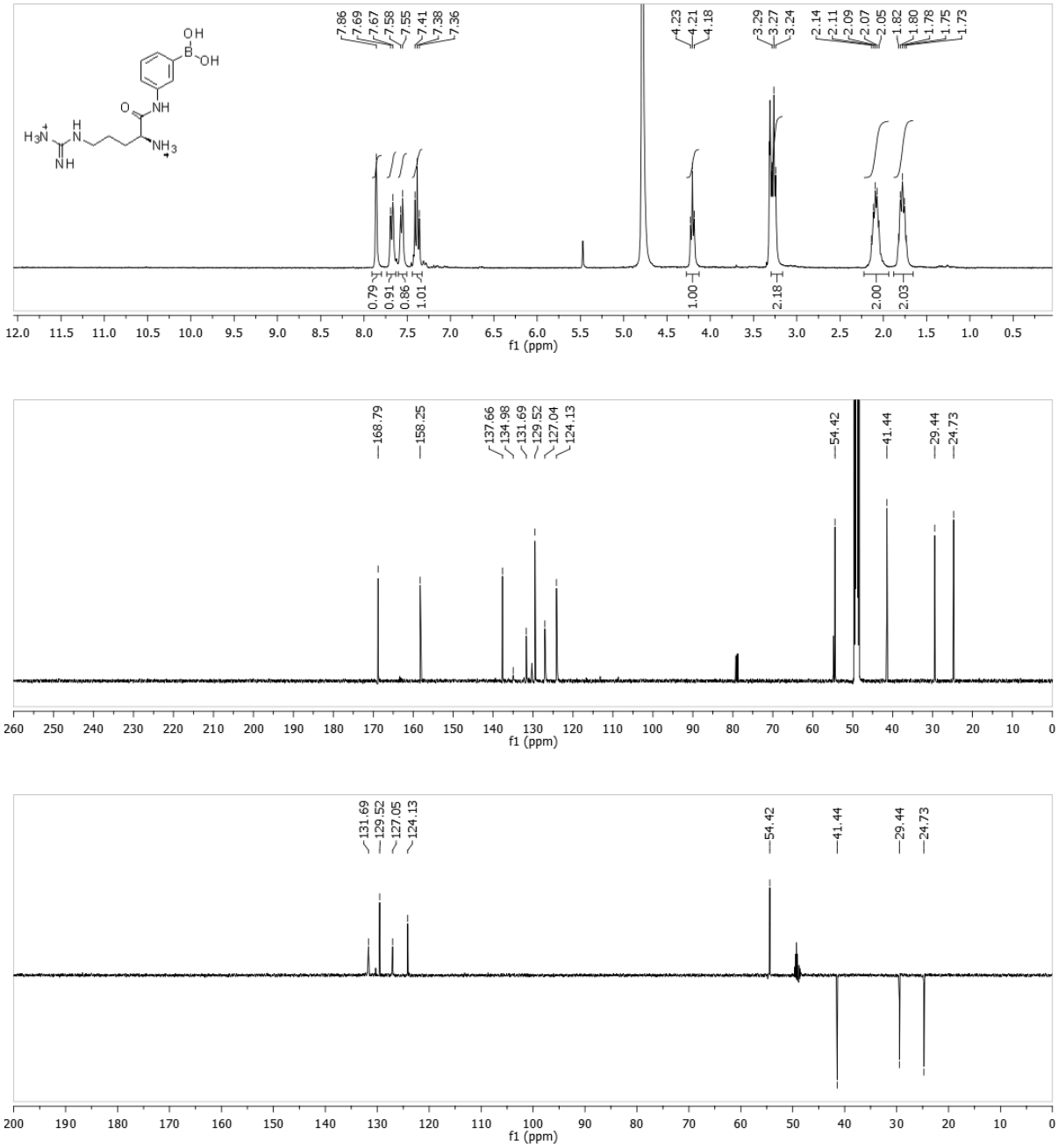


Fig. S13. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **10** in CD_3OD.

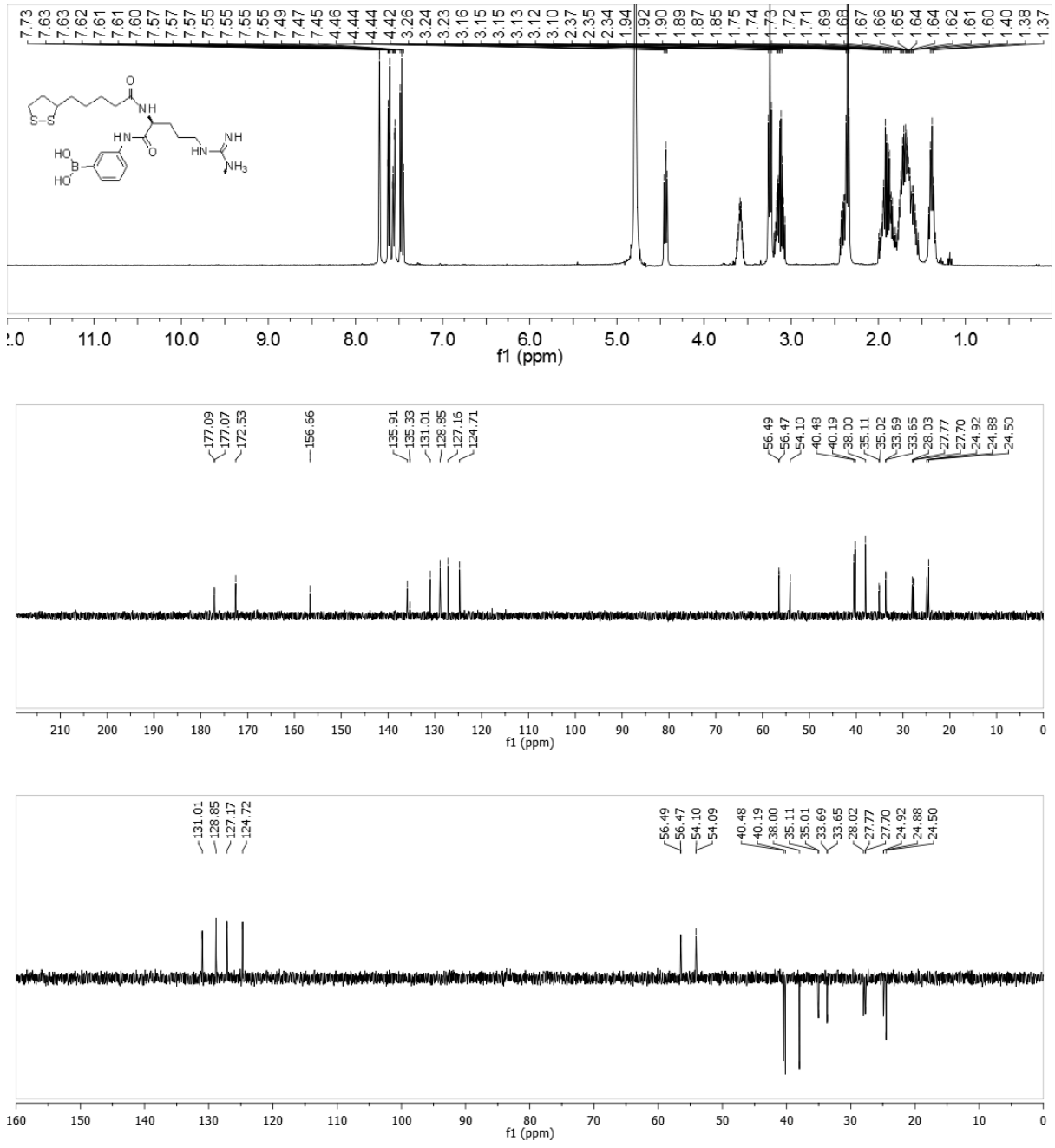


Fig. S14. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of $\mathbf{R}^{\mathbf{Bor}}$ in CD_3OD.

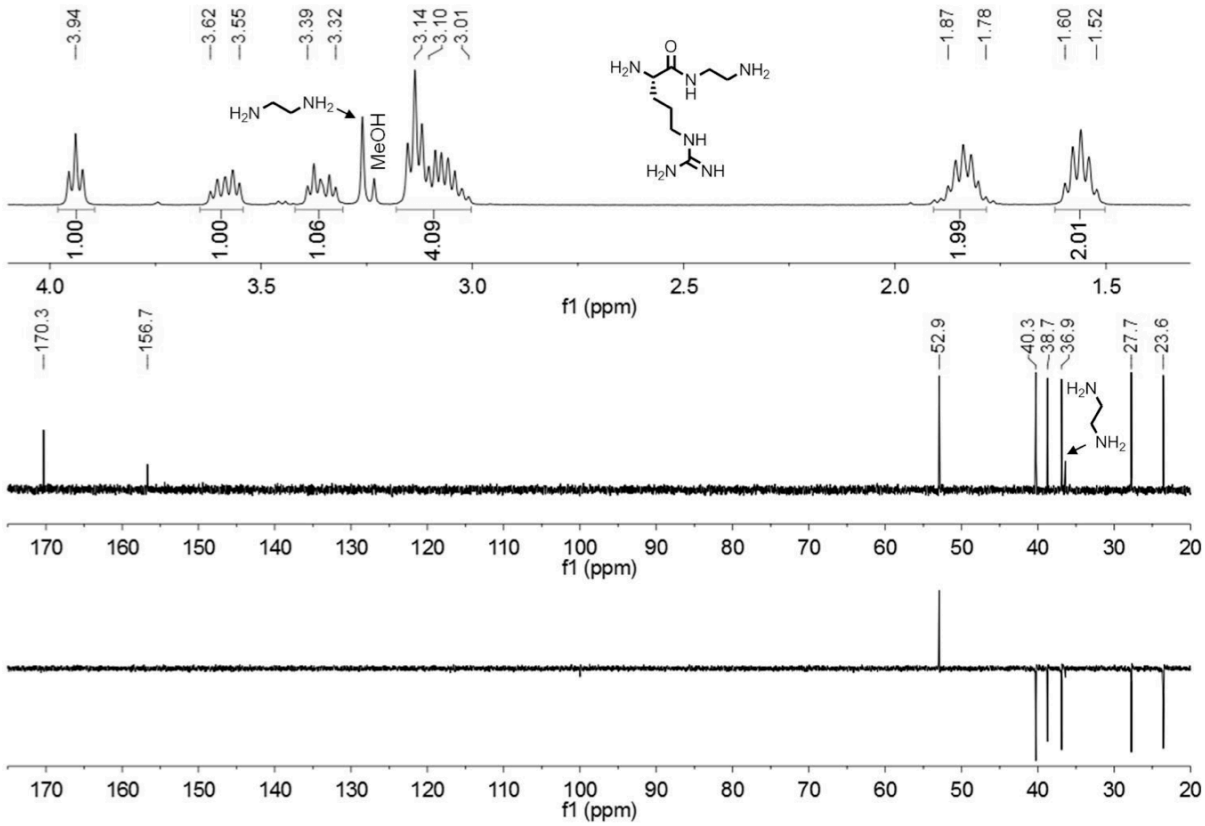


Fig. S15. ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of **11** in D_2O.

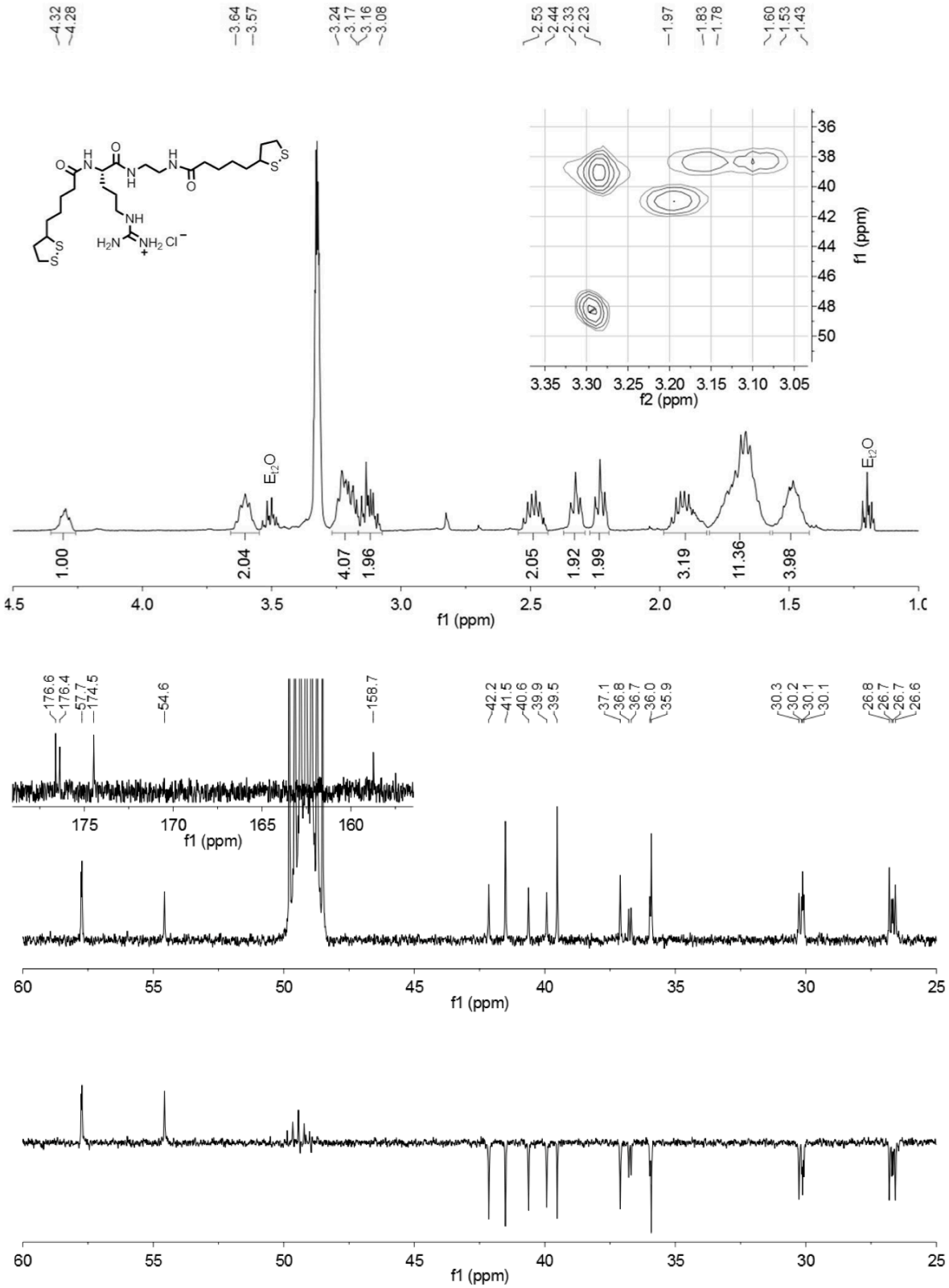


Fig. S16. ^{1}H NMR, HSQC NMR, ^{13}C NMR and DEPT-135 spectra of $\mathbf{R^D}$ in CD_3OD.

6. Supplementary References

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