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Protein delivery with cell-penetrating poly(disulfide)s

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Supplementary information

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1. Materials and methods

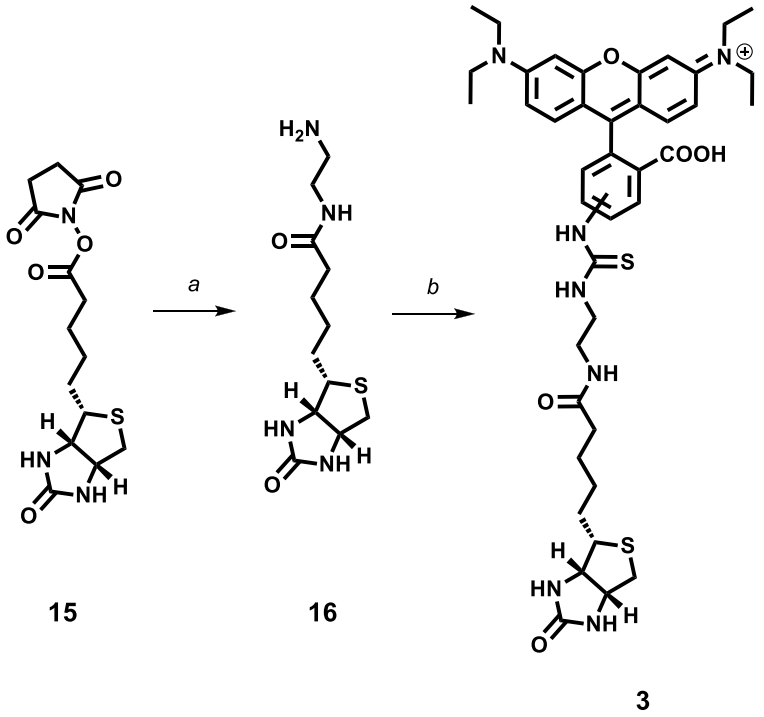
As in ref. S1, Supporting Information. Briefly, reagents for synthesis were purchased from Fluka, Sigma-Aldrich, Apollo Scientific, TCI and Acros, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received.

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) 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 200 Increase 3.2/300 (flow 0.075 ml/min, eluent: 30% ACN in 0.1 M acetate buffer pH = 6.5). Semi-preparative HPLC was performed using JASCO LC-2000 Plus system equipped with quaternary pump (JASCO PU-2089) and UV/Vis detector (JASCO UV-2077 Plus). The chromatographic column used was a Phenomenex Jupiter Proteo (250x10 mm, 4.0 μm particles size, flow 3.0 mL/min with a linear elution gradient from 90% H_2O / 10% ACN + 0.1% TFA to 10% H_2O / 90% ACN + 0.1% TFA in 15.0 min). LC-MS were recorded using a Thermo Scientific Accela HPLC equipped with a Thermo C18 Hypersil GOLD column (50x2.1 mm, 1.9 μm particles size) coupled with a LCQ Fleet three-dimensional ion trap mass spectrometer (ESI, Thermo Scientific) with a linear elution gradient from 95% H_2O / 5% ACN + 0.1% TFA to 10% H_2O / 90% ACN + 0.1% 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). ^1H and ^{13}C spectra were recorded (as indicated) either on a Bruker 300 MHz, 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). 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]). Fluorescence imaging was performed using Leica SP5 confocal microscope, equipped with 63× oil immersion objective lens. Flow cytometry measurements were performed using Beckman Coulter Gallios™ (6 colors 2 lasers) flow cytometer.

Abbreviations. ACN: Acetonitrile; Ac_2O: Acetic anhydride; AcOH: Acetic acid; CDI: 1,1'-Carbonyldiimidazole; CF: Carboxyfluorescein; CF-NCS: Carboxyfluorescein isothiocyanate; DCM: Dichloromethane; DMF: *N*,*N*-Dimethylformamide; DMEM: Dulbecco's modified eagle medium; DMSO: Dimethyl sulfoxide; Et_2O: Diethyl ether; EtOH: Ethanol; FBS: Fetal bovine serum; MEM: Minimum essential media; MeOH: Methanol; NCS: Isothiocyanate; NHS: *N*-Hydroxysuccinimide; PBS: Phosphate buffer saline; PhOH: Phenol; PS: Penicillin / streptomycin; rt: Room temperature; SR: Sytox red; TEA: Triethylamine; TEOA: Triethanolamine; TFA: Trifluoroacetic acid; TIPS: Triisopropylsilane; Trt: Trityl.

2. Synthesis

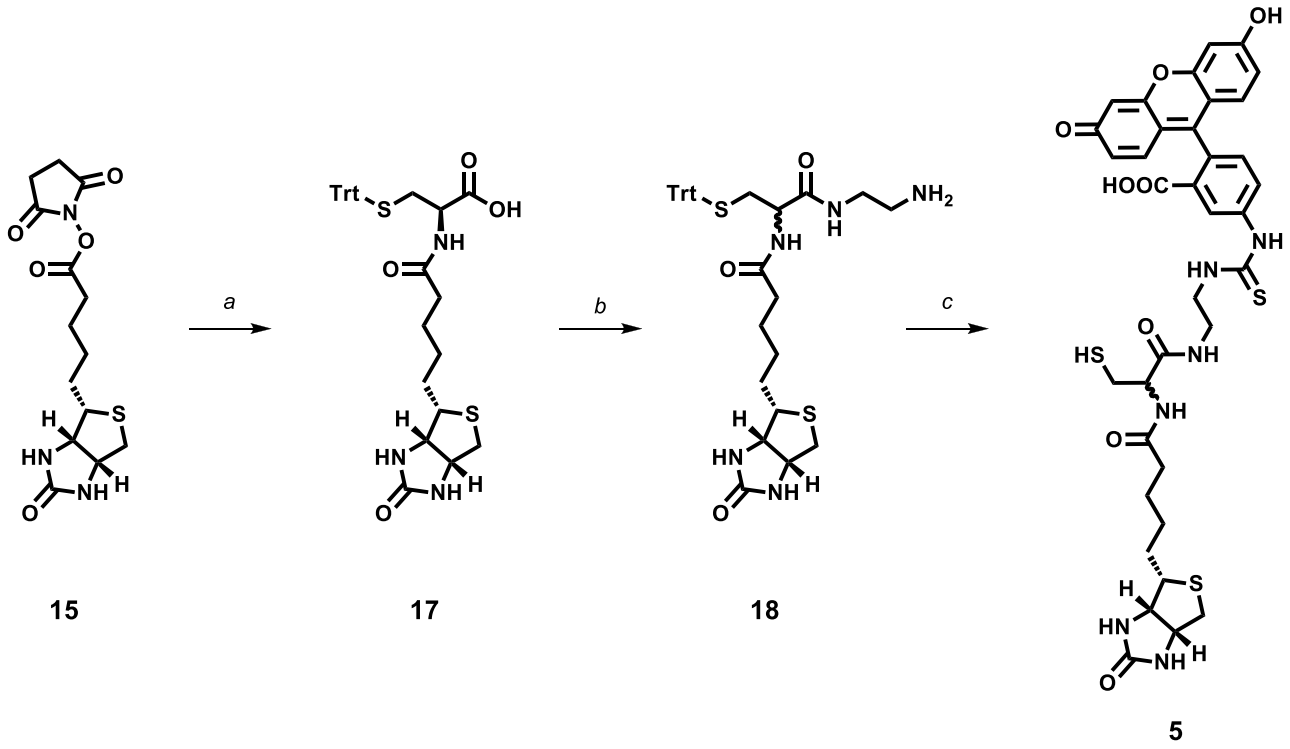


Scheme S1. a) Ethylenediamine, TEA, DMF, 12 h, 70%; b) rhodamine B isothiocyanate, TEA, DMF, 12 h, rt, 42%.

Compound 16. This compound was prepared as reported in the literature.[S2]

Compound 3. *N*-(2-aminoethyl) biotinamide **16** (31.7 mg, 0.111 mmol) and TEA (15 µL, 0.11 mmol) were dissolved in 2 mL of anhydrous DMF. Commercially available rhodamine B isothiocyanate (mixed isomers) (21.9 mg, 40.8 µmol) was dissolved in 1 mL of anhydrous DMF and added to the reaction mixture. The reaction was kept 12 h at rt. TLC control revealed complete formation of the product (DCM/MeOH 8:2 + 2% $NH_{3\ (aq)}$ $R_{f\ isom1}$ = 0.3, $R_{f\ isom2}$ = 0.2). The crude product was purified by flash chromatography (SiO_2) using the same eluent, yielding product **3** as a violet solid (overall mixture of isomers 14.2 mg, 42%). IR (ATR, cm^{-1}): 3248 (br), 2926 (w), 1692 (m), 1644 (m), 1584 (s), 1527 (m), 1462 (m), 1407 (s), 1332 (s), 1270 (m), 1243 (m), 1178 (s), 1127 (m), 1071 (m); ^{1}H NMR isomer 1 (500 MHz, CD_3OD): 8.05 (s br, 1H), 7.88 (dd, ^3J (H,H) = 8.1 Hz, ^4J (H,H) = 1.8 Hz, 1H), 7.37 (dd, ^3J (H,H) = 9.5 Hz, ^4J (H,H) = 0.9 Hz, 2H), 7.24 (d, ^3J (H,H) = 8.1 Hz, 1H), 7.01 (dd, ^3J (H,H) = 9.5 Hz, ^4J (H,H) = 2.4 Hz, 2H), 6.92 (d, ^4J (H,H) = 2.4 Hz, 2H), 4.44 (dd, ^3J (H,H) = 7.7 Hz, ^3J (H,H) = 5.1 Hz, 1H), 4.24 (dd, ^3J (H,H) = 7.7 Hz, ^3J (H,H) = 4.8 Hz, 1H), 3.77 (s br, 2H), 3.66 (q, ^3J (H,H) = 7.1 Hz, 8H), 3.45 (t, ^3J (H,H) = 5.8 Hz, 2H), 3.17 (dt, ^3J (H,H) = 8.6 Hz, ^3J (H,H) = 5.1 Hz, 1H), 2.88 (dd, ^2J (H,H) = 12.8 Hz, ^3J (H,H) = 4.8 Hz, 1H), 2.66 (d, ^2J (H,H) = 12.8 Hz, 1H), 2.29-2.16 (m, 2H), 1.73-1.51 (m, 4H), 1.44-1.38 (m, 2H), 1.29 (t, ^3J (H,H) =

7.1 Hz, 12H); ^{13}C NMR isomer 1 (125 MHz, CD_3OD): 182.8 (C), 176.8 (C), 172.1 (C), 166.1 (2C), 162.7 (C), 159.2 (2C), 156.9 (C), 133.2 (CH), 131.4 (CH), 129.9 (C), 126.1 (CH), 125.7 (CH), 115.1 (C), 114.9 (CH), 97.0 (CH), 63.3 (CH), 61.6 (CH), 56.9 (CH), 46.7 (CH_2), 45.4 (CH_2), 41.1 (CH_2), 39.8 (CH_2), 36.8 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 26.7 (CH_2), 12.8 (CH_3); LC-MS: R_t = 2.54 min, *m/z* = 394 (100, $[M+H]^{2+}$), 787 (60, $[M]^+$).



Scheme S2. a) *S*-Trityl-L-cysteine, TEA, DMF, 2 h, rt, 78%; b) ethylenediamine, CDI, DCM, 2 h, rt, 56%; c) 1. CF-NCS, DMF, 1 h, rt; 2. TFA/H_2O/PhOH/TIPS, 2 h, rt, 56%.

Compound 17. Commercially available S-trityl-L-cysteine (211 mg, 0.580 mmol) was suspended in 7 mL of anhydrous DMF. TEA (162 µL, 1.16 mmol) and biotin-NHS **15** (199 mg, 0.583 mmol) were added sequentially, obtaining a clear solution. The reaction mixture was stirred for 3 h, until TLC control (DCM/MeOH 8:2, R_f = 0.4) showed no traces of starting material. The solvent was evaporated under reduced pressure and 20 mL of water were added to the resulting oily residue, yielding a white precipitate. The crude solid was dissolved in 50 mL of DCM and washed with water (3 × 30 mL). The organic phase was dried over sodium sulphate, filtered and the solvent was evaporated under reduced pressure. The resulting oil was triturated with ACN in order to obtain product **17** as a colourless solid (266.8 mg, 78%). IR (ATR, cm^{-1}): 3278 (br), 3056 (w), 2925 (m), 2853 (w), 1696 (s), 1663 (s), 1489 (m), 1443 (m), 1323 (w), 1233 (w), 740 (s), 698 (s); ^{1}H NMR

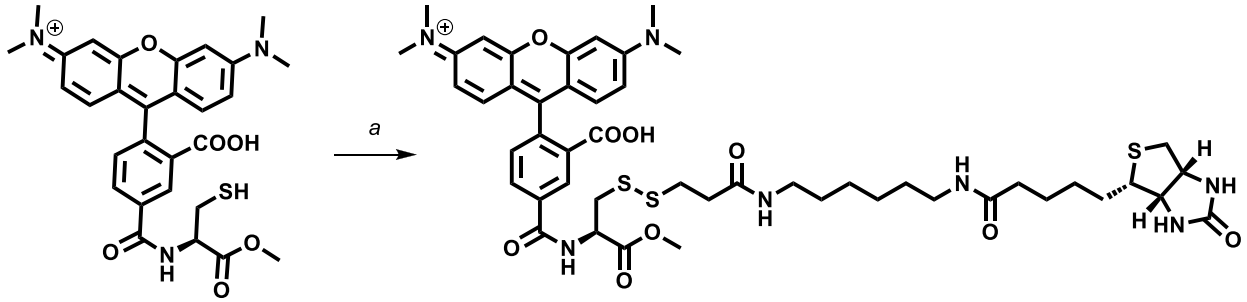
(400 MHz, $CDCl_3$): 7.81 (s, 1H), 7.19-6.99 (m, 15H), 6.72 (d, ^3J (H,H) = 7.9 Hz, 1H), 5.87 (s, 1H), 5.64 (s, 1H), 4.28-4.23 (m, 1H), 4.20-4.17 (m, 1H), 4.02-3.99 (m, 1H), 2.91-2.81 (m, 1H), 2.65-2.61 (m, 1H), 2.52-2.39 (m, 3H), 2.03-1.99 (m, 2H), 1.56-1.21 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$): 172.6 (C), 172.4 (C), 163.4 (C), 144.1 (C), 129.2 (CH), 127.6 (CH), 126.4 (CH), 66.3 (C), 61.2 (CH), 59.7 (CH), 55.1 (CH), 51.0 (CH), 40.1 (CH_2), 35.1 (CH_2), 33.4 (CH_2), 27.6 (2 CH_2), 25.0 (CH_2); LC-MS: R_t = 2.61 min, *m/z* = 1179 (100, $[2M+H]^+$).

Compound 18. Compound **17** (71.1 mg, 0.120 mmol) and CDI (25.8 mg, 0.159 mmol) were dissolved in anhydrous DCM. The resulting clear solution was stirred for 15 minutes at rt, then added dropwise to neat ethylenediamine (1.0 mL, 15 mmol). The reaction mixture was stirred at rt for 2 h. The solvent and excess of ethylenediamine were removed by evaporation under reduced pressure, yielding an yellow oil. The crude product was purified by flash chromatography (SiO_2, DCM/MeOH/TEA 9:1:0.1, R_f 0.13). The obtained yellow oil was dissolved in 1 mL of MeOH and precipitated in 20 mL of Et_2O, yielding product **18** (43.0 mg, 56%), a colourless solid, as a ~1:1 mixture of epimers (oxazolone-mediated cysteine epimerization, obviously irrelevant for the topic of this study, Scheme S2, Figs. 1, 2, compare also **13**). IR (ATR, cm^{-1}): 3260 (br), 3050 (w), 2937 (m), 1670 (s), 1530 (s), 1449 (m), 1261 (m), 1177 (m), 1112 (m); ^{1}H NMR (400 MHz, CD_3OD): 7.40-7.38 (m, 6H), 7.32-7.29 (m, 6H), 7.25-7.22 (m, 3H), 4.3 (td, ^3J (H,H) = 8.3 Hz, ^3J (H,H) = 4.5 Hz, 1H), 4.26-4.20 (m, 1H), 4.14-4.04 (m, 1H), 3.59-3.52 (m, 1H), 3.39-3.32 (m, 1H), 3.16-3.09 (m, 1H), 3.07-2.95 (m, 2H), 2.89-2.83 (m, 1H), 2.69-2.50 (m, 3H), 2.31-2.19 (m, 2H), 1.76-1.41 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): 176.5-176.4 (C, 2 isomers), 173.7 (C), 166.2-166.1 (C, 2 isomers), 145.9-145.8 (C, 2 isomers), 130.7 (CH), 129.1 (CH), 128.0-128.0 (CH, 2 isomers), 68.1-68.1 (C, 2 isomers), 63.3-63.1 (CH, 2 isomers), 61.7-61.6 (CH, 2 isomers), 57.0-56.9 (CH, 2 isomers), 54.6-54.4 (CH, 2 isomers), 41.0-41.0 (CH_2, 2 isomers), 40.8-40.8 (CH_2, 2 isomers), 38.2-38.2 (CH_2, 2 isomers), 36.3-36.2 (CH_2, 2 isomers), 34.4-34.3 (CH_2, 2 isomers), 29.5-29.5 (CH_2, 2 isomers), 29.3-29.3 (CH_2, 2 isomers), 26.7-26.6 (CH_2, 2 isomers); LC-MS: R_t = 2.22 min, *m/z* = 632 (100, $[M+H]^+$), 1263 (10, $[2M+H]^+$).

Compound 5. Compound **18** (45.3 mg, 71.7 µmol) and commercially available CF-NCS (29.6 mg, 76.0 µmol) were dissolved in 2 mL of anhydrous DMF, then TEA (20 µL, 0.15 mmol) was added. The reaction mixture was stirred at rt for 1 h. TLC control confirmed complete consumption of the biotinylated starting material (DCM/MeOH 8:2, R_f = 0.5). The solution was concentrated by evaporation under reduced pressure and the residue added dropwise to 20 mL of Et_2O, obtaining an orange solid. The solid was then dissolved in 1 mL of cleavage mixture (TFA/H_2O/PhOH/TIPS

8.8:0.5:0.5:0.2) and stirred at rt for 2 h. The reaction mixture was added dropwise to 20 mL of cold Et_2O, obtaining an orange precipitate. The crude product was purified by semi-preparative HPLC (R_t 11.6 min), yielding compound **5** as an orange solid (31.4 mg, 56%). IR (ATR, cm^{-1}): 3268 (br), 3063 (w), 2932 (m), 1662 (s), 1600 (s), 1534 (s), 1453 (s), 1384 (m), 1258 (s), 1176 (s), 1114 (s), 993 (w), 848 (m); ^{1}H NMR (500 MHz, CD_3OD): 8.29 (s br, 1H), 8.15-8.14 (m, 1H), 7.79 (d, ^3J (H,H) = 7.7 Hz, 1H), 7.22 (dd, ^3J (H,H) = 8.2 Hz, ^4J (H,H) = 2.2 Hz, 1H), 6.81-6.76 (m, 4H), 6.64-6.62 (m, 2H), 4.47-4.40 (m, 2H), 4.27 (dt, ^3J (H,H) = 8.3 Hz, ^3J (H,H) = 4.2 Hz, 1H), 3.88-3.76 (m, 2H), 3.49 (q, ^3J (H,H) = 5.7 Hz, 1H), 3.19-3.13 (m, 1H), 2.93-2.87 (m, 2H), 2.83-2.78 (m, 1H), 2.67 (dd, ^2J (H,H) = 12.7 Hz, ^3J (H,H) = 4.0 Hz, 1H), 2.36-2.26 (m, 2H), 1.75-1.63 (m, 3H), 1.61-1.53 (m, 1H), 1.47-1.39 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD): 183.2 (C), 176.4-176.4 (C, 2 isomers), 173.2-173.1 (C, 2 isomers), 170.8-170.7 (C, 2 isomers), 166.2-166.1 (C, 2 isomers), 155.0 (C), 142.1 (C), 132.0 (CH), 130.8 (CH), 129.5 (C), 126.5 (CH), 121.4 (CH), 114.4 (CH), 112.2 (C), 103.5 (CH), 63.3-63.2 (CH, 2 isomers), 61.7-61.6 (CH, 2 isomers), 57.5-57.4 (CH, 2 isomers), 57.0-56.9 (CH, 2 isomers), 45.2 (CH_2), 41.0 (CH_2), 40.4-40.2 (CH_2, 2 isomers), 36.6-36.4 (CH_2, 2 isomers), 29.7-29.5 (CH_2, 2 isomers), 29.4-29.3 (CH_2, 2 isomers), 26.8 (CH_2), 26.7- 26.6 (CH_2, 2 isomers); LC-MS: R_t = 1.96 min, m/z = 390 (100, $[M+2H]^{2+}$), 779 (90, $[M+H]^+$).

Compound 6. This compound was prepared as reported in the literature.[S3]



11 **13**

Scheme S3. a) Biotin-HPDP, PBS pH = 7.4, EDTA, DMF, 2 h, rt, 44%.

Compound 11. This compound was prepared as reported in the literature.[S4]

Compound 13. Compound **11** (2.87 mg, 4.92 μmol) was dissolved in 15 mL of PBS buffer at pH = 7.4 (10 mM phosphate, 150 mM NaCl), containing 1 mM EDTA. To this solution Biotin-HPDP (**12**, 2.66 mg, 4.93 μmol), dissolved in DMF, was added. The reaction mixture was stirred at rt for 2 h, TLC control confirmed complete consumption of the starting materials (DCM/MeOH 9:1 + 1% TEA, R_f = 0.4). The solvent was removed by lyophilization and the resulting violet solid was

purified by semi-preparative HPLC (R_t 11.7 min), yielding compound **13** as a violet solid (2.35 mg, 44%). IR (ATR, cm^{-1}): 3293 (br), 2934 (w), 1694 (m), 1648 (m), 1597 (s), 1493 (w), 1412 (m), 1349 (m), 1189 (s), 1132 (m), 928 (w); ^{1}H NMR (500 MHz, CD_3OD): 8.84 (d, ^4J (H,H) = 1.6 Hz, 1H), 8.32 (dd, ^3J (H,H) = 7.9 Hz, ^4J (H,H) = 1.6 Hz, 1H), 7.56 (d, ^3J (H,H) = 7.9 Hz, 1H), 7.17 (d, ^3J (H,H) = 9.4 Hz, 2H), 7.08 (dd, ^3J (H,H) = 9.4 Hz, ^4J (H,H) = 2.4 Hz, 2H), 7.00 (d, ^4J (H,H) = 2.4 Hz, 2H), 5.04 (dd, ^3J (H,H) = 9.9 Hz, ^3J (H,H) = 4.3 Hz, 1H), 4.47 (dd, ^3J (H,H) = 7.9 Hz, ^3J (H,H) = 4.9 Hz, 1H), 4.28 (dd, ^3J (H,H) = 7.9 Hz, ^3J (H,H) = 4.5 Hz, 1H), 3.82 (s, 3H), 3.42 (dd, ^3J (H,H) = 14.0 Hz, ^3J (H,H) = 4.4 Hz, 1H), 3.32 (s, 12H), 3.23-3.13 (m, 6H), 3.03 (t, ^3J (H,H) = 7.1 Hz, 2H), 2.91 (dd, ^2J (H,H) = 12.7 Hz, ^3J (H,H) = 4.9 Hz, 1H), 2.69 (d, ^2J (H,H) = 12.7 Hz, 1H), 2.62 (t, ^3J (H,H) = 7.0 Hz, 2H), 2.17 (t, ^3J (H,H) = 7.2 Hz, 2H), 1.75-1.29 (m, 14H);); ^{13}C NMR (125 MHz, CD_3OD): 175.9 (C), 173.4 (C), 172.5 (C), 168.3 (C), 167.3 (C), 166.1 (C), 160.6 (C), 159.1 (C), 159.0 (C), 138.6 (C), 137.0 (C), 132.9 (C), 132.6 (CH), 132.0 (CH), 131.9 (CH), 131.6 (CH), 115.6 (CH), 114.7 (C), 97.5 (CH), 63.4 (CH), 61.2 (CH), 57.0 (CH), 53.9 (CH), 53.2 (CH_3), 41.1 (CH_2), 41.0 (CH_3), 40.4 (CH_2), 40.3 (CH_2), 40.2 (CH_2), 36.8 (CH_2), 36.6 (CH_2), 35.3 (CH_2), 30.4 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 27.6 (2 CH_2), 27.0 (CH_2); LC-MS: R_t = 2.12 min, *m/z* = 489 (100, $[M+H]^{2+}$).

3. Polymerization

3.1. General procedure for polymerization and purification

The procedures described in ref. S3 were followed to obtain each polymer. Stock solutions of propagator **6** (400 mM in MeOH), initiator **5** (100 mM in MeOH) and terminator (iodoacetamide **7**, 500 mM in water) were freshly prepared and fluxed with N_2 gas. Appropriate amounts of propagator were transferred to an Eppendorf tube and dried *in vacuo*. TEOA buffer (1 M, pH = 7.0) and the solution of initiator were added, yielding the desired final concentration of the reaction mixture (200 mM of propagator and 5 mM of initiator). The sample was kept at 25 °C with vigorous agitation. After the required polymerization time, the reaction mixture was quenched upon addition of the terminator solution (333 mM final concentration of terminator **7**). The obtained mixture was purified within a day by Sephadex G-10 gel-filtration resin. After termination, the polymerization mixture was loaded on a column containing 1.0 mL of swollen resin and eluted using bidistilled water. Fractions of 1 drop were collected and tested with analytical injection in GPC to detect polymer presence. The fractions containing the polymer were then collected and the quantification of the polymer was achieved by UV-Vis measurements, using calibration curves based on initiator **5**. The polymer concentration of the obtained stock solutions were ranging

between 300 and 800 μM. The molecular weight of each polymer was determined by analytical injection in GPC upon conjugation with streptavidin (*vide infra*), using molecular weight standards for calibration. The obtained stock solutions of the polymers were stored at -20 °C.

3.2. Conjugation with streptavidin

Stock solutions of streptavidin **1** (~ 30 μM) and polymer **2** (~ 800 μM) were freshly prepared in water. Stock solutions of fluorescent compound **3**, initiator **5** and bioreducible fluorescent compound **13** were freshly prepared in DMSO, with a concentration ranging from 0.5 to 3 mM. Conjugation with streptavidin was achieved directly mixing the protein stock solution with the desired amount of biotinylated compound solutions. The resulting solution was kept at 25 °C with vigorous agitation for 2.5 h before being used. Substoichiometric amount of biotinylated compounds were used, in order to avoid the presence of unbounded biotinylated compound in solution. The resulting streptavidin adducts were used directly without any further purifications.

Octamer **4**: 50.0 μL of streptavidin solution (2 mg/mL) were mixed with 1.75 μL of compound **3** (1.94 mM) and 3.96 μL of polymer **2** (861.3 μM) solutions. The resulting reaction mixture was kept at 25 °C for 2.5 h.

Octamer **8**: 50.0 μL of streptavidin solution (2 mg/mL) were mixed with 7.70 μL of polymer **2** (861.3 μM) solution. The resulting reaction mixture was kept at 25 °C for 2.5 h.

Octamer **9**: 50.0 μL of streptavidin solution (2 mg/mL) were mixed with 2.38 μL of initiator **5** (2.78 mM) solution. The resulting reaction mixture was kept at 25 °C for 2.5 h.

Octamer **10**: 50.0 μL of streptavidin solution (2 mg/mL) were mixed with 3.42 μL of compound **3** (1.94 mM) solution. The resulting reaction mixture was kept at 25 °C for 2.5 h.

Octamer **14**: 50.0 μL of streptavidin solution (2 mg/mL) were mixed with 7.38 μL of compound **13** (0.46 mM) and 3.96 μL of polymer **2** (861.3 μM) solutions. The resulting reaction mixture was kept at 25 °C for 2.5 h.

3.3. Polymer Characterization

Molecular weight of polymer **2** was determined indirectly upon conjugation with streptavidin. Analytical injections in GPC, equipped with a Superdex 200 Increase 3.2/300 column having molecular weight cutoff between 10-600 kDa, were performed. Commercial molecular weight

standards were used for calibration. After formation of octamer **8**, 10 µL of the reaction mixture were analyzed by GPC. The M_w of polymer **2** was calculated using equation Eq S1:

$$M_{w,\,2} = (M_{w,\,8} - M_{w,\,9}) / 4 \qquad \text{(Eq S1)}$$

Where $M_{w\ oct\ 8}$ is the weight average molecular weight obtained for octamer **8** (streptavidin-polymer **2** adduct) and $M_{w\ oct\ 9}$ is the weight average molecular weight obtained for octamer **9** (streptavidin-initiator **5** adduct). The obtained molecular weight for polymer **2** are perfectly in line with previous published results.[S2]

Table S1. GPC data for **2**, **8** and **9**.

entry	polymerization time (min)	$M_{w,\,8}$ [a] (kDa)	$M_{w,\,9}$ [a] (kDa)	ΔM_w [b] (kDa)	$M_{w\ pol\ 2}$ [c] (kDa)
1	30	119.5	82.0	37.5	9.4
2	60	138.4	82.0	56.4	14.1

a) Molecular weight obtained from analytical GPC. Superdex 200 Increase 3.2/300, flow 0.075 ml/min, eluent: 30% ACN in 0.1 M acetate buffer pH = 6.5. b) Difference in molecular weight between **8** and **9**. c) Molecular weight for **2**, calculated according to equation Eq. S1.

4. Cellular uptake experiments

4.1. Cell culture

Human cervical cancer-derived HeLa Kyoto cells were cultured in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), 1% penicillin / streptomycin (PS) and 1% L-glutamine. The cells were grown on a 25 cm^3 tissue culture flask (TPD corporation) at 37 °C under 5% CO_2. Human breast cancer-derived MCF-7 cells were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin / streptomycin (PS) and 1% L-glutamine. The cells were grown on a 25 cm^3 tissue culture flask (TPD corporation) at 37 °C under 5% CO_2.

4.2. Confocal microscopy

HeLa Kyoto cells were seeded at 6×10^4 cells/well on 35 mm glass-bottomed dishes (MatTek Corporation) and cultured overnight. After removing the medium, the cells were washed twice with PBS and treated with 1 mL of streptavidin adduct solution (500 nM in Leibovitz's medium). The cells were incubated for 1 h at 37 °C, then the media was removed by aspiration. Cells were washed 3 times with PBS and kept in Leibovitz's medium. Distribution of fluorescent compounds was analyzed without fixing using a confocal laser scanning microscope (Leica SP5) equipped with 63×

oil immersion objective lens. Ar laser was used as light source (10-15% laser power) with excitation wavelength 488 nm and emission 498 ~ 535 nm for CF–labelled compounds (Leica HyD™ detector); DPSS laser (5-10% laser power) with excitation 561 nm and emission 571 ~ 650 nm for rhodamine-labelled compounds (Leica HyD™ detector). During CLSM analysis the samples were kept at 37 °C.

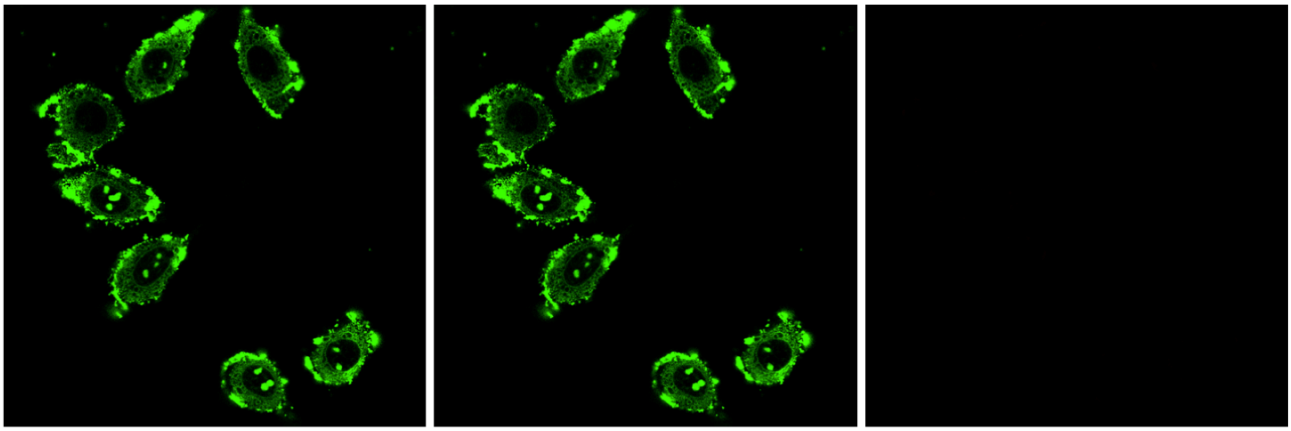


Figure S1. CLSM images of HeLa Kyoto cells after 1 h incubation with 500 nM octamer **8** at 37 °C in Leibovitz's medium, detected by excitation of fluorescein (left) and rhodamine (right). The merged images are displayed in the middle.

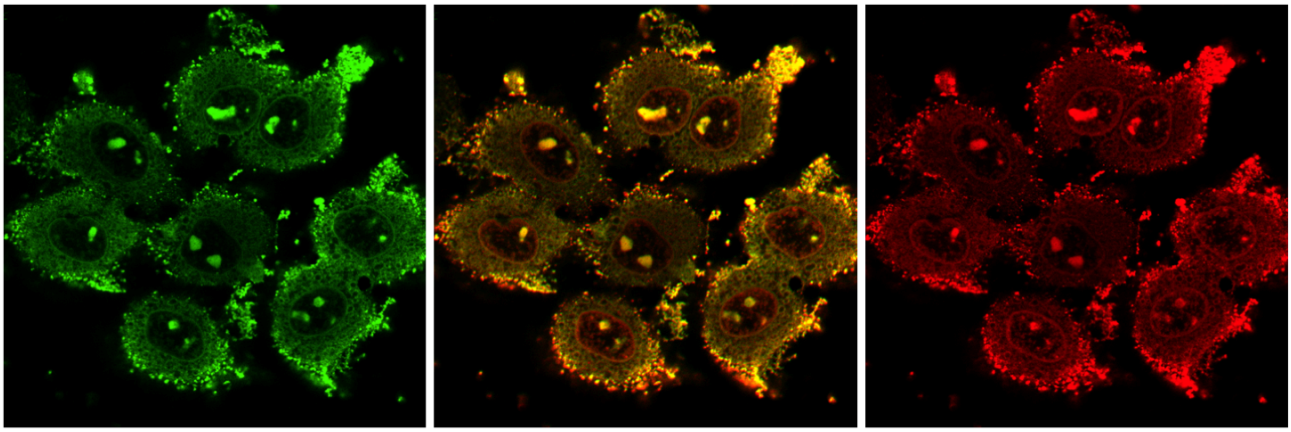


Figure S2. CLSM images of HeLa Kyoto cells after 1 h incubation with 500 nM octamer **14** at 37 °C in Leibovitz's medium, detected by excitation of fluorescein (left) and rhodamine (right). The merged images are displayed in the middle.

4.3. Flow cytometry

HeLa Kyoto cells were seeded at 1×10^5 cells/well in a 6-well plate (BD Falcon) and cultured for 24 h in growing medium. MCF-7 cells were seeded at 6×10^5 cells/well in a 6-well plate (BD Falcon) and cultured for 24 h in growing medium. After removing the medium, the cells were washed twice with PBS and treated with 1 mL of desired streptavidin adduct solution (500 nM in

Leibovitz's medium). The cells were incubated for 1 h at 37 °C, then the media was removed by aspiration. Cells were washed 3 times with cold PBS before detachment by treatment with 1 mL 0.05% trypsin-EDTA at 37 °C for 10 min. The cells were collected and pelleted by centrifugation at 1400 × *g* for 2 min at 4 °C. The supernatant was removed and the cells were washed twice with cold PBS (500 µL). The cells were re-suspended in 500 µL PBS containing 1 µL/mL Sytox Red and 0.02% EDTA. Fluorescent signals in cells were detected by laser excitation at 488 nm (detection at 525±40 nm for CF derivatives and 620±30 nm for rhodamine derivatives) and 635 nm (detection at 669±20 nm, at least 10000 events of live cells were collected) on a Beckman Coulter Gallios cytometer. Cells staining positive for Sytox Red (SR) were excluded from analysis.

Table S2. Flow cytometry data for HeLa Kyoto cells.

entry	compound[a]	emission intensity CF excitation[c] (a. u.)	emission intensity rhodamine excitation[d] (a. u.)
1	untreated[b]	0.26 ± 0.01	3.2 ± 0.1
2	**2**	14.2 ± 0.9	7.9 ± 0.5
3	**4**	7 ± 1	425 ± 47
4	**8**	78 ± 9	23 ± 2
5	**9**	0.39 ± 0.03	2.5 ± 0.2
6	**10**	0.03 ± 0.01	8 ± 1

a) See main text. b) Emission intensity of untreated HeLa Kyoto cells in flow cytometer experimental conditions. c) Emission intensity of HeLa Kyoto cells after incubation for 1 h at 37 °C at 500 nM streptavidin in Leibovitz's media, detected at 525 nm by laser excitation at 488 nm (at least 10000 events of live cells were collected). Values are means ± standard deviations of at least 3 experiments. d) Emission intensity of HeLa Kyoto cells after incubation for 1 h at 37 °C at 500 nM streptavidin in Leibovitz's media, detected at 620 nm by laser excitation at 488 nm (at least 10000 events of live cells were collected). Values are means ± standard deviations of at least 3 experiments.

Table S3. Flow cytometry data for MCF-7 cells.

entry	compound[a]	emission intensity CF excitation[c] (a. u.)	emission intensity rhodamine excitation[d] (a. u.)
1	Untreated[b]	0.8 ± 0.5	1.4 ± 0.5
2	**2**	5 ± 2	4.2 ± 0.7
3	**4**	2 ± 1	89 ± 13
4	**8**	19 ± 8	6 ± 2

a) See main text. b) Emission intensity of untreated HeLa Kyoto cells in flow cytometer experimental conditions. c) Emission intensity of HeLa Kyoto cells after incubation for 1 h at 37 °C at 500 nM streptavidin in Leibovitz's media, detected at 525 nm by laser excitation at 488 nm (at

least 10000 events of live cells were collected). Values are means ± standard deviations of at least 3 experiments. d) Emission intensity of HeLa Kyoto cells after incubation for 1 h at 37 °C at 500 nM streptavidin in Leibovitz's media, detected at 620 nm by laser excitation at 488 nm (at least 10000 events of live cells were collected). Values are means ± standard deviations of at least 3 experiments.

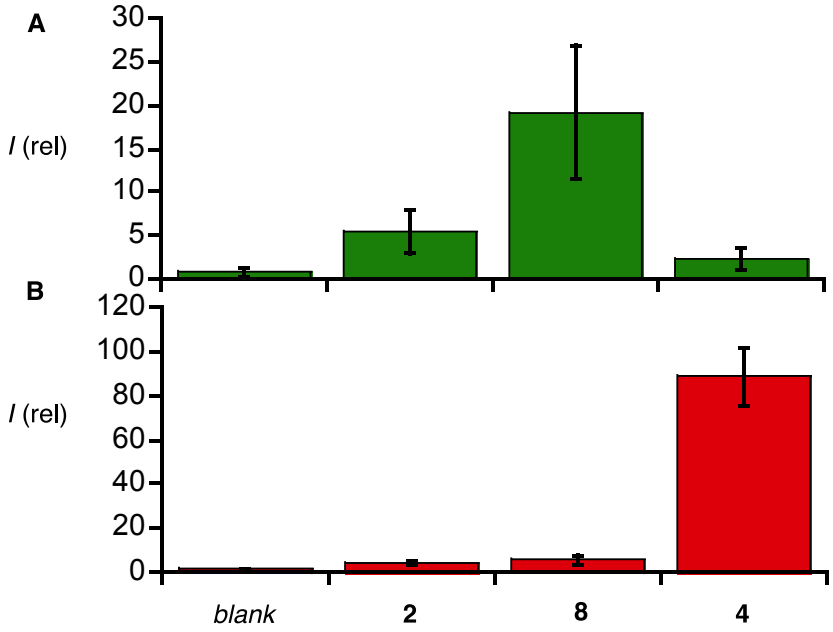


Figure S3. Flow cytometry results for uptake into MCF-7 cells detected by excitation of fluorescein (A) and rhodamine (B).

5. References

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S2 X. Jiang, M. Ahmed, Z. Deng and R. Narain, *Bioconjugate Chem.*, 2009, **20**, 994-1001.

S3 E.-K. Bang, G. Gasparini, G. Molinard, A. Roux, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2013, **135**, 2088-2091.

S4 G. Gasparini, E.-K. Bang, G. Molinard, D. V. Tulumello, S. Ward, S. O. Kelley, A. Roux, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2014, **136**, 6069-6074.

6. NMR spectra

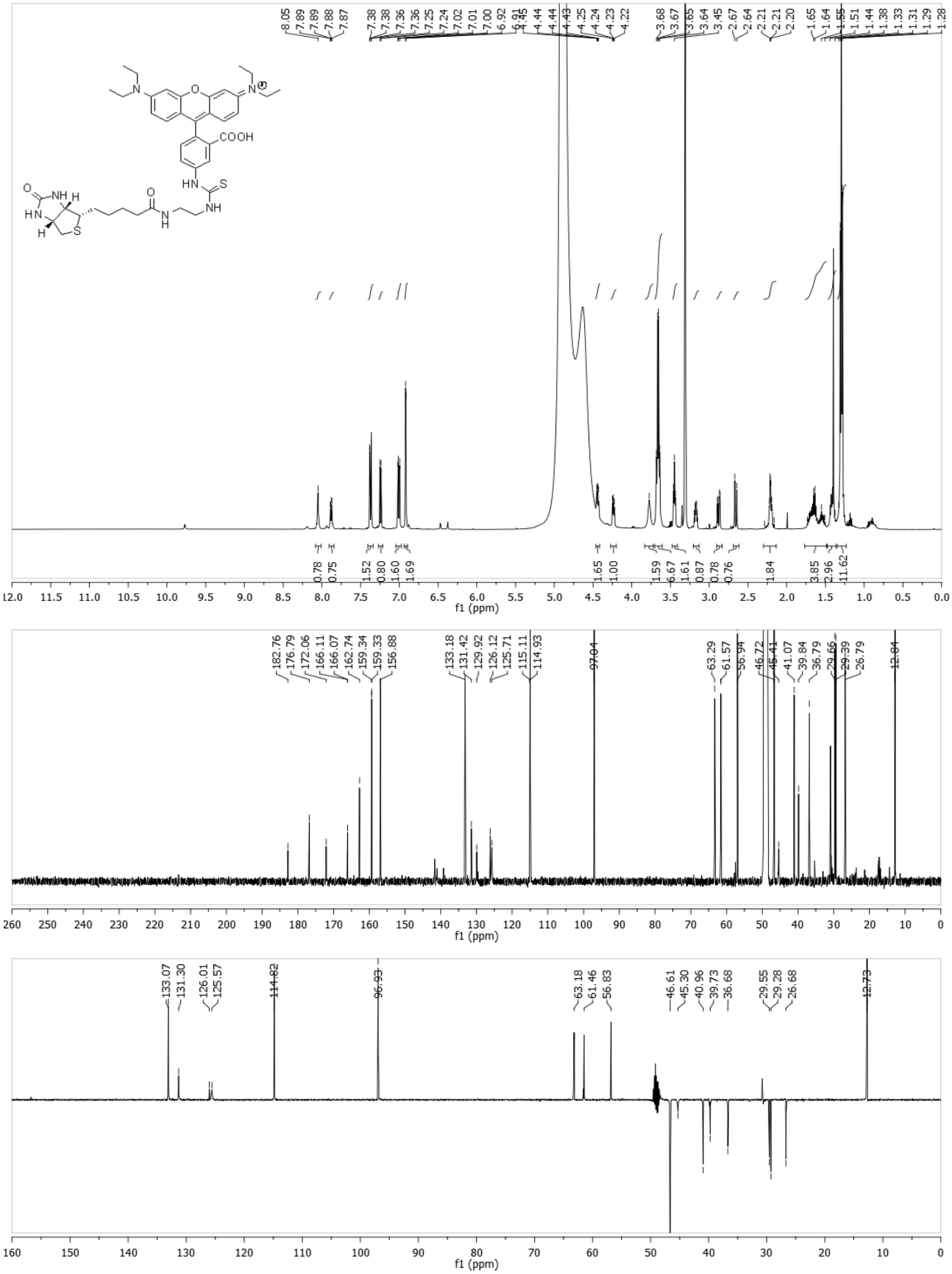

Figure S4. ^{1}H NMR, ^{13}C NMR and DEPT spectra of **3** (isomer 1) in CD_3OD.

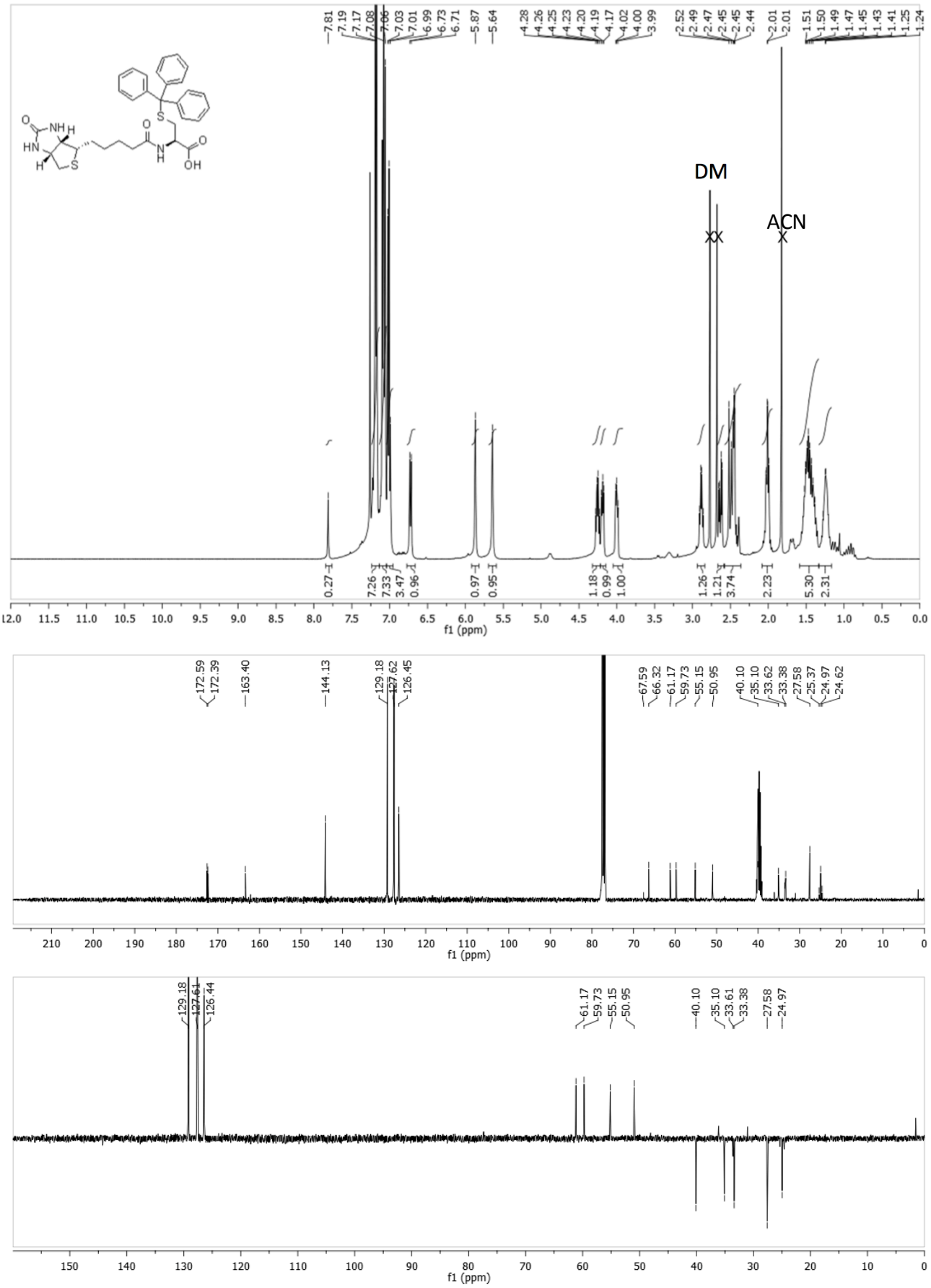

Figure S5. ^{1}H NMR, ^{13}C NMR and DEPT spectra of **17** in $CDCl_3/CD_3OD$.

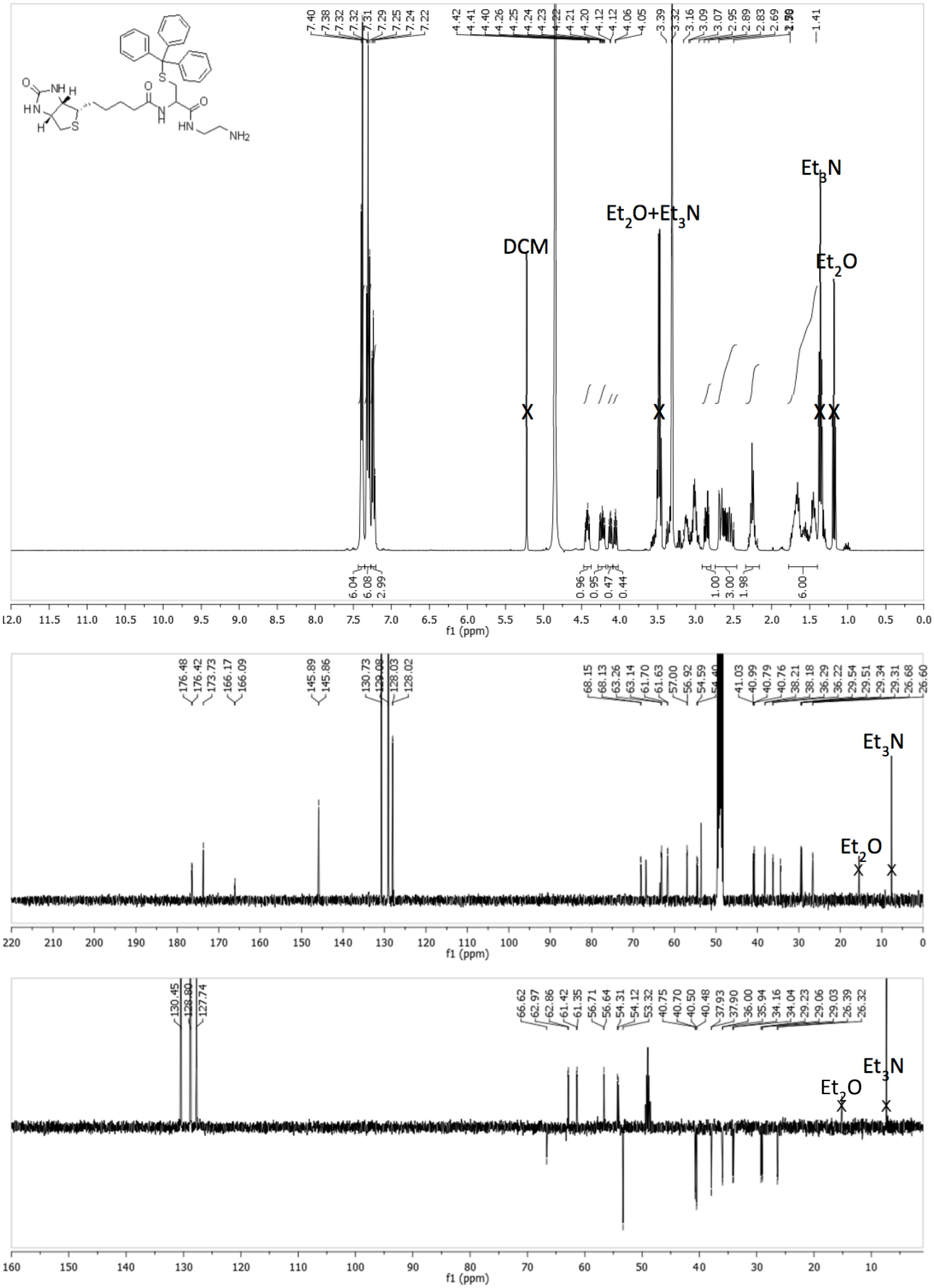

Figure S6. ^{1}H NMR, ^{13}C NMR and DEPT spectra of **18** in CD_3OD (mixture of epimers).

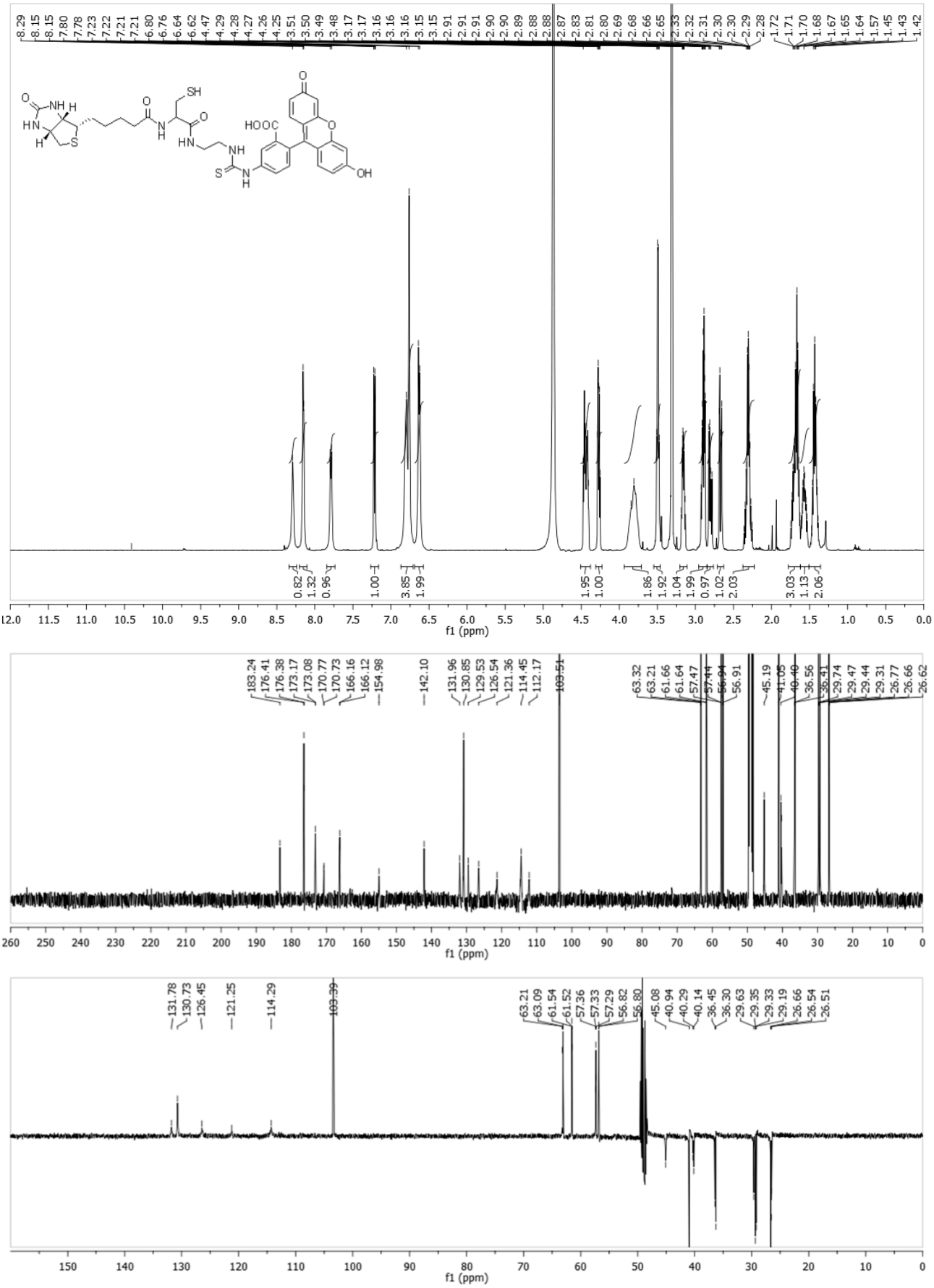

Figure S7. ^{1}H NMR, ^{13}C NMR and DEPT spectra of **5** in CD_3OD (mixture of epimers).

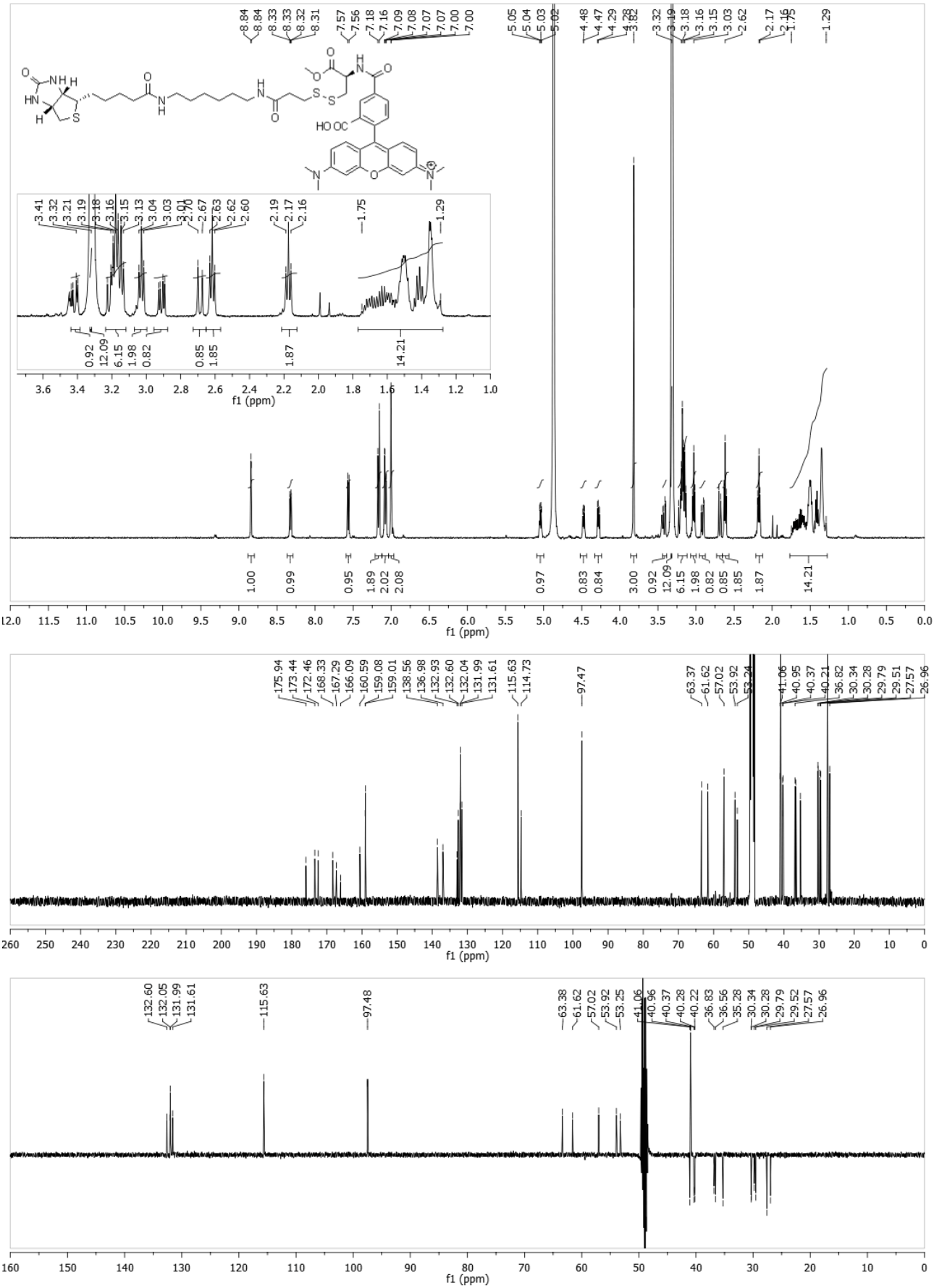

Figure S8. ^{1}H NMR, ^{13}C NMR and DEPT spectra of **13** in CD_3OD.