Anion-π Enzymes

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Supplementary Information

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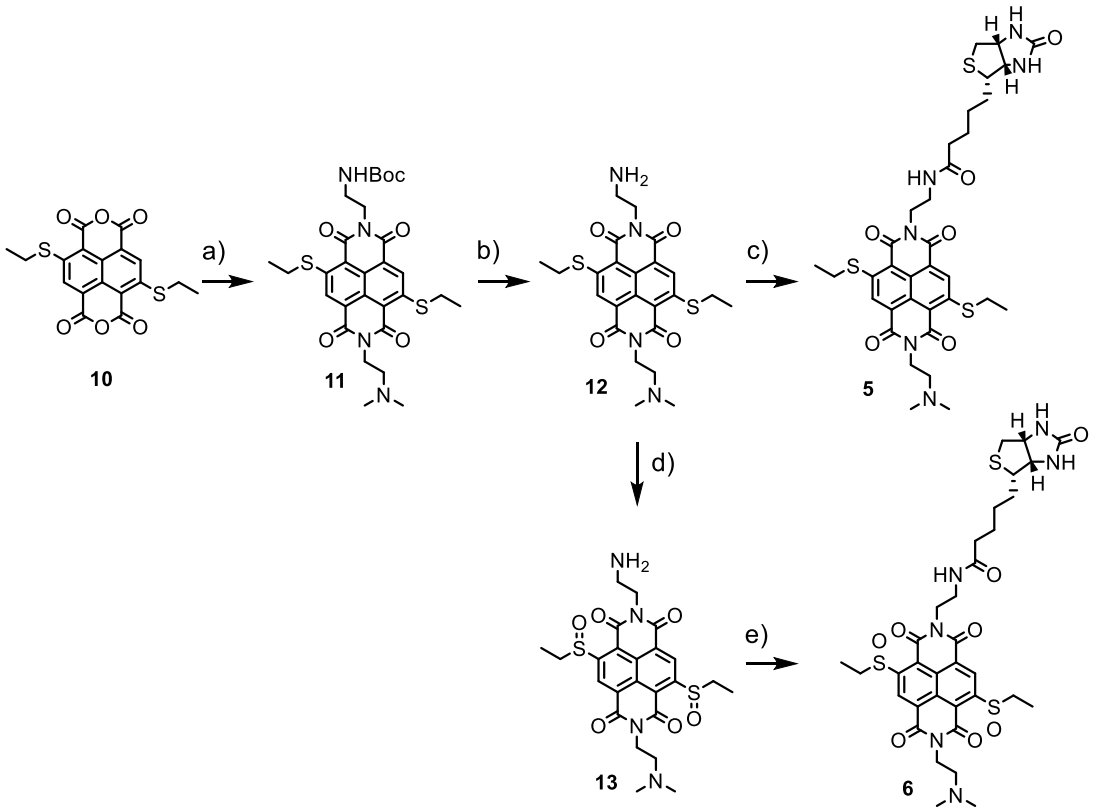
1. Materials and Methods

As in references S1-S3. Reagents for synthesis were purchased from Sigma-Aldrich, Fluka, Acros, Apollo Scientific and Bachem. Unless stated otherwise, column chromatography was carried out on silica gel 60 (SiliaFlash P60, 40-63 µm). Analytical (TLC) and preparative thin layer chromatography (PTLC) were performed on silica gel 60 (Merck, 0.2 mm) and silica gel GF (SiliCycle, 1 mm), respectively. Chiral HPLC were performed on a LC-4000 from JASCO. 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% CH_3CN in 0.1 M acetate buffer pH = 6.5). Melting points (Mp) were measured on a Melting Point M-565 (BUCHI). Circular dichroism spectra were obtained using JASCO J-815 spectropolarimeter and are reported as extremum wavelength λ in nm ($\Delta\varepsilon$ in $M^{-1}cm^{-1}$). pH Values were measured with a Consort C832 multi-parameter analyser equipped with a VWR glass membrane pH electrode calibrated with Tritisol 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, unless stated) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak). ^{1}H and ^{13}C NMR 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 ($\delta = 0$). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t) and quartet (q) with coupling constants (*J*) given in Hz, or multiplet (m). Broad peaks are marked as br. ^{1}H and ^{13}C resonances were assigned with the aid of additional information from 1D and 2D NMR spectra (H,H-COSY, DEPT 135, HSQC and HMBC). ESI-MS were performed on a ESI API 150EX and are reported as *m/z* (%). Accurate mass determinations

using ESI (HR ESI-MS) were performed on a Sciex QSTAR Pulsar mass spectrometer.

Abbreviations. Boc: *tert*-Butoxycarbonyl; DIPEA: Diisopropylethylamine; DMF: *N*,*N*-Dimethylformamide; HCTU: *O*-(6-Chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; *m*CPBA: 3-Chloroperbenzoic acid; rt: Room temperature; TEA: Triethylamine; TFA: Trifluoroacetic acid.

2. Synthesis



Scheme S1. (a) **10**, *N,N*-Dimethylethylenediamine, Boc-ethylenediamine, DMF, Et_3N, 180 °C, 18 h, 34%; (b) TFA, CH_2Cl_2, rt, 2 h, quantitative; (c) biotin, HCTU, DMF, rt, 18 h, 42%; (d) *m*CPBA, CH_2Cl_2, 0 °C, 30 min, 82%; (e) biotin, HCTU, DMF, rt, 18 h, 52%.

Compound 10. This compound was prepared following the literature procedure.[S4]

Compound 11. To a solution of **10** (400 mg, 1.03 mmol) in DMF (20 mL) was added *N,N*-dimethylethylenediamine (165 µL, 1.03 mmol), Boc-ethylenediamine (113 µL, 1.03 mmol) and Et_3N (280 µL). The solution was heated at 180 °C in sealed tube for 18 h. The solvents were removed *in vacuo*. Purification of the residue using silica gel column chromatography (CH_2Cl_2/MeOH 99:1) gave **11** (208 mg, 34%) as a red solid. R_f (CH_2Cl_2/MeOH 95:5): 0.11; Mp: 153 – 155 ºC; IR (neat): 1689 (m), 1647 (s), 1535 (w), 1446 (m), 1371 (w), 1321 (m), 1242 (m), 1207 (m), 1190 (m), 1162 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 8.47 (s, 2H), 5.04 (t, $^3J_{H\text{-}H}$ = 6.0 Hz, NH, 1H) 4.34 – 4.31 (m, 4H), 3.56 – 3.49 (m, 2H), 3.19 (q, $^3J_{H\text{-}H}$ = 7.2 Hz, 4H), 2.65 (t, $^3J_{H\text{-}H}$ = 6.8 Hz, 2H), 2.34 (s, 6H), 1.54 (t, $^3J_{H\text{-}H}$ = 7.2 Hz, 6H), 1.27 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): 163.3 (C), 163.1 (C), 162.4 (C), 162.1 (C), 156.1 (C), 148.7 (C), 148.5 (C), 127.9 (CH), 124.6 (C), 124.5 (C), 123.3 (C), 118.6 (C), 118.5 (C), 79.2 (C), 56.7 (CH_2), 45.7 (CH_3), 40.8 (CH_2), 39.2 (CH_2), 38.7 (CH_2), 29.7 (CH_2), 28.3 (CH_3), 28.2 (CH_3), 26.4 (CH_2), 12.8 (CH_3); MS (ESI, +ve): 601 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{29}H_{36}N_4O_6S_2$ ($[M+H]^+$): 601.2149, found: 601.2137.

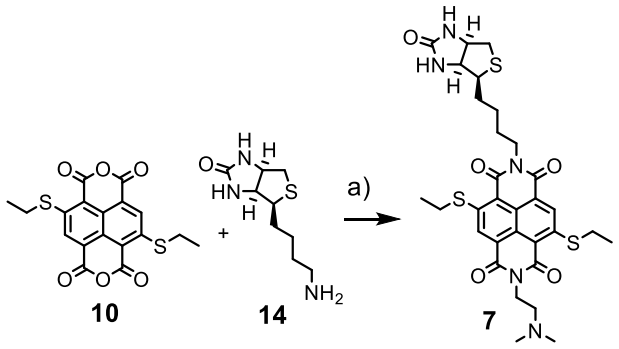
Compound 12. To a solution of compound **11** (90 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) was added TFA (1 mL) at room temperature. The reaction was stirred for 2 h, then the pH was adjusted to 9 with sat. $NaHCO_3$ aqueous solution. The aqueous phase was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and evaporated *in vacuo* to afford compound **12** (72 mg, quantitative) as a red solid. Mp: decomp. >188 °C; IR (neat): 2926 (w), 1693 (m), 1649 (s), 1544 (w), 1443 (m), 1316 (m), 1240 (m), 1104 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 8.65 (s, 2H), 4.36 – 4.31 (m, 4H), 3.24 (q, $^3J_{H\text{-}H}$ = 7.2 Hz, 4H), 3.12 (t, $^3J_{H\text{-}H}$ = 6.4 Hz, 2H), 2.71 (t, $^3J_{H\text{-}H}$ = 6.4 Hz, 2H), 2.37 (t, 6H), 1.56 (t, $^3J_{H\text{-}H}$ = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): 163.6 (C), 163.4 (C), 162.7 (C), 162.4 (C), 148.7 (C), 148.6 (C),

128.2 (CH), 125.0 (C), 123.8 (C), 123.6 (C), 119.1 (C), 118.9 (C), 56.8 (CH_2), 45.7 (CH_3), 43.5 (CH_2), 40.3 (CH_2), 38.7 (CH_2), 29.7 (CH_2), 26.4 (CH_2), 12.9 (CH_3); MS (ESI, +ve): 501 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{24}H_{28}N_4O_4S_2$ ($[M+H]^+$): 501.1625, found: 501.1617.

Compound 5. To a solution of biotin (160 mg, 0.656 mmol) in DMF (10 mL) was added a solution of **12** (65 mg, 0.13 mmol) in DMF (5 mL), followed by HCTU (400 mg, 0.968 mmol) and DIPEA (340 μL). The reaction was stirred at rt for 18 h. The solvents were removed *in vacuo*. Silica gel column chromatography (CH_2Cl_2/MeOH/TFA 90:10:0.1) gave compound **5** as a red oil. To a solution of the obtained product in MeOH/TFA 90:1 was added Et_2O to cause precipitation of TFA salt **5** (46 mg, 42%) as a red solid. R_f (CH_2Cl_2/MeOH/TFA 90:10:0.1): 0.14; Mp: 176 – 177 ºC; CD ($CHCl_3$): 294 (-0.35), 279 (+ 0.34); IR (neat): 1691 (s), 1647 (s), 1547 (w), 1445 (s), 1372 (w), 1321 (m), 1243 (m), 1202 (m); ^{1}H NMR (400 MHz, DMSO-d_6/TFA-*d* 4:1): 8.46 (s, 1H), 8.45 (s, 1H), 4.41 (t, $^3J_{H\text{-}H}$ = 5.2 Hz, 2H), 4.34 (dd, $^3J_{H\text{-}H}$ = 4.8, 7.6 Hz, 1H), 4.20 – 4.13 (m, 2H), 4.08 (dd, $^3J_{H\text{-}H}$ = 4.8, 7.6 Hz, 1H), 3.47 – 3.41 (m, 4H), 3.22 – 3.13 (m, 4H), 3.00 – 2.95 (m, 1H), 2.90 (s, 6H), 2.75 (dd, $^3J_{H\text{-}H}$ = 4.8, 7.6 Hz, 1H), 2.59 (d, $^3J_{H\text{-}H}$ = 7.6 Hz, 1H), 1.94 (t, $^3J_{H\text{-}H}$ = 7.2 Hz, 2H), 1.52 – 1.39 (m, 8H), 1.36 – 1.28 (m, 6H), 1.17 – 1.00 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6/TFA-*d* 4:1): 173.9 (C), 164.3 (C), 163.9 (C), 163.7 (C), 163.3 (C), 162.8 (C), 148.4 (C), 148.0 (C), 128.0 (CH), 127.8 (CH), 125.3 (C), 124.6 (C), 124.2 (C), 119.8 (C), 119.3 (C), 62.3 (CH), 60.8 (CH), 56.0 (CH), 55.7 (CH_2), 43.6 (CH_3), 40.9 (CH_2), 40.4 (CH_2), 37.0 (CH_2), 36.4 (CH_2), 35.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 26.3 (CH_2), 25.9 (CH_2), 13.3 (CH_3); MS (ESI, +ve): 727 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{34}H_{42}N_6O_6S_3$ ($[M+H]^+$): 727.2401, found: 727.2404.

Compound 6. To a solution of **12** (170 mg, 0.339 mmol) in CH_2Cl_2 (10 mL) was added TFA (1 mL) and *m*CPBA (176 mg, 1.02 mmol) at 0 °C. The reaction mixture was stirred for 30 min before

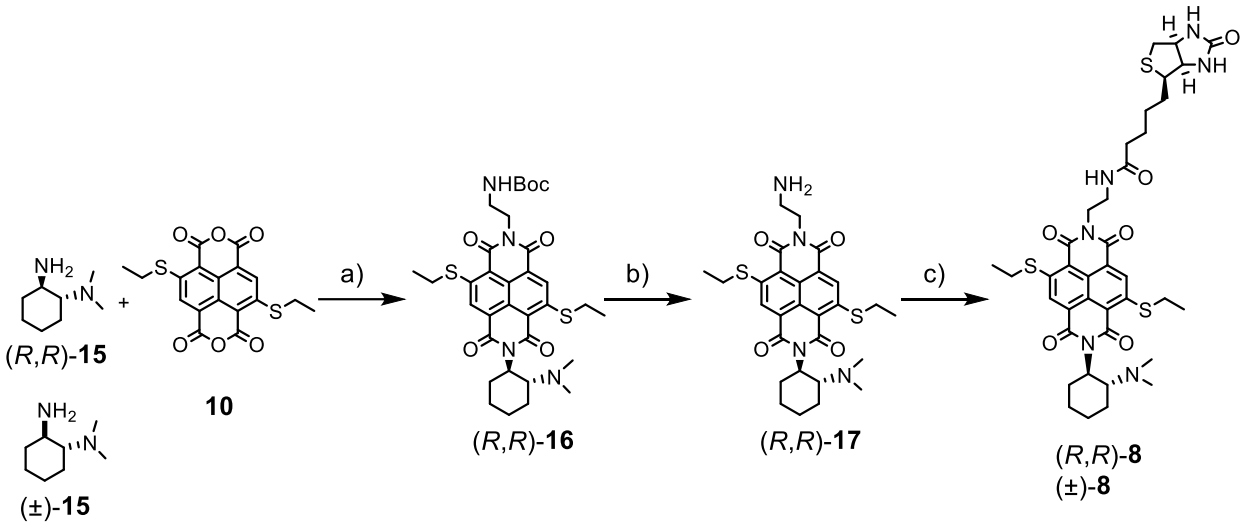
addition of Et_2O (50 mL) to precipitate the residue. The solid was filtered and washed with Et_2O, the residue was then solubilized in CH_2Cl_2 (5 mL) and sat. $NaHCO_3$ aqueous solution (2 mL) was added. The aqueous phase was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **13** (150 mg, 82%) which was directly used in next step without further purification. To a solution of biotin (276 mg, 1.30 mmol) in DMF (10 mL) was added a solution of **13** (120 mg, 0.226 mmol) in DMF (10 mL), followed by HCTU (660 mg, 1.60 mmol) and DIPEA (600 µL). The reaction was stirred at rt for 18 h. The solvents were removed *in vacuo*. Silica gel column chromatography (CH_2Cl_2/MeOH/TFA 85:15:0.1) gave TFA salt **6** (80 mg, 52%) as a yellow solid. R_f (CH_2Cl_2/MeOH/TFA 90:10:0.1): 0.07; Mp: 140 – 142 ºC; CD (MeOH): 429 (-0.37), 276 (+0.62), 253 (-0.82); IR (neat): 1655 (s), 1448 (m), 1311 (m), 1245 (w), 1197 (m), 1128 (m); ^{1}H NMR (400 MHz, DMSO-d_6/TFA-*d* 4:1): 9.23 – 9.20 (m, 2H), 4.50 – 4.42 (m, 2H), 4.37 – 4.32 (m, 1H), 4.25 – 4.10 (m, 3H), 3.59 – 3.48 (m, 3H), 3.40 – 3.32 (m, 3H), 3.06 – 2.95 (m, 3H), 2.93 – 2.90 (m, 6H), 2.82 – 2.76 (m, 1H), 2.60 – 2.57 (m, 1H), 1.95 – 1.86 (m, 2H), 1.56 – 1.41 (m, 1H), 1.37 – 1.27 (m, 3H), 1.21 – 1.13 (m, 11H); ^{13}C NMR (126 MHz, DMSO-d_6/TFA-*d*, n/n stereoisomeric peaks): 174.0 (C), 172.9 (C), 164.4/164.3 (C), 164.0/163.9 (C), 163.8 (C), 163.2/162.9 (C), 153.9 (C), 153.6/153.5 (C), 128.3/128.2 (CH), 127.4/127.3 (CH), 127.1/127.0 (CH), 124.4/124.0 (C), 62.5 (CH), 60.7 (CH), 56.1 (CH), 55.5 (CH_2), 55.3 (CH), 49.0 (CH), 48.6 (CH_2), 43.8/43.7 (CH_3), 43.4 (CH_3), 41.2 (CH_2), 36.9 (CH_2), 36.4 (CH_2), 36.0 (CH_2), 33.1 (CH_2), 32.1/32.0 (CH_2), 29.7/29.6 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 26.1 (CH_2), 25.6 (CH_2), 22.8 (CH_2), 21.2 (CH_2), 14.3 (CH_3), 7.1/7.0 (CH_3), 6.9 (CH_3); HRMS (ESI, +ve) calcd for $C_{34}H_{42}N_6O_8S_3$ ($[M+H]^+$): 759.2299, found: 759.2293.



Scheme S2. (a) **14**, *N,N*-dimethylethylenediamine, DMF, Et_3N, 180 °C, 20 h, 8.5%.

Compound 14. This compound was prepared following the literature procedure.[S5]

Compound 7. To a solution of **10** (235 mg, 0.606 mmol) in DMF (10 mL) was added *N,N*-dimethylethylenediamine (67 μL, 0.61 mmol) and **14** (177 mg, 0.822 mmol). The reaction mixture was heated at 180 °C in a sealed tube for 20 h. The solvent were removed *in vacuo*. Purification of the residue using silica gel column chromatography (CH_2Cl_2/MeOH/TFA 90:10:0.1) gave TFA salt **7** (40 mg, 8.5%) as a red oil. R_f (CH_2Cl_2/MeOH 90:10:0.1): 0.10; CD ($CHCl_3$): 296 (+1.10), 263 (-1.13); IR (neat): 1674 (s), 1650 (s), 1443 (w), 1200 (m), 1126 (s); ^{1}H NMR (300 MHz, $CDCl_3$/TFA-*d*): 8.69 (s, 1H), 8.65 (s, 1H), 4.70 – 4.62 (m, 3H), 4.50 – 4.44 (m, 1H), 4.31 – 4.23 (m, 2H), 3.68 – 3.63 (m, 2H), 3.30 – 3.20 (m, 5H), 3.13 (s, 6H), 3.02 – 2.96 (m, 1H), 2.85 – 2.82 (m, 1H), 1.95 – 1.69 (m, 4H), 1.58 – 1.46 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$/TFA-*d*): 165.1 (C), 163.7 (C), 163.4 (C), 163.3 (C), 162.9 (C), 150.1 (C), 149.5 (C), 138.7 (C), 128.8 (CH), 128.5 (CH), 125.0 (C), 123.8 (C), 122.8 (C), 119.2 (C), 62.5 (C), 61.1 (CH), 57.1 (CH_2), 55.2 (CH), 44.3 (CH_3), 40.5 (CH_2), 40.3 (CH_2), 35.6 (CH_2), 29.7 (CH_2), 27.7 (CH_2), 27.0 (CH_2), 26.2 (CH_2), 26.0 (CH_2), 14.1 (CH_3), 13.7 (CH_3), 12.5 (CH_3); MS (ESI, +ve): 656 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{31}H_{37}N_5O_5S_3$ ($[M+H]^+$): 656.2030, found: 656.2035.



Scheme S3. (a) (*R,R*)-**15**, Boc-ethylenediamine, DMF, Et_3N, 180 °C, 15 h, 39%; (b) TFA, CH_2Cl_2, rt, 2 h, 90%; (c) biotin, HCTU, DMF, rt, 18 h, 46%.

Compound (*R,R*)-**15**. This compound was prepared following the literature procedure.[S6]

Compound (*R,R*)-**16**. To a solution of **10** (200 mg, 0.515 mmol) in DMF (10 mL) was added (*R,R*)-**15** (73 mg, 0.51 mmol), Boc-ethylenediamine (83 µL, 0.51 mmol) and Et_3N (140 µL). The solution was heated at 180 °C in a sealed tube for 15 h. The solvents were removed *in vacuo*. Purification of the residue using silica gel column chromatography (CH_2Cl_2/MeOH 98:2) gave (*R,R*)-**16** (130 mg, 39%) as a red solid. R_f (CH_2Cl_2/MeOH 95:5): 0.17; Mp: 168 – 170 ºC; CD ($CHCl_3$): 297 (+0.83), 257 (-0.37); IR (neat): 1692 (s), 1643 (s), 1545 (w), 1514 (w), 1444 (m), 1366 (w), 1317 (m), 1240 (m), 1211 (m), 1165 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 8.68 – 8.65 (m, 2H), 5.12 – 5.01 (m, 1H), 4.88 (bs, NH, 1H), 4.39 (t, $^3J_{H\text{-}H}$ = 5.2 Hz, 2H), 3.77 – 3.64 (m, 1H), 3.54 (bs, 2H), 3.29 – 3.20 (m, 4H), 2.57 – 2.41 (m, 1H), 2.14 (s, 6H), 1.99 – 1.80 (m, 4H), 1.74 – 1.66 (m, 1H), 1.56 – 1.52 (m, 7H), 1.29 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): 163.9 (C), 163.0 (C), 156.2 (C), 148.8 (C), 128.5 (CH), 127.9 (CH), 125.2 (C), 124.4 (C), 119.8 (C), 79.4 (C), 62.0 (CH), 55.8

(CH_2), 40.6 (CH_3), 39.5 (CH_2), 29.6 (CH_2), 28.4 (CH_3), 26.6 (CH_2), 26.4 (CH_2), 25.4 (CH_2), 23.2 (CH_2), 13.1 (CH_3), 13.0 (CH_3); MS (ESI, +ve): 655 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{33}H_{42}N_4O_6S_2$ ($[M+H]^+$): 655.2619, found: 655.2623.

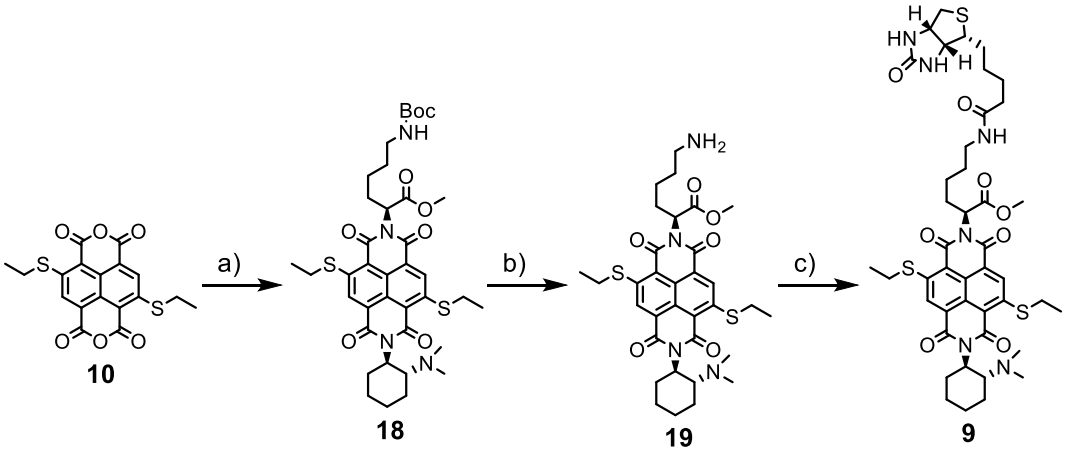
Compound (*R*,*R*)-**17**. To a solution of compound (*R*,*R*)-**16** (120 mg, 0.183 mmol) in CH_2Cl_2 (5 mL) was added TFA (1 mL) at room temperature. The reaction was stirred for 2 h, then the pH was adjusted to 9 with sat $NaHCO_3$ aqueous solution. The aqueous phase was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and evaporated *in vacuo* to afford compound (*R*,*R*)-**17** (90 mg, 90%) as a red solid. Mp: 163 – 164 ºC; CD (MeOH): 260 (-0.33); IR (neat): 1691 (s), 1641 (s), 1542 (w), 1514 (w), 1443 (m), 1320 (m), 1240 (m), 1213 (s), 1166 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 8.67 – 8.64 (m, 2H), 5.13 – 5.00 (m, 1H), 4.30 (t, $^3J_{H\text{-}H}$ = 6.4 Hz, 2H), 3.82 – 3.72 (m, 1H), 3.27 – 3.20 (m, 4H), 3.09 (t, $^3J_{H\text{-}H}$ = 6.4 Hz, 2H), 2.58 – 2.40 (m, 1H), 2.20 – 2.14 (m, 6H), 2.00 – 1.84 (m, 4H), 1.72 – 1.62 (m, 1H), 1.56 – 1.50 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$): 164.4 (C), 164.1 (C), 163.9 (C), 163.4 (C), 163.0 (C), 148.7 (C), 148.5 (C), 147.9 (C), 128.4 (CH), 127.8 (CH), 125.5 (C), 125.2 (C), 124.3 (C), 123.6 (C), 123.4 (C), 120.6 (C), 119.8 (C), 118.7 (C), 62.1 (CH), 53.6 (CH_2), 43.6 (CH_2), 40.5 (CH_2), 32.1 (CH_2), 31.1 (CH_3), 29.8 (CH_2), 29.5 (CH_2), 26.5 (CH_2), 23.4 (CH_2), 13.0 (CH_3), 12.9 (CH_3); MS (ESI, +ve): 555 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{28}H_{34}N_4O_4S_2$ ($[M+H]^+$): 555.2094, found: 555.2071.

Compound (*R*,*R*)-**8**. To a solution of biotin (190 mg, 0.779 mmol) in DMF (10 mL) was added a solution of (*R*,*R*)-**17** (85 mg, 0.15 mmol) in DMF (5 mL), followed by HCTU (523 mg, 1.27 mmol) and DIPEA (450 μL). The reaction was stirred at rt for 18 h. The solvents were removed *in vacuo*. Silica gel column chromatography (CH_2Cl_2/MeOH/TFA 93:7:0.1) gave compound(*R*,*R*)-**8** (100 mg) as a red oil. To a solution of the obtained product in MeOH/TFA 90:1 was added Et_2O to

cause precipitation of TFA salt (*R*,*R*)-**8** (62 mg, 46%) as a red solid. R_f (CH_2Cl_2MeOH 90:10:0.1): 0.37; Mp: 179 – 180 ºC; CD (MeOH): 538 (+0.27), 300 (+0.51), 260 (-1.73); IR (neat): 1690 (s), 1648 (s), 1546 (w), 1445 (m), 1370 (w), 1320 (w), 1243 (m), 1200 (m); ^{1}H NMR (400 MHz, DMSO-d_6/TFA-*d* 4:1): 8.56 – 8.52 (m, 2H), 7.94 (bs, NH, 2H), 5.26 – 5.15 (m, 1H), 4.46 – 4.38 (m, 1H), 4.33 – 4.30 (m, 1H), 4.21 – 4.13 (m, 2H), 4.12 – 4.06 (m, 1H), 3.45 – 3.40 (m, 2H), 3.26 – 3.19 (m, 4H), 3.02 – 2.95 (m, 1H), 2.80 – 2.75 (m, 4H), 2.69 (s, 3H), 2.58 (d, $^2J_{H\text{-}H}$ = 16 Hz, 1H), 2.43 – 2.32 (m, 1H), 2.23 – 2.18 (m, 1H), 1.95 – 1.87 (m, 4H), 1.80 – 1.76 (m, 1H), 1.62 – 1.46 (m, 2H), 1.43 – 1.37 (m, 7H), 1.37 – 1.30 (m, 3H), 1.22 – 1.14 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6/TFA-*d* 4:1):[S13] 173.6 (C), 165.1 (C), 164.7 (C), 164.0 (C), 163.8 (C), 162.8 (C), 148.3 (C), 147.8 (C), 147.7 (C), 128.4 (CH), 127.8 (CH), 127.6 (CH), 125.7 (C), 125.6 (C), 125.5 (C), 125.3 (C), 124.7 (C), 124.5 (C), 120.7 (C), 119.9 (C), 119.6 (C), 64.6 (CH_3), 62.1 (CH), 60.5 (CH), 56.0 (CH), 53.3 (CH), 52.7 (CH), 42.9 (CH_3), 40.8 (CH_2), 40.3 (CH_2), 36.9 (CH_2), 35.9 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 26.2 (CH_2), 26.1 (CH_2), 25.8 (CH_2), 25.1 (CH_2), 24.4 (CH_2), 23.6 (CH_2), 13.4 (CH_3), 13.3 (CH_3); MS (ESI, +ve): 781 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{38}H_{48}N_6O_6S_3$ ($[M+H]^+$): 781.2870, found: 781.2873.

Compound (±)-**8**. This compound was prepared following the procedure used for (*R*,*R*)-**8** but using *trans*-(±)-**15** instead of (*R*,*R*)-**15**. Mp: 181 – 182 ºC; ^{1}H NMR (500 MHz, DMSO-d_6/TFA-*d* 4:1): 8.55 – 8.52 (m, 2H), 5.23 – 5.16 (m, 1H), 4.43 – 4.32 (m, 2H), 4.20 – 4.07 (m, 3H), 3.45 – 3.38 (m, 2H), 3.25 – 3.16 (m, 4H), 3.01 – 2.81 (m, 2H), 2.77 – 2.75 (m, 4H), 2.68 – 2.64 (m, 3H), 2.62 – 2.55 (m, 1H), 2.43 – 2.35 (m, 1H), 2.20 – 2.15 (m, 1H), 1.94 – 1.85 (m, 4H), 1.80 – 1.75 (m, 1H), 1.66 – 1.47 (m, 3H), 1.44 – 1.37 (m, 9H), 1.35 – 1.28 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d_6/TFA-*d* 4:1):[S13] 173.9 (C), 165.2 (C), 164.8 (C), 164.3 (C), 164.2 (C), 164.1 (C), 163.8 (C), 162.9 (C), 162.4 (C), 148.4 (C), 147.9 (C), 147.8 (C), 128.5 (CH), 127.9 (CH), 127.7 (CH), 125.8 (C),

125.7 (C), 125.5 (C), 125.4 (C), 124.7 (C), 124.6 (C), 124.5 (C), 120.7 (C), 119.9 (C), 119.7 (C), 70.7 (CH), 64.8 (CH_3), 64.7 (CH_3), 62.2 (CH), 60.7 (CH), 59.0 (CH_2), 56.7 (CH), 56.0 (CH), 54.5 (CH), 53.9 (CH), 53.4 (CH), 52.8 (CH), 43.1 (CH), 43.0 (CH_3), 40.9 (CH_2), 37.0 (CH_2), 36.9 (CH_2), 36.9 (CH_2), 35.9 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.4 (CH_2), 26.2 (CH_2), 26.1 (CH_2), 25.9 (CH_2), 25.8 (CH_2), 25.7 (CH_2), 25.2 (CH_2), 24.4 (CH_2), 23.8 (CH_2), 23.7 (CH_2), 22.8 (CH_2), 18.5 (CH_2), 17.1 (CH_2), 14.3 (CH_3).



Scheme S4. (a) (*R,R*)-**15**, *N*ε-Boc-L-Lysine methyl ester, DMF, Et_3N, 180 °C, 15 h, 45%; (b) TFA, CH_2Cl_2, rt, 2 h, quantitative; (c) biotin, HCTU, DMF, rt, 15 h, 82%.

Compound (*R,R,S*)-**18**. To a solution of **10** (100 mg, 0.258 mmol) in DMF (5 mL) were added (*R,R*)-**15** (37 mg, 0.26 mmol), H-Lys(Boc)-OMe•HCl (76 mg, 0.26 mmol) and Et_3N (140 µL). The solution was heated at 180 °C in a sealed tube for 15 h. The solvents were removed *in vacuo*. Purification of the residue using silica gel column chromatography (CH_2Cl_2/MeOH 98:2) gave (*R,R,S*)-**18** (88 mg, 45%) as a red solid. R_f (CH_2Cl_2/MeOH 95:5): 0.22; Mp: 155 – 156 ºC; CD (MeOH): 530 (+0.82), 385 (+0.35), 294 (+3.35), 262 (-2.24); IR (neat): 1746 (m), 1692 (m), 1647 (s), 1546 (m), 1436 (m), 1316 (m), 1213 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 8.70 – 8.67 (m, 2H), 5.70

(dd, $^{3}J_{H\text{-}H}$ = 5.6, 8.8 Hz, 1H), 5.10 – 5.00 (m, 1H), 4.49 (bs, NH, 1H), 3.70 (s, 3H), 3.27 – 3.19 (m, 4H), 3.06 – 3.02 (m, 2H), 2.56 – 2.36 (m, 2H), 2.20 – 2.10 (m, 6H), 1.99 – 1.82 (m, 4H), 1.54 – 1.48 (m, 8H), 1.47 – 1.40 (m, 3H), 1.35 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$):[S13] 170.2 (C), 164.3 (C), 163.2 (C), 162.5 (C), 161.9 (C), 156.0 (C), 149.3 (C), 148.5 (C), 147.9 (C), 128.7 (CH), 128.5 (CH), 127.8 (CH), 125.4 (C), 124.5 (C), 123.3 (C), 123.1 (C), 120.8 (C), 120.0 (C), 118.4 (C), 79.1 (C), 63.2 (CH_2), 61.9 (CH), 56.3 (CH), 55.8 (CH), 53.7 (CH_3), 52.7 (CH_3), 40.6 (CH), 40.4 (CH_2), 32.9 (CH_2), 32.0 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 28.7 (CH_2), 28.5 (CH), 26.5 (CH_2), 25.9 (CH_2), 25.5 (CH_2), 23.9 (CH_2), 23.2 (CH_2), 13.0 (CH_3), 12.9 (CH_3); MS (ESI, +ve): 755 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{38}H_{50}N_4O_8S_2$ ($[M+H]^+$): 755.3143, found: 755.3144.

Compound (*R,R,S*)-**19**. To a solution of compound (*R,R,S*)-**18** (80 mg, 0.10 mmol) in CH_2Cl_2 (5 mL) was added TFA (1 mL) at room temperature. The reaction was stirred for 2 h, then the pH was adjusted to 9 with sat. $NaHCO_3$ aqueous solution. The aqueous phase was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and evaporated *in vacuo* to afford compound (*R,R,S*)-**19** (70 mg, quantitative) as a red solid. Mp: 232 – 234 ºC; CD (MeOH): 531 (+0.60), 296 (+1.81), 260 (-1.26); IR (neat): 2925 (m), 1746 (w), 1693 (s), 1649 (s), 1547 (m), 1438 (m), 1371 (w), 1317 (m), 1211 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 8.67 (s, 2H), 5.71 – 5.65 (m, 1H), 5.12 – 4.99 (m, 1H), 3.70 (s ,3H), 3.62 – 3.58 (m, 1H), 3.33 – 3.12 (m, 5H), 2.71 – 2.64 (m, 1H), 2.56 – 2.36 (m, 2H), 2.14 (s, 7H), 1.99 – 1.76 (m, 4H), 1.70 – 1.59 (m, 1H), 1.55 – 1.39 (m, 10H); ^{13}C NMR (101 MHz, $CDCl_3$):[S13] 170.3 (C), 164.3 (C), 164.0 (C), 163.2 (C), 162.5 (C), 149.3 (C), 148.5 (C), 147.9 (C), 128.7 (CH), 128.4 (CH), 127.8 (CH), 125.4 (C), 124.5 (C), 123.3 (C), 123.1 (C), 120.8 (C), 120.0 (C), 118.4 (C), 63.1 (CH_2), 62.0 (CH), 56.2 (CH), 55.7 (CH), 53.8 (CH_3), 53.5 (CH), 52.7 (CH_3), 41.6 (CH_2), 40.5 (CH_3), 39.6 (CH), 32.9 (CH_2), 32.5 (CH_2), 32.0 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 26.5 (CH_2), 26.5 (CH_2), 26.2 (CH_2), 25.9 (CH_2), 25.5 (CH_2), 25.3 (CH_2),

23.9 (CH_2), 23.6 (CH_2), 23.3 (CH_2), 22.8 (CH_2), 13.0 (CH_3), 12.9 (CH_3); MS (ESI, +ve): 655 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{33}H_{42}N_4O_6S_2$ ($[M+H]^+$): 655.2619, found: 655.2609.

Compound (*R*,*R*,*S*)-**9**. To a solution of biotin (107 mg, 0.439 mmol) in DMF (5 mL) was added a solution of (*R*,*R*,*S*)-**19** (65 mg, 0.10 mmol) in DMF (5 mL), followed by HCTU (300 mg, 0.726 mmol) and DIPEA (300 μL). The reaction was stirred at rt for 15 h. The solvents were removed *in vacuo*. Silica gel column chromatography (CH_2Cl_2/MeOH/TFA 93:7:0.1) gave TFA salt (*R*,*R*,*S*)-**9** (82 mg, 82%) as a red solid. R_f (CH_2Cl_2/MeOH/TFA 90:10:0.1): 0.39; Mp: 128 – 130 ºC; CD (MeOH): 538 (+0.35), 372 (+ 0.47), 260 (-2.40); IR (neat): 1649 (s), 1550 (w), 1438 (m), 1318 (m), 1203 (s), 1125 (m); ^{1}H NMR (400 MHz, DMSO-d_6/TFA-*d* 4:1): 8.60 – 8.57 (2H, m), 5.65 – 5.59 (m, 1H), 4.46 – 4.36 (m, 2H), 4.22 – 4.17 (m, 1H), 3.58 (s, 3H), 3.25 – 3.18 (m, 4H), 3.15 – 2.96 (m, 3H), 2.85 – 2.75 (m, 4H), 2.68 – 2.59 (m, 4H), 2.41 – 2.15 (m, 3H), 2.06 – 1.95 (m, 3H), 1.91 – 1.71 (m, 3H), 1.62 – 1.49 (m, 3H), 1.46 – 1.31 (m, 14H); ^{13}C NMR (126 MHz, DMSO-d_6/TFA-*d* 4:1):[S13] 172.7 (C), 170.3 (C), 164.8 (C), 164.4 (C), 163.8 (C), 163.7 (C), 163.7 (C), 163.0 (C), 162.9 (C), 162.3 (C), 162.2 (C), 148.6 (C), 148.6 (C), 148.1 (C), 147.6 (C), 128.6 (CH), 128.3 (CH), 127.5 (CH), 125.7 (C), 125.6 (C), 125.5 (C), 125.2 (C), 124.7 (C), 123.5 (C), 123.4 (C), 120.9 (C), 120.1 (C), 64.4 (CH), 64.3 (CH), 61.8 (CH), 60.1 (CH), 55.8 (CH_3), 53.8 (CH), 52.6 (CH), 52.5 (CH), 42.7 (CH_3), 38.6 (CH_2), 36.7 (CH_2), 35.5 (CH_2), 29.3 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 24.9 (CH_2), 24.8 (CH_2), 24.1 (CH_2), 23.9 (CH_2), 23.3 (CH_2), 13.2 (CH_3), 13.1 (CH_3), 13.0 (CH_3); MS (ESI, +ve): 881 (100, $[M+H]^+$); HRMS (ESI, +ve) calcd for $C_{43}H_{56}N_6O_8S_3$ ($[M+H]^+$): 881.3395, found: 881.3387.

3. Catalysis

Stock solutions of substrates **1** (40 mM), **2** (400 mM) and biotinylated catalysts **5-9** (2 mM) were prepared in CD_3CN. Solutions of substrates **1** should be freshly prepared otherwise decarboxylation product will be present.

Solutions were prepared by mixing successively streptavidin WT or mutants (100 µL, 2 mM, glycine buffer pH 3, 0.2 µmol), biotinylated ligands **5-9** (50 µL, 0.1 µmol), substrates **1** (25 µL, 1 µmol) and **2** (25 µL, 10 µmol) and stirred at 20 °C. After given time, ^{1}H NMR of the mixture, extracted with $CDCl_3$ (0.6 mL), dried over Na_2SO_4, and filtered into NMR tube, was recorded. Integrals associated with the protons alpha to the carbonyl group of the addition product **3b** (δ 3.01 ppm; d, 2H) were compared to the combined integration of all $-OCH_3$ protons present in the substrates and products (i.e., from δ 3.84–3.79 ppm) to obtain conversion of the reaction.

For lower catalysts loading (1%, 0.1%), solutions of streptavidin S112Y (1 mM) and **8** (0.5 mM) were prepared in glycine buffer (pH 3)/CD_3CN 1:1 and diluted 10 or 100 times, followed by substrates **1** (5 mM) and **2** (50 mM). The reactions were followed by ^{1}H NMR spectroscopy and run until completion (72 h at 1% loading, 3 weeks for 0.1% loading).

The spectroscopic data obtained for product **3** were identical to the ones reported in the literature.[S7] Crude mixtures were analyzed by chiral HPLC, (*i.e.*, column: CHIRALPAK ID column; mobile phase: *n*-Hexane/*i*-PrOH 60/40, 0.5 mL/min, rt; detection: 254 nm, Figure S2). To determine absolute configuration of the product **3**, chiral HPLC analysis was conducted under previously reported

conditions. The retention times found for the two enantiomers of **3** were comparable with the ones reported in the literature (Figure S3).[S7]

Inhibiton experiments were carried by preparing solutions of streptavidin S112Y (1 mM), **8** (0.5 mM) and inhibitor (0.01-1M) followed by **1** and **2**. Reactions were monitored by ^{1}H NMR spectroscopy. Inhibition concentration IC_{50} and Hill coefficients n were determined by plotting the *ee* after completion of the reaction as a function of NO_3^- concentration c and fitting them to the Hill equation (Eq S1)

$$Y = ee_0 + (ee_{min} - ee_0) / \{1 + (IC_{50} / c)^n\} \qquad \text{(Eq S1)}$$

where ee_0 is the *ee* without NO_3^-, ee_{min} is the *ee* at NO_3^- saturation, IC_{50} is the concentration of NO_3^- required to inhibit 50% of the decrease in stereoselectivity and n is the Hill coefficient.

Titration of substrate **1** (0.01 M, CH_3CN/H_2O 1:1) by NaOH (0.1 M) was followed by pH meter.

All docking simulations were performed with GOLD (version 5.4) using the graphical interface Hermes 1.8.0, with method similar to that described in *Robles et al.*[S8] Briefly, the method consisted in (i) docking of the catalyst covalently attached to the biotin in a biotin-loaded streptavidin crystal structure, (ii) reconstructing the entire cofactor with this conformation, and (iii) calculating the true interactions cofactor-host. First, the structure of the catalyst was generated using ChemBioDraw Ultra 14.0 and ChemBio3D Ultra 14.0: the biotin of cofactor **8** was deleted (up to the N_{amide} bound to biotin, to afford an NDI flanked with diaminocyclohexane). This structure was optimized using the energy minimization (MM2 force field method) tool of ChemBio3D Ultra 14.0. The structure of the receptor consists of a dimer of streptavidin generated from the PDB structure 3PK2.[S9] In this homotetrameric structure, streptavidin contains the biotinylated [Cp*Ir(Biot-*p*-L)Cl]. Using UCSF Chimera,[S10] two

facing monomers, the solvent molecules and the salts were deleted. In the resulting dimer, both [Cp*Ir(Biot-*p*-L)Cl] cofactors were deleted, except for one biotin (including the C_α atom which corresponds to the aromatic ipso-carbon from the spacer, Figure S8). During the docking procedure, a covalent restraint was set: the C_α atom of the linker of **8** is set to coincide with the C_α of the biotin in the streptavidin dimer. The 10 closest residues from the catalyst were kept flexible and can thus adopt a library of rotamers. These include the positions N49, N118, S112Y, K121 and L124 of both streptavidin monomers. From the resulting structure having the highest ChemScore, the coordinates of the atoms of both the biotin and the docked NDI are pooled in a single file, reconstructing the entire **8** with the minimized docked conformation. The interaction of this latter with the host (with rotamers selected from docking at the flexible positions) is then recalculated using the rescoring tool of GOLD 5.4 with ChemScore method.

The wild-type streptavidin and the various mutants screening herein were expressed and purified according to the previously reported protocol.[S11, S12]

4. Supplementary Figures and Tables

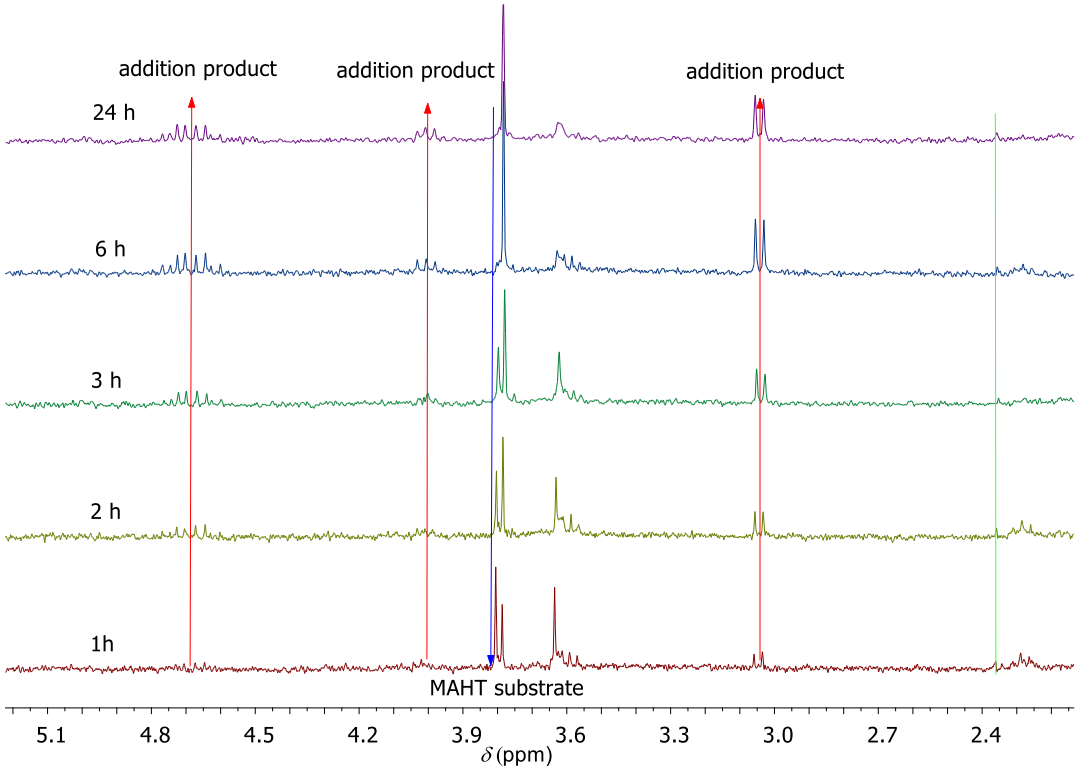


Figure S1. ^{1}H NMR spectra of a mixture of **1** (5 mM), **2** (50 mM) and artificial enzyme S112Y+**8** (10% mol) in glycine buffer/CD_3CN 1:1 at 20 °C extracted in $CDCl_3$. The red arrow shows the formation of product, the blue one shows the consumption of **1**, the green line shows where the decarboxylation product signal should be.

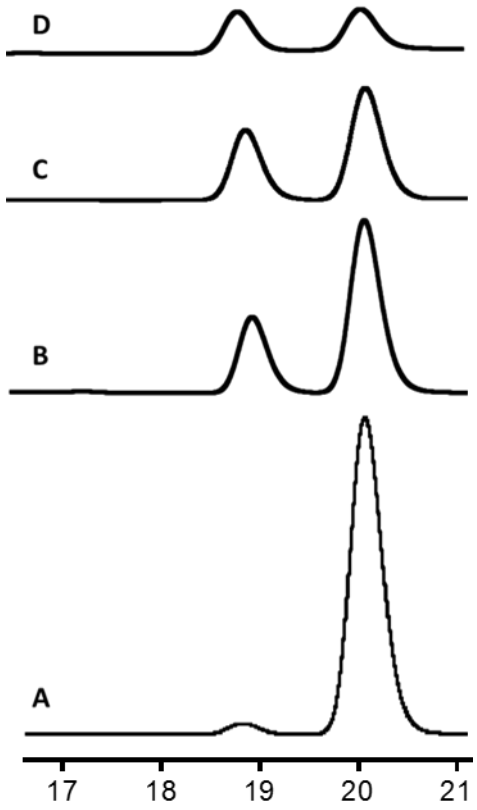


Figure S2. Chiral HPLC traces obtained using Chiralpak ID column (n-hexane/i-PrOH 60:40, rt, 0.5 mL/min, 254 nm) for crude mixture obtained with S112Y+**8** (A), WT+**8** (B), S112Y+**5** (C), K121F+**8** (D).

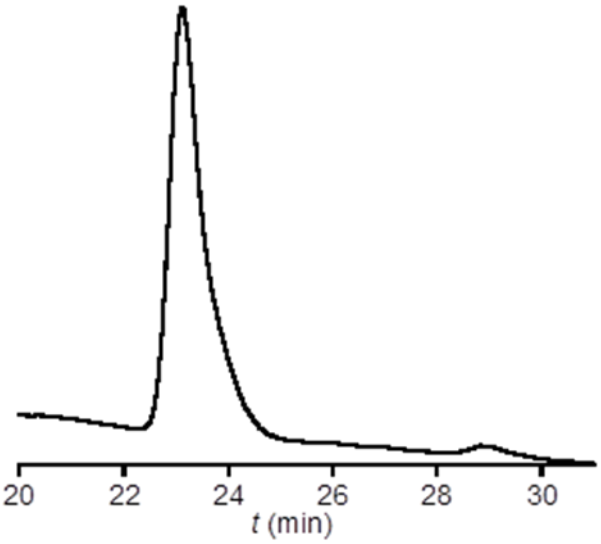


Figure S3. Chiral HPLC chromatogram obtained for product **3** using Chiralcel OD-H column (n-hexane/i-PrOH 1:1, rt, 0.5 mL/min, 254 nm) for crude mixture obtained with streptavidin S112Y+**8**. The retention times are in agreement with previously reported data: t_R: (*S*) = 23.2 min, (*R*) =28.9 min (95% *ee*).[S7]

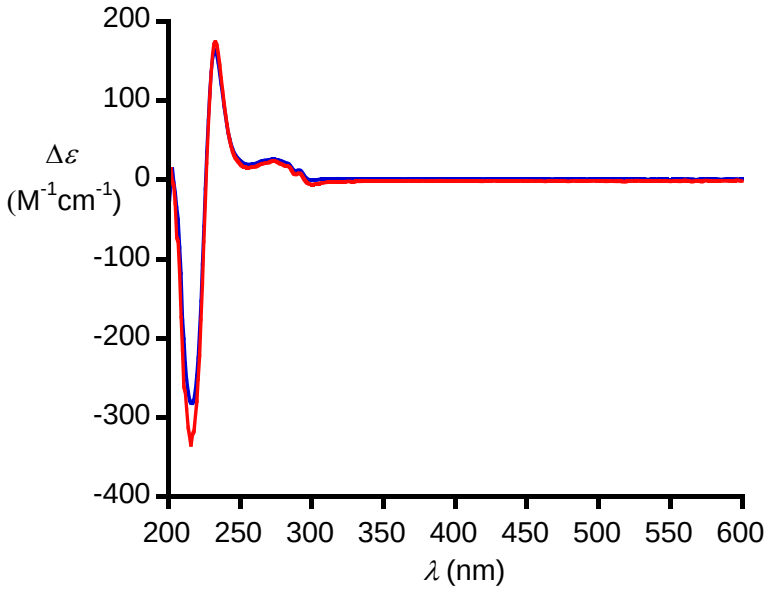


Figure S4. CD spectra of streptavidin WT in CH_3CN/PBS buffer pH =7.4 (1:1 mixture) (blue) and CH_3CN/glycine buffer pH =3.0 (1:1 mixture) (red).

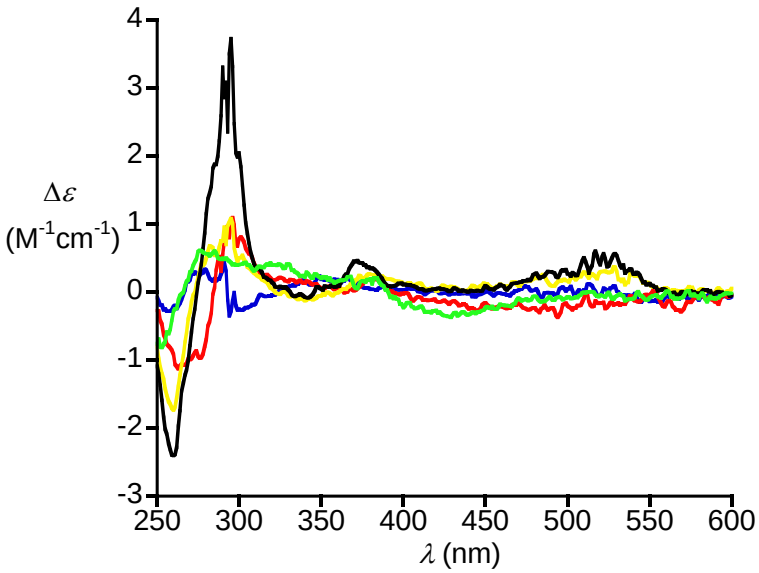


Figure S5. CD spectra recorded in MeOH of **5** (blue), **6** (green), **7** (red), **8** (yellow), **9** (black).

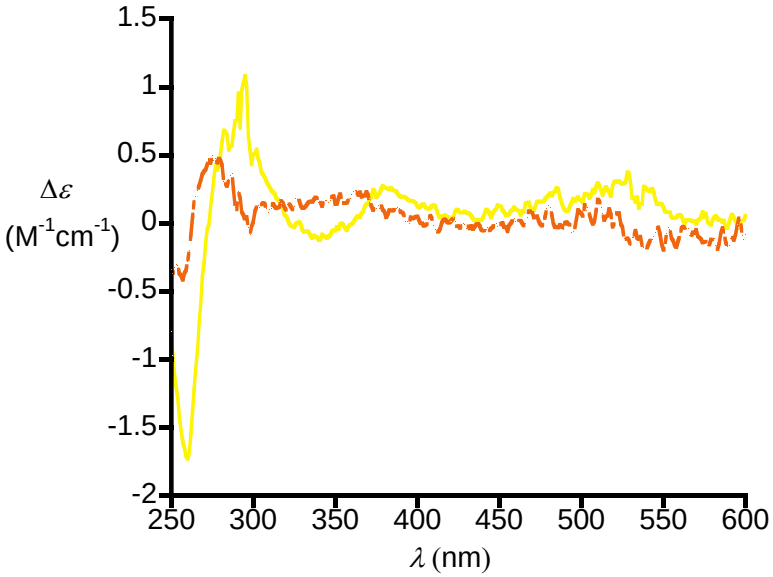


Figure S6. CD spectra recorded in MeOH of (*R*,*R*)-**8** (yellow), (±)-**8** (orange).

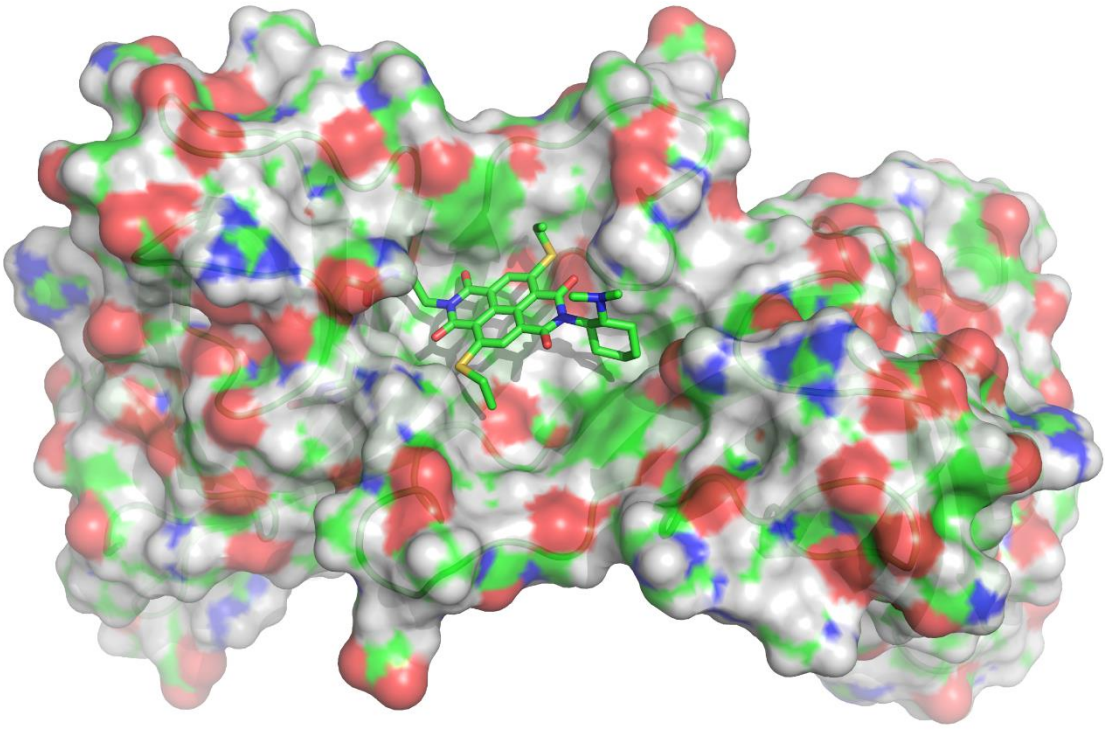


Figure S7. Result of the docking simulation of (*R,R*)-**8** into a dimeric structure of Sav S112Y derived from the X-ray structure 3PK2 (pdbcode). Protein surfaces are rendered with their electrostatic potential (red: negative, blue: positive, green: aromatics), β sheets as faint green arrows. Exposed parts of **8** are in wireframe presentation, C green, N, blue, S yellow, O red.

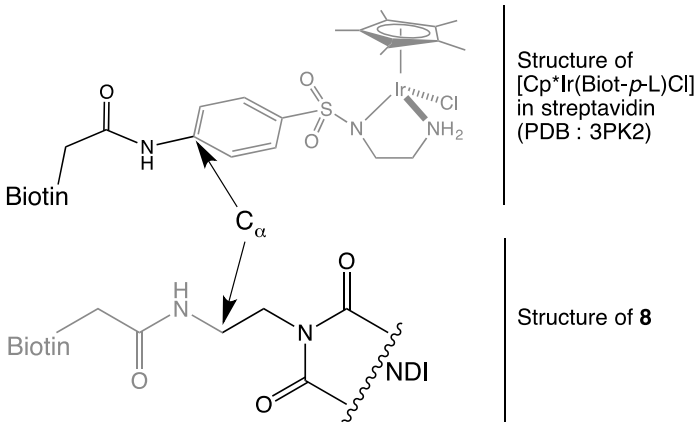


Figure S8. Superimposing protocol to dock the (*R*,*R*)-**8** into the 3PK2 structure containing the [Cp*Ir(Biot-*p*-L)Cl] cofactor.

Table S1. **Catalysis with various equivalent of biotinylated-NDI 8 (1-4 eq)**.

Entry	catalyst concentration	protein	η (%)[b]	A/D[c]	*ee* (%)[d]
1	0.25 mM	WT	34	>30	20
2	0.50 mM	WT	60	>30	41
3	0.75 mM	WT	74	>30	51
4	1.00 mM	WT	75	>30	49

[a]Experiments were performed with streptavidin WT (1 mM) in glycine buffer pH 3/CD_3CN 1:1. [b]Conversion of **3** after 24 h. [b]Ratio of addition product A (**3**) / decarboxylation product D (**4**). [d]Enantiomeric excess.

5. Supplementary References

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(S5) Kastrinsky, D. B.; Barry, C. E. Synthesis of labeled meropenem for the analysis of M. tuberculosis transpeptidases. *Tetrahedron Lett*. **2010**, *51*, 197-200.

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6. NMR Spectra[S13]

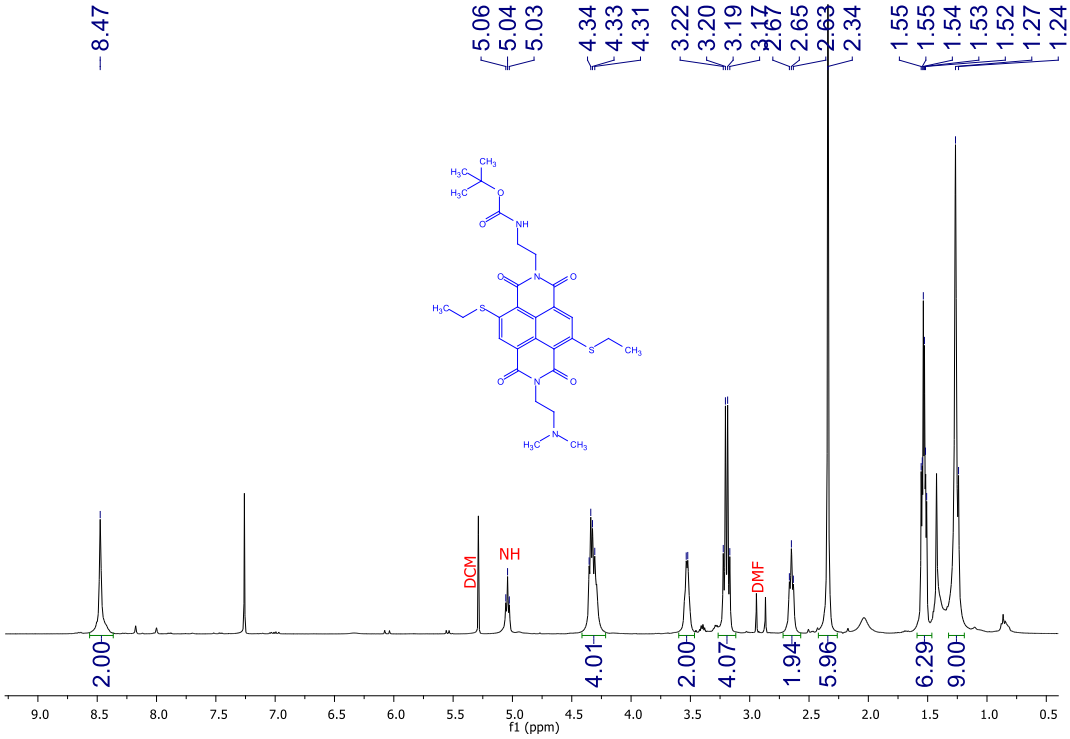

Figure S9. ^{1}H NMR spectrum of **11** in $CDCl_3$.

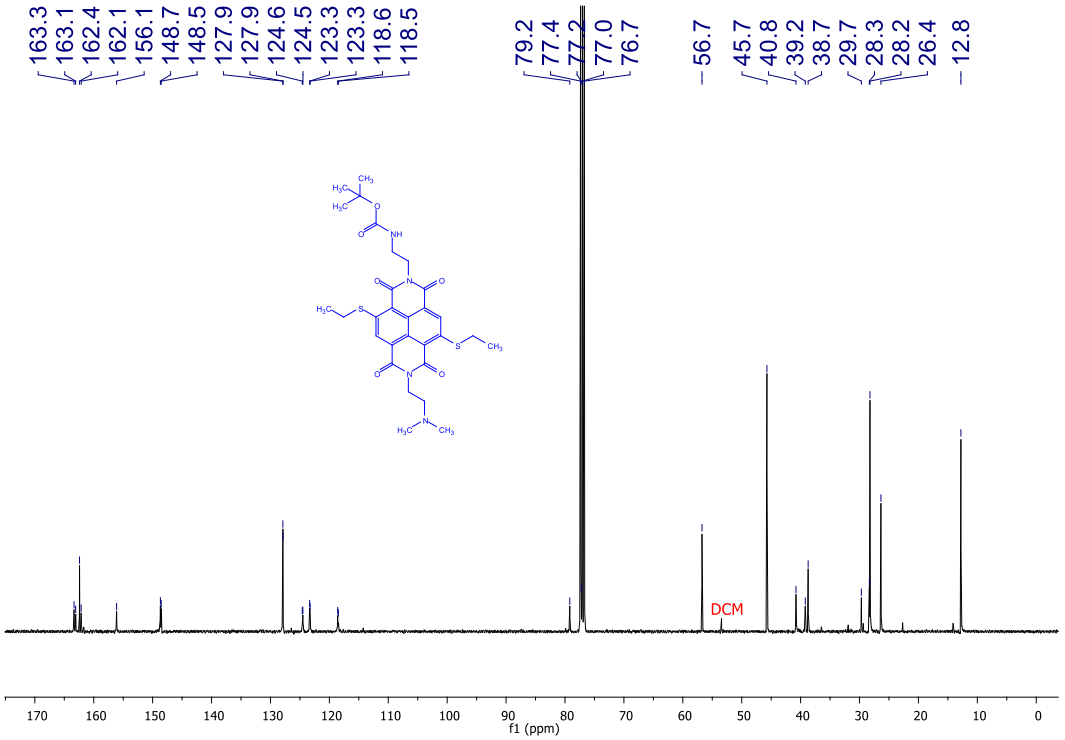

Figure S10. ^{13}C NMR spectrum of **11** in $CDCl_3$.

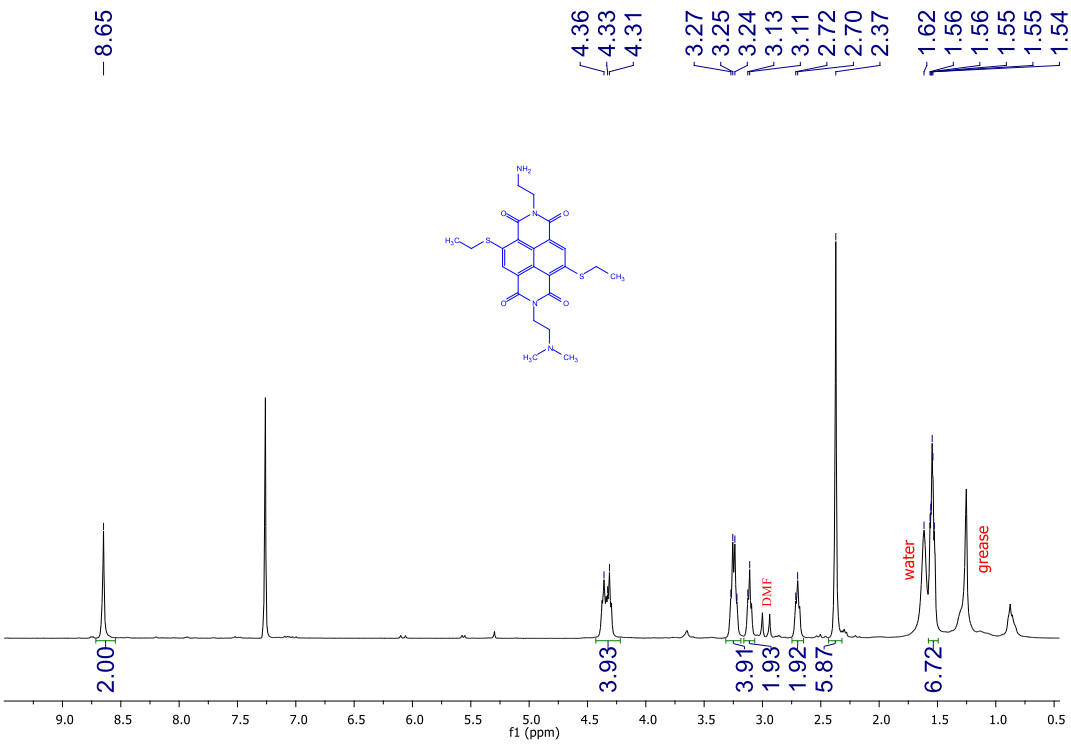

Figure S11. ^{1}H NMR spectrum of **12** in $CDCl_3$.

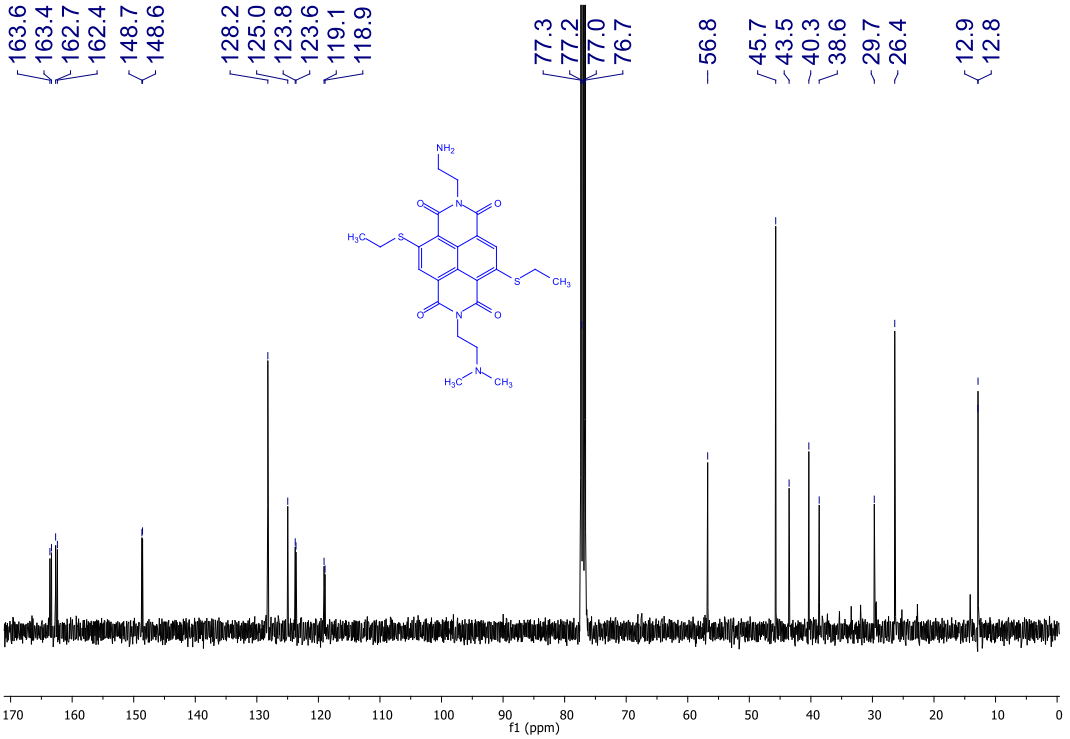

Figure S12. ^{13}C NMR spectrum of **12** in $CDCl_3$.

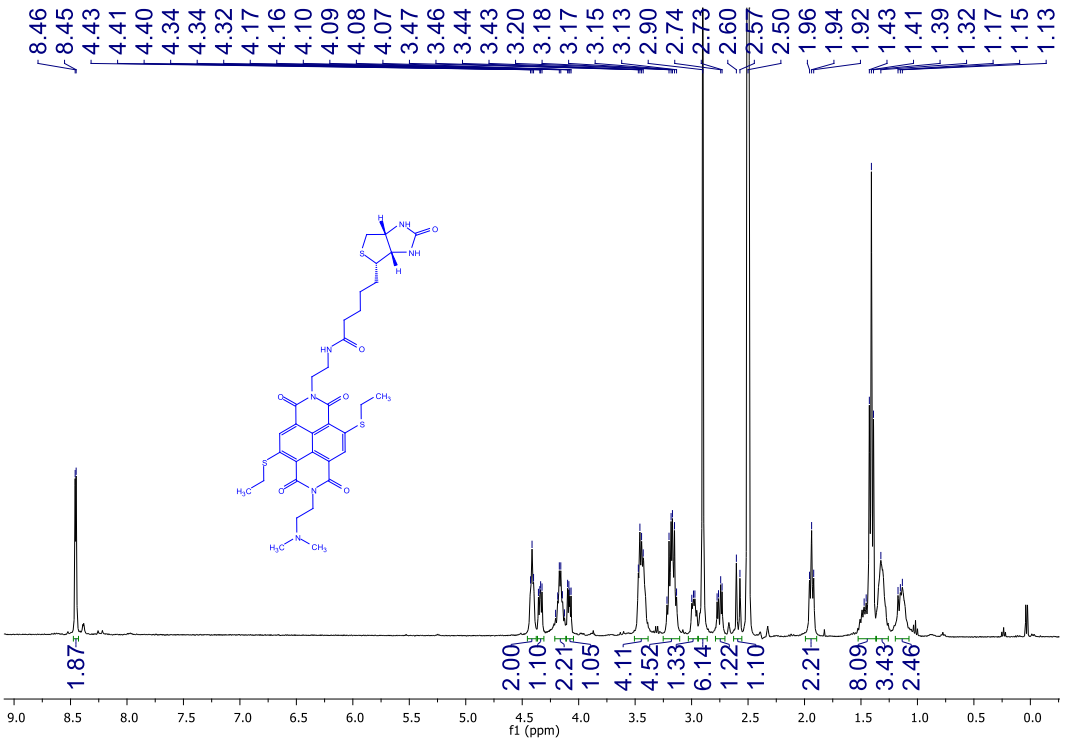

Figure S13. ^{1}H NMR spectrum of **5** in DMSO-d_6/TFA 4:1.

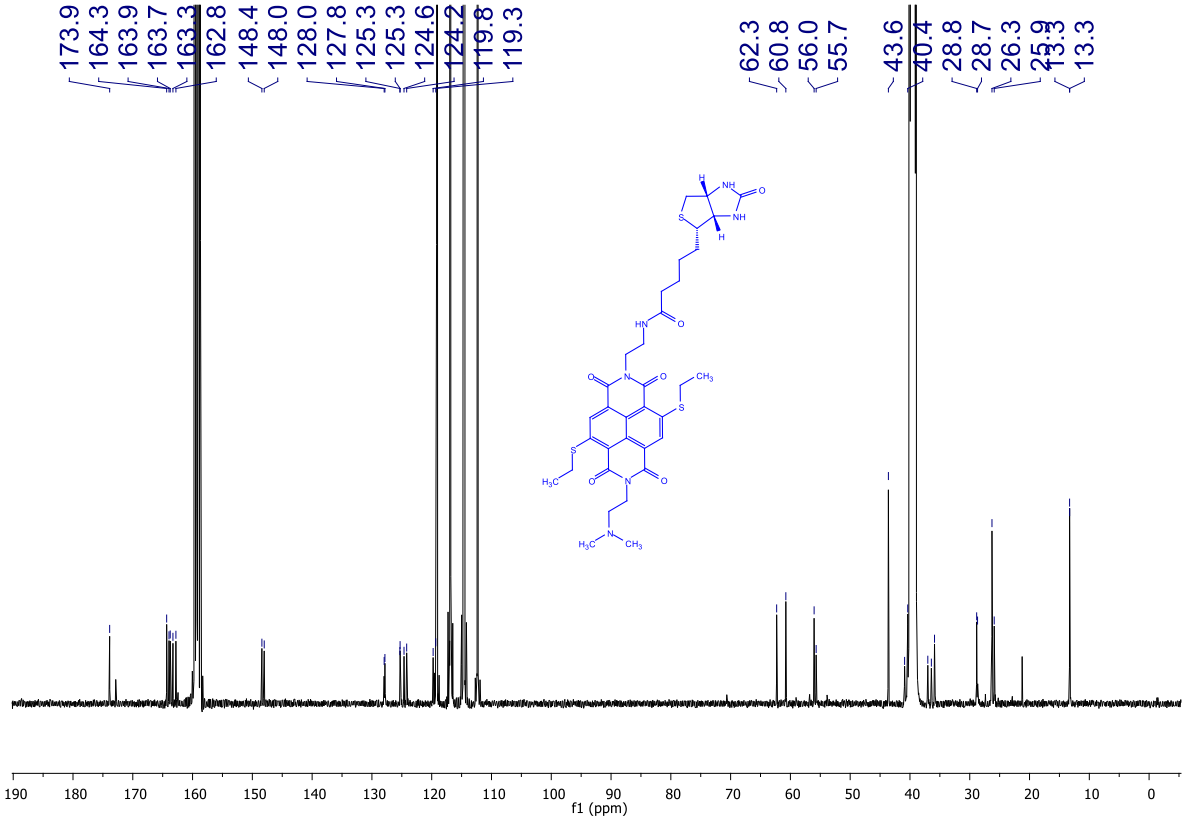

Figure S14. ^{13}C NMR spectrum of **5** in DMSO-d_6/TFA-d 4:1.

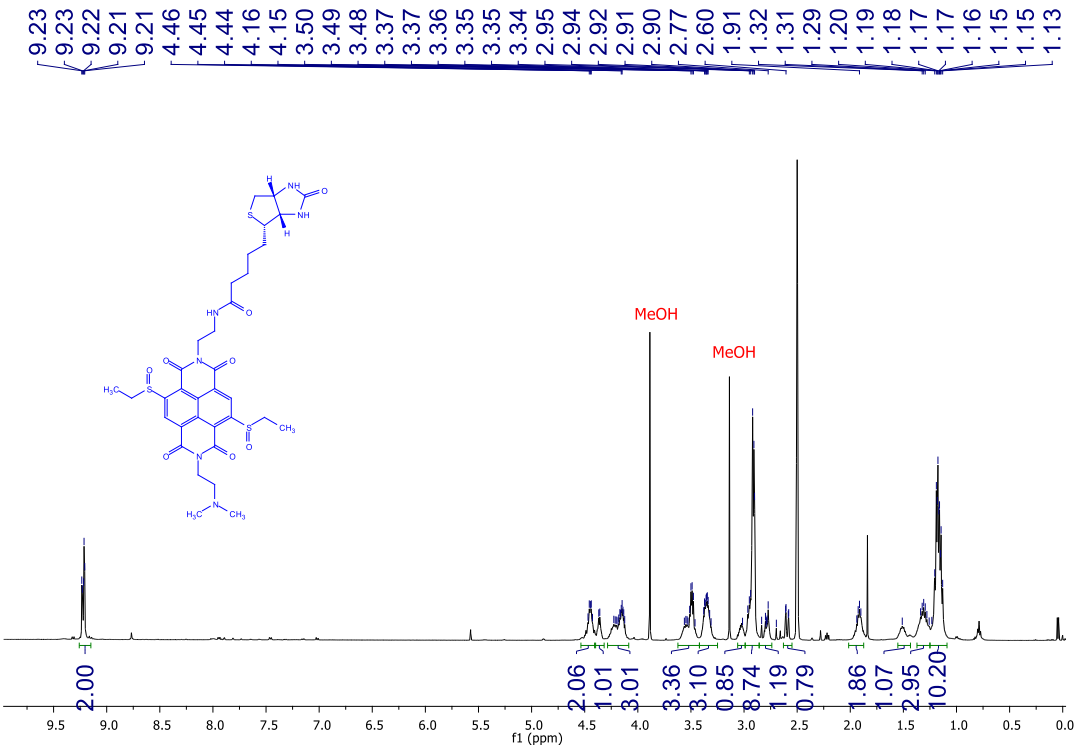

Figure S15. ^{1}H NMR spectrum of **6** in DMSO-d_6/TFA-*d* 4:1.

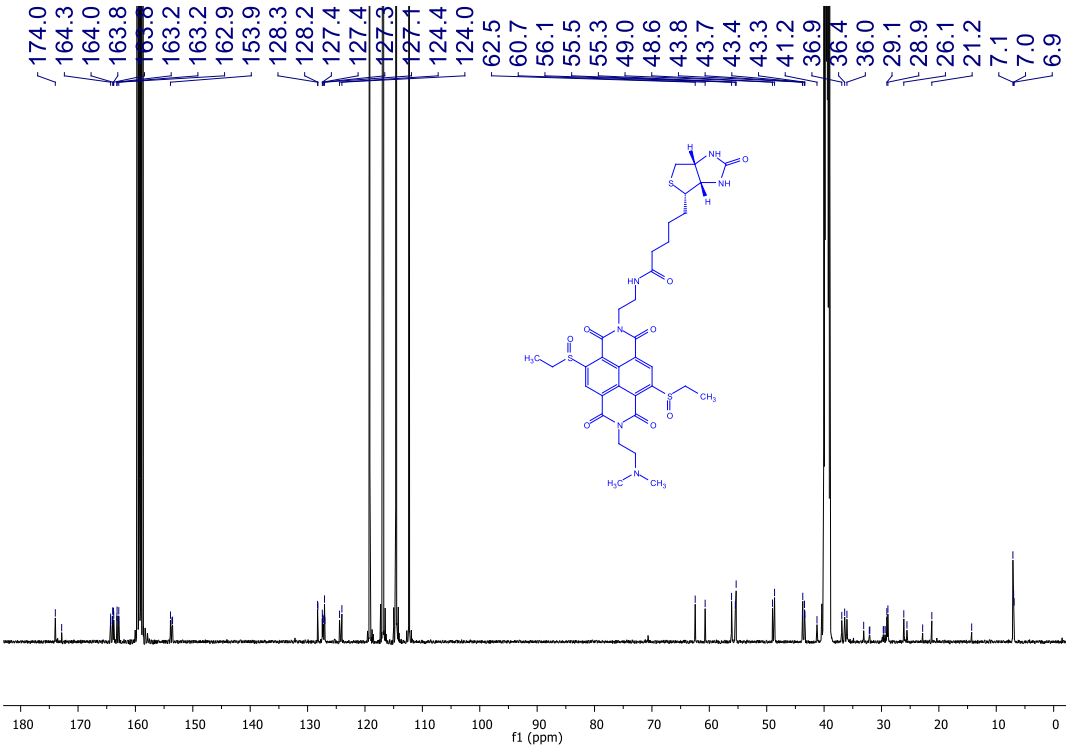

Figure S16. ^{13}C NMR spectrum of **6** in DMSO-d_6/TFA-*d* 4:1.

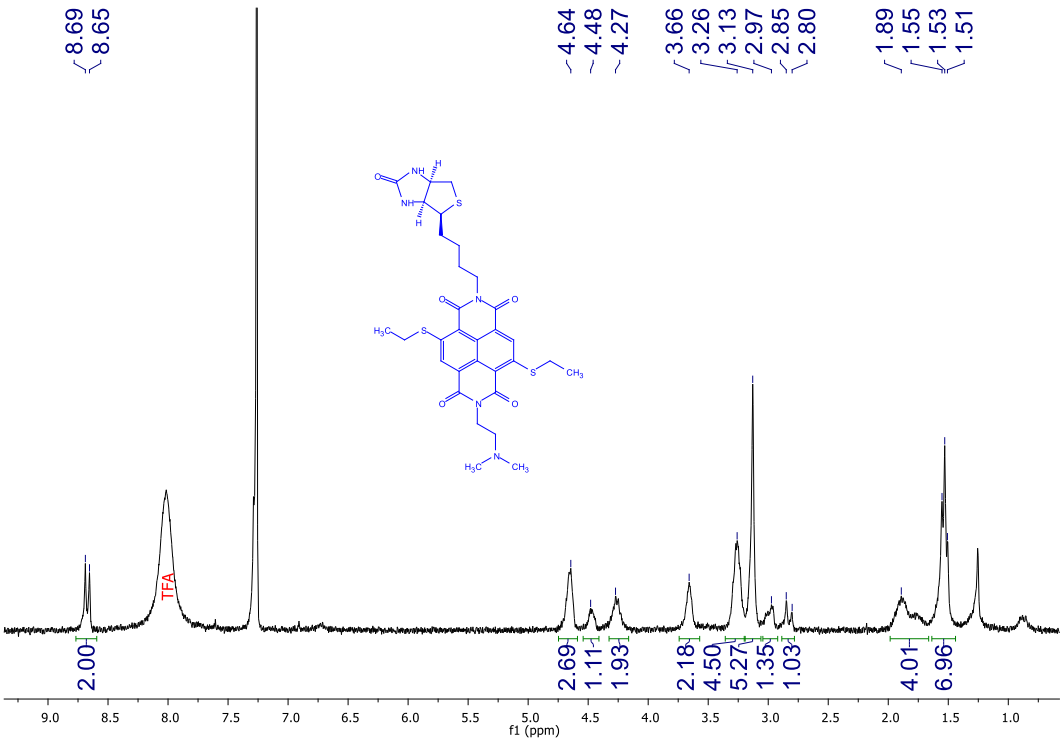

Figure S17. ^{1}H NMR spectrum of **7** in $CDCl_3$/TFA-*d*.

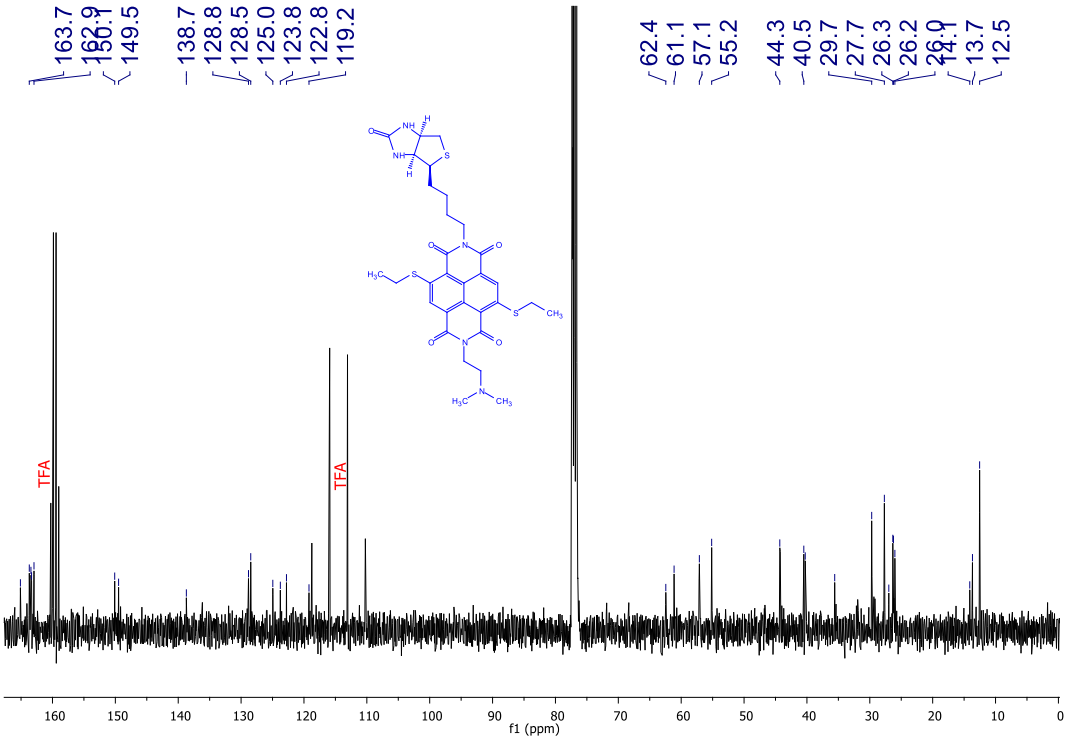

Figure S18. ^{13}C NMR spectrum of **7** in $CDCl_3$/TFA-*d*.

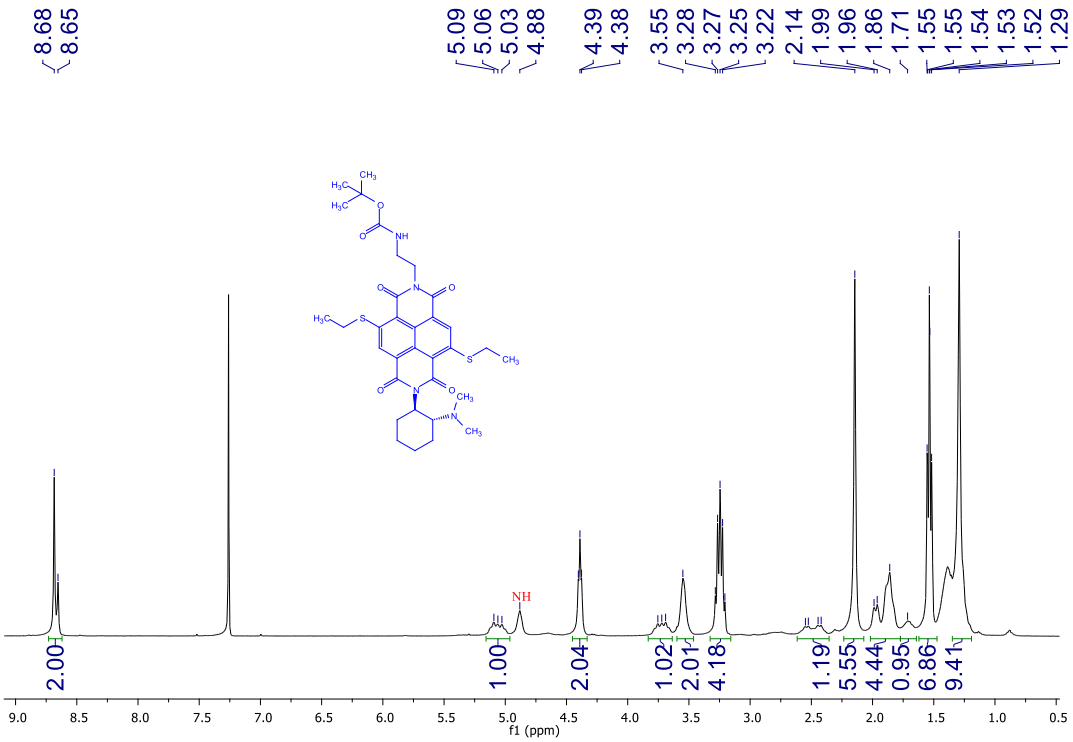

Figure S19. ^{1}H NMR spectrum of (*R*,*R*)-**16** in $CDCl_3$.

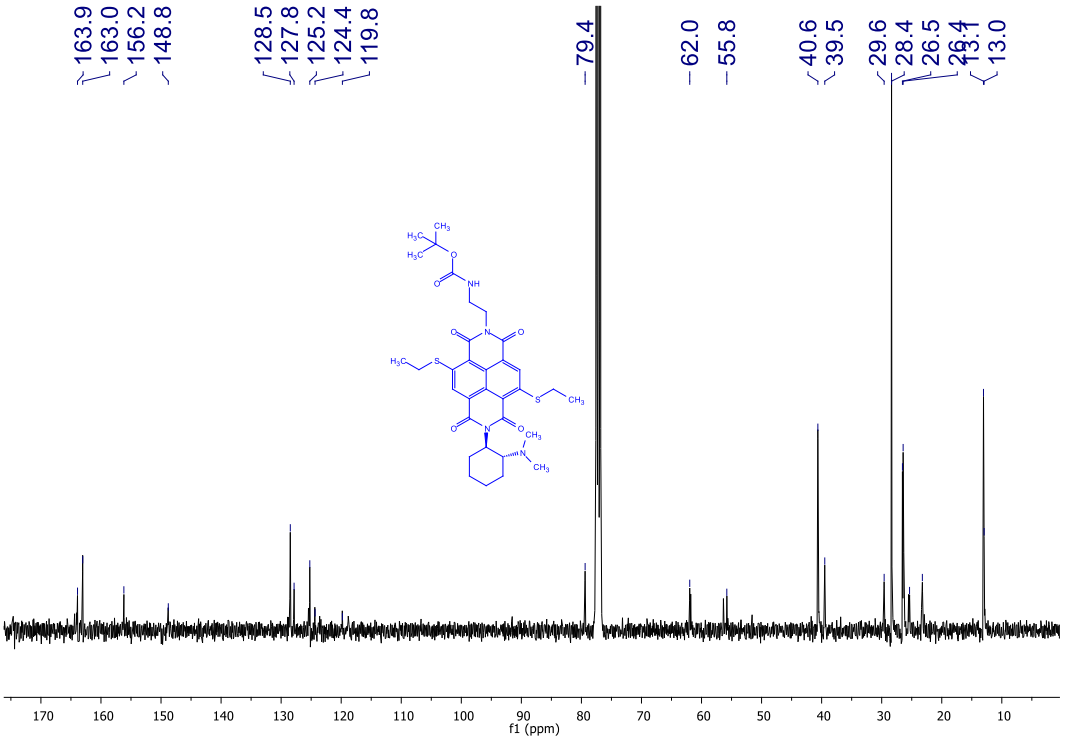

Figure S20. ^{13}C NMR spectrum of (*R*,*R*)-**16** in $CDCl_3$.

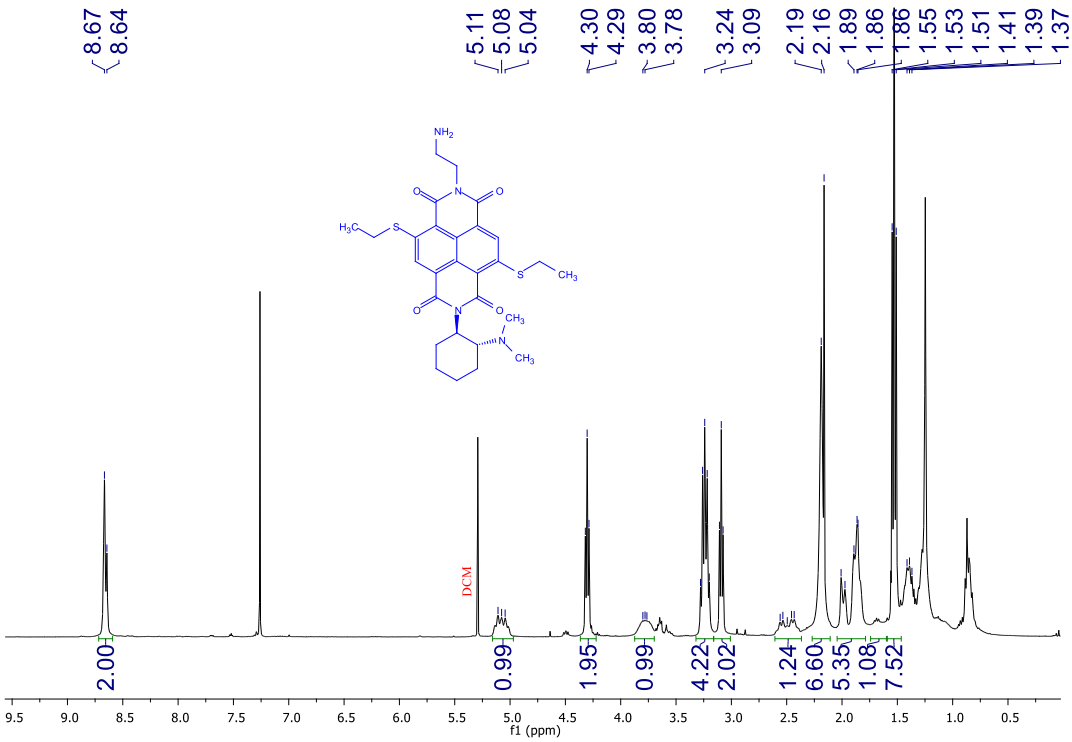

Figure S21. ^{1}H NMR spectrum of (*R*,*R*)-**17** in $CDCl_3$.

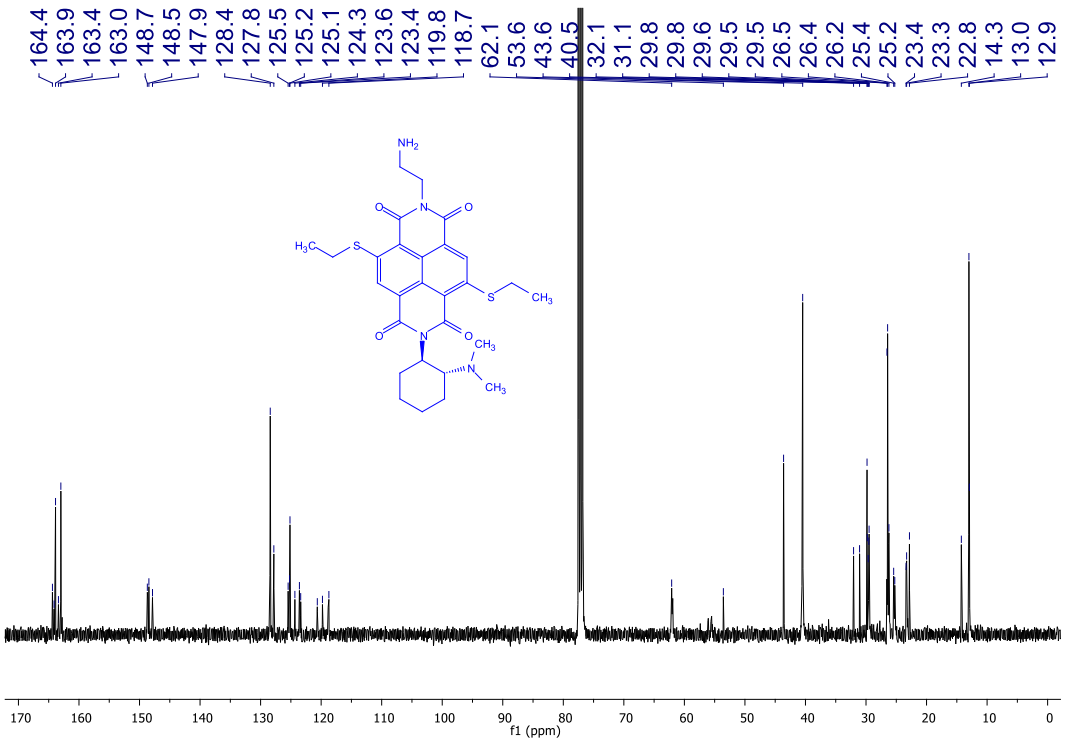

Figure S22. ^{13}C NMR spectrum of (*R*,*R*)-**17** in $CDCl_3$.

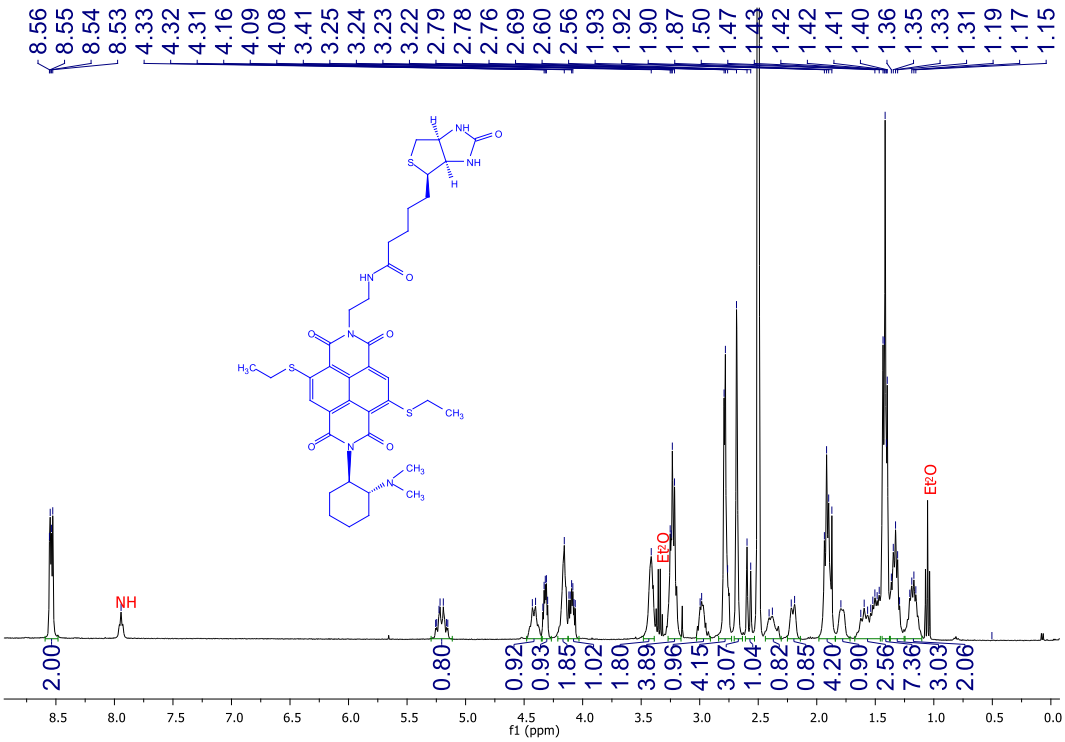

Figure S23. ^{1}H NMR spectrum of (*R*,*R*)-**8** in DMSO-d_6/TFA-*d* 4:1.

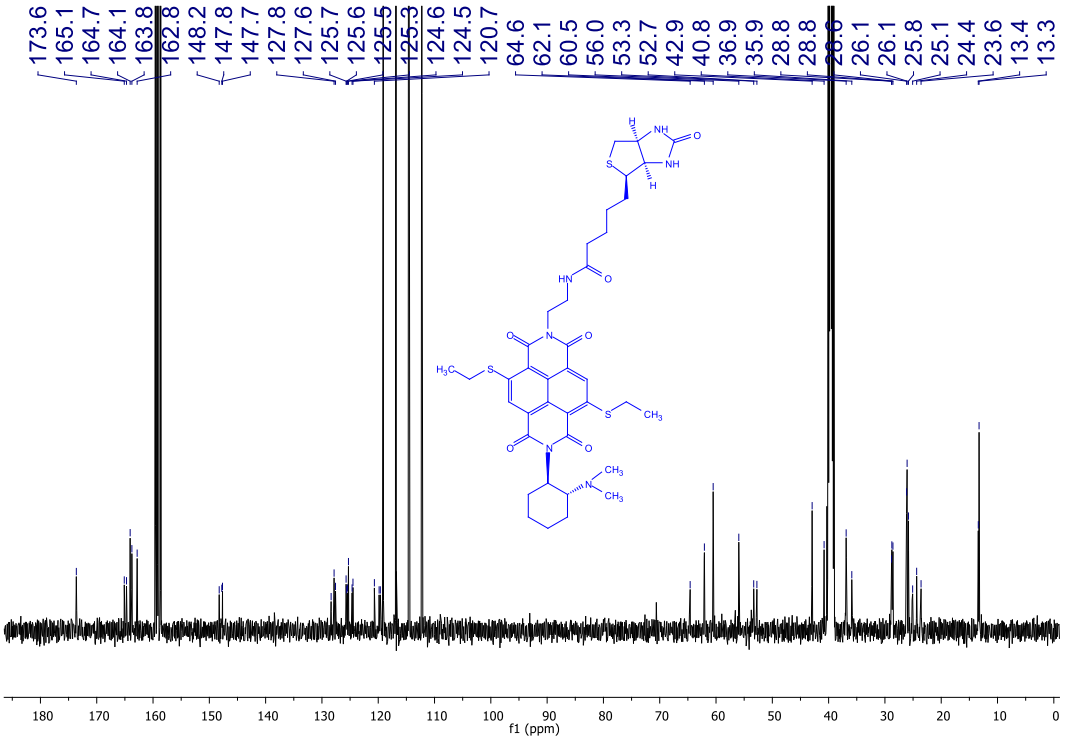

Figure S24. ^{13}C NMR spectrum of (*R*,*R*)-**8** in DMSO-d_6/TFA-*d* 4:1.

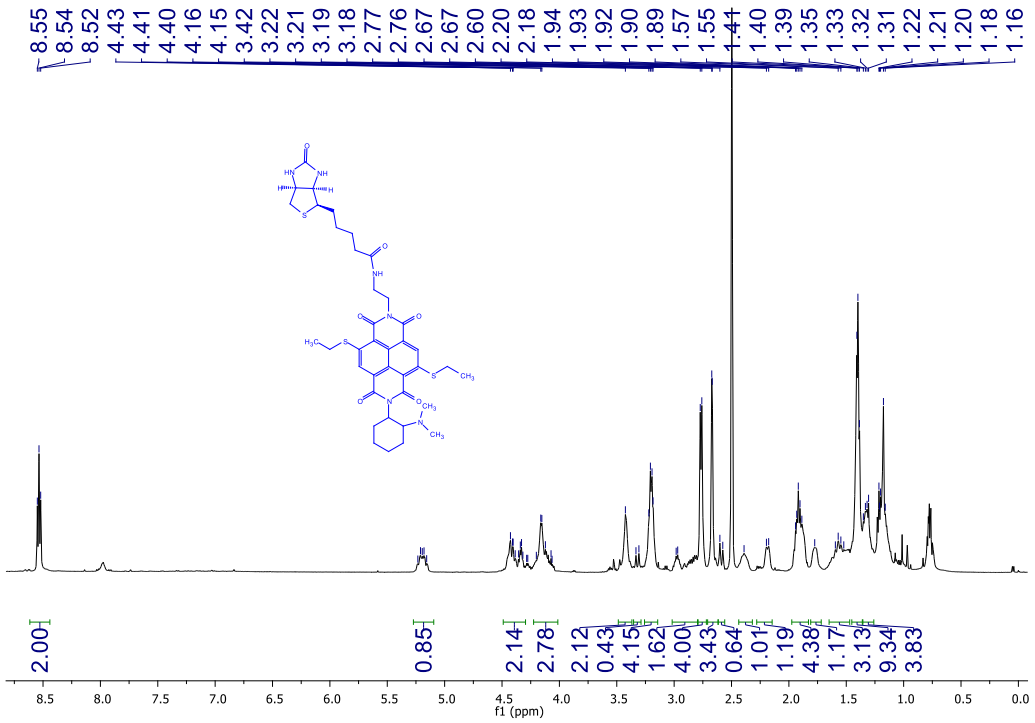

Figure S25. ^{1}H NMR spectrum of (±)-**8** in DMSO-d_6/TFA-*d* 4:1.

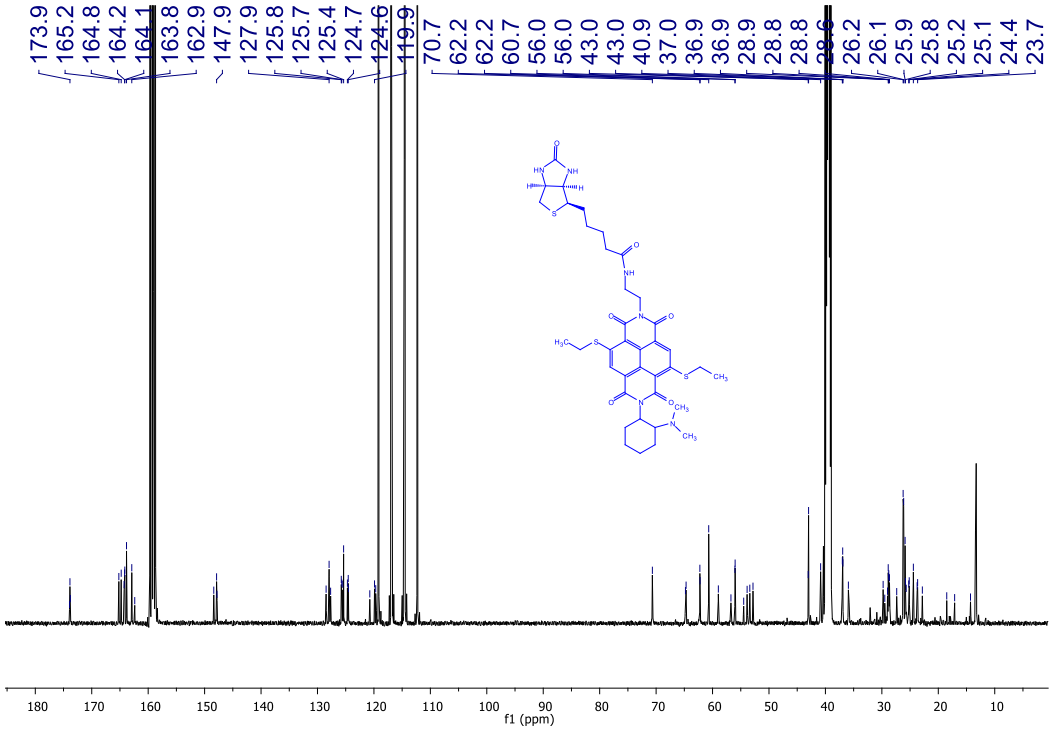

Figure S26. ^{13}C NMR spectrum of (±)-**8** in DMSO-d_6/TFA-*d* 4:1.

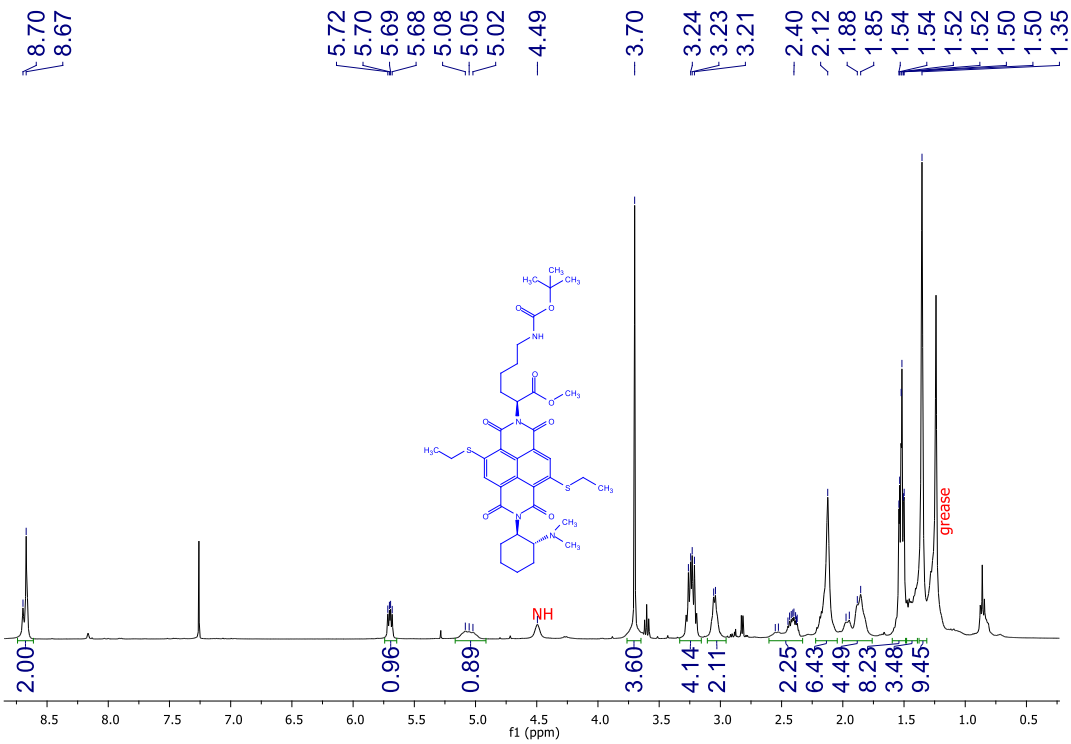

Figure S27. ^{1}H NMR spectrum of (*R*,*R*,*S*)-**18** in $CDCl_3$.

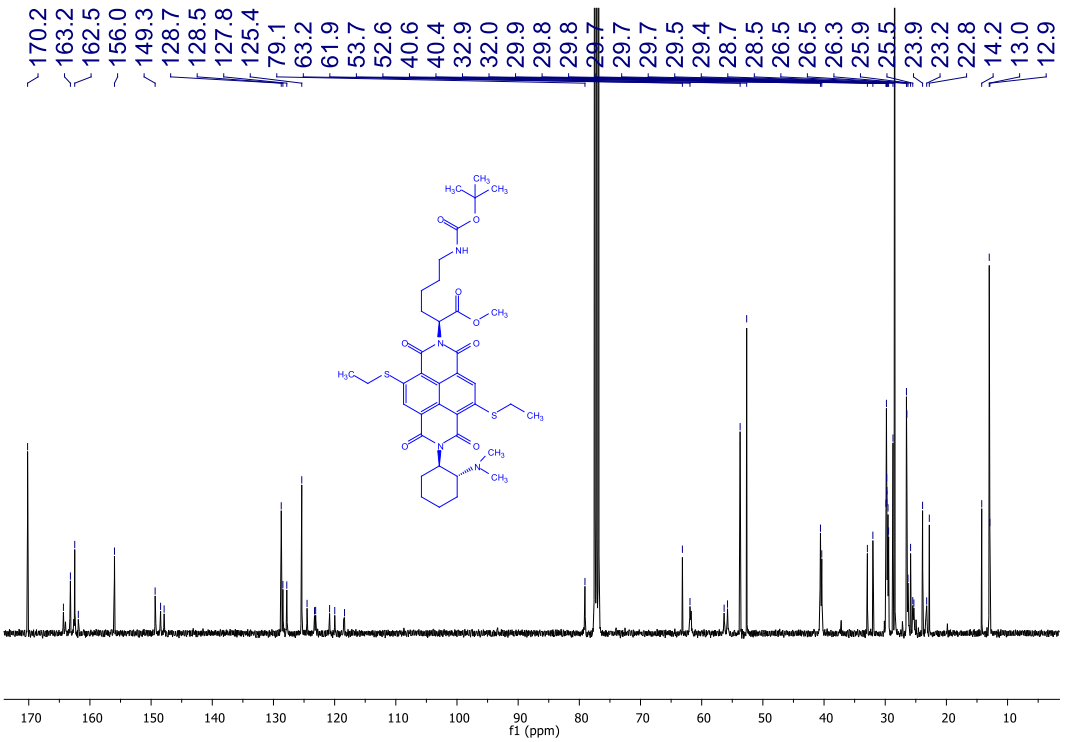

Figure S28. ^{13}C NMR spectrum of (*R*,*R*,*S*)-**18** in $CDCl_3$.

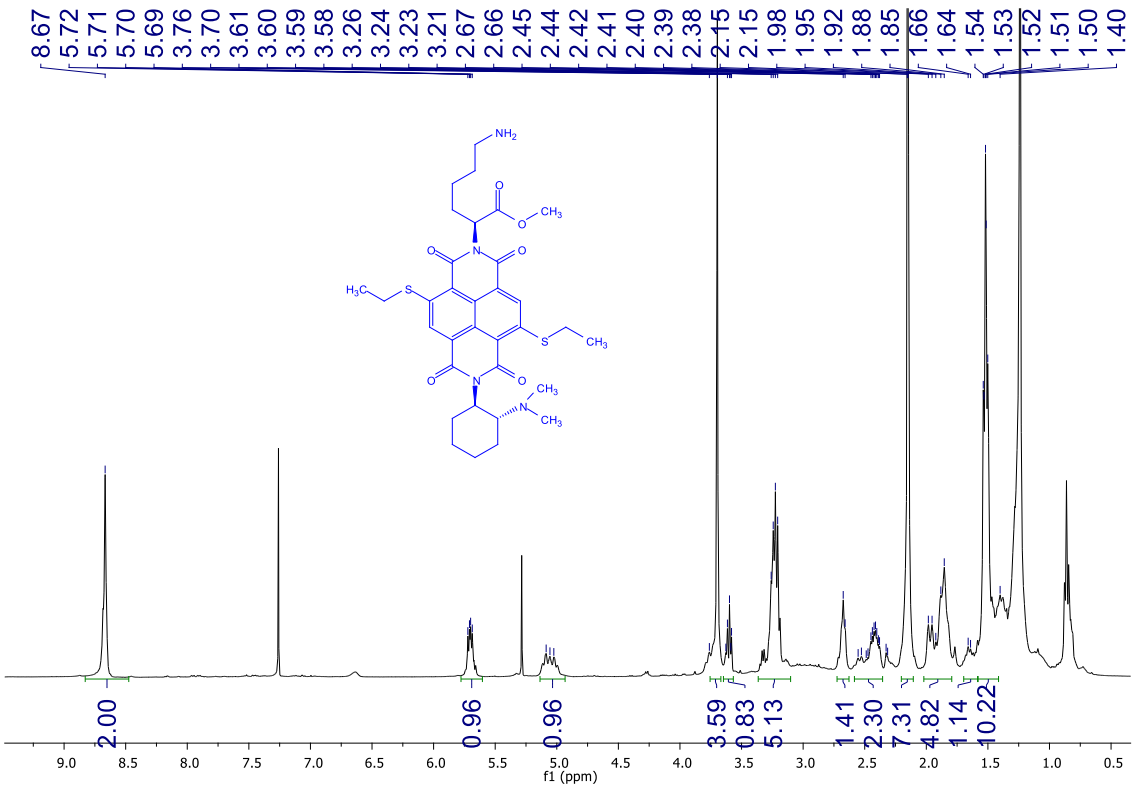

Figure S29. ^{1}H NMR spectrum of (*R*,*R*,*S*)-**19** in $CDCl_3$.

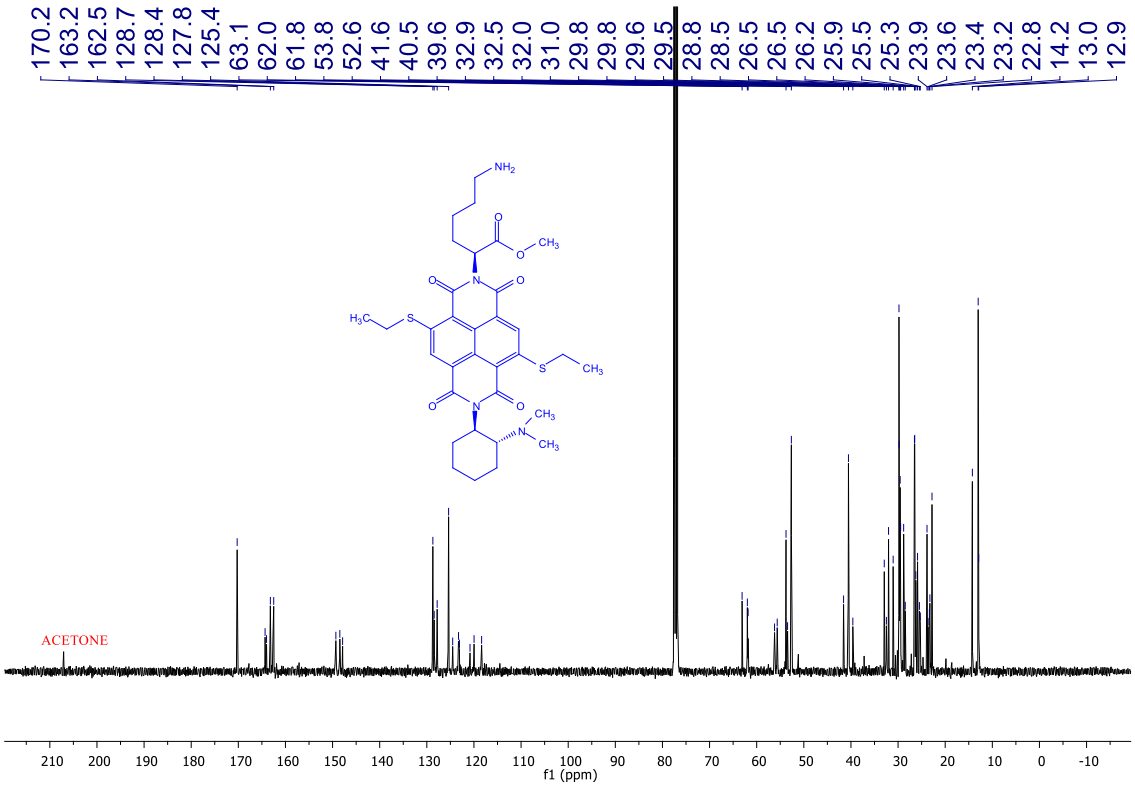

Figure S30. ^{13}C NMR spectrum of (*R*,*R*,*S*)-**19** in $CDCl_3$.

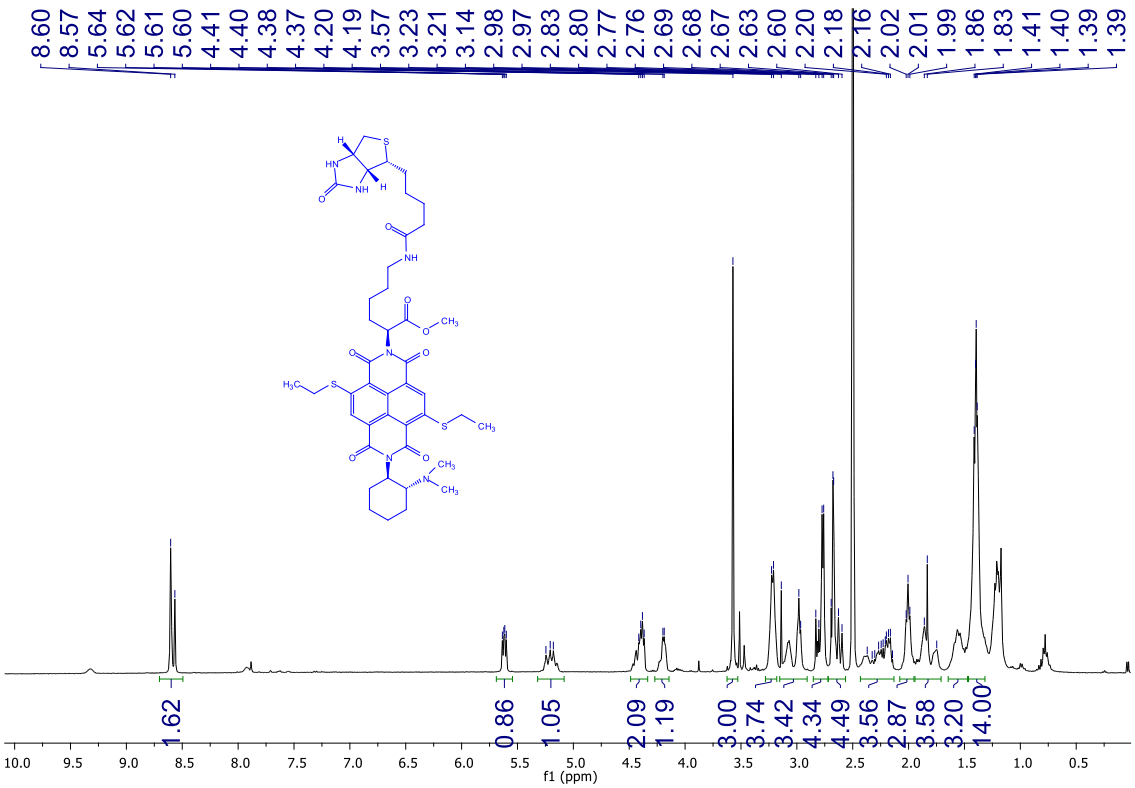

Figure S31. ^{1}H NMR spectrum of (*R*,*R*,*S*)-**9** in DMSO-d_6/TFA-*d* 4:1.

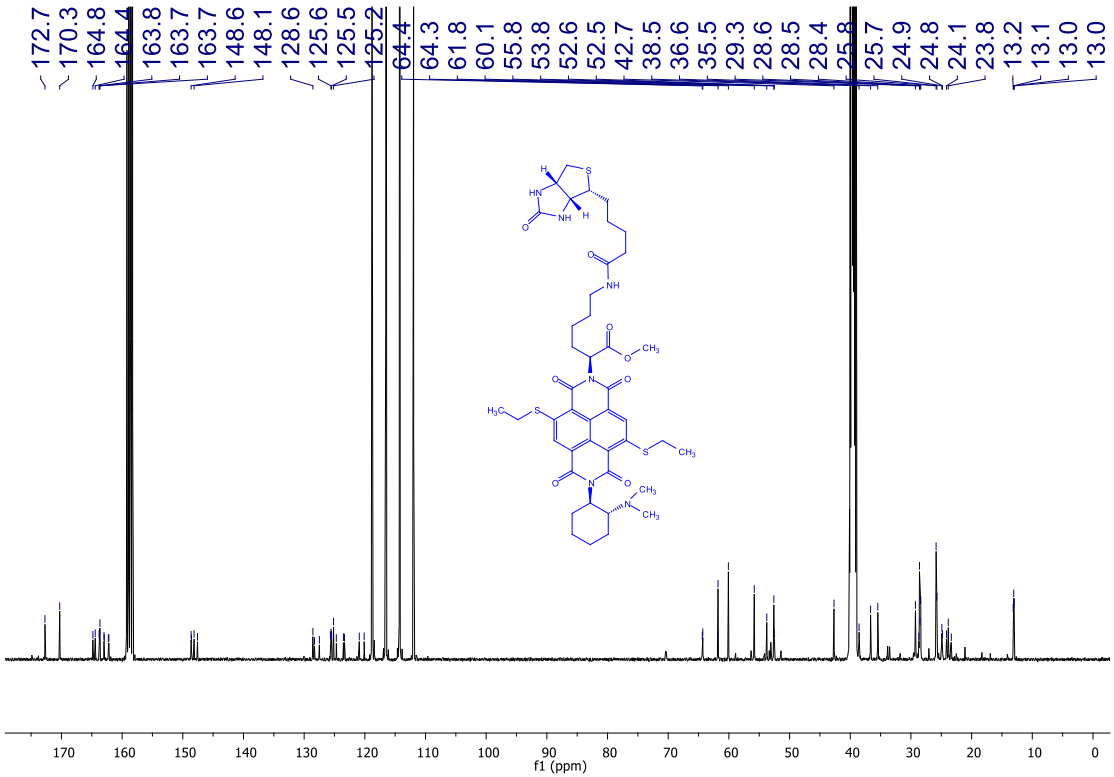

Figure S32. ^{13}C NMR spectrum of (*R*,*R*,*S*)-**9** in DMSO-d_6/TFA-*d* 4:1.