Enantioselective Self-Sorting on Planar, π-Acidic Surfaces of Chiral Anion-π Transporters

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Supporting Information

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1. Material and Methods

As in ref. S1, Supporting Information. Briefly, reagents for synthesis were purchased from Sigma-Aldrich, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received. 8-Hydroxy-1,3,6-pyrenetrisulfonate (HPTS) was from Fluka. Egg yolk phosphatidylcholine (EYPC) and a Mini-Extruder used for vesicle preparation were from Avanti Polar Lipids. All reactions were performed under N_2 or Ar atmosphere. Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical (TLC) and preparative thin layer chromatography (PTLC) were performed on silica gel 60 (Fluka, 0.2 mm) and silica gel GF (Analtech, 1 mm), respectively. Separations of racemates were achieved by preparative HPLC consisting of a NovaPrep 200 pump, a Merck-Hitachi L-7400 UV-detector and a 500 x 50 mm Chiralpak AD column (20 μm material). Analytical HPLC of chiral samples was performed with a Merck-Hitachi L-7100 pump, an L-7455 diode array detector and a Chiralpak AD column (250x4.6 mm; 10 μm material). Melting points (mp) were recorded on a heating table from Reichert (Austria). UV-Vis spectra were recorded on a JASCO V-650 spectrophotometer equipped with a stirrer and a temperature controller (25 ºC) and are reported as maximal absorption wavelength λ in nm (extinction coefficient ε in $M^{-1}cm^{-1}$). Circular dichroism spectra were obtained using JASCO J-815 spectropolarimeter and are reported as extremum wavelength λ in nm (Δε in $M^{-1}cm^{-1}$). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate, neat 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 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 (internal standard, δ = 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). ^{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 was

assigned with the aid of DEPT 135, and reported as s (*C*), d (*CH*), t (CH_2) and q (CH_3). Accurate mass determinations using ESI (HR ESI-MS) were performed on a QSTAR Pulsar mass spectrometer (AB/MDS Sciex). Fluorescence measurements were performed with a FluoroMax-3 spectrofluorometer (Jobin-Yvon Spex) equipped with a stirrer and a temperature controller. All measurements were performed at 25 °C. X-Ray analysis was done on a Nonius Kappa-CCD, a Stoe IPDS (for (*meso*)-**1**) or a Supernova Atlas-CCD (Agilent, for (*M*)-**1**, (*P*)/(*M*)-**2a**, (*P*)/(*M*)-**2b** and (*M*)/(*P*)-**2c**) diffractometer, the first two with Mo-Kα radiation (λ = 0.71073 Å), the third with mirror-monochromated Cu-Kα radiation (λ = 1.54184 Å). Denzo was used for data reduction.

Abbreviations

CF: 5(6)-Carboxyfluorescein; EYPC: Egg yolk phosphatidylcholine; Hepes: *N*-(2-Hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid); HPTS: 8-Hydroxy-1,3,6-pyrenetrisulfonate; LUVs: Large unilamellar vesicles; mCPBA: *m*-Chloroperbenzoic acid; NDI: 1,4,5,8-Naphthalenediimide.

2. Supporting Text

2.1. Synthesis

(*meso*)-4 and (*P*)/(*M*)-4. A mixture of brominated 1,4,5,8-naphthaletetracarboxylic dianhydride including 2,6-dibromonaphthalene-1,4,5,8-tetracarboxylic dianhydride[S1] (2.0 g, < 4.6 mmol) and 2-*tert*-butylaniline (2.7 g, 18.4 mmol) in AcOH (50 ml) was stirred at 80 ºC for 24 h. The solvent was evaporated and the remaining residue was washed with MeOH (50 ml). The crude product was purified by silica gel chromatography (CH_2Cl_2 / petroleum ether 2:1) affording (*meso*)-**4** (410 mg, 13%) and (*P*)/(*M*)-**4** as a yellow solid (400 mg, 12%). (*meso*)-**4:** Mp > 230 ºC; IR: 3063 (w), 2973 (w), 1720 (m), 1677 (s), 1415 (m), 1311 (m), 1217 (s), 754 (m), 714 (m); ^{1}H NMR (300 MHz, $CDCl_3$): 1.29 (s, 18 H), 7.01 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.38 (dt, *J* = 1.5, 7.5 Hz, 2H), 7.49 (dt, *J* = 1.5, 7.5 Hz, 2H), 7.69 (dd, *J* = 1.5, 7.5 Hz, 2H), 9.09 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 31.7 (q), 35.9 (s), 124.7 (d), 125.8 (s), 127.5 (d), 128.3 (s), 128.9 (d), 129.4 (d), 129.7 (d), 130.7 (d), 132.2 (s), 139.5 (s), 146.8 (s), 161.6 (s), 161.8 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m*/*z* calc. for $C_{34}H_{29}N_2O_4Br_2$ $[M + H]^+$: 687.0488, found: 687.0498; (*P*)/(*M*)-**4:** Mp > 230 ºC; IR: 3055 (w), 2983 (w), 1718 (m), 1672 (s), 1416 (m), 1316 (m), 1217 (s), 752 (m), 712 (m); ^{1}H NMR (300 MHz, $CDCl_3$): 1.31 (s, 18 H), 7.05 (dd, *J* = 1.5, 7.5 Hz, 2H), 7.39 (dt, *J* = 1.5, 7.5 Hz, 2H), 7.49 (dt, *J* = 1.5, 7.5 Hz, 2H), 7.70 (dd, *J* = 1.5, 7.5 Hz, 2H), 9.08 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 31.7 (q), 35.9 (s), 124.7 (d), 125.8 (s), 127.6 (d), 128.2 (s), 128.9 (d), 129.3 (d), 129.7 (d), 130.7 (d), 132.3 (s), 139.5 (s), 146.8 (s), 161.6 (s), 161.8 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m*/*z* calc. for $C_{34}H_{29}N_2O_4Br_2$ $[M + H]^+$: 687.0488, found: 687.0507.

(*meso*)-1. A mixture of (*meso*)-**4** (100 mg, 0.15 mmol), EtSH (1 ml) and K_2CO_3 (1.0 g, 7.5 mmol) in $CHCl_3$ (3 ml) was stirred in a sealed tube at 70 ºC for 20 h. After cooling to rt, the solvent and excess EtSH were removed by flow of N_2 gas. The resulting residue was dissolved in CH_2Cl_2

(50 ml) and washed with water (50 ml) and brine (50 ml). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (CH_2Cl_2) affording (*meso*)-**1** as a red solid (81 mg, 85%). Mp > 230 ºC; IR: 2964 (w), 1702 (m), 1660 (s), 1545 (m), 1431(m), 1320 (m), 1219 (s), 754 (m); ^{1}H NMR (300 MHz, $CDCl_3$): 1.30 (s, 18H), 1.49 (t, J = 7.2 Hz, 6H), 3.23 (q, J = 7.2 Hz, 4H), 7.03 (dd, J = 7.5, 1.5 Hz, 2H), 7.36 (ddd, J = 7.5, 7.5, 1.5 Hz, 2H), 7.46 (ddd, J = 7.5, 7.5, 1.5 Hz, 2H), 7.68 (dd, J = 7.5, 1.5 Hz, 2H), 8.80 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 12.6 (q), 26.2 (t), 31.7 (q), 35.9 (s), 119.4 (d), 124.2 (s), 125.6 (s), 127.3 (d), 128.6 (d), 129.3 (d), 130.8 (d), 132.5 (s), 146.9 (s), 149.4 (s), 163.3 (s), 164.2 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m/z* calc. for $C_{38}H_{39}N_2O_4S_2$ $[M + H]^+$: 651.2345, found: 651.2349.

(*P*)/(*M*)-1. In a manner similar to that described above, reaction of (*P*)/(*M*)-**4** (100 mg, 0.15 mmol) with EtSH (1 ml) yielded (*P*)/(*M*)-**1** as a red solid (85 mg, 86%): Mp > 230 ºC; IR: 2968 (w), 1701 (m), 1659 (s), 1547 (m), 1433 (m), 1322 (m), 1229 (s), 759 (m); ^{1}H NMR (300 MHz, $CDCl_3$): 1.30 (s, 18 H), 1.48 (t, J = 7.2 Hz, 6H), 3.18 (q, J = 7.2 Hz, 4H), 7.09 (dd, J = 7.8, 1.5 Hz, 2H), 7.33 (ddd, J = 7.8, 7.8, 1.5 Hz, 2H), 7.45 (ddd, J = 7.8, 7.8, 1.5 Hz, 2H), 7.68 (dd, J = 7.8, 1.5 Hz, 2H), 8.73 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 12.6 (q), 26.2 (t), 31.7 (q), 35.9 (s), 119.3 (d), 124.2 (s), 125.5 (s), 127.4 (d), 128.5 (d), 129.1 (d), 129.2 (d), 130.9 (d), 132.5 (s), 146.8 (s), 149.3 (s), 163.2 (s), 164.1 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m/z* calc. for $C_{38}H_{39}N_2O_4S_2$ $[M + H]^+$: 651.2345, found: 651.2361.

(*P*)/(*M*)-2a, (*P*)/(*M*)-2b and (*P*)/(*M*)-2c. A solution of (*P*)/(*M*)-**1** (100 mg, 0.15 mmol) in CH_2Cl_2 (20 ml) was treated with mCPBA (79 mg, 0.45 mmol) at 0 ºC. After stirring for 0.5 h, the mixture was washed with aqueous $Na_2S_2O_3$ (5%, 50 ml), brine (50 ml) and concentrated in *vacuo*. The crude products was purified by preparative thin layer chromatography (CH_2Cl_2/MeOH/Acetone 100:1:1) affording (*P*)/(*M*)-**2a** (38 mg, 36%), (*P*)/(*M*)-**2b** (55 mg, 52%)

and (*P*)/(*M*)-**2c** (12 mg, 11%) as yellow solid. (*P*)/(*M*)-**2a:** Mp > 230 ºC; IR: 2977 (w), 1711 (m), 1670 (s), 1434 (m), 1315 (m), 1248 (s), 1046 (s), 760 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 1.29 (s, 18H), 1.36 (t, *J* = 7.6 Hz, 6H), 2.95-3.05 (m, 2H), 3.30-3.40 (m, 2H), 7.06 (dd, *J* = 7.6, 0.8 Hz, 2H), 7.41 (ddd, *J* = 7.6, 7.6, 0.8 Hz, 2H), 7.53 (ddd, *J* = 7.6, 7.6, 0.8 Hz, 2H), 7.73 (d, *J* = 7.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 7.0 (q), 31.6 (q), 35.9 (s), 48.9 (d), 123.2 (d), 126.8 (s), 127.5 (d), 127.9 (s), 129.1 (d), 129.5 (d), 130.0 (d), 130.6 (d), 131.6 (s), 147.1 (s), 155.9 (s), 162.1 (s), 163.3 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m*/*z* calc. for $C_{38}H_{39}N_2O_6S_2$ $[M + H]^+$: 683.2244, found: 683.2277. (*P*)/(*M*)**-2b:** Mp > 230 ºC; IR: 2923 (w), 1713 (m), 1666 (s), 1434 (m), 1313 (m), 1247 (s), 1055 (s), 753 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 1.30 (s, 9H), 1.30 (s, 9H), 1.38 (t, *J* = 7.6 Hz, 3H), 1.42 (t, *J* = 7.6 Hz, 3H), 2.92-3.09 (m, 2H), 3.27-3.40 (m, 2H), 7.06 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.08 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.39 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H), 7.40 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H), 7.53 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H), 7.72 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.73 (dd, *J* = 7.6, 7.6, 1.2 Hz, 1H), 9.60 (s, 1H), 9.65 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 7.0 (q), 7.8 (q), 31.7 (q), 31.8 (q), 35.8 (s), 36.0 (s), 48.9 (d), 49.7 (d), 122.8 (s), 123.3 (s), 126.8 (s), 127.0 (s), 127.7 (d), 127.8 (d), 127.9 (s), 128.1 (s), 128.6 (d), 129.2 (d), 129.4 (d), 129.5 (d), 130.0 (d), 130.6 (d), 130.7 (d), 131.5 (s), 131.6 (s), 146.8 (s), 146.9 (s), 155.9 (s), 156.4 (s), 161.9 (s), 162.2 (s), 163.4 (s), 163.6 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m*/*z* calc. for $C_{38}H_{39}N_2O_6S_2$ $[M + H]^+$: 683.2244, found: 683.2272. (*P*)/(*M*)**-2c:** Mp > 230 ºC; IR: 2967 (w), 1709 (m), 1664 (s), 1432 (m), 1312 (m), 1248 (s), 1059 (s), 752 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 1.32 (s, 18H), 1.41 (t, *J* = 7.4 Hz, 6H), 2.89-2.98 (m, 2H), 3.27-3.36 (m, 2H), 7.08 (d, *J* = 7.2 Hz, 2H), 7.41 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.53 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.72 (d, *J* = 7.2 Hz, 2H), 9.65 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 7.7 (q), 31.8 (q), 36.0 (s), 49.7 (d), 123.0 (d), 126.8 (s), 127.9 (d), 128.2 (s), 128.8 (d), 129.5 (d), 130.0 (d), 130.7 (d), 131.5 (s), 146.6 (s), 156.4 (s), 162.1 (s), 163.6 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m*/*z* calc. for $C_{38}H_{39}N_2O_6S_2$ $[M + H]^+$: 683.2244, found: 683.2213.

(*P*)/(*M*)-3. A solution of (*P*)/(*M*)-**1** (100 mg, 0.15 mmol) in CH_2Cl_2 (20 ml) was treated with mCPBA (500 mg, 2.9 mmol) at 25 ºC. After stirring for 5 h, the mixture was washed with aqueous $Na_2S_2O_3$ (5%, 100 ml), brine (50 ml) and concentrated in *vacuo*. The crude products was purified by silica gel chromatography (CH_2Cl_2/MeOH 99:1) affording (*P*)/(*M*)-**3** as light yellow solid (95 mg, 90%): Mp > 230 ºC; IR: 3079 (w), 2968 (s), 1723 (m), 1680 (s), 1419 (m), 1308 (s), 1231 (s), 1133 (s), 753 (m), 709 (m); ^{1}H NMR (500 MHz, $CDCl_3$): 1.33 (s, 18H), 1.37 (t, *J* = 7.5 Hz, 6H), 3.80-3.90 (m, 2H), 3.95-4.06 (m, 2H), 7.08 (dd, *J* = 7.5, 1.2 Hz, 2H), 7.34 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 2H), 7.51 (ddd, *J* = 8.0, 8.0, 1.2 Hz, 2H), 7.72 (dd, *J* = 8.0, 1.2 Hz, 2H), 9.58 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 7.4 (q), 31.8 (q), 36.0 (s), 50.6 (d), 127.4 (s), 127.6 (d), 127.8 (s), 129.4 (d), 129.8 (s), 129.9 (d), 130.8 (d), 131.6 (s), 134.2 (d), 146.2 (s), 147.1 (s), 161.4 (s), 161.5 (s); HRMS (ESI, +ve, CH_2Cl_2/MeOH 1:1) *m*/*z* calc. for $C_{38}H_{39}N_2O_8S_2$ $[M + H]^+$: 715.2142, found: 715.2174.

2.2 Separation of Stereoisomers

Racemates were separated by preparative HPLC, with *n*-hexane/2-propanol 9:1 as mobile phase, an eluting rate of 80 ml/min and peak detection at λ = 242 nm. Pure enantiomers of (*P*)/(*M*)-**1**,**2a**-**c** were collected in the following intervals: (*M*)-**1**: 8-38 min, (*P*)-**1**: 38-100 min; (*P*)-**2a**: 24-39 min, (*M*)-**2a**: 59-80 min; (*P*)-**2b**: 110-154 min, (*M*)-**2b**: 165-200 min; (*P*)-**2c**: 18-35 min, (*M*)-**2c**: 50-100 min. The recovery of separated materials was typically 85-90%. Both enantiomers had ee > 99%, as evidenced by analytical HPLC with *n*-hexane/2-propanol 9:1 as mobile phase, at an eluting rate of 1 ml/min and peak detection at λ = 242 nm.

2.3 Crystal Structure Determination

Single crystals for X-ray diffraction were obtained by slow diffusion of a two-solvent system. The compound was dissolved in $CHCl_3$ and cyclohexane was allowed to evaporate from outer container into the sample solution at 5 ºC. Crystals of (*P*)-**2a**, (*M*)-**2a** and (*P*)-**2b** suitable for X-ray structural analysis were obtained by slow evaporation of their solution in CH_2Cl_2 at +4 ºC. The structures of (*P*)-**2a**, (*M*)-**2a** and (*P*)-**2b** were solved by Direct Methods (Shelxs97). Shelxl97 was used for full-matrix least-squares refinement on F^2. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in geometrically idealized positions and refined using a riding model. Crystals of (*meso*)-**1**, (*M*)-**1**, (*P*/*M*)-**2a**, (*P*/*M*)-**2b** and (*P*/*M*)-**2c** were mounted on quartz fibres with protection oil and measured at T = 180 K. Data were corrected for Lorentz and polarization effects and for absorption. Structures were solved by direct methods (SIR97) and refinement and other calculations were performed with Shelxl97 systems.

CCDC-deposition numbers:

(*P*)-**2a**: 856260

(*M*)-**2a**: 856261

(*P*)-**2b**: 856262

(*meso*)-**1**: 856482

(*M*)-**1**: 856483

(*P*)/(*M*)-**2a**: 856484

(*P*)/(*M*)-**2b**: 856485

(*P*)/(*M*)-**2c**: 856486

2.4 Self-Sorting

Self-sorting was estimated according to literature procedures.[S2] A trace amount of cNDIs was added to another concentrate cNDIs solution, and the chemical shifts of each monomer in the mixture solution were recorded ($\delta_{observed}$) and compared to their chemical shifts in infinitely dilute homogeneous solutions (δ_{dilute}). The cross-assembly value χ_{CA} (or χ_{HETERO}) and χ_{HOMO} was thus obtained according to equations S1 and S2.

$$\chi_{CA} = (\delta_{B,dilute} - \delta_{B,observed}) / (\delta_{A,dilute} - \delta_{A,observed}) \quad \text{(S1)}$$

$$\chi_{HOMO} = 1 - \chi_{CA} \quad \text{(S2)}$$

Dimerization constants of racemate ($K_{D\,(rac)}$) and enantiomer ($K_{D\,(homo)}$) were determined from nonlinear-least-squares fitting of concentration (C_{tot}) dependent ^{1}H NMR chemical shifts to equation S3.[S3] Dimerization constants of heterodimer were obtained from equation S4.[S4] Results were summarized in Table S1.

$$\delta_{obs} = \delta_{mon}[((8K_DC_{tot}+1)^{1/2}-1)/(4K_DC_{tot})] + \delta_{dimer}[((4K_DC_{tot}+1) - (8K_DC_{tot}+1)^{1/2})/4K_DC_{tot}] \quad \text{(S3)}$$

$$K_{D\,(rac)} = (K_{D\,(hetero)} + 2\,K_{D\,(homo)})/4 \quad \text{(S4)}$$

2.4. Partition Coefficient

Partition coefficients of (*P*)-**2b** and (*M*)-**3** were determined according to literature.[S5] A solution of (*P*)-**2b** or (*M*)-**3** (7.5×10^{-5} M) in water was titrated with EYPC LUVs (60 mM lipid in 5 mM Tris buffer, pH 7, prepared as described in section 2.5.1) at 25 ºC. CD spectra were recorded with vesicle concentration varied from 6.5×10^{-2} to 1.8 mM. Concentration dependent CD signals of (*P*)-**2b** at 465 nm and (*M*)-**3** at 400 nm were fitted to equation S5.

$$I = I_{min} + (I_{max} - I_{min})/(1 + [H_2O]/K_x[Lipid]) \qquad (S5)$$

in which, I = response, I_{min} = I without lipids, I_{max} = I at saturation, $[H_2O]$ = concentration of water (55.3M).

2.5. Ion Transport

2.5.1. Vesicle Preparation

EYPC-LUVs ⊃ HPTS.[S6] A thin lipid film was prepared by evaporating a solution of 25 mg EYPC in 2 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (40 °C) and then *in vacuo* overnight. After hydration (> 30 min) with 1.0 ml buffer (10 mM Hepes, 100 mM NaCl, 1 mM HPTS, pH 7.0), the resulting suspension was subjected to >5 freeze-thaw cycles (liquid N_2, 37 °C water bath), and >15 times extruded through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by size exclusion chromatography (Sephadex G-50, Sigma-Aldrich) with 10 mM Hepes, 100 mM NaCl, pH 7.0. Final conditions: ~2.5 mM EYPC; inside: 10 mM Hepes, 100 mM NaCl, 1 mM HPTS, pH 7.0; outside: 10 mM Hepes, 100 mM NaCl, pH 7.0.

EYPC-LUVs ⊃ CF.[S7] A thin lipid film was prepared by evaporating a solution of 25 mg EYPC in 2 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (40 °C) and then *in vacuo* overnight. After hydration (> 30 min) with 1.0 ml buffer (10 mM Hepes, 10 mM NaCl, 50 mM CF, pH 7.0), the resulting suspension was subjected to >5 freeze-thaw cycles (liquid N_2, 37 °C water bath), and >15 times extruded through a polycarbonate membrane (pore size 100 nm). Extravesicular components were removed by size exclusion chromatography (Sephadex G-50, Sigma-Aldrich) with 10 mM Hepes, 107 mM NaCl, pH 7.0. Final conditions: ~2.5 mM EYPC; inside: 10 mM Hepes, 10 mM NaCl, 50 mM CF, pH 7.0; outside: 10 mM Hepes, 107 mM NaCl, pH 7.0.

2.5.2. Determination of Transport Activity with the HPTS Assay (Figs. 6b, S19).[S6]

To 1950 μl gently stirred, thermostated buffer (25°C, 10 mM Hepes, 100 mM NaCl, pH 7.0) in a disposable plastic cuvette, 25 μl EYPC-LUVs ⊃ HPTS were added. The time-dependent change in fluorescence intensity (λ_{em} = 510 nm) was monitored at two excitation wavelengths simultaneously ($I_{t,450}$: λ_{exc} = 450 nm, $I_{t,405}$: λ_{exc} = 405 nm) during the addition of base (20 μl, 0.5 M NaOH) at t = 50 s, transporter (20 μl, DMSO solution) at t = 100 s, and gramicidin D (20 μl, 100 μM in DMSO) at t = 300 s. Time courses of fluorescence intensity I_t were obtained by first, ratiometric analysis (R = $I_{t,450}$ / $I_{t,405}$) and second, normalization according to equation (S6),

$$I_f = (R_t - R_0) / (R_\infty - R_0) \qquad \text{(S6)},$$

where $R_0 = R_t$ right after addition of transporter and $R_\infty = R_t$ after addition of gramicidin D. I_f at 300 s just before addition of gramicidin D was defined as transmembrane activity Y, and analyzed with the Hill equation (S7) to give effective concentration EC_{50} and the Hill coefficient n,

$$Y = Y_\infty + (Y_0 - Y_\infty) / (1 + c / EC_{50})^n \qquad \text{(S7)},$$

where Y_0 is Y in absence of transporter, Y_∞ is Y with excess transporter, and c is the transporter concentration in a cuvette. For compound (*P*)/(*M*)-**2c**, $Y_\infty = 1$ was applied to estimate EC_{50} value. As the activities of compounds (*meso*)-**1**, (*P*)/(*M*)-**1** and (*P*)/(*M*)-**3** did not reach $Y = 0.5$ with up to 50 μM of compounds, EC_{50} values are estimated to be ≥ 100 μM. Complete results for all compounds are shown in table 2.

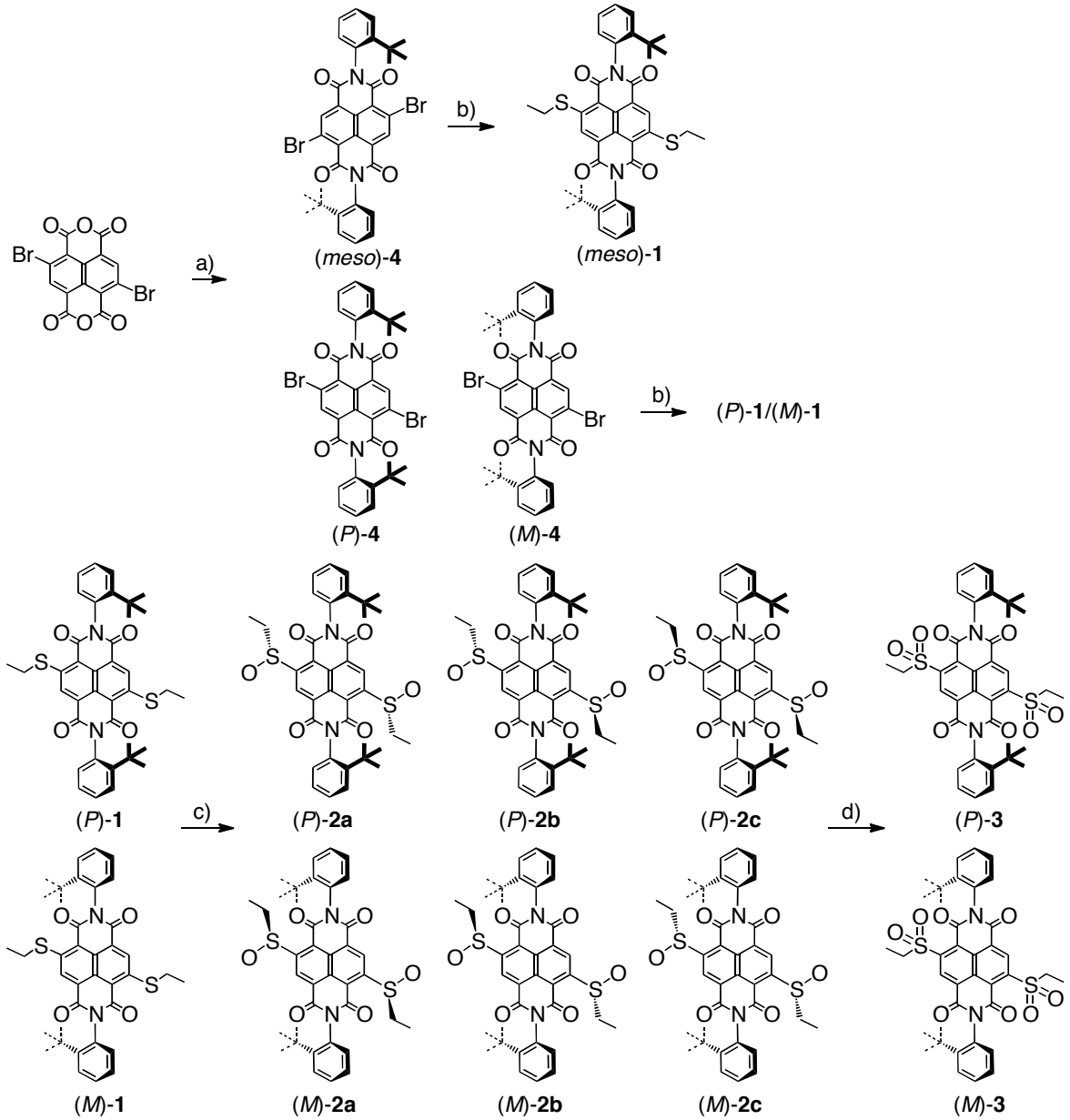
2.5.3. Determination of Non-Specific Leakage with the CF Assay (Fig. S20 - S21).[S7]

To 1950 μl gently stirred, thermostated buffer (25°C, 10 mM Hepes, 107 mM NaCl, pH 7.0) in a disposable plastic cuvette, 25 μl EYPC-LUVs ⊃ CF were added. The time-dependent changes in fluorescence intensity I_t (λ_{exc} = 492 nm, λ_{em} = 517 nm) were monitored during the addition of the transporter at t = 50 s, and the addition of triton X-100 (40 μl 1.2% aq) at t = 250 s. Time courses of I_t were normalized to fractional intensities I_f using equation (S8),

$$I_f = (I_t - I_0)/(I_\infty - I_0) \qquad \text{(S8)},$$

where $I_0 = I_t$ right after the transporter addition and $I_\infty = I_t$ after lysis (Fig. S20). I_f at 250 s just before lysis was defined as transmembrane activity Y and treated the same way as the HPTS assay.

3. Supporting Schemes and Figures



Scheme S1. Synthesis of chiral anion-π- transporters. 2,6-Dibromonaphthalene-1,4,5,8-tetracarboxylic dianhydride was synthesized according to literature procedure.[S1] a) 2-*tert*-butylaniline, AcOH, 80 ºC, 24h, (*meso*)-**4** 13%, (*P*)/(*M*)-**4** 12%; b) EtSH, K_2CO_3, $CHCl_3$, 65 ºC, 20h, (*meso*)-**1** 85%, (*P*)/(*M*)-**1** 86%; c) mCPBA, CH_2Cl_2, 0 ºC, 0.5h, (*P*)/(*M*)-**2a** 36%, (*P*)/(*M*)-**2b** 11%, (*P*)/(*M*)-**2c** 50%; d) mCPBA, CH_2Cl_2, 25 ºC, 20h, (*P*)/(*M*)-**3** 90%.

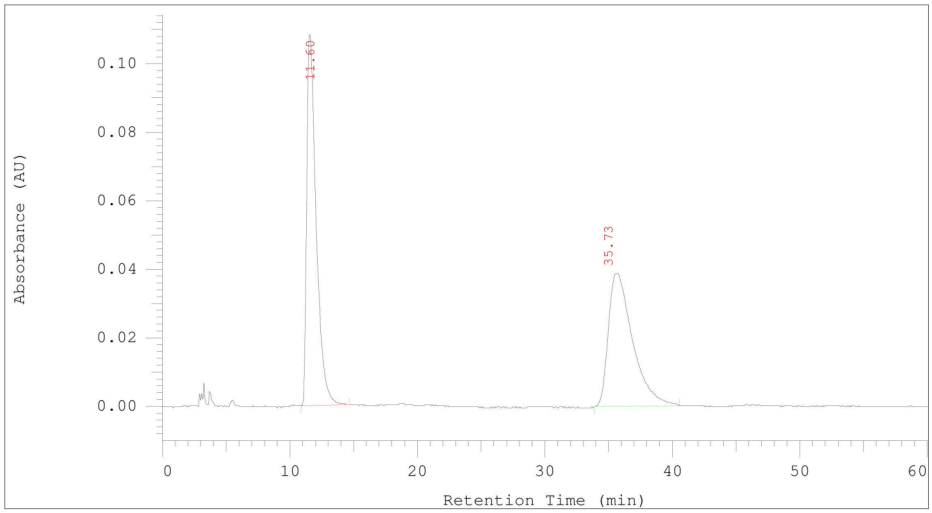


Figure S1. Chiral HPLC of racemate (*P*)/(*M*)-**1**.

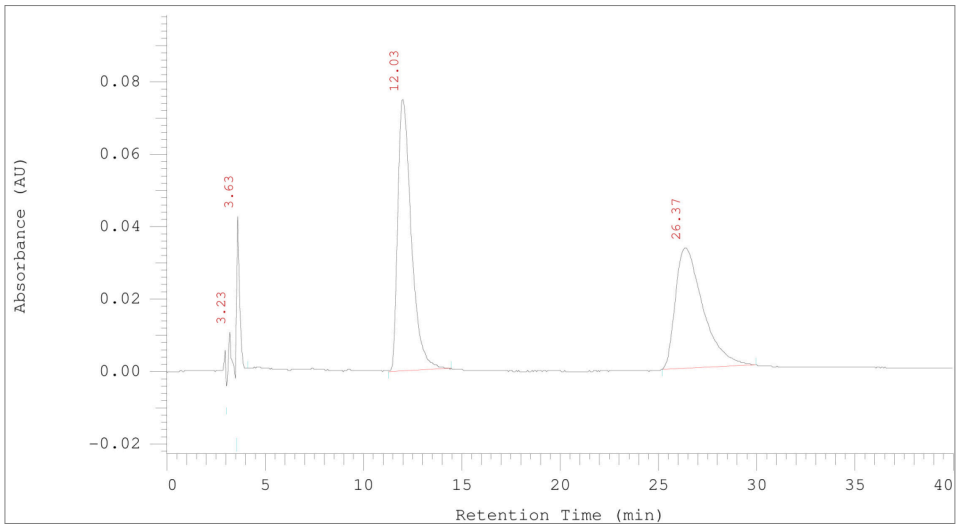


Figure S2. Chiral HPLC of racemate (*P*)/(*M*)-**2a**.

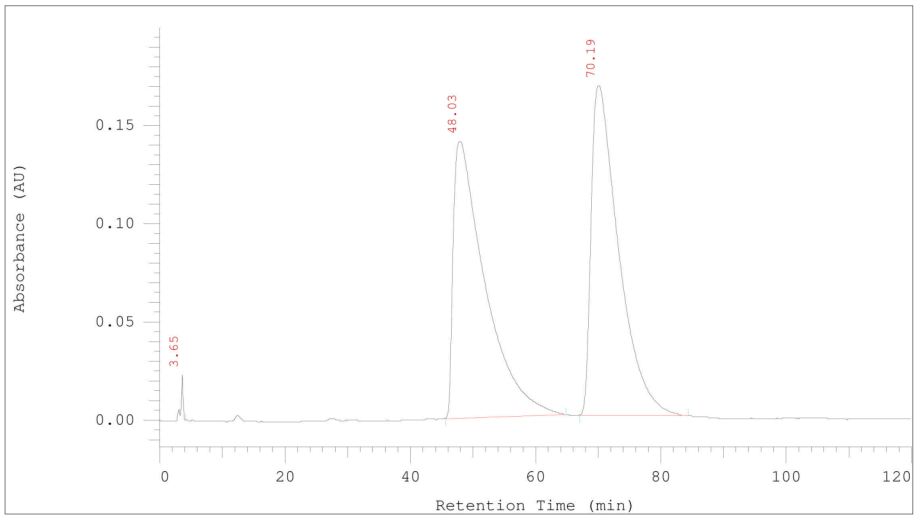


Figure S3. Chiral HPLC of racemate (*P*)/(*M*)-**2b**.

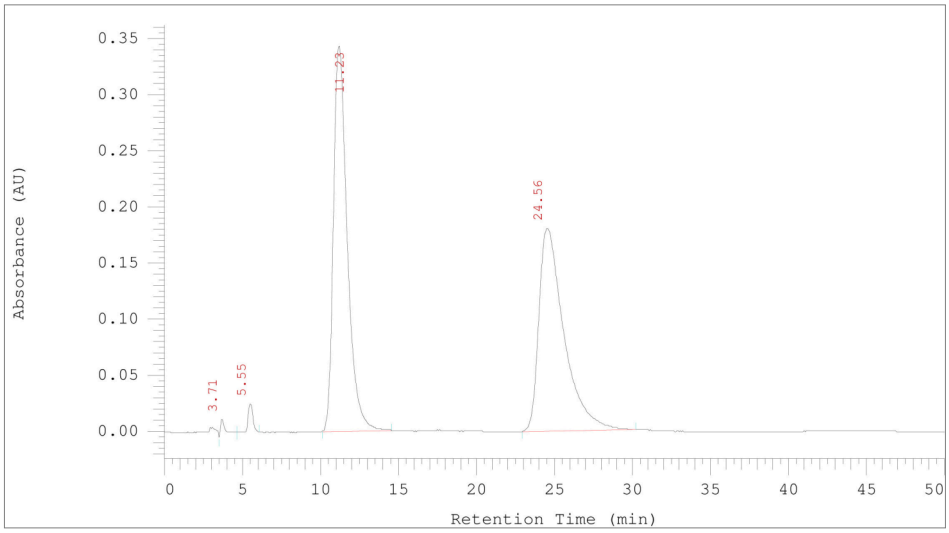


Figure S4. Chiral HPLC of racemate (*P*)/(*M*)-**2c**.

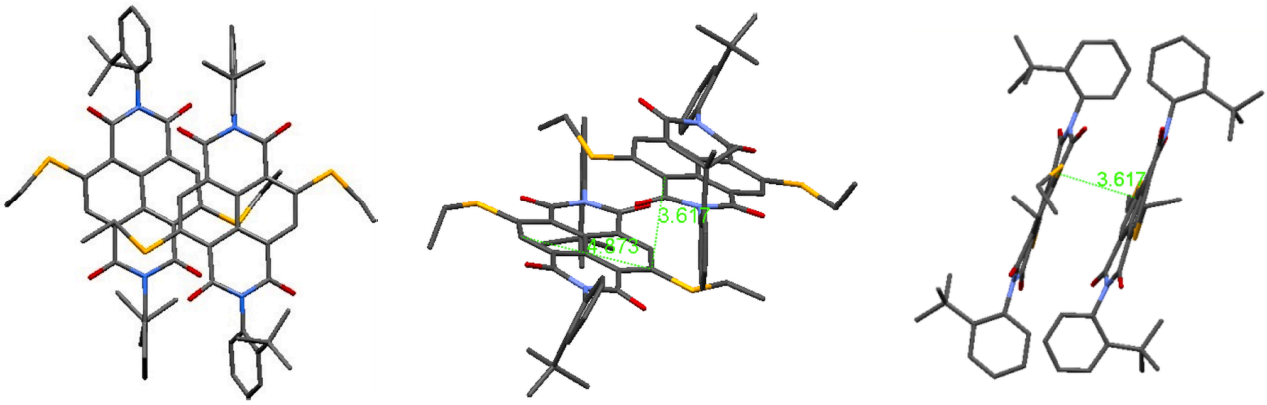


Figure S5. Crystal structure of the first peak in the HPLC of racemate (*P*)/(*M*)-**1**, assigned to (*M*)-**1**.

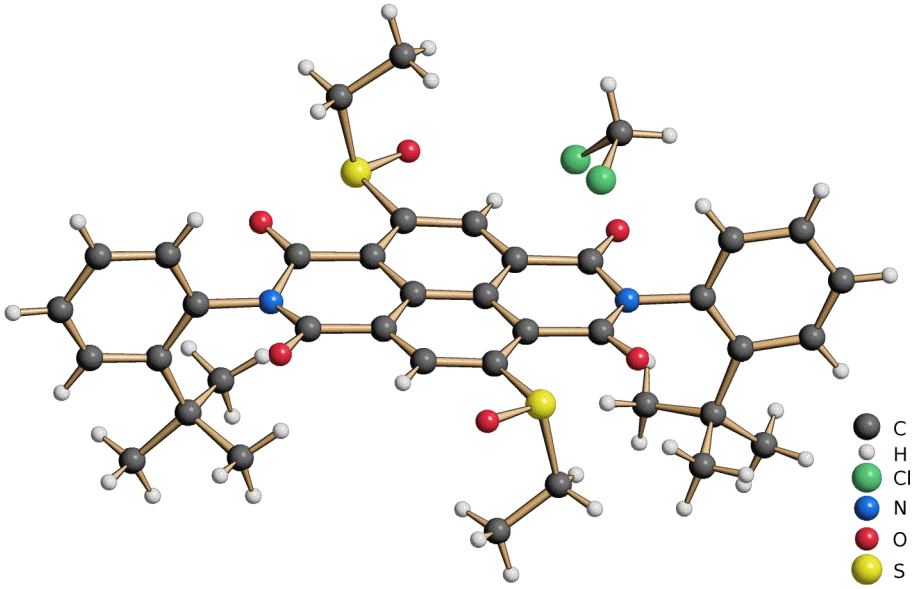
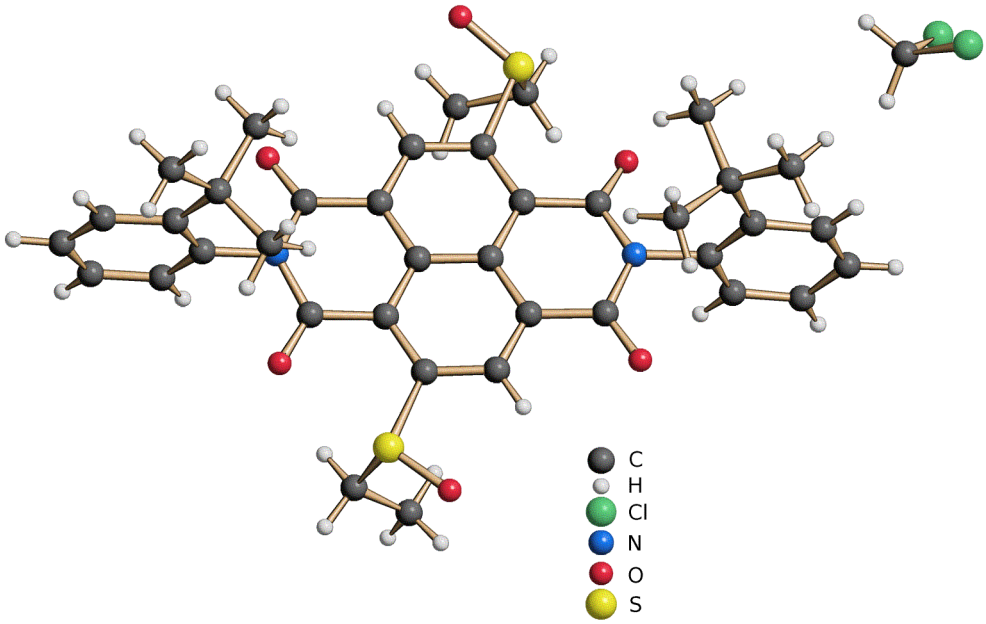


Figure S6. Crystal structure of the first peak in the HPLC of racemate (*P*)/(*M*)-**2b**, assigned to (*P*)-**2b**.



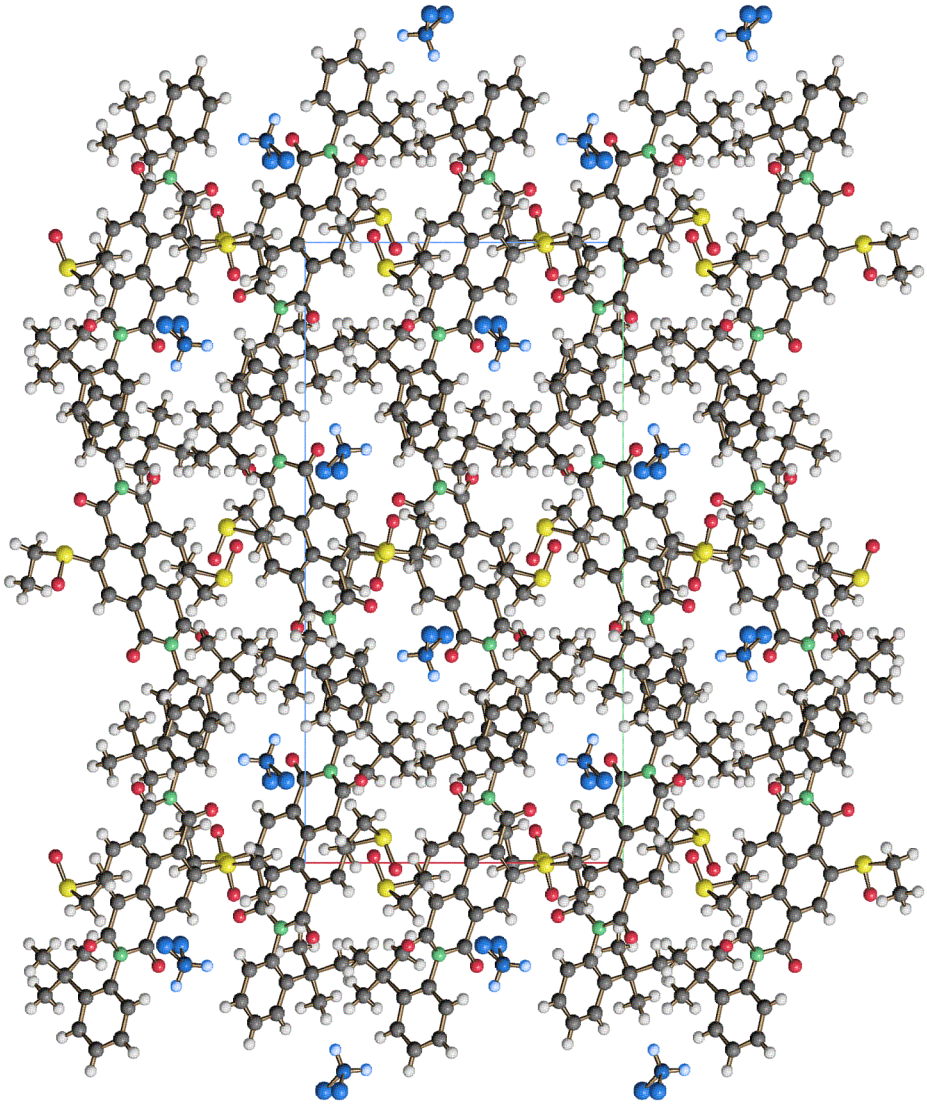


Figure S7. Crystal structure of the first peak in the HPLC of racemate (*P*)/(*M*)-**2a**, assigned to (*P*)-**2a**.

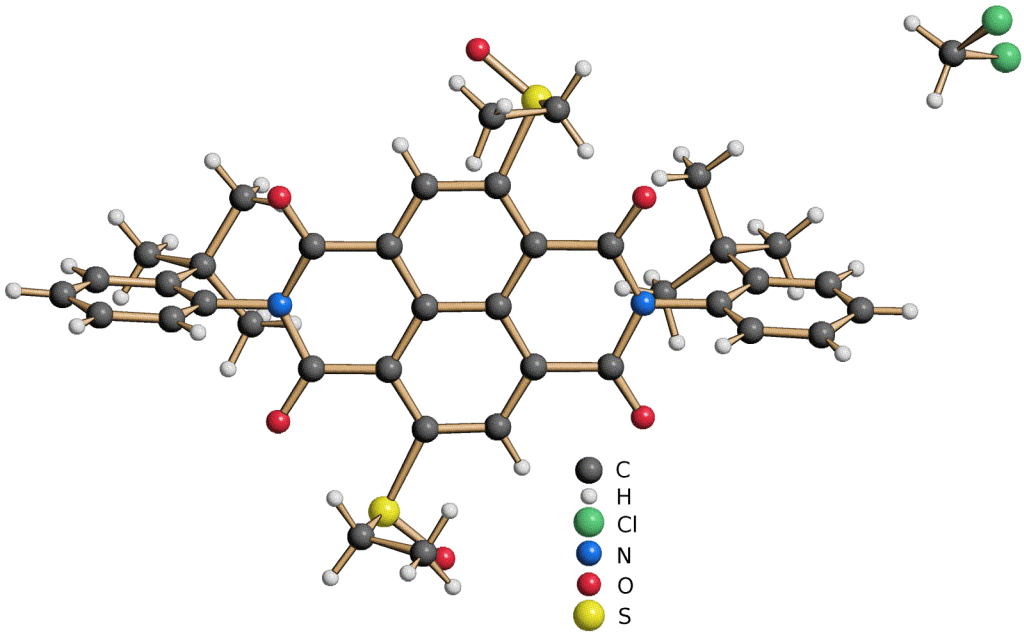


Figure S8. Crystal structure of the second peak in the HPLC of racemate (*P*)/(*M*)-**2a**, assigned to (*M*)-**2a**.

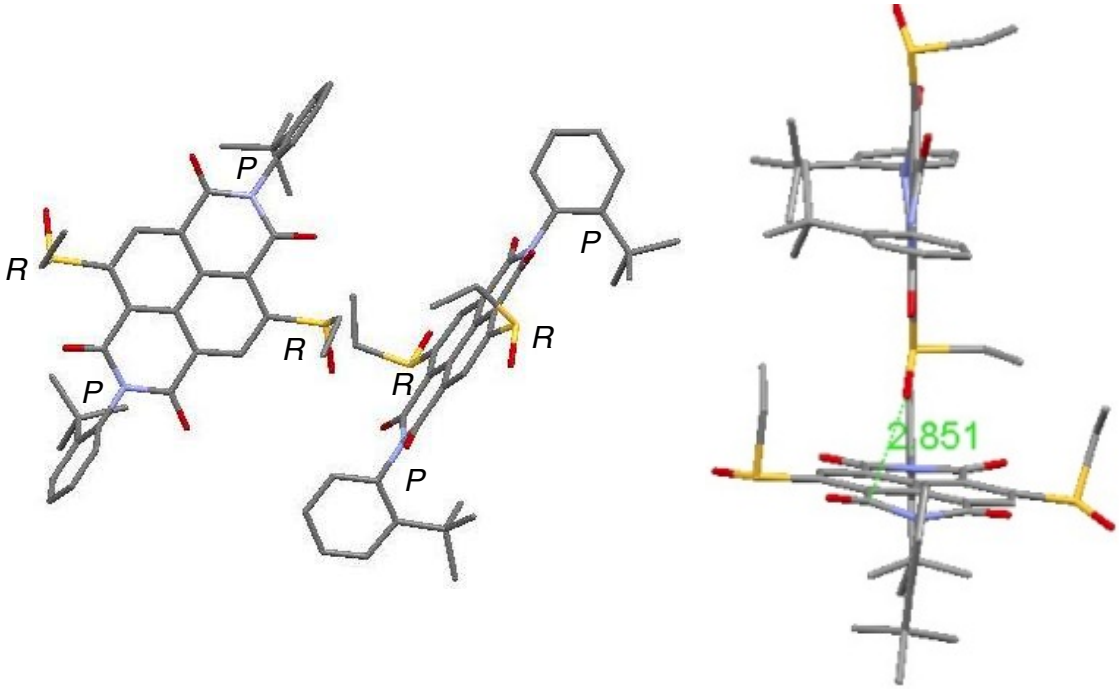


Figure S9. Crystal structure obtained from racemate (*P*)/(*M*)-**2a**, assigned to (*P*)-**2a.**

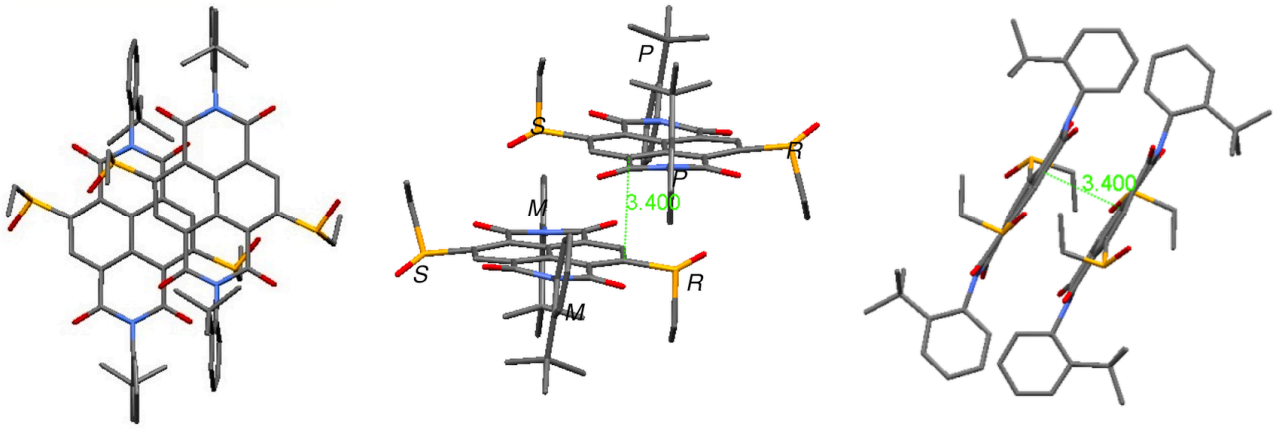


Figure S10. Crystal structure obtained from racemate (*P*)/(*M*)-**2b**, assigned to heterodimers (*P*)/(*M*)-**2b**.

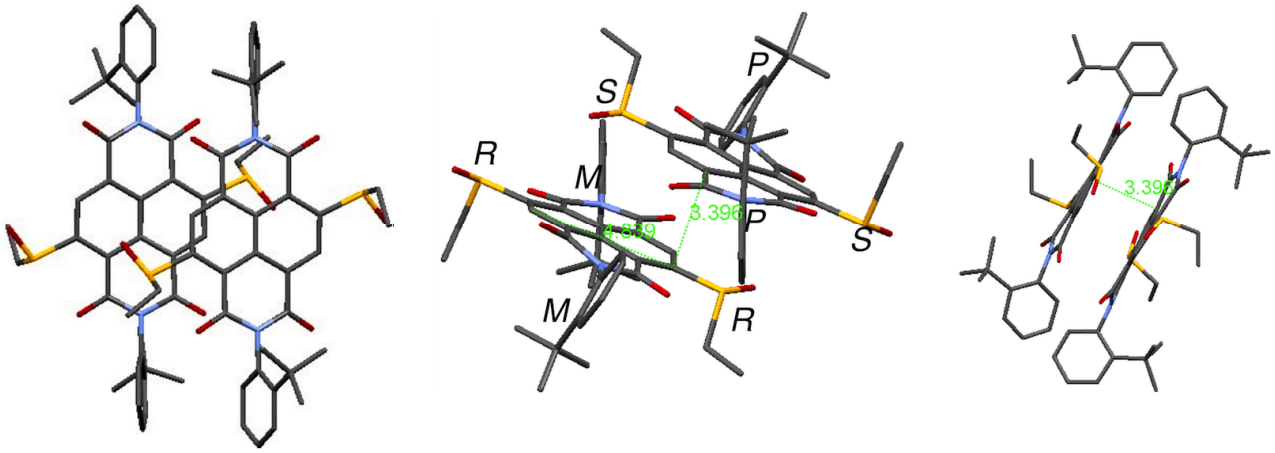


Figure S11. Crystal structure obtained from racemate (*P*)/(*M*)-**2c**, assigned to heterodimers (*P*)/(*M*)-**2c**.

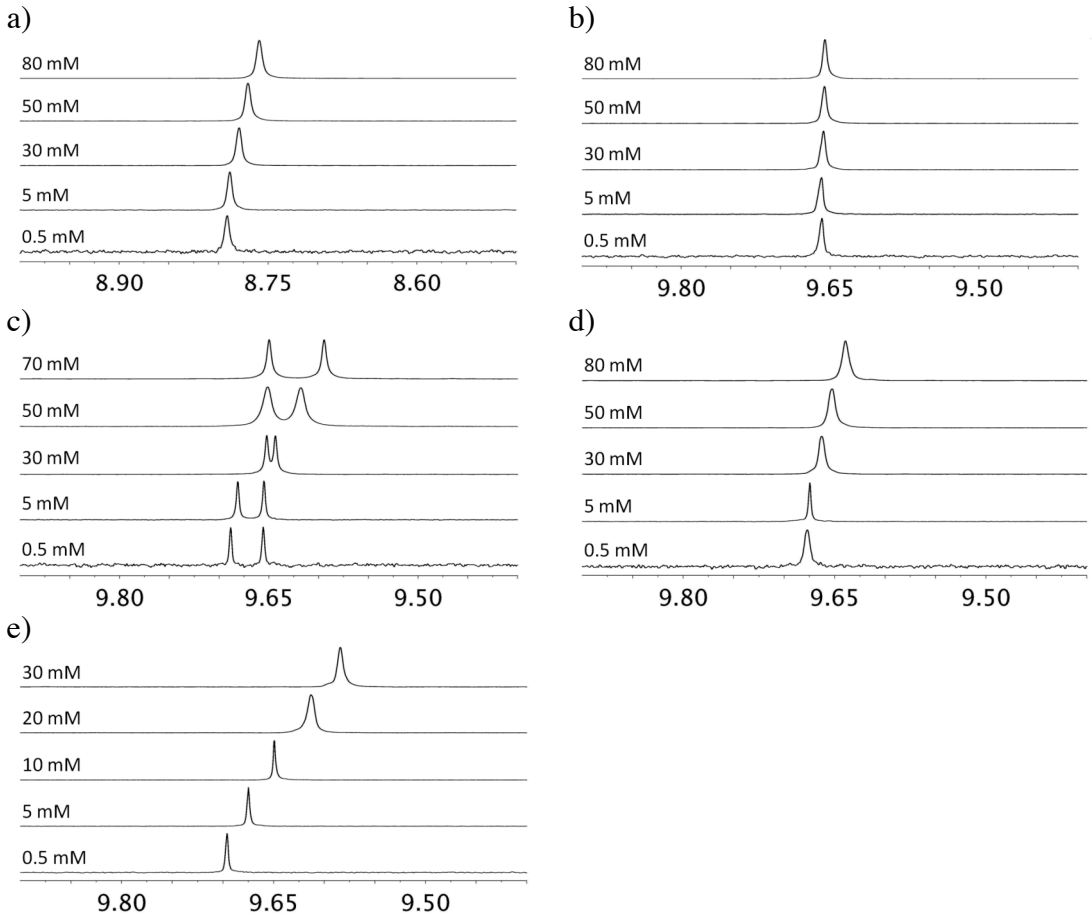


Figure S12. ^{1}H NMR spectra of the aromatic region of a) (*P*)/(*M*)-**1**; b) (*P*)/(*M*)-**2a**; c) (*P*)/(*M*)-**2b**; d) (*P*)/(*M*)-**2c**; e) (*P*)/(*M*)-**3** in $CDCl_3$ at 25 ºC. Chemical shifts were referred to internal TMS. The two hydrogens of (*P*)/(*M*)-**2b** on the core position are diastereotopic.

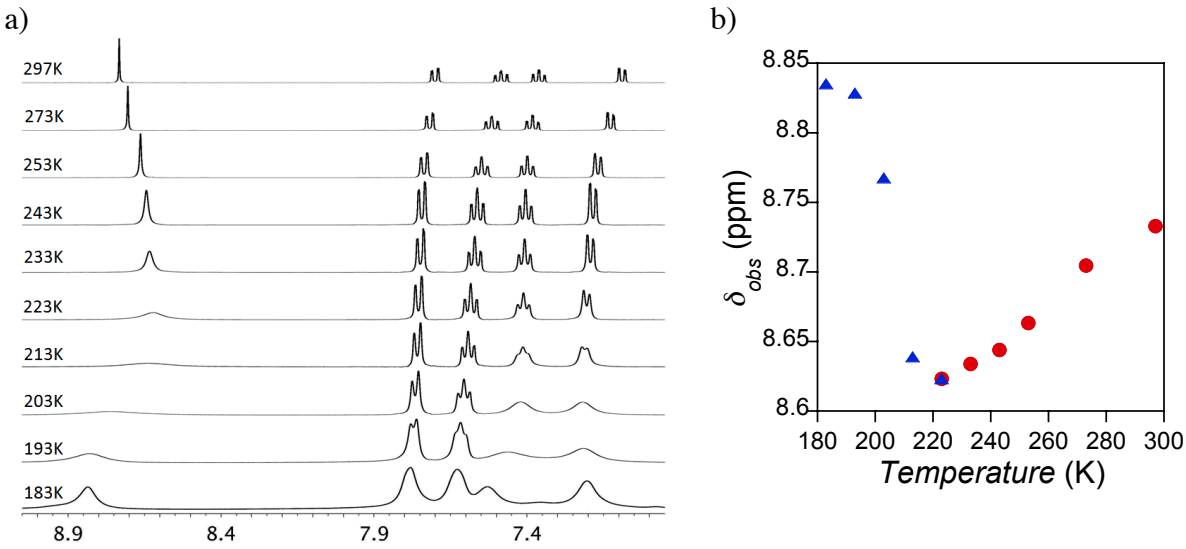


Figure S13. a) Variable-temperature ^{1}H NMR (400 MHz) spectra of racemate (*P*)/(*M*)-**1** in CD_2Cl_2 (c = 0.1 M); b) Temperature-dependent ^{1}H NMR signals of (*P*)/(*M*)-**1**.

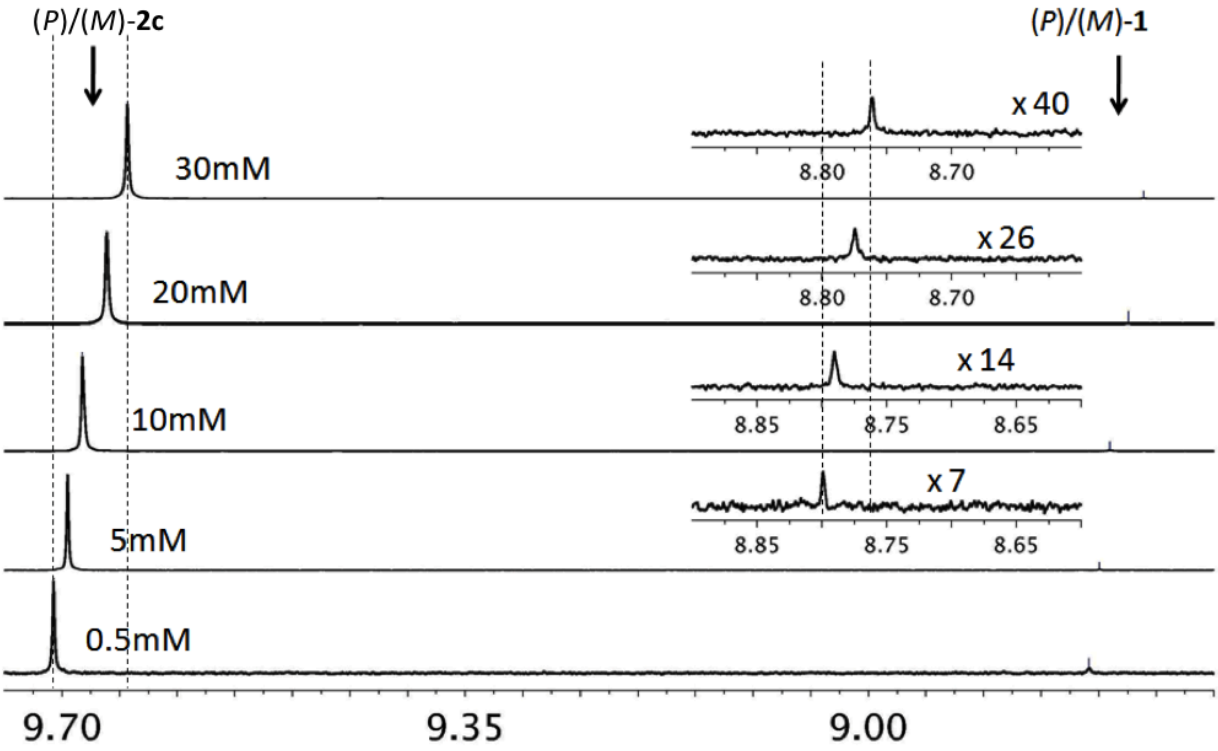


Figure S14. Dimerization of two molecules in solution. A dilute solution of (*P*)/(*M*)-**1** (0.05 mM) was titrated with (*P*)/(*M*)-**2c** in $CDCl_3$/Cyclohexane-d_{12}(3 : 2) at 25 ºC. (*P*)/(*M*)-**1** concentration was kept constant (0.05 mM) and concentration of (*P*)/(*M*)-**2c** varied from 0.5 mM to 30 mM. The (*P*)/(*M*)-**1** protons experience upfield shift as (*P*)/(*M*)-**2c** concentration varied from 0.05 mM to 30 mM indicating the formation of heterodimer in the solution.

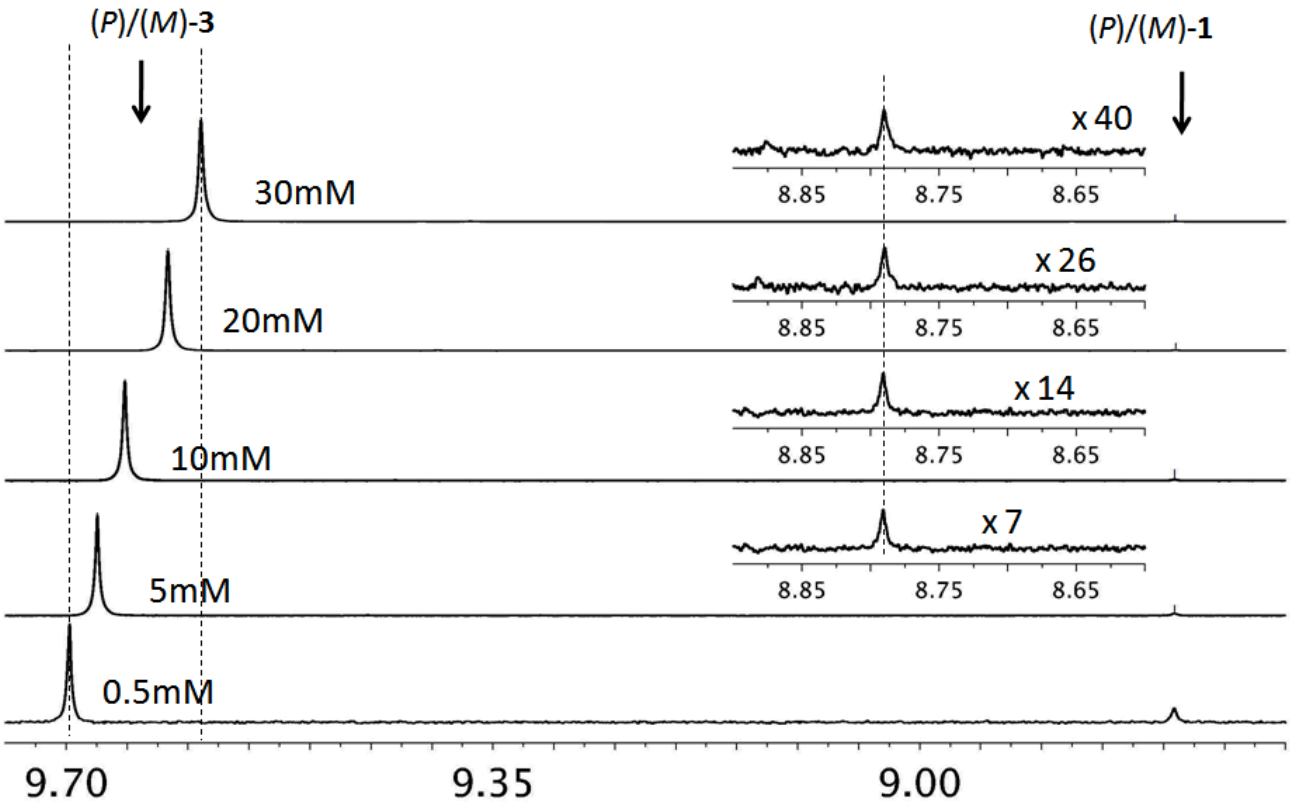


Figure S15. Dimerization of two molecules in solution. A dilute solution of (*P*)/(*M*)-**1** (0.05 mM) was titrated with (*P*)/(*M*)-**3** in $CDCl_3$ at 25 ºC. (*P*)/(*M*)-**1** concentration was kept constant (0.05 mM) and concentration of (*P*)/(*M*)-**3** varied from 0.5 mM to 30 mM. The (*P*)/(*M*)-**1** signal doesn't shift in the presence of increasing (*P*)/(*M*)-**3** suggesting the two molecules are independent in solution.

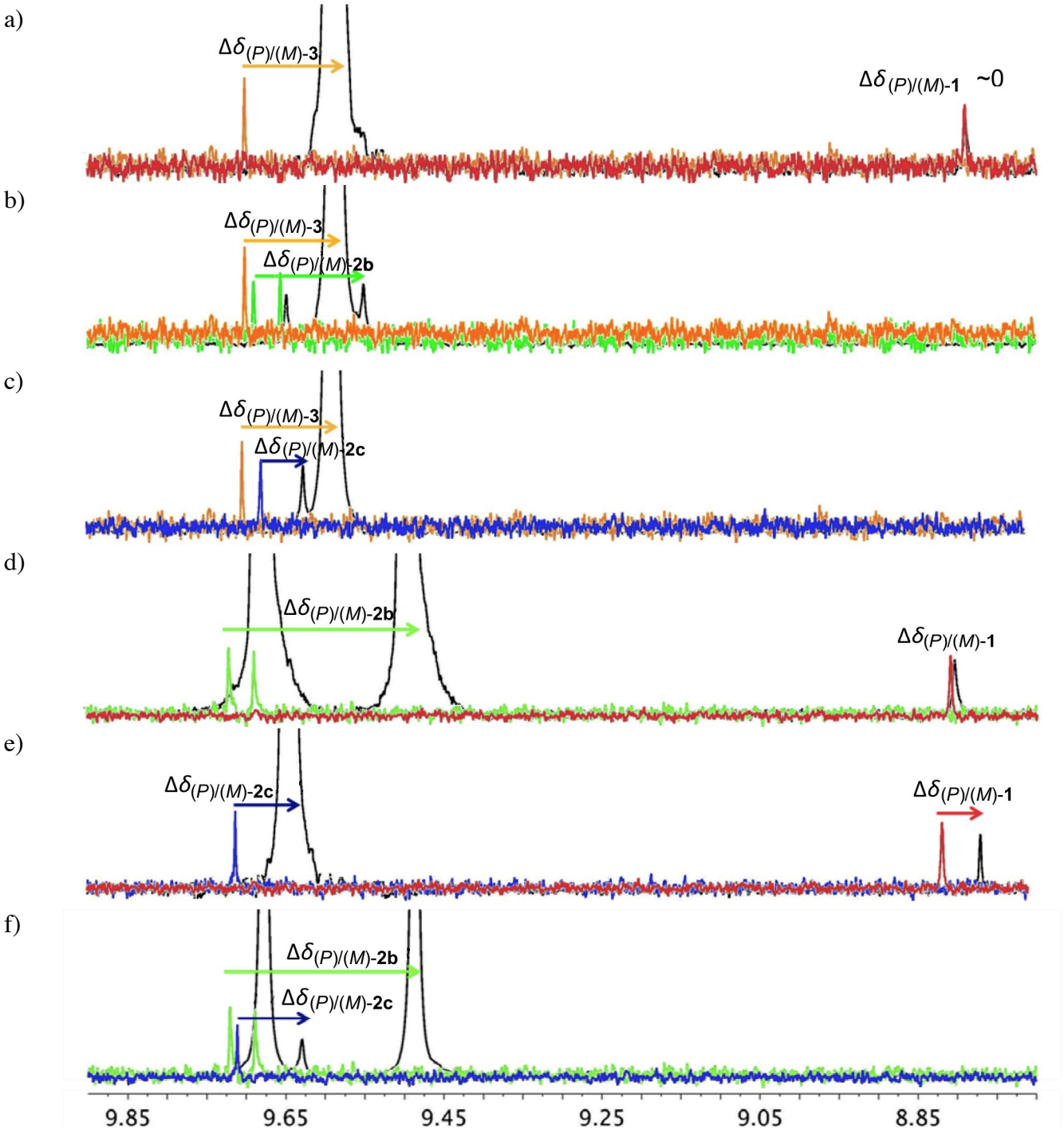


Figure S16. ^{1}H NMR spectra used to estimate extent of self-sorting. A trace amount of NDIs (0.05 mM) was added to another concentrated NDIs (30 mM), chemical shifts of each NDIs in the mixture solution were recorded and compared to their chemical shift in dilute solution. Mixture solutions are shown in black and dilute monomeric cNDIs are shown in color. (a), (b) and (c) in $CDCl_3$ at 25 ºC; (d), (e) and (f) in $CDCl_3$/Cyclohexane-d_{12} 3:2 at 25 ºC.

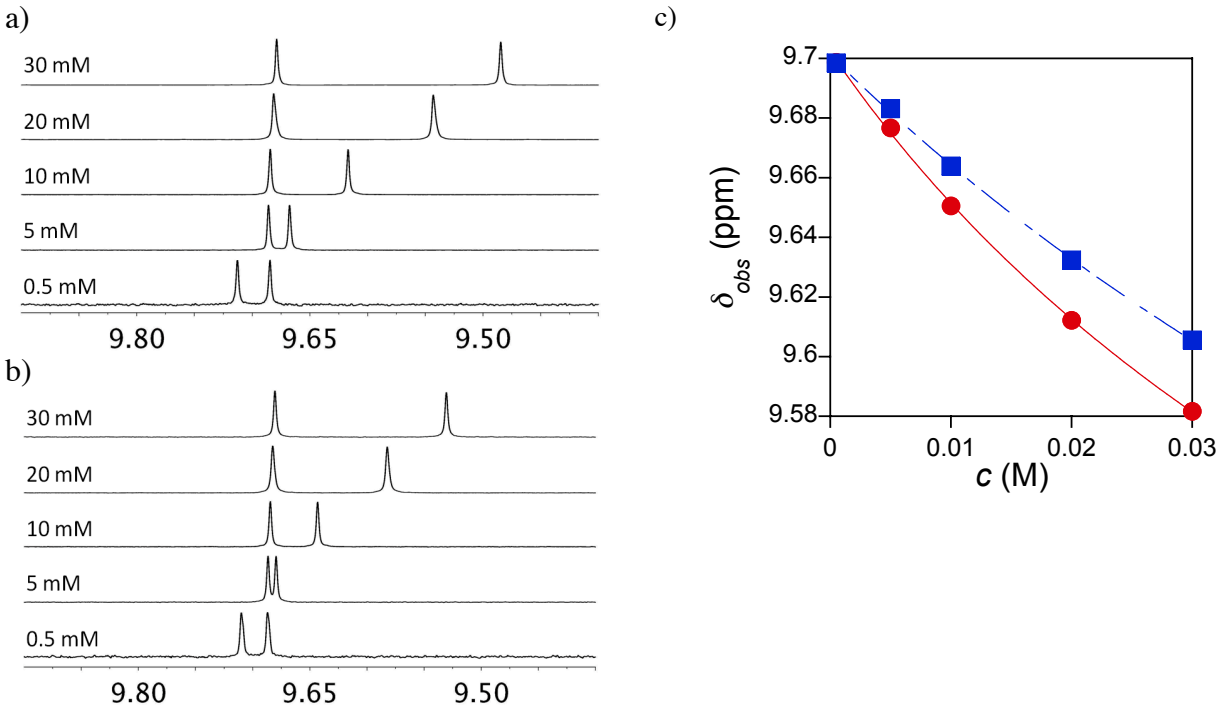


Figure S17. ^{1}H NMR spectra of the aromatic region of a) racemate (*P*)/(*M*)-**2b** and (b) enantiomer (*M*)-**2b** in $CDCl_3$/Cyclohexane-d_{12} (3 : 2) at 25 ºC. c) Concentration dependent ^{1}H NMR signals of enantiomer (■) and racemate (●) in $CDCl_3$/Cyclohexane-d_{12} (3 : 2) at 25ºC. The concentration dependent ^{1}H NMR signals fitted well to the equation S3 and gave dimerization constant as K_{rac} = 4.3 ± 0.9 M^{-1} and K_{homo} = 2.2 ± 0.6 M^{-1} for racemate and enantiomer, respectively.

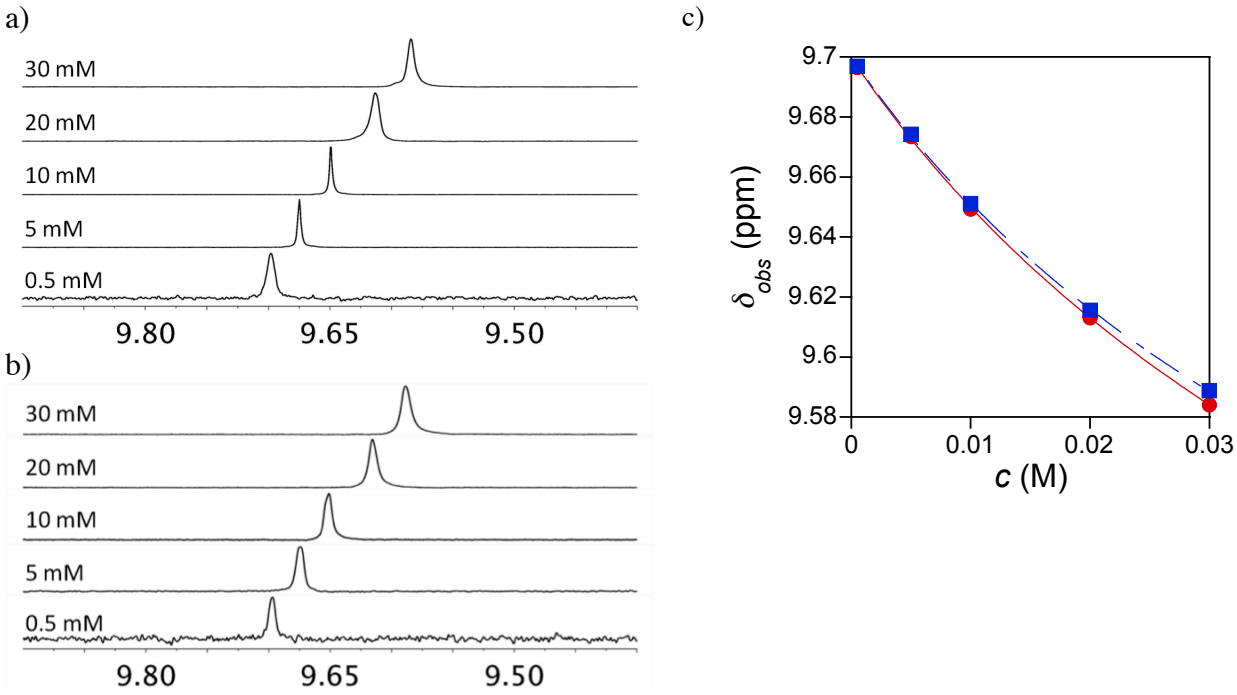


Figure S18. ^{1}H NMR spectra of the aromatic region of a) racemate (*P*)/(*M*)-**3** and (b) enantiomer (*M*)-**3** in $CDCl_3$ at 25ºC. c) concentration dependent ^{1}H NMR signals of enantiomer (■) and racemate (●) in $CDCl_3$ at 25ºC. The concentration dependent ^{1}H NMR signals fitted well to the equation S3 and gave dimerization constant as K_{rac} = 5.4 ± 0.5 M^{-1} and K_{homo} = 5.0 ± 0.5 M^{-1} for racemate and enantiomer, respectively.

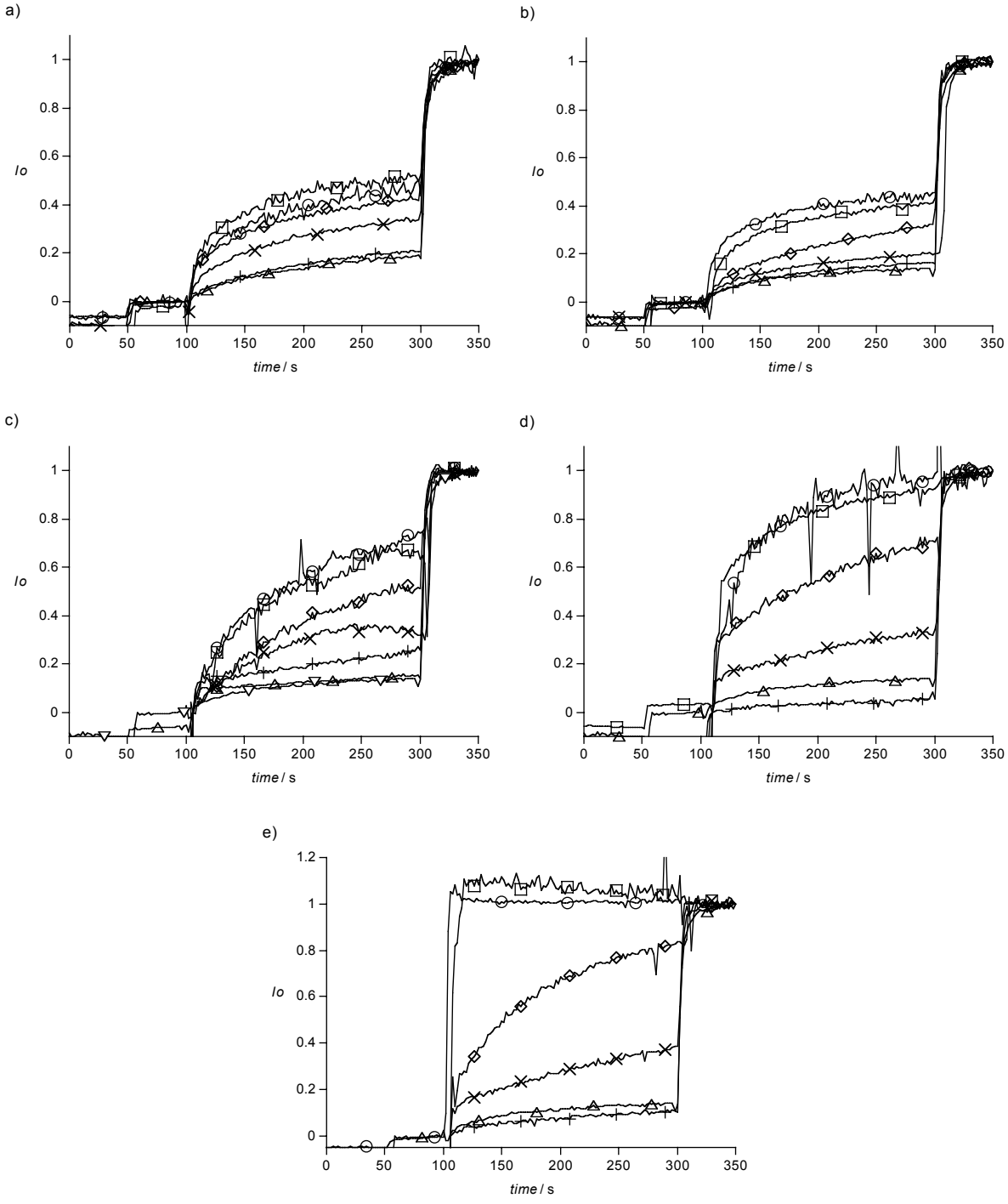


Figure S19. Fluorescence traces for compounds **1** - **2** in the HPTS assay. a) Fractional emission I_F during the addition of NaOH (20 μl, 0.5 M, 50 sec) (*meso*)-**1** (with increasing activity: 0.5 μM (△), 1 μM (+), 5 μM (x), 10 μM (◇), 30 μM (□), 50 μM (○), 40 μl, 100 sec) and gramicidin D (20 μl, 100 μM, 300 sec) to EYPC-LUVs ⊃ HPTS in NaCl (100 mM, 10 mM Hepes buffer, pH 7). b) Same for (*M*)-**1** (with increasing activity: 1 μM (△), 5 μM (+), 10 μM (x), 30 μM (◇), 40 μM (□), 50 μM (○), 40 μl, 100 sec). c) Same for (*M*)-**2c** (with increasing activity: 0.1 μM (▽), 5 μM (△),

10 μM (+), 20 μM (x), 30 μM (◇), 40 μM (□), 50 μM (○), 40 μl, 100 sec). d) Same for (*P*)-**2b** (with increasing activity: 0.1 μM (△), 5 μM (+), 10 μM (x), 15 μM (◇), 20 μM (□), 50 μM (○), 40 μl, 100 sec). e) Same for (*M*)-**2a** (with increasing activity: 0.1 μM (△), 5 μM (+), 7.5 μM (x), 10 μM (◇), 20 μM (□), 50 μM (○), 40 μl, 100 sec).

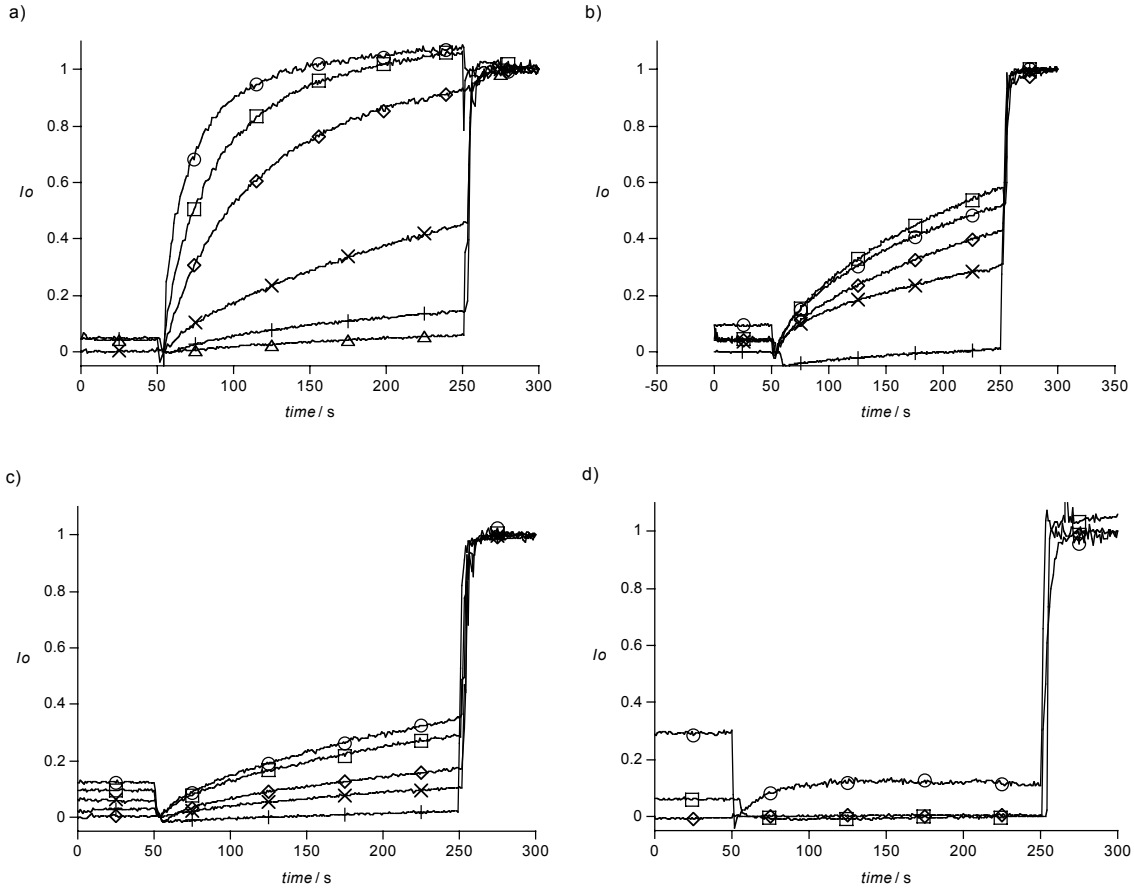


Figure S20. Non-specific leakage (CF assay) for compounds **1 - 3**. a) Fractional emission I_F during the addition of (*M*)-**2a** (with increasing activity: 5 μM (△), 10 μM (+), 20 μM (x), 30 μM (◇), 40 μM (□), 50 μM (○), 20 μl, 50 sec) and Triton X-100 (40 μl, 1.2%, 250 sec) to EYPC-LUVs ⊃ CF in NaCl (107 mM, 10 mM Hepes buffer, pH 7). b) Same for (*P*)-**2b** (with increasing activity: 5 μM (+), 10 μM (x), 30 μM (◇), 40 μM (□), 50 μM (○), 40 μl, 50 sec). c) Same for (*M*)-**2c** (with increasing activity: 5 μM (+), 10 μM (x), 20 μM (◇), 40 μM (□), 50 μM (○), 40 μl, 50 sec). d) Same for (*meso*)-**1** (100 μM, ○), (*M*)-**1** (100 μM, □) and (*M*)-**3** (100 μM, ◇).

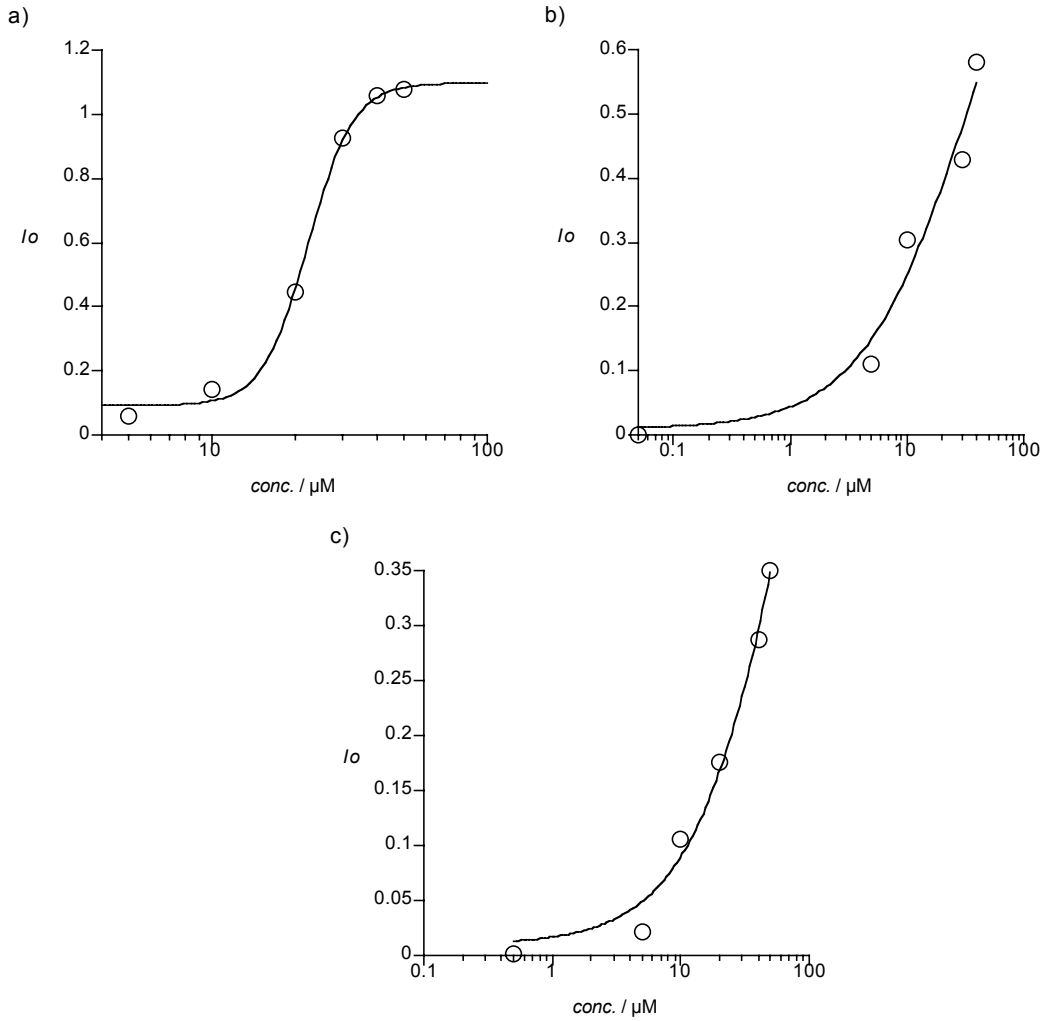


Figure S21. Dose response curves for compounds (*M*)-**2a** (a), (*P*)-**2b** (b) and (*M*)-**2c** (c) in the CF assay (from Figs. S20a, S20b and S20c).

4. Supporting Tables

Table S1. cNDIs dimerization constants at 25ºC.

sample	Solvent $CDCl_3$/CH-d_{12}	$K_{D\ (racemic)}$ [b] / M^{-1}	$K_{D\ (homo)}$ [b] / M^{-1}	$K_{D\ (hetero)}$ [a] / M^{-1}	[hetero]/[homo] $K_{D(hetero)}/2K_{D(homo)}$	% homo
(*P*)/(*M*)-**1**	1:4	28.3	16.9	79.4	2.3	30
(*P*)/(*M*)-**2b**	2:3	4.3	2.2	12.8	2.9	25
(*P*)/(*M*)-**3**	5:0	5.4	5.0	11.6	1.1	48

[a]The values were obtained according to equation S4. [b]Determined from concentration dependent ^{1}H NMR experiments (Figs. 4e, S17, S18) and equation S3.

5. Supporting References

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