Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Length dependent reversible off-on activation of photo-switchable relay anion transporters

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1 Materials and Methods

All reagents and solvents were purchased from commercial sources and used without further purification. Lipids were purchased from Avanti polar lipids and used without further purification. Where necessary, solvents were dried by passing through an MBraun MPSP-800 column and degassed with nitrogen. Triethylamine was distilled from and stored over potassium hydroxide. Normal phase silica gel flash column chromatography was performed either manually using Merck® silica gel 60 under a positive pressure of nitrogen. Where mixtures of solvents were used, ratios are reported by volume. NMR spectra were recorded on a Bruker AVIII 400, Bruker AVII 500 (with cryoprobe) and Bruker AVIII 500 spectrometers. Chemical shifts are reported as δ values in ppm. Mass spectra were carried out on a Waters Micromass LCT and Bruker microTOF spectrometers. Fluorescence spectroscopic data were recorded using a Horiba Duetta fluorescence spectrophotometer, equipped with a Peltier temperature controller and stirrer. UV-Vis spectra were recorded on a V-770 UV-Visible/NIR Spectrophotometer equipped with a Peltier temperature controller and stirrer, using quartz cuvettes of 1 cm path length. Experiments were conducted at 25 °C unless otherwise stated. Vesicles were prepared as described below using Avestin "LiposoFast" extruder apparatus, equipped with polycarbonate membranes with 200 nm pores. GPC purification of vesicles was carried out using GE Healthcare PD-10 desalting columns prepacked with Sephadex G 25 medium.

Abbreviations

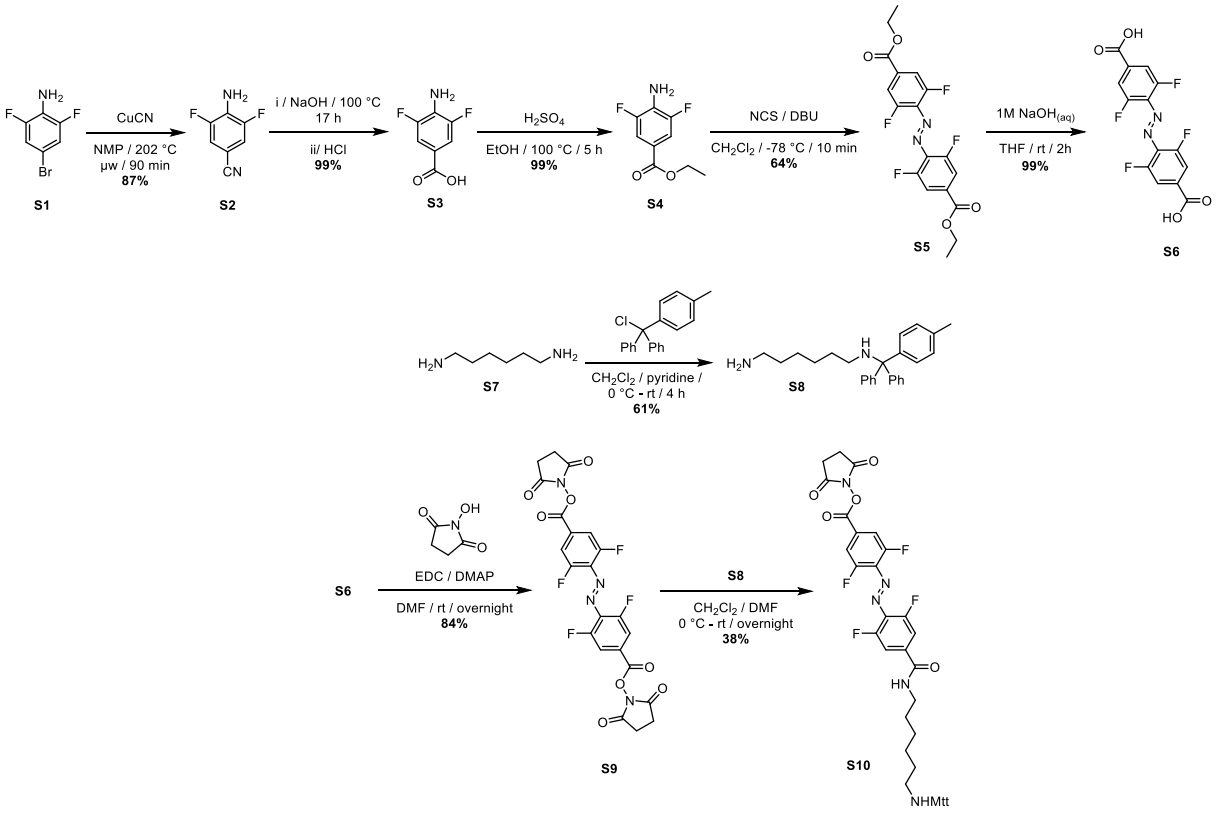
14:0 PC: 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC); **16:1 PC**: 1,2-Dipalmitoleoyl-*sn*-glycero-3-phosphocholine; **18:1 PC**: 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC); **20:1 PC**: 1,2-Dieicosenoyl-*sn*-glycero-3-phosphocholine; **DBU**: 1,8-Diazabicyclo[5.4.0]undec-7-ene; **DIC**: *N*,*N*-Diisopropylcarbodiimide; **DMAP**: 4-Dimethylaminopyridine; **DPPC**: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine; **EDC**: 1-Ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride; **Fmoc**: 9-Fluorenylmethoxycarbonyl; **HEPES**: *N*-(2-Hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid); **HPTS**: 8-Hydroxy-1,3,6-pyrenetrisulfonate; **HRMS**: High resolution mass spectrometry; **LED**: Light-emitting diode; **LUVs**: Large unilamellar vesicles; **Mtt**: 4-Methyltrityl; **NCS**: *N*-Chlorosuccinimide; **NHS**: *N*-Hydroxysuccinimide; **POPC**: 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; **PSS**: Photo-stationary state; **rt**: Room temperature; **μw**: Microwave irradiation.

2 Synthesis and Characterization

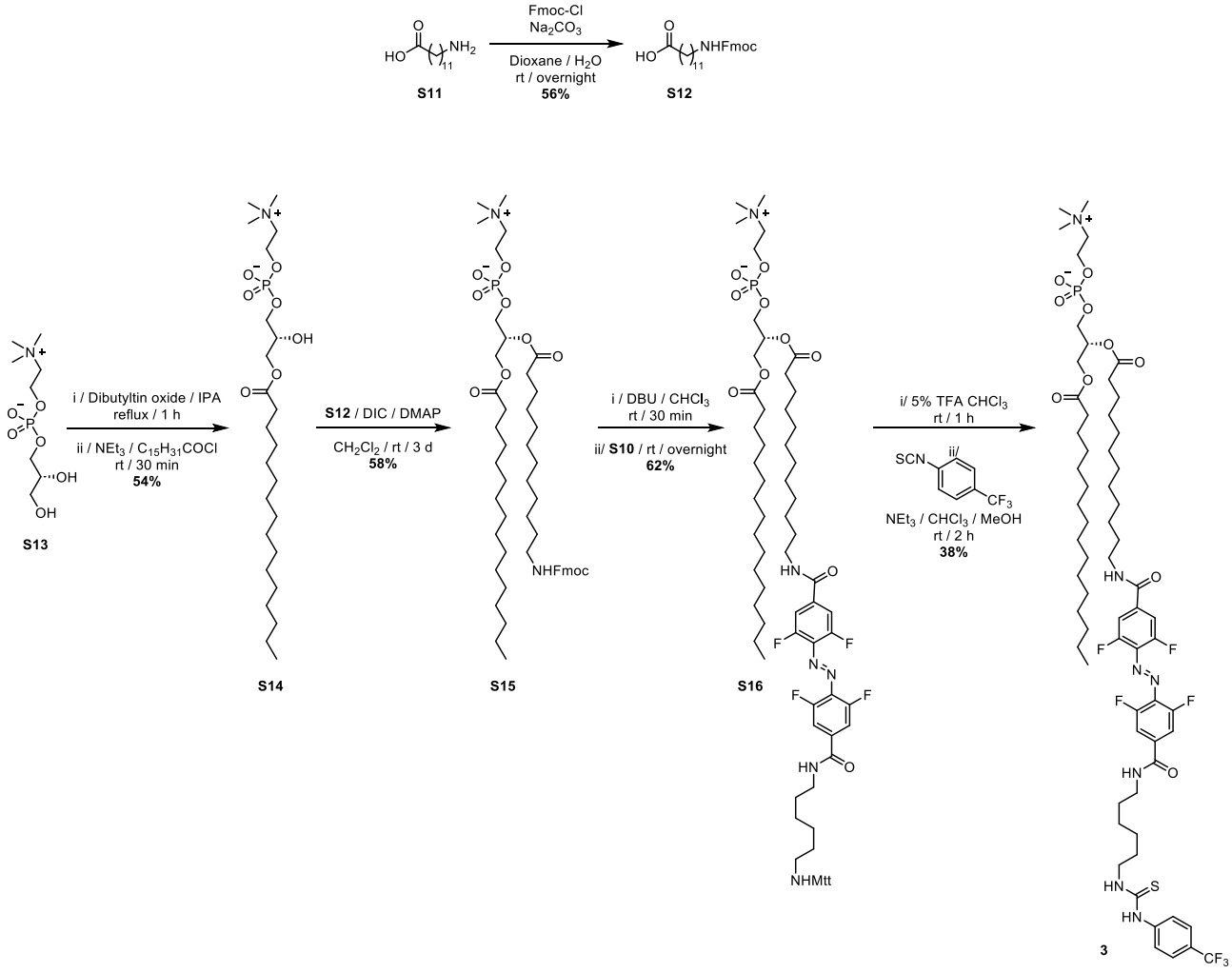
2.1 General comments.

Compounds **S2**,[1] **S3**,[2] **S4**,[2] **S5**,[3] **S6**,[4] **S9**,[5] **S12**,[6] **S14**[7] and **S15**[1] were prepared according to literature procedures.

Peaks for the azobenzene *E* isomer major product are reported in cases where a minor proportion of Z isomer was also formed.

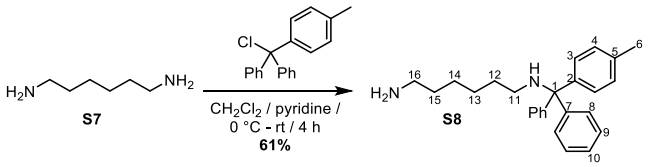


Scheme S1. *Synthesis of azobenzene core* ***S10****.*



***Scheme S2**. Synthesis of longest anion relay transporter **3**.*

2.2 *N*'-[(4-methylphenyl)-diphenylmethyl]hexane-1,6-diamine S8



To a solution of hexane-1,6-diamine **S7** (1.96 g, 16.9 mmol, 9.0 equiv.) in anhydrous CH_2Cl_2 (4 mL) and dry pyridine (2.83 mL) was added 4-methyltrityl chloride (550 mg, 1.88 mmol, 1.0 equiv.) at 0 °C. The reaction mixture was stirred for 4 h at rt, under N_2. The reaction was quenched with MeOH (0.5 mL) and concentrated to dryness *in vacuo*. The crude residue was dissolved in CH_2Cl_2 (1% NEt_3)/H_2O 1:1 (50 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* to afford the title compound as a white solid (425 mg, 1.14 mmol, 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (dt, *J* = 8.2, 1.7 Hz, 4H, H_8), 7.34 (d, *J* = 8.2 Hz, 2H, H_3), 7.26 (td, *J* = 7.7, 3.6 Hz, 4H, H_9), 7.20 – 7.14 (m, 2H, H_{10}), 7.08 (d, *J* = 8.2 Hz, 2H, H_4), 2.71 (t, *J* = 7.2 Hz, 2H, H_{16}), 2.31 (s, 3H, H_6), 2.18 – 2.07 (m, 2H, H_{11}), 1.53 – 1.43 (m, 4H, $H_{12\ \&\ 15}$), 1.34 – 1.24 (m, 4H, $H_{13\ \&\ 14}$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.6, 143.5, 135.8, 128.8, 128.7, 128.6, 127.8, 126.2, 70.7, 43.6, 41.8, 32.5, 31.0, 27.3, 26.9, 21.1.

HRMS-ESI (m/z) Calculated for $C_{26}H_{32}N_2Na$ $[M+Na]^+$, 395.2458; found 395.2458.

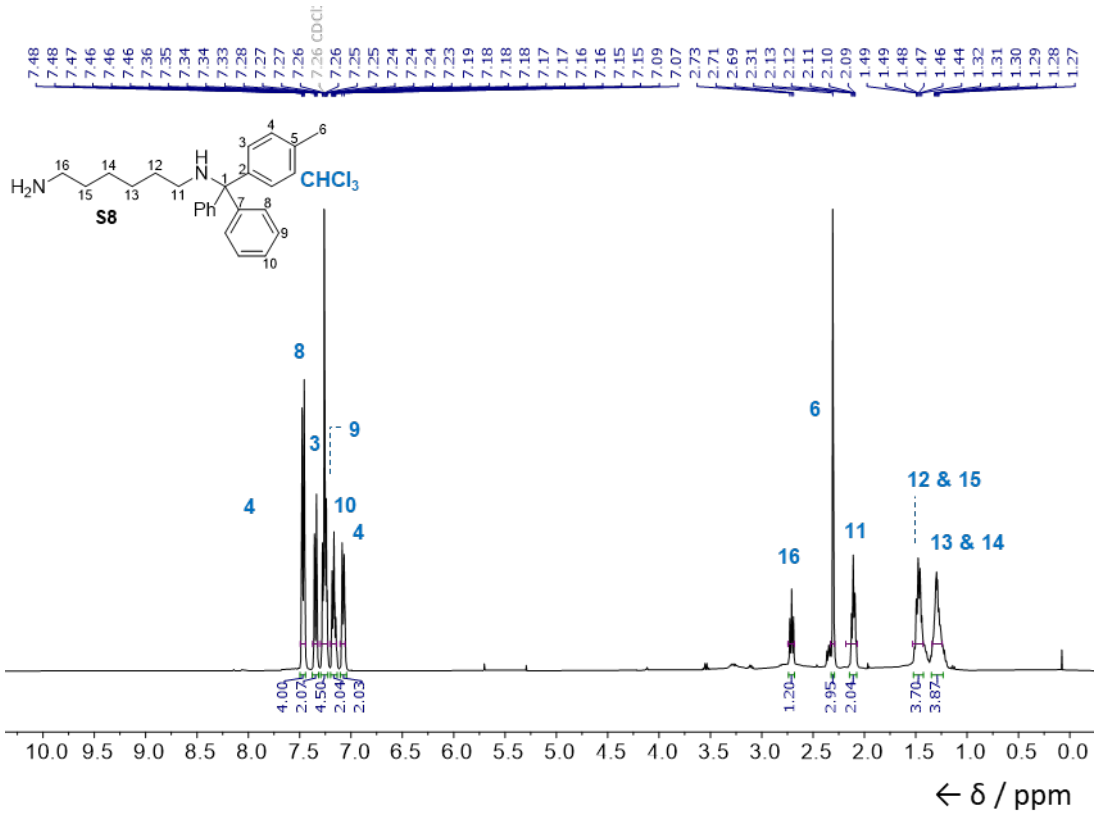


Figure S1. *^{1}H NMR spectrum of compound **S8** ($CDCl_3$, 400 MHz, 298 K).*

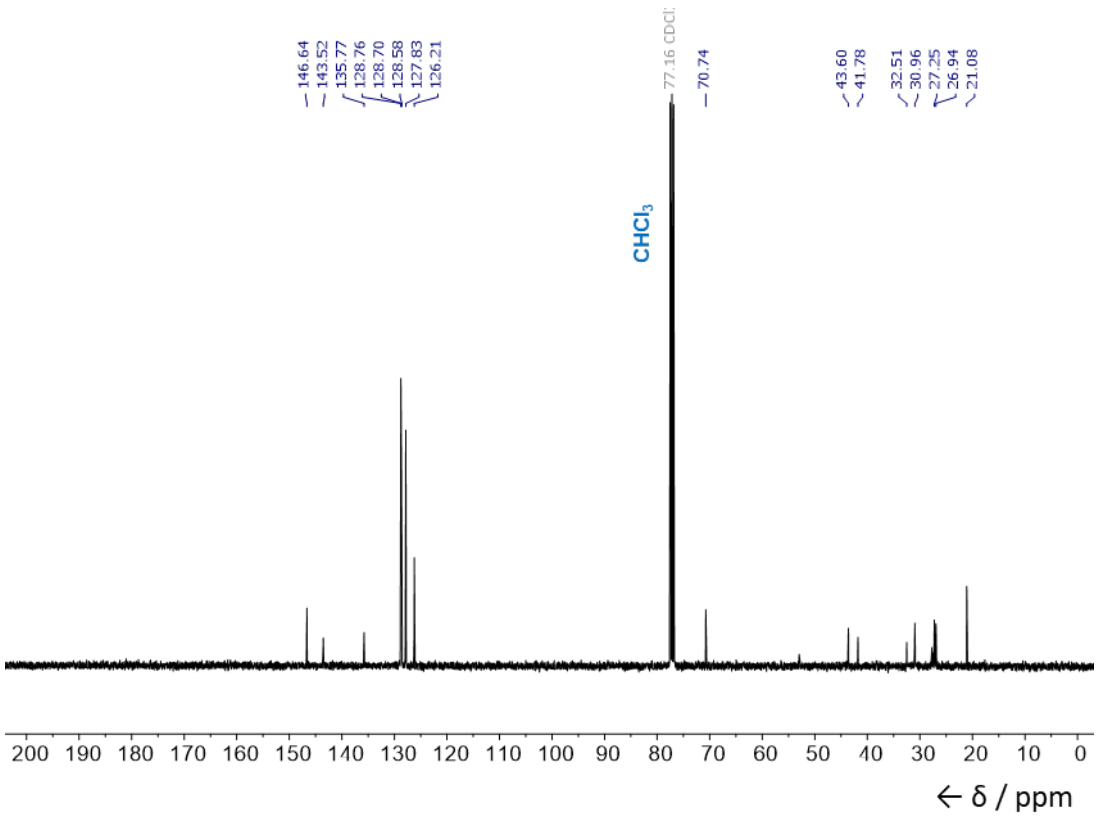


Figure S2. *^{13}C NMR spectrum of compound **S8** ($CDCl_3$, 101 MHz, 298 K).*

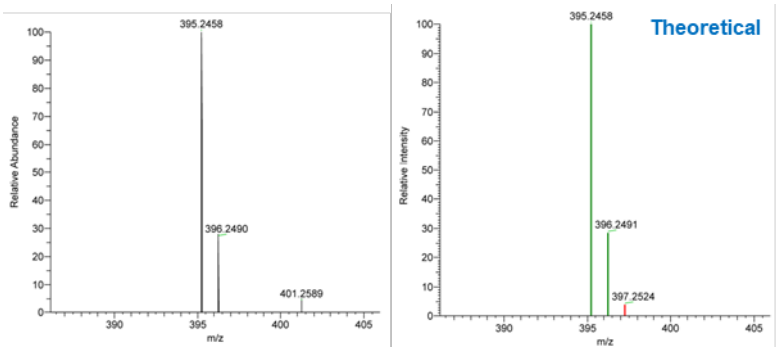
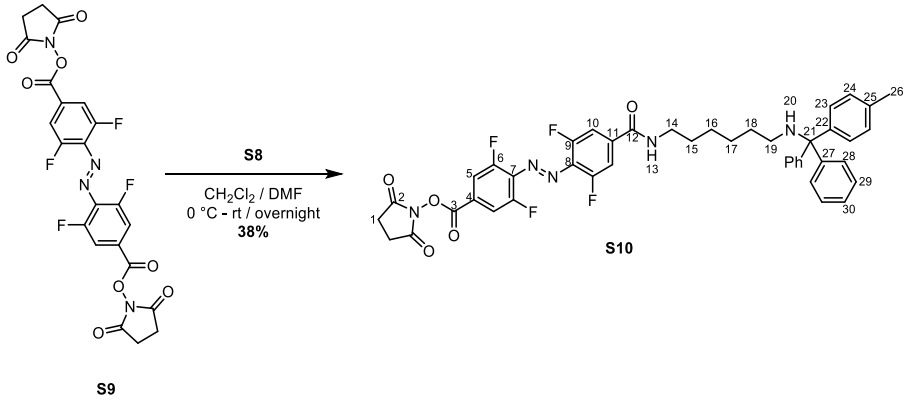


Figure S3. *HRMS spectrum of compound **S8**. HRMS-ESI (m/z) Calculated for $C_{26}H_{32}N_2Na$ $[M+Na]^+$, 395.2458; found 395.2458.*

2.3 Azobenzene S10



Azobenzene **S9** (300 mg, 0.56 mmol, 1.0 equiv.) was dissolved in anhydrous DMF (50 mL) with gentle heating, before being cooled to 0 °C. Amine **S8** (208 mg, 0.56 mmol, 1.0 equiv.) was dissolved in anhydrous CH_2Cl_2 (9 mL) and added to the reaction *via* syringe pump over 2 h. Afterwards the reaction mixture was stirred for 17 h at rt, under N_2. The reaction was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with H_2O (5 × 30 mL), brine (30 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give a crude residue which was purified by silica gel flash chromatography (EtOAc/hexane, 2:1) to afford the title compound as a red solid (169 mg, 0.21 mmol, 38%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.75 (m, 2H, $H_{5\text{ or }10}$), 7.48 – 7.38 (m, 6H, $H_{5\text{ or }10\text{ \& }28}$), 7.34 – 7.27 (m, 2H, H_{23}), 7.25 – 7.19 (m, 4H, H_{29}), 7.12 (t, *J* = 7.2 Hz, 2H, H_{30}), 7.06 – 7.02 (m, 2H, H_{24}), 6.33 (s, 1H, H_{13}), 3.38 (q, *J* = 6.8 Hz, 2H, H_{14}), 2.86 (s, 4H, H_1), 2.26 (s, 3H, H_{26}), 2.10 (q, *J* = 6.0 Hz, 2H, H_{19}), 1.60 – 1.52 (m, 2H, H_{15}), 1.51 – 1.41 (m, 2H, H_{18}), 1.35 – 1.24 (m, 4H, $H_{16\text{ \& }17}$).

^{19}F NMR (377 MHz, $CDCl_3$) δ -117.73 ($F_{6\text{ or }9}$), -118.39 ($F_{6\text{ or }9}$).

HRMS-ESI (m/z) Calculated for $C_{44}H_{40}O_5N_5F_4$ $[M+H]^+$, 794.2960; found 794.2961.

^{13}C NMR spectrum could not be acquired due to poor solubility in a range of solvents, even using a cryoprobe equipped spectrometer. However, the other spectroscopic data for this intermediate compound in the synthesis scheme is consistent with the proposed structure.

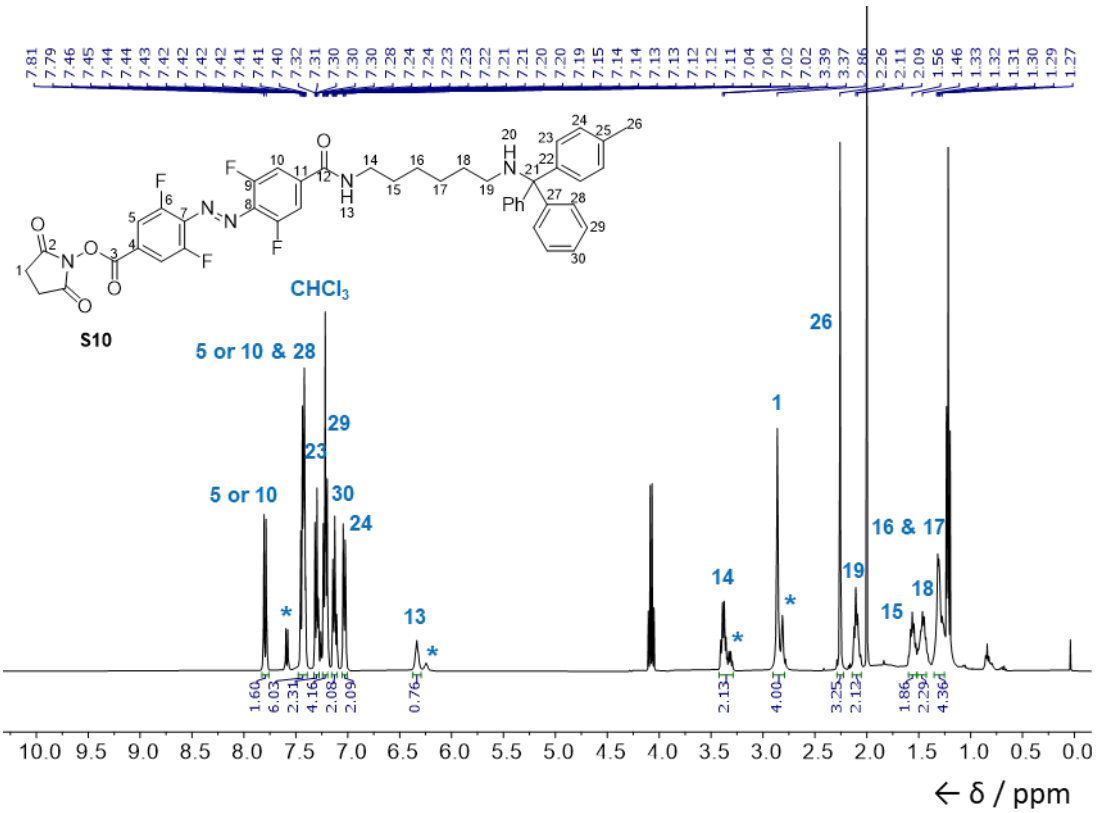


Figure S4. *^{1}H NMR spectrum of compound **S10** ($CDCl_3$, 400 MHz, 298 K).*

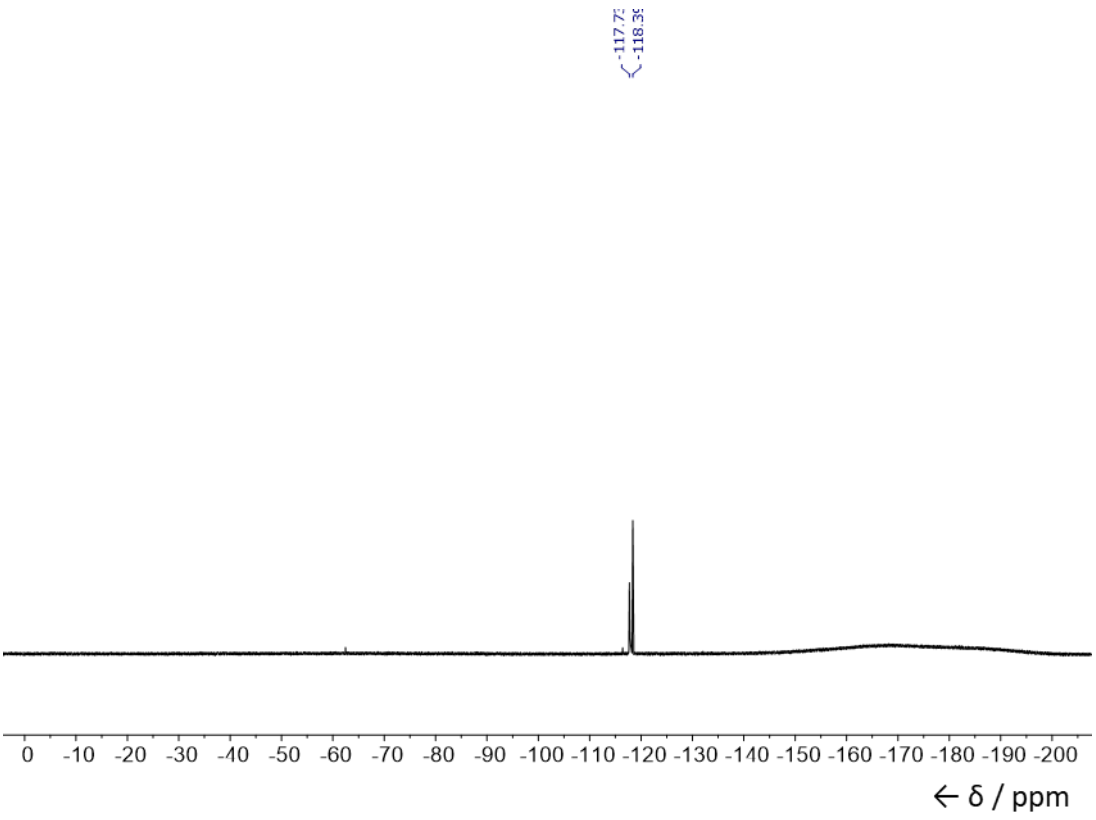


Figure S5. *^{19}F NMR spectrum of compound **S10** ($CDCl_3$, 377 MHz, 298 K).*

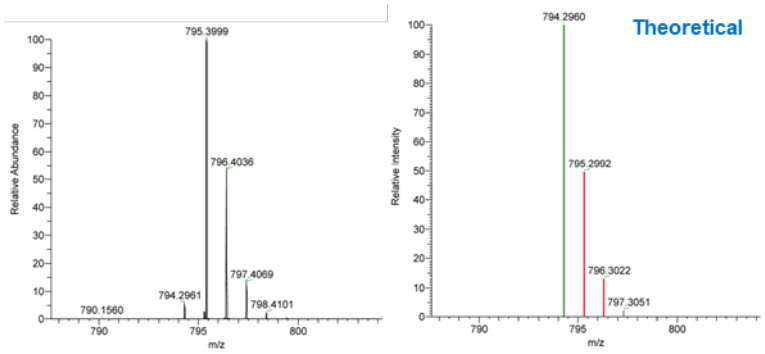
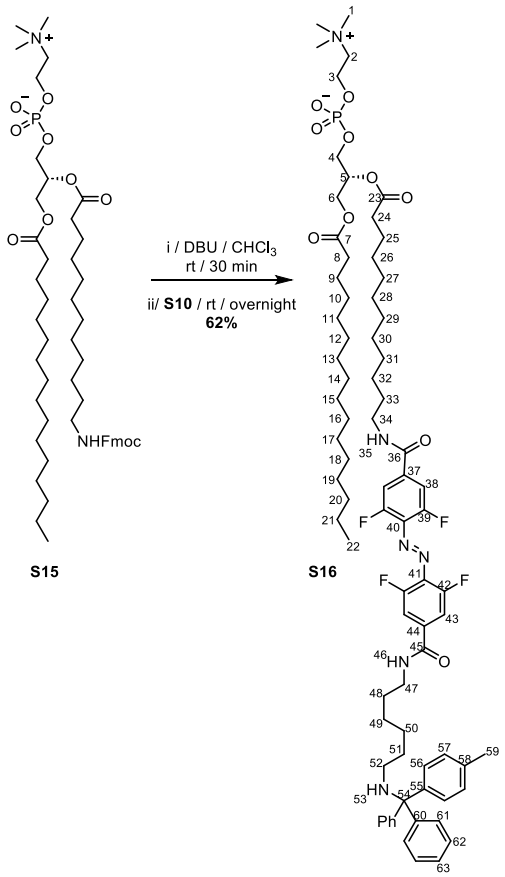


Figure S6. *HRMS spectrum of compound* ***S10****. HRMS-ESI (m/z) Calculated for* $C_{44}H_{40}O_5N_5F_4$ *[M+H]*$^+$*, 794.2960; found 794.2961.*

2.4 Mtt-protected azobenzene lipid S16.



To a solution of compound **S15** (88 mg, 96 µmol, 1.0 equiv.) in anhydrous $CHCl_3$ (2 mL) was added DBU (29.2 mg, 0.19 mmol, 2.0 equiv.). The reaction mixture was stirred for 30 min at rt, under N_2. Compound **S10** (91 mg, 0.11 mmol, 1.2 equiv.) in anhydrous $CHCl_3$ (1.75 mL) was added and the reaction mixture was stirred for 17 h at rt, under N_2. The reaction was concentrated to dryness *in vacuo*. The crude residue was purified by silica gel flash chromatography (0 – 2% H_2O / 25% MeOH / $CHCl_3$) to afford the title compound as a red solid. (81 mg, 59 µmol, 62%).

^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD (2:1)) δ 7.63 – 7.55 (m, 4H, $H_{38\ \&\ 43}$), 7.43 – 7.37 (m, 4H, H_{61}), 7.27 (d, *J* = 8.3 Hz, 2H, H_{56}), 7.22 (dd, *J* = 8.3, 6.8 Hz, 4H, H_{62}), 7.18 – 7.10 (m, 2H, H_{63}), 7.08 – 7.02 (m, 2H, H_{57}), 5.22 – 5.17 (m, 1H, H_5), 4.38 (dd, *J* = 12.0, 3.2 Hz, 1H, H_6), 4.29 – 4.18 (m, 2H, H_3), 4.12 (dd, *J* = 12.0, 6.9 Hz, 1H, H_6), 3.97 (dd, *J* = 6.9, 5.5 Hz, 2H, H_4), 3.61 – 3.55 (m, 2H, H_2), 3.42 – 3.29 (m, 4H, $H_{34\ \&\ 47}$), 3.19 (s, 9H, H_1), 2.34 – 2.24 (m, 7H, $H_{8,\ 24\ \&\ 59}$), 2.15 (t, *J* = 8.2 Hz, 2H, H_{52}), 1.66 – 1.44 (m, 10H, $H_{9,\ 25,\ 33,\ 48\ \&\ 51}$), 1.37 – 1.20 (m, 42H, $H_{10-21,\ 26-32,\ 49\ \&\ 50}$), 0.84 (t, *J* = 6.7 Hz, 3H, H_{22}).

^{13}C NMR (151 MHz, $CDCl_3$:CD_3OD (2:1)) δ 174.4, 174.0, 165.4, 156.5, 154.7, 145.8, 142.6, 138.7, 138.6, 136.7, 133.4, 133.4, 129.0, 129.0, 129.0, 128.3, 127.0, 112.3, 112.1, 71.8, 70.8 (d, *J* = 7.9 Hz, C_5), 66.9 (C_2), 64.1 (d, *J* = 5.1 Hz, C_4), 63.1 (C_6), 59.5 (d, *J* = 4.7 Hz, C_3), 54.5 (t, *J* = 3.5 Hz, C_1), 44.3, 40.8, 40.7, 34.6, 34.6, 34.5, 34.4, 32.3, 30.1, 30.0, 30.0, 29.9, 29.9, 29.9, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 27.4, 27.3, 27.2, 25.6, 25.3, 25.3, 23.0, 21.0, 14.2.

^{19}F NMR (565 MHz, $CDCl_3$:CD_3OD (2:1)) δ -120.07 – -120.12 (m, 4F, $F_{39\ \&\ 42}$).

HRMS-ESI (m/z) Calculated for $C_{76}H_{108}O_{10}N_6F_4P$ $[M+H]^+$, 1371.7795; found 1371.7758.

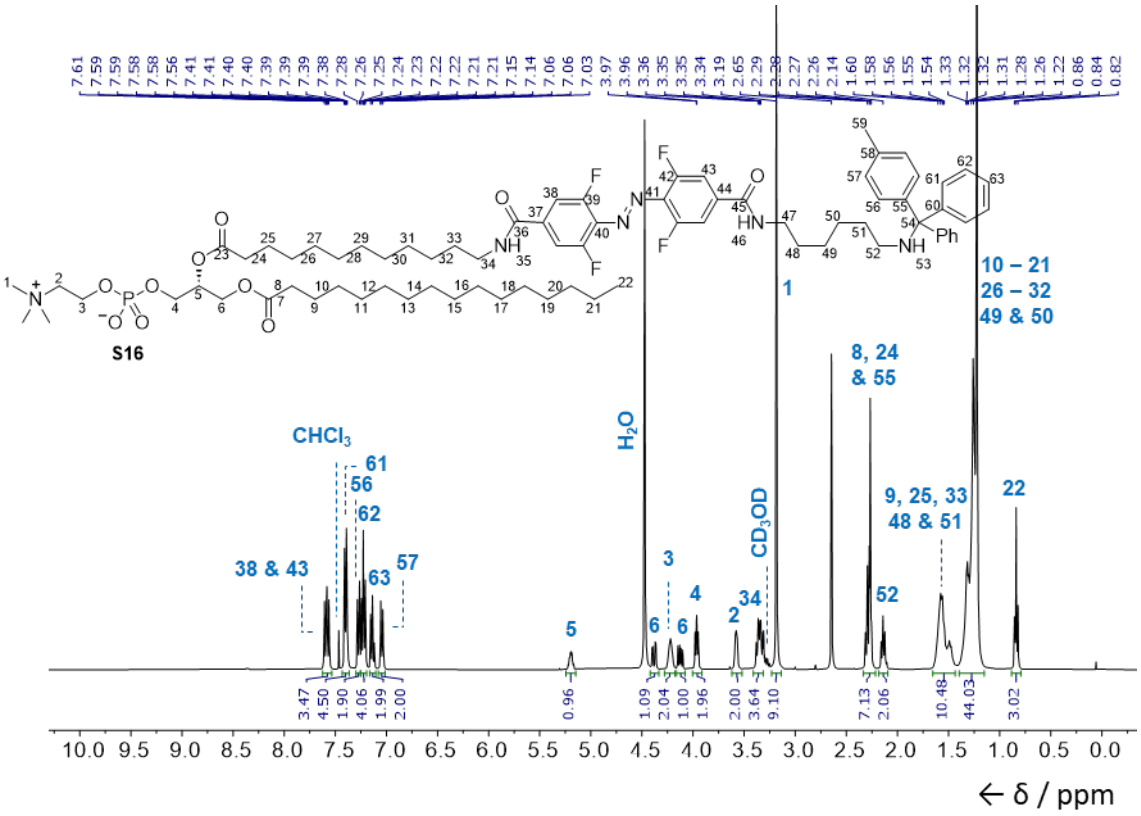


Figure S7. *^{1}H NMR spectrum of compound* ***S16*** *($CDCl_3$:CD_3OD (2:1), 400 MHz, 298 K).*

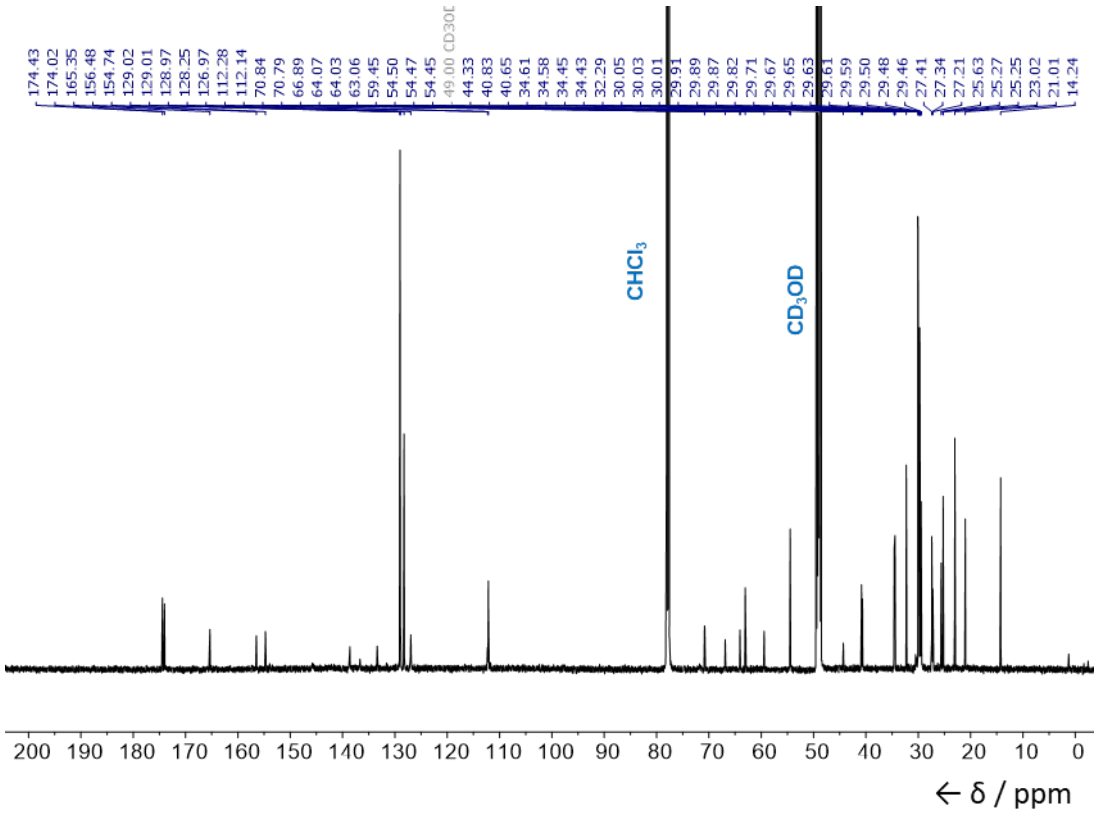


Figure S8. *^{13}C NMR spectrum of compound **S16** ($CDCl_3$:CD_3OD (2:1), 151 MHz, 298 K).*

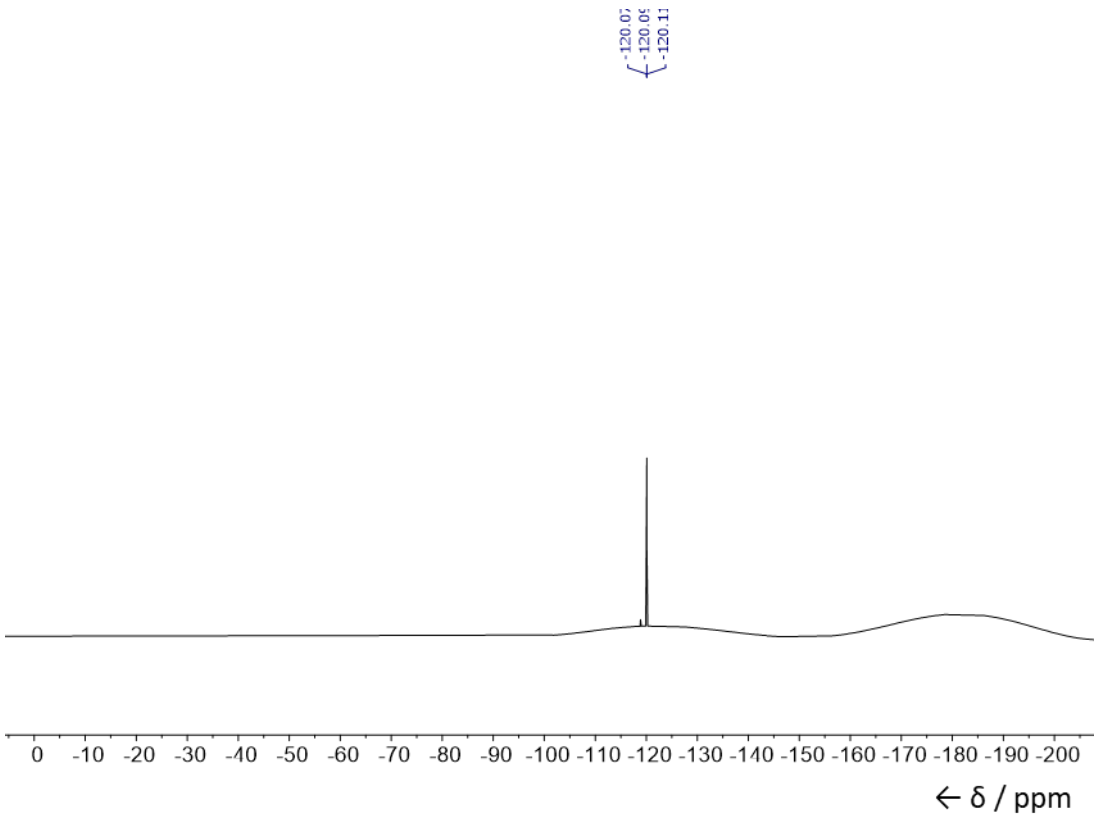


Figure S9. *^{19}F NMR spectrum of compound **S16** ($CDCl_3$:CD_3OD (2:1), 565 MHz, 298 K).*

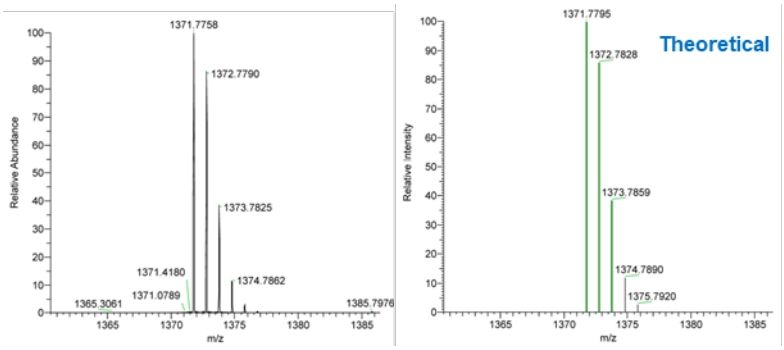
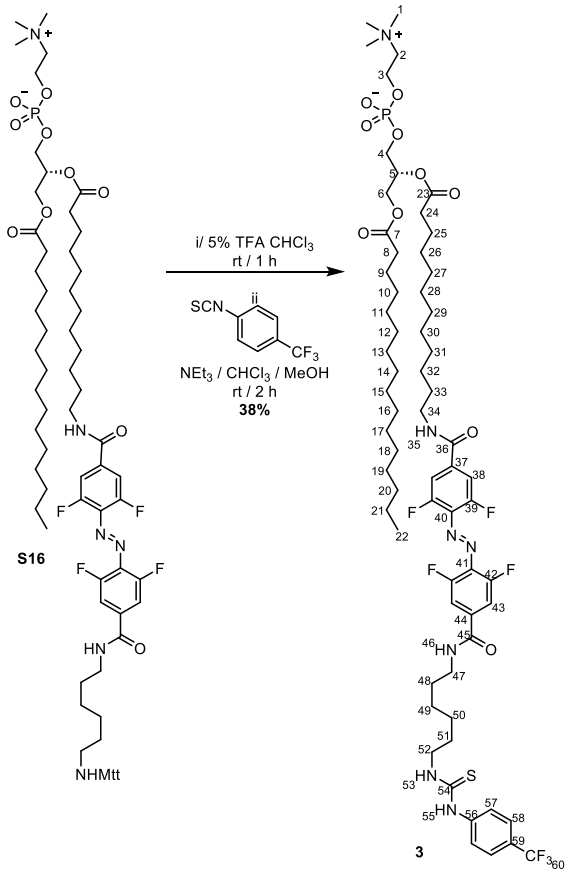


Figure S10. *HRMS spectrum of compound* ***S16****. HRMS-ESI (m/z) Calculated for* $C_{76}H_{108}O_{10}N_6F_4P$ $[M+H]^+$*, 1371.7795; found 1371.7758.*

2.5 Long relay transporter 3.



To a solution of compound **S16** (40 mg, 29 µmol, 1.0 equiv.) in anhydrous $CHCl_3$ (1 mL) was added TFA (50 µL). The mixture was stirred for 1 h at rt, under N_2. After complete deprotection, MeOH (1 mL) was added and the solvent was removed *in vacuo*. The residue was dissolved in anhydrous $CHCl_3$ (1 mL) and NEt_3 (50 µL). 4-(Trifluoromethyl) phenyl isothiocyanate (12 mg, 58 µmol, 2.0 equiv.) was added and the reaction was stirred for 6 h at rt, under N_2. The reaction mixture was concentrated to dryness *in vacuo* and the crude residue was purified by silica gel flash chromatography (0 – 2% H_2O / 25% MeOH / $CHCl_3$) to afford the title compound as a red solid (15 mg, 11 µmol, 38%).

^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD (2:1)) δ 7.61 – 7.53 (m, 8H, $H_{38,\ 43,\ 55\ \&\ 56}$), 5.21 – 5.17 (m, 1H, H_5), 4.23 – 4.19 (m, 2H, H_3), 4.12 (dd, *J* = 12.0, 6.8 Hz, 1H, H_6), 3.98 – 3.95 (m, 2H, H_4), 3.60 – 3.55 (m, 4H, $H_{2\ \&\ 47}$), 3.39 – 3.34 (m, 4H, $H_{34\ \&\ 52}$), 3.18 (s, 9H, H_1), 2.30 – 2.25 (m, 4H, $H_{8\ \&\ 24}$), 1.69 – 1.59 (m, 4H, $H_{48\ \&\ 51}$), 1.59 – 1.53 (m, 6H, $H_{9,\ 25\ \&\ 33}$), 1.45 – 1.40 (m, 4H, $H_{49\ \&\ 50}$), 1.38 – 1.18 (m, 38H, $H_{10\ –\ 21\ \&\ 26\ –\ 32}$), 0.84 (t, *J* = 6.9 Hz, 3H, H_{22}). One proton environment for H_6 is obscured by the H_2O peak.

^{13}C NMR (151 MHz, $CDCl_3$:CD_3OD (2:1)) δ 181.3 (C_{53}), 174.4 (C_7), 174.0 (C_{23}), 165.4, 165.3, 155.59 (dd, *J* = 262.2, 3.9 Hz, 2C, $C_{39\ \&\ 42}$), 142.7, 138.7 (t, *J* = 8.5 Hz), 138.5 (t, *J* = 8.6 Hz), 133.4 (t, *J* = 10.3 Hz), 133.3 (t, *J* = 10.3 Hz), 129.1 (q, *J* = 45.6 Hz), 126.3 (d, *J* = 4.1 Hz), 124.6 (q, *J* = 271.1 Hz), 123.3 (brs), 112.2 (dt, *J* = 22.0, 2.9 Hz, 2C, $C_{38\ \&\ 43}$), 70.8 (d, *J* = 7.6 Hz, C_5), 66.9 (C_2), 64.0 (d, *J* = 5.2 Hz, C_4), 63.0 (C_6), 59.4 (d, *J* = 5.0 Hz, C_3), 54.5 (t, *J* = 3.1 Hz, C_1), 44.8, 40.8, 40.5, 34.6, 34.4, 32.3, 30.0, 30.0, 30.0, 30.0, 29.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.0, 27.4, 26.8, 26.7, 25.7, 25.2, 25.2, 23.0, 14.2.

^{19}F NMR (565 MHz, $CDCl_3$:CD_3OD (2:1)) δ -62.67 (F_{58}), -120.00 (d, *J* = 10.5 Hz, $F_{39\ or\ 42}$), -120.03 (d, *J* = 10.5 Hz, $F_{39\ or\ 42}$).

HRMS-ESI (m/z) Calculated for $C_{64}H_{96}O_{10}N_7F_7PS$ $[M+H]^+$, 1318.6560; found 1318.6524.

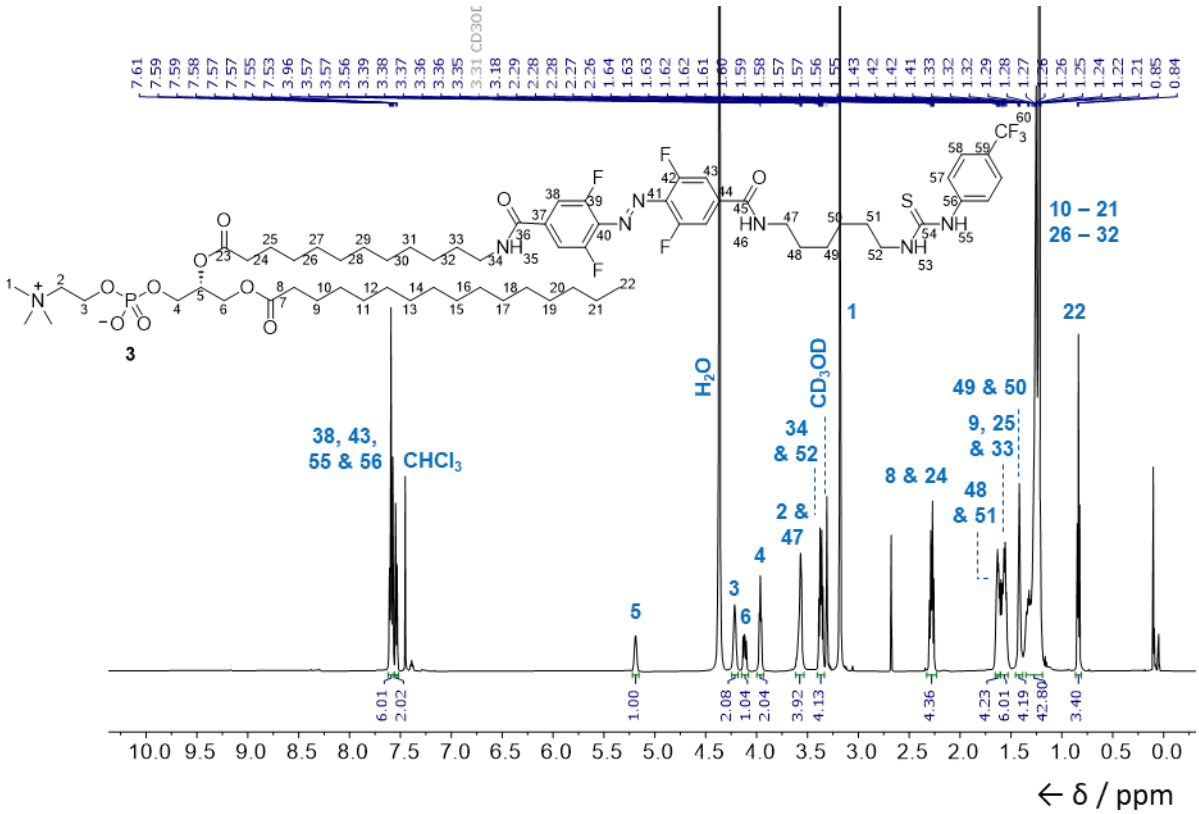


Figure S11. *^{1}H NMR spectrum of compound **3** ($CDCl_3$:CD_3OD (2:1), 600 MHz, 298 K).*

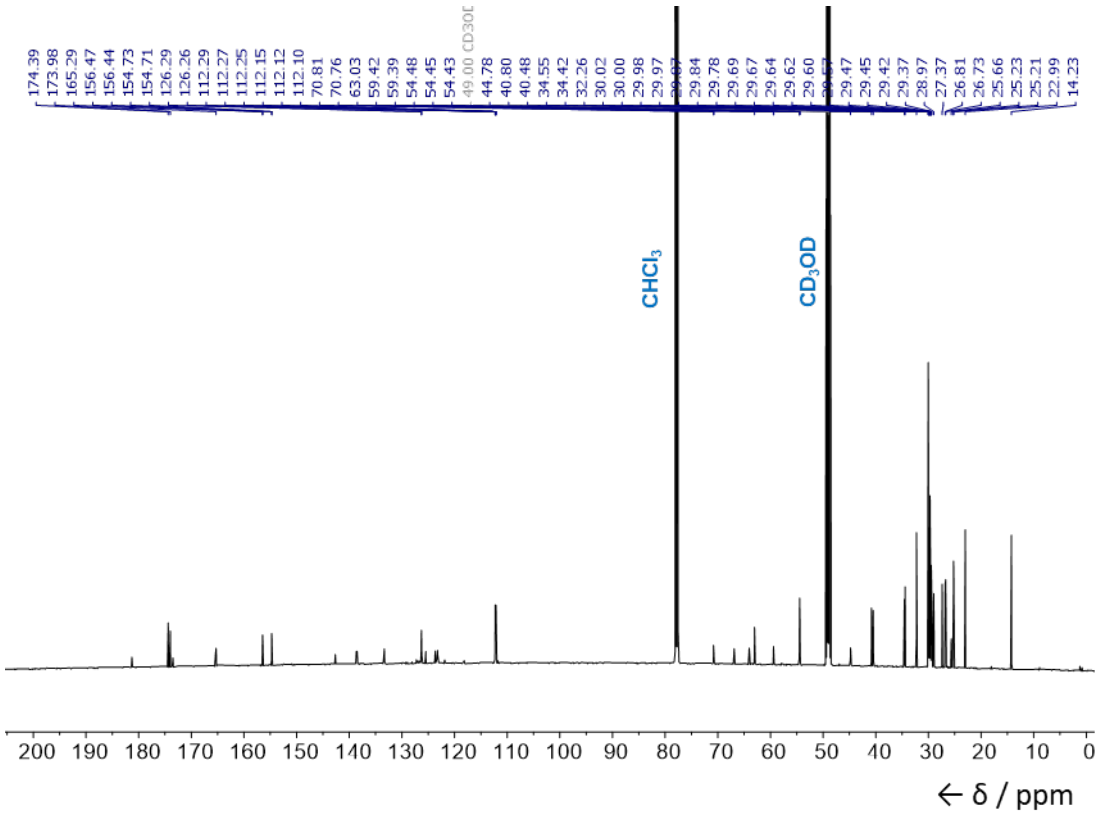


Figure S12. *^{13}C NMR spectrum of compound **3** ($CDCl_3$:CD_3OD (2:1), 151 MHz, 298 K).*

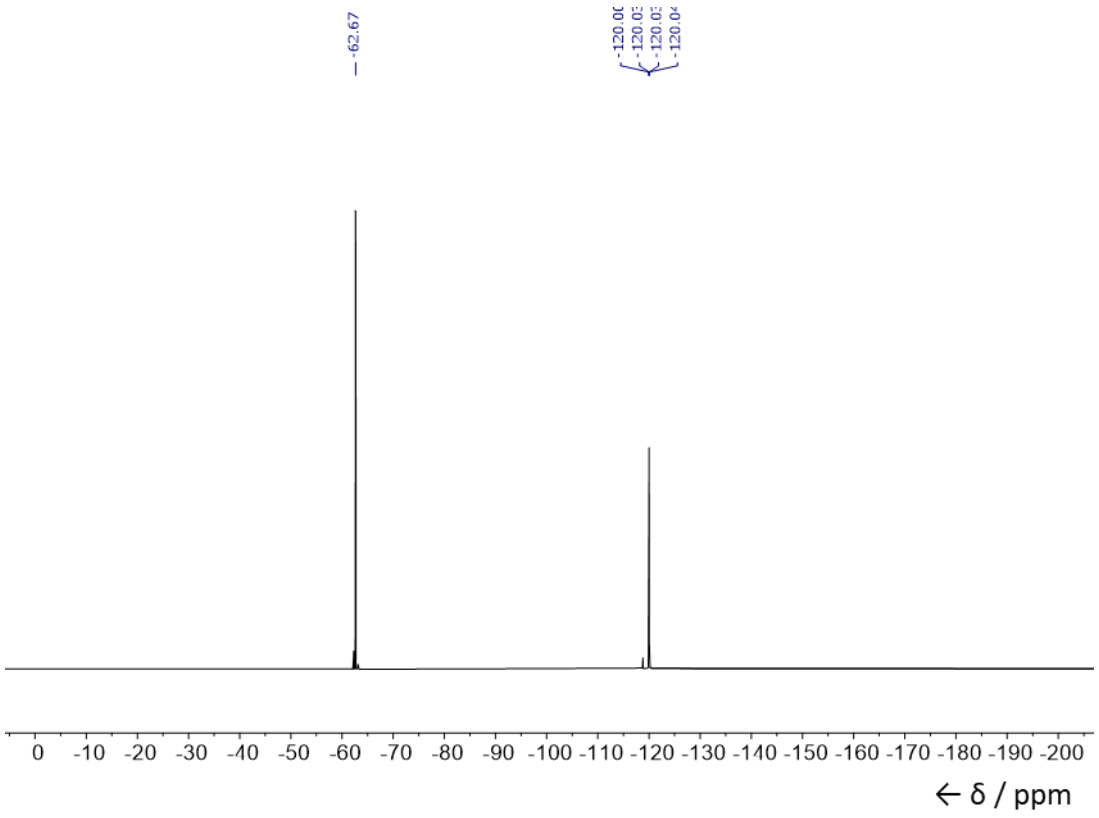


Figure S13. *^{19}F NMR spectrum of compound **3** ($CDCl_3$:CD_3OD (2:1), 565 MHz, 298 K).*

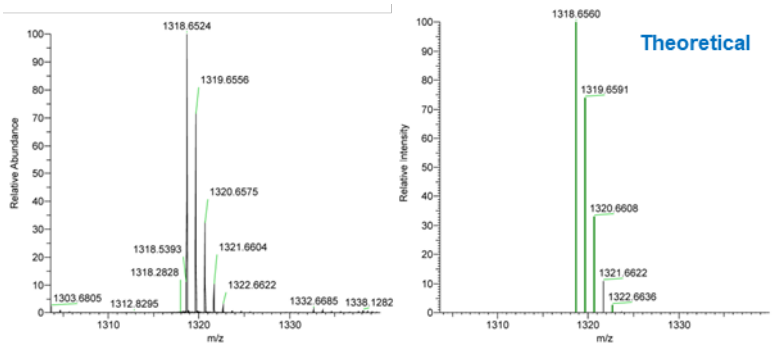


Figure S14. *HRMS spectrum of compound* ***3****. HRMS-ESI (m/z) Calculated for* $C_{64}H_{96}O_{10}N_7F_7PS$ $[M+H]^+$*, 1318.6560; found 1318.6524.*

Photo-isomerisation experiments

Photo-irradiation of liquid samples was carried out using Thorlabs high-power mounted LEDs (models M530L4 (530 nm, 370 mW) and M405L4 (405 nm, 1000 mW)) using an in-house custom built set-up using optical components supplied by Thorlabs, as described previously.[1] For irradiating small vials of samples, NMR tubes and cuvettes, a Thorlabs cuvette holder (CVH100/M) equipped with the mounted LEDs was used. Samples were irradiated for sufficient time to reach the photo-stationary state, as confirmed by ^{1}H NMR or UV-vis experiments.

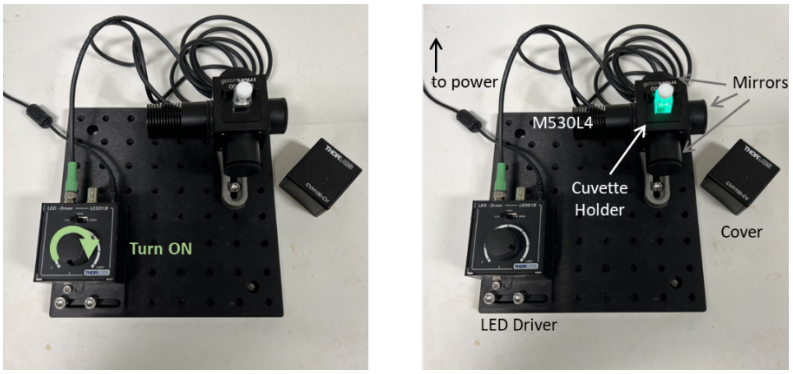


Figure S15. *Apparatus for photo-irradiating cuvettes and liquid samples in small vials.*

2.6 Determination of photo-stationary states (PSS) in solution

The photo-stationary state composition for both 405 nm and 530 nm irradiation was determined by ^{1}H NMR spectroscopy following irradiation of the sample in solution in the NMR tube with LEDs until there was no further change in isomer composition.

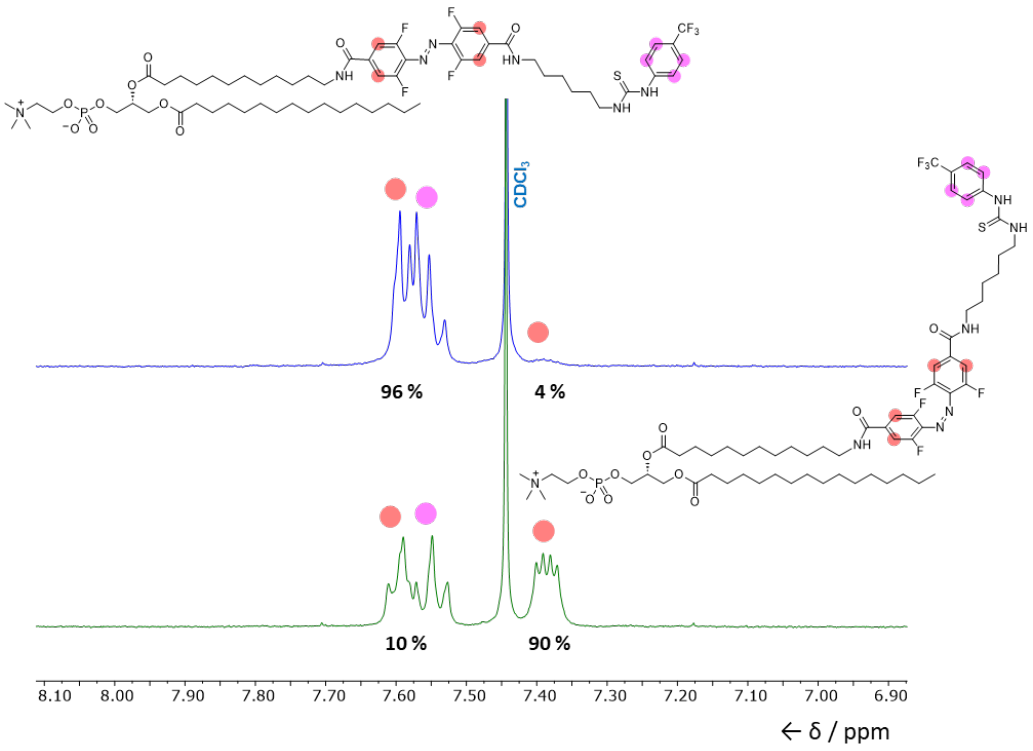


Figure S16. *Partial ^{1}H NMR spectra of **3^E**/**3^Z** ($CDCl_3$:CD_3OD (2:1), 400 MHz, 298 K) showing the PSS achieved by irradiation with blue 405 nm (top, 96% E) and green 530 nm (bottom, 90% Z) light.*

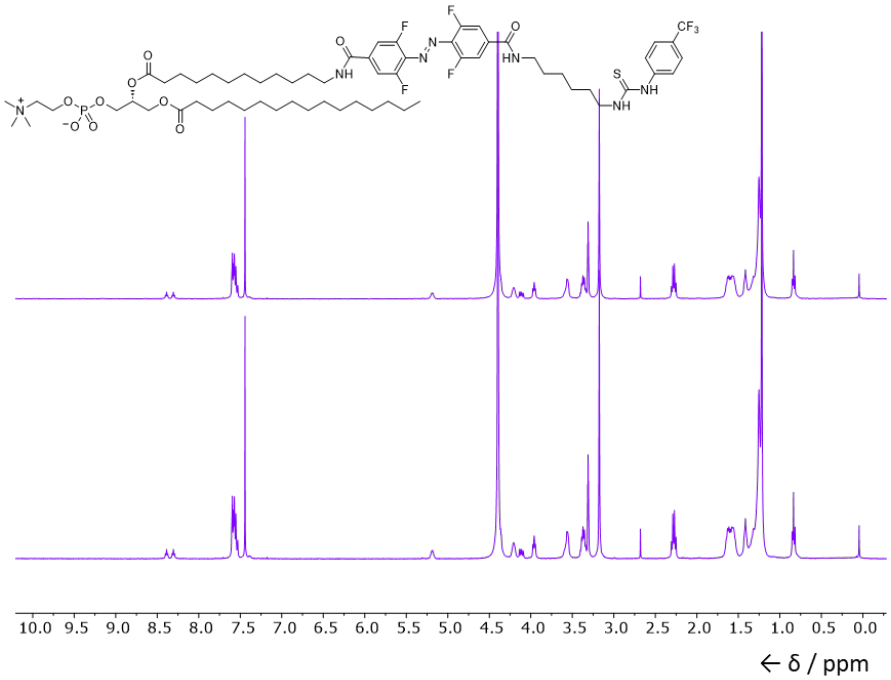


Figure S17. *^{1}H NMR spectra of **3^E** ($CDCl_3$:CD_3OD (2:1), 400 MHz, 298 K) confirming that the PSS_{405} was achieved. Top, spectrum of **3^E** after 10 mins of irradiation with 405 nm LED (1000 mW). Bottom, spectrum of **3^E** following a further 10 mins of irradiation with 405 nm LED (1000 mW). With no observable change in the ^{1}H NMR, this also provides evidence of the photostability of **3** under constant 405 nm irradiation.*

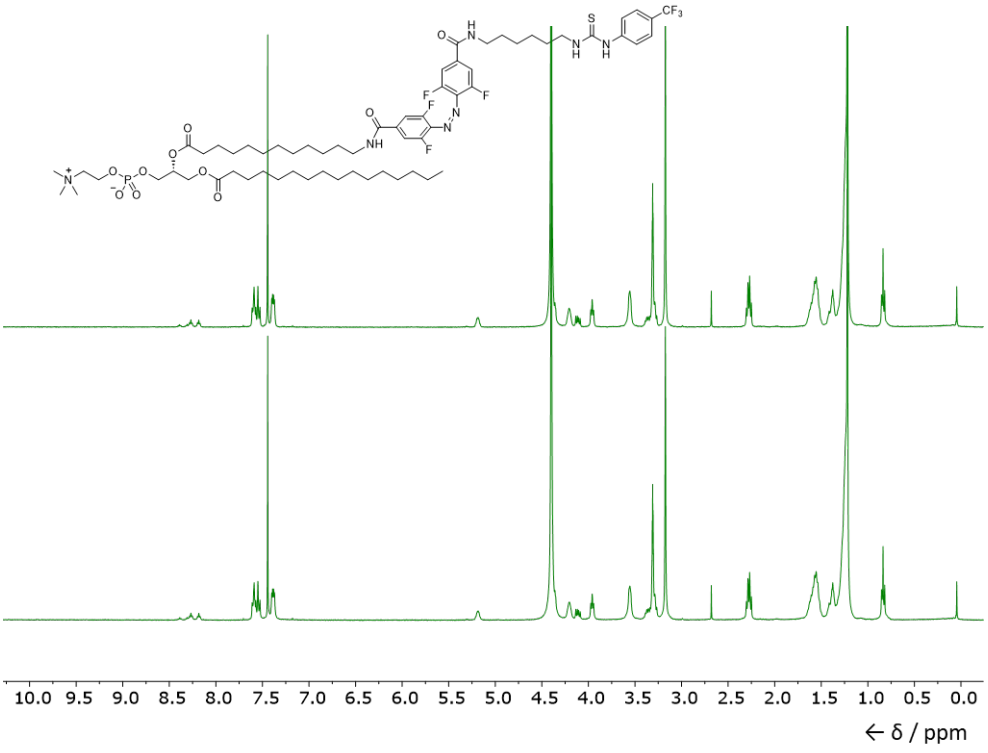


Figure S18. *^{1}H NMR spectra of **3^Z** ($CDCl_3$:CD_3OD (2:1), 400 MHz, 298 K) confirming that the PSS_{530} was achieved. Top, spectrum of **3^E** after 10 mins of irradiation with 530 nm LED (370 mW). Bottom, spectrum of **3^E** following a further 10 mins of irradiation with 530 nm LED (370 mW). With no observable change in the ^{1}H NMR, this also provides evidence of the photostability of **3** under constant 530 nm irradiation.*

3 UV-Visible Absorption Spectroscopy

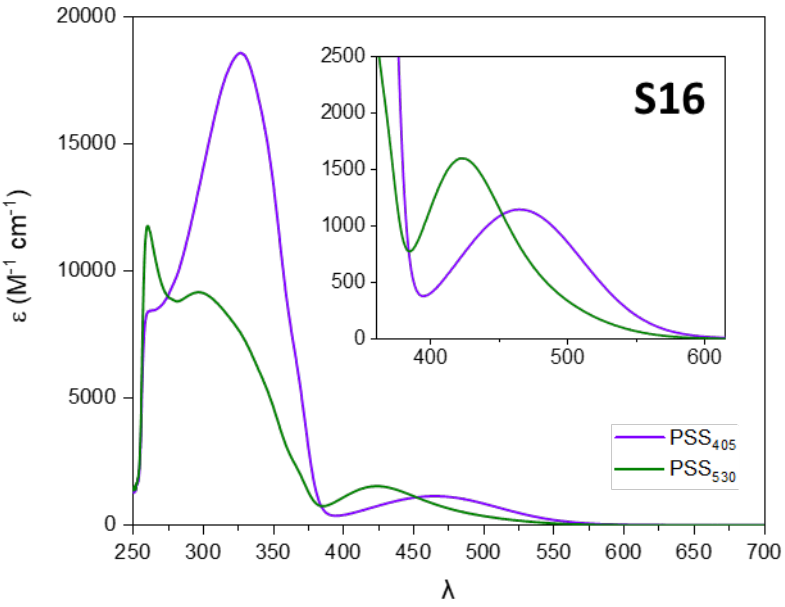


Figure S19. *UV-vis spectra of PSS distributions of* ***S16*** *in DMSO, measured after irradiation with 405 nm or 530 nm light.*

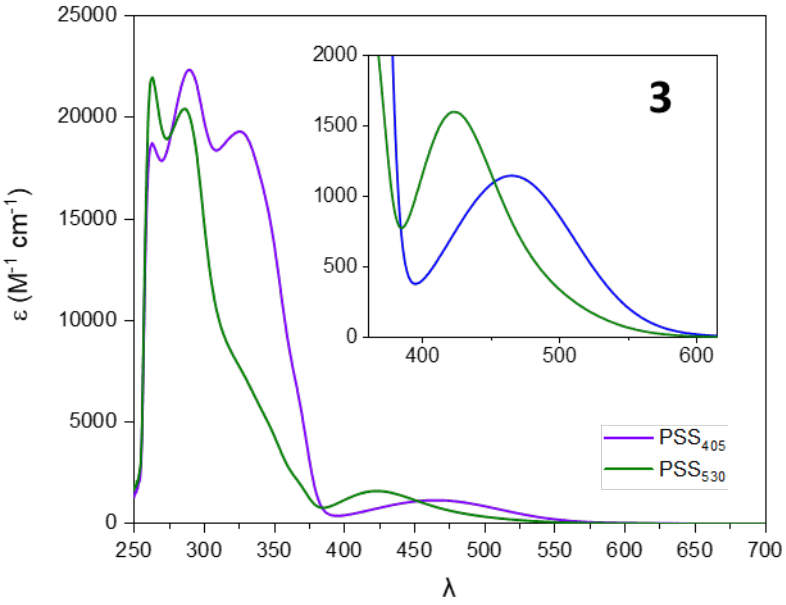


Figure S20. *UV-vis spectra of PSS distributions of* ***3*** *in DMSO, measured after irradiation with 405 nm or 530 nm light.*

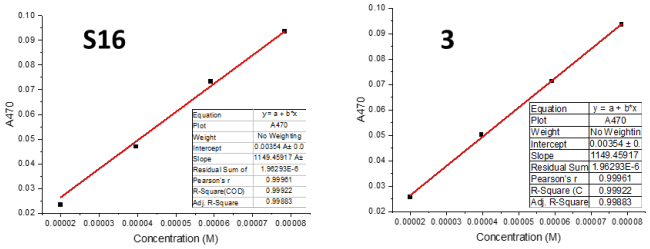


Figure S21. *Beer-Lambert plots for azobenzene derivatives* ***S16*** *and* ***3****.*

4 Anion Transport Experiments

4.1 Vesicle Preparation

A thin film of lipid and transporter in various ratios was formed by evaporating a chloroform solution under reduced pressure on a rotary evaporator (25 °C) and then under high vacuum for 6 hours. The lipid film was hydrated by vortexing with the prepared buffer (100 mM NaCl, 10 mM HEPES, 1 mM 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), pH 7.0). The lipid suspension was then subjected to 5 freeze-thaw cycles using liquid nitrogen and a water bath (40 °C) followed by extrusion 19 times through a polycarbonate membrane (pore size 200 nm). Extra-vesicular components were removed by size exclusion chromatography on a Sephadex G-25 column eluted with 100 mM NaCl, 10 mM HEPES, pH 7.0. Final conditions: LUVs (0.625 mM lipid); inside 100 mM NaCl, 10 mM HEPES, 1 mM HPTS, pH 7.0; outside: 100 mM NaCl, 10 mM HEPES, pH 7.0.

4.2 Transport assays with HPTS

In a typical experiment, the LUVs containing HPTS (100 μL, 0.625 mM) were added to buffer (1880 μL of 100 mM NaCl, 10 mM HEPES, pH 7.0) at 25°C under gentle stirring giving a final lipid concentration of 31.3 μM. A pulse of NaOH (20 μL of 0.5 M solution, final concentration 5 mM) was added to initiate the experiment at t = 0. After 200 s, detergent (25 μL of Triton X-100 in 7:1 (v/v) H_2O-DMSO) was added to lyse the vesicles and calibrate the assay. The fluorescence emission was monitored at λ_{em} = 510 nm (λ_{ex} = 405/460 nm). The fractional fluorescence intensity (I_{rel}) was calculated from Equation S1, where R_t is the fluorescence ratio at time t, (ratio of intensities from 460 nm / 405 nm excitation) R_0 is the fluorescence ratio at time 0 immediately after the base pulse, and R_d is the fluorescence ratio at time 260 s, after the addition of detergent. For each compound as the *E* and *Z* isomer, each individual concentration was repeated at least three times and averaged; error bars represent standard deviations.

$$I_{rel} = \frac{R_t - R_0}{R_d - R_0} \qquad \textbf{(S1)}$$

The k_{ini} values were calculated by fitting the transport kinetics curves (I_{rel} vs t) with the exponential function I_{rel} = a – exp(-kt/b) using Origin 17. k_{ini} (the initial rate of transport at t = 0) is then given by differentiation as k_{ini} = 1/b (s^{-1}).

For the external addition of transporters to pre-formed vesicles, a DMSO solution of transporter (5 μL of stock solution) was added to the vesicle suspension (1875 μl, 31.3 μM lipid concentration) and irradiated with 405 nm or 530 nm light for 2 minutes immediately prior to the transport experiment. The NaOH pulse was added at 90 s, and detergent at 290 s. Data for externally added transporters was normalised based on R_0 (at t = 92 s, immediately after the base pulse) and R_d (at t = 350 s, after the addition of detergent).

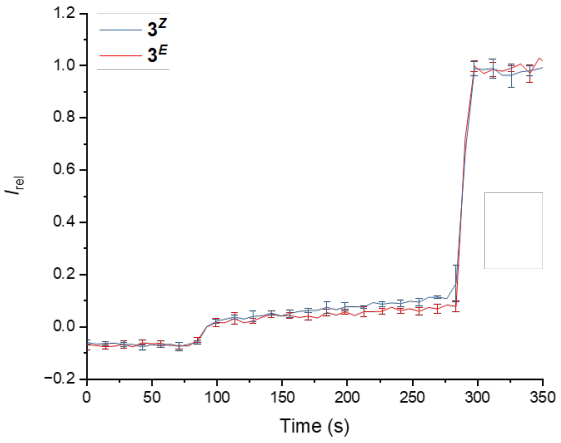


Figure S22. *Ion transport HPTS assay data for* ***3**^E^* *(5 mol% to lipid, PSS_{405} = 96:4 E:Z) and* ***3**^Z^* *(5 mol% to lipid, PSS_{530} = 10:90 E:Z) in POPC LUVs when externally added (5 µL of DMSO stock solution) and then irradiated in the bilayer.*

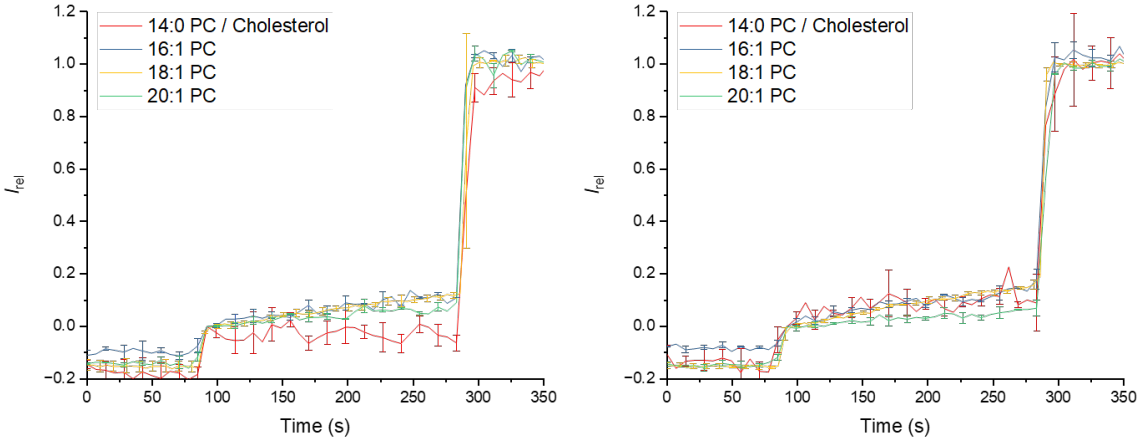


Figure S23. *Ion transport HPTS assay data for* ***3**^E^* *(left, 5 mol% to lipid, PSS_{405} = 96:4 E:Z) and* ***3**^Z^* *(right, 5 mol% to lipid, PSS_{530} = 10:90 E:Z) in LUVs comprised of different lipids (16:1 PC, 18:1 PC, 20:1 PC or 14:0 PC with cholesterol (7:3 ratio)) when externally added (5 µL of DMSO stock solution) and then irradiated in the bilayer.*

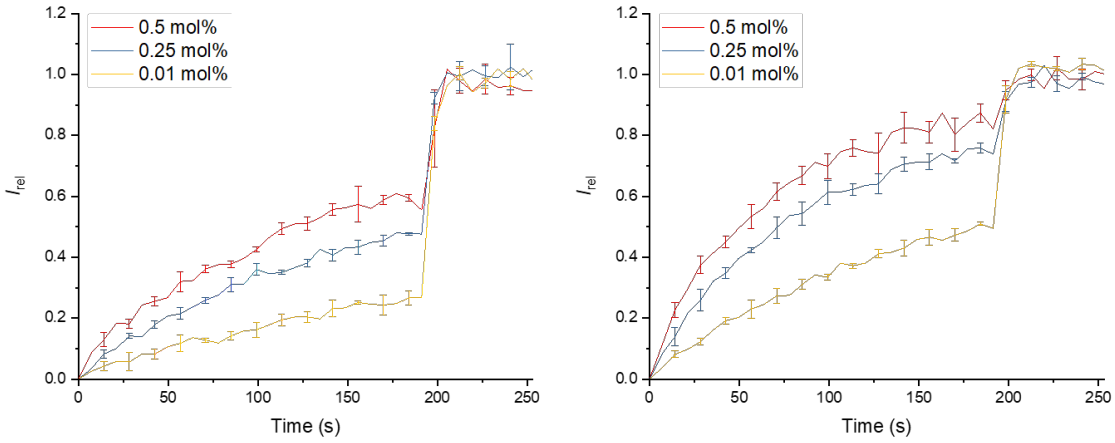


Figure S24. *Ion transport HPTS assay data for* ***3^{E}*** *(left, 0.01–0.5 mol% to lipid, PSS$_{405}$ = 96:4 E:Z) and* ***3^{Z}*** *(right, 0.01–0.5 mol% to lipid, PSS$_{530}$ = 10:90 E:Z) in POPC LUVs.*

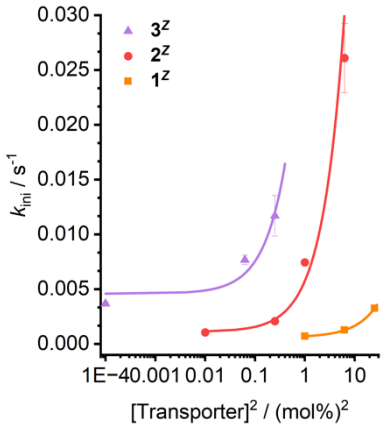


Figure S25. *Dependence of k_{ini} on [Transporter]^2 for the Z-rich PSS generated by in-situ photo-isomerization with 530 nm light for the three relay transporter lengths in POPC LUVs, and fits to a second order rate equation.*

5 References

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