Supporting Information

Tetra-azobenzene extended calix[4]pyrroles: study of the effect of photo-isomerization on the binding and transport activity of chloride anions

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1 General information and instruments

Solvents and reagents were of reagent grade quality and were obtained from commercial suppliers and used without further purification unless otherwise stated. Dry solvents were either obtained from commercial suppliers or taken from a solvent system MB SPS 800 and freshly distilled. Thin-layer chromatography (TLC) was performed with DC-Alufolien Kieselgel 60 F254 (Merck) or neutral Al_2O_3 F254 (Sigma-Aldrich). Column chromatography was performed with silica gel 60 Å for chromatography (Sigma-Aldrich). Routine ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 (300 MHz for ^{1}H NMR), Avance 400 (400 MHz for 1H NMR) or Avance 500 (500 MHz for ^{1}H NMR) ultra-shield spectrometers. Deuterated solvents from Eurisotop are indicated in the characterization and chemical shifts are reported in ppm. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), or triplet (t). Splitting patterns that could not be easily interpreted are designated as multiplet (m) or broad (br). All NMR J values are given in Hz.

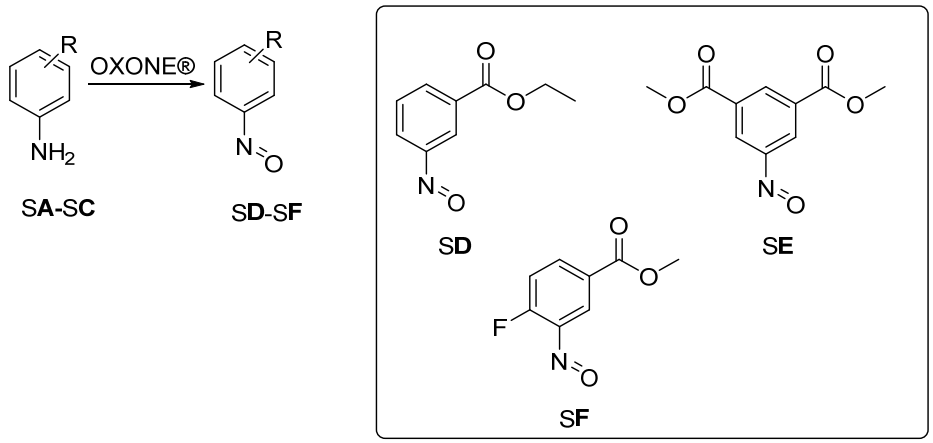
High-resolution mass spectra (HRMS) were obtained on a Bruker HPLC-TOF using ESI as ionization mode. IR spectra were recorded on a Bruker Optics FTIR Alpha spectrometer equipped with a DTGS detectror, KBr beam splitter at 4 cm^{-1} resolution using a one bounce ATR accessory with diamonds windows.

ITC titrations were carried out on a Microcal VP-ITC MicroCalorimeter.

Photo-isomerization studies were performed using a high-power light source purchased from Sahlmann Photochemical Solutions and consisting of 3 LED-diodes from Nichia (365 nm, 241.5 $mW \cdot cm^{-2}$). Samples were irradiated inside the NMR tube or the UV-Vis cuvettes using a custom-made sample holder which located the sample at a 1.5 cm distance from the power source (LED).

2 Synthesis and characterization data

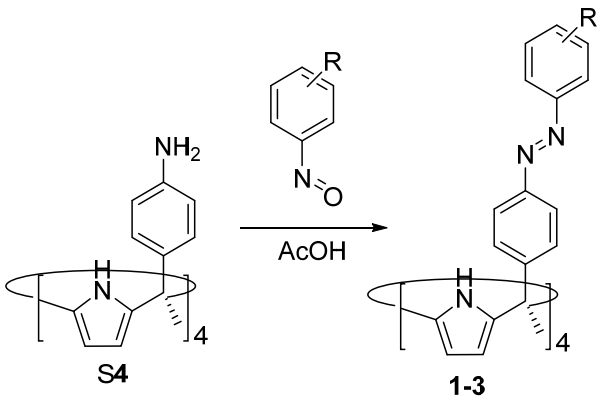
2.1. Nitrosobenzene derivatives



Scheme S 1. General synthesis of nitrosobenzene derivatives S**D-SF**.

Ethyl 3-nitrosobenzoate (S**D**) was synthesized following a described procedure in the literature.[1] Compounds S**E** and S**F** were synthesized using similar conditions described for S**D** but starting from the corresponding aniline derivative. That is, to a DCM solution of the aniline derivatives S**A-SC** (20 mL, 0.3 M) we added 80 mL of an aqueous solution of OXONE® (0.2 mM, 3 equiv.) (DCM:H_2O (2:8)). The mixture was stirred vigorously for 3h at RT. After, the organic phase was washed with water (2x20 mL) HCl 1 N (1x20 mL) followed NaOH 1 N (3x20 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness providing a yellow solid (S**D**: 0.75 g, 70 %; S**E**: 1.1 g, 80 % yield; S**F**: 0.80 g, 74 %). The compounds were used in the synthesis of calix[4]pyrroles **1-3** without further purification.

2.2. Tetra-azobenzene extended calix[4]pyrroles **1-3**



Scheme S 2. General synthetic scheme of the preparation of receptors **1-3**.

Tetra-amine calix[4]pyrrole **4** (**Scheme S 2**) was prepared using a reported procedure.[2] Tetra-amine calix[4]pyrrole S**4** (1 equiv.) and the nitrosobenzene derivative S**D-SF** (5 equiv.) were suspended in acetic acid (5 mL). The mixture was stirred overnight at RT under Ar atmosphere. Afterwards, the reaction was stopped and water (3 mL) was added to the reaction mixture. Then, 5 mL of sat. $NaHCO_3$ solution was added and the product was extracted with AcOEt (3x10 mL). The organic phase was

washed with water (2x10 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (conditions described for each receptor below).

*Tetra-azobenzene extended calix[4]pyrrole **1***

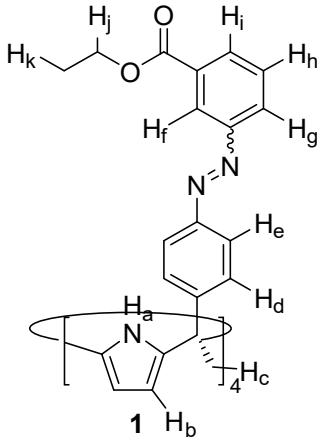


Figure S 1. Line-drawing structure of tetra-azobenzene extended calix[4]pyrrole **1** with the corresponding proton assignment.

Calix[4]pyrrole **1** was purified by column chromatography in silica using as eluent DCM to afford an orange amorphous solid (92 mg, 0.7 mmol, 54% yield; starting from 91 mg of tetra-amine calix[4]pyrrole S**4**). Characterization of the *tttt*-isomer: ^{1}H NMR (500 MHz, DCM-d_2, 298K) δ (ppm) = 8.51 (m, 4H); 8.15 – 8.06 (m, 8H); 7.89 (d, *J* = 8.7 Hz, 8H); 7.59 (dd, *J* = 7.8 Hz, *J* = 7.8 Hz, 4H); 7.37 (d, *J* = 8.7 Hz, 8H); 5.88 (s, 8H); 4.39 (q, *J* = 7.1 Hz, 8H); 2.09 (s, 12H); 1.39 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (126 MHz, DCM-d_2, 298K) δ (ppm) = 166.23; 153.08; 152.07; 151.59; 136.71; 132.24; 131.88; 129.61; 128.77; 128.74; 127.11; 124.05; 122.75; 107.18; 61.68; 50.75; 45.35; 27.90; 14.50. FTIR ν (cm^{-1}) = 3322 (N-H stretching); 2979 (C-H stretching); 1719 (C=O stretching); 1367; 1271; 1181; 757. HR-MS (ESI+) m/z: $[M+Na]^+$ calcd. For $C_{84}H_{76}N_{12}NaO_8$: 1403.5801; found: 1403.5798.

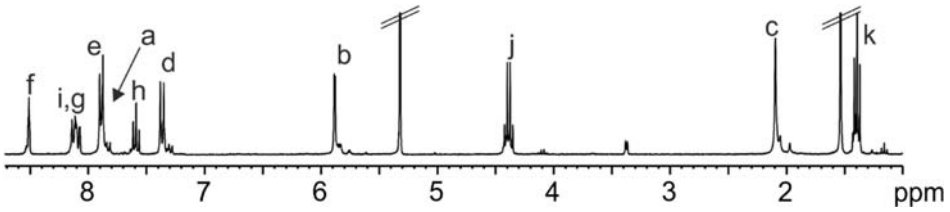


Figure S 2. ^{1}H NMR spectrum (DCM-d_2, 500 MHz, 298 K) of **1**. See **Figure S 1** for proton assignment.

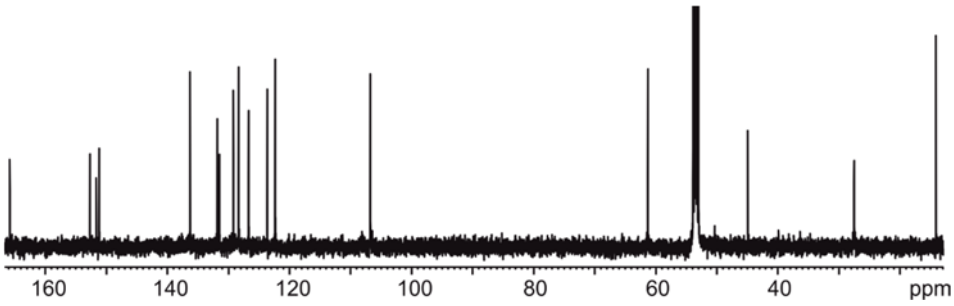


Figure S 3. ^{13}C NMR spectrum (DCM-d_2, 126 MHz, 298 K) of **1**.

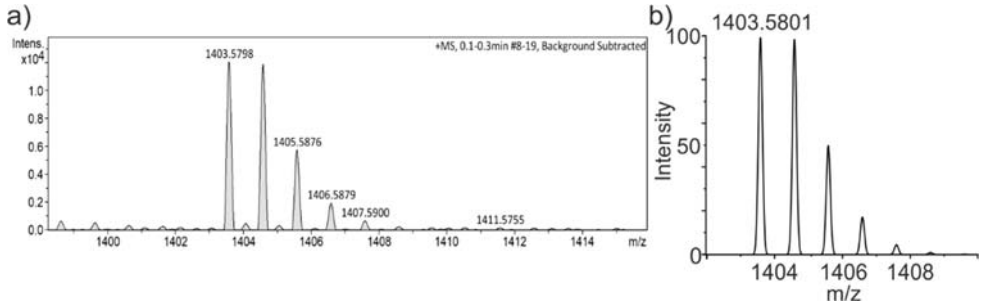
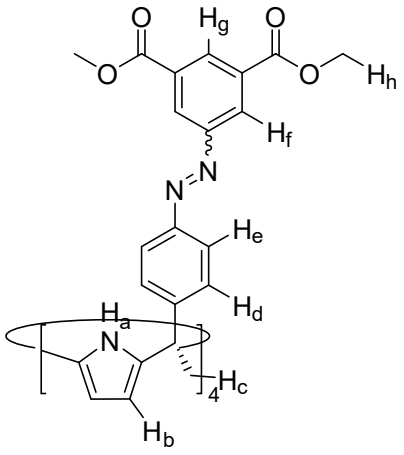


Figure S 4. a) Experimental and b) theoretical isotopic distributions pattern for $[M+Na]^+$ peak of **1**. The exact mass for the monoisotopic peak in a) and b) is indicated.

*Tetra-azobenzene extended calix[4]pyrrole **2***



2

Figure S 5. Line-drawing structure of C[4]P **2** with the corresponding proton assignment.

Calix[4]pyrrole **2** was purified by column chromatography in silica using gradient elution with a mixture DCM:TBME (95:5), to afford an orange solid. Calix[4]pyrrole **2** was purified by dissolving the orange solid in the minimum amount of DCM followed by the slow addition of a layer of MeOH (2:1 DCM:MeOH). The mixture was left in the fridge overnight. Pure compound **2** precipitated from this solvent mixture as an orange solid which was isolated by filtration (31 mg, 0.02 mmol, 21% yield; starting from 70 mg of tetra-amine cali[4]pyrrole S**4**). ^1H NMR (400 MHz, DCM-d_2, 298K) δ (ppm) =

8.71 (t, *J* = 1.6 Hz, 4H); 8.68 (d, *J* = 1.6 Hz, 8H); 7.92 (d, *J* = 8.7 Hz, 8H); 7.85 (br, 4H); 7.38 (d, *J* = 8.7 Hz, 8H); 5.89 (d, *J* = 2.6 Hz, 8H); 3.95 (s, 24H); 2.10 (s, 12H). ^{13}C NMR (101 MHz, DCM-d_2, 298K) δ (ppm) = 165.99; 153.25; 152.51; 151.47; 136.54; 132.34; 132.30; 128.80; 127.86; 122.97; 107.29; 52.85; 45.41; 27.81. FTIR ν (cm^{-1}) = 3402 (N-H stretching); 3351; 2951 (C-H stretching); 1730 (C=O stretching); 1430; 1237; 1002; 757. HR-MS (ESI+) m/z: $[M+K]^+$ calcd. For $C_{88}H_{76}KN_{12}O_{16}$: 1595.5134; found: 1595.5121.

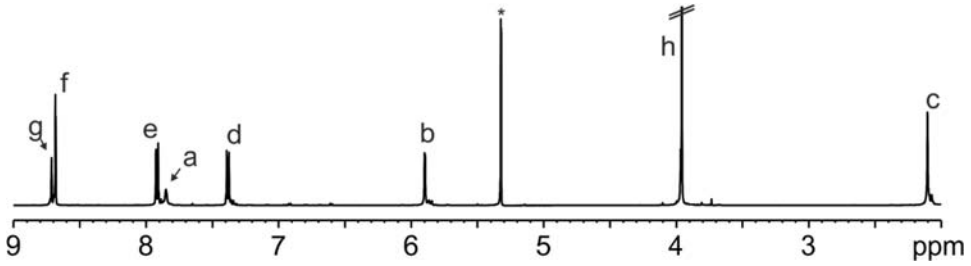


Figure S 6. ^{1}H NMR spectrum (DCM-d_2, 400 MHz, 298K) of **2**. *Residual solvent peaks. See **Figure S 5** for proton assignment.

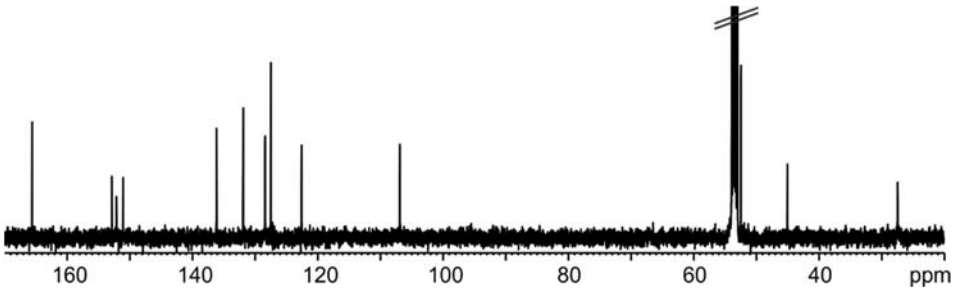


Figure S 7. ^{13}C NMR spectrum (DCM-d_2, 101 MHz, 298K) of **2**.

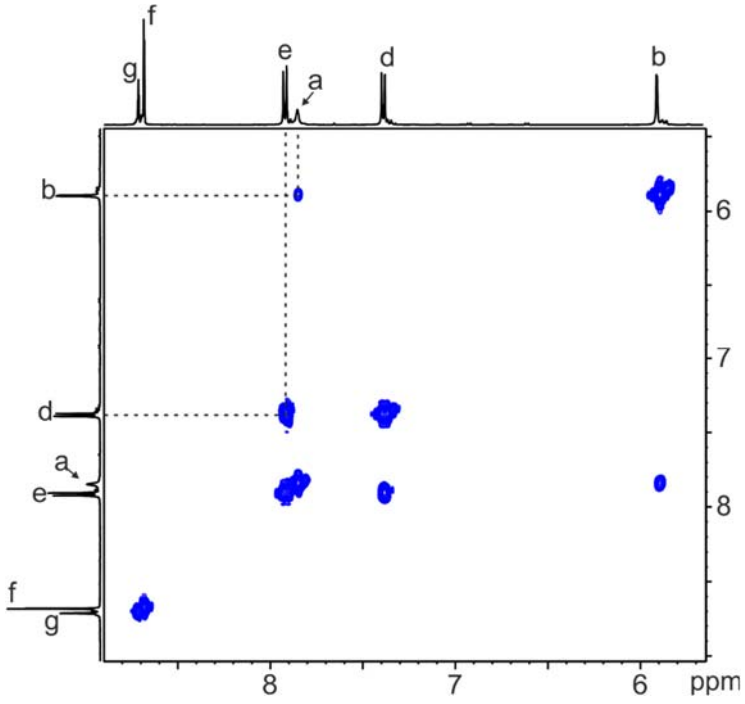


Figure S 8. Selected region of the COSY NMR spectrum (DCM-d_2, 400 MHz, 298K) of receptor **2**. See **Figure S 5** for proton assignment.

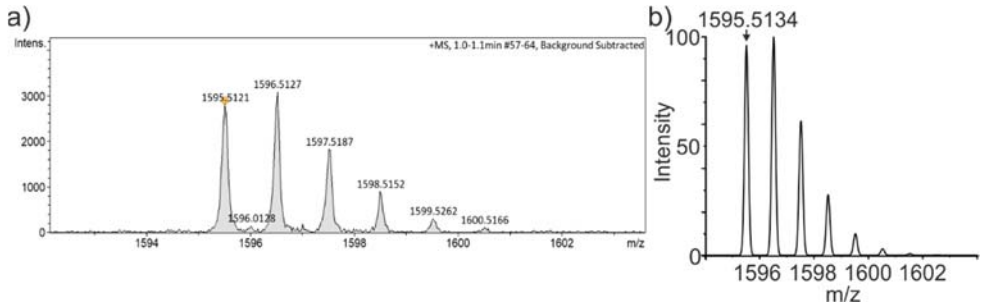


Figure S 9. a) Experimental and b) theoretical isotopic distribution pattern for $[M+K]^+$ peak of **2**. The exact mass for the monoisotopic peak in a) and b) is indicated.

*Tetra-azobenzene extended calix[4]pyrrole **3***

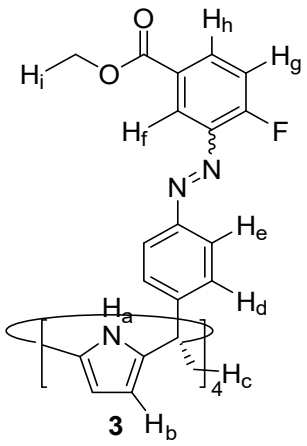


Figure S 10. Line-drawing structure of receptor **3** with the corresponding proton assignment.

Calix[4]pyrrole **3** was purified by column chromatography in silica using gradient elution with a mixture DCM:TBME (98:2), to afford an orange amorphous solid (30 mg, 0.8 mmol, 26% yield; starting from 60 mg of tetra-amine calix[4]pyrrole S**4**). ^{1}H NMR (400 MHz, DCM-d_2, 298K) δ (ppm) = 8.38 (dd, *J* = 7.3, 2.3 Hz, 4H); 8.12 (ddd, *J* = 8.7, 4.7, 2.3 Hz, 4H); 7.91 (d, *J* = 8.7 Hz, 8H); 7.37 (d, *J* = 8.7 Hz, 8H); 7.32 (t, *J* = 10.0, 8.8 Hz, 4H); 5.89 (d, *J* = 2.6 Hz, 8H); 3.90 (s, 12H); 2.09 (s, 12H). ^{13}C NMR (101 MHz, DCM-d_2, 298K) δ (ppm) = 166.06; 164.28; 161.66; 152.65; 151.84; 140.88; 136.64; 133.77; 128.80; 127.38; 123.05; 119.91; 117.76; 117.55; 107.25; 54.38; 54.11; 53.84; 53.57; 53.30; 52.67; 45.42; 27.93. ^{19}F NMR (376 MHz, DCM-d_2, 298K) δ (ppm) = -117.94. FTIR ν (cm^{-1}) = 3346 (N-H stretching); 2950 (C-H stretching); 1723 (C=O stretching); 1599; 1434; 1213; 1112; 761. HR-MS (ESI+) m/z: $[M+Na]^+$ calcd. For $C_{80}H_{64}F_4N_{12}NaO_8$: 1419.4798; found: 1419.4786.

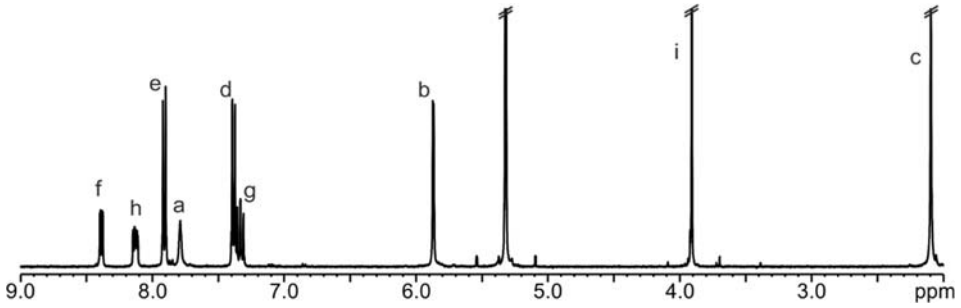


Figure S 11. ^{1}H NMR spectrum (DCM-d_2, 400 MHz, 298K) of receptor **3**. See **Figure S 10** for proton assignment.

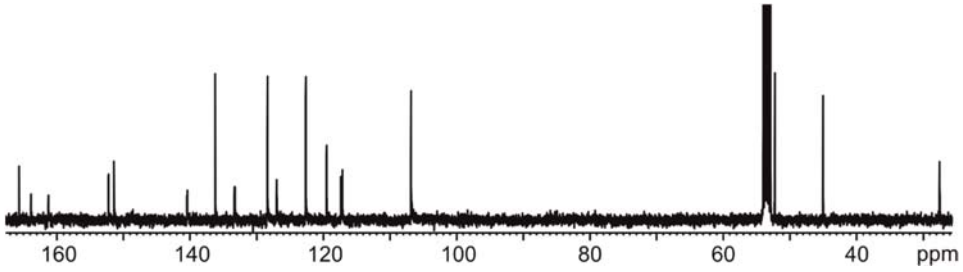


Figure S 12. ^{13}C NMR spectrum (DCM-d_2, 101 MHz, 298K) of receptor **3**.

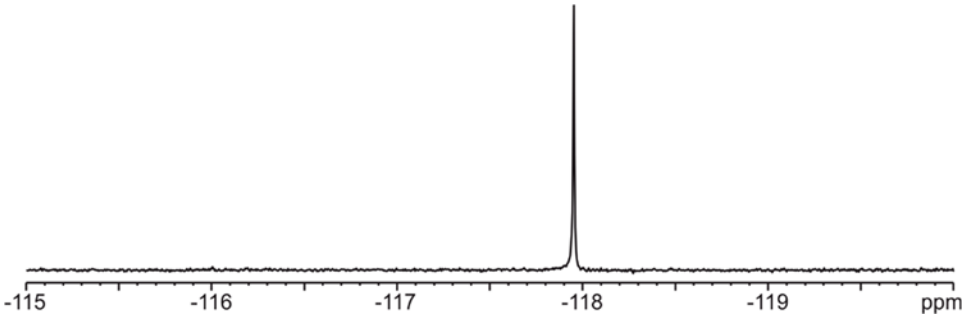


Figure S 13. ^{19}F NMR spectrum (DCM-d_2, 376 MHz, 298K) of receptor **3**.

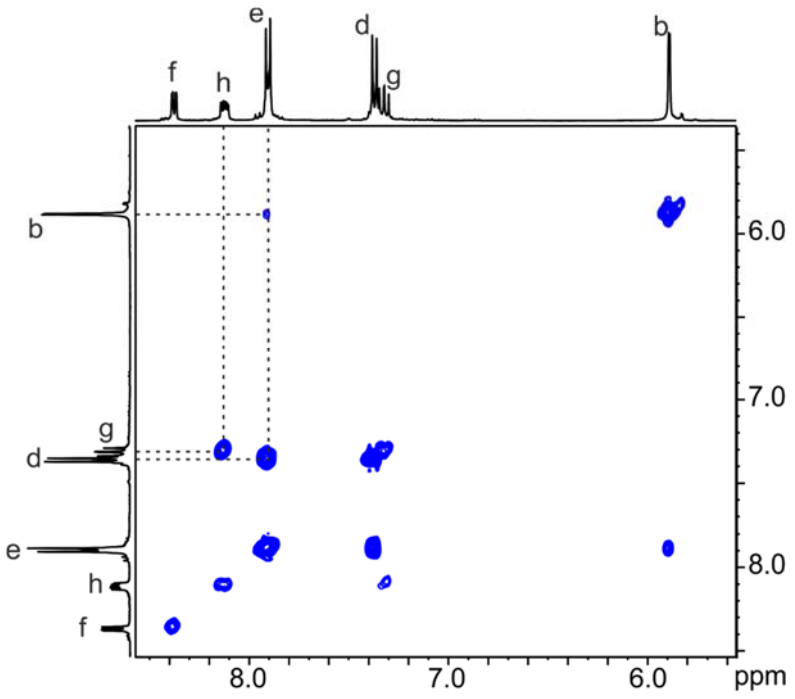


Figure S 14. Selected region of the COSY NMR spectrum (DCM-d_2, 400 MHz, 298K) of receptor **3**. See **Figure S 10** for proton assignment.

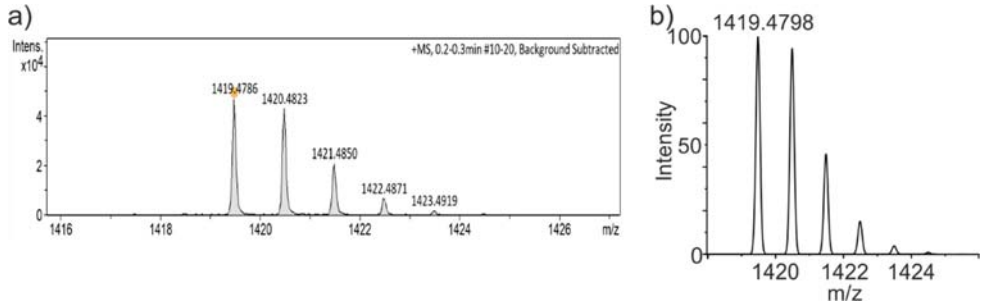


Figure S 15. a) Experimental and b) theoretical isotopic distributions for $[M+Na]^+$ peak of receptor **3**. The exact mass for the monoisotopic peak in a) and b) is indicated.

*Model compound **5***

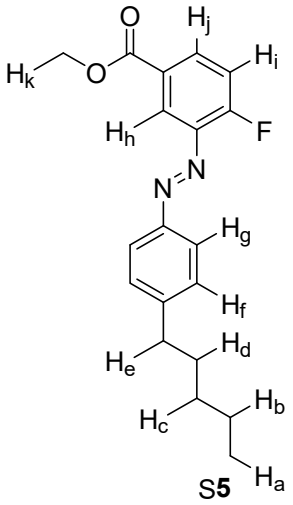


Figure S 16. Line-drawing structure of the model compound S**5** with the corresponding proton assignment.

Compound S**5** was synthetized adapting a procedure reported in the literature.[3] In brief, nitrosobenzene derivative S**F** (0.31 g, 1.7 mmol, 3 equiv.) and 4-pentylbenzenamine (0.09 mg, 0.6 mmol, 1 equiv.) were dissolved in acetic acid (3 mL). The mixture was stirred overnight at room temperature under Ar atmosphere. After, saturated $NaHCO_3$ (10 mL) was added to the reaction mixture. The product was extracted with AcOEt (2x10 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated to dryness. The resulting mixture was purified by column chromatography in silica using as eluent DCM, and affording an orange solid in a 46% yield (85 mg). ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm) = 8.40 (dd, *J* = 7.3, 2.3 Hz, 1H); 8.10 (ddd, *J* = 8.6, 4.8, 2.3 Hz, 1H); 7.89 (d, *J* = 8.3 Hz, 2H); 7.31 (d, *J* = 8.3 Hz, 2H); 7.28 (m, 1H); 3.92 (s, 3H); 2.66 (t, *J* =7.8 Hz, 2H); 1.71 – 1.59 (m, 2H); 1.37 – 1.30 (m, 4H); 0.94 – 0.86 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 298K) δ (ppm) = 165.79; 163.85; 161.22; 151.00; 147.67; 140.48; 133.10; 129.16; 126.74; 123.41; 119.71; 119.69; 117.26; 35.92; 31.47; 30.89; 22.52; 14.01. ^{19}F NMR (376 MHz, $CDCl_3$, 298K) δ (ppm) = -118.29.

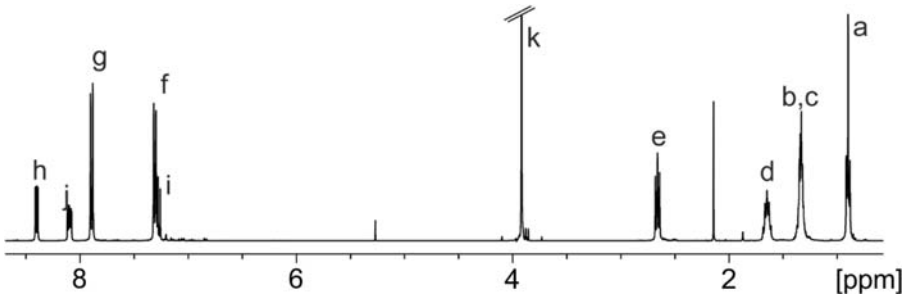


Figure S 17. ^{1}H NMR spectrum ($CDCl_3$; 400 MHz; 298K) of compound S**5**. See **Figure S 16** for proton assignment. *Residual solvent peak.

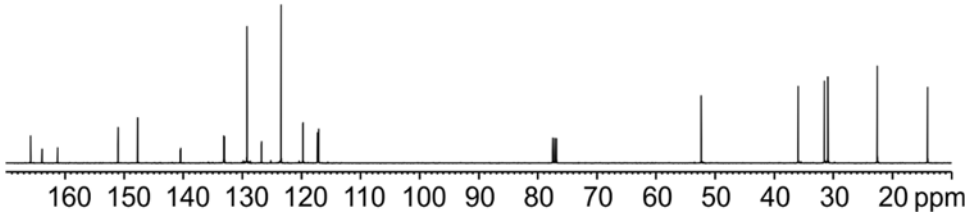


Figure S 18. ^{13}C NMR spectrum ($CDCl_3$, 101 MHz, 298K) of compound S**5**.

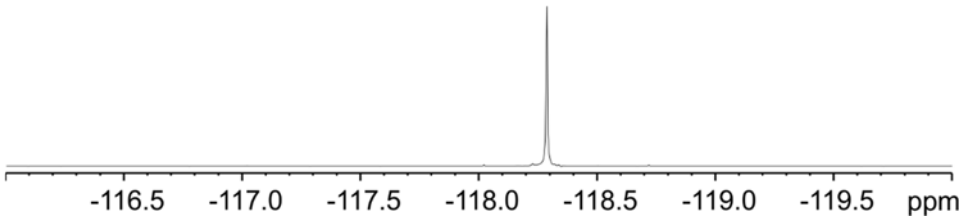


Figure S 19. ^{19}F NMR spectrum ($CDCl_3$, 376 MHz, 298K) of compound S**5**.

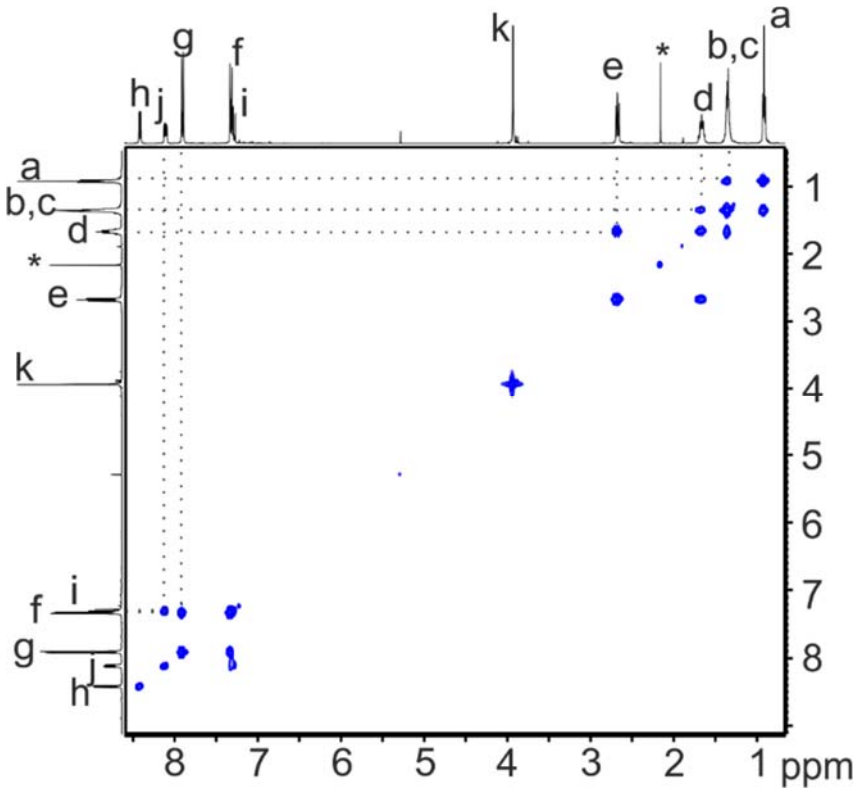


Figure S 20. COSY NMR spectrum ($CDCl_3$, 400 MHz, 298K) of compound S**5**. See **Figure S 17** for proton assignment. *Residual solvent peak.

3 Light irradiation studies

3.1. UV-Vis spectroscopy

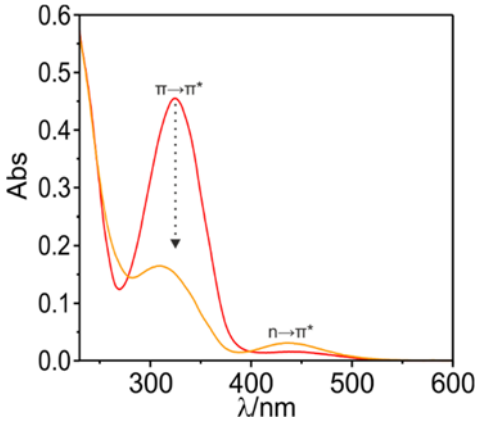


Figure S 21. UV-Vis spectra of a 10 μM DCM solution of receptor **1.** Red line corresponds to a thermo-equilibrated sample; Orange line – corresponds to the same sample after irradiation at 356 nm for 40 s (photo-stationary state, PSS).

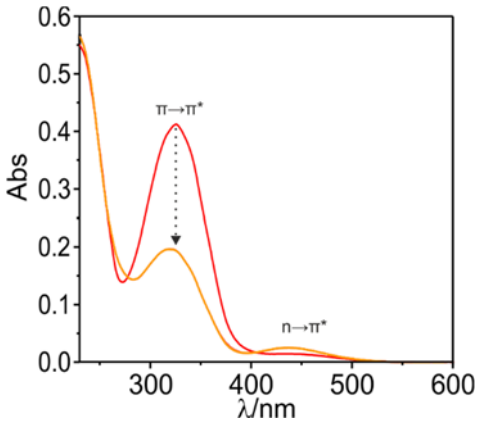


Figure S 22. UV-Vis spectra of a 10 μM DCM solution of receptor **2.** Red line corresponds to a thermo-equilibrated sample; Orange line – corresponds to the same sample after irradiation at 356 nm for 40 s (photo-stationary state, PSS).

3.2. NMR spectroscopy

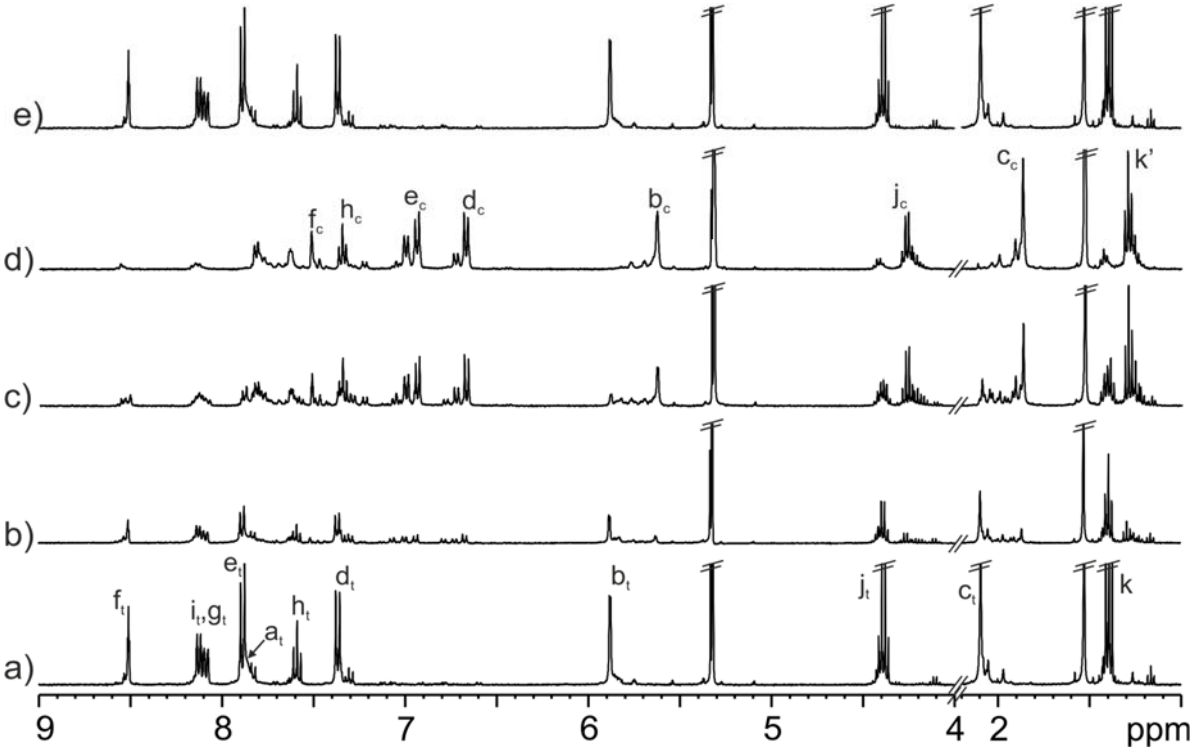


Figure S 23. Selected regions of the ^{1}H NMR spectra at 298K of a DCM-d_2 solution of receptor **1** (2 mM) before (a) and after light-irradiations at 365 nm for b) 10 s; c) 20 s; d) 40 s. Spectrum (e) corresponds to the same mixture after thermo-equilibration in the dark for 2 days at RT. "t" and "c" subscripts refer to tttt-**1** and cccc-**1** isomers, respectively. See **Figure S 1** for proton assignment.

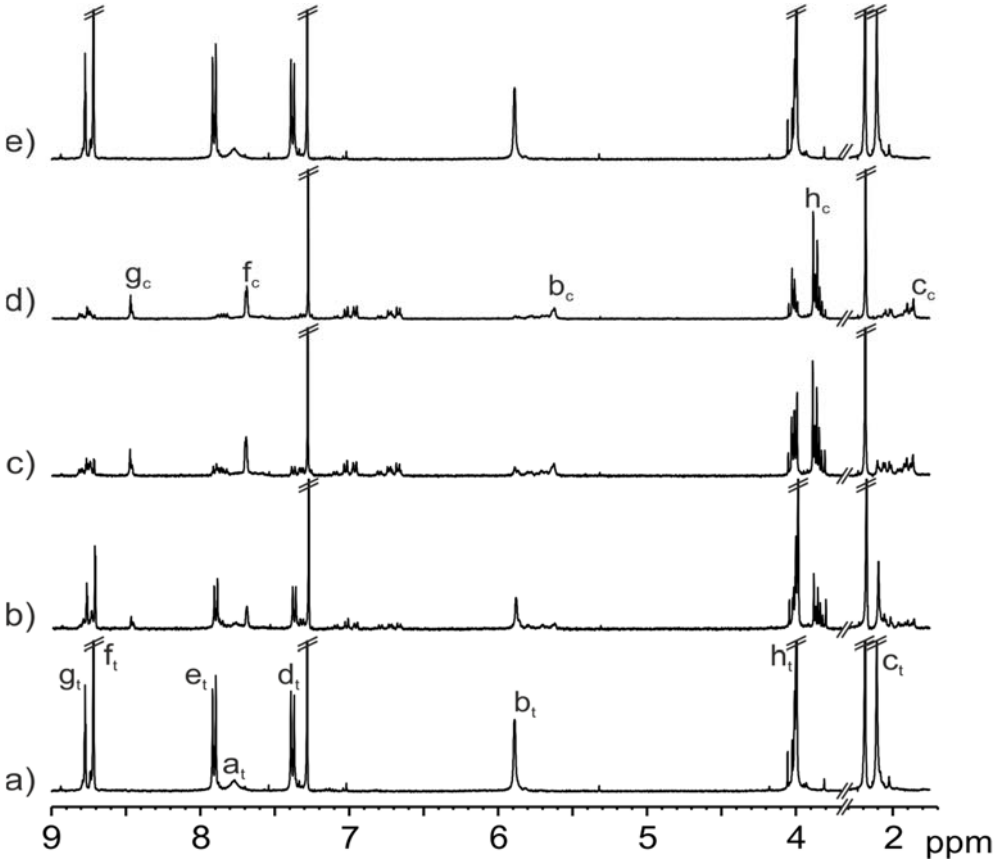


Figure S 24. Selected regions ^{1}H NMR spectra at 298K of a $CDCl_3$ solution of receptor **2** (1.5 mM) before (a) and after light-irradiation at 365 nm for b) 10 s; c) 20 s; d) 40 s. Spectrum e) corresponds to the same mixture after thermo-equilibration in the dark for 2 days at RT. "t" and "c" subscripts refer to *tttt*-**2** and *cccc*-**2** isomers, respectively. See **Figure S 5** for proton assignment.

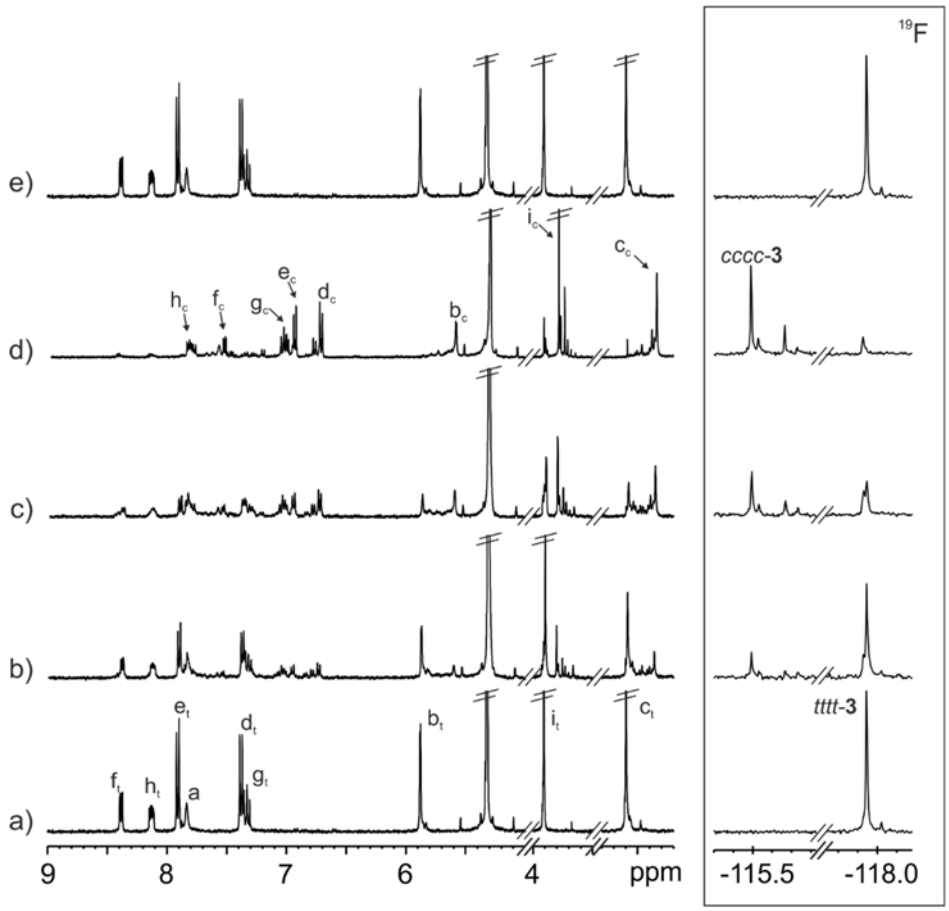


Figure S 25. Selected regions of the ^{1}H and ^{19}F NMR spectra at 298K of a DCM-d_2 solution of receptor **3** (2.4 mM) before (a) and after light-irradiation at 365 nm for b) 10 s; c) 20 s; d) 40 s; Spectrum (e) corresponds to the same mixture after thermo-equilibration in the dark for 2 day at RT. "t" and "c" subscripts refer to *tttt*-**3** and *cccc*-**3** isomers, respectively. See **Figure S 10** for proton assignment.

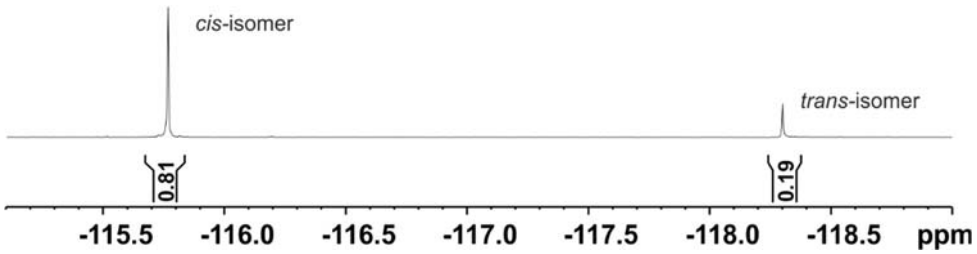


Figure S 26. ^{19}F NMR spectrum at 298K of a DCM-d_2 solution of compound S**5** (5 mM) after light-irradiation at 365 nm for 240 s. Integration of the fluorine signals shows that the *cis:trans* composition in the PSS is 80:20.

4. NMR Binding studies of the tetra-azobenzene extended calix[4]pyrroles with MTOA•Cl in dichloromethane

Receptor tttt-1 + MTOA•Cl

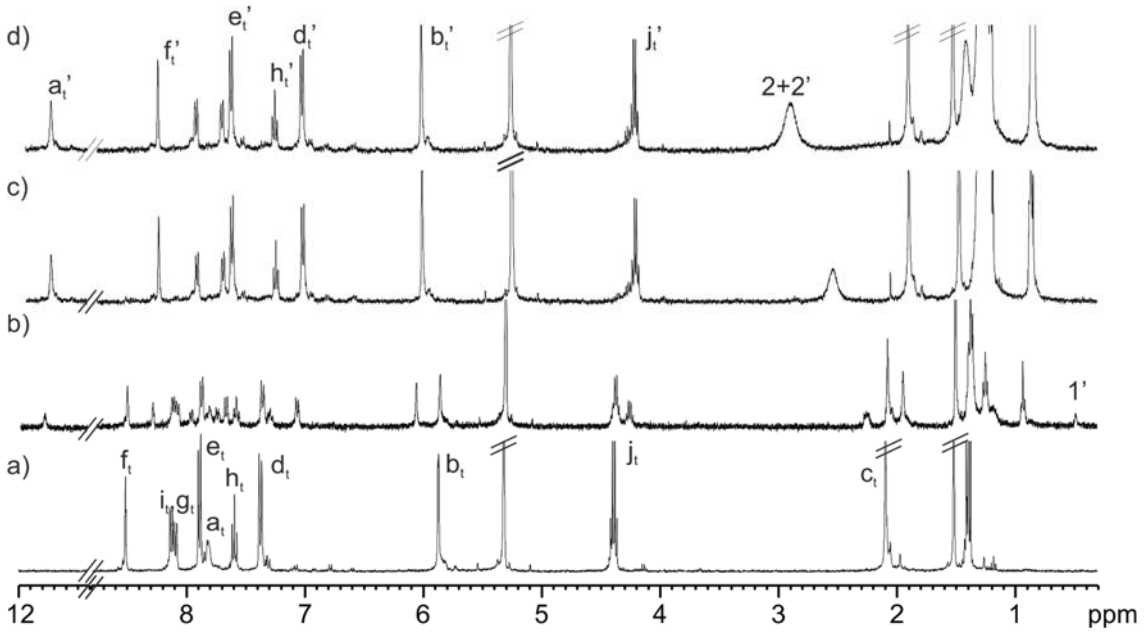


Figure S 27. Selected regions of the ^{1}H NMR spectrum at 298K of a DCM-d_2 solution of receptor **1** (1.6 mM) after addition of a) 0; b) 0.3; c) 0.75 and d) 1.5 equiv. of **MTOA•Cl**. Primed letters correspond to the 1:1 complex (MTOA•Cl)**⊂(*tttt*-1).** Signal H_1 corresponds to the methyl protons alpha to the nitrogen of the tetra-alkylammonium cation. See **Figure S 1** for proton assignment.

Receptor tttt-2 + MTOA•Cl

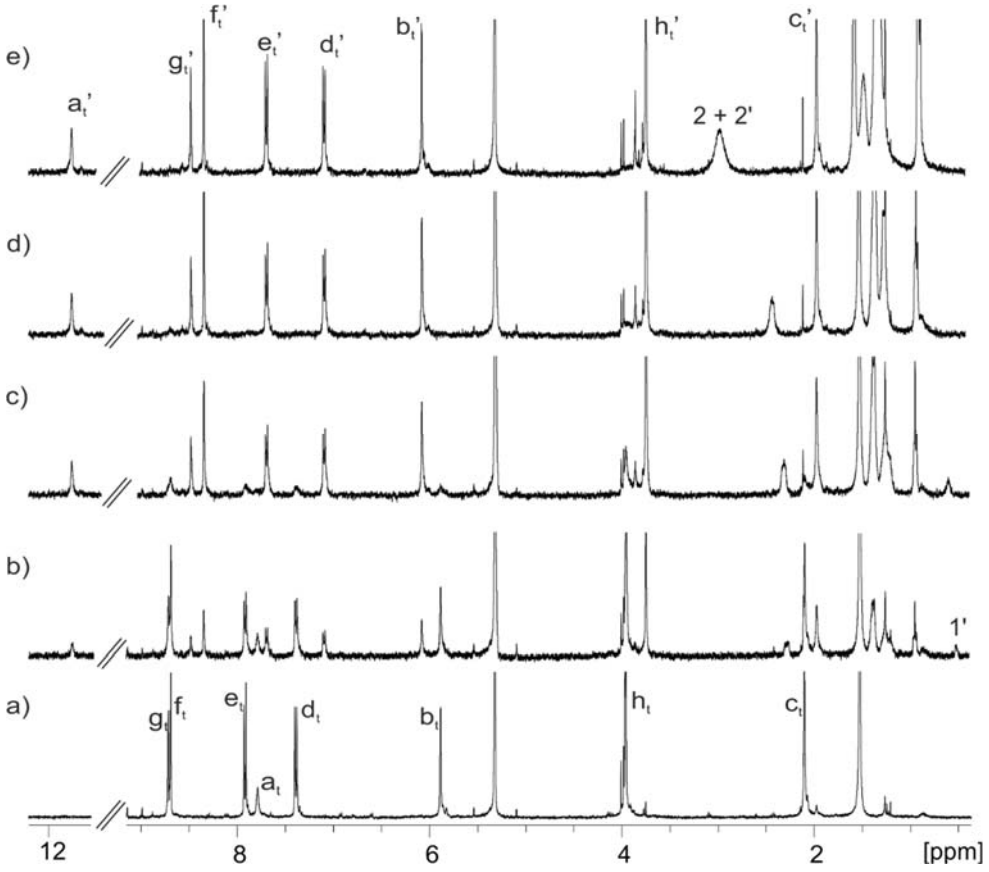


Figure S 28. Selected regions of the ^{1}H NMR spectra at 298K of a DCM-d_2 solution of receptor **2** (1.5 mM) after the addition of a) 0; b) 0.25; c) 0.50; d) 1 and e) 1.5 equiv. of **MTOA•Cl**. Primed letters correspond to the 1:1 complex (**MTOA•Cl⊂(*tttt*-2).** Signals H1 and H2 corresponds to the methyl and methylene protons alpha to the nitrogen of the tetra-alkylammonium cation, respectively. See **Figure S 5** for proton assignment.

Receptor tttt-3 + MTOA•Cl

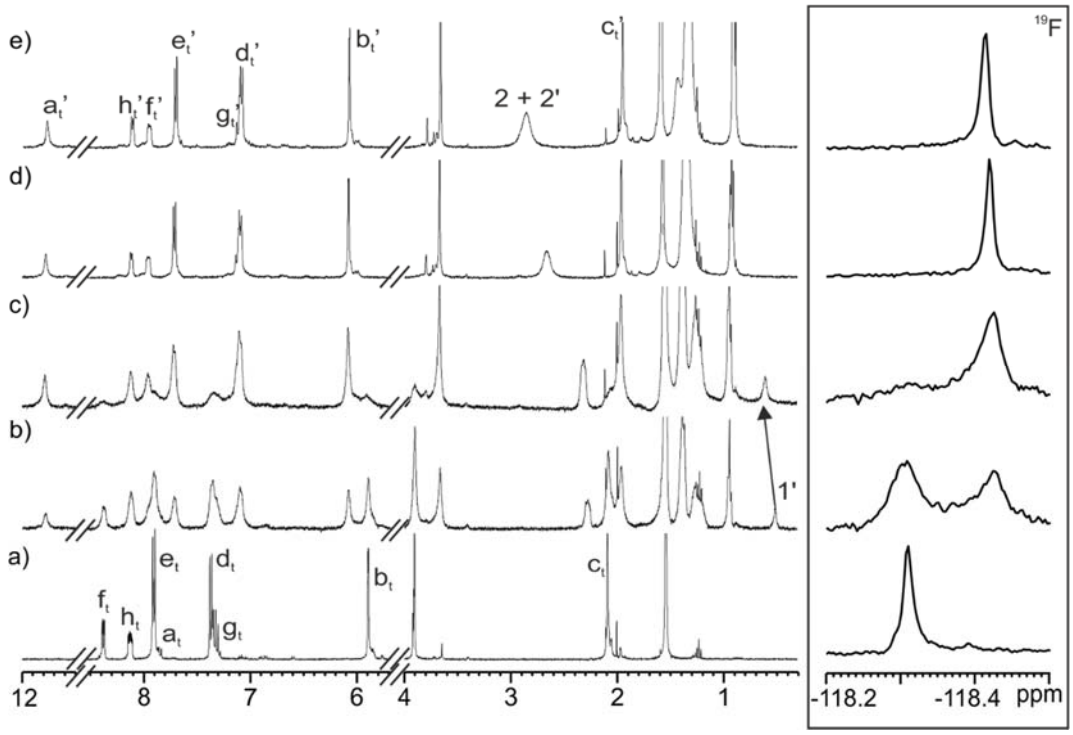


Figure S 29. Selected regions of the ^{1}H and ^{19}F NMR spectra at 298K of a DCM-d_2 solution of receptor **3** (2 mM) after the addition of a) 0; b) 0.4; c) 0.6; d) 1 and e) 1.5 equiv. of **MTOA•Cl**. Primed letters correspond to the 1:1 complex (**MTOA•Cl**)⊂(*tttt*-**3**). Signal H1 and H2 corresponds to the methyl and methylene protons alpha to the nitrogen of the tetra-alkylammonium cation, respectively. See **Figure S 10** for proton assignment.

Receptor cis-enriched-3 + MTOA•Cl

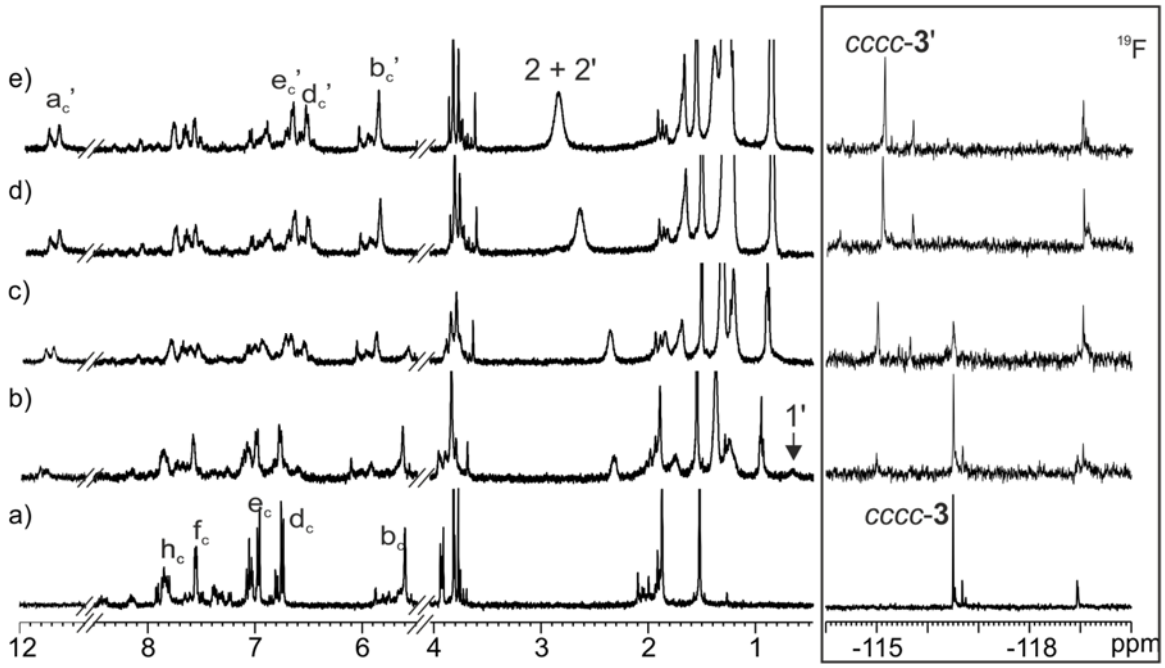


Figure S 30. Selected regions of the ^{1}H and ^{19}F NMR spectra at 298K of a light-irradiated (365 nm, 40 s) DCM-d_2 solution of receptor **3** (1.7 mM, cis-enriched-**3**) after the addition of a) 0; b) 0.25; c) 0.50; d) 1 and e) 1.5 equiv. of **MTOA•Cl**. Signal H1 and H2 corresponds to the methyl and methylene protons alpha to the nitrogen of the tetra-alkylammonium cation, respectively.

4 ITC binding studies of the tetra-azobenzene extended calix[4]pyrroles with MTOA•Cl in dichloromethane

All titrations were performed by injecting small aliquots of dichloromethane or acetone solution of the guests from a computer controlled micro syringe into the solution of the host in the same solvent placed in the cell. The solution of the host was previously thermally equilibrated or photo-irradiated (*cis*-enriched mixture) at 365 nm for 60s. The concentrations of the guest solutions were approximately seven to ten times more concentrated than the receptor ones. The association constants and enthalpy values were derived from the fit of the titration data to a 1:1 binding model using the Microcal ITC Data Analysis module. The ^{19}F NMR spectrum recorded after the ITC experiment of the photo-irradiated receptor **3** with MTOA•Cl confirms that *cis*-enriched-**3** does not isomerize back to the thermodynamically stable *trans*- isomer during the titration. We performed the same experiment for the tttt-**3** isomer for comparison reasons. We assume equal behaviour for the other tetra-azobenzene extended receptors.

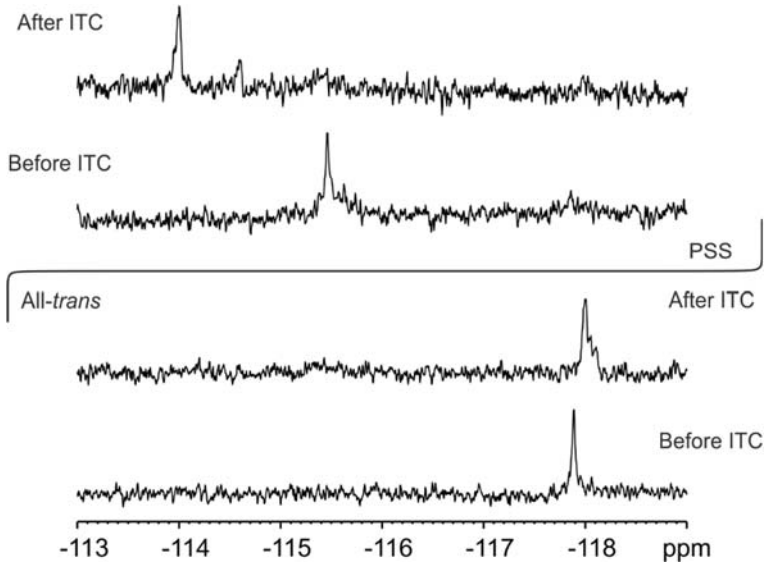


Figure S 31. Selected region of the ^{19}F NMR spectrum before and after ITC binding experiments with MTOA•Cl in CD_2Cl_2 for the *tttt*-**3** thermo-equilibrated receptor (bottom) and the cis-enriched-**3** irradiated sample (top).

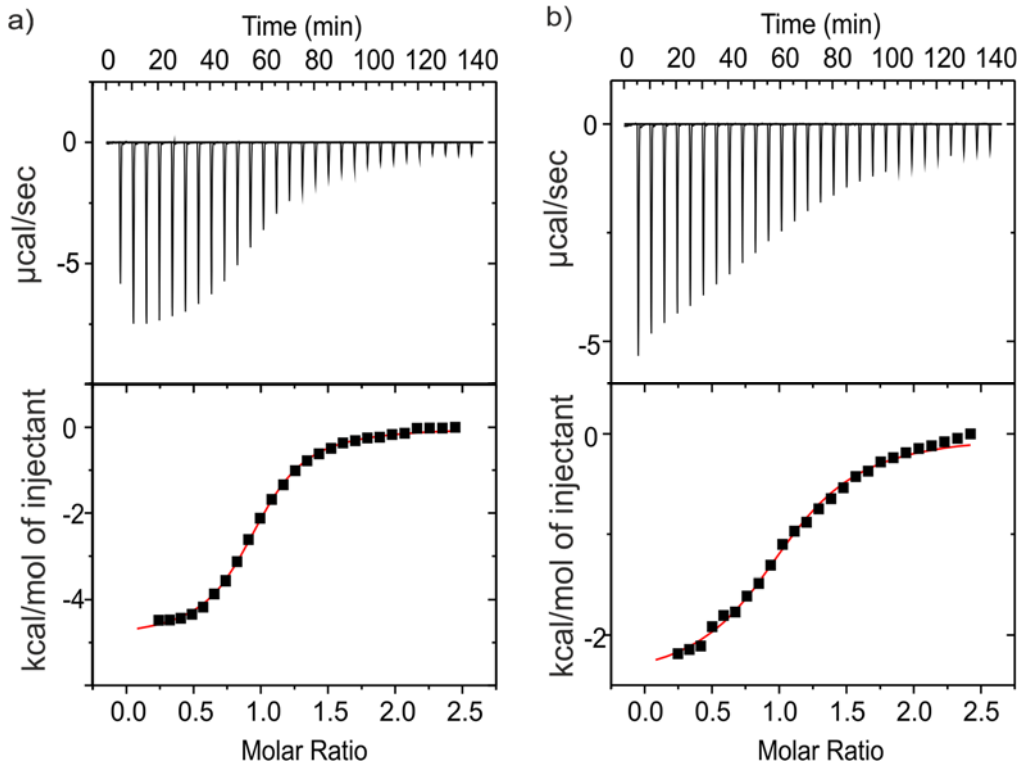


Figure S 32. Top – Trace of the raw data (heat vs time) for the titration experiment of **MTOA•Cl** (3.87 mM) into a dichloromethane solution of receptor **1**: a) *tttt*-**1** (0.34 mM) and b) cis-enriched-**1** (0.34 mM). Bottom – Binding isotherm of the calorimetric titration shown on top. To determine the values of the thermodynamic variables the ITC data was fitted considering a 1:1 binding model (red line).

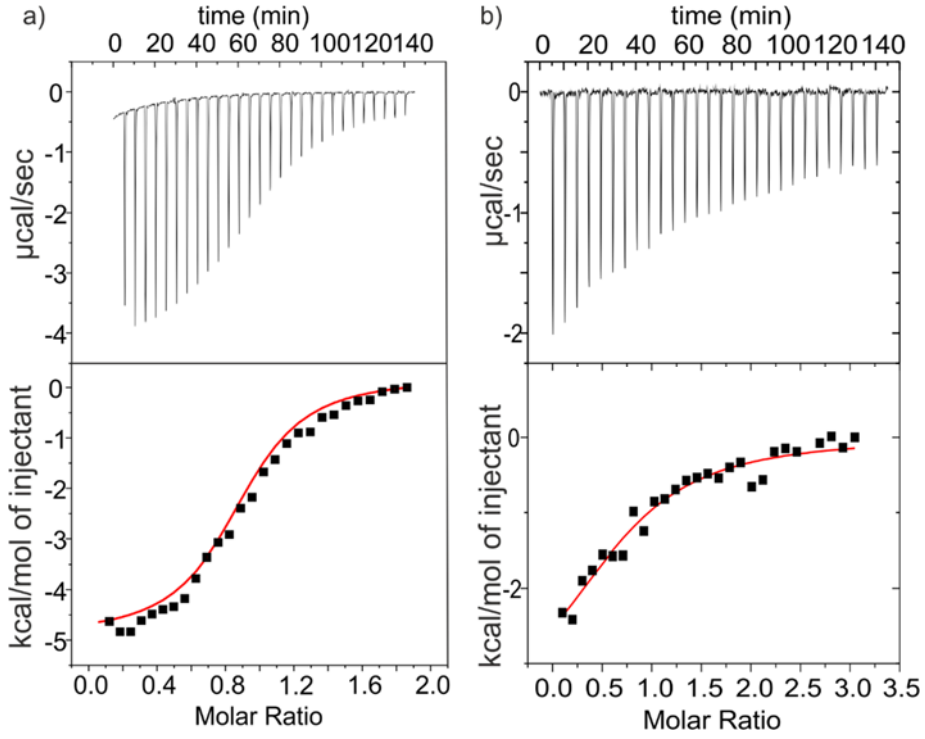


Figure S 33. Top – Trace of the raw data (heat vs time) for the titration experiment of **MTOA•Cl** (1.56 mM) into a dichloromethane solution of receptor **2**: a) *tttt*-**2** (0.18 mM) and b) cis-enriched-1 (0.11 mM). Bottom – Binding isotherm of the calorimetric titration shown on top. To determine the values of the thermodynamic variables the ITC data was fitted considering a 1:1 binding model (red line).

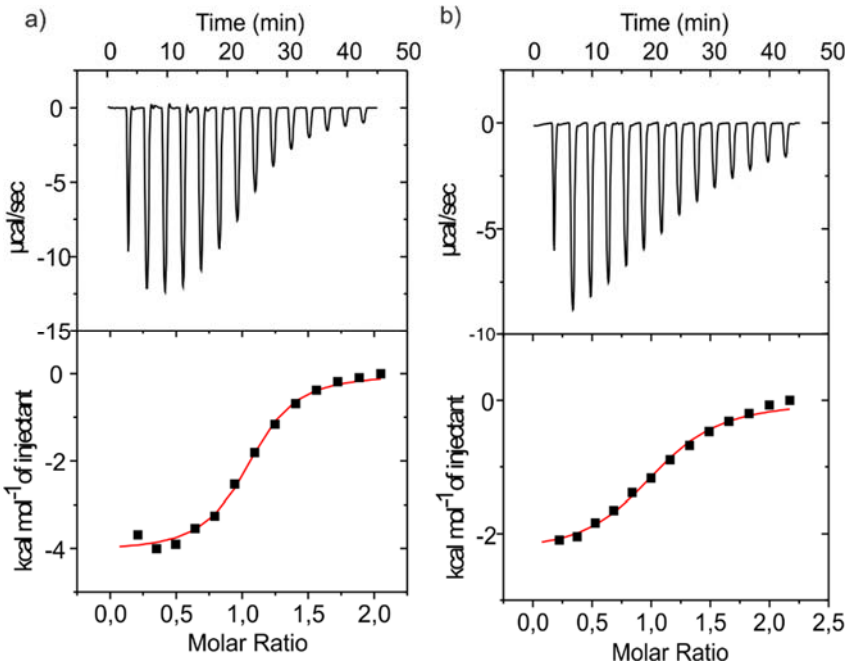


Figure S 34. Top – Trace of the raw data (heat vs time) for the titration experiment of **MTOA•Cl** (2.65 mM) into a dichloromethane solution of receptor **3**: a) *tttt*-**3** (0.29 mM) and b) cis-enriched-**3** (0.29 mM). Bottom – Binding isotherm of the calorimetric titration shown on top. To determine the values of the thermodynamic variables the ITC data was fitted considering a 1:1 binding model (red line).

5 Calculations

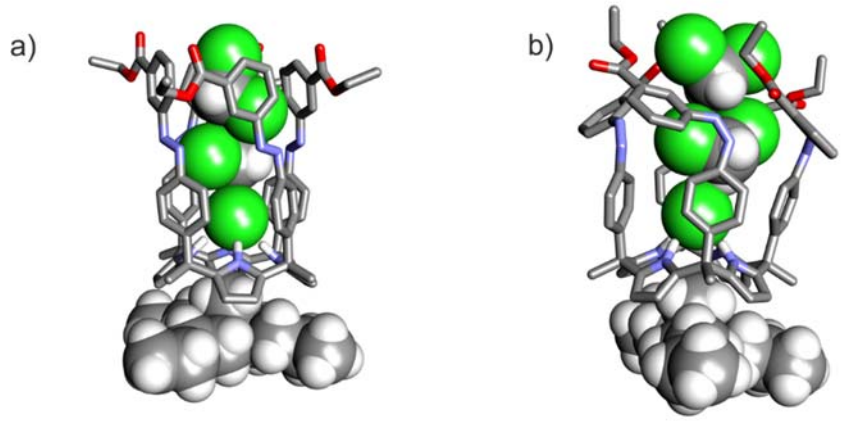


Figure S 35. Energy minimized structures (BP86-D3/def2-SVP) of the 1:1.1 complexes of a) (MTOA•Cl)$^-$⊂*tttt*-**1** and b) (MTOA•Cl)$^-$⊂*cccc*-**1**. We placed 2 implicit molecules of dichloromethane in the cavity of the two ion-paired isomeric complexes.

Dipole moment

Table S 1. Dipole moment calculated for anionic complexes Cl$^-$⊂*tttt*-**1**, Cl$^-$⊂*cccc*-**1**, Cl$^-$⊂*tttt*-**3** and Cl$^-$⊂*tttt*-**3** complexes

	Dipole moment (Debye)
tttt-**1**	2.855
cccc-**1**	0.4221
tttt-**3**	3.523
cccc-**3**	0.7991

Electrostatic Surface Potential (ESP)

The ESPs of the azobenzene model derivatives were calculated by DFT calculation at the BP86-D3/def2-SVP level of theory using GAUSSIAN version 09.

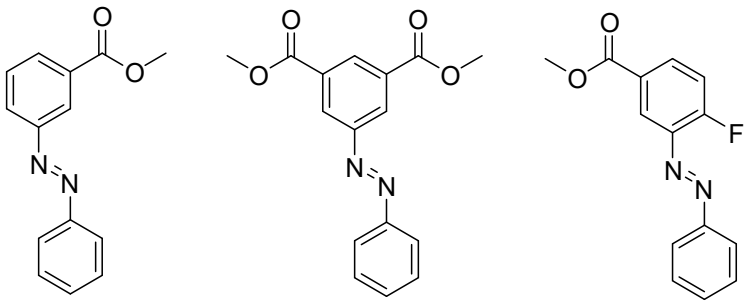


Figure S 36. Line drawing structure of the model compounds **M1, M2** and **M3** used for the ESP calculations.

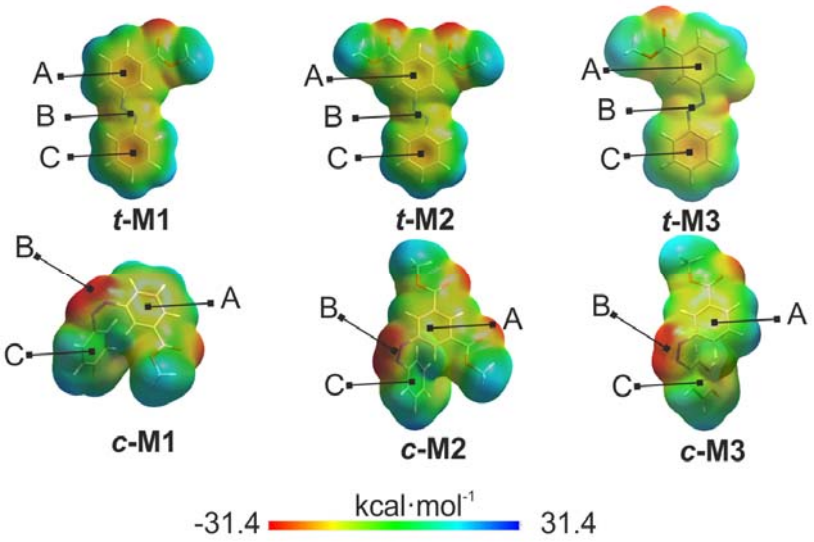


Figure S 37. Calculated ESP surface for the trans and cis isomers of model systems **M1-M3**. ESP cubes mapped at electron density value of 0.004a.u..

Table S 2. ESP values at the centre of aromatic rings (A and C) and at the centre of N=N bond (B) for the computed model system **M1-M3**.

		kcal.mol^{-1}		
		A	**B**	C
M1	*trans*	-19.9	**-13.8**	-21.9
	cis	-20.1	**-34.9**	-21.5
M2	*trans*	-18.6	**-11.7**	-20.4
	cis	-16.8	**-33.1**	-20.3
M3	*trans*	-14.8	**-12.6**	-21.4
	cis	-16.6	**-33.3**	-19.5

6 Anion transport studies

6.1.HPTS Assay

HPTS⊂EYPC-LUV-C[4]P_X (pre-insertion of the carrier): 12.5 mg of egg yolk l-α-phosphatidylcholine (EYPC) were dissolved in 0.5 mL of degassed chloroform. Carrier was then added as chloroform solution to have the respective carrier:EYPC molar ratio. The solvent was removed under reduced pressure at room temperature with continuous rotation to form a thin lipid film. The resulting film was kept in high vacuum overnight. The lipid film was hydrated for 1 h with 1 mL of a buffered HPTS solution (100 mM NaCl; 10 mM HEPES; 1 mM HPTS; Ph 7.0) under continuous stirring. The lipids' suspension was then subjected to eight freeze-thaw cycles and extruded twenty-one times using a polycarbonate membrane of 100 nm pore diameter. Finally; the extravesicular HPTS dye was removed by Sephadex-G50 size exclusion chromatography using buffer (100 mM NaCl; 10 mM HEPES; pH

7.0) as eluent. The lipid concentration of the final solution was estimated considering the initial lipid concentration and the final volume resulting from the column chromatography; Final estimated [EYPC] ≈ 3.4–4.5 mM.

Transport evaluation with LUVs: In a typical transport experiment; freshly prepared HPTS⊂EYPC-LUVs-**C[4]P_X** (10–18 μL for final lipid concentration ≈ 30 μM) were added to gently stirred buffer (2 mL; 100 mM NaCl; 10 mM HEPES; pH 7.0) in a polystyrene cuvette. Fluorescence emission at 510 nm (λex = 450 nm) was monitored during 300 s. NaOH (20 μL; 0.5 mM) was added after 50 s and excess of Gramicidin D (20 μL stock solution in DMSO) was added after 250 s. Continuous stirring and constant temperature were maintained (25.0 ± 0.1 °C with a Peltier device) during the transport experiment.

All fluorescence curves were normalized considering the starting point as 0% F. The 100% F value corresponds to the emission value after the addition of Gramicidin D (out of the transport window). To facilitate data comparison time values were also normalized: initial point of base pulse addition (t = 50 s normalized to t = 0 s) and endpoint of the experiment (just before addition of Gramicidin D; t = 250 s normalized to t = 200 s).

F value just before the addition of Gramicidin was defined as fractional activity Y in order to analyze the experimental data with the Hill equation (**eq. 1**) to get the effective concentration (EC_{50}) and the Hill coefficient (n):

$$Y = Y_i + \frac{Y_i - Y_\infty}{1 + \left(\frac{c}{EC_{50}}\right)^n} \quad (\boldsymbol{eq.1})$$

Y = fractional activity = F just before gramicidin addition (t = 300 s).

Yi = Y in the absence of carrier (control experiment adding DMSO).

Yꝏ = Y at the saturation point (after addition of Gramicidin) (100%).

c = calix[4]pyrrole concentration.

EC_{50} = half-maximum effective concentration

n = Hill coefficient

- *Control experiment (no carrier)*

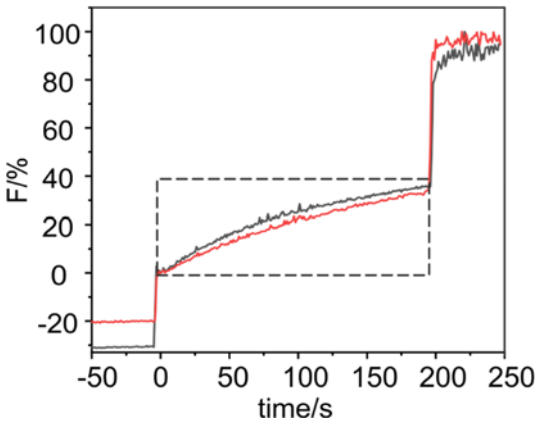


Figure S 38. Fluorescence time-course curves of HTPS chloride transport experiments without carrier to determine Yi. Black line – non-irradiated sample; Red line – light irradiated sample at 365 nm for 60 s.

- *Transport studies with receptor **1***

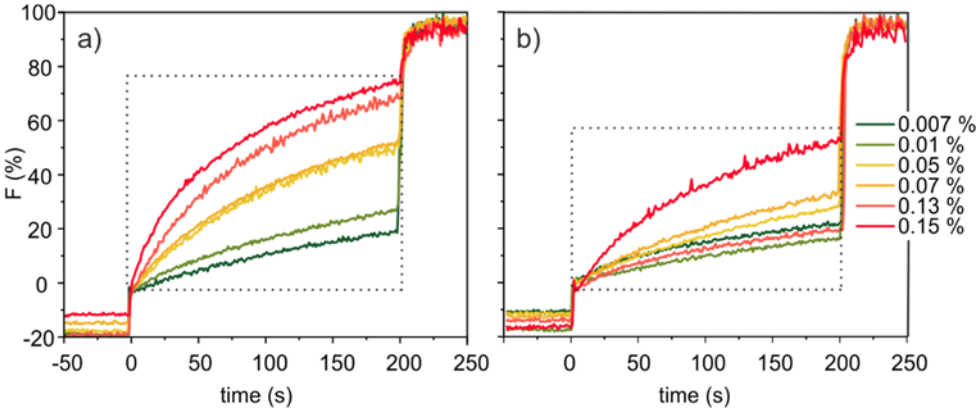


Figure S 39. Fluorescence time-course traces for receptor **1** (0.007 to 0.15%) in the HPTS assay (NaCl). The concentration of carrier is expressed in % carrier/EYPC molar ratio. a) all-*trans* and b) PSS sample of **1**.

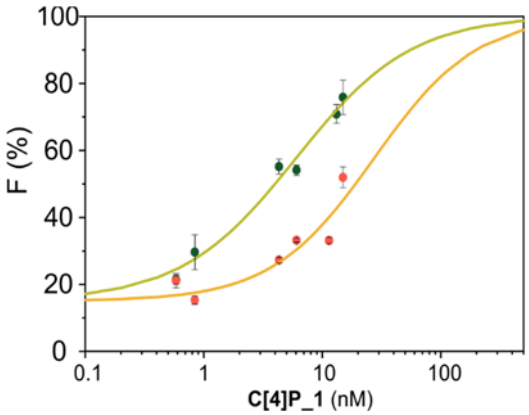


Figure S 40. Dose-response curves for receptor **1** in the HPTS assay. Green points – *all-trans*; Orange points – light-irradiated sample.

- *Transport studies with receptor **2***

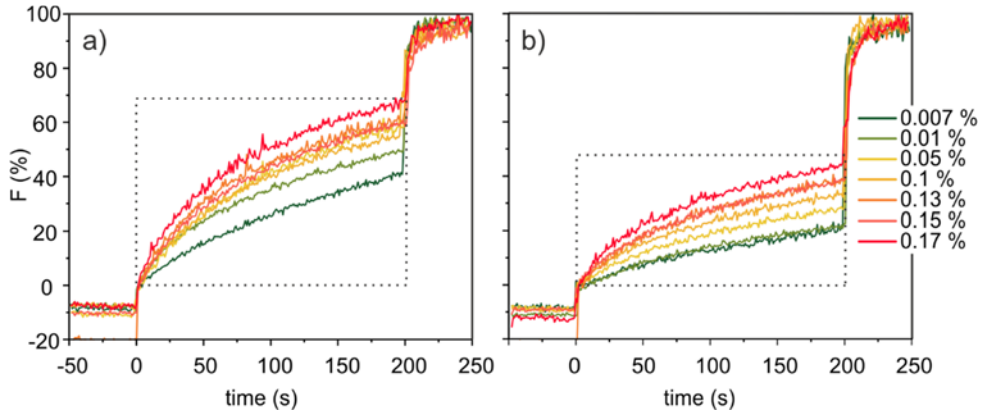


Figure S 41. Fluorescence time-course traces for receptor **2** (0.007 to 0.17%) in the HPTS assay (NaCl). Concentration of carrier is expressed in % carrier/EYPC molar ratio. a) all-*trans* and b) PSS sample of **2**.

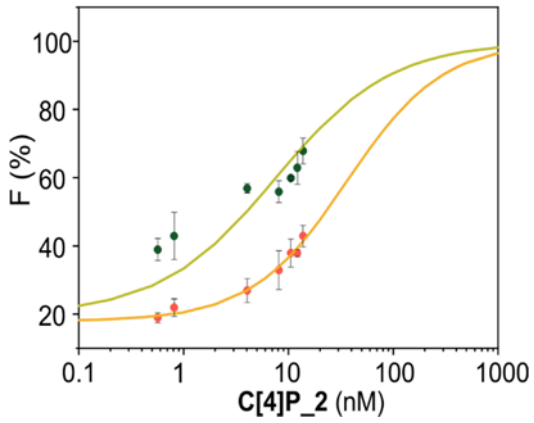


Figure S 42. Dose-response curves for receptor **2** in the HPTS assay. Green points – *all-trans*; Orange points – light-irradiated sample.

- *Transport studies with receptor **3***

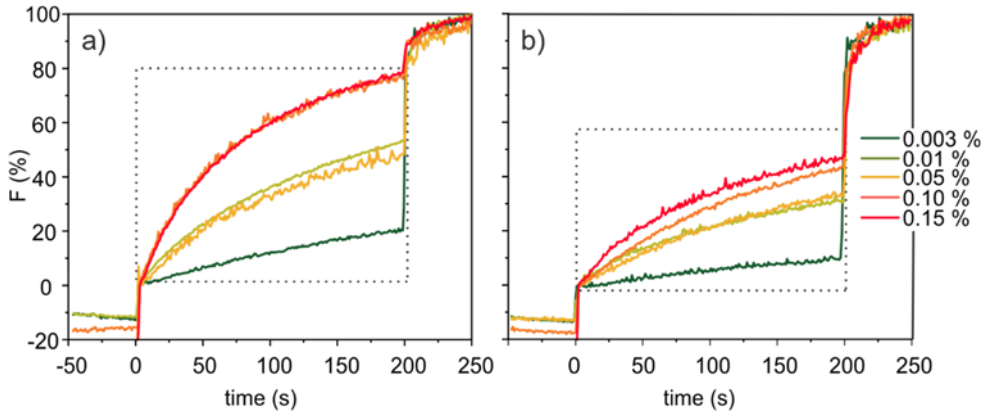


Figure S 43. Fluorescence time-course traces for receptor **3** (0.003 to 0.15%) in the HPTS assay (NaCl). The concentration of carrier is expressed in % carrier/EYPC molar ratio. a) all-*trans* and b) PSS sample of **3**.

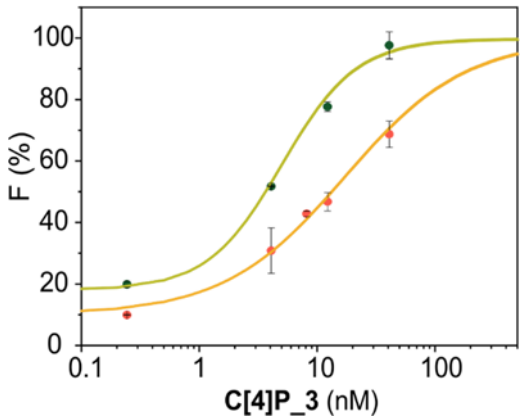


Figure S 44. Dose-response curves for **3** using HPTS assays. Green points – all-*trans*; Orange points – light-irradiated sample.

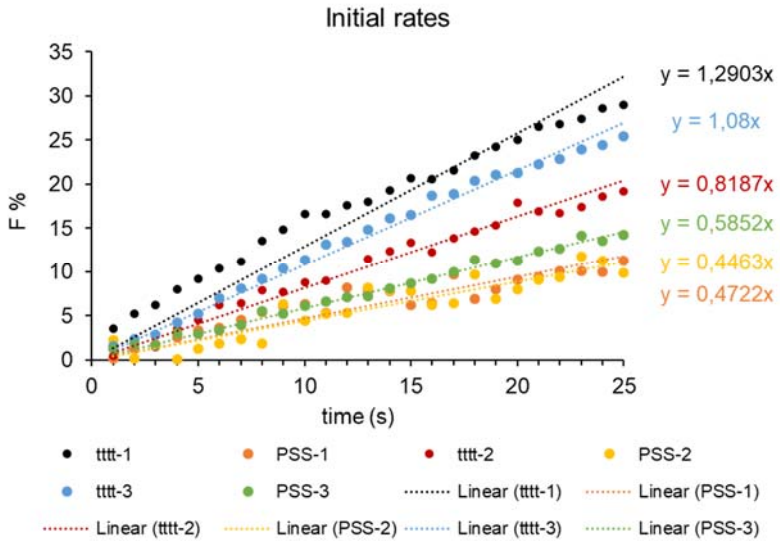


Figure S 45. Initial transport rate determination at 0.15% carrier/lipid molar ratio. Intercept of the linear fit of the initial 25 s was set to 0.

6.2.Lucigenin Assay

Preparation of Lucigenin⊂POPC-cholesterol-LUVs- C[4]P_X: 2.8 mL of a 30 mM 1- palmitoyl-2-oleoylphosphatidylcholine (POPC) solution in degassed chloroform were combined with 1.2 mL of a 30 mM solution of 1-cholesterol in the same solvent to reach a 7:3 POPC:cholesterol molar ratio. The corresponding amount of carrier 1 or 2 was then also added as chloroform solution (to achieve 0.03% and 0.1% carrier:POPC molar ratio) to 0.5 mL of lipid solution (POPC:cholesterol 7:3). The solvent was removed under reduced pressure at room temperature with continuous rotation to form a thin lipid film. The resulting film was kept in high vacuum overnight. The lipid film was hydrated with 0.5 mL of a lucigenin solution (225 mM $NaNO_3$; 0.8 mM lucigenin). The resulting suspension was sonicated for 10 seconds and then left for 1 hour under continuous stirring. The lipids' suspension was then subjected to eight freeze-thaw cycles and extruded twenty-nine times using a polycarbonate membrane of 200 nm pore diameter. Finally, the extravesicular lucigenin dye was removed by Sephadex-G50 size exclusion chromatography using aqueous $NaNO_3$ 225 mM as eluent. The collected LUVs were diluted with $NaNO_3$ solution (225 mM) to obtain a vesicle solution with 0.4 mM lipid concentration.

Transport evaluation with LUVs prepared using the pre-insertion method: In a typical transport experiment; the corresponding previously described freshly prepared Lucigenin⊂POPS-LUVs-**C[4]P_X** (2 mL) were placed in a polystyrene fluorescence cuvette. Fluorescence emission was monitored at λem = 535 nm (λex = 450 nm) during the addition of NaCl (25 mM) (50 uL of 1 M at 50 s). Continuous stirring and constant temperature were maintained (25.0 ± 0.1 °C with a Peltier device) throughout the transport experiment. The experimental data were normalized by dividing the fluorescence values by the fluorescence value before the addition of chloride. Then the normalized traces were averaged as plotted as % fluorescence.

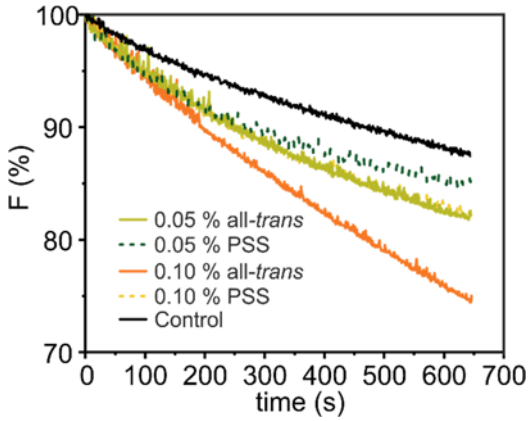


Figure S 46. Fluorescence time-course curves of chloride transport experiments using Lucigenin assay with carrier **3** at two different concentrations (0.05% and 0.1% carrier/POPC molar ratio) all-trans and light-irradiated. No carrier was present in the control curve.

7 References

[1] Escobar, L.; Arroyave, F. A.; Ballester, P., Synthesis and Binding Studies of a Tetra-α Aryl-Extended Photoresponsive Calix[4]pyrrole Receptor Bearing meso-Alkyl Substituents. *Eur. J. Org. Chem.* **2018,** *2018* (9), 1097-1106.

[2] Ballester, P.; Gil-Ramírez, G., Molecular recognition and self-assembly special feature: Self-assembly of dimeric tetraurea calix[4]pyrrole capsules. *Proc. Natl. Acad. Sci.* **2009,** *106* (26), 10455-9.

[3] Knie, C.; Utecht, M.; Zhao, F.; Kulla, H.; Kovalenko, S.; Brouwer, A. M.; Saalfrank, P.; Hecht, S.; Bléger, D., ortho-Fluoroazobenzenes: Visible Light Switches with Very Long-Lived Z Isomers. *Chem. Eur. J.* **2014,** *20* (50), 16492-16501.