Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information for

Imino-Thiolate-Templated Synthesis of Chloride-Selective Neutral Macrocyclic Host with a Specific "Turn-Off-On" Fluorescence Response for Hypochlorite (ClO^-)

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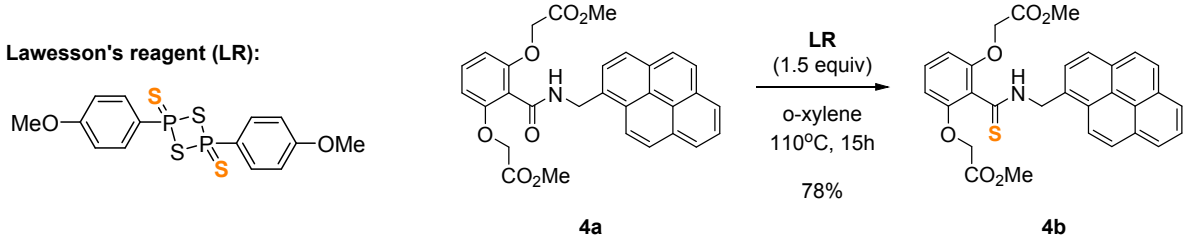
1. General remarks

1.1. Introduction

Commercially available reagents were purchased from Sigma-Aldrich, Alfa Aesar or Th.Geyer, and used without purification as received. Hexanes (65-80˚C fraction from petroleum) and EtOAc were purified by distillation. Thin-layer chromatography was carried out on silica gel 60 F254 (Merck). Compounds were purified using automatic flash chromatography with silica gel 60 (230-400 mesh, Merck). The organic solutions were dried over $MgSO_4$ or Na_2SO_4. The NMR spectra were recorded on Bruker Avance II 400 MHz (at 400 MHz and 100 MHz for ^1H, and ^{13}C NMR spectra, respectively) or Varian VNMRS 600 MHz (at 600 MHz and 151 MHz for ^1H and ^{13}C NMR spectra, respectively) spectrometers using solutions in $CDCl_3$ (^1H NMR σ = 7.26 ppm, ^{13}C NMR σ = 77.26 ppm) or DMSO-d_6 (^1H NMR σ = 2.50 ppm, ^{13}C NMR σ = 39.52 ppm) at 303K. All significant resonances were assigned by COSY (^1H-^1H), HSQC (^1H-^{13}C) and HMBC (^1H-^{13}C) correlations. Mass spectra were measured on Synapt G2-S HDMS (Waters Inc) mass spectrometer equipped with an electrospray ion source and q-TOF type mass analyzer. Elemental analyses were obtained with a Perkin–Elmer 2400 CHN analyzer. Absorption and fluorescence spectra were measured at 298K in MeCN (HPLC grade, Fluka) on Cary 60 (Agilent Technologies) and Cary Eclipse (Agilent Technologies) spectrophotometers, respectively. The compounds **4a** and **5** were prepared as described previously.[1]

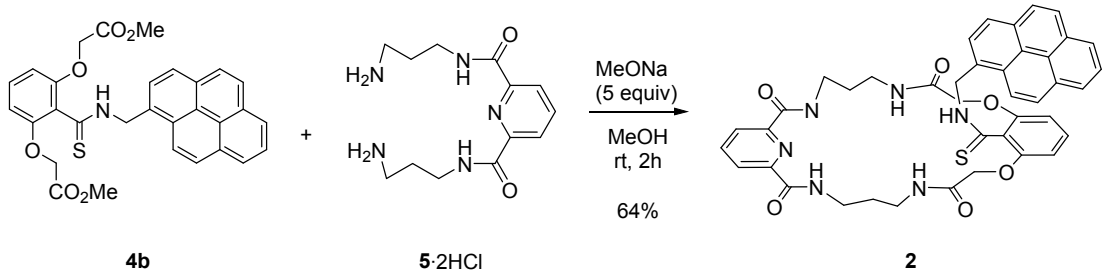
1.2. Synthesis of compound (4b) by thionation of α,ω-diester 4a



The α,ω-diester **4a**[1a] (1.03 g, 2.0 mmol) and Lawesson's reagent (**LR**, 1.20 g, 3.0 mmol, 1.5 equiv) were suspended in toluene (30 mL), purged with Ar, and vigorously stirred under reflux (an oil bath was used for heating) for 15h. After cooling to room temperature silica gel (~5.0 g) was added and the reaction mixture was concentrated under vacuum. The yellow residue was purified by column chromatography[$] to afford thioamide diester **4b** as a yellowish amorphous solid (0.83 g, 78.3%). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.45 (s, 1H), 8.35 (d, J = 12.3 Hz, 1H), 8.26 – 7.97 (m, 9H), 7.10 (t, J = 8.4 Hz, 1H), 6.47 (d, J = 8.4 Hz, 2H), 5.68 (d, J = 4.8 Hz, 2H), 4.59 (s, 4H), 3.31 (s, 6H) ppm. $^{13}C\{1H\}$ NMR ($CDCl_3$, 100 MHz): δ 193.10, 169.53, 154.87, 131.67, 131.41, 130.98, 130.02, 129.88, 129.80, 128.75, 128.31, 128.12, 127.85, 127.57, 126.35, 125.62, 125.57, 125.12, 124.99, 124.71, 123.92, 107.53, 66.35, 52.05, 48.85 ppm. LRMS (ESI) m/z $[M + Na]^+$ Calcd for $C_{30}H_{27}NO_6SNa$ 552.61; Found 552.63.

$ Note: ~60 g SiO_2 was used for filling the column, ~1.0L of hexanes/ethyl acetate 9:1 to 8:2 v/v was used to remove unreacted LR and nonpolar impurities and ~2.0L of hexanes/ethyl acetate 7:3 v/v was used to isolate the product.

1.3. Synthesis of macrocyclic host – 4,10,16,22-tetraoxo-N-[(pyren-1-yl)methyl]-2,24-dioxa-5,9,17,21,30-pentaazatricyclo[23.3.1.1^{11,15}]triaconta-1(29),11,13,15(30),25,27-hexaene-29-carbothioamide (2)



The product was prepared using a synthetic protocol described procedures.[1-2]

To the solution of thioamide α,ω-diester **4b** (200 mg, 0.38 mmol) and dihydrochloride salt of α,ω-diamine **5**[1b] (140 mg, 0.38 mmol) in anhydrous MeOH (100 mL) was added a freshly prepared MeONa (1.9 mmol, 5 equiv) in MeOH (50 mL) and the reaction mixture was stirred for 48 h under ambient conditions. Afterward, silica gel (~2 g) was added and the reaction mixture was concentrated under vacuum. The remaining yellow residue was purified by silica gel chromatography using a gradient of MeOH in DCM (1:99 → 5:95, v/v) as eluent to yield the product **2** (181 mg, 64.1%) as yellowish amorphous solid. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.71 (s, 1H), 8.74 (t, J = 6.0 Hz, 2H), 8.32 – 8.23 (m, 3H), 8.22 – 8.07 (m, 5H), 8.07 – 7.97 (m, 4H), 7.65 (t, J = 5.5 Hz, 2H), 7.31 (t, J = 8.4 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 5.70 (d, J = 5.5 Hz, 2H), 4.71 (s, 4H), 3.25 – 3.03 (m, 8H), 1.62 (s, 4H) ppm. ^{13}C{1H} NMR (DMSO-d_6, 151 MHz): δ 194.19, 167.64, 162.82, 153.56, 148.36, 139.38, 130.68, 130.34, 130.11, 129.65, 128.12, 127.35, 127.23, 126.22, 125.96, 125.35, 125.26, 124.70, 124.01, 123.96, 123.69, 122.81, 122.01, 106.24, 67.52, 47.34, 35.84, 35.51, 28.61 ppm. HR-MS (EI) m/z $[M]^+$ Calcd for $C_{41}H_{38}N_6O_6SNa$ 742.2574; Found 742.2565.

1.4. Titration details

Table S1. Titration details, global stability constants K_a (M^{-1}), and selected maximum signal shifts ($\Delta\delta_{max}$) of amide protons for host **2** with various anions[a]

No	Anion	c_{Host} (M)	c_{Guest} (M)	K_a (M^{-1})	$\Delta\delta_{max}$ (ppm)		
					NH(a)	NH(b)	NH(c)
1	Cl^-	0.00960	0.1897	1128.5±23.4	0.54	0.29	-0.33
2	$MeCO_2^-$	0.01008	0.2772	190.9±7.3	-[b]	0.82	1.12
3	$PhCO_2^-$	0.00960	0.2662	32.2±1.0	-[b]	0.92	-[c]
4	$H_2PO_4^-$	0.00960	0.3110	159.7±6.6	-[b]	1.12	1.18

[a] Determined in DMSO-d_6 + 0.5% H_2O at 303K by ^{1}H NMR titration experiments and nonlinear curve fitting using HypNMR 2008 software;[3] anions added as tetrabutylamonium (TBA) salts. [b] Determination of $\Delta\delta_{max}$ not possible due to broadening of the signal upon addition of the first aliquots of anion. [c] Signal was covered by other peaks during addition of the first aliquots of $TBAPhCO_2$.

1.6. Titration spectra

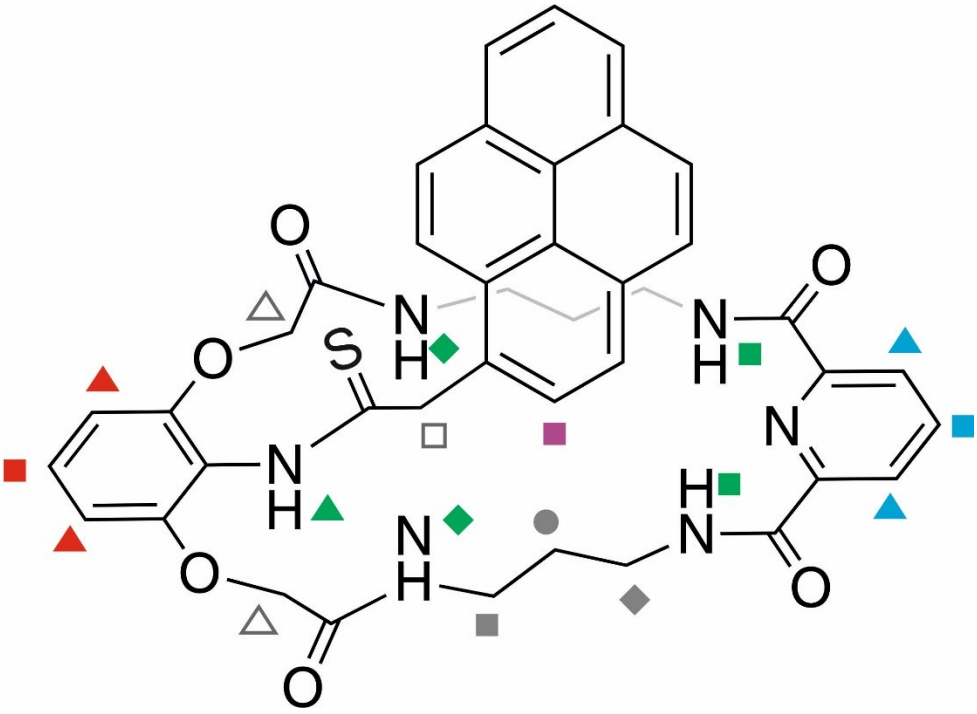


Fig. **S1.** Labeling of the selected protons of the macrocyclic host **2**.

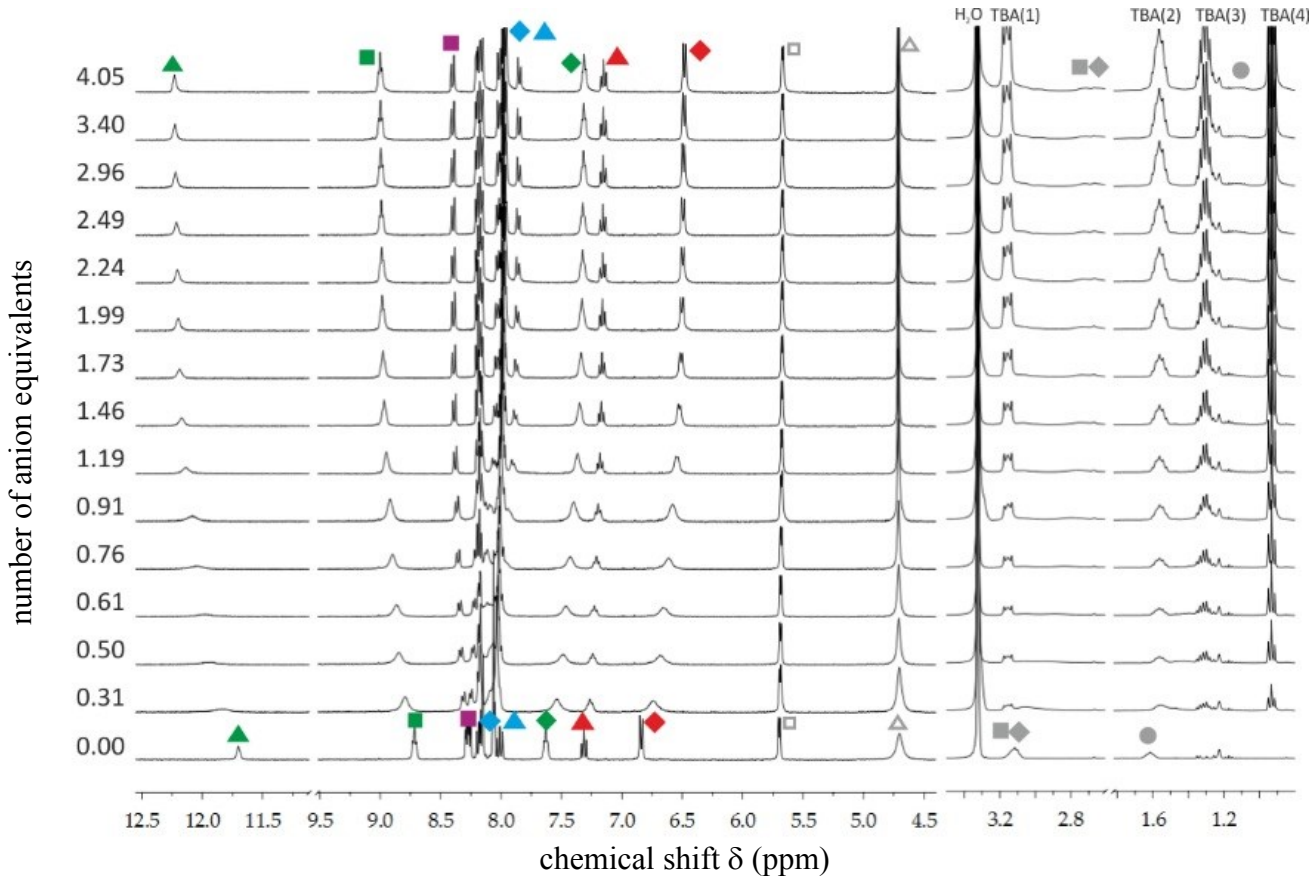


Fig. **S2.** Stacked plot from ^{1}H NMR titration of **2** with increasing amount of TBACl.

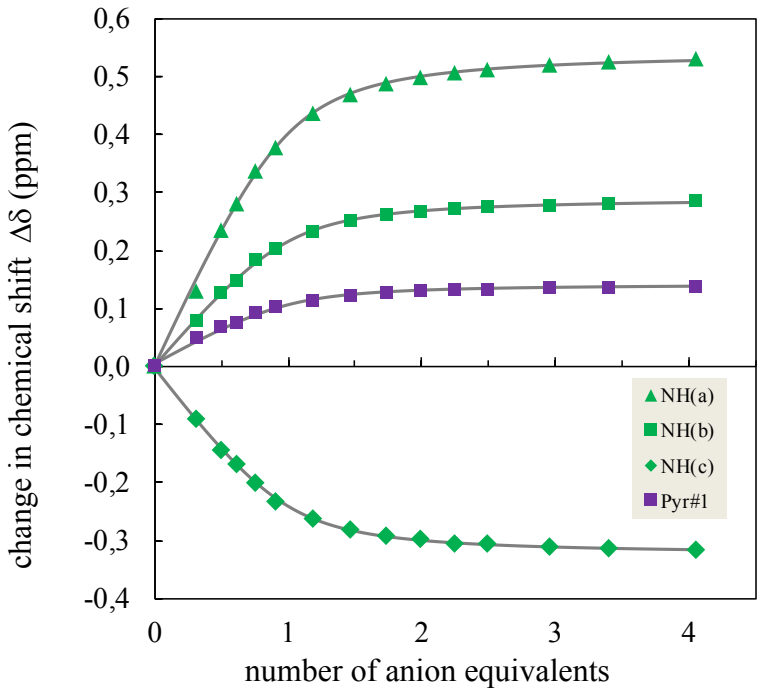


Fig. S3. Experimental chemical shift changes (symbols) and calculated binding isotherms (gray lines) for titration of **2** with TBACl assuming 1:1 binding model.

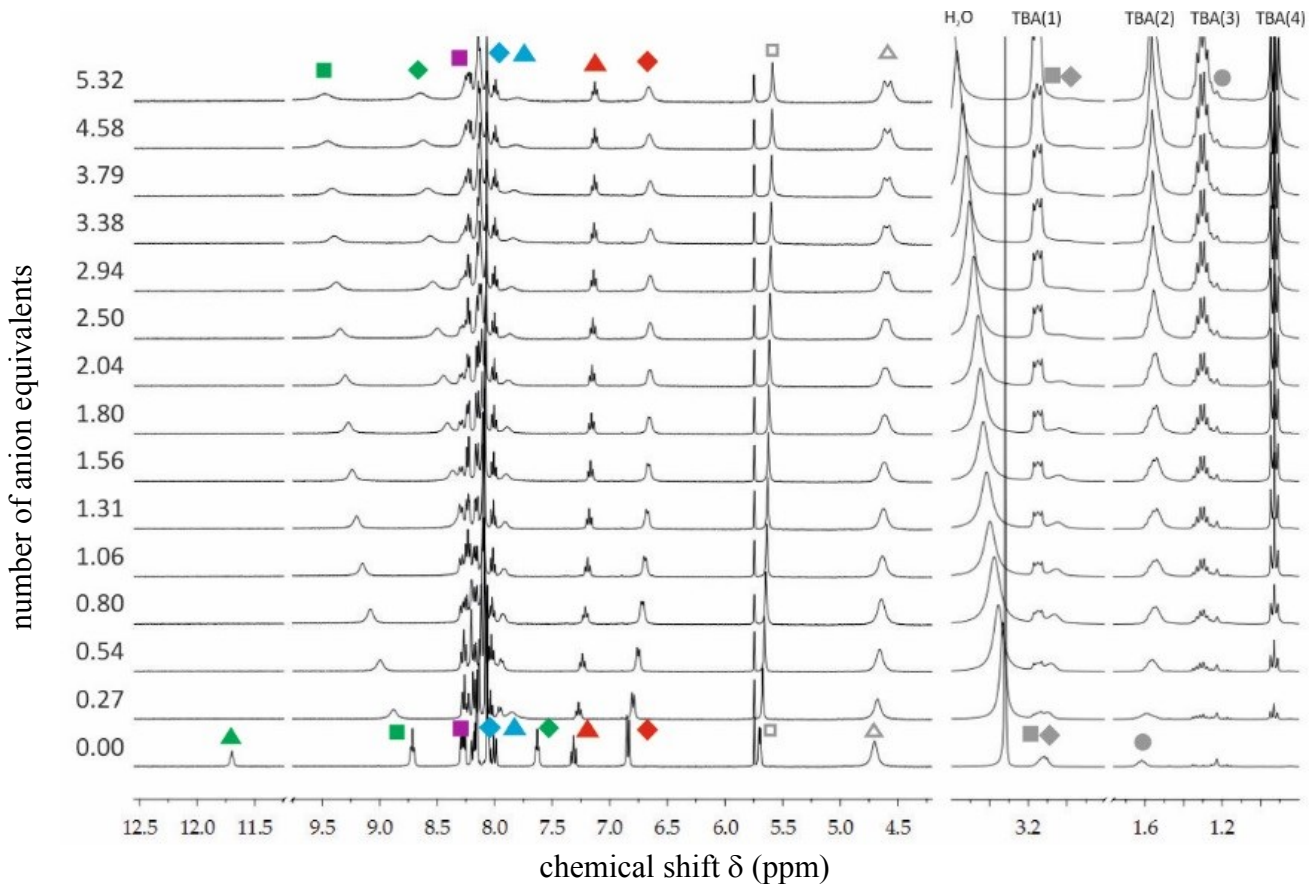


Figure S4. Stacked plot from ^{1}H NMR titration of **2** with increasing amount of $TBAMeCO_2$.

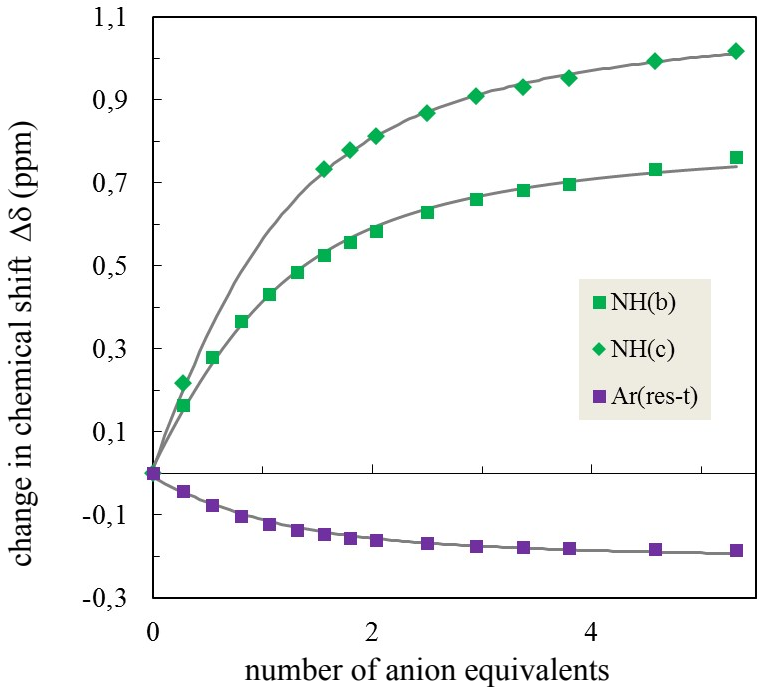


Figure S5. Experimental chemical shift changes (symbols) and calculated binding isotherms (gray lines) for titration of **2** with $TBAMeCO_2$ assuming 1:1 binding model.

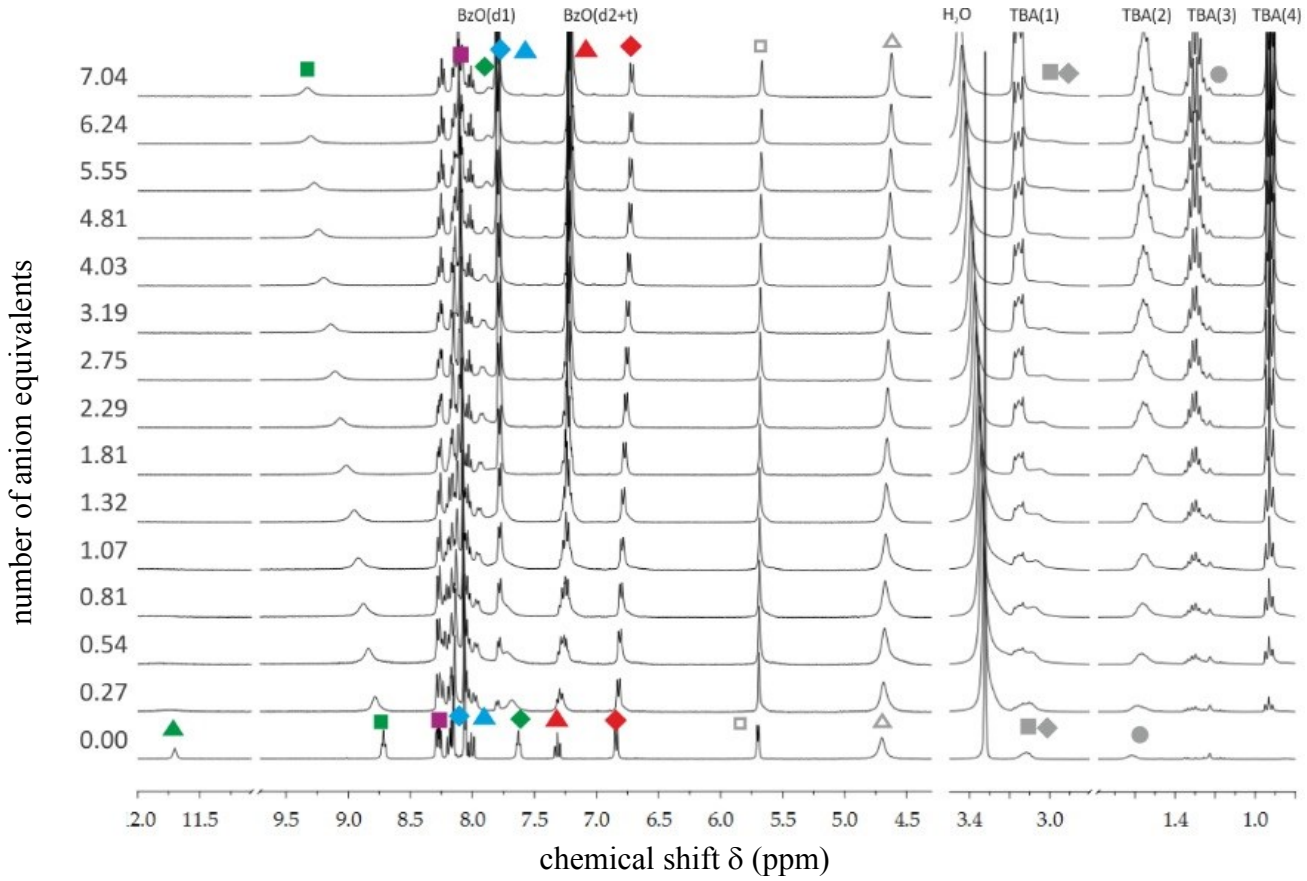


Figure S6. Stacked plot from ^{1}H NMR titration of **2** with increasing amount of TBAPhCO$_2$.

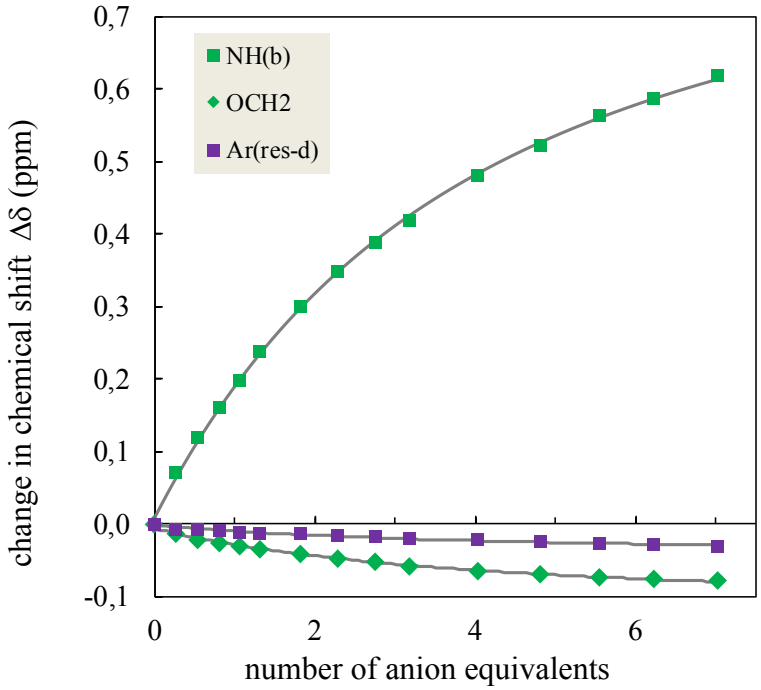


Figure S7. Experimental chemical shift changes (symbols) and calculated binding isotherms (gray lines) for titration of **2** with TBAPhCO$_2$ assuming 1:1 binding model.

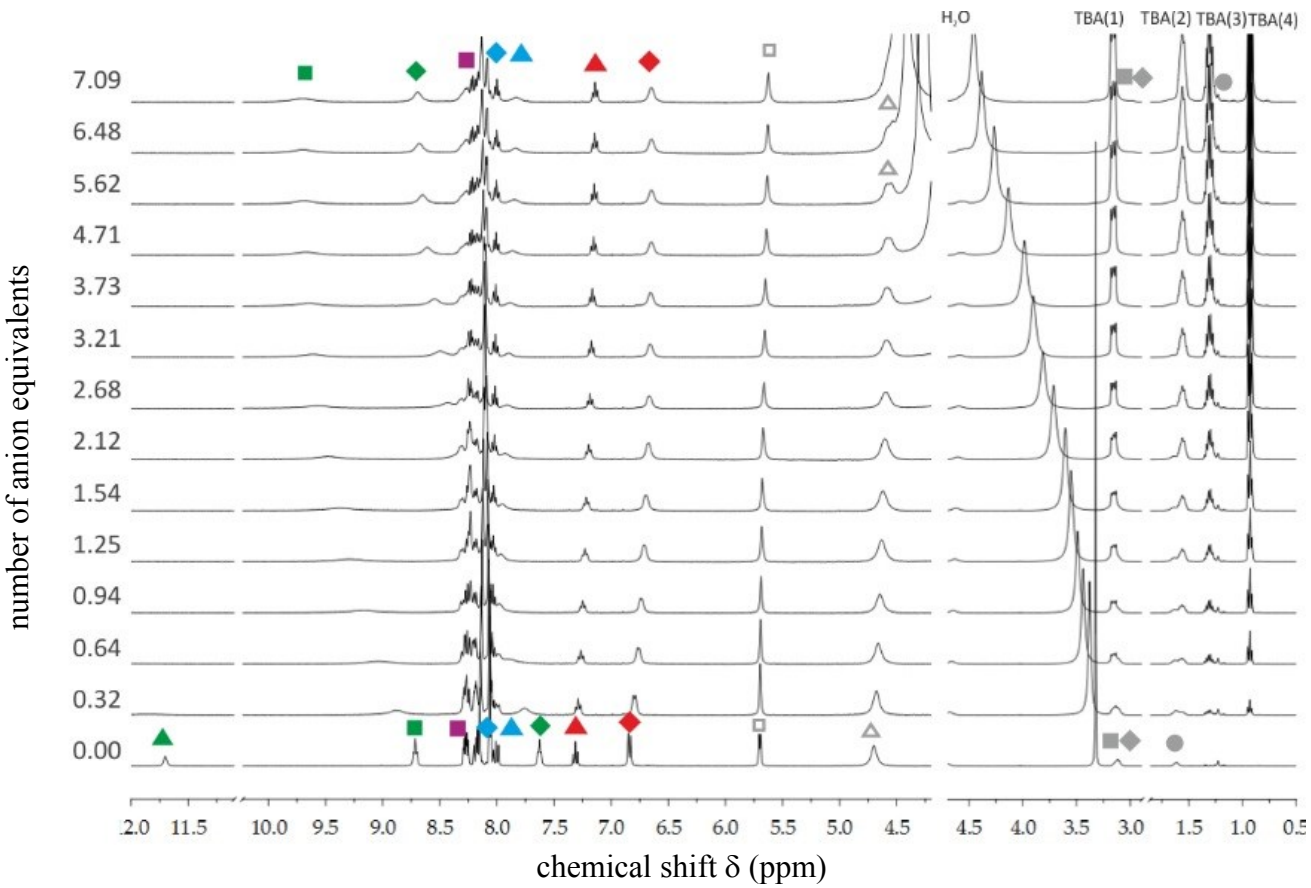


Figure S8. Stacked plot from ^{1}H NMR titration of **2** with increasing amount of $TBAH_2PO_4$.

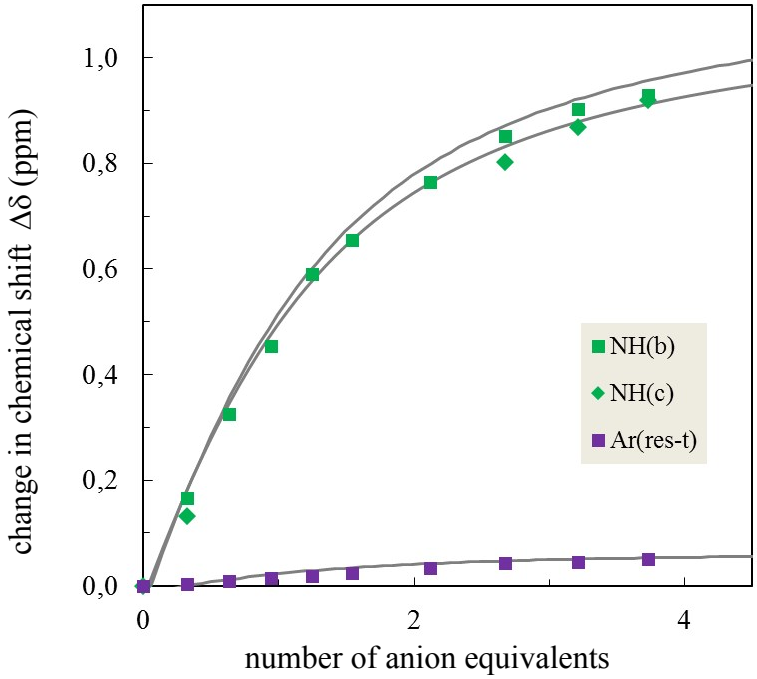


Figure S9. Experimental chemical shift changes (symbols) and calculated binding isotherms (gray lines) for titration of **2** with $TBAH_2PO_4$ assuming 1:1 binding model.

1.6. Crystal data details

Single crystal X-ray diffraction measurements were carried out on a Agilent Supernova diffractometer, at 100K with graphite monochromated Cu Kα radiation (1.54184 Å). The data reduction was made by using CrysAlisPRO software.[4] [1] The structures were solved by direct methods and refined on F^2 by full-matrix least-squares by using SHELXS97 and SHELXL97.[5] All non-hydrogen atoms were refined as anisotropic while hydrogen atoms were placed in calculated positions, and refined in riding mode.

CCDC 2073995 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Table S2. Crystal data and structure refinement details for compound **2**.

Empirical formula	$C_{41}H_{38}N_6O_6S$	
Formula weight	742.83	
CCDC No.	2073995	
Temperature	100 K	
Wavelength	1.54184 Å (CuK_α)	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.8608(2) Å b = 18.6108(4) Å c = 20.1813(4) Å	α = 80.929(2)° β = 84.373(2)° γ = 80.990(2)°
Volume	V = 3601.94(13) Å^3	
Z	4	
Density Calc.	1.370 g/cm^3	
Absorption coefficient	1.282 mm^{-1}	
F(000)	1560	
θ range for data collection	2.4110 – 70.1550°	
Index ranges	$-11 \leq h \leq 11$, $-22 \leq k \leq 22$, $-24 \leq l \leq 21$	
Reflections collected (all/independent)	65064 / 13525 [R_{int} = 0.040]	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	13525 / 0 / 1023	
Goodness-of-fit on F^2	1.031	
Final R indices [$F^2 > 2\sigma(F^2)$]	R_1 = 0.0400, ωR_2 = 0.0932	
R indices (all data)	R_1 = 0.0451, ωR_2 = 0.0963	

1.7. Details of oxidation experiments of compound 2

To study a desulfurization process of the C=S group in compound **2** by common oxidants a fluorescence measurements were employed – increase of fluorescence (related to the formation

of the macrocyclic amide **1**) was monitored in the range 350-500 nm (λ_{max} = 377 nm). During measurements and kinetic experiments the sample was kept at constant temperature (T = 298.0 ± 0.1K) using a Peltier thermostated cell holder.

A 7.44 μM stock solution of thioamide macrocycle **2** was prepared by dissolving 1.4 mg of **2** in 250 mL of HPLC-grade acetonitrile.

To keep a constant pH during experiments with oxidizing agents the aliquots of aqueous phosphate buffer solution (1.25 mL, pH = 7.0, 47.5 mM) was added to the stock solution of **2** (1.25 mL, *c* = 7.44 μM) in the quartz-glass cell (Hellma Analytics, macro-cuvette PN 117.100F-QS, 10 x 10 mm layer thickness), and resulting solution was mixed to give a final host concentration of 3.72 μM and a total volume of 2.5 mL. The spectrum of macrocyclic probe **2** was recorded and 10 equivalents of the freshly prepared stock solution of the corresponding oxidizing agent was added and the spectrum was recorded again after 1h.

The stock solution of NaOCl (*c* = 3.56 mM) was prepared by dilution of the commercially available solution of NaOCl (*c* = 1.67 M, TCI Chemicals) in bicarbonate-carbonate buffer (pH = 10.8).

2. Copies of the NMR spectra

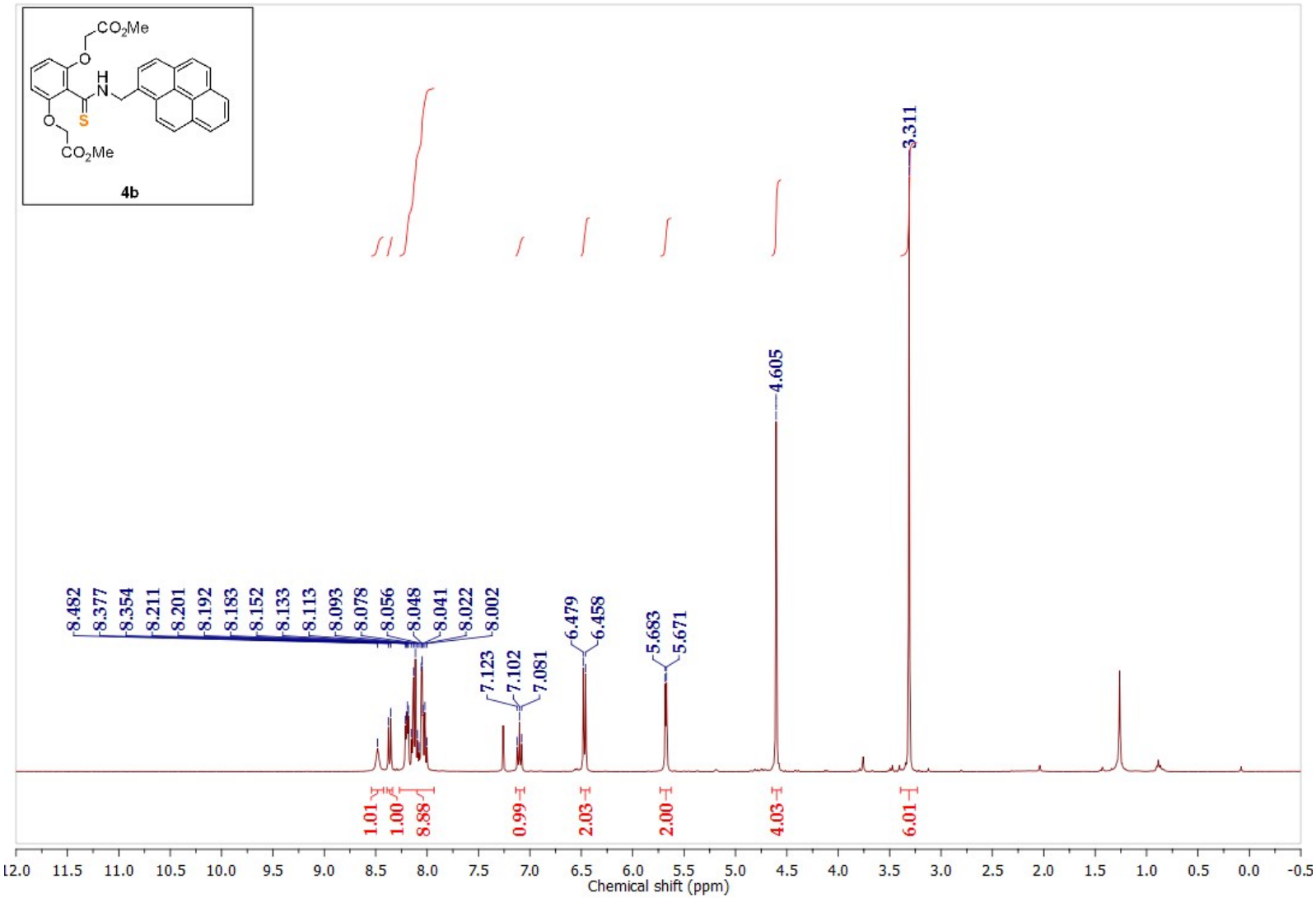


Figure S9. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **4b**.

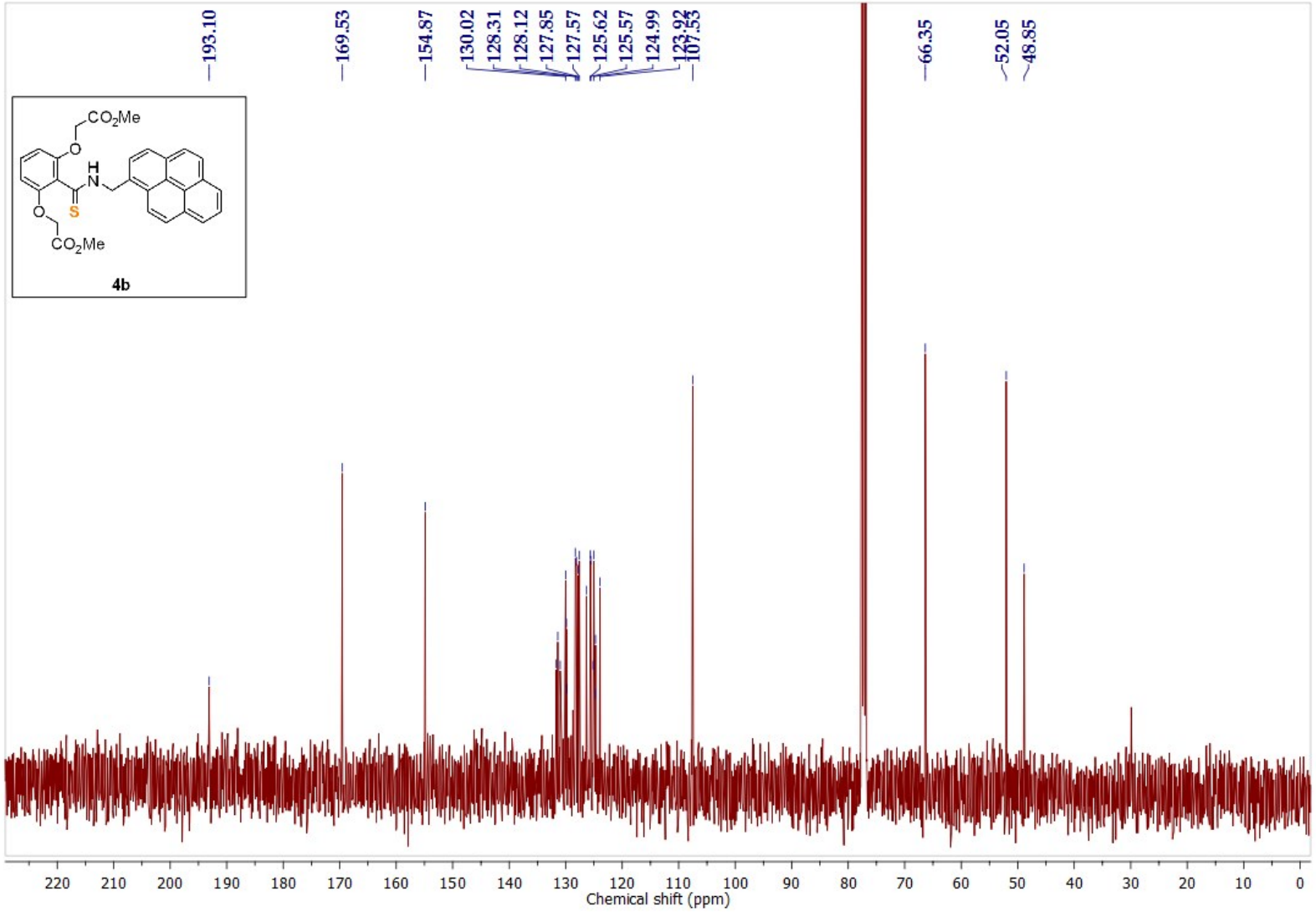


Figure S10. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **4b**.

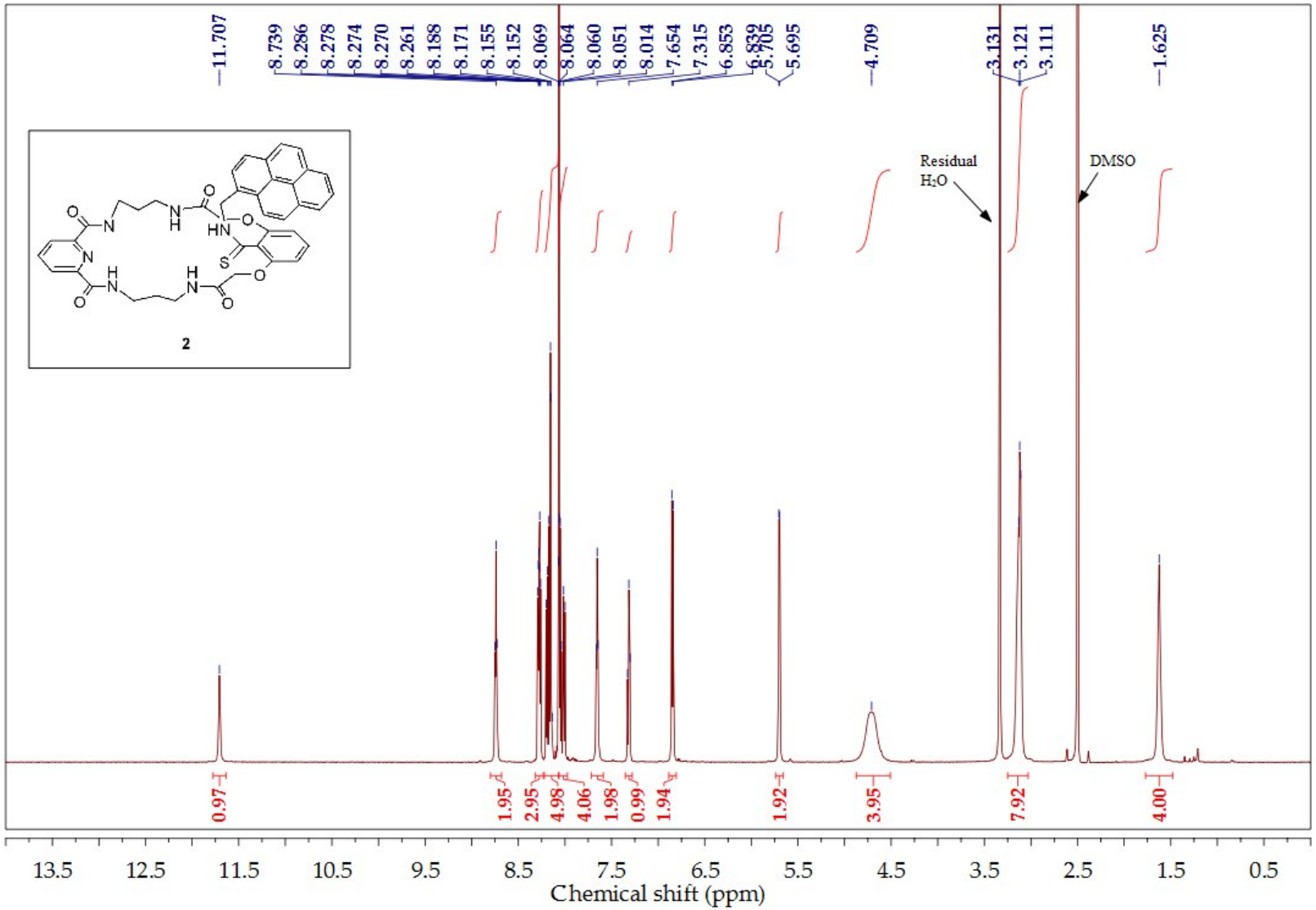


Figure S11. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of macrocyclic host **2**.

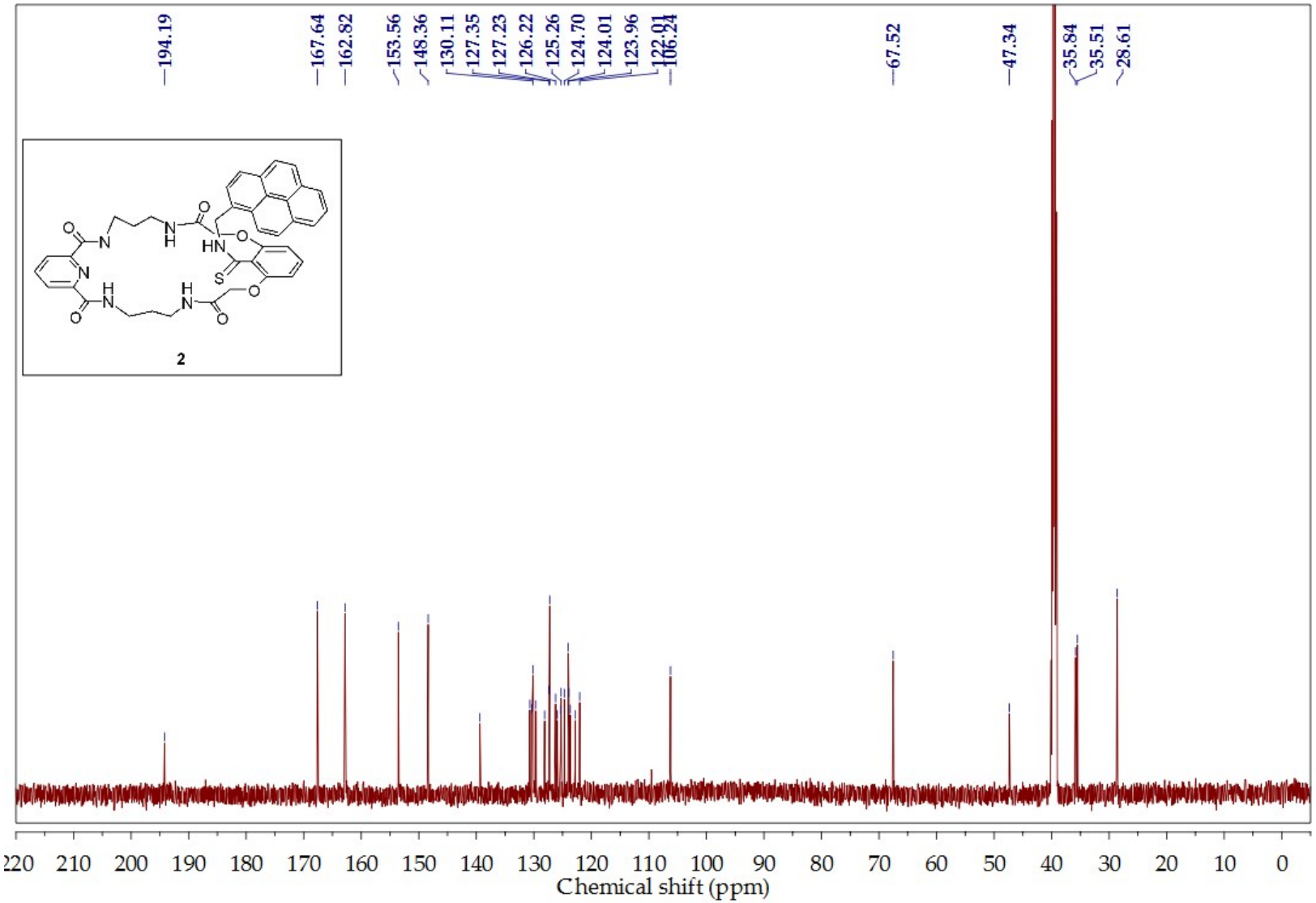


Figure S12. ^{13}C NMR (151 MHz, DMSO-d_6) spectrum of macrocyclic host **2**.

3. References

1. (a) Dąbrowa, K.; Lindner, M.; Wasiłek, S.; Jurczak, J., Selective Recognition of Chloride by a 24-Membered Macrocyclic Host with a Hydrophobic Methylenepyrene Substituent. *Eur. J. Org. Chem.* **2020,** *2020* (29), 4528-4533; (b) Dabrowa, K.; Niedbala, P.; Majdecki, M.; Duszewski, P.; Jurczak, J., A General Method for Synthesis of Unclosed Cryptands via H-Bond Templated Macrocyclization and Subsequent Mild Postfunctionalization. *Org. Lett.* **2015,** *17* (19), 4774-4777.
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