Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Unique binding behaviour of water-soluble polycationic oxacalix[4]arene tweezers towards the paraquat dication

Nadia Manganaro,[a] Gabriele Lando,[a] Claudia Gargiulli,[a] Ilenia Pisagatti,[a] Anna Notti,[a] Sebastiano Pappalardo,[b] Melchiorre F. Parisi,[a] Giuseppe Gattuso*[,a]

E-mail: *ggattuso@unime.it*

[a] Dipartimento di Scienze Chimiche, Università di Messina, viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[b] Dipartimento di Scienze Chimiche, Università di Catania, viale A. Doria 6, 95125 Catania, Italy

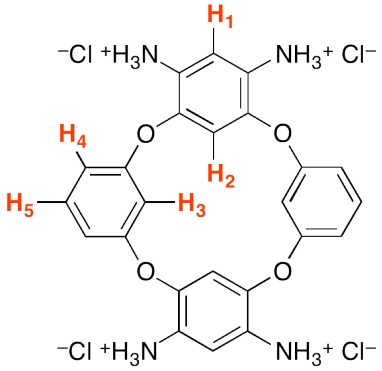
Contents ***page***

General experimental

All chemicals were purchased from Sigma-Aldrich and were used as received. 4,6,16,18-Tetraamino-2,8,14,20-tetraoxacalix[4]arene **1** was prepared according to a published procedure.[S1] ^{1}H and ^{13}C NMR spectra were recorded with the suppression of the HOD peak in D_2O/DCl at 500 and 125 MHz, respectively, on a Varian Vnmrs-500 instrument. Chemical shifts are reported in ppm and are referenced to 1,4-dioxane (δ_H 3.75 ppm, δ_C 67.19 ppm), added as an internal standard. *J* values are given in Hz. UV/Vis absorption spectra were recorded on a Varian Cary 50 UV-Visible spectrophotometer equipped with a fixed path length (1 cm) optic fibre probe, using spectrophotometric grade water. The Fourier Transform Infrared spectrum (FTIR, Perkin Elmer Spectrum 100) was collected in Attenuated Total Reflectance (ATR) configuration.

4,6,16,18-Tetraammonium-2,8,14,20-tetraoxacalix[4]arene tetrachloride (**1**·4HCl)

Aqueous HCl (0.1 M, 5 mL) was added to a stirred solution of tetraamino-oxacalix[4]arene **1** (130 mg, 0.300 mmol) in THF (10 mL). After 10 minutes, the solvents were removed under reduced pressure. The resulting residue was triturated with Et_2O and collected by suction filtration to yield **1**·4HCl as a dark brown solid (164 mg, 0.285 mmol, 95%);mp > 280 °C; ^{1}H NMR (D_2O/DCl, pH 1.6[S2]) δ_H 6.57 (2 H, s, H_2), 6.72 (2 H, t, $J_{3,4}$ 2.2, H_3), 7.10 (4 H, dd, $J_{4,5}$ 8.3, $J_{3,4}$ 2.2, Hz, H_4), 7.43 (2 H, s, H_1), 7.52 (2 H, t, $J_{4,5}$ 8.3, H_5); ^{13}C NMR (D_2O/DCl, pH 1.6[S2]) δ_C 107.6, 108.4, 116.1, 116.6, 120.7, 132.0, 147.7, 156.3. ESI-MS: *m/z* 429.0 ($M+H^+$, 100%). IR: 682, 713, 772, 802, 839, 878, 973, 1052, 1123, 1187, 1223, 1247, 1437, 1482, 1514, 1595, 1629, 2859, 3184, 3344, 3444 cm^{-1}. Elemental analysis: found: C, 49.9; H, 4.2; N, 9.7; Cl, 24.6. $C_{24}H_{24}Cl_4N_4O_4$ requires C, 50.2; H, 4.2; N, 9.8; Cl, 24.7%



1·4HCl

S1 C. Capici, D. Garozzo, G. Gattuso, A. Messina, A. Notti, M. F. Parisi, I. Pisagatti and S. Pappalardo, *ARKIVOC*, 2009, 199–211.

S2 pH values were obtained from pD measurements by applying the appropriate correction factor. See: R. G. Bates, *Determination of pH: theory and practice*, John Wiley & Sons, New York, 1973.

Determination of the protonation constants by UV-Vis spectroscopy

The protonation constants of **1** were determined by means of UV-Vis spectrophotometric titrations. Solutions of **1**·4HCl in the 3×10^{-5} – 5×10^{-6} M concentration range were prepared by dissolving an appropriate amount of the solid sample in aqueous 0.1 M HCl. pH measurements of the resulting solutions were carried out with an ISE-H^+ combined electrode (Metrohm, 6.032.100 model), calibrated immediately before the beginning of the titration with buffer solutions of known pH values. **1**·4HCl solutions (25 mL) were placed in a thermostated cell (T = 298.15±0.1 K) and then titrated with a standard 0.1 M NaOH aqueous solution (typically 10–20 aliquots) to vary the pH in the 1.0 – 10.0 range. UV-Vis spectra were recorded after each NaOH addition in the λ = 250–330 nm spectral window, with baseline subtraction (0.1 M HCl).

Sets of spectra recorded at different pH were analysed with the HypSpec[S3] software, to determine the values of the four protonation constants. Typical absorbance spectra of aqueous solutions of **1**·nH$^+$, recorded at different pH values, are shown in Fig. S1.

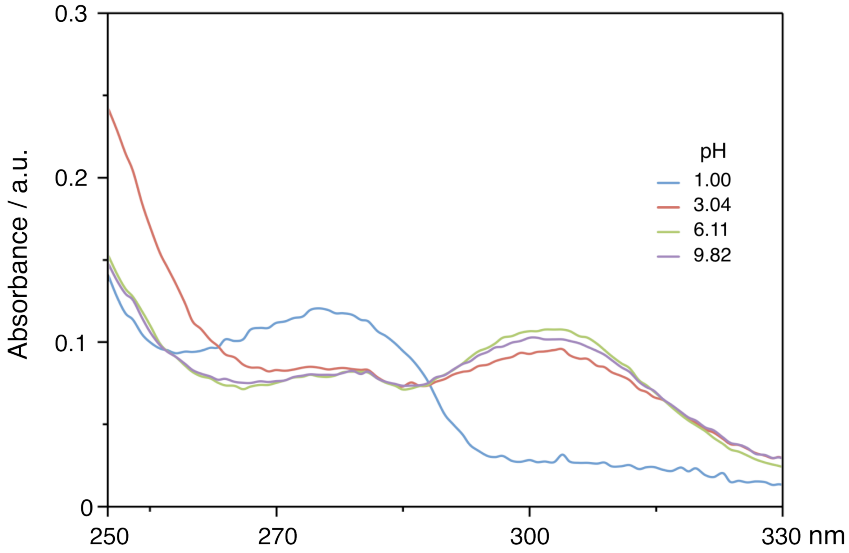


Fig. S1 Absorbance spectra of aqueous solutions of **1**·nH$^+$ at different pH values (see inset); [**1**·nHCl] = 3.0×10^{-5} M (n = 0–4), (T = 298.15±0.1 K).

The spectrum recorded at pH > 9 was confidently assigned to the tetra-amino oxacalixarene **1** and consequently, in the following calculations, the molar extinction coefficient at any given wavelength was obtained from the corresponding absorbance value of a solution of known concentration. Extinction coefficients of the other protonated species (**1**·nH$^+$), on the other hand,

S3 P. Gans, A. Sabatini and A. Vacca, *Ann. Chim. (Rome)*, 1999, **89**, 45–49.

were calculated by the HypSpec software after optimization of the protonation constants. In this way, once the percentage of formation of a given protonated species has been determined, it is then possible to calculate the absorbance of each species. In the case under study here, the fully-protonated **1**·4H$^+$ species displays a characteristic band centred at λ = 276 nm, whereas all the other **1**·*n*H$^+$ species (with *n* = 0–3) show absorbance maxima at λ = 302 nm (Fig. S2).

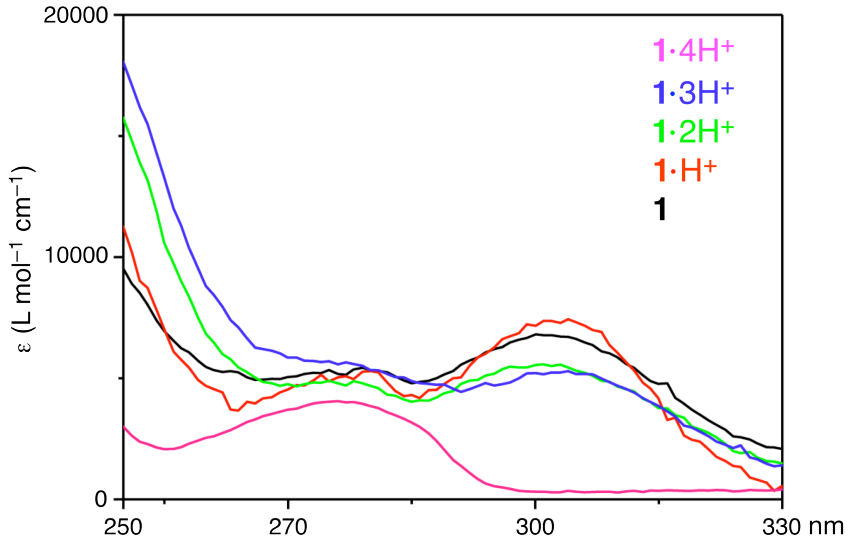


Fig. S2 Calculated molar extinction coefficients (ε) of **1**·*n*H$^+$ species (*n* = 0–4).

HypSpec analysis of the data recorded at different pH values gave the following protonation constant values: log K^H_1 = 4.39±0.04, log β^H_2 = 8.19±0.04, log β^H_3 = 10.74±0.06, log β^H_4 = 12.58±0.06 according to eq. (1) and log K^H_1 = 4.39±0.04, log K^H_2 = 3.80±0.04, log K^H_3 = 2.55±0.06, log K^H_4 = 1.84±0.06 according to eq. (2)

$n\mathrm{H}^+ + \mathbf{1} = \mathbf{1}\cdot n\mathrm{H}^+$ $\quad\quad$ β^H_n (overall) $\quad\quad$ (1)

$\mathrm{H}^+ + \mathbf{1}\cdot(n-1)\mathrm{H}^+ = \mathbf{1}\cdot n\mathrm{H}^+$ $\quad\quad$ K^H_n (stepwise) $\quad\quad$ (2)

Once the protonation constants are known, the **1**·*n*H$^+$ species distribution as a function of the pH can be calculated by using the HySS[S4] software. The distribution diagram of the different **1**·*n*H$^+$

S4 L. Alderighi, P Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coord. Chem. Rev.*, 1999, **184**, 311–318.

protonated species formed, in the 1–7 pH range at T = 298.15 K, from a 10^{-5} M aqueous solution of **1**·4HCl is shown in Fig. S3.[S5]

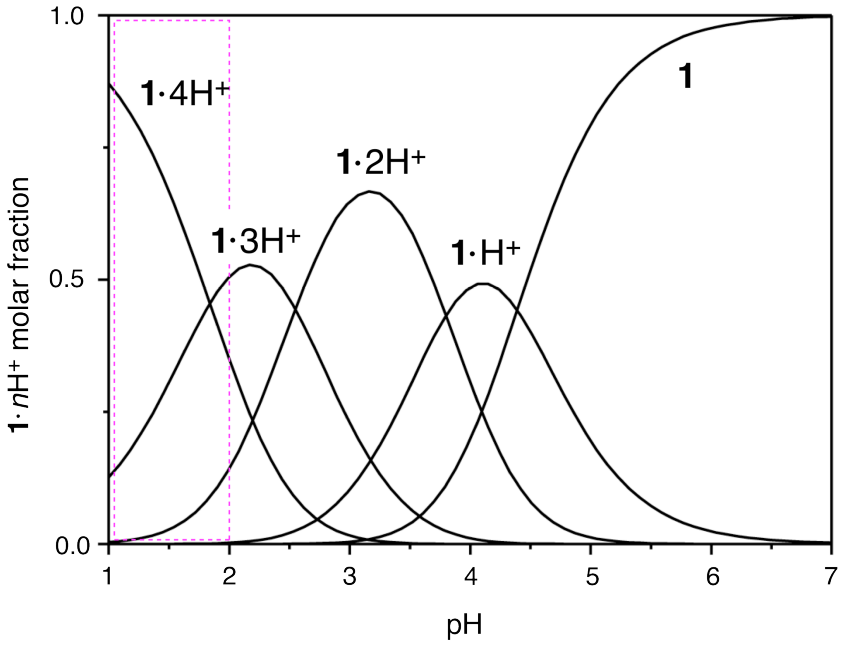


Fig. S3 Distribution diagram of the **1**·nH$^+$ protonated species at [**1**·4HCl] = 10^{-5} M, I = 0.1 M and T = 298.15 K. The magenta dashed box indicates the area that has been expanded in Fig. 1 of the main text.

^{1}H NMR Titrations

The association process of **1**·nH$^+$ with paraquat (PQT^{2+}), expressed as in eqs. (3-4), was investigated by ^{1}H NMR, by carrying out titrations at different pH values. For an easier comparison with the UV-Vis data, given that deuterated chemicals were used throughout the NMR measurements, pH values were recalculated from pD measurements by applying the appropriate correction.[S2]

$n\mathrm{H}^+ + \mathbf{1} + PQT^{2+} = [PQT^{2+}\subset\mathbf{1}\cdot n\mathrm{H}^+]$ $\quad\quad$ β_n (overall) $\quad\quad$ (3)

$\mathbf{1}\cdot n\mathrm{H}^+ + PQT^{2+} = [PQT^{2+}\subset\mathbf{1}\cdot n\mathrm{H}^+]$ $\quad\quad$ K_n (stepwise) $\quad\quad$ (4)

S5 ^{1}H NMR titration experiments of **1** (with a 1.0 M solution of DCl in D_2O) produced an incomplete dataset as a result of the limited solubility of **1**·4HCl in the 2.0–2.5 pH interval (at concentrations suitable for NMR studies, see Fig. S6) and were, therefore, unsuitable for double checking the species distribution data found by absorbance spectroscopy.

Five NMR titration experiments were carried out, in two replicates, at pH = 0.97, 1.35, 1.48, 1.59, 1.99, respectively. Stock solutions of [**1**·*n*HCl] = 10^{-4} M were prepared by dissolving solid **1**·4HCl in DCl/D_2O solutions ([DCl] = 0.1, 0.075, 0.050, 0.025, 0.010 M, respectively). Paraquat dichloride guest solutions ([PQT·2Cl] = 0.025 M) were, in turn, prepared by using as a solvent the above-mentioned **1**·*n*HCl stock solutions so that, upon addition of the titrant, both the host concentration and the pH of the solution would remain constant over the entire titration experiment. Typically, data were collected in the 1:0.25 to 1:140 host/guest ratio interval, by adding 12 aliquots of the above-mentioned PQT·2Cl solution to a given stock solution of the host (Figs. S7–S11, see below).

Data analysis was carried out with the HypNMR[S6] software. Given that titrations were run at fixed pH values (see above), the protonation constants of the **1**·*n*H^+ species can be ignored during the determination of the apparent host-guest formation constant (K_{app}) [eq. (5)]. K_{app} is pH-dependent, as its value depends on the percentage of formation, at a given pH, of the different protonated species of the host and their relative affinity for the guest under consideration (i.e., [PQT^{2+}⊂**1**·3H^+] and [PQT^{2+}⊂**1**·4H^+]).

$$K_{app} = \frac{[PQT^{2+}\subset \mathbf{1}\cdot n H^+]}{[PQT^{2+}]\,[\mathbf{1}\cdot n H^+]} \qquad (5)$$

In equation 5, [**1**·*n*H^+] and [PQT^{2+}⊂**1**·*n*H^+] refer to the total concentration of all the differently protonated species of the host present at the equilibrium in the free and complexed form, whereas [PQT^{2+}] is the unbound guest. The results drawn from this type of data analysis are summarized in Table S1 (alias Table 2 in the main text) together with the relative percentages of coexisting **1**·*n*H^+ protonated species formed, whereas the dependence of the log K_{app} *vs.* pH is shown in Fig. S4.

S6 C. Frassineti, S. Ghelli, P. Gans, A. Sabatini, M. S. Moruzzi and A. Vacca, *Anal. Biochem.*, 1995, **231**, 374–382.

Table S1 Dependence on pH of the log K_{app} of the interaction between 1·*n*HCl and PQT·2Cl at T = 298.15 K.[a]

			% of formation				
[DCl]	pH	log K_{app} [a]	**1**·4H$^+$	**1**·3H$^+$	**1**·2H$^+$	**1**·H$^+$	**1**
0.100	0.97	n.d.[b]	87.06	12.58	0.35	–	–
0.075	1.35	1.44±0.03[c]	74.40	24.08	1.41	0.01	–
0.050	1.48	2.29±0.02	67.85	29.62	2.52	0.01	–
0.025	1.59	1.70±0.04	61.56	34.62	3.80	0.02	–
0.010	1.99	n.d.[b,d]	34.97	50.55	14.25	0.23	–

[a] Average of two measurements. [b] n.d.: not detected (see the Supporting Information for an extended discussion). [c] 95% Confidence interval (C.I.). [d] Precipitation of the oxacalixarene prevented measurement of the log K_{app}.

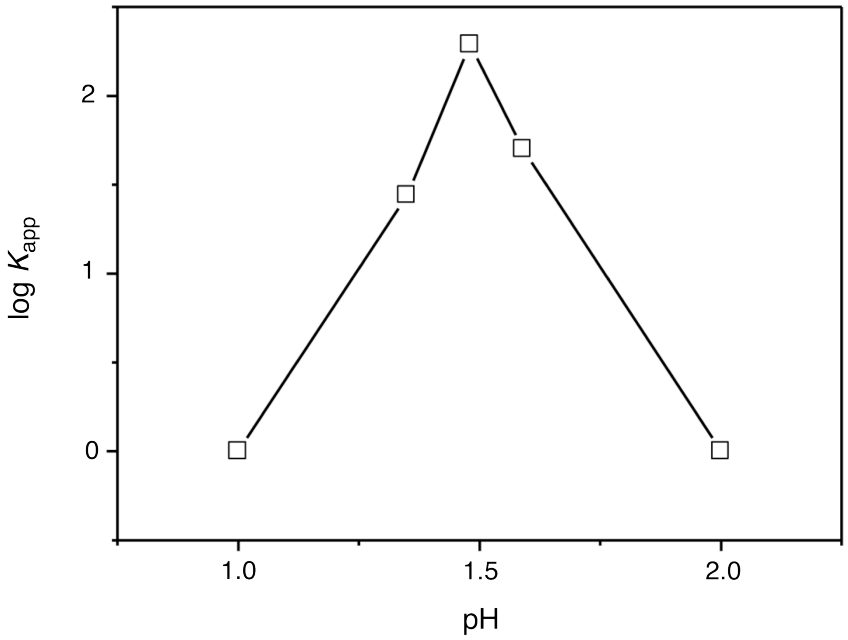


Fig. S4 Dependence of log K_{app} on pH for PQT^{2+}⊂**1**·*n*H$^+$ complexation (T = 298.15 K).

Data in Table S1 and Fig. S4 show that the contribution of the **1**·2H$^+$ species to the binding of PQT^{2+} is negligible, as the value of log K_{app} rapidly drops to zero soon after this species begins to form. This is likely to be caused by a precipitation process observed at pH ≥ 2. Conversely, due to the shape of the function log K_{app} *vs.* pH, the protonated host species that contributes the most to guest binding is **1**·3H$^+$.

Calculation of the formation constants

If only the $[PQT^{2+}\subset\mathbf{1}\cdot 3H^+]$ and $[PQT^{2+}\subset\mathbf{1}\cdot 4H^+]$ species are considered, knowing the mass balance equation of $\mathbf{1}\cdot n H^+$ (both in the free and in the host-guest forms) and knowing that the PQT^{2+} dication does not undergo acid-base equilibria, eq. (5) can be rewritten as follows:

$$K_{app} = \frac{[PQT^{2+}\subset\mathbf{1}\cdot 3H^+ + PQT^{2+}\subset\mathbf{1}\cdot 4H^+]}{[PQT^{2+}]\,[\mathbf{1}\cdot 3H^+ + \mathbf{1}\cdot 4H^+]} \qquad (6)$$

Calculating $[PQT^{2+}\subset\mathbf{1}\cdot 3H^+]$ and $[PQT^{2+}\subset\mathbf{1}\cdot 4H^+]$ from the formation constants of eq. (4) we have:

$$K_{app} = \frac{[PQT^{2+}]\,\{K_3[\mathbf{1}\cdot 3H^+] + K_4[\mathbf{1}\cdot 4H^+]\}}{[PQT^{2+}]\,\{[\mathbf{1}\cdot 3H^+] + [\mathbf{1}\cdot 4H^+]\}} \qquad (7)$$

Simplifying and calculating $[\mathbf{1}\cdot 3H^+]$ and $[\mathbf{1}\cdot 4H^+]$ from the formation constants of eq. (1)

$$K_{app} = \frac{K_3\cdot\beta^H{}_3[H]^3\cdot[\mathbf{1}] + K_4\cdot\beta^H{}_4[H]^4\cdot[\mathbf{1}]}{\beta^H{}_3[H]^3\cdot[\mathbf{1}] + \beta^H{}_4[H]^4\cdot[\mathbf{1}]} \qquad (8)$$

Equation (8) can be simplified as follows:

$$K_{app} = \frac{K_3\cdot\beta^H{}_3[H]^3 + K_4\cdot\beta^H{}_4[H]^4}{\beta^H{}_3[H]^3 + \beta^H{}_4[H]^4} \qquad (9)$$

Knowing the K_{app} at different pH values (Table S1) as well as the protonation constants of the $\mathbf{1}\cdot n H^+$ species (see above), the formation constants of the different host-guest species can be easily calculated by non-linear least square treatment (the LIANA[S7] software was used in this study). Accordingly, K_3 and K_4 were found to be 253±50 and <1 M^{-1}, respectively.

S7 C. De Stefano, S. Sammartano, P. Mineo and C. Rigano, in *Computer Tools for the Speciation of Natural Fluids* in *Marine Chemistry – An Environmental Analytical Chemistry Approach*, ed. A. Gianguzza, E. Pelizzetti and S. Sammartano, Kluwer Academic Publishers, Amsterdam, 1997, pp. 71–83.

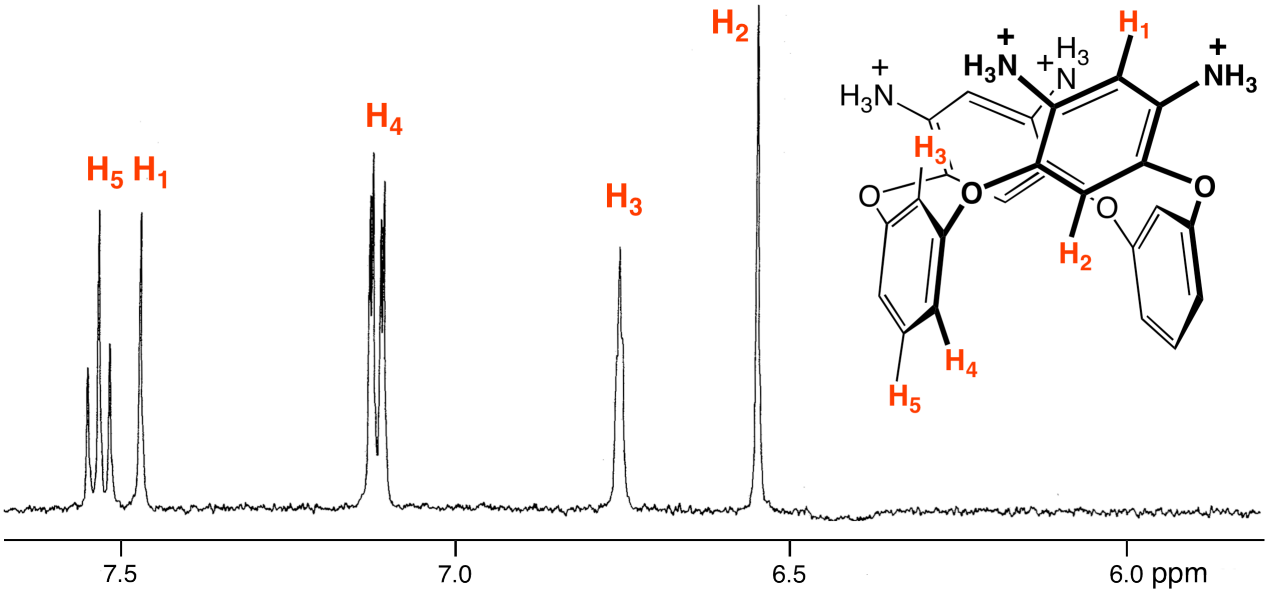


Fig. S5 ^{1}H NMR spectrum (500 MHz, 298 K, D_2O/DCl, pH = 2) of oxacalix[4]arene **1**·4HCl.

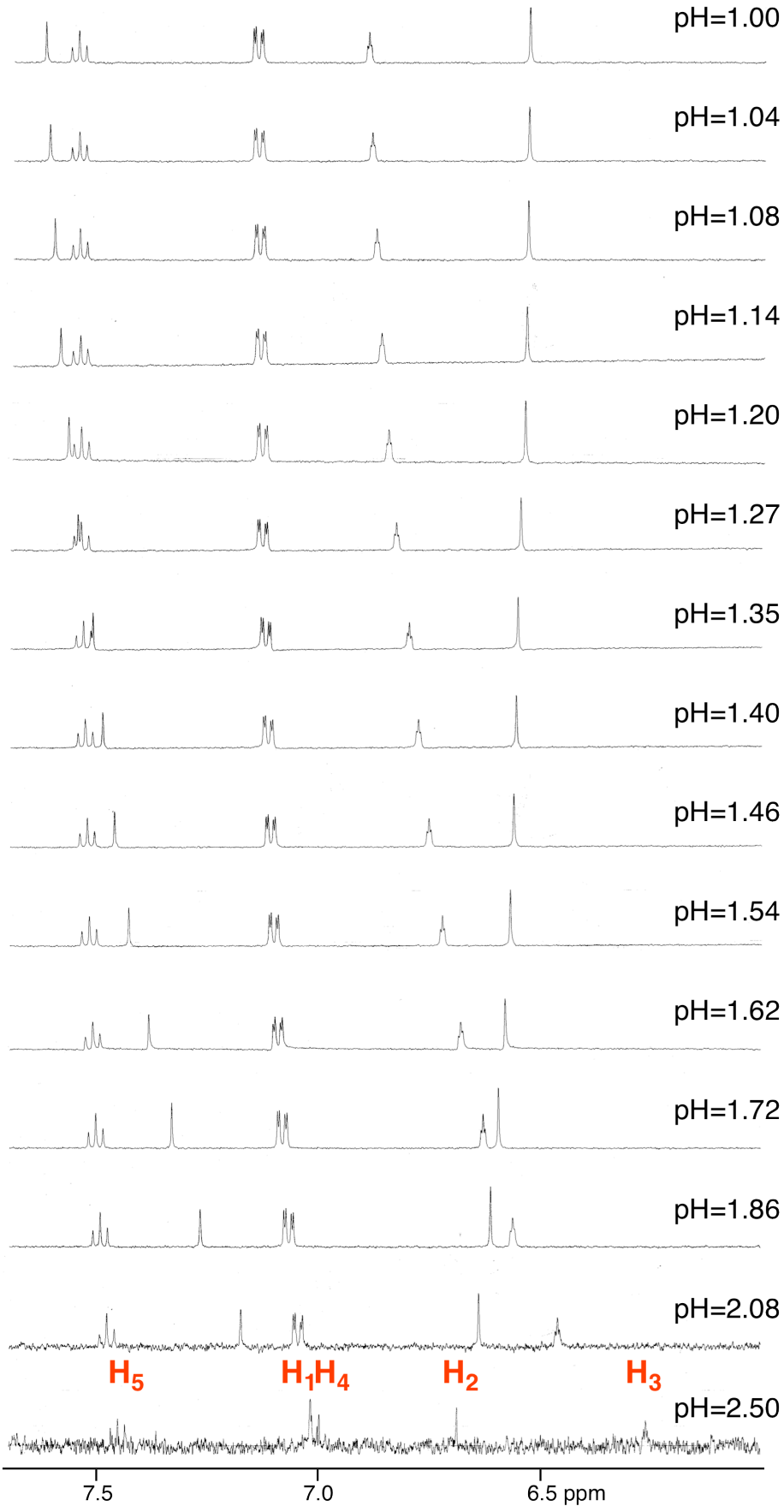


Fig. S6 ^{1}H NMR spectra (500 MHz, 298 K) of a D_2O solution of **1** (7.6×10^{-4} to 6.94×10^{-4} M) upon titration with a 1.0 M DCl solution in D_2O.

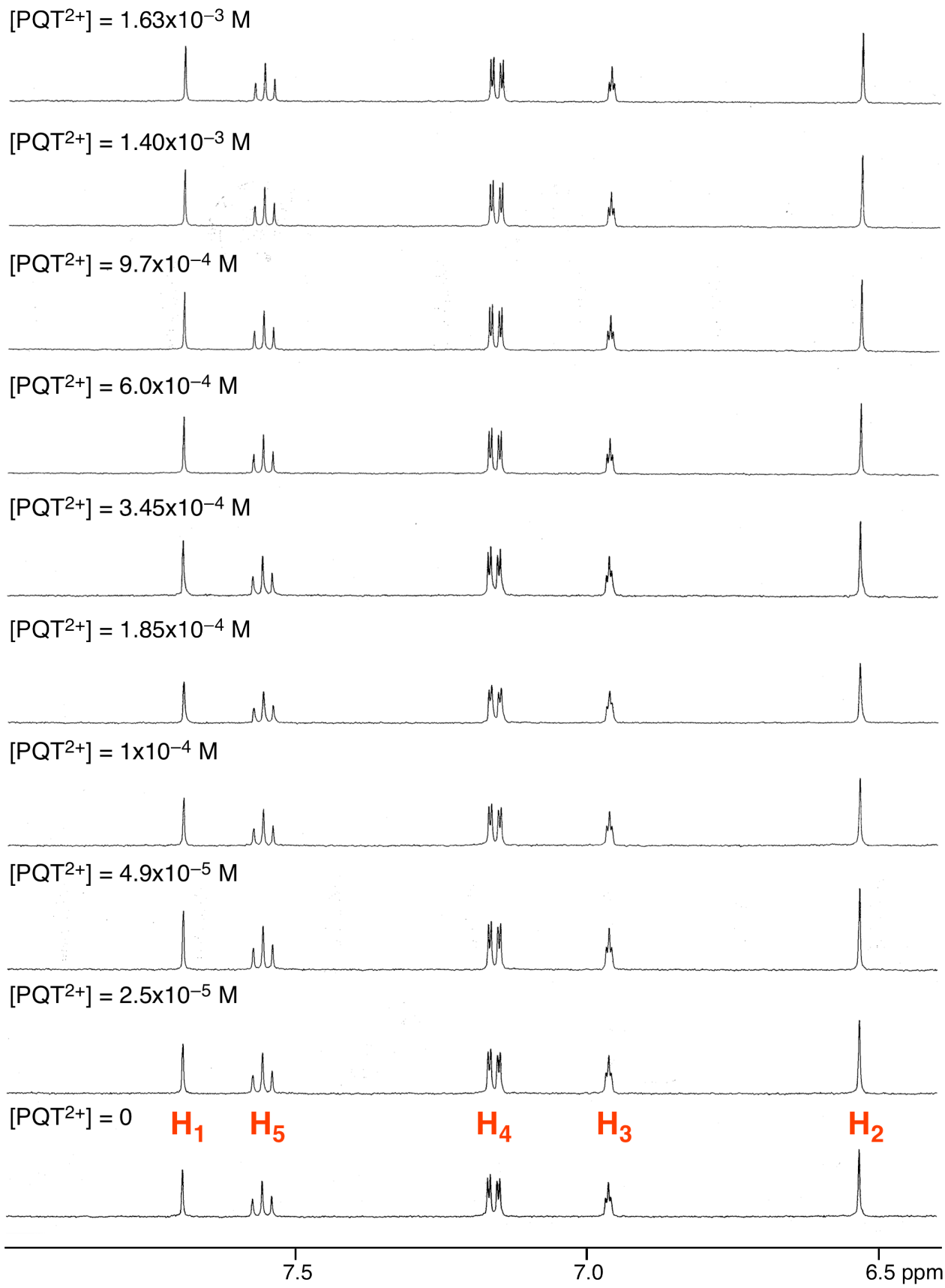


Fig. S7 Sections of the ^{1}H NMR spectra (500 MHz, 298 K, D_2O/DCl, pH = 0.99) obtained upon titration of **1**·*n*HCl (10^{-4} M) with PQT·2Cl (2.5×10^{-3} M).

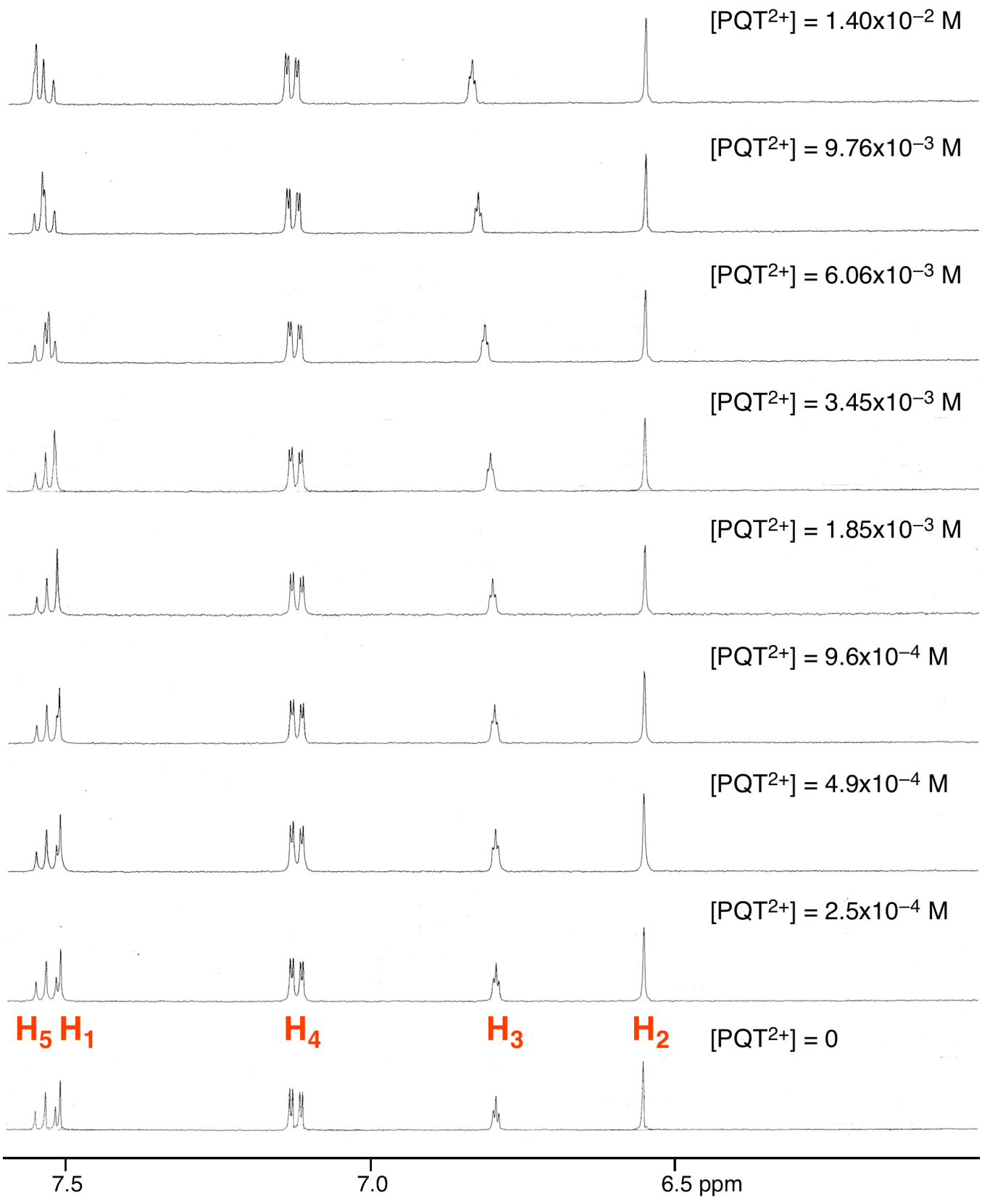


Fig. S8 Sections of the ^{1}H NMR spectra (500 MHz, 298 K, D_2O/DCl, pH = 1.35) obtained upon titration of **1**·*n*HCl (10^{-4} M) with PQT·2Cl (2.5×10^{-2} M).

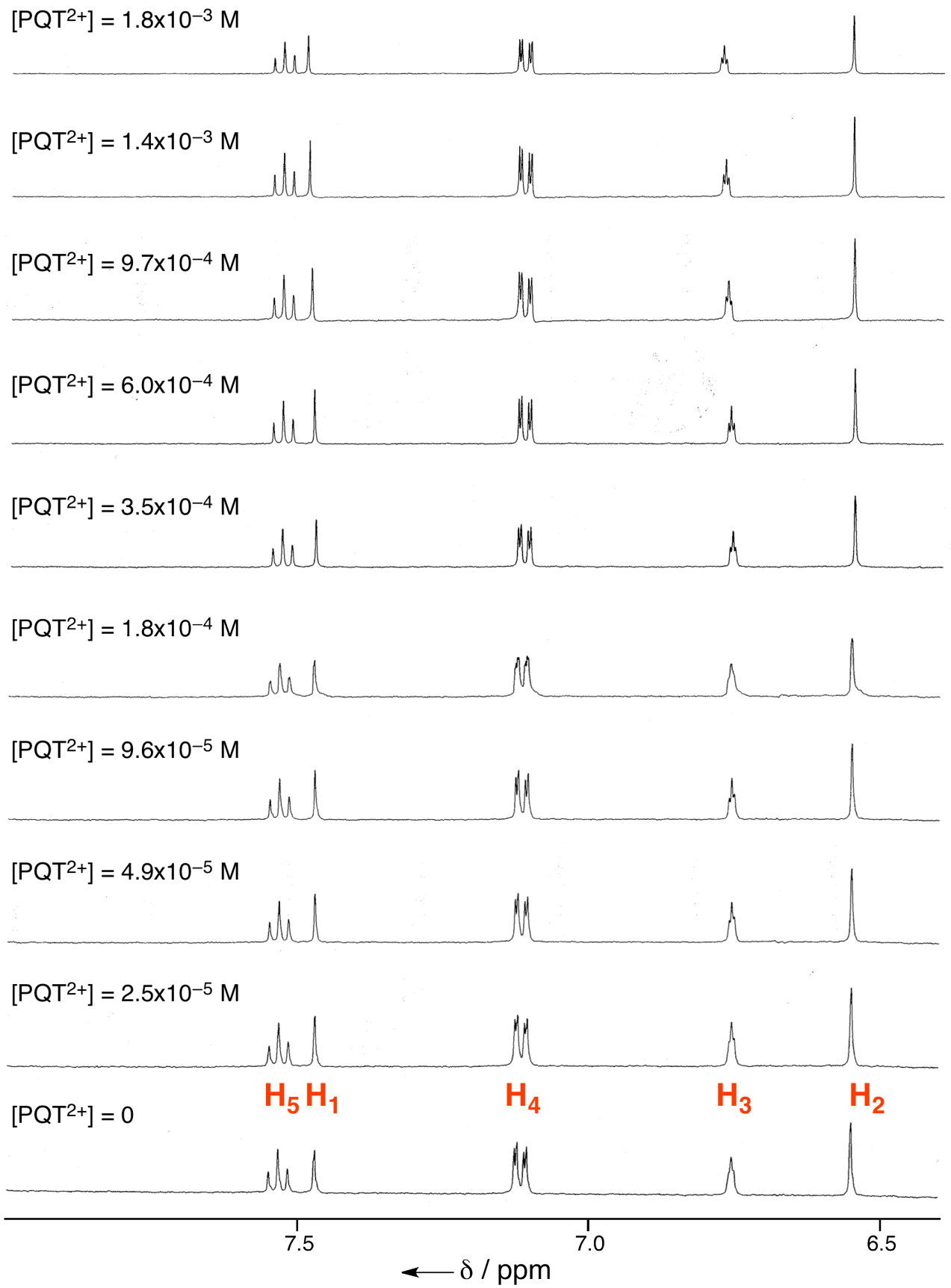


Fig. S9 Sections of the ^{1}H NMR spectra (500 MHz, 298 K, D_2O/DCl, pH = 1.48) obtained upon titration of **1**·*n*HCl (10^{-4} M) with PQT·2Cl (2.5×10^{-3} M).

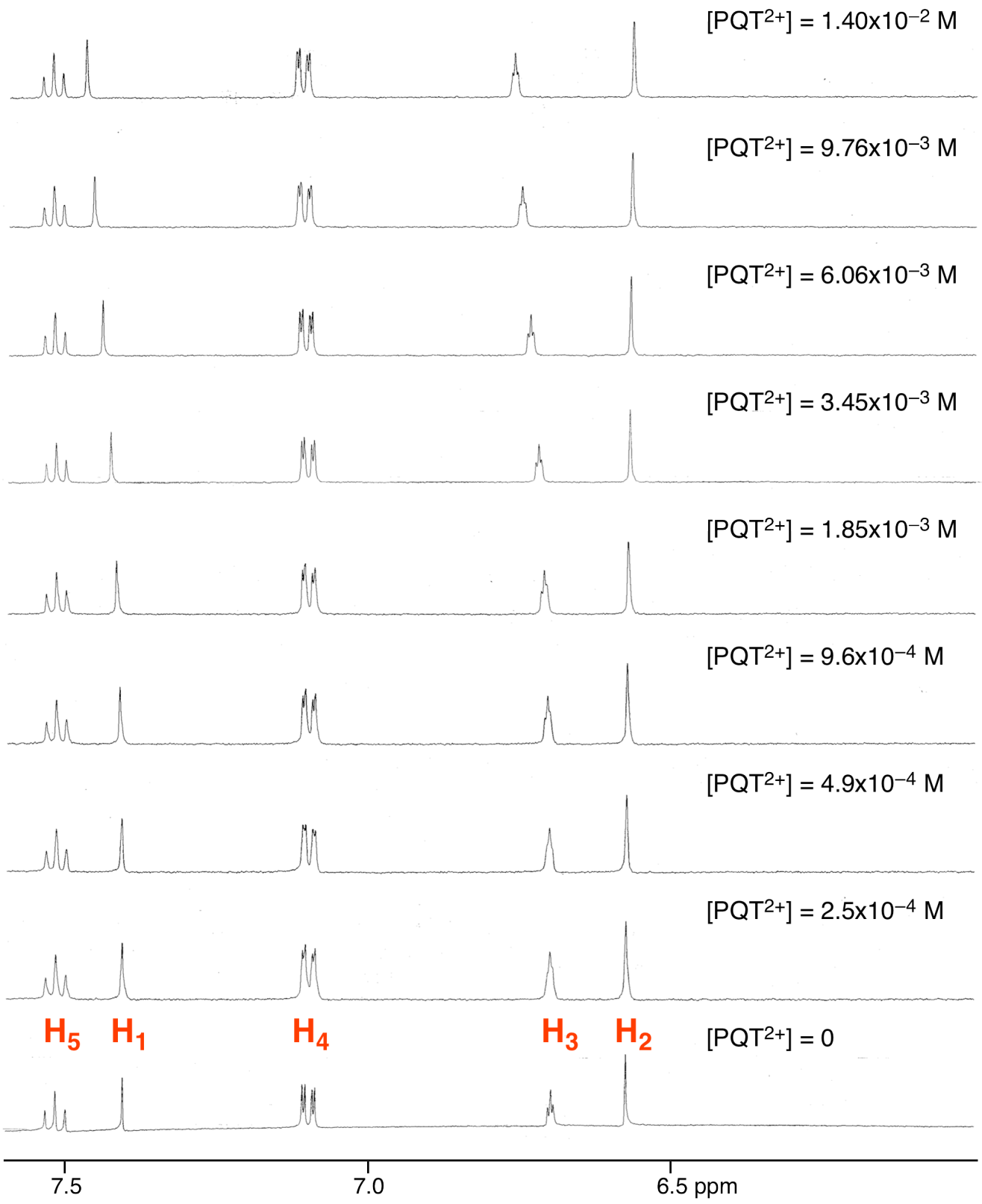


Fig. S10 Sections of the ^{1}H NMR spectra (500 MHz, 298 K, D_2O/DCl, pH = 1.59) obtained upon titration of **1**·*n*HCl (10^{-4} M) with PQT·2Cl (2.5×10^{-2} M).

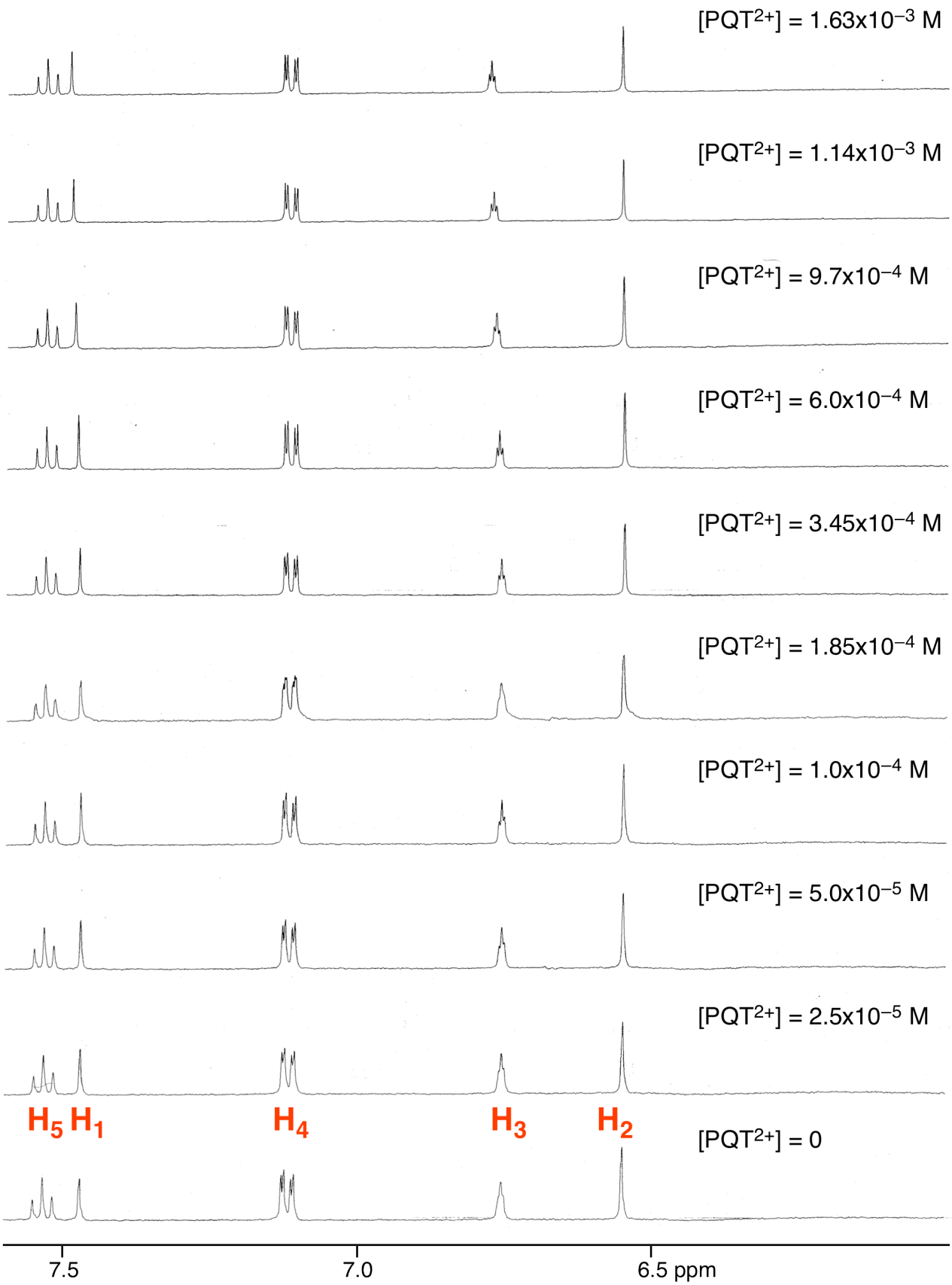


Fig. S11 Sections of the ^{1}H NMR spectra (500 MHz, 298 K, D_2O/DCl, pH = 1.99) obtained upon titration of **1**·*n*HCl (10^{-4} M) with PQT·2Cl (2.5×10^{-3} M).

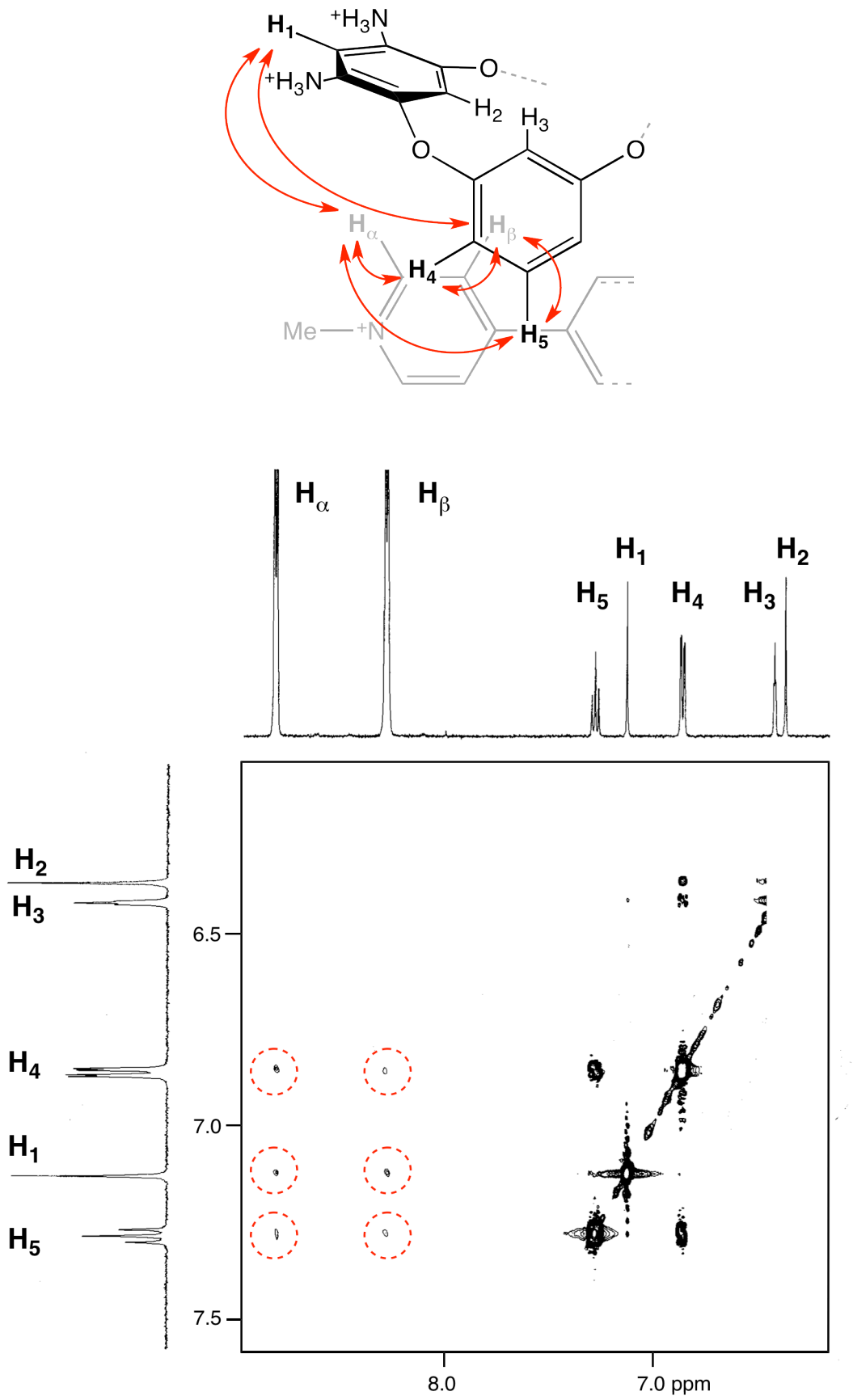


Fig. S12 2D ROESY spectrum (500 MHz, 298 K, D_2O/DCl, pH = 1.6) of the $PQT^{2+}\subset$**1**·nH$^+$ complex: [**1**·nHCl] = 10^{-3} M; [PQT·2Cl] = 4×10^{-3} M.

^{1}H NMR Anion competition experiments

For the anion competition experiments, samples were prepared as follows: concentrated acids (HBr, CF_3COOH or $HClO_4$) were diluted with D_2O (*ca*. 40–45 mM concentration) to a final pH value of 1.52.[S2] 1,4-Dioxane was added as an internal standard (δ_H 3.75 ppm). Stock solutions of [**1**·*n*HCl] = 10^{-4} M were prepared by dissolving solid **1**·4HCl in the stipulated acidic solutions. Paraquat dichloride guest solutions ([PQT·2Cl] = 0.025 M) were, in turn, prepared by using as a solvent the above-mentioned **1**·*n*HCl stock solutions so that, upon addition of the guest, both the host concentration and the pH of the solution would remain constant. Spectra were recorded with the suppression of the HOD peak.

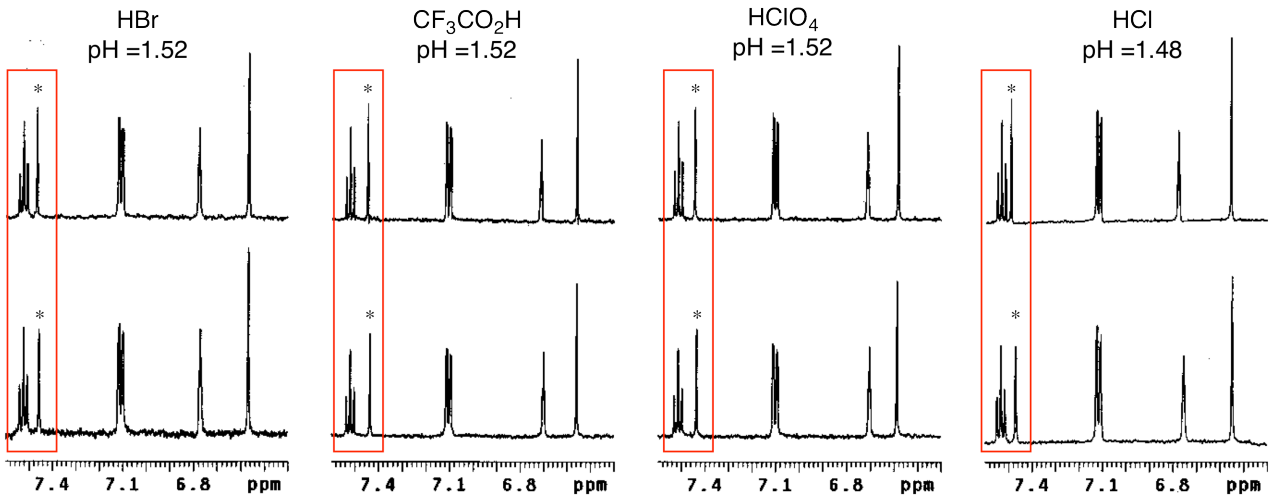


Figure S13. Selected regions of the ^{1}H NMR (500 MHz, D_2O/acid) spectra of [**1**·4HCl] = 0.1 mM prior and after addition of [PQT·2Cl] = 1.83 mM in the presence of different acids. Asterisks indicate H_1 resonances.

DFT Calculations

The conformational analysis of the oxacalix[4]arenes **1**·*n*H$^+$ was carried out with the classical molecular mechanics force field (MMFF) by using the Monte Carlo method to randomly sample the conformational space. The equilibrium geometries were then calculated at the density functional level of theory (DFT, B3LYP functional) using the 6-31G(d) basis set. All quantum mechanical calculations were performed using Spartan'10[S8] (Wavefunction, Inc.).

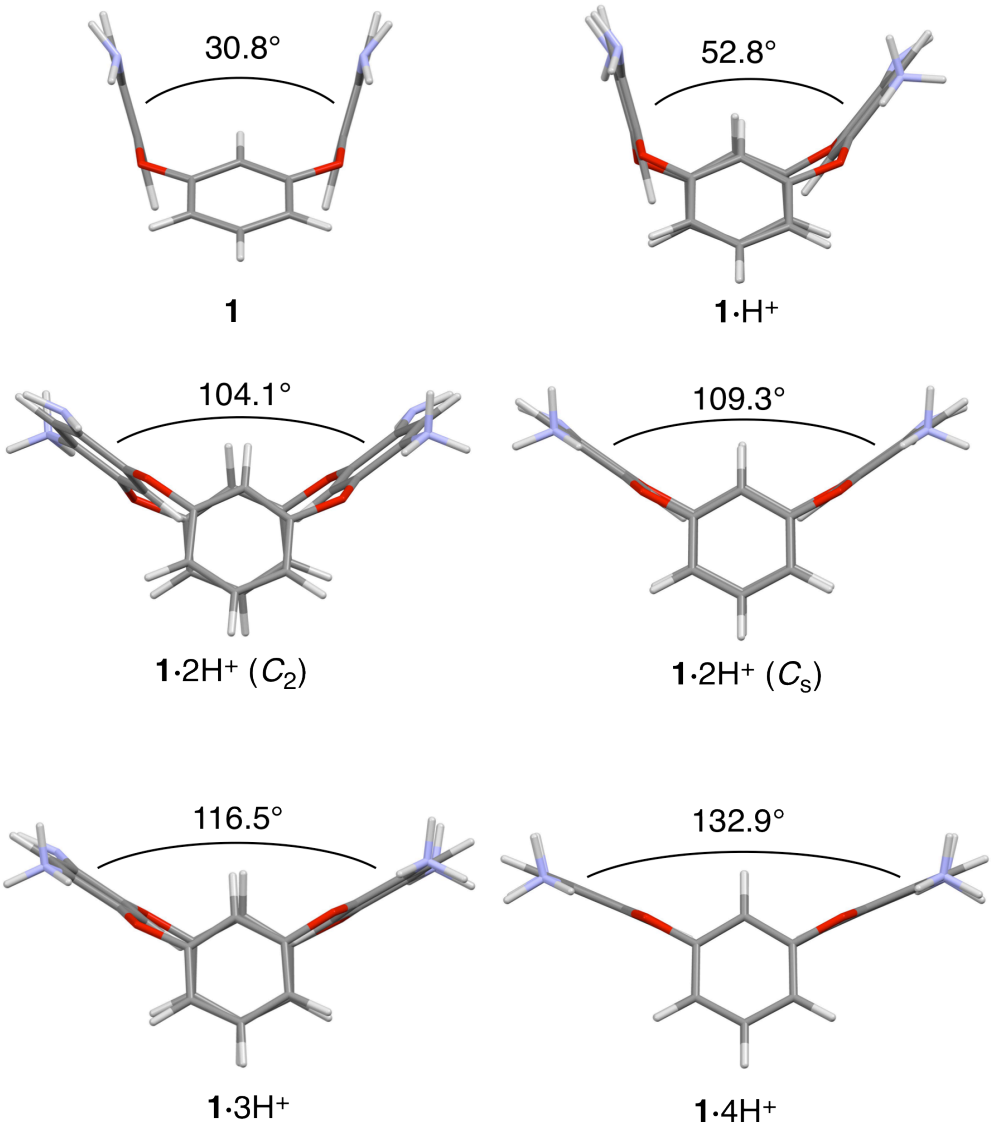


Fig. S14 Additional (side) views of the optimized geometries of oxacalixarenes **1**·*n*H$^+$ (*n* = 0–4).

S8 Y. Shao, L. F. Molnar, Y. Jung, J. Kussmann, C. Ochsenfeld, S. T. Brown, A. T. B. Gilbert, L. V. Slipchenko, S. V. Levchenko, D. P. O'Neill, R. A. Distasio, Jr., R. C. Lochan, T. Wang, G. J. O. Beran, N. A. Besley, J. M. Herbert, C. Y. Lin, T. Van Voorhis, S. H. Chien, A. Sodt, R. P. Steele, V. A. Rassolov, P. E. Maslen, P. P. Korambath, R. D. Adamson, B. Austin, J. Baker, E. F. C. Byrd, H. Dachsel, R. J. Doerksen, A. Dreuw, B. D. Dunietz, A. D. Dutoi, T. R. Furlani, S. R. Gwaltney, A. Heyden, S. Hirata, C.-P. Hsu, G. Kedziora, R. Z. Khalliulin, P. Klunzinger, A. M. Lee, M. S. Lee, W. Liang, I. Lotan, N. Nair, B. Peters, E. I. Proynov, P. A. Pieniazek, Y. M. Rhee, J. Ritchie, E. Rosta, C. D. Sherrill, A. C. Simmonett, J. E. Subotnik, H. L. Woodcock III, W. Zhang, A. T. Bell, A. K. Chakraborty, D. M. Chipman, F. J. Keil, A. Warshel, W. J. Hehre, H. F. Schaefer III, J. Kong, A. I. Krylov, P. M. W. Gill and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2006, **8**, 3172–3191.