Electronic Supplementary Information for

ATP Cleavage by *Cone* Tetraguanidinocalix[4]arene

Riccardo Salvio,[*a] Alessandro Casnati,[*b] Luigi Mandolini,[a] Francesco Sansone[b] and Rocco Ungaro[b]

[a] Dipartimento di Chimica and IMC ‑ CNR Sezione Meccanismi di Reazione, Università La Sapienza, 00185 Roma, Italy. riccardo.salvio@uniroma1.it

[b] Dipartimento di Chimica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy. casnati@unipr.it

Table of Contents

General Experimental Section

Instruments. The ^{31}P NMR spectra were recorded at 121.42 MHz on a Bruker Unity 300 MHz. Chemical shifts are relative to an external reference of 85% H_3PO_4. A capillary filled with D_2O inside the NMR tube was used to lock the deuterium signal.

Materials. DMSO purged 30 min with argon and mQ water were used in the preparation of the mixed solvent used in kinetic and potentiometric experiments. Calixarenes **1**-**4** were available from a previous investigation.[1] Commercial samples of ATPH$_2$Na$_2$, ADPH$_2$Na and Me$_4$ClO$_4$ were used as received.

Potentiometric Titrations

Potentiometric titrations were performed by an automatic titrator equipped with a combined microglass pH electrode. The electrode was calibrated using standard HClO$_4$ and Me$_4$NOH solutions at different concentrations. The time required to obtain a stable pH reading increased from 1 min in acid medium, up to 6 min at pH above 9. The calibration plot of calculated $-\log c_H^+$ values vs experimental pH readings was linear in the range 2-17, with $-\log c_H^+ = a + b \cdot pH_{read}$, and best fit values $a = -0.7852 \pm 1.5\%$, and $b = 0.965 \pm 5\%$. The pK_w values determined in several titrations coincided, within experimental errors, with the value reported in literature.[2] Potentiometric titrations were carried under a nitrogen atmosphere, on 6 mL of 1−3 mM solutions of the compound, in the presence of 0.1 M Me$_4$NClO$_4$, (80% DMSO, 25 °C). A 0.05−0.2 M Me$_4$NOH solution in 80% DMSO was added to the titration vessel in small increments. Analysis of titration plots was carried out by the program HYPERQUAD 2000.[3] Titration plots of ATP, ADP and H$_3$PO$_4$ are shown in Figures 1S, 3S and 5S, respectively. The corresponding distribution diagrams are reported in Figures 2S, 4S and 6S.

Potentiometric titration plots with (CH₃)₄NOH and species distribution diagrams of the given compounds (1-3 mM) in 80% DMSO (25 °C)
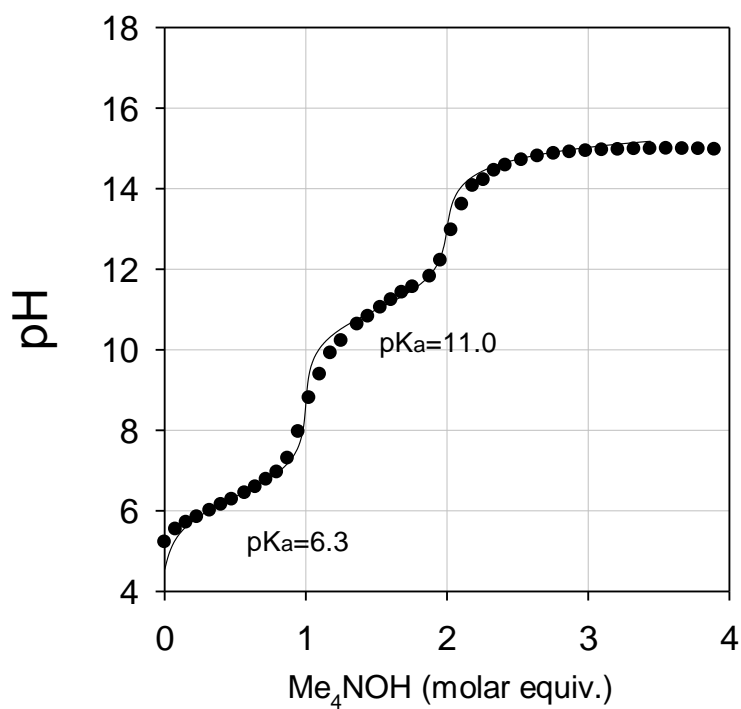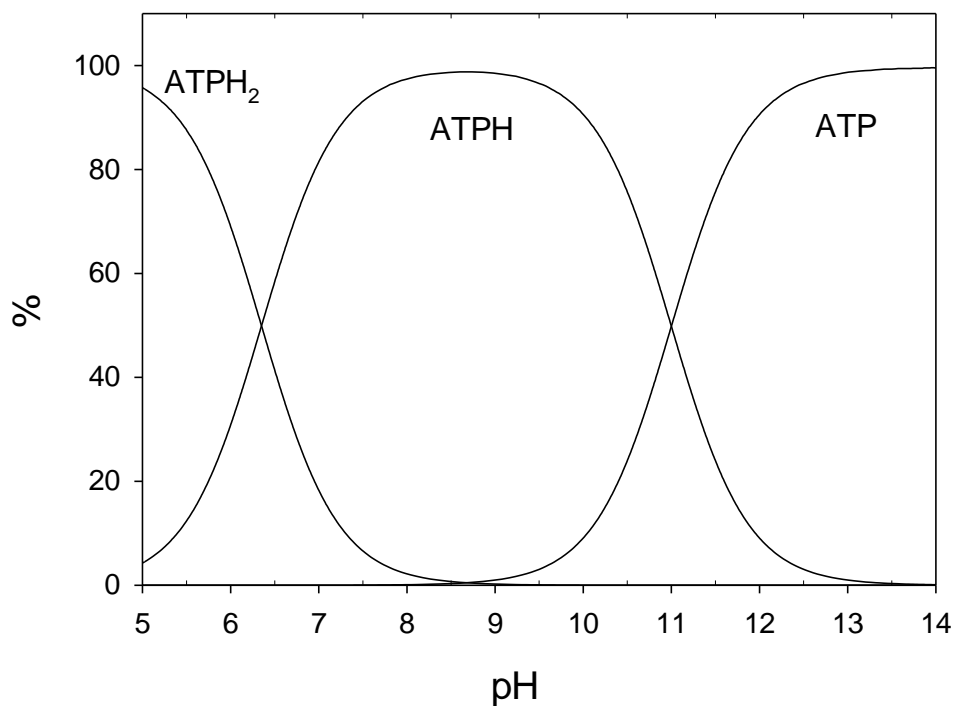

Figure S1 Titration of $ATPH_2Na_2$
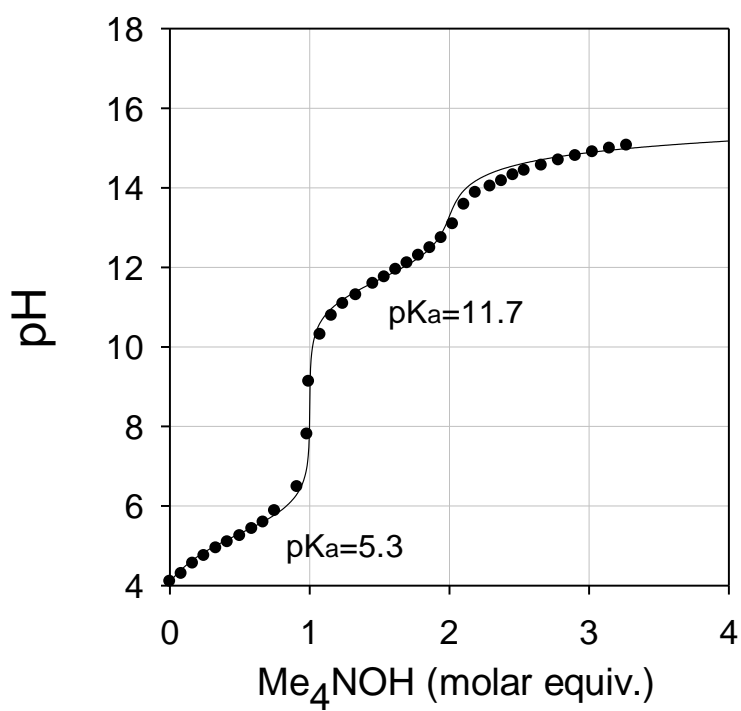

Figure S2 Distribution diagram of ATP

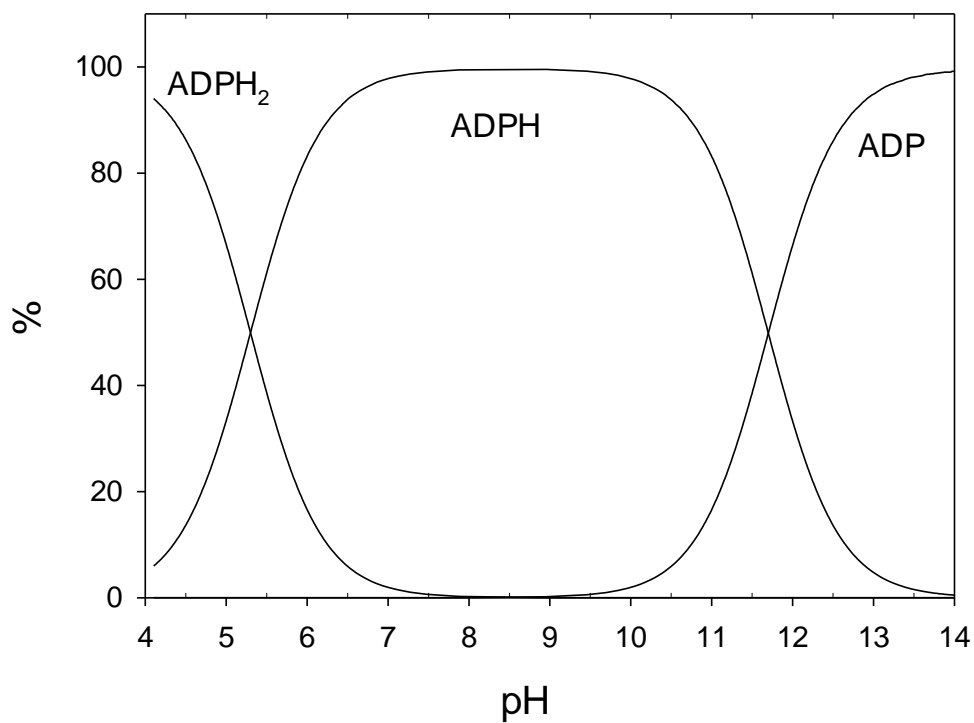**Figure S3** Titration of $ADPH_2Na$

Figure S4 Distribution diagram of ADP
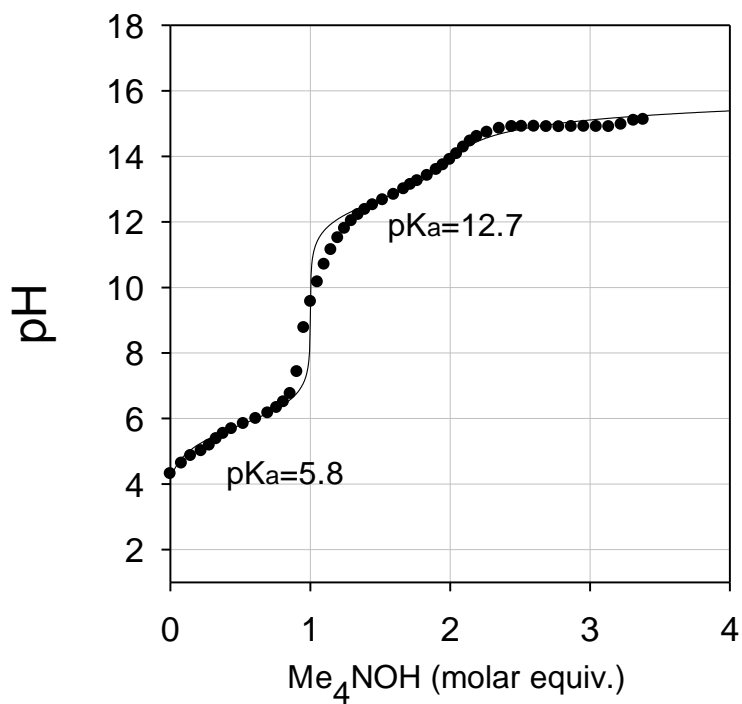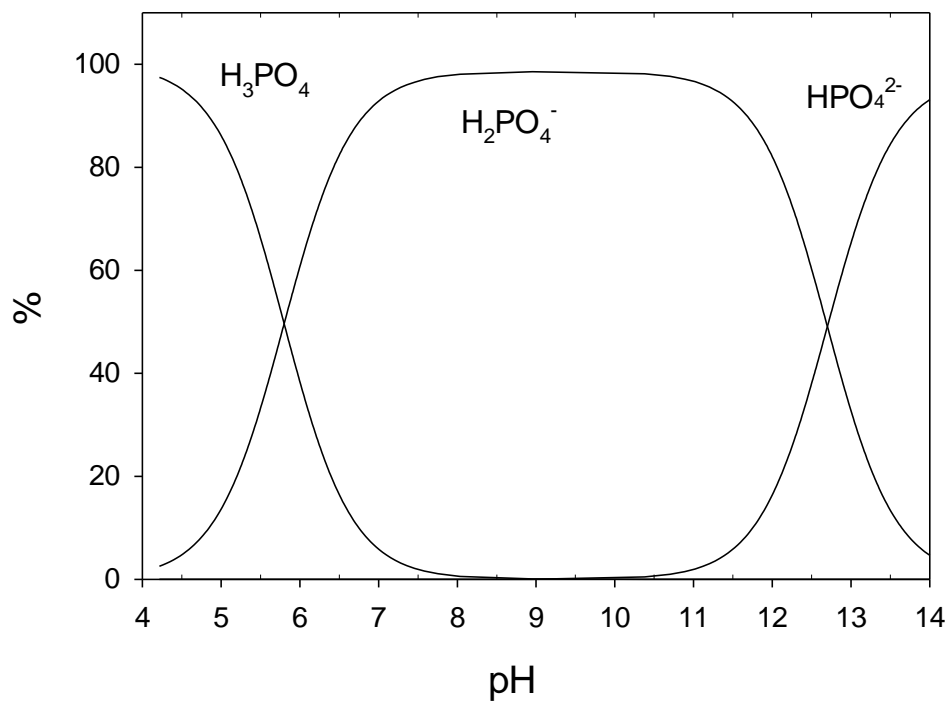
Figure S5 Titration of H_3PO_4

Figure S6 Distribution diagram of H_3PO_4

Rate Measurements

The reaction progress was monitored by following the time dependent changes in the ^{31}P NMR spectra of the signals of ATP, ADP and inorganic phosphate. The NMR acquisition time was set to 12 s to ensure the peak integrals to be proportional to concentrations of the different species. The quantity x in equations (2) and (3) was calculated as follows: $x = I_P/(I_P + I_{\beta-ATP})$, where I represents the integrated intensity of the given signal. Time-course kinetics were obtained for the cleavage of 10 mM ATP in the presence of varying concentrations of **1** (0.10, 0.20 and 0.40 mM) at pH 9.8. Figure S7 shows the poor adherence of a typical run to first-order behaviour and, conversely, the close adherence to the mixed first- and zero-order behaviour consistent with eq (3). Slower reactions were analyzed by an initial rate method. A typical time-concentration plot used in the determination of initial rates is shown in Figure S8.
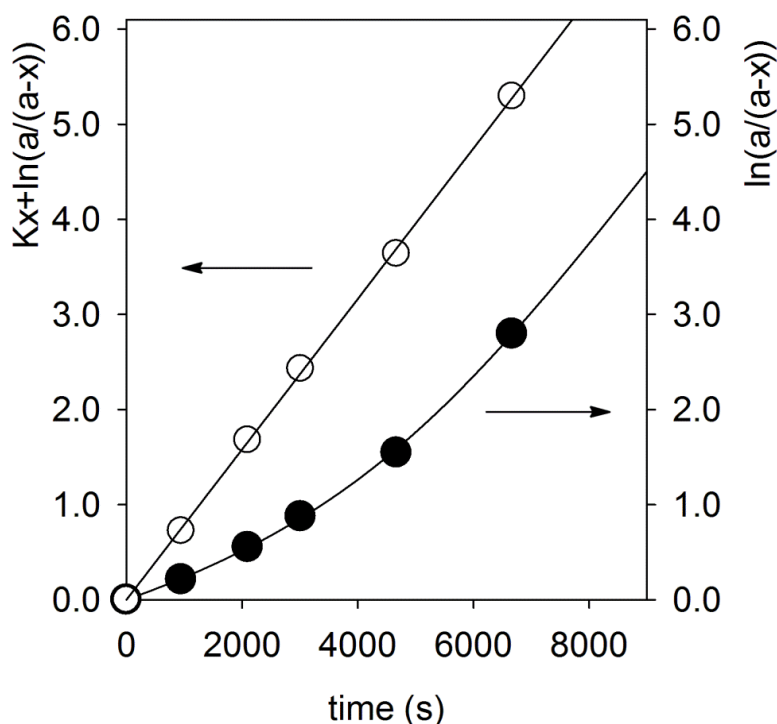

Figure S7 Kinetic data for the cleavage of 10 mM ATP catalyzed by 0.20 mM **1**(H$^+$)$_3$ in 80% DMSO (80 $^{\circ}$C, 0.1 M Me$_4$NClO$_4$). Plots of ln(a/(a-x)) (right) and Kx +ln(a/(a-x)) (left) against time.
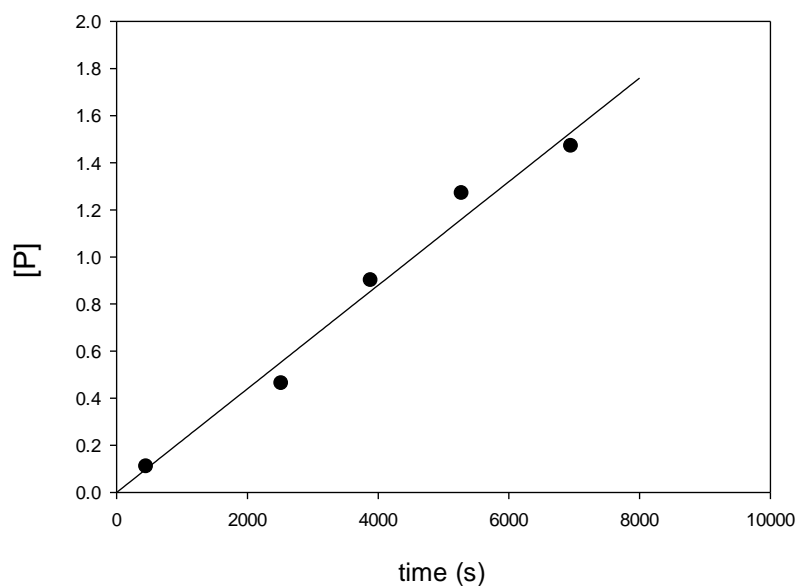
Figure S8 Cleavage of 10 mM ATP in 80% DMSO catalyzed by 0.20 mM $2(H^+)_2$ (T= 80 °C, pH 9.7, 0.1 M Me_4NClO_4).

References

[1] L. Baldini, R. Cacciapaglia, A. Casnati, L. Mandolini, R. Salvio, F. Sansone and R. Ungaro, *J. Org. Chem.*, 2012, **77**, 3381

[2] M. M. Kreevoy and E. H. Baughman, *J. Phys. Chem.*, 1974, **78**, 421

[3] L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coord. Chem. Rev.*, 1999, **184**, 311