LETTER

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Synthesis, characterization and X-ray crystal structures of cyclam derivatives. 7. Hydrogen-bond induced allosteric effects and protonation cooperativity in a macrotricyclic bisdioxocyclam receptor†‡

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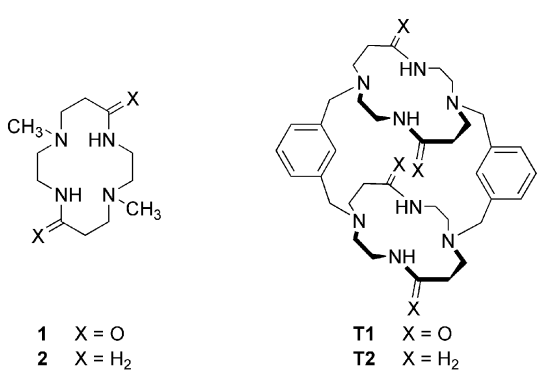
The unprecedented cooperative protonation properties displayed by a barrel-shaped macrotricyclic tetraamine incorporating two 14-membered bisamide rings maintained in a face-to-face arrangement is rationalized in terms of allosteric effects upon binding of the first and third protons.

Allosteric regulation is a well known and an important possibility in biology to control the catalytic activity of enzymes,[1] but it remains a rarity for synthetic systems.[2] Increased understanding of the fundamental principles behind supramolecular chemistry has sparked growing interest in the design of host molecules able to efficiently transmit information through conformational changes mediated by the binding of a molecule or an ion to a second remote coordination site.[3] Association *via* noncovalent interactions of allosteric effectors to artificial receptors not only serve as means to alter the complexation characteristics of the other sites, but furthermore provides a new entry into the realm of molecular switches.[4] In this regard, knowledge of the factors that trigger proton-driven molecular reorganization is of fundamental scientific interest.[5]

Herein, we report the unprecedented cooperative protonation properties exhibited by macrotricyclic receptors of cylindrical topology possessing either two 5,12-dioxocyclam (**T1**) or cyclam (**T2**) units maintained in a face-to-face arrangement (Scheme 1). For the first time, we provide direct experimental evidence (*i.e.* model free) that an abiotic, neutral tetraprotic base is able to add two out of four protons cooperatively in spite of positive charge accumulation.

T1 was prepared in four steps with 26% yield starting from 5,12-dioxocyclam,[6] while the octaamine **T2** could be conveniently isolated by quantitative reduction (BH_3/THF) of **T1**.[7] Alternative synthetic methods of **T2** have also been described,[8] but they do not allow the preparation of heteroditopic tricycles incorporating two rings of different nature or size. En route towards this goal, a more versatile procedure has been devised which enabled **T2** to be obtained in *ca.* 20% yield. The isolated compounds, **T1** · $4H_2O$ and **T2** · $8HCl \cdot 6.5H_2O$ were characterized by elemental analysis and TGA for their water content, FTIR, ^1H and ^{13}C NMR spectroscopy. The MALDI/TOF mass spectrum displayed only the $[M + H^+]$ signal expected for **T1** ($m/z = 661$) and **T2** ($m/z = 605.5$), demonstrating that no oligomers were present. In $CDCl_3$, two sets of sharp ^1H NMR signals, each corresponding to one half of the number of hydrogen atoms, are observed at room temperature. Accordingly, it is inferred that **T1** exists as a mixture of two isomers in slow exchange, a major (*M*) and a minor (*m*), for which the orientation of both dioxocyclam units differs by a 180° rotation around the pivotal tertiary amines. Although a definitive assignment is not possible, one form possesses a C_{2h} and the other a D_2 time-averaged symmetry. With increasing temperature, all signals broaden and start to coalesce above 320 K, indicating a fast tumbling of both rings, while cooling down to 210 K provokes a progressive disappearance of the resonances arising from the *m* isomer. According to a van't Hoff analysis of the low-temperature spectra, the $M \leftrightarrows m$ interconversion equilibrium is characterized by $K_{298} = 0.2$, $\Delta H = 10.2(1)$ kJ mol^{-1}, and $\Delta S = 20.8(3)$ J mol^{-1} K^{-1}.

X-ray diffraction analysis of **T1** · $4H_2O$ provided helpful insights about the conformation adopted by the free-base ligand (Fig. 1). The central cavity, delimited by the phenyl spacers on the lateral side, takes up a twisted arrangement with a dihedral angle between both aromatic rings of 61.2°. The dioxocyclam subunits are rotated by 85.3° with respect to each other so that the amide nitrogen atoms sit almost on the top of the tertiary amines belonging to the facing ring. Most importantly, each amide proton interacts with the *endo*-oriented lone pair of the adjacent tertiary amine, giving rise to two *trans*-



1 X = O
2 X = H_2

T1 X = O
T2 X = H_2

Scheme 1

† For the previous paper in this series, see ref. 10.
‡ Electronic supplementary information (ESI) available: VT ^1H NMR spectra, ^1H–^1H NOESY chart, Curie plots, and ^1H NMR titration of **T1**; full experimental details. See http://dx.doi.org/10.1039/b508076b

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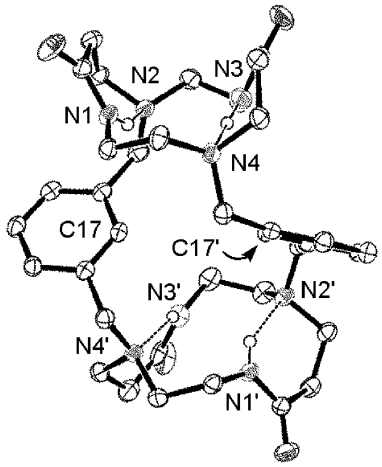


Fig. 1 ORTEP plot (thermal ellipsoids set at 50% probability) of **T1** showing the *trans*-annular hydrogen bonds. Atoms labeled with primes belong to the second half molecular unit, which is generated by a twofold axis placed at $(\frac{1}{2}, y, \frac{3}{4})$. Water molecules and all H(–C) hydrogen atoms are omitted for clarity. Selection of interatomic distances and angles: C17···C17′ = 4.04(2) Å, N1···N4′ = 5.84(2) Å, N2···N3′ = 6.35(2) Å, N2···N2′ = 6.29(2) Å, N2···N4′ = 6.39(2) Å, N4···N4′ = 6.24(2) Å, N2···N4 = 4.50(2) Å, N1···N2 = 2.84(2) Å, N1–H1···N2 = 146.7(2)°, N3···N4 = 2.81(2) Å, N3–H3···N4 147.0(2)°.

annular hydrogen bonds spanning across each U-shaped dioxocyclam ring [H···N = 2.03(2) and 2.07(2) Å].

Hydrogen bonding was also confirmed by the presence of a broad ν_{NH} stretching mode at 3233 cm^{-1} in the solid-state infrared spectrum of **T1**·$4H_2O$. The diagnostic amide I ($\nu_{C=O}$ = 1651 cm^{-1}) and amide II (δ_{CNH} = 1554 cm^{-1}) absorption bands appear in the expected range. Interestingly, the FTIR spectrum recorded in diluted $CDCl_3$ solutions were concentration independent and evidenced no significant shifts, emphasizing that the intramolecular N–H···N hydrogen bonds are preserved in solution. Furthermore, increasing the concentration of **T1** did not lead to the appearance of a low-energy satellite of the C–D stretching band arising from the solvent at 2253 cm^{-1}.[9] This confirms that the tertiary amine's lone pairs are not exposed to the bulk, but rather adopt an *endo*-orientation as evidenced in the solid state.

Additional information on the solution structure and evidence for intramolecular hydrogen bonding was provided by 1D and 2D NMR spectroscopy. Complete assignment of the cross-peaks appearing in the ^1H–^1H NOESY correlation chart further suggests that the main structural features of **T1** seen in the solid state are essentially preserved in $CDCl_3$ (see ESI‡). The *endo* orientation of the amide protons (δ = 8.81 ppm) is attested by NOE connectivities with the γ-CH_2 and the central xylyl protons. Moreover, their engagement in intracyclic hydrogen bonds was confirmed by performing VT NMR experiments. The slopes of the linear Curie plots obtained in pure $CDCl_3$ ($\Delta\delta/\Delta T = -4.33$ ppb K^{-1}) but also in the non-deuterated CH_3OH/H_2O (1 : 1 v/v) mixture ($\Delta\delta/\Delta T = -5.16$ ppb K^{-1} at pH 7) are consistent with solvent-protected N–H groups[10] and fall in the typical range of temperature coefficient values found for the yet stronger NH···O=C interactions in peptides ($\Delta\delta/\Delta T < -5$ ppb K^{-1} in $CDCl_3$).[11]

The binary solvent mixture was mainly chosen for solubility reasons. However, performing these experiments in an aqueous non-deuterated medium offered the possibility to observe the amide proton resonance, which proved to be an outstanding probe for sensing the conformational rearrangements induced by protonation of **T1**. In spite of an obscured aliphatic region, spectral changes occurring in the aromatic part were conveniently monitored as a function of HCl concentration (see ESI‡). At p[H] values (p[H] = $-\log[H_3O^+]$) where the free-base form predominates, NH protons appear as a partially resolved doublet of doublet centred at 9.25 ppm. Most intriguingly, progressive acidification resulted in the vanishing of all resonances arising from the free base and the simultaneous appearance of a new set of peaks that is accompanied with severe line broadening between p[H] 5.5–4.5. This behaviour is consistent with slow proton transfers at the NMR time scale for the partially protonated species, which seems to be a common theme among dioxocyclam-based polycyclic receptors.[10] Evidence for nonhydrogen-bonded amide protons in the fully protonated form is provided by the 0.53 ppm upfield shift experienced by the NH signal. In contrast, the central xylyl protons resonate at much lower field ($\Delta\delta$ = 0.66 ppm) upon protonation, supporting their reorientation from a radial to a tangential position with respect to the cylindrical cavity.

In order to unravel the structural and electronic consequences of the β-alaninamide fragments on the basicity of the neighboring amines, potentiometric titrations of tricycles **T1** and **T2** and their monocyclic *trans*-dimethylated models (**1** and **2**) were carried out in the methanol–water mixed solvent. Special care was taken to ensure that the equilibrium was reached at each titration point. In a first approach, the multiple binding events encountered by these polyprotic bases were evaluated on hand of the occupancy concept and Scatchard analysis.[12] The site occupation factor r, which corresponds to the average number of protons bound per molecule possessing t chemically identical sites, was directly derived from the experimental p[H] readings according to relation (1).

$$r = \frac{[H^+]_{tot} - 10^{-p[H]}}{[L]_{tot}} \quad \text{with } 0 \le r \le t \qquad (1)$$

$$r = \frac{\sum_{i=1}^{t} i\beta_{01i}[H^+]^i}{1 + \sum_{i=1}^{t} \beta_{01i}[H^+]^i} \quad \text{with } \beta_{01i} = \prod_{k=1}^{i} K_{01k} \qquad (2)$$

The Scatchard plots ($r/[H^+]$ *vs.* r) obtained for **T1** and **T2** (Fig. 2) clearly established that only four protons were taken up in the p[H] range 11.9–2.0, although the biscyclam receptor possesses eight protonation sites. Moreover, the hyperbolic shape of the graph corresponding to **T2** contrasted with the downward-curved plot obtained for **T1**, highlighting a drasti-

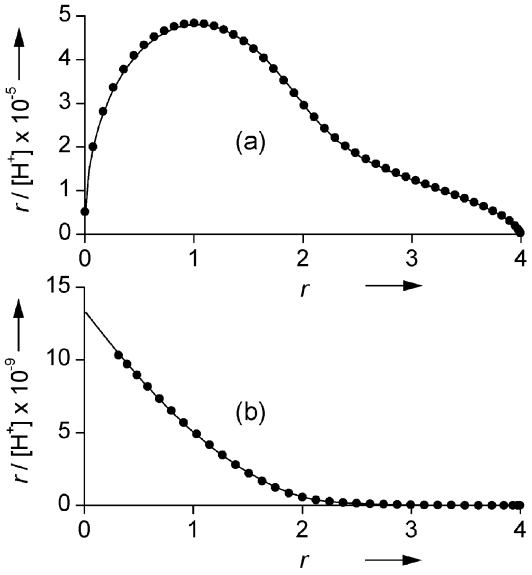


Fig. 2 Scatchard plots for (a) **T1** and (b) **T2**. Experimental p[H] values were converted into site occupation factors (r) using eqn. (1). Solid lines were drawn according to eqn. (2) and the protonation constants reported in Table 1. Solvent: CH_3OH/H_2O (1 : 1 v/v), I = 0.1 (KCl), T = 298.2(1) K.

cally different behavior of both ligands and providing direct experimental evidence for cooperative protonation of **T1**. Indeed, the shape of the plot is an excellent diagnostic tool for detecting multiple-binding cooperativity, the phenomenon by which the coordination of a given substrate enhances the binding strength of the subsequent one. Deviation from linearity (statistical binding) is a sufficient criterion to assess such a positive (downward concavity) or negative (upward concavity) effect.[12] For statistical reasons, the ratio of the successive equilibrium constants K_{i+1}/K_i is less than unity for a polytopic receptor having t identical and independent sites. Quantitatively, positive cooperativity is encountered when the experimental K_{i+1}/K_i ratio is larger than the critical factor for statistical binding.

Once the unusual proton affinity of **T1** has been established, the stepwise protonation constants (K_i) were refined by weighted nonlinear least-squares using the Hyperquad 2000 program.[13] The accuracy of the calculated parameters reported in Table 1 was ascertained by the excellent match between the experimental Scatchard plot and the line derived from the theoretical expression of r as a function of the equilibrium constants.[12] Moreover, Monte-Carlo simulations showed that the shape of the calculated curve is highly sensitive to variations as small as 0.02 log units of the K_i values.

For **T2**, noncooperativity is evidenced by the regular decrease of nearly one logarithmic unit of the four stepwise protonation constants, which is slightly higher than expected for statistical binding. This sequence can be rationalized in terms of charge accumulation and electrostatic repulsive effects. Based on the facts that K_1 and K_2 are close to the values measured for the model ligand **2**, the first two protonation steps of **T2** involve the secondary amines, which are more basic than the bridgehead tertiary nitrogen atoms. Accordingly, proton binding is proposed to take place alternatively on one cyclam subunit and then on the diametrically opposed site belonging to the facing ring. Minimization of coulombic repulsive interactions is best achieved by this sequence, classically observed for polyamines of various topologies.[7,14,15]

As expected, replacement of the secondary amines in **2** and **T2** by electron withdrawing amide groups confers a much weaker basicity to the dioxocyclam derivatives **1** and **T1**.[6] However, the intriguing inversion of both protonation constants in the case of the model compound **1** is more surprising, although protonation studies of 5,12-dioxocyclam also revealed weak cooperativity.[6] Since the relative magnitude of K_2 over K_1 reflects the free-energy cost of protonating an amine in the neighbourhood of a positively charged ammonium group, it is concluded that the coulombic repulsion energy for $\mathbf{1}H_2^{2+}$ is compensated by a favourable change in solvation and/or strain energy.[16]

Noteworthy, the peculiar reversal of protonation constants found for monocyclic 5,12-dioxocyclam derivatives is dramatically exacerbated when such fragments are built in a more constrained tricyclic framework, as highlighted by the exotic acid–base properties of **T1**. The strong cooperativity displayed by this compound is reflected by a most unusual set of protonation constants: K_2 and K_4 are respectively two and one order of magnitude higher than K_1 and K_3. Moreover, the two first and two last constants can be paired, while the second equilibrium constants for **1** and **T1** are virtually identical. According to preliminary results obtained for structural analogues of **T1** that differ by the nature or length of both bridging units, this trend seems to be a common feature. A literature survey revealed only a very limited number of systems where protonation enhances the basicity of the yet unprotonated sites.[15,17–19] Cooperativity has been attributed either to a conformational rearrangement enforcing hydrogen bonding[18] or by the encapsulation of mediating water molecules.[17,20] Curiously, these compounds belong all to the cryptand family, but none revealed such a strong cooperative effect as **T1**.

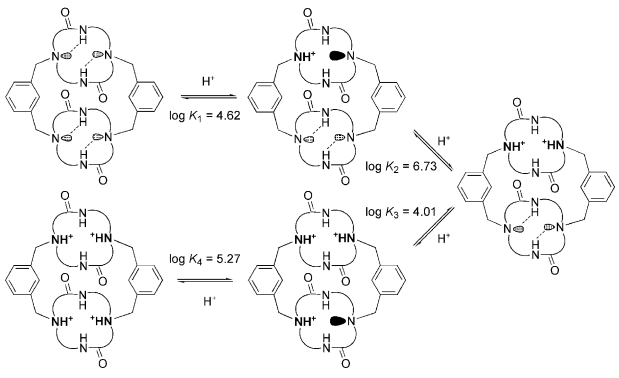
Both FTIR and ^{1}H NMR results clearly support that the free-base form of **T1** retains approximately the solid-state conformation in solution. Moreover, the unexpectedly low K_1 value found for **T1** compared to the monocyclic analogue **1** strongly suggests that the four tertiary amines are still engaged in *trans*-annular N–H···N_{tert} hydrogen bonds in solution. Based on these experimental evidences, allosterism might best account for the peculiar proton affinity. Considering that the first entering proton will necessarily disrupt one of those intracyclic interactions, a conformational rearrangement of the protonated dioxocyclam ring is taking place, which in turn will also break up the second *trans*-annular hydrogen bond. Hence, the thereby disengaged electron doublet becomes more accessible, thus increasing the basicity of the second amine in the already monoprotonated dioxocyclam unit.[16] This proton-triggered allosteric effect accounts for the inversion of K_2 over K_1 but also for the similar K_2 values found for **1** and the tricyclic cage **T1**. We conclude therefore that both amines of the same dioxocyclam subunit are protonated in the $\mathbf{T1}H_2^{2+}$ species and furthermore that the hydrogen-bond network is preserved in the unprotonated ring (Scheme 2).

This allosteric mechanism can operate a second time as the ligand takes up the third and fourth proton, in full agreement with the experimentally observed order $K_4 > K_3$. Due to charge accumulation and electrostatic repulsion, the latter values are slightly lower compared to K_2 and K_1, respectively. It is worth noting that this phenomenon requires two weakly mechanically-coupled subunits, as both must rearrange independently from each other in order to avoid the simultaneous disruption of all hydrogen bonds as the first amine protonates. Flexibility of the tricyclic cage is therefore a prerequisite for observing cooperative protonation in such systems. The abnormal proton and metal-binding properties induced by the β-alaninamide moieties of **T1** and the closely related dioxocyclen and dioxocyclam analogues will be reported in due course.

Table 1 Protonation constants of the various macrocycles[a]

	1	T1	2	T2
log K_1	6.36(1)	4.62(7)	10.41(6)	10.13(1)
log K_2	6.90(2)	6.73(8)	8.53(6)	9.26(1)
log K_3		4.01(3)	<2	7.69(2)
log K_4		5.27(4)	<2	6.62(3)
$\Delta_{1,2}$[b]	−0.54	−2.11	1.88	0.87
$\Delta_{2,3}$[b]		2.72	>6.5	1.57
$\Delta_{3,4}$[b]		−1.26		1.07

[a] Solvent: CH_3OH/H_2O (1 : 1 v/v), $I = 0.1$ (KCl), $T = 298.2(1)$ K. K_i refers to the equilibrium: $LH_{i-1}^{(i-1)+} + H^+ \leftrightarrows LH_i^{i+}$. Values in parenthesis correspond to the standard deviation in the last significant digit. [b] $\Delta_{i,j} = \log K_i - \log K_j$. For statistical binding, the expected values for four identical and independent sites are $\Delta_{1,2} = \Delta_{3,4} = \log 8/3 \approx 0.426$ and $\Delta_{2,3} = \log 9/4 \approx 0.352$.[12]



Scheme 2

Experimental

All experimental details related to the synthesis, spectroscopic, and potentiometric characterization of the described compounds can be found in the Electronic Supplementary Information (ESI‡).

Crystal data for T1·4H_2O

A colourless crystal of prismatic shape (0.32 × 0.25 × 0.20 mm) was obtained by slow evaporation from an ethanol/water (1 : 1 v/v) solution. $C_{36}H_{60}N_8O_8$, $M = 732.92$, orthorhombic, $a = 12.9970(2)$, $b = 18.4860(2)$, $c = 16.1270(3)$ Å, $U = 3874.7(1)$ Å^3, $T = 110(2)$ K, space group $P\,bcn$, $Z = 4$, μ(Mo-Kα) = 0.090 mm^{-1}, 10778 reflections measured, 5657 unique ($R_{int} = 0.0630$) which were used in all refinements on F^2, 255 refined parameters, 4 restrains, $R_1 = 0.0532$, $wR_2 = 0.0992$ [$I > 2\sigma(I)$], $R_1 = 0.1415$, $wR_2 = 0.1288$ (all data). CCDC reference number 233399. See http://dx.doi.org/10.1039/b508076b for crystallographic data in CIF or other electronic format.

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