The unusual protonation constants of cyclam. A potentiometric, crystallographic and molecular mechanics study

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The protonation constants for cyclam (1,4,8,11-tetraazacyclotetradecane) have been determined by glass electrode potentiometry in 0.10 M KCl at 25 °C and yield values of 11.29(2), 10.19(1), 1.61(1) and 1.91(1) for the logarithms of the first, second, third and fourth protonation constants. This result supports previous studies (Micheloni 1978, Thom 1985) which show the unusual sequence of protonation constants where the third protonation constant of cyclam is smaller than the fourth, in contrast with a large number of other workers who report the third to be larger than the fourth protonation constant. The crystal structure of cyclam·4ClO$_4$·2H$_2$O is reported: monoclinic, $P2_1/n$, a = 8.409(2), b = 15.81(3), c = 9.221(1) Å, β = 105.99(1)°, Z = 2, R = 0.0374. A molecular mechanics (MM) analysis of the conformations of cyclam·H$_n^{n+}$, and of trien·H$_n^{n+}$ (trien = 1,4,7,10-tetraazadecane) with differing numbers of protons (n) present, is reported. It is shown that the structures cyclam·H$_4^{4+}$ that have been determined crystallographically correspond to the two lowest energy conformers identified in the MM analysis. The strain energies of the series trien·H$_n^{n+}$ rise smoothly as n increases from 0 to 4, mainly due to increasing electrostatic repulsion between the protons on the nitrogens of the ligand. In contrast, for the cyclam·H$_n^{n+}$ series, there is a sudden large increase in strain energy at n = 3, with a change in conformation to having some of the nitrogens of the ligand *exo*. For n = 4 the rise in strain energy is again not large. These results are discussed in relation to the inversion in order of protonation constants for the cyclam·H$_n^{n+}$ series from n = 3 to n = 4.

Introduction

The protonation constants (pK) of the tetraaza macrocycles [1] differ markedly from those of open-chain tetraamines. The tetraaza macrocycles show large drops in the series of protonation constants after two protons have been added to the macrocycle, compared with the smooth decrease seen for the pK values of open-chain tetraamines as more protons are added:

Ligand	pK_1	pK_2	pK_3	pK_4
2,3,2-tet [2]	10.25	9.50	7.28	6.02
[13]aneN$_4$ [1]	11.0	10.0	2.0	0.8
[14]aneN$_4$ [3] (cyclam)	11.6	10.6	1.61	2.42

For ligand abbreviations, see Fig. 1. pK_1 refers to the equilibrium L + H$^+$ = LH$^+$; pK_2 refers to LH$^+$ + H$^+$ = LH$_2^{2+}$; pK_3 is LH$_2^{2+}$ + H$^+$ = LH$_3^{3+}$, and pK_4 is LH$_3^{3+}$ + H$^+$ = LH$_4^{4+}$ (L = ligand).

Paoletti et al.,[3] and Thom et al.,[4] have reported that for cyclam, the third protonation constant is *smaller* than the fourth. This is in contrast with other workers, who have reported [5-10] that, as is usually the case for tetraaza macrocycles, pK_3 is larger than pK_4. The unusual sequence of protonation constants pK_3 < pK_4 found for cyclam was interpreted [3] in terms of a protonation sequence where the cyclam underwent a major change in conformation on addition of the third proton, from having all the nitrogens *endo* in the macrocyclic ring, to *exo*. The energy required to effect this conformational change was held to account [3] for the small size of pK_3 in cyclam, but once it had been effected, addition of a fourth proton was facilitated, accounting for the larger value of pK_4.

The cyclam·H$_4^{4+}$ cation has two different conformations in the recently reported tetrachloride [11] and tetrakis(trifluoroacetate) [12] salts. During the course of this study we obtained
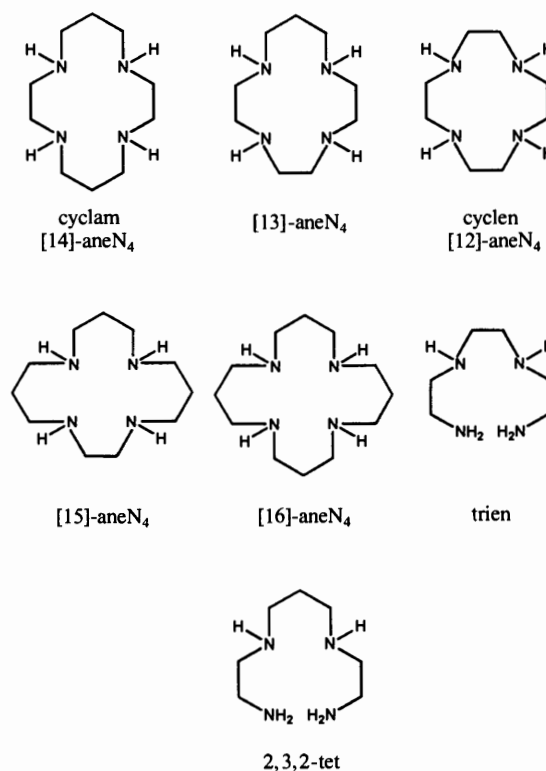

Fig. 1 Formulas of ligands discussed here

crystals of cyclam·4ClO$_4$·2H$_2$O, and determined the structure. This revealed the conformation to be the same as in the tetrakis(trifluoroacetate) salt.[12] We embarked on a molecular mechanics (MM) study of the conformations of cyclam and its protonated forms to try to understand the conformational

Table 1 Protonation constants for cyclam determined here, and reported [1] for other tetraaza macrocycles, at 25.0 °C and ionic strength 0.10

	[12]aneN$_4$[a]	[13]aneN$_4$[a]	[14]aneN$_4$ (cyclam)	[15]aneN$_4$[a]	[16]aneN$_4$[a]
pK_1	10.6	11.02	11.29(2)[b] [11.3][c]	11.1	10.64
pK_2	9.6	9.96	10.19(1)[b] [10.23][c]	10.13	9.49
pK_3	(1.5)	2.0	1.61(1)[b] [1.43][c]	5.23	6.77
pK_4	(0.7)	(0.8)	1.91(1)[b] [2.27][c]	3.62	5.25

[a] Protonation constants from ref. 1. [b] Protonation constants from this work in 0.10 M KCl, 25.0 °C. [c] Literature values from Thom et al.,[4] in 0.10 M NaNO$_3$, 25.0 °C.
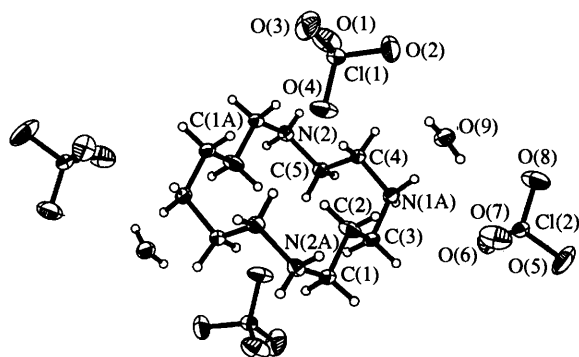

Fig. 2 Structure of cyclam·4HClO$_4$·2H$_2$O, showing the numbering scheme

changes accompanying protonation, and the origin of the reversal pK_3 < pK_4. In this paper is reported a redetermination of the protonation constants of cyclam, a MM analysis of the conformational changes accompanying protonation of cyclam, and a crystallographic study of cyclam·4HClO$_4$·2H$_2$O.

Results and discussion

The potentiometric results for determination of the protonation constants of cyclam are seen in Table 1, together with the protonation constants of the other tetraaza macrocycles from [12]aneN$_4$ to [16]aneN$_4$. The results obtained here for the protonation constants of cyclam display the reverse order pK_3 < pK_4 found by Paoletti et al.[3] and Thom et al.[4] and are in good agreement (Table 1) with the protonation constants found by Thom et al.[4] which are also at ionic strength 0.1, but in 0.1 M NaNO$_3$ rather than 0.1 M KCl. It is felt that the results obtained here confirm the reversal pK_3 < pK_4 found by previous workers,[3,4] and that potentiometric data sets used by other workers [5–10,13] to derive protonation constants that do not show the feature pK_3 < pK_4, possibly contained inaccuracies down in the pH region close to 2, or may have been misinterpreted in calculating the protonation constants. Some workers avoided the issue altogether by reporting only the first two protonations and totally ignoring the 3rd and 4th protonations.[5,7,8,13] Glass electrode potentiometry can be less accurate below pH 2 due to junction potentials,[14] and care is required to obtain good results even a little above this region. In addition, sophisticated computer programs are required to analyse the potentiometric data and discern the presence of the reversal pK_3 < pK_4.

The structure of cyclam·4HClO$_4$·2H$_2$O which is seen in Fig. 2, shows the nitrogens to be exo, and the cation has the same conformation as that found in previous work on the trifluoroacetate salt.[12] There are four alternative conformers for cyclam·H$_4^{4+}$ which have the same exo conformation for the macrocyclic ring found in crystal structures here and in refs. 11 and 12, but which differ in the position of the nitrogens in the ring. These four structures, generated by MM, are seen in Fig. 3. The type found here for the tetraperchlorate, and found previously[12] for the tetrakis(trifluoroacetate), is referred to as type I, and that found[11] for the tetrachloride as type II. With charges calculated by the method of Gasteiger and Marzilli,[15]
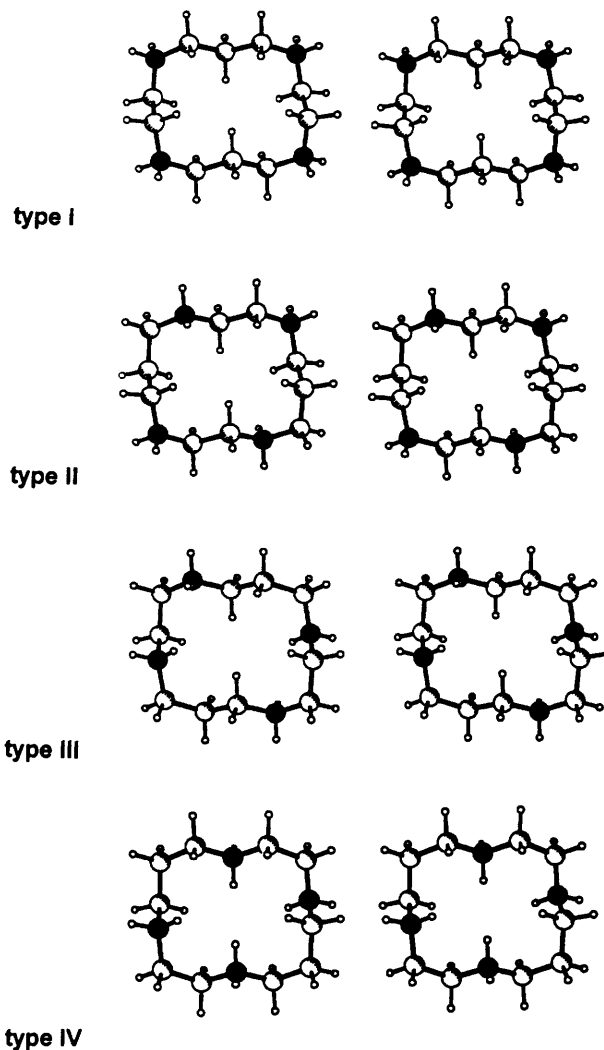

type I

type II

type III

type IV

Fig. 3 Stereoview of structures of the four possible arrangements of the nitrogens of the cyclam·H$_4^{4+}$ cations, the types I through IV conformers, generated by MM calculation. These four types all have the same conformation if the fact is disregarded that the positions of the nitrogens within the macrocylic ring differ. The energies of these four types are given in the text. Type I is that observed in the tetraperchlorate salt (this work) and tetrakis(trifluoroacetate)salt,[13] while type II is observed in the tetrachloride salt.[12] The shaded atoms are nitrogen atoms.

and a dielectric constant (D)† of 1.0, appropriate for a vacuum, the energies of the conformers of type I to IV, shown in Fig. 3, can be calculated. The calculations show type I to be significantly lower in energy, by some 5 kcal mol^{-1}, than the type II conformer, which is, in turn, much lower in energy than the type III and type IV conformers, which have not been observed in crystal structures. (The strain energies of types I, II, III and IV were found to be 145, 150, 161 and 158 kcal mol^{-1}, respectively.) This result can be understood in terms of the

† IUPAC-recommended term: relative permittivity ε.

electrostatic repulsion between the protons on the nitrogens, and the mean separation of the nitrogens bearing the protons. The mean separation is at a maximum in type I, is less in type II, and even less in types III and IV, so that electrostatic repulsion within the macrocycle will increase in the order type I < type II < type VI ~ type III.

The MM calculations showed that the relative energies of conformer I and II are dependent on the dielectric constant (D) assumed in the calculation. If D is set at 1, the value for a vacuum, then the energy of the conformer II is some 5 kcal mol^{-1} higher than for conformer I. As D is increased the difference decreases, until for $D = 80.4$, the dielectric constant of water, the energy of conformer II is slightly lower. It is interesting to note that in the two structures where the cyclam·H$_4^{4+}$ cation has the type I conformation, the hydrogen bonding is not very extensive, but that in cyclam·4HCl·4H$_2$O, there is an extensive network of hydrogen bonds bridging[11] the protons of adjacent cyclam·H$_4^{4+}$ cations with water molecules. It seems reasonable to suggest that the type II structure is adopted by cyclam·4HCl·4H$_2$O because the extensive hydrogen bonding leads to an effectively higher dielectric constant than in the structure of cyclam·4HClO$_4$·2H$_2$O reported here, which has the type I structure.

A point of interest here is the origin of the drop in the size of the protonation constants of cyclam after two protons have been added, and, in particular, the reversal in protonation constants pK_3 < pK_4. One may analyse this effect by comparing the build-up in strain as a function of increased number of protons in the ligand cyclam, and an open-chain ligand such as trien. The calculations were carried out with several values of D between 1 and 80.4, and the overall conclusions are independent of D, so that the results at $D = 1.0$ will be discussed as being representative. In Fig. 4 is shown the variation in strain energy, U, calculated by MM for the minimum energy forms of cyclam·H$_n^{n+}$ and trien·H$_n^{n+}$ species as a function of n, from $n = 0$ to $n = 4$. For the trien series there is a fairly smooth increase in strain energy from $n = 0$ to $n = 4$. In contrast, for the cyclam series, there is an abrupt increase in strain energy from $n = 2$ to $n = 3$, as would be required to account for the very small value of pK_3. The species identified as being of minimum energy for cyclam, cyclam·H$_2^{2+}$, and cyclam·H$_3^{3+}$, are seen in Fig. 5. The minimum strain energy form of cyclam·H$_2^{2+}$ found by MM is the same as that found crystallographically.[16,17] The lowest strain energy form of cyclam·H$_4^{4+}$ is the type IV conformer in Fig. 3, and found here crystallographically (Fig. 2). Also shown in Fig. 5 is the minimum strain energy form of dien·H$_2^{2+}$, which is stabilized by internal hydrogen-bonding. The rapid increase in U for the cyclam H$_n^{n+}$ series at $n = 3$ is observed even for a dielectric constant of 80.4 where electrostatic effects are effectively nullified, so that a significant component of the very low third protonation constant of cyclam derives from the van der Waals repulsions caused by placing three protons within the cavity of the ligand. The minimum energy conformation for the macrocyclic ring of cyclam·H$_3^{3+}$ is not the same as that of cyclam·H$_4^{4+}$. One might have suspected initially (Fig. 5) that the conformations of the macrocyclic rings would be similar. However, with only three protons present, the cyclam·H$_3^{3+}$ cation is able to achieve a lower strain energy with a more folded conformation for its macrocyclic ring.

The results presented in this paper confirm the reversal in the normal sequence of protonation constants, such that pK_4 > pK_3 for cyclam. The crystal structure of cyclam·H$_4^{4+}$ is of the type I conformation, shown by MM calculation to be of the lowest energy. The MM calculations show a rapid rise in strain energy on addition of a third proton to cyclam to give cyclam·H$_3^{3+}$, so that the reversal in the usual order of protonation constants can be understood in terms of the difficulty in fitting a third proton into the cavity of cyclam, with both electrostatic repulsion and van der Waals repulsion
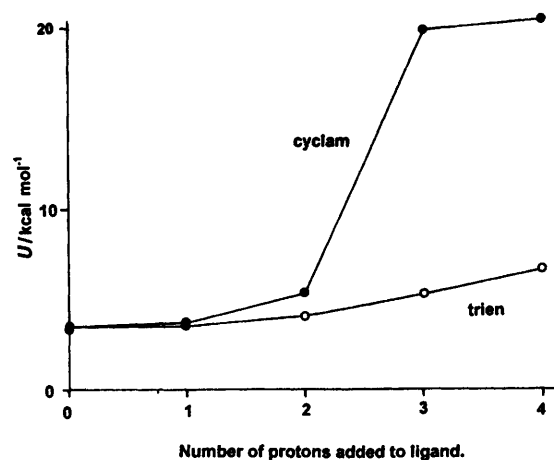

Number of protons added to ligand.

Fig. 4 The strain energy (U), calculated using the program SYBYL,[22] of the series cyclam·H$_n^{n+}$ and trien·H$_n^{n+}$ as a function of n, the number of protons on the ligand
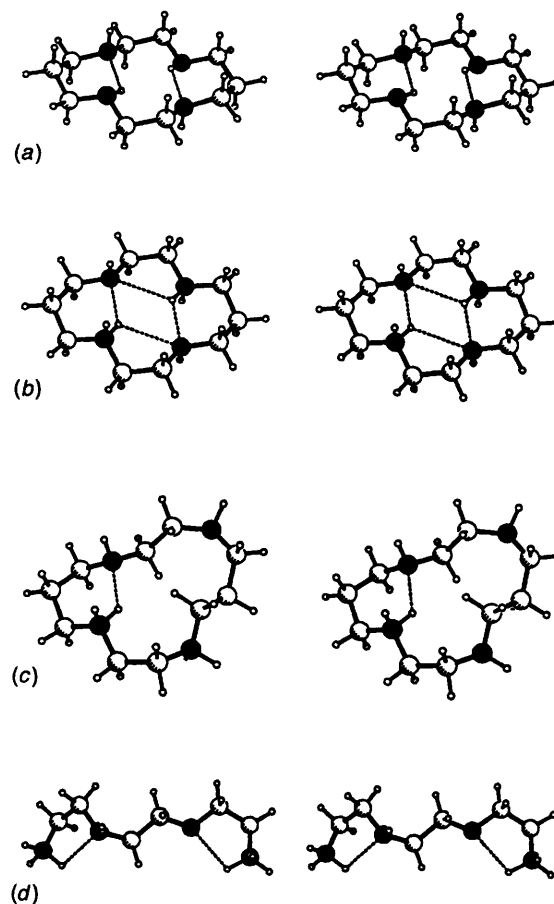

(a)

(b)

(c)

(d)

Fig. 5 The minimum energy conformers of cyclam, some protonated forms of cyclam, and of trien·2H$^+$, generated by molecular mechanics[22] calculation: (a) the free ligand cyclam; (b) cyclam·H$_2^{2+}$, which is the same as observed crystallographically;[16] (c) cyclam·H$_3^{3+}$ and (d) trien·H$_2^{2+}$

between the three protons in the cavity making this arrangement, if the macrocycle retains the same conformation as in[16,17] cyclam·H$_2^{2+}$, very high in energy.

Experimental

Synthesis of cyclam·4HClO$_4$·2H$_2$O

Cyclam (Aldrich) was dissolved in 0.5 M HClO$_4$, which slowly deposited crystals of cyclam·4HClO$_4$·2H$_2$O. Elemental analysis gave: C: 18.56; H, 5.13; N, 8.57%. C$_{10}$H$_{24}$N$_4$·(HClO$_4$)$_4$·2H$_2$O requires: C, 18.87; H, 5.07; N, 8.81%.

X-Ray crystallography

A colourless plate was mounted on a glass fibre at room temperature. Crystal density was measured by flotation in CHCl$_3$–acetone. Preliminary examination and data collection were performed on a Rigaku AFC5 (oriented graphite monochrometer; Mo-Kα radiation) at 163(2) K. Cell parameters were calculated from the least-squares fitting for 25 high-angle reflections ($2\theta > 15°$). Omega scans for several intense reflections indicated acceptable crystal quality. Data were collected for 5.16 to 50.10° 2θ. The scan width for data collection was $1.4 + 0.3$ tan (θ) in ω, with a variable scan rate between 4 and 16 deg min^{-1}. Weak reflections were rescanned (maximum of two rescans) and the counts for each scan were accumulated. The three standards, collected every 150 reflections, showed no significant trends. Background measurement was made by stationary crystal and stationary counter techniques at the beginning and the end of each scan for half the total scan time. Lorentz and polarization corrections[18] were applied to 2231 reflections. A total of 1840 unique observed reflections ($R_{int} = 0.0262$) were used in further calculations. A semiempirical absorption correction was applied.[19] The structure was solved by direct methods.[20] Full-matrix least-squares anisotropic refinement for all non-hydrogen atoms yielded $wR = 0.084$ and $R = 0.043$ at convergence.[21] Hydrogen atoms were placed in idealized positions with isotropic thermal parameters fixed at 0.08 Å.[3] Neutral atom scattering factors and anomalous scattering factors were taken from the *International Tables for X-Ray Crystallography*, Vol. C. The structure of cyclam·4HClO$_4$·2H$_2$O is seen in Fig. 2 and the crystal data are in Table 2. Atomic coordinates, bond lengths and angles, and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre.†

Molecular mechanics calculations

These were carried out using the program SYBYL[22] and the TAFF force field. The charges on the atoms in the calculations were generated by SYBYL using the method of Gasteiger and Marzilli.[15] Trial structures of the various conformers of protonated forms of cyclam were generated using the BUILD module of SYBYL. DYNAMICS runs were carried out to try to find conformations which represent the global minimum for the structures generated.

Trial structures of the cyclam·H$_4^{4+}$ and cyclam·H$_3^{3+}$ cations were generated with the model building facility of SYBYL. These corresponded to the conformations of types I through IV shown in Fig. 3 by cyclam·H$_4^{4+}$. For cyclam·H$_2^{2+}$ the experimentally observed[16] crystal structure was used as a trial structure. For cyclam·H$_3^{3+}$ trial conformers were that of cyclam·H$_2^{2+}$ with an extra proton added, as well as the conformers of cyclam·H$_4^{4+}$ with one proton removed. For trien·H$_2^{2+}$ the maximally extended 'zig-zag' structure found for the linear hydrocarbons was used as a trial structure. Molecular dynamics runs were carried out at room temperature with sampling every 5 fs for a total of 100 000 fs. The structures generated were stored in files, and the graphs of potential energy as a function of time were inspected for structures of low energy, which were then submitted to the molecular mechanics module of energy minimization. No other low energy conformations of the cyclam·H$_4^{4+}$ cations were found. For cyclam·H$_3^{3+}$ the rather irregular conformer shown in Fig. 5(c) was found as the lowest energy form.

Potentiometry

In order to avoid activity complications, the externally thermostatted and argon-protected potentiometric cell and

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† For details of the CCDC deposition scheme, see 'Instructions for Authors (1996),' *J. Chem. Soc., Perkin Trans. 2*, 1996, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 188/15.

Table 2 Crystal data for cyclam·4HClO$_4$·2H$_2$O

Empirical formula	C$_{10}$H$_{32}$Cl$_4$N$_4$O$_{18}$
Formula weight	638.2
Crystal colour and habit	Colourless plate
Crystal dimensions	0.39 × 0.34 × 0.13 mm
T	163(2) K
λ(Mo-Kα)	0.710 73 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
a	8.409(2) Å
b	15.815(3) Å
c	9.221(1) Å
β	105.99(1)°
V	1178.8(4) Å^3
Z	2
Density (calc.)	1.798 g cm^{-3}
Density (obs.)	1.79 g cm^{-3}
Transmission factors	T_{max} 0.999; T_{min} 0.828
Absorption coefficient	0.596 mm^{-1}
$F(000)$	664
θ Range for data collection	2.58–25.05°
Index ranges	$0 \leqslant h \leqslant 10, 0 \leqslant k \leqslant 18, -10 \leqslant l \leqslant 10$
Reflections collected	2231
Independent reflections	2083 [R(int) = 0.0262]
Observation criterion	$> 2\sigma(I)$
Absorption corrections	psi-scans
No. of LS parameters	164
Goodness-of-fit (observed data)	$S = 1.0720$
Goodness-of-fit (all data)	$S = 1.0610$
Final R indices	$R^a = 0.0374, wR^b = 0.0912$
R indices (all data)	$R^a = 0.0465, wR^b = 0.1218$
Largest e-density, peak and hole	0.463 and −0.413

^{a} $R = \Sigma ||F_o| - |F_c||/\Sigma|F_o|$. ^{b} $wR = [\Sigma w(F_o^2 - F_c^2)^2]^{\frac{1}{2}}$. $w^{-1} = \sigma^2 F^2 + [x(F_o^2 + 2F_c^2)/3]^2 + y(F_o^2 + 2F_c^2)/3$.

electrode assembly was calibrated in terms of pH $= -\log[H^+]$ with dilute standard HCl, 25.0 °C, $\mu = 0.100$ (KCl). The linearity of the electrode response was assured in the acid range with a check titration of the 50.0 ml calibration solution using standard CO$_2$-free KOH (0.0986 M). From the starting pH of just above 2 to near the break at just above pH 3, the calculated points were found to agree within ±0.001 pH unit. Furthermore, a Gran's plot ensured a CO$_2$-free environment. The pK_w employed was[1] the standard 13.78 valid under these conditions. The calibration solution was replaced with a 50.00 ml test solution containing 21.8 mg (0.11 mmol) of Aldrich cyclam free base, 0.100 M KCl, to which were added 5.000 ml 0.0960 M standard HCl. After 2 h, the ligand was titrated incrementally until 54 equally spaced data points had been collected spanning the pH range 2.3–11.08. During the course of the refinement of the data with program BEST,[14] four protonation constants were calculated, as well as the molecular weight of the free base. Calculated for C$_{10}$H$_{24}$N$_4$: 200; found: 199. Since the particular calibration employed in this work focuses on the pH region near 2, the two lowest protonation constants obtained should be considered as trustworthy as could be obtained considering the fact that as the titration commences, the species H$_3$L^{3+} starts at 20% and diminishes to about 4% by pH 3 while the species H$_4$L^{4+} starts at only 10% and goes to 0% by pH 3 when 22 data points have already been collected.

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