ORIGINAL ARTICLE

^{13}C longitudinal relaxation time measurements and DFT-GIAO NMR computations for two ammonium ions of a tetraazamacrocyclic *scorpiand* system

Ryszard B. Nazarski

Abstract Spin–lattice relaxation times, T_1s, for ^{13}C nuclei in two cations $H_n\mathbf{1}^{n+}$ ($n = 1$, 5) of N-(2-amino-ethyl)-cyclam (**1**, *scorpiand*) were determined by means of ^{13}C{^{1}H} NMR experiments in aqueous solution at pH 11.5 and 0.2. The theoretical study [modeling with OPLS-AA, B3LYP/6-31G(d) geometry optimizations, dispersion-corrected energies (DFT-D3), and DFT-GIAO predictions of the NMR chemical shifts (including an IEF-PCM simulation of hydration)] was also done for several conformers of the tautomer *iso*-$H_4\mathbf{1}^{4+}$ not investigated before. The binding directions in protonated polyamino receptors necessary for efficient complexation of the nitrate anion(s) were briefly outlined, as well. All these results were discussed in terms of 'abnormal' ^{13}C chemical shift changes found previously for the side-chain carbons of amine **1** in strongly acidic solution (HNO_3). In conclusion, an earlier proposal of its association with NO_3^- at pH <1 was rejected. Instead, the participation of small amounts of a micro-species *iso*-$H_4\mathbf{1}^{4+}\mathbf{D}_{hydr}$ under such conditions can be proposed.

Keywords 'Wrong-way' protonation shift · Amino-pendant cyclams · NMR pH-titration · Protonated polyamines · Nitrate receptors · Dipolar relaxation · OPLS-AA force field · DFT-D3 dispersion correction

Physical image versus molecular structure relation, Part 16. For Part 15, see Ref. [11].

R. B. Nazarski (✉)
Laboratory of Molecular Spectroscopy, Faculty of Chemistry, University of Łódź, ul. Tamka 12, 91-403 Łódź, Poland
e-mail: nazarski@uni.lodz.pl

Introduction

While NMR chemical shifts δ_Xs (where X = C, H, etc.) and coupling constants J_{AB} belong to the most powerful tools available for resolution of various structural issues about organic systems, an increasing interest in the ^{13}C spin–lattice (longitudinal) relaxation time T_1 (hereafter referred to as ^{13}C SLR and ^{13}C T_1) is continually observed. Because such relaxation data vary from milliseconds in macromolecules to several minutes in small objects, the ^{13}C-T_1 value has become an additional spectral parameter of importance to the chemist. Indeed, together with nuclear Overhauser effects arising from ^{1}H decoupling of ^{13}C NMR spectra, the T_1 values of ^{13}C nuclei permit to draw valuable conclusions about SLR mechanisms operative for individual carbon atoms in different (bio)organic systems [1–5]. It follows that they reflect both the inter- and intramolecular mobility of these entities, and so excellently complement the results on their dynamics coming from other NMR techniques such as, e.g., variable-temperature experiments. Hence, ^{13}C T_1s provide a reliable help in the case of some structural problems very difficult (if at all) to solve by use of more conventional methods of an NMR spectroscopy.

In our last study on macrocyclic ligands [6],[1] the overall composite conformations of some protonated forms of a polyamine **1**, i.e., 1-(2-aminoethyl)-1,4,8,11-tetraazacyclo-tetradecane commonly called *scorpiand*, were proposed on the basis of its earlier ^{13}C NMR pH-titration with nitric

---

[1] A certain erroneous explanation given in Ref. [6] about atoms C3–C7 and the N^3 site in two cations $H_n\mathbf{1}^{n+}$ was corrected in the Electronic Supplementary Material (p. S7).

acid [7]. These spectroscopic data were analyzed in the light of GIAO (gauge-independent atomic orbitals) [8 and refs therein, 9] based predictions of δ_Cs made for numerous ammonium ions H_n1^{n+} coexisting in aqueous media. Among other issues, we tried to explain an origin of downfield changes in δ_Cs unexpectedly observed, for atoms C11 and C12 in the N-pendant-arm unit of amine 1 below pH $\sim$3.5 [7], see Fig. S1 in the Electronic Supplementary Material; an atom numbering used here is given in the Formula. As a result, the close proximity of these carbons to adjacent cationic sites at N^1 and N^5 in H_51^{5+} was suggested as one of the possibilities leading to such 'wrong-way' ('abnormally' directed) amino-protonation ^{13}C NMR shifts [6]. In fact, an arrangement of the foregoing N atoms in H_51^{5+} would make possible, in principle, electrostatic and/or H-bonding-type attractive interactions of these cationic centers with a single nitrate anion persisting in a close vicinity of C11/C12. This kind of N^+–H$\cdots$O$^-$–N interactions giving rise to the formation of ion pairs with NO_3^- was reported for $^+H_3NCH_2CH_2NH_3^+$ [10]. After all, it was finally concluded that the second, 'structural' rationalization of the observed ^{13}C trends is perhaps more reliable.

1
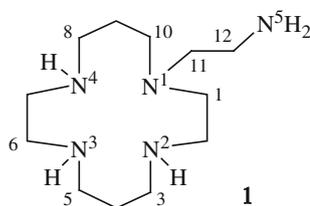
Indeed, these intriguing ^{13}C NMR chemical shift changes were satisfying reproduced in the time-averaged δ_Cs found for GIAO-supported overall shapes of the three subsequently formed polyammoniums H_n1^{n+} ($n = 3$–5) [6 and refs therein]. The composite conformations of these macrocyclic ions were found, however, in a non-standard statistical analysis of the δ_C sets predicted for their unique promising forms. In turn, these conformers were chosen based just on the best agreement of so-computed δ_Cs with the experimental δ_C values. But, according to our recent work [11], large caution must be taken in interpretations of all ^{13}C NMR data-based results on the shapes of molecules being in dynamic equilibrium between more than two distinct forms easily feasible energetically. Because it was also the case of the title ions H_41^{4+} (with non-ionized N^1) [7, 12] and H_51^{5+} existing as ensembles of several fast-interconverting forms [6, 13], the three explanations of 'anomalous' NMR shifts in question should be considered in details (vide infra).

Thus, it became clear that the additional findings, both experimental and theoretical, on some protonated micro-species of the title system 1 were necessary.

Accordingly, two sets of ^{13}C-T_1 times concerning internal dynamics in its 'boundary' ammonium cations H_n1^{n+} ($n = 1$, 5) were determined. In addition, the scarce literature ^{13}C SLR data about pendant-armed tetraaza crowns 2–5 were discussed in the light of current findings on these two ionic scorpiand species. Moreover, several low-energy conformers of the tautomeric cation iso-H_41^{4+} not analyzed before were modeled, initially with the OPLS-AA [14–17] force field and finally at the DFT level, by applying the equilibrium solvation [18] within an IEF-PCM approach [19–23]). All these results were taken into account in a renewed discussion on the origin of 'abnormal' NMR trends mentioned above. To the best of our knowledge, this is the first use of such T_1 data for structural analysis of the protonated states of tetraazamacrocycles. Only the ^{13}C-T_1 based part of this work was presented in a very preliminary form [24].

Results and discussion

Possibilities of H-bonding between cations H_n1^{n+} ($n = 4$, 5) and nitrate anion versus 'wrong-way' evolutions in NMR chemical shifts

It was obvious that host–guest interactions N^+–H$\cdots$O$^-$–N typical of H-bond based polyammonium receptors[2] acting as hard acids versus NO_3^- as a hard Lewis base could be ruled out for the macrocyclic amine 1, because of too small size of its intramolecular hole. Such polyaza hosts (strictly, their protonated states) showing good selectivity towards nitrate are 18- to 24-membered aza [26, 27] or oxaza crowns [28–30]. This monovalent feeble coordinating trigonal oxoanion with poor basicity offers six geometrically preferred H-bond acceptor sites according to the number and spatial arrangement of its oxygen's lone-pair orbitals; slightly unfavorable H-bonds with the softer π-electrons are also possible [31, 32 and refs therein, 33, 34 and refs therein]. In fact, there is an extensive hydration shell around NO_3^- in water [35 and refs therein] as a hard H-bonding Lewis acid [36]. Hence only specially designed macrocyclic ionophores encapsulate this anion in the aforementioned directions, by using the N–H groups in their binding pockets as strong H-bond donors [37–39]. A C_3-symmetric environment in the host was found especially favorable for the NO_3^- binding [37, 38, 40 and refs therein], but this intracavity orientation is not achieved for the majority of such hosts, mainly due to steric hindrance. Indeed, any strong receptor-substrate interactions result

---

[2] For an excellent review in the field of polyammoniums, see e.g., Ref. [25].

from the complementary stereoelectronic arrangement of binding sites in the host and guest [31, 37]. As a result, only half of the six preferred sites in NO_3^- are usually occupied and these enable the two specific H-bonding modes involving all three or only two of its oxygens [32 and refs therein]. Similar molecular-level interactions were found very recently in the crystal structure of $CH_3CH_2NH_3^+NO_3^-$ [41].

It is also true, that while protonated polyaza macrocycles with large internal cavities can enfolded [28, 42, 43] or even encapsulated [28, 44] nitrate(s), most of the single-crystal X-ray results on such systems showed layered structures with NO_3^- hovering above and below the mean planes of relatively flat receptors [26–28, 40, 42, 43]. Just such spatial arrangement was only considered for $H_4\mathbf{1}^{4+}$ most likely existing in the pH range 1-4 [6 and refs therein], which would make potentially possible H-bonds with the NO_3^- oxygens. The fourth protonation of $\mathbf{1}$ occurring at N^3 [7, 12] give rise to the formation of an 'extended' all-out conformation of its macrocyclic unit, which most likely adopts a virtually planar macroring system, with all exocyclic N^+-H bonds in an out configuration defined by Park and Simmons [45]. An outside orientation of the ring NH_2^+ groups was found for several polyammoniums of this type [6, 46–48]; see also Figs. 2, S3, and S4. Hence, a relatively rigid H-bond donor system $N^2/N^3/N^4/N^5$ can be considered for $H_4\mathbf{1}^{4+}$. But, only its cationic site at N^5 would be capable to interact with one discrete NO_3^- ion, due to unfavorable N^+-H bond directions at other N^+ sites. Instead, a dual H-bond donation was likely for two neighboring cationic centers at N^1 and N^5 in $H_5\mathbf{1}^{5+}$. So, it was only possible to think about both these ions (especially, the latter one) as entities potentially engaged in H-bonds of the type $N–H\cdots O^-–N$ leading to the formation of supermolecules $[H_4\mathbf{1}][NO_3]^{3+}$ and, particularly, $[H_5\mathbf{1}][NO_3]^{4+}$ as weak 1:1 nitrate associates (ion pairs).
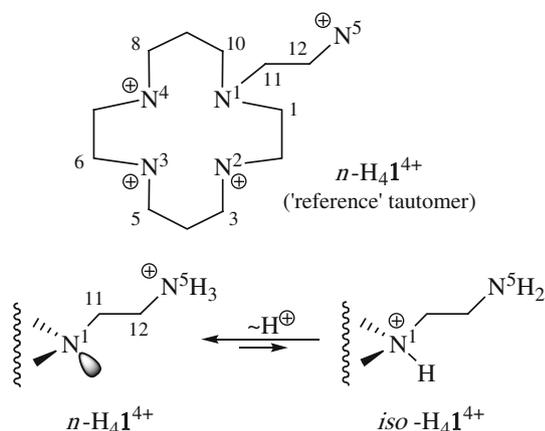
On the other hand, one could discuss about two other events affecting the protonated states of amine $\mathbf{1}$, namely, (i) supporting H-bonding of type $C–H\cdots O^-–N$ found in some crystal structures [49 and refs therein] as an equivalent of interactions $C–H\cdots O^-–X$ (where $X = C$ or P) known from NMR pH-titrations of some biomolecules in aqueous media [50, 51 and refs therein]. Its presence causes 'wrong-way' changes in the δ_H and δ_P data upon protonation to a higher and lower magnetic field, respectively. This phenomenon is perhaps electrostatic in origin and operates through the field. It was recognized as occurring internally, when a highly negatively charged group approaches the CH hydrogen(s) [51].

Moreover, there is the possibility of partial transfer of an electronic charge from N^5 to N^1 of a normal ion n-$H_4\mathbf{1}^{4+}$ with the formation of its isomeric species iso-$H_4\mathbf{1}^{4+}$ (Fig. 1) as a third (ii) explanation of 'abnormal' ^{13}C NMR trends in question. Similar 'wrong-way' evolution in chemical shifts is also seen in ^{15}N NMR pH-titration of unsymmetrical linear pentamines, and is explained just by equilibrium in the protonation of more than one N atom (charge delocalization) [52].

The ^{13}C relaxation times T_1 for cations $H\mathbf{1}^+$ and $H_5\mathbf{1}^{5+}$

In order to receive a more certain answer to the question about an origin of the 'wrong-way' NMR shifts mentioned above, two series of SLR times T_1 of ^{13}C-nuclei in the 14-membered macroring polyamine system $\mathbf{1}$ were evaluated for its aqueous solution at two pH values (11.5 and 0.2). A dedicated proton-decoupled ^{13}C NMR-T_1 approach

Fig. 1 An intramolecular rearrangement possible for the tetraprotonated form of amine $\mathbf{1}$

Table 1 Experimental ^{13}C longitudinal relaxation times, T_1s, determined for the atoms C1–C12 in the ions $H\mathbf{1}^+$ and $H_5\mathbf{1}^{5+}$, s [a]

Carbon no.[b]	$H\mathbf{2}^+$ [pH 11.5]	$H_5\mathbf{2}^{5+}$ [pH 0.2]
1	0.46 (2)	0.29 (2)
2	0.42 (1_5)	0.27 (2)
3	0.45 (2)	nd[c]
4	0.42 (2)	0.38 (4)
5	0.49 (3)	0.29_5 (1_5)
6	0.40 (1)	0.32 (3)
7	0.41 (2)	0.30 (2)
8	0.41 (1)	nd[c]
9	0.39 (2)	0.36 (4)
10	0.47 (2)	0.32 (2)
11	0.49 (4)	0.38_5 (4_0)
12	0.65 (3)	0.67 (6)

[a] Values given in the parenthesis are ± errors in the last significant figure

[b] For atom numbering see Fig. 1

[c] Not determined due to practical overlapping the ^{13}C NMR lines coming from C3 and C8

and exponential data analysis were used; see Methods. The T_1s determined in this way for all well-resolved ^{13}C lines originating from 12 or 10 nonequivalent C sites in the mono- and pentaprotonated form of **1**, i.e., ions H**1**$^+$ (with a protonated atom N^2) [7, 12] and H$_5$**1**$^{5+}$, respectively, are listed in Table 1. These relaxation data afforded valuable information on the molecular mobility of both these species, which were assumed as two ammonium cations mainly existing under such conditions. However, some contribution of the entity H$_4$**1**$^{4+}$ not fully protonated even at pH 0.2 can be inferred from the nonzero slope of ^{13}C NMR pH-titration profiles of **1** in the pH range 0.2–1.0 [6, 7], strongly suggesting not complete protonation. In fact, an exhaustive protonation can be difficult to achievement in some cases due to an unfavorable build-up of positive charges in the macrocycle, especially if its cavity is small [53]. Such an incomplete ionization was found for several aza- and oxaza crowns [54–56].
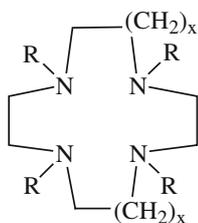
2 x = 1, R = CH$_2$CH$_2$OH
3 x = 2, R = H
4 x = 2, R = CO(CH$_2$)$_4$CH$_3$
5 x = 2, R = SO$_2$C$_{10}$H$_7$

In any NMR experiment, irradiated nuclei transfer their excess spin energy to the surrounding in a process of spin–lattice relaxation (SLR), which rate R_1 can be expressed by the sum of pertinent reciprocal relaxation times T_1^{-1} employing Eq. 1 [1–5].

$$R_1 = 1/T_1 = 1/T_{1,DD} + 1/T_{1,other} \quad (1)$$

Of the four mechanisms of ^{13}C SLR possible for isotropic solutions of diamagnetic systems with typical (spin $I = \frac{1}{2}$) NMR nuclei [dipolar (dipole–dipole, DD), spin-rotation, chemical-shift anisotropy, and scalar coupling], we can expect that an intramolecular DD relaxation provides the dominant effect for pentamine **1** because each of its C atoms carries two attached protons. Indeed, an overwhelming predominance of this mechanism for CH$_2$ carbons in structurally close macrocyclic tetramines **2–5** [57, 58] and polyethers [59] was previously found, by measuring the ^{13}C-{^{1}H} Overhauser enhancement factors ($\eta_{obsd} + 1$) and calculating the purely DD contributions to relevant ^{13}C T_1s, by using Eq. (2) (where $\eta_{max} = \gamma_H/2\gamma_C = 1.988$).

$$T_{1,DD} = T_1(\eta_{max}/\eta_{obsd}) \quad (2)$$

For such molecules rapidly reorienting isotropically in a liquid phase (solution or neat) under ^{1}H decoupling and 'extreme narrowing limit' conditions, the DD relaxation rate of the ^{13}C nucleus i is very well approximated by Eq. 3

$$(1/T_{1,DD})_i = N\hbar^2\gamma_C^2\gamma_H^2 r_{ij}^{-6}\tau_{c,eff} = \text{constant} \times N\tau_{c,eff} \quad (3)$$

in which $\hbar$ is reduced Planck's constant ($\equiv h/2\pi$), γ's are the gyromagnetic ratios of ^{13}C and ^{1}H, τ_c is the molecular correlation time, r_{ij} is an effective C$_i$-H$_j$ internuclear distance (~ 1.09 Å), and N is the number of adjacent protons j, because contributions to ^{13}C SLR from the other protons are practically negligible, due to the r^{-6} dependence [1–3, 5, 60]. But, such an overall tumbling cannot easily by resolved into its components (translation, vibration, rotation) and the average time taken between two reorientations is defined as an effective correlation time τ_c. The proportionality $1/T_{1,DD} \propto \tau_c$ is expressed in a rule *the faster a molecule, the longer is T_1 (and shorter τ_c),* as all carbons within a given system move at the same rate. Most of the nonviscous small and medium-sized rigid objects fulfils this condition. However, conformationally flexible systems are usually anisotropic in their tumbling and related $\tau_{c,eff}$s can be different for each of their C atoms. The NT_1 value is then no longer a constant, but inversely proportional to $\tau_{c,eff}$ (Eq. 4)

$$(NT_1)_i \propto (1/\tau_{c,eff})_i \quad (4)$$

and this quantity can be interpreted as an internal mobility parameter, although only qualitatively and with caution [5]. Indeed, besides an overall tumbling, the flexible molecules (such the system **1**) may have many modes of internal mobility, e.g., segmental dynamics along a side arm or conformational macroring inversions. Each of these motions modulate the DD interaction between coupled nuclei.

From the foregoing, it follows that the calculated $\tau_{c,eff}$ or NT_1 data are generally considered as reliable measures of both the *mobility* [of the whole molecule (overall tumbling) and/or its sub-units (segmental mobility)] and the *ordering* [1–5, 60]. In our case, all numerical ^{13}C T_1 values found for two ions H$_n$**1**$^{n+}$ can be directly compared, because only CH$_2$ groups exist in these species ($N = 2$). Hence, the gross consideration of measured SLR data was applied as completely sufficient for the purpose of our analysis. Moreover, their overall description exclusively in terms of a DD mechanism appears appropriate. The same approach was used in the work [57].

In contrast to the ^{13}C-T_1 results of Wyrwał et al. [58] on non-protonated systems of *cyclam* (**3**) and its two derivatives **4** and **5**, where all macroring backbone carbons can be

treated as dynamically equivalent in CDCl$_3$ solution, analogous atoms in both unsymmetrical ions H$_n$1^{n+} ($n = 1$ or 5) studied here in water are rather diverse in this respect, especially in strongly acidic medium. Generally, the magnitudes of related ^{13}C-T_1 values found for these two ions are between those reported for azacrowns **3** and **4** [58], whereas the shortening of such data for H$_5$1^{5+} relative to H1$^+$ indicates a slower overall tumbling of the former one.

As one can easily see, the mobility of CH$_2$ groups in pendant-arms $^{\alpha}$CH$_2^{\beta}$CH$_2$NH$_2$ and $^{\alpha}$CH$_2^{\beta}$CH$_2$NH$_3^+$ of these ions increases with an increasing distance from the macrocycle center (T_1s becomes longer, Table 1). In both cases, T_1s estimated for α-Cs are equal to the greatest value found for ring carbons, whereas these parameters for β-Cs are identical within the error limits ($\sim$0.66 s) and, simultaneously, they are the longest ones among all of these relaxation rates. Side-chain segmental motion was apparent by the lengthening T_1s along both aminoalkyl groups toward their $^{\beta}$CH$_2$N terminus. A pronounced degree of such motion, typical for open-chains, was also reported for the side arms of **2** and **4** [57, 58]. Indeed, the mean T_1 values for macroring carbons in two ions H$_n$1^{n+} (of 0.43 and 0.32 s for $n = 1$ and 5, respectively) can be expected to approximate the overall T_1s of these species. It was obvious that greater $^1T_1$s found for all four N-pendant-armed systems mentioned above are due to an added internal motion, i.e., an enhanced segmental freedom of their side chains.

The T_1 value of 0.67 ± 0.06 s, i.e., 2.0 × $\sim$0.32 s (estimated for the ring), found for C12 in H$_5$1^{5+} is greater than $\sim$0.48 s predicted from simple comparison with related data for the more mobile H1$^+$ (vide supra). However, this T_1(β-C)/T_1(ring) $\cong$ 2.0 is fully consistent with the analogous T_1/T_1 ratio of 2.25 found in D$_2$O solution for the $^{\alpha}$CH$_2^{\beta}$CH$_2$OH unit of **2** [57]. Moreover, our results indicate much faster internal rotation of the β-CH$_2$ group in **1** at pH 0.2, in agreement with an enhanced mobility awaited for this site in the $^{\alpha}$CH$_2^{\beta}$CH$_2$NH$_3^+$ unit solvated by ion–dipole interactions in strongly polar aqueous solution [60].

The conformational flexibility of an internal hole of H1$^+$ evaluated in this manner is in good agreement with the average experimental vicinal interproton coupling $^3J_{HH}$ of $\sim$5.3 Hz. This J-value, typical of rapidly interconverting cyclic systems, was estimated in a first-order analysis of ring proton multiplets appeared in the 500 MHz ^{1}H NMR spectrum of **1** recorded at pH 11.5 [12]. On the other hand, intramolecular H-bonds to adjacent ring nitrogens (or even being in a dynamic H$^+$-exchange between two such atoms, NH···H$^+$···HN) [61] are highly probable at this protonation state. Consequently, an internal fluctuation of CH$_2$ protons in the macrocyclic backbone of H1$^+$ is always slower than the mobility of such protons in its side-chain.

Similar situation, reflected by comparable magnitudes of ^{13}C T_1s or substantial line broadening of ^{1}H NMR signals, was also reported for other N-pendant-armed azacrowns [58, 62].

In turn, relative small mobility of **1** in its strongly acidic solution is in line with similar observations made for other polyhetero macrocycles, which usually are preorganized structures with specific segmental conformations. To bind metal cations or protons they may change the shape of each ring segment, thereby reducing the T_1s [59 and refs therein]. A low mobility of H$_5$1^{5+} most likely results from strong distance-dependent Coulombic-type repulsions between four positively charged ammonium sites at N^1–N^4 as electrostatic solute ordering effects, which 'fix' its macrocyclic core in a maximally 'extended' form adopting an all-out conformation with ring N-atoms occupying four corners of the molecular polygon and N$^+$-H bonds directed toward the outward of an internal cavity (vide supra). An additional 'ordering' can results from interactions between ring cationic sites and their counter ions or solvent shell of an aqueous surrounding. All such phenomena have a strong effect on the τ_c value [63].

The aminoalkyl side chain of the monoprotonated base, H1$^+$, was recognized previously as its highly mobile fragment. Indeed, the 'medium' coupling $^3J_{HH} \sim$ 7.1 Hz, a signature for the fast conformational interconversion [64], was estimated at pH 11.5 [12]. In other words, there is a typical 'freely' rotating ethane unit [65]. A practical equivalence of T_1s found for terminal atoms C12 in pendant arms of two discussed ions of **1** indicates that the analogously fast rotation also occurs around the single bond CH$_2$-CH$_2$NH$_3^+$ in H$_5$1^{5+}. Obviously, similar mobility of β-CH$_2$ groups in both these species suggests similar solute–solvent interactions of their outer side chains with an aqueous environment. For important implications of this conclusion, see below.

As has already been mentioned, the 'wrong-way' ^{13}C NMR pH-titration shifts found for **1** at pH <3.5 were reproduced quite well by δ$_C$s predicted for effective overall (population-weighted averaged) shapes of the main forms of cations H$_n$1^{n+} ($n = 3$–5) coexisting in an acidic medium [6]. The proposed multicomponent conformations of these composite shapes called H$_3$1^{3+}**ABCD**, n-H$_4$1^{4+}**BC** and H$_5$1^{5+}**ABCD** were, in turn, elucidated by the best fitting measured δ$_C$s to pertinent theoretical δ$_C$ data computed by the GIAO B3LYP/6-31G(d) method. Strictly, the NMR shift of a given C atom, for all of these overall structures, was obtained as a weighted average δ$_C$ value of the same atom in a few preselected forms sampled by a conformational search at the DFT level. For that reason, the whole analysis was a little arbitrary, but it was only one approach possible at this research stage. Nonetheless, in view of the present ^{13}C-T_1 results on internal dynamics in H1$^+$ and

$H_5\mathbf{1}^{5+}$, one can accept that an intermolecular H-bond of type $N^+\!-H\cdots O^-\!-N$ (hypothetically considered before [6], in particular for $H_5\mathbf{1}^{5+}$) does not operate in aqueous solution. Without any doubt, such nitrate complexation, giving rise to the formation of a supermolecule $[H_5\mathbf{1}][NO_3]^{4+}$, would substantially enforce the rigidity of the pendant-arm unit in $H_5\mathbf{1}^{5+}$. However, the anticipated [57, 66 and refs therein] slowdown of internal dynamics of its two constituent CH_2 groups rooted by H-bonding mentioned above, was not found.

The above conclusion is consistent with other considerations. Indeed, a close inspection of low-energy forms of $H_4\mathbf{1}^{4+}$ and, especially, $H_5\mathbf{1}^{5+}$, which were recognized as contributing to their composite shapes $H_4\mathbf{1}^{4+}\mathbf{BC}$ and $H_5\mathbf{1}^{5+}\mathbf{ABCD}$ [6], indicates that ammonium sites in these protonation states of $\mathbf{1}$ do not fulfill the highly specific spatial requirements of the interactions $N^+\!-H\cdots O^-\!-N$ necessary for efficient complexation of nitrate ion (vide supra). Moreover, the supporting H-bonds $C\!-\!H\cdots O^-\!-N$ are not possible.

Prediction of NMR spectra for the tautomer iso-$H_4\mathbf{1}^{4+}$

Amines characteristically exhibit small upfield or even weak downfield protonation shifts for the C atoms α to N atoms and mostly large high field shifts for β-carbons, in ^{13}C NMR spectra [12, 67 and refs therein]. During the protonation of N^1 as a weakest basis center in pentamine $\mathbf{1}$, two β-carbons in the ring, i.e., C2 and C9, show typical upfield changes at pH <1.5 while side-chain atoms C12 and especially C11 behave abnormally [7] (Fig. S1). According to all foregoing facts, a prototropic rearrangement shown in Fig. 1 would excellently rationalize these 'abnormal' trends observed. Indeed, deprotonation of some N atoms, at the expense of protonation of others in close enough proximity and accompanied by differently directed ^{13}C NMR shifts, was reported for both open-chain [68, 69] and macrocyclic [67, 70–72] polyamines. Such type 'wrong-way' protonation effects in the multinuclear NMR pH-titrations were sporadically reported for a great variety of small to large molecules possessing basic sites [73 and refs therein].

Thus, several conformers of a tautomeric ion iso-$H_4\mathbf{1}^{4+}$ not studied to date, with the protonated N^1–N^4, were generated applying the OPLS-AA [14–17] force field successfully used previously for normal ions n-$H_n\mathbf{1}^{n+}$ [6]. The resulting models of iso-$H_4\mathbf{1}^{4+}$ (Table S1) were refined in further quantum–mechanical DFT-level calculations, involving an IEF-PCM hydration simulation, evaluation of DFT-D3 [74] corrected energies, and GIAO-based predictions of NMR spectra (Methods). Because standard density functionals do not describe correctly the intramolecular electron-correlation interactions attributed to van der Waals dispersion forces [74, 75],[3] the adequate DFT-D3 corrections to DFT energies (more precisely, related $\Delta G^o_{298.15}$ data)
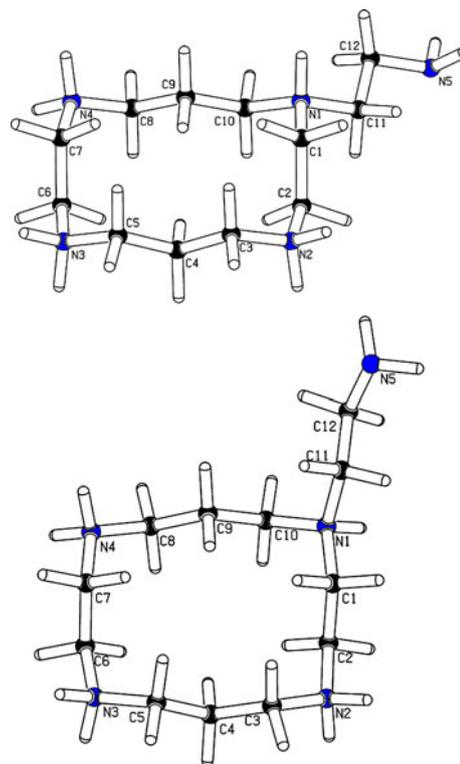

Fig. 2 PLATON views of two B3LYP/6-31G(d)-optimized lowest energy 'hydrated' forms **A** (*top*) and **D** (*bottom*) of iso-$H_4\mathbf{1}^{4+}$; all N atoms are shown in *blue*

were also evaluated for final B3LYP/6-31(d)-optimized structures; similar approach was used in two recent papers [11, 76]. All important results found in this way for the low-energy forms **A**–**D** of iso-$H_4\mathbf{1}^{4+}$ are given in Table S3.

The aforementioned conformers of iso-$H_4\mathbf{1}^{4+}$ were recognized as species of higher energy than related forms of n-$H_4\mathbf{1}^{4+}$ attained in predominant protonation of atoms N^2–N^5. These forms of iso-$H_4\mathbf{1}^{4+}$ with an all-out topology of N–H bonds attached to ring nitrogens were found similar to those established for $H_5\mathbf{1}^{5+}$ [46–48]. But, strongly elongated bond C11–N^1 of $\sim$1.585 Å, shorted bond C12–N^5 ($\sim$1.442$_5$ Å), and slightly flattened amino site at N^5 were unexpectedly found for its lowest-energy form **A** with the outer unit –$CH_2CH_2N^5H_2$ in an equatorial position (Fig. 2). Analogous geometry of the axially oriented side-chain R was found also for iso-$H_4\mathbf{1}^{4+}\mathbf{B}$ ($\Delta E_{tot} = 2.47$ kJ mol^{-1}, $\Delta G^o_{298.15} = 1.81$ kJ mol^{-1}) and two forms **C** and **D** with R_{eq} and R_{ax}, respectively (Table S3). An increase in pyramidality at N^5 on going from **A** to **D**, expressed by the sum of valence angles around this nitrogen,[4] was also remarkable. However, all these results on iso-$H_4\mathbf{1}^{4+}$ were

---

[3] For other papers concerning this topic, see e.g., note 45 in Ref. [11].

[4] Such an approach was used inter alia in Ref. [77].

predicted for a physically unreal case of isolated polyammoniums in the gas phase at 0 K, while experimental data were determined for their strongly polar aqueous solutions at ~294 K. Indeed, various effects of crucial importance such as interactions with counterions, solvation, thermal effects, etc. were completely ignored in this standard approximation 'of the free-molecule'.

Consequently, simulations of an impact of water molecules on the shape of *iso*-$H_4\mathbf{1}^{4+}$ were undertaken, by using an improved IEF-PCM protocol (Methods). As expected, its abnormal gas-phase geometry strongly changed after such 'dissolution' in water. The resulted forms $\mathbf{A_{hydr}}$-$\mathbf{D_{hydr}}$, of *iso*-$H_4\mathbf{1}^{4+}$ possess all above bonds of normal length (Table S3). A large relaxation of their geometry around the $C11$–N^1 bond is noteworthy, in particular.

Obviously, much more important were NMR properties of such constructed conformers of *iso*-$H_4\mathbf{1}^{4+}$. Thus, four pairs (*iso*-$H_4\mathbf{1}^{4+}$, *n*-$H_4\mathbf{1}^{4+}$) of the structurally close ions were considered in order to compute differences in δ_Cs arising from the change *n*-$H_4\mathbf{1}^{4+} \rightarrow$ *iso*-$H_4\mathbf{1}^{4+}$. All forms $\mathbf{A}$–$\mathbf{D}$ of *iso*-$H_4\mathbf{1}^{4+}$ were found as transforming themselves into related conformers $\mathbf{N1}$ and $\mathbf{N2}$ of *n*-$H_4\mathbf{1}^{4+}$ (for their 'hydrated' states, see Figs. S3 and S4) used as 'reference' systems with the same ring geometry, i.e., with protonated N^1 and unprotonated N^5 (Table S2). As a result, two narrow intervals $\Delta\delta_C^{calc}$ of $+(13.9$–$15.5)$ and $-(2.2$–$3.2)$ ppm were in vacuo GIAO-predicted for the atoms $C11$ and $C12$, respectively. This trend was in qualitative agreement with an alteration of $+0.90$ and -0.34 ppm found experimentally for the pH change from 1.02 to 0.24 [6 and refs therein]. But, analogous B3LYP/6-31G(*d*) IEF-PCM (H$_2$O) NMR predictions on 'hydrated' forms $\mathbf{A}$–$\mathbf{D}$, $\Delta\delta_C^{calc}$ of $+(6.6$–$9.8)$ and $-(1.9$–$3.2)$ ppm, were in much better conformity with the experiment, particularly in the magnitude of the trend (Table S3). An impact of the geometry relaxation around $C11$ is evident. In particular, this concerns the thermodynamically preferred forms $\mathbf{A_{hydr}}$ and, especially, $\mathbf{D_{hydr}}$ with the DFT-D3 corrected $\Delta G_{298.15}^\circ$ of 1.4 and 0.0 kJ mol^{-1}, respectively.

Interestingly, both conformers $\mathbf{N1}$ and $\mathbf{N2}$ of $H_4\mathbf{1}^{4+}$ were previously recognized as forms most favored in the gas-phase [6], but they were not proposed finally as existing in the real aqueous medium on the basis of a '*solution environment* (i.e., NMR spectroscopic) *match criterion*' [6, 11, 78]. Indeed, several forms of some multi-component systems initially located as their global energy minima were occasionally not recognized in solutions, by using typical GIAO-supported approaches [6, 78–80]. The majority of discrepancies of this kind was usually explained by specific solute–solvent effects only seldom adequately taken into account in the computational treatment in normal use. Our recent results on multi-conformer mixtures [11] and the present findings on $H_n\mathbf{1}^{n+}$ permit to be skeptical a bit about the quantitative reliability of

current standard experimental versus computational NMR-data-based protocols for some flexible systems, especially those for which only δ_Cs are used in their conformational analysis. For instance, a presumable uncertainty of such labor-consuming evaluations of the compositions of equilibrium mixtures of different forms of $H_n\mathbf{1}^{n+}$ in aqueous solution was of the order of 10–15 % [6].

In view of the foregoing, one can consider the presence of small amounts of the $\mathbf{D_{hydr}}$ and $\mathbf{A_{hydr}}$ forms of *iso*-$H_4\mathbf{1}^{4+}$ equilibrated with the $\mathbf{N1_{hydr}}$ and $\mathbf{N2_{hydr}}$ forms of *n*-$H_4\mathbf{1}^{4+}$, respectively, in the ionic mixture of $\mathbf{1}$ at pH <1. Indeed, the full protonation of this pentamine was only arbitrarily assumed previously (vide supra, see also note 71 in Ref. [6]). On the other hand, a 'structural' rationalization [6] of the discussed ^{13}C trends agreed well with a reasonable postulate that both atoms $C11$ and $C12$ have been in a comparable chemical environment under used measurement conditions. The latter assumption resulted, in turn, from large similarity in the shape of their NMR pH-titration profiles (*resemblance criterion*) [12]. A presumable coexistence of some minor amounts of *iso*-$H_4\mathbf{1}^{4+}$ being in dynamic equilibrium with *n*-$H_4\mathbf{1}^{4+}$ is consistent with such conformational landscape.

Generally, the higher energies of localized forms of *iso*-$H_4\mathbf{1}^{4+}$ in relation to those of *n*-$H_4\mathbf{1}^{4+}$ seem to be the only one alarming aspect of a newly proposed explanation of ^{13}C NMR shifts in question. However, it must be kept in mind that we meet here with the well-known issue of a doubtful trustworthiness of today's computational predictions about multicharged polyammoniums dissolved in highly polar aqueous media. Moreover, the presence of NO$_3^-$ as counterions was neglected. Similar relaxation times T_1 (~0.66 s) estimated for $C12$ of $\mathbf{1}$ at pH 11.5 and 0.2 suggests similarity in their dynamics and so comparable solute–solvent interactions of its pendant arm in two different surroundings. The occurrence of the same molecular unit $^\alpha CH_2^\beta CH_2NH_2$ in $H\mathbf{1}^+$ and *iso*-$H_4\mathbf{1}^{4+}$ would ideally explain practically identical ^{13}C-T_1 values found for their β-CH$_2$ groups.

Conclusion

A crucial role of the ^{13}C spin–lattice relaxation times (^{13}C T_1s) for elucidating internal molecular dynamics was presented in the case of two ammonium cations of a complex tetraazamacrocyclic *scorpiand* ($\mathbf{1}$) system studied by this NMR technique in aqueous medium. These experimental T_1 data, in conjunction with the DFT-level GIAO-based prediction of ^{13}C NMR chemical shifts carried out for several conformers of the ion *iso*-$H_4\mathbf{1}^{4+}$ not studied before, permitted to suggest the presence of minor amounts of this tautomer in solution, as a species co-existing in fast equilibrium with *n*-$H_4\mathbf{1}^{4+}$. Such contribution of *iso*-$H_4\mathbf{1}^{4+}$ to the ionic mixture would rationalize, at least in part, an

'abnormal' ^{13}C NMR trend found previously for the side-chain atoms C11/C12 in **1** below pH 1. At the same time, its earlier working explanation, involving complexation of a single nitrate anion by the perprotonated form of penta-mine **1** was rejected in a definitive manner.

Methods

^{13}C NMR relaxation measurements

Longitudinal relaxation times, T_1s, for ^{13}C nuclei in amine **1** (available from an earlier work [7]) were measured at ~294 K on undegassed samples by the inversion-recovery method [81, 82] on a Varian Gemini 200 BB NMR spectrometer operating at 199.98/50.29 MHz (^{1}H/^{13}C). All experiments were conducted in automation mode under ^{1}H broad band-decoupling conditions achieved with the WALTZ-16 sequence [83], by using pulse program of the software package (version 6.3C) from Varian Associates, Inc. The $(t_d\text{-}\pi\text{-}\tau\text{-}\pi/2\text{-}t_a)_n$ pulse sequence was applied, where t_d, τ, and t_a were the recycle-delay time, relaxation delay, and acquisition time, respectively. Twelve different pulse interval times τ between 0.01 and 20 s were used in arrayed experiments, with t_d 20 s and t_a 4.2 s. Number of scans, n, was between 400 and 900, spectral width 3200 Hz, data size 32 K.

High-precision 5-mm NMR sample tubes were used. The δ_C values, originally measured relative to external liquid tetramethylsilane (TMS) [contained in a coaxially-situated glass NI5CCI-V insert (with the 2-mm-o.d. stem) delivered by Norell, Inc. Landisville, NJ, USA], were corrected by a factor of +0.72 ppm [12], to account for the difference in diamagnetic susceptibilities of both liquids involved ($\Delta\chi_v$) [84 and refs therein]. Roughly 0.01 mol L^{-1} solution of **1** in H$_2$O/D$_2$O (~95:5 vol. %) was applied and HNO$_3$ was employed as titrant; the concentration of **1** and D$_2$O decreased a little, because of dilution of the sample with the added acid. Two solutions of pH values about 11.5 and 0.2 were studied; pH-meter readings were not corrected for a small isotope effect of D$_2$O presents [85]. For details of pH-metric measurements, see Ref [7]. The T_1s for ^{13}C nuclei in the ions H**1**$^+$ and H$_5$**1**$^{5+}$ were estimated with the aid of two-parameter non-linear least-squares fitting program provided by the Varian NMR system. All calculations were carried out on a spectrometer processor.

Molecular modeling and prediction of NMR spectra

An exhaustive molecular-mechanics (MM) exploration of the conformational space of iso-H$_4$**1**$^{4+}$ was performed with the OPLS-AA [14–17] force field as an energy minimizer, by using the Monte Carlo (MC)-type GMMX subroutine of PCMODEL [86]. A randomization [87, 88 and refs

therein, 89] over various macroring conformers and all rotatable bonds in the side chain was performed. The 14.6 kJ mol^{-1} energy window and dielectric constant (bulk relative permittivity), $\varepsilon = 78.36$ [90], were used in a rough simulation of hydration.[5] The returned 25 unique energetically lowest-lying models of iso-H$_4$**1**$^{4+}$, embracing the energy window of 6.2 kJ mol^{-1}, were subjected to a gradient gas-phase geometry refinement, initially at HF/3-21G [91] and then (after some selection) at HF/6-31G(d) and B3LYP/6-31G(d) levels, by applying the Gaussian 09 program [90] with PCMODEL as its graphical interface. Seven HF/3-21G promising trial structures **A-F** of iso-H$_4$**1**$^{4+}$ found in this way are listed in Table S1.[6] In contrast, all input MM models of the likewise examined 'reference' forms **N1** and **N2** of n-H$_4$**1**$^{4+}$ were attained departing from geometries of two structurally close forms of iso-H$_4$**1**$^{4+}$, by their manual deprotonation at N^1.

In addition, frequencies v_i were always computed in harmonic approximation of vibrational modes to verify whether all localized stationary points represented true energy minima (NImag = 0) and to determine differences in standard Gibbs free energies at 298.15 K, $\Delta G^o_{298.15}$. Zero-point energies were evaluated from v_is scaled by a uniform factor of 0.96 [93]. Finally, Grimme's DFT-D3 corrections [74] for dispersion-type interactions (London forces) [74, 75] were applied to so-computed $\Delta G^o_{298.15}$s. These correcting terms were calculated with ORCA [94]. Moreover, simulations of an impact of water molecules on the shape of ions **1** were performed in an improved equilibrium solvation protocol [18] of the polarizable continuum model of solvation (IEF-PCM) [19–23], by using UFF atomic radii. All molecule visualizations were performed employing PLATON [95–97].

Single-point in vacuo GIAO [8, 9] B3LYP/6-31G(d) computations of isotropic magnetic shieldings, σ_Cs, for components of all four ionic pairs of **1** were carried out at their B3LYP/6-31G(d) ground-state structures, by using Gaussian 09. Analogous predictions were also made applying the foregoing hydration model. The ^{13}C NMR chemical-shift value of a given nucleus in all these entities was defined as δ_C^{calcd} [ppm] $= \sigma_C^{stand} - \sigma_C^{calcd}$, where σ_C^{stand}

---

[5] Calculations were executed for up to 210000 MC steps. A search was carried out in seven sets of the MM runs, where every series embraced 30000 MC steps; no new low-energy structure was generated in the last set.

[6] Two starting MM models of iso-H$_4$**1**$^{4+}$, Nos. 22 and 23, with the bent –CH$_2$CH$_2$NH$_2$ unit, underwent rearrangement into forms **X** and **Y** of n-H$_4$**2**$^{4+}$ via the 5-membered cyclic transition state (observed on the monitor screen) with an internal H-bridge of type N···H$^+$···N. A great degeneration of initial MM models of flexible molecules occurs occasionally in the geometry refinement at different ab initio levels. Usually, some changes in relative positions of their energy levels appear in such cases, e.g., #1 → **E**, #2 → **F**, #13 → **A**, #14 → **B** (Table S1); similar observation was reported in Ref. [92].

was of 189.7155 ppm (in vacuo) or 190.1647 ppm (IEF-PCM simulations of H_2O) as respectively evaluated for a used NMR reference standard (TMS with the T_d symmetry) [98]. All final geometry optimizations, frequency calculations, and GIAO predictions at the DFT level were done with the keyword Int(Grid = UltraFine).

References

1. Breitmaier, E., Spohn, K.-H., Berger, S.: ^{13}C spin-lattice relaxation times and the mobility of organic molecules in solution. Angew. Chem. Int. Ed. Engl. **14**, 144–159 (1975)
2. Wehrli, F.W., Wirthlin, T.: Interpretation of carbon-13 NMR spectra, Chapt. 4. Heyden & Son, Ltd., London (1978)
3. Kalinowski, H.-O., Berger, S., Braun, S.: Carbon-13 NMR spectroscopy, Chapt. 5, pp. 621–649. Wiley, Chichester (1988)
4. Levy, G.C., Lichter, R.L., Nelson, G.L.: Carbon-13 nuclear magnetic resonance-spectroscopy, 2nd ed., Chapts. 1 and 9. Wiley, New York (1980)
5. Breitmaier, E., Voelter, W.: Carbon-13 NMR spectroscopy—high-resolution methods and applications in organic chemistry and biochemistry, 3rd rev. edn., Chapt. 3.3. VCH Publishers, Weinheim (1990)
6. Nazarski, R.B.: Physical image versus structure relation. Part 14: an attempt to rationalize some acidic region ^{13}C NMR-pH titration shifts for tetraaza macrocycles throughout the conformational GIAO DFT computational results: a pendant-arm *cyclam* case. J. Phys. Org. Chem. **22**, 834–844 (2009)
7. Sroczyński, D., Grzejdziak, A., Nazarski, R.B.: Physical image vs. structure relation. Part 3: basic properties and protonation mechanism of some tetraaza macrocyclic ligands. J. Incl. Phenom. Macrocycl. Chem. **35**, 251–260 (1999)
8. Wolinski, K., Hilton, J.F., Pulay, P.: Efficient implementation of the gauge-independent atomic orbital method for NMR chemical shift calculations. J. Am. Chem. Soc. **112**, 8251–8260 (1990)
9. Cheeseman, J.R., Trucks, G.W., Keith, T.A., Frisch, M.J.: A comparison of models for calculating nuclear magnetic resonance shielding tensors. J. Chem. Phys. **104**, 5497–5509 (1996)
10. Cascio, S., De Roberts, A., Foti, C.: Protonation of polyamines in NaCl aqueous solution and binding of Cl^- by polyammonium cations. Fluid Phase Equilibr. **170**, 167–181 (2000)
11. Nazarski, R.B., Pasternak, B., Leśniak, S.: Three-component conformational equilibria of some flexible pyrrolidin-2-(thi)ones in solution by NMR data (δ_C, δ_H, and $^nJ_{HH}$) and their DFT predictions: a confrontation of different approaches. Tetrahedron **67**, 6901–6916 (2011)
12. Nazarski, R.B.: Assignment of pH-dependent NMR spectra of the scorpiand macrocycle: an application of titration profiles and spectral linewidths. Magn. Reson. Chem. **41**, 70–74 (2003)
13. Nazarski, R.B.: Physical image vs. structure relation. Part 6. NMR solution studies of dynamic processes in protonated forms of some tetraaza macrocycles. Mol. Phys. Rep. **29**, 176–179 (2000)
14. Jorgensen, W.L., Maxwell, D.S., Tirado-Rives, J.: Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J. Am. Chem. Soc. **118**, 11225–11236 (1996)
15. Rizzo, R.C., Jorgensen, W.L.: OPLS all-atom model for amines: resolution of the amine hydration problem. J. Am. Chem. Soc. **121**, 4827–4836 (1999)
16. Price, M.L.P., Ostrovsky, D., Jorgensen, W.L.: Gas-phase and liquid-state properties of esters, nitriles, and nitro compounds with the OPLS-AA force field. J. Comput. Chem. **22**, 1340–1352 (2001)
17. Kaminski, G.A., Friesner, R.A., Tirado-Rives, J., Jorgensen, W.L.: Evaluation and reparametrization of the OPLS-AA force field for proteins via comparison with accurate quantum chemical calculations on peptides. J. Phys. Chem. B **105**, 6474–6487 (2001)
18. Scalmani, G., Frisch, M.J.: Continuous surface charge polarizable continuum models of solvation. I. General formalism. J. Chem. Phys. **132**(114110), 1–15 (2010)
19. Mennucci, B., Tomasi, J.: Continuum solvation models: a new approach to the problem of solute's charge distribution and cavity boundaries. J. Chem. Phys. **106**, 5151–5158 (1997)
20. Cancès, E., Mennucci, B., Tomasi, J.: A new integral equation formalism for the polarizable continuum model: theoretical background and applications to isotropic and anisotropic dielectrics. J. Chem. Phys. **107**, 3032–3041 (1997)
21. Mennucci, B., Cancès, E., Tomasi, J.: Evaluation of solvent effects in isotropic and anisotropic dielectrics and in ionic solutions with a unified integral equation method: theoretical bases, computational implementation, and numerical applications. J. Phys. Chem. B **101**, 10506–10517 (1997)
22. Cancès, E., Mennucci, B.: New applications of integral equations methods for solvation continuum models: ionic solutions and liquid crystals. J. Math. Chem. **23**, 309–326 (1998)
23. Tomasi, J., Mennucci, B., Cancès, E.: The IEF version of the PCM solvation method: an overview of a new method addressed to study molecular solutes at the QM ab initio level. J. Mol. Struct. (Theochem) **464**, 211–226 (1999)
24. Nazarski, R.B.: pH-Dependent ^{13}C spin-lattice relaxation times for pendant arm *cyclam* (*scorpiand*), poster P-18 at the Vth symposium on NMR in chemistry, physics and biological sciences, Warsaw, Sep. 23–25, 2009; Book of Abstracts, p. P-18
25. Sessler, J.L., Gale, P.A., Cho, W.-S.: Anion receptor chemistry, Chapt. 2. Classic Charged Non-Metallic Systems, pp. 27-130. RSC Publishing, Cambridge (2006)
26. Cullinane, J., Gelb, R.I., Margulis, T.N., Zompa, L.J.: Hexacyclen complexes of inorganic anions: bonding forces, structure, and selectivity. J. Am. Chem. Soc. **104**, 3048–3053 (1982)
27. Wiórkiewicz-Kuczera, J., Kuczera, K., Bazzicalupi, C., Bencini, A., Valtancoli, B., Bianchi, A., Bowman-James, K.: Solid state to solution: crystal structure and molecular dynamics simulations of a polyammonium nitrate host. New J. Chem. **23**, 1007–1013 (1999)
28. Papoyan, K.-J., Gu, G., Wiórkiewicz-Kuczera, J., Kuczera, K., Bowman-James, K.: Molecular dynamics simulations of nitrate complexes with polyammonium macrocycles: insight on phosphoryl transfer catalysis. J. Am. Chem. Soc. **118**, 1354–1364 (1996)

29. Lin, P.K.T., Araujo, A.N., Montenegro, M.C.B.S., Pérez-Olmos, R.: New PVC nitrate-selective electrode: application to vegetables and mineral waters. J. Agric. Food Chem. **53**, 211–215 (2005)

30. Jetzinger, M.: Sensorics new nitrate-ionophore from Sigma-Aldrich. Eliminate interference from competing nitrite, chloride and sulfate ions. Analytix Newsletter, Issue 1, 19–19 (2008)

31. Herges, R., Dikmans, A., Jana, U., Köhler, F., Jones, P.G., Dix, I., Fricke, T., König, B.: Design of a neutral macrocyclic ionophore: synthesis and binding properties for nitrate and bromide anions. Eur. J. Org. Chem. **2002**, 3004–3014 (2002)

32. Hay, B.P., Firman, T.K., Moyer, B.A.: Structural design criteria for anion hosts: strategies for achieving anion shape recognition through the complementary placement of urea donor groups. J. Am. Chem. Soc. **127**, 1810–1819 (2005)

33. Mahoney, J.M., Stucker, K.A., Jiang, H., Carmichael, I., Brinkmann, N.R., Beatty, A.M., Noll, B.C., Smith, B.D.: Molecular recognition of trigonal oxyanions using a ditopic salt receptor: evidence for anisotropic shielding surface around nitrate anion. J. Am. Chem. Soc. **127**, 2922–2928 (2005)

34. Okunola, O.A., Santacroce, P.V., Davis, J.T.: Natural and synthetic receptors for nitrate anion. Supramol. Chem. **20**, 169–190 (2008)

35. Salvador, P., Curtis, J.E., Tobias, D.J., Jungwirth, P.: Polarizability of the nitrate anion and its solvation at the air/water interface. Phys. Chem. Chem. Phys. **5**, 3752–3757 (2003)

36. Pearson, R.G.: Hard and soft acids and bases. J. Am. Chem. Soc. **85**, 3533–3539 (1963)

37. Bisson, A.P., Lynch, V.M., Monahan, M.-K.C., Anslyn, E.V.: Recognition of anions through NH-π hydrogen bonds in a bicyclic cyclophane—selectivity for nitrate. Angew. Chem. Int. Ed. Engl. **36**, 2340–2342 (1997)

38. Niikura, K., Bisson, A.P., Anslyn, E.V.: Optical sensing of inorganic anions employing a synthetic receptor and ionic colorimetric dyes. J. Chem. Soc., Perkin Trans. **2**, 1111–1114 (1999)

39. Blondeau, P., Benet-Buchholz, J., de Mendoza, J.: Enthalpy driven nitrate complexation by guanidinium-based macrocycles. New J. Chem. **31**, 736–740 (2007)

40. Mason, S., Clifford, T., Seib, L., Kuczera, K., Bowman-James, K.: Unusual encapsulation of two nitrates in a single bicyclic cage. J. Am. Chem. Soc. **120**, 8899–8900 (1998)

41. Henderson, W.A., Fylstra, P., De Long, H.C., Trulove, P.C., Parsons, S.: Crystal structure of the ionic liquid EtNH3NO3—insights into the thermal phase behavior of protic ionic liquids. Phys. Chem. Chem. Phys. **14**, 16041–16046 (2012)

42. Clifford, T., Danby, A., Llinares, J.M., Mason, S., Alcock, N.W., Powell, D., Aguilar, J.A., García-España, E., Bowman-James, K.: Anion binding with two polyammonium macrocycles of different dimensionality. Inorg. Chem. **40**, 4710–4720 (2001)

43. Llinares, J.M., Powell, D., Bowman-James, K.: Ammonium based anion receptors. Coord. Chem. Rev. **240**, 57–75 (2003)

44. Motekaitis, R.J., Martell, A.E., Lehn, J.-M., Watanabe, E.-I.: Bistren cryptates of cobalt(II), nickel(II), copper(II), and zinc(II). Protonation constants, formation constants, and hydroxo bridging. Inorg. Chem. **21**, 4253–4257 (1982)

45. Park, C.H., Simmons, H.E.: Macrobicyclic amines. III. encapsulation of halide Ions by in, in-1,(k + 2)-diazabicyclo[k.l.m]alkaneammonium Ions. J. Am. Chem. Soc. **90**, 2431–2432 (1968)

46. Harrowfield, J.M., Miyamae, H., Shand, T.M., Skelton, B.W., Soudi, A.A., White, A.H.: Lewis-base adducts of lead(II) compounds. IX. Synthetic and structural studies of some 1:1 adducts of 'cyclam' and Its hexamethylated derivative 'tet-b' with lead(II) oxoanion salts. Aust. J. Chem. **49**, 1051–1066 (1996)

47. Domagała, M., Olszak, T.A., Nazarski, R.B., Sroczyński, D., Grzejdziak, A.: X-ray structure investigations of (1,1,4,4,8,8,11,11-²H₈)-1,4,8,11-tetraazacyclotetradecane tetranitrate di[²H₂]hydrate. Poster P-52 at the 41st polish crystallographic meeting, Wrocław, June 24–25, 1999; Abstracts, pp. 94–95

48. Harrowfield, J.M., Kim, Y., Lee, Y.H., Nealon, G.L., Skelton, B.W., White, A.H.: Novel and disparate hydrogen-bonding associations in 13-amino-6-hydroxy-13-methyl-1,4,8,11-tetraazacyclotetradecane tetrahydrochloride monohydrate. Aust. J. Chem. **58**, 60–62 (2005)

49. Hanuza, J., Mączka, M., Waśkowska, A., Oganowski, W., Ban-Oganowska, H., Lutz, B., van der Maas, J.H.: Intermolecular = C–H…O–N interaction in 3-bromo-2,6-dimethyl-4-methylthiopyridine derivatives. J. Mol. Struct. **408–409**, 349–354 (1997)

50. Felemez, M., Bernard, P., Schlewer, G., Spiess, B.: Inframolecular protonation process of myo-inositol 1,4,5-tris(phosphate) and related compounds: dynamics of the intramolecular interactions and evidence of C–H···O hydrogen bonding. J. Am. Chem. Soc. **122**, 3156–3165 (2000)

51. Kuad, P., Borkovec, M., Desage-El Murr, M., Le Gall, T., Mioskowski, C., Spiess, B.: Inframolecular protonation process of norbadione A: influence of the ionic environment and stereochemical consequences. J. Am. Chem. Soc. **127**, 1323–1333 (2005)

52. Weisell, J., Hyvönen, M.T., Vepsäläinen, J., Alhonen, L., Keinänen, T.A., Khomutov, A.R., Soininen, P.: Novel isosteric charge-deficient spermine analogue—1,12-diamino-3,6,9-triazadodecane: synthesis, pKa measurement and biological activity. Amino Acids **38**, 501–507 (2010)

53. Sarkar, B., Mukhopadhyay, P., Bharadwaj, P.K.: Laterally nonsymmetric aza-cryptands: synthesis, catalysis and derivatization to new receptors. Coord. Chem. Rev. **236**, 1–13 (2003)

54. Desreux, J.F., Merciny, E., Loncin, M.F.: Nuclear magnetic resonance and potentiometric studies of the protonation scheme of two tetraaza tetraacetic macrocycles. Inorg. Chem. **20**, 987–991 (1981)

55. Geraldes, C.F.G.C., Alpoim, M.C., Marques, M.P.M., Sherry, A.D., Singh, M.: Nuclear magnetic resonance and potentiometric studies of the protonation scheme of a triaza triacetic macrocycle and its complexes with lanthanum and lutetium. Inorg. Chem. **24**, 3876–3881 (1985)

56. Amorim, M.T.S., Ascenso, J.R., Delgado, R., Fraústo da Silva, J.J.R.: Nuclear magnetic resonance studies of the protonation sequence of some oxaaza macrocyclic compounds. J. Chem. Soc., Dalton Trans. 3449–3455 (1990)

57. Grace, D.S.B. Krane, J.: An investigation of the solution structures of the tetrapodand crown imine 1,4,7,10-tetrakis-(2-hydroxyethyl)-1,4,7,10-tetra-azacyclododecane and its complexes with cations using ¹³C longitudinal relaxation time measurements. J. Chem. Res. (M) 1650–1979 (1983); J. Chem. Res. (S) 162–163 (1983)

58. Wyrwał, J., Schroeder, G., Kowalewski, J., Aski, S.N.: The effect of pendant-arm modification and ring size on the dynamics of cyclic polyamines. J. Mol. Struct. **792–793**, 274–279 (2006)

59. Erk, Ç., Zeidler, M.D.: ¹³C NMR relaxation time measurements of macrocyclic ether complexes. Part XII: the association constants of benzo[15]crown-5 and benzo[12]crown-4 with NaClO₄·H₂O. Chem. Phys. **303**, 115–120 (2004)

60. Levy, G.C.: Carbon-13 spin-lattice relaxation studies and their application to organic chemical problems. Acc. Chem. Res. **6**, 161–169 (1973)

61. Fensterbank, H., Berthault, P., Larpent, C.: A tunable one-step N, N′-disubstitution of 1,4,8,11-tetraazacyclotetradecane with acrylamide. Eur. J. Org. Chem. **2003**, 3985–3990 (2003)

62. Inoue, M.B., Oram, P., Inoue, M., Fernando, Q.: ¹H NMR and protonation constants of new metal-chelating macrocycles,

oxocyclopolyamines with pendant carboxymethyl groups, and the stabilities of their Mg^{2+} and Ca^{2+} complexes. Inorg. Chim. Acta **232**, 91–98 (1995)

63. Levy, G.C.: Carbon-13 Spin-lattice relaxation and solvation of organic ions: n-butylamine and n-butylammonium ion. J. Chem. Soc., Chem. Commun. 768–769 (1972)

64. Bifulco, G., Dambruoso, P., Gomez-Paloma, L., Riccio, R.: Determination of relative configuration in organic compounds by NMR spectroscopy and computational methods. Chem. Rev. **107**, 3744–3779 (2007)

65. Agostini, O., Bonacchi, G., Dapporto, P., Paoli, P., Pogliani, L., Toja, E.: Structure and dynamics of theophylline derivatives by X-ray, NMR and molecular mechanics studies. J. Chem. Soc., Perkin Trans. 2 1061–1066 (1994)

66. Młynarz, P., Olbert-Majkut, A., Śliwińska, S., Schroeder, G., Bańkowski, B., Kafarski, P.: 1,4-Phenylene-di(N-L-alanylaminomethylphosphonate) a new diaminophosphonate peptide receptor for lysine and arginine. J. Mol. Struct. **873**, 173–180 (2008)

67. Lacerda, S., Campello, M.P., Marques, F., Gano, L., Kubíček, V., Fousková, P., Tóth, É., Santos, I.: A novel tetraazamacrocycle bearing a thiol pendant arm for labeling biomolecules with radiolanthanides. Dalton Trans. 4509–4518 (2009)

68. Hague, D.N., Moreton, A.D.: Protonation sequence of linear aliphatic polyamines by ^{13}C NMR spectroscopy. J. Chem. Soc., Perkin Trans. 2, 265–270 (1994)

69. Bazzicalupi, C., Bencini, A., Bianchi, A., Fusi, V., Giorgi, C., Paoletti, P., Stefani, A., Valtancoli, B.: Basicity properties of a novel azaparacyclophane receptor and its acyclic precursor: a thermodynamic and structural approach. J. Chem. Soc., Perkin Trans. 2, 275–280 (1995)

70. Bhula, R., Weatherburn, D.C.: ^{13}C nuclear magnetic resonance study of the protonation of 2,2,4-trimethyl-1,5,9-triazacyclododecane. J. Chem. Soc., Perkin Trans. 2, 1161–1164 (1988)

71. Bianchi, A., Escuder, B., García-España, E., Luis, S.V., Marcelino, V., Miravet, J.F., Ramírez, J.A.: Protonation tendencies of azaparacyclophanes. A thermodynamic and NMR study. J. Chem. Soc., Perkin Trans. 2, 1253–1259 (1994)

72. Aguilar, J.A., García-España, E., Guerrero, J.A., Luis, S.V., Llinares, J.M., Ramírez, J.A., Soriano, C.: Synthesis and protonation behaviour of the macrocycle 2,6,10,13,17,21-hexaaza[22]metacyclophane. Thermodynamic and NMR studies on the interaction of 2,6,10,13,17,21-hexaaza[22]metacyclophane and on the open-chain polyamine 4,8,11,15-tetraazaoctadecane-1,18-diamine with ATP, ADP and AMP. Inorg. Chim. Acta **246**, 287–294 (1996)

73. Szakács, Z., Kraszni, M., Noszál, B.: Determination of microscopic acid–base parameters from NMR–pH titrations. Anal. Bioanal. Chem. **378**, 1428–1448 (2004)

74. Grimme, S., Antony, J., Ehrlich, S., Krieg, H.: A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. J. Chem. Phys. **132**(154104), 1–19 (2010)

75. Klimeš, J., Michaelides, A.: Perspective: advances and challenges in treating van der Waals dispersion forces in density functional theory. J. Chem. Phys. **137**(120901), 1–12 (2012)

76. Zakai, U.I., Błoch-Mechkour, A., Jacobsen, N.E., Abrell, L., Lin, G., Nichol, G.S., Bally, T., Glass, R.S.: Synthesis and structure of m-terphenyl thio-, seleno-, and telluroethers. J. Org. Chem. **75**, 8363–8371 (2010)

77. Chęcińska, L., Kudzin, Z.H., Małecka, M., Nazarski, R.B., Okruszek, A.: [(Diphenoxyphosphinyl)methylidene]triphenylphosphorane: the double P+-stabilised carbanion: a crystallographic, computational and solution NMR comparative study on the ylidic bonding. Tetrahedron **59**, 7681–7693 (2003)

78. Michalik, E., Nazarski, R.B.: Synthesis, complete NMR assignments, and NOE versus GIAO data assisted ab initio modelling the overall conformations of amide 3,4'-diquinolinyl sulfides in solution. Another approach to analysis of flexible systems. Tetrahedron **60**, 9213–9222 (2004)

79. Alcântara, A.F.C., Piló-Veloso, D., De Almeida, W.B., Maltha, C.R.A., Barbosa, L.C.A.: Conformational analysis of 8-oxabicyclo[3.2.1]oct-6-en-3-one derivatives by NMR and theoretical calculations. J. Mol. Struct. **791**, 180–185 (2006)

80. Belostotskii, A.M.: Calculated chemical shifts as a fine tool of conformational analysis: an unambiguous solution for haouamine alkaloids. J. Org. Chem. **73**, 5723–5731 (2008)

81. Vold, R.L., Waugh, J.S., Klein, M.P., Phelps, D.E.: Measurement of spin relaxation in complex systems. J. Chem. Phys. **48**, 3831–3832 (1968)

82. Freeman, R., Hill, H.D.W.: High-resolution studies of nuclear spin-lattice relaxation. J. Chem. Phys. **51**, 3140–3141 (1969)

83. Shaka, A.J., Keeler, J., Frenkiel, T., Freeman, R.: An improved sequence for broadband decoupling: WALTZ-16. J. Magn. Reson. **52**, 335–338 (1983)

84. Momoki, K., Fukazawa, Y.: Bulbed capillary external referencing method for proton nuclear magnetic resonance spectroscopy. Anal. Chem. **62**, 1665–1671 (1990)

85. Salomaa, P., Schaleger, L.L., Long, F.A.: Solvent deuterium isotope effects on acid-base equilibria. J. Am. Chem. Soc. **86**, 1–7 (1964)

86. PCMODEL V 8.5, Molecular Modeling Software for Windows Operating System, Apple Macintosh OS, Linux and Unix, Serena Software, Bloomington, August 2003

87. Saunders, M.: Stochastic exploration of molecular mechanics energy surfaces. Hunting for the global minimum. J. Am. Chem. Soc. **109**, 3150–3152 (1987)

88. Saunders, M., Houk, K.N., Wu, Y.-D., Still, W.C., Lipton, M., Chang, G., Guida, W.C.: Conformations of cycloheptadecane. A comparison of methods for conformational searching. J. Am. Chem. Soc. **112**, 1419–1427 (1990)

89. Saunders, M.: Searching for conformers of nine- to twelve-ring hydrocarbons on the MM2 and MM3 energy surfaces: stochastic search for interconversion pathways. J. Comput. Chem. **12**, 645–663 (1991)

90. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, Jr., J.A., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, O., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J.: Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford, 11 Jun 2009

91. Jiao, D., Barfield, M., Hruby, V.J.: Ab initio IGLO study of the φ- and ψ-angle dependence of the ^{13}C chemical shifts in the model peptide N-Acetyl-N'-methylglycinamide. J. Am. Chem. Soc. **115**, 10883–10887 (1993)

92. Cimino, P., Bifulco, G., Evidente, A., Abouzeid, M., Riccio, R., Gomez-Paloma, L.: Extension of the J-based configuration analysis to multiple conformer equilibria: an application to sapinofuranone A. Org. Lett. **4**, 2779–2782 (2002)

93. Curtiss, L.A., Raghavachari, K., Redfern, P.C., Pople, J.A.: Investigation of the use of B3LYP zero-point energies and geometries in the calculation of enthalpies of formation. Chem. Phys. Lett. **270**, 419–426 (1997)

94. Neese, F., et al.: ORCA—An ab initio, DFT and semiempirical SCF-MO package. Version 2.8.0.1, Dec 12, 2010; University of Bonn, Germany; http://www.thch.uni-bonn.de/tc/orca/

95. Spek, A.L.: Single-crystal structure validation with the program *PLATON*. J. Appl. Cryst. **36**, 7–13 (2003)

96. Spek, A.L.: Structure validation in chemical crystallography. Acta. Cryst. **D65**, 148–155 (2009)

97. Spek, A.L.: PLATON, A multipurpose crystallographic tool, Utrecht University, Utrecht, The Netherlands, 1980–2011; http://www.cryst.chem.uu.nl/platon/

98. Foresman, J.B., Frisch, Æ.: Exploring Chemistry with Electronic Structure Methods, 2nd edn., Gaussian, Inc., Pittsburgh (1996) Chapt. 4 + Errata