The effect of pyridinecarboxylate chelating groups on the stability and electronic relaxation of gadolinium complexes†

Nicholas Chatterton,a Christelle Gateau,a Marina Mazzanti,a Jacques Pécaut,b Alain Borel,c Lothar Helmac and André Merbachc

a Laboratoire de Recherche Fondamentale sur la Matière Condensée, CEA-Grenoble, 38054, Grenoble, Cedex 09, France. E-mail: mazzanti@drfmc.ceng.cea.fr
b Laboratoire de Coordination et Chiralité, Service de Chimie Inorganique et Biologique, Département de Recherche Fondamentale sur la Matière Condensée, CEA-Grenoble, 38054, Grenoble, Cedex 09, France
c Laboratoire de Chimie Inorganique et Bioinorganique, Ecole Polytechnique Fédérale de Lausanne, EPFL-BCH, CH-1015, Lausanne, Switzerland

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The ligand \(N,N^\prime\)-bis(6-carboxy-2-pyridylmethyl)ethylendiamine-\(N,N^\prime\)-diacetic acid (H\(_4\)bpeda) was synthesised using an improved procedure which requires a reduced number of steps and leads to a higher yield with respect to the published procedure. It was obtained in three steps from diethylpyridine-2,6-dicarboxylate and commercially available ethylenediamine-\(N,N^\prime\)-diacetic acid with a total yield of ~20%. The crystal structure of the hexa-protonated form of the ligand which was determined by X-ray diffraction shows that the four carboxylates and the two amines are protonated. The crystal structure of the polynuclear complex \([\text{Gd}(\text{bpeda})(\text{H}_2\text{O})_2]_3[\text{Gd}(\text{H}_2\text{O})_6]^2\text{Cl}_3\) (2), isolated by slow evaporation of a 1 : 1 mixture of GdCl\(_3\) and H\(_4\)bpeda at pH ~ 1, was determined by X-ray diffraction. In complex 2 three \([\text{Gd}(\text{bpeda})(\text{H}_2\text{O})_3]_3\) units, containing a Gd(III) ion ten-coordinated by the octadentate bpeda and two water molecules, are connected in a pentametallic structure by two hexa-aquo Gd\(^{3+}\) cations through four carboxylato bridges. The protonation constants (\(pK\)\(_\text{a}^1 = 2.9(1), pK\)\(_\text{a}^2 = 3.5(1), pK\)\(_\text{a}^3 = 5.2(2), pK\)\(_\text{a}^4 = 8.5(1)\)) and the stability constants of the complexes formed between Gd(III) and Ca(II) ions and H\(_4\)bpeda (log\(_{K}\)\(_\text{calc}= 15.1(3); \log\_{K_{\text{calc}}} = 9.4(1)\)) were determined by potentiometric titration. The unexpected decrease in the stability of the gadolinium complex and of the calcium complex of the octant ligand bpeda\(^{18}\) with respect to the hexadentate ligand edta\(^{18}\) has been interpreted in terms of an overall lower contribution to stability of the metal-nitrogen interactions. The EPR spectra display very broad lines (apparent \(A_{\text{H}pp} \sim 800–1200\) G at X-band and 90–110 G at Q-band depending on the temperature), indicating a rapid transverse electron spin relaxation. At X-band, Gd(bpeda) is among the fastest relaxing Gd\(^{3+}\) complexes to date suggesting that the presence of pyridinecarboxylate chelating groups in itself does not lead to slow electron relaxation.

Introduction

The application of gadolinium complexes containing polyaminocarboxylate ligands as contrast agents in magnetic resonance imaging (MRI), a primary medical diagnostic technique, has prompted a large number of studies.† The key property of an efficient contrast agent is its ability to enhance the relaxation rate of solvent water protons (relaxivity, \(r_\text{m}\)). The relaxivity of current clinical contrast agents is much lower than theoretically possible due to lack of simultaneous optimisation of all the parameters determining the relaxation enhancement. Higher relaxivity is required for the next generation of site-specific MRI contrast agents. High relaxivity can be obtained in the presence of a high number of inner sphere water molecules allied with optimized water exchange rate, a long rotational correlation time and a long electronic relaxation time. Furthermore due to the extreme toxicity of the gadolinium ion the stability of MRI contrast agents is critical since the toxicity of contrast agents is directly related to the concentration of free gadolinium \(\text{in vivo}\).

Gadolinium complexes of tripodal or tetrapodal ligands containing pyridinecarboxylate arms have recently shown interesting relaxation properties. Furthermore the facile functionalization of the pyridine rings makes these ligands attractive for the access to macromolecular contrast agents with longer correlation times and therefore higher relaxivity.

We have recently reported the two new tripodal ligands tpa and tpatcn (Scheme 1) containing three pyridinecarboxylate arms which yield low-molecular weight gadolinium complexes with high relaxivity. The heptadentate tripodal ligand tpa (H\(_4\)tpaa = \(\text{H}_{14}\text{tpaa}\) (6-methyl-2-pyridinecarboxylic acid)) contains three pyridinecarboxylate arms connected to a nitrogen atom.† The ligand tpa forms a rather insoluble gadolinium complex which has shown a remarkably higher value of relaxivity \(r_\text{m}= 13.3 \text{mM}^{-1} \text{s}^{-1}\) at 298 K and at 60 MHz than those found in the clinically used contrast agents based on mono-aqua complexes of octacoordinate ligands such as \([\text{Gd}(\text{dota})(\text{H}_2\text{O})_2]^2\text{Cl}_3\) or \([\text{Gd}(\text{dota})(\text{H}_2\text{O})_4]^2\text{Cl}_3\) \((4.3–4.7 \text{mM}^{-1} \text{s}^{-1}, 298\text{ K,} 20\text{ MHz})\).† The observed high relaxivity was attributed to the shorter Gd–Owater distance and to a possible coordination equilibrium between species with two and three bound water molecules. Conversely the similarly high relaxivity \((12.3 \text{mM}^{-1} \text{s}^{-1}, 298\text{ K,} 20\text{ MHz})\) of the bis-aqua complex \([\text{Gd}(\text{ado3a})(\text{H}_2\text{O})_2]^2\text{Cl}_3\) has been interpreted in terms of a substantial second sphere contribution.

† Electronic supplementary information (ESI) available: Tables S1–S3 containing peak-to-peak width and central field as a function of temperature for the EPR spectra of the aqueous [Gd(bpeda)]K complex. See http://www.rsc.org/suppdata/}
nonadentate ligand 1,4,7-tris(6-carboxypyridin-2-yl)methyl-1,4,7-triazacyclononane (tpatcnH3) contains three pyridinecarboxylate units. The ligand H4bpeda has been prepared. While this work was in progress the synthesis of H4bpeda has been reported by Mato-Iglesias and coworkers. The ligand H4bpeda yields nonacoordinated complexes of gadolinium with one water molecule bound to the gadolinium ion. This complex shows water proton relaxivity and water exchange of the coordination sphere on the electronic relaxation. Here we report an improved synthesis of H4bpeda along with EPR spectroscopy of the gadolinium complex.

**Experimental**

**General information**

1H and 13C NMR spectra were recorded on a Varian Mercury 400 spectrometer. Chemical shifts are reported in ppm with solvent or 3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt as internal reference. Mass spectra were obtained with a Finnigan LCQ- ion trap equipped with an electrospray source. Elemental analyses were performed by the Service Central d’Analyses Vernaison, France.

Solutions and starting materials were obtained from Aldrich, Fluka, Acros and Alfa and used without further purification. 6-Chloromethylpyridine-2-carboxylic acid ethyl ester was obtained from the commercially available 2,6-dipicolinic acid according to a published procedure.

**Synthesis of the ligand H4bpeda**

N,N’-Bis[6-carboxypyridin-2-yl)methyl]ethylenediamine-N,N’-diacetic acid. To a solution of 6-chloromethylpyridine-2-carboxylic acid ethyl ester (0.451 g, 2.26 mmol) in anhydrous acetonitrile (10 mL), ethylenediamine-N,N’-diacetic acid (0.181 g, 1.03 mmol), K2CO3 (0.312 g, 2.26 mmol) and KI (0.375 g, 2.26 mmol) were successively added under argon atmosphere. The reaction mixture was refluxed for 16 h. After filtration and evaporation of the solvent, the resulting crude product (0.82 g) was chromatographed (20 mL aluminium oxide, CH2Cl2-ethanol: gradient from 100 : 0 to 90 : 10) to eliminate the inorganic salts and starting material. After evaporation of the solvent a yellow oil was obtained (0.340 g containing the condensation product and a product identified as the lactam in approximately 50 : 50 ratio) which was used without further purification. The oil was dissolved and refluxed overnight in a 6 M HCl aqueous solution (30 mL). After evaporation of the solvent to ~5 ml the resulting precipitate was collected and dried under vacuum to yield H4bpeda-4HCl·2H2O as a white solid (0.206 g, 32%). NMR (D2O, 400 MHz, pH = 7.14); δ 3.22 (s, 4H, H10); 3.44 (s, 4H, H8); 4.19 (s, 4H, H7); 7.55 (d, J = 7.6 Hz, 2H, H5); 7.82 (d, J = 7.6 Hz, 2H, H3); 8.86 (t, J = 7.6 Hz, 2H, H4). 13CN M R (D2O, 100 MHz); δ 51.6 (CH); 56.4 (CH3); 57.8 (CH3); 125.8 (CHpy); 128.4 (CHpy); 142.9 (CHpy); 147.4 (Cpy); 152.0 (Cpy); 166.4 (COOH); 172.1 (COOH). ES-MS: m/z 445.0 [M + Na]+; 447.1 [M + H]+; 403.1 [M + H − COO]−; 401.0 [M + H − COO]−; 357.1 [M − H − 2COO]−. Anal. Calc. for H4bpeda·4HCl·2H2O: C34H30N4O11·4H2O, C20H30N4O8: 38.23, H 4.81, N 8.92%. Found: C 38.49, H 4.88, N 9.00%.

**Preparation of the complexes**

[Ln(bpeda)](Ln = Eu, Gd). A solution of LnCl3·6H2O (Ln = Eu, Gd) (40.7 mmol) in water (2 mL) was added to a solution of H4bpeda (40.7 mmol) in water (2 mL). The pH of the resulting mixture was adjusted (pH ~ 6) by adding aqueous KOH solution (0.445 M). The solution was stirred at room temperature overnight. After evaporation of the water the resulting solid was washed with water. The resulting yellow suspension was refrigerated at 0 °C overnight. KCl was eliminated by filtration. The resulting solution was evaporated to small volume (0.5 ml). Addition of isopropyl alcohol (20 ml) yielded the bpeda complexes of Gd and Eu as yellow microcrystalline solids (65–75%).


**X-Ray crystallography**

All diffraction data were taken using a Bruker SMART CCD area detector three-circle diffractometer (Mo-Kα radiation,
graphite monochromator, $\lambda = 0.71073$ Å. To prevent evaporation of co-crystallised water molecules the crystals were coated with a light hydrocarbon oil. The cell parameters were obtained with intensities detected on three frames with 15 frames with a 180 s exposure time for 1 and 10 s for 2. The crystal detector distance was 5 cm. For three settings of $\Phi$, 1271 narrow data frames for 1 and 2 were collected for 0.3° increments in $\omega$ with a 180 s exposure time for 1 and 10 s for 2. At the end of data collection, the first 50 frames were recollected to establish that crystal decay had not taken place during the collection. Unique intensities with $I > 10\sigma(I)$ detected on all frames using the Bruker SMART program$^{22}$ were used to refine the values of the cell parameters. The substantial redundancy in data allows empirical absorption corrections to be applied using multiple measurements of equivalent reflections with the SADABS Bruker program.$^{32}$ Space groups were determined from systematic absences, and they were confirmed by the successful solution of the structure for 1. For 2 the solution of the structure was attempted in different space groups. The best results were obtained with the space group $P2_1$ (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Crystallographic data for the structures of 1 and 2</th>
</tr>
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<tr>
<td>1</td>
<td>2·0.5MeCN·18.5H$_2$O</td>
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<table>
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<tr>
<th>Formula</th>
<th>$C_{66}H_{127}Cl_3Gd_5N_{12}O_{127}$</th>
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<tr>
<td>$M_r$</td>
<td>1591.40</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic Monoclinic</td>
</tr>
</tbody>
</table>

$\alpha/\AA$ 1.519(1)$
$\beta/\AA$ 15.913(1)
$\gamma/\AA$ 17.363(1)
$\alpha$ 84.739(6)
$\beta$ 87.999(6)
$\gamma$ 90.226(6)
$\gamma /A \times \gamma /A$ 0.9 : 1 metal–ligand mixtures ([L]$= 5.0 \times 10^{-4}$ M) or 1 : 1.09 edta–ligand–metal mixtures ([L]$= 5.0 \times 10^{-3}$ M) were titrated in a thermostated cell (25.0 ± 0.1°C) under a stream of argon with a 0.1 M KOH solution added by means of a 5 mL piston burette (Metrohm). The ion strength was fixed with KCl ($\mu = 0.1$ M).

Titrations were carried out with a Metrohm 751 GPDTitrimeter potentiometer equipped with a combined pH glass electrode (Metrohm). Calibration of the electrode system was performed prior to each measurement. The electromotive force is given by $E = E^0 + \psi[H^+]$ and both $E^0$ and $\psi$ were determined by titrating a known amount of HCl by 0.1 M KOH at $\mu = 0.1$ M (KCl), using the acid range of the titration. The value used for the ion product of water is $\log K_w = 13.77$. More than 70 data points were collected for each experiment.

The data were mathematically treated by the program HYPERQUAD2000.$^{36}$ All values and errors represent the average of at least three independent experiments.

NMR Titrations

$\sim 10^{-2}$ M solutions of H$_4$bpeda were prepared in D$_2$O in presence of tert-butyl alcohol, as reference. Sample solutions with different pH values were prepared by adding dilute NaOD- for dilute DCl-D$_2$O (Alrich). The pH values of the solutions were determined with a MeterLab, PHM 220 pH Meter. The ion strength was not adjusted.

EPR Measurements

A 23 mM [Gd(bpeda)]$^-$ solution was prepared by dissolving 13 mg of K[Gd(bpeda)(H$_2$O)$_2$]·1.5H$_2$O into 0.794 g of bidistilled water. EPR Spectra were recorded on a Bruker Elexsys E500 system operating at X- and Q-band (9.4 and 34 GHz, respectively). The microwave frequency was measured using a frequency counter embedded in the standard microwave bridge (X-band) or an external Hewlett-Packard 5335B frequency counter (Q-band). The temperature was varied between 274 and 340 K using boiling nitrogen flowing over a thermostorer, and measured with a standard substitution technique.

The peak-to-peak widths $\Delta H_{pp}$ and central fields $B_0$ were determined by fitting the digitally recorded spectra to Lorentzian derivatives, with simultaneous baseline and phase correction.$^{37}$

Results and discussion

Synthesis and molecular structure of the ligand bpeda

$N,N'$-Bis[6-carboxy-2-pyryldimethyl]ethylenediamine-$N,N'$-diacetic acid. (H$_4$bpeda) was obtained by the group of Mato-Iglesias in a five-step procedure from dimethylpyridine-2,6-dicarboxylic acid with a total yield of 10%. We have found that the ligand can be obtained in three steps from diethylpyridine-2,6-dicarboxylic acid and commercially available ethylenediamine-$N,N'$-diacetic acid with a total yield of 19.8% (Scheme 2). The 6-chloromethylpyridine-2-carboxylic acid ethyl ester was prepared in 62% yield following the published procedure.$^{38}$ The 6-chloromethylpyridine-2-carboxylic acid ethyl ester was reacted with ethylenediamine-$N,N'$-diacetic acid in the presence of K$_2$CO$_3$ and KI to give a mixture of the expected condensation product and of a byproduct identified as the lactam in approximately 50:50 ratio. Hydrolysis of the raw mixture with amounts of GdCl$_3$·6H$_2$O (Aldrich) in water. The exact Gd$^{3+}$ ion concentration was determined by colorimetric titration in acetate buffer (pH = 4.5) using standardized H$_2$Na$_2$edta solution (Aldrich) and xylene orange as the indicator. Ca(ii) solutions were prepared by dissolving CaCl$_2$·2H$_2$O (Aldrich) in water. The exact Ca$^{2+}$ ion concentration was determined by colorimetric titration in KOH using standardized H$_2$Na$_2$edta solution (Aldrich) and calgonite as the indicators. 20 mL solutions of H$_2$bpeda (5.0 $\times 10^{-4}$ M) alone, acidified (pH = 2–5) 0.9 : 1 metal–ligand mixtures ([L]$= 5.0 \times 10^{-4}$ M) or 1 : 1.09 edta–ligand–metal mixtures ([L]$= 5.0 \times 10^{-3}$ M) were titrated in a thermostated cell (25.0 ± 0.1°C) under a stream of argon with a 0.1 M KOH solution added by means of a 5 mL piston burette (Metrohm). The ion strength was fixed with KCl ($\mu = 0.1$ M).

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HCl (6 M) resulted in the isolation of $\text{H}_4\text{bpeda} \cdot 4\text{HCl} \cdot 2\text{H}_2\text{O}$ in 32% yield.

The synthetic scheme described here requires a reduced number of steps and leads to a higher yield with respect to the published procedure. This synthetic pathway should make it easier to access to functionalised target specific derivatives.

Crystals of $\text{H}_4\text{bpeda} \cdot 2\text{HCl} \cdot 3\text{H}_2\text{O}$, 1, were obtained by slow evaporation of a concentrated aqueous solution of the ligand after adjusting the pH at $\sim 0.5$. The structure is shown in Fig. 1. The structure shows that all the four carboxylates are protonated with C–OH distances ranging from 1.201(4) to 1.215(4) Å. The two remaining protons were located (from difference Fourier electron density map) on the two tertiary amine nitrogens.

**Metal complexes**

The gadolinium and europium complexes of bpeda were prepared by reacting the ligand with LnCl$_3$·6H$_2$O (Ln = Eu, Gd) and adjusting the pH to $\sim 5$. The complexes are very soluble in water, methanol and ethanol. After elimination of the inorganic salts by filtration, the microcrystalline metal complexes isolated from isopropyl alcohol gave a satisfactory elemental analysis in agreement with the formation of analytically pure 1 : 1 complexes. While attempts to grow X-ray quality crystals of the Gd(III) complex of bpeda from various organic solvents and from water (pH $\sim$6) were not successful, slow evaporation of a 1 : 1 mixture of GdCl$_3$ and H$_4$bppez at pH $\sim$1 lead to the isolation of crystals of the polynuclear complex [Gd(bppez)H$_2$O]$_2$[Gd(H$_2$O)$_6$]Cl$_3$ (2). Although, due to the poor quality of the crystals, the structure of 2 is not of very high quality, the coordination environment of the Gd ions is perfectly clear. The isolation of this species can be explained by the partial formation of the complex at very low pH and by the lower solubility of the pentametallic complex. The structure is shown in Fig. 2 and selected average bond distances and angles are set out in Table 2. In complex 2 three [Gd(bppez)H$_2$O]$_2$ units are connected in a pentametallic structure by two hexa-aquo Gd$^{3+}$ cations through four carboxylato bridges (Fig. 3). The [Gd(1)(bppez)(H$_2$O)$_2$] moiety is connected to both [Gd(H$_2$O)$_6$]$^{3+}$ complexes through different bridging carboxylate groups, while the remaining [Gd(bppez)(H$_2$O)$_2$] units are connected through carboxylate bridges each one to a different [Gd(H$_2$O)$_6$]$^{3+}$ complex. This results in a polymeric structure presenting a pseudo-twofold axis passing trough the Gd(1) ion. In the three [Gd(bppez)(H$_2$O)$_2$] units the gadolinium is ten-coordinated by the octadentate bpeda and two water molecules. The coordination geometry can be described as a distorted bicapped square antiprism for the three [Gd(bppez)(H$_2$O)$_2$] units with the capping position occupied by the pyridyl nitrogens. The angle between the two square planes ranges from 4.1 to 4.5°. The average angle N$_{py}$–Gd–N$_{py}$ is 172(2)°. The carboxylate arms are situated alternatively above and below the planes of the pyridyl units. The geometry around the [Gd(H$_2$O)$_6$]$^{3+}$ cation is best described as a distorted dodecahedron.

**Table 2** Average values of selected bond distances (Å) and angles (°) in complex 2

<table>
<thead>
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<th>Bond/Distance</th>
<th>Value</th>
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<tr>
<td>Gd–N$_{py}$</td>
<td>2.61(2)</td>
</tr>
<tr>
<td>Gd–N$_{am}$</td>
<td>2.69(3)</td>
</tr>
<tr>
<td>Gd–O$_{am}$</td>
<td>2.52(2)</td>
</tr>
<tr>
<td>Gd–O$_{py}$</td>
<td>2.46(3)</td>
</tr>
<tr>
<td>Gd–O$_{py}$</td>
<td>2.43(4)</td>
</tr>
<tr>
<td>N$<em>{py}$–Gd–N$</em>{py}$</td>
<td>172(2)</td>
</tr>
</tbody>
</table>

![Fig. 2](image)

**Fig. 2** View of the coordination environment of Gd(2) in the crystal structure of 2 with thermal ellipsoid at 30% probability.

![Fig. 3](image)

**Fig. 3** View of the crystal structure of the complex 2 with thermal ellipsoid at 30% probability.
The average Gd-N<sub>pyridine</sub> distances (2.61(2) Å) are slightly shorter than the average M-N<sub>macro</sub> distances found in other gadolinium polyaminopolycarboxylate complexes (2.64–2.68 Å)\textsuperscript{23,24} but similar to those found in the [Gd(tpaa)] complex (2.56(4) Å) and in the [Gd(tpatcn)] complex (2.55(1) Å). The values of the average Gd-N<sub>macro</sub> bond (2.69(3) Å) are shorter than the values found for the distance Gd-apical amine nitrogen in the [Gd(tpaa)] complexes (2.7886(19) Å), but similar to the values found for the Gd-N<sub>macro</sub> in [Gd(tpatcn)] (2.67(2) Å). The average value of the Gd-O<sub>macro</sub> distances (2.52(2) Å) is in the range of the values found in other gadolinium polyaminopolycarboxylate complexes. The metal-ligand bond distances and the ligand conformation found in complex 2 differ from those found in the structure calculated by Mato-Iglesias and coworkers.\textsuperscript{16} This difference is explained by the difference in nuclearity and coordination number. While the solution structure of the complex [Gd(bpeda)(H<sub>2</sub>O)]K is in agreement with the presence of only one coordinated water molecule,\textsuperscript{16} two water molecules are bound to each Gd(bpeda) unit in complex 2. This is probably a consequence of the presence of the Gd<sup>3+</sup> ion (instead of K<sup>+</sup>) bound to the carboxylate oxygens of the Gd(bpeda) units.

### Protonation constants and stability constants

The deprotonation constants of H<sub>4</sub>bpeda defined as \(K_{a1} = \left[H_{-1},L^{5-}\right]/[H_{-5},L^{1-}][H]^{+}\) were determined to be \(pK_{a1} = 2.9(1)\), \(pK_{a2} = 3.5(1)\), \(pK_{a3} = 5.2(2)\) and \(pK_{a4} = 8.5(1)\). \(0.1\) M KCl, 298 K) by potentiometric titration. The titration curves of \(H_4bpeda\) and of its Gd(III) and Ca(II) complexes are shown in Fig. 4. Variable pH proton NMR spectroscopy of the ligand shows significant variations (0.3 ppm) in the chemical shift of the methylene protons during the third and fourth protonation processes (pH = 5–9) while no variation is observed during the first, second protonation processes (pH = 2–5) (Fig. 5). Significant variations are observed for the chemical shifts of the three pyridyl protons (H4, H5, H3: \(\sim 0.3\) ppm) upon the first and second protonation processes (pH = 2–5). The highest \(pK_{a}(5.2(2), 8.5(1))\) may be identified with the protonation of the two aliphatic amine nitrogens, followed by the protonation of the carboxylates bound to the pyridines (2.9(1), 3.5(1)). The protonation of the two remaining carboxylates occurs at lower pH and the associate \(pK_{a}\) could not be determined. The lowest value of \(pK_{a1}\) (5.2(2)) is associated to the protonation of the second aliphatic amine nitrogen can be explained by the presence of the 6-methyl-2-pyridinecarboxylic acid group. The assignment of \(pK_{a}\) is in agreement with the solid-state crystal structure of the diprotonated ligand H<sub>2</sub>bpeda\textsuperscript{2+}. Very similar values (8.67(8) and 5.53(3)) were found for the highest \(pK_{a}\)'s of the ligand N,N'-bis(2-pyridylmethyl)ethylenediamine-N,N'-diacetic acid (H<sub>2</sub>bpeda) where two of the carboxylate groups on edta are replaced by two pyridylmethyl moieties\textsuperscript{2} (Table 3). The values of \(pK_{a1}\) and \(pK_{a2}\) are consistent with the values found for the protonation of the pyridylmethylicarboxylate groups in the tripodid ligand H<sub>4</sub>bpeda (\(pK_{a1} = 2.5(2)\), \(pK_{a2} = 3.3(1)\), \(pK_{a3} = 4.11(6)\)). The substitution of two of the carboxylate groups on edta with two methylpyridinecarboxylate groups leads to an important decrease of the basicity of the two amine nitrogen (\(pK_{a4} = 6.42, pK_{a5} = 10.08\) for edta\textsuperscript{3+}).

The stability constants of the complexes formed between Gd(III) and Ca(II) ions and H<sub>4</sub>bpeda have been determined by direct titration of 1 : 1 metal-H<sub>4</sub>bpeda (5.0 \(\times 10^{-4}\) M) mixtures in the pH range 2.5–8.5. Titration data could be fitted to eqns. (1) and (2):

\[
\text{Gd}^{3+} + \text{bpeda}^2- \leftrightarrow [\text{Gd}(\text{bpeda})]^2++; \ \log_{\text{calc}} = 15.1(3) \quad (1)
\]

\[
\text{Ca}^{2+} + \text{bpeda}^2- \leftrightarrow [\text{Ca}(\text{bpeda})]^2++; \ \log_{\text{calc}} = 9.4(1) \quad (2)
\]

The values of \(p\text{Gd} = 14.9\) and \(p\text{Ca} = 9.2 \sim [\text{M}]_{\text{free}}) = 7.4, [\text{M}]_{\text{free}} = 1\) M, \([\text{tpaa}]_{\text{total}} = 10\) M, \(\) which allow a straightforward comparison of complex stabilities in physiological conditions are similar to the values found for edta (pGd = 15.3, pCa = 9.0).

The rather unexpected stability constant values show that in spite of the presence of two additional coordinating groups (two pyridyl nitrogens) in the ligand bpeda\textsuperscript{2+} with respect to the ligand edta\textsuperscript{2+} the stability of the gadolinium complex and of the calcium complex is lower for bpeda than edta (log\(\beta_{\text{calc}} = 17.4, \log_{\text{calc}} = 10.5\)). The decrease in stability is consistent with the decrease of the ligand \(pK_{a}\)'s (\(\sum pK_{a} = 20.1\) for H<sub>4</sub>bpeda and 21.9 for H<sub>4</sub>edta) suggesting that the lower stability of bpeda complexes is the result of a overall lower contribution to stability of the metal–nitrogen interactions.\textsuperscript{24}

While the contribution of the 2-pyridylmethyl to stability was evaluated to 2.5 \(\times 10^{-4}\) units for the Gd(III) complex of H<sub>2</sub>bped by Caravan et al.,\textsuperscript{25} this work shows that the pyridyl functions do not

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### Table 3

<table>
<thead>
<tr>
<th>Ligand</th>
<th>(pK_a)</th>
<th>(\log_{\text{calc}})</th>
<th>(\log_{\text{calc}})</th>
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<td>H&lt;sub&gt;4&lt;/sub&gt;bpeda*</td>
<td>8.5(1), 5.2(2), 3.5(1), 2.9(1)</td>
<td>15.1(3)</td>
<td>9.4(1)</td>
</tr>
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<td>12.37(3)</td>
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<tr>
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<td>10.19, 6.13, 2.69, 2.60</td>
<td>17.4</td>
<td>10.5</td>
</tr>
<tr>
<td>H&lt;sub&gt;4&lt;/sub&gt;nta#</td>
<td>9.75, 2.64, 1.57</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>H&lt;sub&gt;4&lt;/sub&gt;tpaa#</td>
<td>4.11(6), 3.3(1), 2.5(2)</td>
<td>10.2(2)</td>
<td>8.5(2)</td>
</tr>
</tbody>
</table>

\* This work. \# From ref. 22. \# From ref. 23 which does not include experimental errors. \# From ref. 8.

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**Fig. 4** Titrations curves (pH vs \(a\); \(a = \text{mol of OH}^- / \text{mol of bpeda}\)) for H<sub>2</sub>bpeda (0.43 mM) and bpeda-M (where M = Ca, Gd) solutions ([bpeda] = 0.43 mM, [M] = 0.38 mM).

**Fig. 5** Variation of the proton NMR chemical shift (ppm) of H<sub>4</sub>bpeda vs. pH at 25 °C.
contribute significantly to the complex stability when included in the 6-methyl-2-pyridinecarboxylic group. The similar values found for the stability constants of the gadolinium complexes of the nonadentate ligand H₅tpaa (log β₃H₃ = 10.2(2)) and of the tetradentate ligand H₅tna (log β₃H₃ = 11.4(3)) are also consistent with a lack of contribution of the pyridyl to the stability in these pyridinecarboxylate systems. This is probably the consequence of a very low basicity of the pyridyl nitrogen due to the electron withdrawing effect of the carboxylate (protonation of the pyridine was not observed). The important effect of the ligand basicity on the thermodynamic stability of Gd(III)pyridinecarboxylate systems. This is probably the consequence of a very low basicity of the pyridyl nitrogen due to the electron withdrawing effect of the carboxylate (protonation of the pyridine was not observed). The important effect of the ligand basicity on the thermodynamic stability of Gd(III) complexes has been recently evidenced by a study on a series of ligands with a similar structural motif.26

Although the stability of the [Gd(bpeda)]⁺ complex is probably rather low for the application as a contrast agent in MRI, the results showed here indicate that an appropriate substitution of the pyridine ring leading to an increased basicity of the pyridyl nitrogen should allow to improve the complex stability. Studies directed to improve the stability of these pyridinecarboxylate complexes through pyridine derivatization are in progress.

**EPR Spectroscopy**

Two typical EPR spectra of Gd(bpeda) in aqueous solution at X- and Q-band are reported in Fig. 6. The X-band spectra display very broad lines (apparent ΔHₚp = 800–1200 G depending on the temperature), centered at g ~ 2 as usual for Gd(III) complexes in aqueous solution. The line shape was notably non-Lorentzian, and could be better described as a superposition of two lines with different intensities, central fields and peak-to-peak widths. The more intense component (double integral of the EPR signal 2–3 times greater than the minor component) is also the broader component (double integral of the EPR signal 2–3 times greater than the minor component), and could be better described as a superposition of two lines with different intensities, central fields and peak-to-peak widths. The more intense component (double integral of the EPR signal 2–3 times greater than the minor component) is also the broader one, with ΔHₚp = 720–840 G, and a g-factor close to 1.93 (B = 3450–3540 G). The minor component is slightly sharper (ΔHₚp = 520–770 G) and appears at a lower magnetic field (g ~ 2.25, B = 2890–3010 G).

![Fig. 6 Baseline-corrected EPR spectra of [Gd(bpeda)]K in aqueous solution: X-band (9.44 GHz) at 297.4 K (left) and Q-band (34 GHz) at 304.5 K (right). The small spikes in the Q-band spectrum are caused by traces of manganese in the clay that seals the EPR capillary tube.](image)

At Q-band, the lines were much sharper, with an approximately Lorentzian shape. The apparent g-factor was about 1.983 (B = 12.250–122.60 G). The peak-to-peak width was between 90 and 110 G depending on the temperature.

A large peak-to-peak width indicates a rapid transverse electron spin relaxation. At X-band, Gd(bpeda) is clearly among the fastest relaxing Gd(III) complexes to date (at X band ΔHₚp = 604 G at room temperature for [Gd(tpa)(H₂O)]⁺).27 At Q-band, its line width is similar to that of [Gd(tpa)(H₂O)]⁺, also fairly broad (103 G at room temperature). The electron spin relaxation has a negative impact on relaxivity, especially for the attractive macromolecular compounds with long rotational correlation times developed in the recent years. Thus our results show that the electronic properties of the Gd(bpeda) chelate are not favorable to its use as a synthon in such an application. This is in qualitative agreement with the NMR data of Muller and coworkers.28 Using the Solomon–Bloembergen–Morgan model modified by Powell et al. to analyze ¹H nuclear magnetic relaxation dispersion (NMRD) and ¹⁷O NMR relaxation rates and chemical shifts, they obtained a transient zero-field splitting magnitude parameters Δ' = 1.2 × 10³ s⁻¹ and correlation time τc = 12.6 ps. With these numbers, one can calculate the transverse electron spin relaxation rates and the peak-to-peak width. Using Powell’s equations we obtain 1053 G at X-band and 279 G at Q-band. With the more rigorous equations of Rast et al.29 the lines are not quite as broad, with ΔHₚp = 796 G at X-band and 237 G at Q-band.

Due to the non-Lorentzian character of the X-band spectra, we could not obtain an acceptable fit of the peak-to-peak widths and central fields using the approach of Rast et al.,28 which can not account for the observed structure of the spectra. Furthermore, the Q-band data on their own are not sufficient since two or more EPR frequencies are required for a reliable analysis. However, the unattractive electronic properties of the complex do not warrant a very high frequency EPR study. A slow electron spin relaxation was estimated from the NMRD profile of [Gd(tpatcn)]²⁺ which has been confirmed by detailed EPR studies displaying for this complex the smallest peak-to-peak widths observed for gadolinium chelates (X-band ΔHₚp = 15 G at room temperature). The remarkably slow electron relaxation of this complex is probably arising from the unusual coordination sphere containing six N-donors associated to the complex symmetry.

However the fast electron spin relaxation observed for the [Gd(bpeda)]K complex indicate that the presence of pyridinecarboxylate chelating groups in itself does not lead to slow electron relaxation. Work in progress is directed to further evaluate the influence on the electronic relaxation of these groups when they are included in different ligand architectures.

**Acknowledgements**

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**References**


