Revisiting Lead(II)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic Acid Coordination Chemistry in Aqueous Solutions: Evidence of an Underestimated Thermodynamic Stability

Marianna Tosato, Luca Lazzari, and Valerio Di Marco*

Cite This: ACS Omega 2022, 7, 15596−15602

ABSTRACT: The complexes formed between Pb²⁺ and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) were reinvestigated in aqueous solutions using a combination of pH potentiometry, UV−vis spectroscopy, and NMR spectroscopy. The thermodynamic data were supported by kinetics assays. Differently protonated complexes, i.e., [PbH₃L]+, [PbH₂L]⁻, [PbHL]⁻, and [PbL]²⁻, were detected, and the corresponding stability constants (logβ) at T = 298 K and I = 0.1 M NaCl were 33.1 ± 0.2, 32.00 ± 0.06, 29.28 ± 0.06, and 25.3 ± 0.1, respectively. Results differed significantly from those previously reported by Chaves et al. (Talanta 1992, 39, 249) and Pippin et al. (Inorg. Chim. Acta 1995, 239, 43) in both the speciation and the overall complex stability; the latter in particular was found to be remarkably higher. The work disclosed herein provides revised data on the Pb²⁺-DOTA complexes, which should be used as a new stability benchmark during the development of lead chelators.

INTRODUCTION

The lead radioisotopes, ²⁰⁳Pb and ²¹²Pb, are attracting growing interest in targeted cancer radiotherapy and radioimaging as they form an ideal theranostic pair.¹−³ Lead radionuclides can be safely driven to the biological target if they are chelated by an appropriate ligand (conjugated to a tumor-seeking vector), forming a highly thermodynamically stable and kinetically inert complex in vivo with Pb²⁺.⁴−⁸

To date, the chelation of lead radioisotopes has been mainly explored with two ligands, the widely used 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and its tetracetamide derivative 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (DOTAM or TCMC) (Figure 1). However, there has been a renewed and increased search for alternative ligands that, better than DOTA and DOTAM, satisfy the stringent demands placed on a metal chelator for use with lead radioisotopes in nuclear medicine applications.⁹−¹⁶ Highly specific Pb²⁺ chelators are also extremely important for lead poisoning prevention and treatment and the remediation of contaminated liquids or environments because of the elevated human toxicity of this heavy metal.¹⁷

When a novel Pb²⁺ chelator is proposed, the first gauge of its performance is given by the thermodynamic properties, i.e., metal−ligand speciation and complex stability constants (logβ), which can be compared with those available in the literature for state-of-the-art DOTA or DOTAM. Data for Pb²⁺-DOTA were given in two rather old manuscripts. In 1992, Chaves et al. detected two 1:1 and two 2:1 metal-to-ligand complexes and reported the corresponding logβ values obtained by potentiometric titrations (Table 1).¹⁸ In 1995, Pippin et al. restudied the Pb²⁺-DOTA complexes via competitive titrations with ethylenediaminetetraacetic acid (EDTA) through UV−vis spectroscopy and detected only two 1:1 complexes (Table 1).² The number, stoichiometry, and stability constants of Pb²⁺-DOTA complexes should be correctly established due to their importance in the above-mentioned research fields. Furthermore, according to our evidence, the stability constant values were underestimated in both studies. Herein, we report a detailed reappraisal of the thermodynamic properties of the Pb²⁺-DOTA complexes in aqueous solutions. A ionic strength of 0.1 M NaCl was chosen

Figure 1. Structures of DOTA and TCMC.

Received: January 19, 2022
Accepted: April 11, 2022
Published: April 29, 2022
Table 1. Acidity Constants of DOTA (pK\textsubscript{a}) and Stability Constants (log\textbeta) of Pb\textsuperscript{2+}-DOTA Complexes Obtained in the Present Work (I = 0.1 M NaCl)a

| reaction | this work | Chaves et al.\textsuperscript{d,e} | Pippin et al.\textsuperscript{e} |
|----------|-----------|-----------------------------------|----------------------------------|
| Pb\textsuperscript{2+} + L\textsuperscript{−} \rightarrow [PbL]\textsuperscript{2−} | 25.3 ± 0.1 \textsuperscript{b} | 22.69 | 24.3 |
| Pb\textsuperscript{2+} + H\textsuperscript{+} + L\textsuperscript{−} \rightarrow [PbHL]\textsuperscript{−} | 29.28 ± 0.06 \textsuperscript{b} | 26.55 | 27.6 |
| Pb\textsuperscript{2+} + 2H\textsuperscript{+} + L\textsuperscript{−} \rightarrow [PbH\textsubscript{2}L]\textsuperscript{−} | 32.0 ± 0.06 \textsuperscript{b} | 31.7 ± 0.1 \textsuperscript{b} | 33.1 ± 0.2 \textsuperscript{b} |
| Pb\textsuperscript{2+} + 3H\textsuperscript{+} + L\textsuperscript{−} \rightarrow [PbHL\textsubscript{2}L]\textsuperscript{−} | 32.8 ± 0.1 \textsuperscript{b} | 32.29 | 25.99 |
| 2Pb\textsuperscript{2+} + L\textsuperscript{−} \rightarrow [Pb\textsubscript{2}L]\textsuperscript{−} | | 29.66 | 16 |
| 2Pb\textsuperscript{2+} + H\textsuperscript{+} + L\textsuperscript{−} \rightarrow [PbH\textsubscript{2}L]\textsuperscript{−} | | | 18 |

\textsuperscript{a}Literature data are reported for comparison. Unless otherwise stated, the reported data are referred to \textit{T} = 298 K. \textsuperscript{b}L denotes DOTA in its fully protonated form. \textsuperscript{c}From ref 20, \textit{I} = 0.15 M NaCl. \textsuperscript{d}From ref 18, \textit{I} = 0.1 M Me\textsubscript{4}NNO\textsubscript{3}. The method was potentiometry. \textsuperscript{e}The reported uncertainty was obtained \textit{T} = 338 K. The method was NMR. \textsuperscript{f}The method was UV−vis spectrophotometric titrations with EDTA. \textsuperscript{g}From ref 2, \textit{I} = 0.1 M NaClO\textsubscript{4}. The method was potentimetry. \textsuperscript{h}The reported data are referred to \textit{C}\textsubscript{Pb\textsuperscript{2+}} = 1 × 10\textsuperscript{−6} M and \textit{C}\textsubscript{DOTA} = 1 × 10\textsuperscript{−5} M at \textit{pH} 7.4.

In this study to more directly relate our results to a blood plasma environment.

\textbf{RESULTS AND DISCUSSION}

**Pb\textsuperscript{2+}-DOTA Formation Kinetics.** The first and obvious requirement to allow rigorous equilibrium data to be obtained in a complex-formation study is that the considered reactions must be at equilibrium when data is collected. This requirement was verified by a preliminary kinetics study in which the time necessary to reach the equilibrium state was qualitatively assessed by mixing Pb\textsuperscript{2+} and DOTA at various pH concentrations. The study was performed using UV−vis spectroscopy. Figures S1 and S2 show the kinetic data obtained at metal and ligand concentrations of 10\textsuperscript{−4} and 10\textsuperscript{−3} M, respectively, which represent the two typical concentration sets employed in the subsequent thermodynamic investigation.

The absorption centered at 258 nm, characteristic of the complexation event, is in agreement with that previously reported in the literature and is assigned to the 6s\textsuperscript{2} → 6p transition of Pb\textsuperscript{2+}. The pH increase in the aqueous medium progressively accelerates the complexation reaction; when the concentration of the two reactants was set to 10\textsuperscript{−4} M, the equilibrium was reached in ~3 h at pH 2.0 and in ~20 min at pH 3.0, while the reaction took place during the mixing time at pH 7.4 (Figure S1). On the other hand, at a concentration of 10\textsuperscript{−3} M, the complexation reaction reached equilibrium in ~10 min at pH 2 and became instantaneous at pH 4.5 (Figure S2). The lower reaction rate at the more acidic pH is ascribed to the increase of the electrostatic repulsion between the metal ion and the protons on the donor sites, which become progressively more protonated (secondary amines of the macrocycle and acetate groups of the side arms).

**Pb\textsuperscript{2+}-DOTA Solution Thermodynamics.** The stability constants (log\beta) of Pb\textsuperscript{2+}-DOTA at \textit{T} = 298 K and \textit{I} = 0.1 M NaCl, summarized in Table 1, were determined using a combination of pH potentiometry, UV−vis spectroscopy, and \textit{1}H NMR spectroscopy. The corresponding distribution diagram is shown in Figure 2, and some typical fitting lines obtained for the potentiometric titrations are shown in Figure S3.

![Figure 2](https://doi.org/10.1021/acsomega.2c00387)

At a concentration of 10\textsuperscript{−3} M, the reaction between Pb\textsuperscript{2+} and DOTA is relatively rapid (vide supra), making the potentiometric method applicable and allowing the speciation model, which involves the presence of differently protonated 1:1 metal−ligand complexes, \textit{i.e.}, [PbH\textsubscript{2}L], [PbHL], and [PbL]\textsuperscript{−}, to be determined (Table 1). The pK\textsubscript{a} values of [PbH\textsubscript{2}L] and of [PbHL] could be determined by potentiometry. However, only a lower limit for the log\beta values can be estimated using this technique due to the high stability of the resulting complexes, which causes the complexation to start at pH levels much lower than 2. The log\beta values were thus determined by UV−vis spectroscopy.

The UV−vis spectra obtained at different pH values from in-batch pH-dependent spectrophotometric titrations are shown in Figure 3. The progressive increase of the absorption band maximum in the investigated pH range (Figure S4) is attributed to the formation of the complexes. The low spectral resolution did not allow evidence of the formation of different protonated species; thus, pK\textsubscript{a} values obtained from pH-potentiometry and NMR (vide infra) were inserted as constant

![Figure 3](https://doi.org/10.1021/acsomega.2c00387)
parameters in the fitting procedure, allowing the determination of logβ value of [PbH,L]+ from which all other formation constants given in Table 1 were computed.

The overall complex stability was evaluated by computing pPb2† (= −log[Pb2†]); a value of 20 was obtained (at pH 7.4, C Pb2† = 10−3 M, and C DOTA = 10−6 M), which was remarkably higher than the values determined using the data of Pippin et al. (pPb2† = 18) and Chaves et al. (pPb2† = 16). According to the speciation data of Pippin et al. and Chaves et al., Pb2† and DOTA should not react significantly at pH ∼2, but our data show that a complex forms in this condition (e.g., see Figures S1 and S2). To further confirm that the complex is stable at acidic pH, thus proving the high complex stability determined in this work, the acid-mediated decomplexation reaction of the Pb2†-DOTA complexes was evaluated by UV–vis spectroscopy. These assays also allowed us to obtain the dissociation rate constants (kobs) and the corresponding half-lives (t1/2) at different HCl concentrations, which are compiled in Table S1. Figure S5 shows the observed variation of the electronic spectra over time at the different pH levels, while representative linear regressions are reported in Figure S6. The Pb2†-DOTA complexes only partially decomplex at pH 2.0, while there is progressively faster and quantitative decomplexation at lower pH, which is in agreement with the thermodynamic data (Figure 2). Starting from a 10−4 M complex concentration, the time necessary to reach the equilibrium conditions was ∼6 h at pH 2.0, ∼3 h at pH 1.0, and ∼7 min at pH 0.0. During the decomplexation at pH 0.0, the time-dependent disappearance of the peak representative of the Pb2†-DOTA complex at 258 nm was accompanied by the appearance of a peak characteristic of the formation of the [PbCl4]2− complex. This was demonstrated by the UV–vis spectra of Pb2†-DOTA solutions recorded in highly acidic pH environments (pH < 1, Figure S7). The maximum absorption at 225 nm was observed at pH 0.8, which was ascribed to the free water ion Pb2†−aq (λmax,Pb2†−aq ≈ 209 nm) undergoing a progressive bathochromic shift up to pH 0.0. At pH 0.0, the maximum absorption is at 245 nm due to lead–chloro complexes [PbCl4]2− (λmax,[PbCl4]2− = 245 nm).

Spectrophotometric titrations were also performed at different metal-to-ligand ratios and different starting ligand concentrations to evaluate the stoichiometric ratio of the metal–ligand complexes. A sharp inflection point was always observed at an equimolar metal-to-ligand ratio (Figure S8). This result confirms the exclusive 1:1 Pb2†-to-DOTA binding stoichiometry, which is in agreement with the speciation obtained by Pippin et al. but in disagreement with that given by Chaves et al.16

The speciation data obtained by potentiometry and UV–vis spectroscopy were further supported by performing a 1H NMR investigation, which also allowed us to gain insight into the aqueous solution structure of the Pb2†-DOTA complexes. NMR was also employed because, to the best of our knowledge, only a very brief note about the NMR properties of Pb2†-DOTA complexes has been provided so far.16

The 1H NMR spectra of free DOTA and Pb2†-DOTA solutions at 298 K are reported in Figures S9 and 4, respectively. The spectra of free DOTA display two signals related to the nitrogen-bound protons of the macrocyclic ring and the methylenic protons of the acetate arms. The addition of Pb2† causes important variations in the chemical shifts and coupling patterns.

![Figure 4](https://doi.org/10.1021/acsomega.2c00387)  
**Figure 4.** 1H NMR spectra of Pb2†-DOTA solutions (400 MHz, T = 298 K, H2O/10% D2O, and C Pb2† = C DOTA = 1 × 10−3 M) at different pH values. The signals related to the free ligand are marked with an asterisk.

These changes were first used to support the stoichiometric results from potentiometry and UV–vis spectroscopy, i.e., the exclusive formation of 1:1 metal-to-ligand complexes. NMR titrations at constant pH and different metal-to-ligand ratios undoubtedly indicate the 1:1 stoichiometry because the spectra display significant changes upon the addition of Pb2† when the metal-to-ligand ratio was below 1:1, whereas no further changes are observed upon the addition of Pb2† equivalents larger than one (Figure S10).

Second, the spectra indicate that the Pb2†-DOTA complexes coexist with the unbound (free) ligand only at very acidic pH levels (Figure 4), which is in agreement with the pH potentiometric and UV–vis data. When the pH increases, the peaks related to free DOTA gradually disappear while those attributed to the metal complexes become progressively more intense. A broad multiplet spanning between 2.85 and 3.24 ppm is noticeable at pH > 1. This resonance is defined progressively with the decrease of the proton content, splitting into three partially overlapped multiplets at 2.80, 3.14, and 3.29 ppm at pH > 2.92; with the aid of 1H-207Pb TOCSY experiment, these multiplets were attributed to the CH2 protons of the polyazaamycrosclof scaffold (Figure S11). At the same time, the singlet attributed to the acetate protons of the free ligand (∼3.91 ppm at pH 1) is gradually replaced by two broad resonances, which experience an increased chemical shift separation (Δδ = 0.1 ppm at pH 2.0 and Δδ = 0.4 ppm at pH 9.92) and end up appearing as two poorly resolved but well-separated multiplets at pH > 2.92.

The presence of many broad signals combined with the absence of 1H-207Pb satellites indicates dynamic coordination.
between the ligand and the metal at 298 K. On the contrary, the exchange with the free ligand does not take place or occurs in a time scale greater than that of the NMR experiment, since the signals related to the free ligand are always recognizable.

At pH > 5, the spectra are unchanged, thus indicating that the same complex exists in solution. According to potentiometry, this species is \([\text{PbL}]^{2-}\). In agreement with the potentiometric data \((\text{vide supra})\), the recognizable peaks that shift toward higher fields and are detected in the range 1.0 \(\leq \text{pH} \leq 5.08\) can be ascribed to the copresence of the differently protonated complexes \((\text{Figure 2})\). However, the presence of the broad multiplets complicates both the peak selection and the relative area calculation, thus preventing the determination of unique \(pK_a\) values for the protonated species.

Variable-temperature \(^1\text{H}\) NMR spectra were thus collected to circumvent this problem and to investigate the intramolecular dynamic exchange processes that occur in the \([\text{Pb}^{2+}]\)-DOTA complexes.

**Figure S12** presents the \(^1\text{H}\) NMR spectra of \([\text{Pb}^{2+}]\)-DOTA solutions at several temperatures \((278 \text{ to } 338 \text{ K})\) and constant pH. All signals become broader as the temperature increases from 298 to 318 K \((\text{coalescence temperature})\), but a further increase in temperature to 338 K leads to less complicated and more resolved spectra when compared to those recorded at 298 K, likely thanks to the faster intramolecular exchange kinetics. Spectra at 338 K were thus collected as a function of the pH, and they are shown in **Figure S13**.

All spectra at pH > 4 are identical to each other, thus indicating the presence of only one complex that, according to the potentiometric data at 298 K, should be \([\text{PbL}]^{2-}\). The two very broad singlets at 2.97 and 3.56 ppm are associated with the NCH\(_2\) and the acetate protons, respectively. These spectral features suggest that \([\text{PbL}]^{2-}\) is a highly symmetric octacoordinated complex and point to a fast interconversion between isomers. In reality, the high symmetry of \([\text{PbL}]^{2-}\) was confirmed in the solid-state by X-ray spectroscopy.\(^{23}\)

Upon lowering the pH, the NCH\(_2\) signals split into two quasi-symmetrical broad multiplets, whereas the acetate signals retained a singlet structure. Nonetheless, all these signals shift remarkably with the decrease in pH \((\text{Figure S13})\). Data fitting allowed us to determine the \(pK_a\) values for \([\text{PbH}_2\text{L}]^+\) and \([\text{PbHL}]^-\) that were associated with the different protonation steps \((\text{Table 1})\). These values are in good agreement with those obtained by pH potentiometry at 298 K, pointing out that the protonation processes are only slightly affected by temperature. The very acidic pH \((<2)\) investigated by NMR spectroscopy allowed to recognize the presence of an additional species with a \(pK_a\) of 1.1, which was assigned to \([\text{PbH}_3\text{L}]^+\). Despite the fact that this species was detected at 338 K, it can be assumed that \([\text{PbH}_3\text{L}]^+\) exists also at 298 K.

Due to the small temperature dependence of the other \(pK_a\) values highlighted above, the \(pK_a\) of \([\text{PbH}_2\text{L}]^+\) can also be set to roughly 1.1 at 298 K.

When the samples are cooled to 278 K, all the signals become sharp \((\text{Figure S14})\) and their multiplicity is recognizable, thus suggesting that these complexes are present only as one conformer. At pH > 4, where \([\text{PbL}]^{2-}\) should exist, the two doublets at 3.78 and 3.40 ppm \((\text{area} \ 4, J = 15 \text{ Hz})\) were attributed to the two CH\(_2\) protons of the acetate side arms, while the two triplets at 3.33 and 3.15 ppm \((\text{area} \ 4, J = 15 \text{ Hz})\) and the two partially overlapped doublets at 2.80 ppm \((\text{area} \ 4, J = 16 \text{ Hz})\) were assigned to the NCH\(_2\) protons of the.

![Figure 5. \(^1\text{H}\) NMR spectra of \([\text{Pb}^{2+}]\)-DOTA solutions (400 MHz, \(T = 338 \text{ K}, \text{H}_2\text{O/10}\% \text{D}_2\text{O}, \text{and} \ C_{\text{Pb}^{2+}} = C_{\text{DOTA}} = 1 \times 10^{-3} \text{M}\)) at different pH values.](https://doi.org/10.1021/acsomega.2c00387)

ring. This spectrum is fairly similar to those reported for the \([\text{PbL}]^{2+}\) complex formed by DOTAM\(^{17}\) and seems consistent with a \([\text{PbL}]^{2+}\) complex in which all the CH\(_2\) protons became non-magnetically equivalent after the metal chelation, differentiating both sides of the cyclen ring. The intramolecular dynamic exchange processes observed for the \([\text{PbL}]^{2-}\) complex of DOTA, which are responsible for the line broadening observed at room temperature, are likely related to the interconversion processes that can occur via pendant arm rotation and macrocyclic chelate ring inversion. The estimated average activation free energies for the dynamic processes \((\Delta G^\ddagger \approx 62-67 \text{ kJ/mol})\) are very close to those determined for DOTAM \((62-64 \text{ kJ/mol})\).\(^{17,24}\) The NMR spectra at 278 K showed a pH-dependent variation of the chemical shift similar to that observed at 338 K \((\text{Figure S15})\), indicating that several protonation states are also present at this low temperature. However, the data could not be fitted and no \(pK_a\) values were here determined.

**EXPERIMENTAL SECTION**

**Materials.** 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was obtained from Chematech. Lead
H2O/10% D2O (Sigma-Aldrich, 99.9% D) at a concentration (Sigma-Aldrich, 99%). All the solutions were prepared in ultrapure water (18.2 MΩ-cm, Purelab Chorus system, Veolia).

Pb2+-DOTA Formation Kinetics. The Pb2+-DOTA formation kinetics was assessed through UV−vis spectrophotometry using a Cary 60 UV−vis spectrophotometer (Agilent). Equimolar amounts of metal ion and ligand were mixed in buffered aqueous solutions at pH 2.0 (1.0 × 10−3 M HCl), 3.0 (1.0 × 10−3 M HCl), 4.5 (acetic acid/acetate), and 7.4 (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid - HEPES) at room temperature. The electronic spectra were collected immediately after mixing and at different time points in the range from 200 to 800 nm using a quartz spectrophotometric cell with a 1 cm path length. Different concentrations ranging from 1 × 10−4 to 1 × 10−3 M were employed.

The complexation reaction was monitored directly over time using the increase of the absorption peak characteristic of Pb2+-DOTA complex formation.

Pb2+-DOTA Solution Thermodynamics. Potentiometry. The experimental procedures and details of the potentiometric apparatus closely followed those in our reported studies. DOTA and lead stock solutions were prepared by the direct standardized using complexometric titrations with ethylenediaminetetraacetic acid (EDTA), and xylenole orange was used as an indicator. The solution was fixed to 0.1 M using sodium chloride (NaCl) as a background electrolyte. Each experiment was performed independently at least three times.

pH-Dependent and Variable-Temperature NMR. NMR spectra of free DOTA and its Pb2+ complexes at different pH levels were collected using a 400 MHz Bruker Avance III HD spectrometer or a 600 MHz Bruker DMX 600 spectrometer. The temperature limits investigated were set between 278 and 318 K. No temperature was considered out of this range to avoid damaging the probe due to the solvent either freezing and breaking the NMR tube or boiling and leaking. Proton chemical shifts (δ) are reported in parts per million (ppm) and are referred to 3-(trimethylsilyl)propionic acid sodium salt (Sigma-Aldrich, 99%). All the solutions were prepared in H2O/10% D2O (Sigma-Aldrich, 99.9% D) at a concentration of ∼10−3 M. The pH was adjusted with small additions of HCl or NaOH and measured with the same setup used for the potentiometric measurements. The water signal was suppressed using an excitation-sculpting pulse scheme.

pH-Dependent UV−vis Spectrophotometric Titrations. UV−vis titrations were carried out using the out-of-cell methods in the pH range 0−12 at 298 K. Stock solutions of DOTA and PbCl2 were mixed in independent vials to obtain a 1:1 metal-to-ligand molar ratio (final concentration of ∼10−4 M), and different amounts of HCl and NaOH were added to adjust the pH. The pH was measured with a Mettler Toledo pH meter equipped with a glass electrode calibrated daily with commercial buffer solutions (pH 4.0 and pH 7.0), whereas in very acidic solutions (pH ≪ 2) it was computed from the HCl concentration (pH = −log [HCl]). The absorption spectra were recorded using the same apparatus described above for the kinetic measurements. The equilibrium was considered to be reached when no variations of the UV−vis spectra or pH were detected.

Titrations at Different Metal-to-Ligand Molar Ratios. Different aliquots of the Pb2+ stock solution were added to the ligand solution buffered at pH 4.8 by acetic acid/acetic acid to obtain a metal-to-ligand ratio that varied from 0:1 to 4:1. Different concentrations were screened, i.e., C(m)/C(d) = 1 × 10−4 M and C(DOTA) = 1 × 10−3 M (final concentration). The UV−vis spectra were recorded, and the stoichiometry of the complex was determined by plotting the absorbance at the characteristic wavelength as a function of the metal-to-ligand ratios (n(Pb2+)/n(DOTA)).

The same experiments were also conducted by collecting the 1H NMR spectra of solutions buffered at pH 4.5 by acetic acid/acetic acid at different lead-to-DOTA molar ratios (C(m)/C(DOTA) = 1 × 10−3 M). Spectra were acquired using the same protocol described above.

Data Analysis. The equilibrium constants were obtained by refining the potentiometric and UV−vis spectrophotometric data using the PITMAP software. All data refer to the equilibria pMn+ + qH+ + rL− ⇌ MnHrLq− + qr, where M is the metal ion and L the nonprotonated ligand. The constants for ligand deprotonation and Pb2+ hydrolysis were taken from the literature.

Pb2+-DOTA Dissociation Kinetics. The dissociation kinetics of the Pb2+-DOTA complex was studied under pseudo-first-order conditions in an aqueous solution at room temperature without control of the ionic strength by adding concentrated aqueous solutions of HCl (0.01, 0.1, and 1 M) to aqueous solutions of the preformed complex. The concentration of the Pb2+-DOTA complex after dilution was 1 × 10−4 M.

The dissociation reaction was followed by the intensity of the absorption band of the complexes decreasing over time. The spectroscopic data were processed to obtain a ln A0 vs ln t” where A0 and A(t) are the absorbances at time t and at the beginning of the reaction, respectively, and kobs is the observed dissociation rate constant. Each measurement was repeated at least in triplicate.

CONCLUSION

Herein, we have reported a thermodynamic reinvestigation of the Pb2+-DOTA complexes through a combination of pH potentiometry, UV−vis spectrophotometry, and NMR spectroscopy, demonstrating that the speciation obtained in the previous studies was uncorrected and that the corresponding formation constants were underestimated. The involvement of 2:1 metal-to-ligand species considered in the case of Chaves et al., and the complicated ternary system employed for the measurements by Pippin et al. (who studied solutions containing Pb2+), DOTA, and EDTA), might at least in part explain the observed differences. This work provides revised and accurate thermodynamic data for the very relevant Pb2+-DOTA complexes, which should be used as a new stability benchmark during the development of lead chelators.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00387.
Example fitting of potentiometric titrations, UV−vis spectra at equilibrium conditions, UV−vis and NMR spectra at constant pH and various metal-to-ligand ratios. UV−vis spectra showing the complexation and decomplexation kinetics between Pb$^{2+}$ and DOTA, decomplexation fittings for the determination of $k_{obs}$ mono- and bidimensional NMR spectra of Pb$^{2+}$−DOTA solutions at equilibrium conditions at various temperatures, example of fitting of NMR titrations, half times, and $k_{obs}$ values (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
Valerio Di Marco − Department of Chemical Sciences, Università degli Studi di Padova, Padua, Padua 35131, Italy; orcid.org/0000-0001-6108-746X; Phone: +390498275219; Email: valerio.dimarco@unipd.it

**Authors**
Marianna Tosato − Department of Chemical Sciences, Università degli Studi di Padova, Padua, Padua 35131, Italy; orcid.org/0000-0002-3726-6174

Luca Lazzari − Department of Chemical Sciences, Università degli Studi di Padova, Padua, Padua 35131, Italy; orcid.org/0000-0003-0415-4042

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c00387

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**
This research work was supported by the P-DiSC#02-BIRD2021-UNIPD project of the Department of Chemical Sciences (University of Padova - Italy).

**REFERENCES**

(1) Bauer, D.; Blumberg, M.; Köckerling, M.; Mamat, C. A Comparative Evaluation of Calix[4]Arene-1,3-Crown-6 as a Ligand for Selected Divalent Cations of RadioPharmaceutical Interest. RSC Adv. 2019, 9 (55), 32337−32366.

(2) Pippin, C. G.; McMurry, T. J.; Brechbiel, M. W.; McDonald, M.; Lambrecht, R.; Milenic, D.; Roselli, M.; Colcher, D.; Gansow, O. A. Lead(II) Complexes of 1,4,7,10-Tetraazacyclododecane-$N,N',N''$'-Tetraacetate: Solution Chemistry and Application to Tumor Localization with 203Pb Labeled Matriol Antibodies. Inorg. Chem. Acta 1995, 239, 43−51.

(3) Ferrier, M. G.; Radchenko, V.; Wilbur, D. S. Radiochemical Aspects of Alpha Emitting Radionuclides for Medical Application. Radioicho. Acta 2019, 107 (9−11), 1065−1085.

(4) Tosato, M.; Asti, M.; Dalla Tiesa, M.; Orian, L.; Häussinger, D.; Vogel, R.; Köster, U.; Jensen, M.; Andrighetto, A.; Pastore, P.; Di Marco, V. Highly Stable Silver(I) Complexes with Cyclen-Based Ligands Bearing Sulfide Arms: A Step Toward Silver-111 Labeled Radiopharmaceuticals. Inorg. Chem. 2020, 59 (15), 10907−10919.

(5) Tosato, M.; Dalla Tiesa, M.; May, N. V.; Isse, A. A.; Nardella, S.; Orian, L.; Verona, M.; Vaccarin, C.; Alker, A.; Mäcke, H.; Pastore, P.; Di Marco, V. Copper Coordination Chemistry of Sulfur Pendant Cyclen Derivatives: An Attempt to Hinder the Reductive-Induced Demetallation in 64/67Cu Radiopharmaceuticals. Inorg. Chem. 2021, 60 (15), 11530−11547.

(6) Ramogida, C.; Orvig, C. Tumour Targeting with Radionuclides for Diagnosis and Therapy. Chem. Commun. 2013, 49 (42), 4720−4739.

(7) Price, E. W.; Orvig, C. Matching Chelators to Radionuclides for Radiopharmaceuticals. Inorg. Chem. 2014, 43 (1), 260−290.

(8) Boros, E.; Packard, A. B. Radioactive Transition Metals for Imaging and Therapy. Chem. Rev. 2019, 119 (2), 870−901.

(9) Lange, J. L.; Davey, P. R. W.; Ma, M. T.; White, A. J. M.; Morgenstern, A.; Bruchertseifer, F.; Blower, P. J.; Paterson, B. M. An Octadentate Bis(Semicarbazone) Macrocyle: A Potential Chelator for Lead and Bismuth Radiopharmaceuticals. Dalton. Trans. 2020, 49 (42), 14962−14974.

(10) Ingham, A.; Kostelnik, T. I.; McNeil, B. L.; Patrick, B. O.; Choudhary, N.; Jaraquemada-Peláez, M. d. G.; Orvig, C. Getting a Lead on Pb$^{2+}$-Amide Chelators for 203/212Pb Radiopharmaceuticals. Dalton. Trans. 2021, 50 (33), 11579−11595.

(11) Regueiro-Figueroa, M.; Ruscáš; E.; Fra, L.; Tircsó, G.; Tóth, I.; de Blas, A.; Rodriguez-Blas, T.; Platas-Iglesias, C.; Esteban-Gómez, D. Highly Stable Complexes of Divalent Metal Ions (Mg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$) with a DOTA-Like Ligand Containing a Picolineantendant. Eur. J. Inorg. Chem. 2014, 2014 (36), 6165−6173.

(12) Lima, L. M. P.; Bleyer, M.; Delgado, R.; Platas-Iglesias, C.; Tripier, R. Investigating the Complexation of the Pb$^{2+}$/Bi$^{3+}$ Pair with Dipicolinate Cyclen Ligands. Inorg. Chem. 2015, 54 (14), 7045−7057.

(13) McNeil, B. L.; Robertson, A. K. H.; Fu, W.; Yang, H.; Hoeher, C.; Ramogida, C. F.; Schaffer, P. Production, Purification, and Radiolabeling of the 203/212Pb Theranostic Pair. EJNMMI Radiopharm. Chem. 2021, 6, 6.

(14) McDonagh, A. W.; McNeill, B. L.; Patrick, B. O.; Ramogida, C. F. Synthesis and Evaluation of Bifunctional [2.2.2]-Cryptands for Nuclear Medicine Applications. Inorg. Chem. 2021, 60 (13), 10030−10037.

(15) Stenberg, V. Y.; Juzeniene, A.; Bruland, Ø. S.; Larsen, R. H. In Situ Generated 212Pb-PSMA Ligand in a 224Ra-Solution for Dual Targeting of Prostate Cancer Sclerotic Stonoma and PSMA-Positive Cells. Curr. Radiopharm. 2020, 13 (2), 130−141.

(16) Chappell, L. L.; Deal, K. A.; Dadachova, E.; Brechbiel, M. W. Synthesis, Conjugation, and Radiolabeling of a Novel Bifunctional Chelating Agent for $^{212}$Ac Radioimmunotherapy Applications. Bioconjug Chem. 2020, 31 (4), 510−519.

(17) Cuenot, F.; Meyer, M.; Espinosa, E.; Bucaile, A.; Burgat, R.; Guilard, R.; Marzial-Westrich, C. New Insights into the Complexation of Lead(II) by 1,4,7,10-Tetra(carbamoylmethyl)-1,4,7,10-Tetrazacyclododecane (DOTAM): Structural, Thermodynamic, and Kinetic Studies. Eur. J. Inorg. Chem. 2008, 2008 (2), 267−283.

(18) Chaves, S.; Delgado, R.; Da Silva, J. J. The Stability of the Metal Complexes of Cyclic Tetra-Aza Tetra-Acetic Acids. Talanta 1992, 39 (3), 249−254.

(19) Kumar, K.; Magerstädt, M.; Gansow, O. A. Lead(II) and Bismuth(III) Complexes of the Polyazacycloalkane-N-Acetic Acids NOTA, DOTA, and TETA. J. Chem. Soc. Chem. Commun. 1989, 3, 145−146.

(20) Di Marco, V.; Pastore, P.; Tosato, M.; Andrighetto, A.; Borgna, F.; Realdon, N. pH-Static Titrations for Kinetic Studies of Metal-Ligand Complex Formation: The Case Example of the Reaction between Strontium(II) and DOTA. Inorg. Chem. Acta 2019, 498, 119147.

(21) Ševčík, R.; Vanek, J.; Lubal, P.; Kotkowsk, Z.; Kotek, J.; Hermann, P. Formation and Dissociation Kinetics of Copper(II) Complexes with Tetraphosphorus Acid DOTA Analogues. Polyhedron 2014, 67, 449−455.

(22) Kasprzyk, S. P.; Wilkins, R. G. Kinetics of Interaction of Metal Ions with Two Tetrazatetraacetate Macrocycles. Inorg. Chem. 1982, 21 (9), 3349−3352.

(23) Nugent, J. W.; Lee, H. S.; Reibenspies, J. H.; Hancock, R. D. Spectroscopic, Structural, and Thermodynamic Aspects of the Stereochamically Active Lone Pair on Lead(II): Structure of the Lead(II) DOTA Complex. Polyhedron 2015, 91, 120−127.

(24) Maunula, H.; Hancock, R. D.; Carlton, L.; Reibenspies, J. H.; Wainwright, K. P. The Amide Oxygen as a Donor Group. Metal Ion Complexing Properties of Tetra-N-Acetamide Substituted Cyclen: A Crystallographic, NMR, Molecular Mechanics, and Thermodynamic Study. J. Am. Chem. Soc. 1995, 117 (25), 6698−6707.
(25) Tosato, M.; Verona, M.; Doro, R.; Dalla Tiezza, M.; Orian, L.; Andrighetto, A.; Pastore, P.; Marzaro, G.; Di Marco, V. Toward Novel Sulphur-Containing Derivatives of Tetraazacyclododecane: Synthesis, Acid–Base Properties, Spectroscopic Characterization, DFT Calculations, and Cadmium(II) Complex Formation in Aqueous Solution. *New J. Chem.* 2020, 44 (20), 8337–8350.

(26) Hwang, T. L.; Shaka, A. J. Water Suppression That Works. Excitation Sculpting Using Arbitrary Wave-Forms and Pulsed-Field Gradients. *J. Magn Reson Ser. A* 1995, 112 (2), 275–279.

(27) Di Marco, V. Study of complex formation between Al(III) and molecules of environmental, pharmacological and physiological interest. PhD Thesis, Univeristy of Padova, Padua, Italy, 1998.

(28) Powell, K.; Brown, P.; Byrne, R.; Gajda, T.; Heffer, G.; Leuz, A. K.; Sjöberg, S.; Wanner, H. Chemical Speciation of Environmentally Significant Metals with Inorganic Ligands. Part 3: The Pb²⁺ + OH⁻, Cl⁻, CO₃²⁻, SO₄²⁻, and PO₄³⁻ Systems (IUPAC Technical Report). *Pure Appl. Chem.* 2009, 81 (12), 2425–2476.