

^{207}Pb – ^1H Two-Dimensional NMR Spectroscopy: A Useful New Tool for Probing Lead(II) Coordination Chemistry

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Despite the fact that lead poisoning is the most common disease of environmental origin in the United States, the spectroscopic properties of aqueous Pb(II) coordination compounds have not been extensively investigated. Spectroscopic techniques that can be used to probe the fundamental coordination chemistry of Pb(II) will aid in both the development of water-soluble ligands that bind lead both tightly and selectively and the characterization of potential biological targets. Here, we report the preparation and characterization of a series of Pb(II) complexes of amido derivatives of EDTA. The ^{207}Pb chemical shift observed in these complexes (2441, 2189, and 1764 ppm for $[\text{Pb}(\text{EDTA})]^{2-}$, $\text{Pb}(\text{EDTA}-\text{N}_2)$, and $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$, respectively) provides an extremely sensitive measure of the local environment and the charge on each complex. These shifts help to map out the lead chemical shift range that can be expected for biologically relevant sites. In addition, we report the first two-dimensional ^{207}Pb – ^1H heteronuclear multiple-quantum correlation (HMQC) nuclear magnetic resonance spectra and demonstrate that this experiment can provide useful information about the lead coordination environment in aqueous Pb(II) complexes. Because this technique allows ^{207}Pb – ^1H couplings through three bonds to be identified readily, ^{207}Pb – ^1H NMR spectroscopy should prove useful for the investigation of Pb(II) in more complex systems (e.g., biological and environmental samples).

Introduction

Lead is a ubiquitous environmental contaminant. Although the use of lead in gasoline and paint has now been banned in most developed countries, lead is still one of the 10 most common contaminants found in clean up sites targeted by the National Priorities List (Superfund) and the Department of Defense.¹ As a result, lead poisoning is the most common disease of environmental origin in the United States, affecting an estimated 1 in 20 preschool-aged children.² Most lead poisoning results from exposure to divalent or “inorganic” lead (Pb(II)) from inhalation or ingestion of leaded paint (PbO), ingestion of Pb(II) that has leached into the water supply from lead pipes and solder, and exposure to contaminated soil.^{3,4}

Despite the prevalence of this problem, several facets of the fundamental aqueous coordination chemistry of Pb(II) have not been investigated. Techniques are needed that will allow the lead coordination environment to be investigated in complex systems (e.g., biological or environmental samples). A better ability to characterize aqueous Pb(II) coordination complexes in situ will aid in the development of ligands that will bind lead both tightly and *selectively* and which could be used to detect lead and treat lead poisoning more effectively. Although the thermodynamics of lead complexation in aqueous solution

has been studied extensively,⁵ relatively few *spectroscopic* studies on the aqueous coordination chemistry of Pb(II) have been reported. Such spectroscopic studies have been stymied by the widespread misconception that Pb(II) ($[\text{Xe}]4f^{14}5d^{10}6s^2$) is spectroscopically silent. However, we have recently reported that Pb(II) complexes can exhibit intense charge-transfer bands when lead is bound to thiolate groups, and that these bands can be used to provide quantitative information about lead complexation in biological systems.⁶ In addition, we discuss here how ^{207}Pb NMR spectroscopy can provide useful insights into lead coordination chemistry: there is one lead isotope with a nuclear spin that can be exploited for NMR spectroscopy (^{207}Pb , $I = 1/2$) that has an excellent receptivity (11.7 times greater than that of ^{13}C), high natural abundance (22.6%), and large chemical shift range (over 16 000 ppm).^{7,8} Although ^{207}Pb NMR spectroscopy has been used extensively to characterize alkyl lead derivatives (Pb(IV)) and solid state Pb(II) compounds, relatively few studies have been conducted on soluble Pb(II) coordination compounds.^{7,9–19} Furthermore, the dependence of the Pb NMR

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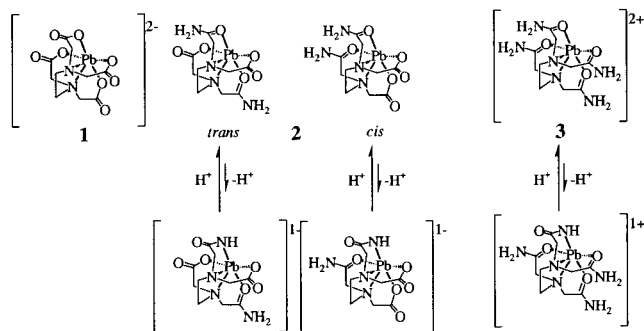


Figure 1. A series of isostructural lead complexes were prepared: Pb ethylenediaminetetraacetic acid ($[\text{Pb}(\text{EDTA})]^{2-}$ **1**),⁴⁵ Pb(ethylenediamine-*N,N'*-bis(acetic acid)-*N,N'*-bis(acetamide)) ($[\text{Pb}(\text{EDTA}-\text{N}_2)]^{2-}$, **2**), and Pb(ethylenediamine tetraacetamide) ($[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$, **3**). Compound **2** can exhibit geometric isomers. In addition, lead could conceivably substitute for an amide hydrogen (N-bound) in either compound **2** and compound **3**, to form a deprotonated N-bound form of these compounds. ^1H and ^{207}Pb NMR studies were conducted to determine whether the N-bound forms of these complexes are present under physiologically relevant conditions.

parameters upon the Pb(II) coordination environment has not been systematically investigated.

Specifically, model systems are needed for lead bound to Ca- and Zn-binding sites in proteins; these interactions are thought to account for lead's toxicity, but have not been systematically characterized using ^{207}Pb NMR spectroscopy. Here, we report ^{207}Pb NMR data for a series of lead complexes that provide good model systems for Ca-binding sites in proteins. Our initial studies focus on lead complexes of amido derivatives of ethylenediaminetetraacetic acid (EDTA) (Figure 1). These complexes were selected not only because they serve as good models for Ca sites in proteins but also because they have potential as chelation therapy agents for lead poisoning.^{20,21} Although CaNa_2EDTA is the most commonly used chelation therapy agent in the United States,²² there is a great deal of room for improvement on this system.²³ In particular, better selectivity for lead and water solubility at neutral pH are needed.⁴

Out of all of the possible derivatives of EDTA, the amido derivatives are of particular interest because Battistuzzi et al. recently reported that amide functionalities can confer specificity for Pb(II) over other metal ions.²⁴ In recent years, a wide range of amide-functionalized chelating agents has been developed for use in magnetic resonance imaging applications. These compounds make excellent pharmacological agents because they generally exhibit low toxicity and good solubility.^{25–27} However, the vast majority of these agents have been designed to bind

Gd(III), which has different chelate ring size and functional group preferences than does Pb(II).²⁸ By contrast, relatively few amido Pb(II) complexes have been reported⁵ and even fewer have been structurally characterized: a search of the Cambridge Structural Database^{29,30} turns up only five structures of Pb(II) bound by an amido moiety.^{31–35} In each of these crystal structures, Pb(II) is bound by the carbonyl oxygen of the amide. This contrasts sharply with the behavior that has been observed for Pd(II), Cu(II), and Ni(II), which have each been observed to substitute for an amido hydrogen, particularly in polydentate compounds.³⁶ These reports raise a series of interesting questions: (1) How does amide functionalization of lead chelating agents affect the lead coordination environment? (2) Does the amido group coordinate to lead via the carbonyl oxygen (O-bound) or via the amide nitrogen (N-bound) in solution under physiologically relevant conditions? (3) Can ^{207}Pb NMR help to address these questions?

To explore these issues, we have prepared a series of amido derivatives of EDTA,^{37,38} characterized their lead complexes, and compared the properties of the amido derivatives to those of $[\text{Pb}(\text{EDTA})]^{2-}$ (**1**). The initial preparation of lead(II) ethylenediamine-*N,N'*-bis(acetic acid)-*N,N'*-bis(acetamide) ($[\text{Pb}(\text{EDTA}-\text{N}_2)]^{2-}$ (**2**)) and lead(II) ethylenediamine tetraacetamide ($[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ (**3**)) and the characterization of these complexes using ^{207}Pb NMR spectroscopy are reported. In addition, we report ^{207}Pb – ^1H heteronuclear multiple-quantum correlation (HM-QC)³⁹ spectra for $[\text{Pb}(\text{EDTA})]^{2-}$ and $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$. These two-dimensional ^{207}Pb – ^1H NMR spectra are unprecedented in the literature. On the basis of these spectra, we are able to make specific predictions about the lead coordination environment of these complexes in solution, which correlate well with the structure of $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ we observe in the solid state (vide infra). Taken together, these studies provide compelling evidence that ^{207}Pb NMR spectroscopy and multidimensional NMR techniques will be a useful methodology for probing lead coordination environments in complex samples.

Experimental Section

General Materials and Methods. Reagents were used as received from Aldrich unless otherwise indicated. Ammonia gas was purchased

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from Mattheson. Methanol was distilled from Mg turnings. 4,4'-Ethane-1,2-diylbis(morpholine-2,6-dione) (EDTA dianhydride)^{27,40,41} and ethylenediaminetetracetic acid tetramethyl ester (EDTA(OMe)₄)⁴² were prepared according to the literature procedures. Lead complexes were prepared by addition of Pb(NO₃)₂ (dissolved in D₂O or H₂O) to a solution of the ligand dissolved in D₂O/H₂O; pH was adjusted using NaOH/NaOD or HNO₃/DNO₃. Isotopically enriched ²⁰⁷Pb(NO₃)₂ (92.4% isotopically enriched) was obtained from Cambridge Isotope Laboratories.

One-dimensional ²⁰⁷Pb spectra were recorded on a Varian Unity Plus 400 MHz NMR spectrometer at 25 °C. Between 12 800 and 128 000 data points were collected in 0.64–1.0 s using a calibrated 90° pulse width. Sweep widths of 10–100 kHz were used to locate the ²⁰⁷Pb resonances. A delay time of 1–3 s was used between cycles (*T*₁ ~ 0.4 s) and 700–2000 transients were acquired. The data were zero filled to 16K or 32K complex points and Fourier transformation with a line broadening of 10–50 Hz. The data for EDTA required a sine bell window function to correct for the nonzero intensity of the FID tail. The lead chemical shifts were referenced to an external 1.0 M Pb(NO₃)₂ sample in 99.9% D₂O at pH* 3.3.

One-dimensional ¹H NMR spectra were collected on a Varian Gemini 300 MHz spectrometer or a 400 MHz Varian Unity Plus spectrometer and are referenced to internal TMS (CDCl₃) or to the residual protonated solvent (for D₂O). Between 4K and 8K complex data points were collected in 0.4–0.9 s using a calibrated 90° pulse width. A low power solvent suppression pulse of 1–1.5 s was applied for suppression of the H₂O and HOD proton resonances. A delay time of 2.5–3 s was used between cycles and 256–512 transients were acquired. The data were zero filled to 16K or 32K complex points and Fourier transformation without further adjustments.

Two-dimensional ²⁰⁷Pb-¹H NMR spectra were recorded on a Varian Unity Plus 400 MHz NMR spectrometer using a 5 mm inverse probe, calibrated pulse widths, and a low power water suppression pulse at 25 °C. For the spectrum of [Pb(EDTA-N₄)]²⁺, a total of 2K data points were acquired for each increment with an acquisition time of 0.41 s (spectral width of 2500 Hz). 256 complex data points were used in the indirect dimension (²⁰⁷Pb) to cover 20 000 Hz. Shifted sine bell window functions were used in both dimensions, and the spectra were baseline corrected in the proton dimension. For the spectrum of [Pb(EDTA)]²⁻, the direct dimension was acquired in 0.128 s with 2K data points (spectral width of 8000 Hz) and 512 complex data points in the indirect dimension (spectral width of 20 000 Hz). A shifted sine bell function and baseline correction were used in both directions. For both spectra, the data were zero filled to a matrix of 8K complex data points in the direct dimension and 2K complex data points in the indirect dimension. The lead chemical shifts were referenced to an external 1 M Pb(NO₃)₂ sample in 99.9% D₂O at pH* 3.3.

Mass spectra were recorded on a Micromass Quattro II electrospray triple quadrupole mass spectrometer in the Analytical Services Laboratory at Northwestern University. Elemental analyses (C, H, N) were performed by Midwest Microlab. X-ray diffraction data were collected on a Bruker SMART-1000 single-crystal X-ray diffractometer with a CCD detector in the Analytical Services Laboratory at Northwestern University.

Safety Note: The chemical shift ²⁰⁷Pb NMR resonances have been referenced to Pb(CH₃)₄ most frequently in the past.⁷ The Pb(CH₃)₄ standard is relatively insensitive to sample conditions, whereas the chemical shift exhibited by Pb(NO₃)₂ is highly sensitive to sample concentration, temperature, and pH.⁴³ Nonetheless, we advocate use of a standardized external sample of Pb(NO₃)₂ (in our case, a 1 M Pb(NO₃)₂ sample in 99.9% D₂O at pH* 3.3, which yields a reference ~-2960 ppm relative to Pb(CH₃)₄⁷ because Pb(NO₃)₂ is significantly less toxic and is safer to handle than Pb(CH₃)₄.

Synthesis. Preparation of Ethylenediamine-*N,N'*-bis(acetic acid)-*N,N'*-bis(acetamide), EDTA-N₂.³⁸ Ammonia gas is bubbled through a solution of EDTA dianhydride (2.38 g, 9.27 mmol) in DMF (225 mL) for 4 h. A white powder is isolated by filtration and recrystallized from CH₃OH:H₂O layered with isobutanol (1.53 g, 5.27 mmol, 56.8% yield). ¹H NMR (99.9% D₂O, pH* 4.7, 25 °C) δ 2.64 ppm (s, 4H, NCH₂CH₂N), 3.21 (s, 4H, NCH₂CO), 3.13 (s, 4H, NCH₂CO). MS (electrospray) M + 1 observed at 291.1 (calculated 290). Anal. Calcd for C₁₀H₁₈N₄O₆: C, 41.38; H, 6.25; N, 19.30. Found: C, 41.28; H, 6.23; N, 18.58.

Preparation of Pb(II) Ethylenediamine-*N,N'*-bis(acetic acid)-*N,N'*-bis(acetamide), Pb(EDTA-N₂). A solution of Pb(NO₃)₂ (92.4% ²⁰⁷Pb) in 99.999% D₂O was added to a solution of EDTA-N₂ to yield a solution with a final concentration of 10 mM Pb(EDTA-N₂); pH was adjusted using NaOD/DNO₃ to a final value of pH* 4.8. ¹H NMR δ 3.1–3.3 ppm (m, 4H, NCH₂CH₂N), 2.5–4.1 (m, 8H, NCH₂CO). ²⁰⁷Pb NMR 2189 ppm (versus 1.0 M Pb(NO₃)₂ standard). MS(electrospray) M + 1 observed at 497 (calculated 496 for ²⁰⁸Pb, all O-bound).

Preparation of Ethylenediamine Tetraacetamide, EDTA-N₄.³⁷ Ammonia gas is bubbled through a solution of EDTA(OMe)₄ (13.79 g, 39.68 mmol) in 100 mL of dry CH₃OH for 16 h. A white powder is collected by filtration and washed with CH₃OH and CH₂Cl₂. Recrystallization from CH₃OH:H₂O layered with isobutanol yields 7.233 g (63%) EDTA-N₄. ¹H NMR (99.9% D₂O, pH* 6.4) δ 2.76 ppm (s, 4H, NCH₂CH₂N), 3.30 (s, 8H, NCH₂CO). MS (electrospray) M + 1 observed at 289.1 (calculated 288). Anal. Calcd for C₁₀H₂₀N₆O₄: C, 41.66; H, 6.99; N, 29.15. Found: C, 41.32; H, 6.94; N, 28.78.

Preparation of Pb(II) Ethylenediamine Tetraacetamide, [Pb(EDTA-N₄)]²⁺. A solution of Pb(NO₃)₂ (92.4% ²⁰⁷Pb) in 99.9% D₂O was added to a solution of EDTA-N₄ to yield a solution with a final concentration of 10 mM [Pb(EDTA-N₄)]²⁺; pH was adjusted using NaOD/DNO₃ to a final value of pH* 6.1. Crystals of [Pb(EDTA-N₄)]²⁺ were prepared by first precipitating the complex in hot water and methanol according to the literature method for preparing complexes with this ligand and other metals.³⁷ A portion of the resulting white powder was redissolved in a minimum of hot H₂O (0.051 g in 600 μL) in a small, clean test tube. The test tube was placed in a beaker containing methanol and tightly covered. Colorless crystals suitable for X-ray crystallographic studies formed in the test tube after 2 weeks. ¹H NMR (D₂O) δ 3.15 ppm (s, 4H, NCH₂CH₂N), 3.98, 3.90 (m, 8H, NCH₂CO, *J* = 15.1 Hz). ²⁰⁷Pb NMR 1764 ppm (versus 1.0 M Pb(NO₃)₂ standard) MS (electrospray) M + 1 observed at 495 (calculated 496.5 for ²⁰⁸Pb, all O-bound).

Crystal Structure Determination. Pb(II) Ethylenediamine Tetraacetamide, [Pb(EDTA-N₄)]²⁺. A colorless plate crystal of **3**, having dimensions of 0.18 mm × 0.13 mm × 0.04 mm was mounted on a glass fiber. All measurements were made on a SMART-1000 (Bruker) CCD area detector with graphite monochromated Mo Kα radiation. The data were collected at a temperature of -120 ± 1 °C. The data were corrected for Lorentz and polarization effects. An integration absorption correction was applied. Maximum and minimum transmission factors were 0.6826 and 0.2019. The absorption coefficient μ was 95.955 cm⁻¹. The highest intensity peaks were found near the Pb(II) atom reflecting the electron density around the metal atom. The structure was solved by direct methods and expanded using Fourier techniques. All calculations were performed using the teXsan crystallographic software package of the Molecular Structure Corp.⁴⁴ The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full matrix least-squares refinement on *F*² was based on 4543 observed reflections. The *R*₁(*F*) and *R*_w(*F*²) factors were 0.036 and 0.094, respectively.

Results and Discussion

Although [Pb(EDTA)]²⁻ (**1**) has been studied extensively,^{9,18,45} the lead complexes of the corresponding amido derivatives, Pb-

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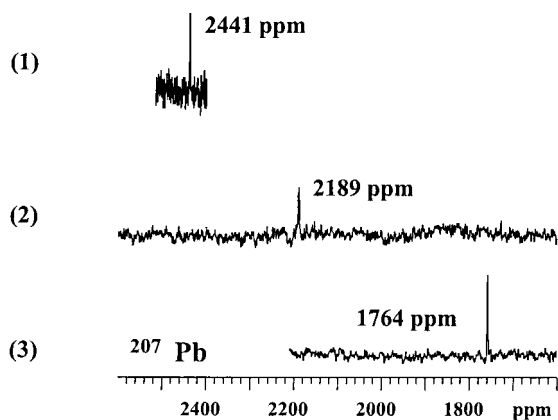


Figure 2. ^{207}Pb resonance shifts upfield as the number of amides in the ligand is increased, suggesting that the charge on the complex becomes more positive as the number of amide groups is increased, not that the number of nitrogen atoms coordinated to the metal. In addition, each of the compounds ($[\text{Pb}(\text{EDTA})]^{2-}$ (1), $[\text{Pb}(\text{EDTA}-\text{N}_2)]^{2-}$ (2), and $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ (3)) exhibits a single ^{207}Pb NMR resonance, suggesting that only the O-bound forms of 2 and 3 are present in aqueous solution at physiologically relevant pH. The lead chemical shifts were referenced to an external 1.0 M $\text{Pb}(\text{NO}_3)_2$ sample in 99.9% D_2O at $\text{pH}^* 3.3$.

($\text{EDTA}-\text{N}_2$) (2) and $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ (3), have not been reported previously. The ligands $\text{EDTA}-\text{N}_2$ and $\text{EDTA}-\text{N}_4$ were prepared using modified versions of the preparations described in the literature.^{37,38} The lead complexes 1, 2, and 3 (Figure 1) were prepared by addition of 1.0–1.1 equiv of either $\text{Pb}(\text{NO}_3)_2$ (natural abundance) or $^{207}\text{Pb}(\text{NO}_3)_2$ (92.4% isotopically enriched, Cambridge Isotope Laboratories) to a solution containing the free ligand in $\text{H}_2\text{O}/\text{D}_2\text{O}$. The pH was adjusted after addition of Pb(II) using $\text{HNO}_3/\text{DNO}_3$ and/or NaOH/NaOD in $\text{H}_2\text{O}/\text{D}_2\text{O}$.

One critical question that arises for these compounds is whether they exist as a single species in aqueous solution at physiologically relevant pH (Figure 1). Compound 2 can exhibit geometric isomers, in which the amido functionalities are either cis or trans to each other. In addition, lead could conceivably substitute for an amide hydrogen (N-bound) in either compound 2 or compound 3, to form a deprotonated N-bound form of these compounds. The corresponding N-bound complexes have been observed for both the Co(II) and Cu(II) complexes of $\text{EDTA}-\text{N}_2$.^{46–48} In these cases, chelation of the metal ion lowers the apparent pK_a of the amide proton to a range that is accessible, resulting in the substitution of the metal for an amide hydrogen at high pH.³⁶ Although Pb(II) clearly has different coordination than either copper or cobalt, it is not clear a priori whether Pb(II) might be capable of substituting for an amide proton once bound to a complex.

To address this question, we obtained the ^{207}Pb NMR spectra of 1, 2, and 3. A single ^{207}Pb NMR resonance is observed for each of the complexes (Figure 2). The observation of a single ^{207}Pb resonance for each compound suggests that either (a) only one form of each compound is observed at this pH in solution, (b) the ^{207}Pb resonances for the different forms of each compound are coincident, or (c) the different isomers are equilibrating rapidly on the NMR time scale.

Because the ^{207}Pb chemical shift is exquisitely sensitive to the number and type of atoms coordinated to lead (Figure 3),⁷

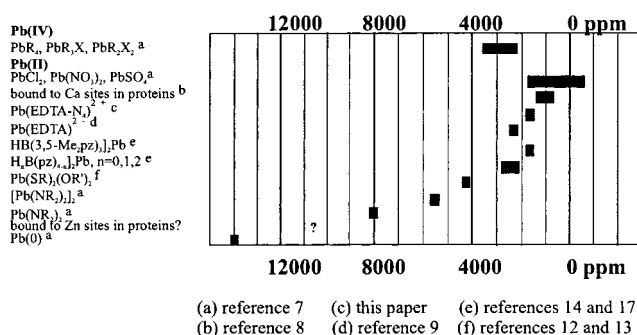


Figure 3. ^{207}Pb chemical shifts cover a range of $\sim 16\,000$ ppm.⁷ Pb(II) compounds cover a much broader range of chemical shifts than do Pb(IV) compounds, but relatively few chemical shifts for Pb(II) compounds have been reported. Those studies that have been reported reveal that the chemical shift is exquisitely sensitive to the lead coordination environment: S bound to lead is more deshielding than N, N is more deshielding than O.^{7–19} The sensitivity of the ^{207}Pb chemical shift to the coordination environment suggests that this should be a useful technique for probing the coordination environment of Pb(II) in complex biological or environmental samples. However, more studies are needed to more fully map out the ^{207}Pb chemical shift range, particularly for thiol-rich sites such as those found in zinc-binding sites in proteins.

explanation (b) is highly unlikely: the N- and O-bound forms of either 2 or 3 are not expected to exhibit the same ^{207}Pb chemical shift. The trend observed for the ^{207}Pb chemical shifts helps to distinguish between these binding modes. Typically, substituting nitrogen for oxygen within the lead coordination sphere tends to increase *deshielding* and shift the ^{207}Pb resonance (Figure 3) *downfield*.⁷ Similar trends have also been observed for ^{113}Cd and ^{199}Hg resonances.^{49,50} Thus, in this series of compounds, if the amide functionality was N-bound to Pb, as the number of nitrogen atoms in the ligand was increased from 1 to 2 to 3, the ^{207}Pb resonance would be expected to shift *downfield*. However, the ^{207}Pb resonance of this series of ligands shifts *upfield* instead (from 2441 ppm, to 2189 ppm, to 1764 ppm; Figure 2, Table 1). This trend suggests that the compounds cannot be predominantly N-bound at physiologically relevant pH, and are most likely O-bound instead. The trend that is observed for the chemical shifts presumably reflects the change going from coordination to negatively charged carboxylates to coordination to neutral amides. As the number of nitrogen atoms in the ligand is increased from $[\text{Pb}(\text{EDTA})]^{2-}$ to $[\text{Pb}(\text{EDTA}-\text{N}_2)]^{2-}$ to $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$, the charge on the metal complex becomes more positive and the ^{207}Pb resonance shifts upfield. These results suggest that it will be important to investigate the effect of charge and site neutrality on ^{207}Pb NMR chemical shifts of lead in other environments as well (e.g., lead bound to proteins).

However, the ^{207}Pb NMR data alone are not sufficient to distinguish between the possibility that only one form of each compound exists (a) and the possibility that the different isomers were equilibrating rapidly on the NMR time scale (c). In the latter case, if the N- and O-bound forms of compounds 2 and 3 were equilibrating rapidly on the NMR time scale (with the equilibrium lying toward O-bound), the observed spectra would reflect the population weighted averages of the spectra of these two species. Furthermore, the ^{207}Pb NMR spectra do not exclude the possibility that compound 2 exhibits more than one O-bound *geometric* isomer that have coincident ^{207}Pb NMR chemical shifts.

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Table 1. Summary of 207Pb NMR Data for Lead Complexes

	δ (207Pb) (ppm)	$J(^{207}\text{Pb}-\text{NCH}_2\text{CH}_2\text{N})$ (Hz)	$J(^{207}\text{Pb}-\text{NCH}_2\text{CO})$ (Hz)	comments
[Pb(EDTA)] ²⁻	2441	17.6	~20	a, refs 9 and 18
Pb(EDTA-N ₂)	2189	b	b	c
[Pb(EDTA-N ₄)] ²⁺	1764	14.3	17.0	d

^a 92.4% ²⁰⁷Pb, 10% D₂O/90% H₂O, pH 7.7, 25 °C. ^b Coupling constants not determined because the peak pattern is too complicated to simulate from one-dimensional ¹H NMR spectrum. ^c 92.4% ²⁰⁷Pb, 99.9% D₂O, pH* 4.8, 25 °C. ^d 92.4% ²⁰⁷Pb, 99.9% D₂O, pH* 6.1, 25 °C.

Table 2. Summary of ¹H NMR Data for Free Ligands and Their Lead Complexes

	$\delta(\text{NCH}_2\text{CH}_2)$ (ppm)	$\delta(\text{NCH}_2\text{H}_2\text{CO})$ (ppm)	$J(\text{H}_\text{A}\text{H}_\text{B})$ (Hz)	comments
EDTA ^a	3.19 (s, 4H)	3.59 (s, 8H)		a, ref 18
[Pb(EDTA)] ²⁻	3.11 (d, 4H)	3.69 (d, 8H)	17.1	b, refs 9 and 18
EDTA-N ₂	2.64 (s, 4H)	3.21 (s, 4H) 3.13 (s, 4H)		c
Pb(EDTA-N ₂)	3.1–3.3 (m, 4H)	3.5–4.1 (m, 8H)	d	f
EDTA-N ₄	2.76 (s, 4H)	3.3 (s, 8H)		e
[Pb(EDTA-N ₄)] ²⁺	3.15 (d, 4H)	3.98 (m, 4H) 3.90 (m, 4H)	15.1	g

^a 10% D₂O/90% H₂O, pH 7.3, 25 °C. ^b 92.4% ²⁰⁷Pb, 10% D₂O/90% H₂O, pH 7.3, 25 °C. ^c 99.9% D₂O, pH* 4.7, 25 °C. ^d Coupling constant not determined because the peak pattern is too complicated to simulate from one-dimensional ¹H NMR spectrum. ^e 99.90% D₂O, pH 6.4, 25 °C. ^f 92.4% ²⁰⁷Pb, 99.9% D₂O, pH* 4.8, 25 °C. ^g 92.4% ²⁰⁷Pb, 99.90% D₂O, pH* 6.1, 25 °C.

To distinguish between all of these possibilities, the ¹H NMR spectra of the lead complexes were examined (Table 2). The most complex spectrum is seen for Pb(EDTA-N₂), which clearly exhibits multiple forms with distinct ¹H NMR resonances under the conditions at which the spectrum was acquired (99.9% D₂O, pH* 4.8).⁵¹ However, it is important to note that these multiple forms, which are distinct on the NMR time scale, exhibited a *single* ²⁰⁷Pb NMR resonance under identical conditions. This observation argues against the possibility that N- and O-bound forms of **2** are equilibrating rapidly on the NMR time scale (possibility (c)). Taken together, these results suggest that the distinct species observed in the ¹H NMR spectra of **2** most likely correspond to different geometric isomers of the O-bound linkage isomer. The mass spectrum (observed, 497; calculated, 496 for ²⁰⁸Pb, all O-bound) of Pb(EDTA-N₂) is also consistent with the assertion that the amido groups are protonated and hence that lead is O-bound.

Each of the other lead complexes **1** and **3** (Table 2) exhibits a single set of proton resonances. In the case of **3**, a complex splitting pattern is observed when the sample is prepared using natural abundance (22.6%) ²⁰⁷Pb(NO₃)₂ (Figure 4a). However, comparison of this spectrum to that obtained for a sample containing enriched (92.4%) ²⁰⁷Pb(NO₃)₂ (no decoupling, Figure 4b; ¹H decoupled, Figure 4c) reveals that the natural abundance spectrum is a superposition of 22.6% [²⁰⁷Pb(EDTA-N₄)]²⁺ (with $J(^{207}\text{Pb}-\text{NCH}_2\text{CH}_2\text{N})$ of 14.3 Hz and $J(^{207}\text{Pb}-\text{NCH}_2\text{CO})$ of 17 Hz) and 77.4% [²⁰⁸Pb(EDTA-N₄)]²⁺ ($I = 0$).⁵² Given the arguments outlined above, **3** most likely exists as a single all O-bound species in aqueous solution.

The two-dimensional ²⁰⁷Pb-¹H NMR spectra further bolster this conclusion and clearly illustrate the sources of the couplings observed in the 1D spectra. Both methylene proton resonances appear as doublets in the spectrum of [Pb(EDTA)]²⁻; the two-dimensional ²⁰⁷Pb-¹H HMQC spectrum of [Pb(EDTA)]²⁻

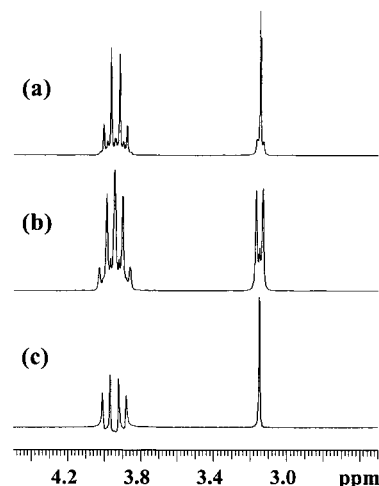


Figure 4. One-dimensional ¹H NMR spectra of [Pb(EDTA-N₄)]²⁺ recorded on a Varian Unity Plus 400 MHz NMR spectrometer at 25 °C. Coupling is seen through three bonds from ²⁰⁷Pb to all methylene protons within the molecule. Comparison of the ¹H NMR of [Pb(EDTA-N₄)]²⁺ prepared using (a) natural abundance Pb(NO₃)₂ (22.6% ²⁰⁷Pb, 99.9% D₂O, pH* 6.3, 25 °C), (b) ¹H NMR of [Pb(EDTA-N₄)]²⁺ prepared using isotopically enriched (92.4%) ²⁰⁷Pb(NO₃)₂ (no decoupling) (99.9% D₂O, pH* 6.1, 25 °C), and (c) ¹H{²⁰⁷Pb} NMR of [Pb(EDTA-N₄)]²⁺ prepared using isotopically enriched (92.4%) ²⁰⁷Pb(NO₃)₂ (99.9% D₂O, pH* 6.1, 25 °C) reveals that the natural abundance spectrum is a superposition of the spectra of 22.6% [²⁰⁷Pb(EDTA-N₄)]²⁺ (with coupling between ²⁰⁷Pb and ¹H) and 77.4% [²⁰⁸Pb(EDTA-N₄)]²⁺ ($I = 0$).

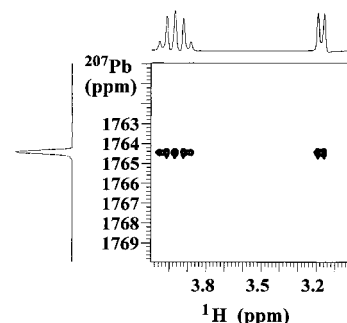


Figure 5. Two-dimensional ²⁰⁷Pb-¹H HMQC NMR spectrum of [Pb(EDTA-N₄)]²⁺ (99.90% D₂O, pH* 6.1, 25 °C). Coupling is observed from ²⁰⁷Pb to ¹H through three bonds. The lead chemical shifts were referenced to an external 1 M Pb(NO₃)₂ sample in 99.9% D₂O at pH* 3.3.

reveals that this splitting arises due to coupling of ²⁰⁷Pb to the methylene protons ($J_{\text{Pb-H}} \sim 20$ Hz).⁵¹ By contrast, the spectrum of [Pb(EDTA-N₄)]²⁺ shows one set of doublets (NCH₂CH₂N) and one multiplet (NCH₂CONH₂) (Figure 5). Direct confirmation that ²⁰⁷Pb is coupled through three bonds to both sets of methylene protons in [Pb(EDTA-N₄)]²⁺ is provided by the ²⁰⁷Pb-¹H HMQC spectrum of [Pb(EDTA-N₄)]²⁺ (Figure 5).

On the basis of all of these NMR data, we predict that in aqueous solution at physiological pH, Pb(EDTA-N₂) is O-bound

(51) See Supporting Information.

(52) The ¹H-¹H and ¹H-²⁰⁷Pb coupling constants were determined from fits to the spectra using Varian's spin simulation program within the VNMR software simulation.

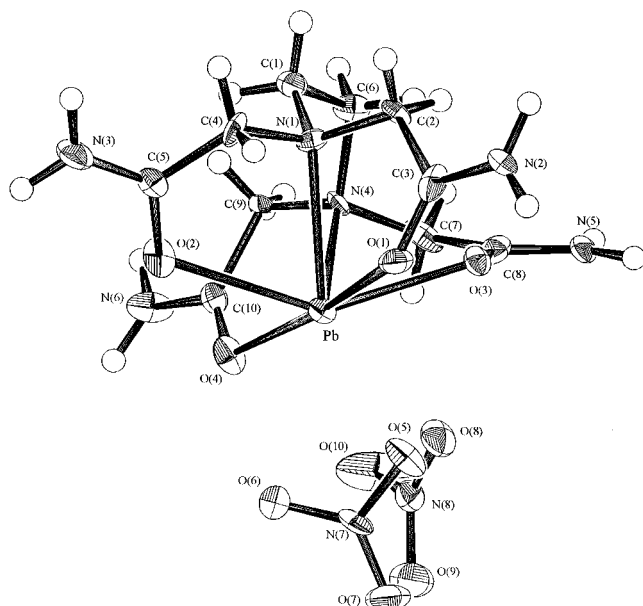


Figure 6. ORTEP view of the Pb atom environment in $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ (**3**) with the atom numbering scheme.

Table 3. Selected Bond Lengths (Å) for $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ in Comparison to $[\text{Pb}(\text{EDTA})]\cdot\text{H}_2\text{O}^a$

$[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$		$[\text{Pb}(\text{EDTA})]\cdot\text{H}_2\text{O}^a$	
Pb–N(1)	2.620(7)	Pb–N	2.518
Pb–N(4)	2.605(8)	Pb–O	2.604
Pb–O(1)	2.693(7)	Pb–O	2.494–2.735
Pb–O(2)	2.597(8)	Pb–O (H ₂ O)	2.967
Pb–O(3)	2.439(7)		
Pb–O(4)	2.632(7)		

^a Reference 45.

and neutral and $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ is O-bound and carries a +2 charge. The prediction that these compounds will bind to Pb(II) via the carbonyl oxygen of the amido functionalities is supported by the structure that we observe for $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ in the solid state (Figure 6). The Pb(II) ion is six coordinate: both ethylenediamino nitrogens are bound, as are all four carbonyl oxygens in the amido functionalities (Table 3). The Pb–N bonds in $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ are more similar in length (2.620 and 2.605 Å) to each other than those observed for Pb(EDTA) (2.518 and 2.604 Å).⁴⁵ Although one of the Pb–O bonds is slightly longer (2.693 Å) than the other three Pb–O bonds, all four bonds are well within the length of previously reported Pb–O interactions.⁵³ The high degree of symmetry observed in the ¹H NMR spectrum of $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ suggests that this irregularity is either absent in aqueous solution or fluxional on the NMR time scale. In the solid state, the ligand is “hemidirected” (i.e., the ligand is not distributed throughout the entire sphere surrounding lead),⁵³ and there is a clearly identifiable gap in the lead coordination sphere, suggesting that $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ contains a stereochemically active 6s lone pair of electrons. Interestingly, this “gap” is occupied by the two negatively charged counterions (NO_3^-). By contrast, in the crystal structure of Pb(EDTA), a smaller gap is observed because the lead recruits additional ligands: either the arm of a neighboring EDTA molecule or a water molecule (Table 3).⁴⁵ Comparison of these structures raises the interesting question of whether these structural differences also exist in solution and

whether the degree to which the lone pair of lead is localized in the complexes correlates in any way with the affinity and selectivity of the ligands for lead.

Conclusions

Taken together, these results provide compelling evidence that one-dimensional ²⁰⁷Pb and two-dimensional ²⁰⁷Pb–¹H HMQC spectroscopies are useful methodologies for studying the aqueous coordination chemistry of Pb(II) complexes. The conclusions drawn by the series of NMR experiments—that Pb(II) is O-bound by the amido functionalities and hence that **3** has a charge of +2—are in agreement with the structure seen for **3** in the solid state. Furthermore, the observation that the coordination mode and net charge on the complex can have a substantial influence on the ²⁰⁷Pb chemical shift suggests that site neutrality may play a critical role in determining ²⁰⁷Pb chemical shifts in biological systems. Since charge neutrality should also play a critical role in defining solubility, bioavailability, and metal-binding selectivity in aqueous solutions, these results suggest that ²⁰⁷Pb NMR spectroscopy should provide valuable insights into the design and properties of improved chelating agents.

The observation of a large (hundreds of ppm) but *systematic* change in the lead chemical shift as a result of a relatively minor change in the complex ion suggests that ²⁰⁷Pb NMR spectroscopy will be a useful methodology for characterizing Pb(II) coordination environments in complex aqueous samples and that factors such as site neutrality may have an important effect on the lead chemical shift. However, the wide range of ²⁰⁷Pb chemical shifts seen for Pb(II) coordination compounds also poses a challenge: it is often difficult to *find* a lead resonance for a new compound unless related systems have been studied. Finding ²⁰⁷Pb resonances becomes all the more challenging when investigating biological or environmental samples, where the species of interest is often fairly dilute (less than 1 mM). The studies presented herein provide two solutions to this problem. First, the compounds investigated provide a useful model system for calcium sites in proteins that have been proposed as targets for lead in vivo.^{8,54,55} Second, we report the first two-dimensional ²⁰⁷Pb–¹H NMR spectra, which should be particularly useful for studying lead in complex samples. The two-dimensional ²⁰⁷Pb–¹H NMR spectrum of $[\text{Pb}(\text{EDTA}-\text{N}_4)]^{2+}$ (Figure 5) demonstrates that couplings from ¹H to ²⁰⁷Pb can be observed through three bonds, suggesting that this methodology should also be useful for identifying the coordination environments of Pb(II) bound proteins, especially when Pb(II) is bound to the thiol group of cysteines (²⁰⁷Pb–S–C–(¹H)₂).^{6,50,56} Furthermore, the improved sensitivity provided by the indirect detection of the analogous one-dimensional HMQC experiment should allow for more rapid screening of the ²⁰⁷Pb chemical shift window when searching for the resonances of new compounds. The studies reported herein demonstrate that this should prove particularly useful for finding ²⁰⁷Pb resonances of lead bound to zinc-binding (thiol-rich) sites in proteins. No model complexes are currently available for these sites, but by analogy to ¹¹³Cd and ¹⁹⁹Hg NMR studies,^{49,50} the chemical shift trends suggest that the ²⁰⁷Pb resonances of thiol-rich sites may be shifted significantly downfield from the resonances reported herein (Figure 1). Thus, these studies lay the foundation for obtaining critical information on the fundamental aqueous solution chemistry of Pb(II) that is needed to design new

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methods for detecting, preventing, and treating lead poisoning. The affinity and selectivity for Pb(II) of EDTA-N₂, EDTA-N₄, and analogues of these compounds are currently under investigation.

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Mass spectrometry studies were conducted in the ASL at Northwestern University by Hoying L. Hung. Elemental analyses were performed by Midwest Microlab.

Supporting Information Available: One-dimensional ^1H NMR spectra (natural abundance, ^{207}Pb -enriched, and ^{207}Pb -decoupled) for Pb(EDTA-N₂) and two-dimensional ^{207}Pb - ^1H HMQC NMR spectrum of [Pb(EDTA)]²⁻. Further crystallographic details and parameters for [Pb(EDTA-N₄)]²⁺ and for the free ligand, EDTA-N₄, are available in CIF file format. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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