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Characterization of the First N_2S(alkylthiolate)lead Compound: A Model for Three-Coordinate Lead in Biological Systems†

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A new N_2S(alkylthiolate)-coordinated Pb^{2+} compound {2-methyl-1-[methyl(2-pyridin-2-ylethyl)amino]propane-2-thiolatolead perchlorate, [PATH-Pb][ClO_4]} has been synthesized and characterized by X-ray diffraction and by ^{207}Pb NMR. [PATH-Pb]$^+$ is the first reported three-coordinate Pb complex with an alkanethiolate ligand and, hence, is a good model for Pb–cysteine interactions in proteins. The Pb center displays distorted trigonal-planar geometry. The Pb–S bond lengths are extremely short (2.590(10) and 2.597(10) Å for two distinct monomers in the unit cell). ^{207}Pb NMR revealed a Pb resonance at 5318 ppm, much further downfield than Pb complexes with N and O ligation. Given recent evidence of three-coordinate Pb-binding in proteins with cysteine-rich metal-binding sites, [PATH-Pb]$^+$ is an important model for Pb sites in biological systems. Crystal data: $C_{12}H_{19}N_2SPbClO_4$, $M_r = 529.99$, monoclinic, $P2_1/n$, $a = 16.8297(9)$ Å, $b = 11.9719(6)$ Å, $c = 17.0868(9)$ Å, $V = 3237.7(3)$ Å^3, and $Z = 8$.

Despite the removal of lead from gasoline and household paint over 2 decades ago, lead poisoning continues to be the most common environmentally caused illness in children in the United States.[1−3] The Centers for Disease Control estimates that in 2001 approximately 454 000 children suffered from elevated blood Pb levels (defined as ≥10 μg/dL or ≥0.5 μM).[4,5] This may even be an underestimate of the number of children affected by lead poisoning: recent studies have shown that Pb has detrimental physiological effects in children at levels of less than 5 μg/dL.[6]

Recent studies have provided fundamental insights into the preferences of Pb for different types of biomolecules and have pointed to the need for coordination compounds that model Pb-binding sites in proteins.[7−10] Studies on model peptides and recombinant proteins have revealed that Pb^{2+} has a particularly high affinity ($\beta_1^{Pb} > 10^{10}$ M^{-1}) for Zn-binding sites in proteins in which Zn is bound by cysteine residues; Pb has the highest affinity for sites with three or more cysteine residues in close proximity.[9] This preference presumably arises from the high affinity of Pb for the thiolate functional group found in cysteine and reflects the high enthalpy of formation for Pb−S bonds. Recent studies suggest that the preferred coordination mode of Pb in thiol-rich sites in proteins is three-coordinate;[9] presumably, Pb assumes a trigonal-pyramidal geometry in these sites, with the fourth "open" coordination site of the tetrahedron occupied by the stereochemically active 6s^2 lone pair.

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A number of fundamental questions about Pb coordination chemistry in biologically relevant sites remain, including the following:

(i) How rapidly does metal binding and substitution occur at these sites?

(ii) How does the ^{207}Pb NMR chemical shift observed for Pb^{2+} compounds depend on the coordination geometry, coordination number, and number of thiolate ligands bound to Pb?

To address these questions, detailed studies on crystallographically well-characterized small-molecule systems with physiologically relevant coordination environments are needed. To date, the best-characterized model system that mimics these environments is the tris(2-mercapto-1-phenylimidazolyl)hydroborato ligand developed by Parkin and coworkers,[11] which binds Pb^{2+} in a trigonal mode via three arylthiolate moieties. However, no biomimetic model compounds containing trigonal Pb bound to alkanethiolate groups have been reported to date, and relatively few other examples of Pb bound to alkylthiolate ligands *in any geometry* have been reported.[12] In addition, mixed N_xS_{3-x} ligands (where $x = 1$ or 2) are needed to model Pb^{2+} binding in other types of structural Zn-binding sites. Here, we report the structure and properties of {2-methyl-1-[methyl(2-pyridin-2-ylethyl)-amino]propane-2-thiolatolead, ([PATH-Pb]$^+$}, which contains Pb bound in a biologically relevant N_2S trigonal environment.

The PATH ligand was synthesized according to previously published methods.[13,14] [PATH-Pb]$^+$ was prepared by adding the ligand to a metal solution at room temperature.[15] Although Pb does have a propensity to form higher-order complexes and even polymeric structures in other ligand systems,[16−19] only the 1:1 PATH/Pb complex was observed. Notably, both the affinity of Pb for PATH ($\log \beta_1^{Pb} = 9.5$) and the relative affinity of Pb versus Zn for PATH ($\log \beta_1^{Pb} - \log \delta_1^{Zn} = -0.9$)[20] are remarkably similar to that of a canonical Zn finger peptide, CP−CCHH ($\log \beta_1^{Pb} = 9.7$; $\log \beta_1^{Pb} - \log \delta_1^{Zn} = -1.5$), the system that we were seeking to model.[10,21,22] (See the Supporting Information for experimental procedures for potentiometric titrations and Table S1 for more detailed formation constants for PATH.)

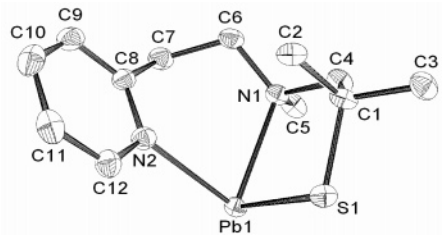

Figure 1. ORTEP representation of [PATH-Pb]$^+$ (drawn with 50% thermal probability ellipsoids). H atoms and ClO_4^- are omitted for clarity.

Table 1. Selected Bond Lengths (Å) and Bond Angles (deg) for the Two Molecules (A and B) in the Unit Cell of [PATH-Pb]$^+$

	A	B		A	B
Pb−S1	2.590(10)	2.597(10)	C4−N1	1.501(5)	1.499(5)
Pb−N1	2.495(3)	2.496(3)	N1−C6	1.490(5)	1.498(5)
Pb−N2	2.531(3)	2.528(4)	C6−C7	1.534(6)	1.524(6)
S1−C1	1.867(4)	1.861(4)	C7−C8	1.515(6)	1.508(6)
C1−C4	1.531(5)	1.536(5)	C8−N2	1.341(5)	1.344(5)
S1−Pb−N1	79.32(7)	79.00(8)	N1−Pb−N2	79.90(11)	80.77(11)
S1−Pb−N2	91.65(8)	91.65(8)			

A diffraction-quality crystal was obtained via slow evaporation of a solution of [PATH-Pb][ClO_4] in acetonitrile. An ORTEP representation of the determined structure is shown in Figure 1. The unit cell contains two [PATH-Pb]$^+$ molecules that are crystallographically distinct (Table 1, unit cell diagram in the Supporting Information). Each [PATH-Pb]$^+$ molecule in the unit cell adopts a distorted trigonal-pyramidal geometry about the Pb ion. The two coordinating N atoms and a single S atom form three of the coordination sites in each molecule; the fourth site is presumably filled by the Pb $6s^2$ lone pair of electrons. The $6s^2$ lone pair of Pb^{2+} is commonly observed to be stereochemically active in low coordination number compounds of Pb^{2+}.[23] The N2−Pb−S1 bond angle is much larger than those for N1−Pb−S1 and N1−Pb−N2 (Table 1), thus describing a distorted trigonal-pyramidal geometry. This geometry is consistent with the preferred trigonal-pyramidal coordination mode for Pb^{2+} in Zn proteins that are targets for Pb in vivo;[10,24−26] steric effects from the pyridinyl ring may prohibit Pb^{2+} from assuming a regular trigonal geometry in this particular

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(15) A total of 212.8 mg of PATH (0.950 mmol) in 20 mL of methanol was added dropwise to a room temperature solution of 450.1 mg of $Pb(ClO_4)_2 \cdot 3H_2O$ (0.978 mmol) in methanol (5 mL). The reaction was allowed to stand at room temperature for several days. Following removal of roughly half of the solvent under reduced pressure, pale-yellow crystals began to form. Washing with cold ether and chloroform yielded white crystals of the pure $C_{12}H_{19}N_2SPbClO_4$. Yield: 99.5 mg (23%). Anal. Calcd for $C_{12}H_{19}N_2SPbClO_4$ ([PATH-Pb]ClO_4): C, 27.11; H, 3.77; N, 5.27. Found: C, 28.28; H, 3.82; N, 4.53. ^{1}H NMR (CD_3CN, 300 MHz): δ 8.780 (d, 1H), 7.984−8.035 (t, 1H), 7.542−7.587 (m, 2H), 4.100−4.144 (d, 2H), 3.364−3.409 (d, 2H), 3.171−3.217 (m, 2H), 2.707 (s, 3H), 1.636 (s, 3H), 1.194 (s, 3H).
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Table 2. ^{207}Pb NMR Data (ppm) for Selected Pb^{2+} Compounds

compound	coordination	chemical shift[a]	ref
$[(PhS)_3Pb](Ph_4As)$	$(ArS)_3$	5828	31
$[PATH\text{-}Pb]^+$	$N_2(RS)$	5318	this work
bis(thiohydroxamato)lead	S_2O_2	~4100−4500	29, 30
$[H_2B(pz)_2]_2Pb$	N_4	2821	18
$[HB(pz)_3]_2Pb$	N_6	2065	18

[a] Relative to $Pb(NO_3)_2$.

compound. Alternatively, the C linker to the S may be too short to accommodate a rigorously trigonal-pyramidal geometry. The bond lengths from Pb to S are 2.5901 and 2.5968 Å in the two crystallographically distinct molecules in the unit cell. These are significantly shorter than any previously reported lengths for Pb−S bonds,[23] including the Pb−S bond (2.62 Å) for the terminal thiolate in the other structurally characterized alkanethiolatolead complex reported to date.[12] Other Pb−S bond lengths reported in the literature range from 2.619 to 3.268 Å.[11,23] In addition to the three short bonds from Pb to S and N, there is also a long contact between Pb and one of the O atoms in perchlorate (2.78 Å) and a long contact between Pb and a S atom from a neighboring complex cation (3.26 Å). (See the Supporting Information, Figures S1 and S2.)

^{207}Pb NMR of $[PATH\text{-}Pb]^+$ in DMF-d_7 revealed a Pb resonance at 5318 ppm relative to $Pb(NO_3)_2$.[27,28] We find that the ^{207}Pb NMR resonance for $[PATH\text{-}Pb]^+$ is further downfield than previously observed either for Pb complexes in which Pb is bound by N atoms only[18] or for bis(thiohydroxamato)lead complexes, which contain both S and O in the coordination sphere[29,30] (Table 2). The ^{207}Pb NMR chemical shift for $[PATH\text{-}Pb]^+$, which contains only a single Pb−alkanethiolate bond, is similar to that of the $[(PhS)_3Pb]$ anion, which contains three arylthiolates bound to Pb.[31] These ^{207}Pb NMR chemical shift differences for the alkyl- and arylthiolatolead complexes presumably arise because the alkanethiolate is less electronegative than an arylthiolate and therefore is more deshielding to the Pb nucleus. These data suggest that the ^{207}Pb NMR resonance for Pb in Zn sites in proteins (which typically contain more than one cysteine residue and, hence, more than one alkanethiolate ligand) will likely be even further downfield [>6000 ppm versus $Pb(NO_3)_2$]. These data constitute important progress toward our goal of providing a chemical shift map of ^{207}Pb NMR resonances in varied biological metal-binding coordination sites.[25] We expect that this compound will also be useful in probing the effect of Pb coordination on the electronic structure in biologically relevant systems.

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Supporting Information Available: Experimental information for potentiometric titrations (including a table of formation constants), experimental information for ^{207}Pb NMR spectroscopy, experimental information for X-ray crystal structures, figures showing long contacts in the crystal structure, tables of crystallographic data, and crystallographic information files (CIF) for PATH-Pb. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) $Pb(NO_3)_2$ (1.0 M) in D_2O (pH 3.3) is used as an external chemical shift reference for ^{207}Pb because of its lower toxicity than $(CH_3)_4Pb$, the previously widely used reference. This lead nitrate solution has a chemical shift of −2960 ppm relative to $(CH_3)_4Pb$.

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