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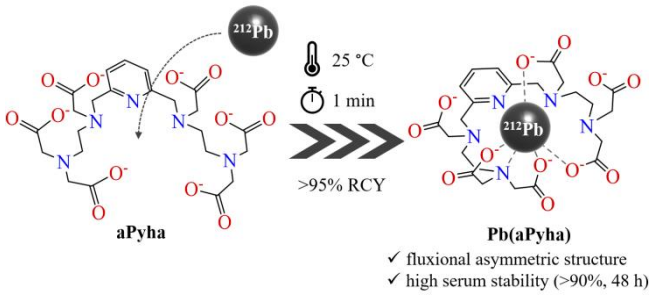
Acyclic pyridine-containing hexaacetate chelator H_6aPyha for lead radionuclides: from solution structure to efficient radiolabeling and serum stability

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We report an optimized synthesis of the acyclic pyridine-hexaacetate chelator H_6aPyha and demonstrate its instantaneous room-temperature radiolabeling with lead radionuclides (>95%), yielding a complex with remarkable kinetic inertness in blood serum (>90% over 48 h). Combined spectroscopic and computational studies reveal that the complex possesses a highly fluxional, asymmetric coordination environment, balancing structural adaptability during metal ion uptake with robust subsequent encapsulation. The results show that H_6aPyha provides a highly desirable dual-affinity platform capable of retaining both the parent ^{212}Pb and daughter ^{212}Bi isotopes, making it an important advancement for targeted alpha therapy.



Keywords: acyclic chelators, lead radionuclides, targeted alpha therapy, solution coordination chemistry, conformational fluxionality, serum stability, DFT calculations.

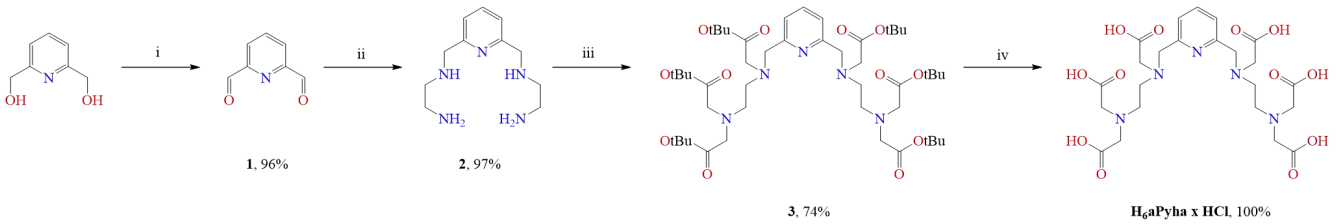
Targeted alpha therapy (TAT) has emerged as a highly promising modality in nuclear medicine, with lead-212 (^{212}Pb, $t_{1/2}$ = 10.6 h) occupying a key position as an efficient *in vivo* generator of the alpha-emitter ^{212}Bi ($t_{1/2}$ = 60.6 min).[1–3] The successful clinical translation of ^{212}Pb-labeled radiopharmaceuticals heavily relies on the development of bifunctional chelating agents that ensure high thermodynamic stability and kinetic inertness of the resulting complexes.[4–6] While macrocyclic ligands often require elevated temperatures for optimal labeling, acyclic architectures are highly attractive due to their rapid coordination kinetics at ambient temperature, which is crucial for preserving the integrity of heat-sensitive biomolecules and minimizing radionuclide decay during synthesis.[7–9]

A critical challenge in ^{212}Pb radiopharmacy stems from its decay mechanism: the sudden β^--transmutation of ^{212}Pb into ^{212}Bi alters the metal oxidation state and coordination preferences, which frequently triggers the release of the toxic daughter radionuclide.[10–12] Consequently, an ideal chelator must possess a dual affinity, effectively retaining both the parent and daughter isotopes.[13–15] Recently, we developed a novel acyclic pyridine-derived hexaacetate ligand, H_6aPyha, which demonstrated outstanding chelating efficiency, rapid kinetics, and high *in vitro* stability when complexed with bismuth radionuclide.[16] The extension of the scope of H_6aPyha to lead isotopes represents a logical and very promising step towards the creation of a coordinated parent-daughter ion chelating system.

Herein, we report an optimized synthetic route to H_6aPyha, as well as a comprehensive study of its complexation with Pb^{2+}. To determine the composition of solutions and the thermodynamic stability of the resulting complexes, mass spectrometry and potentiometric titration were used, and NMR spectroscopy and DFT calculations were used to determine the structure of complexes in aqueous solution. In addition, radiolabeling experiments and serum assays were performed to evaluate the potential of H_6aPyha for targeted radionuclide therapy.

The H_6aPyha synthesis method was optimized compared to our previously described procedure (Scheme 1).[16] Specifically, the pyridine-containing polyamine intermediate (**2**) was prepared from pyridine-2,6-dicarbaldehyde (1), thereby eliminating the need for the environmentally unfavorable 40-fold excess of ethylenediamine required to synthesize intermediate (**2**) from 2,6-bis(chloromethyl)pyridine. Furthermore, the final deprotection step was simplified. The *tert*-butyl ester groups were successfully removed within just 1 hour at room temperature in a hydrochloric acid medium, completely replacing the tedious 5-day refluxing process. This updated approach not only reduces the total synthesis time from weeks to days but also increases the overall yield of the target chelator H_6aPyha.

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Scheme 1 *Reagents and conditions:* i, SeO_2, dioxane, reflux, 24 h; ii, 1) ethylenediamine, MeOH, room temperature, 20 min, 2) $NaBH_4$, MeOH, 0 °C – room temperature, overnight; iii, *tert*-butyl bromoacetate, K_2CO_3, MeCN, reflux, 50 h; iv, 4M HCl, room temperature, 1 hour.

Table 1 Stepwise protonation constants (log *K*) of the ligand, stability constants (log β) of Pb^{2+} complexes (25 °C, I = 0.1 M KNO_3) and pPb value (pPb = −log[Pb]$_{free}$ at [L] = 10 μM, [Pb] = 1 μM at pH 7.4).

Species, L=aPyha^{6-}	log *K*	log β
HL	10.16 [a]	
H_2L	9.36 [a]	
H_3L	5.87 [a]	
H_4L	4.57 [a]	
H_5L	3.22 [a]	
H_6L	1.88 [a]	
LPb		10.6 (1)
HLPb		20.7 (1)
H_2LPb		28.2 (1)
H_3LPb		32.4 (1)
pPb		9.9

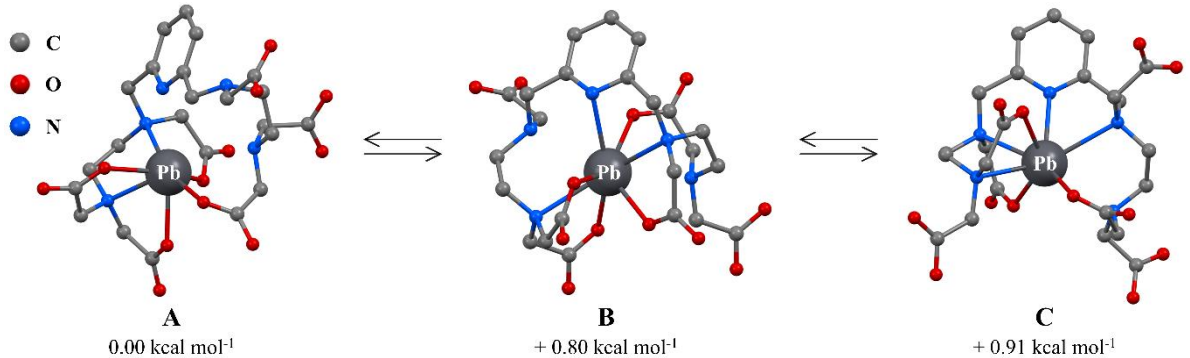
[a] ref. [16]

To obtain preliminary information on the complexing ability of H_6aPyha, the system was analyzed by mass spectrometry (MALDI-TOF) in the presence of a 3-fold excess of $Pb(NO_3)_2$. The mass spectrum revealed the formation of mono-, bi-, and trinuclear species (Figure S1), which reflects the high denticity of the H_6aPyha ligand featuring eleven potential donor centers (four amino groups, one pyridine nitrogen, and six carboxylate groups). However, analysis of potentiometric titration data at a ligand:metal ratio of 1:1 made it possible to reliably determine the formation of exclusively mononuclear complexes (Table 1, Figure S2). The stability constant of the $[Pb(aPyha)]^{4-}$ complex was log K_{LPb} = 10.6, and the calculated value of pPb, taking into account competition with protons under physiological conditions (pH 7.4), was 9.9. These values are significantly lower than the corresponding parameters for the gold-standard macrocycle H_4DOTA (log K_{LPb} = 25.3, pPb = 20.0).[17] Furthermore, it is noteworthy that the first protonation constant of the PbL complex (log K_{HLPb} = 10.1, corresponding to the equilibrium $[PbL]^{4-} + H^+ \rightleftharpoons [PbHL]^{3-}$) closely matches the protonation constant of the free ligand (log K_{HL} = 10.16), suggesting that at least one amine nitrogen atom remains uncoordinated in the complex.

The coordination of H_6aPyha with Pb^{2+} ions in D_2O was investigated using ^{1}H NMR spectroscopy (Figure S3). Comparison with the free, fully deprotonated ligand reveals that all resonances in the spectrum of the Pb^{2+} complex exhibit a distinct downfield shift, driven by the electron-withdrawing effect of the coordinated metal ion and the partial protonation of the ligand, given that the $[PbH_2L]^{2-}$ species predominates in solution at the experimental pH (6.3). Crucially, the pronounced downfield shift of the aromatic signals provides unambiguous evidence for the direct coordination of the pyridine nitrogen atom to the Pb^{2+} ion. At the same time, the spectrum exhibits a highly complicated pattern, pointing to an asymmetric conformation of the bound ligand framework around the large heavy metal ion. Notably, the appearance of sharp singlets in the aliphatic region corresponding to the acetate (*a*) protons indicates that not all carboxylate arms are tightly bound to the metal center. Instead, some acetate groups remain either uncoordinated or involved in a fast fluxional exchange on the NMR timescale.

Additional analysis of the possible structural arrangements of the $[Pb(aPyha)]^{4-}$ complex was performed using DFT calculations. None of the representative conformations exhibit direct coordination of all eleven donor groups to the Pb^{2+} ion; instead, only three or four of the six acetate groups are capable of simultaneous interaction with the metal center. Furthermore, certain amino groups are located far from Pb^{2+}, remaining accessible for protonation. In the lowest-energy conformations, the Pb^{2+} ion adopts a variable coordination number (CN = 6 or 7), binding to 2–3 amino groups, 3–4 carboxylate oxygen atoms, and—in two of these three conformers—the pyridine nitrogen atom (Figure 1).

Small differences in energy between the three local minima (ΔE < 1 kcal mol^{-1}) indicate that the $[Pb(aPyha)]^{4-}$ complex is in rapid dynamic equilibrium in solution. This high conformational fluxionality, characterized by fluctuating CNs and the reversible switching of donor groups, aligns perfectly with both the NMR spectroscopic and potentiometric data. Despite the marked asymmetry of the complex, the metal ion remains effectively encapsulated within the ligand framework, protecting it from the external influence of competing species such as serum proteins or biogenic metal ions.



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Figure 1 The lowest-energy conformers of the $[Pb(aPyha)]^{4-}$ complex calculated by the DFT method. Hydrogen atoms are omitted for clarity. Relative free energies (ΔG_{comp}) are given below the structures relative to the most stable conformer **A**.

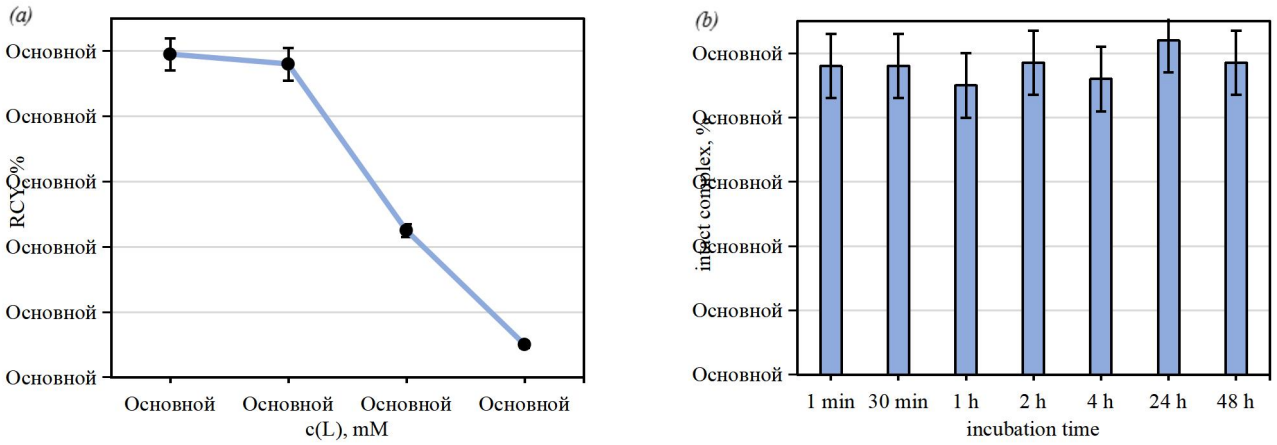


Figure 2 (*a*) Radiochemical yields (RCY, %) of complexes at different ligand concentration (25 °C, 1 min, $c(^{210}Pb)$ = 50 nM, 3 kBq). (*b*) Radiochemical stability (%) of $[^{210}Pb]Pb(aPyha)$ complex in FBS (37 °C, c(L) = 1 mM, V(complex) / V(FBS) = 1/9).

Radiolabeling studies were conducted using the readily available isotope ^{210}Pb. It was demonstrated that, at ligand concentrations exceeding 0.1 mM, the radionuclide undergoes near-quantitative (>95%) binding (Figure 2*a*). Notably, this process occurs instantaneously at room temperature—a significant practical advantage over standard H_4DOTA, which typically requires heating at 37–90 °C for 30–60 minutes and subsequent purification.[18,19] The $[^{210}Pb]Pb(aPyha)$ complex was tested in fetal bovine serum (FBS) to evaluate its kinetic inertness (Figure 2*b*). Remarkably, no dissociation of the complex occurred over a period of at least 48 hours, with the radiochemical purity remaining above 90% throughout the experiment. Thus, the H_6aPyha ligand not only ensures rapid and efficient chelation of the lead radionuclide at ambient temperature but also reliably protects it against unwanted transchelation by serum proteins.

In summary, we have successfully optimized the synthetic route to the acyclic pyridine-derived hexaacetate H_6aPyha chelator, significantly reducing synthesis time while increasing the overall product yield. A comprehensive study, combining potentiometric titrations and ^{1}H NMR spectroscopy, as well as DFT calculations, revealed a unique coordination mode of the $[Pb(aPyha)]^{4-}$ complex. Although its thermodynamic parameters are modest (log K_{PbL} = 10.6, pPb = 9.9), the Pb^{2+} ion undergoes effective encapsulation within a highly dynamic, asymmetric coordination environment characterized by fluctuating coordination numbers (CN = 6 or 7) and rapid fluxional switching of the donor groups. This structural flexibility allows the ligand to accommodate the different stereochemical requirements of both Pb^{2+} and Bi^{3+}, which is highly desirable for the development of dual-affinity platforms. Crucially, radiolabeling experiments with ^{210}Pb demonstrated instantaneous, near-quantitative radionuclide binding (>95%) at room temperature, offering a vital operational advantage over macrocyclic benchmarks. Combined with excellent kinetic inertness in blood serum (>90% stability over 48 h), H_6aPyha emerges as a highly promising and competitive platform for ^{212}Pb targeted alpha therapy, particularly for the labeling of heat-sensitive biological vectors. Future efforts will be directed towards the evaluation of the H_6aPyha chelator with ^{212}Pb and the development of bifunctional derivatives bearing a linker at the pyridine ring for covalent attachment to biomolecules.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:

References

1 Y. Berckmans, J. Kleynhans, S. V. Mechelen, K. Goffin, K. Baete, M. Koole, A. Coosemans, T. E. Cocolios, C. M. Deroose, Y. Seimbille and F. Cleeren, *Theranostics*, 2026, **16**, 2887–2917; https://doi.org/10.7150/thno.126086.

2 J. M. Scaffidi-Muta and A. D. Abell, *EJNMMI Radiopharm. Chem.*, 2025, **10**, 34; https://doi.org/10.1186/s41181-025-00362-7.

3 N. B. Pedersen, N. J. W. Straathof, F. Elvas, M. M. Herth and U. M. Battisti, *Trends Pharmacol. Sci.*, 2026, **47**, 263–275; https://doi.org/10.1016/j.tips.2026.01.001.

4 M. A. Orlova, V. V. Spiridonov, V. Y. Yaryshev, A. A. Evdokimov, A. V. Severin, T. P. Trofimova, A. A. Yaroslavov and S. N. Kalmykov, *Mendeleev Commun.*, 2026, **36**, 376–378; https://doi.org/10.71267/mencom.7972.

5 N. A. Titchenko, S. V. Ermolaev, E. A. Perekina, B. V. Egorova and S. N. Kalmykov, *Mendeleev Commun.*, 2026, **36**, 155–157; https://doi.org/10.71267/mencom.7916.

6 N. A. Titchenko, B. V. Egorova and S. N. Kalmykov, *Mendeleev Commun.*, 2025, **35**, 396–398; https://doi.org/10.71267/mencom.7656.

7 S. Ahenkorah and D. Ramdhani, *Pharmaceutics*, 2025, **17**, 1508; https://doi.org/10.3390/pharmaceutics17121508.

8 K. Yong and M. W. Brechbiel, *Dalton Trans.*, 2011, **40**, 6068; https://doi.org/10.1039/c0dt01387k.

9 H. A. Holik, F. M. Ibrahim, A. A. Elaine, B. D. Putra, A. Achmad and A. H. S. Kartamihardja, *Molecules*, 2022, **27**, 3062; https://doi.org/10.3390/molecules27103062.

10 S. Franchi, V. Di Marco and M. Tosato, *Nucl. Med. Biol.*, 2022, **114–115**, 168–188; https://doi.org/10.1016/j.nucmedbio.2022.06.002.

© Mendeleev Communications.

11 B. Bartoś, K. Lyczko, A. Kasperek, S. Krajewski and A. Bilewicz, *J. Radioanal. Nucl. Chem.*, 2013, **295**, 205–209; https://doi.org/10.1007/s10967-012-2238-4.
12 S. Mirzadeh, K. Kumar and O. A. Gansow, *Radiochim. Acta*, 1993, **60**, 1–10; https://doi.org/10.1524/ract.1993.60.1.1.
13 C. Harriswangler, P. Randhawa, N. Sommariva-Ucha, L. Wharton, L. Valencia, B. L. McNeil, H. Yang, D. Esteban-Gómez, C. Platas-Iglesias and C. F. Ramogida, *Inorg. Chem.*, 2026, **65**, 15150–15163; https://doi.org/10.1021/acs.inorgchem.6c02096.
14 D. Fiaccabrino, T. Masvikeni, B. L. McNeil, B. O. Patrick, M. D. G. Jaraquemada-Peláez, P. Schaffer and C. Orvig, *Dalton Trans.*, 2025, **54**, 11801–11807; https://doi.org/10.1039/D5DT01103E.
15 A. D. Zubenko, A. V. Pashanova, S. P. Mosaleva, E. Y. Chernikova, V. A. Karnoukhova, I. V. Fedyanin, B. V. Egorova, A. A. Shchukina, Y. V. Fedorov and O. A. Fedorova, *Inorg. Chem.*, 2024, **63**, 21652–21669; https://doi.org/10.1021/acs.inorgchem.4c03116.
16 A. D. Zubenko, A. A. Shchukina, E. Y. Chernikova, B. V. Egorova, I. S. Ikonnikova, A. B. Priselkova, A. A. Larenkov, V. B. Bubenshchikov, A. A. Mitrofanov, Y. V. Fedorov and O. A. Fedorova, *Dalton Trans.*, 2024, **53**, 1141–1155; https://doi.org/10.1039/D3DT03451H.
17 M. Tosato, L. Lazzari and V. D. Marco, *ACS Omega*, 2022, **7**, 15596–15602; https://doi.org/10.1021/acsomega.2c00387.
18 K. V. Kokov, B. V. Egorova, M. N. German, I. D. Klabukov, M. E. Krasheninnikov, A. A. Larkin-Kondrov, K. A. Makoveeva, M. V. Ovchinnikov, M. V. Sidorova and D. Y. Chuvilin, *Pharmaceutics*, 2022, **14**, 189; https://doi.org/10.3390/pharmaceutics14010189.
19 M. Asti, M. Tosato, S. Franchi and V. Di Marco, *Coord. Chem. Rev.*, 2026, **566**, 218062; https://doi.org/10.1016/j.ccr.2026.218062.

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