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# Acyclic pyridine-containing hexaacetate chelator $H_6$aPyha for lead radionuclides: from solution structure to efficient radiolabeling and serum stability

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## EXPERIMENTAL SECTION

### *Materials and equipment*

All commercially available reagents and solvents were used without further purification. Reaction progress was followed by TLC using aluminum oxide (Merck, 60 $F_{254}$, neutral). $^1$H NMR and $^{13}$C spectra were recorded at 25 °C on Varian Inova 400 spectrometer. Chemical shifts are reported in parts per million (δ) relative to the deuterated solvent used as the internal reference ($CDCl_3$: δ = 7.27; $D_2O$: δ = 4.75). The coupling constant *J* is given in Hertz (Hz). Electrospray ionization mass spectrometry (ESI-MS) analyses were performed using a Shimadzu LCMS-2020 High Performance Liquid Chromatograph Mass Spectrometer equipped with a single quadrupole detector (desolvation line/heat block temperature 250/400 °C, ionization voltage of 4.5 kV). Electrospray full scan spectra were obtained by infusion of 0.4 mL/min of aqueous solutions of the compounds. A mixture of acetonitrile (99.9+ % HPLC gradient grade) and water (50:50 *v/v*) was used as a mobile phase.

### *Synthesis*

The $H_6$aPyha ligand was prepared (Scheme 1) using a method modified from the previously described procedure.[S1]

*Pyridine-2,6-dicarbaldehyde **(1)***. Selenium dioxide (692 mg, 6.236 mmol) was added to a solution of 2,6-bis(hydroxymethyl)pyridine (600 mg, 4.312 mmol) in dioxane, and the mixture was refluxed for 24 hours. After the reaction was complete, selenium was filtered, the filtrate was evaporated, the residue was dissolved in water and extracted with chloroform. The pure product was obtained as a beige solid (560 mg, 96%). $^1$H NMR ($CDCl_3$, 25 °C, 400 MHz, δ, ppm, *J*/Hz): 8.09 (t, 1H, H(1), *J*=8.3), 8.18 (d, 2H, H(2), *J*=8.1), 10.18 (s, 2H, H(4)). The spectrum corresponds to that reported in the literature.[S2]

*$N^1$,$N^{1'}$-Bis(2-aminoethyl)-2,6-pyridinedimethanamine **(2)***. Dialdehyde **(1)** (50 mg, 0.370 mmol) was added to a solution of ethylenediamine (44 mg, 0.740 mmol) in methanol (3 ml). The mixture was stirred for 20 minutes at room temperature. Then sodium borohydride (182 mg, 4.810 mmol) was added by portions on ice bath and the resulting mixture was stirred overnight at room temperature. The solvent was evaporated and the product was repeatedly extracted with chloroform from the dry residue. After the solvent evaporation the pure product was obtained as a yellow oil (80 mg, 97%). $^1$H NMR ($CDCl_3$, 25 °C, 400 MHz, δ, ppm, *J*/Hz): 2.72 (t, 4H, H(6), *J*=5.4), 2.82 (t, 4H, H(5), *J*=5.4), 3.89 (s, 4H, H(4)), 7.16 (d, 2H, H(2), *J*=7.6), 7.59 (t, 1H, H(1), *J*=7.6). $^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz, δ, ppm): 41.62 (C-6), 52.13 (C-5), 54.95 (C-4), 120.36 (C-2), 136.82 (C-1), 159.32 (C-3).

*Tetra-tert-butyl 2,2',2'',2'''-((((pyridine-2,6-diylbis(methylene))bis((2-(tert-butoxy)-2-oxoethyl)azanediyl))bis(ethane-2,1-diyl))bis(azanetriyl))tetraacetate **(3)***. *tert*-Butyl bromoacetate (308 µl, 2.121 mmol) was added dropwise to a mixture of amine **(2)** (80 mg, 0.353 mmol) and potassium carbonate (585 mg, 4.244 mmol) in acetonitrile (8 ml). The mixture was stirred for 50 hours. After the reaction is complete inorganic salts were filtered, the filtrate was evaporated, the residue was dissolved in chloroform and washed with water. The pure product was obtained by column chromatography ($SiO_2$, $CHCl_3$-MeOH) as a yellow oil (238 mg, 74%). $^1$H NMR ($CDCl_3$, 25 °C, 400 MHz, δ, ppm, *J*/Hz): 1.43 (s, 36H, H(10,14)), 1.46 (s, 18H, H(18)), 2.95 (t, 4H, H(6), *J*=5.4), 3.02 (br.s., 4H, H(5)), 3.44 (s, 8H, H(7,11)), 3.60 (s, 4H, H(15)), 4.18 (s, 4H, H(4)), 7.49 (d, 2H, H(2), *J*=7.3), 7.70 (t, 1H, H(1), *J*=7.6). $^{13}$C NMR ($CDCl_3$, 25 °C, 100 MHz, δ, ppm):

28.11 (C-10, C-14, C-18), 51.36 (C-6), 52.35 (C-5), 55.48 (C-15), 55.80 (C-7, C-11), 59.49 (C-4), 81.13 (C-9, C-13), 81.64 (C-17), 122.16 (C-2), 137.90 (C-1), 156.79 (C-3), 170.46 (C-8, C-12, C-16).

*2,2',2'',2'''-((((Pyridine-2,6-diylbis(methylene))bis((carboxymethyl)azanediyl))bis(ethane-2,1-diyl))bis(azanetriyl))tetraacetic acid hydrochloride* ***($H_6$aPyha·HCl)***. Hydrochloric acid (4 M, 8 ml) was added to a solution of tetraester **3** (238 mg, 0.260 mmol) in a minimal volume of tetrahydrofuran. The mixture was stirred at room temperature for 1 hour. The solvents were evaporated and the product was obtained as a yellow solid (195 mg, 100%). $^1$H NMR ($D_2O$, 25 °C, 400 MHz, δ, ppm, *J*/Hz): 3.28 (br.s., 4H, H(5)), 3.49 (t, 4H, H(6), *J*=4.5), 3.65 (s, 4H, H(11)), 4.05 (s, 8H, H(7,9)), 4.35 (s, 4H, H(4)), 7.82 (d, 2H, H(2), *J*=7.6), 8.30 (t, 1H, H(1), *J*=7.6). $^{13}$C NMR ($D_2O$, 25 °C, 100 MHz, δ, ppm): 49.77 (C-5), 51.92 (C-6), 54.73 (C-11), 56.18 (C-4), 56.61 (C-7, C-9), 125.56 (C-2), 144.29 (C-1), 151.94 (C-3), 170.25 (C-8, C-10), 173.01 (C-12). MS (ESI), *m/z* calcd for $C_{23}H_{33}N_5O_{12}$ + $H^+$: 571.2 $[M+H]^+$; found 572.0; calcd for $C_{23}H_{33}N_5O_{12}$ + $2H^+$: 286.6 $[M+2H]^{2+}$; found 286.6. Anal. Calcd for $C_{23}H_{33}N_5O_{12}$·2.85HCl·4.4$H_2O$: C, 36.60; H, 5.96; N, 9.28. Found: C, 36.61; H, 6.00; N, 9.26.

### *Mass Spectrometric Analysis of the Complexes*

The complex sample for mass spectrometric analysis was prepared by mixing an aqueous solution of the $H_6$aPyha ligand (10 mM, 100 $\mu$L) and an aqueous solution of $Pb(NO_3)_2$ (10 mM, 300 $\mu$L). MALDI-TOF MS measurements were performed using a Shimadzu AXIMA Confidence instrument in reflectron mode, employing an $\alpha$-cyano-4-hydroxycinnamic acid (CHCA) matrix in a 10-fold excess.

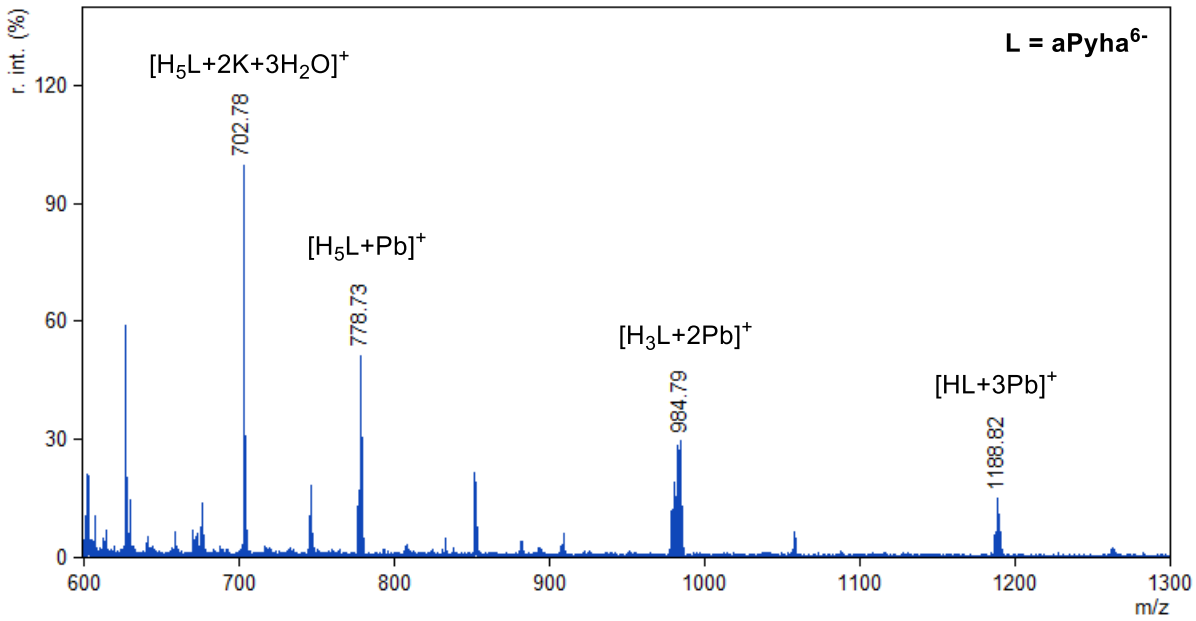



**Figure S1.** MALDI-TOF MS spectrum of $H_6$aPyha in the presence of $Pb^{2+}$ in $H_2O$ using CHCA as a matrix.

### *Potentiometric Titration*

Potentiometric titration experiments were carried out using an 808 Titrando autotitrator (Metrohm) equipped with a 10 mL autoburet, a combined glass-body pH electrode (model HI 1043B, Hanna Instruments), and a water-jacketed titration vessel maintained at 25.0±0.1 °C with a circulation thermostat (model 12108-15, Cole-Parmer). Titration experiments were performed with a constant argon flow through the titration vessel. A $CO_2$-free NaOH titrant solution (approximately 0.1 M) was prepared with deionized water (18.2 MΩ·cm) and standardized by potentiometric titration with dried potassium hydrogen phthalate. A solution of $HClO_4$ (approximately 0.1 M) was prepared using a 70% aqueous solution and standardized with the previously standardized NaOH titrant solution. The combined pH electrode was calibrated as a hydrogen-ion concentration probe by titration of the standardized $HClO_4$ with the standardized NaOH solution, and the equivalence point was determined via Gran's method via the GLEE software,[S3] which gives the standard electrode potential ($E_0$), the electrode function slope (S) and the protolytic impurity level. The ionic product of water $pK_w$ = 13.78 at 25.0 °C for 0.1 M $KNO_3$ was kept constant.[S4] Stock solutions of the ligand (0.005 M)

and $Pb(NO_3)_2$ were prepared in deionized water. The concentration of the metal salt was determined *via* complexometric titration with a standardized $Na_2H_2EDTA$ solution using xylenol orange as an indicator.
The protonation constants of the $H_6aPyha$ ligand were obtained in our previous work.[S1] To determine the stability constants of the $Pb^{2+}$ complex, a 13 mL aliquot of a $7\cdot10^{-4}$ M ligand solution containing $2\cdot10^{-3}$ M HCl and $6\cdot10^{-3}$ M $HClO_4$ in the presence of $7\cdot10^{-4}$ M $Pb(NO_3)_2$ was titrated with NaOH using 0.1 M $KNO_3$ as a background electrolyte. The titrant solution was purged with argon throughout the experiment, stirred with a magnetic stirrer, and thermostated at 25.0±0.1 °C. Electromotive forces were recorded after each addition of 0.03 mL of the NaOH solution. Equilibrium was considered established if the potential change was <0.2 mV/min. Data were collected over the pH range of 2–12 (80–100 experimental points). Stability constants were calculated using the HYPERQUAD2013 software.[S5] Speciation plots (Figure S2) were generated using HySS.[S6]

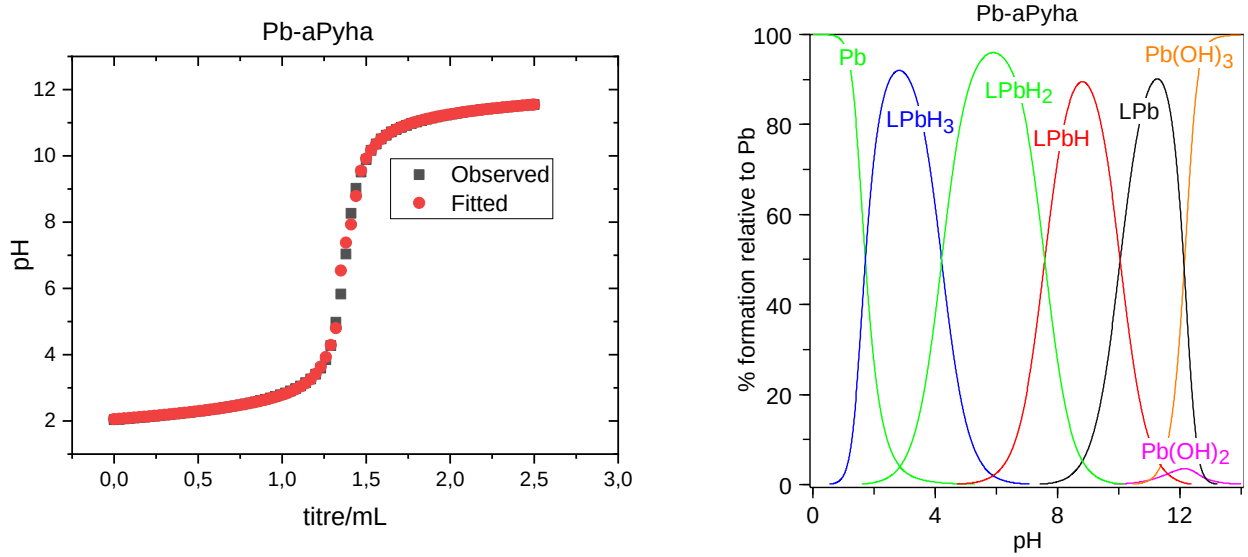



**Figure S2.** Observed (black) and fitted (red) potentiometric titration curves, and the species distribution diagram of the $Pb^{2+}$ (1 mM) – $aPyha^{6-}$ (1 mM) system as a function of pH in 0.1 M $KNO_3$.

***NMR study***

A sample of the $Pb^{2+}$ complex was prepared by dissolving $H_6aPyha$ (0.01 mmol) and $Pb(NO_3)_2$ (0.01 mmol, 1:1 ratio) in $D_2O$ (600 µL), followed by adjusting the solution to a near-physiological pH* value (6.3) using microvolumes of concentrated $HClO_4$. The apparent pH values (pH*) in $D_2O$ were measured directly using a combined glass/AgCl electrode calibrated with standard aqueous buffers.

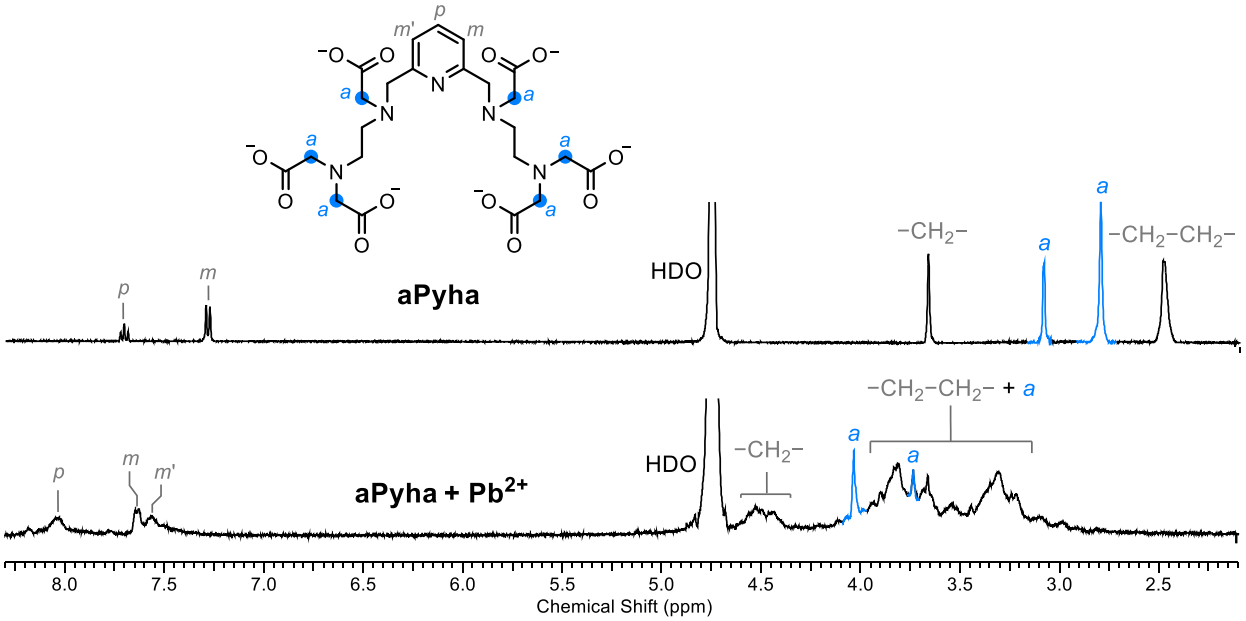



**Figure S3.** $^1H$ NMR spectra of deprotonated ligand $aPyha^{6-}$ (pH = 11.4) and its complex with $Pb^{2+}$ (pH = 6.3) in $D_2O$.

### *DFT calculations*

A conformational search was performed using the GOAT algorithm implemented in ORCA 6.1.1 [S7,S8] at the GFN2-xTB/ALPB($H_2O$) level.[S9,S10] From the resulting ensemble of 267 unique structures, six representative conformers were selected for subsequent DFT optimization based on their relative energies, overall geometric diversity, and differences in the coordination number and N/O donor environment of the $Pb^{2+}$ center. Five structures were selected from within 3.0 kcal $mol^{-1}$ of the lowest-energy GOAT structure, while an additional conformer at 4.03 kcal $mol^{-1}$ was included to represent a distinct nine-coordinate $Pb^{2+}$ geometry. The GOAT/GFN2-xTB energies were used only for conformational search and preselection.

All quantum chemical calculations were performed using the ORCA 6.1.1 software package.[S8] Geometry optimizations and harmonic vibrational frequency analyses were carried out in implicit water using the B3LYP functional [S11,S12] with D3 dispersion corrections and Becke-Johnson damping (D3BJ).[S13,S14] The def2-SVP basis set was employed for light atoms, while Pb was described using the corresponding def2-SVP basis with the associated def2 effective core potential.[S15] The calculations were accelerated by the RIJCOSX approximation [S16] with the def2/J auxiliary basis set.[S17] The solvent effects of water were simulated using the CPCM (water) model.[S18,S19] Frequency analysis confirmed that all optimized structures correspond to local minima on the potential energy surface, showing zero imaginary frequencies.

Final electronic energies were refined by single-point calculations on the optimized geometries using RKS-PBE0-D4/def2-TZVPP [S15,S20,S21] with RIJCOSX/def2/J, CPCM (water), VeryTightSCF convergence criteria, and the DefGrid3 integration grid. Pb was described using the corresponding def2-TZVPP basis with the associated def2 effective core potential. Final relative free energies were estimated using a composite scheme in which the high-level single-point electronic energy was combined with the thermochemical correction obtained from the B3LYP-D3BJ/def2-SVP frequency calculation.

The resulting composite free energies were used for the final ranking of the selected conformers. The three lowest energy-conformers were chosen as the most likely forms of the $[Pb(aPyha)]^{4-}$ complex.

**Table S1.** Electronic single-point energies, thermochemical corrections, and composite relative free energies of the three lowest-energy conformers of the $[Pb(aPyha)]^{4-}$ complex.

| **Conformer** | $\boldsymbol{E_{SP}}$, $E_h$ | $\boldsymbol{\Delta E_{SP}}$, kcal $mol^{-1}$ | $\boldsymbol{(G\text{-}E)_{freq}}$, $E_h$ | $\boldsymbol{G_{comp}}$, $E_h$ | **Final** $\boldsymbol{\Delta G_{comp}}$, kcal $mol^{-1}$ |
|---|---|---|---|---|---|
| **A** | -2261.429376 | 0.00 | 0.446488 | -2260.982888 | 0.00 |
| **B** | -2261.428477 | 0.56 | 0.446870 | -2260.981607 | 0.80 |
| **C** | -2261.427559 | 1.14 | 0.446123 | -2260.981437 | 0.91 |

$E_{SP}$ is the electronic energy from the RKS-PBE0-D4/def2-TZVPP/RIJCOSX/def2/J/CPCM(Water) single-point calculation on the B3LYP-D3BJ/def2-SVP/CPCM(Water)-optimized geometry.

$(G\text{-}E)_{freq}$ is the thermochemical correction obtained from the harmonic frequency calculation at the B3LYP-D3BJ/def2-SVP/CPCM(Water) level.

$G_{comp} = E_{SP} + (G\text{-}E)_{freq}$.

Final $\Delta G_{comp}$ values are given relative to the lowest $G_{comp}$ conformer and were used for final conformer ranking.

All listed structures showed zero imaginary frequencies.

**Conformer A**

| Atom | x | y | z |
|---|---|---|---|
| C | 4.778714 | 2.042141 | 0.002446 |
| C | 3.764897 | 1.208730 | -0.480091 |
| N | 2.583137 | 1.698600 | -0.883753 |
| C | 2.351779 | 3.015624 | -0.846898 |
| C | 3.322773 | 3.919603 | -0.389105 |
| C | 4.548946 | 3.421716 | 0.044791 |
| C | 3.950702 | -0.288130 | -0.589516 |
| N | 2.927706 | -1.049855 | 0.130972 |
| C | 2.999969 | -2.480454 | -0.187750 |
| C | 1.017408 | 3.488844 | -1.379659 |
| N | 0.602182 | 4.764539 | -0.829092 |
| C | 0.260038 | 4.728553 | 0.593507 |
| C | 2.333876 | -2.858519 | -1.505494 |
| N | 0.883553 | -2.637530 | -1.529934 |
| C | -0.883366 | 3.797192 | 1.015566 |
| N | -0.532028 | 2.391204 | 1.221926 |
| C | 0.137557 | -3.544550 | -0.661659 |
| C | 0.377336 | -2.662117 | -2.901421 |
| C | 2.988431 | -0.823078 | 1.571928 |
| C | -0.374847 | 5.460589 | -1.647866 |
| C | -1.742567 | 1.606031 | 1.394024 |
| C | 0.486114 | 2.152928 | 2.254906 |
| C | -1.306127 | -3.086092 | -0.356857 |
| O | -1.588178 | -1.860467 | -0.558517 |
| O | -2.076389 | -3.945402 | 0.098731 |
| C | 0.700886 | -1.397862 | -3.726954 |
| O | 0.844349 | -1.535056 | -4.949754 |
| O | 0.737446 | -0.289648 | -3.093395 |
| C | 1.752706 | -1.322130 | 2.357957 |
| O | 0.644514 | -1.363875 | 1.735767 |
| O | 1.933339 | -1.598912 | 3.555167 |
| C | -0.512402 | 6.995905 | -1.418678 |
| O | 0.312827 | 7.573409 | -0.671058 |
| O | -1.462038 | 7.520833 | -2.051466 |
| C | -2.579576 | 1.443436 | 0.095483 |
| O | -1.944770 | 1.335077 | -1.000184 |
| O | -3.816954 | 1.384133 | 0.232432 |
| C | 0.104557 | 2.698932 | 3.656454 |
| O | -0.562345 | 1.930842 | 4.397931 |
| O | 0.448650 | 3.882721 | 3.917212 |
| H | 5.724725 | 1.615067 | 0.341525 |
| H | 3.086156 | 4.984369 | -0.382968 |
| H | 5.322973 | 4.098496 | 0.416204 |
| H | 3.890200 | -0.558135 | -1.655749 |
| H | 4.967275 | -0.558978 | -0.240109 |
| H | 4.055152 | -2.819849 | -0.220623 |
| H | 2.528782 | -3.036440 | 0.633337 |
| H | 1.132165 | 3.608350 | -2.472456 |
| H | 0.271472 | 2.685612 | -1.230799 |
| H | 1.158162 | 4.492634 | 1.182995 |
| H | -0.009743 | 5.757630 | 0.865457 |
| H | 2.774692 | -2.270549 | -2.324976 |
| H | 2.568772 | -3.920643 | -1.723854 |
| H | -1.344815 | 4.216498 | 1.936265 |
| H | -1.666712 | 3.828872 | 0.241147 |
| H | 0.103310 | -4.573209 | -1.071248 |
| H | 0.636439 | -3.596224 | 0.316092 |
| H | -0.721015 | -2.727106 | -2.862236 |
| H | 0.740183 | -3.549896 | -3.453598 |
| H | 3.051916 | 0.262228 | 1.755691 |
| H | 3.895508 | -1.273312 | 2.022762 |
| H | -1.397753 | 5.027972 | -1.575566 |
| H | -0.086228 | 5.337338 | -2.706025 |
| H | -1.461393 | 0.591776 | 1.730891 |
| H | -2.409791 | 2.012836 | 2.182178 |
| H | 1.435053 | 2.604401 | 1.927016 |
| H | 0.628834 | 1.065937 | 2.317393 |
| Pb | 0.313525 | -0.157386 | -0.613517 |

**Conformer B**

| Atom | x | y | z |
|---|---|---|---|
| C | 5.108142 | 1.745082 | 0.340180 |
| C | 4.120714 | 1.159660 | -0.464048 |
| N | 3.135955 | 1.890764 | -0.993997 |
| C | 3.062967 | 3.207380 | -0.768776 |
| C | 4.005730 | 3.867455 | 0.027055 |
| C | 5.040412 | 3.116797 | 0.593341 |
| C | 4.119484 | -0.312620 | -0.813579 |
| N | 3.000884 | -1.047366 | -0.219667 |
| C | 2.716292 | -2.301305 | -0.921877 |
| C | 1.929560 | 3.914877 | -1.481368 |
| N | 0.596200 | 3.515994 | -1.002730 |
| C | 0.267542 | 4.266015 | 0.217182 |
| C | 1.702831 | -2.168283 | -2.058054 |
| N | 0.361882 | -1.757945 | -1.633276 |
| C | -1.002334 | 3.847549 | 0.953257 |
| N | -0.968618 | 2.524212 | 1.574729 |
| C | -0.309213 | -2.733544 | -0.784200 |
| C | -0.484697 | -1.383266 | -2.761200 |
| C | 3.124393 | -1.190381 | 1.230377 |
| C | -0.414335 | 3.653400 | -2.052883 |
| C | -0.216460 | 2.504813 | 2.825042 |
| C | -2.295116 | 1.914614 | 1.691751 |
| C | -1.331147 | -2.146650 | 0.219337 |
| O | -1.045830 | -1.022883 | 0.743237 |
| O | -2.317702 | -2.850751 | 0.495399 |
| C | -0.053847 | -0.129564 | -3.560779 |
| O | -0.621983 | 0.033325 | -4.657267 |
| O | 0.802853 | 0.640800 | -3.034844 |
| C | 4.318613 | -2.007676 | 1.797132 |
| O | 4.421994 | -1.978570 | 3.048179 |
| O | 5.062023 | -2.624930 | 0.990905 |
| C | -1.717861 | 2.837543 | -1.877987 |
| O | -1.617650 | 1.657762 | -1.418486 |
| O | -2.771850 | 3.377498 | -2.261391 |
| C | 1.318529 | 2.490884 | 2.653098 |
| O | 1.991044 | 3.322010 | 3.297802 |
| O | 1.785056 | 1.595978 | 1.884428 |
| C | -3.345644 | 2.566938 | 2.628963 |
| O | -4.410471 | 1.909996 | 2.750526 |
| O | -3.077622 | 3.672115 | 3.167446 |
| H | 5.906353 | 1.130419 | 0.762034 |
| H | 3.927167 | 4.943378 | 0.197187 |
| H | 5.789877 | 3.599489 | 1.226070 |
| H | 4.024911 | -0.381805 | -1.908437 |
| H | 5.081928 | -0.769735 | -0.523018 |
| H | 3.637538 | -2.750775 | -1.334521 |
| H | 2.357338 | -3.029147 | -0.180804 |
| H | 2.065041 | 5.012438 | -1.417902 |
| H | 2.002290 | 3.634565 | -2.541684 |
| H | 1.125688 | 4.200585 | 0.900681 |
| H | 0.156818 | 5.343315 | -0.037187 |
| H | 2.060159 | -1.414261 | -2.772938 |
| H | 1.664552 | -3.139819 | -2.600688 |
| H | -1.206991 | 4.625154 | 1.713274 |
| H | -1.856352 | 3.883509 | 0.264073 |
| H | -0.815656 | -3.519622 | -1.379294 |
| H | 0.438213 | -3.247020 | -0.160715 |
| H | -1.493884 | -1.158737 | -2.376113 |
| H | -0.605873 | -2.222720 | -3.479122 |
| H | 2.196080 | -1.654467 | 1.607477 |
| H | 3.139077 | -0.187514 | 1.681529 |
| H | 0.028514 | 3.269113 | -2.984687 |
| H | -0.691637 | 4.713844 | -2.217021 |
| H | -0.498220 | 3.344415 | 3.484977 |
| H | -0.472575 | 1.570785 | 3.353540 |
| H | -2.165473 | 0.862682 | 1.990002 |
| H | -2.726995 | 1.863739 | 0.679867 |
| Pb | 0.542044 | 0.766656 | -0.356212 |

**Conformer C**

| Atom | x | y | z |
|---|---|---|---|
| C | 5.227551 | 2.007719 | -1.426216 |
| C | 4.111212 | 1.341419 | -0.898554 |
| N | 3.065483 | 2.021167 | -0.420598 |
| C | 3.029068 | 3.360841 | -0.469196 |
| C | 4.091621 | 4.092549 | -1.009086 |
| C | 5.210234 | 3.400659 | -1.483611 |
| C | 4.030590 | -0.171950 | -0.936568 |
| N | 3.145473 | -0.756496 | 0.060506 |
| C | 2.821192 | -2.158155 | -0.229010 |
| C | 1.801355 | 4.040198 | 0.094567 |
| N | 0.550620 | 3.520909 | -0.468736 |
| C | -0.635117 | 4.051520 | 0.215091 |
| C | 1.871394 | -2.373060 | -1.402008 |
| N | 0.528368 | -1.812429 | -1.205252 |
| C | -0.844615 | 3.546446 | 1.639066 |
| N | -1.128726 | 2.116427 | 1.765514 |
| C | -0.313030 | -2.582215 | -0.285255 |
| C | -0.124535 | -1.485435 | -2.469676 |
| C | 3.657235 | -0.604177 | 1.418129 |
| C | 0.473045 | 3.671307 | -1.925754 |
| C | -0.843073 | 1.608362 | 3.119734 |
| C | -2.466255 | 1.759873 | 1.294924 |
| C | -0.729482 | -4.030371 | -0.676866 |
| O | -0.312195 | -4.504725 | -1.763689 |
| O | -1.464542 | -4.597412 | 0.166433 |
| C | 0.543661 | -0.320161 | -3.231542 |
| O | 0.390155 | -0.284783 | -4.463195 |
| O | 1.171477 | 0.549735 | -2.538700 |
| C | 2.616322 | -0.851014 | 2.539901 |
| O | 1.389895 | -0.720079 | 2.241024 |
| O | 3.078462 | -1.117420 | 3.663819 |
| C | 0.565434 | 5.095718 | -2.542080 |
| O | 0.615876 | 5.112529 | -3.795173 |
| O | 0.572527 | 6.090401 | -1.771005 |
| C | -1.794712 | 2.182978 | 4.205300 |
| O | -1.456095 | 3.273827 | 4.733797 |
| O | -2.850814 | 1.535533 | 4.424006 |
| C | -2.616176 | 1.578705 | -0.234658 |
| O | -3.570444 | 2.147821 | -0.794184 |
| O | -1.776278 | 0.809913 | -0.809160 |
| H | 6.082909 | 1.436877 | -1.794134 |
| H | 4.036562 | 5.181777 | -1.056461 |
| H | 6.060041 | 3.946121 | -1.901941 |
| H | 3.640237 | -0.429546 | -1.933555 |
| H | 5.056531 | -0.591456 | -0.875934 |
| H | 3.748079 | -2.738616 | -0.424215 |
| H | 2.373924 | -2.584807 | 0.678697 |
| H | 1.791182 | 3.858590 | 1.181769 |
| H | 1.882581 | 5.127400 | -0.068746 |
| H | -0.597639 | 5.155967 | 0.246949 |
| H | -1.511824 | 3.796714 | -0.397009 |
| H | 2.297987 | -1.920790 | -2.308555 |
| H | 1.798708 | -3.456480 | -1.597364 |
| H | 0.040949 | 3.766230 | 2.253243 |
| H | -1.663226 | 4.149736 | 2.084246 |
| H | 0.186103 | -2.627287 | 0.696207 |
| H | -1.238974 | -2.007153 | -0.115155 |
| H | -1.148146 | -1.145568 | -2.240952 |
| H | -0.200916 | -2.359148 | -3.137987 |
| H | 4.013608 | 0.430668 | 1.548190 |
| H | 4.529509 | -1.263665 | 1.603696 |
| H | -0.482214 | 3.228827 | -2.253120 |
| H | 1.244468 | 3.042719 | -2.393296 |
| H | -0.915526 | 0.511281 | 3.091841 |
| H | 0.200212 | 1.869301 | 3.358126 |
| H | -3.224089 | 2.494016 | 1.630897 |
| H | -2.736491 | 0.796624 | 1.754619 |
| Pb | 0.703948 | 0.651191 | -0.008769 |

### *Radiochemical Separation of $^{210}$Pb*

Radioactivity measurements were performed using a gamma-ray spectrometer equipped with high-purity germanium (HPGe) detectors: a coaxial ORTEC GEM-C5060P4-B (50% relative efficiency) coupled with an ORTEC DSPec 50 digital signal processor, and a Canberra GR3818 detector. Radiochemical separation of $^{210}$Pb using Sr Resin (Triskem International, France) was carried out according to a previously reported procedure.[S22]
A solution of $^{226}$Ra dissolved in 0.1 M $HNO_3$ was loaded onto a Sr Resin chromatographic column. A 0.1 M $HNO_3$ solution was passed through the column until radium was completely washed out, after which $^{210}$Pb was eluted with 0.05 M ammonium citrate solution, 0.05 M $(NH_4)_3$Cit. The separation of $^{210}$Pb from $^{226}$Ra was monitored by tracking the gamma-ray energy peaks at 46.5 keV ($^{210}$Pb) and 186.2 keV ($^{226}$Ra). The collected fractions of $^{210}$Pb were evaporated to dryness and redissolved in 0.01 M HCl.

### *Radiolabeling Studies*

Radiolabeling was performed at room temperature in a 0.15 M NaOAc buffer at pH 5.0–6.0 using 3 kBq of $^{210}$Pb per sample. Thin-layer chromatography (TLC) was used to determine the radiolabeling yield, employing chromatography paper as the stationary phase and a 10 mM NaOH solution in 0.9 % NaCl as the mobile phase. The distribution of radioactivity on the chromatograms was analyzed via gamma-ray spectrometry.
To determine the optimal ligand concentration required for quantitative complexation, the $^{210}$Pb solution was mixed with 0.15 M NaOAc and varying concentrations of $H_6$aPyha. The radiochemical yield was evaluated using the TLC method described above, followed by gamma-ray counting of the TLC strips.

### *In Vitro Serum Stability*

The radioactively labeled complex at a ligand concentration of 1 mM was added to a ninefold volume excess of fetal bovine serum (HyClone) and incubated at 37 °C. At designated time points, aliquots of the mixture were withdrawn, and the protein fraction was precipitated by adding an excess of cold ethanol (2–4 °C). After centrifugation, the supernatant was separated, and the radioactivity in the solution was quantified using gamma-ray spectrometry.

**References**


S1 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; https://doi.org/10.1039/d3dt03451h.

S2 X. Wu, B. Wang, Z. Yang and L. Chen, *J. Mater. Chem. A*, 2019, 7, 5650; https://doi.org/10.1039/c8ta11447a.

S3 P. Gans and B. O'Sullivan, *Talanta*, 2000, 51, 33; https://doi.org/10.1016/S0039-9140(99)00245-3.

S4 P. L. Brown and C. Ekberg, *Hydrolysis of Metal Ions*, Wiley, Weinheim, 2016.

S5 P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739; https://doi.org/10.1016/0039-9140(96)01958-3.

S6 L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini and A. Vacca, *Coord. Chem. Rev.*, 1999, **184**, 311; https://doi.org/10.1016/S0010-8545(98)00260-4.

S7 B. de Souza, *Angew. Chem., Int. Ed.*, 2025, **64**, e202500393. https://doi.org/10.1002/anie.202500393.

S8 F. Neese, *Wiley Interdiscip. Rev.: Comput. Mol. Sci.*, 2025, **15**, e70019; https://doi.org/10.1002/wcms.70019.

S9 C. Bannwarth, S. Ehlert and S. Grimme, *J. Chem. Theory Comput.*, 2019, **15**, 1652; https://doi.org/10.1021/acs.jctc.8b01176.

S10 S. Ehlert, M. Stahn, S. Spicher and S. Grimme, *J. Chem. Theory Comput.*, 2021, **17**, 4250; https://doi.org/10.1021/acs.jctc.1c00471.

S11 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648; https://doi.org/10.1063/1.464913.

S12 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785; https://doi.org/10.1103/physrevb.37.785.

S13 S. Grimme, J. Antony, S. Ehrlich and H. Krieg, *J. Chem. Phys.*, 2010, **132**, 154104; https://doi.org/10.1063/1.3382344.

S14 S. Grimme, S. Ehrlich and L. Goerigk, *J. Comput. Chem.*, 2011, **32**, 1456; https://doi.org/10.1002/jcc.21759.

S15 F. Weigend and R. Ahlrichs, *Phys. Chem. Chem. Phys.*, 2005, **7**, 3297; https://doi.org/10.1039/b508541a.

S16 F. Neese, F. Wennmohs, A. Hansen and U. Becker, *Chem. Phys.*, 2009, **356**, 98; https://doi.org/10.1016/j.chemphys.2008.10.036.

S17 F. Weigend, *Phys. Chem. Chem. Phys.*, 2006, **8**, 1057; https://doi.org/10.1039/b515623h.

S18 V. Barone and M. Cossi, *J. Phys. Chem. A*, 1998, **102**, 1995; https://doi.org/10.1021/jp9716997.

S19 M. Cossi, N. Rega, G. Scalmani and V. Barone, *J. Comput. Chem.*, 2003, **24**, 669; https://doi.org/10.1002/jcc.10189.

S20 C. Adamo and V. Barone, *J. Chem. Phys.*, 1999, **110**, 6158; https://doi.org/10.1063/1.478522.

S21 E. Caldeweyher, S. Ehlert, A. Hansen, H. Neugebauer, S. Spicher, C. Bannwarth and S. Grimme, *J. Chem. Phys.*, 2019, 150, 154122; https://doi.org/10.1063/1.5090222.

S22 *Lead-210 and Polonium-210 in Water (with Vacuum Box System)*, Eichrom Technologies, LLC, Lisle, IL, USA, 2014.