A 3,2-Hydroxypyridinone-based Decoration Agent that Removes Uranium from Bones In Vivo

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Searching for actinide decoration agents with advantages of high decoration efficiency, minimal biological toxicity, and high oral efficiency is crucial for nuclear safety and the sustainable development of nuclear energy. Removing actinides deposited in bones after intake is one of the most significant challenges remaining in this field because of the instantaneous formation of highly stable actinide phosphate complexes upon contact with hydroxyapatite. Here we report a hydroxypyridinone-based ligand (5LIO-1-Cm-3,2-HOPO) exhibiting stronger affinity for U(VI) compared with the reported tetradentate hydroxypyridinone ligands. This is further revealed by the first principles calculation analysis on bonding between the ligand and uranium. Both in vitro uranium removal assay and in vivo decoration experiments with mice show that 5LIO-1-Cm-3,2-HOPO can remove uranium from kidneys and bones with high efficiencies, while the decoration efficiency is nearly independent of the treatment time. Moreover, this ligand shows a high oral decoration efficiency, making it attractive for practical applications.
**Results**

**Ligand design and synthesis.** In order to weaken the intramolecular hydrogen bond formed between the amide and hydroxide groups found in many tetradeptate HOPO ligands, a ligand [N,N'-oxysbis(ethane-2,1-diy)]bis-[3-(3-hydroxy-2-oxopyridin-1(2H)-yl) acetamide], denoted as 5LIO-1-Cm-3,2-HOPO, was designed by introducing the carboxylic group on the N site of pyridine ring. The distance between the amide and carboxyl group is increased by the addition of a methyl group. 5LIO-1-Cm-3,2-HOPO was obtained from a 4-step synthesis as illustrated in Fig. 125–27. Commercially available 1,2-dihydro-2,3-pyridinediiodol was first alkylated at the nitrogen position of the pyridine ring by reacting it with excess amounts of ethyl bromocacetate at 150 °C to yield the ester (product A). Next, the hydroxyl group in A was protected with a benzyl moiety, and the ester group was activated via hydrolysis to afford product B. 5LIO-1-Cm-3,2-HOPOBn was then obtained via amidation of the carboxyl group with the amine backbone. Benzyl protection was finally removed by 5% Pd/C to obtain 5LIO-1-Cm-3,2-HOPO which was obtained upon amidation of the carboxyl group with the amine backbone.

**Solution thermodynamic studies.** The solution thermodynamic data of 5LIO-1-Cm-3,2-HOPO was first measured to evaluate its complexation behavior with U(VI). The protonation constants of the free ligand, 5LIO-1-Cm-3,2-HOPO (denoted as LH2 in this part), were determined by potentiometric titration. The formation constants could be calculated with equation (1) in the experimental section (Table 1). The two protonation constants,
The stability constants of the UO₂-L complexes, log \( \beta \) constants was calculated with established as log \( \beta _{L} \), indicating that 5LIO-1-Cm-3,2-HOPO is a highly values are significantly lower than those of the previously reported and most optimal ligand, 7.5(7) (Table 1). These formation constants are noticeably higher than those of the previously reported and most optimal ligand, 5LIO-(Me-3,2-HOPO) denoted \( \text{Lc} \), EDTA denoted \( \text{Le} \). Only one mixed formation \( \text{UO}_2\text{LcH}_2\text{O} \) (denoted \( \text{LcH}_2 \)) to 14.9, 15.7.

Table 1 Protonation Constants of Ligands and Uranyl Chelation Stability Constants

| Protonation Constants | EDTA(+LH\textsubscript{4}) | LH\textsubscript{2} | LH\textsubscript{3} |
|-----------------------|--------------------------|----------------|----------------|
| \( pK_{a1} \)         | 2.0(3)                   | 5.91           | 8.3(5)         |
| \( pK_{a2} \)         | 2.7(5)                   | 7.14           | 9.3(4)         |
| \( pK_{a3} \)         | 6.1(1)                   |                |                |
| \( pK_{a4} \)         | 9.8(4)                   |                |                |
| Ligand                | Species                   | \( m, l, h \)  | \( \log {p_{\text{UO}_2}} \) |
| 5LIO-(Me-3,2-HOPO)(L) | \( \text{UO}_2\text{LH}^+ \) | 1, 1, 1        | 24.8(7)        |
|                      | \( \text{UO}_2\text{L}^0 \) | 1, 1, 0        | 18.6(7)        |
|                      | \( \text{UO}_2\text{L}^0\text{OH}^- \) | 1, 1, -1       | 7.5(7)         |
| 5LIO-(Me-3,2-HOPO)(L\textsubscript{e}) \( \text{Lc} \) | \( \text{UO}_2\text{LcH}_2\text{OH}^+ \) | 1, 1, 1        | 18.4           |
|                      | \( \text{UO}_2\text{Lc}^0 \) | 1, 1, 1        | 14.9           |
|                      | \( \text{UO}_2\text{Lc}^0\text{OH}^- \) | 1, 1, -1       | 6.3            |

*Data from ref 29.

\( pM = -\log [M_{\text{free}}]; [M] = 10^{-4} \text{ M and } [L] = 10^{-3} \text{ M} \)

Characterizations of \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO Complex} \)

The \( ^1\text{H NMR and } ^1\text{C NMR spectra of the complex in DMSO-}d_6 \) were collected (Supplementary Figure 1f). A slight difference between the chemical shifts of the carbon atoms from \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO complex} \) and from the ligand can be observed in the \( ^1\text{C NMR spectra collected in DMSO-}d_6 \). More notably, the feature at 8.97 ppm in \( ^1\text{H NMR spectrum} \), which can be observed for the raw ligand and assigned to the hydroxyl group, disappears for the \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO complex} \), initially suggesting the complexion between the ligand and U (VI). In comparison with the FTIR spectrum of \( \text{SIO-1-Cm-3,2-HOPO} \), the spectrum of \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO} \) exhibits an additional peak at 899 cm\(^{-1}\) attributed to the uranyl group (Supplementary Figure 2b). A significant intensity reduction of the peak at 3282 cm\(^{-1}\) assigned to the hydroxyl group was observed for the spectrum of \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO} \), when compared with that of \( \text{SIO-1-Cm-3,2-HOPO} \). Nevertheless, much more powerful evidence comes from the elemental analysis and LC-MS analysis, confirming the formation of \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO complex} \) with a metal to ligand molar ratio of 1:1 (Supplementary Figure 1f).

Extended X-ray adsorption fine structure (EXAFS). To characterize the local coordination environment of the uranyl ion in these complexes, a solid sample of \( \text{UO}_2^-\text{SIO-1-Cm-3,2-HOPO} \) was prepared and characterized for the uranium ion. The EXAFS spectrum of the complex was collected and compared with the spectrum of the ligand. The presence of the uranyl ion was confirmed by the characteristic features in the EXAFS spectrum, including the uranyl peak at 984 cm\(^{-1}\) and the hydroxyl peak at 899 cm\(^{-1}\). These features are consistent with the EXAFS spectrum of the ligand, confirming the presence of the uranyl ion in the complex.
was precipitated from a mixture of methanol and water, and was analyzed using synchrotron radiation EXAFS technique. The EXAFS spectra for the solid samples of UO₂(NO₃)₂ and UO₂-5LIO-1-Cm-3,2-HOPO contain two distinct oxygen coordination shells: axial oxygen, Oₐx, and equatorial oxygen, Oₑq (Supplementary Figure 4). The refinement results are provided in Supplementary Table 2, listing all structural parameters, such as coordination number (CN), bonds distance (R), and the Debye/Waller factor (σ²). Within the experimental error, the coordination environment of uranium contains 2.0–2.2 Oₐx atoms at bond distances ranging from 1.77–1.82 Å, and 4.70 ± 0.60 Oₑq atoms at distances ranging from 2.41–2.48 Å. Notably, the average U–Oₑq bond distance in UO₂-5LIO-1-Cm-3,2-HOPO (2.41 Å) is shorter than that of UO₂(NO₃)₂ (2.48 Å), whereas the U–Oₐx bond distance in UO₂-5LIO-1-Cm-3,2-HOPO (1.82 Å) is longer than that of UO₂(NO₃)₂ (1.77 Å). These come as a result of the change in coordination numbers between UO₂-5LIO-1-Cm-3,2-HOPO (4.7 ± 0.6) and UO₂(NO₃)₂ (5.6 ± 0.6). The calculated bond distances of UO₂-5LIO-1-Cm-3,2-HOPO are in good agreement with the structural data reported of the uranyl hydroxypyridinone compounds²⁵,³²,³³.

Density functional theory (DFT). We performed DFT calculations to reveal the effect of intramolecular hydrogen bonds on the interaction between the ligand and U(VI). In 5LIO-(Me-3,2-HOPO), the strong intramolecular hydrogen bond (approximately 1.96 Å) between the amide group and the hydroxyl group of the pyridinone ring promotes the formation of a planar local structure between the two bonding components (Fig. 2a). This strong hydrogen bond and local planar structure remain intact when chelating a UO₂²⁺ cation (Fig. 2b). On the other hand, for 5LIO-1-Cm-3,2-HOPO, we obtained two different stable structures, one containing –NH···N (pyridine) intramolecular hydrogen bonds and the other containing –NH···O (pyridinone) intramolecular hydrogen bonds [Fig. 2c (State I and State II)]. Stable structure I is more energetically favorable than II by a relatively small energy difference of approximately 0.96 kcal mol⁻¹. The calculated transition state (Fig. 2c) between the two structures shows a 1.37 kcal mol⁻¹ energy barrier for this structural transformation. Such a low energy barrier and small energy difference imply that the C–N and C–C bonds on either side of the methylene group can freely rotate and that the two types of intramolecular hydrogen bonds in 5LIO-1-Cm-3,2-HOPO can spontaneously transform at room temperature. Compared to 5LIO-(Me-3,2-HOPO), the increase in the local degrees of freedom of 5LIO-1-Cm-3,2-HOPO can be attributed to the cooperation of the inversion of the pyridinone ring and the addition of the methylene group, leading to the weakening of the intramolecular hydrogen bonds (Fig. 2c). We then calculated the Gibbs free energy change for the transition state (Fig. 2d).

**Fig. 2** DFT optimized structures, geometric parameters, and relevant energy information. a 5LIO-(Me-3,2-HOPO). b The UO₂-5LIO-(Me-3,2-HOPO) chelate. c State I and State II show two types of stable 5LIO-1-Cm-3,2-HOPO states containing –NH···N (pyridine) and –NH···O (pyridinone) intramolecular hydrogen bonds, respectively. Transition state represents the transition state between State I and State II. The inset energy diagram shows the energy relationship for all states. d Two types of UO₂-5LIO-1-Cm-3,2-HOPO chelates. The left structure contains two –NH···N (pyridinone) intramolecular hydrogen bonds, whereas the right contains one –NH···O (pyridinone) intramolecular hydrogen bond and one pyridine –NH···N intramolecular hydrogen bond. The gray, white, red, blue, and yellow spheres represent C, H, O, N, and U atoms, respectively. The pink dotted lines represent hydrogen bonds. ΔG(depro) in (a, c) denotes the Gibbs free energy changes of the deprotonated reactions. E_b in (b, d) represents the binding energies between the UO₂²⁺ cations and the chelating ligands. The U–Oₑq distances in b and d were calculated by averaging the four U–O distances between uranium and oxygen atoms from the chelating agents. Source Data are provided as Supplementary Data 1–3.
Deprotonation reactions ($\Delta G_{\text{depro}}$) of the hydroxyl group in the pyridinone ring within both ligands. The $\Delta G_{\text{depro}}$ values of 5LIO-1-Cm-3,2-HOPO (38.87 and 37.26 kcal mol$^{-1}$ for I and II, respectively) are both larger than that of 5LIO-(Me-3,2-HOPO) (29.44 kcal mol$^{-1}$), offering a qualitative explanation for the relatively larger $pK_a$ value for 5LIO-1-Cm-3,2-HOPO determined experimentally.

Fig. 2d displays two stable $\text{UO}_2\text{L}_2$ complexes. The red dotted boxes surround the major negative ESP areas. The negative ESP areas contributed by the two oxygen atoms were calculated and the pyridinone ring.

$\text{UO}_2\text{L}_2\text{HOPO}$ cations and the chelator indicates that alteration of the hydrogen-bonding scheme would not drastically affect the binding energy ($-9.23/-9.21$ eV). In contrast, the binding energy difference between $\text{UO}_2\text{L}_2\text{HOPO}$ and $\text{UO}_2\text{L}_2\text{HOPO}$ is $-9.81$ eV vs $-9.23/-9.21$ eV is obvious. Therefore, this calculation result reveals the enhancement of the binding ability of the 5LIO-1-Cm-3,2-HOPO ligand, especially the oxygen denticity.

We propose that the enhanced uranyl binding in 5LIO-1-Cm-3,2-HOPO originates from its intrinsic structural and electronic features. From a classical point of view, the negatively charged oxygen of the deprotonated hydroxyl group is strongly attracted to the hydrogen atom of the nearby amide in 5LIO-(Me-3,2-HOPO), thereby generating a strong $\text{NH}^+\text{O}$ (pyridine) hydrogen bond (Fig. 3a).

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Moreover, the negative ESP area originating from the two oxygen atoms is further broadened over the electron density surface in 5LIO-1-Cm-3,2-HOPO (46.07 Å$^2$) than in 5LIO-(Me-3,2-HOPO) (38.67 Å$^2$). This observation indicates that 5LIO-1-Cm-3,2-HOPO could provide a wider effective landing surface region for $\text{UO}_2^{2+}$ complexation. These calculation results indicate that the exposure of the deprotonated hydroxyl group and the weak and transformable intramolecular hydrogen bond synergistically contributes to the unique distribution of the negative ESP between the two oxygen atoms. To help directly visualize and compare the ESP features, we depicted the two ESP isosurfaces for the two ligands, at a same value of $+/−188.25$ kcal mol$^{-1}$, in Fig. 3e. Clearly, the negative ESP distribution space is much larger in 5LIO-1-Cm-3,2-HOPO than in 5LIO-(Me-3,2-HOPO). In addition, Morokuma scheme energy decomposition analyses (EDA) results show that the E(elstat) term in $\text{UO}_2\text{L}_2\text{HOPO}$ is ca. 1.16 eV larger than that in $\text{UO}_2\text{L}_2\text{HOPO}$. This result confirms our perspective that the electrostatic effect of 5LIO-1-Cm-3,2-HOPO can be fully released.

Most surprisingly, DFT calculations further reveal an important interaction between the axial oxygen of uranyl and the side
chain of 5LIO-1-Cm-3,2-HOPO in the UO2-5LIO-1-Cm-3,2-HOPO complex. For 5LIO-1-Cm-3,2-HOPO, the amide group could simultaneously form an intramolecular hydrogen bond with the oxygen of pyridinone ring, as well as an intermolecular hydrogen bond with the axial oxygen of the uranyl ion (Fig. 4a, b). The bond distance of the intramolecular hydrogen bond (−NH···O (pyridinone), 2.05 Å) is shorter than the intermolecular hydrogen bond (−NH···O (uranyl), 2.39 Å), showing relatively stronger intramolecular hydrogen bonding interactions in the former. This is mainly because of the electrostatic advantage of the oxygen of the pyridinone ring (Fig. 3d). Meanwhile, an intermolecular hydrogen bond between the methylene and the axial oxygen of uranyl can be found in the UO2-5LIO-1-Cm-3,2-HOPO complex. The longer −CH···O (uranyl) hydrogen bond distance (2.71 Å) reveals that this hydrogen bonding interaction is relatively weaker. Furthermore, we performed reduced density gradient (RDG) analyses based on the ground state electron density of the UO2-5LIO-1-Cm-3,2-HOPO complex. The small RDG values (blue areas, arrows point) between H and O (uranyl) clearly confirmed the two types of hydrogen bonding interactions, as shown in Fig. 4c. Formation of the two intermolecular hydrogen bonds are mainly attributed to the longer and more flexible side chain of the 5LIO-1-Cm-3,2-HOPO chelator. In contrast, for 5LIO-(Me-3,2-HOPO), the side chain of this chelator is relative shorter and more rigid, thus only intramolecular −NH···O (pyridine) hydrogen bonds can be formed (Fig. 2b).

In order to quantitatively evaluate the bonding contributions of the intermolecular hydrogen bond, we also performed quantum theory of atoms in molecules (QTAIM), bond critical points (BCP) between relevant H and O atoms were found in the ground state electron densities, and the potential energy density, \( V(r) \), of these BCPs were calculated. According to the relationship between \( V(r) \) and the hydrogen bond energy, \( E^{HB} \), it can be expected that the intermolecular hydrogen bond in UO2-5LIO-(Me-3,2-HOPO) is about −0.36 eV, signifying a relatively stronger hydrogen bond interaction. This intramolecular hydrogen bond was significantly weakened to −0.27 eV in UO2-5LIO-1-Cm-3,2-HOPO. The \( E^{HB} \) values of the intermolecular hydrogen bond interactions (Supplementary Table 3). Although the binding contributions (−0.16 eV in total) of the intermolecular hydrogen bond is relatively small compared to the total binding energy (−9.21 eV), it can be expected that the intermolecular hydrogen bond could provide an additional driving force for the coordination of uranyl and therefore enhance the chelating ability of 5LIO-1-Cm-3,2-HOPO.

**Cytotoxicity of chelating agents.** Given the high affinity of 5LIO-1-Cm-3,2-HOPO for uranyl, the U(VI) sequestration performance and toxicity of 5LIO-1-Cm-3,2-HOPO were evaluated and compared with those of the clinically-used ZnNa3-DTPA and the previously reported most optimal tetradentate ligand 5LIO-(Me-3,2-HOPO) in vitro and in vivo. Renal injury is one of the major concerns in the case of uranium contamination. Therefore, U(VI) uptake and release assays were first conducted using renal proximal tubular epithelial cells from rat (NRK-52E cells). The toxicity assay of U(VI) with chelation therapy agent has been performed, and 12.4 \( \mu \)M was considered as the acceptable concentration for the following cellular assays. Then, a comprehensive toxicity assay of U(VI) and chelating agents was performed by adding 12.4 \( \mu \)M U(VI) and different concentrations of chelating agents ranging from 20.0 to 320.0 \( \mu \)M. The results

![Fig. 4](image-url)
show that the comprehensive toxicity of UO$_2$-5LIO-1-Cm-3,2-HOPO is slightly lower than that of UO$_2$-ZnNa$_3$-DTPA at low dosage, and notably lower than that of UO$_2$-5LIO-(Me-3,2-HOPO) (Supplementary Table 4, Fig. 5a).

**U(VI) uptake and release.** In vitro U(VI) uptake and release assays were conducted to investigate the uranyl removal efficiency of 5LIO-1-Cm-3,2-HOPO at the cellular level. In vitro assay for U(VI) uptake and release from NRK-52E cells were performed by adding 12.4 μM U(VI) solution to cells, followed by 320.0 μM solution of the chelators. Fig. 5b shows that the addition of 5LIO-1-Cm-3,2-HOPO can remove 99.5% of uranium from NRK-52E cells, whereas ZnNa$_3$-DTPA can remove only 12.5% of uranium under the same condition. Clearly, the uranium removal efficiency of 5LIO-1-Cm-3,2-HOPO is much higher than that of ZnNa$_3$-DTPA at an equal molar dosage (Supplementary Table 5). However, since the NRK-52E cells were cultured in medium that contain uranium throughout the experiment, question remains as whether the ligand mainly play the role of preventing the uranium uptake of the cells, or enhancing the release of the intracellular uranium. Therefore, another in vitro assay for U(VI) release from NRK-52E cells was designed by adding 12.4 μM U(VI) solution to cells first, then removing the U(VI) culture medium, followed by the addition of 320.0 μM solution containing the chelators. As shown in Fig. 5b, the treatment of 5LIO-1-Cm-3,2-HOPO resulted in a uranium removal efficiency of 75.8%, illustrating that 5LIO-1-Cm-3,2-HOPO plays a major role for enhancing the U(VI) release from cells (Supplementary Table 6).

**In vivo uranyl decorporation.** Considering the remarkable performance of 5LIO-1-Cm-3,2-HOPO in removing U(VI) at the cellular level, further evaluation of 5LIO-1-Cm-3,2-HOPO, 5LIO-(Me-3,2-HOPO), and ZnNa$_3$-DTPA was conducted via in vivo chelation of U(VI) in mice. Fig. 6 illustrates the procedure of rounded in vivo decorporation assays with different administration methods and time. Three batches of in vivo decorporation assays were designed to study and compare the performance between the three ligands, 5LIO-1-Cm-3,2-HOPO, 5LIO-(Me-3,2-HOPO), and ZnNa$_3$-DTPA, including single dosage group with intraperitoneal (ip) injection, single dosage group with oral administration, and multiple dosage and delayed multiple dosage groups with ip injection.

In the single-dose group with intraperitoneal (ip) injection, the first assay was performed to compare the performance of 5LIO-1-Cm-3,2-HOPO and ZnNa$_3$-DTPA on the decorporation of uranium, where 5LIO-1-Cm-3,2-HOPO and ZnNa$_3$-DTPA (193 μmol kg$^{-1}$, molar ratio to uranium is 92:1) were injected intraperitoneally (ip) three min after an initial intravenous (iv) injection of U(VI) (0.5 mg kg$^{-1}$). At 24 h after this iv injection, the kidneys and femurs from the control group were determined to contain 6.72 ± 0.72 and 2.64 ± 0.36 μg U(VI) per gram of tissue, respectively. The group receiving the ip injection of 5LIO-1-Cm-3,2-HOPO displayed a reduced accumulation of U(VI) in the kidneys and femurs, where uranium levels were decreased by 82.9 and 39.0%, respectively. The groups given ZnNa$_3$-DTPA, however, showed limited removal of U(VI) under identical experimental conditions (20.5% and 9.1%, respectively) (Fig. 6a and Supplementary Table 7). Another assay was performed to directly compare the performance of 5LIO-1-Cm-3,2-HOPO and 5LIO-(Me-3,2-HOPO) (193 μmol kg$^{-1}$, molar ratio to uranium is 92:1) on decorporation of uranium in a similar fashion. The group treated with 5LIO-1-Cm-3,2-HOPO yielded a 86.8% and 47.9% of U(VI) removal efficiency in the kidneys and femurs, respectively. Notably, this ligand shows a removal efficiency in the femurs six times higher than that of 5LIO-(Me-3,2-HOPO), since the group given 5LIO-(Me-3,2-HOPO) showed limited effect on removing U(VI) deposited in femurs under the identical experimental conditions (Removal percentage of 8.0%) (Supplementary Table 8 and Fig. 6b). These results are well consistent with the previous study when 5LIO-(Me-3,2-HOPO) was originally synthesized$^{15,23}$. The results of uranium concentrations in urine and feces for the groups treated with 5LIO-1-Cm-3,2-HOPO and 5LIO-(Me-3,2-HOPO) via ip injection are listed and compared in Supplementary Table 9, showing a clear increase of uranium excretion in the 5LIO-1-Cm-3,2-HOPO treated group. This further supports the observation of the enhanced decorporation efficiency of 5LIO-1-Cm-3,2-HOPO, in comparison with 5LIO-(Me-3,2-HOPO). The other in vivo assays were performed to compare the decorporation ability between 5LIO-1-Cm-3,2-HOPO, 1-Hydroxyethylidene-1,1-diphosphonic acid (HEDP), and NaHCO$_3$, which have been reported to be effective for U(VI) decorporation$^{36,37}$. Supplementary Table 10 lists the U(VI) removal performance in
killed 7 d later. * (molar ratio 30:7:1) with ligands after the iv injection of U(VI) (0.5 mg U(VI) kg \(^{-1}\)) and then were killed 24 h later.

More importantly, in vivo assay with single-dose oral administration was performed to evaluate the oral efficiency of the 5LIO-1-Cm-3,2-HOPO, 5LIO-(Me-3,2-HOPO), and ZnNa\(_3\)-DTPA. Similarly, an assay with single-dose oral administration was first performed to compare the performance of 5LIO-1-Cm-3,2-HOPO

kidneys and femurs following the same experimental procedure. The group treated with HEDP showed reduction of 31.2% of U(VI) deposited in kidneys and 17.4% of U(VI) deposited in femurs, respectively. The group treated with NaHCO\(_3\) showed very limited effect on removing U(VI) from mice.
and ZnNa₃-DTPA. 5LIO-1-Cm-3,2-HOPO and ZnNa₃-DTPA (644 μmol kg⁻¹, molar ratio to uranium is 307:1) were orally administered by gastric tube three min after an initial intravenous (iv) injection of U(VI) (0.5 mg kg⁻¹). The group received the oral administration of 5LIO-1-Cm-3,2-HOPO showed a reduction of 58.2% uranium from kidneys and 39.5% from femurs (Supplementary Table 11 and Fig. 6c). Subsequently, a comparison between 5LIO-1-Cm-3,2-HOPO and 5LIO-(Me-3,2-HOPO) was conducted in a similar fashion. As shown in Fig. 6d, the group given 5LIO-1-Cm-3,2-HOPO displayed similar U(VI) removal percentage in kidneys (68.2%) with the group given 5LIO-(Me-3,2-HOPO) (63.2%), but a much higher U(VI) removal ratio in femurs (30.5%) was observed for the group of 5LIO-1-Cm-3,2-HOPO than the group of 5LIO-(Me-3,2-HOPO) (3.5%) (Supplementary Table 12). Fig. 6e shows the excretion results of uranium concentrations in urine and feces for the groups orally treated with 5LIO-1-Cm-3,2-HOPO and 5LIO-(Me-3,2-HOPO). For the mice given ip injection, 76.8% and 86.9% of uranium were excreted in the group treated with 5LIO-(Me-3,2-HOPO) and 5LIO-1-Cm-3,2-HOPO, respectively, which correspond to enhancement of 2.2- and 2.5-fold comparing to that of the control group (35.1%). For the oral administration group, 53.8% and 78.9% of uranium was excreted in the group treated with 5LIO-(Me-3,2-HOPO) and 5LIO-1-Cm-3,2-HOPO, respectively, corresponding to an enhancement of 1.5- and 2.3-fold in comparison to the oral administration groups of both ligands, consistent with the relatively lower U(VI) decorporation efficiency in kidneys and femurs in the orally treated groups (Supplementary Table 12).

In the multiple-dose group, 5LIO-1-Cm-3,2-HOPO and ZnNa₃-DTPA (97 μmol kg⁻¹; molar ratio to uranium of 46:1) were ip injected at 3 min, 6 h, 24 h, and 48 h after the initial iv injection of U(VI). At 72 h after the iv injection of 0.5 mg U(VI) kg⁻¹, the kidneys and femurs from the control group contained 5.22 ± 1.13 and 3.50 ± 0.39 μg U(VI) per gram of tissue, respectively (Fig. 6f). Compared with the control group, 5LIO-1-Cm-3,2-HOPO significantly reduced U(VI) levels in the femur by 47.4%. Another multiple-dose assay was conducted by iv injection of 5LIO-1-Cm-3,2-HOPO and ZnNa₃-DTPA (97 μmol kg⁻¹; molar ratio to uranium of 46:1) at 1, 7, 25, and 49 h after the initial iv injection of U(VI). Similarly, kidneys and femurs samples were obtained 72 h after the initial U(VI) injection. Notably, 70.9% of U(VI) deposited in the kidneys and 50.0% in femurs were removed in the 5LIO-1-Cm-3,2-HOPO group compared with the untreated U(VI)-injected controls (Fig. 6f). These values are almost identical with those of the multiple-dose group treated 3 min after the U(VI) injection. This achievement overcomes a large hurdle in developing actinide chelation agents and opens up the possibility of complete uranium removal from bones by increasing the dosage further (Supplementary Table 13).

In most nuclear accidents, an immediate response to radionuclide introduction into human bodies is not applicable. Therefore, decoration agents that can still function despite delayed administration are preferred for chelation therapy with elevated significance. To fully study the relationship between the decorporation efficiency and the time of administration, 5LIO-1-Cm-3,2-HOPO (193 μmol kg⁻¹; molar ratio to uranium is 92:1) was given via ip injection at the following three different time intervals: (1) 6, 12, 30, and 54 h; (2) 12, 18, 36, and 60 h; (3) 24, 30, 48, and 72 h, after the initial iv injection of U(VI). The three groups with 6, 12, and 24 h delayed multiple-dose administration display a similar removal level of U(VI) from kidneys of 61.4%, 62.4%, and 65.0%, respectively. For the decorporation efficiency in femurs, all three groups significantly reduced U(VI) accumulation in femurs by 39.6, 24.2, and 30.8%, respectively (Fig. 6g and Supplementary Table 14), suggesting that the decorporation efficiency is nearly independent of the time of administered treatment.

Discussion

The results described above demonstrate that weakening the intramolecular hydrogen bonds between the oxygen donor sites within the HOPO ligand can significantly increase their uranium coordination capabilities. According to this simple idea, we have successfully synthesized a unique chelating ligand, 5LIO-1-Cm-3,2-HOPO. The combination of solution titration studies, EXAFS analysis, FTIR analysis, NMR analysis, LC-MS analysis, elemental analysis, and first principles theoretical analysis demonstrates the coordination mode and the thermodynamic feasibility for decorporating uranium from kidneys and bones. DFT calculations indicate that with the additional acyl derivative on the ring nitrogen, the formation of strong intramolecular hydrogen bond is greatly prohibited, giving rise to strengthened uranium-HOPO interaction consequently. In vitro U(VI) removal assays results show that 5LIO-1-Cm-3,2-HOPO can significantly enhance the release of intracellular U(VI) release from cells. Further, in vivo uranium decorporation assays demonstrate that this ligand not only improve the reduction of U(VI) levels in kidneys, but also show a record high removal efficiency of uranium from bones even for the oral and delayed treatments, while maintaining a low toxicity at the similar level with ZnNa₃-DTPA. Overall, these results signify that 5LIO-1-Cm-3,2-HOPO represents one of the most promising U(VI) decoration agents and its practical application is expected to be realized in the near future.

Methods

Reagents and materials. Caution! ²³⁸UO₂(NO₃)₂·6H₂O used in this study is radioactive and standard procedures for handling radioactive materials were performed throughout these experiments. 1,2-dihydro-2,3-pyridinediol (98%, TCI), 1,5-diamino-3-oxapentane (99%, TCI), benzyl chloride (98%, J & K), ethyl bromoacetate (99%, Adamas), N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (99%, &K), and 5% Pd/C (Adamas) were all used as received. 5LIO-(Me-3,2-HOPO)Br (98%) was purchased from the company of Shandong Huijing Bio-Pharmatech Co., Ltd.

Stock solution of 5LIO-1-Cm-3,2-HOPO and uranyl. 5LIO-1-Cm-3,2-HOPO or 5LIO-(Me-3,2-HOPO) (12.9 mg) was dissolved in DMSO (1.0 mL) and UO₂(NO₃)₂·6H₂O (49.9 mg) was dissolved in 1.0 mL ultrapure water, followed by sterilization with a 0.22 μm sterilization filter. The work solutions containing chelating agent and U(VI) were prepared by diluting with culture medium to the expected concentrations for cell treatment.

Cell line and culture. The NRK-52E cell line (organism: kidney, rat, ATCC® CRL-1571™) was purchased from Maisha Bio-Technology, Ltd. and cultured in a medium mixture of F-12 nutrient mixture (DMEM/F-12) (Hyclone, Thermo Scientific), 10% (v/v) fetal bovine serum (FBS) (Gibco, Invitrogen Technologies),
and 1% Penicillin-Streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. The cells were propagated every two days.

### Ligand synthesis

The ligand, 5LIO-1-Cm-3,2-HOPO, was obtained from a 4-step synthesis (Fig. 1). 1H-dihydro-2,3-pyrindin-1(2H)-yl)acetamide (2.5 g, 90%). 1H NMR (400 MHz, DMSO): δ 8.50 (s, 1H); 7.28 (d, J = 7.2 Hz, 1H); 3.55 (s, 2H); 2.02 (s, 3H); 1.17 (t, J = 7.3 Hz, 3H); 1.07 (s, 6H). 13C NMR (100 MHz, DMSO): 163.2; 157.0; 137.0, 131.8, 128.8, 128.4, 128.3, 116.1, 116.0, 103.9, 103.8, 70.2, 69.1, 51.6, 39.1; ATR-FTIR: 3380 cm⁻¹ (NH), 1591 cm⁻¹ (C=O); Anal. Calcd for C₁₄H₁₃NO₄ (259.08): C, 64.86 (64.76); H, 5.05 (5.582); N, 5.40 (5.74); LC-MS [M + H⁺]/m/z: 259.73 (Supplementary Figure 1 and Supplementary Figure 2a).

Ethyl 2-(3-hydroxy-2-oxopyridin-1(2H)-yl)acetate (A) (10.0 g, 50.0 mmol) was placed into a 500.0 mL round-bottom flask and dissolved in 300.0 mL of 90% methanol; the pH of the solution was adjusted to 12 with NaOH aqueous solution (0.1 M). Benzyl chloride (25.0 g, 0.2 mol) was added to this solution and was refluxed for 12 h, while maintaining a pH above 12 with the addition of NaOH aqueous solution until the reaction completed. The solution was cooled down to room temperature and methanol was removed by rotary evaporation. The aqueous solution was extracted with dichloromethane (3 × 100 mL). 1H NMR (400 MHz, CDCl₃): δ 7.34–7.32 (m, 5H), 7.17 (d, J = 8.6 Hz, 2H), 6.74 (d, J = 7.3 Hz, 1H), 6.15 (d, J = 7.2 Hz, 1H), 5.01 (s, 2H), 4.61 (s, 2H); 13C NMR (100 MHz, CDCl₃): δ 171.5, 157.5, 148.3, 136.7, 131.2, 128.8, 128.4, 116.1, 104.2, 70.2, 51.0; ATR-FTIR: 2929 cm⁻¹ (CH₃), 2857 cm⁻¹ (CH₂), 1648 cm⁻¹ (ν(C=O)); 749 cm⁻¹ (ν(C–O)); Anal. Calcd for C₁₉H₂₁NO₄ (338.39): C, 70.83; H, 7.21; N, 3.89; LC-MS [M + H⁺]/m/z: 338.39 (Supplementary Figure 1b and Supplementary Figure 2a).

A solution of 2-(3-benzoyloxy-2-oxopyridin-1(2H)-yl)acetic acid (B) (10.8 g, 50.0 mmol) was dissolved in this solution, followed by the addition of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (10.0 g) for 4 h. Filtration, the product was re-dissolved in DMF and filtered. The product was dried at 50 °C under vacuum to yield the UO₂-5LIO-1-Cm-3,2-HOPO complex (60 mg, 12%). The solid was filtered under vacuum, washed with H₂O, and dried in a vacuum oven to yield the UO₂-5LIO-1-Cm-3,2-HOPO complex (60 mg, 87%).

The ligand 5LIO-1-Cm-3,2-HOPO and M(II) (1:1, at approx. 10⁻³–10⁻⁴ M) was dissolved in 0.1 M KCl (contains 5% DMSO). The solution’s pH was adjusted to about 2.5 by adding 2.0 mL of 0.1 M HCl and then titrated using 4.0 mL of 0.1 M KOH with a 0.03 mL increment. The minimum and maximum equilibration time between additions of titrant was 60 and 240 s, respectively. The ligand’s protonation constants were determined by three independent experiments. Data analysis was performed using the program GLEE to obtain values of the cumulative constants, pKw, defined by equation (1) (8.35 and 9.34), respectively.

### Potentiometric Titrations

Formation constants for UO₂⁻ were determined by competition titration experiments with EDTA. Initially for the potentiometric experiments, about 5.0 mg of UO₂(NO₃)₂·6H₂O, 4.1 mg 5LIO-1-Cm-3,2-HOPO and 3.7 mg EDTA-Na₂ (1:1:1, at pH around 11) were dissolved in 0.5 mL DMSO and the pH value was adjusted to 12 with 0.1 M KOH solution. The solution was titrated using 4.0 mL of 0.1 M KOH with a 0.03 mL increment. The minimum and maximum equilibration time between additions of titrant was 60 and 240 s, respectively. The ligand’s protonation constants were determined by three independent experiments. Data analysis was performed using the program GLEE to obtain values of the cumulative constants, pKw, defined by equation (1) (8.35 and 9.34), respectively.

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### Preparation of UO₂-5LIO-1-Cm-3,2-HOPO complex

A solution of UO₂⁻ was carried out by competition titration with EDTA (denoted LHO²⁻), only a small quantity of UO₂-L⁻ and UO₂-OH⁻ exist at low pH. The results suggest that EDTA is not able to compete with UO₂-1-Cm-3,2-HOPO (Supplementary Figure 5a).

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ATR-FITR measurement. The ATR-FITR spectra of compounds A, B, 5LIO-1-Cm-3,2-HOPO, 5LIO-1-Cm-3,2-HOPO, 5LIO-(Me-3,2-HOPO) and UO2(NO3)2·6H2O were measured from 4000 to 400 cm\(^{-1}\) on a Bruker VERTX 70 FTIR instrument in the transmission mode.

Extended X-ray Absorption Fine Structure (EXAFS). X-ray absorption spectroscopy measurements were performed at beamline 14 W1 of the Shanghai Synchrotron Radiation Facility with a Si (111) double crystal monochromator in transmission mode for the uranium L\(_3\)-edge spectra. The electron beam energy of the storage ring was 3.5 GeV, and the maximum current was approximately 210 mA. Energy calibration was performed using a zirconium foil (\(\sim 17,998\) eV). The sample was measured thrice, and the spectra were averaged. The uranium L\(_3\)-edge EXAFS data were analyzed using the standard procedures in the Demeter program. Double-electron excitations affect the EXAFS signal and can influence the results of the data analysis. Thus, in the uranium L\(_3\)-edge EXAFS experimental spectra, the double-electron excitations were subtracted as a reflection of the data translated to the position in energy of the excitation using the standard procedures in Demeter. Supplementary Figure 4 shows the uranium L\(_3\)-edge EXAFS data before and after subtracting the double-electron excitation in k- and \(r\)-space, in which the features at very low distances (\(r = 1\) Å) clearly improved. The coordination number of the uranyl center is expected to be influenced, while the bond length is subtly influenced by double-electron excitations. Theoretical EXAFS data were calculated using FEFF 9.0. Fitting procedure was performed with 0.2 mL 238U solution and 0.5 mL ligand solution was administered by intraperitoneal (ip) injection. The single dosage group with intraperitoneal injection: U(VI) solutions (UO2(NO3)2·6H2O, 3.0 mg) were prepared as that the standard dosage (0.5 mg 238U kg\(^{-1}\)) was contained in 20.0 mL of 0.14 M NaCl at pH 4–5; all the chelating agent sample solutions were adjusted to pH 7–8 with 0.1 M NaOH and molar ratio of all the ligands to U(VI) is 92:1, except for the NaHCO3 solution, whose molar ratio to U(VI) is 184:1. The 5LIO-1-Cm-3,2-HOPO (29.0 mg) or 5LIO-(Me-3,2-HOPO) (29.3 mg) was dissolved in 10.0 mL 0.14 M NaCl, and then adjusted the pH to 7–8 with 0.1 M NaOH, finally diluted to 6.0 mL by adding 0.14 M NaCl solution; 3 mL of HEDP (7.4 mg) or NaHCO3 (6.0 mg) solution was prepared following the procedure of ZnNa3-DTPA. For the single dosage group with oral administration, all the chelating agent sample solutions were adjusted to pH 7–8 with 0.1 M NaOH, and molar ratio of all the ligands to U(VI) is 92:1, except for the NaHCO3 solution, whose molar ratio to U(VI) is 184:1. 5LIO-1-Cm-3,2-HOPO (29.0 mg) or 5LIO-(Me-3,2-HOPO) (29.3 mg) was dissolved in 1.0 mL DMSO, 0.2 mL 0.14 M NaCl and a certain volume of 0.1 M NaOH solution, then diluted to 6.0 mL by adding 0.14 M NaCl solution; ZnNa3-DTPA (30.0 mg) was firstly dissolved in 0.14 M NaCl, finally diluted to 3.0 mL by adding 0.14 M NaCl solution; 5LIO-(Me-3,2-HOPO) (48.5 mg) or 5LIO-(Me-3,2-HOPO) (48.1 mg) was dissolved in 1.0 mL DMSO, 1.0 mL 0.14 M NaCl and a certain volume of 0.1 M NaOH solution, then diluted to 3.0 mL by adding 0.14 M NaCl solution; ZnNa3-DTPA (62.3 mg) was firstly dissolved in 1.0 mL 0.14 M NaCl, and then adjusted the pH to 7–8 with 0.1 M NaOH, finally diluted to 3.0 mL by adding 0.14 M NaCl solution. For each dosage groups, 5LIO-1-Cm-3,2-HOPO (50.8 mg, molar ratio to U (VI) is 46:1) was dissolved in 2.0 mL DMSO, 10.0 mL 0.14 M NaCl and a certain volume of 0.1 M NaOH solution, then diluted to 24.0 mL by adding 0.14 M NaCl solution; ZnNa3-DTPA (74.8 mg, molar ratio to U (VI) is 46:1) was firstly dissolved in 10.0 mL 0.14 M NaCl, and then adjusted the pH to 7–8 with 0.1 M NaOH, finally diluted to 3.0 mL by adding 0.14 M NaCl solution. ZnNa3-DTPA (145.0 mg, molar ratio to U (VI) is 92:1) for the 6 h, 12 h, and 24 h delayed multiple dosage groups was dissolved in 5.0 mL DMSO and diluted to 30.0 mL followed the above method. Female Kunming mice (84 to 86 days old, 30 ± 4 g body weight) were obtained for the in vivo uranium decapsulation assay. These animal assays were approved by the Animal Care and Use Committee of Soochow University and were in accordance with the National Institutes of Health guidelines in its guide for the care and use of laboratory animals.

The single dosage group with ip injection: The first assay was carried out to compare the removal efficiency between 5LIO-1-Cm-3,2-HOPO and ZnNa3-DTPA, and the second assay was carried out to compare the removal efficiency between 5LIO-1-Cm-3,2-HOPO and 5LIO-(Me-3,2-HOPO), the third assay was carried out to compare the removal efficiency between ZnNa3-DTPA and NaHCO3. Experimental and control groups of five mice each were intravenously (iv) injected with 238U(VI) solutions. For each group, two (238U) solutions were administered for each intraperitoneal (ip) injection separately. 288U-injected control group were only given 0.5 mL saline. All mice were fed with water and food 4 h after the 238U injection.
injection. Mice were dissected to obtain the kidneys, femur, liver, spleen and muscle samples. The samples of tissues and excreta were digested in aqua regia, and the contents of $^{238}\text{U}$ in each were determined by ICP-MS (Thermo Scientific). The results of these assays are listed in Supplementary Table 11 and 12.

The multiple dosage and delayed multiple dosage groups: two assays were carried out to compare the removal efficiency among $^{239}$U-1-Cm-3,2-HOPO and ZnNa$_3$-DTPA with different treated time and dosage, respectively. For the multiple dosage and 1 h multiple dosage groups, the experimental and control groups of five mice each were intravenously (i.v.) injected with 0.2 mL $^{238}$U(VI) solution, and 0.5 mL ligand solution was administered by gastric tube promptly after. $^{238}$U-injected control group were only given 0.5 mL saline. All mice were fed with water and food 4 h after the initial $^{238}$U injection. Mice were dissected to obtain the kidneys, femur, liver, spleen and muscle samples. The samples of tissues and excreta were digested in aqua regia, and the contents of $^{238}$U in each were determined by ICP-MS (Thermo Scientific). The results of these assays are listed in Supplementary Table 13 and 14.

**Statistical analysis.** All above data were presented as mean ± standard deviation (SD), and obtained from at least three independent experiments in each assay. $p<0.05$ was considered to be statistically significant.

**In vitro detoxification experiments.** The in vitro detoxification experiments were designed to demonstrate and compare the thermodynamic possibility of 5LIO-1-Cm-3,2-HOPO and 5LIO-(Me-3,2-HOPO) to desorb the complexed uranyl from $^{238}$U(VI) solution. Mice were dissected to obtain the kidney and femur samples. For the 6 or 12 or 24 h delayed multiple dosage groups, 0.5 mL ligand solution was given by ip injection at 6, 12, 24, 30, and 54 h after the initial iv injection of $^{238}$U(VI) for the 6 h delayed multiple dosage group, the 12 and 24 h delayed multiple dosage group was given 0.5 mL ligand solution by ip injection at 12, 18, 36, 60 h and 24, 30, 48, and 72 h after the initial iv injection of $^{238}$U(VI), respectively. $^{238}$U-injected control group were only given 0.5 mL saline. All mice were fed with water and food 4 h after the initial $^{238}$U injection. Mice were dissected to obtain the kidney, femur, liver, spleen and muscle samples. The samples of tissues were digested in aqua regia, and the contents of $^{238}$U in each were determined by ICP-MS (Thermo Scientific). These results are listed in Supplementary Table 15.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Data supporting the findings of this work are available within the paper and its Supplementary Information files. A reporting summary for this article is available as a Supplementary Information file. The source data (PDB files) containing the information of coordinates of DFT optimized structures of UO$_2$-5LIO-(Me-3,2-HOPO) (Fig. 1b), UO$_2$-5LIO-1-Cm-3,2-HOPO (Fig. 1d, left), and UO$_2$-5LIO-1-Cm-3,2-HOPO (Fig. 1d, right) are provided as Supplementary Data 1–3. The source data underlying Figs. 5a, 5b, 6a, 6b, 6c, 6d, 6f, 6g, and Supplementary Table 10 are provided as a Source Data file.

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Author contributions
J.D. and S.W. conceived the project; X.W., C.S., L.C., and J.D. performed the synthesis and characterization of the ligand, I. Z., D. Z. and J.W. carried out the extended X-ray adsorption fine structure (EXAFS) studies; X.D. and R.Z. performed the computational studies; X.W., J.W., C.S., X.Y., B.C., K.Y., and X.Y. carried out the in vitro and in vivo studies on uranium decontamination; X.W., C.S., J.D., Z.C., and S.W. analyzed all data; all authors discussed and co-wrote the paper.

Additional information
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Competing interests: A patent “Preparation of hydroxypyridoninate ligand as chelating agent, 2017100998851.X,” on the related content has been filed by Shuao Wang, Juan Diao, Xiaomei Wang, and Soochow University. The remaining authors declare no competing interests.

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