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Original article

Bisphosphonate sequestering agents. Synthesis and preliminary evaluation for *in vitro* and *in vivo* uranium(VI) chelation

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Abstract

A library of bisphosphonate-based ligands was prepared using solution-phase parallel synthesis and tested for its uranium-binding properties. With the help of a screening method, based on a chromophoric complex displacement procedure, 23 dipodal and tripodal chelates bearing bisphosphonate chelating functions were found to display very high affinity for the uranyl ion and were selected for evaluation of their *in vivo* uranyl-removal efficacy. Among them, 11 ligands induced a huge modification of the uranyl biodistribution by deviating the metal from kidney and bones to liver. Among the other ligands, the most potent was the dipodal bisphosphonate **3C** which reduced the retention of uranyl and increased its excretion by around 10% of the injected metal.

Keywords: Actinide; Chelates; Ligand design; Uranium

1. Introduction

The worldwide use of uranium notably as fuel in nuclear power plants (enriched uranium) or for armor-piercing weapons (depleted uranium) is expanding, thus providing increasing opportunities for occupational and environmental exposure. Whatever the chemical form or speciation of the uranium compound incorporated, the uranyl ion, UO_2^{2+}, is the most likely form of uranium species present in body fluids after contamination. Once it is absorbed by the blood, 60% of UO_2^{2+} is rapidly excreted in the urine, while tissues, especially kidney and bones, accumulate uranium for months to years [1,2]. Although no final conclusions can yet be drawn regarding cancer risks associated with uranium exposure, several studies have shown the radiotoxic and chemotoxic properties of this ‘‘heavy’’ metal. Renal injury is one of the major concerns in the case of uranium contamination. In the kidneys, uranium produces chemical damage to renal proximal tubules [3,4], notably through inhibition of ATPase [5] and oxidative stress [6]. Renal toxicogenomic responses to uranyl were also recently studied [7,8]. Uranyl was also found to be cytotoxic to macrophages and CD4+ T-cells [9,10]. Finally, several studies have shown the potential chemical genotoxicity of uranyl [11,12].

Thus, in the case of accidental contamination, an appropriate treatment may be necessary to reduce deposition in target organs, therefore preventing manifestation of the above-

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mentioned adverse effects. Decorporation therapy that uses ligands with strong metal-binding capabilities remains the only way to accelerate excretion of the poisoning metal. Stimulated by the lack of effectiveness of existing treatments [13–19], several specific ligands for UO_2^{2+} (i.e. uranophiles) were prepared and tested for *in vivo* uranium removal. Most of this research has been carried out by K.N. Raymond, P.W. Durbin and co-workers and published in different reviews [2,20,21]. Several multidentate catecholate and hydroxypyridonate ligands were therefore synthesized [22–24] and some of these chelates were found to display significant *in vivo* removal of uranyl [25,26].

In this context, we recently adopted a diversity-based approach that combines parallel synthesis [27] and *in vitro* screening methods [28] to identify new powerful uranyl chelates. A collection of ligands bearing various chelating moieties including polycarboxylates, hydroxamates, catecholates and hydroxypyridonates, was thus screened for their uranyl-binding capabilities. Among the tested candidates, ligands bearing bisphosphonate subunits were found to be the best uranophiles.

The bisphosphonate moiety is a well-known stable analogue of pyrophosphate that able to bind strongly to a range of hard Lewis acid metals due to their high ionization at physiological pH. Bisphosphonates are also known to target bones and generally display low toxicity. These properties make the bisphosphonate core an interesting chelating function for the design of new uranophiles. *In vivo* experiments have shown that administration of simple bisphosphonates, such as 1-hydroxyethane-1,1′-diphosphonic acid (HEDP), counteracts the effect of lethal doses of uranyl nitrate in rats [29,30] and halves the kidney content [19]. The uranyl-binding properties of HEDP and related compounds have been studied *in vitro* in detail and induce the formation of complexes as both 1:1 and 1:2 (metal:ligand) species in aqueous solution [31–33]. UO_2^{2+} is a hard Lewis acid adopting unusual pseudo-planar penta- or hexa-coordinated complexes. Exploiting the well-known chelate effect, our goal is to obtain very powerful uranophiles by synthesizing a series of dipodal and tripodal ligands bearing bisphosphonate functions. Such ligands should be able to efficiently encapsulate the uranyl ion (Scheme 1) and therefore should be valuable candidates for chelation therapy.

Herein we describe the parallel synthesis and metal-sequestering properties of a library of ligands bearing two or three bisphosphonate chelating functions. Preliminary *in vivo* experiments evaluating the efficiency of uranyl removal upon treatment with some of these ligands are described.

2. Chemistry

The bisphosphonate library was prepared by solution-phase parallel synthesis employing Michael addition of amine scaffolds on tetraethyl ethenylidenebisphosphonic ester **26** as a key step (Scheme 3).

A panel of dipodal as well as tripodal amines was first prepared by classic methods (Scheme 2). The amine scaffolds to be synthesized were designed in order to vary several structural features known to be important for chelation: denticity, size of the spacer separating chelating functions and geometry.

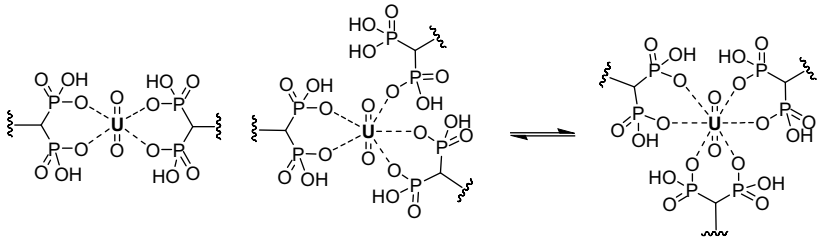
Amines **1–16** were synthesized from bis- or tris-bromomethyl-aryl derivatives by (i) substitution of the bromine atom by cyanide ions followed by reduction of the corresponding nitrile with borane to afford amines **1–3** [34], (ii) treatment with excess diamine to afford amines **4–15** [35], (iii) substitution of the bromine atom by sodium azide and subsequent reduction by PPh_3 to afford amine **16** [36]. The synthesis of amines **17–25** was performed by treatment of the corresponding aromatic esters with excess diamine [37]. All of these procedures provided the desired amine scaffolds with good to excellent yields.

These 25 polyamines and 16 additional commercially available amines were then used as starting material for the two-step parallel synthesis of bisphosphonate-based chelates. After reaction of the 41 amine scaffolds with the Michael acceptor **26**, the resulting secondary amines were quenched by addition of acetic anhydride to avoid the retro-Michael reaction as previously described [38]. The resulting protected chelates were then purified by flash chromatography and the tetraethyl esters were cleaved by treatment with TMSBr [39] affording the bisphosphonate chelates **1A–6A** in moderate to high yields. Each of the 41 products was fully characterized (see Section 6) and their purities (established as >90%) were checked by LC–MS.

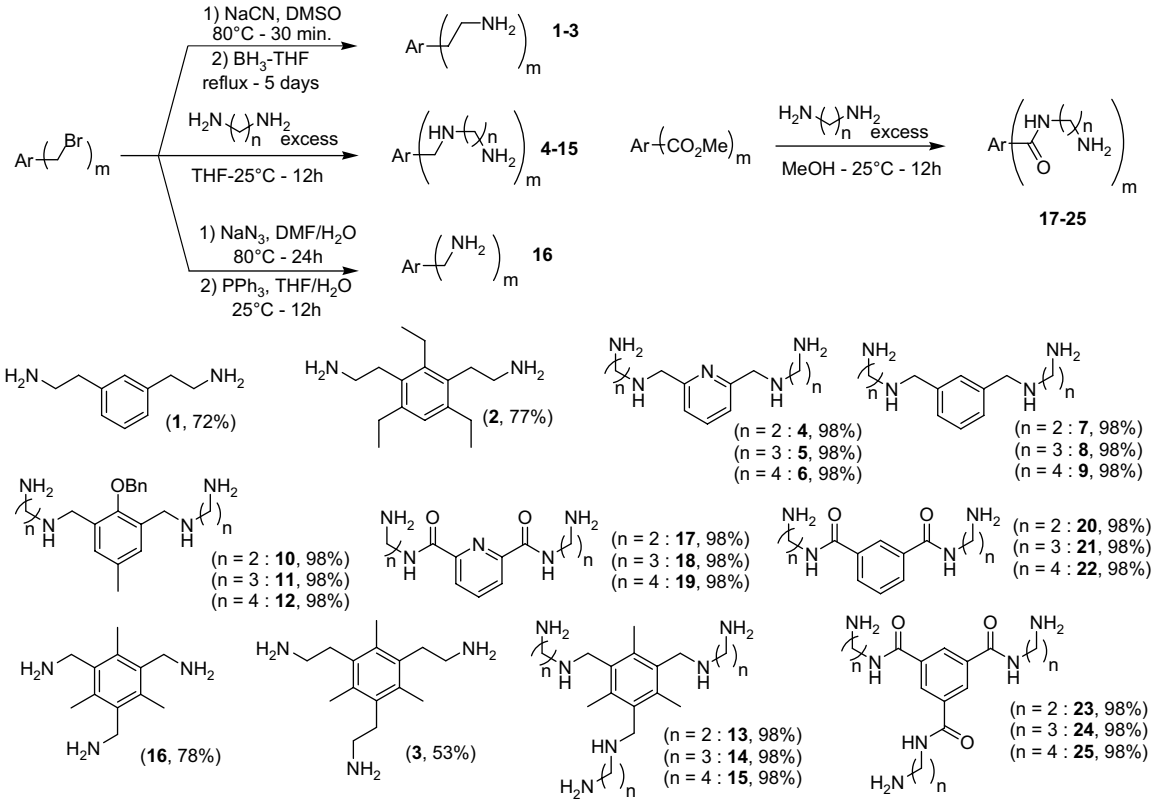
Besides these 41 ligands we also collected 12 additional tripodal bisphosphonate ligands **6B–7E**, whose synthesis was previously published by our group [40–42], to complete our library (Scheme 4). Some of these ligands such as **6H**, **7A** and **7E** possess rigid scaffolds in order to increase preorganization.

3. U(VI)-binding properties of the bisphosphonate library

Before assessing the ability of ligands **1A–7E** to decorporate uranium *in vivo*, we first investigated the UO_2^{2+}-binding properties of the chelate library. This was carried out using



Scheme 1. Hypothetical chelation mode of UO_2^{2+} by di- and tripodal bisphosphonates.



Scheme 2. Synthesis of amine scaffolds.

a displacement method of the preformed sulfochlorophenol–UO_2^{2+} chromogenic complex as we previously described [28]. This method allows a fast determination of the conditional UO_2^{2+}-binding constants (K_{cond}) of candidate ligands by UV–visible spectroscopy. The 53 bisphosphonate ligands were thus screened in a parallel manner on microtiter plates for their uranium-binding properties at pH 7.4 and 5.5 (physiological pH in blood and kidneys) by following the disappearance of the 690 nm absorbance signal of the sulfochlorophenol–UO_2^{2+} complex using a 96-well absorbance reader. All experiments were carried out in duplicate and the results obtained at pH 7.4 are summarized in Fig. 1.

The results offered a rapid readout of the ligand library and highlighted some general trends. First, almost all of the tested ligands displayed remarkable uranyl-binding properties, with K_{cond} varying from 10^{15} to $10^{19.5}$ at pH 7.4. These properties are conserved at pH 5.5, a pH that might be found in the kidney (data not shown). As expected, tripodal bisphosphonate ligands appeared globally more powerful uranophiles than the dipodal ones, the conditional constants being for 1 or 2 orders of magnitude higher. This suggests that the third bisphosphonate subunit of the tripodal ligands participates in the coordination of UO_2^{2+}. Comparison of the results obtained with dipodal bisphosphonates **1A–H** showed the influence of the spacer separating the two chelating moieties (12 atoms separating the bisphosphonate moieties being the optimum). The presence of phenol or pyridine moieties on the amine scaffold did not improve the UO_2^{2+}-binding constants (compare **3E–G**, **3H** and **4A–B** with **3B–D**, Fig. 1). Flexible or rigid ramified tripods were superior to ligands constructed on cyclic backbones (for example compare **6B** and **6H** with **6G**, Fig. 1).

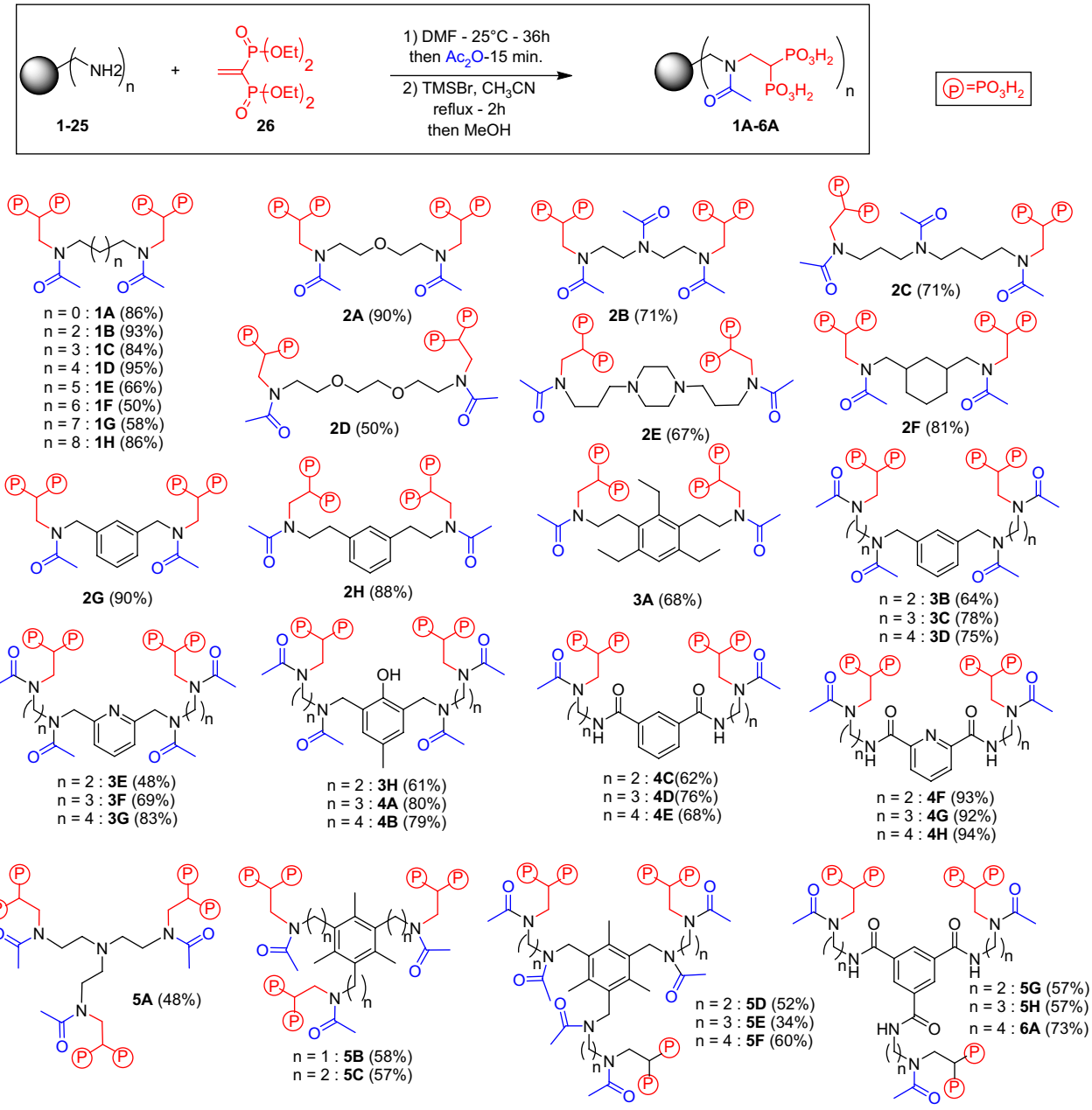
This screening assay allowed us to focus on interesting ligand candidates that can then be selected for *in vivo* experiments. Thus, we selected dipodal bisphosphonates with $K_{cond} > 10^{17}$ and tripods with $K_{cond} > 10^{18}$ at pH 7.4. Finally, 11 dipodal and 14 tripodal ligands were chosen for biological evaluation on the basis of their UO_2^{2+}-binding properties.

4. Preliminary *in vivo* investigations

In order to limit the number of *in vivo* experiments, we first investigated the biodistribution of uranyl after injection of the preformed bisphosphonate chelate–uranyl complexes. Each of 25 UO_2^{2+}–bisphosphonate complexes was therefore prepared *in vitro* by mixing 100 eq. of ligand with 5 kBq of $^{233}U(VI)$ for 30 min and was then intravenously injected into rats at a concentration of 30 μmol kg^{-1}. Urine and feces were collected daily and rats were sacrificed 5 days later. The different organs and tissues were analyzed by liquid scintillation counting, as were urine and feces.

This series of experiments highlighted complexes that can be excreted and stable enough *in vivo* to modify the biodistribution of UO_2^{2+}. Control experiments were carried out without ligand or in the presence of HEDP used as reference chelate (Fig. 2).

As shown in Fig. 2, the tested complexes were globally stable *in vivo*. The injection of these complexes induced significant changes in the biodistribution of UO_2^{2+} compared with control experiments. Unfortunately, in many cases the UO_2^{2+}

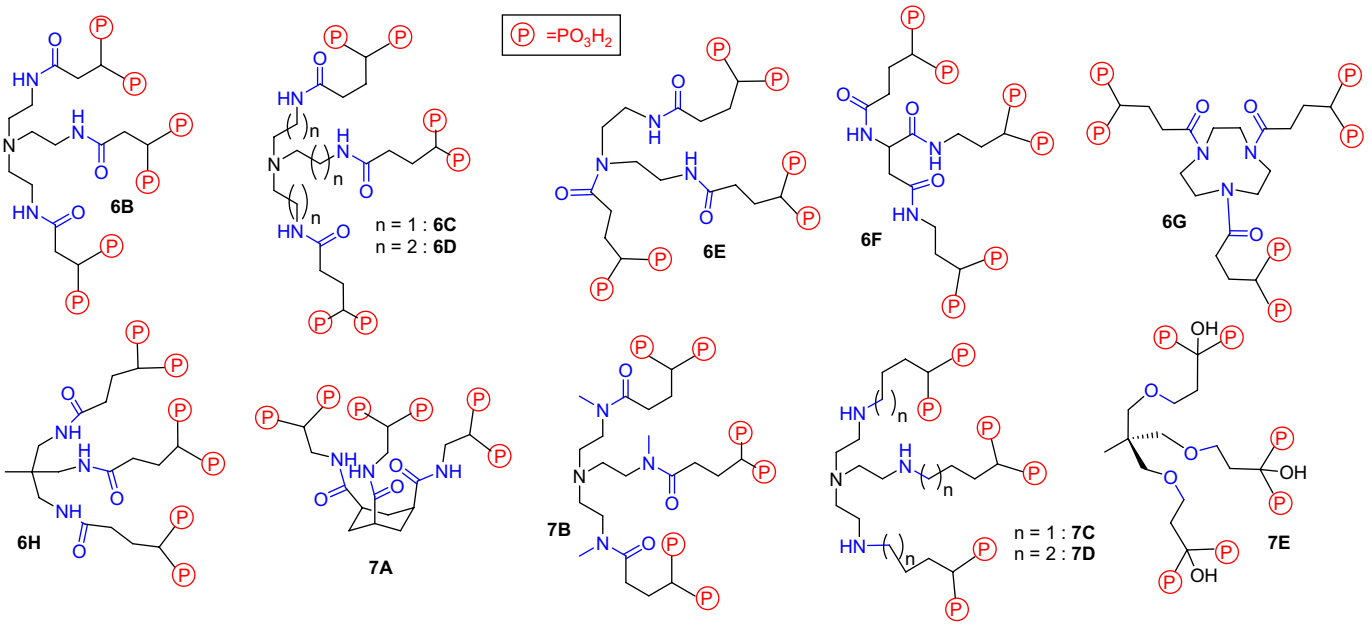


Scheme 3. Synthesis of a library of 41 bisphosphonate ligands.

biodistribution was not modified in the desired direction. For many tripodal ligands, in particular, uranium deposition was increased and partially rerouted from kidney and bones to liver. This phenomenon is particularly important with complexes formed with ligands **3G**, **5C**, **6A**, **6C** and **6H** where more than 40% of the injected uranyl is recovered in the liver. It is noteworthy that very small changes in ligand structure can greatly alter uranyl biodistribution. For example, ligand **7B**, which is the only tripod that does not induce uranyl deposition in the liver, is structurally very similar to ligand **6C,** which accounts for around 40% of hepatic retention. This result might suggest that this hepatic deposition is avoided when *N*-methylamide moieties are present.

Globally, only three bisphosphonate ligands **2H**, **3C**, and **7B** were found to increase excretion of the injected uranyl significantly (arrows in Fig. 2) and not to disturb the normal uranium distribution, and these were therefore selected for further biological evaluation. Two additional tripods (**5C** and **6A**) were also chosen for the study of hepatic retention. HEDP and 5-LICAM(S), a well-known chelating agent for uranyl removal [25,26], were included as reference chelates in this *in vivo* experiment.

The evaluation of the selected ligands as decorporating agents was carried out by prompt injection after uranyl contamination. For each ligand a group of five rats was first contaminated by intraperitoneal injection of ^{233}U(VI) followed 5 min later by injection of 100 eq of ligand (30 μmol kg^{-1}). After 5 days, the animals were sacrificed and the radioactivity associated with collected excreta and main organs was measured. The results are summarized in Table 1.



Scheme 4. Other tripodal BP ligands under investigation.

Compared with the control, only dipodal ligand **3C** significantly increased U(VI) excretion (Table 1). The administration of this ligand allowed a 13% increase in the injected UO_2^{2+} and reduced uranyl deposition in the kidney by around 50%. Several other ligands, particularly tripod **7B**, more effectively reduced uranyl retention in the collected organs, but did not increase excretion. This might be explained by uranyl retention in soft tissues that were not collected. Whatever the ligand used, more than 75% of uranyl excretion occurred during the first day after contamination (Fig. 3).

5. Discussion and conclusion

Chelation therapy for the removal of toxic metals present *in vivo* is first, but not only, a matter of binding competition. To be effective, a candidate ligand should have binding constants at least close to those of biological targets. Unfortunately, and despite the best efforts of researchers in this field [43–45], little is known about the binding strength of the UO_2^{2+}-biological ligands. Transferrin and carbonate ions are known to bind UO_2^{2+} strongly in plasma, with thermodynamic constants $\beta_{1,1} = 10^{16}$ and $\beta_{1,3} = 10^{21.5}$, respectively [46–48], but information is still lacking about the natural ligands present in kidney and bones.

The aim of our work was to synthesize new uranophiles with very high binding constants and use them to compete with the natural targets of UO_2^{2+}. Based on previous findings, we focused our work on bisphosphonate-based ligands and we succeeded in obtaining potent uranophiles. Tripodal bisphosphonate ligands, in particular, were found to display

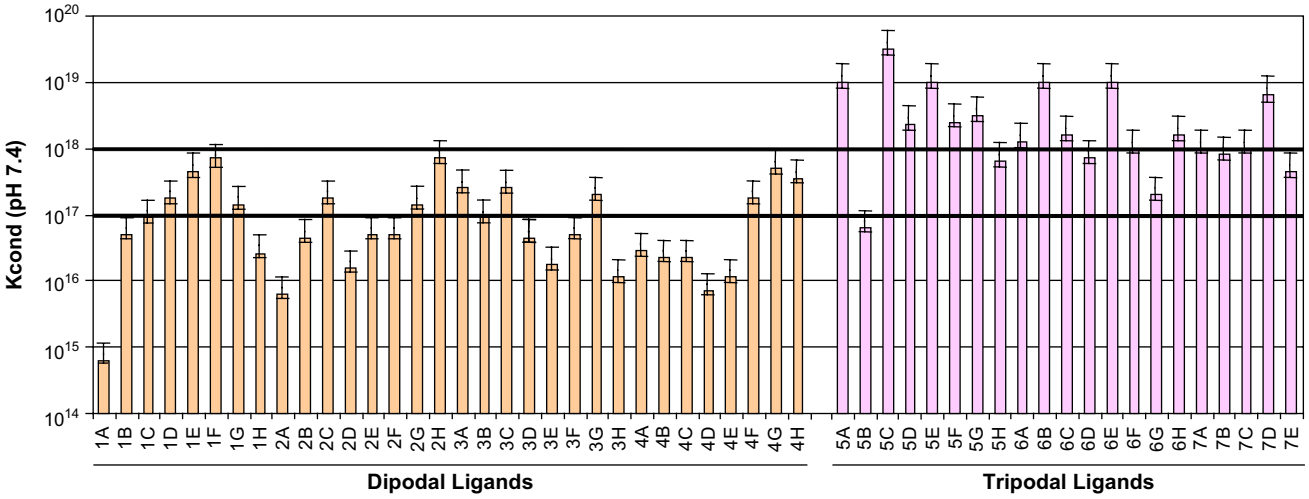


Fig. 1. Conditional constants of BP ligands (pH 7.4).

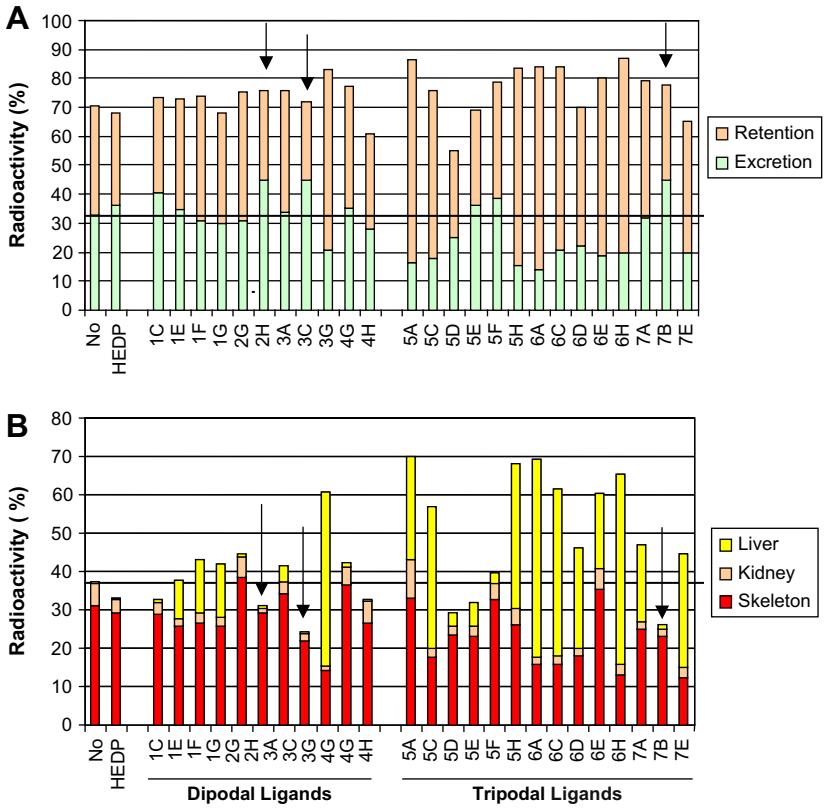


Fig. 2. Biodistribution of uranyl after injection of UO_2^{2+}/L complexes. (A) Global percentages of injected radioactivity on excreta and main organs, (B) detailed percentages of the injected radioactivity in main organs. Complexes were prepared by mixing 100 eq. of ligand with 5 kBq of ^{233}U(VI) and then injected iv (30 μmol kg^{-1}); rats were sacrificed 5 days later.

remarkable UO_2^{2+}-binding capabilities with binding constants up to $K_{cond} = 10^{19.5}$ at physiological pH. These compounds are probably the most powerful uranophiles described to date.

However, while the UO_2^{2+}-binding properties of a metal-sequestering agent candidate are necessary, they are not sufficient to ensure successful chelation therapy. Many other parameters, including the biodistribution and pharmacokinetics of ligands and their metal complexes, are of prime importance and, unfortunately, are difficult to predict. In our library, the ligands inducing the most significant changes in uranyl biodistribution are the tripodal bisphosphonates, but many of them seem to form uranyl complexes that cannot be eliminated from the body. One possible explanation for the observed accumulation of uranyl in liver after treatment with bisphosphonate tripods might be related to complex speciation of the corresponding UO_2^{2+} complexes. Although time-resolved

Table 1
Effect of a prompt injection of bisphosphonate ligands on the biodistribution and excretion of ^{233}U(VI)[a]

Ligand	Percent of injected ^{233}U(VI) ± SD at 5 days				
	Retention			Excretion	
	Kidneys	Skeleton	Liver	Urine	Feces
No (controls)	**8.0** ± 2.3	**34.2** ± 4.5	**0.4** ± 0.0	**40.7** ± 3.4	**1.8** ± 1.0
HEDP	**5.5** ± 0.5	30.2 ± 1.2	0.4 ± 0.1	**50.1** ± 4.3	2.9 ± 1.7
5-LICAM(S)	6.3 ± 0.8	**22.8** ± 4.7	0.5 ± 0.1	**48.0** ± 5.7	2.2 ± 1.2
2H	6.7 ± 1.3	**41.8** ± 3.9	2.8 ± 0.5	31.6 ± 7.8	**4.9** ± 1.3
3C	**3.6** ± 0.6	32.4 ± 1.5	0.3 ± 0.0	**53.5** ± 2.7	2.3 ± 0.5
5C	**3.8** ± 0.7	**28.1** ± 2.6	**13.4** ± 1.0	**25.6** ± 0.8	2.1 ± 0.3
6A	7.5 ± 0.9	**27.9** ± 0.8	**13.5** ± 1.5	31.8 ± 8.5	2.3 ± 1.2
7B	**3.4** ± 0.6	**23.9** ± 0.5	1.5 ± 0.6	**29.7** ± 5.1	1.4 ± 0.6

[a] Ligands were injected ip (30 μmol kg^{-1}, molar ratio 100) 5 min after injection of 9.2 kBq of ^{233}U(VI); animals were sacrificed 5 days later. Data are expressed as % of injected radioactivity, discrepancies are due to rounding. Bold means are significantly different than control mean.

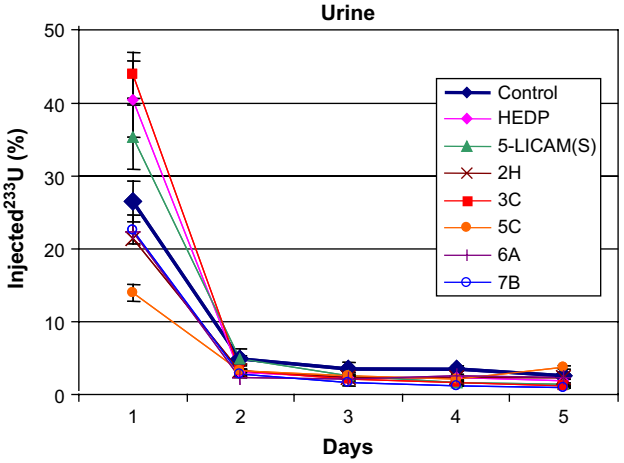


Fig. 3. Kinetic of uranyl excretion.

laser-induced fluorescence (TRLIF) as well as electrospray mass spectroscopy (ES-MS) measurements conducted on selected UO_2^{2+}–tripodal bisphosphonate complexes confirmed a 1:1 stoichiometry [28], one cannot exclude the possibility of *in vivo* formation of high-molecular-weight polymetallic complexes. Preliminary experiments on tripods **5A** [28], **5H** and **5C** (data not shown) highlighted a high level of metal specificity toward UO_2^{2+} over Ca^{2+}, Mg^{2+} and Zn^{2+}, but a very low specificity over Fe^{3+}. All of these critical factors might explain the low level of *in vivo* efficacy of tripodal bisphosphonates.

Dipodal ligand **3C** is the only compound that induces a significant benefit after UO_2^{2+} contamination. Further investigations are under way to define better protocols (increased doses or repeated injection of **3C**).

6. Experimental protocols

6.1. Chemistry

6.1.1. General experimental procedures

Unless otherwise stated, starting materials were obtained from commercial suppliers and used without purification. THF was distilled from sodium/benzophenone ketyl before use. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{31}P NMR (160 MHz) were measured on a Brucker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Electrospray mass spectra were obtained using an ESI/TOF Mariner Mass Spectrometer.

6.1.2. General procedures for the synthesis of amine scaffolds *1–25*

Method I 1,3-Bis-bromomethyl-benzene, 2,4-bis-bromomethyl-1,3,5-triethyl-benzene or 1,3,5-tris-bromomethyl-2,4,6-trimethyl-benzene (3.5 mmol) were reacted with NaCN (10 mmol, 2.8 eq.) in dry DMSO. The mixture was stirred at 80 °C for 30 min. After cooling, ether was added and the organic phase was washed with water and then dried on $MgSO_4$.

The expected di- or tri-cyano compounds were purified by flash chromatography (hexane:ether) and then dissolved in 20 mL of BH_3/THF (1 M, 20 mmol, 10 eq.). The corresponding mixture was refluxed under argon for 3 days. Two milliliter of an aqueous solution of HCl (1 N) were added and the mixture was refluxed for 2 h. The aqueous solution was washed by dichloromethane and then evaporated under reduced pressure. The recovered solid was dissolved in MeOH, precipitated by addition of ether and finally dried over P_2O_5 to afford amines **1–3**.

Method II To 1,3-dibromo or 1,3,5-tribromomethyl-aryl derivatives (1.5 mmol, 1 eq.) in 45 mL of dry THF were added ethane, propane or butane-diamine (60 mmol, 40 eq.). The mixture was stirred at 25 °C for 12 h. Solvent and excess of diamine were removed under reduced pressure. The resulting oil was solubilized in MeOH and KOH (3 mmol, 2 eq.) was added. Inorganic salts were precipitated by addition of ether and removed by filtration. The filtrate was evaporated to afford quantitative yields of amine scaffolds **4–15**.

Method III NaN_3 (36 mmol, 2.34 g) was added to a solution of 2,6-dibromomethylpyridine (3 mmol, 795 mg) in 7 mL CH_2Cl_2/DMF (2/5) and the mixture was warmed at 80 °C for 24 h. Water was added and the hoped 2.6-di(azidomethyl)-pyridine was extracted by CH_2Cl_2, dried over $MgSO_4$ and purified by flash chromatography (CH_2Cl_2:hexane, 1:1).

2.6-Di(azidomethyl)-pyridine (2.65 mmol, 500 mg) was reacted with PPh_3 (13 mmol, 3.57 g) and water (52 mmol, 0.5 mL) in 10 mL THF. After 24 h at room temperature, THF was removed, water was added and the aqueous phase was washed by CH_2Cl_2 and then evaporated to afford 300 mg of amine **16**.

Method IV Isophthalic acid dimethyl ester, pyridine-2,6-dicarboxylic acid dimethyl ester or benzene-1,3,5-tricarboxylic acid trimethyl ester (2.5 mmol) were reacted with ethane, propane or butane-diamine (0.1 mol, 40 eq.) in 45 mL MeOH at 25 °C for 12 h. Solvent and excess of diamine were removed under reduced pressure to afford quantitative yields of amine scaffolds **17–25**.

6.1.3. General procedures for the synthesis of bisphosphonate ligands *1A–6A*

To a solution of amine **1–25** (0.025 mmol, 1 eq.) in 2 mL of dry DMF, was added tetraethyl ethylidenebisphosphonic ester (60 mg, 0.2 mmol, 8 eq. for dipodal amines or 135 mg, 0.45 mmol, 18 eq. for tripodal amine). After reaction at room temperature for 48 h, acetic anhydride (40 μL, 0.43 mmol, 17 eq. for dipodal amines or 60 μL, 0.63 mmol, 25 eq. for tripodal amine) was added. The solution was stirred for 15 min at room temperature, evaporated and then directly filtered through a silica gel (5 g, hexane/acetone 1:1 to remove the excess of acrylate, then acetone/MeOH 7:3) to yield protected chelate. After removal of the solvents, protected chelates were then dissolved in dry CH_3CN and TMSBr (3 eq. per functions) was added under argon. The mixture was stirred under reflux for 3 h and then quenched with water (3 eq. per functions). After refluxing for additional 15 min, the solvents were evaporated and co-evaporated with MeOH. Resulting residues were dissolved in a minimum of MeOH and

precipitated with ether. The resulting slurry was decanted, washed with ether and dried under high vacuum in the presence of P_2O_5 to afford the target chelates.

Ligands **1A**, **1B**, **1D**, **1F**, **1H**, **2G** and **5A** are described in our previous work [28].

*6.1.3.1. Ligand **1C**.* ^{1}H NMR (D_2O): 1.38 (m, 2H), 1.71 (m, 4H), 2.19 (s, 3H), 2.29 (s, 3H), 3.06 (m, 2H), 3.54 (m, 4H), 3.90 (m, 3H), 4.01 (m, 1H); ^{31}P NMR (D_2O): 21.10 and 21.80 (2s, 4P); MS *m/z* 585 (100%, $[M+23]^+$).

*6.1.3.2. Ligand **1E**.* ^{1}H NMR (D_2O): 1.40 (m, 6H), 1.67 (m, 4H), 2.08, 2.20 and 2.30 (3s, 6H), 3.07 (m, 2H), 3.53 (m, 4H), 3.90 (m, 3H), 4.02 (m, 1H); ^{31}P NMR (D_2O): 19.03 and 19.64 (2s, 4P); MS *m/z* 613 (100%, $[M+23]^+$).

*6.1.3.3. Ligand **1G**.* ^{1}H NMR (D_2O): 1.40 (m, 10H), 1.70 (m, 4H), 2.22 and 2.33 (2s, 6H), 3.10 (m, 6H), 3.50 (m, 4H), 3.95 (m, 3H), 4.07 (m, 1H); ^{31}P NMR (D_2O): 18.93 and 19.64 (2s, 4P); MS *m/z* 641 (100%, $[M+23]^+$).

*6.1.3.4. Ligand **2A**.* ^{1}H NMR (D_2O): 2.25 (m, 6H), 2.75 (m, 1H), 3.13 (m, 1H), 3.45 (m, 4H), 3.64 (m, 4H), 3.80 (m, 2H), 3.96 (m, 2H); ^{31}P NMR (D_2O): 16.42, 18.73 and 19.44 (3s, 4P); MS *m/z* 565 (100%, $[M+1]^+$).

*6.1.3.5. Ligand **2B**.* ^{1}H NMR (D_2O): 2.14–2.39 (m, 9H), 2.14–2.39 (m, 9H), 2.86 (m, 1H), 3.13 (m, 1H), 3.59 (m, 2H), 3.71 (m, 4H), 3.81 (m, 2H), 3.93 (m, 3H), 4.08 (m, 1H); ^{31}P NMR (D_2O): 18.63 and 19.34 (2s, 4P); MS *m/z* 606 (100%, $[M+1]^+$).

*6.1.3.6. Ligand **2C**.* ^{1}H NMR (D_2O): 1.60 (m, 4H), 1.87 (m, 2H); 2.19 (m, 9H), 3.06 (m, 2H), 3.43 (m, 8H), 3.82 (m, 3H), 3.97 (m, 1H); ^{31}P NMR (D_2O): 19.74 and 20.45 (2s, 4P); MS *m/z* 648 (100%, $[M+23]^+$).

*6.1.3.7. Ligand **2D**.* ^{1}H NMR (D_2O): 2.10 (s, 6H); 2.62–2.74 (m, 1H), 2.83–3.00 (m, 1H), 3.38–3.47 (m, 4H), 3.51–3.62 (m, 8H), 3.64–3.79 (m, 2H), 3.82–3.98 (m, 2H); ^{31}P NMR (D_2O): 16.02, 18.42 and 19.05 (3s, 4P); MS *m/z* 631 (100%, $[M+23]$).

*6.1.3.8. Ligand **2E**.* ^{1}H NMR (D_2O): 1.83 (m, 4H), 1.90, 2.00 and 2.12 (3s, 6H), 2.20 (m, 4H), 2.85 (m, 2H), 3.37 (m, 8H), 3.56 (m, 4H), 3.77 (m, 4H); ^{31}P NMR (D_2O): 19.22 and 19.82 (2s, 4P); MS *m/z* 661 (100%, $[M+1]^+$).

*6.1.3.9. Ligand **2F**.* ^{1}H NMR (D_2O): 1.30 (m, 2H), 1.46–1.83 (m, 6H), 2.03 (m, 2H), 2.01, 2.12 and 2.24 (3s, 6H), 3.06 (m, 2H), 3.33 (m, 4H), 3.84 (m, 3H), 3.96 (m, 1H); ^{31}P NMR (D_2O): 18.83 and 19.54 (2s, 4P); MS *m/z* 625 (100%, $[M+23]^+$).

*6.1.3.10. Ligand **2H**.* ^{1}H NMR (D_2O): 1.86, 2.09 and 2.27 (3s, 6H), 2.95 (m, 4H), 3.10 (m, 2H), 3.84 (m, 4H), 3.78 (m, 4H), 7.20 (m, 2H), 7.38 (m, 1H); ^{31}P NMR (D_2O): 18.93 and 19.64 (2s, 4P); MS *m/z* 871 (100%, $[M+23]^+$); HRMS: Calcd for $C_{18}H_{33}N_2O_{14}P_4$: *m/z* 625.0882. Found: *m/z* 4625.0908.

*6.1.3.11. Ligand **3A**.* ^{1}H NMR (D_2O): 1.22 (m, 9H), 2.21 and 2.36 (2s, 6H), 2.70 (m, 6H), 3.02 (m, 7H), 3.47 (m, 3H), 3.89 (m, 2H), 3.92 (m, 2H), 4.03 (m, 2H),7.06 (s, 1H); ^{31}P NMR (D_2O): 18.83 and 19.54 (2s, 4P); MS *m/z* 709 (100%, $[M+23]^+$); HRMS: Calcd for $C_{24}H_{45}N_2O_{14}P_4$: *m/z* 709.1821. Found: *m/z* 709.1842.

*6.1.3.12. Ligand **3B**.* ^{1}H NMR (D_2O): 2.12–2.35 (m, 12H), 2.77 (m, 1H), 3.10 (m, 1H), 3.66 (m, 8H), 3.84 (m, 3H) 4.02 (m, 1H), 4.75 (m, 4H), 7.18 (s, 1H), 7.35 (m, 2H), 7.55 (m, 1H); ^{31}P NMR (D_2O): 18.53 and 19.23 (2s, 4P); MS *m/z* 767 (100%, $[M+1]^+$).

*6.1.3.13. Ligand **3C**.* ^{1}H NMR (D_2O): 1.85 (m, 4H), 2.04–2.31 (m, 12H), 2.82 (m, 1H), 3.06 (m, 1H), 3.44 (m, 8H), 3.80 (m, 3H) 3.96 (m, 1H), 4.71 (m, 4H), 7.14 (s, 1H), 7.32 (m, 2H), 7.50 (m, 1H); ^{31}P NMR (D_2O): 18.63 and 19.24 (2s, 4P); MS *m/z* 795 (100%, $[M+1]^+$); HRMS: Calcd for $C_{26}H_{47}N_4O_{16}P_4$: *m/z* 795.1938. Found: *m/z* 795.1937.

6.1.3.14. Ligand 3D. ^{1}H NMR (D_2O): 1.58 (m, 8H), 2.10–2.32 (m, 12H), 3.12 (m, 2H), 3.49 (m, 8H), 3.84 (m, 3H) 3.98 (m, 1H), 4.70 (m, 4H), 7.12 (s, 1H), 7.30 (m, 2H), 7.47 (m, 1H); ^{31}P NMR (D_2O): 18.93 and 19.64 (2s, 4P); MS *m/z* 823 (100%, $[M+23]^+$).

*6.1.3.15. Ligand **3E**.* ^{1}H NMR (D_2O): 2.26 (m, 12H), 2.86 (m, 2H), 3.81 (m, 8H), 3.97 (m, 2H), 4.09 (m, 2H), 5.21 (m, 4H), 7.93 (m, 2H), 8.60 (m, 1H); ^{31}P NMR (D_2O): 18.53 and 19.13 (2s, 4P); MS *m/z* 768 (100%, $[M+1]^+$).

*6.1.3.16. Ligand **3F**.* ^{1}H NMR (D_2O): 2.14 (m, 4H), 2.28 (m, 12H), 3.09 (m, 2H), 3.67 (m, 8H), 3.99 (m, 2H), 4.11 (m, 2H), 5.09 (m, 4H), 7.96 (m, 2H), 8.59 (m, 1H); ^{31}P NMR (D_2O): 18.63 and 19.24 (2s, 4P); MS *m/z* 796 (100%, $[M+1]^+$).

*6.1.3.17. Ligand **3G**.* ^{1}H NMR (D_2O): 1.73 (m, 8H), 2.17, 2.30 and 2.34 (3s, 12H), 2.82 (m, 1H), 3.05 (m, 1H), 3.40–3.70 (m, 8H), 3.87 (m, 3H), 4.02 (m, 1H), 4.99 (s, 4H), 7.90 (m, 2H), 8.55 (m, 1H); ^{31}P NMR (D_2O): 19.64 and 21.85 (2s, 4P); MS *m/z* 824 (100%, $[M+1]^+$).

*6.1.3.18. Ligand **3H**.* ^{1}H NMR (D_2O): 1.73–2.07 (m, 15H), 2.81 (m, 2H), 3.34 (m, 4H), 3.51 (m, 4H), 3.67 (m, 3H), 3.88 (m, 1H), 4.38 (m, 4H), 6.92 (m, 2H); ^{31}P NMR (D_2O): 17.84 (s, 4P); MS *m/z* 796 (100%, $[M+1]^+$).

*6.1.3.19. Ligand **4A**.* ^{1}H NMR (D_2O): 1.58 (m, 4H), 1.73–2.07 (m, 15H), 2.81 (m, 2H), 3.15 (m, 8H), 3.50 (m, 4H), 4.22 (m, 4H), 6.73 (m, 2H); ^{31}P NMR (D_2O): 19.54 and 21.58 (2s, 4P); MS *m/z* 825 (100%, $[M+1]^+$).

*6.1.3.20. Ligand **4B**.* ^{1}H NMR (D_2O): 1.32 (m, 8H; $H_{2,3}$), 1.81–2.08 (m, 15H; $H_{c,7}$), 2.80 (m, 2H; H_a), 3.20 (m, 8H;

$H_{1,4}$), 3.62 (m, 3H; H_b), 3.89 (m, 1H; H_b), 4.30 (m, 4H; H_5), 6.81 (m, 2H; H_6); ^{31}P NMR (D_2O): 18.92 and 19. (2s, 4P); MS *m/z* 853 (100%, $[M+1]^+$).

6.1.3.21. Ligand ***4C***. ^{1}H NMR (D_2O): 1.83, 1.92 and 2.04 (3s, 6H), 2.73 (m, 2H), 3.30 (m, 4H), 3.55 (m, 4H), 3.69 (m, 2H), 3.86 (m, 2H), 7.42 (s, 1H), 7.70 (s, 2H), 8.00 (s, 1H); ^{31}P NMR (D_2O): 17.96 and 19.79 (2s, 4P); MS *m/z* 711 (100%, $[M+1]^+$).

6.1.3.22. Ligand ***4D***. ^{1}H NMR (D_2O): 1.77 (m, 4H), 1.93 and 2.04 (2s, 6H), 2.85 (m, 2H), 3.25 (m, 4H), 3.61 (m, 2H), 3.84 (m, 4H), 3.92 (m, 2H), 7.42 (m, 1H), 7.72 (s, 2H), 7.89 (s, 1H); ^{31}P NMR (D_2O): 18.93, 19.54, 21.65 and 21.85 (4s, 4P); MS *m/z* 739 (100%, $[M+1]^+$).

6.1.3.23. Ligand ***4E***. ^{1}H NMR (D_2O): 1.45 (m, 8H), 1.93 and 2.04 (2s, 6H), 2.83 (m, 2H), 3.22 (m, 4H), 3.37 (m, 4H), 3.64 (m, 3H) 3.77 (m, 1H), 7.39 (m, 1H), 7.68 (s, 2H), 7.85 (s, 1H); ^{31}P NMR (D_2O): 18.35 and 19.26 (2s, 4P); MS *m/z* 767 (100%, $[M+1]^+$).

6.1.3.24. Ligand ***4F***. ^{1}H NMR (D_2O): 2.05, 2.13 and 2.31 (3s, 6H), 3.12 (m, 2H), 3.65 (m, 4H), 3.78 (m, 4H), 3.90 (m, 3H) 4.08 (m, 1H), 8.20 (m, 3H); ^{31}P NMR (D_2O): 19.54 and 21.85 (2s, 4P); MS *m/z* 712 (100%, $[M+1]^+$).

6.1.3.25. Ligand ***4G***. ^{1}H NMR (D_2O): 21.98 (m, 4H), 2.10, 2.19 and 2.35 (3s, 6H), 3.12 (m, 2H), 3.43 (m, 4H), 3.56 (m, 4H), 3.91 (m, 2H), 4.06 (m, 2H), 8.20 (m, 2H); ^{31}P NMR (D_2O): 19.53 and 21.84 (2s, 4P); MS *m/z* 740 (100%, $[M+1]^+$); HRMS: Calcd for $C_{21}H_{38}N_5O_{16}P_4$: *m/z* 740.1264. Found: *m/z* 740.1287.

6.1.3.26. Ligand ***4H***. ^{1}H NMR (D_2O): 1.74 (m, 8H), 2.19 and 2.32 (2s, 6H), 3.12 (m, 2H), 3.59 (m, 8H), 3.92 (m, 2H), 4,03 (m, 2H), 8.17 (m, 3H); ^{31}P NMR (D_2O): 19.64 and 21.85 (2s, 4P); MS *m/z* 768 (100%, $[M+1]^+$).

6.1.3.27. Ligand ***5B***. ^{1}H NMR (D_2O): 1.93–2.27 (m, 18H), 2.39 (m, 9H), 2.98 (m, 3H), 3.24 (m, 3H), 3.32 (m, 3H), 4.91 (m, 6H); ^{31}P NMR (D_2O): 18.73 and 19.43 (2s, 4P); MS *m/z* 898 (100%, $[M+23]^+$).

6.1.3.28. Ligand ***5C***. ^{1}H NMR (D_2O): 1.88 and 1.93 (2s, 9H), 2.17 (m, 9H), 2.50–2.78 (m, 9H), 3.14 (m, 3H), 3.39 (m, 3H), 3.62 (m, 3H), 3.81 (m, 3H); ^{31}P NMR (D_2O): 18.73 and 19.34 (2s, 4P); MS *m/z* 940 (100%, $[M+1]^+$); HRMS: Calcd for $C_{27}H_{52}N_3O_{21}P_6$: *m/z* 940.1519. Found: *m/z* 940.1536.

6.1.3.29. Ligand ***5D***. ^{1}H NMR (D_2O): 1.90–2.43 (m, 27H), 2.95 (m, 3H), 3.32 (m, 12H), 3.80 (m, 4H), 4.05 (m, 2H), 4.91 (m, 6H); ^{31}P NMR (D_2O): 18.53 and 19.33 (2s, 4P); MS *m/z* 1153 (100%, $[M+1]^+$); HRMS: Calcd for $C_{36}H_{67}N_6O_{24}P_6$: *m/z* 1153.2629. Found: *m/z* 1153.2632.

6.1.3.30. Ligand ***5E***. ^{1}H NMR (D_2O): 1.98 (m, 6H), 2.28–2.45 (m, 27H), 2.98 (m, 3H), 3.08 (m, 6H), 3.20 (m, 3H), 3.31 (m, 3H), 3.57 (m, 4H), 3.77 (m, 2H), 4.91 (m, 6H); ^{31}P NMR (D_2O): 18.73 and 19.44 (2s, 4P); MS *m/z* 1195 (100%, $[M+1]^+$).

6.1.3.31. Ligand ***5F***. ^{1}H NMR (D_2O): 1.41 (m, 12H), 2.11–2.44 (m, 27H), 2.95 (m, 3H), 3.74 (m, 3H), 3.11 (m, 12H), 3.94 (m, 3H), 4.91 (m, 6H); ^{31}P NMR (D_2O): 18.83 and 19.44 (2s, 4P); MS *m/z* 1238 (100%, $[M+1]^+$).

6.1.3.32. Ligand ***5G***. ^{1}H NMR (D_2O): 1.25 (m, 36H), 2.09, 2.22 and 2.32 (3s, 9H), 3.08 (m, 3H), 3.77 (m, 12H), 3.98 (m, 6H), 8.29 (m, 3H); ^{31}P NMR (D_2O): 18.63 and 19.44 (2s, 4P); MS *m/z* 1027 (100%, $[M+1]^+$).

6.1.3.33. Ligand ***5H***. ^{1}H NMR (D_2O): 1.95 (m, 6H), 2.19 and 2.31 (2s, 9H), 3.08 (m, 3H), 3.55 (m, 12H), 3.90 (m, 4H), 4.03 (m, 2H); ^{31}P NMR (D_2O): 19.93 and 19.54 (2s, 4P); MS *m/z* 1087 (100%, $[M+1]^+$); HRMS: Calcd for $C_{30}H_{55}N_6O_{24}P_6$: *m/z* 1069.1694. Found: *m/z* 1069.1691.

6.1.3.34. Ligand ***6A***. ^{1}H NMR (D_2O): 1.68 (m, 12H), 2.18 and 2.29 (2s, 9H), 3.08 (m, 3H), 3.50 (m, 12H), 3.88 (m, 4H), 4.01 (m, 2H);^{31}P NMR (D_2O): 18.80 and 19.49 (2s, 4P); MS *m/z* 1134 (100%, $[M+23]^+$).

6.2. In vivo experiments

Adult male Sprague Dawley rats weighing 250 ± 50 g were used for these experiments.

Animal experimentation was run according to national ethical and veterinary guidelines.

6.2.1. Biodistribution after injection of uranyl/ligand complexes

Each rats was given a single 30 μmol kg^{-1} injection (200 μL, 5 kBq, iv) of uranyl/bisphosphonate complexes prepared as followed: to 1 mL of a stock solution of ^{233}U (50 kBq/mL) in HNO_3 1 N was added 100 eq. of bisphosphonate ligand, pH was adjusted to 7.4 in a final volume of 2 mL and the mixture was stirred for 30 min.

Urine and feces samples were periodically collected from individual metabolic cages and rats were killed at day 5. The different organs, tissues as well as urines and feces samples were analyzed by liquid scintillation counting.

6.2.2. Removal of uranyl

Groups of five rats injected ip with 9.2 kBq of ^{233}U(VI) were treated 5 min later by ip injection of bisphosphonates ligands (30 μmol kg^{-1}, molar ratio 100). Urine and feces samples were periodically collected from individual metabolic cages and rats were killed at day 5. The different organs, tissues as well as urines and feces samples were analyzed by liquid scintillation counting.

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