$^{188}Re(CO)_3$-Dipicolylamine-Alendronate: A New Bisphosphonate Conjugate for the Radiotherapy of Bone Metastases

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The palliation of pain due to bone metastases using targeted compounds containing β-emitters such as rhenium-188 (^{188}Re) is an accepted and effective form of treatment. Here, we describe the efficient synthesis and preclinical evaluation of $^{188}Re(CO)_3$-dipicolylamine(DPA)-alendronate, a novel bifunctional bisphosphonate for the palliative treatment of bone metastases. $^{188}Re(CO)_3$-DPA-alendronate can be easily synthesized with high specific activities and yields (18.8 GBq/mg, radiochemical yield $\geq$96%) in two steps using kit-based methodology, and in contrast with the clinically approved bisphosphonate $^{186/188}$Re-HEDP, it forms inert, single species that have been well-characterized. In vivo imaging and biodistribution studies demonstrate that $^{188}Re(CO)_3$-DPA-alendronate is superior to ^{188}Re-HEDP in targeting and accumulating in areas of high metabolic bone activity while having low soft-tissue uptake. In addition to these studies, a simple and convenient new method for purifying its precursor, *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$, is described.

Bone metastases arise most commonly from malignancy of breast, prostate, and lung cancers with an estimated 80% of patients eventually developing these secondary tumors (*1*). Other types of cancers such as melanoma, ovarian, thyroid, kidney, and head and neck are also likely to present these complications. One of the most distressing effects of bone metastases is extensive pain which severely impairs the quality of life of these patients. Palliation of the pain is usually managed by using strong opioid analgesics such as morphine. With ongoing administration, however, these drugs may induce severe side effects such as nausea, respiratory depression, and addiction/withdrawal syndrome. Palliative treatment using external beam radiation therapy is also commonly employed. This modality, however, is not recommended when the metastases have spread to several distant sites of the skeleton, as harmful radiation of essential organs cannot be avoided.

A very effective way of palliating the pain of these tumors is by internal radiotherapy using β^--emitting radionuclides (*2*). In this modality, a targeted radioactive pharmaceutical is taken up selectively by the bone metastases giving a high localized dose of radiotherapy to these areas. Several clinical trials have demonstrated its usefulness. In fact, several β^--emitting radiopharmaceuticals are currently being used in the clinic, of which the most popular are $^{89}SrCl_2$ (Metastron) and ^{153}Sm-ethylenediamine tetra(methylene phosphonic acid) (^{153}Sm-EDTMP or Quadramet) (*3*−*5*). Their clinical efficacy is proven, but they are not without disadvantages. ^{89}Sr and ^{153}Sm can only be produced in specialized nuclear reactors which are decreasing in number. Another concern is economic, as a single dose of one of these agents costs ca. $2900 per dose (*6*). Furthermore, neither is carrier-free, and the low stability of ^{153}Sm-EDTMP requires coadministration of a high concentration of unlabeled EDTMP. As a consequence, there has been a growing interest in developing therapeutic bone-seeking radiopharmaceuticals using generator-produced isotopes. Generator technologies not only provide straightforward availability of radioisotopes in any laboratory or clinical center around the world, but also reduce costs dramatically, as a single generator can potentially provide hundreds of doses.

Rhenium-188 (^{188}Re) is arguably the most promising generator-produced β^--emitting radionuclide for the radiotherapy of bone metastases. ^{188}Re (E_{max} 2.12 MeV, 100%) is readily available using commercially available generators and shows favorable therapeutic properties. It also emits γ-photons (155 keV, 15%) that allow imaging using single photon emission computed tomography (SPECT) (*7, 8*). It has a medium-short half-life (16.9 h) and similar chemistry to technetium-99m (^{99m}Tc), the most widely used radionuclide in biomedical imaging. It was this Tc−Re analogy that drove forward the development of $^{186/188}$Rehydroxyethylidene-1,1-diphosphonate ($^{186/188}$Re-HEDP), the therapeutic "analogues" of the ^{99m}Tc-bisphosphonates (^{99m}Tc-BPs) routinely used as bone imaging agents. ^{186}Re-HEDP and ^{188}Re-HEDP have shown promise as palliative and therapeutic agents for bone metastases in recent clinical trials (*9*−*14*), but their properties are far from optimal from a chemical and pharmaceutical point of view. Their main drawback is that their chemical structures and compositions remain unidentified, although there are clear indications that they are composed of a mixture of anionic polymers (*15*). Also, these complexes are not entirely analogous to their technetium "analogues" since additional nonradioactive Re needs to be added as a "carrier" to achieve bone targeting (*14, 16, 17*). Furthermore, a large fraction of the injected complexes degrade to perrhenate (ReO_4^-) in vivo within 24 h and thus are not retained in bone metastases.

These drawbacks are attributable to poor chemical design. In these complexes, the BP acts as both radionuclide-binding ligand and targeting group, compromising both roles. We believe that to improve upon current Re-BPs a more logical approach is the use of targeted bifunctional ligands in which the targeting (BP) and metal chelating groups are separated within the molecule so that they can each function independently and effectively. A few recent reports describe such a bifunctional

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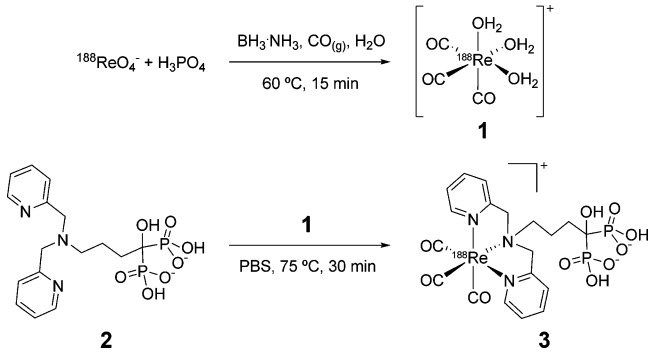
Scheme 1



approach based on BPs (*18*−*20*). These BP conjugates, however, require complicated multistep synthetic strategies, show high plasma protein binding, and often form enantiomeric mixtures. To address these issues, we recently developed dipicolylamine(DPA)-alendronate (**2**, Scheme 1), an easily synthesized and well-characterized bifunctional bisphosphonate that forms single, well-defined isostructural Tc(I) and Re(I) tricarbonyl complexes that efficiently accumulate in bone tissue in vivo (*21*). Herein, we describe the synthesis, characterization, and preclinical in vivo studies of its ^{188}Re complex, $^{188}Re(CO)_3$-DPA-alendronate (**3**, Scheme 1), in comparison with ^{188}Re-HEDP to evaluate its potential as a new improved radiopharmaceutical for the therapy of bone metastases.

3 was easily made from generator-eluted $^{188}ReO_4^-$ in two steps (Scheme 1). The precursor *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$ (**1**, Scheme 1) was synthesized following the method of Schibli et al. (*22*). The radiochemical yields of **1** ranged between 80% and 85%, in agreement with the published method, with the remaining byproduct being unreduced and/or reoxidized $^{188}ReO_4^-$ and colloidal "$^{188}ReO_2$". A new purification method was required, since none was described in the original report. We reasoned that ionic chromatography could be used to separate the two byproducts based on their ionic and colloidal character. Thus, the crude solution was passed through a system composed of two solid-phase extraction columns connected in series, an OnGuard II Ag column (Dionex) to remove chloride ions from the saline solution, followed by a strong anion exchange (SAX) column (SAX Varian Bond Elut 100 mg) to retain $^{188}ReO_4^-$. Using this system, **1** is obtained in the eluate in good radiochemical yields (65%, based on initial $^{188}ReO_4^-$ activity) and excellent purities (≥99%) (Figure 1). The OnGuard II Ag column was proven necessary in order to remove the chloride ions from the saline solution that otherwise compete with $^{188}ReO_4^-$ in the SAX column, at the expense of **1** being retained to some extent in the OnGuard II Ag column (10%). Attempts to release trapped **1** using increasing concentrations of NaCl were unsuccessful, suggesting that the interaction between **1** and the OnGuard II Ag column is not ionic.

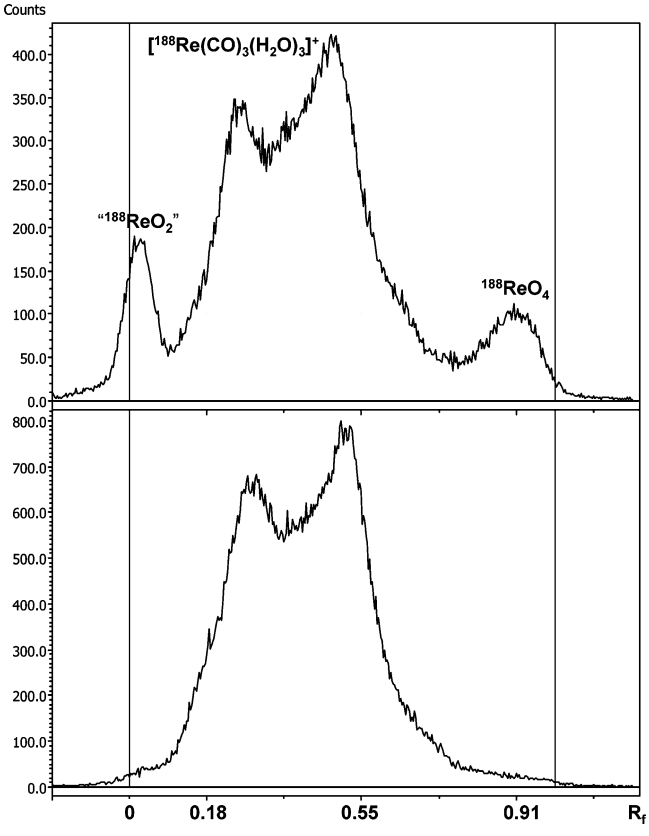


Figure 1. Silica gel TLC analyses of $[^{188}Re(CO)_3(H_2O)_3]^+$ (**1**) in the crude reaction solution (top) and after purification (bottom). Vertical lines indicate origin and solvent front. Using MeOH/HCl_{conc} (99%:1%) as the mobile phase, "$^{188}ReO_2$" colloids appear at $R_F = 0.05$, **1** appears as two broad peaks with $R_F = 0.20-0.50$, and $^{188}ReO_4^-$ appears at $R_F = 0.90$).

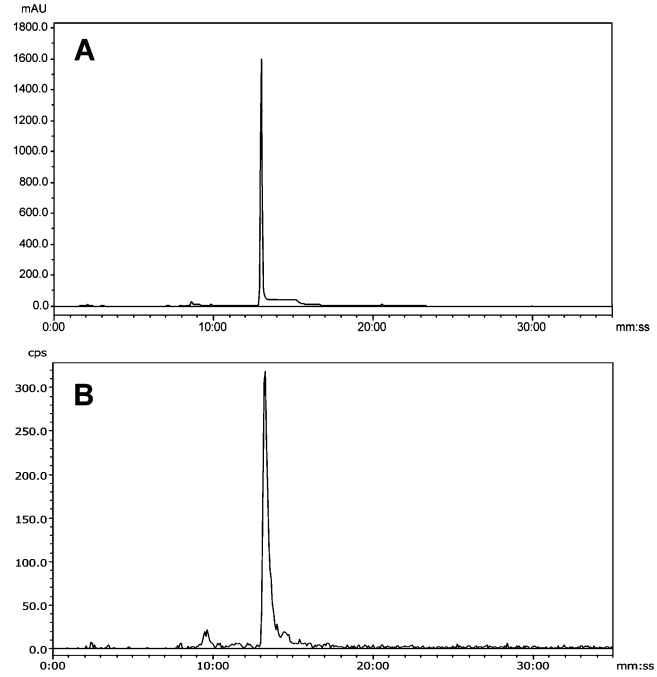


Figure 2. RP-HPLC chromatograms of nonradioactive $Re(CO)_3$-DPA-alendronate (A, UV detection (254 nm), t_R = 13:12 mm:ss) and **3** (B, β detection, t_R = 13:12 mm:ss). The difference in peak width is due to the larger cell volume of the γ/β^- detector.

3 was synthesized by mixing a solution of freshly made **1** (100 μL, 150 MBq) with **2** (0.01 mg/mL in PBS, 100 μL) in a N_2-purged vial followed by heating at 75 °C for 30 min. RP-HPLC analysis of the reaction solution revealed the formation of **3** with a specific activity of 18.8 GBq/mg and ≥96% radiochemical yield with the remainder of the activity being $^{188}ReO_4^-$. In contrast to $[^{99m}Tc(CO)_3(OH_2)_3]^+$, reoxidation of **1** to $^{188}ReO_4^-$ during labeling conditions has been observed previously with other ligands and can be rationalized to be the result of the lower redox potential of Re compared to that of Tc (*22*). Longer reaction times (up to 60 min) and lower reaction temperatures (60 °C) led to lower yields of **3**. A comparison with the chromatogram of the well-characterized nonradioactive $Re(CO)_3$-DPA-alendronate complex, and its ^{99m}Tc analogue, demonstrates the formation of the desired compound as a single species (Figure 2) (*21*).

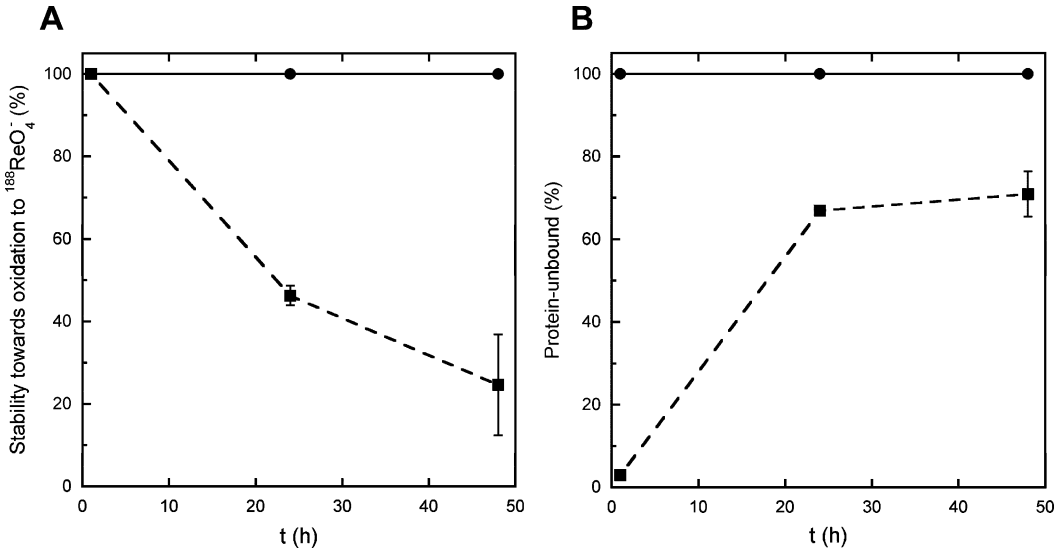


Figure 3. Stability study (toward oxidation to $^{188}ReO_4^-$) in PBS (A) and serum protein binding study (B), of **3** (black circles) and ^{188}Re-HEDP (black squares). See Supporting Information for methods.

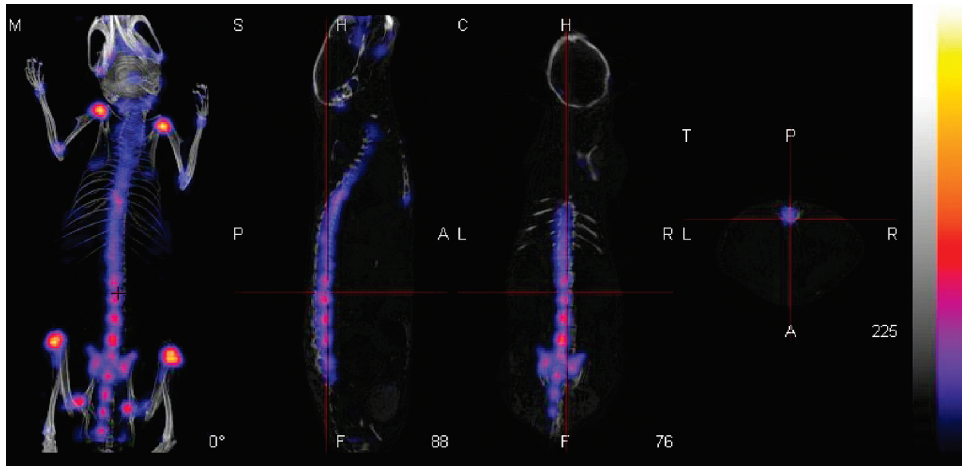


Figure 4. SPECT (color)/CT (grayscale) image taken 24 h postinjection showing the high uptake of **3** in bone tissue, particularly at the joints. From left to right, maximum intensity projection (M), sagittal (S), coronal (C), and transverse (T) sections.

In order to achieve the maximum therapeutic efficiency, ^{188}Re compounds must remain stable and bound to the target during at least one to three half-lives of ^{188}Re (16.9 h). One of the most important drawbacks of ^{188}Re-HEDP is its lack of stability both in vivo and in vitro. In order to assess the in vitro stability of **3** in comparison with ^{188}Re-HEDP, both compounds were incubated in PBS for 48 h at 37 °C. RP-HPLC and TLC analyses demonstrated that **3** did not degrade over this time, whereas most of ^{188}Re-HEDP oxidized to $^{188}ReO_4^-$ (up to 75%) (Figure 3A). Incubation of both compounds in human serum show most of the radioactivity from the ^{188}Re-HEDP sample remains bound to serum proteins during the first 24 h. After this time, ~70% of the radioactivity was free in solution. ITLC analyses demonstrated, however, that the non-protein-bound radioactivity was $^{188}ReO_4^-$, the decomposition product of ^{188}Re-HEDP. **3**, on the other hand, remained non-protein-bound and unmodified throughout the 48 h incubation period (Figure 3B).

In vivo imaging studies with **3** and ^{188}Re-HEDP were carried out at 1, 5, 24, and 48 h postinjection with adult BALB/c female mice (9 weeks old for **3** and 8 weeks old for ^{188}Re-HEDP) using a nanoSPECT/CT scanner (Figure 4). These studies confirmed the ability of **3** to accumulate in areas of metabolically active bone such as the joints, while soft-tissue organ uptake was very low throughout the experiment. Quantification of the images provided an interesting comparison of the pharmacokinetics of each compound (Figure 5). Thus, both compounds show an increase in uptake in the knee for the first 5 h. After this time, however, the uptake of **3** increased during the next 24 h, whereas that of ^{188}Re-HEDP diminished until the end of the 48 h experiment. We speculate that the increased uptake in bone of **3** during the first 24 h is the result of recycling of the unmetabolized, chemically intact complex from soft tissues, coupled with its excellent retention and slow release from bone compared to ^{188}Re-HEDP. This is in agreement with the biodistribution profiles at 48 h (vide infra) as well as with previous in vitro experiments with its ^{99m}Tc analogue, demonstrating the superior capabilities of Re/Tc-DPA-alendronate for binding and remaining bound to the main component of bone mineral (hydroxyapatite) (*21*). Further experiments, however, would be needed to confirm this hypothesis.

Ex vivo biodistribution studies at 48 h demonstrate that **3** exhibits higher uptake in bone tissue than ^{188}Re-HEDP (percentage of injected dose per gram of tissue (% ID/g) in one whole femur: 21.2 ± 6.6% for **3**; cf. 13.4 ± 0.2% for ^{188}Re-HEDP), consistent with its higher stability and/or better targeting properties (Figure 6). As shown in the above-mentioned imaging studies, soft-tissue uptake was very low for both compounds with most organs having an uptake of less than 0.6% ID/g. **3** consistently shows higher uptake than ^{188}Re-HEDP in these organs, especially in the liver (0.96 ± 0.2% ID/g). This may

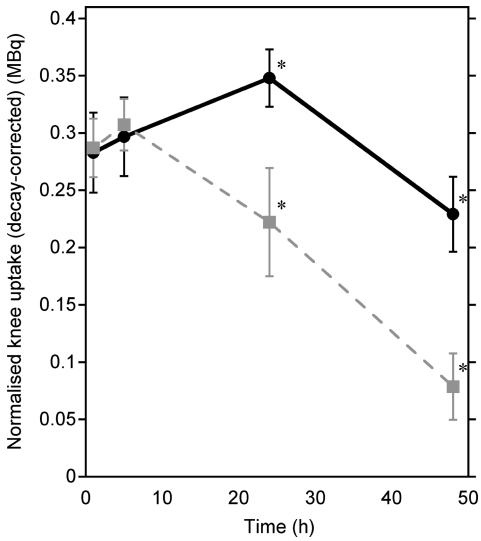


Figure 5. Uptake in the left knee (decay-corrected) after injection of **3** (33 MBq, black circles, continuous line) or ^{188}Re-HEDP (29 MBq, gray squares, dashed line) obtained from ROI analysis of the imaging data. The data from **3** were scaled by a factor of 29/33 to take into account the different injected activity. Values represent the mean ± SD (n = 3 mice). * indicates a significant difference ($P < 0.05$, Student's paired t-test) between the two radiotracers.

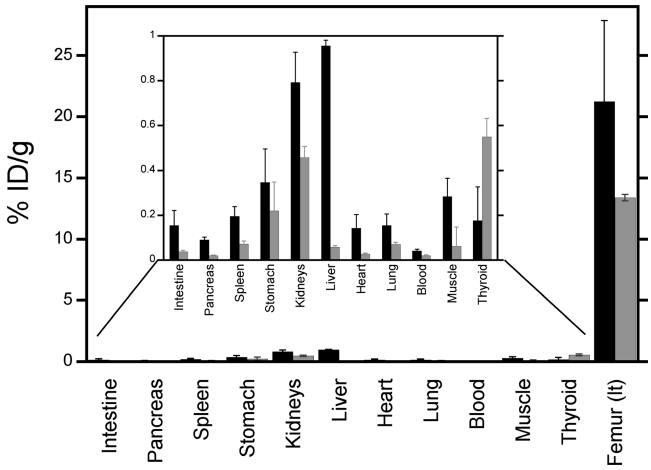


Figure 6. Biodistribution profile of **3** (black bars) and ^{188}Re-HEDP (gray bars) at t = 48 h postinjection. Values represent the mean ± SD (n = 3 mice).

be explained by the lipophilic nature of the carbonyl and dipicolylamine groups. An interesting exception, however, is the lower uptake of **3** in the thyroid. We attribute this to the tendency of ^{188}Re-HEDP, but not **3**, to decompose into $^{188}ReO_4^-$, which is known to be taken up by sodium-iodide symporter (NIS)-expressing organs such as the thyroid (*23*).

In conclusion, we have described a simple and convenient method to purify **1** that will facilitate the labeling of other small molecules and biomolecules such as His-tagged peptides/proteins with ^{188}Re in high radiochemical yields and purities (*24*). We have also described the synthesis of **3** as a new radiopharmaceutical for the radionuclide therapy of bone metastases. **3** can be easily synthesized with high specific activities in two steps using kit-based methodology, and in contrast with the clinically approved $^{186/188}$Re-HEDP, it forms an inert, single species that has been well-characterized. The strategy of using a designed chelating agent for rhenium rather than relying on the chelating properties of the bisphosphonate group is vindicated in that **3** displays superior stability, bone targeting, and retention properties. **3** is therefore an attractive candidate for further clinical studies.

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Supporting Information Available: Detailed experimental details for the synthesis of **1** and **3** and in vitro and in vivo procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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