

Bisphosphonates as radionuclide carriers for imaging or systemic therapy

Elisa Palma, João D. G. Correia, Maria Paula C. Campello and Isabel Santos*

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Bisphosphonates (BP's), biologically stable analogs of naturally occurring pyrophosphates, became the treatment of choice for pathologic conditions characterized by increased osteoclast-mediated bone resorption, namely Paget's disease, osteoporosis and tumor bone disease. Moreover, the clinical success of BP's is also associated with their use in ^{99m}Tc -based radiopharmaceuticals for bone imaging. In addition to the successful delivery of ^{99m}Tc (γ -emitter) to bone, BP's have also been used to deliver β^- -particle emitting radiometals (e.g. ^{153}Sm , $^{186/188}\text{Re}$) for bone-pain palliation. The main goal of this Review is to update the most recent research efforts toward the synthesis, characterization and biological evaluation of novel BP-containing radiometal complexes and radiohalogenated compounds for diagnostic or therapeutic purposes. The structure and *in vivo* properties of those compounds will be discussed and compared to the clinically available ones, namely in terms of image quality and therapeutic effect. We will also mention briefly the use of BP's as carriers of multimodal nuclear and optical imaging probes.

Introduction

The dynamic balance between bone resorption by osteoclasts and bone formation by osteoblasts is the physiologic basis for bone remodeling, and is regulated by a complex system of local and systemic factors.^{1,2} Disruption of that balance, with subsequent bone destruction, is found in various benign and malignant metabolic bone diseases such as osteoporosis,

Paget's disease, tumor-associated hypercalcemia and osteolysis, among others.³ Osteoporosis, a disease characterized by loss of bone tissue that may lead to weak and fragile bones, is currently acknowledged as a major health problem, affecting millions of people worldwide.

Bone metastases may occur in almost all tumors, with prostate, lung, and breast cancer most frequently implicated. Almost 80% of patients with advanced breast or prostate cancer will most likely develop bone metastases.^{4,5} These metastatic malignancies have a high impact in the quality of life of those patients, causing severe complications such as bone pain, pathologic fractures, hypercalcemia, and spinal cord compression.

Unidade de Ciências Químicas e Radiofarmacêuticas, ITN, Estrada Nacional 10, 2686-953 Sacavém, Portugal. E-mail: isantos@itn.pt; Tel: +35 1219946201



Elisa Palma

Elisa Vaz Palma graduated in Analytical Chemistry in 1997 at the University of Aveiro, and received her MSc degree from the University of Lisbon in 2007 with a thesis entitled "Re and Tc Complexes Potentially Interesting for Central Nervous System Imaging", prepared in the group of Prof. I. Santos—Instituto Tecnológico e Nuclear. Since then, she studied new organometallic complexes for bone targeting. This research allowed the preparation of a thesis and currently she is about to obtain her PhD degree at the University of Lisbon.



João D. G. Correia

João D. G. Correia graduated in Pharmaceutical Sciences in 1991 at the Faculty of Pharmacy, University of Lisbon, and received his PhD from Technical University of Munich in 1996 under the direction of Prof. W. A. Herrmann. In 1998 he moved to the Instituto Tecnológico e Nuclear, Portugal, where he was a postdoctoral fellow and an Invited Researcher (2000–2006) in the laboratory of Prof. I. Santos. In 2006 he was appointed as Associated Researcher. His current research

interests include the synthesis and biological evaluation of regulatory peptide analogs and small molecules for nuclear and/or optical molecular imaging or therapy.

Over the past 30 years, bisphosphonates (BP's) were established as an effective new drug class, becoming the treatment of choice for the pathologic conditions characterized by increased osteoclast-mediated bone resorption, namely Paget's disease, osteoporosis and tumor bone disease. In the case of bone metastases, nitrogen-containing BP's were recognized as effective drugs in the prevention or delay of the onset of skeletal-related events (pathologic fractures and spinal-cord compression) and for bone-pain palliation.^{6–10} These compounds emerged also as the leading effective treatment of osteoporosis, increasing bone mass arising from the inhibition of bone resorption, and reducing fracture rates in postmenopausal women.

BP's are biologically stable chemical analogs of naturally occurring pyrophosphates (PPi), in which the labile P–O–P motif has been replaced by a stable P–C–P basic unit (Fig. 1).

These molecules display a strong affinity for binding to hydroxyapatite (HA), namely to the biological well-crystallized apatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, Ca/P = 1.66), which is the main component of the inorganic matrix of bone (69%). Their high affinity for bone is explained by the chelation to Ca^{2+} ions through the deprotonated oxygen atoms of the phosphonate unit in a bidentate coordination mode. The ability of the BP's to bind to HA is enhanced when $\text{R}^1 = \text{OH}$ (Fig. 1), and this is explained by the possibility of a tridentate coordination mode to the Ca^{2+} ions through the deprotonated oxygens of the hydroxyl and phosphonate groups. *In vitro* and *in vivo* studies have shown that such binding to bone prevents both crystal growth and HA dissolution/breakdown, similarly to what has

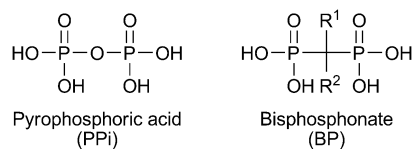


Fig. 1 Pyrophosphoric acid (PPi) and generic structure of bisphosphonates (BP's).

been observed for pyrophosphates.^{6,7,11} In addition to these effects, the antiresorptive properties of BP's have also been assigned to cellular effects on osteoclasts, and the nature of the R^2 substituent on the basic structure of the BP seems to be determinant for inhibition of bone resorption.^{7,12,13}

Additionally, there is also preclinical evidence that BP's may have also antitumor activity, however, such finding must still be confirmed in clinical setting and a general acceptance of this controversial principle is still far from being reached.^{12,14,15} The chemical structures of the most relevant BP's synthesized and biologically evaluated are displayed in Fig. 2.

Alendronate and pamidronate, bisphosphonates containing free primary amines, are 10- to 100-fold more potent than etidronate (HEDP) and clodronate, which have been introduced in the 1970s/1980s and used as antiresorptive drugs.^{7,12} Further structural modifications led to the preparation of compounds bearing tertiary amines, such as the case of ibandronate that is 10-fold more potent than pamidronate. The latest generation of BP's (*e.g.* minodronate, risedronate and zoledronate), bearing nitrogen-containing heterocyclic rings, are the most potent antiresorptive BP's to date. Zoledronate, for instance, is considered to be approximately 10 000-fold more potent than etidronate.^{4,7,8,12} The clinical success of BP's is also associated with their use as radiopharmaceuticals (drugs containing a radioactive element for the diagnosis or therapy of diseases) for Single Photon Emission Computed Tomography (SPECT) or for systemic radiotherapy since the early 1970's. BP's chelate quite efficiently technetium (Tc) and rhenium (Re), while maintaining their superior HA binding properties and, subsequently, their bone-targeting properties.¹⁶ Etidronate was labelled with ^{99m}Tc and successfully used as bone scanning agent.¹⁶ Despite its superior biological properties (*e.g.* high bone accumulation, faster blood clearance and high *in vivo* stability) when compared to ^{99m}Tc-labeled pyrophosphate, the blood clearance was still not as rapid as that of ¹⁸F-fluoride, which had been introduced by Blau *et al.* as a promising bone scanning agent for Positron Emission Tomography (PET).¹⁷



Maria Paula C. Campello

M. P. C. Campello is graduated in Chemistry by the Faculty of Sciences of the University of Lisbon (FCUL) and received her PhD in Chemistry from FCUL in 1998 under the direction of Prof. I. Santos. In 2000, Paula was appointed Assistant Researcher in the Prof. I. Santos group—Instituto Tecnológico e Nuclear (ITN). In the last few years her main research work has been on the synthesis and characterization of new macrocyclic ligands for stabilization of f- and post-transition elements for nuclear molecular imaging or therapy.



Isabel Santos

Isabel R. Santos is graduated in Chemical Engineering by the Technical University of Lisbon, where she has also received her PhD and Aggregation in Chemistry. She teaches at the Faculty of Sciences—University of Lisbon and does research at the Instituto Tecnológico e Nuclear, where she has been appointed as leader of the Radiopharmaceutical Sciences Group in January 2000. The scientific activity of Isabel Santos is in inorganic and organometallic chemistry and on radiochemistry of d- and f-transition elements interesting for Nuclear Medicine applications. Isabel is author or co-author of more than 180 scientific papers, several invited reviews and co-inventor of some patents. She has supervised several MSc and PhD students and Post-doctoral fellows.

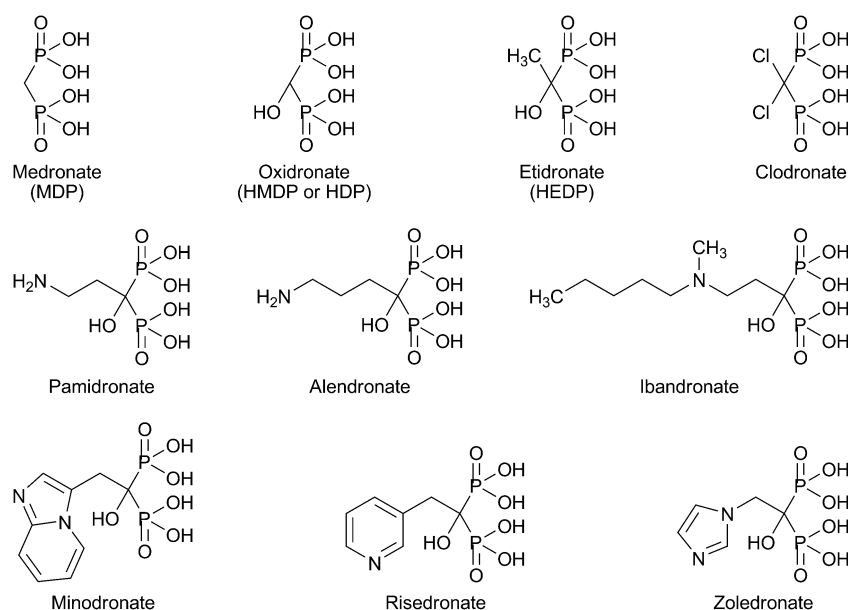


Fig. 2 Bisphosphonate structures.

Such limitation was overcome by the use of methylene diphosphonate (Medronate, MDP) labeled with ^{99m}Tc , which presented a blood clearance equivalent to that of ^{18}F -fluoride. Later on, ^{99m}Tc -labeled hydroxymethylene diphosphonate (Oxidronate, HMDP or HDP) was also successfully introduced as bone scanning agent with improved HA binding properties. Currently, ^{99m}Tc -MDP and ^{99m}Tc -HDP are the only ^{99m}Tc -based radiopharmaceuticals approved for bone imaging to evaluate metastatic disease, for cancer staging, infection, and traumatic injury, among other indications.^{18,19} An important benefit of bone imaging with radiolabelled BP's is its sensitivity. In addition to the successful delivery of γ -emitter radionuclides, such as ^{99m}Tc , for bone imaging, BP's have also been used to deliver β^- -particle emitting radionuclides for bone-pain palliation (Table 1).^{20–23}

Management of painful skeletal metastases is a complex subject and, for the benefit of the patient, a multidisciplinary approach is normally followed. In most of the cases it begins with the use of systemic analgesic drugs, using the “three step ladder” sequence recommended by the WHO.^{25–27} If used correctly this is usually 80–90% effective in palliating pain.

However, the use of analgesics presents serious side-effects, which in some cases limit the patient's functioning more than the pain itself. Management of bone pain includes also hormones, chemotherapeutic agents, steroids, external beam radiation, radiofrequency (RF) ablation, local surgery and therapeutic radiopharmaceuticals.^{5,20,28} When there are extensive multifocal osseous metastases the treatment of bone pain involves the use of hormones, chemotherapy or systemic therapy with β^- -emitting radionuclides such as ^{32}P , ^{89}Sr , ^{153}Sm or $^{186/188}\text{Re}$. Phosphorus-32 (^{32}P , ^{32}P -Phosphate) and strontium-89 (^{89}Sr , $^{89}\text{SrCl}_2$) were the first radionuclides approved for bone-pain palliation. Both compounds have a natural affinity for bone with increased osteoblastic activity, being incorporated into the HA after intravenous injection.²⁰ Owing to several limitations, including high myelotoxicity, the clinical use of ^{32}P -Phosphate has decreased considerably since the 1980's in favor of $^{89}\text{SrCl}_2$, ^{153}Sm -EDTMP (Quadramet[®]), ^{186}Re -HEDP and, more recently, ^{188}Re -HEDP.²⁰ Additionally, the radionuclides ^{177}Lu , ^{166}Ho and ^{223}Ra (Table 1) are also being explored as bone palliative agents, both at the preclinical and clinical level. The α -emitter $^{223}\text{RaCl}_2$ (Alpharadin[®]), with natural

Table 1 Physical characteristics of therapeutic radionuclides and efficacy of radiopharmaceuticals for bone-pain palliation

Radionuclide	Radiopharmaceutical	Half-life/d	β^- -Energy _{max} /MeV	γ -Energy/MeV	α -Energy _{A_v} /MeV	Maximum range/mm	Pain relief
^{32}P	^{32}P -phosphate	14.3	1.71	—	—	8	57–92%
^{89}Sr	$^{89}\text{SrCl}_2$	50.5	1.46	0.91 (0.01%)	—	6.7	57–84%
^{153}Sm	^{153}Sm -EDTMP	1.9	0.81	0.103 (28%)	—	3.4	62–84%
^{186}Re	^{186}Re -HEDP	3.7	1.07	0.137 (9%)	—	4.7	50–92%
^{188}Re	^{188}Re -HEDP	0.7	2.12	—	—	3	60–92%
^{177}Lu	^{177}Lu -EDTMP	6.7	0.497	0.208 (11%)	—	1.8	Preclinical evaluation
	^{177}Lu -DOTP						
^{166}Ho	^{166}Ho -DOTP	1.1	1.86	0.081 (6.2%)	—	8.6	Preclinical evaluation
$^{223}\text{Ra}^{24}$	$^{223}\text{RaCl}_2$	11.4	<4%	<2%	5.64 (94%)	0.045	Phase III
				0.154 (5.6%)			Clinical trial
				0.269 (13.7%)			
				0.323 (3.9%)			

Table 2 Drugs conjugated to bisphosphonates and corresponding therapeutic indication

Drug	Indication
Prostaglandin E ₂ ³⁴	Osteoporosis
Estradiol ^{35–38}	
Synthetic estrogenic agents ³⁹	Osteoarthritis Chronic infection Bone cancer
Nonsteroidal anti-inflammatory drugs ⁴⁰	
Fluoroquinolones ^{41–43}	
Cisplatin ^{44–46}	
Methotrexate ⁴⁷	

affinity for bone matrix, is currently in a Phase III clinical trial to treat bone metastases resulting from prostate cancer.^{29–31}

Following the same concept of radionuclide delivery using BP's as carriers, different types of drugs have been coupled to BP's, and the resulting compounds tested as new agents to treat specifically osteoporosis, osteoarthritis, chronic infections and bone cancer. The drug targeting approach would allow high local concentrations of drug, minimizing systemic adverse effects. This drug-delivery approach has been the subject of several comprehensive surveys,^{11,32,33} and is beyond the scope of this review. For the sake of example, Table 2 lists some drugs that have been already conjugated to BP's and evaluated as therapeutic agents.

The aim of this Review is to update the most recent research efforts toward novel metal-based complexes and organic halogenated compounds containing BP's as carriers of radionuclides for diagnostic or bone-pain palliation purposes. The biological properties of the novel compounds will be discussed and compared with the current available ones, namely in terms of image quality and therapeutic effect. Additionally, we will also mention the use of BP's as carriers of nuclear and optical imaging probes. Despite being out of the scope of this survey, it is also worth mentioning that V. Kubičel and I. Lukes have recently reviewed the use of BP-containing probes for Magnetic Resonance Imaging.⁴⁸

Radioactive metal complexes

Most of the radiopharmaceuticals in clinical use or under investigation are radiometal complexes stabilized by an organic coordinating ligand/chelator, which may bear a pendant biomolecule in the case of target-specific tools.^{49–54} Depending on the nuclear properties of the radiometal, they can be used for diagnostic (γ - or β^+ -emitters) or for delivering therapeutic doses of ionizing radiation to diseased tissues (β^- -emitters).^{20,21,23} Ideally, the latter should accumulate specifically in the target cells and clear rapidly from the non-target organs to minimize radiation damage to healthy tissues. For diagnostic or therapy purposes, the current research efforts are focused on the design of metal-complexes bearing biomolecules (e.g. regulatory peptides, antibodies or small biomolecules) for targeting biomarkers associated to a specific disease.^{55–59} Within this topic, a great deal of work has been conducted towards the study of the coordination chemistry of metals with potential application in Nuclear Medicine, namely on the design of the best suited chelator to give complexes with high thermodynamic stability and kinetic inertness, and the most adequate biological properties.^{51,53,60} Such chelators

must have a donor atom set in accordance with the nature of the metal ion, and may also comprise a functional group for covalent attachment to a targeting biomolecule. These ligands, normally known as “bifunctional chelators” (BFC), may also contain pharmacokinetic modifying linkers for modulation of the biological profile of the final complexes.^{47,49}

Technetium (Tc) and rhenium (Re)

Rhenium and technetium are d-transition metals (group VII elements) with similar coordination chemistry, leading, in most cases, to isostructural complexes. Although Re complexes oxidize more easily and have higher kinetic inertness than the Tc analogs, both metals are stabilized by the same type of ligands.^{49,51,53,54,61} Among the different radioisotopes available for Tc, Technetium-99m (^{99m}Tc) is the most used radionuclide for SPECT-imaging due to its almost ideal nuclear properties (half life = 6.02 h; γ -energy = 140 keV), low-cost and wide-spread availability. This radiometal is responsible for more than 80% of all diagnostic procedures in Nuclear Medicine. Rhenium has two radioisotopes with adequate physical properties for systemic therapy: ¹⁸⁶Re and ¹⁸⁸Re (Table 1). ¹⁸⁶Re is a reactor-produced radionuclide, and ^{99m}Tc and ¹⁸⁸Re are obtained from commercial ⁹⁹Mo/^{99m}Tc and ¹⁸⁸W/¹⁸⁸Re generators, respectively. These radiometals are obtained as $[M^{VII}O_4]^-$ (M = Re, Tc) and are usually reduced to lower oxidation states. The final oxidation state will depend on the reducing agent, nature of the chelator and reaction conditions. Among the oxidation states available for the metal (–I to +VII), ^{99m}Tc(v) has been the most studied in radiopharmaceutical chemistry, and the cores $[^{99m}TcO]^{3+}$, *trans*- $[^{99m}TcO_2]^+$, $[^{99m}TcN]^{2+}$ and $[^{99m}Tc-HYNIC]$ (HYNIC = 6-hydrazinonicotinic acid) the most exploited (Fig. 3).^{45,47,49,50,62}

The $[^{99m}TcO]^{3+}$ core forms square pyramidal Tc(v)–oxo complexes with tetradentate chelators, such as N₂S₂ diamidedithiols (DADS), N₃S triamidethiols, N₂S₂ monoamide-monoaminedithiols (MAMA) and N₂S₂ diaminedithiols (DADT). The *trans*- $[^{99m}TcO_2]^+$ unit has been mainly used in combination with acyclic tetraamine ligands, which form well-defined octahedral Tc(v) dioxocomplexes. In the case of the $[^{99m}TcN]^{2+}$ fragment, the most successful complexes for radiopharmaceutical applications are stabilized by a PXP-type bisphosphine (X = N, S) and by bidentate thiolate-S, amine-N and/or carboxylate-O donors bearing or not a conjugated biomolecule.

With regard to the $[^{99m}Tc-HYNIC]$ core, it can be stabilized by hydrophilic chelating co-ligands like ethylenediamine diacetic acid, gluconate or tricine. The nature of the co-ligand depends on the coordination mode of HYNIC, which can be uni or bidentate. However, the resulting binary mixed-ligand complexes have shown a relatively low stability. Improvement of the stability has been achieved by the introduction of a ternary ligand, such as a water-soluble phosphine, or by using phosphine- and nicotinyl-containing HYNIC derivatives.

The introduction of the organometallic precursor *fac*- $[^{99m}Tc(CO)_3(H_2O)_3]^+$ by Alberto and coworkers brought renewed interest in the design of radiopharmaceuticals based on Tc(I).^{63–69} The easy preparation of the organometallic precursor, the lability of the three water molecules and the

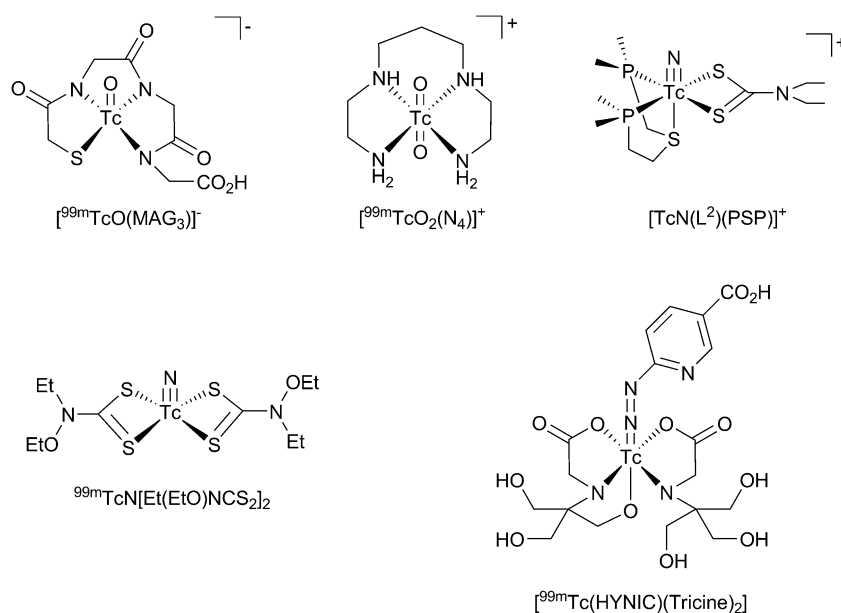


Fig. 3 Examples of Tc(v) complexes with different cores explored in radiopharmaceutical chemistry.

chemical robustness of the *fac*- $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3]^+$ metal fragment offered a great number of advantages for the design of innovative target-specific radiopharmaceuticals (Fig. 4).^{63–75}

BP-containing M complexes (M = Tc, ^{186/188}Re)

As stated before, ^{99m}Tc-MDP and ^{99m}Tc-HMDP are currently the SPECT radiopharmaceuticals of choice for skeletal imaging, being widely used for assessment of bone metastases, especially in breast and prostate cancer.^{20–23,28} The therapeutic analog of the ^{99m}Tc-labeled BP's, ¹⁸⁶Re-HEDP has been approved in some European countries as a pain palliation

radiopharmaceutical for bone metastases²⁸ and ¹⁸⁸Re-HEDP is currently in clinical trials.^{76–80}

In spite of their well-established clinical use as diagnostic or therapeutic agents, ^{99m}Tc- and ^{186/188}Re-labeled BP's present a set of clinical and chemical limitations. The ^{99m}Tc-labeled BP's may lead to false negatives due to their recognized lack of specificity, and display also a relatively slow blood and soft-tissue clearance, delaying the start of the bone-scanning procedure in nuclear medicine centers.⁸¹ With regard to chemical limitations, the exact composition and structure of these radiopharmaceuticals have yet to be determined.^{82,83} At the macroscopic level, and after several attempts, the complex ⁹⁹Tc-MDP has been characterized in the solid state. In fact, by reacting $[\text{TcBr}_6]^{2-}$ with excess of MDP, brown crystals adequate to X-ray diffraction analysis were obtained.⁸⁴ The X-ray analysis showed a polymeric chain structure of stoichiometric composition $[\text{Tc}(\text{OH})(\text{MDP})]^\infty$. As shown in Fig. 5, in such structure each MDP ligand bridges two symmetry related technetium atoms (Fig. 5A), and each technetium atom is bound to two MDP ligands (Fig. 5B). The polymeric repeat unit is completed by an oxygen atom, most probably a hydroxyl group, which bridges the two metal centers. The charge associated with each repeated unit is neutralized by a hydrated lithium cation. The geometry around each metal center is approximately octahedral, but the oxidation state of the metal cannot be unambiguously assigned since it is not clear whether a hydroxyl or an oxo group is bridging the two metal centers.

Since BP's are fairly weak chelates, M-BP complexes tend to oxidize *in vivo* to $[\text{MO}_4]^-$ (M = ^{99m}Tc, Re), resulting in reduced bone uptake with an increased accumulation in non-target soft tissues.⁸⁵ It has also been reported that BP's labeled with ^{186/188}Re may not target bone metastases unless "cold" rhenium is added to the preparation.^{85–87} Altogether, the above mentioned drawbacks prompted several research efforts toward the development of novel M-labeled BP's for

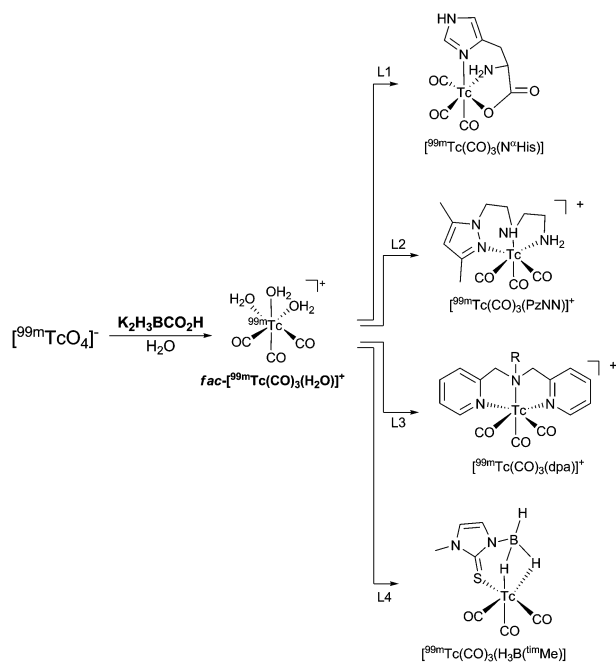


Fig. 4 Synthesis of *fac*- $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and some derivatives.

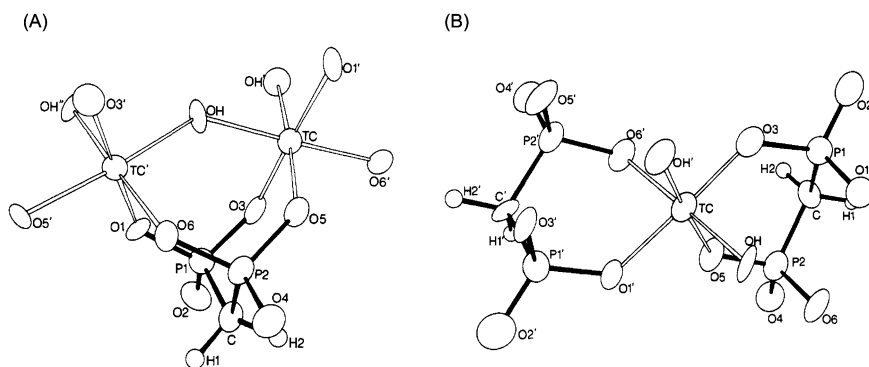


Fig. 5 Perspective views of a portion of the $[\text{Tc}(\text{OH})(\text{MDP})]_{\infty}$ structure showing one MDP ligand bridging two technetium centers (A), and one Tc center bridging two MDP ligands (B). Reprinted with permission from ref. 84. Copyright 1980 American Chemical Society.

bone imaging ($M = {}^{99\text{m}}\text{Tc}$) and therapy (${}^{186/188}\text{Re}$) with well defined chemical structures and improved biological properties.

So far, the most successful strategy took advantage of the so-called *bifunctional chelating approach*. This approach uses a BFC which, simultaneously, stabilizes the radiometal and allows conjugation to the bone-seeking moiety (BP). Using this methodology, the affinity for the bone is expected to increase compared to directly labeled BP's, since the BP moiety would retain full ability for binding to bone surface. Therefore, various BP-containing complexes based on different metal fragments (Fig. 6–8) have been synthesized and explored as bone targeting agents for diagnostic or therapeutic purposes. Some of the nonradioactive Re analogs of these ${}^{99\text{m}}\text{Tc}$ -complexes were also successfully synthesized and characterized by the usual analytical techniques, allowing the full chemical identification of the radioactive compounds.

Using the bifunctional approach, Verbruggen and co-workers have introduced several ${}^{99\text{m}}\text{Tc}$ -oxocomplexes (Fig. 6) stabilized by L,L-ethylene dicysteine derivatives (EC) containing pendant aminomethylenediphosphonate (AMDP) moieties (${}^{99\text{m}}\text{Tc}$ -EC-1; ${}^{99\text{m}}\text{Tc}$ -EC-2; ${}^{99\text{m}}\text{Tc}$ -EC-3).^{73,77,88}

The complexes were prepared by direct labeling of the EC derivatives with $\text{Na}[\text{}^{99\text{m}}\text{TcO}_4]$ in the presence of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, under basic conditions at room temperature. ${}^{99\text{m}}\text{Tc}$ -EC-1 and ${}^{99\text{m}}\text{Tc}$ -EC-2 were obtained as a mixture of isomers. Despite being all stable, none of the radioactive complexes were characterized/identified by comparison with their Re analogs. The most favorable biological results were obtained with ${}^{99\text{m}}\text{Tc}$ -EC-1 that has improved bone uptake in rats (${}^{99\text{m}}\text{Tc}$ -EC-1: 29.4% ID g^{-1} organ; ${}^{99\text{m}}\text{Tc}$ -MDP: 15.4% ID g^{-1} organ, at 2 h postinjection (p.i.)), and blood clearance and urinary excretion rates comparable to ${}^{99\text{m}}\text{Tc}$ -MDP.

The ${}^{186}\text{Re}$ -oxocomplexes ${}^{186}\text{Re}$ -MAMA-BP, ${}^{186}\text{Re}$ -MAMA-HBP and ${}^{186}\text{Re}$ -MAG₃-HBP (MAG₃ = mercaptoacetyl-glycylglycylglycine, N₃S-type chelator) were prepared by Ogawa *et al.* and evaluated as agents for bone palliative treatment (Fig. 7).^{89–93}

${}^{186}\text{Re}$ -MAMA-BP was prepared by ligand exchange reaction using ${}^{186}\text{Re}$ -glucoheptonate as precursor. ${}^{186}\text{Re}$ -MAMA-HBP and ${}^{186}\text{Re}$ -MAG₃-HBP were obtained by direct reaction of the bisphosphonate-containing chelator with $[\text{}^{186}\text{ReO}_4]^-$ and SnCl_2 in citrate buffer. The labeling yields were low, and a purification step was needed to obtain single species with radiochemical purity high enough (>95%) for biological assessment. The radiotracers ${}^{186}\text{Re}$ -MAMA-BP, ${}^{186}\text{Re}$ -MAG₃-HBP, and ${}^{186}\text{Re}$ -MAMA-HBP all presented higher femur to blood ratios than ${}^{186}\text{Re}$ -HEDP.^{89,90} The femur accumulation in mice for ${}^{186}\text{Re}$ -MAMA-HBP was higher than that found for ${}^{186}\text{Re}$ -MAMA-BP, confirming that the presence of the geminal hydroxyl group in the BP confers better bone-targeting properties to the resulting complexes.^{89,90} ${}^{186}\text{Re}$ -MAG₃-HBP inhibited tumor growth in a rat model and attenuated allodynia-induced bone cancer without having critical myelosuppressive side effects.⁹² The same research group prepared the analogs ${}^{99\text{m}}\text{Tc}$ -MAG₃-HBP and ${}^{99\text{m}}\text{Tc}$ -HYNIC-HBP by direct labeling of the BP-containing chelators with $[\text{}^{99\text{m}}\text{TcO}_4]^-$ in the presence of SnCl_2 (Fig. 7). These radiometal complexes exhibited favorable properties in terms of *in vitro* HA affinity and bone uptake in rats.⁹⁴ Despite showing a considerable high protein binding, ${}^{99\text{m}}\text{Tc}$ -HYNIC-HBP still presented a significantly higher bone to blood ratio than ${}^{99\text{m}}\text{Tc}$ -MDP. The isostructural ${}^{99\text{m}}\text{Tc}$ -MAG₃-HBP and ${}^{186}\text{Re}$ -MAG₃-HBP were identified/characterized by comparing their HPLC chromatograms with that of the nonradioactive analog Re-MAG₃-HBP, which was

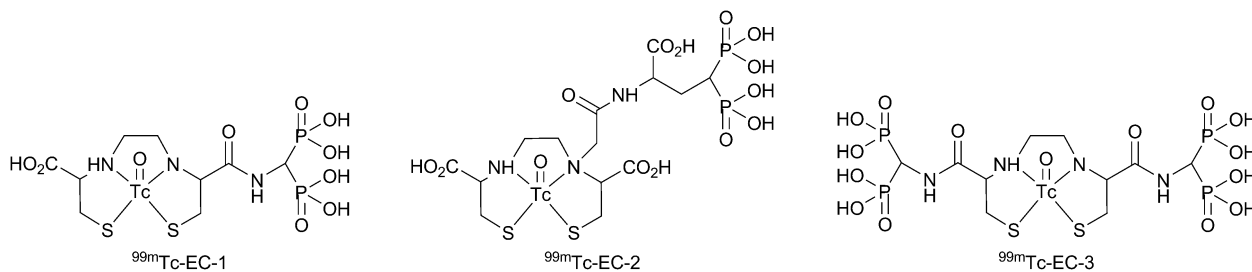


Fig. 6 ${}^{99\text{m}}\text{Tc}(\text{v})$ -oxocomplexes stabilized by L,L-ethylene dicysteine-type chelators (EC) bearing pendant AMDP arms.

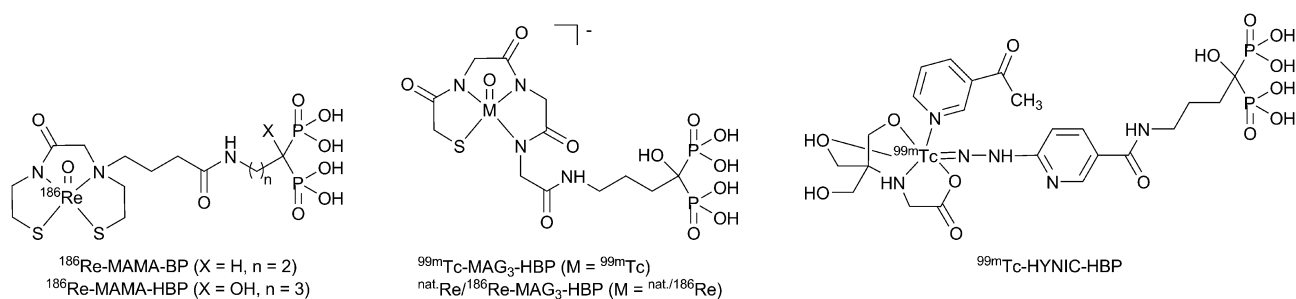


Fig. 7 BP-containing M(v)-oxocomplexes ($M = ^{186}\text{Re}$, $^{99\text{m}}\text{Tc}$) stabilized by N_2S_2 - or N_3S -type chelators, and $^{99\text{m}}\text{Tc-HYNIC-HBP}$.

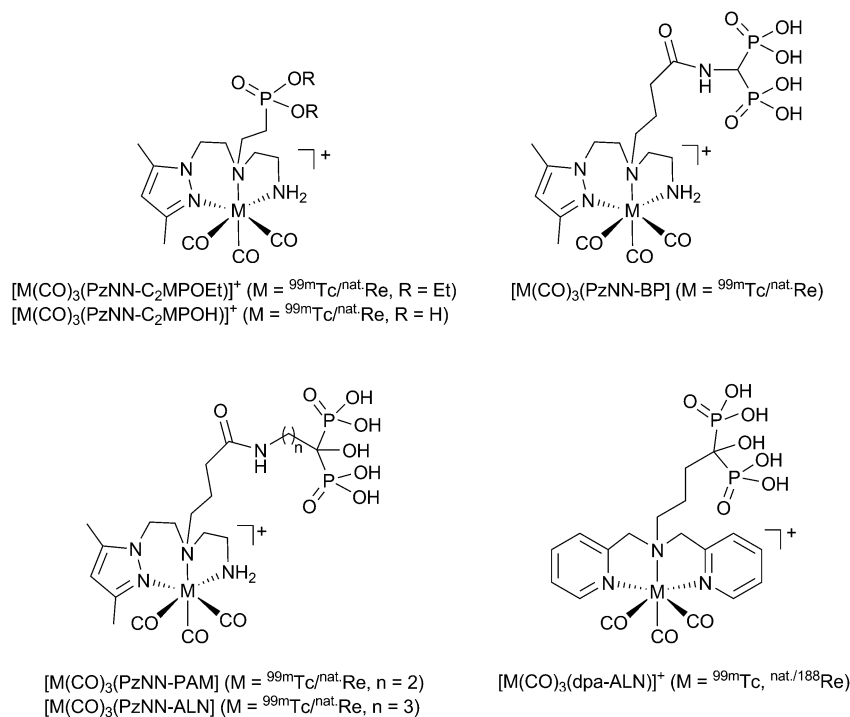


Fig. 8 Re(I) and Tc(I) complexes containing pendant mono- or bis-phosphonate moieties. PzNN = pyrazolyldiamine-containing chelator; PAM = pamidronate; ALN = alendronate and dpa = dipicolylamine.^{95–98}

synthesized by conjugation of the previously synthesized precursor Re-MAG₃ to the BP derivative.⁹¹ The nonradioactive complexes Re-MAMA-BP/HBP were not synthesized but, based on transchelation experiments with MAMA and HEDP, the authors claimed that ¹⁸⁶Re was selectively bound to MAMA with no unspecific interactions between the bisphosphonate moiety and the metal centre.^{89,90}

The bifunctional chelators that have been explored for the design of *fac*-[M(CO)₃]⁺ ($M = ^{99\text{m}}\text{Tc}$, ^{186/188}Re) bone-seeking radiotracers were mono- or bis-phosphonate-containing N₃-tridentate chelators with one ([M(CO)₃(pzNN-C₂MPOEt/H)], [M(CO)₃(pzNN-BP)] and [M(CO)₃(pzNN-PAM/ALN)]) or two aromatic N-heterocycles ([M(CO)₃(dpa-ALN)]) (Fig. 8).

All ^{99m}Tc(CO)₃-complexes were obtained as single species by reacting the precursor *fac*-[^{99m}Tc(H₂O)₃(CO)₃]⁺ with the corresponding chelators, and were evaluated in appropriate animal models.^{95–98} All radiocomplexes showed the same retention times (γ -detection) as those of the corresponding fully characterized non-radioactive Re surrogates (UV/Vis-detection)

in the respective RP-HPLC chromatograms, demonstrating their structural identity.

Santos and coworkers have confirmed the improved ability of bisphosphonates to target bone tissues *versus* mono-phosphonates in a comparative *in vivo* study between [^{99m}Tc(CO)₃(pzNN-C₂MPOH)] and [^{99m}Tc(CO)₃(pzNN-BP)]. In spite of the moderate bone uptake in mice presented by [^{99m}Tc(CO)₃(pzNN-BP)] (3.04 ± 0.47% ID g⁻¹ organ, 4 h p.i.) compared to ^{99m}Tc-MDP (6.40 ± 2.00% ID g⁻¹ organ, 4 h p.i.), the organometallic complex displayed high stability *in vivo*, a favorable bone-to-blood and bone-to-muscle ratios, and high rate of total radioactivity excretion.⁹⁵ This biological behavior encouraged further studies, which resulted in the design of the complexes [^{99m}Tc(CO)₃(pzNN-PAM)] and [^{99m}Tc(CO)₃(pzNN-ALN)] anchored by the same pyrazolyldiamine chelating unit (pzNN), and bearing the pharmacologically relevant BP's pamidronate (PAM) and alendronate (ALN).⁹⁶ Both complexes presented an identical biological pattern in rats: high bone uptake ([^{99m}Tc(CO)₃(pzNN-PAM)]:

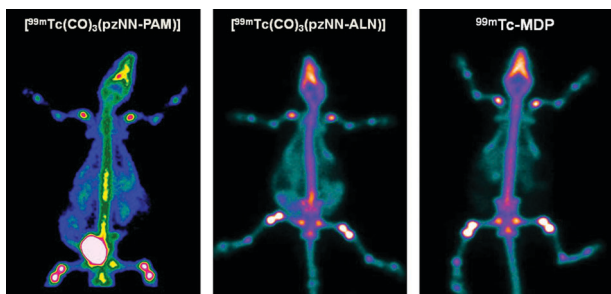


Fig. 9 Planar whole-body gamma camera images of rats injected with $[^{99m}\text{Tc}(\text{CO})_3(\text{pzNN-PAM})]$, $[^{99m}\text{Tc}(\text{CO})_3(\text{pzNN-ALN})]$ and $^{99m}\text{Tc-MDP}$ at 2 h p.i.⁹⁶—Reproduced by permission of The Royal Society of Chemistry. DOI: 10.1039/C0DT01396J.

$18.3 \pm 0.6\%$ ID g^{-1} , $[^{99m}\text{Tc}(\text{CO})_3(\text{pzNN-ALN})]$: $17.3 \pm 6.1\%$ ID g^{-1} , $^{99m}\text{Tc-MDP}$: $17.1 \pm 2.4\%$ ID g^{-1} , at 1 h p.i.), enhanced clearance from most tissues and increased total excretion. The bone-to-blood and the bone-to-muscle ratios are significantly higher than those obtained for $^{99m}\text{Tc-MDP}$. *In vivo* imaging in rats allowed the clear visualization of bone tissue (Fig. 9). Unlike $^{99m}\text{Tc-MDP}$, both ^{99m}Tc (i)-complexes presented negligible serum protein binding.

The complex $[^{99m}\text{Tc}(\text{CO})_3(\text{dpa-ALN})]$ developed by de Rosales *et al.* presented also high bone uptake, comparable to that observed for the gold standard $^{99m}\text{Tc-MDP}$.⁹⁷ Aimed at therapeutic applications, the isostructural analog complex $[^{188}\text{Re}(\text{CO})_3(\text{dpa-ALN})]$ was obtained in high yield ($\geq 96\%$) and high specific activity by reaction of the bisphosphonate conjugate (dpa-ALN) with the precursor *fac*- $[^{188}\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]^+$.⁹⁸ The preparation of the latter is not so straightforward as that of *fac*- $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, which is readily prepared in high yield and radiochemical purity ($>99\%$) using a kit formulation (Isolink[®], Covidean) without any purification step. The precursor *fac*- $[^{188}\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]^+$ is obtained with a radiochemical yield of 80% by reducing $[^{188}\text{ReO}_4]^-$ in the presence of $\text{BH}_3\text{-NH}_3/\text{CO}$ (g) and only after HPLC purification, a radiochemical purity $>99\%$ is achieved.⁹⁹ *In vivo* imaging and biodistribution studies in mice have shown that $[^{188}\text{Re}(\text{CO})_3(\text{dpa-ALN})]$ is superior to

$^{188}\text{Re-HEDP}$ in terms of bone uptake in areas of high metabolic activity, with low soft tissue uptake (Fig. 10).⁹⁸

The complex $[^{186}\text{Re}(\text{CO})_3(\text{CpTR-Gly-PAM})]$, containing a cyclopentadienyl derivative, has been designed as a bone-seeking radiotracer potentially useful as therapeutic agent (Fig. 11).¹⁰⁰ The complex was prepared in 25% yield by conjugation of the fully characterized precursor $[^{186}\text{Re}(\text{CO})_3(\text{CpTR-Gly})]$ to the bisphosphonate derivative.¹⁰⁰ The precursor, obtained in 38% yield, was synthesized by a double ligand transfer reaction of the ferrocene derivative Fer-Gly-OME with $[^{186}\text{ReO}_4]^-$ in the presence of $\text{Cr}(\text{CO})_6$ and SnCl_2 , followed by hydrolysis of the methyl group. The biological evaluation of $[^{186}\text{Re}(\text{CO})_3(\text{CpTR-Gly-PAM})]$ in male ddY mice demonstrated that its bone accumulation was similar to that of $^{186}\text{Re-HEDP}$. When $[^{186}\text{Re}(\text{CO})_3(\text{CpTR-Gly-PAM})]$ was purified by RP-HPLC to increase its specific activity, the resulting purified complex showed better results compared to $^{186}\text{Re-HEDP}$ in terms of plasma stability, HA-binding, bone accumulation and plasma protein binding.

Trivalent and divalent metal ions

Gallium (Ga) and indium (In)

The post-transition metals gallium (Ga) and indium (In) present radioisotopes adequate for SPECT (γ -emitters: ^{67}Ga , ^{111}In) or PET (β^+ -emitter: ^{68}Ga) imaging or for Auger-therapy (^{111}In).^{49–50,54} The oxidation state with relevance for radio-pharmaceutical applications is the +III, for both metals, and due to their chemical features they are stabilized by polydentate chelators with anionic oxygen donor groups, such as acyclic or macrocyclic polyaminocarboxylic ligands (Fig. 12). The different ionic radii of Ga^{3+} (47–62 pm, coordination number = 4–6) and In^{3+} (62–92 pm, coordination number = 4–8) is another important aspect to consider when selecting a proper ligand for labeling a biomolecule with their radioisotopes. BFC's like diethylenetriaminepentaacetic acid (DTPA) or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) derivatives have been used for In^{3+} , and BFC's such as DOTA or

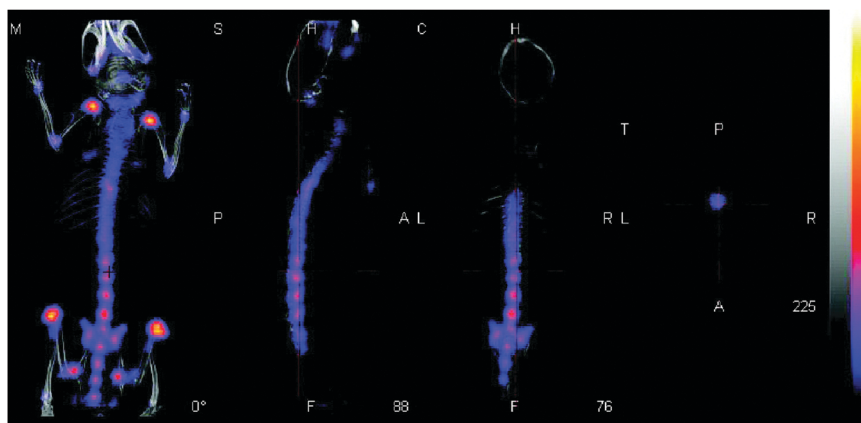


Fig. 10 SPECT/CT image of an adult BALB/c female mouse injected with $[^{188}\text{Re}(\text{CO})_3(\text{dpa-ALN})]$ at 24 h p.i. From left to right, maximum intensity projection (M), sagittal (S), coronal (C), and transverse (T) sections. Reprinted with permission from ref. 98. Copyright 2010 American Chemical Society.

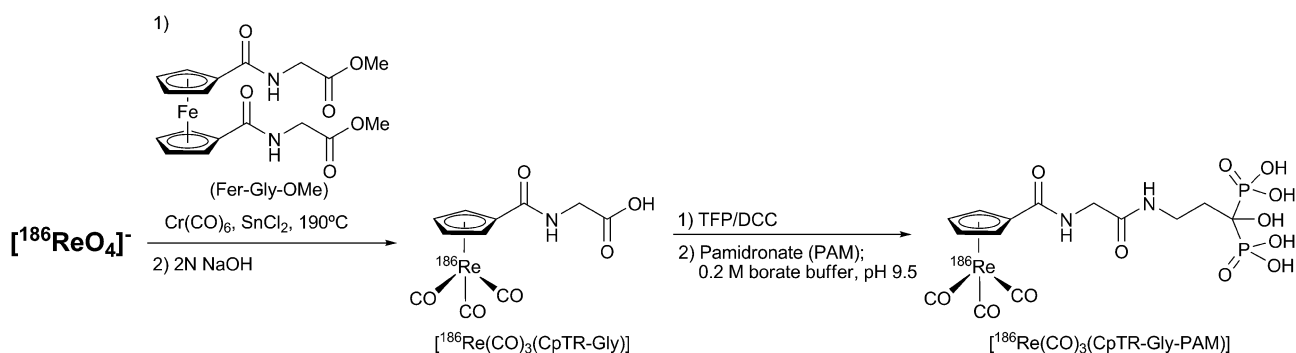


Fig. 11 Synthesis of $[^{186}\text{Re}(\text{CO})_3(\text{CpTR-Gly-PAM})]$.¹⁰⁰

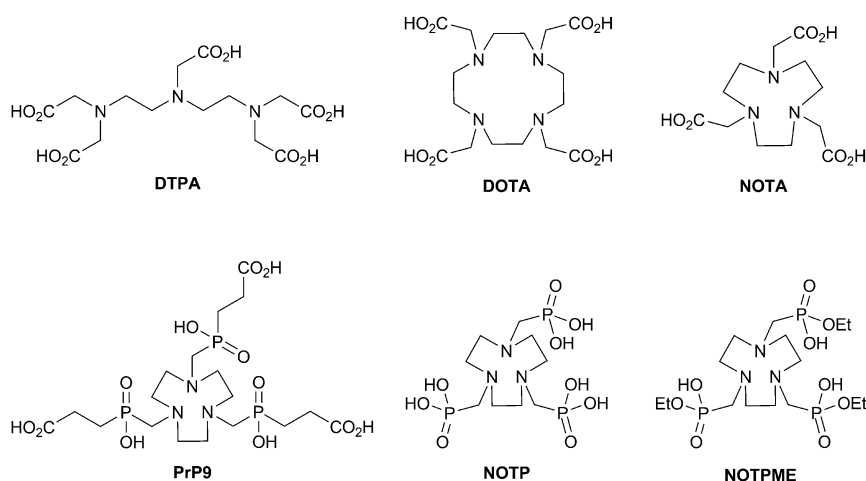


Fig. 12 Acyclic and cyclic polyaminocarboxylic and polyaminophosphonate chelators.

1,4,7-triazacyclononane- N,N',N'' -triacetic acid (NOTA) for Ga^{3+} (Fig. 12).

Several NOTA derivatives have been studied with $\text{Ga}(\text{III})$, and the HA and/or bone uptake of the BP-containing complexes were evaluated.^{49,101–109} Triazacyclononane with methyl-(2-carboxyethyl)phosphinic acid pendant arms (PrP9, Fig. 12) reacted with GaCl_3 yielding $[\text{Ga}(\text{H}_3\text{PrP9})]$, which presented high thermodynamic stability and high kinetic inertness. Besides the characterization in solution by potentiometry, $[\text{Ga}(\text{H}_3\text{PrP9})]$ was also characterized in the solid state by X-ray diffraction analysis (Fig. 13).¹⁰¹ The ligand PrP9 was labeled directly with ^{68}Ga in yields higher than 92% over a wide pH range (1–5) at room temperature. So far, the bone-targeting properties of this complex are unknown.

Other NOTA derivatives, namely 1,4,7-triazacyclononane- N,N',N'' -tris(methylenephosphonic acid) (NOTP) and 1,4,7-triazacyclononane- N,N',N'' -tris(methylenephosphonate monoethyl ester) (NOTPME) (Fig. 12), were labeled with $^{67}\text{Ga}(\text{III})$ and the biological properties of the respective radioactive complexes evaluated in Wistar rats. Despite the high stability and rapid renal excretion properties displayed by ^{67}Ga -(NOTP) and ^{67}Ga -(NOTPME), no significant bone uptake was observed.^{102,103}

NOTA and DOTA derivatives bearing an alendronate pendant arm (NOTA-HBP and DOTA-HBP, Fig. 14) have been labeled with $^{68}\text{Ga}(\text{III})$ (radiochemical yield and purity >95%), and the

bone-targeting properties of the resulting radioactive complexes $[\text{Ga}(\text{H}_3\text{PrP9})]$ and $[\text{Ga}(\text{H}_3\text{PrP9})]$ evaluated *in vitro* (HA binding assays) and in rats.¹⁰⁴ Both radiocomplexes presented HA binding affinities similar to that of $^{99\text{m}}\text{Tc-MDP}$. $[\text{Ga}(\text{H}_3\text{PrP9})]$ emerged as the

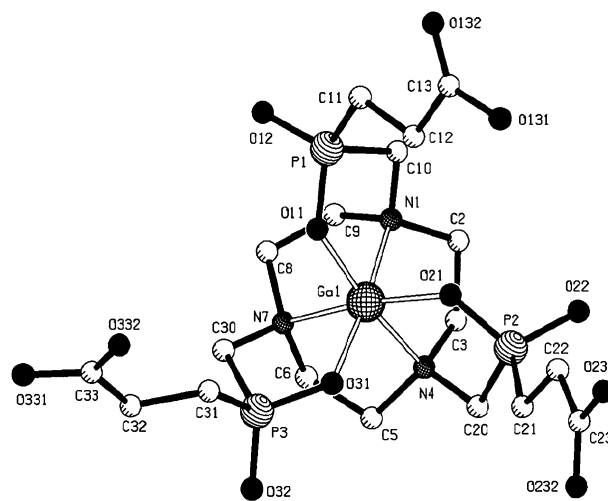


Fig. 13 Molecular structure of the $[\text{Ga}(\text{H}_3\text{PrP9})]$ complex (hydrogen atoms are omitted for clarity).¹⁰¹ Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

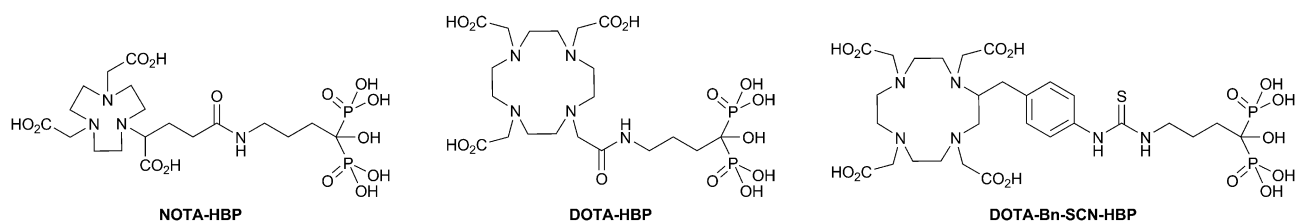


Fig. 14 Structure of NOTA-HBP, DOTA-HBP and DOTA-Bn-SCN-HBP.

most promising, allowing the visualization of the bone structure by micro PET imaging, and displaying the highest bone uptake and bone to blood ratio.¹⁰⁴ Recent studies have shown that [⁶⁸Ga-NOTA-HBP] could detect mouse model bone metastasis by micro-PET imaging at 1 h p.i.¹⁰⁹ The tracer was found to have a high bone affinity and rapid blood clearance, proving its usefulness as a bone-seeking agent for clinical PET.¹⁰⁹

Labeling of the recently described DOTA-like chelator DOTA-Bn-SCN-HBP (Fig. 14), bearing also a pamidronate pendant arm, with ⁶⁷Ga gave the radiocomplex [⁶⁷Ga-DOTA-Bn-SCN-HBP] in high radiochemical yield (95%).¹¹⁰ This radiocomplex has shown promising biodistribution characteristics as a bone scintigraphy agent. The authors claim that these data may be helpful in the design of novel ⁶⁸Ga-based PET bone-imaging tracers.¹¹⁰

The acyclic ligand EDTMP and the 12-membered tetraazamacrocycles DOTP, BPAMD, BPAPD and BPPED (Fig. 15) have been labeled with ⁶⁸Ga, giving radiochemical yields in the range of 50% to 95%.¹⁰⁵

PET imaging on rats revealed moderate bone uptake for [⁶⁸Ga-DOTP] and [⁶⁸Ga-EDTMP]. However, due to the low labeling yield of [⁶⁸Ga-DOTP] and low stability of [⁶⁸Ga-EDTMP], the DOTA-like chelators BPAMD, BPAPD and BPPED emerged as the most promising for bone imaging.

PET imaging using [⁶⁸Ga-BPAMD] as radiotracer showed significant bone uptake and high *in vivo* stability in a rat model^{105–107} These results have been confirmed later on in the clinical setting. Indeed, PET/CT imaging with [⁶⁸Ga-BPAMD] presented intense accumulation in multiple osteoblastic lesions in the central skeleton, ribs and proximal extremities after i.v. injection into a patient with known extensive bone metastases of prostate cancer (Fig. 16).¹⁰⁸

The bisphosphonate-containing chelator DOTA-BP (Fig. 15) was labeled with ¹¹¹In, giving the complex [¹¹¹In-DOTA-BP]. The *in vitro* and *in vivo* studies in normal mice indicated that this complex may find applications in osteoclast-targeted radiotherapy and nuclear imaging of bone diseases due to high bone uptake and long retention, and negligible accumulation in non-target organs.¹¹¹

Yttrium (Y) and lanthanides (Ln)

Yttrium (Y) and lanthanides (Ln) are trivalent metals that offer different β -emitting radioisotopes relevant for therapeutic applications. Among these radioisotopes, ⁹⁰Y, ¹⁵³Sm, ¹⁶⁶Ho and ¹⁷⁷Lu have been the most explored for bone palliation, due to their nuclear properties. The aqueous coordination chemistry of yttrium and lanthanides shows a great similitude due to their common tricationic charge and

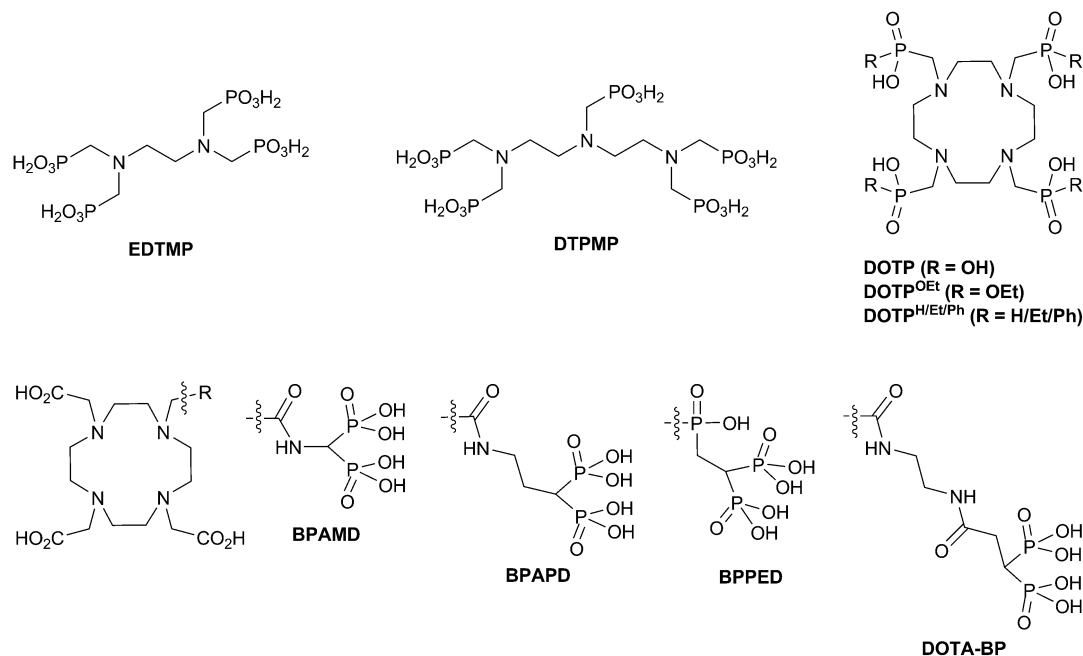


Fig. 15 Acyclic chelators and 12-membered tetraazamacrocycles with phosphonate pendant arms.

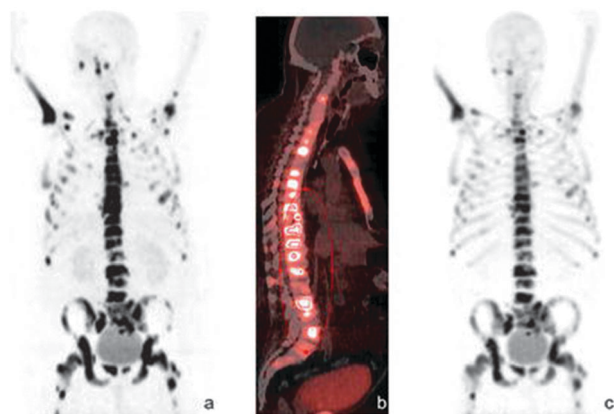


Fig. 16 Patient with known extensive bone metastases of prostate cancer injected i.v. with 462 MBq of [^{68}Ga -BPAMD] (maximum intensity projection 50 min p.i., a = coronal PET, b = sagittal PET/CT) or ^{18}F (c PET, sagittal, maximum intensity projection 90 min p.i., 270 MBq) for comparison. With kind permission from Springer Science + Business Media.¹⁰⁸

similar ionic radii. The Y^{3+} and Ln^{3+} metal ions show a hard acidic character and tend to form complexes with hard donor atom ligands, displaying high coordination numbers, usually 8 or 9. Therefore, most of the studies with these elements have been performed using mainly acyclic or cyclic polyamino ligands bearing carboxylate and/or phosphonate pendant arms, such as DTPA, DTPMP, DOTA, DOTA-HBP, DOTP, *trans*-DO2A2P, DOA3P, TRITP and TETP (Fig. 12, 14, 15 and 17).

The first lanthanide complex approved by the Regulatory Authorities for bone pain palliation was ^{153}Sm -EDTMP (Quadramet[®]), in which the metal is anchored on the linear EDTMP ligand (Fig. 15). Quadramet[®] presents several drawbacks, namely low kinetic inertness, low specific activity and unknown structure, all associated with the nature of the chelator and chemical features of the Ln^{3+} . Indeed, it is well established that metal complexes of macrocyclic chelators are thermodynamically more stable and kinetically more inert

than their non-cyclic counterparts, due to the preorganized nature of the macrocycles.⁶⁰ The use of linear polyamino-polyphosphonate chelators in the preparation of osteotropic radiopharmaceuticals for the treatment of bone metastases has been reviewed elsewhere.¹¹² Therefore, herein, we will focus on the metal complexes stabilized by cyclic polyaminopolyphosphonates (Fig. 12, 14, 15 and 17).

The radiolanthanide complexes [$^{153}\text{Sm}/^{166}\text{Ho}/^{177}\text{Lu}$ -DOTP] emerged as the most promising alternative to ^{153}Sm -EDTMP for bone pain palliation or bone marrow ablation. [^{166}Ho -DOTP] is under clinical evaluation for myeloablative treatment of multiple myelomas or for intravascular radiation therapy.^{22,113–118} The DOTP analog DOTP^{OEt} (Fig. 15) was also labeled with $^{153}\text{Sm}/^{166}\text{Ho}$, yielding (>98%) the stable complexes [^{153}Sm -DOTP^{OEt}] and [^{166}Ho -DOTP^{OEt}], which displayed low binding to HA and negligible bone affinity in a mice model.¹¹⁹ Other DOTP analogs, namely DOTP^H, DOTP^{Et} or DOTP^{Ph} (Fig. 15) have also been explored with lanthanides for bone targeting, however, the resulting complexes exhibited a low stability that hampered their evaluation as bone-targeting complexes.^{120–123}

13-(TRITP) and 14-membered (TETP) tetraazamacrocycles with pendant methylphosphonate arms have been also explored as chelators for the stabilization of ^{153}Sm and ^{166}Ho . The [$^{153}\text{Sm}/^{166}\text{Ho}$ -TRITP] and [$^{153}\text{Sm}/^{166}\text{Ho}$ -TETP] complexes have been obtained in quantitative yield, except in the case of TETP.¹²⁴ The stable $^{153}\text{Sm}/^{166}\text{Ho}$ -TRITP complexes bind to HA, with [^{166}Ho -TRITP] presenting the highest degree of adsorption. Biodistribution studies in mice have shown that [$^{153}\text{Sm}/^{166}\text{Ho}$ -TRITP] accumulate in bone with a high rate of total excretion. The bone uptake found for [^{166}Ho -TRITP] is comparable to the values found for [^{166}Ho -DOTP], making this complex quite promising as potential therapeutic agent.¹²⁴

Considering that the introduction of a pyridine moiety into the macrocyclic backbone would increase the stereochemical rigidity of the resulting complexes and their thermodynamic stability, Marques *et al.* studied 14-membered

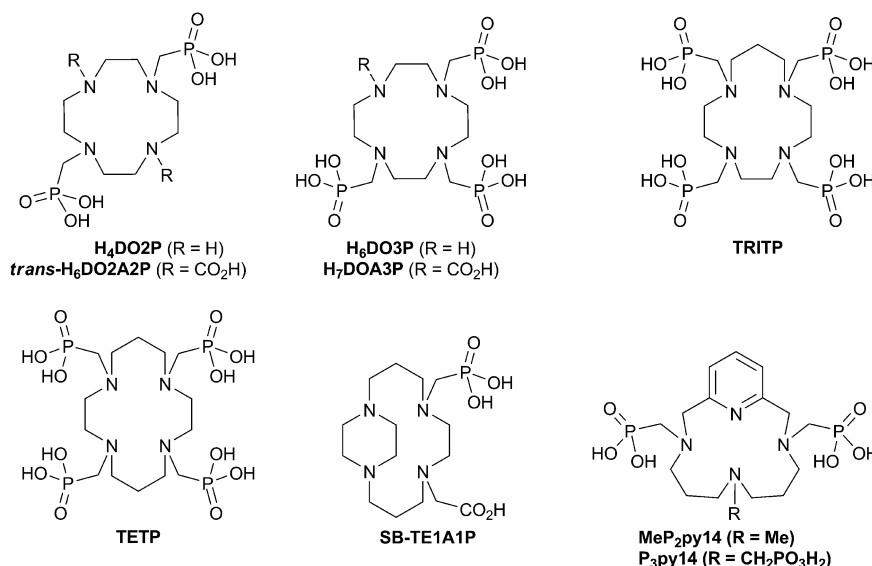


Fig. 17 12-, 13- and 14-membered tetraazamacrocycles with phosphonate pendant arms.

tetraazamacrocycles containing a pyridine and two (MeP2-py14) or three (P3py14) methylphosphonate pendant arms (Fig. 17).¹²⁵ The chelators have been quantitatively labeled with ¹⁵³Sm and ¹⁶⁶Ho at room temperature, being stable in PBS and saline, but unstable in serum. Biodistribution studies in mice indicated a slow rate of clearance from blood and muscle, a high and rapid liver uptake and a very slow rate of total radioactivity excretion.¹²⁵ Negligible bone uptake was observed, which increased with time and number of methylphosphonate groups. The biological profile supports the *in vitro* instability found in serum and is consistent with the low thermodynamic stability constants found for these complexes.

Despite the described promising biological results obtained for both [¹⁵³Sm/¹⁶⁶Ho-DOTP] and [¹⁵³Sm/¹⁶⁶Ho-TRITP] complexes, the relatively slow formation rate, the low selectivity for a specific metal ion and the high osmolarity (due to overall high negative charge) are major drawbacks when considering clinical application. Therefore, aimed at addressing such issues, mixed carboxylate/phosphonate or phosphinate DOTA-like macrocyclic ligands have been proposed as novel chelators. The mixed carboxylate/phosphonate 12-membered tetraazamacrocycles *trans*-H₆DO2A2P and H₇DOA3P (Fig. 17) have been synthesized and, after optimization of the radiolabeling conditions, yielded quantitatively the stable complexes [¹⁵³Sm/¹⁶⁶Ho-DO2A2P] and [¹⁵³Sm/¹⁶⁶Ho-DOA3P].^{126–128} Comparative biodistribution studies in mice have demonstrated that bone uptake correlates directly with the number of methylphosphonate pendant arms, since bone accumulation follows the trend TRITP > DOA3P > DO2A2P. In this study, [¹⁶⁶Ho-TRITP] emerged again as the best bone-targeting complex, comparable to [¹⁶⁶Ho-DOTP].^{126,127}

Aiming to better understand the biological behavior of those radiolanthanide complexes, potentiometric solution studies have been performed with the corresponding non-radioactive lanthanide complexes.¹²⁹ The data obtained have shown that at physiological pH the main species in solution are of the type [ML]. Additionally, the lanthanide complexes prepared with *trans*-DO2A2P were also characterized in the

solid state by X-ray diffraction analysis. For the sake of example, the molecular structure of the lanthanide complex K₂[Sm(HL)]·6.5H₂O is displayed in Fig. 18.¹²⁹

The mixed 12-membered DOTA-like macrocyclic ligands containing methylphosphinate (H₅DO3AP^{PriA} and H₄DO3A-P^{ABn}) or methylphosphonate (H₅DO3AP) pendant arms have been prepared and labeled with ¹⁵³Sm and ¹⁶⁶Ho (Fig. 19).¹³⁰ The biodistribution studies of the stable ¹⁵³Sm- and ¹⁶⁶Ho-complexes in mice have shown a similar biological profile for all complexes, with a rapid clearance from main organs and low bone uptake, in accordance with the low HA binding observed in the *in vitro* assays.¹³⁰

A study performed by T. Vitha *et al.* has demonstrated that the HA affinity of the complex [¹⁶⁰Tb-BPPED] was comparable to that of analog complexes stabilized by chelators with amide bonds (BPAMD and BPAPD, Fig. 15). These studies have shown also that the affinity to HA is dependent on the distance between the bisphosphonic acid group and the macrocyclic unit. The shorter the distance the higher the affinity is.¹³¹ Interestingly, in a subsequent study by the same group, it has been possible to conclude that while the adsorption was very fast with similar adsorption rates for both [¹⁶⁰Tb-BPPED] and [¹⁶⁰Tb-DOTP] complexes, [¹⁶⁰Tb-BPPED] has shown a much higher affinity toward the HA surface than [¹⁶⁰Tb-DOTP].¹³²

Besides ¹⁵³Sm and ¹⁶⁶Ho complexes, also compounds containing other β⁻-emitting radionuclides such as ¹⁷⁷Lu and ⁹⁰Y have been explored for bone-targeted systemic radionuclide therapy.

The chelators BPAMD and BPAPD have been labeled with ¹⁷⁷Lu, and the resulting complexes [¹⁷⁷Lu-BPAMD] and [¹⁷⁷Lu-BPAPD] were evaluated *in vivo* in a rat model.¹⁰⁷ Both radiocomplexes presented high selectivity for newly formed bone and, therefore, were considered promising agents for diagnostic of bone tumors and for bone-pain palliation. Despite presenting similar slow clearance from the skeleton, the bone uptake seems to be slightly slower for [¹⁷⁷Lu-BPAMD] than for [¹⁷⁷Lu-BPAPD].¹⁰⁷

The chelator DOTA-HBP (Fig. 14) was labeled with ⁹⁰Y, yielding [⁹⁰Y-DOTA-HBP] (54–80%). Comparative biodistribution studies in normal mice have shown that both [⁹⁰Y-DOTA-HBP] and [⁹⁰Y-citrate] accumulate rapidly in bone, with the former complex showing better promise as bone-targeting agent. Moreover, [⁹⁰Y-DOTA-HBP] led also to a decrease in the level of unnecessary radiation compared to [⁹⁰Y-citrate].¹³³

Copper (Cu)

Copper (Cu) presents several nontraditional positron-emitting radionuclides suitable for PET imaging, being ⁶⁴Cu the most adequate. ⁶⁴Cu is simultaneously a β⁺ and a β⁻ emitter that presents a moderately long half-life, therefore, it is suitable for PET imaging and for therapy.^{50,134} From the three accessible oxidation states (I–III), Cu(II) has been the most used to obtain ⁶⁴Cu complexes potentially useful as radiopharmaceuticals. This reflects the fact that Cu(III) is relatively rare and difficult to stabilize in aqueous solution, while Cu(II) complexes display an increased kinetic inertness compared to Cu(I) complexes. The ⁶⁴Cu(II) complexes must resist transchelation towards proteins involved in the transport and storage of copper, and must not undergo reduction to Cu(I) to avoid release

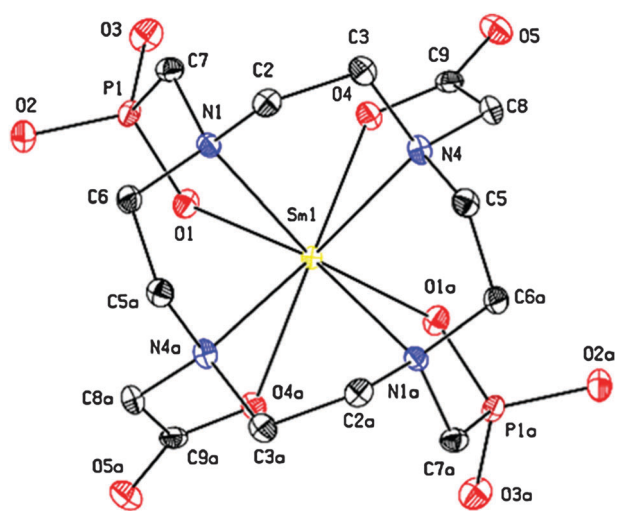


Fig. 18 Molecular structure of K₂[Sm(HL)]·6.5H₂O. Hydrogen atoms are omitted for clarity.¹²⁹ Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

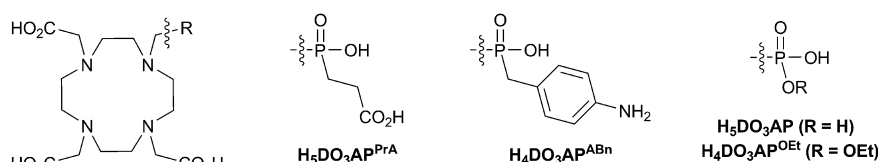


Fig. 19 DOTA-like ligands containing methylphosphinate or methylphosphonate pendant arms.

of the radiometal *in vivo*. DOTA and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) chelators have been widely used for labeling biomolecules with ^{64}Cu , even not being the ideal chelators, as documented by the *in vivo* instability of their Cu(II) complexes. Biodistribution studies in rats have shown that both [^{64}Cu -DOTA] and [^{64}Cu -TETA] undergo transchelation to liver and blood proteins, with this behavior being more pronounced in the case of [^{64}Cu -DOTA].¹³⁵ These macrocyclic complexes present a high thermodynamic stability with almost coincident K_{ML} values (Cu-DOTA: 22.3; Cu-TETA: 21.7),^{136–138} indicating that their kinetic inertness has a more crucial influence on their *in vivo* instability.

Aiming at the preparation of novel PET bone-imaging tracers, Sun *et al.* labeled DOTP, $\text{H}_5\text{DO}_3\text{P}$ and $\text{H}_4\text{DO}_2\text{P}$ (Fig. 15 and 17) with ^{64}Cu , giving the stable complexes [^{64}Cu -DOTP], [^{64}Cu -DO3P] and [^{64}Cu -DO2P], respectively, in high yield (>97%). The biodistribution studies in rats revealed high accumulation in bone. [^{64}Cu -DO2P] presented the most optimal clearance through blood and liver, compared to [^{64}Cu -DO3P] and [^{64}Cu -DOTP], which exhibited high liver uptake.¹³⁹

The ethylene side-bridged cyclam analog SBTE1A1P (Fig. 17) has been labeled with ^{64}Cu , yielding a complex that was stable in human serum. PET imaging in normal mice revealed a rapid whole-body hepatobiliary and renal clearance with minimal hepatic retention, but no bone uptake was observed.¹⁴⁰

BP-containing multimodal imaging probes

The idea of using multiple modalities in conjunction has gained in popularity, and intensive research effort has been undertaken aiming to find new multimodality imaging probes, profiting from the complementary abilities of different imaging modalities.¹⁴¹ The success of fused SPECT/CT and PET/CT instruments, which combine the high sensitivity associated to

nuclear techniques with the high anatomical resolution of CT, is the paradigmatic example of multimodality imaging that paved the way to the exploitation of other multimodality imaging systems and innovative multimodal probes. Fluorescence imaging displays high spatial and temporal resolution, but its sensitivity and quantification suffers from high light absorption and scattering in body tissues and from the autofluorescence of biomolecules in real biological samples.^{141,142} Aiming to overcome such drawbacks, most of the recently explored fluorescent probes work in the far-red or near-infrared (NIR) region. Despite recent achievements, fluorescent probes alone can be detected only at millimetre or centimetre depths in the tissues.

The bone-targeting fluorescent probe Pam-78 (Fig. 20), which combines a pamidronate moiety and the NIR-fluorescent molecule IRDye78 (excitation maximum: 771 nm; emission maximum: 796 nm) displayed high *in vivo* stability, high bone uptake and retention in nude mice.¹⁴³ Despite the low permeability of soft tissues to emitted light, this probe has been used to selectively detect and visualize HA microcalcifications associated with breast cancer, and for imaging calcified vasculature in a transgenic mouse model.^{144,145} Fragoni and co-workers succeeded to synthesize a new dual-modality SPECT/NIR-fluorescent trifunctional probe (Pam- $^{99\text{m}}\text{Tc}$ /Re-800),^{146,147} that contains a pamidronate moiety as HA-binding moiety, the organic fluorophore IRDye800CW (excitation maximum: 778 nm; emission maximum: 799 nm) and a chelating moiety for Tc(V)/Re(V) stabilization. A comparative study between Pam- $^{99\text{m}}\text{Tc}$ /Re-800 and $^{99\text{m}}\text{Tc}$ -MDP has shown that both compounds were identical in terms of total body clearance at 4 h p.i. (70–75% injected dose), with Pam- $^{99\text{m}}\text{Tc}$ -800 presenting the highest uptake in bone and tumor. Pam- $^{99\text{m}}\text{Tc}$ -800 allowed the visualization of breast cancer microcalcifications in a rat model. The authors claimed also that quantification by SPECT provides the “gold standard” by which NIR

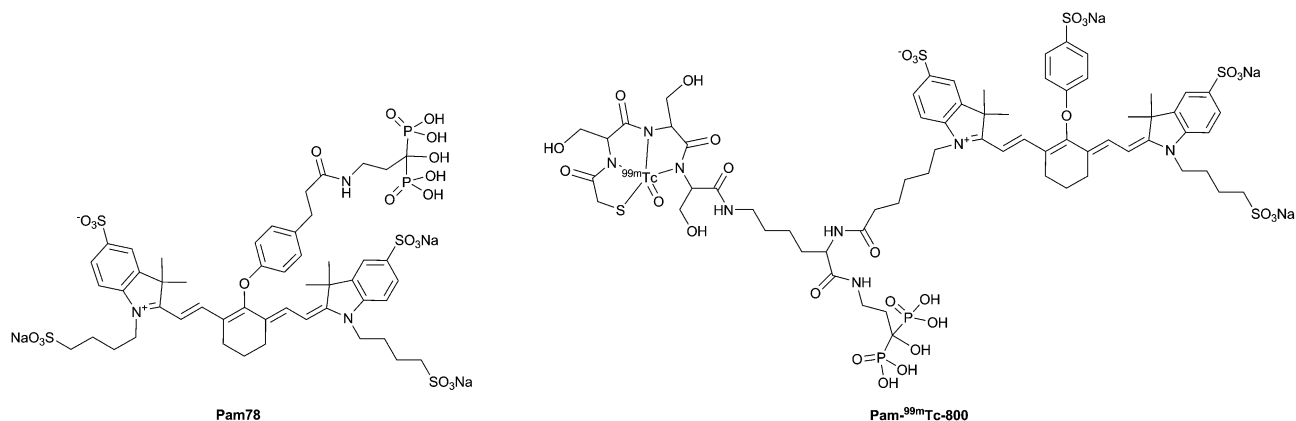


Fig. 20 Bone-targeting fluorescent and multimodal probes.

fluorescence tomography of breast cancer microcalcifications could be compared and optimized.^{146,147}

Radiohalogenated bisphosphonates

BP's have also been labeled with the radiohalogens iodine-131 (¹³¹I, β⁻-emitter, half life = 8.02 days) and/or astatine-211 (²¹¹At, α-emitter, half life = 7.2 h) and biologically assessed in order to explore their potential usefulness in the palliation of pain from osseous metastases. The use of ²¹¹At-based radiopharmaceuticals for targeted radionuclide therapy has been recently reviewed by Zalutsky and co-workers.^{148,149}

In the 1980's, the pioneering work of Eisenhut *et al.* has demonstrated the feasibility of using ¹³¹I-labeled bisphosphonates for bone-pain palliation.^{150,151} The precursor α-amino-(4-hydroxybenzylidene)-diphosphonate (BDP3) was labeled with ¹³¹I by electrophilic aromatic substitution, giving ¹³¹I-BDP3 in 95% radiochemical yield after filtration over AgCl to remove excess radioiodide (Fig. 21). The biodistribution of ¹³¹I-BDP3 in Sprague-Dawley rats showed high bone uptake, with fast blood clearance and renal excretion. These studies demonstrated also the metabolic stability of ¹³¹I-BDP3 regarding radioiodide liberation. A study in 18 patients demonstrated the clinical efficacy of ¹³¹I-BDP3 for the palliative therapy of pain syndromes associated with disseminated bone metastases.^{150,151}

The radiohalogenated amidobisphosphonates I/A-BPB and I/A-PPB (Fig. 22) were obtained in a two-step procedure with high yield (60–97%), by halodestannylation of the active esters *N*-succinimidyl 3-(trimethylstannyl) benzoate and *N*-succinimidyl 5-(trimethylstannyl)-3-pyridinecarboxylate with ²¹¹At or Na^{125/131}I, followed by conjugation to pamidronate (3-amino-1-hydroxypropylidene-1,1-bisphosphonate = APB).

All radiohalogenated compounds presented high *in vitro* stability in mouse, fetal calf, and human serum.^{152–154} Biodistribution studies in Balb/c mice demonstrated that all ¹³¹I- and ²¹¹At-labeled analogs presented relevant bone accumulation with rapid clearance from normal tissues. Bone uptake and bone-to-tissue ratios were better for I/A-BPB compared to I/A-PPB. All compounds are highly resistant to *in vivo* dehalogenation as demonstrated by low uptake in the thyroid gland and stomach. Further biological studies indicated that co- or pre-injection of a non-radioactive bisphosphonate such as pamidronate leads to a significant reduction of uptake in nontarget normal tissues, while bone accumulation and retention is unaffected. Taken together, the study indicated that pretreatment with “cold” BP should be advantageous if radiolabeled BP's are used clinically for bone-pain palliation since it increases the bone to tissue ratios.^{152–154}

Årstad *et al.* labeled a set of arylalkylidenebisphosphonates with ¹²⁵I or ¹³¹I (I–IV) in high yield, under mild conditions,

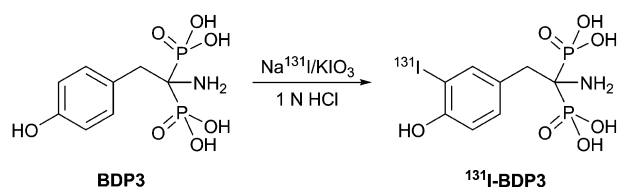


Fig. 21 Radioiodination of α-amino-(4-hydroxybenzylidene)-diphosphonate.

by means of iododesilylation of the respective precursor (Fig. 23).

The biological behavior of the resulting radioiodide compounds was evaluated in adequate animal models.¹⁵⁵ Compounds **IIIa** and **IIIb** presented the highest bone uptake compared to the other compounds in normal Balb/C mice and in a rat model. The therapeutic effect of **IIIa** in two tumor models compared favorably to that observed for the established treatment modalities, demonstrating that radioiodinated bisphosphonates displayed high potential for diagnosis and therapy of bone malignancies.¹⁵⁵

Concluding remarks and perspectives

The concept of BPs as radionuclide carriers has been applied towards the development of a “new generation” of bone-seeking radiopharmaceuticals for skeletal imaging and/or bone-pain palliation. In fact, most of the current research efforts have been focused on the synthesis and biological evaluation of novel radiometal-based compounds with improved stability, fast clearance rate from non-target organs and high bone uptake. M(v)-oxocomplexes (M = ¹⁸⁶Re, ^{99m}Tc) stabilized by N₂S₂- or N₃S-type chelators (*e.g.* MAG₃) with BP pendant arms have been explored as bone-imaging tracers or bone-pain palliation agents. The matched pair ^{99m}Tc-MAG₃-HBP/¹⁸⁶Re-MAG₃-HBP accumulate significantly in bone, and the ¹⁸⁶Re-complex inhibited tumor growth in a rat model, holding promise for further clinical evaluation. The bifunctional approach has also been successfully applied to the organometallic core *fac*-[^{99m}Tc(CO)₃]⁺, and new radiocompounds, with improved biological properties compared to ^{99m}Tc-MDP and ^{99m}Tc-HDP emerged as potential radiopharmaceuticals for bone imaging. Additionally, recent studies demonstrated also that ¹⁸⁸Re(CO)₃-labeled BPs, following also the bifunctional approach, hold great promise as therapeutic agents, which in some cases are comparable to ¹⁸⁸Re-HEDP.

Among the post-transition metal radioisotopes, ⁶⁸Ga-complexes stabilized by DOTA-like chelators with pendant bisphosphonate arms emerged as a quite promising tracer for PET imaging of bone. With the goal of finding novel lanthanide-based compounds with better bone-palliation properties than the approved radiopharmaceutical ¹⁵³Sm-EDTMP, several ¹⁵³Sm- and ¹⁶⁶Ho-complexes stabilized by 12-, 13- and 14-membered macrocyclic chelators with pendant phosphonate arms have been explored. Some of them revealed quite promising at the preclinical level, being superior to ¹⁵³Sm-EDTMP in most cases. ⁶⁴Cu-complexes stabilized by DOTA-like chelators with phosphonate arms presented relevant biological properties at the preclinical level, which envisages their potential utility as PET bone-imaging tracers.

The combination of a bone seeking moiety such as a BP with a γ-(SPECT) or β⁺-emitter (PET) and a fluorescent probe in the same molecule gives multimodal imaging probes, which display the most interesting properties of each technique. This recently introduced strategy, which is still in its infancy, paves the way to the design of more efficient and innovative tools for bone imaging, as well as for detection of microcalcifications associated to cancer. Interestingly, the introduction of this type of probes may help in the optimization of NIR fluorescence

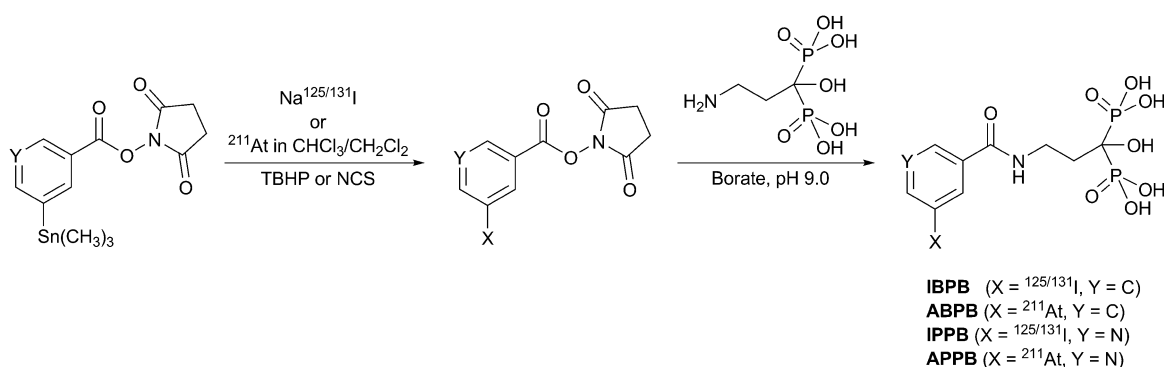


Fig. 22 Synthesis of I/A-BPB and I/A-PPB. APB = 3-amino-1-hydroxypropylidene-1,1-bisphosphonate; TBHP = *tert*-butylhydroperoxide; NCS = *N*-chlorosuccinimide.

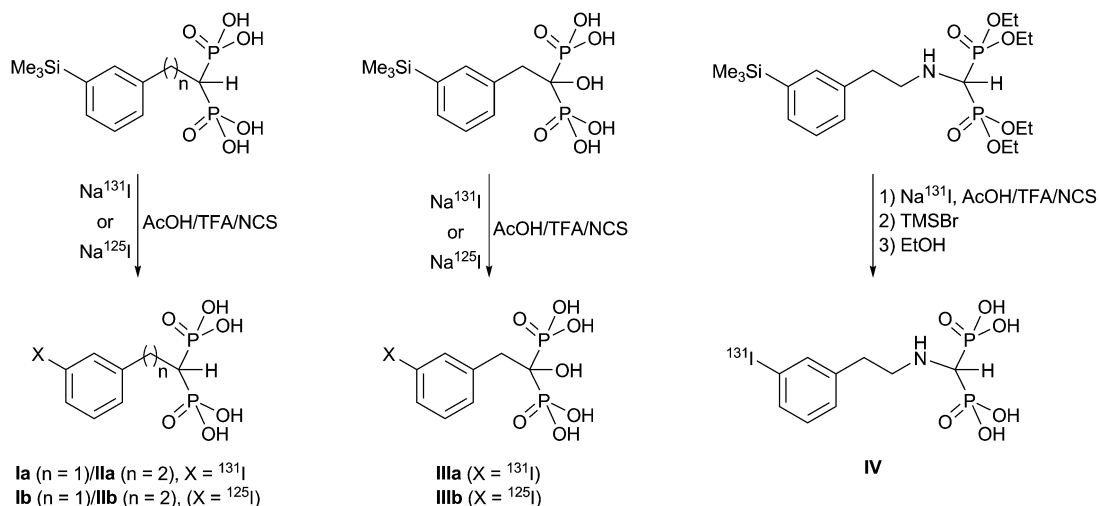


Fig. 23 Synthesis of the radioiodinated compounds I–IV.

tomography since quantification by SPECT or PET may provide the “gold standard” for comparison.

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