Bisphosphonates as radionuclide carriers for imaging or systemic therapy

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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 99mTc-based radiopharmaceuticals for bone imaging. In addition to the successful delivery of 99mTc (γ-emitter) to bone, BP’s have also been used to deliver β−-particle emitting radiometals (e.g., 153Sm, 186/188Re) 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.

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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 Associate 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.5–10 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 hydroxypatite (HA), namely to the biological well-crystallized apatite (Ca_{10} (PO_4 )_6 (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 R^1 = 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 been observed for pyrophosphates.5,7,11 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.5,12,13

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.5,7,8,12 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 ^{18}F-fluoride, which had been introduced by Blau et al. as a promising bone scanning agent for Positron Emission Tomography (PET).17

Fig. 1 Pyrophosphoric acid (PPi) and generic structure of bisphosphonates (BP’s).
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.\textsuperscript{18,19}

An important benefit of bone imaging with radiolabelled BP's is its sensitivity. In addition to the successful delivery of $\gamma$-emitter radionuclides, such as $^{99m}$Tc, for bone imaging, BP's have also been used to deliver $\beta^-$-particle emitting radionuclides for bone-pain palliation (Table 1).\textsuperscript{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.\textsuperscript{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.\textsuperscript{3,20,28} When there are extensive multifocal osseous metastases the treatment of bone pain involves the use of hormones, chemotherapy or systemic therapy with $\beta^-$-emitting radionuclides such as $^{32}$P, $^{89}$Sr, $^{153}$Sm, $^{186,188}$Re. Phosphorus-32 ($^{32}$P, $^{32}$P-Phosphate) and strontium-89 ($^{89}$Sr, $^{89}$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.\textsuperscript{20,22} Owing to several limitations, including high myelotoxicity, the clinical use of $^{32}$P-Phosphate has decreased considerably since the 1980’s in favor of $^{89}$SrCl$_2$, $^{153}$Sm-EDTMP (Quadramet\textsuperscript{\textregistered}), $^{186,188}$Re-HEDP and, more recently, $^{188}$Re-HEDP.\textsuperscript{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 $\alpha$-emitter $^{223}$RaCl$_2$ (Alpharadin\textsuperscript{\textregistered}), with natural

### Table 1

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Radiopharmaceutical</th>
<th>Half-life/d</th>
<th>$\beta$-Energy$_{max}$/MeV</th>
<th>$\gamma$-Energy/MeV</th>
<th>$\alpha$-Energy$_{Av}$/MeV</th>
<th>Maximum range/mm</th>
<th>Pain relief</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{32}$P</td>
<td>$^{32}$P-phosphate</td>
<td>14.3</td>
<td>1.71</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>57–92%</td>
</tr>
<tr>
<td>$^{89}$Sr</td>
<td>$^{89}$SrCl$_2$</td>
<td>50.5</td>
<td>1.46</td>
<td>0.91 (0.01%)</td>
<td>—</td>
<td>6.7</td>
<td>57–84%</td>
</tr>
<tr>
<td>$^{153}$Sm</td>
<td>$^{153}$Sm-EDTMP</td>
<td>1.9</td>
<td>0.81</td>
<td>0.103 (28%)</td>
<td>—</td>
<td>3.4</td>
<td>62–84%</td>
</tr>
<tr>
<td>$^{186}$Re</td>
<td>$^{186}$Re-HEDP</td>
<td>3.7</td>
<td>1.07</td>
<td>0.137 (9%)</td>
<td>—</td>
<td>4.7</td>
<td>50–92%</td>
</tr>
<tr>
<td>$^{188}$Re</td>
<td>$^{188}$Re-HEDP</td>
<td>0.7</td>
<td>2.12</td>
<td>—</td>
<td>0.208 (11%)</td>
<td>3</td>
<td>60–92%</td>
</tr>
<tr>
<td>$^{177}$Lu</td>
<td>$^{177}$Lu-EDTMP</td>
<td>6.7</td>
<td>0.497</td>
<td>0.208 (11%)</td>
<td>—</td>
<td>1.8</td>
<td>Preclinical evaluation</td>
</tr>
<tr>
<td>$^{166}$Ho</td>
<td>$^{166}$Ho-DOTP</td>
<td>1.1</td>
<td>1.86</td>
<td>0.081 (6.2%)</td>
<td>—</td>
<td>8.6</td>
<td>Preclinical evaluation</td>
</tr>
<tr>
<td>$^{223}$Ra</td>
<td>$^{223}$RaCl$_2$</td>
<td>11.4</td>
<td>&lt;4%</td>
<td>0.154 (5.6%)</td>
<td>5.64 (94%)</td>
<td>0.045</td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>$^{223}$RaDOTP</td>
<td>1.1</td>
<td>1.86</td>
<td>0.154 (5.6%)</td>
<td>5.64 (94%)</td>
<td>0.045</td>
<td>Clinical trial</td>
</tr>
</tbody>
</table>

**Fig. 2** Bisphosphonate structures.
Table 2 Drugs conjugated to bisphosphonates and corresponding therapeutic indication

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E&lt;sub&gt;2&lt;/sub&gt;³⁴</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>Estradiol¹⁵⁻³⁸</td>
<td></td>
</tr>
<tr>
<td>Synthetic estrogenic agents¹³⁹</td>
<td></td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory drugs⁴⁰</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones¹⁻⁴³</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>Cisplatin¹⁴⁻⁴⁶</td>
<td>Chronic infection</td>
</tr>
<tr>
<td>Methotrexate⁴⁷</td>
<td>Bone cancer</td>
</tr>
</tbody>
</table>

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. Kubiček and I. Lukes have recently reviewed the use of BP-containing probes for Magnetic Resonance Imaging.

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.

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 (⁹⁹mTc) 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 widespread availability. This radionuclide 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). ¹⁸⁸Re is a reactor-produced radionuclide, and ⁹⁹mTc and ¹⁸⁸Re are obtained from commercial ⁹⁹Mo/⁹⁹mTc and ¹⁸⁸W/¹⁸⁸Re generators, respectively. These radiometals are obtained as [M⁷⁺H₂O]⁻ (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), ⁹⁹mTc(V) has been the most studied in radiopharmaceutical chemistry, and the cores [⁹⁹mTcO]⁺, trans-[⁹⁹mTcO₂]⁺, [⁹⁹mTcN]⁺ and [⁹⁹mTc-HYNIC] (HYNIC = 6-hydrazino nicotinic acid) the most exploited (Fig. 3).⁴⁵,⁴⁷,⁴⁹,⁵⁰,⁶² The [⁹⁹mTcO]⁺ core forms square pyramidal Tc(ν)-oxo complexes with tetradeptate chelators, such as N₂S₂ diamide-dithiols (DADS), N₄S triamide-dithiols, N₅S₂ monoamide monoaminedithiols (MAMA) and N₅S₂ diaminedithiols (DADT). The trans-[⁹⁹mTcO₂]⁺ unit has been mainly used in combination with acyclic tetraamine ligands, which form well-defined octahedral Tc(ν) dioxo complexes. In the case of the [⁹⁹mTcN]⁺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 [⁹⁹mTc-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 phosphate, or by using phosphate- and nicotinyl-containing HYNIC derivatives.

The introduction of the organometallic precursor fac-[⁹⁹mTc(CO)(H₂O)]⁺ 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
chemical robustness of the \( \text{fac-}[\text{Tc}(\text{CO})_3]^{+} \) 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, \(^{99m}\text{Tc}-\text{MDP}\) and \(^{99m}\text{Tc}-\text{HMDP}\) 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 \(^{99m}\text{Tc}\)-labeled BP’s, \(^{186}\text{Re}\)-HEDP has been approved in some European countries as a pain palliation radiopharmaceutical for bone metastases\(^{28}\) and \(^{188}\text{Re}\)-HEDP is currently in clinical trials.76–80

In spite of their well-established clinical use as diagnostic or therapeutic agents, \(^{99m}\text{Tc}\)- and \(^{186/188}\text{Re}\)-labeled BP’s present a set of clinical and chemical limitations. The \(^{99m}\text{Tc}\)-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 \(^{99}\text{Tc}\)-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 \([\text{Tc(OH)(MDP)}]\). 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 \([\text{MO}_4]^{-}\) (\( \text{M} = \text{\text{Tc}}, \text{Re} \)), 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 \(^{186/188}\text{Re}\) 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
bone imaging (M = 99mTc) and therapy ($^{186/188}$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 99mTc-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 99mTc-oxocomplexes (Fig. 6) stabilized by 1,1-ethylene dicysteine derivatives (EC) containing pendant aminomethylenediphosphonate (AMDP) moieties ($^{99m}$Tc-EC-1; $^{99m}$Tc-EC-2; $^{99m}$Tc-EC-3).

The complexes were prepared by direct labeling of the EC derivatives with Na$^{99m}$TcO$_4^-$ in the presence of SnCl$_2$·2H$_2$O, under basic conditions at room temperature. $^{99m}$Tc-EC-1 and $^{99m}$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 $^{99m}$Tc-EC-1 that has improved bone uptake in rats ($^{99m}$Tc-EC-1: 29.4% ID g$^{-1}$ organ, $^{99m}$Tc-MDP: 15.4% ID g$^{-1}$ organ, at 2 h postinjection (p.i.)), and blood clearance and urinary excretion rates comparable to $^{99m}$Tc-MDP.

The $^{186}$Re-oxocomplexes $^{186}$Re-MAMA-BP, $^{186}$Re-MAMAHBP and $^{186}$Re-MAG$_3$-HBP were obtained by direct reaction of the bisphosphonate-containing chelator with [$^{186}$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 $^{186}$Re-MAMA-BP, $^{186}$Re-MAG$_3$-HBP, and $^{186}$Re-MAMAHBP all presented higher femur to blood ratios than $^{186}$Re-HEDP.

The femur accumulation in mice for $^{186}$Re-MAMAHBP was higher than that found for $^{186}$Re-MAMA-BP, confirming that the presence of the geminal hydroxyl group in the BP confers better bone-targeting properties to the resulting complexes. $^{186}$Re-MAG$_3$-HBP inhibited tumor growth in a rat model and attenuated allodynia-induced bone cancer without having critical myelosuppressive side effects. The same research group prepared the analogs $^{99m}$Tc-MAG$_3$-HBP and $^{99m}$Tc-HYNIC-HBP by direct labeling of the BP-containing chelators with [$^{99m}$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. Despite showing a considerable high protein binding, $^{99m}$Tc-HYNIC-HBP still presented a significantly higher bone to blood ratio than $^{99m}$Tc-MDP. The isostructural $^{99m}$Tc-MAG$_3$-HBP and $^{186}$Re-MAG$_3$-HBP were identified/characterized by comparing their HPLC chromatograms with that of the nonradioactive analog Re-MAG$_3$-HBP, which was

Fig. 5 Perspective views of a portion of the [Tc(OH)(MDP)]$^{2-}$ 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.

Fig. 6 $^{99m}$Tc(V)-oxocomplexes stabilized by 1,1-ethylene dicysteine-type chelators (EC) bearing pendant AMDP arms.
synthesized by conjugation of the previously synthesized precursor Re-MAG₃ to the BP derivative. The nonradioactive complexes Re-MAMA-BP/HBP were not synthesized but, based on transchelation experiments with MAMA and HEDP, the authors claimed that Re was selectively bound to MAMA with no unspecific interactions between the bisphosphonate moiety and the metal centre.

The bifunctional chelators that have been explored for the design of \( [M(CO)₃]⁺ \) (\( M = ^{99m}Tc, ^{186/188}Re \)) bone-seeking radiotracers were mono- or bis-phosphonate-containing N₃-tridentate chelators with one ([M(CO)₃(pzNN-C₂MPOH)], [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 \( [fac-[M(CO)₃]⁺ \) (\( M = ^{99m}Tc, ^{186}/^{188}Re \)) bone-seeking radiotracers were mono- or bis-phosphonate-containing N₃-tridentate chelators with one ([M(CO)₃(pzNN-C₂MPOH)], and two aromatic N-heterocycles ([M(CO)₃(dpa-ALN)]) or two aromatic N-heterocycles ([M(CO)₃(pzNN-PAM)])) with the corresponding chelators, and were evaluated in appropriate animal models. All radiocomplexes showed the same retention times (\( \gamma \)-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 monophosphonates 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.

Both complexes presented an identical biological pattern in rats: high bone uptake (\( ^{99m}Tc(CO)₃(pzNN-PAM) \):...
18.3 ± 0.6% ID g⁻¹, [⁹⁹mTc(CO)₃(pzNN-PAM)]; 17.3 ± 6.1% ID g⁻¹, ⁹⁹mTc-MDP: 17.1 ± 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 ⁹⁹mTc-MDP. \textit{In vivo} imaging in rats allowed the clear visualization of bone tissue (Fig. 9). Unlike ⁹⁹mTc-MDP, both ⁹⁹mTc(i)-complexes presented negligible serum protein binding.

The complex [⁹⁹mTc(CO)₃(dpa-ALN)] developed by de Rosales \textit{et al.} presented also high bone uptake, comparable to that observed for the gold standard ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP. \textit{Aim}ed at therapeutic applications, the isostructural analog complex [¹⁸⁸Re(CO)₃(dpa-ALN)] presented also high bone uptake, comparable to that observed for ⁹⁹mTc-MDP.
1,4,7-triazacyclononane-\(N, N, N^\prime\)-triacetic acid (NOTA) for Ga\(^{3+}\) (Fig. 12).

Several NOTA derivatives have been studied with Ga(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 [Ga(H\(_3\)PrP9)], which presented high thermodynamic stability and high kinetic inertness. Besides the characterization in solution by potentiometry, [Ga(H\(_2\)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-\(N,N,N^\prime\)-tris(methylene phosphonic) acid (NOTP) and 1,4,7-triazacyclononane-\(N,N,N^\prime\)-tris(methylene phosphonatemonoethylester) (NOTPME) (Fig. 12), were labeled with \(^{67}\)Ga(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 \(^{68}\)Ga(III) (radiochemical yield and purity >95\%), and the bone-targeting properties of the resulting radioactive complexes \(^{68}\)Ga-NOTA-HBP and \(^{68}\)Ga-DOTA-HBP evaluated \textit{in vitro} (HA binding assays) and in rats.\(^{104}\) Both radiocomplexes presented HA binding affinities similar to that of \(^{99m}\)Tc-MDP. \(^{68}\)Ga-NOTA-HBP emerged as the

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig11}
\caption{Synthesis of \([^{186}\text{Re}(\text{CO})_3(\text{C}p\text{TR-Gly})]\).\(^{100}\)}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig12}
\caption{Acyclic and cyclic polyaminocarboxylic and polyaminophosphonate chelators.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig13}
\caption{Molecular structure of the [Ga(H\(_2\)PrP9)] complex (hydrogen atoms are omitted for clarity).\(^{101}\) Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.}
\end{figure}
most promising, allowing the visualization of the bone structure by micro PET imaging, and displaying the highest bone uptake and bone to blood ratio. Recent studies have shown that \([\text{Ga-NOTA-HBP}]\) could detect mouse model bone metastasis by micro-PET imaging at 1 h p.i. 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.

Labeling of the recently described DOTA-like chelator DOTA-Bn-SCN-HBP (Fig. 14), bearing also a pamidronate pendant arm, with \(\text{Ga}\) gave the radiocomplex \([\text{Ga-DOTA-Bn-SCN-HBP}]\) in high radiochemical yield (95%). 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 \(\text{Ga}\)-based PET bone-imaging tracers.

The acyclic ligand EDTMP and the 12-membered tetraaza-macrocycles DOTP, BPAMD, BPAPD and BPPED (Fig. 15) have been labeled with \(\text{Ga}\), giving radiochemical yields in the range of 50% to 95%.

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

PET imaging using \([\text{Ga-BPAMD}]\) as radiotracer showed significant bone uptake and high in vivo stability in a rat model. These results have been confirmed later on in the clinical setting. Indeed, PET/CT imaging with \([\text{Ga-BPAMD}]\) 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).

The bisphosphonate-containing chelator DOTA-BP (Fig. 15) was labeled with \(\text{In}\), giving the complex \([\text{In-DOTA-BP}]\). 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.

Yttrium (Y) and lanthanides (Ln)

Yttrium (Y) and lanthanides (Ln) are trivalent metals that offer different \(\beta\)-emitting radioisotopes relevant for therapeutic applications. Among these radioisotopes, \(\text{Y}, \text{Sm}, \text{Ho}\) and \(\text{Lu}\) have been the most explored for bone pain-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...
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. The use of linear polyamino-polypophosphonate chelators in the preparation of osteotropic radiopharmaceuticals for the treatment of bone metastases has been reviewed elsewhere. Therefore, herein, we will focus on the metal complexes stabilized by cyclic polyaminopolypophosphonates (Fig. 12, 14, 15 and 17).

The radiolanthanide complexes $[^{153}$Sm$]^{166}$Ho$]^{177}$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 myeloblastic treatment of multiple myelomas or for intravascular radiation therapy. The DOTP analog DOTP$^\text{OEt}$ (Fig. 15) was also labeled with $^{153}$Sm/$^{166}$Ho, yielding (>98%) the stable complexes $[^{153}$Sm$]^{166}$Ho-DOTP$^{\text{OEt}}$ and $[^{166}$Ho-DOTP$^{\text{OEt}}$, which displayed low binding to HA and negligible bone affinity in a mice model. Other DOTP analogs, namely DOTP$^\text{H}$, DOTP$^\text{Et}$ or DOTP$^\text{Ph}$ (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.

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 $[^{153}$Sm/$^{166}$Ho-TRITP] and $[^{153}$Sm/$^{166}$Ho-TETP] complexes have been obtained in quantitative yield, except in the case of TETP. The stable $^{153}$Sm/$^{166}$Ho-TRITP complexes bind to HA, with $[^{166}$Ho-TRITP] presenting the highest degree of adsorption. Biodistribution studies in mice have shown that $[^{153}$Sm/$^{166}$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.

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...
tetraazamacrocycles containing a pyridine and two (MeP2-py14) or three (P3py14) methylphosphonate pendant arms (Fig. 17). The chelators have been quantitatively labeled with $^{153}$Sm and $^{166}$Ho 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. 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 $^{153}$Sm/$^{166}$Ho-DOTP and $^{153}$Sm/$^{166}$Ho-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 tetraaza-macrocycle trans-$\text{H}_2\text{DO2A2P}$ and $\text{H}_2\text{DOA3P}$ (Fig. 17) have been synthesized and, after optimization of the radiolabeling conditions, yielded quantitatively the stable complexes $^{153}$Sm/$^{166}$Ho-DO2A2P and $^{153}$Sm/$^{166}$Ho-DOA3P. 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, $^{166}$Ho-TRITP emerged again as the best bone-targeting complex, comparable to $^{166}$Ho-DOTP.

Aiming to better understand the biological behavior of those radiolanthanide complexes, potentiometric solution studies have been performed with the corresponding non-radioactive lanthanide complexes. 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 K$_2$[Sm(HL)]·6.5H$_2$O is displayed in Fig. 18.

The mixed 12-membered DOTA-like macrocyclic ligands containing methylphosphonate (H$_2$DO3AP$^{\text{P}(\text{Me})_2}$ and H4DO3A-P$^{\text{ABn}}$) or methylphosphonate (H$_2$DO3AP) pendant arms have been prepared and labeled with $^{153}$Sm and $^{166}$Ho (Fig. 19). The biodistribution studies of the stable $^{153}$Sm- and $^{166}$Ho-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.

A study performed by T. Vitha et al. has demonstrated that the HA affinity of the complex $[^{160}\text{Tb-BPPE}]$ 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. 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 $[^{160}\text{Tb-BPPED}]$ and $[^{160}\text{Tb-DOTP}]$ complexes, $[^{160}\text{Tb-BPPE}]$ has shown a much higher affinity toward the HA surface than $[^{160}\text{Tb-DOTP}]$.

Besides $^{153}$Sm and $^{166}$Ho complexes, also compounds containing other $\beta^-$-emitting radionuclides such as $^{177}$Lu and $^{90}$Y have been explored for bone-targeted systemic radionuclide therapy. The chelators BPAMD and BPAPD have been labeled with $^{177}$Lu, and the resulting complexes $[^{177}\text{Lu-BPAMD}]$ and $[^{177}\text{Lu-BPAPD}]$ were evaluated in vivo in a rat model. 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 $[^{177}\text{Lu-BPAMD}]$ than for $[^{177}\text{Lu-BPAPD}]$.

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

**Copper (Cu)**

Copper (Cu) presents several nontraditional positron-emitting radionuclides suitable for PET imaging, being $^{64}$Cu the most adequate. $^{64}$Cu is simultaneously a $\beta^-$ and a $\beta^+$ emitter that presents a moderately long half-life, therefore, it is suitable for PET imaging and for therapy. From the three accessible oxidation states (I–III), Cu(II) has been the most used to obtain $^{64}$Cu 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 $^{64}$Cu(i) complexes must resist transchelation towards proteins involved in the transport and storage of copper, and must not undergo reduction to Cu(0) to avoid release.
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 $[^{64}\text{Cu-DOTA}]$ and $[^{64}\text{Cu-TETA}]$ undergo transchelation to liver and blood proteins, with this behavior being more pronounced in the case of $[^{64}\text{Cu-DOTA}]$.\textsuperscript{135} These macrocyclic complexes present a high thermodynamic stability with almost coincident $K_{ML}$ values (Cu-DOTA: 22.3; Cu-TETA: 21.7),\textsuperscript{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, $H_6\text{DO}_3\text{P}$ and $H_4\text{DO}_2\text{P}$ (Fig. 15 and 17) with $^{64}$Cu, giving the stable complexes $[^{64}\text{Cu-DOTP}]$, $[^{64}\text{Cu-DO}_3\text{P}]$ and $[^{64}\text{Cu-DO}_2\text{P}]$, respectively, in high yield (> 97%). The biodistribution studies in rats revealed high accumulation in bone. $[^{64}\text{Cu-DO}_2\text{P}]$ presented the most optimal clearance through blood and liver, compared to $[^{64}\text{Cu-DO}_3\text{P}]$ and $[^{64}\text{Cu-DOTP}]$, which exhibited high liver uptake\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 auto-fluorescence of biomolecules in real biological samples.\textsuperscript{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.\textsuperscript{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.\textsuperscript{144,145} Fragioni and co-workers succeeded to synthesize a new dual-modality SPECT/NIR-fluorescent trifunctional probe (Pam-$^{99m}\text{Tc}$/$\text{Re}-800$),\textsuperscript{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-$^{99m}\text{Tc}$/$\text{Re}-800$ and $^{99m}\text{Tc}$-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-$^{99m}\text{Tc}$-800 presenting the highest uptake in bone and tumor. Pam-$^{99m}\text{Tc}$-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
fluorescence tomography of breast cancer microcalcifications could be compared and optimized.  

**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.  

In the 1980’s, the pioneering work of Eisenhut et al. has demonstrated the feasibility of using 131I-labeled bisphosphonates for bone-pain palliation. The precursor γ-aminooxybenzylidene)-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.  

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 halodebulkylation of the active esters N-succinimidyl 3-(trimethylstannyl) benzotate and N-succinimidyl 5-(trimethylstannyl)-3-pyridinecarboxylate with 211At or Na211I, followed by conjugation to pamidronate (3-amino-1-hydroxypropyldiene-1,1-bisphosphonate = APB). All radiohalogenated compounds presented high in vitro stability in mouse, fetal calf, and human serum. 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.  

Arstad et al. labeled a set of arylalkyldienediphosphonates with 125I or 131I (I-IV) in high yield, under mild conditions, by means of iododesilylation of the respective precursor (Fig. 23).  

The biological behavior of the resulting radioiodide compounds was evaluated in adequate animal models. 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.  

**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(ν)-oxocomplexes (M = 186Re, 99mTc) 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 99mTc-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 [(99mTc(CO)3]+, and new radiocompounds, with improved biological properties compared to 99mTc-MDTP and 99mTc-HDP emerged as potential radiopharmaceuticals for bone imaging. Additionally, recent studies demonstrated also that 186Re(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, Ga-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 phosphate 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 phosphate 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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