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Bifunctional bisphosphonate complexes for the diagnosis and therapy of bone metastases†

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Abstract

Easily synthesised and structurally well-defined novel imaging/therapeutic radiopharmaceutical agents for bone metastases are described.

1,1-Bisphosphonates (BPs) are a family of compounds extensively used in the management of disorders of bone metabolism.[1] They accumulate in areas of high bone metabolism, such as bone metastases, and consequently have been receiving increasing attention as molecular imaging probes and pain palliation treatments.[2] Imaging bone metastases with BPs using single photon emission computed tomography (SPECT) or planar scintigraphy is one of the most often-performed clinical imaging procedures. β-Emitting analogues capable of producing a therapeutic effect have also been developed.[3] In particular, the rhenium compounds $^{186/188}$Re-hydroxyethylidene-1,1-diphosphonate ($^{186/188}$Re–HEDP) have shown promise as palliative agents for bone metastases in recent clinical trials.[4] The radiochemicals consist of a complex of a BP (*e.g.* methylene diphosphonate, MDP) with γ-(^{99m}Tc) or β-($^{186/186}$Re) emitters.

Despite the proven clinical success of ^{99m}Tc/$^{188/186}$Re–BPs, these radiopharmaceuticals are far from optimal from a chemical and pharmaceutical point of view. For example, despite decades of clinical use, their structures and compositions remain unknown. A critical review of the literature reveals that the ^{99m}Tc–MDP preparation used in the clinic is composed of a mixture of anionic polymers of different properties.[5] A particular concern is that the *in vivo* stability of $^{186/188}$Re–BPs does not adequately match their physical half-life and a large fraction of the injected complex degrades to perrhenate *in vivo* within 24 h, leading to reduced bone uptake and higher soft-tissue doses. Furthermore, $^{186/188}$Re–BPs are not chemically analogous to their Tc-counterparts and do not target bone metastases unless additional "carrier" nonradioactive rhenium is added.[7] Consequently there is a need for

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rational design of $^{186/188}$Re-labelled BP derivatives to improve specificity and reduce soft-tissue and bone marrow doses during radionuclide therapy.

In current Tc/Re–BP complexes the BP acts as both chelator and targeting group. Each role, however, may compromise the other as BPs are excellent bone-seeking agents but poor Re chelators. To improve upon current $^{186/188}$Re–BPs, a more logical approach is the use of targeted bifunctional ligands in which the targeting (BP) and metal chelating groups are separated within the molecule so that they can each function independently and effectively. A few recent reports describe such a BP–chelator bifunctional approach.[8,9] These BP-conjugates, however, require complicated multi-step synthetic strategies, show high plasma protein binding and often form enantiomeric mixtures. Herein we report a new chelator–BP conjugate **3** that is synthesised using mild aqueous conditions in one step from commercially available compounds. In addition, **3** efficiently forms single well-defined isostructural Tc/Re complexes with no detectable protein binding that efficiently accumulate in bone tissue *in vivo*.

Our approach requires the BP part of the molecule to be separated from the chelator by a spacer, to avoid any BP–metal interactions. In addition, the radionuclide must selectively coordinate with the chelating group, not the BP, and must remain inert under *in vivo* conditions. The organometallic precursor *fac*-$[M(CO)_3(H_2O)_3]^+$ (M = Tc, Re) (Scheme 1B, **4**), pioneered by Jaouen *et al.* and Alberto *et al.,* facilitates the latter requirement.[10] When the three labile water molecules are displaced by an appropriate ligand system, the d^6 low-spin octahedral Tc(I)/Re(I) centre formed is protected from oxidation and ligand substitution. Furthermore, imaging probes containing a coordinatively saturated *fac*-$[M(CO)_3]^+$ core have shown high *in vivo* inertness and negligible binding to human serum proteins.[11] Particularly favourable ligands for $[M(CO)_3]^+$ are N_3-tridentate chelators containing two sp^2 N-heterocycles, such as dipicolylamine (DPA).[12] As the targeting vector we selected alendronate (**2**) (Scheme 1A), a clinically-approved BP that binds avidly to hydroxyapatite (HA), the main component of bone mineral.[13] Furthermore, **2** provides an amino group conveniently separated from the BP group by a spacer, allowing facile one-step conjugation of two picolyl units to form a DPA group (Scheme 1A).

Two major obstacles were encountered during the development of DPA-alendronate (**3**, Scheme 1A). First is the insolubility of alendronate in organic solvents, which complicates conjugation reactions. Second, the high basicity of its amino group (pK_a 12.7) inhibits nucleophilic attack by alendronate using standard organic bases.[14] Important factors to overcome these barriers are pH and the concentration of base. Using strong organic bases such as triethylamine was unsuccessful. High concentrations of inorganic bases and high temperatures, however, led to hydrolysis of 2-picolyl chloride (**1**, Scheme 1A) and rearrangements of the BP.[15] We found that using water as solvent and maintaining the pH of the solution at 12 with the minimum amount of NaOH was sufficient to drive the reaction to completion after 36 hours at room temperature, without detectable hydrolysis or BP rearrangements. The yield of **3** was >90% by RP-HPLC.

The complexation of **3** with *fac*-$[Re(CO)_3]^+$, and its solution properties, were examined using HPLC and NMR, MS and IR spectroscopies (Fig. 1 and ESI†). The aim was to

determine whether the organometallic core selectively coordinated the chelating DPA group. NMR/HPLC titration studies revealed that *fac*-$[Re(CO)_3(H_2O)_3]^+$ stoichiometrically binds **3** in the designed facial conformation when less than 1.5 equivalents of *fac*-$[Re(CO)_3(H_2O)_3]^+$ were used. The presence of a single species corresponding to **5** in solution was confirmed by $^1H/^{31}P$-NMR and HPLC (Fig. 1, Fig. S1(ESI†)). HR-ESI-MS also demonstrates the formation of the desired product. Upon addition of 1.5 equivalents or more of the metal reagent, new ^{31}P-NMR signals appeared, accompanied by a general upfield shift of the aromatic protons in the ^1H-NMR spectrum strongly suggesting coordination of metal centres to the BP group. These putative multinuclear species, however, do not form during radiosynthesis as the concentration of ligand always exceeds that of the radionuclide by several orders of magnitude. **3** in concentrations as low as 10^{-5} M (0.7 pg per labelling) can be efficiently labelled with *fac*-$[^{99m}Tc(CO)_3]^+$ in water to form **6** (>98% radiochemical yield, 22 GBq mg^{-1}). RP-HPLC analyses show that **5** and **6** coeluted, demonstrating their analogous structure (ESI†, Fig. S2).

One of the factors that makes **2** one of the most potent BP drugs is its high skeletal uptake and retention, which is directly related to its affinity towards HA.[13] The affinities and selectivities of **6** and ^{99m}Tc–MDP towards several calcium salts were evaluated using an *in vitro* assay. As shown in Fig. 2, **6** binds HA selectively with very high affinity (>80% binding). ^{99m}Tc–MDP, on the other hand, is less selective and binds HA and calcium oxalate (CO) with lower affinity (~40% binding). Remarkably, **6** shows higher affinity for HA despite having a concentration of competitive inhibitor (in the form of non-labelled BP) ~ 10 times higher than in the ^{99m}Tc–MDP preparation.[16]

The fate of a targeted imaging probe or radiopharmaceutical in blood is one of the most important factors during pre-clinical development of radiopharmaceuticals. Strong binding to serum proteins such as albumin often delays blood clearance, leading to low target-to-background ratios.[11] Previous chelator–BP conjugates have shown high binding to serum proteins.[9] Furthermore, human plasma enzymes may decompose exogenous compounds. Complex **6** showed negligible binding to serum proteins and no decomposition after incubation with human plasma for at least 18 h (ESI†, Fig. S4). ^{99m}Tc–MDP, on the other hand, remained mostly bound to serum proteins throughout the 18 h incubation.

In vivo imaging studies with **6** were carried out with adult Balb/C female mice using a nanoSPECT/CT animal scanner. Control imaging studies were also performed with ^{99m}Tc–MDP. **6** shows essentially identical bone uptake to ^{99m}Tc–MDP, demonstrating its usefulness as a bone-seeking agent (Fig. 3A, Fig. S5 (ESI†)). Biodistribution studies were performed *ex vivo* to quantify the uptake of the two tracers in bone and soft-tissue organs (Fig. 3B). As expected, the bone uptake of both compounds was very high, with 27–30% of the injected dose per gram of tissue (% ID g^{-1}) in the femur. Imaging shows that this uptake was in fact confined to the joints, where active remodelling occurs. Liver and lower gastrointestinal uptake, while very low, were slightly higher with **6** (2.5% ID g^{-1}) than with ^{99m}Tc-MDP (0.4% ID g^{-1}), consistent with the more lipophilic nature of the tricarbonyl core compared to MDP. An advantage of using bifunctional compounds is that properties like lipophilicity may be tuned by using, for example, different spacers and/or chelators. Thus, our results demonstrate that **3** forms well-defined, well-characterised and stable bone-targeting agents

with the *fac*-$[M(CO)_3]^+$ (M = Tc, Re) metal core and deserves further evaluation for the diagnosis and therapy of bone metastases. Biological evaluation of the complex of **3** with $[^{188}Re(CO)_3]^+$ compared with ^{188}Re–HEDP is underway to assess the prospects for improving radionuclide therapy by this approach.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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16. The concentration of 3 in the solution of 6 used in these studies was b 10 times higher than that of MDP in the ^{99m}Tc-MDP solution. To test the inhibition properties of 3, the same binding studies were carried out with HA and CO in the presence of an excess of 3, resulting in the complete inhibition of binding of 6.

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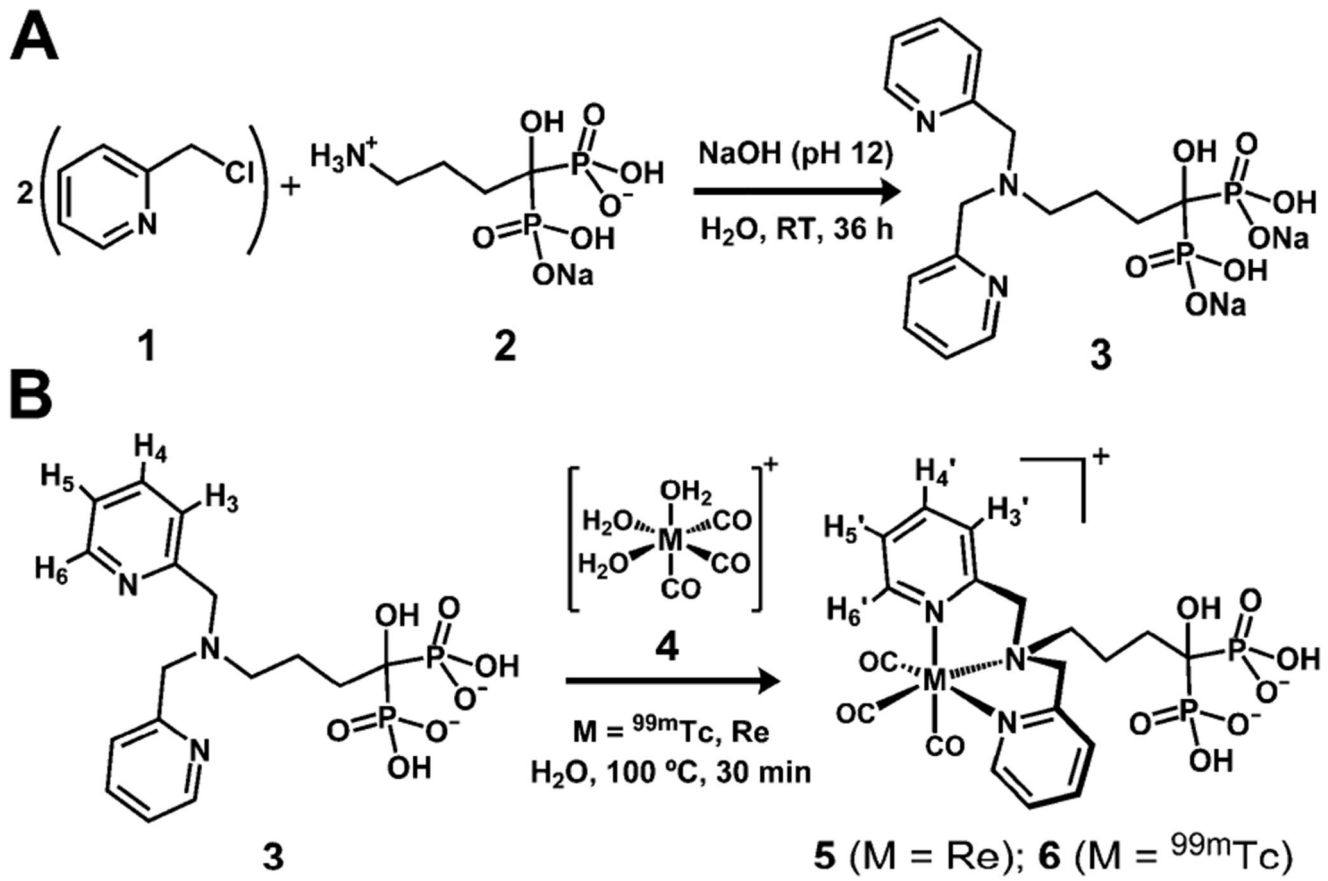

Fig. 1.

NMR titration studies in D_2O showing the ^{1}H- (aromatic region, left) and ^{31}P-NMR (right) of **3** (top row) upon increasing amounts (from top to bottom) of $[Re(CO)_3]^+$ showing the formation of **5** (mid row). See Scheme 1B for ^{1}H-NMR assignments.

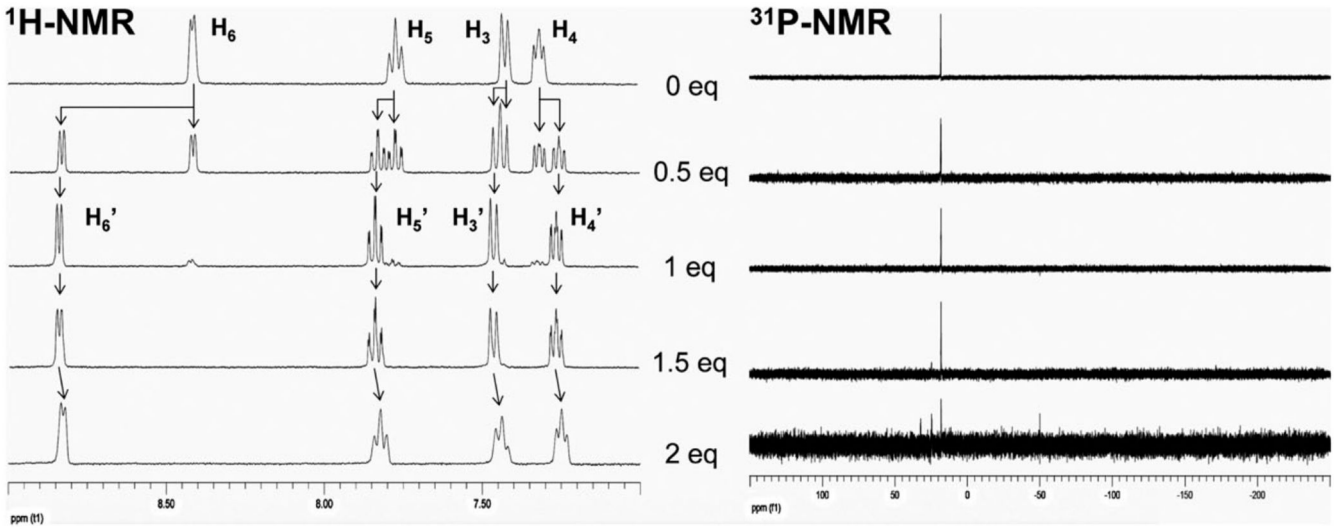

Fig. 2.

In vitro calcium salt binding study in 50 mM TRIS pH 6.9 at room temperature to compare the binding of **6** (black bars) and ^{99m}Tc-MDP (grey bars) to different calcium salts after 1 h (1 mg ml.$^{-1}$): hydroxyapatite (HA); β-tricalcium phosphate (b-CP); calcium phosphate (CP); calcium oxalate (CO); calcium carbonate (CC) and calcium pyrophosphate (CPy).

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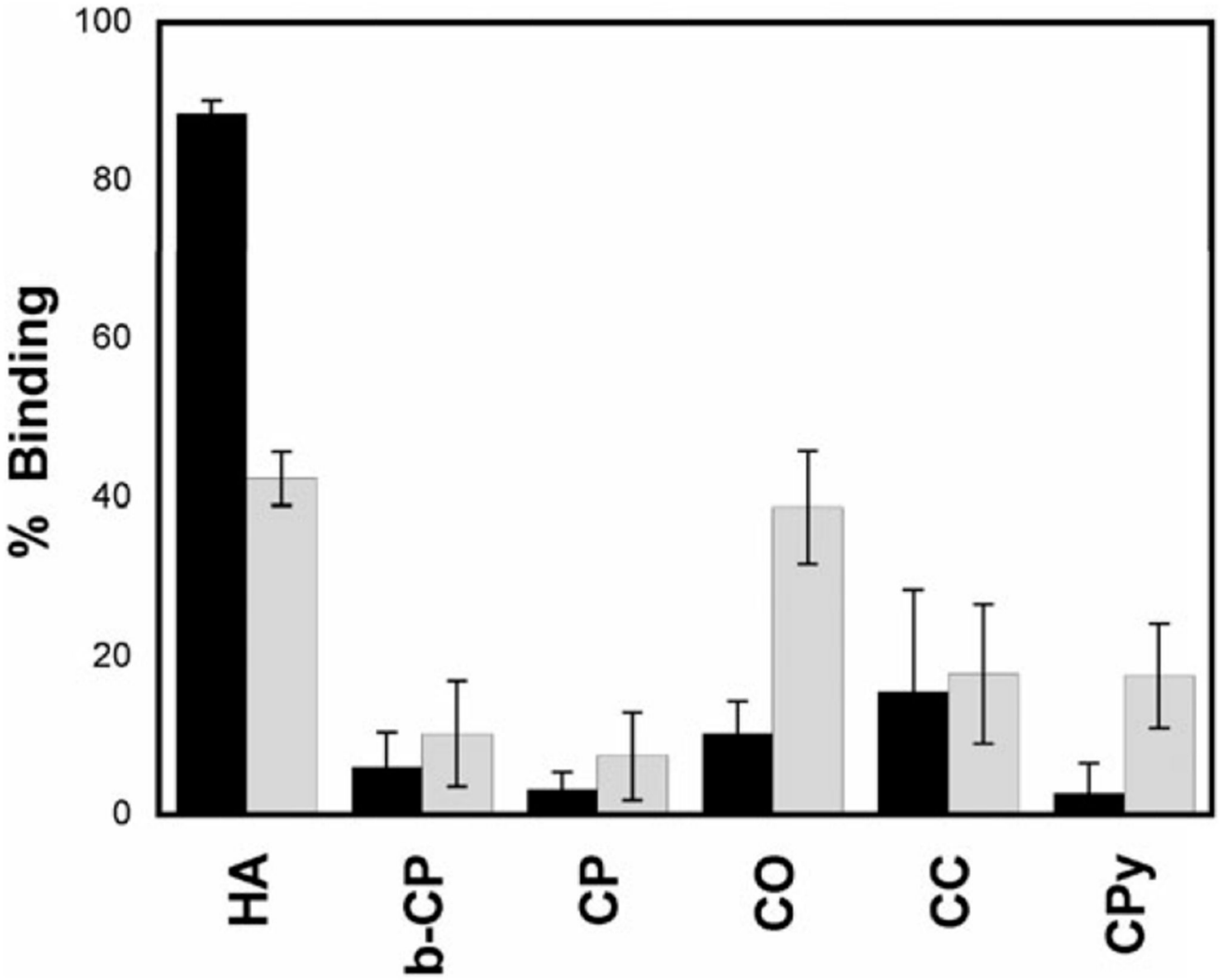

Fig. 3.

(A) SPECT (colour)/CT (greyscale) image showing the high uptake of **6** in bone tissue, particularly at the joints. (B) Biodistribution profile of **6** (black bars) and ^{99m}Tc-MDP (grey bars) at t = 6.5 h post-injection.

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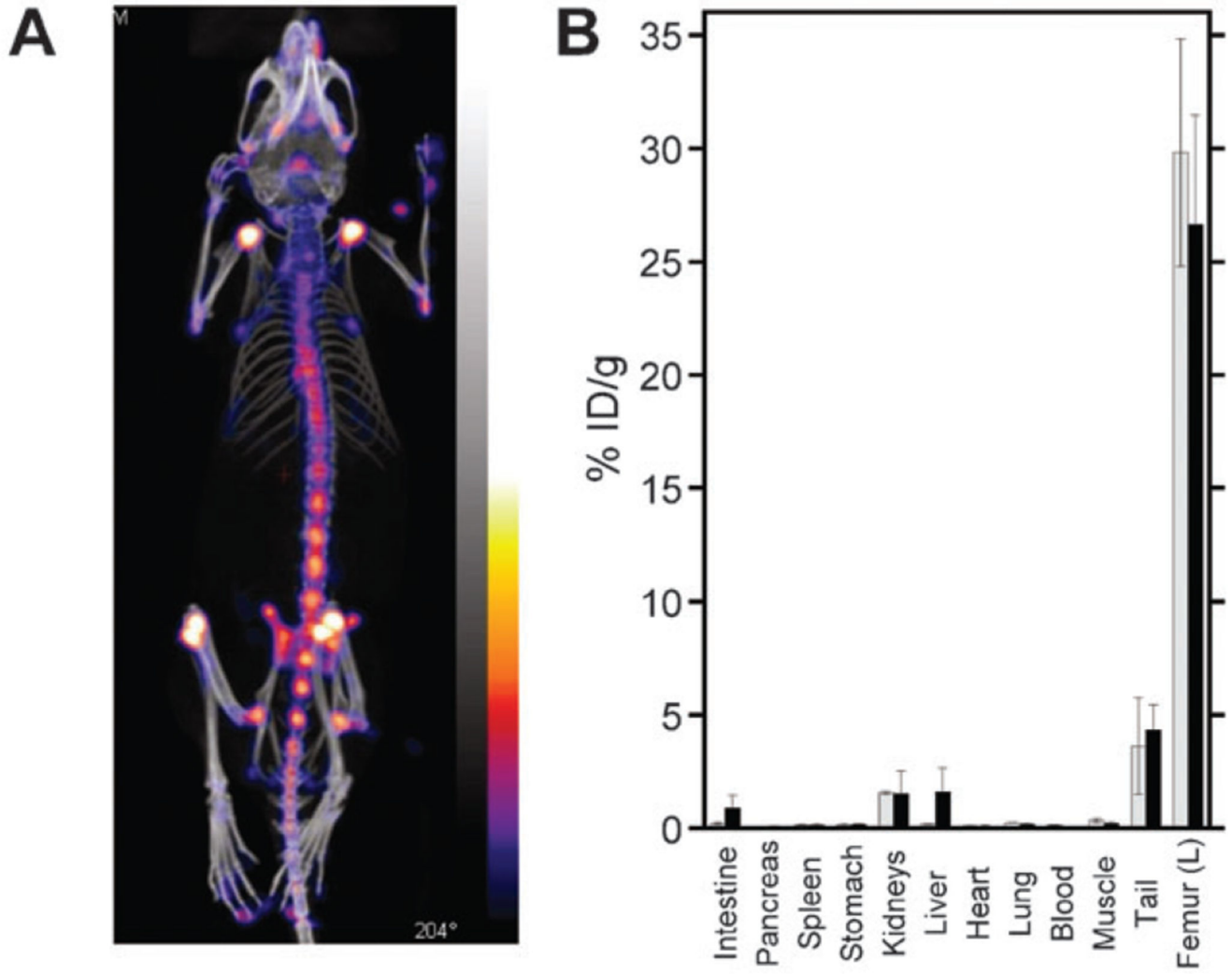

Scheme 1.
Reaction schemes for the syntheses of **3** (A), **5** and **6** (B).