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Unprecedented incorporation of α-emitter radioisotope ^{213}Bi into porphyrin chelates with reference to a daughter isotope mediated assistance mechanism†

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For the first time, α-emitter radioisotope ^{213}Bi has been incorporated into porphyrin chelates, with rates matching with the short period of the radionuclide. An *in situ* transmetalation mechanism involving the daughter isotope ^{209}Pb is expected to boost the ^{213}Bi radiolabeling process.

Compared with β⁻-emitters, which have shown promising results in the radio-treatment of cancers, α-emitters have certain theoretical advantages as α particles (high energy helium nuclei) present higher linear energy transfer and shorter path lengths.[1] These properties make targeted α-therapy appropriate for elimination of minimal residual or micrometastatic diseases. Bismuth α-emitter radioisotope ^{213}Bi is one of the few α-particle emitting radionuclides that meet the parameters for a use in therapy, and clinical studies have demonstrated therapeutic activity of ^{213}Bi labeled immunoconjugates.[2] However, among various complex obstacles, further advances will require improved chelation techniques. Indeed, all studies have been performed with carriers derived from two chelating agents known to complex most of the metals of therapeutic interest, namely CHX-A″ DTPA and C-DOTA.[3] These poly-carboxylic acid ligands were developed in the early 80's, and no innovating carriers have been proposed to clinicians so far. Significant heating is required for the formation of the ^{213}Bi complex of DOTA, thereby limiting its conjugation to small molecules or peptides as targeting vectors. CHX-A" DTPA is the only reported DTPA derivative to form suitably stable complexes with ^{213}Bi *in vivo*.[1b] Thus, improved chelation chemistry still remains to be addressed and, for that, new scaffolds are desirable to be explored. The design of innovative chelating agents for ^{213}Bi should consider not only a high stability of the radiolabeled chelate, but also a fast rate of metal insertion. Indeed, ^{213}Bi nuclide decays with a short half-life of 45.6 min.

The formation of bismuth complexes with unfunctionalized porphyrins generally requires harsh conditions with slow kinetics,[4,5] which makes the basic macrocycle not suitable for applications in α-radiotherapy. However, we have shown that porphyrins functionalized with at least one carboxylic acid group exhibit fast rates of bismuth(III) insertion, *ca.* few tens of minutes at room temperature and instantaneous at 75 °C.[6] The mononuclear bismuth complex of porphyrin **1** (Fig. 1) is easily purified on silica gel and is remarkably resistant to the addition of a large excess of trifluoroacetic acid (ESI†). Both kinetics and stability lie in the coordination of the intramolecular carboxylate group on the bismuth cation (X-ray structure, Fig. 1). Its bifunctional analogue **2** was recently synthesized (Fig. 1), and similar binding properties towards bismuth(III) were observed.[7] Ligand **1** was also shown to complex lead(II) instantaneously at room temperature.[6b] Such coordination properties are of great interest in the context of α-radiotherapy since a ^{212}Pb isotope has been evaluated as an *in-vivo* generator for the production of a ^{212}Bi α-emitter isotope, which results in an artificially extended half-life of ~11 hours.[8] In addition, we found that no demetalation of complex **1Bi** occurred upon

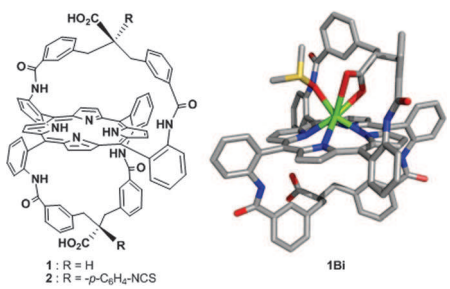


Fig. 1 Left: chemical structures of over-hanged carboxylic acid porphyrins **1** and **2**. Right: X-ray structure of **1Bi.DMSO** (hydrogen atoms removed for clarity).[6b]

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standing 8 hours in cell culture media (ESI†). This allowed us to determine the cytotoxicity of both the free-base and its bismuth complex (prepared with the cold isotope ^{209}Bi) using HT29 colon carcinoma and Hela cervical carcinoma cell lines (ESI†). **1** and **1Bi** have a low cytotoxic effect on both cancer cells with an IC_{50} value for compound **1** between 25–50 μM depending on the cell line, and an IC_{50} value close to 100 μM for compound **1Bi** in both cell lines.

For all these reasons, ligands **1** and **2** were thought to be good candidates for ^{213}Bi complexation assays. It is worth noting that such experiments are scarcely reported due to limited supply of ^{213}Bi isotope. The α-emitter ^{213}Bi isotope was eluted from an ^{225}Ac/^{213}Bi generator with a solution of HCl/NaI (1 : 1, 0.1 M), following a standard protocol. From radioactivity counting, the initial concentration of ^{213}Bi in the eluate was determined as *ca.* 0.3 nM. Nanomolar affinity is obviously difficult to achieve and, in this first study, we used a rather high concentration of ligands (1.2 μM). ^{213}Bi incorporation was monitored by radio-TLC (Fig. 2a). In a CH_2Cl_2/MeOH (9 : 1, v/v) mobile phase, complexes $\mathbf{1}^{\mathbf{213}}\mathbf{Bi}$ and $\mathbf{2}^{\mathbf{213}}\mathbf{Bi}$ are identified as a $R_f \approx 0.5$ spot, whereas the remaining ^{213}Bi salts are observed as a $R_f \approx 0$ spot.

The initial low pH value (~1) of the ^{213}Bi eluate prevents metal incorporation into our porphyrin ligands and pH first has to be adjusted. Four pH values ranging from 5 to 8 were studied at 75 °C while the reactions were monitored over 30 min (Fig. 2b). The first observation concerns the pH 5 value. Whereas this value is optimal for DTPA, with chelate **1**, no incorporation occurred even after 30 min. This observation is consistent with a first protonation pK_a of the macrocycle around 4.5. Conversely, at pH 6, the percentage of incorporation increased at an almost constant value of 25%. To our delight, a significant improvement of the incorporation was measured when pH was raised up to 7 or 8 since, after only 5 min, incorporation was found as high as 80%. These fast kinetics are obviously of great interest considering the short half-life of ^{213}Bi (45.6 min). Over a 30 min period, incorporation has decreased down to 60% and 50% at pH values 7 and 8, respectively. This indicates that pH 7 is an optimal value for this type of porphyrin chelate.

Secondly, the effect of temperature at a fixed, optimal pH value was investigated. For targeted radiotherapy, tumor-specific conjugates are coupled to the chelate of interest prior to radioisotope incorporation. Whereas radiolabeling of hapten-functionalized chelates can be performed at high temperatures (~80 °C), chelate-appended antibodies do not tolerate temperatures above 40 °C. As shown in Fig. 2c, the percentage of ^{213}Bi incorporation into chelate **1** was half as important at 40 °C as at 75 °C. However, approximately 35% of incorporation was observed after only 10 min, which remains quite remarkable. Lowering the temperature down to 20 °C led to a slower rate of incorporation but yet a *ca.* 25% value was found.

These investigations were extended to the related bifunctional chelate **2** (ESI†). No significant differences were observed: at pH 7, up to 75% and 30% ^{213}Bi incorporation values were obtained at 75 °C and 40 °C, respectively, in less than 10 minutes. At this early stage, these results indicate that with such chelates, both targeting strategies mentioned above should be investigated to deliver the radionuclide.

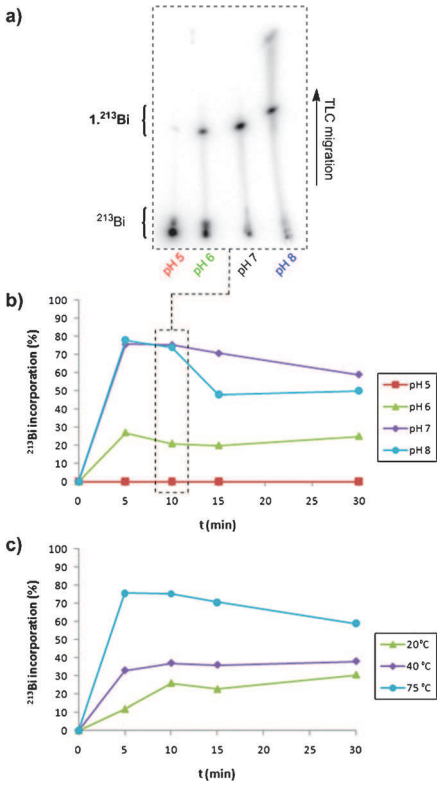


Fig. 2 (a) Radio-TLC at $t = 10$ min corresponding to the monitoring of ^{213}Bi insertion in ligand **1**, at $T = 75$ °C, for different pH values. Influence of pH (b) and temperature (c) on the rate of ^{213}Bi incorporation into ligand **1** (b: experiments performed at 75 °C; c: experiments performed at pH 7).

These fast complexation rates were unexpected working with porphyrin ligands at nanomolar concentration of metal ions. Additional kinetic studies were performed with cold isotopes of bismuth and lead and revealed an interesting metalation mechanism. It should first be reminded that ^{209}Pb is a daughter nuclide of ^{213}Bi, with a half-life of 3.3 h, and therefore accumulates during the radiolabeling process (see decay scheme in ESI†). The rates of Bi(III) and Pb(II) insertion into porphyrin **1** were monitored by UV-vis spectroscopy at μM concentration in dimethylsulfoxide solution. A half-time of reaction of approximately 10 min was found for bismuth (Fig. 3a), while lead insertion proceeded in only a couple of seconds (Fig. 3b, 1st step). Very interestingly, the addition of the bismuth salt to the lead complex of **1** led instantaneously to the formation of the bismuth complex (Fig. 3b, 2nd step): now, the half-time of reaction is lower than 2 seconds! The same behavior was observed with the bifunctional chelate **2** (ESI†). The half-time of reaction for bismuth insertion dropped from ~7 min for the direct complexation down to ~12 seconds when lead was previously inserted. It thus appears that the transmetalation reaction proceeds spectacularly faster, that is with ~300 and ~35 fold increases in the rate of bismuth insertion for ligands **1** and **2**, respectively. In such a lead-mediated bismuth insertion process, two successive mechanisms of activation actually occur: (i) the instantaneous lead complexation likely proceeds *via* a deconvolution mechanism[9] involving a hanged carboxylate group and facilitating a *sitting atop* complex. Indeed, heating is required in the case of related

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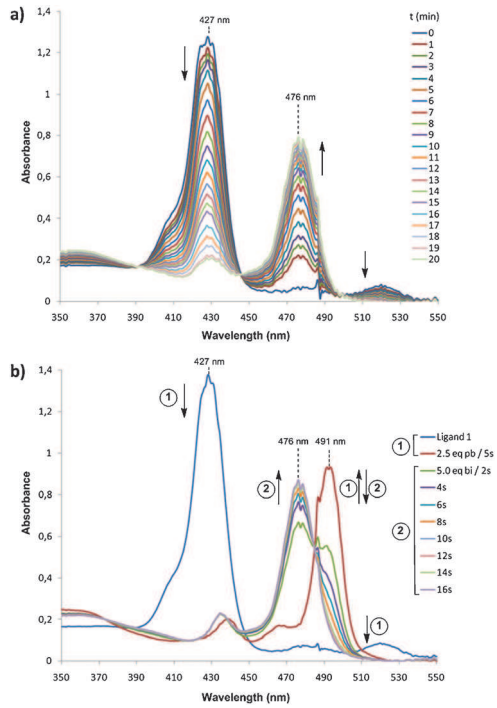


Fig. 3 UV-vis monitoring at room temperature of the formation of the bismuth complex with ligand **1**: (a) direct metalation; (b) transmetalation process. Conditions: DMSO, $[\mathbf{1}]_0$ = 8 μM, 5 equiv. DIPEA; for (a): 5 equiv. of $Bi(NO_3)_3$; for (b): 2.5 equiv. of $Pb(OAc)_2$ (1st step) then 5 equiv. of $Bi(NO_3)_3$ (2nd step).

compounds with hanged ester groups;[10] (ii) we postulate that, in addition to this deconvolution mechanism, the deformation of the porphyrin core induced by the *out-of-plane* coordination of lead[6b] promotes bismuth insertion. Such a distortion-mediated insertion mechanism is involved in chelatase-catalyzed insertions of metal ions into porphyrins.[11] It has also been evaluated for instance in the case of the mercury-mediated zinc insertion,[12] as well as in the case of the lead-mediated zinc and manganese insertion.[13] Here, in contrast to the quasi *in-plane* complexation displayed by *e.g.* zinc, bismuth and lead cations are both complexed significantly *out-of-plane*. However, the bismuth complexes of **1** and **2** are far much stable than their lead counterparts which makes such an assistance mechanism possible.

Based on these results, a similar process might be anticipated for the labeling of ligand **1** with ^{213}Bi, which is a *daughter-mediated parent isotope insertion mechanism* (Fig. 4). Indeed, as mentioned above, the daughter ^{209}Pb isotope accumulates in the eluate from the generator while ^{213}Bi decays. ^{209}Pb is therefore expected to enhance the rate of ^{213}Bi incorporation through an *in-situ* transmetalation process. To our knowledge, such a possibility of having a daughter isotope mediated assistance mechanism has never been described with the classical chelates used for ^{213}Bi complexation (DOTA and DTPA derivatives). For the latter, complexation of lead and bismuth cations has been only studied in the context of an *in-vivo* generator for the production of ^{212}Bi from the parent ^{212}Pb.

In conclusion, we have evidenced for the first time that a suitably functionalized porphyrin macrocycle can be efficiently

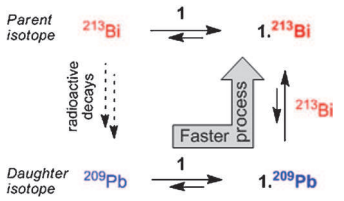


Fig. 4 Schematized assistance mechanism transposed from the cold isotopes to the case of the hot isotopes: ^{213}Bi insertion into chelate **1** mediated by the daughter isotope ^{209}Pb.

labeled with an α-emitter radionuclide of particular interest for cancer radiotherapy. The fast rate of ^{213}Bi incorporation is remarkable and matches with the short half-life of the radionuclide. An unprecedented transmetalation mechanism involving a daughter radionuclide is expected to increase the rate of ^{213}Bi complexation. These first results pave the way for further investigations with related porphyrin chelates, and work towards improved chelation properties and biodistribution assessment is underway. All in all, this contribution shows that innovative chelates for a radioelement might come from a completely divergent approach. We hope that this work also may serve as a source of new inspirations for the design of radiolabeling agents.

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