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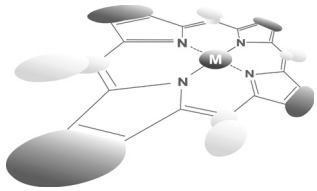

Rapid insertion of bismuth radioactive isotopes into texaphyrin in aqueous media

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ABSTRACT: Radioisotopes ^{213}Bi and ^{207}Bi were successfully inserted into a water-soluble and tumor-targeting texaphyrin ligand. The reaction with $^{207}Bi(OAc)_3$ proceeds in about 15 min in aqueous media without the use of any organic base. The Bi(III) texaphyrin complex formed (**3**) is stable in aqueous media even in the presence of an excess of competitive chelators (EDTA or citric acid), as well as in human blood plasma. These results validate texaphyrins as potential candidates for coordinating bismuth radionuclides and set the stage for their use in targeted alpha therapy (TAT).

KEYWORDS: bismuth radioisotopes, targeted alpha-emitter therapy, texaphyrin.

INTRODUCTION

Alpha-emitting radioisotopes, such as ^{225}Ac, ^{211}At, ^{212}Bi, ^{213}Bi, ^{212}Pb, ^{223}Ra and ^{227}Th, are attractive for the treatment of micro-metastatic disease because of the short penetration range in tissues (50–100 μm) and high linear energy transfer (≈100 KeV/μm) of the emitted α-particles, both of which help restrain the irradiation to the targeted and neighboring cells [1].

One major limitation has been to find a ligand capable of forming a stable complex with one of these radioactive elements. In the particular case of $^{213}Bi^{3+}$, the short $t_{1/2}$ (45.6 min) requires a fast insertion as well as formation of a chemically stable complex. Recently, examples of chelators that are attractive for use in $^{213}Bi^{3+}$ complexation have been described [2]. Unfortunately, the ligands in question lack inherent tumor targeting capability and thus require further conjugation to localizing molecules, such as antibodies, RGD peptide sequences, folate molecules, *etc.* prior to administration [3].

An alternative approach would involve the use of ligands capable of forming in a fast manner stable Bi^{3+} complexes that localize well into tumors. This would eliminate the need for further chemistry focused on the addition of a targeting moiety resulting in less drug processing time and concomitantly, a more effective use of ^{213}Bi given its 45.6 min half-life. Porphyrins have been investigated as ligands for the complexation of bismuth isotopes because they tend to accumulate preferentially in cancerous lesions [4]. While unmodified porphyrins will form complexes with Bi^{3+} [5], the porphyrin core is too small to produce a complex with sufficient stability to be used *in vivo*. One solution has involved the use of elaborate strapped macrocycles. Boitrel and co-workers have been leaders in the development of such systems [6]. In contrast, we have suggested that texaphyrins *per se* would be suitable for $^{213}Bi^{3+}$ complexation.

Texaphyrins are characterized by a central cavity that is nearly 20% larger than those present in porphyrins. This allows for the complexation of larger cations [7]. A number of early preclinical and clinical studies provided support for the suggestion that texaphyrin complexes accumulate selectively in cancerous lesions [8].

◊ SPP full member in good standing

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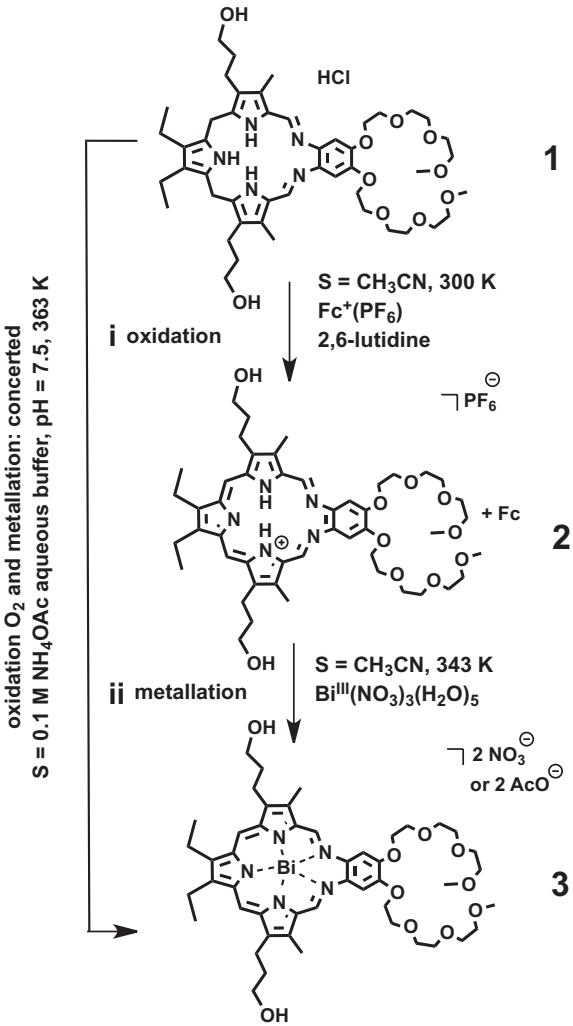
As an initial step towards testing the potential utility of texaphyrins as carriers for $^{213}Bi^{3+}$, we recently reported the complexation of the stable isotope, $^{209}Bi^{3+}$, as well as Pb^{2+}, by a water-soluble texaphyrin macrocycle [9]. The kinetics of insertion were relatively fast (30 min) but still short of ideal for actual ^{213}Bi insertion. Here we report two different protocols that allow for faster Bi^{3+} complexation. The utility of these insertion procedures were tested using cold ^{209}Bi(III) (hydrated nitrate or acetate salts) and the radioisotopes, $^{207}Bi^{3+}$ ($t_{1/2}$ = 31.5 years) and $^{225}Ac^{3+}$ ($t_{1/2}$ = 10.0 days, both as hydrated acetate salts). Both [$^{207}Bi^{3+}$(texaphyrin)] and [$^{213}Bi^{3+}$(texaphyrin)] complexes were rapidly obtained in aqueous media without requiring the use of an organic base.

RESULTS AND DISCUSSION

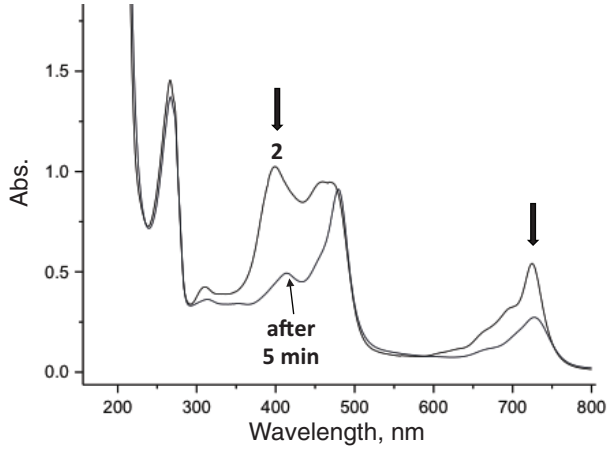
The first approach involves a stepwise insertion carried out in an organic solvent (see Scheme 1). It involves forming initially the oxidized texaphyrin ligand **2** (isolable), followed by preparation of complex **3** *via* the addition of $Bi(NO_3)_3(H_2O)_5$ with warming. This latter step can be completed easily in about 10 to 15 min. The second approach involves use of an aqueous medium. It allows for the formation of the desired complex **3** in about 15 min without the need to add an organic base.

We started by investigating the stepwise method. The metallation of texaphyrins is associated with oxidation and aromatization of the ring. We thus sought to explore whether it would be possible to prepare the oxidized form **2** of the ligand first and then carry out the bismuth insertion. This stepwise approach was expected to improve the insertion kinetics under conditions where the oxidation step is rate limiting step. To test this hypothesis, we used the protocol described previously by our group using a ferrocenium salt as oxidizing agent [10]. The formation of the compound **2** was completed in about 30 min. ^{209}Bismuth(III) in the form of $(NO_3)_3(H_2O)_5$ was added directly to the crude mixture and the reaction mixture heated to 70 °C. After 5 min, about 80% insertion was achieved as inferred from UV-vis spectroscopic and RP-HPLC analyses (see Fig. 1). The reaction went to completion in about 10 to 15 min, and proved roughly 2x faster than the previously reported concerted method that relies on O_2 as the oxidizing agent. For the purposes of analysis, complex **3** was isolated by column chromatography (silica gel, one single peak by RP-HPLC). Unfortunately, this latter chromatographic step, which is necessary to remove the organic impurities and ferrocene, represents a time limitation for possible applications involving ^{213}Bi. Another practical consideration is that most radioactive Bi salts are obtained from, or present in, aqueous solutions. Such precursors are not compatible with protocols such as this one that require the use of organic solvents, such as dry acetonitrile.

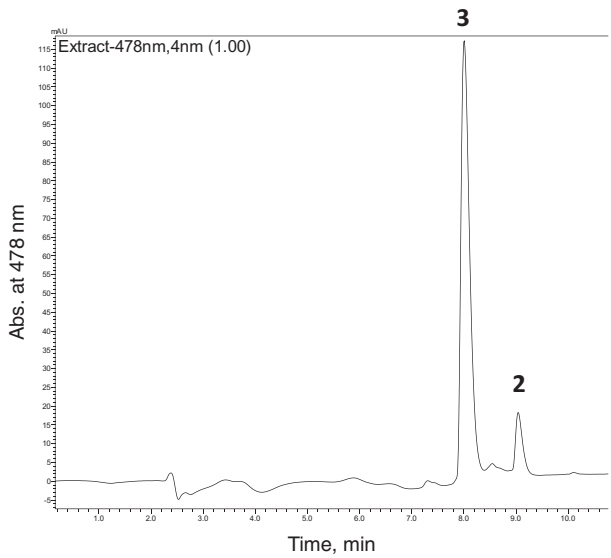


Scheme 1. Protocols for metallation of texaphyrin macrocycles. Method 1: Stepwise strategy ("one pot") that consists in oxidizing **1** first using a ferrocenium salt to form **2** as an intermediate (isolable species), and then inserting the metallic ion Bi^{3+} to form complex **3**; Method 2 (shunting arrow on the left side): Concerted strategy using O_2 as the oxidizing agent

In light of the above considerations, we decided to test a different method with the goal of developing an insertion procedure that might allow the rapid preparation of **3** in buffered aqueous solution in the absence of an organic base, thus obviating its removal from the final product. With this goal in mind, we carried out a Bi(III) insertion analogous to that described above, but in aqueous media under neutral or slightly basic conditions (pH = 7.5). The reaction was warmed to 90 °C and the ligand **1** was present in excess. Under these conditions, complex **3** was formed in about 15 min, a temporal limit considered practical in the case of ^{213}Bi insertions. The Bi(III)-containing product was identified by RP-HPLC and ESI-MS (see Supporting information, Figs S1 and S2), purified and desalted on



(a)



(b)

Fig. 1. UV-vis (CH_3CN, [**1**] = 10 mM) spectra of **2** before and 5 min after addition of $^{209}Bi(NO_3)_3(H_2O)_5$ (2 equiv.) at 70 °C. (a); RP-HPLC chromatogram (UV-vis detector set at 478 nm) of the reaction mixture 5 min after the addition of $^{209}Bi(NO_3)_3(H_2O)_5$ (b)

tC18 column to afford the pure product (one single peak by RP-HPLC, see Fig. S1, chromatogram A).

The fast insertion kinetics and the high stability of **3** led us to test the same protocol using radioactive ^{207}Bi salts, a good model for $^{213}Bi^{3+}$ and much easier to work with because of its long $t_{1/2}$ (31.5 years) and readily observable gamma lines (*e.g.* 569.7 KeV). In this case, the reaction progress was monitored *via* thin layer chromatography (TLC) using two distinct mobile phases. An aqueous mobile phase comprising pH 5.5 citrate gave the free $^{207}Bi^{3+}$ ion at an R_f = 1.0, and the texaphyrin complex (**3***) an R_f = 0. An organic mobile phase was used as a complementary approach; this gave the free $^{207}Bi^{3+}$ ion at an R_f = 0, and the texaphyrin complex an R_f = 1.0. After these plates were developed, they were

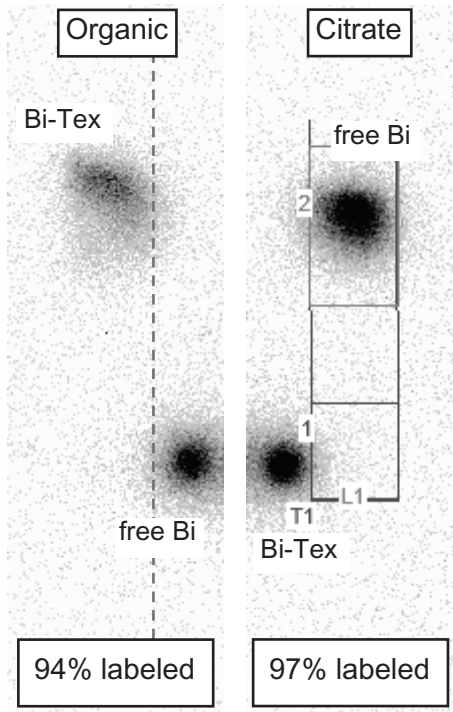


Fig. 2. Radio TLC plates of the product of metal insertion reactions involving **2** + $^{207}Bi(OAc)_3$ (~3 kBq) in aqueous medium. The plates were imaged after a reaction time of 15 min at 95 °C and developed in organic solvent (left, 20% methanol in dichloromethane) or sodium citrate solution (right). Radioactivity was detected using a phosphor imager. Note: Free $^{207}Bi(OAc)_3$ was co-developed to allow for direct comparisons

cut in half to separate free Bi and the bismuth-texaphyrin complex (Bi-Tex), and the activity in each section was measured using a Ge (HPGe) gamma spectrometer to assess radiochemical yields. These TLC plates were also developed with a phosphor imager to visualize the radioactivity on the TLC plates. When a ligand concentration of 0.1 mM was used and 15 kBq of ^{207}Bi was applied, the extent of cation insertion was found to be greater than 95% at room temperature (see Fig. 2).

In an effort to increase the specific activity of the resulting Bi-Tex constructs, the excess free ligand was removed by passing the reaction mixture through a plug made of C18 silica. Since the concentration of ^{207}Bi was initially low (40 nM) in these studies, it was impossible to visually observe the green color characteristic of **3**. Therefore, cold complex **3** was co-loaded with **3*** to allow visual detection of the fractions containing **3** (and **3***). This filter-like isolation procedure required between 5 and 10 min when carried out on a bench scale, for a total reaction and processing time of ~25 min. In a putative clinical setting the mixture of radiolabeled product **3*** and unreacted starting material **1** could likely be co-administrated without a need for separation.

The same protocol was applied to the radioisotope $^{225}Ac^{3+}$. In this case, the complex with $^{225}Ac^{3+}$ was not observed. Our inability to detect the Ac-Tex complex is interesting in light of theoretical calculations that predict Ac^{3+} to form exceptionally stable texaphyrin complexes [11]. However, from our attempted Ac radiolabeling studies, we were able to detect the complex with $^{213}Bi^{3+}$, one of the daughter isotopes on the $^{225}Ac^{3+}$ decay chain.

The stability of **3** (prepared using cold $^{209}Bi^{3+}$) was tested in the presence of a large excess of known chelators, such as EDTA and citric acid, in PBS solution at room temperature. The progress of the putative competition was followed using RP-HPLC while monitoring the characteristic texaphyrin absorptions at 470 and 740 nm, respectively. An RP-HPLC analysis revealed almost no decomplexation in the case of citric acid even after several days (see Fig. S3). In the presence of EDTA, more than 70% of **3** is still present in solution after 3 days (see Fig. S3). These results lead us to suggest that **2** can coordinate the Bi^{3+} ion quickly on the laboratory timescale and form a stable complex. This stability profile is likely due to the aromaticity of **3** (unlike **1**) and to the stabilization of the two schiff bases by the complexation of Bi^{3+}. The stability of **3** was also evaluated in human plasma (containing EDTA) at 37 °C. After a determined time, cold ethanol was added in order to precipitate plasma proteins and the supernatant was analyzed by RP-HPLC. Even at $t = 0$, the protein pellet was dark green leading us to suggest that **3** binds readily to plasma proteins (see Fig. S5). After 24 h, the pellet was still dark green, which is taken as an indication that **3** does not undergo significant degradation (since the typical texaphyrin decomposition products are brown-red). Only about 20% of **3** remained in the supernatant and RP-HPLC analysis of this solution showed only one peak corresponding to the starting material (see Fig. S6). It is currently appreciated that binding to serum proteins, such as albumin, can provide a benefit to drugs in terms of bloodstream half-life [12] and delivery to tumor sites [13]. Indeed, some cancer cells overexpress receptors that are involved in the recognition/internalization of albumin and can mediate the cellular uptake of drug-albumin conjugates [13]. In addition, albumin-bound drugs might benefit (in terms of tumor uptake) from the enhanced permeability and retention (EPR) effect observed in solid tumors [14]. Thus, the binding of **3** to plasma proteins is expected to improve its utility *in vivo*.

In summary, we have developed two methods that allow for the rapid insertion of Bi(III) into a preformed water-soluble texaphyrin core. The kinetics involved and the stability of the product (**3**) augur well for the use of strategies such as those described here to prepare bismuth complexes of the alpha emitter ^{213}Bi that are amenable for use *in vitro*. Efforts to test this hypothesis and optimize conditions to prepare complexes of ^{225}Ac are currently ongoing.

Acknowledgments

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Supporting information

Additional information about synthetic procedures and stability studies are provided. This information is available free of charge *via* the internet at http://www.worldscinet.com/jpp/jpp.shtml.

REFERENCES

1. Dekempeneer Y, Keyaerts M, Krasniqi A, Puttemans J, Muyldermans S, Lahoutte T, D'huyvetter M and Devoogdt N. *Expert Opin. Biol. Ther.* 2016; **16**: 1035–1047.
2. (a) Wilson JJ, Ferrier M, Radchenko V, Maassen JR, Engle JW, Batista ER, Martin RL, Nortier FM, Fassbender ME, John KD and Birnbaum ER. *Nucl. Med. Biol.* 2015; **42**: 428–438. (b) Brechbiel MW, Pippin CG, McMurry TJ, Milenic D, Roselli M, Colcher D, *et al*. *J. Chem. Soc. Chem. Commun.* 1991; 1169–1170. (c) Brechbiel MW and Gansow OA. *J. Chem. Soc. Perkin Trans.* 1992; **1**: 1173–1178. (d) Garmestani K, Yao Z, Zhang M, Wong K, Park CW, Pastan I, *et al*. *Nucl. Med. Biol.* 2001; **28**: 409–418. (e) Lima LMP, Beyler M, Oukhatar F, Le Saec P, Faivre-Chauvet A, Platas-Iglesias C, *et al*. *Chem. Commun.* 2014; **50**: 12371–12374.
3. Kim YS and Brechbiel MW. *Tumor Biol.* 2012; **33**: 573–590.
4. (a) El-Far MA and Pimstone NR. *Cancer Res.* 1986; **46**: 4390–4394. (b) Hill JS, Kahl SB, Kaye AH, Stylli SS, Koo M-S, Gonzales MF, Vardaxis NJ and Johnson CI. *Proc. Natl. Acad. Sci.* 1992; **89**: 1785–1789. (c) Hill JS, Kaye AH, Sawyer WH, Morstyn G, Megison PD and Stylli SS. *Neurosurgery* 1990, **26**: 248–254.
5. (a) Valicsek Z, Horváth O and Patonay K. *J. Photochem. Photobiol. A: Chem.* 2011; **226**: 23–35. (b) Gomes ML, DeFreitas-Silva G, Gomes dos Reis P, Melo MN, Frézard F, Demicheli C and Idemori YM. *J. Biol. Inorg. Chem.* 2015; **20**: 771–779.
6. (a) Le Gac S, Najjari B, Motreff N, Remaud-Le Saec P, Faivre-Chauvet A, Dimanche-Boitrel M-T, Morgenstern A, Bruchertseifer F, Lachkar M and Boitrel B. *Chem. Commun.* 2011; **47**: 8554–8556. (b) Boitrel B, *Bismuth Complexes of Porphyrins and Their Potential in Medical Applications in*

Biological Chemistry of Arsenic, Antimony and Bismuth, Sun H. John (ed.), Wiley & Sons, 2010, Chap. 9, 209–240.
7. Sessler JL, Mody TD, Hemmi GW and Lynch VM. *Inorg. Chem.* 1993; **32**: 3175–3187.
8. (a) Rosenthal DI, Nurenberg P, Becerra CR, Frenkel EP, Carbone DP, Lum BL, *et al*. *Clin. Cancer Res.* 1999; **5**: 739–745. (b) Carde P, Timmerman R, Mehta MP, Koprowski CD, Ford J, Tishler RB, Miles D, Miller RA and Renschler MF. *J. Clin. Oncol.* 2001; **19**: 2074–2083.
9. Preihs C, Arambula JF, Lynch VM, Siddik ZH and Sessler JL. *Chem. Commun.* 2010; **46**: 7900–7902.
10. Hannah S, Lynch VM, Gerasimchuk N, Magda D and Sessler JL. *Org. Lett.* 2001; **3**: 3911–3914.
11. Cao C, Li Q, Moritz A, Xie Z, Dolg M, Chen X and Fang W. *Inorg. Chem.* 2006; **45**: 3444–3451.
12. Zheng Y-R, Suntharalingam K, Johnstone TC, Yoo H, Lin W, Brooks JG and Lippard SJ. *J. Am. Chem. Soc.* 2014; **136**: 8790–8798.
13. (a) Frei E. *Diabet. Met. Syndr.* 2011; **3**: 11–14. (b) Fritzsche T, Schnolzer M, Fielder S, Weigand M, Wiessler M and Frei E. *Biochem. Pharmacol.* 2004; **67**: 655–665.
14. Maeda H, Wu J, Sawa T, Matsumura Y and Hori K. *J. Control. Release* 2000; **65**: 271–284.