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Bismuth– and lead–texaphyrin complexes: towards potential α -core emitters for radiotherapy†

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Abstract

Lead(II)–texaphyrins and the first discrete binuclear μ -oxo bismuth(III)–texaphyrin are reported. The latter was characterized via single crystal X-ray diffraction analysis. Cell proliferation assays using the A2780 ovarian cancer cell line were used to determine the cytotoxicity of the complexes.

Complexes with lead and bismuth are of interest due to the fact that the isotopes ^{212}Bi and ^{213}Bi show potential for use as α -core emitters in the radio-treatment of tumors.¹ However, ^{212}Bi and ^{213}Bi themselves both show very limited half-lives (60.55 min and 45.65 min for ^{212}Bi and ^{213}Bi , respectively). The lifetime of ^{212}Pb is longer; it has a half-life of 10.64 hours and produces ^{212}Bi as its primary decay product along with a β -particle.

Finding suitable ligands for bismuth or lead has proved challenging. An ideal ligand would be one that is able to form rapidly stable complexes with both bismuth and lead and do so under mild conditions. Complexes of bismuth and lead that possess inherent tumor selectivity would be further advantageous since they would allow the radioactive species in question, namely ^{212}Bi , ^{213}Bi , or ^{212}Pb , to be delivered selectively to cancerous tissues. In principle, such localization would maximize efficacy, while minimizing radioactive exposure to healthy tissues *in vivo*. This has made porphyrins, whose selective localization in tumors has been documented in many instances,^{2–4} suitable candidates as ligands for bismuth and lead. Indeed, several porphyrin-systems have already been shown by Boitrel *et al.* to bind the Bi(III) and Pb(II) cations.^{5–11} However, the stable systems reported to date have been structurally complex and a challenge to produce synthetically. The need for elaborate porphyrins reflects the fact that the lead(II) and bismuth(III) cations do not fit inside the cavity of a tetrapyrrolic porphyrin. Bi(III) or Pb(II) complexes of non-elaborated porphyrins generally suffer from stability problems, with decomplexation reactions having been specifically noted in the case of tetrakis(3-pyridyl)porphyrin,¹² *meso*-tetra-*n*-propylporphyrin,¹³ and octaethylporphyrin.¹⁴ This is most likely due to the large distance of

†Electronic supplementary information (ESI) available: Details of synthetic experimental, characterization data, HPLC-spectra, *in vitro* studies, and X-ray crystallography. CCDC 790736 (**2a**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc03528a

the metal center from the plane of the four nitrogen atoms in the porphyrin ligand. Moreover, the insertion kinetics for typical unfunctionalized porphyrins are usually very slow, with heating at reflux in solvents such as methanol, dichloromethane, chloroform or toluene generally being required in the case of bismuth. Even more problematic, partial decomplexation is often observed during the course of purifying the resulting complexes. In an effort to overcome these difficulties, we have explored the use of texaphyrin as a ligand for lead and bismuth. These pentaaza expanded porphyrins have a roughly 20% larger core than their tetrapyrrolic congeners,¹⁵ which allows for the complexation of larger cations.^{16,17} Texaphyrins have been shown to localize to, or be retained selectively in, rapidly growing tissues, including cancerous lesions.¹⁸ One particular gadolinium(III)–texaphyrin complex, known as motexafin gadolinium (MGd), has been studied in considerable detail.¹⁹ It has been shown to be taken up selectively by B cell non-Hodgkins lymphoma, chronic lymphocytic leukemia and myeloma cells,²⁰ as well as non-small cell lung cancer and breast cancer tissues. The ratio of MGd in tumor cells to that in surrounding normal cells is reported to be up to 9 : 1;²¹ this increases to 50 : 1 in the case of metastatic brain tumors.²² All these features inspired the study of texaphyrins as ligands for the bismuth(III) and lead(II) cations. As detailed below, the reduced, sp^3 precursor form of texaphyrin is indeed able to complex these two cations rapidly; in doing so, the first discrete binuclear μ -oxo bismuth(III) macrocyclic complex to be described in the literature is formed as judged by X-ray diffraction analysis. The present Bi(III) and Pb(II) species are chemically stable, a feature that coupled with their ability to be produced in a water soluble fashion has permitted their characterization *in vitro* using the A2780 ovarian cancer cell line.

The chemical structures of the target Bi– and Pb–texaphyrin complexes are shown in Fig. 1. Compounds **1a** (MBi) and **1b** (MPb) bear appended polyethylene glycol units and were expected to display water solubility sufficient to allow for cell studies. In contrast, complexes **2a** and **2b**, with appended methoxy functionalities, were designed to allow for the growth of single crystals suitable for X-ray diffraction analysis. While diffraction grade crystals were obtained in the case of **2a**, unfortunately, to date no crystals of **2b** suitable for X-ray analysis in the solid state could be obtained.

In the case of **1** and **2**, the requisite precursors, the reduced or so-called sp^3 forms of the texaphyrin, are known.^{23–25} These precursors, wherein the pyrrolic subunits are spanned by methylene (as opposed to methine) bridges, typically undergo oxidative metal insertion to give metallotexaphyrins when exposed to labile metal salts in the presence of an organic base and oxygen. This proved true for both Bi(III) and Pb(II). Specifically, exposure of the nonaromatic texaphyrin ligands to commercially available bismuth nitrate or lead nitrate in methanol with addition of triethylamine and in the presence of molecular oxygen leads to the aromatic complexes **1a–b** and **2a–b**, respectively.

The kinetics of metal insertion were monitored by observing the increase in the absorption at $\lambda_{\text{max}} = 478 \text{ nm}$ as a function of time using commercially available bismuth(III) nitrate or lead(II) nitrate as the cation source. When the reaction was carried out at 75 °C in methanol, the appreciable spectral changes for the insertion of Bi(III) were completed within 34 minutes (Fig. 2), leading us to infer that the insertion reaction is also complete. The insertion of Pb(II) was complete after *ca.* 98 minutes. In other words, the insertion time for Bi(III) is about 50% of the half-life of ^{212}Bi and the insertion time for Pb(II) is about 14% of the half-life of ^{212}Pb . This leads us to suggest that texaphyrin may have a role to play as a ligand for ^{212}Bi or ^{212}Pb .

Crystals of **2a** suitable for X-ray diffraction analysis were obtained by slow evaporation from methanol. The complex exists as a binuclear complex in the solid state with a μ -oxo atom bridging the Bi(III) centers ligated by the individual texaphyrin subunits (Fig. 3). One

axial nitrate anion and one methanol molecule are bound to each bismuth cation; the latter is consistent with the oxophilicity of bismuth. The out-of-mean plane distance of the Bi(III) center is roughly 0.3 Å.

To the best of our knowledge, complex **2a** is the first structurally characterized binuclear macrocyclic μ -oxo bismuth complex, and the first stacked expanded porphyrin system to be prepared with a texaphyrin and a non-transition metal. In contrast to what is true for texaphyrins, however, a number of μ -oxo metalloporphyrin complexes are known (albeit not with bismuth). In general, the μ -oxo bridge in these latter complexes can be cleaved by addition of an aqueous acid, such as hydrochloric or hydrobromic acid, to yield, as a general rule, the corresponding monomeric species. In the case of **2a**, however, such treatments led only to decomposition. On the other hand, the use of non-aqueous conditions (trifluoroacetic acid (TFA) in DMSO- d_6) causes a conversion of the binuclear species **2a** to its corresponding mononuclear form (Fig. 4), as inferred from ^1H -NMR spectroscopic analyses (see ESI[†], page S17). This process (conversion to the mononuclear form) is fully reversible. For instance, addition of triethylamine (TEA) serves to regenerate the μ -oxo binuclear complex **2a**.

In accord with design expectations, the water-soluble texaphyrin derivatives coordinating bismuth (**1a**, MBi) and lead (**1b**, MPb) display high levels of stability in water as judged by HPLC analyses. No appreciable decomposition products were observed in aqueous solutions of **1a** and **1b** at pH values ranging from 6 to 7.5. To determine the inherent cytotoxicity of **1a** and **1b** towards potential tumor targets, cell proliferation assays were conducted using an A2780 ovarian cancer cell line, which was recently found to display sensitivity towards MGd.²⁶ As shown in Fig. 5, both MBi and MPb gave IC₅₀ values of 2.2 and 2.9 μM , respectively. While such values are not particularly noteworthy *per se*, they are of interest in that they reflect a 2–3 fold increase in cytotoxicity relative to MGd (IC₅₀ = 6.3 μM in this cell line).

At this point, it is unknown whether the observed cytotoxicity is due to the redox mediation typically noted for texaphyrin complexes^{27,28} or due to heavy metal toxicity, or some combination thereof. However, we favor the former rationale. This is because, in this particular cellular assay, the cytotoxicity (antiproliferative activity) of the free metals (*i.e.* Bi(NO₃)₃ and Pb(NO₃)₂) was significantly lower than that of the corresponding metal complexes. Bismuth(III) nitrate has an IC₅₀ value of 7.9 μM , and under the conditions of our experiments the IC₅₀ value for lead(II) nitrate was found to be between 100 and 300 μM (see ESI[†], page S27). An accurate value for the latter could not be determined due to solubility limits of the metal salt. Further studies to substantiate these observations are currently underway.

In summary, we have shown that both the bismuth(III) and lead(II) cations form stable complexes with texaphyrins. A single crystal X-ray crystallographic analysis of the bismuth(III) complex **2a** reveals that it forms a binuclear μ -oxo sandwich complex²⁹ that can be cleaved to its corresponding monomer by addition of TFA under non-aqueous conditions. The water soluble complexes functionalized with polyethylene glycol groups, **1a** and **1b**, show cytotoxic activity in the A2780 ovarian cancer cell line. On the basis of the present studies and appreciating the tumor-selective properties of texaphyrins, we suggest that this class of ligands would make good complexants for ²¹²Bi, ²¹³Bi, or ²¹²Pb and thus warrant further study as candidates for radiotherapy. The present study also serves to underscore how new coordination modes, namely μ -oxo Bi(III) complexes, can be stabilized using texaphyrins as opposed to, *e.g.*, porphyrins. This provides an incentive to study further the fundamental coordination chemistry of this and other new porphyrin analogues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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29. The crystal data and experimental details for the structural refinement of **2a** are provided in the ESI† section. CCDC 790736.

Fig. 1. Bi(III) and Pb(II)–texaphyrins. Note that ancillary ligands are not shown. In fact, complex **2a** exists in the form of a μ -oxo binuclear complex under most conditions: see text.

Fig. 2. Changes in the UV-Vis spectrum of **1a** produced when the sp^3 precursor was treated with Bi(III) nitrate in methanol at 75 °C under aerobic conditions. Note that the spectrum recorded at 34 minutes is identical to that recorded at 38 minutes.

Fig. 3. Single crystal X-ray structure of **2a** (displacement ellipsoids scaled to 50% probability level). Note that the structure on the right shows only one individual texaphyrin subunit viewed from the top and with the ancillary ligands removed for clarity.

Fig. 4. Reactions involving the μ -oxo bond in **2a** observed upon the addition of TFA and TEA.

Fig. 5.
Cell proliferation vs. complex concentration for MBI and MPb (complexes **1a** and **1b** of this study) using the A2780 ovarian cancer cell line.