Unprecedented Incorporation of α-Emitter Radioisotope ^{213}Bi into Porphyrin Chelates with Reference to a Daughter Isotope Mediated Assistance Mechanism

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Experimental part

Synthesis of 1, 1Bi, 2 and 2Bi

Compound **1** and complex **1Bi** (cold ^{209}Bi isotope) were prepared as previously described.[1] Compound **2** and complex **2Bi** were synthezised as previously reported.[2]

Stability of 1Bi and 2Bi in cell culture media

Two media were used: 1) pure fetal calf serum (FCS); 2) Eagle's minimum essential medium (EMEM) supplemented with 10 % (v/v) FCS and glutamine (2 mM). The metal complex **1Bi** (or **2Bi**) (cold ^{209}Bi isotope) was solubilized in DMSO and added to the cell culture media at final concentrations of 15 µM and 5 µM, respectively. The mixtures were stirred at 37 °C and the stability of the complex was monitored by UV-visible spectroscopy.

Cytotoxic assays

Human HT29 colon carcinoma (mutated p53) and Hela cervical carcinoma (WT p53) cell lines were obtained from ATCC (Rockville, MD, USA) and cultured in Eagle's minimum essential medium (EMEM) (Eurobio, Les Ulis, France) supplemented with 10 % (v/v) fetal calf serum (FCS) (GibcoBRL), glutamine (2 mM). For cytotoxic assays, cells were seeded in 96-well flat bottomed plates at a density of 3×10^4 cells/well (HT29) or 10^4 cells/well (Hela). 24 hours later, cells were treated in sextuplate with increased concentrations of compound **1** or **1Bi** (cold ^{209}Bi isotope) in EMEM medium without FCS for 72 hours. After treatment, cell viability was assessed by a methylene blue colorimetric assay. Briefly, cells were washed 3 times in PBS and were fixed for 30 min in ethanol 95%. Following removal of ethanol, fixed cells were next dried and coloured for 5 min in methylene blue. After 3 washes in tap water, 100 µl of HCl 0.1 N per well was added. Plates were then analyzed with a spectrometer at 620 nm and the % of viability was calculated.

General procedures for ^{213}Bi radiolabelling

All experiments with radioactive ^{213}Bi isotope were performed in a secured and dedicated room with all safety precautions related to the use of α-emitters. The ^{225}Ac-^{213}Bi generator was supplied by the Institute for Transuranium Elements (Karlsruhe, Germany).

The generator was eluted approximately every 2 hours with a 1:1 HCl/NaI solution (0.1 M, 600 µL), following a standard protocol.[3] The initial concentration of ^{213}Bi was determined by radioactivity counting (^{213}Bi activity was measured with a calibrated NaI(Tl) scintillator (RayTest, France)). The average ^{213}Bi concentration was 0.3 nM.

Typical protocol for ligand labeling: to 15 µL of a 159 µM solution of compound **1** in THF/EtOH (1:1 v/v) were added 12 µL of an aqueous NaOH solution (1M), 50 µL of a 2M buffer solution (acetate for pH 5 and 6, and Tris for pH 7 and 8) and 120 µL of the eluate from the generator. The mixture was vortexed and then allowed for standing in a thermoregulated plate. To monitor the rate of ^{213}Bi insertion, aliquots (2 µL) were deposited on a TLC plate (silicagel, Fluka, France) over a period of 30 min (TLC eluent: CH_2Cl_2/MeOH 9:1 v/v). TLC were visualized with a radiosensitive screen scanned with a phosphorimager instrument (Typhoon 9410, Amersham GB). Percentages of ^{213}Bi incorporation were deduced from the ratio of the radioactivity intensities at R_f ~ 0.5 (which corresponds to **1.^{213}Bi**) and R_f ~ 0 (which corresponds to the remaining ^{213}Bi salts). They are average values of at least two experiments (error estimated: +/- 5 %).

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[1] Z. Halime, M. Lachkar, T. Roisnel, E. Furet, J.-F. Halet and B. Boitrel, *Angew. Chem., Int. Ed*. 2007, **46**, 5120-5124.
[2] Z. Halime, S. Balieu, B. Najjari, M. Lachkar, T. Roisnel and B. Boitrel, *J. Porphyrins Phthalocyanines* 2010, **14**, 412-420.
[3] M. R. McDevitt, R. D. Finn, G. Sgouros, D. Ma and D. A. Scheinberg, *Appl. Radiat. Isot.* 1999, **50**, 895-904.

Kinetic studies for the metalation of porphyrins 1 and 2 with cold isotopes

UV-visible spectra were recorded on a SPECORD S600 spectrophotometer (Analitik Jena) with a measuring time of 200 ms.

A 4.0 mM stock solution of ligand **1** was prepared by dissolving 3.0 mg of this compound in 600 µL of DMSO containing 2.0 µL of DIPEA (5 equiv.) (= solution S1). A 3.1 mM stock solution of Bi(III) was prepared by dissolving 6.0 mg of $Bi(NO_3)_3,5H_2O$ in 4.0 mL of DMSO (= solution S2). A 3.3 mM stock solution of Pb(II) was prepared by dissolving 8.7 mg of $Pb(OAc)_2,3H_2O$ in 7.0 mL of DMSO (= solution S3).

For the direct metalation process (5 equiv. of bismuth), 25 µL of S2 were added to 4 µL of S1 in 2 mL of DMSO. UV-visible spectra were recorded every 2 min. For the transmetalation process (2.5 equiv. of lead then 5 equiv. of bismuth), 12 µL of S3 were first added to 4 µL of S1 in 2 mL of DMSO, and UV-visible spectra were recorded every 5 seconds. After 1 min, 25 µL of S2 were added and UV-visible spectra were recorded every 2 seconds.

The same protocol was used for ligand **2**.

Stability of 1Bi vs TFA

The complex **1Bi** in DMSO was formed according to the above described procedure (direct metalation process). After 20 min, 5 µL of trifluoroacetic acid (TFA, 5000 equiv.) were added and UV-visible spectra were recorded over 6 hours.

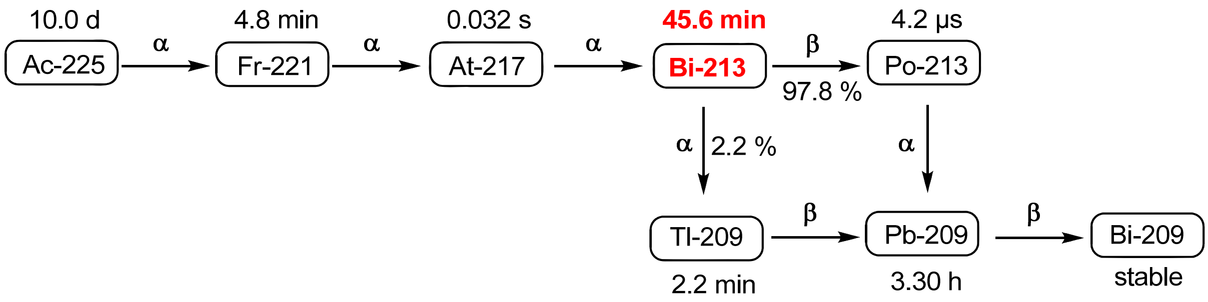

Figure S1. Decay scheme from ^{225}Ac through ^{213}Bi down to stable ^{209}Bi.

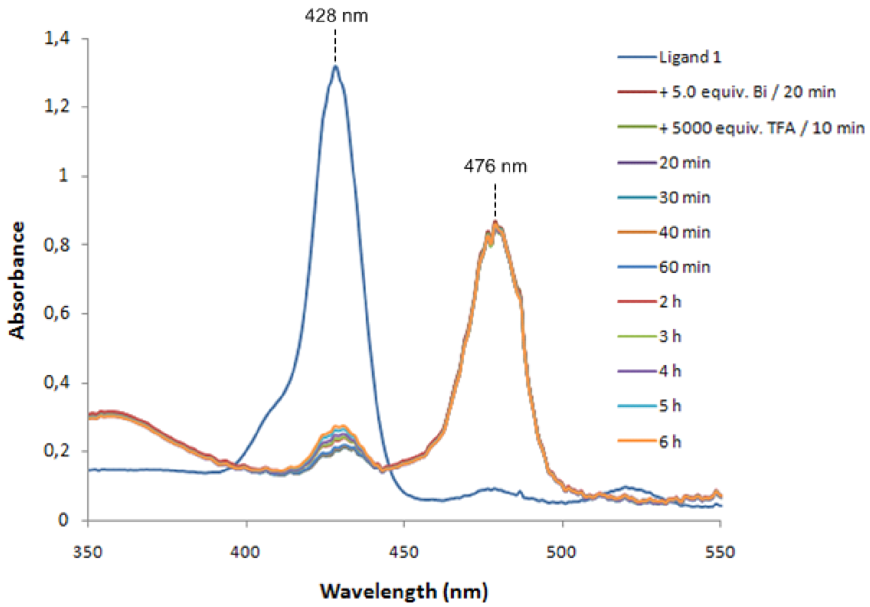

Figure S2. UV-visible monitoring of the stability of complex **1Bi** in the presence of TFA. Conditions: DMSO, [**1Bi**] = 8.0 µM, then TFA (5000 equiv.).

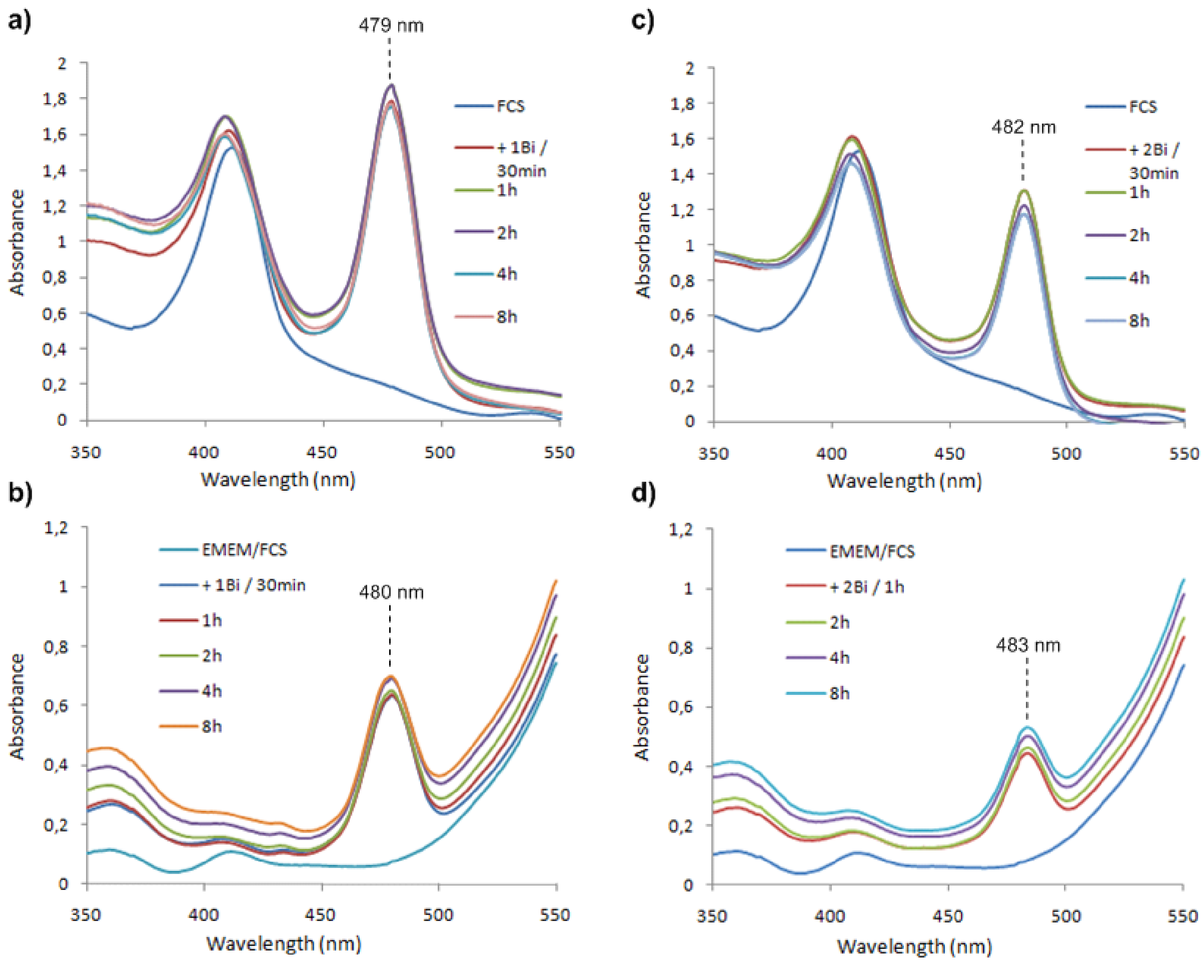

Figure S3. UV-visible monitoring of the stability of complexes **1Bi** and **2Bi** in cell culture media, at 37 °C: (a) **1Bi** in pure FCS; (b) **1Bi** in EMEM supplemented with 10 % (v/v) FCS, glutamine (2 mM); (c) **2Bi** in pure FCS; (d) **2Bi** in EMEM supplemented with 10 % (v/v) FCS, glutamine (2 mM).

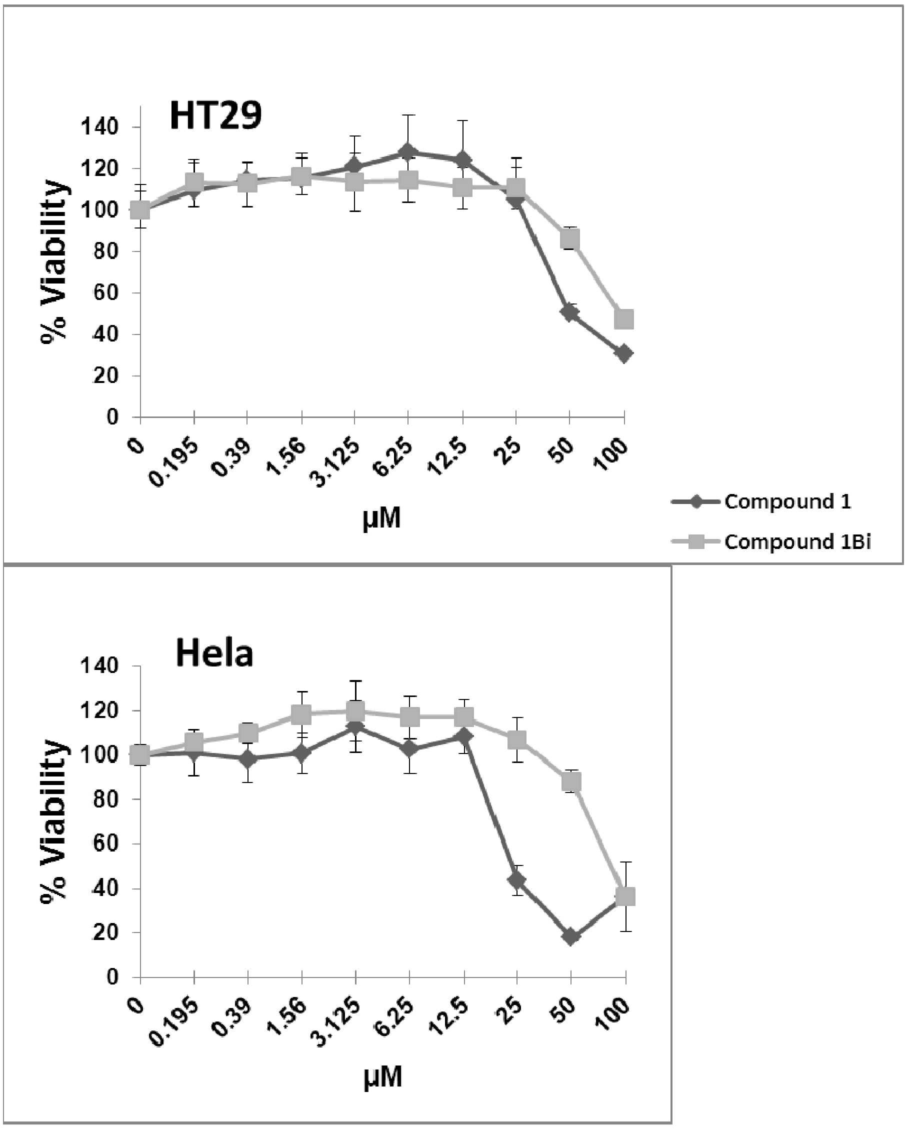

Figure S4. Cytotoxicity of compounds **1** and **1Bi** on human HT29 colon carcinoma and Hela cervical carcinoma cell lines. Error bars correspond to standard deviations.

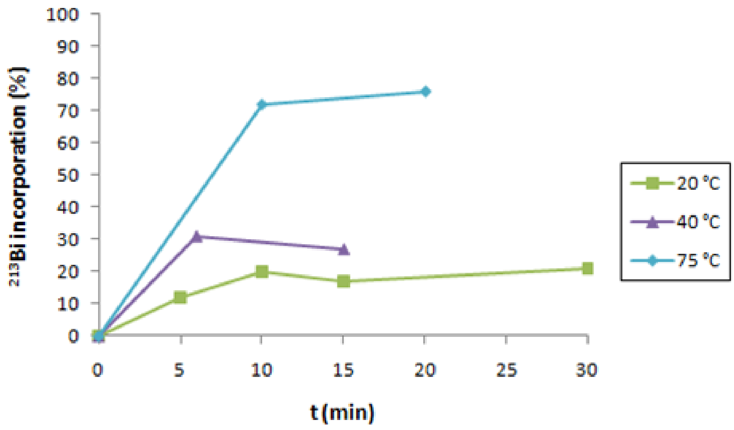

Figure S5. Influence of temperature on the rate of ^{213}Bi incorporation into ligand **2**, at pH 7.

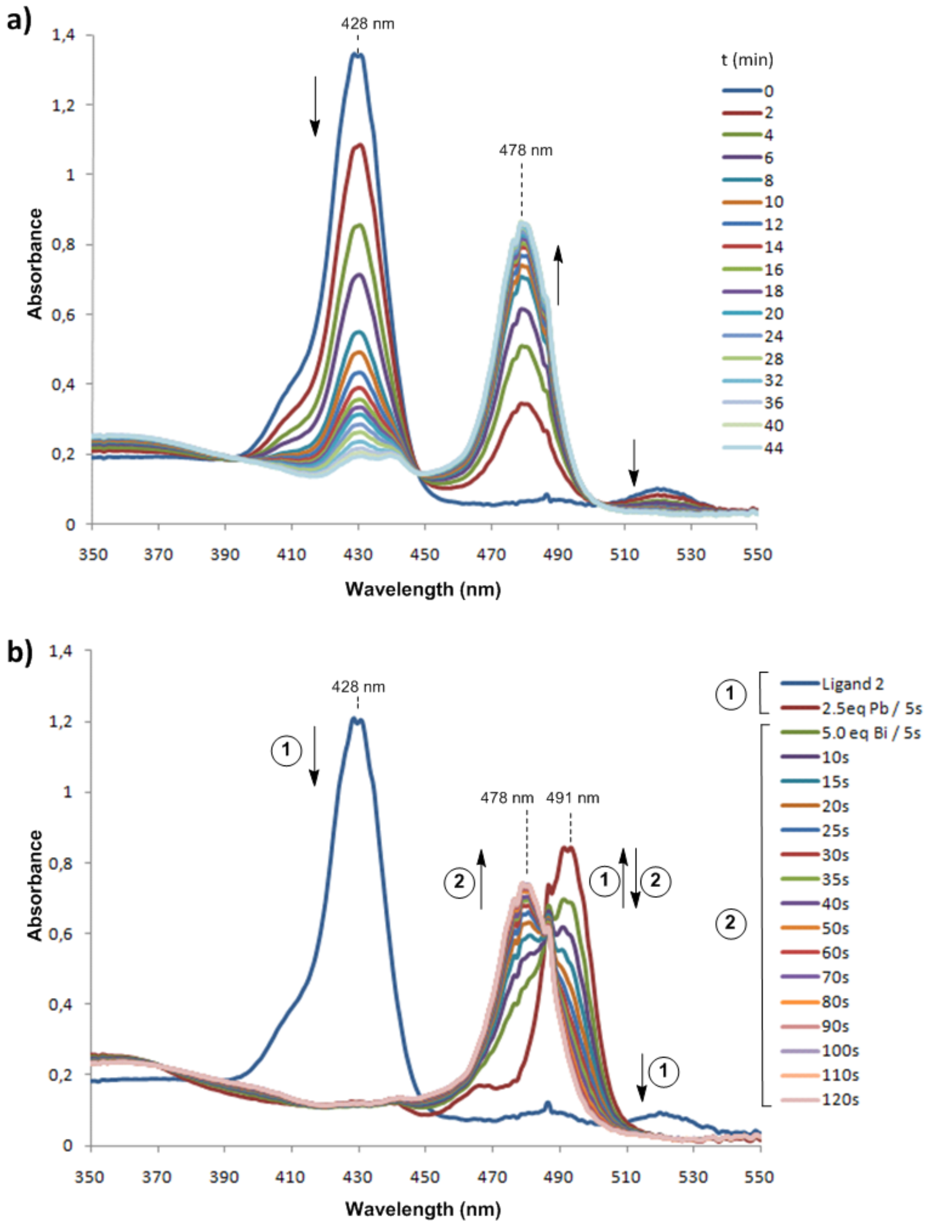

Figure S6. UV-visible monitoring at room temperature of the formation of the bismuth complex with ligand **2**: (a) direct metalation; (b) transmetalation process. Conditions: DMSO, $[\mathbf{2}]_0$ = 8 µM, 5 equiv. DIPEA; for (a): 5 equiv. of $Bi(NO_3)_3$; for (b): 2.5 equiv. of $Pb(OAc)_2$ (1^{st} step) then 5 equiv. of $Bi(NO_3)_3$ (2^{nd} step).