# A cocktail of <sup>165</sup>Er(III) and Gd(III) complexes for quantitative detection of zinc by SPECT and MRI

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# Synthesis:

All commercial reagents and solvents were purchased from different suppliers such as Alfa-Aeser, CheMatech, Fisher Scientific, Sigma-Aldrich and TCIChemicals were used as received from the suppliers unless otherwise indicated. The solvents used for reactions were dried and kept under molecular sieve.

Reactions were monitoring by thin layer chromatography (TLC) plates provided by Merck, aluminium sheets cover with silica gel 60 F254. The TLCs were revealed under the appropriate conditions.

Purifications by flash chomatography were performed on a SPOT Flash II systemfrom Interchim.

<sup>1</sup>H and <sup>13</sup>C and complementary experiments (COSY, HSQC and HMBC) NMR spectra were recorded on a Bruker Advance III HD Spectrometer using a 5 mm BBFO probe. <sup>1</sup>H and <sup>13</sup>C were obtainedrespectively at 600 MHz and 150 MHz. Chemical shifts are reported in  $\delta$  (ppm) and coupling constants (J) are given in Hertz (Hz). The multiplicity of signal are given for the <sup>1</sup>H NMR spectra (s: singulet, d: doublet, t: triplet, q: quadruplet and m: multiplet, bs: broad signal, p: pseudo).

High Resolution Mass Spectrometry (HRMS) spectra were recorded using an electron spray ionization (ESI) technique.



Scheme S1: Synthesis of ligand L. Reagents and conditions: (a) diethyl iminodiacetate,  $K_2CO_3$ ,  $CH_3CN$ , reflux; (b) Nɛ-Boc-L-lysine methyl ester hydrochloride, DIEA,  $CH_3CN$ , reflux; (c) Ethyl bromoacetate, DIEA,  $CH_3CN$ , reflux; (d) TFA,  $CH_2Cl_2$ , rt ; (e) NBS, AIBN,  $CHCl_3$ , reflux; (f) **5**, DIEA, NaI, reflux ; (g) LiOH, THF/H<sub>2</sub>O (1 :1 v/v), rt.

# Compound 1: Diethyl 2,2'-(((6-(bromomethyl)pyridin-2-yl)methyl)azanediyl)diacetate



This compound was prepared as described in the literature.<sup>[1]</sup>

To a solution of 2,6-bis(bromomethyl)pyridine (5.0 g, 19.02 mmol) in acetonitrile (200 mL) were added potassium carbonate (2.2 g, 15.92 mmol) and diethyl iminodiacetate (1.2 g, 6.34 mmol) dropwise over a period of 1 h. The mixture was refluxed for an additional 3h. The solids wereremoved by filtration and the solvent was evaporated. The product **1** was obtained after purification by flash chromatography on silica gel (Dichloromethane: Ethyl Acetate 6:4) as a yellow oil (2.06 g). **Yield: 87 %** 

<sup>1</sup>**H NMR (600 MHz, CDCl<sub>3</sub>)**: $\delta$ (ppm) 7.68 (pt,  ${}^{3}J_{H_{4}-H_{3}}_{and 5}$ = 7.8 Hz, 1H<sub>4</sub>); 7.56 (d,  ${}^{3}J_{H_{3}-H_{4}}$ = 7.8 Hz, 1H<sub>3</sub>); 7.34 (d,  ${}^{3}J_{H_{5}-H_{4}}$ = 7.8 Hz, 1H<sub>5</sub>); 4.53 (s, 2H<sub>7</sub>); 4.16 (q,  ${}^{3}J_{H_{10}-H_{11}}$ = 7.2 Hz, 4H<sub>10</sub>); 4.07 (s, 2H<sub>1</sub>); 3.63 (s, 4H<sub>8</sub>); 1.25 (t,  ${}^{3}J_{H_{11}-H_{10}}$ = 7.2 Hz, 6H<sub>11</sub>).

<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):δ(ppm) 170.7 (C9); 158.8 (C2); 155.7 (C6); 137.3 (C4); 121.9 (C3); 121.6 (C5); 60.1 (C10); 59.4 (C1); 54.4 (C8); 33.7 (C7); 13.9 (C11).

Compound 2: Diethyl 2,2'-(((6-(((6-((tert-butoxycarbonyl)amino)-1-methoxy-1-oxohexan-2-yl)amino)methyl)pyridin-2-yl)methyl)azanediyl)diacetate



A solution of compound **1** (419.2 mg, 1.12 mmol) in acetonitrile (30 mL) was added dropwise over a period of 1h to a mixture of N $\epsilon$ -Boc-L-lysine methyl ester hydrochloride (1g, 3.37 mmol) and diisoproplylethylamine (1.45g, 11.23 mmol) in acetonitrile (70 mL). The mixture was refluxed for 18h. The solvent was evaporated, and the crude product was purified by flash chromatography on silica gel (Ethyl Acetate, 1% triethylamine) to afford compound **2** as a yellow oil (438 mg). **Yield: 71 %** 

<sup>1</sup>**H NMR (600 MHz, CDCl<sub>3</sub>)**: $\delta$  (ppm) 7.63 (pt, <sup>3</sup>J<sub>H1-H2 and 2'</sub> = 7.6 Hz, 1H<sub>1</sub>); 7.46 (d, <sup>3</sup>J<sub>H2-H1</sub> = 7.6Hz, 1H<sub>2</sub>); 7.21 (d, <sup>3</sup>J<sub>H2'-H1</sub> = 7.6 Hz, 1H<sub>2'</sub>); 4.81 (s, 1H<sub>18</sub>); 4.17 (q, <sup>3</sup>J<sub>H7-H8</sub> = 7.2 Hz, 4H<sub>7</sub>); 4.04 (s, 2H<sub>4</sub>); 3.96 (d, <sup>2</sup>J<sub>H9a</sub>-H9b = 15.4 Hz, 1H<sub>9a</sub>); 3.84 (d, <sup>2</sup>J<sub>H9b</sub>-H9a = 15.4 Hz, 1H<sub>9b</sub>); 3.70 (s, 3H<sub>13</sub>); 3.61 (s, 4H<sub>5</sub>); 3.31 (t, <sup>3</sup>J<sub>H10-H11</sub> = 6.6 Hz, 1H<sub>10</sub>); 3.10 (m, 2H<sub>17</sub>); 2.30 (bs, 1H<sub>11</sub>); 1.70 (m, 2H<sub>14</sub>); 1.48 (m, 2H<sub>16</sub>); 1.42 (s, 9H<sub>21</sub>); 1.40 (m, 2H<sub>15</sub>); 1.26 (t, <sup>3</sup>J<sub>H8-H7</sub> = 7.2, 6H<sub>8</sub>).

<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):δ (ppm) 175.4 (C12); 171.0 (C6); 158.5 (C3'); 158.3 (C3); 155.8 (C19); 136.9 (C1); 121.0 (C2); 120.3 (C2'); 78.7 (C20); 60.8 (C10); 60.3 (C7); 59.8 (C4); 54.7 (C5); 53.2 (C9); 51.6 (C13); 40.2 (C17); 32.9 (C14); 29.7 (C16); 28.3 (C21); 22.9 (C15); 14.1 (C8).

HRMS: calc. for  $C_{27}H_{44}N_4O_8$  [M+H]<sup>+</sup> 553.3159 ; found 553.3233

Compound 3: Diethyl 2,2'-(((6-(((6-((tert-butoxycarbonyl)amino)-1-methoxy-1-oxo hexan-2-yl)(2ethoxy-2-oxoethyl)amino) methyl)pyridin-2-yl)methyl)azanediyl) diacetate



A mixture of compound **2** (436 mg, 0.79 mmol), ethyl bromoacetate (171.3 mg, 1.03 mmol) and diisoproplylethylamine (816 mg, 6.31 mmol) in acetonitrile (10 mL) was refluxed for 23h. The solvent was evaporated, and product **3** was isolated after a purification by flash chromatography on silica gel (Dichloromethane: Ethyl Acetate 1:1 + 1% Triethylamime) as a yellow oil (430 mg). **Yield: 85 %** 

<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): $\delta$  (ppm) 7.66 (t,  ${}^{3}J_{H_{1}-H_{2 \text{ and } 2'}} = 7.6 \text{ Hz}$ , 1H<sub>1</sub>); 7.52 (d,  ${}^{3}J_{H_{2}-H_{1}} = 7.6 \text{ Hz}$ , 1H<sub>2</sub>); 7.45 (d,  ${}^{3}J_{H_{2'}-H_{1}} = 7.6 \text{ Hz}$ , 1H<sub>2</sub>); 4.73 (s, 1H<sub>18</sub>); 4.17 (q,  ${}^{3}J_{H_{7}-H_{8}} = 7.1 \text{ Hz}$ , 4H<sub>7</sub>); 4.12 (q,  ${}^{3}J_{H_{23}-H_{24}} = 7.1 \text{ Hz}$ , 2H<sub>23</sub>); 4.03 (s, 2H<sub>4</sub>); 3.97 (d,  ${}^{2}J_{H_{9a}-H_{9b}} = 15.5 \text{ Hz}$ , 1H<sub>9a</sub>); 3.84 (d,  ${}^{2}J_{H_{9b}-H_{9a}} = 15.5 \text{ Hz}$ , 1H<sub>9b</sub>); 3.70 (s, 3H<sub>13</sub>); 3.61 (s, 4H<sub>5</sub>); 3.53 (d,  ${}^{2}J_{H_{11a}-H_{11b}} = 17.6 \text{ Hz}$ , 1H<sub>11a</sub>); 3.24 (d,  ${}^{2}J_{H_{11b}-H_{11a}} = 17.6 \text{ Hz}$ , 1H<sub>11b</sub>); 3.43 (t,  ${}^{3}J_{H_{10}-H_{14}} = 7.5 \text{ Hz}$ , 1H<sub>10</sub>); 3.09 (m, 2H<sub>17</sub>); 1.73 (m, 2H<sub>14</sub>); 1.45 (m, 2H<sub>16</sub>; 2H<sub>15</sub>); 1.43 (s, 9H<sub>21</sub>); 1.26 (t,  ${}^{3}J_{H_{8}-H_{7}} = 7.1, 6H_{8}$ ); 1.24 (t,  ${}^{3}J_{H_{2a}-H_{23}} = 7.1, 3H_{24}$ ).

<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):δ (ppm) 173.4 (C12); 171.5 (C22); 171.1 (C6); 159.0 (C3'); 158.0 (C3); 155.9 (C19); 137.1 (C1); 121.1 (C2); 120.9 (C2'); 78.8 (C20); 63.4 (C10); 60.4 (C7, C23); 59.8 (C4); 57.6 (C9); 54.8 (C5); 52.5 (C11); 51.3 (C13); 40.2 (C17); 29.7 (C14); 29.4 (C15); 28.3 (C21); 23.2 (C16); 14.1 (C8, C24).

HRMS: calc. for  $C_{31}H_{50}N_4O_{10}$  [M+H]<sup>+</sup> 639.3527 ; found 639.3599

Compound 4: Diethyl 2,2'-(((6-(((6-amino-1-methoxy-1-oxohexan-2-yl)(2-ethoxy-2-oxoethyl) amino)methyl)pyridin-2-yl)methyl) azanediyl) diacetate

CF<sub>2</sub>CO

To a solution of compound **3** (0.428 g, 0.67 mmol) in dichloromethane (5 mL) was added trifluroacetic acid (5 mL) and the mixture was stirred for 1 h at room temperature. The solvent and the excess of trifluoroacetic acid were evaporated to give yellow oil (437 mg). **Yield: 100 %** 

<sup>1</sup>**H NMR (600 MHz, D<sub>2</sub>O)**: $\delta$ (ppm) 8.42 (t, <sup>3</sup>*J*<sub>H<sub>1</sub>-H<sub>2</sub></sub> = 7.8 Hz, 1H<sub>1</sub>); 7.89 (d, <sup>3</sup>*J*<sub>H<sub>2</sub>'-H<sub>1</sub></sub> = 7.8 Hz, 1H<sub>2</sub>); 7.84 (d, <sup>3</sup>*J*<sub>H<sub>2</sub>'-H<sub>1</sub></sub> = 7.8 Hz, 1H<sub>2</sub>'); 6.09 (s, 3H<sub>18</sub>); 4.48 (d, <sup>2</sup>*J*<sub>H<sub>9a</sub>-H<sub>9b</sub> = 17,1 Hz, 1H<sub>9a</sub>); 4.44 (s, 2H<sub>4</sub>); 4.43 (d, <sup>2</sup>*J*<sub>H<sub>9b</sub>-H<sub>9a</sub> = 17,1 Hz, 1H<sub>9b</sub>); 4.13 (m, 4H7, 2H<sub>20</sub>); 3.75 (s, 4H<sub>5</sub>); 3.72 (s, 3H<sub>13</sub>); 3.70 (m, 2H<sub>11</sub>); 3.57 (t, <sup>3</sup>*J*<sub>H<sub>10</sub>-H<sub>14</sub> = 7.2 Hz, 1H<sub>10</sub>); 2.91 (t, <sup>3</sup>*J*<sub>H<sub>17</sub>-H<sub>16</sub> = 7.6 Hz, 2H<sub>17</sub>); 1.80 (m, 2H<sub>14</sub>); 1.65 (m, 2H<sub>16</sub>); 1.45 (m, 2H<sub>15</sub>); 1.22 (m, 6H8, 3H<sub>21</sub>).</sub></sub></sub></sub>

<sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD):δ(ppm) 173.0 (C12); 172.3 (C19); 171.5 (C6); 154.3 (C3, C3'); 145.9 (C1); 124.2 (C2); 124.0 (C2'); 64.8 (C10); 60.9 (C20); 60.8 (C7); 55.5 (C5); 55.1 (C4); 53.3 (C9); 52.9 (C11); 51.0 (C13); 39.0 (C17); 29.4 (C14); 26.8 (C16); 22.9 (C15); 13.0 (C8); 13.0 (C21).

HRMS: calc. for  $C_{26}H_{42}N_4O_8$  [M+H]<sup>+</sup> 539.3003 ; found 539.3077

# Compound 5: Ethyl 2-(bromomethyl)nicotinate



A mixture of Ethyl-2-methylnicotinate (2.00 g, 14.6 mmol), N-bromosuccinimide (3.08 g, 17.3 mmol) and AIBN (0.200 g, 1.22 mmol) in chloroform (120 mL) was heated to reflux with vigorous stirring. After 22h, the reaction mixture was cooled to room temperature and the solvent was then evaporated under vacuum. The residue was dissolved in ethyl acetate (10 mL) and the resulting solution was washed with water (40 mL) and brine (20 mL), dried over MgSO<sub>4</sub>, and concentrated under vacuum. Purification of the residue by flash chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>: Ethyl Acetate 93:7) gave the compound **5** as colorless crystals, (1,461 g). **Yield: 41 %** 

<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.70 (dd,  ${}^{3}J_{H_{1}-H_{2}} = 5.0$  Hz,  ${}^{4}J_{H_{1}-H_{3}} = 1.6$  Hz, 1H<sub>1</sub>); 8.29 (dd,  ${}^{3}J_{H_{3}-H_{2}} = 8.0$  Hz,  ${}^{4}J_{H_{3}-H_{1}} = 1.6$  Hz, 1H<sub>3</sub>); 7.34 (dd,  ${}^{3}J_{H_{2}-H_{1}} = 5.0$  Hz,  ${}^{3}J_{H_{2}-H_{3}} = 8.0$  Hz, 1H<sub>2</sub>); 5.04 (s, 2H<sub>6</sub>); 4.44 (q,  ${}^{3}J_{H_{8}-H_{9}} = 7.2$  Hz, 2H<sub>8</sub>); 1.44 (t,  ${}^{3}J_{H_{0}-H_{8}} = 7.2$  Hz, 3H<sub>9</sub>).

<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 165.4 (C7); 157.7 (C5); 152.1 (C1); 139.4 (C3); 125.6 (C4); 123.1 (C2); 61.9 (C8); 32.4 (C6); 14.2 (C9).

**HRMS:**calc. for  $C_9H_{10}BrN_2O_2$  [M+H]<sup>+</sup> 243.9895found 243.9967.

Compound 6: Diethyl 2,2'-(((5-(((6-((bis(2-ethoxy-2-oxoethyl)amino)methyl)pyridin-2-yl)methyl)(2-ethoxy-2-oxoethyl)amino)-6-methoxy-6-oxohexyl)azanediyl)bis (methylene))dinicotinate



A mixture of compound **4** (0.67 mmol), ethyl 2-(bromomethyl)nicotinate (687 mg, 2.8 mmol), sodium iodide (200 mg, 1.34 mmol) and DIEA (1.3 g, 10.05 mmol) was refluxed for 23h. The solids were filtered and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (Ethyl Acetate, 1% triethylamine) and compound **6** was obtained as a yellow oil (438 mg). **Yield: 76** %

<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): $\delta$  (ppm) 8.57 (d, <sup>3</sup>J<sub>H26'H25</sub> = 3.7 Hz, 2H<sub>26</sub>); 7.96 (d, <sup>3</sup>J<sub>H24'H25</sub> = 7.2 Hz, 2H<sub>24</sub>); 7.63 (pt, <sup>3</sup>J<sub>H1'H2 and 2'</sub> = 7.6 Hz, 1H<sub>1</sub>); 7.45 (d, <sup>3</sup>J<sub>H2'H1</sub> = 7.5 Hz, 1H<sub>2</sub>); 7.40 (d, <sup>3</sup>J<sub>H2'H1</sub> = 7.7 Hz, 1H<sub>2</sub>); 7.19 (dd, <sup>3</sup>J<sub>H25'H26</sub> = 4.9 Hz, <sup>3</sup>J<sub>H25'H24</sub> = 4.9 Hz 2H<sub>25</sub>); 4.29 (q, <sup>3</sup>J<sub>H28'H29</sub> = 7.1 Hz, 4H<sub>28</sub>); 4.24 (s, 4H<sub>18</sub>); 4.15 (q, <sup>3</sup>J<sub>H7'H8</sub> = 7.1 Hz, 4H<sub>7</sub>); 4.09 (q, <sup>3</sup>J<sub>H20'H21</sub> = 7.1 Hz, 2H<sub>20</sub>); 4.01 (s, 2H<sub>4</sub>); 3.98 (d, <sup>2</sup>J<sub>H9a'H9b</sub> = 15.6 Hz, 1H<sub>9a</sub>); 3.88 (d, <sup>2</sup>J<sub>H9b'H9a</sub> = 15.6 Hz, 1H<sub>9b</sub>); 3.6 (s, 3H<sub>13</sub>); 3.59 (s, 4H<sub>5</sub>); 3.51 (d, <sup>2</sup>J<sub>H11a'H11b</sub> = 14.8 Hz, 1H<sub>11a</sub>); 3.46 (d, <sup>2</sup>J<sub>H11b'H11a</sub> = 14.8 Hz, 1H<sub>11b</sub>); 3.35 (t, <sup>3</sup>J<sub>H10'H14</sub> = 7.2 Hz, 1H<sub>10</sub>); 2.50 (s, 2H<sub>17</sub>); 1.58 (m, 2H<sub>14</sub>); 1.39 (m, 2H<sub>16</sub>); 1.35 (t, <sup>3</sup>J<sub>H29'H28</sub> = 7.1 Hz, 6H<sub>29</sub>); 1.25 (t, <sup>3</sup>J<sub>H8'H7</sub> = 7.1 Hz, 6H<sub>8</sub>); 1.21 (t, <sup>3</sup>J<sub>H21'H20</sub> = 7.1 Hz, 3H<sub>21</sub>); 1.17 (m, 2H<sub>15</sub>).

<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):δ (ppm) 175.8 (C12); 171.6 (C19); 169.3 (C6); 165.0 (C27); 158.2 (C23); 157.1 (C22); 155.9 (C3, C3'); 148.5 (C26); 135.6 (C24); 135.3 (C1); 125.7 (C25); 119.5 (C2); 119.4 (C2'); 62.0 (C10); 59.3 (C28); 58.6 (C7); 58.5 (C20); 58.0 (C4); 56.2 (C18); 55.9 (C9); 53.0 (C5); 51.7 (C17); 50.5 (C11); 49.4 (C13); 27.7 (C14); 24.3 (C16); 22.1 (C15); 12.3 (C29); 12.2 (C8); 12.2 (C21).

HRMS: calc. for  $C_{44}H_{60}N_6O_{12}$  [M+H]<sup>+</sup> 865.4269 ; found 865.4340

Ligand 2,2'-(((5-(((6-((bis(carboxymethyl)amino)methyl)pyridin-2-yl)methyl) (carboxymethyl)amino)-5-carboxypentyl) azanediyl)bis (methylene))dinicotinic acid



To a solution of compound **6** (0.210 g, 0.281 mmol) in a mixture of THF: H2O (1:1 v:v, 20 mL), was added lithium hydroxide (0.222 g, 5,29 mmol) and the solution was stirred for 1 day at room temperature. The solvent was evaporated and the ligand **L2** was obtained after a purification on C18 phase (MeOH: H2O 1:1 with 0,1 % TFA), as a white solid (0.172 g). **Yield: 90 %** 

<sup>1</sup>**H NMR (600 MHz, D<sub>2</sub>O)**:  $\delta$  (ppm) 8.43 (d, <sup>3</sup>J<sub>H<sub>20</sub>-H<sub>19</sub></sub> = 4.0 Hz, 2H<sub>20</sub>); 8.14 (d, <sup>3</sup>J<sub>H<sub>18</sub>-H<sub>19</sub></sub> = 7.7 Hz, 2H<sub>18</sub>); 8.01 (t, <sup>3</sup>J<sub>H<sub>1</sub>-H<sub>2</sub> and 2'</sub> = 7.5 Hz, 1H<sub>1</sub>); 7.53 (d, <sup>3</sup>J<sub>H<sub>2</sub> and 2'-H<sub>1</sub></sub> = 7.5 Hz, 1H<sub>2</sub> and 1H<sub>2'</sub>); 7.35 (dd, <sup>3</sup>J<sub>H<sub>19</sub>-H<sub>18</sub></sub> = 7.7 Hz, <sup>4</sup>J<sub>H<sub>19</sub>-H<sub>19</sub></sub> = 4.0 Hz, 2H<sub>19</sub>); 4.82 (s, 4H<sub>15</sub>); 4.51 (s, 2H<sub>4</sub>, 2H<sub>4'</sub>); 3.92 (s, 4H<sub>5</sub>); 3.88 (d, <sup>2</sup>J<sub>H<sub>8a</sub>-H<sub>8b</sub></sub> = 15.0 Hz, 1H<sub>8a</sub>); 3.84 (d, <sup>2</sup>J<sub>H<sub>8a</sub>-H<sub>8b</sub></sub> = 15.0 Hz, 1H<sub>8b</sub>); 3.80 (t, <sup>3</sup>J<sub>H<sub>7</sub>-H<sub>11</sub></sub> = 6.5 Hz, 1H<sub>7</sub>); 3.42 (t, <sup>3</sup>J<sub>H<sub>14</sub>-H<sub>13</sub></sub> = 6.8 Hz, 2H<sub>14</sub>); 1.87 (m, 2H<sub>11</sub>); 1.82 (m, 2H<sub>13</sub>); 1.57 (m, 2H<sub>12</sub>).

<sup>13</sup>C NMR (150 MHz, D<sub>2</sub>O):δ(ppm) 172.9 (C10); 171.5 (C9); 170.7 (C6); 168.3 (C21); 151.2 (C17); 151.0 (C3); 150.5 (C3'); 149.7 (C16); 142.3 (C1); 140.6 (C18); 127.2 (C19); 124.8 (C2, C2'); 66.2 (C7); 58.3 (C4, C4'); 57.2 (C15); 56.5 (C14); 55.5 (C5); 53.5 (C8); 27.1 (C11); 22.9 (C13); 21.4 (C12).

HRMS: calc. forC<sub>33</sub>H<sub>38</sub>N<sub>6</sub>O<sub>12</sub> [M+H]<sup>+</sup> 711.2548 ; found 711.2619

### Solution preparation:

The ligand concentrations were determined by adding an excess of lanthanide solution to the ligand solution and titrating the metal excess with standardised  $Na_2H_2EDTA$  in urotropine buffer (pH 5.6 – 5.8) in the presence of Xylenol Orange as an indicator. The concentrations of the metal solutions were determined similarly by complexometric titrations.

The complexes were prepared by mixing 1 eq. of L, with 1 eq. of  $Gd^{3+}$ , and the pH was adjusted to 7.4 either with a buffered solution or by adding KOH or HCl to the solution. The absence of free  $Gd^{3+}$  was checked by the Xylenol orange test.

The concentrations of  $Gd^{3+}$ -containing solutions were also checked by ICP-MS and BMS measurements when possible.

### **Potentiometric titrations :**

Carbonate-free 0.1 M KOH and 0.1 M HCl were prepared from Fisher Chemicals concentrates. Potentiometric titrations were performed in 0.1 mol.L<sup>-1</sup> aqueous KCl under nitrogen atmosphere and the temperature was controlled to  $25\pm0.1$  °C with a circulating water bath. The p[H] (p[H] = -log[H<sup>+</sup>], concentration in molarity) was measured in each titration with a combined pH glass electrode

(Metrohm) filled with 3M KCl and the titrant addition was automated by use of a 702 SM titrino system (Metrohm). The electrode was calibrated in hydrogen ion concentration by titration of HCl with KOH in 0.1 M electrolyte solution.<sup>[2]</sup>A plot of meter reading versus p[H] allows the determination of the electrode standard potential (E°) and the slope factor (f). Continuous potentiometric titrations with HCl and KOH 0.1 M were conducted on aqueous solutions containing 5 mL of L 3.23 mM in KCl 0.1 M, with 2 minutes waiting between successive points. The titrations of the metal complexes were performed with the same ligand solutions containing 1 or 2 equivalents of metal cation, with 2 minutes waiting time between 2 points. Experimental data were refined using the computer program Hyperquad 2008.<sup>[3]</sup> All equilibrium constants are concentration quotients rather than activities and are defined as:

$$K_{mlh} = \frac{[M_m L_l H_h]}{[M]^m [L]^l [H]^h}$$

The ionic product of water at 25 °C and 0.1 molL<sup>-1</sup> ionic strength is  $pK_w = 13.77$ .<sup>[4]</sup> Fixed values were used for  $pK_w$ , ligand acidity constants and total concentrations of metal, ligand and acid. All values and errors (one standard deviation) reported are at least the average of three independent experiments.



FigureS1 : Potentiometric titration of [L] = 3.23 mM, in the presence of 0, 1 or 2 equivalents of metal ion, in KCl 0.1 M, at 298 K.

Log K <sub>H</sub>	L	L1ª	Ру <sup>ь</sup>
Log K <sub>H1</sub>	10.53(9)	8.85	8.95
Log K <sub>H2</sub>	8.75(6)	8.28	7.85
Log K <sub>H3</sub>	8.04 (4)	4.78	3.38
Log K <sub>H4</sub>	3.79 (5)	3.97	2.48
Log K <sub>H5</sub>	2.81 (6)	3.01	
Log K <sub>H6</sub>	2.40 (9)	2.8	
Log K <sub>H7</sub>	1.9 (1)		

Table S1. Protonation constants measured in KCl (0.1 M) at 298 K.

a. From ref <sup>[1]</sup>; b. From ref <sup>[5]</sup>

Table S2. Stability constants of the different complexes measured by potentiometric titration in KCl (0.1M) at 298 K.

Log K	L	L1 <sup>a</sup>	Py <sup>b</sup>
$\log K_{GdL}$	20.1 (1)	17.35	18.60
$\log K_{\rm GdLH}$	8.92 (6)	4.04	
$\log K_{GdLH2}$	3.74 (4)	3.51	
Log K <sub>GdLH3</sub>	2.54 (6)	2.79	
$\log K_{GdLOH}$	10.88 (5)		
Log K <sub>ZnL</sub>	16.5 (1)	14.13	15.84
Log K <sub>ZnLH</sub>	10.03 (9)	6.67	3.81
Log K <sub>ZnLH2</sub>	4.10 (4)	3.98	
Log K <sub>ZnLH3</sub>	3.20 (7)	3.07	
Log K <sub>ZnLH4</sub>	2.4 (1)	2.74	
Log K <sub>ZnLOH</sub>	11.6 (1)		
Log K <sub>Zn2L</sub>	9.9 (1)	6.53	
Log K <sub>Zn2LH</sub>	3.2 (1)	3.60	

a. From ref<sup>[1]</sup>; b. From ref<sup>[5]</sup>

# **Relaxometric Measurements:**

Proton NMRD profiles were recorded on a Stelar SMARTracer Fast Field Cycling relaxometer (0.01-10 MHz) and a Bruker WP80 NMR electromagnet adapted to variable field measurements (20-80 MHz)

and controlled by a SMARTracer PC-NMR console. The temperature was monitored by a VTC91 temperature control unit and maintained by a gas flow. The temperature was determined by previous calibration with a Pt resistance temperature probe. The longitudinal relaxation rates  $(1/T_1)$  were determined in water.

The  $Zn^{2+}$  titrations of GdLalone or GdL in the presence of HSA, and the HSA titrations of GdL or GdLZn were performed at 20 MHz.

# Relaxivity response to zinc :



Figure S2 : <sup>1</sup>H relaxivity measurements in the presence of [GdL] = 0.99 mM in water, pH = 7.4 (Hepes 0.1 M) in the presence of  $Zn^{2+}$  at 20 MHz and 298 K.

A slight increase of relaxivity of 8.5 % is observed upon the addition of 1 eq. of  $Zn^{2+}$ . The formation of a 2/1 GdL/Zn complex can be excluded as the maximum of the increase is obtained for a  $[Zn^{2+}]/[GdL]$  ratio of 1. Moreover, the number of water molecules directly coordinated to  $Gd^{3+}$  remains constant upon  $Zn^{2+}$  addition (see Table S3). Consequently, this small increase could be ascribed to a small change in  $\tau_R$  due to a change of "shape" of the complex upon  $Zn^{2+}$  binding. However, in the conditions of the experiment ([GdL] < [HSA], there is no "free" GdL (not bound to HSA) so this small relaxivity change is not expected to impact the results.

# Response to zinc as a function of GdL concentration:



Figure S3: Paramagnetic relaxation enhancement as a function of GdL concentration in the presence of 0.6 mM of HSA and in the absence ( $\bullet$ ) or in the presence ( $\diamond$ ) of 1 equivalent of Zn<sup>2+</sup> at 20 MHz and 310 K.

Selectivity for physiological anions



Figure S4: <sup>1</sup>H relaxivity measurements in the presence of [GdL] = 0.30 mM, [HSA] = 0.6 mM in the absence or presence of 1 eq. of  $M^{2+}$  or a mixture of 1 eq. of  $Zn^{2+}$  and different amount of  $Cu^{2+}$  at pH = 7.4 (Hepes 0.1 M), at 20 MHz and 310 K.

# Luminescence lifetime measurements:

Europium luminescence lifetimes were recorded on an Agilent Cary Eclipse Fluorescence spectrophotometer by recording the decay of the emission intensity at 616 nm, following an excitation at 263 nm. Measurements were performed in H<sub>2</sub>O and D<sub>2</sub>O solutions at 0.47 mM in Hepes buffers 0.1 M at pH/pD 7. Ten equivalents of citrate or phosphate were added to both solutions. The settings were as follow: gate time: 0.05 ms; delay time: 0.1 ms; flash count: 1; Total decay time: 6 ms; 100 cycle. At least three decay curves were collected for each sample, all lifetimes were analyzed as monoexponential decays. The reported lifetimes are an average of at least three measurements.

The number of water molecules directly coordinated to  $Eu^{3+}$  were obtained using the empirical equation developped by Horrocks et al.<sup>[6]</sup>:

$$q = 1.11 \left( \tau_{H20}^{-1} - \tau_{D20}^{-1} - 0.3 \right)$$

The results obtained are presented in Table S3.

Table S3 :  $Eu^{3+}$  luminescence lifetimes ( $\tau$ ) in the EuL complex (in 0.1 M hepes buffer pH/pD = 7) in the absence and in the presence of 1 equivalent of  $Zn^{2+}$ , and the corresponding calculated *q*-values.

	EuL	EuL-Zn
τ <sub>H2O</sub> (ms)	0.39 (1)	0.38 (1)
τ <sub>D2O</sub> (ms)	2.03(1)	1.82 (1)
q	2.0 (3)	2.0 (3)

# Production, purification and radiolabeling:

<sup>165</sup>Er ( $t_{1/2}$ = 10.36h) was prepared via irradiation of holmium foil by proton beam (16MeV) on a cyclotron available at the laboratory of Conditions Extrêmes et Matériaux : Haute Température et Irradiation (CEMHTI) in Orléans, France. <sup>165</sup>Ho is the single stable isotope of Holmium (100% abundance). <sup>165</sup>Er is produced from the <sup>165</sup>Ho(p,n)<sup>165</sup>Er nuclear reaction with a specific activity of 500 kBq/(µA.h.mg) at end of beam (16 MeV, 2µA, 2h) and decays by EC to <sup>165</sup>Ho. Another nuclear reaction takes place, producing the <sup>166</sup>Ho radioisotope in a low amount.

The separation of <sup>165</sup>Er from the <sup>165/166</sup>Ho was performed by ion-exchange chromatography. In order to optimize the purification step, <sup>165</sup>Er was produced via the 165Ho(d,p)166Ho (deuteron reaction). This increases <sup>166</sup>Ho to reach a <sup>166</sup>Ho/<sup>165</sup>Er activity ratio of 1:8 and allows a better follow-up of the separation Er/Ho, which facilitates the detection of Ho. For regulatory constraints, production of <sup>165</sup>Er at high activities with deuterons beam was not authorized for the moment. The protocol reported by G. J. Beyer et al.<sup>[7]</sup> was optimized. Briefly, the irradiated Ho foil target is dissolved in 0.5 mL of 5 M nitric acid, evaporated and redissolved in 2 mL of 0.3 M nitric acid. This solution is passed through an ion-exchange column, using a 2-ethylhexylphosphic mono 2-ethylhexyl ester LN2 resin (Triskem, Bruz, France), a resin that has been developed specifically for lanthanide separation, instead of the reported Aminex A5 column. An HNO<sub>3</sub> gradient (0.3–1M) was used as eluent, instead of the  $\alpha$ -hydroxy-isobutyric acid. The fractions were eluted with a flow rate of 1 ml/min.

An aliquot of the separated fractions was diluted and their radionuclidic purity was assessed by gamma spectrometry with a HPGe detector. For the data acquisition, the samples were placed at a distance of 5 cm from the crystal. The HPGe detector was calibrated in energy and efficiency for different geometries with certified standard radioactive sources (Cerca France). For activity measurements,  $\gamma$ -ray spectrum analysis software package Genie 2000 (Canberra, USA) was used.

The gamma-spectra of the deuteron irradiated mixture before and after purification are shown in figure S5. In the  $\gamma$ -spectrum of the mixture we identify the corresponding peaks of <sup>165</sup>Er (46.7–55.3 keV) and <sup>166</sup>Ho (80.6 KeV), while the purified sample shows only the peak corresponding to <sup>165</sup>Er.

The ratio <sup>165</sup>Ho/<sup>165</sup>Er is estimated to be between 3.10<sup>4</sup> (measured from the purification issued from a deuteron production) and 10<sup>6</sup> (maximum estimation from the proton production using the limit of detection of the HPGe detector). Indeed, the presence of <sup>165</sup>Ho<sup>3+</sup> is detected through the activity of <sup>166</sup>Ho<sup>3+</sup> knowing the <sup>165</sup>Ho/<sup>166</sup>Ho ratio after irradiation of the foil. For the production via a deuteron reaction an activity of <sup>166</sup>Ho<sup>3+</sup> is always measured allowing to calculate the ratio <sup>165</sup>Ho/<sup>165</sup>Er precisely. From the proton production, similar purification procedures as the deuteron production is used but because <sup>166</sup>Ho<sup>3+</sup> is present in lower quantities, it is not detected anymore. The limit of detection of the HPGe detector gives an overestimation of the ratio <sup>165</sup>Ho/<sup>165</sup>Er of 10<sup>6</sup>.

The concentration of  $^{165}$ Er<sup>3+</sup> produced being 10<sup>-10</sup> M,  $^{165}$ Ho<sup>3+</sup> concentration should be around 3.10<sup>-6</sup> and definitely lower than 10<sup>-4</sup> M, which should indeed not impact the MRI response in the presence of 3.10<sup>-4</sup> M of Gd<sup>3+</sup> complex.

For the radiolabeling, the pH of the <sup>165</sup>Er solution was adjusted between 5-7 with NaOH and 1:30.10<sup>3</sup> molar ratio of L added (the excess of ligand is used to complex the remaining <sup>165</sup>Ho<sup>3+</sup>) using the following procedure: 27  $\mu$ L of L at 1.1 mM (n<sub>L</sub> = 2.97.10<sup>-8</sup> mol) were added to 415  $\mu$ L of <sup>165</sup>Er<sup>3+</sup> (n<sub>Er</sub> = 1.15.10<sup>-12</sup> mol). The mixture is incubated for 10 min at room temperature and the radiochemical purity was followed by thin-layer chromatography (silica plates), using Water/Methanol/Acetic Acid (4:4:0.2) as mobile phase. The TLCs are exposed by impregnation on a multisensitive phosphor screen (Packard, Perkin Elmer, Meriden, USA, and revealed on a Cyclone Storage phosphor system Packard, Perkin Elmer, Shelton, USA). In this system, the free <sup>165</sup>Er migrates (Rf = 0.4) and the <sup>165</sup>ErL has a Rf of 0.1.



Figure S5: Gamma-spectra of the deuteron irradiated mixture before (a) and after (b) purification. In the  $\gamma$ -spectrum a) it is possible to identify the corresponding peaks of <sup>165</sup>Er and <sup>166</sup>Ho; and in the purified sample b) only the peaks corresponding to <sup>165</sup>Er appear.

# Gamma-camera Imaging:

Scintigraphic imaging was performed with a high-resolution gamma camera (Biospace Mesures, Paris, France) equipped with a position-sensitive photomultiplier tube and a parallel collimator (20mm thickness) with 1.7-mm holes. Images were recorded with a 128x128-pixel and 16-bit matrix, with a spectral window centered 20% on the photopeaks of the <sup>165</sup>Er.

# **MRI Imaging :**

All acquisitions were performed on a 1.5T scanner (Signa HDxt, General Electric, Milwaukee, USA) with an homogenous emitting/receiving single element head coil. Acquisitions consisted in a series of conventional single slice (Thickness= 7mm) spin echo sequences with short echo time (TE= 9ms) and different repetition times (TR= 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 500, 1000, 1500 and 2000ms), and a 256x256 matrix. Samples were previously stored in acquisition room, to avoid temperature variation during scanning.

# **Calibration curve :**



Figure S6 : <sup>1</sup>H relaxivity measurements in the presence of [GdL] = 0.50 mM, [HSA] = 0.6 mM, in the presence of increasing amounts of  $Zn^{2+}$  at pH = 7.4 (Hepes 0.1 M), based on T<sub>1</sub>-weighted images acquired at 1.5 T and room temperature.

### Equations to determine Zn<sup>2+</sup> concentrations :

The relaxivity is defined as follows :

$$r_1 = \left(\frac{1}{T_1}\right)_{para} \times \frac{1}{[Gd]} \tag{1}$$

where [Gd] represents the total Gd<sup>3+</sup> concentration in the sample and  $(\frac{T_1}{T_1})_{para}$ , the paramagnetic relaxation enhancement which is expressed by :

$$(\frac{1}{T_1})_{para} = (\frac{1}{T_1})_{exp} - (\frac{1}{T_1})_{dia}$$
(2)

 $(\frac{1}{T_1})_{exp}$  is the experimentally determined relaxation rate and  $(\frac{1}{T_1})_{dia}$  is the relaxation rate determined in the absence of any paramagnetic species.

The relaxivity of the sample can be easily determined using equations (1) and (2), then using the calibration curve Figure S4, the [Zn]/[GdL] ratio can be readily assessed. As the [GdL] concentration is known, the Zn<sup>2+</sup> concentration can be easily determined.

#### Calculations of errors (uncertainties) :

#### On the Gd<sup>3+</sup> concentrations:

For a GdL stock solution (sol. 1), the Gd<sup>3+</sup> concentration is determined by BMS and/or ICP measurements. This GdL stock solution is used to prepare a cocktail GdL/<sup>165</sup>ErL in HSA 0.6 mM (sol. 2). The ratio  $y = [Er^{3+}]/[Gd^{3+}]$  of this cocktail is determined by measuring the activity and knowing the Gd<sup>3+</sup> concentration of the stock solution.

The cocktail GdL/<sup>165</sup>ErL solution (sol. 2) is then diluted to give five solutions, to which unknown concentrations of  $Zn^{2+}$  are added by keeping 0.6 mM HSA concentration (samples 1-5).

For each individual sample (samples 1-5), the  $Er^{3+}$  concentration is determined by the measurement of the activity. Then the  $Gd^{3+}$  concentration of each sample is calculated from the  $Er^{3+}$ concentration using the ratio:  $y = [Er^{3+}]/[Gd^{3+}]$ .

So the error on the Gd<sup>3+</sup> concentration in samples 1-5 is determined as:

$$\frac{s[Gd]_{exp}}{[Gd]_{exp}} = \sqrt{\left(\frac{s[Er]_{exp}}{[Er]_{exp}}\right)^2 + \left(\frac{sy}{y}\right)^2}$$
(3)

The main error in the determination of  $[Er]_{exp}$  comes from the error of the activity measurement which is 11%. Dilution and time measurement errors are negligible; at least a factor of 100 lower than the error coming from the activity measurement.

By definition:

$$\frac{s[Er]_{exp}}{[Er]_{exp}} = \frac{11}{100 * \sqrt{3}} = 0.0635$$

For the ratio y :

$$\frac{sy}{y} = \sqrt{\left(\frac{s[Er]_i}{[Er]_i}\right)^2 + \left(\frac{s[Gd]_i}{[Gd]_i}\right)^2}$$
(4)

Where  $[Er]_i$  and  $[Gd]_i$  represent the concentrations of  $Er^{3+}$  and  $Gd^{3+}$  in the stock solution. The  $Er^{3+}$  concentration is determined as previously by activity measurement with an error of 11%, and the  $Gd^{3+}$  concentration has a an error of 3% (maximized from ICP and BMS determination).

So finally  $s[Gd]_{exp} = 0.0908^*[Gd]$ , and the final error is  $s[Gd]_{exp}^*\sqrt{3}$ , which gives 15.73 %.

# On the Zn<sup>2+</sup> concentrations:

The Zn<sup>2+</sup> concentration of each sample (samples 1-5) is then determined by measuring a longitudinal relaxation time, and by knowing the concentration of Gd<sup>3+</sup>, the concentration can be deduced using a linear calibration curve. Errors in Zn<sup>2+</sup> concentrations therefore originate from errors in Gd<sup>3+</sup> concentrations (previously determined), errors in T<sub>1</sub> measurements (3 to 5 %), and errors in the calibration curve (on the slope value a, and the x-intercept b) given by the fitting analysis. In the calculations, the error in b becomes negligible so that the equation can be simplified as:

$$\frac{s[Zn]_{exp}}{[Zn]_{exp}} = \sqrt{2 * \left(\frac{s[Gd]_{exp}}{[Gd]_{exp}}\right)^2 + \left(\frac{s(^1/T_1)_{exp}}{(^1/T_1)_{exp}}\right)^2 + \left(\frac{sa}{a}\right)^2}$$
(5)

So finally the error in  $[Zn^{2+}]$  concentration is 23 %.

The errors indicated in Table 1 of the manuscript are the maximized errors corresponding to 23 %.

## References

- C. S. Bonnet, F. Caille, A. Pallier, J.-F. Morfin, S. Petoud, F. Suzenet, E. Toth, *Chem. Eur. J.* 2014, 20, 10959-10969.
- [2] A. E. Martell, R. J. Motekaitis, *Determination and use of stability constants*, VCH, **1992**.
- [3] P. Gans, A. Sabatini, A. Vacca, *Talanta* **1996**, *43*, 1739-1753.
- [4] D. S. Raiford, C. L. Fisk, E. D. Becker, Anal. Chem. **1979**, *51*, 2050-2051.
- [5] L. Pellegatti, J. Zhang, B. Drahos, S. Villette, F. Suzenet, G. Guillaumet, S. Petoud, E. Toth, *Chem. Commun.* **2008**, 6591-6593.
- [6] R. M. Supkowski, W. D. Horrocks, Inorg. Chem. 1999, 38, 5616-5619.
- [7] G. J. Beyer, S. K. Zeisler, D. W. Becker, *Radiochimica Acta* 2004, *92*, 219-222.