



## Supplementary Information for

Toward quantification of hypoxia using fluorinated Eu<sup>III/II</sup>-containing ratiometric probes.

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## Supplementary Information Text

## Experimental Procedures

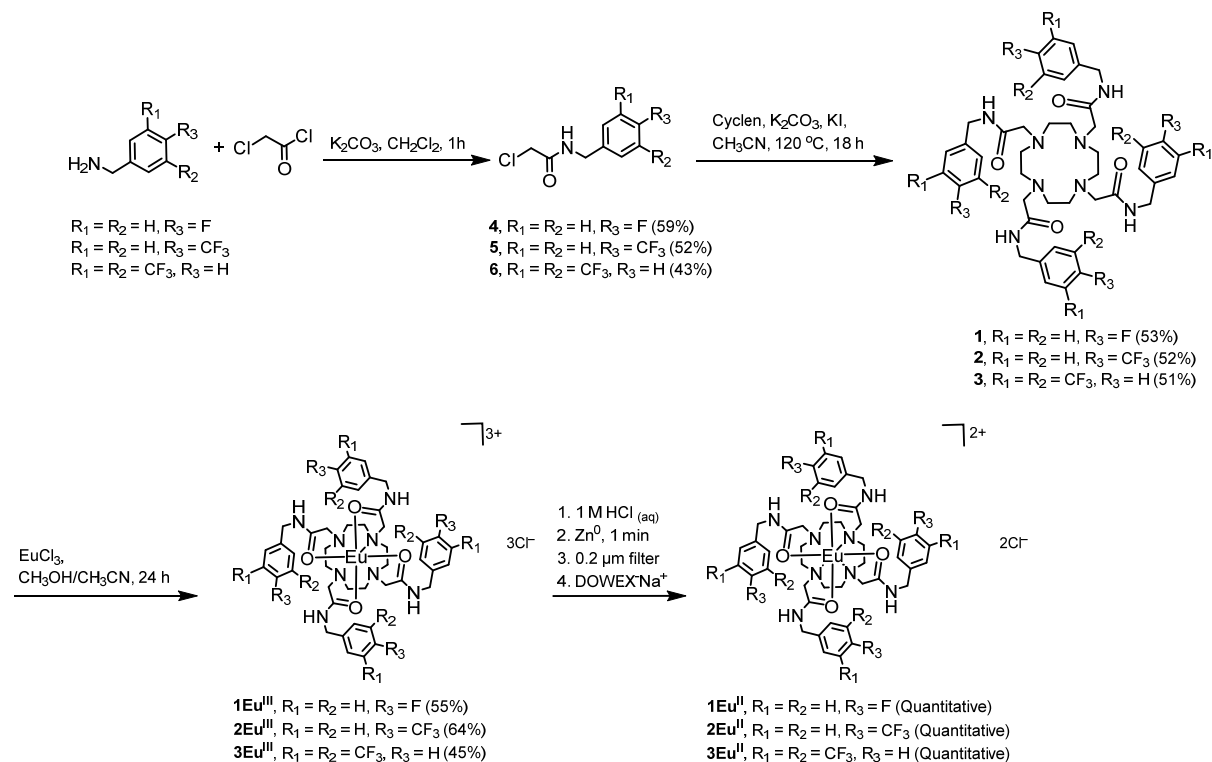
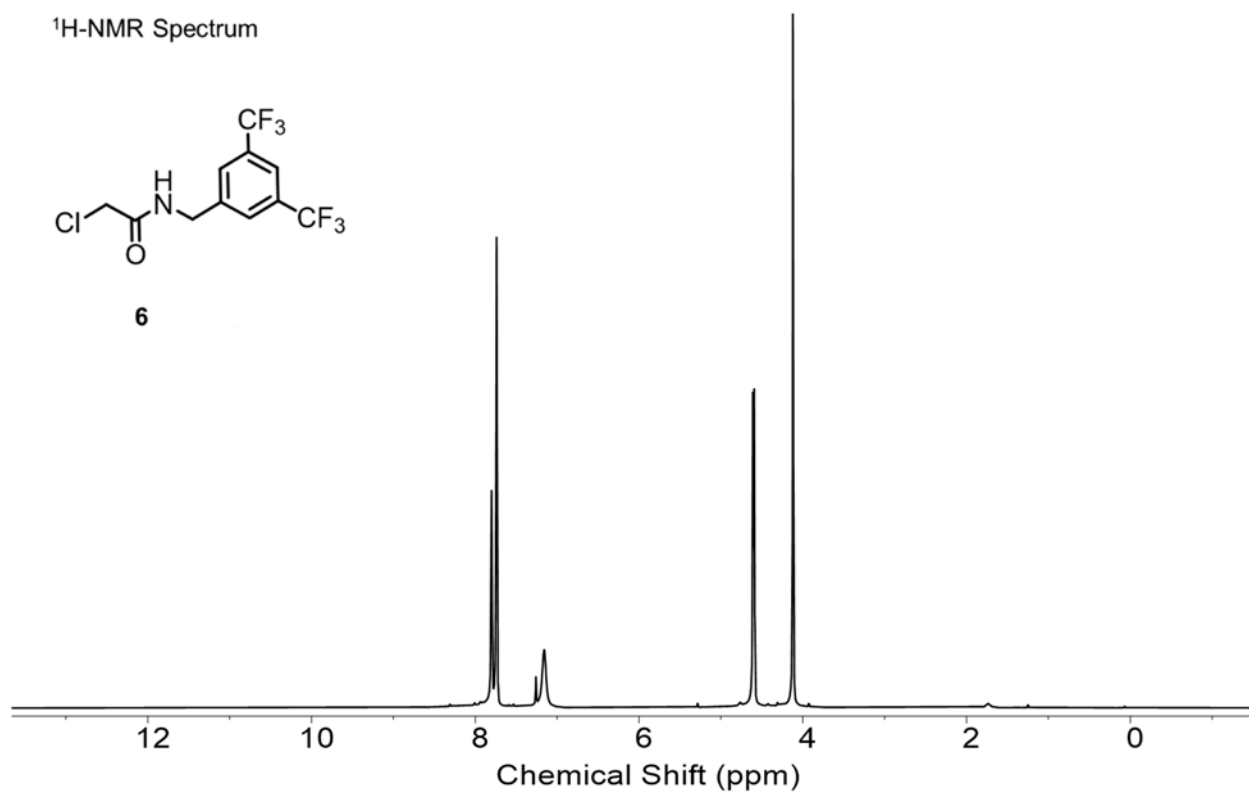
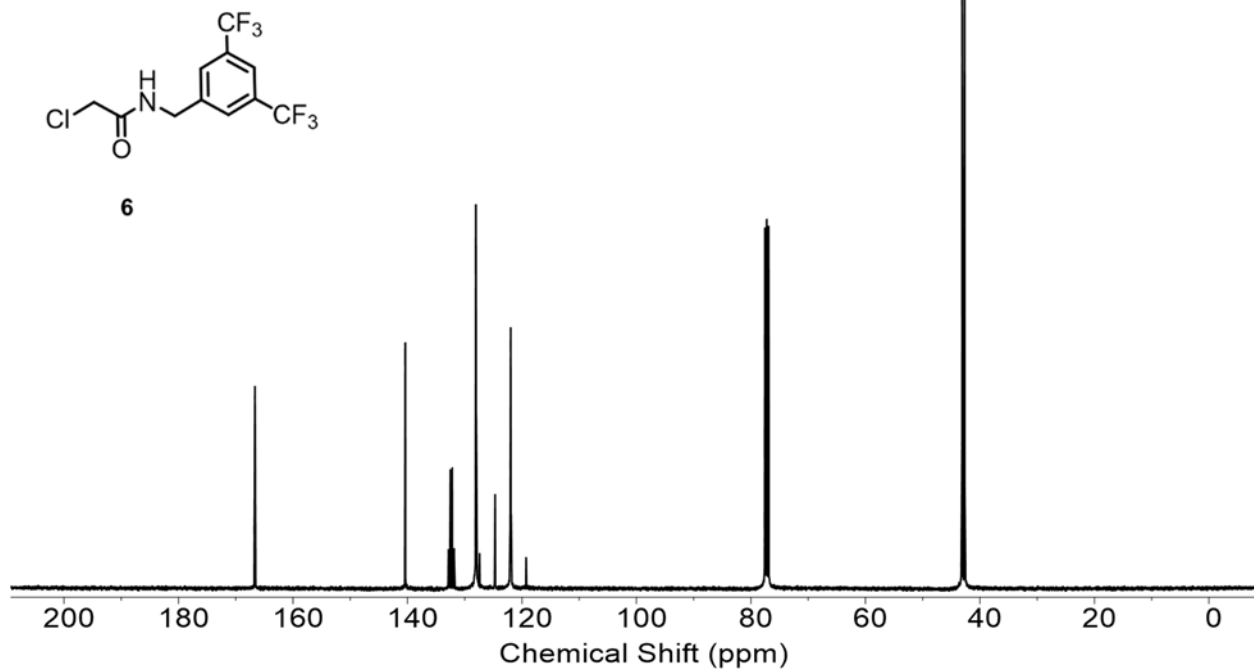
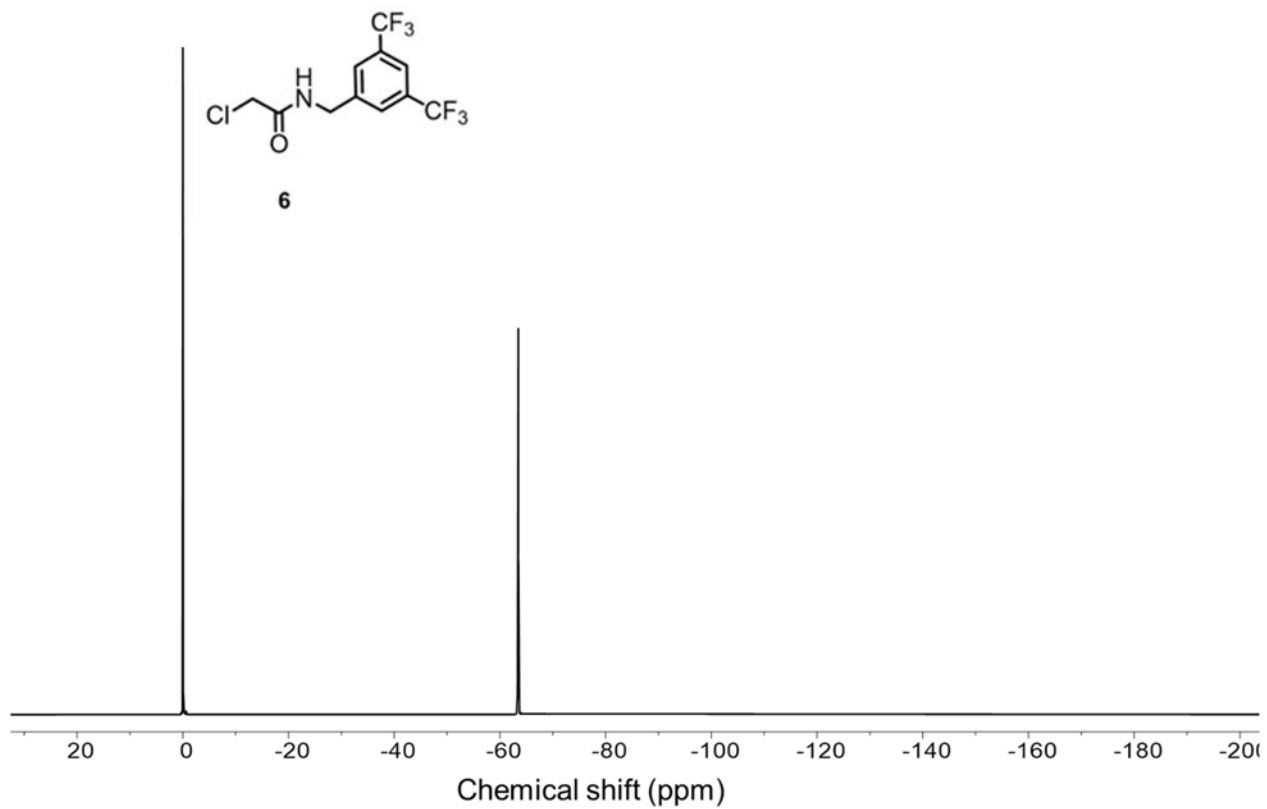
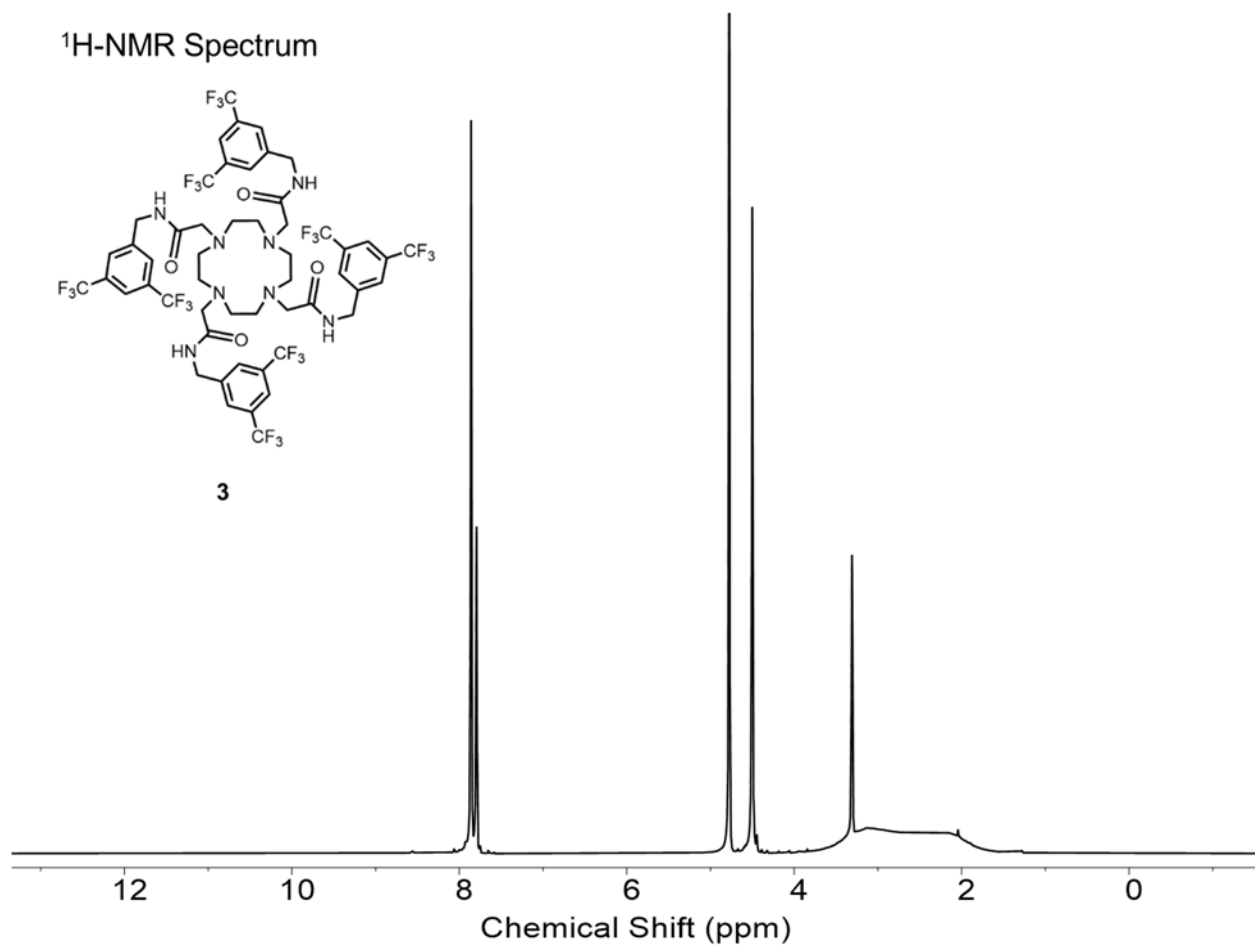
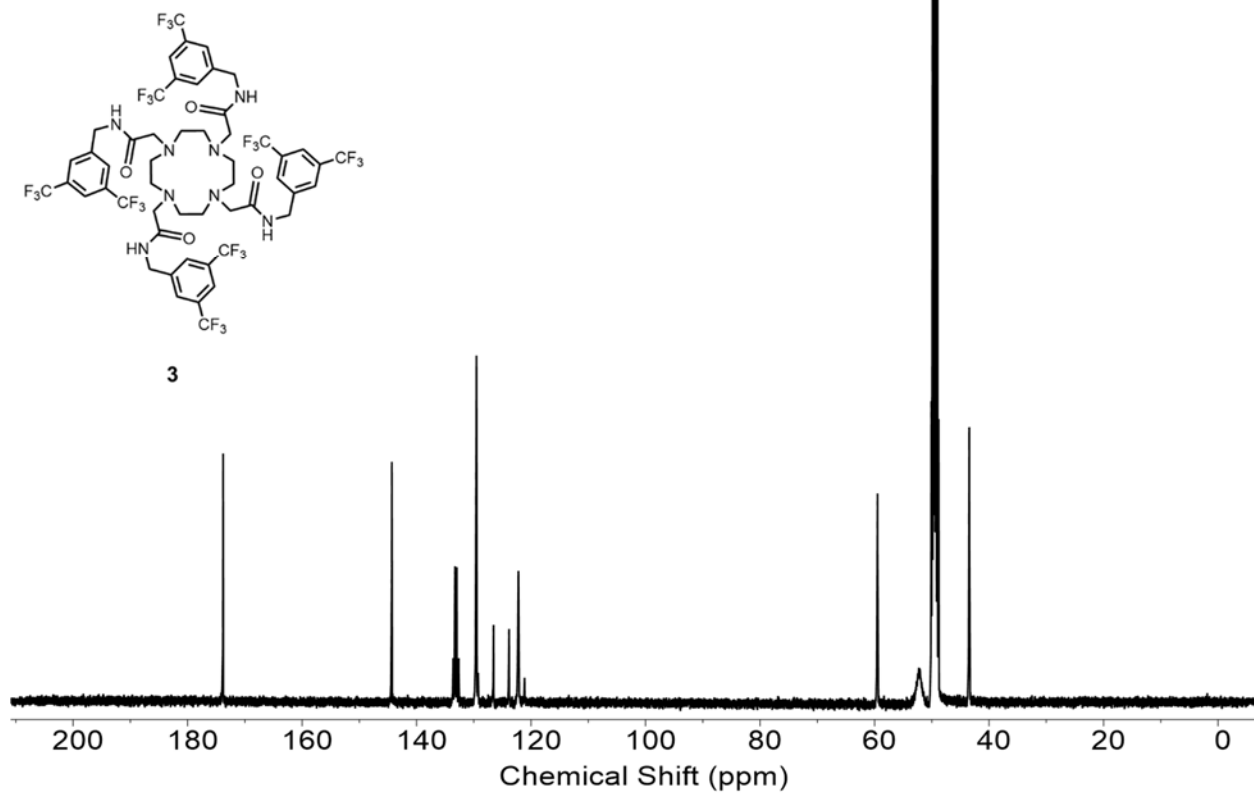
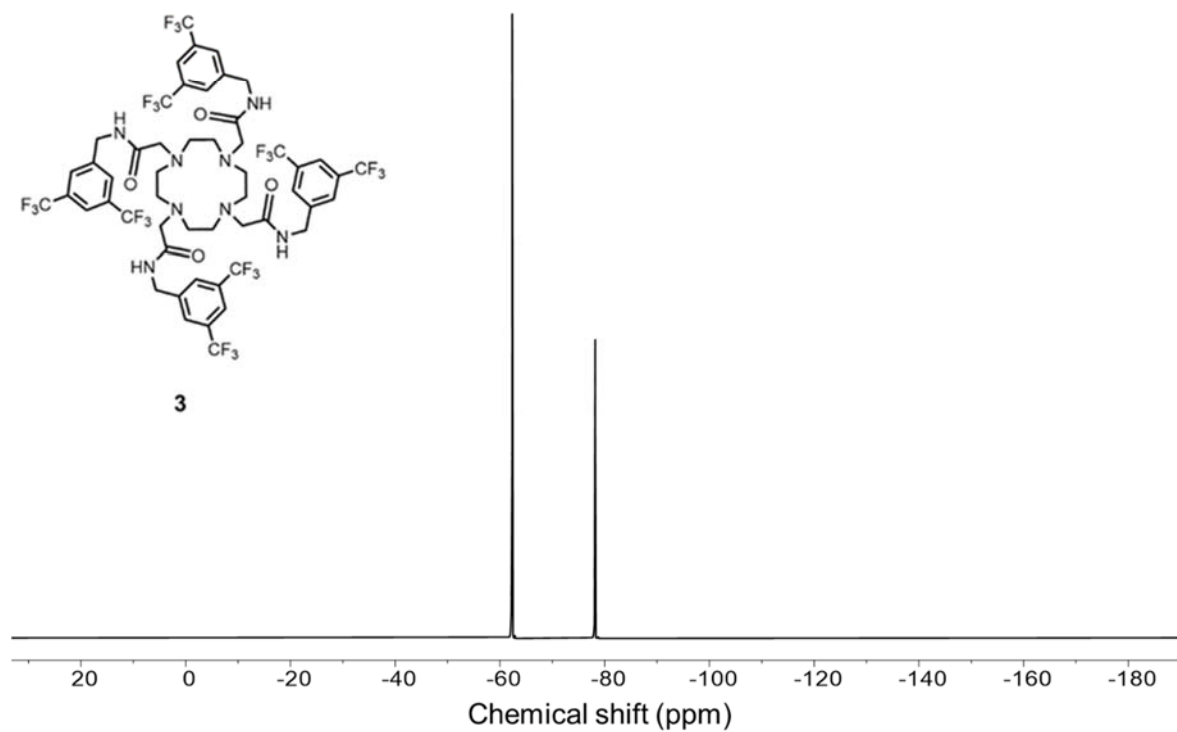


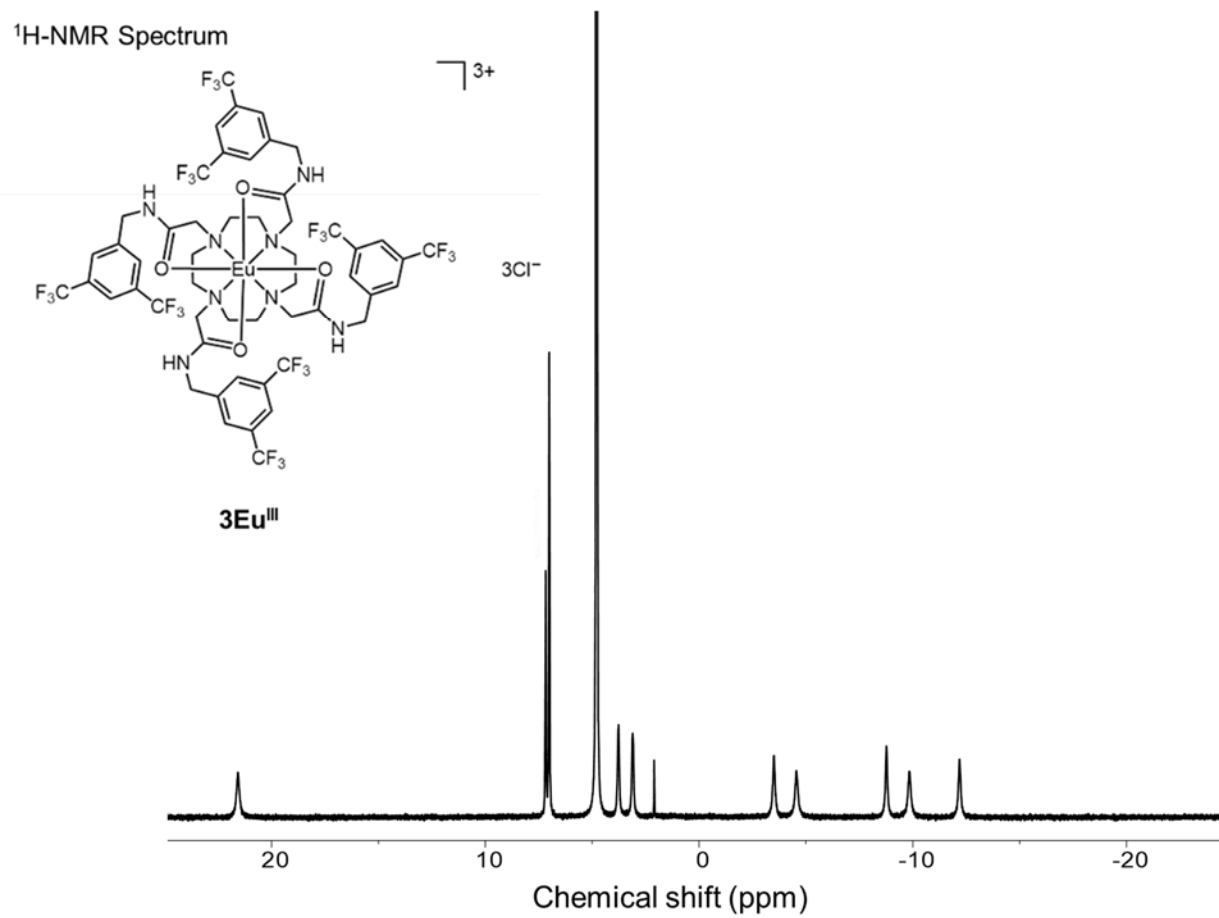
Figure S1. Reaction scheme for complexes studied.

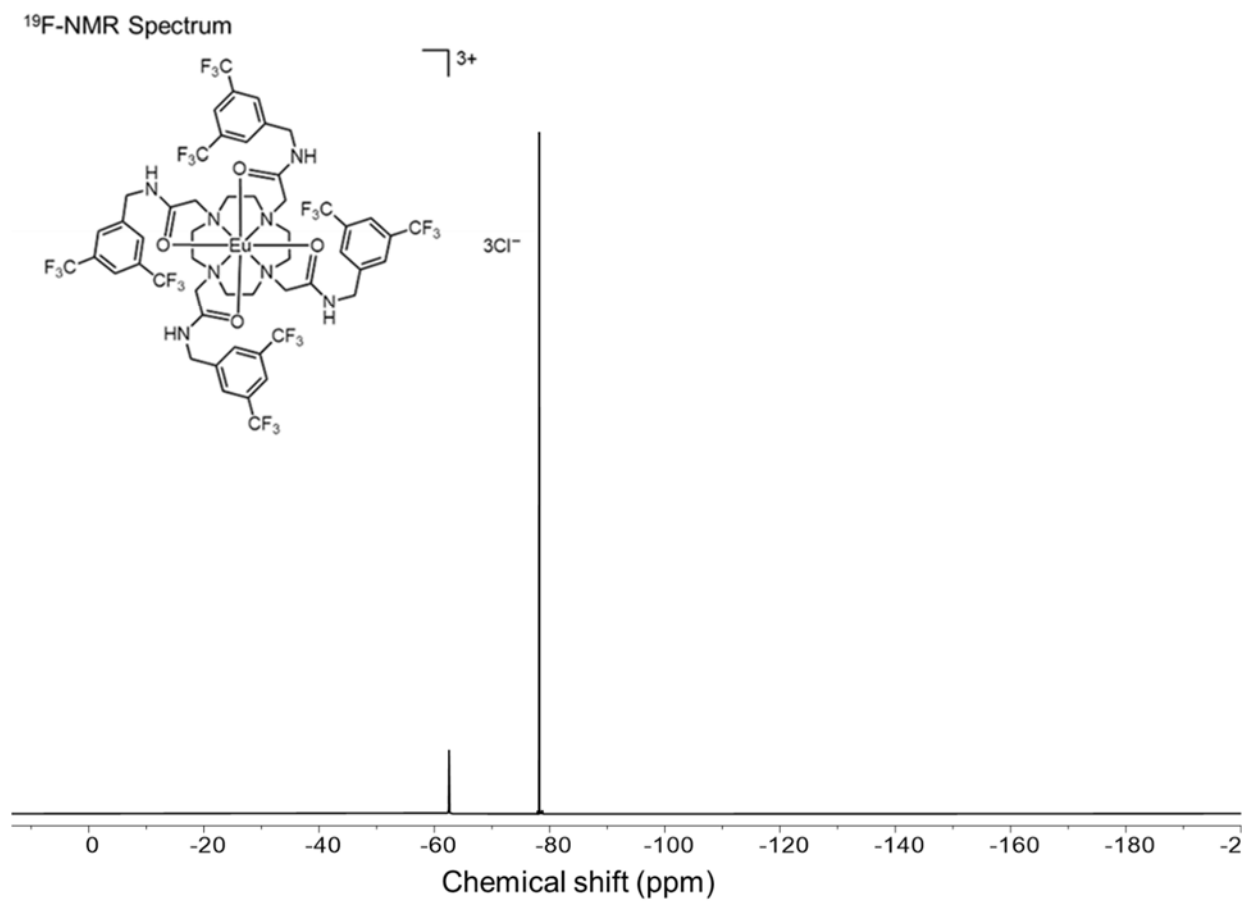
<sup>1</sup>H-NMR Spectrum

<sup>13</sup>C-NMR Spectrum<sup>19</sup>F-NMR Spectrum

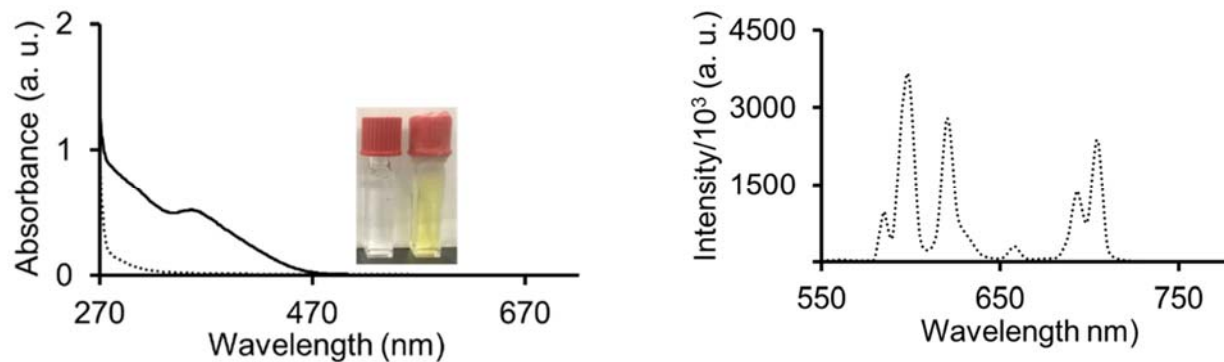


<sup>13</sup>C-NMR Spectrum<sup>19</sup>F-NMR Spectrum

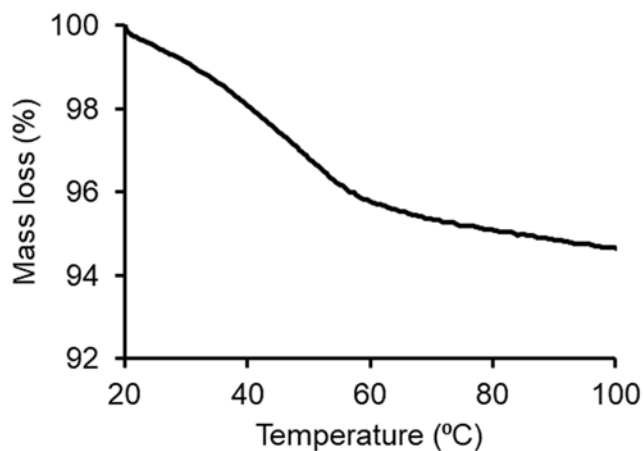








**Figure S2.** (Left) Absorption spectra of  $3\text{Eu}^{\text{II}}$  (solid line) and after oxidation into  $3\text{Eu}^{\text{III}}$  (dashed line). Quartz cuvettes represent solutions of  $3\text{Eu}^{\text{II}}$  (yellow) and  $3\text{Eu}^{\text{III}}$  (colorless) in water. (Right) Luminescence spectra of  $3\text{Eu}^{\text{II}}$  (solid line) and after oxidation into  $3\text{Eu}^{\text{III}}$  (dashed line).



**Figure S3.** Thermal gravimetric analysis of  $3\text{Eu}^{\text{III}}$  indicating the presence of six water molecules per complex.

**Table S1.** Crystallographic properties of **1Eu<sup>III</sup>Cl<sub>3</sub>**, **1Eu<sup>II</sup>Cl<sub>2</sub>**, and **3Eu<sup>III</sup>Cl<sub>3</sub>**

<b>Crystal Data</b>	<b>1Eu<sup>III</sup>Cl<sub>3</sub></b>	<b>1Eu<sup>II</sup>Cl<sub>2</sub></b>	<b>3Eu<sup>III</sup>Cl<sub>3</sub></b>
Empirical formula	C <sub>45.96</sub> H <sub>64.95</sub> Cl <sub>3</sub> EuF <sub>4</sub> N <sub>8.99</sub> O <sub>8.99</sub>	C <sub>46</sub> H <sub>63.25</sub> Cl <sub>2</sub> EuF <sub>4</sub> N <sub>9</sub> O <sub>8.25</sub>	C <sub>53.03</sub> H <sub>59.56</sub> Cl <sub>3</sub> EuF <sub>23.98</sub> N <sub>8.52</sub> O <sub>9</sub>
Formula weight	1221.54	1173.16	1674.22
Crystal size (mm)	0.081 × 0.166 × 0.208	0.20 × 0.23 × 0.23	0.124 × 0.185 × 0.207
Crystal color	colorless	yellow	yellow
Crystal System	Monoclinic	Triclinic	Tetragonal
Space Group	<i>P</i> 2 <sub>1</sub> / <i>n</i>	<i>P</i> $\bar{1}$	<i>I</i> 422
Unit Cell Dimensions	a = 10.4681(6) Å b = 47.867(3) Å c = 11.6815(9) Å  β = 116.529(2)°	a = 20.456(2) Å b = 23.512(3) Å c = 24.127(2) Å α = 82.196(3)° β = 65.036(3)° γ = 89.517(3)°	a = 14.3972(16) Å b = 14.3972(16) Å c = 33.011(4) Å
Volume (Å <sup>3</sup> )	5237.0(6)	10406.6(18)	6842.5(17)
Z	4	8	4
F(000)	2502.0	4810.0	3357.0
Density (g cm <sup>-3</sup> ) (calculated)	1.549	1.498	1.625
<b>Collection Data</b>			
Temperature (K)	100.0	100.0	110.0
Wavelength (Å)	0.71073	0.71073	0.71073
Abs. Coef. (mm <sup>-1</sup> )	1.427	1.382	1.156
R <sub>int</sub> , R <sub>sigma</sub>	0.1758, 0.1566	0.1466, 0.1113	0.0430, 0.0221
2θ range (°)	3.404 to 57.4	1.882 to 51.014	4 to 69.966
Range of h, k, l	-14→14, -64→64, -15→15	-24→24, -28→28, -29→29	-23→23, -22→22, -53→53
Reflections collected	125526	272525	99334
<b>Refinement Data</b>			
No. indep. data	13492	38575	7549
No. restraints	19924	18	770
No. parameters	2222	2565	351
Good of fit	1.050	1.027	1.071
R[F <sup>2</sup> > 2σ], wR(F <sup>2</sup> )	0.0987, 0.2705	0.0597, 0.1611	0.0271, 0.0766
Δρ <sub>max</sub> /Δρ <sub>min</sub> (e Å <sup>-3</sup> )	0.82/-0.72	2.72/-2.01	3.35/-0.88
Flack Parameter	NA	NA	0.039(12)

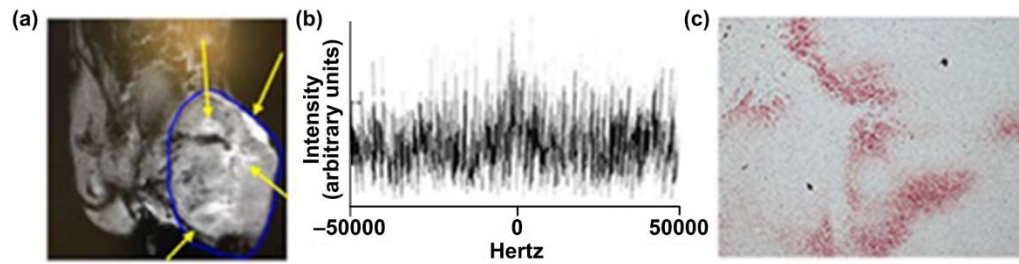
**Table S2.** Comparison of the slopes of the curves of **2Eu<sup>II</sup>/2Eu<sup>III</sup>** system at 1 mM and 6 mM concentrations using a *t*-test.

	<b>6 mM</b>	<b>1 mM</b>
Mean	0.49	0.43
Variance	0.006629	0.002873
Observations	3	3

alpha = 0.05

P(T&lt;=t) two-tail = 0.4860

0.4860 &gt; 0.05



**Figure S4.** (a) Tumor injected with  $1\text{Eu}^{\text{II}}$ . Arrows identify positive  $T_1$ -weighted contrast enhancement, indicating hypoxia; (b) in agreement with  $^1\text{H}$ -MRI,  $^{19}\text{F}$  signal is weak, indicating tumor hypoxia; and (c) hypoxyprobe histological validation of MRI data.