Supplemental Information

A Paramagnetic Chemical Exchange-based MRI Probe Metabolized by Cathepsin D: Design, Synthesis and Cellular Uptake Studies

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Purification and Isolation of Compound 6a

**Figure 1:** HPLC chromatogram of the crude mixture containing conjugate 6a, $t_R = 25.1$ min.
Mass Spectral Characterization of Metalated Conjugates

Figure 2: High resolution mass spectrum (ESI-TOF) of the probe 1 (M$^{5+}$, left). Deconvoluted (MaxEnt 1) spectrum of 1 (right). M=3864.6001, conforms to the formula: $\text{C}_{166}\text{H}_{249}\text{F}_{2}\text{N}_{53}\text{O}_{40}\text{STm}$

Figure 3: High resolution mass spectrum (ESI-TOF) of the fragment 1$_{\text{cleaved}}$ (M$^{3+}$, left). Deconvoluted (MaxEnt 1) spectrum of 1$_{\text{cleaved}}$ (right). M=1906.6001, conforms to the formula: $\text{C}_{81}\text{H}_{101}\text{F}_{2}\text{N}_{15}\text{O}_{24}\text{STm}$
In-vitro Metabolism of 1 by Cathepsin D.

Scheme 1: Metabolism of the dual probe 1 by cathepsin D (Cat D); hydrolysis occurs predominantly where indicated by the asterisk. Structure of pepstatin A (7), an inhibitor of Cat D.

Figure 4: Total ion count spectrograms, positive mode ESI-TOF MS for the in-vitro metabolism of 1 by cathespin D. Top spectrogram: control sample containing cathepsin D (▼) in the presence of pepstatin
A \((8)\) \((t_R \text{ 2.96 min})\). Bottom spectrogram: cleavage of probe \(1\) \((t_R \text{ 2.19 min})\) to yield fragment \(1_{\text{cleaved}}\) \((t_R \text{ 2.42 min})\).
Figure 5: $^1$H NMR spectrum of 4 in CDCl$_3$, full view (top) expanded region 1-4 ppm (bottom), showing extensive line broadening due to conformational rigidity.