Development of Hyperpolarizable ^{13}C-Probes for the Quantification of Metals *In Vivo*

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Introduction

- Disruption of the homeostasis of metals in the body has been correlated to a variety of diseases (cancer,[1,2] Parkinson's Disease,[3] heavy metal poisoning[4])
- Current blood-based methods of metal quantification[5] don't give accurate concentrations of metals in tissue samples[2]
- ^{13}C-tagged EGTA and EDTA probes have experienced a shift in carbonyl peak (singlet in the range of 170 to 180 ppm) unique to the type of metal it bound to (**Figure 1**) via ^{13}C NMR[6]
- The area under the curve of these shifted peaks increased linearly with an increase in concentration of the metal[6]
- Sensitivity of signal was increased through hyperpolarization using dynamic nuclear polarization (DNP)[6]
- Macrocycles tend to have a higher binding affinity than linear molecules due to a higher entropy associated with the chelation process[8]
- Increased binding to metals could give a stronger signal, which could be better for lower concentrations
- **Goal:** Evaluate metal ion-binding properties of ^{13}C-tagged EDTA, and other macrocycles

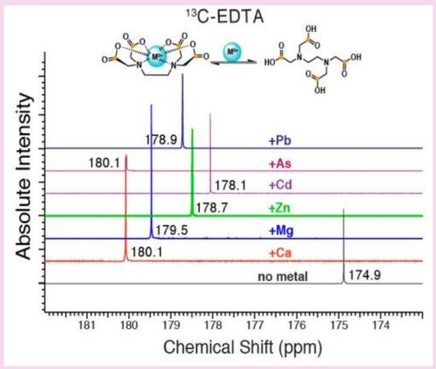
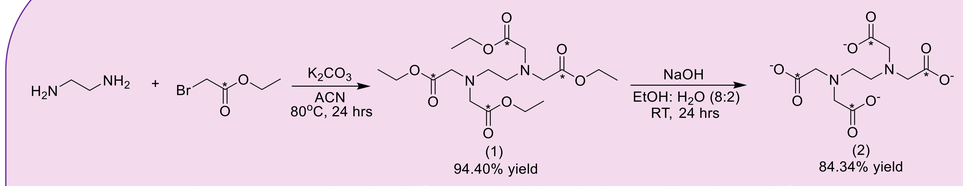


Figure 1. Chemical shifts of ^{13}C-EDTA when bound to different divalent metals via ^{13}C NMR.[6]

Materials and Methods



Scheme 1. Synthesis of ^{13}C-EDTA (2), * indicates location of ^{13}C.

Synthesis of ^{13}C-EDTA

- Synthesized according to **Scheme 1**.
- Ethylenediamine was tetraalkylated with ^{13}C-ethyl bromoacetate
- The resulting intermediate **(1)** was purified via extractions with DCM and deprotected with sodium hydroxide to yield the final product **(2)**
- Final product was adjusted to pH 7.5 and lyophilized
- Identity verified via ^{1}H and ^{13}C NMR spectroscopy.

^{13}C NMR Titration of ^{13}C-EDTA with Ca^{2+}

- ^{13}C-EDTA sample **(2)** (0.0146 g) was dissolved in 1 mL HEPES buffer (0.3 M in D_2O at pH 7.40) to make a stock solution
- 50 µL of this stock was added to 450 µL of the HEPES buffer in an NMR sample tube and a ^{13}C NMR spectrum was acquired
- 4.16 µL (3.94×10^{-7} mol) of a 94.60 mM Ca^{2+} made in the HEPES buffer was added to the sample tube and a ^{13}C NMR spectrum was acquired
- Four more additions of 4.16 µL of the Ca^{2+} stock were made, and after each addition a ^{13}C NMR spectrum was acquired

Results and Discussion

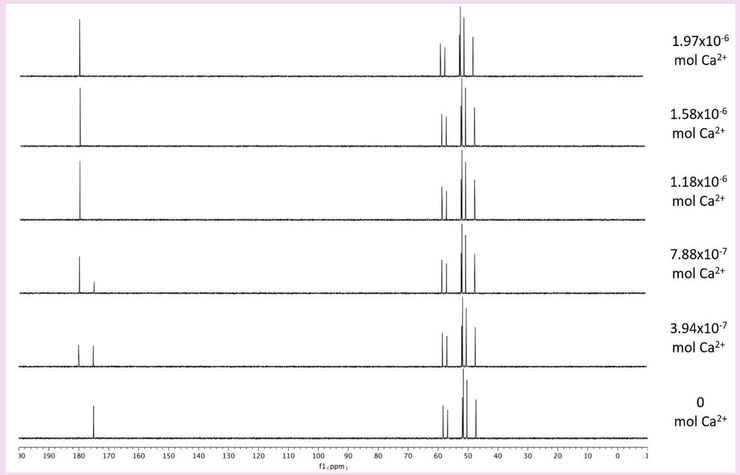


Figure 2. Overlaid ^{13}C NMR spectra from titration of **(2)** with Ca^{2+} in 0.3 M HEPES buffer at pH 7.40 in D_2O acquired at 125 MHz.

- Appearance of a second carbonyl peak at 180.0 ppm was observed (**Figure 2**) upon addition of Ca^{2+}, which matched literature values[1]
- Throughout titration, peaks in the range of 45 to 60 ppm experienced no observable change, these peaks correspond to non-^{13}C-tagged carbons on probe and carbons in HEPES buffer
- As the concentration of Ca^{2+} in the sample increased, the "unbound" peak at 175.0 ppm was observed to decrease in intensity and the "bound" peak increased in intensity

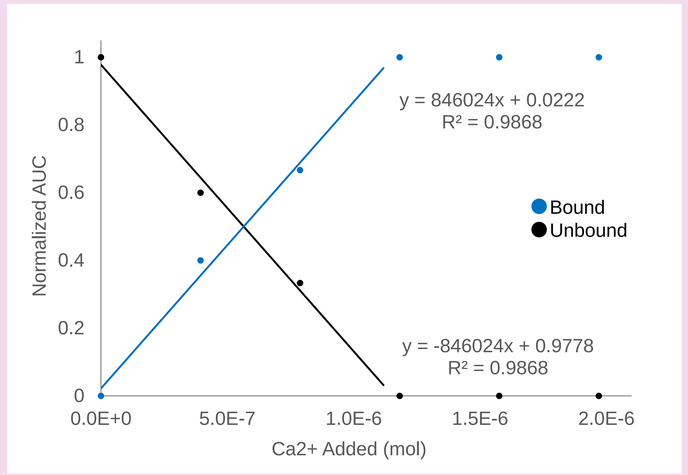


Figure 3. Binding curve of **(2)** with Ca^{2+}. AUC was normalized to total AUC, and ratios of bound and unbound AUC were plotted.

- Carbonyl peaks for bound and unbound ^{13}C-EDTA were integrated and normalized to the total area under the curve (AUC)
- A function of the AUC and moles of Ca^{2+} added was plotted (**Figure 3**)
- Moles Ca^{2+} to fully complex ligand = moles ^{13}C-EDTA, calculated using lines of best fit, used in order to find the percentage of ^{13}C-EDTA in **(2)**
- The synthesized sample **(2)** was calculated to be 46.42% wt ^{13}C-EDTA

Conclusions

- Behavior of ^{13}C-EDTA observed in previous studies[1] was confirmed upon binding Ca^{2+}
- Area under the curve was utilized to determine the concentration of ^{13}C-EDTA in the synthesized sample

Future Work

- Titrate ^{13}C-EDTA with other metal ions to verify chemical shifting when bound to different metals
- Synthesize ^{13}C-tagged macrocyclic ligands
- Investigate specific chemical shifts of ^{13}C-tagged macrocycles upon binding to different metal ions
- Verify ability of ^{13}C-tagged macrocycles to quantify metal concentrations
- Collaborate externally for hyperpolarization studies

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