Synthesis of a Zn(II)-Responsive ParaCEST MRI Agent for Improved Diagnosis of Prostate Cancer

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Introduction

Prostate Cancer

- Prostate cancer accounts for 20 percent of diagnosed cancer cases, the highest rate of diagnosis for all cancers that affect men.[1]
- The Prostate Specific Antigen (PSA) test is used for diagnosis of prostate cancer. If the concentration of PSA in the blood is above a given threshold, the health of the prostate is analyzed with a biopsy.
- 80 percent of positive PSA tests are false positives. The biopsy process is invasive and can cause complications like bleeding and anxiety.[2]
- There is an urgent need for a more accurate diagnostic test for prostate cancer.

Magnetic Resonance Imaging (MRI)

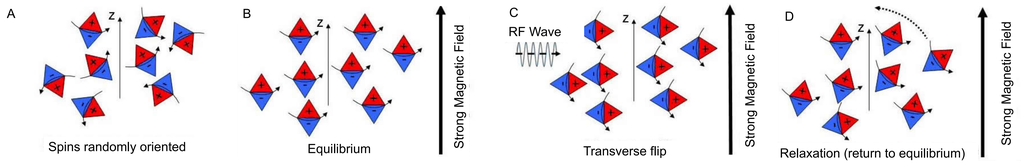

Figure 1: A) random alignment of protons B) application of magnetic field and alignment of protons with field C) application of radio frequency pulse and spinning of protons D) relaxation of protons to alignment with magnetic field

- An MRI is obtained using a magnet to align the protons of the bulk water in the body, then sending a radio wave frequency pulse through the body to spin these protons.
- The time required for the protons to return to their original alignment is the relaxation time (T_1), and its inverse is the relaxivity ($R_1 \propto 1/T_1$), which corresponds to the amount of contrast in an image. [3]

Chemical Exchange Saturation Transfer (CEST) Agents

- CEST Agents represent a new class of MRI contrast agents that induce negative contrast in an image.
- CEST signal is generated through slow exchange of the bound protons on an agent with the bulk water protons in the body.
- Major advantages of CEST agents over clinical MRI contrast agents include:
  - Their ability to be turned “on” and “off” by the application of a radiofrequency pulse.
  - Their sensitivity to the chemical environment in which they are located.
- ParaCEST agents are CEST agents consisting of a lanthanide ion chelated by a multidentate ligand. Use of paramagnetic lanthanides shifts the CEST signal away from the bulk water signal.[5]

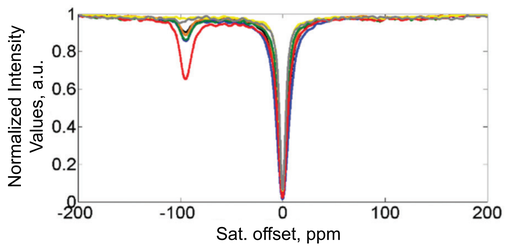

Figure 3: CEST spectrum, observed increase in CEST signal, or bound water signal (left) with decrease in bulk water signal (right). Observed shift of bound water signal away from bulk water signal. [5]

The Zinc Hypothesis and Project Goal

- A healthy prostate has a significantly higher concentration of Zn(II) than other soft tissues in the body.
- Zinc (II) levels have been found to be significantly decreased in the presence of prostate cancer.

- The goal of this project is to synthesize a ParaCEST MRI agent capable of detecting and quantifying zinc in the prostate.
- We hypothesize that binding zinc will alter CEST spectrum, and this will be correlated with the extent of prostate disease. [4]

(nmols/g wet tissue)	Zinc
Normal peripheral zone	3,000-4,500
Malignant peripheral zone	400-800
Normal prostatic fluid	8,000-10,000
Prostate cancer prostatic fluid	800-2,000
Other tissues	200-400
Blood plasma	15

Figure 2: Relationship between prostate health and zinc concentrations (nmol/g wet tissue). Table indicates tremendous decrease in zinc concentration with diminishing prostate health.[4]

Methods

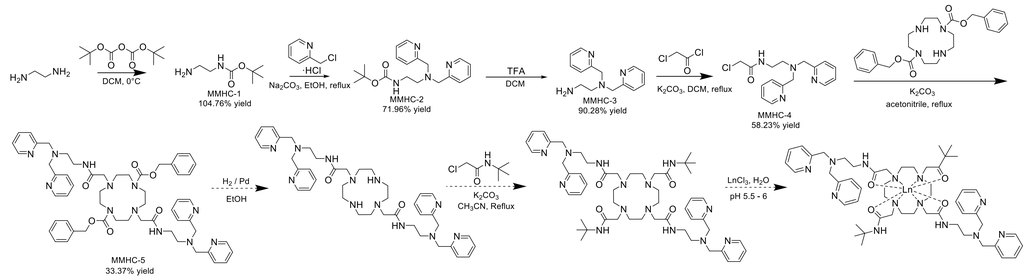

Figure 4: Complete synthetic scheme for desired coordinated complex. The first step shows over 100% yield, attributed to residual solvent.

- All reagents were purchased from commercial suppliers.
- The proposed compounds will be synthesized according to the scheme in Figure 4.
- ^{13}C-NMR and ^{1}H-NMR spectroscopy was used to characterize the intermediate products.
- Flash chromatography was used to purify MMHC-2 and MMHC-5

Results and Discussion

- Compounds MMHC-1 through MMHC-5 have been synthesized successfully and characterized by ^{13}C-NMR and ^{1}H-NMR spectroscopy.
- ^{13}C-NMR and ^{1}H-NMR spectra of compounds MMHC-1 through MMHC-3 are not shown, but were used to verify compound identities.
- The >100 % yield of MMHC-1 was attributed to residual solvent in the final product.

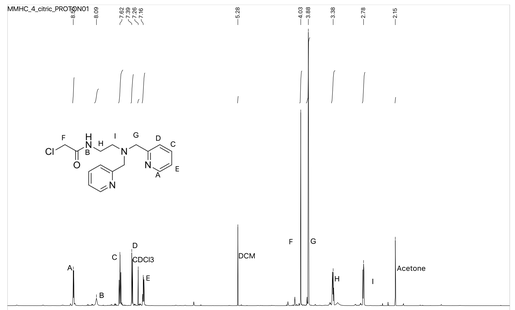

Figure 5: ^{1}H NMR spectrum of MMHC-4 in $CDCl_3$ at 500 MHz

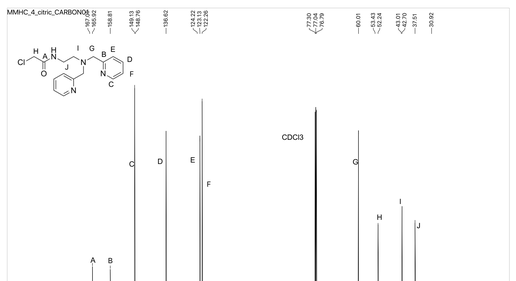

Figure 6: ^{13}C NMR spectrum of MMHC-4 in $CDCl_3$ at 125 MHz

- The ^{1}H-NMR spectrum of MMHC-5 lacks the expected amide proton peak. It is hypothesized that amide protons are exchanging with another species present in the sample.
- The absence of the quaternary and carbonyl carbons in the ^{13}C-NMR spectrum of MMHC-5 is possibly due to the longer T_1 of these carbons.
- An additional ^{13}C NMR of MMHC-5 will be obtained with the intention of reducing baseline noise.

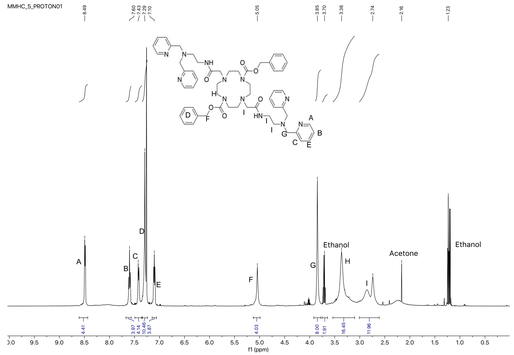

Figure 7: ^{1}H NMR spectrum of MMHC-5 in $CDCl_3$ at 500 MHz

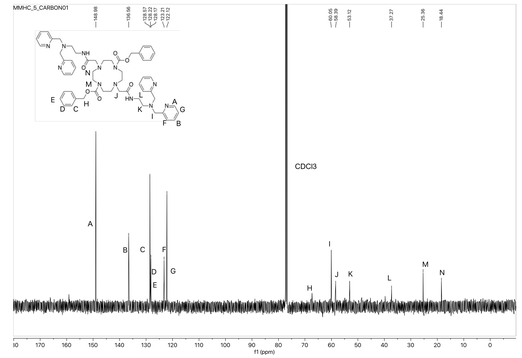

Figure 8: ^{13}C NMR spectrum of MMHC-5 in $CDCl_3$ at 125 MHz

Future Work

- Repeat synthesis for higher yield
- Complexation with lanthanide metal ions
- Titration with $ZnCl_2$, Mg^{2+}, Ca^{2+}, and Cu^{2+} to confirm selective binding to Zn^{2+}
- Analyze CEST spectra of agent in absence and presence of Zn^{2+}

Acknowledgments

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