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Synthesis and Relaxivity of a Target-Specific MRI Contrast Agent

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"Synthesis and Relaxivity of a Target-Specific MRI Contrast Agent"

written by

Callie Clement

and submitted in partial fulfillment of
the requirements for completion of
the Carl Goodson Honors Program
meets the criteria for acceptance
and has been approved by the undersigned readers.

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Table of Contents

Table of Figures and Tables

Introduction

Magnetic resonance imaging (MRI) is a medically important test that provides

a painless, noninvasive way to visualize images of the body by using a strong

magnetic field. The machine quickly changes the direction of the magnetic field in

certain increments and measures the time it takes for protons to change their

direction, lining up with the magnetic field again. The time taken for the protons to

realign is called the overall relaxivity rate (R1). Abnormalities, especially tumors, are

expressed in MRI images because their relaxivity rates are quite different from the

relaxivity rates of normal tissues.

To help enhance MRI images, several contrast agents have been developed in

the past that can be injected into the patient's bloodstream. The contrast agents

studied and synthesized in this research contain a metal called gadolinium. Contrast

agents containing gadolinium can be excreted in the urine by glomerular filtration,

which is a key characteristic of most contrast agents in general. In the last few

decades, the use of contrast agents has drastically increased due to their

enhancement and increased specificity. The most common contrast agents currently used include Magnevist, ProHance, and Omniscan.

For the contrast agent to be useful and effective, it must have several key characteristics. First, it must have an adequate amount of tissue specificity. When injected into the patient, it should go to one area of the body in a higher concentration than anywhere else. The agent must also stay there long enough to allow for image enhancement. Second, the contrast agent must have a long shelf life. If the material isn't stable, not only is it unsafe to use, it won't be available for long-term use. A contrast agent should have a shelf life of years, not months or days. Third, as mentioned before, the body must be able to completely remove the contrast agent from the targeted area or organ. While most contrast agents are excreted in the urine, it must also have low toxicity as to prevent harming the patient. Lastly, to minimize dosage, the contrast agent must be able to alter the image intensity at a low concentration. This helps keep the dosage at a minimum while still producing enhanced images (Contrast Media 454).

In this research several different synthesis reactions were completed and tested for accuracy, using NMR and thin-layer chromatography (TLC). The results ensured that the desired product was produced before proceeding with the next reaction. This thesis describes the synthesis, purification, characterization, and testing of a novel contrast agent, comparing it to current contrast agents being used today. The final product was tested using T1 relaxation rates to compare the newly developed agent, which was synthesized using a piperazine-methoxyphenyl group, to those contrast agents in use today.

Background

While magnetic resonance imaging was first used in the 1970s, it was not

apparent that contrast agents would be helpful in visualizing MRI images until the

1980s. It was discovered that running MRI with contrast agents significantly

improved the visualization and specificity of images obtained. Experimenters focused

specifically on contrast agents containing gadolinium because they have the highest

paramagnetic effect, meaning they enhance the T1 relaxivity rate the most.

The main parameter that determines signal intensity and contrast agent

enhancement is the term relaxivity. The two types of relaxivity rates are termed T1

and T2. The T1 relaxation rate depends on longitudinal relaxation of protons. T2

relaxation is known as transverse relaxation and measures the time taken for the

protons to lose coherency (Preston 2006). This research focused on overall T1

relaxation rates. Figure A below shows the difference between T1 and T2 relaxation
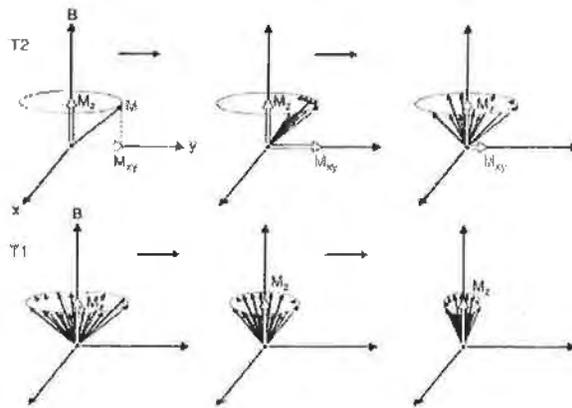
(Themes 2016).

Figure A

A contrast agent that primarily focuses on the T1 relaxivity rate is called a positive relaxation agent because when the T1 relaxivity rate is decreased, a higher intensity and enhancement of the image results (Contrast Media, 455).

In contrast agents, paramagnetic ions such as Gd^{3+} are used the most frequently because they have the highest number of unpaired electrons and the highest susceptibility to being magnetized when placed under a magnetic field (Contrast Media, 455). Of the four categories of magnetic susceptibility, paramagnetic ions give the most enhanced images. Diamagnetic, super-paramagnetic, and ferromagnetic ions don't show as much contrast in MRI images, so they are of little to no interest as contrast agents. In addition, paramagnetic ions have shown the greatest flexibility in design and structure when being used as

contrast agents, so the most attention has been given to paramagnetic agents such as gadolinium, lanthanum, and terbium. Lanthanum and terbium are weakly paramagnetic, where gadolinium is highly paramagnetic. Therefore, because gadolinium is more highly susceptible in a magnetic field, it gives more enhanced T1-weighted images and is used more often. Gadolinium has seven unpaired electrons, giving a high affinity to the chelate. The ion also has a long relaxation time, is completely excreted by the kidneys, and has been proven to give the most enhanced images. Because gadolinium chelates are the main class of contrast agents currently being used, this research was limited to examining T1-weighted paramagnetic contrast agents containing gadolinium.

Along with a paramagnetic compound's ability to become magnetized when being placed under a magnetic field, another important factor to consider is its ability to become demagnetized upon removal. The act of paramagnetic particles losing their magnetization and returning to random orientations between pulses is crucial for MRI imaging- every time the magnetic field pulses and changes directions in specified increments, the particles return to random orientations between each

pulse. This is what the overall T1 relaxivity rate measures- the amount of time it

takes for water protons in the body to realign to the pulsing magnetic field after

being in random orientation (Contrast Media, 455).

When studying or synthesizing contrast agents, another important factor to

be considered is the osmolality of the compound. Injections burn or sting if their

osmolalities differ significantly from the osmolality of blood, so companies that

produce contrast agents want the contrast agent osmolalities to be as close as

possible to the blood osmolality. According to the U.S. National Library of Medicine,

the average blood osmolality is 275-295 mOsmol/kg (milliosmoles per kilogram).

Magnevist has approximately 7 times the osmolality of blood plasma with a value of

1960 mOsmol/kg. ProHance only has 2.2 times more with a value of 630

mOsmol/kg. Omniscan has an osmolality 2.8 times more than that of blood plasma

with a value of 798 mOsmol/kg (Contrast Media p. 462-463).

Most contrast agents are considered to be chelates. Chelates are ligands that

are bound to a central metal molecule at several different points. The first

gadolinium contrast agent to be approved as an MRI contrast agent was Magnevist,

introduced in 1988. (Contrast Media, 462). In Magnevist, the chelate has a

gadolinium atom bound to three different nitrogen atoms in the chelate.

Additionally, the gadolinium (III) ions are bound to three carboxyl groups, which give

the chelate an overall charge of negative two. Magnevist is a linear molecule and

has an osmolality of 1960mOsmol/kg. Magnevist is excreted exclusively in the urine
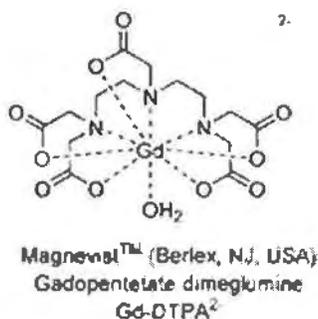
and is shown below in Figure B.

Magnevist™ (Berlex, N.J, USA)
Gadopentetate dimeglumine
Gd-DTPA²

Figure B

ProHance, shown in Figure C, was approved for use in 1992 and was the first

agent with a cyclic structure to be developed. Because of its cyclical structure, it is

very stable *in vivo*. The ligand carries a charge of negative three and the gadolinium

carries a charge of positive three, giving the molecule a net charge of zero. The

osmolality of ProHance is 630 mOsmol/kg, which is approximately 2.2 times that of

the blood. Because of the lower osmolality, there is approval for dosage up to

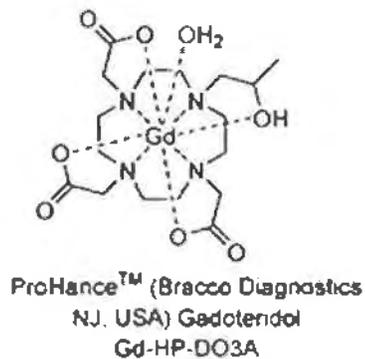0.3mmol/kg (Contrast Media, 463).

ProHance™ (Bracco Diagnostics
NJ. USA) Gadoteridol
Gd-HP-DO3A

Figure C

Omniscan, shown in Figure D, was approved for use in 1993 shortly after

ProHance. Omniscan has a complex structure and is a variant of the linear structure

of Magnevist. The ligand has a charge of negative three and the gadolinium has a

charge of zero, but the molecule achieves neutrality at the cost of a large decrease

in stability. Omniscan's osmolality is 798mOsmol/kg, which is 2.8 times the

osmolality of blood plasma. Because of the complex structure and lack of stability,

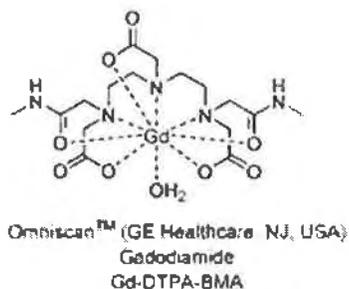Omniscan is only approved for use in adults. (Contrast Media, 463).

Figure D

For the contrast agent to be most effective, it must have an adequate amount of tissue specificity. When injected into the patient, it should go to one area of the body in higher concentration than anywhere else and stay long enough to allow for image enhancement. When designing a contrast agent with higher specificity, WAY-100635, a piperazine drug shown in Figure E, was considered. WAY-100635 is a selective 5-HT1A receptor antagonist that shows high selectivity to a range of central nervous system receptors including dopamine, gamma-amino butyric acid, histamine, and ion receptors. 5-HT1A is a serotonin receptor. When tested in rats, WAY-100635 binds to rodent 5-HT1A receptors in the brain. Therefore, it has been proven that WAY-100635 has the ability to penetrate the blood-brain barrier. Not only can WAY-100635 enter the brain, it has the ability to stay for approximately 30-

13

90 minutes, which is long enough for MRI images to be taken. High specificity was

the primary goal for creating a new contrast agent, so the functional group on the

right side of the molecule was chosen to be synthesized and added to the desired

contrast agent.
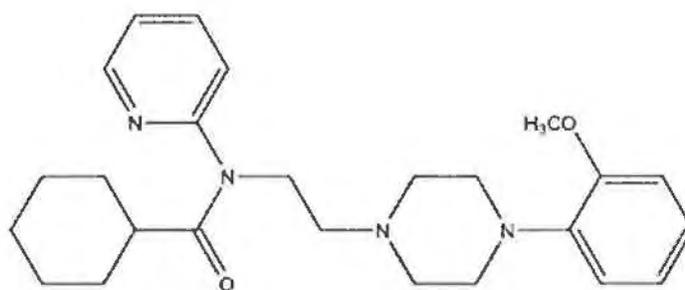
WAY-100635

Figure E

The side effects to gadolinium-based contrast agents are very rare but can

include nausea, taste prevention, dizziness, and hives. These symptoms occurred in

approximately 1% of patients. In previous clinical trials, conclusions were made after

looking at stability *in vivo* that ProHance is the safest agent, while Omniscan is the

least safe (Contrast Media, 462-463). Clinical trials also established that the safest

and most effective dose to administer is 0.1 mmol/kg, which equates to 0.2 ml/kg.

Agents with lower osmolalities can be given in higher doses. However, giving

amounts below the full dose of 0.1mmol/kg caused metastases and other

abnormalities to be easily missed because of lack of enhancement. No gadolinium

contrast agents are approved for use in children under the age of two years.

Methods

The primary goal was to synthesize a contrast agent that was water-soluble, contained gadolinium, and had similar T1 relaxivity rates to other agents already being used. Water solubility was a critical factor in the synthesis of our agent because when contrast agents are given to patients, they are injected into the bloodstream. Blood consists of approximately 92% water, so upon injection, water solubility is critical for the agent to effectively enter the bloodstream and affect contrast enhancement.

The synthesis of the desired contrast agent took approximately eight weeks. Several steps failed to yield the desired product, so procedures were occasionally changed according to the synthesis steps that failed. However, almost everything worked according to plan. Some steps took more time than predicted, but still gave the desired product.

Synthesis of cyclenbromoacetate (1 in Figure F) was begun using cyclen (1,4,7,10- tetraazacyclododecane). A stir bar was added to a three-necked 250mL-round bottom flask. A thermometer, a condenser with a nitrogen inlet, and a funnel

were added to the necks of the flask. 5.0 grams of cyclen, 2.6 grams of acetate

trihydrate, and 20mL of dimethylacetamide were transferred into the flask and the

mixture was stirred for 30 minutes. Using the addition funnel, 2.82mL of

bromoacetate in 10mL of dimethylacetamide was added to the stirred mixture drop-

wise over 30 minutes. After complete addition, the mixture was allowed to stir for 60

hours under nitrogen.

Cyclenbromoacetate was collected and washed with 10mL of cold

dimethylacetamide, suctioned dry, washed twice with 25mL of diethyl ether, and

suctioned dry again. A white, flaky solid resulted and was dissolved in 100mL of

dichloromethane. The solution was washed twice with 15mL of water and once with

15mL of saturated aqueous sodium bromide solution to remove organic materials,

particularly any acetate anions that were still present. The organic phase was

collected and passed through a funnel containing magnesium sulfate. The filtrate

was transferred to a 250mL-round bottom flask and rotavapped to a thin, colorless

oil. The oil was diluted with 80mL of hexane and stirred for three hours.

Crystallization began after five minutes of stirring. After stirring, the mixture was

cooled using an ice methanol bath and stirred for two more hours. Stirring for two

hours yielded a white solid, which was collected and washed with cold

hexane/chloroform (25mL in a 4:1 ratio). The mixture was suctioned dry overnight

and yielded a small amount of a shapeless, white solid (Moore 2008). Upon

characterization testing, it was determined that the cyclic product with tertiary butyl

protecting groups **(3 in Figure F)** had been successfully synthesized. Product was
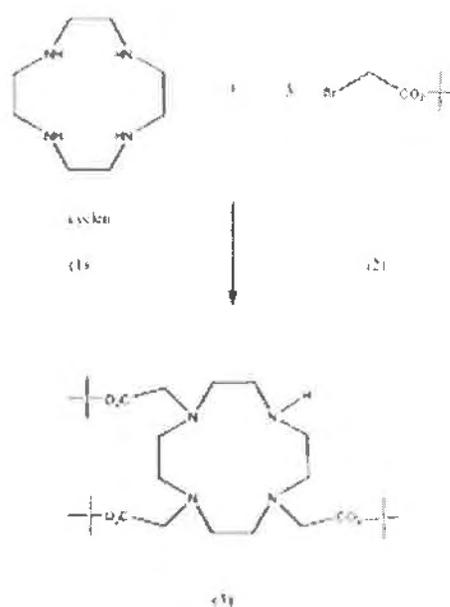
capped and set aside for later use.

Figure F

A separate reaction was started to produce a phenyl-piperazine group to be

added to the protected DO3A molecule. The "DO3A" in the name represents the

cyclic part of the ligand that is bound to the gadolinium. To begin, 1.0 grams of 1-

(2-methoxyphenyl)piperazine, 0.72 grams (472 micro liters) of 3-bromo-1-propanol,

and 0.72 grams of potassium carbonate were added in 30mL of DMF

(dimethylformamide) under nitrogen. The mixture was stirred overnight. The next

day, the reaction was poured onto 100mL of ice and left open to the atmosphere

until the ice melted. The aqueous layer was extracted three times using 80mL of

dichloromethane. The resulting yellow liquid was poured through a funnel

containing magnesium sulfate into a 500mL-round bottom flask. Rotavapping

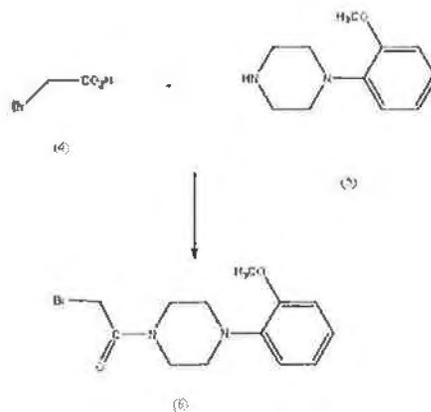resulted in a golden-brown liquid resulted (Figure G).

Figure G

The above product **(6)** was synthesized by reacting 1.38 grams (0.01 mol) of

bromoacetic acid **(4)** with 1.92 grams (0.01 mol) of 1-(2-methoxyphenyl)piperazine

using DCC. Product 6 was obtained as an off-white solid after recrystallization to be

a starting material. NMR was run on product 6 to ensure the desired product was

obtained. From here, 0.14g of product 6 was added to a dry 10mL flask and 0.293g

of the previously synthesized molecule containing the tertiary butyl protecting

groups **(3)** was dissolved in 1mL chloroform and added to the flask. A

chloroform/methanol 5mL mixture was added to the flask (4:1) and the reaction was

stirred at room temperature for 20 hours under nitrogen. After 20 hours the reaction

was rotavapped to a tan solid. The solid product was taken up in 3mL of 50%

dichloromethane/methanol solution. TLC was run in 90%

dichloromethane/methanol. Both the starting products and the rotavapped product

were spotted, and the TLC plate showed a spot representing the desired product **(7)**

in the middle, as expected (see figure H). The dichloromethane/methanol solution

purified by column chromatography filled with Silica gel and rotavapped to a tan,

glassy appearing solid.

The tan, glassy solid was dissolved in 1mL trifluoroacetic acid, covered with a

septum, and stirred for four hours. After stirring, the solution was rotavapped to

remove the trifluoroacetic acid. 25mL of chloroform were added and rotavapped off

to yield the final ligand **(8 in Figure I)**. The ligand was a golden brown, oily,

somewhat syrupy liquid. 0.442 grams of product were obtained. Running NMR

showed that the tertiary butyl protons were removed and the aromatic protons were
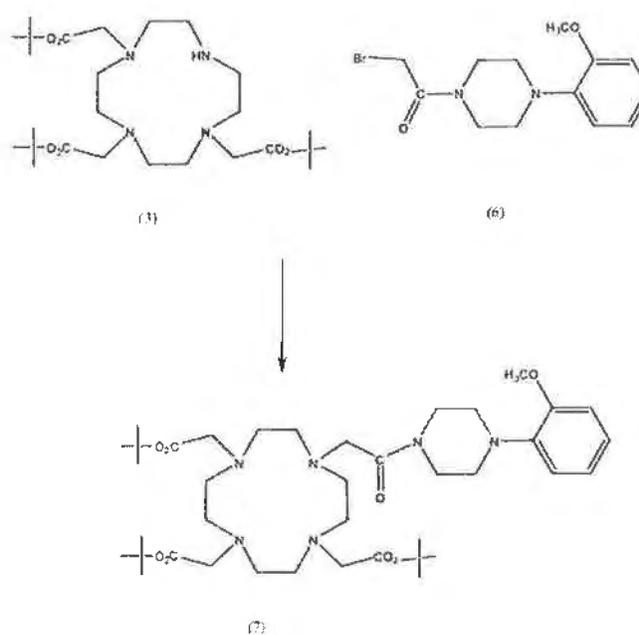
present.

Figure H

The final synthesis step left was to add the gadolinium to the ligand. Based

on the amount of ligand obtained, 0.28 grams of ligand was measured and added

to 3mL of water. Adding 1M NaOH drop-wise adjusted the pH of the mixture to 6.5.

Once the pH reached 6.5, a solution of 0.185 grams gadolinium (III) chloride and

2.5mL water was added drop-wise to the ligand. The pH was brought back to 6.5 by

adding more 1M NaOH drop-wise. The reaction was covered and left to stir

overnight at pH 6.5.

A Chelex-100 column was prepared to remove excess gadolinium. To prepare

the column, 0.5 grams of Chelex-100 was added to a clean beaker. A solution of 1M

NaOH was added to the Chelex-100. The Chelex-100 mixture was then poured into a

column and followed by additional 1M NaOH. The 1M NaOH was eluted off the

column and followed by Milli-Q water until the pH was neutral.

A clean 50mL flask was placed under the Chelex column. Milli-Q water was

poured down the column to ensure the pH was still neutral, then the

ligand/gadolinium mixture was poured through the column. The mixture was eluted

using Milli-Q water, and the water was rotavapped off. 0.246 grams of crystalline,

off-white solid Gd-DO3A-PP was obtained. The "PP" represents the phenyl-

piperazine functional group. HPLC was performed on Gd-DO3A-PP using a Waters

NOVA-PAK C18 column with 100% CH₃CN as the eluting solvent. HPLC indicated
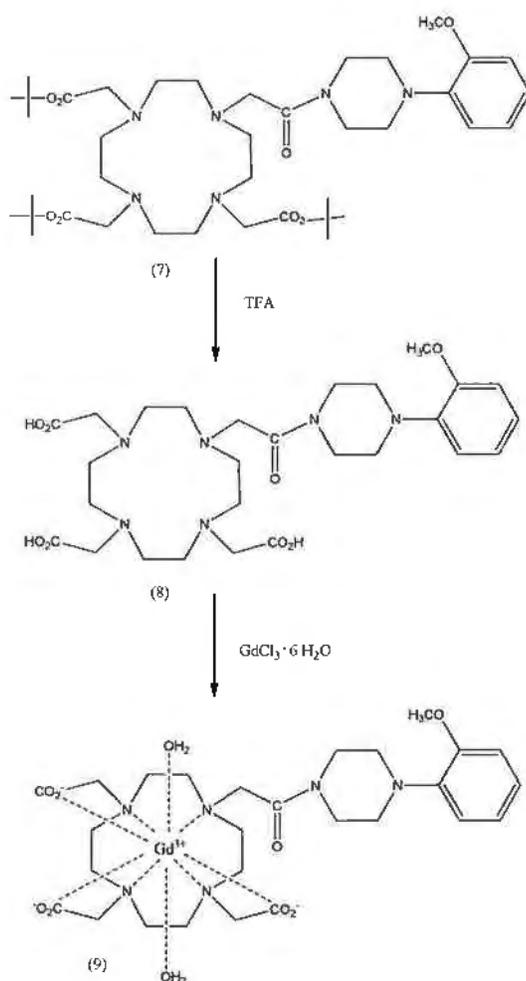
100% purity of the synthesized agent.

Figure I

T1 Relaxation Measurements

ProHance, Magnevist, and Omniscan were measured, using T1 relaxation times

to compare their overall relaxation rates to the rate obtained from Gd-DO3A-PP.

First, a stock solution of 0.001M was made to use in making five dilutions at concentrations of 0.02mM, 0.01mM, 0.025mM, 0.05mM, and 0.1mM for each contrast agent. To make the stock solution, 2µL of contrast agent was measured and added to 998µL of D_2O in a glass vial. The vial was inverted multiple times to ensure proper mixing of the solution. The dilutions were made by diluting the stock solution and using deuterium oxide (D_2O). To obtain the first dilution at 0.002mM, 2µL of stock was mixed with 998µL of D_2O. The second dilution at 0.01mM was obtained by adding 10µL of stock to 990µL of D_2O. The third dilution at 0.025mM was obtained by adding 25µL of stock to 975µL of D_2O. The fourth dilution at 0.05mM was obtained by adding 50µL of stock to 950µL of D_2O. The final dilution at 0.10mM was obtained by adding 100µL of stock solution to 900 µL of D_2O. HPLC vials were used for mixing all solutions and the solutions were transferred to NMR tubes. T1 relaxation studies were run on each agent and the individual relaxation rates for each concentration were measured and graphed. The slope of the line of best fit represented the overall relaxation rate.

A stock solution of Gd-DO3A-PP was made identically to the other contrast

agents and used to make five solutions of increasing concentrations (0.02mM,

0.01mM, 0.025mM, 0.05mM, and 0.1mM) to be tested. The different concentrations

were tested individually to find the relaxation rates at each specific concentration.

The data were graphed and the overall relaxivity rate for Gd-DO3A-PP was taken

from the slope of the line of best fit.

Results

Shown below is Figure J obtained from the HPLC purification step. Because only one peak is shown at 1.5 minutes, Gd-DO3A-PP is deemed to be 100% pure. There were no additional peaks representing other compounds or impurities.
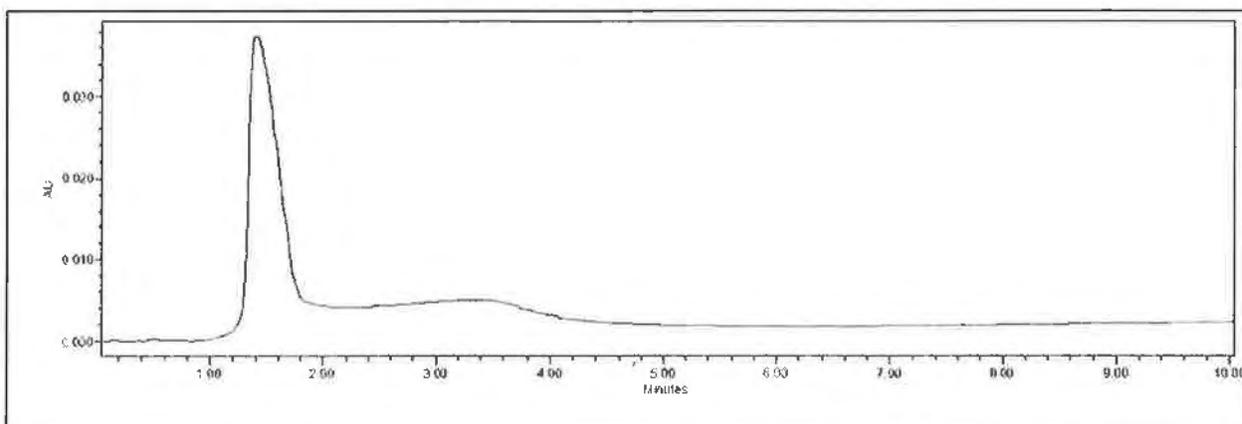

Figure J

Nuclear Magnetic Resonance (NMR) Spectroscopy was used to characterize the products obtained throughout the entire synthesis of Gd-DO3A-PP. The NMR seen in Figure K below was run on cyclen **(1)**, one of the starting materials at the beginning of the synthesis. The large peak around 2.5-3ppm represents eight CH_2 groups in the ringed structure. The peak around 7.5ppm represents chloroform ($CHCl_3$), as the solvent used was deuterated chloroform ($CDCl_3$).
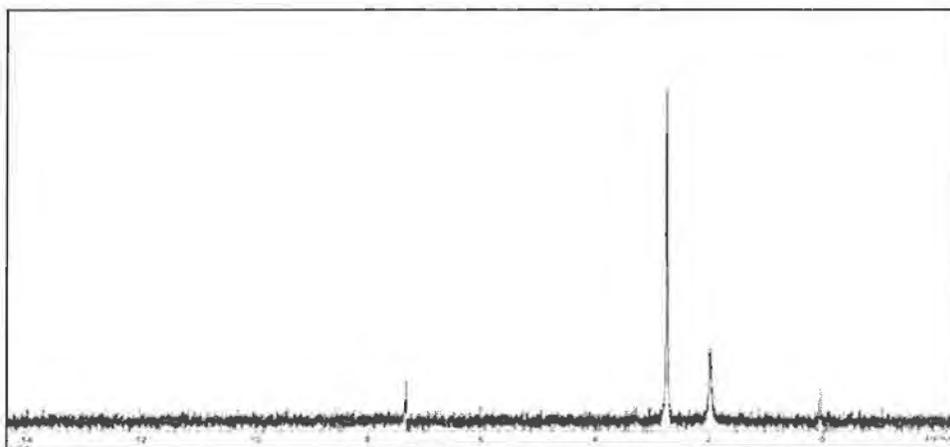
Figure K

NMR was run on bromoacetamide **(6)** and results are shown in Figure L

below. The peak around 7.0ppm represents the aromatic protons that are present.
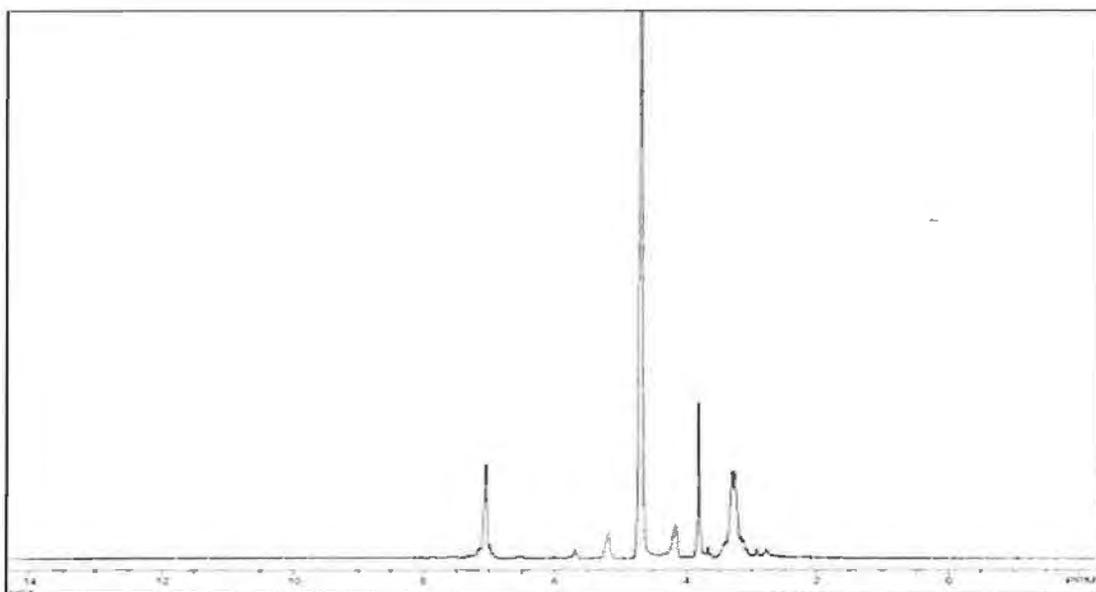

Figure L

NMR was run on the ligand **(7)** before the tertiary butyl groups were removed and

results are shown below in Figure M. The large peak around 1.5ppm represents the

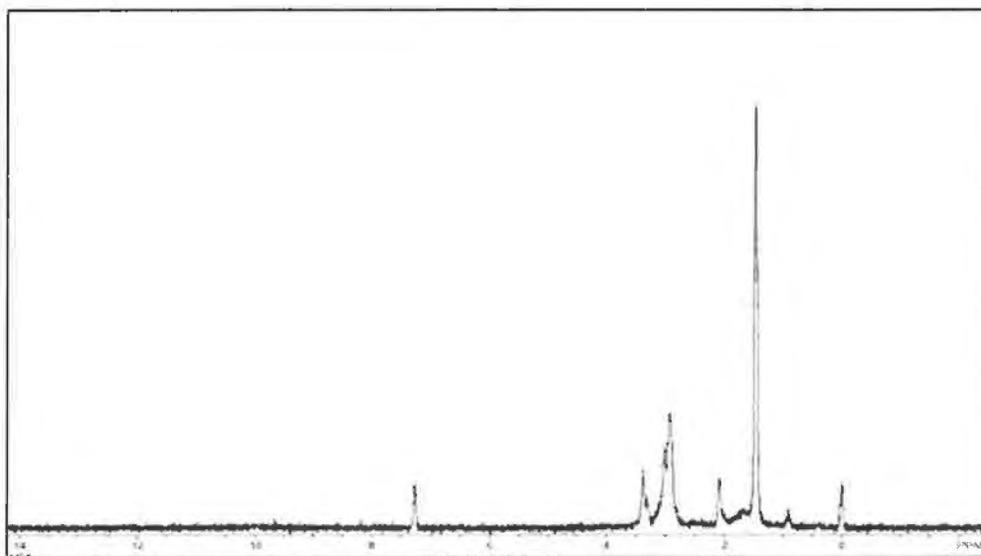tertiary butyl groups that are present.

Figure M

NMR was run on the final ligand **(8 in Figure I)** without gadolinium added

and results are shown below. The peaks show the aromatic signals at ~7.5ppm

along with the hydrogens of the cyclen ring ~3-4ppm. The peak at 1.5ppm is no

longer present, indicating that the tertiary butyl protecting groups were successfully
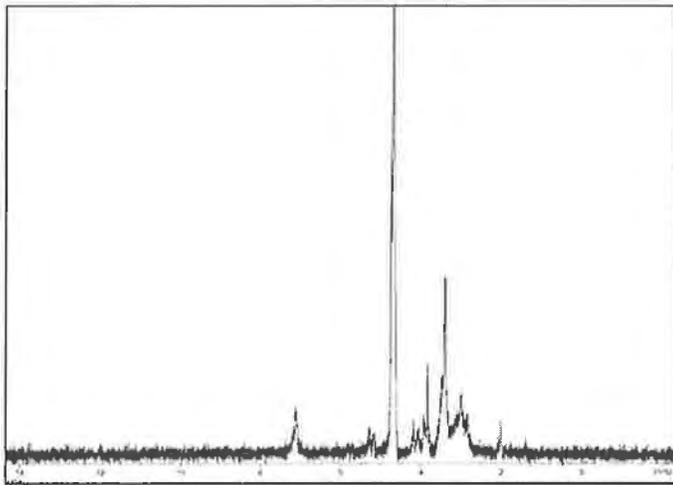
removed.

Figure N

Table 1 below represents the individual relaxivity rates at each concentration

of ProHance. Figure N shows the overall relaxivity of ProHance was measured to be

4.318mM/s.

Concentration (mM)	Relaxation Rate (1/T1)
0	0.08306
0.002	0.08743
0.01	0.14761
0.025	.020516
0.05	0.31618
0.1	0.51622

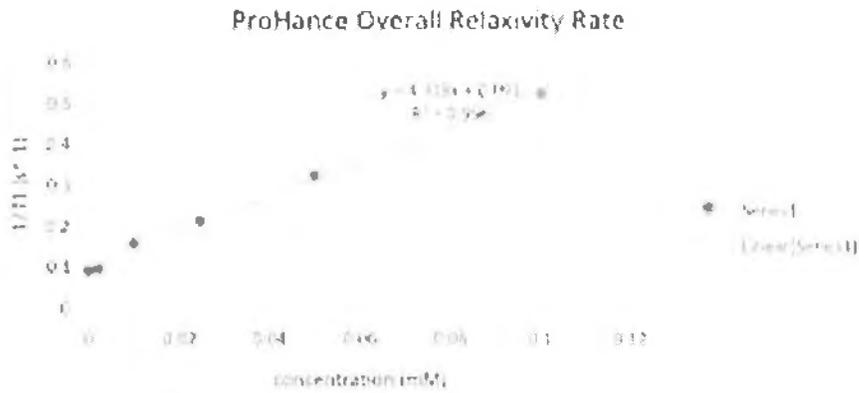**Table 1**

ProHance Overall Relaxivity Rate

Figure O

Table 2 represents the individual relaxivity rates at each concentration of

Magnevist. Figure O shows the overall relaxivity was measured to be 5.053mM/s.

Table 2

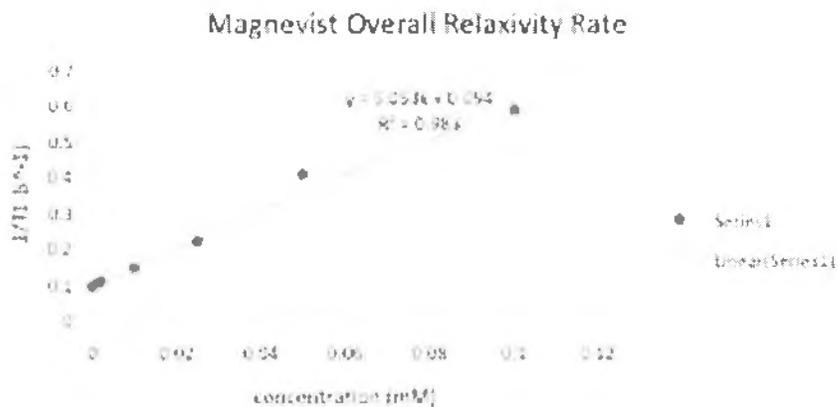| Concentration (mM)	Relaxation Rate (1/T1)
0	0.0873
0.002	0.0996
0.01	0.1360
0.025	0.2141
0.05	0.3973
0.1	0.5774

Magnevist Overall Relaxivity Rate

Figure P

Table 3 below represents the individual relaxivity rates at each concentration

of Omniscan. Figure P shows the overall relaxivity was measured to be 4.697mM/s.

Table 3

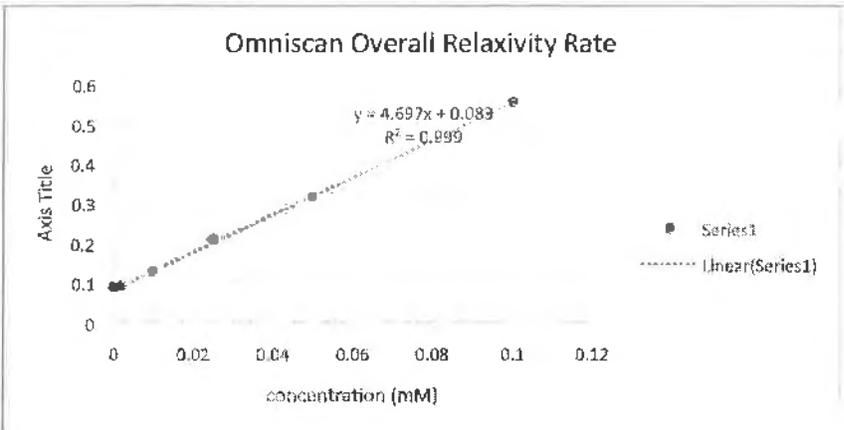| Concentration (mM)	Relaxation Rate (1/T1)
0	0.0873
0.002	0.0925
0.01	0.1261
0.025	0.2047
0.05	0.3141
0.1	0.5550

Figure Q

Table 4 below represents the individual relaxivity rates at each concentration

of Gd-DO3A-PP. Figure Q shows the overall relaxivity was measured to be

5.465mM/s.

Table 4

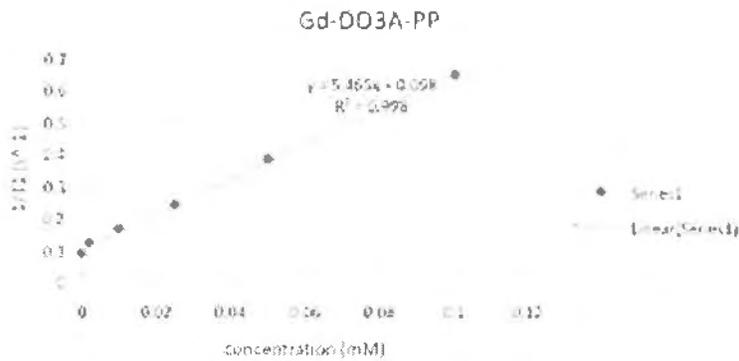| Concentration (mM)	Relaxation Rate (1/T1)
0	0.08413
0.002	0.11477
0.01	0.16143
0.025	0.23650
0.05	.037655
0.1	0.64212

Figure R

Table 5 represents the relaxivity rates obtained from this research. The first

column showing the results at 1.5T represents the overall relaxivity rates measured in

this research. The second column represents values found in literature of relaxivity

rates at 1T, which is the typical magnetic field strength of MRI scanners used in

clinical hospitals. The relaxivity rates are clearly shown to be lower at 1T, but the

relaxivity value for Gd-DO3A-PP should still be higher than those reported.

Table 5

Contrast Agent	Measured Relaxivity Rate (mM/s) @60MHz (1.5T)	Reported Relaxivity Rate (mM/s) @ 1T	Osmolality (mOsmol/kg H_2O)
ProHance	4.318	3.7	630
Magnevist	5.054	3.4	1960
Omniscan	4.698	3.9	798
Gd-DO3A-PP	5.465	-	-

Conclusions and Future Works

This research was successful in synthesizing a novel, water-soluble gadolinium contrast agent with confirmed structure and high purity. The results indicate that, as the dilutions of the contrast agent became more concentrated, the T1 values decreased. This showed a larger relaxivity rate as the concentrations increased, which gave the overall relaxation rate. Gd-DO3A-PP obtained the highest relaxation rate of 5.465mM/s. ProHance acquired the lowest rate of the four compounds at 4.318mM/s.

The next step in this research is for our synthesized agent to undergo testing to determine the overall relaxivity rate at 1T. The osmolality of Gd-DO3A-PP should also be determined. Phantom studies will also be conducted to determine the overall contrast enhancement of MRI images. Specificity and uptake in the brain will be measured through *in vivo* imaging in mice or rats.

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research project. Having the chance to work extensively in a lab, working one-on-

one with my professor, and completing my own research project was a huge step in

my growth as a student, and I am very grateful for the opportunity I was given.

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gained knowledge and experience that will help me in all of my future endeavors in

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