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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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April 24, 2018

Callie Clement

Summer 2017

Synthesis and Relaxivity of a Target-
Specific MRI Contrast Agent

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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).

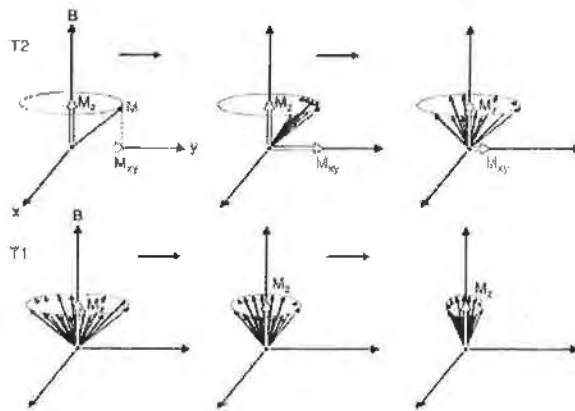


Figure A

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, superparamagnetic, 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.

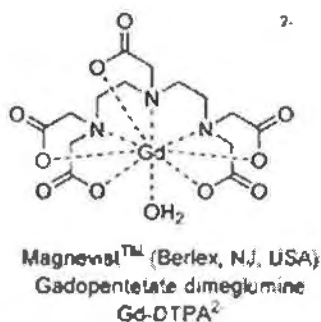


Figure B

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).

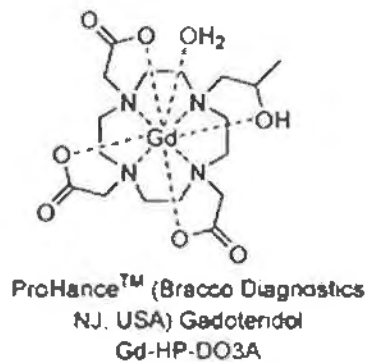


Figure C

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).

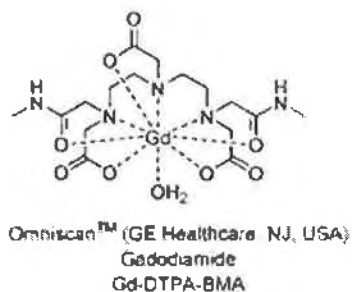
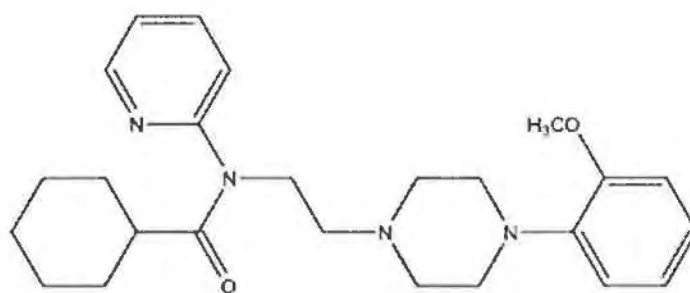


Figure D

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-HT_{1A} 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-HT_{1A} is a serotonin receptor. When tested in rats, WAY-100635 binds to rodent 5-HT_{1A} 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-

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 dropwise 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.

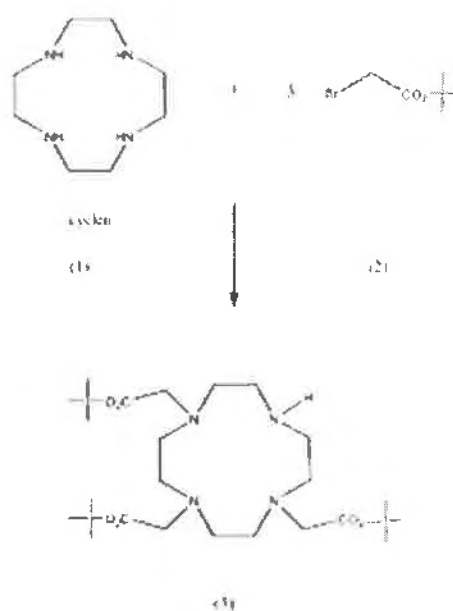


Figure F

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).

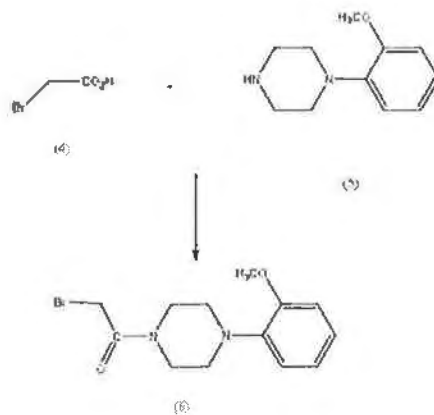


Figure G

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.

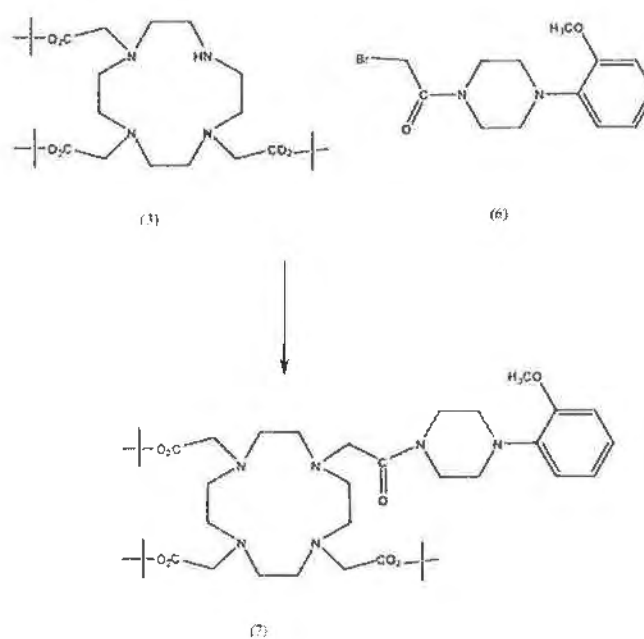


Figure H

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.

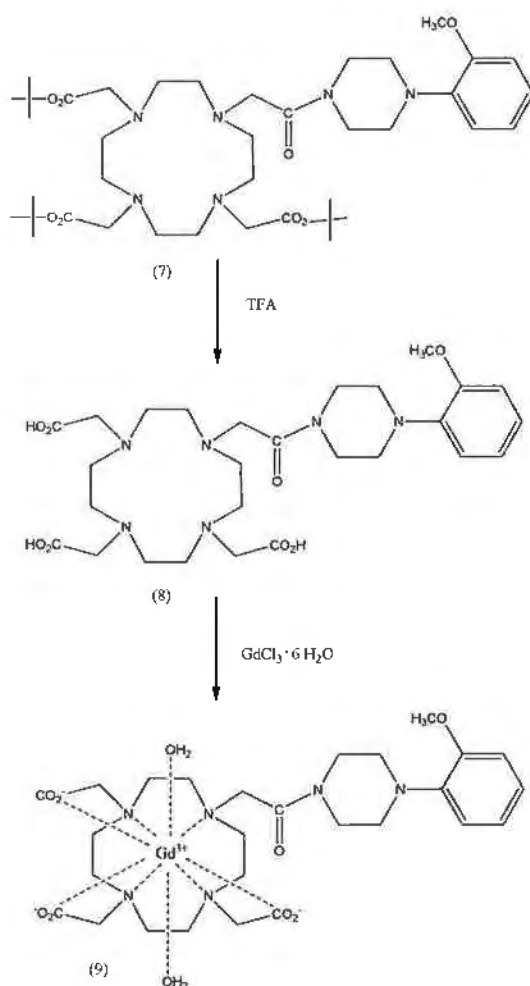


Figure 1

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, 2 μ L of contrast agent was measured and added to 998 μ L of D₂O 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 (D₂O). To obtain the first dilution at 0.002mM, 2 μ L of stock was mixed with 998 μ L of D₂O. The second dilution at 0.01mM was obtained by adding 10 μ L of stock to 990 μ L of D₂O. The third dilution at 0.025mM was obtained by adding 25 μ L of stock to 975 μ L of D₂O. The fourth dilution at 0.05mM was obtained by adding 50 μ L of stock to 950 μ L of D₂O. The final dilution at 0.10mM was obtained by adding 100 μ L of stock solution to 900 μ L of D₂O. 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.

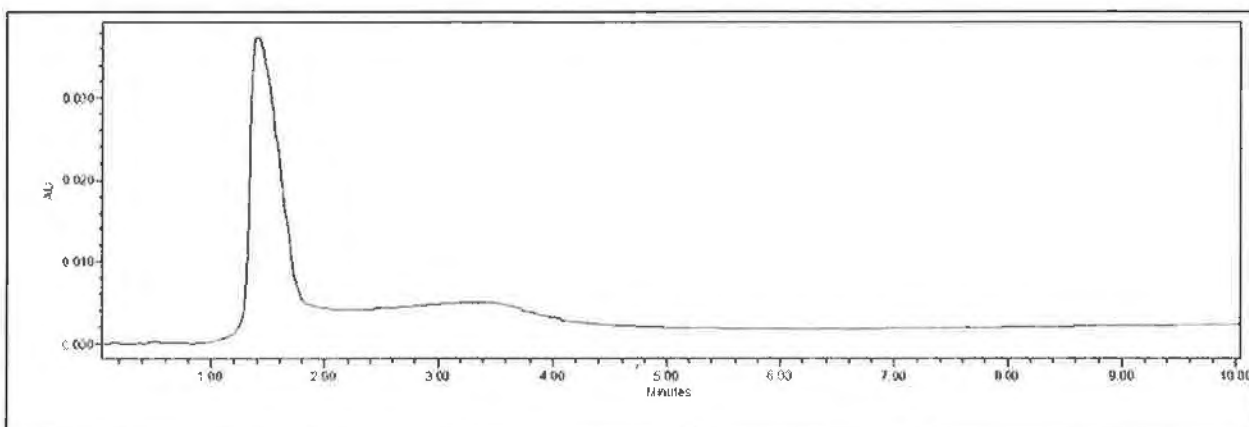


Figure J

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 CH₂ groups in the ringed structure. The peak around 7.5ppm represents chloroform (CHCl₃), as the solvent used was deuterated chloroform (CDCl₃).

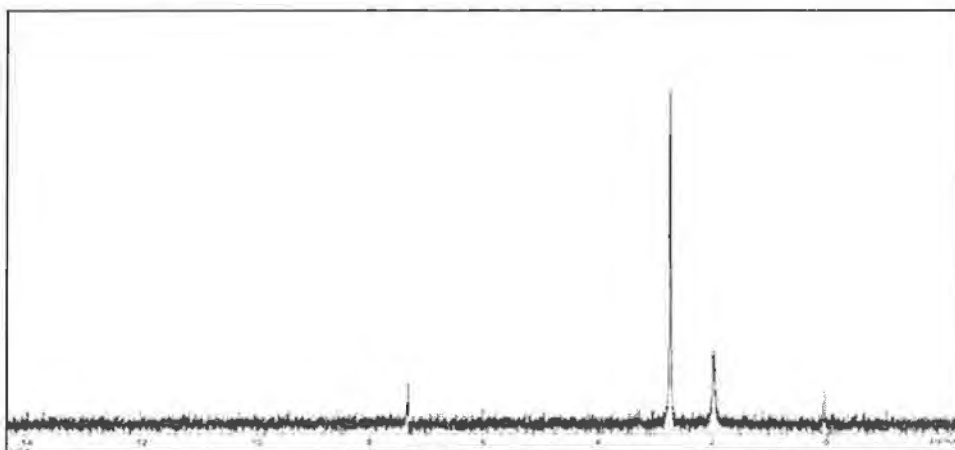


Figure K

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.

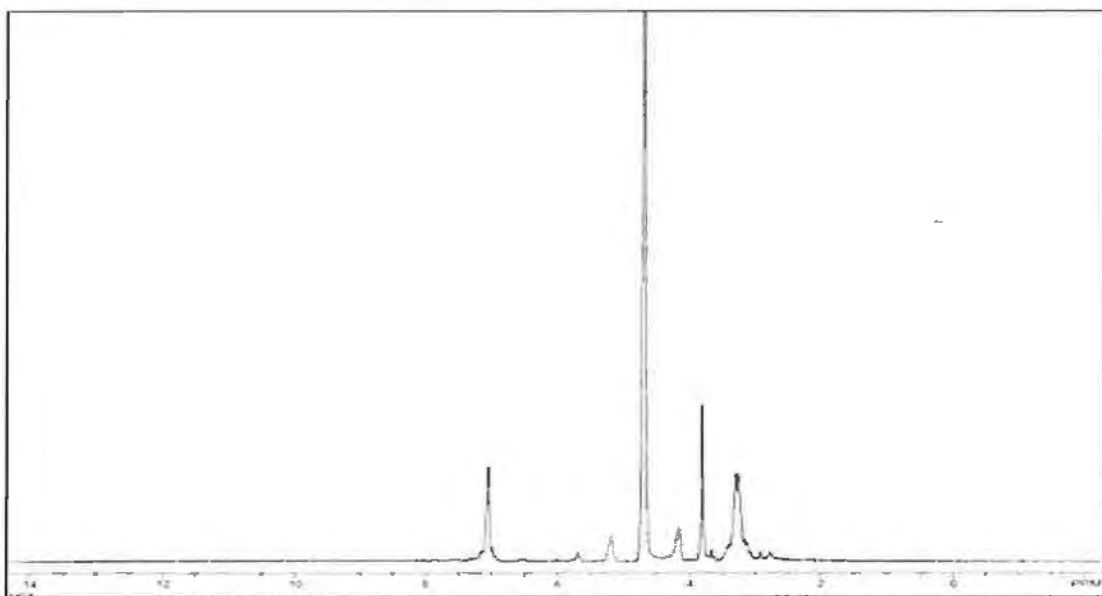


Figure L

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.

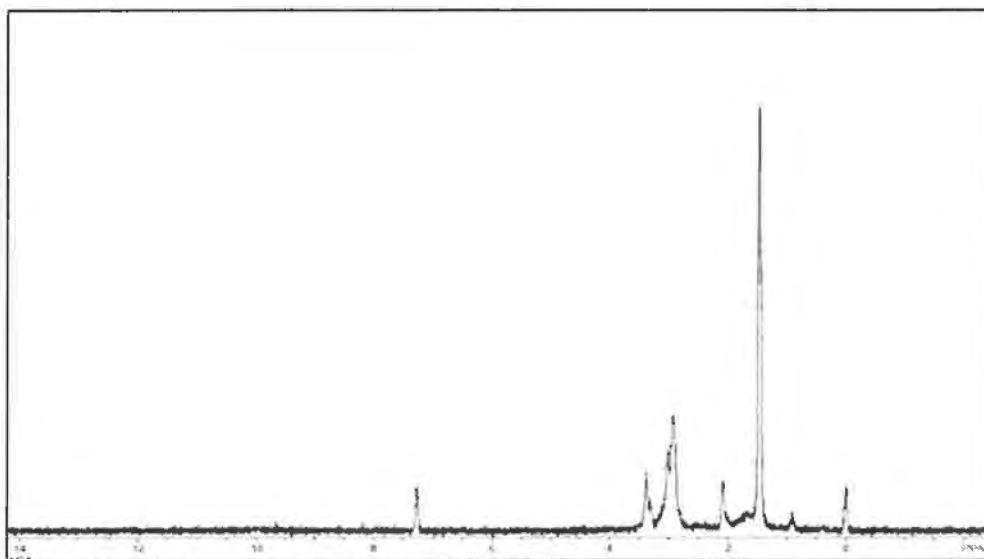


Figure M

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.

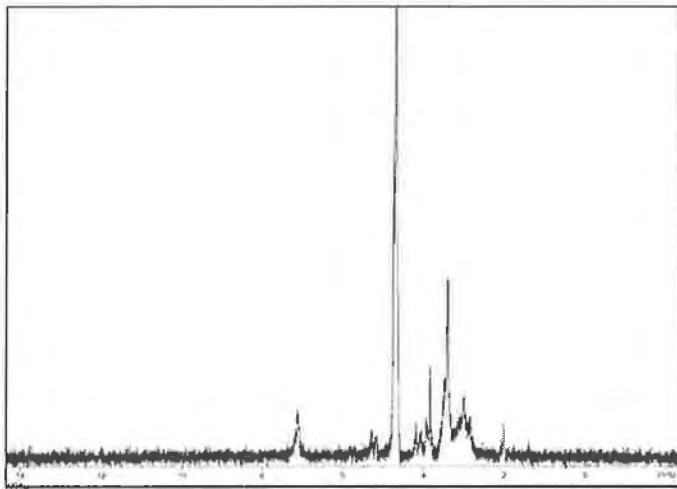


Figure N

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

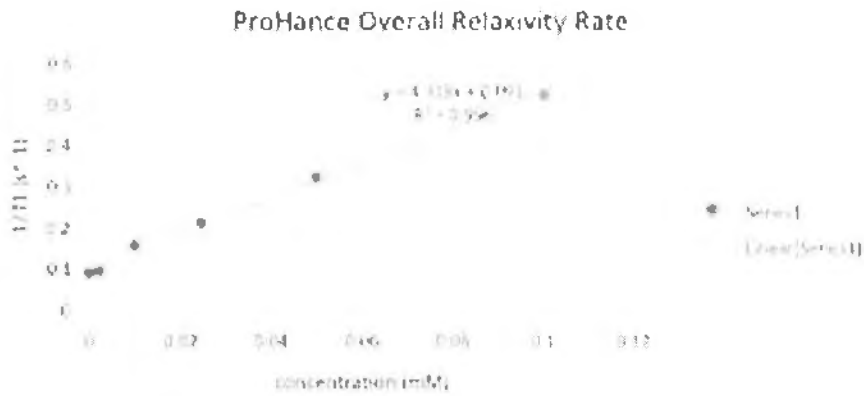


Figure O

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

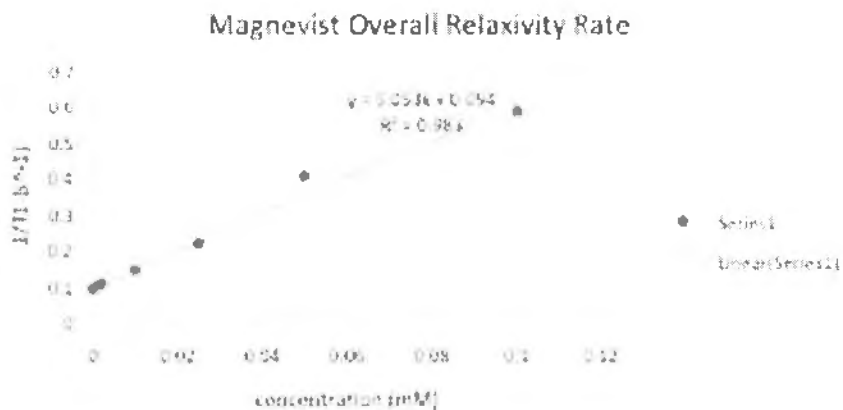


Figure P

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

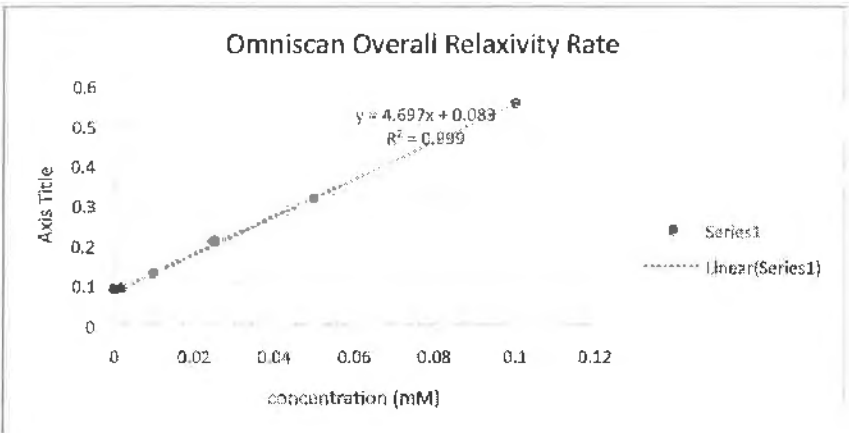


Figure Q

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

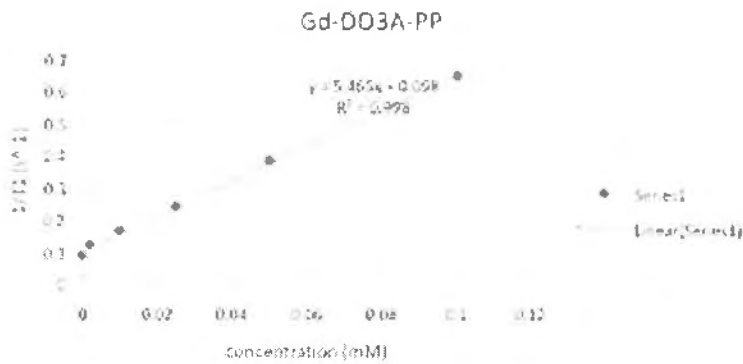


Figure R

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 ₂ O)
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.

Acknowledgements

I would like to thank Dr. Joseph E. Bradshaw for being the primary instructor of this research. He directed me through the project and instructed me in the lab. I would also like to thank Ouachita Baptist University, specifically the Patterson School of Natural Sciences for providing the labs, resources, and equipment necessary to complete this project. Lastly, I would like to thank the J.D. Patterson Summer Research Program for giving me the opportunity to participate in such an interesting 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. Without funding from the J.D. Patterson Summer Research Program, I wouldn't have gained knowledge and experience that will help me in all of my future endeavors in the scientific field.

Works Cited

"Osmolality Blood Test: MedlinePlus Medical Encyclopedia." *MedlinePlus*, U.S. National Library of Medicine, 5 Apr. 2018, medlineplus.gov/ency/article/003463.htm.

Moore, Dennis A. "Selective Trialkylation of Cyclen With tert-Butyl Bromoacetate." *Organic Syntheses*, vol. 85, 2008, pp. 10–14., doi:10.1002/0471264229.os085.02.

Preston, MD, David C. "Magnetic Resonance Imaging (MRI) of the Brain and Spine: Basics." *MRI Basics*, 30 Nov. 2006, [casemed.case.edu/clerkships/neurology/WebNeurorad/MRI Basics.htm](https://casemed.case.edu/clerkships/neurology/WebNeurorad/MRI%20Basics.htm).

Themes, UFO. "Basic Principles and Terminology of Magnetic Resonance Imaging." *Musculoskeletal Key*, 25 May 2016, musculoskeletalkey.com/basic-principles-and-terminology-of-magnetic-resonance-imaging/.

Wang, Cheng-Chung, et al. "Regioselective One-Pot Protection of Glucose." *Nature Protocols*, vol. 3, no. 1, 2008, pp. 97–113., doi:10.1038/nprot.2007.493.

"WAY-100635 Maleate (ab120550)." *WAY-100635 Maleate / Abcam*, Abcam, 14 Apr. 2018, www.abcam.com/way-100635-maleate-ab120550.html.

Zigelboim, Isaac, et al. "Synthesis, Binding Affinity, and Relaxivity of Target-Specific MRI Contrast Agents." *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, vol. 59, no. 3-4, 2007, pp. 323–329., doi:10.1007/s10847-007-9331-2.