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Toward Synthesizing a Selective Dopamine Binding Magnetic Resonance Contrast Agent

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Toward Synthesizing a Selective Dopamine Binding Magnetic Resonance Contrast Agent

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Abbreviations: *Knowledge of chemical symbols and formulas is assumed and are not listed here.*

MRI	Magnetic resonance imaging
CA	Contrast agent
1H-NMR	Proton nuclear magnetic resonance
GC-MS	Gas chromatograph-mass spectrometer
TLC	Thin layer chromatography
DAT	Dopamine transporter

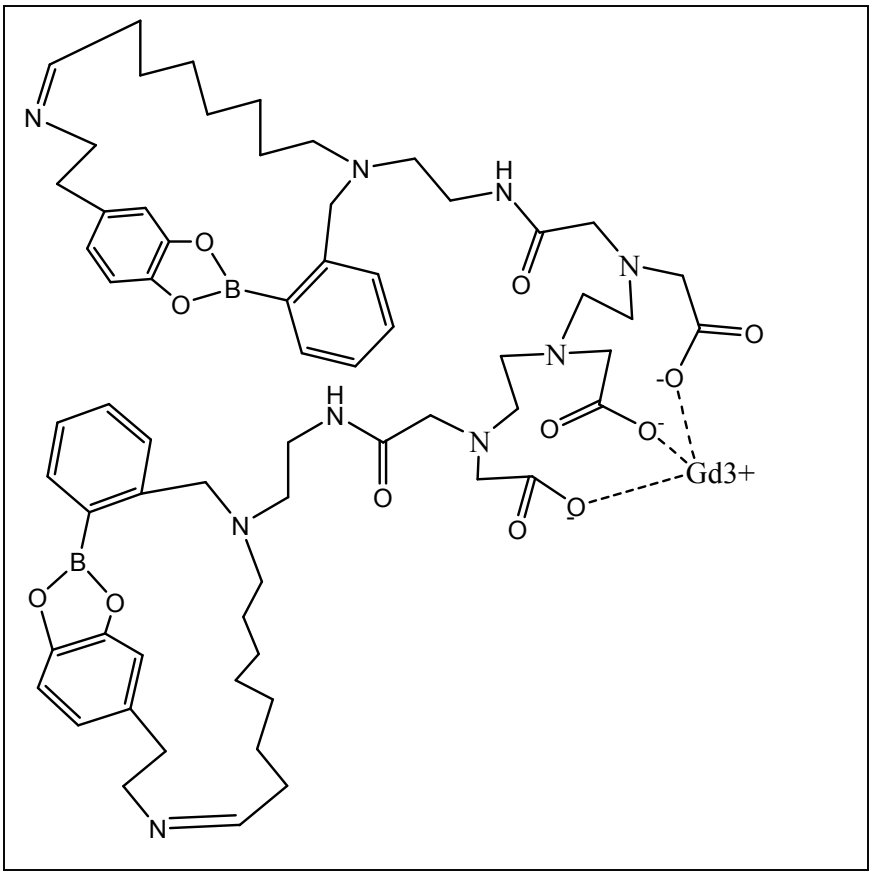
Abstract

Magnetic resonance imaging (MRI) is a non-invasive diagnostic methodology used to provide a two-dimensional view of an internal organ or structure, especially the brain and spinal cord. Magnetic resonance contrast agents are usually injected into necessary parts of the body prior to imaging to increase the differences between different tissues or between normal and abnormal tissue, making it easier for a radiologist or doctor to interpret the image that is taken. The development of new and more efficient, effective, and selective contrast agents for various biological processes or chemicals is a growing field of research and study.

My project allowed me to engage in undergraduate chemistry research in an academic setting under the supervision of an organic chemistry professor, Dr. DeBoef. He designed a molecule that will be developed further to be a magnetic resonance contrast agent, also referred to as an MRI probe. Reactions were carried out that were the beginning steps of the multi-step synthesis, or recipe, if you will, to make the target molecule which also binds gadolinium, making it MRI active (Figure 1). The entire multi-step synthesis is not included in this paper. The purpose of his design is for the molecule to selectively bind dopamine (Figure 2), a neurotransmitter that has been found to be associated with Parkinson's Disease in humans. His molecule's design is based on existing contrast agent structures published in chemical literature but is a new molecule after changes and additions were made. It can be years later before it will be tested for use in MRI as a contrast agent that is safe for use in humans.

Project Objective

The project's objective was to synthesize a ligand via a 6-step synthesis that is not shown in this paper. The ligand's structure was a combination of Frullano et al.'s MRI probe[11] and Feuster et al.'s dopamine sensor[18]. As a result, it is to be a selective dopamine binding magnetic resonance contrast agent. Figure 1 shows how it will serve as a ligand for the paramagnetic gadolinium ion as well as bind to two molecules of dopamine.



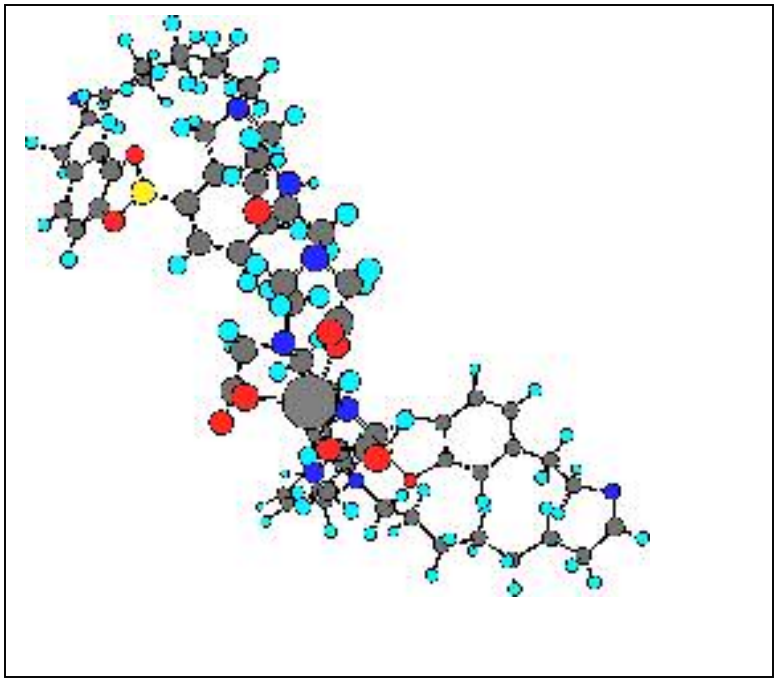


Figure 1. (Top) is a structural drawing of the ligand bound to Gd3+ and also to two dopamine molecules. (Bottom) is a 3-D version of top; Large grey sphere is the gadolinium; Red=Nitrogen; Yellow=Boron; Blue=Oxygen; Light Blue=Hydrogen; Small Grey=Carbon; Note that one boron atom is hidden at the bottom right of the Gd3+. Figures drawn in ChemDraw.

Detection of Dopamine In Its Environment

(This section is written based on Reference 5.)

Scientists are attempting to directly measure dopamine levels in the brains of living organisms. This neurotransmitter is a hot target in biochemical, chemical, and neurological research.

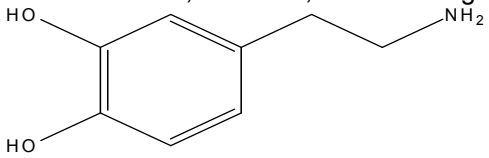


Figure 2. Dopamine

Dopamine is a natural messenger in the brain; it is involved in reward, motivation, and mood.[7] It is involved in motor and cognitive functions. Both dopamine and dopamine transporter (DAT) provide markers for conditions such as Parkinson's disease, attention deficit hyperactivity disorder (ADHD)[8] and schizophrenia. Deficits in dopamine cause Parkinson's Disease in humans. The most serious loss of dopaminergic transmission during Parkinson's disease is in the caudate-putamen; therefore this region has been associated with motor control.[5]

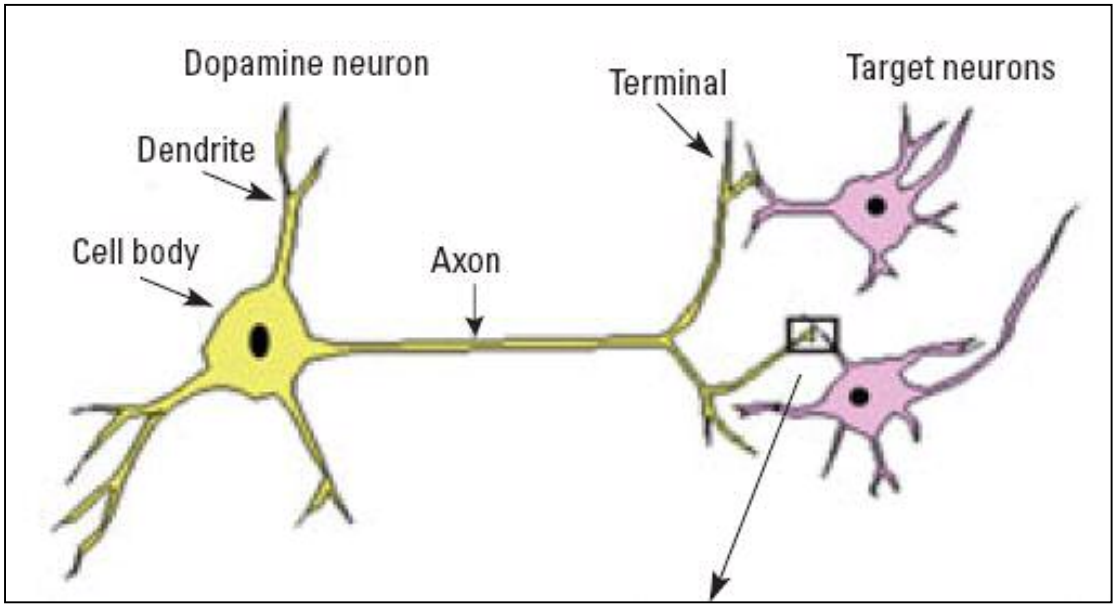
Analytical chemists are working on electrochemical methods and sensors to detect dopamine.[5] The brain, however presents a challenging environment for chemical sensing due to the presence of other compounds including dopamine metabolites, ascorbic acid, and other neurotransmitters such as serotonin, nitric oxide, and norepinephrine.[14] Rapid, selective, and sensitive measurements are critical for real-time detection of chemical changes in the brain.[5]

Figure 3 shows a dopaminergic neuron (taken from Reference 5). Neurons are cells that integrate and control information. Depending on the circuits that they are involved in, they gather, process, and relay specific types of information. Dopaminergic neurons are located in only a few discrete regions of the brain, with the cell bodies found in one area, and the axons projecting in another. They contain dendrites and axons which are projections attached to the cell body. Most cell bodies are in the substantia nigra/ventral tegmental area and their axons project to various regions such as the caudate-putamen or the nucleus accumbens. Projections to nucleus accumbens play a part in the brain reward circuit. There are only ~10,000 dopaminergic neurons on each side of the brain in this pathway, but each axon can branch and form many terminals. These regions cover only a few millimeters in their largest dimension.

Dopamine is synthesized in the neuron and then packaged into membrane-bound packets called vesicles. See Figure 3 again. An electrical impulse called an action potential initiates release of neurotransmitter. Each neuron has a resting membrane potential caused by ionic concentration gradients across the cell membrane. When binding occurs between the appropriate neurotransmitter and the receptors on the dendrites or cell body, ion channels open, initiating an action potential, or "firing." This action potential propagates through the axon to the terminal. Other ionic channels in the terminals are opened, which cause the vesicles to release their contents. This releasing process is called exocytosis and occurs on a millisecond timescale. The extracellular concentration of neurotransmitter following exocytosis depends on the number of vesicles released as well as the amount of neurotransmitter present per vesicle, which may be quite small.[5] Retinal dopaminergic neurons are said to only contain 10^{-21} mole quantities of dopamine.[12] After exocytosis, dopamine rapidly diffuses out of the synaptic cleft to interact with dopaminergic receptors. Dopamine concentrations in extracellular fluid depend on uptake and diffusion.

Dopamine transporter (DAT) is a membrane-bound protein that pumps dopamine from the extracellular space and controls uptake into neurons. Uptake is fast in the caudate-putamen region of the brain, occurring at 4 micromoles per liter per second.[5] Once dopamine gets transported back into the neuron, it is either repackaged into vesicles or metabolized.[5]

Dopamine is released in low concentrations and rapidly cleared from extracellular space,[5] therefore, our MRI probe should be developed for use in intracellular space, if possible. Measuring dopamine concentrations inside the 100-nm synapse has not yet been achieved yet.[5] Analytical chemists are currently trying to minimize the size of the electrodes that they use to approach the synapse as well as minimize tissue damage. Our MRI probe, if successful, would not be invasive to human tissues in this sense.



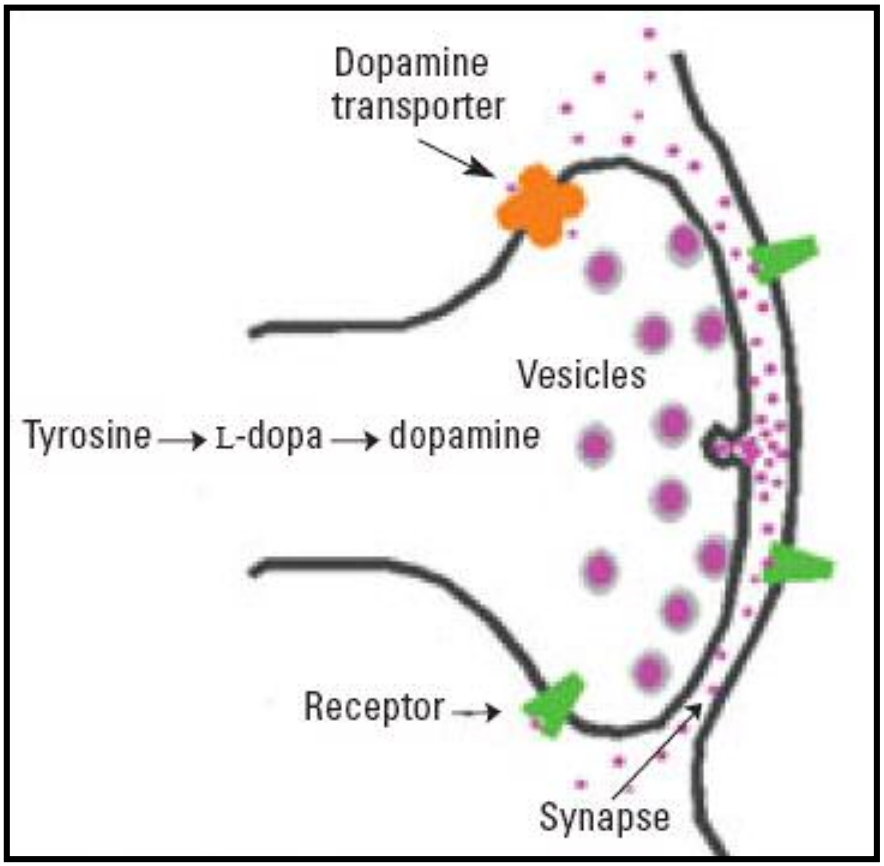


Figure 3. Venton, B. J. and Wightman, R. M. *Anal. Chem.* **2003**, 414A-421A.

Magnetic Resonance Imaging

(This section is based on Reference 13.)

MRI is a noninvasive imaging technique that uses magnets to create an external magnetic field and low-energy radio frequency signals. MRI gathers information from atomic nuclei in the body, specifically hydrogen nuclei, or protons.

The patient is submitted to a strong external magnetic field (B_0). Nuclei adopt to one of two orientations to become either parallel or anti-parallel to the external field. Parallel alignment is the lower energy state and is the preferred alignment. See Figure 5 and 6.

The net magnetization vector (Mz) aligned to the external magnet results from the difference between the two states, which is very small, with a population ratio of 100,000 to 100,006. Individual nuclei that do not line up with B_0 wobble or precess around the direction of the magnetic field. This precessional, or Larmor frequency is given by equation (1).

$$F = \gamma B_0 / 2\pi$$

where F is the precessional frequency, B_0 is the strength of magnetic field, and γ is the gyromagnetic ratio of the nucleus.

Eq (1). From Reference 13.

Commercial systems use magnetic fields that are about 1.5 Tesla, and a Larmor frequency of 63.75 MHz for hydrogen nuclei. Each individual nucleus has a different phase of precession around the axis of the magnetic field. The net magnetization vector from nuclei inside a magnet in equilibrium state is static and does not produce a measurable signal. To obtain information from spins the direction of the Mz must be altered. Precessing spins are excited by applying energy in the form of radio frequency (RF) energy pulses of exactly the Larmor (resonance) frequency. When the RF signal is administered into patient, two things occur: 1) Enough protons absorb energy to jump from the lower energy parallel to higher energy anti-parallel state; 2) Electron are whipped to precess in phase. The effect is that Mz flips 90 degrees from positive z-axis to transverse plane. It then rotates around B_0 at the Larmor frequency. This rotating transverse Mz can be measured because it induces an AC in the receiver coil that is placed around the patient. See Figure 7.

After the RF transmitter is switched off, the equilibrium state will be sought where the high energy decreases to low energy. In other words, the magnetization decays over time; this is represented by a decrease in the magnitude of Mz in the transverse plane. Consequently, the induced signal in the receiver coil will also decrease over time. This decreasing signal is known as Free Induction Decay (FID).

Relaxation time is the time required for the signal to return to equilibrium. Two relaxation processes exist: longitudinal relaxation and transverse relaxation. See Figure 8.They are independent of one another and are characterized as T1 and T2 relaxation times, respectively. Longitudinal relaxation is the process of realignment to the external magnetic field. The T1 relaxation time is the time required for the system to recover to 63% of its equilibrium value after it has been exposed to a 90degree RF pulse. Various human tissues have different T1 values. Transverse relaxation, also known as spin-spin relaxation, depends on the spins precessing around the Mz vector. After excitation by RF pulse the spins initially precess completely in phase. As time passes, the observed signal starts to decrease because the spins start to diphase due to small differences in the Larmor frequency induced by random local magnetic inhomogeneities, spin-spin interactions, and inhomogeneity of the main static magnetic field, B_0. T2 relaxation time is the time it takes for dephasing to decay the signal to 37% of its value. T2 time is different for various tissues but is always shorter than T1.

MRI has the potential to visualize the difference in T1 and T2 of different tissues. Using these differences, contrast between different soft tissues in MRI is superb compared with x-ray computer tomography.[15] See

Figure 4 for different MRI images of the brain that are T1-weighted, proton-density weighted, and T2-weighted.

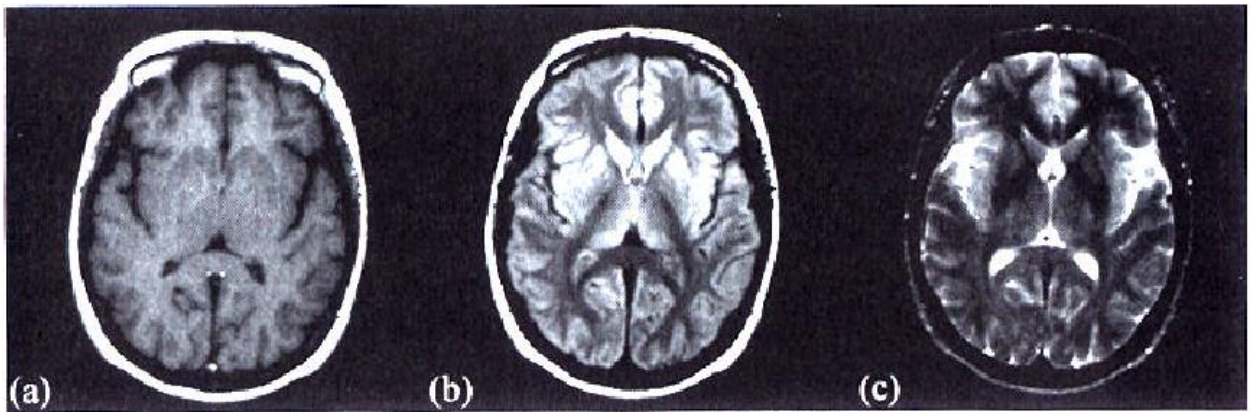
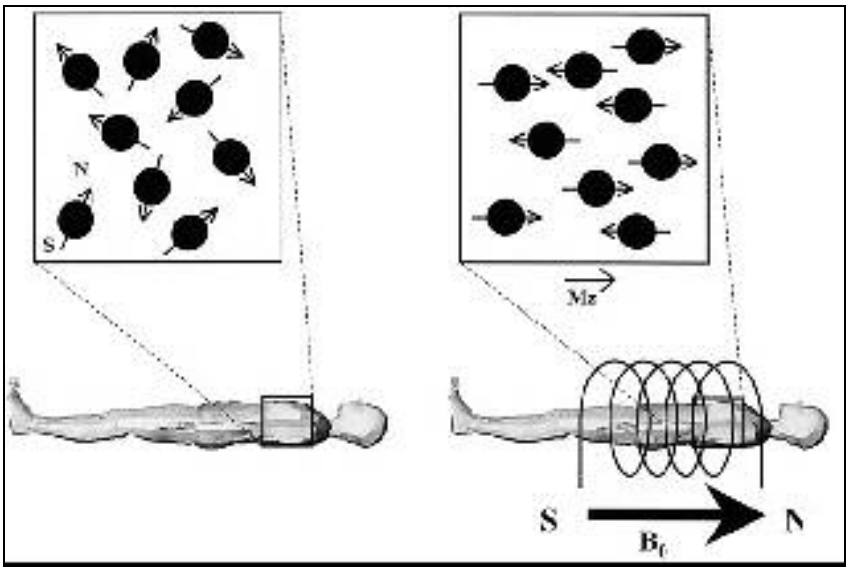


Figure 4. (Taken From Reference 9, Figure 1.3): MRI images of the brain: (a) T1-weighted image; (b) proton-density weighted image; (c) T2-weighted image.

The pictures below were taken from Reference 13.

Figure 5. This shows nuclei in the region of the heart, but imagine it as if we were focused on the brain. The nuclei go into parallel or anti-parallel arrangement once B_0 is applied (right picture).



These pictures were taken from Reference 13.

Figure 6. A. shows the nuclei precessing as B_0 and Mz are present.
B. shows nuclei and their various phases.

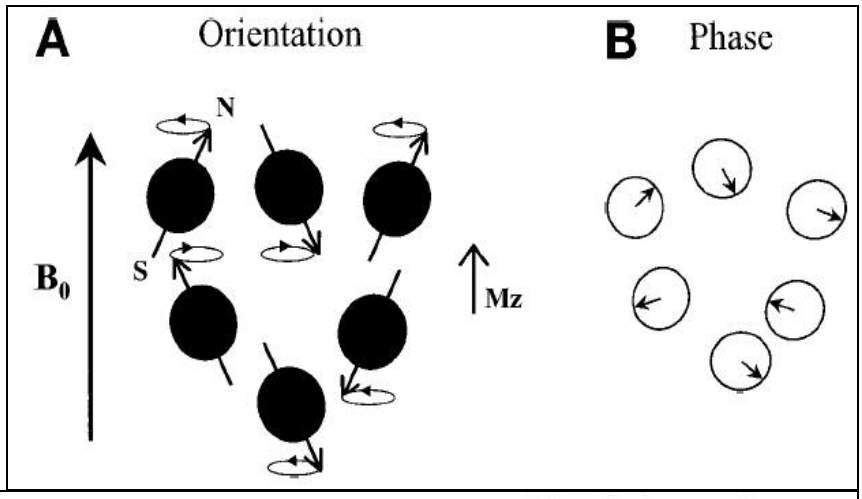


Figure 7. This shows the Mz as RF pulses are administered, as well as the induced AC in the receiver coil.

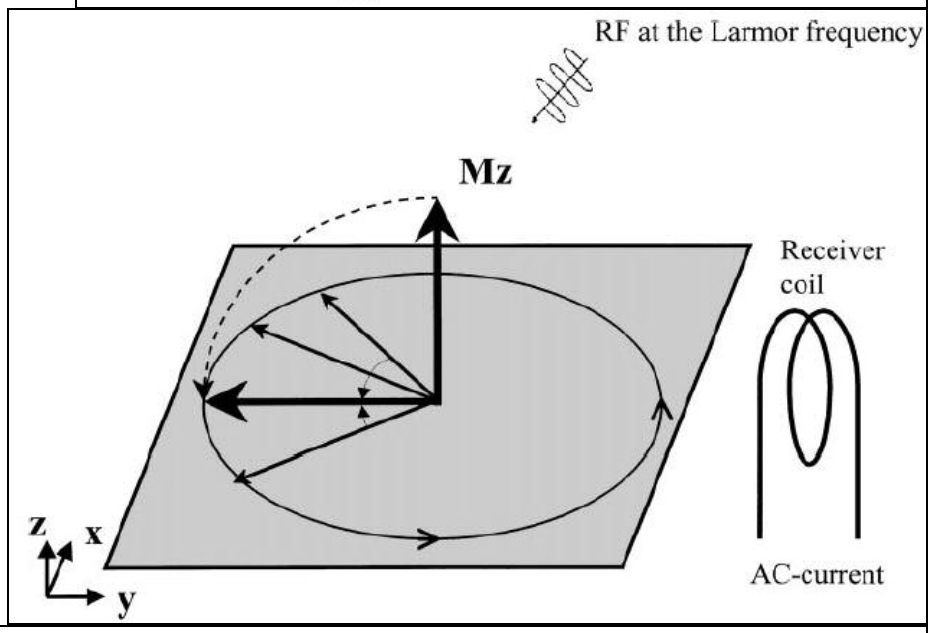
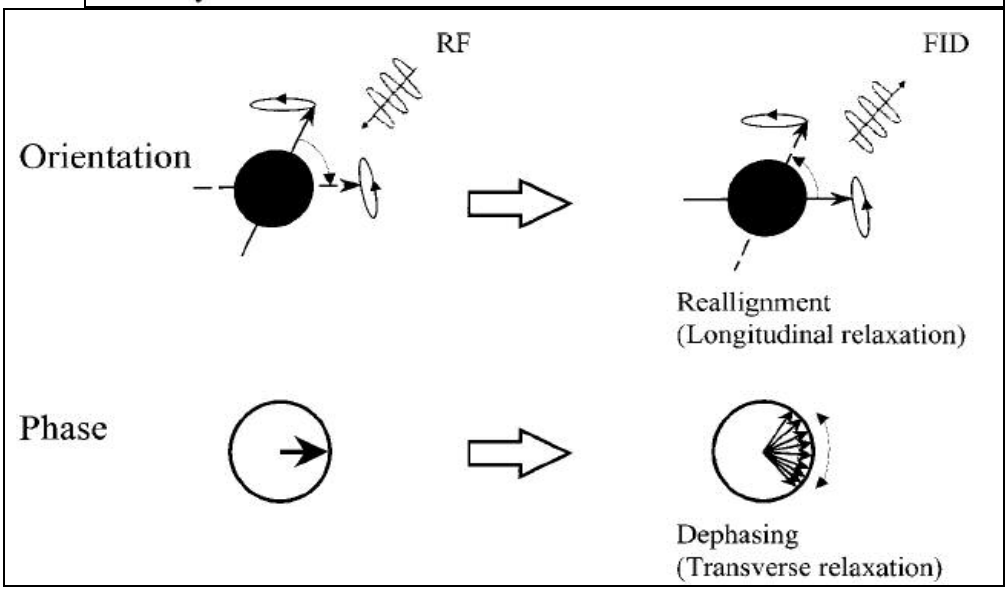


Figure 8. This shows the two types of relaxation as they happen to nuclei. Top row shows longitudinal relaxation and bottom row shows transverse relaxation.



Magnetic Resonance Contrast Agents

Magnetic contrast agents shorten relaxation times of water protons to improve contrast in images. Frullano et al state that current CA's have the following disadvantages: poor efficiency with regard to shortening of T1 and T2 and lack of specificity.[11]They synthesized MRI probes to detect sialic acid. Most CA's use gadolinium. However, it is toxic and cannot be injected as is.[10] The ligands should bind it tightly. Ligands currently used form stable Gd3+ chelates. [10]

Some MR CA's that are currently on the market are listed with with commonly used brand names in parentheses: In Figure 9a is Gadodiamide (Omniscan); In Figure 9b is Gadopentetate dimeglumine (Magnevist); Gadoteridol (Prohance) and Gadoversetamide (OptiMark) are not shown.

Louie and workers created MRI CA's that can indicate reporter gene expression in vivo in opaque animals.[4] Their CA's function due to effects on the first coordination sphere of the chelated paramagnetic ion. The access of water into the first coordination sphere is blocked with a substrate that can be removed by enzymatic cleavage. Following cleavage, the paramagnetic ion interacts with water to increase MR signal. In reference 4 they report galactopyranose as the blocking group, which makes the CA sensitive to expression of the commonly used marker gene, beta-galactosidae. Regions of higher intensity in the MR image correlate with regions expressing the marker enzyme. See Figure 8.

According to Venton and Wightman, after dopamine neuronal activity occurs, changes in blood flow cause local alkaline pH fluctuations which can interfere with dopamine detection by electroanalytical techniques.[5] Likewise, we have to keep this in mind while developing our MRI probe. MRI CA's that are pH sensitive have also been synthesized.[16]

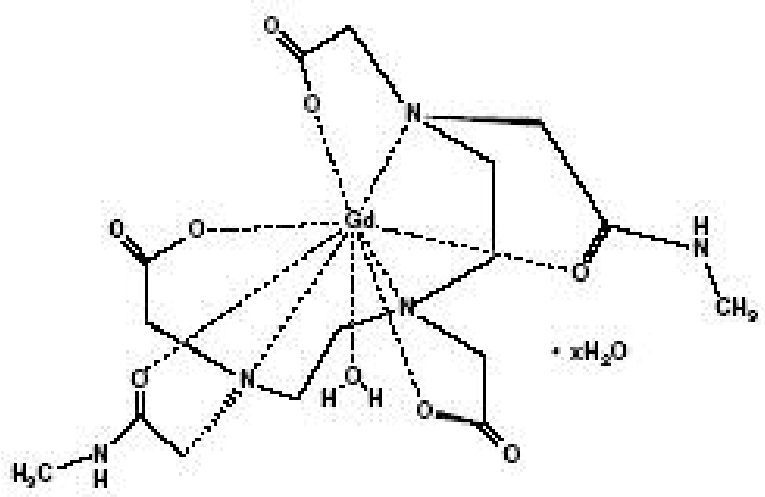


Figure 9a Omniscan (http://www.rxlist.com/cgi/generic3/omniscan.htm)

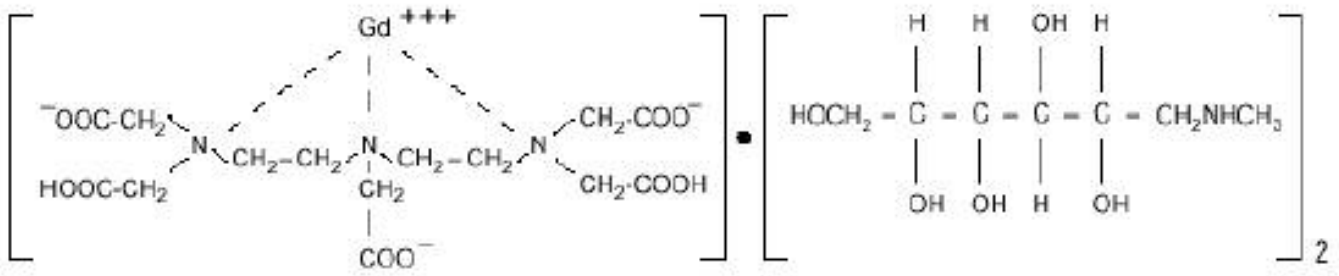


Figure 9b Magnevist (http://www.rxlist.com/cgi/generic4/magnevist.htm)

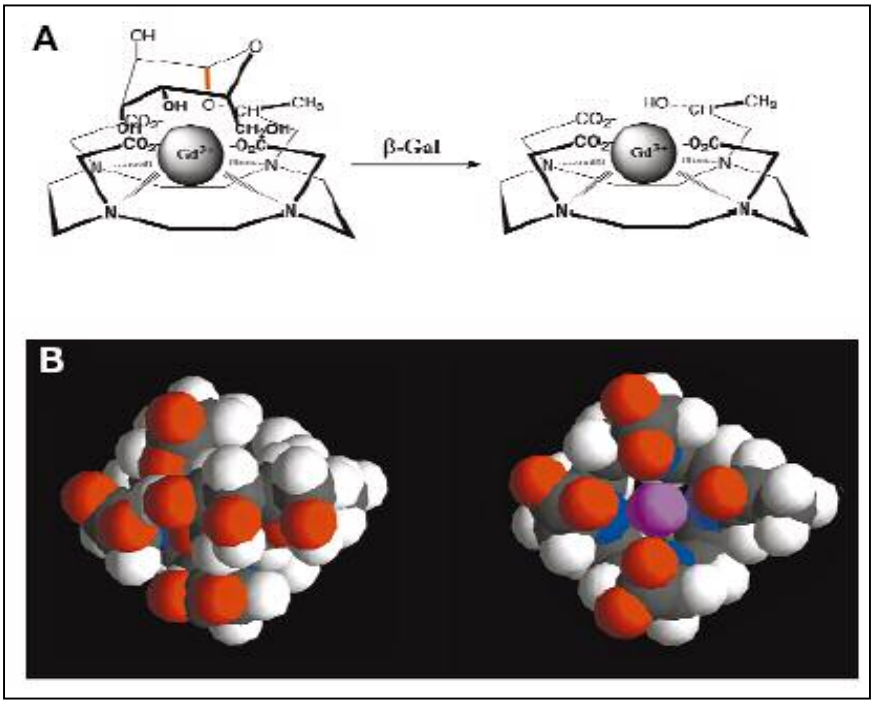
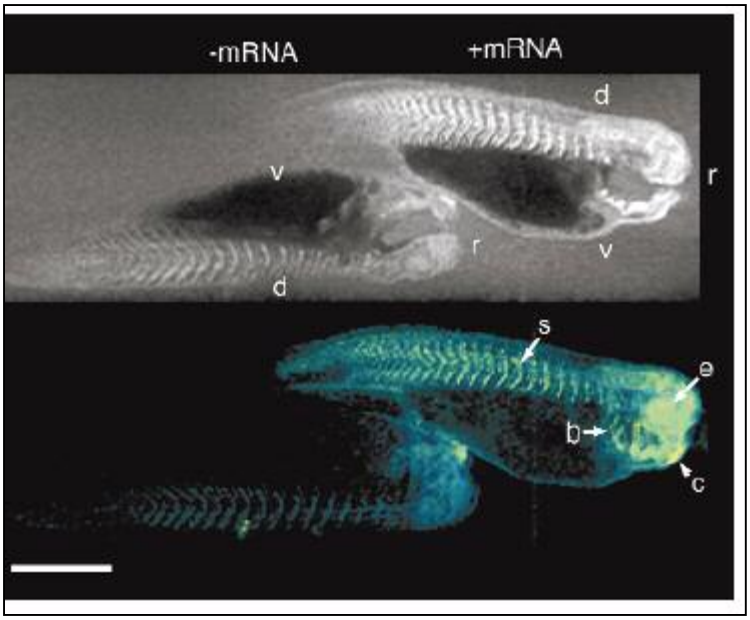
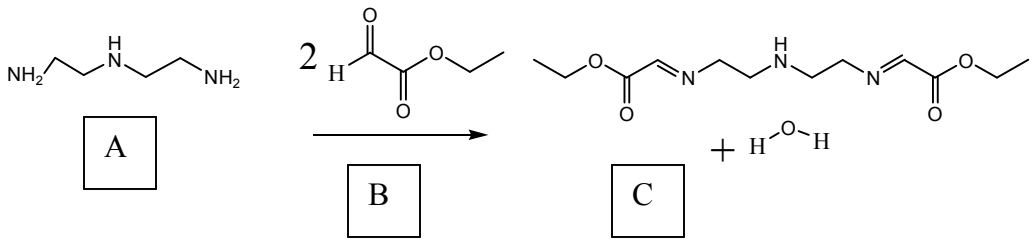


Figure 10. Louie, A. Y., Huber, M. M., Ahrens, E. T., Rothbacher, U., Moats, R., Jacobs, R. E., Fraser, S. E., and Meade, T. J. *Nature Biotechnology*. **2000**, *18*, 321-325.
A. shows the CA being activated by beta-GAL. B. shows the 3-D model. Purple sphere is Gd. Picture on right shows a MR image of a worm-like embryo before CA (top) and after CA (bottom).



Experimental



Scheme 1. Imine Formation

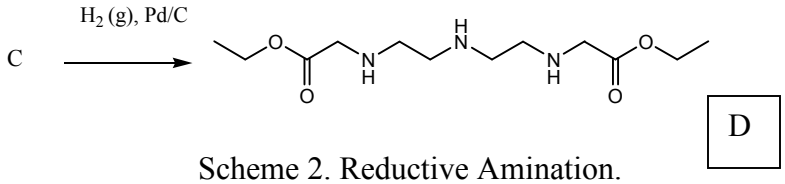
Trial #1

A stir bar was put in a round bottom Flask (300 ml or 500 ml), capped with a septum and put under nitrogen gas (inert atmosphere). See Scheme 1. Reagents and solvent (in this trial, EtOAc)[1] were added with needles and syringes. See Table 1. About 8 minutes after set up the reaction turned cloudy white. It was left to stir over two nights. It turned a cloudy tan color. TLC silica gel plate was spotted with the reaction and two starting materials and then developed with 10 ml of 3% methanol in methylene chloride and 3 drops of ammonium hydroxide. The developed TLC plate was visualized by dipping into a chamber of potassium permanganate. TLC showed that a reaction had occurred.

Table 1. Reagent Table for Step 1: The Imine Formation

Reagent	Mol. wt./density	Amount	mmol	Equiv.	Hazards
Ethyl glyoxylate in toluene (50% w/v solution)	102.09 g/mol 1.003 g/ml	3.78 ml	18.6	2	
Diethylenetriamine, 98.5%	103.17 g/mol 0.960 g/ml	1 ml	9.3	1	
Ethyl acetate **(SOLVENT)**		120 ml			

Proceeded with **Step 2, the reductive amination**. See Scheme 2. Added a spatula tip full of activated palladium on carbon (Pd/C) to reaction flask. A balloon purchased from CVS was filled with completely with hydrogen gas, secured to a syringe and needle; used this to vent reaction flask twice before beginning hydrogenation where two balloons of hydrogen were allowed to flow into reaction flask. Let stir for 3 nights. It was observed that the hydrogen balloons deflated after one day. Filtered reaction and took a TLC with the aforementioned conditions before rotavapping solvent from product. TLC plate showed a concentrated spot in between the two starting materials' spots that was presumed to be product. Rotavapping revealed orange-brown oil. Product was not seen in 1H –NMR spectrum done with 99.6% Chloroform-d.



Scheme 2. Reductive Amination.

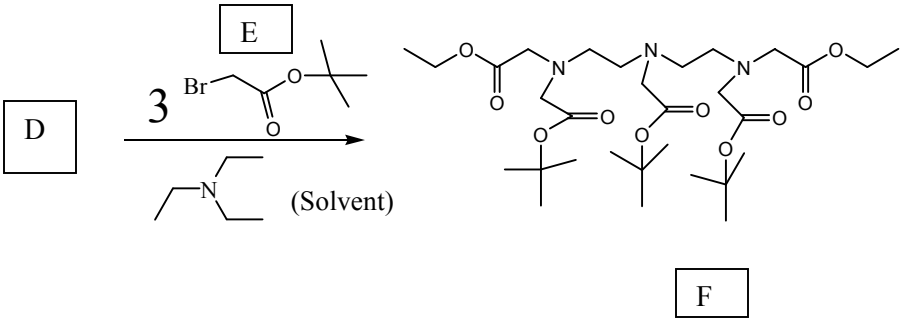
Trial #2

Ethyl acetate was seen as a troublesome solvent as it did not yield the expected product. For Trial#2 the starting materials as listed in Table 1, minus the solvent, were reacted in 120 ml of Methanol instead. The reaction remained clear and stirred for 3 nights. Much of the methanol evaporated from the, then, amber colored reaction. A TLC was taken showing that a reaction had occurred. Proceeded with hydrogenation, which was allowed to stir overnight. Filtered and rotavapped reaction to an amber colored oil. Stored in chemical refrigerator. Took 1H-NMR spectrum of product in 99.6% Chloroform-d, 4 days later, which revealed impure sample. Using 6% methanol in methylene chloride as the eluting solvent, ran a chromatography column with silica gel in attempt to purify the product. Collected 48 fractions in test tubes. The fractions were spotted and developed on TLC plates in 6% methanol in methylene chloride. Fractions developed as streaky spots on the TLC plate. Those that developed with a more concentrated middle spot (assumed to be product) were collected in a clean round bottom flask and rotavapped, revealing a brown oil. The brown oil was stored under vacuum for 6 days. Collected a GC-MS spectrum for the oil. The spectrum was not definitive enough to determine which peaks corresponded to our desired product. On the 7th day, ran a second column with 6% methanol in methylene chloride as the solvent with 3 drops of ammonium hydroxide added per 10 ml of solvent. It was theorized that the ammonium hydroxide might better separation on the column as it did for TLC. Collected fractions. Rotavapped two sets of fractions. One flask held mostly starting material, the other held mostly product. Collected GC-MS and 1H-NMR spectra (99.6% Chl-d) for both. The spectra did not show presence of our desired product. The column was unable to separate the sample, which was labeled “mixture.” Performed Step 3 on mixture as if it were the desired product to get some experience with setting up Step 3’s reaction and with hope that our desired product may be isolated later on.

Table 2. Reagent Table for Step 3: Alkylating the Amine

Reagent	Mol. wt./density	Amount	mmol	Equiv.	Hazards
“Mixture” as if desired product	275 g/mol	0.45 g	1.63	1	
Triethylamine **(SOLVENT)**		1.11 ml			
Methylene Chloride (mixed with Et_3N)		23.7 ml			
t-butyl bromoacetate	195.06 g/mol 1.338 g/ml	0.72 ml	4.93	3	
Methylene chloride (mixed with t-butyl bromoacetate)		7.4 ml			

Scheme 3. Alkylating the 3 amine positions



Step 3 was alkylating each of the three amine positions with t-butyl bromoacetate. See Scheme 3. The N replaces Br where Br is kicked off in an SN2 reaction. The addition of t-butyl bromoacetate was done on a smaller scale than and according to the patent[1]. “Mixture” was dissolved in 1.11 ml of Et_3N and 23.7 ml of CH_2Cl_2. To that we added, dropwise, the 0.72 ml of t-butyl bromoacetate in 7.4ml CH_2Cl_2, while stirring. Took a TLC in 6% MeOH in $CH_2Cl_2$with 3 drops of NH_4OH per 10ml solvent. TLC plate visualized with UV light then with $KMnO_4$. TLC is not a good way to monitor Step 3 because the t-butyl bromoacetate

was not visible to UV light or with $KMnO_4$. The reaction stirred overnight. Took another TLC in same conditions. A reaction may have occurred due to a slight fade in the top spot. Rotavapped and put under vacuum for one hour to remove excess solvent. Took 1H NMR (1% TMS Chl-d) which still showed presence of Et_3N. Left under vacuum overnight before doing a workup according to the patent's procedure. Dissolved brown oil in 30 ml CH_2Cl_2. Washed with 55 ml of 0.2N NaOH in a 500ml separatory funnel. Kept bottom organic layer; discarded upper basic layer. Washed twice with 80 ml distilled water. Washed with 100ml brine. Dried with $MgSO_4$. Filtered to remove $MgSO_4$. Took 1H NMR (1% TMS Chl-d), which did not show the presence of our desired product. Trial #2 was discarded.

Trial #3

Two equivalents of ethylglyoxylate and one equivalent of diethylenetriamine were reacted (see Table 1) in 119 ml of MeOH, our third solvent. It was allowed to stir overnight under N_2(g). Reaction was tinted light yellow. TLC was done with the following solvent systems listed in Table 2 in attempt to find an eluting solvent that will give the Step 1 imine or Step 2 amine a retention factor (R_f) of 0.2-0.4 so that it may be used to run a column for purification. None of them were better than the old solvent system of 6% MeOH in CH_2Cl_2 with 3 drops of NH_4OH per 10ml, so we kept that. The TLC showed the disappearance of ethyl glyoxylate. The imine is assumed to be so polar as to stick to the baseline and therefore is the majority of the baseline spot. Assuming the imine reaction worked, we proceeded with hydrogenation. This time we improved the hydrogenation set up. A balloon was attached to a 3-way stopcock with duct tape and filled with H_2 (g). The stopcock was inserted into reaction flask to which a spatula-tip full of Pd/C catalyst had already been added. The flask was vented with H_2 (g) once to clear air out before hydrogenation. Positive pressure was applied by hooking up the last arm of the stopcock to N_2 (g). See Figure 11.

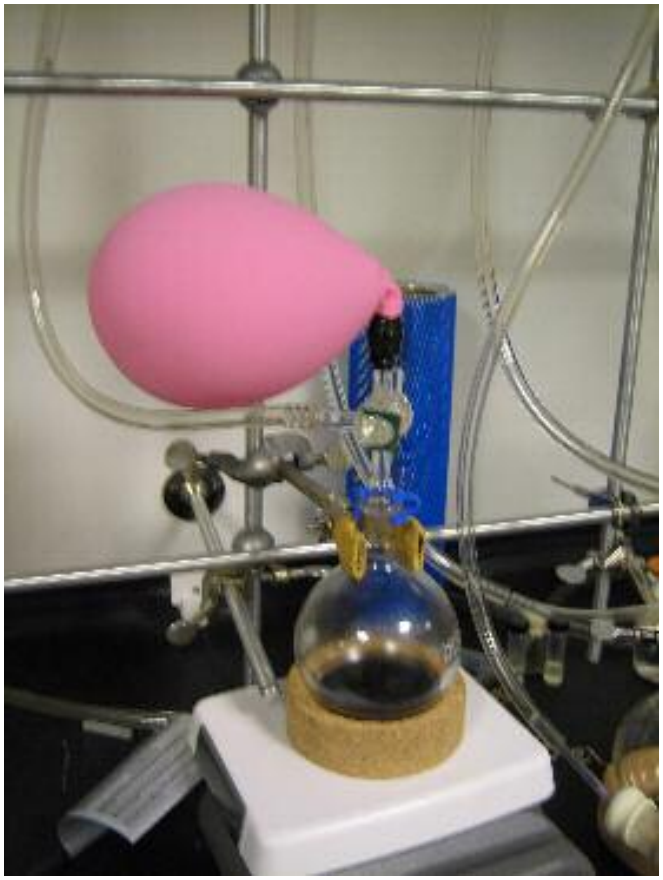


Figure 11. Hydrogenation Set Up. Balloon deflated slightly after a few hours. Photo by J. Lau.

Reaction with first balloon stirred overnight for 15 hours. A second balloon of H_2 (g) was added and then a third. Six days after hydrogenation the reaction was filtered to remove Pd/C. DMF has a high boiling point and cannot be removed from product by rotavapping. To remove it we extracted it several times with water and EtOAc using a 500ml separatory funnel. The product theoretically was retained in EtOAc layers and DMF retained in water layers. Rotavapped EtOAc layers to yield a pale yellow oil. A 1H-NMR showed presence of DMF therefore oil was put on vacuum over two nights to further remove DMF. Another 1H-NMR spectrum was collected but our desired Step 2 product, the amine was not present. The oil was used to try to find TLC conditions for the Step 2 Amine with the hope that if we can find the right solvent system, we would be able to purify it on a column and then isolate our desired product. TLC plates were put under vacuum before developing to get rid of DMF from sample spots. See Table 4 for a list of systems tried but unsuccessful at giving an R_f between 0.2 –0.4 for the amine. Decided not to proceed with Step 3 since all the 1H NMR spectra thus far from the trials were showing similar junk and lack of distinctive peaks for our desired products.

Table 3. Unsuccessful TLC solvent systems to elute Step 1 Imine

1. DMF
2. 50% DMF in CH_2Cl_2
3. 70% Benzene, 20% EtOAc, 10% DMF
4. 30% Hexanes in EtOAc
5. 50% EtOAc in hexanes

Table 4. Unsuccessful TLC solvent systems to elute Step 2 Amine

1. 10% NH_4OH in MeOH
2. 3:7 EtOAc/ MeOH
3. 3:7:1 EtOAc/ MeOH/ NH_4OH
4. 4:5:1 EtOAc/ MeOH/ NH_4OH
5. 8:1:1 EtOAc/ MeOH/ NH_4OH
6. 3:7 MeOH/ CH_2Cl_2
7. EtOAc
8. 10% NH_4OH in EtOAc
9. 5% NH_4OH in EtOAc
10. 0.5:0.5:9 NH_4OH/ MeOH/ EtOAc
11. 1:0.5:8.5 NH_4OH/ MeOH/ EtOAc
12. 2:0.5:7.5 NH_4OH/ MeOH/ EtOAc

Trial #4

Ethanol was used in the patent[1] as the solvent therefore we tried it for Trial #4. The amount of reagents used was calculated based on the patent's scale. See Table 5. Ethyl gyloxylate, diethylenetriamine, and absolute proof ethanol were added to a 300 ml RB flask by syringe under nitrogen to stir for 2 hours under heating at 50 degrees Celsius with a water bath. Reaction was removed from heat and 310 mg of Pd/C catalyst was added for hydrogenation. The reductive amination stirred for 66 hours. Pd/C was filtered out. Reaction was rotavapped. A 1H-NMR was taken for the imine and amine but did not show presence of either.

Table 5. Reagent Table for Trial #4

Reagent	Mol. wt./density	Amount	mmol	Equiv.	Hazards
Ethyl glyoxylate in toluene (50% w/v solution)	102.09 g/mol 1.003 g/ml	4.1 ml	20	2.15	
Diethylenetriamine, 98.5%	103.17 g/mol 0.960 g/ml	1 ml	9.3	1	
Ethanol **(SOLVENT)**		31 ml			
10% Pd/C		0.31 g			

Trial #5

Tried DMF as the solvent again but decreased stir time just in case over-stirring might have negatively impacted the reaction. Step 1 stirred for 22 hours and Step 2 stirred for 21 hours. 1H-NMR still did not show presence of desired products.

Trial #6

Tried EtOAc as the solvent again as well. Step 1 stirred overnight but reaction precipitated out. An 1H-NMR was taken for the rotavapped precipitate to see if imine was in there; however it was not. This confirmed that EtOAc is not a good solvent for our reaction.

Results

The 6 step, multi-step synthesis for our target molecule has not been completed yet. We were only able to try steps 1-3. Step 1 did not work despite changing solvent conditions and stir times. Step 2 and 3 are assumed to be fairly straightforward after successful completion of step 1.

Conclusion

From our lab experience, the best way to monitor our reactions is by taking 1H-NMR spectra. The gas chromatograph-mass spectrometer (GC-MS) is less effective because we are working with large molecules, which fragment too much. TLC silica gel plates are not effective for imine reaction monitoring because they possibly hydrolyze the imines and amines and do not develop well in the many tried solvent conditions. Lee et al recommend Raman spectroscopy for the monitoring of imine synthesis.[6] Most imine syntheses done are with aryl amines, however, whereas in our case it is not.

We can improve the first step of current synthesis adding an acid catalyst such as H_2SO4 or CH_3COOH to facilitate removal of water during imine formation and then heat the reaction to above 150 degrees Celsius. However, if too much acid is added, the reaction will stop. This is because an amine is a base and would react with acid to form the ammonium ion. For every amine molecule that does this, the unshared electron pair has been used to make the N-H bond and is no longer available to act as a nucleophile; it becomes a "benched amine."[3]It is best to compromise by adding fewer acid molecules than there are amines. To ensure removal of water add molecular sieves to absorb the water formed, or use a Dean-Stark trap.

It is best to diversify our strategies for achieving synthesis of this ligand. A different synthesis for same molecule can be attempted using different starting materials.

Given that dopamine is a challenge to measure, the current state of the art is to utilize Technetium derivatives to monitor dopamine transporter (DAT) via positron emission tomography (PET) as confirmed by a current study.[7] Perhaps these derivatives can be altered for use as MRI contrast agents instead by replacing Tc with Gd.

As more lab work occurs, the design of the molecule as well as the corresponding synthetic procedure should be adjusted accordingly to have the greatest affinity to dopamine, to bind dopamine over other biological chemicals in the brain, to be safe for injection into humans, to be MRI active, and to be synthesized via the most efficient and quickest way. Learning about dopamine, its environment in the brain, and how and when it is produced will help with this process. Finally, it is important to understand each step of a multi-step synthesis and to engage in troubleshooting along the way in order to progress in the research.

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