Epoxyaconitic Acid as Prospective Capping Ligand for Magnetite Nanoparticles:

Synthesis and Complexation with Magnetite

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Abstract

The overall purpose of this project is the development and synthesis of novel MRI-contrast and delivery agents based on iron oxide nanoparticles. The focus of this research is the synthesis of a novel organic ligand based on *trans*-aconitic acid, and the complexation of this product, epoxyaconitic acid, with magnetite nanoparticles. At each step of the process, the structure of the intermediates was analyzed in a variety of manners. ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and dynamic light scattering have been used to confirm the structure of the intermediates and the final product. The final complexation of epoxyaconitic acid with magnetite has been discussed.

Background

Magnetic nanoparticles have been the subject of extensive research in recent decades. Attention has specifically been focused on iron oxide nanoparticles. Ranging in sizes of less than 10 nanometers to over 300 nm in diameter, these particles have a huge variety of potential applications (*1*). These nanoparticles are particularly useful because of their superparamagnetic behavior (*1*). This means that in an applied magnetic field, the particles become magnetic (*1*). However, when the field is removed, the particles have no permanent magnetism (*1*). On their own, the nanoparticles have little use in the body. The particles must be coated in an organic ligand to make the nanoparticle biocompatible (*1*). The particles are then functionalized by modifying their surface (*1*). This modification can result in the variety of uses of nanoparticles. As shown in Figure 1, an organic ligand forms a complex with the iron

oxide nanoparticle. For example, this ligand can be citric acid. The structure of complex between iron oxide and citric acid has been proposed in earlier work.

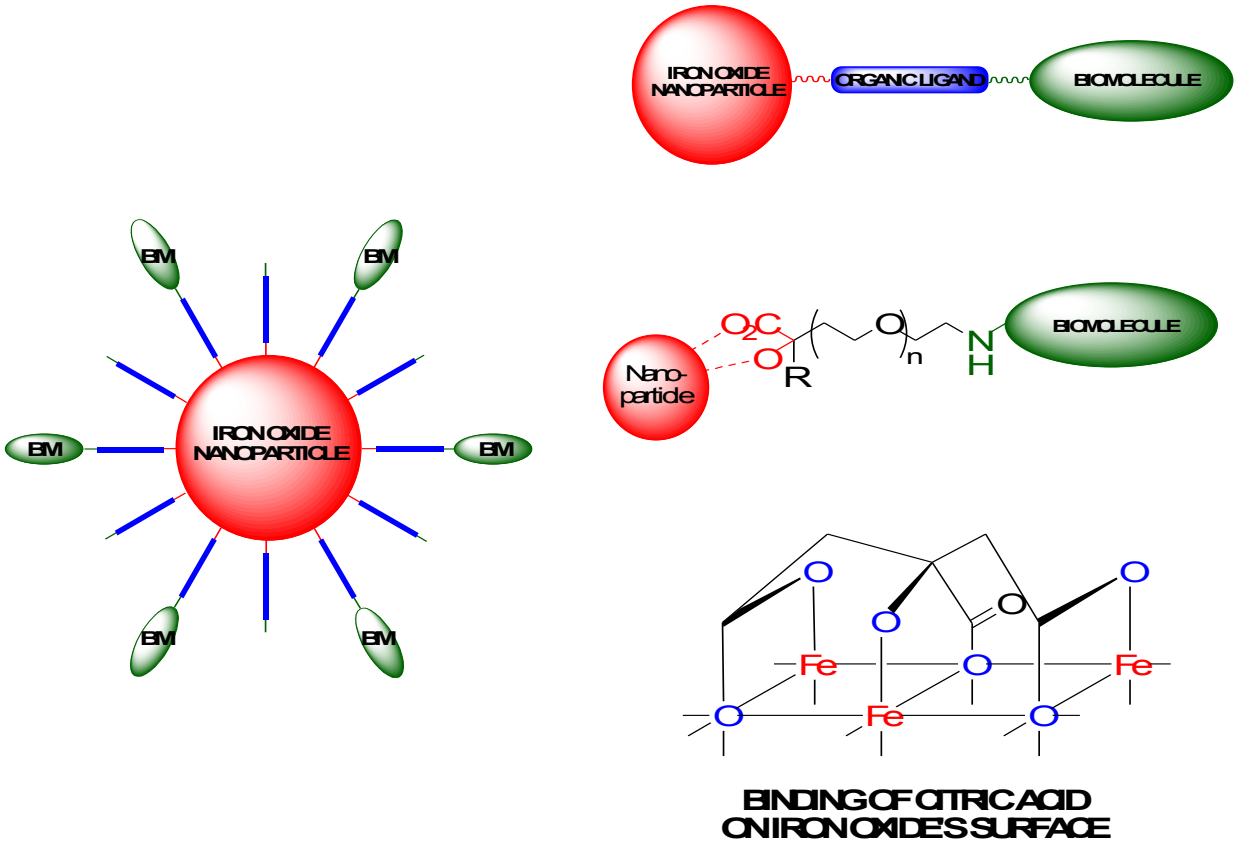

Figure 1. Schematic representation of an iron oxide complex. The pictures show in increasing detail the basic elements of the complex. There is also a proposed model of the complex between iron oxide and citric acid on the surface of the nanoparticle.

Two of the potential applications of these nanoparticle complexes are the use of the nanoparticle as a MRI contrast agent and the use as a drug delivery agent. MRI contrast agents are used to enhance MRI images so that more details can be seen (*1*). Contrast agents need to be both positive (T1) and negative (T2) contrast agents (*2*). Iron oxide particles are already known as good T2 contrast agents (*2*). Additionally, studies of the T1 relaxation time of iron

oxide nanoparticles have shown that they could be possible alternatives to gadolinium based contrast agents currently in use (*3*). These contrast agents have toxic side effects and it would be ideal for them to be replaced (*3*). Making the particles smaller than 5 nm makes iron oxide nanoparticles a better T1 contrast agent (*2*).

Iron oxide nanoparticles can be used for drug delivery agents as well. In this case, the iron oxide would be coated in an organic ligand and the drug would be attached to the nanoparticle. In this case, a magnet field would be applied to the location where the drug is needed, ensuring that more of the medicine is making it to its target (*1*). In that case, the whole complex needs to be small enough to travel throughout the body: it should not be larger than a red blood cell (*4*). It is also important that the complex can hold up in the human body until it reaches its destination. Conversely, it must be able to be broken up for use by the body. Poly α-hydroxycarboxylic acids, such as citric acid, were proposed as ligands due to formation of strong bonds with iron oxide and natural origin of them. *Trans*-aconitic acid was chosen as a prospective precursor for the synthesis of a ligand based on modified citric acid.

Synthetic Approach via Triethyl *trans*-Aconitate

The reaction of the formation of epoxyaconitic acid via the epoxidation of triethyl *trans*-aconitate can be seen in Figure 2. This set of reactions is explained in further detail throughout this section.

Figure 2. Synthesis of Triethyl *trans*-Aconitate, attempted epoxidation, and proposed hydrolysis of the epoxide.

The first step in this synthesis was protection of carboxylates via esterification with ethanol. The acid-catalyzed esterification of *trans*-aconitic acid was completed using an azeotropic distillation of formed water. A mixture of 0.0299 moles of *trans*-aconitic acid and 0.345 moles of ethanol in the presence of 0.714 mmoles of p-Toluenesulfonic acid, as a catalyst, and 100 ml of benzene was refulxed with a backflow condenser and a Dean-Stark adapter until no more water was present. Then 0.559 g (4.04 mmol) potassium carbonate was added to the flask to neutralize the acidic components. Volatile components were removed using a rotary evaporator and the suspension was filtered using vacuum filtration. Then, a vacuum distillation was performed and three separate fractions were collected and analyzed using ESI-MS and ^{1}H NMR. Approximately 2.5 grams of triethyl aconitate were produced, giving a yield of 32%.

Two methods were used to attempt to epoxidize the triethyl *trans*-aconitate. The first method consisted of the following: A solution of 0.275 g (1.07 mmol) of triethyl *trans*-aconitate was prepared in 5 ml of 90% aqueous THF, and combined with 0.292 g (1.26 mmol) of silver (I) oxide and 0.332 g (1.31 mmol) of iodine. The reaction flask was wrapped in aluminum foil and left stirring for several hours at room temperature which resulted in a dark black liquid with a yellow precipitate of silver iodide. The precipitate of inorganic compounds, most likely silver iodide, was separated by filtration and the product was concentrated using a rotary evaporator.

The obtained oil was diluted with 5 ml DCM and filtered twice to remove additional inorganic material. Volatile components were removed again using a rotary evaporator. The final product was a dark brown liquid. The product was analyzed with ^{1}H NMR and mass spectrometry. However, the epoxidized product was not detected.

The second method attempted was epoxidation by dimethyldioxirane (DDO). 0.138 g (0.53 mmol) of triethyl aconitate and 0.798 g (9.50 mmol) of sodium bicarbonate were suspended in 25 ml of 50% aqueous acetone. This mixture was a reddish-orange color. The solution was stirred and placed in an ice bath until the mixture was between 2-5 °C. Then, 0.630 g (1.02 mmol) of oxone was added in portions. As the oxone was added, the solution became colorless. The solution was kept between 2-5 °C for six hours and left stirring overnight. A portion of the solution was prepared for analysis by mass spectrometry. There was a large peak at 259, which indicates a large presence of the starting material. A peak for the epoxide would be expected at 275 and there was no peak present at this location. The epoxide of the triethyl aconitate was not detected.

Had the epoxidation of triethyl aconitate been successful, the next step would have been hydrolysis using potassium hydroxide. Then, this product, epoxyaconitic acid, would be used for coating the iron-oxide nanoparticle.

Synthetic Approach via *trans*-Aconitic Acid

The synthesis of epoxyaconitic acid from the epoxidation of *trans*-aconitic acid can be seen in Figure 3.

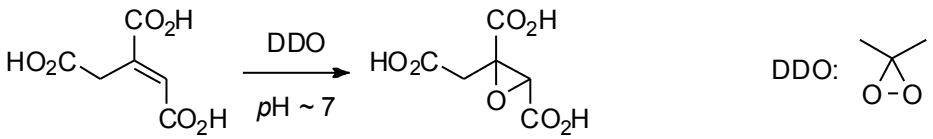

Figure 3. The epoxidation of *trans*-aconitic acid by dimethyldioxirane. Also, the structure of DDO is included.

Dimethyldioxyrane (DDO) is reported to be inefficient reagent for the epoxidation of electronically poor double bonds such as in case of conjugated carboxylates (*5*). However, during this study it was found that in case of *trans*-aconitic acid epoxydation with DDO leads to epoxyaconitic acid. The procedure includes generating DDO *in situ* in the presence of *trans*-aconitic acid and a buffer solution (*p*H ~ 8). A pilot reaction consisted of the following procedure: A suspension of 1.615 g (19.22 mmol) of sodium bicarbonate and 0.192 g (1.103 mmol) *trans*-aconitic acid in 25 ml of 50% aqueous acetone was stirred and chilled to 2-5° C. Then, 1.267 g (2.06 mmol) of oxone was added slowly and the contents were kept at 2-5° C for six hours and then stirred overnight. Analysis of the crude product by mass spectrometry confirmed the presence of epoxyaconitic acid.

This reaction was repeated and scaled up several times. The best results were obtained after performing the same procedure using 10.832 g (0.06221 mol) of *trans*-aconitic acid, 80.718 g (0.9608 mol) of sodium bicarbonate, and 79.915 g (0.12999 mol) of oxone. After six hours in a ice bath, the contents were stirred overnight. The procedure for the isolation of epoxyaconitic acid began with quenching peroxides with 20.368 g (0.166 mol) of sodium sulfite. Then, acetone was removed by rotary evaporation. Then a solution of approximately 30 ml sulfuric acid in 100 ml water was added drop wise and the pH of the solution was lowered to 1.

Then, the solid was placed on the rotary evaporator until dry. The solid was filtered twice using vacuum filtration in methyl-tert-butyl ether (MTBE). The MTBE was evaporated and the epoxyaconitic acid was recrystallized using chloroform. The crystals were collected using vacuum filtration and dried. 6.720 g (0.0353 mol) of epoxyaconitic acid were produced for a yield of 56.8%. This sample was analyzed by NMR and determined to be greater than 95% pure.

A possible explanation of the successful epoxidation by DDO comes from the fact that deprotonation of the carboxylic acid groups takes place in the buffer solution (*5*). This can lead to decreasing of the *–C* -effect of the carboxyl groups that are not strong acceptors anymore, as opposed to the ester groups (*5*).

Complexation of Epoxyaconitic Acid with Magnetite

After the successful synthesis of the epoxyaconitic acid, the next step was to attempt to open the epoxide directly on the surface of the iron oxide nanoparticle in the presence of diethylene glycol (DEG). First, the synthesis of the colloid was performed. Magnetite colloid was prepared according to the scheme below:

$$FeCl_2 + 2FeCl_3 + 8NaHDEG + 4H_2O \rightarrow Fe_3O_4(H_2DEG) + 7H_2DEG + 8NaCl$$

The magnetite colloid was prepared by adding iron oxide precursors to approximately 50 ml of boiling diethylene glycol (DEG) and heated to 190 °C and 210 °C for an hour each, then left stirring overnight. This colloid was then oxidized using oxygen gas to produce an air-stable form according to reaction:

$$2\ Fe_3O_4(H_2DEG) + O_2 \rightarrow 2\ Fe_3O_4(HDEG) + H_2O$$

The final product after the synthesis and oxidation was approximately 97 g of colloid containing 2.231 mmoles of magnetite.

A 0.1 M solution of epoxyaconitic acid in anhydrous diethylene glycol (H_2DEG) was mixed with colloid of magnetite in a ratio of 2 moles of ligand per 5 moles of iron. Under nitrogen, 0.043 g (0.226 mmol) of epoxyaconitic acid was added to 10 ml anhydrous DEG to make a 0.0226 M solution. 1 ml of this solution was combined with 10 ml of the magnetite colloid and stirred for 3 hours at room temperature. Then the stirred reaction mixture was left overnight at 50 °C. Finally, the reaction mixture was heated at 70 °C for 3 hours and chilled down to room temperature. This reaction will possibly proceed via the reaction show in Figure 4.

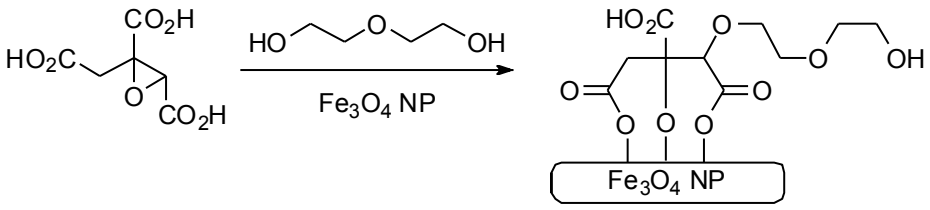

Figure 4. Possible formation of the complex between epoxyaconitic acid, DEG, and the magnetite nanoparticle.

The obtained complex was isolated in the form of brownish powder, which was strongly magnetic. Then, the colloid was washed four times with 15 ml of ethyl acetate to remove the DEG. Washing consisted of adding the solvent, sonicating the solution for five minutes, then placing the flask on a strong magnet to precipitate the solid, then decanting the solvent. The solid was then washed four times with methanol to remove sodium chloride left over from the

colloid synthesis. Lastly, the solid was washed once with 30 ml of MTBE and three more times with 15 ml MTBE.

In order to analyze the complex formed, the iron oxide complex must be decomposed in a basic media. The precipitated magnetite complex was dissolved in 15 ml of deionized water and sonicated. Then it was basified in 2.5 ml of 1 N potassium hydroxide and left stirring for two and a half days. It was then stirred for 2 hours at 60 °C and chilled to room temperature then the magnetite was precipitated on a strong magnet. The almost clear supernatant was filtered through a syringe-type 0.2µm polyproplyene filter two times. The filtrate was acidified by adding approximately 1 N hydrochloric acid dropwise until it reached a pH of 1-2. Then, the solution was evaporated using a rotary evaporator and the solid material was extracted 3 times with MTBE. The organic layers were evaporated using a rotary evaporator. ESMS and NMR analysis proved that diethylene glycol was present in the mother liquor. There were certain peaks in both spectra that could belong to hydroxycitric acid; however integral intensity ratio in proton NMR spectrum was too high for the diethylene glycol peaks. With respect to ESMS, there was no molecular ion peak for the adduct of diethylene glycol and hydroxycitric acid, but there was a peak of (M+1)-28 in positive mode. This peak would correspond to the molecular ion that has lost a carbon monoxide molecule. The fragmentation pattern for this peak resembles the fragmentation for peaks of epoxyaconitic acid, which might be indirect evidence for the hydroxycitric acid-diethylene glycol adduct formation on the surface of the iron oxide nanoparticles.

In order to further analyze the success of the complexation of the epoxyaconitic acid with the iron oxide, this reaction is being repeated with an excess of epoxyaconitic acid. This

time, 0.636 g (3.35 mmole) epoxyaconitic acid was prepared in 10 ml diethylene glycol. This was then added to approximately 80 ml (1.98 mmol) of the colloid. The reaction followed the same procedure as the previous reaction, but with everything scaled up. Hopefully, this will be completed and further analysis will provide evidence of the complex formation.

Future Work

If the synthesis of the magnetite complex with epoxyaconitic acid is successful, the next step would be the incorporation of a different type of bridge such as polyetheleneglycol fragments with amino group on the end or branching with AGE. These reactions can be seen in Figure 5. This would serve as the site where biomolecules could be attached in order to functionalize the nanoparticle.

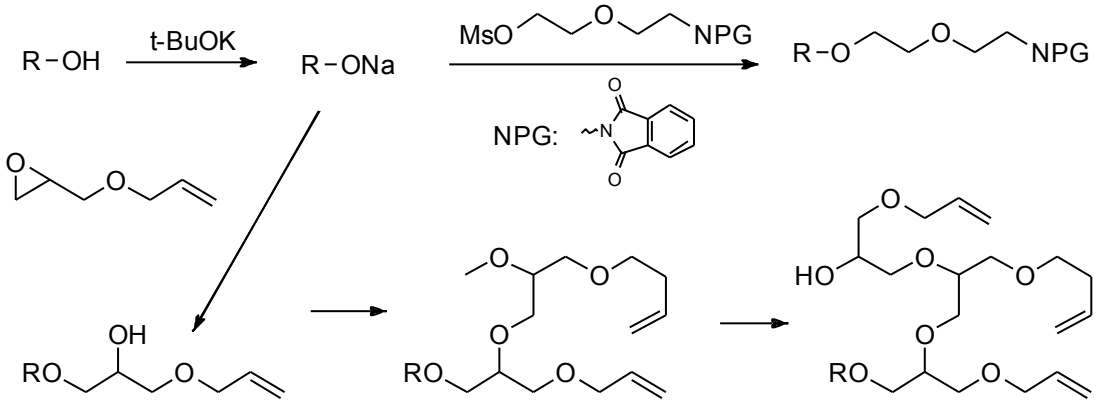

Figure 5. The growth of the organic ligand via polyetheleneglycol fragments with amino group on the end (top) or branching with AGE (bottom).

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