

# SYNTHESIS OF NOVEL BRIDGING LIGANDS FOR NANOPARTICULATE IMAGING AND DELIVERY AGENTS

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Jonathon Watson

Linden Allison

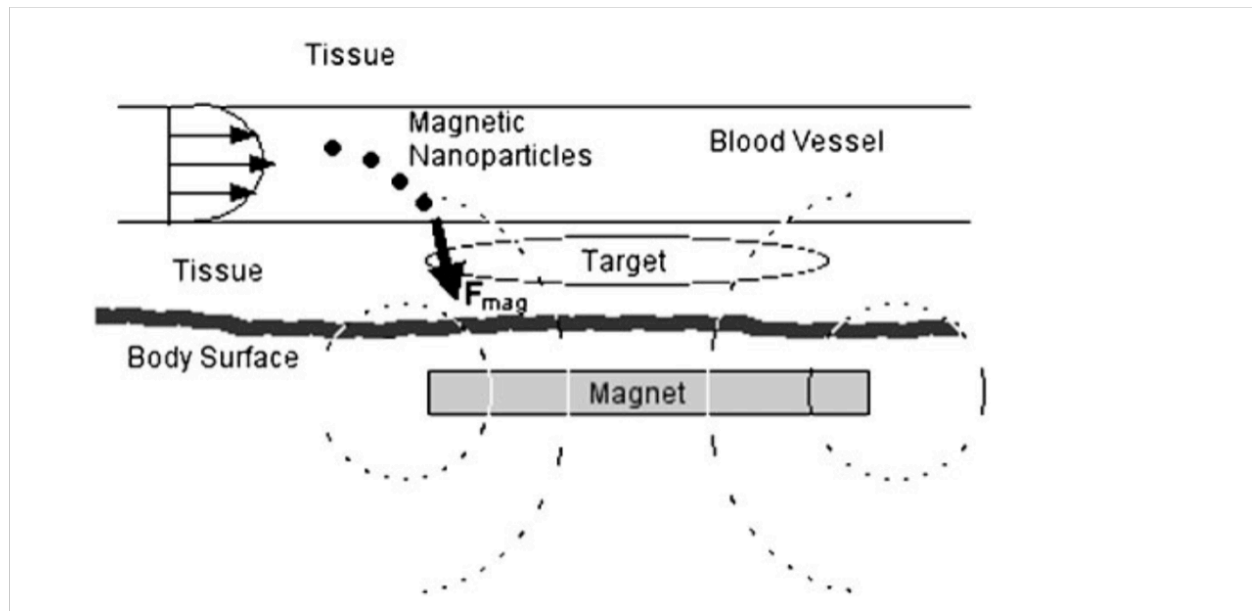
Taylor Gravolet

Xavier University of Louisiana



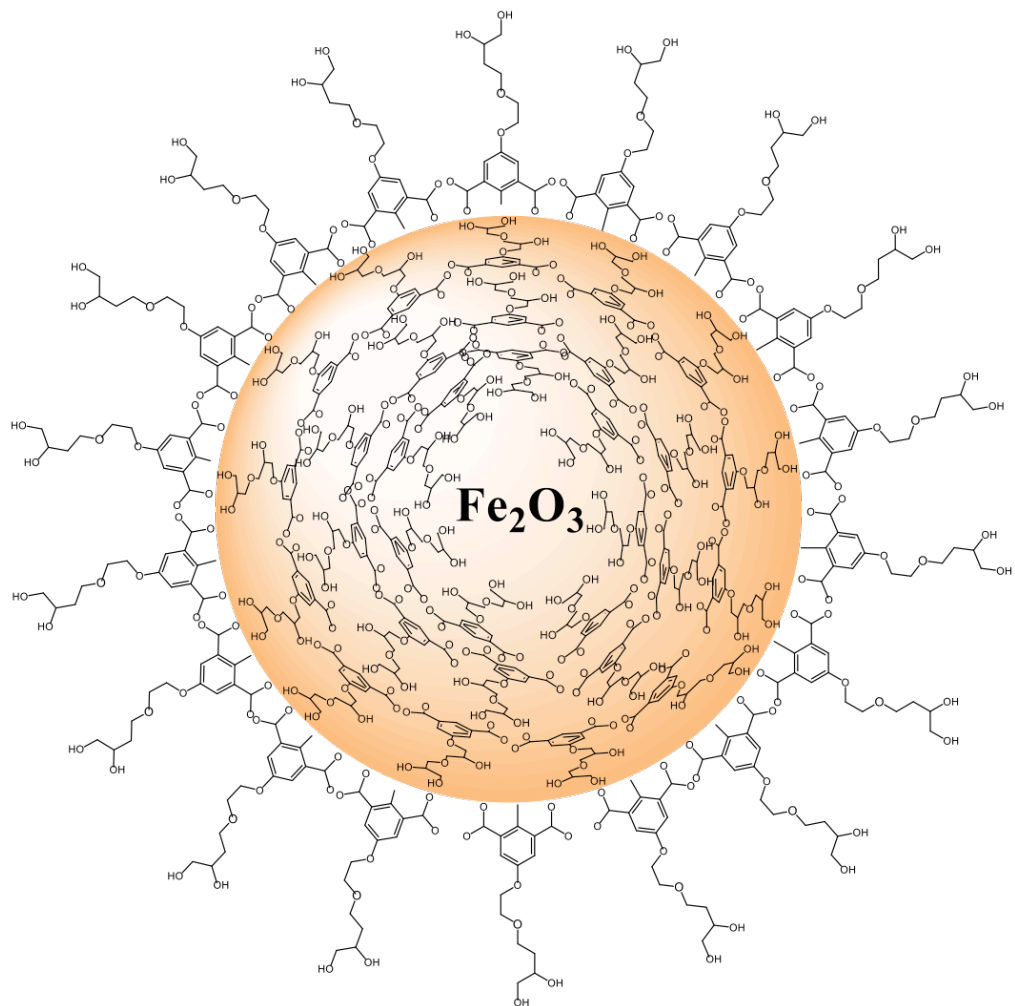
# Applications

- Biomedical field
  - MRI
  - Drug delivery



# MRI Contrasting Agents

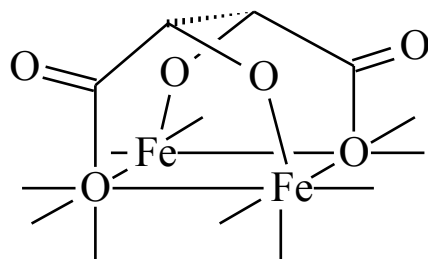
- MRI contrasting agents
  - Used under
    - Magnetic field and
    - Radiofrequency (RF) pulses
  - Work by altering the relaxation rates of water protons that are trying to realign
- 2 types
  - T1
    - Longitudinal relaxation
    - 'positive contrast'
  - T2
    - Transverse relaxation
    - 'negative contrast' (aka dark spots)
- Currently, gadolinium agents are primarily used
  - Paramagnetic
- Polynuclear agents provide an alternative
  - Superparamagnetic



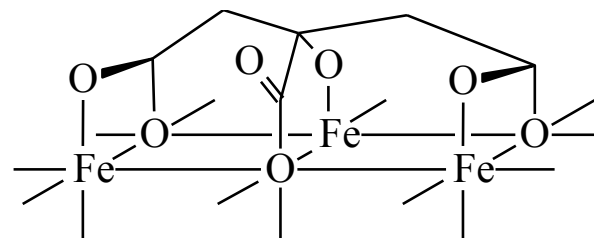
Magnetite nanoparticle encapsulated by an organic ligand yielding enhanced surface stability and increased applicability.



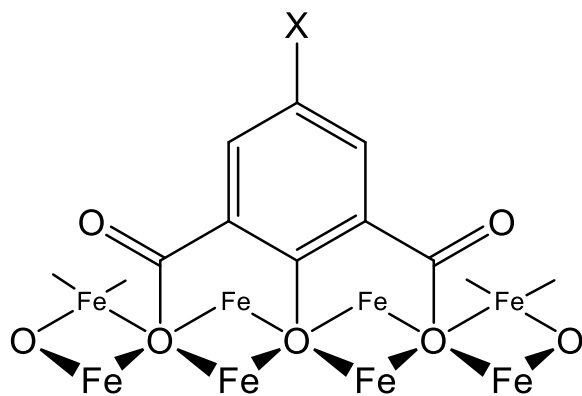
# Challenge



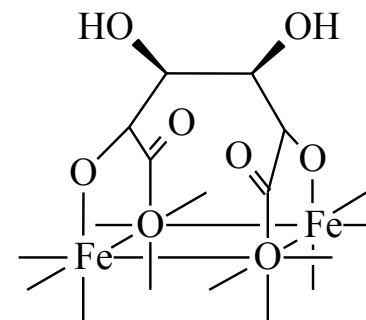
Tartrate



Citrate



Hydroxyisophthalate



Saccarate

# Goals

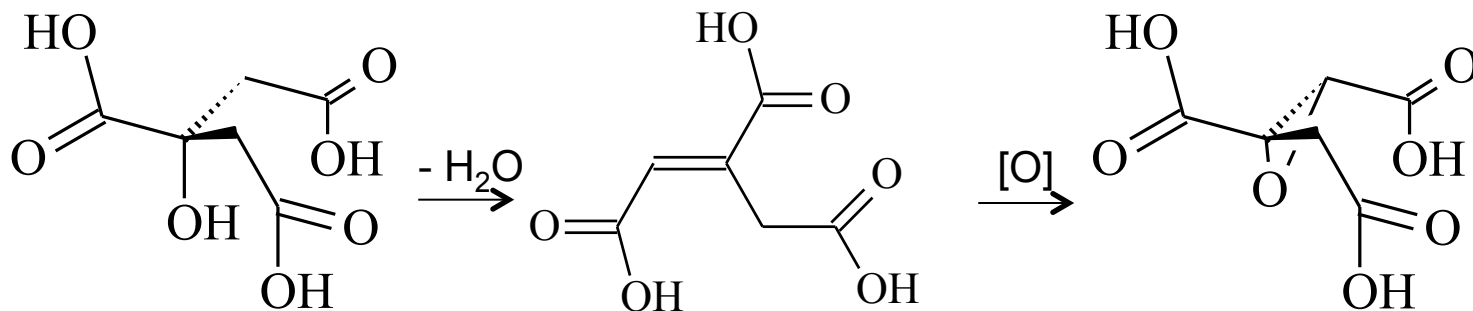
- ✓ Synthesize Novel Iron-Oxide-Based Superparamagnetic Nanoparticles
- Synthesize and fully characterize non-polymeric, polyprotic hydroxycarboxylic acids with bridging capabilities
- ◆ Isolate these organic compounds in pure form and coat the surface of nanoparticles for biomedical application

# NOVEL BRIDGING LIGANDS WITH A FOCUS ON CITRIC ACID

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Jonathon Watson

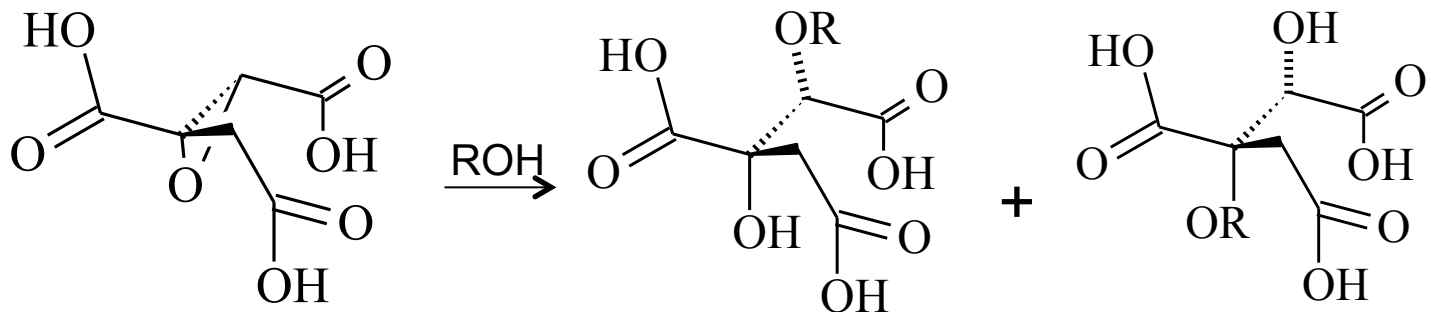
# Approach



Citric acid

*trans*-Aconitic acid

Epoxy-aconitic acid  
exotic and expensive substance  
(Fluka, Sigma Aldrich)

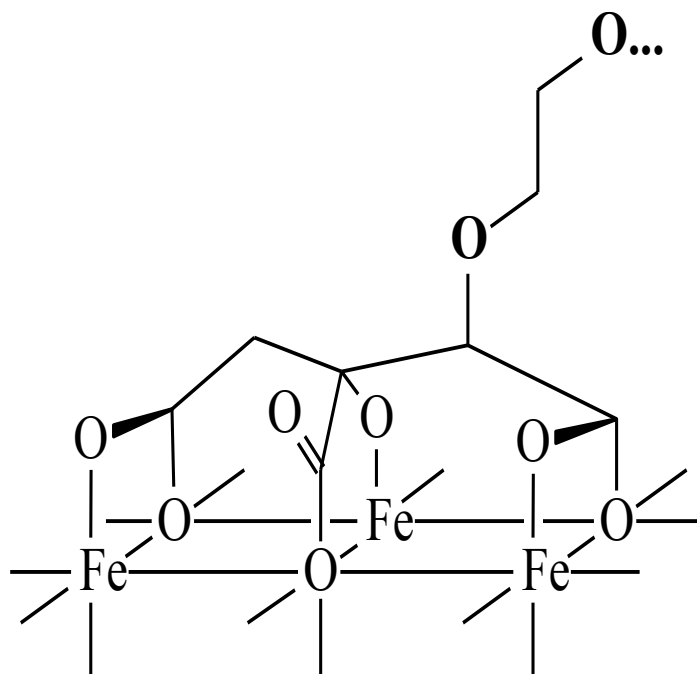


Epoxy Aconitic acid

Alkoxycitrate  
(desired)

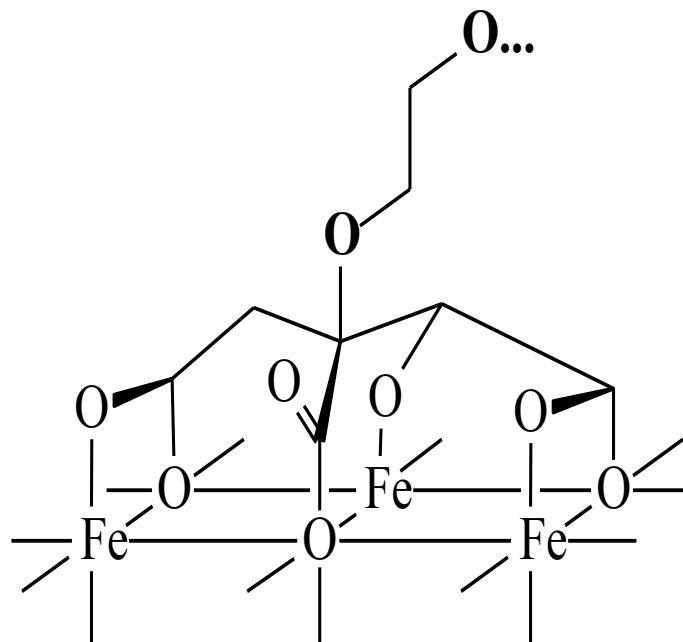
Alkoxyisocitrate  
(undesired)

The desired scenario:



Alkoxycitrate  
No strain – strong complex

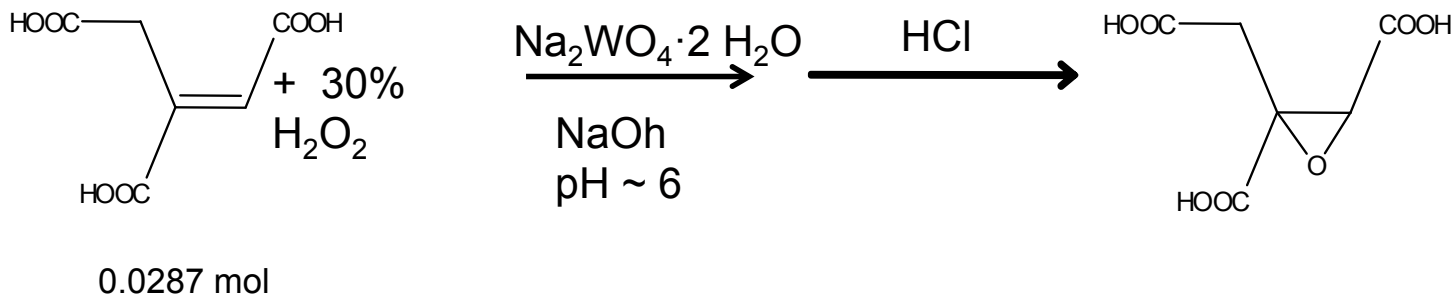
This can happen too:



Alkoxyisocitrate  
Some strain –  
weaker complex

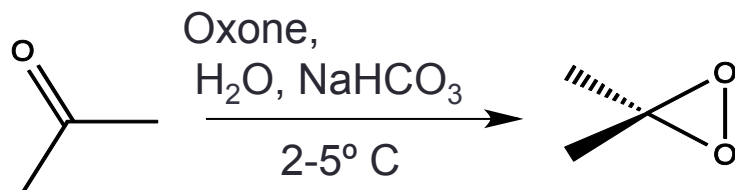
# Synthetic of epoxy-aconitic acid

Previous Work:

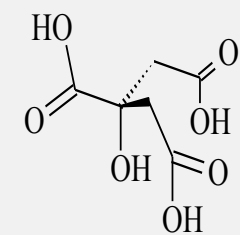


- Unreliable
- Not reproducible

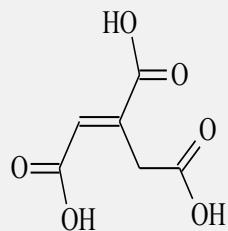
Current Work:



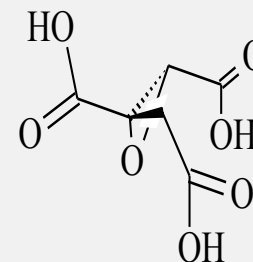
- Cost effective
- Efficient
- Reproducible
- Produced in situ



Citric Acid



*t*-Aconitic Acid

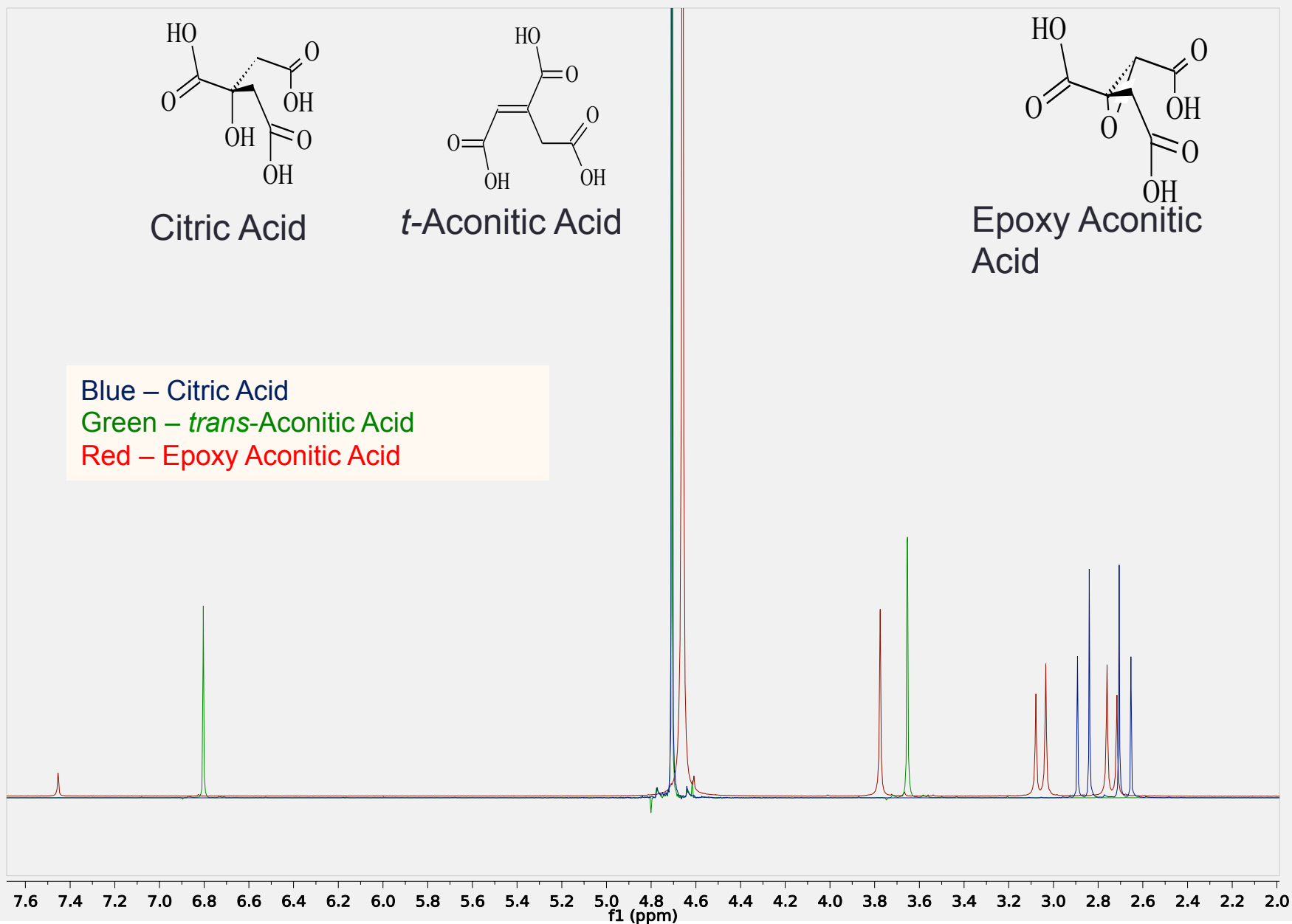


Epoxy Aconitic Acid

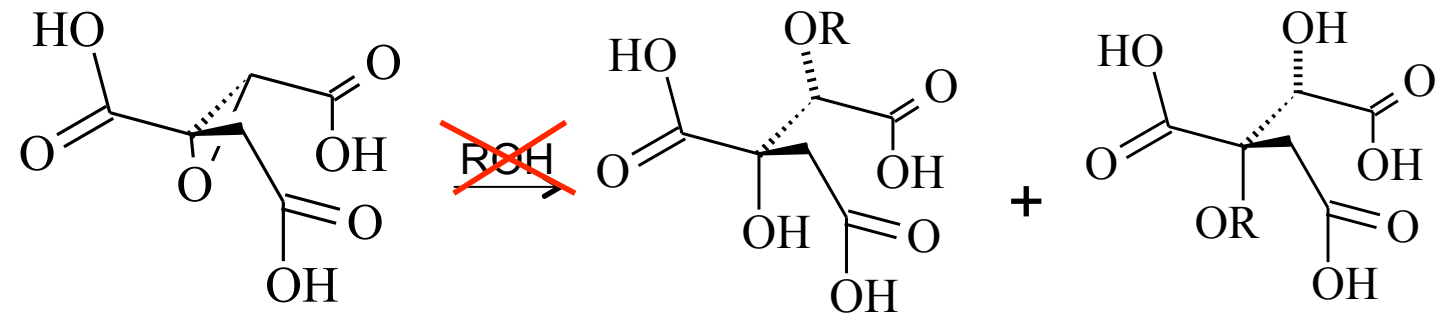
Blue – Citric Acid

Green – *trans*-Aconitic Acid

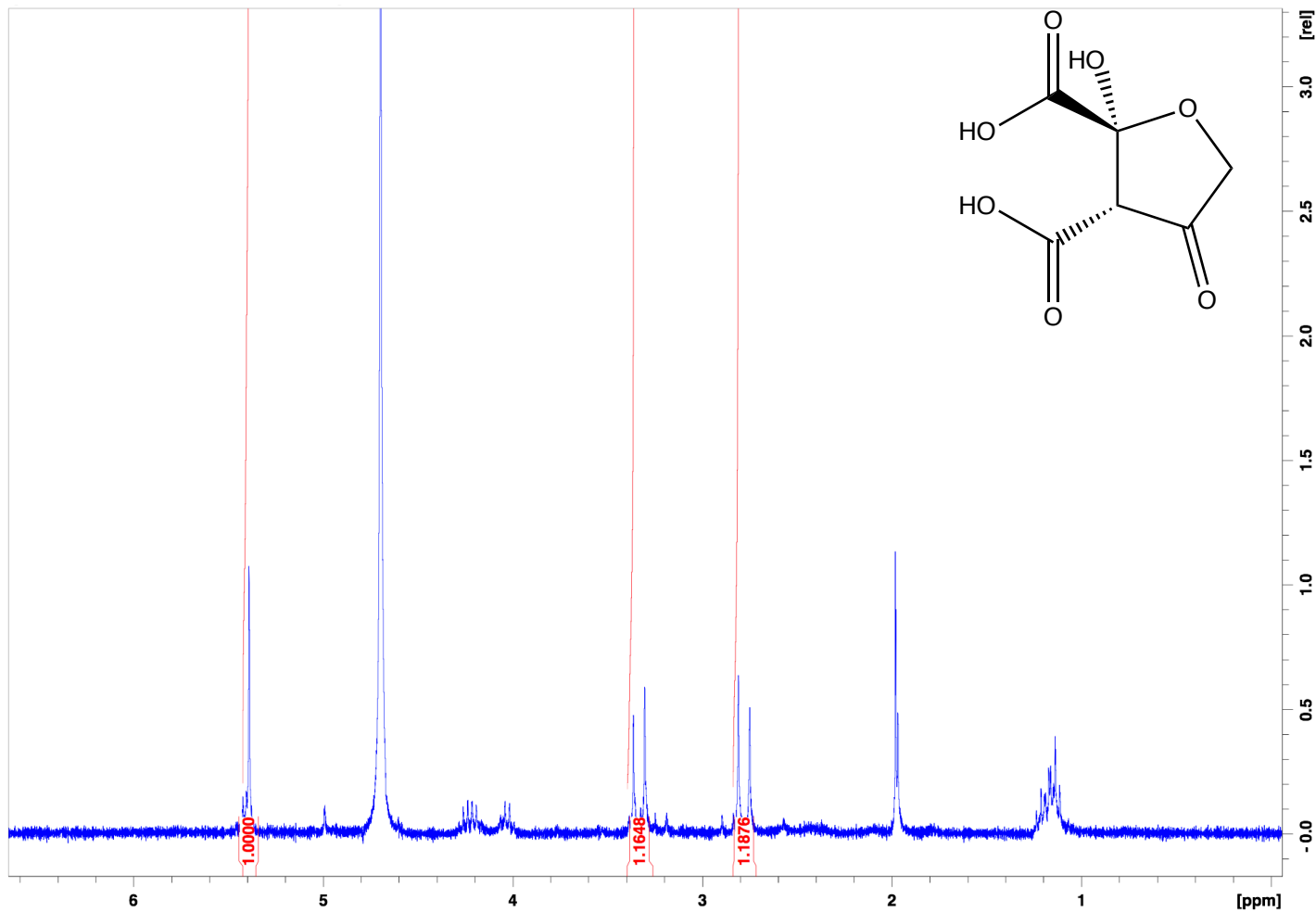
Red – Epoxy Aconitic Acid



# Ring Opening







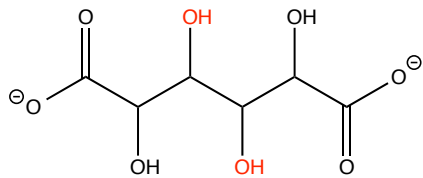
- Proton NMR (400 MHz, Acetone-d, ppm): 5.38 (1H, s), 3.30 (1H, d), 2.80 (1H, d)
- Chemistry, physiological properties.. Appl. Microbiol Biotechnol. (2007) Yamada et al. 75: 977-982
- Chiral Y-buyrolactones related... Ibrahim Ibnusaud et al. Tetrahedron 58 (2002). 4887-4892

# NOVEL SUGAR ACID BRIDGING LIGANDS WITH A FOCUS ON MUCIC ACID

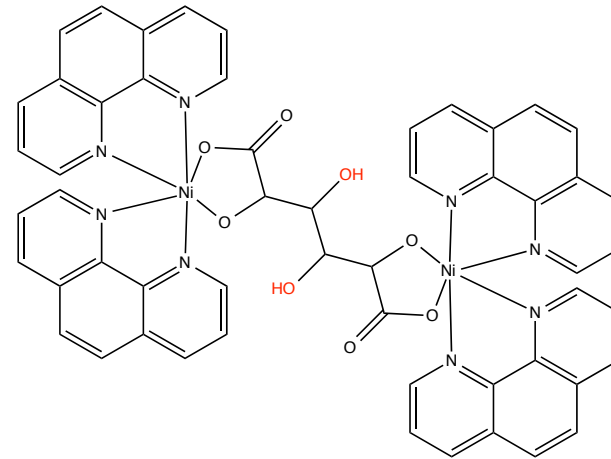
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Linden Allison

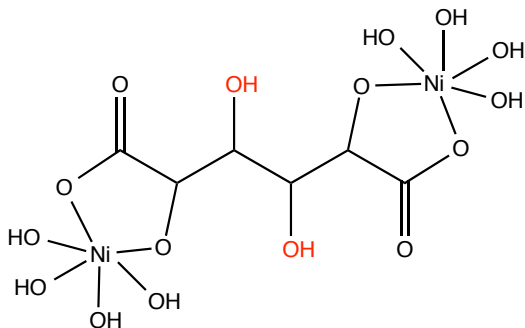
# Mucic acid



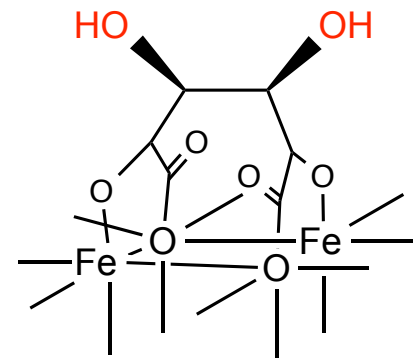
# Nickel phenantroline mucicate



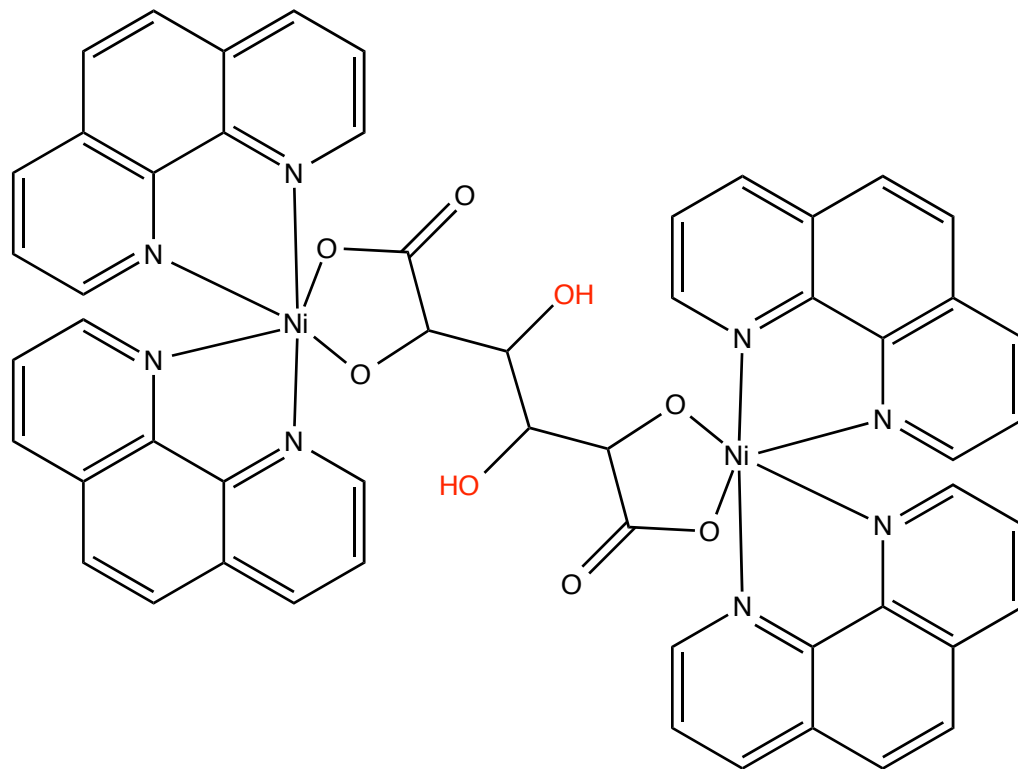
# Nickel mucicate



# Colloidal iron oxide mucicate

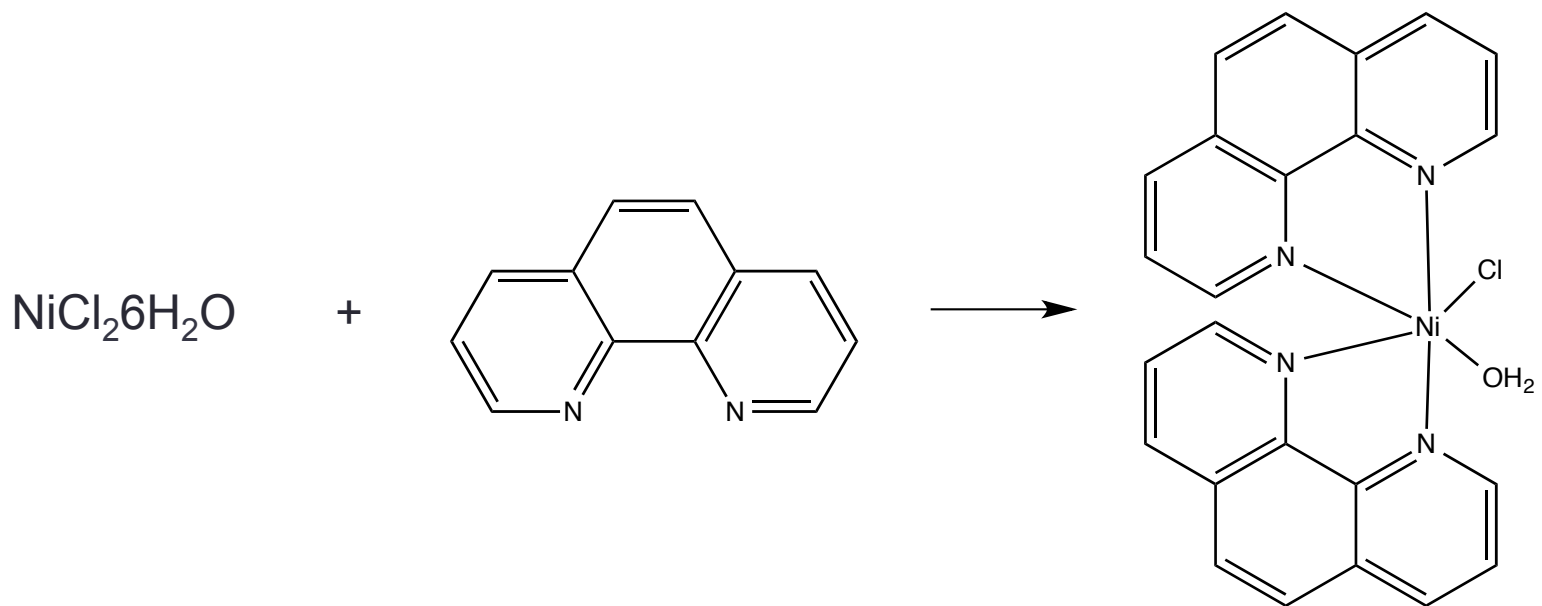


# Nickel Phenantrolino Mucicate



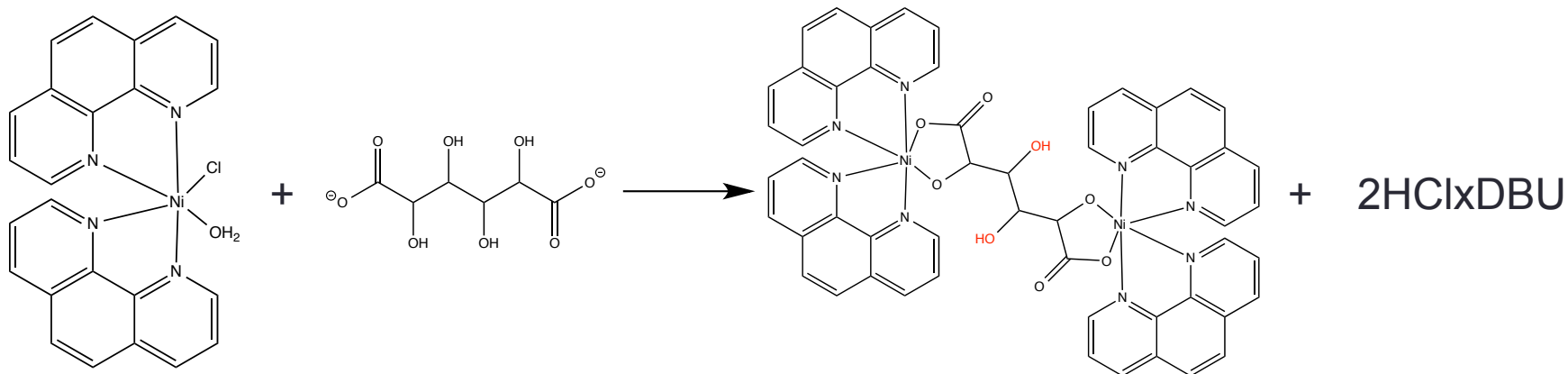
# Nickel Phenantrolino Mucicate Procedure

$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  was reacted with o-phenantroline in a 2:1 ratio in methanol, forming nickel phenantroline.



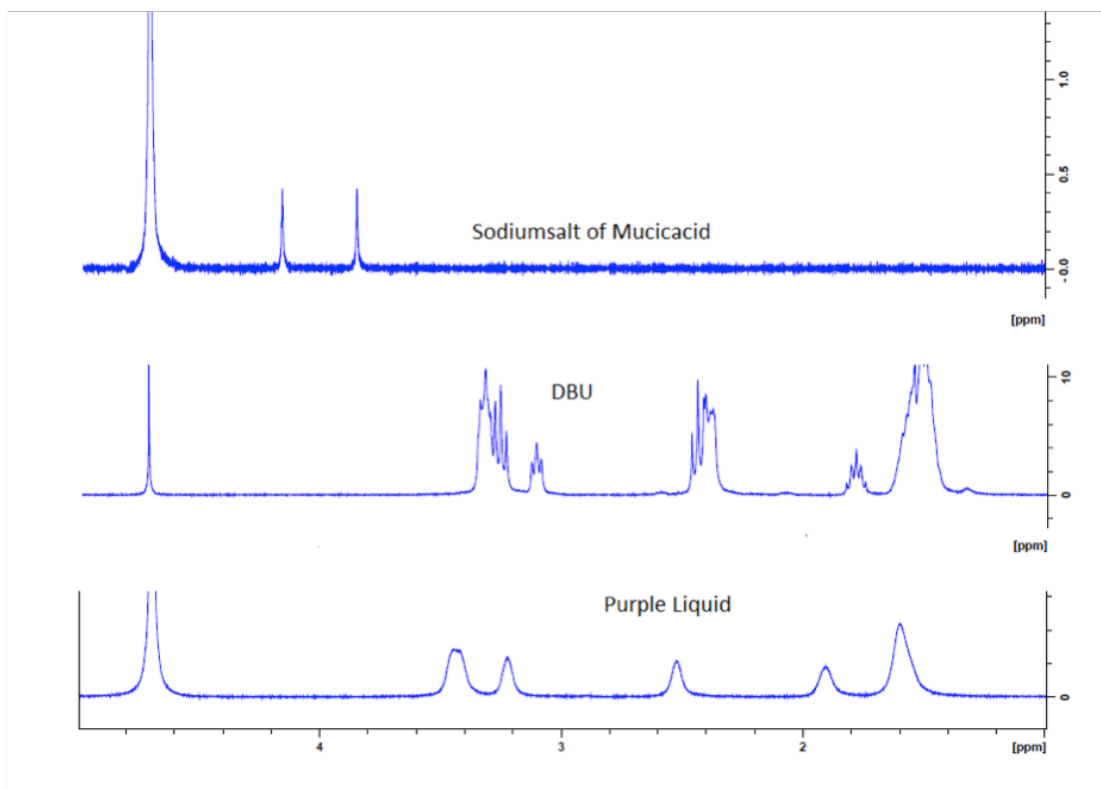
# Nickel Phenantrolino Mucicate Procedure

Nickel phenantroline was reacted with DBU and mucic acid in methanol in a 2:1 ratio forming nickel phenantrolino mucicate with a byproduct of HClxDBU.



# Nickel Phenantrolino Mucicate Procedure

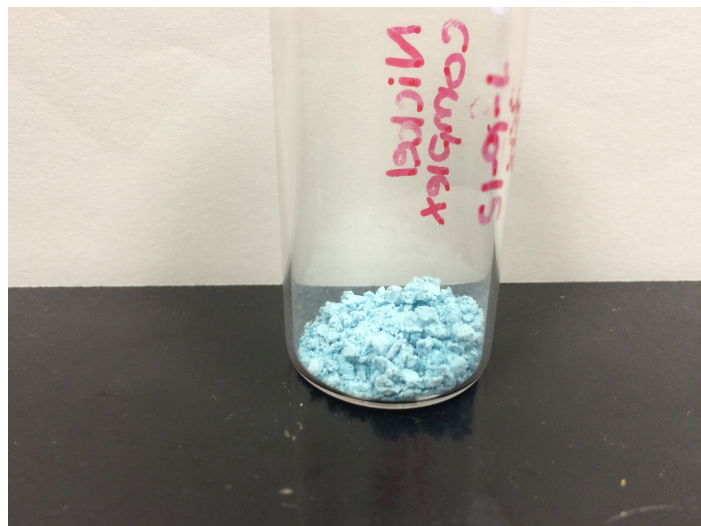
Byproduct was confirmed to be HClxDBU using H<sup>1</sup> NMR analysis.



The peaks in the H<sup>1</sup> NMR were broadened, which suggests that the sample still contains some nickel.

# Nickel Phenantrolino Mucicate

- Two products were formed:



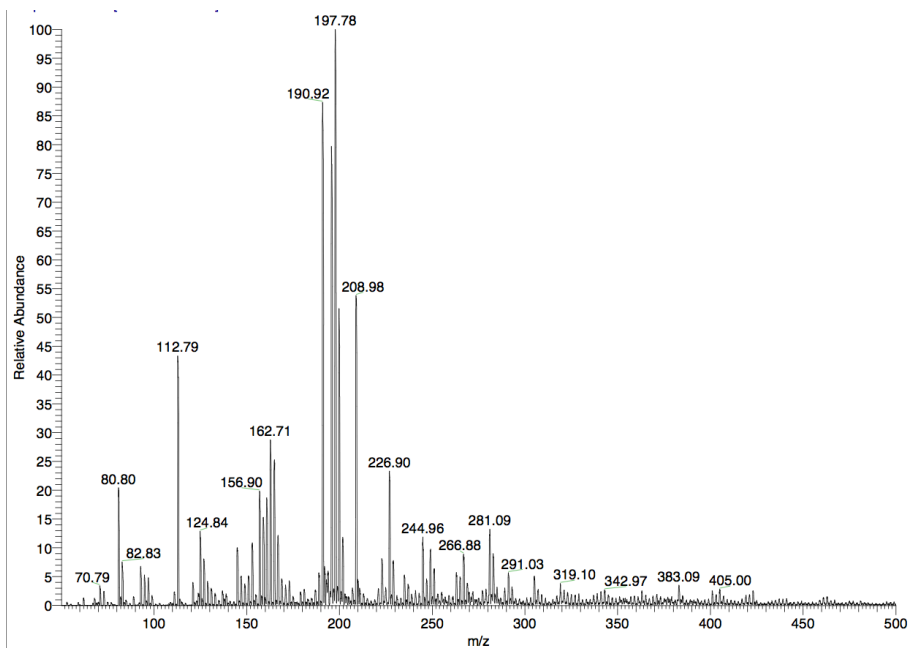
A blue powder that was insoluble



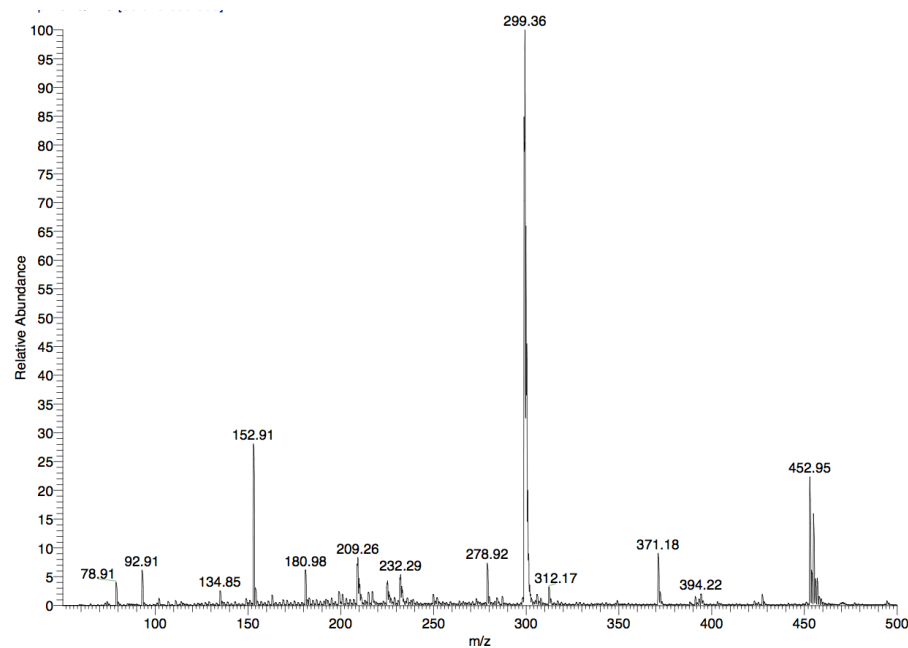
Purple crystals that had a low solubility in methanol



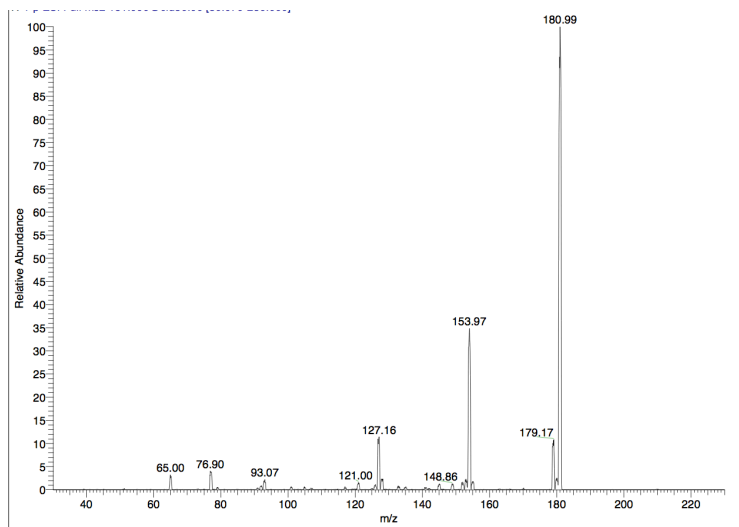
- Products were destroyed in 1 M HCl and characterized using ESI-MS
- Mass spectra showed that the blue powder contained mucic acid and no phenantroline, while the purple powder contained only phenantroline and no mucic acid



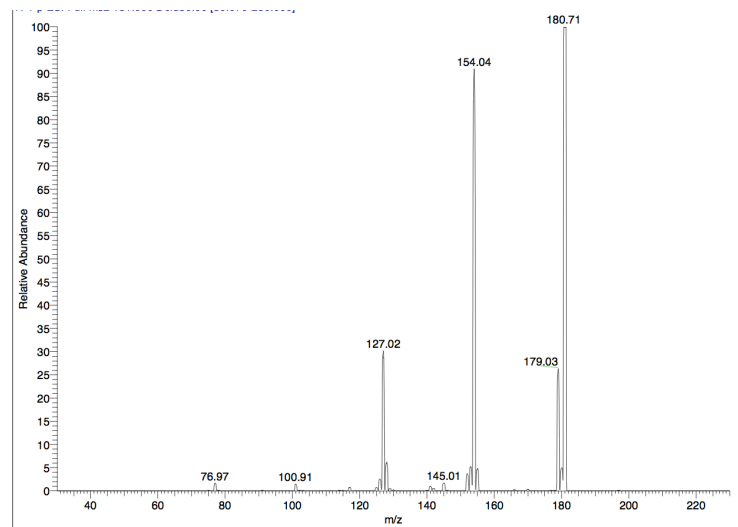
Blue Product



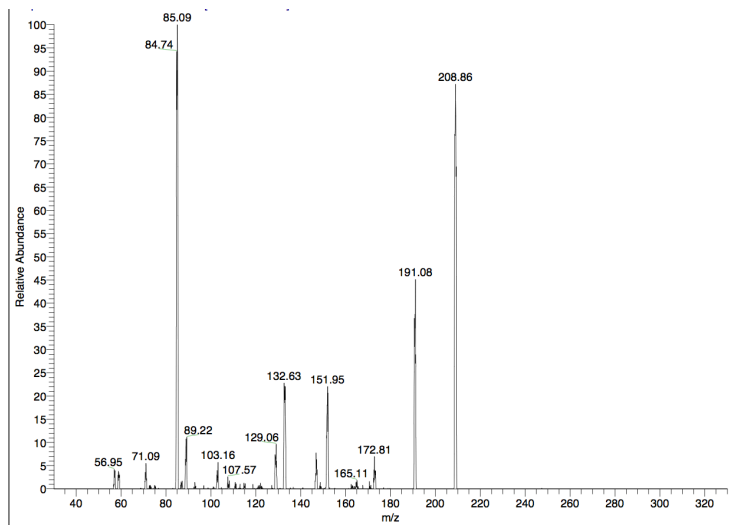
Purple Product



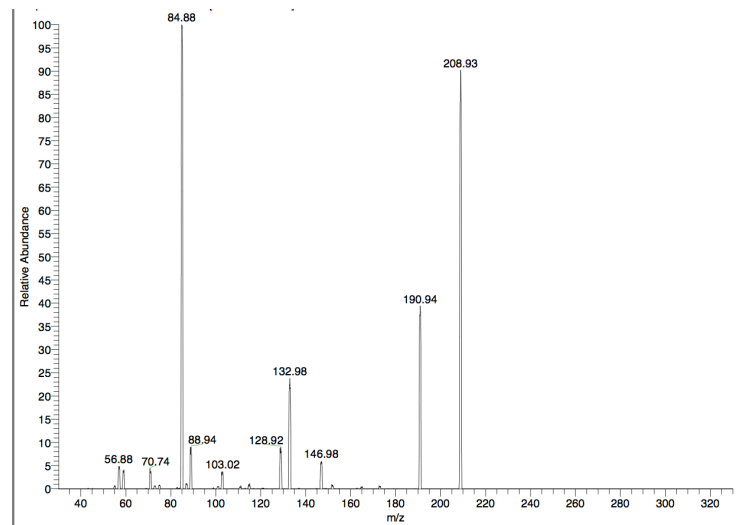
Purple product fractured at peak 181



O-phenantroline fractured at peak 181

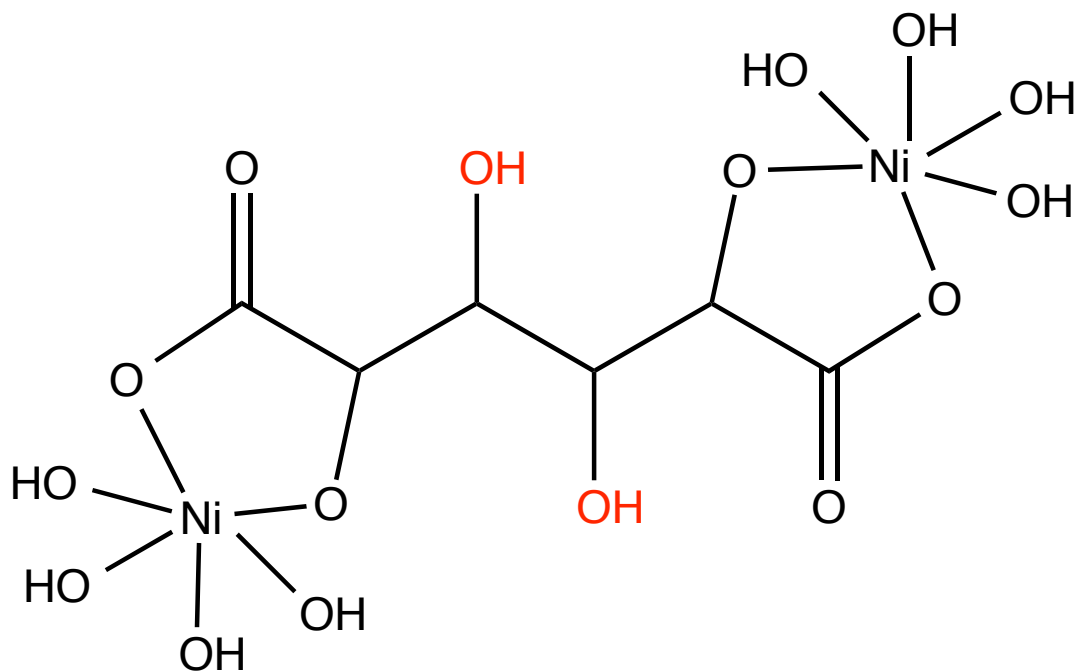


Blue product fractured at peak 209



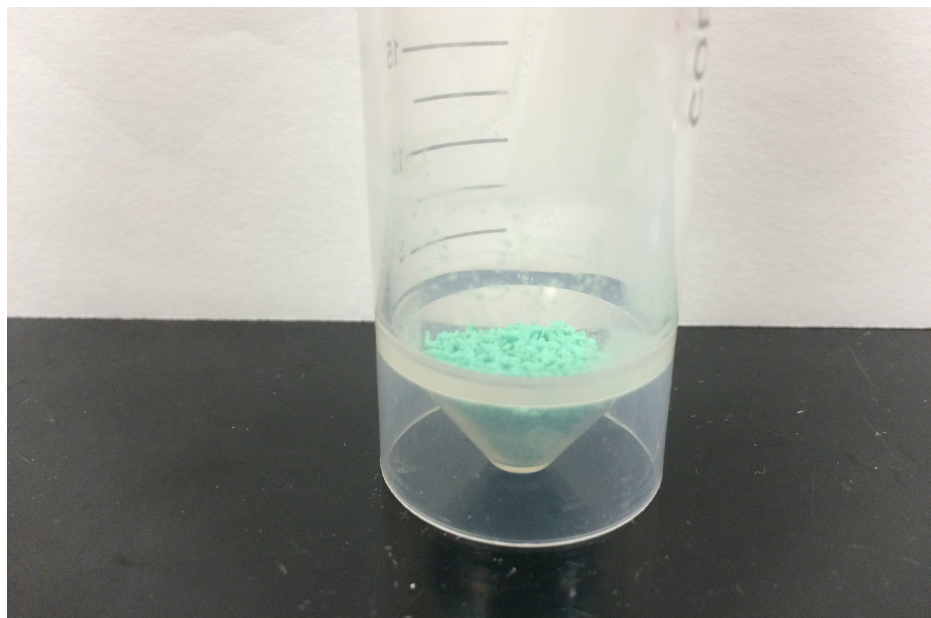
Mucic acid at fractured at peak 209

# Nickel Mucicate

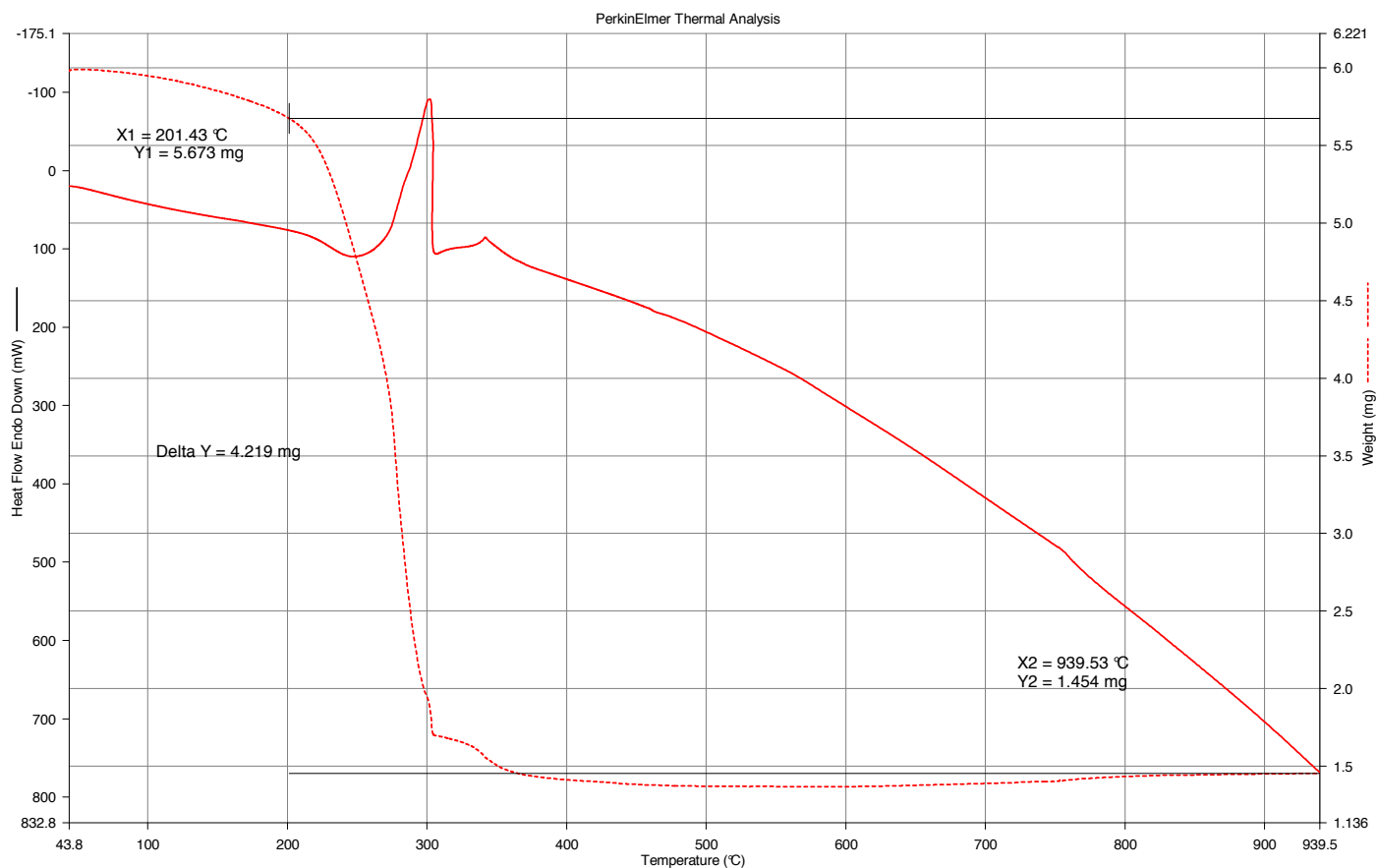


# Nickel Mucicate Synthesis

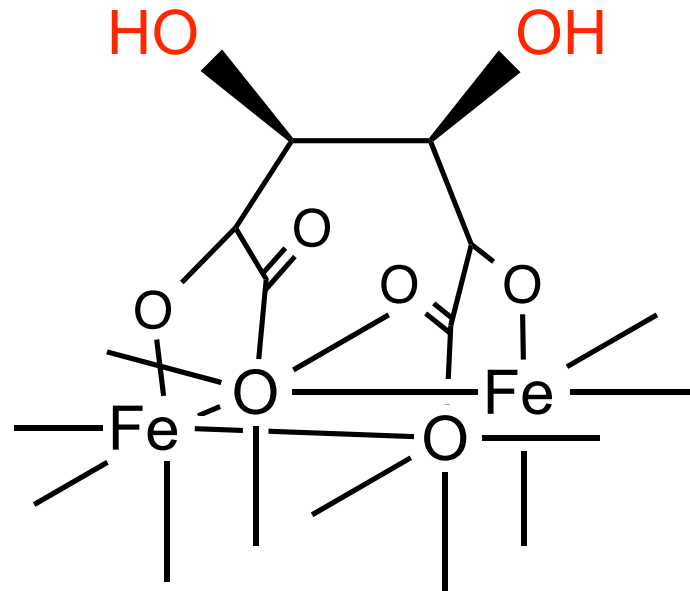
- $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  in methanol was reacted with mucic acid and DBU in a 2:1 ratio.
- A green powder was produced with a byproduct of  $\text{HCl} \cdot \text{DBU}$ . The byproduct could not be confirmed using  $^1\text{H}$  NMR because it was contaminated with excess nickel.



- Using thermo gravimetric analysis (TGA) it was determined through a combustion test that the starting materials were reacting in a 1:1 ratio, forming a coordination polymer instead of the desired product.



# Colloidal Iron Oxide muciccate



# Colloidal Iron Oxide Mucicate Synthesis

Mucic acid was dissolved in diethylene glycol (DEG) and mixed with a previously prepared colloid solution forming colloidal iron oxide mucicate.

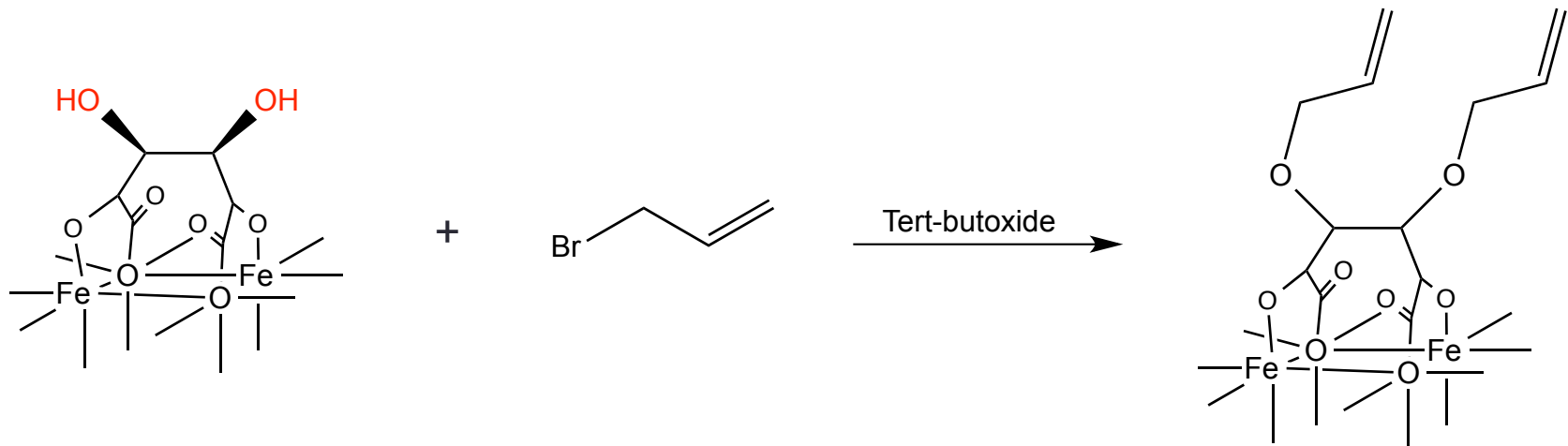


The colloidal muciccate suspension was washed with ethyl acetate, methanol, and isopropanol consecutively. This was done by mixing the solvent with the nanoparticles, agitating the suspension, separating with a magnet, and decanting the solvent.





# Colloidal Iron Oxide Mucicate



- Tert-butoxide was added as a base to the nanoparticle solution followed by 1 molar equivalent of allyl bromide and stirred overnight.
- Methanol was added in order to wash, however the nanoparticles dissolved. The solution was evaporated and 0.01 M of NaOH was added to destroy the complex.
- ESI-MS was performed in order to determine if the linker had attached, but it was inconclusive.

# NOVEL AROMATIC BRIDGING LIGANDS

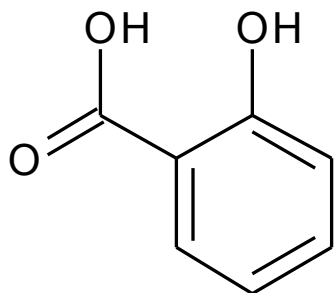
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Taylor Gravolet

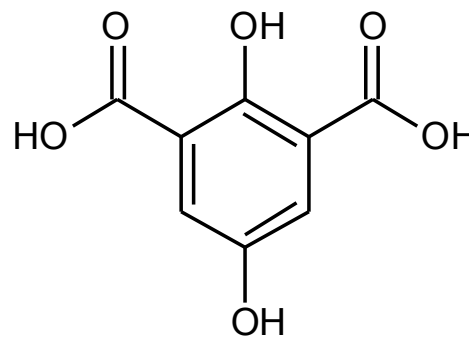
# Novel Aromatic Bridging Ligands as Nanoparticle Colloid Stabilizing Agents

- **Goals:**

- Synthesize 2,5-dihydroxyisophthalic acid linker ligand with multiple coordination sites for nanoparticle attachment.
- Optimize functionalization of the 5-hydroxy position for bioconjugation.
- Further characterize isophthalic acid derivatives

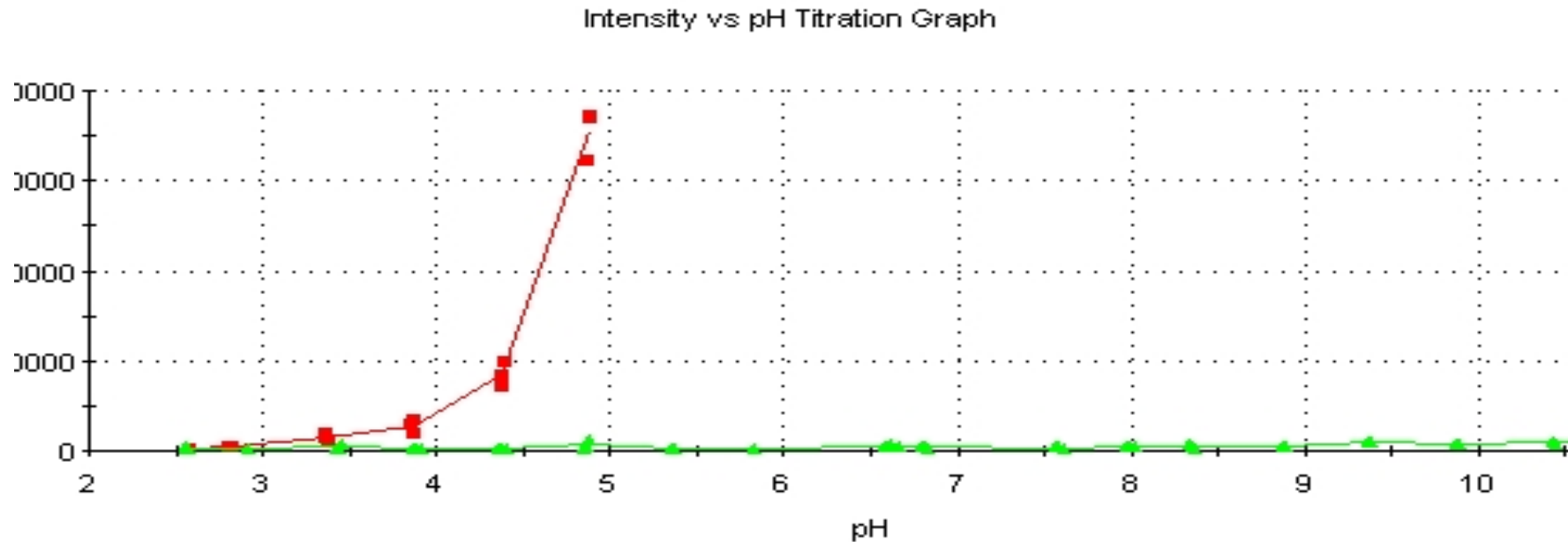


Salicylic Acid



2,5-dihydroxyisophthalic acid

# Salicylic Acid vs. 2-Hydroxyisophthalic Acid



Intensity (Fe(III) + 3H<sub>2</sub>Salicyl)

Intensity (2Fe(III) + 3H<sub>3</sub>Carlsalicyl 2)

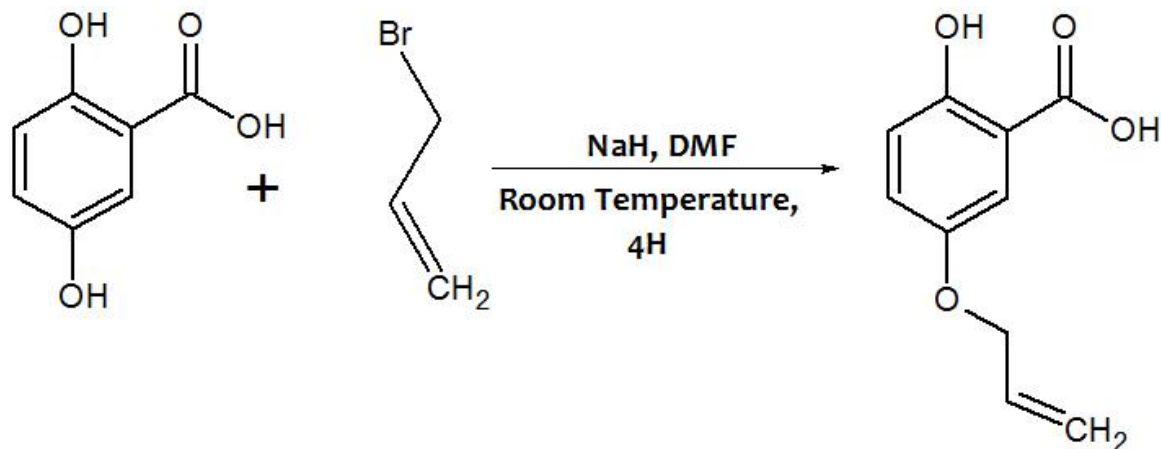
— Weighted Mean Intensity (Fe(III) + 3H<sub>2</sub>Salicyl)

— Weighted Mean Intensity (2Fe(III) + 3H<sub>3</sub>Carlsalicyl 2)

*Preliminary DLS study shows how well the 2-HIP stabilizes magnetite in comparison to salicylic acid by measuring each ligand's ability to inhibit the reaction:  $\text{Fe}^{3+} + 3\text{OH}^- \rightarrow \text{Fe}(\text{OH})_3$  by titrating with sodium hydroxide*

# Synthesis

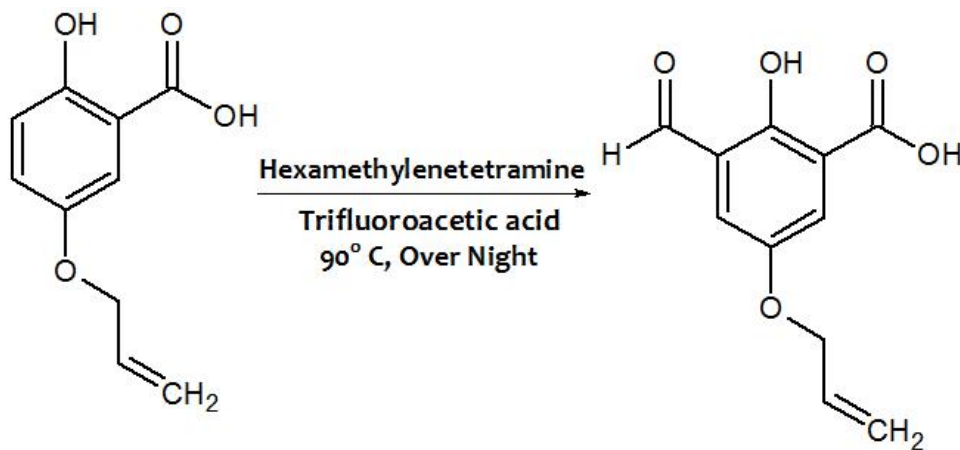
- **Step 1: Coupling**



- 5-hydroxysalicylic acid and NaH in DMF
- Allyl bromide in DMF added drop wise
- DMF removed by a BUCHI Rotovapor R-200
- 1M HCl
- Extracted with ethyl acetate
- Recovered 5-allyloxy salicylic acid (average 66.5% yield)

# Synthesis

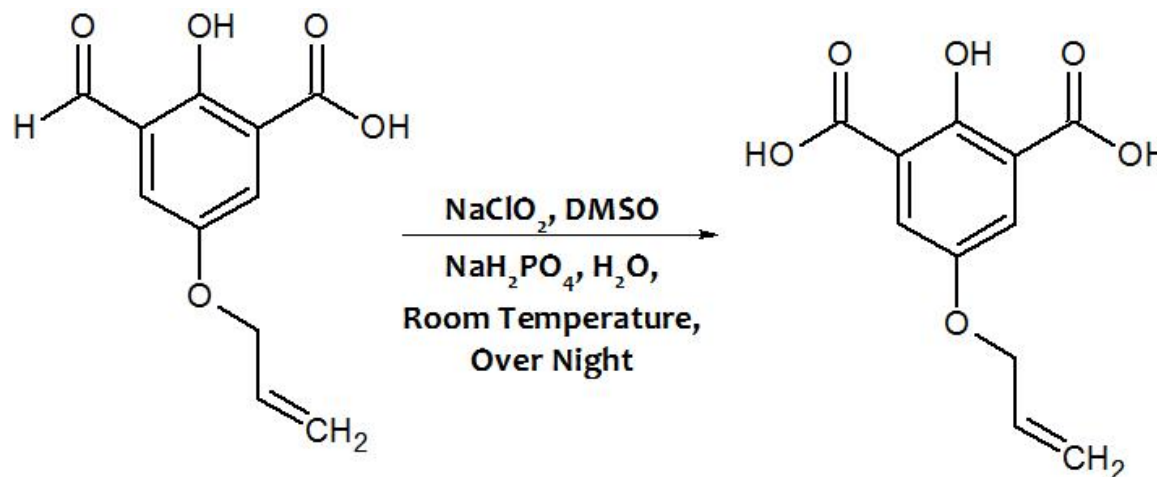
- **Step 2: Formylation**



- Duff reaction of 5-allyloxy salicylic acid with hexamethylenetetramine, and trifluoroacetic acid
- 1M HCl
- Extracted with ethyl acetate
- Recovered 3-formyl-5-prop-2-enoxysalicylic acid (average 62.3% yield)

# Synthesis

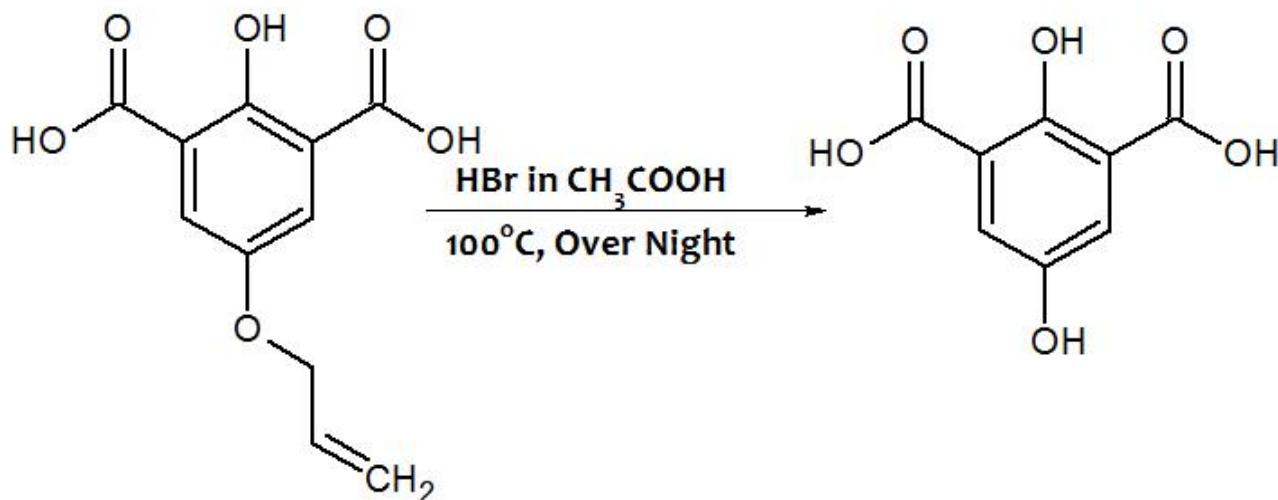
- **Step 3: Oxidation**



- Sodium chlorite solution added drop wise to 3-formyl-5-prop-2-enoxysalicylic acid in DMSO solution buffered with  $\text{NaH}_2\text{PO}_4$
- Sulfuric acid was added until pH  $\sim 1$
- Extracted with ethyl acetate
- Recovered 5-allyloxy-2-hydroxyisophthalic acid (average 68.7% yield)

# Synthesis

- **Step 4: Tail Cleavage**

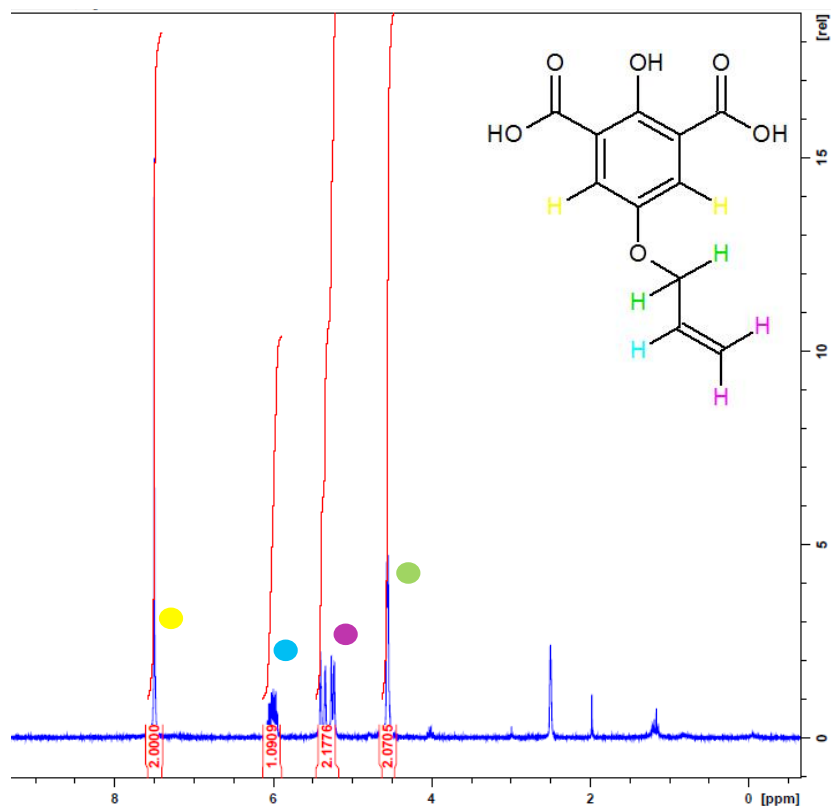


- 5-allyloxy-2-hydroxyisophthalic acid and hydrobromic acid in acetic acid
- Acetic acid removed by cold distillation
- Product recrystallized from water
- Recovered 2,5-dihydroxyisophthalic acid

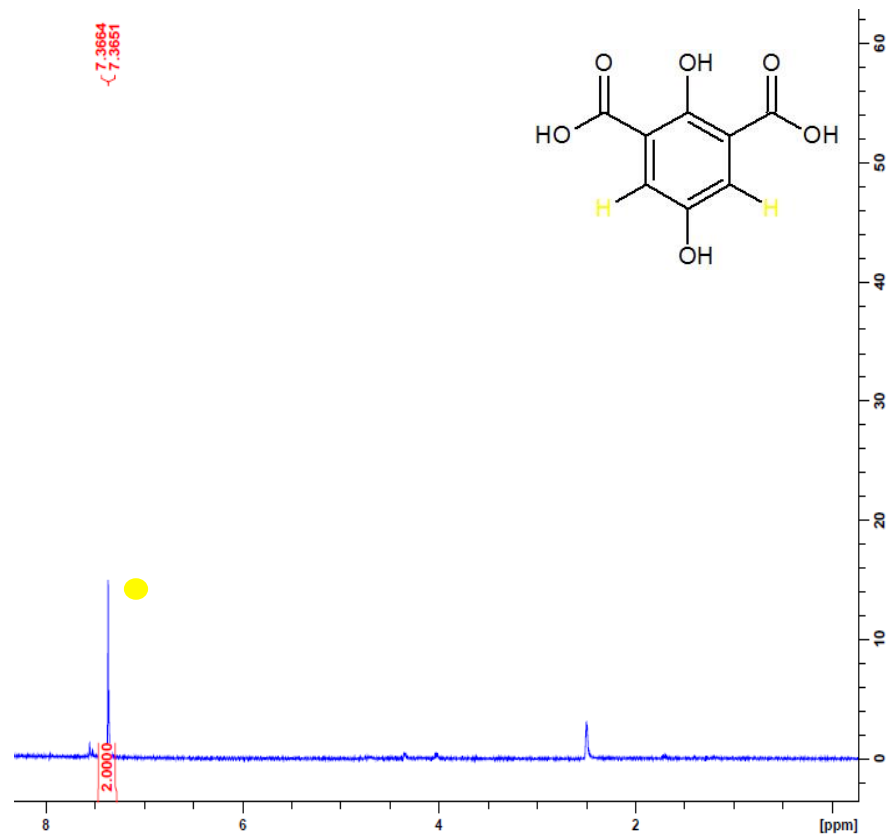


# Characterization

$^1\text{H}$  NMR confirmation of the production of 5-alkoxy-2-hydroxyisophthalic acid. DMSO was used as the solvent contributing to the 2.5ppm peak.



$^1\text{H}$  NMR confirmation of the production of 2,5-dihydroxyisophthalic acid. DMSO was used as the solvent contributing to the 2.5ppm peak.



# Conclusions and future work

- Successfully synthesized 2,5-dihydroxyisophthalic acid. Optimization of tail attachment at the 5-hydroxy position for future biomolecule attachment.
- Mucic acid based ligands:
  - Nickel phenantroline mucicate- unsuccessful
  - Nickel mucicate- coordination polymer
  - Colloidal iron oxide mucicate- inconclusive
  - Currently nickel mucicate is being synthesized in anhydrous conditions
- *Trans*-aconitic acid was successfully oxidized to yield the epoxide product in high purity. Ring opening experiments are ongoing.

# Acknowledgements

- This material is based upon work supported by the National Science Foundation under the NSF EPSCoR Cooperative Agreement No. EPS-1003897 with additional support from the Louisiana Board of Regents.
- Additional thanks to
- Xavier University of Louisiana
- Dr. Vladimir Kolesnichenko
- Dr. Galina Goloverda
- Dr. Rajesh Komati
- Huy Do

# Questions

