Title: The Use of Amino Acid L-Proline as Catalyst for Direct Asymmetric Aldol Reactions

By

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Abstract:

I have explored the asymmetric aldol reaction using natural amino acid L-proline as the catalyst. Amino acid L-Proline mimics class I aldolase enzyme to drive the aldol reaction through enamine-based mechanism. The use of catalyst L-Proline is the first example of amine-based asymmetric class I aldolase mimics. In the first part of this project, we explored the direct asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde in the presence of 30 mol % of L-proline. The reaction was set at room temperature under normal condition, and we got 2 products: aldol and α,β -unsaturated ketone product. Detail characterization of the two products was done using TLC, Column Chromatography, NMR and IR. The percent yield of asymmetric aldol product was 70% which is comparable to the published value of 68%. The yield of α,β-unsaturated ketone was 10.5%. Further in the project, we explored the same aldol reaction using p-Anisaldehyde in place of 4-nitrobenzaldehyde. Only one product was obtained, and the exclusive product was α,β -unsaturated ketone instead of the expected asymmetric aldol product.

Introduction:

Asymmetric aldol reaction is a crucial reaction in biology and chemistry to form new stereoselective C-C bond. The mechanism of how natural catalysts (aldolases) work is remarkably different compared to the mechanism of their synthetic counterparts. The synthetic pathway usually require carbonyl group of a reactant to be modified to form enolate before introducing a chiral catalyst to form stereoselective product. On the other hand, aldolases use a different mechanism to catalyze direct aldolization without modifying the two carbonyl groups. Class I aldolases use an enamine-based mechanism[1], while class II aldolases use zinc cofactor.[2] Research has been done on Class II aldolases mimics[3,4]; however, amine-based asymmetric class I aldolase mimics using enamine-based mechanism has not been described[5]. The goal of the project was to replicate the novel aldol reaction published by JACS in which Proline was found to be the first asymmetric small-molecule aldol catalyst that uses an enamine mechanism.

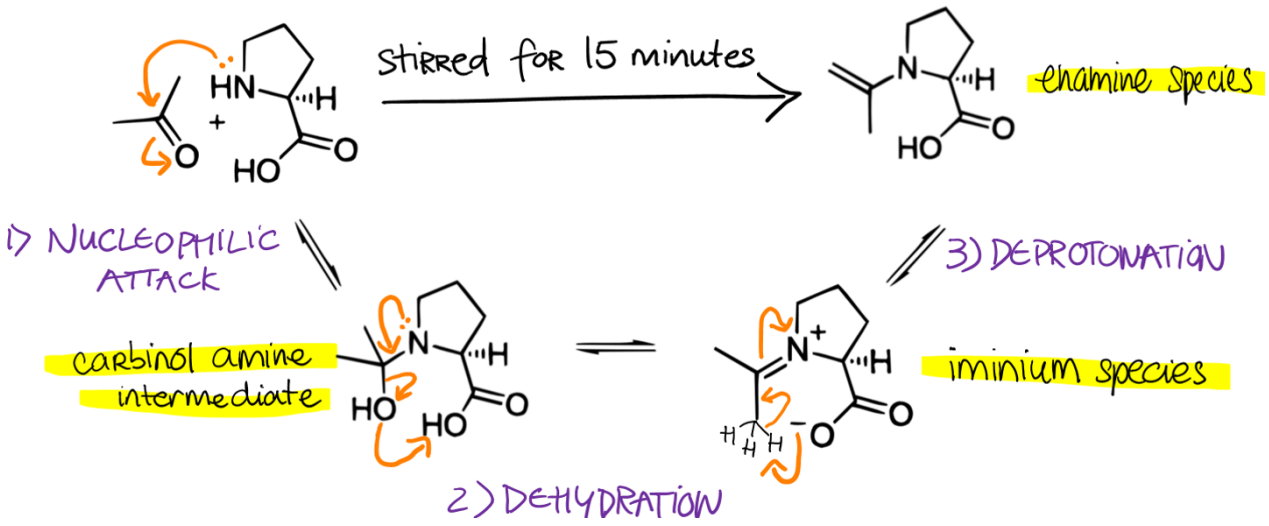
Experimental Section:

Instrumentation: NMR spectra were recorded using NMRReady^{TM} 60 Nanalysis Corporation. $CDCL_3$ was used as a reference/blank and also as solvent for all compounds. IR spectra were recorded using Perkin Elmer Infrared Spectrophotometer (Model 710 B Serial 132636).

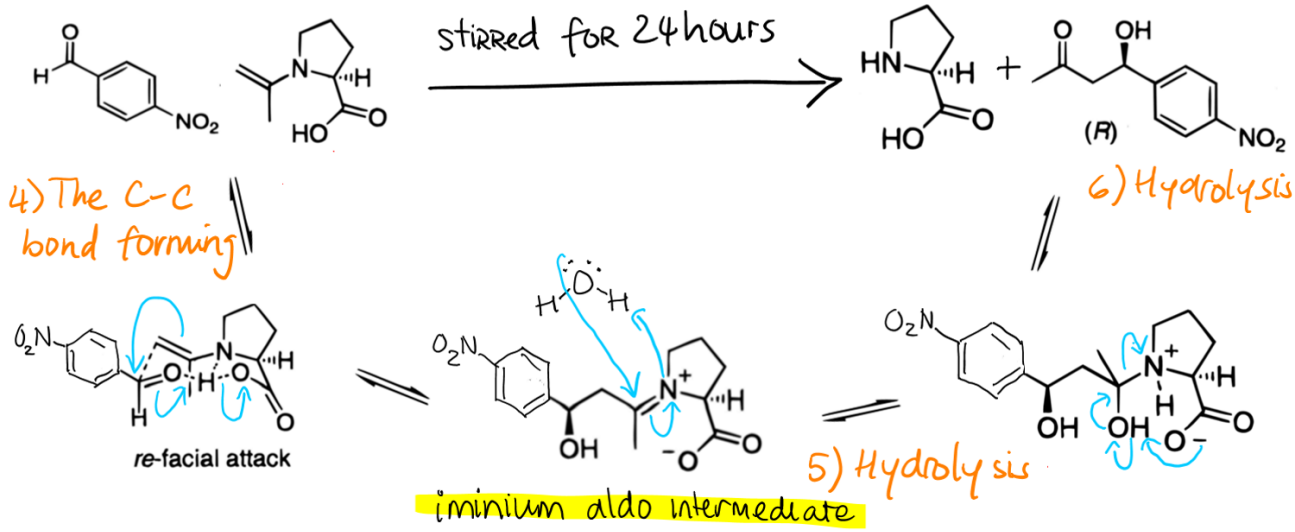
Materials: All materials used for preparation were reagent graded and used without further purification except for p-Anisaldehyde. P-Anisaldehyde was purified using Column Chromatography with silica gel as stationary phase and pentane/ ethyl acetate (3:1) as mobile phase.

Enamine mechanism:

- Step 1: Proline was stirred in DMSO/acetone (4:1) for 15 minutes.



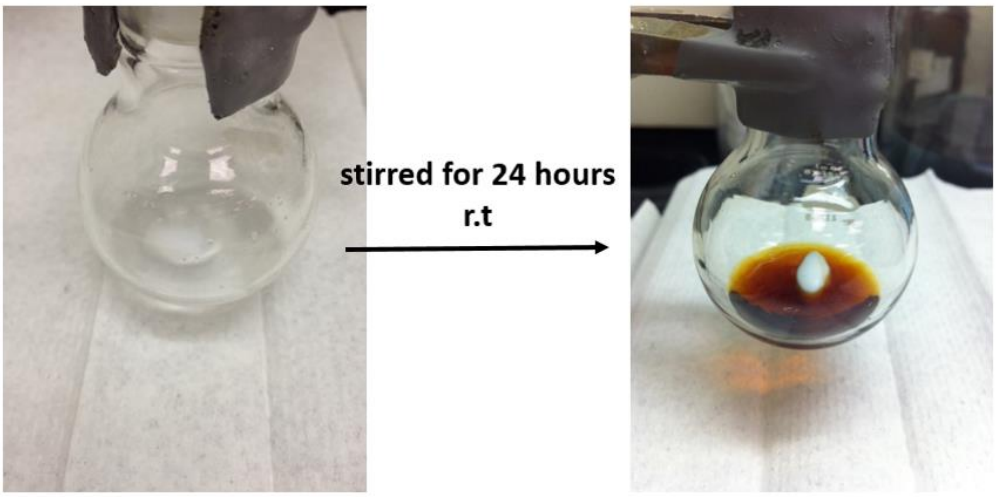
- Step 2: 4-Nitro benzaldehyde was added and the mixture was stirred for 24 hours.



Experiment:

-The amino acid L-Proline (0.16 mmol) was stirred in 4mL of DMSO/acetone (4:1) for 15 minutes.

-After 15 minutes, 4-Nitrobenzaldehyde (0.4mmol) was added and the misture was stirred for 24 hours.



<u>Figure 1.</u> Left: The mixture of Amino acid L-Proline, DMSO, Acetone and 4-Nitrobenzaldehyde at the beginning stage of the reaction. Right: The mixture after 24 hours of stirring under room temperature.

-The crude mixture was treated with 4mL of saturated aqueous ammonium chloride solution and extracted with 3x 20mL of Ethyl Acetate.

<u>Firgure 2.</u> Crude product was extracted with Ethyl actate

-The organic layer was dried using $MgSO_4$ and filtered. Solvent was evaporated using Rotavap.

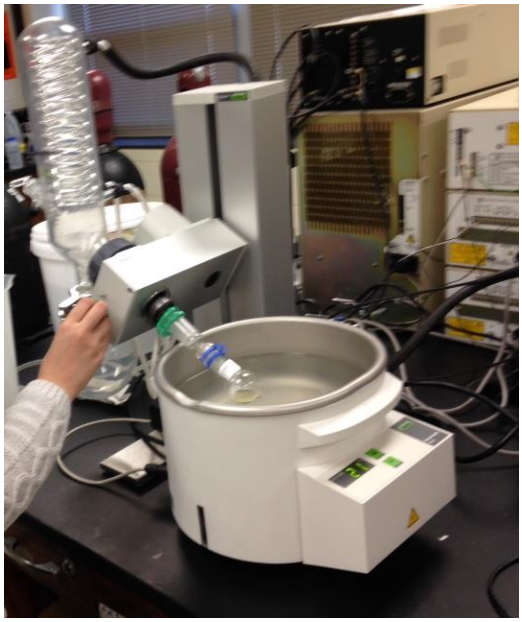


Figure 3. Solvent used in extraction was dried out using Rotavap

-Thin Layer Chromatography and Column Chromatography was done next to isolate the 2 products. The solvent system used for both chromatography was hexanes/ethyl acetate (3:1).

Figure 4. Left: TLC plate result of the crude product and the product after extraction. Right: Column Chromatography to isolate the 2 components in the washed product.

Result and discussion:

-After isolating the 2 products using Column Chromatography, all the test tube fractions were first analyzed using TLC. Solvent system used was also the same with previous TLC and Column Chromatography which was hexanes/ethyl acetate (3:1). The TLC result of all test tube fractions suggested that the minor product ranged from test tube number 4 to 7. Similarly, the major product was in test tube number 12 to 21. The published paper reported that the major product was Aldol product and the minor product was α,β-unsaturated ketone. The percent yield that I got for the Aldol product 70% was comparable to the published number of 68%. The percent yield for α,β-unsaturated ketone was 10.5%. After that, the two products was again dried out by Rotavap and characterized using NMR and IR.

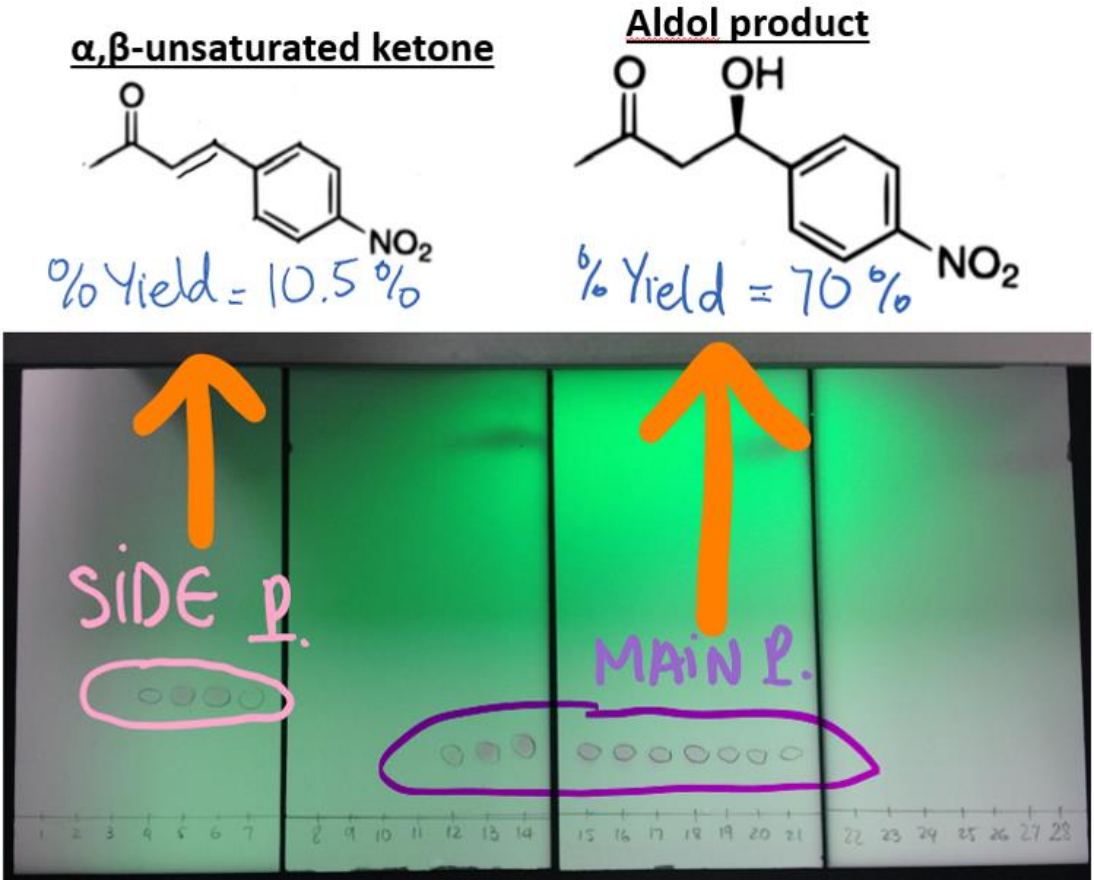
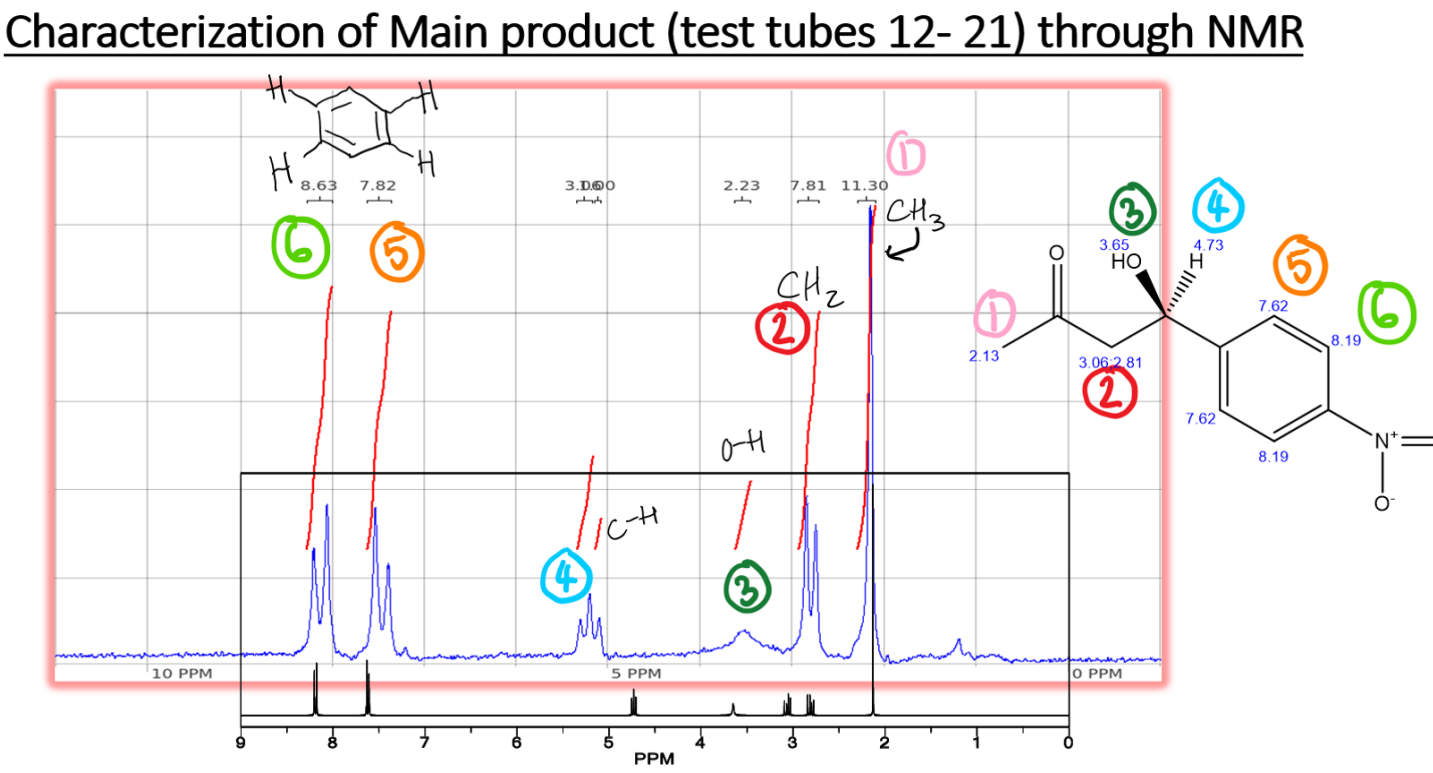


Figure 5. TLC plates of all the collected test tube fractions after isolating with Column Chromatography

-All the characteristic peaks match with the estimated peaks in the computer-generated spectrum. As expected, we see the AB pattern of 4 aromatic protons (proton labeled 5 and 6) around 7 to 8 ppm. A triplet is seen for proton number 4 due to the splitting of 2 neighboring protons at number 2 following n+1 rule. Similarly, a doublet for proton number 2 is obtained due to reciprocal splitting of the single proton at number 4. O-H proton is weak, but it shows up at

number 3. Proton at number 1 which belongs to the methyl group which has no neighboring proton also appears as singlet as expected. All the major characteristic peaks are there which agrees with the published research that Aldol is the major product.



<u>Figure 6.</u> Experimental NMR spectrum of Aldol product (red lining) overlaid with theoretical NMR spectrum generated by computer software.

-The major product was further analyzed through IR. The IR spectrum has a broad OH peak from 3200 to 3500 cm^{-1} and a Carbonyl group at 1706 cm^{-1}. The IR result strongly confirms that Aldol is the structure of the main product.

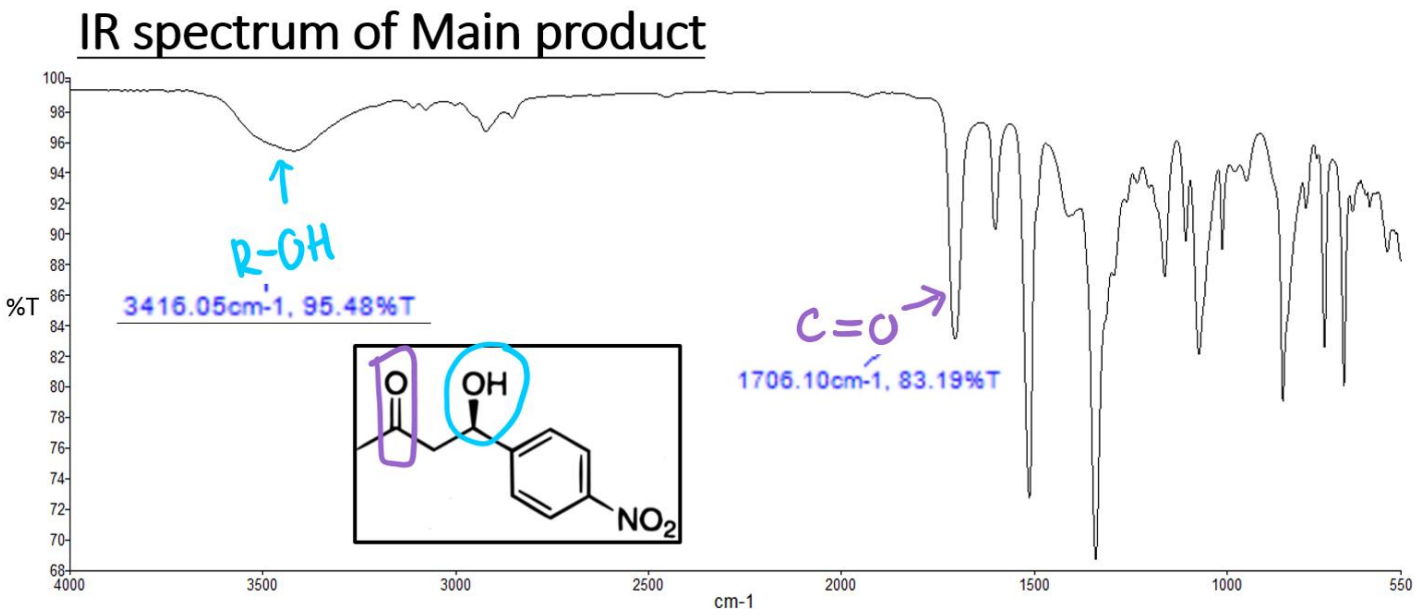


Figure 7. IR spectrum of Aldol product

-The minor product was handled in the same way with the major one. The NMR spectrum of the minor product suggests it is α,β-unsaturated ketone. All the characteristic peaks match with the peaks in the theoretical spectrum. The splitting in the aromatic region 7 to 8 ppm was complex because it has aromatic protons number 4 & 5 and ethylenic proton number 2 & 3 as well. All the protons in this region combined with the low concentration of the minor product and the low resolution of the NMR machine result in the poor NMR spectrum. However, the experimental spectrum match with the computer-generated one. There was some impurities from 1-2 ppm which was analyzed to be the Hexane and Ethyl Acetate solvent used in Column Chromatography.

Characterization of Side product (test tubes 4- 7) through NMR

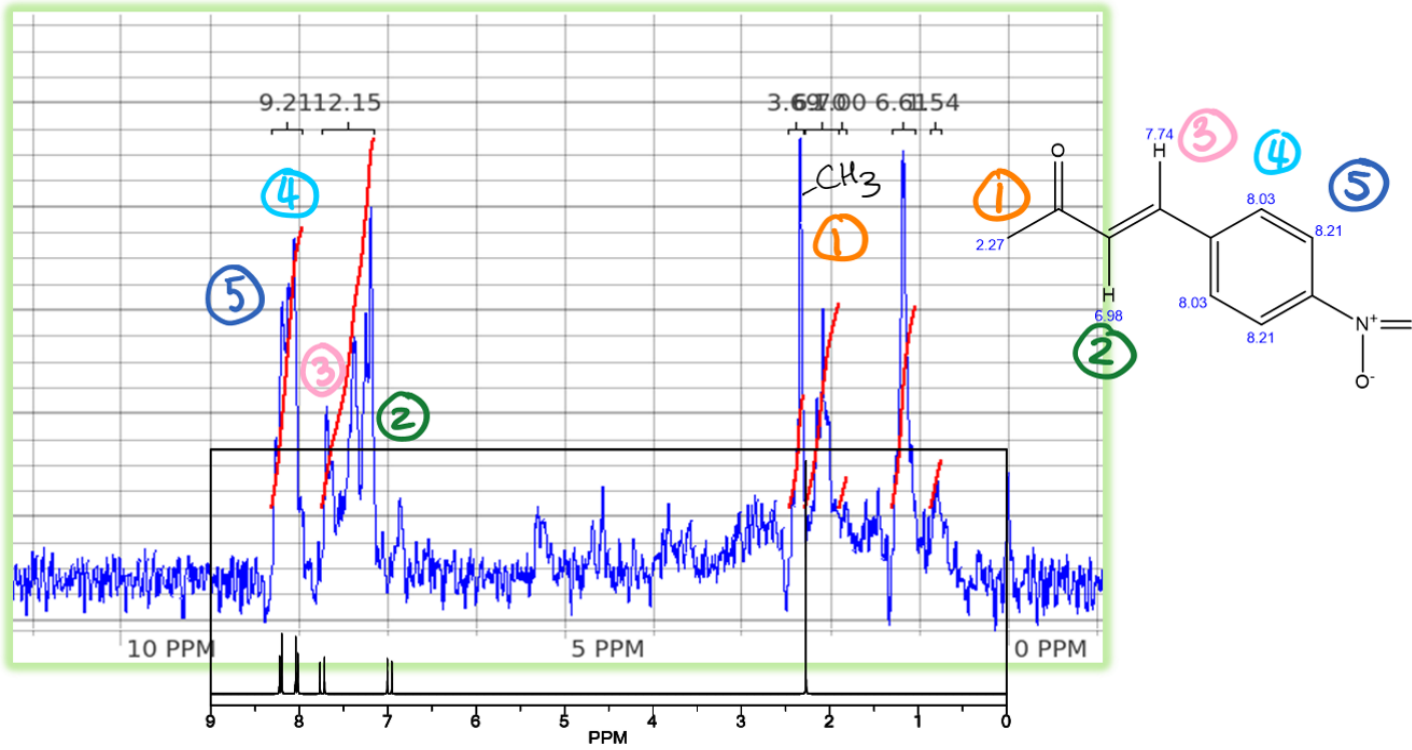
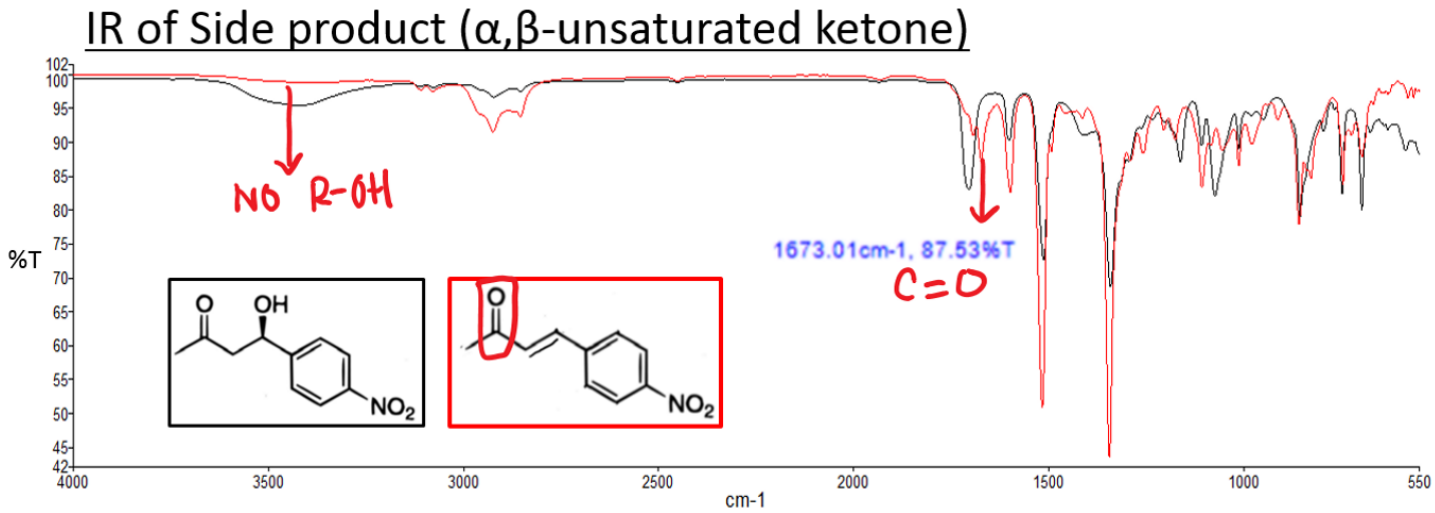


Figure 8. Experimental NMR spectrum of α,β-unsaturated ketone product (green lining) overlaid with theoretical NMR spectrum generated by computer software.

Analyze of Impurities peaks in α,β-unsaturated ketone spectrum

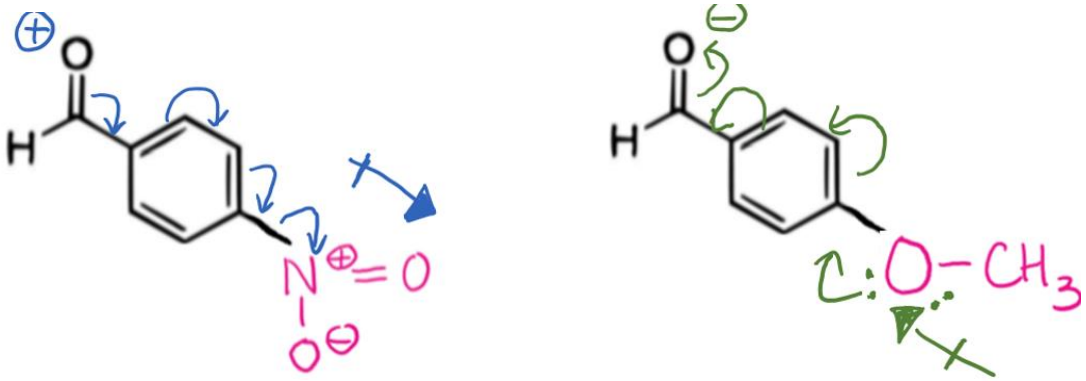
<u>Firqure 9</u>. From top to bottom: NMR spectrum of Hexane, Ethyl Acetate and α,β-unsaturated ketone. The characteristic peaks which do not match with the computer generated peaks come from the solvent system hexane and Ethyl acetate. The impurity peaks are highlighted in colors.

-The minor product was also analyzed through IR. The IR result shows that the structure of the minor product has no alcohol group and a carbonyl group which agree with the structure of α,β-unsaturated ketone.



<u>Figure 10</u>. IR spectrum of α,β-unsaturated ketone (in red) compared to IR spectrum of Aldol product (in black)

-So far we have successfully replicated the published Aldol reaction. We obtained similar result as the one reported. In the next phase of my research, I carried the same reaction using p-Anisaldehyde in place of 4-Nitrobenzaldehyde. The structure of these two are opposite as 4-Nitrobenzaldehyde has an Electron withdrawing group while p-Anisaldehyde has an Electron donating one.

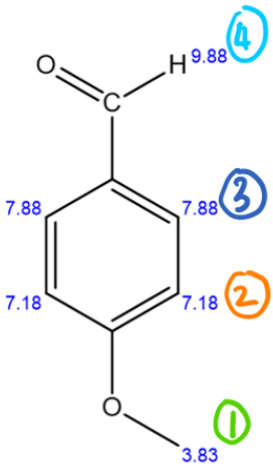


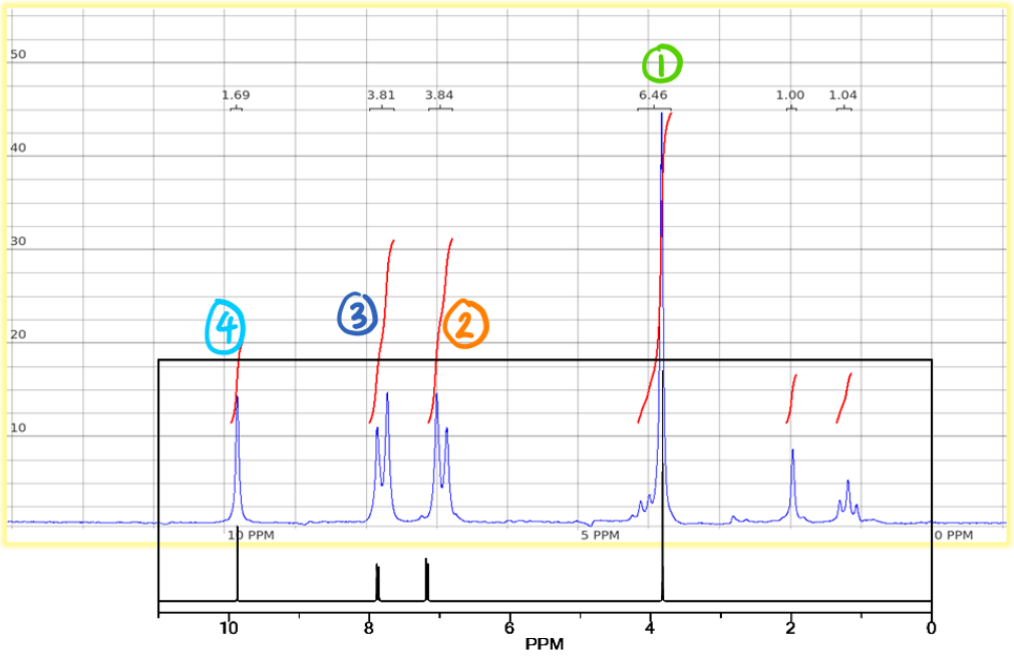
4-Nitrobenzaldehyde	p-Anisaldehyde

-The bottle of p-Anisaldehyde was old; therefore, it was contaminated as the TLC result below shows. The contaminated one was then purified using Column Chromatography to get pure p-Anisaldehyde. NMR spectrum of the purified p-Anisaldehyde was also taken. All the characteristic peaks match with those in suggested by the computer generated spectrum.

<u>Figure 11.</u> TLC plate of contaminated p-Anisaldehyde on the left corner.

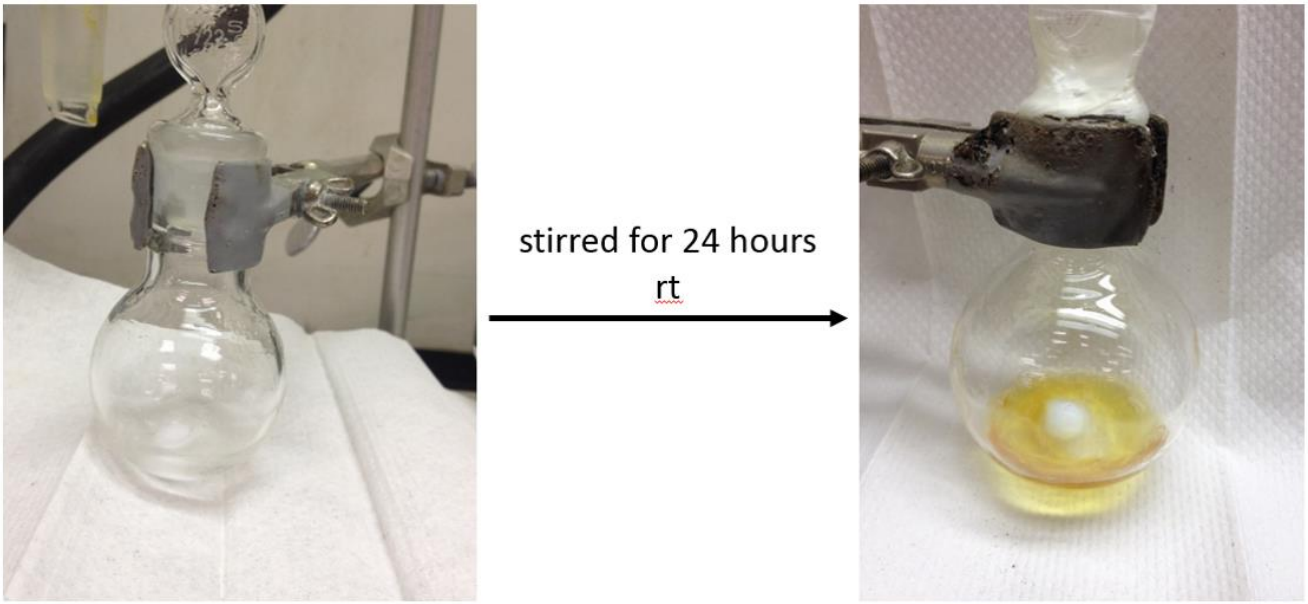
Purified p-Anisaldehyde by Column Chromatography





<u>Figure 12.</u> NMR spectrum of purified p-Anisaldehyde (yellow lining) overlaid with the theoretical NMR spectrum generated by computer software.

-The reaction using p-Anisaldehyde was also run under the similar condition with the previous reaction. Reaction work-ups were carried out in the same way, and there was only one product obtained using p-Anisaldehyde. NMR and IR spectra were also obtained to analyze the structure of the exclusive product. The NMR spectrum of the exclusive product matches with the α,β-unsaturated ketone structure, not the Aldol product as we expected. There were 3 unmatched peaks in the spectrum which comes from the solvent system used in Column Chromatography Petane: Ethyl Acetate (3:1)



<u>Figure 13.</u> Left: The mixture of Amino acid L-Proline, DMSO, Acetone and p-Anisaldehyde at the beginning stage of the reaction. Right: The mixture after 24 hours of stirring under room temperature.

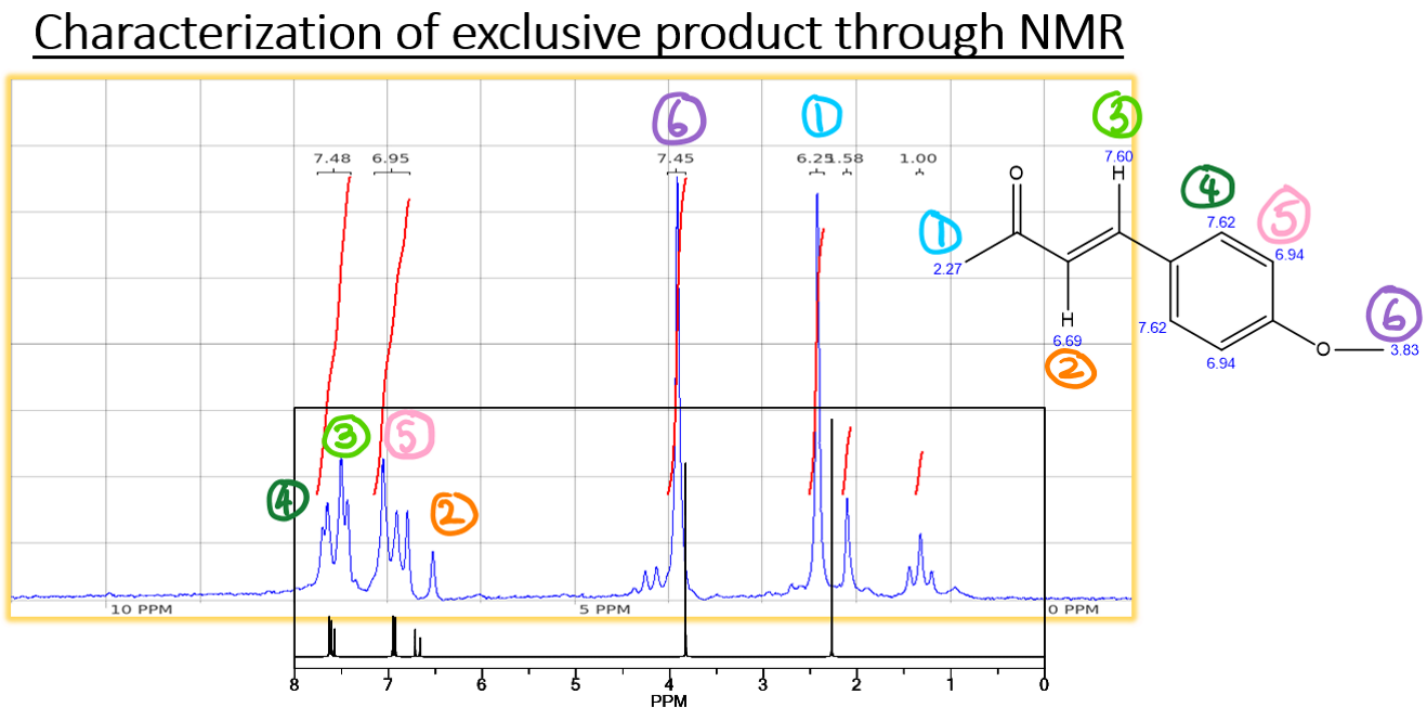


Figure 14. NMR spectrum of exclusive product (yellow lining) overlaid with the theoretical NMR spectrum generated by computer software

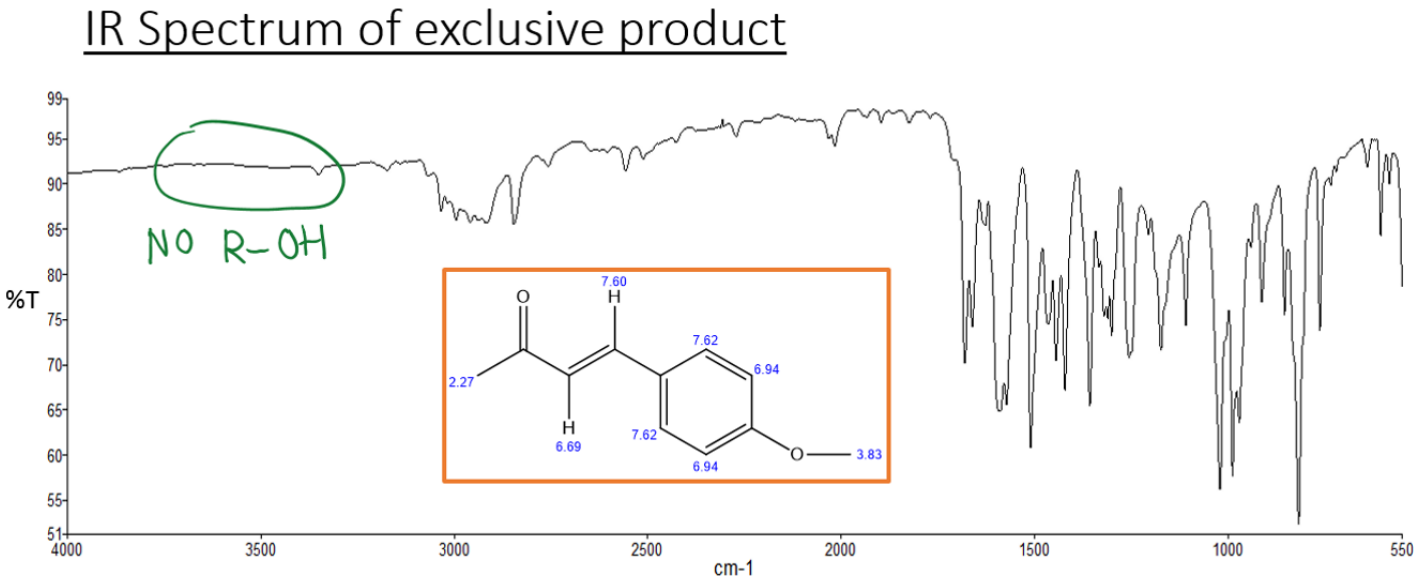
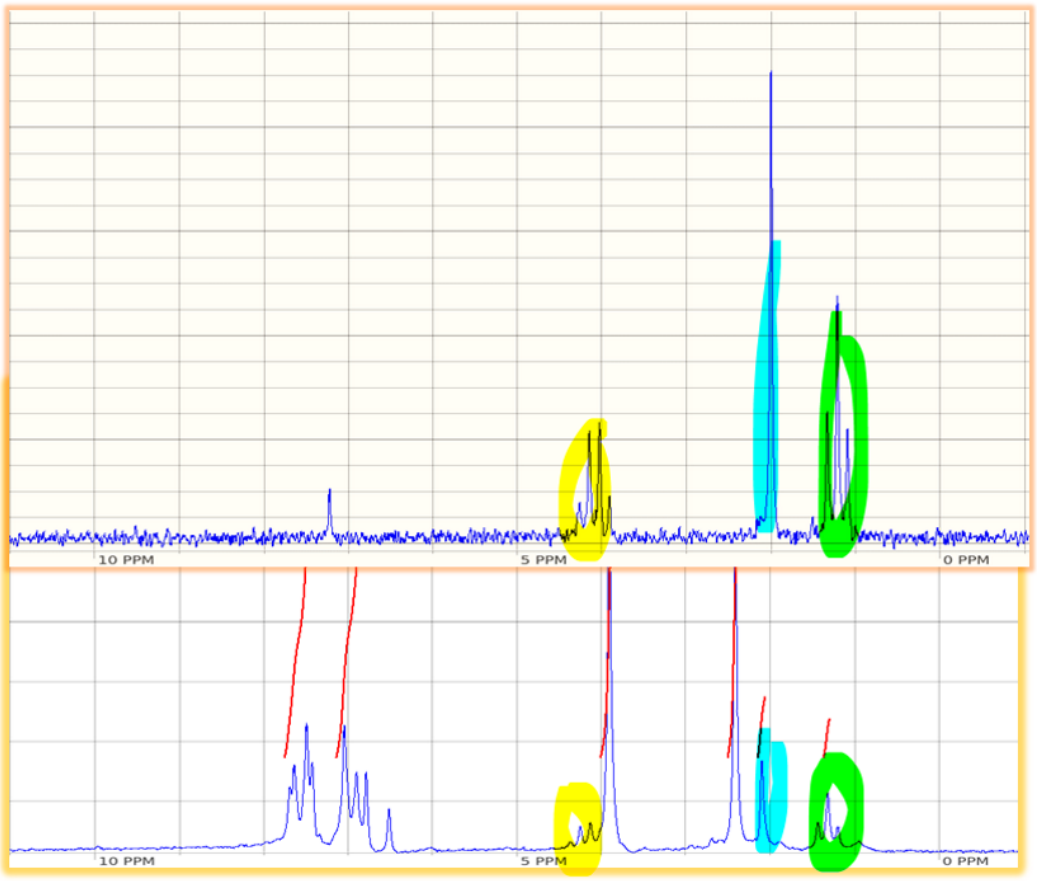


Figure 15. IR spectrum of the exclusive product in next phase of research

Analyze of Impurities peaks in exclusive product's spectrum



Firgure 16. Top: NMR spectrum Ethyl Acetate. Bottom: NMR spectrum of the exclusive product (α,β-unsaturated ketone). The characteristic peaks which do not match with the computer generated peaks come from the solvent Ethyl acetate. The impurity peaks are highlighted in colors.

Conclusion:

In summary, we have replicate successful the asymmetric Aldol reaction in which amino acid Proline was found to be the first example of amine-based asymmetric class I aldolase mimics. We got 2 products: aldol and α,β -unsaturated ketone product. Our percent yield of asymmetric aldol product was 70% which is comparable to the published value of 68%. The yield of α,β-unsaturated ketone was 10.5%. Things that make this reaction stand out were because Proline is nontoxic, inexpensive which mean the reaction could potentially run on industrial scale. The reason also does not require any special condition as it runs at room temperature. One important point is that with a small amount of Proline as catalyst (30 mol %), the reaction proceeds through enamine-based mechanism to form stereoselective product without the prior requirement to modify the carbonyl substrates. Further in the project, we explored the same aldol reaction using p-Anisaldehyde in place of 4-nitrobenzaldehyde. Only one product was obtained, and that exclusive product was α,β -unsaturated ketone instead of the expected asymmetric aldol product. More research needed to be done in the future such as

using other amino acids as catalysts and using a variety of ketones and aldehydes to learn more about the amine-based asymmetric class I aldolase mimics topic.

Acknowlegments:

I would like to thank all of the people listed below. This project could not been done without your support.

- **Dr. Phalguni Ghosh -I have learnt a lot from Dr. Ghosh. He is a very dedicated professor who was always there to guide me and other students through the entire research project.**
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- Dr. Brian Lavey
- Dr. Parag Muley
- MCC Chemistry department
- Bristol-Meyers Squibb
- Chemistry Lab Staff
- Peer Students

References:

(1) (a) March, J. J.; Lebherz, H. G. TIBS 1992, 17, 110. (b) Rutter, W. J. Fed. Proc. Am. Soc. Exp. Biol. 1964, 23, 1248. (c) Lai, C. Y.; Nakai, N.; Chang, D. Science 1974, 183, 1204. (d) Morris, A. J.; Tolan, D. R.; Biochemistry 1994, 33, 12291.

(2) Fessner, W.-D.; Schneider, A.; Held, H.; Sinerius, G.; Walter, C.; Hixon, M.; Schloss, J. D. Angew. Chem., Int. Ed. Engl. 1996, 35, 2219-2221. Phosphoenolpyruvate aldolases use a preformed enolate, phosphoenolpyruvate, to accomplish aldol addition reactions. For studies of this and other aldolase enzymes in organic synthesis see: Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. Chem. ReV. 1996, 96, 443-473

(3) (a) Nakagawa, M.; Nakao, H.; Watanabe, K.-I. Chem. Lett. 1985, 391394. (b) Yamada, Y.; Watanabe, K.-I.; Yasuda, H. Utsunomiya Daigaku Kyoikugakubu Kiyo, Dai-2-bu 1989, 39, 25-31.

(4) (a) Yamada, Y. M. A.; Yoshikawa, N.; Sasai, H.; Shibasaki, M. Angew. Chem., Int. Ed. Engl. 1997, 36, 1871. (b) Yamada, Y. M. A.; Shibasaki, M. Tetrahedron Lett. 1998, 39, 5561. (c) Yoshikawa, N.; Yamada, Y. M. A.; Das, J.; Sasai, H.; Shibasaki, M. J. Am. Chem. Soc. 1999, 121, 4168-4178.

(5) For aldol and retro aldol reactions that are catalyzed by achiral primary amines, see: (a) Pollack, R. M.; Ritterstein, S. J. Am. Chem. Soc. 1972, 94, 5064-5069. (b) Koshechkina, L. P.; Mel'nichenko, I. V. Ukr. Khim. Zh. 1974, 40, 172-174.