**Title:** The synthesis of Chloramphenicol Using L-Proline as the Catalyst

By

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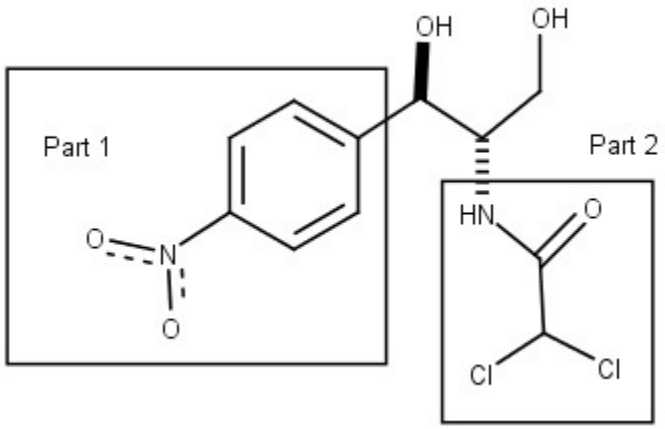
# Chloramphenicol




Molecular Formula: $C_{11}H_{12}Cl_2N_2O_5$

Molecular Weight: 323.13 g/mol

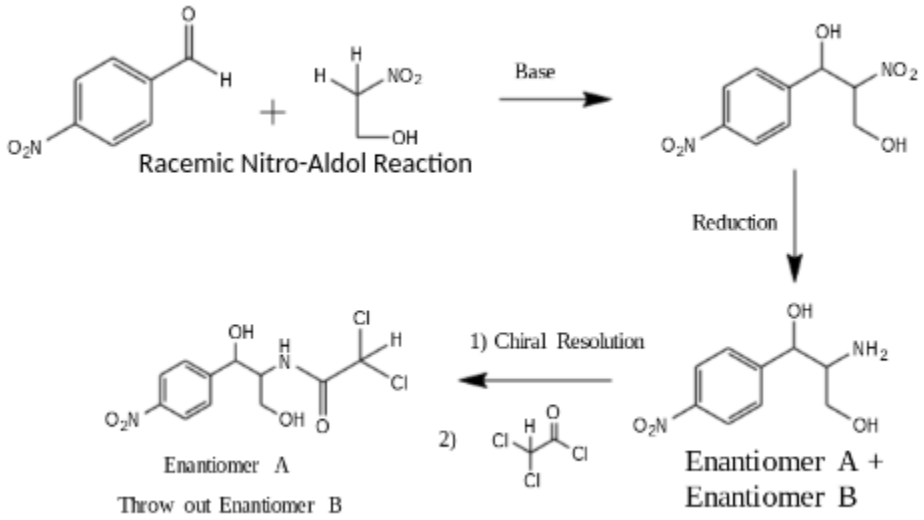
IUPAC Name: 2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide

Chloramphenicol is a broad-spectrum antibiotic. It was discovered in 1947 after being isolated from *Streptomyces venequelae*. A team of scientists at Parke-Davis including Mildred Rebstock published their identification of the chemical structure and their synthesis in 1949. Afterwords, chloramphenicol became the first artificially made antibiotic instead of extracted from a micro-organism.

Chloramphenicol is a bacteriostatic by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. Mechanism: chloramphenicol diffuses through the bacterial cell wall and reversibly binds to the bacterial 50S ribosomal subunit. The binding interferes with peptidyl transferase activity, thereby

prevents transfer of amino acids to the growing peptide chains and blocks peptide bond formation. As a result, bacterial protein synthesis is blocked and delay bacterial cell proliferation.

Industrial synthesis of Chloramphenicol by a nitro-aldol reaction.



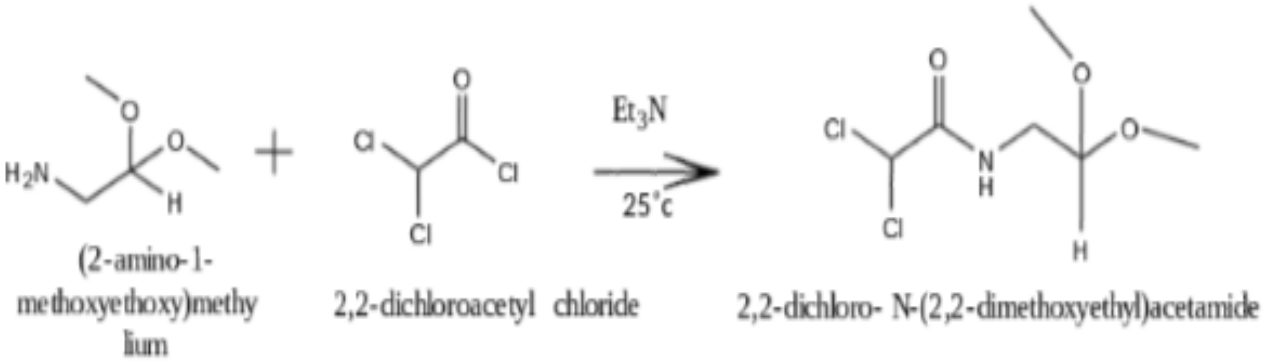

In the large-scale industrial synthesis, 4-Nitrobenzyldehyde reacts with nitro-ethanol in base. Nitro-ethanol is deprotonated by base and becomes an anion, then it attacks the aldehyde's carbonyl carbon. The next step is a reduction of the nitro group to the amine to give both enantiomers as a racemic mixture. The enantiomers are resolved using the method of chiral resolution (chiral separation with a chiral carboxylic acid). The drawback of this method is the loss of half the product as inactive enantiomer.

Dr. Lavey has proposed an alternate way to synthesize chloramphenicol, using a chiral aldol reaction that avoids the resolution step and potentially explosive nitro alkyl compounds.

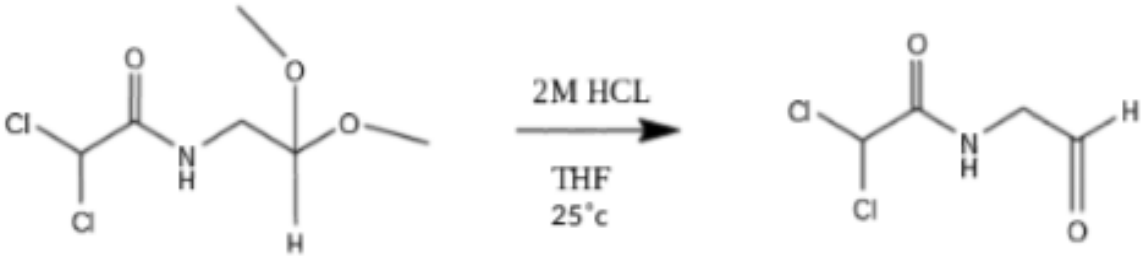
The first step in the proposed synthesis is the acylation of amino group with dichloro acetyl chloride. The acetal is then deprotected to the aldehyde. Proline will then catalyze the

aldol reaction. L-Proline determines the chirality of the products, giving the desired enantiomer as a major product.

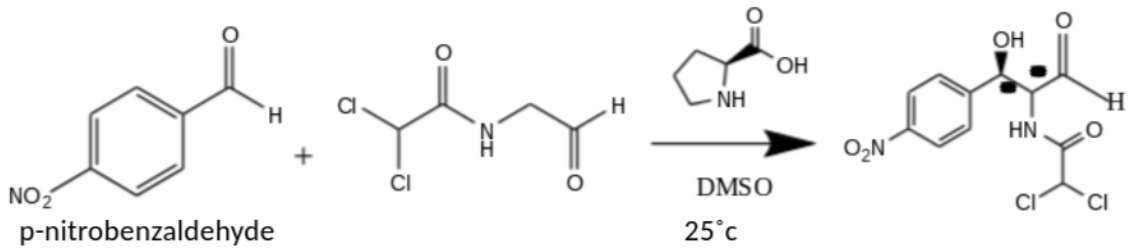
**Step one: Acylation Reaction**



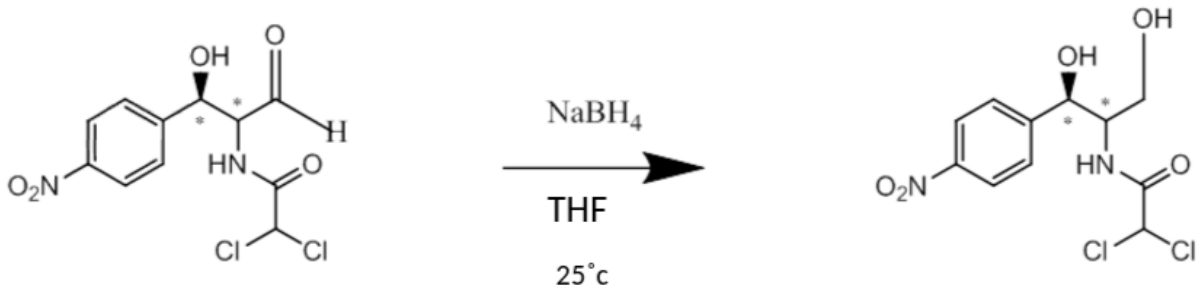

**Step Two: Hydrolysis of di-acetal group**



**Step Three: Aldol reaction catalyzed by L-Proline**




**Step Four: Borohydride Reduction**



# Experimental Procedure

Instrumentation and Materials:

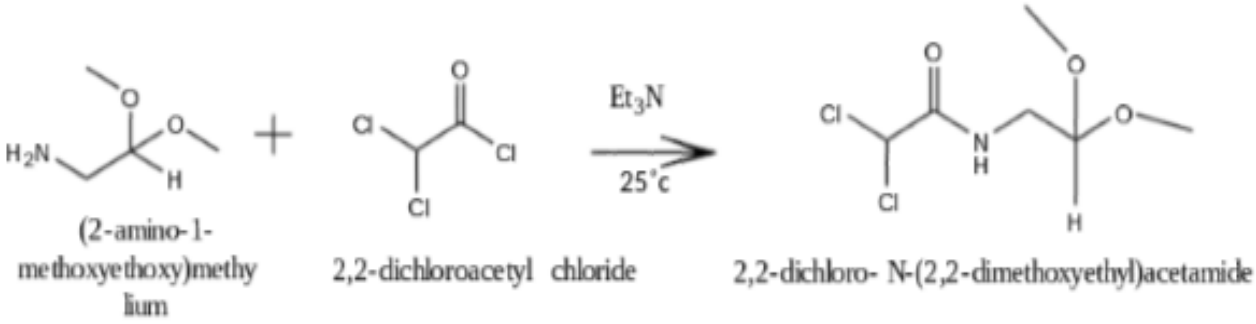
- NMR spectra were recorded using NMRReady$^{TM}$ 60 Nanalysis Corporation.
- $CDCL_3$, Acetonitrile-D3, Methanol-$D_4$ Solvents
- Rotary Evaporator
- Small Column for Flash Chromatography
- TLC Plates, Preparative TLC Plate and Chambers

Chemicals:

- Aminoacetaldehyde Dimethyl Acetal
- Dichloroacetyl chloride
- L-Proline
- P-nitrobenzaldehyde
- Triethylamine
- Sodium Borohydride
- Ethyl Acetate
- N-hexanes
- Tetrahydrofuran (THF)
- 2M HCl
- DMSO
- Methanol

Trial #1

Step one: Acylation Reaction




(2-amino-1-methoxyethoxy)methylium

2,2-dichloroacetyl chloride

2,2-dichloro- N-(2,2-dimethoxyethyl)acetamide

Ratio is 1:1:1, calculations based on MW, Aminoacetaldehyde was used as a limiting reactant

Aminoacetaldehyde – 1.2 ml, Dichloroacetyl – 2 ml, Triethylamine – 1 ml, THF- 10 ml

- The following reagents were mixed in a round-bottomed flask, and stirred with a micro stir bar.
- The flask was covered with a stopper and left overnight to stir.
- A workup procedure was performed using separatory funnel:

  15 ml of water

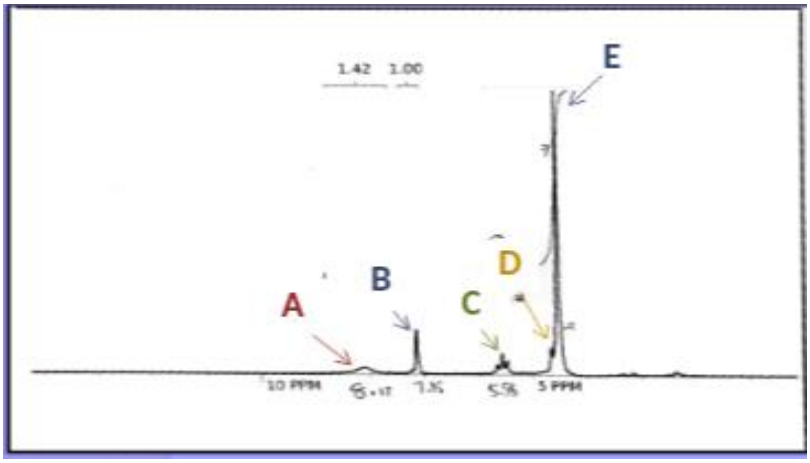
  2x 20 ml Ethyl Acetate

  2x 15 ml 2M $NH_4Cl$

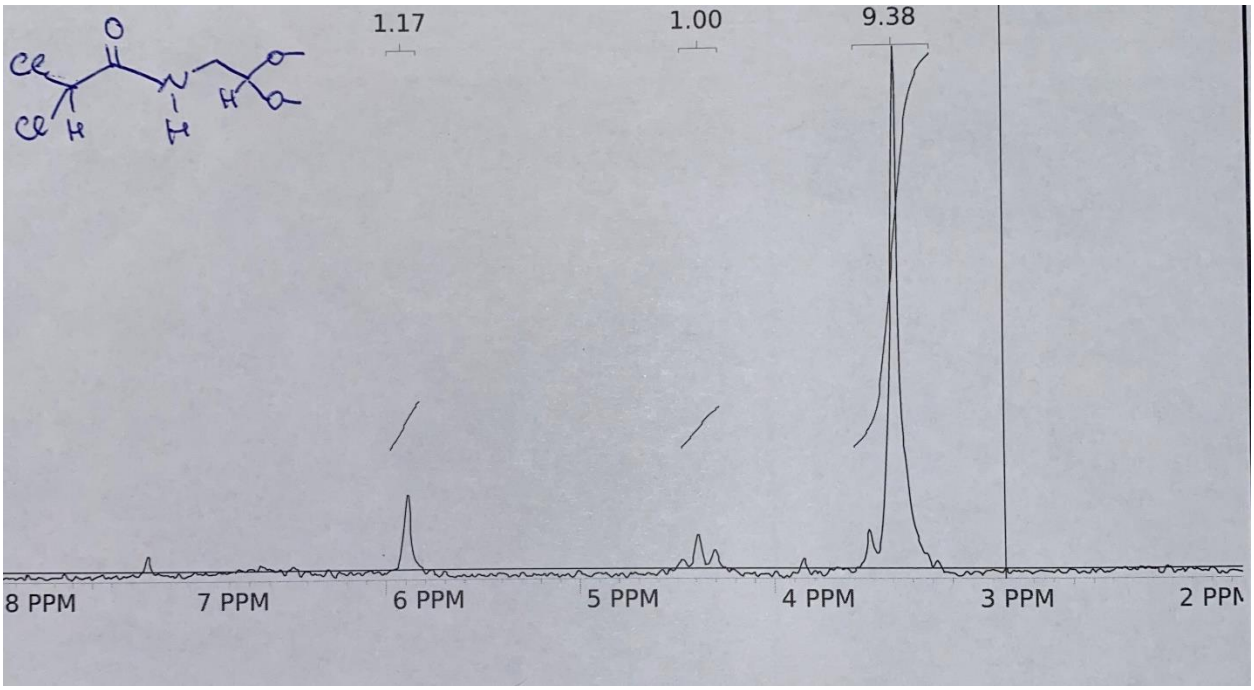
  2x 15 ml water, followed by wash with Brine solution 1x10 ml
- The solution was filtered. The solvent was evaporated using a rotary evaporator.

- The obtained product was set on vacuum pump overnight, to eliminate the remaining solvent. Obtained product was checked by $^{1}$HNMR in $CDCl_3$ solvent.

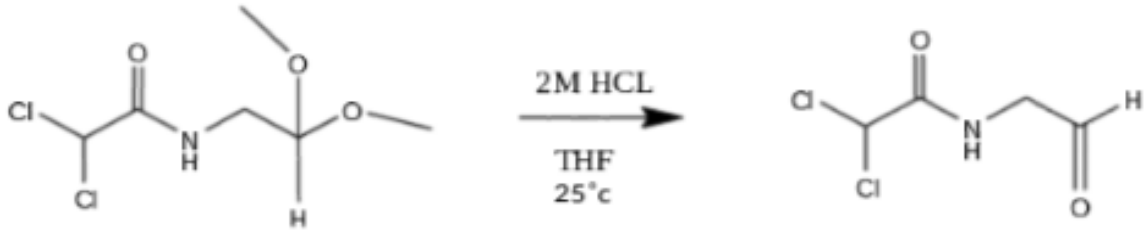
Predicted NMR spectrum by Chemdraw, $CDCl_3$ solvent.




NMR spectrum of the obtained product.



Step Two: Hydrolysis of di-acetal group




Ratio is 1:1

Based on previous experience, the following reaction should be performed with freshly made 2M HCl solution. When older 2M HCl solutions are used, the reaction goes slowly or does not go to completion.

- The obtained product from step 1 was dissolved in 2 mL of HCl and 2 mL of THF
- The reaction was stirred at room temperature overnight.

(Note: the second reaction takes about 2.5h).

- The obtained mixture solution was transferred into a separatory funnel.
- $Na_2CO_3$ was added to neutralize the remaining of acid. Solid $Na_2CO_3$ or saturated solution can be used. The HCl neutralization was checked using pH paper, 8 pH when it turned a green color.
- The following workup procedure was performed:

  Ethyl acetate (20 ml) was added. The layers were separated. The organic top layer was collected and put aside.

The aqueous layer was extracted with 3x 20 ml of Ethel acetate.

Note: bicarbonate in a solid or saturated solution form can create an emulsion. To eliminate this problem put a clean Pasteur pipette in the emulsion and bubble some air through it by squeezing the bulb.

The organic layer was combined with the extracted layers. The combined layer was washed with 1x10 ml of brine solution.

Drying agent ($NaSO_4$) was added to the solution and for 15 min.

- The obtained solution was filtered and set for evaporation on the rotary evaporator.
- The reaction product was checked by TLC plate and $^1$HNMR.

Note: the compound is not UV active. An iodine chamber was used to see the TLC results.

- Column Chromatography was performed to isolate the pure aldehyde compound for the next reaction with Nitrobenzaldehyde catalyzed by L-proline;

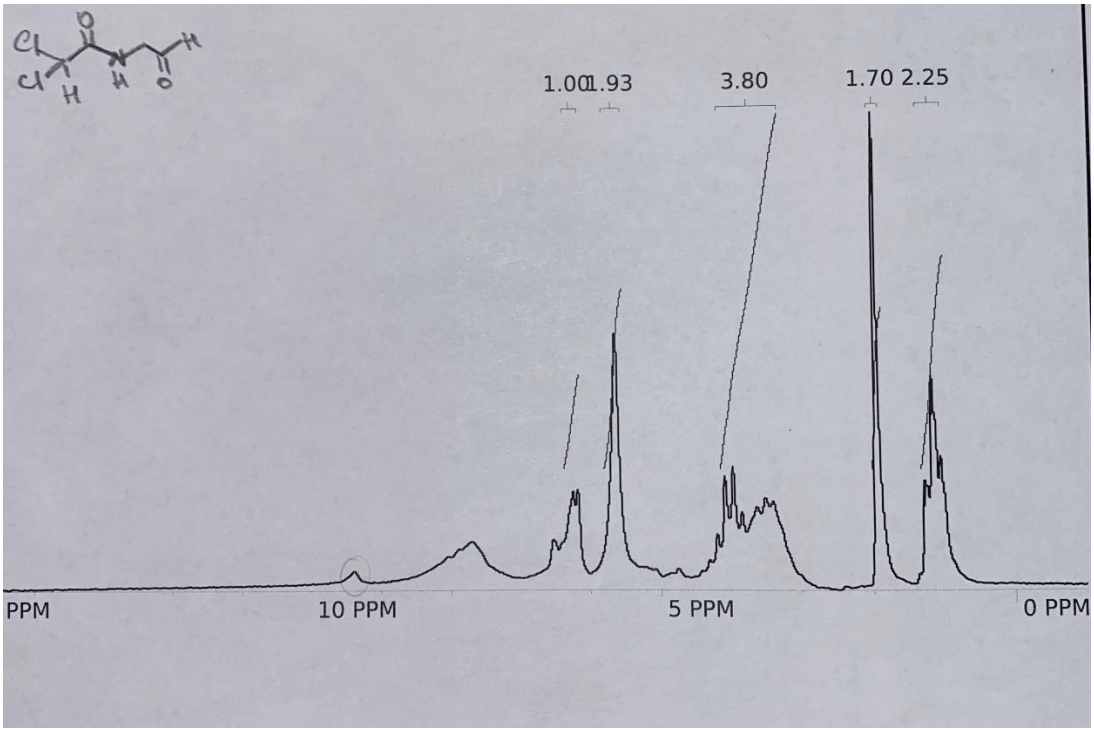
  The used solvent system was 3:7 Ethyl acetate and Hexanes.

  Fractions 5-17 were collected and set for evaporation at $45^0$C. A yellow oil was obtained and set on the vacuum pump overnight.

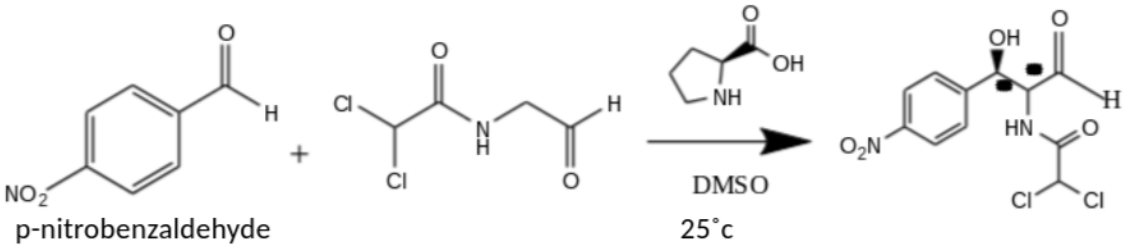
Obtained product weight 0.52 g

Observation: The compound changed the color and consistency from yellow oil to a dark brown sticky material. A small amount was taken for TLC testing. A precipitate formed after dissolving in Ethyl acetate. Dr. Ghosh suggested that the isolated aldehyde product was left on the vacuum pump for too long and got oxidized.

- NMR spectrum was taken using Acetonitrile-$D_3$ solvent. It showed the aldehyde peak around 9.3 ppm.




Step Three: Aldol reaction catalyzed by L-Proline




Ratio 1:1:1

- DMSO (4 ml) was added to the reaction flask with a product from the previous step.
- p-Nitrobenzaldehyde (1 equivalant) and L-Proline (1 equivalant) were added.
- The reaction was stirred overnight.
- Workup was done by adding saturated ammonium chloride solution (1-1.2 ml per 1 ml of DMSO):

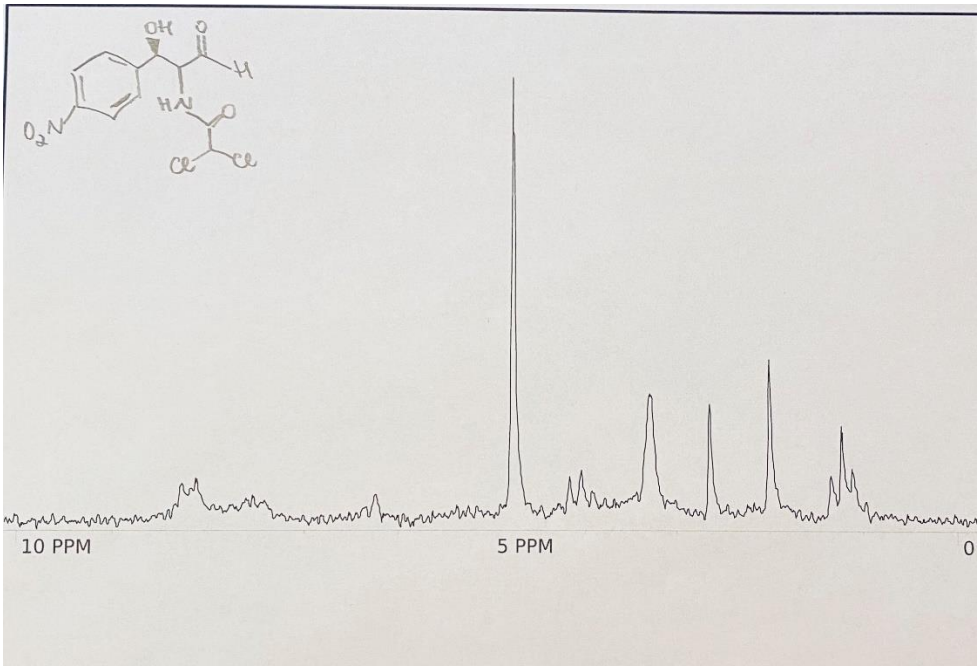
  The solution was extracted with Ethyl acetate, then washed with brine solution.

  The organic layer was dried ($NaSO_4$), filtered, and evaporated.

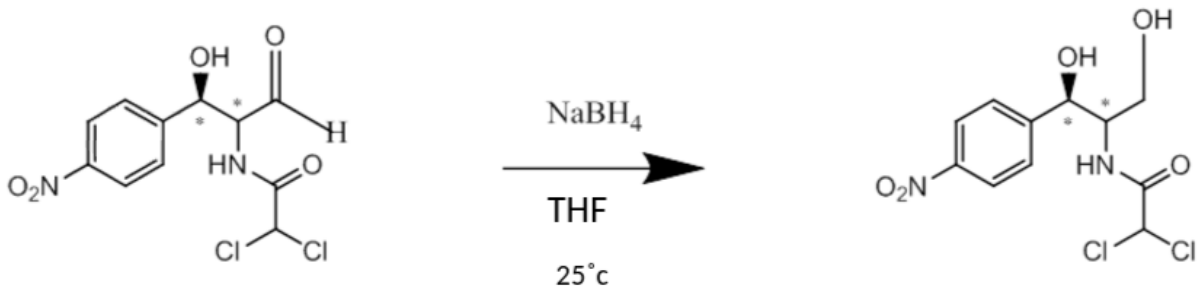
Note: Saturated ammonium chloride solution should be added dropwise, using a Pasteur pipette.

The product compound were UV active. According to the previous experiment performed by other students, the aldol product should be soluble in $CDCl_3$ solvent and move a TLC plate in 3:7 (Ethyl acetate/hexanes) as a mobile phase. The obtained aldol product did not respond to that conditions. The NMR of the product was taken using Methanol-$D_4$. It clearly showed the expected products. Dr. Lavey and I tried to modify the solvent system by adding methanol, in few different percent variations, nothing worked out. Also, two-dimensional TLC showed some evidence of decomposition of a product. The solution was to move on to the next step – reduction by borohydride and do the column chromatography for purification then.

NMR spectrum using Methanol-$D_4$ solvent.




Step Four: Borohydride Reduction




In reaction flask containing 0.52 g of aldol product, was added 15 ml of Methanol. A room temperature water bath was placed under the flask. $NaBH_4$ 0.12 g was added, causing bubbling to occur.

- The reaction was set for stirring overnight.
- The reaction solution was set on the rotovap to evaporate the Methanol.
- Ethyl acetate (20 ml) was added and stirred for 5 min.
- Water (7 ml) was added and stirred for 5 min.
- The reaction solution was transferred to a separatory funnel and the workup was done:

  A small amount of water was added to separate the layers.

  Ethyl acetate (20 ml) was added to the aqueous layer. The water layer was drained and collected.

  The combined Ethyl acetate layer was washed with 10 ml of brine solution.

  The organic layer was dried with $NaSO_4$.

  The filtered solution was set on rotary evaporator.

The product was dissolved in a small amount of dichloromethane, and moved up the TLC plate with 3:7 solvent system.

- Column chromatography was performed twice.

Note: the first column did not show good separation results. However, a lot of contamination products stayed on top of the silica. All fractions were collected and the solvent was evaporated. The TLC plate (3:7 Ethyl acetate/hexanes) showed a good separation of compounds.

- Second Colum chromatography was performed by following system

  150 ml 20% Ethyl acetate 80% Hexanes

  100 ml 25% Ethyl acetate 75% Hexanes

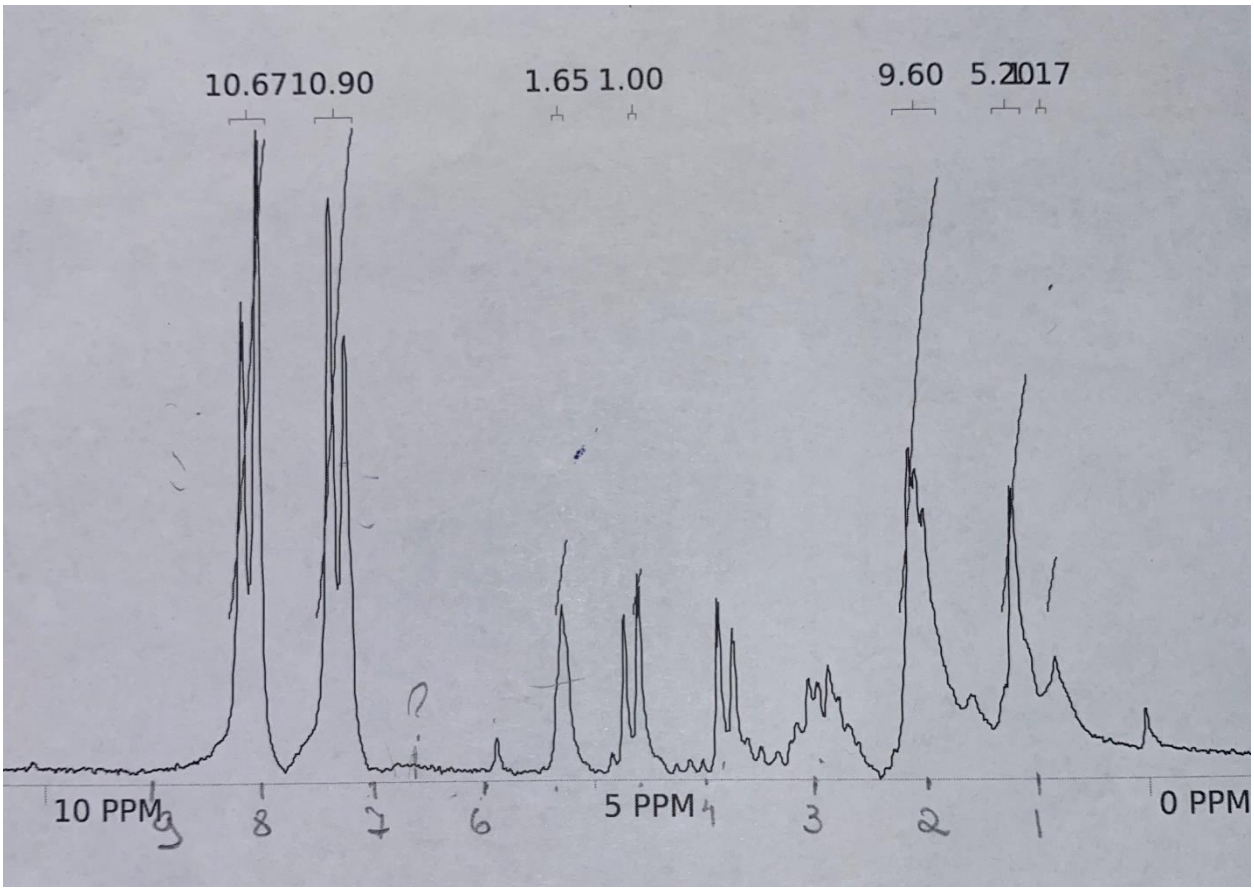
150-200 ml 30% Ethyl acetate 70% Hexanes

It was expected to get two diastereomers of chloramphenicol with $R_f$ values around 0.4, that were separated but close to each other.

Set of fractions 17-24 (80 mg) and 29-34 (30 mg) were collected, evaporated and tested on NMR spectra.

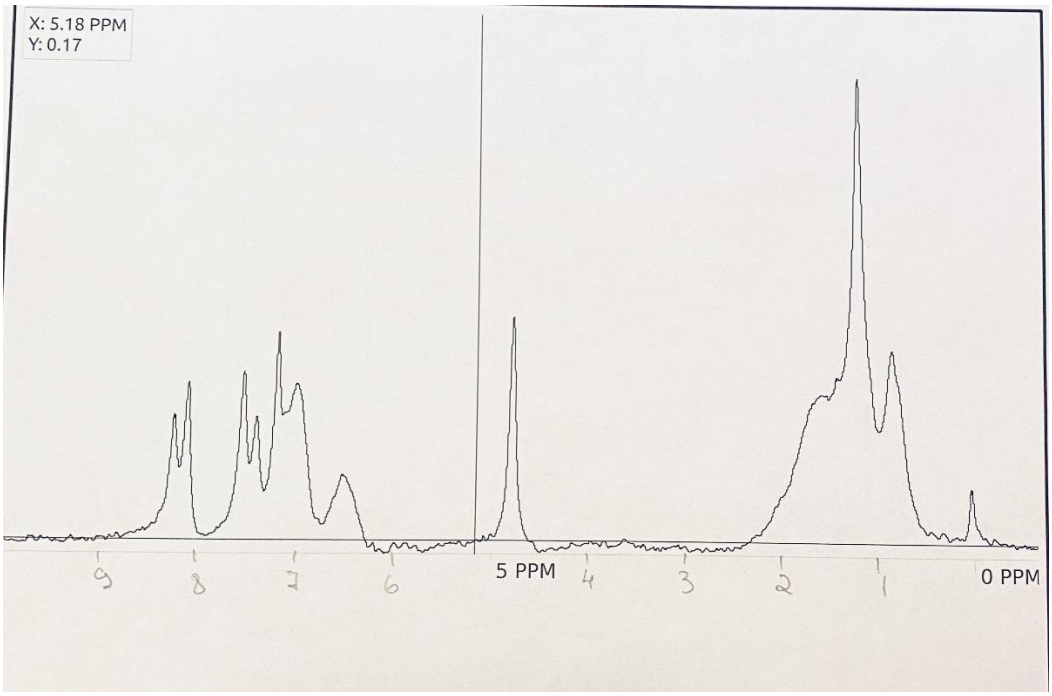
First set 17-24 showed peaks that responded to para substituted aromatic ring regions.

NMR using $CDCl_3$ solvent




Note: Fractions 29-34 contained 2 products.

Preparative TLC plate separation helped to separate the 2 compounds, by using a more polar ratio 1:1 of the same solvent system (Ethyl acetate/hexanes). Both obtained compounds were checked by NMR. Compound A showed picks that responded to para substituted aromatic ring regions as well.



Trial #2

It was decided to repeat the synthesis from step 3. As a starting material I used Dr. Lavey's product from his previous experiment. The pure aldehyde after hydrolysis with 2M HCl. Since the aldehyde compound has been stored in the fridge for some time, the compound was purified by the column chromatography. Almost all fractions were collected.

The plan was to set up the aldol reaction and work it up immediately after it's done. Once the crude product was in hand, it would be reduced with $NaBH_4$ without further purification. Unfortunately, after 3.5 hours the aldol reaction still was not completely done. The TLC plate showed 3 UV active spots including unreacted nitrobenzaldehyde. For reasons of time, it was decided to move on with the Borohydride reaction step, even before the aldol reaction had completely finished.

Ratio kept the same 1:1:1

Starting aldehyde 0.9 g

L-Proline 0.7 g

Nitrobenzaldehyde 0.9 g

DMSO 6.5 ml

First, the starting aldehyde was dissolved in DMSO. p-Nitrobenzaldehyde was added. The reaction solution was stirred for 5-7 min. Catalyst was added after dissolving of the reactants.

- Workup was done, using the same procedure from the previous trial. The solution was treated with 8 mL of saturated ammonium chloride to remove the DMSO and Proline.

- Rotary evaporation and the vacuum pump evaporated the solvent.
- Column chromatography was performed, using the conditions of trial #1.

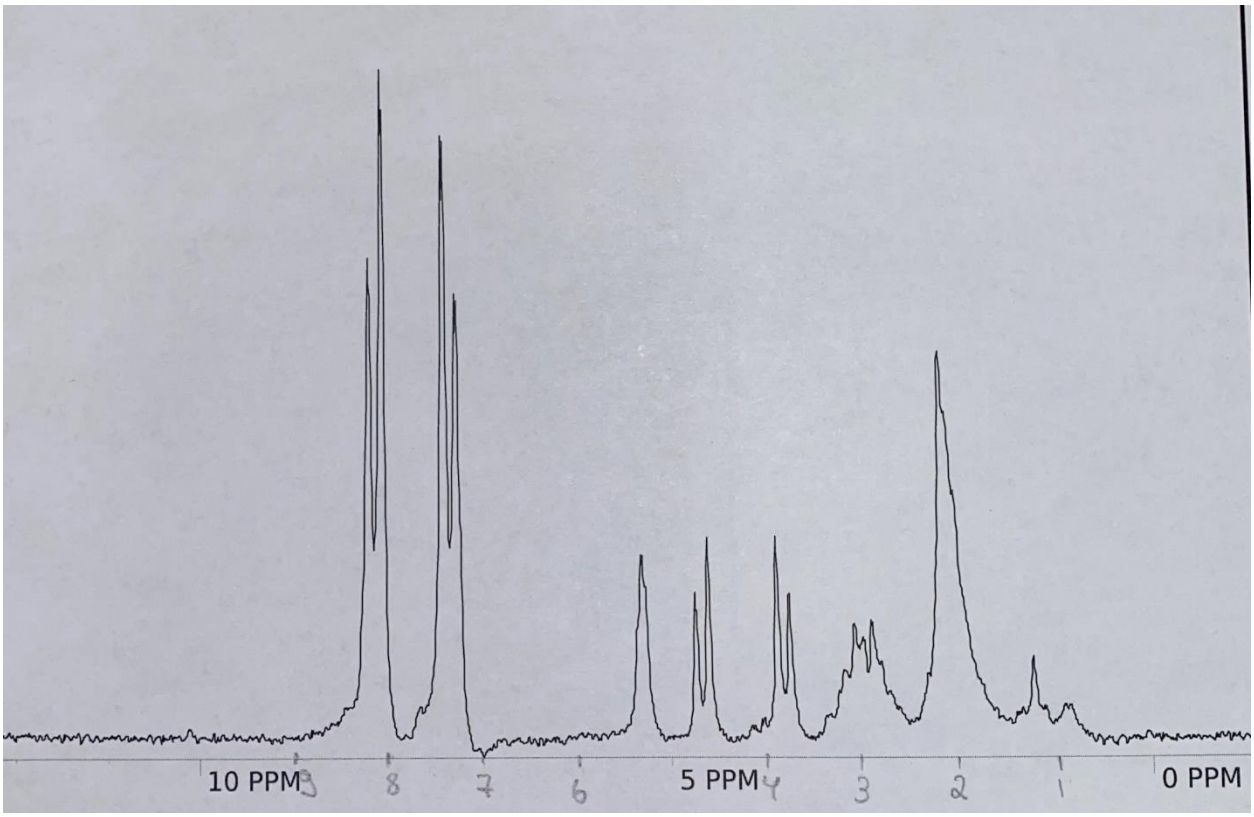
Results:

Set of fractions 9-14 and 21-26 were collected

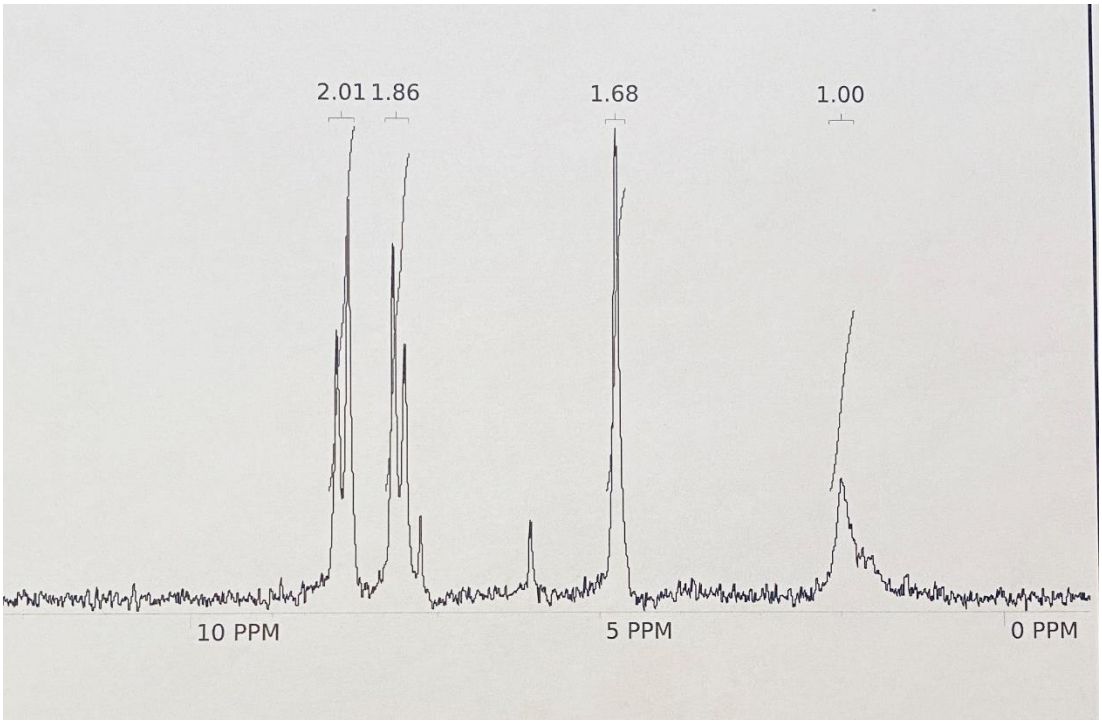
Note: test tubes used for column trial #2 were greater in volume

NMR results are familiar with trial #1 (fractions 17-26)

NMR spectrum of fractions 9-14 showed peaks that responded to para substituted aromatic ring regions.

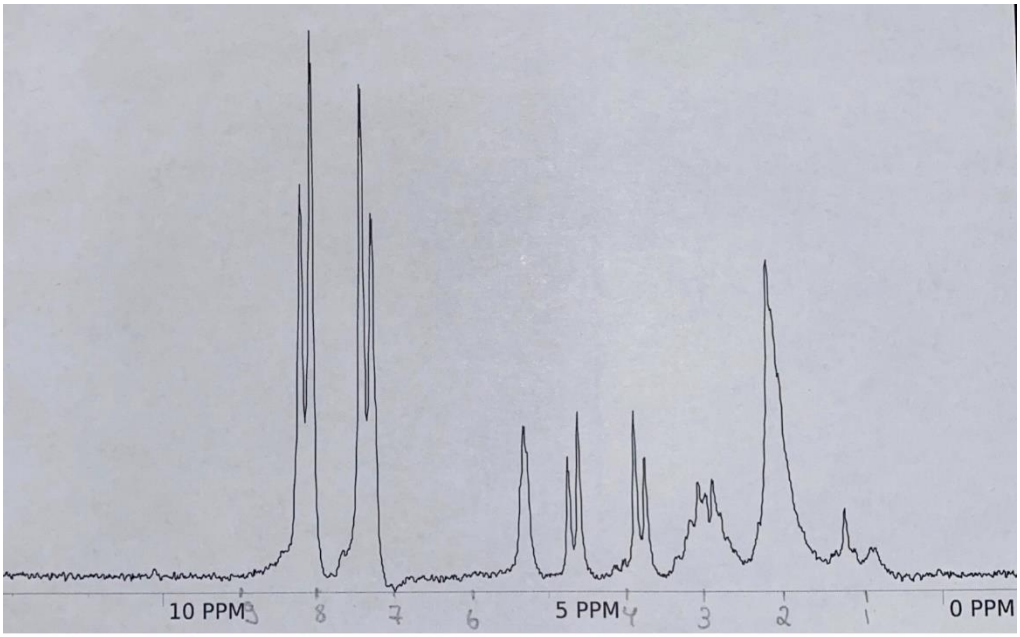


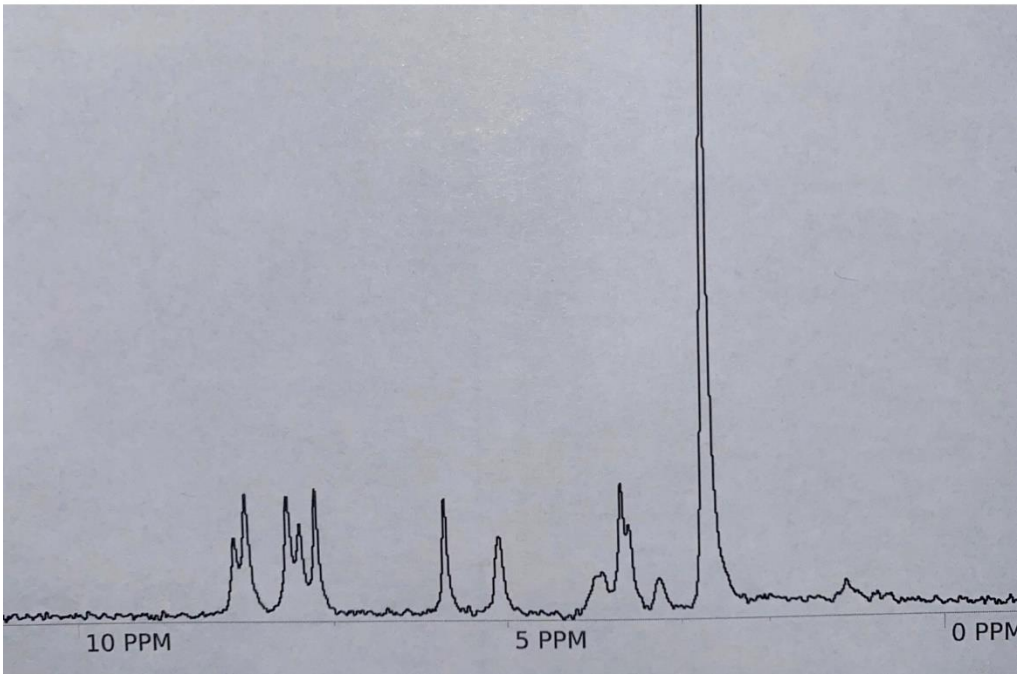
NMR spectrum of fractions 21-26. The spectrum also showed peaks that responded to para substituted aromatic ring regions.




The obtained products from both trials looked like chloramphenicol and responded to conditions from academic articles, such as solubility in $CDCl_3$, light yellow color, $R_f$ values. We obtained a NMR spectrum of an authentic standard of chloramphenicol for comparison. It is difficult to compare the results, because of the poor quality of the NMR Spectrum of the standard. We will try and confirm the Identity of the products using other techniques as well.

NMR spectrum of an authentic Chloramphenicol and NMR spectrum of obtained product from trial #2



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