# ENANTIOMERIC EXCESS DETERMINATION OF PRIMARY AMINES

by

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## 1. Abstract

Certain organic compounds can exist in two variations, known as enantiomers. Such compounds are characterized as being “chiral”. Enantiomers can exist in either R or S configurations, and the relative proportions of one configuration to another can be expressed in terms of something known as enantiomeric excess. When analyzing a particular sample of a compound, determination of the enantiomeric excess is important because enantiomers can display different effects biologically. For example, sometimes an enantiomer can have harmful effects while the other enantiomer has beneficial effects. The types of compounds we are focusing on in this project are known as primary amines. Primary amines play a critical role in various drugs, such as in L-Dopa that is commonly used in the treatment of Parkinson’s disease.[1]

This project sought to develop a method of determining the enantiomeric excess of chiral primary amines. This was to be accomplished by treating the amine with a specific reagent and then analyzing the product of that reaction by means of a technique known as NMR. Synthesis of the reagent has been successful and this reagent was treated with a primary amine to test if it had the ability to determine the enantiomeric excess of the amine.

## 2. Introduction

Complex organic molecules containing carbon can have multiple structures for the same chemical formula, which are called isomers. Isomers can be further labeled as structural isomers or stereoisomers, where the former have different bonding between atoms while the latter have the same bonding between atoms but a distinctive three-dimensional structure. Stereoisomers often have a chiral center, which distinguishes one stereoisomer from another. Chiral centers are places where four different groups are attached to a single carbon. These stereoisomers have subgroups of enantiomers and diastereomers where enantiomers are stereoisomers that are nonsuperimposable mirror images of each other (Fig. 1). An analogy is often used with the left and right hand, they are mirror images of each other but when you place one atop the other they are not the same.[2] Molecules that can exist as a pair of enantiomers are said to be "chiral".

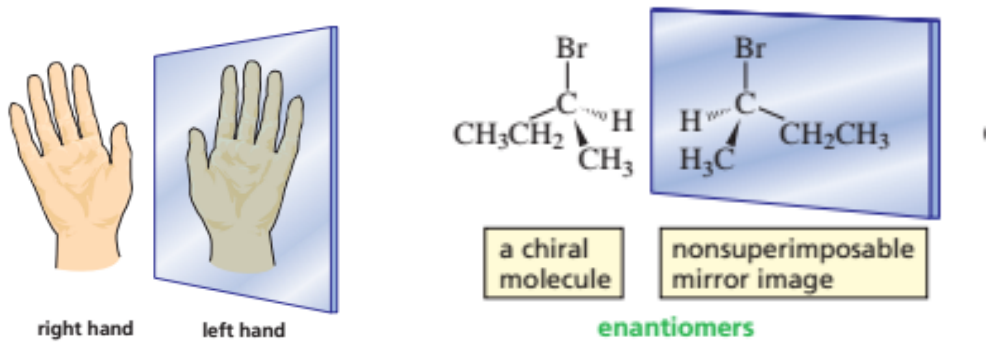


**Figure 1.** An analogous comparison of enantiomers with hands.[2]

What makes enantiomers distinguishable is the positioning of atoms at their chiral centers. A system of nomenclature is utilized to label these enantiomers where the groups attached at a chiral center are ranked and the orientation of these rankings classifies the molecule as an R configuration or an S configuration. When analyzing a particular sample of a compound, determination of the enantiomeric excess is important to determine "how much of an excess of one enantiomer is in the mixture."[2] Enantiomeric excess is calculated via the equation:

$$enantiomeric\ excess\ (ee) = \frac{|R\ configuration - S\ configuration|}{Sum\ of\ R\ and\ S\ configurations} \times 100\%$$

Enantiomeric excess can also be determined via Nuclear Magnetic Resonance (NMR) spectroscopy. Utilizing a chiral auxiliary molecule "that converts the mixture of enantiomers into a diastereoisomeric mixture"[3] a chemical reaction is completed and the solution is analyzed by an NMR spectrometer. The resulting NMR spectrum can be analyzed for the relative amounts of R and S configurations. Knowing a solution's enantiomeric excess is important, especially when the compounds are used in the synthesis of pharmaceuticals. For example, the R configuration of the drug mexiletine is significantly more potent than the S enantiomer.[4] To properly and safely synthesize drugs, it is important to determine the enantiomeric excess of the compounds that they are made up of.

**2.1 Approach**

This project sought to develop a method of determining the ratios for a class of compounds known as primary amines. In the case of L-Dopa the desired enantiomer is in the S configuration, if the R configuration is in excess the patient taking the drug may experience side effects.[1] We began by first synthesizing the reagent. This was accomplished through a series of chemical transformations starting from an available precursor (Fig. 2). This reagent, shown as molecule **5** in Figure 2, was then treated with a primary amine to give molecule **6**, known as a diimine. The NMR spectrum of diimine **6** would be used to determine the enantiomeric excess of

the primary amine used in its preparation.

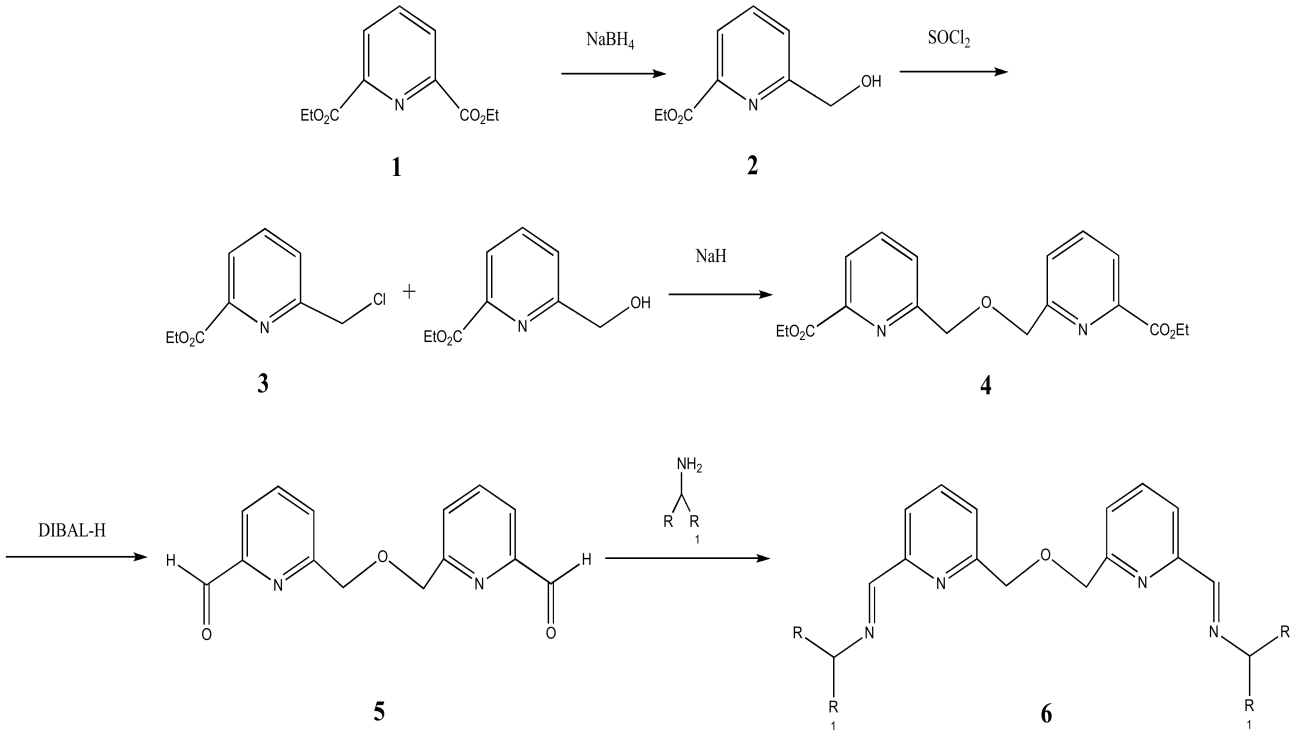


**Figure 2.** Chemical synthesis of the reagent.

The reagent selected for this project was specifically chosen due to characteristics that make its use efficient in the determination of enantiomeric excess. Due to the nature of the molecule **5** seen in Figure 2, the centers where the aldehydes lay are not close in space. But once successfully synthesized, the diimine molecule **6** can be treated with an appropriate metal, causing the molecule to chelate as seen in Figure 3. The formed diimine can undergo chelation due to its ability to bind in multiple sites, namely at the nitrogen atoms present in the molecule, to the metal. Chelation of the molecule will bring the two enantiomeric centers into close proximity so their differences will be exaggerated, making it easier to distinguish them by NMR.

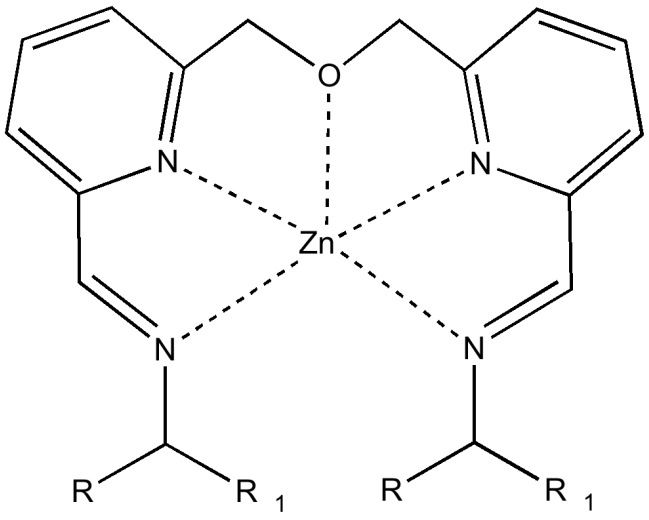


**Figure 3.** An example of the diimine molecule **6** chelated with zinc.

**3. Experimental Methods**

The initial step in the chemical synthesis of the reagent is referred to as a selective reduction of molecule **1**, meaning only one of the carbon groups attached to the six membered amine ring is reduced. After the formation of molecule **2**, the attached OH group is converted to chlorine in the presence of $SOCl_2$. These two starting reagents are then condensed to form molecule **4**, which undergoes a reduction using diisobutylaluminium hydride (DIBAL-H). DIBAL-H is a strong reducing agent in this reaction and due to its nature allows molecule **4** to be reduced to molecule **5** without undergoing further reduction. Finally, molecule **5** is treated with a primary amine to form the diimine **6**.

*Synthesis of **2***: Molecule **1** (1.03 g, 4.6 mmol) was dissolved in 5 mL absolute ethanol. $NaBH_4$ (0.12 g, 3.16 mmol) was slowly added causing gas evolution and an orange color change. The solution was heated to reflux under $N_2$ for 6 hours. The solution was removed from the oil bath, and allowed to stir at room temperature for an additional 17 hours. The solution was diluted with 25 mL distilled water and extracted by dichloromethane (2 x 25 mL). The combined dichloromethane layers were washed with distilled water (50 mL) and saturated sodium chloride

(5 mL). The combined dichloromethane layers were then dried by magnesium sulfate, which was then removed by gravity filtration. The solvent was removed from the filtrate by rotary evaporation under reduced pressure. The white powdery sample was then placed under high vacuum. The percent yield was 55%. This same reaction was also done where the solution was not under $N_2$ during reflux, and had a percent yield of 49%. $^{1}$H NMR ($CDCl_3$) δ: 7.99 (d, 1H), 7.8 (t, 1H), 7.5 (d, 1H), 4.85 (s, 2H), 4.4 (q, 2H), 1.4 (m, 3H). $^{13}$C NMR ($CDCl_3$) δ: 165.0, 160.5, 147.1, 137.6, 123.9, 123.6, 64.5, 61.9.

*Synthesis of **3**:* Molecule **2** (0.46 g, 2.5 mmol) was dissolved in approximately 1 mL $SOCl_2$. The solution was stirred at 0 °C for several hours and then stirred at room temperature for 6 days. Excess $SOCl_2$ was distilled off under reduced pressure. The solution was then diluted with 30 mL dichloromethane, washed with saturated sodium bicarbonate (2 x 25 mL) and saturated sodium chloride (2 x 5 mL). The solution was then dried by magnesium sulfate, which was then removed by gravity filtration. The solvent was removed from the filtrate by rotary evaporation under reduced pressure. The clear, orange liquid sample was then placed under high vacuum. The percent yield was 71%. $^{1}$H NMR ($CDCl_3$) δ: 8.0 (d, 1H), 7.8 (t, 1H), 7.7 (d, 1H), 4.7 (s, 2H), 4.51-4.45 (q, 2H), 1.4 (t, 3H). $^{13}$C NMR ($CDCl_3$) δ: 164.3, 157.0, 156.8, 147.2, 139.6, 139.8, 126.6, 124.5, 62.2, 45.8.

*Synthesis of **4**:* Molecule **3** (0.24 g, 1.2 mmol) was dissolved in approximately 5 mL dry THF. Molecule **2**, (0.21 g, 1.2 mmol) was added followed by the addition of 60% NaH (0.10 g, 4.2 mmol). Upon addition of the 60% NaH, there was gas evolution as well as a dark brown color change. The solution was refluxed under $N_2$ for 22 hours. The solution was diluted with 25 mL diethyl ether and washed with distilled water (2 x 25 mL) and saturated sodium chloride (5 mL). The solution was then dried by magnesium sulfate, which was then removed by gravity filtration.

The solvent was removed from the filtrate by rotary evaporation under reduced pressure. The dark brown liquid sample was then placed under high vacuum. The percent yield was 43%. $^{1}$H NMR ($CDCl_3$) δ: 8.0 (t, 1H), 7.8 (m, 1H), 7.7 (m, 1H), 4.9 (s, 2H), 4.5 (q, 2H), 1.4 (t, 3H).$^{13}$C NMR ($CDCl_3$) δ: 165.1, 158.8, 147.7, 137.6, 124.4, 123.9, 61.9, 29.7, 25.6.

*Synthesis of **5**:* Molecule **4** (0.10 g, 0.30 mmol) was dissolved in approximately 5 mL dry dichloromethane. DIBAL-H in heptane (0.9 mL, 1M) was slowly added to the solution and the solution was stirred at -78°C under $N_2$ for 2.5 hours. Methanol (1 mL) was added to the solution followed by the addition of 1 M NaOH (2.5 mL) at -78°C. The solution was brought back to room temperature and stirred for 16 hours. The solution was diluted with 25 mL dicholoromethane and washed with distilled water (25 mL) and saturated sodium chloride (5 mL). The solution was then dried by magnesium sulfate, which was then removed by gravity filtration. The solvent was removed from the filtrate by rotary evaporation under reduced pressure. The orange liquid sample was then placed under high vacuum. The percent yield was 90%. $^{1}$H NMR ($CDCl_3$) δ: 10.06 (s, 1H), 8.2 (t, 1H), 8.0 (m, 1H), 7.8 (m, 1H), 4.9 (s, 2H). $^{13}$C NMR ($CDCl_3$) δ: 193.2, 158.8, 152.2, 137.8, 125.7, 120.6, 29.7.

*Synthesis of **6**:* (±)-α-methylbenzylamine (0.16 g, 1.3 mmol) was dissolved in approximately 10 mL toluene. Molecule **5** was then added to the solution. A Dean-Stark trap was filled with toluene and attached to the reaction flask, and the solution refluxed for 5 hours. The solution was diluted with 25 mL diethyl ether and washed with saturated sodium bicarbonate (1x25 mL). The solution was then dried by magnesium sulfate, which was then removed by gravity filtration. The solvent was removed from the filtrate by rotary evaporation under reduced pressure. The orange liquid sample was then placed under high vacuum. The percent yield was 57%. $^{1}$H NMR

($CDCl_3$) δ: 8.4 (s, 1H), 8.0 (t, 1H), 7.8 (m, 1H), 7.4 (t, 1H), 7.34-7.21 (m, 1H)  4.8 (s, 2H), 2.2 (q, 1H), 1.5 (d, 3H).

*Alternative Synthesis of **6**:* Molecule **5** (0.19 g, 0.73 mmol) was dissolved in approximately 10 mL dichloromethane. (±)-α-methylbenzylamine (0.18 g, 1.5 mmol) was added to the reaction flask followed by sufficient magnesium sulfate addition for dry reaction conditions. The solution was stoppered and stirred at room temperature for 24 hours. The magnesium sulfate was removed by gravity filtration. The solvent was removed from the filtrate by rotary evaporation under reduced pressure. The orange liquid sample was then placed under high vacuum. The percent yield was 27%. $^1$H NMR ($CDCl_3$) δ: 7.8 (s, 1H), 7.4 (d, 1H), 7.3 (t, 1H), 7.2 (d, 1H), 4.8 (q, 1H), 4.1 (q, 1H), 2.2 (s, 2H), 1.5 (d, 3H), 1.4 (d, 3H).

**4. Results**

We were able to successfully synthesize the starting molecules **2** and **3** with 55% and 52% yields respectively. Treatment of these molecules with 60% NaH produced the ether, **4,** with a 43% yield. This molecule was then treated with three equivalents of commercially obtained diisobutylaluminium hydride (DIBAL-H) to synthesize **5** with a 90% yield. The dialdehyde reagent, **5**, was then treated with  (±)-α-methylbenzylamine in two separate reaction conditions. The second synthesis was done under mild conditions and showed evidence that it was successful.

**5. Conclusions**

This project has found a successful way to synthesize pure dialdehyde reagent **5** by treating the reagent with 3 equivalents of DIBAL-H under $N_2$ at -78°C. This dialdehyde reagent has been treated with a primary amine and the NMR spectrum from the product suggested that the diimine was synthesized. Unfortunately, the crude product obtained from this reaction

contained several impurities and could not be used directly. An alternative synthesis to produce diimine **6** was tentatively shown to be successful. NMR data suggests that the molecule was made, but it cannot be definitively claimed that the diimine was formed. Further investigation with the product made under this alternative synthesis must be done to confirm the molecule's identity. Once diimine **6** is successfully purified, its spectrum will be used to determine enantiomeric excess.

## 6. References

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