ASYMMETRIC REDUCTIONS OF KETONES, IMINES, AND OXIMES USING BIOCATALYTIC ENZYMES FOUND IN PEA PLANTS

Honors Research Thesis

Presented in partial fulfillment of the requirements for graduation
with honors research distinction in Chemistry in the undergraduate colleges of The Ohio State University

by

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ABSTRACT

In chemistry today, there is an ever growing and inevitable need to mass produce optically active starting materials for areas such as pharmaceutical and natural product synthesis. It is also important to modern chemists that these reactions are carried out under “green” conditions, which implies a significant reduction in the environmental impact of hazardous chemical production, storage, transportation, and waste disposal. This project employs the use of biocatalytic enzymes to generate chiral alcohols and amines via the asymmetric reduction of ketones, imines, and oximes. Initial reductions with prochiral ketones have yielded enantiomeric excess of >98%, with selectivity for either the (R) or (S) enantiomer depending on pea type. In order to further examine the enzymatic reduction capabilities of various types of pea plants, a library of imines and oximes has been synthesized. The library consists of prochiral imines, and oximes with an assortment of substituents, including molecules with electron donating and electron withdrawing groups. Initial reductions of the aryl imines reveal that hydrolysis is a major competing reaction. Biocatalytic reductions of the oximes have thus far been very successful and produced >99% e.e. with selectivity depending on pea type. This is the first reported instance of biocatalytic oxime reductions.

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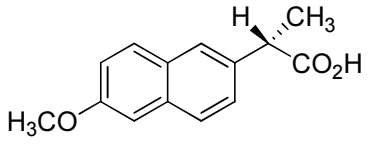
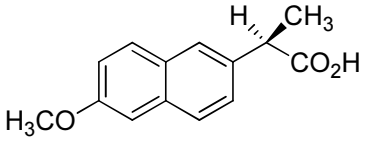
CHAPTER 1: INTRODUCTION AND BACKGROUND

The natural world of biological life is composed of a seemingly infinite complex intertwined with the building blocks of proteins and amino acids. Enzymes function in this complex to actively assemble, disassemble, and modify implicit protein structures, which in return act as templates for biological processes. Arguably the most crucial characteristic which ties the branches of the processes of life together is that of the innate chirality existent in plants, animals, humans, and all living things. The concept of chirality, meaning the inability to be superimposed on its mirror image, is what dictates the functionality of a molecule, based on how it is oriented in three dimensional spaces. It is often described as providing structures with a handedness, and most importantly is a key feature of what defines a molecule's biological activity.

Particularly in the human body, where pharmaceutical applications are concerned, chirality is a major part of what dictates how drugs will interact with the body. Therefore, in order for pharmaceutical drugs to successfully fulfill their purpose, they need to posses the appropriate chirality which will cooperatively interact with the natural biological chirality. For example, as shown in Figure 1.1, simple inversion of the chiral center causes completely



(S)-Naproxen



(R)-Naproxen

Figure 1.1. Naproxen enantiomers.

different functionalities for the Naproxen enantiomers. While (S)-Naproxen is commonly used as an anti-inflammatory drug with analgesic and antipyretic properties, (R)-Naproxen is ineffective at combating these symptoms and is known to cause liver damage.[1]

In the *Organic & Biomolecular Chemistry* journal, a study entitled "Analysis of the Reactions Used for the Preparation of Drug Candidate Molecules" examines details of the characterization and synthesis of drugs which were pre-selected by Medicinal Chemists.[2] Of the 128 drug candidate molecules analyzed, greater than 90% are small molecules (molecular weight less than 550 g/mol), greater than 90% contain a nitrogen atom, and 54% are chiral in nature. Of the 54% that are chiral, 97% are asymmetric, meaning that only one enantiomer is present. It is common practice for pharmaceutical companies to have reactors that are utilized for multiple purposes, and therefore it is easier to facilitate the simple reactions of multiple complex fragments, rather to than build a singular more complex molecule in small fragments. Chirality is then most commonly introduced in the early stages of synthesis via small molecules being added to the complex fragments. These small molecules are most often purchased from industrial chemical companies.[2] Therefore, as the applications of chirality in natural product synthesis increases, the demand for a larger, more diverse library of easily synthesized small, chiral, asymmetric molecules continues to grow and be of even greater importance (Figure 1.2).

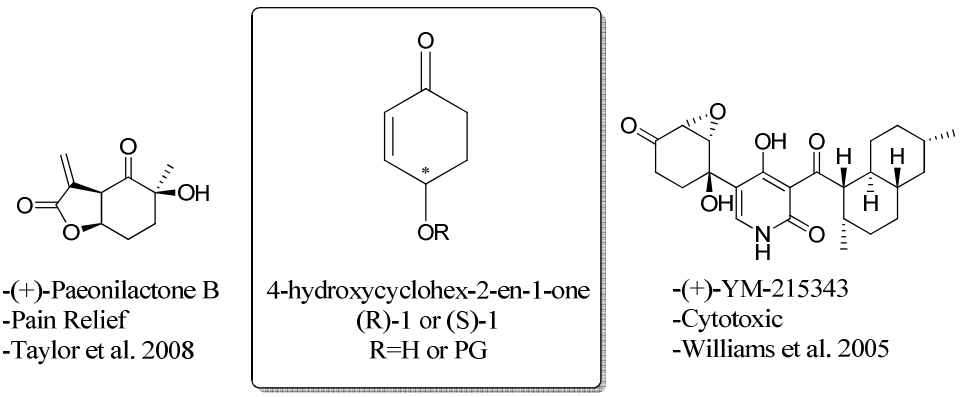
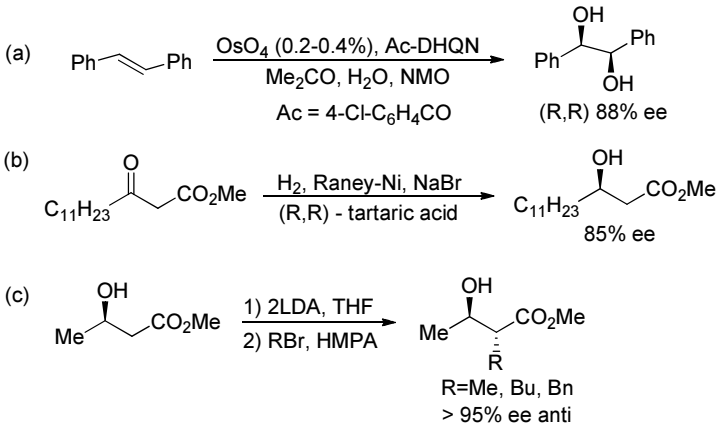


Figure 1.2. Examples of how the small chiral molecule 4-hydroxycyclohex-2-en-1-one is commonly used in natural product synthesis.[3]

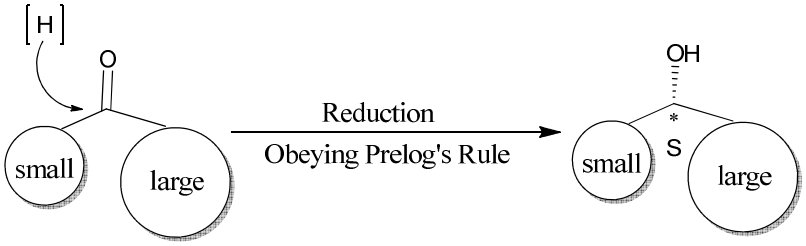
Historically, methods that are capable of achieving a specific stereochemistry are termed stereospecific, and include both enantioselective and diastereoselective reactions. These types of reactions are carried out in the presence of chiral additives and chiral auxiliaries, or involve the transfer of an achiral species. Chiral additives and chiral auxiliaries consist of a broad range of substances and include cosolvents, photosensitizers, and catalysts.[4] In order for a chrial additive or chiral auxiliary to be ideal, it must generate high selectivity, be easily removable from the desired product, have both pure enantiomers readily available, and lastly, be inexpensive to purchase. Reactions that involve the transfer of an achiral species are termed "self-immolative" and are usually utilized in redox processes. Common reaction methods which exploit the ability to induce stereoselectivity include: oxidations with osmium tetroxide, hydrogenations with Raney Nickel, and alkylations with LDA and alkyl halides (Scheme 1.1).[4]



Scheme 1.1. (a) Example of stereoselective oxidation. (b) Example of stereoselective hydrogenation. (c) Example of stereoselective alkylation.

By the 1970's, the full importance of stereochemical reactions had been realized. This realization was also partly due to the development of the necessary analytical tools to characterize the success of a reaction's stereochemical control. These instruments, which for example include HPLC and NMR, revolutionized chemistry as a whole and especially served to further reinforce the role that asymmetric synthesis plays in past, present, and future natural product synthesis.[4]

Of the asymmetric processes, reduction is perhaps the most valued and currently studied tool. One of the first chemists to explore the asymmetric potential of various reductions was Vladimir Prelog. Prelog's rule for reduction states that the hydride attacks from the re-face only of the prochiral ketone to furnish only (S) alcohols (Scheme 1.2).[5] Implementation of this rule

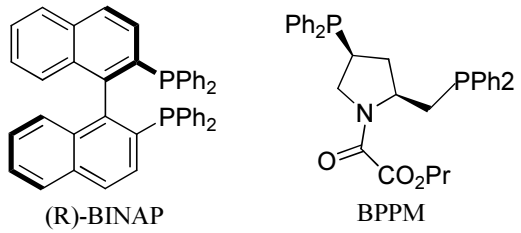


Scheme 1.2. Asymmetric reduction of ketones according to Prelog's Rule.[6]

leads to the prediction of stereochemical outcomes when biocatalytic whole cells are used in the reduction. The ability to predict the resulting enantiomer in a reduction is extremely valuable

when determining the best method to synthesize a desired enantiomer. Vladimir Prelog and John Cornforth shared the Nobel Prize for their work in this area in 1975.[7]

The processes of chemical catalytic asymmetric reductions have evolved and essentially centralized around rhodium complexes. As early as 1966, Wilkinson found that the complex, chlorotris(triphenylphosphine)rhodium, functions as a catalysts in the hydrogenation of various alkene and alkyne substances.[8] His group was the first to report a rhodium complex used as a true catalyst, where as in studies previous to this, the metal was not capable of catalysis without rapid deposition occurring.[8] Shortly after this discovery, research involving catalytic rhodium rapid expanded to encompass reduction as well. Today, the research surrounding rhodium catalysts has the goal of producing high selectivity in reductions of a broad range of molecules with various properties.[4] The majority of the most common rhodium catalysts used in asymmetric reduction are rhodium-chiral phosphine catalysts which contain various ligand groups such as BINAP and BPPM (Figure 1.3).[4] In addition to rhodium being used as a catalyst,

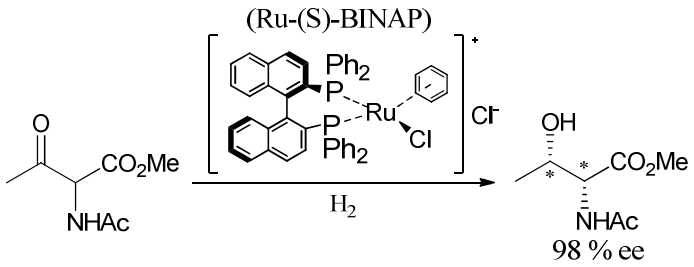


(R)-BINAP BPPM

Figure 1.3. Common ligands that complex with rhodium and ruthenium to catalyze asymmetric reductions.

more recently ruthenium has been found to be effective at facilitating asymmetric reductions as well. Ruthenium also is most effective when used in correlation with various chiral phosphine ligands.[9] Particularly Ru-BINAP catalysts are extremely valuable in the production of enantiomerically pure ketones because configuration is very predictable, meaning that the

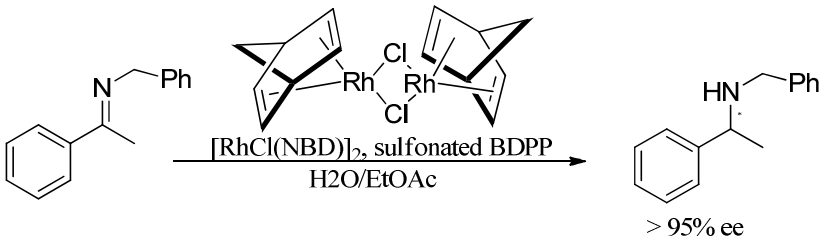
catalyst used with (S)-BINAP usually gives (S)-alcohols in excellent ee of greater that 99% (Scheme 1.3).[4] In the catalytic reduction process ligands generally serve the purpose of



Scheme 1.3. Asymmetric reduction with ruthenium-BINAP catalyst complex.

donating electrons to the coordinated central metal, and thereby activating it to bind to the substrate. As for as reaction conditions for these types of reactions, all are carried out under hydrogen pressure, in dichloromethane or aqueous methanol as solvents and in some cases high temperatures of up to 100 °C shortened reaction time without significant loss of selectivity.[9]

In addition to asymmetric reductions of ketones, the asymmetric reduction of imines to afford optically active amines is a very valuable, under studied process. Like alcohols, amines are also precursors in many important natural product syntheses, meaning the need for enantiomerically pure amines is just as high. Various rhodium complexes have proven to be successful in the reduction of imines, particularly the $[RhCl(NBD)]_2$ complex with a mixture of sulfonated BDPP ((-)-(2S,4S)-2,4-bis(diphenylphosphino)pentane) at 20 °C in water-ethyl acetate under hydrogen pressure provides greater than 95% e.e. (Scheme 1.4).[9]



Scheme 1.4. Rhodium complex used in enantioselective reduction of an imine.

Another commonly used method of reducing imines utilizes trichlorosilane with N-picolinoylpyrrolidine or N-formyl derivatives as organic activators.[10] These various derivatives provided up to 90% yield, with 80 % ee. It is speculated that the basis for the successful coordinating of trichlorosilane to the organic activator is that the Si atom of the complex must coordinate with both the nitrogen atom of the picolinoyl group and the carbonyl oxygen (Figure 1.4).[10]

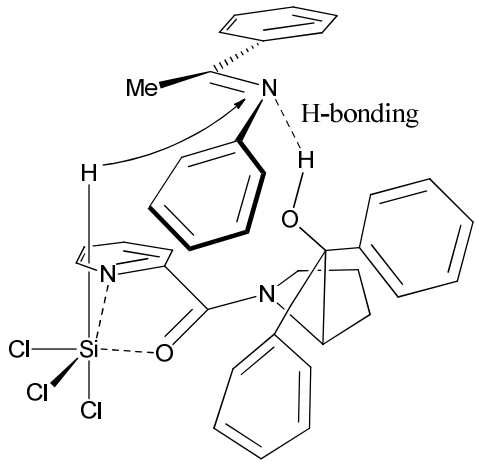
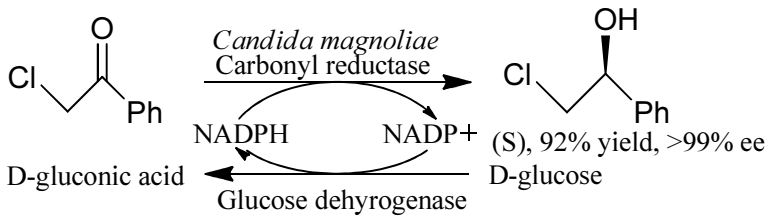


Figure 1.4. Imine reduction mechanism with trichlorosilane and picolinoyl activator.

While chemical catalysis still maintains a vital role in asymmetric synthesis, a more recent trend in chemistry has turned towards exploring the potentials of biocatalysts. Biocatalysts have proven not only to be effective synthetic tools, but they are representative of a new category of approaches which require minimally toxic reagents, and do not produce hazardous waste. The modern realization of the importance of minimizing the negative effects that humans have on the environment has impacted chemistry in a huge way and thus, "Green Chemistry" is now being explored more than ever. The wide implementation of this type of chemistry would mean a significant reduction in the environmental impact of hazardous chemical production, storage, transportation, and waste disposal.

In many cases green, biocatalytic, synthetic methods cannot only be successfully implemented in place of chemical catalysis, but are in fact useful in some cases for carrying out processes which are difficult to accomplish with chemical catalysis. Over the past decade, the usage of biocatalysts has drastically increased, and in 2009, it was estimated that approximately 10% of total drug synthesis processes depend on biocatalysts.[11] In order to screen potentially suitable biocatalytic enzymes for reactivity, it is crucial to understand how they function. For the reduction process, the coenzymes, NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate) and an enzyme function together to catalyze the reaction. In the initial step of the process, the coenzyme and oxidized substrate bind to an enzyme. Next, the substrate is reduced while simultaneously the coenzyme is oxidized. In the final step, the coenzyme and reduced product detach from the enzyme and in effect the coenzyme is recycled and ready for the process to begin again.[11]

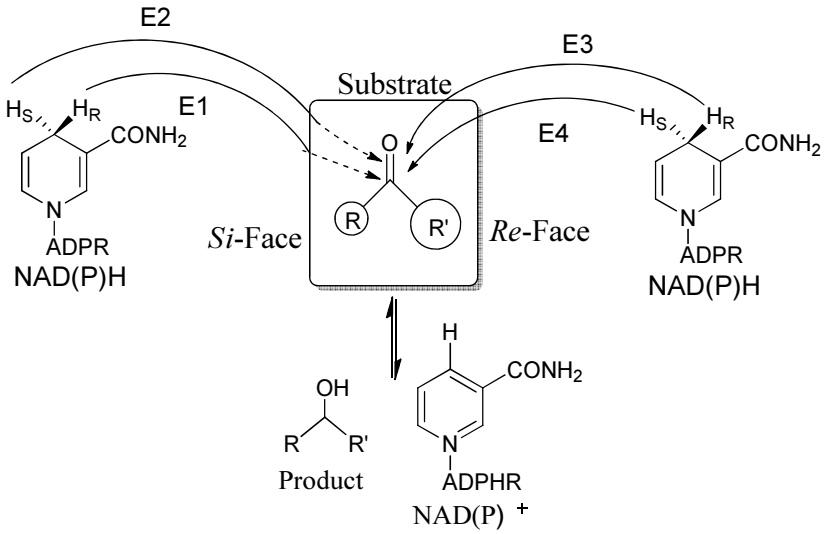
The process by which the coenzyme is recycled back to its reduced form can vary depending on which hydrogen sources are most readily available in the reaction. Some common example of hydride sources available to recycle the the coenzyme via reduction are alcohols, sugars, formic acid, certain amino acids, and also electric and light power sources. For example, in the reduction of ketones by *Candida magnolia*, glucose and glucose dehydrogenase functioned to regenerate an active coenzyme by reduction (Scheme 1.5)[11]. Photochemical methods also



Scheme 1.5. Recycling of NADPH using sugar for the reduction of a ketone.

provide an environmentally friendly process to regenerate NAD(P)H, however, a few of the other methods utilized for this process are more environmentally hazardous and not economically preferable. For example, in order for electrochemical regeneration to be effective it requires an expensive and chemically hazardous rhodium complex.[11]

As for the delivery of the hydride ion to the substrate being reduced, it can either attack the *si*-face or *re*-face of the carbonyl or imine group. The face which is attacked depends on the distinctive orientation of the substrate-enzyme bond. For different enzymes and different substrates, this orientation may be reversed, and therefore the selectivity for the (R) or (S) product can be controlled (Figure 1.5).[11]



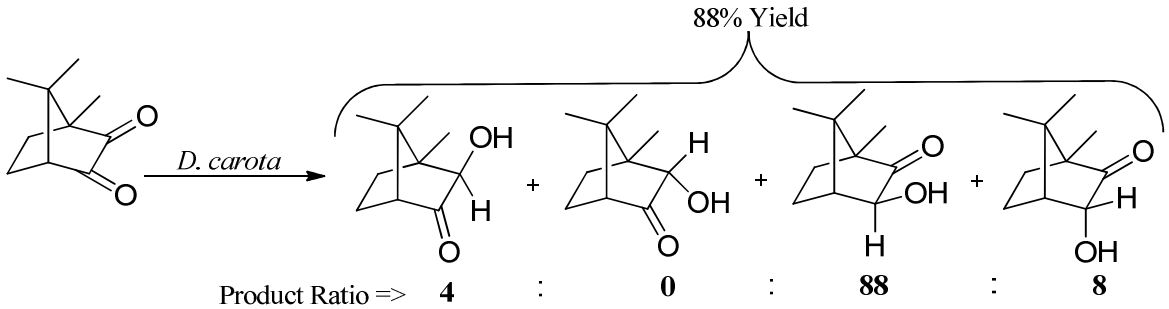
-E1, E2, E3, and E4 represent different types of enzymes.

Figure 1.5. Example of hydride transfer from coenzyme, NAD(P)H, to a carbonyl compound.

A more in depth look into the enzymes that are used to provide selectivity shows that there is much to be investigated and developed. One of the first biocatalysts to be studied extensively for its applications in asymmetric reductions is baker's yeast (*Saccharomyces cerevisiae*). The reductase enzyme present in the yeast has been isolated, purified, and tested to

show that it is very effective in the asymmetric reduction process for a wide variety of ketone substrates.[12] More recent research with baker's yeast has eliminated the extensive purification steps and the reductase genes have been expressed and cloned in *Escherichia coli*. The asymmetric reduction of 20 ketones have been successfully carried out with these recombinant *E. coli* cells and 11 of the alcohols produced were enantiomerically pure.[13]

While the utility of baker's yeast and many other microorganisms have been thoroughly studied and screened for use as biocatalysts, the enzymes derived from plants are virtually untouched potentially very valuable resources. So far, most of the few plants that have been studied posess unique enzymes with interesting capabilities. For example, vegetables such as carrot root (*Ducus carota*) have successfully been tested for the reduction of (+)-camphorquinone to produce secondary alcohols in 88% yield (Scheme 1.6).[14] However, a



Scheme 1.6. Reduction of (+)-camphorquinone by *Ducus carota.*

common problem that arises when using vegetables as biocatalysts is the effect of microbial contamination. A recent study showed that when bacterial inhibitors were added to the reaction mixture, there was a sharp decrease in percent conversion and selectivity. It can therefore be concluded that endophytic microorganisms, or microorganisms that share some sort of symbiotic relationship with the host organism, may be involved in the asymmetric reduction process when

certain plants are utilized as the enzyme carrier.[11] This is a problem in biocatalysis because it means that not all vegetables of the same type, which contain the same enzymes, may be equally effective in asymmetric reduction due to a lacking presence of the second party microorganisms.

In addition to *D. carota*, other common plants that have successfully been screened and tested as potential biocatalysts for the asymmetric reduction of ketones include: arracacha roots (*Arracacia xanthorrhiza*), beet roots (*Beta vulgaris*), yam tubers (*Dioscorea alata*), coriander roots (*Coriandrum sativum*), and turnip roots (*Brassica rapa*). These are a few of the more well known plant biocatalysts and in the reduction of acetophenone derivatives, they provide decent percent conversion with typically high enantiomeric selectivity (Table 1.1).[15] In order to achieve these high quality transformations, the plants were first washed with water prior to being soaked in a 5% sodium hypochlorite aqueous solution for 20 minutes. They were then rinsed with ethanol, peeled and cut to increase the surface area of the biocatalysts that the substrate could come into contact with.[15]

Biocatalyst	**% Conversion**	**% ee and config.**
arracacha roots *Arracacia xanthorrhiza*	91	66 (S)
beet roots *Beta vulgaris*	83	87 (R)
yam tubers *Dioscorea alata*	47	94 (S)
coriander roots *Coriandrum sativum*	56	99 (S)
turnip roots *Brassica rapa*	10	99 (S)

Table 1.1. Biocatalytic reductions of acetophenone with various plants.

In another recent study, sprouted green peas (*Pisum sativa*) were used for the first time as biocatalysts in the enantioselective reduction of prochiral ketones. In correlation with the

previous study, these tests also examined the reduction of acetophenone derivatives in water. However, the procedure for these reductions utilized an aqueous buffer pH 7.0, and the reactions were only carried out for 24 hours in order to furnish the corresponding *S*-alcohols with ee ranging from 80-98%.[5] Lastly, under similar conditions, other reductions of the same acetophenone derivatives with soaked *Phaseolus aureus L* as the biocatalyst showed that the presence of electron donating substituents on the attached aromatic rings of the ketones slow the reaction and produce relatively lower yields with moderate selectivity (Table 1.2). However, this study successfully tested their method of reduction on a multi-gram scale, which consisted of using 500 g of the biocatalysts.[16]

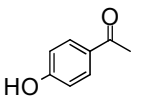
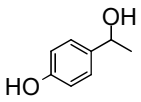
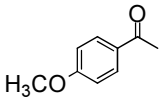
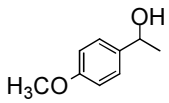
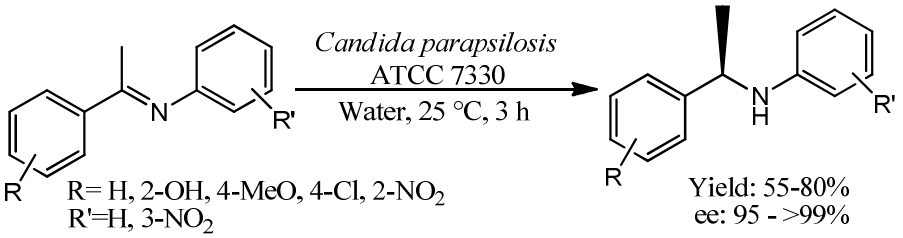
Substrate	Product	% ee and config.	% Yield
		72 (S)	23
		90 (S)	28

Table 1.2. Examples of decreased reaction rates observed for substrates with electron donating groups.

There is still much to discover regarding the biocatalytic activity of plants in the reduction of ketones to produce optically active alcohols, however, this area of research is expanding and only very recently have these procedural methods been applied to substrates other than ketones. The asymmetric reduction of C=N bonds to generate enantiomerically pure amines via biocatalytic methods is virtually unexplored. Efforts to change this fact have recently surfaced out of the sheer value and need for more efficient methods to produce enantiomerically pure amines for their vast applications in the pharmaceutical and agricultural industries.[17] One of

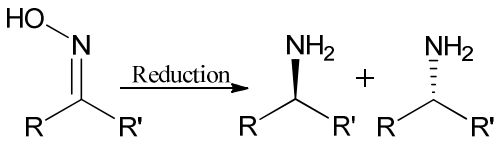
these studies utilizes the enzyme *Candida parapsilosis* ATCC 7330 in order to reduce a library of aromatic imines (Scheme 1.7). The reductions were carried out using



Scheme 1.7. Asymmetric reduction of aromatic imines using the whole cells of *Candida parapsilosis* ATCC 7330.

whole cells of the biocatalyst in an aqueous medium with no additional cofactor. The results provided yields of up to 80% and e.e. of greater than 99% which was determined by chiral High Performance Liquid Chromatography (HPLC). It should also be noted that substrates with electron withdrawing and electron donating groups were still produced in high yields with excellent ee.[17]

While the biocatalytic studies over the reductions of imines are extremely minimal and underdeveloped, the literature on biocatalytic oxime reduction is seemingly nonexistent. The reduction of oximes to primary amines requires the cleavage of the N-O bond in the molecule (Scheme 1.8). Even catalytic methods capable of oxime reduction are fairly scarce. However, a



Scheme 1.8. Reduction of oximes to primary amines.

few well known methods involve processes using lithium aluminium hydride, sodium borohydride in the presence of various derivatives, diboranes with zinc and acetic acid, and a

more recent method utilizes catalytic indium metal in solution with acetic anhydride, acetic acid and THF to facilitate reduction.[18] Although, these methods have proven to be successful, if it is found in the future that a simple, nonhazardous, biocatalyst is capable of reducing oximes to their corresponding amines, it would most definitely become the preferred method.

The world of chemistry as a whole and especially synthetic chemistry are developing at continually increasing rates each year, and as the modern world turns toward a "greener" more eco-friendly future, chemistry must turn there as well. Therefore, further exploration of biocatalytic pathways is a major part of the chemistry of the future. More extensive testing and implementation of these biocatalytic methods will help to fulfill the need that industry has for substrates such as, alcohols and amines, which are easily obtainable and enantiomerically pure. With the development of these processes, complex synthesis will be able to be carried out in an efficient, practical, and non-environmentally hazardous way.

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CHAPTER 2: BIOCATALYTIC REDUCTIONS OF PROCHIRAL KETONES

Results and Discussion

As previously discussed, the utility of enantiomerically pure chiral alcohols extends deep into the core infrastructure of natural product synthesis. The ability to mass produce a wide variety of enantiomerically pure secondary alcohols is extremely valuable and furthermore to accomplish this in a "green", environmentally friendly way is an even more highly coveted methodology. This portion of the project explored the biocatalytic activity of a variety of pea seeds in the reductions of an assortment of ketones (Table 2.1, Figure 2.1).

Pea	Common Pea Name	Family : *Genus : Species*
A	Pigeon Peas	Fabaceae : *Cajanus : cajan*
B	Black Beans	Fabaceae : *Phaseolus : vulgaris*
C	Navy Beans	Papilionaceae : *Phaseolus : vulgaris*
D	Garbanzo Bean	Fabaceae : *Cicer : arietinum*
E	Garden Peas (Split)	Fabaceae : *Pisum : sativum*
F	Black Eyed Peas	Fabaceae : *Vigna : unguiculata*

Table 2.1. Pea and beans seeds tested as potential biocatalysts.

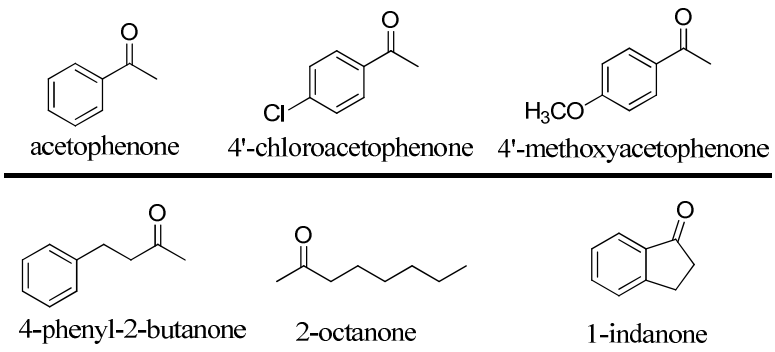
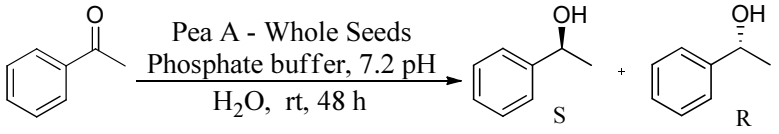


Figure 2.1. Ketone substrates selected for experimental reductions.

While all of the peas tested provided high e.e. values, certain peas were more successful than others depending on the substrate being reduced. In addition to excellent e.e. it was determined that the resulting stereochemistry could be controlled based on pea type. It was also

determined that using a phosphate buffer in the reactions increased percent conversion. Without the use of the buffer, the reaction mixture became fairly acidic and it was found that the enzymes responsible for reduction were not fully activated below a pH of 5. Optimal reactions were determined to have a pH between 5.5 and 7.5.

For all of the subsequent experimental reactions, conversion was determined via gas chromatography (GC), yield was taken after column chromatography purification, % e.e. was determined with chiral GC, and all values were averaged over 20 trials. The first ketone substrate reduced was acetophenone and the peas responsible for the best results in selectively producing each of the enantiomers was, pea F which gave 78% conversion and 97% e.e. of the (S) enantiomer, and pea B which gave 78% conversion and 97% e.e of the (R) enantiomer (Scheme 2.1, Table 2.2).



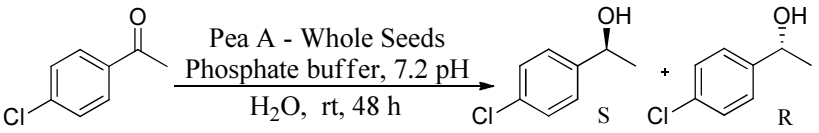
Scheme 2.1. Example reduction of acetophenone.

Pea	% Conversion	% Yield	% e.e.
A	88%	81%	95% (S)
B	81%	77%	**97% (R)**
C	90%	85%	90% (S)
D	74%	70%	93% (S)
E	80%	77%	88% (S)
F	79%	74%	99% (S)

Table 2.2. Acetophenone reductions with the biocatalytic peas.

In the reduction of 4-chloroacetophenone the pea responsible for yielding the most enatiomerically pure (S) alcohol varied from that of acetophenone. In this case, the most

successful reductions came from, pea A which gave 82% conversion and 97.5% e.e of the (S) alcohol, and pea B which gave 81% conversion and 95% e.e. of the (R) alcohol (Scheme 2.2, Table 2.3).

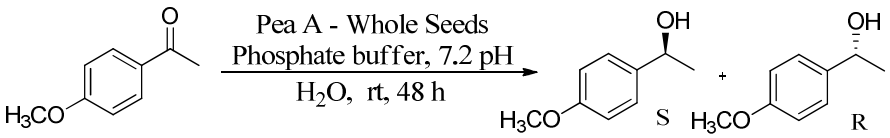


Scheme 2.2. Example reduction of 4-chloroacetophenone.

Pea	% Conversion	% Yield	% e.e.
A	82%	81%	97.5% (S)
B	81%	71%	**95% (R)**
C	68%	55%	88% (S)
D	71%	60%	91% (S)
E	78%	64%	88% (S)
F	79%	69%	92% (S)

Table 2.3. 4-Chloroacetophenone reductions with the biocatalytic peas.

For the reduction of 4-methoxyacetophenone, pea A was the most effective at producing the (S) enantiomer and furnished 88% conversion with 98% e.e. The highest selectivity for the (R) enantiomer was again achieved by pea B which yielded 85% conversion and 93% e.e (Scheme 2.3, Table 2.4). It should also be noted that in this case, the presence of the methoxy electron donating substituent did not seem to influence the effectiveness of either the progression of the reaction or its selectivity. A previous study in the biocatalytic, asymmetric reduction of ketones with soaked *Phaseolus aureus L* attested that only moderate selectivity could be achieved with low yields when electron donating groups were present on the attached aromatic ring of the ketone.[1]

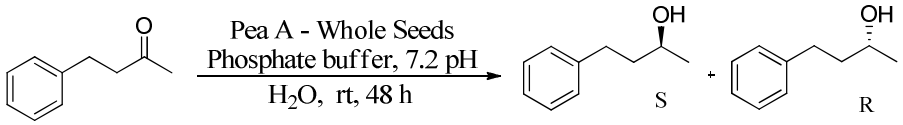


Scheme 2.3. Example reduction of 4-methoxyacetophenone.

Pea	% Conversion	% Yield	% e.e.
A	88%	83%	98% (S)
B	85%	76%	**93% (R)**
C	81%	73%	88% (S)
D	74%	68%	93% (S)
E	71%	60%	94% (S)
F	80%	71%	96% (S)

Table 2.4. 4-Methoxyacetophenone reductions with the biocatalytic peas.

In order to explore a wider variety of ketones that were not acetophenone derivatives, the reductions of 4-phenyl-2-butanone were examined (Figure 2.5). For these reactions, pea F gave the highest e.e. of 94% with 80% conversion for the (S) enantiomer, and pea B yielded the best results for the (R) enantiomer with 90% e.e. and 78% conversion (Table 2.5). These particular ketone reductions generally demonstrated slightly lower % e.e values than the previously studied acetophenone derivatives. This is most likely attributed to the implicit structural properties of the ketone itself. By having the longer alkane chain separating the phenyl and ketone functionalities, there is more opportunity for free rotation meaning, less steric restrictions which function to retain the rigidity of the molecule. Retention of the rigidity of the molecule enables the enzyme complex to maximize the selectivity of its approach to the ketone substrate, therefore, the less rigid the molecule being reduced, the less selective the reduction process.

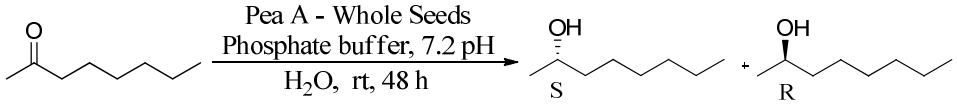


Scheme 2.4. Example reduction of 4-phenyl-2-butanone.

Pea	% Conversion	% Yield	% e.e.
A	86%	83%	87% (S)
B	78%	70%	**90% (R)**
C	83%	73%	82% (S)
D	78%	68%	77% (S)
E	72%	60%	90% (S)
F	80%	73%	94% (S)

Table 2.5. 4-Phenyl-2-butanone reductions with the biocatalytic peas.

In the reductions of 2-octanone, pea A provided the best results for the (S) enantiomer with 87% e.e. and 65% conversion, while pea B provided 70% e.e. with 78% conversion for the (R) enantiomer (Figure 2.6, Table 2.6). As such was the case with 4-phenyl-2-butanone, the enantiomeric purity of the reductions were lower for 2-octanone as compared to what was achieved with the acteophenone derivatives. Of the six ketone substrates examined, 2-octanone afforded the lowest e.e. values, which is again most likely due to the lack of rigidity of the molecule. The linear chain alkane structure of 2-octanone has diminished conversion and e.e. which could be due to poor binding to the enzyme active site.

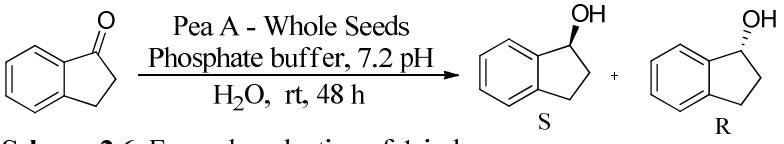


Scheme 2.5. Example reduction of 2-octanone.

Pea	% Conversion	% Yield	% e.e.
A	65%	50%	87% (S)
B	78%	70%	**70% (R)**
C	50%	43%	80% (S)
D	43%	37%	79% (S)
E	48%	41%	82% (S)
F	51%	45%	80% (S)

Table 2.6. 2-Octanone reductions with the biocatalytic peas.

In the reductions of 1-indanone, pea C afforded the highest selectivity for the (S) enantiomer with 99% e.e. and 79% conversion, and pea B the highest selectivity for the (R) enantiomer with 94% e.e and 78% conversion (Figure 2.7, Table 2.7). As compared to the previous two non-acetophenone derivatives, 1-indanone has a cyclic structure bound to a phenyl ring, which maintains and fairly strict rigidity, and therefore extremely high % e.e. values were obtained for a variety of the pea enzymes tested.



Scheme 2.6. Example reduction of 1-indanone.

Pea	% Conversion	% Yield	% e.e.
A	72%	61%	87% (S)
B	78%	70%	**94% (R)**
C	79%	72%	99% (S)
D	65%	60%	88% (S)
E	58%	52%	92% (S)
F	71%	60%	94% (S)

Table 2.7. 1-Indanone reductions with the biocatalytic peas.

Another interesting observation is that the same pea type did not produce all of the highest % e.e. values for the (S) alcohols. This indicates that different enzymes from the particular peas are interacting with each ketone substrate in a unique way. For example, because pea F furnished the highest % e.e. in the reduction of acetophenone to its (S) alcohol, and pea A furnished the highest % e.e in the reduction of 4-chloroacetophenone to its (S) alcohol, it can be concluded that the enzyme or active site responsible for reduction varies from pea to pea and therefore, different enzymes are optimal for the reduction of different substrates. If the particular enzymes responsible for reduction were known, hypotheses could potentially be made based

upon steric interactions or electronic effects, however, further research into the implicit enzyme properties would need to be understood first. This leaves a huge potential for further research into more of the enzymatic functionalities and properties which control these reactions.

The obvious anomaly that appeared in all of the ketones tested was that of the reverse selectivity achieved by pea B. It is very well known, according to Prelog's Rule for reduction that the hydride attacks from the re-face of the prochiral ketone to furnish only (S) alcohols.[2] However, the enzymes in pea B seem to have defied this concept by producing the (R) alcohols. In order to obtain an inversion of the chiral center, there needs to be a property of the enzyme to substrate binding complex which blocks the re-face of the prochiral center and makes the si-face more favorable for hydride attack. Again, without structural knowledge of the enzyme itself, it is difficult to make assumptions about its particular binding functionalities, but it is clear the inherent structural properties of the reductase enzymes in pea B poses unique qualities from that of the other pea reductase enzymes explored.

The first step towards isolating the enzymes responsible for the reduction process was to determine which part of the pea itself contains the enzymes. This was done by running the reduction reactions with the pea cores and pea shells separately. These experiments resulted in reductions that were run with pea core yielding up to 99% e.e. and 83% conversion to the (R) enantiomer with pea F. On the other hand, the reductions run with pea shells only yielded 20% e.e and 68% conversion to the (R) enantiomer with pea F. This data clearly demonstrates that more of the enzymes responsible for reduction reside in the pea core and not the pea shell.

In addition to exploring the location of the enzymes responsible for reduction, potential pathways to minimize the ratio of pea to millimole of substrate were also explored. In the

methodology utilized for these experiments, it is speculated that there is actually excess enzyme present within the 25 g of peas than what is being utilized to reduce the 1 mmol of substrate, however, the concentration of coenzyme (NAD(P)H) is depleting at a faster rate. Without a source of coenzyme or the means to regenerate it, the catalytic process stalls.[3] While adding excess coenzyme or employing a means to regenerate it would potentially help to maximize the usage of the total enzyme present, these techniques are either expensive or environmentally hazardous.

The most interesting and innovative result that came from these various reductions is the ability to control selectivity for a particular enantiomer based solely on pea type used. The ability to effectively control selectivity is one of the most highly regarded methodologies in chemistry and to be able to do it simply by switching the type of pea seed used is by far more efficient, more cost effective, and less environmentally hazardous than any other method previously available. For example, in the chemical, asymmetric, reductions of ketones, the commonly used toxic rhodium catalyst, Benzeneruthenium(II) chloride dimer, cost $153.40 per 0.5 g according to Sigma-Aldrich.[4] It is therefore feasible that with further development of the enzymatic properties of the peas, that biocatalytic pea reductions could prove to be extremely applicable.

Experimental

General Method for Biocatalytic Reduction of Ketones.

The ketone (1.00 mmol) was added to a suspension of peas (25-30 g) in water (50 mL) or phosphate buffer (50 mL, pH 7.1). The container was sealed and shaken for 48 h. The reaction mixture was vacuum filtered to remove the pea/bean solids. The pea/bean solids were washed with water (2 x 20 mL) and ethyl acetate (2 x 20 mL). The biphasic filtrate solutions were combined and extracted with ethyl acetate (2 x 20 mL). The organic layer was isolated, dried with Na_2SO_4, filtered and evaporated under reduced pressure. The crude oil was analyzed for percent conversion and e.e. The oil was purified by column chromatography (hexanes:EtOAc) to yield the purified alcohols. All spectroscopic data for the alcohols was compared to literature data for the previously reported compounds.

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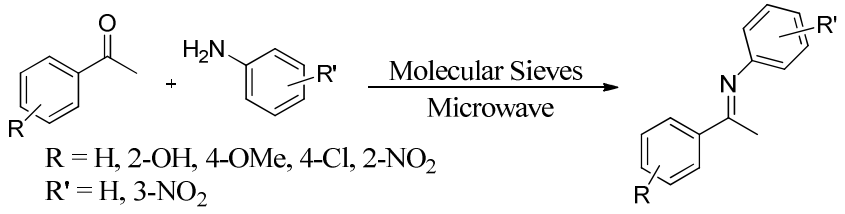
CHAPTER 3: PREPARATION OF IMINES AND OXIMES AND BIOCATALYTIC REDUCTIONS

Results and Discussion

In contrast to biocatalytic reductions of ketones, there is only one reported literature reference for the biocatalytic reduction of imines, and there is not a current literature reference for the biocatalytic reduction of oximes. This means that there is a tremendous amount of uncharted territory in the area of exploring potential biocatalytic enzymes and their effectiveness in asymmetric reductions of imines and oximes.

In order to examine the capabilities of the pea enzymes toward asymmetric reduction of imines, the imines first needed to be synthesized. The methodology used for the synthesis of five biaryl imines was derived from a previously reported methodology which utilized a microwave reactor to condense various acetophenone and aniline derivatives (Scheme 3.1).[1] However, the literature proved to not be as effective as reported in our hands, and in reality, multiple microwave heating cycles and multiple additions of the aniline derivatives supplied the best yields of up to 80%.

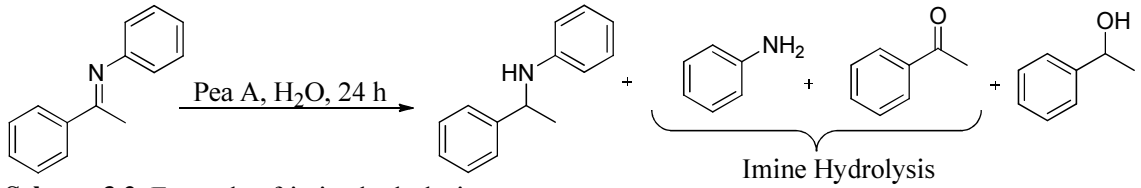


Scheme 3.1. Previously reported synthesis of aryl imines.

While the microwave condensation method gave a broad range of yields, once developed, it was a fairly facile synthesis and extremely economically efficient as compared to other previously reported methods which required expensive catalysts and harsh reaction conditions. For example, a previous method required that the reaction to be carried out with dry solvents

under nitrogen pressure in the presence of a titanium tetrachloride catalyst at -30 °C.[2] Expensive reagents, harsh conditions, and long reaction times were not required for the microwave condensations which, in some cases, were even prepared neat and reaction times consisted of only three 15 minute heating cycles.

In testing the capabilities of the biocatalytic enzymes in peas toward asymmetric reduction of the synthesized imines, hydrolysis back to the respective acetophenone and aniline derivatives was consistently found to be the primary reaction taking place, not reduction (Scheme 3.2).



Scheme 3.2. Example of imine hydrolysis.

Hydrolysis was the primary reaction because the mixture became too acidic during the shaking period, which caused formation of an iminium ion, making it highly susceptible to attack from water. The reaction mixture became acidic because the enzymes, coenzymes, and plant materials in the peas were slowly released into solution during the course of reaction. In order to combat hydrolysis by stabilizing the pH of the mixture, a variety of buffers were tested. The buffers tested include sodium phosphate buffers at 0.1 M and 0.2 M concentrations and a Tris buffer. Of the numerous trials conducted utilizing these buffers with a variety of pea types and imine substrates, the only instance that showed slight reduction of the imine to the secondary amine was observed with the 0.2 M sodium phosphate buffer (Figure 3.1). The GC-MS figure also shows that after hydrolysis took place, the pea enzymes did function to reduce the acetophenone

that formed to produce the corresponding alcohol. This evidence shows that the pea enzymes capable of reduction are present and active however, the issues are arising with the imine substrate itself.

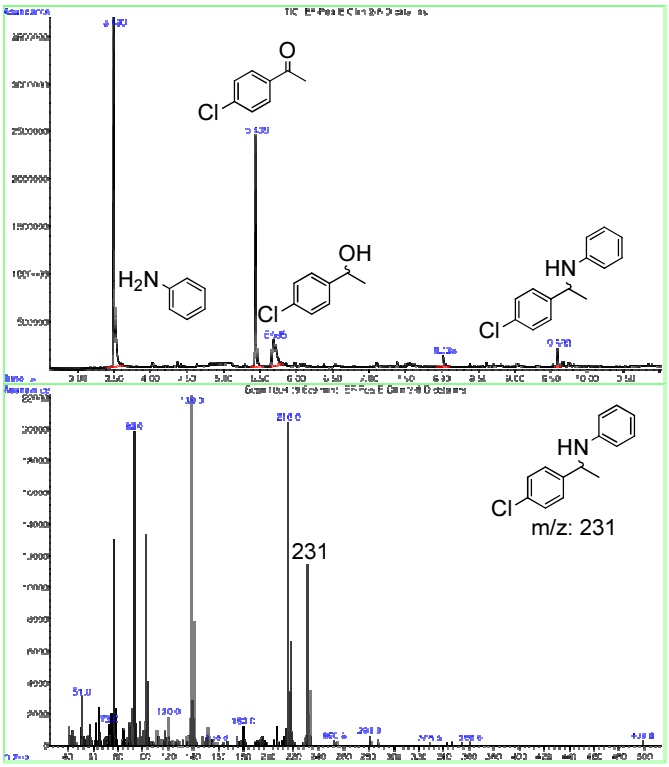
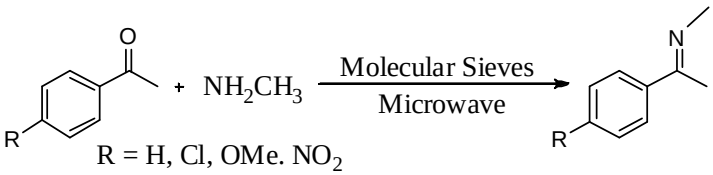


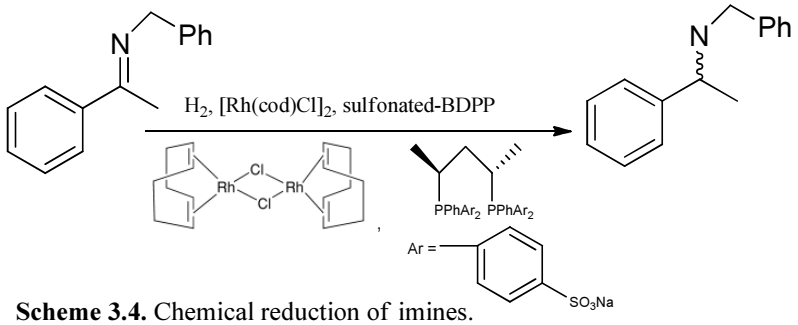
Figure 3.1. Biocatalytic imine reduction GC-MS.

Another possibility as to why the imines are not reducing is that they are potentially too sterically hindered for the enzyme complex to bind properly in order for reduction to take place. Therefore, the biaryl groups are not ideal for biocatalytic reductions for two reasons; the phenyl groups function to further activate the imine nitrogen which leads to hydrolysis, and there are larger groups which may be sterically preventing the desired reduction reaction all together. Future research entails the synthesis of less electron donating, less sterically hindered imines, for example using methyl amine instead of aniline (Scheme 3.3).



Scheme 3.3. Examples of less sterically hindered imines.

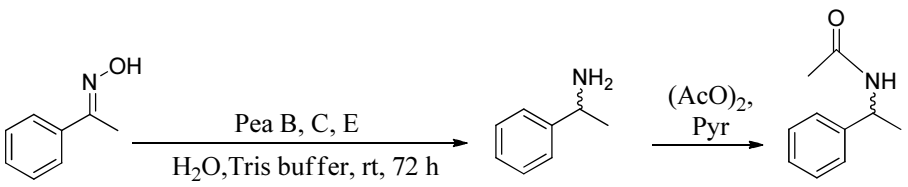
Should the approach of using less sterically hindered amines to synthesize imines afford decent reduction results, the biocatalytic method would be superb in many ways, as compared to chemical asymmetric reduction methodology. For example, a previous chemical method utilized the chiral catalyst, cyclooctadiene (cod) dimer $[Rh(cod)Cl]_2$ and sulfonated-BDPP under hydrogen pressure (70 bar) to asymmetrically reduce imines (Scheme 3.4).[3] According to Sigma-Aldrich the chiral catalyst costs $207.00 per 0.5 grams and the BDPP prior to sulfonation costs $147.50 per 0.5 grams.[4,5]



Scheme 3.4. Chemical reduction of imines.

As for synthesis of the oximes, they were easily produced in high yields of up to 98%.[6] In the biocatalytic reductions, the reaction mixture again became acidic, however oximes are not as prone to hydrolysis as biaryl imines. The oxime structure is also not as sterically hindered around the C=N bond as compared to the biaryl imine structures, which may aid in making it less resistant to reduction. Because the reaction solution became acidic during the reduction, when the oxime reduced to its corresponding free amine, the amine became protonated and therefore water soluble. In order to ensure that the amine could be fully extracted from the reaction, the

solution was neutralized to pH 10 prior to extraction (Scheme 3.5, Table 3.1). Thus far, only three peas (B, C, and E) have been tested and up to 95 % e.e. has been achieved utilizing pea E. As such was the case with the previously examined ketones, pea B appears to again demonstrate reverse selectivity by providing the (R) enantiomer, while the other peas appear to produce fairly selectively the (S) enantiomer. Configurational assignments were determined by optical rotation measurements as compared to authentically prepared samples.



Oxime: **2a** Amides: **4a**

Scheme 3.5. Biocatalytic oxime reduction and acylation process.

Pea	% Conversion	% e.e.	Configuration
B	TBA	85	R
C	83	67	S
E	72	95	TBD

Table 3.1. Oxime 2a reduction results for peas A, B, and C.

Future work with the oximes will consist of bringing the data up to par with what was obtained for the ketone reductions. This consists of exploring the effects that the electron donating and electron withdrawing substituents have on the reduction process, and if the trend of reverse selectively continues for all pea B reactions. Again, the ability to selectively control which enantiomer is produced simply by changing the pea type used in the reaction, is by far more simplistic than other reported methodologies which utilize expensive chiral catalysts.

To make this biocatalytic process applicable for industrial purposes, collaborations with biochemists would need to be made in order to further explore the enzymatic properties of the peas. If the enzymes responsible for reduction were isolated, then they could potentially be

cloned and expressed in bacteria such as *E.coli*. The reductase infused *E. coli* could then be replicated to produce large portions of pure active enzyme. This process has been successfully completed on the enzyme responsible for reduction in baker's yeast.[7] With successful completion of this process would come the ability to effectively reduce a large amount of substrate with only very small portions of the pure enzyme.

While this project has taken steps toward uncovering the vast capabilities of biocatalytic enzymes, there is still much to be discovered and much work to be done. There are still countless enzymes to be screened and processes to test. The use of the biocatalytic enzymes in peas toward asymmetric reduction thus far has not only proven to be effective, but it represents a new era of "green chemistry". The implementation green processes into routine synthetic reactions is instrumental for the future of modern chemistry and is a field only at the brink of exploration.

Experimental

General. All microwave reactions were carried out utilizing a CEM Explorer microwave at power 150. Molecular sieves (4 Å) were first activated prior to use in all microwave reactions. Reactions were monitored by GC-MS (Agilent 6850, 5975C detector, Agilent HP-5MS, 30 m x 0.25 mm, 0.25 mm film column) and TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by staining with *p*-anisaldehyde. Chromatography was performed on silica gel 60 (40-60) μM). All GC-MS spectra were run with an equivalent method, as shown in Appendix 1. Solvents were evaporated under reduced pressure at below 45 °C (bath). ^{1}H NMR spectra were recorded at 250 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 62.9 MHz, and are referenced to TMS (0.0, $CDCl_3$). The general procedure for the imine synthesis reactions was developed from a previously reported methodology.[1] Specific reaction variations in imine synthesis are given in Table 3.2. Product yields and appearances of imines are given in Table 3.3. Imines **1a**, **1b**, and **1c** have previously been synthesized and characterized.[1] The procedure used to synthesize the oximes has been previously reported.[6] Product yields for oximes are given in Table 3.4. All oximes have been previously synthesized and characterized.[8, 9, 10]

Imine Synthesis

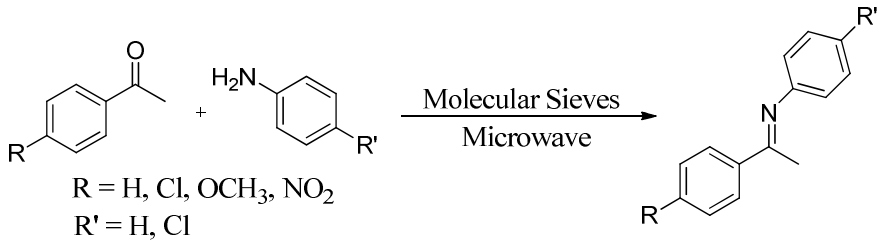
Method A. Acetophenone (25.6 mmol) and aniline (38.5 mmol) in the presence of molecular sieves (4 Å) were condensed using a microwave oven (Power 150) (Scheme 3.6, Table 3.2). After each heating cycle, aniline (25.6 mmol) was again added to the reaction and heated for another cycle. The progression of the reaction was monitored by GC-MS and TLC for the disappearance of acetophenone. The crude product was purified via chromatography (1%

triethylamine in petroleum ether) to yield the imine products **1a** or **1b** (Table 3.3). For best results, the silica gel column was first prepared with 10% triethylamine in petroleum ether and allowed to set for 24 h prior to flushing the column with the 1% triethylamine solution.

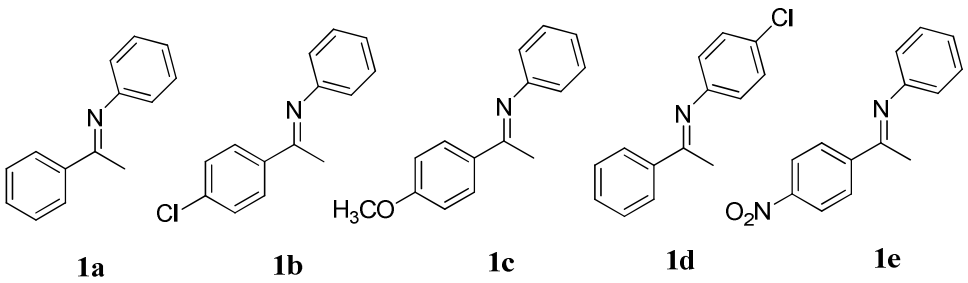
Method B. Acetophenone (25.6 mmol) and aniline (38.5 mmol) in the presence of molecular sieves (4 Å) were condensed in toluene (3 mL) using a microwave oven (Power 150) (Scheme 3.6, Table 3.2). After each heating cycle, aniline (25.6 mmol) was again added to the reaction and heated for another cycle. The progression of the reaction was monitored by GC-MS and TLC for the disappearance of acetophenone. The crude product was purified via chromatography (1% triethylamine in petroleum ether) to yield the imine product **1c** or **1d** (Table 3.3). For best results, the silica gel column was first prepared with 10% triethylamine in petroleum ether and allowed to set for 24 h prior to flushing the column with the 1% triethylamine solution. **(1d)** as a mix of E and Z isomers: R_f 0.89 (7:1, petroleum ether: EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$, δ_H) 7.88-7.84 (m, 2 H), 7.37-7.23 (m, 3 H), 7.22-7.13 (m, 2 H), 6.65-6.50 (m, 2 H), 2.13-2.12 (m, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$, δ_C) 166.2, 150.2, 139.2, 130.7, 129.0, 128.5, 128.4, 127.2, 120.8, 17.4.

Method C. Acetophenone (25.6 mmol) and aniline (38.5 mmol) in the presences of molecular sieves (4 Å) were condensed in toluene (3 mL) using a microwave oven (Power 150) (Scheme 3.6, Table 3.2). After the heating cycle, aniline (25.6 mmol) was again added to the reaction and heated for another cycle with the progression of the reaction being monitored by GC-MS and TLC for the disappearance of acetophenone. The crude product was purified via chromatography (1% triethylamine in petroleum ether) to yield the imine product **1e** (Table 3.3). For best results, the silica gel column was first prepared with 10% triethylamine in petroleum ether and allowed to set for 24 h prior to flushing the column with the 1% triethylamine solution.

(**1e)** as a mix of E and Z isomers: R_f 0.74 (7:1, petroleum ether: EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$, δ_H) 8.32-8.26 (m, 2 H), 8.16-8.05 (m, 2 H), 7.41-7.34 (m, 2 H), 7.17-7.10 (m, 1 H), 6.82-6.70 (m, 2 H), 2.29-2.28 (m, 3 H); ^{13}C NMR (250 MHz, $CDCl_3$, δ_C) 163.6, 150.8, 148.9, 145.0, 129.1, 128.1, 123.9, 123.5, 119.1, 17.5.



Scheme 3.6. Synthesis of imines 1a-1e.



1a **1b** **1c** **1d** **1e**

Figure 3.2. Imines synthesized (1a-1e).

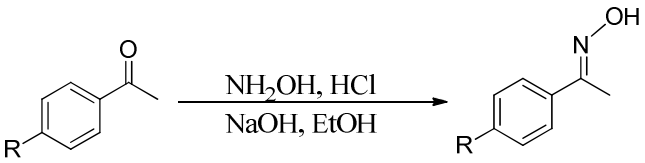
Product Number	R	R'	Reaction Temperature	Reaction Time	Procedural Method
1a	H	H	200 °C	15 min	A
1b	Cl	H	200 °C	15 min	A
1c	OCH_3	H	160 °C	15 min	B
1d	H	OCH_3	160 °C	15 min	B
1e	NO_2	H	160 °C	15 min	C

Table 3.2. Synthesis conditions of imines 1a-1e.

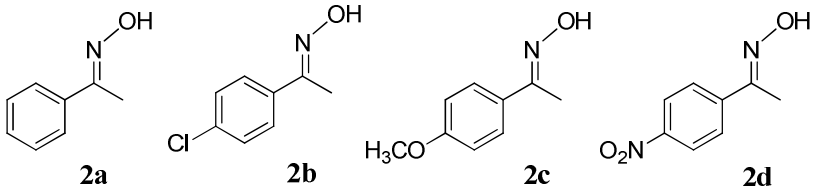
Product Number	R	R'	Product Mass	Yield %	Appearence
1a	H	H	1.2622 g	73	pale yellow solid
1b	Cl	H	1.5447 g	80	pale yellow solid
1c	OCH_3	H	1.1351 g	60	white soid
1d	H	Cl	3.0302 g	61	yellow solid
1e	NO_2	H	2.1406 g	43	bright yellow solid

Table 3.3. Synthesis yields of imines 1a-1e.

Oxime Synthesis. A mixture of acetophenone (37.0 mmol), hydroxylamine hydrochloride (57.8 mmol), 95% ethanol (10 mL) , and water (2 mL) was stirred at rt. To this mixture crushed sodium hydroxide pellets (7.26 g, 181.48 mmol) were added in small portions with vigorous stirring. The reaction was refluxed (5 min) and after cooling was poured into a solution of concentrated hydrochloric acid (6 mL) and water (40 mL). The resulting precipitate was vacuum filtered, washed with cold water (2 x 10 mL), and dried to yield oxime **2b**, **2c**, or **2d** (Scheme 3.7, Figure 3.3, Table 3.4).



Scheme 3.7. Synthesis of Oximes.



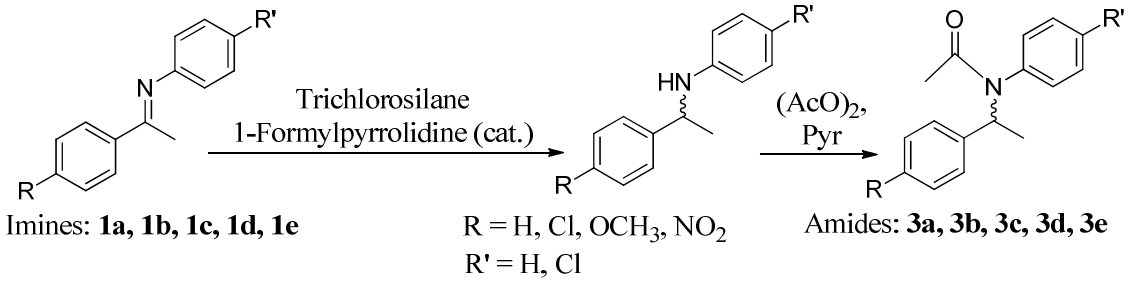
2a **2b** **2c** **2d**

Figure 3.3. Oximes (2a-2d).

Product Number	R	Product Mass	Yield %	Appearence
2a	H	purchased	purchased	white soild
2b	Cl	4.9989 g	99	white solid
2c	OCH_3	4.8623	97	white solid
2d	NO_2	4.9251 g	98	brown solid

Table 3.4. Synthesis yields of oximes 2a-2d.

Racemic Reductions of Imines. Cl_3SiH (1.5 mmol) was added to a solution of **1a**, **1b**, **1c**, **1d**, or **1e** (1.0 mmol), and 1-formylpyrrolidine (0.1 mmol) in dicholoromethane (5.0 mL). The resulting solution was allowed to let stir at rt for 5 days. The reaction solution was then neutralized with sodium bicarbonate (5 mL) and extracted with dichloromethane (2 x 50 mL). The organic layer was dried with Na_2SO_4 and concentrated to dryness. The resulting amine, aceticanhydride (2.0 eq), pyridine (2 mL), and dichloromethane (3 mL) were reacted in a microwave for 10 min at 60 °C. The resulting solution was quenched with water (10 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness. The crude product was purified via chromatography (5:1, petroleum ether/EtOAc) to yield amide **3a**, **3b**, **3c**, **3d**, or **3e** (Scheme 3.8, Figure 3.4, Table 3.5). This methodology was previously reported in the literature.[11]



Scheme 3.3. Example of racemic reduction and acylation process.

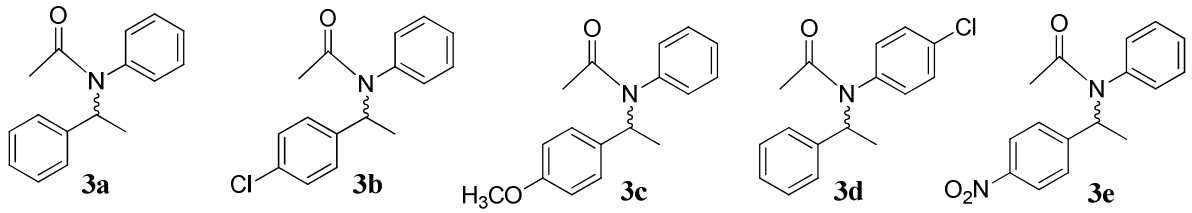
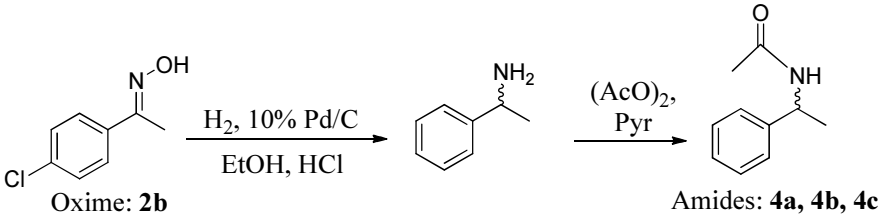


Figure 3.3. Amides 3a-3e.

Product Number	R	R'	Product Mass	Yield %
3a	H	H	0.0170 g	28
3b	Cl	H	0.1562 g	79
3c	OCH_3	H	0.1239 g	75
3d	H	Cl	0.1576 g	75
3e	NO_2	H	0.1155 g	98

Table 3.5. Racemic reduction and acylation of imines 1a-1e to form amides 3a-3e.

Racemic Reduction of Oximes. A solution of oxime **2b** (1.38 mmol) in ethanol (25 mL) and concentrated hydrochloric acid (1 mL) was hydrogenated at atmospheric pressure in the presence of 10% Pd/C (50 mg) for 24 h. The reaction mixture was filtered through Celite and concentrated to dryness. The product was then taken up in ethanol (25 mL) and again concentrated to dryness.[12] The resulting product, acetic anhydride (2.0 eq), and pyridine (2 mL) were reacted in a microwave for 10 min at 60 °C. The solution was quenched with water (10 mL) and extracted with dichloromethane (2 x 50 mL). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness. The crude product was purified via chromatography (5:1, petroleum ether/EtOAc) to yield 1-phenylethanamine as a racemic mixture (Scheme 3.9).



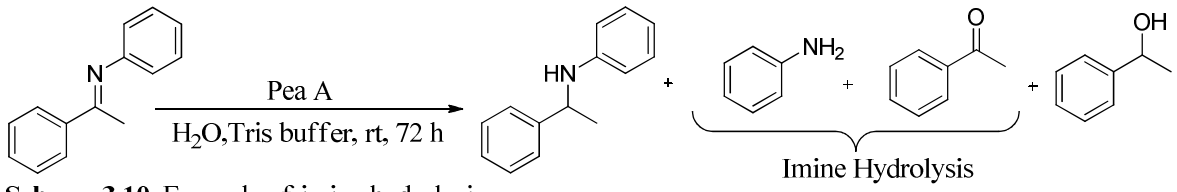
Oxime: **2b** Amides: **4a, 4b, 4c**

Scheme 3.9. Example of racemic oxime reduction and acylation process.

General Method for Biocatalytic Imine Reductions. Pea A, B, C, D, E, or F (25.0 g) was added to water (100 mL) and allowed to soak for 24 h (Table 3.6). The water was then decanted and imine **1a**, **1b**, **1c**, **1d**, or **1e**, tris buffer (10 mL), and water (40 mL) were added to the pea mixture and it was placed on an orbital shaker for 72 h. The liquid solution was decanted away from the peas and then neutralized with sodium hydroxide to pH 10. The solution was then extracted with ethyl acetate (2 x 50 mL) and the organic layer was separated, dried with Na_2SO_4, filtered and concentrated. The resulting products consisted of the respective acetophenone and aniline derivatives. The results of these hydrolysis reactions were confirmed by GC-MS and TLC (Scheme 3.10).

Pea	Common Pea Name	Family : *Genus : Species*
A	Pigeon Peas	Fabaceae : *Cajanus : cajan*
B	Black Beans	Fabaceae : *Phaseolus : vulgaris*
C	Navy Beans	Papilionaceae : *Phaseolus : vulgaris*
D	Garbanzo Bean	Fabaceae : *Cicer : arietinum*
E	Garden Peas (Split)	Fabaceae : *Pisum : sativum*
F	Black Eyed Peas	Fabaceae : *Vigna : unguiculata*

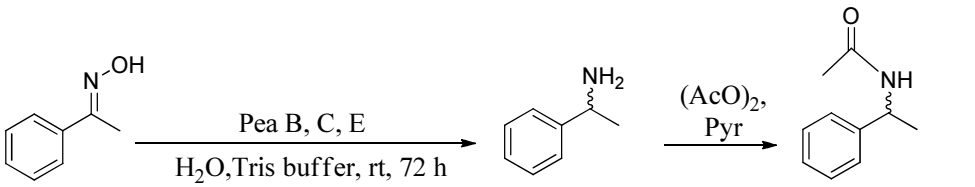
Table 3.6. Pea and beans seeds tested as potential biocatalysts.



Scheme 3.10. Example of imine hydrolysis.

General Method for Biocatalytic Oxime Reductions. Pea B, C, or E (25.0 g) was added to water (100 mL) and allowed to soak for 24 h (Table 3.6). The water was then decanted and oxime **2a**, tris buffer (10 mL), and water (40 mL) were added to the pea mixture and it was placed on an orbital shaker for 72 h. The liquid solution was decanted away from the peas and

then neutralized with sodium hydroxide to pH 10. The solution was then extracted with ethyl acetate (2 x 50 mL) and the organic layer was separated, dried with Na_2SO_4, filtered and concentrated. The resulting amine was confirmed by GC-MS and TLC. Next, the amine, acetic anhydride (2.0 eq), pyridine (2 mL), and dichloromethane (3 mL) were reacted in a microwave for 10 min at 60 °C. The resulting solution was quenched with water (10 mL) and extracted with dichloromethane (2 x 50 mL). The organic layer was dried with Na_2SO_4 and concentrated to dryness. The crude product was purified via chromatography (3:1, petroleum ether/EtOAc) to yield amides **4a**, **4b**, or **4c** (Scheme 3.11, Table 3.7). Enantiomeric excess was determined by Shimadzu LC-10 standard phase HPLC using low pressure gradient mixing and manual injection of 5 μL on a Ciracel AD-H column from Daicel Chemical Industires, LTD. All samples were resolved under AS-H, 40°C, 5% iPrOH/Hex, 1 mL/min conditions.



Oxime: **2a** Amides: **4a, 4b, 4c**

Scheme 3.11. Example of biocatalytic oxime reduction and acylation process.

Product Number	Pea	Product Mass	% Yield	% Conversion	% e.e.	Configuration
4a	B	0.0306 g	19	TBD	85	R
4b	C	0.0761 g	46	83	70	S
4c	E	TBD	TBD	72	95	TBD

Table 3.7. Biocatalytic reduction and acylation from oxime 2a to form amides 4a-4c.

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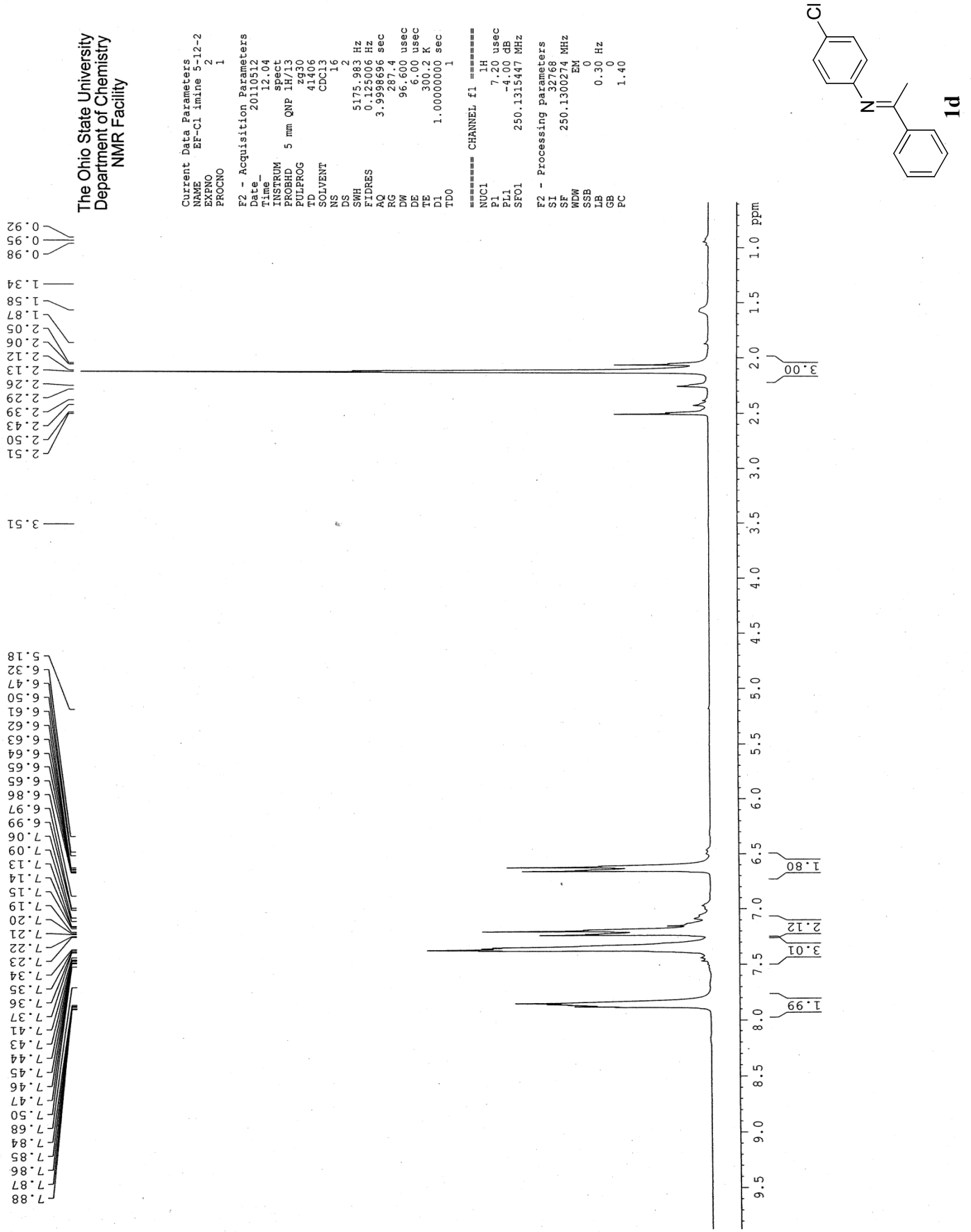
APPENDIX I: GC-MS METHOD PARAMETERS

OVEN
Initial temp: 50 'C (On) Maximum temp: 325 'C
Initial time: 0.00 min Equilibration time: 0.50 min
Ramps:
Rate Final temp Final time
1 20.00 250 6.00
2 0.0(Off)
Post temp: 0 'C
Post time: 0.00 min
Run time: 16.00 min
INLET (SPLIT/SPLITLESS) DETECTOR (NO DET)
Mode: Split Temperature: 250 'C (On)
Initial temp: 250 'C (On)
Pressure: 9.70 psi (On)
Split ratio: 100:1
Split flow: 119.2 mL/min
Total flow: 123.1 mL/min
Gas saver: On
Saver flow: 20.0 mL/min
Saver time: 2.00 min
Gas type: Helium
COLUMN SIGNAL
Capillary Column Data rate: 0.1 Hz
Model Number: Agilent 19091S-433E Type: col comp
HP-5MS 5% Phenyl Methyl Siloxane Save Data: Off
Max temperature: 325 'C Zero: 0.0 (Off)
Nominal length: 30.0 m Range: 0
Nominal diameter: 250.00 um Fast Peaks: Off
Nominal film thickness: 0.25 um Attenuation: 0
Mode: constant flow
Initial flow: 1.2 mL/min
Nominal init pressure: 9.71 psi
Average velocity: 40 cm/sec
Source: Inlet
Outlet: MSD
Outlet pressure: vacuum
COLUMN COMP
Use: Valve Box Heater
VALVES POST RUN
Post Time: 0.00 min
TIME TABLE
Time Specifier Parameter & Setpoint
MIKE 16 MIN.M Thu May 12 17:06:14 2011 Page: 2
GC Injector

Front Injector:
Sample Washes 1
Sample Pumps 2
Injection Volume 1.00 microliters
Syringe Size 10.0 microliters
PreInj Solvent A Washes 2
PreInj Solvent B Washes 2
PostInj Solvent A Washes 2
PostInj Solvent B Washes 2
Viscosity Delay 0 seconds
Plunger Speed Fast
PreInjection Dwell 0.00 minutes
PostInjection Dwell 0.00 minutes
Back Injector:
No parameters specified
Column 1 Inventory Number : AB001
MS ACQUISITION PARAMETERS

Solvent Delay : 2.50 min
EMV Mode : Gain Factor
Gain Factor : 1.00
Resulting EM Voltage : 1188
[Scan Parameters]
Low Mass : 40.0
High Mass : 700.0
Threshold : 150
Sample # : 1 A/D Samples 2
Plot 2 low mass : 50.0
Plot 2 high mass : 550.0
[MSZones]
MS Source : 230 C maximum 250 C
MS Quad : 150 C maximum 200 C

APPENDIX II: ^{1}H, ^{13}C NMR


The Ohio State University
Department of Chemistry
NMR Facility
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PROCNO 1
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Time_ 12.04
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PULPROG zg30
TD 41406
SOLVENT CDCl3
NS 16
DS 2
SWH 5175.983 Hz
FIDRES 0.125006 Hz
AQ 3.9998696 sec
RG 287.4
DW 96.600 usec
DE 6.00 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
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NUC1 1H
P1 7.20 usec
PL1 -4.00 dB
SFO1 250.1315447 MHz
F2 - Processing parameters
SI 32768
SF 250.1300274 MHz
WDW EM
SSB 0
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GB 0
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Cl
N
1d
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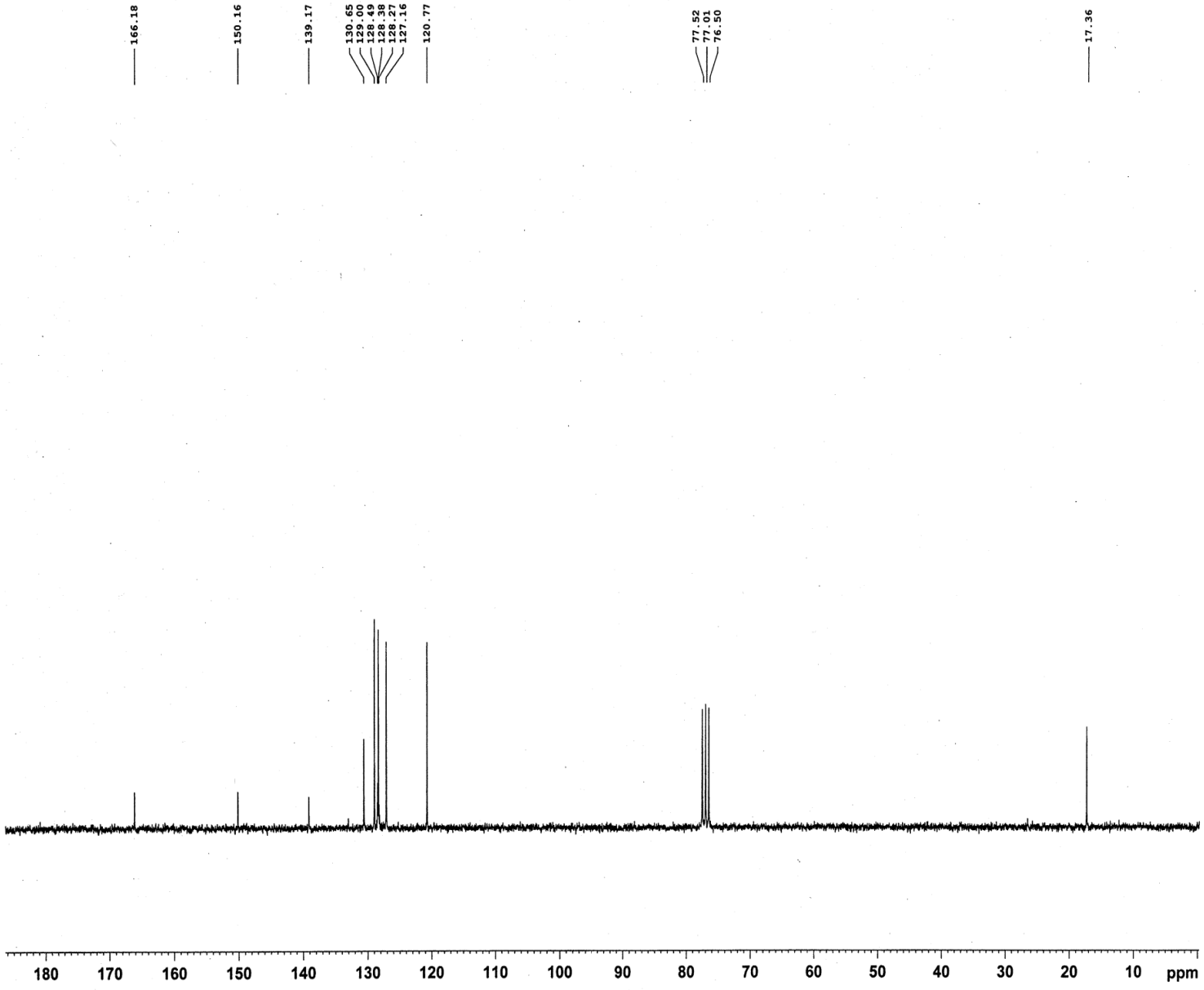
1.99

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AQ            1.0879476 sec
RG                  256
DW               33.200 usec
DE                6.00 usec
TE               300.2 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                   1

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P1                 8.00 usec
PL1                0.00 dB
SFO1         62.9015280 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PL2              -4.00 dB
PL12             16.00 dB
PL13            120.00 dB
SFO2        250.1310005 MHz

F2 - Processing parameters
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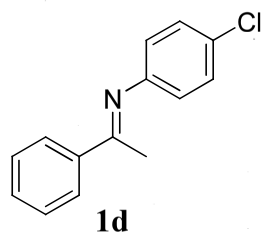

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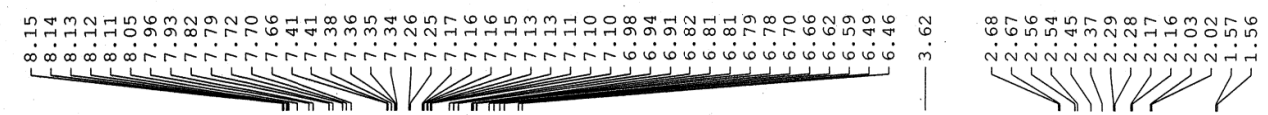

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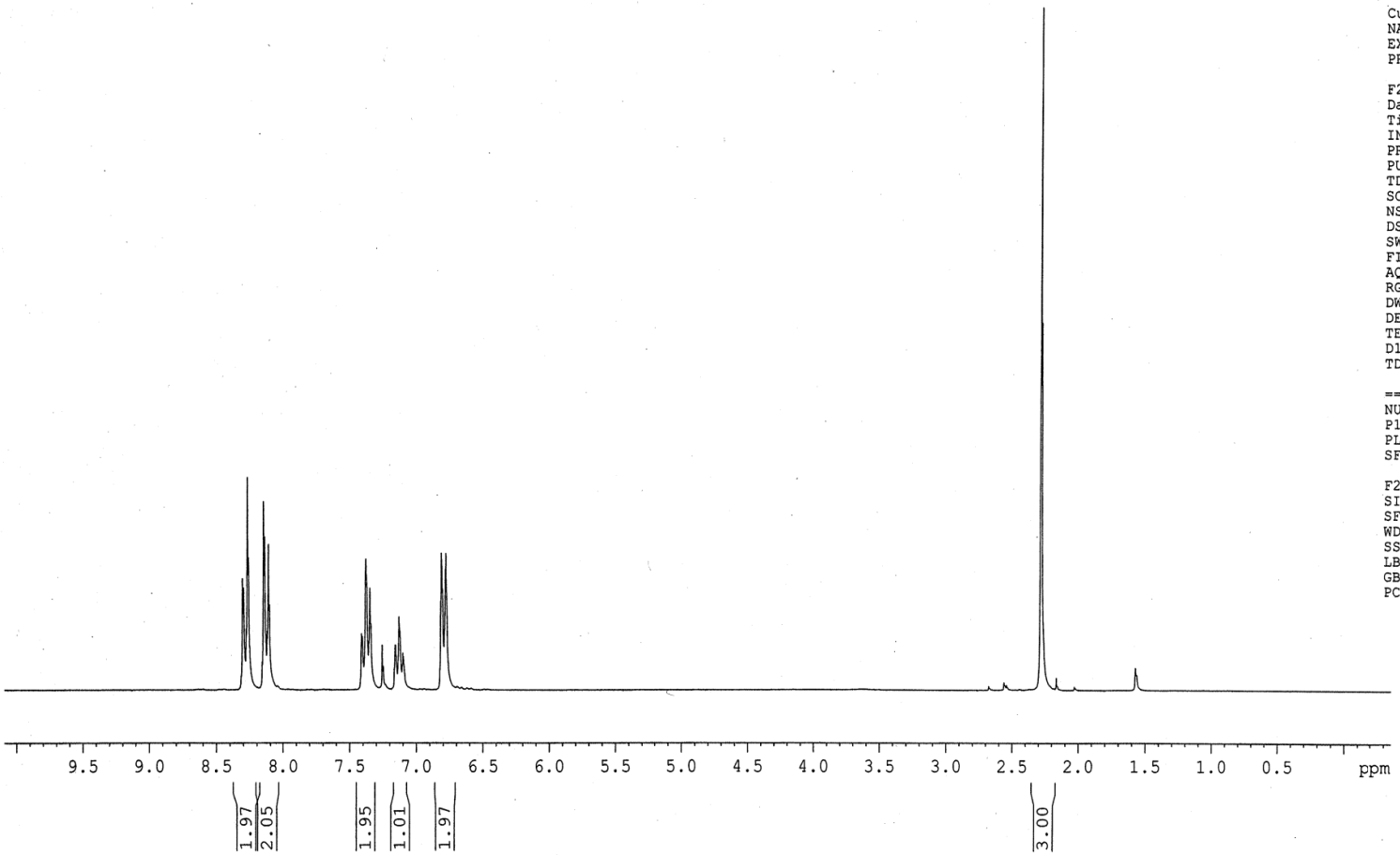
NMR Facility

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PL1              -4.00 dB
SFO1        250.1315447 MHz

F2 - Processing parameters
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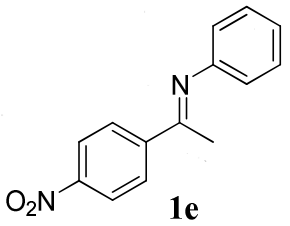
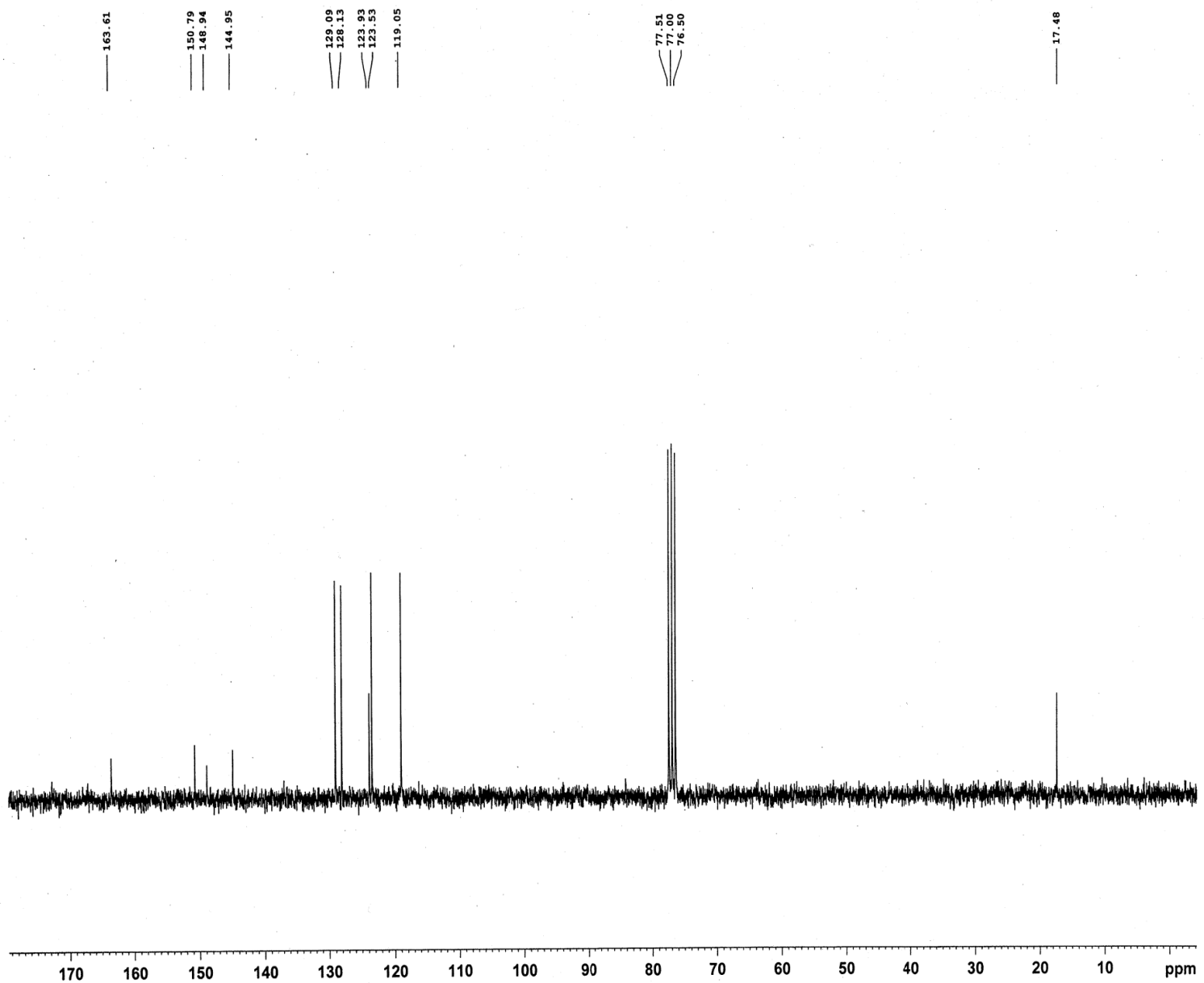
SSB                 0
LB              0.30 Hz
GB                  0
PC                1.00
```



1e



BRUKER

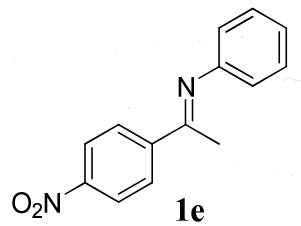
```
Current Data Parameters
NAME      EF-NO2 imine Carb 200 5-13
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110513
Time              13.21
INSTRUM           spect
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                32768
SOLVENT           CDCl3
NS                  200
DS                    4
SWH           15060.241 Hz
FIDRES         0.459602 Hz
AQ             1.0879476 sec
RG                  256
DW               33.200 usec
DE                6.00 usec
TE                300.2 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                8.00 usec
PL1               0.00 dB
SFO1         62.9015280 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2            80.00 usec
PL2              -4.00 dB
PL12             16.00 dB
PL13            120.00 dB
SFO2        250.1310005 MHz

F2 - Processing parameters
SI                32768
SF           62.8952386 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```



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