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Conversion of Racemic Ibuprofen to (S)-Ibuprofen

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DEDICATION

Dedico esta disertación a mi querida esposa Karina Lujan, a mis padres

Rosalía Flores y Marcos Chávez, a mis hermanos

Noel y Eliud,

y a mis hijos Valentina y Mateo

que son la más grande inspiración en mi vida.

CONVERSION OF RACEMIC IBUPROFEN TO (*S*)-IBUPROFEN

by

DAVID CHAVEZ-FLORES, M. S.

DISSERTATION

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ABSTRACT

Ibuprofen is a common Non-Steroidal Anti-Inflammatory Drug (NSAID) sold as a racemic or equal mixture of (*R*) and (*S*) mirror-image enantiomers. Without patent protection, ibuprofen is an orphan drug even though one enantiomer, (*S*)-ibuprofen, provides relief three times faster than its racemic mixture with fewer side effects. The goal of this research was to develop a facile, environmentally benign (no waste of ibuprofen or catalysts), and inexpensive process to convert a commercially available racemic mixture of ibuprofen to the therapeutically active (*S*)-ibuprofen.

After many attempts, the most successful conversion of racemic ibuprofen to (*S*)-ibuprofen was the *in situ* racemization and enantioselective hydrolysis (a dynamic kinetic resolution) of the methyl ester of racemic ibuprofen in the presence of *Candida rugosa* lipase and 20 % DMSO at pH 9.8. All reactions started with quantitative extraction of ibuprofen from inexpensive commercial pills. Both esters could be quantitatively isolated after Fischer esterification of racemic ibuprofen with the corresponding alcohol.

The kinetics of the dynamic kinetic resolution of the racemic methyl ibuprofen ester to (*S*)-ibuprofen were fit to a "consecutive reactions with a reversible step" model to give 0.02583 ± 0.0042 and 0.05253 ± 0.00454 h^{-1} rate constants for the racemization and hydrolysis steps, respectively. The hydrolysis under these conditions was twice as slow as most hydrolyses where no racemization was occurring. After 144 hours, 94 % of racemic ibuprofen by weight was converted to (*S*)-ibuprofen with an *ee* of 94 %.

To complete the purification, racemic ibuprofen was crystallized in methanol from the enriched product of the dynamic kinetic resolution to isolate (*S*)-ibuprofen in a 93.2 % yield and an *ee* of 99.7 %. Combining the dynamic kinetic resolution and crystallization steps, and considering that isolation of ibuprofen from pills and forming its racemic methyl ester is also quantitative, the overall conversion of (*S*)-ibuprofen from racemic ibuprofen pills, is 88 %, with the possibility of recovering all other reagents.

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CHAPTER I

INTRODUCTION

Ibuprofen, acetaminophen and aspirin are some of the most commonly used over-the-counter analgesics. Ibuprofen is the active ingredient in the brand name drugs Advil, Medipren, Motrin, Nuprin, PediaCare Fever, and Midiron. Ibuprofen belongs to the group of profen drugs that consist of propionic acids bearing various substituted aromatic groups at the 2-position, also named α-Arylpropanoic acids. All the α-Arylpropanoic acids possess a chiral center and are an important class of Non-Steroidal Anti-Inflammatory Drugs (NSAID) that exist as (*R*)- and (*S*)-enantiomers, which have been used for at least 3 decades. [**1**] The therapeutic efficacy of this class of drugs is well demonstrated by the introduction and extensive use of more than a dozen compounds exemplified by Ibuprofen, Naproxen, Ketoprofen and Flurbiprofen, to mention just a few, **Figure 1.1.**

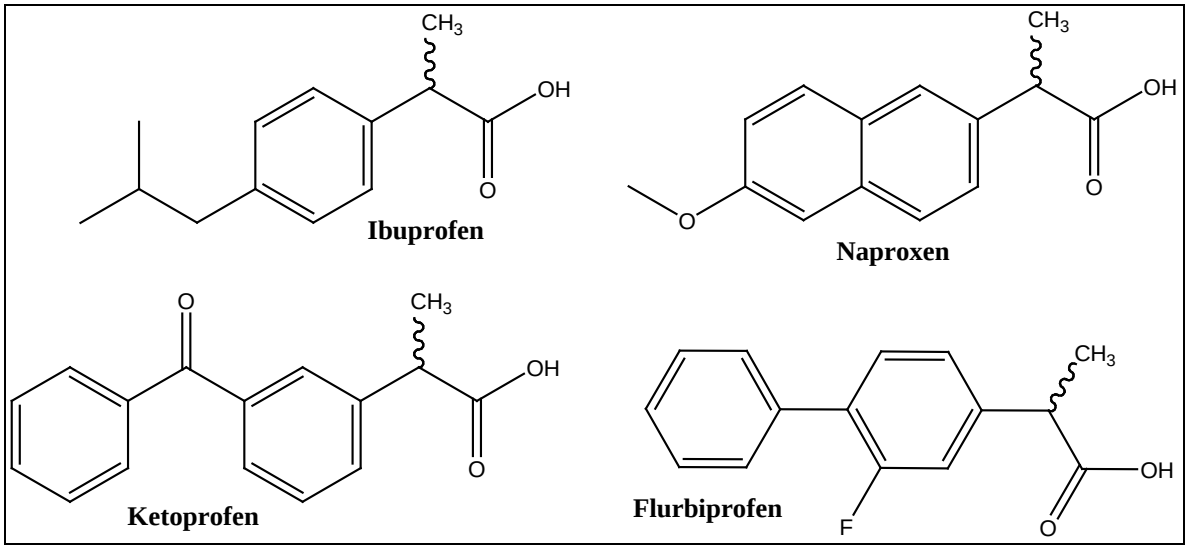
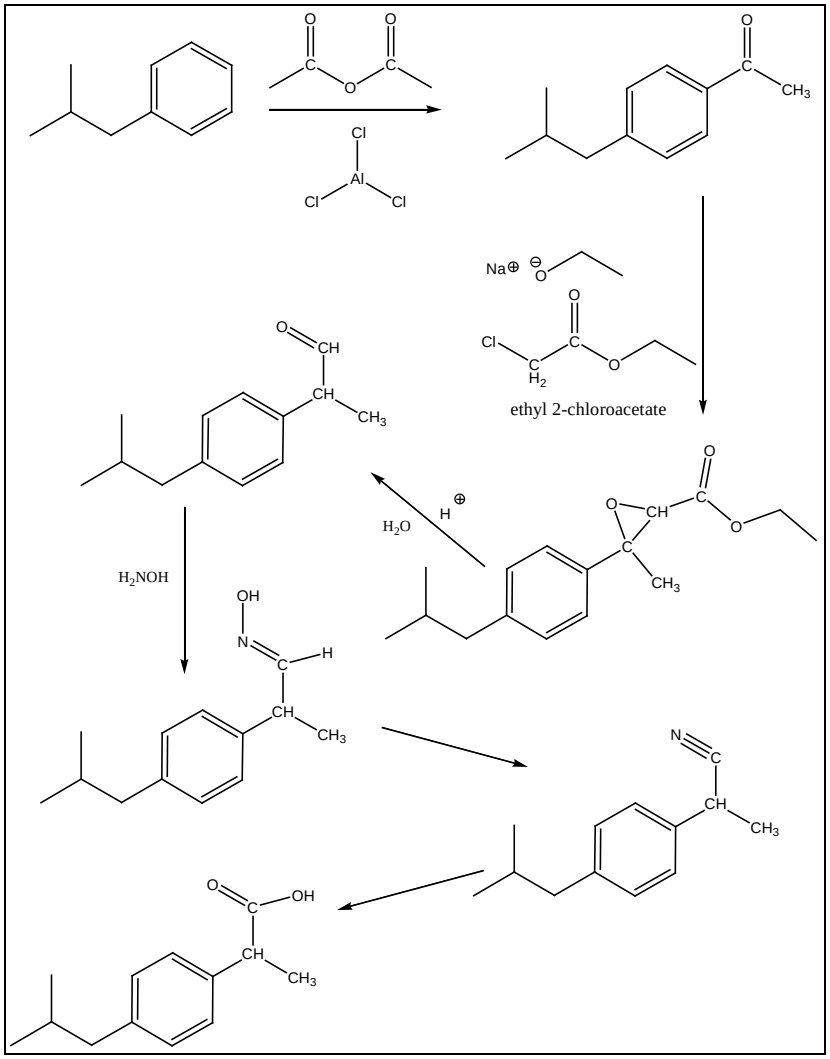


Figure 1.1. Chemical Structures of Racemic Ibuprofen, Naproxen, Ketoprofen and Flurbiprofen

Synthesis of ibuprofen was patented (U.S. Patent 3,385,886) by Boots Company PLC of Nottingham in the 1960s and became available on the drugstore shelves without prescription in the UK on May 1984 due to its patent expiration. Prior to its introduction, non-prescription pain relief was mainly provided by acetaminophen (introduced in 1955) and aspirin (marketed since 1899). [**2**] It was the first new over-the counter pain-relief medication to enter the marketplace in a generation. In spite of the 50 years since its discovery, its industrial preparation remains essentially the same, **Scheme 1.1.**



Scheme 1.1 Traditional Synthesis of Racemic Ibuprofen

Ibuprofen is commercially available as a racemic mixture (an equal mixture of (*R)* and (*S)*-enantiomers) and is commonly used for the treatment of rheumatoid arthritis. Gastrointestinal ulceration and hemorrhage are the most frequent side effects associated with the profen consumption. The (*R*)-enantiomer is often the major cause of side effects. [**3**] The biological activity of these compounds is known to reside almost exclusively in the (*S*)-enantiomer [**4 and 5**] as it has been demonstrated to be 100 times more active than (*R*)-ibuprofen. [**6]** Recently, the development of new techniques to separate or enrich the single (*S*)-enantiomer of ibuprofen have been claimed.

As non-super imposable mirror images, enantiomers of a racemic mixture have identical properties in a symmetric environment, but different properties in an asymmetric or chiral environment such as biological systems. It is common that enantiomers have different therapeutic effects, potency, pharmacological activity and pharmacokinetic profiles since the receptors with which they interact in a biological system are also chiral. Within biological systems, the metabolism of one enantiomer may be via a different pathway or occur at a different rate from that of the other enantiomer. The enantiomers from a racemic mixture may have nearly identical qualitative pharmacological activity, but quantitatively different potency. [**7**]

Thalidomide is the most common example as to the importance of realizing the different therapeutic effects of enantiomers. Thalidomide is a chiral molecule, its racemic mixture, **Figure 1.2,** was prescribed as a sedative and to prevent morning sickness in pregnancy during the late 1950's and the earlier 1960's in almost 50 countries including West Germany and Britain, where it became popular. In the mid 1960's it was

realized that the drug causes congenital disorders; when taken in early pregnancy. About 20% of fetus presented phocomelia (defective development of the limbs) and other deformities. 5000 to 10000 such babies were born. It was reported that (*R*)-Thalidomide was the effective enantiomer against morning sickness and that the (*S*)-enantiomer was the cause of the tetarogenic effects. Although it is an important starting point in the discussion of the different biological activity of enantiomers, which led to legislation, the fact that Thalidomide enantiomers have been demonstrated to easily interconvert or racemize under physiological conditions [**8 and 9**], it is doubtful that administering only the (*R*)-enantiomer would have avoided the Thalidomide tragedy.

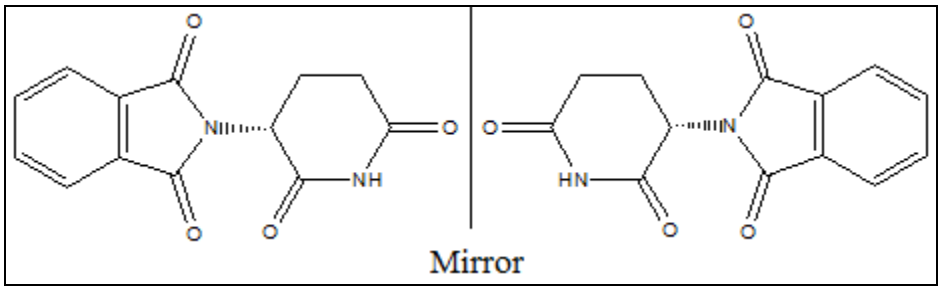


Figure 1. 2. Thalidomide Enatiomers

Recently the interest of pharmacological and toxicology activity of the enantiomers of chiral drugs has been increasing. This interest in drug stereochemistry has resulted from the considerable advances in the synthesis, analysis and separation of chiral molecules, together with an increased appreciation of the potential significance of the different biological properties of the chiral drugs administered as racemates. Thus, the sale of pure enantiomers has gained full acceptance as shown by the substantial number of single isomer pharmaceuticals. [**10 and 11**] The motivation for this single isomer

trend has been provided in part by the FDA (Food and Drug Administration) and in part by the production of a host of single enantiomer pharmaceuticals previously protected as racemic mixtures by patents that may last around 20 years. FDA requirements include:

- pharmacological properties of the individual enantiomers and of the racemic mixture
- assays which determine enantiomeric purity
- the need to produce as single isomer
- economic incentives to develop separation methods for existing racemic mixtures

Those particular chiral drugs, whose patents are expiring, are attracting a multitude of oversea producers. This would provide pricing competition and increase the "generic brand" availability from producers with large scale capacities. [**12**] Today exist a considerable number of drugs, which are eligible for a racemic to single enantiomer switch as shown in **Table 1.1.** In the last years several racemic mixtures have been reformulated in to their respective single enantiomer drugs that are now on the market. This is a method used by companies to extend a patent for several years.

As an example of a racemic switch, omeprazole is a proton pump inhibitor that blocks the enzyme in the wall of the stomach that produces acid. This drug was patented by AstraZeneca on 1989 under the names Losec® or Prilosec®. Omeprazole contains a tricoordinated sulfur atom in a pyramidal structure and therefore can exist in equal amounts of both the (*S*)- and (*R*)-enantiomers, **Figure 1.3.** [**13**]

Table 1.1. Candidates for Racemic to Single Enantiomer Switch. [14]

Cardiovascular		***Anti-inflammatory and analgesic***	
Acebutolol	Alprenolol	Cicloprofen	Corticosteroids
Atenolol	Betaxolol	Dihydroxythebaine	Feribufen
Bisoprolol	Bopindolol	Fenoprofen	Flurbiprofen
Bucumolol	Bufetolol	Ibuprofen	Indoprofen
Bufuralol	Bunitrolol	Ketoprofen	Minoxiprofen
Bupranolol	Butofilolol	Pirprofen	Suprofen
Carazolol	Carvedilol	Triamcinolone	
Disopyramide	Dobutamine		
Indenolol	Mepindolol	***Anticancer***	
Meupranolol	Metoprolol	Cytarabine	
Nadolol	Nicardipine		
Oxprenolol	Pindolol	***Antibitoics, anti-infectives, and anti-virals***	
Propranolol	Sotalol	Ciprofloxacin	Norfloxacin
Toliprolol	Verapamil	Ofloxacin	
Xibenolol			
Central Nervous System		***Hormones and genitourinary***	
Dobutamine	Fluxetine	Benzyl glutamate	Butoconazole
Ketamine	Lorazepam	Calcitonin	Estradiol
Meclizine	Oxaprotiline	Fluorogestone	Gonadorelin
Phenylpropanolamine	Thioridazine	Norgestrel	Testosterone
Polychloramphetamine	Tomoxetine		
Toloxatone	Viloxazine	***Antihistamines and cough-cold***	
		Astemizole	Terfenadine
Respiratory			
Albuterol	Metaproterenol		
Terbutaline			

Source: Technology Catalysts International

A study demonstrated that the treatment with (*S*)-omeprazole (esomeprazole) resulted in higher values in the blood of the two main metabolites (5-hydroxy and sulphone) than with either (*R*)-omeprazole or racemic omeprazole after both single and repeated doses due to a lower metabolic rate of (*R*)-omeprazole than (*S*)-omeprazole and, consequently, racemic omeprazole. Since February of 2001, AstraZeneca marketed the enantiopure (*S*)-omeprazole as Nexium®. AztraZeneca patented Nexium® just a few months before the expiration of the patent of the racemic mixture, Prilosec®.

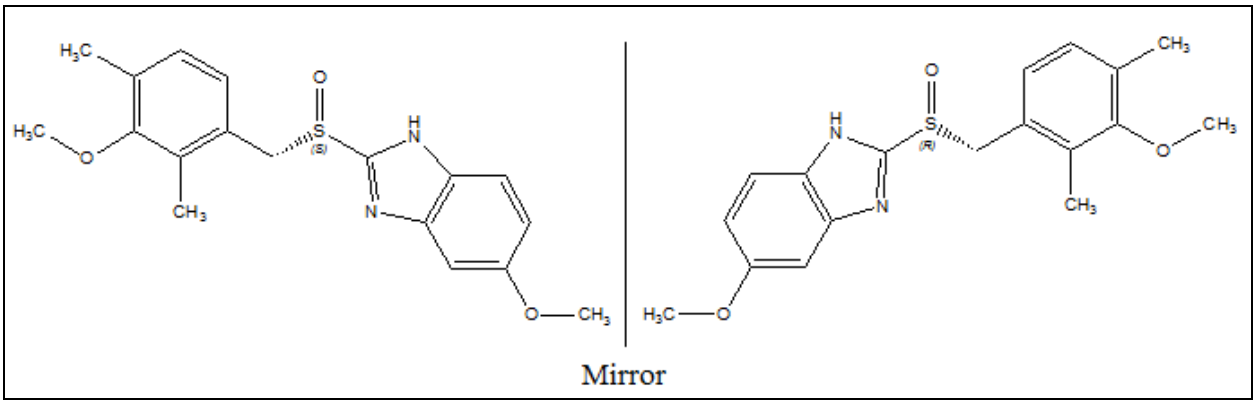


Figure 1.3. (*R*,*S*)-Omeprasole Enantiomers

Albuterol or salbutamol is a short acting β_2-adrenergic receptor agonist, "first choice" or preferred as a treatment for both the relief of acute symptoms and the prevention of exercised-induced bronchial spasms. [**15**] This compound was marketed in 1968 by Allen & Hanburys under the brand name Ventolin®. It has been established that the bronchodilating effect resides with the (*R*)-albuterol enantiomer and the nonactive enantiomer is (*S*)-albuterol, these are referred to as the eutomer (the active enantiomer) and distomer (the less active enantiomer), respectively, **Figure 1.4.** [**16**] There are several reports indicating that (*S*)-albuterol induces airway hyperreactivity and may cause adverse effects in asthmatics possibly leading to fatalities. *(R)*-Albuterol inhibits histamine release from human basophils whereas (*S*)-albuterol may increase histamine release. [**17**] This difference in metabolic rate of sulfation and elimination when the racemate is given may result in accumulation of (*S*)-albuterol and subsequent increased bronchial reactivity without bronchodilatary protection. Today enantiopure (*R*)-albuterol can be purchased by prescription. It was released by Sepracor Inc. on January of 2002 under the commercial name Xopenex®. Some studies demonstrated that there is

no enhancement of activity on (*R*)-albuterol than the racemic mixture but it was demonstrated that side effects including tachycardia, tremors, and nervousness can be avoided by taking just the (*R*)-enantiomer.

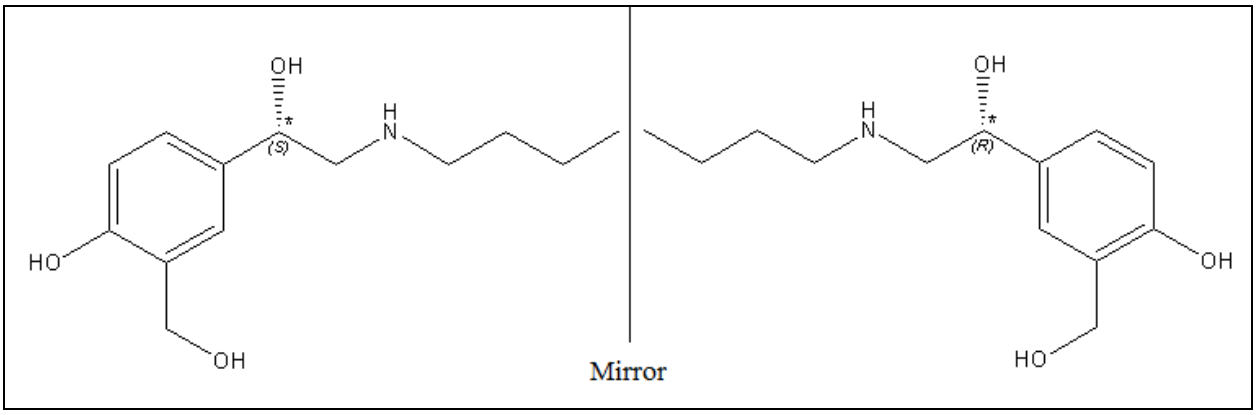


Figure 1.4 . (*R*,*S*)-Albuterol Enantiomers

Another example is the histamine H_1-receptor antagonist cetirizine (Zyrtec®). Zyrtec is the racemic mixture of levocetirizine and dextrocetirizine**, Figure 1.5.** This commercial drug is used in patients with urticaria and allergic rhinitis. Optically pure cetirizines show different pharmacological effects. Dextrocetirizine is more useful for the treatment of urticaria and levocetirizine is more useful for the treatment of allergic disorders while avoiding the adverse effects associated with the racemic mixture of cetirizine. [**18**] The reason of the pharmacological difference is not known yet. In Europe and the USA, the desired enantiomer for treatment of allergies is now available under the trademark Xyzal®. Some studies have demonstrated that Xyzal is up to 4 times more potent than the racemic mixture. Side effects such as dry mouth, wheezing,

coughing, urticarial eruptions, and hypersensitivity are diminished substantially when the pure enantiomer is administered.

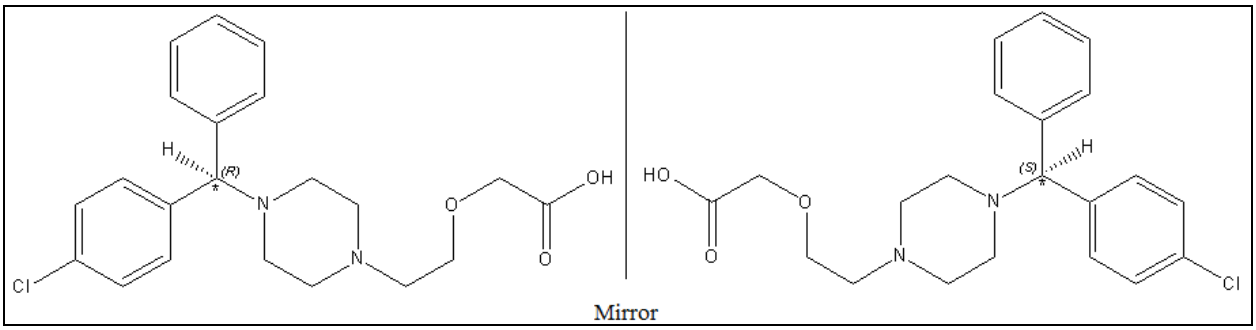


Figure 1.5. (*R*,*S*)-Cetirizine Enantiomers

Enantiopure drugs represent a large share of the pharmaceutical market. Global revenues from chiral technologies soared from $6.63 billion in 2000 to $16.03 billion in 2007, growing at a compound annual rate of 13.4% during that period. Approximately 80% of all products currently in development for the pharmaceutical industry are based on chiral building blocks. [**19**] Below, **Table 1.2** outlines the top ten drugs sold in the United States during 2008, all of which are chiral.

In the last 2 decades, the synthesis and/or separation of chiral drugs and their intermediates have received much attention. Besides synthesizing chiral drugs from natural chiral building blocks (e.g., carbohydrates and amino acids) or chiral auxiliaries, the most common procedures for enantiomer separation are formation of diasteromeric salts of chiral amines or carboxylic acids and crystallization, chiral chromatography, enzymatic catalytic resolution (kinetic resolution), and lately by dynamic kinetic resolution. [**20, 21, 22 and 23**]

Table 1.2 Top Ten of Chiral Drug Sales in 2008

Top 10 drugs by sales in 2008					
Rank	**Product**	**Company**	**Therapeutic Subcategory**	**Form of Ingredient**	**WW sales ($ millions)**
1	Lipitor	Pfizer + Astellas + Almirall	Anti-hyperlipidaemics	Single enantiomer	13,507
2	Plavix	BMS + Sanofi-Aventis	Platelet aggregation inhibitors	Single enantiomer	9,447
3	Advair	GlaxoSmithKline	Other bronchodilators	Single enantiomer	7,828
4	Enbrel	Wyeth + Amgen + Takeda	Other anti-rheumatics	Recombinant product	6,455
5	Diovan	Novartis + Ipsen	Angiotensin II antagonists	Single enantiomer	5,825
6	Rituxan	Roche	Anti-neoplastic MAbs	Monoclonal antibody	5,481
7	Remicade	SGP + J&J + Mitsubishi Tanabe	Other anti-rheumatics	Monoclonal antibody	5,293
8	Nexium	AstraZeneca	Antacids & anti-ulcerants	Single enantiomer	5,200
9	Epogen/Procrit	J&J + Amgen + Kirin	Anti-anaemics	Recombinant product	5,162
10	Avastin	Roche	Anti-neoplastic MAbs	Monoclonal antibody	4,818

Due to its operational simplicity the majority of industrial scale syntheses, employ a kinetic resolution via enantioselective enzymatic reactions to separate the desired enantiomer from the racemic mixture. [**24**] However, this methodology has the inherent disadvantage of limiting the yield to a maximum of 50%, which can have an impact on the environment and on the economic viability of the procedure due to the production of 50% waste. [**25**]

The disadvantage of a maximum 50% yield of the desire enantiomer and a 50 % yield of "waste" has lead to the work described in this dissertation on the racemization or conversion of the undesired enantiomer to the desired enantiomer, in the case of ibuprofen, in-situ conversion of (*R*)-ibuprofen to (*S*)-ibuprofen. This conversion has been previously attempted by many methods.

One approach involved the enzymatic resolution of ibuprofen enantiomers, separation of (*S*)-ibuprofen, followed by brute force racemization of (*R*)-ibuprofen by at least an 8 hour reflux in a 1:1 solution of dimethylsulfoxide (DMSO) and 2 M NaOH. These harsh reaction conditions are required to deprotonate the α-position of a carboxylate salt to form a planar double enolate intermediate. This method is inefficient by requiring the geometric repetition of the resolution and racemization steps until "enough" (*S*)-ibuprofen has been produced and by the complexity of the experiments for an industrial scale. [**26**]

Other research groups have attempted to produce pure (*S*)-profens by enzymatic hydrolysis of the racemic mixture of their trifluoroethyl thioesters (such as that of naproxen and suprofen) with *in situ* racemization catalyzed by organic bases. Up to this point, the resulting (*S*)-profens were produced in low conversion and with low enantiomeric purity because the bases can also hydrolyze the esters to profens without enantioselectivity. Besides the expense and complexity of reagents that mimic in vivo sulfur containing isomerases, the other major limitation of these reactions is that the enzyme must be encapsulated to avoid degrading it under harsh pH conditions. [**27 and 28**]

This dissertation describes several attempts and eventual success to overcome the 50 % yield limitation of traditional kinetic resolutions by *in situ* racemization. These techniques are referred to as dynamic kinetic resolutions. The goal of this research was to develop a facile, environmentally benign (no waste of ibuprofen or catalysts), and

inexpensive process to convert a commercially available racemic mixture of ibuprofen to the therapeutically active (*S*)-ibuprofen.

CHAPTER 2

RESULTS

After initial isolation from commercial pills, racemic ibuprofen was converted to racemic ibuprofen esters under different conditions. The esters were subjected to different pHs and solvent conditions in the presence of *Candida rugosa* lipase to induce *in situ* racemization and enantioselective hydrolysis, converting most of the racemic ibuprofen to (*S*)-ibuprofen in high enantiomeric excess. Pure (*S*)-ibuprofen was isolated in high yield. Other attempts to convert racemic ibuprofen to pure (*S*)-ibuprofen are also briefly described.

2.1 Ibuprofen Extraction

Ibuprofen was extracted with acetone from commercial ibuprofen tablets (200 mg/pill Member's Mark) because it was readily available, inexpensive ($12 per gram Aldrich, 5 ¢ per gram Sam's Club) and of the quality available to the consumer. The active ingredient (ibuprofen) was quantified to be on average as advertised. For example, 5.964 g of ibuprofen were isolated from 30 200 mg pills, a 99.4 % recovery, **Figure 2.1.** Chiral HPLC was used to analytically separate enantiomers and to verify the racemic composition of ibuprofen.

Figure 2.1 Commercial Ibuprofen Tablets, Extracted Ibuprofen and Racemic Ibuprofen Chiral HPLC Chromatograph

2.2.1 Racemic Ibuprofen Esters Synthesis

Initially, the methyl ester of racemic ibuprofen was synthesized from the acid chloride of ibuprofen, **Figure 2.2.** Thionyl chloride and racemic ibuprofen were reacted to produce the acid chloride followed by evaporation of excess thionyl chloride. The resultant acid chloride was reacted with methanol producing the methyl ester, which was isolated by extraction with dichloromethane and solvent evaporation to give a 93 % yield.

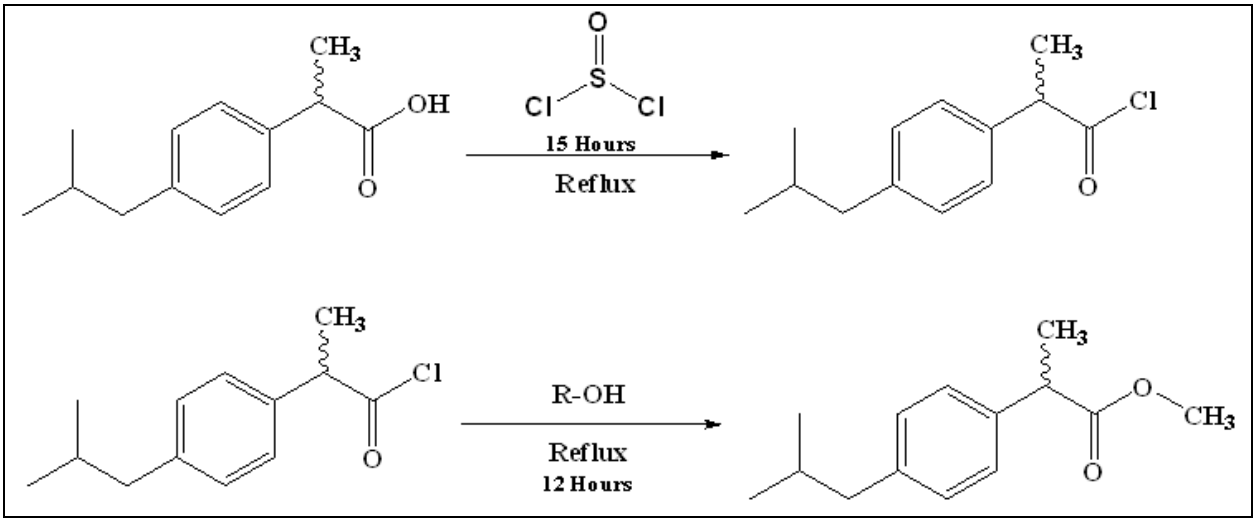


Figure 2.2. Traditional Racemic Ibuprofen Ester Synthesis

In order to avoid the caustic conditions of the acid chloride route, the racemic ibuprofen esters were also synthesized by Fischer esterification. For all the reactions, into a 100 mL round bottom flask was added 10 mmol of racemic ibuprofen, 0.5 mL of concentrated H_2SO_4 and 40 mL of different alcohols. The reactions were stirred at 40 °C for 5 hours and were monitored by chiral HPLC.

Each ester required a different purification method. In the methyl ester case, hexanes extraction was sufficient to purify the product because methanol is insoluble in this solvent. In all other cases, more hexanes (80 mL) and washing with water was necessary because larger alcohols are amphiphilic or soluble in both polar and non-polar phases. A rotatory evaporator was used to remove the hexanes and volatile alcohols, and the purification was completed by bulb-bulb distillation. The isolation of all esters by the Fischer reaction was quantitative.

Chiral HPLC analysis confirmed that all the esters were pure but only the enantiomers of the propyl ester were baseline resolved, **Figure 2.3.** All esters, being less polar than ibuprofen, eluded closer to the void time, **Figure 2.4.**

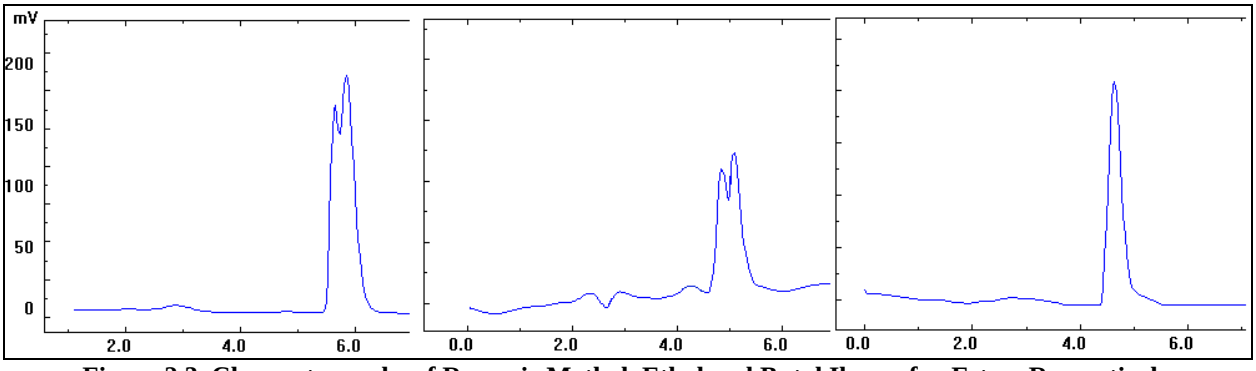


Figure 2.3. Chromatographs of Racemic Methyl, Ethyl and Butyl Ibuprofen Esters Respectively

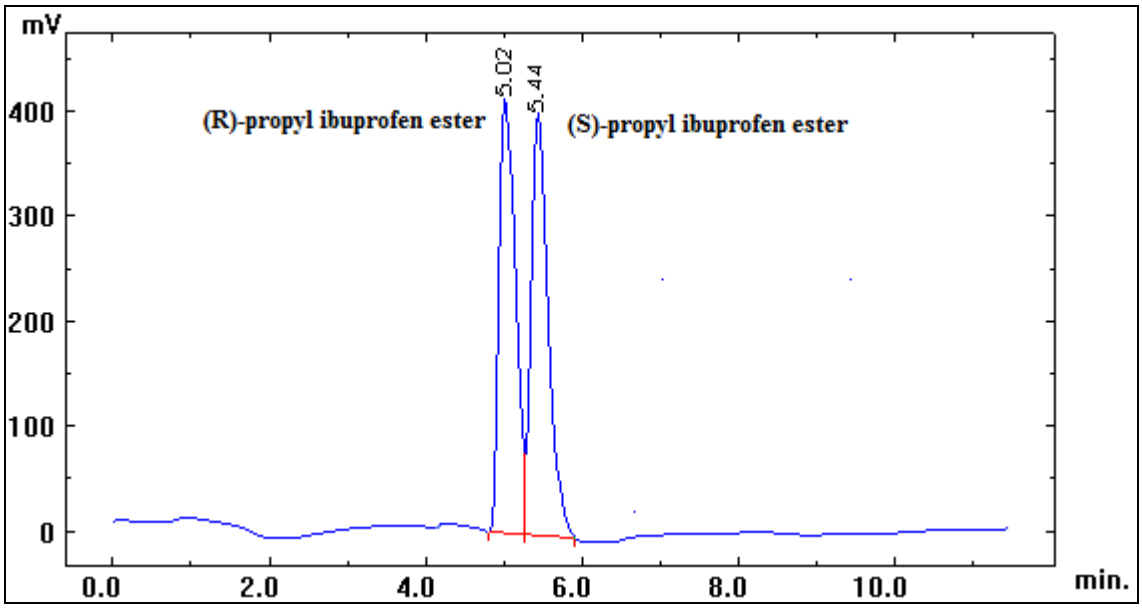


Figure 2.4. Chromatograph of Racemic Propyl Ibuprofen Ester

2.2.2 Kinetic Resolution of Racemic Methyl Ibuprofen Ester

Initially *in situ* racemization of ibuprofen esters was attempted with the methyl ester under different pH conditions. Though not successful, this was also the method of separating ibuprofen enantiomers that would be used for further studies.

The methyl ester of racemic ibuprofen was hydrolyzed by *Candida rugosa* lipase in a buffered aqueous media at 40 °C and different pH (5.3, 7.2 and 9.8) . The reaction was monitored at different time intervals. Alkaline samples were acidified with 0.25 M HCl solution. All samples were extracted with hexanes and analyzed by chiral HPLC. The enantiomeric excess (*ee*) of the ibuprofen product was calculated by **Equation 2.1.**

$$ee = \frac{[S]-[R]}{[S]+[R]} = \text{ enantiomeric excess of product}\ldots\ldots\ldots\ldots\ldots\ldots\textbf{(2.1)}$$

The percent composition of each compound, was calculated directly from the chiral HPLC spectral areas, since the molar absorptivity of (*R*)-ibuprofen, (*S*)-ibuprofen and the ester products are equal because phenyl is the major chromophore at the 256 nm wavelength of the UV detector. [**32**]

The Enantiomeric Ratio (*E*) describes how well the enzyme discriminates between the enantiomers of a substance under given reaction conditions as given in **Equation 2.2,**

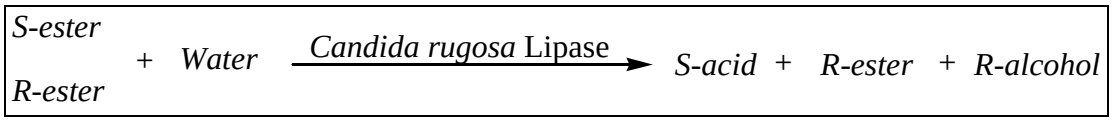
where conversion (*c*) was determined by **Equation 2.3** and *ee* is the enantiomeric excess of the product in decimal form.

$$E = \ln[(1-c)(1-ee)]/\ln[(1-c)(1+ee)]\textbf{.....(2.2)}$$

$$c = 1 - \frac{[S_{ester}]+[R_{ester}]}{[S_{ester}]_0+[R_{ester}]_0} = \text{Conversion}\ldots\ldots\ldots\ldots\ldots\ldots\textbf{(2.3)}$$

At all pHs, mostly the (*S*)-ibuprofen ester was hydrolyzed to (*S*)-ibuprofen and no major *in situ* racemization was observed, **Scheme 2.1.** Though this hydrolysis reaction

involves two substrates, water and the racemic methyl ibuprofen ester, because water is in excess, the reactions behaved like a single substrate mechanism and look linear (time is proportional to the conversion), **Figure 2.5.** Statistically the differences in the rate and the enantioselectivities (*E*) of the reactions at different pHs were not significant, **Table 2.1.**



Scheme 2.1 Kinetic Resolution of Racemic Ibuprofen Ester

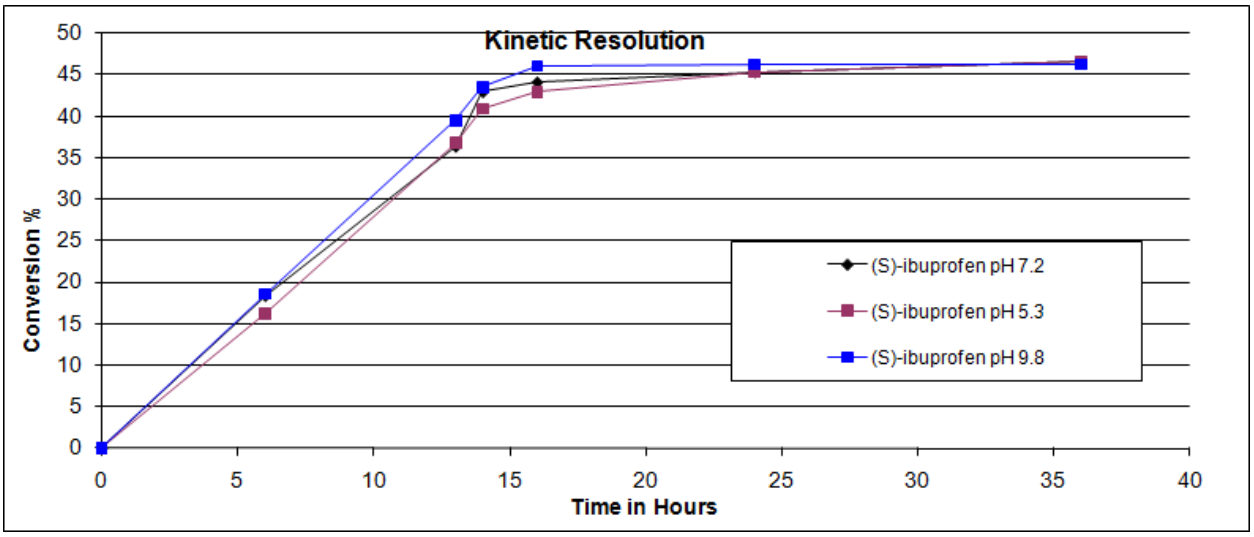


Figure 2.5. Enzymatic Enantioselective Hydrolysis of Racemic Ibuprofen at Different pH

For the hydrolysis of the racemic methyl ibuprofen ester at different pHs, *E* values are given in **Table 2.1**. Although several studies of the kinetics of *Candida rugosa* lipase reactions have been carried out [**33 and 34**], the most common procedure is the use of the *pseudo*-first order model [**35**]. Due to the high enantioselectivity in other and this

ibuprofen ester hydrolysis reactions [**36**], it can be assumed that only the (*S*)-methyl ibuprofen ester was hydrolyzed.

Under this hypothesis, using data from **Table 2.1,** different kinetics were tested, and the hydrolysis of the racemic methyl ibuprofen ester under the optimal conditions (40 ºC, barometric pressure and stirring magnet agitation) was fitted to first-order kinetics, time versus ln[(*S*)-ibuprofen], **Figure 2.6.**

Table 2.1. Time, Conversion and Enantiomeric Excess Values for the Kinetic Resolutions at Different pH

KR at pH 5.3				**KR at pH 7.2**				**KR at pH 9.8**			
Time	***Conversion***	***ee***	***E***	**Time**	***Conversion***	***ee***	***E***	**Time**	***Conversion***	***ee***	***E***
0	0			0	0			0	0		
6	16.254	97.6	5	6	20.648	99.2	6	6	18.579	94.4	5
13	36.768	94.5	13	13	40.261	98.6	17	13	39.515	96.8	19
14	40.912	94.2	19	14	42.908	98.3	32	14	43.474	97.9	34
16	42.932	93.8	25	16	42.974	97.5	36	16	46.013	96.7	50
24	45.276	93.8	36	24	45.285	95.8	40	24	46.200	96.6	52
36	46.573	93.7	49	36	46.564	95.5	54				

The hydrolysis reaction for racemic methyl ibuprofen ester at pH 9.8 is properly described by *pseudo*-first order kinetics, **Equation 2.4 and Equation 2.5,** as indicated by a linear least-squares fit with goodness of fit $R^2 = 0.9753$. The *pseudo* first-order rate constant (k) was found to be $0.0975 \pm .0012 h^{-1}$ from the slope of the fit, **Equation 2.6**.

$$-d[(S)-Ibuprofen]/dt = k[(S)-Ester]_{To} \quad \textbf{.....(2.4)}$$

$$\ln[(S)-Ibuprofen]_{Tx} = -kt + \ln[(S)-Ester]_{To} \text{ ……(2.5)}$$

$$y = 0.0961x + 2.3725 \text{ ……..……..(2.6)}$$

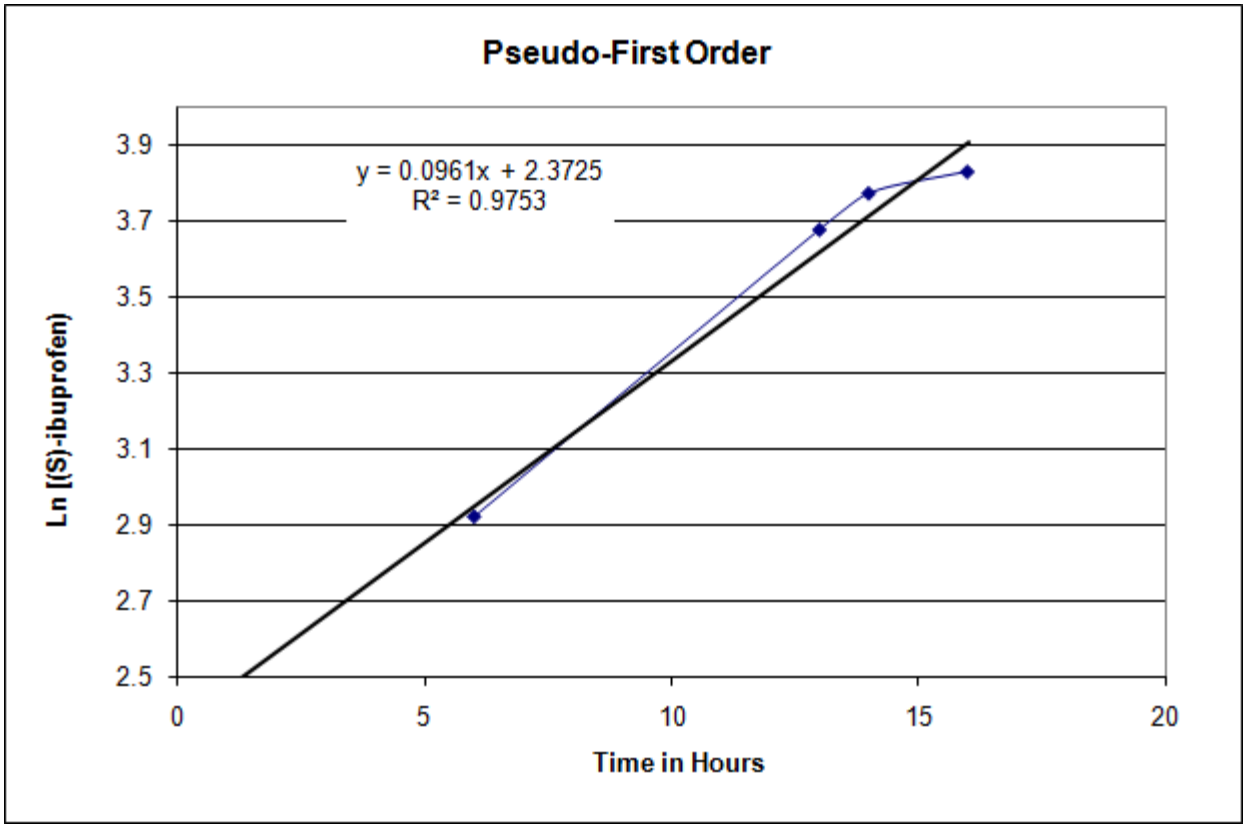


Figure 2.6 Determination of the *Pseudo*-First-Order-Kinetic

2.2.3 Addition of DMSO as Co-Solvent to Increase the Enantioselectivity of *Candida rugosa* Lipase

The addition of ionic liquids such as DMSO has been reported to enhance the enantioselectivity of *Candida rugosa* lipase towards hydrolysis of chiral esters. [**37**] The addition of polar compounds can change the lipase conformation from the less hydrophobic closed form to a more hydrophobic open form favoring the binding of the hydrophobic substrate to the active site of the enzyme, **Figure 2.7.** [**38**] This enhanced enantioselectivity has been confirmed for ibuprofen by adding DMSO (20 % volume) to the previously described hydrolysis reactions under neutral conditions. For example, after 45 % conversion, the *ee* of ibuprofen obtained was 98 % versus 96 %, with and without DMSO added, respectively (**Figure 2.8**) without decreasing the general reactivity of the enzyme.

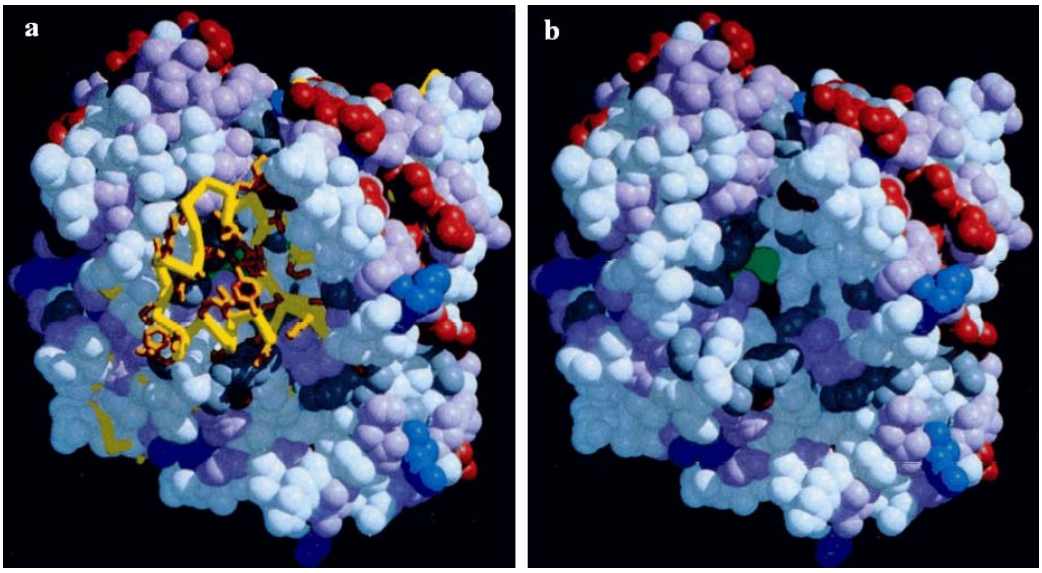


Figure 2.7. Open Structure of *Candida rugosa* Lipase. [39]

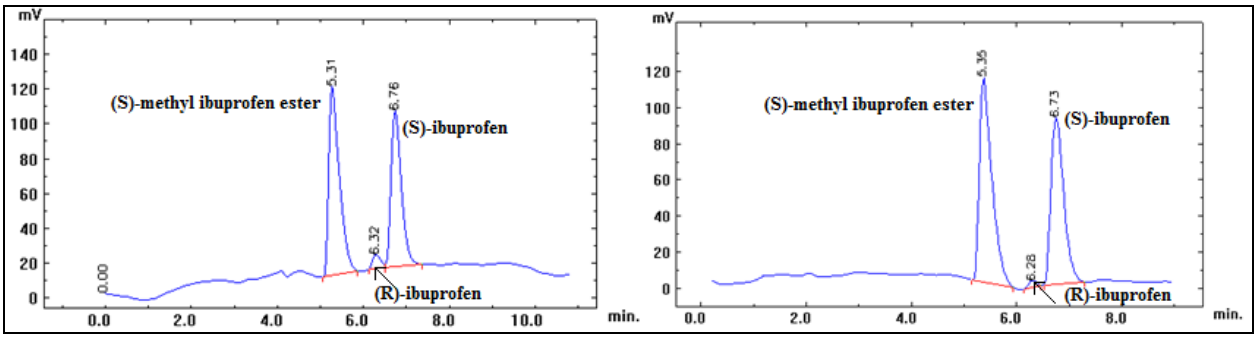
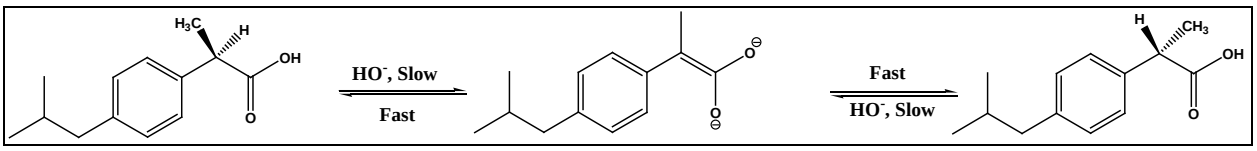


Figure 2.8. HPLC Chromatogram of Enzymatic Racemic Ibuprofen Hydrolysis at pH 7.2 without (*ee* = 95.5) and with DMSO Addition Respectively

Following the observation that the activity of *Candida rugosa* lipase is not greatly affected by pH or DMSO in an aqueous buffer, and knowing that DMSO has been used to catalyze the brute force racemization of ibuprofen under highly alkaline conditions, [**26**] it was hypothesize that DMSO might allow the racemization of ibuprofen esters under mild alkaline conditions because enolization of esters only requires one deprotonation as opposed to the two deprotonations required for racemization of ibuprofen, **Scheme 2.2.** It was hypothesized that DMSO might allow racemization because it allows non-polar compounds to better dissolve in an aqueous media.



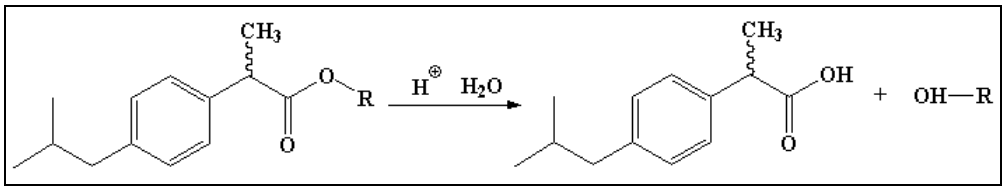
Scheme 2.2. Brute Force Racemization of (*R*)-Ibuprofen

The following reactions were performed to test the hypothesis that DMSO can catalyze racemization of ibuprofen esters under acidic or basic conditions while allowing enantioselective enzymatic hydrolysis of (*S*)-ibuprofen esters to (*S*)-ibuprofen. Coupling

these two steps together would allow for conversion of racemic ibuprofen to (*S*)-ibuprofen.

2.2.4 Racemization of (*R*)-Methyl Ibuprofen Esters in Different Buffers pH with DMSO as Co-Solvent

The (*R*)-methyl ibuprofen ester was isolated from a previously described enantioselective enzymatic hydrolysis reaction. The ester was reacted at 40 °C in a 20% DMSO/buffer solutions at different pHs (5.31, 7.1 and 9.8). The reaction was sampled every 24 hour for five days. At pH 5.3, acid catalyzed hydrolysis with and without DMSO over the prolonged reaction period. The production of racemic ibuprofen was observed, **Scheme 2.3 and Figure 2.9.**



Scheme 2.3 Acid Hydrolysis of Esters

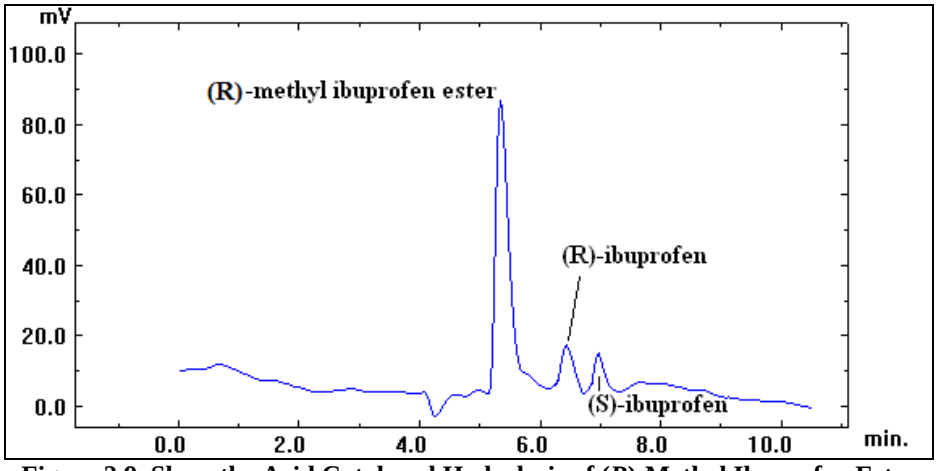


Figure 2.9. Show the Acid Catalyzed Hydrolysis of (*R*)-Methyl Ibuprofen Ester

At pH 7.2, no racemization or hydrolysis reactions occurred with or without DMSO. At pH 9.8, complete racemization without hydrolysis was observed after 5 days for the reaction with 20% DMSO, **Figure 2.10**. At this pH, no racemization or hydrolysis occurred without DMSO present. The racemization under basic conditions is explained by the effective deprotonation at the α-position giving a planar enolate in DMSO as showed in **Scheme 2.4**.

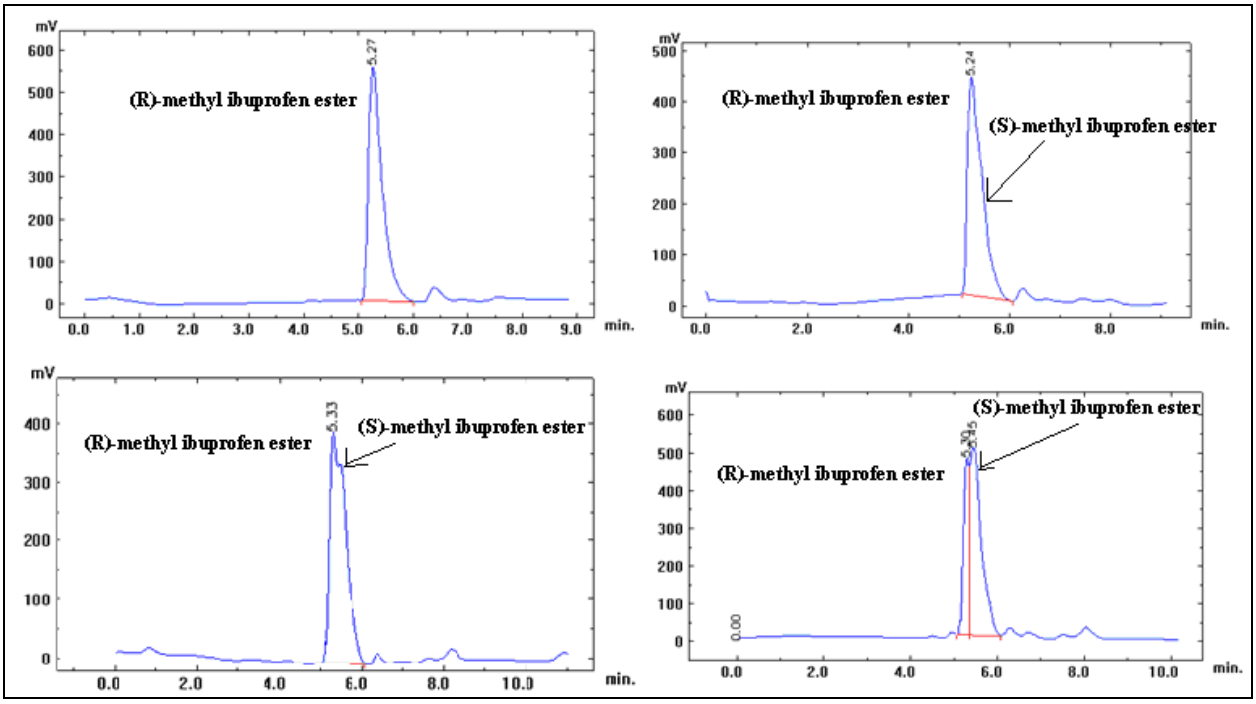
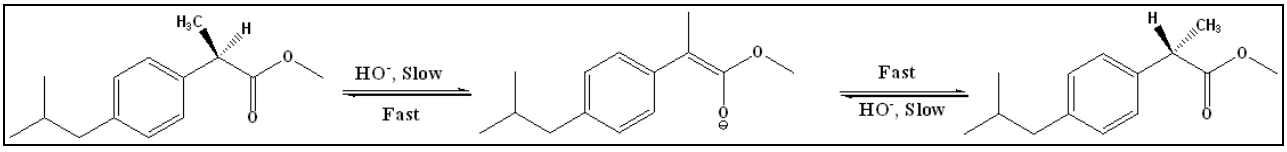


Figure 2.10. HPLC Analysis of *(R)*-Methyl Ibuprofen Ester Racemization, Samples From Day 1, 3, 4 and 5 From Left to Right and Top to Bottom Respectively



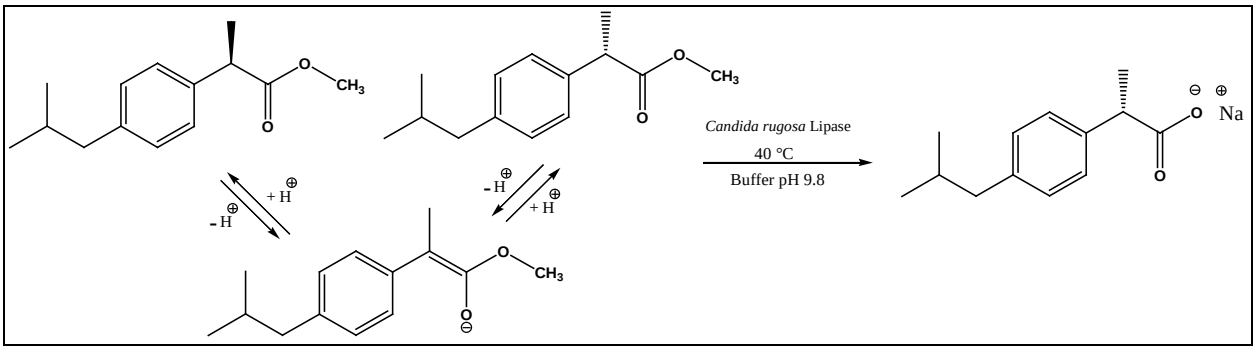
Scheme 2.4. Racemization Reaction of the (*R*)-Methyl Ibuprofen Ester Under Basic Conditions

2.2.5 Racemization of (*R*)-Ibuprofen at pH 5.3 Buffer

In order to determine whether racemization occurred to the ibuprofen ester, ibuprofen or both at pH 5.3, a separated experiment was performed. Enriched *(R)*-ibuprofen with *ee* of 98.6 % was stirred and heated to 40 C° in a 20 % DMSO/ pH 5.3 aqueous buffer. The reaction was monitored daily for a period of 6 days with no racemization observed.

2.2.6 Dynamic Kinetic Resolution of Racemic Ibuprofen Esters

The enantioselective enzymatic hydrolysis of the racemic methyl ibuprofen ester with *in situ* racemization of the unreacted the (*R*)-methyl ibuprofen ester was tried at different pHs (5.3, 7.2 and 9.8) with 20% DMSO by volume as co-solvent and the respective control reactions (no DMSO), **Scheme 2.5.** All reactions were stirred at 40 ºC and monitored at different time intervals by chiral HPLC until the reaction velocity slowed or conversion stopped.



Scheme 2.5. Dynamic Kinetic Resolution of Racemic Methyl Ibuprofen Ester. (Enantioselective Enzymatic Hydrolysis of Racemic Methyl Ibuprofen Ester in 20% DMSO / pH 9.8)

Figure 2.11 shows the progress of the dynamic kinetic resolution process at different pHs. At acidic and neutral pHs, the reaction proceeded up to 45 % conversion in 24 hours and then stopped asymptotically at 47 %, even after several days. This confirms the previous observation, that the (*R*)-methyl ibuprofen ester does not racemize and that only the (*S*)-methyl ibuprofen ester is hydrolyzed.

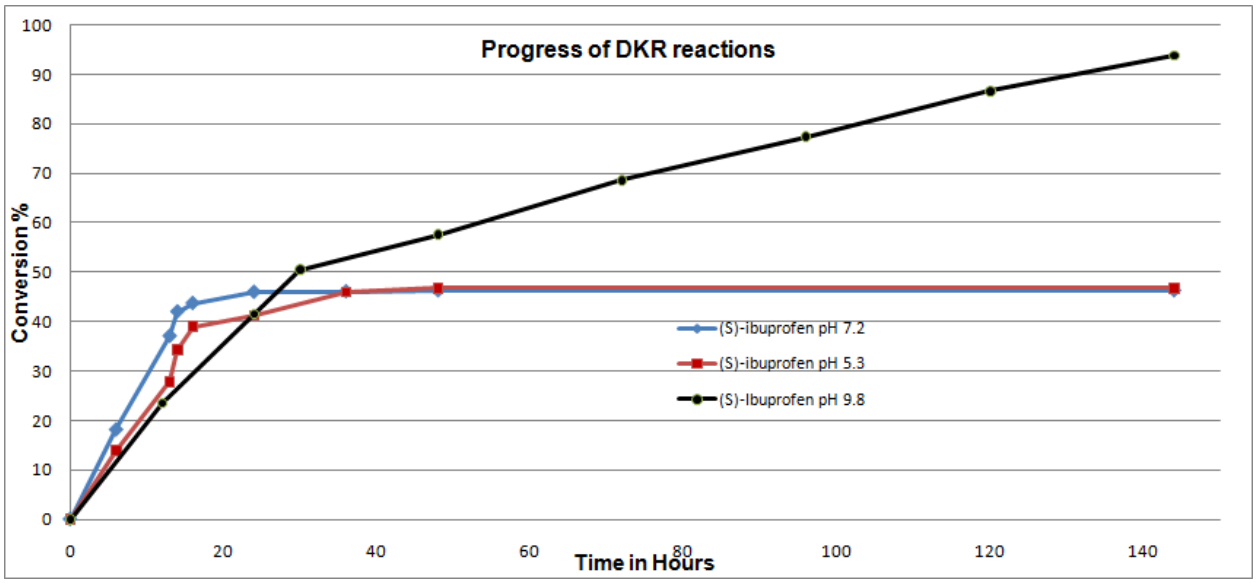


Figure 2.11. Progress of Dynamic Kinetic Resolution Reaction

The reaction at pH 9.8 overcame the 50 % conversion limit, confirming that racemization is occurring between methyl ibuprofen ester enantiomers, followed by enantioselective hydrolysis of the (*S*)-methyl ibuprofen ester, as shown in **Table 2.2 and Figure 2.12**.

Table 2.2. Time, Conversion and Enantiomeric Excess Values for the Kinetic Resolutions at Different pH

DKR at pH 5.3			DKR at pH 7.2			DKR at pH 9.8		
Time	***Conversion***	***ee***	**Time**	***Conversion***	***ee***	**Time**	***Conversion***	***ee***
0	0		0	0		0	0	
6	13.879	95.1	6	19.911	99.1	12	23.568	100.0
13	27.876	93.9	13	38.966	98.4	24	41.524	98.6
14	34.235	89.6	14	40.803	98.3	30	50.468	98.4
16	38.987	88.7	16	41.663	98.3	48	57.56	97.8
24	41.209	86.8	24	43.993	98.2	72	68.562	97.2
36	45.995	87.1	36	45.342	98.1	96	77.358	95.5
48	46.761	86.7	48	46.231	98.0	120	86.598	94.4
						144	93.823	94.1

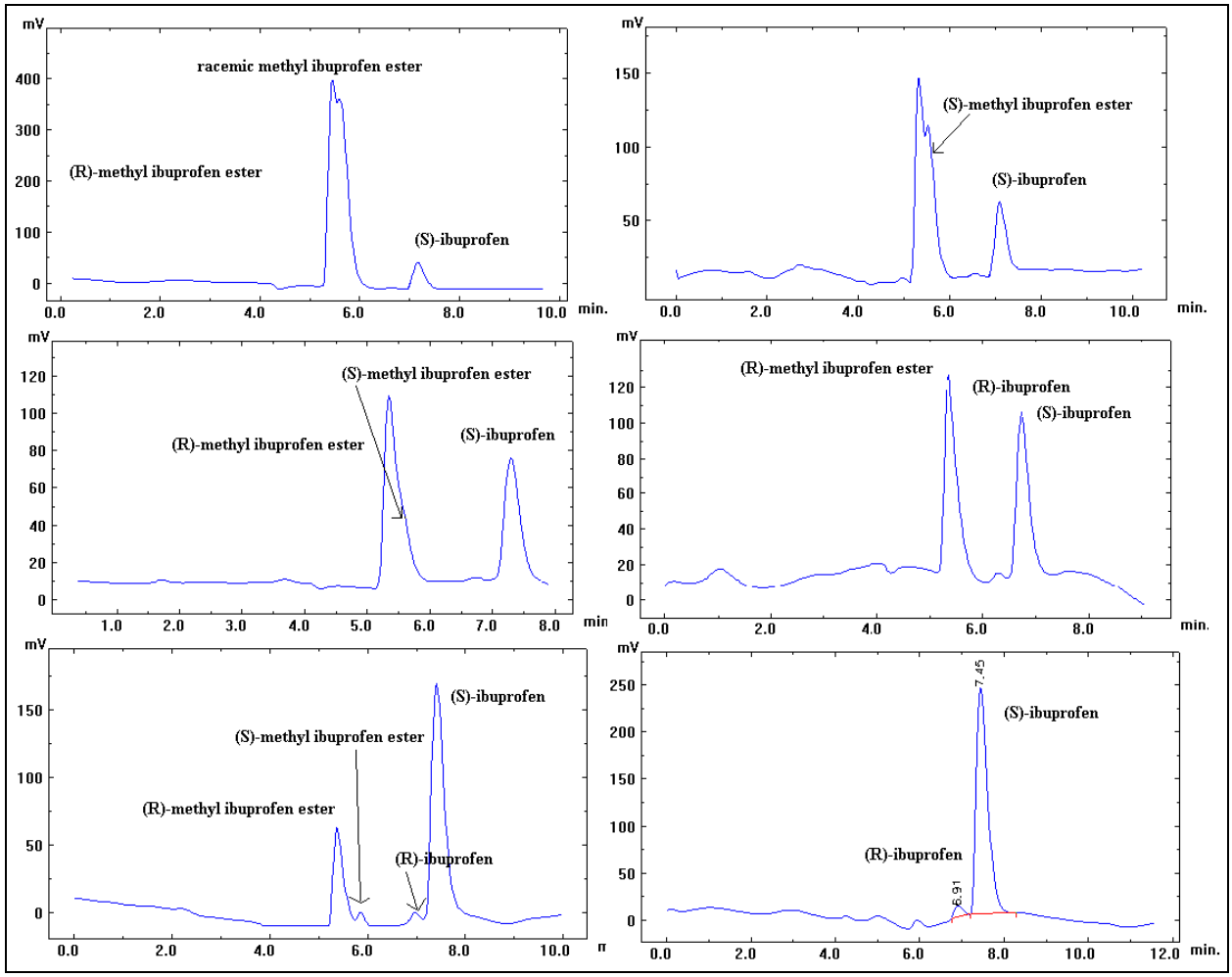


Figure 2.12. HPLC Analysis of (*R,S*)-Methyl Ibuprofen Ester Hydrolysis with *in situ* Racemization of the Un-Reacted *(R)*-Methyl Ibuprofen Ester on 20% DMSO/Buffer pH 9.8. Sample Order From Left to Right and Top to Bottom Respectively

After 5 days, 94 % by weight of the original racemic ibuprofen was isolated by acidifying the reaction, extraction with hexanes, and evaporation as enantiomerically enriched (*S*)-ibuprofen. This represents a 188 % yield of (*S*)-ibuprofen from the original amount. The *ee* of the isolated (*S*)-ibuprofen was also 94 %.

Due to the high enantioselectivity of *Candida rugosa* lipase observed in previous ibuprofen esterification and hydrolysis studies [**26**], it can be assumed that only the (*S*)-methyl ibuprofen ester was hydrolyzed. Under this hypothesis and the data from **Table 2.3** different kinetic models were developed.

Although several studies of the *Candida rugosa* lipase kinetics have been carried out [**33**], the most common procedure is the use of the *pseudo*-first order model [**34 and 35**]. According to **Figure 2.11**, there are two major phases of the dynamic kinetic resolution as delineated by a discontinuity. The first phase from 0 to 30 hours is when most of the (*S*)-methyl ibuprofen ester is being hydrolyzed. **Equation 2.7** fits the first phase of the reaction as shown in **Figure 2.13** with an $R^2 = 0.9929$. This implies that the rate of hydrolysis for this reaction is 0.043 h^{-1}, slower than observed when no racemization was occurring.

$$y = 0.043x + 2.6565. \text{……..……..} \textbf{(2.7)}$$

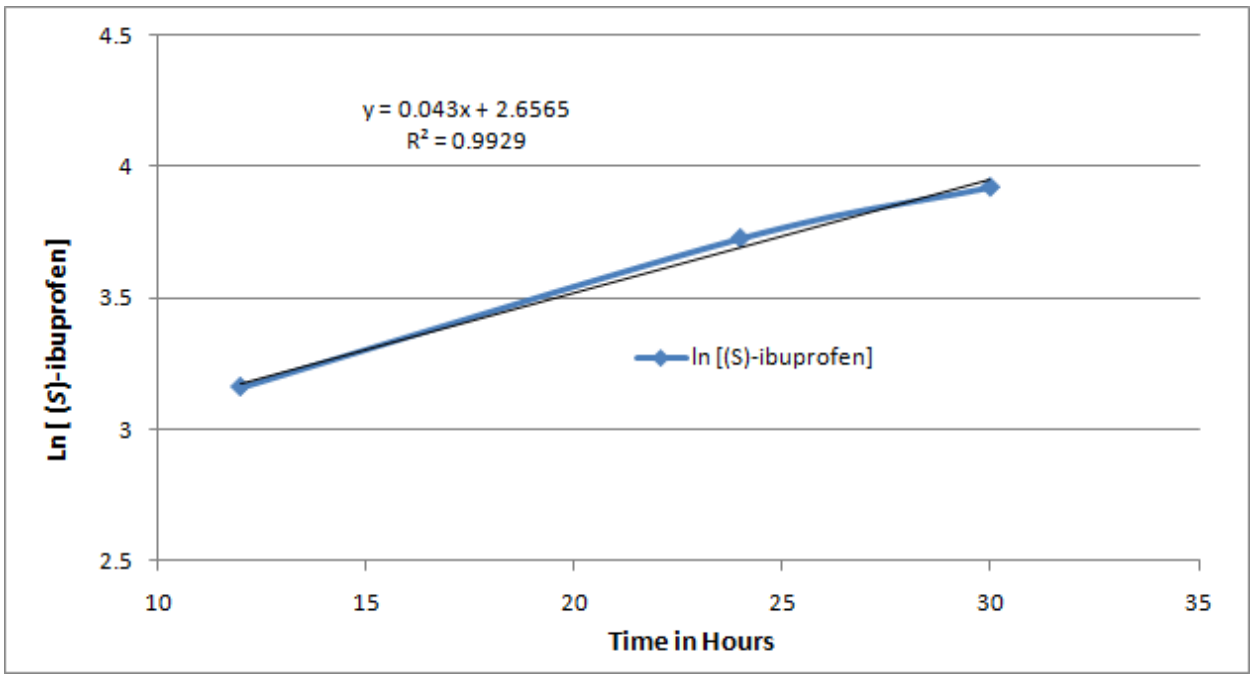


Figure 2.13. Determination of the *Pseudo*-First-Order-Kinetic at the First Part of the DKR Reaction

In the second phase of the dynamic kinetic resolution from 30 to 144 hours, most of the (*S*)-methyl ibuprofen ester has been hydrolyzed and the kinetics represents mostly the racemization of the (*R*)-methyl ibuprofen ester. **Equation 2.8** fits this region with *pseudo*-first order kinetics and $R^2 = 0.9921$. This implies that the rate of racemization for this reaction is 0.0044 h^{-1}, a magnitude of order slower than the rate of hydrolysis, **Figure 2.14**.

$$y = 0.0044x + 3.9205 \text{ ……..……..(2.8)}$$

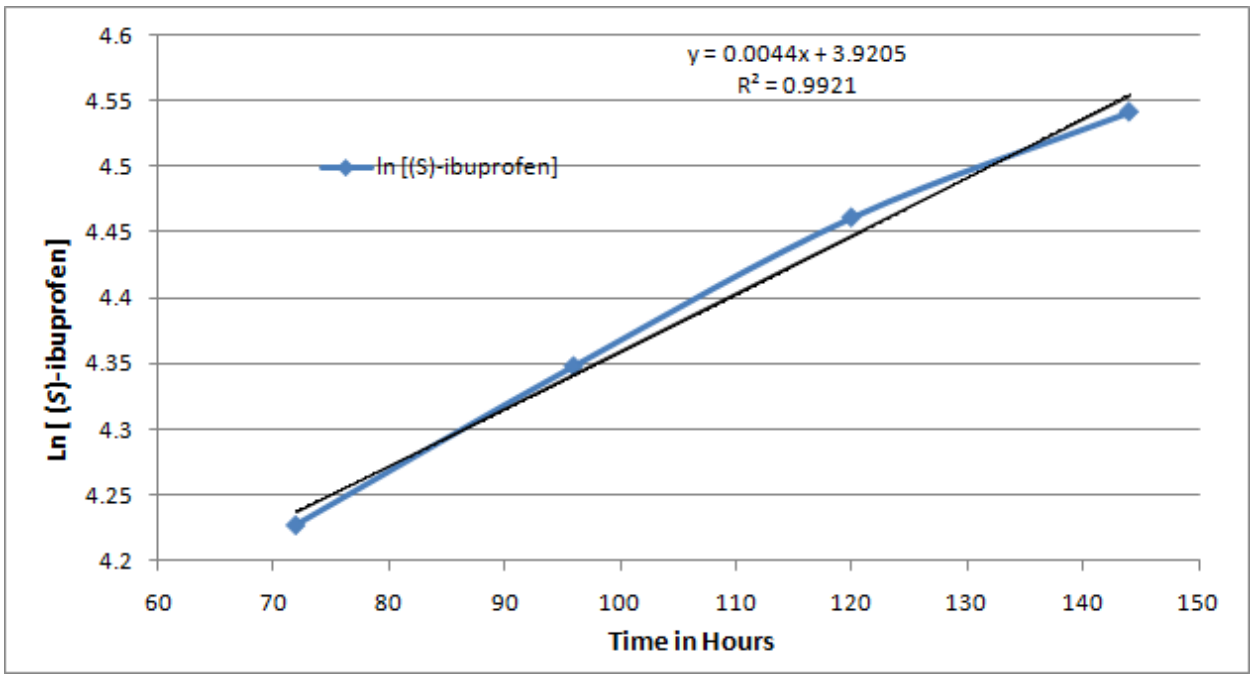
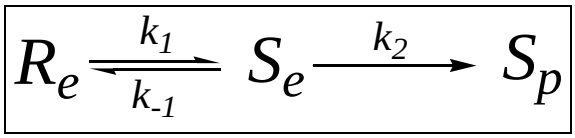


Figure 2.14. Determination of the *Pseudo*-First-Order-Kinetic at the Second Part of the DKR Reaction

Unfortunately, the data cannot be fit so simply by *pseudo*-first order kinetics since the first phase of the dynamic kinetic resolution does not solely represent the hydrolysis of the (*S*)-methyl ibuprofen ester but also the racemization of the ibuprofen ester enantiomers. A more complete kinetic model is given by **Scheme 2.6**, a consecutive reaction with a reversible step, ignoring the enolate intermediate between ester enantiomers and the complexation of esters with *Candida rugosa* lipase. k_1 and k_{-1} are the rates of racemization as before, which are assumed to be equal. K_2 is the rate of hydrolysis. **Equation 2.9** represents the rate of formation of the (*R*)-methyl ibuprofen ester, R_e. **Equation 2.10** gives the rate of formation of the (*S*)-methyl ibuprofen ester, S_e. **Equation 2.11** represents the rate of formation of the sodium salt of ibuprofen, S_p.



Scheme 2.6. Consecutive Reaction with a Reversible Step

$$\frac{dR_e(t)}{dt} = -k_1 R_e(t) + k_1 S_p(t) \text{ .....(2.9)}$$

$$\frac{dS_e(t)}{dt} = k_1 R_e(t) - (k_1 + k_2) S_e(t) \text{ .....(2.10)}$$

$$\frac{dS_p(t)}{dt} = k_2 S_e(t) \text{ .....(2.11)}$$

Maxima software was used to solve these differential equations exactly assuming that the initial concentrations of R_e and S_e were 0.5 and zero for S_p. [**40**] The exact solutions are given in **Equations 2.12 through 2.14**, where %e is Maxima's notation for exponential base *e*.

$$R_e(t) = e^{\left(\frac{-\left(\frac{(k_2+2k_1)t}{2}\right)\left(2\left(\frac{k_2}{2}+k_1\right)-\frac{k_2+2k_1}{2}\right)\sinh\left(\frac{sqrt\left(k_2^2+4k_1^2\right)t}{2}\right)\cosh\left(\frac{sqrt\left(k_2^2+4k_1^2\right)t}{2}\right)}{sqrt(k_2^2+4k_1^2)}\right)}$$

Equation 2.12. Represents the Rate of Formation of the (*R*)-Methyl Ibuprofen Ester, R_e

$$S_e(t) = e^{\left(\frac{-\left(\frac{(k_2+2k_1)t}{2}\right)\left(2k_1-\left(\frac{k_2+2k_1}{2}\right)\right)\sinh\left(\frac{sqrt\left(k_2^2+4k_1^2\right)t}{2}\right)\cosh\left(\frac{sqrt\left(k_2^2+4k_1^2\right)t}{2}\right)}{sqrt(k_2^2+4k_1^2)}\right)}$$

Equation 2.13. Represents the Rate of Formation of the (*S*)-Methyl Ibuprofen Ester, S_e

$$S_p(t) = e^{\left(\frac{-\left(\frac{(k_2+2k_1)t}{2}\right)\left(2\left(-\left(\frac{k_2}{2}\right)-2K_1\right)+2k_1\right)\sinh\left(\frac{sqrt\left(k_2^2+4k_1^2\right)t}{2}\right)\cosh\left(\frac{sqrt\left(k_2^2+4k_1^2\right)t}{2}\right)}{sqrt(k_2^2+4k_1^2)}\right)}$$

Equation 2.14. Represents the Rate of Formation of the Sodium Salt of Ibuprofen, S_p

These equations were graphed using Microsoft Excel Software, **Figure 2.15**, and a macro program to vary k_1 and $k2$ to fit the data in **Table 2.3** by the least-squares criterion. The average k_1 and k_2 values for three experiments were found to be 0.02583 ± 0.0042 and 0.05253 ± 0.00454 h^{-1}, respectively, indicating that the rate of racemization is only about half of the rate of hydrolysis.

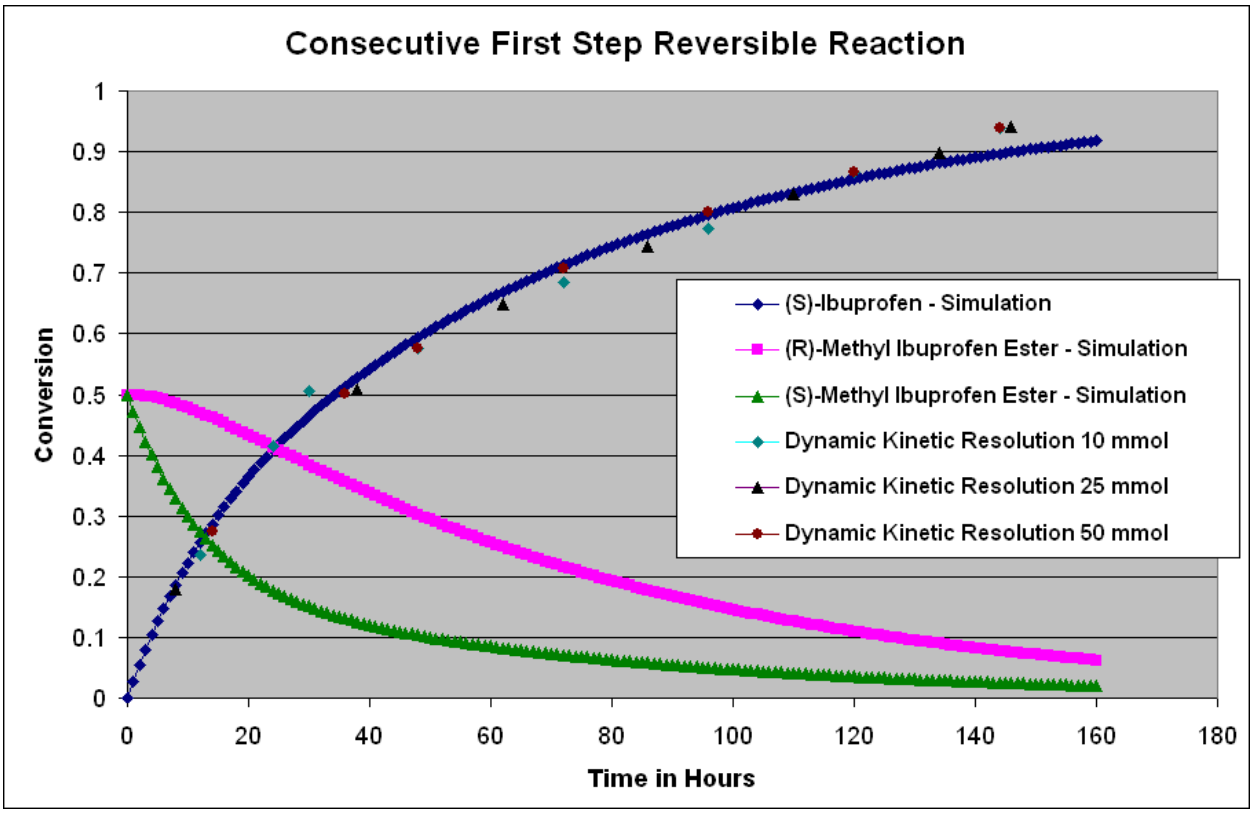


Figure 2.15. Progress of the Dynamic Kinetic Resolution of Ibuprofen

In order to test the generality of the developed dynamic kinetic resolution, racemic propyl ibuprofen ester was synthesized and used in the same reaction. At pH 5.3, the reaction proceeded up to only 46 % conversion and the *ee* of the product was 80%. When the buffer pH was 7.2 the reaction stopped at 46 % conversion with an *ee* of 82 %. Finally, for the reaction at pH 9.8 the conversion stopped at 56% with a product *ee* of 93% after 120 hours, **Figure 2.16.** Therefore, the dynamic kinetic resolution is too slow for larger ibuprofen esters than methyl.

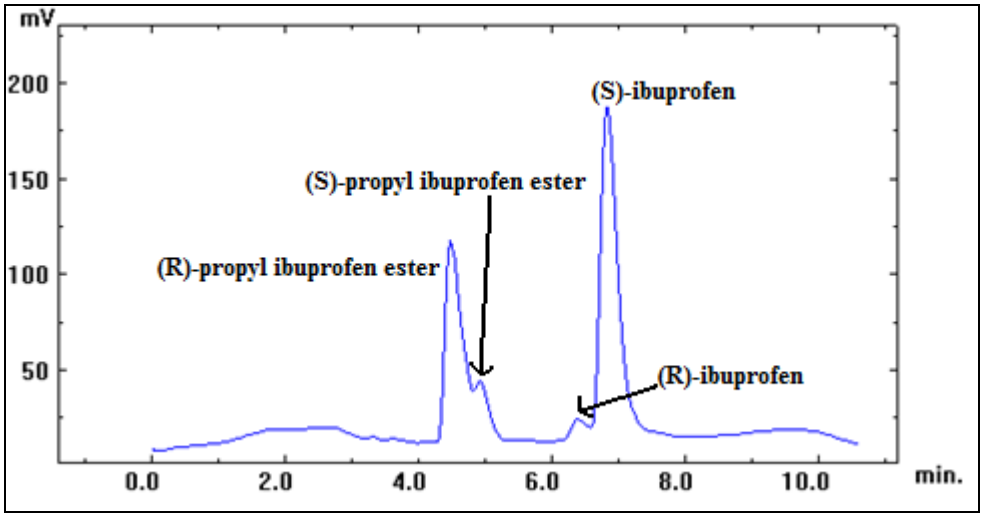


Figure 2.16. Dynamic Kinetic Resolution with Propyl Ibuprofen Ester at pH 9.8

2.2.7 Crystallization of Enriched (*S*)-Ibuprofen to Enhance the Enantiomeric Excess

Repeated enantioselective reactions is the most common way to increase the enantiopurity or the enantiomeric excess of the desired product, (*S*)-ibuprofen in this case. However, this method requires the used of more time, catalyst, reagents, solvents, equipment, energy, purifications, and complexity. In order to increase the *ee* of the

product of the dynamic kinetic resolution, different crystallization techniques were attempted.

In separate experiments, 2 g of pulverized enantiomerically enriched (*S*)-ibuprofen (*ee* = 94%), a magnetic stir bar, and 10 mL of anhydrous methanol, ethanol and isopropanol, respectively, were added to a 25 mL round bottom flask. The flasks were heated to 45 ºC in a water bath and were stirred until the solutions were clear. Then the temperature of the water bath was reduced to 35 ºC. When the flasks were cooled, racemic ibuprofen crystals were added as seed, and the flasks were placed in an ice bath and kept in a refrigerator for 12 hours. After crystallization occurred for the isopropanol and ethanol cases, the crystals were filtered and air-dried to give 87 and 76 %, respectively, of recovered ibuprofen. The crystals were analyzed by chiral HPLC and were determined to have an *ee* of 94 to 95 % of (*S*)-ibuprofen for the isopropanol and ethanol cases, respectively.

In the case of methanol, the recrystalization solution was only turbid. The solution was filtered and analyzed by chiral HPLC indicating it had an *ee* of 99.7 %. The methanol was evaporated to give (*S*)-ibuprofen in a 93.2 % yield. The optical rotation of this material was $[\alpha]^{25}_{D} = 59.52\ °$ (ethanol 95%, c = 1), indicating an *ee* of at least 99.2 %. The melting point of 55 °C agreed well with the literature value of 55.5 °C. [**41**]

2.3 Other *In Situ* Racemization Attempts

2.3.1 Attempted *In Situ* Racemization of Ibuprofen with Organic Acids in Cyclohexane

A series of organic acids (*p*-toluenesulfonic, *p*-toluenesulfonyl chloride, phenyl dimethyl chlorosilane, boric acid, pyruvic acid, isolute Si-propylsulfonic acid, and isolute Si-TsOH) were added to the reaction of racemic ibuprofen, decan-1-ol, and *Candida rugosa* lipase in cyclohexane. In no case was racemization of ibuprofen observed, whereas the esterification of (*S*)-ibuprofen was greatly reduced with concentration of acid to enzyme greater than 5 % by weight.

2.3.2 Attempted *In Situ* Racemization of Ibuprofen with Organic Bases in Cyclohexane

A series of organic bases (tripentylamine, tributylamine, diethylamine, methyldiethylamine and ethyldisopropylamine) were added to the reaction of racemic ibuprofen, decan-1-ol, and *Candida rugosa* lipase in cyclohexane. In no case was racemization of ibuprofen observed, whereas the esterification of (*S*)-ibuprofen was greatly reduced with concentration of base to enzyme greater than 10 % by weight.

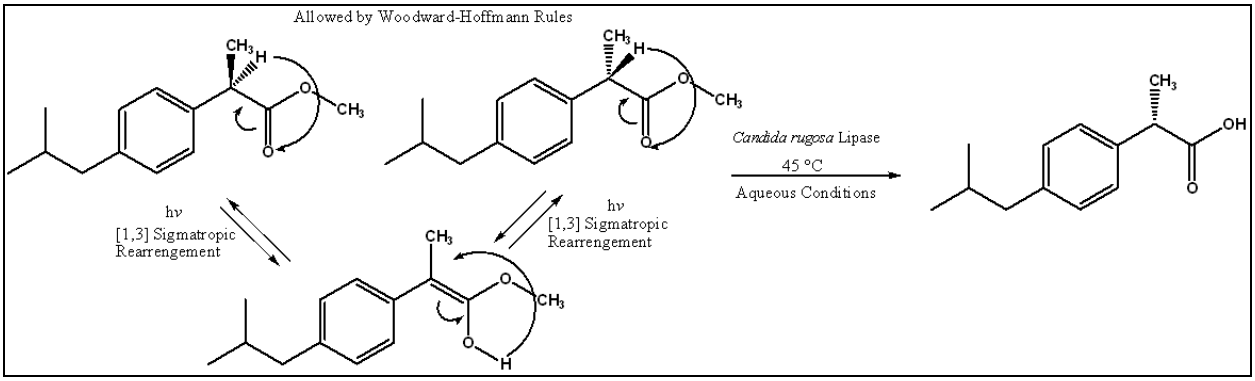
2.3.3 Attempted Racemization of Methyl Ibuprofen Esters with Light

In order to induce the *in situ* racemization of the racemic methyl ibuprofen ester with enantioselective hydrolysis of the (*S*)-methyl ibuprofen ester, a photochemically allowed 1,3-sigmatropic rearrangement of ester to enol was attempted as shown in

Figure 2.17, Scheme 2.8. Chiral HPLC analysis, **Figure 2.18**, showed the development of several byproducts including one that initially was thought to be an increase of (*S*)-ibuprofen ester. In addition, after several attempts, it was determined that ibuprofen esters easily evaporate with or without water present.

It is important to note that ibuprofen esters are volatile, easily evaporating if left out in the open. Ibuprofen esters also generate small amounts of good chromophores with visible and ultraviolet light exposure that often confused the HPLC analysis of their reactions. Over prolonged exposure, these esters often turned yellow.

Figure 2.17. Photochemical Reactors



Scheme 2.7. Expected 1,3 Sigmatropic Reaction for the Racemization of *(R)*-Methyl Ibuprofen Ester

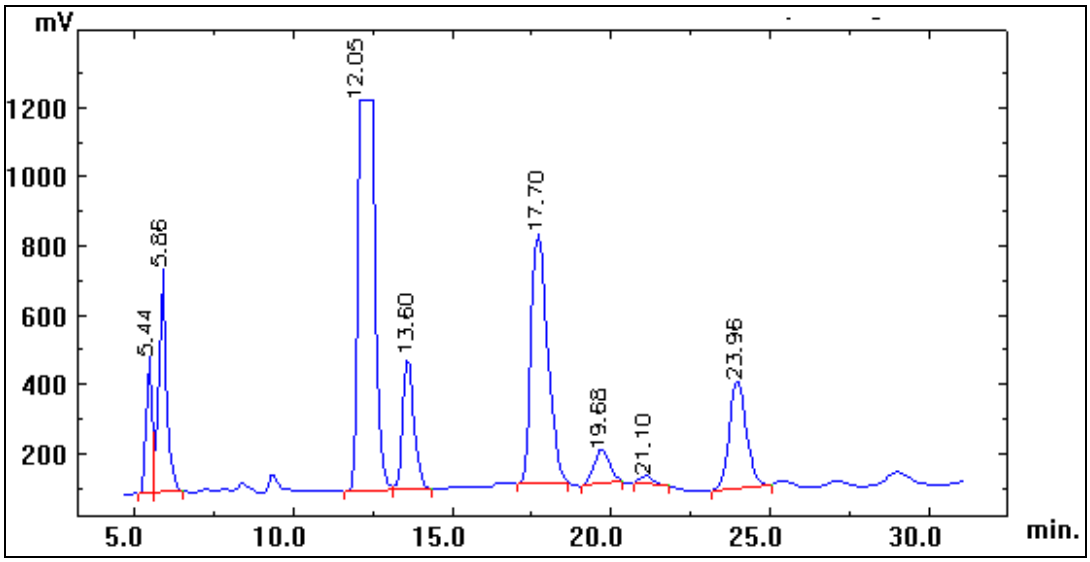


Figure 2.18. Chromatograph of Photochemical Racemization Attempt

2.3.4 Attempted Racemization of Ibuprofen in a Two-Phase Continuous Reactor

Racemization of racemic ibuprofen was attempted in the presence of *Candida rugosa* lipase, methanol, aqueous base, and cyclohexane in the left part of **Figure 2.19**. The idea was that any (*S*)-methyl ester formed would dissolve in cyclohexane and would be transported to the right receiver flask. The left flask was heated to 40 °C. The right flask was heated to 60 ºC to recycle the methanol and cyclohexane. The pH of the aqueous portion was 8.6. Chiral HPLC analysis showed some methyl ester being formed for the first 8 hours. Thereafter racemic ibuprofen was also transported.

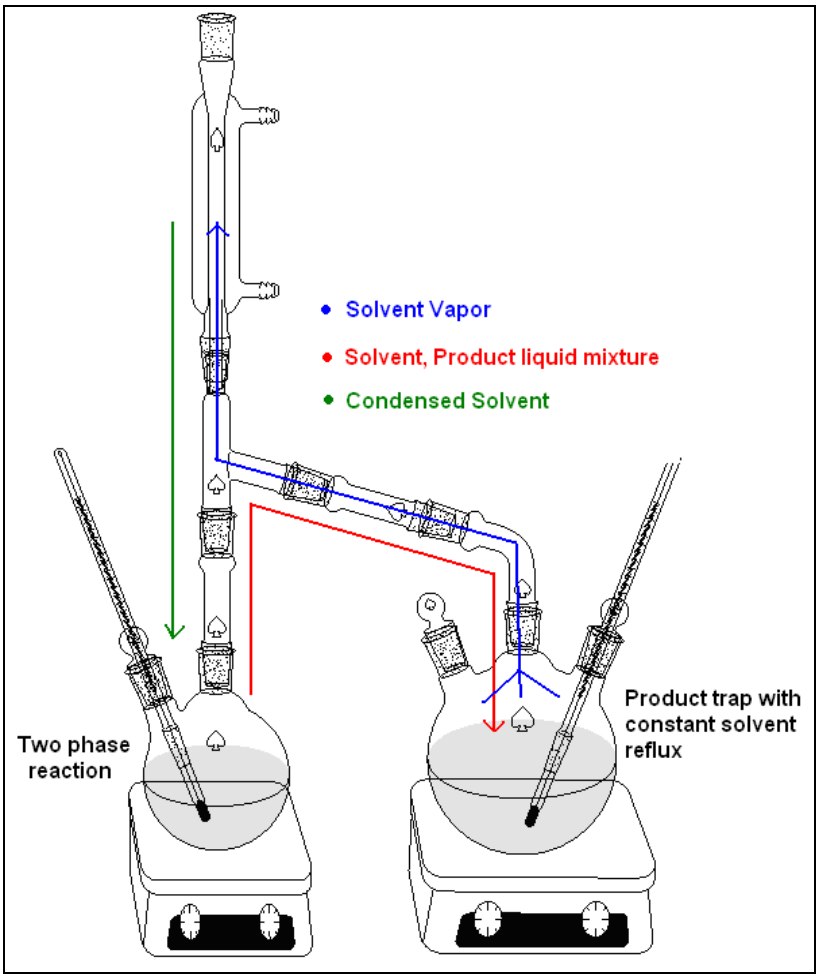


Figure 2.19. Reactor used for the Conversion of Racemic Ibuprofen to *(S)*-Ibuprofen

2.3.5 Attempted Racemization of Methyl Ibuprofen Esters with a Three-Phase Membrane Separated Reactor

To avoid hydrolyzing ibuprofen esters, they were exposed to sodium hydroxide pouches suspended in cyclohexane. The pouches were made from either filter paper or Whatman Nucleopore filters. Enantioselective enzymatic hydrolysis catalyzed by *Candida rugosa* lipase was expected to occur below in the aqueous phase as shown in **Figure 2.20.** For both pouch types, solid appeared to form on their surface, which

eventually dropped into the lower aqueous phase to increase the pH. The reactions were not studies beyond 10 % conversion and 48 hours.

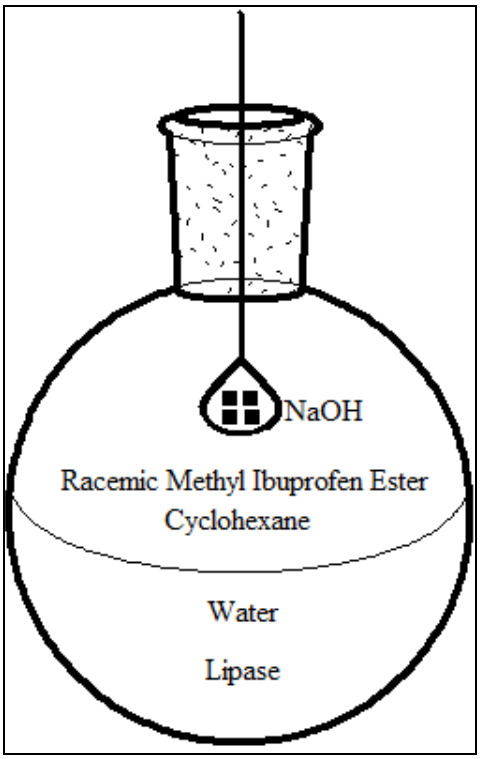


Figure 2.20. Two-Phase Hydrolysis Reaction with Racemization of the Un-Reacted Ester at the Non-Polar Phase

2.3.6 Attempted Acid Catalyzed Esterification, Racemization and Enantioselective Hydrolysis in a Two Reactor System

To separate the racemization and enantioselective enzymatic reactions that were believe to have incompatible environments; racemic ibuprofen, methanol and sulfuric acid were placed in one flask, and *Candida rugosa* lipase and an aqueous buffer were place in a second flask. The flasks were connected by a distillation head and were heated to 40 °C in the same sand bath/heater. Racemization and ester formation were to occur in

the left flask, **Figure 2.21**, while enantioselective hydrolysis was to occur in the right flask as the methyl ibuprofen ester distilled over. Because of the differences in vapor pressure of the solvents used, the left flask always overfilled and contaminated the right flask with acid.

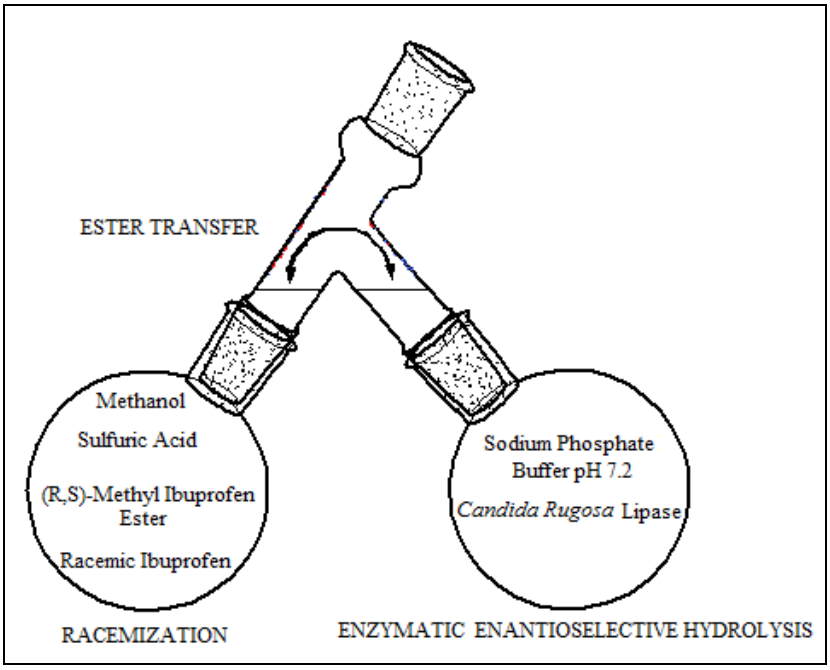


Figure 2.21. Two Reactor System

CHAPTER 3

CONCLUSIONS

Though extraction of racemic ibuprofen from commercial pills with inexpensive acetone is not a traditional way of obtaining laboratory reagents, this step represents a time and cost effective way of starting this research. Thus the overall conversion of racemic ibuprofen to (*S*)-ibuprofen should be overall cost effective, environmentally benign, and facile.

To avoid generating waste reagents like sulfur dioxide and hydrogen chloride in the synthesis and use of acid chlorides, the Fischer esterification of ibuprofen was investigated using sulfuric acid as a reusable catalyst. It was found that most esters could be generated quantitatively but only the methyl ester was easily separated because methanol and sulfuric acid are insoluble in the extracting solvent, hexanes. The Fischer esterification was also less time consuming and easier to run than using thionyl chloride to make the acid chloride of ibuprofen.

From previous work, *Candida rugosa* lipase had enatiomeric ratios *E* of 86 and 130 for esterifications of racemic ibuprofen with butyl and decyl alcohols [**24**]. Though operating in the opposite direction, this work confirms that this lipase is less selective towards hydrolysis of the racemic methyl ibuprofen ester with a maximum *E* value of 52. This is believed to be due to the greater interaction between larger esters and the lipase active site channel. [**42**] Nevertheless, an *E* value of 52 is much greater than the minimum (10) believe to be required to effectively select between enantiomers. [**43**]

It is interesting to note that the effect of acids and bases on the activity of *Candida rugosa* lipase is much different in aqueous versus non-polar solvents. Whereas the rate of hydrolysis of racemic methyl ibuprofen esters did not change significantly in acidic, neutral or basic aqueous buffers; the activity of the lipase toward esterification was greatly reduced at acid or base concentrations greater than 5 or 10 % by weight respectively, when added in cyclohexane. This is probably because organic acids and bases are polar compounds that are more attracted to the lipase in cyclohexane and denature it. Though no racemization was observed, surprisely, racemic methyl ibuprofen esters were not hydrolyzed either by aqueous acid or base conditions.

Once DMSO was added, the desired racemization reactions started to be observed for acidic and basic conditions because of the known ability of ionic liquids to allow polar and non-polar reagents to mix. At pH 5.3, 14.8 % of the (*R*)-methyl ibuprofen ester was hydrolyzed without any lipase present giving ibuprofen with an *ee* of 12 %. That (*R*)-ibuprofen does not racemize under the same reaction conditions demonstrates that it is the more electron rich ester that is protonated, enolized and undergoes racemization.

Under basic conditions and DMSO present, *in situ* racemization of racemic ibuprofen esters was observed along with the hydrolysis of the (*S*)-methyl ibuprofen ester to (*S*)-ibuprofen in a 94 % conversion by weight of original ibuprofen after 144 hours. To our knowledge, this is the best dynamic kinetic resolution observed for ibuprofen in terms of % conversion, *ee*, complexity, and cost. [**26 and 27**]

The kinetics of this reaction was very interesting and two phased as shown in **Figure 2.10.** In the first part of the reaction, the hydrolysis rate depends on the abundance of the (*S*)-methyl ibuprofen ester. In the second part, the hydrolysis reaction is limited or depends on the reaction rate of racemization of the (*R*)-methyl ibuprofen ester because little (*S*)-methyl ibuprofen ester is present. This kinetics was confirmed by fitting a "consecutive reactions with a reversible step" model to the hydrolysis data. Compared with all other reactions, the hydrolysis reaction at pH 9.8 with DMSO present was almost twice as slow. The lipase functions in both aqueous and highly non-polar solvents such as cyclohexane but is denatured by solvents of intermediate polarity such as DMSO has been noted.

Although the dynamic kinetic resolution gave (*S*)-ibuprofen with an *ee* of 94 % (97 % (*S*) to 3 % (*R*)-ibuprofen), racemic mixtures can be less soluble than pure enantiomers. When crystallization was tried racemic ibuprofen crystallized and the (S)-ibuprofen was still in solution (the best estimate being 99.7 % *ee*) and it was easily isolated by evaporation. This seems to contradict that a mixture of two different molecules may have a stronger interaction than that of a pure compound but this phenomena is often observed for racemic mixtures versus pure enantiomers. Combining the dynamic kinetic resolution and crystallization steps, and considering that isolation of ibuprofen from pills and forming its racemic methyl ester is also quantitative, the overall conversion of (*S*)-ibuprofen from racemic ibuprofen pills, is 87.60 %, with the possibility of recovering all other reagents.

Using research laboratory prices, which are much more expensive than prices available to industry, a mole of (*S*)-ibuprofen (206 g) could be synthesized for $112. This would be 5 ¢ per pill from a pill that originally cost 1 ¢. Considering that $80 of this cost per mole are reusable solvents and reagents, the price could go down to less than 2 ¢.

The final question is whether the pharmaceutical industry is willing to adopt this orphan drug and if the consumer is willing to pay more for pure (*S*)-ibuprofen that works three times as fast as racemic ibuprofen with less side effects.

Considering that in the United States, Gastro Intestinal (GI) complications due to NSAIDs intake have been estimated to cause approximately 300,000 deaths and to cause around 1,700,000 people to have been hospitalized at a cost of $38.8 billion, the price of producing *(S)*-ibuprofen may be worth it, **Figure 3.1**.

In perspective, 300,000 people dying from NSAID GI bleeding is a greater number than the number of dead (234,000) from the Revolutionary War, the War of 1812, the Mexican War, the Spanish-American War, World War I, the Korean War, the Vietnam War, and the Persian Gulf Wars combined**. [44]**

	USA				Great Britian			Germany		Total	
Year	Deaths	Hospitalizations	Ref	Cost	Deaths	Hospitalizations	Ref	Deaths	Ref	Deaths	Hospitalizations
1984											
1985	0										
1986	1,245			764,628,371							
1987	2,516			828,416,436							
1988	3,787			897,525,933							
1989	5,058			972,400,794							
1990	6,329	7,572		1,053,521,987							
1991	7,600	20,000	*	1,141,410,604							
1992	8,871	32,428		1,236,631,207							
1993	10,142	44,856		1,339,795,457							
1994	11,413	57,284		1,451,566,042							
1995	12,684	69,712		1,572,660,934							
1996	13,955	82,140		1,703,858,000							
1997	15,226	94,568		1,846,000,000							
1998	16,500	107,000	*	2,000,000,000							
1999	16,500	107,000		2,000,000,000	4,000	12,000	*	1,650	*		
2000	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2001	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2002	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2003	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2004	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2005	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2006	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2007	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2008	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
2009	16,500	107,000		2,000,000,000	4,000	12,000		1,650			
Total	296,826	1,692,560		38,808,415,765	44,000	132,000		18,150		358,976	1,824,560
* Actual numbers from studies											
Estimated Yearly NSAID deaths and hospitilizations											
1,271 - Deaths delta per year (estimate) (16,500 - 8,900) / 7								45 per day die (estimate)			
12,428 - Hospitalization delta per year (estimate) (107,000-20,000)/7								293 per day are hospitalized (estimate)			
Estimated delta % from 1991 to 1998 is 7.7%											

Figure 3.1 Estimated Yearly NSAID Deaths and Hositalization in the Last 25 Years

Given the long list of racemic profens (2-phenylpropionic acid, fenoprofen, flurbiprofen, indoprofen, ketoprofen and pranaprofen) that should be converted to their pharmacological active (*S*)-enantiomer, this successful research on the "Conversion of Racemic Ibuprofen to (*S*)-Ibuprofen" should be extended to other profens. The major obstacle of extending this methodology will be the solubility of other esters in the aqueous hydrolysis step. For example, when the racemic propyl ibuprofen ester was subjected to the same reaction conditions, only 56 % of the ester was hydrolyzed in 5

days. The *ee* of the ibuprofen was 93 %. Other ionic liquids and esters must be tested to see if they will increase the miscibility of the esters with the aqueous media and make this conversion faster and more general.

CHAPTER 4

MATERIAL, EQUIPMENT AND EXPERIMENTAL SECTION

4.1 Materials

Racemic ibuprofen (α-methyl-4-[isobutyl]phenylacetic acid) was isolated from inexpensive commercial tablets (200 mg Member's Mark, Sam's Club). *Candida rugosa* lipase (706 units / mg solid), methanol, propan-1-ol, butan-1-ol, pentan-1-ol, octan-1-ol, decan-1-ol, *p*-toluenesulfonic acid, phenyl dimethyl chlorosilane, boric Acid, pyruvic Acid, solute Si-Propylsulfonic Acid, isolute Si-TsOH, *p*-toluene sulfonyl chloride, sodium hydroxide, sodium bicarbonate, tributyl amine, tripental amine, trioctyl amine, diethyl amine and ethyl diisopropylamine were purchased from Aldrich Chemical Company. Cyclohexane, hexanes and isopropanol were purchased from Mallinkcrodt Reagents. PL-HCO3 MP Resin, PL-OH MP Resin and PL-CO3 MP Resin were purchased from Polymer Laboratories. Magnesium sulfate, potassium phosphate monobasic and potassium phosphate dibasic were purchased from Spectrum Chemical Company. All other chemicals and analytical grade reagents were from commercial sources.

4.2 Equipment

4.2.1 Rotavapor

A Rotavapor rotary evaporator (**Figure 4.1**) (BÜCHI, LABORATORLUMS-TECHNIK AG, 70 Switzerland) was used to remove volatile solvents from reactions and separations.

Figure 4.1 Rotavapor Used for Removes Solvents

4.2.2 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) was performed with a Chiracel OJ chiral column (Diacel Chemical Industries, LTD) **Figure 4.2**, a Spectra-Physics Spectra System P1500 gradient pump, UV2000 detector, and Winner for Windows Software. The mobile phase normally used was 95% hexanes and 5% isopropanol by volume with helium solvent de-gassing. The flow rate was set to 1.00 mL/min. and the detector was set to a 256 nm wavelength, all at room temperature. HPLC was used to monitor the esterification and hydrolysis reactions of ibuprofen. Usually only 1.0 μL of analyte was used.

Figure 4.2 Chiracel OJ, HPLC Chiral Column

4.2.3 Thin Layer Chromatography (TLC)

Silica gel 60 F254, size 2.5 cm x 7 cm TLC glass plates were used to monitor reactions. A model UVGL-15 Mineralight® lamp, Multiband UV 254/366 nm (115 V, 60 Hz, 0.16 amps) lamp (**Figure 4.3**) was used to visualize TLC plates.

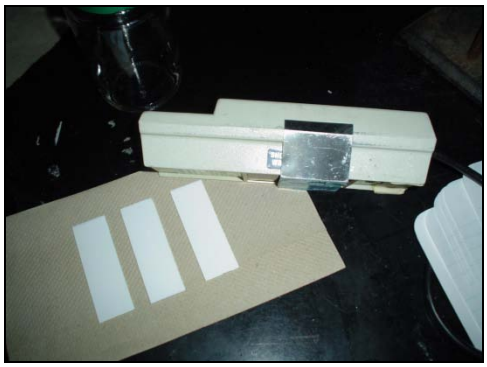


Figure 4.3 UV Lamp and TLC Plates Used to Monitor Reactions

4.2.4 Melting Points

Melting point measurements for starting materials and products were determined with a Melt-Temp II (Laboratory Devices USA) melting point apparatus, **Figure 4.4**. A

standard Celsius thermometer was inserted into the melting point apparatus without calibration. A small amount of compound was packed into a melting point tube.

Figure 4.4 Melt-Temp II Laboratory Devices USA Equipment

4.2.5 Nuclear Magnetic Resonance (NMR)

NMR spectra were recorded with a Bruker Spectrospin Avance 300 Spectrometer and JEOL 600 MHz. The solvent used for these experiments was deuterated chloroform ($CDCl_3$) relative to the internal tetramethylsilane standard (0.03 % v/v). All spectra matched literature references.

4.2.6 Optical Polarimeter

The optical activities were measured using and Atago 5291 AP-300 Fully Automatic Digital Polarimeter (**Figure 4.5**) at 22°C at 589 nm using samples with concentrations of g/100 mL in ethanol.

Figure 4.5 Atago 5291 AP-300 Fully Automatic Digital Polarimeter

4.2.7 ORION Model 420A Simple pH/mV/ORP/Temperature Benchtop Meter

The pH measurements were made using an Orion pH meter, **Figure 4.6,** by introducing the pH electrode into the solution for a few seconds until the instrument was stable on its reading.

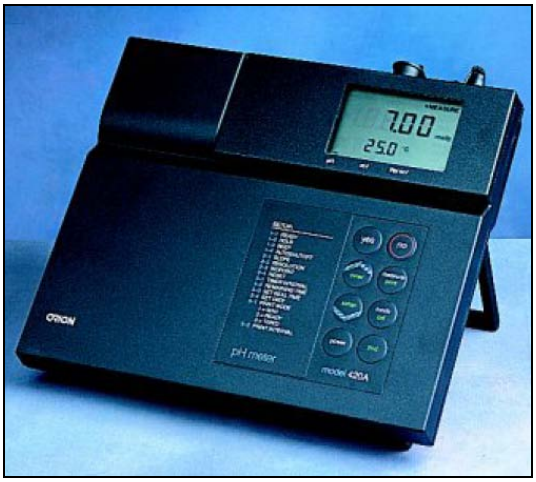


Figure 4.6. Orion 420 A pH Meter

4.3 Experimental

4.3.1 Extraction of Racemic Ibuprofen

Racemic ibuprofen was extracted from commercial tablets by braking and suspending the tablets in acetone for 30 minutes in a beaker and filtering the insoluble coating and filler. The filtrate solvent was removed under reduced pressure to recover the amount of ibuprofen indicated on the bottle label quantitatively.

4.3.2 Racemic Ibuprofen Esters Synthesis

To a dry 125 mL round bottom flask was added 2.06g (10 mmol) of racemic rbuprofen , 0.5 g sulfuric acid (H_2SO_4, 5 mmol) and 40 mL 990 mmol) of methanol. The mixture was stirred at 40 ºC and monitored by chriral HPLC until reach the conversion was greater than 99 %, which was about 5 hours. The reaction mixture was extracted with 2 x 40 mL portions of hexane. The organic layers were separated and evaporated under reduced pressure to determine the conversion . More alcohols were used for the synthesis of larger esters but just in the methyl ester case, hexanes extraction was sufficient to purify the product because methanol is insoluble in this solvent. In all other cases, more hexanes (80 mL) and washings with water were necessary because larger alcohols are amphiphilic or soluble in both polar and non-polar phases.

4.3.3 Sodium Phosphate Buffer Preparation

A 2 M monobasic sodium phosphate solution was prepared in a 100 mL volumetric flask. In a separate 100 mL volumetric flask, a 2 M dibasic sodium phosphate solution was prepared.

To prepare the buffer solution at pH 5.3, to a 250 mL Erlenmeyer flask was added 90.5 mL of the monobasic solution and 9.5 mL on the dibasic solution.

For the preparation of the pH 7.2 buffer solution, to a 250 mL Erlenmeyer flask was added 28 mL of the monobasic solution and 72 mL on the dibasic solution.

4.3.4 Sodium Bicarbonate/Sodium Hydroxide Buffer Preparation

A 0.5 M sodium bicarbonate solution was prepared in a 100 mL volumetric flask. In a separate 100 mL volumetric flask a 1 M sodium hydroxide solution was prepared.

To prepare the pH 9.8 buffer solution, to a 250 mL Erlenmeyer flask was added 100 mL of the sodium bicarbonate solution and 15.2 mL of the sodium hydroxide solution.

4.3.5 Addition of DMSO as Co-solvent to Increase the Enantioselectivity of *Candida rugosa* Lipase

In a 25 mL round bottom flask; 1.1 g (5 mmol) of racemic methyl ibuprofen ester, 0.84 g of *Candida rugosa* lipase, 2 mL of DMSO, and 8 mL of pH 7.2 aqueous buffer were placed. The mixture was stirred with a magnetic Teflon™ coated stir bar at 40 ºC.

A control reaction was run at the same time without DMSO. Both reactions were analyzed by chiral HPLC every 24 hours. The reaction was monitored for up to 172 hours.

4.3.6 Racemization of (*R*)-Methyl Ibuprofen Ester in 20 % DMSO/Buffer pH 9.8.

This reaction was performed at a 10 mL scale. In a typical reaction, to a 25 mL dry round bottom flask were added 1.1 g (5 mmol) of enantiomeric eriched (*R*)-methyl ibuprofen (as indicated by chiral HPLC), 2 mL of DMSO, and 8 mL of pH 9.8 aqueous buffer. The reaction was stirred at 40 ºC. The mixture was analyzed by chiral HPLC every 24 hours. The reaction was monitored for up to 172 hours.

4.3.7 Racemization of (*R*)-Ibuprofen at pH 5.3 Buffer

In order to determine whether racemization occurred to the ibuprofen ester, ibuprofen or both at pH 5.3, a separated experiment was performed. In a 20 mL vial 0.50 g of (*R*)-ibuprofen 4 mL of pH 5.3 buffer and 1 mL of DMSO were placed. The reaction was stirred and heated to 40 ºC for 5 days. Chiral HPLC was used for the daily monitoring.

4.3.8 Kinetic Resolution of the Racemic Methyl Ibuprofen Ester

To a dry 125 mL round bottom flask was added 2.2 g (10 mmol) of racemic methyl ibuprofen ester, 1.67 g of *Candida rugosa* lipase, and in three separate reactions 40 mL of aqueous buffers at pH 5.3, 7.2 and 9.8, respectively. The mixtures were stirred at 45 ºC for 144 hours. The reactions were monitored at different time intervals. The

basic samples were acidified to pH 5 with 1 molar HCl solution. When a reaction was stopped, the reaction mixture was extracted with two 40 mL portions of hexanes. The combined organic layers were dried with magnesium sulfate and the conversion of methyl ibuprofen ester to ibuprofen was determined by a chiral HPLC analysis.

4.3.9 Dynamic Kinetic Resolution of Racemic Ibuprofen Ester

This reaction was run at different scales. In a dry 125 mL round bottom flask was placed 2.20 g (10 mmol) of racemic methyl ibuprofen ester, 1.67 g of *Candida rugosa* lipase, 8 mL of DMSO, and in three separate reactions 32 mL of aqueous buffers at pH 5.3, 7.2 and 9.8, respectively. The mixtures were stirred at 40 ºC and monitored (acidifying the basic samples with 1 molar HCl solution) by chiral HPLC for 144 hours. The mixtures were extracted in two 40 mL portions of hexanes. The combined organic layers were dried with magnesium sulfate and the conversions were determined by a chiral HPLC analysis.

4.3.10 Crystallization of Enriched (*S*)-Ibuprofen to Enhance the Enantiomeric Excess

In three different experiments 2.000 g of pulverized enantiomerically enriched (*S*)-ibuprofen (*ee* = 94%) were added to 10 mL of methanol, ethanol, and isopropanol in a 25 mL round bottom flask. The flasks were placed in a water bath at 45 ºC and stirred with a magnetic bar until the solution was clear; then the temperature of the water bath was reduce to 35 ºC. When the flasks were cooled, some crystals of pulverized (*S*)-ibuprofen

or racemic ibuprofen were added to the flask to act as nuclei (seeds). Then the flasks were placed in a water bath at 25 ºC and the temperature was gradually reduced to 0 ºC in about 6 hours. For maximum crystal recovery, the samples were allowed to stand at 0 ºC in a refrigerator overnight.

The ibuprofen crystals were collected by vacuum filtration on a Buchner funnel and dried at room temperature for further analysis. For ethanol and isopropanol, most of the ibuprofen was recovered as filtered crystals with no enrichment.

For methanol, the solution was turbid and very little crystals formed. The filtrate contained the desired enriched (*S*)-ibuprofen product, as indicated by chiral HPLC, and was then evaporated to give solid product.

4.4 Other *In Situ* Racemization Attempts

4.4.1 Attempted *In Situ* Racemization of Ibuprofen with Organic Acids in Cyclohexane

In a typical reaction, 2.06 g (10 mmol) of racemic ibuprofen was dissolved on 40 mL of cyclohexane, 6.66 g of *Candida rugosa* lipase, and 1.58 g (10 mmol) of decan-1-ol and 0.026, 0.052, 0.103, 0.206, 0.412, 0.824 and 1.648 g of *p*-toluenesulfonic acid in separate reactions. The reactions were monitored by chiral HPLC to determine the racemization rate caused by the acid addition. The same procedure was followed using

different acids or acid sources: *p*-toluenesulfonyl chloride, phenyl dimethyl chlorosilane, boric acid, pyruvic acid, isolute Si-propylsulfonic acid, and isolute Si-TsOH.

4.4.2 Attempted *In Situ* Racemization of Ibuprofen with Organic Bases in Cyclohexane

In a typical reaction, 2.06 g (10 mmol) of racemic ibuprofen was dissolved on 40 ml of cyclohexane, 6.66 g of *Candida rugosa* lipase and 1.58 g (10 mmol) of decan-1-ol and 0.026, 0.052, 0.103, 0.206, 0.412, 0.824 and 1.648 g of trioctylamine in separate reactions. The reaction were monitored by chiral HPLC to determine the racemization rate caused by the acid addition. The same procedure was followed using different bases: tripentylamine, tributylamine, diethylamine, methyldiethylamine and ethyldisopropylamine.

4.4.3 Attempted Racemization of Methyl Ibuprofen Esters with Light

In a dry 125 mL Erlenmeyer flask was added 2.2 g (10 mmol) of racemic methyl ibuprofen ester, 1.67 g of *Candida rugosa* lipase, and 40 mL of potassium phosphate buffer solution at pH 7.2. A UVc lamp was inserted into the reaction to induce photochemical reactions. The mixture was stirred at 45 ºC for 144 hours and monitored at different time intervals. The reaction mixture was extracted with two 40 mL portions of hexane. The combined organic layers were dried with anhydrous magnesium sulfate and the conversion from racemic methyl ibuprofen ester to (*S*)-ibuprofen was determined by a

chiral HPLC analysis. Some photochemical reactions were developed in a 7880 photochemical reactor from ACE photochemical reactors company.

4.4.4 Attempted Racemization of Ibuprofen in a Two-Phase Continuous Reactor

A two-phase reactor was designed to improve the esterification reaction with *in situ* racemization of the unreacted (*R*)-ibuprofen. On the left side, to a 50 mL round bottom flask was added 20 mL of distillated water, 3.33 g of *Candida rugosa* lipase and 1.03 g of racemic ibuprofen. The pH was adjusted to 8.6 by adding pulverized NaOH. Cyclohexane and methanol 9/1 v/v were added to fill the flask. The left flask was adjusted to 45 ºC with magnetic agitation and the right flask (product trap) was heated to 65 ºC to return the mixture of cyclohexane and methanol to the left flask. The samples were taken from the right flask and analyzed by chiral HPLC at different interval times.

4.4.5 Attempted Racemization of Methyl Ibuprofen Esters with a Three-Phase Membrane Separated Reactor

In a 250 mL round bottom flask, 80 mL potassium phosphate buffer solution at pH 7.2 and 13.12 g of *Candida rugosa* lipase were added to form the bottom aqueous phase. 80 mL of cyclohexane containing 4.4 g of racemic methyl ibuprofen ester were added making the top phase of the two phase reaction. One gram of NaOH wrapped in either a filter paper or Whatman Nucleopore filters was suspended by string in the organic phase. The objective was the racemization of the unreacted (*R*)-methyl ibuprofen ester to

increase the production of (*S*)-ibuprofen through the enantioselective enzymatic reaction on the surface of the base pouch.

4.4.6 Attempted Acid Catalyzed Esterification, Racemization and Enantioselective Hydrolysis in a Two Reactor System

Using two 50 mL round bottom flasks connected with a three way adapter two different reactions were run. In a left flask 20 mmol of racemic ibuprofen, 1 g of concentrate sulfuric acid were added and the rest of the volume was filled with a 10% methanol-water solution (the minimum methanol concentration to produce the esters). In the right flask, 3.33 g of *Candida rugosa* lipase were placed and the flask was completely filled with pH 7.2 sodium phosphate buffer solution. The two flask were connected by a distillation adapter and the reactions were heated at 40 °C and stirred at a 100 rpm.

CHAPTER 5

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EDUCATION AND ACADEMIC DEGREES

2010 Ph. D. Chemistry, University of Texas at El Paso
Dissertation: "Conversion of Racemic Ibuprofen to (*S*)-Ibuprofen"
The goal of this research was to develop the dynamic kinetic resolution of (*S*)-ibuprofen from its racemate. This research is environmentally benign and involved the use of enzymes as enantioselective catalysts in organic and aqueous solvents, racemization and low temperatures. Enzymes are the promising catalyst for the transformation and production of chemicals due to the high efficiency and ability to catalyze reactions such as hydrocarbon oxidation, desulfurization, dehydration, esterification, hydrolysis, etc., enzymes are appealing for the environmental chemistry (Green Chemistry).

2006 M. S. Chemistry, University of Texas at El Paso
Thesis: "Commercial viable resolution of (*S*)-ibuprofen"
The goal of this research was to develop and environmentally friendly kinetic resolution process for racemic ibuprofen; however the maximum percent yield of the product obtained was 50 percent based on the racemic starting substrate.

2002 B. S. Chemical Engineering with emphasis on Foods, University of Chihuahua Mexico
Thesis: "Design and Building of a Cooling Tower"
This research involved the design and building of a cooling tower to reduce water waste and energy use in the cooling and pasteurization process for cheese industry in the Chihuahuan desert.

PROFESSIONAL EXPERIENCE

2004 - 2010 Teaching Assistant of Organic Chemistry Laboratories in the Chemistry Department at the University of Texas at El Paso. During my graduate studies at UTEP, I worked as Teaching Assistant of the Organic Chemistry Laboratories, lecturing for the pre-lab and monitoring the laboratory sessions for chemistry major students.

2006 to 2008 Research Assistant in the Development of Materials World Modules and a web page design for UG Science Courses ARMY. My research assistantship requires that I develop and edit a scientific web page.

http://materialsworld.utep.edu

May - June 2007 — Sustainability and Green Chemistry Summer School, Pan-American Advanced Studies Institute (PASI), Universidad Iberoamericana. Mexico City, MEXICO. Organized by ACS Green Chemistry Institute. I attended a 3-week summer course that brought together international scientists to teach and interact with graduate students from around the world. All the courses and laboratories were focused on Green Chemistry and Sustainability.

May–Dec. 2005 — Internship at the ExxonMobil Research and Engineering Company with the Project "Preparation of Hybrid (Organic-Inorganic) Membranes for Distillate Separation and Solvent Separation from Extraction". I worked in the Advanced Separation Division (Catalyst Technology), where reactions involving different inorganic materials and organic membranes were developed with the purpose of produce new hybrid materials with superior chemical, thermal stability and selectivity. As result, we obtained a membrane able to remove solvents from the extraction mixture and a membrane able to upgrade the distillate quality to produce diesel from FCC which reduced costs of the solvent recovery and diesel separation.

1998 - 2002 — During my bachelors on Chemical Engineering I made three internships and the Community Service developing chemistry and chemical engineering tasks as next:
Internship in DELPHI: Quality control and design of new products.
Internship in Harinas de Chihuahua: Quality control and analysis of final product.
Community Service Chihuahua Health Department: Taking samples of foods from restaurants in Chihuahua Mexico city for future microbiological and physical analysis.
Internship in Cuproquim of Mexico: Quality Control.

CONFERENCES, CONGRESS AND ACHIEVEMENTS

October 2009 — Oral Presentation at the SACNAS National Conference: "Improving the Human Condition: Challenges for Interdisciplinary Science" in Dallas Texas.

Publication: Karina Castillo, J.G. Parsons, David Chavez, Russell R. Chianelli. Oxidation of Dibenzothiophene to Dibenzothiophene-sulfone using Silica gel. *Journal of Catalysis 268 (2009) 329–334*

June 2009 Oral Presentation at the 13th Annual Green Chemistry and Engineering Conference in College Park MD. from June 22 to June 25, 2009.

Oral Presentation at the XI Congreso Mexicano de Catálisis in Ensenada Baja California Mexico from June 2 to June 5, 2009.

April 2009 Publication: Chavez-Flores, D. and Salvador, J.M., Commercially Viable Resolution of Ibuprofen. *Biotechnol. J. 2009, 4, 1222–1224*

October 2008 Poster Presentation at the SACNAS *(*Society for Advancement of Chicanos and Native Americans in Science*)* National Conference: "International Polar Year: Global change in Our Communities", at Salt Lake City, UT.

June 2008 Winner of the Poster presentation Award at the 12th Green Chemistry and Engineering National Conference in Washington, DC. Organized by ACS Green Chemistry Institute. At this conference, nearly 160 posters were presented covering an array of issues, from Bio-based Materials & Processes to Solvents and Solvent Systems, to green educational ideas. Only two students received poster prizes ($1000), sponsored by Royal Society of Chemistry and American Institute for Chemical Engineers and Institute for Sustainability, one was awarded for my research "Commercial Viable Resolution of (*S*)-Ibuprofen".

April 2008 Oral Presentation at the I National Congress of Sustainable Chemistry and II National Congress on Microscale Chemistry at the Autonomous University of Yucatan, Mexico.

October 2007 Oral Presentation at the SACNAS National Conference: "Stretching the Imagination to Support Leadership and Sustainability" in Kansas City MO.

March 2007 Poster Presentation at ACS (American Chemical Society) National Conference in Chicago Il.

October 2006 Poster Presentation at the SACNAS National Conference: "Science Revolution in Minority Communities: What Progress Have We Made" in Tampa Bay FL.

March 2006 Poster Presentation ACS (American Chemical Society) National Conference in Atlanta GA.

SKILLS PROFILE

Chemistry	Fourier Transform Infrared (FTIR) spectroscopy, Ultraviolet Visible (UV-vis) spectroscopy, Flame Atomic Absorption Spectroscopy, Chromatography techniques (GC, HPLC and HPLC-MS) and organic molecules analysis by different techniques on NMR.
NMR Maintenance	On the last 5 years I helped on the maintenance of the NMR instruments (300 MHz Bruker and 600 MHz JEOL), Nitrogen and Helium fills.
Languages	I am bilingual and proficient in writing in both English and Spanish
Computer Skills	Good Skills in data acquisition systems and software, database handling, statistical data analysis, high proficiency in Microsoft Office, scientific graphics, and publishing software