## ABSTRACT

## SYNTHESIS AND ENZYMATIC RESOLUTION OF AMINO ACID ESTERS IN "GREEN" SOLVENTS — IONIC LIQUIDS

**By**
**Hua Zhao**

Chiral separation has attracted tremendous attention in pharmaceutical and chemical fields, especially in the area of chiral drug development. Chiral amino acids are among the most important intermediates in the asymmetric synthesis of modern drugs. This research has developed an effective method for obtaining optically pure amino acids by enzymatic resolution using a "green" solvent, named an ionic liquid.

Ionic liquids, a new type of "green" solvents, have been used in many organic reactions and other chemical processes (such as liquid-liquid extraction) with improved performance. This is mainly due to their favorable properties for chemical applications, such as low vapor pressure, low melting point, catalytic features, chemical and thermal stability, nonflammability, and high ionic conductivity, etc. In this study, three different ionic liquids were prepared, 1-ethyl-3-methylimidazolium tetrafluoroborate ($[EMIM]^+[BF_4]^-$), *N*-ethyl pyridinium tetrafluoroborate ($[EtPy]^+[BF_4]^-$) and *N*-ethyl pyridinium trifluoroacetate ($[EtPy]^+[CF_3OO]^-$). They were used as "green" solvents in the enzymatic resolution and as catalysts for the esterification reactions.

Amino acid L-(-)-piperazine-2-carboxylic acid was prepared with 98.1% enantiomeric excess (*ee*) and 40.6% resolution yield ( max 50% possible) by several steps, including *N*-protection, esterification, and enzymatic resolution in an organic solvent by the enzyme *Bacillus licheniforms* alcalase (*BL*-alcalase). Another important unnatural amino acid *N*-acetyl homophenylalanine ethyl ester was synthesized by a three-

step-reaction strategy. Further, L-(+)-homophenylalanine hydrochloride with 92.4% *ee* was obtained by an enzymatic resolution in acetonitrile-water mixture using enzyme *BL*-alcalase.

The solvent effects on the kinetic resolution of *N*-acetyl homophenylalanine ethyl ester were systematically investigated using the enzyme *BL*-alcalase in several organic solvents and ionic liquids ($[EMIM]^+[BF_4]^-$, $[EtPy]^+[BF_4]^-$, and $[EtPy]^+[CF_3OO]^-$). It has been shown that a high concentration of ionic liquids can decrease the performance of the enzyme, while low content of ionic liquids might increase the activity of the enzyme *BL*-alcalase. The enzymatic resolution reaction was also studied at different reaction temperatures and reaction times. High *ee* and yield achieved in ionic liquids indicate that ionic liquids can be ideal substitutes for organic solvents in the kinetic resolution of amino acid esters.

For the first time, it has been shown that the ionic liquid $[EtPy]^+[CF_3COO]^-$ can be used as a "green" catalyst in the synthesis of amino acid esters, including unnatural amino acid esters. Satisfactory conversion was achieved for the formation of amino acid esters under mild conditions. This straightforward process provided starting racemic amino acid esters for the kinetic resolution studies.

Furthermore, the ionic liquid $[EtPy]^+[CF_3OO]^-$ was applied as a "green" solvent for the kinetic resolution of several other *N*-acetyl amino acid esters using different enzymes: *BL*-alcalase and porcine pancreas lipase (*PPL*). High optical purity and yield were generally achievable under low concentration of ionic liquids. It also shows that this method could be a general process in the production of chiral amino acids for pharmaceutical and biotechnology applications.

# SYNTHESIS AND ENZYMATIC RESOLUTION OF AMINO ACID ESTERS IN "GREEN" SOLVENTS — IONIC LIQUIDS

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To my beloved family

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# CHAPTER 1

# INTRODUCTION AND OBJECTIVES

## 1.1 Chiral Synthesis, Resolution and Separation of Amino Acids

Chiral technology has played an increasingly important role in pharmaceutical and chemical industries. Chirality can offer entirely different biological properties to molecules that are alike in every aspect except that their mirror image is not superimposable. Chiral technology is extremely valuable to the pharmaceutical industry. As shown in Figure 1.1, single isomer (chiral) drug sales reached $115 billion worldwide in 1999, 16% up from $99 billion in 1998 (Stinson, 2000). This figure has exceeded $133 billion in 2000 and could hit $200 billion in 2008 (Stinson, 2001). Also, 40% of all dosage-form drug sales in 2000 were of single enantiomers. In 1999, the share was one-third (Stinson, 2001).

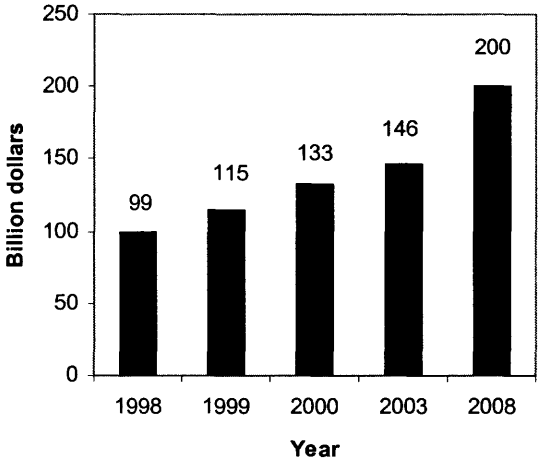



**Figure 1.1** Annual chiral drug sales and predictions reported by C&EN (Stinson, 2001).

This importance is simply because enantiomers have the ability to confer different chemical properties in the presence of an optically active biological system. For example, dextromethorphan is an over-the-counter (OTC) drug sold as a cough suppressant, however, its stereoisomer, levomethorphan is a controlled narcotic (Ahuja, 1997).

Most α-amino acids have a relatively simple structure, with one or two asymmetric centers, and are amenable to a variety of chemical transformations (Coppola and Schuster, 1987). The amino acid functionality is particularly versatile and may be used in many different ways. L-α-amino acids are increasingly becoming the first compounds to be considered when planning a synthesis leading to a homochiral target. Commercial availability has followed market needs, a classic example being the development of low cost production of L-aspartic acid and L-phenylalanine to meet the demand for the artificial sweetener aspartame. Similarly, L-proline, available from fermentation, is the key raw material for a variety of angiotensin-converting enzyme (ACE)-inhibitor drugs (Sheldon et al., 1991) including Captopril, Enalapril, and Lisinopril. For the latter two products, L-alanine and L-lysine are also key raw materials, respectively. The mirror-image D-enantiomers are less common, however, they are becoming increasingly available. For example, production of D-alanine has increased to meet the market demand for it as the raw material for Pfizer's new dipeptide sweetener, Atitame (Sheldon, 1993).

Single stereoisomers can be obtained mainly by two different methods, i.e., asymmetric synthesis and resolution also known as chiral separation. Chiral separations tend to be more difficult compared with traditional separation methods (such as distillation, centrifugation, etc.) because enantiomers have identical physical properties.

In the following sections, a brief review will be given for each method on chiral synthesis and separations.

A number of practical methodologies have been developed for the synthesis of optically pure α-amino acids, especially for nonproteinogenic (unnatural) class of amino acids. Generally, two major routes can be followed: (1) direct asymmetric synthesis of α-amino acids by modern organic synthesis methods using appropriate chiral auxiliaries; (2) resolution of racemic α-amino acids by chemical and enzymatic methods.

### 1.1.1 Asymmetric Synthesis

Asymmetric synthesis is the process by which a substrate containing no chiral elements is transformed by route of an asymmetric step into the desired chiral product. Asymmetric synthesis has the advantage that theoretically all of the substrate can be converted into the desired single stereoisomer. Basically, there are two asymmetric synthesis methods: non-enzymatic methods (Scott, 1989) and enzymatic methods (Crosby, 1991). However, the decision to implement an asymmetric synthesis approach should be based on an assessment of efficiency and cost. The factors to consider include: (1) the catalyst efficiency (i.e., the number of product molecules produced per molecule of the catalyst); (2) the availability of the metal, the ligand, and the starting materials (especially critical for low-value products); (3) reaction conditions, such as low temperatures or high pressures, and reaction kinetics.

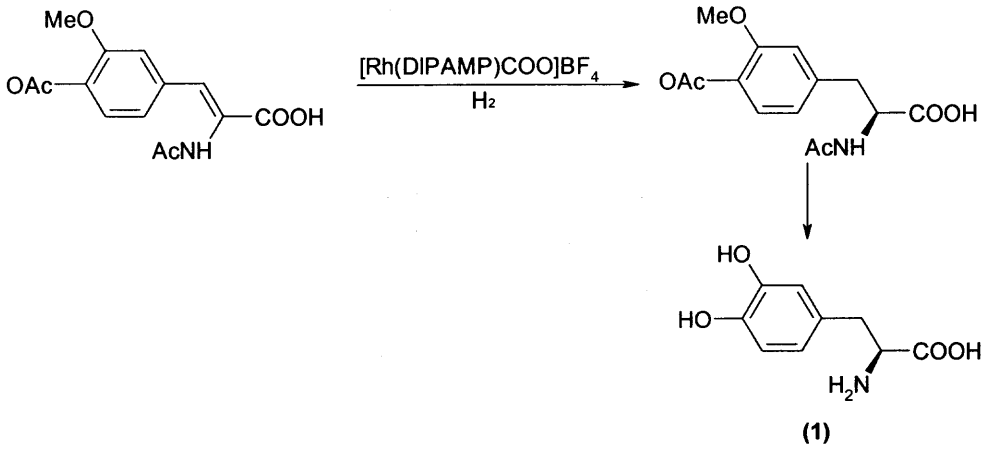



**Figure 1.2** Asymmetric hydrogenation using modified Wilkinson catalyst.

**1.1.1.1 Non-enzymatic Catalytic Asymmetric Synthesis.** Asymmetric hydrogenation has its origins in the soluble Wilkinson catalyst modified with chiral phosphine ligands; this led to the Monsanto process for L-DOPA (**1**) (Figure 1.2) commercialized in the early 1970s. This was a landmark in industrial asymmetric synthesis (Knowles, 1983) and a spur for a huge amount of other industry-based research. Another commercial asymmetric hydrogenation is the Enichem synthesis of (S)-phenylalanine (Ojima et al., 1989).

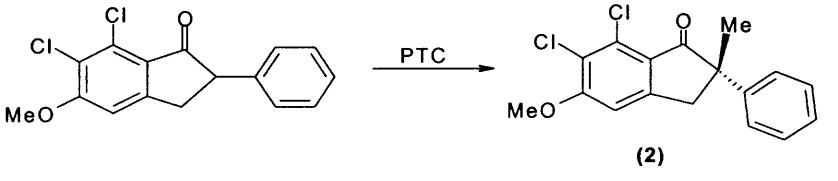



**Figure 1.3** Alkylating indanone by asymmetric phase transfer catalysis.

Asymmetric phase transfer catalysis (PTC) has proved a difficult area with false dawns (Dehmlow, et al., 1981) before the signal achievement by the Merck Group of Dolling et al. who obtained the alkylated indanone (**2**) (Figure 1.3) (Dolling et al., 1984) in 92% *ee* and 95% yield. More recently O'Donnell et al. (1989) also using a quaternised cinchona alkaloid as the catalyst, alkylated the Schiff base ester (Figure 1.4) to realize an α-amino acid synthesis. Although by most standards the *ee* of the product of the alkylation step would be considered low for a practical synthesis (66%), recrystallisation leads in one step to optically pure product (in the filtrate); to date this process has only been run on a multigramme scale.

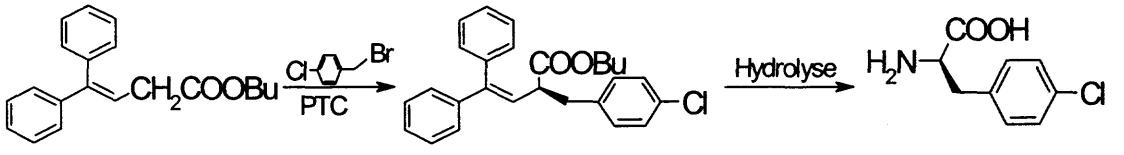



**Figure 1.4** Alkylating Schiff base ester using quaternised cinchona alkaloid as catalyst.

**1.1.1.2 Enzymatic Asymmetrical Synthesis.** This section is concerned with the use of microorganisms or isolated enzymes to catalyze single transformations, rather than multi-step sequences from basic feed stocks such as carbohydrates.

Reductive amination

Figure 1.5 shows an interesting example developed by Wandrey at the Institute of Biotechnology, Nuclear Research Center, Julich; the process has been commercialized by Degussa. The continuous operation reactor system, which had a capacity of about 250

tonnes per year of amino acids (Wandrey, 1987), is achieved through use of an ultrafiltration membrane to retain the soluble enzyme. The necessary cofactor is also retained by the membranes by binding it to a water-soluble polymer, polyethylene glycol (PEG) in order to increase its molecular weight. One of the first materials to be produced commercially using this technique was L-tertiary leucine in 1986.

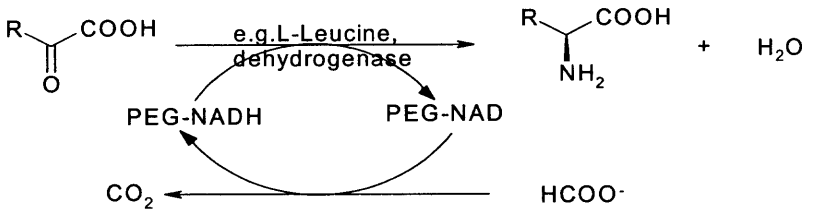
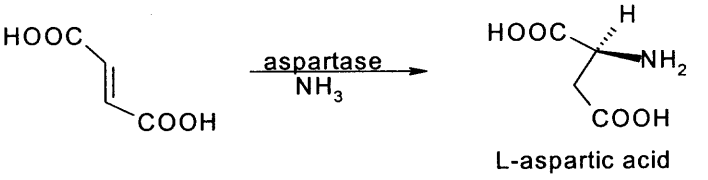



**Figure 1.5** Continuous production of amino acids through reductive amination.

(a)

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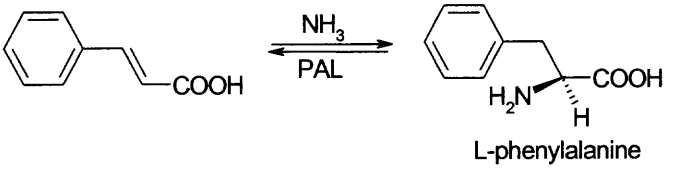


(PAL=L-phenylalanine ammmoniolyase)

**Figure 1.6** Production of L-aspartic acid and L-phenylalanine by ammonia addition.

Ammonia addition

Production of L-aspartic acid (Figure 1.6a) is an industrial important example using enzyme addition of ammonia to a prochiral substrate, fumaric acid. The production of pharmaceutical grade material was about 4,000 tonnes per annum in 1987 (Calton, 1987). It is possible to immobilize the enzyme and still retain virtually all (97%) of the aspartase activity; the half life of such an immobilized catalyst was estimated at 3-4 years (Calton, 1987). Commercialization of a corresponding process to L-phenylalanine (Figure 1.6b) was achieved by Genex (Evans, 1989). This is a more difficult reaction because of low conversion, poor stability of the enzyme, and substrate inhibition.

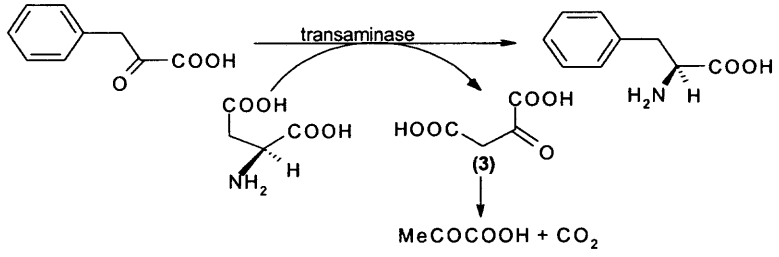


**Figure 1.7** Synthesis of L-phenylalanine by transamination from aspartic acid.

Transamination

Transaminases have been studied extensively for over 60 years and have frequently been investigated in biotransformation approaches for the production of amino acids and chiral amines (Christen and Metzler, 1985; Calton et al., 1986). Another large scale (600 tonnes per year) approach to L-phenylalanine, developed by Purification Engineering (Anon, 1984) is transamination from aspartic acid to phenylpyruvic acid (Figure 1.7). The initial

byproduct, (3), decarboxylates yielding pyruvic acid. A recent review of production of unnatural amino acids using transaminases has been given by Taylor et al (1998).

### 1.1.2 Resolution by Enzymes and Chiral Reagents

A promising development for the resolution of amino acids is the use of enzymes. It was shown that the enzymes used for this purpose are often purified, and in immobilized, cell-free, or whole-cell forms. The enzymes of amidase, acylase, hydantionase, and esterases are being proven effective in the production of optically active α-amino acids.

The case of preparing racemic α-amino acids by the Strecker synthesis (Stockenius, 1878) has made use of selective **Amidase** in effecting a kinetic resolution of considerable importance. The review of **Acylase I** as a broadly applicable enzymatic catalyst for the kinetic resolution of unnatural and rarely occurring α-amino acids was given by Chenault et al. (1989). **Lipase** has been a popular enzyme for chiral separations, whose kinetics in organic-water solvent for chiral reaction (Mohapatra and Hsu, 1997) and for resolution of amino acids (Houng et al., 1996) have been extensively investigated. **Proteases** have also been studied for the enzymatic resolution of non-protein amino acids (Miyazawa et al., 1992 and 1994). An industrial alkaline protease *Bacillus licheniforms* **alcalase** (*BL*-alcalase) has been found to be very stable in organic solvents and efficient for resolution of *N*-protected amino acids.

The kinetic resolution of different amino acids by *BL*-alcalase has been studied in water (Kijima et al., 1994) and mixtures of organic solvent + water, such as **acetone**-water (Kijima et al., 1994), **acetonitrile**-water (Kijima et al., 1994), **ethanol**-water (Kijima et al., 1994), **1-propanol**-water (Kijima et al., 1994), **tetrahydrofuran**-water

(Kijima et al., 1994), **dioxane**-water (Chen et al., 1991), **t-butanol**-water (Chen et al., 1991), **2-methyl-2-propanol**-water (Chen et al., 1994), and **DMF** (Chen et al., 1986), etc. Kinetic resolution has revealed many promising results. It has been successfully used to separate chiral alcohols, acids, esters, amino acids, diols, and diesters, etc (Ahuja, 1997).

In addition, recent work on cross-linked enzyme crystals (CLECs) (St Clair and Bavia, 1992) has demonstrated that enzymes in this form acquire high stability while preserving their activity in high-water mixtures (Persichetti et al., 1995) and organic solvents (Khalaf et al., 1996). It was also reported that cross-linked crystals of *Candida rugosa* lipase (CRL) show high enzymatic activity for the resolution of chiral esters (Lalonde et al., 1995). It is believed that the cross-linked enzyme crystal (trademarked CLEL) technology will increase the enzyme stability without losing enzyme activity, and could make the scale-up of biocatalytic processes possible (Lalonde, 1997).

Using chiral reagents is another strategy to achieve enantiomeric compounds without using enzymes. Diastereomers are usually formed by an enantiomeric compound and a chiral reagent. The diastereomers are isolated by either crystallization or chromatography. During the last three decades, various chiral derivatizing reagents have been developed for determination of enantiomeric purity and absolute configuration of optically active compounds by NMR and HPLC. Among these reagents, Mosher acid (MTPA, α-methoxy-α-trifluoromethylphenylacetic acid) has been most widely used (Dale et al., 1969). Later, Ishikawa and his co-workers provided two such reagents, perfluoro-2-propoxypropionic acid (PPPA) (Kawa and Ishikawa, 1980) and prefluoro-2-isopropoxypropionic acid (PIPA) (Kawa et al., 1982). CFPA (α-cyano-α-

fluorophenylacetic acid) was reported (Takeuchi et al., 1991) to give very large $^{19}F$ NMR shift for the diastereomers. A new reagent α-methoxy-α-trifluoromethylpropionic acid (MTPr) has been studied for capillary gas chromatographic separation of enantiomeric amino acids at considerably low column temperature (Yasuhara et al., 1992). Large-scale chiral separation of amino acids was considered possible by using chiral reagents of (S)-2-*N*-(*N'*-benzylprolyl) aminobenzaldehyde series (Ryzhov and Belokon, 1994). The enantiomeric separations of amino acids using micellar electrokinetic chromatography (MEKC) after pre-column derivatization with chiral reagent FLEC (1-(9-fluorenyl)-ethyl chloroformate) was investigated by Chan et al. (1995). The drawbacks of using chiral reagents are the high cost of the agents and the usual difficulties connected with using ion-exchange technology for separation of amino acids from solution after decomposition of the complex.

### 1.1.3 Chiral Separation by Chromatographic Methods (HPLC, TLC, and GC)

**1.1.3.1 High Performance Liquid Chromatography (HPLC).** One of the most powerful techniques for separating enantiomers is using high performance liquid chromatography (HPLC). There are major approaches: (1) pre-column derivatization with a chiral reagent followed by the separation of the resulting diastereomeric derivative (Yamada et al., 1989a,b); (2) direct separation of enantiomers on a chiral column. The latter process is more active in recent research. In this case, the racemic mixture passes through a column containing a chiro-selective stationary phase or a column using a chiro-selective mobile phase. As the racemic mixture flows through the column, its enantiomers are separated according to their affinities to the chiral selector. Difference in

binding and repulsive interactions between the chiral selector and the enantiomers is what causes the separation.

Mobile phase chiral selectors have been used to resolve amino alcohols (such as alprenolol), carboxylic acids (such as tropic acid and naproxen), and amino acids (such as tryptophan) (Ahuja, 1997). An overview of this method has been published by Lindner and Pettersson (1985). Three major approaches to the formation of diastereomeric complexes are transition metal ion complexes (ligand exchange), ion pairs and inclusion complexes. Each method is based on the formation of reversible complexes and uses an achiral chromatographic packing. The chiral ion-pair systems are not stable. The chromatography can be affected by the water content of the mobile phase, temperature, pH and other variables. In addition, the counterions often absorb in the UV region, reducing the sensitivity of the system; indirect photometric detection (Allenmark, 1986) or other detection methods must be used.

On the other hand, the use of chiral selectors in the stationary phase has also shown some potential and is among the fastest growing areas in chiral separations. The first commercially available chiral stationary phase (CSP) for HPLC was introduced by Pirkle in 1981 (Pirkle et al., 1981), and today many kinds of chiral phases are commercially available. The separation of enantiomeric compounds on a CSP is due to differences in energy between temporary diastereomeric complexes formed between the solute isomers and the CSP; the larger the difference, the greater the separation.

There are several types of stationary phases available: (1) Pirkle-type and related CSPs; (2) derivatized cellulose; (3) cyclodextrins and other inclusion-complex-based CSPs; (4) ligand exchangers, which is a promising method developed in recent years for

the separation of enantiomers (Davankov, 1989). Several columns have thus far become available for this purpose. Among such columns is the one packed with octadecylsilanized silica coated with *N*, *S*-dioctyl-D-penicillamine as a chiral ligand-exchange phase (Sumichiral OA-5000). The enantiomeric separation of proteinogenic amino acids, including a few nonprotein amino acids, has been achieved by making use of this column (Oi et al., 1992; Miyazawa et al., 1997). (5) Protein CSPs. The most important criterion for choosing a CSP is obviously chiral selectivity, expressed by the separation factor $\alpha$. Nevertheless, when a preparative separation is required, other parameters such as loading capacity, solubility of the solute in the mobile phase, or chemical stability of the CSP can outweigh selectivity.

However, using chromatography for chiral separation was considered inefficient. At first, the undesired enantiomers that make up to 50% of the substrate usually went to waste. Also, the large quantities of solvents required made chromatography an expensive option. Fortunately, new developments have improved many of the stated weaknesses. For example, the undesired enantiomer is now being treated and recycled back into the system, which has substantially recycled the usage of raw materials.

Another new chromatographic technique called simulated moving bed (SMB) has also compensated for many of the known deficiencies. In this technique, the front and back end of the column are united giving it a circular shape. The feed is intermittently injected in a circular fashion around the column. The overall attempt is to simulate a backwards moving bed. This technique, which was initially developed in the 1960s (Broughtonand and Gembicki, 1962), is already applied in various industrial processes, and it is now available on the laboratory scale. SMB chromatography allows the

continuous introduction of the feed and fully utilizes the available mass-transfer area, so throughput is higher. Moreover, the technique is particularly appropriate for the separation of binary mixtures such as racemates. Both the principle and the design of the SMB system have recently been reviewed (Barker and Ganestos, 1987; Hashimoto et al., 1993; Kubota and Hayashi, 1994). In view of its scale-up potential, its range of applicability can be expected to increase rapidly. Besides saving the mobile phase, SMB chromatography has the advantage of being highly productive with even modest amounts of CSP, thus reducing the costs for the generally very expensive chiral sorbents.

**1.1.3.2 Thin-Layer Chromatography (TLC).** Other than HPLC, Thin-Layer chromatography (TLC) is also a popular separation method because of its simplicity, low cost, low solvent consumption and wide versatility and applicability. TLC is used extensively in amino acid, peptide, and protein research, since its reproducibility makes it ideal for microscale analysis and separation of amino acids (Jentsch, 1986). However, it is not suitable for large-scale separation in industry.

**1.1.3.3 Gas Chromatography (GC).** In contract to the development of CSPs for HPLC, there are also some developments and commercialization of CSPs for GC. Schuring (1994) recently published an excellent review article on GC CSPs. Enantioselective GC offers several other advantages over HPLC (Konig, 1993): speed of analysis, separation of nonderivatizable compounds, low detection limits for enantiomeric impurities, universal detectors, easy resolution of most volatile compounds, durability, and high absolute sensitivity that is independent of functional groups. The disadvantage of using GC and volatile amino acid derivatives is that the analyte of interest must be derivatized twice to be sufficiently volatile and selective.

### 1.1.4. Other Chiral Separation Methods

**1.1.4.1 Micellar-Enhanced Ultrafiltration (MEUF).** Organized assemblies of amphiphilic molecules or surfactants have recently found applications in a number of analytical separations that require large contact areas for resolution. Separation techniques using amphiphilic molecules include micellar chromatography, micelle-based membrane separations, foam fraction, and surfactant-mediated extractions. Micellar–enhanced ultrafiltration (MEUF), a micelle-based membrane separation system, has been used to remove organic molecules from aqueous solutions by solubilization in the dydrophobic core of the micelle (Dunn and Scamehorn, 1985; Gibbs et al., 1987). More recently, MEUF is investigated as a large-scale technique for separating amino acid enantiomers. Creagh et al. (1994) used L-5-cholesterol glutamate, a chiral ligand-exchange cosurfactant, together with a nonionic surfactant to form mixed micelles that preferentially bind D-phenylalanine over L-phenylalanine in the presence of copper (II). Operational selectivities (ratio of the distribution coefficients of D- and L-enantiomer between the permeate and the retentate) as high as 4.2 were obtained.

**1.1.4.2 Capillary Electrophoresis (CE).** Capillary Electrophoresis (CE) is a powerful analytical approach for the analysis of trace amounts of amino acids (Bergman and Jornvll, 1992) and for other chiral separation purposes. CE can be used as an alternative method for the separation of many compounds (both polar and nonpolar). The short separation time (minutes) is convenient, however, the small amount of solvent used (nanoliters) is considered a disadvantage for preparative-scale separations and detection sensitivity. Types of CE include capillary zone electrophoresis, capillary electrokinetic chromatography, capillary gel electrophoresis, capillary isotachophoresis, and capillary

isoelectric focusing (Ward, 1994). A good review of chiral separations by capillary zone electrophoresis and micellar electrokinetic capillary chromatography is given by Kuhn and Hoffstetter-Kuhn(1992). As mentioned before, CE is not good for large-scale chiral separation purposes because of its limitations.

**1.1.4.3 Resolution by direct Crystallization.** This is an attractive but infrequently used method; auxiliaries and reagents, other than a solvent are not required. The treatise by Jacques et al. (1981) gives a detailed exposition of the theory. There are a number of variations in the way of resolution by direct crystallization that may be affected in practice. In the first method, simultaneous crystallization of two enantiomers is carried out in a special apparatus (Crosby, 1991). This was in essence the method used in the Merck process for the antihypertensive methyldopa (Anon, 1965). Another method consists of taking alternate crops of each of the enantiomers using a simple vessel; this is the so-called method of resolution by entrainment (Amiard, 1956). A more esoteric variant has been reported (Eur. Pat. 325965) in which the supersaturated solution of racemate is seeded simultaneously with large crystals of one enantiomer and very small crystals of the other. After crystallization has occurred, the products are separated by sieving. Botsaris et al. (1999) recently reported that the use of sodium chlorate crystallization as a tool for probing nucleation had shed new light in the understanding of secondary nucleation.

Another attraction of direct crystallization is that unlike classical resolution it is not necessary for substrates to possess any other particular functionality for it to work. However, it is of limited, and unpredictable applicability. Note, for example, that of the naturally occurring α-amino acids virtually all are resolvable either directly or as

derivatives (Jacques et al., 1981). Uniform quality of feedstock, preferably of high chemical purity, will be required in order to achieve reproducible crystallizations.

### 1.1.5 Summary

There are currently 528 synthetically derived chiral drugs in the market, of which 88% are sold as racemic mixture (Ahuja, 1997). The consultants of *Technology Catalysts* predicted that by the year 2000 almost three-quarters of all man made drugs would be composed of single enantiomers (Richard and McCagne, 1997). The actual figure is only 40% in 2000, however, still a big increase from one-third in 1999 (Stinson, 2001). This large move from racemates to single stereoisomers is the main reason for the rapid growth of chiral technology. Obviously, chiral technology will be extremely important and must have a promising future. However, at present, different kinds of chiral separation methods face different difficulties for practical uses in industry. Such problems as efficiencies, costs, and scale-up, etc. must be solved in order to satisfy the increasing market demands. It seems that direct asymmetric synthesis, HPLC, kinetic resolution, and MEUF will be the most effective tools for manufacturing single enantiomers.

## 1.2 Ionic Liquids and their Physical Properties

### 1.2.1 Introduction and Applications

An ionic liquid (IL) is a liquid containing only ions, however, it is different from the classic definition of a molten salt (Seddon, 1997). A molten salt is usually defined as a highly-melting, highly viscous and highly corrosive liquid, while an ionic liquid is a

liquid at low temperature (< 100 °C) and has lower viscosity. As the introduction of cleaner technologies is becoming a major concern in both industry and academia, using alternatives to the most damaging solvents has become a high priority. Organic solvents are high on the list of damaging chemicals for two simple reasons: (1) they are used in huge amounts, and (2) they are usually volatile, flammable and toxic liquids that are difficult to handle. An ionic liquid is considered as a substitute for those volatile organic solvents, not only because of its low vapor pressure and thus being environmental friendly, also it may show the ability of catalysis as a solvent or auxiliary. Moreover, ionic liquids also have many other attractive features, including chemical and thermal stability, nonflammability, high ionic conductivity, and a wide electrochemical potential window.

Ionic liquids usually consist of nitrogen-containing organic cations and inorganic anions. In fact, ionic liquids can now be produced with melting point of room temperature or below (as low as –96 °C), which is an important reason that ionic liquids have been explored in many applications (Seddon, 1997). Their chemical and physical properties can be finely tuned for a range of applications by varying the cations or anions (Freemantle, 1998). Alkylpyridinium ($RPy^+$) chloroaluminate based ambient temperature ionic liquids have been known since the 1950s (Hurley and Wier, 1951). However, it was the discovery of ethylimidazolium ($EMIM^+$) based chloroaluminate ionic liquids in 1982 (Wilkes and Zaworatko, 1992) that afforded the impetus for a dramatic increase in activity in this area.

Reviews of current and potential applications of ionic liquids as solvents for catalytic synthesis, are available (Seddon, 1997; Welton, 1999; Wasserscheid and Keim,

2000). Ionic liquids have many favorable properties, such as good solvents for a wide range of inorganic, organic and polymeric materials, adjustable polarity, and catalytic effects, etc. Therefore, they have been investigated as reaction media, co-catalysts or catalysts in many organic and organometallic syntheses, including Diels-Alder reactions (Jaeger and Tucker, 1989; Earle et al., 1999), Heck reactions (Welton, 1999; Camichael, 1999a; Xu et al., 2000a), Friedel-Crafts reactions (Surette et al., 1996; Welton, 1999; Stark et al., 1999), alkylation (Welton, 1999; Badri et al., 1992; Chen et al., 1999), hydrogenation (Chauvin and Oliver-Bourbigon, 1995; Suarez et al., 1997; Steines et al., 2000), hydroformylation (Welton, 1999; Chauvin and Oliver-Bourbigon, 1995), oxidation (Song and Roh, 2000), dimerization of butadiene (Silva et al., 1998; Ellis et al., 1999), benzoin condensation (Davis and Forrester, 1999), acylative cleavage of cyclic and acyclic ethers (Green et al., 2000), and polymerization of methyl methacylate (Carmichael et al., 2000a). They have also been used as solvents for extraction (Huddleston et al., 1998; Sheng et al., 1999), multiphase bioprocess operations (Cull et al., 2000), and electrolytes in electrochemistry (Fuller et al., 1998; Kosmulski et al., 2000).

Recently, great attention has been focused on the enzymatic reactions in ionic liquids. As early as 1984 (Magnusson et al., 1984), it was observed that the enzyme alkaline phosphatase is relatively stable in a 4:1 (v/v) mixture of triethylammonium nitrate and water. Erbeldinger *et al* presented the first enzymatic catalysis of formation of Z-aspartame in the ionic liquid $[BMIM]^+[PF_6]^-$ (BMIM=1-butyl-3-methylimidazolium) containing 5% (v/v) water (Erbeldinger et al., 2000). The enzyme *thermolysin* exhibited

excellent stability in the ionic liquid and displayed a competitive rate in comparison to that of enzymatic synthesis in an organic solvent.

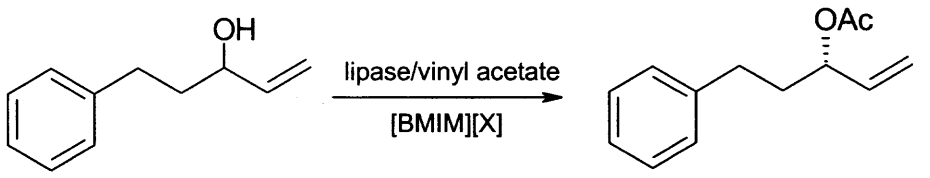


**Figure 1.8** Dynamic kinetic resolution of 1-phenylethanol lipase in an ionic liquid.

The enzyme lipase has been frequently reported as a biocatalyst in organic reactions using ionic liquids. Nine lipases were investigated for dynamic kinetic resolution of 1-phenylethanol by transesterification in various ionic liquids (Schofer *et al.*, 2001). Improved enantioselectivity and activity in ionic liquids were observed compared with those reactions carried in MTBE (Figure 1.8). Kim *et al* (2001) also noticed that enhanced enantioselectivity of lipase was obtained in the transesterifications of alcohols in $[BMIM]^+[BF_4]^-$ and $[BMIM]^+[PF_6]^-$.

The lipase-catalyzed enantioselective acylation of allylic alcohols in the ionic liquid $[BMIM]^+[X]^-$ (X = $PF_6$, TFA, OTs, $SbF_6$) was performed (Itoh et al., 2001). It was found that the center anion of the imidazolium salts had significant influence on the reaction (Figure 1.9).

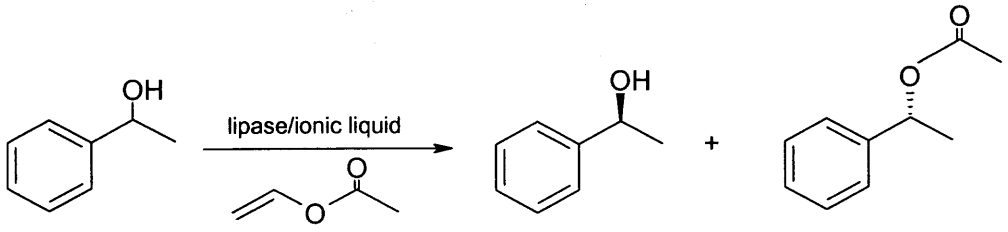


**Figure 1.9** Lipase-catalyzed acylation of allylic alcohols in an ionic liquid.

More systematic studies on lipase-catalyzed enantio- and regioselective acylations were conducted by Park and Kazlauskas (2001) in several imidazolium and *N*-alkyl-pyridinium based ionic liquids. In their research, enantioselective acylation of 1-phenylethanol with vinyl acetate was catalyzed by lipase from *pseudomonas cepacia* (PCL) with very high enantioselectivity (E > 200). Regioselective 6-O-acetylation of β-D-glucose in ionic liquids yielded only 6-O-acetyl glucose (>13:1 and up to > 50:1), while the acetylation in an organic solvent gave 3,6-O-diacetyl glucose (2-3:1 mixture). The epoxidation of cyclohexene by peroxyoctanoic acid, generated in situ by the immobilized enzyme (Novozyme 435) — catalyzed the reaction of octanoic acid with 60% aqueous $H_2O_2$, was performed successfully (Litjens et al., 1999). More recently, a study (Kragl et al., 2001) shows that the enantioselectivity of a lipase-catalyzed kinetic resolution can be increased at higher temperature. This study indicates that for a galactosidase catalyzed synthesis of a disaccharide the secondary hydrolysis is suppressed doubling the yield. It was also observed that three different lipases exhibit both excellent activity and stability in $[BMIM]^+[PF_6]^-$ for the synthesis of an ester (Husum et al., 2001).

### 1.2.2 Compositions of Ionic Liquids

The most commonly used cations in room temperature ionic liquids are alkylammonium, alkyphosphonium, *N*, *N'*- dialkylimidazolium (abbreviated as **[RR'IM]**, for example, 1-ethyl-3-methylimidazolium as [EMIM]), and *N*-alkypyridinium (abbreviated as **[RPy]**) cations (Figure 1.10). The most commonly used alkyl chains are methyl, ethyl, butyl, hexyl, octyl, and decyl, etc.

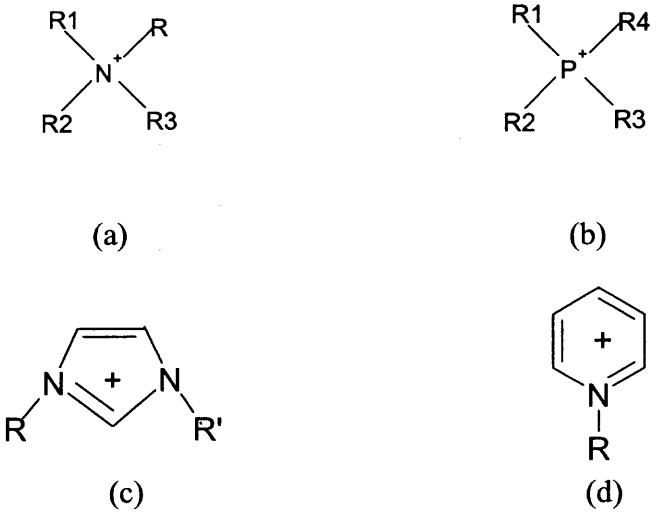


**Figure 1.10** Important types of cations in ionic liquids: (a) Tetraalkyl-ammonium, (b) Tetraalkyl-phosphonium, (c) *N*, *N'* - dialkyl-imidazolium and (d) *N*-alkyl-pyridinium cations.

**Table1.1** A List of Anions in Ionic Liquids and their References

| Anions | References | Anions | References |
|---|---|---|---|
| $BF_4$ | (Wilkes and Zaworatko, 1992) | $(CF_3SO_2)_2N$ | (Bonhote et al., 1996) |
| $PF_6$ | (Fuller et al., 1994) | $CF_3CO_2$ | (Bonhote et al., 1996) |
| $SbF_6$ | (Chauvin and Oliver-Bourbigon, 1995) | $B(Et_3Hex)$ | (Ford et al., 1973) |
| $CH_3CO_2$ | (Wilkes and Zaworatko, 1992) | OTs $TS=H_3CC_6H_4SO_2$ | (Karodia et al., 1998) |
| $HSO_4$ | (Keim et al., 2000) | $AuCl_4$ | (Hasan et al., 1999) |
| $NO_3$ | (Wilkes and Zaworatko, 1992) | $AlCl_4$ | (Fannin et al., 1984) |
| $NO_2$ | (Wilkes and Zaworatko, 1992) | Carborane anions | |
| $CF_3SO_3$ | (Bonhote et al., 1996) | $(1\text{-}R\text{-}CB_{11}H_6Cl_6)$ | (Larsen et al., 2000) |

Several anions have been investigated as listed in Table 1.1. Various combinations of cations and anions provide finely designed ionic liquids for different applications.

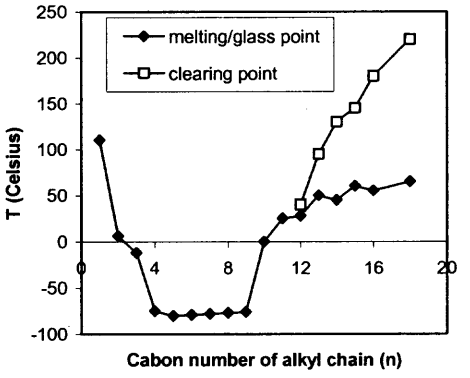


**Figure 1.11** Melting, glass and clearing transitions[†] of 1-($C_nH_{2n+1}$)-3-methylimidazolium tetrafluoroborate (Holbrey and Seddon, 1999).

### 1.2.3 Melting Point of Ionic Liquids

Low-melting-point is an important criterion that make ionic liquids popular as a mediumin organic reactions and other chemical processes. Molten salts usually have very high melting points (for example, NaCl has m.p. = 803 °C), while ionic liquids usually have melting points below 100 °C and many of them are just liquids at room temperature. This characteristic of low-melting-point can be explained as replacing the simple inorganic cations by unsymmetrical organic cations (Seddon, 1997). From the discussion

[†] The term liquid crystal is applied to any compound that exhibits an intermediate state of matter (1-/2-D order) which exists between the crystalline solid (3-D order) and the isotropic liquid (zero order). The intermediate state of matter is termed the mesophase, mesomorphic state or liquid crystalline state and possesses certain properties characteristic of the crystalline solid, the isotropic liquid and some, which are unique to the mesophase. The **clearing point** is the transition from mesophase to isotropic liquid.

of the melting point data below, it can be seen that both cations and anions in the ionic liquids contribute to this low-melting-point feature.

The phase diagram of 1-($C_nH_{2n+1}$)-3-methylimidazolium tetrafluoroborate is shown as a function of carbon number in the alkyl chain in Figure 1.11 (Holbrey and Seddon, 1999). The salts only crystallize on cooling when n = 1 and n > 9. The ionic liquids (n = 2-9) have a strong tendency to supercool, resulting in more viscous fluids, and eventually glasses. When n > 12, the liquid crystalline mesomorphism can be observed. When n ≤ 5, the melting/glass transition points decrease as the chain length increases. It was explained (Larsen et al., 2000) that the alkylation pattern of the imidazolium cation affects the melting point and the packing inefficiency of the cation is the intrinsic reason for the low melting point. As n > 5 in Figure 1.11, the melting/glass transition points increase when the carbon chain is larger. The reason is that when the chain is so big, the alkyl chain itself becomes the main structure of the cation, not the imidazolium part. It is known that alkanes have higher melting points for longer chain molecules. The same pattern can be found in the melting point data listed in Tables 1.2 and 1.3.

**Table 1.2** Comparing Melting Points of Imidazolium Based Ionic Liquids

| Salts | R | R' | Anion | m.p. (°C) | Reference |
|---|---|---|---|---|---|
| **1** | $CH_3$ | $CH_3$ | Cl | 124.5-128 | (Wilker et al., 1982) |
| **2** | $CH_3$ | $C_2H_5$ | Cl | 82-87 | (Wilker et al., 1982) |
| **3** | $CH_3$ | n-$C_3H_8$ | Cl | 58-66 | (Wilker et al., 1982) |
| **4** | $CH_3$ | n-$C_4H_9$ | Cl | 65-69 | (Wilker et al., 1982) |
| **5** | $CH_3$ | $C_2H_5$ | $AuCl_4$ | 58 | (Hasan et al., 1999) |
| **6** | $CH_3$ | n-$C_3H_8$ | $AuCl_4$ | 50 | (Hasan et al., 1999) |

**Table 1.3** Melting Points of Alkoxymethyl Type Imidazolium Based Ionic Liquids (Pernak and Czepukowicz, 2001)

| salt | R | R' | m.p. (°C) | salt | R | R' | m.p. (°C) |
|---|---|---|---|---|---|---|---|
| | **With anion: $BF_4$** | | | | **With anion: $PF_6$** | | |
| **7** | $CH_3$ | $C_3H_7OCH_2$ | liquid | **35** | $CH_3$ | $C_3H_7OCH_2$ | liquid |
| **8** | $CH_3$ | $C_4H_9OCH_2$ | liquid | **36** | $CH_3$ | $C_4H_9OCH_2$ | liquid |
| **9** | $CH_3$ | $C_5H_{11}OCH_2$ | liquid | **37** | $CH_3$ | $C_5H_{11}OCH_2$ | liquid |
| **10** | $CH_3$ | $C_6H_{13}OCH_2$ | liquid | **38** | $CH_3$ | $C_6H_{13}OCH_2$ | liquid |
| **11** | $CH_3$ | $C_7H_{15}OCH_2$ | liquid | **39** | $CH_3$ | $C_7H_{15}OCH_2$ | 37-38 |
| **12** | $CH_3$ | $C_8H_{17}OCH_2$ | liquid | **40** | $CH_3$ | $C_8H_{17}OCH_2$ | liquid |
| **13** | $CH_3$ | $C_9H_{19}OCH_2$ | liquid | **41** | $CH_3$ | $C_9H_{19}OCH_2$ | 47-49 |
| **14** | $CH_3$ | $C_{10}H_{21}OCH_2$ | 56-57 | **42** | $CH_3$ | $C_{10}H_{21}OCH_2$ | 46-47 |
| **15** | $CH_3$ | $C_{11}H_{23}OCH_2$ | 61-62 | **43** | $CH_3$ | $C_{11}H_{23}OCH_2$ | 52-53 |
| **16** | $CH_3$ | $C_{12}H_{25}OCH_2$ | 62-64 | **44** | $CH_3$ | $C_{12}H_{25}OCH_2$ | 61-63 |
| **17** | $C_6H_{13}$ | $C_3H_7OCH_2$ | liquid | **45** | $C_6H_{13}$ | $C_3H_7OCH_2$ | liquid |
| **18** | $C_6H_{13}$ | $C_4H_9OCH_2$ | liquid | **46** | $C_6H_{13}$ | $C_4H_9OCH_2$ | liquid |
| **19** | $C_6H_{13}$ | $C_5H_{11}OCH_2$ | liquid | **47** | $C_6H_{13}$ | $C_5H_{11}OCH_2$ | liquid |
| **20** | $C_6H_{13}$ | $C_6H_{13}OCH_2$ | liquid | **48** | $C_6H_{13}$ | $C_6H_{13}OCH_2$ | liquid |
| **21** | $C_6H_{13}$ | $C_7H_{15}OCH_2$ | liquid | **49** | $C_6H_{13}$ | $C_7H_{15}OCH_2$ | liquid |
| **22** | $C_6H_{13}$ | $C_8H_{17}OCH_2$ | liquid | **50** | $C_6H_{13}$ | $C_8H_{17}OCH_2$ | liquid |
| **23** | $C_6H_{13}$ | $C_9H_{19}OCH_2$ | liquid | **51** | $C_6H_{13}$ | $C_9H_{19}OCH_2$ | liquid |
| **24** | $C_6H_{13}$ | $C_{10}H_{21}OCH_2$ | 37-39 | **52** | $C_6H_{13}$ | $C_{10}H_{21}OCH_2$ | liquid |
| **25** | $C_6H_{13}$ | $C_{11}H_{23}OCH_2$ | liquid | **53** | $C_6H_{13}$ | $C_{11}H_{23}OCH_2$ | 29-31 |
| **26** | $C_4H_9OCH_2$ | $C_3H_7OCH_2$ | liquid | **54** | $C_4H_9OCH_2$ | $C_3H_7OCH_2$ | 47-49 |
| **27** | $C_4H_9OCH_2$ | $C_4H_9OCH_2$ | liquid | **55** | $C_4H_9OCH_2$ | $C_4H_9OCH_2$ | 56-58 |
| **28** | $C_4H_9OCH_2$ | $C_5H_{11}OCH_2$ | liquid | **56** | $C_4H_9OCH_2$ | $C_5H_{11}OCH_2$ | 36-40 |
| **29** | $C_4H_9OCH_2$ | $C_6H_{13}OCH_2$ | liquid | **57** | $C_4H_9OCH_2$ | $C_6H_{13}OCH_2$ | 49-51 |
| **30** | $C_4H_9OCH_2$ | $C_7H_{15}OCH_2$ | liquid | **58** | $C_4H_9OCH_2$ | $C_7H_{15}OCH_2$ | 56-58 |
| **31** | $C_4H_9OCH_2$ | $C_8H_{17}OCH_2$ | liquid | **59** | $C_4H_9OCH_2$ | $C_8H_{17}OCH_2$ | liquid |
| **32** | $C_4H_9OCH_2$ | $C_9H_{19}OCH_2$ | liquid | **60** | $C_4H_9OCH_2$ | $C_9H_{19}OCH_2$ | 49-51 |
| **33** | $C_4H_9OCH_2$ | $C_{10}H_{21}OCH_2$ | 15-17 | **61** | $C_4H_9OCH_2$ | $C_{10}H_{21}OCH_2$ | 53-55 |
| **34** | $C_4H_9OCH_2$ | $C_{11}H_{23}OCH_2$ | 21-23 | **62** | $C_4H_9OCH_2$ | $C_{11}H_{23}OCH_2$ | 59-62 |

The inorganic anion also accounts for the low melting point of an ionic liquid as well, and in some cases it even makes the most significant contribution for lowering the melting point. It has been argued that increasing size of the anion with the same charge can contribute to the decreasing of the melting point (Wasserscheid and Keim, 2000). It was also concluded that the C-alkylation of the anion could lead to low melting point by

the introduction of a packing inefficiency (Larsen et al., 2000). Table 1.4 compares the impact of anions on the melting points of some important ionic liquids.

**Table 1.4** Impact of Anions on the Melting Points of Ionic Liquids

| Salt | Compounds | m.p. (°C) | Ref |
|---|---|---|---|
| **63** | [EMIM][$CoCl_4$] | 100-102 | (Hitchcock, 1993) |
| **64** | [EMIM][$NiCl_4$] | 92-93 | (Hitchcock, 1993) |
| **65** | [EMIM]Cl | 87 | (Wilker et al., 1982) |
| **66** | [EMIM][$SO_4$] (monohydrate) | 70 | (Wilkes and Zaworatko, 1992) |
| **67** | [EMIM][$PF_6$] | 58-60 | (Fuller et al., 1994) |
| **68** | [EMIM][$AuCl_4$] | 58 | (Hasan et al., 1999) |
| **69** | [EMIM][$NO_2$] | 55 | (Wilkes and Zaworatko, 1992) |
| **70** | [EMIM][$NO_3$] | 38 | (Wilkes and Zaworatko, 1992) |
| **71** | [EMIM][$AlCl_4$] | 7 | (Fannin et al., 1984) |
| **72** | [EMIM][$BF_4$] | 6 | (Holbrey and Seddon, 1999) |
| **73** | [EMIM][$CF_3SO_3$] | -9 | (Bonhote et al., 1996) |
| **74** | [EMIM][$CF_3CO_2$] | -14 | (Bonhote et al., 1996) |
| **75** | [EMIM][$CB_{11}H_{12}$] | 122 | (Larsen et al., 2000) |
| **76** | [EMIM][$CB_{11}H_6Cl_6$] | 114 | (Larsen et al., 2000) |
| **77** | [EMIM][$CB_{11}H_6Br_6$] | 139 | (Larsen et al., 2000) |
| **78** | [EMIM][1-$CH_3$-$CB_{11}H_{11}$] | 59 | (Larsen et al., 2000) |
| **79** | [EMIM][1-$C_2H_5$-$CB_{11}H_{11}$] | 64 | (Larsen et al., 2000) |
| **80** | [EMIM][1-$C_3H_7$-$CB_{11}H_{11}$] | 45 | (Larsen et al., 2000) |
| **81** | [EMIM][1-$C_4H_9$-$CB_{11}H_{11}$] | 49 | (Larsen et al., 2000) |
| **82** | [OMIM][$CB_{11}H_{12}$] | 70 | (Larsen et al., 2000) |
| **83** | [OMIM][$CB_{11}H_6Cl_6$] | 67 | (Larsen et al., 2000) |
| **84** | [EDMIM][$CB_{11}H_{12}$] | 156 | (Larsen et al., 2000) |
| **85** | [EDMIM][$CB_{11}H_6Cl_6$] | 137 | (Larsen et al., 2000) |
| **86** | [BDMIM][$CB_{11}H_{12}$] | 129 | (Larsen et al., 2000) |
| **87** | [BDMIM][$CB_{11}H_6Cl_6$] | 101 | (Larsen et al., 2000) |

*Note: EMIM = 1-ethyl-3-methylimidazolium; OMIM = 1-octyl-3-methylimidazolium; EDMIM = 1-ethyl-2, 3-dimethylimidazolium; and BDMIM = 1-butyl-2, 3-dimethylimidazolium.*

In conclusion, low symmetry and packing efficiency may lead to a low melting point of an ionic liquid. However, other factors may have impact on the melting point as

well, such as weak intermolecular interaction (such as less hydrogen bonding) (Elaiwi et al., 1995; Bonhote et al., 1996), and an average distribution of charge in the cation (Stegemann et al., 1992), etc.

### 1.2.4 Vapor Pressure and Chemical/Thermal Stability

Clean technology requires the design of safe and environmentally benign chemical processes and thus reduces the waste from an industrial process to its minimum. Ionic liquids do not posses measurable vapor pressure, which makes them good candidates for "green"[†] chemistry (Appendix D). This attractive feature is one of the important reasons that ionic liquids are emerging as novel alternatives for volatile organic compounds (VOCs) traditionally used as industrial solvents. Also, due to this reason, in a distillation process, the azeotrope formation between the solvent and products does not occur.

The chloroaluminates based ambient temperature ionic liquids are water-sensitive and produce a lot of HCl while exposed to air (Freemantle, 1998). Therefore, this type of ambient ionic liquid has to be rigorously protected from moisture and other oxide impurities. The chloroaluminates based ionic liquids have thus been limited to a narrow selection of organic substrates, such as Friedel-Crafts substrates (Wilkes, 1987). On the other hand, the tetrafluoroborates, hexafluorophosphates and other newly developed ionic liquids are air and water stable. This development gives the ionic liquid more potential applications in a very wide range of chemical processes.

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[†] "Green" Chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products (Anon, 2000).

Ionic liquids are also thermally stable. This stability depends on the strength of the heteroatom-carbon and heteroatom-hydrogen bonds (Wasserscheid and Keim, 2000). While 150 °C is considered as a maximum working temperature for most quaternary ammonium chloride salts, $[EMIM]^+[BF_4]^-$ (1-ethyl-3-methylimidazolium tetrafluoroborate) is stable up to 300 °C (Mutch and Wilkes, 1998) and $[EMIM]^+[(CF_3SO_2)_2N]^-$ (m.p. -3°C) is stable to more than 400 °C (Bonhote et al., 1996). Table 1.5 illustrates the temperature ($T_d$) of 10% weight loss of ionic liquids during a heating scan from room temperature by using thermogravimetry, which evidently indicates that ionic liquids are thermal stable.

**Table 1.5** Thermal Properties of Ionic Liquids

| Ionic Liquid | $T_d$ (K) | Reference |
|---|---|---|
| $[EMIM][BF_4]$ | 664 | (Noda et al., 2001) |
| [EMIM][TFSI] | 690 | (Noda et al., 2001) |
| $[EMIM][CF_3COO]$ | 475 | (Bonhote et al., 1996) |
| $[BuPy][BF_4]$ | 615 | (Noda et al., 2001) |
| [BuPy][TFSI] | 677 | (Noda et al., 2001) |

*Note: TFSI = bis(trifluoromethylsulfonyl)imide; BuPy = 1-butylpyridinium.*

### 1.2.5 Polarity

Since solvent polarity and polarizability are the critical indexes of solvent strength, they may have significant influences on the chemical reactions. Although different characteristic parameters (for example, dielectric constant, dipole moments, refractive index, and polarizabilities, etc) reflect the solvent strength from different aspects, solvent polarity can not be simply described by any one of those terms because the individual interactions between a solvent and a solute are not accounted for. Therefore, the studies of empirical scales of solvent polarity are adapted. As a common approach, fluorescent

probes are applied to determine the solvent strength of organic solvents and has been recently used for some ionic liquids. For instance, three different fluorescent probes (e.g. pyrenecarboxaldehyde, pyrene and bromonaphthalene) were studied to establish the solvent properties of the ionic liquid $[EMIM]^+[TFSI]^-$ (1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide) (Bonhote et al., 1996). It was concluded that the solvent strength of $[EMIM]^+[TFSI]^-$ is less than that of 1-hexanol using pyrenecarboxaldehyde, while similar to ethanol's using pyrene.

**Table 1.6** Fluorescence Properties of Probe DAP in Ionic Liquids or Organic Solvents (Aki et al., 2001)

| Solvent | $\lambda_{fluo}^{max}$ | $E_T(30)$ (kJ/mol) |
|---|---|---|
| hexane | 437 | 129.7 |
| $[BuPy][BF_4]$ | 495 | 187.9 |
| Acetonitrile | 490 | 189.5 |
| $[C_8MIM][PF_6]$ | 503 | 196.0 |
| $[BMIM]]PF_6]$ | 512 | 205.1 |
| ethanol | 523 | 217.1 |
| $[BMIM][NO_3]$ | 525 | 218.2 |
| Methanol | 534 | 230.1 |
| water | 562 | 264.0 |

The solvent strength and polarity of four imidazolium and pyridinium based ionic liquids have been measured by two fluorescent probes AP and DAP (Aki et al., 2001). In this study, the microscopic polarities are expressed in terms of the absorption energy of the betaine dye, $E_T(30)$, and can be calculated from the fluorescent lifetime ($\lambda_{fluo}^{max}$) based on the correlation relationship. The larger value of $E_T(30)$ means stronger solvent strength and polarity. Table 1.6 lists the fluorescent properties of DAP in ionic liquids or organic solvents at room temperature and the estimated $E_T(30)$ values (Aki et al., 2001).

The four studied ionic liquids in Table 6 have close or stronger polarity than acetonitrile but are less polar than methanol.

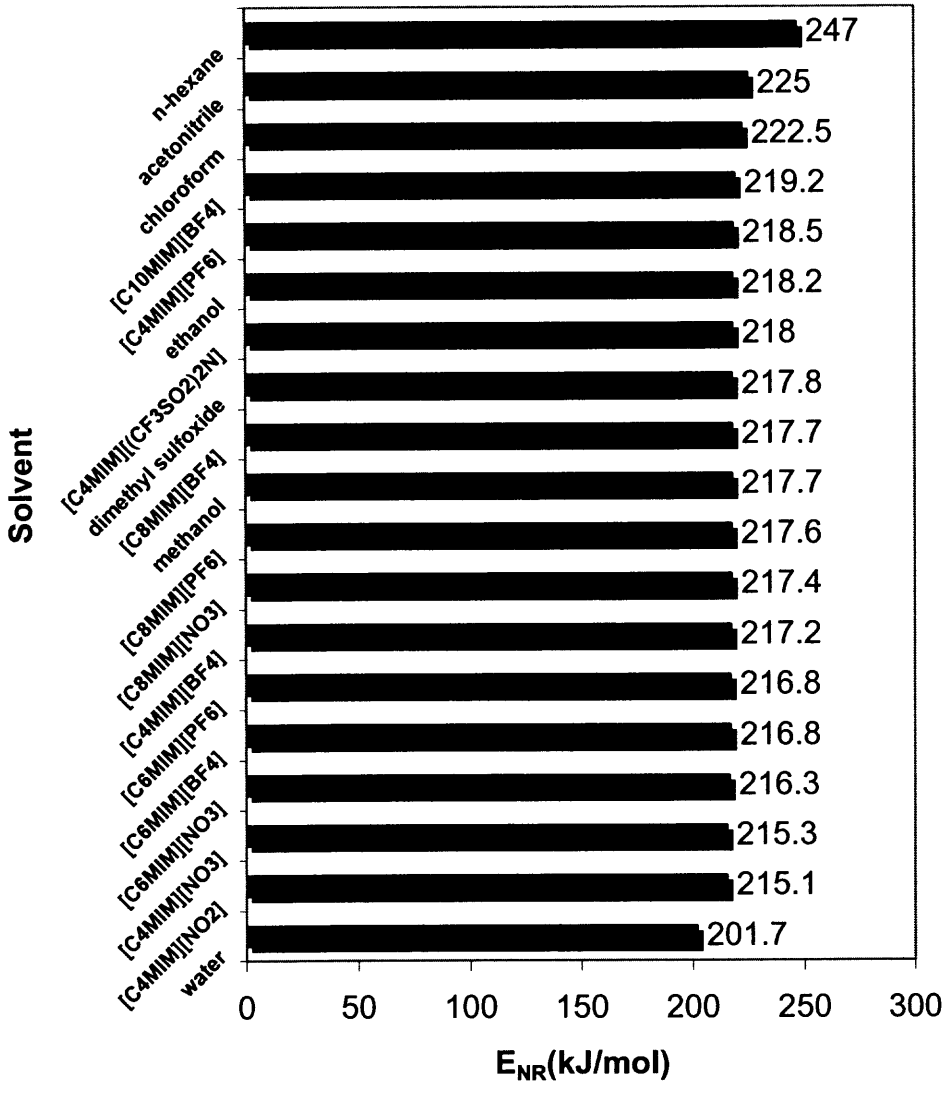



**Figure 1.12** $E_{NR}$ values for $[C_nMIM][X]$ ionic liquids and organic solvents (Carmichael and Seddon, 2000b).

More systematical polarity studies of 1-$C_nH_{2n+1}$-3-methylimidazolium type ionic liquids have been reported by the Seddon research group (Carmichael and Seddon, 2000b). In their research, the wavelength of maximum absorption ($\lambda_{max}$) was measured

and the molar transition energies ($E_{NR}$) were calculated for the solvatochromic dye Nile Red dissolved in the ionic liquids (Figure 1.12). In this case, the smaller value of $E_{NR}$ indicates the stronger polarity that a solvent has.

From Figure 1.12, the polarity of $[C_4MIM]^+$ type salts declines with the increasing size of anions $NO_2$, $NO_3$, $BF_4$, and $PF_6$. This is due to the spreading of negative charge on the anion, and thus less charge is available for interactions with the solute Nile Red (Carmichael and Seddon, 2000b). However, $[C_4MIM]^+[(CF_3SO_2)_2N]^-$ is an exception due to the negative charge partially delocalized within the anion (Golding et al., 1998).

While the 1-alkyl chain was varied for both $BF_4^-$ and $PF_6^-$ type ionic liquids, the polarity increases with the increasing of n from 4 to 6, and thereafter, it falls with further longer alkyl chains. This observation correlates with the phenomena of maximum melting point which occurs at n = 6 for these salts, as described in the melting point section previously.

As a tentative conclusion, the polarity of 1-alkyl-3methylimidazolium ionic liquids is determined by the anion for those consisting of short 1-alkyl chains and by the cation for those consisting of long 1-alkyl chains (Carmichael and Seddon, 2000b). It was also observed that the longer alkyl groups on both cations and anions lead to great lipophilicity of the ionic liquids (Shetty et al., 1987). However, since more types of ionic liquids are actually involved in current organic reactions than summarized here, more consistent and comprehensive studies have to be done on the solvent strength and polarity.

### 1.2.6 Miscibility

Ionic liquids are considered good solvents for a wide range of inorganic, organic and polymeric compounds (Seddon, 1997). The solubility of ionic liquids in water and organic solvents can be finely tuned by changing the structure of cation and anion. The influence of cation structure on the miscibility may be explained by two factors: (a) the similarity of the polarity between the ionic liquid and the other fluid; (b) free space between molecules caused by large side chains. For example, 1-alkyl-3-methylimidazolium tetrafluoroborate ($[RMIM]^+[BF_4]^-$) salts are miscible with water at 25 °C when the alkyl group length is less than 6 carbons, while they form a second phase with water at or above 6 (Earle and Seddon, 2000). Since a longer alkyl chain means less polarity and more hydrophobicity, the solubility of ($[RMIM]^+[BF_4]^-$) is thus reduced in the polar solvent water. The behavior is actually beneficial to the extraction operation due to this adjustable solubility of ionic liquids in the extraction phase. A successful example is the use of $[BMIM][PF_6]$ ($[BMIM]^+$ = 1-butyl-3-methylimidazolium), which forms a triphasic mixture with alkanes and water (Earle and Seddon, 2000). $[BMIM]^+[PF_6]^-$ was applied for the liquid-liquid extraction of erythromycin-A and for the *Rhodococcus* R321 catalyzed biotransformation of 1,3 dicyanobenzene (1,3- DCB) in a liquid-liquid two phase system (Cull et al., 2000).

Another instance of the cation influence on the miscibility is the investigation of the solubility of 1-octene in four different tri-n-alkylmethylammonium tosylate salts at 80 °C (Wasserscheid and Keim, 2000). With the increase of carbon number, the solubility of 1-octene accordingly increases. This may be because of the low polarity of 1-octene and larger free space between molecules for longer chain ionic liquids. Newly developed 3-

alkoxymethyl-1-alkyl based ionic liquids do not mix with water because of their antielectrostatic properties, however, are miscible with acetone, chloroform, THF, ethyl acetate and DMF (Pernak and Czepukowicz, 2001).

**Table 1.7** Miscibility of Some Ionic Liquids in Water/Organic Solvents (Bonhote et al., 1996)

| | $H_2O$ | $CH_2Cl_2$ | THF | Ethyl acetate | toluene | 1,4-dioxane |
|---|---|---|---|---|---|---|
| Dielectric constant (ε) | 80.10 | 8.93 | 7.58 | 6.02 | 2.38 | 2.01 |
| [EMIM][$CF_3SO_3$] | M | m | m | m | im | im |
| [EMIM][$CF_3CO_2$] | M | m | m | pm | im | im |
| [EMIM][n-$C_3F_7CO_2$] | | m | m | pm | im | im |
| [BMIM][$CF_3CO_2$] | M | m | m | m | im | im |
| [BMIM][n-$C_3F_7CO_2$] | M | m | m | m | im | im |

*Note: m: miscible; pm: partial miscible; im: immiscible.*

On the other hand, the anion in an ionic liquid usually shows a more impressive influence on the miscibility property. Generally, ionic liquids consisting of the anions $[CH_3COO]^-$, $[CF_3COO]^-$, $[NO_3]^-$, $Cl^-$, $Br^-$, $I^-$, $[Al_2Cl_7]^-$, and $[AlCl_4]^-$ etc are water soluble, while those containing the anions $[PF_6]^-$, $[(CF_3SO_2)_2N]^-$, $[BR_1R_2R_3R_4]^-$ form biphasic mixture with water. The solubility of $[BF_4]^-$, $[CF_3SO_2]^-$ type mostly depends on the properties of the cation (Seddon et al., 2000). According to Bonhote's research (Bonhote et al., 1996), ionic liquids may be completely miscible with organic solvents if the dielectric constants of ionic liquids are larger than a certain limit. This limit depends on the combination of each cation-anion pair (Table 1.7) (Bonhote et al., 1996). In this case, the anion has much stronger influence on the miscibility than the changing of alkyl chain length in the cation.

### 1.2.7 Density

The density of an ionic liquid depends on the length and type of substituents in the cation, and also on the kind of the anion. Figure 1.13 clearly illustrates that with the lengthening of the alkyl chain, the density of 3-$C_nH_{2n+1}OCH_3$-1-methylimidazolium tetrafluoroborate salts declines linearly (Pernak and Czepukowicz, 2001). As a general conclusion, the density of comparable ionic liquids decreases as the bulkiness of the cation grows. This is due to the poor crystal packing between the bulky cation and the weakly complexing symmetrical anion (Fuller et al., 1994).

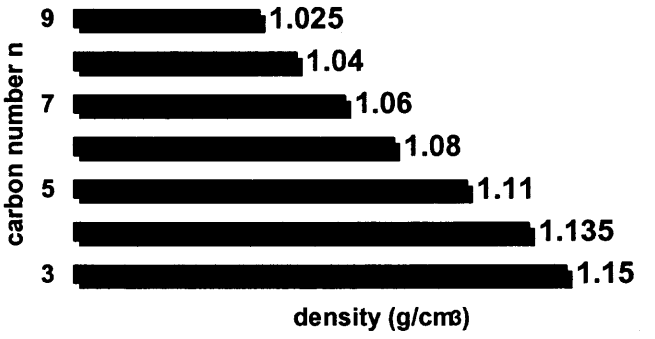



**Figure 1.13** Density of 3-$C_nH_{2n+1}OCH_3$-1-methylimidazolium tetrafluoroborate as a function of carbon number n (Pernak and Czepukowicz, 2001).

Varying the structure of the anion also changes the density of ionic liquids. Figure 1.14 shows the density of 1-ethyl-3-methylimidazolium carborane type ionic liquids varies with the bulky size (Larsen et al., 2000). Replacing the hydrogen atom by heavier elements such as F, Cl, Br, also increases the density of ionic liquids. This can also be verified by the data in Table 1.8, where chain bulkiness and number of fluorine atoms affect the liquid density significantly.

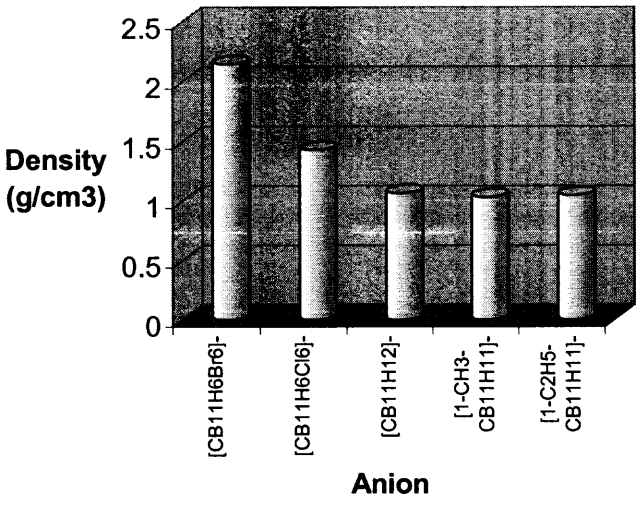



**Figure 1.14** Influence of anion on the density of the ionic liquid [EMIM][X] (Larsen et al., 2000).

**Table 1.8** Influence of Fluorine Atoms on the Density of Ionic Liquids (22 °C)

| [BuMIM] [X] | Density ($g/cm^3$) | Reference |
|---|---|---|
| $CF_3CO_2^-$ | 1.209 | (Koryta et al., 1993) |
| $CF_3SO_3^-$ | 1.290 | (Kissinger and Heineman, 1984) |
| n-$C_3F_7CO_2$- | 1.333 | (Bonhote et al., 1996) |
| $(CF_3SO_2)_2N^-$ | 1.429 | (Bockris and Reddy, 1970) |
| $C_4F_9SO_3^-$ | 1.473 | (Bonhote et al., 1996) |

The density of ionic liquids is also temperature dependent. Figure 1.15 shows the temperature dependence of density of [EMIM][$BF_4$] (Noda et al., 2001). As temperature changes from 293 to 313 K, the density decreases linearly as the temperature increases.

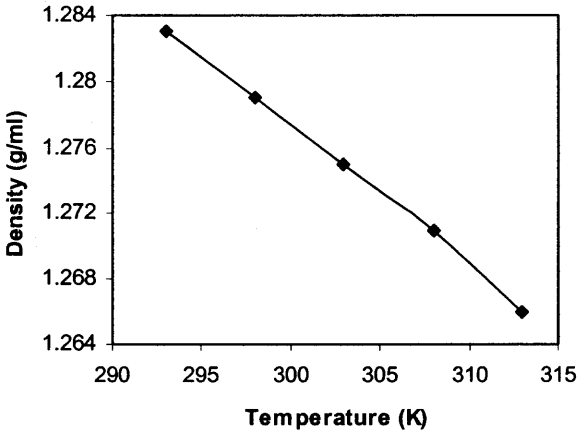



**Figure 1.15** Variation of density of $[EMIM]^+[BF_4]^-$ with temperature (Noda et al., 2001).

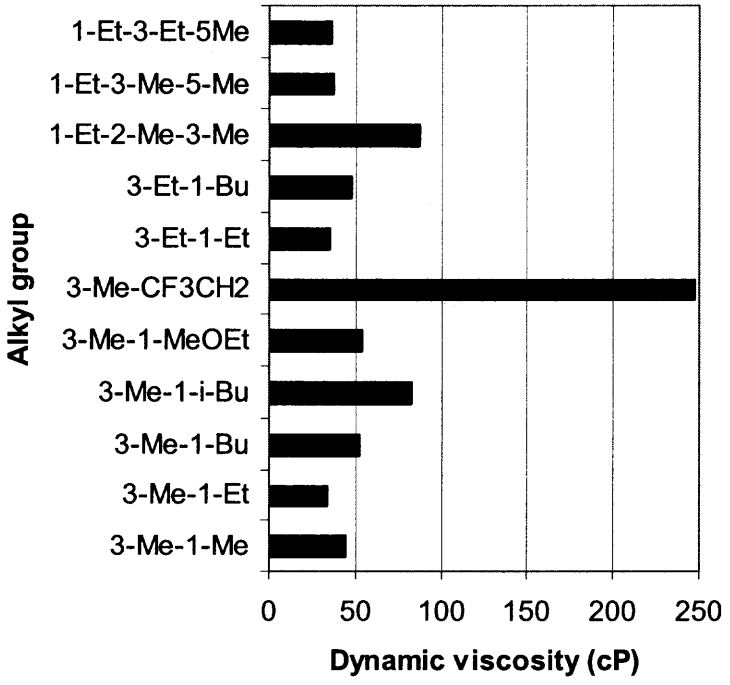



**Figure 1.16** Dynamic viscosity at 20 °C of imidazolium based $(CF_3SO_2)_2N$ salts which are liquids or supercooled liquids at that temperature (Bonhote et al., 1996).

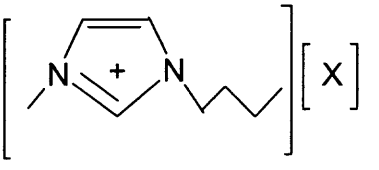
### 1.2.8 Viscosity

Generally, it is considered that the viscosity of ionic liquids is determined by Van der Waals forces and hydrogen bonding. Alkyl chain lengthening or fluorination in the cation increases the viscosity of the ionic liquids because of the stronger Van der Waals interactions (Figure 1.16). Methylation at the C-2 position makes the ionic liquids more viscous due to the hydrogen bonding suppression.

The viscosity of 1-n-butyl-3-methylimidazolium ([BMIM]) type ionic liquids with different fluorine containing anions is listed in Table 1.9 (Bonhote et al., 1996). The significant increase of viscosity from $CF_3SO_3^-$ to n-$C_4F_9SO_3^-$, and from $CF_3COO^-$ to n-$C_3F_7COO^-$, indicates stronger Van der Waals interactions than the hydrogen bonding suppression in the case of n-$C_4F_9SO_3^-$ and n-$C_3F_7COO^-$. However, from $CF_3SO_3^-$ to $(CF_3SO_2)_2N^-$, the hydrogen bonding suppression slightly overcompensates the increase caused by the Van der Waals attraction.

**Table 1.9** Dynamic Viscosity η of Various $[BMIM]^+$ Ionic Liquids (20 °C) (Bonhote et al., 1996)

| [BuMIM][X] | η (cP) |
|---|---|
| $CF_3SO_3^-$ | 90 |
| $C_4F_9SO_3^-$ | 373 |
| $CF_3CO_2^-$ | 73 |
| n-$C_3F_7CO_2^-$ | 182 |
| $(CF_3SO_2)_2N^-$ | 52 |




Viscosity-temperature data of several ionic liquids have been well fitted by the Vogel-Tamman-Fulcher (VTF) equation (Noda et al., 2001) and the equation parameters are listed in Table 1.10.

**Table 1.10** Vogel-Tamman-Fulcher Equation Parameters for Viscosity Data (Noda et al., 2001) $\eta = \eta_0 \exp[B/(T - T_0)]$

| | $\eta_0$ (× 0.1 mPa·s) | B (× 100 K) | $T_0$ (× 100 K) |
|---|---|---|---|
| [EMIM][$BF_4$] | 2.0 | 7.5 | 1.5 |
| [EMIM][TFSI] | 1.5 | 8.4 | 1.4 |
| [BPy][$BF_4$] | 2.3 | 7.2 | 1.8 |
| [BPy][TFSI] | 1.2 | 8.5 | 1.6 |

*Note: TFSI = bis(trifluoromethylsulfonyl)imide; BuPy = 1-butylpyridinium.*

A recent study (Seddon et al., 2000) indicates that the chloride impurities in the ionic liquids increase the viscosity, while the presence of water or other cosolvents decreases the viscosity. Fortunately, the influence of the addition of cosolvents can be fitted to mathematical equations, and thus can be accounted for in the design calculation precisely (Seddon et al., 2000).

### 1.2.9 Summary

In summary, as a substitute for traditional volatile organic solvents in clean technologies, ionic liquids have potential applications in organic reactions, chemical processes, and even in enzymatic reactions. Elemental analysis (Holbrey and Seddon, 1999), $^1$H NMR chemical shifts study (Holbrey and Seddon, 1999), X-ray crystallography (Fuller et al., 1994), and X-ray absorption fine structure (XAFS) (Carmichael et al., 1999b), are some general techniques for studying the structure of ionic liquids. With the further study of microstructure of ionic liquids, better understanding of the physical properties of ionic liquids will be available. More systematic studies are needed in order to have a complete ionic liquid database.

## 1.3 Objectives

Based on the advantages of enzymatic resolution and ionic liquids discussed above, this research focuses on conducting enzymatic resolution reactions by using ionic liquids as solvents. The advantages of using ionic liquids as reaction media will possibly be: (1) clean compounds could be produced by using ionic liquids as "green" reaction media; (2) room temperature ionic liquids are usually moisture-stable and thermally stable, therefore, they are suitable candidates as reaction media and catalysts; (3) ionic liquids could be recovered and reused again simply by evaporating the water and organic compounds under vacuum. Unless specified, the term "amino acid" in this research means α-amino acid. The possible research objectives and research approach will be discussed in the following.

1) Preparations of Racemic Amino Acids and their Esters

In order to study the kinetic resolution, amino acid esters are needed for the enzymatic hydrolysis. Some of the amino acids and their esters may be synthesized in the lab. The reaction strategy and conditions will be investigated in detail in this research.

2) Synthesizing Economical Room-Temperature Ionic Liquids

Although many room-temperature ionic liquids have been applied in chemical reactions, few reports have been found using ionic liquids for enzymatic reactions. Meanwhile, some ionic liquids are expensive to prepare although they are easy to synthesize, and some of them are not commercially available. This research will try to find and

synthesize economical ionic liquids that can be practically used in industry. The detailed synthetic method of preparing ionic liquids will be studied.

3) Enzymatic Resolution in Organic Solvents and Ionic Liquids

By comparing the enzymatic reaction in organic solvents, water and the ionic liquid-water mixtures, the effectiveness of using ionic liquids as reaction media in the enzymatic resolutions of amino acids will be discussed. A suitable ionic liquid-water ratio will be determined. The desired situation is the use of a small amount of ionic liquids (instead of organic solvents) can have at least the equivalent reaction rates or have other advantages (e.g. easy to separate the product).

4) Studies on Enzyme Activity and Enantioselectivity

The soluble enzyme activities will be studied in the ionic liquid, water, ionic liquid-water mixtures and organic solvents during different reaction conditions, such as temperature, solvent ratio and reaction time, etc. The enzyme activity differences between organic solvents and ionic liquid/water solutions will then be compared. The optimum condition for sustaining enzyme activity will then be determined.

5) Reaction Optimization

Based on the studies mentioned above, reaction conditions (reaction time, solvent ratio, buffer solutions, temperature, etc.) will be studied to optimize the resolution processes using ionic liquids as solvents. This generalized method will be further investigated on the kinetic resolution of different amino acids and the results will be compared with those using an organic solvent.

# CHAPTER 2

# SYNTHESIS OF AMINO ACIDS AND ENZYMATIC RESOLUTION IN ORGANIC SOLVENTS

## 2.1 Synthesis of L-(-)-Piperazine-2-carboxylic Acid

### 2.1.1 Introduction and Strategy

Piperazine-2-carboxylic acid **1** is a conformationally restricted nonproteinogenic amino acid with medicinal applications. Its enantiomer (L)-piperazine-2-carboxylic acid can be used for the synthesis of the HIV protease inhibitor Crixivan from Merck (Askin et al., 1994), an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Bigge et al., 1992), and a cardioprotective nucleoside transport blocker (Bruce et al., 1995). Currently the pure enantiomers of this amino acid are separated through classical resolution of the racemate by fractional crystallization of diastereomeric salts (Aebischer et al., 1989; Shiraiwa et al., 1991). An asymmetric synthesis of orthogonally protected *N*, *N'*-(L)-piperazine-2-carboxylic acid has been achieved in four steps from *N*-tert-Boc-L-serine β–lactone (Warshawsky et al., 1997) with 40% overall yield. (L)-piperazine-2-carboxylic acid has also been obtained by enzymatic resolution of racemic 4-(tert-butoxycarbonyl)piperazine-2-carboxamide with leucine aminopeptidase (Bruce et al., 1995). Recently, a kinetic resolution of racemic piperazine-2-carboxamide was reported by using whole cells of wild-type microorganisms that contain stereospecific amidases (Eichhorn et al., 1997). The disadvantages of these processes are that the preparation of the racemic starting materials is complicated and the availability of the biocatalysts for large scale production is limited.

This study will report an efficient synthesis and kinetic resolution of methyl 4-(tert-butyroxycarbonyl)-piperazine-2-carboxylate **3** to the corresponding enantiomerically pure carboxylic acid using readily available starting materials and a low-cost enzyme *BL*-alcalase. The process has the potential to produce a large quantity of material. The reaction scheme is presented in Figure 2.1.

### 2.1.2 Materials

All the organic and inorganic reagents were purchased from Aldrich. The enzyme *BL*-alcalase from Novozymes was distributed by Aldrich with a specific activity of 2.4 AU/g[†] for hydrolysis of dimethyl casein at 50 °C and pH 8.3. It was used without further purification.

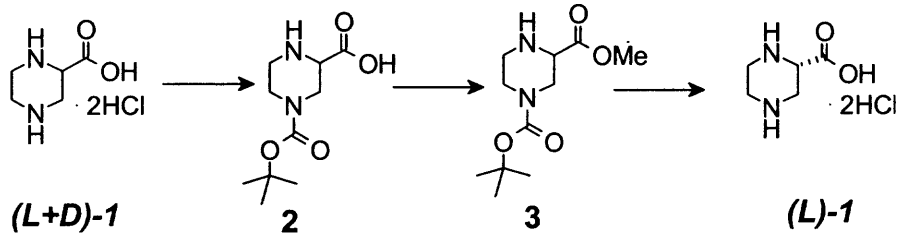



**Figure 2.1** Synthesis and enzymatic resolution of piperazine-2-carboxylic acid.

[†] The major enzyme component in *BL*-alcalase is subtilisin Carlsberg. According to Novozymes, one Anson Unit [AU] is the amount of enzyme which, under standard conditions, digests haemoglobin at initial rate liberating per min an amount of tricholoroacetic acid (TCA) soluble product, which gives the same color of phenol reagents as 1 mequiv of tyrosine. Thus, 1 AU = 1000 U, 1U = 1 mmol of L-tyrosine methyl ester hydrolyzed per min.

### 2.1.3 Experimental

4-t-Butoxycarboxyl-piperazine-2-carboxylic acid (**2**)

A solution of 6.4 g of potassium hydroxide (114.8 mmol) in 20 ml of methanol was added into the suspension of 11.6 g (57.4 mmol) of piperazine-2-carboxylic acid hydrochloride in 140 ml of methanol and the mixture was stirred at room temperature for 1 hr. After removing methanol, the residue was dissolved in a solvent consisting of 20 ml of water and 60 ml of 1,4-dioxane to give free piperazine-2-carboxylic acid solution. A solution of 12.6 g of di-t-butyldicarbonate in 20 ml of 1,4-dioxane-water (v/v, 3/1) was added. The reaction mixture was stirred overnight at room temperature. Insoluble white solid was filtered off. 4.8 g, yield 36.36% $\delta_H$ (DMSO), 4.1 (1H, sbr, NH), 3.9-2.9 (7H, m, $CH_2CH_2$, $CH_2CH$), 1.4 (9H s, $3CH_3$) ppm. MS, m/e 231 ($M^+$5.1), 229 ($M^+$-1). The solvent was removed from the mother liquor, and the aqueous phase was extracted. Finally, the ethyl acetate was evaporated to give di-protected piperizine-2-carboxylic acid. 2.6 g yield 13.73%. $\delta_H$ 3.8-2.9 (7H, m, $CH_2CH_2$, $CH_2CH$), 1.4 (18H, s, $9CH_3$ppm). MS m/e 330 ($M^+$).

Cesium 4-t-Butoxycarboxyl-piperazine-2-carboxylate

2.4 g (10.44 mmol) of 4-t-Butoxycarboxyl-piperazine-2-carboxylic acid **7** and 2.0 g (61.5 mmol) of cesium carbonate were added into 40 ml of *N*, *N'*-dimethylformamide. The resulting suspension was stirred at room temperature for 5 hrs. Water was added until the suspension turned into a clear solution. This solution was stirred at 45°C for an additional 5 hrs and then poured into 120 ml of ethanol. Filtration gave 3.7 g of the cesium salt with

a yield of 98.04%. The cesium salt was used in the next esterification without further purification.

Methyl 4-t-Butoxycarboxyl-piperazine-2-carboxylate (**3**)

A suspension of 3.7 g (10.2 mmol) of cesium 4-t-Butoxycarboxyl-piperazine-2-carboxylate in 40 ml of *N, N'*-dimethyl formamide was heated at 60°C for 1 hr. 16 g (113.5 mmol) of iodomethane was added and the mixture was maintained at 60°C overnight. The solvent *N, N'*-dimethylformamide was distilled off. The residue was extracted with ethyl acetate at the boiling temperature of ethyl acetate. Subsequently, concentrating the extract gave a brown thick semi-solid. Recrystallization of the semi-solid from ethyl acetate gave 560 mg of light yellow solid with a yield of 22.50%. mp 159-160 °C. $\delta_H$ (DMSO) 4.3-3.5 (7H, m, $CH_2CH_2$, $CH_2CH$), 3.66 (3H, c, $OCH_3$), 1.50 (9H, s, $3CH_3$) ppm. MS, m/e 244 ($M^+$). Anal, calcd. C, 54.08%, H 8.25%, N 11.47%. Found C, 53.69%, H, 8.02%, N, 11.98%. Comparison of TLC results of monoprotected ester in the solvent ($CHCl_3/CH_3OH$=2/0.5 v/v) $R_f$=1.45/3.55=0.41 with diprotected ester' $R_f$=2.95/3.55=0.83 indicated the presence of the diprotected ester in the mother liquid.

L-Piperazine-2-carboxylic acid dihydrochloride ((L)-**1**)

A suspension of 488 mg (2 mmol) of methyl-4-t-butoxycarboxyl-piperazine-2-carboxylate, 163 mg (2.2 mmol) of sodium bicarbonate and 0.54 ml of *BL*-alcalase (with a specific activity of 2.4 AU/g for hydrolysis of dimethyl casein at 50 °C and pH 8.3) in 10 ml of acetonitrile and 20 ml of water was stirred overnight. The suspension turned into a clear solution after 3 hrs and then gradually turned back to a suspension. The precipitate

was collected by filtration, washed with distilled water to pH 7 and dried. The white solid (146 mg, 0.59 mmol) was dissolved in 30 ml of 4 N HCl and stirred overnight. The solid was completely dissolved. Hydrogen chloride gas was bubbled into the solution to get D-piperazine-2-carboxylic acid hydrochloride, 85 mg, yield 83.74% based on methyl-D-4-(t-butoxycarbonyl)piperazine-2-carbonate (yield 41.87% based on methyl—L, D-4-(t-butoxycarboxyl) piperazine-2-carbonate). $\delta_H$ (DMSO), 10.1 (1H, sbr, $CO_2H$), 4.2 (2H, dd, 2NH), 3.6-3.2 (7H, m $CH_2CH_2$, $CH_2CH$) ppm, MS, m/e 203 ($M^+$).

The filtrate was concentrated to about 5 ml and was acidified to pH 4 by aqueous HCl solution at room temperature. The light brown solution was stirred overnight and concentrated to about 10 ml. Hydrogen chloride gas was carefully bubbled into the solution at 0°C to precipitate the hydrochloride salt. L-piperazine-2-carboxylic acid hydrochloride was then filtered. The crude product was dissolved in 3 ml of water. Any insoluble solid was removed by filtration. Hydrogen chloride was bubbled into the filtrate to get pure product 165 mg, yield 81.28% (based on Methyl-L-4-(t-butoxycarboxyl)-piperazine-2-carboxylate). The 98.1% enantiomeric excess (*ee*) of (L)-**1** was obtained by chiral HPLC (Chiralpak WH column[†]). $^1$H-HMR (400 MHz, $D_2O$), δ=3.41-3.56 (m, 2H), 3.56 (dd, 1H), 3.71-3.83 (m, 2H), 4.01 (dd, 1H). 4.46 (dd, 1H); $^{13}$C NMR (75 MHz, $D_2O$) ppm 42.21, 43.21, 44.43, 56.03, 169.88; IR (KBr) 3700-2000, 1760, 1220 $cm^{-1}$; MS, m/e 131 ($M^+$); $[\alpha]^{20}_D$= -4.88 (c 1.2; $H_2O$).

[†] The Chiralpak WH column was produced by Chiral Technologies, INC (France) with internal diameter 4.6 mm, column length 250 mm, partical size 10 μ and adsorbent of ligand exchange.

### 2.1.4 Results and Discussion

L-(-)-piperazine-2-carboxylic acid with 98.1% *ee* was synthesized by the above effective method using the industrial enzyme *BL*-alcalase. The first reaction was to add a butoxycarboxyl (BOC) *N*-protecting group on the amino acid piperazine-2-carboxylic acid hydrochloride. The purpose of this reaction is to free the carboxyl group for further esterification by breaking the zwitterion structure of this amino acid. Since position 4 nitrogen atom on piperazine-2-carboxylic acid contributes most to the zwitterion structure, protection of this nitrogen atom would be sufficient to free the carboxyl group. Meanwhile, position 1 nitrogen atom is close to the carboxyl group, therefore, position 4 nitrogen provided more free space for the protection reaction to take place. The following reaction was to esterify the *N*-BOC-piperazine-2-carboxylic acid via cesium salt, which is an effective method for synthesizing the amino acid ester (Wang, et al., 1977). Enzyme *BL*-alcalase again showed its powerful kinetic resolution on methyl 4-t-butoxycarboxyl-piperazine-2-carboxylate.

## 2.2 Synthesis and Kinetic Resolution of Homophenylalanine

### 2.2.1 Introduction and Reaction Strategy

L-(+)-homophenylalanine ((S)-2-amino-4-phenylbutanoic acid) is a vital component of angiotensin-converting enzyme (ACE) and renin inhibitors (Johnson et al., 1985; Hayashi et al., 1989). Many ACE inhibitors such as Benazepril, Enalapril and Lisinopril, have been intensely studied as a medicinal target for the treatment of hypertension and heart failure (Ondetti and Cushman, 1981). There are several strategies available for the synthesis of L-(+)-homophenylalanine including selective crystallization of

diastereomeric salts (Miyazawa), enzymatic resolution of racemic derivatives (Hayashi et al., 1989), asymmetric syntheses (Williams et al., 1988), and chemical transamination of 2-oxo-phenylbutanoic acid (Senuma et al., 1989). Among them, asymmetric synthesis method seems to attract a good deal of interest. For example, L-(+)-homophenylalanine with 13% overall yield and 80% enantiomeric excess (*ee*) was synthesized in four steps from α-*t*-butyl, β-methyl ester of *N*-benzyloxycarbonyl-(S)-aspartate (Baldwin et al., 1989); chiral homophenylalanine derivatives were also obtained via enantioselective hydrogenation catalyzed by rhodium complexes (Li et al., 1999; Xie et al., 2000); more recently, it was reported that L-(+)-homophenylalanine could be synthesized from *N*-phthaloyl-L-(-)-aspartic anhydride through three steps in 55% overall yield with 99% *ee* (Xu et al., 2000b). However, asymmetric synthesis usually involves an expensive catalyst, unusual starting materials, and even critical reaction conditions.

Most of the racemic amino acids used in this study were purchased from Sigma-Aldrich. Due to the large quantity needed in kinetic resolution reactions, homophenylalanine **6** was synthesized by a simple three-step-reaction as shown in Figure 2.2.

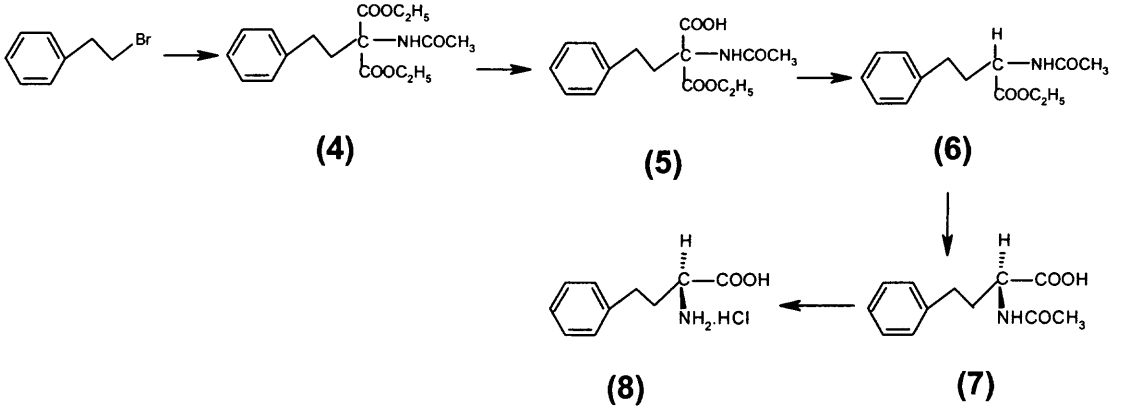



**Figure 2.2** Synthesis and kinetic resolution of homophenylalanine.

### 2.2.2 Materials

All organic reagents were purchased from Aldrich and the metal sodium was provided by ChemPacific Company. The enzyme *Bacillus licheniforms* alcalase (*BL*-alcalase) was produced by Novozymes A/S and distributed by Sigma-Aldrich with a specific activity of 2.4 AU/g for hydrolysis of dimethyl casein at 50 °C and pH 8.3. It was used without further purification.

### 2.2.3 Experimental

Diethyl-4-phenyl-2-acetamidomalonate (**4**)

2.484 g (0.108 mol) sodium was dissolved in 220 ml anhydrous ethanol. After the sodium completely dissolved, 23.47 g (0.108 mol) diethyl acetamidomalonate was added to the solution. The resulting pale yellow solution was stirred at room temperature for 40 minutes. Then 19.2 ml (0.140 mol, 30% excess) 2-bromoethyl benzene in 30 ml anhydrous ethanol was dropped into the solution within 4 hours while the mixture was refluxed for 5 hours. The precipitate (NaBr) was filtered off after the solution cooled to room temperature. The solvent was further evaporated by Rotavapor under vacuum. The product was extracted by ethyl acetate, followed by washing with water and drying with sodium sulfate. After evaporating ethyl acetate, the product was washed by hexane three times. The white crystal product weighted 27.83 g, yield 80.2%. $^1$H NMR ($CDCl_3$) δ 1.26 (t, 6H, $OCH_2CH3$), 1.98 (s, 3H, $COCH_3$), 2.28-2.10 (m, 2H, $ArCH_2CH_2$), 2.60-2.71 (m, 2H, $ArCH_2$), 4.18 (q, 4H, $OCH_2CH_3$), 4.66 (m, 1H, NCH), 5.62 (d, 1H, J 6.8, NH), 7.29-7.32 (m, 5H, Ar-H); MS *m/z* 322.1 ($M^+$); Anal. calcd for $C_{17}H_{24}NO_5$: C, 63.35; H, 7.45; N, 4.35; Found: C, 64.06; H, 7.51; N, 4.20.

Ethyl-2-carboxy-4-phenyl-2-acetamidomalonate (**5**)

20.0 g (0.0623 mmol) **4** was dissolved in 250 ml ethanol, followed by adding 31.2 ml 6 N NaOH. The mixture was stirred at room temperature for 2 hours. The suspension solution was diluted by 20 ml distilled water. After the pH of the solution was adjusted to 6-7 by 6 N HCl, the ethanol was evaporated under vacuum. 6 N HCl was further added until pH 2-3, and a white precipitate formed in the solution. Followed by filtering off the liquid, the crystals were washed with water and hexane three times respectively. Product weighted 15.25 g, yield 83.6%. $^1$H NMR ($CDCl_3$) δ 1.25 (t, 3H, J 7.2, $OCH_2CH_3$), 1.98 (s, 3H, $COCH_3$), 2.29-2.11 (m, 2H, $ArCH_2CH_2$), 2.60-2.71 (m, 2H, $ArCH_2$), 4.18 (q, 2H, J 7.2, $OCH_2CH_3$), 4.66 (m, 1H, NCH), 5.61 (d, 1H, J 6.8, NH), 7.29-7.32 (m, 5H, Ar-H); MS *m/z* 294.1 ($M^+$); Anal. calcd for $C_{15}H_{20}NO_5$: C, 61.22; H, 6.81; N, 4.76; Found: C, 62.32; H, 6.80; N, 4.82.

Ethyl-4-phenyl-2-acetamidomalonate (**6**)

8.00 g **5** was dissolved in 100 ml anhydrous 1, 4- dioxane, and the mixture was refluxed for 1.5 hour. The solvent was evaporated by Rotavapor under vacuum. Ethyl acetate was added to extract the product, followed by washing with saturated sodium bicarbonate and distilled water. The organic phase was dried with sodium sulfate for 30 minutes, followed by filtering off the solid and evaporating the solvent. The product was washed with hexane twice. Product weighted 5.85 g, yield 86.1%. $[\alpha]_D^{20}$= +2.2 (c=1.0, EtOH); mp 78-80 °C; $^1$H NMR ($CDCl_3$) δ 1.26 (t, 3H, J 7.2, OCH2C$H_3$), 1.98 (s, 3H, $COCH_3$), 2.28-2.10 (m, 2H, $ArCH_2CH_2$), 2.60-2.70 (m, 2H, $ArCH_2$), 4.19 (q, 2H, J 7.2, $OCH_2CH_3$), 4.65 (m, 1H, NCH), 5.60 (d, 1H, J 6.9, NH), 7.30-7.33 (m, 5H, Ar-H); IR (KBr) 3326, 1751,

1652, 1539 $cm^{-1}$; MS *m/z* 250.0 ($M^+$); Anal. calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.68; N, 5.62; Found: C, 67.33; H, 7.72; N, 5.56.

S-(+)-homophenylalanine Hydrochloride (**8**)

2.0961 g **6** was dissolved in 8.0 ml acetonitrile ($CH_3CN$), followed by adding sodium bicarbonate ($NaHCO_3$) solution (1 g $NaHCO_3$ in 72.0 ml water). After 1.0 ml *BL*-alcalase was added, the solution was stirred at room temperature and under nitrogen environment for 24 hours. Then the solvent acetonitrile was evaporated under vacuum and the D-isomer was precipitated. After filtering off the D-isomer, the alkaline solution was extracted with ethyl acetate to remove the trace D-isomer in the solution. The alkaline solution was then evaporated under vacuum to remove trace ethyl acetate. While 6 N HCl solution was dropped into the solution until pH 2-3, L-acid was precipitated. After filtering off water, the *N*-acetyl-L-(+)-homophenylalanine **7** was purified by washing with distilled water and hexane three times respectively. L-isomer **7** weighted 0.89 g and yield is 48.0 %.

The *N*-acetyl group in *N*-acetyl-L-(+)-homophenylalanine **7** was taken off by refluxing it with 2 M HCl solution for 4 hours. The 92.4% enantiomeric excess (*ee*) of amino acid 5 was obtained by chiral HPLC (Chiralpak WH column). $[\alpha]_D^{20}$= 44.3 (c=0.6, 3N HCl) [The results from Weller and Gordon (1982): $[\alpha]_D^{20}$= 45.0 (c=0.6, 3N HCl)]; mp > 260 °C; $^1H$ NMR ($D_2O$+TFA) δ 1.82-2.00 (m, 2H, Ar-$CH_2CH_2$), 2.38-2.45 (m, 2H, $ArCH_2$), 3.72 (t, 1H, J 6.2, NCH), 6.09-7.03 (m, 5H, Ar-H); IR (KBr) 3436, 1655, 1622, 1582 $cm^{-1}$; Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82; Found: C, 66.86; H, 7.36; N, 7.88.

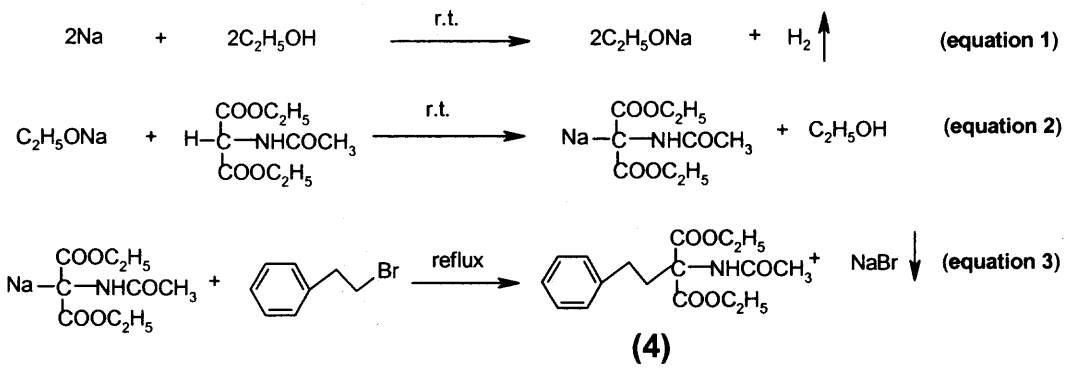



**Figure 2.3** Three steps for synthesis of diethyl-4-phenyl-2-acetamidomalonate (**4**).

### 2.2.4 Results and Discussion

**2.2.4.1 Formation of Diethyl-4-phenyl-2-acetamidomalonate (4).** A critical requirement for the formation of the diester **4** reaction was to maintain an anhydrous condition in the reaction system. Metallic sodium was first reacted with anhydrous ethyl alcohol to produce a strong base $(C_2H_5COO)Na$ as shown in Eq. (1) of Figure 2.3. If any water was present in the system, the sodium metal would react with it. If that happened, it would not only waste the use of expensive metallic sodium, but also decrease the efficiency of the following reactions.

After adding diethyl acetamidomalonate into the base solution of $(C_2H_5COO)Na$, a nucleophile $(COOC_2H_5)_2C(Na)(NHCOOCH_3)$ was formed after stirring at room temperature for 40 minutes (Eq. (2) of Figure 2.3). The rate of this reaction could be influenced by any water in the system. The third step in Figure 2.3 involved addition of 2-bromoethyl benzene to form a diester diethyl-4-phenyl-2-acetamidomalonate. 2-bromoethyl benzene was used in 30% excess. The reason is that a side reaction of 2-

bromoethyl benzene may occur to form styrene in strong alkali solution. Another way of minimizing the side reaction is to slowly add the 2-bromoethyl benzene solution instead of adding the solution all at once. The reflux time needed for this reaction was monitered by GC and TLC. As indicated in Figure 2.4, the total reflux time of 5 hours was sufficient for completing this reaction.

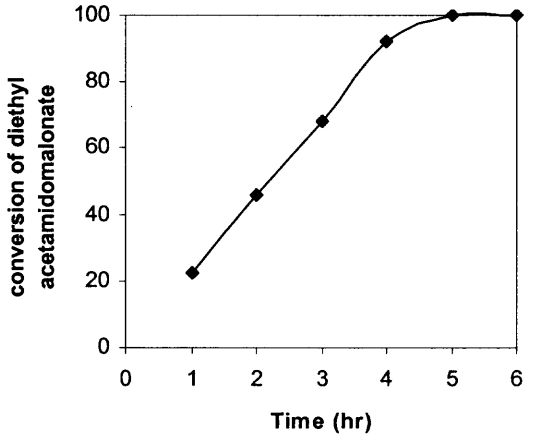
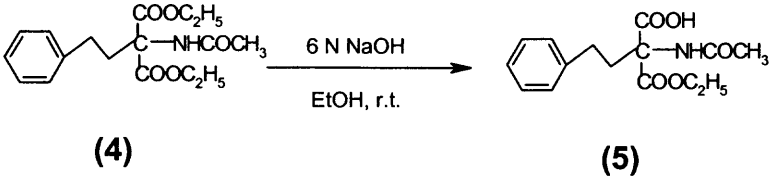



**Figure 2.4** Reflux time for the formation of diethyl-4-phenyl-2-acetamidomalonate (**4**).




**(4)** **(5)**

**Figure 2.5** Hydrolysis of diethyl-4-phenyl-2-acetamidomalonate (**4**).

**2.2.4.2 Hydrolysis of Diethyl-4-phenyl-2-acetamidomalonate (4).** There are two ester groups in the diester diethyl-4-phenyl-2-acetamidomalonate **4**. An important adjustable factor for this reaction is the reaction time if the concentration of sodium

hydroxide is a constant. Longer reaction would make sure the hydrolysis of one ester group was complete (Figure 2.5). However, if the reaction was too long, the second ester group might get hydrolyzed. Figure 2.6 illustrates that the optimum reaction time for hydrolyzing one ester group would be about 2 hours.

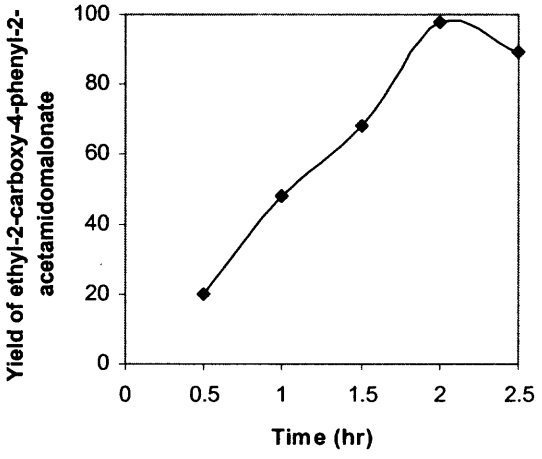
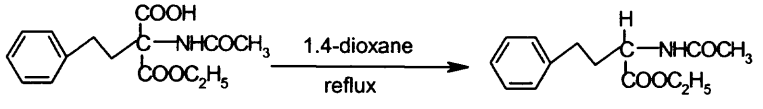



**Figure 2.6** Reaction time for hydrolysis of diethyl-4-phenyl-2-acetamidomalonate (**4**).




**(5)** **(6)**

**Figure 2.7** Decarboxylation of ethyl-2-carboxy-4-phenyl-2-acetamidomalonate (**5**).

**2.2.4.3 Decarboxylation of Ethyl -2- carboxy -4- phenyl -2- acetamidomalonate (5).** Anhydrous conditions are also required for conducting this decarboxylation reaction (Figure 2.7). The reaction can be monitored by TLC and the product was confirmed by NMR, MS, IR, etc. An important purification step is to wash the product with saturated

sodium bicarbonate ($NaHCO_3$). The reason is that any un-decarboxylated acid would dissolve in the alkali solution and could be separated from the product monoester **6**.

**2.2.4.4 Kinetic Resolution of *N*-acetyl-Homophenylalanine Ester (6).** The kinetic resolution of the amino acid ester was carried out in the organic solvent acetonitrile and water solution using an effective enzyme *BL*-alcalase. The detailed studies on the reaction conditions of kinetic resolution will be presented in the following chapter.

## 2.3 Summary

The industrial enzyme *BL*-alcalase shows highly effective resolution on the amino acid (piperazine-2-carboxylic acid) ester. L-(-)-piperazine-2-carboxylic acid was produced with 98.1% *ee* and 40.6% of resolution yield. An economical and simple reaction strategy was developed to synthesize L-(+)-homophenylalanine hydrochloride with over 90% *ee*. The overall yield of the three-step synthesis is 58% and the yield of the kinetic resolution is 48% (maximum 50%). From this study, it also clearly indicates that racemic amino acid esters with high purity are required for the kinetic resolution reactions. Chapter 4 will describe an effective method of producing amino acid esters catalyzed by a new ionic liquid.

# CHAPTER 3

# KINETIC RESOLUTION OF HOMOPHENYLALANINE ETHYL ESTER IN IONIC LIQUIDS BY THE ENZYME *BL*-ALCALASE

## 3.1 Background Information

Obtaining optically pure α-amino acids, especially unnatural α-amino acids, have been extensively studied in recent years due to their significance as starting materials in chiral synthesis of modern drugs. Enzymatic resolution of amino acid derivatives is one of the simplest and most efficient methods of synthesizing enantiomerically enriched amino acids. Traditionally, a mixture of organic solvents and water has been used as the reaction media for the kinetic resolution of amino acids. For example, the kinetic resolution of different amino acids by the enzyme *Bacillus licheniforms* alcalase (*BL*-alcalase) has been studied in acetone-water (Kijima et al., 1994), acetonitrile-water (Kijima et al., 1994), ethanol-water (Kijima et al., 1994), 1-propanol-water (Kijima et al., 1994), tetrahydrofuran-water (Kijima et al., 1994), dioxane-water (Chen et al., 1991), t-butanol-water (Chen et al., 1991), 2-methyl-2-propanol-water (Chen et al., 1994), and DMF (Chen et al., 1986), etc.

Due to their environmental friendly nature, ionic liquids have gained a lot of attention as "green" solvents in organic synthesis and other chemical processes. Ionic liquids can now be produced with melting point of room temperature or below (as low as –96 °C), which is an important reason why ionic liquids are becoming a more attractive substitute for volatile and toxic organic solvents (Seddon, 1997). Ionic liquids have many favorable properties, such as good solvents for a wide range of inorganic, organic and polymeric materials, adjustable polarity, and catalytic effects, etc. More recently, ionic

liquids have been used in the studies of enzymatic systems, such as lipase catalysed kinetic resolution of 1-phenylethanol in ionic liquids (Schofer et al., 2001), enzymatic catalysis of formation of Z-aspartame in ionic liquids (Erbeldinger et al., 2000), catalyzing alcoholysis, ammoniolysis and perhydrolysis reactions by lipase in ionic liquids 1-butyl-3-methylimidazolium tetrafluoroborate or hexafluorophosphate (Lau et al., 2000), etc. Markedly enhanced enantioselectivity was achieved in ionic liquids for the lipase-catalyzed transesterifications (Kim et al., 2001).

Chapter 2 has reported the synthesis of homophenylalanine ester and piperazine-2-carboxylic acid ester, as well as the enzymatic resolution of the esters to obtain the single enantiomer (Zhao et al., 2002; Wu et al., 2002). With the foreseeable advantages and goal of finding a proper ionic medium to substitute organic solvents, a study on the resolution of homophenylalanine ester was initiated. In this study, very promising results have been obtained and several suitable ionic solvents have been used to achieve this enzymatic resolution by the enzyme *BL*-alcalase.

## 3.2 Materials and Methods

### 3.2.1 Materials

*Bacillus licheniforms* alcalase (*BL*-alcalase) is a commercially available endoproteinase of the serine type, the major component of which is subtilisin A (Subtilisin Carlsberg). *BL*-alcalase was produced by Novozymes and distributed by Sigma-Aldrich as a brown liquid with a specific activity of 2.4 AU/g for hydrolysis of dimethyl casein at 50 °C and pH 8.3. It was used without further purification. The *N*-acetyl homophenylalanine ethyl ester was prepared through a three-step-reaction strategy (Zhao et al., 2002). All the

solvents have purity higher than 99%. Ionic liquids 1-ethyl-3-methylimidazolium tetrafluoroborate ($[EMIM]^+[BF_4]^-$), *N*-ethyl pyridinium tetrafluoroborate ($[EtPy]^+[BF_4]^-$) and *N*-ethyl pyridinium trifluoroacetate ($[EtPy]^+[CF_3OO]^-$) were prepared using methods given in the literature (Holbrey and Seddon, 1999). Those ionic liquids were dried at 65 °C under vacuum. *N*-ethyl pyridinium bromide is from Alfa Aesar, A Johnson Matthey Company. All other materials for preparing ionic liquids are from Sigma-Aldrich.

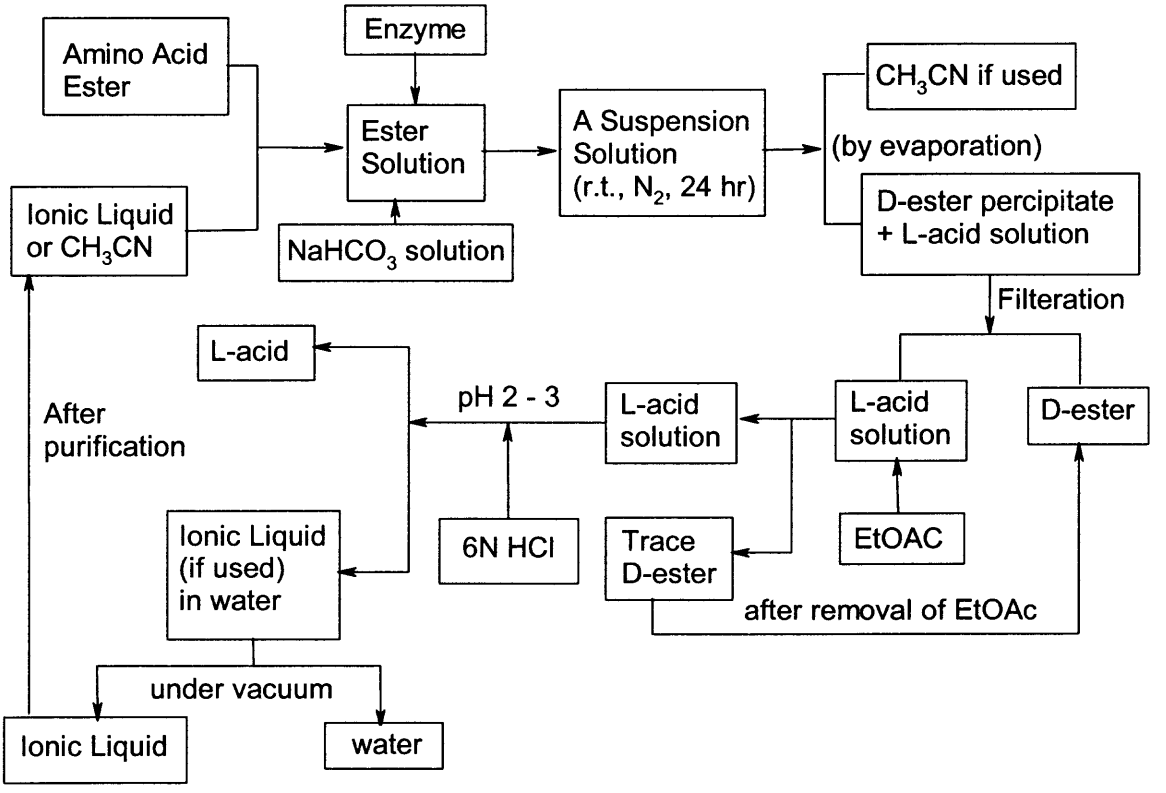



**Figure 3.1** Flow chart of enzymatic resolution of amino acid esters.

### 3.2.2 General Procedures of the Kinetic Resolution

General method (Figure 3.1): 0.5 g of racemic *N*-acetyl amino acid ethyl ester was suspended or dissolved in 60 ml of a mixed solvent containing water and ionic liquid; 0.5 g $NaHCO_3$ was dissolved in the reaction mixture while the pH of the solution was about 8. 1.0 ml *BL*-alcalase was then added. The reaction mixture was gently stirred at 25 °C for 24 hrs under a nitrogen atmosphere. The *N*-acetyl D-ester was extracted by ethyl acetate three times. 6 N HCl was added and the pH lowered to 2-3. The remaining trace ethyl acetate in aqueous solution was evaporated and the solution was further concentrated until the precipitate appeared. Removal of water gave *N*-acetyl L-acid. The *N*-acetyl D-acid was obtained by hydrolyzing *N*-acetyl D-ester in 6 N NaOH solution for 2 hrs. The *N*-acetyl group in the L- and D-acid was taken off by refluxing with 3 N HCl for 3 hrs.

### 3.2.3 Analytic Methods

The enantiomeric excess (*ee*) was calculated from the specific optical rotation which was measured in 3 N HCl using an AUTOPOL IV polarimeter from Rudolph Research Analytical Company. Also, the *ee* measurements were confirmed by HPLC with a Chiralpak WH column. The densities of ionic liquids were measured by mass-volume method. The IR spectrum of ionic liquids was measured by Spectrum One FT-IR System from PerkinElmer Corporation.

### 3.3 Preparation of Ionic Liquids

The symbol $R^+X^-$ is a general formula for the starting material used to prepare imidazolium or pyridinium based ionic liquid $R^+B^-$. For example, R = 1-ethyl-3-methylimidazolium (EMIM), or R = *N*-ethylpyridinium (EtPy), and X = Cl, Br, etc (structure of cations in Figure 1.10). A general approach for preparing the imidazolium or pyridinium based ionic liquid $R^+B^-$ can be described by the following two reactions.

$$Ag_2O + 2\,HB \longrightarrow 2\,AgB + H_2O \qquad (1)$$

$$AgB + RX \longrightarrow RB + AgX\downarrow \qquad (2)$$

**Figure 3.2** Schema of preparation of ionic liquids by a two-step-reaction.

In reaction (1) of Figure 3.2, silver (I) oxide is dissolved in an acid forming a homogeneous solution. The ionic liquid $R^+B^-$ is then produced in the second reaction by adding $R^+X^-$ to form a AgX precipitate. Detailed experimental steps were modified from the literature method (Holbrey and Seddon, 1999) and described in the following.

#### 3.3.1 Synthesis of $[EMIM]^+[BF_4]^-$

Tetrafluoroboric acid (22.34 ml, 0.171 mol, 48% solution in water) was slowly added to a stirred slurry of silver (I) oxide (19.83 g, 0.0855 mol) in 50 ml distilled water over a period of 10 minutes. To avoid photodegradation of silver (I) oxide, the reaction mixture was fully covered with aluminum foil. Until the silver (I) oxide was completely reacted, a

solution of 1-ethyl-3-methylimidazolium chloride (25.0 g, 0.171 mol) in 150 ml distilled water was added to the reaction mixture and stirred at room temperature for 2 hours. The white precipitate of silver (I) chloride was filtered off, and the solvent was removed at 65 °C under vacuum. The resulting salt is a pale yellow liquid. Yield 30.5 ml, 90%.

### 3.3.2 Synthesis of $[EtPy]^+[BF_4]^-$

Tetrafluoroboric acid (30.0 ml, 0.230 mol, 48% solution in water) was slowly added to a stirred slurry of silver (I) oxide (26.66 g, 0.115 mol) in 60 ml distilled water over 10 minutes. To avoid photodegradation of silver (I) oxide, the reaction mixture was fully covered with aluminum foil. Until the silver (I) oxide was completely reacted, a solution of *N*-ethyl-pyridinium bromide (43.2 g, 0.230 mol) in 150 ml distilled water was added to the reaction mixture and stirred at room temperature for 2 hours. The yellow precipitate of silver (I) bromide was filtered off, and the solvent was removed at 65 °C under vacuum. The resulting salt is a light brown liquid. Yield 29.0 ml, 92%.

### 3.3.3 Synthesis of $[EtPy]^+[CF_3COO]^-$

Trifluoroacetic acid (13.3 ml, 0.1726 mol) was slowly added to a stirred slurry of silver (I) oxide (20.0 g, 0.0863 mol) in 50 ml distilled water over 10 minutes. To avoid photodegradation of silver (I) oxide, the reaction mixture was fully covered with aluminum foil. Until the silver (I) oxide was completely reacted, a solution of *N*-ethyl-pyridinium bromide (32.46 g, 0.1726 mol) in 150 ml distilled water was added to the reaction mixture and stirred at room temperature for 2 hours. The yellow precipitate of

silver (I) bromide was filtered off, and the solvent was removed at 65 °C under vacuum. The resulting salt is a brown liquid. Yield 30.1 ml, 91%.

### 3.3.4 Purification of Ionic Liquids

It was reported (Swartling et al., 2000) that any color and impurities present in the synthesized ionic liquids could be removed by dissolving the ionic liquids in water and passing the solution through a charcoal column. In this study, a certain amount of charcoal was added into the ionic liquid solution and kept overnight. Charcoal was then removed by filtration and water was evaporated under vacuum. The resulting ionic liquids are usually colorless or light brown.

## 3.4 Results and Discussion

### 3.4.1 Density and IR Spectroscopy of Ionic Liquids

The densities of three ionic liquids were measured by mass-volume method and recorded in Table 3.1. As illustrated in Hagiwara and Ito (2000)'s work, the densities of most ionic liquids at ambient temperature (16-28 °C) are in the range of 1.1-1.6g/ml.

**Table 3.1** Density of Three Ionic Liquids (20 °C, 1 atm)

| Ionic Liquid | Experimental Density (g/ml) | Literature Density (g/ml) | Reference |
|---|---|---|---|
| $[EMIM]^+[BF_4]^-$ | 1.296 | 1.282 | Noda *et al.*, 2001 |
| $[EtPy]^+[BF_4]^-$ | 1.302 | N/A | |
| $[EtPy]^+[CF_3COO]^-$ | 1.273 | N/A | |

In the region 4000–2000 $cm^{-1}$, C–H stretching vibrations were observed. The peaks at > 3100 $cm^{-1}$ in the spectrum can be attributed to the ring C–H stretch, while

those below 3000 $cm^{-1}$ can be attributed to aliphatic stretches. Other bands are given in Table 3.2.

**Table 3.2** Main IR Bands ($cm^{-1}$) Assigned According to Tait and Osteryoung (1984)

| Wavelength of the band, $cm^{-1}$ | Vibration |
|---|---|
| 3171, 3124 | ν(C–H) aromatic, str[a] |
| 2966, 2939, 2878 | ν(C–H) aliphatic, str |
| 1575, 1467 | ν(ring), str sym |
| 1431, 1386 | MeC–H , asym |
| 1170 | (ring), str sym |
| 3490, 1650, 700 | O-H in $H_2O$ |

[a] Abbreviation: str, stretching; sym, symmetrical; asym, asymmetrical.

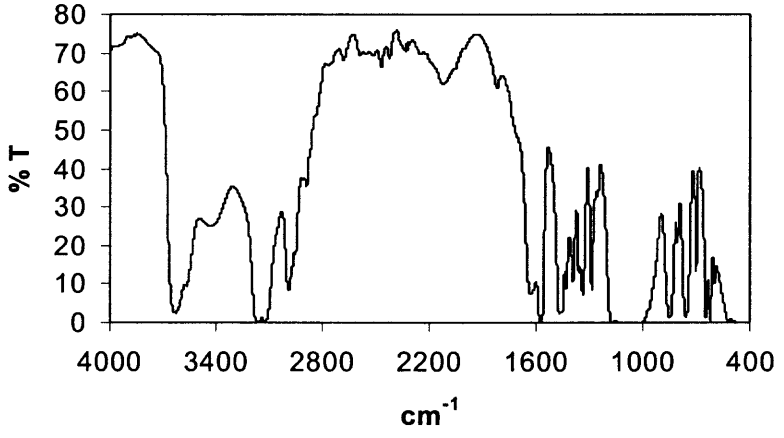



**Figure 3.3** IR spectrum of ionic liquid $[EMIM]^+[BF_4]^-$.

The anion $[BF_4]^-$ is a weakly complexing anion and is not expected to participate in strong hydrogen bonding. Indeed, the IR spectrum of $[EMIM]^+[BF_4]^-$ (Figure 3.3) and $[EtPy][BF_4]$ (Figure 3.4) show that no hydrogen bonding bands in the region 3000–3100 $cm^{-1}$ where C–H...Cl– interactions on imidazolium chloride were observed previously (Tait and Osteryoung, 1984). However, anion $[CF_3COO]^-$ is a stronger complex and participates in the formation of hydrogen bonding, which has been confirmed in Figure 3.5 by a band in the region of 3000–3100 $cm^{-1}$ in the ionic liquid $[EtPy]^+[CF3COO]^-$.

Therefore, IR results are consistent with a lack of hydrogen bonding in salts containing weakly complexing anions. This means that cation–anion coulombic attraction is driving the overall structure, with local steric effects influencing the final orientation of ions (Fuller *et al.*, 1994). It is also possible that the orientation of alkyl groups plays a role in hydrophobic effects.

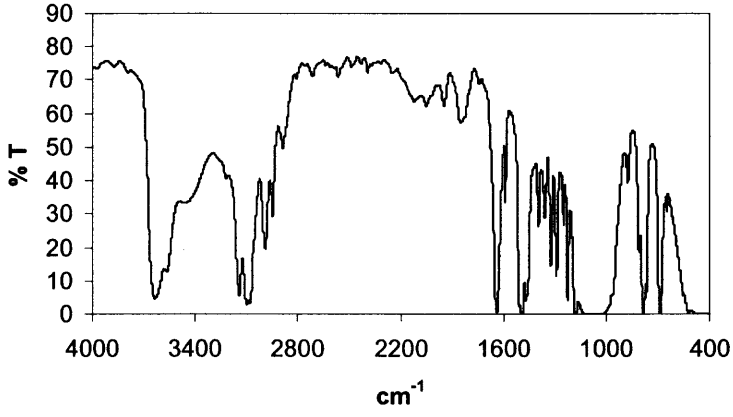



**Figure 3.4** IR spectrum of ionic liquid $[EtPy]^+[BF_4]^-$.

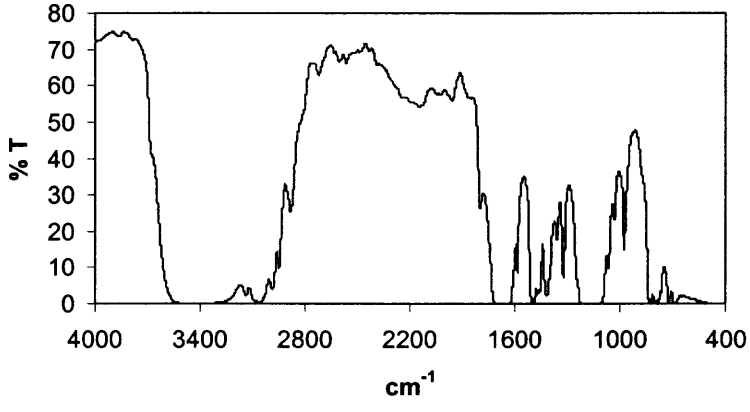



**Figure 3.5** IR spectrum of ionic liquid $[EtPy]^+[CF_3COO]^-$.

In the case of moisture stable ionic liquids, IR spectroscopy helps to reveal impurities in the liquids. Evidence for the presence of hydrogen bonding can be obtained

from IR spectroscopy. The presence of a band in the region of 3050 – 3080 $cm^{-1}$ is regarded as diagnostic of the presence of a strong, discrete C(2)...X hydrogen bond (Abdul-Sada *et al.*, 1986). In all the three infrared spectra (Figure 3.3, 3.4, 3.5), this situation does not occur that indicates the complete consumption of the starting material RX, therefore, no discrete C(2)...X hydrogen bond. The complete removal of water and the absence of other –OH species can be confirmed by the lack of O–H stretching bands from 3400 to 3800 cm–1 in the infrared spectra of the final melts. However, in this study, mixed solvent of ionic liquid and water will be used, therefore, complete removal of water is not so critical as long as it does not significantly affect the calculation of ionic concentration.

### 3.4.2 HPLC Studies on the Kinetic Resolution of Homophenylalanine Ethyl Ester

Several chiral homophenylalanine samples after kinetic resolution in acetonitrile and ionic liquids were analyzed by HPLC with a chiral column (Chiralpak WH). High resolution was achieved for those samples under the following HPLC operation conditions:

| | |
|---|---|
| Column: | Chiralpak WH[†] |
| Mobile phase: | 30% $CH_3OH$, 70% $H_2O$, 0.75 mM $CuSO_4$ |
| Flow Rate: | 1.5 ml/min |

[†] The Chiralpak WH column was produced by Chiral Technologies, INC (France) with an internal diameter 4.6 mm, column length 250 mm, partical size 10 μ and adsorbent of ligand exchange.

UV: 230 nm

Temperature: 50 °C

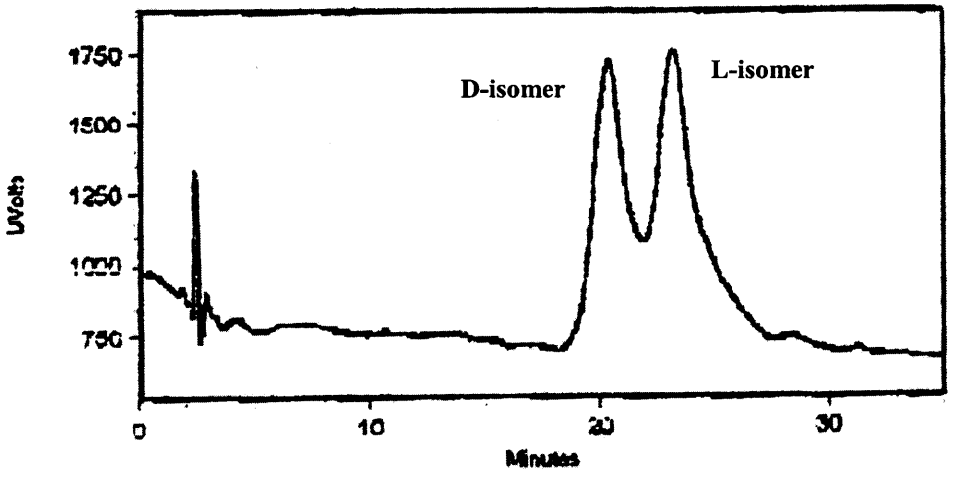



**Figure 3.6** HPLC profile of racemic D, L- homophenylalanine.

Figure 3.6 shows that racemic homophenylalanine can be well resolved by HPLC under the operating conditions listed above. The D-enantiomer of homophenylalanine was eluted first, followed by the L-enantiomer. This profile will be used as a reference for the on-coming analysis of enantiomeric homophenylalanine samples.

The enzymatic resolution of homophenylalanine ethyl ester was carried out in acetonitrile and water mixture (66% v/v water) by using the enzyme *BL*-alcalase. The HPLC profile of the L-isomer sample is shown in Figure 3.7. The reaction conditions were followed by the figure. The L-isomer has a much larger peak compared with the D-isomer. The *ee* of the L-isomer is 92.4% according to the integration of the HPLC peaks.

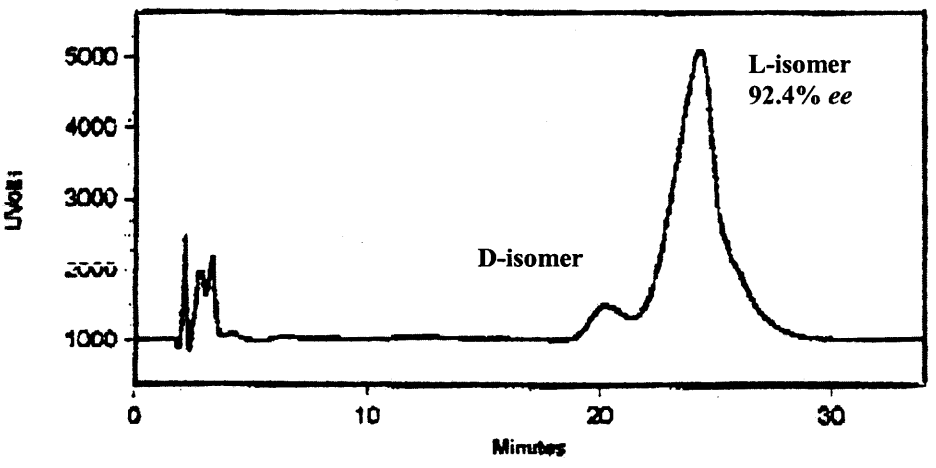



**Figure 3.7** HPLC profile of kinetic resolution in acetonitrile and water mixture (0.5 g ester, 0.5 g $NaHCO_3$, 20.4 ml acetonitrile, 39.6 ml water, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

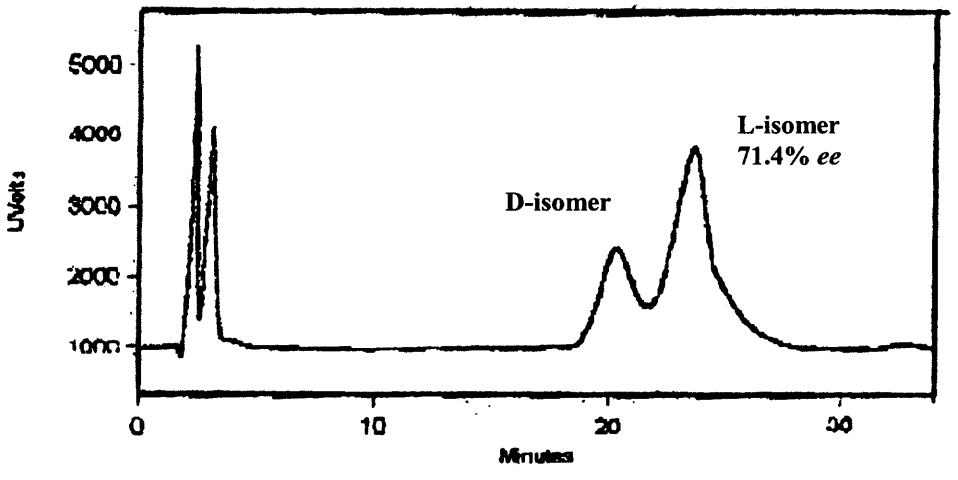



**Figure 3.8** HPLC profile of kinetic resolution in $[EMIM]^+[BF_4]^-$ and water mixture (0.5 g ester, 0.5 g $NaHCO_3$, 20.4 ml $[EMIM]^+[BF_4]^-$, 39.6 ml water, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

In order to compare the impact of ionic liquids on the resolution reaction, ionic liquids $[EMIM]^+[BF_4]^-$ and $[EtPy]^+[BF_4]^-$ were used as "green" solvents while the water

content was kept the same (66% v/v). Figure 3.8 and 3.9 illustrate the HPLC profiles of the L-enantiomer by using the ionic liquid $[EMIM]^+[BF_4]^-$ and $[EtPy]^+[BF_4]^-$ respectively. Lower optical purity was obtained when the ionic liquids were used in the kinetic resolution. The *ee* was 71.4% when using the ionic liquid $[EMIM]^+[BF_4]^-$ and 52.5 % when using the ionic liquid $[EtPy]^+[BF_4]^-$. It is obvious that when a large amount of ionic liquids was added into the reaction mixture, the enantioselectivity was decreased due to the influence of salt concentration on the active sites on the enzyme. Therefore, high concentration of ionic liquid is not suggested for use in the enzymatic reactions.

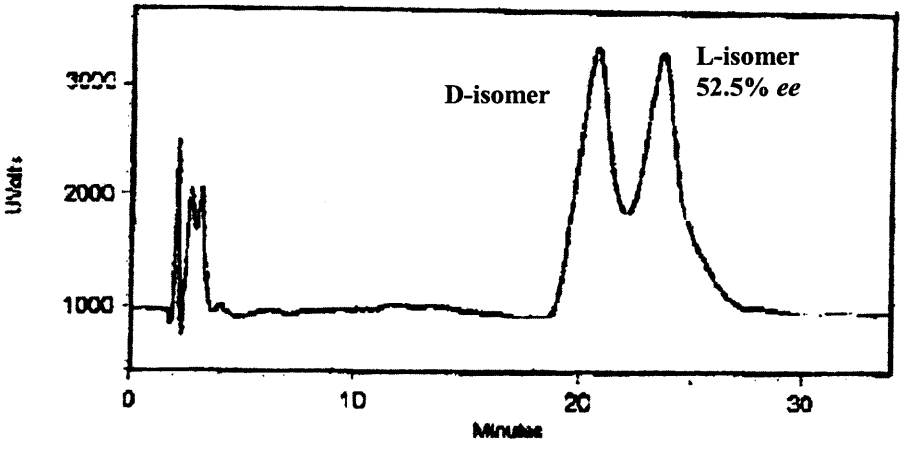



**Figure 3.9** HPLC profile of kinetic resolution in $[EtPy]^+[BF_4]^-$ and water mixture (0.5 g ester, 0.5 g $NaHCO_3$, 20.4 ml $[EtPy]^+[BF_4]^-$, 39.6 ml water, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

However, when the use of the ionic liquid $[EtPy]^+[BF_4]^-$ was decreased to 83% (v/v), a significant increase of *ee* (97.4%) was observed in the kinetic resolution of homophenylalanine ethyl ester (Figure 3.10). This indicates that in low concentration of ionic liquid, enzyme *BL*-alcalase shows high enantioselectivity in the kinetic resolution

reaction. Therefore, low concentration of ionic liquids will be used through out the current investigation of enzymatic resolution of amino acid esters.

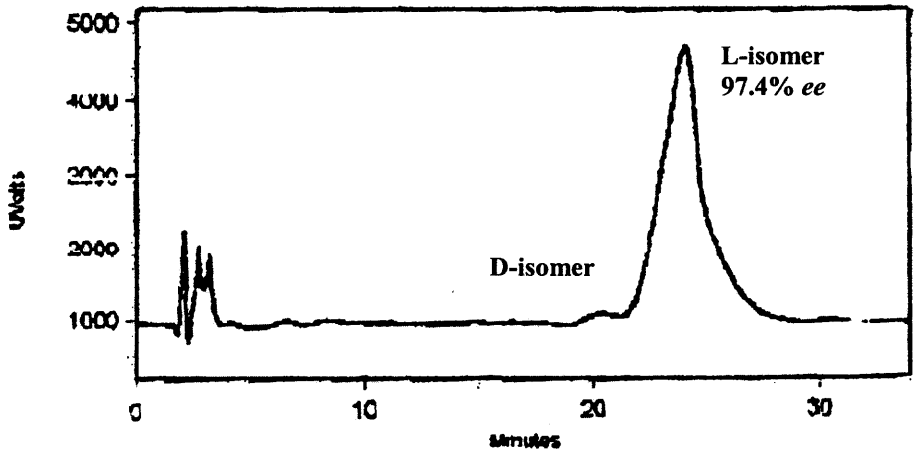



**Figure 3.10** HPLC profile of kinetic resolution in $[EtPy]^+[BF_4]^-$ and water mixture (0.5 g ester, 0.5 g $NaHCO_3$, 10.2 ml $[EtPy]^+[BF_4]^-$, 49.8 ml water, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

### 3.4.3 Solvent Effect on the Kinetic Resolution of Homophenylalanine Ethyl Ester

The study by Kijima et al. has shown that the effect of organic solvents is significant on the activity and enantioselectivity of protease for hydrolyzing ester or peptide (Reslow et al., 1987). The effect of different organic solvents on the resolution of DL-3,4-dihydroxyphenylalanine ethyl ester has been studied by Kijima et al (1994). In this study, the resolution reactions of the DL-ester mixture with a series of organic solvents were conducted using the enzyme *BL*-alcalase. As shown in Table 3.3, the resolution could be achieved with varied enantioselectivity using different organic solvents. The results show that optically pure product can be obtained in acetone, acetonitrile, and 1,4-dioxane and poor resolution is given in methanol and ethanol. In this study, organic solvents also

show a strong effect on the resolution of *N*-acetyl DL-homophenylalanine ethyl ester (Table 3.3). Acetonitrile, 1-propanol and ethanol are good solvents for obtaining L-enantiomer with high optical purity and fairly good yield.

**Table 3.3** Effect of Solvents on the Resolution of Homophenylalanine Ethyl Ester (0.5g ester, 85% (volume) water and 15% (volume) solvent, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C, 24 hours)

| Solvents | Dielectric constant* | e.e. (%) of L-enantiomer | Yield (%) based on L |
|---|---|---|---|
| Acetonitrile | 36.64 | 95.0 | 39.0 |
| $[EtPy]^+[BF_4]^-$ | N/A | 94.8 | 48.0 |
| Acetone | 21.01 | 77.8 | 40.0 |
| 1-Propanol | 20.1 | 93.3 | 31.0 |
| Ethanol | 25.3 | 90.1 | 33.3 |
| Methanol | 33.0 | — | — |
| $[EMIM]^+[BF_4]^-$ | N/A | 89.0 | 32.0 |
| Water | 80.10 | 100.0 | 49.0 |
| $[EtPy]^+[CF_3COO]^-$** | N/A | 96.0 | 40.0 |

* data from literature (Weast, 1967).
** polarity data unknown.

Three ionic liquids, $[EMIM]^+[BF_4]^-$, $[EtPy]^+[BF_4]^-$ and $[EtPy]^+[CF_3COO]^-$, were used in this study. The order of solvents in Table 3.3 was based on the polarity from recent research on ionic liquids (Aki et al., 2001; Carmichael and Seddon, 2000b). One exception is that the polarity data of $[EtPy]^+[CF_3COO]^-$ is unknown. As Table 3.3 shows, high *ees* and comparable yields of L-isomer were obtained when these solvents were used in only 15% by volume. In general, a less polar solvent seems beneficial in improving the optical purity and yield of the L-enantiomer. With these promising results, a systematic study will be conducted using an ionic liquid and water in varied concentrations.

The water content in the mixing solvent seems to be a strong factor influencing the enantioselectivity of the enzyme *BL*-alcalase. Figure 3.11 illustrates that the water

content influences the kinetic resolution of *N*-acetyl homophenylalanine ethyl ester by using enzyme *BL*-alcalase. It was observed that a change in concentration of acetonitrile did not significantly affect the *ee* of the L-isomer though the yield changed. However, lower *ee* is expected at high concentration of organic solvent because high concentration of organic solvent may inhibit the activity of enzymes. Although in this case pure water is a good solvent for enzymatic resolution, it is not always a suitable medium for the resolution of amino acids (Kijima et al., 1994). The results in Figure 3.11 are similar to those shown by Kijima et al (1994), where the *ee* declines sharply while the water content is above 70% (v/v) for the kinetic resolution of DL-tyrosine ethyl ester in acetonitrile-water.

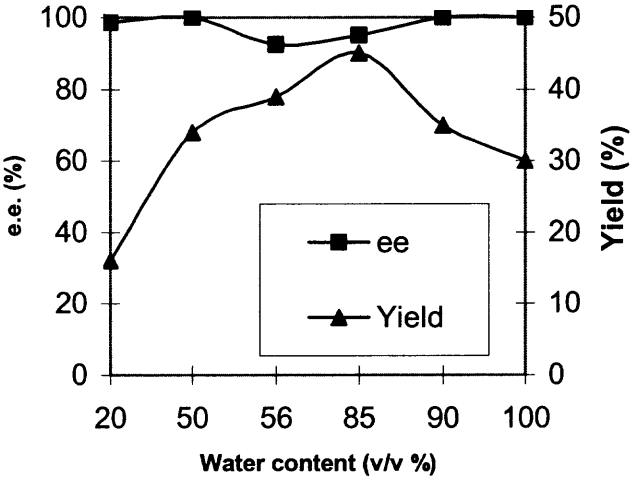



**Figure 3.11** Effect of acetonitrile and water ratio on the kinetic resolution of DL-homophenylalanine ethyl ester (0.5 g ester, 60 ml mixing solvent, 0.5 g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

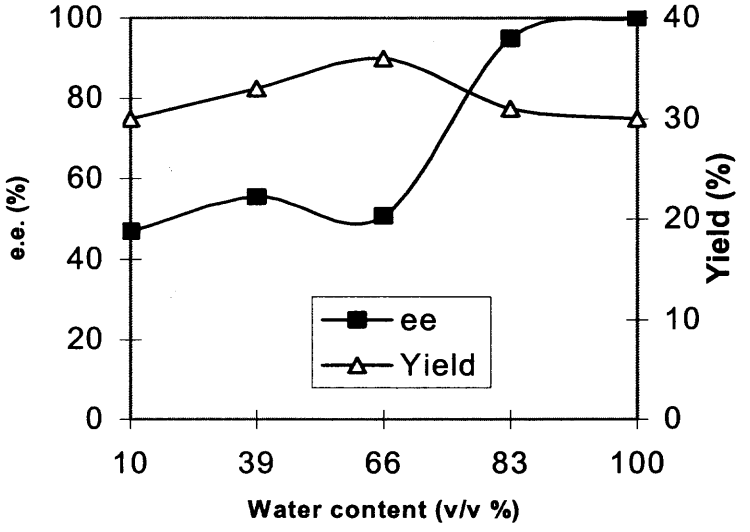



**Figure 3.12** Effect of ionic liquid $[EMIM]^+[BF_4]^-$ and water ratio on the kinetic resolution of DL-homophenylalanine ethyl ester (0.5 g ester, 60 ml mixing solvent, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

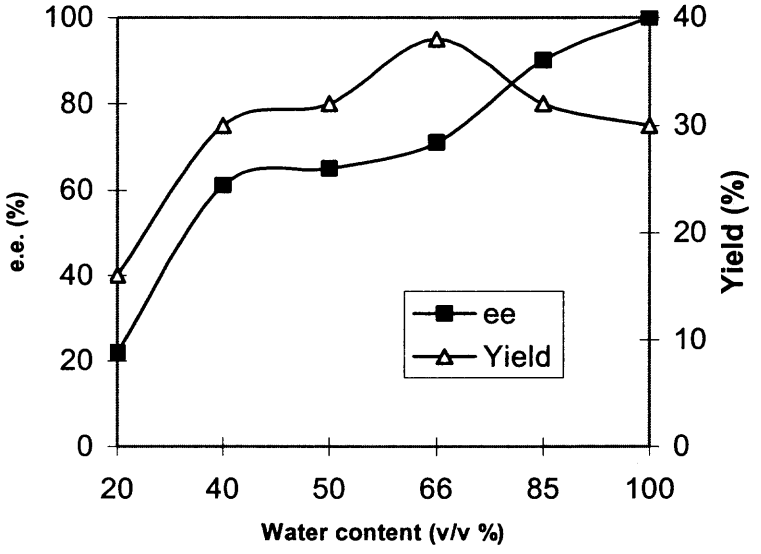



**Figure 3.13** Effect of ionic liquid $[EtPy]^+[BF_4]^-$ and water ratio on the kinetic resolution of DL-homophenylalanine ethyl ester (0.5 g ester, 60 ml mixing solvent, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

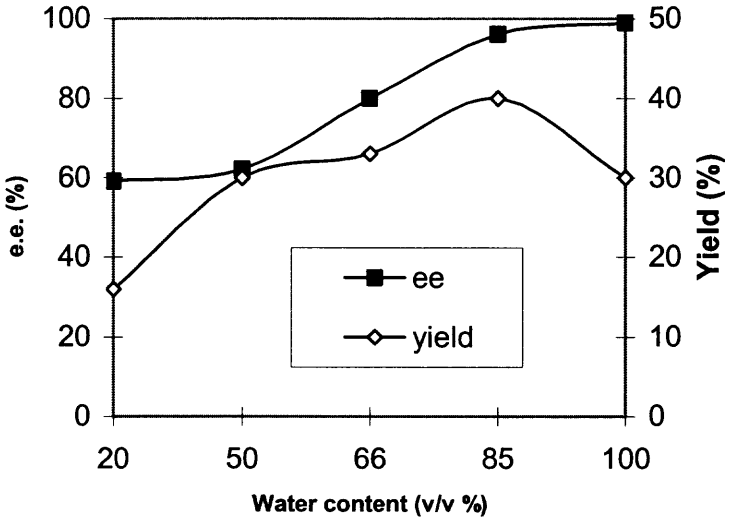



**Figure 3.14** Effect of ionic liquid $[EtPy]^+[CF_3COO]^-$ and water ratio on the kinetic resolution of DL-homophenylalanine ethyl ester (0.5 g ester, 60 ml mixing solvent, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C, 24 hours).

However, ionic liquids have a different pattern of affecting the kinetic resolution of amino acids. Figure 3.12, 3.13 and 3.14 show the kinetic resolution of *N*-acetyl homophenylalanine ethyl ester in three different ionic liquids $[EMIM]^+[BF_4]^-$, $[EtPy]^+[BF_4]^-$ and $[EtPy]^+[CF_3COO]^-$ respectively. The *ee* of the L-isomer declines with the increase of ionic liquid concentration. A high concentration of the ionic liquid causes high ionic strength of the reaction media, which may denature the enzyme and thus decrease the enantioselectivity of the enzyme. An increased concentration of ionic liquid may also increase the non-enzymatic hydrolysis of the ester. However, a high yield of L-enantiomer does not occur in high concentration of ionic liquid, nor high water content. This phenomenon is similar to the case in the organic solvent acetonitrile, which indicates ionic liquids can boost the enantioselectivity and activity of the enzyme *BL*-alcalase at a

different range of concentration. However, the ionic liquid $[EtPy]^+[CF_3COO]^-$ shows more promising applications among those three ionic solvents since both *ee* and yield of the desired product maintain high values in the presence of 85% (v/v) water (Figure 3.14). In addition to this advantage, the ionic liquid $[EtPy]^+[CF_3COO]^-$ is also easy to prepare from inexpensive starting materials. Therefore, it will be selected as a suitable candidate in the enzymatic resolution of other amino acid esters.

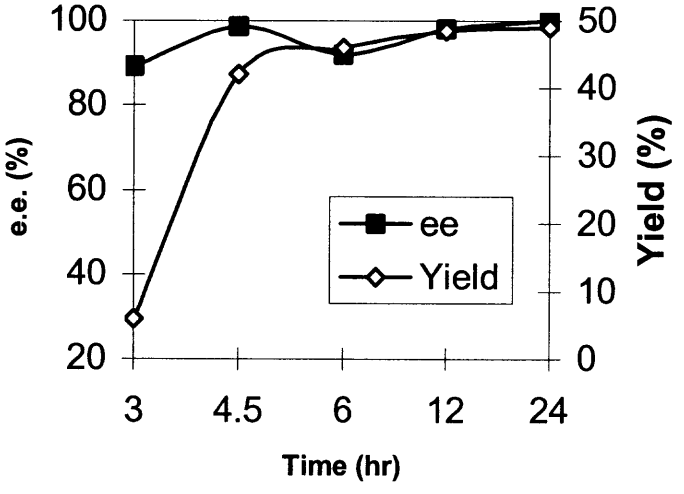



**Figure 3.15** Effect of reaction time on the kinetic resolution of DL-homophenylalanine ethyl ester in acetonitrile + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C).

### 3.4.4 Time Effect on the Kinetic Resolution

Since L-homophenylalanine ethyl ester is preferred for hydrolysis by the enzyme *BL*-alcalase compared with the D-ester, longer reaction time leads to the higher product yield. Kinetic resolutions in acetonitrile-water (Figure 3.15), ionic liquid $[EtPy]^+[BF_4]^-$-water (Figure 3.16), and $[EtPy]^+[CF_3COO]^-$-water mixture (Figure 3.17) indicate that high optical purity was achieved in these cases regardless of reaction time, and increasing product yield was obtained with longer reaction time. However, in acetonitrile-water

shorter time is needed for high yield (4.5 hrs with yield 42%), while 24 hours is generally necessary for complete enzymatic resolution in the ionic liquid and water mixture.

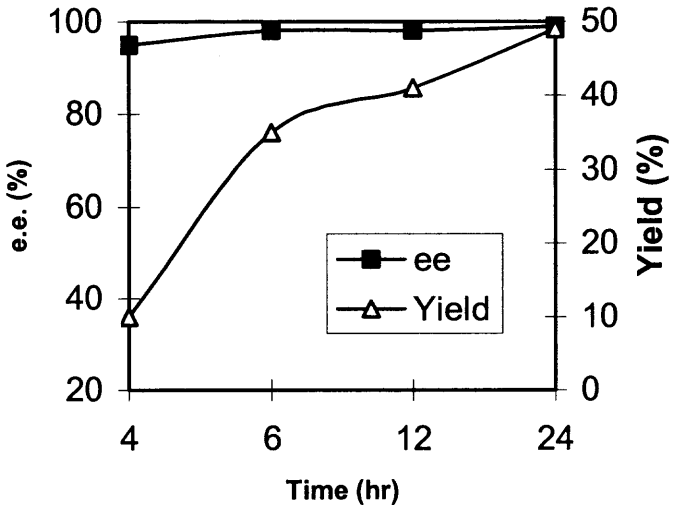



**Figure 3.16** Effect of reaction time on the kinetic resolution of DL-homophenylalanine ethyl ester in $[EtPy]^+[BF_4]^-$ + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C).

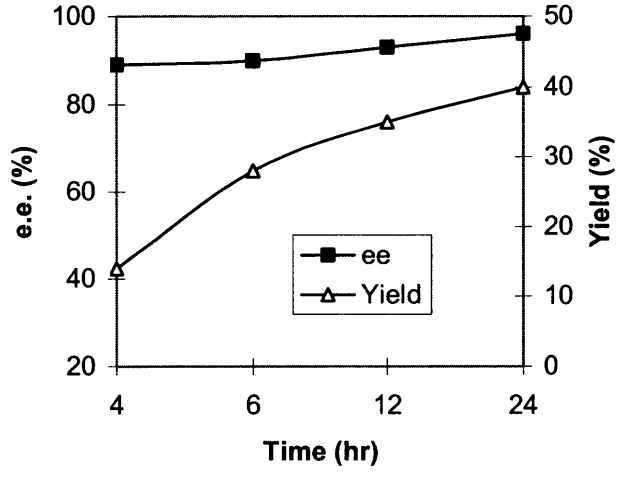



**Figure 3.17** Effect of reaction time on the kinetic resolution of DL-homophenylalanine ethyl ester in $[EtPy]^+[CF_3COO]^-$ + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 25 °C).

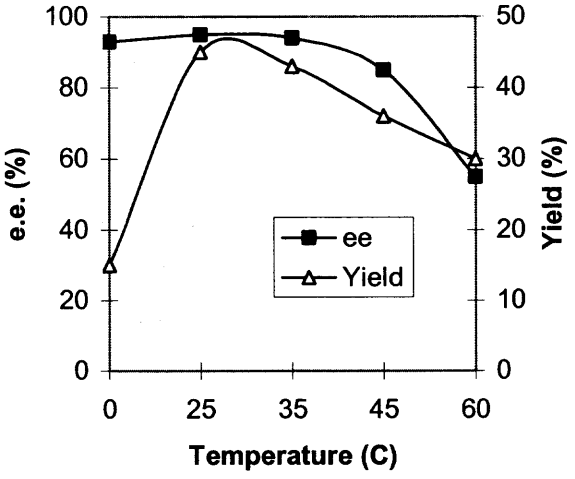



**Figure 3.18** Effect of reaction temperature on the kinetic resolution of DL-homophenylalanine ethyl ester in acetonitrile + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 6 hrs).

### 3.4.5 Temperature Effect on the Kinetic Resolution

Temperature plays an important role in controlling the product optical purity and yield. Figure 3.18 illustrate the influence of temperature on the kinetic resolution of *N*-acetyl homophenylalanine ethyl ester. The *ee* of the L-enantiomer does not have a significant change until the temperature increases beyond 35 °C. It is obvious that high temperature causes the substrate molecules to move more frequently instead of binding to a specific enzyme active site, which decreases the enantioselectivity of the reaction. 0 °C seems too low to activate the kinetic resolution reaction and too high a temperature may denature the enzyme and decrease its activity. In this case, room temperature (25 °C) seems an ideal temperature for obtaining high *ee* and yield.

The effect of temperature on the kinetic resolution of homophenylalanine ethyl ester in different ionic liquids and water mixture was also illustrated Figure 3.19,

($[EMIM]^+[BF_4]^-$), 3.20 ($[EtPy]^+[BF_4]^-$), 3.21 ($[EtPy]^+[CF_3COO]^-$) respectively. For these ionic liquids, maximum optical purity and yield occur at an optimum temperature. This temperature is 45 °C for $[EMIM]^+[BF_4]^-$, 25 °C for $[EtPy]^+[BF_4]^-$ and $[EtPy]^+[CF_3COO]^-$. It is interesting that at 0 °C, both *ee* and yield were very low while using an ionic liquid as a reaction medium. The possible reason could be that the ionic liquid might have a strong interaction with the enzyme active site at very low temperature, and therefore, inhibit the substrates to attach with the enzyme. When the temperature rises, this ionic liquid-enzyme interaction could be eliminated due to the movement of ionic liquid molecules. In an ionic liquid, the enzyme is more sensitive to temperature changes. At a higher temperature than 50 °C, the enzyme *BL*-alcalase loses its enzymatic activity significantly, indicating denaturation of the enzyme by heat. It has been known that the optimum temperature for *BL*-alcalase in aqueous solution is about 60 °C; this study shows lower stability of *BL*-alcalase in ionic liquids compared to aqueous solution.

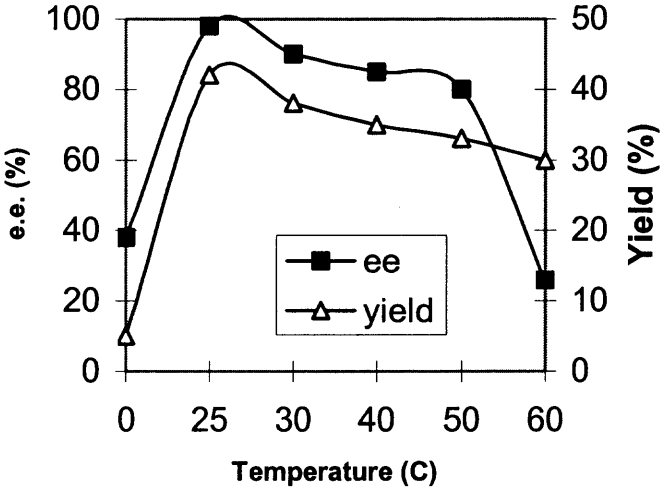



**Figure 3.19** Effect of reaction temperature on the kinetic resolution of DL-homophenylalanine ethyl ester in $[EMIM]^+[BF_4]^-$ + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 6 hrs).

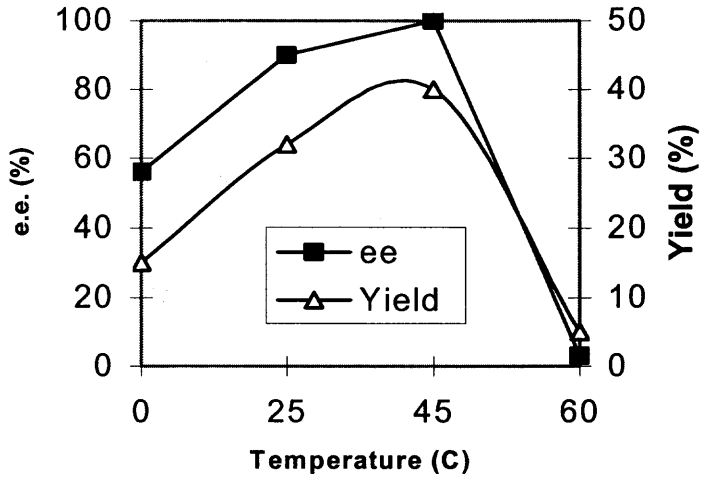



**Figure 3.20** Effect of reaction temperature on the kinetic resolution of DL-homophenylalanine ethyl ester in $[EtPy]^+[BF_4]^-$ + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 6 hrs).

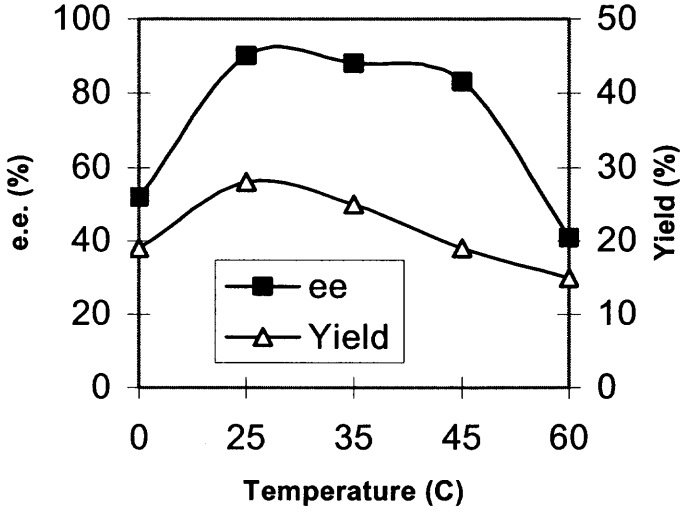



**Figure 3.21** Effect of reaction temperature on the kinetic resolution of DL-homophenylalanine ethyl ester in $[EtPy]^+[CF_3COO]^-$ + water (0.5 g ester, 85% $H_2O$, 0.5g $NaHCO_3$, 1.0 ml *BL*-alcalase, 6 hrs).

The enantioselectivity of the kinetic resolution reaction generally declines with the rise of reaction temperature. This phenomenon is consistent with the common understanding of enantiospecificity of enzymes: the enzyme enantioselectivity decreases

with increase of temperature. If the rate constant $k_S$ of the reaction leading to the *S* product is greater than $k_R$ leading to the *R* product, then the product with configuration *S* at the new stereogenic center will predominate, and vice versa. The enantiomeric ratio is simply the ratio of the rate constant:

$$\frac{[P_S^*]}{[P_R^*]} = \frac{k_S}{k_R} \tag{3.1}$$

The Arrhenius equation shows the relationship between the rate constant and the activation energy.

$$k_S = Ae^{E_S/RT} \qquad k_R = Ae^{E_R/RT} \tag{3.2}$$

Therefore,

$$\frac{[P_S^*]}{[P_R^*]} = e^{-(E_S - E_R)/RT} = e^{-\Delta E/RT} \tag{3.3}$$

The greater the difference in the activation energy of the two reactions, the greater the selectivity. When the activation energy of the reaction giving *S* product is the same as that of the reaction to the *R* product, there is no enantioselectivity, and the ratio is 1:1. There is no need for a very large difference in activation energy to achieve high selectivity. For example, at 300 K a difference of 2 kcal/mol (8.4 kJ/mol) produces an enantiomeric ratio of 96:4 (or 92% *ee*).

It is important to understand the dependence of the enantioselectivity on the reaction temperature. For a certain value of ΔE, lowering the temperature benefits higher selectivity, but also leads to a slower reaction rate (hence might cause decreased product

yield). Many asymmetric reactions are conducted well below the room temperature, in some cases as low as –120 °C, in order to obtain the maximum enantioselectivity.

In the current study, since the reaction medium does not change, the difference in the activation energy does not change either. With the increase of temperature, the enantiomeric ratio decreases according to the above equation. Lowering the temperature will lead to increased selectivity, but also cause slower reaction rate. Since room temperature can satisfy both requirements of enantioselectivity and reaction rate, also, no extra operation cost is needed for room temperature reaction, room temperature will be chosen as a reaction condition for the enzymatic resolution of amino acid esters in the current research.

### 3.5 Recovery of Ionic Liquids

After the reactions, the ionic liquids remained in the aqueous phase. Under vacuum and heating, water in the mixture was evaporated and ionic liquids were left behind due to their higher boiling point.

Table 3.4 illustrates the recovery percentage of ionic liquid $[EtPy]^+[CF3COO]^-$ and the effect of using recycled ionic liquid on the enzymatic resolution. The ionic liquid $[EtPy]^+[CF3COO]^-$ can be recycled at very high recovery efficiency (over 95%). The most important feature is that the recycled ionic liquid can be reused. There is no significant impact on the *ee* and yield of the enzymatic resolution using the recycled ionic liquid $[EtPy]^+[CF3COO]^-$. This also indicates that the operating cost of enzymatic resolution using an ionic liquid can be reduced due to its reusability; thus, ionic liquid $[EtPy]^+[CF3COO]^-$ may be considered as a part of "green" chemistry process.

**Table 3.4** Recovery of $[EtPy]^+[CF3COO]^-$ and Its Effect on Enzymatic Resolution (0.5g ester, 85% (volume) Water and 15% (volume) Solvent, 0.5g $NaHCO_3$, 1.0 ml *BL*-Alcalase, 25 °C, 24 hours)

| Run | Recovery percentage | e.e. (%) of L-enantiomer | Yield (%) based on L |
|---|---|---|---|
| 1 | 100% | 96.0 | 40.0 |
| 2 | 95% | 93.0 | 39.3 |
| 3 | 96% | 95.0 | 40.5 |

## 3.6 Summary

Kinetic resolutions of the homophenylalanine ethyl ester in three ionic liquid ($[EMIM]^+[BF_4]^-$, $[EtPy]^+[BF_4]^-$, and $[EtPy]^+[CF_3COO]^-$) solutions are compared with the results in the organic solvent acetonitrile. Systematic studies have been conducted on the kinetic resolution of *N*-acetyl homophenylalanine ethyl ester by varying reaction parameters, such as solvent concentration, reaction time and reaction temperature. The enzyme usually shows high enantioselectivity at a low concentration (15%) of ionic liquid in water; 24 hours reaction time would be sufficient to complete the enzymatic reaction; based on the experimental data and mechanistic considerations, room temperature will be generally used as the reaction temperature for kinetic resolutions. Among those ionic liquids studied, $[EtPy]^+[CF_3COO]^-$ has the best performance in achieving high *ee* and yield at the same time. High *ee* and yield achieved in ionic liquids indicate that ionic liquids can be an ideal substitute for organic solvents in the kinetic resolution of amino acid esters.

# CHAPTER 4

# ESTERIFICATION OF AMINO ACIDS USING AN IONIC LIQUID AS A "GREEN" CATALYST

## 4.1 Background Information

Amino acid esters are very important intermediates for the chemical and pharmaceutical industry (Sheldon, 1993). For instance, various methyl and ethyl esters of amino acids were resolved enzymatically in order to obtain chiral amino acids (Houng et al., 1996). D-phenylglycine and D-*p*-hydroxy-phenylglycine esters have been investigated in the enzymatic process to semi-synthetic penicillins and cephalosporins (Bruggink et al., 1998). Phenylalanine methyl ester was used in the Holland Sweetener Company (DSM-Tosoh joint venture) for enzymatic synthesis of the artifical sweetener aspartame (Hanzawa, 1999). This study also requires various amino acid esters for the purpose of enzymatic resolution.

The earliest method of esterifying amino acids was developed by Fischer (Jakubke and Jeschkeit, 1977). By continuously passing hydrogen chloride gas into ethanolic suspensions of amino acids, the amino acids dissolve and the solution becomes homogeneous. After adding base to the reaction mixture, the resulting ester hydrochlorides can be isolated and extracted with ether. In principle, this is not a very efficient method since it is an equilibrium reaction. However, it can be made useful by manipulation of the equilibrium. For example, using an excess of alcohol and removal of water as it is formed may force the reaction to the direction of forming esters. Another disadvantage of this method is the difficulty of handling toxic hydrogen chloride gas.

Another chemical method (Bodanszky and Bodanszky, 1984) of synthesizing amino acid esters is to form acyl halides by reacting amino acids with thionyl chloride

first. Then the appropriate alcohol is added to produce the ester. A facile synthetic method under mild conditions was studied via cesium salts to yield amino acid esters (Wang et al., 1977). This process not only involves expensive cesium salts, but also produces non-racemic amino acid esters (one enantiomer in rich).

Lipases have been used for esterification of acids for a long time (Langrand et al., 1986), however, attempts to esterify amino acids have failed (Kirchner et al., 1985). A commercially available enzyme, *papain* was extensively investigated for the esterification of *N*-protection amino acids (Chen and Wang, 1988; Cantacuzene and Guerreiro, 1989). The average yield usually ranges from 60 to 80%. However, the resulting esters were enriched in one of the enantiomers, which is undesirable for some applications.

Recently (Wegman et al., 2001), the acid form of ultrastable zeolite Y (H-USY) has been studied as a solid catalyst in the reaction of α-amino acids with methanol at 100-130 °C (15-20 bar). The yields of those amino acids were DL-homophenylalanine, 68%, D-phenylglycine, 86%, L-phenylalanine, 77%, and D-*p*-hydroxyphenylglycine, 14%. This method uses a catalyst that is complicated in preparation, and the reaction uses high pressure.

With the rapid applications of ionic liquids as novel solvents and catalysts in organic reactions (Welton, 1999), it would be fascinating to try ionic liquids as catalysts for the synthesis of organic esters. The potential advantages of applying ionic liquids in this process are: (1) mild condition will be necessary for the esterification reaction; (2) clean esters could be produced by using ionic liquids as environmentally friendly ("green") reaction media; (3) most of the resulting esters could easily be separated due to

their immiscibility with ionic liquids; (4) room temperature ionic liquids are usually moisture-stable and thermal-stable; therefore, they are suitable candidates as catalysts; (5) ionic liquids could be recovered and reused again. A representative esterification reaction of amino acid is shown in Figure 4.1 and the reaction process is presented in Figure 4.2.

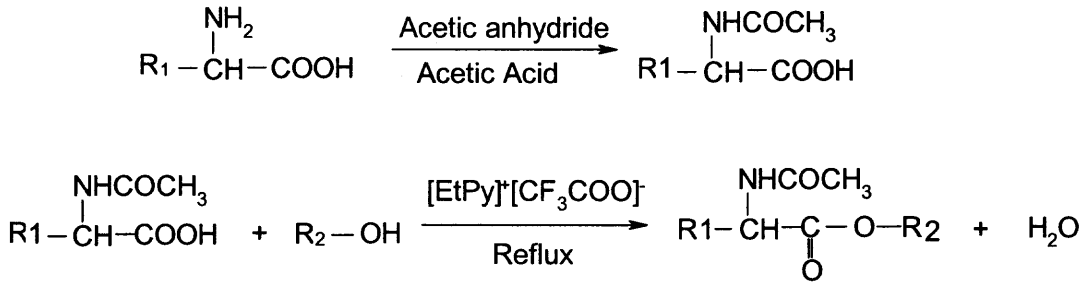



**Figure 4.1** Schema of acetylation and esterification of amino acid.

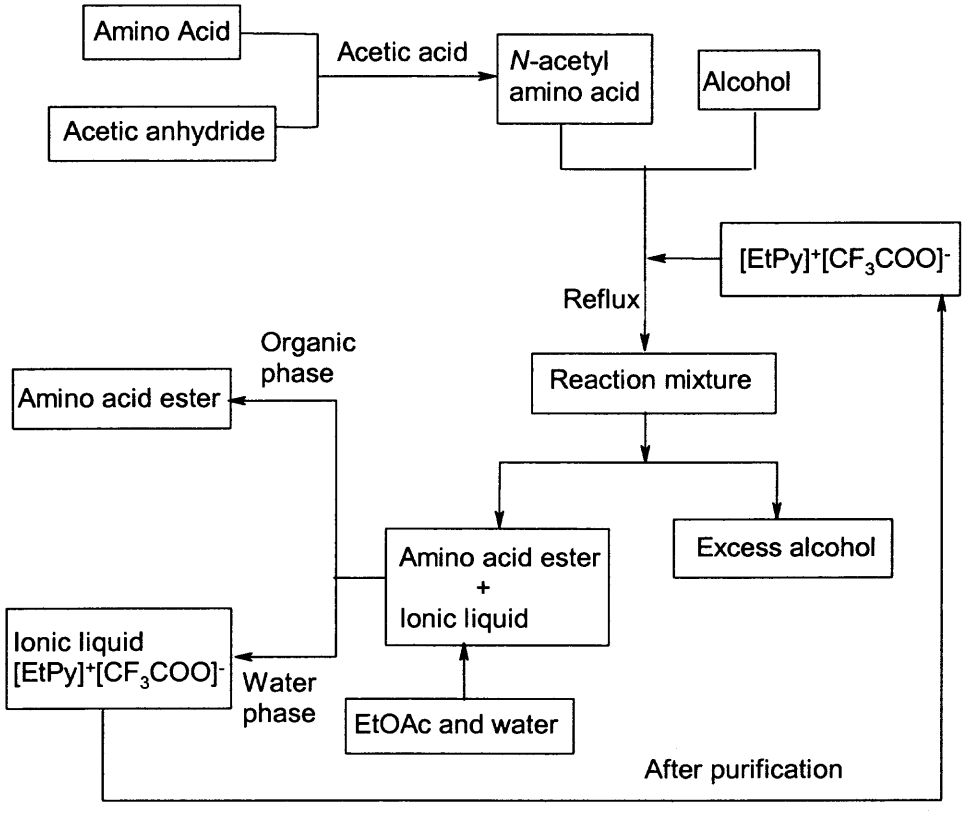



**Figure 4.2** Flow chart of esterification of amino acids catalyzed by $[EtPy]^{+}[CF3COO]^{-}$.

## 4.2 Materials and Methods

### 4.2.1 Materials

Amino acids, alcohols, acetic acid and acetic anhydride were purchased from Sigma-Aldrich. The preparation of the ionic liquid *N*-acetyl pyridinium trifluoroacetate ($[EtPy]^+[CF_3COO]^-$) was based on the literature method (Holbrey and Seddon, 1999).

### 4.2.2 Methods

The reaction mechanism of *synthesizing ionic liquid* $[EtPy]^+[CF_3COO]^-$ (structure of the cation in Figure 1.10) has been discussed in detail in Chapter 3. The following discusses experimental procedures for preparing this ionic liquid: Trifluoroacetic acid (13.3 ml, 0.1726 mol) was slowly added to a stirred slurry of silver (I) oxide (20.0 g, 0.0863 mol) in 50 ml distilled water for a period of 10 minutes. To avoid photodegradation of silver (I) oxide, the reaction mixture was fully covered with aluminum foil. Until the silver (I) oxide was completely reacted, a solution of *N*-ethyl-pyridinium bromide (32.46 g, 0.1726 mol) in 150 ml distilled water was added to the reaction mixture and stirred at room temperature for 2 hours. The yellow precipitate of silver (I) bromide was filtered off, and the solvent was removed at 65 °C under vacuum. The resulting salt is a brown liquid. Yield 30.1 ml, 91%. 1 g of charcoal was added to the ionic liquid solution and kept overnight to remove any color and impurities. Charcoal was then removed by filtration and water was evaporated under vacuum. The resulting liquid is colorless or light brown.

The *preparation of N-acetyl amino acids* followed a literature method (Chenault et al., 1989): 5.0 g of amino acid was suspended and stirred in 80 ml of glacial acetic acid, followed by adding 1.2 molar equivalent of acetic anhydride. The mixture was

stirred at room temperature until the solid disappeared. If the amino acid does not appear to react to form a homogeneous phase, a gentle heat is supplied. The solvent was removed by rotary evaporation under vacuum, and the residue was taken into acetone and filtered. Rotary evaporation of the filtrate gave the *N*-acetyl amino acid.

The general *esterification process* can be described as the following: 5 g of *N*-acetyl amino acid was dissolved in 100 ml of anhydrous alcohol, followed by adding 1 ml of the ionic liquid. The solution was stirred and refluxed for several hours. The reaction progress was monitoring by a Varian GC CP-3800. When the reaction was complete, the solvent was evaporated under vacuum by Rotavapor. The residue was dissolved in ethyl acetate and washed twice with distilled water. Rotary evaporation of the dried organic phase gave *N*-acetyl amino acid ester. If needed, the acetyl group was taken off from the amino acid by refluxing it in 3N HCl for ca. 3 hrs.

## 4.3 Results and Discussion

### 4.3.1 Reaction Strategy and Reaction Time

Esterification of amino acids is a difficult reaction to perform because amino acids exist as zwitterions (dipolar ions) and the carboxyl group is not a free group (it is an anion). Since the esterification reaction is an equilibrium process, factors that shift the reaction toward the products will benefit the formation of the amino acid ester. First, the purpose of acetylation of amino acids before the esterification reaction are: (1) to make the amino acid soluble in organic solvents like alcohol; (2) to free the carboxyl group by protecting the amino group; (3) to prevent possible polymerization and other side reactions caused by the amino group. This step effectively increases the concentration of amino acid in the

reaction phase which benefits the esterification reaction. Other amino protecting groups may be used if they can promote the esterification progress. Second, the alcohol is used in excess in order to push the equilibrium to the products. Third, the reaction system is initially anhydrous which helps to shift the equilibrium by reducing the water content in the reaction medium. However, studies to understand the reaction mechanism of using an ionic liquid as a catalyst in the esterification process is underway.

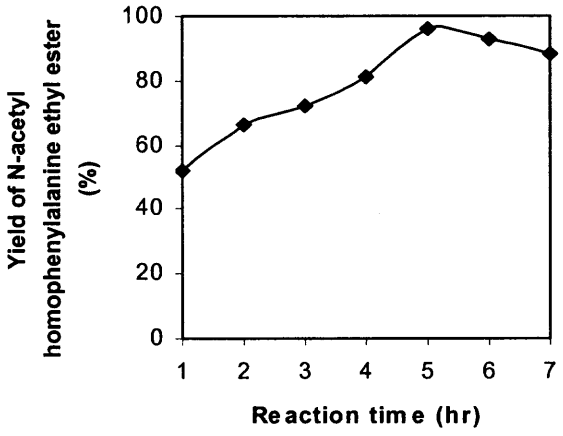



**Figure 4.3** Time course of synthesizing *N*-acetyl-homophenylalanine ethyl ester catalyzed by $[EtPy]^+[CF_3COO]^-$.

Figure 4.3 and 4.4 show the effect of refluxing time on the yield of amino acid ethyl esters *N*-acetyl homophenylalanine ethyl ester and *N*-acetyl 4-chlorophenylalanine ethyl ester, respectively. At first, increasing reaction times increases the reaction conversion as expected. However, extending the reaction time further decreases the product yield which may be understood as follows: (1) the esterification reaches an equilibrium state at a certain point; after that, further refluxing may cause the reaction to shift left; (2) side reactions may occur. Based on the amino acids studied in this work

(Table 4.1), approximately 3 to 5 hours refluxing time was sufficient for many amino acids to be completely consumed in the reaction.

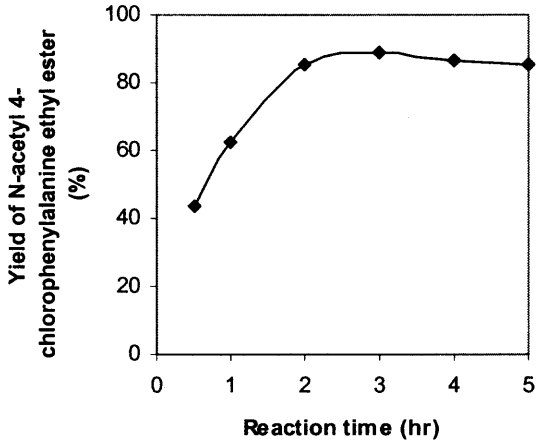



**Figure 4.4** Time course of synthesizing *N*-acetyl-4-chlorophenylalanine ethyl ester catalyzed by $[EtPy]^+[CF_3COO]^-$.

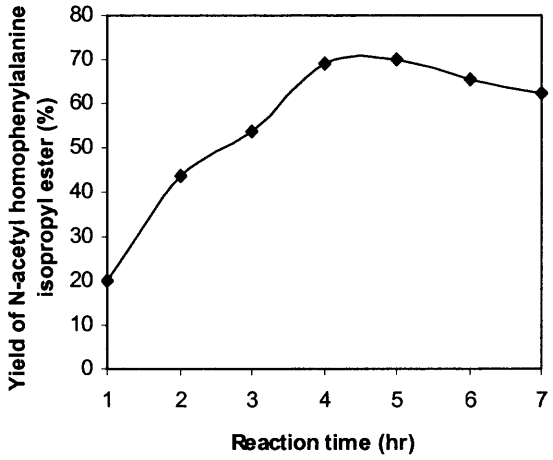



**Figure 4.5** Time course of synthesizing *N*-acetyl-homophenylalanine isopropyl ester catalyzed by $[EtPy]^+[CF_3COO]^-$.

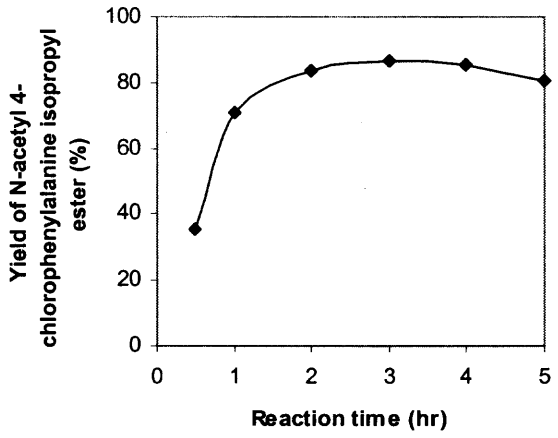
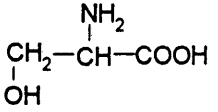
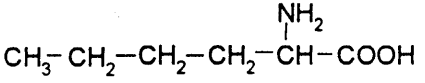
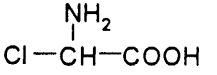
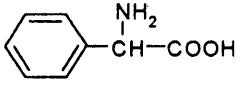
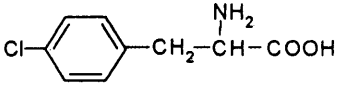
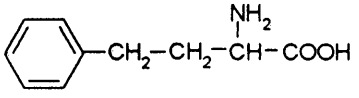
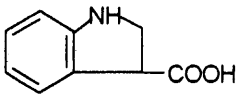
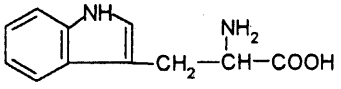



**Figure 4.6** Time course of synthesizing *N*-acetyl-4-chlorophenylalanine isopropyl ester catalyzed by $[EtPy]^+[CF_3COO]^-$.

The isopropyl esterifications of homophenylalanine and 4-chlorophenylalanine were also analyzed during the course of reactions, as illustrated in Figure 4.5 and 4.6 respectively. In general, the trends are similar to the time course of ethyl esterification. The reaction proceeded to an optimum conversion and thereafter the reverse reaction or side reactions take place.

**Table 4.1** Esterification of Amino Acids Using the Ionic Liquid (IL) $[EtPy]^+[CF_3COO]^-$ as A Green Catalyst

| Amino Acid | | Molar ratio of | Ethyl Ester | | Isopropyl Ester | |
|---|---|---|---|---|---|---|
| Name | Structure | amino acid/IL | Time (hr) | Yield (%) | Time (hr) | Yield (%) |
| Serine |  | 20/1 | 3 | 25.0 | 3 | 12.3 |
| Norleucine |  | 20/1 | 3 | 70.1 | 3 | 72.1 |
| 2-Chloroglycine |  | 18/1 | 3.5 | 93.2 | 3.5 | 51.9 |
| 2-Phenylglycine |  | 19/1 | 4 | 72.1 | 4 | 78.1 |
| 4-Chlorophenylalanine |  | 20/1 | 3 | 88.9 | 3 | 86.8 |
| Homophenylalanine |  | 19/1 | 5 | 76.9 | 5 | 70.2 |
| Indoline-2-carboxylic acid |  | 20/1 | 3 | 78.5 | 3 | 90.8 |
| Tryptophan |  | 19/1 | 3 | 94.8 | 3 | 54.4 |

### 4.3.2 Esterification of Different Amino Acids

Syntheses were conducted with ethyl and isopropyl alcohol for each amino acid. As listed in Table 4.1, the reaction time and conversion were given for each ester. The molar ratio of amino acid to ionic liquid $[EtPy]^+[CF_3COO]^-$ is about 20:1, which means that trace quantity of ionic liquid is needed for the esterification reaction. When the $R_1$ group in the amino acid (Table 4.1) is small, such as in serine, the conversion is low. While in norleucine, the bulkiness of $R_1$ increases, the yield of corresponding ester is improved. This could be due to: (1) the ionic liquid $[EtPy]^+[CF_3COO]^-$ shows a better catalytic effect on amino acids with large side chains; (2) the bulky group increases the homogeneity of the reaction system by increasing the solubility of amino acids and interaction with alcohol.

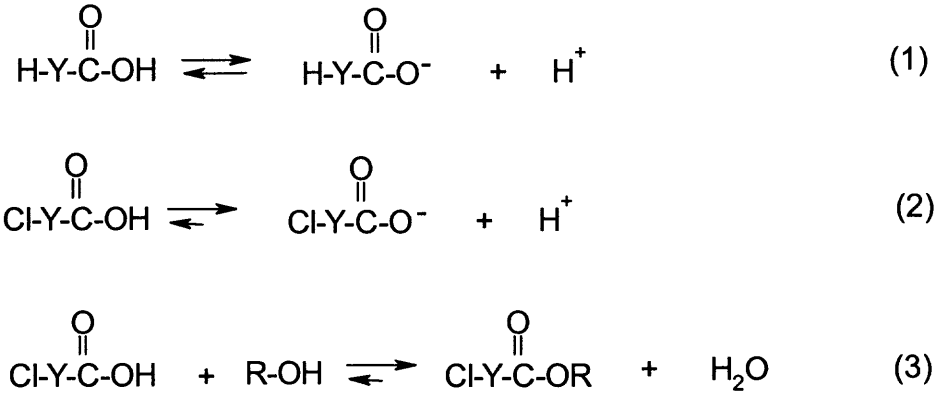



**Figure 4.7** Effect of halide substitute on the esterification of amino acid.

Comparing the results of 2-chloroglycine with serine, also 4-chlorophenylalanine with 2-phenylglycine and homophenylalanine, suggests that a halide substituting group, such as chloride, improves the substrate activity and therefore, increases the reaction conversion. By replacing the hydrogen by chloride, due to the strong electron

withdrawing ability of chloride, the acidity of the amino acids is increased which benefits the formation of esters (as shown in Figure 4.7).

Amino acids with side chains containing the benzyl and indoline groups usually show good performance in the esterification. In general, higher yield is obtained for ethyl esters compared with isopropyl esters. One exception is indoline-2-carboxylic acid, where the yield of isopropyl ester is much higher that of ethyl ester. It is also believed that the *N*-acetyl ethyl esters are more stable than the *N*-unprotected ethyl esters and butyl esters (Greenstein and Winitz, 1961), which implies that the *N*-acetyl ethyl esters studied are very valuable for further applications.

### 4.3.3 A Proposed Mechanism for the Ionic Liquid-Catalyzed Esterification

A mechanism for the esterification of amino acids catalyzed by an ionic liquid is presented in Figure 4.8. Initially, the cation in the ionic liquid $[EtPy]^+[CF_3COO]^-$ interacts with the carbonyl oxygen in the amino acid, which gives a delocalized carbocation and increases the reactivity of the carbonyl group. This makes the carbonyl carbon susceptible to the nucleophilic attack by an alcohol molecule. Proton transfer from the alcohol oxygen to a nearby oxygen in the hydroxyl group is a crucial step, because it can lead to two different ways. First, it can lose the alcohol molecule and reverse back to the amino acid. The second path is the elimination of water and the yield of the ester. All the steps are reversible, therefore, either excess use of alcohol or removal of water benefits the formation of the ester by shifting the equilibria.

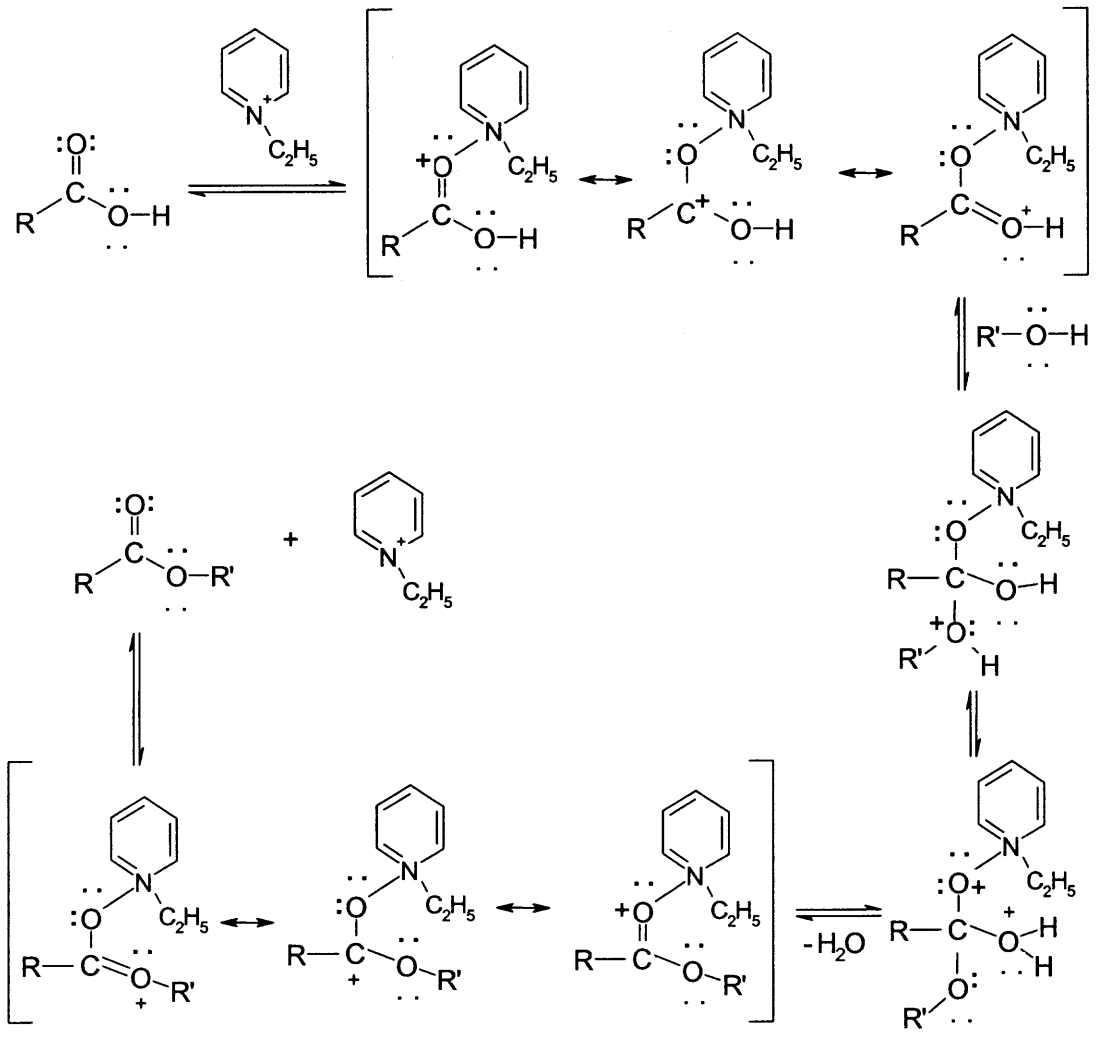


**Figure 4.8** A proposed mechanism for the esterification catalyzed by the ionic liquid $[EtPy]^+[CF_3COO]^-$.

## 4.4 Summary

A "green" catalyst, ionic liquid $[EtPy]^+[CF_3COO]^-$, was used for the first time for the synthesis of amino acid esters including unnatural amino acid esters. The reaction time for the esterification was monitored by GC. The data suggested that a reflux time of 3 to 5 hours would be sufficient for completing the esterification reaction. Further extending

the reaction time might cause the reverse reaction or side reactions, which decreased the product yield.

The results also indicate that under mild reaction conditions, satisfactory conversion can be achieved for the formation of amino acid esters. Compared to those esterification methods using enzymes, this straightforward reaction process provides racemic amino acid esters instead of those products with one enantiomer in enriched, which are more valuable for the pharmaceutical and chemical applications.

# CHAPTER 5

# ENZYMATIC RESOLUTION OF DIFFERENT AMINO ACID ESTERS USING AN IONIC LIQUID AS A "GREEN" SOLVENT

## 5.1 Background Information

Room-temperature ionic liquids have attracted tremendous attention in chemical transformations over the past few years (Welton 1999). More recently, ionic liquids have also been used as a reaction medium for enzymatic systems, such as lipase catalyzed kinetic resolution of 1-phenylethanol (Schofer *et al.* 2001), catalysis of formation of Z-aspartame (Erbeldinger *et al.* 2000), catalyzing alcoholysis, ammoniolysis and perhydrolysis reactions by lipase in ionic liquids 1-butyl-3methylimidazolium tetrafluoroborate or hexafluorophosphate (Lau *et al.* 2000), etc. More recently, ionic liquids were used as reaction media for butyl butyrate synthesis catalyzed by free *Candida antarctica* lipase B (Lozano *et al.* 2001). In this study, enhanced synthetic activity was observed in all ionic liquids in comparison with two organic solvents (hexane, and 1-butanol). Meanwhile, the continuous operation of lipase with all the assayed ionic liquids showed over-stabilization of the enzyme. In addition to the advantage of being environmental benign, ionic liquids could enhance the selectivity of biocatalysts (Kim *et al.* 2001). Due to those promising features of ionic liquids and high market values of chiral amino acids, a new ionic liquid *N*-ethyl pyridinium trifluoroacetate will be used for the kinetic resolution of amino acid esters and satisfactory results have been achieved by using this novel solvent. In this note, successful data were obtained to illustrate the effect of the ionic liquid on the enzymatic resolution of amino acid esters.

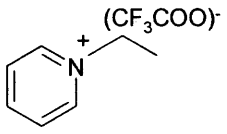



**Figure 5.1** Structure of ionic liquid $[EtPy]^+[CF_3COO]^-$.

## 5.2 Materials and Methods

### 5.2.1 Material Preparations

The ionic liquid *N*-ethyl pyridinium trifluoroacetate $[EtPy]^+[CF_3COO]^-$ ($[EtPy^+]$ = *N*-ethyl pyridinium) (Figure 5.1) was developed for this study. The preparation of this ionic liquid was based on the method of Holbrey and Seddon (1999). The enzyme *Bacillus licheniforms* alcalase (*BL*-alcalase) was produced by Novozymes and distributed by Sigma-Aldrich as a brown liquid with a specific activity of 2.4 AU/g for hydrolysis of dimethyl casein at 50 °C and pH 8.3. It was used without further purification. Porcine pancreas lipase (*PPL*), also named Lipase-3126, was obtained from Sigma-Aldrich as a crude powder. One unit of *PPL* will hydrolyze 1.0 microequivalent of fatty acid from a triglyceride in one hour at pH 7.4 and 37 °C. The *N*-acetyl homophenylalanine ethyl ester was prepared by three reaction steps as described previously (Zhao *et al.* 2002). Some *N*-protected amino acid esters were prepared based on the method in Chapter 4. Some other amino acid esters were purchased from Sigma-Aldrich and acetylated in the lab. The enantiomeric excess (*ee*) was calculated from the specific optical rotation which was measured by an AUTOPOL IV polarimeter from Rudolph Research Analytical Company. Also, the *ee* measurements were confirmed by HPLC with a Chiralpak WH column. The

yield calculation was based on the total racemic mixture, therefore, the maximum yield that one enantiomer can have is 50%.

### 5.2.2 Acetylation of Amino Acid Esters

The preparation of *N*-acetyl amino acid esters was based on a literature method (Chenault et al., 1989): 1.0 g amino acid ester was dissolved in 20 ml glacial acetic acid, followed by adding 1.2 molar equivalent of acetic anhydride. The mixture was stirred at room temperature for 1 hr. The solvent was removed by rotary evaporation under vacuum, and the residue was taken into acetone and filtered. Rotary evaporation of the filtrate gave *N*-acetyl amino acid ester.

### 5.2.3 General Method of Kinetic Resolution

Racemic amino acid ester 0.5g was suspended or dissolved in 60 ml mixed solvent of water (85% v/v) and ionic liquid. To this 0.5 g $NaHCO_3$ was dissolved in the reaction mixture while the pH of the solution was about 8. 2 ml *BL*-alcalase or 0.25 g *PPL* was added to the mixture. The reaction mixture was gently stirred at 25 °C for 24 hrs under a nitrogen atmosphere. The *N*-acetyl D-ester was extracted by ethyl acetate three times. 6 N HCl was added to the aqueous solution until the pH was lowered to 2-3. The remaining trace ethyl acetate in the aqueous solution was evaporated and the solution was further concentrated until the precipitate appeared. Removal of water gave *N*-acetyl L-acid. The *N*-acetyl D-acid was obtained by hydrolyzing *N*-acetyl D-ester in 6N NaOH solution for 2 hrs. The *N*-acetyl group in the L- and D-acid was taken off by refluxing in 3 N HCl for 3 hrs.

## 5.3 Results and Discussion

### 5.3.1 Effect of Ionic Liquid Concentration on the Kinetic Resolution

The reasons for acetylation of amino acid esters are: (1) making separation of L-amino acids from solution easier after the reaction is completed; (2) preventing possible polymerization and other side reactions caused by the amino group.

Ionic liquid concentration in the mixed solvent seems to be a strong factor influencing the kinetic resolution of amino acid esters. Figure 5.2 illustrates the effect of $[EtPy]^+[CF_3COO]^-$ concentration on the *ee* and yield of L-homophenylalanine. The *ee* of L-enantiomer declines with the increase of ionic liquid concentration. High concentration of ionic liquid causes high ionic strength of the reaction medium, which decreases the enantioselectivity of the enzyme. An increased concentration of ionic liquid increases the nonenzymatic hydrolysis of the ester. However, high yield of L-enantiomer occurs at 15% (v/v) of ionic liquid in water. This phenomenon indicates that the ionic liquid $[EtPy]^+[CF_3COO]^-$ can boost the activity of the enzyme *BL*-alcalase at a certain range of concentration.

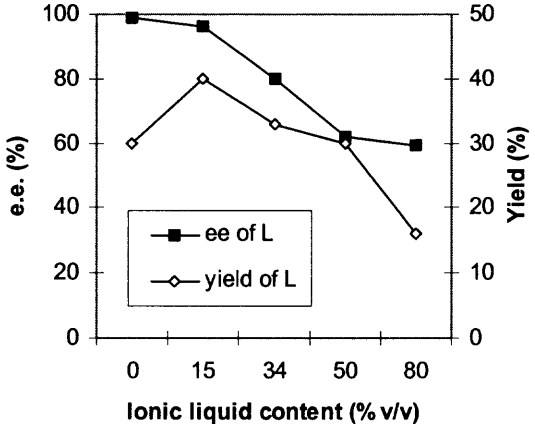


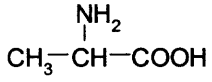
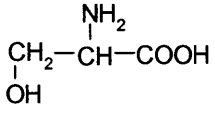
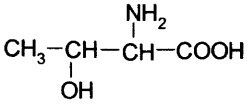
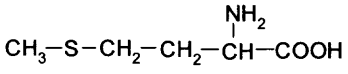
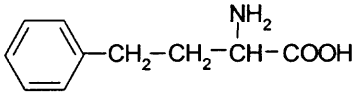
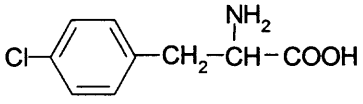
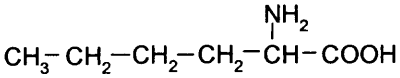

**Figure 5.2** Effect of ionic liquid $[EtPy]^+[CF_3COO]^-$ and water ratio on the kinetic resolution of *N*-acetyl DL-homophenylalanine ethyl ester (0.5 g ester, 60 ml mixed solvent, 0.5g $NaHCO_3$, 3.0 ml *BL*-alcalase, 25 °C, 24 hours).

### 5.3.2 Kinetic Resolution of *N*-acetyl Amino Acid Esters by the Enzyme *BL*-Alcalase

The data described above for the resolution of *N*-acetyl DL-homophenylalanine ethyl ester indicates that at low concentration of ionic liquid, the enzymatic resolution reaction may be boosted by using an ionic liquid due to its special stereo structure and possible interaction with substrate and enzyme. Due to pharmaceutical interest, various amino acids have been studied using this reaction strategy and the results are shown in Table 5.1. Both acetonitrile and the ionic liquid $[EtPy]^+[CF_3COO]^-$ were investigated as reaction medium for the kinetic resolution of various amino acid esters.

In general, an ionic liquid improves the optical purity and yield of the resulting L-amino acids by comparing the results with those from using the organic solvent acetonitrile. In the acetonitrile-water system, high *ee* was achieved for L-homophenylalanine and L-threonine, but the yield of L-threonine was very low (15%). For other amino acid esters, only moderate optical purity and yield were obtained while using acetonitrile as a solvent. In two cases, two L-amino acids (L-serine and L-4-chlorophenylalanine) were not achievable in acetonitrile + water solvent using the enzyme *BL*-alcalase. However, the resolution reactions were successful by using the ionic liquid $[EtPy]^+[CF_3COO]^-$ instead of acetonitrile. By comparing the results obtained for alanine, threonine and norleucine, the larger bulky side chain in amino acids seems beneficial to the high optical purity while the ionic liquid is used as reaction solvent. The reason could be the bulkiness of the side chain in amino acids might improve the specific interaction among substrate, enzyme and ionic liquid for L-isomer.

**Table 5.1** Kinetic Resolution of *N*-acetyl Amino Acids in $[EtPy]^+[CF_3COO]^-$ by *BL*-Alcalase (0.5 g ester, 0.5g $NaHCO_3$, 15% Solvent in Water, 3.0 ml *BL*-alcalase, 25 °C, 24 hours)

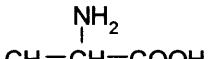
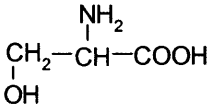
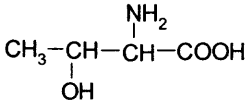
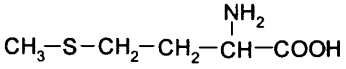
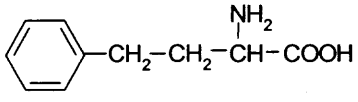
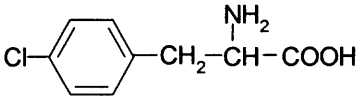
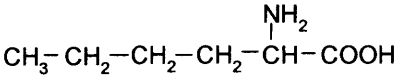
| Amino Acid | | Acetonitrile | | [EtPy][$CF_3COO$] | |
|---|---|---|---|---|---|
| Racemic Ester (each with *N*-acetyl) | Acid Structure | *ee* (%) | yield (%) | *ee* (%) | yield (%) |
| Alanine ethyl ester |  | 63 | 31 | 86 | 33 |
| Serine methyl ester |  | — | — | 90 | 35 |
| Threonine methyl ester |  | 92 | 15 | 97 | 36 |
| Methionine methyl ester |  | 83 | 30 | 89 | 29 |
| Homophenylalanine ethyl ester |  | 95 | 35 | 93 | 38 |
| 4-Chlorophenylalanine ethyl ester |  | — | — | 96 | 39 |
| Norleucine methyl ester |  | 18 | 32 | 88 | 30 |

### 5.3.3 Kinetic Resolution of *N*-acetyl Amino Acid Esters by the Enzyme *PPL*

Lipases have been widely investigated in the synthesis of chiral synthons and optically pure compounds including amino acids (Miyazawa, et al., 1989; Chiou, et al., 1992;

Houng et al., 1996). Among the various lipases, *PPL* was considered having the best performance on the enantioselective resolution of amino acids in general, especially for aromatic amino acids (Houng et al., 1996). Therefore, this study will conduct further research of kinetic resolution in $[EtPy]^+[CF_3COO]^-$ by using enzyme *PPL*.

**Table 5.2** Kinetic Resolution of *N*-acetyl Amino Acids in $[EtPy]^+[CF_3COO]^-$ by *PPL* (0.5 g ester, 0.5g $NaHCO_3$, 15% solvent in water, 3.0 ml *PPL*, 25 °C, 24 hours)

| Amino Acid | | Acetonitrile | | [EtPy][$CF_3COO$] | |
|---|---|---|---|---|---|
| Racemic Ester (each with *N*-acetyl) | Acid Structure | *ee* (%) | yield (%) | *ee* (%) | yield (%) |
| Alanine ethyl ester |  | 68 | 26 | 81 | 30 |
| Serine methyl ester |  | 35 | 21 | 78 | 28 |
| Threonine methyl ester |  | 36 | 19 | 89 | 24 |
| Methionine methyl ester |  | 62 | 31 | 86 | 29 |
| Homophenylalanine ethyl ester |  | 92 | 33 | 95 | 39 |
| 4-Chlorophenylalanine ethyl ester |  | 95 | 26 | 98 | 41 |
| Norleucine methyl ester |  | 18 | 32 | 73 | 30 |

The *ee* and yield of various L-amino acids were obtained by using the enzyme *PPL* in two different medium acetonitrile and ionic liquid $[EtPy]^+[CF_3COO]^-$ respectively (Table 5.2). Lipase *PPL* shows more specific resolution for amino acids with an aromatic side chain and has moderate resolution on other types of amino acids. Serine ester and 4-chlorophenylalanine ester have no significant resolution in acetonitrile when using the enzyme *BL*-alcalase, as shown in Table 5.1. While using the enzyme *PPL*, there are different degrees of resolution even in acetonitrile. The *ee* was 35% for L-serine and 95% for L-4-chlorophenylalanine.

In general, the ionic liquid $[EtPy]^+[CF_3COO]^-$ improves the optical purity and product yield on the kinetic resolution while using the enzyme *PPL*. Aromatic amino acids are also preferred for this enantioselective resolution using *PPL*. Other types of amino acids have moderate *ee* and yield compared with the results using the enzyme *BL*-alcalase. However, while dealing with amino acids with an aromatic side chain, *PPL* might become the first choice in conducting the enzymatic resolution either in organic solvents or ionic liquids.

## 5.4 Summary

The ionic liquid $[EtPy]^+[CF_3COO]^-$ was investigated as a "green" solvent in the enantioselective resolution of various amino acids. Improved optical purity and yield were generally achievable compared with reactions using the organic solvent acetonitrile. In summary, the ionic liquid $[EtPy]^+[CF_3COO]^-$ can be a good substitute for organic solvents used in kinetic resolution of *N*-acetyl amino acid esters. Using an ionic liquid

may enhance the enantioselectivity and activity of the enzyme, therefore, a highly enantiomeric amino acid may be obtained.

Two different industrial enzymes, *BL*-alcalase and *PPL*, were studied separately as a biocatalyst in the kinetic resolution of amino acid ester. Both enzymes maintain high catalytic activity and enantioselectivity at low concentration of ionic liquid. *BL*-alcalse is generally adaptable for various amino acids, but *PPL* has its advantage in the resolution of aromatic amino acids.

# CHAPTER 6

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

1. In this study, three different ionic liquids were prepared, including 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][$BF_4$]), *N*-ethyl pyridinium tetrafluoroborate ([EtPy][$BF_4$]) and *N*-ethyl pyridinium trifluoroacetate ([EtPy][$CF_3OO$]). They were purified and used as "green" solvents or catalysts in the kinetic resolution and esterification reactions.
2. L-(-)-piperazine-2-carboxylic acid was prepared with 98.1% *ee* and 40.6% resolution yield by several steps, including *N*-protection, esterification, and enzymatic resolution in an organic solvent using *Bacillus licheniforms* alcalase (*BL*-alcalase). Another important unnatural amino acid, *N*-acetyl homophenylalanine ethyl ester was synthesized by a three-step-reaction strategy. Further, L-(+)-homophenylalanine hydrochloride with 92.4% *ee* was obtained by kinetic resolution in acetonitrile-water mixture using the enzyme *BL*-alcalase.
3. Solvent effects on the kinetic resolution of *N*-acetyl homophenylalanine ethyl ester were systematically studied in several organic solvents and ionic liquids ([EMIM][$BF_4$], [EtPy][$BF_4$], and [EtPy][$CF_3OO$]) by using the enzyme *BL*-alcalase. It has been proved that a high concentration of ionic liquid can decrease the performance of enzyme, while a low content of ionic liquid might increase the activity of the enzyme *BL*-alcalase. The enzymatic resolution reaction was also studied in different reaction temperatures and reaction times. High *ee* and yield

achieved in ionic liquids indicate that ionic liquids can be an ideal substitute for organic solvents in the kinetic resolution of amino acid esters.

4. The ionic liquid [EtPy][$CF_3COO$], a "green" catalyst, was used first time in the synthesis of amino acid ester, including unnatural amino acid esters. Satisfactory conversion was achieved for the formation of amino acid esters under mild conditions. This straightforward process provided starting racemic amino acid esters for the kinetic resolution studies.
5. Furthermore, the ionic liquid [EtPy][$CF_3OO$] was applied as a "green" solvent for the kinetic resolution of several other *N*-acetyl amino acid esters using the enzyme *BL*-alcalase and *PPL*. High optical purity and yield were generally achievable under low concentration of ionic liquid. Lipase *PPL* shows high enantioselectivity on the aromatic type amino acids. It also implies that this method could be a general process in the production of chiral amino acids for pharmaceutical and biotechnology applications.

## 6.2 Recommendations

1. Systematic studies on kinetic resolution can be made on ionic liquids from various combinations of cations and anions. For example, by changing different *N*-substitute groups in the pyridinium cation, the resulting ionic liquids may have strong effects on enzyme activity and enantioselectivity.
2. Various enzymes may be investigated for the kinetic resolution reaction and their activity in ionic liquids can be compared.

3. More amino acids may be investigated systematically. The structure and other physical properties may have influences on the enzymatic resolution reactions. The mechanism of substrate-enzyme-solvent interaction could be explored when the structure-property relationship is fully understood.
4. The enzymatic resolution could be conducted on a larger scale in order to measure its adaptability as an effective chiral separation method in the chemical and pharmaceutical industry.

## APPENDIX A

## SOME CONCEPTS IN CHIRAL CHEMISTRY

The following terms are the most often used concepts in asymmetric chemistry:

1. **CHIRAL**: C*hirality* is a fundamental asymmetry property of three-dimensional objects. An object is *chiral* if it can not be superimposed upon its mirror image.
2. **ENANTIOMER**: Many compounds may be obtained in two different forms in which the molecular structures are constitutionally identical but differ in the three-dimensional arrangement of atoms such that they are related as mirror images. In such a case the two possible forms are called *enantiomers* and are said to be *enantiomeric* with each other.
3. **ENANTIOMERIC EXCESS (*ee*)** is defined as the proportion of the major enantiomer less that of the mirror enantiomer and is commonly expressed as a percentage.
4. **DIASTEREOMER** is a chiral molecule with more than one stereogenic unit (center, axial and planar).
5. **SPECIFIC ROTATION** is defined as

$$[\alpha]_{\lambda}^{t} = 100\ \alpha'/l\ c$$

where $\alpha'$ is observed rotation, $l$ is the cell path length in dm, $c$ is the concentration of sample in g per 100 $cm^3$, $t$ is temperature is Celsius, and $\lambda$ is the wavelength of incident light (nm).

6. **RACEMATE (or RACEMIC MIXTURE)** is corresponds to a 1:1 mixture of enantiomers with *ee* of 0%.

## APPENDIX B

## APPROVED DESCRIPTIVE TERMS IN CHIRAL CHEMISTRY

The following is a summary of approved terms for describing chiral molecules:

| For description of: | Approved terms | Older or disfavored terms |
|---|---|---|
| Enantiomers | (+)-/(-)- | *d/l* |
| Enantioselectivity | % *ee* | |
| Diastereoselectivity | % *de* or diastereomer ratio | % *ds* |
| Absolute configuration | *R/S* | *D/L* |
| Relative configuration | *l/u*, or *R*, R*/R*, S**, etc. | *erythro/threo* |
| Enantiotopic faces or groups | *Re/Si* | *proR/proS* |
| Topicity of enantioselective reactions | *Re/Si* | |
| Relative topicity of diastereoselective reactions | *lk/ul* | |

## APPENDIX C

## LIST OF ABBREVIATIONS

The following is a list of abbreviations used in the previous chapters:

**ACE** — angiotensin-converting enzyme

***BL*-alcalase** — *Bacillus licheniforms* alcalase

**BMIM** — 1-butyl-3-methylimidazolium

**BOC** — Butoxycarboxyl

**CE** — Capillary Electrophoresis

**CFPA** — α-cyano-α-fluorophenylacetic acid

**EMIM** — 1-ethyl-3-methylimidazolium

**EtPy** — *N*-ethyl pyridinium tetrafluoroborate

**FLEC** — 1-(9-fluorenyl)-ethyl chloroformate

**MEKC** — micellar electrokinetic chromatography

**MEUF** — Micellar–enhanced ultrafiltration

**MTPr** — α-methoxy-α-trifluoromethylpropionic acid

**OTC** — over-the-counter

**PAL** — L-phenylalanine ammmoniolyase

**PEG** — polyethylene glycol

**PIPA** — prefluoro-2-isopropoxypropionic acid

***PPL*** — porcine pancreas lipase

**PPPA** — perfluoro-2-propoxypropionic acid

## APPENDIX D

## 12 PRINCIPLES OF "GREEN" CHEMISTRY

"Green" Chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products (Anon, 2000). "Green" solvents are generally environmentally friendly, recyclable and reusable, and they do not contaminate the products. The 12 principles of "green" chemistry (Anastas and Warner, 1998) have been proposed by the American Chemical Society and are listed in the following.

**1. Prevention**

It is better to prevent waste than to treat or clean up waste after it has been created.

**2. Atom Economy**

Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

**3. Less Hazardous Chemical Syntheses**

Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

**4. Designing Safer Chemicals**

Chemical products should be designed to affect their desired function while minimizing their toxicity.

**5. Safer Solvents and Auxiliaries**

The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

**6. Design for Energy Efficiency**

Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

**7. Use of Renewable Feedstocks**

A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

**8. Reduce Derivatives**

Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

**9. Catalysis**

Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

**10. Design for Degradation**

Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

**11. Real-time analysis for Pollution Prevention**

Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

**12. Inherently Safer Chemistry for Accident Prevention**

Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

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