UNIVERSITI PUTRA MALAYSIA

FACILE SYNTHESIS, CHARACTERIZATION AND BIOCATALYTIC APPLICATION OF IMIDAZOLIUM-BASED CHIRAL IONIC LIQUIDS

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FP 2008 25

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2008

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By

NG SHIE LING

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Master of Science
MARCH 2008

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science.

FACILE SYNTHESIS, CHARACTERIZATION AND BIOCATALYTIC APPLICATION OF IMIDAZOLIUM-BASED CHIRAL IONIC LIQUIDS

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Chairperson: Mohd. Basyaruddin Abdul Rahman, PhD.

Faculty: Science

In view of the emerging importance of ionic liquids as reaction media in organic synthesis, researchers have recently focused on the synthesis of chiral ionic liquids (CILs) for their particularly potential applications to chiral discrimination. The study of the application of CILs in asymmetric synthesis is not only an opportunity but also a challenge for researchers. Herein, the synthesis of new CILs based on alkyl-imidazole as the cation and four different chiral acids as the anion were reported.

Eighteen chiral ionic liquids were synthesized and characterized by a variety of physico-chemical techniques. Four different chiral acids chosen were L-lactic acid, L-tartaric acid, (R)-(-)-camphor-10-sulfonic acid and L-malic acid. Imidazole was chosen because they are easier to prepare and less toxic compared to thiazole and pyrrolidine which contain sulfur and nitrogen compounds respectively. These salts were prepared using simple ion-exchange reaction which gave good overall yield (> 95 %) at room temperature. All the CILs synthesized are hygroscopic. Their enantiomeric purity was analyzed using ^{1}H NMR spectroscopy. The effect of alkyl

substituents bonded to the nitrogen on imidazolium cation on the physical properties especially its melting point was also examined and observed. The melting points for bulkier ionic liquids are higher as compared to those of small ionic liquids. For the solid CILs, their solubility in organic solvents were tested and followed by recrystallization. Their three dimensional network of cation-anion and hydrogen bonding were analyzed by single-crystal X-ray diffraction analysis. Each CILs optical polarity was measured using polarimeter.

An example of the application of CILs is in biocatalysis. Chiral ionic liquid coated-enzyme (CILCE) was prepared by coating *Candida rugosa* lipase with 1-hydrogen-3-hexylimidazolium hydrogen-tartrate. CILCE was then used to catalyze some non-chiral and chiral esterification reactions. For non-chiral esterification, we found that CILCE gave higher percentage of conversion compared to native enzyme for short and medium chain acids, where all acids were reacted with oleyl alcohol. For chiral esterification, enantioselective esterification of (±)-menthol with butyric anhydride was studied. Percentage conversion of menthyl butyrate in CILCE (81.91 %) was better than in CRL (28.19 %). However, its enantiomeric excess (ee) from CILCE was low compared to CRL with 1.1 and 3.3, respectively calculated from its enantiomeric value, E.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Master Sains.

FASIL SINTESIS, PENCIRIAN DAN APLIKASI DALAM BIOMANGKIN BAGI CECAIR IONIK KIRAL YANG BERDASARKAN IMIDAZOLIUM.

Oleh

NG SHIE LING

MARCH 2008

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Dewasa ini, kepentingan cecair ionik sebagai media tindak balas dalam sintesis organik semakin berkembang. Kini, para penyelidik mula memfokuskan kepada sintesis cecair ionik kiral (CILs) yang berpotensi dalam aplikasi diskriminasi kiral. Kajian aplikasi CILs dalam sintesis asimetri bukan hanyalah satu peluang malah juga adalah satu cabaran bagi para penyelidik. Dalam kajian ini, sintesis CILs baru yang berasaskan alkil-imidazol sebagai kation dan pelbagai asid kiral sebagai anion dilaporkan.

Lapan belas CILs telah disintesis dan dicirikan menggunakan pelbagai teknik kimia-fizikal. Empat asid kiral berlainan yang dipilih adalah L-asid laktik, L-asid tartarik, (R)-(-)-asid camphor-10-sulfonik dan L-asid malik. Imidazol telah dipilih kerana ia mudah disediakan dan kurang toksik jika dibandingkan dengan thiazol dan piroldinium yang mengandungi sulfur dan nitrogen. Kesemua garam ini telah disediakan menggunakan kaedah tindak balas penukargantian-ion mudah yang memberikan hasil yang tinggi (> 95 %) pada suhu bilik. Kesemua cecair ionik kiral

yang disintesis adalah higroskopik. Ketulenan enantiomerik CILs dicirikan menggunakan ^{1}H spektroskopi magnetik resonan nuklear (NMR). Kesan penukargantian alkil dalam ciri-ciri fizikal khususnya terhadap takat leburnya juga telah diuji dan diperhatikan. Takat lebur bagi cecair ionik yang lebih besar saiznya adalah lebih tinggi jika dibandingkan dengan cecair ionik yang lebih kecil. Bagi cecair ionik pepejal, keterlarutan dalam pelarut organik diuji, diikuti oleh penghabluran semula. Rangkaian tiga dimensi antara kation-anion dan ikatan hidrogen dianalisis menggunakan X-ray serakan kristal tunggal. Polariti optik bagi setiap cecair ionik diukur dengan menggunakan polarimeter.

Salah satu contoh aplikasi CILs adalah dalam biopemangkinan. Enzim bersalut cecair ionik kiral (CILCE) disediakan dengan menyalut lipase *Candida rugosa* dengan 1-hidrogen-3-heksilimidazolium hidrogen-tartrat. CILCE kemudiannya digunakan untuk memangkin beberapa tindak balas pengesteran mudah tak-kiral dan kiral. Bagi pengesteran tak-kiral, CILCE memberikan peratusan penukaran yang lebih tinggi berbanding enzim asli bagi asid berantai pendek dan sederhana, di mana semua asid ditindak balaskan dengan olil alkohol. Bagi pengesteran kiral, pengesteran enantioselektif bagi (±)-menthol dengan butirik anhidrida telah dikaji. Peratus penukaran menthil butirat dalam CILCE (81.41 %) adalah lebih bagus daripada dalam CRL (28.19 %). Walau bagaimanapun, lebihan enantiomerik, (ee) daripada CILCE adalah rendah berbanding CRL dan masing-masing memberikan 1.1 dan 3.3 bagi nilai enantiomeriknya, E.

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my heartfelt thanks to both of my supervisors, Associate Professor Dr. Mohd. Basyaruddin Abd Rahman from Universiti Putra Malaysia and Professor Kenneth Richard Seddon from Queen's University of Belfast, for their help, advice and tireless encouragement throughout this period of study. I would also like to thank them for giving me the opportunity to come to Belfast; a small city with cold weather, but very warm people.

To my research group, CREAM in UPM, thank you all especially to Professor Abu Bakar Salleh and Professor Mahiran Basri for your valuable advice and suggestions you gave during our weekly meeting. I would also like to express my deepest gratitude to Professor Jim Swindall OBE and Dr. Sarah Thompson for all the paperwork that they had to deal with in order to get me there to Belfast. I really appreciate it. And of course, thanks too, to both super-secretaries, Deborah Poland and Louis Porter.

To all my friends in Malaysia, thanks for always being there for me. I want to thank all of you for the good time we shared together. Your names alone would fill the rest of this thesis, and more importantly, would put me in very difficult position of having to decide on an order of preference. And to Nora, I could never thank you enough for extending a helping hand with all my leftover work during the period when I was in Belfast. Without you, I would not have made it this far.

Special thanks go to Isaac Nyambiya for all his guidance, patience and fruitful chemistry discussions. None of this could have happened without you. To all the

QUILL labmates; Alina, Natasha, Gosia, Geetha, Lynsey, Zheng Xi, Jamie, Laurent, Kris, Marcus, Manuella, Tayeb, Martin, Martyn Earle and Angela, thank you for all your help, support and also the lovely coffee breaks we had together at 12 noon everyday. They were the best! And also to the many people who has already left QUILL, especially Alberto, José Santiago, Marianna and my Scottish buddy, Craig, thank you so much for making my stay at QUILL so enjoyable.

To my Belfast best friend and labmate, Monica Sanches, you will always have a special place in my heart. I will always remember our 3 hours lunch break that ended up in the city centre and all the traveling we did together these past few months. And because of you, I got to meet some of the coolest people on Earth, the IAESTE bunch. To all my IAESTE buddies; Jacobo, Katherina, Stephanie, Anna, Sylvia, Athula, Robin, Diana, Sabrina, Anna, Ewa, Monica (Poland), Qasha, Tom, Sebastian, Manuel, Jørgen, Manuel, Karun, Maria (Austria), Maria (Malta) and Ben, thank you for the lovely times we have had together, for being my friends, for all the trips that we made together and most importantly, for the many experiences that have taught me to be a better person. You are really a wonderful group of people. A million thanks also go out to all my flat-mates and friends in Guthrie House for their food sharing and the party.

Last but definitely not least, to my family, especially my mom, there is no way I can adequately express my gratitude in words, for the support, love and encouragement you have shown me throughout the years and for the many sacrifices that you have made for me. I will always cherish it in my heart and soul.

I love you all.

I certify that an Examination Committee met on 24 March 2008 to conduct the final examination of Ng Shie Ling on her Master of Science thesis entitled "Facile Synthesis, Characterization and Biocatalysis Application of Imidazolium-Based Ionic Liquids" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the degree of Master of Science, MSc.

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The thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been acknowledged. I also declare that is has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NG SHIE LING

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

ILs	Ionic liquids
RTIL	Room-temperature ionic liquid
CILs	Chiral ionic liquids
$[H_2\text{-im}]^+$	1,3 dihydrogenimidazolium
$[H\text{-mim}]^+$	1-hydrogen-3-methylimidazolium
$[H\text{-bim}]^+$	1-hydrogen-3-butylimidazolium
$[H\text{-him}]^+$	1-hydrogen-3-hexylimidazolium
$[bmim]^+$	1-butyl-3-methylimidazolium
$[emim]^+$	1-ethyl-3-methylimidazolium
$[lac]^-$	Lactate
$[tar]^-$	Tartrate
$[H\text{-tar}]^-$	Hydrogen-tartrate
$[sulf]^-$	Camphor-10-sulfonate
$[mal]^-$	Malate
$[H\text{-mal}]^-$	Hydrogen-malate
$[BF_4]^-$	tetrafluoroborate
$[PF_6]^-$	hexafluorophosphate
$[Tf_2N]^-$	bis(trifluoromethylsulfonyl)imide
$[Ms_2N]^-$	bis(methanesulfonyl)imide
DABCO	1,4-diazabicyclo[2.2.2]octane
VOC	Volatile organic compounds
ee	Enantiomeric excess
E	Enantiomeric ratio

ATRP	Atom transfer radical polymerization
MMA	Methyl methacrylate
DMSO	Dimethyl sulphoxide
DABCO	1,4-diazabicyclo[2.2.2]octane
TBAB	Tetrabutylammonium bromide
PTFE	Polytetrafluoroethylene
CILCE	Chiral ionic liquid-coated enzyme
ILCE	Ionic liquid-coated enzyme
CRL	*Candida rugosa* lipase
CHNS	Carbon hydrogen nitrogen sulphur
DSC	Differential scanning calorimetry
TLC	Thin layer chromatography
GC	Gas chromatography
QUILL	Queen's University Ionic Liquid Laboratory
ASEP	The Analytical Services and Environmental Projects Unit

LIST OF APPENDICES

CHAPTER 1

1. INTRODUCTION

As we advanced into the 21st century, it has become more evident that chiral drugs have become a major focus of most pharmaceutical companies. The increasing demand for many organic esters and chiral drugs creates the need for developing highly specific catalyst and for this reason, the application of enzymes as the catalyst for the synthesis of esters is undergoing rapid development (Garcia *et al*., 1996; Gryglewicz, 2003). Enzymatic synthesis offers various advantages over chemical synthesis such as lower energy requirement and enhanced selectivity and quality of the product. Moreover, enzymes especially lipases have high specificity towards ester bond and hence eliminating the presence of undesirable side-reactions and by-products in esterification reaction (Yadav and Devi, 2003).

Chiral analysis is an important subject in science as well as in technology. Enantiomeric forms of many compounds are known to have different psychological and therapeutic effects. Very often, only one form of an enantiomeric pair is pharmacologically active. The other form of the same enantiomeric pair can reverse or otherwise limit the effect of the desired enantiomer. Recognizing the importance of chiral effects, the Food and Drug Administration (FDA) in 1992 issued a mandate requiring pharmaceutical companies to verify the enantiomeric purity of chiral drugs that are produced. It is, thus, hardly surprising that the pharmaceutical industry needs effective methods to determine enantiomeric purity and high enantiomeric excess (ee) (Tran *et al.*, 2006).

Room temperature ionic liquids (ILs) are novel and promising materials for a variety of chemical applications. They have unique chemical and physical properties; including possessing a high solubility power and virtually no vapour pressure (Earle *et al.*, 2000 and Earle and Seddon, 2000). Because of these two properties, they can serve as an alternative to the volatile organic compounds that are traditionally used as industrial solvents. In view of the emerging importance of ILs as reaction media in organic synthesis, researchers have focused on the synthesis of chiral ionic liquids (CILs) for their particularly potential applications to chiral discrimination, including asymmetric synthesis and optical resolution of racemates (Wang *et al.*, 2005). Chiral ionic liquids therefore may offer a solution for determining enantiomeric purity and high ee in asymmetric reactions.

The popularity stems from the fact that it is possible to use CILs to replace the organic solvent for the enantiomeric purity determination method. Specifically, the CILs with its high solubility power should dissolve many different types of analytes (Zhao and Malhotra, 2002). Its chirality may produce the needed diastereomeric interactions with both enantiomeric forms of an analyte. Unfortunately, despite their potentials, CILs have been synthesized and the synthesis of reported CILs required rather expensive reagents and elaborates synthetic schemes. Because of these limitations, the study and applications of CILs have been severely hindered (Tran *et al.*, 2006). Therefore, it is of particular importance to develop a novel synthesis by which CILs can be simply and easily prepared from commercially available reagents by researchers.