UNIVERSITI PUTRA MALAYSIA

SYNTHESIS AND CHARACTERIZATION OF NEW TETRAETHYLAMMONIUM-BASED CHIRAL IONIC LIQUIDS FOR BIOCATALYSIS APPLICATION

KHAIRULAZHAR BIN JUMBRI

FS 2009 43

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

SYNTHESIS AND CHARACTERIZATION OF NEW TETRAETHYLAMMONIUM-BASED CHIRAL IONIC LIQUIDS FOR BIOCATALYSIS APPLICATION

By

KHAIRULAZHAR BIN JUMBRI

November 2009

Chairman : Mohd Basyaruddin Abdul Rahman, PhD

Faculty : Science

Chiral ionic liquids (CILs), molten salts at temperature below than 100 ºC are unique liquids having different characteristics from ordinary liquids. Since these CILs are prepared by coupling various organic ions, there are many chances to create novel functionalities by changing structure of components ions. These liquids combine with their ability to perform a task with their "green" character, which makes them environmental friendly solvents. Lately, researchers focused to synthesize CILs for their potential applications in many reactions such as chiral discrimination.

In this study, eleven new CILs derived from chiral amino acids and plant acid have been synthesized and characterized. They are tetraethylammonium L-serinate, tetraethylammonium L-prolinate, tetraethylammonium L-threoninate, tetraethylammonium L-isoleucinate, tetraethylammonium L-asparaginate, tetraethylammonium L-glutaminate, tetraethylammonium L-glutamate, tetraethylammonium L-methioninate, tetraethylammonium L-histidinate, tetraethylammonium L-lysinate and

tetraethylammonium L-malate. Meanwhile, one compound derived from chiral plant acids (tetraethylammonium L-tartarate) can't be classified as ILs due to high melting point above 100 °C.

All of these salts were prepared using simple neutralization reaction which gave good overall yield (>85 % yields for CILs derived from amino acids and 98 % for tetraethylammonium L-malate) at room temperature. ^{1}H NMR and elemental analysis were carried out to identify the molecular structure and purity of CILs produced. Colour of each CILs's produced depending on the anions used. All CILs were hygroscopic and easily dissolved in water and polar organic solvents. The new CILs synthesized from plant acid have higher melting point (T_m 87.7 ± 0.4 °C) compared to CILs derived from amino acids (T_m 54.3 ± 1.0 °C for tetraethylammonium L-histidinate and T_m 58.7 ± 1.0 °C for tetraethylammonium L-asparaginate).

The thermal properties were studied by using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC analysis showed that CILs in a liquid form had no melting point (T_m) and glass transition temperature (T_g). Meanwhile, in the TGA study, it was found that CILs derived from amino acids have a slightly low decomposition temperature (T_{onset} 168 to 210 °C) compared to tetraethylammonium L-malate (T_{onset} 210 °C), but all CILs were stable up to 160 °C.

Single crystal X-ray diffraction was used to solve the crystal structures of tetraethylammonium L-malate and tetraethylammonium L-tartarate. The crystal systems for both compounds were monoclinic. Analysis also carried out in order to reveal an extensive series of hydrogen bonds between H-atoms on the cation and the anion meanwhile each CILs' optical polarity was measured using polarimeter. Their viscosity

and ionic conductivity for CILs in a liquid form were also determined and observed by using viscometer and conductivity meter. The strong correlation between viscosity and ionic conductivity was observed.

In biocatalysis application, chiral ionic liquid-coated enzyme (CILCE) was employed in the esterification of oleyl alcohol with various fatty acids. CILCE was prepared by simple method involving coating of *Candida rugosa* lipase with tetraethylammonium L-asparaginate and was found to give a better percentage of conversion of ester (60 – 81 %) compared to native enzyme (36 – 70 %) for all fatty acids from short, medium and long alkyl chains.

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

SINTESIS DAN PENCIRIAN CECAIR IONIK KIRAL YANG BAHARU BERASASKAN TETRAETILAMONIUM UNTUK APLIKASI BIOMANGKIN

Oleh

KHAIRULAZHAR BIN JUMBRI

November 2009

Pengerusi : Mohd Basyaruddin Abdul Rahman, Ph.D

Fakulti : Sains

Cecair ionik kiral (CILs), garam lebur pada suhu di bawah 100 ºC adalah cecair unik yang mempunyai ciri yang berbeza daripada cecair biasa. Disebabkan cecair ini disediakan melalui pemadanan pelbagai ion organik, terdapat banyak kemungkinan untuk mencipta fungsi CILs yang baharu dengan menukar struktur komponen ion. Cecair ini digabungkan dengan kemampuannya untuk melakukan tugas dengan ciri "hijaunya" yang mana membuatkan cecair ini pelarut yang mesra alam. Kebelakangan ini, ramai penyelidik menumpukan perhatian terhadap sintesis CILs untuk melihat potensi kegunaannya di dalam banyak tindak balas seperti diskriminasi kiral.

Dalam kajian ini, sebelas cecair ionik kiral baharu yang berasal dari kiral asid amino dan asid tumbuhan telah disintesis dan dicirikan. Sebatian tersebut adalah tetraetilamonium L-serinat, tetraetilamonium L-prolinat, tetraetilamonium L-threoninat, tetraetilamonium L-isoleucinat, tetraetilamonium L-sasparaginat, tetraetilamonium L-glutaminat, tetraetilamonium L-glutamat, tetraetilamonium L-methinonat, tetraetilamonium L-histidinat, tetraetilamonium L-lysinat dan tetraetilamonium L-malat. Sementara itu, satu

sebatian lain yang disintesis daripada kiral asid tumbuhan (tetraetilamonium L-tartarate) tidak diklasifikasikan sebagai cecair ionic kiral kerana mempunyai takat lebur melebihi 100 °C.

Kesemua garam CILs ini telah disediakan menggunakan tindak balas penulenan mudah yang memberikan hasil keseluruhan yang baik (>85 % hasil untuk CILs yang berasal daripada kiral asid amino dan 98 % hasil untuk tetraetilamonium L-malat) pada suhu bilik. ^{1}H resonan magnetic nuklear (RMN) dan analisis elemen dilakukan untuk mengenalpasti struktur molekul dan ketulenan CILs yang dihasilkan. Warna untuk setiap CILs yang terhasil adalah bergantung kepada anion yang digunakan. Semua CILs didapati bersifat higroskopik dan mudah larut di dalam air dan pelarut organik polar. Satu CILs baharu yang disintesis daripada kiral asid tumbuhan mempunyai takat lebur yang tinggi (T_m 87.7 ± 0.4 °C) berbanding CILs yang berasal daripada kiral asid amino (T_m 54.3 ± 1.0 °C untuk tetraetilamonium L-histidinat dan T_m 58.7 ± 1.0 °C untuk tetraetilamonium L-asparaginat).

Sifat termal telah dipelajari menggunakan kalorimeter pengimbasan kebezaan (DSC) dan analisis thermogravimetrik (TGA). Analisis DSC menunjukkan CILs dalam bentuk cecair tidak mempunyai takat lebur (T_m) dan suhu peralihan gelas (T_g). Sementara itu, dalam kajian TGA, didapati bahawa CILs yang berasal daripada kiral asid amino mempunyai suhu penguraian yang sedikit rendah (T_{onset} 168 hingga 210 °C) berbanding tetraethylammonium L-malate (T_{onset} 210 °C), tetapi kesemua CILs stabil sehingga suhu 160 °C.

Penyerakkan sinar-X kristal tunggal telah digunakan untuk membuktikan struktur sebatian tetraetilammonium L-malat dan tetraetilammonium L-tartarat. Didapati bahawa sistem kristal bagi kedua-dua sebatian ini adalah monoklinik. Analisis juga telah dilakukan untuk

menunjukkan rangkaian ikatan hidrogen di antara atom hidrogen kation dan anion manakala polariti optik bagi setiap CILs ditentukan dengan menggunakan polarimeter. Kepekatan dan konduktiviti ionik untuk CILs dalam bentuk cecair juga ditentukan dan diamati menggunakan alat kepekatan dan meter konduktiviti. Perhubungan yang kuat di antara kepekatan dan konduktiviti ionik telah diamati.

Dalam aplikasi biomangkin, enzim bersalut cecair ionic kiral (CILCE) telah digunakan dalam tindakbalas pengeksteran olil alkohol dengan pelbagai asid lemak. CILCE telah disediakan melalui kaedah yang mudah dengan menyalut lipase daripada *Candida rugosa* dengan tetraetilamonium L-asparaginat dan penggunaan CILCE telah dikaji dan memberikan peratus penukaran ester yang lebih baik (60 – 81 %) berbanding enzim asli (36 – 70 %) bagi semua asid lemak daripada asid lemak berantai pendek, sederhana dan panjang.

ACKNOWLEDGEMENTS

Alhamdulillah, praise to ALLAH s.w.t., for giving me the strength to endure all challenges and complete this research. Firstly, I would like to thank my supervisor, Prof. Dr. Mohd Basyaruddin Abdul Rahman, who gave me the opportunity to conduct this research, for the help, advice and directed me through nearly two years of thesis work. His supportive discussions and enthusiasm for chemistry always replenished my energy to work. I'm also indebted to my co-supervisor, Prof. Dr. Mahiran Basri and Dr. Kamaliah Sirat for their invaluable guidance, encouragement and criticism, which kept me in a right track. Without these people, this thesis would not have been possible.

To my research group, Enzyme and Microbial Technology Research Group UPM, especially thanks to Prof. Dato Dr. Abu Bakar Salleh and Prof. Dr. Raja Noor Zaliha Raja Abd Rahman for their valuable advice and suggestions during weekly meeting. I would like also thanks to Dr. Emilia Abdul Malek and Dr. Bimo Ario Tejo for their opinion, support and discussion during completion of this thesis.

I want to thanks all of my lab mates in lab 401 especially to Kak Emmy, Kak Nora, Casey, Salwa, Naz, Intan, Azlan, Alif, Fairuz, Rizal, Asrul, Peter, Zati and Naimah that participated in their own way during my research. Without all of you, I would not have made it this far.

To all my housemates, thank you for the support during my thesis period.

Special thank to my family, especially my mom and dad, 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 many sacrifices that you have made for me. Last but not least, thank to Ministry of Science, Technology and Innovation Malaysia (MOSTI) for scholarship *National Science Fellowship* (NSF) and Universiti Putra Malaysia for financial support and facilities.

I certify that a Thesis Examination Committee has met on 17th November 2009 to conduct the final examination of Khairulazhar Jumbri on his thesis entitled "Synthesis and Characterization of New Chiral Ionic Liquids for Biocatalysis Application" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science Degree

Members of the Thesis Examination Committee were as follows:

Irmawati Ramli, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Nor Azah Yusuf, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Tan Yeng Peng, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Yatimah Alias, PhD
Associate Professor
Department of Chemistry
Faculty of Science
Universiti Malaya
Malaysia
(External Examiner)

_____

BUJANG KIM HUAT, Ph.D
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 Febuary 2010

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

Mohd. Basyaruddin Abdul Rahman, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mahiran Basri, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Kamaliah Sirat, PhD
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 17 March 2010

DECLARATION

I hereby 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 institutions.

_____

KHAIRULAZHAR JUMBRI

Date: 18 January 2010

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

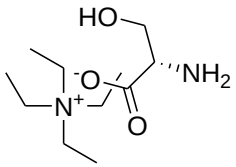
LIST OF ABBREVIATIONS

ILs	ionic liquids
RTILs	room temperature ionic liquids
CILs	chiral ionic liquids
MeOH	methanol
T_m	melting temperature
T_g	glass transition
T_{start}	start temperature
T_{onset}	onset temperature
EtOH	ethanol
dd	doublet of doublets
t	triplet
s	singlet
q	quartet
m	multiplet
asn	asparagine
gln	glutamine
glu	glutamic acid
his	histidine
ile	isoleucine
lys	lysine
mal	malic acid
met	methionine
pro	proline
ser	serine
thr	threonine
$[BF_4]^-$	tetrafluoroborate
$[PF_6]^-$	hexafluorophosphate
$[Tf_2N]^-$	bis(trifluoromethylsulfonyl)imide

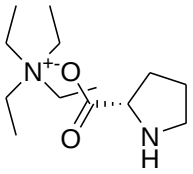
$[N_{2222}]$	tetraethylammonium
VOCs	volatile organic compounds
VOSs	volatile organic solvents
CILCE	chiral ionic liquid-coated enzyme
ILCE	ionic liquid-coated enzyme
CRL	*Candida rugosa* lipase
CHNS/O	carbon hydrogen nitrogen sulphur/oxygen
DSC	Differential Scanning Calorimetry
TGA	Thermogravimetric Analysis

OVERVIEW OF THE CHEMICAL STRUCTURES

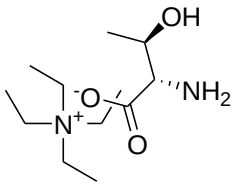
1. [N_{2222}][ser]: tetraethylammonium L-serinate



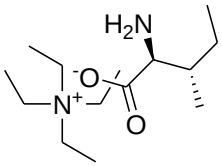
2. [N_{2222}][pro]: tetraethylammonium L-prolinate



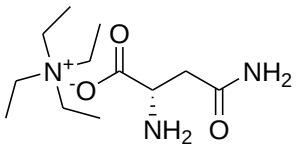
3. [N_{2222}][thr]: tetraethylammonium L-threoninate



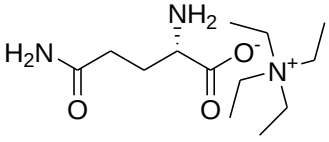
4. [N_{2222}][ile]: tetraethylammonium L-isoleucinate



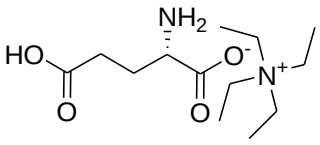
5. [N_{2222}][asn]: tetraethylammonium L-asparaginate



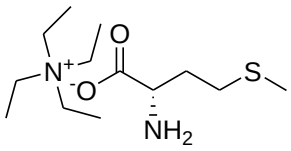
6. [N_{2222}][gln]: tetraethylammonium L-glutaminate



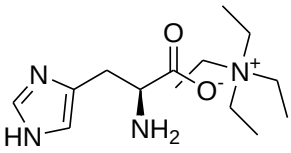
7. [N_{2222}][glu]: tetraethylammonium L-glutamate



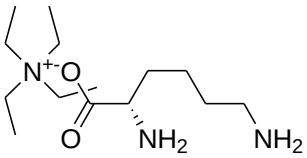
8. [N_{2222}][met]: tetraethylammonium L-methioninate



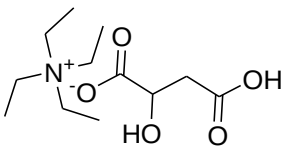
9. [N_{2222}][his]: tetraethylammonium L-histidinate



10. [N_{2222}][lys]: tetraethylammonium L-lysinate



11. [N_{2222}][mal]: tetraethylammonium L-malate



CHAPTER 1

INTRODUCTION

Lately, ionic liquids (ILs) are attracting a number of overwhelmed science and industrial communities as a reaction media, extraction solvents, electrolytes and in life sciences (Kagimoto *et al.,* 2007). These liquids that contain ions show good and tunable solubility properties with negligible vapor pressure and excellent thermal stability have rapidly found as valuable substitutes for many volatile solvents (Welton, 1999 and van Rantwijk *et al.,* 2003).

ILs are a new class of solvents that currently receiving much attention for their wide range of applications especially in catalysis and biocatalysis, dissolving polar to non-polar substrates and almost anything including coal, plastics, metal and even rock (Obliosca *et al.,* 2007). They are not as flammable as the volatile organic solvents (VOSs) therefore, making process safety and environmental concerns less of an issue. The thermodynamics and kinetics of reactions carried out in ILs can possibly varied to those in traditional VOSs and therefore creating great interests amongst chemists in their potential as solvents, co-solvents and catalysts.

A wide range of ILs consisted of inorganic/organic cations and anions have been successfully synthesized. In view of the emerging importance of ILs as reaction media in organic synthesis, researchers have turn their attraction 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). Several examples of CILs were mentioned in the literature and partial information can be

found in some reviews reported by Baudequin *et al.* (2005) and Tran *et al.* (2006). Due to their ease of synthesis and their particular chiral properties, these new CILs should play a central role in enantioselective research and expand the scope of chiral solvents. A significant transfer of chirality in these solvents can be expected due to their high degree of organization.

The specific properties of CILs should perform the classical chiral solvents for asymmetric induction. Studies about the application of CILs in asymmetric synthesis are not only an opportunity but also a challenge for researchers. It is interesting, meaningful and necessary to synthesize different kinds of CILs from different starting materials, especially from the chiral pool. Good chemical and configurational stability, are some of the most important criteria for synthesis of CILs and necessary properties for their application to chiral discrimination.

Another great property of ILs is their insolubility in most organic solvents which led us to envisage that they might be suitable as coating materials for immobilizing enzymes or cells. Interestingly, van Rantwijk *et al.* (2003) observed that ILs can enhance the selectivity of an enzyme. It was also demonstrated that they are useful as a media for the enzymatic reaction of polar substrates, which are difficult to dissolve in conventional organic solvents (Park and Kazlauskas, 2003). The very first ionic liquid coated enzyme (IL-CE) was reported by Lee and Kim (2002), which is easily prepared and exhibits markedly enhanced enantioselectivity and stability.

The aim of the study was to synthesis and characterizes a series of new CILs, and used the suitable CILs as biocatalysts in esterification reactions. To do this, the selected CILs, tetraethylammonium L-asparaginate was coated onto *Candida rugosa* lipase to form chiral

ionic liquid-coated enzyme (CILCE). CILCE was later tested for esterification reactions of oleyl alcohol with various fatty acids in order to figure out their amazing properties.

1.1 Objectives of the Research

This research embarks on the following objectives:

1) To synthesize twelve new tetraethylammonium-based chiral ionic liquids (CILs) derived from amino acids and chiral plant acids.
2) To characterize the physico-chemical properties of CILs.
3) To identify the suitable CILs for coating with *Candida rugosa* lipase (CRL) to form chiral ionic liquid-coated enzyme (CILCE).
4) To determine the enzymatic activity of CILCE in esterification reactions.