



UNIVERSITI PUTRA MALAYSIA

**CHEMO-ENZYMATIC EPOXIDATION OF 1-NONENE,
1-HEPTENE AND STYRENE**

MAHASHANON ARUMUGAM

FS 2013 5

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NONENE, 1-HEPTENE AND STYRENE**



MAHASHANON ARUMUGAM

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2013



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**CHEMO-ENZYMATIC EPOXIDATION OF 1-NONENE, 1-HEPTENE AND
STYRENE**

By

MAHASHANON ARUMUGAM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

March 2013

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

**CHEMO-ENZYMATIC EPOXIDATION OF 1-NONENE, 1-HEPTENE AND
STYRENE**

By

MAHASHANON ARUMUGAM

March 2013

Chair: Emilia Abd Malek, PhD

Faculty: Science

In view of the emerging importance of enzyme as a promising biocatalyst in fine organic synthesis, we focused on the synthesis of epoxides for their potential applications in chiral synthesis, including asymmetric synthesis and optical resolution of racemates. Epoxides are increasingly used as intermediates in many industrial processes and have wide applications in food, polymer and pharmaceutical formulations due to their excellent ability to facilitate ring opening reactions to various desirable functional groups. The enzymatic synthesis of epoxides has received much attention for its clean production due to its high-regioselectivity and low production of by-products. Furthermore, hydrogen peroxide (H_2O_2) as green oxidant and mild operating temperature, hence, low energy requirement, are becoming two important advantages of this enzymatic reaction.

Herein, we describe an improved enzyme-facilitated epoxidation of a simple alkene (1-nonene) using a conventional water bath shaker. The lipase was used to catalyse the formation of peroxy acids instantly from H_2O_2 and various perhydrolysis

substrates. The peroxy acid generated was then utilised directly for *in-situ* oxidation of 1-nonene to 1-nonene oxide. Various reaction parameters affecting the synthesis of epoxide, including the nature of peroxy acids, organic solvents, enzyme's sources, enzyme concentrations, reaction temperatures, initial concentrations and rate additions of the H₂O₂, stirring rates (rpm), and amounts of H₂O₂ and peroxy acid, were investigated.

Highest conversion was achieved using phenylacetic acid as an oxygen carrier. 1-nonene was converted most efficiently with maximum yield of 97% by Novozym 435, an immobilised *Candida antarctica* lipase B (CALB) and chloroform as reaction media. A minimum amount (1.4% w/w, 19 mg) of Novozym 435 was needed to maintain the catalytic activity (190.0 U g⁻¹). The highest yield was successfully obtained within 12 h reaction time at optimal synthesis conditions (35°C, 4.4 mmol of H₂O₂ (30%) in a single step addition, stirring rate 250 rpm and 8.8 mmol of phenylacetic acid). Subsequently, the optimised conditions were employed for the epoxidation of an array of aliphatic (1-heptene) and aromatic (styrene) alkenes which gave 94% to 99% yield and quantitative purity. In addition, a simple and rapid gas chromatography – mass spectrometry (GC-MS) selective ion monitoring (SIM) method was developed using an HP-5ms column for determining the epoxide yields. For 1-nonene oxide, the method was found to be linear in the range of 29.9 - 298.8 mg/L with R² = 0.9960.

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

PENGEPOKSIDAN KIMO-ENZIM 1-NONENA, 1-HEPTENA DAN STIRENA

Oleh

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Pengerusi: Emilia Abd Malek, PhD

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Memandangkan kepentingan enzim sebagai satu bio-pemangkin yang berpotensi dalam proses sintesis organik halus, kami telah memberi fokus kepada sintesis epoksida di atas potensi aplikasinya dalam pemillihan molekul kiral, termasuk sintesis asimetrik dan resolusi optik rasemat. Penggunaan epoksida semakin meningkat sebagai perantara dalam banyak proses industri dan aplikasinya meluas dalam pembentukan makanan, polimer dan farmaseutikal disebabkan oleh tindak balas pembukaan gelangya yang baik kepada pelbagai kumpulan berfungsi yang dikehendaki. Tindakan enzim dalam pengoksidaan telah menarik perhatian ramai kerana pengeluarannya yang bersih, disebabkan oleh keregipilihan yang tinggi dan pengeluaran produk sampingannya yang rendah. Tambahan pula, hidrogen peroksida (H_2O_2) sebagai bahan pengoksidaan hijau dan suhu pengoperasian yang rendah, yang seterusnya membawa kepada keperluan tenaga yang rendah, telah menjadi dua kebaikan penting tindak balas enzim ini.

Di sini, kami menghuraikan proses pengoksidaan alkena yang mudah, yang dibantu oleh enzim yang sudah dipertingkatkan (1-nonena) dengan menggunakan alat penggetar air rendaman konvensional. Lipase telah digunakan untuk memangkinkan pembentukan asid peroksi dengan serta-merta dari H₂O₂ dan pelbagai substrat perhidrolisis. Asid peroksi yang dihasilkan kemudiannya digunakan terus untuk proses pengoksidaan *in-situ* 1-nonena kepada oksida 1-nonena. Berbagai parameter tindak balas yang memberi kesan kepada sintesis epoksida, termasuk keadaan semulajadi asid peroksi, pelarut organik, sumber enzim, kepekatan enzim, suhu tindak balas, kepekatan awal dan kadar tambahan H₂O₂, kadar pengacauan (rpm), dan kandungan H₂O₂ dan asid peroksi, telah dikaji.

Pertukaran tertinggi telah dicapai menggunakan asid fenilasetik sebagai pembawa oksigen. 1-Nonena telah ditukar dengan baik dengan 97% hasil maksimum oleh Novozym 435, satu lipase B *Candida antarctica* (CALB) yang tidak bergerak dan dengan klorofom sebagai medium tindak balas. Sebanyak (1.4% w/w, 19 mg) Novozym 435 diperlukan untuk mengekalkan aktiviti pemangkin (190.0 U_g⁻¹). Hasil tertinggi telah berjaya dicapai dalam masa 12 jam masa tindak balas pada keadaan sintesis yang optimal [35°C, 4.4 mmol of H₂O₂ (30%) dalam tambahan satu-langkah, kadar pengacauan 250 rpm dan 8.8 mmol asid fenilasetik. Seterusnya, keadaan optima digunakan untuk pengoksidaan berjenis alkena alifatik (1-heptena) dan aromatik (stirena) memberikan hasil 94% hingga 99% dan ketulenan kuantitatif. Sebagai tambahan, satu kaedah mudah dan cepat pemantauan ion selektif (SIM) gas kromatografi-spektrometri jisim (GC-MS) telah dikembangkan menggunakan kolum HP-5ms untuk menentukan hasil-hasil epoksida. Untuk epoksida 1-nonena, kaedah didapati linear dalam julat 29.9 - 298.8 mg/L dengan R² = 0.9981.

ACKNOWLEDGEMENTS

I would have never been able to complete my thesis without the proper guidance and support of my supervisory committee members of Enzyme and Microbial Technology Research Group (EMTECH), helps from laboratory mates and friends as well as support from my family.

First and foremost, my deepest gratitude to my supervisor, Dr. Emilia Abd Malek because for accepting me as a Master student and also for her invaluable support and guidance throughout the completion of this study. Thanks also for the Research Assistant-ship and for being patience in correcting the thesis and English throughout these years.

My sincere appreciation also goes to co-supervisors, Prof. Dr. Mohd Basyaruddin Abdul Rahman and Prof. Dr. Mahiran Basri for their fruitful comments during our weekly meetings and for financially supporting my research by using the Green Chemistry research grant.

I would like to extend my sincere thanks to my fellow friends, Mohammad Hesham Abu Alrub and Balakrishnan Shanmugasamy for guiding my research especially in GC-MS method development. Accordingly, many thanks also to Dr. Intan Safinar Ismail and Prof. Dr. Md. Jelas Haron for their numerous discussions on chromatography.

I also would like to acknowledge my thank you to the Lab 401 seniors, kak Emmy, kak Nora, abang Asrul and Saadi Bayat for their precious advices and suggestions throughout the duration of my study.

Special thanks to Mr. Lim Chaw Jiang who as a good friend, is always willing to help and give his best suggestions and Hanim Salami for being such a great person to work with. To all my other colleagues in Lab 401 and BASL 105, thank you for the wonderful time we shared together in our lab. Our joyful moments have made the lab environment more pleasant.

I also wish to thank Encik Mohd. Johadi Iskandar Che' Jamil and kak Syarina for assisting me in running samples with the Nuclear Magnetic Resonance (NMR).

To my beloved family, I would like to express my deepest affection for their constant support and inspirations in coming so far and to accomplish these challenging years of study.

Not forgotten, financial support from the Ministry of Higher Education (MOHE) through Fundamental Research Grant Scheme (FRGS) and Mini Budget, and a Graduate Research Fellowship (GRF) from the Universiti Putra Malaysia (UPM) are also gratefully acknowledged.

Last but not least, thanks to the almighty GOD, for blessing me the strength and health in order to complete the study.

I certify that a Thesis Examination Committee has met on **date** to conduct the final examination of Mahashanon Arumugam on his thesis entitled "Chemo-enzymatic Epoxidation of Alkenes" in accordance with the Universities and University Colleges 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.

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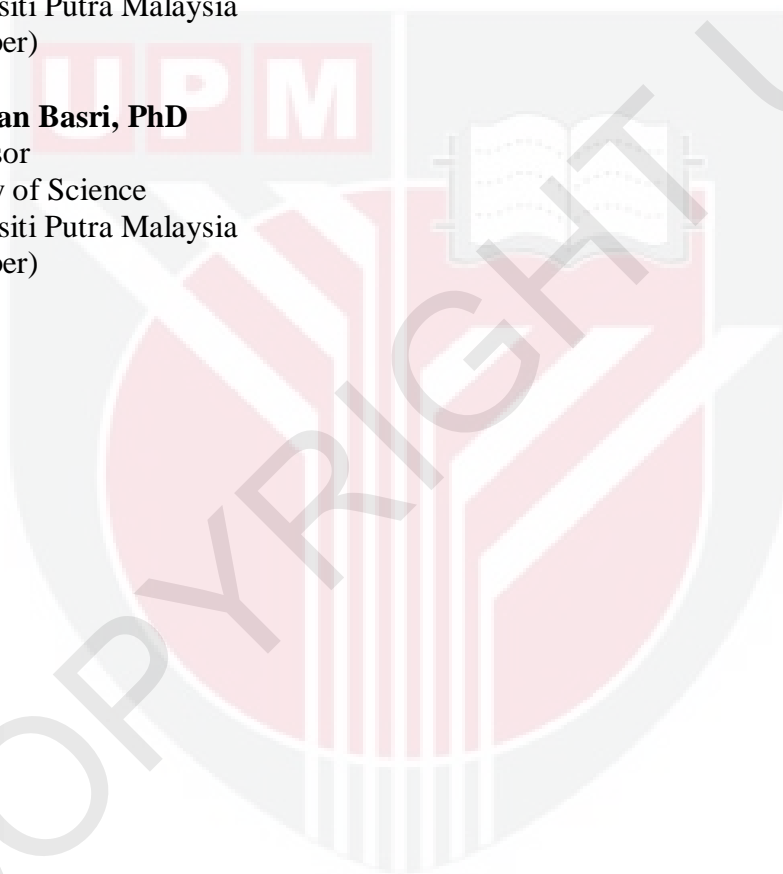
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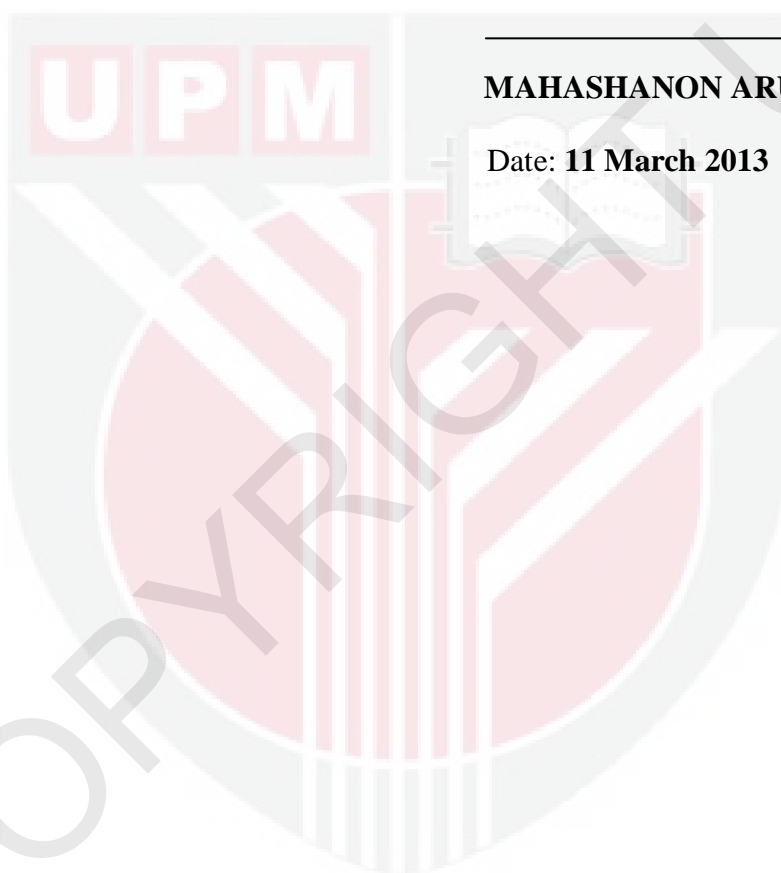
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DECLARATION

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



MAHASHANON ARUMUGAM

Date: 11 March 2013

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