



UNIVERSITI PUTRA MALAYSIA

***RATIONAL DESIGN OF MIMETIC PEPTIDES BASED ON PROMISCUOUS
ALDO-KETOREDUCTASE ENZYME AS ASYMMETRIC CATALYSTS IN
ALDOL AND MICHAEL REACTIONS***

SAADI BAYAT

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By

SAADI BAYAT

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the degree of Doctor of Philosophy**

March 2014

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the degree of Doctor of Philosophy

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March 2014

Chairman: Mohd Basyaruddin Abdul Rahman (PhD)

Faculty: Science

The asymmetric aldol and Michael reactions, as the most prominent carbon-carbon bond formation reactions, are the central study issues in the field of asymmetric synthesis. In this study, promiscuous aldo-ketoreductase (AKR) used to catalyze aldol reaction. between aromatic aldehydes and ketones. Good yield (up to 75%), moderate enantioselectivity (60%), and high diastereoselectivity (dr) up to 93/7 (anti/syn) were obtained. Several mimetic peptides from AKR's active site were designed and synthesized as asymmetric catalysts in the aldol and Michael reactions. Mimetic peptides PE16aa (**1**), PH16aa (**2**), 16aa (**3**), 8aa (**4**), 8aa-z (**5**), 5aa (**6**), 3aa (**7**), Fmoc-KLH-R (**8**), K(z)LH-R (**9**), PYE (**10**), PEY (**11**), PHE (**12**), PEH (**13**), LFV (**14**) **4a** and **4b** were successfully synthesized using manually solid phase peptide synthesis protocol. Then, all of these mimetic peptides were employed to catalyze aldol reactions and peptides **2**, **4**, **4a**, **4b**, **10**, **11**, **12**, and **13** were selected to catalyze Michael reactions. In the aldol and Michael reactions, peptide **4** exhibited the best results (up to 97% yield, up to 99.9% ee and dr up to 99/1). Peptide **1** produced a good yield (88%), moderate enantioselectivity (68%), and excellent diastereoselectivity (dr = 99/1). Peptide **2** afforded the desired anti aldol product in 95% yield, 86% ee and 95/5 dr. Peptide **3** exhibited moderate yield (67%) but poor enantioselectivity (39% ee). Peptide **5** showed good catalytic activity and produced high yield (89%) and enantioselectivity (86%). Pentapeptide **6** catalyzed aldol reaction in high diastereo- and enantioselectivity (dr = 99/1 and 90% ee). PHE showed the best reactivity and selectivity amongst four tripeptides (PYE, PEY, PEH, PHE) up to 94% ee and up to 95/5 dr. Peptide **2** afforded corresponding Michael reaction up to 89% yield, 44% ee, and 99/1 dr. Peptide **4** generated desired Michael product up to 95% yield, 84% ee and 95/5 dr. Mechanism study demonstrated that enamine intermediate and hydrogen-bonding interaction are very important for obtaining high enantiomeric excess. The reusability of peptide **4** as the best catalyst was also conducted for 10 times. Peptide **4** is able to hydrolysis esters in a good to excellent yields of up to 99.7%. All mimetic peptides exhibited to be active in terms of reactivity and selectivity in c-c bond forming reactions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

REKABENTUK RASIONAL PEPTIDA MIMETIK BERDASARKAN ENZIM ALDO-KETOREDUCTASE RAMBANG SEBAGAI MANGKIN ASYMMETRIC DALAM TINDAKBALAS ALDOL DAN MIHAEL

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Tidakbalas tidak simetri aldol dan Michael adalah isu yang paling menonjol dalam kajian tindakbalas sistesis kimia bagi pembentukan ikatan karbon-karbon. Dalam kajian ini, aldo-ketoreductase (AKR) digunakan untuk memangkin tindakbalas aldol diantara aromatik aldehida dan keton. Keputusan yang diperoleh menunjukkan peratusan hasil yang baik (sehingga 75%), enantioselektiviti yang sederhana(60%), dan kadar diastereometrik yang tinggi (*dr*) sehingga 93 /7 (anti / syn). Peptida yang meniru hormon AKR telah direka dan disintesis sebagai pemangkin tidak simetri dalam tindak balas Michael dan aldol. Peptida PE16aa (1), PH16aa (2), 16AA (3), 8aa (4), 8aa-z (5), 5aa (6), 3aa (7), Fmoc-HKL-R (8), K(z) LH-R (9), PYE (10), PEY (11), PHE (12), PEH (13), LFV (14), 4a dan 4b telah berjaya disintesis secara manual mengikut kaedah sintesis pepejal peptida. Kemudian, kesemua peptida yang meniru hormon tersebut digunakan sebagai pemangkin tindakbalas aldol dan hanya peptida 2, 4, 4a, 4b, 10, 11, 12, dan 13 dipilih sebagai pemangkin untuk tindakbalas Michael. Melalui tindakbalas aldol dan Michael, peptida 4 memberikan hasil yang terbaik (peratusan hasil sehingga 97 %, sehingga 99.9% ee dan *dr* sehingga 99 /1). Peptida 1 pula memberikan hasil yang bagus (88 %), enantioselectiviti sederhana (68 %), dan diastereoselectiviti baik (*dr* = 99 /1). Hasil produk Peptida 2 adalah sebanyak 95%, 86% ee dan 95/5 *dr*. Peptida 3 memberikan hasil yang sederhana (67 %) dan peratusan enantioselectiviti yang rendah (39% ee) . Peptida 5 menunjukkan aktiviti pemangkinan yang baik dan menghasilkan produk hasil yang tinggi (89%) dan enantioselectiviti (86 % ee). Manakala pentapeptida 6 menjadi mangkin untuk tindakbalas aldol dengan diastereo - dan enantioselectiviti yang tinggi (*dr* = 99 /1 dan ee 90%). PHE menunjukkan kadar kereaktifan yang terbaik di kalangan empat tripeptida yang lain (PYE, PEY, PEH, PHE) dengan kadar peratusan 94 % ee dan 95/5 *dr*. Dalam kajian ini, peptida 2 memberikan hasil tindakbalas Michael sehingga 89% , 44% ee, dan 99/1 *dr*. Peptida 4 memberikan hasil produk sebanyak 95% , 84% ee dan 95/5 *dr*. Kajian terhadap mekanisma tindakbalas menunjukkan bahawa enamine yang bertindak sebagai pengantara dan juga ikatan hidrogen adalah sangat penting untuk mendapatkan peratusan enantioselectiviti yang tinggi. Kebolegunaan peptida 4 sebagai pemangkin yang paling bagus dapat diguna semula sebanyak 10 kali. Peptida 4 ini juga mampu menghidrolisis ester kepada hasil produk yang lebih baik sehingga 99.7 %. Semua peptida yg meniru-niru dipamerkan untuk aktif dari segi kereaktifan dan pemilihan dalam bon cc membentuk tindak balas.

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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENTS	iv
APPROVAL	v
DECLARATION	vii
LIST OF FIGURES	xii
LIST OF TABLES	xvi
LIST OF ABBREVIATIONS	xviii
CHAPTER	
1 INTRODUCTION	1
1.1 Problem Statements	3
1.2 Goal and objectives of the study	4
2 LITERATURE REVIEW	5
2.1 Promiscuous Biocatalyst in the C-C Bond Forming Reaction	5
2.2 Organocatalysts As Asymmetric Catalyst	6
2.2.1 Organocatalysis Based on Covalent Catalysis	6
2.2.2 Iminium Activation Catalysis	9
2.2.3 SOMO Activation Catalysis	11
2.3 Organocatalysis Based on Non-Covalent Catalysis	12
2.3.1 Hydrogen-Bonding and Bronsted acid Activation Catalysis	12
2.4 Structural Requirements of Enamine Catalysis	13
2.5 Asymmetric Aldol reaction catalyzed by organocatalysts	14
2.6 Peptide as Asymmetric Catalyst	17
2.7 Peptides for Hydrolysis of Esters	28
2.8 Industrial Application of Organocatalyst	28
3 MATERIALS AND METHODS	30
3.1 Materials	30
3.1.1 Sources of Amino Acids, Resin And Coupling Reagents	30
3.1.2 Chemicals for Asymmetric Aldol and Michael Reactions	30
3.1.3 Solvent Sources	30
3.1.4 Instrumentations	30
3.2 Method	31
3.3 BasicLocal Tool (Alignment Search BLAST)	33
3.4 Computational Modeling to Design And Draw Transition State	33
3.5 Peptides derived from AKR	33

3.6	Kaiser Test	35
3.6.1	Preparation of Kaiser (A) and (B) Solutions	35
3.7	General protocols for the synthesis of solid phase peptide	36
3.8	Protocol : Manual Peptide Synthesis Using HCTU/DIEA	37
3.8.1	Synthesis of Lys-Leu-His-NH ₂ as a Model peptide	37
3.8.2	Purity Analysis of Peptides	40
3.9	General Procedure for C-C Bond Forming Reactions	41
3.9.1	Preparation of Standard Racemic Product	41
3.9.2	General Procedure for Asymmetric Aldol Reaction	41
3.10	General Procedure for Asymmetric Michael Addition	43
3.11	General Procedure for Hydrolysis of Ester	46
3.12	Screenings	46
3.12.1	Scope and Limitation Study	46
3.12.2	Solvent Screening	46
3.12.3	Effect of Catalyst Loading	46
3.13	Product Analysis	47
3.13.1	Thin layer Chromatography (TLC)	47
3.13.2	Nuclear Magnetic Resonance Spectroscopy (NMR)	47
3.13.3	Fourier-Transform Infrared Spectroscopy (FT-IR)	47
3.13.4	Mass Spectrometry (MS)	47
3.13.5	Optical Rotation	48
3.13.6	Circular Dichroism (CD)	48
3.13.7	General Procedure for Aldol and Michael Products Purification	48
3.14	Determination of Enantiomeric Excess	49
3.15	Recyclability of the Catalyst	50
3.16	Experimental Section	50
3.16.1	Spectroscopic data of peptides	51
3.16.2	Characterization of Corresponding Aldol and Michael Products	56
4	RESULT AND DISCUSSION	57
4.1	Promiscuous Aldo-Ketoreductase (AKRs)	57
4.2	AKR1A1 for Aldol Reaction	59
4.2.1	Optimisation of Synthesis Reaction	59
4.2.2	Scope and Limitation of Substrates	62
4.2.3	Proposed Mechanism of the Aldol Reaction Catalyzed by AKR1A1	63
4.3	Peptides for Aldol Reaction	64

4.4	Peptide Structural Studies	66
4.4.1	PE-16aa (1) as an Asymmetry Catalyst	75
4.4.2	PH-16aa (2) as an Asymmetry Catalyst	77
4.4.3	Peptide 3 as an Asymmetric Catalyst	79
4.4.4	Peptides derived from Peptide 3	79
4.4.5	Mechanism Study	84
4.4.6	Optimisation of Synthesis reaction catalyzed by peptide 4	86
4.4.7	Short Polar Peptide as Asymmetric Catalysts	92
4.5	Peptides for Michael Reaction	97
4.5.1	Peptide 4 Catalyzed Asymmetric Michael Reaction	98
4.5.2	Shot Polar Peptides for Michael Reaction	104
4.5.3	Peptide 2 for Michael Reaction	108
4.6	Peptides for Hydrolysis of Esters	110
4.6.1	Hydrolysis of <i>p</i> -Nitrophenyl Acetate (PNPA) by Peptide 4	111
4.6.2	Effect of Solvents on Ester Hydrolysis	112
4.6.3	Scope and Limitation Study	114
4.6.4	Mechanism Study	116
4.6.5	Effect of Solvents Mole Ratios on Hydrolysis	119
5	CONCLUSIONS AND RECOMMENDATIONS	121
5.1	Conclusions	121
5.2	Recommendation for Futher Reaesrch	122
	REFERENCES	123
	APPENDICES	133
	BIODATA OF STUDENT	167
	LIST OF PUBLICATIONS	168

LIST OF FIGURES

Figure	Page
2.1.[a] (S)-Proline catalyzed intermolecular direct asymmetric aldol reaction [b] Imidazolidinone salt catalyzed enantioselective Diels-Alder reaction.	7
2.2. General mechanism for the amine-catalyzed α -functionalization of carbonyls	7
2.3. Stereochemical models enamine reactivity (a) List-Houk model, (b) Steric model, and (c) Seebach model.	8
2.4. The proline catalytic cycle with a pivotal role of oxazolidinones	9
2.5. General mechanism for the amine-catalyzed β -functionalization of α,β -unsaturated carbonyls	10
2.6. Stereochemical outcome of the amine catalyzed Michael addition to enals.	10
2.7. Representative mechanistic cycle for asymmetric organo-somo activation catalysis	11
2.8. Asymmetric catalysis by <i>N</i> -heterocyclic carbene (NHC)	12
2.9. Classification of the activation mode in organocatalysis	13
2.10. Hajos-Parrish-Eder-Sauer-Wiechert reaction catalyzed by (S)-proline	14
2.11. Proposed mechanism for intermolecular aldol reaction catalyzed by proline.	15
2.12. Aldol reaction catalyzed by aminodisulfonamide analogue	16
2.13. Diastereoisomeric catalysts for the reaction of <i>p</i> -nitrobenzaldehyde with acetone.	16
2.14. [a] Diketopiperazine catalyzed hydrocyanation of benzaldehyde. [b] Julia-colonna epoxidation using poly-L-Leu as catalyst.	17
2.15. [a] peptide catalyzed desymmetrization by selective acylation. [b] enantioselective Pictet-Spengler reaction catalyzed with thiourea-based catalyst.	18
2.16. Tetrapeptide-based iminium catalysis: asymmetric nitro-Henry reactions of cyclohex-2-en-1-one and nitroalkanes	18

2.17. Peptides bearing a secondary primary amine at the <i>n</i> -terminus	19
2.18. Some instances about different dipeptides catalyzed aldol reaction	20
2.19. Direct aldol reaction proline-based small peptides	20
2.20. Various derivatives of histidine in the aldol reaction	21
2.21. Catalytically active H-Pro-Pro-Asp-NH-resin-tenta and PEG Gel on the aldol reaction between <i>p</i> -nitrobenzaldehyde and acetone	22
2.22. Sample of aldol reaction catalyzed by di-and tripeptides immobilized	22
2.23. Proline derivatives asymmetric catalysts in the michael reactions	23
2.24. Derivatives of proline as organocatalysts in the michael reaction	24
2.25. Michael Addition Catalyzed by Peptide	25
2.26. 1,4 Addition between aldehyde and nitrostyrene catalyzed by short peptides.	26
2.27. Michael addition of cyclohexanone to <i>trans</i> - β -nitrostyrene catalyzed by peptides on water	26
2.28. α -Hydroxycarboxylate synthesis by peptides.	27
2.29. Organocatalytic aldol reactions by thiopeptides	27
2.30. Enamine catalyzed Michael addition in Nicolaou's total synthesis of biyouyanagin A	29
3.1. Flow diagram of the experimental work	32
3.2. All synthesized peptides derived from AKR using manually procedure on solid phase	34
3.3. Synthesized peptides driven from AKR	35
3.4. Removal of Fmoc Group from RAAR	37
3.5. Kaiser test (A) The resin before deprotection;(B) The resin after removing fmoc group turned to blue.	38

3.6. Synthesis of representative tripeptide (TFA.H-Lys-Leu-His-NH ₂) according to solid phase synthesis protocol	39
3.7. Condition of linear AB gradient	40
3.8. Normal phase HPLC using chiral column to identify enantiomer excess percentage	49
4.1. Secondary structure of the AKRs (PDB = 1VBJ) enzyme	57
4.2. Similarity of AKRs (1VBJ) And AKR1A1 by BLAST.	58
4.3. Proposed mechanism to produce stereoselective compound in the aldol reaction catalyzed by AKR1A1	64
4.4. All amino acid residues in AKR's active site	65
4.5. Secondary structure of mimetic peptides based on AKR predicted by LOMETS (Local Meta-Threading-Server)	66
4.6. CD spectrum of peptide 4, in water and 1%SDS	67
4.7. Assignment of FT-IR spectrum of peptide 4	68
4.8. FT-IR spectrum of peptide 4	69
4.9. CD spectrum of ph16aa, 2, in water and 1%SDS	70
4.10. FT-IR spectrum of peptide 2 to determine secondary structure	71
4.11. CD spectrum of PE16aa (peptide 1)	72
4.12. FT-IR spectrum of peptide 1 to determine secondary structure	73
4.13. CD spectrum of 16aa (peptide 3)	74
4.14. FT-IR spectrum of peptide 3	75
4.15. Analytical HPLC of peptide 4	80
4.16. HPLC analysis to identify enantiomeric excess of 2-(hydroxy(4-nitrophenyl)methyl)cyclohexanone catalyzed by peptide 4	80

4.17. Comparison of HPLC results of fragmented peptides catalyzed aldol reaction referred to Table 4.8	84
4.18. Proposed aldol reaction mechanism catalyzed by peptide 4	85
4.19. Proposed mechanism of aldol reaction catalyzed by peptide 4, drawn by pymol in different view	86
4.20. Proposed transition state model for the aldol reaction catalyzed by four different tripeptides	97
4.21. (4a) Protected peptide attached to rink-amide-am-resin (4b) Peptide without side chain protection group but attached to the resin (4) Cleavage peptide from resin.	99
4.22. Michael reaction in the presence of peptide 4	101
4.23. Proposed transition state models for Michael reaction catalyzed by peptide 4	102
4.24. Michael reaction catalyzed by catalyst 4c	103
4.25. Proposed mechanism for the michael reaction catalyzed by 4c	103
4.26. Proposed freely rotation peptide bond C9–N8 bond and C9–C10(O) bond and transition state for catalyst 11	106
4.27. Proposed transition state mechanism of Michael reaction catalyzed by peptides, 10, 11, 12, 13	107
4.28. Micheal addition catalyzed by peptide 2	108
4.29. (A) Hydrolysis of PNPA in the presence of peptide 4 in water after 24h (B) Hydrolysis of PNPA in the presence of peptide 4 in water after two days	112
4.30. HPLC of hydrolysis of PNPA in the biphasic solvents	113
4.31. A series of esters which hydrolysed by peptide 4	114
4.32. Samples of hydrolysis reactions of esters catalyzed by peptide 4 in biphasic solvent (CHCl ₃ /H ₂ O) (A) PNPA, (B) PNPB, (C) PNPD	115
4.33. Catalyst and Ester Arrangement in Biphasic Solvent	118
4.34. Proposed mechanism of ester hydrolysis catalyzed by peptide 4	119

LIST OF TABLES

Table	Page
3.1. Aldol reaction with different substrates and major products	42
3.2. Michael adduct reaction with different substrates and major products	44
3.3. ¹ HNMR spectroscopy data of peptide 4	50
3.4. ¹³ CNMR spectroscopy data of peptide 4	50
4.1. Effect of pH on the aldol reaction catalyzed by AKR1A1- in phosphate–sodium buffer: iPrOH	59
4.2. Effect of mole ratio on the model aldol reaction	60
4.3. The enzyme loading for the AKR1A1–catalyzed aldol reaction	61
4.4. Effect of solvent on the aldol reaction catalyzed by AKR1A1	61
4.5. Investigation of the reactant scope of the AKR1A1-catalyzed asymmetric aldol reaction in CHCl ₃ :H ₂ O	63
4.6. Direct aldol reaction catalyzed by peptide 1 in aqueous media	76
4.7. Results of reaction of various aldehydes with cyclohexanone catalyzed by peptide 2	78
4.8. Aldol reaction catalyzed by fragmented peptides, 3, 4, 5, 6, 7, 8 and 9	82
4.9. Solvent ratio investigation in aldol reaction catalyzed by peptide 4	87
4.10. Effect of solvents on aldol reaction catalyzed by peptide 4	88
4.11. Optimization studies of aldol reaction catalyzed by peptide 4	89
4.12. Direct aldol reaction catalyzed by peptide 4 in aqueous media	91
4.13. Investigated reusability of peptide 4	92
4.14. Effect of the solvent ratio on the catalytic aldol reaction between cyclohexanone and <i>p</i> -nitrobenzaldehyde catalyzed by 10	93
4.15. Aldol reaction catalyzed by different short polar peptides	94

4.16. Catalytic aldol reactions between cyclic and acyclic ketones and aromatic aldehydes catalyzed by 13	96
4.17. Michael addition in the presence of different catalysts and substrates	101
4.18. Reusability studies of peptide 4 catalyzed Michael addition in the reaction between nitrostyrene and propanal	104
4.19. Michael addition reactions catalyzed by peptides 10, 11, 12 and 13	105
4.20. Optimization screening of Michael adduct reaction	108
4.21. Examples of peptide 2 catalyzed nitro-Michael additions of different aldehydes and ketone to nitroolefins	110
4.22. Effect of solvent on hydrolysis of PNPA catalyzed by peptide 4	113
4.23. Hydrolysis of different esters catalyzed by peptide 4	115
4.24. Hydrolysis of PNPA catalyzed by fragmented short peptides from peptide 4	116
4.25. Effect of ratio of $\text{CHCl}_3/\text{H}_2\text{O}$ on PNPA hydrolysis catalyzed by 4	120

LIST OF ABBREVIATIONS

δ	Chemical Shift
$[\alpha]_D$	Specific Optical Rotation
Ala (A)	Alanine
aq	Aqueous
Asp (D)	Aspartic Acid
Boc	<i>Tert</i> -Butyl-Oxycarbonyl
Bu	<i>N</i> -Butyl
<i>c</i> / conc.	Concentration / Concentrated
calcd	Calculated
Cbz (Z)	Carboxybenzyl
CD	Circular Dichroism
Cy	Cyclohexyl
<i>d</i>	Days
DEPT	Distortionless Enhancement By Polarization
DIC	Diisopropylcarbodiimide
DCC	Dicyclohexylcarbodiimide
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
<i>dr</i>	Diastereomeric Ratio
<i>ee</i>	Enantiomeric Excess
<i>eq</i> / equiv.	Equivalents
ESI	Electrospray Ionisation
Et	Ethyl
Fmoc	9-Fluoromethoxycarbonyl
FT	Fourier Transformation
GC	Gas Chromatography
Glu (E)	Glutamic Acid
Gly (G)	Glycine
<i>h</i>	Hours
HCTU	O-(1H-6-Chlorobenzotriazole-1-Yl)-1,1,3,3-Tetramethyluronium Hexafluorophosphat
His (H)	Histidine
HOBt	1-Hydrobenzotriazole
HOMO	Highest Occupied Molecular Orbital
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectroscopy
<i>i</i> -Pr	<i>Iso</i> -Propyl
IR	Infrared (Spectroscopy)
<i>J</i>	NMR Coupling Constant
Leu (L)	Leucine

LOMETS	Local Meta-Threading-Server
M	Molar
Me	Methyl
min	Minutes
MS	Mass Spectroscopy
NMM	<i>N</i> -Methylmorpholine
NMP	<i>N</i> -Methylpyrrolidone
NMR	Nuclear Magnetic Resonance
BDP	Protein Data Bank
Ph	Phenyl
Phe (F)	Phenylalanine
Pr	<i>n</i> -Propyl
Pro (P)	Proline
RT	Room Temperature
Ser (S)	Serine
SOMO	Singly Occupied Molecular Orbital
SDS	Sodium dodecyl sulfate
<i>t</i> -Bu / <i>t</i> Bu	<i>Tert</i> -Butyl
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
<i>t</i> R	Retention Time
Trt / Trityl	Triphenylmethyl

CHAPTER 1

INTRODUCTION

Life is impossible without the miraculous role of catalytic reactions in plants, animals, and human beings. Billions of vital reactions in living organisms are carried out by biocatalysts (enzymes), and they take place within seconds. Without enzymes these reaction would possibly take centuries. Not only nature applies catalysts, it is also keystone of the chemical industry. Approximately 90% of all manmade chemicals and materials are produced using catalysis at one stage or another. Human application of catalysis began in prehistory (Ojima, 2004). For example, the ancient Sumerians unknowingly applied catalysis many millennia ago to produce their beer. Even today, many catalytic processes are found using a hit-and-miss approach. Rational design of desired catalysts followed by synthesis seems to be difficult yet. As demand for optically active pharmaceutical compounds has grown in recent years, much research progress has been made towards the development of asymmetric catalysts.

Until recently, the asymmetric catalysts utilized for enantioselective synthesis of organic compounds, fall into two general categories – transition metal complexes and enzymes (Dalko et al., 2004). In 2001 the Nobel Prize in chemistry was awarded to William R. Knowles and Ryoji Noyori for their work on the chiral hydrogenation catalyzed reactions, and K. Barry Sharpless for his work on chirally catalyzed oxidation reactions. For all three winners the development of chiral transition metal catalysts was the key to success. It has been a long-standing belief that only man-made transition metal catalysts can be tailored to produce either two product enantiomers whereas the enzymes cannot. This dogma has been challenged in recent years by the immense advances in the field of biocatalysis, for example, the discovery of preliminarily useful enzymes of novel microorganisms, and the optimization of enzyme performance by selective mutation or evolutionary methods (Berkessel et al., 2006).

Recently, researchers vividly demonstrated the highly competitive head to head race between transition metal catalysis and enzymatic catalysis in contemporary industrial production of enantiomerically pure fine chemicals. Therefore, biocatalysis is considered to be one of the available approaches to achieve green chemistry owing to its high selectivity, mild condition, low energy requirements, and few by-products (Wu et al., 2006).

Enzymes are usually very selective with regard to chemo-, diastereo-, and enantioselectivity, due to their complex three-dimensional structure allowing only specific target molecules to interact with the active site of the enzyme. Problems may arise with the low tolerance of changes in operational parameters, such as pH values or temperature, preference for water as a reaction medium, and their well recognized severe dependence on their natural cofactor, often making them too costly for stoichiometric use. Another drawback of enzymes is that they are produced by nature in only one enantiomeric form, and their antipodes cannot simply be made from all D-amino acids to yield the opposite stereoisomer in a given, chemical transformation. In other words, enzymes are generally a specific catalyst (Aleu et al., 2006).

In recent years, some hydrolytic enzymes have demonstrated high activity for unnatural substrates and alternative chemical transformation, namely, biocatalytic promiscuity, which provides a new tool for organic synthesis and largely extends the application of enzymes. To overcome the specificity properties of enzymes, the chemists and biochemists involved in the asymmetric catalyst field have given a lot of attention to promiscuous enzyme (Svedendahl et al., 2005). Catalytic promiscuity refers to the ability of a single active site to catalyze more than one chemical transformation (Li et al., 2010). These transformations may differ in terms of the functional group involved; that is, the type of bond formed or cleaved during the reaction (Kazlauskas, 2005).

Though this field of study is new, the obtained results have exhibited that both yield and stereoselectivity are moderate. Although today the vast majority of asymmetric reactions catalysis continue to rely on organometallic complexes, this picture is changing, and between the extremes of transition metal catalysis and enzymatic transformations, a third approach to the catalytic production of enantiomerically pure organic compounds has emerged –namely, organocatalysis. Since 1970, organocatalysts have become popular for the synthesis of chiral compounds. organocatalysis is becoming an increasingly important segment of organic chemistry, offering a number of advantages over metal-based and biocatalyst methods. In general, organocatalysis can be used in wider range of solvents and for a broader scope of substrates compared to enzymes (Revelou et al., 2012). In addition, they are typically less toxic and less sensitive to oxidation and moisture than most organometallic based reagents. Given the sheer number of amino acids in a given enzyme, it is possible to achieve a near infinite amount of structural diversity.

However, for a given enzyme, the active site is usually extremely specific allowing for a limited substrate scope. One rapidly-growing subtopic of organocatalysis, peptide-based catalysis, is providing an interesting perspective into the nature of both low molecular weight and enzymatic catalysis (Jarvo et al., 2002). By examining the nature of small peptide based catalysis, it is possible to study amino acid-mediated binding events while “tuning out” some of the more complex interactions that are inherent to enzymatic interactions. In addition, it allows for an interesting entry into peptide engineering. With careful selection of each residue within a peptide catalyst, one may create a system which affects a catalytic transformation with a high level of selectivity while employing a bare minimum of amino acids. One of the most significant applications of asymmetric organocatalysis is the construction of carbon-carbon and carbon-heteroatom bond (Pedrosa et al., 2010).

Therefore, asymmetric aldol and Michael reactions are known as the fundamental methods for producing one or two stereocenter organic compounds, which are quite applicable for pharmaceutical purposes (Milhazes et al., 2006). Peptide-based organocatalysis can catalyze these reactions asymmetrically to afford functionalized, optically active compounds bearing quaternary stereocenter with the benefits of high enantioselectivities, excellent yields, and high atom economy. Due to the increasing number of chiral drugs in the pharmaceutical industries, organocatalysis and particularly, peptide-based biocatalysts, can play significant roles as asymmetric catalysts in organic reactions to produce highly efficient stereogenically useful compounds (Simon et al., 2012). However, a major drawback of organocatalysis is low activity compared to organometallic catalysts, therefore,

requiring a larger quantity of catalyst, at least 10 mol % for the reaction. Organocatalysts are basically categorized as either Lewis base, Lewis acid, Brønsted base or Brønsted acid mediated. An important class of Lewis base catalysis is asymmetric enamine catalysis which is regarded as the catalysis of electrophilic substitution reactions in the α -position of carbonyl compounds by primary and secondary amines occurring via enamine intermediates. The versatility of enamines in stoichiometric reactions has been confirmed for α -functionalisation of carbonyl compounds (Stork et al., 1963). However, many scientists have reported employing amino acids and short peptides, especially, proline as an asymmetric organocatalyst. This area of study is still challenging. A few studies have been disclosed usage of emulated peptides of enzymes, particularly, promiscuous enzymes, as organocatalysts in organic reactions.

Peptide can be used as a multipurpose catalyst. Thus, it might catalyze different types of organic reactions. For a long time, hydrolysis of esters has been dominated by acid and base. However, for the last decade, peptides have emerged as important organocatalysts for hydrolysis of esters. . Due to their structural diversity peptides are becoming known as a versatile catalyst with a remarkable ability to catalyze hydrolysis of esters (Tsutsumi et al., 2004).

1.1 Problem Statements

Promiscuous enzymes can only generate stereospecific products with moderate yield and stereoselectivity of carbon-carbon bond forming reaction. Therefore, design and synthesis of mimetic peptides derived from active site of promiscuous enzymes in this research have been considered to enhance stereoselectivity. One of the problems of current organocatalytic methods is the use of high catalyst loading (up to 30 mol%). A large excess of aldehydes or ketones (normally 10-20 equiv) are also required to achieve good catalytic activity and selectivities. Therefore, reaction optimization, design and development of highly active organocatalysts are needed in order to overcome these limitations.

Current study strongly investigated the rational design of several peptides with different length as asymmetric catalyst in aldol and Michael reactions. The role of hydrophilic and hydrophobic amino acids residues and also position of residues have been investigated. Hydrolysis of esters by acids and bases is caused to change configurations of chiral compounds to racemic in the organic synthesis and trouble for industry. Therefore, peptides are excellent alternative to replace of acid and base due to their multifunctionality and mild reaction which is similar to enzymes.

1.2 Goal and objectives of the study

The main goal of this study is to investigate the catalytic activity of mimetic oligopeptides based on a promiscuous enzyme in the carbon-carbon bond forming reactions and also hydrolysis of esters. The objectives were set as follows:

- 1- To evaluate the reactivity and selectivity of promiscuous aldo-ketoreductase (AKRs) enzyme in aldol reaction.
- 2- To design and synthesize mimetic peptides based on AKR active site.
- 3- To utilize the mimetic peptides as asymmetric catalysts in aldol and Michael reactions.
- 4- To optimize the reaction conditions with respect to different parameters, such as solvents and catalyst loading
- 5- To investigate the reusability of the best peptide as asymmetric catalyst in the aldol and Michael reaction.
- 6- To use the best mimetic peptide in hydrolysis of esters.

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