

# Asymmetric Organic Synthesis with Enzymes

*Edited by*

*Vicente Gotor, Ignacio Alfonso, and Eduardo García-Urdiales*



WILEY-VCH Verlag GmbH & Co. KGaA

## Contents

**Preface** X1

**List of Contributors** XIII

1	<b>Methodology</b>	1
1	<b>Medium Engineering</b>	3
	<i>Giacomo Carrea and Sergio Riva</i>	
1.1	Introduction	3
1.2	Modulation of Enzyme Enantioselectivity by Medium Engineering	5
1.2.1	Selectivity Enhancement by Addition of Water-Miscible Organic Cosolvents	5
1.2.2	Selectivity Enhancement in Organic Media with Low Water Activity	7
1.2.2.1	Organic Solvent Systems	7
1.2.2.2	Enzyme Properties in Organic Solvents	8
1.2.2.3	Medium Engineering	9
1.2.3	Rationales	12
1.2.4	Modulation of Enzyme Selectivity: New Trends of Research	14
1.2.4.1	Ionic Liquids	14
1.2.4.2	Additives	16
1.3	Conclusions and Outlooks	17
	References	17
2	<b>Directed Evolution as a Means to Engineer Enantioselective Enzymes</b>	21
	<i>Manfred T. Reetz</i>	
2.1	Introduction	21
2.2	Molecular Biological Methods for Mutagenesis	23
2.3	High-throughput Screening Methods for Enantioselectivity	27

2.4	Examples of Enhancing the Enantioselectivity of Enzymes by Directed Evolution	28
2.4.1	Lipase from <i>Pseudomonas aeruginosa</i> (PAL)	28
2.4.2	Other Lipases	38
2.4.3	Esterases	38
2.4.4	Hydantoinases	39
2.4.5	Nitrilases	39
2.4.6	Epoxide Hydrolases	41
2.4.7	Phosphotriesterases	45
2.4.8	Aminotransferases	45
2.4.9	Aldolases	46
2.4.10	Cyclohexanone and Cyclopentanone Monooxygenases as Baeyer–Villigerases and Sulfoxidation Catalysts	48
2.4.11	Monoamine Oxidases	54
2.4.12	Cytochrome P450 Enzymes	55
2.4.13	Other Enzymes	55
2.5	Conclusions and Perspectives	56
	References	56
<b>3</b>	<b>The Search for New Enzymes</b>	<b>65</b>
	<i>Jean-Louis Reymond and Wolfgang Streit</i>	
3.1	Introduction	65
3.2	Mechanism-based Enzyme Design	66
3.2.1	Catalytic Antibodies	66
3.2.2	Rational Design of New Catalysts on Enzyme and Protein Basis	69
3.2.3	Synthetic Enzyme Models	71
3.3	Metagenomics	71
3.3.1	Construction of Metagenome-derived DNA Libraries	72
3.3.1.1	Selection of the Environment	72
3.3.1.2	Cloning Strategies	73
3.3.1.3	Screening and Detection Technologies	73
3.3.1.4	Major Problems that Need to be Addressed	74
3.3.2	The Genomes of Not Yet Cultured Microbes as Resources for Novel Genes	75
3.3.2.1	Polysaccharide Degrading/Modifying Enzymes	75
3.3.2.2	Lipolytic Biocatalysts	77
3.3.2.3	Vitamin Biosynthesis	77
3.3.2.4	Nitrilases, Nitrile Hydratases, and Amidases	78
3.3.2.5	Oxidoreductases/Dehydrogenases	79
3.3.2.6	Proteases	79
3.3.2.7	Glycerol Hydratases	79
3.3.2.8	Antibiotics and Pharmaceuticals	79
3.4	Conclusion	80
	References	80

II	<b>Synthetic Applications</b>	87
4	<b>Dynamic Kinetic Resolutions</b>	89
	<i>Belen Martín-Matute and Jan-E. Bäckvall</i>	
4.1	Introduction	89
4.1.1	Synthesis of Enantiomerically Pure Compounds	89
4.1.2	Kinetic Resolution (KR) and Dynamic Kinetic Resolution (DKR)	90
4.1.3	Enzymes in Organic Chemistry	91
4.2	Metal-Catalyzed Racemization	92
4.2.1	DKR of Allylic Acetates and Allylic Alcohols	93
4.2.2	DKR of <i>sec</i> -alcohols	94
4.2.3	DKR of Amines	98
4.3	Base-Catalyzed Racemization	98
4.3.1	DKR of Thioesters	99
4.3.2	DKR of Activated Esters	100
4.3.3	DKR of Oxazolones	100
4.3.4	DKR of Hydantoins	101
4.3.5	DKR of Acyloins	101
4.4	Acid-Catalyzed Racemization	101
4.5	Racemization through Continuous Reversible Formation–Cleavage of the Substrate	102
4.5.1	DKR of Cyanohydrins	103
4.5.2	DKR of Hemithioacetals	103
4.6	Racemization Catalyzed by Aldehydes	104
4.7	Enzyme-Catalyzed Racemization	106
4.8	Racemization through S <sub>N</sub> 2 Displacement	106
4.9	Other Racemization Methods	107
4.9.1	DKR of 5-Hydroxy-2-(5 <i>H</i> )-Furanones	107
4.9.2	DKR of Hemiaminals	107
4.9.3	DKR of 8-Amino-5,6,7,8-tetrahydroquinoline	108
4.10	Concluding Remarks	109
	References	110
5	<b>Deracemization and Enantioconvergent Processes</b>	115
	<i>Nicholas J. Turner</i>	
5.1	Introduction	115
5.2	Deracemization Processes	116
5.2.1	Cyclic Oxidation–Reduction Systems	116
5.2.2	Microbial Deracemization of Secondary Alcohols Using a Single Microorganism	122
5.2.3	Deracemization of Alcohols Using Two Enzyme/Microorganism Systems	124
5.2.4	Epoxides	126

5.2.5	Carboxylic Acids	126
5.3	Enantioconvergent Processes	127
5.3.1	Epoxide Hydrolysis	128
5.3.2	Sulfatases	129
5.4	Conclusions and Future Prospects	130
	References	130
<b>6</b>	<b>Transesterification and Hydrolysis of Carboxylic Acid Derivatives, Alcohols, and Epoxides</b>	<b>133</b>
	<i>Robert Chênevert, Pierre Morin, and Nicholas Pelchat</i>	
6.1	Introduction and General Aspects	133
6.1.1	Scope	133
6.1.2	Reaction Conditions	133
6.1.3	Kinetic Resolution, Dynamic Kinetic Resolution, and Desymmetrization	134
6.1.4	Enantioconvergent Transformation	137
6.2	Enantioselective Biotransformations of Carboxylic Acid Derivatives	137
6.2.1	Ester Hydrolysis	137
6.2.2	Ester Alcoholysis	140
6.2.3	Esterification of Carboxylic Acids	140
6.2.4	Lactones	142
6.2.5	Anhydrides	143
6.2.6	Hydrolysis of Nitriles	144
6.2.7	Hydrolysis of Amides, Lactams, and Hydantoins	146
6.3	Enantioselective Enzymatic Transformations of Alcohols	150
6.4	Hydrolysis of Epoxides	157
6.5	Conclusion	162
	References	163
<b>7</b>	<b>Aminolysis and Ammonolysis of Carboxylic Acid Derivatives</b>	<b>171</b>
	<i>Vicente Gotor-Fernández and Vicente Gotor</i>	
7.1	Introduction	171
7.2	Mechanism of Enzymatic Ammonolysis and Aminolysis Reactions	172
7.3	Aminolysis and Ammonolysis of Carboxylic Acids	174
7.4	Aminolysis and Ammonolysis of Esters	176
7.4.1	Preparation of Nonchiral Amides	176
7.4.2	Resolution of Esters	178
7.4.3	Resolution of Amines, Diamines, and Aminoalcohols	180
7.4.4	Desymmetrization of Diesters	184
7.5	Kinetic Resolution of Secondary Amines	185
7.6	Toward the Synthesis of $\beta$ -Aminoacid Derivatives	186
7.7	Summary and Outlook	188
	References	189

<b>8</b>	<b>Enzymatic Reduction Reaction</b>	193
	<i>Kaoru Nakamura and Tomoko Matsuda</i>	
8.1	Introduction	193
8.2	Hydrogen Source for Coenzyme Regeneration	193
8.2.1	Alcohol as a Hydrogen Source of Reduction	194
8.2.2	Sugar as a Hydrogen Source of Reduction	194
8.2.3	Formate as a Hydrogen Source of Reduction	194
8.2.4	Molecular Hydrogen as a Hydrogen Source of Reduction	195
8.2.5	Light Energy as a Hydrogen Source of Reduction	196
8.2.6	Electric Power as a Hydrogen Source of Reduction	198
8.3	Methodology for Stereochemical Control	199
8.3.1	Screening of Microbes	199
8.3.2	Modification of Biocatalysts by Genetic Methods	201
8.3.2.1	Modified Yeast	201
8.3.2.2	Overexpression	202
8.3.2.3	Coexpression of Genes for Carbonyl Reductase and Cofactor-Regenerating Enzymes	203
8.3.2.4	Modification of Biocatalysts: Directed Evolution	204
8.3.3	Modification of Substrates	205
8.3.4	Modification of Reaction Conditions	206
8.3.4.1	Acetone Treatment of the Cell	206
8.3.4.2	Selective Inhibitor	207
8.3.4.3	Reaction Temperature	208
8.4	Medium Engineering	209
8.4.1	Organic Solvent	209
8.4.1.1	Soluble Organic Solvent	209
8.4.1.2	Aqueous-organic Two-phase Reaction	209
8.4.2	Use of Hydrophobic Resin, XAD	211
8.4.3	Supercritical Carbon Dioxide	213
8.4.4	Ionic Liquid	215
8.5	Synthetic Applications	216
8.5.1	Reduction of Aldehyde	216
8.5.2	Reduction of Ketone	216
8.5.3	Dynamic Kinetic Resolution and Deracemization	221
8.5.3.1	Dynamic Kinetic Resolution	221
8.5.3.2	Deracemization through Oxidation and Reduction	223
8.6	Conclusions	224
	References	225
<b>9</b>	<b>Biooxidations in Chiral Synthesis</b>	229
	<i>Marko D. Mihovilovic and Dario A. Bianchi</i>	
9.1	Introduction	229
9.2	Oxidations of Alcohols and Amines	231
9.2.1	Regioselective Oxidation of Alcohols	231
9.2.2	Desymmetrizations of Diols	233

9.2.3	Kinetic Resolution of Primary and Secondary Alcohols	234
9.2.4	Deracemization of Secondary Alcohols	235
9.2.5	Deracemization of Amines	237
9.3	Oxygenation of Nonactivated Carbon Centers	237
9.3.1	Hydroxylations by Wild-Type Whole-Cells	238
9.3.2	Hydroxylations by Recombinant Cytochrome P450 Monooxygenases	239
9.3.3	Hydroxylation via Hydroperoxide Formation	241
9.4	Enzymatic Epoxidation	241
9.5	Baeyer–Villiger Oxidations	243
9.5.1	Chemoselectivity	245
9.5.2	Desymmetrizations	246
9.5.3	Kinetic Resolutions	248
9.5.4	Regiodivergent Biooxidations	251
9.6	Heteroatom Oxidations	253
9.6.1	Sulfide Oxidation	253
9.6.2	Oxidation of Dithio-Compounds	256
9.6.3	Oxidation of other Heteroatoms	256
9.7	Aryl Dihydroxylations	257
9.7.1	Dioxygenation in ortho- and meta-Position	257
9.7.2	Dioxygenation in ipso- and ortho-Position	262
9.8	Halogenation Reactions	263
9.9	Summary and Outlook	265
	References	265
<b>10</b>	<b>Aldolases: Enzymes for Making and Breaking C–C Bonds</b>	<b>275</b>
	<i>Wolf-Dieter Fessner</i>	
10.1	Introduction	275
10.2	Mechanistic Classification	276
10.3	Pyruvate Aldolases	278
10.3.1	N-Acetylneuraminic Acid Aldolase	278
10.3.2	Related Pyruvate Aldolases	281
10.4	Dihydroxyacetone Phosphate Aldolases	284
10.4.1	Fructose 1,6-Bisphosphate Aldolase	285
10.4.2	Related DHAP Aldolases	286
10.4.3	Preparative Applications	288
10.5	Transketolase and Related Enzymes	302
10.6.	2-Deoxy-D-Ribose 5-Phosphate Aldolase	305
10.7	Glycine Aldolases	308
10.8	Summary	311
	References	311