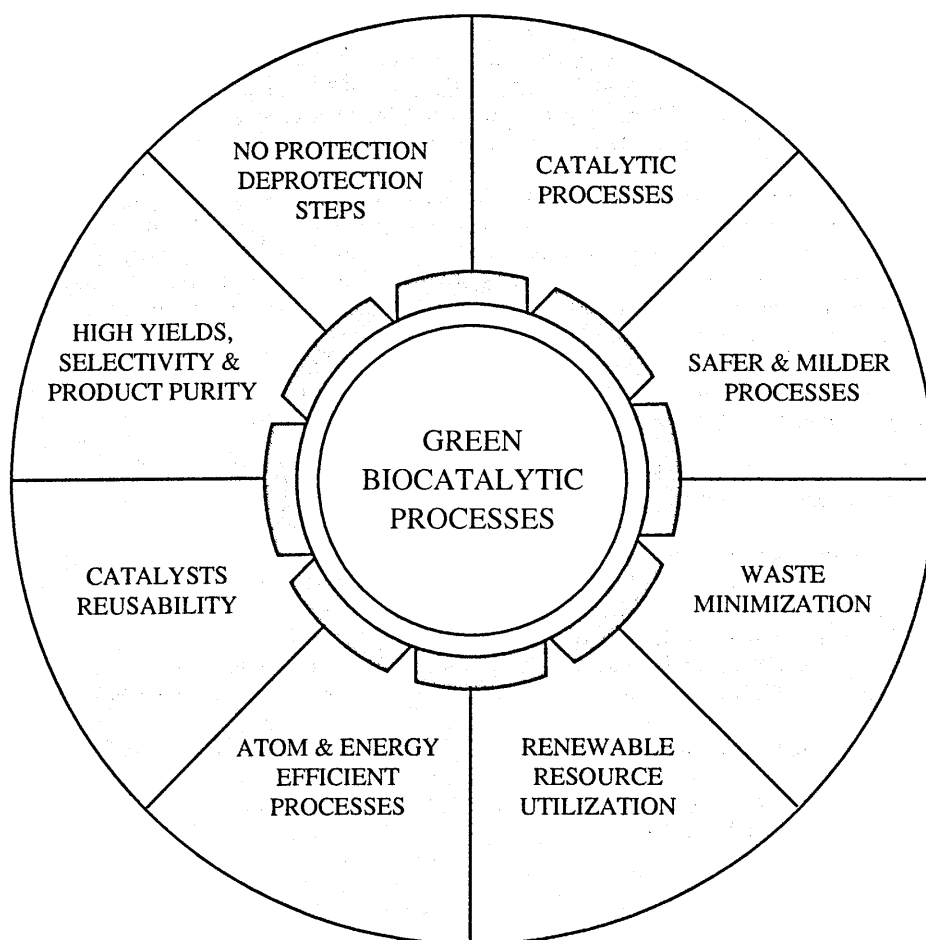


GREEN BIOCATALYSIS

Edited by
Ramesh N. Patel



WILEY

Contents

Preface xix

About the Editor xxiii

Contributors xxv

Chapter 1	<i>Biocatalysis and Green Chemistry</i> <i>Roger A. Sheldon</i>	1
1.1	Introduction to Sustainable Development and Green Chemistry	1
1.2	Green Chemistry Metrics	2
1.3	Environmental Impact and Sustainability Metrics	4
1.4	Solvents	5
1.5	The Role of Catalysis	6
1.6	Biocatalysis and Green Chemistry	6
1.7	Examples of Green Biocatalytic Processes	8
1.7.1	A Chemoenzymatic Process for Pregabalin	8
1.7.2	A Three-Enzyme Process for Atorvastatin Intermediate	8
1.7.3	Enzymatic Synthesis of Sitagliptin	11
1.7.4	Biocatalytic Synthesis of the Fragrance Chemical (–) Ambrox (Ambrafuran)	12
1.8	Conclusions and Future Prospects	13
	References	13
Chapter 2	<i>Enzymatic Synthesis of Chiral Amines using ω-Transaminases, Amine Oxidases, and the Berberine Bridge Enzyme</i> <i>Eduardo Busto, Robert C. Simon, Nina Richter, and Wolfgang Kroutil</i>	17
2.1	Introduction	17
2.2	Synthesis of Chiral Amines using ω -Transaminases	18
2.2.1	ω -Transaminases: Definition and General Facts	18
2.2.2	Stereoselective Transformations Involving ω -TAs	18
2.2.3	Asymmetric Amination of Ketones	19
2.2.4	Asymmetric Amination of Linear Ketones	20
2.2.5	Asymmetric Amination of Cyclic Ketones	21
2.2.6	Application in the Synthesis of Pharmaceutically Active Ingredients	22
2.2.7	Amination of Ketones in Organic Solvents	24
2.2.8	Asymmetric Amination of Keto Acids: Synthesis of Nonnatural Amino Acids	25
2.2.9	Amination of Aldehydes	26

2.2.10	Cascade Reactions Involving ω -TAs	27
2.2.11	Cascades Initiated by ω -TAs: Synthesis of Chiral Heterocycles	27
2.2.12	Multienzyme Cascades Involving ω -TA-Catalyzed Amination of Ketones	30
2.2.13	Deracemization of Primary Amines	32
2.2.14	Perspective	34
2.3	Amine Oxidases	34
2.3.1	Amino Acid Oxidases	35
2.3.2	Cascade Reactions Involving AAOs	38
2.3.3	Monoamine Oxidases	41
2.3.4	Cascade Reactions Involving Monoamine Oxidases	47
2.3.5	Perspective	49
2.4	Berberine Bridge Enzymes	50
2.5	Conclusions	52
	References	53

Chapter 3	<i>Decarboxylation and Racemization of Unnatural Compounds using Artificial Enzymes Derived from Arylmalonate Decarboxylase</i>	59
	<i>Kenji Miyamoto</i>	

3.1	Introduction	59
3.2	Discovery of a Bacterial α -Aryl- α -Methylmalonate Decarboxylase	61
3.3	Purification and Characterization of the Decarboxylase (AMDase)	61
3.4	Cloning of the AMDase Gene	62
3.5	Stereochemical Course of AMDase-Catalyzed Decarboxylation	62
3.6	Directed Evolution of AMDase to an Artificial Profen Racemase	63
3.7	Inversion of Enantioselectivity Dramatically Improves Catalytic Activity	65
3.8	Future Prospects	68
	References	69

Chapter 4	<i>Green Processes for the Synthesis of Chiral Intermediates for the Development of Drugs</i>	71
	<i>Ramesh N. Patel</i>	

4.1	Introduction	71
4.2	Saxagliptin: Enzymatic Synthesis of (S)-N-Boc-3-Hydroxyadamantylglycine	71
4.3	Sitagliptin: Enzymatic Synthesis of Chiral Amine	72
4.4	Vanlev: Enzymatic Synthesis of (S)-6-Hydroxynorleucine	73
4.5	Vanlev: Enzymatic Synthesis of Allysine Ethylene Acetal	74
4.6	Vanlev: Enzymatic Synthesis of Thiazepine	74
4.7	Tigemonam: Enzymatic Synthesis of (S)- β -Hydroxyvaline	76
4.8	Autoimmune Diseases: Enzymatic Synthesis of (S)-Neopentylglycine	76
4.9	Atazanavir: Enzymatic Synthesis of (S)-Tertiary Leucine	77
4.10	Thrombin Inhibitor (Inogatran): Synthesis of (R)-Cyclohexylalanine	78
4.11	Gamma Secretase Inhibitor: Enzymatic Synthesis of (R)-5,5,5-Trifluoronorvaline	79
4.12	NK1/NK2 Dual Antagonists: Enzymatic Desymmetrization of Diethyl 3-[3',4'-Dichlorophenyl] Glutarate	80
4.13	Pregabalin: Enzymatic Synthesis of Ethyl (S)-3-Cyano-5-Methylhexanoate	81
4.14	Chemokine Receptor Modulator: Enzymatic Synthesis of (1S,2R)-2-(Methoxycarbonyl)-Cyclohex-4-ene-1-Carboxylic Acid	82

- 4.15 Enzymatic Synthesis of (3*S*,5*R*)-3-(Aminomethyl)-5-Methyloctanoic Acid 82
- 4.16 Atorvastatin (Lipitor): Enzymatic Desymmetrization of 3-Hydroxyglutaronitrile 83
- 4.17 Anticancer Drugs: Enzymatic Synthesis of Taxane Side Chain 84
- 4.18 Antidiabetic and CNS Drugs: Enzymatic Hydrolysis of Dimethyl Bicyclo[2.2.1] Heptane-1,4-Dicarboxylate 85
- 4.19 Clopidogrel (Plavix): Enzymatic Preparation of 2-Chloromandelic Acid Esters 85
- 4.20 Antiviral Drug: Regioselective Enzymatic Acylation of Ribavirin 86
- 4.21 Anticholesterol Drug: Enzymatic Acylation of Alcohol 87
- 4.22 Saxagliptin: Enzymatic Synthesis of (5*S*)-4,5-Dihydro-1*H*-Pyrrole-1,5 Dicarboxylic Acid, 1-(1,1-Dimethylethyl)-5-Ethyl Ester 88
- 4.23 Montelukast: Synthesis of Intermediate for LTD4 Antagonists 89
- 4.24 Atazanavir: Enzymatic Synthesis of (1*S*,2*R*)-[3-Chloro-2-Hydroxy-1 (Phenylmethyl) Propyl]-Carbamic Acid,1,1-Dimethyl-Ethyl Ester 90
- 4.25 Atorvastatin: Enzymatic Synthesis of (*R*)-4-Cyano-3-Hydroxybutyrate 91
- 4.26 Antianxiety Drug: Enzymatic Synthesis of 6-Hydroxybuspirone 92
- 4.27 Protease Inhibitor: Enzymatic Synthesis of (*R*)-3-(4-Fluorophenyl)-2-Hydroxy Propionic Acid 93
- 4.28 Dermatological and Anticancer Drugs: Enzymatic Synthesis of 2-(*R*)-Hydroxy-2-(1',2',3', 4'-Tetrahydro-1',1',4',4'-Tetramethyl-6'-Naphthalenyl) Acetate 94
- 4.29 Antipsychotic Drug: Enzymatic Reduction of 1-(4-Fluorophenyl)4-[4-(5-Fluoro-2-Pyrimidinyl)1-Piperazinyl]-1-Butanone 95
- 4.30 Cholesterol-Lowering Agents: Enzymatic Synthesis of (3*S*,5*R*)-Dihydroxy-6-(Benzyloxy) Hexanoic Acid, Ethyl Ester 95
- 4.31 Antimigraine Drugs: Enzymatic Synthesis of (*R*)-2-Amino-3-(7-Methyl-1*H*-Indazol-5-yl) Propanoic Acid 96
- 4.32 Antidiabetic Drug (GLP-1 Mimics): Enzymatic Synthesis of (5)-Amino-3-[3-{6-(2-Methylphenyl)} Pyridyl]-Propionic Acid 97
- 4.33 Ephedrine: Synthesis of (*R*)-Phenylacetylcarbinol 98
- 4.34 Zanamivir: Enzymatic Synthesis of *N*-Acetylneuraminic Acid 99
- 4.35 Epivir: Enzymatic Deamination Process for the Synthesis of (2'*R*-*cis*)-2'-Deoxy-3-Thiacytidine 100
- 4.36 HMG-CoA Reductase Inhibitors: Aldolase-Catalyzed Synthesis of Chiral Lactol 101
- 4.37 Boceprevir: Oxidation of 6,6-Dimethyl-3-Azabicyclo[3.1.0]Hexane by Monoamine Oxidase 102
- 4.38 Crixivan: Enzymatic Synthesis of Indandiol 103
- 4.39 Potassium Channel Opener: Preparation of Chiral Epoxide and *trans*-Diol 104
- 4.40 Epothilones (Anticancer Drugs): Epothilone B and Epothilone F 105
- 4.41 β -Adrenergic Blocking Agents: Synthesis of Intermediates for Propranolol and Denopamine 106
- 4.42 Conclusion 106
- References 107

Chapter 5 | *Dynamic Kinetic Resolution of Alcohols, Amines, and Amino Acids*

115

Jusuk Lee, Yoon Kyung Choi, Jaiwook Park, and Mahn-Joo Kim

- 5.1 Introduction 115
 - 5.1.1 Kinetic and Dynamic Kinetic Resolution 115
 - 5.1.2 Enzymes as the Resolution Catalysts for DKR 115
 - 5.1.3 The Enantioselectivity of Enzymes in DKR 116
 - 5.1.4 Metal (Complexes) as the Racemization Catalysts for DKR 117
- 5.2 Dynamic Kinetic Resolution of Secondary Alcohols 119
- 5.3 Dynamic Kinetic Resolution of Amines and Amino Acids 133
- 5.4 Applications of Dynamic Kinetic Resolution 139
- 5.5 Summary 145
- Appendix: List of Abbreviations 145
- References 146

Chapter 6	<i>Recent Developments in Flavin-Based Catalysis: Enzymatic Sulfoxidation</i>	149
	<i>Patricia B. Brondani, Marco W. Fraaije, and Gonzalo de Gonzalo</i>	
6.1	Introduction	149
6.2	Enzymatic Sulfoxidation Catalyzed by Flavoprotein Oxidases	150
6.3	Use of Flavoprotein Monooxygenases for the Synthesis of Chiral Sulfoxides	151
6.3.1	Sulfoxidations Catalyzed by Baeyer–Villiger Monooxygenases	152
6.3.2	Oxidative Processes Employing Styrene Monooxygenases	159
6.3.3	Enzymatic Sulfoxidations Catalyzed by Flavin-Containing Monooxygenases	159
6.4	Asymmetric Sulfoxidation using Flavins as Catalysts	160
6.5	Summary and Outlook	162
	References	163
Chapter 7	<i>Development of Chemoenzymatic Processes: An Industrial Perspective</i>	165
	<i>Rajesh Kumar, Carlos Martinez, Van Martin, and John Wong</i>	
7.1	Introduction	165
7.2	Synthetic Route Design and Integration of Biocatalysis	166
7.3	Screening and Biocatalyst Selection	169
7.4	Chemoenzymatic Process Development	169
7.4.1	Reaction Engineering versus Enzyme Engineering	169
7.4.2	Product Isolation	171
7.4.3	Scale-Up of Enzymatic Processes	172
7.4.4	Enzyme Supply Scenarios	173
7.4.5	Manufacture of APIs using Enzymes: Quality and Safety Aspects	174
7.5	Conclusions	176
	References	176
Chapter 8	<i>Epoxide Hydrolases and their Application in Organic Synthesis</i>	179
	<i>Alain Archelas, Gilles Iacazio, and Michael Kotik</i>	
8.1	Introduction	179
8.2	Sources and Reaction Mechanism of EHs	181
8.2.1	Sources of EHs	181
8.2.2	Heterologous Expression of EHs	182
8.2.3	Reaction Mechanisms of EHs	182
8.3	Directed Evolution and Genetic Engineering of EHs	183
8.4	Immobilized EHs and Reactions in Nonaqueous Media	186
8.4.1	Immobilization of EHs	186
8.4.2	EH-Catalyzed Reactions in Organic Solvent- or Ionic Liquid-Containing Media	188
8.5	Monofunctional Epoxides as Chiral Building Blocks for the Synthesis of Biologically Active Compounds	188
8.5.1	Monosubstituted Aromatic Epoxides	189
8.5.2	Disubstituted Aromatic Epoxides	194
8.5.3	Nonaromatic Epoxides	197
8.5.4	<i>meso</i> -Epoxides	203
8.6	Preparation of Valuable Chiral Building Blocks for the Synthesis of Biologically Active Compounds Starting from Bifunctional Epoxides	204
8.6.1	Halogenated Epoxides	204
8.6.2	Epoxyamide	206
8.6.3	Protected Epoxy Alcohols	206

8.6.4	Epoxy Ester	208
8.6.5	Epoxy Aldehyde	208
8.7	Application to Natural Product Synthesis	210
8.7.1	Disparlure	210
8.7.2	Linalool	210
8.7.3	Bisabolol	211
8.7.4	Frontalin	211
8.7.5	Mevalonolactone	212
8.7.6	Myrcenediol and Beer Aroma	212
8.7.7	Pityol	213
8.7.8	Pestalotin: Jamaican Rum Constituent	214
8.7.9	Panaxxytriol	214
8.7.10	Fridamycin E	215
8.8	Bienzymatic Process Implying One Epoxide Hydrolase	216
8.9	Conclusions	219
	References	220

Chapter 9	<i>Enantioselective Acylation of Alcohol and Amine Reactions in Organic Synthesis</i>	231
	<i>Vicente Gotor-Fernández and Vicente Gotor</i>	

9.1	Introduction	231
9.1.1	General Considerations for Hydrolase-Catalyzed Reactions	231
9.1.2	Serine Hydrolase Mechanism for the Acylation of Alcohols and Amines	232
9.1.3	Use of Organic Solvents for Hydrolase-Catalyzed Acylation Reactions	233
9.2	Enantioselective Acylation of Alcohols	234
9.2.1	Classical Kinetic Resolution of Racemic Alcohols	235
9.2.2	Dynamic Kinetic Resolution of Racemic Alcohols	240
9.2.3	Desymmetrization of Diols	242
9.2.4	Selected Examples of Acylation Reaction with Interest for the Pharmaceutical Industry	243
9.3	Acylation of Amines	248
9.3.1	Kinetic Resolution of Racemic Amines	248
9.3.2	Dynamic Kinetic Resolution of Racemic Amines	252
9.3.3	Selected Examples of Acylation Reactions with Interest for the Pharmaceutical Industry	257
9.4	Conclusions	260
	References	260

Chapter 10	<i>Recent Advances in Enzyme-Catalyzed Aldol Addition Reactions</i>	267
	<i>Pere Clapés</i>	

10.1	Introduction	267
10.2	Pyruvate-Dependent Aldolases	269
10.2.1	N-Acetylneuraminic Acid Aldolase	270
10.2.2	Other Pyruvate-Dependent Aldolases	271
10.2.3	Structure-Guided Pyruvate Aldolase Modification	275
10.3	Dihydroxyacetone Phosphate (DHAP)-Dependent Aldolases, D-Fructose-6-Phosphate Aldolase (FSA) and Transaldolases	276
10.3.1	DHAP-Dependent Aldolases	276
10.3.2	Iminocyclitol, Pipecolic Acids, Homoiminocyclitols, and Aminocyclitol Synthesis	278
10.3.3	Synthesis of Polyhydroxylated Pipecolic Acids and Homoiminocyclitols	281
10.3.4	Aminocyclitol Synthesis	281

- 10.3.5 DHA-Utilizing Enzymes 281
- 10.3.6 Iminocyclitol, Pipecolic Acid, Homoiminocyclitols, and Aminocyclitol Synthesis 284
- 10.3.7 Carbohydrates, Deoxysugars, and Sugar Phosphate Synthesis 284
- 10.4 Threonine Aldolases 287**
 - 10.4.1 2-Deoxy-D-Ribose 5-Phosphate Aldolase 291
- 10.5 Aldol Type Reactions Catalyzed by Non-Aldolases 293**
- 10.6 Computational De Novo Enzyme Design 294**
- 10.7 Conclusions and Perspectives 295**
- References 295**

Chapter 11 | **Enzymatic Asymmetric Reduction of Carbonyl Compounds**

307

Tomoko Matsuda, Rio Yamanaka, and Kaoru Nakamura

- 11.1 Introduction 307**
- 11.2 Mechanisms 307**
- 11.3 Preparation of Biocatalysts 309**
 - 11.3.1 Screening of Enzymes from Culturable Microorganisms 309
 - 11.3.2 Screening of Enzymes using Metagenomes 310
 - 11.3.3 Screening of Enzymes of Microorganisms of Known Genome Data 310
 - 11.3.4 Mutation of Enzymes 311
 - 11.3.5 Hyperthermophilic Enzyme as a Biocatalyst 312
 - 11.3.6 Photosynthetic Organism as a Biocatalyst “Photobiocatalyst” 312
- 11.4 Solvent Engineering 316**
 - 11.4.1 Organic Solvent 316
 - 11.4.2 CO₂ 316
 - 11.4.3 Ionic Liquid 317
- 11.5 Examples for Biocatalytic Asymmetric Reductions 317**
 - 11.5.1 Reduction of Ketones 317
 - 11.5.2 Reduction of Diketones 322
 - 11.5.3 Dynamic Kinetic Resolution Through Reduction 322
- 11.6 Conclusions 325**
- References 326**

Chapter 12 | **Nitrile-Converting Enzymes and their Synthetic Applications**

331

Ludmila Martínková

- 12.1 Introduction 331**
- 12.2 Screening Methodology 332**
 - 12.2.1 Screening Metagenomic Libraries 332
 - 12.2.2 Database Mining 332
 - 12.2.3 Construction of Enzyme Variants 332
- 12.3 Nitrilases 333**
 - 12.3.1 Arylacetonitrilases 334
 - 12.3.2 Aromatic Nitrilases 337
 - 12.3.3 Aliphatic Nitrilases 338
 - 12.3.4 Plant Nitrilases and their Bacterial Homologues 339
- 12.4 Nitrile Hydratases 340**
 - 12.4.1 Fe-type Nitrile Hydratase 340
 - 12.4.2 Co-type Nitrile Hydratase 342
- 12.5 Conclusions 343**
- Acknowledgements 343**
- References 344**

Chapter 13	<i>Biocatalytic Epoxidation for Green Synthesis</i>	351
	<i>Hui Lin, Meng-Yu Xu, Yan Liu, and Zhong-Liu Wu</i>	
13.1	Introduction	351
13.2	Enzymes for Asymmetric Epoxidation	352
13.2.1	Monooxygenases	352
13.2.2	Chloroperoxidases	354
13.3	Application of Bioepoxidation in Organic Synthesis	354
13.3.1	Asymmetric Epoxidation of Aliphatic Alkenes	354
13.3.2	Asymmetric Epoxidation of Aromatic Alkenes	359
13.4	Protein Engineering for Biocatalytic Epoxidation Reaction	362
13.4.1	Screening Methods	363
13.4.2	Examples of Engineered Enzymes for Biocatalytic Epoxidation Reactions	366
13.5	Conclusions and Outlook	367
	Acknowledgments	368
	References	368
Chapter 14	<i>Dynamic Kinetic Resolution via Hydrolase–Metal Combo Catalysis</i>	373
	<i>Pilar Hoyos, Vittorio Pace, María J. Hernáiz, and Andrés R. Alcántara</i>	
14.1	Introduction	373
14.2	DKR of Secondary Alcohols	374
14.2.1	Racemization Catalysts for DKR of <i>sec</i> -Alcohols	374
14.2.2	Synthetic Applications of the DKR of <i>sec</i> -Alcohols	377
14.3	DKR of Amines	386
14.3.1	Racemization Catalyst for the DKR of Amines	387
14.3.2	Synthetic Applications of the DKR of Amines	388
14.4	Conclusion	391
	References	391
Chapter 15	<i>Discovery and Engineering of Enzymes for Peptide Synthesis and Activation</i>	397
	<i>Ana Toplak, Muhammad I. Arif, Bian Wu, and Dick B. Janssen</i>	
15.1	Introduction	397
15.2	Classification of Enzymes for Peptide Coupling	399
15.3	Serine and Cysteine Proteases for Peptide Synthesis	402
15.3.1	Chymotrypsin, Trypsin, and Related Enzymes	402
15.3.2	Subtilisin-Like Enzymes	404
15.3.3	Other Serine Hydrolases	405
15.3.4	Aminopeptidases	405
15.3.5	Peptidases Accepting β -Amino Acids	405
15.3.6	D-Amino Acid-Specific Peptidases	405
15.3.7	Sulfhydryl Peptidases	406
15.3.8	Sortase	407
15.3.9	Metalloproteases in Peptide Synthesis	407
15.3.10	Aspartic Proteases in Peptide Synthesis	408
15.4	Protease Discovery	409
15.4.1	Metagenomics	409
15.4.2	Proteases from Thermophiles	409
15.4.3	Solvent-Tolerant Proteases	409
15.4.4	Proteases from Salt-Resistant Organisms	410
15.5	Proteases Engineered for Improved Synthesis	410
15.5.1	Solvent-Resistant and Thermostable Subtilase Mutants	410
15.5.2	Thermostable Thermolysin Variants	411

- 15.5.3 Increasing Aminolysis to Hydrolysis Ratio by Protein Engineering 411
- 15.5.4 Protein Engineering of Trypsin-Like Proteases 411
- 15.5.5 Computational Design 412
- 15.6 Enzymes for Peptide Terminal Modification 412**
 - 15.6.1 Subtilisins for C-Terminal Peptide Modification 412
 - 15.6.2 C-Terminal Activation by Lipase 413
 - 15.6.3 Peptide Deformylase 414
 - 15.6.4 Peptide Amidases for C-Terminal Modification 414
 - 15.6.5 Enzymes for N-Terminal Modification 415
 - 15.6.6 Enzymes for Peptide Cyclization 415
- 15.7 Conclusions 415**
- References 416**

Chapter 16 | **Biocatalysis for Drug Discovery and Development** 421

Youyun Liang, Mingzi M. Zhang, Ee Lui Ang, and Huimin Zhao

- 16.1 Introduction 421**
- 16.2 Single Enzymatic Reactions 423**
 - 16.2.1 Hydrolytic Reaction 423
 - 16.2.2 Reduction 431
 - 16.2.3 Oxidation 432
 - 16.2.4 C—C Bond-Forming Reaction 434
 - 16.2.5 Michael-Type Reaction 434
 - 16.2.6 Diels–Alder Reaction 435
 - 16.2.7 Pictet–Spengler Reaction 435
 - 16.2.8 Terpene Cyclization 435
 - 16.2.9 Transfer Reaction 436
 - 16.2.10 Fluorination 436
 - 16.2.11 Other Reactions 437
 - 16.2.12 Bifunctional Enzymes 437
- 16.3 Multienzyme Biocatalytic Reactions 437**
 - 16.3.1 One-Pot Cascade Reactions 438
 - 16.3.2 Whole-Cell Biocatalysts 440
 - 16.3.3 Multistep Biocatalytic Conversions 444
- 16.4 Future Perspective: Biocatalysts for the Pharmaceutical Industry 445**
 - 16.4.1 Biocatalyst Discovery: New Enzymes, New Chemistries 446
 - 16.4.2 Biocatalyst Development: Improvement of Desired Properties 447
 - 16.4.3 Integration of Biocatalytic Processes 448
- 16.5 Conclusion 448**
- References 449**

Chapter 17 | **Application of Aromatic Hydrocarbon Dioxygenases** 457

Watumesa A. Tan and Rebecca E. Parales

- 17.1 Introduction 457**
- 17.2 Challenges in Aromatic Hydrocarbon Dioxygenase Applications 457**
- 17.3 Protein Engineering to Improve Enzymatic Activity and Alter Substrate Specificity 459**
- 17.4 Protein Engineering for the Production of Specific Chemicals 464**

17.5	Strain Modification for the Development of New Biodegradation Pathways	467
17.6	Phytoremediation: The Expression of Bacterial Dioxygenases in Plant Systems for Bioremediation Purposes	468
17.7	Concluding Remarks	469
	Acknowledgments	469
	References	469
Chapter 18	<i>Ene-reductases and their Applications</i>	473
	<i>Tanja Knaus, Helen S. Toogood, and Nigel S. Scrutton</i>	
18.1	Introduction	473
18.2	Substrate Classes and Industrial Applications	474
18.3	Multienzyme Reactions	478
18.4	Alternative Hydride Sources	479
18.5	Improvements of Productivity, Stereoselectivity, and/or Conversion	482
	References	486
Chapter 19	<i>Recent Developments in Aminopeptidases, Racemases, and Oxidases</i>	489
	<i>Yasuhisa Asano, Seiji Okazaki, and Kazuyuki Yasukawa</i>	
19.1	Aminopeptidase	489
19.1.1	Discovery of D-Stereospecific Aminopeptidase and its Utilization for Dynamic Kinetic Resolution	489
19.1.2	Discovery of D-Aminopeptidase, D-Amino Acid Amidase, and Alkaline D-Peptidase	489
19.1.3	Structure of D-Aminopeptidase (DAP)	490
19.1.4	Structure of D-Amino Acid Amidase (DaaA)	491
19.2	Racemase	492
19.2.1	Synthesis of D-Amino Acids by Optical Resolution and Dynamic Kinetic Resolution	492
19.2.2	Structure of ACL Racemase	494
19.2.3	<i>In Silico</i> Identification of ACL Racemases	495
19.3	Amino Acid Oxidase	495
19.3.1	Development of Novel R-Stereoselective Amine Oxidase	495
19.3.2	Design of R-Stereoselective Amine Oxidase	497
19.3.3	Deracemization Reaction with R-Stereoselective AOx	498
19.3.4	Structure of the Mutant Porcine Kidney D-Amino Acid Oxidase (Y228L, R283G)	499
	References	500
Chapter 20	<i>Biocatalytic Cascades for API Synthesis</i>	503
	<i>John M. Woodley</i>	
20.1	Introduction	503
20.2	Multienzymatic Biocatalysis	504
20.2.1	Rationale	504
20.2.2	Biocatalytic Cascade Concepts	505
20.3	Process Aspects for Multistep Biocatalysis	506
20.3.1	Balancing Reaction Schemes	507
20.3.2	Biocatalytic Reactor Options	507
20.3.3	Process Intensification	508
20.3.4	Continuous Processes	508
20.3.5	Process Integration	509

20.4	Process Development	511
20.5	Biocatalytic Cascade Examples	512
20.5.1	Linear Cascades	512
20.5.2	Parallel Cascades	513
20.5.3	Cyclic Cascades	513
20.5.4	Orthogonal Cascades	513
20.5.5	Linear–Parallel	514
20.5.6	Linear–Cyclic	514
20.5.7	Complex Cascades	514
20.5.8	Convergent Parallel Cascade	514
20.6	Future Outlook	515
20.6.1	Protein Engineering	515
20.6.2	Flow Chemistry and Process Intensification	516
	References	516

Chapter 21	<i>Yeast-Mediated Stereoselective Synthesis</i>	519
	<i>René Csuk</i>	

21.1	Introduction	519
21.2	Reductions of Aldehydes and Ketones	521
21.3	Reduction of Thiocarbonyls or Sulfur-Containing Compounds	524
21.4	Reduction of Functionalized Carbonyl and Dicarboxyl Compounds	524
21.5	Reduction of Keto Esters	527
21.6	Hydrolysis of Esters	529
21.7	Immobilized Baker's Yeast	530
21.8	Whole-Cell Biocatalysis in Ionic Liquids and Deep Eutectic Solvents	531
21.9	C–C Bond-Forming and Breaking Reactions	532
21.10	Miscellaneous Reactions	533
21.11	Conclusions	534
	References	534

Chapter 22	<i>Biocatalytic Introduction of Chiral Hydroxy Groups using Oxygenases and Hydratases</i>	545
	<i>Jun Ogawa, Makoto Hibi, and Shigenobu Kishino</i>	

22.1	Introduction	545
22.2	Regio- and Stereoselective Hydroxylation of Propylbenzene and 3-Chlorostyrene by Cytochrome P450 BM-3 and its Mutant	546
22.3	Regio- and Stereoselective Hydroxylation of Aliphatic Amino Acids by Fe(II)/α-Ketoglutarate-Dependent Dioxygenases	547
22.3.1	L-Isoleucine 4-Hydroxylase	547
22.3.2	Fe/ α KG-DOs Closely Homologous with L-Isoleucine 4-Hydroxylase	548
22.3.3	L-Leucine 5-Hydroxylase	549
22.3.4	N-Succinyl L-Leucine 3-Hydroxylase	549
22.3.5	Catalytic Properties of the Aliphatic Amino Acid Hydroxylases	550
22.3.6	Practical Use of Fe(II)/ α -Ketoglutarate-Dependent Dioxygenases Coupled with Cosubstrate Generation System	550
22.4	Regio- and Stereoselective Hydration of Unsaturated Fatty Acids by a Novel Fatty Acid Hydratase	551
22.4.1	Linoleic Acid Δ 9 Hydratase	552
22.4.2	Efficient Enzymatic Production of Hydroxy Fatty Acids by Linoleic Acid Δ 9 Hydratase	553
22.5	Conclusion	553
	Acknowledgment	553
	References	553

Chapter 23	<i>Asymmetric Synthesis with Recombinant Whole-Cell Catalyst</i> <i>Harald Gröger, Werner Hummel, and Severin Wedde</i>	557
23.1	Introduction	557
23.2	The Design/Construction of Whole-Cell Catalysts	558
23.3	Biotransformations with Whole-Cell Catalysts	561
23.3.1	Hydrolysis Reactions	561
23.3.2	Hydration and Dehydration Reactions	563
23.3.3	C–C Bond-Forming Reactions	565
23.3.4	Reduction Reactions	568
23.3.5	Oxidation Reactions	576
23.4	Conclusion	581
	References	581
Chapter 24	<i>Lipases and Esterases as User-Friendly Biocatalysts in Natural Product Synthesis</i> <i>Kenji Mori</i>	587
24.1	Introduction	587
24.2	Desymmetrization of Prochiral or <i>meso</i> -Diols and Diacetates	587
24.2.1	Desymmetrization of <i>meso</i> -Compounds with 1,2-Stereogenic Centers	588
24.2.2	Desymmetrization of <i>meso</i> -Compounds with 1,3- and 1,5-Stereogenic Centers	590
24.2.3	Desymmetrization of Prochiral Compounds with a Single Stereogenic Center	591
24.3	Kinetic Resolution of Racemic Alcohols	592
24.3.1	Kinetic Resolution of (±)-Primary Alcohols	592
24.3.2	Kinetic Resolution of Acyclic (±)-Secondary Alcohols	593
24.3.3	Kinetic Resolution of Cyclic (±)-Secondary Alcohols	596
24.4	Preparation of Enantiopure Intermediate(s) from a Mixture of Stereoisomers	599
24.4.1	(1 <i>S</i> ,4 <i>R</i>)-4- <i>t</i> -Butyldimethylsilyloxy-3-Chloro-2-Cyclopenten-1-ol (54)	599
24.4.2	(4 <i>R</i> ,5 <i>S</i>)-5-Hydroxy-4-Methyl-3-Hexanone (55)	599
24.4.3	(3 <i>R</i> ,14 <i>R</i> ,26 <i>R</i>)-3,26-Diacetoxy-14-Methyl-1,2-bis(trimethylsilyl)octacos-4,24-Diene-1,27-Diyne (60)	600
24.5	Conclusion	601
	Acknowledgments	601
	References	601
Chapter 25	<i>Hydroxynitrile Lyases for Biocatalytic Synthesis of Chiral Cyanohydrins</i> <i>Romana Wiedner, Helmut Schwab, and Kerstin Steiner</i>	603
25.1	Introduction	603
25.2	Discovery of Hydroxynitrile Lyases: Bioprospecting	604
25.2.1	Screening Plants Based on Detection of Activity	605
25.2.2	Isolation of HNL Proteins and Identification of the Encoding Genes	605
25.2.3	Database Mining	605
25.2.4	Heterologous Expression	609
25.3	Applications of Hydroxynitrile Lyases	609
25.3.1	Cyanohydrins	609
25.3.2	β-Nitro Alcohols	610
25.4	Structural and Mechanistic Aspects	611
25.5	Engineering of Hydroxynitrile Lyases	612
25.5.1	Substrate Scope, Activity, and Enantioselectivity	612
25.5.2	Stability	619
25.5.3	Expression	619
25.5.4	New Catalytic Activities	620

25.6	Reaction Engineering and Reaction Systems	620
25.6.1	Reaction Systems	620
25.6.2	Immobilization of HNLs	622
25.7	Conclusion	623
	Acknowledgment	623
	References	624

Chapter 26	<i>Biocatalysis: Nitrilases in Organic Synthesis</i>	629
	<i>Jin-Song Gong, Jin-Song Shi, and Zheng-Hong Xu</i>	

26.1	Introduction	629
26.2	Nitrilase Discovery	630
26.2.1	Conventional Screening	630
26.2.2	Metagenomic Mining	630
26.2.3	Genome Mining	630
26.3	Nitrilase Improvement	631
26.3.1	Culture Optimization	631
26.3.2	Nitrilase Reengineering	632
26.4	Applications in Organic Synthesis	635
26.4.1	Production of Glycolic Acid	635
26.4.2	Production of Iminodiacetic Acid	635
26.4.3	Production of Indole-3-Acetic Acid	636
26.4.4	Conversion of Phenylacetonitrile and its Derivates	636
26.4.5	Regioselective Hydrolysis of Dinitriles	637
26.4.6	Degradation of Benzonitrile Herbicides	638
26.5	Conclusions and Future Prospects	638
	Acknowledgments	639
	References	639

Chapter 27	<i>Biotechnology for the Production of Chemicals, Intermediates, and Pharmaceutical Ingredients</i>	643
	<i>Hans-Peter Meyer</i>	

27.1	Introduction	643
27.2	Value Chains and Markets	645
27.2.1	Pharmaceuticals	647
27.2.2	Medical Technology (MedTech)	650
27.2.3	Food and Feed	650
27.2.4	Flavor and Fragrance	652
27.2.5	Cosmetics and Personal Care	653
27.2.6	Polymers	654
27.2.7	Surfactants and Lubricants	657
27.2.8	Commodity Chemicals	658
27.2.9	Energy	659
27.2.10	Other Markets and Products	660
27.3	The Toolbox	661
27.3.1	The Current Toolbox	661
27.3.2	The Future Toolbox	662
27.4	Sustainability, Green Premium Pricing, and Subsidies	665
27.5	Regulatory Aspects and Public Perception	667
27.6	Innovation (Not Only in the Laboratory!)	669
27.7	Conclusions	670
	Acknowledgments	671
	References	671

Chapter 28	<i>Microbial Transformations of Pentacyclic Triterpenes</i>	675
	<i>Robert Azerad</i>	
28.1	Introduction	675
28.2	Typical Biotransformations in the Lupane Family	677
28.3	Typical Biotransformations in the Oleanane Family	680
28.4	Typical Biotransformations in the Ursane Family	692
28.5	Microbial Transformations of Other PTs	704
28.6	Glycosylations and Deglycosylations	704
28.7	Conclusion and Perspectives	710
	References	710
Chapter 29	<i>Transaminases and their Applications</i>	715
	<i>Sarah-Marie Dold, Christoph Syltatk, and Jens Rudat</i>	
29.1	Introduction	715
29.2	General Properties of Transaminases	715
29.2.1	Classification as Pyridoxal-5'-Phosphate-Dependent Enzymes	716
29.2.2	Classification Based on Substrate Scope	716
29.2.3	Reaction Mechanism	717
29.2.4	Enantioselectivity of Transaminases	718
29.3	Synthesis Strategies with Transaminases	719
29.3.1	Synthesis of Chiral Amines	720
29.3.2	Synthesis of Canonical and Noncanonical Amino Acids	729
29.3.3	Synthesis of β -Amino Acids	731
29.3.4	Synthesis of Amino Alcohols	733
29.3.5	Transaminase-Catalyzed Reactions with Whole Cells	733
29.4	Approaches to Optimize the Transaminase-Catalyzed Reactions	735
29.4.1	Protein Engineering by Rational Enzyme Design	736
29.4.2	Protein Engineering by Directed Evolution	736
29.4.3	Immobilization of Transaminases	738
29.4.4	Process Development: A Fast Way to Identify Appropriate Transaminases	741
29.4.5	ω -Transaminases in Organic Solvents	742
29.5	Conclusion	743
	References	743
	<i>Index</i>	747