Contents

Preface *XV*

List of Contributors *XVII*

1 Fluorescence Assays for Biotransformations *1*
Jean-Louis Reymond
1.1 Introduction *1*
1.2 Alcohol Dehydrogenases (ADHs) and Aldolases *2*
1.2.1 Chiral Fluorogenic ADH Substrates *2*
1.2.2 Fluorogenic Aldolase Probes *3*
1.2.3 Transaldolases and Transketolases *4*
1.2.4 Enolase Probe *4*
1.3 Lipases and Esterases *5*
1.3.1 Assays on Solid Support *6*
1.3.2 The Clips-O Substrates with Periodate *8*
1.3.3 Esters of Fluorogenic Cyanohydrins and Hydroxyketones *9*
1.3.4 Fluorogenic Acyloxymethyl Ethers *10*
1.3.5 FRET-Lipase Probes *11*
1.4 Other Hydrolases *11*
1.4.1 Epoxide Hydrolases *11*
1.4.2 Amidases and Proteases *13*
1.4.3 Phosphatases *14*
1.5 Baeyer–Villigerases *15*
1.6 Conclusion *15*
Acknowledgment *16*
References *16*

2 Immobilization as a Tool for Improving Enzymes *21*
Ulf Hanefeld
2.1 Introduction *21*
2.2 Adsorption/Electrostatic Interactions *22*
2.2.1 Van der Waals Interactions *22*
2.2.2 Hydrogen Bonds *25*

Modern Biocatalysis: Stereoselective and Environmentally Friendly Reactions.
Edited by Wolf-Dieter Fessner and Thorleif Anthonsen

ISBN: 978-3-527-32071-4

2.2.3 Ionic Interactions *27*
2.3 Encapsulation *31*
2.4 Covalent Binding/Cross-linking *33*
2.5 Conclusion *38*
Acknowledgments *38*
References *39*

3 Continuous-flow Microchannel Reactors with Surface-immobilized Biocatalysts *43*
Malene S. Thomsen and Bernd Nidetzky
3.1 Introduction *43*
3.2 Biocatalytic Synthesis Using Microreaction Technology with Free and Immobilized Enzymes *44*
3.3 Novel Microfluidic Immobilized Enzyme Reactors *45*
3.3.1 Microreactor Design *45*
3.3.2 Enzyme Immobilization *47*
3.4 Enzymatic Hydrolysis of Lactose *48*
3.4.1 Catalytic Effectiveness of Immobilized CelB *48*
3.4.2 Continuous Conversion of Lactose *48*
3.5 Biocatalytic Process Intensification Using Microreaction Technology *50*
3.6 Conclusions and Outlook *51*
Acknowledgements *52*
References *52*

4 Activity and Stability of Proteases in Hydrophilic Solvents *55*
Lars Haastrup Pedersen, Sinthuwat Ritthitham, and Morten Kristensen
4.1 Introduction *55*
4.2 Activity and Selectivity of Proteases in Synthesis of Carbohydrate Fatty Acid Esters *56*
4.3 Enzyme Stability and Conformation *59*
4.4 Solvent Engineering *63*
4.5 Conclusion *64*
References *65*

5 Importance of Enzyme Formulation for the Activity and Enantioselectivity of Lipases in Organic Solvents *67*
Francesco Secundo
5.1 Introduction *67*
5.2 Lipase Formulations and their Activity and Enantioselectivity in Neat Organic Solvent *68*
5.3 Why do Additives Affect the Activity and Enantioselectivity of Lipases in Organic Solvent? *73*
5.4 Conclusions *76*
References *76*

6 Direct Esterification with Dry Mycelia of Molds: a (Stereo)selective, Mild and Efficient Method for Obtaining Structurally Diverse Esters *79*
Francesco Molinari, Diego Romano, Raffaella Gandolfi, Lucia Gardossi, Ulf Hanefeld, Attilio Converti and Patrizia Spizzo
6.1 Mycelia and Biotransformations in Organic Media *79*
6.2 Screening and Microbiological Aspects *79*
6.3 Production of Acetate *81*
6.4 Stereoselective Esterifications of Racemic Alcohols *83*
6.5 Stereoselective Esterifications of Racemic Carboxylic Acids *85*
6.6 Partition Phenomena and Equilibrium of Esterification Reactions *88*
6.7 Conclusions *91*
References *91*

7 Factors Affecting Enantioselectivity: Allosteric Effects *93*
Elisabeth Egholm Jacobsen and Thorleif Anthonsen
7.1 How to Provide Enantiopure Compounds *93*
7.1.1 Kinetic Resolution of Racemic Mixtures Catalyzed by Enzymes *94*
7.1.2 Absolute Configurations in Resolution *95*
7.2 Factors Affecting the Enantiomeric Ratio *E* *96*
7.2.1 Is the *E*-value Really Constant? *96*
7.2.2 Influence of the Reaction Medium on the *E*-value *96*
7.2.3 Influence of Enzyme Immobilization on the *E*-value *97*
7.2.4 Enzyme Inhibition *97*
7.2.5 Enantioselective Inhibition and Activation: Allosteric Effects *97*
7.2.6 The *E*-value of CALB is Influenced by R-Alcohols *99*
7.2.7 Is a Changing *E* Caused by the Slow or the Fast Enantiomer? *102*
7.3 Asymmetrization of Prochiral Compounds *103*
7.3.1 Asymmetrization of Prochiral Dicarboxylates: Single-Step Process *103*
7.3.2 Asymmetrization of Prochiral Diol: Double-Step Process *105*
7.3.3 Is the e.e. Constant During Asymmetrization Reactions? *105*
7.4 Conclusions *106*
References *107*

8 Kinetic Resolution of *Sec*-alcohol in Non-conventional Media *109*
Maja Habulin, Mateja Primožič and Željko Knez
8.1 Introduction *109*
8.2 SCFs – Replacement for Organic Solvents in Biocatalysis *111*
8.3 Effect of Pressure *112*
8.4 Effect of the Acyl Donor/Alcohol Molar Ratio *114*
8.5 ILs – Solvents for Sustainable Technology in Biocatalysis *114*
8.6 ILs, Based on the N, N'-Dialkylimidazolium Cations as Reaction Media *116*
8.7 ILs/SCFs Biphasic Systems as Promising Media for Biocatalysis *117*

8.8 The [bmim][PF_6]/SC-CO_2 System as a Reaction Medium *117*
8.9 Effect of Acyl Donor Concentration *119*
8.10 Conclusion *120*
References *120*

9 Strategies for the Biocatalytic Lipophilization of Phenolic Antioxidants *123*
Maria H. Katsoura, Eleni Theodosiou, Haralambos Stamatis and Fragiskos N. Kolisis
9.1 Introduction *123*
9.2 Materials and Methods *125*
9.2.1 Materials *125*
9.2.2 Enzymatic Acylation Procedure *125*
9.2.3 Analytical Methods *125*
9.2.4 Purification and Chemical Structure Determination of Esters *125*
9.3 Results and Discussion *125*
9.3.1 Modification of Natural Antioxidants in Organic Solvents *126*
9.3.1.1 Enzymatic Acylation of Rutin and Silybin with Dicarboxylic Acids *126*
9.3.1.2 Effect of Organic Solvent *127*
9.3.1.3 Effect of Substrate Concentration *127*
9.3.2 Modification of Natural Antioxidants in Ionic Liquid Media *128*
9.3.2.1 Enzymatic Acylation of Natural Polyhydroxylated Compounds *128*
9.3.2.2 Effect of Substrate Concentration *130*
9.3.2.3 Effect of Acyl Donor Nature: Synthesis of Hybrid Antioxidants *130*
9.4 Conclusions *131*
References *132*

10 Biocatalysis Applied to the Synthesis of Nucleoside Analogs *135*
Vicente Gotor
10.1 Introduction *135*
10.2 Chemoenzymatic Modification of the Sugar *136*
10.3 Resolution and Anomeric Separation *143*
10.4 Biotransformations that Modify the Base *145*
10.5 Transglycosylation for the Synthesis of Nucleosides *147*
10.6 Summary *149*
References *150*

11 Efficient Fructooligosaccharide Synthesis with a Fructosyltransferase from *Aspergillus aculeatus* *153*
Francisco J. Plou, Miguel Alcalde, Iraj Ghazi, Lucía Fernández-Arrojo and Antonio Ballesteros
11.1 Introduction *153*
11.2 Purification of Fructosyltransferase in Pectinex Ultra SP-L *155*
11.3 Properties of Fructosyltransferase from *A. aculeatus* *157*

11.3.1 Substrate Specificity *157*
11.3.2 Effect of pH and Temperature *158*
11.3.3 Influence of Chemicals *158*
11.3.4 Kinetic Behavior *159*
11.3.5 Fructooligosaccharide Production *159*
11.4 Immobilization of Fructosyltransferase from *A. aculeatus* *161*
11.4.1 Sepabeads EC-EP as Immobilization Carriers *161*
11.4.2 Effect of pH and Ionic Strength on Immobilization *162*
11.4.3 Application of Immobilized Biocatalysts to Fructooligosaccharide Synthesis *164*
11.5 Fructooligosaccharide Production Using Sugar Beet Syrup and Molasses *164*
11.5.1 Sugar Beet Syrup and Molasses as Low-cost Feedstock for Fructooligosaccharide Synthesis *164*
11.5.2 Batch Production of Fructooligosaccharide *167*
11.6 Conclusions *168*
Acknowledgments *168*
References *168*

12 Hydantoin Racemase: the Key Enzyme for the Production of Optically Pure α-Amino Acids *173*
Francisco Javier Las Heras-Vázquez, Josefa María Clemente-Jiménez, Sergio Martínez-Rodríguez and Felipe Rodríguez-Vico
12.1 Introduction *173*
12.2 Search for New Hydantoin Racemases and Molecular Characterization *175*
12.3 Biochemical Characterization of Hydantoin Racemase Enzymes *180*
12.4 Substrate Enantioselectivity and Kinetic Analysis of Hydantoin Racemases *181*
12.5 Proposal for a Reaction Mechanism of Hydantoin Racemase Enzymes *183*
12.6 Design of a Tailormade Recombinant Biocatalyst Including Hydantoin Racemase Enzymes for Optically Pure D-Amino Acid Production *187*
Acknowledgments *192*
References *192*

13 Chemo-enzymatic Deracemization Methods *195*
Davide Tessaro, Gianluca Molla, Loredano Pollegioni and Stefano Servi
13.1 Introduction *195*
13.2 Deracemization Methods for α- and β-Hydroxy Acids *196*
13.2.1 Deracemization of Hydroxy Acids by DKR (Hydrolytic Enzymes + Ruthenium-based Racemization Catalysts) *197*
13.2.2 Deracemization of Hydroxy Acids by DKR with a Two-enzyme System *198*
13.2.3 Deracemization of Hydroxy Acids by Stereoinversion *199*

13.2.4 Deracemization of Hydroxy Acids by Microbial Stereoinversion *200*
13.3 Deracemization of α-Hydroxy Nitriles *201*
13.4 Deracemization of α-Amino Acids *202*
13.4.1 Deracemization of α-Amino Acids by Stereoinversion *202*
13.4.1.1 Deracemization by Stereoinversion via the Two-enzyme System D-Amino Acid Oxidase and L-Amino Transferase *202*
13.4.1.2 Deracemization by Stereoinversion via the Two-enzyme System D-Amino Acid Oxidase and L-Leucine Dehydrogenase *204*
13.4.1.3 Deracemization by Stereoinversion via the Three-enzyme System L-Amino Acid Oxidase, D-Amino Transferase and Amino Acid Racemase *204*
13.4.2 Deracemization of α-Amino Acids via DKR *205*
13.4.2.1 Deracemization of α-Amino Acids via Enzyme-catalyzed DKR Coupled with *In Situ* Racemization *205*
13.5 Useful Enzymes for Deracemization Methods *213*
13.5.1 Amino Acid Oxidases *213*
13.5.1.1 D-Amino Acid Oxidase (EC 1.4.3.3) *213*
13.5.1.2 L-Amino Acid Oxidase (EC 1.4.3.2) *216*
13.5.2 Amino Acid Racemases *217*
13.5.2.1 PLP-dependent Racemases *217*
13.5.2.2 PLP-independent Racemases *220*
13.5.2.3 Mandelate Racemase (EC 5.1.2.2) *221*
13.5.3 Transaminases *221*
13.5.3.1 L-Amino Transferases (EC 2.6.1.x) *222*
13.5.3.2 D-Amino Transferases (EC 2.6.1.21) *223*
13.6 Summary and Outlook *223*
References *223*

14 Nitrilases from Filamentous Fungi *229*
Ludmila Martínková, Vojtech Vejvoda, Ondrej Kaplan, Vladimír Kren, Karel Bezouška and Maria Cantarella
14.1 Introduction *229*
14.2 Distribution and Evolutionary Relationship of Fungal Nitrilases *230*
14.2.1 Molecular Genetic Analysis *230*
14.2.2 Selection and Screening of Nitrilase Activity *232*
14.3 Structural Properties *234*
14.4 Catalytic Properties *236*
14.4.1 Reaction Mechanism *236*
14.4.2 Substrate Specificity *238*
14.4.3 Activity and Stability *240*
14.5 Conclusions and Outlook *242*
Acknowledgment *243*
References *243*

15 Nitrilase- and Nitrile Hydratase-catalyzed Enantioselective Preparation of Non-proteinogenic Amino Acids *247*
Norbert Klempier and Margit Winkler
15.1 Introduction *247*
15.2 Nitrile Hydratase/Amidase Biotransformations *249*
15.2.1 Protecting Groups for Amino Nitriles *249*
15.2.2 Enantioselective Hydrolysis of β-Amino Nitriles *250*
15.3 Nitrilase Biotransformations *253*
15.3.1 Enantioselective Hydrolysis of β-Amino Nitriles *253*
15.3.2 Enantioselective Hydrolysis of γ-Amino Nitriles *255*
15.3.3 Nitrile Hydratase Activity of Nitrilases *257*
References *258*

16 Nitrilases in the Enantioselective Synthesis of α-Hydroxycarboxylic Acids *261*
Fred van Rantwijk, Cesar Mateo, Andrzej Chmura, Bruno C. M. Fernandes and Roger A. Sheldon
16.1 Routes to Enantiomerically Pure α-Hydroxycarboxylic Acids *261*
16.2 Nitrilase-mediated Hydrolysis of Cyanohydrins *262*
16.3 A Bienzymatic Approach to Enantiopure 2-Hydroxycarboxylic Acids *264*
16.4 Stabilization of NLases as Cross-linked Enzyme Aggregates *265*
16.5 Hydrocyanation and Hydrolysis in a Bienzymatic Cascade *265*
16.6 Nitrilases Acting as Nitrile Hydratases *267*
16.7 Conclusion *270*
Acknowledgments *271*
References *271*

17 UF-Membrane Bioreactors for Kinetics Characterization of Nitrile Hydratase–Amidase-catalyzed Reactions: a Short Survey *273*
Maria Cantarella, Alberto Gallifuoco, Agata Spera, Laura Cantarella, Ondřej Kaplan and Ludmila Martínková
17.1 Introduction *273*
17.2 Experiment Design *275*
17.3 Temperature Dependence of the Nitrile Hydratase–Amidase Cascade System *275*
17.4 CSMR Investigations *277*
17.5 Substrate Concentration Effects on the Reaction Rate, Enzyme Stability, Substrate Conversion, and Reactor Capacity *279*
17.6 Concluding Remarks *283*
Acknowledgments *285*
References *285*

18 Enzymes Catalyzing C—C Bond Formation for the Synthesis of Monosaccharide Analogs *287*
Laurence Hecquet, Virgil Hélaine, Franck Charmantray and Marielle Lemaire
18.1 Introduction *287*
18.2 Recent Syntheses Involving Transketolase and Fructose-1,6-*bis*phosphate Aldolase *287*
18.2.1 DHAP Syntheses *288*
18.2.1.1 DHAP Synthesis from Dihydroxyacetone *289*
18.2.1.2 DHAP Synthesis from Rac-Glycidol *290*
18.2.2 Synthesis of Aminocyclitols *291*
18.2.3 Synthesis of 5-D-Xylulose and 5-D-Xylose Analogs *293*
18.2.3.1 Synthesis of 5-halo-D-xylulose *293*
18.2.3.2 Synthesis of 5-thio-D-xylopyranose 21 *293*
18.3 Modification of Substrate Specificity of Yeast Transketolase *295*
18.4 Conclusion *296*
References *297*

19 Novel Strategies in Aldolase-catalyzed Synthesis of Iminosugars *299*
Pere Clapés, Georg A. Sprenger and Jesús Joglar
19.1 Introduction *299*
19.2 DHAP-Aldolase-mediated Synthesis of Iminosugars from *N*-Cbz-amino Aldehydes *301*
19.2.1 Reaction Media *301*
19.2.2 Aldolase-catalyzed Aldol Additions of DHAP to N-Cbz-Amino Aldehydes *301*
19.2.3 Effect of N-Protecting Groups *304*
19.2.4 Synthesis of Iminosugars: Reductive Amination *306*
19.3 D-Fructose-6-Phosphate Aldolase as Catalyst for Iminosugar Synthesis *307*
19.4 Summary and Outlook *309*
References *309*

20 Biocatalytic Asymmetric Oxidations with Oxygen *313*
Roland Wohlgemuth
20.1 Introduction *313*
20.2 Biocatalytic Asymmetric Oxidations with Oxidases *317*
20.3 Biocatalytic Asymmetric Oxidations with Peroxidases *319*
20.4 Biocatalytic Asymmetric Oxidations with Dehydrogenases *320*
20.5 Biocatalytic Asymmetric Oxidations with Monooxygenases *321*
20.6 Biocatalytic Asymmetric Oxidations with Dioxygenases *325*
20.7 Biocatalytic Asymmetric Oxidations with Other Enzymes *328*
20.8 Outlook *331*
Acknowledgments *331*
References *332*

21 Second Generation Baeyer–Villiger Biocatalysts *339*
Veronique Alphand, Marco W. Fraaije, Marko D. Mihovilovic and Gianluca Ottolina
21.1 Introduction *339*
21.2 BVMO Enzyme Platform *341*
21.3 Engineering of BVMOs *342*
21.4 Baeyer–Villiger Biooxidation in Synthetic Chemistry *347*
21.4.1 Chemoselectivity *347*
21.4.2 Dynamic Kinetic Resolutions *350*
21.4.3 Regio- and Stereoselectivity *351*
21.4.4 Natural Product and Bioactive Compound Synthesis *354*
21.5 BVMOs in Stereoselective Sulfoxidations *357*
21.6 Towards a Technology Platform *358*
21.6.1 Fermentation Up-Scaling *358*
21.6.1.1 Whole Cells *358*
21.6.1.2 Enzyme *361*
21.6.2 Immobilization of BVMOs *361*
21.6.3 Self-sufficient Fusion Protein BVMOs *361*
21.7 Outlook *363*
References *363*

Index *369*