

## Contents

### Preface *xi*

|          |                                                                                                  |          |
|----------|--------------------------------------------------------------------------------------------------|----------|
| <b>1</b> | <b>Enzymatic Synthesis of Nucleoside Analogues by Nucleoside Phosphorylases</b>                  | <b>1</b> |
|          | <i>Sarah Kamel, Heba Yehia, Peter Neubauer, and Anke Wagner</i>                                  |          |
| 1.1      | Introduction                                                                                     | 1        |
| 1.1.1    | Nucleosides and Nucleoside Analogues                                                             | 1        |
| 1.1.2    | Enzymes Involved in the Enzymatic Synthesis of Nucleoside Analogues                              | 3        |
| 1.2      | Nucleoside Phosphorylases                                                                        | 3        |
| 1.2.1    | Classification and Substrate Spectra of Nucleoside Phosphorylases                                | 3        |
| 1.2.1.1  | Nucleoside Phosphorylase-I Family                                                                | 4        |
| 1.2.1.2  | Nucleoside Phosphorylase-II Family                                                               | 6        |
| 1.2.2    | Limitations in the Current Classification                                                        | 7        |
| 1.2.3    | Reaction Mechanism                                                                               | 8        |
| 1.2.4    | Domain Structure and Active Site Residues of Nucleoside Phosphorylases                           | 9        |
| 1.2.4.1  | NP-I Family Members                                                                              | 9        |
| 1.2.4.2  | NP-II Family Members                                                                             | 10       |
| 1.3      | Enzymatic Approaches to Produce Nucleoside Analogues Using Nucleoside Phosphorylases             | 11       |
| 1.3.1    | One-pot Two-Step Transglycosylation Reaction                                                     | 11       |
| 1.3.2    | Pentofuranose-1-phosphate as Universal Glycosylating Substrate for Nucleoside Phosphorylase (NP) | 12       |
| 1.3.2.1  | Nucleoside Synthesis from Chemically Synthesized Pentose-1P                                      | 12       |
| 1.3.2.2  | Nucleosides Synthesis from D-Glyceraldehyde-3-phosphate                                          | 13       |
| 1.3.2.3  | Nucleoside Synthesis from D-Pentose                                                              | 13       |
| 1.3.2.4  | Nucleoside Synthesis from Enzymatically Produced Pentose-1P                                      | 13       |
| 1.4      | Approaches to Produce Nucleoside Analogues                                                       | 14       |
| 1.4.1    | Whole Cell Catalysis                                                                             | 14       |
| 1.4.2    | Crude Enzyme Extract                                                                             | 15       |
| 1.4.3    | Application of Purified Enzymes                                                                  | 15       |
| 1.4.3.1  | Immobilized Enzymes                                                                              | 16       |
| 1.4.3.2  | Enzyme Reactors                                                                                  | 17       |

- 1.5 Upscaling Approaches for the Production of Nucleoside Analogues 18
- 1.6 Production of Pharmaceutically Active Compounds by Nucleoside Phosphorylases 18
- 1.7 Outlook for the Application of Nucleoside Phosphorylase in the Production of Nucleoside Analogues 19
- References 20
  
- 2 Enzymatic Phosphorylation of Nucleosides 29**  
*Daniela Ubiali and Giovanna Speranza*
  - 2.1 Introduction 29
  - 2.2 Nonspecific Acid Phosphatases (NSAPs) 30
  - 2.3 Deoxyribonucleoside Kinases (dNKs) 33
  - 2.4 Conclusion 37
  - References 37
  
- 3 Enzymatic Synthesis of Nucleic Acid Derivatives Using Whole Cells 43**  
*Elizabeth S. Lewkowicz and Adolfo M. Iribarren*
  - 3.1 Introduction 43
  - 3.2 Nucleoside Synthesis Mediated by Microbial Nucleoside Phosphorylases 45
  - 3.3 Nucleoside Analogues Synthesis by the Combined Action of Microbial Nucleoside Phosphorylases and Other Enzymes 48
    - 3.3.1 Nucleoside Phosphorylases Coupled to Deaminases 48
    - 3.3.2 Nucleoside Phosphorylases Coupled to Phosphopentomutase 48
    - 3.3.3 Nucleoside Phosphorylases Coupled to Phosphopentomutase and Other Enzymes 49
    - 3.3.4 Nucleoside Phosphorylases Coupled to Other Enzymes 51
  - 3.4 Chemoenzymatic Preparation of Nonconventional Nucleoside Analogues Involving Whole Cell Biocatalyzed Key Steps 51
    - 3.4.1 L-Nucleosides 52
    - 3.4.2 Carbocyclic Nucleosides 55
    - 3.4.3 C-Nucleosides 56
  - 3.5 Nucleoside Prodrugs Preparation by Whole Cell Systems 57
    - 3.5.1 Acynucleosides 57
    - 3.5.2 Nucleoside Phosphates 59
  - 3.6 Other Nucleoside Derivatives 61
    - 3.6.1 NDP 61
    - 3.6.2 NDP-sugar 61
  - 3.7 Perspectives 65
  - References 65
  
- 4 Enzymatic Synthesis of Nucleic Acid Derivatives by Immobilized Cells 79**  
*Jorge A. Trelles, Cintia W. Rivero, Claudia N. Britos, and María J. Lapponi*
  - 4.1 Introduction 79

|         |                                                                                          |     |
|---------|------------------------------------------------------------------------------------------|-----|
| 4.2     | Nucleic Acid Derivatives                                                                 | 81  |
| 4.3     | Whole Cell Immobilization: Generalities                                                  | 85  |
| 4.4     | Synthesis of Nucleosides by Immobilized Cells                                            | 86  |
| 4.4.1   | Natural Nucleoside Synthesis                                                             | 87  |
| 4.4.2   | Nucleoside Analogues Synthesis                                                           | 88  |
| 4.4.3   | Nucleoside Analogues Derivatives Synthesis                                               | 92  |
| 4.5     | Conclusion                                                                               | 98  |
|         | References                                                                               | 98  |
| <br>    |                                                                                          |     |
| 5       | <b>Enzymatic Synthesis of Nucleic Acid Derivatives by Immobilized Enzymes</b>            | 107 |
|         | <i>Jesús Fernández-Lucas and Miguel Arroyo</i>                                           |     |
| 5.1     | Introduction                                                                             | 107 |
| 5.2     | Immobilized Glycosyltransferases                                                         | 108 |
| 5.2.1   | Immobilized Nucleoside Phosphorylases                                                    | 108 |
| 5.2.1.1 | Stabilization of Nucleoside Phosphorylases by Immobilization                             | 108 |
| 5.2.1.2 | Synthesis of Nucleosides Catalyzed by Immobilized Nucleoside Phosphorylases              | 109 |
| 5.2.2   | Immobilized Nucleoside 2'-Deoxyribosyltransferases                                       | 111 |
| 5.2.2.1 | Stabilization of Nucleoside 2'-Deoxyribosyltransferases by Immobilization                | 113 |
| 5.2.2.2 | Synthesis of Nucleosides Catalyzed by Immobilized 2'-Deoxyribosyltransferases            | 114 |
| 5.2.3   | Immobilized Nucleobase Phosphoribosyltransferases                                        | 116 |
| 5.3     | Immobilized Nucleoside Oxidase                                                           | 117 |
| 5.4     | Immobilized Hydrolases                                                                   | 118 |
| 5.4.1   | Immobilized Lipases                                                                      | 118 |
| 5.4.2   | Immobilized Proteases                                                                    | 120 |
| 5.4.3   | Immobilized Esterases                                                                    | 121 |
| 5.4.4   | Immobilized Deaminases                                                                   | 121 |
| 5.4.5   | Immobilized S-Adenosylhomocysteine Hydrolases                                            | 122 |
| 5.5     | Immobilized Phosphopentomutases                                                          | 122 |
| 5.6     | Immobilized Deoxyribonucleoside Kinases                                                  | 123 |
|         | References                                                                               | 124 |
| <br>    |                                                                                          |     |
| 6       | <b>Synthesis of Nucleic Acid Derivatives by Multi-Enzymatic Systems</b>                  | 129 |
|         | <i>Qingbao Ding</i>                                                                      |     |
| 6.1     | Multi-Enzymatic Systems in Biosynthesis                                                  | 129 |
| 6.2     | General Overview of Multi-Enzymatic Synthesis of Nucleic Acid Derivatives                | 131 |
| 6.3     | Multi-Enzymatic Synthesis of Nucleosides and Their Derivatives                           | 132 |
| 6.3.1   | Multi-Enzymatic Synthesis of Nucleosides and Their Analogues by Nucleoside Phosphorylase | 132 |
| 6.3.2   | Transglycosylation Coupled with Xanthine Oxidase                                         | 134 |
| 6.3.3   | Transglycosylation Reactions Coupled with Deamination                                    | 135 |

|       |                                                                   |     |
|-------|-------------------------------------------------------------------|-----|
| 6.3.4 | ADase in Combination with Lipase                                  | 136 |
| 6.3.5 | Esterification of Nucleosides                                     | 138 |
| 6.3.6 | Multi-Enzymatic Synthesis of Fluorine Nucleosides                 | 140 |
| 6.3.7 | Multi-Enzymatic Synthesis of Nucleosides via R5P                  | 142 |
| 6.3.8 | Other Reactions                                                   | 144 |
| 6.4   | Multi-Enzymatic Synthesis of Nucleotides and Their Derivatives    | 145 |
| 6.4.1 | Multi-Enzymatic Synthesis of NMPs and dNMPs                       | 146 |
| 6.4.2 | Multi-Enzymatic Synthesis of NTPs and dNTPs                       | 147 |
| 6.4.3 | Multi-Enzymatic Synthesis of NDP-Sugars and Other NDP Derivatives | 148 |
| 6.5   | Conclusion                                                        | 150 |
|       | References                                                        | 151 |

## 7 Enzymatic Synthesis Using Polymerases of Modified Nucleic Acids and Genes 159

*Elena Ereemeeva and Piet Herdewijn*

|         |                                                |     |
|---------|------------------------------------------------|-----|
| 7.1     | Introduction                                   | 159 |
| 7.2     | Types of XNA Biomolecules                      | 161 |
| 7.3     | Enzymatic Synthesis of XNA and DNA Polymerases | 161 |
| 7.4     | Base-Modified XNAs (Base-XNAs)                 | 167 |
| 7.4.1   | Nucleobase Analogues                           | 167 |
| 7.4.1.1 | Non-Canonical Nucleotides                      | 167 |
| 7.4.1.2 | Amino-acid-Like Groups                         | 174 |
| 7.4.1.3 | Functional Tags                                | 176 |
| 7.4.2   | Unnatural Base Pairs                           | 177 |
| 7.4.2.1 | Hydrogen-Bonding Base Pairs                    | 177 |
| 7.4.2.2 | Hydrophobic Base Pairs                         | 179 |
| 7.5     | Sugar-Modified XNAs (Sugar-XNAs)               | 180 |
| 7.5.1   | Pentose-XNA                                    | 180 |
| 7.5.2   | 2'-Ribose-XNA                                  | 182 |
| 7.6     | Phosphodiester Backbone-XNA                    | 183 |
| 7.7     | A Mirror-Image L-DNA                           | 184 |
| 7.8     | Conclusions                                    | 184 |
|         | References                                     | 185 |

## 8 Synthetic Approaches to the Fleximer Class of Nucleosides – A Historic Perspective 195

*Therese C. Ku and Katherine Seley-Radtke*

|       |                                   |     |
|-------|-----------------------------------|-----|
| 8.1   | Distal Fleximers                  | 198 |
| 8.1.1 | Ribose Distal Fleximers           | 198 |
| 8.1.2 | 2'-Deoxyribose Distal Fleximers   | 201 |
| 8.1.3 | 2'-Modified Distal Fleximers      | 209 |
| 8.2   | Proximal Fleximers                | 209 |
| 8.2.1 | Ribose Proximal Fleximers         | 209 |
| 8.2.2 | 2'-Deoxyribose Proximal Fleximers | 215 |
| 8.2.3 | Carbocyclic Proximal Fleximers    | 216 |

|           |                                                                                                              |            |
|-----------|--------------------------------------------------------------------------------------------------------------|------------|
| 8.2.4     | Proximal Fleximers from Other Groups                                                                         | 218        |
| 8.3       | "Reverse" Fleximers                                                                                          | 222        |
| 8.4       | Acyclic Fleximers                                                                                            | 226        |
| 8.5       | Conclusion                                                                                                   | 228        |
|           | References                                                                                                   | 229        |
| <b>9</b>  | <b>Synthesis of Oligonucleotides Carrying Nucleic Acid Derivatives of Biomedical and Structural Interest</b> | <b>237</b> |
|           | <i>Ramon Eritja, Anna Aviñó, Carme Fàbrega, Adele Alagia, Andreia F. Jorge, and Santiago Grijalvo</i>        |            |
| 9.1       | Introduction                                                                                                 | 237        |
| 9.2       | Oligonucleotides Carrying the DNA Lesion $O^6$ -Alkylguanine                                                 | 238        |
| 9.3       | The Effect of Chemical Modifications in Non-Canonical DNA Structures                                         | 240        |
| 9.3.1     | Triplex-Forming Oligonucleotides                                                                             | 241        |
| 9.3.2     | G-quadruplex-Forming Oligonucleotides                                                                        | 243        |
| 9.3.3     | Oligonucleotides Forming i-Motif Structures                                                                  | 245        |
| 9.4       | Modified siRNAs for Gene Silencing                                                                           | 246        |
| 9.4.1     | Modifications of the 3'-Overhangs                                                                            | 246        |
| 9.4.2     | Modifications of the 5'-End                                                                                  | 249        |
|           | References                                                                                                   | 251        |
| <b>10</b> | <b>Synthesis of Carbohydrate-Oligonucleotide Conjugates and Their Applications</b>                           | <b>259</b> |
|           | <i>Juan C. Morales</i>                                                                                       |            |
| 10.1      | Introduction                                                                                                 | 259        |
| 10.2      | Synthesis of COCs                                                                                            | 260        |
| 10.2.1    | On-Support Synthesis                                                                                         | 260        |
| 10.2.1.1  | Phosphoramidite Chemistry                                                                                    | 261        |
| 10.2.1.2  | Derivatization of Nucleoside Base Residues                                                                   | 261        |
| 10.2.1.3  | Oximation Chemistry                                                                                          | 263        |
| 10.2.1.4  | Amide Chemistry                                                                                              | 263        |
| 10.2.1.5  | Urea Chemistry                                                                                               | 264        |
| 10.2.1.6  | CuAAC Chemistry                                                                                              | 264        |
| 10.2.2    | Solution-Phase Conjugation                                                                                   | 265        |
| 10.2.2.1  | Disulfide Formation                                                                                          | 265        |
| 10.2.2.2  | Nucleophilic Addition on Unsaturated Carbon                                                                  | 265        |
| 10.2.2.3  | Carbonyl Addition-Elimination Reaction                                                                       | 267        |
| 10.2.2.4  | CuAAC Chemistry                                                                                              | 267        |
| 10.2.2.5  | Diazocoupling Reaction                                                                                       | 267        |
| 10.2.2.6  | Amide Bond Formation                                                                                         | 267        |
| 10.2.2.7  | Enzymatic Incorporation of Saccharides or Nucleotides                                                        | 268        |
| 10.3      | Synthesis of Glycocluster Oligonucleotides                                                                   | 268        |
| 10.3.1    | dsDNA Scaffolds                                                                                              | 269        |
| 10.3.2    | Non-Canonical DNA Scaffolds (G4 and three-Way Junction)                                                      | 269        |
| 10.3.3    | Organic Spacer Scaffolds                                                                                     | 270        |

|           |                                                                                                                       |            |
|-----------|-----------------------------------------------------------------------------------------------------------------------|------------|
| 10.3.4    | Biomolecules as Scaffolds                                                                                             | 271        |
| 10.4      | Applications of COCs                                                                                                  | 273        |
| 10.4.1    | Improving Cellular Uptake                                                                                             | 273        |
| 10.4.2    | Molecular Interactions Probes                                                                                         | 279        |
| 10.4.3    | Lectin Binding and Glycoarrays                                                                                        | 280        |
| 10.5      | Outlook                                                                                                               | 281        |
|           | References                                                                                                            | 281        |
| <b>11</b> | <b>Advances in Light-Directed Synthesis of High-Density Microarrays and Extension to RNA and 2'-F-ANA Chemistries</b> | <b>291</b> |
|           | <i>Jory Lietard, Masad J. Damha, and Mark M. Somoza</i>                                                               |            |
| 11.1      | Introduction                                                                                                          | 291        |
| 11.2      | Phosphoramidite Chemistry Applied to the Photolithographic Synthesis of Microarrays                                   | 293        |
| 11.3      | Recent Improvements in the Synthesis of DNA Microarrays                                                               | 295        |
| 11.4      | Synthesis of RNA Microarrays                                                                                          | 300        |
| 11.5      | Enzymatic Approaches to RNA Array Synthesis                                                                           | 305        |
| 11.6      | Synthesis of 2'-F-ANA Microarrays                                                                                     | 306        |
| 11.7      | Conclusion and Outlook                                                                                                | 309        |
|           | References                                                                                                            | 310        |
| <b>12</b> | <b>SAMHD1-Mediated Negative Regulation of Cellular dNTP Levels: HIV-1, Innate Immunity, and Cancers</b>               | <b>313</b> |
|           | <i>Tatsuya Maehigashi, Dong-Hyun Kim, Raymond F. Schinazi, and Baek Kim</i>                                           |            |
| 12.1      | Cellular dNTP Concentrations                                                                                          | 313        |
| 12.2      | SAMHD1 and Negative Regulation of Cellular dNTPs                                                                      | 314        |
| 12.3      | SAMHD1 Substrates, Activators, and Inhibitors                                                                         | 316        |
| 12.4      | SAMHD1 and HIV-1 Reverse Transcription                                                                                | 318        |
| 12.5      | SAMHD1 Mutations and Innate Immunity                                                                                  | 318        |
| 12.6      | SAMHD1 and Cancers                                                                                                    | 321        |
| 12.7      | Summary                                                                                                               | 321        |
|           | Acknowledgment                                                                                                        | 322        |
|           | References                                                                                                            | 322        |
|           | <b>Index</b>                                                                                                          | <b>327</b> |