Protocols for Oligonucleotides and Analogs
Methods in Molecular Biology

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Preface

When first conceived, not only was the aim of Protocols for Oligonucleotides and Analogs to provide wide coverage of the oligonucleotide chemistry field for readers who are well versed within the field, but also to give investigators just entering into the field a new perspective. The very first book on this topic was edited and published by Michael Gait in 1984, in whose laboratory I encountered the newer aspects of oligonucleotide chemistry. Since then, oligonucleotide research has developed to such an extent that its uses extend far beyond basic studies, and now find wide application throughout clinical science as well.

Until recently, the major application of oligonucleotides has been in the area of DNA-based diagnostic and “antisense oligonucleotide”-based therapeutic approaches. However, oligonucleotides are now also being used as therapeutic agents and are thus frequently found in clinical trials in humans.

Synthesis of unmodified oligonucleotides using automated synthesizers has become a common practice in numerous laboratories. However, improvements on the existing techniques and the introduction of ever newer methods for oligonucleotide synthesis is constantly driving ahead in the leading research laboratories. And several new oligonucleotide analogs have been synthesized and studied for their individual properties in recent years. The present volume strives to bring the readers the most up-to-date information on the newest aspects of synthesis of oligonucleotides and their analogs. A separate volume covers synthesis of oligonucleotide conjugates, along with most of the analytical techniques presently used for analysis of oligonucleotides.

The first chapter of Protocols for Oligonucleotides and Analogs provides a sense of the history of the development of oligonucleotide synthesis. This is followed by three chapters (Chapters 2–4) describing three main approaches to the synthesis of unmodified oligonucleotides. The phosphoramidite and H-phosphonate approaches have become the
two main avenues of choice for synthesizing oligonucleotides. Chapters 5 and 6 provide protocols for synthesizing oligonucleotides and their 2'-analogs, respectively. There are chapters describing the synthesis of phosphate analogs of oligonucleotides including methylphosphonates (Chapter 7), phosphorothioates (Chapter 8), phosphorodithioates (Chapter 9), and phosphotriesters (Chapter 10). There is a description of several newer phosphate modifications, e.g., boronates (Chapter 11) and phosphofluoridates (Chapter 12). Chapter 13 describes the protocols for synthesizing a-oligonucleotides. The stereospecific synthesis of chiral oligonucleotides is discussed and described (Chapter 14). Oligonucleotide analogs containing nonphosphate backbones are presently being studied and their synthetic chemistry is being worked out; Chapters 15 and 16 provide the synthetic chemistry of these oligonucleotide analogs. Included are two chapters describing various aspects of large-scale oligonucleotide synthesis using the solution phase (Chapter 17) as well as the solid phase approach (Chapter 18). Also included are descriptions of some of the supports that have been used in oligonucleotide synthesis, including protocols for their derivatization and handling (Chapter 19).

I am, of course, deeply indebted to all authors of the various chapters of this book for their dedication, hard work, and patience. To them goes credit for the book's teachings and uses, since the field itself either originated from their laboratories or was contributed to significantly by it. The high quality of the various manuscripts has made my role as editor an easy one. Let me thank Drs. Paul Zamecnik, Thoru Pederson, and Dan Brown for encouragement, guidance, and discussions. I would like to thank Dr. John Walker, Methods in Molecular Biology Series Editor for his help, guidance, and encouragement at all stages of editing. Finally, many thanks go to Mr. Thomas Lanigan, Ms. Lucia Read, and Ms. Bonnie Gustafsson of Humana Press, whose hard work and diligence have made this book a reality.

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Foreword

Since the discovery of the nucleic acids in the latter part of the last century, several decades elapsed before the structures of the constituent purines and pyrimidines, the corresponding nucleosides, and the nature of the internucleotide bonds in the polynucleotides were clarified. By 1952, the chemical structures of both classes of nucleic acids, RNA and DNA, had been established. Soon followed the Watson-Crick proposal for the DNA structure and this ushered in a new era in chemical, biochemical, and molecular biological studies.

As with other classes of biological macromolecules, interest in the synthesis of oligo- and polynucleotides increased very rapidly and systematic studies on the synthesis of polynucleotides were undertaken in the mid-1950s. Indeed, strategies were successfully developed for the synthesis of oligonucleotides of defined sequences. These, in conjunction with enzymatic approaches enabled the synthesis of high molecular weight polymers that were successfully used in the elucidation of the genetic code and later in the total laboratory synthesis of genes fully functional in vivo.

In more recent years new developments in synthesis have advanced the synthetic technology to a highly efficient level. In particular, synthesis on polymer supports, a concept first developed by Merrifield in polypeptide synthesis, has resulted in enormous improvements and speed in synthesis of large size polynucleotides.

Dr. Agrawal is to be congratulated on having produced two authoritative books with contributions from experts in different aspects of polynucleotide synthesis. These monographs should be extremely useful to those who are already practitioners in the field and also those who want to enter this field without any particular background.

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