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# Preface

Nucleic acids chemistry has been fundamental to molecular biology and recently it has moved into therapeutic areas. The evolution of this chemistry has been tremendous during the last ten years and new techniques are regularly introduced and existing protocols are ameliorated. It is not possible to give an overview of the evolution of the different aspects of nucleic acids chemistry and their applications within the context of one book. The aim of *Oligonucleotide Synthesis: Methods and Applications* is to give the readers an insight into new key developments and to deliver protocols and critical comments for the practical execution of the experiments. Inside details from the inventors of protocols has often proven to be of the utmost importance for the successful application of a new technique.

*Oligonucleotide Synthesis: Methods and Applications* covers new developments in the fundamental chemistry of nucleic acids as well as new applications of nucleic acids with tremendous potential, such as RNA interference.

High-throughput DNA synthesis is normally done by the phosphoramidite four-step process. Now, a novel two-step cycle, developed by Marvin Caruthers, enables a higher purity DNA to be obtained in a less costly way. New discoveries, such as siRNA, have driven the scientific community to come up with synthetic methods to obtain RNA as efficiently as DNA. Thanks to the development of new reagents, the classical RNA synthesis using a 2'-*O*-tert-butyldimethylsilyl protecting group has reached the level of 99% coupling yields. The synthesis and purification of RNAs by this method is described by Brian Sproat. New methods have also been developed in the field of RNA synthesis. One of these methods uses an inverse protection scheme (acid labile group in the 2'-*O*-position and fluoride labile group in the 5'-*O*-position), and this protocol is covered by William Marshall. Using this method, the oligonucleotide is stored in its 2'-*O*-protected form and final deprotection is carried out before use.

The most widely used oligonucleotides in antisense technology are phosphorothioates, mainly because of the susceptibility of the target RNA to be cleaved by RNase H (when hybridized with phosphorothioate oligonucleotide). The synthesis of phosphorothioates has been improved considerably by the introduction of new sulfur transfer reagents, as described by Yogesh Sanghvi. The guidelines to monitor the RNA cleavage reaction using ribonucleases H have been written by Masad Damha, in which illustrative examples on selecting particularly potent, enzyme eliciting AON are provided by the 2'-fluoroarabinonucleic acids (2'F-ANA) and their analogs.

Although the phosphoramidite approach has become the method of choice for oligonucleotide synthesis, the H-phosphonate alternative, as described by Jurek Stawinski and Roger Strömberg, remains a useful alternative, especially for solution-phase synthesis and when only a small excess of building blocks are used. The original phosphotriester approach to oligonucleotide synthesis has no longer a routine application, but is still very useful, for example for the ring closure of circular oligonucleotides. The synthesis of circular oligonucleotides is described by Enrique Pedroso, and he points to the many potential applications of circular DNA.

The antisense technology has stimulated research on modified oligonucleotides that hybridize very strongly with complementary RNA, mainly as a result of the preorganization of the carbohydrate moiety of the building units of these oligonucleotides into a “northern-type” conformation. The most strongly binding oligonucleotides are LNA, whose synthesis is summarized by Jesper Wengel. The HypNA–pPNA chimers of Vladimir Efimov are water soluble PNA-like hybrids with DNA/RNA binding properties very close to that of PNA itself. Besides sugar modification, duplex stability may also be increased by base modifications. The 7-substituted 8-aza-7-deazapurine base, as described by Frank Seela, can bring the stability of a dA–dT base pair to the same level as for dG–dC pair. The synthesis of hypermodified nucleotides and their incorporation into oligonucleotides is a recent field of research that is of particular importance for investigation on the local structure and the specificity of tRNA. Darell Davis describes here several examples of hypermodified nucleoside phosphoramidites and their incorporation into RNA.

The topic of conjugate chemistry is covered by several important examples. Peptide conjugation, as described by Michael Gait, is an extremely important method to improve cellular delivery, as well as cell-specific targeting of oligonucleotides. Biotin-labeled oligonucleotides are widely used for oligonucleotide detection and isolation. The synthesis of biotin phosphoramidites with extraordinarily long tether and biotin-labeled oligonucleotide is covered by Nikolai Polushin. Ali Laayoun describes the synthesis of reactive reporter groups for labeling of nucleic acids on their phosphates and the results obtained with high-density DNA chip analysis.

Triple helix formation is a unique approach that uses oligonucleotides to target double-stranded DNA, this allows interaction at an early stage of gene expression. Jian-Sheng Sun describes the synthesis of conjugates between triple helix-forming oligonucleotides and camptothecin, which is a way to direct the cleavage activity of topoisomerases.

Before moving to the application section, a chapter is introduced where Michael Göbel describes fluorescence-based on-line detection in RNA elec-

trophoresis. The importance of developing fluorescence technology is that, sooner or later, all techniques in molecular biology and biochemistry that use radioisotope-based protocols, will be replaced by less hazardous (radiation protection) and more user friendly methods (waste disposal, faster analysis).

One of the most widely used techniques for the quantification of nucleic acids is the real-time polymerase chain reaction assay, which uses molecular beacons to monitor the amplification process. Jacqueline Vet describes this procedure in a stepwise fashion. An important factor for the quality of an amplification reaction, is the selection of optimized primers (choice of sequences and adjustment of reaction conditions). Nikolai Polushin and his group have developed modified primers or fimers to direct sequencing of genomic DNA.

The “RNA world” hypothesis has stimulated research on the nonenzymatic template-directed synthesis of RNA. The contribution of Michael Göbel is aimed at providing reliable protocols for carrying out these experiments, using nonradioactive labeling procedures for monitoring the process. The nuclease footprinting technique, initially developed to study protein–DNA interactions, is now widely used to investigate the sequence-selective binding of small molecules to DNA. This technique is described by Christian Bailly, using actinomycin D as a model compound. The search for strong and selective DNA binding ligands is a continuing process, given the need for molecules that may control gene expression. One of the most unique tools for interfering with the function and metabolism of nucleic acids is PNA. The most important properties of PNA and some of their analogs are reviewed by Peter Nielsen.

It is becoming impossible to cover modern techniques in science and overlook library approaches. Depending on the application, the desired library may vary from very low sequence variation, to very high diversity. Seven techniques to construct nucleic acids libraries of different diversity are described by Peter Unrau, whereas Andres Jäschke is concentrating on protocols describing combinatorial nucleic acid libraries applied in the field of ribozymes accelerating a Diels-Alder reaction. The SELEX approach for aptamer design against RNA and protein targets is covered by Jean-Jacques Toulmé.

The last chapter, written by Jean-Remi Bertrand, deals with the most exiting new area of oligonucleotide applications, i.e., the use of small interference RNA (siRNA) as an inhibitor of gene expression. Although the mode of action of siRNA is not fully understood, the use of siRNA as an inhibitor of gene expression (both in vitro and in vivo), has changed the strategy for the analysis of gene functions and the control of their expression.

We hope that *Oligonucleotide Synthesis: Methods and Applications* will give the reader a flavor of several important new directions in nucleic acids chemistry and their applications, as well as provide detailed protocols for carrying out

these experiments. Some topics are more specific, other are more general. Rather than presenting a laboratory manual dealing with one specific topic of nucleic acids chemistry, we have decided to cover a broad field of research to make the manual useful for a large audience and attract the interest of scientists in this expanding field of research. The book starts with a new protocol for DNA synthesis and finishes with details of RNA interference experiments.

Most of all, I would like to cordially thank all our contributors, appreciating the time they have taken to write the chapters and knowing that none of them are looking for additional paper work that only keeps them away from their experiments.

***Piet Herdewijn***