Preface

The development of the phosphoramidite method for the synthesis of natural nucleic acids (DNA and RNA) made possible the production of oligonucleotides on a large scale for biotechnological purposes. In parallel, it provided organic chemists with the opportunity to synthesize artificial oligonucleotides for a wide range of applications. Initially, these modified oligonucleotides were synthesized as potential antisense constructs, targeted against particular regions of mRNAs to potentially inhibit the translation process. Such oligomers should have high RNA affinity to hybridize stably with the target strand. Therefore, the focus of this research was to develop oligonucleotides that were conformationally pre-organized for an A-form double helix and in addition stable against enzymatic degradation. Subsequently, the research efforts gradually shifted from the synthesis of simple backbone-modified oligonucleotides (such as the DNA phosphorothioates) to more complicated bicyclic and tricyclic sugar-modified oligonucleotides. Chemical modifications were introduced into the phosphate backbone, the sugar, and the nucleobases, and classical peptide chemistry also entered the field (PNA synthesis). As far as therapeutic and diagnostic applications of artificial oligonucleotides are concerned, the original antisense approach underwent a significant expansion and now includes the use of oligonucleotides as antigene constructs, aptamers, decoys, ribozymes, siRNA, and miRNA.

A second line of research on artificial oligonucleotides focused on etiological questions. Here, the chemical variations in the oligonucleotide analogs that were explored were driven more by the feasibility of a particular pairing system in the prebiotic context. This research yielded fundamental insights into the ways that the structures of the repeating nucleotide units that build up the artificial nucleic acid affect the stability and geometry of duplexes. The accumulated data have not yielded an answer to the question about the precise origins of the natural information systems, but they have paved the way to a more fundamental understanding of how the sugar moiety influences the strength and specificity of base pairing, the chemistry of templated polymerization, and chirality. In addition, the landscape of possible duplex structures has been broadened beyond the classical A- and B-forms toward varied geometries, including left-handed, unwound, and ladder-like as well as right-handed duplexes.

The availability of chemical methods to synthesize artificial oligonucleotides with a virtually unlimited repertoire of modifications has also had a major impact on other areas of research involving chemically altered nucleic acids, such as investigations directed at DNA damage and genome maintenance, oligonucleotide-templated (combinatorial) chemistry (see the contribution of *Berger* and *Oberhuber*), and, more recently, applications in synthetic biology and systems chemistry (see the chapter by *Plöger* and *von Kiedrowski*). The nucleoside and nucleotide research community has profited from developments in the field of artificial oligonucleotides by being provided

with a range of new chemical approaches to the synthesis of modified nucleosides and nucleotides. Artificial nucleic acids have become an important tool for scientists conducting research in chemical biology or studying nucleic acid- and nucleotide-metabolizing enzymes. Moreover, structural biologists have used artificial nucleic acids for a better understanding of the relationship between structure and function, and to gain insights into mechanism as well as for technical improvements in X-ray crystallography, NMR, and computational simulations.

This book is a compilation of review articles on the chemistry and biology of artificial nucleic acids, written by the scientists who originally performed the research. Some chapters mainly deal with synthesis, others with structural aspects or with the biological and therapeutic applications of artificial nucleic acids, but many of the chapters are a mix of multiple themes.

Phosphorothioate DNA (PS-DNA) is a representative of the first generation of antisense oligonucleotides (AONs) and numerous PS-DNAs were tested in the clinic or are currently being assessed in clinical trials, whereby many of the evaluated AONs contain other modifications besides a PS backbone. *Guga* and *Koziołkiewicz* provide an overview of the PS modification and report on recent progress in the synthesis and applications of P-chiral oligonucleotides, including phosphoroselenoates. Selenium modification of nucleic acids is also the focus of the contribution by *Sheng* and *Huang* who discuss incorporation of Se at various sugar-phosphate backbone and nucleobase sites from the perspectives of crystallographic phasing and functional investigations. Unlike in PS-DNA in which a non-bridging phosphate O-atom is replaced by a S-atom, the modification in N3′ → P5′ phosphoramidate DNA concerns the bridging 3′-O-atoms, and *Gryaznov*'s update on its chemistry and therapeutic potential offers us a first-hand account of the fascinating properties of this analog as well as the thiophosphoramidates (NPS-DNAs).

Several chapters focus on modifications of the sugar moiety and/or the inter-nucleoside linkages. *Vasseur* and colleagues describe the pairing properties of α-phosphodiester oligonucleotides, and chimeric α- and β-anomeric species. The role of conformational preorganization looms large in several contributions, including the one by *Veedu* and *Wengel* who discuss the chemistry and key properties of locked nucleic acid (LNA) and LNA's application in therapeutics and biotechnology. Additional 2′,4′-bridged sugar modifications and 2′-modified nucleotides in general as well as their role in the development of antisense therapeutics are reviewed in the chapter by *Prakash*. More rigid sugars like hexoses and the supposedly less restrained compounds represented by acyclic moieties occupy opposite ends of the conformational preorganization spectrum. *Herdewijn* provides a comprehensive overview of the chemistry and biology of nucleic acid analogs with six-membered carbohydrate moieties in their backbone. *Chaput* and colleagues take a look at the structural and functional properties of nucleic acids lacking a cyclic sugar and highlight insights from such analogs gained in the prebiotic arena and their potential in nanotechnology. In his review on peptide nucleic acids (PNA), *Nielsen* looks back at almost two decades of research following their introduction on the use of PNA in fields including molecular biology, diagnostics, therapeutics, and prebiotic chemistry.

In the realm of base-modified nucleic acids, *Budow* and *Seela* describe the synthetic route to 2-azapurine nucleosides, their incorporation into oligomers, and the ensuing

chemical and biological properties of modified oligonucleotides. *Chiba* and *Inouye* use their chapter to take a wide-ranging look at the chemistry and pairing properties of artificial nucleic acids with non-natural bases, and they examine the structural diversity of these non-canonical nucleic acids. DNA Base modifications caused by diverse environmental and endogenous agents, and their biological consequences are the focus of the review by *Stone* and colleagues. The effects of adducted bases on pairing and stability are discussed and the conformational properties of oligonucleotides featuring such lesions examined by NMR. The latter technique is also applied in studies described by *Ebert* and *Jaun* and directed at analogs investigated in the context of a chemical etiology of nucleic acids, such as pyranosyl RNA and TNA. X-Ray crystallographic analyses of chemically modified nucleic acids are at the center of the review by *Egli* and *Pallan* who look back at the impact of structural work for an improved understanding of the consequences of modifications for conformation and stability and the design of novel analogs with tailor-made properties. *D'Alonzo* and colleagues expand on the role of chirality in nucleic acid recognition and in their chapter discuss studies exploring the structural factors underlying the formation of stable heterochiral complexes by incorporating β-L-nucleotides into natural DNA and RNA duplexes.

The book thus covers a broad range of topics, from anionic to cationic oligonucleotides, from acyclic oligonucleotides to nucleic acids with a six-membered carbohydrate moiety, from classical *Watson–Crick* pairing systems to non-natural metallo base pairs. It is meant as an introduction to the field for students of the chemical and biological sciences as well as a reference book for the specialists in the field.