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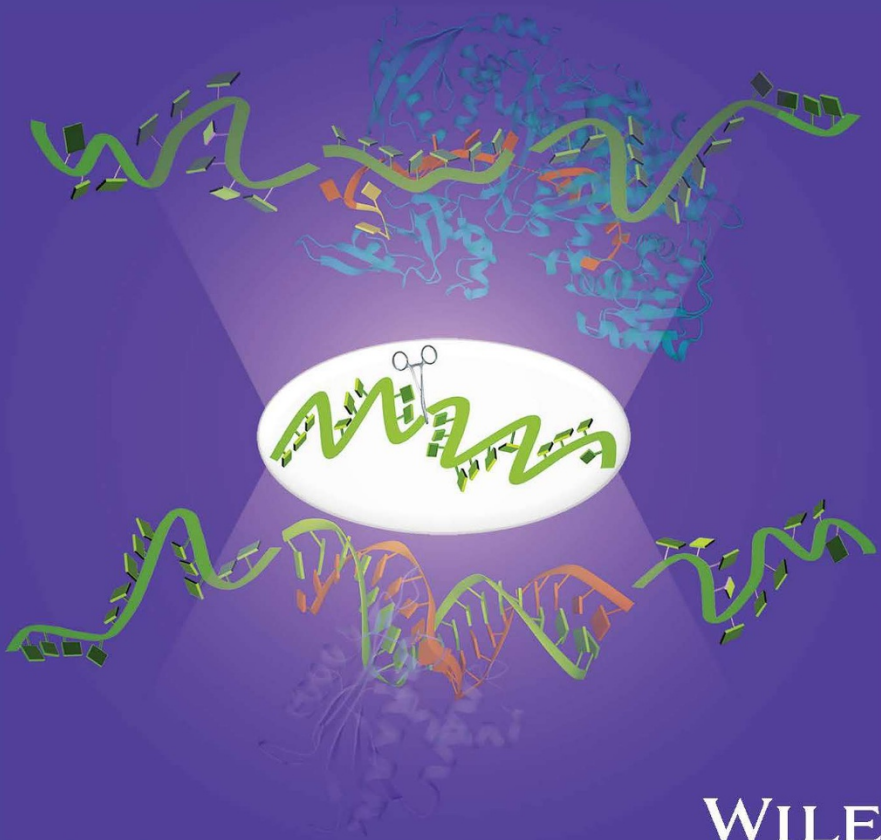
Nucleic Acids in Medicinal Chemistry and Chemical Biology

Drug Development and Clinical Applications

Edited by
Lihe Zhang | Xinjing Tang | Zhen Xi | Jyoti Chattopadhyaya



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NUCLEIC ACIDS IN MEDICINAL CHEMISTRY AND CHEMICAL BIOLOGY

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NUCLEIC ACIDS IN MEDICINAL CHEMISTRY AND CHEMICAL BIOLOGY

DRUG DEVELOPMENT AND CLINICAL APPLICATIONS

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PREFACE

As is often the case, research progress involving a given class of compounds can be limited by its availability. As a consequence of extensive investigations carried out by many laboratories, it is now possible to acquire (modified) DNAs and RNAs of diverse structure commercially, or by following detailed preparative descriptions in the literature. This certainly now includes the nucleic acids that encode many proteins, facilitating the enhanced study of both classes of molecules. The increased accessibility of such molecules is clear from the range of topics addressed in the current volume.

One would expect advances of understanding in many areas of inquiry to be enabled by ready access to nucleic acids and molecules with which they interact. The chapters in this volume certainly provide this increased level of insight into biological systems and their modes of function. Perhaps somewhat more surprising is the large increase in the number of applications reflected in the individual chapters of this volume. These now extend well beyond the development of new therapeutic strategies to a great variety of new tools to interrogate detailed elements of biological and biochemical processes. A number of these applications are introduced briefly below as part of this preface to illustrate the expanded reach of current studies in nucleic acids.

The current volume addresses several aspects of ongoing research activities pertinent to the medicinal chemistry of nucleic acids. Three chapters deal with nucleoside and nucleotide analogues as physiological mediators. Among these is Chapter 1, which deals with nucleoside analogues that function in biological systems as phosphorylated species. Such nucleoside analogues are generally not internalized by cells when phosphorylated due to the accompanying negative

charge and often cannot be phosphorylated efficiently intracellularly. An alternative is the administration of neutral phosphate and phosphonate prodrugs. These can be unmasked within cells using any of several strategies. Chapter 2 discusses the structures of the cyclic dinucleotides and the mechanisms that they use to produce an immune response. This includes the STING (stimulator of interferon) pathway and the identification of hSTING protein, which has 379 amino acids and for which 3 structural domains have been defined. There are potential applications in anticancer therapy (via IFN, cytotoxic T-lymphocytes, and natural killer cells). The cyclic dinucleotides are good candidates for adjuvant chemotherapy with numerous therapeutic agents. Cyclic dinucleotides are also potentially useful for the treatment of autoimmune diseases resulting from STING gain-of-function mutations. Chapter 3 discusses cyclic ADP-ribose and NAADP, two Ca-mobilizing messengers. They have numerous biological functions, including Ca trafficking processes and the transfer of mitochondria from human astrocytes to glioma cells. The chapter also discusses their enzymatic synthesis (involving ADP-ribosyl cyclase and CD38). Type III CD38 may also be involved in the endogenous production of cyclic ADP-ribose, while type II CD38 is implicated in the endogenous production of NAADP. Recently, it was shown that protein SARM1, not at all similar to CD38, is expressed in neuronal tissue that biosynthesizes both mediators.

Two additional chapters are also concerned with medicinal chemistry. These include Chapter 14, which details the use of structurally distinct forms of G-quadruplexes as targets for selective binding by small molecules. The small molecules include derivatives of indoles and bis-indoles, which have been functionalized with side chains that can be protonated to enable specific types of G-tetrad interactions. Recently discovered ligands include copper metallocrowns and trisubstituted fan-shaped trinuclear Pt(II) complexes. Additionally, carbohydrates have been used to functionalize G-tetrad binders, imparting increased affinity and selectivity, as well as improved solubility. Finally, Chapter 8 describes the use of DNA-encoded chemical libraries as a strategy for drug discovery. There is a surprisingly large number of strategies that can be employed to utilize DNA encoding for the discovery of small molecules potentially useful as drugs, and this chapter cites several small-molecule clinical candidates discovered from DNA-encoded libraries.

Of continuing interest to the nucleic acids community are the numerous studies that employ molecules capable of interacting selectively with nucleic acids. These studies have provided very important insights in the past and promise to continue to do so. Chapter 5 discusses work that has been done to define and utilize compounds that can bind to chromosomal DNA in a sequence-specific fashion. As noted, intervening at the level of cellular DNA is very attractive conceptually because the number of target molecules is minimal. The authors compare several natural, mostly protein-based systems (including CRISPR/Cas9, TALENs, ZNF, and TFOs) to synthetic peptide nucleic acids (PNA) and pyrrole-imidazole polyamides, noting key differences and issues. The chapter

notes recent initiatives, including targeted mutagenesis, homologous recombination, and the use of PNA for dsDNA invasion, as well as potential applications (e.g. gene editing, gene modulation via epigenetics, and PNA regulation of gene structure). Chapter 12 provides a highly informative summary of the basics of gene editing with CRISPR/Cas9 for therapeutic applications. It also includes the details of a clinical trial to counter the effects of HIV infection by ablating the *CCR5* gene. Yet another example of molecules that interact selectively with nucleic acids is provided in Chapter 10, which deals with the fluorescence hybridization imaging of neurological disease mRNAs in live neuronal cells. MAOs occur in the mitochondrial outer membrane. The imaging probes included MAOA-specific PNA dodecamers, separated by an N-terminal spacer to a μ-opioid receptor targeting peptide, with a spacer and fluorophore at the C-terminus. These were delivered into SH-SY5Y neuroblastoma cells through μ-opioid receptor-mediated endocytosis.

Two strategies that have been pursued for a number of years continue to receive substantial attention. One of these, oligonucleotide therapeutics, has arguably become even more active in the past several years. The comprehensive review of this topic in Chapter 11, comparing ASOs and siRNAs, includes a discussion of adjuvant delivery by GalNAc and lipids. Antibodies have also been used as conjugates. Oligonucleotide drugs can work by active mechanisms involving degradation of endogenous mRNA target sequences (by RNase H or argonaute-2). An additional mechanism involves physical blocking, e.g. by translation arrest or modulation of RNA processing. Finally, Chapter 13 describes the properties functions and applications of aptamers, single-stranded DNA or RNA that can bind selectively to a specific target. Aptamers occur in nature as riboswitches and can be selected to mediate enzyme inhibition, growth factor neutralization, immune function, and antiviral activity.

The remaining four chapters deal with modified nucleic acids. There are numerous modified nucleosides that appear both in RNA and DNA, and these have a variety of functions. High density CpG methylation of DNA promoter regions can block transcription factor binding and diminish gene expression. Likewise, post-transcriptional modification of mRNA can affect its stability and expression efficiency. There is a strong link of epigenetic modifications and disease, as well established for cancers. Chapter 7 outlines techniques used to detect and characterize such modifications. Also of interest is Chapter 6, which describes the preparation of modified genomes and organisms. Such modified genomes contain new structural features, such as the elimination of repetitive sequences to improve stability, the minimization of genome size, and recoding (simplification) of genomes. The crystallization of nucleic acid structures and their characterization by X-ray crystallography is greatly facilitated by the introduction of Se atoms at specific positions. As described in Chapter 4, there are now methods for effecting substitutions throughout the entire DNA oligonucleotide structure. Finally, the study of DNA origami has become very popular and has led to a variety of techniques for preparing highly specialized

structures. Chapter 9 focuses on the preparation of nucleic acid-based nanocarriers that can be used for drug delivery, including three-dimensional cages. These include bio-orthogonal conjugation-induced functionalization of DNA nanocages. RGD-conjugated DNA structures showed high binding affinity to HeLa cells, and a cell-penetrating peptide (CPP)-modified DNA nanostructure developed to facilitate endocytosis-mediated uptake into target cells.

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FOREWORD

NUCLEIC ACID-BASED THERAPEUTICS: A TOUR DE FORCE

As the serial editor, it gives me special pleasure to write a foreword for this volume focused on nucleic acids in the *Wiley Series in Drug Discovery and Develop* to showcase past accomplishments and envision future opportunities. This is the second edition of a monograph published in 2011. This new edition includes both updates of some earlier chapters and new chapters that discuss various aspects of nucleic acid-based therapeutics. The foreword by Professor Sidney Hecht provides comprehensive highlights of this new edition. Thus, this foreword aims to complement by providing some context and background for this monograph. First, the *Wiley Series in Drug Discovery and Develop* published its inaugural book in 2004. Since then, about 30 volumes have been published covering a broad range of subjects that impact drug discovery and development. The monograph on nucleic acid is especially important because of the central roles that this field has played in advancing biomedical science. Since the historical work by many on DNA structures culminating with the proposal of the double helix structure for DNA by Watson and Crick in the 1950s, discoveries of milestone nature have continued in the nucleic acid field. The human genome project was a monumental undertaking that has fundamentally transformed the biomedical field. Further, the nucleic acid field has been recognized with the Nobel Prize no less than seven times in the past 20 years in areas including telomeres and chromosomal protection, RNA interference, split genes, genome editing, DNA repairs, DNA chemistry (PCR and site-directed mutagenesis), and ribosomal structures and functions. With all the advancements in the nucleic

acid field as the background, the first edition of this book was a tremendous success, which led to the commissioning of the second edition nine years later. Furthermore, the nucleic acid field has seen much progress including CRISPR gene editing, antisense therapy, DNA-encoded library, and nanoparticle-based delivery, which need to be reflected in a new edition, especially in relation to their application in developing new therapeutics.

In terms of medicinal chemistry work in the field of nucleosides, nucleotides, and nucleic acids, there has been an impressive number of success stories from nucleoside-based small-molecule drugs. However, oligonucleotides and nucleic acids are somewhat different. On one hand, they present exciting opportunities in precision and specificity in addressing the various underlying molecular mechanisms of diseases. On the other hand, the development of oligonucleotide-/nucleic acid-based therapeutics also faces unique challenges in delivery. Nevertheless, it is exciting to see the tremendous progress that has been made along this line. One transformative example is the recent success of mRNA-based vaccines for the SARS-CoV-2 virus, which are poised to rescue the world from the possibly catastrophic consequence of a pandemic that at times seemed to be out of control. The success of these mRNA-based vaccines also helps to break the myth of "drug-likeness" that has hovered over the field of nucleic acid-based therapeutics for a long time. At a time when nucleic acid-based approaches are positioned to bring a large number of unique solutions to issues of human health and suffering, this volume timely focuses on the medicinal chemistry and therapeutic application aspects of oligonucleotides and nucleic acids including important drug delivery approaches. It is quite a *tour de force* covering past accomplishments and, yet more importantly, it envisions future opportunities for much more to come. It is hoped that this volume will help to stimulate increasing interests in this field in order to accelerate the development of nucleic acid-based therapeutics.

On a personal note, the lead editor, Professor Zhang, and his wife, Professor Peiying Zhang, were my professors when I was an undergraduate student at Peking University Health Sciences Center (then Beijing Medical College). Thus, it is with special personal gratitude that I write this foreword to express my appreciation of Professor Zhang's efforts in leading a group of four editors in organizing this book and very importantly of his contributions to the development of the nucleic acid chemistry field in China and worldwide.

With much progress made in the past few decades, the time has come for nucleic acids to be a major force in the development of therapeutic options with precision and specificity!

Binghe Wang
April 11, 2021, On Lake Allatoona

INTRODUCTION

In 2011, we invited some scientists to contribute their research progress in areas of nucleic acid medicinal chemistry and published a collection of these articles, named by ***Medicinal Chemistry in Nucleic Acids*** by ***Wiley*****.** As nine years passed since the publishing of above book, and many new developments have been achieved in nucleic acid medicinal chemistry and chemical biology, we think that it's right time to publish a new edition in this field.

Nucleoside, nucleotide, and nucleobase analogues have been widely utilized for the treatment of viral pathogens, neoplasms, and in anticancer chemotherapy for decades. Nucleoside analogues are known to be incorporated into DNA and RNA, producing the replication and transcription inhibition, respectively. It is believed that nucleoside transporter proteins are responsible for cellular uptake of these nucleoside derivatives. Once they enter cells, some kinases phosphorylate nucleoside analogues to the corresponding mono-, di-, and triphosphates. Nucleoside triphosphate analogues are regarded as the active cytotoxic form that leads to cell apoptosis as these are substrates for DNA polymerases and can be incorporated into DNA. When they integrated into an elongate viral DNA chain, the nucleotide inhibitor works as a chain terminator to terminate viral DNA synthesis [1–4]. Moreover, certain nucleoside 5-O-triphosphate analogues also can be incorporated into RNA to inhibit RNA synthesis. Currently, thousands of nucleoside and nucleotide derivatives are synthesized and their synthetic strategy is highlighted [5].

The attractive progress in this field would be new anti-HBV and anti-HCV drugs approved for market. The first-generation anti-HBV drug is Entecavir, which was approved by FDA in 2005 [6]. Tenofovir (TFV) was originally

described as anti-HIV agent in 1993 under the name of (R)-PMPA. Then many TFV derivatives were synthesized through modification of phosphate moiety such as tenofovir disoproxil fumarate (TDF). Tenofovir alafenamide (TAF) can be considered a new prodrug of TFV with enhanced delivery to lymphoid and hepatocytes. Its active diphosphate metabolite can target RNA-dependent DNA polymerase (reverse transcriptase) of both HIV and HBV. TAF is equally potent as an antiretrovirus agent at a 30-fold lower dose than TDF. TAF (Vamlidy), approved by FDA in 2016, is not only described for the treatment and prevention of HIV infections but also shows promise for the treatment of HBV infections [7] (Figure 1).

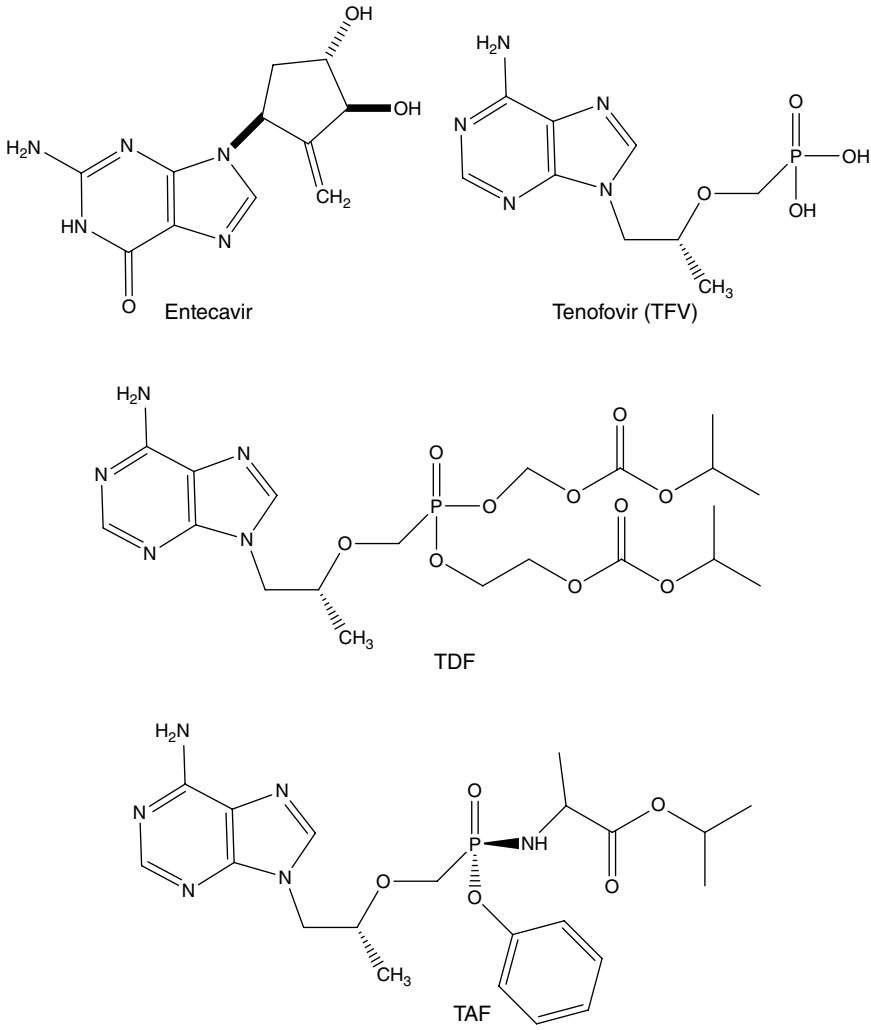

Figure 1 Structures of entecavir, tenofovir (TFV), and its derivatives TDV and TAF.

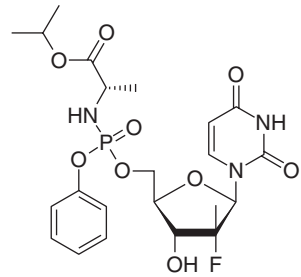

Figure 2 Structure of Sofosbuvir.

HCV is a potentially life-threatening disease and may lead to progressive liver damage and liver failure consequently. The first generation of anti-HCV drug is sofosbuvir. It undergoes the intracellular metabolism to its pharmacologically active form, uridine analogue triphosphate (GS-461203). This active metabolite is then incorporated into HCV RNA by NS5B RNA-dependent RNA polymerase and leads to the chain termination of HCV RNA. *In vitro*, the polymerase activity of the recombinant NS5B from several different HCV genotypes can be inhibited by GS-461203. This magic anti-HCV drug is a fixed-dose combination tablet containing sofosbuvir and NS5A/B protease inhibitors velpatasvir and voxilapavir for oral administration [8]. Another fixed dosage combination tablet named Harnovi in market, where NS5A/B protease inhibitor ledipasvir is used instead of velpatasvir and voxilapavir (Figure 2).

Oligonucleotides targeting RNA have attracted substantial interest from both academic institutions and pharmaceutical companies. Antisense, Aptamer, and siRNA oligonucleotides are designed based on different mechanisms and developed to be new agents for the sequence-specific gene silencing of target cellular mRNAs or selective interaction with target proteins. And the potential of mRNA technology for rapid vaccine development is valuable in light of COVID-19 pandemic [9].

The first antisense oligonucleotide drug is Vitravene, which was developed by Isis Pharmaceutics and approved by FDA in 1998 for the specific treatment of CMV Retinitis. Followed by Vitravene, an aptamer drug (Pegaptanib) was approved by FDA in 2003 targeting of VEGF (vascular endothelial growth factor) for the treatment of diabetic macular edema (DME). However, the *in vivo* therapeutic potency of the aptamer drug is crucially limited by their inherent physicochemical characteristics which may affect pharmacokinetic properties. These effects result in metabolic instability, rapid renal filtration, rapid distribution from the plasma compartment into the tissues (for example the liver or spleen), nonspecific immune activation, and polyanionic effects [10]. Pegaptanib has been superseded by VEGF-specific monoclonal antibodies that show improved therapeutic effects. Another antisense oligonucleotide Kynamro that targets messenger RNA for apolipoprotein B was developed and approved by FDA (2013) to treat homozygous familial hypercholesterolemia, FoFH.

The wide adoption of RNA-based therapeutics across the biopharmaceutical industry takes a few more years. Recent research achievements have begun to open a new area for RNA-based therapeutics, especially new delivery systems are developed for oligonucleotide drugs, such as *N*-acetylgalactosamine (GalNAc) conjugation [11] and lipid nanoparticle for the delivery drugs into hepatocytes. And several candidates were further developed for the treatment of hepatic viruses, liver-centric genetic diseases, and cardiometabolic disorders. Drug formulations of RNA-based therapeutics have made significant progress including pharmacodynamics-related challenges in targeting specificity, off-target RNAi activity and immune-mediated cytotoxicity, and pharmacokinetics-related challenges in systemic circulation, cellular uptake, and endosomal escape [12, 13]. In 2016, two antisense oligonucleotides, Eteplirsen (Exondys 510) and Spinraza (Nusinersen) were also approved by FDA for the treatment of Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA), respectively.

RNA interference (RNAi) is a hot field in nucleic acid drug development by the use of small inhibitory double-stranded RNA(siRNA) to target degradation of the sequence-specific cellular mRNAs, and as a result to silencing gene expression. With the more recent development of RNAi in mammalian systems, investigators have opened a new therapeutic approaches in human genetics and/or infectious diseases. Patisiran is the first RNAi therapeutics, approved by FDA in 2018, targeting transthyretin (TTR) for the treatment of polyneuropathy in adult patients with hereditary transthyretin-mediated (hATTR) amyloidosis, a progressive, debilitating, chronic, and often fatal disease. Another antisense oligonucleotide Givosiran for the treatment of hATTR has also been approved in 2019. Very recently, Novartis receives positive opinion for Leqvio (Inclisiran) from Committee for Medicinal Products for Humen Use (CHMP) of European Medicines Agency (EMA), a potential first-in-class siRNA for the treatment of high cholesterol. Inclisiran has already been approved by FDA now. In 23 November 2020, FDA approved another siRNA drug, Oxlumo (Lumasiran), for the treatment of primary hyperoxaluria type 1 (PH1) to lower oxalate in urine. Oxlumo works by degrading hydroxyacid oxidase 1(HAO1) messenger RNA and reducing the synthesis of glycolate oxidase, which inhibits hepatic production of oxalate, the toxic metabolite responsible for the clinical manifestations of PH1. Up to now, 431 RNA-based therapeutics are in different stage for clinic trials [9]. A blowout growth of RNA-based therapeutics will be expected in the future drug market.

Nucleic acid detection has shown a wide range of applications, including diagnostics, biosensing and bioimaging, affinity isolation, biomarker discovery. Recent advances in the sequence-specific detection of nucleic acids now provide a number of options for rapid and cost-effective diagnostics, such as nanomaterial-based nucleic acid detection approaches [14] and point-of-care CRISPR/Cas nucleic acid detection [15]. With the more recent development of sgRNA in CRISPR/Cas systems, investigators attempt the development of new

therapeutic approaches in human genetics and/or infectious diseases. On another hand, precision medicine and gene therapy became a very hot field. Recent technological and analytical advances in genomics have made it possible to rapidly identify and interpret the genetic variation underlying a single patient's disease. The current COVID-19 pandemic presents a serious public health crisis, and a better understanding of the spread scope and pathway of the virus would be aided by more widespread testing. Nucleic-acid-based tests currently offer the most sensitive and early detection of COVID-19 [16].

RNA epigenetics is another hot research area in RNA chemical biology [17]. Many research contributions are still trying to figure out how and why around 170 different chemical modifications impact RNA biology. Now, increasing evidences suggest that RNA methylation plays the key role in diseases from cancers to infectious diseases. RNA methylation to form N^6-methyladenosine (m^6A) in mRNA accounts for the most abundant mRNA internal modification and has emerged as a widespread regulatory mechanism that controls gene expression in diverse physiological processes. For DNA, RNA, and histones alike, three factors, writers, readers and erasers, are most important for these regulation process: "writers" add post-synthesis modifications that alter the molecular structure to either recruit or repel "readers." Readers interpret those marks to alter transcription in the case of DNA and histones, or to modulate translation and degradation in the case of RNA. And "erasers" remove the modifications, restoring the unaltered functions of the source materials. For histone biology, drug developers have already made inroads against all three components of the writer–reader–eraser paradigm. The same things are going to do in RNAs, small-molecule inhibitors of the METTL3–METTL14 complex, which regulates epigenetic marks on RNA, have been developed for clinic trials [18]. On the another hand, many RNAs fold into structures that can be selectively targeted with small molecules, which makes it possible to develop sequence-based design of ligands targeting RNAs [19].

Drugs are often divided into the broad classes of small organic molecules and "biologics," the latter of which are most often medicinal preparations of proteins such as natural and recombinant antibodies. In general, drug discovery is the identification of novel active chemical compounds that typically target a desired protein using a biological assay. RNA-based therapeutics, targeting directly to the desired mRNA, will open a new field for the clinical medicine as well as medicinal chemistry. In this book, 14 research groups are invited to contribute the recent progresses in nucleic acid medicinal chemistry and chemical biology. I would like to take this opportunity to express my most sincere thanks for their contributions and also for the hard work of ***Wiley*** Publishers.

Li He Zhang
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31 December 2020

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CHAPTER 1

DESIGN, SYNTHESIS, AND APPLICATIONS OF NUCLEOSIDE PHOSPHATE AND PHOSPHONATE PRODRUGS

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1.1. INTRODUCTION

For decades, numerous nucleoside analogs have been developed as drugs or drug candidates [1, 2] for the treatment of various cancers and infections by different viruses, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), and human cytomegalovirus (HCMV). In cells, nucleoside analog molecules are converted to the corresponding phosphates by nucleoside or nucleotide kinases [3, 4] and then are incorporated into viral or tumor DNA/RNA where they serve as chain terminators. For example, the anti-HIV nucleoside drug azvudine (FNC) [4–6] is further phosphorylated *in vivo* to the active nucleoside triphosphate (FNC-TP, Figure 1.1), which inhibits the viral replication. However, the structural differences between the nucleoside analogs and the natural nucleosides may affect the phosphorylation processes, thus decreasing the pharmacological activity of the compounds [7, 8]. Due to their poor chemical stability and high polarity, the corresponding phosphates themselves cannot be used directly as drugs

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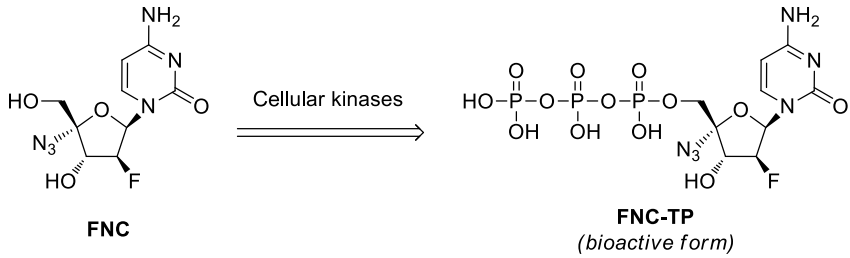

Figure 1.1 Formation *in vivo* of the active triphosphate (TP) of FNC.

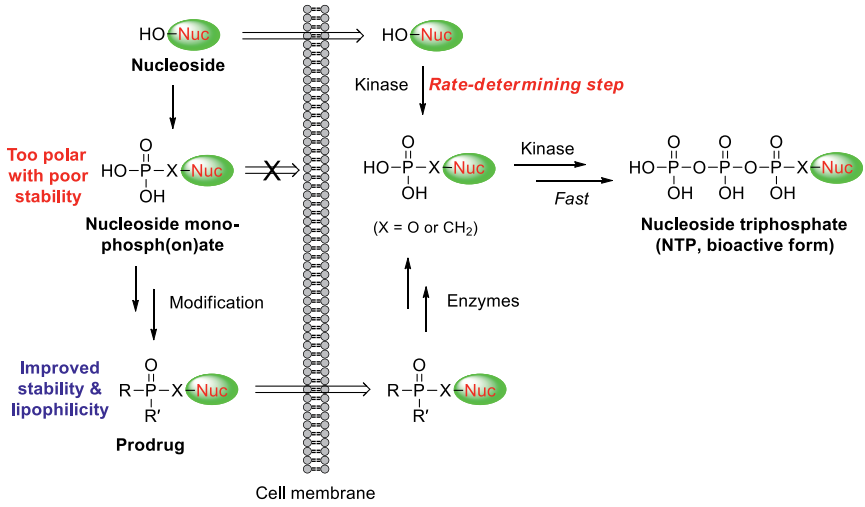

Figure 1.2 Design and mechanism of action of nucleoside phosph(on)ate prodrugs.

(Figure 1.2). To address these issues, diverse prodrugs of nucleoside (nucleotide) phosph(on)ate analogs have been designed and synthesized in drug discovery [3, 9–14].

Introduction of phosph(on)ate esters as prodrugs (Figure 1.2) has emerged as a very useful tool in the design and discovery of nucleoside and nucleotide analog drugs for the treatment of cancers and viral infectious diseases. The features of a prodrug may include (1) improved chemical stability; (2) increased lipophilicity for better bioavailability; (3) oral availability (the parent compound may often be administered only by injection); and (4) an improved therapeutic effect with reduced toxicity *via* targeted drug delivery. In recent years, considerable effort has been devoted to the design, synthesis, and biological evaluation of nucleoside phosph(on)ate prodrugs and more than twenty drug candidates have entered clinical development. Of these, several prodrugs have received FDA approval for clinical use. These include

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