

Biocatalytic Synthesis of Antiviral Nucleosides, Cyclic Dinucleotides, and Oligonucleotide Therapies

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Cite This: JACS Au 2023, 3, 13–24



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ABSTRACT: Nucleosides, nucleotides, and oligonucleotides modulate diverse cellular processes ranging from protein production to cell signaling. It is therefore unsurprising that synthetic analogues of nucleosides and their derivatives have emerged as a versatile class of drug molecules for the treatment of a wide range of disease areas. Despite their great therapeutic potential, the dense arrangements of functional groups and stereogenic centers present in nucleic acid analogues pose a considerable synthetic challenge, especially in the context of large-scale manufacturing. Commonly employed synthetic methods rely on extensive protecting group manipulations, which compromise step-economy and result in high process mass intensities. Biocatalytic approaches have the potential to address these limitations, enabling the development of more streamlined, selective, and sustainable synthetic routes. Here we review recent achievements in the biocatalytic manufacturing of nucleosides and cyclic dinucleotides along with progress in developing enzymatic strategies to produce oligonucleotide therapies. We also highlight opportunities for innovations that are needed to facilitate widespread adoption of these biocatalytic methods across the pharmaceutical industry.

KEYWORDS: biocatalysis, nucleosides, cyclic dinucleotides, oligonucleotides, nucleic acid therapeutics



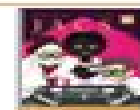
INTRODUCTION

Nucleotides are a central building block of life. They make up our DNA and RNA that in turn encode the proteins required to control biochemical processes and are also used as enzyme cofactors and as a source of energy. Synthetic analogues of nucleosides, nucleotides, and oligonucleotides, herein referred to as nucleic acids, have been developed as therapeutics to target diverse processes including DNA replication, transcription, translation, and cell signaling (Figure 1).^{1–6} Nucleosides, comprising a ribose sugar and nucleobase, are well established as anticancer and antiviral therapies.^{1–3} For example, lamivudine and nelarabine are nucleoside prodrugs that are converted to the corresponding nucleotide triphosphates (NTPs) *in vivo* and incorporated into DNA by polymerases. These noncanonical nucleotides inhibit further chain extension, thus preventing DNA replication and inhibiting cell or viral proliferation. Cyclic dinucleotides (CDNs) are secondary signaling molecules which control many processes in both prokaryotes and eukaryotes and are comprised of two ribonucleic acid monophosphates linked via 3'–5' and 2'–5' phosphodiester bonds.^{6–9} CDNs activate the stimulator of interferon genes (STING) protein to elicit an immune response and are currently being evaluated as potential cancer treatments.^{10–12} While nucleoside and

nucleotide analogues commonly target proteins to elicit their therapeutic effects, oligonucleotides target mRNA to modulate the production of disease related proteins.^{7–9} Therapeutic oligonucleotides, which are typically 20 nucleobases in length and can be single stranded (antisense oligonucleotides) or double stranded (siRNAs), have attracted much attention in recent years. Although initially developed to treat rare diseases, therapies for more common disorders have started to emerge, as highlighted by the recent approval of inclisiran for the treatment of atherosclerotic cardiovascular disease.^{13,14}

Nucleic acid therapeutics contain chemical modifications to the ribose sugar, nucleobase and/or phosphate backbone, which confer favorable selectivity, binding affinity, metabolic stability, and toxicity profiles.^{15,16} Common modifications include 2'-ribose substitutions, designed to enhance metabolic stability and promote a stable RNA-like C3'-endo sugar conformation for improved binding affinity. CDNs and

Received: September 1, 2022
Revised: November 11, 2022
Accepted: November 11, 2022
Published: November 30, 2022



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