

Chapter 13

Nucleoside Phosphorylation and Related Modifications

Natural nucleic acids (DNA, RNA) contain phosphodiester groups, dinucleoside pyrophosphates (as intermediates in nucleic acid ligation), and dinucleoside triphosphates (mRNA caps). Nucleoside triphosphates are substrates for the biochemical synthesis of nucleic acids, and nucleoside analog triphosphates are frequently used as substrates for the synthesis of modified nucleic acids and as components in tools for molecular techniques in nucleic acid biology. Derivatives and analogs of natural nucleosides are frequently studied as their mono-, di-, and triphosphate derivatives in studies of biochemical processing. In many cases these studies stem from interest in these analogs as therapeutics, in other cases as tools for probing and dissecting the function of phosphorylated nucleoside intermediates in biochemical pathways including nucleoside metabolism, co-factor function, and signal transduction.

There are many effective chemical reagents and strategies for transforming nucleosides to nucleoside monophosphates (nucleotides). However, the transformation of nucleotides or nucleotide derivatives to their di- and triphosphates is often a challenge. The nature of the nucleobase as well as the presence of certain kinds of substituents can strongly influence the outcome of both chemical and enzymatic phosphorylation. In addition, there is interest in analogs derived from modified phosphates, including phosphorothioates and phosphoramidates. There is no one optimal way to construct all of the different types of phosphorylated nucleosides. The goal of this chapter will be to provide a cross-section of the best methods for different analogs and situations.

The first unit, *UNIT 13.1*, provides a complete overview of nucleoside phosphate and polyphosphate synthesis. The chapter critically discusses the issues and problems associated with these syntheses. Classical chemical methods for synthesis of nucleoside monophosphates are discussed, and strategies for synthesis of diphosphates, triphosphates, and cyclic phosphates are presented. A substantial portion of the chapter is devoted to nucleotide analogs of interest to nucleic acid chemists, including thio-phosphorous derivatives, phosphonates, and imidophosphates. A section on ^{32}P -radiolabeled derivatives is included as well. Finally, there are sections on phosphosulfate and enzymatic nucleotide synthesis.

The most common rapid method for synthesis of nucleoside triphosphates is the Yoshikawa procedure that is often done as a single continuous operation. One of the most effective versions of this process is provided in *UNIT 1.5*. However, analogs containing sensitive modified bases cannot always be phosphorylated by the Yoshikawa procedure. In *UNIT 13.2*, Wu et al. outline effective procedures for the synthesis of ribo- and deoxyribonucleoside 5'-triphosphates ofazole carboxamide-containing nucleoside analogs using a combination of chemical and enzymatic methods.

Donald E. Bergstrom

Contributed by Donald E. Bergstrom

Current Protocols in Nucleic Acid Chemistry (2004) 13.0.1

Copyright © 2004 by John Wiley & Sons, Inc.

**Nucleoside
Phosphorylation
and Related
Modifications**

13.0.1

Supplement 16