CHAPTER 1
Synthesis of Modified Nucleosides

INTRODUCTION

This chapter will focus first on nucleosides modified in the heterocyclic base. Base-modified nucleosides have a wide variety of uses, often after incorporation into oligonucleotides, such as attachment to reporter groups and studies of base-pairing altered by mutagens, by carcinogens, or by design (Beaucage and Iyer, 1993). In keeping with *Current Protocols* format, the goal of this chapter is not to review the subject, but to provide a set of specific and detailed procedures leading to specific compounds. Within this framework, however, the procedures should enable researchers to undertake syntheses of related compounds that have been reported in the chemical literature. The compounds produced may themselves be key intermediates from which other modified nucleosides of interest can be prepared. For example, the palladium-mediated C5 substitution of pyrimidines pioneered by Bergstrom has proved over the years to be a uniquely valuable route to these important compounds (Goodchild, 1990). An understanding of the synthetic procedure detailed in *UNIT 1.1* will provide access to a large number of C5-modified pyrimidines. Similarly, *UNIT 1.8* describes the synthesis and use of aminoalkyl-modified purine analogs.

The enzymatic coupling methods in *UNIT 1.2* and *UNIT 1.6* use enzymatic transglycosylation for synthesis of 2′-deoxyribonucleosides and ribonucleosides, respectively. Although nucleoside 2′-deoxyribosyltransferase is not commercially available at this time, the alternative enzymes (thymidine phosphorylase and purine nucleoside phosphorylase) are available. These routes then provide access to a wide variety of both 2′-deoxyribo- and ribonucleosides.

Modified nucleosides containing reactive functionality, which have been denoted as "convertible" nucleosides when incorporated into oligonucleotides, are an increasingly important class of compounds, of which the 2-fluoro-2′-deoxyinosine derivative described in *UNIT 1.3* is a timely example (Huang et al., 1999). *UNIT 1.4* provides a discussion on the topic of unnatural nucleotides with unusual base-pairing properties and of universal nucleotides. *UNIT 1.5* continues this topic by providing step-by-step procedures for synthesizing *N*- or *C*-nucleosides with a number of specific base analogs. *UNIT 1.7* describes the synthesis of 2′-deoxy-2′-fluoroarabinonucleosides. Oligonucleotides containing these modified bases form stable heteroduplexes with RNA that are substrates for RNase H, which is important to antisense applications.

UNIT 1.9 addresses the use of nucleosides with a modified sugar to create hexitol nucleic acids (HNAs). This unit decribes the synthesis of 1,5-anhydrohexitol nucleoside monomers as well as their starting sugar compound. These can be used in automated oligonucleotide synthesis with standard phosphoramidite chemistry to synthesize HNAs, which hybridize to RNA and DNA in a sequence-specific manner and have applications in antisense and antiviral approaches.

To a very different end, *UNIT 1.6* provides procedures for the specific ^{15}N-labeling of adenosine and guanosine. These nucleosides are useful in NMR studies that probe local interactions at nitrogen atoms, such as hydrogen bonding, stacking, and protonation.

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LITERATURE CITED

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