CHAPTER 2
Protection of Nucleosides for Oligonucleotide Synthesis

INTRODUCTION

Since the discovery of the helical structure of DNA by Watson and Crick in 1953, tremendous strides in the chemical synthesis of oligonucleotides have been made in an attempt to produce substantial quantities of synthetic DNA or RNA of defined sequences. The availability of synthetic oligonucleotides has facilitated investigations on gene structure and function, and has intensified research efforts in deciphering the nature of nucleic acid interactions in complex cellular functions.

The essence of DNA and RNA synthesis is the correct formation of internucleotide phosphodiester linkages. This is complicated by the presence of reactive functional groups within nucleotide monomers that require protection prior to incorporation into DNA or RNA oligonucleotides. The presence of two and three hydroxyls in deoxyribonucleosides and ribonucleosides, respectively, demands proper protection of these groups to ensure formation of internucleotide linkages with precise directional polarity during chain assembly. In addition to hydroxy groups, the functional groups of the nucleobases require protection to prevent formation of side products during internucleotide coupling reactions.

One objective of this chapter is to provide literature overviews of the functional groups that have been used for nucleobase protection along with those employed as protecting groups for the 5′- and 2′-hydroxyls of nucleosides. For example, *UNIT 2.1* outlines protective groups for the imide/lactam function of thymine/uracil and guanine, respectively. This class of protecting groups prevents irreversible nucleobase modifications that may occur in the presence of alkylating or condensing reagents; these are commonly used in nucleoside protection strategies and during oligonucleotide synthesis. *UNIT 2.1* also identifies protecting groups for the exocyclic amino function of cytosine, adenine, and guanine. In this context, the overview addresses oligonucleotide depurination during synthesis, and examines purine *N*-protecting groups that have been shown to minimize this problem. Finally, *UNIT 2.1* explores recent trends in nucleobase protection that would permit reliable oligonucleotide synthesis and facile removal of *N*-protecting groups under very mild conditions.

UNIT 2.2 relates to 2′-hydroxyl protection of oligoribonucleotides, which, in fact, dictates the selection of both nucleobase and 5′-hydroxyl-protecting groups. The unit surveys in exquisite detail the various types of protecting groups that have been used in the past and those that are currently being used in the synthesis of oligoribonucleotides. The requirements that a protective group must satisfy to become the 2′-hydroxyl-protecting group of choice, in regard to effective oligoribonucleotide synthesis, are delineated in the unit. Furthermore, the issue of 2′-*O*-acyl and 2′-*O*-silyl group migration to the 3′-hydroxy function of ribonucleosides during protection along with the consequences of the conditions used for their removal on the stability of internucleotide linkages are authoritatively presented. The wealth of information emerging from *UNIT 2.2* shall allow one to undertake oligoribonucleotide synthesis with confidence.

Methods for protecting the 5′-hydroxy function of nucleosides are equally well reviewed in *UNIT 2.3*. Acid-labile protecting groups that can be used as hydrophobic ligands in the

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Current Protocols in Nucleic Acid Chemistry (2004) 2.0.1-2.0.2

purification of synthetic oligonucleotides are particularly useful and compatible with the most popular oligonucleotide synthesis protocols. Alternatively, 5′-hydroxyl protection of nucleosides with base-labile protecting groups is particularly attractive because it eliminates the risk of oligonucleotide depurination encountered in the stepwise deprotection of acid-labile 5′-*O*-protecting groups during synthesis. Base-labile 5′-*O*-protecting groups enable the development of orthogonal protection systems for oligoribonucleotides by expanding the choice of acid-labile 2′-*O*-protecting groups that can be used. The utilization of the 9-fluorenylmethoxycarbonyl (Fmoc) group for 5′-*O*-protection and 4-methoxytetrahydropyran-4-yl for 2′-*O*-protection of ribonucleosides is an excellent example of orthogonal protection in oligoribonucleotide synthesis. This method is detailed in *UNIT 2.4*.

In addition to acid- and base-labile protecting groups, 5′-*O*-silyl-blocking groups that are removable by fluoride ions, photolabile 5′-*O*-protecting groups, and other functional groups that can be removed under near neutral conditions have been carefully examined in *UNIT 2.3*. The overviews presented in this chapter will provide anyone interested in oligonucleotide synthesis with the basic knowledge to proceed with the preparation of properly protected nucleoside phosphoramidites or *H*-phosphonate monomers for incorporation into oligonucleotides. In this regard, a number of synthetic protocols delineating the step-by-step preparation of nucleosides functionalized with *N*-, 5′-*O*-, and 2′-*O*-protecting groups will eventually be included in this chapter to illustrate the versatility of protecting group combinations that allow, in a number of ways, the synthesis of oligonucleotides with well-defined physicochemical properties. Syntheses of *N*-protected ribonucleosides having a 4,4′-dimethoxytrityl (DMTr) group for 5′-*O*-protection and a photolabile 2-nitrobenzyloxymethyl (NBOM) group or a fluoride-sensitive *tert*-butyldimethylsilyl (TBDMS) group for 2′-*O*-protection are outlined in *UNIT 2.5*, and are representative examples of such synthetic protocols. An elegant method for the highly regioselective synthesis of 2′-*O*-TBDMS purine ribonucleosides is presented in *UNIT 2.8*. A creative modification of the NBOM group has led to the development of the fluoride-sensitive [(triisopropylsilyl)oxy]methyl (TOM) group for 2′-*O*-protection of ribonucleosides. The detailed preparation of *N*-protected 5′-*O*-DMTr-2′-*O*-TOM ribo-nucleosides is provided in *UNIT 2.9*. Alternatively, *UNIT 2.10* addresses the preparation of *N*-protected 5′-*O*-benzhydroxy-bis(trimethylsilyloxy)silyl-2′-*O*-bis(acetoxyethyloxy)-methyl ribonucleosides and their phosphoramidite derivatives as innovative precursors to the solid-phase synthesis of oligoribonucleotides. The preparation of RNA oligonucleotides suitable for small interfering RNA applications using the precursors will be presented in a forthcoming unit.

The conversion of suitably protected deoxyribonucleosides and ribonucleosides to *H*-phosphonate derivatives is carefully described in *UNIT 2.6*. Alternatively, the conversion of protected deoxyribonucleosides to phosphoramidite derivatives functionalized with groups different than the conventional 2-cyanoethyl group for *P*-protection is delineated in *UNIT 2.7*. The generality of these methods ensures the reliable preparation of monomeric nucleoside building blocks for oligonucleotide syntheses.

It should be noted that with the advent of oligonucleotide microarrays as powerful diagnostic tools, deoxyribonucleoside phosphoramidites functionalized with photosensitive groups for 5′-/3′-hydroxyl protection have been developed to enable the synthesis of oligonucleotides on planar glass surfaces. To this end, the preparation of 3′-*O*-[2-(2-nitrophenyl)propoxycarbonyl] deoxyribonucleoside 5′-phosphoramidites is reported in *UNIT 12.3*.

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