CHAPTER 5
Methods for Cross-Linking Nucleic Acids

INTRODUCTION

Characterization of synthetic oligonucleotides shapes much of our understanding of native, higher-molecular-weight DNA and RNA molecules. Although of immense utility, oligonucleotides usually possess lower structural and thermal stability and have greater end effects than the larger nucleic acid constructs they are intended to model. Hence, the physiochemical and biological properties of oligonucleotides may not always compare favorably to those of larger nucleic acids. One of the most successful strategies to stabilize oligonucleotides is to connect the strands that comprise elements of helical structure with an interstrand cross-link. Cross-linked oligonucleotides are often very resistant to denaturation (induced thermally or by changes in pH, salt, or oligonucleotide concentration) relative to their unmodified constructs. A wide variety of novel chemistries exist to introduce cross-links into nucleic acids. In nearly all of these methods, solid-phase synthesis of oligomers site-specifically labeled with modified nucleosides bearing the appropriate reactive functional groups is used to generate cross-links in high yield.

In addition to providing increased structural stability, cross-links have been used to probe the geometry and conformational dynamics of both medium- and large-sized nucleic acids and have been used to explore the topology of protein-ligand complexes. In the units presented in this chapter, along with those in forthcoming supplements, the reader will be provided with state-of-the art protocols to form cross-links within nucleic acids and nucleic acid–ligand complexes. To provide the reader with a comprehensive array of techniques, the chapter will present the chemistry to synthesize both interstrand and intrastrand cross-links, cyclic nucleic acids, and nucleic acid–ligand complexes.

UNIT 5.1 presents protocols to postsynthetically modify 2-amino-containing oligoribonucleotides with either an alkyl-phenyl disulfide or an alkyl thiol group. These groups react under mild conditions to form disulfide cross-links by thiol-disulfide interchange. When incorporated on opposite faces of a short, continuous RNA helix, these reactants, as expected, do not form a disulfide bond. In contrast, when these reactive groups are placed in proximity, disulfide cross-links form rapidly. In addition, by incorporating these groups at various positions of large RNAs through semisynthesis, the dynamics of thermal motions can be detected. Such motions are believed to be linked to biological function, and the protocols presented are among the few simple ways to assess such dynamics.

Methods to synthesize small circular oligonucleotides for use in diagnostic, therapeutic, and laboratory operations are presented in *UNIT 5.2*. These systems have gained considerable attention in recent years because they form unusually strong and specific complexes with RNA and DNA strands. In addition to their properties as molecular recognition agents, synthetic circular DNAs 20 to 200 nucleotides in size can also serve as catalysts for the amplified synthesis of DNA and RNA, a process termed "rolling circle synthesis."

One of the most convenient methods for generating oligonucleotides possessing either intrastrand or interstrand cross-links is through incorporation of oligo(ethylene glycol) bridges by solid-phase synthesis as described in *UNIT 5.3*. Many of the reagents needed are either commercially available or can be prepared in a few easy synthetic steps. Unlike many other DNA and RNA cross-links, aspects of the structural and thermodynamic impact of modifying nucleic acids with oligo(ethylene glycol) has been studied.

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In *UNIT 5.4*, a second group of methods for incorporating disulfide cross-links within RNA structure is presented. These protocols describe methods for the synthesis of alkylthiol-modified ribonucleosides, their incorporation into synthetic RNA, and the formation of intramolecular disulfide bonds in RNA by air oxidation. The disulfide bonds can be formed in quantitative yields between thiols positioned in close proximity in either RNA secondary or tertiary structure. Disulfide cross-links are useful tools to probe solution structures of RNA, to monitor dynamic motions, to stabilize folded RNAs, and to study the process of tertiary structure folding.

In *UNIT 5.5*, site-specific cross-links are introduced into oligodeoxyribonucleotides by electrophilic substitution. A nucleophilic base (deoxythiouridine) is incorporated into an oligodeoxyribonucleotide, and an electrophilic tether is used to cross-link that base to a complementary DNA strand. A variety of different electrophilic DNA strands can be generated from the same deoxythiouridine-containing oligodeoxyribonucleotide by changing the nature of the electrophilic tether.

In *UNIT 5.6*, the preparation of short endcapped DNA duplexes is presented. Using this approach, the 5′ terminus of one strand of a duplex is cross-linked to the complementary 3′ strand with a hydrophobic or hydrophilic linker. Several different linkers are presented along with methods for their incorporation into DNA during solid-phase synthesis.

Finally, in *UNIT 5.7*, terminal disulfide cross-links are generated by substituting the terminal bases of oligodeoxyribonucleotides with a modified thymidine residue. The usefulness of this approach is discussed, with a demonstration that the cross-links do not modify the structure of the oligodeoxyribonucleotides, and examples of applications.

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