High-Yielding Method for On-Column Derivatization of Protected Oligodeoxynucleotides and Its Application to the Convergent Synthesis of 5′,3′-Bis-conjugates

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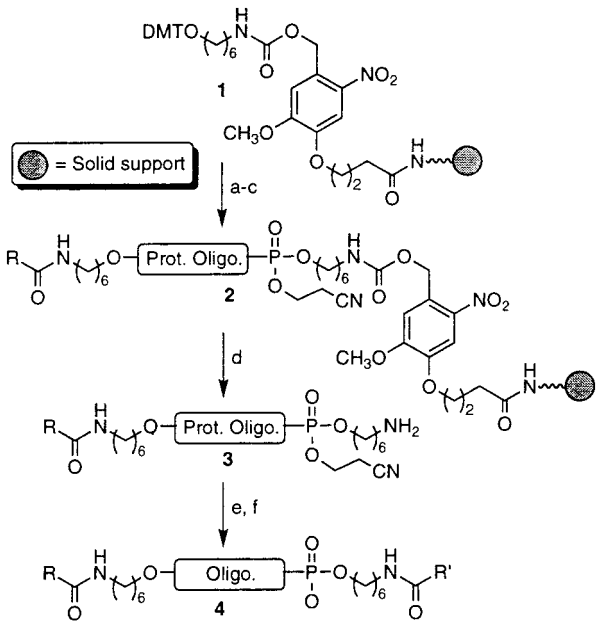
We recently reported on highly efficient methods for synthesizing 3′-oligodeoxynucleotide conjugates.[1] High yields of homogeneous products are obtained by using protected oligodeoxynucleotides in solution as substrates.[2] The protected oligonucleotides are prepared using photolabile solid-phase synthesis supports under conditions that do not damage the biopolymers and are compatible with commercially available phosphoramidite reagents.[1,2] These methods enjoy a number of advantages over previously reported strategies for preparing oligodeoxynucleotide conjugates, including short reaction times, mild reaction conditions, and the need for a relatively small excess of reagents.[3,4] In addition, the method is convergent, enabling one to readily prepare multiple conjugates from a single oligodeoxynucleotide synthesis. This latter feature is particularly useful for preparing libraries of oligodeoxynucleotides.[5] In addition to their utility as components of libraries, biopolymer conjugates are generally of increasing interest to a variety of chemists, due to their utility as diagnostic and mechanistic probes, as well as their potential therapeutic applications.[6,7] Consequently, we and others have continued to develop methods for their synthesis.[1,8] We now wish to report on a simple, highly effective method for modifying oligodeoxynucleotides at their 5′-termini. When coupled with our previously reported strategy for synthesizing 3′-oligodeoxynucleotide conjugates, this method provides a facile route for preparing 5′,3′-bis-conjugates of oligodeoxynucleotides in a convergent manner (Scheme 1).[9]

A number of methods exist for modifying the 5′-termini of oligodeoxynucleotides with biologically relevant molecules.[1,6,8] The most common approaches to introducing such modifications involve either preparing the appropriate phosphoramidite or postsynthetic conjugation of a fully deprotected oligodeoxynucleotide containing a 5′-alkylamine to an electrophilic form of the desired modifying molecule.[6]

Scheme 1[a]



[a] Key: (a) standard oligonucleotide synthesis; (b) demonomethoxytritylation; (c) RCO_2H, PyBOP, DIEA; (d) *hν*; (e) $R'CO_2H$, PyBOP, DIEA; (f) concentrated aqueous ammonia.

These methods can be accompanied by distinct disadvantages, including low yields, time-consuming reagent preparation, and impure products.[3,4,10,11] A method employing isolated activated carboxylic acid esters was recently reported for derivatizing protected oligodeoxynucleotides containing 5′-terminal amines while the biopolymer was still bound to its solid-phase support.[12] Although the yields of modified oligodeoxynucleotides were not explicitly reported, yields of peptide nucleic acids modified by this method were believed to be greater than 80%.

We sought to develop a method for on-column modification of the 5′-amino terminus of an oligodeoxynucleotide that would not require any prior synthetic manipulation of the electrophile. Using our recent experience with developing solution-phase conjugations of protected oligodeoxynucleotides containing 3′-alkylamines as a guide, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) was chosen as the activating agent for the carboxylic acid in solution.[1b] The initial oligodeoxynucleotide substrate, composed of a sequence useful for preparing triplex DNA and a 6-amino-*n*-hexyl linker at its 5′-terminus, was prepared on a standard 3′-succinato long-chain alkylamine controlled pore glass (CPG) support.[14,15] Following removal of the monomethoxytrityl group from the 5′-alkylamine terminus, the CPG-bound oligodeoxynucleotide was coupled

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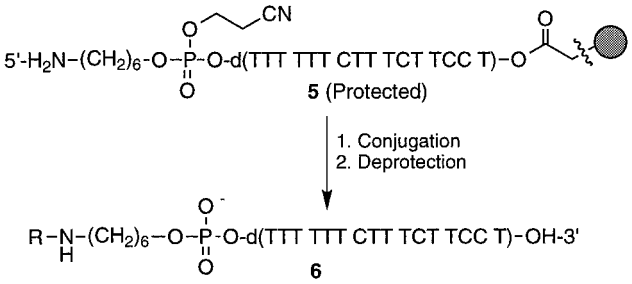
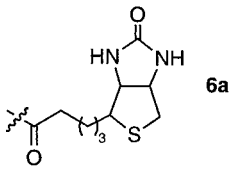
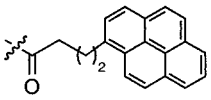
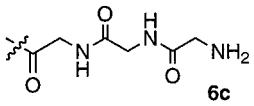
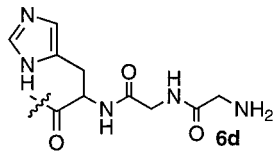
Table 1. Coupling Yields of Oligodeoxynucleotide Conjugates Prepared by On-Column Derivatization



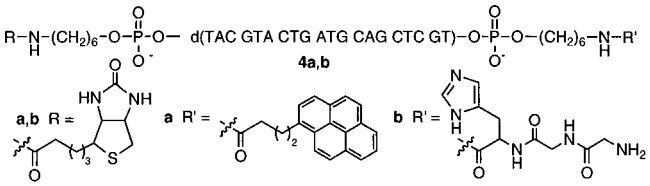
R	Isolated Yield (%)[a,b]
 **6a**	99 ± 4
 **6b**	104 ± 3
 **6c**	100 ± 4
 **6d**	86 ± 4

[a]Isolated yields were determined via comparing the amount of oligonucleotide obtained from a comparable amount of unconjugated material subjected to the identical deprotection, purification, isolation conditions. [b]Yields represent an average of a minimum of two reactions ± standard deviation from this value.

to biotin using PyBOP and diisopropylethylamine in DMF. On the basis of previous results of solution-phase conjugation, we found that 5 molar equiv of reagents relative to oligodeoxynucleotide were sufficient to effect conjugation in 15 min at room temperature.[13] Following removal of the excess reagents, deprotection with concentrated aqueous ammonium hydroxide, and purification by denaturing polyacrylamide gel electrophoresis, **6a** was obtained in essentially quantitative yield (Table 1). In addition, no diminution in yield of the biotin conjugate (**6a**) was detected when using as few as 2 equiv of reagents during the same reaction time. However, the isolated yield of **6a** dropped to 86 ± 2% (the remainder of material was unreacted starting material) when only 1 equiv of biotin and PyBOP were employed. The efficiency of the conjugation reaction was general, with the lowest isolated yield being obtained for coupling the tripeptide bis-Fmoc-Gly-Gly-His (Table 1). The high isolated yields of oligodeoxynucleotide conjugates obtained following alkaline treatment are consistent with highly specific reaction at the primary amine and the absence of electrophilic attack at other sites within the biopolymer. While we have previously shown that oligodeoxynucleotides containing a single reactive functional group do not undergo any undesirable modifications under these reaction conditions, all products were found to be homogeneous by electrospray mass spectrometry, providing further affirmation of the selectivity of the conjugation reaction.[1b]

With this simple, efficient method of preparing 5′-oligodeoxynucleotide conjugates in hand, we sought to expand the scope of this process to the synthesis of 5′,3′-bis-conjugates (Scheme 1). Oligodeoxynucleotides modified at both of their termini have many potential applications.[9] For instance, the presence of a molecule such as biotin at one terminus can provide a means of cellular transport or as a capture reagent for the oligodeoxynucleotide by providing a binding site for streptavidin.[16] Alternatively, the simultaneous incorporation of a polycyclic aromatic molecule or metal binding peptide may enhance the binding affinity of an oligodeoxynucleotide for its target or enable the biopolymer to behave as a nuclease mimic, respectively.[3,17]

To apply this approach to the synthesis of bis-conjugates, we prepared **2** on an orthogonal support (**1**, Scheme 1). This allows the release of 3′-(alkylamino)oligodeoxynucleotides into solution with retention of their protecting groups.[2] Biotin was conjugated to the support-bound biopolymer in quantitative yield, using 5 equiv of reagents as described above.[18] Following photolytic cleavage of **2** from its support, the crude protected oligodeoxynucleotide (**3**) was conjugated at its 3′-terminus in solution as previously described.[1b,2] 3′-Oligodeoxynucleotide conjugates of pyrene (**4a**, quantitative yield) and the tripeptide bis-Fmoc-Gly-Gly-His (**4b**, 87 ± 6%) were obtained using 10 molar equiv of activating agents (PyBOP, diisopropylethylamine) and the appropriate carboxylic acid in 2 h at 25 °C.[18] To our knowledge, such



coupling yields surpass those previously reported for postsynthetic modification of oligodeoxynucleotides. Furthermore, the ease with which this method is reduced to practice should make it a very useful tool for the synthesis of a variety of modified oligodeoxynucleotides from a single DNA synthesis.

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Supporting Information Available: General procedure for conducting conjugation reactions and electrospray mass spectra of **3**, **4a**,**b**, and **6a**−**d** (8 pages).

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(18) The yields were determined as described in the footnote to Table 1 and in refs 1 and 2.