Communications to the Editor

Palladium(0)-Catalyzed Modification of Oligonucleotides during Automated Solid-Phase Synthesis

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Oligonucleotides modified with functional groups have diverse and important research and clinical applications, including primers for DNA sequencing, hybridization probes for detecting DNA, antisense and antigene oligonucleotides for therapy, and spectroscopic probes for DNA structure and function studies.[1−19] Synthetic strategies toward these complex oligonucleotides focus primarily on either synthesizing the labeled phosphoramidite for subsequent incorporation into the nucleic acid strand or modifying the synthesized nucleic acid single strand after solid-phase synthesis.[1] The major hurdles with the first procedure are the multiple synthetic and purification steps to the desired phosphoramidite, which is moisture-sensitive and often possesses limited solubility in common solvents, followed by the low coupling yield on the DNA synthesizer and chromatographic purifications of the oligonucleotide derivative. The second procedure, oligonucleotide postmodification, does require fewer synthetic steps. This solution-phase coupling, however, of the functional group to the oligonucleotide is often hampered by a low coupling yield, side reactions, and chromatographic purifications to isolate the final product. Consequently, there is a need for new and alternative procedures that offer greater flexibility and synthetic ease to modify oligonucleotides. Herein, we report the site-specific modification of oligonucleotides on solid support using a novel procedure that combines the advantages of solid-phase DNA and Pd(0) cross-coupling chemistries.

To demonstrate the generality of this new oligonucleotide modification procedure, three structurally and electronically different functionalities were directly incorporated into an oligonucleotide *during* standard automated DNA solid-phase synthesis.[20−22] Alkynyl-derivatized amines, biotin, and a transition-metal complex, each of which possesses unique properties and synthetic requirements, were attached to an oligonucleotide using the Sonogashira Pd(0) cross-coupling reaction.[23−26] These alkynyl precursors (**2**, **3**, **5**, **8**) were synthesized as shown in Scheme 1. The protected amines, *N*-trifluoroacetyl propargylamine (**2**) and *N*-*tert*-butyloxycarbonyl propargylamine (**3**) were prepared by reacting propargylamine with ethyl trifluoroacetate in MeOH and di-*tert*-butyl dicarbonate in water/$CHCl_3$, respectively.[27,28] The biotin analogue, 6-((6-biotinoylamino hexanoyl)aminohexanoyl)-propargylamine (**5**), was synthesized from the biotin aminohexanoylaminohexanoic acid succinimidyl ester (biotin-NHS ester) and propargylamine in dry DMF.[29] Reaction of $Ru(bpy)_2Cl_2$ with 4′-methyl-2,2′-bipyridine-4-carbonylpropargylamine afforded bis-(2,2′-bipyridine)(4′-methyl-2,2′-bipyridine-4-carbonylpropargylamine) ruthenium(II)bis-(hexafluorophosphate), **8**. Incorporation of these groups into the oligonucleotide also required the preparation of a 5-iodo-substituted pyrimidine nucleoside for subsequent Pd(0) cross-coupling reaction. Specifically, 5′-DMT-3′-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite-2′-deoxy-5-iodouridine was synthesized in two steps. In the first step, 2′-deoxy-5-iodouridine was treated with DMT-Cl in dry pyridine to afford 5′-DMT-2′-deoxy-5-iodouridine.[22,27] This DMT-protected nucleoside was then reacted with 2-cyanoethylchloro-*N*,*N*′-diisopropylphosphoramidite, in the presence of diisopropylethylamine, to yield 5′-DMT-3′-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite-2′-deoxy-5-iodouridine, **9**.[30]

The oligonucleotide syntheses were performed on a commercial ABI 395 DNA/RNA synthesizer from the 3′ to 5′ end using standard automated DNA synthesis protocols as shown in Scheme 2 (0.2 and 1.0 μmol scale). A 0.1 M solution of 5′-DMT-3′-cyanoethyl-*N*,*N*′-diisopropyl phosphoramidite-2′-deoxy-5-iodouridine in dry acetonitrile was prepared and installed on the DNA synthesizer in a standard reagent bottle. All solid-phase syntheses were performed in such a manner that the sequence was stopped after incorporation of the 5′-DMT-3′-cyanoethyl-*N*,*N*′-diisopropyl phosphoramidite-2′-deoxy-5-iodouridine, and without deprotecting the 5′-hydroxyl or cleaving the oligonucleotide from the resin. The column was subsequently removed from the synthesizer and

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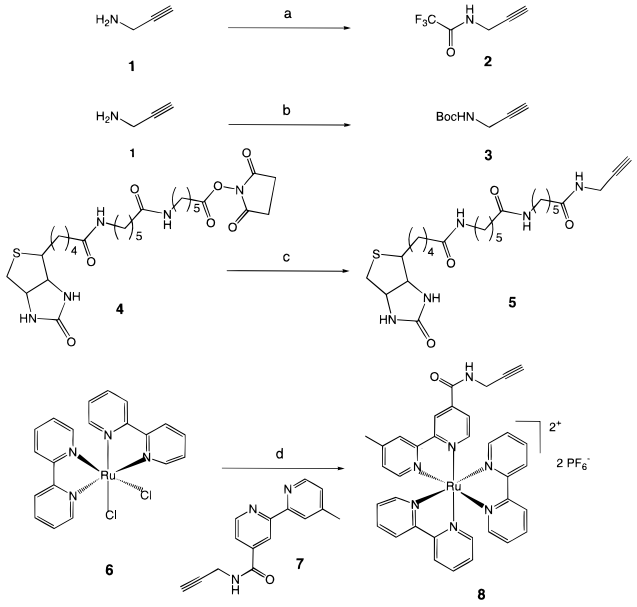
Scheme 1[a]



[a] Key: (a) ethyl trifluoroacetate, MeOH, 25 °C, 3 h, yield 82%; (b) water, $NaHCO_3$, di-*tert*-butyl dicarbonate, 25 °C, 3 h, yield 87%; (c) propargyl amine, DMF, 25 °C, 24 h, yield 83%; (d) 70% ethanol/water, reflux, 10 h, yield 82%.

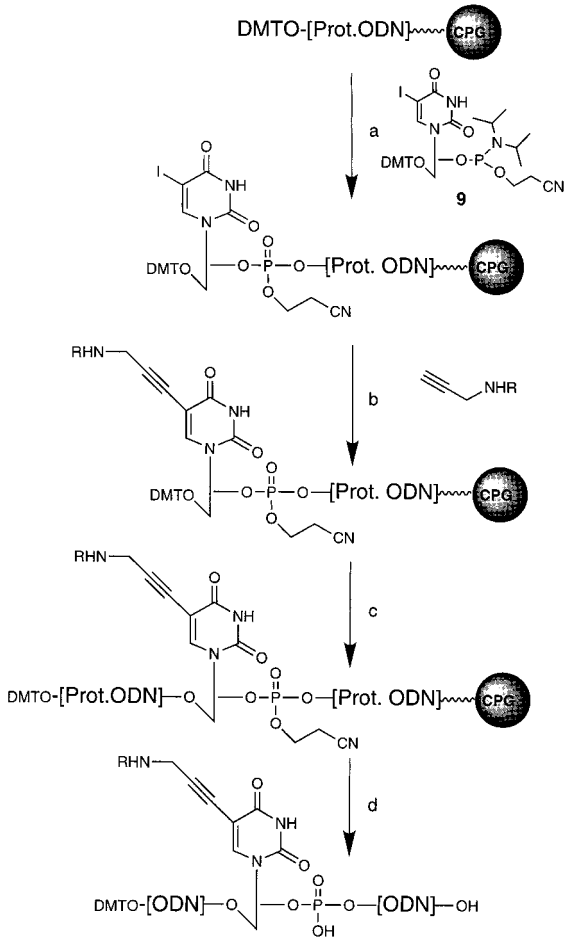
Table 1. Site-Specifically Modified Oligonucleotides Synthesized

no. sequence 5′−3′	functional group (alkynyl group, no.)	DNA coupling (% yield)	Pd(0) coupling (% yield)
10, CTU*AGCA	amine, **2**	98%	85%
11, DMT-TU*GCA	Boc-amine, **3**	98%	89%
12, GTTU*GA	biotin, **8**	98%	92%
13, TAC ATC CTA U*CT	biotin, **8**	98%	89%
14, GGT CTT ATT CAC CAC AAT AAC CTC AGT U*CT	biotin, **8**	98%	92%
15, DMT-TU*CA	$Ru(bpy)_3^{2+}$, **5**	96%	89%

sparged with N_2 to maintain the anhydrous condition. Next, the column was attached to a syringe and the alkyne-derivatized functional group, $Pd(Ph_3P)_4$, CuI, and 150 μL of dry solvent DMF:Et_3N (3.5:1.5) were added. After 3 h, the column was washed with DMF:Et_3N (9:1; 10 mL) and acetonitrile (40 mL), dried with N_2 for 30 min, and then reinstalled on the synthesizer. Solid-phase synthesis was resumed, and additional DNA bases were added.

Collection and analysis of the trityl fractions during automated synthesis showed efficient phosphoramidite coupling throughout the procedure, with both the standard pyrimidine and purine nucleosides as well as with 5-iodouridine (>95%; Table 1). Finally, the modified oligonucleotide was collected and incubated at 55 °C in NH_3 overnight, to completely deprotect the oligonucleotide. Analysis of the HPLC traces of the crude oligonucleotide products showed efficient Pd(0) cross-coupling reactions for each functional group (yields ranged from 85 to 92%; Table 1). Enzymatic digestion of the modified oligonucleotides showed selective coupling to 5-iodouridine with no side reactions observed with the other bases. The free amine, **10**, or Boc-protected amine, **11**, modified oligonucleotides were synthesized from groups **2** and **3**, respectively. Three oligonucleotides derivitized with biotin, **5**, were synthesized (**12**, **13**, and **14**). An oligonucleotide, **15**, modified with a transition-metal complex, $Ru(bpy)_3^{2+}$, was also

Scheme 2[a]



[a] Key: (a) DNA synthesis; (b) off-synthesizer, $Pd(Ph_3P)_4$ CuI, TEA, DMF, 3 h, R = $CF_3C(O)$, $(CH_3)_3COC(O)$, biotin-C(O), and $Ru(bpy)_2(bpy\text{-}(CH_3)\text{-}C(O))^{2+}$; (c) resume DNA synthesis; (d) 30% NH_3, 55 °C,16 h. ODN = oligodeoxynucleotide. Prot. = protected.

synthesized. Importantly, the type, size, three-dimensional shape, or charge of the functional group did not significantly affect the Pd(0) cross-coupling reaction or the ability to synthesize both short and long modified oligonucleotides.

In conclusion, a simple and convenient procedure for the derivatization of oligonucleotides using solid-phase nucleic acid and Pd(0) cross-coupling chemistries is described. The advantages of this *on-column derivatization* method include: (1) fewer overall synthetic steps, (2) efficient Pd (0) cross-coupling reactions, (3) practical solid-phase reaction conditions, (4) ease of oligonucleotide purification, and (5) wide functional group tolerance. This new protocol is an attractive alternative to the synthesis and use of highly functionalized and specialized phosphoramidites for the preparation of modified oligonucleotides at the nucleobase. These favorable reaction conditions will facilitate the development and design of new oligonucleotide derivatives which are of research and clinical interest.

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Supporting Information Available: Detailed experimental information and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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