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Versatile 5′-Functionalization of Oligonucleotides on Solid Support: Amines, Azides, Thiols, and Thioethers via Phosphorus Chemistry

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Received December 2, 2003

Although the preparation of conjugates of oligonucleotides is by now commonplace, existing methods (usually utilizing thiols or primary amines) are generally expensive, and often require postsynthetic reaction with the DNA followed by a separate purification. Here we describe simple procedures for a broad set of direct 5′-end (5′-terminal carbon) functionalizations of DNA oligonucleotides while they remain on the synthesizer column. 5′-Iodinated oligonucleotides (prepared by an automated cycle as previously reported) are converted directly to 5′-azides, 5′-thiocarbamates, and alkyl and aryl 5′-thioethers in high yields. Further, we demonstrate high-yielding conversions of DNA-azides to 5′-amines, and of thiocarbamates to 5′-thiols. Finally, we report a new, one-pot conversion of naturally substituted 5′-OH oligonucleotides (again on the solid support) to 5′-amino-oligonucleotides. All of the above reactions are demonstrated in multiple sequence contexts. Most of the procedures are automatable.

Introduction

A large fraction of DNA biotechnology requires the attachment of reactive groups or reporter groups to synthetic DNA oligonucleotides. This is true, for example, for most DNA sequencing applications[1−3] and microarray applications.[4−7] Simple conjugates, employing small groups such as biotin[8−10] and larger groups such as peptides and proteins,[11−15] continue to be employed widely as well. In addition, chemistries for the joining of DNA strands have found use in multiple laboratories.[16−23] Moreover, there continues to be new development in the applications of chemistry carried out on DNA strands, including a broad array of organic synthetic steps and strategies.[24−34]

Virtually all of these derivatization and conjugation methodologies require the addition of functional groups to synthetic DNAs. While it is now a simple matter to add amine and thiol functional groups to the ends of oligonucleotides via commercially available phosphoramidite reagents, such groups are nearly always attached via flexible linkers, thus placing any conjugated group at some distance from the DNA. In addition, further attachment of functional or reporter groups then requires

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10.1021/jo035765e CCC: $27.50
Published on Web 03/05/2004

reaction and purification steps after DNA is released from the solid support, which significantly complicates the process.

A number of recent reports describe on-column derivatization reactions, which potentially simplifies conjugations significantly. In general, reactions that are carried out on solid support have several advantages over the more common solution-phase reactions: speed, ease of purification, and the possibility of application in combinatorial library synthesis. Sonogashira couplings have been carried out on solid support to incorporate amines, biotin, and transition metal complexes site-specifically into oligonucleotides.[29−31] A similar strategy to couple oligonucleotides to anthraquinone, biotin, and fluorescein via a 5-ethynyl-2′-deoxyuridine residue has also been described.[28] In a different approach, Greenberg's laboratory has used photolabile phosphoramidite units which after incorporation into an oligonucleotide can be selectively cleaved and subsequently coupled to a variety of labels.[32−34] While these methods do offer the advantage of ease of purification (i.e., by filtration), they do not offer much functional group diversity, and rely on relatively flexible linkers to the DNA.

We previously reported that the 5′-terminal hydroxyl group of oligonucleotides can be converted to 5′-iodide via the on-column application of $(PhO)_3PCH_3I$.[35] The iodo group can be useful in ligations of two oligonucleotides for detection of genetic sequences in solution, and in constructing long or nonnatural oligomers, thus showing its utility as an electrophile.[35−37] However, nucleophilic groups attached to DNA also remain highly useful in conjugation of reporters and biomolecules, and many electrophilic reagents are commercially available for reacting with them.[38] As a result, we undertook studies aimed at broadening the application of phosphorus chemistry, with the goal of generating reactive thiol and amine groups directly at the 5′-terminal carbon, without an intervening tether. Our methods make extensive use of phosphorus chemistry developed by Staudinger and Rydon and subsequently applied to nucleoside synthesis by Moffatt and others.[39−44] In the process, we also developed simple procedures for forming thioethers and azides at this position. Finally, we developed a simple and rapid one-pot approach to conversion of the 5′-hydroxyl group in oligonucleotides to a primary amine.

Results

Conversion of 5′-Iodides to Aryl and Alkyl Thioethers. We investigated whether commercially available thiols could react with 5′-iodo-substituted oligonucleotides without losing the protecting groups or releasing the oligonucleotide from the solid support under the basic conditions. To test the reactivity of aryl thiols with CPG-bound 5′-iodo-oligonucleotides, the sequences 5′-I-dNTT (N = G, A, C, T) were reacted with 1.0 M 2-naphthalenethiol in the presence of 1.1 equiv of diisopropylethylamine at room temperature. With use of DMF as solvent, all reactions were complete within 5 min. Yields, as determined by HPLC, were very good, ranging from 97.3% (dTTT) to 88.7% (dATT) (Table 1 and Figure 1) and mass spectral data indicated the desired products were formed (Supporting Information). No significant side-product formation was observed. HPLC chromatograms of the reaction solutions revealed that no measur-

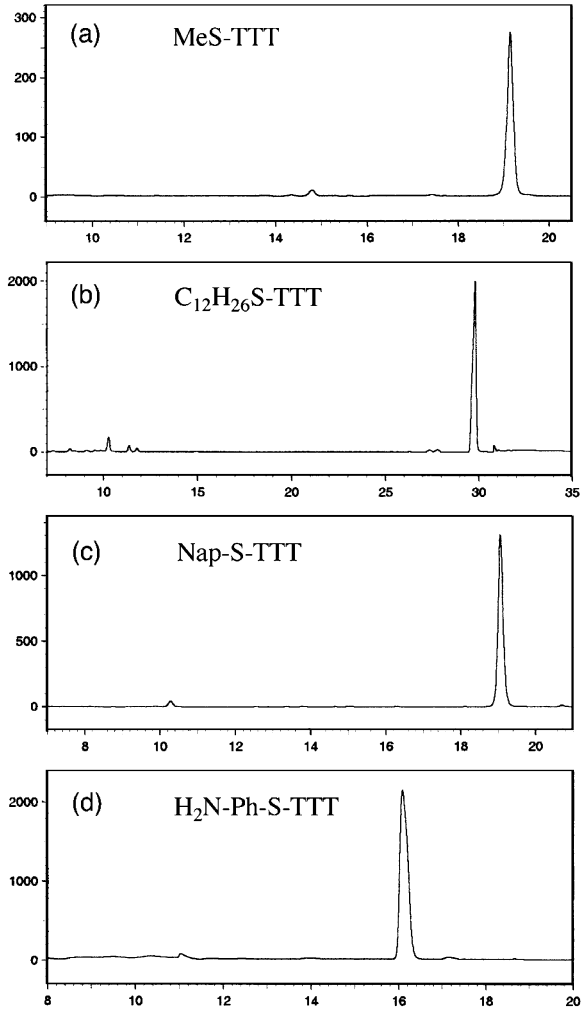

FIGURE 1. HPLC chromatograms of crude products in reactions of 5′-I-dTTT-CPG with (a) sodium thiomethoxide, (b) dodecanethiol with sodium methoxide, (c) 2-naphthalenethiol with diisopropylethylamine, and (d) 2-amino benzene thiol with diisopropylethylamine.

TABLE 1. Effect of Varying 5′-Nucleobase on Conversion of I-dNTT to 2-Naphthyl-S-dNTT

oligomer	time (min)	yield (%)
TTT	2	97.3
GTT	2	92.6
ATT	5	88.7
CTT	5	90.5

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TABLE 2. Effects of Varied Conditions on Conversion of I-dNTT-CPG to RS-dNTT-CPG (R = alkyl)

oligomer	R	time (min)	temp (°C)	base[a]	solvent	result[b]
dGTT	Et	60	rt	DIPEA	DMF	NR
dGTT	Et	60	60	DIPEA	DMF	NR
dCTT	Et	60	rt	NEt_3	DMF	NR
dCTT	Et	60	60	NEt_3	MeOH	NR
dCTT	*n*-$C_{12}H_{26}$	60	rt	DIPEA	EtOH	NR
dCTT	*n*-$C_{12}H_{26}$	60	60	DIPEA	EtOH	NR
dTTT	Et	5	rt	NaOMe	MeOH	58.3%
dTTT	Bu	2	rt	NaOMe	MeOH	7.1%
dTTT	Me	2	rt	N/A[c]	MeOH	8.6%
dTTT	Me	2	rt	N/A[c]	pyridine	NR
dTTT	Me	2	rt	N/A[c]	CH_3CN	NR
dTTT	Me	2	rt	N/A[c]	DMF	97.5%
dTTT	n-Pr	4	rt	NaOMe	DMF	94.3%
dTTT	*n*-$C_{12}H_{26}$	6	rt	NaOMe	DMF	92.1%
dGTT	Me	3	rt	N/A[c]	DMF	89.7%
dATT	Me	2	rt	N/A[c]	DMF	86.8%
dCTT	Me	2	rt	N/A[c]	DMF	86.9%
dCTT	n-Pr	3	rt	NaOMe	DMF	85.4%

[a] DIPEA = diisopropylethylamine. [b] NR = no reaction. [c] Reaction did not require base because commercially available sodium thiomethoxide was used.

able amount of oligonucleotide was cleaved from the solid support during the reactions.

Alkanethiols failed to react with the 5′-iodo oligonucleotides under the conditions used for the aryl thiols. A variety of solvents and bases were subsequently tested (Table 2) and it was found that displacement readily occurred when the reactions were carried out in DMF at room temperature with 0.1 equiv of the stronger base sodium methoxide. Reactions of all four dNTT sequences were complete within 5 min, using propanethiol or dodecanethiol with sodium methoxide or with commercially available sodium thiomethoxide (Figure 1). Yields ranged from 97.5% for dTTT to 86.8% for dATT with sodium thiomethoxide. Mass spectral data revealed the expected masses (Supporting Information). Again, HPLC chromatograms of the reaction solutions showed that no oligonucleotide was cleaved from the CPG support during the reactions, despite the presence of the relatively strong base.

A 20-min procedure was written for Applied Biosystems DNA synthesizers to generate 5′-thioethers from the 5′-hydroxy oligomers. After synthesizing the oligomer with use of a standard trityl-off cycle, the "thioether" cycle was run, converting the 5′-hydroxyl to a 5′-iodo intermediate, and then to the thioether. The cycle consists of two 5-min iodination steps with 0.5 M $(PhO)_3PCH_3I$, followed by a series of rinse steps and then a 2-min incubation with a 1.0 M thioate solution. This cycle was used to synthesize all four 5′-MeS-dNTT and 5′-naphthyl-dNTT oligomers, and yields were comparable to those obtained when run manually (data not shown).

Two-Step Conversion of 5′-Iodides to 5′-Thiols. The current most commonly used procedures for introducing thiol functional groups into oligonucleotides require the synthesis of modified phosphoramidites or the use of a limited number of commercially available but costly modified phosphoramidites.[45−48] These thiol linkers generally contain a phosphoramidite moiety linked to a poly-methylene or poly-ethylene glycol chain, at the end of which is a protected thiol. For many purposes, e.g., tethering the oligomer to a solid support, these thiol linkers work sufficiently well. However, there are often instances in which the few available linkers would not be appropriate, especially when closer attachment to the DNA is desired. We therefore explored the possibility of developing a method to introduce thiol functionality to any oligonucleotide, regardless of the identity of the 5′-base.

Our initial strategy for 5′-thiol synthesis called for displacement of the 5′-iodo group by thioacetate, with subsequent release of the free thiol during cleavage/deprotection. However, despite the use of scavengers during the deprotection/cleavage step, protecting group addition to the thiol prevented us from obtaining consistently high yields. We therefore focused on a method that would separate the base-deprotection step from the thiol-deprotection step.

We suspected that the diethylamino dithiocarbamate $-SCSNEt_2$ would not be hydrolyzed using normal deprotection/cleavage conditions. We thus reacted 1.0 M $NaSCSNEt_2$ with 5′-I-dNTT oligomers on solid support in DMF and found that the reactions were complete within 30 s at room temperature (Figure 2, Table 3). Deprotection and cleavage with NH_4OH at 60 °C yielded the unhydrolyzed 5′-dithiocarbamate as evidenced by HPLC chromatograms (Figure 2), mass spectral data (Supporting Information), and NMR data (Supporting Information). Following evaporation of the ammonia solution, the protected thiols were dissolved in a 0.1 M solution of $AgNO_3$ in 0.1 M TEAA buffer and heated to 95 °C for 12 min. A slight excess of DTT was then added to precipitate the silver ion, the samples were spun in a centrifuge, and the supernatant was injected directly into an HPLC. Using this method, we regularly achieved very good yields when dG or dT was the 5′-base and good to fair yields when dA or dC was in the 5′-position (Figure 2, Table 3). NMR and mass spectral data confirmed the identity of the products (Supporting Information). When the cleavage reactions were run on the oligonucleotides 5′-I-dTCTGTAT (designated T7N), 5′-I-dACAGATA (A7N), 5′-I-dGCGAGTG (G7N), and 5′-I-dCACGCTC (C7N), we found that yields were comparable to those obtained with the trimers, suggesting that the lower relative yields for dA and dC are due to the identity of the 5′-base and not to the A/C content of the oligonucleotides. Automated synthesis of the protected thiol from the 5′-alcohol is also possible on a DNA synthesizer by using the cycle written for thioether synthesis, and yields for the thiocarbamate products are typically greater than 90%.

Conversion of 5′-Iodides to 5′-Azides and Amines. Like 5′-thiol-modified oligonucleotides, the most popular methods for producing 5′-amine-modified oligonucleotides involve the use of phosphoramidites.[45,46,49] Similar in structure to the thiol-modified phosphoramidites, the amine-modified phosphoramidites are expensive and limited in diversity. We therefore focused on developing a general method to add amine functionality directly to

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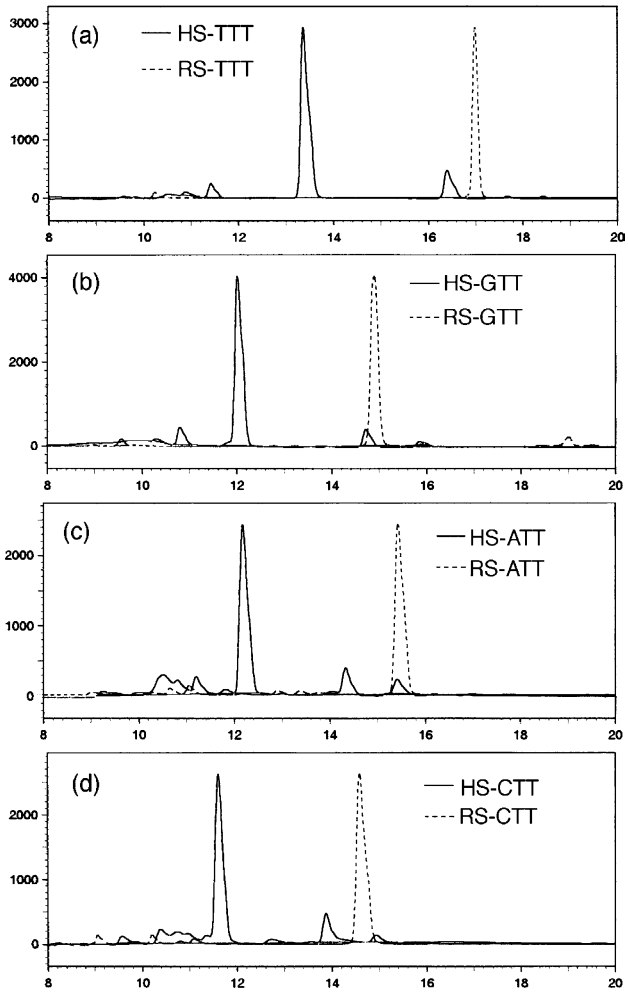

FIGURE 2. HPLC chromatograms of unpurified thiocarbamate (dashed lines) and thiol (solid lines) reaction products. R = $CSNEt_2$.

TABLE 3. Conversion of 5′-Iodides to Dithiocarbamates and Thiols

		yield (%)	
oligo	time (min)	thiocarbamate	thiol
dTTT	2	100.0	84.2
dGTT	3	94.3	80.1
dATT	3	90.3	69.6
dCTT	1.5	91.7	63.4
T7N	2	92.1	85.8
G7N	2	81.7	66.4
A7N	2	85.7	56.8
C7N	2	86.9	71.1

the 5′-end of oligomers via the 5′-iodide intermediate. Oligonucleotides with a direct 5′-amino terminus have found multiple applications in the literature, but were previously prepared by much more laborious synthesis of 5′-aminonucleoside phosphoramidite reagents.[13−15,50−55]

We found that the conversion of 5′-I-dNTT to the respective azides proceeds to completion within 1 h at 60 °C. Mass spectra and NMR confirmed the identity of the reaction products (Supporting Information). Several reagents were tested for their ability to reduce the azide to the amine without cleaving the oligonucleotide from the solid support. $NaBH_4$ (1.0 M) in EtOH or DMF

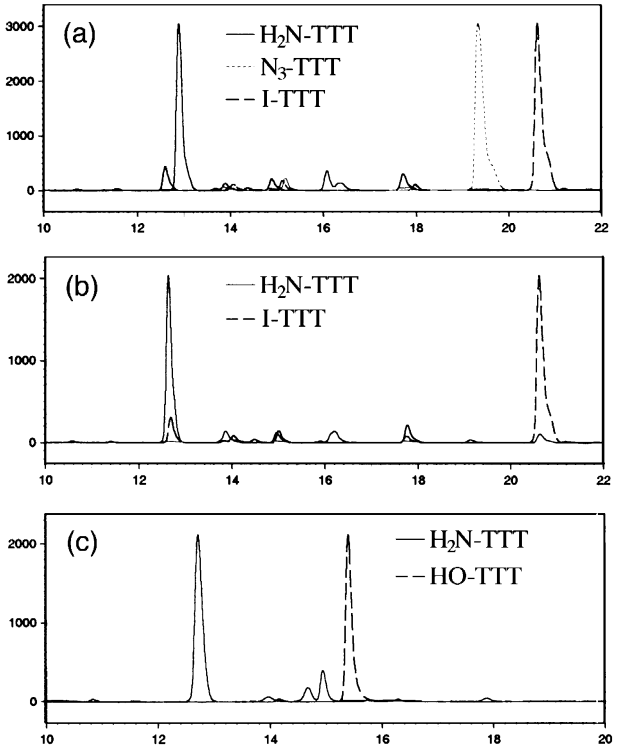

FIGURE 3. HPLC chromatograms of intermediates and products of 5′-amination reactions with 5′-dTTT: (a) 3-pot reaction; (b) 2-pot reaction; and (c) 1-pot reaction.

cleaved oligomers from the solid support before significant reduction took place, while the phosphine alkylimide produced with $POEt_3$ was not hydrolyzed to the amine under normal deprotection/cleavage conditions. PPh_3 (1.0 M) in DMF, however, resulted in conversion to the iminophosphorane in 1 h at 60 °C and was hydrolyzed to the primary amine during deprotection/cleavage with NH_4OH. Mass spectral and NMR data of the reaction products indicated the desired 5′-amines were obtained (Supporting Information). 5′-dTTT consistently gave the best yields (70−80%) while the other dNTT sequences generally resulted in yields near 45% (Figures 3 and 4, Table 4). When pentamers of each of the four bases were used, similar yields were obtained (Table 4), suggesting that the lower yields are due to the identity of the 5′-base and not the base composition.

Because the overall transformation of the 5′-hydroxyl to amine required three individual steps, and therefore may not be practical for some purposes, we checked to see if the last two steps could be combined, thus proceeding directly from the iodide to the iminophosphorane. We found that using 0.25 M NaN_3 and 1.0 M PPh_3 in DMF at 60 °C for 1 h resulted in yields slightly better than those obtained in three steps (Table 4, Figure 3, Figure 4). Encouraged by these results, we then decided to test the feasibility of converting the 5′-hydroxyl directly to the 5′-iminophosphorane. Various ratios of PPh_3, I_2, imidazole, and NaN_3 were tested and it was found that when their respective concentrations were 1.5, 0.38, 3.0, and 0.77 M, yields comparable to those obtained in the two-pot reactions were achieved (Table 4, Figure 3, Figure 4).

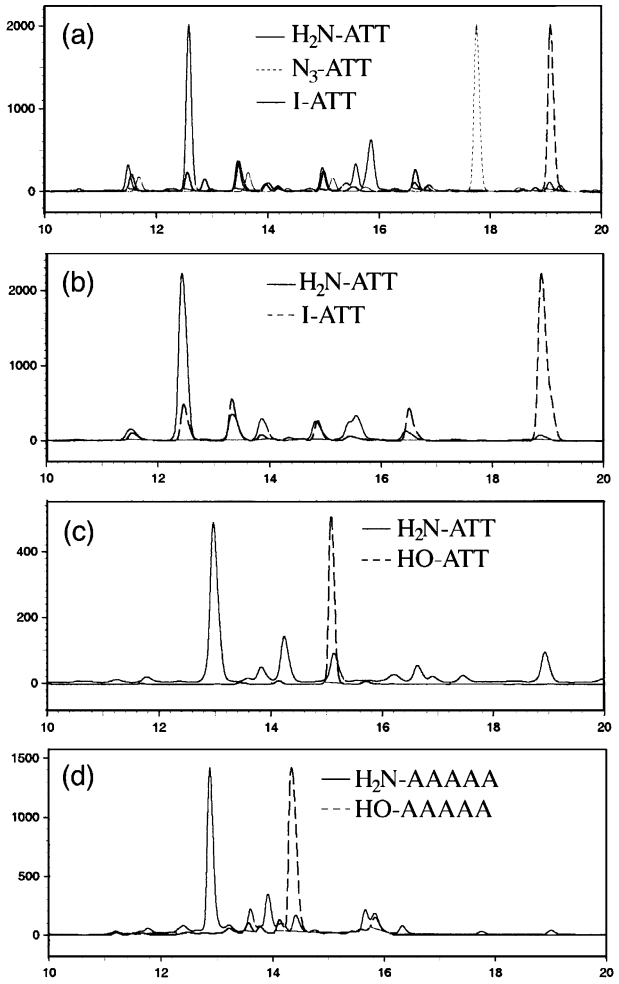

FIGURE 4. HPLC chromatograms of intermediates and products of 5′-amination reactions with 5′-dATT and 5′-dAAAAA: (a) 3-pot conversion from 5′-HO-dATT; (b) 2-pot reaction of 5′-dATT; (c) 1-pot reaction of 5′-dATT; and (d) 1-pot reaction from 5′-HO-dAAAAA.

TABLE 4. Conversion of 5′-Hydroxy to 5′-Amine

		yield (%)	
oligo	steps	azide	amine
dTTT	3	94.1	78.0
dGTT	3	84.8	46.9
dATT	3	77.0	43.5
dCTT	3	76.5	47.3
dTTT	2	N/A	75.0
dGTT	2	N/A	56.8
dATT	2	N/A	53.0
dCTT	2	N/A	54.4
dTTT	1	N/A	78.1
dGTT	1	N/A	56.2
dATT	1	N/A	54.3
dCTT	1	N/A	65.2
dT_5	1	N/A	61.5
dG_5	1	N/A	49.8
dA_5	1	N/A	51.1
dC_5	1	N/A	58.1

Recent reports on the utility of azides in bioconjugate chemistry[56−62] prompted us to investigate the synthesis of 5′-azides further. The small differences in yields achieved for the 5′-N_3-dNTT trinucleotides prompted us to test the azide-forming reaction on longer oligomers, particularly those containing higher proportions of G, A, and C. When run on the heptamers T7N, A7N, G7N, and C7N, we found that yields dropped only slightly (Table 5). Reactions were performed four times for each oligomer and average yields were reasonably good for T7N, G7N, and C7N (83% to 69%) but were somewhat lower for A7N (56%).

TABLE 5. Conversion of 5′-I-N7N to 5′-N_3-N7N

oligo	av yield[a] (%) of azide
T7N	83.3
G7N	70.0
A7N	55.6
C7N	69.1

Discussion

Whereas the vast majority of modifications have been introduced to oligodeoxynucleotides (ODNs) via modified phosphoramidites, it has become clear that solid-supported protected ODNs are suitable substrates for a variety of organic transformations. The advantages to this approach are numerous: First, the reactions are general, and can be performed with any of the four bases at the 5′-terminus without syntheses of individual modified nucleosides. Second, the chemistry is inexpensive. Since the reactions are run on a micromole scale, even a 1000-fold excess of reagent only amounts to 1 mmol. Also, all of the reagents used for these experiments are inexpensive and commercially available. Compared to the typical multistep synthesis of modified phosphoramidites, this represents a great improvement in terms of cost and time. Third, the reactions are rapid. Of the cases described here, reaction times varied from 1 h to less than 1 min. Fourth, the methods are extremely simple. Most of the reactions described here only require that a reagent be pushed (via syringe or synthesizer) onto a DNA synthesis column, and the reaction workup only entails rinsing the reagent off. In addition, most of the reactions can be automated. A DNA synthesizer can convert an unmodified oligonucleotide to a thioether or protected thiol in less than 20 min. Thus, synthesizer technicians with little training in organic chemistry could use the

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procedures presented here as readily as methods requiring modified phosphoramidites. Fifth, the reaction products are easily purified by using reverse-phase HPLC. Short oligomers (<13mers) modified at the 5′-position as amine, thiol, various thioethers, azide, and iodide are easily separated from the 5′-hydroxy starting materials via reverse-phase HPLC. Finally, a wide variety of functional groups can be incorporated. One can imagine, for example, reacting substituted aryl thiols with 5′-iodides to install any functional group that is not reactive with DIPEA or a thioate. Indeed, preliminary results with such a method to install arylamines and thiols have shown that yields in excess of 90% can be achieved (data not shown).

A modified deoxythymidine phosphoramidite in which an amino group is attached directly to the 5′-carbon has been used to synthesize oligomers for a variety of purposes.[13−15,50−55] Smith et al. conjugated 5′-amino oligonucleotides in solution to fluorescent markers to use in DNA sequence analysis.[50] Richert's laboratory has added a variety of groups including palmitoyl, quinolones, and steroids to the 5′-amine while still on solid support.[51−53] Additionally, a number of methods have been devised to conjugate oligonucleotides to amino acids or polypeptides.[13−15,] Finally, Lynn's group has used 5′-amino-modified oligonucleotides in template-directed ligation reactions.[18,55] The current approach to 5′-amines offers a substantial savings in time and effort for these applications.

Similarly, phosphoramidites bearing a thiol group on the 5′-carbon have been synthesized and used for a number of purposes.[63−65] Sproat et al. first synthesized all four 5′-mercapto-2′,5′-dideoxyribonucleoside phosphoramidites which were used to conjugate fluorescent labels to primers for nonradioactive DNA sequencing.[63] They also successfully labeled 5′-thio-modified oligonucleotides with heavy metals to use in X-ray crystallography studies.[63] Additionally, 5′-thio-modified oligonucleotides have been used in template-mediated ligation reactions to couple oligonucleotides to both peptides and other oligonucleotides.[64,65] As with the 5′-amine methodology, our approach to 5′-thiols is considerably simpler and should be quite useful for the above applications.

Recent reports on the utility of azides in conjugating peptides suggests they may be useful as well in ligating appropriately modified DNAs.[56−62] Thus, a system in which a 5′-azide-modified oligonucleotide is ligated to a 3′-thioester-modified strand upon binding to a complementary template may be envisioned. Alternatively, 5′-azide-modified oligonucleotides may be conjugated to a variety of alkynes via a 3+2 Huisgen cyclization. In fact, Lee et al. recently coupled 85 different azides to an alkyne-modified guanosine diphosphate in an effort to identify inhibitors of human α-1,3-fucosyltransferase.[66]

A review of the literature revealed only one instance of the use of thioether linkages to conjugate groups to oligonucleotides.[67] This is probably because electrophilic functional groups on DNA are rare. However, our approach to thioethers is quite efficient and rapid, and the stable C−S linkage is very desirable for many applications. Of particular benefit, the thioether linkage is inert to many reaction conditions, but is easily and selectively cleaved by some metal salts such as $AgNO_3$. Also, this method of conjugation is attractive because a wide variety of thiols are commercially available, and many others can be synthesized in just a few steps. Because the thioether in the current strategy is introduced in the final step, it avoids oxidation by oxidizing agents during the oligonucleotide synthesis cycle.

In conclusion, we have described a new and diverse set of on-column derivatization reactions that significantly increases the repertoire of possible DNA conjugation chemistries. Future studies will be aimed at developing new applications using these functional groups.

Experimental Section

5′-Alcohol to Iodide. 5′-Iodo oligodeoxyribonucleotides were prepared as previously described via both manual and automated procedures.[32]

5′-Iodide to Aryl Thioether. A DNA synthesis column containing 5′-iodo oligodeoxyribonucleotide on CPG resin was fitted with a 1-mL syringe containing anhydrous DMF. The DMF was pushed through the column to remove any water adsorbed to the resin. A 1-mL syringe containing 1.0 mL of 1.0 M aryl thiol with 1.1 equiv of diisopropylethylamine in DMF was immediately attached to the other end of the column and the solution was passed between the syringes for 2−5 min. The solution was then removed from the column and the resin was washed with 10 mL of DMF and 5 mL of CH_2Cl_2 and then blown dry with air. Deprotection and cleavage from the solid support were effected with concentrated NH_4OH at 60 °C for 17 h.

5′-Iodide to Methyl Thioether. Synthesis of 5′-methyl thioether was achieved via a procedure similar to that used to synthesize the 5′-aryl thioether except that a 100 mM solution of sodium thiomethoxide in DMF was used.

5′-Iodide to Alkyl Thioether. Synthesis of 5′-propyl thioether was achieved via a procedure similar to that used to synthesize the 5′-aryl thioether except that a 1.0 M solution of 1-propanethiol with 0.1 equiv of sodium methoxide in DMF was used and the reaction was carried out at room temperature for 3−4 min. Synthesis of 5′-dodecyl thioether was achieved via a procedure similar to that used to synthesize the 5′-aryl thioether except that a 0.5 M solution of 1-dodecanethiol with 0.1 equiv of sodium methoxide in DMF was used and the reaction was carried out at room temperature for 6 min.

5′-Iodide to Dithiocarbamate. The 5′-dithiocarbamate was afforded via a procedure similar to that used to synthesize the 5′-thioether except that a 1.0 M solution of sodium diethyldithiocarbamate trihydrate in DMF was used and the reaction was carried out at room temperature for 0.5−5 min. Deprotection and cleavage from the solid support was effected with NH_4OH at 60 °C for 17 h.

5′-Dithiocarbamate to Thiol. Following deprotection and cleavage of the 5′-dithiocarbamate-modified oligodeoxyribonucleotide, the aqueous ammonium hydroxide was evaporated under reduced pressure. The oligonucleotide was dissolved in 0.9 mL of 100 mM TEAA and 100 μL of 1.0 M $AgNO_3$ was added. The solution was heated to 95 °C for 12 min, and then 105 μL of 1.0 M DTT was added to precipitate the silver. H_2O (400 μL) was then added and the mixture was vortexed briefly

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and then spun at 14 000 rpm for 5 min. The supernatant was removed and injected directly onto the HPLC or was extracted twice with ethyl acetate to remove excess DTT prior to injection.

5′-Iodide to Azide. A DNA synthesis column containing 5′-iodo oligodeoxyribonucleotide on CPG resin was fitted with a 1-mL syringe containing anhydrous DMF. The DMF was pushed through the column to remove any water adsorbed to the resin. A 1-mL syringe containing 1.0 mL of saturated NaN_3 in DMF was immediately attached to the other end of the column and the solution was passed between the syringes several times. The junctions between the column and syringes were sealed with Parafilm and the reaction was placed in a shaker at 60 °C. After 1 h, the reaction was removed and the solution was pushed from the column. The resin was washed with 10 mL of DMF and 5 mL of CH_2Cl_2 and then blown dry with air.

5′-Azide to Amine. A DNA synthesis column containing 5′-azido oligodeoxyribonucleotide on CPG resin was fitted with a 1-mL syringe containing anhydrous DMF. The DMF was pushed through the column and a 1-mL syringe containing 1.0 mL of 1.0 M PPh_3 in DMF was immediately attached to the other end of the column. The PPh_3 solution was then passed between the syringes several times and the junctions between the column and syringes were sealed with Parafilm. The reaction was agitated for 1 h at 60 °C. The reaction was then removed from the incubator and the solution was pushed from the column. The resin was washed with 10 mL of DMF and 5 mL of CH_2Cl_2 and then blown dry with air. Cleavage and deprotection with concentrated NH_4OH at 60 °C for 17 h afforded the 5′-amine.

5′-Alcohol to Amine, 2 Pots. (a) Alcohol to Iodide. 5′-Iodo oligodeoxyribonucleotides were prepared as previously described via both manual and automated procedures.[32] **(b) Iodide to Amine.** A procedure identical with that used to synthesize the 5′-azide was used to obtain the 5′-amine from the iodide, except that the NaN_3 solution was also 1.5 M in PPh_3. Cleavage and deprotection with concentrated NH_4OH at 60 °C for 17 h yielded the 5′-amine.

5′-Alcohol to Amine, 1 Pot. Protected oligodeoxyribonucleotide attached to CPG resin was placed into a 1.5-mL vial with 0.77 mmol NaN_3. PPh_3 (1.0 mL, 1.5 M), 3.0 M imidazole, and 0.375 M I_2 in DMF were added to the vial, which was then immediately sealed with a screw-cap containing a rubber O-ring. The vial was briefly vortexed and then put on a shaker at 60 °C. After 1 h, the vial was removed from the shaker and the beads were transferred to a DNA column with a pipet. The resin was rinsed with 10 mL of DMF and 5 mL of CH_2Cl_2 and then blown dry with air. Deprotection and cleavage from the solid support were effected with concentrated NH_4OH at 60 °C for 17 h.

Automated Synthesis of 5′-Thioethers and Dithiocarbamates. A procedure was written for ABI 392 and 394 DNA synthesizers to convert the 5′-alcohol to a thioether or dithiocarbamate (Supporting Information). Following oligonucleotide synthesis with standard phosphoramidite chemistry, the terminal DMT group is removed and the free hydroxyl is allowed to react with a 0.5 M solution of $(PhO)_3PCH_3I$ in DMF. After two 5-min iodination steps, a 1.0 M thioate or dithiocarbamate solution in DMF is sent to the column and allowed to react for 0.5−5 min. Excess reagent is removed by a series of rinse steps with DMF and CH_2Cl_2. The CPG beads are then removed from the column and deprotected with concentrated NH_4OH at 60 °C for 17 h.

Acknowledgment. We thank the U.S. Army Research Office (DAAD19-03-1-0066) and the U.S. National Institutes of Health (GM068122 and GM067201) for support.

Supporting Information Available: NMR spectra and HPLC chromatograms of representative compounds, HPLC conditions and retention times, MS data, and programmed procedure for automated conversion to thioethers and dithiocarbamates. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035765E