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Synthesis of Modified Oligodeoxyribonucleotides on a Solid-Phase Support via Derivatization of a Selectively Revealed 2′-Amino-2′-deoxyuridine

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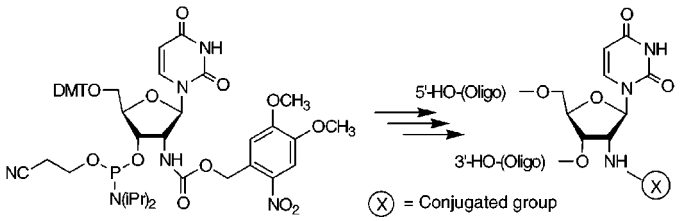
ABSTRACT



High yields of oligodeoxyribonucleotides modified at the C2′-position of site specifically incorporated 2′-amino-2′-deoxyuridine are obtained by photolytically unmasking the nucleophile in an otherwise protected solid-phase support-bound biopolymer.

As the applications for modified oligonucleotides increase, so do the methods for their chemical synthesis.[1] Postsynthetic modification of oligonucleotides is one general approach to this family of biomolecules. This strategy is attractive from the standpoint that a single nucleotide triphosphate,[2] phosphoramidite,[3] or solid-phase support[4] that is compatible with polymerase enzymes or chemical synthesis can be used to synthesize a variety of modified oligonucleotides. One potential downside of postsynthetic modification is that the conditions for carrying out the conjugation reaction, yield, and/or product purity can be less than optimal. Recently, we reported methods for conjugating protected oligodeoxyribonucleotides in solution or on solid-phase supports ("on-column") that produce high yields of products in short reaction times, using modest excesses of reagents.[5,6] This

(1) For recent reviews see: (a) Beaucage, S. L. In *Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: Oxford, U.K., 1996; pp 36−74. (b) O'Donnell, M. J.; McLaughlin, L. W. In *Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: Oxford, U.K., 1996; pp 216−243. (c) Miller, P. S. In *Bioorganic Chemistry: Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: Oxford, U.K., 1996; pp 347−374.

(2) Sakthivel, K.; Barbas, C. F. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2872.

(3) For some recent examples see: (a) Kohgo, S.; Shinozuka, K.; Ozaki, H.; Sawai, H. *Tetrahedron Lett.* **1998**, *39*, 4067. (b) Haginoya, N.; Ono, A.; Nomura, Y.; Ueno, Y.; Matsuda, A. *Bioconjugate Chem.* **1997**, *8*, 271. (c) Markiewicz, W. T.; Gröger, G.; Rösch, R.; Zebrowska, A.; Markiewicz, M.; Klotz, M.; Hinz, M.; Godzina, P.; Seliger, H. *Nucleic Acids Res.* **1997**, *25*, 3672.

(4) Hovinen, J.; Guzaev, A.; Azhayeva, E.; Azhayev, A.; Lönnberg, H. *J. Org. Chem.* **1995**, *60*, 2205.

(5) (a) McMinn, D. L.; Matray, T. J.; Greenberg, M. M. *J. Org. Chem.* **1997**, *62*, 7074. (b) McMinn, D. L.; Greenberg, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3289.

(6) (a) Kahl, J. D.; McMinn, D. L.; Greenberg, M. M. *J. Org. Chem.* **1998**, *63*, 4870. (b) Kahl, J. D.; Greenberg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 597.

(7) (a) Adamic-Matulic, J.; Beigelman, L.; Dudycz, L. W.; Gonzalez, C.; Usman, N. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2721. (b) Hendrix, C.; Devreese, B.; Rozenski, J.; Van Aerschot, A.; DeBruyn, A.; Van Beeumen, J.; Herdewijn, P. *Nucleic Acids Res.* **1995**, *23*, 51. (c) Manoharan, M.; Prakash, T. P.; Barber-Peoc'h, I.; Bhat, B.; Vasquez, G.; Ross, B. S.; Cook, P. D. *J. Org. Chem.* **1999**, *64*, 6468.

(8) Sigurdsson, S. T.; Eckstein, F. *Nucleic Acids Res.* **1996**, *24*, 3129.

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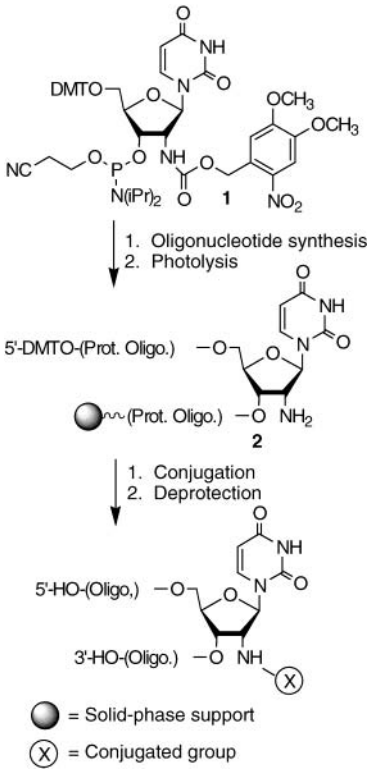
approach to modified oligodeoxyribonucleotides has now been extended to introducing functionality at the 2′-position of a nucleotide by substituting for the pro-*R* hydrogen in 2′-deoxyuridine (Scheme 1). Previously, oligonucleotides

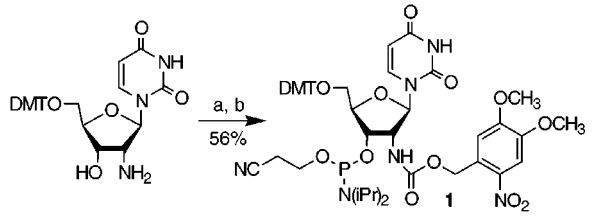
Scheme 1



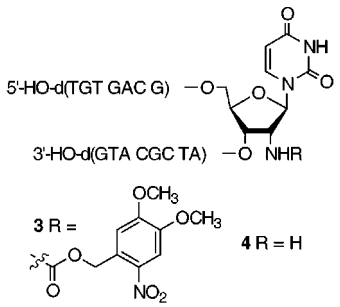
functionalized at the 2′-position have utilized individually synthesized phosphoramidites or have been prepared postsynthetically using fully deprotected oligonucleotides containing an appropriate functional group.[7,8]

The requisite phosphoramidite (**1**) designed for this problem utilizes the *o*-nitrobenzyl photoredox process to selectively unmask the 2′-amino group and was synthesized in a straightforward manner from 5′-O-dimethoxytritylated 2′-amino-2′-deoxyuridine (Scheme 2).[9] A protected hexadecanucleotide containing the nucleotide derived from **1** was prepared using β-cyanoethyl phosphoramidites containing *N*-isobutyryl protecting groups for deoxyadenosine, deoxycytidine, and deoxyguanosine, to eliminate transamidation of the support-bound protected oligodeoxyribonucleotide.[10] The steric bulk of the carbamate at the 2′-position of **1** hindered its coupling during automated solid-phase synthesis. Using standard coupling times **1** coupled in only 35% yield. However, the coupling yield was improved to 87% overall by reacting **1** twice with the support-bound primary hydroxyl and extending the reaction time to 10 min. Deprotection using concentrated aqueous ammonium hydroxide, followed by detritylation and purification by denaturing polyacrylamide gel electrophoresis of this hexadecanucleotide prior to photolysis, produced the carbamate-containing oligodeoxyribonucleotide **3** without any loss of the *o*-nitrobenzyl moiety, as determined by electrospray ionization mass spectrometry (ESI-MS).[11]

Scheme 2[a]



[a] Legend: (a) 4,5-dimethoxy-2-nitrobenzyl chloroformate (1.3 equiv), diisopropylethylamine (2 equiv), THF; (b) 2-cyanoethyl diisopropylchlorophosphoramidite (1.5 equiv), diisopropylethylamine (4 equiv), CH_2Cl_2.



Photolytic unmasking of the 2′-amino group was carried out under conditions (λ_{max} 365 nm, 2 h, 25 °C) previously shown to be optimal for the deprotection of a primary amine in support-bound protected oligodeoxyribonucleotides.[6b,12] Furthermore, these photolysis conditions have been shown not to impart any measurable damage on oligonucleotides.[13] Following concentrated aqueous ammonia deprotection (6 h, 55 °C) and detritylation, the crude oligonucleotide was purified by denaturing polyacrylamide gel electrophoresis (PAGE). Although the 3′-amino-containing oligonucleotide (**4**) migrates more quickly than **3** in a denaturing gel, **4** was the only compound observed by denaturing polyacrylamide gel electrophoresis as well as by ESI-MS.[11] On the basis of these observations, we concluded that the photoconversion of the *o*-nitrobenzyl protecting group was complete.

(9) McGee, D. P. C.; Vaughn-Settle, A.; Vargeese, C.; Zhai, Y. *J. Org. Chem.* **1996**, *61*, 781.

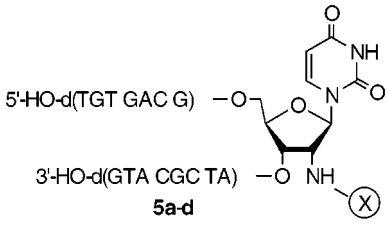
(10) McMinn, D. L.; Greenberg, M. M. *Tetrahedron Lett.* **1997**, *38*, 3123.

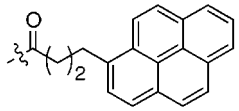
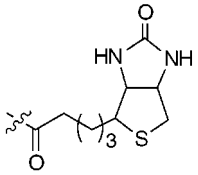
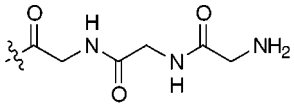
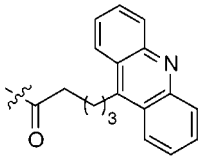
(11) See the Supporting Information.

(12) **Photolytic Deprotection of Solid-Phase Supported Oligonucleotides Containing Modified Nucleotides.** Oligodeoxyribonucleotide bound to support (18 mg) was added to a Pyrex tube containing a stir bar constructed from a standard (white) pipe cleaner (in order to not crush the CPG containing the oligonucleotide) and CH_3CN/H_2O (16 mL, 9:1 by volume). The tube was fitted with a rubber septum and sparged with Ar for 30 min, after which the needle was raised well above the surface of the solvent. Photolyses were carried out with a VWR Chromato-Vue transilluminator (λ_{max} 365 nm) for 2 h. It is important to maintain the temperature at ≤25 °C using a fan. The resin was filtered, washed with EtOAc (10 mL) followed by CH_2Cl_2 (10 mL), collected, and placed in a screw-capped vial for storage.

(13) Venkatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525.

Table 1. Isolated Yields of Oligodeoxyribonucleotide Conjugates Prepared via Coupling with **2**



	X	Isolated Yield (%)[a,b]
a		96 ± 2 (3)
b		98 ± 11 (3)
c		100 ± 9 (4)
d		86 ± 3 (3)

[a] Isolated yields were determined by comparing the amount of oligodeoxyribonucleotide obtained from a comparable amount of unconjugated material subjected to the identical deprotection, purification, and isolation procedures. [b] Yields represent an average of separate reactions plus or minus the standard deviation from this value. The numbers of reactions run are noted in parentheses.

Conditions for the conjugation of carboxylic acids to **2** were optimized using 4-pyrenebutyric acid as substrate. Initial experiments were carried out using conditions previously established in our group for on-column conjugation studies as a guide.[6] Following deprotection, the slower moving conjugates were separated from any unreacted **4** by denaturing PAGE, as discussed above. However, under these initial conjugation conditions (1 h, 5 equiv of PyBOP and carboxylic acid substrate relative to **2**) the steric hindrance surrounding the 2′-amine of **2** resulted in only an 82 ± 2% yield of **5a**. Longer reaction times (3 h) and a greater excess of reagents (10 equiv) were required in order to consistently obtain yields of **5a** well in excess of 95% (Table 1).[14] The homogeneity of **5a** was analyzed by ESI-MS and reverse-phase HPLC analysis of the nucleosides released upon enzymatic digestion.[11,15] As in all previous studies involving the conjugation of protected oligonucleotides, these methods did not detect any impurities in the modified oligodeoxyribonucleotide.[5,6] The generality of these conditions (10 equiv, 3 h, 25 °C) was demonstrated using other carboxylic acids whose structures are representative of families of molecules typically used to modify the physical and biological properties of oligonucleotides (Table 1).

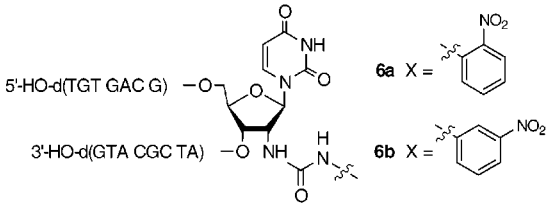


Figure 1. Oligonucleotide conjugates (**6a**,**b**) formed via coupling of **2** with isocyanates.

The utility of the solid-phase supported oligodeoxyribonucleotide containing a single 2′-amine was further demonstrated in its conjugation to isocyanates. 2′-Amino-substituted nucleotides in deprotected oligonucleotides have been conjugated to isocyanates postsynthetically in previous studies.[8] In these experiments high yields were reported following careful optimization of the reaction conditions so as to avoid reaction between the oligonucleotides' exocyclic amines and the electrophilic reagents. Because the nucleobases remained protected, such side reactions were not a concern with **2**, and conjugation (10 equiv, 3 h) with 2-nitrophenyl isocyanate and 3-nitrophenyl isocyanate proceeded essentially quantitatively (**6a**,**b**) at room temperature (Figure 1).

In summary, a convenient, convergent method for introducing modifications in oligodeoxyribonucleotides at the 2′-position of a defined nucleotide has been developed. High yields of homogeneous oligodeoxyribonucleotide conjugates (**5**, **6**) have been obtained by selectively unmasking the 2′-amino substituent in an otherwise protected biopolymer that is covalently bound to the solid-phase support on which it was prepared via automated synthesis. This oligonucleotide conjugate synthon facilitates the synthesis of modified oligonucleotides and should also be amenable to the chemical synthesis of libraries of such molecules.

(14) **General Procedure for Conjugation of Protected Resin-Bound Oligodeoxyribonucleotides**. A solution (20 mM) of the coupling reagents (8 mg PyBOP and 5.2 μL of diisopropylethylamine (2 molar equiv)) in DMF (500 μL) was prepared in an oven-dried 1 dram vial equipped with a septum. A solution (20 mM) of carboxylic acid (0.01 mmol in 500 μL of DMF) was prepared in a second oven-dried vial. The resin-bound DNA (2 mg, containing ~60 nmol of DNA, based on trityl response) was treated with 60 μL (10 molar equiv) of a 1:1 mixture (by volume) of the PyBOP and carboxylic acid solutions. The reaction mixture was capped and shaken at room temperature for 3 h. The resin was washed with MeOH (3 × 1 mL) and dried in vacuo. Detritylation was effected by transferring the resin to a standard oligonucleotide synthesis column and passing the standard trichloroacetic acid solution (3 × 10 s) through the column, followed by CH_3CN and drying under vacuum. The free-flowing resin was treated with 28% aqueous ammonia (600 μL) for 6 h at 55 °C and concentrated under vacuum. Purification of the conjugated oligonucleotide was carried out via 20% polyacrylamide denaturing gel electrophoresis. Isolated yields were obtained (o.d. 260 nm; 350 nm for **5a**) by comparing the amount of conjugated oligonucleotide to the amount of unconjugated material subjected to identical deprotection, purification, and isolation conditions.

(15) Hwang, J. T.; Greenberg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 4311.

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Supporting Information Available: ESI-MS data for **3**, **4**, **5a**−**d**, and **6a**,**b** and an HPLC chromatogram of the enzyme digest of **5a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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