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Articles

Synthesis of 2′-Modified Oligodeoxynucleotides via On-Column Conjugation

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Oligodeoxynucleotides modified at the 2′-position of 2′-amino-2′-deoxyuridine or uridine were prepared in high yield and purity using phosphoramidites **2** and **3**, respectively. Oligodeoxynucleotide conjugates were prepared on the solid-phase synthesis support following selective unmasking of the nucleophile incorporated in these phosphoramidites. Synthesis of oligodeoxynucleotides modified at the 2′-position of an internal nucleotide provides molecules that are complementary to those previously prepared via a similar approach using C5-substituted pyrimidines. The efficiency of functionalization of the 2′-*O*-alkylamino-uridine derived from **3** in a protected oligodeoxynucleotide was less susceptible to steric hindrance than the 2′-amino-2′-deoxyuridine in the same polymeric substrate. However, the greater reactivity of the 2′-*O*-alkylamine containing nucleotide gave rise to undesired acetamide formation resulting from nucleophilic attack on the 5′-terminal acetate in capped failure sequences. This problem was overcome by using 2,2,2-trimethylacetyl anhydride as a capping agent during the automated synthesis cycles. Finally, the efficiency of the photochemical unmasking of the support bound alkylamine on a 1 μmole scale was improved by using two 20 min photolysis cycles, coupled with removing reaction byproducts between cycles.

The incorporation of suitably functionalized nucleotides at defined sites of oligonucleotides facilitates the parallel synthesis of modified nucleic acids containing diverse structural features. Modified oligonucleotides are finding increasing uses that include structural/diagnostic probes, therapeutic candidates, and artificial enzymes.[1−3] Structural diversity can be incorporated via individually prepared phosphoramidites, using the fully deprotected polymer as a substrate in solution, or in chemically synthesized oligonucleotides that retain their exocyclic amine, phosphate diester, and 5′-terminal hydroxyl protecting groups.[4−7] Postsynthetic modification of protected

(1) O'Donnell, M. J.; McLaughlin, L. W. In *Bioorganic Chemistry Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: Oxford, 1996.

(2) (a) Reed, M. W.; Wald, A.; Meyer, R. B. *J. Am. Chem. Soc.* **1998**, *120*, 9729. (b) Robles, J.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1997**, *119*, 6014.

(3) (a) Santoro, S. W.; Joyce, G. F.; Sakthivel, K.; Gramatikova, S.; Barbas, C. F., III *J. Am. Chem. Soc.* **2000**, *122*, 2433. (b) Wiegand, T. W.; Janssen, R. C.; Eaton, B. E. *Chem. Biol.* **1997**, *4*, 675. (c) Tarasow, T. W.; Tarasow, S. L.; Eaton, B. E. *Nature* **1997**, *389*, 54.

(4) Beaucage, S. L. In *Comprehensive Natural Products Chemistry, Volume 7 DNA and Aspects of Molecular Biology*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999.

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(6) (a) Ghosh, S. S.; Musso, G. F. *Nucleic Acids Res.* **1987**, *15*, 5353. (b) Bischoff, R.; Coull, J. M.; Regnier, F. E. *Anal. Biochem.* **1987**, *164*, 336. (c) Erout, M.-N.; Troesch, A.; Pichot, C.; Cros, P. *Bioconjugate Chem.* **1996**, *7*, 568.

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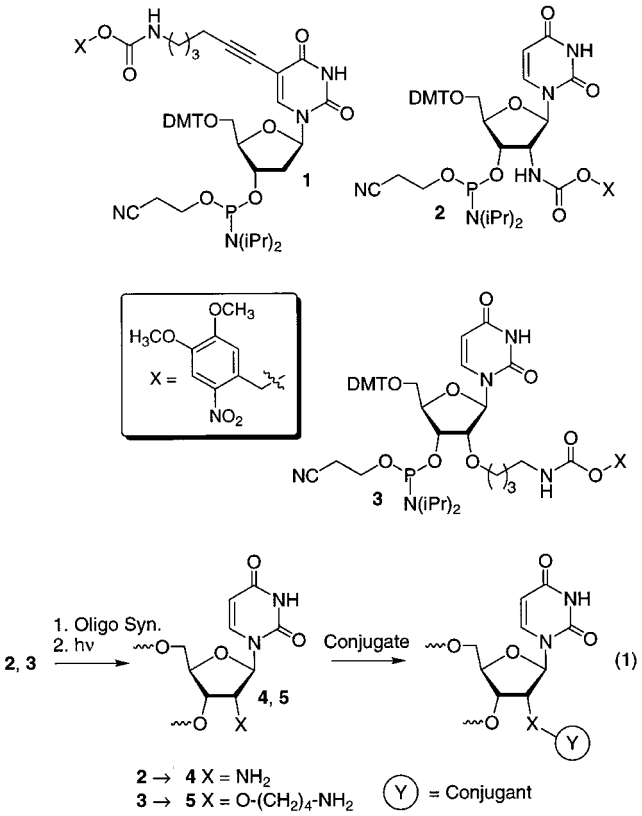
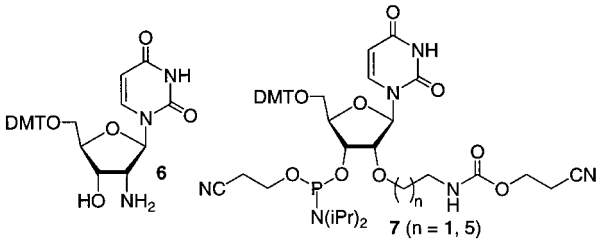
oligonucleotides has been carried out in solution or while the biopolymer substrate is still covalently linked to the support on which it was synthesized.[7−9] We have reported on a general strategy that utilizes photolabile protecting groups to selectively release electrophiles or nucleophiles at the 3′-termini of oligodeoxynucleotides, as well as at internal positions.[10−12] In the latter, masked reactive sites are introduced using modified phosphoramidites (e.g., **1**, **2**), and the photochemical deprotection is orthogonal with respect to the other families of protecting groups present throughout the oligonucleotide. *o*-Nitrobenzyl-protected monomers **1** and **2** are complementary to one another in the sense that hybridization of oligonucleotides containing them via Watson−Crick base pairing allows one to introduce structural modifications in the major and minor grooves of the duplexes, respectively. We now wish to report further on the scope and limitations of **2** and related phosphoramidite **3** for the synthesis of oligodeoxynucleotides that are modified at the 2′-position of a pyrimidine nucleoside (eq 1).



As mentioned above, we have developed methods for preparing modified oligonucleotides in solution, or while the chemically synthesized biopolymer is still bound to its solid support. The general strategy has utilized the *o*-nitrobenzyl photochemical reaction to selectively reveal an electrophile or nucleophile in an otherwise completely protected oligonucleotide.[10−13] Compared to methods for postsynthetic modification of deprotected oligonucleotides, this approach often proceeds in higher yield and uses shorter reaction times and relatively modest excesses of reagents. Furthermore, the use of a single phosphoramidite or solid phase support as a precursor for the preparation of multiple modified oligonucleotides is also more efficient than individually synthesizing phosphoramidites which are then incorporated into oligonucleotides during the linear synthesis process.

Results and Discussion

Preparation of Oligodeoxynucleotides Containing Amine Nucleophiles at the 2′-Position of a Nucleotide Using 2′-Amino-2′-deoxyuridine. Phosphoramidites **2** and **3** were designed to facilitate the functionalization of oligonucleotides at the 2′-position of (2′-deoxy)uridine, such that Watson−Crick complexation of these biopolymers would introduce the modification into the minor groove of the duplex.[14] Although one could envision a plethora of derivatives to prepare, we chose two molecules that would produce oligonucleotide conjugates differing in their degrees of freedom (and consequently steric hindrance) at the site where the modification is introduced (eq 1). Phosphoramidites **2** and **3** were readily prepared from known precursors. Although **2** was previously reported by us, details of its preparation from **6** are provided in the Experimental Section.[12,15] Similarly, 2′-*O*-alkylamine derivatives of uridine, such as the cyanoethyl carbamate phosphoramidites (**7**), have been reported.[14b] Hence, the synthesis of **3** was also



straightforward from **8** (Scheme 1).[16] The greater steric hindrance at the phosphorus center in **2** compared to **3** was reflected in the required coupling conditions during the synthesis of protected oligonucleotides **10** and **14**. With the exception of **2** and **3**, *N*-isobutyrylated phosphoramidites were used for preparing the oligonucleo-

(7) (a) Kremsky, J. N.; Pluskal, M.; Casey, S.; Perry-O'Keefe, H.; Kates, S. A.; Sinha, N. D. *Tetrahedron Lett.* **1996**, *37*, 4313. (b) Habus, I.; Xie, J.; Iyer, R. P.; Zhou, W.-Q.; Shen, L. X.; Agrawal, S. *Bioconjugate Chem.* **1998**, *9*, 283. (c) Khan, S. I.; Grinstaff, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 4704.

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

(9) (a) Allerson, C. R.; Chen, S. L.; Verdine, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 7423. (b) Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4328. (c) Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *J. Org. Chem.* **1992**, *57*, 3839.

(10) (a) McMinn, D. L.; Greenberg, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3289. (b) Kahl, J. D.; McMinn, D. L.; Greenberg, M. M. *J. Org. Chem.* **1998**, *63*, 4870. (c) Kahl, J. D.; Greenberg, M. M. *J. Org. Chem.* **1999**, *64*, 507.

(11) Kahl, J. D.; Greenberg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 597.

(12) Hwang, J.-T.; Greenberg, M. M. *Org. Lett.* **1999**, *1*, 2021.

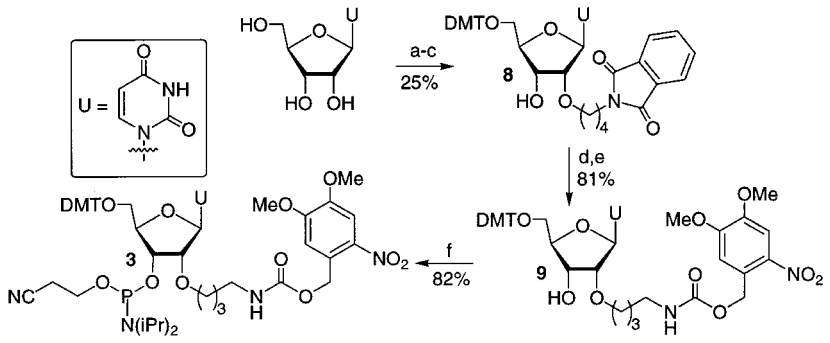
(13) (a) Walker, J. W.; Reid, G. P.; McCray, J. A.; Trentham, D. R. *J. Am. Chem. Soc.* **1988**, *110*, 7170. (b) Pillai, V. N. R. *Synthesis* **1980**, 1. (c) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333.

(14) For other examples of introducing modifications at the C2′-position of oligonucleotides, see ref 4 and: (a) Salo, H.; Virta, P.; Hakala, H.; Prakash, T. P.; Kawasaki, A. M.; Manoharan, M.; Lönnberg, H. *Bioconjugate Chem.* **1999**, *10*, 815. (b) 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. (c) Sigurdsson, S. T.; Eckstein, F. *Nucleic Acids Res.* **1996**, *24*, 3129.

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

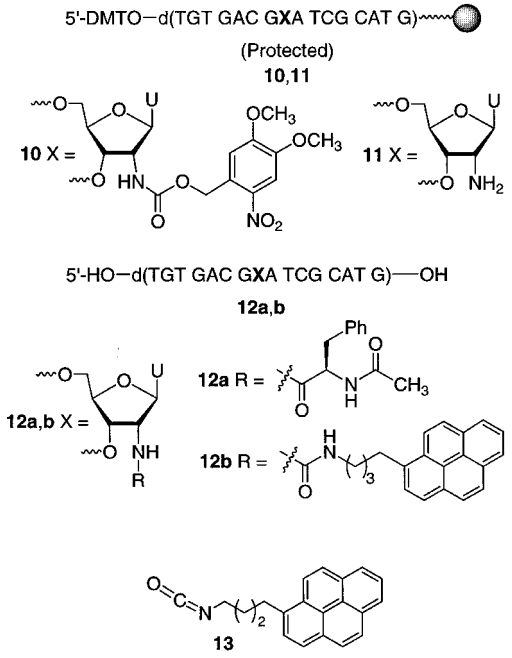
(16) (a) Wagner, D.; Verheyden, J. P. H.; Moffat, J. G. *J. Org. Chem.* **1974**, *39*, 24. (b) Manoharran, M.; Tivel, K. L.; Andrade, L. K.; Cook, P. D. *Tetrahedron Lett.* **1995**, *36*, 3647.

Scheme 1[a]

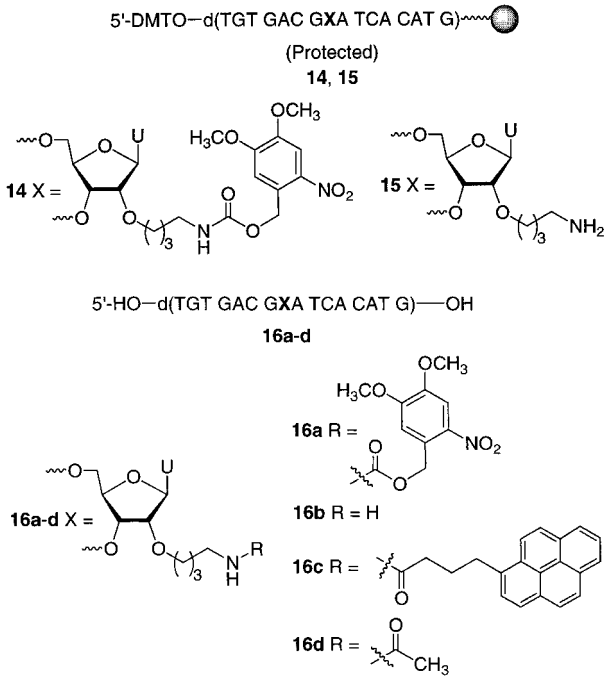


[a] Key: (a) dibutyltin oxide, MeOH; (b) *N*-4-bromobutylphthalimide, NaI, DMF; (c) DMTCl, pyridine; (d) hydrazine, MeOH; (e) 4,5-dimethoxy-2-nitrobenzylchloroformate, Hünig's base, THF; (f) Hünig's base, *N*,*N*-diisopropylaminocyanoethyl phosphonamidic chloride, CH_2Cl_2.

tides.[17] Standard oligonucleotide synthesis cycles were employed for coupling the phosphoramidites of native nucleotides. However, it was necessary to double-couple **3** (0.05 M) for 5 min each cycle, resulting in a 95% yield (as determined via dimethoxytrityl cation release); whereas only an 87% yield was obtained upon double-coupling **2** (0.1 M, 10 min/cycle).[12]



Moreover, the steric hindrance at the amino position of 2′-amino-2′-deoxyuridine (**4**, eq 1) manifested itself during conjugation reactions of **11**, and provided further impetus for the synthesis of **3**. High yields of conjugates were obtained when **11** was reacted with aryl isocyanates, or alkyl carboxylic acids containing methylene groups at their α-carbons.[12] However, attempted coupling of **11** with carboxylic acids containing tertiary α-carbons, such as *N*-acetylphenylalanine, under the identical conditions employed previously (10 equiv of PyBOP and carboxylic acid, 3 h, 25 °C) produced **12a** in only an average yield of 23%. Increasing the number of equivalents of reagents relative to **11** to 50 and the reaction time to 5 h increased the yield of **12a** to only 37%. Similarly, reaction of **11** with alkyl isocyanate **13** only yielded the respective conjugate (**12b**) on average in 43% yield, even when 10 equiv of the isocyanate was allowed to react with **11** for 3 h.

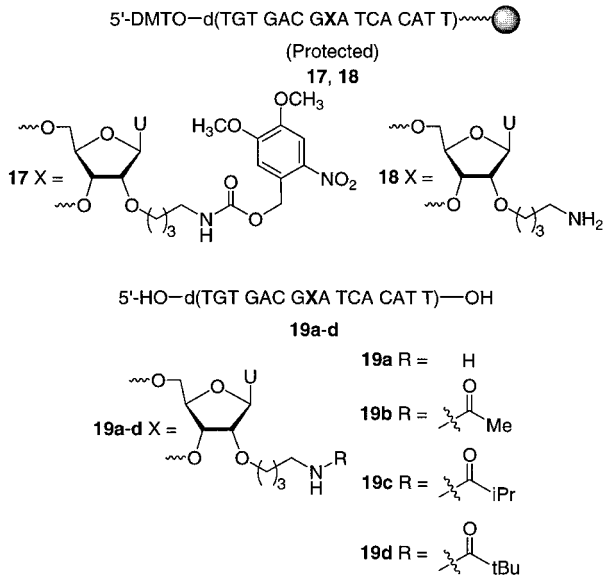


It was assumed that oligodeoxynucleotide **14** prepared from **3** would yield a conjugation substrate (**15**) that is more amenable to reaction with less reactive electrophiles. Initial attempts at conjugating **15** to pyrenebutyric acid (5 equiv, 3 h) produced **16c** in only modest yields, which did not increase significantly when reactions were run in the presence of a greater number of equivalents (10) of acid and activating agent (PyBOP). Analysis of the crude mixture by anion exchange HPLC revealed the presence of a previously unobserved product that did not coelute with the carbamate (**16a**), or conjugate (**16c**), and eluted slightly later than the free amine (**16b**).[18] Isolation and characterization of this product by ESI-MS indicated that it was the acetamide (**16d**).[18] Based upon the observations that the photolabile carbamate is stable to concentrated aqueous ammonia deprotection conditions, and that the acetamide was only

(17) McMinn, D. L.; Greenberg, M. M. *Tetrahedron Lett.* **1997**, *39*, 1103.

(18) See the Supporting Information.

observed in oligodeoxynucleotides that were photolyzed prior to deprotection, we hypothesized that this product was derived from nucleophilic attack by the photochemically revealed amine while the otherwise protected substrate (**15**) was covalently bound to the long chain alkylamine−controlled pore glass (LCAA-CPG) support on which it was synthesized. Attempts at eliminating this undesired product by changing the solvent conditions during the photochemical release of the alkylamine met with limited success. Removing water from the solvent mixture routinely used during photolyses (CH_3CN:H_2O, 9:1), or changing to the less polar solvent, benzene increased the ratio of **16a**:**16d** from 1:1 to as much as 2.5:1 (benzene).

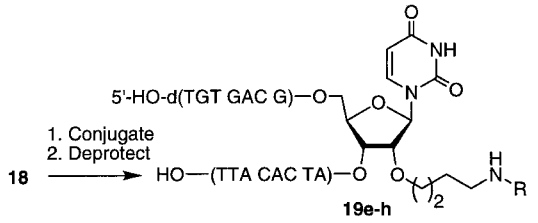


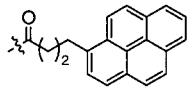
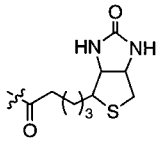
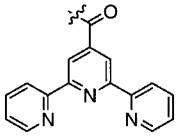
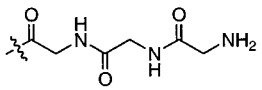
It was determined that the acetates introduced upon capping of failure sequences produced during oligonucleotide synthesis gave rise to the acetamide product described above (**16d**). This information was gleaned from experiments in which LCAA-CPG support containing 5′-*O*-dimethoxytritylthymidine was prepared using 2,2,2-trimethylacetyl chloride as capping agent for the underivatized alkylamines. Isobutyryl anhydride was substituted for acetic anhydride (maintaining the same concentration, 1 M) in the capping step of the automated synthesis cycle during the synthesis of **17**. Anion exchange HPLC analysis of the crude mixture obtained upon photolysis and subsequent ammonia deprotection still revealed the presence of an additional product that eluted slightly later than **19a**. The intensity of this product relative to that of **19a** was approximately 50%. Isolation and analysis by ESI-MS revealed that this product was the isobutyryl amide (**19c**), indicating that the acetate esters introduced during the capping of failure sequences were the predominant source of the acetyl group. The respective acetamide product (**19b**) was not observed. Formation of the undesired product(s) were subsequently reduced to <5% by synthesizing the oligodeoxynucleotides on commercially available LCAA-CPG support using 2,2,2-trimethylacetyl anhydride (1 M) as capping agent for the primary hydroxyls that failed to couple during the automated synthesis cycles. The identity of the minor impurity remaining was ascertained via ESI-MS, and was still found to be the isobutyrylated product (**19c**). No pivaloylated material (**19d**) was detected. (The HPLC chromatograms and ESI-MS spectra can be found in the Supporting Information.) The only possible source of isobutyrylated material was the exocyclic amine protecting groups of 2′-deoxyadenosine, 2′-deoxycytidine, and 2′-deoxyguanosine. These protecting groups have been observed to undergo transamidation to a small extent in other studies.[11]

Having solved this unexpected problem, we briefly turned our attention to optimizing the photochemical unmasking of the alkylamine in **17**. Through several previous studies involving photolabile solid-phase synthesis supports and phosphoramidites (including **1** and **2**), we had established irradiation conditions (365 nm, 2−3 h) that did not impart detectable damage on the oligodeoxynucleotides.[10−12] We were able to improve the efficiency of the photochemical conversion of **17** by taking advantage of the covalent linkage of the product (and substrate) to the solid support. Conversion of the carbamate in **17** was determined by measuring the amount of carbamate present in fully deprotected material (**19n**) by anion exchange HPLC. However, none of this product was observed following two 20 min irradiations (CH_3CN) in which the resin was filtered and washed between photolysis periods on scales as large as 1 μmole. Increased photochemical efficiency is attributed to removal of the nitroso substituted aromatic aldehyde byproduct released during the *o*-nitrobenzyl photoredox reaction, which may be acting as a filter for the process.

Conjugation Reactions of Oligodeoxynucleotide Containing 5 (18). Under the best circumstances, satisfactory conjugation of 2′-amino-2′-deoxyuridine (**4**) required 10 equivalents of reagents and a 3 h reaction time.[12] Using pyrene butyric acid as a standard, we found that excellent isolated yields of **19e** could be obtained using 5 equivalents of acid and PyBOP relative to oligonucleotide for 2 h at room temperature (Table 1). As expected, based upon previous conjugation experiments utilizing protected oligonucleotides as substrates, ESI-MS, as well as enzymatic digestion (followed by reverse phase HPLC analysis) indicated that the conjugate was of high purity.[18] Comparable yields and purity (as determined by ESI-MS analysis) of oligonucleotide conjugate products were also obtained using carboxylic acids such as biotin (**19f**), the tripeptide *N*-Fmoc-gly·gly·gly-CO_2H (**19h**), and a terpyridine (**19g**) (Table 1). Interestingly, the ESI-MS of **19g** revealed that the oligonucleotide conjugate was isolated as a mixture of the free ligand and its copper complex.[18] Isolated yields of conjugates in the 80−90% range (Table 2) were also obtained from reaction of **18** with more hindered substrates such as *N*-acetylphenylalanine (**19i**), which did not couple efficiently with the oligonucleotide containing the more hindered 2′-amino substituted nucleotide (**11**). The isolated yield of **19i** improved slightly (90 ± 1%) by increasing the number of equivalents of reagents to 10, and the reaction time to 3 h. These latter conditions yielded very good isolated yields of the respective oligonucleotide conjugates of diphenyl acetic acid (**19j**) and a tripeptide containing proline at its C-terminus (**19k**). Finally, coupling of the aryl isocyanate, *m*-nitrophenyl isocyanate (**19l**) to **18** also required shorter reaction time (2 h) and fewer equivalents (5 equiv) of isocyanate than did the more hindered substrate (**11**). However, only

Table 1. Yield of Oligonucleotide Conjugates from the Reaction of 18 with Unhindered Carboxylic Acids



	R	Isolated Yield (%)[a,b]
e		83 ± 1 (3)
f		91 ± 7 (3)
g		93 ± 11 (3)
h		81 ± 6 (3)

[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, and isolation conditions. [b] Yields represent an average of separate reactions ± standard deviation from this value. The number of reactions run are noted in parentheses.

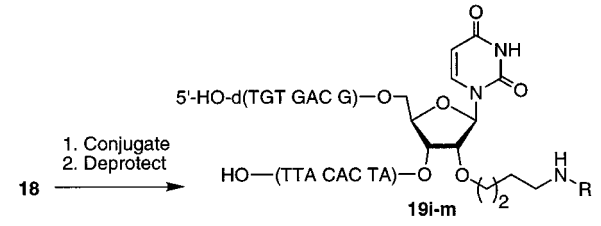
modest yields of **19m** were obtained when coupling **18** to the alkyl isocyanate (**13**), even when as many as 10 equiv were employed for 3 h at 55 °C. The reasons for the poor coupling of **13** to **18** are unknown at this time.

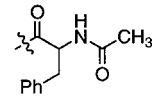
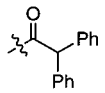
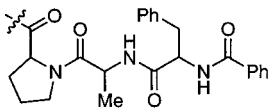
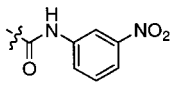
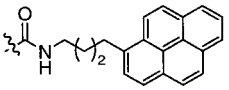
Summary. Oligonucleotides containing modifications at the 2′-position of (2′-deoxy)uridine can be prepared in high yields via conjugation to protected nucleic acids containing a single unmasked nucleophile. Conjugation of hindered electrophiles requires the use of a 2′-*O*-alkylamine substituted uridine. These phosphoramidite reagents provide a simple, efficient method for introducing structural diversity in the minor groove of duplexes composed of such chemically prepared modified oligonucleotides.

Experimental Section

Oligonucleotides were synthesized using standard 1μmol cycles, except where noted, and β-cyanoethyl protected phosphoramidites. The exocyclic amines of deoxyadenosine, deoxycytidine, and deoxyguanosine were protected as their isobutyryl amides. With the exception of polystyrene solid-phase support and *N*-isobutyryl phosphoramidite for deoxycytidine (Pharmacia), all DNA synthesis reagents were obtained from Glen Research, Inc. Benzotriazole-1-yloxy-tri-pyrrolidinophosphonium hexafluorophosphate (PyBOP) was obtained from NovaBiochem. Calf intestine alkaline phosphatase was obtained from New England Biolabs. Nuclease P1 and snake venom phosphodiesterase were from Boehringer Mannheim. Dimethylformamide (DMF), diisopropylethylamine (DIEA), and CH_3CN were freshly distilled from CaH_2. THF was distilled from sodium/benzophenone ketyl. Electrospray samples were prepared by precipitating from NH_4OAc. Preparative oligonucleotide separations was carried out using 20% polyacrylamide denaturing gels (5% cross-link, 45% urea (by weight)).

Table 2. Conjugation of Isocyanates and Hindered Carboxylic Acids to 18



	R	Isolated Yield (%)[a,b]	Conditions[c]
i		83 ± 4 (2) 90 ± 1 (3)	5 eq., 2 h, 25 °C 10 eq., 3 h, 25 °C
j		71 ± 2 (3)	10 eq., 3 h, 25 °C
k		69 ± 5 (3)	10 eq., 3 h, 25 °C
l		79 ± 3 (3)	5 eq., 2 h, 25 °C
m		44 ± 7 (3) 42 ± 3 (3)	5 eq., 2 h, 25 °C 10 eq., 3 h, 55 °C

[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, and isolation conditions. [b] Yields represent an average of separate reactions ± standard deviation from this value. The number of reactions run are noted in parentheses. [c] For amide bond formation, equal number of equivalents of PyBOP are added.

2′-*N*-(4,5-Dimethoxy-2-nitrobenzylcarbonyl)amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine. To a solution of 4,5-dimethoxy-2-nitrobenzylchloroformate (0.16 g, 0.6 mmol) in THF (15 mL) was added diisopropylethylamine (0.12 g, 0.92 mmol), followed by addition of 2′-deoxy-2′-amino-5′-*O*-dimethoxytrityluridine (**6**)[15] (0.25 g, 0.46 mmol) at room temperature. After being stirred for 30 min, the reaction was diluted with dichloromethane, washed with saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The solvent was removed to give crude product as a brownish foam which was purified on silica gel (CH_2Cl_2/EtOAc, 4:1 to 2:1, containing 1% Et_3N) to yield the carbamate as white loaves (0.28 g, 78%): ^{1}H NMR ($CDCl_3$) δ 9.45 (bs, 1H), 7.70 (d, 1H, J = 8.1 Hz), 7.66 (s, 1H), 7.50−7.20 (m, 9H), 7.02 (s, 1H), 6.87 (d, 4H, J = 8.4 Hz), 6.29 (d, 1H, J = 7.5 Hz), 6.20 (d, 1H, J = 8.1 Hz), 5.46 (m, 3H), 4.40 (m, 2H), 4.24 (s, 1H), 3.96 (s, 6H), 3.92 (s, 3H), 3.81 (s, 6H), 3.46 (m, 2H); IR (film) 3650 (br), 3316, 3064, 2359, 2341, 1693, 1607, 1520, 1509 cm-1; ^{13}C NMR ($CDCl_3$) δ 163.5, 158.7, 156.1, 153.7, 151.6, 148.1, 144.2, 140.3, 139.5, 135.3, 135.1, 130.2, 128.7, 128.1, 127.7, 127.2, 113.4, 110.4, 108.1, 103.2, 87.3, 86.0, 85.7, 76.8, 71.9, 64.2, 63.9, 58.4, 56.6, 56.4, 55.4; HRMS (FAB) calcd for $C_{40}H_{40}N_4O_{13}$ 784.2592 (M^+), found 784.2600.

2′-*N*-(4,5-Dimethoxy-2-nitrobenzylcarbonyl)amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 3′-*O*-[(2-Cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (2). To a cloudy solution of 2′-*N*-4,5-dimethoxy-2-nitrobenzylcarbonylamino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (0.1 g, 0.13 mmol) in

dichloromethane (10 mL) was added diisopropylethylamine (67 mg, 0.52 mmol), followed by addition of *N,N*-diisopropylaminocyanoethyl phosphonamidic chloride (48 mg, 0.2 mmol) at 0 °C. After stirring for 2 h at room temperature, additional phosphitylating reagent (16 mg, 0.07 mmol) was added to the reaction mixture. After being stirred for an additional 2 h at room temperature, the reaction was quenched with propan-2-ol (0.24 g, 3.9 mmol). The solution was stirred for 1 h at room temperature, whereupon a solution of 5% Na_2CO_3 (5 mL) was added. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude phosphoramidite was purified on silica gel (CH_2Cl_2/EtOAc, 10:1 to 1:1, containing 1% Et_3N) to yield a mixture of diastereomers of **2** as a pale brownish foam (0.1 g, 79%): ^{1}H NMR ($CDCl_3$) δ 7.70 (m, 2H), 7.42−7.25 (m, 9H), 7.06 (s, 1H), 6.86 (d, 4H, J = 8.7 Hz), 6.14 (t, 1H, J = 9.2 Hz), 5.97 (d, 1H, J = 7.5 Hz, minor), 5.62 (dd, 1H, J = 11.1, 25.5 Hz), 5.45 (m, 2H), 4.80−4.35 (m, 2H), 4.30 (d, 1H, J = 54.3 Hz), 4.01 (s, 3H), 3.96 (s, 3H), 3.85 (m, 1H), 3.81 (s, 6H), 3.81−3.54 (m, 3H) 3.43 (m, 2H), 2.63 (m, 1H), 2.51 (m, 1H), 1.21 (m, 12H); ^{31}P NMR ($CDCl_3$) δ 152.44, 150.31; IR (film) 3232, 3065, 2967, 2252, 1694, 1607, 1519 cm^{-1}; HRMS (FAB) calcd for $C_{49}H_{57}N_6O_{14}P$ 985.3749 (M^+ + H), found 985.3766.

2′-*O*-(4-Phthalimidobutyl)-5′-*O*-(4,4′-dimethoxytrityl)-uridine (8). A suspension of uridine (2.5 g, 10.3 mmol) and dibutyltin oxide (2.86 g, 11.2 mmol) in methanol (500 mL) was heated under reflux for 1 h, and the resulting clear solution was evaporated to yield 2′,3′-*O*-dibutylstannyleneuridine as a white solid.[16] The crude product was dried further under high vacuum. To a solution of 2′,3′-*O*-dibutylstannyleneuridine (4.92 g, 10.3 mmol) in DMF (60 mL) were added *N*-4-bromobutylphthalimide (8.10 g, 28.7 mmol) and NaI (1.1 g, 7.3 mmol). The reaction mixture was heated at 130−140 °C for 24 h, after which time it was poured into a mixture of ether (100 mL) and water (100 mL). The aqueous layer was extracted with ether. The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo to give a crude product mixture (55:45 ratio of 2′- and 3′-isomers as determined by ^{1}H NMR) as an oil which was used in the next step without purification. To a solution of the mixture of 2′ and 3′-alkylated isomers, which was coevaporated with pyridine, in pyridine (30 mL) in an ice−water bath was added 4,4′-dimethoxytrityl chloride (3.56 g, 10.5 mmol). The reaction mixture was warmed to room temperature. After the mixture was stirred overnight, the pyridine was removed under reduced pressure to give a syrup, which was purified by flash column chromatography (hexanes/EtOAc, 1:2 to 1:1, containing 1% Et_3N). The 2′-alkylated product (**8**) eluted first as a pale brownish foam (1.94 g, 25%), followed by the 3′-alkylated product as a white foam (1.34 g, 18%). 2′-Alkylated product: ^{1}H NMR ($CDCl_3$) δ 9.70 (br, 1H), 8.09 (d, 1H, J = 8.1 Hz), 7.86 (dd, 2H, J = 3.0, 5.7 Hz), 7.73 (dd, 2H, J = 3.3, 5.4 Hz), 7.45−7.20 (m, 9H), 6.85 (d, 4H, J = 8.7 Hz), 5.99 (s, 1H), 5.31 (d, 1H, J = 8.7 Hz), 4.52 (br, 1H), 4.09 (m, 2H), 3.94 (d, 1H, J = 5.1 Hz), 3.83 (s, 6H), 3.78 (m, 3H), 3.60 (s, 2H), 3.03 (br, 1H), 1.86−1.66 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 168.5, 163.7, 158.7, 150.2, 144.5, 140.1, 135.4, 135.2, 134.0, 132.1, 130.3, 130.2, 128.2, 128.1, 127.2, 123.3, 113.4, 102.1, 87.7, 87.1, 83.3, 82.7, 70.6, 68.6, 61.3, 55.4, 37.8, 26.6, 25.5; IR (film) 3467 (br), 3193 (br), 3058, 1770, 1712, 1608, 1509 cm^{-1}; HRMS (FAB) calcd for $C_{42}H_{41}N_3O_{10}$ 747.2792 (M^+), found 747.2677. 3′-Alkylated product: ^{1}H NMR ($CDCl_3$) δ 9.65 (br, 1H), 7.90 (d, 1H, J = 8.1 Hz), 7.86 (dd, 2H, J = 2.7, 5.4 Hz), 7.72 (dd, 2H, J = 3.0, 5.4 Hz), 7.45−7.20 (m, 9H), 6.88 (d, 4H, J = 9.0 Hz), 5.97 (d, 1H, J = 3.9 Hz), 5.44 (d, 1H, J = 8.1 Hz), 4.39 (t, 1H, J = 4.2 Hz), 4.23 (m, 1H), 4.10 (m, 1H), 3.81 (s, 6H), 3.75−3.50 (m, 5H), 3.42 (m, 1H), 1.86−1.60 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 168.5, 163.4, 158.7, 150.7, 144.3, 140.2, 135.4, 135.2, 134.0, 132.1, 130.1, 128.2, 128.1, 127.2, 123.3, 113.4, 102.5, 90.0, 87.1, 81.4, 77.4, 74.0, 70.3, 62.4, 55.4, 37.6, 26.9, 25.4

2′-*O*-(4-Aminobutyl)-5′-*O*-(4,4′-dimethoxytrityl)uridine. 2′-*O*-(4-Phthalimidobutyl)-5′-*O*-(4,4′-dimethoxytrityl)-uridine (1.46 g, 1.95 mmol) was dissolved in methanol (30 mL). Hydrazine monohydrate (0.50 g, 9.96 mmol) was added, and the reaction mixture was heated to reflux. After 5 h, the solvent was removed to give a crude product as a foam, which was purified on silica gel (CH_2Cl_2/MeOH, 10:1, containing 1% Et_3N) to yield a brownish foam (1 g, 83%). ^{1}H NMR ($CDCl_3$) δ 8.15 (d, 1H, J = 7.8 Hz), 7.44−7.26 (m, 9H), 6.87 (d, 4H, J = 9.3 Hz), 6.00 (d, 1H, J = 1.5 Hz), 5.33 (t, 1H, J = 4.1 Hz), 4.07 (m, 1H), 3.93 (m, 2H), 3.82−3.64 (m, 8H), 3.56 (m, 2H), 2.79 (br, 2H), 1.80−1.54 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 164.2, 158.8, 150.8, 144.5, 140.0, 135.4, 135.2, 130.3, 130.2, 128.3, 128.1, 127.2, 113.4, 102.4, 87.6, 87.2, 83.6, 82.9, 71.0, 68.7, 61.7, 55.4, 41.5, 29.5, 27.2; IR (film) 3357 (br), 3160, 3058, 2933, 1694, 1633, 1509 cm^{-1}; HRMS (FAB) calcd for $C_{34}H_{39}N_3O_8$ 618.2815 (M^++ H), found 618.2814.

2′-*O*-[*N*-((4,5-Dimethoxy-2-nitrobenzyl)carbonyl)-4-aminobutyl]-5′-*O*-(4,4′-dimethoxytrityl)uridine (9). To a solution of 4,5-dimethoxy-2-nitrobenzylchloroformate (0.20 g, 0.73 mmol) in THF (15 mL) was added diisopropylethylamine (0.14 g, 1.1 mmol), followed by addition of 2′-*O*-(4-aminobutyl)-5′-*O*-(4,4′-dimethoxytrityl)uridine (0.34 g, 0.55 mmol) at room temperature. After being stirred for 30 min, the reaction was diluted with dichloromethane, washed with saturated $NaHCO_3$ and brine, and dried over Na_2SO_4. The solvent was removed to give a crude product as a brownish powder which was purified on silica gel (CH_2Cl_2/EtOAc, 4:1 to 2:1, containing 1% Et_3N) to yield a white foam (0.46 g, 97%): ^{1}H NMR ($CDCl_3$) δ 9.98 (br s, 1H), 8.07 (d, 1H, J = 8.4 Hz), 7.68 (s, 1H), 7.20−7.48 (m, 9H), 7.02 (s, 1H), 6.87 (d, 4H, J = 9.0 Hz), 5.95 (s, 1H), 5.58−5.40 (m, 3H), 5.32 (d, 1H, J = 5.7 Hz), 4.50 (m, 1H), 4.10−3.88 (m, 9H), 3.82 (s, 6H), 3.73 (m, 1H), 3.57 (m, 2H), 3.29 (m, 2H), 3.06 (br, 1H), 1.71 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 163.6, 158.71, 158.68, 156.1, 153.5, 150.5, 148.1, 144.4, 140.1, 139.9, 135.3, 135.1, 130.3, 130.2, 128.2, 128.1, 127.2, 113.3, 110.5, 108.2, 102.2, 87.7, 87.1, 83.3, 82.7, 77.4, 70.9, 68.5, 63.6, 61.3, 56.52, 56.49, 55.4, 53.6, 40.9, 26.8, 26.6; IR (film) 3314 (br), 2939, 2865, 1702, 1697, 1618, 1581 cm^{-1}.

2′-*O*-[*N*-((4,5-Dimethoxy-2-nitrobenzyl)carbonyl)-4-aminobutyl]-5′-*O*-(4,4′-dimethoxytrityl)uridine 3′-*O*-[(2-Cyanoethyl)-*N,N*-diisopropyl]phosphoramidite (3). To a cloudy solution of 2′-*O*-[*N*-((4,5-dimethoxy-2-nitrobenzyl)carbonyl)-4-aminobutyl]-5′-*O*-(4,4′-dimethoxytrityl)uridine (0.4 g, 0.47 mmol) in dichloromethane (15 mL) was added diisopropylethylamine (0.24 g, 1.88 mmol), followed by the addition of *N,N*-diisopropylaminocyanoethyl phosphonamidic chloride (0.14 g, 0.7 mmol) at 0 °C. After being stirred overnight at room temperature, the reaction was quenched with propan-2-ol (0.79 g, 14.1 mmol) and stirred for 1 h at room temperature, at which time a solution of 5% Na_2CO_3 (5 mL) was added to the reaction mixture. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude phosphoramidite was purified on silica gel (CH_2Cl_2/EtOAc, 10:1 to 1:1, containing 1% Et_3N) to yield a pale brownish foam (0.4 g, 82 mmol): ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 1H), 7.71(s, 1H), 7.20−7.50 (m, 9H), 7.04 (m, 1H), 6.87 (m, 4H), 5.95 (m, 1H), 5.60−5.2 (m, 4H), 4.70−4.40 (m, 1H), 4.25 (m, 1H), 3.88−4.08 (m, 7H), 3.88−3.65 (m, 8H), 3.65−3.20 (m, 8H), 2.68 (t, 1H, J = 6.3 Hz), 2.46 (t, 1H, J = 6.3 Hz), 1.70(br, 4H), 1.05−1.33 (m, 13H); ^{31}P NMR ($CDCl_3$) δ 150.67, 150.06; IR (film) 3325, 3198, 3057, 2966, 2935, 2872, 2368, 2342, 1693, 1682, 1519, 1277, 1252 cm^{-1}; HRMS (FAB) calcd for $C_{53}H_{65}N_6O_{15}P$ 1057.4324 (M^++ H), found 1057.4355.

Preparation of the 5′-*O*-Dimethoxytrityl-3′-succinatothymidine LCAA-CPG Capped by Trimethylacetic Anhydride. LCAA-CPG (0.1 g), PyBOP (2.9 mg, 5.5 μmol), diisopropylethylamine (2 mL, 12 μmol), DMAP (0.7 mg, 5.7 μmol), and 5′-*O*-dimethoxytrityl-3′-succinatothymidine (3.5 mg, 5.4 μmol) were combined in a screw-capped vial with acetonitrile (2 mL). The reaction mixture was shaken at room temperature for 20 min. The resin was filtered, washed with MeOH and CH_2Cl_2, and then dried under high vacuum. The amount of nucleoside loading on the support (~30 μmol/g) was determined by trityl analysis.[19] Unreacted alkylamines were

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capped by reacting the DMT-thymidine-CPG (0.1 g), trimethylacetyl chloride (14 μL, 113 μmol), DMAP (6.7 mg, 55 μmol), pyridine (18 μL, 220 μmol), and acetonitrile (2 mL) in a screw-capped vial. The reaction mixture was shaken at room temperature for 2 h. The resin was filtered, washed with MeOH followed by CH_2Cl_2, and dried under high vacuum.

Photolytic Deprotection. Oligodeoxyribonucleotide bound to support (30 mg) was added to a Pyrex tube containing a stir bar constructed from a standard (white) pipe cleaner and CH_3CN (20 mL). The tube was fitted with a rubber septum and the solution was sparged with Ar for 20 min, after which the needle was raised well above the surface of the solvent. Photolyses were carried out with a VWR Chromato-Vue transilluminator ($\lambda_{max} = 365$ nm) for 20 min. The resin was filtered, washed with MeOH, followed by CH_2Cl_2, collected, and dried under high vacuum. The resin was transferred to a tube in CH_3CN (20 mL), and the sparging and photolysis procedures were repeated. The resin was filtered, washed with MeOH, followed by CH_2Cl_2, dried under vacuum, and placed in a screw capped vial for storage. Note: It is important to maintain the temperature during photolysis at ≤25 °C using a fan.

General Procedure for Conjugation of Protected Resin-Bound Oligodeoxyribonucleotides. A solution (12 mM) of the coupling reagents (3.2 mg of PyBOP and 2.1 μL of diisopropylethylamine (2 molar equiv) in DMF (500 mL) was prepared in an oven-dried 1 dram vial equipped with a septum. A solution (12 mM) of carboxylic acid (6 μmol in 500 mL 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 50 μL (5 molar equiv) of a 1:1 mixture (by volume) of the PyBOP and carboxylic acid solutions. The reaction was capped and shaken at room temperature for 2 h. The resin was washed with MeOH 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 biotin-conjugated oligonucleotide was carried out via 20% polyacrylamide denaturing gel electrophoresis. The others was purified by anion-exchange HPLC column (Vydac 301VHP575; A, 10 mM Tri, pH 8.0; B, 10 mM Tri, 0.5 M NH_4Cl, pH 8.0; 0−80% B linearly over 24 min; 80% B over 10 min for pyrenebutyryl and terpyridinyl conjugated oligonucleotides; 0−52% B linearly over 43 min; 52−80% B linearly over 3 min for other conjugated oligonucleotides; flow rate, 1 mL/min). Isolated yields were obtained (o.d. 264 nm; 352 nm for pyrenebutyryl conjugated products) by comparing the amount of conjugated oligonucleotide to the amount of unconjugated material.

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Supporting Information Available: Electrospray mass spectra of **12b**, **16a**−**d**, **19a**,**c**,**e**−**n**. Anion-exchange HPLC chromatograms of oligonucleotides. Experimental procedure for the synthesis of 2′-*O*-[(1-pyrenebutyryl)-4-aminobutyl)]-uridine. Procedure for, and reversed-phase HPLC chromatogram of, enzyme digest of **19e**. Spectra (^{1}H, ^{13}C, ^{31}P) of the molecules prepared in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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