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Introducing Structural Diversity in Oligonucleotides via Photolabile, Convertible C5-Substituted Nucleotides

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Abstract: Chemically synthesized oligonucleotides are functionalized at defined sites while in their protected form on solid-phase supports via the incorporation of nucleotides containing masked alkyl carboxylic acids or alkylamines. The reactive functional groups are selectively revealed upon 365 nm irradiation of the appropriate *o*-nitrobenzyl based protecting group. For the photolabile nucleotide that reveals alkylamines, a combination of analytical methods revealed that very high yields (≥94%) of the oligonucleotide containing a single reactive functional group were obtained by using photolysis conditions that do not damage the biopolymer. Either functionalized nucleotide is efficiently coupled to the corresponding reaction partner via PyBOP mediated amide bond formation. Isolated yields of the conjugated oligonucleotides are ≥90%. This method is compatible with introducing multiple modified sites sequentially and is amenable to the synthesis of libraries of oligonucleotides in a combinatorial fashion containing nonnative nucleotides while bound to their solid supports.

Modified oligonucleotides are finding increasing application in biological and biophysical studies. These biomolecules have been equipped with functional molecules including fluorophores, enzymes, and nuclease mimics.[1−3] In addition, RNA and DNA molecules containing naturally occurring and nonnative nucleotides show promise as ligands for nonnucleic acid receptors and as catalysts.[4−8] The C5-position of (deoxy)uridine is a popular position for introducing structural diversity in oligonucleotides when designing functional biopolymers for applications as diverse as sensors, sequence selective nucleic acid binding and/or damaging agents, and aptamers.[9−14] Part of the allure of utilizing C5-substituted pyrimidines is that large substituents can be incorporated at this position without perturbing the syn/anti equilibrium about the glycosidic bond, or the overall structure of the biopolymer. The absence of such perturbations often enables one to incorporate C5-modified pyrimidine nucleotides enzymatically via their respective triphosphates. The compatibility of C5-modified nucleotide triphosphates with polymerase enzymes has proven particularly useful in expanding the diversity of libraries of nucleic acid ligands.[9] Developments in the deconvolution of such libraries

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(2) Truffert, J.-C.; Asseline, U.; Brack, A.; Thuong, N. T. *Tetrahedron* **1996**, *52*, 3005.
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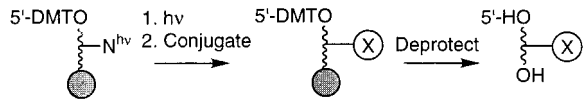
Scheme 1[a]



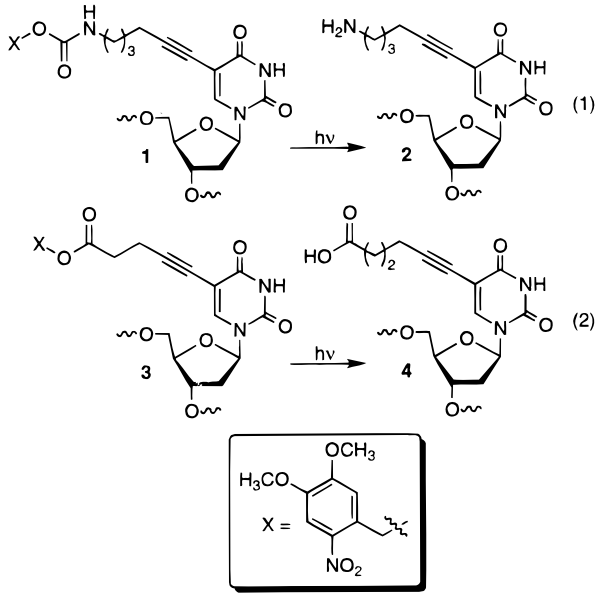
[a]Key: $N^{h\nu}$, photolabile nucleotide; (X), modified nucleotide

by mass spectrometry could provide further impetus for the incorporation of modified nucleotides in oligonucleotide libraries.[15]

Independent synthesis of the requisite nucleotide triphosphates and phosphoramidites (for chemical oligonucleotide synthesis) can be a lengthy process, although a one-step process that is applicable for some C5-modified pyrimidines has been reported.[9] In an alternative method, a single C5-substituted nucleotide is modified following its incorporation into an oligonucleotide.[10] The use of such convertible nucleosides has the advantage that only a single phosphoramidite (or nucleotide triphosphate) needs to be synthesized, making the method convergent and potentially suitable for constructing libraries of modified oligonucleotides. Convertible nucleosides functionalized at positions other than C5 have also been employed for introducing structural diversity in oligonucleotides.[16−18] In general, the previously reported methods utilizing convertible nucleosides require large excesses of functionalizing agent (e.g., alkylamines) and reaction periods as long as 3 days.[17] We wish to describe an alternative method for introducing structural diversity in oligonucleotides from convertible nucleosides in which the functionalization reaction is rapid, utilizes reagents efficiently, and produces high yields of modified oligonucleotides. Convertible nucleotides containing photolabile triggers are incorporated at defined sites in chemically synthesized oligonucleotides (Scheme 1). The support bound, protected oligonucleotides are conjugated following photolysis, and then deprotected and purified in the usual fashion. A further advantage of carrying out the conjugation chemistry while the polymer is on the solid support is the ease of separation of the product from activating agents and other reactants. In addition to facilitating the synthesis of oligonucleotides containing modified nucleotides, the method is compatible with the chemical synthesis of oligonucleotide libraries containing nonnative nucleotides.

Results and Discussion

The strategy presented below capitalizes on developments for selectively unmasking a single functional group within a protected oligonucleotide, and for conjugating oligonucleotides to small organic molecules in solution or while anchored to a solid-phase support.[19−22] Oligonucleotides containing 3′-alkylamines or 3′-alkyl carboxylic acids which retain their nucleobase, phosphate, and 5′-hydroxyl protecting groups are obtained from photolabile, orthogonal solid-phase synthesis supports that are based upon the *o*-nitrobenzyl photoredox process.[21,23] Solution-phase coupling of these protected oligonucleotides to a variety of small organic molecules and tripeptides via amide bond formation proceeds in no less than 83% isolated yield, and quite often in yields that are in excess of 90%. Although a variety of activation methods have been employed, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) is the activating agent of choice. In addition, reactions are typically carried out in ≤2 h between 25 and 55 °C with ≤10 equiv of activating agent and small molecule reactant relative to oligonucleotide substrate. More recently, even more efficient conjugation has been effected between support-bound protected oligonucleotides containing 5′-alkylamines and small molecules.[20]



Phosphoramidite and Oligonucleotide Synthesis. We anticipated that incorporation of C5-substituted molecules containing alkylamines (**1**) or alkyl carboxylic acids (**3**), protected with photolabile groups as their respective phosphoramidites (**12**, **17**), would facilitate the postsynthetic introduction of functional groups at defined sites in chemically synthesized biopolymers and would enjoy the benefits of the convertible nucleoside approach mentioned above (eqs 1 and 2). While one can envision a variety of tethers between the C5-position of the nucleoside and the conjugatable functional group(s), we chose an alkyl alkynyl linker to demonstrate this method. The selection of this linker is based upon its ease of synthesis via Pd(0) chemistry, and the sufficient length of the alkyl chain which was expected to provide a sterically unhindered environment in the region of the reactive functional group.[24]

The silylated alkynol (**5**) served as a common intermediate for the preparation of **12** and **17**. However, one should note that the resulting electrophilic (**4**) monomer contains one less methylene unit in its alkyl tether than its nucleophilic complement (**2**). Conversion of **5**, which was readily prepared from 5-iododeoxyuridine, to the respective alkylamine was achieved via reduction of the azide (**7**, Scheme 2). Due to difficulties in purifying the alkylamine, it was carried on in crude form to the bis-silyl protected photolabile carbamate (**9**). Synthesis of the

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(19) (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.
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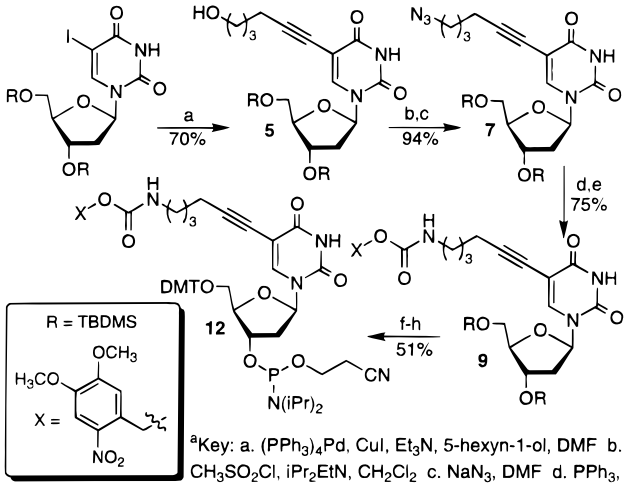
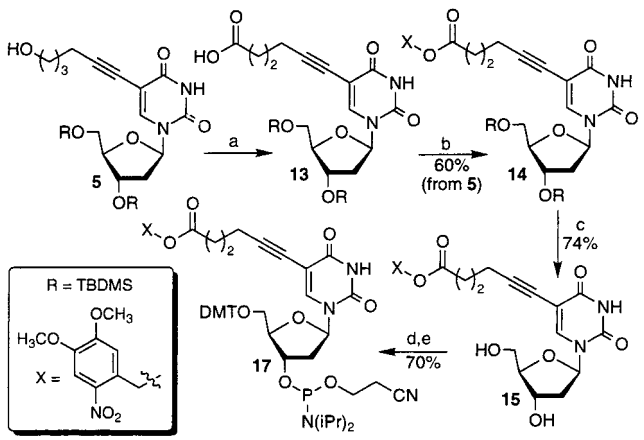
Scheme 2[a]



[a]Key: a. $(PPh_3)_4Pd$, CuI, Et_3N, 5-hexyn-1-ol, DMF b. CH_3SO_2Cl, iPr_2EtN, CH_2Cl_2 c. NaN_3, DMF d. PPh_3, THF e. 4,5-Dimethoxy-2-nitrobenzyl chloroformate, iPr_2EtN, THF f. TBAF, DMF g. DMTCl, pyridine h. 2-Cyanoethyl diisopropylchlorophosphoramidite, CH_2Cl_2

Scheme 3[a]



[a]Key: a. PDC, DMF b. Dimethoxynitrobenzyl alcohol, DCC, DMAP, CH_2Cl_2 c. $Et_3N{\bullet}3HF$, THF d. DMTCl, pyridine e. 2-Cyanoethyl diisopropylchlorophosphoramidite, iPr_2EtN, CH_2Cl_2

carboxylated analogue was accomplished via oxidation of **5**, and esterification of the bis-silylated carboxylic acid (**13**, Scheme 3). The respective photolabile bis-silyl compounds (**9**, **14**) were carried on to **12** and **17** via standard dimethoxytritylation and phosphitylation, following desilylation.

On the basis of previous observations with respect to photostability and propensity for phenoxyacetyl protected exocyclic amines of nucleobases to undergo transamidation, the respective *N*-isobutyryl protected phosphoramidites of dA, dC, and dG were employed during the synthesis of oligonucleotides containing **1** and **3**.[23,25,26] The oligonucleotides containing the photolabile monomers at defined sites were prepared via standard automated cycles on either 0.2 or 1.0 mmol scale. No differences were observed in the coupling of **12** and **17** compared to the standard, commercially obtained phosphoramidites.

Characterization of the Photodeprotection of 1 in Oligonucleotides. Treatment of carbamate **10** with concentrated aqueous ammonia at 55 °C for 24 h revealed that this functional group was stable to the oligonucleotide deprotection conditions. Consequently, it was possible to analyze the efficiency of the photochemical deprotection following incorporation of **12** in a chemically synthesized oligonucleotide (**18**) by a variety of methods. The deprotected alkylamine (**21b**) and acetylated alkylamine (**21c**, produced via capping and/or acetylation during detritylation) oligonucleotides isolated following photolysis and deprotection/cleavage were separable by gel electrophoresis and anion exchange HPLC from carbamate (**21a**) containing oligonucleotide. Irradiation of the support-bound, protected biopolymer (**18**) was carried out for 1 h with use of a transilluminator at 365 nm, as previously described for the photochemical cleavage of protected oligonucleotides from solid-phase supports.[23,26] Subsequent anion exchange HPLC analysis of **21** following deprotection indicated that photoconversion of **1** to **2** was complete. Similarly, complete conversion of **1** to **2** was observed upon photolysis of **19** for 1 h. In contrast, gel electrophoretic analysis of photodeprotected oligonucleotide containing **1** at position 17 (**20**) revealed that a small amount of carbamate remained after photolysis for 1 h, but none after 2 h of irradiation. Isolated yields of oligonucleotides (obtained as a mixture of **23b** and **23c**), which were 92% and 98% following irradiation for 1 and 2 h, respectively, were consistent with these observations. The less efficient photolysis of **1** when it is positioned closer to the 3′-terminus (and consequently the solid-phase support) is consistent with the observations that photolabile solid-phase supports containing *o*-nitrobenzyl linkers require longer irradiation times than do **1** to achieve comparable photoconversion, and that oligonucleotide array synthesis becomes more efficient as the photolabile nucleotides are incorporated further from the solid support.[23,26,27]

5′-DMTO−d(TAC GTA CTG A**1**G CAG CTC GT)〰●
(Protected)
18

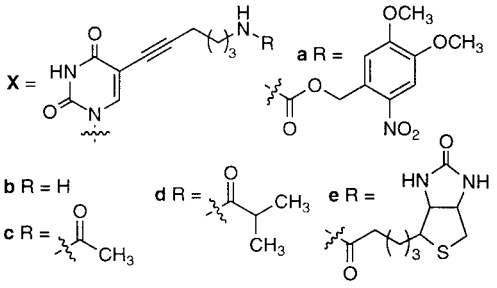
5′-DMTO−d(TAC G**1**A CTG ATG CAG CTC GT)〰●
(Protected)
19

5′-DMTO−d(TAC GTA CTG ATG CAG C**1**C GT)〰●
(Protected)
20

5′-HO−d(TAC GTA CTG A**X**G CAG CTC GT)—OH
21a-h

5′-HO−d(TAC G**X**A CTG ATG CAG CTC GT)—OH
22a-e

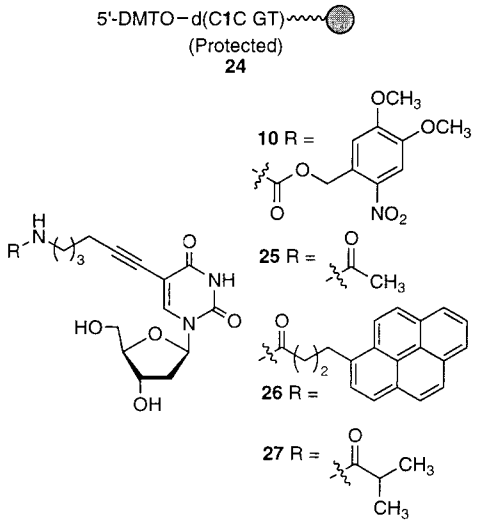
5′-HO−d(TAC GTA CTG ATG CAG C**X**C GT)—OH
23a-e



An alternative method for the quantitative analysis of the photochemical deprotection process (eq 1) involved examination of the free nucleosides released upon enzymatic digestion of the polymer obtained starting from **24**. For this method of analysis, the oligonucleotides containing photochemically re-

(25) McMinn, D. L.; Greenberg, M. M. *Tetrahedron Lett.* **1997**, *38*, 3123.
(26) Venkatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525.
(27) McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081.

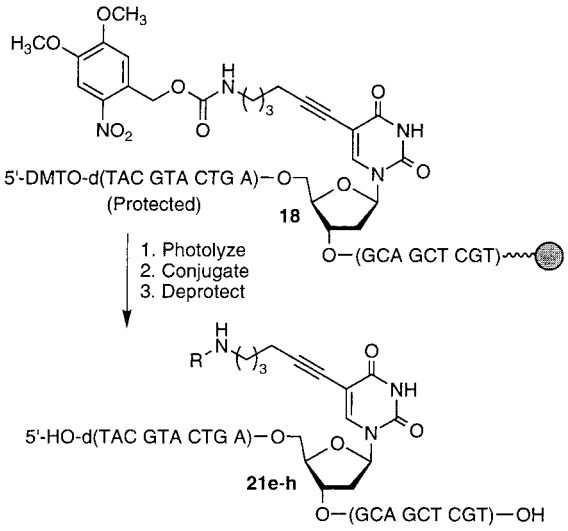
leased alkylamine groups were capped with acetic anhydride (to facilitate subsequent nucleoside analysis), and the biopolymer was deprotected with concentrated aqueous ammonia. Following enzymatic digestion, the free nucleosides were analyzed by reverse-phase HPLC.[28] On the basis of detection limits established by using an internal standard and measured response factors of independently synthesized **10** and **25**, it was determined that a 2 h photolysis yielded ≥94% conversion of the carbamate to the reactive alkylamine. The conclusion drawn from these experiments was that regardless of the position where **1** was incorporated in an oligonucleotide, the carbamate could be completely converted to the conjugatable alkylamine (**2**) via a 2 h photolysis at 365 nm by using a readily available UV-light source. One should note that these photolysis conditions have been shown previously to not damage the oligonucleotides.[23,26]

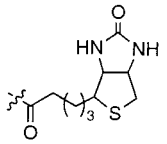
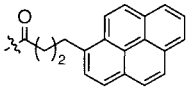
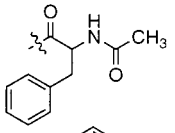
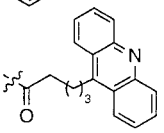


On-Column Conjugation of C5-Substituted Deoxyuridines. C5′-Terminal primary amines in oligonucleotides, which are bound to their solid supports, serve as suitable substrates for bioconjugate formation.[20,22] We recently reported that oligonucleotide conjugates can be efficiently prepared from alkylamines via PyBOP mediated on-column coupling in 15 min at 25 °C, using as few as 2 equiv (typically 5 equiv) of the appropriate carboxylic acid. This method was extended to conjugating **2** to a series of alkyl carboxylic acids (eq 1, Table 1). In all cases, average coupling yields exceeded 90% when carried out for 1 h at 25 °C with 5 equiv of the respective carboxylic acid and activating agent relative to oligonucleotide. Conjugate **21f** was subjected to enzymatic digestion. Quantitative analysis of the nucleosides released indicated that dA, dC, dG, T, and **26** were present in the expected ratios.[28]

The dependency of the efficiency of the coupling process on the position of the convertible nucleotide in the biopolymer was investigated by using two eicosameric oligonucleotides which differed from **18** with respect to which thymidine was substituted by **1**. By using the conjugation conditions described above, biotin was coupled to **2**, generated at position 5 (from **19**), in essentially quantitative yield (**22e**, 100 ± 2%).[29] However, the isolated yield of **23e**, in which **1** is incorporated at position 17 (**20**), was consistently slightly lower (84 ± 5%) than at other positions in comparable oligonucleotides.[29] Increasing the number of equivalents of activating agent and biotin relative to oligonucleotide to 10 did not lead to any improvement in the isolated yield of **23e** (80 ± 1%).[29] Three possible explanations were considered for the lower isolated yield of conjugate compared to that obtained at other sites of incorporation of **1**. Analysis of the crude conjugate by gel electrophoresis confirmed that the lower coupling yield at the fourth position was not due to the presence of unconverted carbamate (**23a**). However, this method could not distinguish between unreacted alkylamine (or the respective acetate which would be formed upon detritylation, **23b** and **23c**) and isobutyryl amide (**23d**), as the respective products were inseparable by gel electrophoresis. Although it had not been observed previously during conjugation reactions with this approach, the isobutyrylated product (**23d**) could arise via transamidation following photolysis.[25] The third explanation was that a minor population of the alkylamine at the fourth position from the 3′-terminus in a protected eicosamer bound to its solid support is inaccessible, and unreactive. To distinguish

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



	R	Isolated Yield (%)[a,b]
e		91 ± 7 (6)
f		92 ± 8 (7)
g		98 ± 4 (3)
h		91 ± 2 (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.

(28) See Supporting Information.

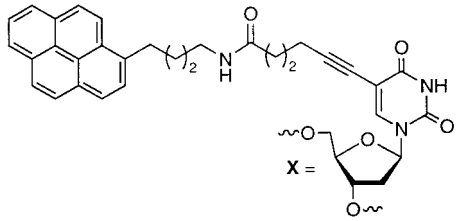
(29) The yields were determined as described in the footnote to Table 1 and in refs 19a and 19b.

(30) Eaton, B. E.; Gold, L.; Hicke, B. J.; Janji'c, N.; Jucker, F. M.; Sebesta, D. P.; Tarasow, T. M.; Willis, M. C.; Zichi, D. A. *Bioorganic Med. Chem.* **1997**, *5*, 1087.

between the latter two explanations, an unpurified sample of **23**, which had been photolyzed, conjugated to biotin, and deprotected, was digested. Provided that photolyses of **23** were cooled with a fan, no isobutyrylated nucleoside (**27**) was observed by reverse-phase HPLC analysis. However, a small amount of the *N*-acetylated nucleoside (**25**) was detected. On the basis of this observation, we hypothesize that **25** is produced from oligonucleotide containing unreacted free amine upon detritylation. The decreased reactivity at the 17th position in an eicosamer may be attributable to lower accessibility when it is buried closer to the support as part of a longer oligonucleotide.

5'-DMTO−d(TAC GTA CTG A3G CAG CTC GT)~●
(Protected)
28

5'-HO−d(TAC GTA CTG A**X**G CAG CTC GT)—OH
29

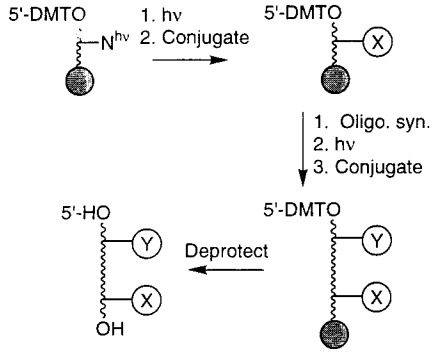


Having established the general reactivity of alkylamine-containing nucleotides in solid-phase supported oligonucleotides, attention was turned to reversing the polarity of the bond formed during the conjugation reaction. It had previously been demonstrated that there is no statistical difference in the yields of conjugates when protected oligonucleotides containing either 3′-alkylamines or 3′-alkyl carboxylic acids are coupled in solution.[21] We anticipated that support bound oligonucleotides would exhibit similar reactivity patterns. Consequently, **3** was incorporated into an oligonucleotide at the 11th position of an eicosamer (**28**) that was otherwise identical to **18**. Indeed, conjugation to pyrene butylamine (5 equiv), following photolysis of the protected oligonucleotide on its solid-phase support for 2 h, yielded **29** in 97 ± 3%.[29]

Using Photoconvertible Nucleotides To Introduce Structural Diversity in Oligonucleotides. The efficiency of the photolysis/coupling process presented above suggested that this method would be useful for preparing libraries of chemically synthesized oligonucleotides containing nonnative nucleosides. For example, this method could be useful for expanding the diversity of a nucleic acid ligand that had been selected via enzymatic synthesis for which one or more nucleotides have been identified as being desirable for modification.[30] The advantage of this chemical process over enzymatic methods is that one can readily control the site(s) where the biopolymer is diversified. A further advantage of employing a convertible nucleotide is that one does not need to chemically synthesize and purify multiple phosphoramidites. In addition, since coupling is achieved "on-column" without affecting other functional (protecting) groups in the biopolymer, this method is compatible with introducing diversity at multiple sites in the oligonucleotide sequentially (Scheme 4). Other convertible nucleosides are not amenable to this, because these molecules typically involve introducing chemical diversity via nucleophilic aromatic substitution.[16−18] The nucleophiles can be expected to at least partially deprotect the biopolymer, making subsequent chemical oligonucleotide synthesis impossible.

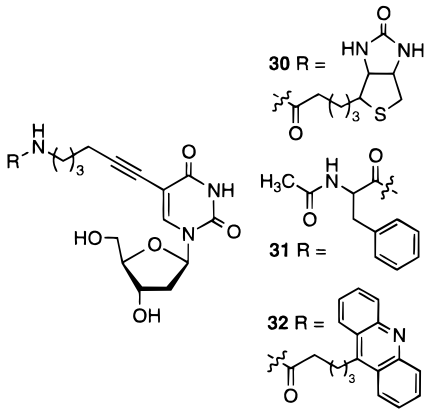
The principle was demonstrated by preparing a two-site, four-component library. A pentameric oligonucleotide (**24**) containing

Scheme 4[a]



[a]Key: N[hν], photolabile nucleotide; (X), (Y), mixtures of modified nucleotides

1 at position 2 (position 17 in the ultimate eicosamer, **33**) was synthesized. Following photolytic unmasking of the primary alkylamine, an equimolar mixture of biotin and *N*-acetyl phenylalanine was conjugated to the oligonucleotide as described above. After washing away the excess reagents, the pentamer was manually acetylated by using commercially available automated DNA synthesis capping reagents. An aliquot of the resin was removed, and the oligonucleotide was enzymatically digested following standard deprotection/detritylation. HPLC analysis revealed that the expected amide coupling products (**30**, **31**) were present in equimolar ratio. Carbamate (**10**), acetate (**25**), and isobutyrylated (**27**) nucleosides were not observed. The remainder of the resin was placed back on the oligonucleotide synthesizer, and the preparation of the protected eicosamer containing **1** at the fifth position of the eicosamer was completed. The second site was coupled to pyrene butyric acid and 9-(5-pentanoic acid)acridine, followed by capping. The crude mixture of deprotected oligonucleotides (**33**) was enzymatically digested and analyzed by reverse-phase HPLC.[28] In addition to **30** and **3l** being present in a 1:1 ratio, the second set of deoxyuridine derivatives (**26**, **32**) were also present in equal amounts to one another. In addition to the conjugated products, the digest of the crude eicosameric mixture contained a small amount of **25**, but carbamate (**10**) and isobutyrylated (**27**) nucleosides were not observed. The presence of small amounts of unreacted molecules is inconsequential for preparing a library. The mixture of oligonucleotides was further characterized by electrospray mass spectrometry, following their separation from the typical deletions produced during automated synthesis.



5'-HO−d(TAC G**X**A CTG ATG CAG C**Y**C GT)—OH
33 X = **26**, **32**; **Y** = **30**, **31**

Summary. Oligonucleotides containing modified C5-deoxyuridines can be prepared in high yields via "on-column"

conjugation of protected oligonucleotides containing a single reactive functional group. Oligonucleotide substrates containing alkylamines or alkyl carboxylic acids at defined sites are obtained by utilizing the appropriate photolabile phosphoramidite. The reactive functional groups are revealed in an orthogonal manner under photolysis conditions that do not damage the oligonucleotides. In addition to simplifying the synthesis of modified oligonucleotides, this method is directly applicable to expanding the diversity of oligonucleotide libraries.

Experimental Section

General Methods. All reactions were carried out in oven-dried glassware under an argon atmosphere, unless noted otherwise. Diisopropylethylamine, CH_2Cl_2, pyridine, and DMF (aspirator pressure) were distilled from CaH_2. THF was distilled from Na°/benzophenone ketyl. Oligonucleotides were synthesized by using an Applied Biosystems Incorporated 380B automated synthesizer on standard succinato long chain alkylamine controlled pore glass support purchased from Glen Research. Isobutyryl protected phosphoramidites were purchased from Glen Research and Pharmacia Biotech. Reverse-phase HPLC was carried out on a Rainin C18-Microsorb column (4.6 × 25 mm). Anion exchange HPLC was carried out on Vydac No. 301VHP575. Anion exchange HPLC conditions: solvent A, H_2O; solvent B, aqueous NaCl (1 M); 100% A to 100% B linearly over 40 min.

General Procedure for Photolytic Deprotection of Solid-Phase Supported Oligonucleotides Containing Modified Nucleotides (1, 2). Oligonucleotide bound to support (5−8 mg) was added to a Pyrex tube containing a stir bar constructed from a standard pipe cleaner (in order to not crush the CPG containing the oligonucleotide) and CH_3CN/H_2O (12 mL, 9:1 by volume). The tube was fitted with a rubber septum and sparged with N_2 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 (λ_{max} = 365 nm) for 2 h. It is important to maintain the temperature at ≤25 °C with a fan. The resin was filtered, washed with EtOAc (15 mL), followed by Et_2O (15 mL), collected, and placed in a screw-capped vial for storage.

Sample General Procedure for Conjugation of Protected Resin-Bound Oligonucleotides. A solution (33 mM) of the coupling reagents (11.2 mg of PyBOP and 8 μL of diisopropylethylamine (2 molar equiv)) in DMF (653 μL) was prepared in an oven-dried 1 dram vial equipped with a septum. A solution (33 mM) of biotin (7.1 mg in 746 μL of DMF) was prepared in a second oven-dried vial. The resin-bound DNA (2 mg, containing ∼66 nmol of DNA, based on trityl response) was treated with 20 μL (5 molar equiv) of a 1:1 mixture (by volume) of the PyBOP and biotin solutions. The reaction was capped and shaken at room temperature for 1 h. The resin was washed with DMF (3 × 50 μL) and dried in vacuo. The free-flowing resin was treated with 28% aqueous ammonia (600 μL) for 6 h at 55 °C and concentrated under vacuum. Detritylation was effected by adding 80% aqueous acetic acid (100 μL) to the resin for 20 min, at which time the reaction was quenched with absolute EtOH (100 μL) and concentrated to dryness. Purification of the conjugated oligonucleotide was carried out via 20% polyacrylamide denaturing gel electrophoresis. Isolated yields were obtained (O.D. 260 nm) by comparing the amount of conjugated oligonucleotide to the amount of unconjugated material subjected to identical deprotection, purification, and isolation conditions.

Synthesis of an Oligonucleotide Library. The pentameric oligonucleotide (**24**) was synthesized on a 1 μmol scale with standard DNA synthesis cycles. Resin corresponding to 0.2 μmol of the original loading was subjected to the photolysis and conjugation conditions described above, with the exception that biotin and *N*-acetyl phenylalanine were each present in 5 molar equiv relative to oligonucleotide. Following conjugation and washing of the support with DMF (3 × 100 μL) and CH_3CN (3 × 100 μL) sequentially, the support was placed back on the DNA synthesizer and extended to the fully protected eicosamer containing **1** at position 5. The resin was then photolyzed and conjugated to pyrene butyric acid and the acridine carboxylic acid as described. Deprotection was carried out via standard methods following removal of the excess reagents from the resin and washing, as described above.

Enzymatic Digestion of Modified Oligonucleotides. The oligonucleotide substrate (1 O.D. unit) was evaporated to dryness in a sterilized Eppendorf tube. The residue was resuspended in 1 M $MgCl_2$ (10 μL), (10×) calf intestine alkaline phosphatase buffer (10 μL), nuclease P1 (0.3 U/μL, 10 μL), snake venom phosphodiesterase (3×10^{-3} U/μL, 2 μL), and calf intestine alkaline phosphatase (10 U/μL, 2 μL) and diluted to a total volume of 100 μL with autoclaved, doubly deionized H_2O. The digest was incubated at 37 °C for 36 h. An aliquot of nuclease P1 (5 μL) was added after each 12 h incubation period. The digest was filtered through a 0.45 μM Nylon filter. The Eppendorf tube was rinsed with MeOH (5 × 100 μL), which was passed through the Nylon filter and combined with the original digest material. The mixture was evaporated to dryness, resuspended in MeOH (80 μL), and analyzed by reverse-phase HPLC. HPLC conditions: solvent A, 2.5% CH_3CN in KH_2PO_4 (10 mM, pH 6.0); solvent B, 72% CH_3CN in KH_2PO_4 (10 mM, pH 6.0); time 0, 100% A; linear gradient to 28% B over 15 min; hold at 28% B for 20 min; linear gradient to 100% B over 5 min; hold 100% B for 15 min.

Preparation of 5. To a solution of 3′,5′-*O*-bis-(*tert*-butyldimethylsilyloxy)-5-iododeoxyuridine (701 mg, 1.20 mmol) in degassed DMF (10 mL) were added with stirring $(PhP_3)_4Pd$ (139 mg, 0.12 mmol), CuI (46 mg, 0.24 mmol), Et_3N (243 mg, 2.4 mmol), and 5-hexyn-1-ol (353 mg, 3.6 mmol). The reaction mixture was allowed to stir for 18 h at 25 °C, at which time it was diluted with EtOAc (50 mL) and poured into a separatory funnel containing saturated $NaHCO_3$ (25 mL). The aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (1:4, EtOAc:Hex to 2:3, EtOAc:Hex) yielded **5** (461 mg, 70%) as a light yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.99 (s, 1H), 7.89 (s, 1H), 6.27 (dd, 1H, J = 6.6, 6.3 Hz), 4.38 (m, 1H), 3.89 (m, 2H), 3.71 (m, 3H), 2.39 (t, 2H, J = 6.3 Hz), 2.27 (m, 2H), 1.99 (p, 2H, J = 7.5 Hz), 1.68 (m, 4H), 0.91 (s, 9H), 0.86 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), 0.05 (s, 3H), 0.05 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 162.1, 149.2, 141.5, 100.7, 95.1, 88.3, 85.6, 72.2, 62.9, 62.2, 41.9, 31.9, 26.0, 25.7, 24.6, 19.4, 18.4, 18.0, −4.7, −4.9, −5.4, −5.5. IR (film): 3423, 3189, 3069, 2929, 2857, 1698, 1626, 1462, 1279, 1255, 1104 cm^{-1}. Anal. Calcd for $C_{27}H_{48}N_2O_6Si_2$: C, 58.66; H, 8.76; N, 5.07. Found: C, 58.64; H, 8.68; N, 4.91.

Preparation of the Mesylate of 5 (6). To a solution of **5** (462 mg, 0.84 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added with stirring diisopropylethylamine (152 mg, 1.18 mmol) and methanesulfonyl chloride (115 mg, 1.0 mmol) dropwise over 5 min. The mixture was warmed to room temperature over a 20 min period, at which time TLC analysis (4:1, EtOAc:Hex) indicated that the starting material had been completely consumed. The reaction was diluted with Et_2O (15 mL) and poured into a separatory funnel containing saturated $NaHCO_3$ (10 mL). The aqueous layer was extracted with Et_2O (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (3:7, EtOAc:Hex to 3:2, EtOAc:Hex) yielded the mesylate **6** (520 mg, 99%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 8.81 (s, 1H), 7.90 (s, 1H), 6.26 (dd, 1H, J = 7.2, 6.3 Hz), 4.37 (m, 1H), 4.25 (t, 2H, J = 6.0 Hz), 3.94 (m, 1H), 3.87 (dd, 1H, J = 11.4, 2.1 Hz), 3.73 (dd, 1H, J = 11.4, 2.1 Hz), 2.98 (s, 3H), 2.42 (t, 2H, J = 6.6 Hz), 2.27 (m, 1H), 3.01−1.84 (m, 4H), 1.67 (m, 3H), 0.91 (s, 9H), 0.96 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 161.8, 149.2, 141.8, 100.5, 93.8, 88.3, 85.6, 72.3, 69.5, 63.0, 42.0, 37.4, 28.3, 26.0, 25.7, 24.3, 19.1, 18.4, 18.0, −4.7, −4.9, −5.4, −5.5. IR (film): 3186, 3065, 2952, 2929, 2856, 1712, 1461, 1354, 1279, 1255, 1173, 1104 cm^{-1}.

Preparation of 7. To a solution of **6** (340 mg, 0.54 mmol) in DMF (5 mL) was added NaN_3 (207 mg, 2.7 mmol). The reaction mixture was heated to 35 °C for 2 h, after which it was diluted with EtOAc (15 mL) and poured into a separatory funnel containing saturated $NaHCO_3$ (10 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (1:4, EtOAc:Hex) yielded **7** (303 mg, 95%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 1H), 7.89 (s, 1H), 6.27 (dd, 1H, J = 7.5, 6.0 Hz), 4.38 (m, 1H), 3.95 (m, 1H), 3.88 (dd, 1H, J = 11.4, 2.4 Hz), 3.74 (dd, 1H, J = 11.4, 2.1 Hz), 3.30 (t, 2H, J = 6.6 Hz), 2.40 (t, 2H, J = 6.6

Hz), 2.27 (m, 1H), 1.74−1.58 (m, 5H), 0.92 (s, 9H), 0.86 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 161.7, 149.2, 141.6, 100.5, 94.0, 88.2, 85.6, 72.3, 72.1, 62.9, 51.0, 41.9, 28.1, 25.9, 25.6, 25.5, 19.2, 18.4, 18.0, −4.7, −4.9, −5.4, −5.6. IR (film): 3186, 3066, 2953, 2929, 2857, 2359, 2341, 2096, 1715, 1624, 1576, 1558, 1546, 1506, 1458, 1404, 1360, 1322, 1279, 1255, 1196, 1104, 1068, 1030 cm^{-1}. Anal. Calcd for $C_{27}H_{47}N_5O_5Si_2$: C, 56.12; H, 8.20; N, 12.13. Found: C, 55.96; H, 7.96; N, 11.84.

Preparation of 9. To a solution of **7** (302 mg, 0.51 mmol) in THF (5 mL) was added triphenylphosphine (141 mg, 0.53 mmol). After 6 h water (14 mL, 0.77 mmol) was added. The reaction was stirred for an additional 6 h, after which the volatiles were removed under vacuum to afford a white foam. Due to difficulties with purification, the crude amine (**8**) was carried on as is. To a solution of **8** (287 mg, 0.51 mmol) in THF (10 mL) was added diisopropylethylamine (92 mg, 0.77 mmol), followed by neat 4,5-dimethoxy-2-nitrobenzyl chloroformate (155 mg, 0.56 mmol). The reaction was protected from light and stirred at 25 °C for 1 h. The reaction mixture was diluted with Et_2O (15 mL) and poured into a separatory funnel containing saturated $NaHCO_3$ (15 mL). The aqueous layer was extracted with Et_2O (2 × 10 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (2:3, EtOAc:Hex) afforded the carbamate **9** (309 mg, 75% yield over two steps) as a light yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.46 (s, 1H), 7.91 (s, 1H), 7.68 (s, 1H), 7.02 (s, 1H), 6.26 (t, 1H, J = 6.3 Hz), 5.48 (s, 2H), 5.30 (m, 1H), 4.38 (m, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 3.88 (dd, 1H, J = 11.4, 1.8 Hz), 3.24 (m, 2H), 2.38 (t, 2H, J = 6.3 Hz), 2.27 (m, 1H), 1.99 (m, 1H), 1.64 (m, 6H), 0.91 (s, 9H), 0.86 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 162.1, 155.9, 153.5, 149.1, 147.9, 141.6, 139.6, 128.7, 109.9, 108.0, 100.5, 94.6, 88.3, 85.6, 72.2, 72.0, 63.3, 62.9, 56.4, 56.3, 41.9, 40.4, 28.9, 25.9, 25.7, 25.2, 19.2, 18.4, 17.9, −4.7, −4.9, −5.4, −5.6. IR (film): 3343, 3183, 3067, 2952, 2930, 2856, 2359, 2340, 1713, 1621, 1581, 1521, 1461, 1327, 1277, 1255, 1220, 1124, 1067 cm^{-1}. Anal. Calcd for $C_{37}H_{58}N_4O_{11}Si_2$: C, 56.18; H, 7.39; N, 7.08. Found: C, 56.24; H, 7.17; N, 6.92.

Preparation of Free Carbamate Nucleoside (10). To a solution of **9** (309 mg, 0.38 mmol) in DMF (1 mL) at 25 °C (protected from light) was added a solution (0.5 M) of tetrabutylammonium fluoride (1.68 mL, 0.84 mmol) in DMF.[31] After 1 h, the reaction was diluted with EtOAc (25 mL) and poured into a separatory funnel containing saturated $NaHCO_3$ (15 mL). The aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (1:19, MeOH:EtOAc) yielded the free nucleoside **10** (200 mg, 80%). ^{1}H NMR (MeOH-d_4): δ 8.14 (s, 1H), 7.72 (s, 1H), 7.14 (s, 1H), 6.18 (t, 1H, J = 6.3 Hz), 5.44 (s, 2H), 4.36 (m, 1H), 3.94 (s, 3H), 3.89 (s. 3H), 3.79 (dq, 2H, J = 11.4, 1.8 Hz), 3.17 (m, 2H), 2.41 (t, 2H, J = 6.3 Hz), 2.20 (m, 2H), 1.61 (m, 4H). IR (film): 3361, 3066, 2929, 1697, 1521, 1457, 1324, 1270, 1221, 1059 cm^{-1}. Anal. Calcd for $C_{25}H_{30}O_{11}N_4$: C, 53.36; H, 5.38; N, 9.96. Found: C, 53.50; H, 5.55; N, 9.72.

Preparation of 5′-*O*-Dimethoxytritylated 10 (11). To a solution of **10** (200 mg, 0.33 mmol) in pyridine (6 mL) protected from light was added dimethoxytrityl chloride (134 mg, 0.40 mmol). The reaction was allowed to stir overnight at 25 °C, after which it was quenched with excess methanol. The reaction mixture was diluted with EtOAc (30 mL) and poured into a separatory funnel containing saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (EtOAc) afforded **11** (241 mg, 81%), which was contaminated with a small amount of inseparable pyridinium salts. The material was used in this form during the phosphoramidite preparation (next step). ^{1}H NMR ($CDCl_3$): δ 8.62 (s, 1H), 8.06 (s, 1H), 7.69 (s, 1H), 7.46 (d, 2H, J = 1.46 Hz), 7.33 (m, 8H) 7.05 (s, 1H), 6.84 (d, 4H, J = 8.8 Hz) 6.34 (t, 1H, J = 7.3 Hz), 5.48 (s, 2H), 5.39 (t, 1H, J = 5.5 Hz), 4.54 (m, 1H), 4.11 (m, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.79 (s, 6H), 3.43 (dd, 1H, J = 8.1, 2.9 Hz), 3.25 (dd, 1H, J = 8.1, 2.9 Hz), 3.07 (m, 2H), 2.50 (m, 1H, J = 6.3 Hz), 2.26 (m, 1H), 2.10 (t, 1H, J = 6.9 Hz), 1.42 (m, 2H), 1.27 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 166.7, 157.5, 154.0, 151.5, 149.0, 144.7, 143.5, 140.0, 137.1, 135.2, 131.1, 125.5, 124.0, 123.6, 122.5, 108.8, 105.7, 103.6, 96.3, 91.6, 82.5, 81.9, 81.0, 67.8, 66.7, 58.9, 58.8, 55.9, 51.9, 50.7, 37.0, 35.9, 24.3, 20.6, 16.6, 14.6, 9.7. IR (film) 3374, 2936, 2359, 1698, 1607, 1581, 1509, 1461, 1328, 1277, 1249, 1220, 1176, 1066 cm^{-1}.

Preparation of 12. To a solution of **11** (241 mg, 0.28 mmol) in CH_2Cl_2 (10 mL) protected from light was added diisopropylethylamine (180 mg, 1.4 mmol). The reaction was cooled to 0 °C and 2-cyanoethyl diisopropylchlorophosphoramidite (69.3 mg, 0.29 mmol) was added dropwise over 15 min. After 30 min, the reaction was quenched with methanol (12 mL, 0.32 mmol). The mixture was diluted with freshly distilled EtOAc (15 mL) and poured into a separatory funnel containing saturated Na_2CO_3 (15 mL). The organic layer was washed with brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. Chromatography (9:1, EtOAc:Hex) yielded **12** (286 mg, 78%) as a light yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.94 (br, 1H), 8.16 (s, 0.5H), 8.11 (s, 0.5H), 7.72 (s, 1H), 7.72−7.24 (m, 9H), 7.07 (s, 1H), 6.86 (m, 4H), 6.11 (m, 1H), 5.51 (s, 2H), 5.12 (m, 1H), 4.60 (m, 1H), 4.22−4.12 (m, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.89−3.45 (m, 8H), 3.31−3.28 (m, 5H), 3.05 (p, 1H, J = 6.6 Hz), 2.67−2.5 (m, 2H), 2.33 (p, 2H, J = 6.1 Hz), 2.08 (p, 2H, J = 6.1 Hz), 1.45−1.35 (m, 2H), 1.31−1.18 (m, 10H), 1.06 (t, 3H, J = 6.6 Hz). ^{31}P NMR (MeOH-d_4, H_3PO_4 reference): δ 149.6, 149.2. HRMS (FAB) calcd 1065.4375 (M + H), found 1065.4373.

Preparation of 13. Pyridinium dichromate (3.93 g, 10.5 mmol) was added to a solution of **5** (1.15 g, 2.1 mmol) in DMF (10 mL) with stirring. The reaction was allowed to stir at room temperature for 3 h, after which two drops of trifluoroacetic acid were added. TLC analysis (4:1, EtOAc:Hex) indicated that the starting material was completely consumed within 15 min. The reaction was diluted with EtOAc (60 mL) and poured into a separatory funnel containing H_2O (40 mL). The aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the crude acid as an orange oil. Typically, the crude product (**13**) was carried on without purification. However, **13** could be purified by chromatography (4:1, EtOAc: Hex to EtOAc) ^{1}H NMR ($CDCl_3$): δ 9.09 (s, 1H), 7.98 (s, 1H), 6.32 (t, 1H, J = 6.3 Hz), 4.44 (m, 1H), 4.0 (m, 1H), 3.94 (d, 1H, J = 11.1 Hz), 3.79 (d, 1H, J = 11.4 Hz), 2.54 (m, 4H), 2.34 (m, 1H), 2.08 (m, 1H), 1.96 (m, 2H), 0.97 (s, 9H), 0.93 (s, 9H), 0.18 (s, 3H), 0.17 (s, 3H), 0.12 (s, 3H), 0.11 (s, 3H). IR (film): 3188 (bd), 3064, 3033, 2922, 2850, 2358, 1697, 1497, 1449, 1381, 1337, 1309, 1295, 1272, 1214, 1170 cm^{-1}. HRMS (FAB) calcd 567.2922 (M + H), found 567.2921.

Preparation of 14. To a solution of **13** (1.18 g, 2.1 mmol) in CH_2Cl_2 (25 mL) was added DMAP (26 mg, 0.21 mmol), DCC (431 mg, 2.31 mmol), and dimethoxynitrobenzyl alcohol (446 mg, 2.31 mmol). The reaction mixture was protected from light. After the solution was stirred for 24 h, the reaction was filtered through a pad of Celite (2 cm), and the filter cake rinsed with additional CH_2Cl_2 (15 mL). The filtrate was poured into a separatory funnel containing EtOAc (75 mL) and saturated $NaHCO_3$ (25 mL). The aqueous layer was extracted with EtOAc (2 × 25 mL) and the combined organic layers washed with brine (25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a yellow oil. Chromatography (3:2 EtOAc:Hex) afforded **14** (0.95 g, 60% overall from **5**) as a light yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.43 (s, 1H), 7.93 (s, 1H), 7.73 (s, 1H), 7.04 (s, 1H), 6.29 (t, 1H, J = 6.2 Hz), 5.53 (s, 2H), 4.42 (m, 1H), 4.0 (s, 3H), 3.99 (m, 1H), 3.97 (s, 3H), 3.91 (dd, 1H, J = 11.4, 1.2 Hz), 3.77 (dd, 1H, J = 11.7, 1.8 Hz), 2.63 (t, 2H, J = 7.2 Hz), 2.50 (t, 2H, J = 7.2 Hz), 2.33 (m, 1H), 2.01 (m, 3H), 0.93 (s, 9H), 0.91 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H), 0.09 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 172.6, 161.7, 153.8, 149.3, 148.4, 142.1, 140.1, 127.4, 110.5, 108.4, 100.6, 93.7, 88.6, 85.9, 72.6, 63.4, 63.2, 56.7, 56.6, 42.2, 33.2, 26.2, 26.0, 23.8, 19.3, 18.6, 18.2, −4.4, −4.6, −5.2, −5.3. IR (film): 3186, 3067, 2953, 2930, 2856, 2359, 1715, 1622, 1581, 1523, 1462, 1327, 1279, 1256, 1221, 1067 cm^{-1}. HRMS (FAB) calcd (M + H) 762.3455, found 762.3467.

Preparation of 15. To a solution of **14** (0.95 g, 1.7 mmol) in THF

(31) Majetich, G.; Casares, A.; Chapman, D.; Behnke, M. *J. Org. Chem.* **1986**, *51*, 1745.

(5 mL) at room temperature (protected from light) was added triethylamine trihydrofluoride (1 mL, 8.5 mmol). After the mixture was stirred for 24 h, the reaction was quenched with saturated $NaHCO_3$ (15 mL). The mixture was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (5% MeOH, EtOAc) afforded **15** (0.51 g, 74%) as a light yellow foam. ^{1}H NMR (MeOH-d_4): δ 8.18 (s, 1H), 7.77 (s, 1H), 7.19 (s, 1H), 6.23 (t, 1H, J = 6.3 Hz), 5.51 (dd, 2H, J = 25.2, 15 Hz), 4.42 (m, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.83 (dd, 1H, J = 12.0, 3.3 Hz), 3.75 (dd, 1H, J = 10.2, 3.3 Hz), 2.67 (t, 2H, J = 6.9 Hz), 2.52 (t, 2H, J = 6.6 Hz), 2.27 (m, 2H), 1.97 (m, 2H). IR (KBr) 3446, 3217, 3065, 2943, 2857, 2563, 2356, 1698, 1578, 1523, 1458, 1327, 1273, 1218, 1147, 1060 cm^{-1}. HRMS (FAB) calcd (M + H) 534.1724, found 534.1735.

Preparation of 5′-*O*-Dimethoxytritylated 15 (16). To a solution of **15** (438 mg, 0.80 mmol) in pyridine (6 mL) protected from light was added dimethoxytrityl chloride (335 mg, 1.0 mmol). After the mixture was stirred overnight at room temperature, the reaction was quenched with MeOH (5 mL). The reaction mixture was poured into a separatory funnel containing saturated $NaHCO_3$ (50 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. Chromatography (EtOAc) afforded **16** (562 mg, 82%) as a light yellow foam. ^{1}H NMR ($CDCl_3$): δ 9.25 (s, 1H), 8.04 (s, 1H), 7.74 (s, 1H), 7.45 (d, 2H, J = 7.5 Hz), 7.34 (m, 7H), 7.04 (s, 1H), 6.88 (d, 4H, J = 12 Hz), 6.34 (t, 1H, J = 6 Hz), 5.50 (s, 2H), 4.56 (m, 1H), 4.13 (m,1H), 4.0 (s, 3H), 3.97 (s, 3H), 3.80 (s, 6H), 3.40 (m, 2H), 2.74 (s, 1H), 2.53 (m, 1H), 2.49 (t, 2H, J = 7.5 Hz), 2.31 (m, 1H), 2.22 (t, 2H, J = 7.2 Hz), 1.69 (p, 2H, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$): δ 172.3, 161.8, 158.5, 153.5, 149.4, 148.1, 144.5, 141.8, 139.8, 135.5, 129.9, 129.0, 127.9, 127.2, 126.9, 112.2, 110.2, 108.1, 100.7, 93.6, 86.9, 85.6, 72.3, 71.8, 63.5, 63.1, 56.5, 56.3, 55.2, 41.4, 32.8, 23.3, 18.8. IR (film) 3482, 3183, 3067, 2941, 2835, 2314, 1713, 1689, 1607, 1583, 1510, 1462, 1327, 1274, 1245, 1221, 1177, 1066, 1028 cm^{-1}. HRMS (FAB) calcd (M + H) 836.3032, found 836.3014.

Preparation of 17. To a solution of **16** (182 mg, 0.22 mmol) in CH_2Cl_2 (10 mL) protected from light was added diisopropylethylamine (140 mg, 1.1 mmol). The reaction was cooled to 0 °C, and 2-cyanoethyl diisopropylchlorophosphoramidite (54 mg, 0.23 mmol) was added dropwise over 15 min. After the mixture was stirred for an additional 30 min, the reaction was quenched with MeOH (9 mL, 0.28 mmol). The mixture was diluted with freshly distilled EtOAc (15 mL) and poured into a separatory funnel containing saturated Na_2CO_3 (15 mL). The organic layer was washed with brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. Chromatography (9:1, EtOAc: hexane) afforded **17** (191 g, 85%) as a light yellow foam. ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 0.5H), 7.99 (s, 0.5H), 7.67 (s, 1H), 7.40 (m, 2H), 7.26 (m, 7H), 6.95 (s, 0.5H), 6.94 (s, 0.5H), 6.80 (m, 4H), 6.25 (m, 1H), 5.43 (s, 2H), 4.55 (m,1H), 4.13 (m, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H), 3.71 (m, 1H), 3.54 (m, 1H), 3.38 (m, 1H), 3.26 (m, 1H), 2.58 (t, 1H, J = 7.0 Hz), 2.50 (m, 1H), 2.39 (t, 1H, J = 7.0 Hz), 2.26 (m, 2H), 2.11 (m, 2H), 1.56 (m, 2H), 1.22 (m, 2H), 1.11 (m, 8.5H), 1.03 (d, 3.5H, J = 6.9 Hz). ^{31}P NMR (MeOH-d_4, H_3PO_4 reference): δ 148.8, 148.4. Anal. Calcd for $C_{54}H_{62}N_5O_{14}P$: C, 62.58; H, 6.03; N, 6.76. Found: C, 62.49; H, 6.20; N, 6.52.

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Supporting Information Available: Table of HPLC response factors for nucleosides; experimental procedures for the preparation of **25**−**27** and **30**−**32**; reverse-phase HPLC chromatograms of enzyme digests; electrospray mass spectra of oligonucleotides (**21a**−**h**, **29**). This material is available free of charge via the Internet at http://pubs.acs.org.

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